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Biomass productivity of snow algae and model production algae under low temperature and low light conditions

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ABSTRACT

This study was designed to determine biomass productivities of specific algal species under low temperature and low light conditions. The algal species examined in this study included two psychrophilic algal species (*Chlamydomonas yellowstonensis* and *Chlamydomonas augustae*). These species are commonly known as “snow algae” due to their ability to grow in low temperature water bodies including ice and snow deposits. Additionally, two model production algal species used in high biomass productivity pilot studies (*Scenedesmus bijuga* and *Chlorella sorokiniana*) were evaluated. Currently, temperature dependent growth data within known optimal limits exists for these model production species but there is no detailed information about their biomass productivity under low temperatures. In addition, little information can be found about the potential for productivity of these species under limited light exposure. This study examined biomass productivity of these four species at four relatively low temperatures (5, 10, 15, and 20 °C) with three relatively low light exposures (50, 100, and 300 μmol/m^2 s). It was hypothesized that the two psychrophilic algae species would produce more biomass per day than model production algal species under these limiting conditions. This study found that both snow algae species performed better than model production species at the lowest temperature (5 °C) and two lower light intensities (50 and100 μmol/m^2/s). *C. augustae* growth rate was shown to have a positive correlation with temperature and a *negative* correlation with light intensity for the values observed in this study. This finding has significant implications for the use of *C. augustae* as a cool-season algal crop and a source of valuable genetic material for future engineering of algae. This could lead to the development of cool-season algal crops for sustainable, year-round, industrial production of algae in temperate climates. Furthermore, both of the snow algae species studied here showed inhibited growth at the highest light intensity studied here.

1. Introduction

An apparent conflict arising in sustainability analyses of algal systems is the question of the efficacy of cool-season operation. From a greenhouse gas (GHG) life cycle analysis (LCA) perspective, the literature suggests that cool-season yields are not sufficient to maintain sustainability criteria necessary to meet critical metrics such as those required for advanced biofuels designation in the United States. Specifically, it has been suggested that systems that incorporate cool-season growth using current accepted yields under these conditions are unable to meet the required 50% reduction in GHG emissions as compared to petroleum diesel necessary to achieve advanced biofuel status [3,11]. In contrast, when one examines the systems using technoeconomic analysis (TEA) it is predicted that it is necessary to run algal systems continuously throughout the year as consistent operation and production is necessary to maintain steady revenues required for financial sustainability. A highly regarded TEA analysis suggested that the number of days of operation is one of the most influential parameters on the economic success of algal production operations and achieving these goals [4]. Likewise, an analysis of over 40 algae system TEAs and LCAs conducted by Quinn and Davis [10] suggests that seasonal and regional variability in productivity can have a profound effect on the overall economic viability of algal production systems.

Ultimately, the conflict faced when trying to achieve economic and environmental sustainability in algal systems will need to be addressed if production of biofuels from algae is to become a viable component of the global energy mix. Reconciling these issues will be achieved by increasing cool-season algal productivity to provide sustained biomass production leading to both the desired reduction in GHG emissions and economic viability. One pathway that may lead to resolving the

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inherent conflict between economic and environmental sustainability of algal systems is the development of cold-tolerant and psychrophilic algal species as cool-season crop alternatives to model production algal species.

Production algae perform best at optimal conditions, which often include warm temperatures (> 20 °C) and exposure to high levels of sunlight (> 150 µmol/m^2/s) [15,16]. Based on these conditions, ideal geography for the outdoor, open-pond based production of algae has often been identified as locations that maintain temperate conditions for most of the year, for example, the Desert Southwest of the United States. However, one factor, which needs to be considered in the siting of algal farms, is the availability of water. Open-pond systems require a tremendous amount of makeup water to compensate for rapid evaporation from the high surface area required for production of algae [2]. This has resulted in a reconsideration of ideal sites for these facilities. A study by Venteris et al. [15] examined algal water demand as well as algae production and utilization infrastructure availability, water suitability (salinity), etc. This study produced a GIS based map that identified the Gulf of Mexico area as ideal for two model production species (*Anthrospira* and *Sphaeropleales*). The Florida peninsula was identified as the most suitable location in the U.S. Further analysis from Venteris et al. [16] identified a contiguous band from eastern Georgia and Florida to Southwestern Texas as a high productivity region for production of several algal species including *Nannochloropsis* and *Chlorella*. If cool-season adapted algae were identified that maintained modest biomass production, it is possible that the growing season of algae could be extended in these regions to provide more biomass from algal crops.

This study was designed to identify algal species that have the potential for production in cooler months to provide the possibility of continuous production of algae for biomass at an industrial scale. It was determined that such algal species would be identified within a group of psychrophilic algae known as “snow algae” named for their discovery in hard pack snow and glaciers of Antarctica, mountain ranges and other extremely cold environments [6,8,12,13]. Through a literature search and subsequent screening process snow algae strains were identified as suitable for this study. This study ultimately examined the biomass productivity of four species at four low temperatures (5, 10, 15, and 20 °C) and three low light intensities (50, 100, and 300 µmol/m^2/s). Two snow algae species were selected due to their psychrophilic nature (*Chlamydomonas yellowstonensis* and *Chlamydomonas augustae*). Two species that serve as model laboratory organisms, *Scenedesmus bijuga* and *Chlorella sorokiniana*, were selected to serve as a baseline for comparison.

2. Materials and methods

2.1. Species selection

The species selected for this study are shown in Table 1 along with data on their temperature and light dependent growth where available.

The species were selected based on two sets of criteria. *S. bijuga* and *C. sorokiniana* were native species isolated from wastewater from Dalton, GA and have been demonstrated as well-adapted to growth in the Southeastern United States. These algae have served as model organisms for the production based algal research at the University of Georgia's Bioconversion Research and Education Center (Athens, GA). *C. yellowstonensis* and *C. augustae* were selected based on literature review of “snow algae” which are algae often found in glaciers or snow deposits. Many of these species have adapted to grow at lower temperatures near the freezing point of water. These particular species were found to be available from the UTEX algal repository and met initial screening criteria demonstrating growth across a wide range of temperatures in modified BG-11 growth media.

Several other snow algae were screened for suitability for this study including: *Chodatia tetrallantiodea, Chlorommonas brespevina, Chlorosarcinopsis sempervirens and Raphidonema nivale. C. sempervirens* and *R. nivale* demonstrated irregular, low productivity growth under the screening protocol used here. *C. tetrallantiodea* and *C. brespevina* showed no growth at all in modified BG-11 media at four relatively low temperatures (5, 10, 15, and 20 °C).

2.2. Temperature and light controlled algae growth

Scenedesmus bijuga (SB), *Chlorella sorokiniana* (CSO), *Chlamydomonas yellowstonensis* (CY) and *Chlamydomonas augustae* (CA) were grown in 100 mL cultures of modified BG-11 media [9] in 250-mL Erlenmeyer flasks which were placed in a fisher scientific 436 incubated orbital shaker operating at 100 rpm. The lid of the shaker was removed and the shaker was placed in a temperature and humidity controlled growth chamber with a constant humidity of 70–75%. Cultures were grown at 5, 10, 15, and 20 °C with light exposure of 50, 100 and 300 µmol/m^2/s providing for a total of 12 treatments (conditions) per species with 3 replications (i.e., flasks) for each treatment, totaling 144 experimental units. Temperature was maintained within ± 1 °C for all treatments except 300 µmol/m^2/s treatments, which experienced a temperature drift of ± 4 °C due to heat generated from the lighting system. Light was cycled to provide 12 h light and 12 h dark conditions. Cultures were grown in triplicate for a minimum of 14 days. 500 µL of each culture was removed every 30 h under sterile conditions and optical density (OD) @750 nm was measured for each sample using BioTek Synergy H1 Hybrid Reader. OD readings were converted to algal cell concentrations (mg/L) using standard curves plotted from the OD of known biomass concentrations (mg/L) for each algal species. Growth curves (concentration vs. time) were plotted using the daily biomass data and biomass productivity rates (mg/L/day) which were determined as the slope of a linear regression of the linear phase of these growth curves.

The 144 data points (4 algal species × 4 temperatures × 3 light intensities × 3 replicates) generated above were then compared across the 4 species under each treatment regime to identify differences in growth patterns between snow algae and model production organisms. Growth models based on the treatment and species matrix were built using ANOVA and/or Linear Regression (where possible) using methodology described below and the most accurate models were identified. These models and the observed behavior of the algal species were used

Table 1
Snow algae and model laboratory algae used in temperature and light regulated growth studies.

Species	Optimal temperature	Known growth temperature range	Source	Reference(s)
Scenedesmus bijuga	TBD	5[1]–40 °C[2]	UTEX[a] 2980	[1]Our Preliminary Data [2]Bajaj and Srivastava [1]
Chlorella sorokiniana	38 °C[3]	10[4]–38 °C[3]	UGA[b]	[3]Franco et al. [5] [4]Our Preliminary Data
Chlamydomonas yellowstonensis	16 °C[5]	0–25 °C[5]	UTEX[a] SNO134	[5]Hoham [7]
Chlamydomonas augustae	11–18 °C[6]	4–32 °C[6]	UTEX[a] SNO155	[6]Teoh et al. [14]

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