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A novel horizontal photobioreactor for high-density cultivation of microalgae

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HIGHLIGHTS

- A novel low-cost floating horizontal photobioreactor (HBR) for algae was designed.
- Successful cultivation of the microalgae *Nannochloris atomus* in the HBR.
- High algae biomass concentration was achieved without contamination issues.
- Biomass productivity doubled, when light intensity tripled.
- High productivity achieved during semi-continuous outdoor operation over 165 days.

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ABSTRACT

Microalgae are a promising source of biofuels and bioproducts, as they consume CO_2 to grow, multiply quickly, and can be cultivated in wastewater and on marginal land. Development of low-cost and high-efficiency microalgal cultivation systems is important to the cost-competitiveness of algae. A floating horizontal photobioreactor (HBR) was developed that is inexpensive and scalable, as it is manufactured from inexpensive plastic film and is modular. Its performance was successfully tested using the marine microalgae *Nannochloris atomus* Butcher CCAP 251/4A in a 65-L prototype unit. High biomass concentration of 4.0 g L^{-1} and productivity of 12.9 g m^{-2} d^{-1} was achieved indoors under artificial illumination of 31.3 *k*lux (435 μmol m^{-2} s^{-1}). Outdoors, during semi-continuous operation in Florida, the HBR achieved over the course of 165 days a maximum biomass concentration of 4.3 g L^{-1} and an average biomass productivity of 18.2 g m^{-2} d^{-1} without any contamination issues.

1. Introduction

Microalgae are a promising aquatic biomass source of sustainable biofuels and bioproducts. They have significantly higher growth rates than terrestrial biomass and conventional crops, as most strains can double their cell mass within 24 h. Other advantages of microalgae include the option to cultivate them on arid or poor-quality land and the ability of many species to grow in saline or brackish water (Amaro et al., 2011; Chisti, 2007; Griffiths and Harrison, 2009). These features of algae eliminate the need for agricultural land and fresh water, hence enhancing sustainability. Algal biomass has been produced at industrial scale for various applications, such as human food supplements, animal feed, nutraceuticals, and pigments, as well as in wastewater treatment (Borowitzka and Moheimani, 2013; Wang et al., 2012). In recent years, algal biofuels are viewed as a promising alternative to conventional fuels and interest has grown in biodiesel and aviation fuel production from microalgae (Amaro et al., 2011; Chisti, 2007).

Currently, microalgae are cultivated at large scale mostly in open ponds due to their low capital and operating costs. However, such systems provide little control on operational conditions, which leads to low biomass productivities, and are constantly exposed to potential contaminants increasing the risk of culture collapse. (Borowitzka and Moheimani, 2013). On the other hand, 'closed' cultivation systems or photobioreactors (PBR) provide higher photosynthetic efficiency, hence can achieve higher biomass productivity and concentration (Wang et al., 2012), but at much higher cost because of high energy use (mixing, cooling, and embodied energy) and capital cost (Zittelli et al., 2013). In the last few years, various photobioreactor designs have been proposed, most of them aiming at reducing costs. Besides improvements of the classic tubular and flat panel designs, some new concepts have been proposed, like hybrid systems combining open

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Abbreviations

HBR	horizontal bioreactor
PE	polyethylene
DO	dissolved oxygen
OD	optical density
DCW	dry cell weight
NO_3-N	nitrate nitrogen
PO_4-P	phosphate phosphorus
T	PE transparency percentage
I_0	light intensity without the PE piece in front of the light sensor
I_{PE}	light intensity with the PE piece in front of the light sensor
R^2	correlation coefficient
SE	standard error
X	biomass concentration ($g\,L^{-1}$)
N	cell density ($cells\,mL^{-1}$)
μ_m	maximum cell growth rate (d^{-1})
R_N	average NO_3-N consumption rate ($mg\,L^{-1}\,d^{-1}$)
R_P	average PO_4-P consumption rate ($mg\,L^{-1}\,d^{-1}$)
P_v	average volumetric productivity ($g\,L^{-1}\,d^{-1}$)
P_a	average areal productivity ($g\,m^{-2}\,d^{-1}$)
Y_I	biomass yield on light energy (g biomass per mol photons)
Y_N	biomass yield on nitrogen (g biomass per g NO_3-N)
Y_P	biomass yield on phosphorus (g biomass per g PO_4-P)
$X_{outdoor}$	biomass concentration ($g\,L^{-1}$) during outdoor cultivations,
X_{indoor}	biomass concentration ($g\,L^{-1}$) calculated from indoor OD-DCW linear correlation

ponds and photobioreactors, and floating photobioreactors, which aim at exploiting water bodies, instead of competing for land, for algae cultivation (Zittelli et al., 2013). While some floating PBR designs have been proposed (Albus et al., 2012; Berzin et al., 2011; Bussell, 2010; Muller-Feuga and Lemar, 2011; Patel et al., 2011), very few have been deployed outdoors, like the OMEGA reactor developed by NASA, reactors designed by Algasol Renewables, and submerged systems by Solix (Zittelli et al., 2013). However, the NASA reactor depends on offshore waves for culture mixing and aeration, which are intermittent, and all reactors are limited to deployment on water.

In an effort to advance the status of algae cultivation systems with a readily scalable bioreactor of low cost but high productivity, we developed a horizontal bioreactor (HBR) that combines the advantages of open ponds and PBRs in a cost-effective way and can be used on both water and ground, depending on the end user's particular needs. Among marine microalgae, we tested the HBR performance with the genus *Nannochloris*, which is known to grow well in defined media and to accumulate significant amounts of intracellular lipids (Griffiths and Harrison, 2009; Takagi et al., 2000) that are important for biofuel production. The cultivation of *Nannochloris atomus* Butcher CCAP 251/4A in a 65-L floating HBR was successfully demonstrated first indoors under artificial illumination and then outdoors in semi-continuous mode over a 6-month period (165 days).

2. Methods

2.1. The horizontal bioreactor (HBR)

The HBR consists of two plastic films forming the top and bottom surfaces of the horizontal raceway, sealed to each other and connected to two vertical airlift units (Fig. 1). The plastic film was fabricated from inexpensive transparent polyethylene (PE) sheet 0.15 mm thickness. The HBR prototype unit was 133.5 cm long × 68 cm wide and the raceway had a low depth of 5 cm to enable increased light exposure of the culture liquid and reduce water use. The effective surface area of the HBR was 0.94 m^2. Culture mixing was accomplished by the two airlift sections made from acrylic pieces of 0.5 cm thickness, each providing air enriched with CO_2 at a flow rate of 10 $L\,min^{-1}$ (Fig. 1). For heat dissipation, the HBR unit was floated on water inside a tub (indoors) or in an artificial pond (outdoors). Buoyancy of the unit was achieved by inexpensive foam pieces attached to the airlifts and bubble-wrap strips under the PE sheet. The strips did not fully cover the reactor bottom sheet in order to allow sufficient contact with the surrounding water for heat dissipation. A pump was used to transfer liquids via silicone tubing into and out of the bioreactor. The HBR was equipped with a submergible pH probe with automatic temperature compensation (Cole-Parmer, USA) connected to a digital controller (pH 200 Series, Eutech Instruments, USA). For indoor experiments the photobioreactor was exposed to the light of two Aqualite™ metal halide lamps (Ushio America Inc., USA).

2.2. Microorganism and growth medium

The green microalgae strain *N. atomus* Butcher CCAP 251/4A was selected to evaluate the algae cultivation performance of the HBR. The strain was obtained from Culture Collection of Algae and Protozoa (CCAP, U.K.). *N. atomus* was grown in artificial seawater medium, as defined in Table 1. The Instant Ocean™ solution was freshly prepared prior to inoculation per manufacturer's instructions. The macronutrient, trace metal, and vitamin B12 stock solutions were prepared, filtered (0.45 μm), and stored in a refrigerator in the dark for up to a month. For the pre-culture preparation, the Instant Ocean solution and appropriate volumes of macronutrient

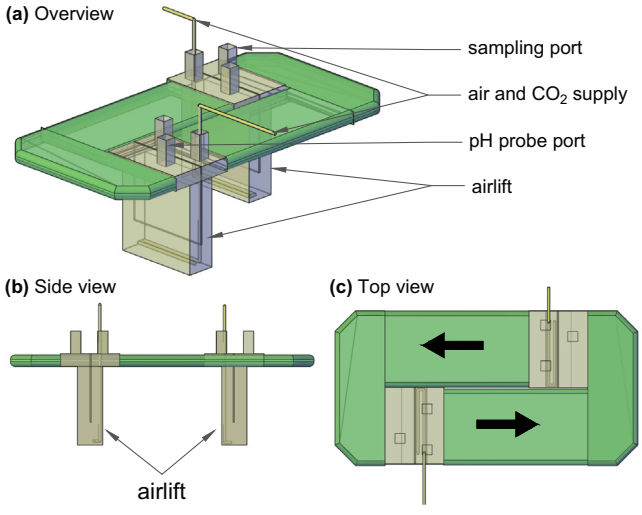

Fig. 1. Drawing of the floating HBR system for the cultivation of microalgae: (a) overview, (b) side view, and (c) top view. Arrows indicate the flow direction of the algal culture.

Table 1
N. atomus growth medium initial composition. Nitrate (KNO_3) and phosphate (KH_2PO_4) were supplemented when needed to maintain their levels in the 0–1000 and 0–80 mg L^{-1} range, respectively.

Component	Concentration
Seawater[a]	g L^{-1}
Instant Ocean™	35
Macronutrients[b]	mg L^{-1}
KNO_3	506
$NaHCO_3$	420
KH_2PO_4	27.2
Vitamin[b]	mg L^{-1}
B12 (cyanocobalamin)	0.50
Trace metals[c]	mg L^{-1}
$ZnSO_4{\cdot}7H_2O$	10.1
$MnCl_2{\cdot}4H_2O$	1.98
$Na_2MoO_4{\cdot}2H_2O$	1.21
$Co(NO_3)_2{\cdot}6H_2O$	0.087
$CuSO_4{\cdot}5H_2O$	0.075
$Na_2EDTA{\cdot}2H_2O$	44.7
H_3BO_3	24.7
$FeSO_4{\cdot}7H_2O$	6.95

Sources
[a] Manufacturer recipe.
[b] Ben-Amotz (1987).
[c] McLachlan (1973).

and trace metal stock solutions were autoclaved (120 °C, 30 min) separately and mixed afterwards just prior to inoculation. For HBR cultivation runs the medium was sterilized in situ using commercial concentrated bleach (8% sodium hypochlorite) at a dose of 0.25 mL per L of medium for 6 h. Afterwards the bleach was neutralized with sodium thiosulfate solution at a 1:1 molar dose between sodium thiosulfate and sodium hypochlorite. Vitamin B12 was added to the medium aseptically through sterile filtering (0.2 μm). Nitrate (KNO_3) and phosphate (KH_2PO_4) were supplemented throughout the cultivations aseptically by sterile filtering (0.2 μm) directly into the bioreactor, when needed, to maintain their concentrations in the 0–1000 and 0–80 mg L^{-1} respectively. All chemicals for growth medium preparation were of analytical grade and supplied by Fisher Scientific (USA). Compressed pure CO_2 gas was supplied by Airgas (USA).

2.3. Experimental setup

2.3.1. Inoculum preparation

The inoculum for the 65-L HBR was prepared in a custom-made acrylic vertical flat-panel photobioreactor with a 7.5-L working volume. More specifically, *N. atomus* cultures were initially prepared in 2-L Erlenmeyer flasks with 1 L of medium in a rotary shaker (Excella E24, New Brunswick, USA), operated at 180 rpm and 23 °C for 14 days. A 10% (v/v) inoculum (750 mL) was transferred from the flask to the 7.5-L flat-panel photobioreactor and was supplemented with fresh medium (Table 1) to a final volume of 7.5 L. The bioreactor was sterilized using sodium hypochlorite (common household bleach) overnight and neutralized for 1 h with sodium thiosulfate. The culture was bubbled with air mixed with CO_2. CO_2 addition was employed as a means of maintaining the pH of the medium at 7.50 ± 0.10 using a pH-stat system. The flat-panel bioreactor operated in an air-conditioned lab at 22 °C. Samples were taken regularly for growth calculation and nutrient monitoring.

2.3.2. HBR cultivation

The bioreactor and fresh growth medium were sterilized using sodium hypochlorite overnight and neutralized for 1 h with sodium thiosulfate. When the flat-panel bioreactor culture (inoculum) reached high density (OD_{680} = 7.1), while still in the exponential phase, a 10% (v/v) inoculum (6.5 L, 1.4×10^7 cells mL^{-1}) was transferred to the 65-L floating HBR for the cultivation test. The pH of the culture was maintained at 7.50 ± 0.10 by adjusting the CO_2/air mix between 2% and 3% (v/v) CO_2 in air. During indoor experiments, the HBR operated in an air-conditioned lab at 22 °C. Outdoor semi-continuous cultivations were conducted in the Lakeland area in Central Florida, where the HBR operated in a temperature-regulated pool. Samples were taken regularly to determine algae growth, macronutrient consumption, salinity, and dissolved oxygen measurements. In order to compensate for water evaporation, sterile (autoclaved at 120 °C, 30 min) deionized water was added to the bioreactor after each sampling based on the measured salinity change.

2.4. Light intensity

A portable light meter (Model CA813, AEMC Instruments) was used to measure the light intensity in lumens per meter (lux) on the surface of the photobioreactors both indoors and outdoors. A 6- and 12-point measurement across the vertical (flat panel) and the horizontal (HBR) photobioreactor surfaces, respectively, was employed to estimate the average illumination. The available photon flux (μmol m^{-2} s^{-1}) was calculated from the measured light intensity (in *k*lux), based on the approximate conversion value of 1 lux = 0.014 μmol m^{-2} s^{-1} for radiation at 400–700 nm from metal halide lamps, (Sager and McFarlane, 1997). *N. atomus* stock flask cultures were initially prepared under continuous illumination by two 40-Watt Philips fluorescent lights providing approximately 1 *k*lux. The vertical flat panel bioreactor was exposed to 10.0 *k*lux of light on a 16:8 h light-dark cycle. When indoors, the HBR was irradiated with either 11.2 or 31.3 *k*lux on a 16:8 h light-dark cycle.

2.5. Analytical methods

Optical density (OD) was measured in duplicate at 680 nm in a spectrophotometer (DU 730, Beckman Coulter, USA). For linearity, the samples were diluted appropriately to measure the OD in the 0.100–0.400 range. Dry cell weight (DCW) of each sample was measured after filtering 10 mL of culture volume through preweighed 0.45 μm Whatman nylon filters and drying at 100 °C to constant weight in a moisture analyzer (MB25, Ohaus, USA). DCW (g L^{-1}) was calculated by subtracting the dry weight of the empty filter from the dried weight of the loaded filter and dividing by the volume of the filtered sample. The initial and residual amounts of nitrogen (NO_3-N) and phosphate (PO_4-P) were calculated from nitrate and phosphate concentrations in the filtrate (0.45 μm) of the reactor samples. The amounts of nitrate and phosphate were estimated colorimetrically with nitrate and orthophosphate test kits, respectively, following instructions supplied by the kit manufacturer (Hach, USA). Cell density was measured directly under the microscope using an Improved Neubauer hemocytometer. Cell counts were conducted in duplicate for each sample, and a minimum of 200 cells was counted for statistical reliability.

Dissolved oxygen (DO) content was measured regularly, immediately after sampling, with a portable dissolved oxygen Meter HI 9147 (HANNA Instruments, Romania) calibrated at 30‰ salinity following the manufacturer's instructions. The salinity of the culture medium was measured in each sample with a portable salinity meter SALT6+ (OAKTON, USA) calibrated with a 3.00 (w/v) NaCl solution following the manufacturer's instructions.

2.6. Measurement of bleached PE transparency and possible effect on algal growth

To account for any possible effect that bleach sterilization of the HBR could have on the transparency of the PE material and on algal growth, three 10 cm × 10 cm pieces of the original PE sheet were prepared and their light transmittance was measured before (control) and after bleaching using the same conditions as described for HBR sterilization (see Section experimental setup). The PE pieces were exposed to 5 different light intensities by varying their distance from a metal halide bulb in front of a portable lightmeter (Model CA813, AEMC Instruments, USA) and each intensity was recorded. The transparency (T) was calculated using Eq. (1). In addition, a wavelength scan of the PE pieces was performed in a spectrophotometer (DU 730, Beckman Coulter, U.S.A.) and the absorbance of the PE pieces at 680 and 420 nm was measured.

$$T = \frac{I_0 - I_{PE}}{I_0} \times 100 \quad (1)$$

where T is the transparency percentage, I_0 is the light intensity without the PE piece and I_{PE} is the intensity with the PE piece in front of the light sensor.

The effect of the bleached PE material on algal growth was tested with *N. atomus* flask cultures in duplicate. Appropriate-size PE pieces were inoculated in 250-mL Erlenmeyer flasks (100 mL culture liquid) to mimic the contact of the culture with the PE surface in the HBR, followed by the same procedure for bioreactor bleach sterilization. The flasks were inoculated with *N. atomus* culture (about 1.6×10^7 cells mL^{-1}). Control flasks without PE pieces were also included in duplicate. Samples were taken after 7 and 14 days for growth measurement.

2.7. Calculations and statistical analysis

The maximum specific growth rate μ_m (d^{-1}) of algae during indoor HBR cultivations was calculated during the exponential phase from the slope of the linear regression curves ($R^2 = 0.97 - 0.98$, $P < 0.001$) of the natural logarithm of cell density ($\ln N$) versus cultivation time (t). The volumetric productivity, P_v ($g\,L^{-1}\,d^{-1}$), was calculated from the change in biomass concentration, X ($g\,L^{-1}$), within a certain cultivation period (d). The average areal productivity, P_a ($g\,m^{-2}\,d^{-1}$), was calculated based on the HBR's surface area (0.94 m^2). The total biomass yield on light energy (Y_I), nitrogen (Y_N) and phosphorus (Y_P) were calculated by dividing the final algae biomass concentration by the incident mol of photons, the consumed grams of NO_3-N or the consumed grams of PO_4-P, respectively. Calculations and statistical analysis were performed using Microsoft Excel 2011 and SYSTAT Sigmaplot 12, respectively.

3. Results and discussion

3.1. Transparency of and algae compatibility with bleached polyethylene

The HBR and the growth medium were chemically sterilized using sodium hypochlorite (common household bleach) and neutralized with sodium thiosulfate. The possible effects of bleach sterilization on the PE's light transmittance and of the bleached PE on algae growth were determined in flask experiments. The mean light transmittance of the control (original PE) was 92.8% (SE = 0.7), whereas the bleached PE sample allowed 92.0% (SE = 0.9) of light to pass through (data not shown). In addition, a wavelength scan of the PE pieces was performed. Their absorbance at 680 nm (red light) and 420 nm (blue light) was measured because red and blue light are more preferable by green algae for photosynthesis (Emerson and Lewis, 1943). At both wavelengths the difference in the absorbance between the control and the bleached sample was less than 4% (data not shown). The results indicate that the effect of the chemical sterilization on the light transmittance of the PE material is negligible. Therefore, visible light in general as well as photosynthesis-relevant (red and blue) light are transmitted efficiently through the bleached PE of the HBR.

The possible effect of bleached PE on algal growth was tested with *N. atomus* cultures, which were inoculated in the absence or presence of bleached PE pieces (Fig. 2). The results showed that the addition of bleached PE did not cause any growth inhibition to *N. atomus*, based on OD_{680} measurements, and hence chemical sterilization of the HBR is safe for algal cultivation.

3.2. Indoor HBR cultivation experiments

The large number of gathered growth data ($n = 52$) enabled us to derive correlations among the growth parameters measured in the HBR (Fig. 4). Strong linear correlations were identified between OD_{680}, DCW, and cell concentration of *N. atomus* during the cultivation. Hence, the algal cell concentration (N, cells mL^{-1}) could be efficiently calculated ($R^2 = 0.998$, $P < 0.0001$) from the readily measured OD_{680} by using Eq. (2):

$$N = 1.66 \cdot 10^7 \times OD_{680} \quad (2)$$

Cell concentration (N, cells mL^{-1}) could also be used ($R^2 = 0.961$, $P < 0.0001$) to calculate the biomass concentration (X, $g\,L^{-1}$), based on Eq. (3).

$$X = 1.98 \cdot 10^{-8} \times N \quad (3)$$

Finally, the OD_{680} of an algae sample could be used to confidently estimate ($R^2 = 0.979$, $P < 0.0001$) the algal biomass concentration (X, in $g\,L^{-1}$), as described in Eq. (4).

$$X = 0.348 \times OD_{680} \quad (4)$$

The findings indicate that spectrophotometric methods, which are in general rapid and not labor intensive, are adequately accurate. As a result, OD_{680} was selected for monitoring cell growth of *N. atomus* during subsequent outdoor HBR operations and was used as a proxy for rapidly estimating biomass concentration and productivity in the HBR via Eq. (4).

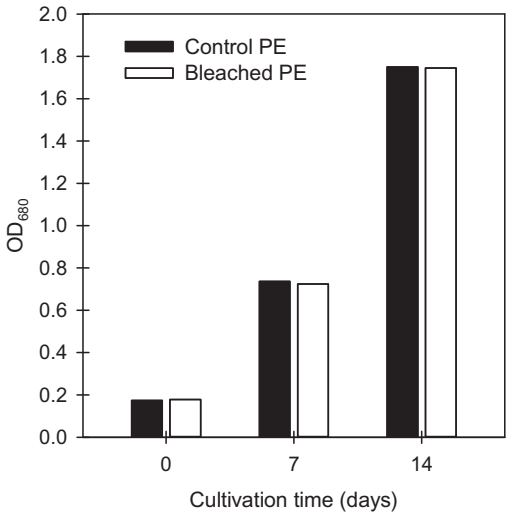

Fig. 2. Cultivation of *N. atomus* in the presence of original PE (control) and bleached PE pieces to account for any adverse effect of PE bleaching on cell growth.

The HBR is modular and is manufactured from inexpensive plastic film. It can operate on a body of water using adequate buoyancy aids, thus eliminating land use and providing temperature control through heat dissipation into the surrounding water. It can also be used on the ground, including unutilized or underutilized land, whose value for crop growing or other beneficial use is diminished. The HBR is designed to reduce water usage by over 4-fold (depth of only 5 cm) and hence diminish energy use (pumping and processing) over traditional systems.

It is equipped with airlifts placed on opposite sides (Fig. 1) to provide adequate culture mixing and increase mass transfer of CO_2 and nutrients, hence boosting microalgae productivity under real-world conditions. Compressed air mixed with pure CO_2 was directed into each airlift unit. The velocity of the liquid flow was mapped at different locations of the HBR using small lightweight acrylic chips. When the airflow in each airlift was set at 15 L min^{-1}, the culture flow in the HBR averaged 20 cm s^{-1}, while at an airflow of 10 L min^{-1} the velocity was 15 cm s^{-1}. The airlift units functioned properly at airflow of 10 L min^{-1} or higher, hence that was considered as the lower limit for HBR operation. According to Borowitzka (2005), culture circulation at 5 cm s^{-1} should prevent algae cells from settling, provide uniform light and avoid thermal stratification phenomena in raceway ponds. However, the flow is usually not uniform due to friction in the channel and corners, hence higher velocities should be attained (Borowitzka, 2005). Therefore, the attained velocity in the HBR of 15 cm s^{-1} at 10 L min^{-1} airflow should provide sufficient culture mixing at the lowest energy consumption level capable to operate the unit efficiently.

Another important factor for optimum growth is the dissolved oxygen (DO) levels in the culture. Oxygen (O_2) is generated during photoautotrophic growth of microalgae and a build-up can have negative effects on productivity as it competes with CO_2 as a substrate of ribulose 1,5-*bis*phosphate carboxylase/oxygenase, thus inhibiting photosynthesis and leading to photorespiration (Borowitzka and Moheimani, 2013). High O_2 levels can be prevented by introducing air into the culture, which removes O_2 (Giordano et al., 2005). The airlift units of the HBR, in addition to culture mixing, were designed to provide a means of reducing the O_2 concentration in the culture and hence improving productivity. DO concentrations above 300% of air saturation could be detrimental to algal cells and therefore could reduce productivity (Molina Grima et al., 2001). DO measurements of HBR samples ranged between 80% and 93% of air saturation throughout the cultivations, so no loss of productivity should have occurred.

Culture temperature and pH values on the controller were manually recorded at each sampling. The HBR was passively cooled during indoor operations in an air-conditioned lab at 22 °C. The maximum observed bioreactor temperature was 30 °C at the highest light intensity of 31.3 *k*lux. The pH was adjusted to 7.50 daily by manually varying the CO_2 input. As the culture progressed increased volumes of CO_2 were required to maintain the pH at 7.50. At low light intensity (11.3 *k*lux) initially the CO_2:air ratio was 0.8% (at 0.08 L min^{-1} of CO_2), while towards the stationary phase (highest cell concentration) the CO_2 demand increased by 50% (1.2% CO_2:air ratio at 0.12 L min^{-1} of CO_2) to maintain a pH of 7.50. At high light intensity (31.1 *k*lux) the CO_2:air ratio had to be increased from 0.8% (at 0.08 L min^{-1} of CO_2) to 2.4% (at 0.24 L min^{-1} of CO_2) in order to keep the pH around 7.50. This change in the CO_2 demand at high light intensity can be justified by the expected higher algal photosynthesis rate, which would consume more dissolved CO_2 than during cultivation at lower light intensity.

To assess the performance of the HBR, the cultivation of *N. atomus* was first performed indoors under controlled conditions. The bioreactor was floated in a pool and light was provided by two metal halide lamps. The desired light intensity was set by adjusting the position of the lamps relative to the HBR surface. Two different illumination levels were applied, 11.2 and 31.1 *k*lux, or 157 and 435 μmol m^{-2} s^{-1}, respectively, in order to understand the effect of light intensity on algal growth. At the start of the experiment light intensity was set at a lower level (6.5 *k*lux) to prevent any photoinhibition phenomena on the low cell density inoculum. After 2 days of cultivation and a very brief lag phase, the culture cell density doubled from 0.8 to 1.8 × 10^7 cells mL^{-1} (OD_{680} of 0.9) and illumination was increased to 11.2 *k*lux (Fig. 3). At that point the culture entered exponential growth phase until day 22, when it reached stationary phase and was harvested. A brief slowdown occurred around days 16–18, but growth resumed afterwards. The maximum reached OD_{680} was 6.96, which corresponded to algal biomass of 2.3 g L^{-1} on a dry basis and final cell concentration of 1.05 × 10^8 cells mL^{-1}.

After the 1st growth cycle at low light intensity reached stationary phase, 90% (v/v) of the culture was harvested and the residual 10% (v/v) was used as inoculum for a 2nd successive cycle, but this time at a higher light intensity. No lag phase was observed, as the culture entered immediately exponential phase. The growth continued up to days 47–48, when it entered stationary phase until the 52nd day, at which point the bioreactor was shut down. Interestingly, the OD_{680} continued to increase slightly even after cell concentration and DCW stopped rising, until it leveled off towards the end of both growth cycles (Fig. 3). The 2nd cycle at high light intensity led to a higher maximum OD_{680} of 11.46 and a higher algal biomass concentration of 4.0 g L^{-1} on a dry basis, which corresponded to a cell concentration of 1.93 × 10^8 cells mL^{-1}.

Algal concentrations and productivities achieved in the floating HBR system at different illumination levels are summarized in Table 2. During low light the estimated maximum growth rate, μ_m, was 0.20 d^{-1}, and doubled (μ_m = 0.41 d^{-1}) when light intensity increased threefold. Increasing the light intensity led to higher nutrient consumption by the algae cells. Nitrogen consumption rate, $R_{m,N}$, increased by 42% and phosphorus consumption rate, $R_{m,P}$, by 35% during the high illumination cycle. The total biomass yields on nitrogen, Y_N, and phosphorus, Y_P, followed the same trend as their consumption rates. When switching to higher illumination, Y_N increased by 59% and Y_P by 41% compared to low illumination yields, which indicates that the nutrients (N and P) were more efficiently utilized when more light was available.

The higher photon flux provided at the 31.1 *k*lux setup in conjunction with the higher nutrient consumption resulted in an 80% increase in algal biomass productivity. The highest average volumetric productivity, P_v, 0.18 g L^{-1} d^{-1}, which was observed during the cultivation at 31.1 *k*lux, corresponds to areal productivity, P_a, of 12.9 g m^{-2} d^{-1}. As mentioned above, a 3-fold increase in light intensity raised productivity by only 2-fold. Moreover, the total biomass yield on light energy, Y_I, at the high illumination cycle was about 50% lower compared to the low light cycle. These results indicate a diminishing effect of light on algal biomass production after a certain level. Huertas and Lubián (1998) reported that photosynthesis of *N. atomus* saturated at photon flux densities up to 1000 μmol m^{-2} s^{-1}, which is double the highest intensity used in our study.

3.3. Outdoor semi-continuous HBR operation

The algae cultivation performance of the 65-L HBR outdoors was demonstrated at our facility in central Florida. The unit was floated in a water pool with active temperature regulation of the pool required occasionally via an external chiller to assist with heat dissipation due to the small volume – hence low heat storage capacity – of the demonstration pool. The average culture temperature was 20.8 ± 1.8 °C, and did not exceed 26 °C during the

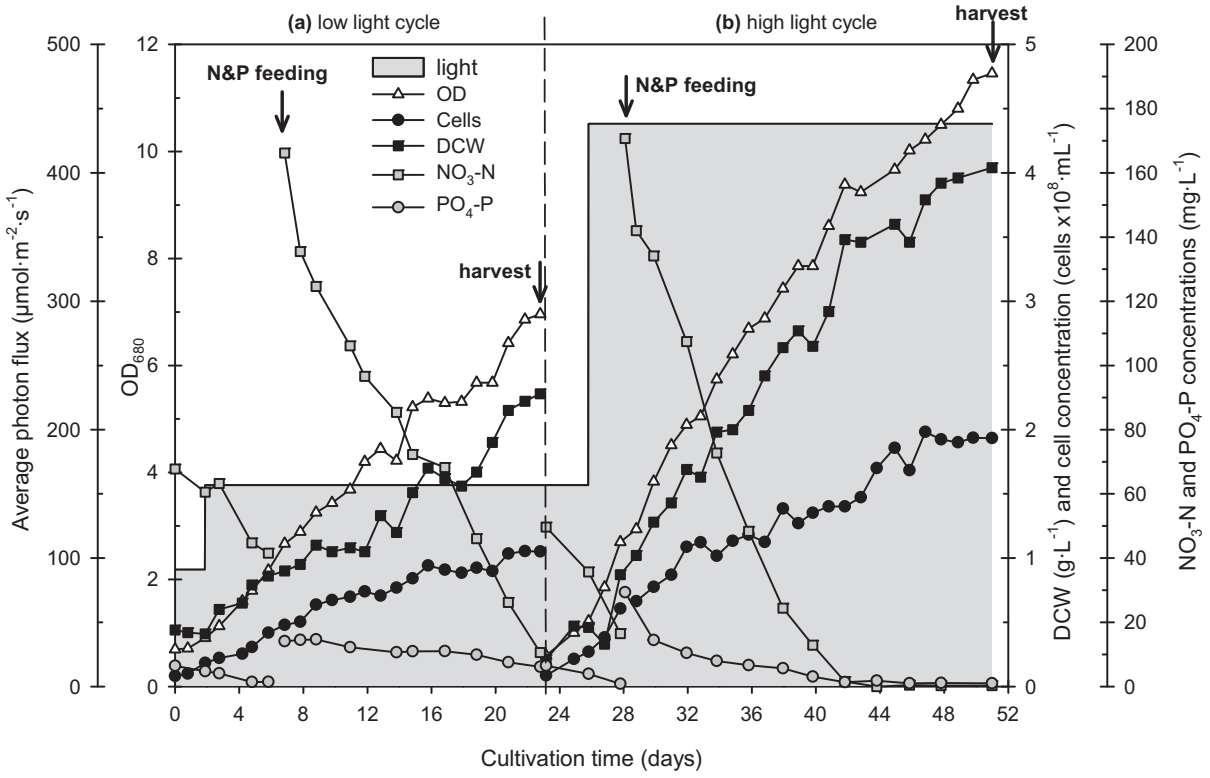

Fig. 3. Growth and nutrient consumption of *N. atomus* during the two cultivation cycles, 0–23rd day and 23rd–52nd day, in the floating HBR under two artificial illumination levels of (a) 11.3 *k*lux and (b) 31.1 *k*lux. After the 1st cycle (growth in low light) 90% (v/v) of the culture was harvested and the residual 10% (v/v) was used as inoculum for the 2nd cycle (growth in high light). The arrows mark either nutrient (nitrate and phosphate) feeding or culture harvesting.

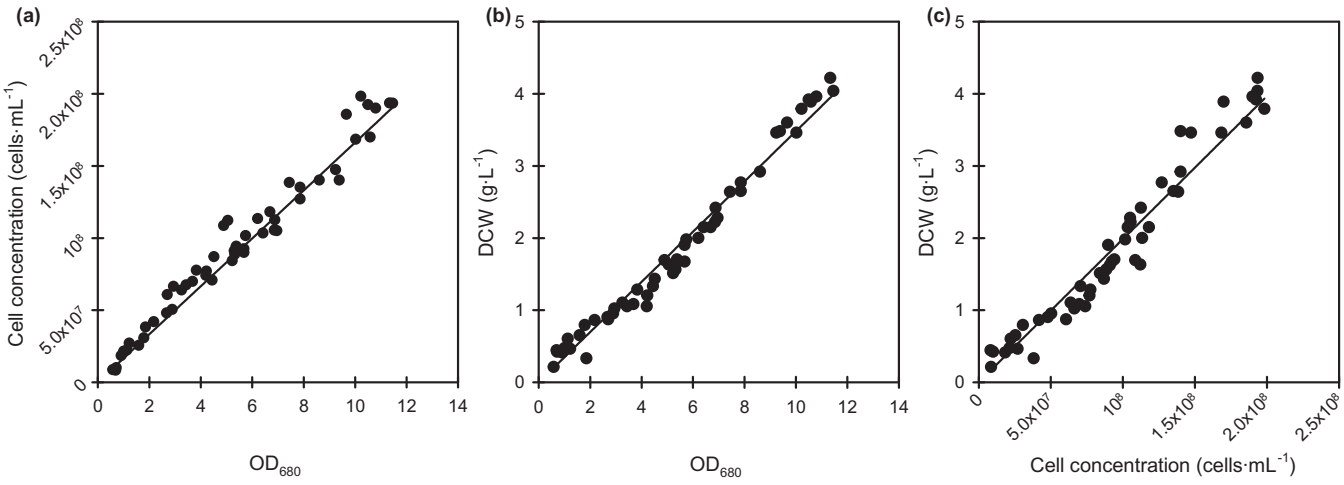

Fig. 4. Linear correlations among *N. atomus* growth parameters: (a) OD_{680}-cell count; (b) OD_{680}–DCW; and (c) cell count-DCW. Data derived from indoor HBR growth experiments.

outdoor operation. Semi-continuous cultivation of *N. atomus* over the course of 165 days was performed from March until September with no contamination problems. The daily algal growth (OD_{680}), bioreactor volume harvested% (v/v), and biomass concentration during each harvest cycle are presented in Fig. 5.

During the outdoor operation 21 consecutive algae growth-and-harvest cycles were conducted with harvest ranging from 31% to 77% (v/v) of the working HBR volume. The dry biomass concentration of the harvested algae cells was measured as described before. A total of 745 L of culture was collected, which corresponded to 2.265 kg of algal biomass produced over the 165-day operation of the 65-L HBR with no contamination. Consistently high biomass concentration was measured at harvest with the lowest being 2.07 g L^{-1} and the highest 4.3 g L^{-1} (Fig. 5). This yield is comparable to typical biomass concentrations achieved in more expensive PBRs, where 2-8 g L^{-1} are common (Kumar et al., 2015; Quinn et al., 2012; Wang et al., 2012; Zhu, 2015), and is considerably higher than algae concentrations in open raceway ponds, which typically range between 0.1 and 0.5 (Kumar et al., 2015; Zhu, 2015), but can reach up to 1.4 g L^{-1} (Ashokkumar et al., 2014; Ketheesan and Nirmalakhandan, 2012). Achieving high biomass density is critical for process economics, as it significantly reduces

Table 2
Algal biomass concentrations and productivities achieved in the floating HBR system at different levels of artificial illumination.

Parameter	Low illumination cycle	High illumination cycle	Unit
Cultivation Period	23	28	days
I	11.2 (157)	31.1 (435)	*k*lux (μmol·m^{-2}·s^{-1})
X_m	2.3	4.0	g L^{-1}
N_m	1.05×10^8	1.93×10^8	cells mL^{-1}
μ_m	0.20	0.41	d^{-1}
$R_{m,N}$	9.1	12.9	mg L^{-1} d^{-1}
$R_{m,P}$	0.79	1.07	mg L^{-1} d^{-1}
P_v	0.10	0.18	g L^{-1} d^{-1}
P_a	7.0	12.9	g m^{-2} d^{-1}
Y_I	0.71	0.36	g per mol photons
Y_N	12.3	19.6	g per g NO_3-N
Y_P	92.2	130.0	g per g PO_4-P

downstream processing costs (algae dewatering and recovery) and improves the overall economics of algae production (Molina Grima et al., 2003).

The recorded daily optical densities at 680 nm ranged between 2.3 and 16.0. Using the strong correlation established between OD and DCW during indoor cultivations, as described in Eq. (4), the daily outdoor HBR productivity data was calculated. The biomass concentration was measured after each harvest of the outdoor culture and was compared with the calculated values from indoor experiments (Fig. 6a). A linear correlation ($R^2 = 0.927$, $P < 0.0001$) was observed between indoor OD–DCW correlated data and actual outdoor DCW data. As a result, the outdoor daily productivities were calculated using the correlation equation Eq. (5) with outdoor daily biomass concentration.

$$X_{outdoor} = 0.851 \times X_{indoor} \quad (5)$$

where $X_{outdoor}$ is the biomass concentration (g L^{-1}) during outdoor cultivations and X_{indoor} is the biomass concentration (g L^{-1}) calculated from the indoor OD–DCW linear correlation.

The average monthly productivities were calculated and their variation is shown in Fig. 6. The productivity increased from March up to June, where it peaked at 23.9 g m^{-2} d^{-1}. Subsequently, it started declining dipping down to 7.3 g m^{-2} d^{-1} in September. While this trend is in agreement with the high sun irradiance of the summer season, there seemed to be some inconsistency in May's productivity, which was lower than March's. The overall average productivity (based on the actual biomass measurements at harvest) during the entire HBR outdoor operation was 18.2 g m^{-2} d^{-1}, which is comparable to the maximum sustainable productivities of 20–25 g m^{-2} d^{-1} achieved in open pond systems (Borowitzka and Moheimani, 2013), but with the invaluable added benefit of no culture contamination and crashes over a long period of time.

3.4. Cost considerations

The motivation behind designing the HBR was to reduce the cost of algal biomass production and hence help advance the current status of the algal biofuels and bioproducts industry. To achieve that goal, the HBR was designed to combine the advantages of open ponds and PBRs in a cost-effective way and reduce the cost per kg of algal biomass by (a) increasing productivity through an enclosed design that creates barriers to contamination; (b) reducing capital and operating costs with the use of inexpensive plastic film for the HBR body; and (c) using less water (shallow depth) to achieve lower energy consumption during operation and reduce dewatering costs during downstream processing. Furthermore, the land usage cost can be eliminated if the unit is operated on a body of water. Although the cultivation system is still a prototype of small scale, a preliminary cost projection was performed to estimate the capital cost of the HBR and the cost per kg DCW based on the achieved productivities reported in this study.

Based on cost of materials and manufacturing labor extrapolations of the current 65-L (0.94 m^2) prototype, the capital cost of the HBR at full production is estimated to be $25,000 per hectare using scaled-up units, each with a surface area of 1000 m^2. This

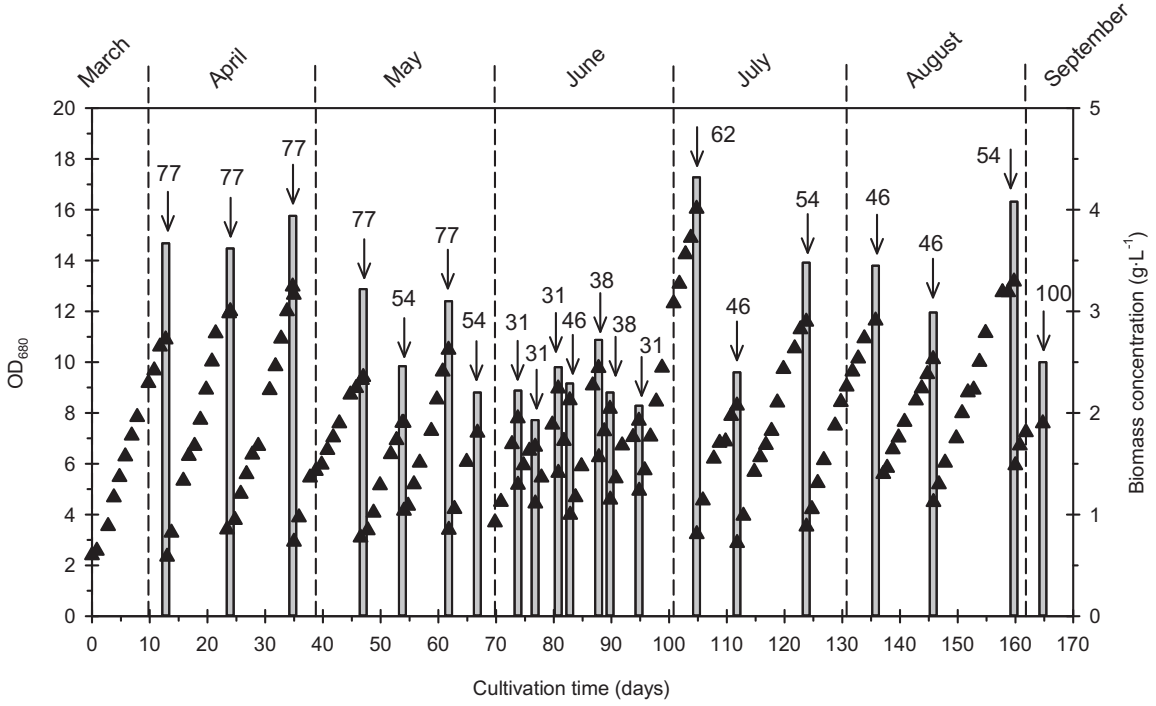

Fig. 5. Cultivation of *N. atomus* in the floating 65-L HBR during outdoor semi-continuous operation in central Florida. Daily growth as monitored by OD_{680} (▲); arrows and their adjacent labels mark culture harvesting and % of total reactor volume harvested, respectively; gray bars show the biomass concentration at harvest.

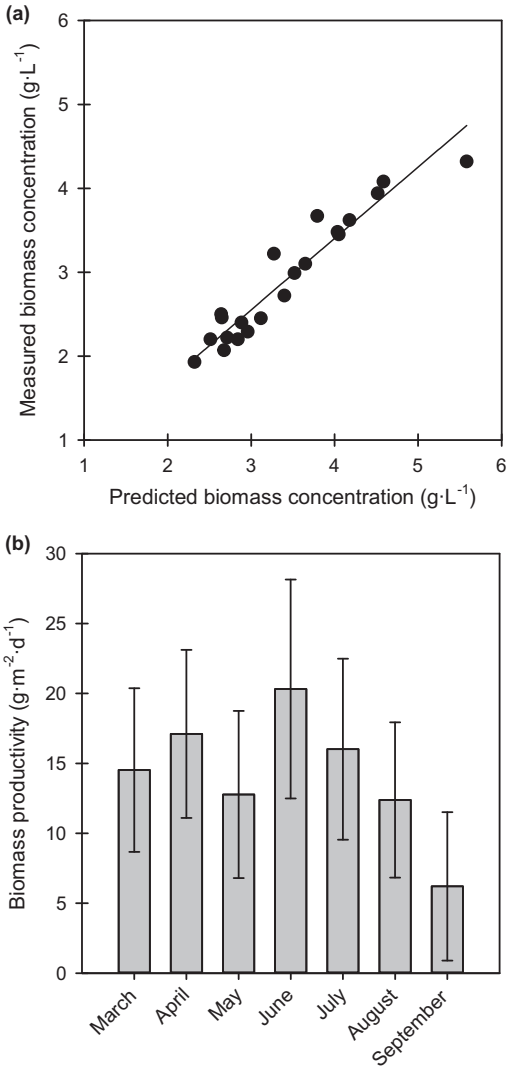

Fig. 6. (a) Linear correlation between biomass concentration predicted from OD–DCW indoor correlations and measured biomass concentration outdoors at harvest; (b) Monthly biomass areal productivity (g m^{-2} d^{-1}) in the floating 65-L HBR during outdoor semi-continuous cultivation of *N. atomus* in central Florida. Average productivities are shown ($n = 2 - 26$) with the error bars denoting standard deviation.

capital cost includes only materials, labor, and overhead costs for manufacturing the 1000 m^2 HBR units. It does not include site preparation or reactor installation, as these will vary widely depending on the end-user location. There is a limited availability of detailed and up-to-date large-scale algae production cost data in the literature, and high variability on costs is reported for similar systems (Davis et al., 2011; Norsker et al., 2011; Richardson et al., 2012; Rogers et al., 2014). A capital cost comparison between the HBR and common cultivation systems (open ponds and PBRs) for algal biomass production is summarized in Table 3. The reported capital cost of open ponds ranges from about $10,000 to almost $79,000 per hectare taking into account the costs of the liner and the paddlewheel. The cited cost for manufacturing the PBRs, regardless of tubular or flat panel design, was 4–9 times higher than the average cost of open ponds. Based on the above analysis the cost of the HBR is closer to that of open ponds and considerably lower than that of closed PBR systems. This difference in cost is mainly due to the use of inexpensive thin plastic film for constructing the HBR body compared to the thick plastic tubes or panels used in PBRs.

At the achieved average productivity of 18.2 g m^{-2} d^{-1} in the 65-L HBR prototype and 330 days of operation per year, the estimated capital cost for algal biomass production is \$0.42 kg^{-1} DCW, when taking into account the HBR plastic film and airlift units. Similar calculations were performed for the cultivation systems cited in the aforementioned literature (with average productivities of 15-25 g m^{-2} d^{-1}) as shown in Table 3. In general, the lower capital cost of open ponds translates into lower cost per produced kg DCW compared to the PBRs. However, wide differences in reported productivities lead to significant cost variability for both open ponds and PBRs. The projected capital cost per kg of produced algal biomass in the HBR is estimated to be 2–8 times lower than that of PBRs and comparable to that of open ponds. Unlike open ponds, however, no incidents of culture contamination or crashing were experienced with the HBR. It should be noted that the HBR technology, although promising for reducing algal production costs, is still at a small scale. Ongoing design improvements and scale-up studies will allow a detailed techno-economic analysis of algal biomass production in the HBR.

4. Conclusions

The development and long-term operation of a scalable and low-cost horizontal photobioreactor for microalgae production was presented. A 65-L floating HBR prototype was successfully deployed for cultivation of the marine strain *N. atomus*. Indoor experiments confirmed the HBR's ability to promote high-density algal cultivation. Outdoor semi-continuous operation, performed successfully over 165 days, resulted in high density and productivity without any contamination problems, thus validating the functionality of the HBR design under real-world conditions. Next, the novel bioreactor will be scaled up to test its performance with a variety of microalgae for biofuel and bioproduct manufacture.

Table 3
Comparison of reactor and produced algal mass capital costs for open ponds, PBRs, and the proposed HBR cultivation system.

Cultivation system	Reactor (per ha) and algal mass (per DCW) capital costs			Data referenced from
	Capital items included	\$ ha^{-1}	\$ kg^{-1} DCW	
Open pond	Liner, paddlewheel	76,132	1.39[a]	Rogers et al. (2014)
Open pond	Liner, paddlewheel	10,111	0.12[a]	Richardson et al. (2012)
Open pond	Pond	15,373	0.19[a]	Davis et al. (2011)
Open pond	Liner, paddlewheel	78,792	0.58[b]	Norsker et al. (2011)
Tubular PBR	Liner, pump, tube, blower	262,814	3.19[a]	Richardson et al. (2012)
Tubular PBR	PBR system	189,606	2.30[a]	Davis et al. (2011)
Tubular PBR	Pump, tube, blower	303,461	1.03[b]	Norsker et al. (2011)
Flat panel PBR	Panel, blower	422,759	0.92[b]	Norsker et al. (2011)
HBR	Film, airlift	25,000	0.42[a]	This study

[a] Calculated from reported algal biomass productivities per ha and reactor cost per hectare.
[b] Reported by the authors in € and converted to \$ based on an exchange rate of \$1.1 per €.

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