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Evaluation of internally illuminated photobioreactor for improving energy ratio

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The internally illuminated photobioreactor (IIPBR) design has been shown to be more efficient in utilizing the incident light energy than the externally illuminated designs. This study evaluated (i) optimal sparging of the IIPBR with CO_2-enriched air (CEA) to enhance biomass productivity; and, (ii) single-stage and two-stage operation of the IIPBR to enhance lipid productivity. Growth data from two algal cultures-*Scenedesmus* sp. and *Nannochloropsis salina*, cultivated in an 18-L prototype version of the IIPBR were used to establish the optimal conditions for the two goals in terms of the energy ratio. Based on the optimized results under sparging with CEA, the energy ratio in the IIPBR in the first stage with *Nannochloropsis salina* was at least 6 times higher due to optimal performance of the IIPBR at lower energy input than typical literature results for other PBR designs, whereas the energy ratios in the second stage were comparable to literature results.

[**Key words:** Internally illuminated photobioreactor; Net energy ratio; Lipid content; Fatty acid methyl esters content; Two stage operation]

Compared to traditional fuel crops, microalgae have been identified as a more viable feedstock for biodiesel production due to their higher oil content, faster growth rates, and lower land requirements for cultivation. Besides its potency as a renewable fuel, combustion of algal biodiesel results in lower net carbon emissions than petroleum-based fuels and soybean-derived biodiesel (1). Despite such advantages, microalgal biodiesel is not commercially viable yet (2,3) due to poor photosynthetic efficiency and low oil yields. It has been recommended that energy-efficient photobioreactors with high light harvesting capability coupled with algal strains capable of higher lipid accumulation and faster growth rate under optimized supply of carbon/nutrients can render algal biodiesel production economically feasible (4).

Traditionally, performance of PBRs has been assessed and compared on the basis of volumetric or areal biomass productivity, with little regard to the energy associated with the process. Recent studies (5–7) have begun to consider the energy input to the process and the energy that can be harvested from the biomass in assessing and optimizing PBRs for biofuel production. These studies have suggested energetic measures such as biomass productivity per unit energy input, net energy ratio, and net energy gain as more appropriate ones to evaluate PBRs and to assess the returns from energy intensive cultivation practices (such as CO_2-enrichment, nutrient enrichment and starvation) that have been suggested to maximize biomass and oil production.

In this study, the performance of an internally illuminated photobioreactor (IIPBR) is optimized in terms of the energy ratio. The design features and advantages of this IIPBR, which can serve as an energy-efficient parent reactor for seeding mass scale systems, have been presented earlier (8). The energy ratio that we propose to adapt in this study is calculated as the ratio of the energy output to the energy input.

Energy input to the cultivation process includes energy expended for illuminating the cultures and that for mixing the cultures and providing the CO_2 supply. In artificially illuminated PBRs, the former is significantly higher than the latter. For a given incident light energy and a given algal species, efficiency of conversion of light energy to biomass is a function of the reactor geometry; higher conversions could be achieved with higher incident area per unit culture volume and shorter light path length. For example, for a given incident area per unit culture volume, the IIPBR geometry has been shown to have a smaller footprint, shorter light path length, higher biomass density, and higher biomass productivity per unit energy input than the traditional externally illuminated bubble column PBR (BCPBR) (8).

From the perspective of algal biodiesel production, the energy that can be harvested from the biomass can be quantified in terms of lipid content or, more specifically in terms of fatty acid methyl esters (FAME) content. Microalgal strains are known to accumulate lipids [mostly triacylglycerols (TAGs), which are saturated and mono-unsaturated fatty acids] when subjected to stressing conditions such as nutrient limitation or altered growth conditions such as fluctuations in light intensity or temperature (9–11). However, such stressing conditions are known to retard biomass growth rate. Since lipid (or FAME) productivity is equal to the lipid content (or FAME content) times biomass productivity, maximizing lipid (or FAME) productivity has remained a challenge. To achieve high biomass productivity and high lipid content, two-stage cultivation has been proposed, where growth-stimulating conditions are

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maintained in the first stage followed by stressing conditions in the second stage to raise the lipid content (12).

For example, Su et al. (12) had evaluated two-stage cultivation of *Nannochloropsis oculata*, with the following stressing approaches in the second stage to maximize lipid content: nitrogen limitation, varied inoculum concentrations, salinity, and irradiance. The maximum lipid content achieved in the second stage was 2.8 times that in the first stage at an inoculum concentration of 2.3 g L^{-1}, salinity of 35 g L^{-1}, and illumination of 500 μ Einsteins m^{-2} s^{-1} (12). Solovchenko et al. (10) had investigated accumulation of fatty acid content under nutrient stressing at three light intensities of 35, 200 and 400 μ Einsteins m^{-2} s^{-1}. The total fatty acid content (TFA) of the cultures grown under 400 μ Einsteins m^{-2} s^{-1} and sufficient nitrogen was found to be higher than those under nitrogen starved cultures at lower light intensities (10).

Under environmental stresses, microalgae have the ability to modify lipid metabolism allowing them to endure extreme conditions (13–16). During unfavorable nutrient limitation such as nitrogen deprivation, microalgae modify their metabolism to support the synthesis of lipid bodies, which are largely comprised of triacylglycerols (15,17,18). Both lipid production and algal biomass compete for photosynthetic assimilate (15). While similar studies had evaluated the effect of nutrient-stress on algal growth and lipid accumulation, not many have studied the feasibility of accumulation of lipid and fatty acids and compositional changes under carbon-limitation in the second stage. We evaluated the energy ratio during lipid productivity under CO_2 limitation reducing de novo synthesis of fatty acids and assessing the lipid accumulation based on the organism's ability to undergo lipid remodeling of its resources.

The ultimate goal of this study was to improve the energy ratio in the IIPBR by evaluating the following two premises: (i) biomass productivity could be maximized by optimizing the carbon supply; and (ii) lipid content of biomass could be improved through stressing the cultures under nutrient starvation.

To validate the first premise, growth of two algal species in the IIPBR under sparging at various CO_2-air ratios was evaluated. Studies in the past have identified carbon, which constitutes 40–50% by mass of algae, as one of the main limitations to algal growth besides light (19). Carbon supply should be optimized so that it neither limits nor inhibits the growth; the level is strain-specific. For example, in a bubble columns study, Hsueh et al. (20) reported increase of 135% in the biomass under sparging with 5% CO_2-enriched air (CEA) compared to sparging with ambient air; and, increase of 200% under sparging with 8% CEA. Further increase in enrichment to 10% resulted in inhibition, with pH falling below 5. In another study, Ryu et al. (21) evaluated the effect of CEA of 0.5%, 1%, 2% and 5% with *Chlorella* sp. cultures and reported that, compared to the biomass obtained at CEA of 0.5%, biomass increased by 34% at 1% CEA; by 55% at 2% CEA; and by 75% at 5% CEA. While sparging with CO_2-enriched air is seen to increase productivity, the related costs also will increase. As such, an optimal enrichment has to be provided so that growth can be maximized. Thus, the first part of this study was to optimize the operation of the IIPBR in terms of the CO_2-air ratio in the sparging gas.

To validate the second premise, two alternate strategies were evaluated: (i) stressing under carbon-rich/nutrient-limiting condition in a single-stage; and (ii) carbon-limited/nutrient-limited condition in the second stage of a two-stage cultivation scheme. Several studies have shown that light intensity and nutrients such as nitrates, phosphates, CO_2 supply not only aid in cell and chlorophyll formation, but also alter the biochemical pathways and formation of cellular components such as proteins, lipids, and carbohydrates (15,22). Stressing the cultures by limiting the light and nutrients can alter the biochemical composition of algae, favoring storage of energy-rich metabolites (15). To the best of our knowledge none of the previous studies had considered carbon-limitation as a means of stressing and the duration of stressing as a means of altering the fatty acid composition and maximizing the energy ratio.

Since the intent of this study was to evaluate and compare the effects of specific operating conditions, the tests were conducted under laboratory conditions under fixed light input to avoid the variations typically encountered under outdoor conditions. As such, the energy ratios estimated in this study including the light energy input, are quite low. Nevertheless, the absolute values of the energy ratios served as a rational measure, not only to assess the outcomes of different test conditions in this study, but also to compare the results of this study with those from several laboratory studies reported in the literature.

MATERIALS AND METHODS

Algal strains The two algal strains chosen in this study were *Scenedesmus* sp. and *Nannochloropsis salina*. Growth media for two species were prepared as described elsewhere (6) and autoclaved. The inocula for both algal cultures were grown in an incubator. The first-stage reactor (IIPBR) was seeded with the inoculum diluted with the nutrient media to achieve initial optical density at 750 nm (OD_{750nm}) greater than 0.15.

Internally illuminated PBR The details of the IIPBR tested in this study and its principle of operation have been described previously (6). In this study, the culture volume was fixed at 18 L; mixing was provided by sparging with CO_2-enriched air from the bottom of the annular space via four porous silica air diffusers. The air supply was sterilized (0.2 μm Millipore filter paper) and measured with gas proportioner (EW-03218-50 Cole Parmer flow meter system). CO_2 gas flow rate was measured by a CO_2 mass flow meter (00261BY, Cole Parmer) in standard mL min^{-1}. The CO_2-air ratio was a variable in the experiments.

Algal growth measurements Algal growth was tracked daily in terms of OD_{750nm} and converted to dry weight through previously established correlations. Culture samples from the IIPBR were diluted with deionized water (23,24) prior to O.D. measurements to ensure that the spectrophotometer readings were below 0.5. Algal dry weight was determined by centrifuging the wet algal cells as described previously (6). In the case of the marine algae, *N. salina*, same procedure was repeated except for washing and centrifuging the settled algal samples twice to minimize salts. Besides growth measurement, pH and temperature were monitored using Mettler Toledo M300 pH transmitter placed in the culture. During all the tests under laboratory conditions, the temperature ranged between 26 °C and 27 °C. Light measurements were taken daily as described previously (6).

Lipid and fatty acid methyl ester (FAME) analysis Total lipids, which are otherwise defined as organic solvent (such as hexane, ether) soluble fraction of a matrix of the algal cultures were estimated by the extraction of organic soluble solutes from dried tissue using a mixture of $CHCl_3$ and MeOH based on the Folch method of lipid extraction (25). The lipid extraction is performed using an accelerated solvent extraction (ASE) system using Dionex 350 (Dionex Corporation, Salt Lake City, UT, USA) by Mulbry et al. (26). Briefly, 0.25 g of dried algal samples were mixed with 30 g of Ottawa sand and loaded into 33 mL sample cells. The mixtures of algae and sand were extracted using chloroform:methanol (2:1, v/v) at 120 °C and pressure of ~1500 psi for 5 min and transferred to a pre-weighed 60 mL collection vials. The increased temperature was adapted from ASE method (26). The extraction is performed under an inert atmosphere to prevent lipid oxidation. The collection vials with extract solutions were dried under a stream of nitrogen to estimate the lipid content gravimetrically. Within the context of this study we opted for optimal lipid yield and productivity with disregard to co-product production. All lipid extracts were stored under nitrogen at −20°C. The fatty acid profiles of the cultures were analyzed base catalyzed direct transesterification of algal tissue. The results represent the fatty acid profile of the bound esterified fatty acids and not the free fatty acid content. Briefly, 10 μL of glycerol tritridecanoate (13:0 FAME standard at 20 mg mL^{-1} in hexane) is added to 50 mg of dry algal tissue. Then 5 mL of 0.2 N KOH in MeOH was added and the mixture vortexed for 20 s and finally placed in hot water bath at 65°C for 10 min (with additional vortexing for 30 s). These last two steps were repeated three times total. To quench the reaction, 1 mL of 1-M acetic acid was added to each sample and vortexed for 20 s. Two milliliters of hexane with an internal standard methyl tricosanoate at 50 mg L^{-1} was added to each sample vial. Each sample was vortexed for 20 s and two phases are separated by centrifugation. The top hexane layer was taken for the GC–MS analysis. For GC/MS, helium was used as the carrier gas with a 2-μl injection volume. The temperature ramp started at 80°C and ramped 20°C min^{-1}–220°C and held for 6 min for a total run time of 13.3 min. The instrument was tuned with a standard spectra auto tune method, and a calibration curve was made from a Supelco component FAME mixture (cat no. 47885-U, Sigma Aldrich, St. Louis, MO, USA).