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ENGINEERING SCIENCES

Development and evaluation of low-cost flat plate photobioreactors for microalgae and cyanobacteria cultivation with biotechnological potential

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Abstract: The high performance of biomass and metabolite biosynthesis by photosynthetic microorganisms is directly influenced by the cultivation system employed. Photobioreactors (PBRs) stand out as controlled and fundamental systems for increasing the production of biocompounds. However, the high costs associated with these systems hinder their viability. Thus, a more practical and economical approach is necessary. Accordingly, this study aimed to design and evaluate low-cost flat-panel photobioreactors on a laboratory scale for the cultivation of photosynthetic microorganisms, using economical materials and instruments. Additionally, internal optimization of the low-cost system was aimed to maximize growth and biomass production. The PBRs were designed and built with uniform dimensions, employing 4 mm translucent glass and agitation through compressors. The internally optimized system (PBR-OII) was equipped with perforated acrylic plates used as static mixers. To evaluate the performance of the low-cost PBR-OII, a comparison was made with the control photobioreactor (PBR-CI), of the same geometry but without internal optimization, using a culture of *Synechocystis* sp. CACIAM 05 culture. The results showed that the PBR-OII achieved maximum biomass yield and productivity of 6.82 mg/mL and 250 mg/L/day, respectively, values superior to the PBR-CI (1.87 mg/mL and 62 mg/L/day). Additionally, the chlorophyll concentration in the PBR-OII system was 28.89 ± 3.44 µg/mL, while in the control system, the maximum reached was 23.12 ± 1.85 µg/mL. Therefore, low-cost photobioreactors have demonstrated to be an essential tool for significantly increasing biomass production, supporting research, and reducing costs associated with the process, enabling their implementation on a laboratory scale.

Key words: Biomass productivity, closed systems, optimization, static modules, *Synechocystis*.

INTRODUCTION

The increasing consumption of non-renewable resources has driven the search for alternative sources of green energy. One of these bioeconomic and promising sources is the cultivation of photosynthetic microorganisms, such as cyanobacteria. Cyanobacteria are considered biological factories due to their rapid growth rates and ability to produce various biocompounds with high added value, among other characteristics. The biomass produced by cultivating these microorganisms can be applied in

various industries, such as energy, pharmaceuticals, and nutraceuticals, among others (Clagnan et al. 2022, Merlo et al. 2021).

Commonly, these microorganisms are cultivated in open tanks or closed photobioreactors (PBRs) (Wang et al. 2014). However, open systems are vulnerable to contamination and unfavorable weather conditions. On the other hand, for effective and quality production, aiming at obtaining products with high added value, it is necessary to cultivate in highly controlled systems, such as photobioreactors (Gómez-Pérez et al. 2015, Milano et al. 2016, Sun et al. 2016).

Photobioreactors with flat plates have been widely used to cultivate photosynthetic microorganisms due to their simple structure, large illuminated surface area (A) in relation to volume (V), shorter light/dark (L/D) cycle, and easy scaling, among other parameters (He et al. 2016, Hinterholz et al. 2019, Tamburic et al. 2011, Vasumathi et al. 2012, Wang et al. 2014). However, many problems hinder the use of these systems due to their high cost, making the competitiveness of the products obtained unfeasible in the market. Therefore, it is necessary to build systems that are easy to operate and that can provide high biomass yields (Guo et al. 2015, Huang et al. 2017, Milano et al. 2016, Wang et al. 2014).

The growth of these microorganisms requires a certain amount of light energy. However, light intensity inside the systems tends to decrease with the distance from the irradiated surface. Thus, mixing conditions play an important role in optimizing photobioreactors (Huang et al. 2015). The static modules intensify the performance of the process, promoting the movement of cells between the lighted zones (areas with good lighting) and dark zones (areas with low lighting). Therefore, the better the light/dark cycle of a cropping system, the greater the possibility of high yields through photosynthetic microorganisms (Wang et al. 2014, Zhang et al. 2013). Huang et al. (2015) developed a cultivation system with static modules, intending to improve the system's mixing, aiming to reduce the L/D cycle, and obtained maximum biomass concentrations by cultivating *Chlorella pyrenoids* of 32.8%, corresponding to 0.89 g/L. In the same way, Zhang et al. (2013) optimized horizontal flat plate PBR with inclined baffles and obtained biomass productivity of *Chlorella* sp., about 29.94% higher than the control.

Synechocystis sp. is a freshwater cyanobacterium belonging to the phylum of unicellular cyanobacteria, classified in the class *Cyanophyceae* and in the family *Merismopediaceae*. It is recognized as a species of significant importance and has been the subject of extensive study since the sequencing of its genome. *Synechocystis* has rapid growth and is often employed as a model microorganism in various photosynthetic studies and for potential biotechnological applications (Bahman et al. 2022, Hagemann & Hess 2018, Liu et al. 2019). Aboim et al. (2019) used several strains of Amazonian cyanobacteria for the production of biofuels and biomass, including the strain *Synechocystis* sp. CACIAM05, and found that this strain, when subjected to light stress and supplemented with $NaNO_3$, demonstrated great potential for biomass and biodiesel production.

In this context, the present study aimed to design and evaluate low-cost flat plate photobioreactors on a laboratory scale. Additionally, internal system optimization was sought with static mixers to improve the internal flow performance of the PBR, aiming to reduce the C/E cycle and increase biomass productivity through cultivation of the cyanobacterium *Synechocystis* sp. CACIAM 05. However, it is worth noting that the low-cost system can be used for a variety of photosynthetic organisms.

MATERIALS AND METHODS

Specifications of the design and construction of photobioreactors

The flat plate photobioreactors developed in this work represent a low-cost approach to cultivating photosynthetic microorganisms on a laboratory scale. A schematic diagram of the systems is shown in Figure 1. The photobioreactors were designed and built from 4 mm translucent glass. Both have the same dimensions, 0.35 m in length, 0.3 m in height, and 0.05 m in width, with a useful volume of 4.2 L and an area/volume ratio of 20 m^{-1} (Figure 1a and 1c). To increase biomass productivity, a system was internally optimized with static modules (PBR-OII) (Figure 1b). In this study, the static modules were designed to be easy to install, use, maintain, and clean. The four modules were constructed from 3 mm acrylic, with a length of 21 cm and a height of 4.25 cm. The modules have three rows of evenly spaced holes with a diameter of 0.3 cm. They were spaced equidistantly from each other at 6 cm with an alternating configuration forming five regions. The performance of the PBR-OII was evaluated against a control photobioreactor (PBR-CI) of the same size and working volume, but without internal modules.

The photobioreactor's artificial lighting was obtained through the construction of a panel with five cold white tubular fluorescent lamps (OuroLux, T10, 20 W, 6400 K). The lamps were arranged horizontally, with an average distance of 4 cm from each other. The five lamps emit a maximum light intensity of 800 µmol photons m^{-2} s^{-1}. The system was stirred by injecting air into the lower part of the photobioreactors through aquarium compressors (Model S series silent, Air Pump, S-510s, 3 W), with a flow rate of 4 L/min connected by a PVC hose (3/16" x 1 mm) to an air curtain with microholes along its length (Model Air curtain - Boyu). The photobioreactor lids were designed with gas inlets and outlets, with a diameter of 2 cm, and the sides of the PBRs were equipped with an outlet for sampling.

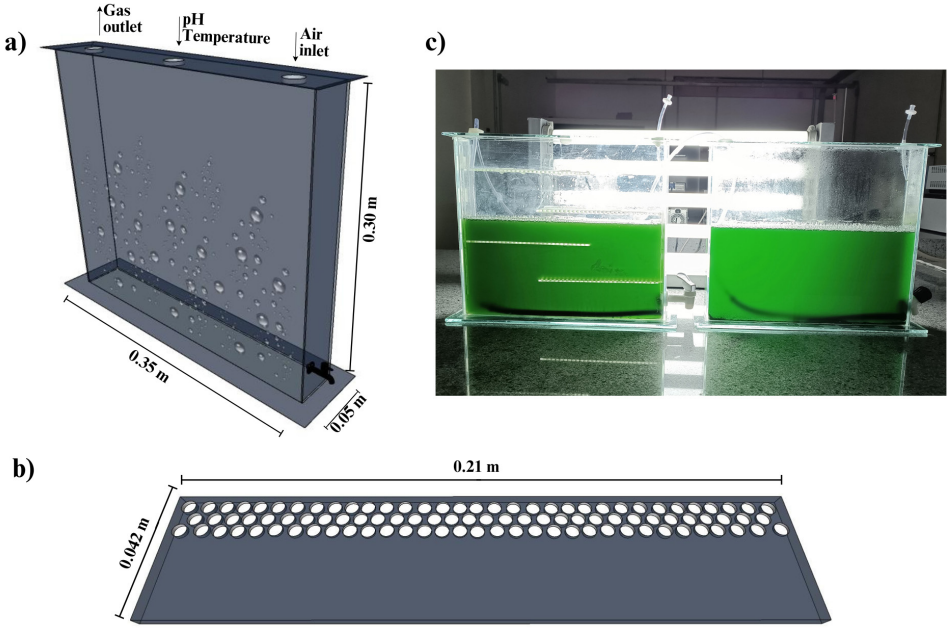

Figure 1. Design configurations of the low-cost flat-plate photobioreactor. (a) 3D scheme of low-cost photobioreactors, 3D scheme of internal optimization modules (b), and (c) photobioreactor with internal optimization and control, respectively.

Microorganism, inoculum and growth conditions

In this research, the lineage *Synechocystis* sp. CACIAM 05 (GenBank accession number: MG272377.1), belonging to the Amazonian Collection of Cyanobacteria and Microalgae (CACIAM), provided by the Biomolecular Technology Laboratory (LTB), of the Universidade Federal do Pará (UFPA), collected from Lake Bolonha, located in the metropolitan region of Belém-PA, was used to verify the reliability and performance of the designed low-cost photobioreactors. The sterilized BG-11 growth medium (Allen 1968) was used during the experiments, and the stock culture was cultivated in 1 L Erlenmeyer flasks containing 350 mL of BG-11 medium for a period of 12 days. The experiments were conducted in batch mode over 20 days, with a photoperiod of 13:11 hours (light:dark), at a temperature of 20 ± 1°C. The light intensity used was 100 µmol photons m^{-2} s^{-1}, measured using a digital luxmeter (model MLM-1011, Minipa), and the growth medium was supplemented with 2 g/L of sodium nitrate ($NaNO_3$) (Aboim et al. 2019). Each photobioreactor was inoculated with 350 mL of inoculum and 2 L of BG-11 medium. The initial biomass concentration was 0.092 mg/mL. The cultivation was carried out in a batch for 20 days, the growth of the cyanobacteria was expressed by the concentration of chlorophyll *a* measured every 2 days, and the system's productivity was estimated through analysis of dry biomass.

Analytical methods

Analysis of chlorophyll content

The determination of growth by quantification of chlorophyll *a* was performed using the protocol of Meeks & Castenholz (1971) with modifications of Fiore et al. (2000). Thus, 3 mL aliquots were collected from each container and centrifuged for 4 minutes at 6.000 rpm. Subsequently, the supernatant was discarded and the pellet collected. 3 mL of a 9:1 methanol and water solution were added to the pellet. The sample was then homogenized and left for 15 minutes in a dark environment for maximum chlorophyll *a* extraction, then, the contents were centrifuged at 6.000 rpm for 15 minutes. Absorbance was analyzed at a wavelength of 663 nm using a UV-Visible spectrophotometer (Model Thermo Scientific Evolution Array). All procedures were performed in triplicate. The chlorophyll *a* /methanol ratio was determined by equation 1. In which C is the concentration of chlorophyll *a* present in the sample, A is the absorbance obtained by the analysis in the spectrophotometer, and 12.7 is the absorbance coefficient determined for the extraction of chlorophyll with methanol.

$$C\ (\mu g/mL) = A \times 12.7 \quad (1)$$

Determination of biomass and specific growth rate

The biomass of the systems was determined by means of dry-weight analyses. In which, 2 mL aliquots were collected, washed with ultrapure water in the same proportion, and centrifuged for 10 minutes at 6000 rpm. Subsequently, the supernatant was discarded, and the *pellet* was suspended with 100 µL of ultrapure water. The sample was then transferred to a previously weighed container and dried for 24 h at 40 °C until a constant weight was obtained. The dry weight of the biomass was obtained by weighing the sample on a precision balance and determined by subtraction. After obtaining the dry weight, the biomass productivity of both systems was calculated using equation 2 (Fiore et al.

2000). The specific growth rate (μ, d^{-1}) was determined according to equation 3 based on the biomass concentration in an appropriate growth phase (Shahid et al. 2021).

$$P\ (mg/L/day) = (X_t - X_0)/(t - t_0) \quad (2)$$

Where X_t and X_0 represent the biomass concentration at the beginning (t_0) and at the end of the experiments (t), respectively.

$$\mu = \ln (X_t/X_0)/(t - t_0) \quad (3)$$

Statistical analysis

Measurements were performed in triplicate and data were represented as mean ± standard deviation. For the analysis of the variance of the obtained data, the *t*-test with a p-value ≤ 0.05 was used. Statistical calculations and graphical representations were performed using *Minitab software*.

RESULTS

Evaluation of growth in low-cost systems

To assess the performance of the low-cost systems PBR-OII and PBR-CI, the growth dynamics of the cyanobacterium *Synechocystis* sp. CACIAM 05, in terms of chlorophyll *a* (Chl *a*) concentration, were monitored for 20 days (Figure 2). From the graphs analyzed, the system's internal characteristics directly influenced the cultivation of cyanobacteria. Thus, the PBR with static modules (PBR-CI), demonstrating significant promoting effects on growth ($p \leq 0.05$) from the 2nd day of cultivation. The maximum Chl *a* content was attained in PBR-OII after 14 days of cultivation (28.89 ± 3.44 μg/mL). This represented a significant difference compared to PBR-CI, which reached a concentration of 23.12 ± 1.85 μg/mL in the same period. After this period, the growth rate of the cultures gradually decreased, reaching the end of the batch with 21.17 ± 1.28 μg/mL and 16.13 ± 3.50 μg/mL, respectively.

Low-cost photobioreactors promoting biomass yield

The biomass production continuously increased in the low-cost photobioreactors until the 14th day, after which the culture entered a decline phase. On the 14th day, within the PBR-OII system, the strain CACIAM 05 reached a maximum biomass yield of approximately 6.82 ± 0.79 mg/mL (Figure 3), with a biomass productivity of 250 mg/L/day and a daily growth rate of 0.21, showing significant promoting effects ($p<0.05$). This value was approximately three times higher than that obtained by the PBR-CI system, which recorded a production of 1.87 ± 0.14 mg/mL, productivity of 60 mg/L/day and a daily growth rate of 0.08. By the end of the batch (20th day), the internally optimized system with static modules experienced a reduction in biomass, reaching a concentration of 5.10 ± 0.58 mg/mL and a productivity of 170 mg/L/day. Meanwhile, the control photobioreactor achieved 1.25 ± 0.12 mg/mL and 32 mg/L/day, respectively.

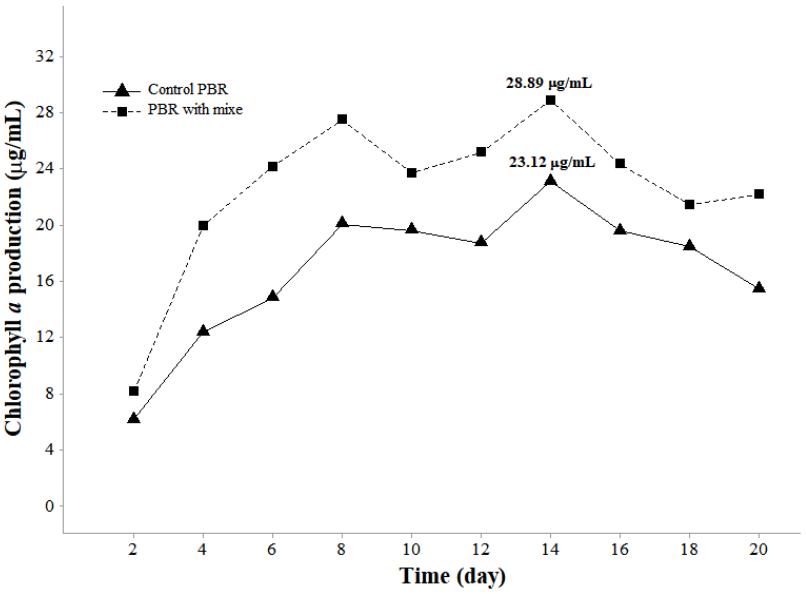

Figure 2. Growth curve by extraction of chlorophyll *a*, 20 days of batch time for the internally optimized photobioreactor and control. Data are mean values of three replicate cultures.

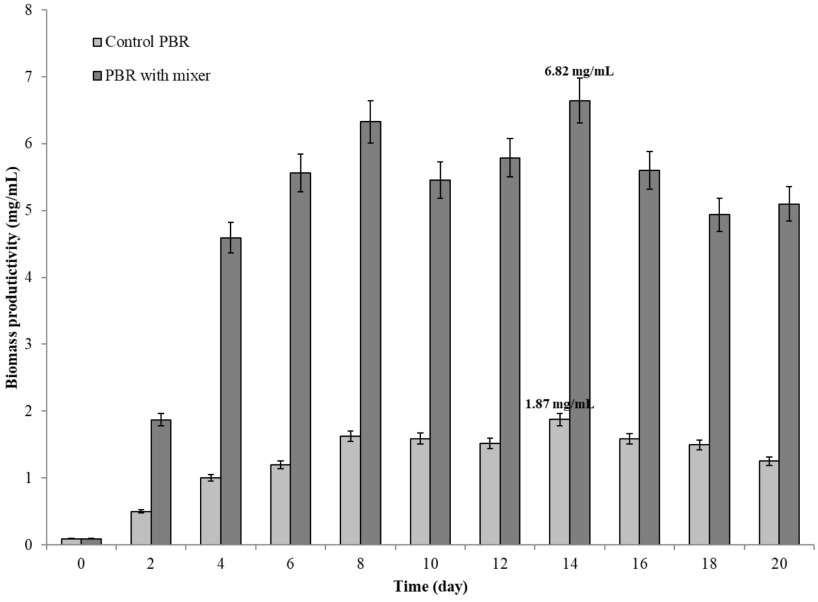

Figure 3. Dry weight concentration for the photobioreactor with static module and control. Data are mean values of three replicate cultures ± standard deviation. The average value of biomass production for the photobioreactor with static modules and the control represent statistical significance, $p \leq 0.05$.

DISCUSSION

Photobioreactors are highly controlled systems in which it is possible to maintain ideal growth conditions. However, these systems come with a high cost, which directly influences the process's feasibility (Milano et al. 2016, Huang et al. 2017). Therefore, this study presented low-cost photobioreactors at a laboratory scale capable of sustaining high biomass yields. Cyanobacteria have been gaining great notoriety due to the possibility of obtaining bioproducts from their cultivation and biomass production (Fernandes et al. 2020, Koller et al. 2014, Kothari et al. 2017). Cyanobacterial pigments, such as chlorophylls, play essential roles in the development of photosynthetic

microorganisms, being crucial in light absorption and its conversion into biochemical energy (Ma et al. 2019, Zhou et al. 2022).

Chlorophyll *a* is one of the main growth indicators. This pigment is directly related to the photosynthetic efficiency of the cultivated microorganism. They are vital for the absorption of light in the photosynthesis process, as well as for the adaptation of the microorganism to different environments (Lakatos et al. 2021, Lau et al. 2022).

The results showed that internal optimization directly influenced the growth of the cyanobacterium CACIAM 05. In which, the PBR-OII reached a maximum chlorophyll concentration of 28.89 ± 3.44 µg/mL corresponding to the 14th day. Meanwhile, in the same period, the control system reached 23.12 ± 1.85 µg/mL (Figure 2).

This increase in growth can be attributed to the ability of optimization modules to enhance light utilization efficiency, directly influencing system mixing and contributing to reducing the disparity in algae growth. In environments where the light source is distant, a light gradient resulting from light absorption and scattering is observed, leading to a distinction between a well-lit area (light zone) and a less illuminated area (dark zone) within the photobioreactor. Unfavorable lighting conditions in the dark zone are attributed to increased optical path length, resulting in a longer path for light. Therefore, the more efficient the light/dark cycle, the higher the algae productivity that can be achieved (Ye et al. 2020, Huang et al. 2015, Wang et al. 2014). However, after the 14th day, a decline in the growth of the cyanobacterium *Synechocystis* sp. CACIAM 05 was observed in both systems, indicating the onset of the cellular decline phase.

It was also observed that the internally optimized low-cost system with static modules achieved a higher biomass concentration (6.82 ± 0.79 mg/mL) compared to the control (1.87 ± 0.14 mg/mL) during the same period (Figure 3). Additionallly, the maximum productivity in PBR-OII was 250 mg/L/day, approximately 4.18 times higher than the productivity of the PBR-CI system (60 mg/L/day), demonstrating that the use of static modules not only improves the capture of light energy by cyanobacteria but also increases the chances that cells can move from the dark region to the illuminated surface, thus having a continuous flow in the system and higher productivity. The data found are consistent with the literature (Table I).

Wang et al. (2014), investigating the impact of static deflectors on validating flat plate photobioreactors using *Chlorella vulgaris* 31 culture, achieved a maximum chlorophyll *a* concentration of 0.153 µg/mL in the optimized photobioreactor. In contrast, in the control photobioreactor, the concentration reached was 0.081 µg/mL. Additionally, the maximum biomass productivity in the optimized bioreactor was 1.88 times higher than that obtained in the bioreactor without optimization modules. Huang et al. (2015) achieved a maximum cell concentration in the internally optimized flat plate photobioreactor, reaching a biomass of 0.89 g/L, representing an 11.3% increase compared to the non-optimized photobioreactor. Therefore, experimental results suggest that cultivating cyanobacteria in low-cost laboratory-scale photobioreactors using static deflectors is more effective than in non-optimized photobioreactors. The incorporation of deflectors in the photobioreactor has a direct impact on the growth of photosynthetic microorganisms and biomass production, optimizing light distribution, speed, and homogenization of the culture medium.

Table I. Comparison of biomass and chlorophyll production in photobioreactors (PBR) with internal optimization with literature.

PBR	Strain	Maximum chlorophyll concentration		Maximum biomass concentration		Reference
		PBR with optimization	PBR without optimization	PBR with optimization	PBR without optimization	
Flat panel	*Synechocystis* sp. CACIAM 05	28.89 µg/mL	23.12 µg/mL	6.82 mg/mL	1.87 mg/mL	(This study)
Flat panel	Chlorella vulgaris 31	0.154 µg/mL	0.0018 µg/mL	-	-	(Wang et al. 2014)
Flat panel	*Chlorella pyrenoidosa*	-	-	0.89 mg/mL	0.67 mg/mL	(Huang et al. 2015)

CONCLUSIONS

The results showed that the static modules in the low-cost photobioreactor not only effectively influence and enhance productivity but also can standardize the growth conditions of microorganisms in the system. The biomass concentration and maximum productivity obtained through the low-cost flat plate photobioreactor with static modules were approximately 3.65 and 4.18 times higher, respectively than the control. Therefore, it can be inferred that internal optimization and low-cost systems are of great value for the effective obtainment of photosynthetic microorganism biomass at a laboratory scale .

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RUTILÉIA J. PAIVA: design and construction of photobioreactors, conceptualization, methodology, data analysis, and writing. DEBORAH T. DE OLIVEIRA: methodology and data analysis. VANESSA A. DE MESCOUTO: conceptualization, experiments, and draft writing. AGENOR V. SANTOS and EVONNILDO C. GONÇALVES: structure and experimental part. RENATA C. R. NORONHA: visualization, writing revision, financing. LUÍS ADRIANO S. DO NASCIMENTO: conceptualization, write review, structure, and financing.