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PAPER

Slab waveguide photobioreactors for microalgae based biofuel production†‡

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Microalgae are a promising feedstock for sustainable biofuel production. At present, however, there are a number of challenges that limit the economic viability of the process. Two of the major challenges are the non-uniform distribution of light in photobioreactors and the inefficiencies associated with traditional biomass processing. To address the latter limitation, a number of studies have demonstrated organisms that directly secrete fuels without requiring organism harvesting. In this paper, we demonstrate a novel optofluidic photobioreactor that can help address the light distribution challenge while being compatible with these chemical secreting organisms. Our approach is based on light delivery to surface bound photosynthetic organisms through the evanescent field of an optically excited slab waveguide. In addition to characterizing organism growth-rates in the system, we also show here, for the first time, that the photon usage efficiency of evanescent field illumination is comparable to the direct illumination used in traditional photobioreactors. We also show that the stackable nature of the slab waveguide approach could yield a 12-fold improvement in the volumetric productivity.

Introduction

Concerns surrounding global climate change and foreign energy dependency have led to substantial interest in increasing biofuel production. Biofuels can be produced from a number of different feedstocks, including corn, soybean, canola and palm oil. As described by Chisti,[1] meeting 50% of the US transport fuel demands with current biodiesel production technology from these feedstocks would require more than 100% of the existing US cropping area. By contrast, microalgae could meet this demand with sunlight collected from between 1% and 3% of this land.[1] This vast difference in required land space comes from the fact that microalgae have high oil yields (10 to 30 times higher than oil palm, which is the next highest-yielding oil crop[1]) and the fastest growth-rate among plants (their doubling time is measured in hours[2]).

Despite these advantages, there are major challenges that prevent wide-spread, commercially viable, microalgae-derived biofuel production. One major challenge is the non-uniform distribution of light within existing photobioreactors[3] (*e.g.* open-to-air pond reactors or closed-systems tube reactors[4–6]). In dense microalgae cultures, shading of incoming light by the algae themselves prevents light penetration into the reactor. This results in overexposure of the organisms near the reactor surface and underexposure of the organisms away from the surface.[7] Overexposure generates reactive oxygen species capable of damaging photosynthetic machinery and underexposure provides insufficient light to sustain high volumetric productivities.[8,9] To better distribute light within photobioreactors, innovative designs that incorporate larger surface areas,[10] enhanced light-scattering by integrating light guiding structures,[11,12] and surface-plasmon-based light backscattering using silver nanoparticles[13] have been proposed and demonstrated.

A second major challenge is traditional biomass processing (*e.g.* dewatering and filtering to harvest the microalgae and cell disruption to extract the oil[14,15]). Algal harvesting contributes to 20–30% of the total biomass production cost[16,17] and the oil extraction from algae requires expensive chemicals and energy-intensive processes.[17] To address these problems, a number of studies have generated genetically-modified bacteria that directly secrete fuels, or chemical precursors to fuels, without requiring the biomass itself to be processed.[18,19] For example, Atsumi *et al.*[20,21] have demonstrated the production of isobutyraldehyde (a precursor to isobutanol) directly from a modified unicellular photosynthetic bacterium *Synechococcus elongatus* (*S. elongatus*) PCC7942. While this approach can provide a potential solution to the economic limitation of biomass processing, commercialization of this technique is limited by current photobioreactor architecture which is designed for the biomass production, and not for direct fuel production.

The combination of microfluidic transport and guided wave optics, which is the hallmark of Optofluidics,[22] may provide a

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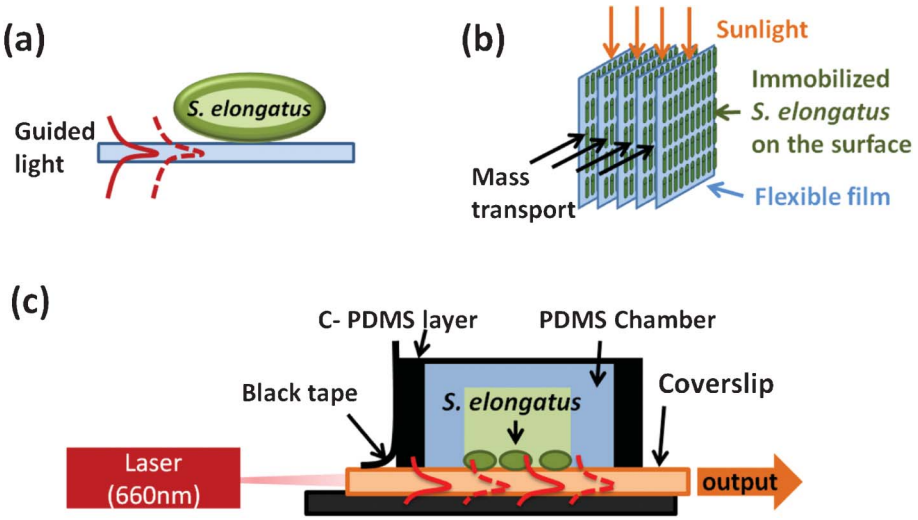

Fig. 1 Schematic showing evanescent illumination of *S. elongatus*, the stackable slab waveguide photobioreactor concept, and the experimental device used here (**a**) *S. elongatus* on the surface of the waveguiding structure obtain optical energy from the evanescent field of the guided light. (**b**) Slab waveguides with immobilized *S. elongatus* on the surface can be stacked leaving a gap for fluid-based reactant/product transport. (**c**) In this paper we use a PDMS chamber containing *S. elongatus* solution. A coverslip is used as a slab waveguide. To block scattered and transmitted light from laser, the PDMS chamber containing *S. elongatus* solution was surrounded by C-PDMS layer and black tape.

combined solution to these limitations. A recent review article by Erickson *et al.*[23] proposed a technique incorporating the use of waveguiding structures to deliver light *via* evanescent waves to photosynthetic organisms and microfluidics to introduce reactants and remove products. Fig. 1(a) and 1(b) illustrate the evanescent excitation approach. Briefly, when light is guided through a waveguiding structure, an evanescent field is generated on its surface and decays exponentially a few hundred nanometers into the surrounding media. Such a method could take advantage of coupling light directly into the photosynthetic machinery near the surface of the organism rather than illuminating the entire system. Moreover, synergetic effects can be expected by incorporating the evanescent excitation with the genetically-modified bacteria that produce fuel directly. Use of these organisms reduces the need for biomass harvesting and having them fixed on a surface facilitates the controlled transport of carbon to the organisms and extraction of oxygen, fuel, and potentially restrictive end products. The first demonstration of evanescent-field-derived photosynthesis was reported by Ooms *et al.*[24] in 2012. They demonstrated that the evanescent excitation generated by the total internal reflection of light off a prism can be used to direct light into the photosynthetic membrane of *S. elongatus* ATCC 33912 and drive photosynthesis. To date, however, this effect has not been demonstrated using a waveguiding system that is compatible with upscaling to a pilot-scale photobioreactor.

Here, for the first time, we demonstrate and characterize photosynthetic growth in the evanescent field of a slab waveguide. As part of this, we also demonstrate quantitatively that the optical energy consumed by *S. elongatus* PCC7942 using evanescent illumination is as efficient as a direct illumination method. The advantages of using slab waveguides are that they are easily stackable inside a reactor and could result in dense cultures that minimize shading effects and facilitate mass transport in the interstitial space between the waveguides. As outlined in our previous review[23] increases in culture density can result in cost-effective bioreactors with lower operational costs and reduced water and energy consumption.

Materials and methods

Photosynthetic organism cultures and optical density measurements

S. elongatus PCC7942, which was kindly provided by the Liao group at UCLA was used for all experiments. It was maintained in BG-11 media under yellow fluorescent light (discharge lamp, emission spectrum ranging from 500 nm to 700 nm) maintained at an intensity of 30 μE m^{-2} s^{-1} and maintained at room temperature. The optical density of the culture, which is proportional to the cell concentration, was used as an evaluation parameter for *S. elongatus* growth and biomass accumulation.[25] The optical density was measured at 750 nm (OD_{750}) by a spectrometer (Spectramax plus 384) and normalized to the OD_{750} of fresh BG11 media. Polystyrene spectrophotometry cuvettes (Perfector Scientific) were used for all measurements. A fresh pre-culture was maintained at the exponential growth phase (*i.e.* OD_{750} to be around 0.2) by regular inoculation of the solution. Prior to any experiments the culture was kept in the dark for at least a day to reduce carryover growth from the preculture. To create the initial surface phase culture on waveguides, reactors were inoculated with a dilution of the preculture into the PDMS chamber through holes on top of the chamber using a syringe.

Reactor fabrication

The PDMS chambers to contain *S. elongatus* were fabricated from a plastic master (*W*: 1 cm × *L*: 4.5 cm × *H*: 6 mm) that was formed by laser cutting. Uncured PDMS was poured over the plastic master and cured for 2 h in an 80 °C convection oven. The resulting chambers had a 2.5 mL capacity. To block the

scattered, uncoupled light from entering the reactor, the clear PDMS chamber was surrounded by a carbon black-embedded PDMS layer (C-PDMS layer) with a thickness of 5 mm, and the coverslip was placed on a slide glass wrapped with black tape. The light absorption layers (C-PDMS layer) consisted of carbon black, PDMS base, and PDMS curing agent in the ratio 0.01 : 1 : 0.1 by weight.[26] The C-PDMS was poured around the cured PDMS chamber, leaving a window on top of the chamber. The C-PDMS surrounded chamber was cut out from the master to have the 5 mm thick C-PDMS layer. No transmitted light could be detected behind the C-PDMS layer. A coverslip (width of 2.5 cm, length of 6 cm, and thickness of 150 μm) was used as the slab waveguide and support for the PDMS chamber. The laser light (laser diode, Newport LPM660-30C) used in experiments had a wavelength of 660 nm. The fabricated chamber was bonded to a coverslip by epoxy. PDMS chambers and coverslips used in experiments were pre-cleaned with 70% Isopropyl Alcohol.

Measurement of absorbed power

To quantify the amount of optical energy absorbed by *S. elongatus* suspending in the reactor, the power difference measured on the top and the bottom of the reactor filled with plain media using an optical power meter (Thorlabs PM100D) was subtracted from that of the reactor filled with the *S. elongatus*. The power absorbed by *S. elongatus* on the waveguide was measured in a similar manner. The output measured at the end of the slab waveguide when *S. elongatus* had settled on the waveguide was subtracted from the output measured when the chamber was filled with plain media.

Results and Discussion

S. elongatus PCC7942, which is an unicellular cyanobacterium with a diameter of 1 μm and length of 5 μm, belongs to a phylum of bacteria that harvest optical energy for photosynthesis using photosynthetic thylakoid membranes peripherally located within a few hundred nanometers of their outer surfaces.[27] A number of recent studies have suggested the potential application of cyanobacteria for the generation of clean energy *via* converting sunlight into biomass,[28,29] electricity[30] and chemicals such as isobutyraldehyde[21] and ethanol.[19]

Fig. 1(c) shows the conceptual schematic of the optofluidic slab waveguide photobioreactor used here. Although sunlight or broadband illumination could be coupled into the waveguides from lens type solar collectors[31,32] or luminescent solar concentrators,[33] for this work a laser was used as a light source to ease the optical coupling and facilitate quantification of the results. The laser light used had a wavelength of 660 nm, which is within the range of the photosynthetically active radiation (λ = 400–700 nm). *S. elongatus* has absorption peaks at 450, 630 and 680 nm as shown in ESI Fig. S1†. Light in the red region is absorbed by photosystems I and II (630 nm and 680 nm respectively) and is therefore particularly well suited for driving photosynthesis.[34,35] We observed about 98% of *S. elongatus* in the chamber settling down onto the cover slip surface within 24 h, resulting in a monolayer on the surface of the slab waveguide. Although gravity-based settling was sufficient for our horizontal experiments here, we note that cell adhesives[36] could be used to form bacterial layers for vertical bioreactors.

Fig. 2(a) and 2(b) show photographs of the slab waveguide-based photobioreactors used in this study. The reactor containing *S. elongatus* solution was illuminated only by the evanescent field of the coupled laser light as shown in Fig. 2(a). All other lights except the laser light were blocked during experiments by the C-PDMS layer around the reactor and a black plastic film cover on top of the reactor as shown in Fig. 2(b). To evaluate the *S. elongatus* growth rate at various locations on the slab waveguide, a transparent plastic film with 1 mm × 1 mm grids was integrated under the chip as shown in Fig. 2(c). Fig. 2(d) shows sample static images of photosynthetic growth following evanescent field illumination over the course of 5 days. Here, *S. elongatus* solutions with OD_{750} of 0.2 were diluted down 1 : 100 to place about 100 to 150 cells mm^{-2} on the slab waveguide. As we will quantify below, the absorbed optical power by *S. elongatus* along the length of the waveguide was about 12 μW for an input power of 5 mW. Under these conditions, the number of *S. elongatus* in the same region increased approximately 20 fold in 5 days. Experiments were terminated after 5 days because by that point *S. elongatus* began to grow densely which may influence growth. We note that although our approach is designed to work with product secreting photosynthetic organisms, here we quantify photosynthetic activity through biomass accumulation rather than fuel production due to the relative ease of making this measurement in cultures with low numbers of organisms.

The growth rates of *S. elongatus* at different locations in our reactor were characterized as shown in Fig. 3 for the evanescent field generated by 1 mW and 5 mW of coupled optical power. Fig. 3(a) and 3(b) show the *S. elongatus* density across the width

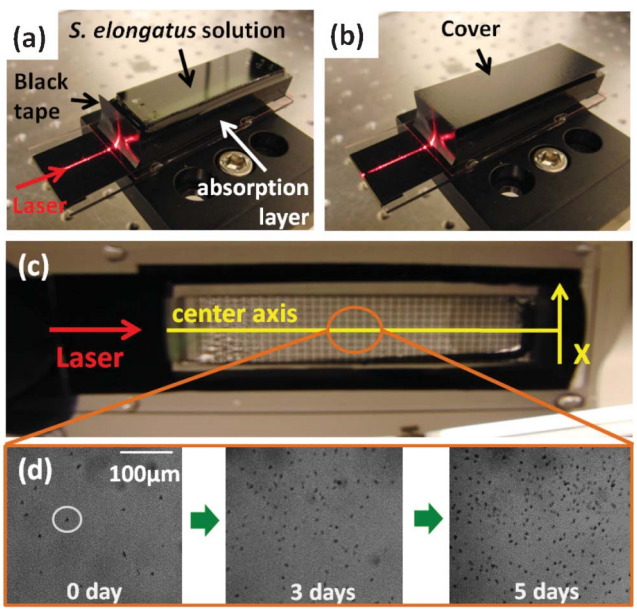

Fig. 2 Fabricated device and *S. elongatus* growth through evanescent illumination. (**a** and **b**) Fabricated photobioreactor without and with a cover to block external illumination, respectively. (**c**) Photobioreactor with grid on the bottom for counting *S. elongatus*. (**d**) Experimental images showing the change in the *S. elongatus* density following 5 days of evanescent illumination.

of the chip (X-direction in Fig. 2(c)) at different locations after 5 days of the evanescent illumination (center axis in Fig. 2(c), red: 1 cm, yellow: 3 cm, green: 5 cm away from the start of the coverslip). The increase in the coupled power from 1 mW to 5 mW resulted in 5-fold increase in the *S. elongatus* density (Fig. 3(a) and 3(b)). This indicates that the growth rate was governed by the intensity of the laser power in the range we tested. The *S. elongatus* density increased from 120 cells mm^{-2} to 2900 cells mm^{-2} at the center of the slab waveguide where the evanescent field was strongest (the beam width at the entrance of the waveguide was 2 mm), whilst the areas at the edge showed no significant change in *S. elongatus* density after 5 days. Controls with no illumination (black lines in Fig. 3(a) and 3(b)) showed no growth compared to the initial density (100 to 150 cells mm^{-2}). Fig. 3(c) and 3(d) show the change in the *S. elongatus* density along the center axis of the chip as a function of time. Increase in the coupled laser power from 1 mW to 5 mW also increased the growth rate[37] of *S. elongatus*. The specific growth rate (*i.e.* $\ln(N_2/N_1)/(t_2,t_1)$ where N_2 and N_1 are the cell densities at time t_2 and t_1)[37] was 0.32 day^{-1} with 1 mW and 0.63 day^{-1} with 5 mW (resulting in doubling times of 2.3 days and 1.1 days respectively). The cells of *S. elongatus* located at the entrance of the chip (red) grew faster than the ones at the exit (green). The growth rate of *S. elongatus* at the entrance was 0.634 day^{-1} (doubling time 1.1 days) and the growth rate at the exit was 0.431 day^{-1} (doubling time 1.6 days) with the coupled laser light of 5 mW. The doubling time of *S. elongatus* PCC 7942 in standard culture is reported as several hours under optimal growth conditions,[38] indicating the performance of our device can be improved by optimizing growth conditions (*e.g.* light intensity, temperature, and nutrient supply). The difference in growth rate at the entrance and the exit of the slab waveguide was most likely the result of the lowering of the optical power density along the length of the chamber due to the expanding laser light across the width of the slab waveguide.

Fig. 4 compares the efficiency of direct and evanescent illumination at driving the photosynthetic process. For these studies we quantify the efficiency by the ratio of the change in OD_{750} to the optical energy consumed. To measure OD_{750} in the waveguide based reactors we agitated the reactors to suspend the *S. elongatus* back into solution (from their settled state) and removed the homogenized solution for measurement. Following measurement, the *S. elongatus* were returned to the chamber and allowed to settle back onto the excited waveguide. This measurement was done once per day and required removal from the illumination source for less than 10 min. The same devices from previous experiments were used for both evanescent and direct illumination method. For the evanescent illumination, 5 mW of laser light was coupled into the slab waveguide and for the direct illumination, a yellow fluorescent lamp (discharge lamp, emission spectrum ranging from 500 nm to 700 nm) was used as a light source and positioned directly above the reactor. The emission spectrum of the lamp is given in the supplementary information. Fig. 4(a) shows the change in OD_{750} values over the course of 5 days. The average OD_{750} value in the evanescently illuminated reactor changed from 0.195 to 0.218 while that in the directly illuminated reactors changed to 0.49 under 3 W m^{-2} and 0.46 under 15 W m^{-2} of relative optical power at 660 nm (see Fig. S1† for details of the calculation of relative optical power). The result showed that the OD_{750} value did not change and

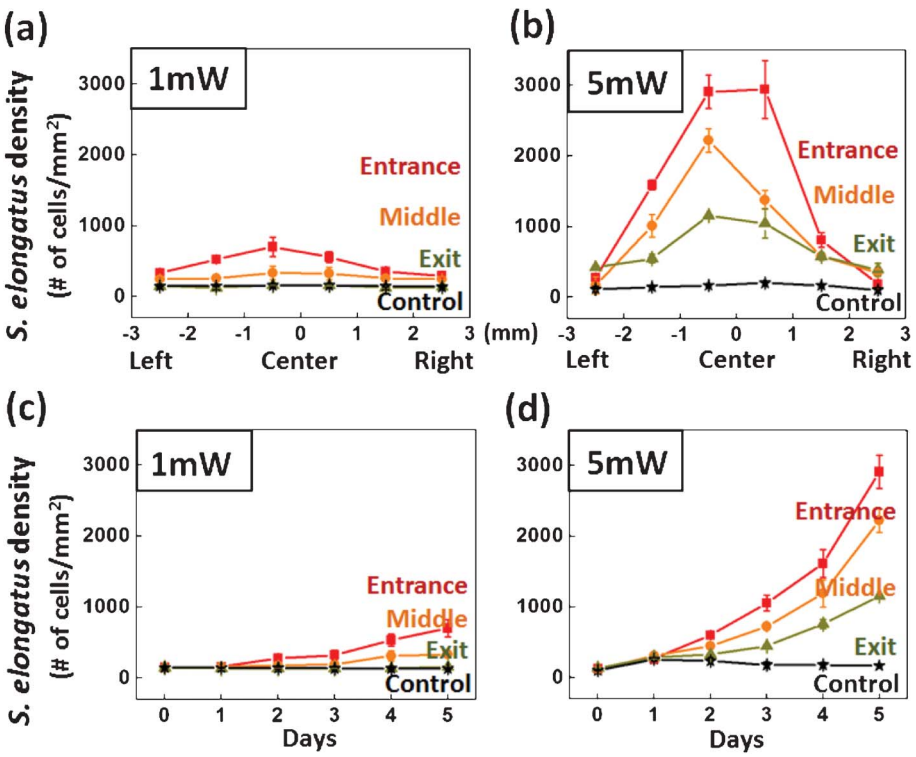

Fig. 3 *S. elongatus* density at different locations and times in the photobioreactor. (**a** and **b**) *S. elongatus* density (number of cells mm^{-2}) at different locations (entrance = red, middle = orange, exit = green) across the width of the chip after 5 days of the evanescent illumination of 1 mW and 5 mW coupled laser power. (**c**) and (**d**) Density at different locations along the center axis of the chip as a function of time.

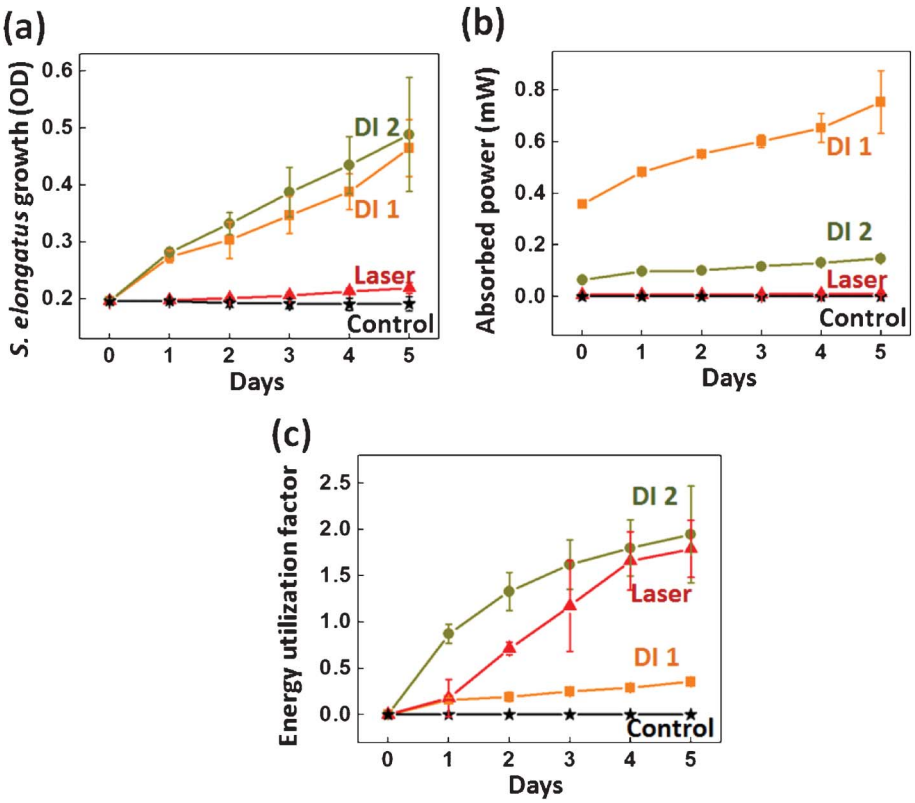

Fig. 4 Optical energy utilization for evanescent and direct illumination methods. (**a**) OD_{750} values for evanescent illumination (Laser, red), direct illumination (DI_1 = 15 W m^{-2} (orange), DI_2 = 3 W m^{-2} (green) and unilluminated control (black)) as a function of time. (**b**) Absorbed power as a function of time. (**c**) Energy utilization factor as a function of time.

remained equivalent to no additional increase in the *S. elongatus* concentration in the culture media, despite the 5-fold increase in the direct illumination power beyond the optimal light intensity for the *S. elongatus* growth. OD_{750} values of the control reactors showed no significant change over the initial value as shown in Fig. 4(a). Fig. 4(b) shows the total amount of optical energy absorbed by *S. elongatus* in both the direct illumination and evanescently coupled photobioreactors. We measured 0.15 mW and 0.75 mW of relative optical power at 660 nm were absorbed by the culture on the fifth day when the reactor was directly illuminated by 3 W m^{-2} and 15 W m^{-2} respectively. When the reactor was illuminated by the evanescent field, 0.012 mW of optical power was consumed by *S. elongatus* on the fifth day. Details of the measurement of absorbed powers are provided in the Materials and Methods section. The energy efficiencies of photobioreactors were evaluated by an "energy utilization factor", which we defined as the ratio of the OD_{750} change to the absorbed optical power. Fig. 4(c) shows calculated energy utilization factors. The energy utilization factor of the evanescent illumination was similar to that of 3 W m^{-2} direct illumination, while the 15 W m^{-2} direct illumination was 5-fold lower than the previous two. This result implies that the optical energy utilized by *S. elongatus* to grow photosynthetically, and therefore the energy efficiency, is comparable for evanescent and direct illumination. It is possible, however, that the energy utilization factor of the evanescent illumination was underestimated because several hours were needed for *S. elongatus* to settle on the slab waveguide to access the evanescent field after the daily OD measurement, whereas the direct illumination culture did not require this setting time. Also, given the non-uniformity of the light field, removing productive organisms for OD measurement and redistributing uniformly on the waveguide may set back growth leading to further underestimation.

The stackable nature of the slab waveguide evanescent illumination approach developed here could enable much higher volumetric productivity than existing photobioreactor designs. The volumetric productivity can be estimated by comparing the ratio of the change in OD over time (discussed above) to the volume utilized to grow *S. elongatus*. A monolayer of *S. elongatus* would require approximately 5 μm to grow, and therefore the efficacy of the evanescent field to support growth is practically limited to this region, as demonstrated by Ooms *et al.*[24] Including the thickness of the slab waveguide used here (150 μm) and leaving space between slab waveguides for mass transport (50 μm) as shown in Fig. 1(b), total volume required to grow *S. elongatus* using the slab waveguide photobioreactor is approximately 200 μm × surface area of the slab waveguide. For the proof-of-concept system demonstrated here the volumetric productivity (δOD_{750}/utilized volume) would be 0.03/(200 μm × surface area of the slab waveguide). For a system in which the organisms are grown on both sides of the waveguide, this value would double. The volumetric productivity of the direct illumination method would be 0.285/(6 mm × surface area). The evanescent illumination with organisms on both sides of waveguide would yield a 6-fold increased volumetric productivity compared to the direct illumination method. This could be

greatly improved however by uniformly distributing light across the width of the slab waveguide. As shown in Fig. 2 and quantified in Fig. 3(a) and (b), in the prototype system the light is much more intense in the middle than the edges, and thus the waveguide area was not fully utilized. Expanding the light (*e.g.* matching the beam profile to the geometry of the inlet of the waveguide) so as to achieve the optimal growth rate over the entire slab waveguide, would improve the volume productivity another 2-fold to give an approximately 12-fold improvement (for a double sided waveguide) of the direct illumination method.

Conclusions

In this work, we have demonstrated a photobioreactor that utilizes the evanescent field of a slab waveguide to culture photosynthetic organisms. The fastest doubling time of *S. elongatus* that we achieved here using the evanescent illumination was 1.1 days. We also demonstrated for the first time that the optical energy consumed to grow organisms using the evanescent illumination was at least as efficient as that using direct illumination. The advantage of this slab waveguide approach is that it is inherently "stackable", enabling us to create high density cultures that would otherwise be impossible due to poor light penetration. The system we demonstrate here enables us to obtain a 6–12 fold improvement in volumetric productivity compared with traditional photobioreactors. The optofluidic approach demonstrated here can also provide an appropriate design for a new type of biofuel production reactor that utilizes the genetically-modified photosynthetic organisms to produce fuel directly.

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