Bioresource Technology 192 (2015) 845–849

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Integrated hollow fiber membranes for gas delivery into optical waveguide based photobioreactors

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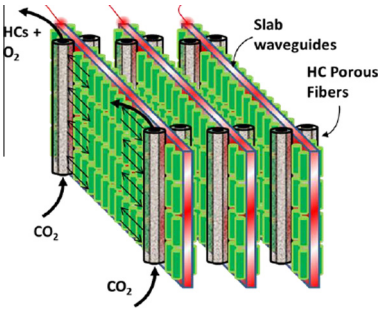
HIGHLIGHTS

- Integrated hollow fiber gas membranes and light-delivery via waveguides in novel PBRs.
- Characterized PBRs for fuel secreting bacteria for various light and flow conditions.
- Measured optimal flow rates of 66 mL/min corresponding to "gas-mixing" energy of 1 mW/L.
- Carbonated gas streams (1% CO_2) increased ramp production rates by two times.
- Under optimal gas flow, productivity increased at least 20% across light intensities.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 April 2015
Received in revised form 4 June 2015
Accepted 5 June 2015
Available online 11 June 2015

Keywords:
Algae
Photobioreactors
Scatterers
Waveguides
Hollow fiber membranes

ABSTRACT

Compact algal reactors are presented with: (1) closely stacked layers of waveguides to decrease light-path to enable larger optimal light-zones; (2) waveguides containing scatterers to uniformly distribute light; and (3) hollow fiber membranes to reduce energy required for gas transfer. The reactors are optimized by characterizing the aeration of different gases through hollow fiber membranes and characterizing light intensities at different culture densities. Close to 65% improvement in plateau peak productivities was achieved under low light-intensity growth experiments while maintaining 90% average/peak productivity output during 7-h light cycles. With associated mixing costs of ~1 mW/L, several magnitudes smaller than closed photobioreactors, a twofold increase is realized in growth ramp rates with carbonated gas streams under high light intensities, and close to 20% output improvement across light intensities in reactors loaded with high density cultures.

1. Introduction

Amongst potential sources of alternative energy, there has been considerable interest in biomass from algae. Algae are seen as a promising feedstock due to their: high areal/volumetric productivity (Chen et al., 2011; Chisti, 2007; Zhao et al., 2013); independence from arable land (Wijffels and Barbosa, 2010); potentially fast growth rates with high oil content (Rodolfi et al., 2009); and possibility of producing high-value food and pharmaceutical co-products (Janssen et al., 2003).

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http://dx.doi.org/10.1016/j.biortech.2015.06.028
0960-8524/

The most common method to cultivate algae involves the use of circular raceway ponds and tubular reactors (Chisti, 2007). These conventional photobioreactors (PBRs) are unable to efficiently distribute nutrients, such as carbon dioxide, and remove wastes, such as oxygen, uniformly due to poor mixing (Grima et al., 1999; Janssen et al., 2003); and are unable to distribute light in the bioreactor uniformly due to self-shading so that only a thin layer of the pond receives the optimal light intensity while the top layer receives too much light which destroys their photo-synthetic machinery by creating radical species (Bosma et al., 2007) and the bottom layer does not get enough light to sustain growth (Lee, 1999). To overcome these limitations and to achieve high-density cultures, bioreactors require energy-intensive mixing to cycle individual algae to the optimal light zones from the non-optimal zones and to better distribute carbon dioxide and remove oxygen (Chisti, 2007; Grima et al., 1999; Janssen et al., 2003; Lehr and Posten, 2009; Posten, 2009). Even in simple closed PBR systems, the energy required for mixing is close to the energy that is eventually harvested from the system; therefore, the energy return on investment (EROI), which is a measure of energy input *versus* output, is very low for such systems and has not been convincingly shown to be greater than one (Beal et al., 2012). Furthermore, the cost of mixing amounts to a considerable 13–52% of total construction and operating costs (Norsker et al., 2011).

To address the light delivery problems, ultracompact waveguide photobioreactors have recently been developed and demonstrated (Jain et al., 2015). By delivering light through the side of the waveguides where it is transmitted throughout the bioreactor, the light can be released uniformly into the culture using embedded scatterers (Ahsan et al., 2014). In addition, stacking waveguides closely to reduce the light path between each parallel waveguide enables near-uniform light distribution throughout the culture volume (Jung et al., 2014). The use of such compact stacked scattering waveguide (SW)-PBRs has enabled up to 8-fold increase in biomass productivity and enabled the possibility of sustaining high-density algal cultures (Jain et al., 2015). Previous work has also optimized such SW-PBRs systems, while achieving a carrying capacity of OD_{730} 20 (Jain et al., 2015). In addition, the integration of this system has been demonstrated with a genetically modified algal strain capable of secreting ethylene that would simplify post-processing steps including harvesting and product extraction that conventionally account for 50% of total production cost (Jain et al., 2015; Jung et al., 2014). Finally, SW-PBRs managed to improve ethylene production rates to 937 $\mu g\ L^{-1}\ h^{-1}$, which represented a 4-fold improvement compared to a conventional flat plate PBR (Jain et al., 2015).

Previous SW-PBRs did, however, suffer from productivity bottlenecks due to gas nutrient exchange. In high-density cultures, there is a particularly strong need for high-gas exchanges because of the high volumetric reaction rates; adequate carbon dioxide needs to be supplied while oxygen needs to be constantly removed to avoid toxic conditions. One possible strategy to accomplish this is through the use of hollow-fiber membranes (HFMs) for gas exchange. Previous works have demonstrated the efficacy of HFMs for gas-exchange in sustaining algal cultures (Kalontarov et al., 2014). HFM enabled reactors showed both an increase in the specific growth rate by 15% and the surface density by 35% and managed to eliminate any gradients along the length of the fibers (Kalontarov et al., 2014). Other work with HFM integrated reactors also showed minimal loss of CO_2 to the atmosphere and independent control over the pH and the growth of photoautotrophic biomass (Kim et al., 2011).

In this work, HFM stacked waveguide photobioreactors were built and investigated. The HFMs are used to alleviate oxygen build-up and deliver carbon dioxide while removing ethylene as a gas, which is collected at the output to determine system productivity. The reactor performance was investigated under three different input gas stream conditions: passive flow, active flow with atmospheric air, and active flow with carbonated air. Compared to SWPBRs without HFMs, SWPBRs with HFM have significantly greater photosynthetic-efficiency in producing ethylene while consuming on the order of ~1 mW/L for aeration energy, which is considerably smaller than conventional bioreactors. In addition, active aeration improves steady-state ethylene production significantly by 35% under high light intensity and even at low light intensities by over 60%. It was also shown that integrating HFMs also allows for higher production capacities in high OD_{730} experiments.

2. Methods

2.1. Reactor design and assembly

The reactor frames were printed using a 3D printer with a photocurable resin (VeroClear, Objet Geometries Inc.). The dimension on the frame was 7.5 cm × 2.5 cm × 3 cm (length × width × height). The reactor frames consisted of 10 parallel slots reactor separated by 2 mm along the height of the reactor for each waveguide. In addition, there were holes separated by 1 mm in each waveguide layer for HFMs (model No. MHF304KM purchased from the Mitsubishi Rayon Co., Ltd.) placed transverse along the width of the reactor where they were bunched through nozzles on each layer for easy gas delivery and removal. The reactor had a chimney connecting each waveguide layer to better allow fluid transport between layers and two ports: – one for influent media on the bottom and one for effluent media on the top. After assembly of the reactor frame with the waveguides and HFMs, Polydimethylsiloxane (PDMS) (Dow Chemicals, Midland, Michigan, USA) was used to fix all the parts in place including the fibers and their nozzles and then coated with parylene C to ensure airtight conditions.

2.2. Waveguide fabrication

Waveguides were fabricated on 1-mm borosilicate glass slides. The glass slides were coated with a glass-etching paste (purchased from Armour Etch) for 7 h to produce etched surfaces with a characteristic roughness to randomly scatter the internally transmitted light. The slides were then affixed with 80-μm coverslips and sealed in place with PDMS to create air cladding at the interface to better scatter the light. The light intensity was determined from control SWPBRs using methods reported by previous work.

2.3. Organism

A genetically modified cyanobacteria *Synechocystis* sp. PCC 6803 2× EFE was used for experiments and served as the model organism. Semi-batch cultures were cultivated for the inoculum in the PBRs in flasks and maintained in their growth phase at OD_{730} of 1–2. The culture media consisted of standard BG-11 medium (5×), 20 mM $NaHCO_3$ as an additional carbon source, 4.6 g/L TES buffer, 25 mg/L spectinomycin, and 200 mg/L kanamycin. The antibiotics were used to ensure that the modified strain, which is resistant to antibiotics, would not revert to its wild-type strain.

2.4. Experimental conditions

The reactors were illuminated during the light cycles by LED banks placed on both sides of the reactor about 1 cm away. The light was delivered through the side of the glass slides and released into the culture volume in a relatively uniform intensity

distribution. The reactors were run for 6–8 h of light cycles each day depending on the pH of the culture volume. If pH increased beyond 9.5 in a particular run (indicating carbon-limited conditions), the light cycle hours were reduced to alleviate this problem. The algal culture was removed from the bioreactor, centrifuged, and re-suspended in fresh carbonated media daily. This ensured the presence of an extra carbon source and other nutrients necessary for growth. Unfortunately, because of the close packing of the fibers and the tendency of the algae to settle, it was hard to remove a representative sample to monitor OD_{730} throughout the operating period. Instead, the increase in system ethylene productivity was used as a proxy for biomass densities.

2.5. Gas flow and sampling

During the experiment, three different gas stream flows were studied: passive aeration, room air flow, and flow with 1% CO_2 in air (Airgas, Elmira, NY). Because a measurement of the system production required air to be actively pumped through the HFMs, all the runs, even the ones with passive aeration, started with pressurized flow for the first hour at the end of which several mL of gas were collected at the gas sampling port. For the passive aeration runs, the pressurized flow would then be closed for the rest of the day to best approximate SWPBR systems. For the room air flow and flow with 1% CO_2 in air, the pressurized flow with room air continued to the end of the light cycle. These measurements monitored system peak productivity that were collected for the growth rate data.

3. Results and discussion

3.1. Measurement details

The ethylene concentration was sampled after one hour from the start of each light cycle. To calculate the ethylene production at this point, the ethylene concentration was multiplied with the flow rate to determine productivity rates. Once the LED banks were turned on, it took about 20–25 min for the reactor to start producing at peak productivities (Fig. 1(a)). This was expected as it would take some time for the bacterial cells to start producing ethylene, which was consistent with what was observed in flat-plate reactors (not reported). The beginning of the run starting from half an hour onwards for several hours corresponded to the peak productivity rates. This seems reasonable because prior to the start of each run, the algae was centrifuged and re-suspended in fresh carbonated media. As the run continued (generally lasting for several hours), it was hypothesized that the oxygen build-up and the carbon depletion would result in lower productivities.

This was confirmed in actively aerated gas stream runs (Fig. 1(d)) at relatively high light intensity conditions of 100 $\mu E\ m^{-2}\ s^{-1}$. There was significant difference between the two gas streams investigated for life cycle productivities (Fig. 1(d)). Both gas streams were studied when reactor productivity was close to 400 μg/L/hr at flow rates of 200 ± 100 mL/min. Under gas streams consisting of pressurized room air, the PBRs performed at peak productivity for 2 h. Afterwards, it was observed that the PBR productivity decreased sharply over 1 h to around 20% peak productivity at 3 h. In contrast, when the PBRs were fed a gas stream of 1% CO_2 in air, their ethylene productivities remained constant for almost the entire duration of the light cycle except for the last hour where it decreased to ~55% of peak productivity. Even though the actively aerated gas stream should alleviate oxygen build-up, it probably also removes the carbon source resulting in depleted productivities in the later hours of the light cycle. For the average 7-h light cycle used in the experimental runs to grow the algae, this would correspond to an average productivity of 43% that of the peak productivity if a minimum productivity of 20% was assumed. In contrast, the gas stream with 1% CO_2 should not only alleviate the oxygen build-up but also resupply the carbon dioxide into the media. As a result, the average productivity over the light cycle corresponds to over 90% the peak productivity measured after an hour. It is believed that the decrease in productivities past the 6th h is probably due to wear and damage to the photosynthetic machinery of the individual cells from usage due to relatively high light intensity conditions even when the bottlenecks associated with carbon limitation and oxygen build-up are alleviated.

3.2. Flow rate optimization

The productivities of the photobioreactor compared to different flow rates during the first 2 h of the life cycle run were measured (after waiting half an hour for the flow rate effects to equilibrate). The results (Fig. 1(c)) indicated that increasing flow rates beyond a 66 mL/min made little difference to improve ethylene collection into the fiber membranes from the reactor culture. The optimal flow rate should in principle optimize the energy output *versus* the energy required for the gas flow. Previous experiments (Federspiel et al., 1996) have determined that the operating pressure to be roughly 2 mPSI, and assuming a flow rate of 66 mL/min, the energy required for gas exchange is close to 14 mW for these reactors of 15 mL, which scales to ~1 mW/L for mixing. This is an order of magnitude lower in terms of energy required for mixing in most PBR systems where even simple systems use ~3 $W\ m^{-2}$ (Posten and Schaub, 2009) at significantly lower culture densities.

3.3. System performance under different gas streams

The different gas streams were investigated in experimental runs at low and high light intensity conditions to observe productivity growth in the *Synechocystis* sp. PCC 6803 2× EFE cultures (Fig. 1(e–h)). Because of the tightly-packed nature of the reactors and the settling tendency of the algae, one could accurately measure the cyanobacteria count through a simple OD_{730} measurement. Rather, the ethylene production was monitored to extrapolate for growth. Generally, the productivity of the culture should depend on the OD_{730} of the culture as well as the healthiness of the culture so that the steady-state productivity should correspond directly to the culture density of the system at the time.

At 23 $\mu E\ m^{-2}\ s^{-1}$, the growth of the cultures was observed under passive room air aeration from close to nominal ethylene production to ~245 μg/L/hr while under actively aerated room air, this production rose to 405 μg/L/hr representing a 65% improvement. It is suspect this increase was due to the ability of the aerated gas stream to remove toxic oxygen build-up; it is also possible that the system was carbon-limited even at such low light-intensities starting with daily suspension of 20 mM $NaHCO_3$ as the pH of the culture went well beyond pH 9.5. Even so, the addition of carbonated air (not shown here) did not make a significant difference in the steady-state production rates for ethylene. The peak ethylene production efficiency for the aerated runs at these low intensities was markedly higher than that in the SW PBR by close to 35%. The ethylene production efficiencies were close to 0.16% calculated in terms of lower heating value of ethylene over inputted light energy.

At 100 $\mu E\ m^{-2}\ s^{-1}$, a passive aeration gas stream and an actively aerated 1% CO_2 gas stream were investigated. The PBR under passive aeration grew from nominal ethylene production values to 420 μg/L/hr although the ramp rate did not vary significantly from either of the runs at 23 $\mu E\ m^{-2}\ s^{-1}$ all of which were close to ~45 μg/L/hr/day for the first half of the run. In comparison, under

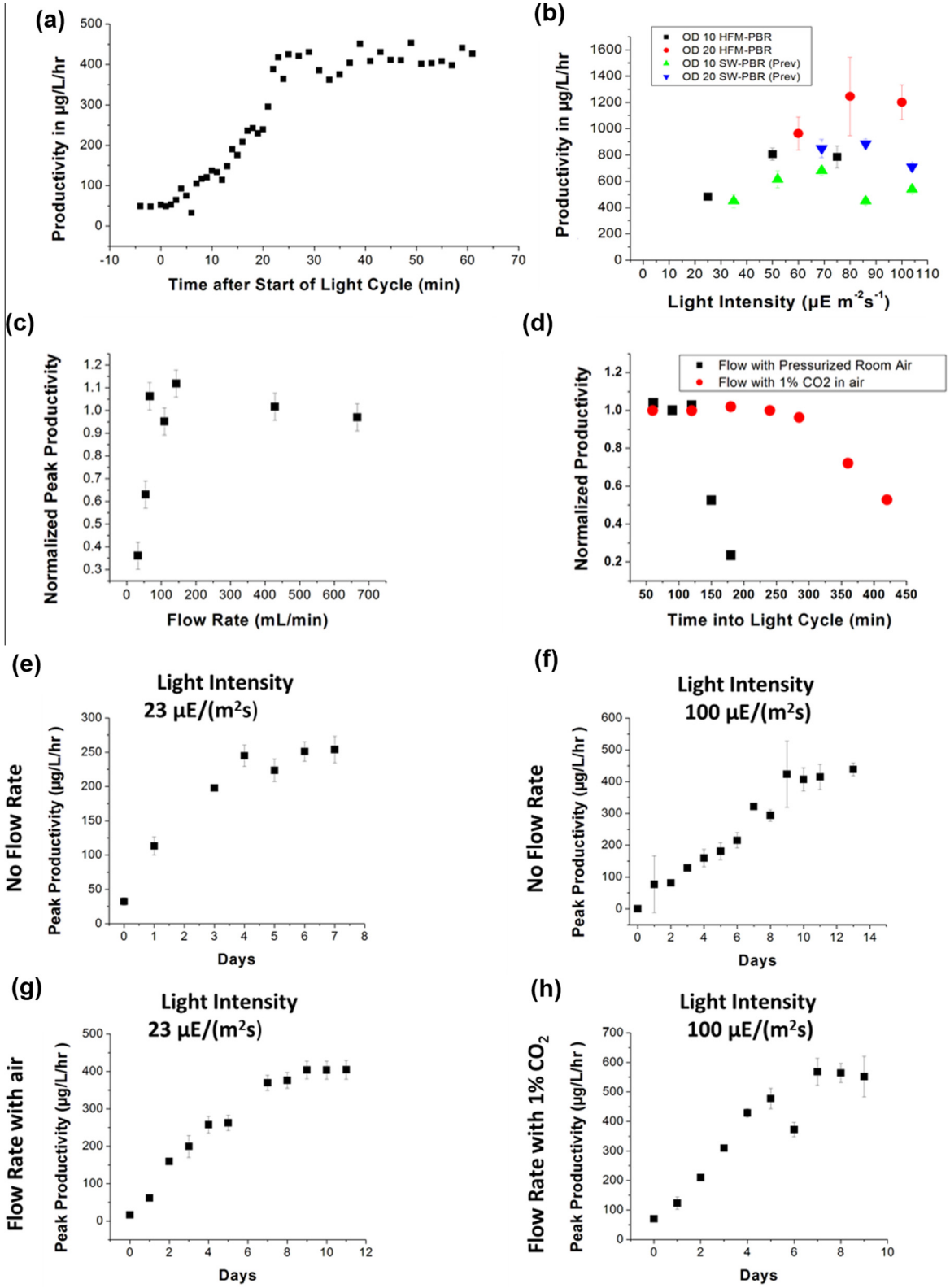

Fig. 1. (a) Transient behavior at beginning of light cycle; (b) peak productivity with carbonated gas stream at different intensities loaded with high OD_{730} cultures; (c) normalized productivity to flow rate; (d) normalized productivity over course of light cycle for different gas streams. Growth experiments performed at (e) low light intensity and passive aeration; (f) high light intensity and passive aeration; (g) low light intensity and active aeration with air; (h) high light intensity with active aeration with 1% CO_2.

an actively aerated gas stream with 1% CO_2, the ethylene production rose to 560 µg/L/hr with much faster ramp rates of ~90 µg/L/hr/day. This corresponded to an improvement in plateau peak productivities of close to 33%. While this is quite significant of an improvement, it is smaller than the improvements seen at lower light intensities by a factor of two. It is believed that this is the case because while active aeration is able to alleviate oxygen build-up in the system, it is able to do little about the damage to the

photosynthetic machinery by the higher flux of photons, which may be a more significant factor.

3.4. Optimal PBR productivities at different OD_{730} and light intensities

While the transient culture densities could not be determined by an OD_{730} measurement, reactors were inoculated with cultures grown and kept in the exponential phase of known OD_{730}. These experiments were then run under 2 different light–dark cycles of 2 h each that particular day after which they retired and new inoculum was used for the following experiment. This was to ensure that there was no significant difference on the OD_{730} caused because of growth. Ethylene productivities measured in these HFM PBRs run with 1% CO_2 in air outperformed their SWPBR counterparts (Jain et al., 2015) when accounting for average productivities as opposed to peak productivities.

At OD_{730} 10, peak productivities were in excess of 800 μg/L/hr at 50 μE m^{-2} s^{-1} as compared to 660 μg/L/hr for the SWPBR amounting to a 21% improvement (Fig. 1(b)). While the general production to intensity relationship of the two reactors was roughly the same, the HFM reactor counterpart seems to augment productivities across the entire range of intensities. The same general characteristic held for experiments performed with OD_{730} 20 inoculum. The productivities were in excess of 1200 μg/L/hr at both 80 and 100 μE m^{-2} s^{-1} yielding a 28% increase in maximum productivities over the SWPBRs.

4. Conclusion

In this work, an integrated hollow fiber membrane stacked waveguide photobioreactor was built and tested. A genetically modified ethylene-secreting cyanobacteria *Synechocystis* sp. PCC 6803 2× EFE strain was used. It was realized that system productivity plateaued with flow rates in excess of 66 mL/min, which corresponded to a "gas-mixing" input energy of close to ~1 mW/L with average to peak productivities of close to 90% for carbonated gas streams. In addition, a twofold increase in production ramp rates with carbonated gas streams, and close to 20% output improvement across light intensities in reactors with high density cultures were shown.

Acknowledgements

This work was supported by the Advanced Research Project Agency – Energy (DE-AR0000312) to D.E. and L.T.A. This work was performed in part at the Cornell NanoScale Facility, a member of the National Nanotechnology Infrastructure Network, which is supported by the National Science Foundation (Grant ECCS-0335765). We thank Jianping Yu, PhD (NREL, Golden, CO) for providing the *Synechocystis* sp. PCC 6803 2× EFE algal strain. We thank Rose Harmon for assistance with gas analyses.

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