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DEVELOPMENT OF A SCALABLE CULTIVATION SYSTEM FOR SUSTAINABLE PRODUCTION OF ALGAL BIOFUELS

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ABSTRACT: Algae are a promising source of renewable jet and diesel fuels and can play a significant role in climate change mitigation as a low-carbon fuel source. Currently, most industrial microalgae cultivation systems are open ponds because of their low capital and operating costs, but they suffer from low biomass productivity and high risk of contamination. We describe the development of a novel, cost-effective, and modular horizontal bioreactor (HBR) for algae cultivation. It was designed and engineered to keep costs low, while minimizing water and energy use and enhancing CO_2 and nutrient uptake. The selected marine microalgal strain, *Nannochloris oculata*, has shown potential for biofuels production. Algal growth was first optimized indoors before testing the HBR performance outdoors under real-world conditions. The indoor study showed that urea and potassium nitrate yield comparable results, when used as nitrogen source, whereas ammonium chloride was less effective. Varying inoculum size from 10% to 15% to 20% (v/v) had no effect on lag time, so the lowest level was selected. The 150-L HBR prototype was tested outdoors with *N. oculata* using the indoor optimal conditions. High-density growth was consistently achieved in the HBR without contamination problems over extended periods of time outdoors in central Florida.
Keywords: microalgae, bioreactor, cultivation, biofuel, sustainability.

1 INTRODUCTION

The production of renewable transportation fuels has become an international priority in recent years, as the European aviation industry and the US and European transportation sectors seek ways to reduce carbon emissions and their reliance on imported fossil fuels. Algae are a promising source of renewable jet and diesel fuels and can play a significant role in climate change mitigation as a low-carbon fuel source. Many algal species grow readily in culture, double their mass within a few days, and grow sustainably using wastewater and CO_2 from industrial emissions [1-3]. Algal lipids and whole algae cells can then be converted to fuels, while algal proteins may serve as animal feed and fish meal, and algal carbohydrates may be used in nutraceuticals, thereby achieving life-cycle sustainability and profitability.

Currently, most industrial microalgae cultivation systems are open ponds because of their low capital and operating costs, but they often suffer from low biomass productivity and contamination issues [4]. Closed bioreactor systems can support higher biomass concentrations and productivity, but they often come at a considerably higher cost.

We describe a cost-effective and modular horizontal cultivation system for algal biofuel production developed by a US-EU public-private partnership. The horizontal bioreactor (HBR) was designed and engineered to keep costs low, while enhancing CO_2 and nutrient uptake by algae cells. A green microalgal species, *Nannochloris oculata*, was selected for demonstration of the HBR performance. *N. oculata* has shown potential for biofuel production based on growth rate, ability to grow readily in culture, and high lipid production [2, 5]. First, we ran a series of controlled indoor growth experiments to identify the appropriate inoculum size and nitrogen source. Then, we cultivated *N. oculata* in the HBR under real-world conditions in central Florida, USA and documented the bioreactor's performance.

2 MATERIALS AND METHODS

2.1 The Horizontal Bioreactor (HBR)

The HBR unit is an enclosed system constructed from low-density polyethylene (Fig. 1). It has a working volume of 150 L and a low depth of just 5 cm, which enables increased light exposure and minimal water use. Mixing of the culture was accomplished by an 8-blade paddlewheel at the center of the HBR. CO_2 was diffused directly through a high-efficiency ceramic micro-diffuser with a valve controlling its delivery based on a culture pH set-point of 7.50 ± 0.05. The unit was equipped with a pH sensor with automatic temperature compensation.

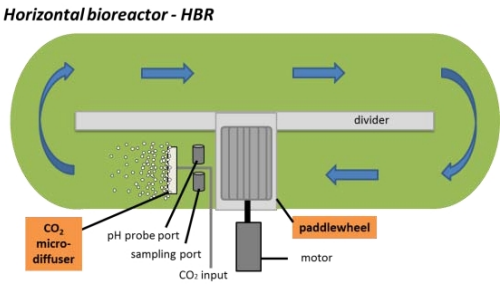

Figure 1: Schematic of the horizontal bioreactor for the production of algal biomass (top view). Mixing of the culture is facilitated by an 8-blade paddlewheel and CO_2 diffusion by a high-efficiency ceramic micro-diffuser.

2.2 Microorganism and growth conditions

The marine strain of green microalga *Nannochloris oculata* LB 1998 was selected to demonstrate the performance of the HBR. The strain was obtained from the UTEX Culture Collection of Algae at the University of Texas at Austin and grown in artificial seawater medium containing: (a) Instant Ocean at 35 $g \cdot L^{-1}$; (b) a nitrogen source providing 22.6 mM of N in the form of KNO_3, urea or NH_4Cl; (c) KH_2PO_4 at 0.129 $g \cdot L^{-1}$; (d)

$NaHCO_3$ at 0.42 g·L^{-1}; (e) trace metals mix II [6]; and (f) vitamin B12 (cyanocobalamin) at 0.5 mg·L^{-1}.

For the indoor experiments the inoculum culture was prepared in flasks maintained at 23°C in a rotary shaker operating at 150 rpm under continuous LED illumination. A 10% (v/v) inoculum was transferred from the flask cultures to bleach-sterilized vertical photobioreactors with a working volume of 3.5 or 7.5 L for growth optimization experiments. These flat-panel photobioreactors were bubbled with air enriched with CO_2 for culture mixing and carbon source provision. The pH was controlled at 7.50 ± 0.05 by automatically varying the CO_2-air mix through a controller. Samples were taken daily for growth measurement and nutrient monitoring. The flat-panel bioreactor was exposed to artificial LED light at approximately 10 *k*lux on a 16:8 hour light:dark cycle and was operated in an air-conditioned lab at 22°C.

Outdoor growth experiments were performed in a 150-L HBR prototype unit in central Florida. A 10% inoculum was transferred to the HBR from the indoor flat-panel photobioreactor after the culture reached a high density of OD_{680} 8.6, as described above. The pH was controlled at 7.50±0.05 by automatically regulating the CO_2 input. Samples were taken regularly for growth measurement and nutrient monitoring.

2.3 *N. oculata* indoor small-scale cultivation

Growth optimization experiments were conducted indoors in vertical flat-panel photobioreactors with working volume of 3.5 L in duplicate. The effect of inoculum size on *N. oculata* growth was studied by varying the inoculum concentration from 10% to 15% and 20% (v/v) in 3.5-L vertical photobioreactors in duplicate. Inoculum was provided from *N. oculata* cultivations in 7.5-L vertical photobioreactors, as described above.

The effect of the nitrogen source on the growth of *N. oculata* was investigated by comparing urea and ammonia (compounds often found in wastewaters) to potassium nitrate (control) in order to assess the suitability of wastewater as a potential inexpensive and plentiful nitrogen source. The 3 nitrogen sources (urea, ammonium chloride, and potassium nitrate) were added to the growth medium at levels that generated a final elemental nitrogen concentration of 22.6 mM. Based on *N. oculata* ammonium toxicity tests performed in our lab (data not shown), the NH_4Cl was not added at full strength to the reactors, but in 8 daily doses to minimize growth inhibition. In order to buffer pH changes expected from the addition of NH_4Cl and urea, 8mM of HEPES buffer was added by filter sterilization to all cultures.

2.4 Algae growth and nutrient analysis

Optical density (OD) was measured in duplicate at 680 nm using a spectrophotometer (DU 730, Beckman Coulter, USA). Cell concentration was measured in duplicate using an automated cell counter (Auto X4, Nexcelom, USA). Dry cell weight (DCW) was determined by filtering 5 mL of culture volume through pre-dried and pre-weighed 0.47 µm Whatman nylon filters and then by using a moisture analyzer (MB25, Ohaus, USA) at 100°C. For HBR samples, cell concentration (cells·mL^{-1}) was used as a proxy for daily DCW determination after establishing a strong correlation between cell concentration and DCW measurements (R^2=0.999, P<0.0001).

Nitrate and phosphate concentrations were measured in the sample filtrate (0.47 µm) using UV spectroscopy at 220 nm [7] and phosphate test kits (Hach, USA), respectively. Urea and ammonium chloride were measured in the sample filtrate (0.47 µm) using Total Nitrogen test kits (Hach, USA).

2.5 Statistical analysis

Data were analyzed using t-tests (nitrogen source) and analysis of variance, ANOVA (inoculum size).

3 RESULTS AND DISCUSSION

3.1 Growth optimization – effect of inoculum size

The marine microalgae strain *N. oculata* was selected to demonstrate the algae cultivation performance of the HBR. In order to ensure the strain was cultivated properly, a set of experiments was first conducted indoors in the lab to determine key cultivation parameters using vertical flat-panel photobioreactors with working volume of 3.5 or 7.5 L. Residual nitrogen (N) and phosphorus (P) concentrations in the medium were monitored, and N and P sources were supplied to ensure that algal growth was not nutrient-limited throughout the cultivations.

Three inoculum sizes, 10%, 15% and 20% (v/v), were tested to determine their effect on culture growth and lag time. Maximum optical density after 14 days was 12.6 ± 0.43, 10.8 ± 0.27, and 12.0 ± 0.16 for cultures provided with 10%, 15% and 20% inoculum, respectively (Fig. 2). There was deviation in OD after the 9th day between the two bioreactors that started with 15% inoculum. This was most likely due to a malfunction we experienced with the light source of one of these two bioreactors, which resulted in half the normal light output after the 9th day. Still, there were no significant differences between maximum optical density among inoculum sizes (pairwise t-tests not significant; P>0.05). Regarding the effect on lag phase, pairwise t-tests run for Days 1-5 showed no significant differences between the three inoculum sizes, indicating that culture lag time was not reduced, when the cultures started with more inoculum.

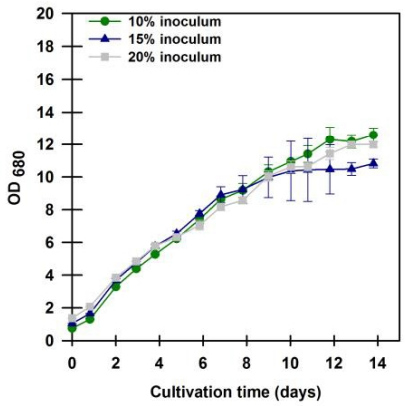

Figure 2: Effect of inoculum level on growth (OD_{680}) of *N. oculata* in 3.5-L vertical flat-panel photobioreactors. Error bars represent standard deviation between duplicate bioreactors. Light intensity was approximately 10 *k*lux on a 16:8 hour light:dark schedule.

Other researchers have reported that higher initial population size results in increased biomass production

and that the final dry weight of a culture is strongly affected by inoculum size, when using a *Nannochloropsis* sp. [8, 9]. However, based on the results from our inoculum size experiment, we determined that increasing inoculum size to 15% or 20% did not affect the growth significantly, nor it reduced culture lag time in *N. oculata*. Therefore, to trim the cost associated with larger inoculum size at large scale we used a 10% (v/v) level for subsequent indoor and outdoor *N. oculata* cultivations.

3.2 Growth optimization – effect of nitrogen source

Potassium nitrate, urea, and ammonium chloride were screened as nitrogen sources for the growth of *N. oculata* and supplied at the same elemental N concentration. As shown in Fig. 3, average maximum optical density at 680 nm was 14.9 ± 0.58 on Day 19 for KNO_3, 15.6 ± 0.65 on Day 19 for urea, and 7.7 ± 0.31 on Day 13 for NH_4Cl. Optical density varied between KNO_3 and NH_4Cl (t: 2.04; P: 0.05) and between urea and NH_4Cl (t: 2.12; P: 0.04), but did not differ between KNO_3 and urea (t: -0.29; P: 0.77). The cultures supplied with NH_4Cl as a nitrogen source reached stationary phase earlier, at about half the cultivation time compared to the cultures in the other two nitrogen sources, and no further growth was observed even after 14 days (Fig 3).

There were no significant differences in optical density between cultures grown on KNO_3 and urea; thus, urea from wastewater may well be a viable and sustainable source of nitrogen for algal cultures [4, 10]. NH_4Cl, on the other hand, exhibited some toxicity and/or resulted in dramatic decreases in culture pH to the extent that the cultures did not grow well. Eustance et al. [11] reported that two species of green algae were able to grow using ammonium as a nitrogen source, but required the use of synthetic buffers (PIPES and HEPES) or KOH dosing, when CO_2 was supplemented [11]. Using NH_4Cl as a nitrogen source from wastewater is possible [2, 11, 12], but additional research is needed to determine a means of adequately and cost-effectively buffering pH variations and reducing toxicity to the cultures.

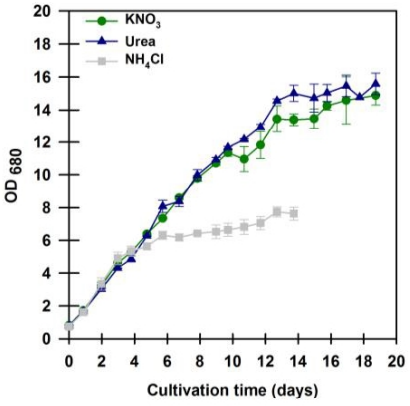

Figure 3: Effect of nitrogen source (KNO_3, urea or NH_4Cl) on growth (OD_{680}) of *N. oculata* in 3.5-L vertical flat-panel photobioreactors. Error bars represent standard deviation between duplicate bioreactors with the same nitrogen source. Light intensity was approximately 10 *k*lux, on a 16:8 hour light:dark schedule.

3.3 HBR operation and algae cultivation

The 150-L HBR prototype passed hydraulic testing, leak test, and wind and rain resistance tests. The enclosed reactor was designed with an 8-blade paddlewheel that was rotated by a low-speed motor. The paddlewheel was able to ensure adequate mixing with a very low energy consumption of just 5 W per 150 L of culture. The motor operated continously without any overheating issues.

The algae cultivation performance of the HBR was demonstrated by growing *N. oculata* over extended periods of time. A high-density (OD_{680} of 8.6) inoculum (10% v/v, 15 L) from the indoor flat-panel bioreactors was transferred to the HBR, which was operated outdoors under real-world conditions in central Florida for two consecutive cycles of 34 days each (Figs. 4 and 5). After the 1st growth cycle, 90% of the culture was harvested and the remaining 10% acted as inoculum for the 2nd cycle. The HBR facilitated highly reproducible algae growth. The 2nd growth cycle resulted in slightly higher algae productivities and biomass, probably due to algae adaptation to the outdoor environment.

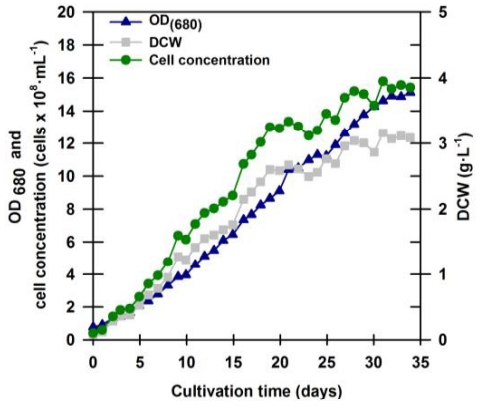

Figure 4: 1st cycle of cultivation of the marine microalga *N. oculata* in the 150-L HBR. Growth parameters (OD_{680}, DCW, and cell concentration) are shown. At the end of the cultivation, 90% (v/v) was harvested and the residual 10% was used as inoculum for the 2nd cycle.

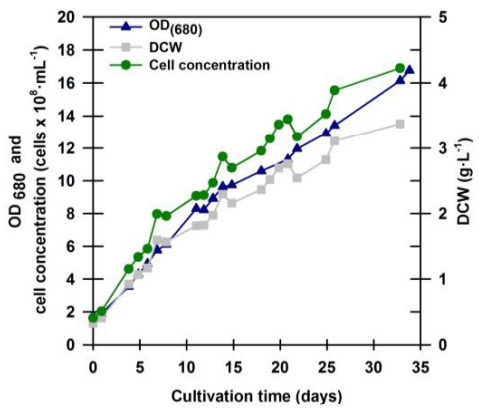

Figure 5: 2nd cycle of cultivation of the marine microalga *N. oculata* in the 150-L HBR. Growth parameters (OD_{680}, DCW, and cell concentration) are shown. The 2nd cultivation cycle was initiated with the use of a 10% inoculum from the 1st cycle of the same HBR.

The culture was supplemented with nutrients (nitrate and phosphate) as the cultivation progressed to prevent nutrient limitations and hence a drop in biomass productivity. The maximum culture OD_{680} observed was

16.8 after 34 days of cultivation. The average productivity was 7.9 $g \cdot m^{-2} \cdot d^{-1}$ or 0.16 $g \cdot L^{-1} \cdot d^{-1}$ and resulted in a significant final cell concentration of 3.5 $g \cdot L^{-1}$ on a dry basis (Table I).

Table I: Algae biomass concentration and productivity achieved with *N. oculata* in the 150-L HBR during outdoor cultivation cycles.

	1st cycle	2nd cycle
Cultivation time (days)	34	34
Average areal productivity ($g \cdot m^{-2} \cdot d^{-1}$)	7.17	7.90
Average volumetric productivity ($g \cdot L^{-1} \cdot d^{-1}$)	0.14	0.16
Max biomass concentration ($g \cdot L^{-1}$)	3.16	3.50

The maximum cell concentration achieved was $1.75 \cdot 10^9$ cells$\cdot mL^{-1}$. Such high-density algae growth was achieved without any contamination issues over long periods of time. The enclosed design of the HBR reduces considerably the chances of contamination compared to open systems.

4 CONCLUSIONS

We designed and successfully operated a novel low-cost modular horizontal bioreactor (HBR) for the cultivation of microalgae for biofuel production. High-density growth of the marine green algae *N. oculata* was achieved in the HBR without any contamination issues over long periods of time outdoors under real-world conditions. Algae growth was highly reproducible in consecutive cultivation cycles (semi-continuous mode) and resulted in high algae productivity and final concentration, while reducing cost and water and energy use significantly compared to convention systems.

The HBR runs were preceded by indoor studies of *N. oculata* to optimize key growth parameters, such as nitrogen source and inoculum level, which were subsequently applied outdoors. Urea, a commercial and rather inexpensive source of nitrogen, matched the performance of the costlier potassium nitrate and could therefore serve as nitrogen source in commercial HBR operations. On the other hand, ammonia was found to be toxic and therefore its usefulness as a nitrogen source (e.g. in wastewaters) for cultivating algae may be limited. Increasing the inoculum size by 50 or 100% did not result in higher growth yields and did not reduce culture lag time. Therefore, using a small inoculum (10%), which leads to higher throughput at commercial scale, is recommended.

We continue our research on HBR design and algal growth optimization. Through algae adaptation and longer operating experience we expect the HBR to achieve even higher productivity. Most importantly, the HBR has the potential to make algae cultivation more sustainable and cost-effective and could hence advance the potential of algal biofuels as a renewable energy source for the transportation sector.

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