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(Article begins on next page)

1 **Design and characterization of a new pressurized flat panel photobioreactor for**
2 **microalgae cultivation and CO₂ bio-fixation.**

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12 **Abstract**

13 Microalgae-based biorefinery processes are gaining particular importance as a
14 biotechnological tool for direct carbon dioxide fixation and production of high-quality
15 biomass and energy feedstock for different industrial markets. However, despite the many
16 technological advances in photobioreactor designs and operations, microalgae cultivation
17 is still limited due to the low yields achieved in open systems and to the high investment
18 and operation costs of closed photobioreactors. In this work, a new alveolar flat panel
19 photobioreactor was designed and characterized with the aim of achieving high
20 microalgae productivities and CO₂ bio-fixation rates. Moreover, the energy efficiency of
21 the employed pump-assisted hydraulic circuit was evaluated. The 1.3 cm thick alveolar
22 flat-panels enhance the light utilization, whereas the hydraulic design of the
23 photobioreactor aims to improve the global CO₂ gas-liquid mass transfer coefficient
24 (k_{LaCO_2}). The mixing time, liquid flow velocity, and k_{LaCO_2} as well as the uniformity
25 matrix of the artificial lighting source were experimentally calculated. The performance
26 of the system was tested by cultivating the green microalga *Acutodesmus obliquus*. A
27 volumetric biomass concentration equal to 1.9 g L⁻¹ was achieved after 7 days under

28 controlled indoor cultivation conditions with a CO₂ bio-fixation efficiency of 64 % of
29 total injected CO₂. The (gross) energy consumption related to substrate handling was
30 estimated to be between 27 and 46 Wh m⁻³, without any cost associated to CO₂ injection
31 and O₂ degassing. The data suggest that this pilot-scale cultivation system may constitute
32 a relevant technology in the development of microalgae-based industrial scenario for CO₂
33 mitigation and biomass production.

34 **Keywords**

35 Flat panel photobioreactor, CO₂ mass transfer coefficient, CO₂ bio-fixation efficiency,
36 Hydrodynamic characterization, *Acutodesmus obliquus*.

37

38 **1. Introduction**

39 Since the 20th century, the concentration of greenhouse gases in the atmosphere
40 continued to increase as result of anthropogenic activities related to the use of fossil fuels,
41 deforestation, and agricultural activities. The annual global average carbon dioxide
42 concentration at Earth's surface in 2020 has reached 412.5 ± 0.1 ppm, the highest value
43 in modern atmospheric records, increasing by 2.5 ± 0.1 ppm from 2019, a value
44 comparable to the average rate of increase during the last decade (Blunden and Boyer,
45 2021). Therefore, the development of renewable and clean technologies is needed to
46 sustain a considerable fraction of the global economy and to reduce the impact of human
47 activities. In parallel, major efforts are needed to improve CO₂ Capture and Utilization
48 (CCU) technologies that can effectively reduce carbon dioxide emissions mainly from
49 power plants and different industrial processes.

50 In this context, microalgae-based refinery concepts have gained importance over the
51 last few decades. Microalgae are considered promising biochemical factories and
52 excellent CO₂ fixers (Brown and Zeiler, 1993). Their simple cellular structure, large

53 surface-to-volume ratio, and aquatic lifestyle allow these organisms an easy access to
54 water, CO₂ and other nutrients, and thus a more efficient conversion of solar energy into
55 chemical energy, showing 10-50 higher CO₂ fixation rates than land plants (Carlsson et
56 al., 2007; Goli et al., 2016; Rosenberg et al., 2011). Moreover, compared to higher plants,
57 microalgae also show faster growth rates and their cultivation does not compete for arable
58 lands (Greenwell et al., 2010; Khan et al., 2009). As a result of CO₂ fixation, microalgae
59 accumulate significant amounts of carbohydrates, proteins, lipids, and other valuable
60 compounds, such as pigments and vitamins. Hence, microalgal biomass is considered a
61 promising energy feedstock with multifaceted applications in the production of dietary
62 supplements, cosmetics, food and animal feed and biofuels (Cheah et al., 2015; Francisco
63 et al., 2010; Gimpel et al., 2015; Vanthoor-Koopmans et al., 2013). Algal cultivation
64 technologies are traditionally classified as open or closed systems (photobioreactors,
65 PBRs). Open cultivation systems (*e.g.*, artificial ponds, raceways, thin layer) produce
66 algal biomass at lower costs thanks to their lower investment and management costs in
67 terms of Capital Expenditure (CapEx) and Operating Expense (OpEx) (Benemann, 2013).
68 The open raceway pond is currently the most frequently used and cheapest cultivation
69 system for commercial production of microalgae (Acién et al., 2017). Despite this, the
70 open pond technology is limited by several disadvantages such as low biomass
71 productivities, mainly related to poor mixing, low CO₂ mass transfer, high risk of
72 biological and chemical contamination and high consumption of water. Moreover, its
73 dependence on climatic conditions limits its application to tropical and subtropical
74 regions (Chini Zittelli et al., 2013; Pandey et al., 2014; Tredici et al., 2010). On the other
75 hand, closed photobioreactors allow precise control of the operating conditions and show
76 higher biomass productivities. The confined space limits contaminations and assures
77 higher biomass quality, but requires higher CapEx and OpEx (Adesanya et al., 2014;
78 Norsker et al., 2011). Currently, tubular photobioreactors are the most common closed

79 system configurations for industrial-scale microalgae cultivation, mainly related to high-
80 value applications (Acién et al., 2017; Chini Zittelli et al., 2013; Torzillo and Zittelli, 2015).
81 However, all types of closed systems present major constraints in the process scale-up,
82 mainly due to the difficulty in increasing the sizes of PBRs while keeping optimal culture
83 and hydrodynamic parameters. The choice of the circulating device and design of the
84 PBR influences important operating parameters such as mixing time, turbulence degree,
85 O₂ build-up, and CO₂ supply, thus impacting on both the overall performance of the
86 process (Torzillo et al., 2003) and the final cost. An extensive comparison of the strengths
87 and limitations of the different cultivation systems, together with the importance of
88 illumination and hydrodynamic parameters, has been thoroughly analysed in several
89 reviews (Acién et al., 2017; Carvalho et al., 2006; De Vree et al., 2015; Torzillo and Zittelli,
90 2015; Tredici et al., 2010; Yadav and Sen, 2017). It is important to note that, although many
91 PBRs and open system setups have been proposed at laboratory and industrial levels,
92 there is no optimal design for all applications. Moreover, the overall negative energy
93 balance of the processes still poses limitations in the scale-up of microalgal culture
94 technologies, making them profitable only for applications with high added value goods
95 such as dietary supplement and cosmetic raw materials (Acién et al., 2012; Slade and
96 Bauen, 2013; Tredici et al., 2015). Recently, several studies have focused on the
97 development of many simulation approaches coupling computational fluid dynamics,
98 mass transport phenomena and microbial growth kinetics, to identify the best conditions
99 to maximize microalgae productivity (del Rio-Chanona et al., 2019; Solimeno et al.,
100 2017; Tan et al., 2020; Vasile et al., 2021; Weise et al., 2019). Likewise, several new
101 design of open, closed, and hybrid PBRs setups based on different hydrodynamics, mass
102 transfer mechanisms and illumination strategies have been proposed to improve the
103 global cultivation efficiency and the scale-up feasibility (Chen et al., 2016; Chiaramonti et
104 al., 2013; Cuaresma et al., 2009; Estrada-Graf et al., 2020; Li et al., 2014; López-Rosales et

105 al., 2019; Massart et al., 2014; Reyna-Velarde et al., 2010; Rodolfi et al., 2009; Sierra et al.,
106 2008; Tan et al., 2020; Tredici et al., 2015; Yadav et al., 2015). Particularly, more attention
107 was given to flat panel PBRs due to the high photosynthetic efficiencies that can be
108 reached (Jorquera et al., 2010). However, currently employed flat panel PBRs still present
109 some limitations mainly due to the low mixing efficiency, being the air bubbled mainly
110 directly from the bottom of the panels, which brings to high energy costs and can lead to
111 the occurrence of serious biofouling (Sierra et al., 2008; Tredici et al., 2015). Moreover,
112 the use of compressed air for mixing constitutes a major part of the PBR energy
113 consumption. Indeed, the power supply associated to air compression is function of the
114 type of compressor, gas pressure, type of blower, and the aeration rate (Norsker et al.,
115 2012). Centrifugal pumps, which are considerably more efficient than air compressors,
116 have been mainly employed in tubular PBRs, although the concern for cell shear damage
117 has led more frequently to the use of air-lift pumps. Nevertheless, as pointed out by
118 Norsker and co-authors, the use of centrifugal pumps would be not recommended only
119 for the cultivation of very shear sensitive algae (Norsker et al., 2011). To our knowledge,
120 no literature reports are present concerning pilot-scale flat panel PBRs with a centrifugal
121 pump. In this research, it is presented the design and characterization of a novel alveolar
122 flat panel photobioreactor with a pump-assisted hydraulic circuit, that constrains
123 microalgae to flow inside a positive-pressurized serpentine directly exposed to the
124 artificial light source. The system has been realized to maximize the distribution of light
125 through the microalgae culture as the width of the flat panels is equal to 1.3 cm. The main
126 purpose of this work was to assess the hydrodynamic and cultivation performances of the
127 proposed PBR. The results will help to improve the actual proposed technology and to
128 evaluate its possible use in an industrial scenario, in which the application of energy-
129 efficient technologies is nowadays a priority.

130 Therefore, the hydraulic, lighting and energetic behaviour of the new flat-panel PBR
131 were investigated through the experimental characterization of the hydraulic flow, mixing
132 time, CO₂ gas-liquid mass transfer coefficient, and irradiance matrix. The biological
133 performances of this new prototype were tested by cultivating the green microalga
134 *Acutodesmus obliquus*.

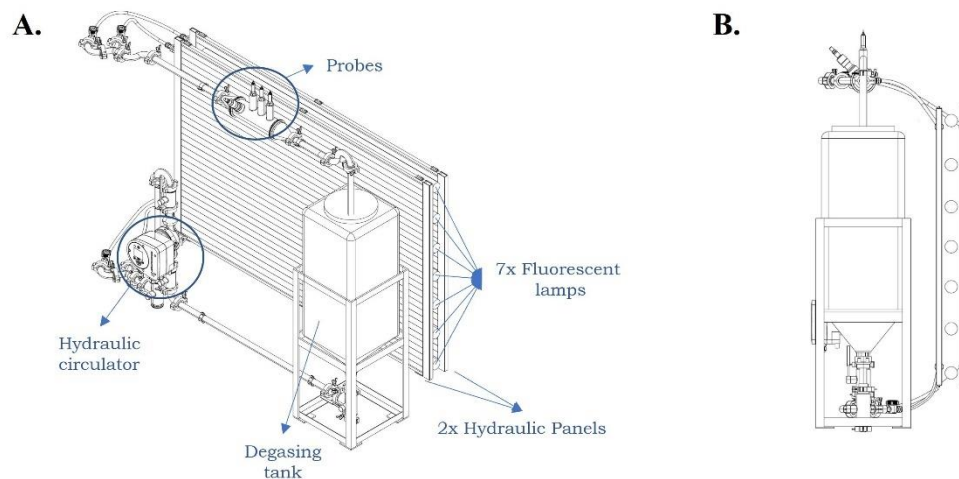
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136 **2. Materials and Methods**

137 **2.1 The flat panel photobioreactor**

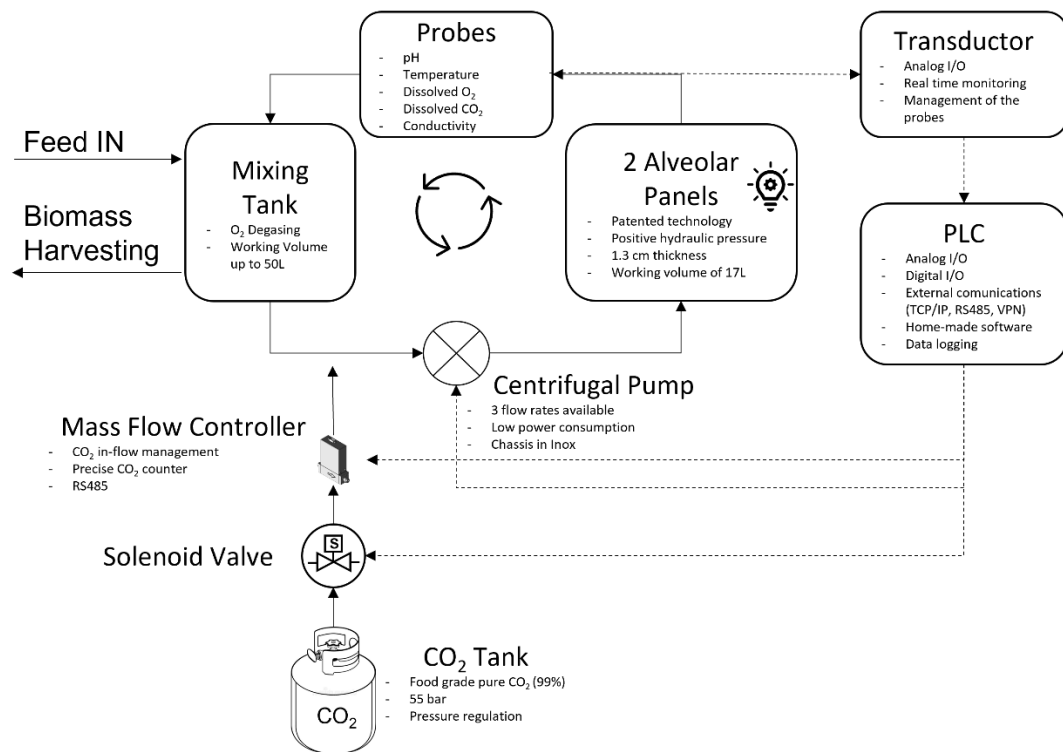
138 The photobioreactor used in this research was kindly provided by Arcobaleno
139 Cooperativa Sociale (Turin, Italy), the patent holder of the PBR (EP2830413A1). A
140 representative scheme of the PBR is reported in Figure 1A. Briefly, it is composed by
141 two interconnected units: a photostage loop and a mixing tank. The photostage loop
142 consists of two parallel alveolar flat panels illuminated by an interposed array of seven
143 fluorescent lamps (58W, OSRAM, Germany). The alveolar flat panels are made of
144 transparent polycarbonate with a light exposed surface area of 1.5 m² each and an
145 internal path of 13 mm. Each panel is partitioned into 28 internal channels (alveoli) for
146 a total length of the illuminated path of about 40 m and total volume of about 17 L. The
147 mixing tank is made up with a darkened HDPE (High density polyethylene) material
148 with a total volume of 50 L. Its truncated-cone shape has been designed to minimize
149 biomass sedimentation (Fig. 1B). The tank is equipped on its top of a hydraulic inlet
150 and a removable lock cap. A hydraulic circulator (ALPHA1 L - 45W, Grundfos,
151 Denmark) is connected at the bottom of the mixing tank and upstream of the photostage
152 loop. The hydraulic circulator drives the liquid flow into both flat panels, from the
153 bottom to the top. Moreover, the hydraulic circulator allows to manually set up three
154 different factory-defined liquid flow rates operating with constant performance curves.
155 Gas inlet coming from a food grade CO₂ tank is located between the bottom of mixing

156 tank and the hydraulic circulator. This configuration maximizes gas dissolution in the
157 liquid phase thanks to the turbulence generated by the circulation system. CO₂ flow
158 rates are finely regulated by a thermal flow meter (Red-y smart controller GSC, Vögtlin
159 Instruments GmbH Switzerland), whereas temperature/pH, conductivity, dissolved
160 oxygen, and carbon dioxide are constantly monitored by a InPro 325Xi pH electrodes,
161 Four-electrode conductivity sensor, InPro 6000 Optical O₂ sensor and a InPro 5000i
162 CO₂ sensor (Mettler-Toledo®, USA), respectively. The probes are located at the output
163 of the flat panels. The PBR is equipped with a Mettler-Toledo® multi-parameter
164 transducer M800 and the signals from the sensors are transmitted to a Programmable
165 Logical Controller (PLC, Unitronics, Israel) through industrial standard analogic signal
166 protocol (4-20 mA). The PLC controls a dedicated solenoid valve for the CO₂ injection,
167 allowing the regulation of the CO₂ flow based on pH or carbon dioxide concentration
168 threshold, according to the experimental setup. A schematic overview of the whole PBR
169 working process is reported in Figure 2.



170
171 **Figure 1:** Schematic representation of the flat panel photobioreactor. A) 3D isometric
172 view of the whole PBR. B) Schematic view of the mixing tank.

173



174

175 **Figure 2:** Schematic process diagram of the flat panel photobioreactor.

176

177 2.2 Microalgae and cultivation conditions

178 *Acutodesmus obliquus* strain 276-3b, formally *Scenedesmus obliquus* (Turpin) Kützing,
 179 was obtained from the SAG Culture Collection of Algae (Göttingen, Germany). The
 180 inoculum preparation was carried out in batch mode and axenic conditions in 2 L
 181 disposable culture chambers by using a 1.5 L volume of sterile BG-11 medium (Stanier
 182 et al., 1971). The cultures were maintained at constant temperature ($23^{\circ}\text{C} \pm 2$), pH 7.00-
 183 7.50, and under constant (24/24 h of illumination) artificial illumination ($120 \mu\text{mol m}^{-2} \text{s}^{-1}$)
 184 ¹⁾ by fluorescence tubes (Osram, Germany). Aeration and mixing of the cells were
 185 guaranteed by flowing air at the bottom of the chambers. The microalgal cells were used
 186 to inoculate the flat panel photobioreactor when they just reached the stationary phase.
 187 The PBR was inoculated with BG-11 medium and microalgae cells for a total volume of
 188 60 L, corresponding to a surface area-to-volume ratio (S_f/V) of 50 m^{-1} , and an initial cell
 189 concentration of 0.25 g L^{-1} of dry weight. Each experiment in the PBR was conducted in

190 batch mode for 7 days. The injection of CO₂ was carried out with a flow rate of 0.12 L
191 min⁻¹, keeping constant the CO₂ concentration threshold in the PBR at 25 mg L⁻¹ using
192 the combination of solenoid valve and mass flow meter.

193

194 **2.3 Biomass concentration measurements**

195 Microalgae growth was gravimetrically quantified as dry biomass concentration as
196 previously reported (Perin et al., 2017). Briefly, 10-20 mL of microalgae culture was
197 filtered using pre-weighted 1.5 µm pore size glass fibre filters (Hahnemühle, Germany).
198 The filters were then dried using a thermobalance (MLS-N, Kern, Germany) until stable
199 weight, and then weighted with an analytical balance (Kern, Germany).

200 The biomass volumetric productivity (P_x) was then calculated as [Eq. 1]:

$$P_x = \frac{X_t - X_0}{t - t_0} \quad (1)$$

201 where X_t is the biomass concentration [g L⁻¹] at time t [d], and X_0 is the biomass
202 concentration [g L⁻¹] at time t_0 [d].

203

204 **2.4 Hydraulic flow determination**

205 The hydraulic flow rate of the hydraulic circulator was measured using an
206 electromagnetic flow meter (FD-Q20C. Keyence, Japan). All measurements were carried
207 out by placing the flow meter at the outlet of the hydraulic panels.

208

209 **2.5 CO₂ mass transfer coefficient (k_{LaCO_2})**

210 In order to estimate gas-liquid transfer efficiencies of the photobioreactor, the k_{LaCO_2}
211 was measured using 60L of distilled water (without microalgal cells) to avoid
212 interferences of biological activity. The test was conducted at room temperature and
213 atmospheric pressure ($25 \pm 2^\circ\text{C}$ and 101.325 Pa).

214 The tests were conducted by blowing a constant and continuous flow rate of CO₂ inside
215 the PBR and monitoring, through the CO₂ probe, the carbon dioxide concentration over
216 time. Different carbon dioxide flow rates were tested at different hydraulic circulator
217 powers (different hydraulic flow rate). As indicated in paragraph [2.1], the PBR employs
218 a circulation system that allows to choose between three different levels of power flows
219 corresponding to three different liquid rates. For each circulation level, the k_La value was
220 identified for different carbon dioxide flow rates.

221 The $k_La_{CO_2}$ parameter was determined using the following equation [Eq. 2](Sierra et
222 al., 2008):

$$\frac{dC}{dt} = k_La * (C^* - C) \quad (2)$$

223

224 integration for $C = C_0$ at $t = 0$ lead to [Eq. 3]:

$$\ln\left(\frac{C^* - C}{C^* - C_0}\right) = -k_La * t \quad (3)$$

225

226 where C_0 is the initial CO₂ concentration [mg L⁻¹], C is the dissolved CO₂ concentration
227 [mg L⁻¹] at time t , C^* is the CO₂ saturation concentration in water [mg L⁻¹] and t is time
228 [min]. Since dissolved carbon dioxide is in equilibrium with carbonate and bicarbonate
229 species, to determine the exact concentration of carbon dissolved into the aqueous phase
230 the relevant equilibrium and corresponding equilibrium constants were calculated as
231 previously reported (Chen et al., 2016).

232

233 **2.6 CO₂ fixation yield**

234 The CO₂ fixation yield (η_{CO_2}) expresses the CO₂ bio-fixation rate of the culture in terms
235 of percentage (Lim et al., 2021). It is calculated as the ratio between the kilograms of
236 carbon accumulated within the algal biomass at the end of the cultivation and the kg of

237 carbon supplied to the microalgae through the injection of CO₂ at a known flow rate [Eq.
238 4], as previously reported (Lim et al., 2021).

$$\eta_{CO_2} = \frac{W_{C \text{ biomass}}}{W_{C \text{ in}}} * 100 \quad (4)$$

239

240 With $W_{C \text{ biomass}}$ and $W_{C \text{ in}}$ deriving respectively from [Eq. 5] and [Eq. 6]:

$$W_{C \text{ biomass}} = W_{\text{biomass}} * C_{C \text{ biomass}} \quad (5)$$

$$W_{C \text{ in}} = (W_{CO_2 \text{ in}} - W_{CO_2 \text{ water}}) * (M_C / M_{CO_2}) \quad (6)$$

241

242 Where $W_{C \text{ biomass}}$ is the kg of carbon accumulated in the biomass; W_{biomass} the kg of
243 biomass obtained during the cultivation; $C_{C \text{ biomass}}$ is the fraction of carbon within the cells
244 obtained through experimental elemental analysis of *A. obliquus*; $W_{C \text{ in}}$ is the kg of carbon
245 injected into the PBR as CO₂ flow; $W_{CO_2 \text{ in}}$ is the total kg of CO₂ injected; $W_{CO_2 \text{ water}}$ is the
246 kg of CO₂ dissolved in water at the end of the batch; M_C and M_{CO_2} represent respectively
247 the molar mass of carbon (12 g mol⁻¹) and carbon dioxide (44 g mol⁻¹).

248

249 **2.7 Mixing time**

250 The mixing time [t_m] is defined as the time required to attain a given uniformity close
251 to the fully mixed state after the injection of a tracer (Yang and Mao, 2014) and it was
252 evaluated by a pH tracing test. To estimate the mixing time of the photobioreactor, the
253 experiments were performed using 60 L of distilled water (without microalgal cells) to
254 avoid interferences of biological activity. Diluted hydrochloric acid (5 mL HCl; with a
255 final concentration in the PBR of 10⁻³ M) was poured into the mixing tank of the
256 photobioreactor, and the pH was recorded every minute by the pH probe located at the

257 output of the flat panels. The t_m was determined as the time required to reach the 95% of
258 complete homogeneity after the injection of the HCl solution (Chisti, 1989).

259

260 **2.8 Energetic measurements**

261 The energy consumption of the PBR main components (hydraulic circulator and
262 lighting system) was measured using a digital multimeter (Siglent SDM3065X-SC,
263 Germany). Voltage and current intensity measured for each unit were used to calculate
264 the power input expressed in Watt [W]. These data, together with the hydraulic flow
265 measurements, were further computed to evaluate the net energy consumption for the
266 fluid handling as Wh m⁻³.

267

268 **2.9 Radiance matrix**

269 The PBR was illuminated by an artificial lighting system based on an array of seven
270 fluorescent tubes interposed between the two flat panels. To evaluate the uniformity of
271 incident light on both exposed surfaces of the panels, the light intensity was determined
272 by a PAR spectroradiometer (PLA 20, Everfine, China). A matrix array was imposed on
273 the artificial lighting system to fix and homogeneously distribute the sampling points
274 along the radiant surface. The data obtained from the measurements were interpolated
275 using the software Matlab[®] to create the pattern of light intensity for the whole exposed
276 panel surface.

277 The light uniformity coefficient was calculated using the following equation:

$$U_I = \frac{I_{min}}{I_{mean}} \times 100 \quad (7)$$

278

279 where U_I is the light uniformity coefficient [%], I_{min} is the minimum value of light
280 intensity [$\mu\text{mol m}^{-2} \text{s}^{-1}$] and I_{mean} is the mean value of light intensity [$\mu\text{mol m}^{-2} \text{s}^{-1}$].

281 **3. Results and discussion**

282 **3.1 Hydrodynamics flow description**

283 This study has described, for the first time in literature, the hydrodynamics, mass
284 transfer and mixing time parameters of an innovative and patented flat panel
285 photobioreactor. The experimental characterization of biological performance parameters
286 has been also reported. Key hydrodynamic parameters have been explored in order to
287 compare the pump-assisted setup of the cultivation system here reported to traditional flat
288 panel photobioreactors. In the latter, mixing and liquid flow is typically achieved by air
289 bubbling or through an external airlift system. Whereas the hydrodynamics of the
290 described PBR is function of the pump-driven culture flow, and thus is linked to the gross
291 power of the hydraulic circulator. Therefore, the hydrodynamics characterization of the
292 reactor included the measurements, at the three default pump setups and at various CO₂
293 injection rates, of the different liquid flow rates and the effects on the mixing time and
294 the CO₂ volumetric mass transfer coefficient (k_{LaCO_2}).

295 The hydraulic flow rates calculated using the available standard setups on the circulator
296 system (hereafter named level I, II, III), and their variations at increasing flow of injected
297 CO₂ gas were measured. As shown in Table 1, the experimental results showed that,
298 without any gas injection, the hydraulic flow varies greatly from 4 L min⁻¹ for the lower
299 configuration of the circulator to values of 14 and 18 L min⁻¹ for level II and level III,
300 respectively. The calculated liquid flow velocity (U_L) within the alveoli is 0.17, 0.60 and
301 0.77 m s⁻¹ at level I, II and III, respectively.

302

303

304

305

306 **Table 1:** Variation of hydraulic flow rates according to the manually chosen level of the
 307 hydraulic circulator and the CO₂ flow injection. Liquid flow rates for the higher levels of
 308 the circulation system, at low CO₂ flow rates, were not measured assuming a negligible
 309 reduction of the rates according to the data for level I. Liquid flow rates (L min⁻¹) are
 310 shown as the average of three replicates ± standard deviation. U_L = liquid flow velocity
 311 (m s⁻¹).

Liquid flow rate [L min⁻¹]			
CO₂ flow rate [NL min⁻¹]	Circulator level I $U_L = 0.17 \text{ m s}^{-1}$	Circulator level II $U_L = 0.60 \text{ m s}^{-1}$	Circulator level III $U_L = 0.77 \text{ m s}^{-1}$
None	4.05 ± 0.03 (100%)	14.11 ± 0.15 (100%)	18.10 ± 0.33 (100%)
0.06	3.66 ± 0.11 (90.4%)	n.d.	n.d.
0.12	3.69 ± 0.09 (91.1%)	n.d.	n.d.
0.48	3.58 ± 0.02 (88.4%)	13.52 ± 0.31 (95.8%)	n.d.
0.60	3.62 ± 0.05 (89.4%)	11.23 ± 0.61 (79.6%)	n.d.
0.84	n.d.	8.61 ± 0.09 (61%)	18 ± 0.05 (99.4%)
1.08	n.d.	n.d.	16.95 ± 0.56 (93.6%)

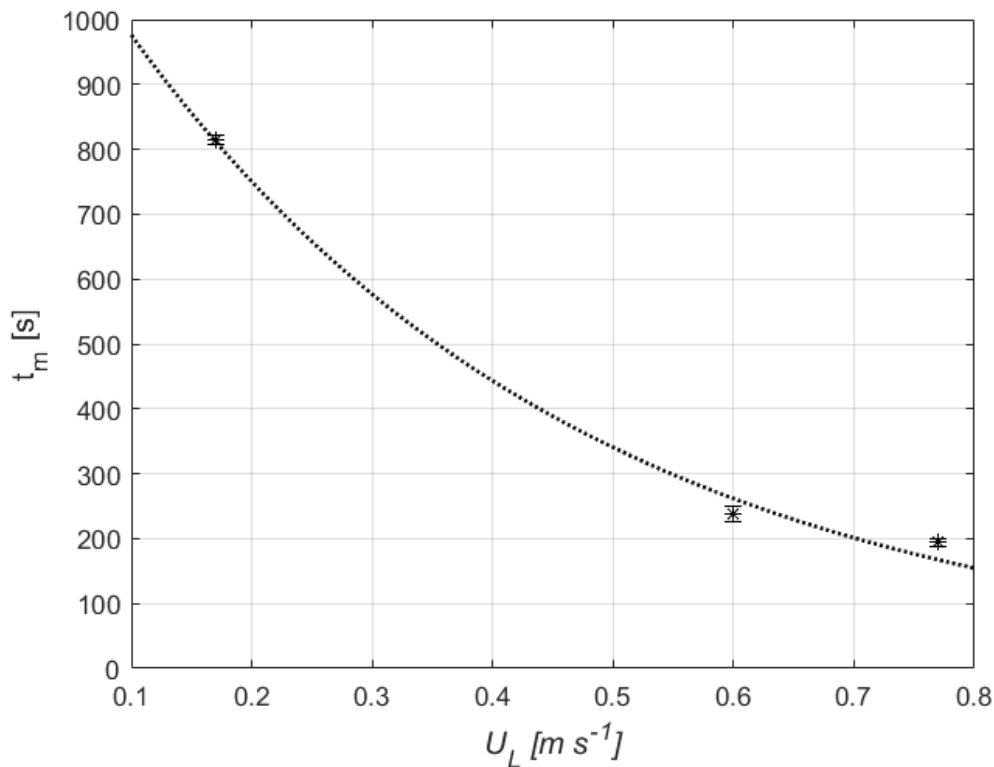
312
 313 Since the CO₂ is injected immediately upstream the circulator, the hydraulic flow is
 314 affected by the gas flow, and it is reduced as the gas flow rate increases. This is mostly
 315 evident at the level II of the circulator. Gas flow rates above 0.48 NL min⁻¹ reduce the
 316 hydraulic flow rate to values below the 90% of the hydraulic load without CO₂ injection.
 317 On the contrary, the liquid flow rate at level III (18 L min⁻¹) is not significantly reduced
 318 by even higher CO₂ rates. Whereas no significant reductions in the liquid flow occur at
 319 the lower configuration of the circulator (level I). In the latter case, the low liquid flow (4
 320 L min⁻¹) could favor a gas leak toward the tank rather than being swallowed up by the
 321 circulator and dissolved within the bulk flow to the hydraulic panels.

322 3.2 Mixing characterization

323 The mixing time (t_m) has been calculated at the three nominal liquid velocities, without
324 CO₂ gas injection, to identify the relationship between t_m and U_L in the characterized
325 cultivation system. The data showed that, by increasing the liquid velocity from 0.17 m
326 s⁻¹ (level I) to 0.60 m s⁻¹ (level II), the mixing time decreases from 814 s to 246 s. Whereas
327 above $U_L = 0.60$ m s⁻¹ the slope of the curve decreases, and the t_m is not consistently
328 reduced (194 s) (Fig. 3). The best fitting curve of the experimental data has an exponential
329 trend (Fig. 3).

330 The computed energy consumption for the fluid handling at the three different liquid
331 velocities was calculated to be 46, 27 and 38 Wh m⁻³, respectively. Therefore, the reported
332 power consumption values suggest that working at U_L values above 0.60 m s⁻¹, the
333 inflexion point of t_m and U_L curve, is energetically disadvantageous since mixing time
334 remains comparable with the one measured at 0.60 m s⁻¹ (level II).

335 The reactor hydrodynamic behavior of other flat panel PBRs described in literature is
336 based on different engineering solutions that affect hydrodynamic characteristics
337 (Massart et al., 2014; Sierra et al., 2008). The authors reported the trend of mixing time in
338 correlation with the gas velocity which drives the liquid handling. In the PBR prototype
339 here described, the photosynthetic loop can be assimilated mainly to a plug flow reactor,
340 although a continued stirred situation may be assumed since the estimated Reynolds
341 number within the alveoli ($Re \sim 10,000$) indicates a turbulent state. Meanwhile, the
342 mixing tank can be described as a CSTR (Continued Stirred Tank Reactor). Despite the
343 different liquid handling solution, adopted in this study, with respect to the reported
344 literature, the t_m values measured at 0.60 (level II) and 0.77 m s⁻¹ (level III) fall into the
345 ranges of mixing times reported in the above-mentioned works (Massart et al., 2014; Sierra
346 et al., 2008).



347

348 **Figure 3:** Influence of liquid flow velocity on mixing time. Data are shown as the
 349 average of three replicates \pm standard deviation. Dotted line represents the exponential
 350 fitting ($R^2 = 0.9946$) obtained with the software Matlab®.

351

352 3.3 CO₂ mass transfer

353 Carbon dioxide uptake is one of the main target of microalgae cultivation technologies
 354 exploitation, therefore the CO₂ mass transfer coefficient ($k_{LA}CO_2$) in the new PBR
 355 prototype was investigated. As described in section [2.1], CO₂ is directly injected
 356 upstream of the circulation system and the bubbles are swallowed up and broken by the
 357 circulator to smaller sizes. Moreover, the gas circulates within the fluid in the flat panels
 358 with a CO₂ bubbles residence time that is linked to the total length of the alveoli in the
 359 photostage loop (around 40 m).

360 The injection of CO₂ causes a reduction in the hydraulic flow rate since the gas flow
 361 interferes with the liquid handling of circulator, as discussed in section [3.1]. Therefore,
 362 k_{LA} values were not measured for the CO₂ flow rates reducing the hydraulic flow below

363 the 90% of nominal value (Table 1) and the results are reported in Table 2. As expected,
364 the data showed a significant increase of $k_{La}CO_2$ values from $1.21 \cdot 10^{-5}$ to $2.99 \cdot 10^{-4} s^{-1}$
365 along with the increase of the CO_2 flow injected for each hydraulic flow. The lowest k_{La}
366 values, meaning a more efficient CO_2 mass transfer, were achieved at the lowest CO_2
367 flows (0.06 and 0.12 NL min^{-1}) for all the circulator flow rates. However, at the circulator
368 level I (0.17 m s^{-1}) the measurements of $k_{La}CO_2$ may be affected by a gas leak toward the
369 mixing tank. Furthermore, such low liquid flow velocity determines the longest mixing
370 time of the PBR system (section [3.2]), which could affect the microalgae growth *e.g.*, by
371 favoring the sedimentation of biomass. The decrease of $k_{La}CO_2$ at level III, with respect
372 to level II, can be justified by the higher speed of the pump that favors the breaking and
373 mixing of CO_2 gaseous bubbles. However, circulator level III (0.77 m s^{-1}) did not show a
374 relevant improvement of hydraulic and mass transfer performances and it has a higher
375 energy consumption with respect to circulator level II (section [3.2]). Therefore, the
376 circulator and CO_2 flow setups showing the best performances in term of hydrodynamics,
377 CO_2 mass transfer and energy consumption were identified to be circulator level II with
378 the injection of CO_2 flow rates of 0.06 and 0.12 NL min^{-1} .

379 In the presented prototype, being the system's hydrodynamics based on a mechanical
380 circulation of the liquid, there is no need to inject air to support the movement of the
381 liquid culture and guarantee a certain degree of mixing and gas transfers. Thus, it is
382 possible to inject only pure low CO_2 flow rates achieving a high solubilization in the
383 photostage loop. In this way, CO_2 losses in atmosphere are limited, and the only cost to
384 obtain an efficient mixing and gas transfer is the one associated to the hydraulic circulator,
385 as in real scale application pure CO_2 comes already pressurized by previous industrial
386 stages. In literature, few examples of $k_{La}CO_2$ experimental measurements were reported.
387 The only comparisons with the system described in this work can be done against flat
388 panel air-lift PBRs (Chen et al., 2016; Massart et al., 2014). In these works, the authors

389 have used gas for the liquid-biomass mixing, and the reported $k_{LA}CO_2$ values were higher
 390 (lower CO_2 solubilization) with respect to the data showed in the present manuscript.

391 **Table 2:** Dependency of CO_2 gas-liquid mass transfer coefficient (k_{LA}) on the liquid flow
 392 velocities and CO_2 flow rates tested.

$k_{LA} CO_2 [s^{-1}]$			
CO₂ flow rate [NL min ⁻¹]	Circulator level I * $U_L = 0.17 \text{ m s}^{-1}$	Circulator level II $U_L = 0.60 \text{ m s}^{-1}$	Circulator level III $U_L = 0.77 \text{ m s}^{-1}$
0.06	$1.21 \cdot 10^{-5}$	$1.89 \cdot 10^{-5}$	$1.64 \cdot 10^{-5}$
0.12	$1.47 \cdot 10^{-5}$	$3.43 \cdot 10^{-5}$	$3.20 \cdot 10^{-5}$
0.48	n.d.	$1.30 \cdot 10^{-4}$	$1.24 \cdot 10^{-4}$
1.08	n.d.	n.d.	$2.99 \cdot 10^{-4}$

393 * k_{LA} values for level I may be affected by the leak of CO_2 toward the tank (see section [3.1]).
 394

395 The configuration of the presented PBR applies an innovative liquid handling solution
 396 that makes difficult to directly compare it with other flat panel PBRs described in
 397 literature, in terms of hydrodynamics and mass transfer efficiencies. Nevertheless,
 398 comparisons could be possible by addressing the problem from an energy point of view,
 399 in terms of energy required per unit volume operating in the unit of time.

400 In the PBR here described, the energy consumption related to the substrate handling
 401 was evaluated as effective power (at the wall socket) amounting to 27 Wh m^{-3} for level II
 402 of the hydraulic circulator, without any further cost associated to the CO_2 supply and
 403 stripping-out of O_2 .

404 On the contrary, in the majority of flat panel PBRs described in literature, air is injected
 405 directly from the bottom of the flat-panels with the triple function of provide adequate
 406 mixing, CO_2 and favour the stripping-out of O_2 . Therefore, the air supply parameter
 407 governs the energy consumption and the mass transfer capacity (Leupold et al., 2013;
 408 Sierra et al., 2008). A large volume of compressed air is therefore consumed to achieve
 409 the described triple function.

410 A power supply of 53 W m^{-3} was estimated by Sierra et al., (2008) to reach a mass
411 transfer rate high enough to avoid excessive O_2 accumulation. More recently, Li et al.,
412 (2014) proposed a new design of flat panel PBR coupled to an external airlift module to
413 overcome the major limitation of the mixing and improve the energy efficiency of the
414 process by estimating a power supply of 31.6 W m^{-3} . However, the effective energy
415 consumption of the system must include the efficiency of the associated air compressor,
416 as also highlighted by Norsker et al., (2012). Therefore, direct comparisons with
417 traditional flat-panel PBRs are difficult due to the absence of gross energy data
418 concerning the power consumption of compressed air flat panels.

419

420 **3.4 Light homogeneity**

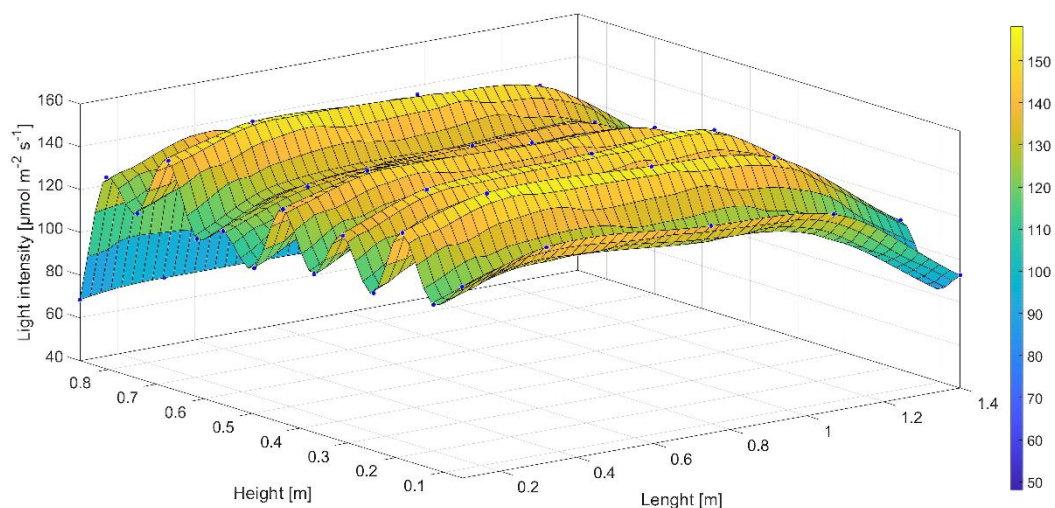
421 The lighting exposure (natural or artificial light) in photoautotrophic cultivation systems
422 is a major factor influencing the performances of the process. Therefore, in the present
423 study, we investigated the homogeneity of the artificial lighting system of the PBR
424 prototype in order to assess the average light intensity at which microalgae cells are
425 exposed. Light measurements on the surface exposed to the incident light have been used
426 to build up a radiant matrix [Fig. 4]. The computation of the different measurements on
427 the surface established a mean incident light intensity of $120 \mu\text{mol m}^{-2} \text{ s}^{-1}$ with a light
428 uniformity coefficient U_I of 40%. The trend of light distribution presents a wave shape,
429 with the peaks corresponding to the fluorescent tubes positioning along the surface.
430 Considering the light uniformity coefficient of 40%, a linear liquid velocity of 0.60 m s^{-1}
431 (level II), and the length of each alveolus within the flat-panels (around 1.5 m),
432 microalgae cells take about 10 s to move between two adjacent light intensity peaks.
433 Therefore, it can be concluded that light fluctuations within the photostage loop do not
434 significantly affect the overall biomass growth performance.

435 The use of fluorescent tubes as artificial lighting system has some disadvantages.
436 Fluorescent tubes are omnidirectional light sources, emitting at 360°. Therefore, only half
437 of the emitted light is directed to the microalgae suspension, whereas the other half might
438 need to be redirected to the desired area with the use of reflecting surfaces, requiring
439 additional accessory parts. Moreover, their emitting light intensity, as well as the light
440 spectrum quality, cannot be tuned if not by increasing the number of tubes (for the light
441 intensity).

442 The energy consumption of the lighting system, measured as described in section [2.8],
443 was found to be 455 Wh, a value which must be considered in order to estimate the global
444 efficiencies of the cultivation system, and to investigate alternative artificial light sources
445 to overcome the above-reported disadvantages reducing the energy requirements.

446 Further investigations are undergoing to evaluate the implementation of Light Emitting
447 Diodes (LEDs) as directional light source, due to their overall higher energy efficiency
448 compared to other commercially available lighting systems.

449



450

451 **Figure 4:** Graphical representation of the light intensity in a 3D space of the fluorescent
452 source. $f(x,y)$ = piecewise cubic surface computed with the software Matlab® from p
453 (structure coefficient), where x is normalized by mean 0.728 and std 0.4788 and where y
454 is normalized by mean 0.4625 and std 0.2507.

455

456 **3.5 Microalgae growth and CO₂ bio-fixation efficiency**

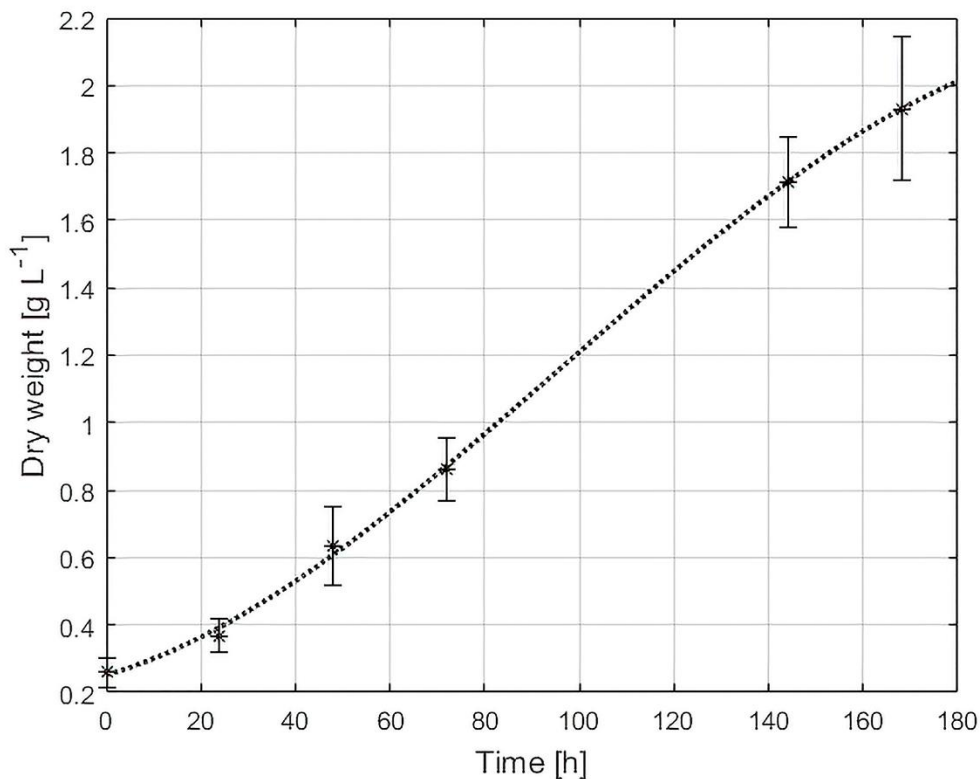
457 Based on the data obtained for what concern the system's hydrodynamics and energy
458 consumption, it has been decided to assess the growth of the green microalga
459 *Acutodesmus obliquus* (*Scenedesmus obliquus*) operating at a liquid velocity of 0.60 m s⁻¹
460 (level II) and with a CO₂ flow rate of 0.12 L min⁻¹. As mentioned in previous sections,
461 these hydraulic parameters have been identified among the best performing setups.
462 Moreover, it has been decided to work at constant dissolved CO₂ concentration of 25 mg
463 L⁻¹, corresponding to a carbon dioxide partial pressure (P_{CO₂}) of 0.017 bar. This value has
464 been chosen to guarantee a relatively high CO₂/O₂ ratio, which may reduce the potential
465 negative effects of O₂ accumulation via photorespiration, as suggested by (Sousa et al.,
466 2012), and to assure a constant control on pH in the range between 7 and 7.5.

467 After 7 days of cultivation the cells reached a final concentration of 1.9 g L⁻¹ (Fig. 5).
468 The analysis of the growth curves showed a mean daily volumetric productivity (P_x) of
469 0.21 g L⁻¹ d⁻¹ ± 0.01 (n = 3). The CO₂ fixation yield (η_{CO_2}), calculated as in Eq. 5 and
470 based on elemental composition analysis of *A. obliquus* previously performed in our
471 laboratory with the same cultivation setup (C 53.8 %, H 8.88 %, O 37.3% by weight),
472 accounted for the 64% of the total CO₂ injected.

473 The cultivation of *A. obliquus* was successful in the PBR, and an axenic homogeneous
474 cell culture could be developed to high cell concentrations. Using fluorescent microscopy,
475 microalgae cells were found in good shape for the whole batch cultivation (data not
476 shown), indicating no apparent shear stress despite the use of the mechanical circulating
477 device set to obtain $U_L = 0.60$ m s⁻¹. Finally, as shown in section [3.2], the average light
478 intensity on the surface of the flat panels was about 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and the biomass
479 yield on light energy ranged from 0.3 to 0.6 g mol⁻¹ during the whole cultivation period,

480 values comparable with data reported for several green microalgae grown in flat panel
481 reactors (Kliphuis et al., 2010; Li et al., 2014).

482 A recently published review by Lim and co-authors extensively analyzed the CO₂ bio-
483 fixation results available in literature (Lim et al., 2021). The authors analyzed several
484 approaches to evaluate the effective CO₂ bio-fixation by microalgae culture and reported
485 that most of the scientific literature investigated the CO₂ fixation only at laboratory scale.
486 In this work, for CO₂ bio-fixation calculations we used the elementary analysis approach.
487 Moreover, the experiments described in this work were performed on volumes higher
488 than laboratory scale, and additionally a pure CO₂ stream was used to feed the PBR in a
489 way that could be compatible with already available industrial streams. The obtained
490 results place the new flat-panel PBR among the most promising prototypal technology to
491 implement a microalgae CO₂ fixation approach at industrial scale. Furthermore, the
492 hardware components of the PBR, equipped with a multi-probes system connected via
493 transducer to an integrated PLC (see section [2.1]), make this pilot-scale prototype
494 already suitable for remote monitoring and control of cultivation parameters to
495 incorporate the Internet of Things (IoT). This, as extensively highlighted in recent
496 literature, is becoming a relevant aspect as both academic research and industry are
497 gradually moving towards process automation and remote operation (Tham et al., 2022;
498 Wang et al., 2022).



499

500 **Figure 5:** Biomass concentration of *Acutodesmus obliquus* in the flat panel
 501 photobioreactor. Stars represent the dry weight measurements \pm standard deviation ($n =$
 502 3). Dotted line represents the third-degree polynomial interpolation ($R^2 = 0.9985$)
 503 obtained with the software Matlab®.

504

505 4. Conclusion

506 This study provides the characterization of a new flat panel PBR prototype regarding
 507 the main hydrodynamic parameters, the CO₂ supply strategy and the artificial lighting
 508 system. An innovative liquid handling strategy based on a pump-assisted circulation has
 509 been proposed and tested in this work. The liquid culture is moved by a centrifugal pump
 510 that allows to achieve appropriate culture mixing and enhance CO₂ mass transfer with a
 511 lower power consumption compared to the majority of flat-panels described in the
 512 literature. The biological performances of the system have been successfully tested by
 513 cultivating the green microalgae *Acutodesmus obliquus*, which showed a mean daily
 514 volumetric productivity (P_v) of 0.21 g L⁻¹ d⁻¹ and a biomass yield on light energy
 515 comparable to those reported for several green microalgae in flat-panel PBRs.

516 Furthermore, the CO₂ bio-fixation efficiency was found to be higher (64%) than those
517 reported in literature for several microalgae species grown in large-scale setups. Taken
518 all together, the energetic and biological performances of this pilot-scale PBR may
519 constitute an important step toward the development of industrial-scale technologies to
520 mitigate CO₂ while obtaining high quality microalgal biomass. Future work will be
521 carried out in order to further reduce the energy consumption, optimize CO₂ and light
522 supply strategies, as well as several other cultivation parameters (e.g., media composition,
523 temperature, light quality), to further optimize the overall photobioreactor CapEx and
524 OpEx.

525

526 **Declaration of Competing Interest**

527 The authors declare that the research was conducted in the absence of any commercial or
528 financial relationships that could be construed as a potential conflict of interest.

529

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535

536 **Author contributions**

537 M.C., A.O., M.Z. and V.A.R. designed research; M.C., D.A., V.C., and C.D. performed
538 research; M.C. and V.C. analysed data; M.C. and V.A.R. wrote the article.

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