Bioresource Technology 171 (2014) 495–499

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Stacked optical waveguide photobioreactor for high density algal cultures

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HIGHLIGHTS

- An ultracompact photobioreactor with planar scattering light waveguides was developed.
- Alleviates the problem of non uniform light distribution in traditional bioreactors.
- Eightfold increase in algal biomass accumulation over a control without waveguides.
- The photobioreactor supported consistent production of ethylene over 45 days.
- Twofold volumetric productivity increase over a conventional flat plate bioreactor.

ARTICLE INFO

Article history:
Received 16 June 2014
Received in revised form 20 August 2014
Accepted 21 August 2014
Available online 28 August 2014

Keywords:
Photobioreactor
Biofuel
Microalgae cultivation
Algal-based biorefineries
Optical waveguides

ABSTRACT

In this work, an ultracompact algal photobioreactor that alleviates the problem of non-optimal light distribution in current algae photobioreactor systems, by incorporating stacked layers of slab waveguides with embedded light scatterers, is presented. Poor light distribution in traditional photobioreactor systems, due to self-shading effects, is responsible for relatively low volumetric productivity. The optimal conditions for operating a 10-layer bioreactor are outlined. The bioreactor exhibits the ability to sustain uniform biomass growth throughout the bioreactor for 3 weeks, and demonstrates an 8-fold increase in biomass productivity. Using a genetically engineered algal strain, constant secreted ethylene production for over 45 days is also demonstrated. Since the stacked architecture leads to improved light distribution throughout the volume of the bioreactor, it reduces the need for culture mixing for optimum light distribution, and thereby potentially reducing operational costs.

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1. Introduction

Biofuels derived from algae represent a promising source of alternative fuel that could help meet ever increasing energy demands and address rising concerns with regards to carbon emissions leading to global warming (Chisti, 2007). Microalgal systems are an attractive feedstock for biofuel due to their independence from soil fertility (*i.e.* they do not compete with arable land area or forest ecosystems for their development) (Chisti, 2008; Stephens et al., 2010), relative independence from seasonal cycles allowing for year round production, high oil content (as percentage of biomass) (Chisti, 2007), and significantly higher productivity rates as compared to oilseed crops (Brennan and Owende, 2010; Chisti, 2008).

Currently, the most popular systems for commercial production of algal biomass for biofuels are so-called open systems – including natural, circular, or raceway ponds (Chen et al., 2011; Zittelli et al., 2013). The relatively low capital cost associated with the pond bioreactors, adds to their popularity, but they do suffer from a number of operational and control problems related to evaporation, temperature fluctuations, sensitivity to culture contamination, and other environmental factors such as rainfall (Zittelli et al., 2013). These issues have led to development of closed PBR systems, which provide a more controlled environment. A number of closed PBRs have been developed including flat, tubular, manifold, and biofilm bioreactors (Chen et al., 2011; Zittelli et al., 2013). Generally speaking the increased capital costs of these reactors are offset by the operational advantages outlined above (Chisti and Yan, 2011). In all these bioreactors, adequate light supply and distribution across the bulk of the algal culture is a key factor affecting productivity

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http://dx.doi.org/10.1016/j.biortech.2014.08.093
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(Chisti, 2007). An overabundance of light, typically at the illuminated surface, can lead to photoinhibition (Brennan and Owende, 2010; Janssen et al., 2003), while insufficient penetration of light into the culture (*e.g.* due to self-shading effects) leads to optically dark regions incapable of supporting optimal growth. To avoid this, PBRs require high surface to volume ratios allowing light to be distributed to as large a fraction of the culture as possible. Most PBR designers attempt to resolve the illumination problem by actively mixing the culture volume to expose the algae, on an average, to sufficient number of photons (Janssen et al., 2000; Molina Grima et al., 1999). However, these active mixing mechanisms tend to be energetically demanding and lead to higher operational costs (Chisti, 2007; Molina Grima et al., 1999). This is one of the reasons that the energy return on investment (EROI) – ratio of energy produced to energy input to the system – for algal biosystems is relatively low compared to other fuel sources, and in some cases has been estimated to be less than one (Beal et al., 2012b). Development of PBRs that can provide optimal light delivery with a reduced requirement for active mixing would, therefore, be of significant interest (Chisti and Yan, 2011).

To mitigate the effect of poor light distribution, PBRs with internal light distribution and guiding structures have been developed. Techniques that have been demonstrated include the use of: surface plasmon based light back scattering (Torkamani et al., 2010), LED array panels (Choi et al., 2013), optical fibers (Chen et al., 2006), and planar waveguides (Dye et al., 2011). Most of the studies were applied to relatively low density algae cultures (~OD 3), whereas high production systems necessitate high-density cultures. Jung et al. (2012) recently demonstrated single layer slab-waveguide systems that used near surface evanescent fields for algal growth and characterized the spatial–temporal growth patterns. The "stackable" nature of these single-layer systems is attractive in that it allows for increased productivity on a limited land area. However, the shallow depth of the evanescent field near the waveguide surface limits overall achievable biomass accumulation, thereby requiring a large number of stacks, which significantly increases capital costs.

Leveraging the advantages of short-light path design and eliminating the limitations of evanescent field illumination, a 10-stack PBR with integrated slab waveguides was developed. Light was allowed to escape the waveguide surface *via* scattering from an etched surface and penetrate deep inside the bioreactor. The performance of the bioreactor is primarily quantified in two ways: biomass accumulation evaluated by measurement of surface coverage and optical density (OD) of the bacterial colonies and volumetric ethylene production rates from a genetically engineered strain (Ungerer et al., 2012).

2. Methods

2.1. Fabrication of the 10-stack photobioreactor and light scattering waveguides

The bioreactor consisted of a frame to hold 10 slab waveguides for delivering light to microalgae, and a photomask to block uncoupled light from entering the bioreactor (Fig. S1(a) and (b)). A 3D printer (Connex 500, Objet Geometries Inc.) was used to print a frame of the 10 stack PBR. The frame was printed using a photocurable resin (VeroClear, Objet Geometries Inc.) and then coated by parylene C to prevent gas and liquid leakage. The photomask was also printed using the same photocurable resin and was subsequently covered by aluminum foil to reflect light that was not coupled into the slab waveguides. Optical waveguides were fabricated using standard borosilicate glass slides (VWR VistaVisio Microscope Slides) and cover slips (VWR Micro Cover Glasses). The borosilicate glass slides were chemically etched to create a scattering surface for allowing the light within the waveguide to escape out (Fig. S1(c)–(g)).

2.2. Growth of the inoculum culture (*Synechocystis* sp. PCC 6803 2× EFE)

A genetically modified strain (Ungerer et al., 2012) of cyanobacteria *Synechocystis* sp. PCC 6803, which are often also called blue-green algae (Sharma et al., 2011), was used for experiments and served as a model organism for continuous biofuel production. Semibatch cultures providing the initial inoculum for the stacked PBRs were grown, and subsequently maintained at OD_{730} of 60, at 30 °C and 100 μE m^{-2} s^{-1} broad-spectrum light, in gastight 1 L Schott bottles under a headspace containing a 5% CO_2 atmosphere. The culture media consisted of standard BG-11 medium, augmented with 20 mM $NaHCO_3$ as an additional carbon source, and 4.6 g/L TES as buffering agent. All cultures were grown in media with 25 mg/L spectinomycin and 200 mg/L kanamycin (Ungerer et al., 2012). Antibiotics are added to prevent the ethylene producing strain, which has been genetically modified to be resistant to the antibiotics, from reverting back to the wild-type strain or being outcompeted by the latter.

2.3. Image analysis

The surface coverage of bacterial colonies, *i.e.* the ratio of the surface of the waveguide covered by bacterial colonies to the entire surface area of the waveguide, was measured by obtaining fluorescent images of the cyanobacteria. The cyanobacteria were cultured on the waveguide for 21 days and quantitatively analyzed by the method published previously (Kalontarov et al., 2013). The surface coverage was calculated by first subdividing the obtained fluorescence images in 128-by-128 pixel squares. In each square the number of pixels which contained bacteria was identified (*i.e.* pixels that have higher grey values than a threshold value of fluorescent signals from bacteria), which was then divided by the total number of pixels in the square. This calculation yielded the local surface density expressed as a percentage.

2.4. Gas extraction and analysis

After each experimental run, the gaseous products were collected through the septum at the output port using syringes, while the influent port was connected to a bottle containing displaced algal culture. As the gas was pulled out from the reactor using the syringe, the liquid culture displaced earlier was pulled back into the bioreactor, ensuring that there was no dilution of the gas samples in the reactor by ambient air. The entire volume of the gaseous products was thus collected. Gas samples were analyzed using a gas chromatograph (GC) with alumina–silica column (181 °C, He carrier gas at 20 mL/min), to obtain the ethylene concentration in the sample.

2.5. Conventional flat plate photobioreactor (FPR)

A 4.5-L FPR with a light-path of 3 cm was run semi-continuously and used as a benchmark for the ultra-compact bioreactors. During the operating period, the PBR was continuously aerated with ambient air at a gas flow rate of 1.5 L h^{-1} to provide the culture with CO_2 and to ensure sufficient mixing of the culture. The FPR was subjected to light–dark cycles (0.5 h dark followed by 0.5 h light) at 200 μE m^{-2} s^{-1} .FPR was fed semi-continuously when mineral nutrient composition was found insufficient, while the pH was monitored daily and adjusted manually to pH 8.5 ± 0.25 using 1 M HNO_3.

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