

## REVIEW

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## A review on photobioreactor design and modelling for microalgae production

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A microorganism culture process is a complex system in which physical operating parameters and biological responses strongly interact. Mathematical formulation and modelling of the different phenomena involved in the process enable a better understanding of the behaviour of the process, and therefore enable the process parameters to be defined accordingly. The contribution of a model, even a simple one, is highly beneficial to the understanding of the process. The definition of a model for the particular case of photobioreactors is not easy, however, and requires the integration of multiple and often complex knowledge. This article reports a review on the biological aspects of the photosynthetic microorganisms culture necessary to model kinetic growths, the designs of photobioreactor used for deep analysis of the physiologic aspects of the microalgae culture and for the industrial culture. The different approaches to model the kinetic growth are described together with the modelling of the radiative field and its coupling to a simple biological model in order to illustrate the particular influence of the light factor, which is the main specific feature of photobioreactors.

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## Introduction

Photosynthetic microorganisms, through cyanobacteria, are at the origin of the oxygen contained in the atmosphere. These microorganisms have also been used since thousands of years, to feed populations thanks to their nutritional quality. The interest in photosynthetic microorganisms has only been increasing, in particular due to the diversity of existing species and metabolites of interest they contain. The composition of microalgae is of interest in many fields, such as human and animal food, cosmetics, health, or energy.<sup>1,2</sup> Many researches are turning to so-called third-generation fuels from photosynthetic microorganism biomass (microalgae and cyanobacteria, *etc.*).<sup>3</sup> This possible valorisation is in addition to the many ways of use microalgal biomass. Agri-food applications (dietary supplements for food or feed),<sup>4</sup> environmental (water pollution and smoke control, *etc.* ...), medical (dietary deficiencies, anti-cancer drugs)<sup>5–8</sup> can thus be cited. Compared to other plant resources, the exploitation of photosynthetic microorganisms has several advantages: their high growth rate gives access to higher yields than the terrestrial plants and their biodiversity combined with their ability to orientate their metabolism to promote the synthesis of a compound, by imposing specific growing conditions offer

a wide range of applications. Large-scale cultivation of microalgae is nowadays mainly reserved for certain species, known as extremophilic, and is mainly carried out in open ponds. However, research into the production of microalgae in closed systems is still in progress. This is particularly the case in recent years, especially due to the increasing scarcity of fossil fuels, to produce third-generation biofuel from microalgal biomass. This application of microalgae certainly appears to be the most important today, given the objectives and constraints that this imposes. There is a strong need for research, particularly on closed cultivation systems. Indeed, these culture systems equipped with instrumentation allow the maintenance of optimal conditions for growth (reduction of the risks of contamination, control of physico-chemical parameters such as pH, temperature, *etc.* ...). These systems called photobioreactors (PBRs) thus make it possible to convert energy biomass light *via* the photosynthesis mechanism more efficiently than open systems. Biomass composition, growth rate, and metabolites depends strongly on the strain and on the cultivation conditions. Important factors are light intensity, composition of the medium, temperature, pH, carbon dioxide. The complex interactions between the different parameters affecting the biomass growth could be investigated through the development of models which are able to predict the biomass productivity. There are several ways to grow photosynthetic microorganisms, depending on the desired application. Two modes of culture can be operated: autotrophy and heterotrophy. Autotrophy consists of the addition of an inorganic carbon source while heterotrophy uses an organic

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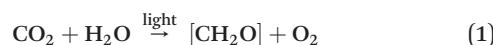
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carbon source. Mixotrophy is obtained when the two sources are mixed. In this article, only autotrophic cultures are considered. Two main families of culture systems can be distinguished: open reactors and closed reactors (PBR). Although expensive due to their high level of technology, the latter allow total control of the reactor and growing conditions and give access to performances far superior to those of the obtained in extensive cultivation systems (raceway type). This is mainly due to that PBRs allow a certain control level of operating conditions obtaining reproducible conditions for the culture<sup>9</sup> and all nutritional limitations can be deleted with the exception of light. The growth rate of the microorganisms is then controlled solely by the thermodynamic efficiency of photon use,<sup>10</sup> corresponding to photolimited growth. This mode of cultivation makes it possible to obtain performances close to maximum productivity on the surface (mass of biomass produced per unit area). For a reduction of the impacts of biomass production, several ways are being studied, such the recycling of industrial flue gases as a source of carbon, the recycling of the culture media to limit water consumption, the use of waste water as a basis for culture media with in addition environmental benefits. In fact, the CO<sub>2</sub> from industrial plants can be fixed for mitigation of the greenhouse gas emission as well as nitrogen or phosphorus removal in wastewater treatment processes.<sup>11</sup> The majority of the culture systems are built in a small scale medium (maximum a few ha) for a total worldwide biomass production around 30 000–50 000 tonnes of dry matter per year. This production is obviously not enough to meet the demand for biofuel, an insufficient quantity for the real worldwide demand.<sup>12,13</sup> Moreover, the production costs of biofuels from microalgae are too high to compete in energy markets. To further reduce the production cost of the system, it is necessary to employ the use of advanced control strategies to ensure an optimization of the system is a way to reduce the production cost.<sup>14</sup> To control the pH of the culture and reducing significantly the cost and the CO<sub>2</sub> losses of the system, techniques based on model predictive control have obtained successful results in this target.<sup>15–17</sup> It is necessary to take into account all the system variables to have a cost reduction by a good use of the resources. For example, Ifrim *et al.* (2013)<sup>14</sup> developed a nonlinear multivariable controller based on dynamical model with an exact feedback linearization to control biomass concentration and pH by acting on the dilution rate and the injected carbon dioxide gas flow rate. Depending on the control complexity, different types of system models are developed. Fernández *et al.* (2014)<sup>18</sup> has developed a dynamic model for microalgae production in tubular photobioreactor for the prediction of the main parameters influencing the microalgae growth rate: temperature, pH, dissolved oxygen and biomass concentration. Calibration and validation tests have been made in an outdoor tubular photobioreactor. A review on the use of Computational Fluid Dynamics (CFD) for the optimization of bioreactor design and for the study of the interaction of hydrodynamics, light supply, heat and mass transfer with biological kinetics has been done by Pires *et al.* (2017).<sup>19</sup>

In the first part, a reminder of photosynthesis and the associated production of microalgae is given, as well as the factors limiting their growth. Then, some examples of culture system design are described, with focus on laboratory culture systems, which are necessary to study the behaviour of microalgae and to get information on growth kinetics, on open, closed and developing systems. The last part concerns the different modelling approaches.

## Photosynthesis and production of microalgae

The term “microalgae” includes, strictly speaking, any microscopic algae. This includes microalgae (eukaryotes) as well as prokaryotic organisms (cyanobacteria) and photosynthetic bacteria. Three modes of culture are to be distinguished: autotrophy, which consists in the feeding of an inorganic carbon source, heterotrophy, an organic carbon source, and mixotrophy, when inorganic and organic sources are mixing. In this article only autotrophic cultures, then cultivation by photosynthesis, are considered. Photosynthesis is described by the following equation:



This process converts light energy into chemical energy (ATP and NADPH) which is then used by cells to synthesize organic carbon from inorganic carbon (CO<sub>2</sub> or other dissolved inorganic carbon). This conversion is made possible by the succession of two types of reactions: (i) light (or photochemical) reactions and dark (or biochemical) reactions. The light reactions take place in the thylakoids. Thylakoid membranes have photosynthetic pigments grouped into photosystems. Two photosystems called PSI and PSII absorb light at slightly different wavelengths. Incident photons having a wavelength within a domain of radiation, called photosynthetically active radiation (PAR, 400 nm < λ < 700 nm) are picked up by a photon collecting antenna, named here light harvesting complex II (LHCII), composed of pigments (chlorophyll a and b and protective carotenoids (PPC, photo-protective carotenoids)). The energy of photons having an energy equivalent higher than that of a photon emitted at 680 nm or 700 nm ( $E = hc/\lambda$ ) is degraded to the same energy level accepted by the corresponding photosystem. Excess energy is dissipated in the form of heat or fluorescence by the collector antenna. The energy equivalent to one photon emitted at 680 nm (PSII) or 700 nm (PSI) is then transmitted to the reaction centers (primary electron donors), called P680 and P700 by proteins D1 and D2. This contribution of energy at the reaction center will change it from a P680 state to an excited state noted P680\*, thus releasing an electron. The latter will be transferred by the plastoquinone (PQ) which will take two protons available in the stroma (inside the chloroplast) to switch to its oxidized state PQH<sub>2</sub>. The electrons recovered by the PQ are removed from the water by oxidation (photolysis of water). This

reaction takes place at the level of the tyrosine Z complex and allows the release of 4 protons, 4 electrons and 1 oxygen molecule per molecule of oxidized water. The protons are released in the lumen (inside the thylacoid). The PQ transmits its two electrons to the cytochrome b6f and rejects two protons into the lumen. The cytochrome b6f also pumps two protons from the stroma into the lumen, thus contributing to accentuate the proton gradient between the inside (highly charged proton) and the outside of the thylacoid (weakly charged). The two electrons are then transferred to a second electron carrier, the plastocyanine (PC). The PC will transmit one electron at a time to the P700 (PSI). The P700 is similar to P680, except that it operates at an energy equivalent of one photon at 700 nm. The P700 will accumulate two electrons, which will take it to its excited state P700\*. He will then give up his electrons to ferredoxin (Fd). The ferredoxin transfers its electrons to the enzyme ferredoxin NADP reductase (FNR) which will allow the reduction of  $\text{NADP}^+$  to  $\text{NADPH}, \text{H}^+$ , which induces the pumping of an additional proton from the stroma to the lumen. The proton gradient generated by the protolysis of the water is used as a proton-motor force for the synthesis of ATP by the enzyme ATP synthase at from ADP and inorganic phosphate. There are two electron transfer paths: an acyclic pathway, leading to the synthesis of ATP and  $\text{NADPH}, \text{H}^+$  by photophosphorylation (Z-schema), and a second, called cyclic electron transfer. This is a direct transfer of an electron from ferredoxin to PQ. This results in the pumping of protons by the cytochrome, which increases the proton gradient and promotes the production of ATP, without producing of  $\text{NADPH}, \text{H}^+$ . The specific production rates of ATP and  $\text{NADPH}, \text{H}^+$  directly affect the metabolism and growth of a photosynthetic microorganism. The ratio of these two terms (defined as the  $P/2e^-$  by Cornet *et al.* (1998)<sup>20</sup>) will reflect the adjustment of the cells energy metabolism. Similarly, the phthosynthetic quotient, QP, is denoted, as the ratio between the production rate of one mole of oxygen and the consumption rate of one mole of carbon dioxide. Similarly, the ratio  $QR_{\text{O}_2}$  (quantum requirement for oxygen production) denotes the number of photons necessary to produce one mole of oxygen. Theoretically this value is eight photons per oxygen molecule.<sup>21</sup> The ATP and  $\text{NADPH}, \text{H}^+$  molecules will then be used for Calvin cycle (dark reactions). The dark reactions are related to carbon fixation take place through a cycle called Calvin cycle. This cycle takes place in the stroma and uses the energy stored during the light phase in the form of ATP and  $\text{NADPH}, \text{H}^+$  to fix inorganic carbon and synthesize organic carbon. The first phase is the carbon fixation by the synthesis of 3-phosphoglycerate (3PGA) from  $\text{CO}_2$  and ribulose-1,5-biphosphate (RuBP), a reaction triggered by an enzyme, RuBisCO. The second phase is the reduction phase, during which each 3PGA molecule receives an additional phosphate from ATP (synthesized in clear phase), then an electron pair released by the  $\text{NADPH}, \text{H}^+$  molecule reduces the 1,3-biphosphoglycerate molecule to D-glycéraldéhyde-3-phosphate (G3P), releasing a phosphate

group. The G3P molecule is a three-carbon sugar. For every twelve moles of G3P synthesized, only two are transported to the metabolic pathway of sugars, the remainder being directed to the third phase of the cycle, the regeneration of RuBP. This last phase completes the Calvin cycle by synthesizing RuBP molecules from G3P under the action of ATP to regenerate the first  $\text{CO}_2$  acceptor.

The efficiency of photosynthesis is directly related to the amount at the photon rate received at a point. This rate is called irradiance ( $G$ ) and its unit is the micromole of photons absorbed per square meter of surface area per second ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Three behaviours are distinguished: (i) a photolimited regime, in which photosynthesis increases with irradiance, (ii) a second, so-called photosaturated regime, in which photosynthesis is independent of the light received, and (iii) a photoinhibition regime in which the efficiency of photosynthesis decreases with the increase in irradiance received by the microorganism. It should be added to this that below a certain irradiance value, called compensatory irradiance, and characterized by a zero oxygen balance at the cell level, the phenomenon of respiration is predominant over photosynthesis. This is characterised by negative biomass production (consumption of carbon reserves).

## Factors limiting growth of microalgae

Many parameters can affect the productivity of a photobioreactor. Biological parameters such as bacterial and fungal contamination, predation by protozoa, or even sometimes contamination by another microalgae than the desired one.<sup>22</sup> Several physico-chemical parameters are also influential: light energy supply, temperature, pH, salinity and the supply of nutrients necessary for growth (including inorganic carbon). The homogeneity of temperature, pH and salinity, as well as the nutrient accessibility to microalgae and light access in the system are controlled by the hydrodynamics of the system, making it a key parameter for the optimization of PBR production. The dynamics of operation under solar conditions brings an additional particularity, a notion of instability over time, which does not allow the system to operate at the optimum throughout the day. This applies in particular to light, or even temperature and pH depending on the control conditions of the culture system.

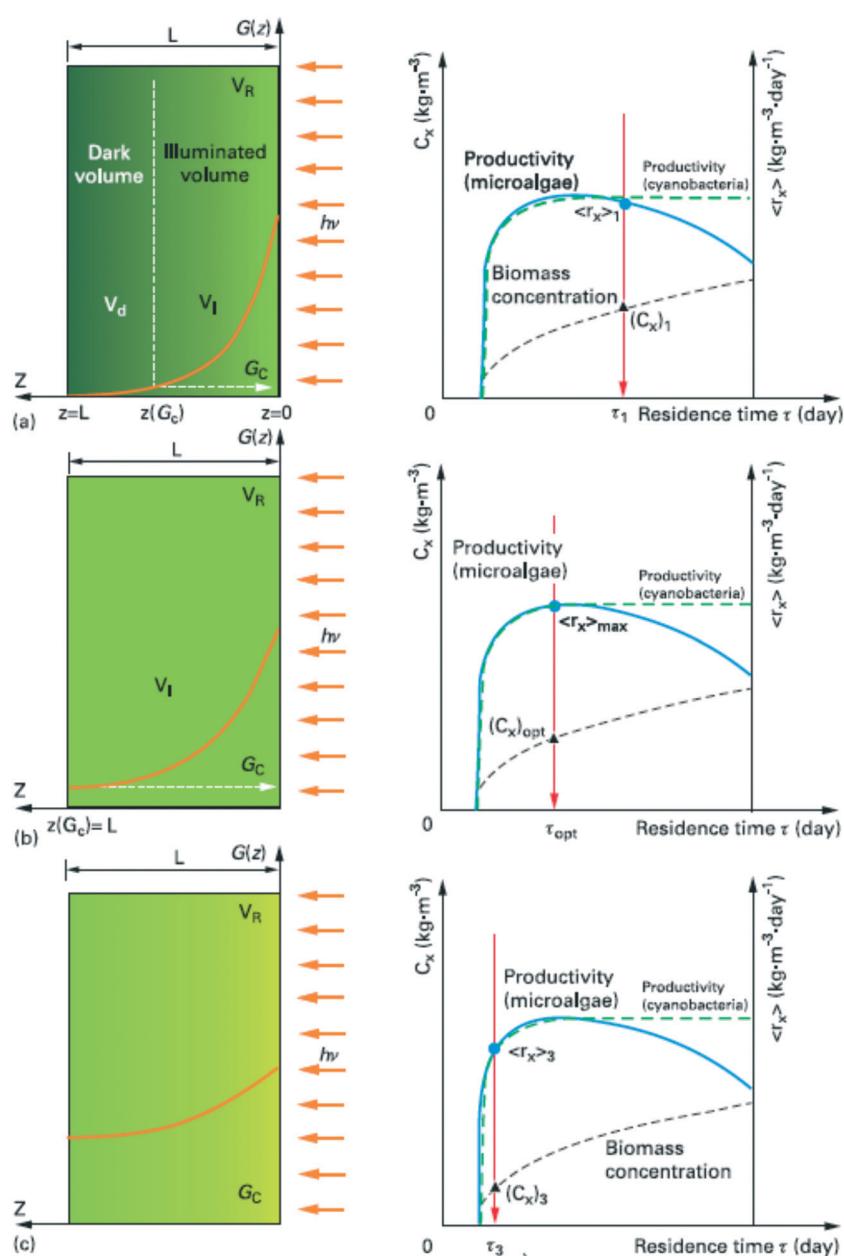
### Light energy in photobioreactors

The efficiency of photosynthesis is directly related to the amount of light absorbed by the microalgae. At the cell level, it is possible to know the quantity of light necessary to obtain the maximum performance of the microalgae. When considering the photobioreactor system, it is easy to understand that if microalgae absorb part of the light transmitted to it, the rest of the microalgae contained in this system will share the remaining flux. As a consequence, light energy is heterogeneous in the volume of the photobioreactor. It will be then impossible to maintain maximum performance for all the microorganisms in the PBR. When considering a flat PBR, the

attenuation of light, when transmitted homogeneously to the PBR, occurs in only one dimension, in the thickness of the culture. The light attenuation profile depends on several parameters: (i) the amount of light transmitted at the surface of the PBR also called PFD (photon flux density in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), (ii) the concentration of microalgal biomass ( $C_x$  in  $\text{g x l}^{-1}$ ) and (iii) the radiative properties of the microalgae, depending in particular on the pigment content of the microalgae ( $w_{\text{pig}}$  in %) and the shape of the cultured microalgae.<sup>23</sup> Pruvost and Cornet (2012)<sup>24</sup> identified three light attenuation regimes corresponding to distinct performances. These are differentiated by the parameter  $\gamma$ , representing the illuminated volume fraction in a PBR. In the literature, this is defined by

considering the light received by the microalgae in the reactor, characterized by irradiance  $G$ . This parameter  $\gamma$  is the ratio of the illuminated volume to the total volume of the PBR. The boundary between these two zones is then given by the compensating irradiance ( $G_c$ ). When the local irradiance is below this value, the zone is considered to be a dark volume, in which the phenomenon of respiration is predominant, and thus negatively impacts biomass productivity. The three operating regimes are as follows (Fig. 1):

- Photo-limited regime (case a): in this case  $\gamma < 1$ , the entire photon flux transmitted to the crop is absorbed. A dark zone is present due to the high biomass concentration. This is achieved by a high residence time of the microalgae and



**Fig. 1** Light attenuation profiles for a planar PBR and associated productivities.<sup>24</sup> (a) case for  $\gamma < 1$ , (b) case for  $\gamma = 1$ , (c) case for  $\gamma > 1$  (this figure was published in J. Pruvost and J.-F. Cornet, *Microalgal Biotechnology: Potential and Production*, 2012, De Gruyter, Berlin, Germany, 181–224, Copyright De Gruyter).

does not allow maximum biomass productivity due to the negative impact of respiration.

- Strict physical limitation or luminostat regime (case b): here  $\gamma = 1$ , *i.e.* all the light transmitted to the system is absorbed by the microalgae without the appearance of a respiration zone ( $G(L) = G_c$ ). It is this regime that leads to the maximum performance of a PBR for a given light flux.

- Kinetic regime (case c): here  $\gamma > 1$ , *i.e.* part of the light is not absorbed in the culture volume and is therefore transmitted. This translates in energy terms into biomass productivity below the maximum achievable productivity (because not all the transmitted energy is converted). This regime is very particular because the residence time is low, which leads to a decrease in biomass concentration and a risk of culture leaching. Moreover, the system performance can be reduced by poor absorption of the luminous flux. Indeed, due to the low light attenuation, the amount of energy absorbed by the microalgae is important, which can induce a phenomenon of photosynthesis oversaturation or even photoinhibition, damaging the photosynthetic apparatus of the microalgae and significantly reducing the performance of the PBR.

### Temperature and microalgal growth

A non-optimal culture temperature affects the growth of microalgae. Although not directly affecting photochemical reactions, the temperature at which the microorganism grows plays a major role in enzyme activity.<sup>25</sup> This has the consequence of reducing the growth capacity of a given microorganism when growth deviates from the optimum, and in some cases even leading to cell death. In addition, a change in the temperature of the medium can force the microalgae to change its composition. This has been shown for example on lipids.<sup>25</sup> For the culture of the study microalga, *C. vulgaris*, the optimal growth temperature is estimated to be between 20 and 30 °C.<sup>26–28</sup> Most microalgae are so-called mesophilic, *i.e.* they have an optimal growth temperature between 15 and 40 °C. For the latter, a temperature of the environment surrounding the microalgae above 45 °C for more than 24 hours can have an irreversible effect on the culture.<sup>29</sup> There are other strains which, due to their natural environment, have totally different optimal growth temperatures, such as the so-called psychrophilic ( $T_{opt} < 15$  °C) and thermophilic ( $T_{opt} > 50$  °C) microalgae.<sup>28</sup> No photosynthetic microorganisms with an optimal growth temperature above 75 °C have so far been identified, probably due to the instability of chlorophylls at this temperature.<sup>28</sup> Generally, the temperature control must be addressed for the evaluation of the technical feasibility of large-scale algae production.<sup>30</sup>

When producing microalgal biomass under solar conditions, the night period is often a period when no production takes place. However, the loss of biomass due to respiration can be major, sometimes up to 20% biomass loss for a ten-hour night.<sup>31</sup> The night period is generally accompanied by a

consumption of the cell's carbohydrates (energy source) in the metabolism of cell maintenance and protein synthesis.<sup>32</sup> Photosynthetic microorganisms are unequal to the phenomenon of respiration. Indeed, cyanobacteria will lose on average less biomass at night than the eukaryotic cells.<sup>33</sup> Moreover, within eukaryotic microorganisms, there is a great disparity in respiration rates at night depending on the cultured.<sup>31</sup> The most influential parameter on the loss of biomass in the dark phase of a given microalgae is temperature. Edmundson and Huesemann (2015)<sup>31</sup> showed that when the temperature at night was reduced from 25 °C to 10 °C, it was possible to reduce biomass decrease. The most impressive case is that of the microalga *Nanochloropsis salina*, which loses 20% of its dry matter concentration after ten hours in the dark at 25 °C, whereas reducing the temperature to 10 °C over the same period only results in a decrease in concentration of around 2%. The slowing down of metabolic activity, and therefore of the decrease in biomass by temperature reduction has been confirmed by numerous studies on numerous microorganisms.<sup>32–35</sup>

In order to guarantee a good thermal management in a PBR (to maximise production) and to be able to estimate the energy needs linked to the control of a PBR, it is essential to be able to represent the different exchanges taking place within it. Knowing the exchanges taking place in a growing system allows to model the temperature evolution in a given culture system throughout the year. It also serves as a tool for dimensioning the thermal exchanger necessary for the regulation of a solar PBR (and for estimating the associated costs), and thus as a tool for comparing temperature-regulated closed PBRs. Solar radiation is responsible for the heating of microalgae cultures, but other thermal exchanges take place between a PBR and its environment.

### Nutrients and carbon supply

For a photosynthetic microorganism, essential nutrients are needed, at least a source of nitrate (or ammonium), sulphates and phosphates ions. These nutrients are provided under chemical salts dissolved in aqueous solution. The composition of the culture medium is adjusted according to the specific needs of the cultured microorganisms. Nutrients can be supplied in three different ways. A high dose is only given at the beginning of the cultivation, this is called a batch culture. The solution is brought continuously to the culture, a part of the culture is then continuously renewed), it is called continuous culture mode. The fed-batch mode corresponds to the addition of new medium is done punctually during the cultivation process. For the continuous mode, two cases can be distinguished: the chemostat mode for which the supply of the medium is fixed by the flow rate of the feed pump, and the turbidostat mode for which the supply of the medium is regulated according to the quantity of biomass in the culture.

Carbon is the majority element in the biomass, it represents about half of the dry mass of the microalgae. In autotrophic culture, it is brought into the medium in the

form of inorganic salts dissolved in the culture medium ( $\text{HCO}_3^-$  or  $\text{CO}_3^{2-}$ ) or in gaseous form ( $\text{CO}_2$ ) transferred to the culture. When grown in an open system, the liquid is in equilibrium with the atmosphere containing a very low quantity of  $\text{CO}_2(\text{g})$ , *i.e.* about 400 ppm. Consequently, if the medium contains dissolved carbon in a quantity greater than that given by the equilibrium with the atmosphere, a desorption of  $\text{CO}_2$  in its aqueous form towards its gaseous form will be generated by this concentration gradient. In addition, the form in which inorganic carbon is present is pH dependent. With a pH above 9, desorption is low because only the aqueous form of  $\text{CO}_2$  is in equilibrium with the gaseous phase. Therefore, at high pH, it is not necessary to cover a culture system to avoid carbon limitation, this explains why *Spirulina platensis* culture is generally grown in an open system. As is the case with temperature, each microalga has an optimal pH for growth. For example, *Chlorella vulgaris* grows at pH values between 5 and 9 with an optimal pH between 7 and 8.<sup>36</sup> For the cyanobacterium *Anabaena*, this optimum is between 9 and 10. During cultivation, when growth occurs, the total carbon concentration decreases. Since  $\text{CO}_2$  is an acid, the pH then increases.<sup>37</sup> Increasing this pH may reduce growth. One method of ensuring the presence of carbon (avoiding limitation) and maintaining the pH at the optimal level is therefore to occasionally add gaseous (acidic)  $\text{CO}_2$  to the culture when the pH exceeds the set value.

### Mixing and microalgal growth

Although this parameter is not directly a factor inhibiting or promoting growth, it acts at the reactor scale as an overall parameter acting on all the other parameters presented above. Indeed, all the considerations concerning thermal, salinity, pH, biomass concentration (and consequently light attenuation) are valid only if agitation makes the medium, in which the microalgae grow, homogeneous. Since the 1950s, Richmond and Hu (2013)<sup>29</sup> concluded after simple trials (culture with and without agitation) that the growth of a dense culture of microalgae was promoted by setting the microalgae in motion. This can be explained, for example, by the presence of thermal stratification or a pH gradient within the culture system in the absence of agitation. There are different mechanical and non-mechanical systems for the movement of microalgae in a PBR. Non-mechanical stirring is bubble stirring. This type of agitation has two advantages: the shear is generally low, which will not generate any agitation stress; moreover, it promotes the transfer of dissolved oxygen from the liquid phase to the gas phase, which reduces the risk of growth inhibition due to too high an oxygen concentration in the culture medium. Circulation by mechanical system can be done *via* a recirculation pump, a marine propeller, a paddle wheel or by manual agitation depending on the application and the geometry of the culture system used. To ensure agitation for maximum performance of a culture system regardless of geometry, the average

agitation speed should be around  $15 \text{ cm s}^{-1}$ .<sup>38</sup> In a raceway-type basin, the preferred agitation system is the paddle wheel for several reasons.<sup>36</sup> The presence of vertical velocities allows good agitation and distribution of biomass in all dimensions. Moreover, this system causes very low shear and is suitable for all microalgae strains, including the most fragile. Finally, the electrical consumption linked to agitation with this type of system remains low (around  $6 \text{ W m}^{-2}$ ). Nevertheless, recent studies highlight the interest of stirring raceway systems by airlift, which would be up to three times less energy consuming than the paddle wheel, while maintaining the same quality of agitation.<sup>39</sup>

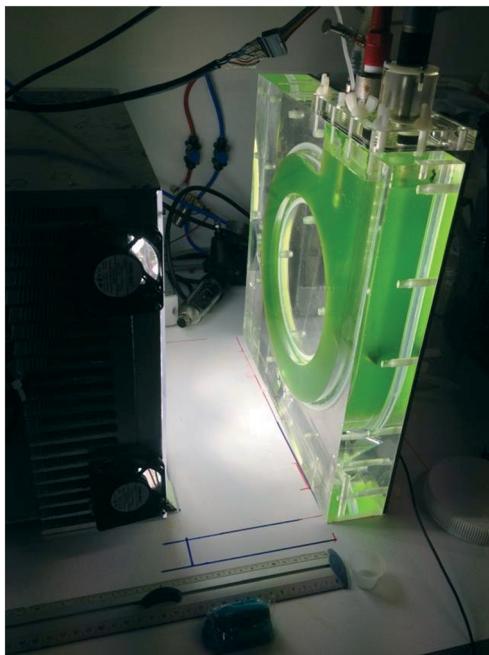
A wide variety of PBRs technologies exist, such as tubular, cylindrical or flat panel systems with specific mixing problems. However, the criteria for a "good" mixing can be stated: avoid sedimentation of the biomass, give the same story about the light received to the whole algal population, satisfy heat transfer to maintain an optimal temperature and gas-liquid mass transfer for  $\text{CO}_2$  supply and  $\text{O}_2$  removal, avoid biofilm formation and shear rates too high in relation to cell fragility. From the conception point of view, different criteria have to be taken into account: cost, limitation of the dark zones, limitation of the thickness of the culture media to increase the volumic productivity, easiness to scale up and energy efficiency. The various PBR technologies available have advantages and limitations in terms of hydrodynamics conditions and biomass productivity relatively to the construction cost.<sup>50,69,76,81</sup>

### Design of photobioreactors

The photobioreactors are most often categorized according to their size, agitation mode (when available), light source (solar or artificial), culture depth, or shape (flat or tubular).<sup>22,29,40–42</sup> This highlights the complexity and thus the non-universal nature of the microalgae culture bioprocess. Indeed, the choice of a given technology will depend on the application (energy, food, cosmetics...), the geographical area, the available resources (surface area, electricity...), and the cultivated strain (extremophilic, fragile...). Changing one of these parameters may favour the use of a different system.

### Labscale photobioreactors

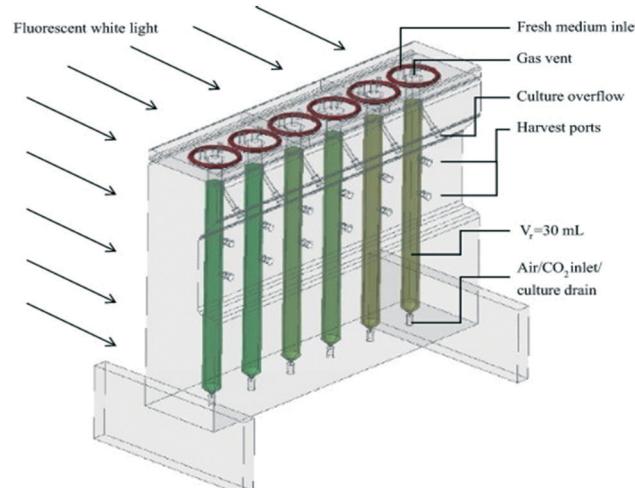
Lab-scale cultures are made in photobioreactors with artificial lighting. To study the impact of a parameter on the growth or production of a particular metabolite, it is necessary to perfectly control all the parameters acting on production, *i.e.*: light intensity, pH, temperature, nutrient concentration, agitation and biomass concentration. The most classic of the chemical or biological reactors is the perfectly stirred reactor, because of the possibility to get a homogeneous mixture, including biomass. In contrast to common chemical nutrients, it is not possible to distribute the light homogeneously in whole reactor volume, because of the light attenuation caused by the light absorption by the microalgae.



**Fig. 2** Torus photobioreactor (with courtesy of GEPEA laboratory – UMR CNRS 6144).

Csögör *et al.* (1999)<sup>43</sup> have developed a PBR, which combine the advantages of a stirred reactor and a plate reactor to reduce the light path length. Another example is the torus-shaped PBR, which was designed<sup>44</sup> for lab-scale experiments requiring a full control of culture conditions (Fig. 2). The culture is circulated in a torus-shape tank by the rotation of a marine impeller. The combination of the culture rotation and the torus configuration allows good mixing without dead volume and with reasonable shear stress.<sup>44</sup> The light-supplying device (LED panel) is placed in front of the PBR. The plane front surface and the square-sectioned torus channel prevents optical distortion along the light emission direction. As a consequence, light attenuation occurs along only one main direction, leading to facilitate calculation of light transfer and light attenuation conditions.

The torus-shaped PBR has been used to model and optimize microalgal biomass productivity<sup>23,45</sup> and to investigate the coupling between hydrodynamics and photosynthetic conversion for the “light/dark cycles effect”.<sup>46</sup> Another example can be found in Martzolff *et al.* (2012).<sup>47</sup> The possibility to control of mixing time with respect to the circulation time and the plug-flow behavior was used for isotopic nonstationary <sup>13</sup>C-metabolic flux analysis. The characterization of the kinetics of <sup>13</sup>C-labeling incorporation allows to define the biochemical reaction network of *C. reinhardtii*.<sup>48</sup> For the screening of operating conditions for a given strain or comparing microalgae strains in the same conditions, a cultivation system, named efficient overproducing screening system-photobioreactor (EOSS-PBR), was developed by Taleb *et al.* (2015).<sup>49</sup> It consists of six fully automated small-scale bubble columns PBRs operated in



**Fig. 3** EOSS-PBR (with courtesy of GEPEA laboratory – UMR CNRS 6144).

parallel (Fig. 3). Each column has a volume of 30 ml and could be operated in batch and semi-continuous conditions.

Other photobioreactor geometries have been developed for the same research use. The geometries found in the literature are: (i) cylindrical geometry, inspired by stirred-tank fermenters with internal or external illumination,<sup>50,51</sup> (ii) tubular geometry,<sup>52</sup> (iii) plane geometry,<sup>52,53</sup> (iv) bubble column,<sup>54,55</sup> (v) airlift column,<sup>56,57</sup> (vi) helical tubular,<sup>58</sup> (vii) conical.<sup>59</sup>

### Open industrial systems

The first use of microalgae by humans dates back more than 2000 years, to China, where the cyanobacterium *Nostoc* was harvested in response to famine.<sup>1</sup> Nowadays there are many commercial applications, which is why microalgae, originally harvested in their natural environment, are now produced in real farms or even factories. It should be noted that although research into large-scale production dates back to the 1950s,



**Fig. 4** *D. salina* ponds at Hutt Lagoon (Australia) (<http://www.bsb.murdoch.edu.au/groups/beam/BEAM-AppI0.html>, with permission of Professor Michael A. Borowitzka).

the term “farming” appeared in the 1960s.<sup>22</sup> The first systems dedicated to the cultivation of microalgae were artificial ponds, also called lagoons. This is the simplest production system. It consists of a body of water between 20 and 40 centimeters deep without agitation. This system was used by the Cognis company in Hutt Lagoon (Australia) to cultivate the microalga *Dunaliella salina* (Fig. 4). These microalgae are cultivated for their high carotene content. Today, the global company BASF farms these 700 hectares of land, making it the largest production site in the world.<sup>60</sup>

Subsequently, agitated basins were developed in order to avoid sedimentation and thus optimize light distribution in the culture volume. Moreover, this avoids the presence of concentration gradients or thermal stratification, reducing the overall performance of the process.<sup>42</sup> Thus, in order to guarantee homogeneity in the culture basin, Andersen (2005)<sup>61</sup> recommends an average velocity between 20 and 30 cm s<sup>-1</sup> to avoid the risk of sedimentation. This generally guarantees minimum local velocities in the basin greater than 10 cm s<sup>-1</sup>. This velocity is defined as the velocity at which the risks of biomass sedimentation appear (for most strains). The most popular open agitated systems on a large scale are circular and raceway-type systems.<sup>114</sup> Circular systems are agitated *via* an arm with a size of the radius of the basin set in motion by the axis. This type of basin has two major disadvantages, a non-optimal surface area (due to the circular shape) and difficulty in extrapolating beyond 1000 m<sup>2</sup>.<sup>62</sup>

Notoriety of circular systems remained relatively low as they were supplanted by basins called raceways because of their shape. Initially developed in the field of wastewater treatment by Oswald and Goleuke (1967)<sup>63</sup> and Benemann and Oswald (1996),<sup>64</sup> this technology consists in a loop (or sometimes several interconnected loops) consisting of two straight zones and two turning zones (Fig. 5) in which a thin layer of microalgae culture (about 20 to 40 centimeters for industrial systems) is set in motion, usually by means of a paddle wheel.

A final type of open system was developed in the 1960s in the Czech Republic, in Trebon<sup>66,67</sup> The aim of this system is



Fig. 5 Raceway of 3000 m<sup>2</sup> in NBT, Israël (<https://www.israel21c.org/wp-content/uploads/2016/02/algae-NBT.jpg>)<sup>65</sup> (this figure was published in P. J. Harvey and A. Ben-Amotz, *Algal Research*, 2020, 50, 102002, Copyright Elsevier).

to reduce the thickness of the culture as much as possible for the same ground surface area. For this purpose, a slightly inclined plane system was developed (inclination of 1.7%,<sup>67</sup> on which the microalgae culture flows down and is then reinjected at the reactor head *via* a pump (Fig. 6).

This cultivation system reduces the costs of biomass production and processing. Indeed, since production is only dependent on the lighting area, a reduction in thickness will have under identical conditions no impact on the quantity of biomass produced. Agitation and separation of biomass from water will therefore be cheaper due to the small amount of water to be treated. An industrial version of this concept has been implemented in Portugal by the company A4F in 2014. This system comprises a succession of four inclined planes for a total production area of 3000 m<sup>2</sup> (Fig. 7).

Even today, open systems are still the most widely used on an industrial scale because of their low manufacturing cost and ease of extrapolation. However, they are not suitable for all types of production. Indeed, these systems are reserved for the production of extremophilic strains, because of the risk of contamination by airborne microorganisms. There is no problem for strains such as *Dunaliella salina* or *Arthrospira platensis* because these two photosynthetic microorganisms grow in conditions where most contaminants cannot proliferate (hypersalinity and highly alkaline medium, respectively). The second point limiting production in this type of system is the input of inorganic carbon. Since these systems are in contact with air (containing very little CO<sub>2</sub>), the dissolved inorganic carbon in the basins will tend to equilibrate with the atmosphere (except in the case of highly alkaline pH). This difference in inorganic carbon concentration will induce desorption of the latter from the basin into the ambient air, which may lead to a limitation in nutrients, which will tend to significantly reduce the



Fig. 6 Inclined plane system (200 litres) developed in Trebon, Czech Republic<sup>68</sup> (original figure was published in J. R. F. Malapascua, C. G. Jerez, M. Sergejevová, F. L. Figueroa and J. Masojídek, *Aquat Biol.*, 2014, 22, 123–140, under the Creative Commons CC-BY License).



**Fig. 7** Cascade of inclined plane photobioreactor of A4F in Pataias, Portugal (reproduced with the permission of A4F company, [www.a4f.pt](http://www.a4f.pt)).

maximum performance of the reactor in terms of productivity. Some performance data for *Chlorella vulgaris* are reported in the following table for different culture technologies (Table 1).

The results presented in Table 1 are for the most part maximum productivities obtained during the summer periods. Tredici (2003)<sup>72</sup> estimates that the average annual surface productivity of any open basin is around 40 t ha<sup>-1</sup> per year. An annual culture of the cyanobacterium *A. platensis* has been carried out in Spain,<sup>73</sup> with an annual surface productivity of 30 t ha<sup>-1</sup> per year. In their report, Benemann and Oswald (1994)<sup>64</sup> reduce the crop yield in temperate zones to 10–20 t ha<sup>-1</sup> per year, which is confirmed by an experiment carried out by Tredici over several years obtaining a productivity on Spirulina of 20 t ha<sup>-1</sup> per year.<sup>74</sup>

### Closed industrial systems

Photobioreactors have many advantages over open ponds: (i) contamination can be controlled because there is no contact with outside, so it is sufficient to sterilize the PBR before cultivation; (ii) desorption is also limited because of the low exchange with the ambient air, which avoids the risk of carbon limitation; (iii) water consumption is low compared to open systems because evaporation is almost nil. There are many types of closed PBRs, and these culture systems will be presented in three categories: (i) conventional systems with artificial lighting, (ii) conventional systems with solar lighting (pilot or large-scale) and (iii) breakthrough systems (at the development stage).

**Table 1** Surface productivity of *C. vulgaris* in open culture systems

Culture system	Surface productivity (t ha <sup>-1</sup> per year)	Ref.
Lagoon	3.6	Richmond and Hu (2013) <sup>29</sup>
Raceway	43–47	Richmond and Hu (2013) <sup>29</sup>
Raceway/circular	36–91	Pulz (2001) <sup>69</sup>
Cascade	40–84	Doucha and Lívanský (2006) <sup>70</sup>
Cascade	91	Chen (2009) <sup>71</sup>

Because of its ease of extrapolation, the tubular photobioreactor is the most widely used system under solar conditions.<sup>38,42,50,62,69,72,75–78</sup> On this same basis of construction, several alternatives can be distinguished according to: (i) the mode of agitation (airlift or mechanical by means of a pump), (ii) the regulation system (internal via a concentric tube, external by immersing the tubes in a swimming pool,<sup>76</sup> by spraying water on the surface of the tubes or without thermal regulation), (iii) the construction material (PVC, PMMA, glass...), (iv) the type of construction (PVC, PMMA, glass...). One of the most world's largest closed tubular PBR is in Klötze (Germany) and operated by Roquette. The system consists of 20 independent modules with a total volume of about 600 m<sup>3</sup> with 500 km of glass tubes arranged in a 1.2 hectare greenhouse (Fig. 8).

Alga Technologies, Ltd, based in Israel, the world leader in the production of naturally occurring astaxanthin has also chosen tubular PBR. Microalgae are cultivated in 300 kilometres of tubes on a production area of more than one hectare.<sup>79</sup>

As regards flat systems, although often considered to be more efficient<sup>78</sup> due to ease of light attenuation management, they are more complicated to scale up than tubes, due in particular to their lower resistance to hydrostatic pressure. Nevertheless, research on this geometry has led to the marketing of various industrial production systems. This is the case of the Green Wall Panels developed by Tredici *et al.* (2015),<sup>80</sup> the second version of which is now on the market (Fig. 9).

Other vertical PBRs are marketed by the German company Subitec. These have a variable thickness depending on the height in order to promote mixing. There are also, on the same principle as the submerged tubular system, two types of planar systems sold respectively by Proviron<sup>81</sup> and Solix,<sup>82</sup> in which airlift planar PBRs are immersed in a layer of water to increase the thermal inertia of the cultivation system.

The GICON® Photobioreactor, also called the Christmas tree reactor due to its truncated conical shape, was designed for providing optimum light supply thanks to its geometry. Self-shading of algae could be prevented, depending on the biomass concentration obtained in the culture system, and light incidence could be maximized by allowing variable angles of radiation.

### PBRs under development

A new tubular photobioreactor design based on the Fibonacci equation is proposed by Diaz *et al.* (2019).<sup>83</sup> The idea is to

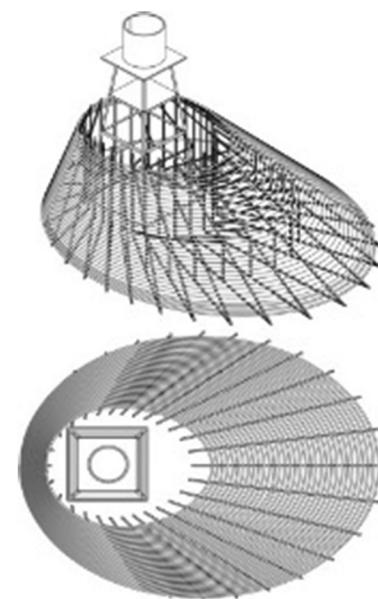


**Fig. 8** Tubular photobioreactor in greenhouse in Klötze, Germany<sup>69</sup> (agreement from Springer Nature in the framework of Copyright Clearance Center).

mimic plants, which develop varied leaf distribution geometries to optimize light absorption. Fibonacci-type tubular photobioreactor is based on a helical spiral and could be considered as artificial photosynthetic trees (Fig. 10). The design of the PBR allows up to a 1.4-times increase in intercepted solar radiation with respect to that received on a horizontal surface.

SunOleo firm develops photobioreactors, which are simple tanks with immersed inflatable light wells, which bring sunlight up to 6 m deep in the tanks (Fig. 11) and theoretically allow to increase surfacic productivity with respect of raceway ponds.

Photobioreactor technology is still evolving. In order to reduce consumption (especially water consumption) and increase the efficiency of photosynthesis in photobioreactors, new cultivation systems currently under development could be the conventional technologies of tomorrow. This is the case of the Algofilm photobioreactor.<sup>84</sup> This system is an intensified photobioreactor, as it allows biomass



**Fig. 10** Scheme of the Fibonacci-type tubular photobioreactor<sup>83</sup> (this figure was published in J. P. Diaz, C. Inistrosa and F. G. Acien Fernandez, *Process Biochemistry*, 2019, **86**, 1–8, Copyright Elsevier).

concentrations up to 100 times higher than in a raceway-type PBR, for the same productivity on the illumination area. This can be achieved by reducing the thickness of the culture, which increases the ratio of illumination area to culture volume. This PBR works on the principle of a falling film with small inclination angle, making it possible to obtain a culture thickness of 1.5 mm. The culture of microalgae on biofilm is also one of the solutions to reduce the amount of water used.<sup>85–87</sup> Indeed, microalgae are fixed and grow on a support, nutrients are then provided by immersing this film in water supplemented with minerals essential to the growth of microalgae. Although this process consumes little water and energy for harvesting, it is difficult to manage the light attenuation in a film of immobilized microalgae and therefore to optimize productivity. Cornet<sup>88,112</sup> has developed a PBR called DiCoFluV (Solar Flux Volume Controlled Dilution) based on the dilution of the luminous flux in the culture volume. Based on the principle that the efficiency of photosynthesis is inversely proportional to the luminous flux captured, they have developed a culture system in which a sheath of optical fibres brings energy to the culture. The aim is to reduce the luminous flux sent to the culture for the same overall amount of energy supplied to the system. This principle makes it possible to approach the maximum thermodynamic limit of conversion of light energy into biomass through photosynthesis.

PRIAM (Internal Radiation Photobioreactor and Modular Layout) photobioreactor, which was patented by the CNRS – ENSCCF University of Nantes,<sup>89</sup> is based on the principle of internal lighting by flat panels, these panels delimiting volumes of culture whose repetition allows, in a design mode close to the filter press, to extrapolate in volume by



**Fig. 9** PBR green wall panels in Sesto Fiorentino, Italia<sup>80</sup> (this figure was published in M. R. Tredici, N. Bassi, M. Prussi, N. Biondi, L. Rodolfi, G. Chini Zittelli and G. Sampietro, *Applied Energy*, 2015, **154**(September), 1103–1111, Copyright Elsevier).



**Fig. 11** SunOleo photobioreactor (with the kind permission of the company SunOleo).

simply increasing the number of panels (Fig. 12). This conception brings many advantages on key points in the culture of photosynthetic microorganisms, namely (i) an optimised contribution of light within the culture itself, with thus an optimal exploitation of the photons emitted, (ii) a design limiting the risks of adhesion on the lighting structures, easily dismantled and facilitating cleaning, and (iii) a modular production that can be simply extrapolated in volume while maintaining constant surface and volume productivity.

New types of photobioreactor with a coupling between a photobioreactor and a photovoltaic cell are currently in development.<sup>115,116</sup> A pilot-scale spectrally-selective, insulated-glazed photovoltaic flat plate photobioreactor was developed with an infrared reflecting system embedded in the illumination surface for the thermal regulation of outdoor photobioreactors. The interest is to produce both microalgal biomass and electricity and to increase the net energy ratio.<sup>117</sup>

## Modelling of the PBR

### Growth models

The overall approach generally consists of a mathematical law that correctly translates the experimental behaviour

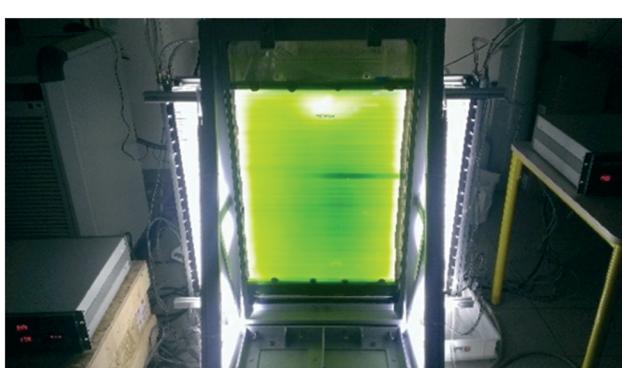
observed. The parameters of the model are adjusted for each case, but are not necessarily representative of the physical, chemical or biological reactions that take place. The most widely used are those of Monod (1942)<sup>90</sup> and Andrews-Haldane.<sup>91</sup> Monod's model is commonly used to describe bacterial growth. This model is based on the assumptions that the yield of growth (in g of biomass per g of substrate) of conversion of a given substrate (concentration of this substrate noted  $S$ , in g l<sup>-1</sup>) into biomass remains constant over time, and that the evolution of the specific growth rate ( $\mu$ , in h<sup>-1</sup>) with the evolution of the substrate concentration considered follows a hyperbolic trend:

$$\mu = \mu_{\max} S / (K_S + S) \quad (2)$$

where  $\mu_{\max}$  is the maximum specific growth rate and  $K_S$  represents the half-saturation constant of the microorganism with respect to the substrate under consideration. This constant is dependent on the microorganism and its culture conditions, which express the affinity of the microorganism to the substrate. A limitation of the Monod model is the non-inclusion of the inhibitor effect of a substrate present in excess. The Andrews-Haldane model<sup>91</sup> introduced a term for the substrate inhibition:

$$\mu = \mu_{\max} S / (K_1 + S + S^2 / K_2) - \mu_m \quad (3)$$

where  $\mu$  is the specific growth rate, in h<sup>-1</sup>,  $\mu_{\max}$  the maximum specific growth rate, in h<sup>-1</sup>,  $S$  the substrate concentration in the extracellular medium, in g l<sup>-1</sup> or mol l<sup>-1</sup>,  $K_1$  the limiting constant, in g l<sup>-1</sup> or mol l<sup>-1</sup>,  $K_2$  the inhibition constant, in g l<sup>-1</sup> or mol l<sup>-1</sup>. The term  $\mu_m$  corresponds to the term maintenance of microorganisms (respiration), which allows access to the negative values of biomass productivity observed for low substrate values. As for the Monod model, the modeling of the growth of photosynthetic microorganisms photolimited by the Andrews-Haldane model will be done by replacing the substrate,  $S$ , by irradiance,  $G$ , for local values of growth rate, or an averaged value,  $G_{\text{moy}}$ , over the whole photon flux culture for an average



**Fig. 12** PRIAM (internal radiation photobioreactor and modular layout) photobioreactor prototype patented by CNRS – University of Nantes<sup>89</sup> (with courtesy of GEPEA laboratory – UMR CNRS 6144).

value of the growth rate. The type of model is often used for the good approximation of the experimental behaviours,<sup>92,93</sup> although its formulation is problematic in the case of photosynthetic microorganisms. There is thus no proportionality between respiration and luminous intensity.<sup>94</sup>

Some approaches are based on artificial intelligence with deep learning technology and data-driven surrogate modeling framework.<sup>95</sup> A stochastic optimization algorithm was used in order to develop models from hydrodynamic and biochemical kinetic results to optimise biosystems and obtain decision-making for choosing the best parameters for microalgae production.

### Predictive models

A dynamic semi-predictive model for microalgal culture in tubular photobioreactor have been developed by Fernández *et al.* (2014).<sup>18</sup> The model is based on a photosynthesis rate equation taking into account light intensity and the others most important variables (temperature, pH, and dissolved oxygen) that influence the growth and performance of the culture in any microalgal production system. The thermodynamic approach of the irreversible processes was applied by Stucki (1978)<sup>96</sup> to describe the biological reactions observed in microorganism cultures. This approach consists in considering that, in a complex biological process such as photosynthesis, the average state of the measured reactions (not thermodynamically stable in instantaneous value, due to the autocatalytic steps of enzyme activation and inhibition) is stable on a appropriated time scale.<sup>97</sup> Cornet's analysis of the Z-scheme of photosynthesis has led to the establishment of a growth model, which was compared with experiments on cultures of two microorganisms: *Arthospira platensis* and *Rhodospirillaceae* sp.<sup>10,98</sup> This predictive method requires a high degree of theoretical knowledge, since it is based on the precise analysis of the major phenomena governing growth, as the energetics of photosynthetic conversion, anabolism and catabolism.

**Flux metabolic modeling.** Metabolic flux analysis allows the modeling of intracellular metabolism in response to genetic and/or environmental variations in a system biological. All modeling is based on the reconstruction of a metabolic network associating each reaction involved for the conversion of a given substrate into products of interest. Cogne *et al.* (2003)<sup>99</sup> established the metabolic network of autotrophic growth of *Arthospira platensis*; 121 reactions associated with 134 metabolites made it possible to reconstruct a metabolic network linked to the production of C-phycocyanin. Cogne *et al.* (2011),<sup>48</sup> based on two computational approaches of metabolic flux, modeled of the behavior of the eukaryotic microalgae *Chlamydomonas reinhardtii* grown under photoautotrophic conditions. The reconstruction of a metabolic network comprising 280 metabolic reactions linked to 278 metabolites was carried out. The study was able to demonstrate a reorientation of

metabolism in response to changes in the illumination conditions. Current state of the art of constraint-based modeling and computational method development are discussed by Tibocha-Bonilla *et al.* (2018).<sup>100</sup>

**Light attenuation modelling.** Light attenuation can be represented by the Beer-Lambert equation using an extinction coefficient representing the biomass absorption coefficient.<sup>101</sup> Beer-Lambert's law is a relatively simple law allowing to model correctly the exponential attenuation of monochromatic radiation through a homogeneous medium, absorbent and non-diffusive. It is a law which is very widely used in spectrophotometry, especially for the determination of chemical species in solution. This law does not take into account the phenomena of radiation diffusion. Its simplicity of use means that this law, even if it neglects an important phenomenon, namely diffusion, is still widely used for modeling, in the first instance, the approximation, light transfer to PBR.<sup>101-104</sup>

However, this model does not take into account the effects of light scattering by microalgae. For this reason, Cornet *et al.* (1992, 1995)<sup>97,105</sup> used a different approach to accurately describe light attenuation in a photobioreactor. By adapting Schuster's (1905)<sup>106</sup> model describing the behaviour of light in a foggy atmosphere, the proposed radiative transfer model describes light attenuation by taking into account light absorption by pigments and light scattering throughout the cell. The assumptions are as follows: the medium is assumed to be absorbing, scattering and non-fluorescent. Therefore, it is sufficient to determine three parameters to characterize the light path: the mass coefficient of light absorption ( $Ea_\lambda$  in  $\text{m}^2 \text{ kg}^{-1}$ ), the mass coefficient of scattering ( $Es_\lambda$  in  $\text{m}^2 \text{ kg}^{-1}$ ) and the coefficient of backscattering ( $b_{2\lambda}$ , without unit). When this model considers the light attenuation as monodirectional (on the z-axis), it is called a two-flux model, and takes into account the propagation of light, for a given wavelength (in nm) along the z-axis and in two opposite directions,  $I_\lambda^+$  and  $I_\lambda^-$ .<sup>97</sup> The sum of these two specific intensities gives the local irradiance  $G$  (eqn (4)):

$$G_\lambda = \iint_{4\pi} I_\lambda d\omega = I_\lambda^+ + I_\lambda^- \quad (4)$$

where  $d\omega$  represents the solid angle defining a radiation beam. The system of differential equations to be solved as a function of culture depth is as follows:

$$\frac{dI_\lambda^+}{dz} = -Ea_\lambda \cdot C_X \cdot I_\lambda^+ - Es_\lambda \cdot \bar{b}_{2\lambda} \cdot C_X \cdot (I_\lambda^+ + I_\lambda^-) \quad (5)$$

$$\frac{dI_\lambda^-}{dz} = -Ea_\lambda \cdot C_X \cdot I_\lambda^- - Es_\lambda \cdot \bar{b}_{2\lambda} \cdot C_X \cdot (I_\lambda^- + I_\lambda^+) \quad (6)$$

The boundary conditions for a planar system, illuminated by a collimated light source at normal incidence, having a rear face of reflectivity  $\rho$  are as follows ( $q_{\lambda,0}$  is the surface incident flux of the PBR):

$$\begin{cases} z = 0, & I_\lambda^+ = q_{\lambda,0} \\ z = L, & I_\lambda^- = \rho I_\lambda^+ \end{cases} \quad (7)$$

Finally, the expression of irradiance as a function of the thickness of a planar PBR is given by Pottier *et al.* 2005:<sup>107</sup>

$$\frac{G_\lambda(z)}{q_{\lambda,0}} = 2 \frac{[\rho(1 + \alpha_\lambda)e^{-\delta_\lambda L} - (1 - \alpha_\lambda)e^{-\delta_\lambda L}]e^{\delta_\lambda z} + [(1 + \alpha_\lambda)e^{\delta_\lambda L} - \rho(1 - \alpha_\lambda)e^{\delta_\lambda L}]e^{-\delta_\lambda z}}{(1 + \alpha_\lambda)^2 e^{\delta_\lambda L} - (1 - \alpha_\lambda)^2 e^{-\delta_\lambda L} - \rho(1 - \alpha_\lambda^2)e^{\delta_\lambda L} + \rho(1 - \alpha_\lambda^2)e^{-\delta_\lambda L}} \quad (8)$$

with:

$$\alpha_\lambda = \sqrt{\frac{Ea_\lambda}{Ea_\lambda + 2\bar{b}_{2\lambda} \cdot Es_\lambda}} \quad (9)$$

$$\delta_\lambda = C_X \sqrt{Ea_\lambda(Ea_\lambda + 2\bar{b}_{2\lambda} \cdot Es_\lambda)} \quad (10)$$

The coefficients  $\alpha_\lambda$  and  $\delta_\lambda$  are referred to as linear diffusion modulus and extinction coefficient, respectively. Pruvost and Cornet (2012)<sup>24</sup> modelled the light attenuation of an PBR under solar illumination, distinguishing between the collimated ( $q_{//}$ ) and diffuse ( $q_{\cap}$ ) parts of the luminous flux and taking into account the angle of inclination of the PBR (noted  $\beta$ ) and the angle between the position of the sun and the normal to the PBR (noted  $\beta$ ). This leads to the following equations:

$$\frac{G_{\text{col}(z)}}{q_{//}} = \frac{2}{\cos \theta} \frac{(1 + \alpha)e^{-\delta_{\text{col}}(z-L)} - (1 - \alpha)e^{\delta_{\text{col}}(z-L)}}{(1 + \alpha)^2 e^{\delta_{\text{col}}L} - (1 - \alpha)^2 e^{-\delta_{\text{col}}L}} \quad (11)$$

$$\frac{G_{\text{dif}(z)}}{q_{\cap}} = 4 \frac{(1 + \alpha)e^{-\delta_{\text{dif}}(z-L)} - (1 - \alpha)e^{\delta_{\text{dif}}(z-L)}}{(1 + \alpha)^2 e^{\delta_{\text{dif}}L} - (1 - \alpha)^2 e^{-\delta_{\text{dif}}L}} \quad (12)$$

with

$$\delta_{\text{col}} = \frac{\alpha C_X}{\cos \theta} (Ea + 2b \cdot Es) \quad (13)$$

$$\delta_{\text{dif}} = 2\alpha C_X (Ea + 2b \cdot Es) \quad (14)$$

The local irradiance is then determined by:

$$G(z) = G_{\text{col}(z)} + G_{\text{dif}(z)} \quad (15)$$

In order to locally determine the amount of light absorbed by the microalgae, it is necessary to know the biomass concentration ( $C_X$ ), the intensity and spectrum of the light source and the optical properties of the microorganism under study.

### Radiation properties of microalgae

Radiation properties are necessary to determine the radiation field. The determination of the radiative properties of microalgae can be carried out using the Lorenz-Mie solution, a theory of light diffraction using Maxwell's equations, and applying to spherical particles between 0.1

and 10 times the wavelength of the received radiation. To solve these equations, the following parameters are required:<sup>107,108</sup> (i) the complex refractive index of the particle  $m_\lambda$ , (ii) its size, and (iii) the refractive index of the medium surrounding the particles  $n_{m,\lambda}$ . The complex

refractive index of the microorganism is composed of two parts (eqn (13)): the first real part  $n_\lambda$  represents the scattering part of the index,<sup>107</sup> and the second  $\kappa_\lambda$  complex part represents the absorption part of the index and is strongly wavelength-dependent.<sup>107</sup>

$$m_\lambda = n_\lambda + i\kappa_\lambda \quad (16)$$

The determination of the complex index is based on the absorption properties of the pure pigments as well as their proportion in the cell and the index of the refractive anchor point. Once the characteristics of the light radiation have been determined (absorption, scattering and phase function per cell), they are converted into mass coefficients using the biomass water fraction  $x_w$ , the density of the dry biomass and the Sauter  $D_{32}$  diameter.<sup>108</sup> Considering that the microalga behaves like a double concentric sphere, Kandilian (2016)<sup>108</sup> was able to determine the radiative properties of the microalga *Chlorella vulgaris* as a function of its pigmentary material and compare them with experimental values obtained using an integrating sphere spectrophotometer.

### Characterization of the amount of light absorbed by microalgae

As previously mentioned, irradiance  $G$  is the parameter used to characterize the local growth of a microalgae culture. It represents the photon flux available locally but does not give information on the flux absorbed by the microalgae. Indeed, depending on its shape, size or pigmentary material, microalgae will absorb the available light differently. To characterize the behavior of a microalgae culture it is important to analyse in terms of absorbed flux and not in terms of available flux. This has already been done in the literature to characterize growth in an PBR or the production of metabolites.<sup>20,109,110</sup> The photon absorption rate can then be studied per unit mass of microalgae and called MRPA (mean rate of photons absorption, noted in  $\mu\text{mol kg}^{-1} \text{s}^{-1}$ ), noted  $\langle A \rangle$ . Mass absorption cross section (Ea) ( $\text{m}^2 \text{ kg}^{-1}$ ) enable to convert irradiance to rate of photon absorption. Kandilian<sup>108,110</sup> showed, for several strains, that mass absorption cross section is linearly linked to the pigment content in the microalgal biomass. MRPA represents the specific rate of conversion of photons into biomass and takes into account: the light flux received at the surface, the pigment composition as well as the biomass concentration. Its expression, in the case of a planar PBR, is as follows:

$$\langle \mathcal{A} \rangle = \frac{1}{L} \int_0^L \mathcal{A} = \frac{1}{L} \int_0^L \overline{Ea} G(z) dz \quad (17)$$

The MRPA can also be determined by carrying out a balance on the photonic phase in the reactor volume.<sup>109</sup>

$$\langle \mathcal{A} \rangle = \frac{S}{VC_X} (q_0 - q_L) = \frac{a_s}{C_X} (q_0 - q_L) \quad (18)$$

where  $a_s$  represents the specific surface area of the PBR, *i.e.* the ratio between the illuminated surface area and the volume of the PBR (in  $m^{-1}$ ),  $q_0$  and  $q_L$  represent the photon flux density (PFD) at the input and output of the system, respectively. When the entire light flux is absorbed in the PBR, this expression can be summed up to:

$$\langle \mathcal{A} \rangle \approx \frac{q_0 a_s}{C_X} \quad (19)$$

Note that the latter relationship is particularly interesting. Following this parameter in dynamic conditions becomes indeed trivial. It is sufficient to know the flux received at the surface of PBR (*via* a weather station for example), the biomass concentration (by measuring the dried biomass) and the culture thickness.

### Engineering factors driving PBR productivity

When all parameters are kept at the optimum operating level, the output depends solely on the amount of light absorbed. Pruvost *et al.* (2015)<sup>111</sup> modelled the growth of microalgae under these conditions based on their biological and optical properties. Locally, the specific oxygen production rate  $J_{O_2}$  (in  $\text{mol}_{O_2} \text{ kg}^{-1} \text{ s}^{-1}$ ) is determined by the equation:

$$J_{O_2} = \left[ \rho_M \bar{\phi}'_{O_2} \frac{K \cdot \mathcal{A}}{K + \mathcal{A}} - \frac{J_{NADH_2}}{v_{NADH_2-O_2}} \frac{K_r}{K_r + \mathcal{A}} \right] \quad (20)$$

where  $\rho_M$  represents the maximum photon conversion efficiency,  $\bar{\phi}'_{O_2}$  the molar quantum oxygen efficiency of the Z scheme of photosynthesis (in  $\text{mol}_{O_2} \mu\text{mol}^{-1}$ ),  $K$  the half-saturation constant of photosynthesis (in  $\mu\text{mol} \text{ kg}^{-1} \text{ s}^{-1}$ ),  $K_r$  the saturation constant describing respiration to light (in  $\mu\text{mol} \text{ kg}^{-1} \text{ s}^{-1}$ ),  $J_{NADH_2}$  the specific rate of regeneration of respiratory chain co-factors (in  $\text{mol}_{NADH_2} \text{ kg}^{-1} \text{ s}^{-1}$ ) and  $v_{NADH_2-O_2}$  the stoichiometric coefficient of regeneration of respiratory chain

$$\overline{S_{X \max}} = (1 - f_d) \rho_M \bar{\phi}_X \frac{2\alpha}{1 + \alpha} \left[ \bar{\chi}_d K \ln \left[ 1 + \frac{q_0 E_a}{K} \right] \right]$$

co-factors. Each parameter depends on the microalgae growth. The saturation constant  $K_r$  is dependent on the rest of the parameters of the kinetic model by the equation:

$$K_r = \frac{\mathcal{A}_c}{\frac{J_{NADH_2}}{v_{NADH_2-O_2} \rho_M \bar{\phi}'_{O_2} E_a} \left[ \frac{1}{\mathcal{A}_c} + \frac{1}{K} \right] - 1} \quad (21)$$

The volumetric local growth rate  $r_X$  (in  $\text{kg}_X \text{ m}^{-3} \text{ s}^{-1}$ ) is related to the specific rate of oxygen production  $J_{O_2}$  by the formula:

$$r_X = \frac{J_{O_2} C_X M_X}{v_{O_2-X}} \quad (22)$$

where  $M_X$  and  $v_{O_2-X}$  represent, respectively, the C-molar mass of the biomass ( $\text{kg}_X \text{ mol}^{-1}$ ) and the stoichiometric coefficient of oxygen production. In the reactor volume ( $V_R$ ), the MRPA value,  $A$ , obtained from the radiative transfer model (eqn (14)–(16)), changes as the light attenuates. The average volumetric velocity is then calculated by integrating the local volumetric growth rate over the reactor volume:

$$\langle r_X \rangle = \frac{1}{V_R} \int \int \int r_X dV \quad (23)$$

In a photobioreactor, the balance equation giving the temporal evolution of growth as a function of time is written:

$$\frac{dC_X}{dt} = -D \cdot C_X + \langle r_X \rangle \quad (24)$$

Solving this equation predicts the volume productivity of the PBR:

$$\overline{P_X} = \langle r_X \rangle = D \cdot C_X \quad (25)$$

where  $D$  is the dilution ratio of the photobioreactor (in  $\text{h}^{-1}$ ), related to the passage time  $\tau_p$  and the biomass outflow rate  $Q_s$  by:

$$D = \frac{1}{\tau_p} = \frac{Q_s}{V_R} \quad (26)$$

The productivity of a PBR for a given strain depends, as mentioned above, on many parameters (temperature, agitation, light attenuation...). Maximum performance for a given strain is therefore reached when all these parameters are maintained at the optimum and the entire light flux is absorbed in the culture without the appearance of dark zones (luminostat regime). In order to determine the maximum performance of a photobioreactor under constant incident flux, Takache *et al.* (2010)<sup>45</sup> proposed a simplified engineering law for PBR sizing:

$$\overline{S_{X \max}} = \rho_M \bar{\phi}_X \frac{2\alpha}{1 + \alpha} \frac{K}{E_a} \ln \left[ 1 + \frac{q_0 E_a}{K} \right] \quad (27)$$

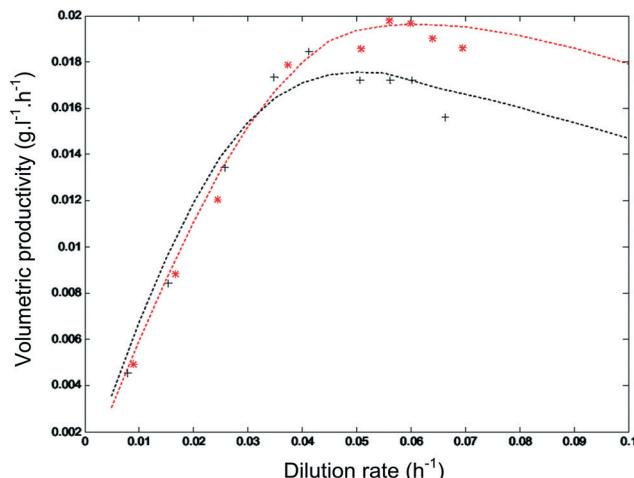
When growing microalgae under solar conditions the formula becomes:<sup>112</sup>

$$\overline{S_{X \max}} = \left( 1 - f_d \right) \rho_M \bar{\phi}_X \frac{2\alpha}{1 + \alpha} \left[ \frac{2q_0 E_a}{K} + (1 - \bar{\chi}_d) \cos \theta \frac{K}{E_a} \ln \left[ 1 + \frac{q_0 E_a}{K \cos \theta} \right] \right] \quad (28)$$

where  $f_d$  is the dark fraction of the reactor,  $\theta$  the angle formed between the position of the sun and the normal with respect to the PBR and  $\bar{\chi}_d$  is the diffuse fraction of the incident flux. In addition, the volume productivity of a PBR is related to the area productivity by the specific surface area of the PBR ( $a_s$ ):

$$\overline{P_X} = a_s \overline{S_X} \quad (29)$$

The latter equation emphasizes the independence of the production potential of a PBR from its geometry. Thus, the larger the specific surface area of the photobioreactor, the



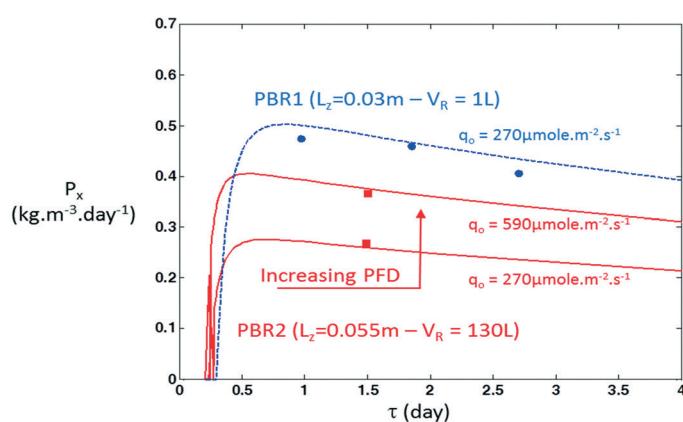
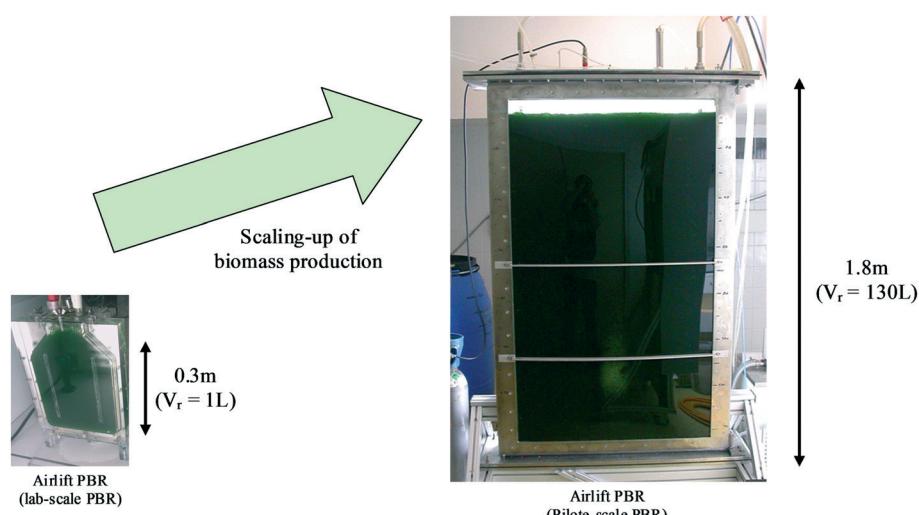
**Fig. 13** Comparison between experimental data and theoretical predictions for *C. vulgaris* under white (experimental: black points; theoretical: black dashed line) and red (experimental: red points; theoretical: red dashed line) radiations of volumetric productivity.

higher the volume productivity will be and in the case of a planar system, the maximum volume productivity ( $\text{kg}_x \text{ m}^{-3} \text{ d}^{-1}$

or  $\text{g l}^{-1} \text{ h}^{-1}$ ) increases with decreasing culture thickness ( $a_s = S/V_R = 1/L$ ). This results in a reduction in the costs of circulating the culture and post-treatment of the biomass, hence the growing interest in PBR with high volumic productivity. The model for the volumic productivity (eqn (27) and (29)) has been tested for the cultivation of *Arthrospira platensis*.<sup>10</sup> A deviation of less than 15% was observed,<sup>10</sup> despite the different growing conditions: batch and continuous conditions, annular, cylindrical and plate photobioreactors. The same model was used to predict with an average relative error less than 10% (Fig. 13) the productivity of *Chlorella vulgaris* for two light sources from a LED panel, white LED with mean emissive wavelength at around 440 nm and red LED (maximum emissive peak at 660 nm).<sup>11,3</sup>

The engineering factor are also very useful to scale-up the biomass productivity in photobioreactors of different sizes (Fig. 14). The lab-scale PBR has a volume of 1 l and a thickness of 3 cm and the pilot scale PBR a volume of 130 l and a thickness of 5.5 cm. A cultivation of *Neochloris oleoabundans* was made in the two PBR for two values of the incident PFD.<sup>24</sup>

A maximal deviation of 15% was found for the prediction of biomass productivity as a function of PBR geometry and



**Fig. 14** Comparison of the volumic productivity in PBR of different sizes and comparison the model of eqn (27)–(29) with for *Neochloris oleoabundans*:  $\rho_M = 0.8$ ,  $\varphi_M = 1.83 \times 10^{-9}$  kg per  $\mu\text{mole}$ ,  $K = 90 \mu\text{mole per m}^2 \text{ s}^{-1}$ .

PFD. The positive effect of increasing the PFD on biomass productivity and the negative effect of increasing the depth of culture can be seen.

## Conclusion

The development of microalgae culture processes requires knowledge of their physiology, and more particularly their ability to capture light and the associated metabolism, and their behaviour according to the experimental conditions, in particular the limiting factors. The growth of photosynthetic microorganisms is a complex process, affected by many environmental factors such as light, mixing, gas-liquid transfer, temperature, and pH. Depending on the use, different types of photobioreactor have been designed. For laboratory studies, photobioreactors must be designed to control the various parameters that influence biomass productivity as well as possible. The results obtained in perfectly controlled conditions are essential for modelling culture systems in order to predict their productivity. No perfect type of PBR exists for the mass cultivation of microalgae, because of the need to make a compromise between investment and operating costs. In this article, some examples of photobioreactors, open systems to the atmosphere, or closed systems with a culture confined in the photobioreactor, are described. The different modelling approaches were discussed, distinguishing between models based on parameters fitted from experiments and predictive models that take into account radiative models to predict the distribution of light in photobioreactors.

## Conflicts of interest

There are no conflicts to declare.

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