Hollow fibre membrane arrays for CO$_2$ delivery in microalgae photobioreactors

Michael Kalontarov,$^a$ Devin F. R. Doud,$^b$ Erica E. Jung,$^a$ Largus T. Angenent$^b$ and David Erickson*$^{a}$

Microalgae can serve as a carbon sink for CO$_2$ sequestration and as a feedstock for liquid biofuel production. Methods for microalgal biomass and biofuel cultivation are progressing, but are still limited in the efficiency of light delivery and gas exchange within cultures. Specifically, current gas exchange methods are very energy intensive since they rely on mixing algal cultures at high flow rates. One method that can improve gas exchange within photobioreactors without excessive mixing is the use of hollow fibre membranes, which enable simultaneous transport of gases deep into the reactor and rapid exchange with the culture media. Here we demonstrate the optimal geometric and operational conditions for CO$_2$ transport to planar cultures of Synechococcus elongatus via hollow fibre membrane arrays. Specifically, we investigated the effects of inter-fibre spacing and active/passive aeration on the growth rate, planar surface density, and total biomass accumulation. We show that spacing in excess of 3 times the fibre diameter lead to significant variations in the uniformity of the surface density and spatially resolved growth rate, whereas spacing of 3 times the fibre diameter supported culture surface densities nearing 90%, which were maintained for 17 days without decreasing. Active aeration with the fibres showed an increase in the specific growth rate and the average surface density with respect to passive aeration by approximately 15% and 35%, respectively, while also eliminating gradients in localized growth rates along the length of the fibres.

Introduction

Concerns about the impact of climate change, CO$_2$ emissions, and energy security have led to widespread interest in the production of biofuels from microalgae.$^1$ Microalgae have higher CO$_2$ fixation efficiencies and growth rates than other plant-based feedstocks$^{1,2}$ and the potential to utilize waste-water or industrial gas wastes as nutrient sources.$^3$ The most developed method for extracting biofuels from microalgae is converting their stored lipids into biodiesel,$^4$ which utilizes a separation process that is very energy intensive.$^5$ This has prompted the research and development of many engineered strains of cyanobacteria to directly secrete fuels such as hydrogen,$^6$ ethanol,$^7$ iso-butyraldehyde,$^8$ and other high value products.$^9$

To take advantage of these engineered strains, innovative photobioreactor (PBR) designs are required that can sustain high density cultures while enabling efficient light delivery and gas exchange.$^{10}$ The most common reactors used in algal cultivation are open raceway ponds and tubular-type enclosed reactors.$^{11}$ While these designs do have respective advantages,$^{12}$ both are faced with fundamental limitations in delivery of sufficient light and CO$_2$ and extraction of products to maintain high photosynthetic rates.$^{13}$ The former of these problems has received a significant amount of attention as of late.$^{14}$ Uneven light distribution causes the culture to be overexposed at the surface and underexposed below the light penetration depth.$^{15}$ To counteract this problem many approaches have been investigated including the integration of optical fibres,$^{16}$ interaction with evanescent$^{17}$ and plasmonic fields,$^{18}$ and planar waveguides.$^{19}$

In parallel to the problem of light delivery, limitations in gas exchange and transport are also being addressed. Traditionally, gas exchange in PBRs is provided by bubbling or passive exposure to the atmosphere.$^{20}$ Though easy to implement, these methods constrain optimal PBR geometries, operation and limit possible culture densities. CO$_2$ delivery is limited by uneven distribution throughout the reactor volume.$^{21}$ Maintaining a uniform distribution is important for efficient volume utilization since regions with low CO$_2$ concentration suffer from lower rates of photosynthesis.$^{22}$ Turbulent flow mixing is a common mechanism by which CO$_2$ concentration is equilibrated. This condition requires that a large amount of energy is spent on mixing the algal cultures,$^{23}$ up to 41% of the cultivation energy budget in some cases, and contributes to the already high energy costs of the algal cultivation process.$^{24}$ This has motivated research into various methods for improving gas

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$^a$Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, New York, 14853, USA. E-mail: de54@cornell.edu
$^b$Department of Biological and Environmental Engineering, Cornell University, Ithaca, New York, 14853, USA
A recent advance in enhancing gas exchange has been the integration of hollow fibre membranes (HFMs) modules into PBRs. A HFM consists of hollow fibres with membranes that allow for gas exchange between the media inside and outside the fibre. HFMs have been used in the chemical, petrochemical, pharmaceutical and galvanic industries, and applied in such varied applications as wastewater treatment, drinking water treatment, tissue engineering, and the development of artificial organs. Recently, HFMs have shown potential to address the gas exchange challenges faced by PBRs and several lab scale reactors incorporating HFM modules have been reported. These studies have verified the potential benefits of integration of HFMs into PBRs by reporting increased biomass production, improved gas exchange, regulation of pH, and promotion of CO₂ fixation. In previous work, we have characterized the effectiveness with which a single HFM fibre, which was applied independently of a module, can provide the necessary gas exchange to locally sustain growth in a carbon-limited reactor with no circulation and only passive gas transport.

Here, we performed a study to determine the optimal operating and geometric conditions for the use of hollow fibre arrays as a method for delivery of atmospheric levels of CO₂ to planar cultures of photosynthetic organisms. We report three experiments that investigated the effects of inter-fibre spacing and active/passive aeration on the organism growth rate, planar surface density, and total biomass accumulation. In the first experiment, we have characterized what array spacing might be most effective by studying a two fibre unit cell. In the second experiment, we fabricated full HFM arrays and measured the behaviour of the organisms for an extended period of time (30 days). In the third experiment, a study characterizing the effect of adding active gas flow through the fibres was conducted.

**Materials and methods**

**Investigation of optimal spacing with two fibres**

To characterize the effects of the spacing in a HFM fibre array we first conducted experiments on a unit cell of two fibres. Miniature reactors were fabricated, inoculated, and operated in the manner described in our previous work, however, as seen in Fig. 1(a), two fibres were inserted instead of one. The dimensions of the sealed miniature reactors were 40 mm × 16 mm × 6 mm and the HFM fibres (model no. MHF304KM purchased from the Mitsubishi Rayon Co., Ltd.) were centred on the bottom of the reactors along the primary axis. The reactors consisted of an epoxy sealed polydimethylsiloxane (PDMS) chamber sandwiched between a microscope slide and a large cover slip (Fig. 1(a) and (b)). KwikWeld Epoxy (J-B Weld Company) was used as a sealant to make the reactors as gas tight as possible. The reactors were inoculated with *S. elongatus* SA665 (obtained from the Liao lab at UCLA) and modified BG-11 medium. The medium was modified by the addition of 50 mg L⁻¹ thiamine and a 50 mM phosphate buffer (pH 7.0), the removal of bicarbonate, and by sparging with N₂ for 30 min in an anaerobic serum bottle prior to re-suspension and inoculation of *S. elongatus*. This was performed to make the initial culture carbon free and to achieve CO₂ limited growth conditions in the reactor. The reactors were placed under two fluorescent lamp strips (American Fluorescent Plug-In Light Strip), which provided a photon flux density of 75 µE s⁻¹ m⁻² (Fig. 1(c)). The ambient temperature in the laboratory was measured to be 25 °C. The ends of the fibres where open to the atmosphere to allow passive gas exchange to occur through the lumens of the fibres.

Three different unit cell spacing values were tested: 870 µm, 1740 µm, and 3480 µm. The diameter of the HFM fibres was ~290 µm, thus, these spacing distances correspond to 3, 6, and 12 fibre diameters, respectively. A 3D printed (OBJET Connex 500) frame was used to position the fibres on the glass slide and ensure the proper gap between them; epoxy was used to fix the fibres in place. Four reactors were fabricated for each spacing distance. The space between the fibres was imaged by using fluorescence microscopy at the time point of inoculation and then again after a seven-day operating period. As can be seen in Fig. 1(a), the observed region was offset from the edges of the reactor to minimize boundary effects on our measurement (the observed region was 26 mm long). The captured images were
processed to map the surface density of *S. elongatus* distribution. Briefly, surface density is the percentage of the two-dimensional area, in this case between the two fibres, that is covered by the bacteria (a more thorough description of imaging setup and processing algorithm is available in our previous publication⁹).

**Expanded HFM fibre arrays**

To conduct experiments with larger arrays of fibres, we fabricated reactors using the same processes as described above (Fig. 2), except two large cover slides (75 mm × 50 mm × 1 mm) were used to sandwich the epoxy sealed PDMS chamber.

The area of the reactor was increased to 58 mm × 38 mm × 6 mm. The fibre array was placed on the bottom of the reactor and centred; the fibres were arranged parallel to the primary axis of the reactor and were 75 mm in length. The fibre array spanned 20 mm of the reactor width and was offset from the walls of the reactor by about 1 cm on each side so that edge effects did not interfere with the growth in the array. The dimensions of the observed region were 20 mm × 40 mm, and the region was marked by a grid placed in the centre of the reactor as seen in Fig. 2(a) and (b). The grid served as a reference for imaging using a fluorescence microscope. We fabricated three reactors for two unit-cell types: 19 fibres were used to make the 3-diameter spacing array and 11 fibres were used to make the 6-diameter spacing array (Fig. 2(a)). After inoculation (using the same procedure as for the unit-cell experiments), the reactors were placed under the fluorescent light strips as utilized in the previous experiment (Fig. 2(b)). The experiment was conducted for a 30-day operating period and the reactors were imaged daily on day 0 – day 5, and again on day 10, 17, and 30. Images were taken in the same manner as previously discussed. A total of 360 images were taken per reactor to map the growth distribution around the fibre array and images were stitched together to produce surface density maps.

**Active gas delivery**

The goal of this experiment was to study the effect of active aeration on the growth of *S. elongatus* in HFM array reactors; all other conditions such as the organism, media, light conditions, etc. were kept the same. To incorporate active flow into these reactors, we built a setup that allowed us to pump in compressed atmospheric air under controlled flow and pressure conditions (Fig. 3). Three actively aerated reactors were compared to the same number of passively aerated and non-fibre control reactors. The actively aerated reactors were operated at a pressure of 3.45 kPa, which corresponds to a flow rate of ~50 mL min⁻¹. The reactors were constructed similarly as in the previous experiment except the fibre ends were bundled and inserted into a coupler to interface them with tubing. The fibres used in these experiments were approximately 10 cm long. The 3-fibre diameter spacing was chosen for the fibre array in these reactors. A cantered 20 mm × 40 mm region was imaged on a daily basis using fluorescence microscopy in each reactor and analysed with the previously described methods. Using these images the surface density of the bacteria in each reactor was calculated. The experiment was allowed to run for 5 days and upon completion the organisms were harvested from the reactors. Optical density at 750 nm was measured for these samples to compare final bacteria concentration achieved in the three different types of reactors.

**Results and analysis**

**Investigation of optimal fibre spacing**

After seven days of growth, the space between the fibres in each of the two fibre unit-cell reactors was imaged and the average surface density was calculated. Sample images of the inter-fibre spacing are presented in Fig. 4. *S. elongatus*, which appears white in these fluorescence microscopy images, was inoculated at a low surface density (Fig. 4(a)). By day 7 of the operating period (Fig. 4(b)), the bacteria covered most of the available area except for a small gap. The local surface density measurements were averaged together to get the surface density in the observed region for each reactor, with four reactors for each fibre spacing. The dependence of the surface density on the two fibre unit-cell spacing is show in Fig. 4(c). The surface density was highest for the 3-diameter spacing, reaching an average of 80% ± 4.5% coverage, while the surface density decreased to 43% ± 1.5% for the 6-diameter gap and 24% ± 2.3% for the 12-diameter gap. The amount of reactor surface area not taken up by the fibres for each unit cell type depends on the fibre spacing. For each unit-cell type the percentage of reactor area supported for photosynthetic growth by the fibres is 75%, 83%, and 92%, for the 3, 6, and 12-diameter gaps, respectively. Multiplying the respective measured surface densities and available area percentages yielded the better comparison metric to identify which type of
unit cell was more effective. This calculation yields adjusted surface densities (accounting for the reactor area taken up by the fibres themselves) of 60%, 36%, and 22% for the 3-, 6-, and 12-diameter gap unit-cells, respectively. Based on these results we decided to further explore the 3- and 6-diameter unit-cells by fabricating fibre arrays with these gap distances.

HFM fibre arrays

The growth distribution that developed in the 3- and 6-diameter fibre arrays differed from each other both spatially and temporarily. In Fig. 5, we present the surface density maps of the growth distribution on day 0, 3, 5, 10, 16, and 30 of the operating period for both array types. In both cases, the initial distribution is uniform and at a low surface density of 1.35% ± 0.4%. By day 3, the surface density maps show clear evidence of bacterial growth, which was initiated adjacent to the fibres in both cases. However, by day 5 there was greater coverage in the 3-diameter spaced array compared to the 6-diameter array. Additionally, the quality of this coverage was also superior as the bacteria were at a higher surface density in the tighter array. By day 10, most of the available area in the 3-diameter spaced array is covered by a dense fluorescent layer, however, in the 6-diameter spaced array the bacteria are at a comparable surface density only directly adjacent to the fibres. The bacteria layer continues to get denser in the 3-diameter array until the layer completely covers all of the available area at an average surface density of 88%. In the 6-diameter array the bacteria were limited to bands of high surface density directly next to the fibres with an area of restricted growth at further distances from the fibre. The measurements for day 30 show that surface density of the bacteria distribution decreases in the centre of the 3-diameter array, while for the 6-diameter array the bacteria bands next to the fibres expand slightly.

To visualize the growth layers, which we have so far represented by surface density maps, we present several fluorescent microscopy images of *S. elongatus* in these reactors in Fig. 6. The surface density maps are made by processing multiple images and stitching the results together to map out the whole 20 mm × 40 mm observed region in each reactor. Looking at the day 17 map for the 3-diameter spaced array (Fig. 6(a)), very high surface density regions are seen on the left and right panels. The image in Fig. 6(a) depicts a layer that formed in between the fibres; note that there is minimal empty space between the bacteria. The right panel is taken at the centre of the observed region and depicts a layer that has a lower local surface density region inside it. For the 6-diameter spaced array (Fig. 6(b)), we would like to illustrate how the size of the bacteria bands

![Fig. 3](image-url) Setup for flow experiments: the setup consists of a compressed air source, manifold, and pressure regulators to allow for the active aeration of 3 miniature reactors. The actively aerated reactors are compared to 3 passively aerated reactors, and 3 control reactors with no fibres.
formed next to the fibre differs at two locations. On the left, the panel depicts a location closer to the observed region edge and larger surface coverage is seen. On the right, an image from the centre is shown and here a thinner bacterial band is observed. The bands are asymmetric relative to the fibres and this is consistent with our observations of growth around a single fibre.

In Fig. 5, we illustrate the spatial variation in the bacteria distributions that developed in the two types of tested array designs. Data pertaining to the development of these distributions with respect to time is presented in Fig. 7. This data describes the trends in the total surface density of the bacteria in the observed region for the complete set of reactors \( (n = 3 \text{ for each type}) \). The total surface density is a representation of the fraction of the observed region covered by bacteria. Though the initial conditions for both types of reactors are virtually identical, the measured values for the surface density quickly diverge. In the reactors with 3-diameter arrays, a large proportion of the available area becomes covered with bacteria in an exponential manner; after 5 days 63% ± 10% of the surface is covered by a bacterial layer. Growth is slower in the 6-diameter array reactors and only 30% ± 9% of the surface area is covered by the bacteria after 5 days. In the next phase, the rate at which the surface density grows decreases in both reactor types. The bacteria layers in the 3-fibre diameter spaced arrays reach a maximum average surface density of 88% ± 3%. This high surface density persists until day 17, but measurements made on day 30 reveal that the density of these layers does not persist indefinitely and a decrease is observed. In reactors with 6-fibre diameter spaced arrays, the average surface density has been observed to increase at a slow rate through the course of the experiment.

By performing an exponential fit on the total surface density data for days 0–4, we can obtain the specific growth rate for the bacteria that developed in these reactors. The average specific growth rate in the 3-fibre diameter spaced array and 6-fibre diameter spaced array reactors was found to be 0.041 ± 0.004 (h⁻¹) and 0.031 ± 0.003 (h⁻¹), respectively. We also mapped the specific growth rate as a function of location in the observed region by applying the same method to the local...
surface density maps. Such maps are presented in Fig. 8. When comparing the maps for the two types of arrays, we observed two trends. In the tighter arrays the growth rates are distributed in a continuous manner, however, in the sparser array the areas of high growth rate are concentrated next to the fibres. This of course parallels the observations of the surface density maps presented earlier.

Another trend is that in both cases the growth rates are higher closer to the edges of the observed region. This is an artefact of the fact that these reactors are passively aerated. CO₂ is entering the reactors from the open ends on both sides of the reactors and a reduced amount is delivered to the centre, thus, limiting the growth rate there. The extent of the higher growth rate region is a function of CO₂ diffusion rates in the fibre and the media and the rate of CO₂ depletion by the bacteria.

Fig. 6 Micrographs of bacteria layers in HFM fibre array reactors. Images taken at the edge and centre of the observed region in a 3 fibre diameter spaced reactor (a) and 6 fibre diameter spaced reactor (b).

Fig. 7 Total surface density in the observed region for the 3 fibre diameter spaced and 6 fibre diameter spaced reactors (n = 3 for each type). The error bars represent the standard error of the mean.

Fig. 8 Local specific growth rate maps for the two types of fibre arrays with passive aeration. In the tighter array the growth rate distribution is more continuous than in the sparser array. Growth is also higher at the edges as expected for passive aeration.
Active aeration of HFM array reactors

To determine if the limitations observed in the passive aeration experiments could be overcome, we compared how bacteria grew in actively aerated and passively aerated reactors with 3-fibre diameter spaced fibre arrays versus a set of control reactors without fibres. The total surface density of the observed regions for the complete set of reactors (n = 3 for each type) is plotted in Fig. 9. The starting density for this experiment was 3.0% ± 0.9%. The control reached the lowest surface density after 5 days, 17% ± 3%, due to being limited by gas transport through the liquid volume and exchange through the reactor walls. The passively aerated reactors behaved as before, exhibiting close to exponential growth and reaching 54% ± 2% coverage during the course of the experiment. The actively aerated reactors reached 74% ± 8% average surface density, which was the highest out of this set of reactors. The specific growth rates for the actively and passively reactors were calculated by performing an exponential fit on the total surface density data for the first four days. The average specific growth rates for the actively and passively reactors were 0.037 ± 0.002 (h⁻¹) and 0.032 ± 0.004 (h⁻¹), respectively.

We also present spatial distribution of the local specific growth rates for actively and passively aerated reactors in Fig. 10. In the actively aerated reactor, the specific growth rate is uniformly distributed in the direction of gas propagation in the observed region. However, in the passively aerated reactor we see higher growth rates toward the left and right edges of the observed region as previously discussed. Finally, at the conclusion of the experiment we extracted the liquid volume from the reactors and measured the optical density (OD) at 750 nm to compare the bacteria concentrations in these reactors. We present this data in Fig. 11. The initial OD for the experiment was 0.005 ± 0.001. The control reactors were found to have an OD of 0.05 ± 0.006, due to growth at the edges of the reactors. In the passively aerated reactors, the OD of the bacteria solution reached 0.076 ± 0.008. The OD measurements for these two types of reactor are closer than the final surface density values, since
the fibre array only covers ~53% of the available reactor area. The OD found in the actively aerated reactors was 0.214 ± 0.010. The surface density values for the actively and passively aerated reactors were much closer together due to the fact that even though in both types of reactors the bacteria layers covered a similar amount of area, the bacteria layer in the actively contained a larger number of bacteria due to being stacked to a larger degree in the third dimension.

### Discussion

**Performance of fibre array reactors**

We have shown that the localized effect of a single HFM fibre on bacteria growth could be extended to a large area by properly arraying multiple fibres. Table 1 allows us to compare the results of these experiments. For context, *S. elongatus* observed in a bubble-aerated, continuously rapidly mixed, culture flasks maintained at a temperature of 35 °C and a similar light flux (50 μE s⁻¹ m⁻²) as used in our experiments was reported to be ~0.058 (ref. 40). The growth rates observed in our experiments range from 53–71% of growth rate in well mixed culture flasks but were observed without any mixing. In the single fibre experiments, the growth rates observed depended on the initial surface density. The same trend is seen in the array reactors, as the experiments started at 3.0% initial surface density have somewhat lower growth rates suggesting that CO₂ is already a limiting factor at these surface densities. For the same initial surface density, however, using the 3-fibre diameter spacing in the arrays increased the growth rate by 30% and more than doubled the surface density after 5 days with respect to the 6-fibre diameter array spacing.

Additionally, our 30-day experiments with the 3 fibre diameter spaced array reactors showed that the surface density of the bacteria layers increased even further after day 5 and can be maintained at these high values without mixing or media replenishment for at least 17 days without decreasing. The area spanned by the array in our experiments was 800 mm², but it could be increased if more fibres were added to the array while still delivering the same performance, as long as the fibres are maintained at the same length. By properly spatially arranging the fibres we were able to achieve the goals of distributing CO₂ and providing channels for gas transport throughout a PBR.

**Active aeration to address limitations of passive aeration**

To apply HFM arrays over any area both the number of fibres and the length of the fibres have to be increased. If passive aeration is used, the fibre length is limited by gradients in the CO₂ concentration along the fibre. The effects of this constraint can be observed in the specific growth rate maps for the passively aerated reactors, which were presented in Fig. 8 and 10. In both those cases the hotspots for growth were close to the edges of the observed region. Over the length tested in our reactors (7.5–10 cm) the growth in the middle was diminished but still allowed for a uniform distribution of bacteria to develop over time, as seen in Fig. 5. If longer fibres were used the gradients in bacteria growth rates would have increased, potentially to the point that growth in the centre would be inhibited. To mitigate this problem we have considered actively aerating the reactors. When compared to passively aerated reactors in the same experiment, active aeration improved the initial specific growth rate and the final average surface density by approximately 15% and 35%, respectively (Table 1). Furthermore, a more uniform distribution in the growth rate was observed (Fig. 10); no discernible gradients along the fibre direction indicate that active aeration is a way to overcome this constraint on length. The uniformity of the distribution could be maintained for longer fibres if the flow rate, input gas CO₂ concentration, and desired bacteria concentration are properly matched. The proper combinations of these conditions will be explored in future experiments. Lastly, OD measurements revealed that adding aeration also greatly increased the amount of bacteria in the layers that developed around the fibres by providing better access to CO₂ than passive aeration, as indicated by the 3 times greater final OD in the actively aerated reactors.

### Conclusions

Eliminating the need to mix and circulate the bacteria culture throughout a PBR would greatly reduce the operational energy costs. In this work we have directly applied HFM fibres to a planar culture of cyanobacteria to provide gas exchange and facilitate growth without circulation or media replenishment. We have demonstrated that a high surface density bacteria layer can be attained and maintained with passive aeration through a HFM array. While passive aeration can potentially eliminate the need to spend energy on gas exchange, it limits the area to which a fibre array can be applied. We have shown that active aeration can be a way to address this limitation since it decreases the gradients in the growth along the fibre length. Though active aeration through the fibre array does require additional energy we have observed that it also leads to higher reactor productivity. Further work is necessary to understand
how the geometric and operational conditions for the fibre arrays would change with increased CO₂ concentrations, light intensity, gas flow rates through the fibres, and bacteria concentrations. In summary, fibre arrays have been demonstrated as a possible way to provide gas exchange and facilitate photosynthetic growth over any surface area.

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