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Effect of temperature control on green algae grown under continuous culture

Carolann M. Knutson[a,b], Evelyn M. McLaughlin[b], Brett M. Barney[a,b,*]

[a] Biotechnology Institute, St. Paul, MN 55108-6130, United States of America
[b] Department of Bioproducts and Biosystems Engineering, 1390 Eckles Avenue, St. Paul, MN 55108-6130, United States of America

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ABSTRACT

The large-scale cultivation of algae of commercial value requires a variety of important energy-related inputs to achieve the proposed yields required for economic viability. One specific energy input of interest is temperature control and the associated costs of this control, both in terms of initial capital investment and costs associated with continued cooling or heating. In this work, the green algae *Neochloris oleoabundans* and *Scenedesmus dimorphus* were grown in tightly-controlled turbidostat-based photobioreactors to determine the potential benefits of temperature control for biomass production of these organisms versus allowing these cultures to experience temperature fluctuations similar to what would be found in uncontrolled, outdoor open ponds or closed bioreactors. The results of these studies indicate stark differences between these two strains, with *N. oleoabundans* yielding improved growth rates under conditions of stringent temperature control at 22 °C, while *S. dimorphus* yielded slightly higher growth under conditions where temperature was allowed to fluctuate based on modeled natural temperature profiles. Further analysis reveals that the utilization of non-conventional temperature profiles could enhance growth yields further for *N. oleoabundans*, allowing it to overcome the detrimental effects of the natural temperature fluctuation. These results and a discussion of the potential for turbidostat-based algal culture growth are presented.

1. Introduction

Microalgae comprise an important class of photosynthetic organisms that are able to fix carbon through photosynthesis and have the potential to serve as a source of pigments, lipids, proteins, and carbohydrates [1,2]. The large-scale commercial culture of algae for utilization as a source of either commodity or high-value products will require a variety of important capital cost investments and ongoing energetic inputs to achieve the yields required for economic viability [1,3]. For this reason, studies to elucidate the importance of various energy intensive inputs are invaluable in assessing the viability and economic feasibility of a particular strain of algae for commercial use. These potential high-cost inputs may be evaluated through a variety of low-cost approaches to determine how essential each input is to the culture productivity and evaluate the associated cost for growing that strain, both monetarily and energetically.

One specific energy input of interest is associated with the requirements for temperature control, as it has been shown to have a dramatic impact on algal growth [4,5]. Most large-scale algal bioreactors and open ponds or raceways are designed to operate outdoors, where the culture could be exposed to large fluctuations in internal temperature as a result of ambient air temperature, evaporation and internal heating of the culture related to radiant thermal energy absorption. While various approaches to bioreactor or pond design could mitigate issues associated with temperature fluctuations in the culture, the importance of these design parameters need to be assessed for each strain. Evaluations of the effect of temperature on other algal strains such as *Phaeodactylum tricornutum* [6] and *Dunaliella salina* [7] have been completed under batch growth conditions, while considerably less information is currently available related to growth in turbidostat grown reactors.

In this report, continuous culture experiments were conducted to determine the importance and role that temperature control played in the culture of two strains of green algae with potential commercial value. The two strains of algae selected for this study were *Neochloris oleoabundans* and *Scenedesmus dimorphus*, as the first has been targeted for lipid production and biofuels [8,9], and the second, grown for various high-value co-products such as chlorophyll [10]. To accomplish the goals of this study, side by side photobioreactors, operated as turbidostats, were constructed to provide simple, programmable and

* Corresponding author at: Biotechnology Institute, St. Paul, MN 55108-6130, United States of America.
E-mail address: bbarney@umn.edu (B.M. Barney).

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strictly-controlled laboratory experiments. Additionally, the studies described in this report illustrate the use of a turbidostat as a potential tool to evaluate algal growth under non-typical temperature profiles. Turbidostats are a valuable tool for many non-photosynthetic organisms, where they have been used to assist in research related to bacterial antibiotic resistance and in synthetic circuit characterization [11,12]. Application of this combined turbidostat/photobioreactor system to algae has allowed us to grow strains under static and highly fluctuating temperature conditions where energy inputs could be altered and their effect characterized. The studies described in this report utilized controlled, turbidostat-operated, photobioreactors monitored over the duration of several days to assess the effects of maintaining a constant temperature versus allowing the culture to experience large temperature fluctuations. The potential benefits in elucidating the effects of temperature of this simple reactor system are described.

2. Materials and methods

2.1. Algae strains and media

Neochloris oleoabundans UTEX 1185 and *Scenedesmus dimorphus* UTEX 417 were obtained from the UTEX Culture Collection of algae. Strains were grown on a low nitrogen freshwater medium containing 1.85 mM K_2HPO_4, 0.35 mM $MgSO_4{\cdot}7H_2O$, 0.15 mM $CaCl_2{\cdot}2H_2O$, 0.61 mM NaCl, 1.51 mM Na_2SO_4, 0.07 mM Ferric Ammonium Citrate, 5.88 mM $NaNO_3$, and adjusted to pH 7.6. Trace elements were added at a concentration of 2 mL/L of the freshwater medium. The composition of the trace element solution (per L) was 1 g boric acid, 1 g sodium EDTA, 200 mg $MnCl_2{\cdot}4H_2O$, 20 mg $ZnCl_2$, 15 mg $CuCl_2{\cdot}2H_2O$, 15 mg $Na_2MoO_4{\cdot}2H_2O$, 15 mg $CoCl_2{\cdot}6H_2O$ and 10 mg KBr. Several drops of polypropylene glycol (approximately 50 μL) was added to each liter of medium to minimize foaming. All chemical reagents were obtained from Sigma Aldrich (St. Louis, MO) or Thermo Fisher Scientific (Pittsburgh, PA), unless stated otherwise.

2.2. Growth conditions

Cultures were maintained as pure stocks by multiple passages on solid medium prior to transfer via sterile loop to the initial medium for inoculation of the reactor through the medium input line (Fig. 1). Algal strains were subsequently grown in side-by-side, turbidostat operated, 1.2-L glass tubular reactors (51-mm inner diameter) with 1-mm inner diameter glass capillary tubes to provide constant aeration at a flow rate of 0.3 L air per minute per L of culture medium. Compressed air combined with 0.2% CO_2 was provided via mass flow controllers (Alicat, Tucson, AZ). The flow of the gases provided CO_2, gas exchange and mixing to the culture. Additional 1-mm inner diameter glass capillary tubes were used to provide fresh medium required for dilution of each culture. Each culture was provided light by three independent fluorescent light banks containing two T8 fluorescent lamps (2 ft. length) in a stepwise fashion intended to mimic natural lighting conditions, and were placed on a timer to deliver a 14:10-h light:dark cycle. The step function used for the three light banks is depicted on each figure. Reactors and light banks were held in place by a custom polycarbonate and wood structure and covered by white curtains to block external environmental lighting and reflect internal lighting. Under full artificial lighting, the surface of the reactors were receiving approximately 200 μmols $m^{-2} s^{-1}$ of light (MQ-200, Apogee Instruments, Logan, UT), with each light bank providing roughly 1/3 of the light.

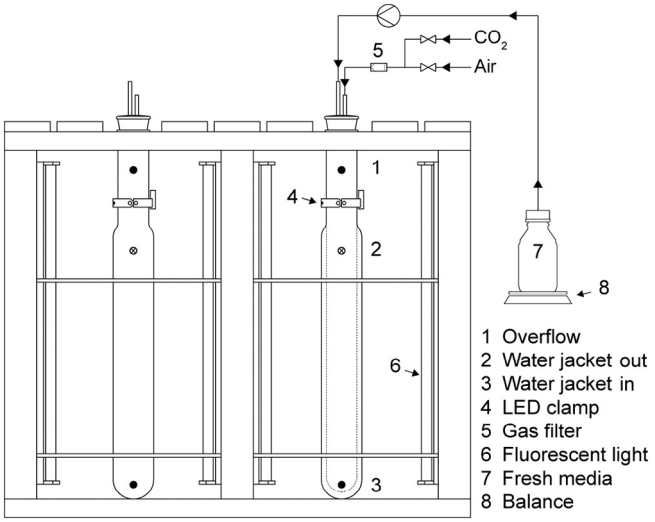

Fig. 1. Schematic of the turbidostat system.

Temperature control was provided via water circulation through the reactor jackets by two programmable water baths (VWR, Radnor, Pennsylvania). Initially, two temperature conditions were selected, termed constant and sinusoidal; the sinusoidal temperature profile was modeled after outdoor data collected from an open raceway pond operated during the months of May through July in Logan, Utah, so as to mimic natural temperature fluctuations. A constant temperature of 22 °C was selected as a control, as these cultures are typically maintained at room temperature in the laboratory. After observing differences in growth rates between the constant and sinusoidal profiles for *N. oleoabundans*, further optimization was investigated with this specific culture. The optimal growth temperature of *N. oleoabundans* and *S. dimorphus* were determined through a temperature optimization experiment, in which the strain was subjected to a range of temperatures based on an 8 h dark/light cycle; where each 8-h period consisted of a four-hour dark period followed by a four-hour light period. Cultures were maintained at that temperature for at least three consecutive dark/light cycles, following one cycle to allow the culture to acclimate to the current temperature setting before taking any measurements. Based on the findings from the temperature profile of *N. oleoabundans*, we developed two rapid temperature profiles for this strain, termed *Rapid 1* and *Rapid 2*. In each of the laboratory experiments presented, excluding the temperature optimization test, cultures were grown for multiple days, with each day serving as a single replicate from 0 to 24 h. In most cases, the first day of data was excluded from the analysis, as it was used to allow the culture to synchronize to the light and temperature cycles.

2.3. Turbidostat control system

Reactor parameters were controlled and monitored via LabVIEW (National Instruments, Austin, TX) and operated under continuous culture conditions as a turbidostat, in which a constant density was targeted. To accomplish this, the LabVIEW program controlled the supply of power to a constant delivery peristaltic pump (Cole Parmer, Vernon Hills, IL) and, if culture density exceeded the set point, the culture would undergo dilution. The amount of fresh medium used for dilution was determined by storing the fresh medium reservoir on a balance, as depicted in Fig. 1. To obtain in situ density measurements of the culture, an external “clamp” was designed in Tinkercad (Autodesk, San Rafael, CA) and 3D printed (MakerBot, New York City, NY). Five white LEDs (P/N 754, Adafruit, New York, NY) were utilized in conjunction with a photodetector (P/N 1143, Phidgets, Calgary, Canada), as shown in Fig. 2, to measure the culture density through the reactor wall once every minute. Density readings were averaged over 30 events to reduce fluctuations in the data. The clamp was positioned on the neck of each reactor, above the jacketed region and secured with wire wrapped around the “anchor posts”, to keep the clamp in place around the tube during the experiments.