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Characterization of split cylinder airlift photobioreactors for efficient microalgae cultivation

Bruno D. Fernandes *, André Mota, António Ferreira, Giuliano Dragone, José A. Teixeira, António A. Vicente

CEB—Centre of Biological Engineering, University of Minho, Braga, Portugal

HIGHLIGHTS

- A novel photobioreactor (SCAPBR) was developed.
- SCAPBR has up to 53% more illuminated area comparing to a bubble column.
- Mass transference in SCAPBR was very efficient (K_La up to 0.003 s^{-1}).
- Maximum biomass productivity was obtained in SCAPBR 50 at U_{Gr}=0.004 m s^{-1}.

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ABSTRACT

An extensive characterization of photobioreactors (PBRs) must be made in order to optimize their operational conditions, operate design improvements and perform scale-up. In this work, a hydrodynamic characterization of liquid and gas phases was performed, as well as the determination of the mass transfer coefficient of three different PBRs (bubble column – BC – and two Split Cylinder Airlift Photobioreactors – SCAPBRs – featuring two different riser-to-downcomer cross sectional area ratios: SCAPBR 75 and SCAPBR 50). The effect of these parameters on biomass productivity was also evaluated. The developed SCAPBRs proved to be extremely suitable for microalgae cultivation. The design of the PBR, particularly the designed gas sparger, allowed meeting the needs of microalgae in terms of mixing and mass transfer (efficient supply and removal of CO_2 and O_2, respectively). SCAPBR 50 (with a superficial gas velocity of 0.0044 m s^{-1}) showed, among the tested PBRs, the highest value of biomass volumetric productivity (0.75 g L^{-1} d^{-1}). This result is probably due to a higher PBR illuminated surface area, and a more regular flow pattern between the illuminated and dark zones verified in SCAPBR 50, which allows exposing cells to regular light–dark periods.

1. Introduction

The driving force for the development of microalgae-related technology is the ability of these microorganisms to efficiently convert solar energy to chemical energy via carbon dioxide (CO_2) fixation. In recent years microalgae became one of the most promising feedstocks for biofuel, bioplastics, cosmetics, pharmaceutical and human nutrition markets. In spite of the huge interest in microalgae cultivation, the economic aspects of the process are still not satisfactorily solved, especially at large-scale. Assuming that the best microalgal specie for the process is identified and selected, the next quest remaining is an optimal design of the microalgae cultivation system to increase cultivation productivity as a whole, reducing the cost of the production process.

In general, the cultivation systems that have been proposed or used for microalgae growth are, inefficient, complex or too costly to be applied in large-scale production. Enclosed photobioreactors (PBRs) have several advantages over open pond production and these advantages are even more important if the desired product is to be used in pharmaceutical applications or if the microalgae require a culture environment that is not highly selective (Mirón et al., 2003) and consequently liable to contaminations. Generally, closed PBRs can be divided in horizontal and vertical PBRs.

Vertical PBR orientation has been proposed to enhance productivity by reducing the photosaturation (Cuaresma et al., 2011). This photosaturation reduction is achieved by an effect of light dilution, since the sunlight falling on a given ground area is spread over a larger reactor surface area when the PBRs are placed vertically. As a result, more algae are exposed to lower intensities,

* Corresponding author. +351 963141775.
E-mail address: brunofernandes@deb.uminho.pt (B.D. Fernandes).

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being able to maximize their photosynthetic efficiency (Posten and Schaub, 2009). Cuaresma et al. (2011) tested outdoor vertical and horizontal PBRs and concluded that the highest photosynthetic efficiency was found for the vertical simulation, 1.3 g of biomass produced per mol of PAR photons supplied, against 0.85 g mol^{-1} of horizontal PBR and the theoretical maximal yield (1.8 g mol^{-1}). In addition, it is known that under low light intensity a vertical orientation captures more reflected light (Sánchez Mirón et al., 1999). The same authors also concluded that vertical PBRs performed better than horizontal PBRs because they are supposedly more suited for scale-up, require less energy for cooling because of the low surface to volume ratio, and overall outperform horizontal reactors throughout the year. Moreover, vertical column PBRs are characterized by their high volumetric gas transfer coefficients. This is caused by the bubbling of gas from the bottom, which enables not only efficient CO_2 utilization, but also optimal O_2 removal (Wang et al., 2012). The main factor that affects microalgae growth in vertical PBRs is the limited efficiency of light utilization.

It is well known that both the quantity and the quality of the light delivered to the cells are significant to the cells' growth (Fernandes et al., 2010). For dense cultures, in certain periods of the day, the regions close to the surface are subject to high light intensities. These are often greater than the saturation value of the main microalgae species causing photoinibition (Wu and Merchuk, 2004). On the other hand some zones in the reactor may remain in the dark due to optical absorption and self-shading of the cells, causing photolimitation. Thus, it is necessary to prevent high residence time of microalgae cells under these conditions, which is achieved through a constant but regular cell circulation. It is known that the conversion of light energy to biomass can be enhanced if microalgal cells are made to repeatedly move between the well-lit exterior and the dimly lit interior of the photobioreactor (Janssen et al., 2003). Ordered mixing forces the cells to experience periodical light/dark cycles. The effect of the light/dark cycles has been studied previously (Merchuk et al., 1998), and it was found that periodical light/dark cycles might enhance growth (Wu and Merchuk, 2004). However, random mixing does not appear to enhance productivity as much as a regular light–dark cycle (Degen et al., 2001). According to Janssen et al. (2003) fast light/dark cycles on a microsecond–millisecond scale improve microalgal photosynthetic efficiencies. The same authors state that photosynthetic efficiencies can be increased with light/dark cycles of 1–4 s, but these improvements were less evident at the longest cycles. On the other hand, utilization of light/dark cycles of several seconds to tens of seconds does not appear to result in an improvement of the photosynthetic efficiency. Therefore the microalgal photosynthetic efficiency seems to be influenced by the frequency of light/dark cycles, which is determined by liquid circulation velocity, which in turn depends on reactor design and superficial gas velocity (Janssen et al., 2003).

According to Wang et al. (2012), airlift PBRs can sustain better biomass production of different microalgae in comparison to other vertical column PBRs. This might be due to this regular mixing, as opposed to random mixing found in bubble columns. The concentric tube airlift is the most commonly used airlift for microalgae cultivation. However some limitations are evidenced, such as difficult temperature control and large fraction of dark zones inside the PBR, mainly due to the presence of the internal column, which limits light penetration.

In this work a novel Split Column Airlift Photobioreactor (SCAPBR) is proposed as a very promising microalgae cultivation system. The novel SCAPBR has the potential to overcome the limitations of the concentric tube airlift (integrated temperature control system and transport of light to the centre), while maintaining all the benefits inherent to an airlift PBR. In order to provide the best conditions for microalgae growth in SCAPBR, it is of interest to determine and optimize all the parameters that characterize SCAPBR operation. At this stage it is necessary to prove some of the assumptions on which the design was based. SCAPBRs characterization in terms of hydrodynamics and mass transfer characteristics includes the determination of: mass transfer coefficient (K_La), mixing time, liquid velocity, gas bubble velocity and gas hold-up. The nutritional and light requirements of photosynthetic microorganisms may be covered in PBRs with larger light paths, if hydrodynamic and mass transfer conditions are optimized in these PBRs. Only taking into account this point it will also allow predicting the effects of scale-up on the performance of the SCAPBR. In this work a full characterization of two different SCAPBRs designs (SCAPBR 50 and SCAPBR 75) and a bubble column (BC) (used as a control PBR) will be carried out, as well as the evaluation of the effect of the hydrodynamic characteristics and design on biomass productivity.

2. Material and methods

2.1. Photobioreactors

In the proposed SCAPBRs (Fig. 1), a flat plate splits the diameter of the column and separates the column into two parts (riser and downcomer), acting also as a heat exchanger and an internal light guide. The choice for a SCAPBR and the options made in the project design had as main objective to overcome some of the limitations of existing microalgae cultivation systems.

The flat plate that splits the column is made of a transparent material and fully filled with water and acts as a light conductor and distributor inside the SCAPBR (Fig. 1). Therefore the PBR illuminated surface significantly increases. Thus, the central area of the PBR which normally would be completely devoid of light (especially for higher cell concentrations) will have a continuous supply of light. The presence of this central baffle also allows using diameters in the SCAPBR scale-up that would be otherwise unviable due to a substantial increase of dark zones within the PBR. Finally the central wall of the PBR also functions as heat exchanger (Fig. 1), ensuring an efficient cooling of the medium without the need of a large technical apparatus nor the use of large amounts of water.

Considering all the characteristics presented, the SCAPBR proposed has the potential to provide conditions for an ideal microalgae cultivation: proper exposure to light energy, good mass exchange between gas and liquid, flow mixing, low shear stress over the cells and a proper temperature control.

Three different PBRs were tested: a bubble column (BC) and two different SCAPBRs, as shown schematically in Fig. 1.

All vessels were made of 3.8 mm thick, transparent poly (methyl methacrylate) with 90 mm of internal diameter. The liquid height was 600 mm, for a working volume of 3.7 L. All the three PBRs have a total height of 700 mm. The riser-to-downcomer cross sectional area ratio was 1.0 for the SCAPBR 50 and 3.0 for the SCAPBR 75. The baffles, with 4.0 mm of thickness, were located 50 mm from the bottom of the PBRs and 50 mm below the liquid level and were also made of transparent poly(methyl methacrylate) to allow light penetration (Fig. 1).

2.1.1. Aeration system

To ensure an efficient mass transfer inside the SCAPBR an aeration system was developed. The fluid was mixed by sparging CO_2-enriched air (2% v/v CO_2) through a sparger composed by 45, 26 and 19 uniformly spaced needles (with an inner diameter (d_n) of 0.25 mm) in the BC, SCAPBR 75 and SCAPBR 50, respectively. In all the spargers, needles were placed with a spacing (L_n) of

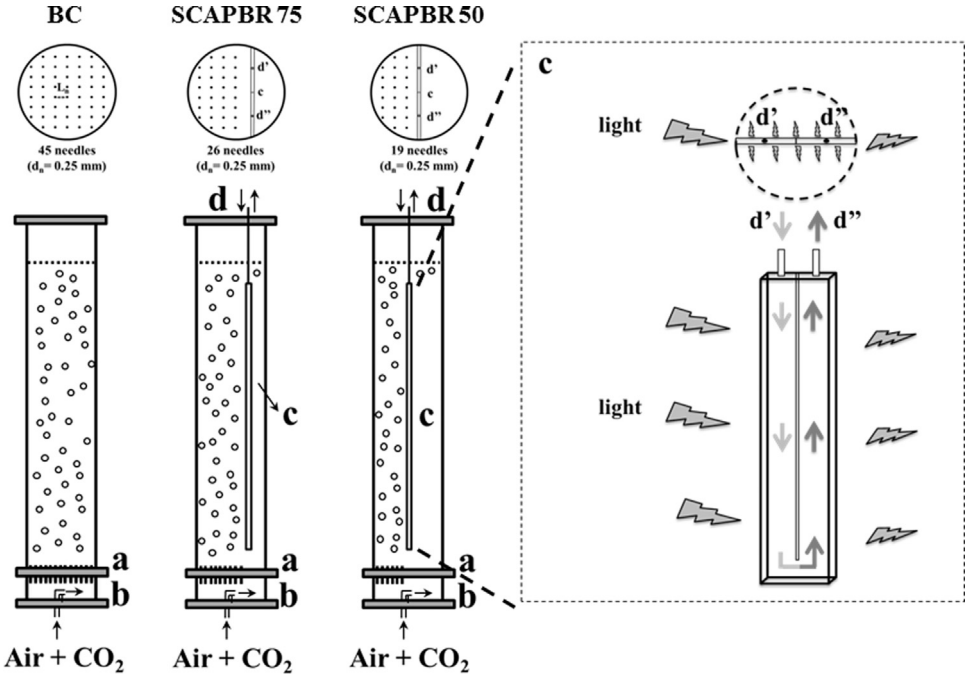

Fig. 1. The geometry of tested photobioreactors (BC, SCAPBR 75 and SCAPBR 50) and respective air spargers (frontal and top view). Sparger (a); pressure chamber (b); baffle (c); heat exchanger inlet and outlet (d). Detailed schematic representation lateral and top view of SCAPBRs baffle (c), acting as heat exchanger and light guide. Cold water inlet (d′); warm water outlet (d″).

5.0 mm between them (Fig. 1). The shape and size of the needles ensure the formation of small and well-defined bubbles. Needles' disposition enables a uniform bubble distribution along the PBRs which, in theory, optimizes mass transfer and enhances the suspension of low-density solids.

The aeration system comprises two mass flow controllers that determine CO_2 and air flow rates; these gases are subsequently mixed in a gas mixing chamber and filtered before being injected into the PBR through the sparger. Between the filter and the gas sparger there is a pressure chamber (Fig. 1), which allows a homogeneous gas distribution through all needles even at low flow rates.

2.2. Hydrodynamic and mass transfer characterization of PBRs

All the hydrodynamic and mass transfer determinations were performed at different superficial gas velocities (U_{Gr}) (0.001–0.009 m s^{-1}) based on the riser cross-section of the reactors. The superficial gas velocity U_{Gr} is easily derived from the air-flow rate by dividing this value by the cross-sectional area of the aerated zone.

All the measurements were made at 25 °C with tap water and microalgae growth medium (described in Section 2.3). Viscosities of growth medium and tap water were approximately the same (0.998×10^{-3} Pa s). The viscosities were measured at 25 °C using a Cannon–Fenske viscometer. The surface tension of both fluids was also approximately the same (72.3×10^{-3} N m^{-1}). The surface tension was measured at 25 °C using a tensiometer (Kruss K6 GmBH, Germany). The conductivity (Conductivity Meter LF 538, WTW, Germany) of water and growth medium was 2.07 and 516.67 μS cm^{-1}, respectively.

2.2.1. Liquid phase characterization

2.2.1.1. Mixing and circulation time. For determination of mixing time and circulation time, 1 mL of saturated NaCl aqueous solution was injected (using a syringe) as a pulse near the bottom of the riser in the central region through a 1 mm stainless steel capillary. The tracer influence in the system was assessed by a conductivity probe (Conductivity Meter LF 538, WTW, Germany) placed near the top of the riser. For each operating condition, experiments were run five times. The liquid phase was changed after each three runs.

Mixing time was defined as the time needed to reach 95% of complete mixing. The circulation time was computed by averaging the time spans between maximum consecutive peaks in the conductivity probe response curve (Freitas et al., 2000).

2.2.1.2. Liquid circulation velocity. The mean liquid circulation velocity in the riser was obtained using a thermal tracer method, which provides the fastest response time among the various tracer methods available. The thermal tracer method involves injecting a pulse of 5 ml of hot water into the flowing liquid and plotting the time–temperature profile at two given points in the riser by means of two thermocouples connected to a computer. The liquid linear velocity in the riser was then obtained by the ratio of the distance between the two thermocouples and the differences in response times between the two sensors (García-Calvo et al., 1999).

2.2.2. Gas phase characterization

2.2.2.1. Gas holdup. Riser gas holdup in the PBRs was determined by the use of a monofibre optical probe technology described by Mena et al. (2008) The optical probe is used to locally detect the presence of the gas phase in a multiphase system. A monochromatic light is transmitted through an optical fiber to the tip of the probe. When the tip is dipped into a gas phase, the light is mainly reflected, travels back to the detector through a Y junction and is converted into an electrical signal (high level signal). This signal is converted to a digital signal that is subsequently interpreted by the So2_4 software (Mena et al., 2008), which finally provides the values of gas holdup. In order to obtain values with a statistical meaning, about 2000 bubbles were analyzed for each experimental condition.

2.2.2.2. Bubble characterization. In order to obtain the bubble size distribution (Sauter mean diameter (d_{32})), bubble elongation (F_{max}/F_{min}) and bubble complexity degree (B_{CD}), a chamber with a flat straight section filled with water was coupled to the PBRs. The chamber used during bubble size measurements was designed in order to minimize the problems related with the effect of the optical distortion of bubbles caused by the round surface of the photobioreactors. Sets of images, obtained at 200 mm from the gas sparger, were grabbed with a black and white high speed digital video camera (frame rate of 250 images s^{-1}) connected to a PC, and used to study the bubble shape and size distribution. After the acquisition of a set of images (about 5 images s^{-1}), these were automatically treated and the bubbles were identified and classified. For that, the image analysis technique and the discriminant factorial analysis were combined as described by Ferreira et al. (2012). In order to obtain values with a statistical meaning, about 600 bubbles were analyzed for each experimental condition, this number is in accordance with the values presented elsewhere (Ferreira et al., 2012).

Bubble gas velocity in the BC and in the SCAPBRs riser were also measured by means of the optic probe technique, previously described by Mena et al. (2008) and used for gas holdup determination. In order to obtain values with a statistical meaning, about 2000 bubbles were analyzed for each experimental condition.

2.2.3. Mass transfer coefficient (K_La) of carbon dioxide

In autotrophic microalgae cultivation, gas–liquid mass transfer of CO_2 is of major importance, because CO_2 is the main carbon source. Therefore, it is necessary to determine the volumetric mass transfer coefficient K_La (CO_2) that allows characterizing the CO_2 transfer rate between gas and liquid phases.

According to the literature (Baquerisse et al., 1999), the physical properties of the liquid, liquid flow, as well as system and gas injector geometries are the factors that determine volumetric mass transfer coefficients. Thus, the calculation of K_La of CO_2 has been done from the determination of the K_La of O_2

$$K_La\,(CO_2) = \sqrt{\frac{D_{O2}}{D_{CO2}}}\,K_La\,(O_2), \tag{1}$$

where K_La (CO_2) is the CO_2 mass transfer coefficient (s^{-1}), K_La (O_2) is the O_2 mass transfer coefficient (s^{-1}); D_{O2} is the O_2 diffusion coefficient ($m^2\,s^{-1}$) and D_{CO2} is the CO_2 diffusion coefficient ($m^2\,s^{-1}$).

Oxygen mass transfer experiments were performed in a two-phase system at different superficial aeration velocities (U_{Gr}) (0.001–0.008 m s^{-1}) and liquids (water and mineral growth medium). Air was used as gas phase. The liquid height was h_0= 600 mm for all experiments (no liquid throughput). Firstly, the liquid was deoxygenated by bubbling nitrogen, then, when the dissolved oxygen concentration was practically zero, humidified air was fed into the column. Dissolved oxygen concentration values were measured online using an O_2 electrode (CellOx 325, WTW, Germany), located 200 mm from the gas sparger and 30 mm from the wall, and recorded directly in a PC, through a data acquisition board. Dissolved oxygen concentration data versus time, t, were obtained, and K_La was calculated according to Ferreira et al. (2012). The experimental results are reproducible with an average relative error of 5% and are not influenced by the dynamics of the oxygen electrode since its response time, less than 16 s for a 95% confidence interval (technical data), was smaller than the mass transfer time of the system (ranging from 25 to 500 s). Additionally, the first-order time constant of this probe is 6 s (as measured by Vasconcelos et al. (2003)), therefore indicating its possible application in the present system (Ferreira et al., 2013).

2.3. Evaluation of PBRs biomass productivity

Chlorella vulgaris (P12) obtained from the Culture Collection of Algal Laboratory (CCALA, Czech Republic), was used for cultivation. The inoculum for the photobioreactors was grown under artificial light (250 μmol $m^{-2}\,s^{-1}$ light flux at the PBR's surface) in a 1 L bubble column aerated at 0.5 vvm. The preculture medium was identical to that used in the final reactor cultivation. The carbon source and agitation during cultivation of microalgae were supplied by bubbling CO_2-enriched air (2% v/v CO_2) through a needle sparger (Fig. 1).

The three PBRs tested were placed in a fully closed compartment with controlled temperature, in order to maintain cultures at 30 °C. Illumination was provided by 8 fluorescent lamps (Sylvania Standard 36 FW) placed equidistant from the PBRs surface (200 mm) and equidistant from each other (60 mm) on one side of the photobioreactors, at an irradiance level of 250 μmol $m^{-2}\,s^{-1}$, measured using a LI-COR Quantum/Radiometer/Photometer Model LI-250 Light Meter (San Diego, CA, USA). The incident photon flux density was measured (in triplicate) at 5 different points (different heights) at the surface of the PBRs.

Also the light radiating from the central baffle to the interior of both SCAPBRs was measured. The SCAPBRs were filled with water and externally covered with opaque paper except in the line that represents the intersection of the central baffle with the SCAPBR column, allowing the penetration of light there. In both SCAPBRs the light flux conveyed by the central baffle was measured to be ca. 25 μmol $m^{-2}\,s^{-1}$. This value is the average of the measurements (in triplicate) in 5 different points of the baffle and is independent of cell concentration, since the baffle was filled with water. Like the PBR walls, baffle internal walls were considered as a continuously illuminated PBR surface.

The growth medium based on chemical components present in the microalgal biomass (Fernandes et al., 2013) had the following composition (mM): 18.32 $(NH_2)_2CO$, 1.74 KH_2PO_4, 0.83 $MgSO_4\cdot H_2O$, 0.79 $CaCl_2$, 0.11 $FeNa–C_{10}H_{12}O_8N_2$, 0.017 $MnCl_2\cdot 4H_2O$, 0.013 H_3BO_3, 0.009 $ZnSO_4\cdot 7H_2O$, 0.004 $CuSO_4\cdot 5H_2O$, 0.002 $CoSO_4\cdot 7H_2O$, 0.0001 $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and 0.0001 $(NH_4)VO_3$ in distilled water. The medium was inoculated using inoculum in the late exponential growth phase after cell synchronization and the pH was adjusted to 7. Biomass concentration in the freshly inoculated PBRs was about 0.05 g L^{-1}.

In order to determine how the hydrodynamic and mass transfer parameters affect the system productivity, microalgae cultivations were carried out (in triplicate) at 3 different conditions (U_{Gr}=0.0011; 0.0044 and; 0.0077 m s^{-1}) in each of the 3 different tested PBRs.

Biomass concentration was estimated by cell dry weight after centrifugation of the sample (8750 g for 10 min), washing with distilled water and drying at 105 °C until constant weight.

Biomass productivity (P_{max}, g $L^{-1}\,d^{-1}$) during the culture period was calculated from

$$P_{max} = (X_t - X_o)/(t_x - t_0), \tag{2}$$

where X_t is the biomass concentration (g L^{-1}) at the end of the exponential growth phase (t_x) and X_0 is the initial biomass concentration (g L^{-1}) at t_0 (day).

3. Results and discussion

Liquid and gas phase characterization and volumetric mass transfer coefficient determination (K_La) were performed for all PBRs at different values of superficial gas velocity in the riser (U_{Gr}) using tap water and growth medium. No statistically significant differences in these parameters were found between water and

growth medium suggesting that changes in ionic strength within a certain range of values did not significantly affect liquid and gas phase parameters as well as mass transfer. This is in agreement with the results obtained by other authors (Sánchez Mirón et al., 2004; Sánchez Mirón et al., 1999). Thus, the results presented refer only to results obtained with water.

3.1. Liquid phase characterization

It is known that the level of mixing in a reactor strongly contributes to the growth of microalgae (Suh and Lee, 2003). Mixing improves biomass productivity by increasing the frequency of cell exposure to light and dark volumes of the reactor and by increasing mass transfer between nutrients and cells, maintaining uniform pH and eliminating thermal stratification. Liquid phase characterization was performed for the 3 PBRs (Fig. 2).

3.1.1. Liquid circulation velocity

The liquid circulation velocity is an important factor to assess airlift reactors' mixing efficiency. Mixing time in these reactors is expected to be affected by the relative velocity between the gas and the liquid phases. Moreover liquid velocity is also a parameter often used during the scale-up of airlift reactors. Liquid circulation velocity is a meaningless parameter in bubble columns (in this range of U_{Gr} values), thus it was only determined for the SCAPBRs (Fig. 2A).

In both SCAPBRs the increase of U_{Gr} causes a pronounced increase in liquid circulation velocity for $U_{Gr} < 0.005$ m s^{-1}, meaning by this that liquid circulation velocity is very dependent on U_{Gr}. However, for higher values of U_{Gr}, liquid circulation velocity appears to be nearly independent of U_{Gr}. This relation between U_{Gr} and liquid circulation velocity has been previously reported in airlifts reactors (Klein et al., 2003; Lu et al., 1995).

Fig. 2A also shows that SCAPBR 50 presented higher liquid circulation velocity than SCAPBR 75 for all values of U_{Gr}, which is most probably explained by differences in the PBRs geometry, namely the different riser:downcomer ratios. Gavrilescu and Tudose (1996) showed that riser:downcomer ratio affects the circulation liquid circulation velocity because it modifies the resistance to flow by varying the fraction of the total volume contained in downcomer and riser. Riser:downcomer ratio has proven to be the main factor which determines the friction in the reactor which means that for higher riser:downcomer ratios the liquid circulation velocity is lower (Gavrilescu and Tudose, 1996).

3.1.2. Mixing and circulation time

Mixing times *vs* U_{Gr} data for the three PBRs are shown in Fig. 2B.

For a given U_{Gr}, the BC always has a lower mixing time compared with the SCAPBRs. These results are in agreement with other authors who reported that analysis of mixing in bubble columns showed that they have shorter mixing times than airlift reactors (Guieysse and Munoz, 2001). In fact, compared with the chaotic flow in the bubble column, the organized cyclic flow in the airlift reactors inhibits bulk mixing (Sánchez Mirón et al., 2004). In the three tested PBRs, the general tendency was a decline of mixing time with increasing U_{Gr}. As expected at low aeration flow rates, the mixing time in the SCAPBRs was much more sensitive to U_{Gr} than at high aeration rates. In the bubble column the mixing time was almost flow-independent for gas flow rates above 0.003 m s^{-1}(Fig. 2B).

Comparing the two SCAPBRs it is clear that, for $U_{Gr} <$ 0.002 m s^{-1} mixing times are almost the same, whereas for $U_{Gr} > 0.002$ m s^{-1} SCAPBR 75 requires less time to achieve complete mixture. It is possible to assume that, in general, SCAPBR 75 could guarantee a more efficient transport of nutrients for the cells than SCAPBR 50 at higher U_{Gr}. The reason for the lower mixing times of SCAPBR 75 can be its higher riser:downcomer ratio, that allows a more chaotic flow in its riser, promoting bulk mixing and, consequently, reducing mixing time. These observations are in close agreement with results of other authors (Gavrilescu and Tudose, 1996).

The dependence of circulation time on U_{Gr} in SCAPBRs (Fig. 2C) was quite similar to mixing time profiles in the same reactors (Fig. 2B). Circulating time is defined as the average time needed for particles to circulate one cycle in the bioreactor; it can be also used to evaluate mixing performance of bioreactors (Oldshue, 1976). Circulation time is a meaningless parameter in bubble columns, thus it was only determined for SCAPBRs (Fig. 2C).

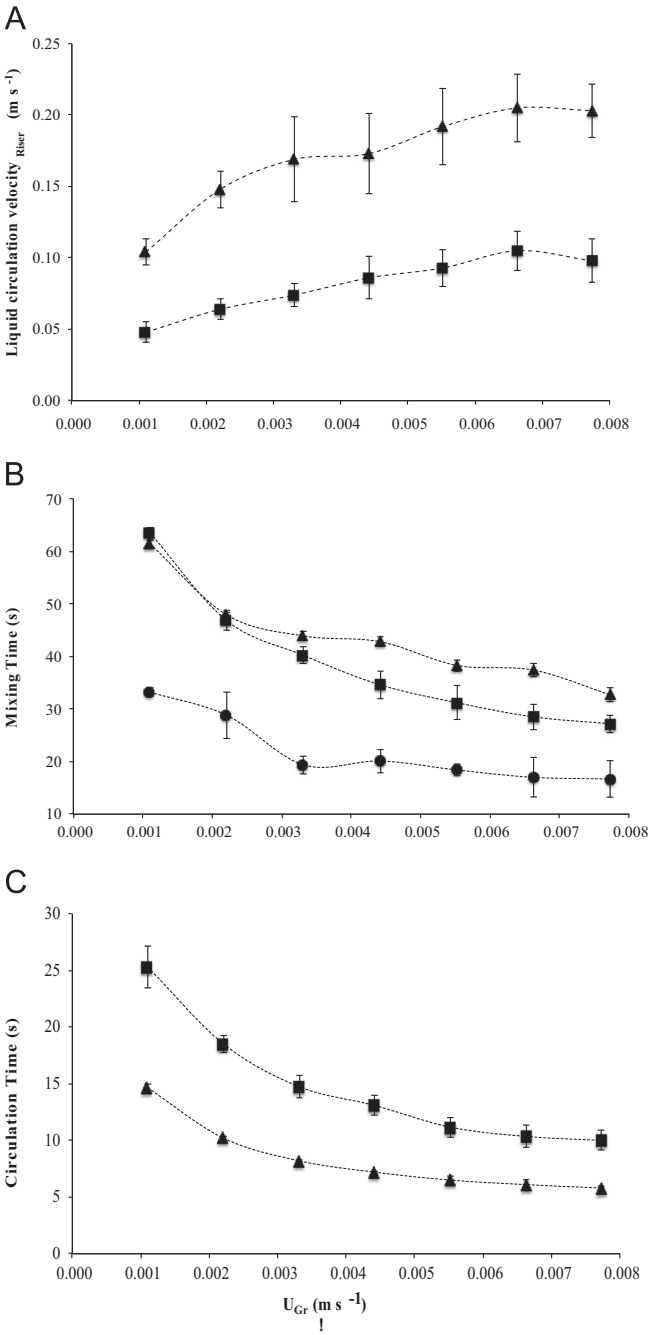

Fig. 2. Liquid circulation velocity (A); mixing time (B); circulation time (C), for SCAPBR 50 (▲), SCAPBR 75 (■) and BC (●), at different values of U_{Gr}.

At gas flow rates lower than 0.005 m s^{-1}, the circulation time decreased sharply with increasing U_{Gr}. However, for $U_{Gr} >$ 0.005 m s^{-1} circulation time dependence on U_{Gr} becomes very small. This observation is common to both SCAPBRs and is quite typical of airlift reactors (Sánchez Mirón et al., 2004). This phenomenon is associated with micronization of gas bubbles because of increasing turbulence and a consequent build-up of these smaller bubbles in the downcomer zone. The consequently reduced difference between gas holdup values in the riser and downcomer reduces the driving force for liquid circulation (Erickson, 1990; Sánchez Mirón et al., 2004). Although the shape of the curves circulation time versus U_{Gr} is similar in both SCAPBRs, circulation time values differ considerably, since SCAPBR 50 shows lower circulation times for all values of U_{Gr} tested. However, it is clear that the difference between the circulation time of these two SCAPBRs decreases with increasing U_{Gr}.

A comparison between Fig. 2B and C suggests that, in each SCAPBR, mixing time improves when circulation time is reduced. This is because rapid cycling causes the fluid to pass more frequently through the relatively well-mixed head zone of the reactor (*i.e.*, the zone above the upper edge of the baffle) (Erickson, 1990; Sánchez Mirón et al., 2004). However, comparing the two SCAPBRs shows that this relationship between the mixing time and circulation time is not the same for the two cases. SCAPBR 50, despite having a lower circulation time (Fig. 2C), shows a higher mixing time (Fig. 2B) than SCAPBR 75. Again, the explanation possibly lies in the fact that SCAPBR 75 allows bulk mixing in a greater extent due to its larger riser, which behaves somehow as a bubble column. Although it reduces mixing time when compared to SCAPBR 50, this bulk mixing in the riser of SCAPBR 75 increases the residence time in the riser and consequently increases the circulation time.

It is known that mixing and circulation times depend primarily on reactor geometry (Chisti and Moo-Young, 1988). Although connected, mixing and circulation times do not necessarily vary in the same way with the geometry which might explain the observed differences in mixing and circulation times between the three tested PBRs.

3.2. Gas phase characterization

3.2.1. Gas holdup

Gas holdup (ε) is one of the most important parameters characterizing PBRs hydrodynamics since the difference between gas holdup in the riser and in the downcomer is the driving force for the circulation inside the reactor. In this work, gas holdup was measured in the riser, for different U_{Gr} (Fig. 3).

In all the three PBRs tested riser gas holdup increased almost linearly with the increase of U_{Gr}. Due to its large riser fraction, SCAPBR 75 shows very similar ε values in comparison to that obtained in the BC, while SCAPBR 50 displays considerably lower ε values. The differences between the 2 SCAPBRs can be explained by other authors who claim that in airlift reactors, holdup is also influenced by the induced liquid circulation velocity (Fig. 2A) that depends on the geometry of the flow path (Xu et al., 2002).

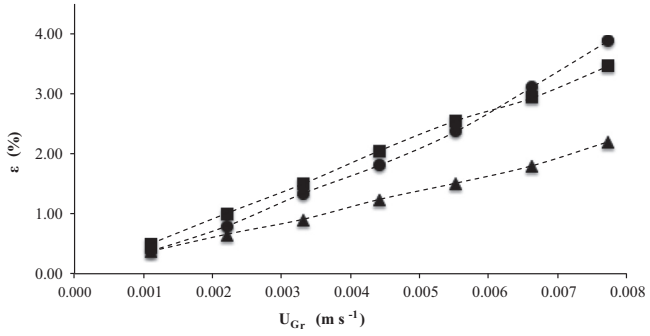

Fig. 3. Riser gas holdup for SCAPBR 50 (▲), SCAPBR 75 (■) and BC (●), at different values of U_{Gr}.

3.2.2. Bubble characterization

In combination with gas holdup, bubble size and shape influence the gas–liquid interfacial area available and consequently the mass transfer coefficient (Erickson, 1990). Interfacial area may be enhanced either by increasing gas holdup or by decreasing the prevailing bubble size. However there are limits for bubble size decrease since it is known that small bubbles induce more shear stress to cells than larger bubbles.

3.2.2.1. Sauter mean diameter (d_{32}). Bubble size is a crucial factor to minimize shear damage to cells and optimize mass transfer. Rocha et al. (2003)grew *Nannochloropsis gaditana* using small *vs*. large bubbles and they found better microalgal growth with larger bubbles and as air flow rate was increased the cells suffered more shear with smaller than with larger bubbles. In practice, there is a diameter distribution of bubble sizes in the PBR. The Sauter mean bubble diameter (d_{32}) is frequently used as a bubble size quantification parameter. The d_{32} refers to the diameter of a sphere with the same volume-to-surface ratio as the gas bubble (Erickson, 1990). For all tested PBRs, bubbles' Sauter mean diameter (d_{32}) generally increases with U_{Gr} (Fig. 4A).

The dependence of d_{32} to U_{Gr} is lower for higher values of U_{Gr}. Although the difference is not very defined, it is possible to conclude that the largest bubbles are observed in BC and the smallest in SCAPBR 50 for all tested values of U_{Gr}.

3.2.2.2. Elongation (F_{max}/F_{min}). Maximum (F_{max}) and minimum (F_{min}) Feret diameters (Feret diameter is the smallest distance between two parallel tangents to the object, the tangent position being defined by the angle between them and the horizontal axis) were obtained in order to calculate the elongation (F_{max}/F_{min}) of bubbles (Ferreira et al., 2012).

It is known that the shape of bubbles is influenced by the superficial gas velocity. Depending on U_{Gr}, bubbles can be more or less elongated. Fig. 4B shows the F_{max}/F_{min} ratio (*i.e.*, elongation), which gives the bubble shape for different U_{Gr} in the three tested PBRs.

In the three tested PBRs, it was found that for $U_{Gr} < 0.005$ m s^{-1} bubble shape (in terms of F_{max}/F_{min} ratio) is strongly dependent on the U_{Gr} value. However, for $U_{Gr} > 0.005$ m s^{-1} bubble shape becomes constant. In BC, bubbles have a slightly higher elongation than in both SCAPBRs, which can be attributed to different mixing patterns between BCs and SCAPBRs. According to F_{max}/F_{min} values and using the classification previously described (Mena et al., 2005), the bubbles present in all tested PBRs are classified as flattened spheroids. Flattened spheroids are known to have higher oscillation amplitudes that influence mass transfer. Montes et al., 1999 showed that oscillating bubbles improve mass transfer due to the variation of contact times and concentration profiles surrounding the bubbles.

3.2.2.3. Bubble complexity degree (B_{CD}). The complexity of the bubble system can be determined through the parameter "bubble complexity degree" (B_{CD}). The higher the value of B_{CD}, the higher the tendency of bubbles to flow in groups which typically, above certain B_{CD} levels, means that mass transfer is reduced (Ferreira et al., 2012).

The values of B_{CD} of the three tested PBRs (Fig. 4C) increased almost linearly with the increase of U_{Gr}, with the exception of BC at U_{Gr} higher than 0.07 m s^{-1}. The SCAPBR 50 shows for all U_{Gr} a lower value of BCD, whereas the BC has, for $U_{Gr} < 0.07$ m s^{-1} higher BCD values than those obtained in SCAPBRs. For $U_{Gr} >$ 0.07 m s^{-1} SCAPBR 75 shows the higher bubble complexity degree.

3.2.2.4. Gas bubble velocity. Gas bubble velocity determination (Fig. 4D) shows that in SCAPBR, this parameter is almost independent of U_{Gr} since it remains almost constant over the different values of U_{Gr}. In the BC, bubble velocity decreases with the increase of U_{Gr} (probably due to an increase of turbulence caused by the higher number of bubbles), keeping constant from $U_{Gr} > 0.03$ m s^{-1} onwards. These results are not in agreement with what would be expected by the analysis of Fig. 4A. Typically, larger bubbles tend to have higher rising velocities, which was not observed in this case. Possibly the fact that bubbles in BC have a more flattened geometry (as shown in Fig. 4B) has a major influence in the calculation of their size: they are actually sized as being bigger than they effectively are. When comparing bubbles' velocity in the different PBRs, it is expected that the BC shows the best mass transfer performance followed by SCAPBR 75 and SCAPBR 50.

This happens because the lower bubbles' velocity means a higher residence time in the PBR and, consequently, a better mass transfer. These differences between BC and SCAPBR are in agreement with those reported by other researchers (Contreras et al., 1998).

3.3. Mass transfer of CO_2

Microalgae cultivation systems, especially at a large scale, are limited by the transfer of CO_2 from the gas to the liquid phase. Mass transfer can be evaluated by means of the volumetric mass-transfer coefficient (K_La). The results shown in Fig. 5 are in close agreement with results obtained for mixing time (Fig. 2B), gas holdup (Fig. 3) and gas bubble velocity (Fig. 4D).

Among these factors, gas holdup seems to be the one that most influences K_La, since as for gas holdup (Fig. 3), also K_La increases almost linearly with U_{Gr}. The increase verified in Sauter mean diameter (Fig. 4A) (perhaps because the variation was not very significant) does not appear to have had a negative effect on K_La.

The very high K_La values obtained for all PBRs must be highlighted in this work. K_La values ranged between 0.007–0.04 s^{-1} in BC; 0.005–0.03 s^{-1} in SCAPBR 75 and; 0.003–0.02 s^{-1} in SCAPBR 50. Through literature review (Table 1) it was not possible to find such high values of K_La in microalgae cultivation systems. The key to these K_La values seems to lie in the aeration system developed (Fig. 1) more than in PBRs design, since comparing K_La values obtained in the BC with the results obtained by Merchuk et al. (1998) (also with a bubble column) there is nearly one order of magnitude difference in the values.

The improved mass transfer capability of all the three PBRs obtained in our study may contribute to an efficient CO_2 delivery to cells, and an effective removal of O_2 from the culture. These results

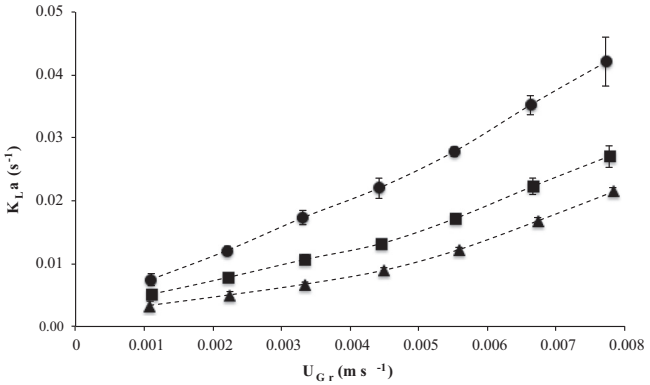

Fig. 5. K_La for SCAPBR 50 (▲), SCAPBR 75 (■) and BC (●), at different values of U_{Gr}.

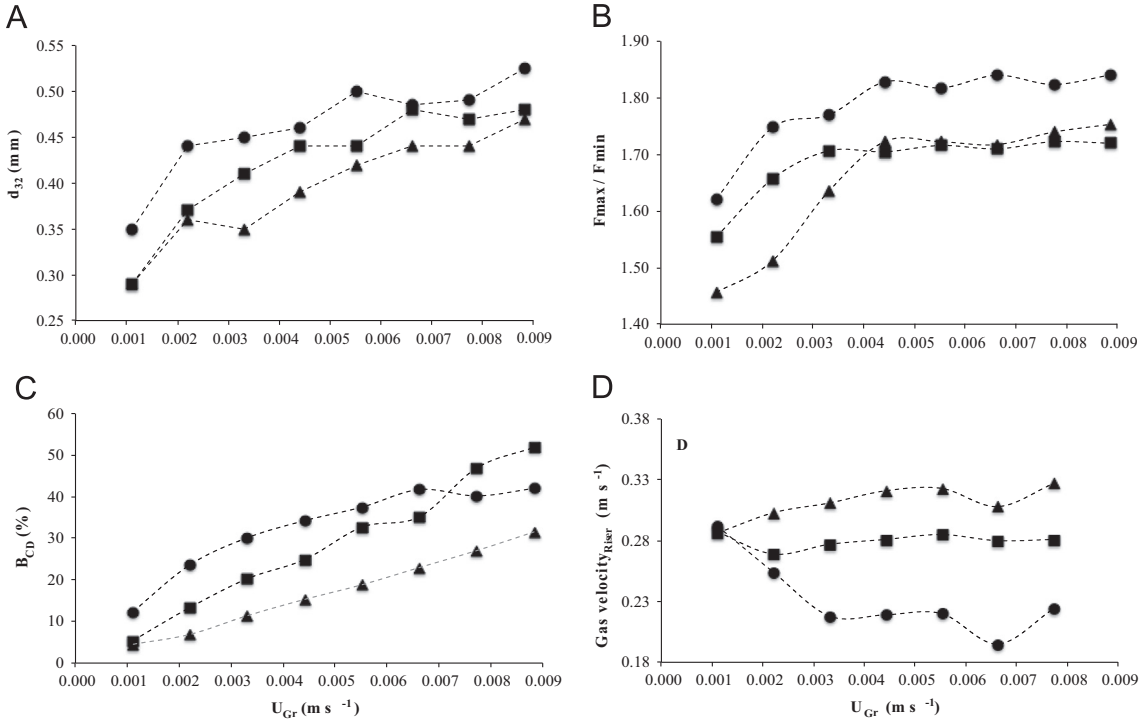

Fig. 4. Bubble Sauter mean diameter (d_{32}) (A); bubbles elongation (B); bubbles mean complexity degree (B_{CD}) (C); gas velocity$_{Riser}$ (D), for SCAPBR 50 (▲), SCAPBR 75 (■) and BC (●), at different values of U_{Gr}.

Table 1
K_La values obtained in different cultivation systems (adapted from Ugwu et al. (2003).

PBR	U_G ($m\ s^{-1}$)	K_La (s^{-1})	Reference
Concentric tube airlift	0.055	0.02	Contreras et al. (1998)
Stirred tank	0.009	0.02	Ogbonna et al. (1998)
Inclined tubular	0.02	0.003	Ugwu et al. (2002)
Bubble-column	0.008	0.005	Merchuk et al. (2000)
Flat plate	0.009	0.002	Zhang et al. (2002)
Split cylinder airlift	0.024	0.009	Vega-Estrada et al. (2005)
Horizontal tubular airlift	0.16	0.014	Camacho et al. (1999)
Tubular external-loop airlift	0.25	0.006	Acién Fernández et al. (2001)

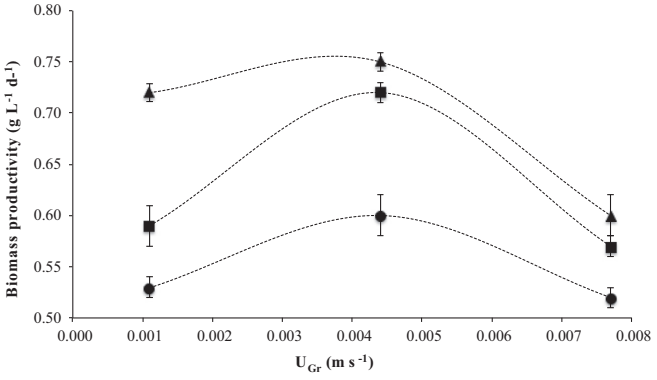

Fig. 6. Biomass productivity for SCAPBR 50 (▲), SCAPBR 75 (■) and BC (●), at three different values of U_{Gr}.

suggest that the CO_2 and O_2 mass transfer will not be a limiting factor to the microalgae growth in 3 tested PBRs, which is an important improvement in relation with other cultivation systems.

3.4. Biomass productivity

Although extremely important for characterization, optimization and scale-up of PBRs, none of the previously analyzed parameters allows concluding which PBR is more suitable for microalgae cultivation. To make that evaluation, *C. vulgaris* was grown in the three PBRs (BC, SCAPBR 75 and SCAPBR 50) at three different values of U_{Gr} (0.0011, 0.0044 and, 0.0077 $m\ s^{-1}$).

The maximum biomass productivities (P_{max}) obtained in each of the conditions are reported in Fig. 6. Contrary to what the results of liquid and gaseous phase characterization as well as mass transfer suggested (Figs. 2A–5), the highest volumetric productivities (0.60–0.72 $g\ L^{-1}\ d^{-1}$) were obtained by SCAPBR 50, exceeding BC's volumetric productivities in 15–36% and SCAPBR 75's volumetric productivities in 5–22%, depending on the U_{Gr}. Additionally it was found that in all the PBRs the highest value of Pmax was achieved at $U_{Gr}=0.0044\ m\ s^{-1}$, and the lowest at $U_{Gr}=0.0077\ m\ s^{-1}$. In all the situations the maximum cell concentration was around 6 $g\ L^{-1}$ (data not shown), which was most likely due to nutrient depletion given the concentration of medium components available. Also, no major pH differences were verified during the different growth experiments, where a maximum pH value of 7.6 ± 0.2 was obtained.

Published literature shows no unanimous conclusions when it comes to comparing the performance of airlift and bubble column PBRs. In a review by Janssen et al., the authors concluded that bubble column and air-lift reactors, in general, appear to have similar productivities, however bubble column reactors perform better at $U_{Gr} > 0.05\ m\ s^{-1}$ (Janssen et al., 2003).

Pilot scale (0.19 m column diameter, 2 m tall, 0.06 m^3 working volume) outdoor bubble column and airlift PBRs (a split-cylinder and a draft-tube airlift device) were used for *Phaeodactylum tricornutum* cultivation (Sánchez Mirón et al., 2002). The three PBRs produced similar biomass versus time profiles and final biomass concentration (~4 $g\ L^{-1}$). In a different study (Mirón et al., 2003), using the same PBRs and the same microalgae but with different growth conditions, the volumetric productivity of the three PBRs was approximately 0.30 $g\ L^{-1}\ d^{-1}$ with $U_{Gr}=0.01\ m\ s^{-1}$. Other researchers (Krichnavaruk et al., 2007) examined the cultivation of *Chaetoceros calcitrans* in airlift and bubble column PBRs and biomass productivity was about the double in an airlift device than in a bubble column. These differences in the conclusions of different studies show that it is not possible to establish one particular type of PBR as being the most suitable for microalgae cultivation. PBR performance depends on factors such as PBR geometry, aeration system and operational conditions (*e.g.*, U_{Gr} or light supply).

The results shown in Fig. 6 seem to indicate that none of the previously discussed parameters (Figs. 2A–5) appear to be the limiting factor to PBR productivity, since SCAPBR 50 showed the least favorable values for all parameters, while apparently being the one with the best results in terms of Pmax. The only factor that has remained unchanged was the fact that the SCAPBR 75 is invariably displayed as the intermediate element between the two "extremes" in terms of design (SCAPBR 50 and BC). This contributes to the conclusion that the SCAPBR 75 shows characteristics between an airlift and a bubble column.

The fact that the highest value of P_{max} was obtained for all PBRs at $U_{Gr}=0.0044\ m\ s^{-1}$ followed by a sharp decrease in productivity for $U_{Gr}=0.0077\ m\ s^{-1}$ does not seem to find an explanation in the parameters previously discussed, since higher values of U_{Gr} and K_La usually lead to higher biomass productivities (Zhang et al., 2002). None of the parameters analyzed before (Figs. 3–5) showed an inversion of behavior at $U_{Gr}=0.0044\ m\ s^{-1}$ (or indeed at any value of U_{Gr}).

One of the possible explanations for the decline in biomass productivity at $U_{Gr}=0.0077\ m\ sm\ s^{-1}$ is the shear stress caused by a higher flow rate, but this does not seem plausible because the tested values of U_{Gr} are below the values reported in the literature as being capable of causing stress in microalgae (Camacho et al., 2001) and *C. vulgaris* is described as very robust and resistant to shear stress. Thus, the hydrodynamic stress may be discarded as a factor that led to a decrease in biomass productivity in all the PBRs.

From the results it is clear that mass transfer was not a limiting factor in the present work, since SCAPBR 50 is the PBR with lower values of K_La while the BC shows the highest values for this parameter (Fig. 5). Additionally, there is a decrease in productivity between $U_{Gr}=0.0044$ and $0.0077\ m\ s^{-1}$, which is in opposite direction to K_La variation in all PBRs (Fig. 5).

Thus, it seems reasonable to conclude that none of the parameters discussed earlier (Figs. 2A–5) is by itself a limiting factor for the tested PBR productivities. It is also plausible to conclude that provided conditions (mainly in terms of mixing, mass transfer and hydrodynamic stress) are very suitable, in all three PBRs, and cannot be considered as limiting factors as a whole to obtain higher productivities in these PBRs.

The only parameters that have not been extensively studied were the light distribution inside PBRs and microalgal cells light history (frequency and pattern). However, it is known that these factors may have an important role to define PBRs' productivity.

As described previously, the light conveyed by the central baffle to the interior of SCAPBRs was measured and the light flux was found to be around 25 $\mu mol\ m^{-2}\ s^{-1}$ in both SCAPBRs. This value is independent of cell concentration and, therefore, baffle internal walls are considered as continuously illuminated PBR surfaces.

Thus, while the surface area that is continuously illuminated in the BC is 0.190 m^2, in SCAPBR 75 the illuminated surface is 39% higher (0.265 m2) and in the SCAPBR 50 the illuminated surface area is 0.290 m^2 which is 53% and 9% higher than those presented by BC and SCAPBR 75, respectively. At low cell concentrations this difference is not very important, since some light can still reach the central region of the BC, but at high cell concentrations the SCAPBR enables delivery of light to regions that in the BC are in complete darkness. This extra continuously illuminated surface area, provided by the central baffle, supplies a relatively low light flux (25 μmol m^{-2} s^{-1}), however at high cell concentrations the cells only have access to light when they circulate along the PBRs walls (in all the PBRs) and along both sides of the central wall (in SCAPBRs only). Consequently, these differences in illuminated PBR surface can be an important part of the justification for differences between the PBRs in terms of biomass productivity and one justification to the highest productivities verified in SCAPBR 50 for all tested conditions.

Regarding to the light/dark cycles frequency, as Janssen et al. (2003) state, this parameter is determined by liquid circulation velocity, which depends on reactor design and superficial gas velocity. Also, it is known that higher light/dark cycles frequencies lead to higher productivities. However, the bell-shaped curve presented in Fig. 6 seems to show that the dependence between all these parameters is not linear. The results seem to show that when U_{Gr} is increased it does not mean that the light/dark cycles frequencies are, forcibly, also increased. This is probably due to an increase in average residence time of cells in the connections between the riser and the downcomer (top and bottom sections). In fact if the residence time increases in these sections, the light/dark cycles frequency can decrease and not the opposite, which can also explain the productivity values obtained. Analyzing mixing and circulation times (Figs. 2B and 2C) it is possible to see that for high values of U_{Gr}, the increase of U_{Gr} is not followed by a significant decrease of mixing and circulation time, which indicates that probably the medium (and the cells) become "trapped" somewhere. Another fact that also suggests this was the verification of bubbles "trapped" in the top and bottom sections (where the dark volume is higher) when working at higher values of U_{Gr}. These results and observations make us believe that in the SCAPBR, from certain values of U_{Gr} onwards, it is not possible to establish such a straightforward relation between the increasing of U_{Gr}, mixing rate and light/dark cycles. The existence of higher light/dark cycles frequencies in U_{Gr}= 0.0044 m s^{-1} together with higher residence time of cells in the top and bottom of SCAPBR (darker zones) can be the part of the justification to the occurrence of higher productivities observed at U_{Gr}=0.0044 m s^{-1}.

For more robust conclusions, an extensive study should be carried out in order to evaluate the light distribution inside PBRs (at different cells' concentration) and the microalgal cells light history (frequency and pattern). This evaluation will be the subject of future works.

4. Conclusions

The developed SCAPBRs proved to be suitable for microalgae cultivation. The design of PBRs, particularly the gas sparger, allowed meeting the needs of microalgae in terms of mixture and mass transfer. SCAPBR 50 (U_{Gr}=0.0044 m s^{-1}) showed, among the tested PBRs, the highest value of P_{max} (0.75 g L^{-1} d^{-1}). This result is probably due to a higher PBR illuminated surface area, and a more regular flow pattern between the illuminated and dark zones.

These results provide important indications to predict the effects of scale-up on the performance of the SCAPBR.

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