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Microalgae Culture Using the DASGIP® PBR4 Module for Illumination with a New Brunswick™ CelliGen® 310 Stirred-tank Bioreactor

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Abstract

The number of bioprocess applications for microalgae has increased in recent years, particularly in the field of biofuel production. The combination of the New Brunswick CelliGen 310 stirred-tank bioreactor and the DASGIP LED Illumination System creates a bioreactor setup which is capable of supporting high density microalgal growth. Using the stand-alone Eppendorf DASGIP PBR4 Module, LED illumination spectra and intensities can be controlled for optimal support of all types of chlorophylls and carotenoids. For this study in which high density culture of up to 1.5 x 10^7 cells/mL was achieved, the unicellular freshwater alga, *Dunaliella tertiolecta*, was used.

Introduction

Up to half of a microalgae's composition by weight is lipid, making it an ideal and almost limitless source of biofuel[1]. Grown in large pond-like open systems or in closed bioreactors, algae are perfect non-fossil fuel alternatives for a number of reasons. First, growing algae is beneficial to the environment since CO_2 is removed and oxygen is generated. In addition, since they do not devote resources to producing leaves and other cellulose-based structures like terrestrial plants, they grow far faster and require less input than other fossil fuel alternatives such as soybean and corn. In fact, aside from sunlight, these unicellular phototrophs require only minimal salts, trace elements, a nitrogen source and CO_2 to grow. Finally, downstream processing of dried algal biomass is simple and involves either a pressing procedure or an organic extraction wherein up to 100 % of the lipid content is harvested. Recent advancements in algal downstream processing is further simplifying this process[2].

Despite the growing list of advantages of microalgae over other biodiesel sources, raising large quantities of algae remains cost prohibitive[1]. Government subsidies for algae farms do not equal those already in place for the production of crops such as corn. Moreover, supplementing algal cultures with CO_2 and powering the agitation mechanisms required to ensure that the growing culture is exposed to enough light remains expensive. There is a clear need for the development of a strain with more self-sufficiency and less dependence on these interventions.

Figure 1: Equipment used to assemble the bioreactor – CelliGen 310 (left) and DASGIP PBR4 Module with LED Illumination Devices (right)

The DASGIP PhotoBioreactor combined with PBR4 LED Illumination System has been previously shown to support the growth of two algae strains. As Figure 2 illustrates, *Dunaliella tertiolecta* and *Tetraselmis sp.* were successfully cultivated in a glass DASGIP photobioreactor with the LED Illumination System[3]. In this work, we show that *D. tertiolecta* can be grown in the New Brunswick

Table 1: Materials, media and cells

Material	Supplier	Order no.
Dunaliella tertiolecta	Provasoli-Guillard National Center for Marine Algae and Microbiota	CCMP362
f/2 culture medium supplements	Provasoli-Guillard National Center for Marine Algae and Microbiota	f/2 medium
TAP growth medium	Gibco®	A1379801
1x Dulbecco's Phosphate buffered saline	Life Technologies®	14190-144
Instant Ocean®	United Pet Group, Inc.	SS15-10
250 mL PC vent cap Erlenmeyer flask	VWR®	89095-266

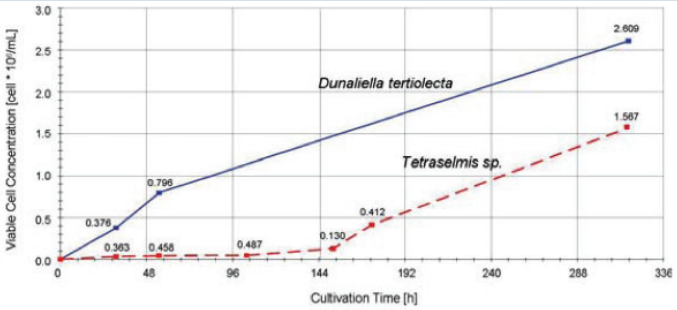

Figure 2: Growth of two algae strains using a DASGIP PhotoBioreactor combined with the PBR4 LED Illumination System

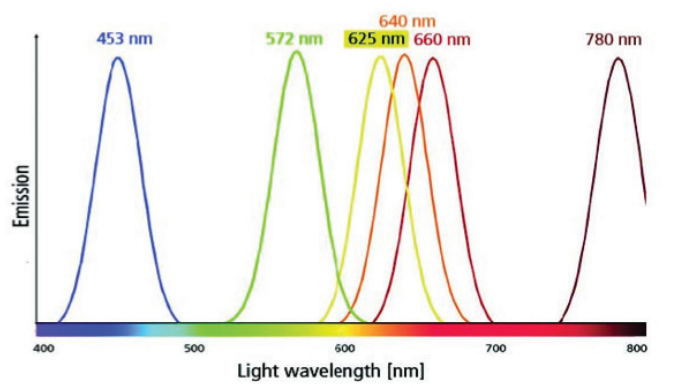

Figure 3: Available wavelengths of LED lights are illustrated – light spectra of the LEDs are optimized to meet various photosynthesis requirements with 453, 572, 625, 640, 660 and 780 nm available

CelliGen 310 stirred-tank bioreactor with the addition of the DASGIP LED Illumination system without the use of a DASGIP photobioreactor (Figure 1). This bioreactor setup is able to generate high-density microalgae cultures which can be used for strain engineering and other research and development endeavors. The stand-alone DASGIP PBR4 Module for precise control of illumination settings allows growing plant cell suspensions, green or brown algae as well as phototrophic bacteria like cyanobacteria or green sulfur bacteria on variable lighting conditions. The DASGIP LED Illumination Devices were designed to provide the most effective light supply for the highest photosynthesis and growth rates (Figure 3). Up to 4 devices per bioreactor can be installed using the industry-standard PG13.5 headplate port and serve optimized light spectra with defined wavelengths to meet the specific photosynthesis requirements. The spectral composition of the LEDs supports all types of chlorophylls and carotenoids (Figure 3).

Materials and Methods

Consumable Materials

Table 1 details the consumable reagents and materials that were used in this study.

Algae Inoculum Culture

D. tertiolecta (Figure 4) was cultured in vent cap flat bottom Erlenmeyer flasks (VWR®, USA) in a New Brunswick Innova® 44R shaker outfitted with the programmable photosynthetic light bank (Eppendorf, Inc., USA). Sterile Instant Ocean (United Pet Group, Inc., USA) was used as a base to which f2 media supplements were added at the concentrations recommended by the kit protocol (Provasoli-Guillard National Center for Marine Algae and Microbiota, USA). Instant Ocean with f2 supplements is referred to as f2 medium – so named because it represents a reduced-

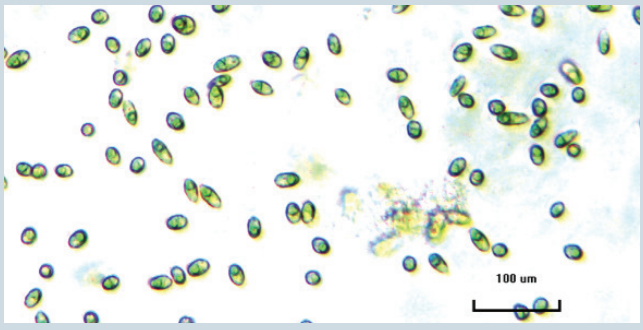

Figure 4: *D. tertiolecta* grown in bioreactor conditions – scale bar represents 100 µm, photographed at 100x using an Olympus® IX51 inverted microscope (OLYMPUS, Japan) equipped with an Infinity2 CCD camera (Lumenera®, Canada)

strength version of the original "f medium."[4] While f2 medium provides trace metals, a nitrogen and phosphorous source, and vitamins, Tris acetate phosphate (TAP) medium provides optimal salinity and buffer conditions for algae growth. Robust microalgal growth was observed using a combination of f2 and TAP, formulated as follows: 1 part f/2 medium and 9 parts TAP medium (f2:TAP). Cultures were inoculated at an approximate density of 1 x 10^6 cells/mL and maintained at 23 °C with a shaking speed of 100 rpm in constant light conditions. Culture density was monitored using a Vi-CELL® automated cell counter (Vi-CELL XR; Beckman Coulter, Inc., USA #731050).

Bioreactor culture

Log phase *D. tertiolecta* were used to inoculate a CelliGen 310 1 L glass water-jacketed vessel (Eppendorf, Inc., USA). The vessel was outfitted with a standard gel-filled pH sensor, a pitched-blade impeller and three DASGIP LED Illumination Devices (DASGIP GmbH, Germany). An inoculation density of 1 x 10^6 cells/mL in a final working volume of 1.8 L of the f2:TAP combination described above was used. The vessel was filled to maximum working volume of 1.8 L to allow as much LED light into the culture medium as possible. Since algal cultures tend to become more alkaline over time, CO_2 gas was used to maintain pH without base addition. When CO_2 was directly sparged into the culture, the pH dropped too rapidly and resulted in a reduction in viability (data not shown); therefore, pH control was accomplished using the overlay option installed on the CelliGen 310 controller (Eppendorf, Inc., USA). The pH control was cascaded to overlay CO_2 flow. A pH deadband of 0.2 was used.

The DASGIP PBR4 Module was used to control LED light delivery to the growing culture. This module allows individual control of three independent wavelength channels emitting light of 6 different wavelengths, as shown in Table 2. For green algae, LEDs in the red, orange and blue ranges were used which is appropriate for an organism containing chlorophyll A and B (Table 2). Setpoints for the LED illumination sticks are shown in Table 3.

Table 2: Wavelengths of PBR4 channels

Channel	Description	Available wavelengths (nm)
A	Bright red, near infared	660, 780
B	Green, yellow, orange	572, 625, 640
C	Blue	453

Table 3 illustrates the setpoints used for these experiments. Two independent trials were performed using similar conditions with one exception: In Run 1, the blue LED light (453 nm) intensity was reduced by 50 % to 2.15 µmol photons/sec, because blue wavelength LEDs tend to emit very high levels of photons. In Run 2, the blue LED was used at maximum intensity (4.30).

Table 3: Parameter setpoints for microalgal growth in bioreactor conditions

Parameter	Setpoint
Agitation (rpm)	100
Temperature (°C)	23
DO	Not controlled
pH	7.6
pH deadband	0.2
Overlay CO_2	Cascade to pH Start SP: 0 @pH Start Out %: 0 End SP: 1.00 SLPM @pH End Out %: -100
PBR4 light control [µmol photons/sec]	Continuous light setting A: 3.19 B: 1.87 C: Run 1: 2.15 Run 2: 4.30

Results and Discussion

As shown in Figure 5, densities as high as 1.5x10^7 cells/mL were achieved using the current experimental parameters with high viability in both bioreactor runs. In Run 2, a shorter lag phase was noted, which could be due to the higher viability and overall health of the inoculum culture (inoculum viability was 96.2 % for Run 2 while viability was 86 % for Run 1). Although these conditions were not optimized, it is clear that the DASGIP PBR4 provides sufficient light for the culture of microalgae in a standard glass stirred-tank bioreactor.

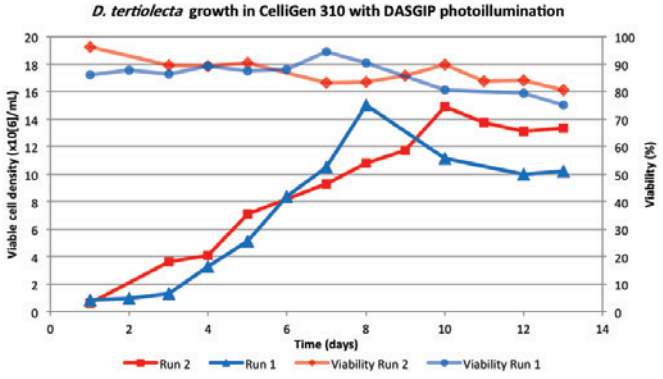

Figure 5: Growth and viability of *D. tertiolecta* in bioreactor culture – this graph shows the viable cell density and % viable cells at each timepoint during two consecutive bioreactor runs

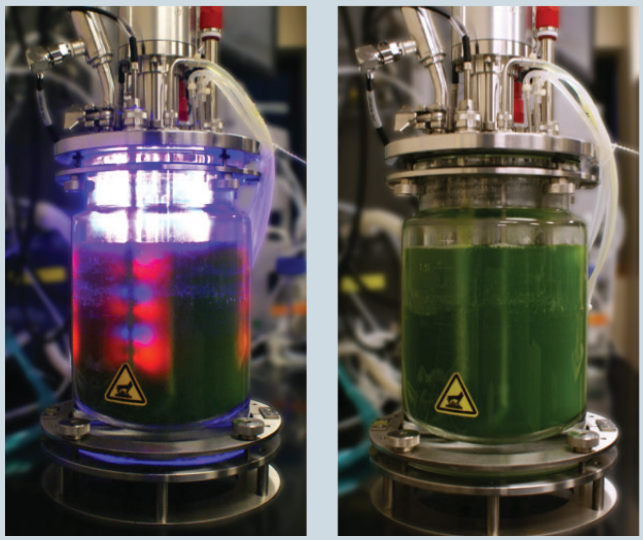

Figure 6: Bioreactor at high density – setup with LED illumination on (left) and the same experiment with lights off (right) to illustrate the highly turbid green microalgal culture

The utility of this setup to the field of microalgal bioprocess is clear. Not only are there direct applications to the field of algal biofuel production, but also to the fields of nutraceutical and pharmaceutical bioprocess. Strains such as *Chlorella vulgaris* have been used extensively in the nutraceutical bioprocess field and are currently marketed as "clean" alternative protein sources to the human diet. This microalgae's diminutive size (≤10 µm in diameter) and extremely high chlorophyll and protein content (up to 80 % by weight) have made it an attractive choice for dietary supplementation for a number of decades[5,6]. Microalgae such as *Chlamydomonas reinhardtii* have been used for R&D protein production for many years. Recent expression of the E7 protein from Human papilloma virus in *C. reinhardtii* chloroplast highlights the role that this organism has to play in pharmaceutical bioprocess manufacturing[7].

Conclusions

We have shown that pairing a stirred-tank bioreactor such as the New Brunswick CelliGen 310 with the DASGIP PBR4 LED Illumination Module can be used to successfully grow high density microalgal cultures. The utility of this setup is widespread since the DASGIP PBR4 can be added to any industrial bioreactor which is equipped with a standard PG13.5 port and the field of microalgal bioprocess continues to grow at a rapid pace. From biofuels to dietary supplements to viral vaccines, microalgae represent a growing market full of opportunities.

References

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Ordering information

Description	Order no.
CelliGen® 310, 4 TMFC (0.1-5 SLPM) With factory installed Overlay option	Call for ordering info
1 L Vessel Kit w/motor	M1287-0300
DASGIP® PhotoBioreactor Illumination Module, for 4 vessels, w/o LED Illumination Devices, incl. EasyAccess Software	76DMPBR4
DASGIP® PhotoBioreactor LED Illumination Devices, for 1 vessel, 220 mm, type S (4 sticks w/ 453/572/625/640/660/780 nm)	76DGLED220S

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