A NOVEL BENCH-SCALE PHOTOBIOREACTOR FOR CONTINUOUS CULTIVATION OF MICROALGAE

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There are different types of carbon intensive industries. While some operate with daily intervals, others must be maintained in continuous operation, sometimes for weeks or months. Processes that depend on microbiological activity are usually of the second category, resulting in the continuous production of CO_2 during extensive periods of time. In order to help mitigate climate change, alternative methods of carbon capture into added-value products have been the focus of research. Autotrophic microalgae cultures can be employed to sequester the carbon present in these streams, generating new products, while increasing process sustainability. However, to sequester these emissions microalgal bioreactors must also function under continuous constant conditions, requiring photobioreactors (PBRs) that can act as chemostats for long periods of time. Moreover, there is currently a lack of studies and design alternatives using microalgal chemostats. Most works tend to focus on batch assays or semi-continuous processes, presenting different responses depending on the growth stage of the culture, or the daytime. The present work is centred on the development of a novel continuous bench-scale PBR. This system uses an innovative recirculation concept to combine three different units (retention vessel, photocollector and degasser) that operate as a single autotrophic chemostat, allowing the study of carbon sequestration from a biogenic CO_2-rich constant air stream. The novel PBR was tested by cultivating the microalga *Haematococcus pluvialis* at different dilution rates (0.1–0.5 d^{-1}), while using as sole carbon source an air stream containing ≈0.35 vol% of CO_2 (produced by a coupled heterotrophic bacterial chemostat). The results obtained revealed that the system could operate as a chemostat, allowing the production of stable cultures with proportional responses to the changes in dilution rates for more than 3 months, reaching a maximum biomass productivity of 183 mg/L/d, with a carbon fixation efficiency of ≈39% at 0.3 d^{-1}. This makes the PBR prototype a promising tool to study/optimise integrated heterotrophic and autotrophic continuous processes, or constant sequestration of stable CO_2-rich streams, making it easier to gather data for future scale-up.

Material and Methods

Microalga and culture medium: *Haematococcus pluvialis* Flotow seed culture used in this study was supplied from "ALISU" – Coleção de Algas da Universidade de Lisboa (FCUL, Lisbon, Portugal). Stock cultures were propagated and maintained in synthetic Bold's Basal Medium (BBM), without vitamin solution (Stein, Handbook of Phycological methods. Culture methods and growth measurements. 1980, Cambridge University Press) at 25°C and low photon flux density (≈20 µmol/m^2/s) with a photoperiod of 14 h. This culture medium was used for all experiments performed.

Source of biogenic CO_2: The biogenic CO_2 used was generated by a continuous culture of the bacterium *Gordonia alkanivorans* strain 1B, under the conditions described by Tavares et al. (Colloids Surf. B. 2021, 208, 112111). Atmospheric air was continuously fed to the aerobic heterotrophic bacterial chemostat, to provide oxygen for culture development, generating a constant flow of 0.95 L/min of exhaust gases containing an average of 0.35 ± 0.03 vol% CO_2 and 20.5 ± 0.1 vol% O_2, which was supplied to the PBR system to be used as sole carbon source.

Photobioreactor design and assembly: The closed PBR developed in this study, illustrated in Fig. 1 of Results & Discussion section, integrated three main units: a retention vessel (Fig. 1, 10), a photocollector unit comprising 6 bubble columns, *i.e.,* 6 x 1 L vertical transparent glass cylinder (H 475 mm; Ø 70mm; NS 45/40), with a hexagonal base and fitted with a gas wash head (Fig. 1, 12), and a degasser container (Fig. 1, 14). In the experiments carried out along this work, the PBR was operated in continuous mode. About 0.6 L of microalgae culture were used to inoculate the PBR, totalizing 6 ± 0.2 L of working volume. The inoculated system was left to grow in batch mode, with a recirculation rate of about 0.67 L/min and an aeration input of 0.158 vvm, corresponding to an air flow of 0.95 L/min. Temperature and pH were maintained at 21 ± 1°C and 7.5 ± 0.2, respectively, and the culture was continuously illuminated with a light intensity of about 2000 lux (≈50 µmol/s/m^2) measured at the surface of each photocollector cylinder (Tavares et al., J. Environ. Manage. 2023, 332, 117418). The selected conditions were previously defined as appropriate to produce *H. pluvialis* green cells (García-Malea et al., Biotechnol. Bioeng. 2009, 102, 651–657; Fábregas et al., J. Biotechnol. 2001, 89, 65–71). The growth in the microalgae culture was monitored through optical density at 600 nm (OD_{600}) measurements, until exponential phase was achieved. Thereafter, the PBR was continuously fed with fresh BBM medium, at different dilution rates (D) ranging from 0.1 to 0.5 d^{-1}, corresponding to a hydraulic residence time (HRT) between 240 h and 48 h, respectively. Conditions were maintained for at least the time necessary for three complete turnovers of total working volume, to ensure that the response was stable and the chemostats achieved steady state. Throughout the assays, the growth parameters were monitored, and inlet and outlet gases were analyzed daily. At least four analytical replicates from at least two independent culture samples were considered for the analysis of each steady state (±SD).

Results and Discussion

1. PBR advantages

- A key aspect of this novel PBR is the unique recirculation process between its three main units (**Fig. 1**). The recirculation between the retention vessel (10), the photocollector system (12) and the degasser (14) is kept constant throughout the tests, continuously, regardless of the dilution rate, since it is controlled by a Brushless Water Pump, 12V, 19W (5), allowing the integrated system to function as a single chemostat and not as several sequential continuous vessels.

2. Continuous cultivation of *Haematococcus pluvialis*

2.1. Growth kinetic parameters

- Kinetic parameters for the growth of *H. pluvialis* in continuous cultivation in the new recirculating PBR system, at the steady state of each dilution rate tested (0.10 – 0.50 d^{-1}).

Kinetic parameters	Dilution rates (d^{-1})					
	0.10	0.15	0.20	0.30	0.40	0.50
DCW (g/L)	0.99 ± 0.01	0.93 ± 0.08	0.81 ± 0.08	0.61 ± 0.02	0.42 ± 0.02	0.39 ± 0.06
Px (g/d)	0.56 ± 0.00	0.82 ± 0.07	0.94 ± 0.09	1.06 ± 0.03	0.99 ± 0.04	1.04 ± 0.11
Pv (g/L/d)	0.096 ± 0.00	0.142 ± 0.01	0.163 ± 0.02	0.183 ± 0.00	0.171 ± 0.01	0.180 ± 0.02
AC (g/L/d)	0.386 ± 0.04	0.463 ± 0.00	0.463 ± 0.06	0.502 ± 0.01	0.463 ± 0.07	0.568 ± 0.02
PC (g/L/d)	0.337 ± 0.00	0.337 ± 0.00	0.337 ± 0.00	0.337 ± 0.00	0.337 ± 0.00	0.337 ± 0.00
P_{CO2} (g/d)	2.32 ± 0.21	2.78 ± 0.00	2.78 ± 0.37	3.01 ± 0.08	2.78 ± 0.39	3.41 ± 0.10
CFE (%)	32.7 ± 3.3	31.4 ± 2.7	36.0 ± 1.8	38.5 ± 0.7	39.9 ± 1.1	36.7 ± 3.4
Y (g/g)	0.24 ± 0.02	0.30 ± 0.02	0.34 ± 0.05	0.35 ± 0.01	0.36 ± 0.05	0.31 ± 0.03

DCW – Dry cell weight of biomass; Px – Biomass productivity; Pv – Volumetric productivity; AC – CO_2 assimilation capacity; PC – Daily O_2 production capacity; P_{CO2} - Daily CO_2 fixation; CFE – Carbon fixation efficiency; and Y – Biomass yield (Biomass/CO_2).

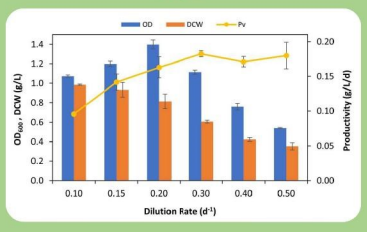
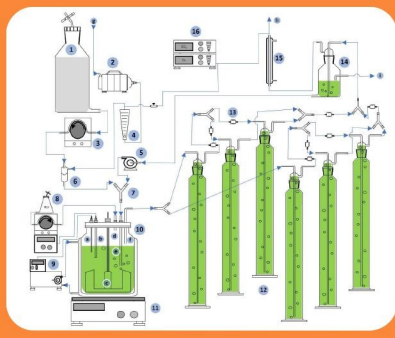


Fig. 2. The optical density (OD_{600}), biomass concentration (DCW) and volumetric productivity (Pv) of *H. pluvialis* in continuous culture within the new recirculating PBR, as a function of increasing dilution rates.

PBR prototype



(1) bottle of fresh medium; (2) air compressor: g) air flow from a heterotrophic bioreactor (CO_2 inflow) ; (3) peristaltic pump; (4) precision rotameter; (5) culture recirculation pump; (6) drip chamber; (7) Y-connector; (8) pH controller; (9) thermostatic water bath; (10) retention vessel: a) and b) temperature and pH sensors, respectively, c) magnetic stirring propeller, d) pH controller inlet, e) air and culture inlet, f) air driven outflow leveling; (11) magnetic stirrer; (12) photocollector - bubble columns; (13) coupling sockets/plugs; (14) degasser: i) overflow tube to harvest (sampling/ collection bottle); (15) condenser: h) exhaust airflow; (16) gas analyzer.

Fig. 1. Schematic diagram of the benchtop PBR.

2.2. Kinetics of CO_2 consumption and O_2 release

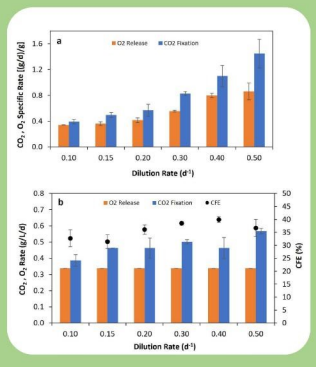


Fig. 3. Dynamics of CO_2 sequestration and O_2 release (a - specific rate; b - rate) by *H. pluvialis*, as a function of the dilution rate, in continuous culture in PBR.

- **Fig. 3a** demonstrates that increasing dilution rates resulted in an increase in terms of oxygen production rate per gram of microalga (O_2 release specific rate = A_{O2}) and CO_2 consumption rate per gram of microalga (CO_2 fixation specific rate = A_{CO2}). In both cases, the lowest values were obtained with 0.1 d^{-1} dilution rate (0.342 $g_{O2}/d/g_{DCW}$ and 0.392 $g_{CO2}/d/g_{DCW}$, respectively); however, while A_{O2} increased 2.5 times, from 0.1 to 0.5 d^{-1} (to 0.86 $g_{O2}/d/g_{DCW}$), A_{CO2} increased 3.7 times (to 1.45 $g_{CO2}/d/g_{DCW}$), for the same dilution range.
- **Fig. 3b:** CO_2 fixation rate (or CO_2 assimilation capacity = AC) ranged from 0.386 g/L/d at the lowest dilution rate (0.1 d^{-1}) to 0.568 g/L/d at the highest dilution rate tested (0.5 d^{-1}), which seems to indicate that higher dilution rates result in increased fixation rates.
- CFE ranged from 31.4 ± 3.3% at 0.15 d^{-1} dilution rate to 39.9 ± 1.1% at 0.4 d^{-1} dilution rate. Overall, the range of CFE values obtained are consistently better than those reported for phototrophic microalgae cultures in most closed PBRs, which are usually less than 30% (Kumar et al., Trends Biotechnol. 2010, 28 (7), 371–380).

Conclusions

- The PBR system developed stands out from commonly reported single-vessel benchtop PBRs for its innovative recirculation concept, which integrates three different units (retention vessel, photocollector system composed by 6 bubble columns and degasser) in a single recirculating system.
- Using a constant air flow rich in biogenic CO_2, the novel PBR was able to operate under chemostat conditions for more than 100 days, producing a stable microalgae culture that generated proportional responses to the stimuli it was subjected to, attaining a maximum biomass productivity of 183 mg/L/d with a carbon fixation efficiency of ≈39 % at 0.3 d^{-1}. These results reinforce the effectiveness of this PBR system, making it suitable for laboratory-scale studies of continuous photoautotrophic microalgae cultivation.
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