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Internally illuminated photobioreactor for algal cultivation under carbon dioxide-supplementation: Performance evaluation

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ABSTRACT

This study evaluated an internally illuminated photobioreactor (IIPBR) design for improving biomass productivity through better utilization of light and carbon dioxide-supplementation. Growth of *Scenedesmus* sp. and *Nannochloropsis salina* in an 18-L version of this design was evaluated under artificial light at varying CO_2–air (vol/vol) ratios, but at constant air supply rate of 0.8 L min^{-1} corresponding to gas-to-culture volume ratio of 0.044 min^{-1}. CO_2–air ratios of 4% and 1% were found as the optimal for *Scenedesmus* sp., and *N. salina*, yielding volumetric productivities of 0.40 and 0.104 g dry L^{-1} d^{-1}, respectively. Under continuous operation with regular harvesting at the optimal CO_2–air ratios, constant biomass levels of 1.40 and 0.52 g dry L^{-1} were maintained with average biomass productions of 2.53 and 0.93 g dry day^{-1}, for the two species, respectively. Based on volumetric biomass productivity per unit light energy input per unit incident area, performance of this IIPBR design is shown to be comparable to that of bubble column and airlift designs reported in the literature, but at much lower gas-to-culture volume ratio.

1. Introduction

Open raceway ponds that have been widely used for mass cultivation of algae are known to suffer from several shortcomings such as low biomass density, low biomass productivity, high evaporation loss, and potential for contamination. Engineered photobioreactors (PBRs) facilitate cultivation of high quality cultures under controlled growth conditions with minimal potential for contamination [1]. Other advantages of engineered PBRs over the open raceway ponds include higher biomass densities, smaller footprint, efficient harvesting, longer production cycles, and minimal labor input, albeit at a higher initial cost [2,3]. Since biomass concentration in engineered PBRs can be nearly 30 times than that in open raceways, biomass recovery and downstream processing with PBRs can be more efficient and could offset their higher initial cost [3].

Several types of photobioreactor designs such as flat plate, tubular, bubble column, airlift reactors have been evaluated for culturing microalgae [3–5]. Volumetric productivities in such engineered PBRs have been reported to be an order of magnitude greater than those in raceway systems. For example, Pushparaj et al. [6] reported productivity of 2.1 g L^{-1} d^{-1} in a tubular reactor vs. 0.18 g L^{-1} d^{-1} in a raceway system; Chisti [3] has reported 1.54 g L^{-1} d^{-1} for a tubular PBR vs. 0.12 g L^{-1} d^{-1} for raceways, both under outdoor conditions. Even though volumetric productivity has often been used to assess and compare different PBR designs, a more functional measure of PBR performance could be volumetric biomass productivity per unit light energy input per unit incident area to reflect the light utilization efficiency and the production costs.

Previous studies have identified that, other than light, supply of carbon to algal cultivation systems is one of the principal limitations. For optimal growth, CO_2 levels have to be maintained above the minimum nutritional requirements and below the inhibitory level; the range depends on the species. Benemann et al. [7] have reported that supply and transfer of CO_2 into the broth account for nearly 1/3 the cost of algal cultivation. Most engineered PBRs have relied on sparging with CO_2–enriched air (CEA) to improve biomass growth. In batch bubble columns, for example, Hsueh et al. [8] found that, compared to sparging with ambient air (0.035% CO_2), biomass (*Nannochloropsis oculta*) increased by 135% under sparging with CEA containing 5% CO_2; and by 200%, with CEA containing 8% CO_2. Growth was fully inhibited with CEA containing 10% CO_2, as the pH fell below 5. Another study by Ryu et al. [9] evaluated the growth of *Chlorella* sp. in bubble columns with CEA at varying CO_2-to-air ratios of 0.5%, 1%, 2% and 5%. Compared to the run under

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CO_2-to-air ratio of 0.5%, increase in biomass of 34% was noted at 1% CO_2; increase of 55% at 2% CO_2, and increase of 75% at 5% CO_2.

In the current study, it is hypothesized that an annular, internally illuminated photobioreactor (IIPBR) configuration could be more energy-efficient and cost-effective than the conventional externally illuminated bubble column and airlift designs. This hypothesis is based on the following premises: first, IIPBR configuration could be more compact than externally illuminated bubble column and airlift designs. It can be shown that, an annular IIPBR of external diameter of D and internal diameter of $0.62D$ would have the same illuminated surface area/unit culture volume as an externally illuminated bubble column of diameter D (See Appendix). As such, the overall footprint of the IIPBR will be smaller than that of an externally illuminated bubble column of equal illuminated surface area/unit culture volume.

A second premise is that the light utilization efficiency of the IIPBR design could be more than that of an externally illuminated bubble column. In the two examples considered above, the light path length of the annular IIPBR would be $0.19D$ while that of the externally illuminated bubble column would be $0.5D$. Shorter light path lengths can accommodate higher culture densities, beneficial in reducing cultivation and downstream processing costs. Thirdly, better mixing could be achieved in the IIPBR by a combination of the effects of the rising bubbles in the sparged zones (where the four diffusers are located, Fig. 1, Appendix) and the internal circulation by the airlift action induced between the sparged and unsparged zones (in between the diffusers, Fig. 1, Appendix). Such improved mixing has been shown to improve light penetration in dense cultures [10]. Finally, the IIPBR design can be deployed outdoors to take advantage of natural illumination with optimal spacing between the IIPBR units and their alignment with respect to the sunlight, augmented by artificial internal illumination if necessary, during cloudy days, for example.

The primary goal of this study was to validate the above hypothesis by evaluating biomass growth and lipid production of two algal species in an 18-L IIPBR design, sparged with carbon dioxide-enriched air (CEA) in batch and fed-batch modes, under constant light intensity. A secondary goal of this study was to compare the performance of this IIPBR design with that of bubble column and airlift PBR designs reported in the literature, considering volumetric productivity, (P_X, g L^{-1} d^{-1}) per unit light energy input per unit incident area (E, W m^{-2}). Two microalgal species were selected for assessing the performance of the IIPBR: *Scenedesmus* sp., a freshwater algae, and *Nannochloropsis salina*, a marine algae. Based on our preliminary tests, *Scenedesmus* sp. was tested in this study at CO_2-to-air ratios of 1%–5%; and *N. salina*, at CO_2-to-air ratios of 0.5%, 1% and 2%.

2. Materials and methods

2.1. Photobioreactor

The IIPBR tested in this study was fabricated out of two concentric acrylic columns as described previously [11]. The culture was grown in the annular space between the two columns, with the lights placed inside the inner column provided radial illumination. Nominal diameter of the inner column was 10 cm and that of the outer column was 19.7 cm; both columns were 90 cm high, with a working culture volume of 18 L. The culture was sparged with CO_2–enriched air from the bottom of the annular space through four porous silica air diffusers placed 90° apart at 800 mL min^{-1}; the gas flow rate per culture volume (G/L) was the same in all experiments at 0.044 min^{-1}. The air supply was filtered (0.2 μm Millipore filter paper) prior to mixing with carbon dioxide gas using gas proportioner (EW-03218-50 Cole Parmer flow meter system).

2.2. Strain and nutrient media

Growth of chlorophyceae *Scenedesmus* sp. (S) and *N. salina* (N1) was investigated in this study. *Scenedesmus* sp. strain was cultivated using Bold's basal medium [12,13] and *N. salina* was grown with modified *f*/2 media as described elsewhere [11]. pH of the *Scenedesmus* sp. culture medium was adjusted to 6.7 as per the recipe after autoclaving by adding few drops of acid (dilute 1 M H_2SO_4) or base (1 M KOH). Both inocula were diluted with the nutrient media to achieve the initial optical density (OD) at 750 nm greater than 0.15.

All tests were conducted under laboratory conditions where, the temperature ranged from 26 to 27 °C. Algal growth was quantified in terms of OD at 750 nm measured with a spectrophotometer (Thermo Scientific GENESYS™ 10 Visible Spectrophotometer). Culture samples were diluted prior to OD measurements to ensure that the spectrophotometer readings were below 0.5. Correlation between optical density and dry weight was established by determining the dry weight by centrifuging and oven drying as detailed previously [11].

2.3. Experimental scheme

In the case of *Scenedesmus* sp., experiments were conducted with ambient air and with CEA at CO_2–air ratios of 1, 2, 3, 4 and 5% (vol/vol); the light source, culture depth, and the gas flow rate were kept constant for all the tests. Initial biomass concentration in all these tests ranged between 0.1 and 0.2 g L^{-1} corresponding to OD at 750 nm greater than 0.15. Due to the lower growth rates observed with ambient air and with CEA at CO_2–air ratio of 1%, these two runs were conducted only in batch mode; the runs with CEA at CO_2–air ratios of 2, 3, 4 and 5% were conducted in batch mode for the first 4 days, and switched to fed-batch mode thereafter. The 4-day batch period was selected based on saturation of growth as discussed elsewhere [11].

In the case of *N. salina*, experiments were conducted with ambient air and with CEA at CO_2–air ratios (vol/vol) of 0.5, 1, and 2%; the light source and culture depth were kept constant for all the tests. Initial biomass concentration in all these tests was greater than 0.085 g L^{-1} corresponding to OD at 750 nm greater than 0.2. The runs with 0.5, 1 and 2% CEA were operated in fed-batch mode; the run with ambient air was run under batch mode.

During the fed-batch operation in both cases, 10% of the culture was harvested, and the culture volume was replenished immediately with equal volume of fresh media. Harvesting cycle was repeated only when the biomass concentration equaled or surpassed the concentration preceding the harvest. The fed-batch runs at each of these CO_2-to-air ratios were continued until a steady state concentration was achieved in each run.

2.4. Lipid extraction

Lipids were extracted from 0.25 g of dried algal samples (in triplicate) with a Dionex 350 accelerated solvent extraction (ASE) system (Dionex Corporation, Salt Lake City, UT, USA). Samples were mixed with 30 g Ottawa sand and loaded into 33 mL sample cells. Extraction conditions were adapted from Mulbry et al. [14]. Briefly, sand/algae mixtures were extracted with chloroform:methanol (2:1, v/v) for 5 min at an extraction temperature of 120 °C and pressure of ~1500 psi and then dispensed to a pre-weighed 60 mL collection vial. Extract solutions were dried under a stream of nitrogen immediately after extraction to determine the extractable lipid gravimetrically. Lipids were analyzed at the end of each run during steady state.