Chapter 3

# Considerations for Photobioreactor Design and Operation for Mass Cultivation of Microalgae

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Additional information is available at the end of the chapter

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**Abstract**

Microalgae have great biotechnological potential for production of substances through photosynthesis. Light capture process and electron transportation imply energy losses due to reflection, fluorescence emission, and energy dissipation as heat, giving a maximum theoretical value of 8-9% for microalgae energy capture efficiency and conversion to biomass. For development of full potential of microalgae the knowledge of the light capture process is required. High yields can only be obtained linking photobioreactor design with biological process taking place inside. In massive microalgae cultures, light gradients are generated and this depends on the biomass concentration, cellular types, cells sizes, and pigment content, and also on geometry, hydrodynamic, and light conditions inside the photobioreactor. In the present chapter we explain the relationship between light energy capture process and photobioreactor design and operation conditions, like turbulence, gas exchange, and nutrient requirements. Finally, the productivity and costs are discussed, and the parameters that determine the economic viability of any microalgae culture.

**Keywords:** photobioreactor design, photosynthesis efficiency, nutrients, mixing, gas exchange

## 1. Introduction

From a biotechnological point of view, the term microalgae refers to unicellular organisms capable of carrying out oxygenic photosynthesis, they contain Chlorophyll *a* (Chl-a) and/or other similar photosynthetic pigments. This definition takes into account a very large and diverse group of photosynthetic prokaryotic and eukaryotic organisms, capable to catalyze

the process of carbon dioxide ($CO_2$) fixation and its conversion into organic matter. The number of species of microalgae has been estimated from 1 to 10 million [1, 2], these organisms are ubiquitous and have managed to colonize almost every habitat known on the planet, from the tropic to the poles.

### 1.1. Advantages of culturing microalgae

Microalgae possess great biotechnological potential for the production of a wide variety of compounds such as polysaccharides, lipids, proteins, carotenoids, and other pigments, vitamins, steroids, among others. Dozens of algal species are used to produce animal feed, human nutrition, cosmetics, and pharmacy industry components. They also find application as wastewater treatment, $CO_2$ fixation, and greenhouse gas emissions reduction. Microalgae can be used to produce biofuels, hydrocarbons, and hydrogen [3–5]. They can be a clean and renewable energy source because of their high yield and low spatial requirements, if compared to terrestrial plants. Some authors consider microalgae as biodiesel feedstocks for the future [4].

The only natural process that allows the production of biomass using only sunlight as the energy source and $CO_2$ is photosynthesis. Unicellular photoautotrophic organisms are capable of using sunlight in a more efficient way than superior plants [6]. The advantages of microalgae culture compared to superior plants are listed [2, 6, 7]:

1. Microalgae biological systems are considered the most efficient for solar light capture, and the production of compounds through the photosynthetic process.
2. Whole microalgae biomass can be harvested and used because they lack complex reproductive organs and have no vascular systems.
3. Many algal species produce and accumulate particular compounds of high commercial value can be induced, for example, proteins, carbohydrates, lipids, and pigments.
4. The isolation, genetic selection, and strain studies is relatively easy and less time consuming because microalgae reproduce themselves by simple cellular division and can fulfill their life cycles in just a few hours or days.
5. Microalgae can be cultivated with low inorganic nutrients concentration. These make them of particular interest as a protein source, assuring protein availability in regions of low agriculture productivity due to the lack of water and nutrient poor soils.
6. Systems for biomass production can be adapted or scaled up to different operation levels, allowing later incorporation of these systems to fully automated facilities for large scale production.

### 1.2. Beginning of mass culture of microalgae

Large scale culture of microalgae began in the early 1960s in Japan with the culture of *Chlorella* [8], this development was followed by the establishments of other facilities, for example in the 1970s it established the culture of *Spirulina* in the Texcoco lake in Mexico city by the Sosa Texcoco Co. [9]. In 1977 Dai Nippon Ink and Chemicals Inc. established a commercial *Spirulina*

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production facility in Thailand and by 1980 there were 46 large scale facilities in Asia that produced more than 1000 Kg of microalgae per month, mainly *Chlorella* [10] and in 1996 near 2000 tons of *Chlorella* were commercialized only in Japan [11]. The commercial production of *Dunaliella salina* as a source of β-carotene was the third microalga of industrial production when Western Biotechnology Ltd. and Betatene Ltd. installed a production facility in Australia in 1986. These were followed by the installation of other production facilities in Israel and in the United States of America [5].

Commercial production success of microalgae in large scale facilities depends on many factors, among which, we can mention the development of large scale culture systems of economic feasibility, and development of these systems has been a gradual process [5].

Productivity of microalgae biomass is affected by factors like photosynthetic pigments efficiency of the in capturing and converting light energy to chemical energy, accumulation of dissolved oxygen produced by photosynthesis, insufficient $CO_2$ mass transfer rate, depletion of nutrients, and photoinhibition [12, 13].

## 2. Light capturing process and photosynthetic efficiency

Autotrophic growth of microalgae depends on photosynthesis, which involves light electromagnetic radiation energy absorption and conversion by photosystems I and II (PSI and PSII) into electrochemical potential and chemical energy (NADPH and ATP). Energy is later used in the $CO_2$ fixation process [14]. Thermodynamic efficiency over the PAR region of systems working with low light regimes (100–300 μmole $m^{-2}$ $s^{-1}$) can be below 5%, decreasing to 2% under large solar irradiance (>500 μmole $m^{-2}$ $s^{-1}$). In addition, under outdoor conditions around 95% of the captured total light spectrum energy is converted into heat [15].

In an ideal case, a photobioreactor (PBR) must capture all light available in the environment and transfer it into the culture to be used for biomass production [16]. Although in normal conditions this does not happen [17] because photosystems are exposed to an amount of light energy below or greater than the amount that can be transformed into chemical energy. If biomass concentration is too low, some of the light is transmitted through the culture. Conversely, if biomass concentration is too high, a dark zone appears. Maximal productivity will require the exact condition of full absorption of all light received but without a dark zone in the culture volume. This is called luminostat regime. But maintaining luminostat regime over the year in outdoor conditions has no interest in practice as it cannot be applied in actual operating conditions due to the disconnect between the dynamics of irradiation conditions (below 1 h) and biomass concentration changes (days) [15].

Under sunlight, biomass growth rate is insufficient to compensate for the rapid changes in sunlight intensity. Consequently, light attenuation conditions that are fixed by biomass concentration are never optimal. This is why determining the maximum photosynthetic efficiency and the upper limits of biomass production through photosynthesis has been a central topic of investigation in mass culture of microalgae [18] since its beginning.

### 2.1. Phothosynthetic efficiency

Only a fraction of the energy of sunlight can be used to build up biomass and derived products. One form of measuring the overall light usage for biomass production is known as photon conversion efficiency (PCE) [19], also called energy conversion efficiency (η) [17, 20]. The conversion of light energy is limited by several factors; some due to the physical nature of light itself and others inherent to the photosynthetic process [19]. First, light must travel from the Sun to the Earth surface, losing one part of the energy just by passing through the atmosphere, for the remaining amount of energy, only the part that has a wavelength between 400-700 nm can be used for photosynthesis because this wavelength can be captured by the photosynthetic pigments and is known as photosynthetic active radiation (PAR).

Solar energy conversion efficiency ($\eta_{solar}$) depends on three factors: 1) Light-Harvesting Yield (LHY), 2) Fractional Energy Yield (FEY) of the redox products of PSII, and 3) quantum yield (QY), therefore $\eta_{solar}$=LHY·FEY·QY. LHY value depends on the coordinated functioning of the anthena complex, which absorbs light energy through dozens or even hundreds of protein bound pigments, and the oxygen liberating complex of PSII. This last requires Mn as cofactor to split the water molecules and liberate electrons and generate an excitation of the Chlorophyll (P680*) in the reaction centre. LHY has a maximum value of 34% under ideal conditions. The FEY depends on the electron transfer chain of the PSII, from P680* to quinone B (QB) and also has a maximum value of approximately 34%, again in ideal conditions. The QY denotes the probability that P680* formation results in product formation along the main path of redox chemistry and has an accepted value of approximately 0.875 [17].

In the light absorption process and the electronic transport chain (FHY and FEY), most of the energy is lost due to reflection, fluorescence emission, and energy dissipation as heat by photosynthetic pigments, this makes that the PCE or η decreases to a value of 12.6%. Also, the conversion of light energy into biomass diminish even more the energetic yield, making the light energy capture and conversion to biomass efficiency of roughly 8-9% [17, 19, 20]. Important is to highlight that this last value is the theoretical maximum accepted for microalgae. Maximum light energy conversion efficiency and its conversion to biomass in higher plants have an actual value of just 5% [17].

### 2.2. Light and culture conditions

Exposure of photosynthetic cells to an excessive amount of light could lead to photoinhibition and to a decrease in the growth rate. On the other hand, the self-shading effect between individual cells presented in mass cultures of microalgae causes a productivity decrease, even when the amount of light is sufficient for the population of microalgae in the PBR [21]. The quality of light, which means the light wavelength that is used in photosynthesis by the microalgae cultures, also affects the culture performance [22, 23]. During batch culture, or where light is constant, cells can experience photoinhibition at the beginning of the culture, and the limitation of light when a high cell concentration is reached [24]. This can be avoided using fed batch cultivation or continuous culture mode. For example, Garcia-Cañedo [25] have reported that photosynthetic efficiency is maximum when nutrients like nitrogen are supplied,

and fed-batch culture mode application promotes a high maximum photosynthetic efficiency, close to the reported maximum theoretical value.

To develop the full potential of photosynthetic organisms, that can be economically feasible and similar to heterotrophic eukaryotic organisms, like yeasts or filamentous fungi, it is required to identify the "bottle neck" of the bioprocess. The growth of microalgae requires appropriate light capture and conversion into biomass, therefore, it required novel PBR designs with geometries not commonly used for heterotrophic organisms in different operation modes that promote higher photosynthetic efficiencies [25, 26].

## 3. Photobioreactor design

The fundamental principle for photobioreactor design is a high surface area to volume ratio in order to use light energy efficiently, and is a requirement to obtain high values of PCE (**Figure 1**). Higher photosynthetic efficiency can result in higher biomass productivity and concentration, but at much higher cost because of high energy use (mixing, cooling, and embodied energy) and capital cost [27]. PBR design must include a short light path, which can be obtained using different geometries and low level of liquid to minimize the energy used for mixing the culture [19]. At high liquid level, the water column could generate high hydrostatic pressure and require higher energy input for mixing of the culture inside the PBR with air injection.

### 3.1. Opens systems: open ponds

Normally, microalgae and cyanobacteria large scale mass cultivation is done in shallow open ponds tanks, of circular or raceway type, with solar light. One of the major advantages of using open systems is that they are easy to build, operate, and they have lower costs than closed systems. Even though it has been demonstrated that open pond culture is economically feasible, they still have some disadvantages and limitations, they use light in a very inefficient manner, have evaporation water loses, low $CO_2$ mass transfer rate from the atmosphere, due to its inefficient mixing mechanisms; open ponds also require a large area of land for the culture due to its shallow depth. Additionally, open systems can be contaminated with predators or fast growing microorganisms like bacteria that can compete with microalgae for nutrients, this is why open systems are only used for organisms that can tolerate extreme conditions [28–30], like high salinity or pH. The scaling up of open ponds culture systems can only be performed by increasing the area, because increasing depth will not increase light penetration leading to lower productivities.

### 3.2. Closed systems: photobioreactors

To overcome the problems detected in open systems it has been proposed the use of closed photobioreactors (PBRs). The former are more appropriate for strains that cannot tolerate extreme environments or when final product is highly susceptible to degradation or contamination. Closed systems also allow the prevention of contamination, allowing the operation in

culture modes like photoautotrophic, heterotrophic, or mixotrophic. Also, closed systems can obtain up to three times more biomass than open systems, thus reducing harvesting costs [31]. Despite the great advances that have been achieved in the construction and operation of PBRs, its technology is still in development. Around 90% of current biomass production worldwide is obtained in open systems, despite the fact that PBR technologies offer greater potential in terms of productivity, control of culture conditions, and applicability to cultivate various strains [15].

Several closed PBR designs that are in operation are in laboratory, pilot plant levels, and even some have been successfully scaled up to an industrial level. One of this successful closed PBR design is the tubular type, in this the tubes configuration where the culture is hold is one of the main factors affecting productivity of photosynthetic biomass [32]. Tubular PBRs can be built with plastic materials like rigid transparent polyvinyl chloride (PVC), polycarbonate or flexible plastic bags, among other materials. They can be arranged in vertical, horizontal, conical, and inclined form, with degasifying units that allow the removal of the $O_2$ produced during photosynthesis [30], the tubes can also be arranged in an annular form [19, 33], each of these forms affect the productivity expected in this type of systems.

Appropriate design of vertical tubular PBRs can reduce the culture area and distribute photosynthetic organisms in vertical columns. Vertical reactors can increase the exposure of the organisms to light, and also the contact time between gas and liquid, thus increasing residence time of $CO_2$ and the efficiency of $CO_2$ assimilation [13, 28, 34]. Vertical columns can be compact, low cost, easy to operate aseptically [35], and very promising for large scale culture. It has been reported that vertical PBRs, vertical columns, and airlift type PBRs of even 0.19 m of diameter can reach a final biomass concentration and a specific growth rate comparable to values reported for PBRs of smaller diameters [30, 35]. The main disadvantages of vertical systems are light reflection and/or incidence of light at the peak hours of the day when the sun is in the summit due to the incidence angle of light on the PBR surface, also vertical form can generate hydrodynamic and shear stress if the height of the PBR is too large.

Horizontally displayed tubular PBRs are considered appropriate for mass cultivation of microalgae because they possess a large illuminated area and have better usage of light at sun summit. Some have been successfully scaled up to a volume of 4000 L or more [36, 37]. Even though this PBR design generate oxygen accumulation, when used outdoors can present photoinhibition [38]. When scaling up these systems, it must also be considered that increasing the diameter of the tubes will decrease the area to volume ratio, and the increase of the length of the tubes could generate $CO_2$ and nutrient gradients and oxygen concentration that could rise up to toxic levels [30, 38]. Formerly described designs are combined in inclined tubular PBRs, which have lower hydrodynamic stress and better illumination because the incidence light angle can be adjusted with the inclination of the PBR, also mixing is better than in horizontal tubular PBRs [38].

Flat panel PBRs have also been studied in order to make an efficient use of light for algal biomass production [39, 40]. These PBRs have a large illumination surface and the advantage of high area to volume ratio, and therefore optimum illumination of the cells and low oxygen concentration can be achieved. There are varieties with flat and curved semicircular bottom.

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In this last form, mixing dead zones is avoided and favour biomass accumulation [41, 42]. Although, it is difficult to achieve efficient biomass productivity per area of land using flat panel PBRs. Factors affecting biomass productivity in this type of reactors are the angle, direction of flat panels, and the number of panels per land unit [16], also their scale up requires the addition of compartments and support materials for the PBR [30]. Generally flat panels are displayed in vertical form but they can also be arranged inclined. Examples of different types of PBRs can be observed in **Figure 1**.

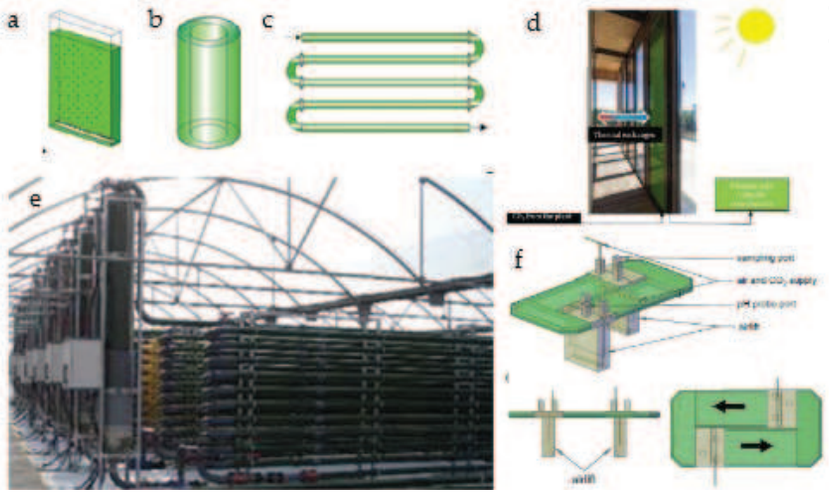


**Figure 1.** Examples of different photobioreactor designs. Basic designs: a) flat panel, b) vertical tubular, c) horizontal tubular [19], d) Flat panel building integrated PBR [15], e) Mass cultivation 28, 000 Liters PBR in operation in Spain [36], f) Floating horizontal PBR [27].

### 3.3. Photobioreactor design considerations: power and cost effective designs

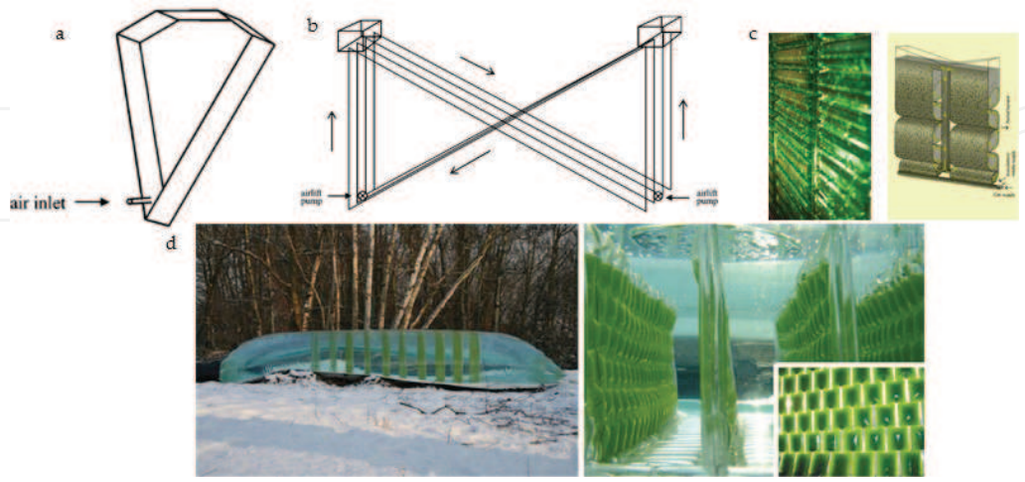


**Figure 2.** Examples of unconventional photobioreactor designs. a) Conic flat panel; b) alfa form; c) Subitec flat panel PBR; d) Proviron plastic bag PBR. Images taken from [44–49], http://subitec.com/en/flat-panel-airlift-fpa-photobioreactor and http://www.proviron.com/node/39.

Unconventional designs have been proposed, which include alveolar type [43], alpha form (**Figure 2a**) [44], flat panel and tubular conic form (**Figure 2b**) [45, 46], and also spiral tubes [47]. When proposing new design forms it must be considered to combine a high productivity with a low need for auxiliary power [19]. A German enterprise called Subitec [48] has developed a flat panel airlift PBR of 180 L (Patents EP 1 169 428 B1 and EP 1 326959 B1) for outdoors culture (**Figure 2c**), that consists of two fine layers of sealed plastic bags, this design includes baffles to generate vortex that enhance turbulence and mixing of the culture inside the reactor, improving light utilization. This company claims that the energy consumption of their PBR is only 200 W $m^{-3}$, consumption verified in pilot plant scale. Company Proviron [49], has developed plastic bag PBR in multiple vertical panels of 1 cm thick, this reactor is displayed unrolling a plastic bag film and does not require additional support; Proviron claims that in their design, it is possible to achieve a biomass concentration of 10 g $L^{-1}$ with a low investment cost of only 20 Euros $m^{-2}$, the most important issue about this design is its low auxiliary power of 2 W $m^{-2}$, which represents approximately half of the maximum value that allows economic feasibility in Central Europe [19].

More recently, buoyant inexpensive plastic film PBRs has been developed. It consists of two plastic films forming the top and bottom surfaces of the horizontal raceway, sealed to each other and connected to two vertical airlift units. This design combines the advantages of open ponds and closed systems in a cost-effective way and can be used on both water and ground, depending on the end user's particular needs [27].

There is significant incentive to design and operate algal PBRs with high biomass productivity and conversion efficiency. Although many factors affect performance of PBRs, such as the type of PBR, culture media, temperature, pH, microorganism used, $CO_2$ mass transfer, $O_2$ accumulation, mixing, light intensity, and light/dark cycles. Among these, the major limiting factors for growth of microalgae are usually light availability and interphase mass transfer [50].

## 4. Turbulence and light capture

Nutritional and light requirements of photosynthetic microorganisms can be covered in high light path PBRs [42], higher than 0.10 m, if the design and operation characteristics are adequate, for example low mixing time and high axial dispersion. Among the advantages of using high light path PBRs are the decrease on construction cost and in energy expenditure, also, it can contain more liquid quantity in less land area.

In dense microalgae cultures, incident light intensity on the PBR surface (Io) decreases with culture depth, and in a certain depth it reaches an intensity equal to the saturation of photosystems (Is), this is why all the light that penetrates further will be used with maximum efficiency [51]. Grobbelaar [20] suggested that in a PBR with dense culture of microalgae exists a light gradient and several illuminated regions designated as follows: 1) Light limited region, is the deepest part of the PBR, 2) Light saturated region, is the region where all the penetrating light can activate photosynthesis and saturation of photosystems is achieved, 3) Photoinhibi-

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tion region, presented in some cases in intense light conditions, and corresponds to the outer part.

### 4.1. Light gradient determination

The exact dimensions of each region depends on the concentration of biomass, geometry, hydrodynamic, and light conditions of the photobioreactor, this will be influenced by cellular size, forms, and pigments content [24, 52]. The light penetration depth in a photobioreactor can be calculated using the modified Evers model [53] (**Figure 3**):

$$PFD(s)=\frac{PFDin}{\int_{0.5\pi}^{1.5\pi}\cos(\theta+\pi)d\theta}\left[\int_{0.5\pi}^{1.5\pi}\cos(\theta+\pi)exp\left[-a_{chl-a}\cdot\left[chl-a\right]\cdot b\right]d\theta\right]$$

$$b=(r-s)\cdot cos\theta+\left[r^2-(r-s)^2\cdot sen\theta^2\right]^{0.5}$$

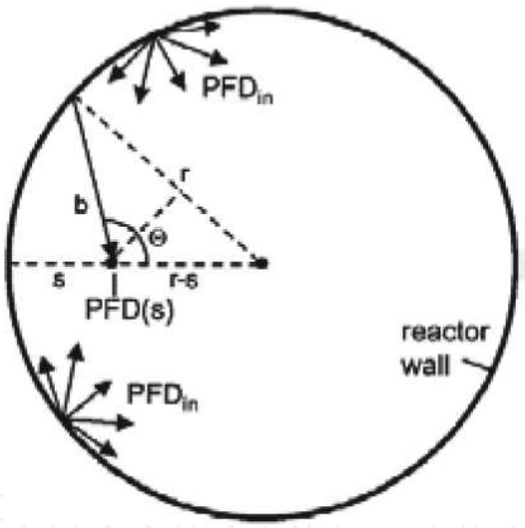


**Figure 3.** Light gradient calculus illustration according to the modified Evers Model [52, 53].

where PFDin is the photon flux density of the incident light intensity on the photobioreactor surface, PFD(s) photon flux density that saturates the photosystems, $a_{chl\text{-}a}$ wavelength dependent Chl-a specific absorption coefficient, Chl-a concentration, r radial distance, and s distance between the PBR surface and the hypothetical point where light saturation is reached at a certain PFD.

From above, it can be deduced that the microalgae inside a PBR will be moving between the three light regions due to the turbulence generated by aeration. Higher aeration rate generates more turbulence, and higher turbulence could generate faster movement of microalgae between the light regions.

If the nutritional requirements of a mass culture of microalgae are met, and the culture conditions do not limit growth, then a design aimed to create a turbulent flow will be the most important requisite to obtain higher biomass yields [54].

### 4.2. Biomass production enhancing: turbulence and mixing effects

Turbulence and mixing of cultures have three main effects: 1) prevent microalgae sedimentation, 2) avoid formation of nutritional $CO_2$ and $O_2$ gradients, and 3) moving cells through light gradients, where the quantity and quality of light received by cells vary [20].

The turbulence can be measured in two forms, one is using the Reynolds number (Re) [20], and the other form is using the Swirl number (Sn) [55]. From the definition of the Swirl number [56], the average turbulence or liquid movement inside a PBR can be calculated using the following expression:

$$Sn_v = \frac{\int_0^L \int\int_S UVrdSdz}{\int_0^L \int\int_S U^2 rdSdz}$$

where U is the mean axial velocity component; V mean circumferential velocity component; r is the radial distance from z-coordinate; and L is the photobioreactor length [47].

The use of Re is recommendable when the liquid properties and change with time are known, for example viscosity, but it is difficult to determine when the geometry of the photobioreactor is "special", this is with less common geometries or with baffles that help to create more turbulence, in those cases the use of the Sn is recommendable.

If a mass culture of microalgae has good mixing, then the cells will be exposed to a light gradient in their movement through the PBR [52]. Near the reactor irradiated surface, algal radiative exposure is usually adequate or in excess, whereas a dark volume with insufficient light for photosynthesis to occur often resides only a few centimeters or less from the irradiated surface, depending on the cell concentration [50].

Normally photosynthetic systems become saturated with light irradiance values of approximately 200 μmol photon $m^{-2}$ $s^{-1}$, this value is equivalent to 10% of the maximum light irradiance in the summer of approximately 2000 μmol photon $m^{-2}$ $s^{-1}$. The saturation of light is considered one of the main limitations of using solar light efficiently [51]. The former has awaken the interest of studying energy light usage with the objective of maximizing photoautotrophic organisms culture productivity [24], through the design of PBRs with geometries that can enhance and make better use of turbulence.

It has been proposed that the control of light irradiance to the culture can be made by giving the necessary amount of light based in cell concentration, aimed to maintain a luminostatic environment inside the PBR. In this sense "specific light uptake rate, qe" has been studied:

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$$q_e = \frac{(E_{in} - E_{out})A}{VxC}$$

where A and V are the surface and volume of the column or columns that contain the culture, C is the cell concentration. $E_{in}$ is the input light energy to the PBR and $E_{out}$ is the outgoing light energy from the PBR, these are quantified calculating the average value of the light intensity measures on 16 points, every $\pi/8$ radians, on the inner and outer surface of the PBR [24].

Although, luminostatic operation is efficient to generate high cell density cultures, it is evident that an amount of light applied to the exterior of a PBR will only be effective if it is combined with good mixing and turbulence (Re ≥ 3300) of the culture inside the PBR. For this reason, a key factor in the design of PBRs is the incorporation of mechanisms to periodically transport or expose cells between light and dark regions of the reactor (mixing-induced light/dark cycles) [50].

One alternative to make better use of light is providing internal illumination with light guides, which can increase the illuminated surface in the same volume of PBR [16] and, therefore, resembles artificial leafs described by Janssen et al. [52]. Solar light is captured by lenses and then transported to the PBR interior with the use of optic fibers [57] or with the usage of light guides of Polymethylmethacrylate (PMMA), which is a plastic that has more light transmittance compared with other plastics, this is why it is considered the ideal material for the construction of light guides. PMMA has a refraction index of 1.49-1.50 for visible light spectrum; much greater than the required 1.415, assuring a total reflection of the light inside the guide when it is surrounded by air, while it limits light reflection in the upper part of the guide [16].

Optical fibers have been previously used to provide internal illumination, better light dispersion, and increase illuminated surface per volume unit of PBR [57–59]. Unfortunately, large quantities of optical fibers are needed to achieve an increase on the illuminated surface to volume ratio in comparison with externally illuminated PBR. Costs and construction considerations for large scale cultivation systems using optical fiber further limited their application [57] because PBRs containing large quantities of optical fibers will not allow achieving good turbulence (Re ≥ 3300) and mixing at a low cost. Multiple light guides of PMMA seem to be more promising. One displayed after the other can increase the illuminated surface in large scale PBR because the effect of self shading inside the PBR, common in flat panel and column systems, will be reduced in internally illuminated systems, therefore, the potential of light capture and usage can be increased, achieving optimal conditions with a more uniform illumination.

Mixing, which governs the movement of the cells between the illuminated and the dark zones, can considerably enhance the productivity for a wide range of operational conditions, as it can create beneficial light fluctuations onto the cells. Mixing induced light/dark (L/D) cycles usually occur at frequencies on the order of 1 Hz or less, which is significantly lower than the minimum frequencies required to produce the flashing light effect (>25 Hz). Nevertheless, it has been demonstrated that photosynthesis can be enhanced by low frequency L/D cycles.

Mixing time decreases with the increase of the superficial gas velocity. Superficial gas velocity not only enhances the mixing and the light–dark cycle of microalgae, but also increases the rate of shear in the reactor, which is harmful. Mixing and the shear stress should be balanced carefully when a suitable superficial gas velocity is adopted. It has been reported that an optimal superficial gas velocity of 8.333 $x10^{-4}$ m $s^{-1}$ for the cultivation of the *Chlorella vulgaris*. Economic analysis has estimated that mixing accounts for 53% of the total costs in some types of PBRs, and one critical challenge to algae biofuel generation was its poor energy balance due to high auxiliary energy requirements for the mixing and the mass transfer [50, 60].

## 5. Gas exchange, carbon dioxide and oxygen

Aeration rate is a key parameter to improve the growth of microalgae cell. Gas supplied to the culture increases the mass transfer coefficient, avoiding deficiency of $CO_2$, control the toxic level of dissolved $O_2$ and the inhibitory level of $CO_2$, reduce nutrients gradient, avoids cell sedimentation, clumping, fouling, and dead zones [31], can create an optimized light/dark cycle that can enhance the photosynthesis. However, excessive aeration may produce cell damage due to mechanical shear forces in susceptible microalgae. Also a high aeration rate will lead to high running costs. A deep knowledge of the fluid dynamics and the mass transfer is needed for the PBR rational design and optimization. It is necessary to understand the interplay among gas holdup, liquid circulation velocity, mixing, and gas–liquid mass transfer [31, 60].

### 5.1. Carbon dioxide mass transfer in photobioreactors

$CO_2$ consumption is proportional to microalgae growth rate; this consumption can be increased by increasing the light irradiation to the culture, but only when light is limiting the photosynthetic process. Because carbon represents approximately half of the dry weight of microalgae biomass, the $CO_2$ demand for cellular growth will be lower than the maximum demand at low light intensities. For example, the maximum demand of $CO_2$ in a flat panel PBR considering a maximum radiation of 1000 μmol photon $m^{-2}$ $s^{-1}$, was a demand of $CO_2$ for photosynthesis that will require a value of $CO_2$ specific mass transfer rate ($K_La$ $CO_2$) of only 4-6 $h^{-1}$ (0.0011-0.0016 $s^{-1}$), due to the growth rate of microalgae [61]. Therefore, if the gas contains a low concentration of $CO_2$, it will require a high $K_La$ to satisfy the $CO_2$ demand during microalgae growth [61]. The former $K_La$ values are very low and most of the designed PBRs under normal operation conditions present a $K_La$ of $CO_2$ from 10 to 100 times superior to this requirement [30], because $CO_2$ can be added to the air supplied to the PBR.

Even though from the economic point of view, a high aeration rate will increase costs, it is not recommendable for large scale production PBRs. It is necessary to establish a minimum aeration rate for each culture conditions [61]. An aeration rate of 0.05 vvm is appropriate for cell production, and is recommended for an efficient PBR [60].

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### 5.2. Avoiding $O_2$ deleterious effects

Considering a carbon fraction in the biomass of 0.45 and $CO_2$ as the only carbon source in the medium, it must be provided a minimum of 1.65 g of $CO_2$ to generate 1 g of microalgae biomass [19]. Additionally, to this stoichiometric aspect, the competitive inhibition of $O_2$ and $CO_2$ for the active site of the Rubisco enzyme (Ribulose-1, 5-diphosphate carboxylase oxygenase) must be considered. This is why $O_2$ removal from the medium is very important. $O_2$ can accumulate to levels that can be toxic to microalgae. Oxygen concentrations above 35 mg $L^{-1}$ are toxic to most of the microalgae species [31]. In this sense, dissolved $O_2$ concentration of 100% is equal to 8.6 mg $L^{-1}$, this means that dissolved $O_2$ concentrations above 300% of air saturation can be detrimental to algal cells and therefore could reduce productivity [27, 62].

In closed systems like, for example, horizontal tubular PBRs of 5 cm of internal diameter with exponential growth of *Spirulina,* the oxygen concentration can increase up to 70-80 mg $L^{-1}$, even when there is ventilation every 50 seconds [51]. Oxygen build up, generated by photosynthesis, is a particular serious problem in closed PBRs with high area to volume ratio [31] and can generate a decrease of the biomass productivity of up to 33%, this imposes strong limitation to tubes length in tubular PBRs and scale up of PBRs [51].

To overcome this problem, some authors have proposed the use of degasifiers [63–66] (**Figure 4**), even though, to achieve an efficient gas separation from the liquid, the distance between the entrance and exit to the degasifier unit must be of a magnitude that allows the smallest gas bubbles enough time to separate from the liquid [31]. Another possible solution is the use of low altitude vertical PBR like for example flat panel PBRs [41, 42] or alveolar systems that allows more contact between the liquid and the air [67].

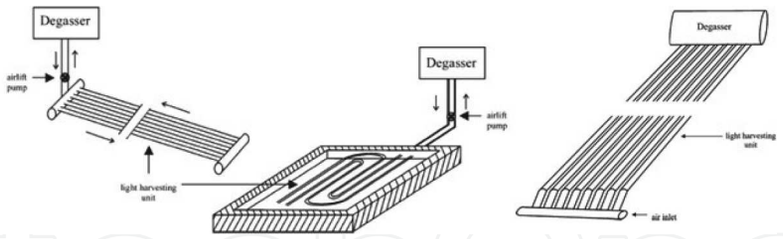


**Figure 4.** Degasifiers units coupled to horizontal PBRs designs [31].

### 5.3. $CO_2$ utilization efficiency

Another aspect that must be considered is $CO_2$ utilization efficiency. Daily fixated $CO_2$ as g $CO_2$ fixated per $g^{-1}$ of injected $CO_2$ $d^{-1}$ can be calculated with the formula FD = $(FA_{(t+1)} - FA_t)$*$mid^{-1}$, where $FA_{(t+1)}$ is the accumulation of $CO_2$ fixed during t + 1(d), $FA_t$ the accumulation of $CO_2$ during t(d) and mid(g) the mass in grams of $CO_2$ injected each day. It has been demonstrated that is possible to fixate even 80% of the introduced $CO_2$ (FDmax%) in an air current with 0.04% of $CO_2$ using vertical tubular PBRs connected in serial three stage manner, and up to 40% of the introduced $CO_2$ in an air current with 12% of $CO_2$ [28]. Therefore a high aeration rate results in low $CO_2$ utilization efficiency, indicating that it could be highly

expensive in an industrial facility to use $CO_2$ supplementation. Aeration conditions must be optimized considering biomass productivity and supplemented $CO_2$ utilization efficiency.

Despite all the presented alternatives are very ingenious, at this moment there is no universal unit to achieve an optimal degasification, the selected choice will depend on the cultured microalga and the preselected objective of this culture (for example, biomass, pigments, $H_2$ production, etc.).

## 6. Nutrients

Nutrient supply like nitrogen and phosphate is another factor of special interest. The dynamics of these nutrients are strongly coupled to each other, and to the metabolic processes present in the PBRs. Exploring the fate of nitrogen and other nutrients through the different biological pathways during cultivation in PBRs is a valuable tool for designing such systems for full scale with an ever growing demand for more efficient nutrient removal systems. For this, knowing the true metabolism of nutrients in PBRs and its effects on algae growth is vital [62]. For example, in nitrogen-deprived culture of *Haematococcus pluvialis* the photosynthetic electron transport chain is heavily damaged due to the significant reduction of cytochrome b6/f complex [68]. To prevent cells being overreduced by photosynthesis, a correct amount of nitrogen needs to be supplied.

### 6.1. Culture media development considerations

Nutrients are needed to generate biomass and high productivities. Concentration of macronutrients in the medium has a wide range and micronutrients have a narrower range [20]. Minimum requirements for medium composition are obtained from elemental mass balance [19]. Requirements for the development of culture media are enlisted as follows:

- The total salt content is determined by the habitat where the microalga was isolated.
- First, the composition of the major ionic components must be considered.
- Nitrogen sources are mainly nitrate, ammonium, and urea.
- The carbon source could be $CO_2$, $HCO_3^-$ or organic carbon, like acetate or glucose.
- pH is generally required above 7 for maximum specific growth rate.
- Trace elements and chelated components, and vitamin requirements are considered last [69].

Medium nutrients can be manipulated in order to obtain a different responses from microalgae, for example, nutritional stress can be a strategy for the production of specific compounds like carotenoids and fatty acids that are produced when there is a nitrogen deficiency in the medium [25, 70, 71], low nitrogen conditions promotes the synthesis of these compounds while the synthesis of proteins and nucleic acids is inhibited.

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Also, it must be considered that the fact that some microalgae have the capacity of consuming nutrient in an excessive manner and store them, a phenomena known as "luxury uptake" [20], this type of consumption presents when cells have been exposed to a medium with low concentration of certain nutrients ("starvation") or when cells have the capacity of accumulating nutrients, in this last case a previous starvation is not required [72, 73]. It is important to highlight that luxury uptake is desirable in a strain used in waste water treatment, because it allows the removal of nutrients or contaminants from waste water without generating large quantities of biomass as a secondary product.

### 6.2. Importance of nutrient in the photosynthetic process

All organisms have minimum optimal and maximum nutrient requirements, and the nutrient level in the media affects the growth rate [20], media usually used on laboratories generally are not suitable to obtain high biomass concentration because high concentration of their components can inhibit growth or precipitate [19]. Fed-batch cultivation mode can be used to maintain adequate nutrient concentrations in the media, even though, very few works have been published in this theme [25, 70–73] and most of these works have been focussed on the fed of organic carbon source [74, 75], but heterotrophic cultivation of microalgae using an organic carbon source is not suitable for all species, also some strains often change their chemical composition under heterotrophic culture [5].

It is important to mention that microalgae productivity can be dependent on nutrients like nitrogen, phosphorous, magnesium, iron, and manganese because these are required during the photosynthethic process. Nitrogen is found in the form of proteins that form antenna complexes (LHC), reaction centers, and the enzymes that participate in the photosynthesis process. Phosphorous is required in phosphate form to store captured light energy as chemical energy in the form of NADPH and ATP. The magnesium in the porphyrin ring of the Chlorophyll molecule, iron is part of the ferredoxin molecule, this later is an electron transporter of the PSI, and last manganese is important because it acts as a cofactor in the oxygen liberator complex, this has the function of liberating electrons from the water molecule [76, 77].

Beside nutrients, there are other factors that can be manipulated in a PBR during its operation like the pH and temperature, but because each of these factors deserves its own review, they are not part of the discussion on the present chapter.

## 7. Productivity and costs

### 7.1. Maximum biomass productivity

In PBR design it is important to define the upper limits of light capture efficiency. Maximum biomass productivity has been determined to be 14.31 g dry biomass $m^{-2}$ $d^{-1}$, considering an average solar irradiation of 1104 μmol photons $m^{-2}$ $s^{-1}$. But some regions in the world possess

a higher solar irradiation, for example in some regions of the United States of America and Mexico, maximum solar irradiation ranges between 1450 and 2300 μmol photon $m^{-2}$ $s^{-1}$, this can be equal to productivities as high as 29.81 g dry biomass $m^{-2}$ $d^{-1}$ [20, 78]. Main objective in the industrial-scale deployment of this new technology today is to decrease PBR costs without compromising system performances [15].

### 7.2. Biomass productivity in different photobioreactors

Reported biomass productivities per unit of land area for different PBR are limited by the sub-optimal conditions of light inside the PBR, this limits the biological photosynthetic efficiency, or even the design is the limiting factor that affects light usage inside of it. Therefore, high yields can only be obtained linking PBR design with the biological process occurring within. PBR efficiency is determined by factors like capture, transportation, distribution, and use of light energy [16], and also by the overall use of other main nutrients like carbon, nitrogen, phosphorous, magnesium, and manganese.

It has been reported for an outdoor cultivation in a facade PBR run on a whole-year basis, an expected yearly production of around 25–30 tons biomass per ha with *Chlorella vulgaris* (i.e. average daily productivity of 7.68 g $m^{-2}$ $day^{-1}$), which corresponds to around 40–50 tons of $CO_2$ fixed per year per ha [15].

A cascade type open culture system with high cellular densities has been developed since 1970 and is still in use for *Chlorella* cultivation in the city of Trebon, Czech Republic. It can reach cellular concentrations as high as 10 g $L^{-1}$, with high growth rates and biomass productivity of even 25 g dry biomass $m^{-2}$ $d^{-1}$. Despite its high yields, the implemented system at Trebon is very expensive because of the material (glass) used to give the PBR slope, but using other materials a similar system can be built at a significant lower cost. Also, in the geographic location of Trebon, it is only possible to use this PBR during a short period of time during the year because of climate conditions [79].

For economic feasibility of microalgal biorefinery, every cell components of microalgae need to be utilized as much as possible. Continuous or semi-continuous mode of cultivation for a long period helps to improve microalgae cultivation as commercially successful [15].

A semicontinuous system was used to produce *Chlorella* in a lagoon build up with plastic, near the city of Dongara located in Western Australia. This system had an average productivity near to 25 g dry biomass $m^{-2}$ $d^{-1}$, because of the optimal climate conditions. Unfortunately, technical problems in its scale up led to the closure of this facility [5].

A narrow light path from 1.2-12.5 cm allows reaching cellular concentrations of up to 20 g $L^{-1}$ and a volumetric biomass productivity of 0.25-3.64 g $L^{-1}$ $d^{-1}$ in outdoors cultures operated in fed batch mode. Ironically, the biomass productivity per area of land unit in a PBR displayed horizontally was 25.0-27.8 g $m^{-2}$ $d^{-1}$ [63, 80–8282] and was not superior to the reported for an open pond system, this last with a productivity of 25 g $m^{-2}$ $d^{-1}$ [79]. The same had been observed in alveolar type vertical panels [67]. Volumetric productivity in an inclined PBR, with a light

path of 1.2-1.3 cm, was only 1.5-1.7 times superior to the one obtained in open cultures of 1 cm of depth [82].

### 7.3. Economical experiences in previous designs

Based on cost of materials and manufacturing labour extrapolations it has been estimated the capital cost of wall PBR at full production to be $25,000 per hectare. Open ponds ranges approximately from about $10,000 to almost $79,000 per hectare taking into account the costs of the liner and the paddlewheel [27].

Raceway type open ponds are used in Israel, United States of America, China, and other countries. It has been reported that this type of PBR can maintain a cellular concentration of 0.5 g $L^{-1}$ and a productivity of 25 g $m^{-2}$ $d^{-1}$ [63]. Despite their low construction and operation costs, the average cost of these systems is $8-15 U.S. dollars per Kg of dry biomass [82]. The system that is more widely used in large scale facilities (near 1000 L) are the ones that use sterile plastic bags near to 0.5 m of diameter, adapted with an aeration system. These systems require intensive labour and generally present poor mixing. This makes very expensive to produce microalgae biomass. The costs are nearly $50 U.S. dollars per Kg of dry microalgae biomass, for smaller cultures the costs can raise up to $300 and even to $600 U.S. dollars per Kg. Costs are very high and superior to the estimated for the production of *Chlorella*, *Spirulina,* and *Dunaliella*, which are between $9 and $25 dollars per Kg [5].

Productivities and cost are important questions for an algal industry whose economic survival depends on production, and vary accordingly to cultivation methodology. From the perspective of scaling between laboratory to large-sized outdoor facilities, differences might arise in products or co-products expected if cultivation methods are not the same [25, 83].

## 8. Conclusions

Microalgae autotrophic growth is first limited by the photosynthethic process itself, and by the process of light energy captures and $CO_2$ conversion into biomass. This is why PBR design must consider this biological process and focus on providing light, $CO_2$, and other nutrients at a low cost.

Microalgae mass cultures can only be achieved with PBRs designs aimed to improve photosynthetic efficiency in light capture, maintaining at the same time adequate turbulence conditions that can promote cells movement through the different illuminated regions, a high mass transfer rate and high usage efficiency of supplied $CO_2$, allowing efficient $O_2$ removal produced by photosynthesis, and avoiding the generation of nutrients gradients; besides supply adequate nutrient quantities in the moment they are required, in order to improve their use by the culture. This can be done using PBRs with a long light path operating in fed-batch cultivation mode, if the design and operation characteristics are adequate.

Therefore, current designs of PBR still can be improved with the objective of lowering costs, increase efficiencies, and maintain high productivities. New PBR materials and different

culture modes need to be investigated and evaluated because responses of specific strains cannot be inferred from other PBRs or culture conditions. Future investigations must consider microalgae as systems and aim to evaluate interactions between photosynthetic efficiency, $CO_2$ and nutrient assimilation under different culture modes and operation conditions.

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### Abbreviations

| | |
|---|---|
| $CO_2$ | carbon dioxide |
| PSI | photosystem I |
| PSII | photosystem II |
| NADPH | reduced nicotinamide adenine dinucleotide phosphate |
| ATP | adenosine-5'-triphosphate |
| PAR | photosynthetic active radiation |
| PCE | photon conversion efficiency |
| $\eta$ | energy conversion efficiency |
| $\eta_{solar}$ | solar energy conversion efficiency |
| LHY | light harvesting yield |
| FEY | fractional energy yield of the PSII redox products |
| QY | quantum yield |
| P680* | excited PSII reaction center |
| $Q_B$ | quinone B |
| PBRs | photobioreactors |
| Io | incident light intensity on the photobioreactor surface |
| Is | photosystems saturation light intensity |
| PFDin | photon flux density incident light intensity on the photobioreactor surface |

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| | |
|---|---|
| PFD(s) | photon flux density that saturates the photosystems |
| $a_{chl-a}$ | wavelength dependent Chlorophyll a specific absorption coefficient |
| [Chl-a] | Chlorophyll a concentration |
| r | Photobioreactor radial distance |
| s | Distance between the photobioreactor surface and the hypothetical point where light saturation is reached at a certain PFD |
| Re | Reynolds number |
| Sn | Swirl number |
| U | Mean axial velocity component |
| V | Mean circumferential velocity |
| L | Photobioreactor length |
| S | cross-sectional surface area |
| dS | differential cross-sectional surface area |
| Fv/Fm | Maximum photosynthetic efficiency of dark adapted cells |
| Fq'/Fm' | Operational photosynthetic efficiency of light adapted cells |
| $q_e$ | specific light uptake rate |
| A | photobioreactor superficial area |
| V | photobioreactor operational volume |
| C | Cellular concentration |
| $E_{in}$ | input light energy to the photobioreactor |
| $E_{out}$ | outgoing light energy of the photobioreactor |
| PMMA | polymethyl methacrylate |
| FD | daily $CO_2$ fixation in g $CO_2$ fixed per g $CO_2$ injected per day |
| $FA_{(t+1)}$ | accumulation of $CO_2$ fixed during t + 1 (d) |
| $FA_t$ | accumulation of $CO_2$ during t (d) |

mid mass in grams of $CO_2$ injected each day (g).

FDmax % overall maximum percentage daily $CO_2$ fixation

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