Algal Research 21 (2017) 178–190

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Simulation of outdoor pond cultures using indoor LED-lighted and temperature-controlled raceway ponds and Phenometrics photobioreactors

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ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 24 September 2016
Accepted 19 November 2016
Available online xxxx

Keywords:
Phenometrics photobioreactors
ePBR
climate-simulation raceway ponds
Chlorella sorokiniana
Picochlorum soloecismus
flow cytometry

ABSTRACT

Two innovative culturing systems, the LED-lighted and temperature-controlled 800 liter indoor raceways at Pacific Northwest National Laboratory (PNNL) and the Phenometrics environmental Photobioreactors™ (ePBRs) were evaluated in terms of their ability to accurately simulate the microalgae growth performance of outdoor cultures subjected to fluctuating sunlight and water temperature conditions. When repeating a 60-day outdoor pond culture experiment (batch and semi-continuous at two dilution rates) conducted in Arizona with the freshwater strain *Chlorella sorokiniana* DOE 1412 in these two indoor simulators, it was found that ash-free dry weight based biomass growth and productivity in the PNNL climate-simulation ponds was comparatively slightly higher (8–13%) but significantly lower (44%) in the ePBRs. The difference in biomass productivities between the indoor and outdoor ponds was not statistically significant. When the marine *Picochlorum soloecismus* was cultured in five replicate ePBRs at Los Alamos National Laboratory (LANL) and in duplicate indoor climate-simulation ponds at PNNL, using the same inoculum, medium, culture depth, and light and temperature scripts, the optical density based biomass productivity and the rate of increase in cell counts in the ePBRs was about 35% and 66%, respectively, lower compared than in the indoor ponds. Potential reasons for the divergence in growth performance in these pond simulators, relative to outdoor raceways, are discussed. In conclusion, the PNNL climate-simulation ponds provide reasonably reliable biomass productivity estimates for microalgae strains cultured in outdoor raceways under different climatic conditions.

1. Introduction

In response to increasing concerns about global climate change and the need to produce carbon-neutral transportation fuels, there has been renewed interest in utilizing microalgae for the generation of biodiesel and jet-biofuel [29]. In order to develop an economically viable microalgae biofuels production process, it is imperative to identify crop rotation strains that collectively exhibit high annual biomass productivities (> 30 g/m^2-day) in outdoor culture systems [30]. Significant campaigns have been initiated by industry, academia, and other research organizations to find promising new microalgae strains, either by prospecting, genetic engineering or other improvement strategies, which might be suitable for large-scale, economic, biofuel production.

A major challenge is the identification and selection of strains that have the potential to exhibit high annual biomass productivities in outdoor ponds. Even when a strain grows well in laboratory cultures under particular incubation conditions (e.g., room temperature and relatively low light intensities), there is no guarantee that it will perform satisfactorily in outdoor pond cultures that are subjected to a wide range of daily and seasonal water temperature and light fluctuations. Therefore, in order to evaluate whether a particular promising strain is able to generate biomass at a sufficiently high productivity in outdoor ponds at any geographic location of choice, it would be useful to test these strains in lab-scale culture systems capable of simulating the respective fluctuating light and temperature environment present in outdoor raceways. If successful, such indoor pond culture simulators would significantly reduce the time, effort, and costs associated with building and operating outdoor ponds in different locations, and thereby accelerate the screening and identification of strains with superior productivity.

Until very recently, to the best of the authors' knowledge, such pond simulators did not exist. While numerous photobioreactor studies have been carried out over the years to determine the effects of temperature, light intensity and different light:dark cycle periods on biomass growth and productivity [9,10,20,27], these experiments did not simulate the variable light and temperature conditions observed in outdoor ponds.

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http://dx.doi.org/10.1016/j.algal.2016.11.016
2211-9264/

There have been several reports of using indoor ponds for research on microalgae. Grobbelaar and Soeder [12] quantified biomass losses due to dark respiration in indoor ponds (260 L volume, 2 m^2 surface area, 0.13 m culture depth) operated at constant temperature and light intensity via illumination with four high intensity 500 W incandescent lights. Several studies on microalgae biomass production and growth modeling were also performed in indoor hydraulically integrated serial turbidostat algal reactors (HISTAR, 1158 L volume each, 1.67 m^2 surface area, 0.71 m depth) operated at constant temperature and illuminated at constant light intensity using 1000 W metal halide lamps [4,5,26]. Radmann et al. [24] used very small indoor raceway ponds (6 L culture volume, 0.7 m length, 0.2 m width, 0.075 m maximum depth) maintained at constant 30 °C and illuminated 12 h per day with a 40 W (3000 lx) fluorescent lamp to identify optimum culture conditions for *Spirulina platensis*. However, since none of these systems were designed to vary the water temperature and light intensity during the cultivation experiments, these indoor ponds were not suitable for simulating the fluctuating light and temperature environment encountered in outdoor ponds.

We report here on the performance of two different indoor pond culture simulators that have recently become available. The first is the bench-top environmental photobioreactor (ePBR) marketed by Phenometrics, Inc. [17]. The second is the indoor climate-simulation raceway developed by Pacific Northwest National Laboratory. Both systems were tested for their ability to replicate the growth performance of microalgae cultured in outdoor ponds.

2. Material and methods

2.1. Microorganisms and Media

Chlorella sorokiniana strain DOE 1412 was isolated by Dr. Juergen Polle at Brooklyn College, NY [19] and grown at pH 7 in freshwater BG-11 medium containing 17.6 mM nitrate and 0.66 mM phosphate [1,25]. *Picochlorum soloecismus* was isolated from a mixed culture at Los Alamos National Laboratory and grown at pH 7 in saltwater f/2-Si medium containing 8.8 mM nitrate and 0.254 mM phosphate [1,13]. A high concentration of N and P in both media was used, with the aim of maintaining nutrient replete conditions. Cultures were periodically monitored for nitrate and phosphate concentrations during the experiment (see below).

2.2. Indoor climate-simulation raceway ponds

State-of-the-art LED-lighted and temperature-controlled indoor fiberglass raceway ponds, similar in dimension to the outdoor ponds used in this study (see below), were designed and built at PNNL. These ponds enable the cultivation of microalgae strains under climate-simulated conditions which reproduce the light and water temperature fluctuations encountered in outdoor ponds at any geographic location in the world where metereological data are available (Fig. 1). Lighting is provided by an array of multi-colored high intensity LEDs (ca. 4500 per pond) which closely simulate the full spectrum (PAR) of sunlight (Fig. S1). The light intensity can be varied in one second time increments, if needed, from 0 to 2700 μmol/m^2-*sec* to simulate daily fluctuations in sunlight intensity, including those caused by passing clouds. The light calibration procedure is described in detail in Exhibit 1 in the Supplemental Section. Using electric heaters, chillers, and heat exchangers surrounding the outside of the pond fiber glass shell, the water temperature can either be kept constant (ca. 6 °C to 38 °C) or can be increased ($r_{max\text{-}heat}$ ~ +4 °C/h) and decreased ($r_{max\text{-}cool}$ ~ −1.3 °C/h) to simulate diurnal water temperature fluctuations in outdoor ponds. Labview™ software is used to control the water temperature according to the temperature scripts that are generated for a

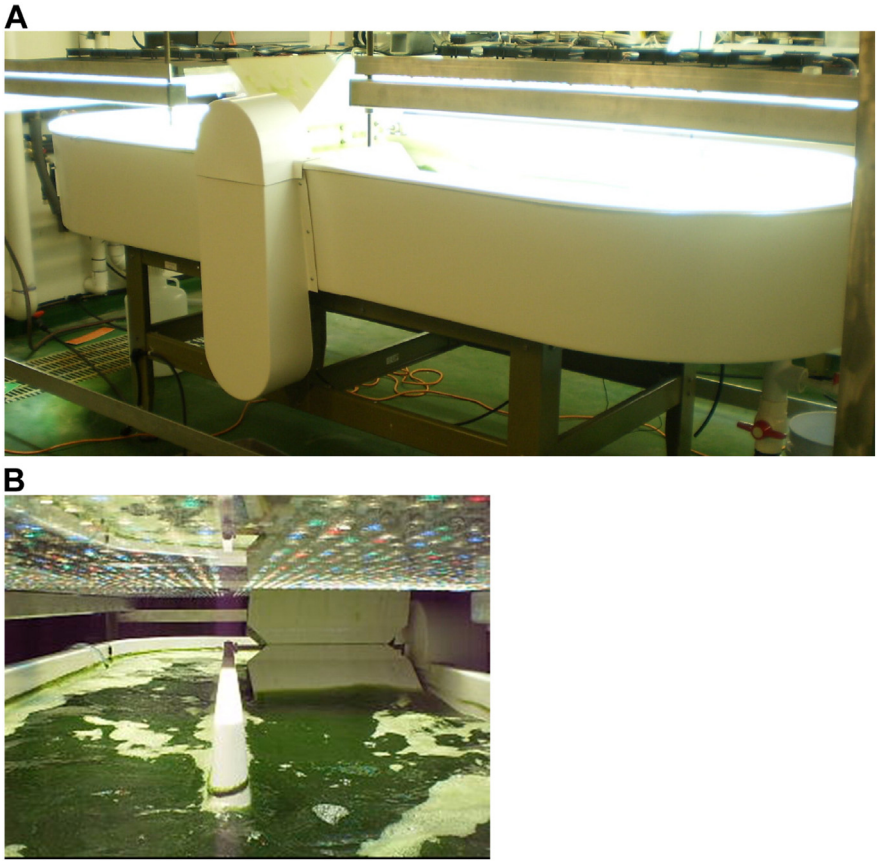

Fig. 1. PNNL indoor climate simulation raceway pond. A. Side view. B. View of the LED panels and growing *Chlorella sorokiniana* DOE 1412 culture.