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Reduction of water and energy requirement of algae cultivation using an algae biofilm photobioreactor

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ABSTRACT

This paper reports the construction and performance of an algae biofilm photobioreactor that offers a significant reduction of the energy and water requirements of cultivation. The green alga *Botryococcus braunii* was cultivated as a biofilm. The system achieved a direct biomass harvest concentration of 96.4 kg/m^3 with a total lipid content 26.8% by dry weight and a productivity of 0.71 g/m^2 day, representing a light to biomass energy conversion efficiency of 2.02%. Moreover, it reduced the volume of water required to cultivate a kilogram of algal biomass by 45% and reduced the dewatering energy requirement by 99.7% compared to open ponds. Finally, the net energy ratio of the cultivation was 6.00 including dewatering. The current issues of this novel photobioreactor are also identified to further improve the system productivity and scaleup.

1. Introduction

Cultivation of algae is a promising method for producing renewable hydrocarbon feedstock for biofuel production as (i) select algae species can produce about two orders of magnitude more oil per acre than from soybeans, (ii) algae cultivation does not require arable land, and (iii) can use marginal sources of water not suitable for drinking or irrigation (Chisti, 2007). However, a cost effective algae cultivation technology that can be scaled up to sizes large enough to make a significant contribution in reducing our dependence on foreign oil has yet to be realized (Chisti, 2007; Molina Grima et al., 2003). In part, this stems from cultivation of dilute biomass concentrations in conventional systems, such as raceway ponds as well as flat plate and tubular photobioreactors (PBRs), where algae cells are suspended in the liquid phase (Pulz, 2001). These technologies require (i) in excess of 6000 gallons of water to cultivate 1 gallon of algae oil, (ii) a large amount of energy for pumping and circulating a dilute algae suspension as large as 385.71 MJ/kg of cultivated algae, and (iii) energy intensive dewatering and biomass concentration processes for downstream use of the biomass resulting in energy requirements of up to 82 MJ/kg algae biomass produced (Jorquera et al., 2010; Chisti, 2007; Molina Grima et al., 2003; Uduman et al., 2010). To address these challenges, this study reports the design, operation, and performance of a novel photobioreactor based on algal biofilm cultivation that reduces the water and energy requirements of algae cultivation for economic and sustainable biofuel production.

2. Current state of knowledge

2.1. Current algae cultivation technologies

The type of system used for cultivating algae depends on the requirements of the organism being cultivated and on the nature of the product being harvested. Most current technologies cultivate the algae as planktonic cells, suspended in liquid nutrient media. These include open systems such as raceway ponds and closed systems such as flat panel and tubular PBRs which are being used for high value products such as β-carotene, astaxanthin, and C-phycocyanin which have prices ranging from $310 to $10,000/kg (Brennan and Owende, 2010). In the case of biofuel production, strict limitations on the cost as well as the energy and water requirements are imposed due to (i) low value of biofuel as a product, (ii) biofuel production requiring larger than unity net energy ratio (NER), and (iii) potentially large scale of operation.

Raceway ponds are constructed as artificial ponds having a depth of about 0.3 m (Chisti, 2007). The algae cultivated in these ponds are kept suspended through continuous agitation with a paddlewheel (Chisti, 2007). The main advantage of these systems is that they are relatively inexpensive to build and operate. In raceway ponds, the maximum biomass concentration ranges from 0.1

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Nomenclature

A_s	cultivation surface area (m^2)
G_{in}	the incident light energy (W/m^2)
t_b	algal biofilm thickness (m)
V_H	volume of the direct algal biomass harvest (m^3)
E_{aux}	auxiliary energy input for pumping and dewatering (MJ)
E_B	heating value of algal biomass (MJ/kg)
E_L	heating value of algal neutral lipids (MJ/kg)
R_A	areal biomass productivity (kg/m^2-day)
W_{dry}	total dry weight of biomass harvested (kg)
W_{net}	net dry weight of biomass produced (kg)
X_H	density of direct algal biomass harvest (kg/m^3)
x_L	the mass fraction of neutral lipids in the biomass

Greek symbols

Δt	total duration of experiments (days)
η_B	light to biomass energy conversion efficiency (%)
η_L	light to lipid energy conversion efficiency (%)

to 0.5 kg/m^3, while photosynthetic efficiency can range from 1% to 4% (Borowitzka, 1999; Stephens et al., 2010). Moreover, areal biomass productivities ranging from 4 to 21 g/m^2-day have been reported for raceway ponds (Hase et al., 2000). Due to low biomass concentration, these photobioreactors require large footprint areas and large volumes of water (Chisti, 2007). Moreover, they require energy intensive harvesting and dewatering processes necessary for downstream processing of algae in the biorefinery (Chisti, 2007). According to Molina Grima et al. (2003) the harvesting costs associated with algae biomass production is on the order of 20–30% of the total cost. Another difficulty associated with open systems is the loss of water through evaporation. Evaporation losses as high as 10 L/m^2-day, amounting to about 410 kg water loss through evaporation per kg of algal biodiesel produced have been reported for raceway ponds (Sheehan et al., 1998). Although evaporation helps in buffering the temperature of the system, large volumes of fresh water has to be supplied to the ponds to keep the water chemistry and nutrient balance under control for maximum productivity. Furthermore, increased use of fresh water not only increases the water intensity but also increases the auxiliary energy input for biofuel production.

To address some of these limitations, closed systems have been developed. The most common types of closed photobioreactors reported in the literature are tubular and flat panel types. It is also possible to find photobioreactors which have adopted different fermenter designs in research stage. Biomass concentrations in the range from 2 to 8 kg/m^3 are attainable in closed photobioreactors (Pulz, 2001). Areal biomass productivities ranging from 13 to 47.7 g/m^2-day, and from 10.2 to 22.8 g/m^2-day have been reported for tubular and flat panel reactors, respectively (Brennan and Owende, 2010). Moreover, photosynthetic efficiencies ranging from 1.3% to 6.9% have also been reported for these reactors under outdoor conditions (Eriksen, 2008). However, these systems are much more expensive to build and operate than open pond systems and require large amount of auxiliary energy input for cultivation of algae (Jorquera et al., 2010). For instance in tubular PBRs, typical pumping energy inputs on the order of 350–400 MJ/kg of algae biomass is required (Jorquera et al., 2010; Morweiser et al., 2010). Flat panel reactors use gas flow for mixing within the system to ensure high mass transfer (Morweiser et al., 2010). Although this high mass transfer increases the biomass productivity, an air pumping energy of 15–20 MJ is required per kg of algae biomass output which is about 15 times more than the energy required for mixing in open ponds (Jorquera et al., 2010; Morweiser et al., 2010). A technical report by U.S. Department of Energy indicated that the capital cost associated with closed photobioreactors were 2–10 times larger than that of open ponds which was about $\$20/m^2$ (Benemann and Oswald, 1996). Moreover, it was reported that the production cost of algae ranged from \$8 to \$15/kg dry algae biomass in open ponds whereas it was as large as \$50/kg algae biomass in closed photobioreactors due to large operating costs (Lee, 2001; Borowitzka, 1999).

2.2. Research on immobilized algae cultivation systems

Cultivation of algae as benthic systems where cells are immobilized on surfaces has been attracting attention as these systems offer the potential to lower the energy and water requirements. Several research efforts can be found in the literature investigating immobilized algae cultivation systems in biofuel production as well as in environmental remediation applications. Akin et al. (1993) studied carbon dioxide sequestration using the green algae *Botryococcus braunii* immobilized on agar surfaces. Using 10% by volume CO_2 enriched air and 0.24 mol/L $NaHCO_3$ as the carbon sources, oil contents ranging from 15% to 47% by dry cell weight were achieved. However, the authors did not report or compare the biomass production rates of suspended and immobilized cultivation systems. Bailliez et al. (1985) reported an increase as large as 23% in lipid production of *B. braunii* cells immobilized in calcium alginate gels compared to cells suspended in liquid media. However, biomass production rate was decreased by 21% in the log phase of growth. The decrease in biomass production was attributed to (i) a switch in metabolic activity from growth to hydrocarbon production as well as (ii) to steric stress on the encapsulated cells.

Moreover, a membrane photobioreactor was developed to mitigate CO_2 emissions from a fossil-fired power plant using a thermophilic cyanobacteria species (Kremer et al., 2006). The photobioreactor system consisted of vertically hung membranes contained in a closed chamber that were illuminated by optical fibers delivering light from solar collectors. Carbon dioxide from stacks was supplied to the chamber while the nutrient medium was delivered to the membranes using a drip system. The cyanobacteria grew on the membranes as immobilized cells and were washed off at the time of harvest. Biomass productivities up to 55 g/m^2-day were achieved with simulated flue gas emissions (Kremer et al., 2006). However, this cyanobacteria was not capable of accumulating appreciable amount of lipids. The economic analysis of the system indicated that the sophisticated solar collection system utilized made this reactor economically unfeasible either for carbon dioxide sequestration or for biofuel production.

In addition, algae biofilms have also been applied for removal and recovery of nutrients from wastewater. Craggs et al. (1997) studied removal of ammonium and orthophosphate from primary sewage effluent using monocultures of the diatom *Phaeodactylum tricornutum* and the cyanobacteria *Oscillatoria* sp. grown attached over polyethylene corrugated raceways. Removal efficiencies of 100% were achieved for both ammonium and orthophosphate with both species using seawater diluted wastewater in the ratio of 1:1 by volume. Mono-cultures were maintained throughout the experiments which lasted for 4 months for both species (Craggs et al., 1997). Also, algal turf scrubbers (ATSs) were investigated at both lab and pilot scale for recovery of dairy manure nutrients to produce protein supplements for farm animals (Mulbry et al., 2008). The ATS consisted of black polyethylene mesh as the support for attached growth of mainly filamentous microalgae. A tipping

bucket was used to flush the wastewater over the algae mat to increase the mass transport of nutrients into the biomats. These systems were not inoculated with any specific strain and local mix cultures of filamentous algae dominated the algal mats. Biomass productivity of up to 25 g/m^2-day were reported with a pilot scale unit operated under out-door conditions (Mulbry et al., 2008).

Johnson and Wen (2010) investigated the use of *Chlorella* sp. biofilms to produce biofuels. The algae were cultivated on polystyrene foam immersed in dairy manure wastewater and agitated with a rocking shaker. The authors reported biomass yield as large as 25.65 g/m^2 and biomass productivity of 2.57 g/m^2-day. This study gave encouraging results for the use of algal biofilms in biofuel production. However, it did not provide any energy or water use analysis of this method or offer a photobioreactor system that can potentially be implemented outdoors and scaled up.

Cao et al. (2009) proposed the use of floating conveyor belts made out of laser textured stainless steel for cultivation of algal biofilms. The authors reported better attachment of *Scenedesmus dimorphus* on microdimpled stainless steel surfaces compared to non-textured ones.

In a more recent study, Christenson and Sims (in press) developed an algal biofilm reactor using cotton cords as immobilization surfaces and reported the operation under both indoor and outdoor conditions. The system consisted of cotton cords wrapped around rotating drums that were partly submerged in wastewater. The authors reported a biomass production rate of 5.5 g/m^2-day for bench scale tests. Moreover, biomass and fatty acid methyl ester productivities of up to 31 and 2.5 g/m^2-day were reported, respectively, for pilot scale tests under outdoor conditions. The system developed had a positive energy balance with a net energy output of up to 6.3 W/m^2. Finally, the system had a concentrated biomass harvest with a solid content ranging from 12% to 16%.

Development of an inexpensive, efficient, and scaleable photobioreactor system based on immobilized algae cultivation can significantly reduce the energy and water requirements of the process, bringing algae based biofuel production closer to being energetically, environmentally, and economically viable technology. This paper presents the design and performance of an algal biofilm based photobioreactor that addresses these issues. It provides a detailed analysis of the energy and water requirements of the system, identifies its key advantages and as well as shortcomings requiring further research and development.

3. Methods

3.1. Inoculum preparation

In this study the green algae *B. braunii* (LB 572) was employed due to its capability to produce biofilms and capacity to accumulate hydrocarbons (Chisti, 2007). The strain was obtained from UTEX culture collection at the University of Texas at Austin. The inoculum was cultivated in the autotrophic nutrient medium BG-11 due to its superiority over BBM, BBMa, and modified Chu13 media in terms of biomass and lipid productivity (Dayananda et al., 2007). The culture was continuously sparged with air containing 1% by volume CO_2 and illuminated with fluorescent light bulbs (Home Light Cool White Plus, Philips, The Netherlands) at 100 μE/m^2-s (21 W/m^2) in the photosynthetically active radiation (PAR) measured with a quantum sensor (Li-COR, Model Li-190SL; LICOR Inc., Lincoln, NE, USA).

3.2. Photobioreactor construction and operation

Fig. 1 shows the schematic of the algae biofilm photobioreactor. The system consists of (i) a biofilm growth surface, (ii) a nutrient medium recirculation system, and (iii) an illumination system. The biofilm growth surface was an 8 mm thick concrete layer (Commercial Grade Quikrete Quick Settling Cement, ATL, USA) over a wood support plate and had an active cultivation area of 0.275 m^2. The initial pH of the concrete was 13 which served to sterilize the immobilization surface. In order to decrease the surface pH to a more favorable level for algal growth, the concrete surface was carbonated with 1.9 M $NaHCO_3$ solution. At the completion of the carbonation the surface pH was stabilized at 8.3.

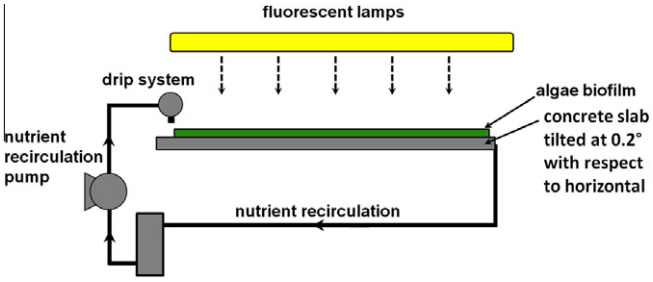

Fig. 1. Schematic of the algae biofilm photobioreactor system.

The nutrient medium BG-11 was delivered by dripping nozzles (Adjustable Dripper, DIG Irrigation Products, CA, USA) located above the concrete surface at a total rate of 150 mL/min. The growth surface was tilted by 0.2° with respect to the horizontal to enable the flow of nutrient medium over the algae biofilm by gravity. At the end of the growth surface the nutrient medium was collected and delivered to the reservoir by gravity. Finally, the medium was pumped to the dripping system located 3.6 cm above the reservoir using a peristaltic pump (Master Flex L/S 7524–40 and HV-0701452, Cole-Parmer Instrument Company, IL, USA). During the operation of the photobioreactor, the liquid volume over the growth surface as well as the liquid volume in the rest of the system was 300 mL each giving a total volume of 600 mL. The growth surface was illuminated with four 32 W fluorescent lamps (Philips, Home Light Cool White Plus, Netherlands) providing 55 ± 3 μE/m^2-s (11.55 ± 0.63 W/m^2) irradiation in the PAR measured with a quantum sensor (Li-190SL, Li-COR Inc., NE, USA). Spectral irradiance of the fluorescent bulbs was measured using a lock-in amplifier (SR830, Stanford Research Systems, CA, USA) and a monochromator (Cornerstone 260, Newport, CA, USA). The spectrum indicated that the fluorescent bulbs had major emission peaks at 435, 490, 545, 585, and 615 nm in PAR.

The carbonated concrete surface was inoculated with 500 mL of *B. braunii* culture during its exponential growth phase at a concentration of 0.50 kg/m^3 giving an initial inoculation of 0.90 ± 0.01 g/m^2. In order to promote cell attachment, nutrient media was not circulated for 5 days and the cells were allowed to settle and attach on concrete. The biofilm photobioreactor was operated for 35 days under continuous illumination. Moreover, samples from the biofilm were taken at regular intervals and observed under optical microscope (Eclipse 80i, Nikon, Japan) to check for contamination by other species. Over the course of experiments no contamination of the culture was observed.

3.3. Biofilm thickness, direct harvest biomass density measurement and areal biomass productivity

At the end of the 35th day, the algae biofilm was divided into 9 subsections and biomass was harvested from the concrete surface by gentle mechanical scraping with a squeegee. This method does not incorporate any contaminating chemicals, such as for flocculants, thus eliminates any additional purification process. The biomass and lipid productivities of each area was determined individually to evaluate the variation in performance across the

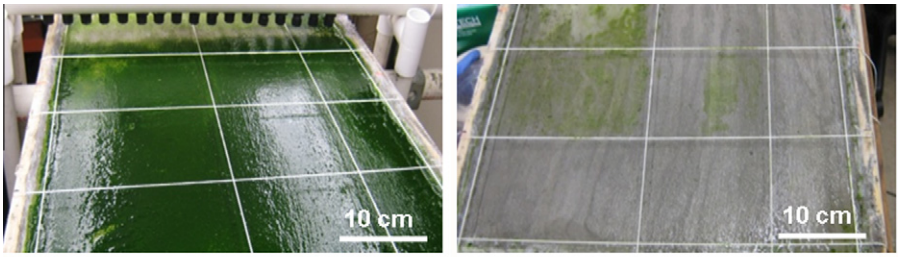

Fig. 2. (a) Biofilm photobioreactor cultivating algae, (b) biofilm photobioreactor after harvesting the algal biofilm.

bioreactor. Fig. 2 shows the picture of the biofilm photobioreactor before and after harvest. First the volume of the direct harvest V_H was quantified using a pipette. Using the total volume of the harvest and the cultivation surface area A_S the algal biofilm thickness t_b was estimated as $t_b = V_H/A_S$. Then, the biomass was dried in preweighed aluminum weighing boats at 60 °C in a vacuum oven (Isotemp Vacuum Oven, Model 280A, Fisher Scientific, NH, USA). The dry biomass was weighed using an analytical scale (Model AB135-S/FACT, Mettler Toledo, Switzerland) over time to ensure the weight did not vary. Using the dry weight W_{dry}, the biomass density of the direct harvest X_H was estimated as $X_H = W_{dry}/V_H$.

Moreover, the areal productivity R_A was estimated as the ratio of the net dry biomass weight produced over the total cultivation surface area, W_{net} over the entire cultivation period Δt as, $R_A = W_{net}/(A_S \cdot \Delta t)$.

3.4. Lipid extraction and analysis

To determine the neutral lipid content of the cultivated algae, part of the dried biomass was weighted and homogenized in mortar and pestle with gas chromatography grade *n*-hexane (H307-4, Fisher Chemical, PA, USA) for 15 min and the supernatant obtained from centrifugation was dried under nitrogen flow according to Rao et al. (2007). The weight of the extracted lipids were determined gravimetrically using the analytical balance to obtain the weight fraction of neutral lipids x_L. In addition, using the remaining part of the biomass total lipids, both polar and nonpolar, were extracted by Folch method and quantified gravimetrically.

3.5. Light to biomass and neutral lipid energy conversion efficiency

The light to biomass energy conversion efficiency η_B is computed according to,

$$\eta_B = \frac{W_{net} E_B}{G_{in} A_S \Delta t} \quad (1)$$

where E_B is the heating value of the dry biomass equal to 28.3 MJ/kg dry weight for *B. braunii* (USDOE, 1984), G_{in} is the irradiation, and Δt is the total duration of the experiment. Finally, the light to neutral lipid energy conversion efficiency η_L of the system was estimated as,

$$\eta_L = \frac{x_L W_{net} E_L}{G_{in} A_S \Delta t} \quad (2)$$

where x_L is the mass fraction of neutral lipids in the biomass, and E_L is the heating value of the algal lipids equal to 37.5 MJ/kg (Sa et al., 2000).

3.6. Net energy ratio

The net energy ratio of the photobioreactor system was quantified according to

$$NER = \frac{W_{net} E_B}{E_{aux}} \quad (3)$$

where E_{aux} is the auxiliary energy input associated with pumping and dewatering and does not include energy associated with the light input as ultimately sun is envisioned as the light source.

4. Results and discussion

Table 1 summarizes the key system parameters obtained for cultivating *B. braunii* in the algal biofilm photobioreactor. It indicates that the algal biofilm grew to a thickness of 278 ± 21 μm over the 35 day cultivation period yielding a net dry biomass yield of 24.94 ± 2.07 g/m^2. This corresponds to a light to biomass energy conversion efficiency of 2.02 ± 0.17%. Moreover, the direct harvest from the biofilm photobioreactor yielded a biomass concentration of 96.4 ± 6.8 kg/m^3. Based on the lipid extraction and analysis, 9.81 ± 0.81% of the dry biomass was neutral lipids which corresponds to a light to neutral lipid energy conversion efficiency of 0.26 ± 0.03%. In addition, based on Folch extraction, the total lipid content of the biomass was 26.80 ± 2.05% by weight. Finally, during the operation of the photobioreactor, a total of 32.29 ± 0.47 kJ of energy was used in the nutrient recirculation, and evaporative loss rate was 1.09 ± 0.05 L/m^2-day for the system.

Based on these results the productivity, energy and water use of the biofilm photobioreactor were estimated. Table 2 summarizes these results and compares them with those of a raceway pond, flat-plate and tubular photobioreactors. Due to lack of consistent set of data for these photobioreactors cultivating *B. braunii*, the comparison is given with respect to systems cultivating *Nannochloropsis* sp. reported by Jorquera et al. (2010). The results indicate that the areal productivity of the biofilm photobioreactor was 0.71 ± 0.06 g/m^2-day which was 15 and 35 times less than that of an open pond and closed photobioreactor cultivating *Nannochloropsis*, respectively, and about one quarter of the highest areal biomass productivity reported for *B. braunii* cultivated in suspension at a lab setting (Jorquera et al., 2010; Mata et al., 2010). The reasons for the observed low areal productivity can be attributed to (i) *B. braunii* being a notoriously slow grower with a doubling time ranging from 40 h to 6 days compared with *Nannochloropsis* sp. having a doubling time of about 29 h (Grifiths and Harrison, 2009) (ii) to low irradiation used in this experimental system having a magnitude of only 11.55 W/m^2 compared with the outdoor systems cited receiving daily irradiation of 109.5 W/m^2, and (iii) to mass transport limitations of the biofilm (Vasudevan and Briggs, 2008). Indeed, the light to biomass energy conversion efficiency of the algal biofilm photobioreactor in this study was 2.02 ± 0.17% based on PAR which was comparable with those of other systems. For instance, areal biomass productivities corresponding to 2% solar energy conversion efficiency under outdoor conditions are on the order of 30–40 g/m^2-day (Richmond, 1992). It should be noted that photosynthesis is light limited at low and light inhibited at large intensities (Chisti, 2007). The incident light intensity and

Table 1
Parameters on start up, cultivation and harvest of the biofilm.

Parameter	Value and uncertainty
Irradiance (W/m^2)	11.55 ± 0.63
Ambient temperature (°C)	25 ± 1
Total liquid volume of the system (L)	0.60 ± 0.01
Total area of the system (m^2)	0.275 ± 0.010
Total duration of the experiment (days)	35.0 ± 0.5
Evaporative loss rate (L/m^2 day)	1.09 ± 0.05
Total pump energy used (70% pump efficiency) (kJ)	32.29 ± 0.47
Net biomass yield (g/m^2)	24.94 ± 2.07
Total lipid fraction of biomass (Folch method) (%)	26.80 ± 2.05
Biomass thickness at the time of the harvest (μm)	278 ± 21
Initial biomass inoculation (g/m^2)	0.90 ± 0.01

spectral quality are critically important for the cultivation of algal biofilms as (i) biomass productivity is directly proportional to the rate of photosynthesis and (ii) the light intensity is attenuated exponentially across the biofilm (Kuhl et al., 1996; Stevenson et al., 1996). Indeed the intensity of light can decrease by two orders of magnitude within a photosynthetic biofilm thickness of 1 mm (Kuhl et al., 1996). Due to this gradient at high irradiance while the cells on top of the biofilm are photoinhibited, the cells at the subsurface can be exposed to optimum or limited irradiances based on thickness of the biofilm (Stevenson et al., 1996; Dodds et al., 1999). Thus, when the whole biofilm community is considered irradiances much larger than typically used in planktonic systems can result in increase in the total rate of photosynthesis (Stevenson et al., 1996; Dodds et al., 1999). For instance, Dodds et al. (1999) studied the rate of photosynthesis of a mixed culture algal biofilm collected from a river and reported increase in overall photosynthesis rate with increasing irradiance of up to 6000 $\mu E/m^2$-s (1260 W/m^2). For comparison it should be noted that solar irradiance goes up to about 2000 $\mu E/m^2$-s (420 W/m^2) at noon time. Thus it is expected that under larger irradiance, the algal biofilm photobioreactor productivity can be further increased. To circumvent any possible photo-oxidative damage to the cells, the cultivation surface can be corrugated to work with reflected light over larger surface area rather than with direct irradiation. This strategy will not only minimize photoinhibition, but also (i) increase the surface to volume ratio for O_2 desorption and (ii) increase the biofilm growth area per reactor footprint increasing areal productivity.

One of the reasons responsible for lowering the productivity of the current system compared to suspended systems is the mass transport limitation. Due to no slip condition for fluid flow over the biofilm surface hydrodynamic and concentration boundary layers form (Stevenson et al., 1996). These boundary layers reduce the nutrient flux from the liquid phase to the biofilm resulting in diffusion dominated nutrient transport (Stevenson et al., 1996). To increase the mass transfer of nutrients the bulk flow velocity of nutrient solution and concentration of its constituents can be increased. Indeed, increases in biological activity and growth rate have been reported for increased flow velocities and nutrient concentrations in mixed culture benthic algae (Stevenson et al., 1996).

Temperature is another critical parameter significantly affecting the biomass productivity of algae cultivating systems. The thermal effects including culture temperature and evaporative losses in algae bioreactor has recently been reported by Murphy and Berberoglu (2012). The authors reported that biofilm photobioreactors are more prone to culture temperature fluctuations as these systems contain significantly smaller quantities of water than planktonic systems which help buffering the system temperature (Murphy and Berberoglu, 2012). In order to reduce these fluctuations, the authors suggested using selective covers that are transparent in PAR while opaque in infrared radiation or increasing thermal capacitance of the system.

Based on the neutral lipid fraction of the biomass the areal lipid productivity was estimated to be 277 ± 32 L/ha-year, corresponding to a light to lipid energy conversion efficiency of 0.26 ± 0.03%. The reason behind the low productivity is the combination of low biomass productivity combined with relatively low neutral lipid content of *B. braunii*. Thus, in future systems a strain capable of benthic growth with higher biomass and neutral lipid productivities should be investigated. Moreover, nitrogen starvation and other nutrient stresses can more easily be imposed in the benthic system to further increase the lipid productivity as the biomass is decoupled from the nutrient medium.

The water requirement of the biofilm photobioreactor was 1618 L/kg biomass produced. This is encouraging when compared to that of an open pond system which is 2857 L/kg (Jorquera et al., 2010). In the biofilm system more than 95% of the water requirement was due to the evaporative losses. This can be attributed to the large surface to volume ratio of these photobioreactors which is on the order of 550 m^2/m^3 compared to open ponds which have 6.67 m^2/m^3 (Pulz, 2001). While evaporation is undesirable as it increases the fresh water and auxiliary energy inputs for biofuel production, it is critical for buffering the temperature of the system. In order to decrease the evaporative losses and maintain optimal temperature ranges within the system, the PBR can be closed with a transparent film capable of blocking infrared radiation.

Finally, the biofilm photobioreactor required 4.71 ± 0.62 MJ of energy per kg dry biomass produced. This corresponds to a net energy ratio (NER) of 6.01 ± 0.49. To bring this achievement in

Table 2
Comparison of the biomass concentration, neutral lipid content, areal biomass productivity, energy requirement of cultivation, net energy ratio (NER) on biomass, and NER on biomass including dewatering of the biofilm photobioreactor used in the current study compared with those of raceway ponds, flat plate and tubular PBR reported in the literature.

	Raceway Ponds	Flat-plate PBR	Tubular PBR	Biofilm PBR[f]
Biomass concentration (g/L)	0.35[a]	2.7[a]	1.02[a]	96.4 ± 6.8
Neutral lipid content (%)	29.6[b]	29.6[b]	29.6[b]	9.81 ± 0.81
Areal biomass productivity (g/m^2 day)	11[a]	27[a]	25[a]	0.71 ± 0.06
Energy input to produce one kg biomass (MJ/kg)	9.18[a,c]	16.96[a,c]	385.71[a,c]	4.71 ± 0.62
NER on biomass (direct harvest)	3.44[a]	1.86[a]	0.08[a]	6.01 ± 0.49
NER on biomass including dewatering with tangential flow filtration to prepare an algae cake ready for lipid extraction[c]	1.06 [d]	1.61[d]	0.08 [d]	6.00 ± 0.56[e]

[a] Based on data compiled by Jorquera et al. (2010).
[b] Based on lipid content reported by Rodolfi et al. (2009).
[c] Based on energy requirement for 24 h of daily pumping within the system as suggested by Chisti (2007).
[d] Based on Uduman et al. (2010) for calculation of dewatering energy requirement.
[e] Based on an uncertainty of ±10% for biomass dewatering energy requirement.
[f] Results obtained in this study.

perspective let us consider the energy requirements of raceway ponds, flat plates and tubular photobioreactors which are 9.18, 16.96, and 385.71 MJ/kg, respectively, corresponding to a NER of 3.44, 1.86, and 0.08, respectively, based on 24 h of daily pumping (Jorquera et al., 2010; Chisti, 2007). It should be noted that these NER values only take into account the energy required for biomass cultivation and exclude those of harvesting. The direct algal biomass harvest from the biofilm photobioreactor in this study yielded a concentration of 96.4 kg/m^3, which is about 275, 35 and 95 times more concentrated than the biomass concentration of the direct harvest from a raceway pond, flat plate and tubular photobioreactor systems, respectively (Jorquera et al., 2010). For downstream processing algae biomass harvest need to be concentrated to an algae cake with a solid content of 15–25% by weight (Uduman et al., 2010). For this tangential flow filtration or centrifugation are being used which require an energy input of 2 and 8 kWh/m^3 of algae suspension processed, respectively (Uduman et al., 2010). To produce a kilogram of algae cake using tangential flow filtration, 2.86, 0.37, 0.98 m^3 of suspension has to be treated requiring an energy input of 21, 2.7, 7.1 MJ for raceway ponds, flat plate and tubular photobioreactors, respectively. On the other hand, the dewatering of the biomass harvested from the biofilm PBR requires only 0.075 MJ of additional energy which is about 0.3% of the dewatering energy requirement of the raceway pond harvests. When filtration based harvesting and dewatering are included in the system boundaries of NER calculation, the NER of raceway ponds, flat plate and tubular photobioreactors, decrease to about 1.06, 1.61, and 0.08, respectively, for algal biomass production. Thus, open ponds and tubular photobioreactors consumes more or similar amounts of energy compared to the energy output from these systems. However, even with the additional harvest energy requirement, the NER for the biofilm photobioreactor is equal to 6.00 ± 0.56. These results indicate that even at a biomass productivity of 0.7 g/m^2-day attained with the current system, the net energy output is equal to that from an open pond with a productivity of 9.3 g/m^2-day indicating the importance of biomass harvest concentrations.

Finally, algal biofilm photobioreactors are not mature technologies but hold potential to be further developed for achieving energy and water efficient algae cultivation targeted for biofuel production. To optimize the productivity and performance of these systems (i) light, mass, and thermal energy transport in these systems should be investigated to identify and mitigate the major bottlenecks through system design, (ii) species capable of benthic growth that have higher biomass and lipid productivities should be identified and incorporated, (iii) biofilm photobioreactors with proper corrugation, maximizing photon use, should be designed, and (iv) strategies for minimizing evaporative losses and contamination should be developed. Moreover, to bring these systems closer to practical implementation, pilot scale biofilm photobioreactors should be tested under outdoor conditions, and life cycle analysis of these systems should be conducted for assessing their energy, economic, and environmental impacts.

5. Conclusion

The novel algae biofilm photobioreactor reported in this study was capable of producing direct algal harvest density of 96.4 kg/m^3 which is more than 35 times more concentrated than the largest reported direct harvest, making the downstream process integration easier and less energy intensive. Moreover, the system achieved a net energy ratio of 6.00 while that of open ponds was 1.06. Also, the light to biomass conversion efficiency was 2.02%, comparable with that of planktonic systems. Finally, the system is open for further improvement through research on thermal management, mass and light transfer optimization as well as algal species selection.

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