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Algae Cultivation for Carbon Capture, Waste Management, and Supplement Development

by

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Abstract

Algae research is attractive for biofuel, bioplastic, pigment, and livestock feed production as well as carbon capture and waste mitigation. This project investigates the growth of algae for sustainable purposes. This includes the examination of *Scenedesmus acutus* and *Coelastrella sp.* under various growth and stress conditions. The first section of this project includes a media screening for *Coelastrella sp.* growth and astaxanthin production under brackish conditions, light intensity, nitrogen deprivation, and sodium bicarbonate exposure. The second section includes *S. acutus* growth with sodium bicarbonate and carbonate pH control, a cobalt catalyst, amine solution, various nitrogen sources, and ammonia gas sparging. *Coelastrella sp.* growth benefitted from BG-11 media opposed to urea, and tolerated all salinities within brackish concentrations, although productivity was higher at lower concentrations for the algal species. Increased astaxanthin productivity was observed in *Coelastrella sp.* for a combination of nitrogen deprivation with 5 g/L salinity, increased light intensity, and light intensity paired with sodium bicarbonate. A cobalt catalyst provided shading, which inhibited *S. acutus* growth. Ammonium bicarbonate and carbonate resulted in higher algal growth in comparison to urea, but equal growth compared to BG-11 with 25% $NaNO_3$. *S. acutus* productivity was improved by a CO_2/NH_3 gaseous ratio of 10 compared to other ratios, BG-11, and urea, resulting in algal biomass with high protein content. The results offer a foundation for further optimization of algal growth based on either the desired mitigation strategy (wastewater, CO_2 uptake, or NH_3 gas scrubbing) or the desired harvested biomass utilization.

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INTRODUCTION

Algae species currently account for nearly 50% of earth's oxygen production and algae (along with coral and other vegetation) store 93% of earth's CO_2 [1]. Thus, algae have a highly relevant impact on earth's atmospheric composition. In turn, algae are also highly impacted by earth's atmosphere. Climate change is shifting components of earth's climate including increasing temperatures, decreased light availability, and ocean acidification, all of which significantly impact algae diversity, physiology, and proliferation [1]. Further, the effects of climate change will lead to an increase in the toxicity of algal species, amplify the impacts of algal blooms, and cause ecosystem disruption [1]. Climate change is a prominent issue within the algae research field.

One proposed method of addressing the issue of high atmospheric CO_2 concentration is the growth of algae as a mitigation strategy and as biofuel feedstock. Biofuel has potential to significantly impact the global energy crisis, and algae utilization as a biofuel feedstock is an advantageous option because algae can produce 300x more oil than crop plants [2]. Algae cultivation is also beneficial due to their adaptability, low ecological impact (when properly performed), bioremediation effects, and commercial competitivity [2].

Coelastrella sp. productivity and astaxanthin production

The first aspect of algae cultivation to be explored in this review is the production of carotenoids in *Coelastrella sp.* for supplement development. There are currently 750 known carotenoid compounds which exhibit a yellow, orange, and red pigmentation [3]. The keto-carotenoid focused on in this study was astaxanthin, a red carotenoid pigment produced by *Coelastrella sp.* to protect the algae cell from UV damage and other stressors.

Astaxanthin, along with other carotenoids, is in high demand and has high market value because carotenoids are antioxidants that function as nutraceutical supplements. The conjugated double bond is the primary structural element that results in their coloration and antioxidant properties [3]. The health benefits of a carotenoid nutraceutical supplements include a preventative function in liver protection, antibacterial properties, and skin health [3].

Media. The ability of microalgae to prosper in a variety of environments is one aspect that intrigues researchers to investigate their cultivation, especially considering microalgal applications such as biofuels, bioplastics, supplements, livestock feed production, carbon capture and much more. The investigation of microalgal media could also lend itself to applications in bioremediation and waste management and utilization. In the spirit of a circular economy, *Scenedesmus sp.* has been cultivated with BG-11 and 50% industrial wastewater for a high CO_2 capture efficiency of 446 ±150 mg CO_2/L/day [4]. *Acutodesmus dimorphus* cultivated in raw dairy wastewater was able to produce 5-6 times more biomass than BG-11, consisting of 25% lipid (biodiesel applicable) and 30% carbohydrate (bioethanol applicable), producing a commercially attractive management technique for dairy waste [5]. Wheat bran, a fibrous by-product of industrial wheat processing typically disposed of by an air-polluting burning process, has been evaluated as a zero-cost media alternative (supplemented by garlic powder for fungal pathogen control) to produce high biomass and pigment productivity as well as low biomass and pigment production costs [6]. Alternative feed sources are widely studied for different algal strains to reduce production costs and increase desired productivity.

Media sources have been studied for cost and productivity optimization for many microalgae. *Scenedesmus abundans* cultivated in 0.32 g KNO_3/L-modified CHU-13 media was found to produce the highest biomass compared to Bold's Basal Medium (BBM) and BG-11

media, although all media cultures had promising productivity for bio-energy potential [7]. BG-11 is often studied since it is known to increase productivity despite not being viable for industrial-scale algae cultivation. Urea, a relatively low-cost agricultural-grade chemical, is a primary growth medium determined as the cheapest nitrogen source for *Scenedesmus dimorphus* pigment productivity compared to ammonium sulfate and sodium nitrate [6]. Studying the viability of urea and BG-11 in *Coelastrella sp.* builds a basis for understanding the optimum conditions for algal growth and pigment production.

Brackish conditions. Algae cultivation utilizes substantial amounts of water and media, so reducing this usage is important to enhance the economic benefits of algae cultivation in light of the global water crisis. To address this issue, media recycling has been studied to reduce cost and water use, but often inhibits algal growth. Optimization of the media recycling process has been reviewed and studied, with the conclusion that certain robust algae strains, extraction during the exponential growth phase, and induced flocculation conditions are often uninhibited by media recycling [8, 9, 10, 11]. However, this practice still presents limitations and requires optimization for large-scale algae cultivation.

Further considering the consumption of freshwater by algal cultivation, investigating the utilization of salt-water that is not biologically available for human consumption is of interest. Although sea water, typically ~35 g/L NaCl, is not conducive to freshwater microalgae growth, a mixture of fresh water and saltwater could reduce freshwater consumption within algae cultivation. This mixture, termed brackish water, consists of salt concentrations anywhere from 0.1 to 35 g/L. Understanding a freshwater algae strand's ability to utilize brackish water could result in a large reduction of the freshwater demand that algae cultivation requires, enabling the practice to be more sustainable in light of the global water crisis.

Light intensity and nitrogen deprivation. Light exposure, nitrogen deprivation, and high salt concentration increase the production of carotenoids in *Coelastrella sp.*. This increase in production has been attributed to the singlet oxygen quenching and radical scavenging enabled by substitutions at the hydrocarbon molecule [3]. Examining which stressors significantly increase the astaxanthin production in *Coelastrella sp.* could aid in increasing the profitability of algae cultivation.

Sodium bicarbonate and light stress. Sodium bicarbonate has been studied in algal species to enhance fatty acid methyl esters (FAMEs) production. FAMEs are the constituents of biodiesel, which can be derived from triacylglycerol (TAG) [12]. *Scenedesmus* sp. strain WC-1 grown rapidly in a green phase with KOH-adjusted pH of 8.7, BBM, 14:10 h light-dark cycling, and 5% CO_2 bubbling (doubling growth rate but lowering pH), caused cessation of cell replication and rapid TAG accumulation once a nitrogen depletion-signaled red stage was initiated with the addition of 50 mM sodium bicarbonate and ambient air bubbling [12]. *Scenedesmus obliquus* growth rate decreased at higher sodium bicarbonate concentrations, primarily due to pH changes [13]. So, sodium bicarbonate introduction is primarily considered during a stress phase following a previous growth phase.

The addition of sodium bicarbonate during a red phase could also be beneficial for astaxanthin production. *Haematococcus pluvialis* was cultured with 2.5 mM sodium bicarbonate, under pH control conditions, enabling cells to produce astaxanthin faster, especially when preceding a stress phase with high concentrations of sodium bicarbonate (50 mM), light intensity, and nitrogen deprivation [14].

A combination of sodium bicarbonate, nitrogen depletion, and light intensity stressors has been shown to increase TAG accumulation in microalgal species and astaxanthin production in red algal cells. Examining the effect of the combination of these stressors in *Coelastrella sp.* could be beneficial to further increase astaxanthin productivity in microalgal cells.

Scenedesmus acutus productivity

Sodium carbonate and sodium bicarbonate pH control with cobalt catalyst and amine supplementation. Improving the CO_2 capture capabilities of microalgae can be pursued by the use of catalysts or a combination of supplements.

This strategy is known to be effective, but the extent to which algae cultivation can utilize atmospheric CO_2 could still be improved. Catalysts can be used to increase CO_2 uptake in chemical processes. This study focused on the use of a cobalt catalyst to improve the ability of *S. acutus* to absorb CO_2 with the aid of sodium carbonate and sodium bicarbonate to control pH. To address the high demand of oil from alternative feedstocks, cobalt catalysts have been researched as a method for refining diesel-range hydrocarbons to gasoline-range hydrocarbons since they enable selectivity of gasoline-range hydrocarbons [15]. Cobalt catalysts also have industrial interest in converting CO_2 emissions to usable carbon structures. Three structurally rigid, robust, and reusable cobalt (II) metal-organic frameworks have been developed to capture gaseous CO_2 at atmospheric pressure with high yield and selectivity [16]. A low-cost cobalt-coordinated conjugated microporous polymer was developed that could convert CO_2 to cyclic carbonates with high efficiency at ambient temperature and atmospheric pressure, which is useful in the production of pharmaceuticals, chemicals, and lithium batteries [17]. A cobalt catalyst, Co-Tpy-C, was shown to efficiently convert CO_2 to CO with a faradaic efficiency over 90%, so CO could

be sold to metal and chemical manufacturing industries upon purification [18]. Cobalt (II) and zinc catalysts in an amine-based solution improved carbon capture from flue gas [19].

To supplement algal cells with cobalt for carbon capture, it is important to consider the uptake of cobalt into the cells or the effect of cobalt on the environment. Cobalt naturally occurs in cobalamin (also known as vitamin B_{12}) and is involved in the enzymatic reaction of inorganic carbon assimilation assisted by methylmalonyl CoA [2]. Not all forms of cobalamin are biologically available to all strains of algae and may interact differently with each species [2]. However, the direct availability of cobalt in solution can increase the production of cobalamin in algal cells [20]. Examining the interaction of *S. acutus* and a cobalt catalyst is important not only to understand this interaction, but to determine if cobalt availability increases the capacity of algae cells to absorb CO_2. Using cobalt could also make use of coal ash in which cobalt is present [20].

S. acutus naturally contains 30 total amines, ten of which are majorly present, researched, and quantified thoroughly [21]. Polyamines are known to indicate vitality and have been shown to increase as a response to environmental stress like that caused by light intensity, which stimulates non-photochemical quenching as a defense mechanism that converts excess light energy to heat, a response observed in *Scenedesmus obliquus* [22]. This suggests that an excess of amine groups in solution could trigger an increased stress tolerance in algal cells. This possibility, along with the CO_2 capture increase that has been observed in cobalt catalyst-containing amine solutions, could enable *S. acutus* to increase tolerance and CO_2 capture at higher pH levels.

Finally, pH control within this study is crucial because high pH conditions have been observed to increase cobalt absorption by algal species *Chlorella salina* [20]. Two different pH control strategies were utilized in this study, including sodium bicarbonate and sodium carbonate. Saline conditions have also been shown to increase cobalt uptake in algal cells, so these conditions could be worth studying in a continuation of this study [20].

Ammonium nitrogen sources. Microalgae can tolerate stressful conditions and utilize a variety of nutrients for nourishment. Understanding the range of nitrogen sources which can nourish algae is important for understanding what possible gaseous or dissolved solid pollutants algae could capture and utilize to manage chemical waste and/or to reduce the cost of media sources for algal biomass production.

Identifying alternative nitrogen sources is one of the primary methods through which media are optimized for algal growth since biologically available nitrogen sources are a primary cost concern, nitrate sources often being more expensive than ammonia. One study of *Scenedesmus sp.* BR003 exemplified that the algal strand could utilize commercial-grade ammonium-based media, although high ammonium concentrations altered coagulation [23]. The ability of algae to thrive off commercial-grade ammonium sources supports the potential of agricultural-grade media, further reducing the price of an already low-cost alternative to BG-11 [23]. Here, Soares et al. also demonstrates the robust nature of Scenedesmus to handle different nitrogen sources, further justifying the selection of *S. acutus* for this study [23].

The use of medias with ammonium-based nitrogen sources has drawbacks. One study that cultivated *Scenedesmus rubescens* in indoors photobioreactors (PBRs) and outdoor raceway ponds with ammonium carbonate, urea, and sodium nitrate as nitrogen sources, found that

cultures grown using media with ammonium-based nitrogen sources had the fastest growth rate during the first five days but fell behind in the following days [24]. Another study that cultivated *Scenedesmus sp.* LX1 with various nitrogen sources found that ammonium-based nitrogen sources led to a decrease in productivity and nitrogen efficiency rate compared to nitrate and urea, which was due to acidic solution conditions resulting from NH_4^+ releasing H^+ ions upon dissolution [25]. The use of ammonium-based media during algae cultivation still needs to confront these challenges and be optimized.

Ammonium bicarbonate and ammonium carbonate have their own chemical considerations when introduced to algal systems. Ammonium bicarbonate tends to crystalize in ammonia-based CO_2 capture processes, so it is important to consider the solubility of ammonium bicarbonate in algae cultures, which are primarily aqueous and bubbled with CO_2 [26]. NH_3 scrubbing is another technique researched for CO_2 capture involving aqueous solutions of ammonium carbonate and bicarbonate. Aqueous ammonium ion solutions are exposed to CO_2 to capture the latter and the reaction between NH_3, H_2O, and CO_2 has been studied [27]. Being aware of NH_3 scrubbing is important since this CO_2-absorbing chemical reaction could potentially be competing with the algal cells.

Ammonia gas sparging. Ammonia gas is a pollutant and a toxic emission resulting from agriculture and livestock, but also partially attributed to industrial processes. Ammonia and CO_2 emissions are a large concern for the livestock industry. *Scenedesmus dimorphus* had a removal efficiency >95% with an ammonia feed of 41-42 mg/L/day, the resulting biomass displaying a high protein content which would render it ideal for animal feed applications. Ammonia has been shown to be low-cost, efficient, and recyclable when used to induce flocculation during

harvesting. High concentrations of ammonia stunt growth by inhibiting the photosynthetic process, but protein content increases and lipid and carbohydrate content decreases [28]. Microalgae has also been employed in the treatment of high-ammonia wastewater [28]. Algae may be able to utilize this toxic gas from emission streams as a nitrogen source to mitigate its effects on the environment while also capturing carbon and producing biomass. Ammonia is attractive for its low cost and low viscosity. *Scenedesmus acutus* was selected due to previous work in which this strain exhibited both resilience and productivity under stressful conditions, including alkaline pH.

OBJECTIVES

Coelastrella sp. productivity and astaxanthin production

To investigate factors that contribute to *Coelastrella sp.* growth and astaxanthin production.

Media. A screening was conducted to compare urea and BG-11 to determine the best growth medium for *Coelastrella sp..*

Brackish conditions. To provide a foundation for the light intensity and nitrogen deprivation experiment, a preliminary study was conducted to examine the tolerance of *Coelastrella sp.* to various salt concentrations.

Light intensity and nitrogen deprivation. The intersectional effect of a brackish conditions with stress conditions (increased light exposure or nitrogen deprivation) was examined in terms of algae productivity and carotenoid production.

Sodium bicarbonate and light stress. The productivity of *Coelastrella sp.* and the production of astaxanthin were studied by stressing the algae with increased light intensity and sodium bicarbonate concentrations during a red phase preceded by a green growth phase.

Scenedesmus acutus productivity

To investigate factors that contribute to *S. acutus* growth and CO_2 uptake.

Sodium carbonate and sodium bicarbonate pH control with cobalt catalyst and amine supplementation. In this experiment, algae were cultured in BG-11 medium with a cobalt catalyst and amine to study the productivity of *S. acutus* using sodium bicarbonate and sodium carbonate for pH control.

Ammonium nitrogen sources. In this experiment, *S. acutus* was cultured in both urea and BG-11 media without the traditional nitrogen source to determine strand productivity with different nitrogen sources.

Ammonia gas sparging. In this experiment, *S. acutus* was cultured in BG-11 medium without the traditional nitrogen source to examine the effect of a range of CO_2/NH_3 mole ratios as a carbon and nitrogen source for *S. acutus* as well as variations in total gas flowrate.

METHODS

Coelastrella sp. productivity and astaxanthin production

Media. 10 L PBRs were seeded with *Coelastrella sp.* cultures which were grown in urea media since 2015. The cultures were exposed to continuous lighting, bubbled with a blend of gaseous N_2 and CO_2, and kept at ambient temperature. A media comparison was conducted between urea, BG-11, and BG-11 brackish media (0.5g/L of NaCl added) with continuous light

exposure. Sampling was conducted 3 days per week and measured pH, UV-vis spectroscopy, and dry mass weight [29].

Brackish conditions. Since *Coelastrella sp.* thrived in BG-11, a further media screening was conducted incrementally increasing salinity concentration. These concentrations included 0.5, 1, 5, 10, 20, and 30 g/L of added NaCl. Outdoor experimentation was conducted in parallel, which consisted of two raceway ponds cultivating *Coelastrella sp.* with BG-11 and 0.5 g/L added NaCl. This involved a 50% dilution once per week to achieve consistent productivity. Sampling was conducted 3 days per week and measured pH, UV-vis spectroscopy, and dry mass weight [29].

Light intensity and nitrogen deprivation. To examine the stress response of *Coelastrella sp.* in terms of carotenoid production, *Coelastrella sp.* was cultured in BG-11 medium before exposure to stressors. This required two steps: a growth step (green phase) and stress step (red phase). Each phase included triplicates of a control (freshwater) group and a saltwater group. The PBRs for the control group were seeded from 12 L of algae that had been centrifuged to remove excess salt. The remaining 9 PBRs were seeded in salt solutions of 10, 20, and 30 g/L, from 30 L of algae previously cultured in a 10 g/L salt solution. All reactors were filled to 10 L total volume. Algae samples from the control group and 10 g/L group were maintained in small, dense cultures. Algae in each group was grown in the green phase for 12 days, during which sampling was conducted every 2 days and measured pH, UV-vis spectroscopy, and dry mass weight [29].

The green phase was conducted twice, and each run was fundamentally the same leading up to the red phase. Each red phase lasted for one week and involved analyses for density through gravity filtration and light absorbance at wavelengths of 680 nm and 450 nm via UV

spectroscopy to evaluate chlorophyll A and astaxanthin production, respectfully. In red phase 1, conditions were the same as the green phase except all reactors were exposed to double the initial light exposure. In red phase 2, conditions were the same as the green phase except at the beginning of the period, when the algae was given a nutrient medium without the main nitrogen source (sodium nitrate).

The reactors were thoroughly cleaned before each green phase. The reactors for the second red phase were seeded with small cultures grown from samples taken in green phase 1 and allowed to grow dense for one week.

Figure 1 below is a graphic representation of the methodology employed.

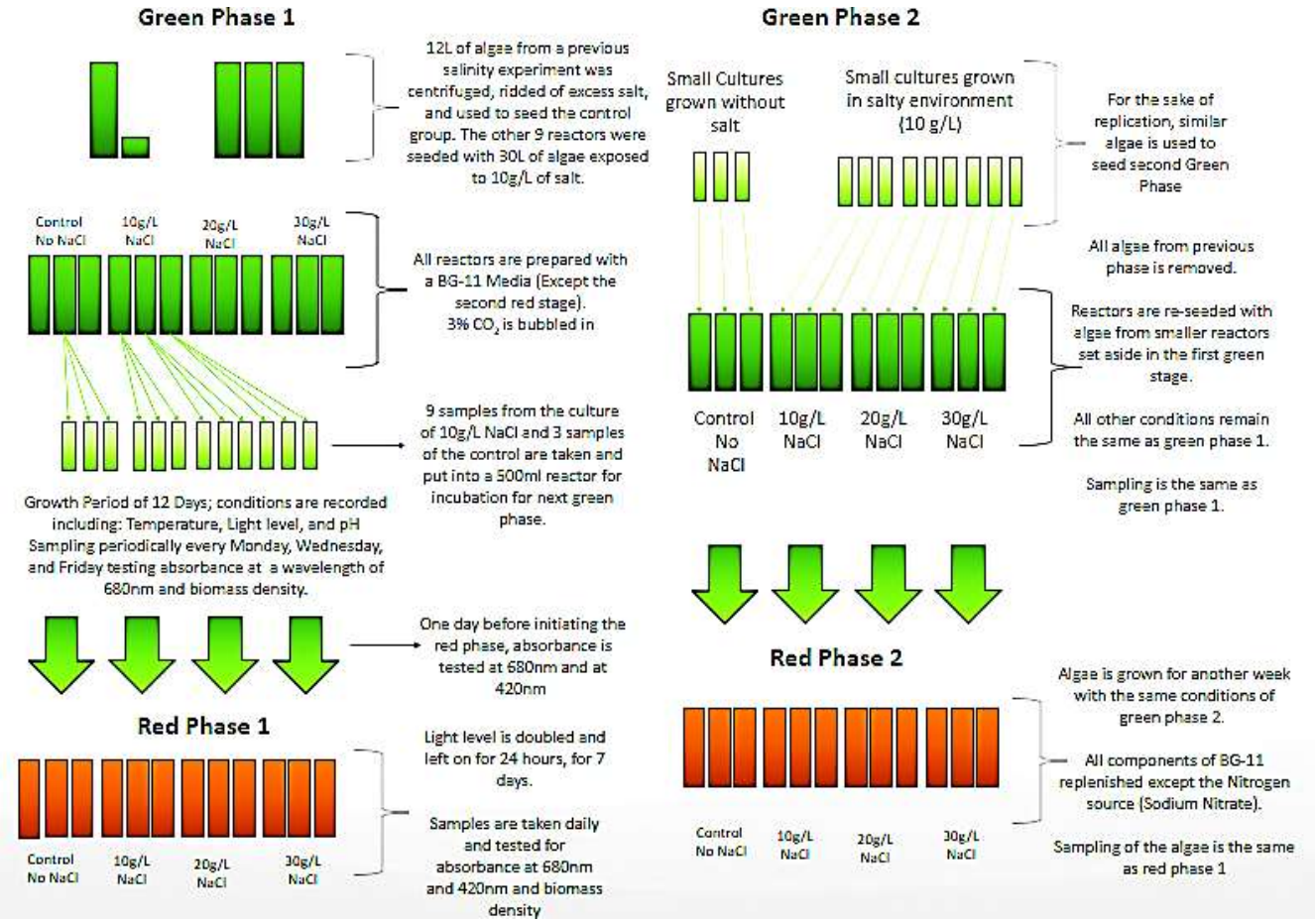

Figure 1. Flowchart depicting the experimental setup of the *Coelastrella sp.* brackish stressing experiment.

Sodium bicarbonate and light stress. A 10 L PBR culture of *Coelastrella sp.* was grown in BG-11 (25% $NaNO_{3)}$ prior to experimentation. Algal cultures were seeded from this culture in 800 mL PBRs and supplemented with BG-11 (25% $NaNO_3$) for growth in the green stage. Each PBR was bubbled with a blend of gaseous house N_2 and CO_2/N_2. The culture temperature was 24.5±0.1˚C. Gaseous flow was regulated to maintain adequate mixing within the PBRs. A continuous light source was provided by T5 35-watt cool white fluorescent bulbs (90 μmol/m^2/s). The experimentation period was conducted over one growth cycle. Water was UV-sterilized tap water. Nutrient media solutions were autoclaved for sterilization purposes, the point at which sterilization occurred varied depending on chemical constraints.

The red stage was initiated on day 16, which was assumed to be a nitrogen depleted environment due to the typical length of the *Coelastrella sp.* growth period. The stressing phase consisted of increased light intensity and the addition of 0 (control), 2.5, 50, and 100 mM $NaHCO_3$.

Sampling occurred three days a week. Analyses included pH and temperature monitoring, UV-vis spectrophotometry (λ = 680 nm in green and red stage and λ = 450 nm in red stage), and dry mass measurements [29]. These analyses indicate the health, productivity, and astaxanthin production in the algal culture.

Scenedesmus acutus (UTEX B72) productivity

Sodium carbonate and sodium bicarbonate pH control with cobalt catalyst and amine supplementation. Both sodium carbonate and sodium bicarbonate were added to the cultures for pH control. Algae can only utilize aqueous CO_2 or bicarbonate for growth in alkaline conditions, and not carbonate. At pH 10, for example, most of the solution carbon absorbed will

be in the bicarbonate form, not carbonate, while the reverse has been observed at pH 8 [30]. The relationship between carbonate/bicarbonate concentration and alkaline pH has been well described and correlated to CO_2 capture and algal growth [30]. Air was bubbled with 400 ppm CO_2 which enables some carbonate to convert to bicarbonate, but only slowly. A combination of sodium carbonate and bicarbonate were used in this study to achieve pH 10.4. The following experiments were based off this idea and used a combination of sodium carbonate and bicarbonate to achieve a pH of 9 and 10.

Cobalt Catalyst and pH treatment. All algal cultures were seeded with *S. acutus* grown in BG-11 (25% $NaNO_3$) in 800 mL PBRs for one week prior to the addition of any supplements. At this time no new media was added. Absorbance and pH data were recorded; however, dry mass density measurements were not conducted since the mass of the cobalt catalyst would interfere with the weighing of dry algal cells. Instead, dry mass was estimated by converting absorbance readings to density estimations using a previously established method for *S. acutus* [31]. Sodium carbonate and bicarbonate amounts were estimated in an initial test and additions were made in a stepwise manner to adjust the pH value. Each culture was bubbled with air and 350 ppm CO_2, which enabled good mixing. Each culture was maintained in a continuous light exposure environment.

The following table displays the final masses of each supplement added to the 800 mL PBRs.

Table 1. The treatments for pH adjustment and cobalt catalyst addition for each experimental group displayed as masses added to 800 mL PBRs.

Cobalt Catalyst and pH Treatment			
Treatment Type	$NaHCO_3$ (g)	Na_2CO_3 (g)	Cobalt Catalyst (g)
Control	———	———	———
pH 10 + Catalyst	0.086	0.71	0.9
pH 10	0.086	0.71	———
pH 9	0.063	0.265	———

Cobalt Catalyst and Amine. All algal cultures were seeded with *S. acutus* grown in BG-11 (25% $NaNO_3$) for one week prior to the addition of supplements in 800 mL PBRs. At this time no new media was added. UV-vis spectrophotometry (λ = 680 nm) and pH data were recorded, as well as dry mass density for all trials [29]. The cobalt catalyst mass was assumed to be negligible since the concentration in this experiment was less than in previous trials. However, this likely still interfered. Hemocytometer counts were conducted to complement dry mass measurements on days of seeding and harvesting. Each culture tube was bubbled with air and ~400 ppm CO_2, which enabled good mixing. Continuous light exposure was utilized, and sodium carbonate and bicarbonate amounts were used to raise pH to ~10.

The DMG solid was provided as DMG with HCl, starting at a pH value of ~1. The sample (7.21 g amine) was dissolved in 120 mL DI water and neutralized with 2.81 g NaOH. The addition of NaOH generated NaCl and H_2O when in contact with the HCl, so the algae would not be harmed by this addition. This resulted in a final DMG solution pH of 9.36. This amine solution was utilized for each group involved in amine experimentation.

Table 2 displays the treatment masses and volumes given to each treatment group per 800 mL PBR.

Table 2. The treatments for pH adjustment as well as cobalt catalyst and/or amine addition for each experimental group displayed as masses and volumes added to 800 mL PBRs.

Treatment	$NaHCO_3$ (g)	Na_2CO_3 (g)	Cobalt Catalyst (g)	DMG solution (mL)
Control	0.13	0.493		
Catalyst	0.13	0.493	0.6	
Catalyst + Amine	0.249	0.867	0.6	20
Amine	0.241	0.86		20

Amine continued. Two harvest cycles were completed after the initial cycle in an experiment in which only the control and amine groups were examined. All parameters were kept the same, but the catalyst groups were omitted.

Double Amine. An experiment was conducted in which the amine concentration previously utilized was doubled. The same parameters were followed in terms of *S. acutus* seeding, no addition of new media, bubbling gases, continuous light exposure, sodium carbonate and bicarbonate determination, and sampling procedures. The following table describes the samples used to examine the algae culture behavior with double amine concentration.

Table 3. The treatments for pH adjustment and double amine addition for each experimental group displayed as masses and volumes added to 800 mL PBRs.

Treatment	$NaHCO_3$ (g)	Na_2CO_3 (g)	*DMG solution (mL)
Control	0.0845	0.338	---
Amine	0.65	0.26	40

Ammonium nitrogen sources. Algae cultivation involved *S. acutus* grown in standard BG-11 media prior to experimentation. Both modified standard agriculture-grade algae media and modified BG-11 media were studied.

Agriculture-grade media. Agriculture-grade media was modified to compare ammonia-based nitrogen sources to urea in the standard fertilization formulation. This experiment was conducted in 10 L PBR triplicates, each seeded from 800 mL PBR stock cultures. Table 4 below

summarizes the chemical components and masses for 10 L PBR triplicates for the agriculture media formulation.

Table 4. Urea and ammonium nitrogen sources masses and standard agriculture-grade fertilization media chemical makeup for 30 L total *S. acutus* cultivation.

	Nitrogen Source (g)	TSP (g)	POT (g)	Sprint 330 (g)
Urea	4.22	3.93	2.44	0.78
Ammonium Carbonate	7.68	3.93	2.43	0.78
Ammonium Bicarbonate	12.41	3.93	2.48	0.79

Modified BG-11 with urea control. BG-11 media was modified to compare ammonia-based nitrogen sources to typical BG-11 (25% $NaNO_3$) nitrogen source. A urea control group was also included in this trial. This experiment was conducted in 800 mL PBR triplicates, seeded from a 10 L PBR stock culture. Table 5 summarizes the chemical components and masses for 800 mL PBRs for BG-11 modifications.

Table 5. BG-11 media concentrations without nitrogen source with the column highlighted green representing the utilized masses for 800 mL PBRs.

Compound	**dose/L (mL)**	**Solution Concentration (g/L)**	**dose/ L (g)**	**dose/800mL Algae (g)**	**dose/800mL Algae (mg)**
Dibasic potassium phosphate	2.5	16	0.04	0.032	32
Magnesium sulfate heptahydrate	2.5	30	0.075	0.06	60
Calcium chloride dihydrate	2.5	14.4	0.036	0.0288	28.8
Citric acid monohydrate	2.5	2.4	0.006	0.0048	4.8
Ferric ammonium citrate	2.5	2.4	0.006	0.0048	4.8
EDTA, disodium salt, dihydrate	2.5	4	0.01	0.008	8
Sodium carbonate	2.5	8	0.02	0.016	16

This standard BG-11 recipe was supplemented by the typical trace metal solution. The nitrogen component masses are included below in Table 6.

Table 6. BG-11 nitrogen source modifications for 800 mL PBRs.

Nitrogen source/800mL Algae (g)		mol/L	mg/L
0.25 $NaNO_3$	0.3	0.00441204	375
AC: $(NH_4)_2CO_3$	0.205	0.002666771	256.25
ABiC: NH_4HCO_3	0.33	0.00521782	412.5
Control: Urea			

Each PBR was bubbled with a blend of gaseous house N_2 and CO_2/N_2. The culture temperature ranged from 23.6-24.7 ˚C. Gaseous flow was regulated to maintain adequate mixing within the PBRs. A continuous light source was provided. The experimentation period was conducted over the course of multiple growth cycles, with supplement adjustment, water addition, and media replenishment at the start of each cycle. Harvesting was performed biweekly and reduced algae concentration to ~0.2 g/L. Water initially supplied and added after each harvest was UV-sterilized tap water. Nutrient media solutions were autoclaved for sterilization purposes, the point at which sterilization occurred varied depending on chemical constraints.

Sampling occurred three days a week. Analyses included pH and temperature monitoring, UV-vis spectrophotometry (λ = 680 nm), and dry mass measurements [29]. These analyses indicate the health and productivity of the culture.

Ammonia gas sparging. *S. acutus* was cultivated in 800 mL PBRs seeded from a 10 L PBR culture, grown with BG-11. Good mixing was achieved in each PBR by bubbling 0.1 L/min of a gaseous blend of N_2, 0.25% CO_2/N_2 and 0.1% NH_3/N_2 (providing C and N for 0.2 g/L/day algae growth rate) stored at ambient temperature. This blend was manipulated to achieve each CO_2/NH_3 mole ratio (from 7 to 18) by decreasing the N_2 input to increase CO_2 for multiple harvest cycles. Then, mole ratio CO_2/NH_3 = 10 was established and total gas flowrate was manipulated for experimentation. Each harvest was conducted when algae concentration reached

~1 g/L and were reduced by ~80% to achieve an algae density of ~0.2 g/L and remain in the log growth phase.

Water and nutrients were provided at the beginning of each trial and replenished following harvesting. Water supply was filtered through a 1 μm filter with a 10" whirlpool canister prior to being UV sterilized, a similar sterilization method being employed for the nutrient solution. Culture temperature was maintained at 23.3±0.6 °C and continuous light was provided. Three times a week, samples were analyzed for pH and temperature monitoring, UV-vis spectrophotometry (λ = 680 nm), dry mass measurements, and residual CO_2 using a refinery gas analyzer [29]. The exit gas was also analyzed with Kitagawa tubes (minimum detection level of 0.2 ppm) for some trials to test for NH_3 (which was never detected). Productivity (g/L/day) and CO_2 fixation rate (g/L/day) were then calculated.

NH_3 and NH_4^+ Concentration. Ammonium ions were analyzed in triplicate with an Ion Chromatography (IC) system (Dionex ICS-3000) and manual peak integration was performed with Dionex Chromeleon chromatography management system software version 6.80. Calibrations were conducted with 50x water-diluted certified ammonium standard obtained from Environmental Express (Charleston, SC).

Equation 1 and 2 below were used to calculate free ammonia concentration in solution from the NH_4^+ ion concentration.

$$X_{NH3} = \frac{1}{10^{pKa+pH} + 1} \qquad \text{Eqn. 1}$$

$$pK_a = 0.0902 + \frac{2729.92}{T_k} \qquad \text{Eqn. 2}$$

X_{NH3} is the mole fraction of free ammonia, T_k is the temperature in Kelvin, and pH refers to the measured pH of the sample.

Analysis of algae biomass. Biomass was submitted for elemental analysis, moisture, and ash content. Previous work has established that multiplying the nitrogen content by a 4.78 conversion factor allows for the protein content to be estimated [34]. CO_2 and NH_3 utilization were calculated with Equations 3 and 4.

$$\eta_{CO2} = \frac{100 * m_{SA} * X_C}{m_{CO2}} \quad \text{Eqn. 3}$$

$$\eta_{NH3} = \frac{100 * m_{SA} * X_N}{m_{NH3}} \quad \text{Eqn. 4}$$

η_{CO2} is the $\%CO_2$ utilization, m_{SA} is the mass of algae produced, X_C is the C fraction in algae, and m_{CO2} is the mass of CO_2 fed. η_{NH3} is the $\%NH_3$ utilization, X_N is the N fraction in algae, and m_{NH32} is the mass of NH_3 fed.

Direct extraction and transesterification of harvested algae enabled lipid profiling following a previously published procedure [32]. Transesterification reaction products were analyzed via Gas Chromatography (GC) for additional carbon utilization analysis.

RESULTS

Coelastrella sp. productivity and astaxanthin production

Media. *Coelastrella sp.* was cultured in 10 L PBRs using urea, plain BG-11 media, and BG-11 media with 0.5 g/L NaCl. Figure 2 below displays the calculated density of each media group. The urea group displayed steady growth increasing by only .2 g/L over the seven-day period. The BG-11 group, with and without added NaCl, displayed a short acclimation period

where minimal growth was observed, then after day 3, a sharp spike in growth was observed in both groups.

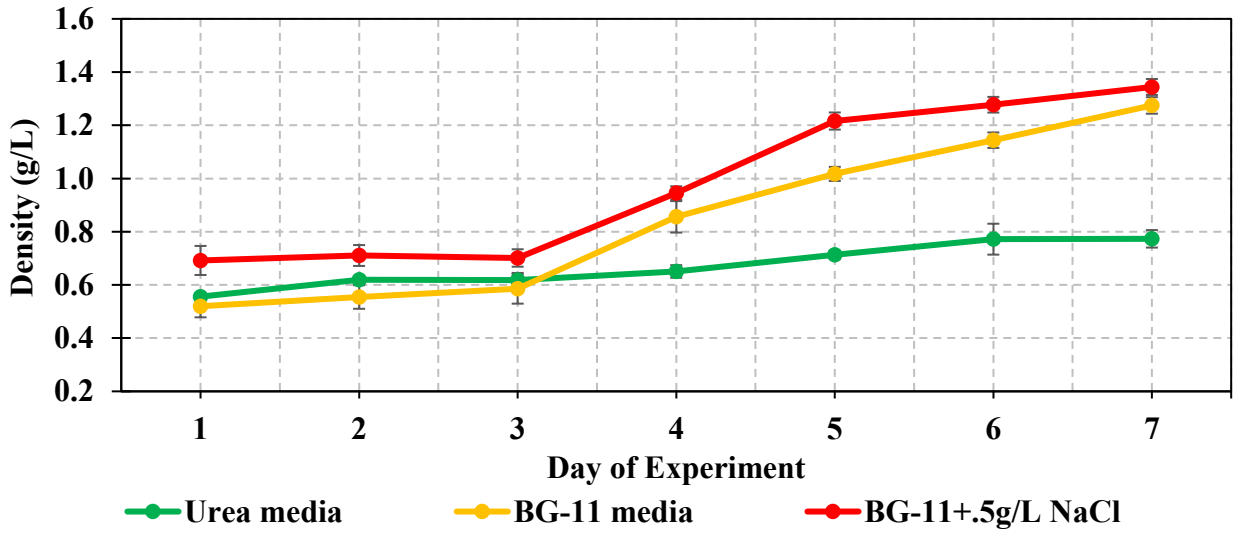

Figure 2. Density of *Coelastrella sp.* cultured in urea, BG-11, and BG-11 with .5 g/L NaCl. Error bars represent standard deviations of triplicate trials.

Brackish conditions. *Coelastrella sp.* was cultured in 10 L PBRs using BG-11 media with various concentrations of NaCl. Figure 3 below displays the calculated density achieved with each salinity, normalized so that all growth trends appear to start from the same initial density. It is apparent that the greater the NaCl concentration, the less growth is observed. However, *Coelastrella sp.* was able to grow in all salt concentrations tested.

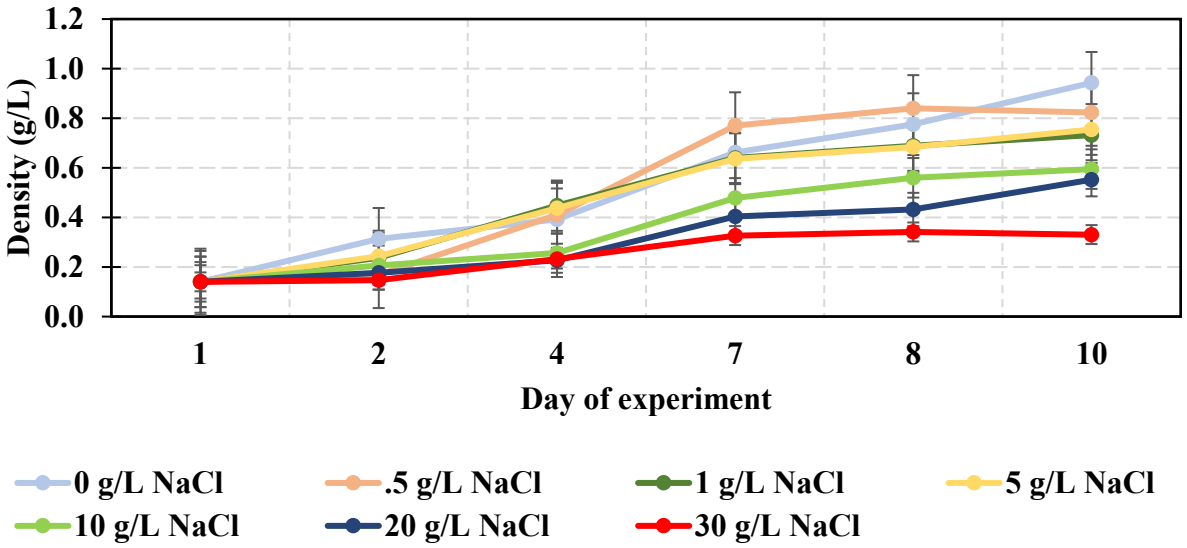

Figure 3. Normalized density of *Coelastrella sp.* cultured with BG-11 and various NaCl concentrations. Error bars represent standard deviations of triplicate trials.

Figure 4 below displays the calculated density of both raceway ponds harvested on day 11 and day 25. The trends mirror each other with sudden dips or peaks throughout a growth cycle due to weather-related factors. *Coelastrella sp.* was able to grow adequately outdoors with 0.5 g/L of NaCl.

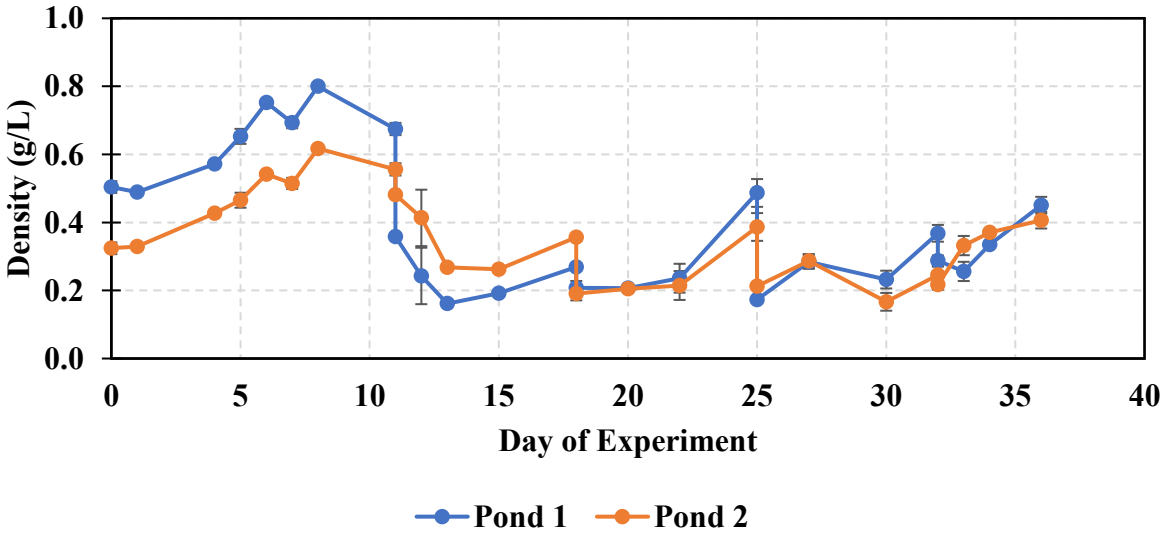

Figure 4. Density of *Coelastrella sp.* cultured with BG-11 and 0.5 g/L NaCl in two outdoor raceway ponds. Error bars represent standard deviations of triplicate samples.

Light intensity and nitrogen deprivation. Both stressors were tested following a growth period in different salt concentrations.

Light intensity. *Coelastrella sp.* was cultured with BG-11 media in a green stage then stressed with increased light intensity and concentrations of 0, 10, 20, and 30 g/L NaCl. Figure 5 below displays the normalized density values calculated using the measured dry mass of samples in the red stage. The samples with less salt continued to grow during the red stage, while the trials with higher salt concentrations only increased slightly in culture density in the presence of increased light intensity.

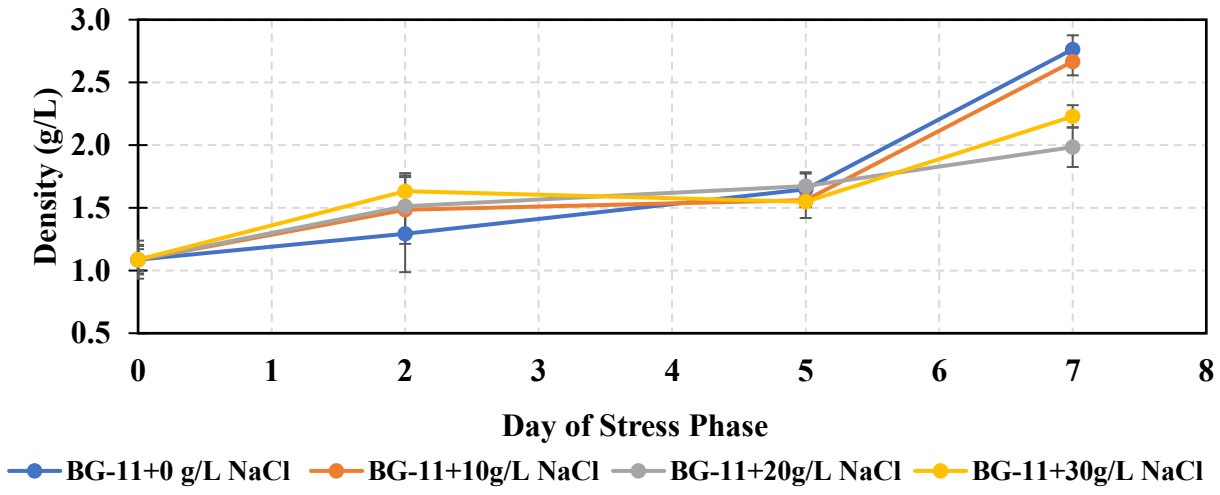

Figure 5. Normalized calculated density of *Coelastrella sp.* cultured with BG-11 in the red stage achieved by stressing with increased light intensity and different NaCl concentrations. Error bars represent standard deviations of triplicate trials.

Figure 6 below displays the normalized absorbance values measured at $\lambda = 680$ nm (to indicate the production of Chlorophyll A) in the red stages induced with increased light intensity and different salt concentrations. The samples with 10 g/L NaCl showed the highest chlorophyll A production while the other samples remained relatively constant.

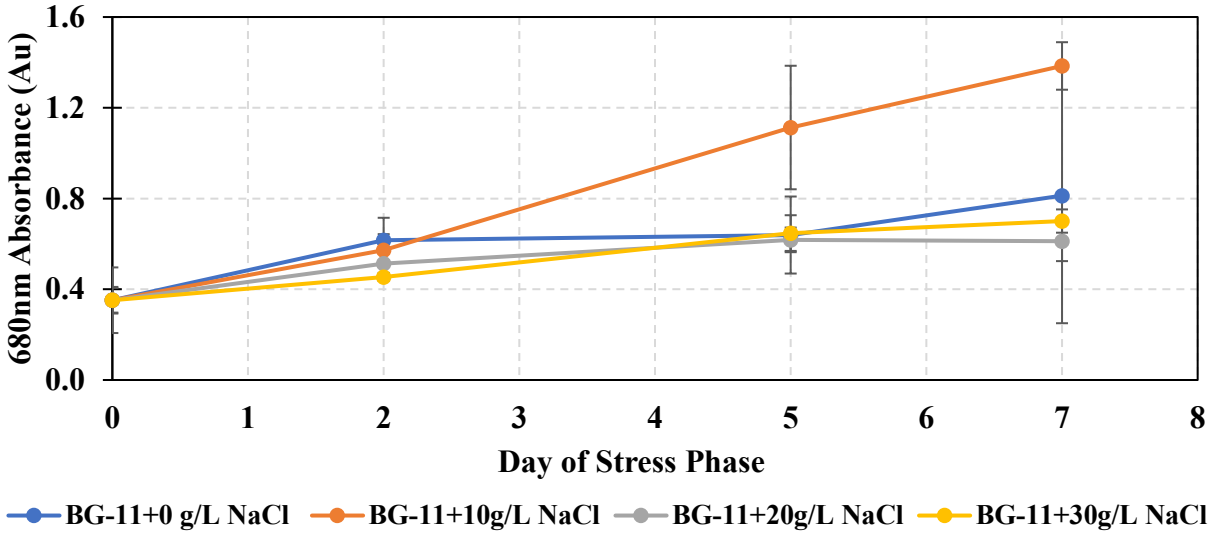

Figure 6. Absorbance (λ = 680 nm) of *Coelastrella sp.* cultured with BG-11 in the red stage achieved by stressing with increased light intensity and different NaCl concentrations. Error bars represent standard deviations of triplicate trials.

Figure 7 below displays the measured absorbance values obtained at λ = 450 nm (to indicate the production of astaxanthin) in the red stage for all samples. The samples with less salt had the highest astaxanthin concentrations, which may be partially attributed to their productivity.

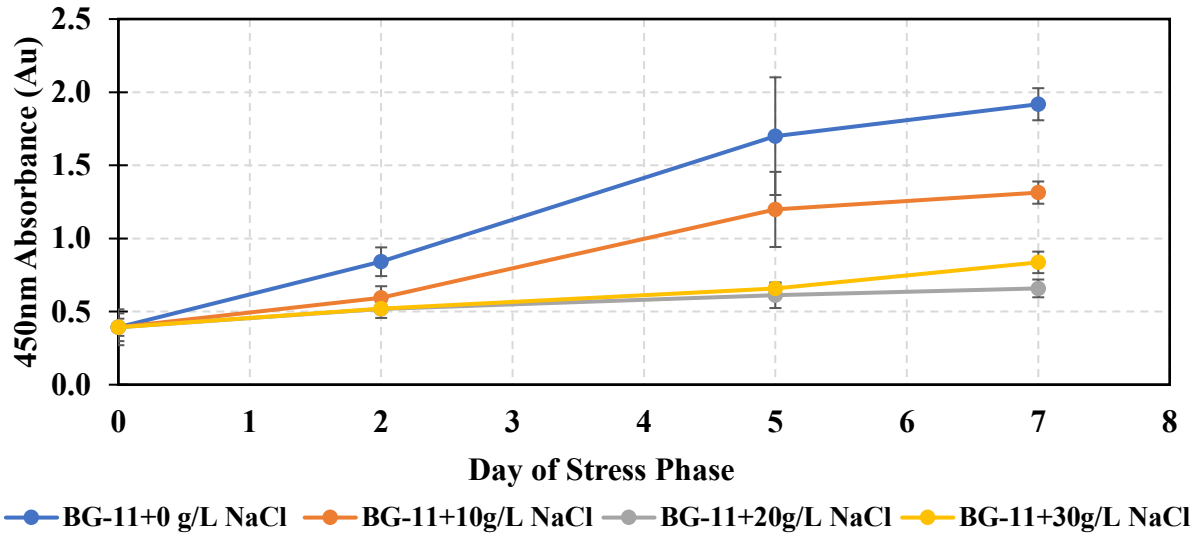

Figure 7. Absorbance (λ = 450 nm) of *Coelastrella sp.* cultured with BG-11 in the red stage achieved by stressing with increased light intensity and different NaCl concentrations. Error bars represent standard deviations of triplicate trials.

Nitrogen deprivation. *Coelastrella sp.* was cultured using BG-11 media in a green stage before being stressed in a red stage with nitrogen deprivation and various NaCl concentrations. Figure 8 below displays the normalized density values calculated using dry mass measurements in the red stage. The BG-11 samples with no added salt and 20 g/L of added salt had the highest productivity and continued to grow despite nitrogen deprivation, but the other groups did not.

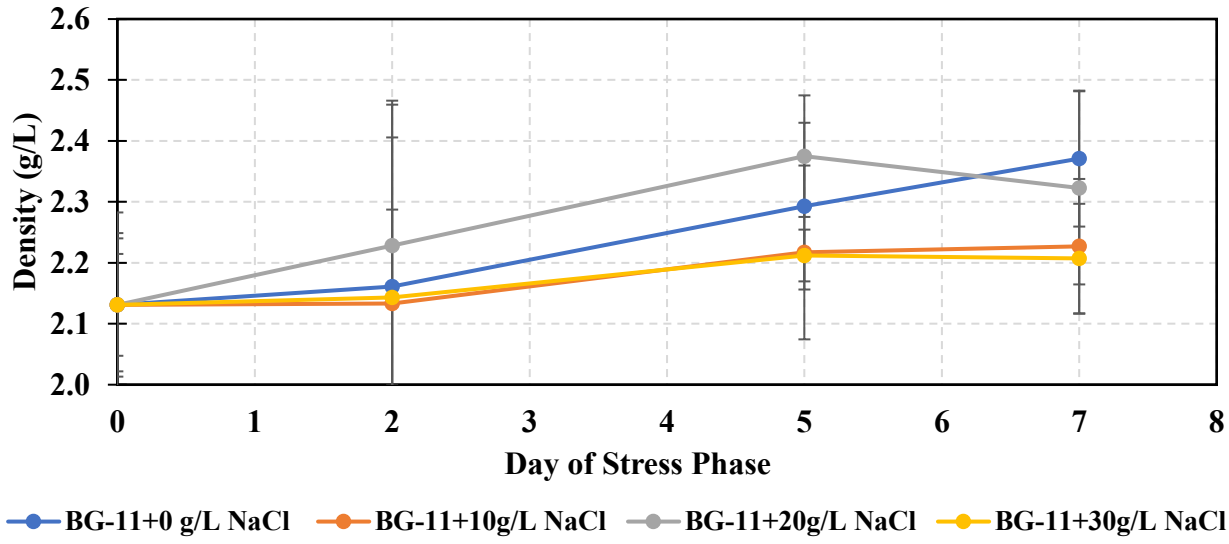

Figure 8. Calculated density of *Coelastrella sp.* cultured with BG-11 in the red stage (day 14+) achieved by stressing with different concentrations of $NaHCO_3$. Error bars represent standard deviations of triplicate trials.

Figure 9 below shows the normalized absorbance values measured at λ = 680 nm in the red stage for all samples. The samples corresponding to the highest NaCl concentration displayed the lowest absorbance, which also correlates with their low productivity.

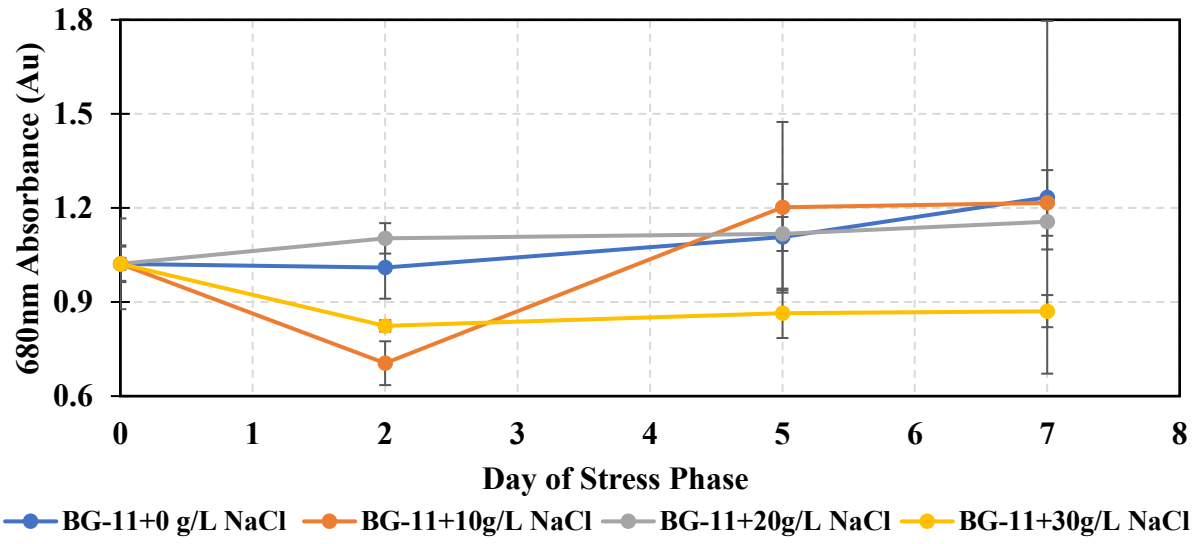

Figure 9. Normalized absorbance (λ = 680nm) of *Coelastrella sp.* cultured with BG-11 in the red stage achieved by stressing with nitrogen deprivation and different NaCl concentrations. Error bars represent standard deviations of triplicate trials.

Figure 10 below displays the absorbance values measured at λ = 450 nm in the red stage for all samples. In this case, the samples with no added NaCl had the lowest increase in astaxanthin production from the green phase. The group which was given 10 g/L NaCl showed the highest astaxanthin production.

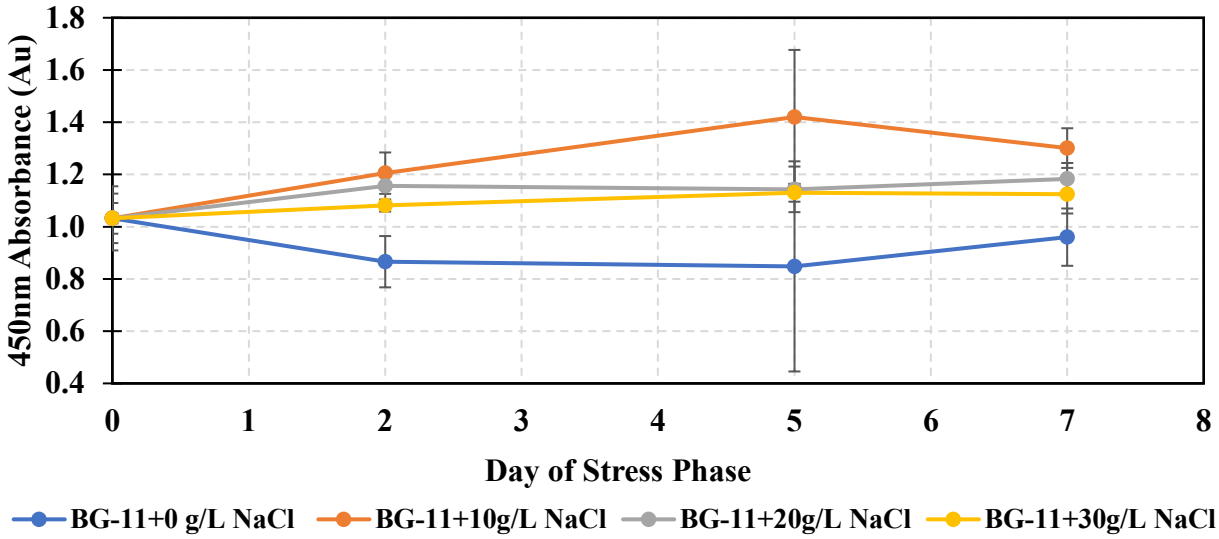

Figure 10. Absorbance (λ = 450nm) of *Coelastrella sp.* cultured with BG-11 in the red stage achieved by stressing with nitrogen deprivation and different NaCl concentrations. Error bars represent standard deviations of triplicate trials.

Sodium bicarbonate and light stress. *Coelastrella sp.* was cultured using BG-11 media (25% $NaNO_3$) in a green stage with 2.5 mM sodium bicarbonate and then stressed in a red stage with various sodium bicarbonate concentrations and increased light intensity. Figure 11 below displays the measured pH of each set of samples. All cultures displayed similar pH values and trends during the green stage, with an initial pH value of ~6.1 and a final pH value of ~6.5. Once the red stage began on day 14, the groups which were given a higher concentration of sodium bicarbonate (50 mM and 100 mM) had a dramatic spike in pH, which reached a pH value of ~7.6 for 50 mM and a pH value of ~7.8 for 100 mM. The pH slightly increased in the control and 2.5

mM group, but both maintained a pH value of ~6.7. The trend observed below exemplifies the increase in pH associated with high concentrations of sodium bicarbonate.

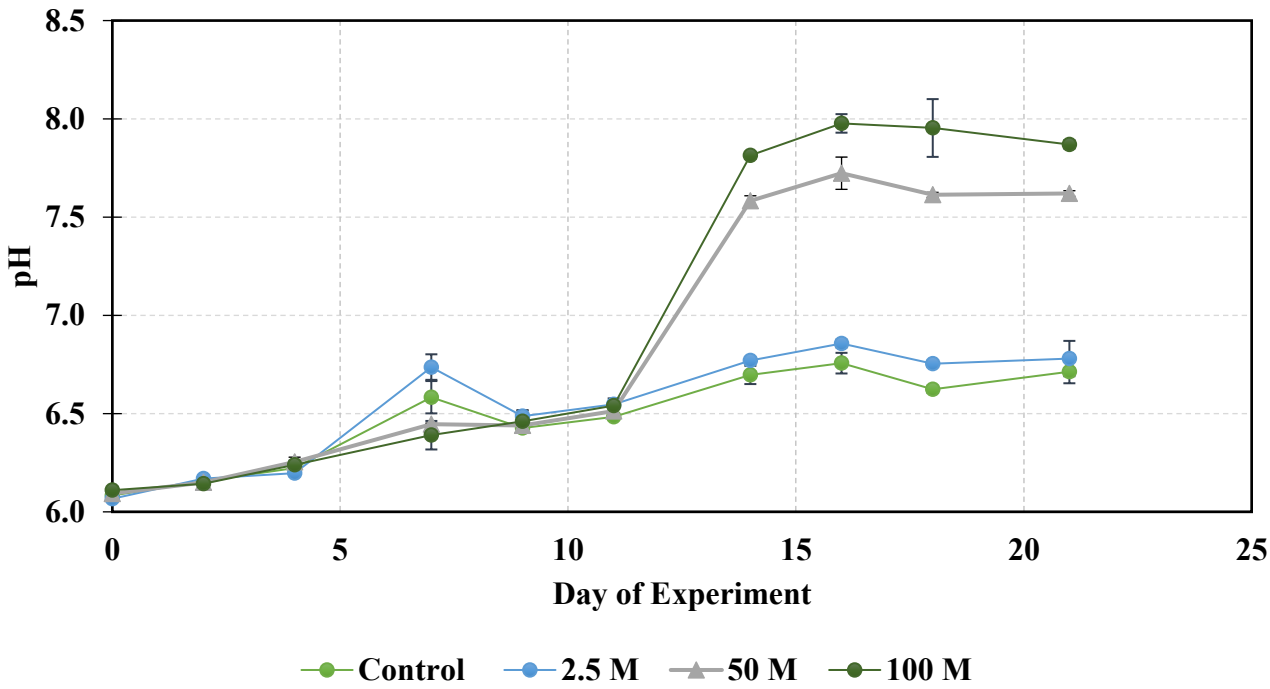

Figure 11. pH of *Coelastrella sp.* cultures with BG-11 (25% $NaNO_3$) in green and red stages (day 14+) achieved by stressing with different molar concentrations of $NaHCO_3$. Error bars represent standard deviations of triplicate trials.

Figure 12 below displays the absorbance values measured at $\lambda = 680$ nm in both the green and red stages for all samples. All cultures displayed similar absorbance values and trends during the green stage, with an initial absorbance value of ~0.2 AU and a final value of ~1.1 AU. Once the red stage began on day 14, the samples with higher sodium bicarbonate concentration (100 mM) had the lowest absorbance values, which began to decrease at the end of the growth period. The highest absorbance values were seen in the control group and the 50 mM stress group.

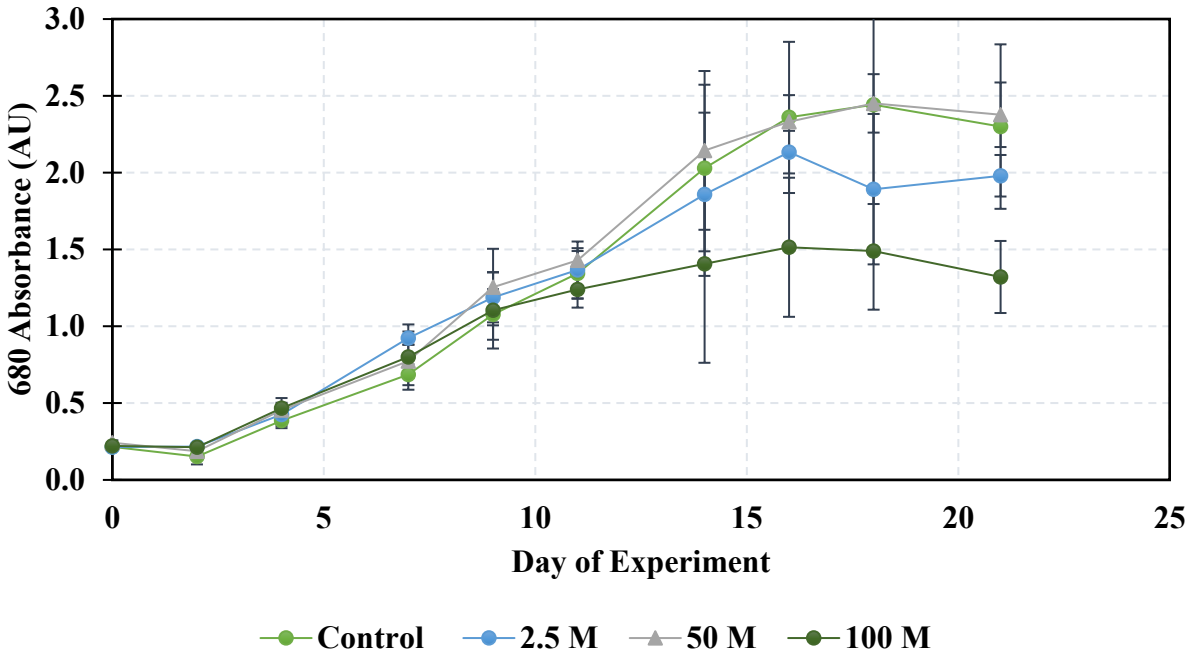

Figure 12. Absorbance (λ = 680nm) of *Coelastrella sp.* cultured with BG-11 in green and red stage achieved by stressing with different concentrations of $NaHCO_3$. Error bars represent standard deviations of triplicate trials.

Figure 13 below displays the absorbance values measured at λ = 450 nm in the red stage for samples. The cultures displayed a large variation in absorbance. Across the three-day red stage, the absorbance did not deviate from initial values in any experimental group. The 50 mM and control group had the highest absorbance with a value maintained at ~2.7 AU. The samples with the highest sodium bicarbonate concentration (100 mM) displayed the lowest absorbance, which was maintained at ~1.6 AU.

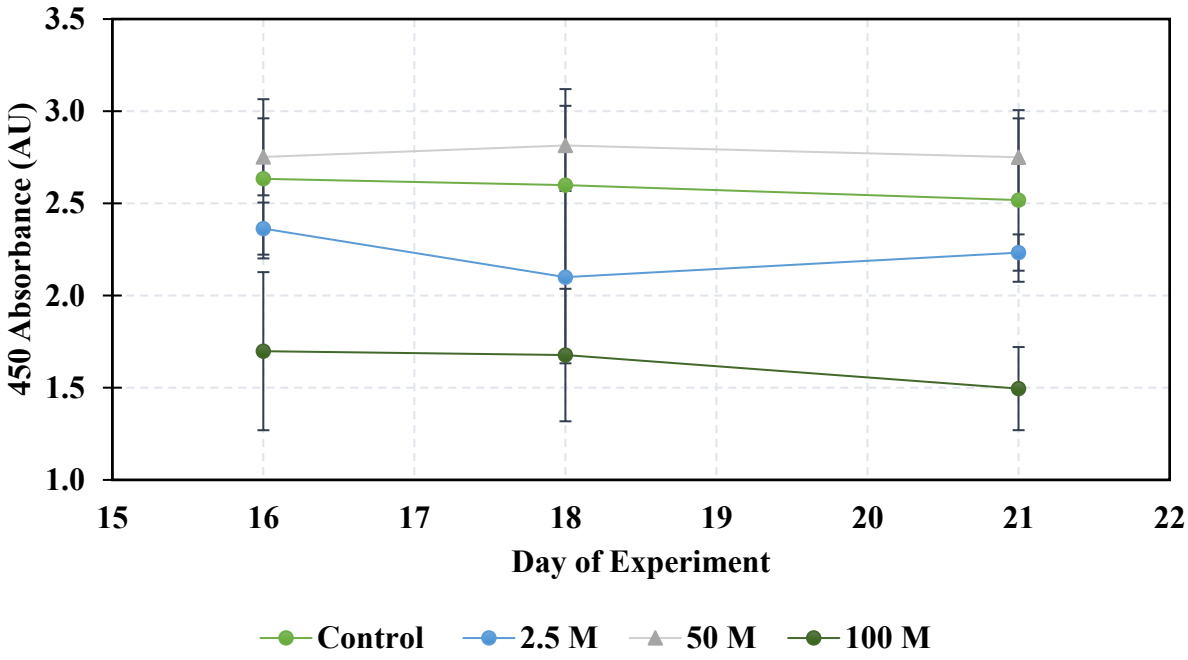

Figure 13. Absorbance (λ =450 nm) of *Coelastrella sp.* cultured with BG-11 (25% $NaNO_3$) in green and red stage (day 14+) achieved by stressing with different concentrations of $NaHCO_3$. Error bars represent standard deviations of triplicate trials.

Figure 14 below displays the density values calculated using dry mass measurements for each experimental group in the green and red stage. All cultures displayed similar density values and trends during the green stage with an initial culture density of ~0.15 g/L and a final density of ~1.15 g/L. Once the red stage began on day 14, all cultures increased in density, which was unexpected as no additional media was added. This may have been due to the increased light intensity stressor. The 50 mM sodium bicarbonate group and control had the highest increase in density, while the 100 mM group exhibited the lowest increase in density, a similar trend being observed in the 2.5 mM stress group.

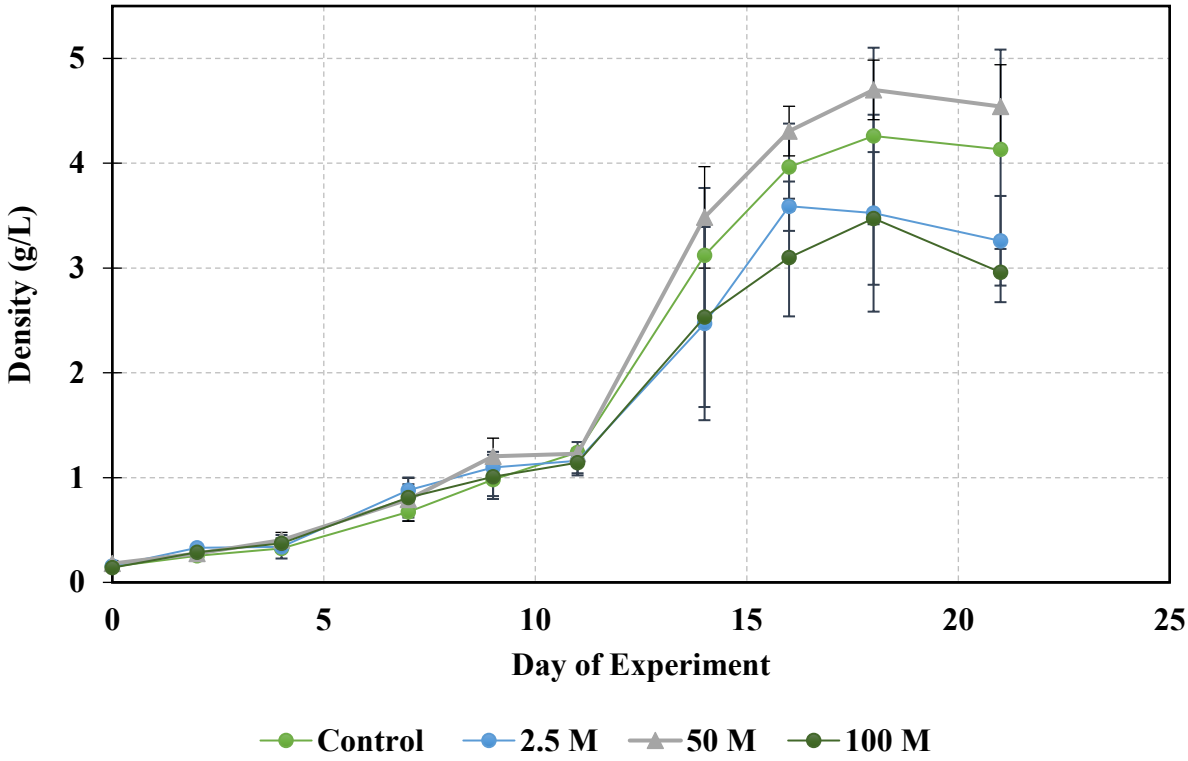

Figure 14. Calculated density of *Coelastrella sp.* cultured with BG-11 (25% $NaNO_3$) in green and red stage (day 14+) achieved by stressing with different concentrations of $NaHCO_3$. Error bars represent standard deviations of triplicate trials.

Scenedesmus acutus productivity

Sodium carbonate and sodium bicarbonate pH control with cobalt catalyst and amine supplementation. *S. acutus* was cultured using BG-11 media (25% $NaNO_3$) with pH adjustment, cobalt catalyst, and amine supplementation.

Cobalt Catalyst and pH treatment. *S. acutus* was cultured for one week using BG-11 (25% $NaNO_3$) prior to pH adjustment using sodium bicarbonate and carbonate as well as cobalt catalyst addition for one growth cycle. Figure 15 below displays the measured pH values of each treatment group. The cultures each began at the desired pH values. The BG-11 control group began at a pH value of 6.7 due to the pH of the media. The pH seemed to be buffered by the

cultures and converged to a value between ~7.4 and ~8 by day 5. The pH then steadily increased for the remainder of the experimental period. The pH stabilization is attributed to cellular processes of the algal cells also responsible for the robustness and acclimation properties characteristic of *S. acutus.*

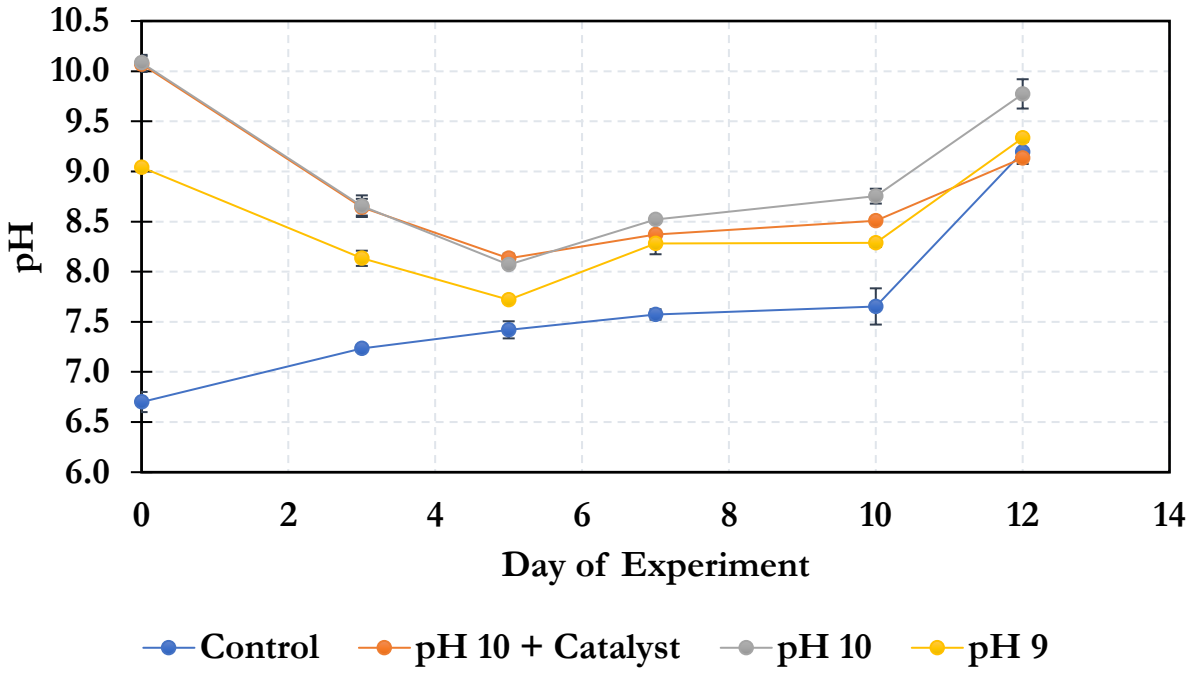

Figure 15. pH of the cultures grown with BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and cobalt catalyst. Error bars represent standard deviations of triplicate trials.

Figure 16 below displays the absorbance values measured at λ = 680 nm of each treatment group. The cultures all displayed the same initial absorbance value of ~0.5 AU. The absorbance remained constant for the cobalt catalyst group and steadily increased over the growth period in all other samples.

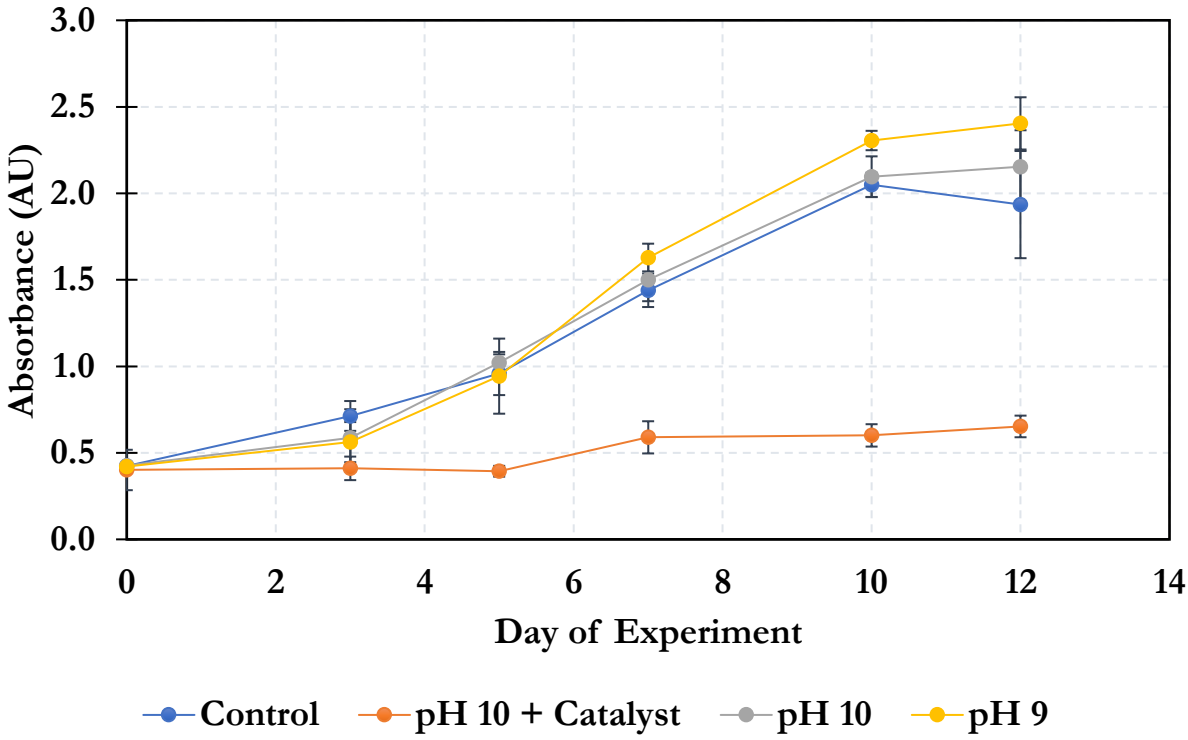

Figure 16. Absorbance (λ = 680 nm) of the cultures grown with BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and cobalt catalyst. Error bars represent standard deviations of triplicate trials.

Figure 17 below displays the estimated density values based on absorbance readings as described previously. All cultures displayed the same initial density value just below ~0.2 g/L. The density remained constant for the cobalt catalyst group and steadily increased over the growth period in all other samples. This follows the same trend as absorbance since density estimations are based on absorbance values. Nevertheless, it is still valuable to study this trend to compare the growth rate observed with typical growth behavior.

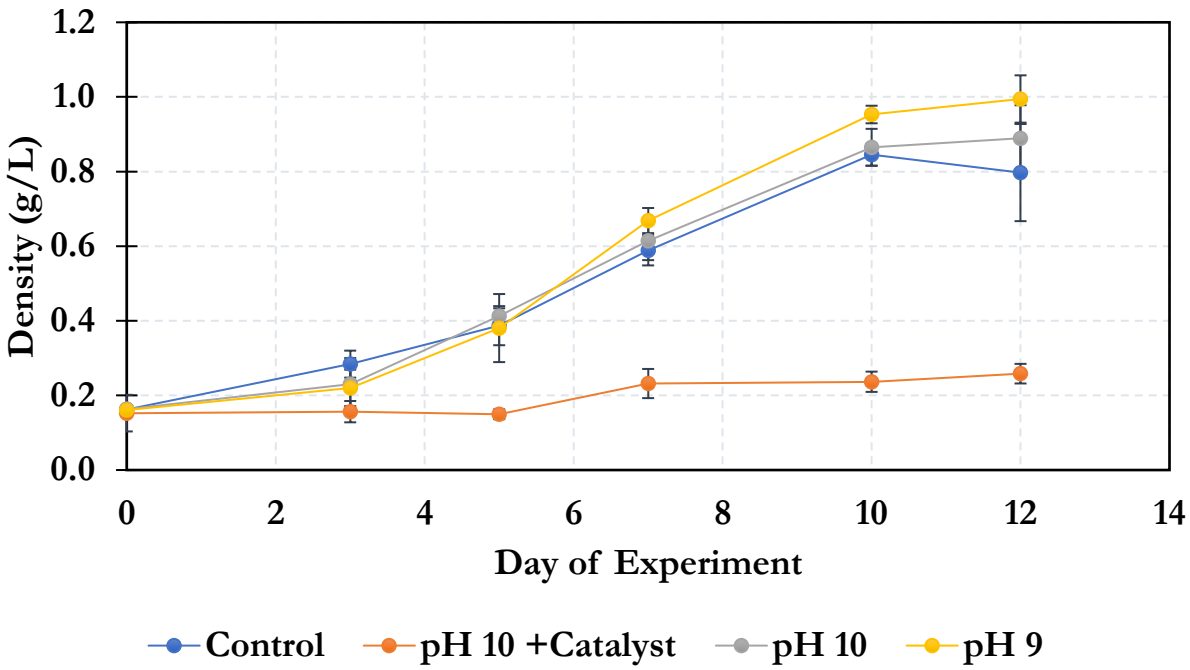

Figure 17. Calculated density of the cultures grown with BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and cobalt catalyst. Error bars represent standard deviations of triplicate trials.

Cobalt Catalyst and Amine. *S. acutus* was cultured for one week using BG-11 (25% $NaNO_3$) prior to pH adjustment with sodium bicarbonate and carbonate, as well as supplementation with a cobalt catalyst and amine for one growth cycle. Figure 18 below displays the pH values measured in each treatment group. All cultures started near the desired pH values, although exact pH control was difficult to achieve for the amine groups using sodium carbonate and bicarbonate. The control group began at the desired pH value of 10 and pH of the other groups clustered around 9.5. The pH seemed to be buffered by the cultures and converged to a value between ~8.4 and ~8.9 by day 5. The pH then slightly increased for each group for the remainder of the experimental period.

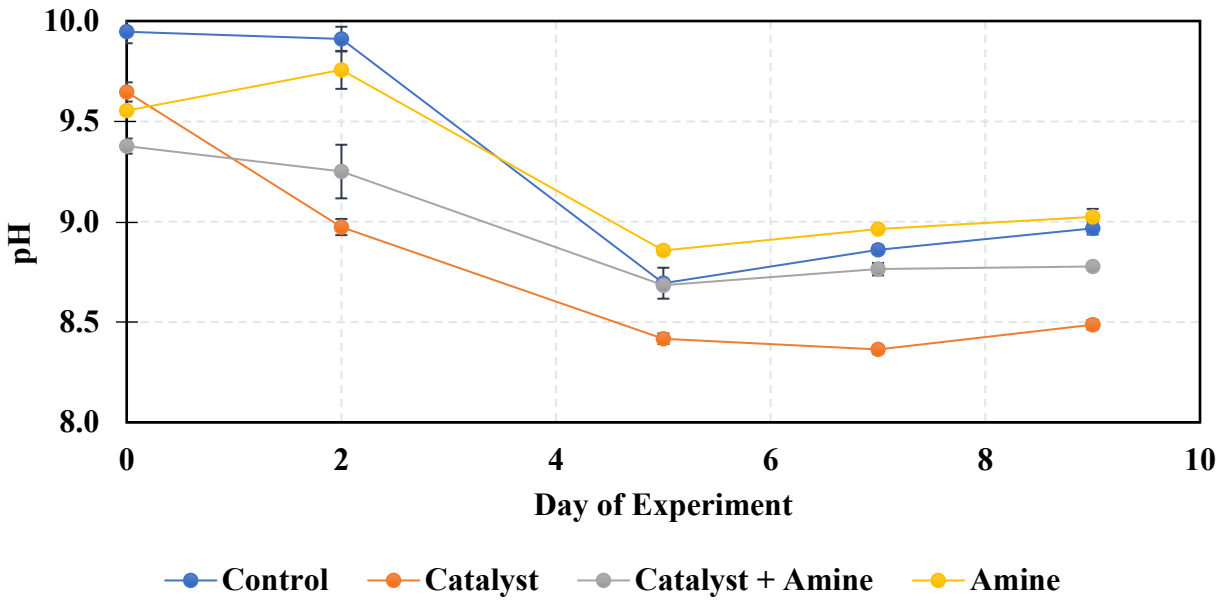

Figure 18. pH of the cultures grown with BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, cobalt catalyst, and amine. Error bars represent standard deviations of triplicate trials.

Figure 19 below displays the absorbance values measured at λ = 680 nm of each treatment group. All cultures displayed the same initial absorbance value of ~0.7 AU. The absorbance steadily increased over the growth period for the control group and amine-only group but decreased slightly and then remained constant for the cobalt catalyst groups despite amine addition.

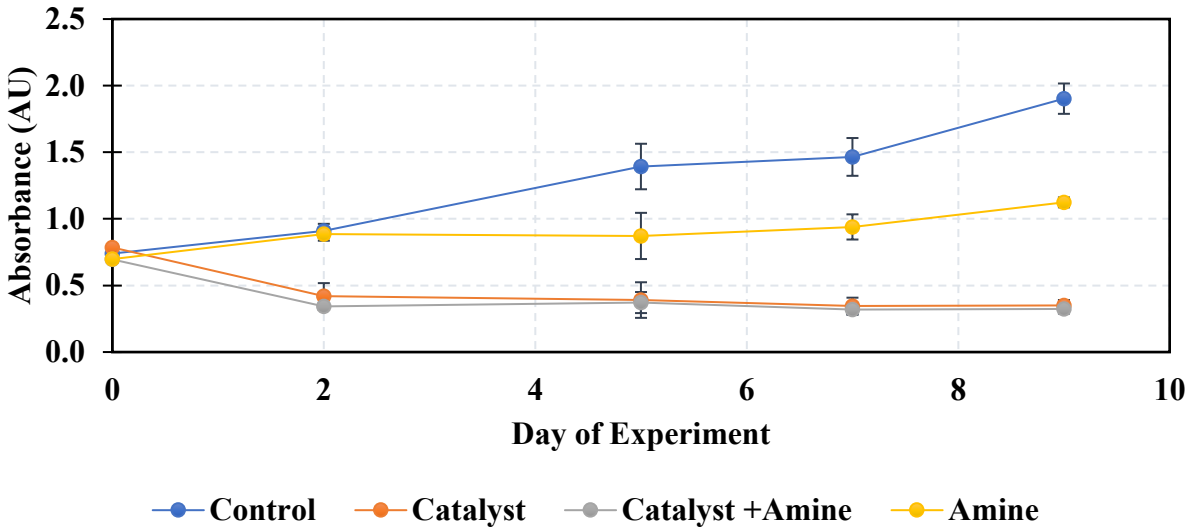

Figure 19. Absorbance (λ = 680nm) of the cultures grown using BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, cobalt catalyst, and amine. Error bars represent standard deviations of triplicate trials.

Figure 20 below displays the density values calculated from dry mass measurements. The density of the control and amine-only groups started at ~0.5 g/L and the cobalt catalyst groups started ~0.9 g/L. Density increased steadily over the growth period for the control group but decreased slightly and then remained constant for the cobalt catalyst groups despite amine addition. This follows the same trend as the absorbance values, which is to be expected given the relationship between growth rate and cellular Chlorophyll A production.

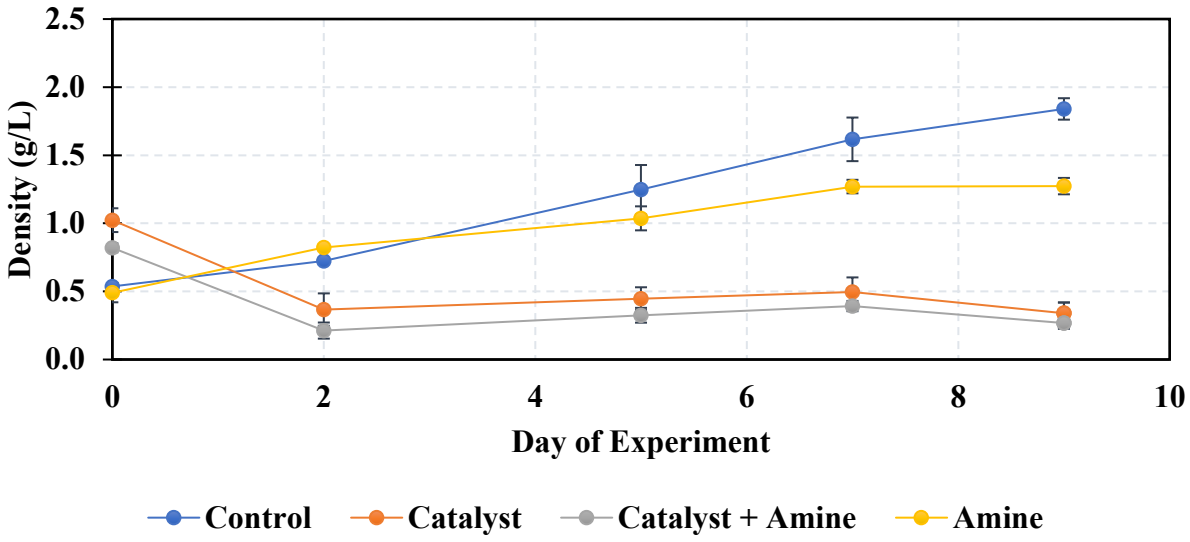

Figure 20. Calculated density of the cultures grown using BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, cobalt catalyst, and amine. Error bars represent standard deviations of triplicate trials.

Amine continued. Two additional harvest cycles were completed with the control and amine groups since growth ceased in the cobalt catalyst group, likely due to shading. Figure 21 below displays the observed pH trends. After each harvest the control group started at the desired pH (~10) and the amine group started at ~9.7. Generally, each group steadily decreased in pH along the growth cycle, aside from a divergence observed in the control group towards the end of the experiment. A gas flow issue or other unidentified experimental error(s) may have caused this divergence.

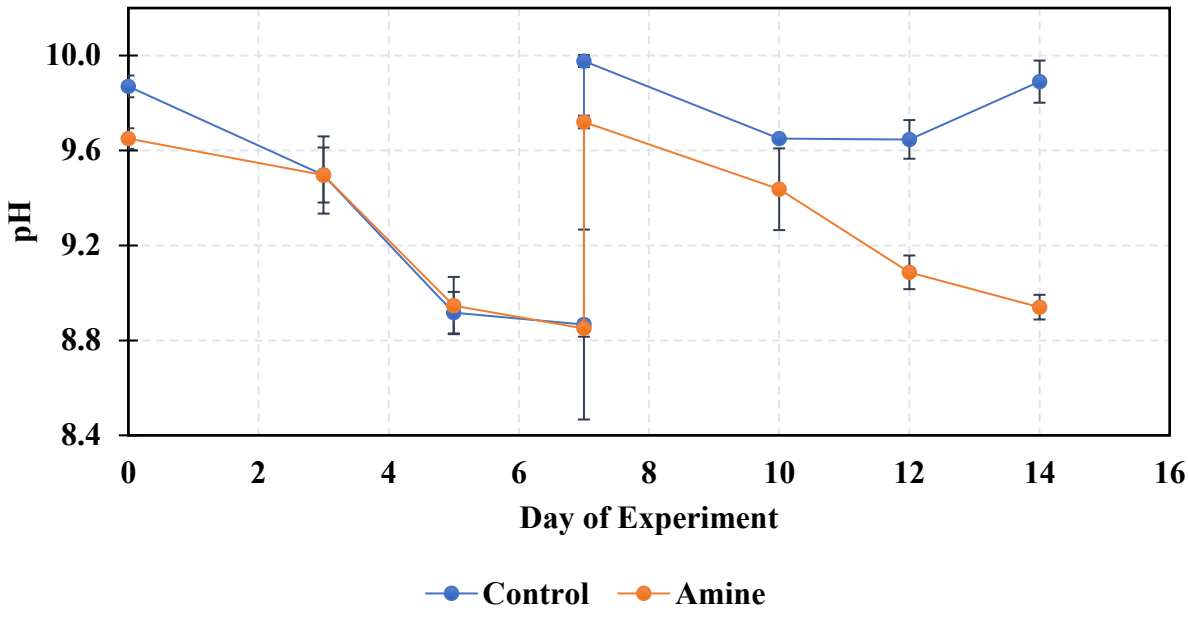

Figure 21. pH of the cultures grown using BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and amine. Error bars represent standard deviations of triplicate trials.

Both Figure 22 and 23 below display the Chlorophyll A productivity and growth rate trends exhibited by the control culture and the amine-only culture.

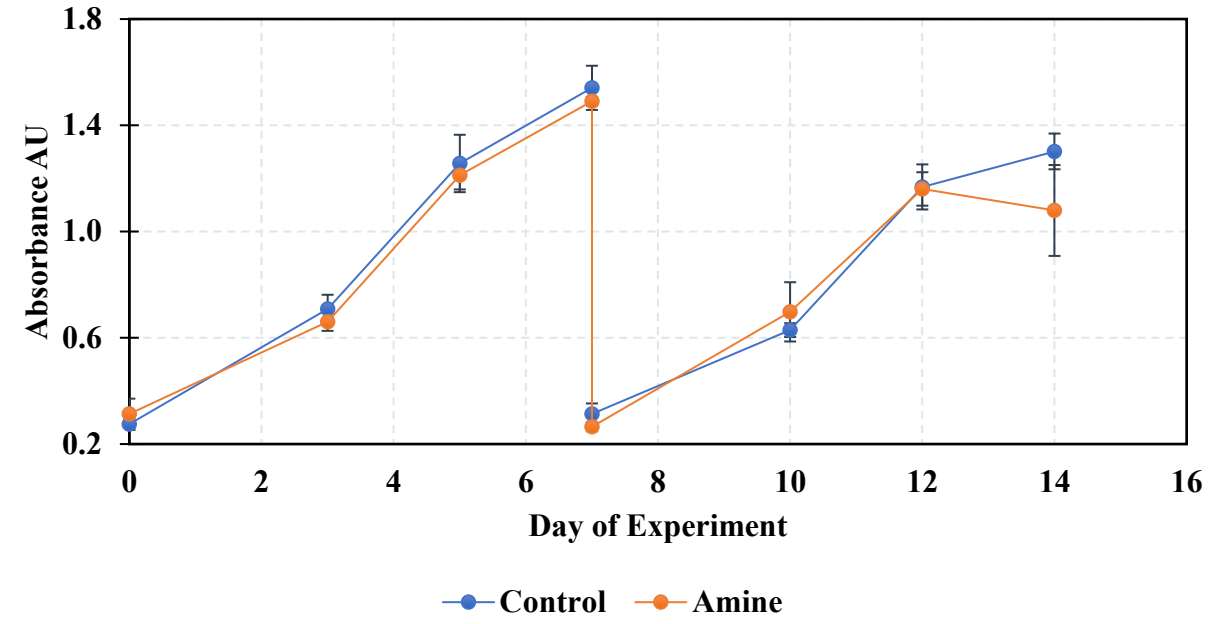

Figure 22. Absorbance (λ = 680 nm) of the cultures grown using BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and amine. Error bars represent standard deviations of triplicate trials.

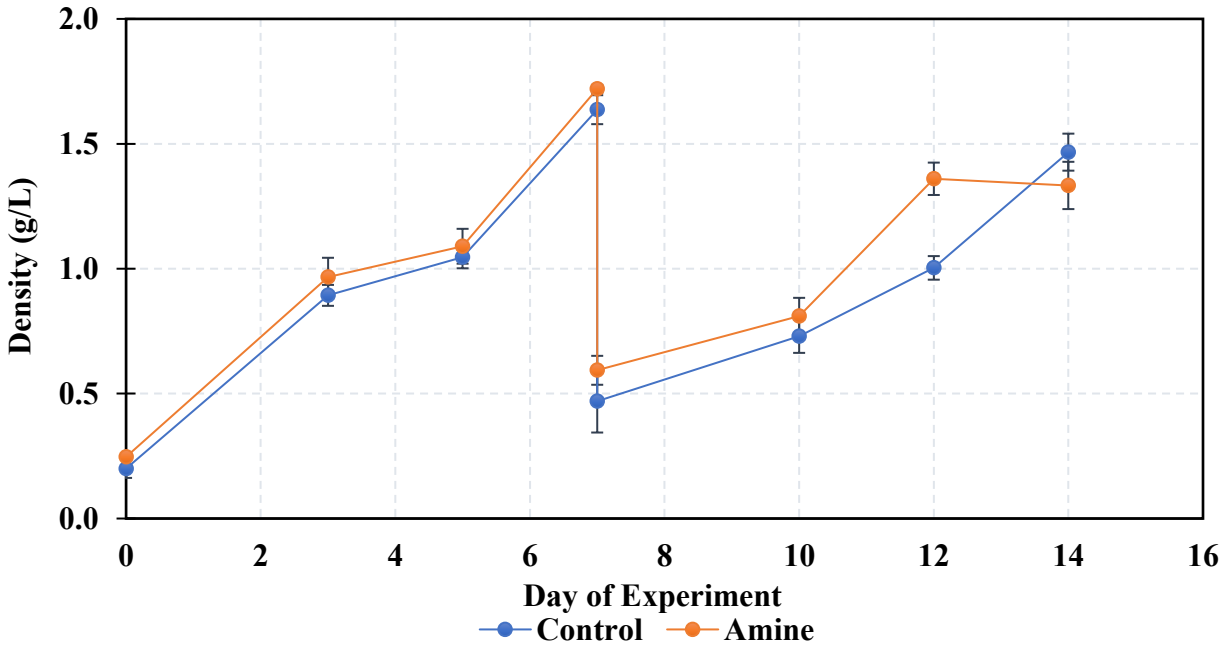

Figure 23. Calculated density of the cultures grown using BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and amine. Error bars represent standard deviations of triplicate trials.

Double Amine. The adequate productivity displayed by the amine group inspired another experiment in which the amine concentration was doubled. Figure 24 below displays the pH trends observed, which are similar for both culture groups. The higher pH the control group displayed throughout relative to the amine group is simply attributed to the difference in the starting pH of the groups.

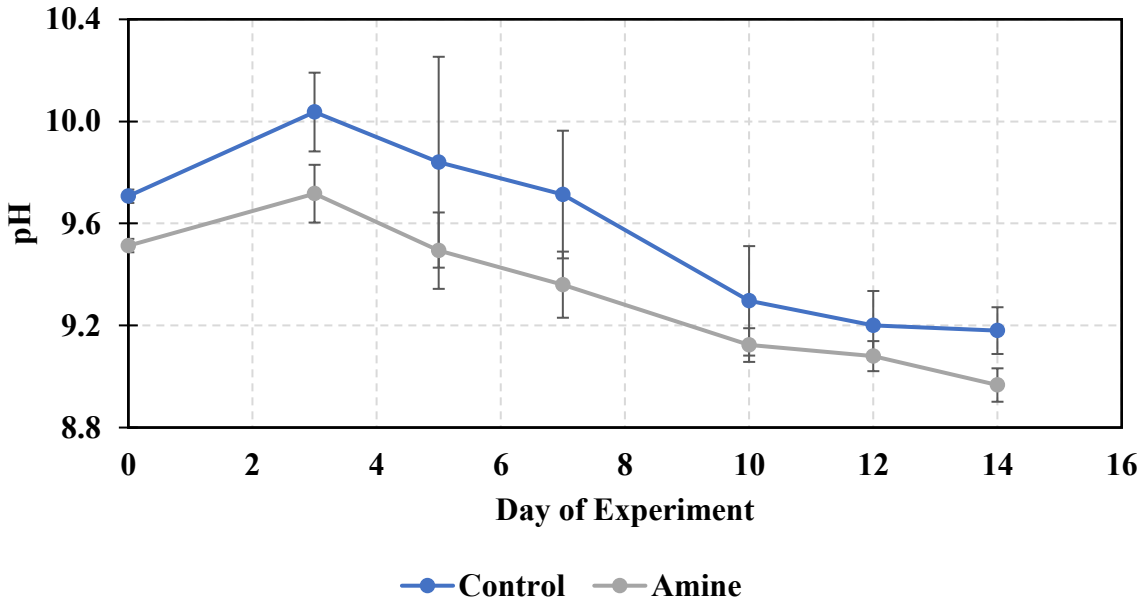

Figure 24. pH of the cultures grown using BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and doubled amine concentration. Error bars represent standard deviations of triplicate trials.

Both Figure 25 and 26 below display Chlorophyll A productivity and growth rate trends exhibited by the control culture and the double amine culture, which indicate that an excessive amount of amine in solution can inhibit *S. acutus* growth and health.

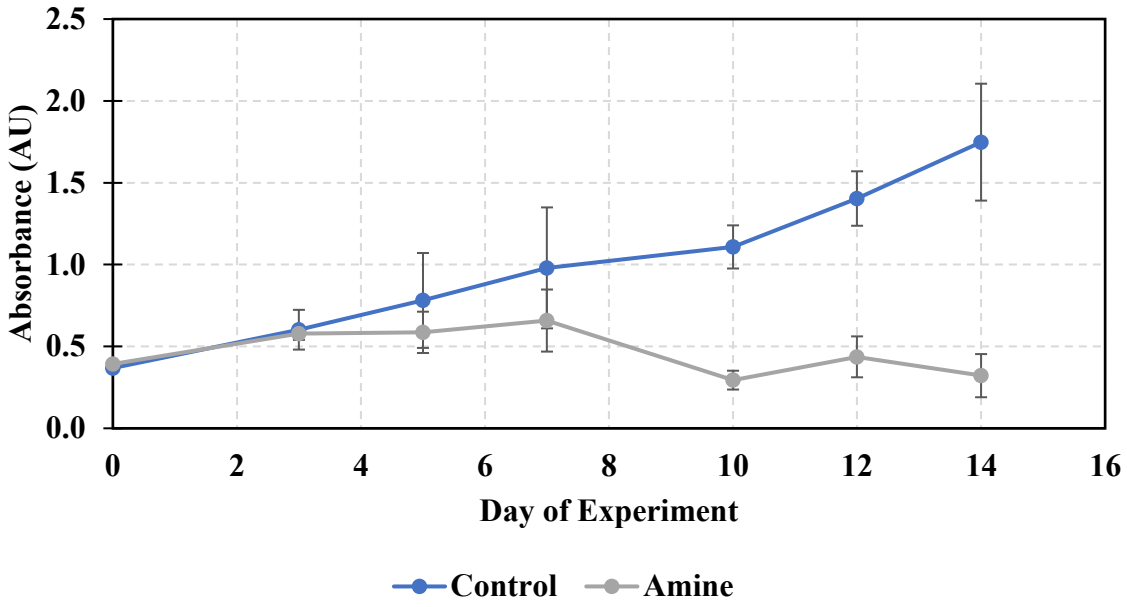

Figure 25. Absorbance (λ = 680 nm) of the cultures grown using BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and double amine concentration. Error bars represent standard deviations of triplicate trials.

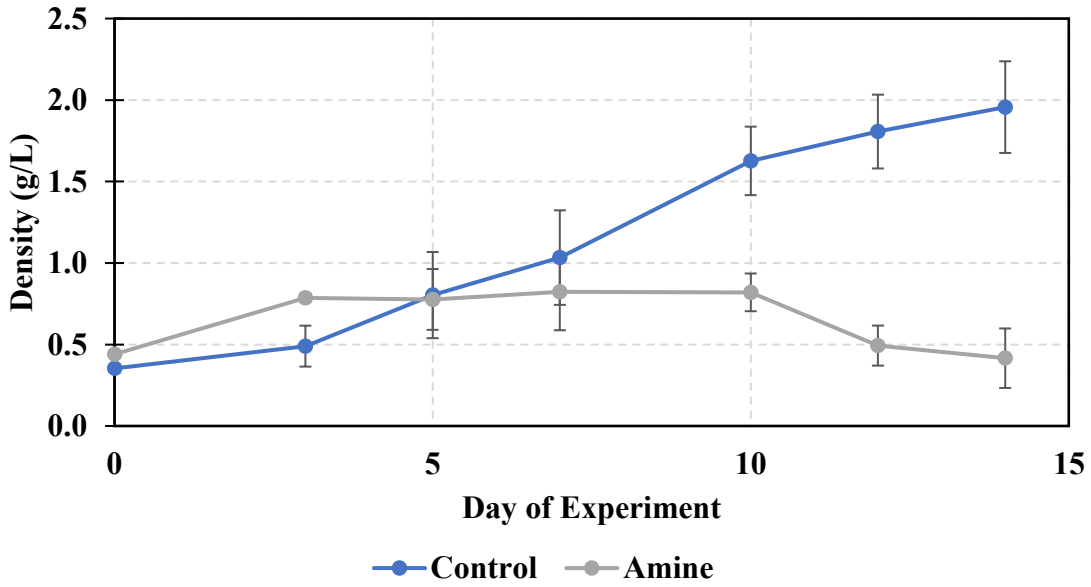

Figure 26. Calculated density of the cultures grown using BG-11 (25% $NaNO_3$), ammonium (bi)carbonate, and doubled amine concentration. Error bars represent standard deviations of triplicate trials.

Ammonium nitrogen sources. *S. acutus* was cultured in agriculture-grade media and BG-11 media with various nitrogen sources.

Agriculture-grade media. *S. acutus* was cultured with agriculture-grade media with various nitrogen sources including urea, ammonium carbonate, and ammonium bicarbonate over the course of a 25-day period. Figure 27 below displays the measured pH values of each sample. All cultures displayed the same initial pH value of ~6.2. The pH steadily decreased for the samples using ammonium as the nitrogen source. The pH abruptly dropped from the initial value and then steadily increased for the sample using urea as the nitrogen source.

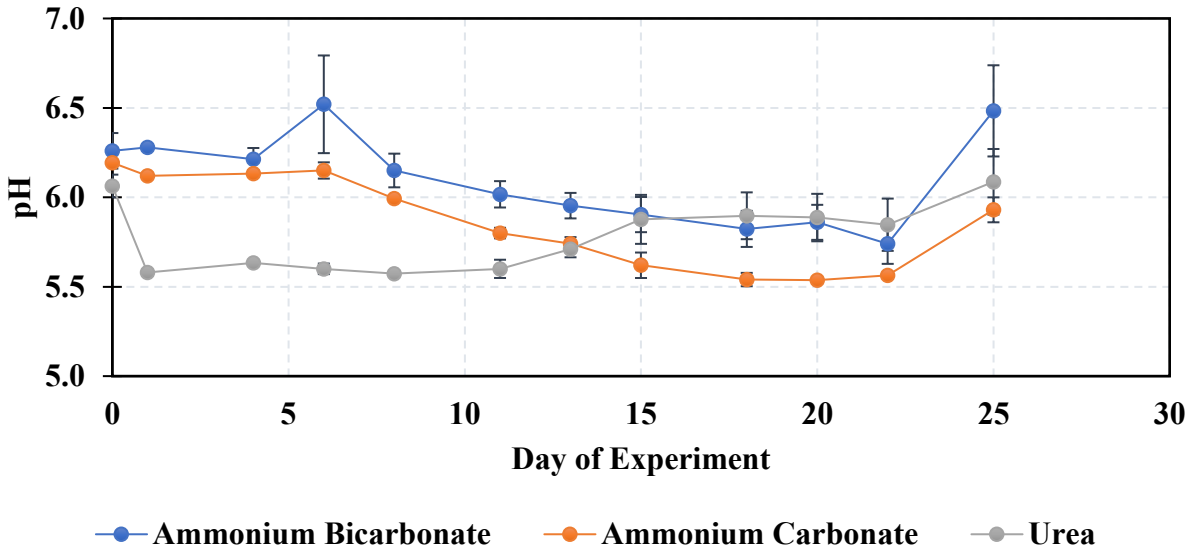

Figure 27. pH of the cultures grown using agriculture-grade fertilizer with urea, ammonium carbonate, and ammonium bicarbonate as the nitrogen source. Error bars represent standard deviations of triplicate trials.

Figure 28 below displays the absorbance values measured. All cultures displayed the same initial absorbance value of ~0.1 AU. Absorbance steadily increased over the growth period for all nitrogen sources. However, the absorbance values increased significantly more for the ammonium-based formulations.

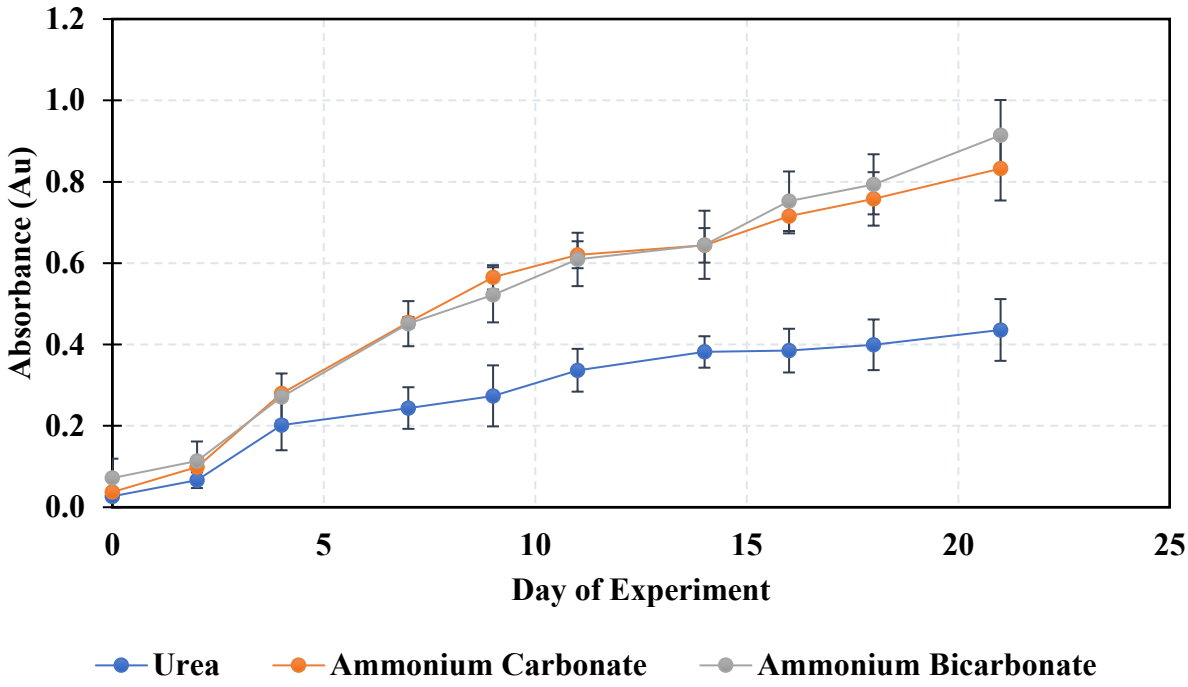

Figure 28. Absorbance (λ = 680 nm) measurements of the cultures grown using agriculture-grade fertilizer with urea, ammonium carbonate, and ammonium bicarbonate as the nitrogen source. Error bars represent standard deviations of triplicate trials.

Figure 29 below displays the density values calculated based on dry mass measurements. All cultures displayed the same initial density value of ~0.02 g/L. The density steadily increased over the growth period for all nitrogen sources. However, the density values increased significantly more for the ammonium-based formulations. This reflects the trend observed in absorbance, which is to be expected.

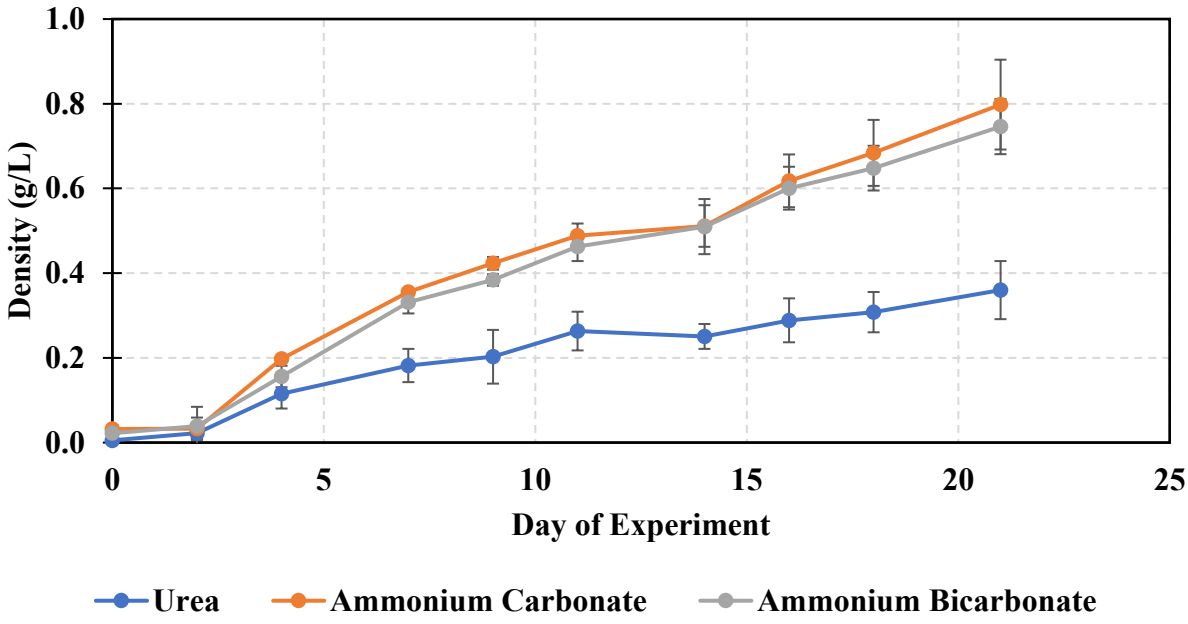

Figure 29. Density measurements for the cultures grown using agriculture-grade fertilizer with urea, ammonium carbonate, and ammonium bicarbonate as the nitrogen source. Error bars represent standard deviations of triplicate trials.

Modified BG-11 with urea control. *S. acutus* was cultured with BG-11 media with various nitrogen sources including 25% of the standard $NaNO_3$, ammonium carbonate, and ammonium bicarbonate, along with urea control over the course of a 72-day period. Figure 30 below displays the measured pH values. The ammonium nitrogen sources started at a significantly higher pH that BG-11 and urea. Trends were mostly reproducible across growth-harvest cycles. The pH steadily decreased for the ammonium nitrogen sources across all cycles other than the final cycle. The pH rose dramatically in each cycle within the trial involving BG-11 with 25% $NaNO_3$. The pH rose steadily for the trials involving urea, replicating what was observed in a previous experiment.

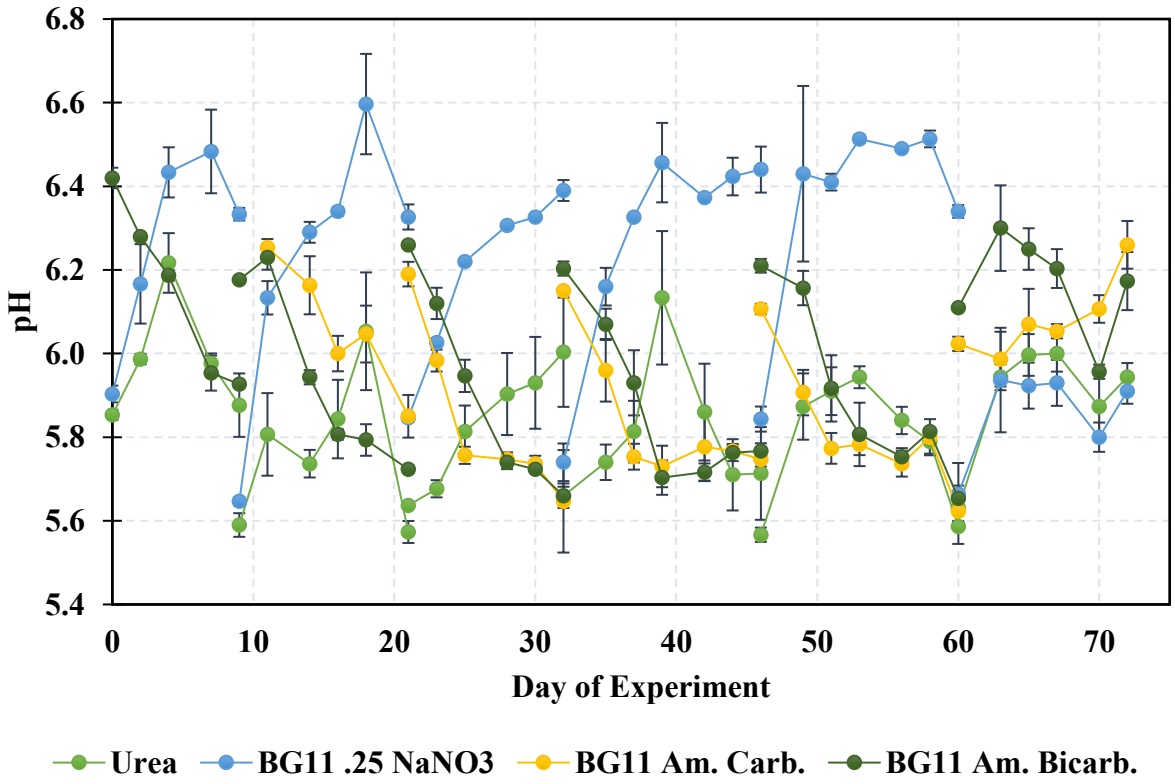

Figure 30. pH of the cultures grown using BG-11 modified media (25% $NaNO_3$), ammonium carbonate, ammonium bicarbonate, and urea control. Error bars represent standard deviations of triplicate trials.

Figure 31 below displays the absorbance values measured. All cultures displayed the same initial absorbance value of ~0.3 AU. The absorbance steadily increased over the growth period for all samples; however, the absorbance trends varied throughout harvest cycles. The ammonium bicarbonate group consistently displayed the highest absorbance.

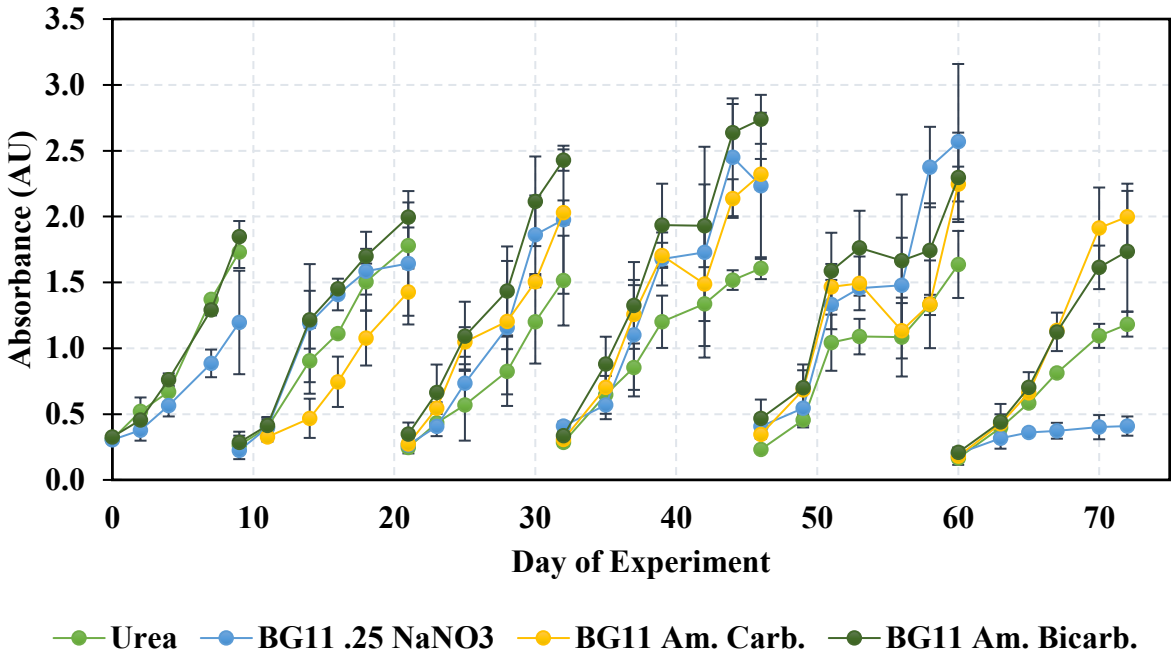

Figure 31. Absorbance (λ = 680 nm) measurements of the cultures grown using BG-11 (25% $NaNO_3$), ammonium carbonate, ammonium bicarbonate, and urea control. Error bars represent standard deviations of triplicate trials.

Figure 32 below displays the density values calculated based on dry mass measurements. All cultures displayed the same initial density of ~0.3 g/L, although this value varied throughout growth-harvest cycles. The density trends mostly reflect the absorbance trends. BG-11 with ammonium bicarbonate usually achieved the highest density, although this varied throughout growth-harvest cycles. Urea typically displayed the lowest density, but this also varied throughout growth-harvest cycles.

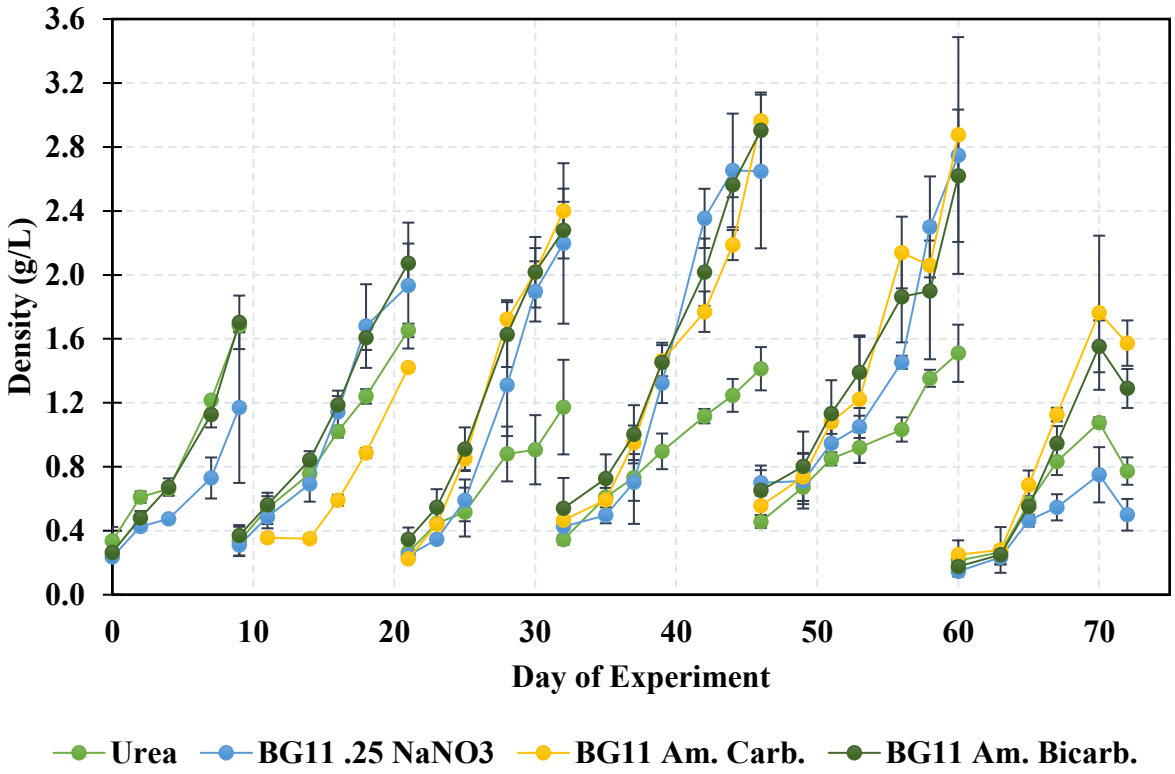

Figure 32. Density measurements of the cultures grown using BG-11 (25% $NaNO_3$), ammonium carbonate, ammonium bicarbonate, and urea control. Error bars represent standard deviations of triplicate trials.

Ammonia gas sparging. *S. acutus* was cultured in BG-11 without $NaNO_3$, with gaseous CO_2 and NH_3.

Various ratios. Various CO_2 and NH_3 ratios were evaluated for *S. acutus* cultivation in BG-11 without $NaNO_3$. For all trials and growth-harvest cycles, there was an initial increase in pH due to the bubbling of the CO_2/NH_3 gas in the absence of algae, resulting in an NH_3/NH_4^+ accumulation. For all trials, pH decreased from seed to harvest date due to algal cells utilizing the accumulated NH_4^+. The graphic below displays the harvest and maximum pH values observed for each CO_2/NH_3 ratio.

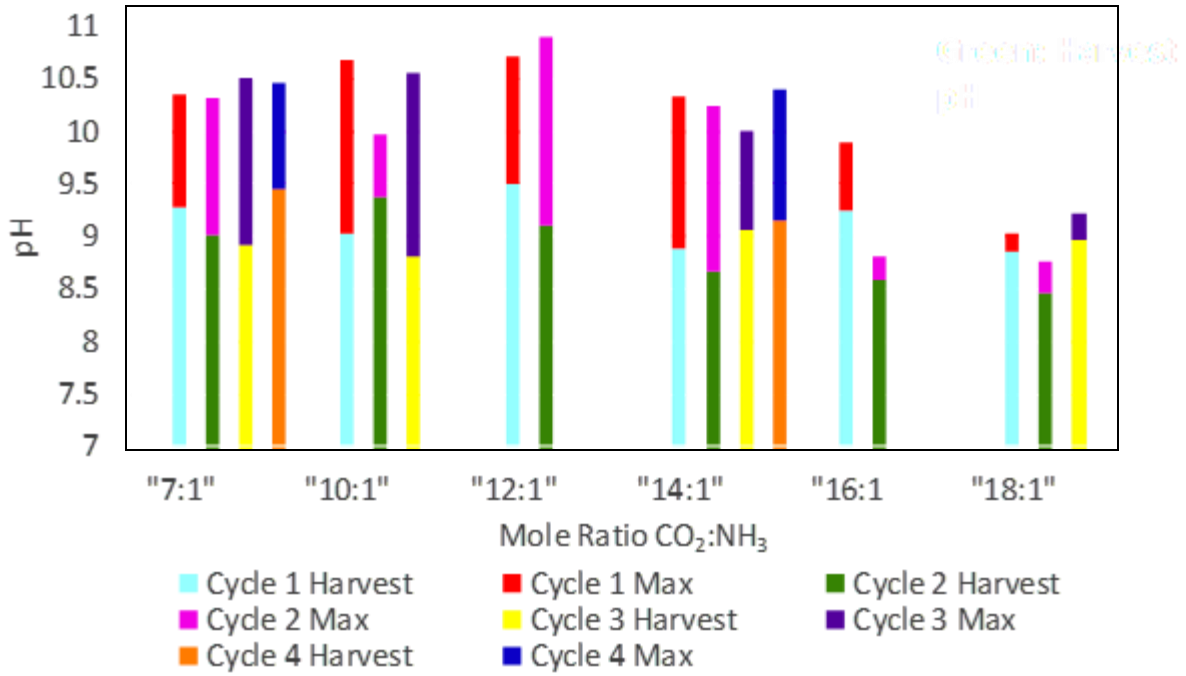

Figure 33. Maximum culture pH and pH at harvest as a function of molar CO_2/NH_3 ratio and harvest cycle number.

Figure 34 below displays the productivity values based on the culture density calculated from dry mass measurements at each ratio. Overall, the productivity of each ratio was adequate, indicating that *S. acutus* can survive and prosper using gaseous CO_2/NH_3 as the C- and N-source. Observing the differences between the various ratios, a ratio of 10 seems to be ideal for *S. acutus.* Indeed, a CO_2/NH_3 ratio of 10 exhibited a productivity of 0.17 g/L/day, which exceeds previous productivity values for *S. acutus* grown with 1% CO_2/N_2 and urea (0.13 g/L/day) or BG-11 (0.1 g/L/day). One interesting point in these data are the unexpectedly low productivities displayed in the CO_2/NH_3 = 12 experiment, which also exhibited higher maximum pH values than expected. Therefore, further investigation and repetition is warranted for this ratio.

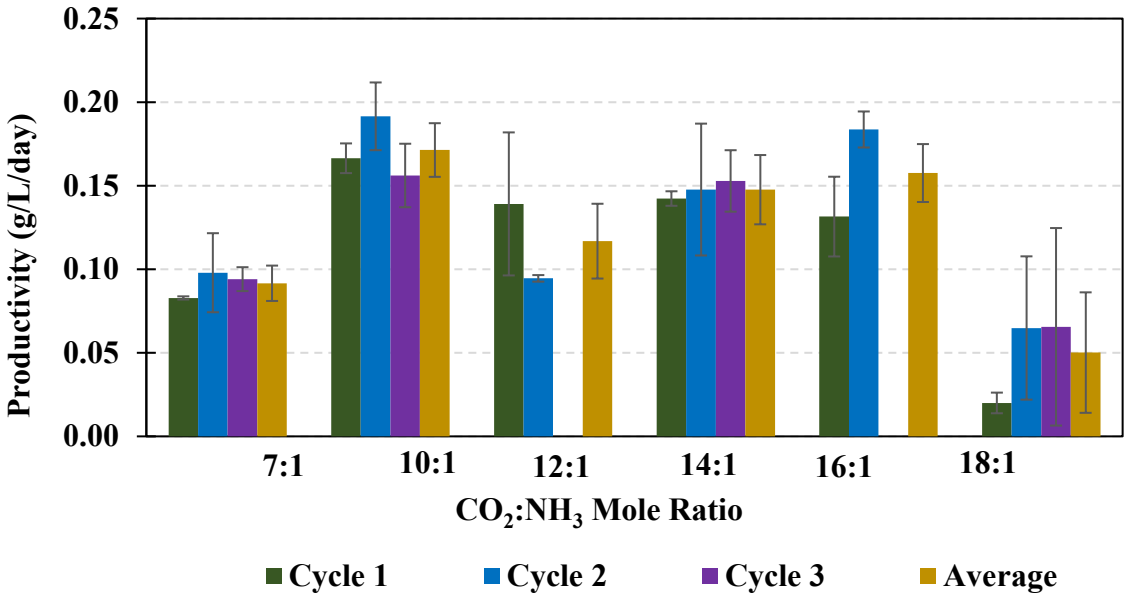

Figure 34. Algae culture productivity (in grams per liter per day) observed with different CO_2/NH_3 mole ratios across several growth-harvest cycles.

At $CO_2/NH_3 = 7$, CO_2 and NH_3 utilization efficiencies were 44.3% and 50.0%, respectively. As the ratio increased, CO_2 and NH_3 utilization both increased to 59-72% and >90%, respectively. Once the ratio tested rose to 14:1 and 18:1, CO_2 utilization decreased (partially due to excess CO_2) and NH_3 utilization decreased (partially due to lower algae growth). These calculations, which were based on GC measurements, did not consider any dissolved NH_3 in the culture left unconsumed by the algae. While good agreement was observed in carbon utilization calculations based on CO_2 utilization estimates from GC data for the 10:1 ratio, this was not the case for the 18:1 ratio. Therefore, carbon and nitrogen utilization estimates were based on IC results. The IC results are displayed below in Table 7.

Table 7. Carbon and nitrogen utilization (calculated from mass of C and N fed daily to culture and measured in harvested samples via elemental analysis) by algae cultures grown with different CO_2/NH_3 ratios across several growth-harvest cycles.

CO_2/NH_3 Mole Ratio	Cycle no.	Total in Algae (g/day)		Total in Feed (g/day)		Utilization (%)		Ash (wt%)
		C	N	C	N	CO_2	NH_3	
7	1	0.082	0.015	0.191	0.032	44.3	50	4.43
10	1	0.166	0.031	0.273	0.032	60.9	97.8	2.89
	2	0.196	0.036	0.273	0.032	71.7	~100	3
	3	0.155	0.03	0.273	0.032	56.8	94.5	3.58
14	1	0.148	27	0.382	0.032	39	83.8	2.77
18	3	0.06	0.01	0.491	0.032	12.1	31.3	3.19

The nitrogen content for the 10:1 ratio, calculated using the cellular nitrogen content observed in Table 7, consistently amounted to ~9.8 wt%, which corresponds to a high protein content of 47 wt%. A low ash content (< 3.6 wt%) was also observed in the biomass grown using a 10:1 ratio. The biomass grown using the 18:1 mole ratio had a lower N content (8.6 wt%), which corresponds to a protein content similar to that of *S. acutus* grown in urea [29].

Various Flowrates. Various gas flowrates of $CO_2/NH_3 = 10$ were researched to cultivate *S. acutus* in BG-11 without $NaNO_3$. At a 0.1L/min (x1.0) flow, a productivity between 0.12 and 0.15 g/L/day was consistently observed. Increasing the feed rate inhibited algal growth, as shown in Figure 35 below.

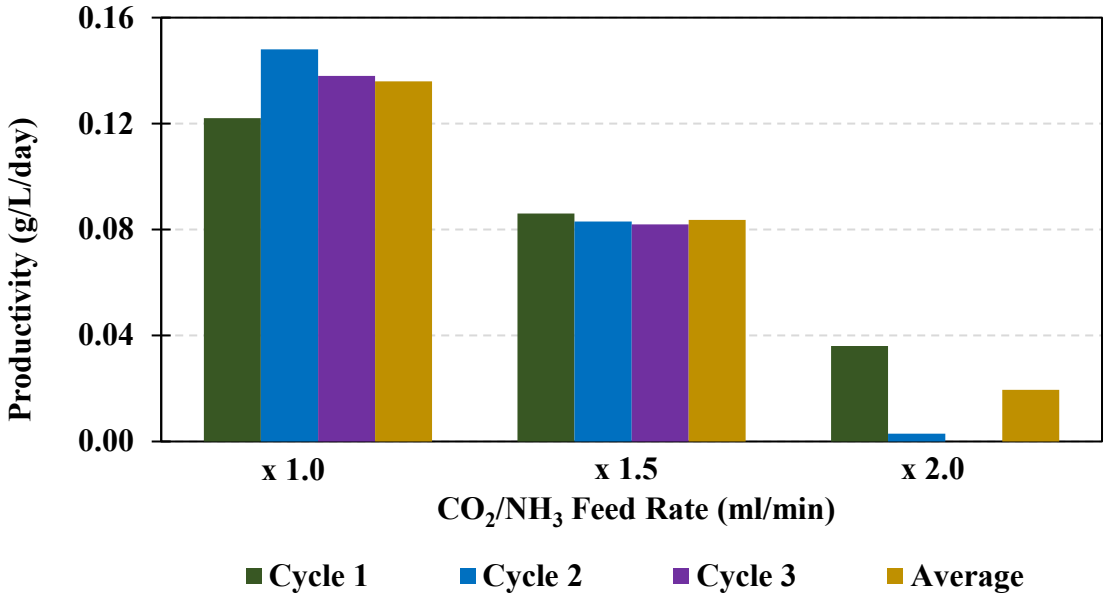

Figure 35. Algae culture productivity (in grams per liter per day) observed with different CO_2/NH_3 ratios across several growth-harvest cycles.

GC analyses revealed a higher CO_2 uptake at the lower feed rates (x1.0 and x1.5) compared to the x2.0 experiment, a result consistent with algal growth trends. IC was used to measure ammonium ion concentrations from which free ammonia concentrations were calculated. Figure 36 below displays these results for each feed rate on the ninth day of the second cycle. Both NH_4^+ and free NH_3 concentrations increased with the feed rate.

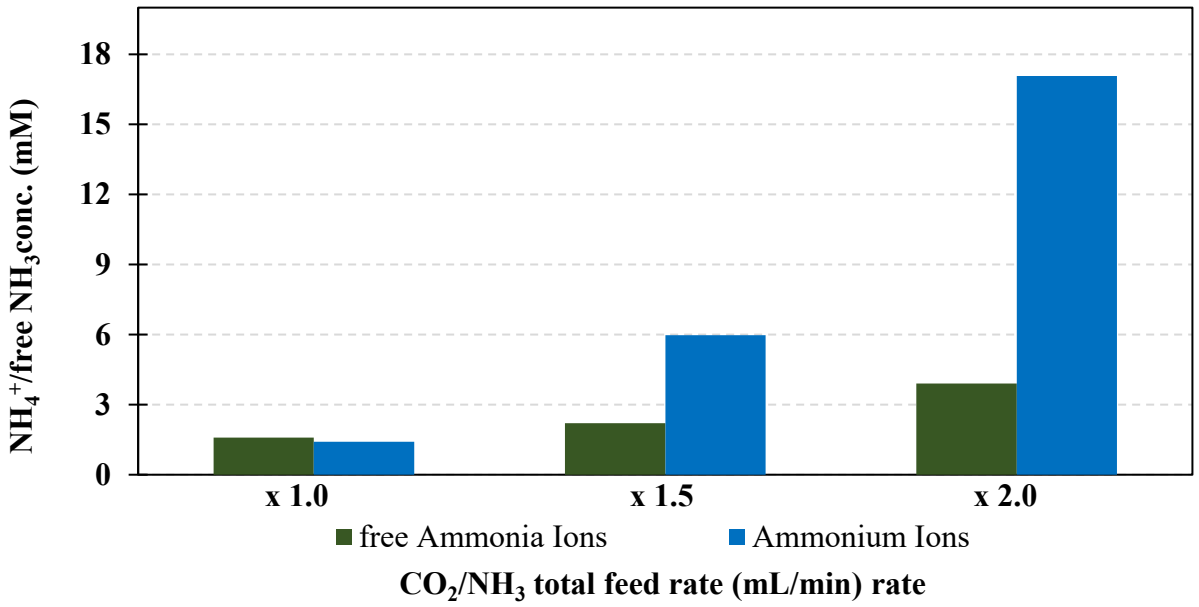

Figure 36. NH_4^+ and free NH_3 concentration observed with different total gas flow rates during the ninth day of the second growth-harvest cycle.

DISCUSSION

Coelastrella sp. productivity and astaxanthin production

Media. The media screening indicated that *Coelastrella sp.* displayed higher productivity in BG-11 compared to urea; however, BG-11 is more expensive and not ideal for large-scale algae production.

Brackish conditions. Representative levels of salinity spanning the brackish water range were evaluated and the freshwater algae *Coelastrella sp.* was able to maintain growth in all salinity levels; however, growth was higher at the lower concentrations, which was to be expected. It is plausible to cultivate *Coelastrella sp.* in lower salinity level which would lead to a decrease in freshwater usage.

Light intensity and nitrogen deprivation. Brackish conditions can increase pigment productivity during a stress phase, but not during an initial growth phase. Nitrogen deprivation in addition to brackish conditions was a particularly effective means of increasing pigment production, specifically the carotenoid astaxanthin.

Sodium bicarbonate and light stress. 50 mM of sodium bicarbonate added during a stress phase following a growth phase exhibited the highest growth rate and the highest astaxanthin productivity of all conditions evaluated, including a control group which was a close second. The samples to which 100 mM sodium bicarbonate was added had the lowest productivity and pigment production, indicating that there is an optimum supplementation of sodium bicarbonate to *Coelastrella sp.* and that sodium bicarbonate addition above this optimum could be toxic to the algae. Light intensity is also effective in increasing productivity during a stress phase.

Scenedesmus acutus productivity

Sodium carbonate and sodium bicarbonate pH control with cobalt catalyst and amine supplementation. Cobalt catalyst addition caused shading, which inhibited algal growth. Amine supplementation allowed algal growth, but the growth observed was not statistically higher than that of a control. pH control was achieved with the use of sodium bicarbonate and carbonate without a toxicity effect at alkaline pH conditions. The acclimation tendency of *S. acutus* was also exemplified by the results in that the pH was buffered and stabilized throughout all experimental group regardless of treatment group. This further exemplifies the robust nature of *S. acutus.*

Ammonium nitrogen sources. Ammonium bicarbonate and ammonium carbonate used as a nitrogen source with other agricultural-grade media components performed better than urea. However, in BG-11, ammonium bicarbonate and ammonium carbonate performed with the same productivity as BG-11 with 25% $NaNO_3$.

Ammonia gas sparging ratios. *S. acutus* exhibited the greatest growth at a CO_2/NH_3 ratio of 10 (exceeding that attained using 1% CO_2/N_2 and $NaNO_3$ or urea), averaging a 0.17 g/L/day growth rate. High CO_2 utilization (59% to 72%) and high NH_3 utilization (>90%) was observed, which shows promise for the utilization of gaseous emissions. High protein (47 wt%) and low ash (< 3.6 wt%) content was observed in algae grown at this CO_2/NH_3 ratio, which is favorable for the use of algal biomass in the production of bioplastics or as animal feed. The NH_4^+ and free NH_3 concentrations increased with the total gas feed rate, which resulted in lower algae growth rates and suggests that excess NH_3 is toxic to *S. acutus*. This is consistent with previous results, which indicate that high concentrations of free NH_3 can inhibit growth in *Scenedesmus obliquus* and other types of algae [33, 34, 35]. Hence, for practical applications, a CO_2/NH_3 feed control strategy would be required that considers both the ammonium ion concentration in solution as well as the pH to avoid excessive concentrations of free ammonia.

CONCLUSIONS

The past few years of algal research studying *S. acutus* and *Coelastrella sp.* have indicated that algae cultivation shows great promise for CO_2 mitigation, waste control, and astaxanthin production. Nitrogen deprivation and 50 mM sodium bicarbonate stressors have shown an increase in pigment productivity in *Coelastrella sp.*. Brackish water can sustain *Coelastrella sp.* growth, although growth is better at lower salt concentrations when paired with BG-11, which affords higher productivity than urea although at a higher cost and complexity.

Tests with various nitrogen sources, including ammonium bicarbonate, indicate that *S. acutus* can utilize ammonium ions in solution as a nitrogen source, providing a basis for investigating the use of gaseous ammonia to feed algal cultures. Gaseous bubbling of CO_2 and NH_3 in a molar ratio of 10:1 to *S. acutus* could lead to both agricultural waste mitigation and optimal algal biomass characteristics for bioplastics and livestock feed production.

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