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Review article

Enclosed “non-conventional” photobioreactors for microalga production: A review

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ABSTRACT

Recent years have witnessed a growing interest in microalgae, namely toward production of lipids for biodiesel, upgrade of effluents, and synthesis of high added-value compounds. However, industrial production remains a major issue – chiefly due to the constraints posed by classical cultivation systems. Common system designs for microalga cultivation exhibit major bottlenecks, with regard to such specific processing parameters as light, shear stress, gas exchange, and biofouling. Non-conventional photobioreactor arrangements have meanwhile arisen in response to those constraints – yet most of them raise challenges for practical use, despite a number of advantages.

A detailed description and discussion of such non-conventional photobioreactor configurations is provided, including a qualitative comparison on their performance – after a brief introduction on parameters that affect photobioreactor performance, and the most common designs suitable for microalga cultivation.

1. Introduction

In the latest decades, developments in microalgal culture technology have taken place to a considerable extent. Microalgae are known for producing a wide range of fine chemicals and bulk products, such as lipids, sugars, proteins, pigments, dyes, antioxidants, biopolyesters and several added-value biological derivatives [1–4]. From an environmental point of view, they are attractive alternatives for clean exploitation of energy sources and bioremediation [5]; they have been indeed recognized as feedstock for third-generation renewable biofuels and energy (i.e. biodiesel, bioethanol, biobutanol, biohydrogen, bioelectricity) [3,6–9], along with their ability to biofixate or mitigate CO_2, remove toxic compounds (i.e. nitric and sulfur oxides) from flue gases [10–12], and treat wastewaters [13]. Microalgae hold indeed a strong potential as cell factories, which overpasses their terrestrial plant counterparts. When compared to the latter, microalgae offer such advantages as higher rates of photosynthetic growth (100-fold those of traditional food crops) and more efficient degree of CO_2 biofixation (of the order of 10–50 fold), while not competing with agricultural lands for food production [14].

The global demand for microalga has been steadily increasing, and the yearly production in this niche segment is estimated as ca. 20,000 ton – with prices ranging between 30 and 300 € per kilogram [15,16]. Nevertheless, the full potential of microalgae is still constrained by existence of still less expensive alternatives in the market.

Photoautotrophic cultivation is by far the most commonly used mode of production of microalgae, and the only technically feasible strategy at present to obtain biomass at large scale [5,12,17]; this is because sunlight is a free, renewable, and clean source of energy. However, cultivation methods resorting to mixotrophic, heterotrophic or

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Abbreviations: A/V, Area to volume ratio; S/V ratio, Surface area-to-volume ratio; CO_2, Carbon dioxide; CO_3^{2-}, Carbonate; DIC, Dissolved inorganic carbon; DS-PBR, Dome-shaped photobioreactor; FP-PBR, Flat-plate photobioreactor; HCO_3^-, Bicarbonate; H_2CO_3, Carbonic acid; HFM, Hollow fiber membrane; k_la, Volumetric mass transfer coefficient; LDPE, Low-density polyethylene; O_2, Oxygen; OMEGA, Offshore Membrane Enclosures for Growing Algae; PAR, Photosynthetically active radiation; PBR, Photobioreactor; PMMA, Polymethyl methylacrylate; PVC, Polyvinyl chloride; PVDF, Polyvinylidene fluoride; VAP, Vertical alveolar panel; FAP, Flat panel-airlift.

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photoheterotrophic growth can also be employed, depending on the desired product and species available, although incurring in higher costs.

Open systems, such as circular and raceway ponds, are the most frequently used devices employed at industrial level, and account for 90% of the overall microalga annual production [18]. Nevertheless, most microalgae cannot stand extensive periods in open space environments, for their susceptibility to contamination by faster-growing microorganisms – except for a few established species (e.g. *Spirulina platensis*, *Chlorella* sp. and *Dunaniella salina*) that are maintained via selective media, or exhibit unusually high growth rates [15].

Within this context, closed cultivation systems – the so-called photobioreactors (PBRs), have been developed to face the growing demand for, and interest in microalgae. While open systems are relatively cheap to build and easy to operate, PBRs tend to be more complex and expensive. However, they can appropriately overcome the main drawbacks of open systems, namely, poor mass transfer rates (i.e. low diffusion of CO_2 in the culture), high extent of water evaporation, difficult assurance of stable culture conditions, and high susceptibility to contamination, apart from requiring extensive land surface for installation [2,3,19]. Conversely, closed photobioreactors allow for a better control of growth environment, in terms of nutrients, temperature, pH and lighting; hence, cultivation of single-species of microalgae for extended periods becomes possible, with a much lower risk of external contamination [20,21]. Furthermore, those systems are designed to reduce mutual shading and distribute light over a large surface area (via high surface-to-volume ratios), thus minimizing photo-inhibition and photo-oxidation [22,23]. Cultivation in closed systems is also compatible with specialty products, sought under stricter market specifications as ingredients for the cosmetic and pharmaceutical industries. Closed PBRs are an excellent option when safety and environmental issues are relevant. For instance, dinoflagellates are able to synthesize compounds with remarkable anti-tumor and anti-fungal bioactivities [4,24,25]; however, many such chemicals have deleterious effects upon animals or plants, if exposed to the open environment. Other advantages of PBRs include prevention of water losses, enhancement of gas transfer, better O_2 stripping, and higher areal productivities [26–28].

Several types of photobioreactors have been developed for isolation and cultivation of green microalgae, at small or pilot scales, and in laboratory as well as outdoors [15,26,29]. Conventional closed PBR configurations encompass a few standard designs [26], including flat-plate [30–32], tubular (horizontal) [15,30,33,34], and column-type [35–37]; the latter are, in turn, subdivided into stirred tank-type PBR and aerated columns (bubble column or airlift).

Performance of conventional closed PBRs in practice still lags far behind the theoretical maxima extrapolated by computational simulation from laboratory data [26,38]; major difficulties arise from attempts to scale-up capital costs [20]. Different PBR geometries and dedicated methods of operation are determined by the intrinsic features of the microalga selected (in terms of energy demand and growth kinetics), the target biocompounds or allowed sub-products, and the local conditions available [19,26]. Furthermore, both quantity and quality of the biomass and final target product attainable, have to be put into perspective toward final selection of the best PBR configuration [20].

Microalgae are inherently photosynthetic microorganisms, and thus able to harness light and biofixate carbon dioxide; hence, production of biomass and buildup of metabolites of interest is strongly dependent on how light and carbon sources (i.e. CO_2) are made available. Light distribution/penetration and mode of CO_2 supply are accordingly, essential factors in PBR design – and critical to achieve high yields of microalgae. However, efforts to obtain efficient PBR designs for commercial exploitation are not restricted to optimizing light or CO_2 supply. Photobioreactors are normally complex devices, so such other points as geometrical design in terms of surface area-to-volume ratio, quality and quantity of light supplied, hydrodynamics and mixing patterns, gaseous exchanges of both CO_2 supply and O_2 stripping, nutrient provision, material of construction, contamination control and growth kinetics [19,29] are taken into account. Those factors closely interact with each other, and may as a whole dictate success or failure of PBR performance [29]. Other important physicochemical parameters affecting PBR operation include temperature, pH, dissolved oxygen and CO_2, and velocity pattern. For instance, temperature requirements of phototrophic microalgae range within 25–35 °C, with optima pH usually around 7–8 (except for *Chlorella* and *Spirulina* that require high pH values for biomass production) [39]. Integrated temperature/pH controllers are thus vital to assure high performance by a given PBR, and avoid growth limitations throughout microalga cultivation.

Classical PBR configurations pose hurdles that ought to be overcome – e.g. light demand, shear stress, gas transfer and mixing, cooling and biofouling; unfortunately, the associated costs may jeopardize affordability of the overall biotechnological process. Implementation of a universal microalga cultivation system has not been attained as well; a single conventional PBR design or strategy is not likely to address all needs of an existing microalga species, or respond with the intended productivity in terms of a target product(s) with commercial interest [20]. In the last years, several progresses have been possible pertaining to light transfer/distribution into the PBR and photosynthetic efficiency, hydrodynamics and growth kinetics [40]; novel PBR designs, including alternative “non-conventional” designs, have therefore arisen – most of them conceived for specific research purposes or small-scale applications [15].

Such modern PBR designs/strategies derive normally from classical geometries of cultivation, and accumulated know-how on improvement of microalga biomass production. Examples encompass mounting of baffles or static mixers inside flat or column PBRs to enhance mixing, and promotion of more frequent passage of cells under the light source. Other layouts adopt distinct approaches, such as internal illumination – with a light source placed inside the reactor chamber; alternatives to better reach individual cells include non-typical means, such as optical fibers or light diffusers. All of them exhibit specific design geometries and/or unusual operational arrangements.

The concept of photobioreactor itself has also evolved; large-scale exploitation of microalgae appears to be an opportunity to reduce capital or operational costs if integrated approaches entailing bioenergetics, environment and bioremediation are sought; this is the case of façade-PBR or floating-type PBR [41,42].

The general parameters that affect performance of a photobioreactor for microalga production, will be discussed below to some length, departing from existing classical designs employed for microalga cultivation as reference. A detailed description of “non-conventional” configurations of photobioreactors will follow afterward – underlying their features, key strengths and potential drawbacks; but always with a focus on improvements of conventional designs (flat panel, tubular, column PBRs). New concept applications, still undergoing development but with an anticipated industrial potential, will deserve some attention in the end.

2. General parameters affecting PBR performance

The performance of photobioreactor cultivation systems at large is strongly dependent on both chosen design and interrelation of environmental factors with biological response [26]. Hence, a good understanding of the engineering concepts behind design criteria and a deep knowledge of a number of aspects of cell physiology and behavior are crucial to implement a successful PBR.

In addition to physicochemical parameters (i.e. temperature, pH, dissolved O_2, CO_2 availability, shearing and nutrient availability), physical and operational factors have considerable influence upon performance of a photobioreactor. Light requirements (e.g. spectral width, attenuation and distribution), surface area-to-volume ratio, mixing/agitation patterns, rate of exchange of CO_2 and O_2, nutrient provision and renewal, temperature and pH control, quality of construction material (e.g. transparency of casing material) and biofouling are decisive for proper operation of a PBR. Some of those parameters interact with each other, which complicates design of an effective PBR as a cultivation system [26]. The next section will provide a brief discussion of their importance; and how they generally affect performance of a PBR.

2.1. Light and surface area-to-volume ratio

Efficient light supply and wavelength range available are crucial factors to attain effective photoautotrophic cultivation of microalgae. Under photosynthesis, most microalgae process the available energy only within 400–700 nm – the so called photosynthetically active radiation (PAR). Their (photosynthetic) activity can be performed based on natural solar light (outdoor PBR systems), or on artificial light sources (i.e. fluorescent lamps, LED) in indoor systems [43]. Light sources that deviate from regular PAR may provide poorer light spectral quality for efficient photosynthetic processes, so reduction in biomass productivity is expected. For example, ultraviolet light (i.e. 218–400 nm) is reported to negatively affect photosynthetic performance [44,45]. Sunlight accounts for ca. 50% of PAR [28]; the remainder is wasted as fluorescence or heat [46]. In the most common outdoor mass cultivation systems, solar light is used as inexpensive source of energy for microalga growth. However, unmanaged or increased irradiances at the surface of a PBR – measured by photon flux density [FDP, in $\mu mol_{photons}.m^2.s^{-1}$], may lead to a high degree of photoinhibition, or even photo damage. Such a phenomenon reduces photosynthetic ability of the cells and decreases their net growth [47], especially near the transparent walls of a PBR (or near the light source). On the other hand, the cells inside the core of the photobioreactor, namely the central region of tubular or vertical PBRs, are susceptible to dimmed light environments, and exhibit poor light intensity distribution; a light gradient (with negative slope) is indeed established toward the center of the PBR. This event is strongly dependent on PBR geometry (i.e. diameter) and cell-shading effect (especially in high-density cultures). Inside the reactor, microalga cells absorb, scatter and redistribute light [29]; hence, cells become light-limited, which may negatively impact biomass production.

Surface area-to-volume ratio (S/V ratio) is an important aspect affecting PBR performance; light distribution/dilution over the PBR surface relates to the total transparent surface area available. In general, the higher the S/V ratio, the higher the portion of light allowed through the PBR surface, with a simultaneous improvement in photosynthetic efficiency, and thus in biomass and metabolite productivities [15,28,48]. Therefore, short light path PBR designs are preferred (i.e. flat panel or alveolar PBR), as they are more robust and prone to attain higher cell densities [26]. Most conventional vertical designs (i.e. column PBRs) entertain an increased light path due to a lower A/V ratio (area to volume ratio) i.e. ratio of sectional area to volume of PBR, which accordingly impairs light distribution in the core. For structural reasons, however, diameter cannot be excessively reduced, otherwise the column may collapse. Exposing cells to light/dark cycles (i.e. by stirring) is a good strategy to minimize the effect of light attenuation inside a PBR [49,50]. In this way, microalgae cells are exposed to periods of light and dark in a cyclic (or random) manner, so light distribution and thus photosynthetic efficiency become more balanced and uniform. Those light/dark cycles should not be extended for a too long time (i.e., < 10 s), because cell growth and light utilization may be negatively affected [51].

Interestingly, microalga cell cultures can also benefit from use of light shifting materials in PBR composition. This means that light quality can be improved by specific materials (i.e. semiconductors, phosphorescent or fluorescent compounds), able to provide wavelength spectral conversion that shifts non-PAR to PAR wavelengths. There are three main principles underlying spectral converters: i) reduction of high energy photons that split this energy in two or more photons with lower energy (down-conversion); ii) shift of photons into a targeted wavelength region (photoluminescence); and iii) more than one photon with lower energy is converted to a photon with higher energy (up-conversion). Such materials could be applied to enhance use of solar energy, and are claimed to possess high absorption coefficient, high quantum conversion efficiencies, different emission and absorption wavelength bands, and extended photo-stability associated to a low cost [52,53].

2.2. Mixing and agitation

Most microalga cultivation systems operate with cells in suspension in the broth medium. Therefore, mixing becomes an important parameter – not only to favor homogenization of culture, but also to prevent settling or cell clumping with each other and onto in PBR walls, especially in horizontal tubular PBRs [26]. Mixing also contributes to a more uniform distribution of nutrients, and assures reduction of pH and temperature gradients (heat dispersion). Furthermore, mixing is critical to enhance gas-liquid mass transfer, thus ensuring a more competent biofixation of $CO_{2,}$ and enhancing light utilization by microalgae cells due to the underlying light/dark cycles. Cells are indeed transported in a cyclic manner from the interior and dimmed light regions, to more well-lit regions near the PBR walls (and vice versa); this favors balanced and shorter light/dark cycle periods [54], which promotes biomass generation and improves PBR performance [55].

Poor mixing or agitation permits buildup of undesired gradients of nutrient and pH, concomitant with biofouling on the walls and oxygen increase in the medium [55]. To prevent such events, moderate mixing/aeration rates are required, via turbulent flow patterns brought about inside the PBR. In these cases, mixing time decreases with increased surface gas velocities – thus supporting faster mass transfer rates [56].

The most commonly employed mixing/recirculation systems in microalgae PBRs are pumping, mechanical stirring, and airlift type – in which CO_2-enriched air or gas are sparged or bubbled so as to create turbulent mixing and/or recirculation [57]. It is important to highlight that the nature of the mixing/agitation system may influence the flow patterns in a PBR, depending on its geometry. Non-mechanical aeration systems, such as CO_2/air spargers or pumps are commonly employed in vertical columns (i.e. bubble-column or airlift), and flat-plates able to enhance mass transfer upward. Such systems require much more kinetic and mechanical energy than mechanical agitation-type systems, in which a setup of paddles, static mixers or impellers are used to stimulate agitation/mixing (i.e. raceway pond or stirred tank). A combination of both mechanical and non-mechanical methods can also be applied [28,58]. Mixing will be improved by using baffles or static mixers inside the reactors [59–62]; their presence assures a defined circulation flow path, so microalga cells become more likely to benefit from regular light flashing effects [57].

Nevertheless, levels of turbulence that surpass cell shear-resistance may lead to inhibition of metabolic activity, cell damage or even cell disruption. It is believed that microalgae shear-stress is induced by fluid circulation, micro-eddies and bubble rupture on the cell surface [63]. The microalgae cells should be half or less in size than eddy length scale. Therefore, the imposed hydrodynamic forces resulting from combination of high surface gas velocity and intense mixing must be taken into account. The goal here is to find the degree of mixing that assures sufficient mixing and aeration, and thus reasonable or optimal cell growth, without compromising cell integrity; this goal will be particularly challenging when ultrasensitive species are at stake (i.e. dinoflagellates) [63].

2.3. Gas exchange

The rate of gas exchange (i.e. CO_2 supply and O_2 stripping) is another nuclear feature of PBRs. Delivery by CO_2 is crucial to establish microalga cultivation, as this nutrient is essential for photosynthesis. Hence, the CO_2 concentration in the culture medium should not fall at any point below some threshold required for microalga growth – so that photosynthesis will not get limited [64].

The main problem associated to CO_2 supply derives from mass transfer limitations. Open systems face several constraints due to low atmospheric partial pressure of CO_2, corresponding to a volumetric fraction of a mere 0.035% (*v*/*v*) [64]. Consequently, microalgae cannot biofixate CO_2 directly in gaseous form; enhanced CO_2 mass transfer rates require CO_2 be previously dissolved in a liquid phase, with recommended partial pressures above 0.2 kPa, to ensure satisfactory growth [29,65].

Closed and conventional PBRs (i.e. bubble-column, airlift) employ sparging or bubbling of CO_2-enriched air, in order to provide the amounts of carbon dioxide required by efficient photosynthesis. According to Lam et al. (2012) [12], systems relying on dispersion of microbubbles permit a better dispersion of gas in the liquid phase than systems that generate macrobubbles. The former provides indeed a great area-to-volume ratio, as it promotes better dispersion and dissolution of gas. Conversely, macrobubbles are less effective, as they tend to rise up with higher velocity and readily burst at the surface of the culture, with reduced chance for interfacial mass transfer during their path. Besides bubble size, the rate of dissolution of gaseous CO_2 depends on residence time of gas in the medium, level of CO_2 saturation in the medium, operating pressure and temperature of the PBR [64]. The parameter normally utilized to measure diffusional limitations is the volumetric mass transfer coefficient, represented by k_la – which lumps the intrinsic mass transfer rate, k_l, with a specific area, a. This parameter sets the rate of CO_2 uptake by cells under steady-state [19], and directly influences cell growth rates.

Other sources of carbon may be made available to the culture, namely bicarbonate or carbonate [66]; however, such salts add extra costs to the microalga cultivation process, when compared to gaseous CO_2 enriched-streams [64]. Supply of flue gas from industry has proven an appropriate source of CO_2, besides being rather cost-effective – especially to those species possessing higher CO_2 tolerance; however, several chemical contaminants (e.g. NO_x or SO_x) of flue gases may affect the overall process. On the other hand, CO_2 can be tolerated by microalga cells up to a certain threshold, after which it becomes detrimental for growth [67]; hence, dissolved CO_2 concentrations, as well as concentration of dissolved species of inorganic carbon (i.e. CO_3^{2-}, HCO_3^-, H_2CO_3) in the medium should be carefully controlled as a whole. High levels of dissolved carbon dioxide in the medium may trigger undesired effects in some microalgae species via unwanted decrease in pH [68].

Accumulation of O_2 has to be taken into consideration in PBR design; remember that O_2 is generated during photosynthesis. Phototrophic microalgae possess *Rubisco* enzyme that behaves as both a carboxylase and an oxygenase; this means that its binding site is able to bind CO_2 and O_2, respectively. However, this enzyme exhibits a higher affinity to O_2; when high levels of oxygen are present, CO_2 has to actively compete to bind to its active center. This event leads to switch from photosynthesis to a process known as photorespiration – and significantly affects photosynthetic efficiency. In addition, excessive amounts of dissolved oxygen (DO) in the broth may reach inhibitory levels, which would turn out detrimental to microalgal cells; if combined with intense light irradiance and temperature, reactive oxygen species can form that are likely to impair the physiological state of the microalgal culture [69]. That is why O_2 removal constitutes a major issue in PBR design. Closed PBRs – especially those holding a horizontal tubular configuration, are more prone to oxygen accumulation; this problem worsens when it comes to large-scale cultivation. To circumvent this difficulty, provision of separated aeration and degassing devices has been pointed out as a reasonable solution for O_2 stripping [70–72]. Headspace dimensions, in vertical PBRs, are vital to assure adequate degrees of gas exchange [28]. All in all, control systems to monitor O_2 and CO_2 concentrations are crucial, in order to maximize CO_2 supply while minimize the deleterious effects of O_2.

2.4. *Nutrient provision*

Provision of nutrients in the medium is crucial to assure adequate performance of a PBR. Aside from carbon, nitrogen and phosphorous are the most important macronutrients implicated in process regulation at cellular level, namely photosynthesis (i.e. pigment and protein synthesis) and energy transfer [73]. The dynamics of take-up of such nutrients are coupled to each other in regular PBRs [56]. Limitation on those nutrients may severely impact microalga growth. It is well-known that nitrogen depletion slows down microalga growth rate, while lipid/carbohydrate production is favored under those conditions [74–76]. Hence, knowledge of how nutrients are modulated during PBR operation is essential to maintain adequate nutrition profiles in microalga cultures – obviously envisaging the metabolite(s) of interest.

Microalga cultures are commonly operated in batch mode (especially at lab scale), so nutrients are essentially consumed in full by the end of a run; the inlet of nutrients can be manipulated if one resorts to continuous, semi-continuous or fed-batch operation processes [74]. Continuous and semi-continuous operation cultures are characterized by continuously or intermittently (at fixed intervals) removal of microalga broth, along with replenishing with fresh medium – to ensure that the nutrients are sufficient for regular microalgae growth at any time. An adequate regulation of dilution rate (typically low, as microalgae possess relatively low growth rates) is essential to keep steady-state conditions. When high dilution rates are employed, cell washout may occur, thus compromising performance of a PBR – with a sharp decrease in growth, and even leading to culture collapse [74].

Nutrient provision can also be performed via fed-batch operation, in which nutrients are accordingly added without harvesting any portion of the culture. Since nutrient is added to prevent depletion thereof, it will hardly become limiting; however, if fresh nutrients added to the medium are not diluted sufficiently fast, an excessive concentration (of nutrients) may build-up locally, and inhibitory effects upon microalga growth may arise [74,77].

2.5. *Temperature control*

Temperature control is a significant operational parameter in PBR performance; it greatly influences the growth rate of microalgae [68]. The efficiency of photosynthesis depends on a balance between light and temperature [78]. Most microalgae can adjust to a wide range of environmental temperatures and light fluctuations, yet response of the photosynthetic process might vary within a range of time scales – few seconds, to minutes, or even hours and days [79]. Despite their ability to adapt to changes in their surroundings through a number of mechanisms of regulation of photosynthesis and acclimation [79], microalgae hold an optimum temperature interval – that should be sought a priori. This is essential to promote effective light harnessing and CO_2 biofixation, and thus reach high biomass productivities. Despite the said optimum temperature range depending, for most species, on intrinsic features of the species/strain and geographic location [68], optimum temperatures typically range within 20–24 °C. Nevertheless, most microalga are able to tolerate temperatures between 16 and 35 °C [29,80]; values below the lower bound or above the upper bound will obviously lead to significant decrease in cell growth – and, in extreme cases, to overall decline of the culture itself. Suboptimal temperatures hamper uptake of a few nutrients essential for microalga growth (i.e. carbon, nitrogen), mainly because cells undergo structural alterations – e.g. rigidity of cell wall and viscosity of cytoplasm; the photosynthetic machinery can also be affected, with consequent photoinhibition [68]. On the other hand, microalga cells exposed to elevated temperatures in tandem with high light intensities (beyond their optimum) may undergo serious physiological damage in their protein structure [68], leading to photobleaching and degradation of their photosynthetic apparatus [79]. The problem of temperature control is particularly critical in outdoor, large-scale systems – because they are exposed to a wide range of day/night and seasonal thermal differences. Mehlitz (2009) has shown that PBRs without temperature control may experience temperature variations between 10 and 30 °C in summer [81]; he outlined the importance of employing additional (and effective) cooling mechanisms to maintain favorable growth rates inside the PBR.

Several thermoregulation methods have been described in closed PBRs: incorporation of heat-exchanger, surface water spraying, shading nets, pool water immersion, overlapping tubes, or regulation of feed stream [8,48]. Tredici et al. (2007) [48] claimed that the cost-effectiveness of some of those methods is doubtful – for example, shading can in fact decrease productivity, because up to 80% of the PBR illuminated surface has to be covered, while immersion in water pool or water spraying demand an excessive water footprint.

More recently, various forms of thermal insulation of PBRs have been tested. Development of new composite materials characterized by low thermal conductivities, such as hollow glass microspheres [82], insulated-glazed photovoltaic glass [83], or infrared blocking films appear to be promising solutions [84] – since the amount of energy required to control broth temperature is reduced, without dramatic reduction in culture performance.

2.6. pH control

An effective control system for pH is also important in PBR performance – since pH outside the optimum range may have a severe impact upon several components of microalga cells involved in uptake of carbon dioxide and/or other important nutrients (e.g. iron), thus compromising health of the culture as a whole [20]. The pH is strongly affected by the concentration ratio of dissolved carbon species (i.e. CO_3^{2-}, HCO_3^-, CO_2) [68]; full account of this complex interrelation has been reported elsewhere [11,27,68]. The balance between supply and mass transfer of CO_2 to the liquid phase, and carbon uptake by microalga cells, determine the total dissolved inorganic carbon (DIC) in the medium – and is a key-factor in attempts to control pH. Acidic pH shifts (< 7) can occur when DIC appears chiefly in the form of CO_2; alkaline pH shifts (i.e. $>$ 10) are frequent when the main form of DIC is carbonate (i.e. CO_3^{2-}), and HCO_3^- is considered to predominate in DIC form in neutral systems (i.e. pH 7–9). A delicate equilibrium between carbon dioxide and derived dissolved species should be established in a PBR – with the cultured microalga species and the desired products; such equilibrium also depends on temperature, carbon transfer rates, and velocity of carbon consumption rate of microalga cells. Hence, pH is a relevant factor affecting PBR, especially when large culture volumes are at stake. Current practice in pH regulation includes use of buffers in the medium (e.g. sodium bicarbonate) and injection of pure CO_2 into the culture [20,85]. The latter resorts to plain on-off switching controllers for convenience: it is simple, and absence of appropriate valves for gas injection at low flow rates does not pose a problem [85]. However, the cost associated to addition of pure CO_2 for pH control is high [86]. Finding suitable pH system control solutions implies normally an integrated PBR design-approach.

2.7. Quality of construction material

The choice of material to build a PBR is an important issue – as far as it influences durability and resistance thereof over its lifespan; furthermore, the quality of materials chosen and their chemical properties are of importance to avoid potential adverse effects upon microalga cells. The bottom line is assuring metabolite stability, and anticipating undesired chemical interactions with PBR wall surface in contact with the microalgae. Good mechanical strength, some flexibility (but not excessive, to avoid changing light or mixing patterns) and high transmittance of light are considered essential features regarding material selection, in attempts to establish a competent microalga cultivation process.

Concerning transparency of materials: polycarbonate, silicate, glass, polymethyl methylacrylate (PMMA), polyvinyl chloride (PVC), acrylic-polyvinyl chloride and polyethylene are the most frequently chosen [29,39]. Other types of materials have also been reported, such as polystyrene, polyethylene terephthalate or (transparent) polyurethane [39]. However, each choice holds pros and cons – in terms of optical properties, mechanical strength, and thermal and chemical properties. For instance, PMMA has a high effective transmittance, although is easily scratched and exhibits poor chemical resistance [39]. Glass also has an excellent transparency, along with and a unique chemical stability and increased lifespan; however, it is very fragile, and so requires supporting structures. In a recent approach, Wiley et al. (2013) have reported an innovative system (Offshore Membrane Enclosures for Growing Algae, OMEGA) manufactured from low density polyethylene (LDPE) – in which microalga cultures are confined, in floating-bag PBRs, and deployed to marine environments [87]. The material chosen seems adequate for that purpose, as LDPE is intrinsically transparent – and, in particular, has low absorption of UV and infrared rays.

Durability and mechanical strength are key parameters, further to porous features that permit partial exchange of CO_2 and O_2. The material surface should also be assessed for its likeliness of forming biofilms (biofouling). In general, such phenomena raise an additional challenge to PBR performance – depending on the design elected (especially in the case of tubular PBRs).

2.8. Biofouling

One of the major drawbacks of PBRs (especially the closed ones) is occurrence of biofouling – a phenomenon consisting on aggregation and adherence of cells onto its inner walls. This can negatively affect performance of the cultivation system, by decreasing the extent of light penetration into the inner layers of the system – thus reducing availability of photosynthetic light for cells, and consequently attaining poor biomass concentration. Therefore, the construction material of the walls and the PBR geometry play a major role upon biofouling. Such designs as flat-plate PBR, which have a more cuboidal geometry, are in general more accessible for cleaning and maintenance than tubular reactors. Integration of efficient mixing and recirculation systems should entail an effective strategy to mitigate this negative effect. PBRs that hold continuous turbulent regimes, due to random stirring or gas bubbling, exhibit indeed low tendency for biofouling. In addition, fluid radial flow patterns (e.g. cylindrical vessels), brought about via rotational stirring, prove better than axial flow only.

On the other hand, tubular PBRs (especially horizontal ones) have severe handicaps, because fluid motion relies on the power of a pump. Biofouling is more likely to occur in the bending parts of the tubes. Several strategies have been put forward to tackle this particular issue in tubular designs; integration of foam balls made of poly(urethane), or scouring pad into the tubes (especially in the case of helical arrangements) proved successful in preventing settling of culture onto the inner walls of helically-arranged systems [88,89].

3. Classical PBR design and configuration (general features)

3.1. Flat-plate PBR

The conventional configuration of flat-plate PBR (FP-PBR) has been in use since early 1950s [91] – being well-suited for both indoor and outdoor operation. FP-PBR appears as a compact and robust design, characterized by a cuboidal shape – with two wide flat surfaces or sheets, lying on top of each other and ranging from a few mm up to 70 mm [26], and illuminated on both sides (Fig. 1i). This design of FP aims at taking the best advantage of harvesting light via solar collectors, and by reducing light decay along the light path, especially in high-density cultures [40]; note that the light path cannot be increased indefinitely because of cell shading, which contributes to decrease photosynthetic activity. Hence, a sufficiently small light path assures a high illuminated surface S/V [28], and thus, an effective light

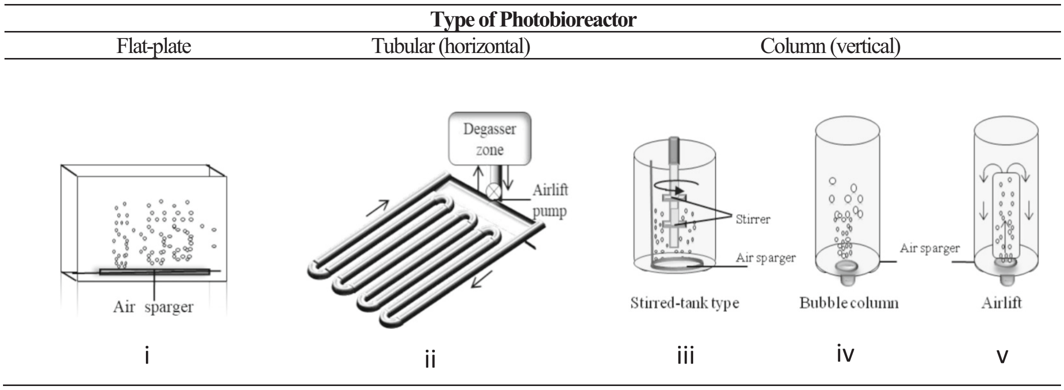

Fig. 1. - Configurations of classical enclosed PBRs employed for microalga cultivation: i) flat-plate; ii) tubular (horizontal); and column (vertical): iii) stirred tank; and aerated columns – iv) bubble and v) airlift types. (Adapted from [19,22,90]).

penetration, distribution and utilization [15,92]. However, a flat design implies a high risk of photoinhibition, especially in outdoor cultures or at early stages of growth (diluted cultures). Under high levels of irradiation, cells become inhibited owing to light oversaturation; this can severely affect photosynthetic systems and cellular metabolism, and may ultimately lead to culture collapse.

FP-PBR have vertical orientation by default, but can also be inclined relative to the ground as per an angle commonly called tilt angle [28]. The optimal tilt angle is chosen based on the light source position (i.e. sun), so as to maximize incident light in attempts to increase biomass productivity [93].

Agitation is provided by either of two methods: pump-driven or airlift (via bubbling of air at the base); baffles are often included to improve mixing efficiency. In general, oxygen build-up is not a problem, because an effective open gas disengagement system (i.e. open head space in the top of PBR) can be provided – except when a vertical alveolar panel is considered. Water spraying or internal heat exchangers are strategies followed for temperature control. Such type of PBRs, especially those containing baffles, may undergo some degree of wall growth, and accordingly raise cleaning issues. Scale-up can also become a problem; the bigger the volume of the reactor, the higher the prevailing hydrostatic pressure therein [94].

3.2. Tubular PBR

Conservative tubular PBRs are made of transparent tubing (glass or polyethylene); the most common configuration appears as a serpentine loop, arranged in a single plan [34] (Fig. 1ii). Such configurations are displayed horizontally, and are the most popular choice for outdoor mass culturing [27]. Apart from the tube arrangement, tubular PBRs differ in tube length and diameter, flow velocity, form of recirculation, and geometric shape of the light receiver. The tubes utilized are normally between 0.1 and 0.6 cm in diameter [26], while their lengths can go up to several hundred meters; photosynthetic activity will basically set tube length between liquid degassing points. Diameters of 0.1 cm or less have been reported to bring about higher productivities [26].

Tubular PBRs are seen as solar collectors, because of microalga flow through a large solar-illuminated surface. The so-called "lens effect", or "focusing effect" assures that incident light is distributed evenly, since it is diluted along the circumference and flows in a radial direction – thus focusing on the axis of the tube. Such effect reduces mutual shading, and allows increase of radiation intensity [26]. The great advantage of such configuration is the high S/V ratio, particularly adequate for efficient light harvesting – with photo-inhibition being minimized [26,28]. In addition, structural integrity is not easily compromised when compared to their vertical counterparts; large tubular setups are organized into outdoors modules, thus offering the possibility for operation with different volumes of culture, and allowing improved control of operating parameters. Extension of tubular PBRs cannot occur indefinitely, either due to high land requirements, or the extra costs associated with the equipment attached to the solar collector, viz. degasser units, pumps for medium, reservoirs for nutrient mixing, and CO_2 supply systems (such as carbonation towers).

The supply of gas can take place at the end/beginning of the tube system. When gas bubbles are released into the culture, they coalesce and form an interface between liquid, gas and PBR wall. The surface area between gas and liquid is reduced, so poor mass transfer rates are observed [95]. Nevertheless, this way of providing gas may cause accumulation thereof, and pH axial gradients owing to low gas transfer. Such a poor efficiency creates problems of O_2 build-up and/or CO_2 depletion, so biomass productivities may be severely affected when compared to bubble column or airlift PBRs [96] – resulting in occurrence of photorespiration and cell oxidative damage effects.

Excess of CO_2 can be supplied via the gas stream, thus avoiding CO_2 limitation. As large losses may occur, a common approach to overcome this problem is to supply CO_2 at multiple points along the tubular path [28]. Furthermore, gas exchange and nutrient addition normally take place in a separate vessel (degasser unit or stripper vessel) – thus allowing better O_2 stripping, whereas culture circulation can be achieved by a pump or an airlift [15,34].

Scale-up issues exist for such type of reactors, as the A/V decreases with the increasing diameter; this may result in less light-cell harvesting, cell shading-effects or poor mass transfer. Depending on the configuration, a stronger tendency for a fouling effect and microalga growth on the inner walls may arise [22].

Owing to the increased S/V ratio, large amounts of metabolic heat can be generated [28]; temperature control thus turns difficult, and those configurations often run the risk of overheating – which calls for expensive cooling, especially in regions with significant thermal ranges. Shading, heat exchanger coils, water spraying, or even submerged tubular arrays in water pools are strategies adopted to meet with temperature requirements – but none of these options is fully feasible at scale-up, which compromises for now the associated economic feasibility [97]. The major advantages and drawbacks of such reactors are illustrated in Table 1.

3.3. Vertical column PBR

Vertical column PBRs can be organized into stirred-tank vessels (Fig. 1iii) and aerated columns, such as bubble column (Fig. 1iv) or airlift (Fig. 1v). The central regions of such type of reactors usually appear as dark or dimly lit environments – thus causing overall limitations to cell exposure to light along the axis. Photosynthetic efficiency can be negatively affected, thus impacting upon microalga biomass production and productivity [54]. In general, the relatively low A/V ratio hampers scale-up [8,26]. The main advantages and drawbacks of

Table 1
- Comparison features of most common enclosed photobioreactor configuration employed for microalga biomass cultivation. (Adapted from [18,20,39]).

	Type of photobioreactor	Advantages	Limitations	Applicability/Observations
	Flat-plate	- High area to surface ratio - Large illuminated surface area - Good light path - Moderate biomass yields	- Expensive construction materials - Easily subjected to photo-inhibition - High shear stress from aeration - Hard temperature control - Some degree of cell wall attachment - Scale-up problems (require numerous modules and support elements) - High shear stress owing to mechanical pumping	- Suitable for outdoors and indoors - Application to algal strains with high lipid content (under nutrient limitation) - Inadequate for photo-sensitive microalga or more sensitive to hydrodynamic stress
	Tubular (horizontal)	- High S/V ratio - Effective in capture of solar radiation - Possibility of arrangement with adequate angles to harvest sunlight - Relatively low cost to built	- Poor mass transfer - Requirement of separate degassing units - High risk of pH gradient and O_2 build-up - Risk of photo-inhibition or photo-oxidation - Susceptibility to biofouling - Risk of overheating - High land surface area requirement - High energy requirements	- Well suited for cultivation outdoors - Well suited for industrial cultivation of most common microalgae species (i.e. *Nannochloropsis*, *Haematococcus*, *Chlorella*) and production of valuable dyes (i.e. astaxanthin)
Column-type	Column (vertical) Stirred-tank	- Precise monitoring of each culture parameter - Used for optimization studies	- Limited to laboratory scale - Low area-to-volume ratio - Poor efficiency in light conversion - Low productivity	- Ideal for production of added value compounds - Cultivation of biomass for wastewater treatment - Limited to heterotrophic microalgae (opaque walls)
	Aerated columns (bubble column and airlift)	- Cheap and compact - Low maintenance cost - High mass transfer - Good mixing - Efficient CO_2 supply and O_2 removal - Good photosynthetic efficiency - Exposure to ligh/dark cycles - Low fouling - Low land requirements -Disengagement zone separate from gassed liquid and gas phase	- Risk of high shear stress upon cultures - Small illumination area (depending on light incidence angle) - Increased light path with increasing column diameter -Photo-inhibition problems - Deficient scale-up	- Unstable for microalgae prone to flotation and/or species highly sensitive to shear stress (e.g. dinoflagellates)

both stirred-tank type and aerated column PBRs are tabulated in Table 1.

3.3.1. *Stirred-tank type PBR*

Stirred-tank type PBR is a commercial stirred tank bioreactor, routinely made of steel, glass or organic glass; it is often employed in industry, to produce fine chemicals or pharmaceutical products (Fig. 1iii). This system is particularly suitable for heterotrophic growth of microalgae, when using appropriate organic carbon sources. The precision and control accuracy of every operating parameter, along with the minimization of contamination by heat-sterilization are indeed the main advantages offered by such apparatuses. By using wall-transparent arrangements, they can be used for phototrophic cultivation; but also for photomixotrophic and photoheterotrophic modes, as long as an external light source is provided (e.g. fluorescent lamp, LED or even sunlight). Although it exhibits a quite low A/V ratio, the said configuration is useful at laboratory scale (indoors) for optimization processes [19]; it has indeed been quite useful to obtain high value added compounds (e.g. pigments, carotenes, polysaccharides, polyunsaturated fatty acids). Agitation is a must, and easily provided via mechanical means using an impeller (marine or ribbon type), or resorting to magnetic stirring (in smaller units) [98,99]; however, this mode of homogenizing the mixture entails increasing costs when reactor volume increases, owing to the associated energetic demand. Air and CO_2 are supplied to the cultures through spargers, and temperature culture control can be provided via cooling jackets or coiled heat-exchangers.

The effective degree of light absorption is undeniably small, and production is in fact limited because of low throughputs (biomass productivities lie within the range 30–50 $mg.L^{-1}.d^{-1}$) [18]. A strategy to shorten light path and increase photosynthetic efficacy is via internal illumination; use of several, or both natural and artificial light sources has been possible in novel types of combined photobioreactors, such as the internally-illuminated PBRs.

3.3.2. *Aerated columns-PBR*

Common aerated column-PBRs consist of transparent glass or plastic, vertical cylinders – usually no >0.2 m in radius or/and 4 m in height [28,35] (Fig. 1 iv and v). Smaller radii have been suggested to overcome cell-shading effects – especially when the culture attains high cell concentrations, as the A/V ratio is increased. The core regions of such type of reactors are usually dark or dimly lit environments – thus creating overall limitations in terms of cell exposure to light along the axis; hence, photosynthetic efficiency will be hampered [54]. Owing to the relatively low A/V ratio, problems with scale-up issues have been referred to quite often [8,26]. Furthermore, the limited height is related with both structural reasons – as strength of transparent materials may not support tall PBR columns; and gas transfer limitations – as CO_2, O_2 (and also pH) gradients may be a problem, when going beyond a reference height [28]. However, convenient headspace dimensions may provide efficient gas exchange and effective gas stripping. In addition, the movement of axially dispersed bubbles in the liquid broth contributes to efficiently mix the culture, with less shear stress than when impellers or centrifugal pumps are used [63]. However, care should be taken with regard to high superficial velocities; those may lead to increased turbulent conditions, and induce high shear rates – with detrimental effects upon microalga cells, especially those less shear-tolerant [4,24,63]. Column hydrodynamics and mass transfer depend entirely upon break up and redistribution of bubbles, produced and released from a sparger or perforated plate; when a turbulent regime is imposed to the culture, large circulatory flows arise – with the high gas hold-up driving the liquid upward along the axis, with a corresponding down flow of liquid near the walls [22,49]. Conversely, low gas flow rates favor even distribution of bubbles across the column cross-section, with little or no back mixing of the gas phase [22].

Aerated columns are usually classified as bubble column (Fig. 1iv) or airlift reactors (Fig. 1v), depending on their mode of liquid motion [22]. In both layouts, agitation and mixing are provided by gas or CO_2 sparging at the bottom of the PBR. This provides good overall mixing, and sufficient gas transfer rates, of the order of 0.006 s^{-1} [26,39], across the cultures. In bubble column, hydrodynamics and mass transfer depend entirely upon break up and redistribution of bubbles (as emphasized above); hence, airlift systems have more defined liquid flow patterns [100] in view of employing internal draft tubes (internal loop-airlifts) that contribute to enhance mixing efficiency [36,51,101]. The loop-airlift working principle is based on gas flow through a sparger – where two different interconnecting regions of gas may be distinguished: the riser (commonly represented by a physical separation), in which the gas is driven upwards to the top of the liquid; and the downcomer, in which heavier bubble free liquid undergoes downward movement [90,102]. The density of liquid between riser and downcomer result in liquid motion inside the reactor, with the fluid dynamics being significantly influenced by gas hold-up between the two zones [100].

4. “Non-conventional” PBR designs

Mass production of microalga requires appropriate culturing systems – and chief bottlenecks are found from design (i.e. geometry, hydrodynamics, shadowing) and strict operational requirements (i.e. carbon dioxide and other nutrient supply) thereof. Careful adaptation of PBR set-ups to the specific kinetic and metabolic requirements exhibited by microalga will offer an opportunity for novel nutrient regimes, as needed for enhanced microalga biomass productivities during cultivation [55].

A compilation of all photobioreactor configurations described in the literature is hard to come up with. Photobioreactors have appeared in various shapes and sizes – and the most recent configurations may fall into more than one category of PBR configuration. This is so because some of the different or unconventional arrangements have been combined with several alternate strategies, not only to improve light distribution in the culture broth but also to modulate hydrodynamics that directly affects rate of mass transfer of nutrients.

An educated overview on the microalga modes of culturing will be provided below – with a focus on “non-conventional” PBRs, and associated new advances. Most of them are based on the most common used geometries and arrangements, i.e. flat-plate and tubular or column PBR; hence, they are grouped accordingly. Other types of PBR do not fall into the most “conventional” arrangements, either because they have geometries completely out of the box (i.e. pyramid or torus-shaped reactor), or because they hold specific features – e.g. modified to improve light distribution or quality of light (i.e. internally-diffused PBR, LED-based PBRs), or because they are designed to enhance gas-liquid mass transfer (i.e. membrane-based PBR), or even because of usage of unconventional materials – e.g. disposable-based PBRs.

Immobilized PBR and hybrid-PBR systems will be described – as an illustration of emerging technologies; the first use immobilized microalgae combined with the advantages of one or two of basic designs (i.e. open ponds, flat-plate, bubble-column, stirred-tank) [9].

It should be emphasized that several arrangements to be described have not reached pilot- or industrial-scale – and thus still remain at laboratory or research level; however, their contribution as case studies appears instructive to advance development of novel and more suitable designs for bulk production of microalga biomass and metabolites. The various types of “non-conventional” designs are compared in Fig. 2.

4.1. Geometry/fluid motion issues

4.1.1. Flat-plate PBR

The designation flat-plate (FP), or panel PBR includes configurations arranged in a way that mimics the light harvesting strategy of leafs from plants. A short light path is assured by a thin cuboidal-shape geometry; however, a few special arrangements are included in this category, namely the curved-chamber, the V-shape, the tilted flat-plate and the flat-plate combined with airlift (see Fig. 2). Each such configuration holds particular features that favor high surface-to-volume ratio or else mixing (Table 2).

4.1.1.1. Curved-chamber PBR. The curved-chamber PBR (Fig. 2) was developed by Tredici et al. [30] – and consists of an adaptation of a conventional FP. This configuration consists basically on a vertical chamber, made of 0.6 cm-thick Plexiglas sheet, with an aperture on top for gas disengagement; the transparent front and rear walls of the PBR are molded, so as to form a nearly hemispherical dome. This design was developed for artificial illumination, and to specifically assess the effect of spatial light dilution upon growth and photosynthetic efficiency – when compared to the classical (cuboidal) FP; it exhibited a higher photosynthetic efficiency, along with a lower volumetric productivity though (Table 2).

4.1.1.2. V-shaped PBR. A V-shaped PBR appears as an unconventional FP-PBR – in that it bears an (untypical) enclosed V-shaped vessel of glass (Fig. 2); its basic format was inspired by a fluidized bed reactor [103]. Bubbling is provided at the bottom of the bioreactor, and its engineering features include elimination of escape corners – thus allowing enhancement of mixing rates, without developing high shear; while restraining cell growth on the reactor walls, and exhibiting large exposed areas suitable for light harvesting by cells [39,103] (Table 2).

4.1.1.3. Alveolar panel PBR. Vertical alveolar panel (VAP) was also pioneered by Tredici et al. [104,105] – and consists of flat vessels, of parallelepipedal shape with internal compartments that form narrow divisions (*alveoli*); they are made of transparent PVC, PMMA and/or polycarbonate sheets (Fig. 2), [28,39]. These PBRs can be set vertically, or at an angle to the ground floor – aimed at circumventing some of the flaws of conventional PF-PBRs, namely, difficulties in culture flow control and construction with inappropriate materials [28]. Alveolar panels assure a more adequate light dilution within the culture broth; mixing is, in general, provided as compressed air, pumped through nozzles placed at the bottom of the reactor, and relevant to induce cell cyclic flow [31] (Table 2).

Some modifications have been attempted with this type of PBR; for instance, Pulz et al. (1995) have compacted flat panel devices in a modular way, made of transparent polyacrylic polymers – with several parallel plastic plates closely packed, and plates ca. 25 mm apart from each other. Circulation was driven by a mechanical pump; since the *alveoli* were designed to form horizontal channels, the culture moves along an alternating path through the module [106]. This configuration has allowed circulation of 6 m^3 microalga culture along a 100 m^2 ground area – yet accounting for an enlarged total illuminated area of ca. 500 m^2 [31]. Hu and Richmond (1996) [107] have reported modifications on the original VAP, and built a PBR with glass sheets and silicon rubber – in which *alveoli* were eliminated, so as to favor free turbulent flow [104]. Hu and coworkers (1996, 1996a, 1998) have extensively worked on this FP-PBR [108–110], and significantly improved productivity of *Arthrospira platensis* biomass – with a maximum threshold of 30 g.L^{-1} (indoors). This entails an ultra-high cell density system, with a reduced light path (< 2 cm) – and able to handle biomass densities above 10 g.L^{-1} [40].

High S/V ratio and uniform distribution of light are indeed the major advantages of the aforementioned systems; however, they are prone to develop O_2 build-up, if air flow rates are not sufficient through the culture – partly due to existence of *alveoli* (especially in the vertical design), and concomitant with high photosynthetic activity [19]. Some susceptibility to shear stress may arise, because microalga cells are subjected to strong hydrodynamic forces during liquid motion, and can be projected against the surface of the PBR outer and inner divisions. Unfortunately, this specific design suffers from some degree of cell adhesion onto the walls of the PBR – which pose difficulties in cleaning.

4.1.1.4. Tilted rocking flat-plate PBR. This type of PBR was developed specifically to avoid production of specific metabolites by microalga culture – while promoting production of hydrogen; it bore a design similar to that of tilted FP-PBR (i.e. an inclined FP toward the light-emitting source) (Fig. 2).

This PBR is a panel with a (teflon) frame, compacted into two (acrylic) sheets with neoprene gaskets. To overcome the problem of poor agitation degree, the system was placed on top of an electric motor with eccentric motion, thus providing the pulsating mixing input along with a minor consumption of energy. A degasser helps in gas exchange; when in complete balance, the reactor does not require net energy for displacement (Table 2). Maximum biomass concentrations (for the photosynthetic bacteria *Rhodobacter sphaeroides*) attained were of the order 0.7 g.L^{-1}, with an average production of hydrogen of 6.8 mL.L^{-1}.h^{-1} [111]. Despite the aforementioned promising results, this configuration is not appropriate for large scale biomass microalgae, owing to scale-up difficulties [39].

4.1.1.5. Flat panel-airlift (FPA) with static mixers. Flat pane airlift (FPA) has been recently developed by Subitec GmbH Co. – and is considered as the last generation of FP-PBRs. This system combines the advantages of the flat-plate with internal airlift loop reactor (Table 2); it consists of two deep-drawn plastic half-shells, welded together to form internal static mixers (Fig. 2) [39].

The fluid motion is based on the same principle of the airlift-loop reactor – in which injected air (or gas) creates a pressure differential that displaces the culture broth bottom up; followed by downward motion, in a loop, via vertical downcomers. The airlift operation assures the level of turbulence necessary for efficient gas exchange between microalga cells and dissolved gas, without creating relevant shear forces near the cells. Owing to the small layer thickness, in tandem with fluid recirculation as induced by the static mixers, the cells are able to attain optimal light supply. The microalga cells can be transported from the illuminated part to the dark zone of the PBR at a frequency of ca. 1 Hz (https://subitec.com/en/flat-panel-airlift-bioreactor-technology). Such type of PBR holds improved productivities of cyanobacteria *Spirogyra* sp., of the order of 1.15 g.L^{-1}.d^{-1} – the highest ever reported for this microalga [112]. In fact, the application of baffles supports established circulation paths that expose the cells to regular light/dark periods – a strategy suitable to improve PBR performance; this led to this or other uncommon configurations, by combining a flat-plate geometry with an airlift system (Fig. 2) [59,113]. Degen and et al. (2001) developed a FPA-PBR, where a rectangular channel airlift (made with Plexiglas) permitted the improvement of *Chlorella vulgaris* biomass productivities. The downcomer zone is placed on one side of the reactor, and the riser sub-compartmented into interconnected chambers by horizontal baffles. These baffles are, in turn, alternatively placed at the front, and at the larger flat faces of the reactor – thus creating the advantage of a defined mixing pattern, able to induce regular light cycling and thus promote biomass generation. Sparging of compressed air occurs in the riser zone. The temperature is controlled by circulating cooling water, through a transparent jacket located on the front of the reactor [59]. Baffles were also successfully applied by Huang et al. (2014, 2015), to improve FPA performance, aided by computational fluid dynamics. They built three transparent (polyacrylamide), 15 L-bioreactor with novel static mixers, besides a row of four fluorescent lights installed on both front and back sides of the unit (parallel and horizontal, regarding the surface). The downcomer was provided with inclined baffles (at a 75° angle), forming trapezoid chambers through which fluid motion took place. The gas was provided centrally at the bottom; this configuration increased the frequency of light/dark cycles, which led to an improved growth rate of *Chlorella pyrenoidosa*. A maximum biomass concentration of 1.2 g.L^{-1} (average) was indeed achieved when compared to the control (i.e. plain FP, without baffles), corresponding to an approximate increase of 32% [113,114].

A novel arrangement of a 4 L-FPA was recently proposed – in which a rectangular container (0.2 m × 0.2 m × 0.1 m), made of plastic transparent material, was set-up with an inclined reflective fluid circulation guide. The interior of the vessel was divided in two distinct cross-sectional areas – the riser and the downcomer; the former was set like a column in one side of the vessel, whereas the latter was set-up like an inclined plaque (75°) crossing the reactor, in order to favor a defined circulation path. Sparging was provided by a mechanical pump placed at the bottom of the riser section, and light was provided by fluorescent lamps mounted above the vessel. This arrangement combines good mixing with better light distribution – sufficient to improve the biomass concentrations attained by *Desmodesmus subspicatus* up to 1.5 g L^{-1}, and lipid productivities up to 0.052 g.L^{-1}.d^{-1} [115].

According to Huang et al. (2017), the FPA designs possess several advantages from an economic point of view; they have a high S/V ratio, so a high illuminated area is feasible. Temperature is easy to control by spraying with water or submerging it in a water pool; the hydrodynamics is improved, and the cells are subjected to low to moderate degree of shear or mechanical stress, as for the airlift loop – all leading to low energy consumption. On the other hand, the problem of cell adhesion or settling can be avoided, via high enough culture velocities. Installation of baffles, or circulation guides may provide a synergistic effect in outdoor photobioreactors – eventually leading to higher biomass concentrations [115].

4.1.1.6. Dome-shaped PBR. A dome-shaped PBR (DS-PBR) bioreactor bears an unconventional geometry, derived from the FP one – in that a semi-spherical geometry results, with two hemispheric transparent vessels, one after the other (Fig. 2). Light can be provided by sunlight or some artificial means, as there is some free space inside the dome. This design entails a dome-shaped culture chamber, possessing a high surface-to-volume ratio suitable for effective light harvesting. Mixing and supply of CO_2 are provided by several nozzles placed at the bottom, on the lateral side around the dome. A particular device – called "train", can be installed inside the culture chamber at the bottom; it helps enhance mixing of the culture broth. In addition, an air tube can be connected to this moving apparatus, which continuously sweeps the inner surface of the PBR – thus avoiding the negative effects of cell adhesion onto the dome walls [116]. A cylindrical aperture on the top of the external dome is provided for gas exchange. Cooling is obtained via water spraying on top of the reactor – and this device can be placed below the dome, and integrate the solar light supply. The major drawbacks arise when attempting to apply such a system at commercial scale – as several large units would be necessary, with high land requirements and posing difficulties for cleaning up [48,116] (Table 2).

4.1.2. Tubular PBR

Tubular PBRs backed up non-conventional designs based on modifications leading to high S/V ratios; this is the case of the helical, and conical or inclined tube arrangements (i.e. α-shaped PBR or near horizontal PBR). Furthermore, introduction of static mixers has brought about hydrodynamic improvements.

4.1.2.1. α-Shaped PBR. The α-shaped PBR is considered an unconventional form of tubular PBR; first described by Lee et al. (1995), it consists of two sets of parallel polyvinylchloride transparent tubes (25 m in

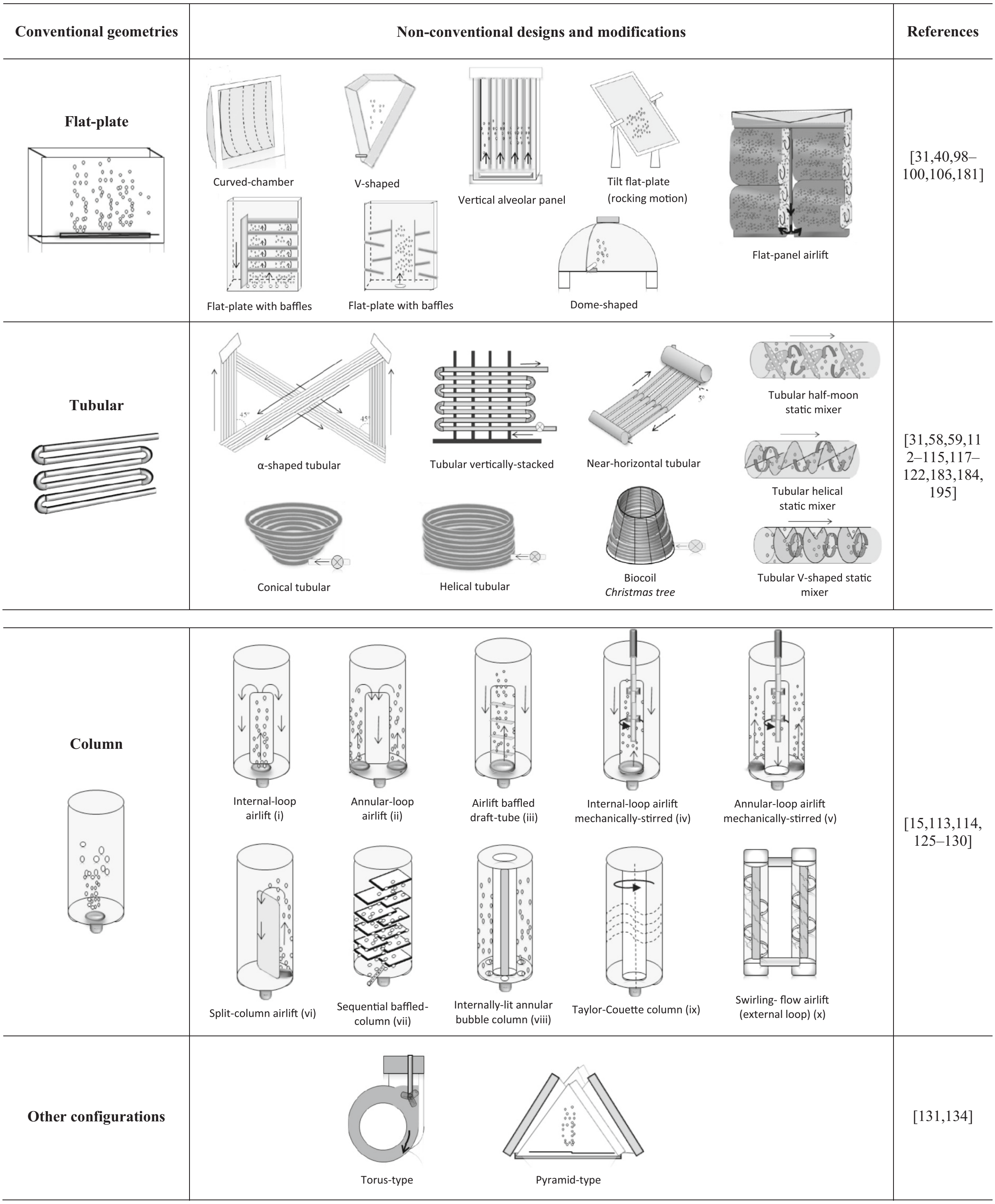

Fig. 2. - Configuration of non-conventional enclosed photobioreactors employed for microalga cultivation. (Adapted from [19,22]).

length and 2.5 cm in internal diameter, for 300 L in volume), placed at an angle of 45° toward sunlight (Fig. 2). Each set of tubes is connected to a receiver tank (in the upper part), while the base is connected to vertical air-riser tubes. The underlying principle is to propel the microalga culture up to the receiver tank – which will descend through an opposite set of tubes, forming a loop-recirculation. The unidirectional flow (except in the airlift tubes) favors high flow rates, at the expense of relatively low air supply rates in the rising tubes; this led to a biomass concentration of ca. 10 $g_{DW}\,L^{-1}$ in the case of *Chlorella pyrenoidosa*. Despite the high cell densities achieved, severe foam formation can occur – thus enforcing addition of an antifoaming agent (which may become costly) or renewal of the culture every single day, in attempts to sustain high biomass concentrations [117] (Table 2).

4.1.2.2. Vertically-stacked horizontal PBR. In the vertically-stacked horizontal PBR, tubular manifold rows look like fence systems (Fig. 2). This arrangement is beneficial to achieve a high S/V ratio – yet a limited number of vertical rows are allowed, because the tubes at the bottom would not receive an adequate amount of light, and would then suffer from a shading-effect caused by the taller tubes. At least a distance of

0.5 m is to be kept between vertical loops, so as to guarantee good microalga productivities [33,118]. In addition, North-South orientation of the bioreactor is particularly important, so as to prevent shading from the rows next to each other. Degassing is made by a column stripper (airlift system), in which air is sparged at the bottom. High liquid velocities are required to prevent accumulation of photosynthetically produced oxygen – which decreases the residence time through the tubes. In this kind of bioreactor, sharp edges should be avoided, since tubular arrays are prone to develop biofilms [118]. De Vree et al. (2016) have studied the effect of biomass concentration on areal productivity and photosynthetic efficiency of *Nannochloropsis* sp., using three outdoor pilot-scale photobioreactors (raceways, vertically and horizontally stacked, and plain horizontal tubular PBR) operated continuously. They found higher areal productivities in vertically stacked units in days characterized by high light intensities (20 $g.m^{-2}.d^{-1}$), as well as highest average photosynthetic efficiencies (3%) under low light intensities. Efficient light interception is probably one of the main advantages of such a type of tubular configuration [119] (Table 2).

4.1.2.2.1. Near-horizontal/tilted tubular PBR. This type of tubular arrangement was proposed by Tredici and Zitelli (1998), and consists of a set of 6 parallel (Plexigas) tubes (3.4-cm internal diameter, 0.3-cm wall thickness) – connected, at both bottom and top ends, by tubular (Plexiglas) manifolds for air injection, and a degassing column [30]. The system is placed facing South and exhibits a superior S/V ratio; it is laid down and tilted 5° relatively to the ground area (Fig. 2**)**. The inclination of the system has proven effective in reducing gas hold-up and removing oxygen, thus resulting in high volumetric productivity (1.26 $L^{-1}.d^{-1}$) and photosynthetic efficiency in the case of *Arthrospira platensis* [30]. According to Carvalho et al. (2006), the maximum volume tested in such configuration was 4000 L – using a set of 8 parallel tubes of 44 m in length, but achieving considerably lower mean productivities (0.7 $g.L^{-1}.d^{-1}$) in the case of *Nannochloropsis* sp. [19]. Despite the aforementioned considerable area-to-surface ratio and the easy scale-up, the poor temperature control (via water spraying) and the low rate of gas transfer – owing to its large length and small diameter, remain as major drawbacks [19,57] (Table 2).

4.1.2.3. Helical/conical tubular PBR. A helical tubular PBR deviates from a conventional tubular PBRs owing to its helical shape design; it was developed to enhance S/V ratio, while maximizing light capture (Fig. 2). Such a configuration consists of a series of coiled or looped tubes, made of flexible plastic, which entail a helical or conical framework (Fig. 2). Suitable for use outdoor with sunlight, it is more commonly operated indoors under artificial light – which adds to the operating cost of that apparatus [89]. The fluid is projected in ascending mode provided by a centrifugal pump. The system usually contains a separate unit of gas exchange, and is coupled to a heat exchanger. The cylindrically-shaped helical tubular PBR, mostly known as *Biocoil*, was developed and patented by Biotechna Grasser A.P., London, UK (European patent No. EPO239272, March 6, 1987). It has a large S/V ratio that allows efficient delivery of light through the culture system; however, in low-latitude sites – owing to the high angle of sunlight and the direct solar irradiance at midday, the light incidence may create a shadow effect in its central area. To overcome the aforementioned limitation, a conical version was proposed by Watanabe and Hall (1995) [120]. The conical unconventional design has the advantage of reducing loss of radiant light energy, since the funnel shape provides a larger photo-receiving area (Fig. 2). In fact, Morita et al. (2000) have reported a maximal photosynthetic efficiency of 6.84%, under a 60° cone angle, in the helical layout [71]. A similar photosynthetic efficiency was reported in an earlier study encompassing *Arthrospira platensis* [30].

The *Christmas Tree Reactor* (developed by the GICON Advanced Environmental Technologies GmbH:http://www.gaet.gicon.com/en/products-services/microalgae-cultivation/giconrphotobioreactor.html) (Fig. 2) is a good example of how a truncated conical geometry helps minimize cell self-shading zones. This particular design is compact, and resorts to flexible plastic – which provides the desired inclination relative to light harvesting. It is often coupled to external gas degasser and heat exchanger units. Moreover, supply of gas is possible along with a low energy input; and a high rate of radial mixing avoids biofouling on the walls [89].

Despite its notable S/V ratio, conical shape designs are, in general, not easy to scale-up (except for the *Biocoil* case). The only way to keep high photosynthetic rates is to increase the number of light harvesting units, which also leads to larger energy losses in the complicated branches of the flow networks; hence, the land area productivity will be significantly reduced [19]. The main shortcomings of both designs – helical or conical, are the use of an air pump that recirculates the culture broth; this approach may increase the shear stress upon the cells, and eventually damage their wall – which constrains high biomass concentrations. Therefore, microalga species more sensitive to shear stress (i.e. dinoflagellates) are not suitable for such type of arrangements – while others may be trapped in the tubes, thus promoting biofouling [19]. On the other hand, oxygen build-up can be a problem – and it is prone to increase proportionally to increasing number of coils [94] (Table 2).

Traviesio (2001) operated a helical tubular PBR with an airlift, instead of a centrifugal pump [121]. Carlozzi and Pizani (2005) also resorted to an airlift column, and a peristaltic pump to help in recirculation of the culture; their innovation consisted of application of a heat exchanger mandrel that allowed wiring of flexible PVC tubes around it – thus acquiring a helical-coiled shape, and permitting a better control of culture temperature. The coil-light receiver was placed on a 90° V-solar collector, and included a white reflecting polyethylene sheet to improve light harvesting [122]. The culture was circulated between the degasser vessel and the light harvesting unit – so the airlift device contributed not only to recirculation, but also to limit accumulation of O_2. The incoming air facilitates stripping of dissolved oxygen; while the gas-liquid separator, at the top of the airlift, prevents gas bubbles from recirculating in the system [28]. Remember that the airlift device is a non-expensive system, and can also be useful to avoid mechanical cell damage caused by flow through the pump [122].

4.1.2.4. Tubular with static mixers. The placement of static mixers has converted classical tubular arrangements into “non-conventional” forms (Fig. 2**)**. By resorting to this simple engineering strategy, the poor rates of mass transfer can be overcome, via improvement of the underlying hydrodynamics; hence, enhancement of biomass productivities is expected to stem from establishment of well-defined and more regular light/dark cycles. However, the said benefits are not straightforward, as they depend on reactor arrangement (i.e. inclination), number of baffles and geometry thereof [123]; if a number of such requirements are not satisfied, cell entrapment in stagnation zones within the liquid culture may raise problems (Table 2).

Ugwu et al. (2002) have developed an outdoor inclined tubular PBR, equipped with motionless baffles; two tubular tubes (a riser and a downcomer) were inclined at 45° relative to the ground plane – joined at the bottom by a gassing chamber and on the top by a degasser unit. Use of V-cutted static mixers was effective toward mass transfer rates (increases over 140% were indeed reported), and gas hold-up (increases of 65% were also possible) – with a positive effect upon biomass productivities of *Chlorella sorokiniana*, which almost reached 1.5 $g.L^{-1}.d^{-1}$, i.e. well above straight tubular designs [123]. Following this approach, several other novel tubular designs have been proposed resorting to static mixers [60,61,123–129] (Fig. 2**)**. Most such works took advantage of computational fluid dynamics to simulate, and subsequently improve performance of hydrodynamic patterns, along with light/dark cycles and mass transfer issues; several configurations included helical static mixers [61,126]. An illustration is provided by Zhang et al. (2013), who have demonstrated that motionless helical mixing can improve biomass productivity of *Chlorella* sp. up to 37% relative to a tubular reactor without mixer. A more recent approach has employed an helical self-rotor (spiral blades) inside a tubular arrangement; under the motion of the liquid culture, the spiral blades are forced to rotate, which establishes a swirling flow with enhanced turbulence that promotes better mixing and faster mass transfer – yet shear-stress issues have arisen [128].

Cheng et al. (2016) designed new static mixer arrangements, consisting of two crossed half-moon-shaped blades with pores in each; based again on computational fluid dynamics, they run simulations of mixing patterns associated to the light regime – resulting in settings for liquid motion and light/dark cycles susceptible of improving performance [130] (Fig. 2**)**. Another novel design was put forward, consisting of two concentric tubes – in which aeration was provided in parallel to the axis of the reactor, through radial pores. The biomass productivity increased by 43.6% and 107.4%, respectively, compared to concentric double tubes with axial aeration at both ends, or common tubular bioreactor without aeration, respectively – and maintained a lower level of dissolved oxygen [131].

Despite implementation of static mixers in PBR conventional tubular designs being seen as a relatively novel strategy to improve performance, in terms of liquid circulation and light penetration, care should be taken regarding the shear-stress imposed upon the microalga cells. A compromise between high velocity imposed upon culture flow with generation of turbulent eddies, and not too low velocity to minimize the risk of cell attachment onto the PBR walls is to be sought.

4.1.3. Column PBR

Column PBR modifications have been proposed ever since their first use; researchers soon realized that the photosynthetic efficiency inside column-PBRs had to be improved – owing to their low lit zones, besides their low A/V ratio. Attempts to diminish the light path inside the reactor have led to an annular column PBR, able to circumvent low-irradiated areas. Other technical developments focused on light flashing effects (enhancement of light/dark cycles), hydrodynamics and mass transfer rates; for instance, introduction of horizontal baffles or creation of swirling flow combined with airlift were tested in such PBRs, with the goal of assuring better mixing, adequate light/dark cycles, reduction of gas hold-up, and less extensive shear stress in the cultivation broth.

4.1.3.1. Airlift column with static mixers. Considering that column PBR categories are intrinsically related to specific flow motion patterns, some non-conventional modifications have used known engineering strategies to improve hydrodynamics, gas liquid-mass transfer, and more regular light/dark cycles of cells inside the column (Fig. 2**)**.

Two main forms of airlift column are known: the internal-loop airlift PBR, and its external-loop counterpart. The former comprises different types of arrangements: concentric draft-tube (the most common) – known as internal loop airlift (Fig. 2i**)**, in which a typical transparent internal column becomes the riser zone, and the annular space becomes the downcomer. Although this configuration may present distinct *modus operandi*, the gas is normally sparged in the annular space (riser), whereas the downcomer region becomes the center of the column – yielding an annular-loop airlift (Fig. 2ii). To improve mixing efficiency, concentric draft tubes can be integrated with static mixers or baffles (airlift baffled draft-tube) (Fig. 2iii). Other non-conventional arrangements are possible, in which mechanical stirrers are added inside the draft tube – and air is directly sparged through the inner cylinder (Fig. 2iv), or sparged in the annular space (Fig. 2v**)** [132]; or a split tube, in which a flat-plate or baffle splits the diameter of the column – thus separating the column into two parts, the riser and the downcomer regions (Fig. 2vi).

Integration of sequential baffles inside the bubble-column [133] also led to a non-conventional format. For instance, Lam and Lee (2014) have design a sequential-baffled column (Fig. 2vii), made of transparent acrylic material, in attempts to increase the residence time of gas bubbles (CO_2) inside the PBR – while providing the necessary homogenization of nutrients during mixing. Installation of baffles as static mixers inside the column-PBR, concomitant with application of high aeration rates apparently improved gas dispersion into the liquid phase; under these circumstances, the mass transfer coefficient of CO_2 tends to increase when compared to the conventional bubble-system without baffles [133]. This appears to be a good strategy to obtain better light flashing effects – and may indeed improve performance of the cultivation system; however, care should be exercised to avoid development of high shear rates (Table 2).

4.1.3.2. Annular bubble column. Another non-conventional design is the annular bubble column – which consists of two cylinders made of Plexiglass, one inside the other, so as to form an annular chamber. A perforated plastic tube is placed in the said annular chamber, to provide mixing and gas exchange – as well as serve as gas diffuser for CO_2 supply and pH regulation. Illumination can be provided by either natural or artificial light [134,135]; in general, the light apparatus is placed inside the inner cylinder, thus helping reduce the light path – with a better use of artificial photon flux by microalga cells. The internally lit annular configuration (Fig. 2viii), accordingly improved photosynthetic efficiency, and conveyed a satisfactory microalga biomass production [134] (Table 2).

Another non-common configuration of annular bubble column for microalgae cultivation is Taylor-Couette photobioreactor (Fig. 2ix) [136,137]. This particular configuration is composed by an inner central rotating chamber, inside a main cylindrical vessel that induces the so-called Taylor's vortex flow. The cells are cultivated in the annular chamber, and their mixing is promoted by such vortices, promoted by rotational displacement of the inner cylinder. It was claimed that such a type of fluid motion helped improve mass transfer, yet the associated ordered mixing also induces regular light/dark cycles – thus enhancing photosynthetic efficiency. In addition, as fluid agitation does not depend on bubble turbulent flow, much lower CO_2 flow rates need to be used, thus reducing operational costs; and a significantly higher CO_2 uptake can be achieved than in conventional microalga reactors (Table 2).

Within the set of non-conventional arrangements, columns can be adapted via insertion of a draft-tube inside, with sequential baffles attached to its inner wall; they can as well be adapted to a mechanical mixer, with air sparged directly through the inner cylinder or in the annular space.

Another non-typical configuration is known as swirling flow airlift-PBR [135] (Fig. 2x). By combining the advantages of an airlift system, the annular geometry, and the swirling flow, this PBR assures effective light penetration and enhanced mass transfer rates. The annular shape allows housing of artificial light tubes in the inner vessels, and an extended S/V ratio. The external cylindrical vessels made of PPMA (transparent material) are connected by two flanges, at top and bottom. The swirling motion is generated by tangential inlet flow, in an annular cavity separating two static cylinders to improve mixing efficiency and homogenization; however, some foam formation (in the degasser zone) and microalgal deposit on the walls of the downcomer were reported [135] (Table 2).

4.1.4. Alternative PBR designs

4.1.4.1. Torus-type PBR. The torus-type PBR is a configuration (Fig. 2**)** used specifically in studies of cultivation of microalga for hydrogen production, and the effect of mixing upon phototrophic microorganisms [138]; however, it was also investigated in attempts to control the photoautotrophic growth process of *Chlamydomonas reinhardtii*, by applying a nonlinear multivariable controller [138,139].

In general, this system is characterized by a round configuration, made of transparent material (e.g. PPMA) in which flow occurs in a loop.

The light is provided by external tubes parallel to the illuminated surface; the system is completely automated, and suitable for operation either in batch- or continuous mode [138]. The major advantage relates to high dispersion of liquid, because a mechanical propeller (marine impeller) can be used to generate a swirl motion forming Dean's vortices in the reactor bends [138,140]. Low impeller rotation can achieve good mixing efficiencies, but the flow is heterogeneous around the mechanical stirrer; hence, some shear stress can be generated [138,140]. Torus-type PBR design have been limited to lab-scale, and its alternative geometry makes it very difficult to scale-up (Table 2).

4.1.4.2. Pyramid-PBR. A pyramid-PBR is one of the most recent cultivation systems bearing a pyramidal shape-geometry (Fig. 2). This type of configuration, seldom described in the literature, was developed by Soley Biotechnology Institute – and is specifically made of medical acrylic, a non-toxic material that prevents cell adhesion to the PBR walls. The tetrahedron configuration endows a high illuminated surface area on the outer surface, but also allows integration of an artificial light system in the back surface walls (Table 2).

An airlift system allows recirculation of the cell fluid and enhances mixing, thus avoiding high shear rates – with the advantage of reducing pump costs as well [141]. The system is fully automated – with pH, temperature and DO probes; and advanced materials and technologies are used to save costs and avoid overheating. It permits sterilization by an UV lamp placed inside the vessel; although still under experimental stage, this system appears a promising candidate for high areal productivities. According to Płaczek et al. (2017) [18], the maximum productivities of biomass clearly exceed those in open ponds, and may go beyond productivities in all other tubular PBRs.

4.2. Light enhancement issues

4.2.1. Internally-illuminated PBR

As mentioned earlier, some designs (e.g. column-PBRs, stirred-tank type PBRs) suffer from inherent constraints toward successful light distribution inside microalga cultures. To mitigate the adverse effects of irregular light distribution, new configurations have been sought that resort to illumination devices placed inside the culture vessels – which led to development of internally-illuminated PBRs. According to Olivieri et al. (2014), a first generation of internally irradiated PBRs arose when fluorescent tubes were submerged, or integrated inside bubble column or airlift PBRs. The second generation of internally illuminated PBRs was characterized by use of optical fibers as light transmitters, and diffusers inside the cultivation vessel. The external light source (solar or artificial, or both combined) can be collected, concentrated and distributed by optical fibers, or other waveguide devices such as glass or quartz bars. Several studies have covered irradiated PBRs integrating optical fibers, combined with diverse external light sources [142–146].

Ogbonna et al. (1996) [143] developed a typical first generation, internally-illuminated PBR – by resorting to a conventional stirred-tank PBR, with 4 internal fluorescent lamps mounted inside (Fig. 3i). Air and CO_2 were supplied by spargers, and a modified impeller was installed to achieve good mixing, and thus a high rate of mass transfer inside the reactor – concomitant with a relatively low degree of shear stress. This type of photobioreactor was latter adapted for use of solar and artificial light, by resorting to an optical fiber system for light transmission, and then to light radiators inside the culture [144] (Fig. 3ii). Analogous PBR types, resorting to optical fibers, were reported to improve light irradiation inside the culture, and thus improve biomass productivities. For instance, Mori (1985) [147] has developed a bioreactor in which solar light was collected by Fresnel lenses, transmitted through optical fibers, and finally dispersed by ca. 100 light radiators inside the bioreactor. In a similar approach, and using the same type of PBR, an internally illuminated-PBR was created in which light provided by a metal halide lamp (to mimic sunlight) was transmitted and diffused by the surface of optical fibers [142]. Ponte et al. (2016) [146] have adapted a cylindrical reservoir to a simple system of air inflow (by concentric tube spargers), with integration of concentrically arranged polished optical fibers connected to LED lamps as external light source. Other PBR-types, such as airlift systems, were also tested with optical fibers [148,149], yet problems of leakage and cell adhesion were found. Despite those drawbacks, enhancement in biomass productivity of two different microalgae species – *Spirulina platensis* and *Scenedesmus dimorphus*, up to 43% and 38%, respectively, was achieved under light frequencies over 10 Hz, when compared to traditional airlift ones [148]. The integrated PBR system developed by Hincapie and Stuart (2015) [149] has, in turn, led to a growth rate twice as high, under optimal mixing rate conditions [50].

Photobioreactors employing fiber optics are expected to markedly overcome the structural limitations posed by externally illuminated PBRs. Optical fibers can indeed be coupled to tailor-made distributor plates, made of similar material or directly immersed in the cultivation system, thus allowing a high S/V ratio and a more uniform scattering of light inside the PBR, concomitant with low heat generation rates [5,150]. They also allow control of illumination and duration of light periods, and can be equipped with several artificial light apparatuses (i.e. LED) to supply light with specific wavelength distributions, besides solar light using the full spectrum and a solar-photon collector. The last option, with delivery system through fiber optics, would be desirable to both optimize microalga growth and reduce system footprint in large-scale cultivation systems. However, use of optical fibers in most cases implies use of concentrating devices (i.e. Fresnel lens), which can increase the initial capital expenditure and operational cost of the PBR [148,151] (Table 2).

4.2.2. Light-diffused PBR

Instead of using optical fibers, several recent PBR designs have adopted such other strategies of light dispersion as transparent panels, reflective surfaces, or waveguide devices to redistribute light through microalga suspensions [151–156]. The concept of light diffused PBR was pioneered by El-Shishtawy et al. (1997) [157]. They developed a two-plate compact system: one part was made of transparent Plexiglas sheet, treated with dots to help in light dispersion; and the other sheet was made of poly(ethyleneterephthalate) and placed on the printed surface, to aid in homogenous diffusion of light (from halogen lamps) through the culture vessel. This system was used to produce hydrogen by photosynthetic bacteria, but similar concepts emerged using new light sources (i.e. LED) to enhance light diffusion. As an illustration, Sun et al. (2016) [151] resorted to embedded hollow polymethyl methacrylate (PMMA) tubes as light guides, to improve light distribution across a flat plate; part of the incident light from fluorescent lamps could be transmitted to the interior of the PBR, while the PMMA tubes served as in-static mixers to promote bulk turbulent flow. In this study, *Chlorella vulgaris* cells were able to receive light, even in the more deficient-regions – with an increment of 23% in biomass production when compared to conventional FP. Another example is the ultracompact PBR proposed for ethylene production [153]. Stacked layers of borosilicate slides were treated chemically to form etched surfaces, which functioned as waveguides to allow penetration of light within the cultures. Consistent production of ethylene was achieved over a period of days, and (genetically modified) *Synechocystis* sp. increased its biomass by 8-fold relative to a conventional airlift system.

An innovative airlift cuboidal vessel was proposed, in which planar waveguides doped with nanoparticles were employed. In this case, the reactor was constructed in a modular way, and LED light was supplied to help scatter light over the *Chlorella vulgaris* cultures; a maximum biomass concentration of 3.05 $g.L^{-1}$ was reached [152].

In a new concept, Pierobon et al. (2016) [158] have developed a non-conventional PBR in which a breathable waveguide was used – not only to distribute light, but also deliver CO_2 by permeation. This special waveguide, made of transparent cellulose acetate butyrate, permitted cultivation of cyanobacteria with different concentrations of light and CO_2 – and a two-fold increase in growth was achieved, when compared to impermeable waveguides.

The PBR designs with planar waveguides have attracted increasing attention because they represent an efficient strategy to improve incident light; their “stackability” and simple structure is easy to scale up and available at a low-cost, besides not needing to bypass secondary processing light (unlike optical fibers) [52,152]. Other light guides (or non-waveguide) PBR designs [151,157], characterized by employing reflective surfaces or transparent structures inside to disperse light and reduce light path, are impractical at large scale; they can occupy a high fraction of PBR volume, and run the risk of developing a biofilm. Moreover, microalga cells very close to incident light surfaces can be severely affected by photo-inhibition [52] (Table 2).

4.2.3. LED-based PBR

As seen above, light distributors (e.g. light waveguides and optical fibers) have been used to modify PBRs, so as to manage light path and increase photosynthetic efficiency; in addition, the type of light sources/technologies used with the said PBR were also addressed, in attempts to improve photosynthetic efficiency. LEDs have indeed been gaining ground over conventional lamps, and this technology has become more cost-competitive in recent years – along with dramatic improvements in performance and efficiency [159]. LEDs possess a potentially high energetic efficiency and are long-lasting, when compared to traditional fluorescent lamps; they present narrower wavelength bands that are more suitable for higher photosynthetic efficiencies; and are claimed not to generate excessive heat – which can be a problem in conventional indoor PBRs, as overheating of the culture will take place [160,161]. According to Glemser et al. (2016), use of LED-based PBRs has been claimed as having technical advantages toward microalga growth. However, attempt to use PBRs with different LED wavelengths (i.e. blue light vs. red-light, or use of white light proper) to enhance microalgal growth supported inconsistent results across the literature, being highly species/strain dependent [161]. For instance, use of white LED can lead to misleading comparisons among distinct studies, because information about the emission spectra is rarely presented [162]. In addition, LED efficiency is highly dependent on type of LED used, current supplied, and actual color spectrum. Furthermore, it is reported that LED photon output efficacy is far better than conventional incandescent lights, but has the same order of magnitude of fluorescent light sources; not to mention that initial fixtures costs are up to 4-fold those of fluorescent lamps [161,162].

However, a significant number of innovative structures and PBR designs have been adapted to LED light through the years [146,163–169]. For instance, Hu and Sato (2017) [166] have proposed a configuration with a round vessel internally provided with a stainless steel-helical frame, with alternated red and blue LED lights. Its novelty resides in the hexagonal closest-packed structure of the LED-light source, which aims at enhancing flash light-effects. A maximum biomass concentration of *Dunaliella tertiolecta,* as model microalga, was 1.32 $g.L^{-1}$; numerical simulations, assuming ideal light-source distance and intensity, have established a maximum concentration of 19.80 $g.L^{-1}$, more than one order of magnitude larger.

4.3. Gas exchange enhancement

4.3.1. Membrane-based PBR

Gas exchange is one of the most important parameters pertaining to PBR performance. In fact, efficient gas-liquid mass transfer is essential to avoid limitation in carbon availability to microalga cells or/and a high accumulation of inhibitory O_2 (released by photosynthesis) in the medium [19,40]. Furthermore, poor gas-liquid mass transfer is often found in conventional PBRs (i.e. tubular), especially at larger scale.

Non-conventional PBR configurations entailing improvements in gas-liquid mass exchange systems (especially those for CO_2 delivery) are based on bubble-free cultures [40].

Membrane based-PBRs are a specific type of enclosed photobioreactor, in which large surface areas of membrane (as contactors) are provided to facilitate gas exchange between gas and liquid [28] – with the extra advantage of avoiding high shear rates that may damage microalga cells. It is well-known that carbon supply is a key-parameter affecting microalga cultivation, and most times it is supplied by bubbling continuous air or CO_2 enriched-air – with relatively low gas transfer rates, along with high shear rates (Table 2). Membrane-based PBRs circumvent these limitations, owing to their larger k_la’s and reduced formation of eddies.

The use of hollow fiber membrane (HFM) modules was recently suggested as a dispersion/permeation device – aimed at increasing retention time of CO_2 along the PBR, while improving mass transfer rates [170]. Those commercial apparatuses consist of bundles of semi-permeable polymeric hollow fibers (diameter between 50 and 250 μm), arranged in parallel within a tubular housing [19]. They can be implemented in one of the two basic ways: i) integrated within the reactor via an internal sparging system [171]; or ii) installed externally to the PBR, as a plain membrane contactor, connected to a peristaltic pump [154,163,172–175].

Ideally, the HFM employed in PBR should present such unique material properties as: hydrophobicity to resist acid and alkaline conditions, and prevent fluid impregnation of the membrane itself; homogenous and porous surface, to assure even distribution of gas (CO_2) through the culture; and anti-biofouling features, to minimize cell attachment, and thus cleaning needs.

Fan et al. (2007) [171] have designed an enclosed membrane-photobioreactor to improve CO_2 fixation by *Chlorella vulgaris*; the PBR consisted of a cylindrical airlift tube, with a cooling water jacket and a hollow fiber membrane module, made of polyvinylidene fluoride (PVDF) fitted inside the PBR as gas sparger. When compared to conventional PBRs, the CO_2 biofixation rate was ca. 5.4× higher, under optimal operating conditions.

A similar study was conducted by Cheng et al. (2006) [173] with *Chlorella vulgaris*, but using a polypropylene hollow fiber membrane module integrated externally with this reactor. The researchers have observed an improvement in CO_2 gas retention time from 2 to 20 s, in tandem with an increase of 3.25-fold CO_2 fixation rate – and a reduction of dissolved oxygen level, when compared to common PBRs. Fan et al. (2008) [174] have proposed another configuration of membrane-PBR – more specifically, a membrane-sparged helical tubular PBR. A helical tube was used to capture the maximum light possible, and internally adapted with hollow fiber membranes that resulted in improvements of CO_2 fixation rates of 58% with *Chlorella vulgaris*.

A more integrated approach produced a waveguide-based bio-ultracompact reactor, with HFM apparata incorporated to characterize different gas regimes (passive flow, active flow with atmospheric air, and active flow with CO_2-enriched air); the performance of this PBR was tested in terms of production of ethylene by genetically modified *Synechoccus* sp. [144]. Compared to ultracompact reactor without HFM, those authors have shown a higher photosynthetic efficiency; whereas use of the HFM reduced mixing costs by orders magnitude, when compared to classical closed photobioreactors. In addition, HFM active aeration with an enriched-CO_2 stream enhanced growth rates by 2-fold – thus clearly demonstrating its positive influence upon mass gas transfer rates.

Sano and co-workers employed instead a silicon hollow fiber membrane module as PBR itself [175]. They took advantage of the cylindrical shape and transparency of the said module to cultivate microalga cells, combined with LED lighting as external light source. An enhancement of CO_2 mass transfer rate resulted, leading to an increment *Chlorella vulgaris* growth by 3-fold when compared to a non-membrane PBR.

Both increase in gas hold-up and in CO_2 fixation rate by microalga cells are viewed as the main advantages of membrane-PBRs. However, a few limitations may arise, especially in PBRs where membrane modules

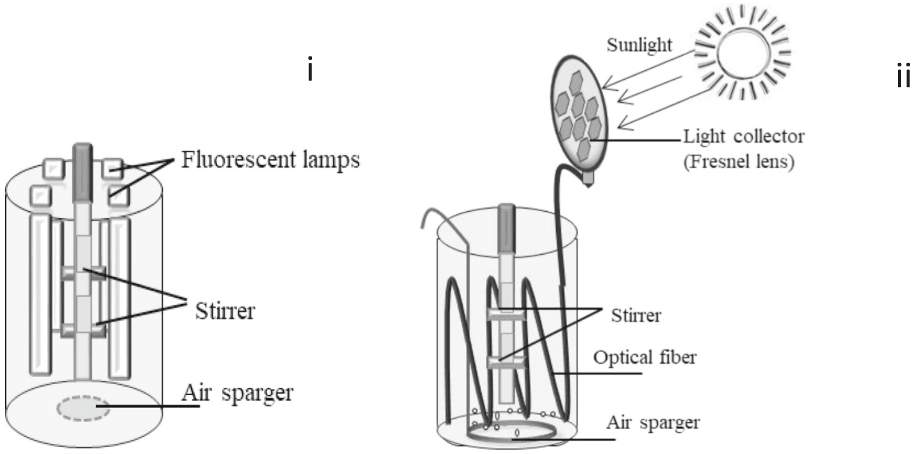

Fig. 3. - Configurations of internally illuminated PBR employed for microalga cultivation – (i) stirred-tank with internal florescent lamps and (ii) stirred-tank connected to a Fresnel lens for collection of light and optical fibers for conduction of thereof. (Adapted from [143,144]).

are integrated internally. If the gas hold-up is high, dispersion of cloudy bubbles is favored, so light penetration is reduced – and the growth rate of microalgae cells will be compromised [174].

The strategies of using membrane modules externally or using them as a photobioreactor proper can be useful to improve mass transfer rates of CO_2 (and stripping of O_2), while maintaining a high S/V ratio and an efficient light distribution. The excess CO_2 gas can be recirculated, thus allowing lower gas pressures to be used – while decreasing operating costs. However, care should be exercised in applying those systems, as CO_2 and light-rich periods may not coincide with each other (both in space and time), which will reduce growth of microalgae and production of target metabolites [19].

4.4. *Cultivation strategy issues*

4.4.1. *Immobilized PBR-systems*

Most microalga cultivation systems are operated as suspension media – yet the spatial distribution of light over the cells, in terms of conventional growth systems, is a classical constraint (as seen above). Hence, research on immobilized microalga systems has attracted attention in recent years as another non-conventional technology – resorting, in particular to biofilm PBRs [176].

By attaching or immobilizing microalgae cells as dense thin layers (as biofilms) and recalling the goal of a short light path, a number of configurations have been experimented with, and yielded promising results – especially in wastewater treatment for potential removal of pollutants (i.e. phosphate, nitrogen), besides production of oil for biodiesel manufacture and value-added compounds [177–185].

The most successful approaches immobilize microalga cells on sheet-like surfaces, by growing them on artificial supports or structures (i.e. membrane, filters, nylon mesh, cotton rope, polystyrene foam, polyethylene screen, steel mesh, concrete layers) [186]. This allows light scattering in a more uniform way, and is thus susceptible of better photosynthetic efficiencies – while the water and nutrient supplies are provided in a semi-continuous or continuous way to improve growth rates [187]. The control of medium flow is essential, in order to avoid cell wash-out, which means a lower shear stress upon the microalga cultures; the water requirements were proven to be much lower than suspended based-PBRs [188] (Table 2).

Biofilm-based PBRs can be operated in two different ways: i) by totally submerging the biofilm [178,184,185] (Fig. 4i) or ii) by cultivating the biofilm in a semi-permeable membrane (or porous substrate layer) [179,180,189] (Fig. 4ii). A variant from the former is intermittently submerge the biofilm; Christenson and Sims (2012) [178] have accordingly developed a biofilm PBS intermittent approach, consisting of a cotton cord wrapped around rotating drums for microalga attachment – and partly submerged in wastewater. Biomass productivities of 20–31 $g.m^{-2}.d^{-1}$, and a positive energy net balance were found – meaning a reduction of water and energy requirements.

Several examples have been reported in the literature, encompassing

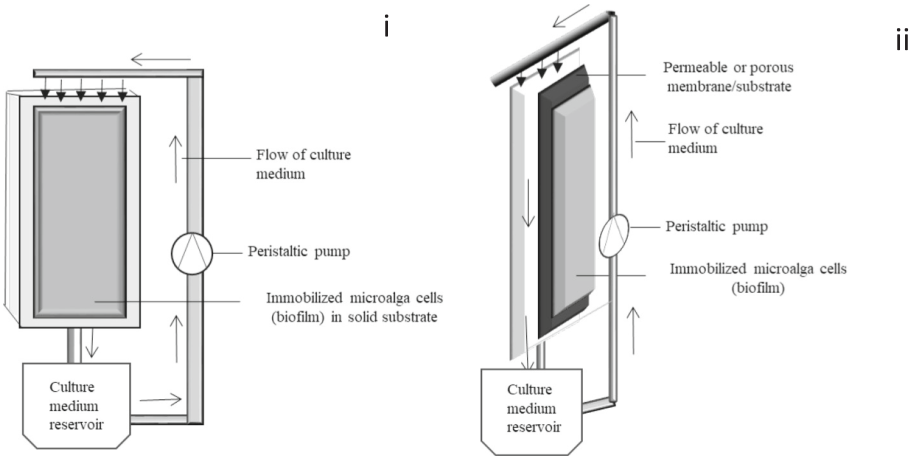

Fig. 4. - Configuration of immobilized-based photobioreactor for microalga cultivation – (i) totally submerging algal biofilm; (ii) cultivating biofilm in a porous or permeable membrane or substrate by allowing nutrient exchange through the permeable material.

the second approach – as is the case of a multilayer PBR [179,180,189] (Fig. 4ii). In this case, the photobioreactor is compartmented into intercalated arrays of thin biofilm, attached to an artificial support (solid-phase) and the liquid media (liquid phase); both are separated by semipermeable and transparent membranes. Light is provided and diffused along the clear medium partition, thus assuring adequate light transmittance on both sides of the microalga biofilms [20]. In a more recent study, Xu et al. (2017) [190] developed a new strategy of producing microalgae via a capillary-driven photobioreactor – and supplying nutrients in the carriers enriched via capillary action; microalga productivities reached ca. 10 g m^2 d^{-1}, with a footprint area of ca. 121 g m^2.

4.4.2. Falling-film based-PBR

Falling-film based PBRs (or thin layer based-PBR) set on the principles of using high specific illuminated surface area with a thin culture depth (1.5–8 mm) [191], so as to enhance microalga volumetric productivities. The culture is injected at the top of a tilted surface and flows down, being collected and pumped back through a tube and re-injected at the top – thus closing the loop. This principle is chiefly used in open systems [65,192,193]; however, a recent work by Pruvost et al. (2017) described adaptation of this system to an enclosed version (AlgoFilm©) [191]. The tilted surface was covered with a glass plate, thus turning it to a fully enclosed geometry. A small flow of air is injected through the PBR headspace, and a peristaltic pump helps circulate the culture with minimum low shear – with a LED panel installed above the PBR to provide lighting thereto [191].

Years before, Doucha and Livansky (1995) [192] worked on the same concept – and accordingly developed the so called outdoor thin-layer PBR (or cascade system). This is the largest system of this kind: it is composed by two tilted surfaces (tilt angle of 1.7%), interconnected to create a circulating loop (like a cascade), and a carbon reservoir to feed the culture, with an illuminated area of 224 m^2 and a working volume of 2000 L. After several modifications (e.g. removal of baffles, initial tilt angle), it is still in operation to produce *Chlorella* in fed-batch mode, with a culture depth <1 cm – and reaching areal productivities up to 10 g^{-2}d^{-1}, while maintaining high growth rates [191–193].

Despite their high productivities, such systems are poorly competitive in terms of cost; they need improvement of materials used, because the sloping culture made of glass is quite expensive [194] (Table 2). According to Doucha (personal communication), preliminary studies indicate that sunnier placement, use of paddle-wheel raceway systems, and replacement by cheaper materials will make such system very competitive in a near future [194]. Use of flue-gas was already tested, prone to minimize the cost of pure or air-enriched carbon supply [195].

4.4.3. Hybrid PBR designs

Hybrid systems can be viewed as emerging technologies – and clearly different from conventional ones. In general, a hybrid combines one or two of basic designs (e.g. flat-plate, bubble-column, stirred-tank), to complement each other and overcome the disadvantages of each one [9]. The most common approach is to combine the advantages of both open ponds and enclosed PBR – in attempts to find a compromise between the low cost of open ponds and the good control of conditions of enclosed PBRs. A two-stage approach is normally anticipated: enclosed PBRs are used to increase biomass production in aseptic environments, and then open ponds or raceways (usually shielded to reduce the chance for contamination) are employed to expose the cells to the stress of nutrient lack – which should trigger synthesis of desired metabolites [18]. An example of this two-stage system has been reported for production of both oil and astaxanthin by *Haematococcus pluvialis* – with an average production rate above 10 ton ha^{-1} [196].

Several new hybrid designs have been suggested in the literature [174,197–201]. For instance, a new hybrid PBR consisting of an open tank – connected to a transparent flat plate-PBR arranged in parallel and two vertical bubble columns arranged in series, was used to assess biomass productivity of *Chlorella homosphaera*. The system was interconnected with piping for algal recirculation (via a pump) and gas bubbling. All system components were made of transparent materials, and the light system was provided over the PBR tank to enhance irradiance distribution. Flow dynamics and gaseous transfer were improved, resulting in higher algal densities – up to 3.6 g.L^{-1} (3-fold those of standard open tanks, under similar photoautotrophic conditions) [198].

Another promising, low-cost, durable and integrated translucent bubble-mixed hybrid configuration was patented by Mottahedeh and Tredici (2012) [197]. It consists of a C-shaped, open-top bioreactor chamber enclosed by semi-rigid, rollable glass-reinforced plastic (fiberglass) sheet, and adjusted into height-shaped sustaining supports; this system allows irradiance of sunlight in all directions. An external and removable cover, also made of fiberglass material, is provided to create a closed chamber and improve light irradiance – as it can have a solar reflector, and assure a low-cost temperature control. The choice of fiberglass is claimed to assure a long time span for the PBR (of at least 25 years) (Table 2).

4.5. Construction issues

4.5.1. Disposable PBR designs

The concept of disposable PBR has arisen in recent years, and gained more attention with regard to commercial production of microalga biomass [208]. The faster installation, higher ease of operation and lower cost of materials employed make such configurations more attractive than typical compact PBRs, in view of their inherent disadvantages – namely poor flexibility, complexity of operation and high energy demands. Furthermore, they can offer cost savings because in situ sterilization and cleaning are not required. Several disposable PBRs can be fixed vertically in a metal stand – such as the vertical sleeve PBR-like cultivation system at pilot scale, mounted by Abomohra et al. (2014) [209], composed of 20 bags, each with ca. 16 L capacity and occupying 1.9 m^2 land area. Other designs entail metal frames associated to a short light path [210]; this includes the flat-panel concept – e.g. a 250 L-polyethylene disposable PBR placed between two metal frames, with aeration provided by a tube running from side to side, and a heat-exchanger inside the bag. Another form of disposable PBR exhibits a special X-shaped arrangement [211]; this configuration possess a working volume of 20 L meant to operate outdoors, and is made of low-cost polypropylene. The integrated airlift (i.e. downcomer in the middle column, and riser in the side columns) has been found to enhance light distribution over the microalga cells through appropriate average cycles, and to develop a homogeneous laminar flow pattern. This design has been reported to create low shear stress upon microalgae, which apparently improved biomass productivities of *Chlamydomonas reinhardtii* (ca. 1.35 g L^{-1} d^{-1}) and associated lipid productivities – likely due to absence of challenge on their cell membranes, associated with Kolmogorov's eddy length scales. Another disposable-system, based on baffled FP-PBR, was proposed for culturing of *Chlorella* sp. and *Scenedesmus dimorphus* at pilot-scale [212]; the PBR was placed horizontally on the ground – with the upper surface illuminated by sunlight, and the non-illuminated downsurface composed by several inclined baffles placed in a zigzag pattern. A pump ensured recirculation of the bulk medium, whereas baffles established a spiral-like flow – helpful to obtain flashing light effect upon the microalga cells. The maximum areal biomass produced was of the order of 11.0 and 21.9 g.m^2.d^{-1} for *Chlorella* sp. and *Scenedesmus* sp., respectively; an increase of 25% in biomass productivity of FP-PBR with baffles was recorded relative to absence thereof.

Other studies focused on single-use bioreactors, characterized by sealed plastic clear chambers filled with microalga culture, agitated in a rocking motion or a shaking device, and illuminated by an external light source (i.e. LED) [208,213]. The gentle agitation provided in those systems is attractive for cultivation of shear-sensitive microalgae with commercial interest [208], like dinoflagellates or diatoms; however,

Table 2
- General comparison of "non-conventional" photobioreactors used for microalga cultivation, in terms of main features, advantages and disadvantages.

General type of photobioreactor	Particular features of system										
	S/V ratio	**Main source of light**	**Agitation system**	**Temperature control**	**Gas exchange**	**Mixing efficiency and gas transfer**	**Shear stress**	**Scalability**	**Advantages**	**Disadvantages**	**Ref.**
Flat-panel											
Curved-chamber PBR	Medium	Artificial	Air bubbling	Thermostated fluid	Opening aperture on the top	Moderate (both)	Moderate	Very low	High S/V; high photosynthetic efficiency	Scale-up issues, prone to biofouling	[30]
V-shaped	Medium	Solar	Air bubbling	None reported	Exchange headspace	High (both)	Moderate	Limited	High S/V; enhanced mixing rates; mitigation of biofouling; low cost material	Scale-up issues due to non-typical geometry	[103]
Alveolar panel	High	Solar	Air bubbling	Water circulation in upper part	Exchange headspace	High (both)	Moderate	Limited	High S/V; uniform distribution of light	High risk of cell wall attachment; Scale-up issues	[31,104,105]
Tilted flat-plate (rocking motion)	High	Solar	Pulsating motion	Heat exchange coils	Exchange headspace	Moderate (both)	Low	Very limited	High S/V; good mixing	Scale-up issues owing to pulsating form	[111]
Dome-shaped	High	Solar/ Artificial	Air bubbling	Water spraying	Opening aperture on top	Low (both)	Low	Very limited	High S/V; Low degree of biofouling	Scale-up issues; high land requirements	[202,203]
Flat-panel airlift + static mixer	Medium	Artificial	Airlift/Air bubbling	Cooling water jacket	Exchange headspace	High (both)	Moderate to high	Moderate (modules)	High S/V; regular light/dark cycle effect	Scale-up issues; prone to shear stress	[39,59,113]
Tubular PBR											
α-shaped	High	Solar	Airlift	N/a	Air sparging at vertical units Degasser at top	High (both)	Low to medium	Limited	Unidirectional flow, high flow rate, high S/V	Poor temperature control; foam formation	[117]
Vertically stacked	Very high	Solar	Centrifugal pump/airlift column	Heat exchange coils inside airlift	Air sparging/ column stripper	Moderate mixing/ low gas transfer	High	High	High S/V; efficient light capture; high areal productivity	High liquid velocity; risk oxygen build-up and biofouling	[116,117]
Near-horizontal (inclined)	Very High	Solar	Air bubbling	Water spraying	Air sparging; degasser at top	Low mixing/ medium gas transfer	Low	High	Inclined angle favors high S/V; high photosynthetic efficiency; low gas-hold up	Poor temperature control	[30]
Helical	High	Solar, artificial light or combined	Centrifugal pump/airlift column	Heat exchanger	Degasser unit	Moderate mixing/low gas transfer	High	High (modules)	Inclined angles favor high S/V	High risk of shear stress; high risk of oxygen build-up and biofouling	[30,89,122]
Conical	High	Solar, artificial light, or combined	Centrifugal pump	Heat exchanger	Degasser unit	Moderate mixing/ low gas transfer	High	High (modules)	High S/V: efficient light capture	Scale-up issues; High risk of shear stress, oxygen build-up and biofouling	[120,204,205]
Tubular with static mixers	High	Solar	Centrifugal pump/air bubbling	Water sparging	Degasser unit	High (both)	Very high	Medium	Efficient mixing Improved gas hold-up; efficient light/dark cycles	Inadequate baffles (number, geometry) leading to cell entrapment or liquid stagnation	[58,59,117–121]
Column PBR											
Column baffled	Low	Artificial	Air bubbling	Possible	Exchange headspace	Very high (both)	Very high	Limited	Increase residence time of gas/improved gas dispersion	High risk of shear stress	[133]
	Medium		Air bubbling	Possible			High	Medium	Reduced light path	High risk of shear stress	[15]

(continued on next page)

Table 2 (*continued*)

General type of photobioreactor	Particular features of system										
	S/V ratio	**Main source of light**	**Agitation system**	**Temperature control**	**Gas exchange**	**Mixing efficiency and gas transfer**	**Shear stress**	**Scalability**	**Advantages**	**Disadvantages**	**Ref.**
Annular bubble column		Solar, artificial and combined			Exchange headspace	Moderate to high (both)					
Taylor Couette column	Low	Artificial	Air bubbling	Possible	Exchange headspace	High (both)	Moderate	Limited	Taylor vortex flow; regular light/dark cycles; low CO_2 input	Foam formation; cell wall attachment	[136,137]
Swirling flow airlift-column	Low	Artificial	Air bubbling	Possible	Exchange headspace	Very high (both)	High	Limited	Reduced light path; improved gas-hold-up and light/dark cycles	Scale-up issues; high risk of shear stress	[135]
Other arrangements											
Torus-type PBR	Low	Artificial	Mechanical impeller	Ambient air blowing or cooling water jacket	N_2 or air injection/ continuous air sparging	Moderate (both)	High	Limited	High liquid flow (swirl motion) Fully automated	Scale-up issues; high risk of shear stress	[138]
Pyramid-PBR	High	Solar, artificial	Airlift	Thermo-isolated materials	Exchange headspace	Possibly moderate (both)	Moderate	Limited	High S/V; fully automated system; potential for high areal productivities, low degree of biofouling	Possible scale-up issues	[206]
Internally illuminated PBR (optical fibers)	Low to medium	Solar, artificial or combined	Centrifugal pump; mechanical impellers; magnetic bars (for stirred-tank)	Exchange coils; circulation thermostated water	Air sparging or degasser unit	Moderate (both)	High	Limited	Reduced light path; accurate control of operational parameters (stirred tank configuration)	Scale-up issues; high risk of biofouling and oxygen build-up; elevated costs of light diffusing apparatus.	[144,148]
Light-diffused PBR (waveguides)	Very high	Solar, artificial	Air bubbling	N/a	Degasser unit Air sparging or HFM	Low (both)	Low to moderate	Very limited	Reduced light path; more effective light penetration	Scale-up issues; high risk of biofouling; elevated costs of light apparatus	[106]
Membrane-based PBR	Low	Artificial	Peristaltic pump or air sparging	Circulation of thermostated water	HFM modules (air diffusion)	Low mixing, high transfer	Low	Limited	Increased gas hold-up; high CO_2 fixation rates; low shear stress	Risk of clogging effect; scale-up issues; elevated costs of PBR operation and membrane apparatus	[163,172,174,207]
Immobilized-PBR systems (biofilm PBR)	High	Solar, artificial	N/a	N/a	Direct (semi-permeable membrane)	N/a	Low	Low to moderate	Thin film; effective light penetration; controlled medium supply; less effective shear	Scale-up issues; risk of cell wash-out and/ or photoinhibition.	[178,179,182,183]
Fall-film based PBR (thin-layer based PBR)	High	Solar, artificial	Peristaltic/ centrifugal pump	Air blow	Exchange headspace	Moderate /low gas transfer	Low to moderate	Moderate	High S/V; thin film (<1 cm); high areal productivities	High land requirements; elevated cost of PBR operation (i.e. pump) and materials	[191–193]
Hybrid-PBR	Medium to high	Solar, artificial	Centrifugal pump, mechanical impellers or air bubbling	Variable – depends on the system (solar reflector)	Dependent on system (i.e. gas sparging)	High (both)	Dependent on system	Moderate	Good A/V ratio; low-cost and durable materials; improved fluid motion and gas transfer	High land requirements; possibly elevated costs of operation.	[197,198]

large scale production is still far away [213].

Novel and unconventional systems of disposable PBRs have appeared recently, urged by the excessive costs associated with cooling of classical PBR configurations. An example is Proviron Co., developed the ProviAPT PBR, and covered by EP Patents 2,039,753 (2009) and 2,203,546 (2011) – an arrangement of multiple vertical 1 cm-thick transparent panels, submerged in a unique water-filled clear polyethylene compartment that acts as temperature buffer and helps diffuse radiant energy [214]. No additional scaffold is required in this case – and the inventors claimed a maximum of 10 $g.L^{-1}$ for biomass concentration.

The disposable-PBR concept also applies to novel floating-PBR systems designed for the ocean or open sea – with improved biomass production, and without extra land or energy demands [42,87].

In general, similar challenges are raised to disposable PBR and classical PBR designs, namely, efficient light supply to the microalga cells [208]. Photolimitation may arise, in particular, due to distortion of the bag; in addition, they are made of more fragile materials, and thus more prone to leakage and subsequent contamination. On the other hand, the indiscriminate use of plastic PBRs may raise serious environmental issues in the near future: owing to their reduced lifespan, disposable PBRs will generate huge amounts of plastic waste, and this will translate into disposal problems – with likely additional costs when scaling up to industrial processing.

4.5.2. *Façade-PBR*

Reducing capital and operational costs, without compromising performance is a major goal in microalga biomass production. This may lead to the opportunity of exploiting culturing systems based on non-typical approaches – with potential applications in bioenergy production, and environmental and bioremediation. One illustrative example is the PBR-façade system, to be integrated in city buildings using live microalga cells; this takes advantage of the high surface-to-volume ratio of incident light on buildings, and of the sufficient light for microalga photosynthetic growth. Notwithstanding the concept of low-cost operation, the idea is not to merely produce biomass or metabolites – but also to improve building shade, thermal conditions, and indoor air quality, besides generating renewable energy along with integration in the façade architecture [215].

Façade-PBRs consist of a large number of interconnected, single flat panel modules – designed in such a way that microalga in the (bioreactor) façades grow faster in bright sunlight, while providing natural internal shading [89]. Buildings normally exhibit surface-to-volume ratio and high illuminated areas, able to provide adequate light for photosynthetic growth. Microalgae will thus be able to produce energy, increase biomass, and ultimately synthetize metabolites of interest – e.g. oils for biofuel production, or added-value compounds. This strategy could reduce capital cost regarding PBR installation, while conveying an opportunity to explore new architectural concepts and providing the building with a lower energy consumption or even effluent generation. Furthermore, gas exchange can be provided between the PBR and the building itself – thus allowing thermal regulation of both, and regulation of nutrient needs, especially elimination of CO_2 waste used as nutrient for the cells. Façade PBR's are expected to produce 25–30 tons biomass per ha per year – corresponding to 40–50 tons of CO_2 fixed per year [41]. This means significant energy savings, and reduction in greenhouse gas emissions to the atmosphere, concomitant with a high-quality way of living.

Masojídek et al. (2003) [216] also developed a similar approach, in which a closed tubular reactor – termed "penthouse roof PBR", was installed with both outdoor and indoor features. Solar light was collected by Fresnel lens concentrators, and mounted on a roof climate-controlled greenhouse. This PBR arrangement turned out to be a functional biomass cultivation system – with sufficient mixing, cooling, and efficient stripping of oxygen that could ultimately, be used to improve the air quality inside the building; a maximum of 2.2 $g.L^{-1}$ biomass of *Arthrospira platensis* was attained – with surplus solar energy used to heat service water.

A PBR-integrated building façade (or roof) solution still faces several technical challenges: efficient integration of infrastructures to supply nutrients, water, light and CO_2, along with microalga harvesting and extraction systems; besides the substantial (initial) investment encompassing PBR construction, and operating costs, further to maintaining the culture in suspension and supplying the necessary nutrients for growth thereof [215].

4.5.3. *Floating-type PBR*

The trend of reducing PBR operation costs, concomitant with the potential of microalga-based cultures be extended to offshore, has led to the innovative concept of floating-PBR systems – for the management of wastewaters, concomitant with bio-oil production; they do not indeed compete with land demands by food supply.

One example is the floating-type PBR, a technology that challenges the most traditional concepts and design. The major illustration on the table is the (proposed) OMEGA Project (Offshore Membrane Enclosures for Growing Algae), funded by NASA – and consisting of a tubular PBR, made of clear and flexible low-density polyethylene (LDP). The transparent solar-collector contains regularly spaced swirl vanes – to create helical flow and mixing within the circulating culture, while improving light harvesting. The LDP material is connected with cam-lock fittings to a U-shaped PVC manifold. The ends of the LDP-light harvesting unit are connected with flexible PVC tubes, on which two pumps, a gas-exhange and a harvesting column are mounted. The system is complemented with an instrumentation control device, to manage/stripe the excessive amounts of dissolved oxygen generated by photosynthesis; supply of CO_2 takes place through a diffuser, and a spot is provided to both add fresh wastewater to the culture and harvest the aggregated microalgae – while suspended microalgae are returned to the PBR.

The above concept was presented primarily toward wastewater treatment, in floating infrastructures located offshore (near wastewater plants); it simultaneously constitutes an opportunity for production of bio-oils by microalgae (for biofuels), or other derived-compounds with commercial interest, aside from mitigation of CO_2 from flue gas plants (near coastal offshore). Furthermore, there is no land requirement that might directly compete with arable land, or disrupt urban infrastructures in the vicinity of wastewater treatment plants. An average microalgae productivity 14.1 ± 1.3 $g.m^2.d^{-1}$ was claimed; supplemental CO_2 was converted to biomass with an efficiency above 50% – while >90% of ammonia-nitrogen was recovered from secondary effluents.

The aforementioned configuration has only been tested within a small-scale unit, namely a seawater tank – so optimization parameters of hydrodynamics, pumping and mixing remains to be done, so that feasibility at large scale can be equated [87].

A related microalga biomass system has been developed in parallel, using floating-PBR made of flexible plastic enclosures with a semi-permeable nature; lightweight, compact bags, bearing a high surface-to-volume ratio, have been tested as enhancers of radiant energy provided by sunlight. Domestic nutrient-rich waters can be used as source of CO_2 to grow microalgae inside this floating PBR-type system in marine environments, but resorting to freshwater microalga species. A principle based on plain reverse osmosis will be in action: a basic gradient will build up between freshwater inside the PBR and saltwater outside – so domestic water will be removed and cleared via a semi-permeable membrane (forward osmosis), and released into the marine environment. Microalga growth will be promoted by nutrient concentration, and biomass harvesting will be facilitated. No external energy for agitation will be needed, and gentle wave motion will be enough; furthermore, cooling requirements will not be an issue, as the surrounding water will help stabilize PBR temperature (https://www.nasa.gov/centers/ames/research/OMEGA/index.html).

However, this is considered an intermittent approach, because the reactors are limited to the frequency of offshore waves; a careful choice of materials will also be critical for this type of PBR – since it will be subjected to adverse weather conditions (e.g. storm, rain), water currents or other potential damaging threats that will menace material integrity. It should be stressed that sea surface covered by this type of PBR is not an unlimited resource, nor can sea be treated as an unlimited dumping ground; such a realization has surely constrained further development of this layout at present.

5. Challenges and opportunities

Most "non-conventional" PBR designs have been proposed in attempts to overcome the major bottlenecks of classical configurations; modifications have indeed improved light conduction, hydrodynamic patterns, mass transfer, and controllability – yet several challenges remain to be addressed.

Light harvest improvements consisted basically of one of the following approaches: increasing photosynthetic efficiency by expanding the S/V ratio, increasing light path or light dilution inside the reactor, of improving features of the light source itself. For instance, PBRs bearing unconventional geometries have been claimed to permit more efficient light collection, due to high S/V ratio and larger illuminated surfaces; α-shaped, V-shaped, vertically-stacked, helical, curved-chamber, pyramid, or even dome-shaped PBRs are indeed promising geometries for improved biomass cultivation. However, scale-up issues are still to be considered especially when interaction takes place, primarily in tubular shapes; oxygen build-up and CO_2 supply can become a hurdle; and when employed outdoors, effective temperature control systems are a must, which will add to cost.

Configurations of PBRs specifically designed to reduce light path (and increase the surface area-to-volume ratio), such as internally illuminated PBRs and light-diffused PBR's, entail promising advances in improving light distribution inside the system – a major problem arising when handling high density microalga cultures. Moreover, optical fibers and light guide devices in column vessels and compact systems are quite difficult to scale-up, in terms of both cost-effectiveness and long-term performance [26,55]. The inherent complexity of the underlying configurations; the poor volumetric productivity – as a major portion of the reactor is occupied by light sources or radiators; and the poor mechanical agitation – owing to static internal illumination systems may lead to microalga adhesion to the wall and to the surface of optical fibers [217], are shortcoming to be addressed in future approaches.

In terms of light enhancement, lighting technologies based on LEDs have gained importance in novel PBRs, and the supporting technology has experienced considerable advances in recent years. Their implementation, especially indoor, appears advantageous, because they are compatible with light supply within only the most suitable wavelength bands – and thus can be tailor-made to each specific microalga species/strain. Furthermore, the associated production of heat is marginal, and such heat can be readily dissipated without damaging the microalgae. As cooling requirement and temperature control are quite expensive in microalga cultures, LEDs offer an opportunity to manage spectral light quality and decrease cooling costs. However, LED-based PBRs are still expensive, although their cost exhibits a declining tendency; hence, economic viability comes along at present only with microalga-mediated production of high-added metabolites. R&D efforts are still deserved to expand fundamental and applied knowledge on PBR optics, including in-situ light generation by specialty polymers.

Enhancing the hydrodynamics, with tailor-made internal flow patterns, and developing effective flashing light effects and light/dark cycles have proven useful to improve microalga biomass productivities. Induction of active mixing has accordingly been found to enhance microalga performance when exposed to intercalated illumination and dark cycles [49,50]. PBR designs focusing on this approach have resorted to bubbling gas or stirring, as in airlift systems, or use of static mixers to promote light and momentum transfer throughout the reactor [26,60–62,114,218,219]. Such geometries as torus-shaped and annular PBRs take advantage of vortex Taylor flow regimes, promoted by rotational inner flows – which hold great advantages toward improved mixing. Despite these advantages, PBRs based on active mixing raise a few problems as to the effects of shear stress upon microalga cell integrity, thus compromising scale-up.

Active mixing can also be attained by pulsated rocking motion – but this configuration is hardly suitable for large scale, due to the elevated cost of equipment needed.

Membrane-based PBR technology has been successful in improving gas-liquid mass exchange; effective CO_2 supply to the culture can indeed be attained through HFM modules, owing to their inherently high k_la values when compared to conventional bubbling systems. However, coincidence of the period of CO_2 supply with the period of light supply is hard to assure, despite constraining balanced photosynthetic metabolism [19]. In addition, use of membranes suffers from serious risks of clogging. The expenditure with membrane apparatuses, operation and maintenance are key-aspects to consider; process scalability will, nevertheless, be quite limited.

Immobilized PBR-systems take advantage of growth of non-suspended cultures, or biofilms that gain from an effective light penetration within cells – and, consequently, from a high photosynthetic performance. Furthermore, cells are concentrated in a small foot-print area using cheap and available materials, which helps reduce building costs. Immobilized PBRs also raise lower water requirements (for the same amount of biomass produced in suspended culture), of the order of 10% those of conventional suspended-based PBR [188]. However, such systems are mostly of a laboratory scale, so scale-up issues are anticipated, because such surfaces cannot be increased indefinitely.

Falling film based-PBRs seem susceptible of improvement, yet those systems are not cost-competitive because of their materials (i.e. glass) – despite the large areal productivities shown. Replacement of such materials by cheaper ones, concomitant with use of alternative carbon sources (i.e. flue gas), as well as combination with paddle-wheel raceways to circulate the culture could contribute to make this reactor economically more feasible in the near future [194].

In hybrid PBRs, the two-stage approach appears to have good future perspectives, in both economic terms and technical feasibility. A recent study on hybrid PBRs unfolded an economically viable strategy, by providing continuous and consistent inoculum for a short period of time (which prevented the biological system from crashing) [220]; the initial expenditures could be slightly high (particular in the case of the enclosed PBR), yet sustained production would overrun them and contribute to economic feasibility at large-scale.

Disposable PBRs have appeared as an opportunity for use of low cost materials (i.e. plastic), along with easy operation. Transparency of many low-cost plastics favors transmittance of light into the microalga cultures – yet photo-limitation may be induced. Less-expensive, yet more fragile materials are more susceptible to leakage and contamination. They can undergo photo-degradation when exposed to UV-radiation (combined with exposure to high temperatures) [221], so plastic optical properties can be compromised and eventually impair regular growth and performance of microalgae. Conversely, the impermeability of many plastics makes them suitable for use outdoors in compartmented chambers, where microalgae can be placed to grow. Regulatory reduction in extent of use of plastic materials, along with the reduced life span of the PBRs constructed therefrom may lead to disposal issues, and thus raise serious environmental concerns.

All in all, development of novel PBR still faces a number of challenges as unconventional configurations for microalga cultivation – especially pertaining to scale-up, in view of the high construction and operational costs incurred in. Investigation/development in this field appears, nevertheless, as a major opportunity to combine both empirical experience and theoretical fundamentals, in attempts to produce economically more feasible and environmentally more sustainable systems.

6. Final remarks

Classical PBR configurations do not entirely respond to the unique requirements of microalga cultivation, and several bottlenecks have been identified concerning light irradiance/penetration, gas transfer, mixing efficiency and cooling requirements – which, as a whole, limit their performance. In recent years, a number of improvements of classical configurations have been tested, namely, changes in geometry and fluid motion pattern, light enhancement, improved gas transfer, and type of construction material; unfortunately, all exhibit advantages along with disadvantages – and major challenges remain to be addressed in full, e.g. scalability, operating costs and complex arrangements. There is not, in fact, a universally ideal PBR – and the best design still depends on the microalga species at stake, and the final metabolite envisaged.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

CRediT authorship contribution statement

Joana Assunção: Conceptualization, Formal analysis, Investigation, Writing - original draft. **F. Xavier Malcata:** Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

The authors hereby declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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