Bioresource Technology 209 (2016) 391–396

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Breathable waveguides for combined light and CO_2 delivery to microalgae

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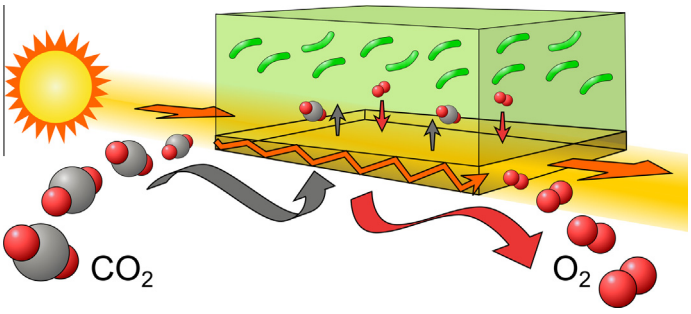
HIGHLIGHTS

- A permeable waveguide is used for combined light and CO_2 delivery to microalgae.
- CO_2 delivery using permeation led to double the growth as compared to a control.
- Cellulose acetate butyrate was a suitable breathable waveguide material.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 January 2016
Received in revised form 27 February 2016
Accepted 1 March 2016
Available online 7 March 2016

Keywords:
Microalgae
Cyanobacteria
Waveguide
Membrane contactor
Photobioreactor

ABSTRACT

Suboptimal light and chemical distribution (CO_2, O_2) in photobioreactors hinder phototrophic microalgal productivity and prevent economically scalable production of biofuels and bioproducts. Current strategies that improve illumination in reactors negatively impact chemical distribution, and vice versa. In this work, an integrated illumination and aeration approach is demonstrated using a gas-permeable planar waveguide that enables combined light and chemical distribution. An optically transparent cellulose acetate butyrate (CAB) slab is used to supply both light and CO_2 at various source concentrations to cyanobacteria. The breathable waveguide architecture is capable of cultivating microalgae with over double the growth as achieved with impermeable waveguides.

1. Introduction

Microalgal-based photobioreactors (PBRs) are a promising technology for renewable biofuel and high-value bioproduct generation. (Angermayr et al., 2015; Kumar et al., 2011; Savakis and Hellingwerf, 2015). Given the strong dependence of microalgal photosynthesis on light intensity and chemical concentration (CO_2, O_2), optimal production necessitates tight control of these conditions throughout (Carvalho et al., 2011; Kumar et al., 2011; Savakis and Hellingwerf, 2015). While open pond PBRs benefit from design simplicity (Medipally et al., 2015), they have not been cost-effective due to light and chemical supply limitations that cause gradients throughout the reactor that limit photosynthetic efficiency and culture density (Brennan and Owende, 2010; Norsker et al., 2011; Richardson et al., 2012). In contrast, closed PBRs allow greater photosynthetic productivities and densities (Brennan and Owende, 2010; Norsker et al., 2011; Olivieri et al., 2014; Posten, 2009), due to improved light distribution and CO_2 and O_2 transport (Posten, 2009). However, strategies that benefit light distribution often negatively impact chemical control, and vice versa (Norsker and Barbosa, 2012).

Abbreviations: PBR, photobioreactor; OD_{750nm}, optical density at 750 nm; CAB, cellulose acetate butyrate; PET, polyethylene terephthalate.

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http://dx.doi.org/10.1016/j.biortech.2016.03.016
0960-8524/

Delivering the optimal amount of light to cells is critical to reactor design (Graham et al., 2015). Illumination control in closed PBRs has traditionally been accomplished by turbulent perfusion (Norsker and Barbosa, 2012; Norsker et al., 2011). Turbulent mixing and bulk flow moves cells through a light gradient for time-averaged intensity exposure (Posten, 2009). While effective, this fluid circulation is energy-intensive, and is impractical at large scales (Nagarajan et al., 2013; Norsker and Barbosa, 2012). For example, in tube pilot plant reactors, liquid pumping requires 53–60% of operational power (Acién et al., 2012; Norsker and Barbosa, 2012). These approaches bring cells to light. An alternative illumination strategy is to distribute light to cells via waveguides in reactors. Waveguiding distributes light through large volumes of culture, and shifts operational cost (pumping) to capital cost (material). Recently, reactors using planar waveguides have demonstrated improved light distribution, photosynthetic productivity, cell density, and modular scalability (Ahsan et al., 2015; Pierobon et al., 2014). However, waveguides developed to date occupy significant reactor volumes (5–33%), limiting volumetric productivity (Ahsan et al., 2015; Dye et al., 2011; Xue et al., 2013). Furthermore, all waveguide reactor strategies reported to-date are impermeable, choking cultures of vital CO_2.

Control of the chemical environment is also critical to photobioreactor operation. One traditional approach is to bubble CO_2 at a single node and circulate the culture through the reactor loop. This approach suffers from chemical hold-up in the reactor, that is, CO_2 depletion and O_2 accumulation. The alternative approach is to bubble CO_2 throughout reactor panels. Bubbling throughout the illuminated culture in flat panel reactors effectively removes chemical gradients and enables the greatest productivities, densities and photosynthetic efficiencies. However, bubbles on the top surface of the reactor panels scatter light strongly. Thus, improved chemical transport is achieved at the expense of light distribution and usage (Janssen et al., 2003). Bubbling is also expensive. In tube pilot plant reactors, active aeration and CO_2 costs are respectively 7–16% and 15–44% of nutrients-plus-utilities costs (Acién et al., 2012). Further, aeration and CO_2 costs are each 5–10% of capital-plus-operation costs (Nagarajan et al., 2013; Norsker et al., 2011). Alternative aeration architectures based on nanoporous membrane contactors achieve single-phase gas-to-liquid mass transfer, and cost-effective CO_2 delivery and utilization (Bilad et al., 2014; Gabelman and Hwang, 1999; Markov et al., 1995). Recently, hollow fiber membrane contactors have demonstrated enhanced mass transfer of CO_2 to cells in photobioreactors (Ahsan et al., 2015; Kalontarov et al., 2014; Kim et al., 2011). With high density cultures, however, a high density of hollow fibers is required to avoid CO_2 limitations (Ahsan et al., 2015; Kalontarov et al., 2014). While membrane contactors clearly show promise for photobioreactors, and have been deployed at scale in waste water treatment (Bilad et al., 2014; Judd, 2011), all current efforts involved opaque membrane materials that block light.

In this work, an integrated illumination and aeration architecture is demonstrated, using a single transparent, hydrophobic, nanoporous material – a breathable waveguide – to simultaneously distribute light and CO_2 throughout a microalgal culture. Use of a single surface for combined delivery leverages the similar length scale of light and CO_2 dispersion (mm to cm) in high density cultures (Janssen et al., 2003; Ni and Pereira, 2000). The phototrophic biomass growth of batch cultures of *Synechococcus elongatus* were evaluated in carbon-limited and -replete conditions, using cellulose acetate butyrate first as a contactor for CO_2 permeation alone, and then as a breathable waveguide. Positive growth response to CO_2 permeation was observed and double the growth was achieved with waveguide illumination and breathability as compared to waveguide illumination alone.

2. Methods

2.1. Microorganism and cultivation

The microalgae used for all experiments was a T2SE-null mutant of the freshwater cyanobacteria *S. elongatus* PCC 7942 with kanamycin resistance, donated by Professor Rakefet Schwarz (Bar-Ilan University, Israel). The mutation permits biofilm growth, but this capacity was not used. This species was selected for its high-affinity carbon uptake in CO_2-rare media, which enabled clear evaluation of CO_2-limited growth (Price et al., 2004; Woodger et al., 2005). Additionally, *S. elongatus* is a model organism in photosynthetic studies, and is one of four microalgal species that have received great attention as direct producers of high-value biofuels and bioproducts, due to their amenability to genetic modification. Ethanol, isobutyraldehyde, and free fatty acid production in *S. elongatus* has been demonstrated (Atsumi et al., 2009; Ruffing and Jones, 2012; Savakis and Hellingwerf, 2015; Schwarz et al., 2011; Woods et al., 2004).

The culture was grown in modified BG-11 media (Sigma–Aldrich, C3061), supplemented with 50 μg/mL kanamycin and 80 mM HEPES buffer, pH-adjusted to 8.0 with NaOH and kept at a temperature of 27 ± 1 °C. The culture was maintained in a shaken incubator flask with ambient 0.05 ± 0.01% CO_2 headspace. Culture depth and 0.1 optical density at 750 nm (OD_{750nm}) were maintained by daily dilution with fresh media. The incubator and experiment reactors were illuminated with red light (632–638 nm), which enables strong growth response in lieu of the solar spectrum, and is standard practice when using monochromatic sources (Cuaresma et al., 2009; Graham et al., 2015; Jain et al., 2015). In the incubator, an array of 632 nm LEDs (OSRAM Opto Semiconductors) and light-diffusers provided uniform area illumination.

2.2. Breathable waveguide material

Cellulose acetate butyrate (CAB) was chosen as a breathable waveguide material for this study, due to its optical transparency, biocompatibility, relatively high gas permeability (32 and 6.2 Barrer to CO_2 and O_2, respectively), and relatively low cost and water impermeability. A comparative table of permeabilities and costs of a selection of other candidate breathable waveguide materials is presented in Supplementary material.

2.3. Breathable reactor design and operation

Five breathable reactors were fabricated, as shown in Fig. 1(a). For each reactor, constant output from a 634 ± 14 nm LED (Avago Technologies) and collimator lens (Thorlabs) provided uniform-area intensity top-down through 13 mL of culture in a black-anodized 25 mm ID aluminum cylinder. A glass window above the culture sealed 3 mL of initially ambient atmosphere (0.05% CO_2), such that 0.7 ± 0.1 mM of total inorganic carbon (herein Ci – the sum of $CO_{2\,(aq)}$, H_2CO_3, HCO_3^- and CO_3^{2-} species) was initially available to cells. The bottom of the culture contacted a highly gas-permeable, white, hydrophobic membrane (Pall, Versapor 200 R), which reflected diffuse light into the culture. A test material could be fastened directly below. The Versapor membrane served to maintain identical illumination conditions, independent of membrane test material. Both membranes sealed the culture against a polyethylene terephthalate (PET) sheet, with a CO_2 permeability of 0.27 Barrer (Goodfellow, ES303020). Flowing CO_2 diffused into the culture from below. Ambient pressure, air-balanced CO_2 mixtures were flowed at 1.4 L/min with 75 ± 2% relative humidity. Water vapor added to the gas stream mitigated water loss due to permeation (<5%).