Biomass and Pigment Production for Arthrospira platensis via Semi-Continuous Cultivation in Photobioreactors: Temperature Effects

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Abstract

his study describes the response of Arthrospira platensis to a variety of temperature conditions as reflected in variations of photosynthetic parameters, pigmentation, and biomass productivity in indoor photobioreactor (PBR) cultivations. These experiments are designed to better understand the impact of temperature, seasonal variations, and acclimation effects on outdoor biomass production. The irradiance levels and temperature range (20 – 39°C) are chosen to enable modeling of semi-continuous operation of large-scale outdoor PBR deployments. Overall, the cultivations were quite stable with some pigment-related instabilities after prolonged high temperature exposure. Changes in productivity with temperature, as reflected in measured photosynthetic parameters, are immediate and mainly attributable to the temperature dependence of the photosaturation parameter, a secondary factor being variation in pigment content. This study confirms that temperature is critical for optimal performance of Arthospira platensis for both biomass and pigment production and provides a basis for risk assessments related to temperature mitigation for large-scale outdoor cultivations. Finally, the biomass productivities in a semi-continuous operation mode are quantitatively reproduced with a productivity model incorporating the photosynthetic parameters measured herein. Those productivities are in good agreement with extensive outdoor testing in PBR arrays at large scale (24,000 L) and over long time periods (up to 18 months).

Introduction

The filamentous cyanobacterium *Arthrospira platensis (Spirulina)*is an oxygenic photosynthetic organism able to grow in tropical and subtropical environments, and one of only a few microalgal systems that has been successfully commercialized and approved by United States Food and Drug Administration (FDA) as a food supplement (Trabelsi et al., 2009). *Arthrospira* cultivation and processing yields valuable biochemical components including protein, carbohydrates, fatty acids and pigments such as phycocyanin (PC), which can be used in nutritional, pharmacological, and cosmetic products. Due to these high value applications, as well as relatively easy harvesting and extraction processes, *Arthrospira* cultivation has been deployed commercially at moderate scale (10 – 100 acre open ponds) for many decades (Lu et al., 2011). It is important to note that *Arthrospira* is an extremophile, in that it can maintain high productivity under high alkalinity, high pH conditions; this limits predation and competition sufficiently to allow commercial production in open pond systems. Algal cultures are influenced by various abiotic variables such as temperature, irradiance levels, and nutrient availability, all of which play a significant role in regulating photosynthetic activity, biomass composition and overall productivity. Under outdoor cultivation conditions, temperature and light intensity are the two key external factors that determine photosynthetic activity and biomass growth rates. Obviously, both factors are highly variable on a daily and seasonal basis in the natural environment, and spatially within the culture as well (Chaiklahan et al., 2007; Vonshak and Novoplansky, 2008). Typically,

Arthrospira is cultivated outdoors for mass production in raceway ponds, where cells encounter fluctuating environments in terms of irradiance, temperature, and nutrient supply. Though the PBR environment tends to be more homogeneous, similar fluctuations are present and temperatures are generally higher due to absorptive heating and the absence of evaporative cooling. Outdoor algal cultures are subjected to high light intensity as well as possible high temperature stress that can negatively impact photosynthetic activity (Torzillo et al., 1991b). These factors can change both the photosynthesis and respiration rates, thereby directly influencing the growth and the chemical composition of the biomass produced (Trabelsi et al., 2009).

Overall, the existing literature is consistent with an optimal temperature range for stable production of roughly 20-35 °C. Our screening studies are consistent with that range and also consistent with an activation energy of about 60 kJ $mole^{-1}$ (Q10 ˜2) under saturating light conditions over that temperature range. It is well-known that productivity is enhanced in semi-continuous operation where the impact of photosaturation effects are lessened. We know of no detailed studies dealing with the effect of temperature and acclimation response on growth and pigment content of*Arthrospira* in a semi-continuous production mode for extended time scales under tightly controlled (laboratory) conditions. The intention here is to determine what portion of previous learnings translate to semi-continuous operation and the dynamic (light/temperature) conditions experienced outdoors. Therefore, in the present work we will examine temperature effects at a longer time scale, and carry out the experiments in semi-continuous operation mode in PBRs at 20 °C, 30 °C and 35 °C. In subtropical conditions, the outdoor culture temperature in the summer months can be very high in PBRs, reaching up to 35-45 °C for several hours. We have only a limited understanding of temperature impacts on photosynthetic parameters, and pigment accumulation in that outdoor environment. Thus, the scope of this work includes *Arthrospira* growth under a variety of temperature conditions with a work plan that includes assessment of temperature response and recovery, and quantification of the dynamic change in biomass and pigment content of *Arthrospira* during the experiments. The work was performed in three phases (Figure 1a): Phase I employs constant temperature conditions (same for day and night cycles), Phase II shifts the Phase 1 cultures to opposing temperature conditions, and Phase III continues the examination under dynamic summer temperature profiles (hourly variations) in a semi-continuous operation mode in PBRs. The experimental setup, shown in Figure 1b, involves vertically oriented tubular photobioreactors, designed to be predictive of outdoor performance in large PBR arrays.

Material and Methods

Algal Strain and culture condition

The algal strain used in this study was *ArthrospiraPlatensis* maintained in Zarrouk's medium with the following macro and micro ingredients (mM): $NaHCO_3$ (200), K_2HPO_4 (3.7), $NaNO_3$(30), K_2SO_4 (5.7), NaCl (18), $CaCl_2$ $2H_2O$ (0.27), $FeSO_47H_2O$ (0.036), Na_2EDTA $2H_2O$ (0.215), NaOH (0.1), H_3BO_3 (0.045), $MnCl_24H_2O$ (0.009), $ZnSO_4$ $7H_2O$ (0.001), $NaMoO_4$ $2H_2O$ (0.000083), and $CuSO_4$ $5H_2O$ (0.00032) (Zarrouk, 1966). Unless stated otherwise, Zarrouk´s medium with 200 mM $NaHCO_3$ was used for all culture cultivations. The seed culture was sourced from a private collection and cultivated at 30 °C for one week to reach a biomass concentration of 0.75 gDW L^{-1} (optical density at 750nm, OD_{750}, equal to 1.5). Under most conditions the DW to OD_{750}ratio was about 0.5 gDW L^{-1} per OD_{750}which is regarded as normal for this organism. Although some variation within the relationship between OD and DW can occur with differing temperature conditions (Jahnke et al., 2011; Torzillo et al., 1991a), such variations were generally minor in the experiments reported here and only occurred at the latter stages of growth at 35 °C where the cultures displayed clear instabilities in pigmentation. The DW measurements involve collecting algal cells on pre-rinsed glass fiber filters (1.5 µm pore size, 47 mm ProWeigh Filters, Cole-Parmer) by filtration, washing three times in deionized water, and then drying to a constant weight at 60 °C for 48 h. The remaining salt content after this procedure was generally less than 2%, and to that extent our DW is equivalent to ash-free DW (ASFW).

Arthrospira platensis , the most common commercial strain, is interchangeably referred to as "*Spirulina* " in the literature. We have used *Arthrospira* throughout this article to refer to the organism under study here

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and literature results cited for *Arthrospira platensis* .

0.35-liter PBR cultivation conditions

For 0.35 L PBR operation, the reactors were inoculated with the *Arthrospira* strain grown at 30 °C to a concentration of 0.5 gDW L^{-1}. The pH value was maintained between 9.2-9.8 with an aeration rate of 80 mL min^{-1} supplying 0.75 % CO_2 during the light phase and 0.20 % CO_2 in the dark phase for cultures grown at 30 °C and 35 °C. The cultures grown at 20 ° C were supplied with 0.38 % CO_2 during the light phase, and 0.2 % CO_2 in the night phase. The light regime for the cultures was a 12 h light:12 h dark cycle with light intensity of 230 µmol m^{-2} s^{-1} from one side of the PBRs using fluorescent lamps (Plusrite). This irradiance level was chosen to model annual average conditions for typical vertical PBR arrays deployed in Fort Myers, Florida. For CO_2 aeration and mixing of the cultures, a custom designed porous air diffuser (0.5 mm diameter) was used in order to generate mm-size gas bubbles for aeration at a constant flow rate of 80 mL min^{-1}.

Semi-continuous cultivation and experimental set-up in PBR

For semi-continuous cultivation, culture dilution with Zarrouk´s medium was carried out on alternate days maintaining OD_{750} = 2.0 (˜1 gDW L^{-1}) as the starting point for the next production cycle. The experiment was continued over a time course of 43 days in this semi-continuous operation mode. The experimental program was divided into three phases with eight PBRs designated in alphabetical order from A to H. These eight PBRs were divided in four sets of duplicate PBRs indicated as 'AB' for A and B, 'CD' for C and D, etc. (Figure 1). OD750nm was monitored as a surrogate for biomass concentration, with the OD-concentration relationship periodically monitored. That relationship (typically DW in g L^{-1} = 0.5*OD750nm) varied only slightly during the course of these experiments as discussed above.

In Phase I, four reactors each were cultivated at constant 20 °C (AB, CD) and 35 °C (EF, GH) for 15 days. In Phase II, the four reactors at 20°C were shifted to 30 °C (CD) and 35 °C (AB) whereas the other set of four reactors at 35°C were shifted to 20 °C (EF) and 30°C (GH). The Phase II shift to opposing temperatures was started on the 15^{th} day and kept in place until the 33^{rd} day. To acclimate the cultures in the newer temperature conditions and examine the acclimation effect, cultures were grown in batch mode for four days (15 – 19) without performing any dilution. In the final Phase III, the culture conditions were shifted from constant temperature to dynamic summer temperature profiles (derived from historical climate data for Fort Myers, Florida) with hourly changes in temperature over the course of 24 hours. This phase lasted from day 33 to day 43. In this phase, the cultures grown at constant 20 °C (EF) and 30 °C (CD) in Phase II were shifted to average summer profile with 35/21 °C as maximum/minimum temperatures, the culture at constant 35 °C (AB) was moved to extreme summer profile with 39/26 °C as maximum/minimum temperatures, and final set of two reactors cultured at constant 30°C (GH) were shifted to constant day (31 °C) and night (22 °C) temperature based on summer average of day and night temperatures. Summer is defined as June 1 through August 31 for creating these profiles from historical data.

Determination of chlorophyll and phycocyanin

Chlorophyll-a (Chl-a) was measured using a standard methanol-based methodology (Marsac and Houmard, 1988).

PC extraction and quantification was carried out using repeated freeze-thaw cycles based on Yoshikawa and Belay (2008). Briefly, this method extracts PC from fresh biomass using a repeated freeze-thaw and soaking regime and then quantifies PC spectro-photometrically based on absorbance at three wavelengths: 620 nm, 650 nm and 680 nm. PC content is calculated following the Yoshikawa equation, where cPC is C-phycocyanin and aPC is allophycocyanin:

$$\text{cPC, mg/mL} = 0.162 \times OD_{620} - 0.098 \times OD_{650} \quad (1)$$

$$\text{aPC, mg/mL} = 0.180 \times OD_{650} - 0.042 \times OD_{620} \quad (2)$$

Photosynthetic parameters:

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The oxygen PI (O_2 production vs irradiance) curves were determined in Algenol's custom-designed system. The system is comprised of a white light LED source, photosynthetically active radiation (PAR) light sensor, and an O_2 sensor, all in a temperature-controlled cassette (Legere, 2017). The optical path length, d, is 1 cm. For measurement of O_2, needle-type oxygen sensors (OXR50, Pyroscience) composed of fiber-optical cable connected to FireSting O_2 sensors were used. The temperature is controlled to ±1 °C over the range from 10 °C to 50 °C. The culture samples were taken from the PBR and incubated at 30 °C for 1.5 hr for dark acclimation, and then diluted to an absorptivity of exp(-kd)=0.1 (concentration˜ 1 mg Chl.a L^{-1}) using fresh Zarrrouk's media, where k is the absorption coefficient for the whole cell *Arthrospira* over the PAR range (400-750 nm). In determining k, spectra are first corrected for scattering (approximately) by subtracting at all wavelengths the absorbance at 750 nm (OD_{750}). The first 10 min of oxygen uptake data in the dark is used to calculate the dark respiration rate (R_d). The light is then ramped up to 1000 µE m^{-2} s^{-1} stepwise with 3 min at each step (typically 15 steps). The oxygen evolution rate is fitted with a Monod model form to report photosynthetic parameters: α, E_k, and P_{max} (limited quantum yield in low light limit, photosaturation parameter, and max photosynthetic rate in high light limit). The Monod model is used to be consistent with the approach taken for the Algenol Productivity Model. All measurements are carried out in duplicate.

Productivity Modeling:

Annual ethanol productivity from cyanobacteria has been successfully modeled by Algenol using a Monod modeling approach (Chance and Roessler, 2019). The Algenol Productivity Model was developed in this manner and used in conjunction with PI-derived photosynthetic parameters to estimate expected average outdoor productivities for PBR deployments in Fort Myers (and elsewhere around the world). The daily biomass volumetric productivity can be described as:

$$P_{\text{Biomass}} = \alpha E_k \gamma \lambda \nu \left[\frac{E_k + E_s}{E_k + E_s e^{-kD}}\right] \frac{t_1}{D} - R_0 C_0 \gamma t_2 (3)$$

where α is the quantum yield in the low light limit (mol C /mol photons), E_k is the photosaturation parameter (µE m^{-2} s^{-1}), γ is the conversion between fixed C to dry weight biomass (gDW mol C^{-1}), E_s is the incident light intensity (µE m^{-2} s^{-1}), k is the absorptivity coefficient of biomass (1/m), D is the effective light path (m), R_0 is the specific respiration rate (µmol C mgChl.a^{-1} min^{-1}), $C_0$is the Chl.a concentration (mgChl.a m^{-3}), t_1 is the time for light-on (sec), and t_2 is the time for respiration load (min). Light-on time (t_1) is about half of the respiration load time (t_2) for outdoor cultivation. For the indoor PBR experiment, 12 hr light/12 hr dark cycle, E_s is constant at 230 µE m^{-2} s^{-1}, and the average light path (D) is approximately the radius of the reactor tubes with illumination from one side. The productivity data are quoted as the mean values ± SD ($n = 2$) for the two independent replicate cultures.

Results and Discussion

Cell growth characteristics

This study targets an assessment of temperature effects on*Arthrospira* cultivation with three difference approaches to temperature conditioning. Accordingly, results for the three work phases are described in the next three sections. Cell growth was evaluated based on the determination of optical cell density (OD_{750}), converted to dry weight (DW). Pigment content was determined as described above. All results reflect duplicate measurements, plus at least two biological replicates.

Startup: Phase I

The biomass growth profiles of *Arthrospira* under photoautotrophic conditions in three phases are shown in Figure 2. In Phase I, the growth response to constant low temperature (LT) (20 °C±1) (AB & CD) and high temperature (HT) (35°C±1) (EF & GH) was assessed. PBRs were cultivated in batch mode for initial 5 days to reach OD_{750} 2.0 and beyond, and then operated in semi-continuous mode maintaining OD_{750} = 2.0 starting point from day 5 to 15 with harvest/dilution every alternate day.

For the chosen temperatures of 20°C and 35°C, the average pre-dilution concentrations of the cultures were

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1.30 gDW L^{-1}(OD_{750} = 2.6) and 1.62 gDW L^{-1}(OD_{750} = 3.2) respectively (Figure 2) yielding biomass growth rates 0.17 gDW L^{-1} d^{-1} and 0.20 gDW L^{-1} d^{-1} at 20 °C and 35 °C, respectively. These results are summarized in Table 1. As can be seen in the table, good reproducibility is found for all results (including pigment contents to be discussed later).

Transition: Phase II

In Phase II the cultures were shifted to opposing temperatures and grown from day 15 to 33. The cultures grown previously at 20 °C were shifted to 35 °C (AB) and 30 °C (CD), and cultures at 35 °C were shifted to 20 °C (EF) and 30 °C (GH). As a recovery phase and to acclimate the cultures after transition from Phase I to Phase II, the cultures in the newer temperature conditions were grown in batch mode without dilution for four days from day 15-19. The responses to temperature change were assessed at the end of the fourth day. As expected, the culture shifted from lower temperature (LT) (20 °C) to higher temperatures (HT) (30 °C and 35 °C) showed higher growth to 1.90 gDW L^{-1} (from 0.93 to 1.90 gDW L^{-1} over 4 days). The cultures shifted from higher temperature (HT) (35 °C) to low temperature (LT) (20 °C and 30 °C) grew more slowly, with biomass concentration reaching around 1.71 gDW L^{-1} (from 1.17 to 1.71 gDW L^{-1}) on day 19 after four days of batch cultivation (Figure 2 a and b). The time scale of temperature response for *Arthrospira* , as judged from these OD750nm measurements, is essentially instantaneous within the noise of these measurements.

During semi-continuous operation in Phase II, different algal growth patterns are found under the chosen temperature conditions. The average pre-dilution DW biomass concentrations for temperature treatments are shown in Table 1. The 20 °C average is the only one that is clearly distinguishable. The most favorable temperature appears to be 30 °C, which is close to the optimum temperature for *Arthrospira* for achieving maximum productivity under our growth conditions. The results are consistent with that of Colla et al., (2007), where high temperatures had a negative effect on *Arthrospira platensis*biomass production. An optimization study carried out by Sánchez-Luna et al., (2007) in fed-batch cultivation reported 29 degC as best growth temperature. For the two cultures in our Phase II study maintained at 30 degC (one originating from the 20 degC Phase I experiment and the other from the 35 degC Phase I experiment), the results are essentially the same. Thus, the extreme of temperatures and prolonged exposure to high temperatures at 35 degC in Phase I is thought to have caused some stress to the cells, and that usually results in decline in biomass production and protein content, with simultaneous accumulation of carbohydrate and EPS (Panyakampol et al., 2015; Trabelsi et al., 2009). There was little, if any decline in growth rate at 35 degC (though a stress response is clear in the pigment content, as discussed below). Noticeable decline in growth and a lower cell density were observed in the cultures that were shifted from the extreme HT to LT (35 degC-20 degC). This decline in growth is normal temperature dependence (Kumar et al., 2011) and, not surprisingly, the lowest growth and dilution rates were observed at 20 degC. The relative dilution rates were 0.12 day^{-1} at 20 degC, 0.16 day^{-1} at 30 degC, and 0.13 day^{-1} at 35 degC. The highest dilution rate, and therefore productivity, was seen at 30 degC. This agrees with the results of Trabelsi et al., (2009) where maximum growth rate for*Arthrospira platensis* was found at 30 degC.

Outdoor Simulation: Phase III

Phase III involved exposing the cultures to dynamic temperature profiles with hourly changes in temperature that are representative of outdoor summer culture temperature profiles (Appendix Table A1). This final phase of cultivation was carried out from day 33-43 with scheduled alternate day dilutions and with no adaptation period. The cultures grown at constant 20 degC (EF) and one set of the constant 30 degC (CD) cultures were shifted to average summer profile (AvSP) (appendix, Table 2) with 35 degC/21 degC as maximum/minimum temperatures during the course of day/night temperature ramping. The culture cultivated at constant 35 degC (AB) was shifted to extreme summer profile (ExSP) (Table 2) with 39 degC/26 degC as maximum/minimum temperatures, and the final set of two photobioreactors at constant 30 degC (GH) were shifted to constant summer profile (CtSP) where day/night temperature were maintained at constant 31 degC/21 degC, selected based on Fort Myers summer profile and averaging the day and night temperatures separately.

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The average pre-dilution cell concentration in gDW $L^{-1}$for the temperature profile treatments are shown in Table 1. The relative dilution rates were about 0.16 day^{-1} in AvSP (CD), AvSP (EF) and CtSP (GH), and about 20% lower (0.135 day^{-1}) for ExSP (AB).

From visual observations, it is worth noting that during the processing of biomass samples for the various analyses, agglomeration or clumping of the algal cultures occurred for cultures grown at higher temperature (constant 35 degC and ExSP). This is attributed to a stress response. In addition, the dried sample used for the DW measurement showed a flaky texture on the dry weight plate membrane surface. In cyanobacteria, high temperature stress can result in a rise in fluidity of membranes which can cause disintegration of the lipid bilayer and many other alterations in the physical properties of the cells that result in the loss of functionality of photosynthetic machinery (Panyakampol et al., 2015; Panyakampol et al., 2016).

Biomass Productivity

Table 1 summarizes the results obtained in different phases of temperature treatment. In Phase I, the average biomass productivity of cultures at constant 20 degC (in reactors AB & CD) and 35 degC (in reactors EF and GH) were 0.17 g L^{-1} day^{-1} and 0.21 g L^{-1} day^{-1}, respectively. In Phase II, the cultures were shifted to contrasting temperature conditions. Average biomass productivities are given in Table 1 and show an apparent peak at 30 degC. In Phase III, the cultures were shifted to dynamic summer temperature profiles; average biomass productivities are shown in Table 1. The lowest biomass productivities were obtained in the ExSP (AB) treatment where the culture was shifted from 20 degC-35 degC-ExSP. Figure 3 summarizes the productivity results. The variation from 20 degC to 30 degC is less than the commonly observed Q10 = 2 behavior. This is due to the irradiance level (230 μE $m^{-2}s^{-1}$) being substantially below P_{max} as shown below. The slight downturn at 35 °C and higher in Figure 3 is attributed to a stress response that is more apparent in the pigment results discussed below. The downturn in productivity and pigmentation was enhanced for batch experiments at 35 °C to 45 °C that are not discussed here.

The choice of 230 μE m^{-2} s^{-1} was based on the average annual irradiance at the exposed culture surfaces of PBR arrays in Fort Myers, Florida with a height-to-spacing ratio chosen to maximize productivity (Legere, 2017). To convert the quoted biomass productivities from g $L^{-1}d^{-1}$ to g m^{-2} d^{-1}, multiply by a geometric factor of 95 L m^{-2}. Thus, taking 30 °C as a reasonable estimate of the annual average daytime temperature in Fort Myers, a biomass productivity of 21 g m^{-2} day^{-1} is obtained. This is very close to the observed annual average of 23 g $m^{-2}day^{-1}$ found experimentally for large PBR arrays (24,000 L culture) tested for over 1 year at the Algenol site in Fort Myers (Chance and Roessler, 2019).

Chlorophyll and Phycocyanin Production

During the cultivations, chlorophyll (Chl-a) content and phycocyanin (PC) content for *Arthrospira* were monitored. The two main components of the PC content were determined separately: allophycocyanin (aPC) and C-phycocyanin (cPC) via spectroscopic determination (Yoshikawa and Belay, 2008). In addition, whole cell (WC) absorption spectra were measured (Figure 4) for qualitative pigment analysis. Results for pigment content are summarized in Table 1 and displayed in detail in Figure 5. All quoted contents are expressed as a percentage of whole cell dry weight.

First, from the Phase I results in Table 1, good reproducibility between the biological duplicates (AB-CD and EF-GH) is found for all the measurements. It can also be seen from Figure 5 that the AB and CD experiments at 20 °C are very stable in their pigment content, both PC and Chl-a. That is not the case for the 35 °C results where both PC and Chl-a are decreasing steadily throughout the Phase I residence time in Figure 5c.. The decline in PC is about 20% and the decline in Chl-a is about 40% over the course of the Phase I experiments at 35 °C. It is unlikely that the degradation in pigment content in either case is due to thermal damage to the pigments, as these pigments are known to be stable to much high temperatures. It is more likely due to a slow alteration of the photosynthetic apparatus. The decrease in PC content could in fact be a consequence of the decrease in Chl-a content as the light harvesting machinery re-balances the optimal ratio for these pigments.

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In Phase II, the AB culture goes from 20 °C to 35 °C with an initial sharp increase in PC content followed by a slow decline (Figure 5a). The Chl-a content stays constant initially and then declines slowly over the Phase II residence time, the overall decrease being similar to that for EF in Phase I (Figure 5c). The CD culture, which transitioned from 20 °C to 30 °C (Figure 5b), shows much more stable behavior, with a slower increase in PC content before reaching an apparent steady concentration of about 12%. The Chl-a concentration is stable at about 1.7%, similar to Phase I at 20 °C. There is no indication of instability at 30 °C. The CD culture, transitioned from 35 °C to 20 °C (Figure 5c), shows a steady decline in PC to the expected level for 20 °C production (˜8 %). The Chl-a content increases slowly, eventually reaching the level expected for 20 °C production (˜1.5%). The time scale for these changes are consistent with the expected time scale for culture turnover (roughly 20-30% per 2 day cycle). At the end of Phase II, the EF culture is almost exactly at the expected pigment contents found in the AB and CD Phase I experiments. The GH culture, transitioned from 35 °C to 30 °C (Figure 5d), achieves an overall increase in PC content and Chl-a content, with a slightly enhanced time scale for reaching stable levels for both pigments.

Phase III observations from Figure 5 are consistent with the above observations. AB (35 °C to ExSP) shows an initial decline in pigment contents and then some recovery over time. CD shows essentially no change in going from 30 °C to AvSP. EF shows expected changes in going from 20 °C to AvSP. GH (30 °C to CtSP) shows little or no change in pigment content.

According to the literature for shorter duration experiments, there is a narrow temperature range between 35 °C and 37 °C for optimal growth with 40 °C being definitely detrimental for *Arthrospira* (Kumar et al., 2011; Torzillo et al., 1991b). Our results suggest extended periods at 35 °C are also not favorable for sustained growth, though the effects are largely reversible and most of the variation is in pigment production. A similar trend was seen in whole cell spectrum in Figure 4, where relatively higher peak at ˜680nm (Chl-a) and lower peak at 620 nm (cPC) was found at lower temperatures, and thus indicates a higher Chl-a to PC ratio for low temperature cultures compared to those after prolonged high temperature exposure. The culture at 35 °C turned bluish green with Chl-a reduction (by >50 %) after prolonged exposure to this modestly elevated temperature. The spectra in Figure 4 are consistent with this visual observation. These results are generally consistent with Watras et al., (2017) where a progressive decrease in chlorophyll and phycocyanin fluorescence with increasing temperature was reported in most of the cultures of green and blue-green algae (e.g., *Scenedesmus dimorphus, Selenastrum minutum, and Synechococcus leopoliensis*).

Photosynthetic parameters

Photosynthesis-irradiance (PI) curves have been extensively used to evaluate the photosynthetic response to various abiotic stresses experienced by algae (Falkowski and Raven, 2007). Photosynthetic parameters, including P_{max} (µmol O_2L^{-1} hr^{-1}), α' (mol O_2 mol $photon^{-1}$), R_0' (µmol O_2L^{-1} min^{-1}) E_k (µE m^{-2} s^{-1}), were estimated at different temperature treatments during Phases I and II using PI curves (Figure 6 and Table 2) to test consistency with the above observations for changing conditions and provide parameters for productivity modeling. The culture samples from different temperature treatments were first incubated at 30 °C under dark conditions for 1 h. This testing for the different treatments was conducted at a single temperature (30 °C) to avoid the normal temperature dependence wherein $P_{max}$and E_k display a $Q_{10} = 2$ dependence (about 60 kJ mol^{-1}). The PI curves were measured (in duplicate) for all treatments, with average values reported. The photosynthetic response patterns from cultures grown at 20 °C, 30 °C and 35 °C Phase I are shown in Figure 6, with results summarized in Table 2. It is clear that with this experimental protocol none of the samples in Figure 6 shows a significant difference from the others, the only possible exceptions being the AB-ExSP sample exposed to the most severe summer profile conditions and the A sample from Phase II (constant 35 °C). PI curves measured at 20 °C for culture samples from Phase I (20 °C treatment) yield a P_{max} of 240 µmol O_2L^{-1} hr^{-1} and E_k as 85 µE m^{-2} s^{-1}, which is roughly Q_{10} of 2 when compared to results from PI curves measured at 30 °C. In fact a more extensive testing (not presented here) of PI curves measured over the temperature range 15-35 °C yields and activation energy for P_{max} of 62 kJ mol^{-1}, which is roughly $Q_{10} = 2$. As noted earlier, this observation is typical of a temperature response in photosynthetic organisms (Falkowski and Raven, 2007). The constant exposure to 35 °C, also measured at 35 °C, (Table

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2) yields photosynthetic parameters similar to the other tests at 30 °C. In Phase I there is some indication of a stress response at sustained high temperatures (HT) in these results, though this is not as clear as the pigment variation. There is no indication in Phase III of dynamic HT exposure having an adverse effect. These observations are consistent those made in conjunction with biomass and pigment production.

There was no significant difference in values of α' (limited quantum yield for O_2 production) which were close to ~ 0.070 mol O_2/mol photon for all the treatments. The lowest R_0'(respiration rate) of 0.12 μmol O_2/L-min was observed at 20°C while the maximum of 0.55 μmol O_2/L-min was found at 35°C. R_0'determinations have effects due to the light exposure history (Falkowski and Raven, 2007). Little temperature dependence is expected for α', consistent with the results from this study. The ratio α'/α is the photosynthetic quotient (O_2 per fixed carbon) which is expected to be in the range 1.1-1.3 (Falkowski and Raven, 2007). We will use 1.2 for the modeling analysis to follow. The same value applies to R_0, the respiration rate on a carbon basis required for application in the Algenol Productivity Model, Equation 3.

It is noteworthy that large changes in pigment content and light absorption level are seen with very little change in biomass productivity, whether measured directly or inferred from the PI curves. This is consistent with the relatively minor impacts of low pigment mutants on productivity in other organisms (Kirst et al., 2014; Lea-Smith et al., 2014).

Productivity Modeling Analysis

The Algenol Productivity Model (Legere, 2017; Chance and Roessler, 2019) is used to analyze these indoor PBR experiment results, and determine if a set of photosynthetic parameters can be developed to adequately represent all experimental results. A representative model parameter set for the productivity model is derived from the PI data sets with [α, E_k, R_0] = [0.061 fixed C/photon, 240 μE m^{-2} s^{-1}, 0.1 μmol C mgChl.a^{-1} min^{-1}] at 30 °C providing a reasonable representation of the entire data base. The R_0 value at the reference 30 °C temperature was taken as 0.1 μmol C mgChl.a^{-1} min^{-1}consistent with conclusions from outdoor experiments on a carbon basis (Legere 2017; Chance and Roessler, 2019) and recognizing that R_0' determinations from PI curves will show an irradiance-related enhancement (Falkowski and Raven, 2007). To model temperature effects, E_k is set as a function of temperature (activation energy 60 kJ mol^{-1} which is roughly $Q_{10} = 2$), and the respiration rate (R_0) was modeled as a function of temperature (activation energy as 27 kJ mol^{-1}), with the estimates based on previous studies (Legere, 2017). Table 4 gives a summary of the model parameter values. Comparison between the modeled and experimental productivities are shown in Figure 7. The model results are in good in agreement with experiment results for all cases considered here. Even at 35 °C, where clear changes in pigmentation are seen, the agreement is quite good. For example, with temperature increased from 20 °C to 30 °C, the biomass productivity increases by 28% (experimental) and 26% (productivity model). At higher light intensities >>E_k, an increase of 100%, or Q_{10} = 2, would be expected. Good agreement between biomass productivities for the small, L scale, experiments reported here and the large, 24000 L scale, outdoor experiments (Chance and Roessler 2019) was noted earlier. This consistency can be extended to the PI experiments (mL scale) where the derived photosynthetic parameters are in good agreement with those deduced from model fits to the large scale outdoor experiments (Chance and Roessler, 2019).

Conclusions

We have provided here a detailed study of temperature impacts on *Arthrospira platensis* biomass production in semi-continuous operation. This temperature study of *Arthrospira platensis* in photobioreactor cultivations demonstrates that temperatures in the 20 - 35 °C range were favorable for achieving consistent productivities, though long term exposure to 35 °C caused some modest changes in productivity and more obvious changes in pigmentation. Exposure to simulated conditions for summer temperature profiles for Southwest Florida showed some issues for the most extreme conditions but a general tolerance for the short term, mid-day exposures to higher temperatures. The response of the cultures to abrupt changes in temperature was immediate for biomass production and quantitatively consistent with the temperature dependence observed for P_{max} in smaller scale photosynthetic response experiments. Pigment variations with abrupt changes in temperature occurred on a time scale that was essentially the same as that expected for turnover of the cell

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population under semi-continuous operation. These results all involve annual average irradiance conditions. Extension of this study to higher irradiance conditions in the summer may cause additional issues in combination with extreme temperature exposures. Productivity modeling based on photosynthetic parameters derived from periodic sampling of the cultures provided excellent agreement with experiment and consistency with the performance of large scale outdoor PBR cultivations.

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Tables

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Parameters/Temperature treatments	**AB**	**CD**	**EF**	**GH**
Phase I: 0-15 Days (n=4)	**20°C**	**20°C**	**35°C**	**35°C**
Biomass Concentration (g L^{-1})	1.29±0.07	1.30±0.05	1.62±0.05	1.61±0.08

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Biomass Productivity (g L^{-1} day^{-1})	0.17±0.03	0.17±0.02	0.20±0.04	0.21±0.03
PC % (%DW)	8.08±0.3%	8.09±0.4%	9.95±1.1%	9.73±0.8%
aPC % (%DW)	2.85±0.09%	2.83±0.10%	2.68±0.21%	2.61±0.15%
cPC % (%DW)	5.22±0.22%	5.26±0.35%	7.27±0.57%	7.11±0.42%
Chl-a %	1.61±0.10%	1.61±0.13%	0.98±0.17%	1.0±0.12%

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Phase II: 15-33 Days (n=4)	**35°C**	**30°C**	**20°C**	**30°C**
Biomass Concentration (g L^{-1})	1.57±0.17	1.48±0.18	1.40±0.09	1.58±0.14
Biomass Productivity (g L^{-1} day^{-1})	0.18±0.04	0.21±0.02	0.15±0.03	0.23±0.06

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PC % (%DW)	11.50±0.8%	11.31±0.6%	7.45±0.3%	10.35±1.0%
aPC% (%DW)	3.20±0.13%	3.49±0.15%	2.22±0.08%	2.91±0.23%
cPC% (%DW)	8.30±0.36%	7.82±0.39%	5.23±0.23%	7.44±0.45%
Chl-a %	1.32±0.05%	1.70±0.11%	1.14±0.23%	1.42±0.18%
Phase III: 33- 43 Days (n=4)	**Ext. Summer (39/26°C)**	**Ave. Summer (35/21°C)**	**Ave. Summer (35/21°C)**	**Ct. Summer (31/21°C)**

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Biomass Concentration (g L^{-1})	1.59±0.03	1.52±0.02	1.45±0.05	1.58±0.04
Biomass Productivity (g L^{-1} day^{-1})	0.22±0.02	0.25±0.02	0.24±0.02	0.25±0.02
PC % (%DW)	10.85±0.4%	12.42±0.5%	11.85±0.6%	11.49±0.4%

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Table 1. Summarized results indicating various growth and pigment parameters in response to temperature conditions in Phase I, II and III. Phase I starts with 20 °C for both AB and CD, shifted to 35 °C (AB) and 30 °C (CD) in Phase II, then to ExSP (AB) and AvSP (CD) in Phase III, whereas culture EF and GH start with 35 °C in Phase I, shifted to 20 °C (EF) and 30 °C (GH) in Phase II, then to AvSP (EF) and CtSP (GH) in Phase III, respectively. Biomass Concentration is the concentration prior to dilution, averaged over the relevant Phase period (semi-continuous operation regions only). Error bars are +/- one standard deviation.	**Table 1**. Summarized results indicating various growth and pigment parameters in response to temperature conditions in Phase I, II and III. Phase I starts with 20 °C for both AB and CD, shifted to 35 °C (AB) and 30 °C (CD) in Phase II, then to ExSP (AB) and AvSP (CD) in Phase III, whereas culture EF and GH start with 35 °C in Phase I, shifted to 20 °C (EF) and 30 °C (GH) in Phase II, then to AvSP (EF) and CtSP (GH) in Phase III, respectively. Biomass Concentration is the concentration prior to dilution, averaged over the relevant Phase period (semi-continuous operation regions only). Error bars are +/- one standard deviation.	**Table 1**. Summarized results indicating various growth and pigment parameters in response to temperature conditions in Phase I, II and III. Phase I starts with 20 °C for both AB and CD, shifted to 35 °C (AB) and 30 °C (CD) in Phase II, then to ExSP (AB) and AvSP (CD) in Phase III, whereas culture EF and GH start with 35 °C in Phase I, shifted to 20 °C (EF) and 30 °C (GH) in Phase II, then to AvSP (EF) and CtSP (GH) in Phase III, respectively. Biomass Concentration is the concentration prior to dilution, averaged over the relevant Phase period (semi-continuous operation regions only). Error bars are +/- one standard deviation.	**Table 1**. Summarized results indicating various growth and pigment parameters in response to temperature conditions in Phase I, II and III. Phase I starts with 20 °C for both AB and CD, shifted to 35 °C (AB) and 30 °C (CD) in Phase II, then to ExSP (AB) and AvSP (CD) in Phase III, whereas culture EF and GH start with 35 °C in Phase I, shifted to 20 °C (EF) and 30 °C (GH) in Phase II, then to AvSP (EF) and CtSP (GH) in Phase III, respectively. Biomass Concentration is the concentration prior to dilution, averaged over the relevant Phase period (semi-continuous operation regions only). Error bars are +/- one standard deviation.	**Table 1**. Summarized results indicating various growth and pigment parameters in response to temperature conditions in Phase I, II and III. Phase I starts with 20 °C for both AB and CD, shifted to 35 °C (AB) and 30 °C (CD) in Phase II, then to ExSP (AB) and AvSP (CD) in Phase III, whereas culture EF and GH start with 35 °C in Phase I, shifted to 20 °C (EF) and 30 °C (GH) in Phase II, then to AvSP (EF) and CtSP (GH) in Phase III, respectively. Biomass Concentration is the concentration prior to dilution, averaged over the relevant Phase period (semi-continuous operation regions only). Error bars are +/- one standard deviation.
aPC% (%DW)	2.81±0.07%	3.61±0.18%	3.57±0.14%	3.34±0.12%
cPC% (%DW)	8.04±0.40%	8.81±0.39%	8.28±0.54%	8.15±0.33%
Chl-a %	1.08±0.12%	1.83±0.16%	1.93±0.09%	1.86±0.04%

Table 2. Summary of photosynthetic parameters at different temperature treatments in the three phases obtained from Mo

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Temperature treatments
PI Temperature
sOD750
Chl.a-Extract/sOD (mg/L)
$\Pi_{\mu\alpha\xi}$ ($\mu\mu o\lambda O_2/\Lambda$-$\eta\rho$)
α' ($\mu o\lambda O_2/\mu o\lambda$ $\pi\eta o\tau o\nu$)
$E_{\varkappa}$ ($\mu E/\mu^2$-ς)
P_0 ($\mu\mu o\lambda O_2/\Lambda$-$\eta\rho$)

Table 3 : Productivity Model Parameters

Model Parameters	**Unit**	**Culture Density 1 gDW/L**
α	*mol C/mol photon*	0.061
E_k	$\mu E/\mu^2$-ς	*240 @ 30C*
R_0	$\mu\mu o\lambda$"/$\mu\gamma\eta\lambda$.a-$\mu\iota\nu$	*0.1 @30C*
F	-	*1*
E_s	$\mu E/\mu^2$-ς	*230*
D	*m*	*0.0254*
k	*1/m at 1 gDW/L*	*175*
kD	-	*4.45*
C_0	*mgChl.a/m^3 at 1 gDW/L*	*18,000*
g	*gDW/molC*	*22.68*
t_1	*sec*	*43,200*
t_2	*sec*	*86,400*

Figure legends

Figure 1. a) Experimental program illustrating timing for the three experimental phases and the sequencing of the eight reactors. The temperature profiles for Phase III are based on historical climate data in Fort Myers Florida. The Extreme Profile is based on summer temperatures only; the average profile is based on annual average. Both involve hourly temperature variations in the reactors. The Constant Profile has contant values on a 12-12 cycle based on annual day and night averages. b) Photobioreactor setup for cultures in Phase I where Reactors A, B and C, D were cultivated at 20 °C and (b) Reactors E, F and G, H were cultivated at 35 °C. Dye-1 and Dye-2 were the dummy reactors used for monitoring temperature, and reactors W-1 and W-2 were connected to the reactor exhaust and used as waste collectors due to minor foaming and evaporation loss. Reactors are brought outside of the incubator for sampling and photograph.

Figure 2. OD (750 nm) results for cultures in three different phases (a) AB and CD and (b) EF and GH at various temperature treatments. Results shown are the average of two determinations with error bars showing the range of values. Temperature conditions for the various phases are shown.

Figure 3. Average biomass productivities of *Arthrospiraplatensis* at various temperature treatments in three phases: Phase I temperature were 20 and 35 °C; in Phase II temperature were 20, 30 and 35 °C and in Phase III 31 °C (CtSP), 32 °C (AvSP) and 36 °C (ExSP), where temperature designations are the average daytime values. Error bars are +/- one standard deviation for the averages over multiple days of semi-continuous operation.

Figure 4 . Representative whole cell (WC) spectra of cultures at different temperature treatments in the three phases of the experiment (a) Phase I at 20 °C (A and C) and 35 °C (E and G), (b) Phase II at 35

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°C (A), 30 °C (C) 20 °C (E) and 30 °C (G), and (c) Phase III with Extreme Summer, ExSP (A), Average Summer, AvSP (C) and Constant Summer, CtSP (G). The absorbance values are normalized to 1 gDW L^{-1}.

Figure 5. PC and Chl-a content (%, DW) in the three phases of the experimental plan at different temperature conditions. Experiments were performed in duplicate.

Figure 6 . Photosynthesis irradiance (PI) response curves (measured at 30 °C) for the algal cultures at different temperature treatments in (a) Phase II on day 27, A-35 °C, C-30 °C, F-20 °C, and H-35 °C, and (b) Phase III on day 43, AB-ExSP, CD and EF-AvSP and GH-CtSP.

Figure 7 . Experiment results of biomass productivity in comparison to the productivity model simulation. The productivity model parameter set, representative of the PI database at 30 °C, is [α, E_k, R_0] = [0.061 fixed C/photon, 240 µE/m^2-s, 0.1 µmolC/mgChl.a-min].

Figure 1

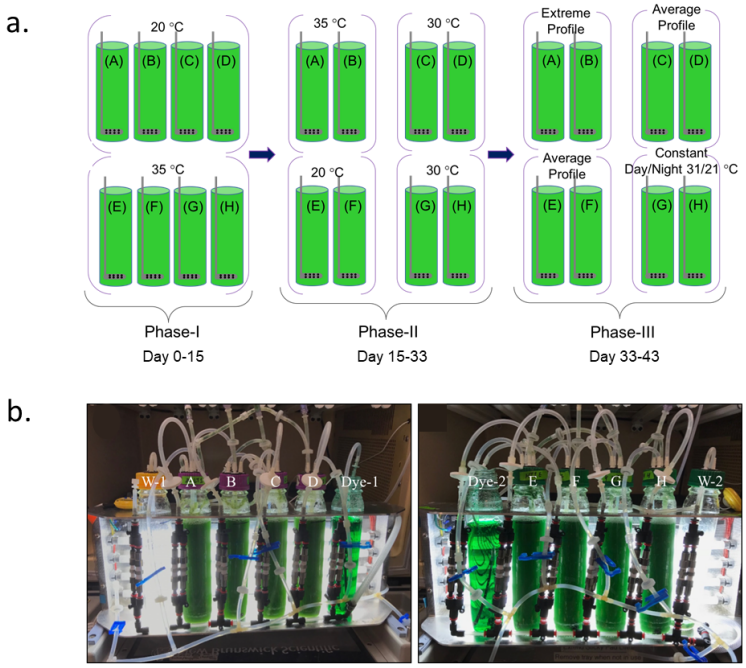

Figure 2

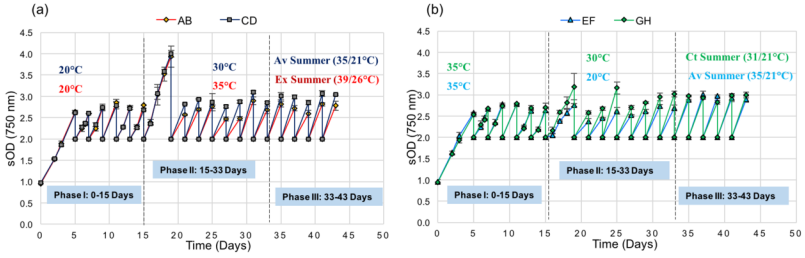

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Figure 3

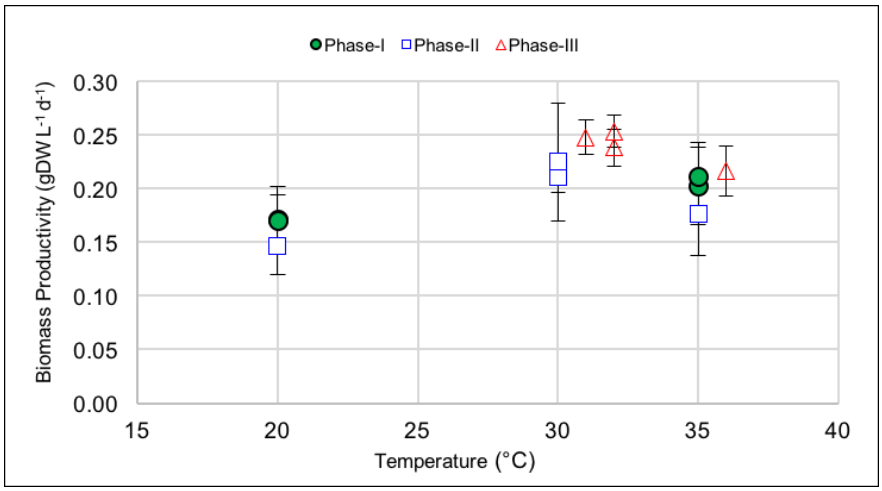

Figure 4

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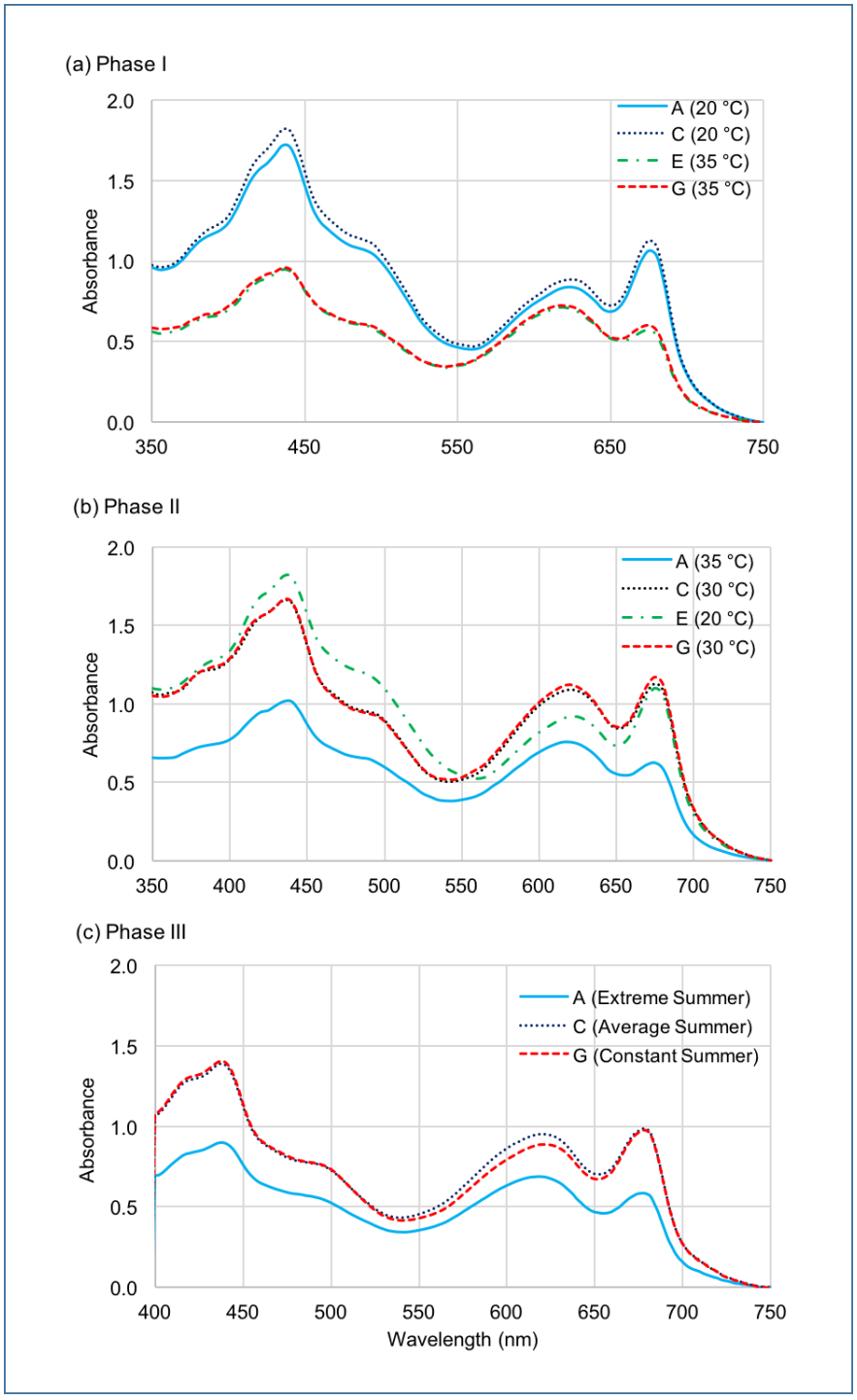

Figure 5

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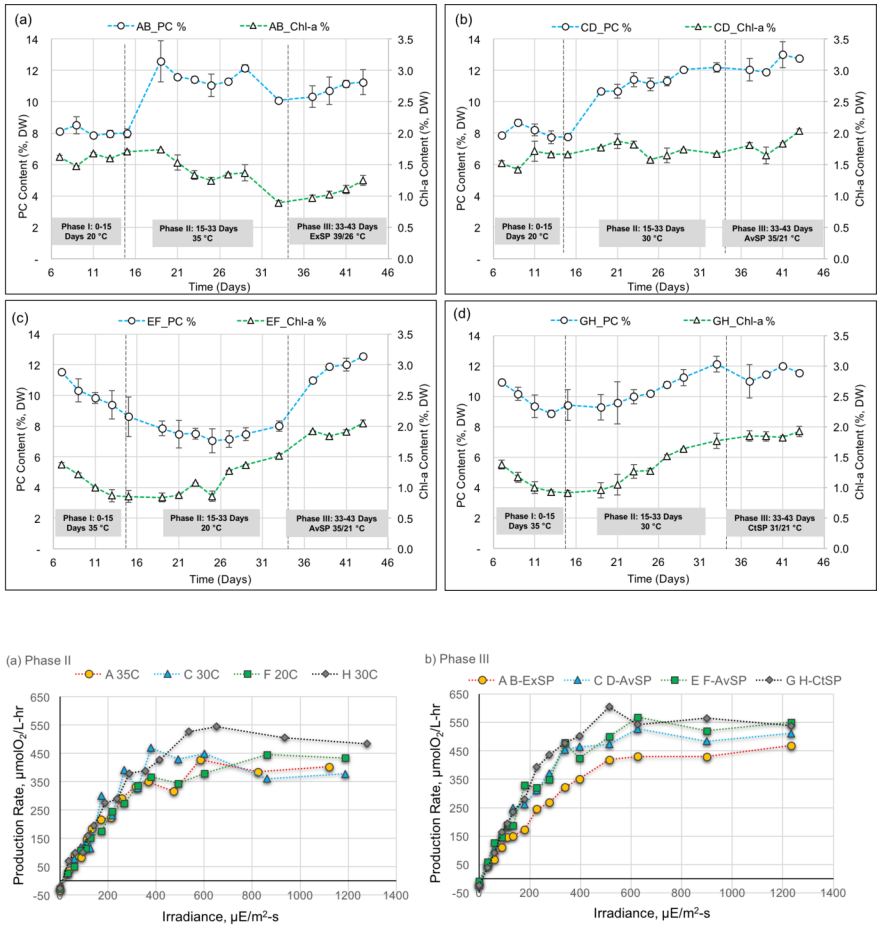
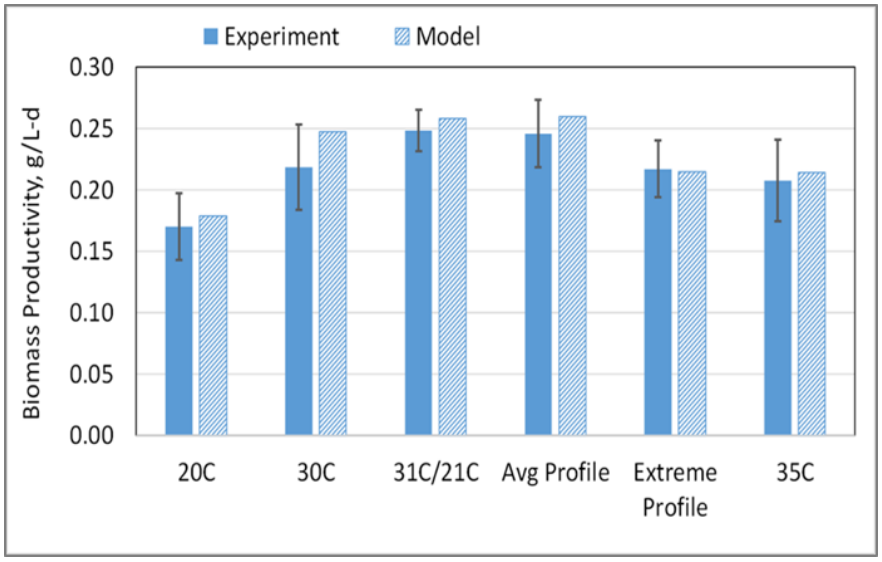


Figure 6

Figure 7



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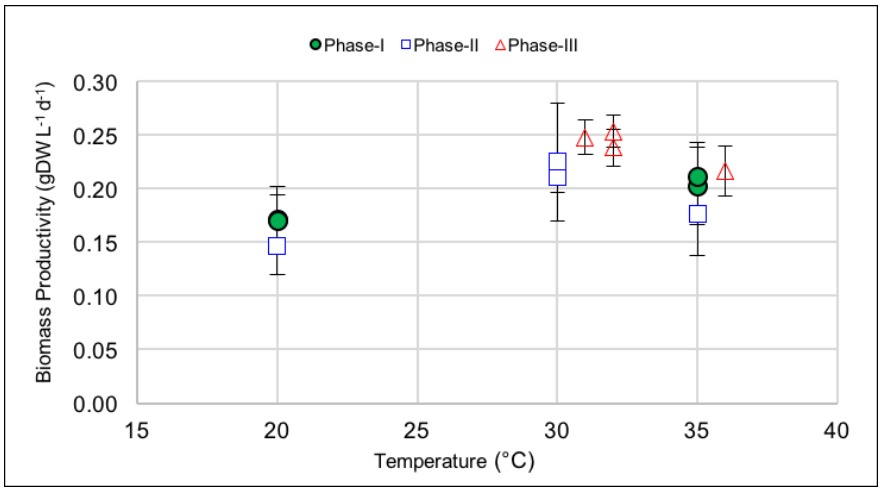

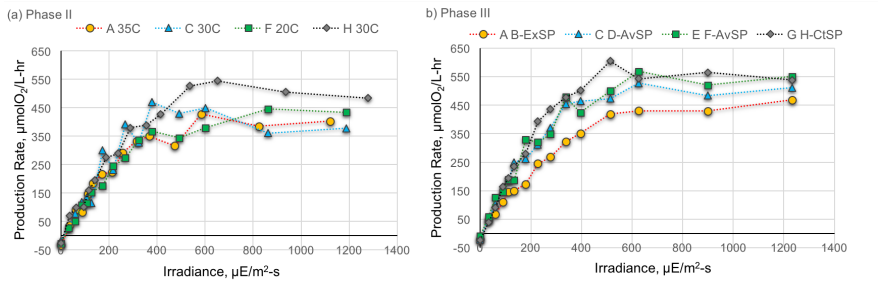

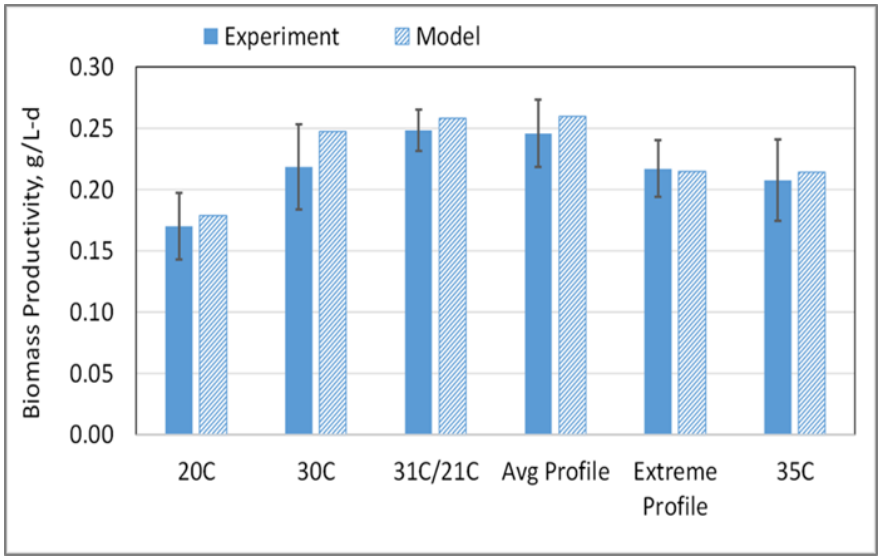

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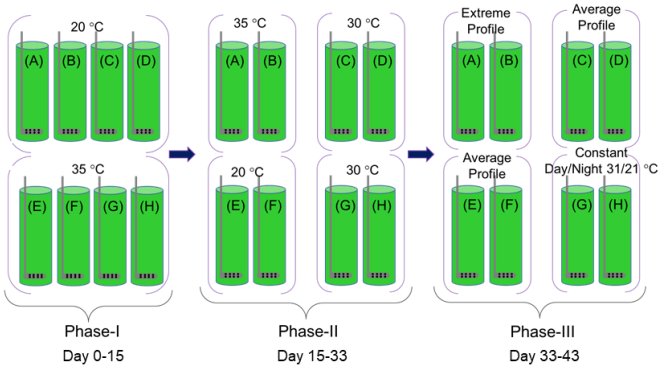
a.



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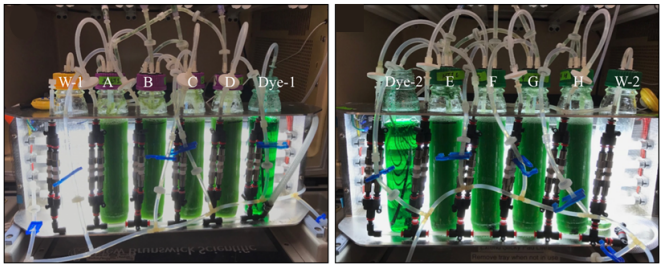

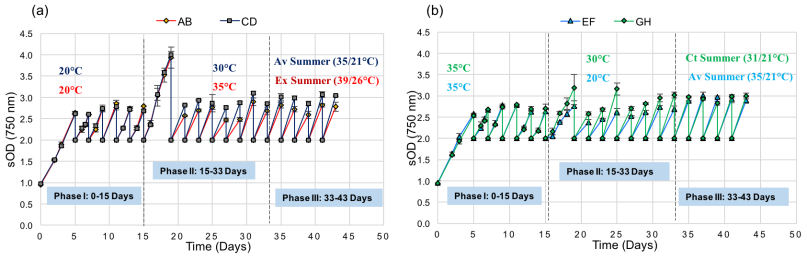

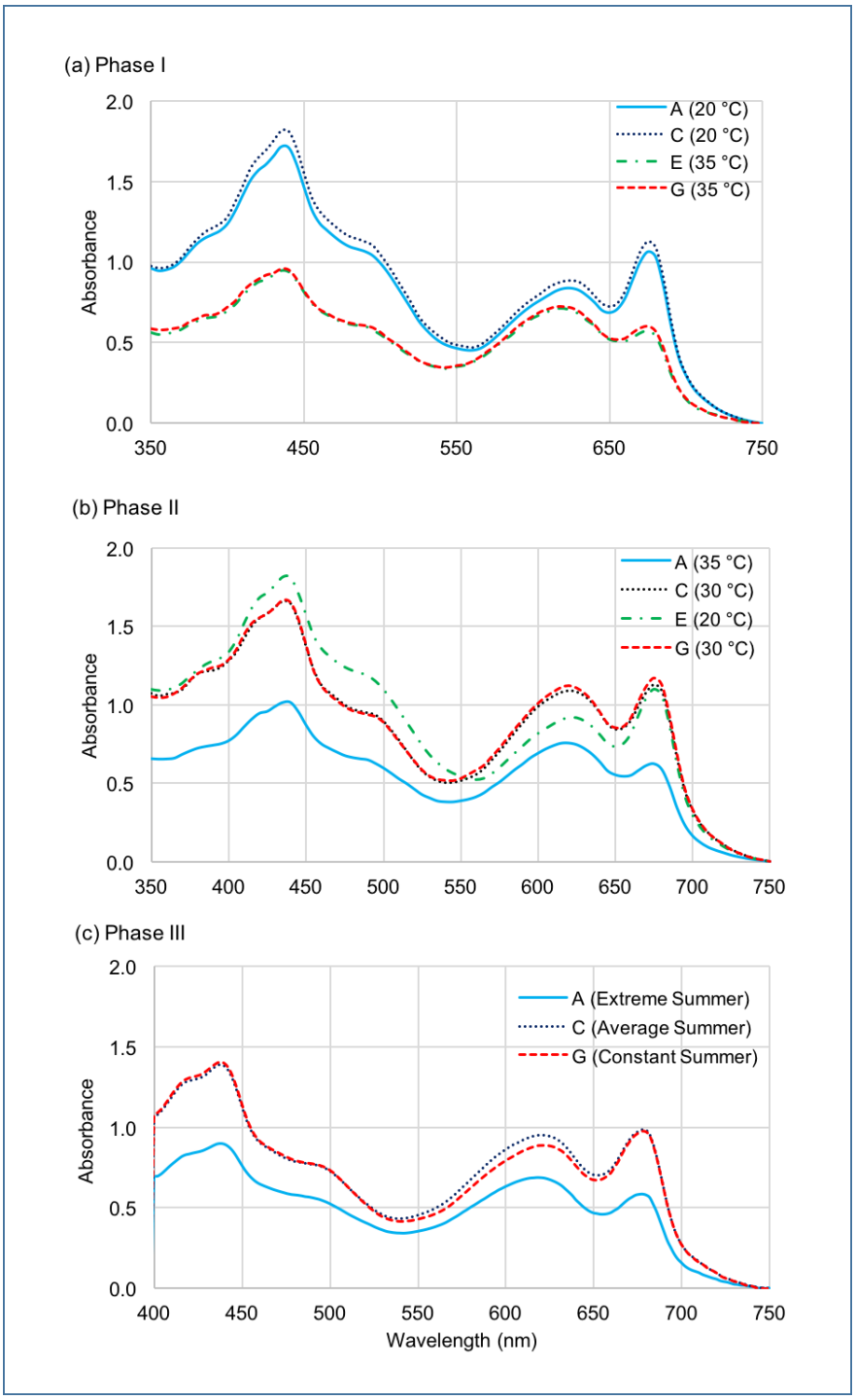

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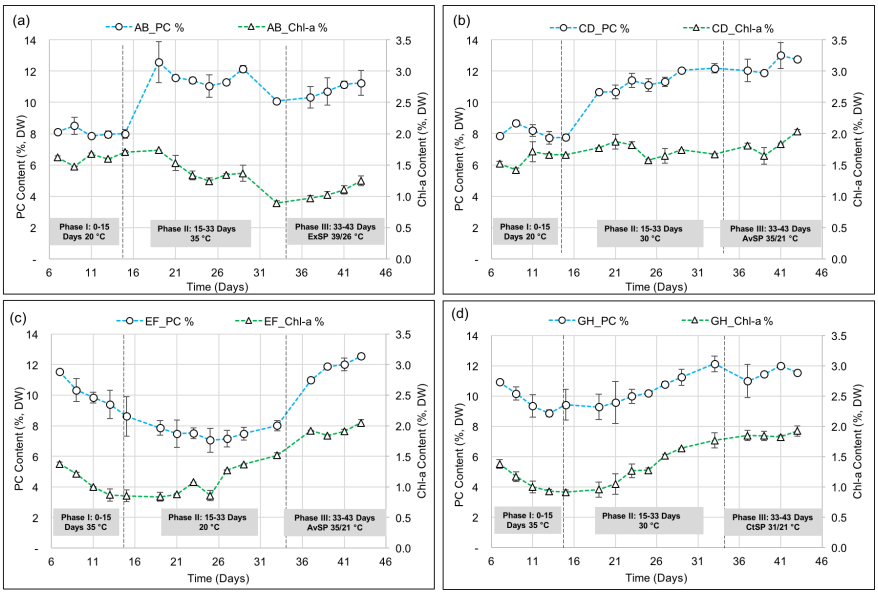

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