Microalgae Bioreactors

João C. M. Carvalho, Marcelo C. Matsudo, Raquel P. Bezerra, Lívia S. Ferreira-Camargo and Sunao Sato

Abstract Photobioreactor design and operation mode are essential steps to ensure a high overall microalgae yield and cell productivities, making viable the commercial production. For this reason, there are trends of research in the field of microalgae that encompass design and development of reactor systems towards maximum productivity with minimum operation costs. In the literature, various photobioreactor designs have been employed such as open ponds, bubble column, flat plate, and tubular (conical, helical, etc.). Open ponds are the most commonly applied photobioreactor design in industrial processes. On the other hand, studies have been focused on tubular photobioreactors due to the possibility of achieving high volumetric productivity and better biomass quality. Therefore, in this chapter, some photobioreactor designs and their characteristics such as geometrical configuration, building material, and cell circulation systems will be discussed. Moreover, the operation mode, such as temperature and pH control, nutrient feeding, CO_2 addition systems, flow rate, light supply, mixing, cultivation process and cleanness will also be considered to be important parameters in this field.

Keywords Photobioreactors · Microalgae · Photobioreactors operation · Microalgae culture circulation · Microalgae cultivation processes

Acronyms

DHA	Docosahexaenoic acid
DO	Dissolved oxygen
DW	Dry weight
H/D	Height to diameter ratio
HDPE	High Density Polyethylene
LDPE	Low Density Polyethylene
NER	Net Energy Ratio
PBR	Photobioreactor
PBRs	Photobioreactors
PEP	Photosynthetic efficiency
PMMA	Rigid acrylic

J. C. M. Carvalho (✉) · M. C. Matsudo · R. P. Bezerra · L. S. Ferreira-Camargo · S. Sato
Department of Biochemical and Pharmaceutical Technology, University of São Paulo, Av. Prof. Lineu Prestes 580, Bl. 16, São Paulo 05508-900, SP, Brazil
e-mail: jcmdcarv@usp.br

R. Bajpai et al. (eds.), *Algal Biorefineries,* DOI 10.1007/978-94-007-7494-0_4,
© Springer Science+Business Media Dordrecht 2014

PVC Poly Vinyl Chloride
TRC Transparent rectangular chamber
UV Ultraviolet
VAP Vertical alveolar panels
VFPP Vertical flat-plate photobioreactor

1 Introduction

Microalgal production has been studied for decades, given the wide variety of potential metabolic products, such as protein, lipids, starch, vitamin, enzymes, polymers, and pigments that can be used as food supplements, feed, biodiesel, and "green energy" products.

Different reactor configurations and cultivation strategies are still targets of intense research to improve biomass growth, since all have some inherent problems, such as control of light intensity, CO_2 introduction, pH control, inoculum size, proper flue gas rate, optimal nutrition level, oxygen concentration control, culture mixing, water loss, contamination, temperature control, nutrients addition, huge land area, algae collection and algae aggregation and adhesion.

Most of problems are either unresolvable or may require a lot of costly modifications that are not very much favored by microalgae producers. Among main designs there are: open ponds, bubble column, flat plate, and tubular photobioreactors. Each design has its own problems which place the efficiency far behind theoretical calculations and wishful expectations.

Commercial-scale culture of microalgae generally requires the ability to economically produce ton quantities of algal biomass. Acién et al. (2012) showed that the cost of photobioreactors is the major factor in the production cost for large scale and that the reduction of the photobioreactor cost dramatically decreases the biomass unit production cost.

Mode of culture circulation of culture, providing light availability, CO_2, O_2 removal, pH control, temperature and nutrient feeding, materials utilized for constructing photobioreactor and mode of cleaning are several basic features that must be considered when building a photobioreactor. In this chapter the main characteristics of different configurations of photobioreactors will be considered.

2 Photobioreactors

Different photobioreactor configurations have been designed, built, and described depending on the ultimate goal. Important design aspects include lighting, mixing, water consumption, CO_2 consumption, O_2 removal, nutrient supply, temperature maintenance, material for construction, and cleaning. In general, sophisticated photobioreactors are more versatile but more expensive to construct and more

complicated to operate. The choice depends on several factors, including the microalgal growth mode, the culture medium composition and the value of the final product. The photobioreactor geometry must maximize production of microalgal bioproducts while minimizing the land surface occupied.

2.1 Types of Photobioreactors

Several types of photobioreactors have been constructed to support the demands of microalgal products such as open ponds, illuminated stirred tank reactors, tubular or flat plate photobioreactors. Although there are several types of photobioreactors described in the literature, there are still several researchers proposing new types of photobioreactors in order to increase productivity and reduce costs of industrial production. Many challenges include: (i) minimizing contamination (ii) efficient supply of carbon dioxide and light; (iii) controlling cultivation conditions; (iv) reducing capital and production costs; and (v) minimizing space requirements (Marxen et al. 2005; Molina Grima et al. 1999). This item is going to address some of the main types found in the literature.

In general, we can classify the several types of photobioreactors as:

1. Indoor or outdoor systems
2. Open or closed systems
3. Photobioreactor configurations: Open ponds, flat plate, vertical-column, tubular and their variations such as horizontal, inclined, vertical, spiral, conical, helical; manifold and serpentine.

2.1.1 Indoor or Outdoor Systems

Several types of lighting have been used to produce large quantities of phototrophic algae such as sunning, artificial light or both. Outdoor systems are where light is supplied as sunlight and the cells are subject to environmental variations. Sun light is free-cost, the cheapest light source available, whereas artificial light sources may be very expensive. Solar energy contains the full spectrum of light energy, and, through a specific UV filter, it can provide a suitable absorption wavelength for both microalgae cell growth and target product production (Chen et al. 2008). Efficient utilization of solar energy can simultaneously solve the problems of a high operating cost, electricity consumption and environmental pollution. Outdoor system has been utilized in industrial scale to Chlorella and *Arthrospira* (*Spirulina*) production for health food, *Dunaliella salina* for β-carotene, *Haematococcus pluvialis* for astaxanthin, *Crypthecodinium cohniia* and *Schizochytrium* sp. for docosahexaenoic acid (DHA). It is worth mentioning that although according to Silva et al. (1996) the correct scientific designation for *Spirulina platensis* is *Arthrospira platensis,* in this chapter, the denomination given by the authors of the referenced articles was maintained.

Generally microalgae cultures in photobioreactors are suitable for outdoor cultures. Most outdoor photobioreactors are characterized by largely exposed illumination surfaces and are subject to changes in time of day, weather, season, and geography. Commercial outdoor cultivation is generally restricted to tropical and subtropical zones in regions of low temperature change, rainfall and low cloud cover. Unfortunately, all outdoor reactors using natural light are subject to the absence of light during night time, what suggest that studies focusing the night light supplying with solar energy accumulated during the day could be useful for the development of this technology. In fact, According to Chisti (2007), biomass losses might reach as high as 25 % during the night, depending on the light intensity during the day, the temperature during the day, and the temperature at night.

Several indoor microalgal cultivations have been extensively studied in laboratory-scale photobioreactors. However, scientific information about outdoor cultivations is still scarce, even though those are so important for industries.

Some care need to be taken into account when attempting to change the type from indoor to outdoor. Scoma et al. (2012) related that the H_2 production from *Chlamydomonas reinhardtii* is possible after a previous cell acclimatizing to sunlight, a condition that caused a number of physiological changes, namely: (i) a decrease in the chlorophyll content per unit of dry weight; (ii) an increase in the photosynthesis and respiration rates, and (iii) a higher induction of the xanthophylls cycle pigments as compared to non-acclimated cultures.

In the indoor systems, light is supplied by electric lights and it allows control over illumination and temperature in the photobioreactor cultivation. Laboratory-scale photobioreactors are usually artificially illuminated with fluorescent or other types of lamps, which can require high power consumption and high operating cost. This system is more used in laboratory scale since energy used to keep the light on can endear to industrial production. On the other hand, artificial lighting techniques can provide to researchers an insight into how algae respond to varying light conditions and other parameters. In addition, it is also possible to transmit solar energy from outside to illuminate indoor photobioreactors, such as solar-energy-excited optical fiber systems. The major obstacle to their practical application is the high power consumption and operating cost due to the need for artificial light sources.

Lopez-Elias et al. (2005) related that outdoor cultivation of diatom *Chaetoceros muelleri* growing may be as safe and reliable as indoor one, and that the savings caused by the lower power consumption may mean a reduction of more than 40 % of the annual operating cost of the area of microalgae production.

2.1.2 Open or Closed Systems

Open systems are mostly uncovered ponds and tanks or natural lakes. These systems have almost always been located outdoor and rely on natural light for illumination. Although they are inexpensive to install and run, open systems suffer from many problems: cultures are not axenic so contaminants may outcompete the desired algal species; predators like rotifers can decimate the algal culture; weather

variations can hinder proper control of nutrients, light intensity, and CO_2; poor light utilization by the cells; evaporative losses of water and others volatile compounds to the atmospheres; and requirement of large areas of land (Carvalho et al. 2006).

As open system is more readily contaminated than closed culture vessels such as tubes, flasks, carboys or bags, it is limited to a relatively small number of algae species that control contamination by using highly alkaline, saline selective environments or other strategies. The open raceway pond reactor can be used to strains cultivation that, by virtue of their weed-like behavior (e.g. *Chlorella* sp.) or by their ability to withstand adverse growing conditions as *Spirulina* (*Arthrospira*) sp. or *Dunaliella* sp., can outcompete other microorganisms (Del Campo et al. 2007).

Open ponds systems are made of leveled raceways 2–10 m wide and 15–30 cm deep, running as simple loops or as meandering systems. Each unit covers an area of several hundred to a few thousand square meters. Turbulence is usually provided by rotating paddle wheels, which create a flow of the algal suspensions along the channels at a rate of 0.2–0.5 m s^{-1} (Del Campo et al. 2007).

Cultivation of microalgae using natural and man-made open-ponds is technologically simple, but not necessarily cheap due to the high downstream processing cost and low cell productivity. Products of microalgae cultured in open-ponds could only be marketed as value-added health food supplements, specially feed and reagents for research (Lee 2001) and energy source.

An alternative to open ponds are closed photobioreactors which provide better options to grow every microalgae strain, protecting the culture from invasion of contaminating organisms and allowing exhaustive control of operation conditions. Moreover, in closed photobioreactors, the higher ratio between illuminated area and culture volume leads to higher final biomass concentration compared to open-pond culture.

The technical difficulty in sterilizing closed photobioreactors has hindered their application for the production of high value pharmaceutical products. Cultivation of microalgae in heterotrophic mode in sterilizable fermentors has achieved some commercial success (Lee 2001).

Closed photobioreactors can adopt a variety of designs and operation modes. But only a few attempts have been made for scale-up photobioreactors to commercial size. They offer higher productivity and better quality of the generated biomass (or product), although they are certainly more expensive to build and operate than the open systems.

Closed photobioreactors are considered the most productive systems but to be competitive their cost must be reduced below the cost of the current open raceways reactors. In addition, large facilities capable of producing more than 150 t/ha·year must be operated with low labor costs, using flue gases as carbon source and wastewater as growth medium in the largest possible extent (Acién et al. 2012). But, even aiming to diminish the cost, it is necessary to keep in mind the application of the product to be produced.

New design has been done by improving shaping of the photobioreactor (PBR), controlling environmental parameters during cultivation, aseptic designs, cleanness mode and operational approaches to overcome rate-limiting of growth, such as pH,

temperature, light supply, nutrient addition, mixing, and gas diffusion. The main final goal of any PBR is reduction in biomass production costs and/or allowing mixotrophic or heterotrophic cultivation of microalgae. A feasible alternative for phototrophic cultures in PBRs, but restricted to a few microalgae species, is the use of their mixotrophic or heterotrophic growth.

Algae have also been cultivated in bag type reactors. However, according to Borowitzka (1999), the big bag system suffers from the need to be operated indoors for adequate temperature control. In addition, if installed indoors, the large bags cannot be sufficiently illuminated by artificial lighting, and mixing is generally insufficient (Kunjapur and Eldrigde 2010). Thus, it is necessary additional studies considering this type of photobioreactor, including temperature maintenance and mixing systems.

2.1.3 Photobioreactors Configurations

Many configurations of photobioreactors have been devised and built. They range from ponds to tubular and cylindrical systems to conical systems to flat-sided vessels. The most natural method of growing algae is through open-pond. For convenience, the closed photobioreators are described herein by categories:

1. Vertical column photobioreactor
2. Tubular photobioreactor
3. Flat panel photobioreactor

There are some works on high performance photobioreactors of closed type. Flat-plate, horizontal and inclined tubular photobioreactors are among the most suitable types for mass cultivation of algae because they have large illumination surface (Chisti 2006), but there are difficulties in scaling them up. Though large-scale designs are the focus, the majority of experiments comparing the designs were performed at laboratory scale.

For any type of photobioreactor, the mixing is an essential parameter to be considered. Some preliminary comments are presented here. More detailed study will be presented in posterior sections.

The mixing systems most described in the literature are those mechanically agitated with paddle wheels found mainly in open ponds and agitation with air as found in bubble column and air-lift systems. Choice of mixing system depends on the bioreactor configuration, type of cell and medium composition properties. Liquid flow velocity must be sufficiently high to ensure a turbulent flow so that cells do not stagnate in the interior darker portion of the tube for long. However, excessive turbulence can damage cells and this poses an upper limit on the culture velocity. The trichomes of *Arthrospira platensis* are sensitive to shear when mechanical agitation systems are used, so pneumatic system is most suitable for this type of cultivation. Markl et al. (1991) showed that hydrodynamic stress reduced the size of the cyanobacterial trichomes of *Spirulina platensis.* Sánchez Pérez et al. (2006) related

that the average shear rate is related with the superficial aeration velocity and the rheological properties of the fluid.

The stirred reactors have paddle wheels which consist of simple partial-depth blades or high speed rotors whereas in bubble column and airlift photobioreactors, the agitation occurs due to gas added that causes agitation in the culture. One of the costs of growing algal cultures in airlift and bubble column reactors is that of the added gases. Merchuk et al. (1998) compared *Porphyridium* culture in an airlift photobioreactor with that in a bubble column reactor. By adding a helical flow promoter to the airlift system, the cost of gases for the production per kg microalgal biomass was 50 % of that in the bubble column to achieve the same specific growth rate.

Photobioreactors such as bubble-column, and stirred-tank have good scalability though their use in outdoor cultures is limited since they have low illumination surface areas (Ugwu et al. 2008), but they have great potential for industrial bioprocesses, because of the low level and homogeneous distribution of hydrodynamic shear. One growing field of application is the flue-gas treatment using algae for the absorption of CO_2.

Degen et al. (2001) proposes airlift photobioreactor with baffle to induce a regular light cycling of microalgae and increase the cell productivity. The reactor is based on the airlift principle and baffles to induce a regular light cycling of microalgae. The use of baffles can contribute to productivity enhancement in several possible ways as (i) the baffles increase the residence time of gas bubbles in the reactor and this can affect the mass transfer rates of carbon dioxide and oxygen. However, because oxygen did not accumulate and CO_2 was supplied on demand so that it was never a limiting factor, the mass transfer effects do not explain the better performance of the baffled reactor; (ii) the baffles affect mixing; however, both reactors were always mixed sufficiently to supply the cells with dissolved nutrients. Thus, it seems that the baffles provided the better light cycling of the microalgae, thus improving the performance of the cultivation system.

2.2 Characteristics of Photobioreactors

There are a number of different implementations of photobioreactors. Some characteristics of main photobioreactors cited in the literature such as open ponds, vertical column and tubular photobioreactors will be described in this section.

Open ponds Several types of open ponds have been developed for large-scale outdoor algal culture. The most common is the “raceway pond”, an oval for resembling a car-racing circuit (Lee 2001; Pulz 2001; Chisti 2007) (Fig. 1). These cultivation ponds present relatively low construction and operating costs and can be constructed on degraded and non agricultural lands, avoiding use of high-value lands and crop producing areas (Chen 1996; Tredici 2004).

Two other types of open ponds more common are circular ponds with a rotating arm or long channel ponds, single or connected to each other that are mixed with a paddle wheel.

Fig. 1 Laboratory scale open tanks

Open ponds are the simplest algae growing systems, which consist of open vessels using outdoor sunlight. Individual ponds are up to 1 ha in area with an average depth of about 20–30 cm and are mixed via a paddle wheel that circulates water with nutrients and microalgae. Paddle wheels seem to be the most efficient device for mixing the algal cultures and are the easiest to maintain. The design of the paddle wheel also affects flow rate and energy requirements. Pond size affects water circulation, which in turn affects the design and operating cost of the circulation/mixing system (Borowitzka 2005).

Besides paddle wheels, earlier designs also used air lifts, propellers, and drag boards (Becker 1994). Drag board is a wooden board that closes the pond in cross-section except for a slot of only some centimeters above the bottom. It is dragged through the culture pond to create turbulence (Valderrama et al. 1987).

Open ponds may have several drawbacks: (i) Growth parameters depend on local weather conditions, which may not be controlled, causing seasonality to the production; rainfall may also be a problem, significantly diluting the medium in the pond and favoring rapid invasion by predators; (ii) Monoculture of the desired microalgae is difficult to maintain for most microalgae species because of constant contamination by the air, except for extremophile species; (iii) reduced light diffusion inside the pond, decreasing with depth and causing self-shading; consequently shallow depth is required for ponds and they have a low volume to area ratio; (iv) CO_2 is not used efficiently; (v) a large area of land is required, so only unproductive or waste land can be used; (vi) biomass productivity is lower than that in closed cultivation systems; (vii) harvesting is laborious, costly, and sometimes limited for low cell densities; (viii) continuous and clean water is needed; and (ix) production of food

or pharmaceutical ingredients is very limited or even not viable (Vonshak 1997b; Perez-Garcia et al. 2011; Xu et al. 2009).

The few commercial species that are currently being successfully cultured in large open ponds are extremophiles growing in a highly selective environment (high pH, salinity, or temperature). These conditions preclude the growth of most other algae and even many bacteria (Xu et al. 2009). Single, rectangular ponds with a paddle wheel (raceway ponds) are the most widely used for the production of *Arthrospira* due to high alkalinity, *D. salina* due to high salinity, and *Haematococcus* sp., and represent the most efficient design for the large-scale culture of most microalgae. Large inoculum amounts is another approach that can be used for fast-growing species such as *Chlorella* (Borowitzka 1999).

When installing open ponds, source and quality of water, and availability and cost of land are important factors to be considered, as well as near-optimal climatic conditions. If the land costs are high, other types of reactors, such as tubular photobioreactor, may be more convenient. The larger the pond area, the greater the amount of water lost by evaporation and when cultivating marine or hypersaline algae, natural source of seawater or saltwater is desirable for the cost of salt is prohibitive. Moreover, water sources may also contain heavy metals or other contaminants, and a final product contamination makes the microalgae culture inappropriate (Borowitzka 2005).

The final product is certainly an important factor to take into account when choosing a photobioreactor. Depending on the purpose of the use of the biomass, a "cleaner" production may be required and open ponds may become inadequate. On the other hand, if the biomass is used for animal feed or if a specific bio-product is extracted from the algae and contamination is not a concern, open ponds can be utilized.

Vertical-column photobioreactor Vertical tubular photobioreactors were among the first enclosed algal mass culture systems described in the literature, but their high cost discouraged their use (Miyamoto et al. 1988).

Vertical columns are frequently used especially in larger laboratory scale for indoor experiments. Diameters of 20 cm and more are necessary to work with sufficient volume. This leads to considerable high dark fraction in the middle of the cylinder. This part does not contribute to productivity or has even detrimental effects on growth (Posten 2009). To leave this part out of the internal reactor space the so-called annular column has been developed.

Various designs and scales of vertical-column photobioreactors have been tested for cultivation of algae (Choi et al. 2003; Vega-Estrada et al. 2005; García-Malea et al. 2006; Kaewpintong et al. 2007). Vertical-column photobioreactors are compact, low-cost, and easy to operate monoseptically (Sanchez Miron et al. 2002). Furthermore, they are very promising for large-scale cultivation of algae. It was reported that bubble-column and airlift photobioreactors (up to 0.19 m in diameter) can attain a final biomass concentration and specific growth rate that are comparable to values typically reported for narrow tubular photobioreactors (Sanchez Miron et al. 2002). Some bubble column photobioreactors are equipped with draft tubes

to improve the circulation. In this case, mixing occurs between the riser and the downcomer zones of the photobioreactor through the walls of the draft tube (Ugwu et al. 2008).

Vertical tubular reactors have the inherent advantage, over horizontal or serpentine reactor, of allowing continuous gassing of the cultures, resulting in efficient gas transfer (CO_2 in and O_2 out) and mixing of the cultures (Miyamoto et al. 1988). These reactors are mainly used because of their simple construction; excellent heat and mass transfer properties (Lau et al. 2010; Kaidi et al. 2012), better handling of solids, low operating costs and easier to operate, high height to diameter ratio (H/D) that minimize the effect of gas distributors where small bubbles are generated. It was found that the gas distributor design depended on the system properties (Haque et al. 1986). In bubble columns, the source of agitation is the pneumatic power input.

Extensive research on mixing has been carried out in bubble column reactors and empirical correlations have been given for gas holdup, however the flow inside these reactors remains complex. Often three flow regimes have been assumed in bubble columns (homogeneous, transition and heterogeneous) with the increase of gas velocities (Chen et al. 1994). Comprehension of the hydrodynamics inside the vertical column reactors is important for modeling and optimization of gas-liquid reactors.

The bubble size was the most important parameter for better understanding the dispersion of the gas inside the bubble column reactor. The variation in the average bubbles diameter depends on the type of sparger (Shah et al. 1982) and increases slightly with the increase of superficial gas velocity (Kaidi et al. 2012).

Gas hold up is an important parameters characterizing the gas–liquid systems. It is necessary to the hydrodynamic design in different industrial processes because it governs gas phase residence time and gas–liquid mass transfer. It depends mainly on the superficial gas velocity and the type of sparger.

It is worth mentioning that vertical tubular reactors can be efficiently mixed distributing Rushton turbines along the photobioreactor (Ávila-Leon et al. 2012), thus improving both the light cycling and the mass transfer.

Tubular photobioreactors (PBR) Tubular photobioreactors are the most popular closed systems, being basically constituted by tubes arranged in multiple possible orientations such as vertical, horizontal, inclined, spiral, helicoidal and their variations, but all orientations have basically the same work way. Besides the arrangement of tubes, tubular photobioreactors differ in the tube length, flow velocity, circulation system, and geometric configuration of the light receiver.

Tubular photobioreactor is a good option to obtain large microalgae biomass. It is increasing the number of facilities that produce microalgae using tubular photobioreactors aiming to get the advantages of this system, like high productivity and minor area required to cell growth if compared with open ponds. Tubular reactors are made of transparent tubes (rigid or flexible) arranged in parallel lines, inclined or not, at the ground or disposed at a difference of height, coupled by manifolds.

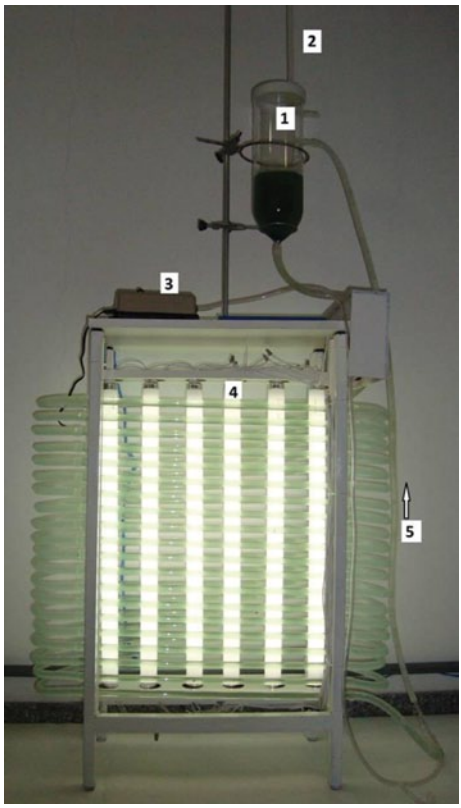

Fig. 2 Tubular photobioreactor. (*1*) Degasser; (*2*) Condenser tube; (*3*) Air pump; (*4*) 20 W Fluorescent lamps; (*5*) Airlift system (Carvalho et al. 2013). (With kind permission from Springer Science+Business Media)

The single tubes can be straight, they can follow a meandering course either flat on the ground or ordered in panels or coils, also called helical reactor. The tubes have diameters of 10 to maximum 60 mm, and lengths of up to several hundred meters. The employment of tubes leads to a quite high surface to volume ratio over 100 m^{-1}, which is one of the main advantages of this design (Posten 2009). Increasing tube diameter results in a decrease in the surface/volume ratio, and this factor has a strong impact on the culture.

Horizontal tubular photobioreactor (Fig. 2) allow a better use of light (relative to vertical orientation) requiring however a large area installed. The horizontal arrangement of the tubes also raises difficulties in introducing CO_2 and removing O_2.

Culture circulation through the tubes can be done by various methods; use of airlift circulators is especially common. High levels of mixing are necessary to reach a turbulent flow of the culture, in order to optimize the light regime, nutrients availability, and to avoid biofouling. Mechanical pumping and/or high culture velocity can produce some cell damage due to hydrodynamic stress, limiting the species possible to cultivate in this type of system.

The tubular design allowed the possibility of overcoming some of the problems, such as contamination, pH control, harvesting and introduction of nutrients, but light intensity, mixing, aggregation and temperature control are not resolved yet.

Large-scale PBRs have some disadvantages that make them uneconomical for low-cost end-products: At operational volumes of 50–100 L or higher, it is no longer possible to disperse light efficiently and evenly inside the PBR (Chen 1996; Pulz 2001); development of algal biofilm fouls PBR surfaces and thereby limiting light penetration into the culture. The algae may stick to the inside surface of the tubes and block sunlight, and tubes may get too hot. Photoinhibition, difficulty to control temperature, and accumulation of dissolved oxygen along the tubes are common occurrence. When scaled up, mass transfer becomes a problem and light distribution is not very effective.

Flat-plate Photobioreactor The flat plate reactors are surely the most robust design in which algae are cultivated. They are made of two sheets that have to be glued together and with any desired light path length in the range from a few mm up to 70 mm (Posten 2009). Compared to tubular bioreactors, however, there are some advantages with respect to compactness: their narrow U-turns may use less space than coiled tubes, and their wall thickness can be thinner than tubular bioreactor (Pulz and Scheibenbogen 1998).

The panels/plates are made of transparent materials for maximum utilization of solar light energy. It has been reported that high photosynthetic efficiencies can be achieved with flat-plate photobioreactors. Flat-plate photobioreactors are very suitable for mass cultures of algae. Accumulation of dissolved oxygen concentrations in flat-plate photobioreactors is relatively low compared to horizontal tubular photobioreactors. It has been reported that with flat-plate photobioreactors, high photosynthetic efficiencies can be achieved due to large illumination surface area (Hu et al. 1996; Richmond 2000). It is relatively cheap, easy to clean; suitable for outdoor cultures and due to their modular design convenient for scale-up. Flat Plate reactors also consume less power than tubular reactors to achieve similar or greater mass transfer capacity. In fact, power consumption is another important criterion for comparisons among reactor types (Kunjapur and Eldridge 2010).

Although high biomass concentrations (up to 80 g L^{-1}) can be reached in narrow light path flat panels (Hu et al. 1998), there are some limitations. Scale-up requires many compartments and support materials, shows difficulty in controlling culture temperature, some degree of wall growth, and exhibits possibility of hydrodynamic stress to some algal strains resulting from aeration, a problem that has never been reported in tubular reactors (Raehtz 2009).

Thin-layer photobioreactor This kind of photobioreator has been designed and operated for a long time, with the aim of increasing productivity and reducing production cost. In a thin-layer photobioreactor well-mixed microalgal suspension flows continuously in a very thin layer (6–8 mm) on inclined lanes, construed by transparent material, and arranged in meandering way (Doucha and Lívanský 2006, 2009). This configuration allows to decrease algal layer exposed to the light to as low values as technologically possible and, consequently, increase the light/dark

periods of single cells, thus increasing the efficiency of light utilization and decreasing the photoinhibitory effect up to very high solar light intensities (Doucha and Lívanský 2006). In addition, thin-layer photobioreactor reaches a very high algal density at harvest (Doucha and Lívanský 2006) up to 40–50 g (DW) L^{-1}, corresponding to an areal density of 240–300 g DW m^{-2} (Doucha and Lívanský 2009). These authors related that thin-layer culture technology is suitable for the production of biomass as a feedstock for bioethanol and, under climate conditions, yields of 80–100 t DW *Chlorella* biomass per 1 ha area for a 300-day culture season can be reached.

In thin-layer photobioreactor the high production rates and possibility of achieving high cell density decrease the presence of undesirable algal species and operational costs. Moreover besides providing better distribution of light among the cell, control of cultivation parameters like temperature, for instance, is also favored (Doucha and Lívanský 2006). Thin cell layer is also easily heated up by high light intensity, but on the other hand it is also spontaneously cooled by water evaporation at higher temperatures (Masojídek et al. 2011).

2.3 Material of Photobioreactors

The materials for the construction of photobioreactors represent a significant practical issue both from the standpoint of the investment cost and biological performance. The materials used for the reactor include glass, concrete, bricks, compacted earth, plexiglass, Low Density Polyethylene (LDPE), acrylic, High Density Polyethylene (HDPE), Rigid acrylic (PMMA) and Poly Vinyl Chloride (PVC).

The choice of materials for pond construction depends on many factors such as temperature, composition, pH, and salinity of the medium culture and high light resistant material. Several coating materials are available, and the most appropriate choice depends on its cost, durability, and effect on algal quality and growth (Borowitzka 2005).

Light is the most important parameter in the design and construction of a photobioreactor. Wherever possible, the surface of the photobioreactor should be designed to minimize reflection of light to increase the light capture by the cell. Photobioreactors made with tightly curved surfaces like tubes will have less light available than those made with flat surfaces (Tredici and Zittelli 1998). Some designs have incorporated sophisticated parabolic light collection devices, fiberoptics, or light guides (Ogbonna et al. 1999; Janssen et al. 2002).

Large open ponds can be built of glass, plastic, concrete, polyethylene, PVC bricks, or compacted earth in a variety of shapes and sizes and have been used successfully for algal production. They are the most cheaply constructed ponds.

Ponds can be constructed on ground with walls of bricks, concrete blocks or other resistant material and floors of either concrete or some other suitable line. In addition, pond reactor can be excavated and lined with impermeable material. Considering cost of construction and operation, concrete blocks appear to be the

most effective material for building ponds. On a leveled ground, the pond may be constructed by building the external walls and the central divider of concrete blocks and then installing a suitable impermeable coating material (Borowitzka 2005).

A well-manufactured polyvinyl chloride (PVC) liner of 0.75 mm lasts about 5 years in temperate desert climates, and has been used for microalgae cultivation. But flexible PVC tubing can be damaged by UV rays and it tends to break down. PVC when attacked by UV rays will discolor the surface of the pipe limiting the light availability in the medium. Besides, PVC can lead to decrease in microalgae growth (Dyer and Richardson 1962; Blankley 1973).

PVC linings are frequently stabilized by lead, and it can be accumulated by the microalgae cells as already evidenced for some studies. An alternative to PVC is chlorinated polyethylene (CPE). CPE has little effect on algal growth (Bernhard et al. 1966; Blankley 1973) and does not appear to present any human health hazard.

Photobioreactors must be built with fully translucent material without any loss in transparency over time. Glass and acrylic are widely used in the construction of photobioreactors. Ultraviolet (UV)-stabilized acrylic is more appropriate because it is lighter, more flexible, stronger, and easier to machine, cut, bond, and so on.

Glass can be a suitable material to build photobioreactors. It requires a supporting structure and many more connection fittings as lengths of more than a few meters. In addition, this structure is difficult to transport and assemble. Among the types of glasses, borosilicate (Pyrex) glass is commonly used in the solar hot water collector of PBR. For a tubular photobioreactor, glass has considerably higher NER (Net Energy Ratio) than rigid polymers like acrylic material (polymethyl methylacrylate). Clear acrylic tubing with outer diameter in the range of 30 to 60 mm and wall thickness of 3 to 5 mm has been used in a number of prototype photobioreactor systems, (Tredici and Zittelli 1998). Studies indicate that the acrylic sheet transmits 95 % of the incident light from 390 to 800 nm. Based on its excellent transmittance in the Photosynthetic Activity Radiation, acrylic is suitable construction material for photobioreactors (Berberoglu et al. 2008).

Metals and metal alloys are used primarily as structure of the photobioreactors (support of the photobioreactors). The connections between the different materials can be made by various techniques such as bolted joints, bonded joints and welding. It is important to choose materials that are suitable to seal joints and adhesives with chemical compositions which are compatible with the environment to which they will be subjected, and do not react with the environment or release harmful substances to the culture.

The photobioreactors can also be made of large bags made of low density polyethylene. They are cheaper and generally used in the production of inoculum for larger volumes of culture or for the production of microalgae for feeding bivalves and crustaceans in aquaculture

As described above, a variety of materials is available for photobioreactor construction which not only have considerable benefits but also show a few drawbacks. Therefore it is important to select the best material that satisfies all the criteria for designing an efficient PBR.

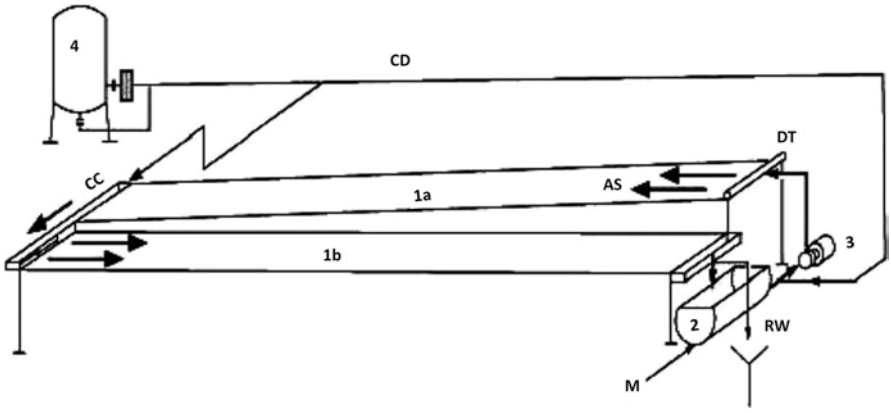

Fig. 3 (*1a* and *1b*) culture area; (*2*) retention tank; (*3*) pump; (*4*) CO_2 storage tank; (M) aeration air; (*RW*) rain water; (*DT*) distribution tube; (*CD*) carbon dioxide; (*CC*) connecting channel (Doucha and Lívanský 2006). (With kind permission from Springer Science+Business Media)

2.4 Circulation Devices

The success of growing photosynthetic microorganisms inside photobioreactors also depends on the applied circulation device, which will be different for each reactor type or configuration. It is important to consider that there is a small variety of devices used to circulate the cells in open artificial ponds. In these kinds of photobioreactors the culture is usually driven by paddle wheels to furnish the necessary turbulence that will put the cells in contact with the light and nutrients (Pulz 2001; Rosa et al. 2011). Doucha and Lívanský (2006) proposed the use of a pump, as circulation device, and inclined cultivation sheets, which is composed by two glass sheets inclined in opposite directions; these sheets are connected by a channel, through which the culture flows from the upper to the lower area; at the end of the lower sheet there is a retention tank, where a pump, to connect the upper edge of the culture area, is located (Fig. 3). This circulation device is applied to an outdoor open photobioreactor for a 2000 L culture cultivation of a termophilic strain of *Chlorella* sp. that was well grown using a flow rate of 60 cm s^{-1} furnished by the pump.

Closed photobioreactors are more versatile than the open ones when talking about different configurations, which give the possibility of using various types of circulation devices. One of the most common circulation devices, mainly for tubular reactors, are the air pumps, which circulate the culture through the so called airlift system. In this kind of system, the air is injected inside the reactor to drive the culture in a given path, differently of a bubble column reactor where the culture flows randomly, through the air injection at the bottom of the photobioreactor (Siegel and Robinson 1992). There are a lot of authors that use the airlift mechanism to cultivate microalgae in tubular photobioreactors, as Soletto et al. (2008) who granted the circulation of *Spirulina platensis* culture by mixing the air from an air pump to uplift this culture using a flow rate of 1 L min^{-1} in helical photobioreactor;

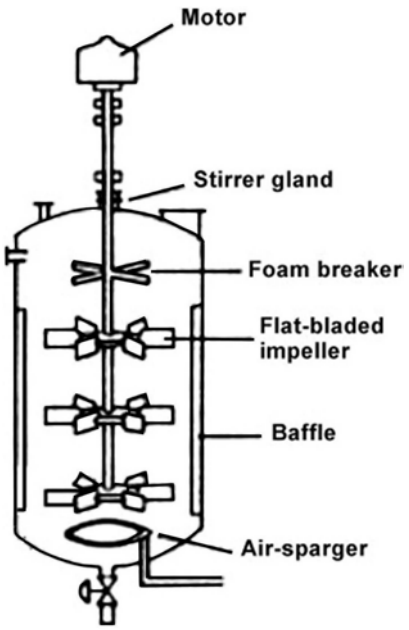

Fig. 4 Stirred tank photobioreactor (Reprinted from Renew Sustain Energy Rev; Singh and Sharma 2012, with permission from Elsevier)

they used different light intensity from fluorescent lamps and different carbon dioxide concentrations from cylinder and obtained the highest cell productivity value of about 0.25 g L^{-1} d^{-1}. There are other researchers which equally, through air pumps by the airlift system, cultivated: *Arthrospira platensis* using sunlight as the energy source (Carlozzi 2003); *Phaeodactylum tricornutum* in a tubular outdoor photobioreactor and, also using the sunlight (Fernández et al. 2001); *Chlamydomonas reinhardtii* in a closed and novel geometric configuration of airlift photobioreactor, using fluorescent lamps (Loubiere 2011); and, *Dunaliella salina* cultivated in a 250 L airlift loop photobioreactor using artificial light (Zimmerman et al. 2011). All these microorganisms achieved maximum cell concentration of about 0.3–2.5 g L^{-1} using air pumps as circulation device. Although the source of air can be air pumps, in large scale conditions, the compressed air could be used for culture circulation.

Other mechanism for cell circulation inside closed reactor is mechanical agitation, in which impellers and baffles are used. Sassano et al. (2010) verified the influence of nitrogen source supply rate on protein and lipid contents of *Arthrospira platensis* biomass. The cultivations were carried out in a vertical, cylindrical glass tank, with 20.7 cm internal diameter (V = 9.0 dm^3), four turbines, and four 2.00 cm-wide chicanes, which ensured a great homogeneity and consequently, efficient mixing. This vertical cylindrical glass tank is one of the most conventional reactors also named stirred tank, where the agitation is the mechanical one. This reactor type contains impellers that are responsible for circulating cells inside the photobioreactor, and baffles to avoid vortex formation (Singh and Sharma 2012) (Fig. 4) or even break up the gas bubbles that increase $k_L a$ values (volumetric mass transfer coefficient).

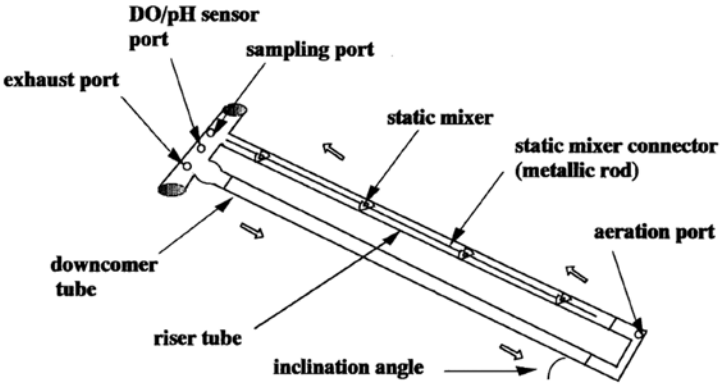

Fig. 5 Scheme of the inclined tubular photobioreactor with internal static mixers (Ugwu et al. 2002). (With kind permission from Springer Science+Business Media)

Ugwu et al. (2002) used static mixers inside an inclined tubular photobioreactor to cultivate *Chlorella sorokiniana*. The culture was circulated by the injection of air through an orifice at the bottom of the riser tube, where the static mixers were located, and then the culture went to the downcomer tube (Fig. 5). The static mixers have a v-cut and an orifice to permit, respectively, gas dispersion and liquid circulation, which resulted in better mixing when compared to not using the static mixers, and a maximum biomass productivity of 1.47 g L^{-1} d^{-1}.

Masojídek et al. (2003) applied a peristaltic pump as circulation apparatus to mix *Spirulina platensis* culture inside a tubular inclined photobioreactor using a working volume of 65 L and sunlight as the energy source. The peristaltic circulation pump takes the culture from the degasser flask, which is located at the lowest position of the cultivation system and, it is connected to the inlet tube of the highest position of the reactor. In this highest position a frequency inverter, to vary the flow speed of suspension, is placed. Finally, the culture goes down through the tubes to reach again the degasser flask (Fig. 6). The pump was applied to flow the culture at 20 cm s^{-1} and they obtained a cell productivity of 0.5 g L^{-1} d^{-1}, which was considered a relatively high value by the authors.

Other different circulation devices were tested by Ferreira et al. (2012a) who compared three different cell circulation systems: motor driven pumping, airlift and pressurized ones (Fig. 7). In the first system, a motor pump was used to circulate the cells in the photobioreactor, as in the pump, submersed into the degasser flask (located at the top of the reactor), propels the culture to move downwards through the external silicon tube and, upwards through the glass tubes. In the second system, an air pump was used to move the culture oppositely to the previous system. In the pressurized one, the cells were moved down or upwards depending on the activation of a solenoid valve. The authors evaluated various parameters and concluded that the traditional airlift one could be substituted by the others to cultivate *Arthrospira platensis* in a tubular photobioreactor.

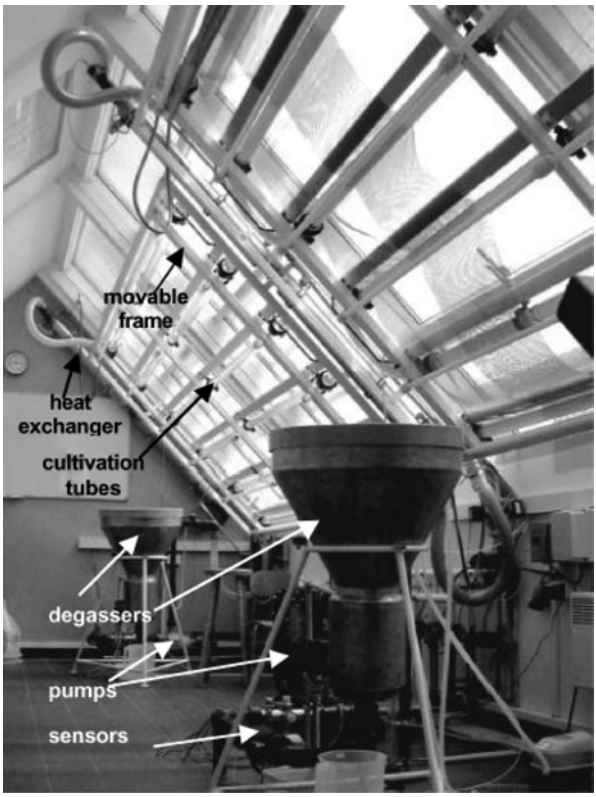

Fig. 6 The main parts of tubular inclined photobioreactor: movable frame, heat exchanger, cultivation tubes, degasser, pump, sensors (Masojídek et al. 2003). (With kind permission from Springer Science+Business Media)

3 Photobioreactor Operation

For several decades, numerous efforts have been addressed to make possible the exploitation of microalgae on a technological scale as a source of food, feed, pharmaceuticals, fertilizers and several other compounds like vitamins, pigments, and lipids (Becker 1994).

As in conventional heterotrophic cultivations, in microalgal biotechnology high volumetric productivities are required to reduce the size of cultivation system and consequently reduce production and downstream processing costs (Barbosa 2003).

Cultures with high-cell density can be achieved in microalgae biotechnology with a proper reactor design and process optimization (Richmond 2000). It is important to improve the knowledge in this field, emphasizing the most critical scale-up and operational parameters: light, mass transfer, shear and mixing rates, etc. These parameters are intimately interrelated and highly influence the productivity and efficiency of the system.

Microalgal cultivations require continuous monitoring. One kind of monitoring is regular microscopic examination to detect any unusual morphological changes and the presence of contaminants, such as undesirable algae and protozoa. Regular

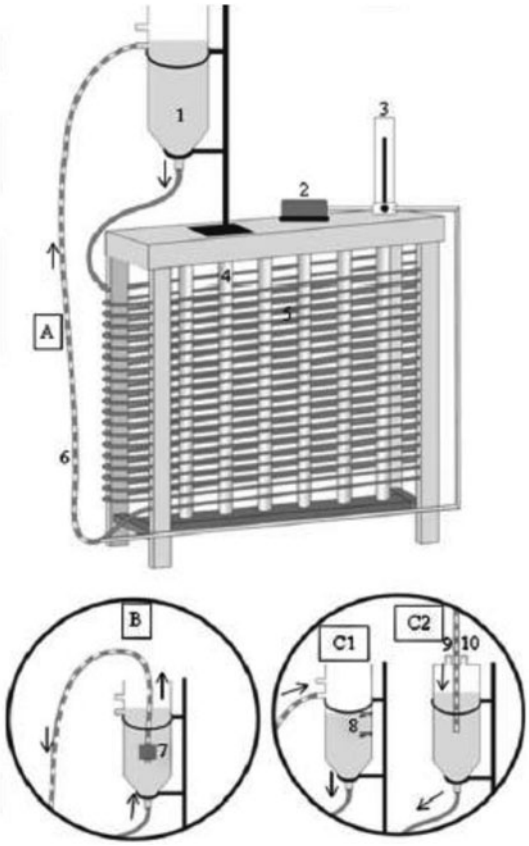

Fig. 7 Scheme of the photobioreactor and the three cell circulation systems. (*1*): degasser; (*2*): air pump; (*3*): rotameter; (*4*): 20 W fluorescent lamps; (*5*): glass tubes; (*6*): external silicon tube; (*7*): motor pump; (*8*): level sensors; (*9*): connected air pump; (*10*): connected solenoid valve. **a** airlift system; **b** motor-driven pumping system; **c1** pressurized system (glass flask on the top); **c2** pressurized system (glass flask on the bottom). (Ferreira et al. 2012a)

verification on the nutrient concentration must also be carried out to avoid unexpected nutrient deficiencies. Nowadays, there are different ways of automatically controlling cultivation conditions like pH, temperature and nutrients, that can be applied for photosynthetic microorganisms reactors, even with devices for on-line data acquisitions (Barbosa 2003).

Many reports in the literature point out that changes in nutrient levels, pH, temperature, and light intensity can cause alterations in the growth and secondary metabolism of algae. For instance, many algae can channel carbon into storage biomolecules, either lipid or carbohydrate, when nitrogen is limited but carbon is still available (Behrens 2005).

For the microalgae cultivation four basic requirements are very important: carbon, water, light, and space. By maximizing the quality and quantity of these requirements, it is possible to maximize the quantity of biomass and the return on investment (Demirbas and Demirbas 2010).

Investigations in the field of applied phycology have led to the design and construction of several types of cultivation plants capable of producing quantities of algae ranging from kilograms to tons. But, it seems that, at least until now, only

raceway ponds and tubular photobioreactors are practicable methods for large scale production (Molina Grima 1999; Molina Grima et al. 1999; Sánchez Mirón et al. 1999).

The production of algal biomass can also be distinguished in three different systems, depending on the raw materials and the destination of the products (Becker 1994):

I. Open system in which a specific algal strain is cultivated using fresh water, mineral nutrients and additional carbon sources, in a so-called clean process. The resultant biomass is suitable to be utilized mainly as food supplement.
II. System in which sewage or industrial waste waters are used as culture medium, without addition of mineral and external carbon. Here, it's difficult to maintain a unialgal population and the biomass will probably have more than one species in the presence of high amounts of bacteria. It's also possible that certain algae adapt their metabolism from autotrophy to heterotrophy, so that they are able to utilize both inorganic and organic compounds from the medium, in a mixotrophic culture.
III. Enclosed systems to cultivate microalgae, using sunlight or artificial light, growing preferably under autotrophic metabolism. It can be considered to employ more sophisticated biotechnologies with a high input of equipment and energy for the growth of specific microalgal strains for the production of specific biochemicals such as enzymes, pigments and therapeutic agents, and the large initial investment costs are justified by the product quality.

Large-scale outdoor culture of microalgae and cyanobacteria in open ponds, raceways, and lagoons is well established. It is commercially used in the USA, Japan, Australia, India, Thailand, China, Israel, and elsewhere to produce algae for food, feed, and extraction of metabolites (Becker 1994).

The use of open ponds requires wider cultivation areas and may hamper an appropriate control of some culture conditions like temperature, light intensity, gases, etc. In fact, according to Brennan and Owende (2010), fluctuations in temperature and light availability due to diurnal cycles and seasonal variations are a major problem for open systems. This aspect is possibly responsible for a lower productivity.

Closed photobioreactors are certainly a very good alternative for microalgae cultivation. In this case, the algae fluid remains in a closed environment to enable accelerated growth and better control of environmental conditions. These glass or plastic enclosures, frequently operated under modest pressure, can be mounted in a variety of horizontal or vertical configurations and can take many different shapes and sizes (Demirbas and Demirbas 2010).

In closed photobioreactors it is possible to achieve up to fivefold higher productivity with respect to reactor volume. Besides saving water, energy, and chemicals, closed bioreactors have many other advantages that are increasingly making them the reactor of choice for microalgal biomass production, as their costs are lower (Schenk et al. 2008).

Closed photobioreactors may be required for special purposes. The high value of certain algal products and the need for good manufacturing practices as well as

the necessity for sterile reactors, or controlled unialgal reactors, justify the operation of such installations (Becker 1994). One of the main advantages of such closed systems is that they are not as subject to contamination with whatever organism happens to be carried in the wind (Demirbas and Demirbas 2010) as it would happen in the case of open ponds.

Enclosed photobioreactors have been employed to overcome problems with contamination and evaporation encountered in open ponds (Molina Grima et al. 1999; Matsudo et al. 2012). Nevertheless, as expected, there are some limitations for closed systems, particularly for the tubular ones: The long pathways of some of these reactors result in significant hydraulic problems as well as depletion of CO_2 in the culture. Another major limiting factor is the accumulation of photosynthetically produced oxygen in the tube (Torzillo 1997).

Schenk et al. (2008) have also proposed a hybrid system, in which both open ponds as well as closed bioreactor system are used in combination for better results. Open ponds are lucrative and skilled method for algae cultivation, but they become contaminated with unwanted species quickly. Therefore, a combination of both systems may be the most coherent choice for cost-effective cultivation of high productive strains. In this sense, open ponds are inoculated with a specific strain that had invariably been cultivated in a closed bioreactor, whether it is as simple as a plastic bag or a high-tech fiber-optic bioreactor. It is important to note here that the size of the inoculums has to be large enough for the desired species to establish in the open system before undesired species. Anyway, for minimizing contamination, cleaning or flushing the ponds routinely is very important.

It is also worth mentioning that production plants for algal biomass cultivation have to be preferably located in areas with suitable climatic conditions. Nevertheless, unfortunately, it seems to be difficult to find places where all the involved parameters are optimal.

When a photobioreactor is going to be designed, parameters like reactor size, light requirements, flow rate, culture condition, algae species, economic value and reproducibility have to be considered. Depending on the reactor size, installation locality, and local climate, these parameters can determine the type of cultivation system needed (open or closed). This reactor design should allow good mixing properties, efficiency, and reproducibility and also be easy to maintain and sterilize. An efficient photobioreactor has to provide good productivity and the possibility of cultivating multiple strains of algae. The performance of this photobioreactor can be measured by volumetric productivity, areal productivity, and productivity per unit of illuminated surface. Volumetric productivity is defined as a concentration of biomass per unit volume of bioreactor per unit of time. Areal productivity is a function of biomass concentration per unit of occupied land per unit of time. Productivity per unit of illuminated surface is measured as biomass concentration per area per unit of time (Demirbas and Demirbas 2010). The operation of the photobioreactors, irrespective to their configurations must be accomplished during the whole period of the cell growth, and, in order to obtain the best performance of the cultivation process, some physico-chemical controls of the culture in the photobioreactors are imperative. Such controls are discussed below.

3.1 Temperature Control

Temperature is one of the most important factors affecting metabolic rate in living organisms. It is worth to test different temperature values in laboratory bench scale for each specific microalgae, and the starting point may be the temperature of the environment where the strain was isolated from. It seems that for the many tropical/subtropical algae, a temperature range of 20–25 °C is the most suitable for growth.

For temperature monitoring, classical mercury thermometers are useful but, unfortunately, easily breakable. Recently, indoor-outdoor, water-resistant, max-min, digital thermometers are proving very useful, durable, and economical (West 2005).

Constant temperatures throughout cultivations are desired. In a laboratory, it is possible to have thermal stability in a temperature-controlled room, even using air conditioner equipment or a thermostatically controlled heater for higher temperatures (Morocho-Jácome et al. 2012). And it is also important to remember that in air-conditioning units, often the temperature near the light will be 1–2 °C above the room ambient level. Another way of controlling temperature in bench scale is using a water jacket connected to a water bath operated at a desirable temperature (Barbosa 2003).

Nevertheless, for larger scales, the control of the temperature can be an important challenge, and a suitable system for temperature control may have a high impact on operational cost. For instance, Santos et al. (2012) describes the use of flat-plated reactors, which they considered inexpensive and easy to construct and maintain, but the large surface area presented scale-up problems, including difficulties in controlling temperature, besides carbon dioxide diffusion rate and the tendency of algae adhering to the walls.

Tubular photobioreactors can be advantageous in areas with moderate temperatures but become problematic in warm climates because the temperature in the tubes may reach values 10 ~ 15 °C higher than the ambient air temperature during the day. Therefore it is necessary to set up a cooling system. Several different possibilities have been tested to prevent overheating of the algal suspension:

I. shading of the tubes with dark-colored sheets. However, to achieve a significant shading effect, up to 80 % of the surface area has to be covered, which causes a large reduction of the illumination and consequently in the yield of biomass (Torzillo 1997);
II. cooling of the culture by spraying water on the surface. Experiments performed in Italy maintaining the temperature of the algal suspension between 33 and 35 °C demonstrated that the amount of water lost by evaporation ranges between 1 and 2 L d^{-1} m^{-2} (Torzillo 1997; Becker 1994);
III. Floating the tubular system on a large body of water. This solution ensures sufficient heat exchange besides providing agitation through wave action and reduction of support structures. In a French bioreactor, thermal regulation is ensured by flexible tubes mounted beyond the cultivation tubes, which may be inflated with air so that the culture tubes are lifted during periods of tolerable temperatures or are immersed when the temperature in the culture tubes becomes too warm or too cold (Becker 1994);

IV. use of Aluminum or stainless steel water jackets to the external surfaces (usually the bottom to not interfere with illumination) of the reactor (Barbosa et al. 2003);
V. use of stainless steel coiling coils submerged in the culture medium. Employing a refrigerated water source, a temperature probe, connected to a temperature controller, operates a solenoid valve to regulate cooling water flow. It is recommended to acquire a "normally open" solenoid valve for granting an overcool instead of an overheating, in the case of a controller failure. The only problem here is that cooling coil may interfere with the circulation within the reactor (Barbosa 2003).

In contrast, at night, temperature can become a limiting factor of algae production at some locations, reducing the extension of the cultivation period during seasons with low ambient temperatures. In this case, there is a possibility of building a larger covered system for maintaining relatively higher temperatures at night. However, there is evidence that the maximum increase in algal biomass by providing increase night temperatures seems to be less than 20%, a questionable benefit when compared with the costs for that cover (Becker 1994).

Also for larger scale in colder weather, Becker (1994) describes a method of adjusting temperature of a tubular photobioreactor (40 mm inner diameter and 79 m length), with a water-jacketed 2 m long tube. In this heat exchanger, it was possible to achieve maximum of 38 °C temperatures inside the reactor with a water bath temperature of 60 °C.

Another possibility of heat exchanger is a design in which to improve the unfavourable geometry of the tower for illumination, a thinner glass tube ("finger") has been inserted from above, eliminating the central space, which is poorly illuminated in dense cultures, but can be filled by water, connected to a thermostat, for controlling the temperature of the culture (Becker 1994).

3.2 pH Control

The pH of the culture medium is another important factor in algal cultivation. Fortunately, it is a very simple parameter to control using existing technology (Sonnleitner 1999). Nowadays, there are several commercially available pH controllers.

The value of pH determines the solubility of minerals and carbon dioxide and influences the metabolism of the microalgae. Algae exhibit a clear dependency on the pH of the growth medium and different species vary greatly in their response to pH. *Cyanidium,* for instance, has its optimum growth at pH 2.0 (Becker 1994), whereas *A. platensis* grows well at pH 9.5 (Sanchez-Luna et al. 2007).

Careful control of pH and other physical conditions for introducing CO_2 into the ponds allow for more than 90% utilization of injected CO_2 (Demirbas and Demirbas 2010), and it is also important for optimizing the uptake of ammoniacal nitrogen sources, like ammonium salts and urea, when they are employed (Ávila-Leon et al. 2012; Rodrigues et al. 2011; Carvalho et al. 2013).

The pH of algal cultures can be influenced by various factors such as composition and buffering capacity of the medium, amount of dissolved CO_2, temperature (which controls CO_2 solubility) and metabolic activity of the algal cells. It was found that in actively growing *A.platensis* cultures, the pH continuously increased to values up to 11, mainly because of the depletion of the anions NO_3^- and HCO_3^- from the medium (Vieira et al. 2012).

A more sophisticated method for maintaining the pH at a desired value is the introduction of pH controllers (pH states), which measure the actual pH of the medium and, as soon as a preselected pH is exceeded, open a solenoid valve that releases CO_2 (Matsudo et al. 2011) or turn on a peristaltic pump to add an inorganic acid, normally HCl solution, to the medium until the desired pH is reached. One should note though that in the case of inorganic acid addition, it led to loss of the carbon source in the form of CO_2, which points out the importance of addition of CO_2 instead of acid solution to control pH.

Employing a tubular photobioreactor, Matsudo et al. (2011) evaluated the use of CO_2 released from alcoholic fermentation, without any prior treatment, for pH control and carbon source replacement, in continuous cultivation of *A. platensis*. They used a continuously operating pH controller coupled to a solenoid valve to release CO_2 when necessary for maintaining constant pH value (9.5 ± 0.2). Irrespective of the carbon source used (pure CO_2 or from alcoholic fermentation), similar behavior in cell growth was observed, and no difference was observed in the protein content of the dry biomass.

When an automated pH controller is not available, pH maintenance may be done by adding pulses of CO_2 or even in a constant flow, employing a small ratio with the air. This ratio or the duration and number of pulses in a day may be predetermined by preliminary tests.

Moreover, in continuous cultivation, it is possible to modify the proportion of nutrients for adjusting the initial pH in the feeding medium (Sassano et al. 2007). For instance, Ávila-Leon et al. (2012) employed Schlösser (1982) culture medium, which has high concentration of carbonate and bicarbonate, for continuous cultivation of *A. platensis* with urea as nitrogen source. Since the optimum pH for its cultivation is around 9.5, they adjusted the proportion of carbonate and bicarbonate salts to achieve a pH of 9.3, but keeping the same overall carbon amount. With this procedure, the pH inside the bioreactor kept constant between 9.5 and 10.3, depending on the dilution rate and urea concentration.

On the other hand, it should be kept in mind that, in the case of outdoor large scale open reactors, high pH could prevent night-time losses of respiratory CO_2 allowing the maintenance of reserves of this nutrient.

3.3 Nutrients

Once the composition of a culture medium is optimized for a specific or a group of microalgae, another challenge is to maintain adequate levels of main nutrients throughout a cultivation.

For this reason, in commercial scale production, it is possible to set up instrumentation for measuring selected nutrients and controlling by automated compensation of them. Nevertheless, it leads to an elevated initial investment and operational cost. Alternatively it is possible to estimate the required quantity of nutrient addition during the cultivation, taking into account a standard cultivation.

The major absolute requirements include carbon, phosphorus, nitrogen, sulfur potassium and magnesium. Elements like iron and manganese are required in small amounts. Various other elements like cobalt, zinc, boron, copper and molybdenum are essential trace elements. In addition to these basic minerals, several algae may require additional organic substrates (vitamins, nucleic acids, growth factors) for their growth (Becker 1994).

Carbon source About 50 % of the algal biomass consists of carbon (Cornet et al. 1998), therefore an adequate supply of carbon is of utmost importance for its cultivation, what is justified by the low solubility of CO_2 in the medium and its high demand in cultivations with high final cell concentrations. Even in systems in which it is possible to have higher reserves of CO_2 (alkaline media), it is important to consider that the higher the carbon uptake, the higher the pH of the cultivation medium, what certainly leads to deficient production of the microalgae. Carbon can be supplied as an inorganic substrate in the form of gaseous CO_2, as in the case for most photoautotrophic forms (organisms that obtain the energy for their metabolism from light and all the elements from inorganic compounds), or in the form of bicarbonate. Concerning CO_2 sources, pure CO_2 is commonly used, although there are works that have been focused on use of CO_2 from burn of organic materials and/or from industrial wastes (Carvalho et al. 2009; Matsudo et al. 2011; Ferreira et al. 2012b; Demirbas and Demirbas 2010).

There is also the possibility of exploring the mixotrophic metabolism, nutritional mode in which the energy is derived either through photosynthesis or by chemical oxidation (of sugars, for example), and both organic and mineral carbon sources are required.

In several algal species, the mode of carbon nutrition can be shifted from autotrophy to heterotrophy by modifying the carbon source, based on the ability of the naturally photoautotrophic algae to utilize both inorganic and organic carbon substrates. Becker (1994) describes that different sugars are able to promote the growth of *Scenedesmus obliquus*. The highest increase in biomass was obtained with glucose, followed closely by mannose, whereas sucrose, fructose and galactose had much lower effect, even allowing higher biomass concentrations, in comparison with control cultures, with no sugar addition.

The cultivation of *Spirulina* was studied by Márquez et al. (1993) who showed that it is able to grow heterotrophically in a glucose containing medium in aerobic and dark conditions but also mixotrophically if light is provided. Chen et al. (2006) also showed that acetate may be employed as carbon source for mixotrophic cultivation of *Spirulina,* even for the production of several photosynthetic pigments.

It is very common to alter culture growth conditions, like deprivation of essential nutrients (mainly nitrogen) for enhancing lipid content in microalgae, but starving

the cell to enhance lipid content requires time, and will in the end result in lowered biomass productivity. In this sense, the employment of an organic carbon source could accelerate cell growth, and moreover it seems that mixotrophic growth for several species of microalgae, when grown in the presence of an appropriate carbon source, has been shown to result in higher intracellular lipid levels than those grown only under photoautotrophic conditions (Das et al. 2011).

On the other hand, it seems that *Dunaliella* is unable to grow heterotrophically. Using acetate or glucose as the sole source of carbon (Ben-Amotz and Avron 1989), it was impossible to grow this alga in the night, allowing the conclusion that this microorganism is an obligate photoautotrophic.

Especially in developing countries, for developing an economic algal mass cultivation, where the use of pure CO_2 is unfeasible due to the high cost, it is necessary to explore the possibilities of utilizing low cost carbon sources such as industrial or agricultural by-products (Becker 1994). As it is going to be explored in a later section, there are some studies about the use of CO_2 from alcoholic fermentation for the cultivation of *Arthrospira* (*Spirulina*) *platensis* (Carvalho et al. 2009; Matsudo et al. 2011; Ferreira et al. 2012b). In these cases, the authors had success in using this exhaust gas directly from the fermenter, but for other photosynthetic microorganisms, it is important to consider that this CO_2 can include some volatile organic compounds that, depending on the concentration, can serve as organic carbon source or even inhibit the microalgal growth.

Lodi et al. (2005) tested different organic carbon sources for *A. platensis* in fed-batch mixotrophic process, under continuous illumination. For avoiding carbon source accumulation in the medium, acetate and propionate were added by pulse feeding equimolar amounts about 12 h after their complete depletion. In this sense, acetate was added every 3.4 days, propionate every 4.0 days, and glucose once a day. The results indicated that glucose was metabolized faster than the other 2 carbon sources for algal growth.

Nitrogen source Besides carbon, nitrogen is the most important element in algal growth, since approximately 10 % of the algal biomass may consist of this element (Cornet et al. 1998). It is a key element for cell growth and reproduction, since it is an essential building block of nucleic acids and proteins. Therefore, it is important to choose a suitable and economic nitrogen source. Nitrate, ammonia and urea are widely used, dependent on the species and the optimum pH. Certain cyanobacteria are also capable of assimilating nitrogen in its elemental form from the atmosphere. Changes in the nitrogen supply essentially influence the metabolic pathways in the alga and subsequently the overall composition of the organism (Becker 1994).

Xu et al. (2012) observed that nitrogen significantly affected the algal growth and oil production of a microalga in batch culture, and they found out that it is possible to improve algal oil production from *Brotryococcus braunii* by feeding nitrate up to its initial concentration 15 days after the beginning of the cultivation. They also observed that low nitrate concentration limited microalgal growth and high nitrate concentration inhibited hydrocarbon accumulation, which justify the need of controlling nutrients concentration throughout a cultivation. In fact Li et al.

(2008) observed that sodium nitrate in a concentration of 15 mM or higher seemed to be inhibitive for *Neochloris oleoabundans* cell growth, and around 10 mM was the optimum concentration, but the nitrogen sources was depleted after 3 days of cultivation.

It is known that the preferred nitrogen supply is in the form of ammonia or urea, either of which is economically more favorable than nitrate, which is more expensive and requires considerable metabolic energy for its assimilation (Sassano et al. 2004; Carvalho et al. 2013). It is generally assumed that, before assimilation, nitrate-nitrogen is reduced to ammonium-N in two reducing steps catalyzed by the enzymes nitrate reductase and nitrite reductase (Hattori and Myers 1966).

Excessive ammonium may be deleterious for microalgae. Diatoms were found to be damaged when ammonium-N exceeded 5 mmol L^{-1}, particularly when the pH was above 8.0 (Azov and Goldman 1982).

Several studies are available in the literature dealing with the use of alternative nitrogen sources, such as urea and ammonium salts, for reducing production cost by replacing the traditionally used sodium or potassium nitrate. It is a particular kind of cultivation because of the alkalinity. In high pH medium, ammonia is easily assimilated by the microorganism, since ammonia uptake involves simple diffusion followed by trapping through protonation (Boussiba 1989). It is worth remembering that urea is hydrolyzed to ammonia in alkaline conditions (Danesi et al. 2002) and/or by urease (Shimamatsu 2004).

However, it has been reported that ammonia concentrations are acceptable only in a certain limit, depending on the microorganism, for its toxicity, especially in combination with pH. For instance, 10 mM of ammonia can be toxic for the *Spirulina platensis* (Belkin and Boussiba 1991). Therefore, if ammonium salts or urea are added in large amount at the beginning of the process, the excessive ammonia released may provoke cell death.

Notwithstanding, the addition of these nitrogen-containing compounds by fed-batch or continuous process can be a very promising for low-cost microalgal biomass (Danesi et al. 2002; Sánchez-Luna et al. 2004; Sassano et al. 2004; Bezerra et al. 2008; Matsudo et al. 2012; Ferreira et al. 2010; Matsudo et al. 2011). Moreover the simultaneous use of nitrate and urea or nitrate and ammonium showed to be even more advantageous (Rodrigues et al. 2010; Vieira et al. 2012).

Considering the efficiency of microalgae for uptaking nitrogen and phosphorus from culture medium, algae cultivation can be coupled to another type of environmental remediation that will enhance productivity while mitigating pollution. High nutrient wastewater from domestic or industrial sources, which may already contain nitrogen and phosphate salts, can be added to the algal growth medium directly (Schneider 2006). This allows for inexpensive improvement in algae production along with simultaneous treatment of wastewater.

By evaluating the annual productivity of *Spirulina* and its ability to remove nutrients in outdoor raceways treating anaerobic effluents from pig farm wastewater, Olguín et al. (2003) also showed that this photosynthetic microorganism can be used for ammonia removal from wastewater in processes in which the wastewater is fed periodically, diluting it in a mixture of sea and fresh water.

Trace elements Trace elements are found to be essential and are mostly supplied in very small quantities from stock solutions. For this reason, they are difficult to be chemically analyzed and monitored in a cultivation.

Fresh water could be a valuable natural resource of the salts and minerals needed, but it is common to have stock solution of minerals which is added in the culture medium in very little amount. Alternatively, salt water can be used, either from a saline aquifer or seawater. This means that competition for water will be low (Demirbas and Demirbas 2010).

It is worth mentioning that phosphates may complex with metal ions, even in trace concentrations, and not all the added phosphorus is bio-available. Therefore, phosphorus must be supplied in significant excess in the culture medium (Belay 1997).

3.4 CO_2 Addition Systems

Culture media containing salts as bicarbonate and carbonate (carbon sources) are frequently used in laboratory cultivations (Danesi et al. 2002; Rodríguez-Maroto et al. 2005; Bezerra et al. 2008; Rodrigues et al. 2010; Vasumathi et al. 2012). CO_2 gas is other carbon source form used by photosynthetic microorganisms, and it has been applied to cultivations in order to reduce costs by using carbon dioxide gas from cylinders or from industrial waste gases. It is interesting to highlight the importance of using CO_2 gas from wastes, since it will contribute to greenhouse gases and global warming mitigation. There are some examples, as the successful use of CO_2 from ethanol fermentations in *Arthrospira platensis* cultivations in tubular photobioreactors when compared to the use of CO_2 from cylinders (Ferreira et al. 2012b; Matsudo et al. 2011); or, the use of flue gas into microalgae (*Chlorella vulgaris*, *Scenedesmus* sp., *Botryococcus braunii*) cultivations by Yoo et al. (2010) who obtained great results of biomass, productivity and a better C-fixation ability for *Scenedesmus* sp. when using ambient air enriched with 10 % of flue gas.

The amount of CO_2 to be added to an algal culture depends on the efficiency of gas sparging, CO_2 loss from microalgae culture and CO_2 consumption by algal cells (Doucha and Lívanský 2006). These variables are related to CO_2 addition systems and, consequently, to photobioreactor type and its configurations.

Open photobioreactors present disadvantages as example, a short way to the gas-liquid transfer, which results in higher gas loss if compared to closed photobioreactors. Some researchers reported that about 13–20 % of supplied carbon dioxide was absorbed in open tanks (Richmond and Becker 1986; Becker 1994). However, Doucha and Lívanský (2006) cultivated *Chlorella* sp. in open reactors and they concluded that, from all CO_2 supplied to the culture, about 70 % of CO_2 was utilized for photosynthesis. These authors cultivated the microalga using a gaseous CO_2 from a storage tank, where liquid CO_2 was kept under pressure; this gas was supplied into the suction pipe of the suspension circulating pump, and when the CO_2 content was detected in excess, the magnetic valve for CO_2 delivery was switched off.

Binaghi et al. (2003) have also cultivated a photosynthetic microorganism (*Spirulina platensis*) inside an open photobioreactor, where pure CO_2 from cylinder was bubbled into open tanks resulting in maximum cell concentration of 1.5 g/L as the higher value obtained in this work, but still lower than that obtained by cultivations in closed photobioreactors (Ferreira et al. 2010; Molina et al. 2001).

Closed photobioreactors are more efficient in the case of CO_2 addition due to the higher time of the gas in contact with the culture medium. There are a lot of researchers that use CO_2 gas to maintain the pH at an optimum value and also as carbon source for photosynthetic microorganisms cultivations. Masojídek et al. (2003), when cultivating *Spirulina platensis* in tubular photobioreactor, fed pure carbon dioxide into the cyanobacterium suspension before the inlet to the circulation pump according to the pH value, and they obtained biomass productivity of about 0.5 g L^{-1} d^{-1}.

Sobczuk et al. (2000), when using the same kind of photobioreactor, injected CO_2-air mixtures directly into the culture, and studied the CO_2 uptake efficiency by *Phaeodactylum tricornutum*. They found that the CO_2 uptake efficiency was 63 % when the CO_2 concentration in the gas inlet was 60 % v/v. The CO_2 loss during the photosynthetic period was about 10–20 % of the added amount, and the highest biomass productivity value was 2.47 g L^{-1} d^{-1}.

It is interesting to highlight that the success of CO_2 addition system also depends on other parameters as the flow rate, since the higher the flow rate the lower the coalescence of CO_2 bubbles (Doucha and Livanský 2006). Zhang et al. (2002) observed that the amount of CO_2 added depends on k_La, i.e., on mass transfer and mixing. Accordingly, mixing methods and devices have to be considered for high CO_2 utilization efficiency.

3.5 Cultivation Processes

Process optimization is a very important aspect in the development of technologies for microalgal production, overcoming the obstacles and making it economically feasible. The improvement of the bioprocess is very important to link the discovery and the commercialization and should take place after the identification of a strain and the product of interest.

Microorganism cultivations may be carried out under different processes, according to the microorganism, kinds of nutrients, the desired product, etc. In both closed or open systems, microalgae can be cultivated by batch (Watanabe and Hall 1995), fed-batch (Danesi et al. 2002; Torre et al. 2003; Carvalho et al. 2004; Bezerra et al. 2008; Matsudo et al. 2009), semi-continuous (Lee and Low 1992) or continuous processes (Matsudo et al. 2011; Ávila-Leon et al. 2012; Matsudo et al. 2012).

In batch process, neither nutrient is added after the initial charge nor the product is removed until the end of the process. Conventional batch operation can provoke inhibitory concentration of substrate or even formation of undesired products through direct metabolic pathway of the organism but, on the other hand, represents

the most safety one when facing problems with asepsis. Besides, it shows flexibility of operation, better control of genetic stability of the strain and permit the traceability of all materials related to a specific lot, which is of utmost importance in pharmaceutical industries, for example (Carvalho and Sato 2001). A variant of this process is fed-batch process.

Fed-batch process is defined as a technique applicable in microbial processes in which one or more nutrients are added to the reactor throughout the cultivation, while cells and products remain until the end of the operation. The culture volume may vary, depending on the nutrient concentration and evaporation of the system (Carvalho et al. 2013).

The advantages of this process include: deviation of cell metabolism for the synthesis of the desired product; prevention of release of toxic substances in microbial metabolism or catabolite repression; and control of the specific growth rate (Yamane and Shimizu 1984).

Fed-batch process was particularly important in the studies of *Arthrospira* cultivations. In this case, it was used to prevent or reduce substrate-associated growth inhibition by controlling nutrient supply. Since both overfeeding and underfeeding of nutrient is detrimental to cell growth and/or product formation, the development of a suitable feeding strategy is critical in fed-batch cultivation. Fed-batch operations can be the best option for some systems in which the nutrients or any other substrates are only sparingly soluble or are too toxic to add the whole requirement for a batch process at the start (Carvalho et al. 2013).

In the fed-batch mode of operation, nutrient feeding during a fed-batch process can be done utilizing either constant of variable mass flow rate (Danesi et al. 2002; Bezerra et al. 2008), and by pulses or continuous mode (Sánchez-Luna et al. 2004).

More recently, Ferreira et al. (2010) observed that parabolic protocol for ammonium sulfate addition appeared to be the best one for *A. platensis* biomass production in tubular photobioreactor. Additionally, due to elevated cell growth in the cultivations in such photobioreactor, the nitrogen amount required was extremely high, reaching values around 12 mM per day. Considering that the inhibitory levels of ammonia is around 6 mM (Carvalho et al. 2004), in this case, the addition of 12 mM of ammonium sulfate per day in a single daily addition would probably lead to cell death. Thus, the daily addition was divided into eight separate additions, providing very promising results.

Still, the fed-batch cultivation can be carried out as a repeated fed-batch process, that can avoid unproductive times of the photobioreactors. Once the cultivation reaches a certain stage, where cell concentration is almost stable or processes the end of the logarithmic growth, a portion of the culture medium is removed from the reactor and replaced by fresh nutrient medium. As it happens in semi-continuous process, it is possible to keep part of the medium in the reactor at the end of cultivation, reusing the exponentially growing cells for the following runs, granting high starting cell levels, and avoiding long stopping of the process (Carvalho et al. 2013). In this sense, Matsudo et al. (2009) showed that this mode of operation can be successfully exploited for long-term *A. platensis* cultivation with urea as nitrogen source, in open ponds, obtaining high cell productivity and high protein content biomass.

In semi-continuous process (repeated batch process), a batch process is initiated, and once the cell concentration is stabilizing, part of the culture medium is removed, and the remaining part is used for a subsequent cultivation, by the addition of fresh culture medium, replacing the withdrawn volume. In this sense, the cell suspension remaining in the reactor serves as inoculum for the following cultivation (Borzani 2001). This process, as the repeated fed-batch, can be used to avoid any unproductive time of the photobioreactor as well, mainly in conditions in which it is not necessary to feed any nutrient to the photobioreactor during the cell growth. Continuous process is carried out by continuous feeding of fresh medium to the reactor, in a specific flow rate, and continuous withdrawal of exhausted medium in the same flow rate for maintaining a constant volume and aiming to achieve a "steady-state" condition (Pamboukian 2003). In this case, the cultivation may last long-term, having several advantages in comparison with batch process.

Cultures with high cell concentration of photosynthetic microorganisms may have the shadowing effect (Vonshak et al. 2000), which reduces the light available for each cell and, consequently, negatively affects carbon fixation. This drawback can be overcome withdrawing, periodically, part of the medium from the reactor at the end of cultivation, reusing the cells for subsequent runs, in a semi-continuous or repeated fed-batch process (Matsudo et al. 2009) or maintaining constant optimum cell concentration range, by continuous process (Matsudo et al. 2011; Ávila-Leon et al. 2012; Matsudo et al. 2012).

According to Maxon (1954), a disadvantage of continuous process could be the possibility of contamination by undesired microorganisms. In fact, this kind of process is more recommended for extremophiles organisms, like *Arthrospira*, which is cultivated in high pH and salinity. Besides these characteristics, not only the use of inorganic medium but also the use of ammoniacal salts and closed photobioreactors may help to avoid contamination.

In general, mixotrophic culture experiments for microalgae have been conducted under sterile conditions in closed laboratory systems, mainly because addition of organic substrates in a open system is likely to induce growth of undesired heterotrophic bacteria, resulting in a low microalgal biomass yield.

3.6 Light Supply

Microalgae obtain their metabolic energy by photosynthesis process, which shows the great importance of light supply for their growth. There are a lot of photobioreactor configurations with internal or external illumination used for microalgae cultivation. Tank reactors, plate or tubular are examples of photobioreactors with different illumination which will influence on the way the cells, inside the reactor, will receive the provided light (Perner-Nochta and Posten 2007).

Open photobioreactors are the most commercially used for microalgae cultivations, because of their advantages, as example, the ease of construction and low cost. Hsieh and Wu (2009) developed an effective system for enhancing light utili-

zation in an open photobioreactor. In this proposed system, transparent rectangular chamber (TRC), made of transparent acrylic, was placed inside an open rectangular reactor to provide a larger area of illumination, which conduct the light deep into the culture. The authors obtained, as the main result, higher biomass value (56 % higher) for *Chlorella* sp. cultivations in open photobioreactor with TCR when compared to that without the chamber.

The open reactors resemble the microalgae natural habitat and the water depths are, usually, 15–20 cm (Pulz 2001). This measure is the length that the light has to go through to reach the photosynthetic cells, and this is the major difference when compared to the closed photobioreactors.

Notwithstanding, Doucha and Lívanský (2006, 2009) proposed the employment of a thin-layer culture technology for the production of microalgal biomass. In this outdoor photobioreactor, with a total volume of 2,000 L, the microorganisms are grown in a 6–8 mm thick layer, allowing a higher efficiency of light utilization. Indeed, Doucha and Livanský (2009) showed that it was possible to achieve a cell density higher than 40 g DW L^{-1} in less than 10 days.

There are many closed photobioreactor configurations used for laboratory experiments. They are composed of thin structures (tubes or flat plates, as examples) where the culture flows. Converti et al. (2006) cultivated *Arthrospira platensis* in tubular reactors, whose tubes have 1.2 cm of internal diameter. In this photobioreactor, the light had to go through 1.2 cm as a maximum to reach the cells, and the result was a high photosynthetic efficiency (PE), i.e., a high efficiency of light conversion by the cells, (PE = 8.1 % for the best experiment) when compared to a cultivation in an open tank using the same strain and the same light intensity (water depth of 5 cm) (PE = 7.1 % for the best experiment).

It is important to notice that there are techniques and apparatus used to increase the better use of light by the cells, both in open or closed photobioreactors. Ugwu et al. (2002), as mentioned before, have studied the use of static mixers, which were able to improve mixing and, probably, result in better capture of light by *Chlorella sorokiniana*. As this last paper, all the researches previously mentioned, in the section named “Circulation Devices”, were about the use of apparatus, which can change circulation inside the photobioreactor and, consequently, can improve capture of light energy by photosynthetic cells. Another applied technique was the success of changing the angle illumination at the surface of a conical photobioreactor (photo-redistribution), which resulted in efficient light use and high microalgae productivity (Morita et al. 2000).

It is important to remark that low light intensities can result in photolimitation, that is, lack of light energy for photosynthetic cell growth; and on the other hand, high light intensities can lead to photoinhibition, i.e., a reduction in the photosynthesis rate of cells when the light is provided in excess (Vonshak 1997a). Through this last knowledge, Cuaresma et al. (2011) studied microalgae cultivation in an effort to improve light capture by cells, and also to avoid the photoinhibition effect. For this purpose they cultivated *Chlorella sorokiniana* in a vertical panel photobioreactor with 14 mm of light path and, by an outdoor condition simulation, different light intensities were tested during the day (higher light intensity was provided at midday).

When cultivating photosynthetic microorganisms, it is important to note that the use of narrow transparent tubes can result in high microalgae productivities, whereas the excess light intensity on these tubes could result in photoinhibition and low light conversion efficiencies. Photoinhibition can be decreased if at a constant incidence light intensity, light absorbed per unit cell will be very low. Ugwu et al. (2003) observed that even at relatively low aeration rate, the use of static mixers modify the broth flow from plug flow to turbulent circulation, which results in a good culture homogenization inside the closed photobioreactor. Therefore, static mixers utilization will ensure that the light will reach more the cells resulting in better light utilization efficiency.

The increase in photosynthetic rates and light utilization efficiencies was already found by Grobbelaar et al. (1996) who applied light/dark periods and observed that the higher the light/dark frequencies the higher the photosynthetic rates. According to Merchuk et al. (1998), the introduction of dark period can avoid long exposure to high light intensities and also reduce photoinhibition effect; however, if the dark cycle is higher than 50 % of the cycle time, the light utilization efficiency is reduced (Janssen et al. 2001). This influence of light/dark cycles shows the importance of controlling periods of light both in outdoor (sunlight) and indoor (artificial light) cultivations.

Finally, independently on the reactor configuration (open or closed), apparatus, structures, and techniques utilization, the efforts have to be made to provide the required light energy to the microalgae growth.

3.7 Mixing

Mixing is the most important requisite to obtain constant high yields of microalgae biomass, when considering that the environmental conditions are not limiting. The most important reason to mix cells is to maintain a good suspension. The high turbulence around the cells results in high nutrients and gas gradients, which are responsible for the achievement of a dense algal culture (Torzillo 1997). Mixing inside any kind of photobioreactor can influence the oxygen tension, hydrodynamic stress, light cycling and gaseous transfer in the culture medium.

Microalgae are photosynthetic microorganisms, i.e., they obtain energy from light and release oxygen gas. The amount of dissolved oxygen (DO) in microalgae culture medium may vary depending on the photobioreactor type and mixing. It is known that high dissolved oxygen levels are toxic to most algae and may lead to photooxidative culture death (Richmond 1986). This problem could be solved by using vigorous mixing, which, according to Richmond and Grobbelaar (1986), will result in less oxygen build-up in the culture. For *Spirulina*, for example, Vonshak (1997b) observed that in small ponds, in which high flow rates can be employed, oxygen concentration can reach levels not higher than 200 % of air saturation ($12 \sim 14$ mg. L^{-1}), but in larger ponds, where water flow is limited, oxygen concentration as high as 500 % of air saturation can be observed.

Inside open photobioreactors the accumulation of oxygen generated by photosynthesis leads to a severe cell growth inhibition. Vonshak (1997a) observed in their studies that when DO concentrations were higher than 20–22 mg L^{-1} the cyanobacterium photosynthetic activity was reduced. It is known that the paddle wheels, used to circulate cell cultures inside tanks (open reactors), are more efficient on removing oxygen gas from reactors when compared to air bubbling applied in closed ones (Fontes et al. 1989), which suggests the ability of mixing and circulation devices on gases removal.

The problem of dissolved oxygen becomes dramatic when the microalgae cultivation is carried out in closed photobioreactors, in which oxygen gas accumulates during the loop cycle and is removed only at the degassing flasks. Among closed reactors, tubular ones are still disadvantageous due to the long way the gas has to go through the tubes until exit the system. However, there are a lot of methods used for oxygen removal in tubular photobioreactors, for example: cell circulation systems, like air bubbling by a system called airlift (Converti et al. 2006) or the utilization of static mixers inside the reactor tubes (Ugwu et al. 2002). Moreover, Torzillo (1997) constructed a tubular photobioreactor with several tubes laid side by side on a white polyethylene sheet and joined by PVC bends to form a loop, and in each bend, a narrow tube was incorporated for allowing the release oxygen.

P. tricornutum was cultivated in outdoor tubular photobioreactor and its results showed that DO percentage in the culture medium changed according to the hour of the day and season when the cultivation was carried out, i.e., it is related to light intensity. This paper also showed that the photosynthetic activity, i.e., cell growth, clearly was reduced at dissolved oxygen concentrations higher than 100 % of saturation, and at dissolved oxygen of 475 %, the photosynthesis rate was reduced by 55 % (Molina et al. 2001).

There are great variety of closed photobioreactor configurations and one of them is the vertical alveolar panels (VAP) used by Tredici et al. (1991) to carry out *Anabaena azollae* cultivations. They reported that microalgae yield decreased when the dissolved oxygen accumulated to 400 % of saturation. This reactor configuration (VAP) cannot be considered a typical closed system since it presents the inherent advantage of allowing a better degas action of air.

It is worth mentioning that studies on mixing have to be done to avoid hydrodynamic stress, which may be affected by geometry of the bioreactor involved, type of pump utilized, morphology of algal cells, physiological conditions of microalgae. Some authors have already mentioned that circulation devices can damage photosynthetic cells, for example, utilization of screw-pumps for *Porphyridium* cultivation (Gudin and Chaumont 1991), and even air pumps for airlift systems used to cultivate *Dunaliella* sp., whose cells were increasingly sensitive to high specific bubble rates (Silva et al. 1987).

Another relevant point to be considered about hydrodynamic stress is the applied flow rate. According to Torzillo (1997), the cell stress depends mostly on the rate at which cells flow, since the higher the flow rate the greater the damage to cells. In fact, a culture flow rate of about 0.3 m/s was not enough to cause damage to *Arthrospira platensis* cells, but when it was increased to 0.8 m s^{-1}, the biomass concentration was reduced by 16 %.

The hydrodynamic, mass and gaseous transfers are related to vigorous mixing, which can: increase flashing-light effect, remove excess dissolved oxygen, improve CO_2 supply, and, consequently, result in higher cell productivity. In order to study hydrodynamic and mass transfer in a flat-panel photobioreactor with high light path (0.15 m) for *Spirulina* sp. cultivation, Reyna-Velarde et al. (2010) evaluated the volumetric mass transfer coefficient ($k_L a$), the gas hold-up and the mixing time as a function of superficial gas velocity. The results suggested that this photobioreactor is more efficient than the tubular and flat-plate configurations, thus indicating the possible use of photobioreactors with higher light paths than yet proposed.

Zhang et al. (2002) described the effect of CO_2 flow rate on microalgal productivity and the ability of gaseous transfer in a vertical flat-plate photobioreactor (VFPP) for *Synechocystis aquatilis* cultivation. According to these authors, increasing the height of the VFPP, both the CO_2 mass transfer and the illumination conditions could be improved, which suggests the possibility of scaling up. They also tested different CO_2 aeration rate, and observed that a rate lower than 0.005 vvm resulted in mass transfer rate from the aerated CO_2 as the main limiting factor for microalgae growth. If low concentration of CO_2 is supplied to a culture, a high critical $k_L a$ value is necessary to reach the CO_2 requirement for microalgae growth.

Ugwu et al. (2003), also studied gaseous transfer and verified that the use of static mixers inside a closed photobioreactor could increase mass transfer, which is proved by the increase of gas hold up and $k_L a$ values.

Lastly, an efficient mixing system in microalgae photobioreactors require methods or apparatus to move both the culture and the gases, between the upper and lower parts of the reactor. One of the variables for microalgae cultivation success is a well mixing, which will contribute to good gaseous transfer, besides avoiding hydrodynamic stress.

3.8 Cleanness

To develop a culture of microalgae in monoculture, it is necessary to prevent the contamination by other organisms, as this can compromise the productivity of the cultivation and the safety of the product, mainly in the case in which the whole biomass will be used as food supplement. The main types of contaminants in clean algal cultures are, bacteria, other algal forms, zooplankton, viruses, fungi and insects, depending on the local conditions, the algal species cultivated, and the particular cultivation system (Becker 1994).

For many phototrophs, gross contamination by bacteria, fungi and protozoa is not a significant problem because there is generally very little free organic carbon to support their growth. A higher concern is to prevent contamination of the photobioreactor by other phototrophs.

To avoid the contamination of a microalgal cultivation with other phototrophs, the best strategies are high concentration of the inoculum, periodic cleaning of the ponds, and creating specific environmental conditions that favor the growth of the desired algal species, for example cultivation with high salt concentration in

Dunaliella sp. cultures, high pH to *Arthrospira* sp. cultivation, absence of nitrogen source to cultivation of nitrogen-fixing cyanobacteria. These types of photosynthetic microorganisms are common in open ponds cultivation which does not require expensive cleaning or sterilization processes.

Photobioreactors are usually made of optically clear materials (e.g., glass or acrylic) which do not allow themselves to sterilization by steam, and the size of most photobioreactors go over what can be settled in an autoclave. It could be possible to use systems with ozone for sterilization, but they are expensive and difficult to use. Therefore sanitization rather than sterilization is recommended, which can be easily performed with bleach. Air pumps, analyzers, and other equipment can be kept free of other microorganisms by the use of the proper prefilters. (Behrens 2005).

In the Large-scale photobioreactor, the disinfection and sterilization can be done with solutions containing peroxyacetic acid (5%, 20 min) or chlorine which is an oxidizing agent (sodium hypochlorite). The choice of sodium hypochlorite is due to the fact that it is cheap, effective, and it is also possible to neutralize the residual chlorite solution with sodium thiosulfate ($Na_2S_2O_3$), if necessary. According to De Beer et al. (1994), sterilization should be performed after assembly of all components of the reactor through the circulation of chlorine-based solutions. Finally, the PBR was washed with distilled water several times to remove organic and inorganic residues. At the end, the system was emptied and left to dry.

In mixotrophic and heterotrophic cultivation, mainly, the culture medium must also be sterilized. Two general methods are filter sterilization and steaming. Filter sterilization is effective for small volumes or for heat-sensitive components such as vitamins. Steam sterilization of culture medium is more common. The culture medium can be either sterilized and aseptically transferred to the vessel or sterilized in the vessel (Quesnel 1987; Demain and Davies 1999).

4 Scaling-up and Final Considerations

When thinking about developing a microalgal production, the starting point may be the selection of an appropriate and commercially suitable algal strain. It can be performed in small volumes laboratory cultures. The emphasis of this step have to be addressed for optimization of medium composition and culture conditions, analyses of biomass chemical composition, development of product analysis methods and harvesting process, and finally, extraction procedures. Particular interesting photosynthetic micro-organisms are those that grow under particular or stress conditions, which helps in the cultivation in large scale.

When the optimization in the laboratory scale is successfully concluded and the microalgal strain is suitable for being produced in a large scale, the next step is the scale up, either in open ponds or in closed photobioreactor. If preferred, it can be done in small outdoor units, before going to larger ones. Moreover, it should be mentioned that if the desired product is a high value compound produced by the microalgae and it is released to the medium, it is worth evaluating the possibility

of cultivating this microorganism indoor, in small units, for better control of cultivations parameters and mainly the asepsis.

When scaling-up microalgal cultivation, environmental factors like temperature, contamination, agitation, and aeration deserve much attention but it seems that light is one of the most common problems encountered in large-scale microalgae cultivations. Light is rapidly attenuated inside the photobioreactor leading to light intensities heterogeneity inside the photobioreactor. When scaling-up, illumination surface area per unit volume is often used as a photobioreactor design criterion (Ogbonna and Tanaka 1997). In this sense, an efficient photobioreactor has a high surface area-to-volume ratio and, therefore, a pond, for example, should be as shallow as possible. This must also be the reason for the existence of several studies about tubular and panels-like photobioreactors, which allow high surface area-to-volume ratios.

Large scale microalgal production may require great capital and operating costs. Open ponds, mainly mixed raceway ones, are cheaper to build and operate and the most common method of choice for commercial microalgae production, but may require large land areas. But considering the drawbacks such as high susceptibility for contamination, temperature limitations, and light availability, it is worth to intensify the efforts in developing outdoor photobioreactors for effectively producing high amounts of good quality microalgal biomass, meeting the demand that must gradually increase in coming years.

References

Acién FG, Fernández JM, Magán JJ, Molina E (2012) Production cost of a real microalgae production plant and strategies to reduce it. Biotechnol Adv 30:1344–1353

Ávila-Leon I, Matsudo MC, Sato S, Carvalho JCM (2012) Arthrospira platensis biomass with high protein content cultivated in continuous process using urea as nitrogen source. J Appl Microbiol 112:1086–1094

Azov Y, Goldman JC (1982) Free ammonium inhibition of algal photosynthesis in intensive culture. Appl Environ Microbiol 43:435–739

Barbosa MJGV (2003) Microalgal photobioreactor: scale-up and optimization. Ph.D. Thesis. Wageningen University, The Netherlands

Barbosa MJ, Hoogakker J, Wijffels H (2003) Optimisation of cultivation parameters in photobioreactors for microalgae cultivation using the A-stat technique. Biomol Eng 20:115–123

Becker EW (1994) Microalgae—Biotechnology and Microbiology. Cambridge University Press, Cambridge, p 293

Behrens PW (2005) Photobioreactors and fermentors: the light and dark sides of growing algae In: Andersen RA (ed) Algal culturing techniques. Elsevier academic press, London, p 189–204

Belay A (1997) Mass culture of Spirulina Outdoors In: Vonshak A (ed) Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology. Taylor and Francis, London p 131–158

Belkin S, Boussiba S (1991) High internal ph conveys ammonia resistance in S. platensis. Bioresour Technol 32:167–169

Ben-Amotz A, Avron M (1989) The biotechnology of mass culturing Dunaliella for products of commercial interest. In: Cresswell RC, Rees TAV, Shah N (eds) Algal and Cyanobacterial Biotechnol. Longman Scientific & Technical, Essex p 91–114

Berberoglu H (2008) Photobiological hydrogen production and carbon dioxide sequestration. University of California, Los Angeles
Bernhard M, Zattera A, Filesi P (1966) Suitability of various substances for use in the culture of marine organisms. Pubblicazioni della Stazione Zoologica di Napoli 35:89–104
Bezerra RP, Matsudo MC, Converti A, Sato S, Carvalho JCM (2008) Influence of the ammonium chloride feeding time and the light intensity on the cultivation of Spirulina (Arthrospira) platensis. Biotechnol Bioeng 100:297–305
Binaghi L, Del Borghi A, Lodi A, Converti A, Del Borghi M (2003) Batch and fed-batch uptake of carbon dioxide by Spirulina platensis. Process Biochem 38:1341–1346
Blankley WF (1973) Toxicity and inhibitory materials associated with culturing. In: Stein JR (ed) Handbook of phycological methods. I. Culture methods and growth measurements. Cambridge University Press, New York, p 207–229
Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. J Biotechnol 70:313–321
Borowitzka MA (2005) Culturing microalgae in outdoor ponds. In: Andersen RA (ed) Algal culturing techniques. Elsevier academic press, London, p 205–218
Borzani W (2001) Fermentação semicontínua. In: Schmidell-Neto W, Almeida-Lima U, Aquarone E, Borzani W (eds) Biotecnologia Industrial, vol. 2. Edgar Blücher, São Paulo, p 219–222
Boussiba S (1989) Ammonia uptake in the alkalophilic cyanobacterium Spirulina platensis. Plant Cell Physiol 30:303–308
Brennan L, Owende P (2010) Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 14:557–577
Carlozzi P (2003) Dilution of solar radiation through "culture" lamination in photobioreactor rows facing south–north: a way to improve the efficiency of light utilization by cyanobacteria (Arthrospira platensis). Biotechnol Bioeng 81:305–315
Carvalho JMC, Sato S (2001) Fermentação descontínua alimentada. In: Schmidell-Neto W, Almeida-Lima U, Aquarone E, Borzani W (eds) Biotecnologia Industrial, vol. 2 Edgar Blücher, São Paulo, p 205–218
Carvalho AP, Meireleles LA, Malcata FX (2006) Microalgal reactors: a review of enclosed system designs and performances. Biotechnol Prog 22:1490–1506
Carvalho JCM, Bezerra RP, Matsudo MC, Sato S (2013) Cultivation of Arthrospira (Spirulina) platensis by fed-batch process. In: Lee JW (ed) Advanced Biofuels and Bioproducts. Springer, New York, p 781–805
Carvalho JCM, Francisco FR, Almeida KA, Sato S, Converti A (2004) Cultivation of Arthrospira (Spirulina) platensis by fed-batch addition of ammonium chloride at exponentially-increasing feeding rate. J Phycol 40:589–597
Carvalho JCM, Sato S, Converti A, Bezerra RP, Matsudo MC, Vieira DCM, Ferreira LS, Rodrigues MS(applicants) (2009) Método de aproveitamento de dióxido de carbono e seu uso no cultivo de microrganismos fotossintetizantes. Brazil Patent Application Nº 0805123-2, Publication Nº 1998, p 82 (published Apr. 22, 2009)
Chen F (1996) High cell density culture of microalgae in heterotrophic growth. Trends in Biotechnol 14(11):421–426
Chen RC, Reese J, Fan LS (1994) Flow structure in a three-dimensional bubble column and three-phase fluidized bed. Adv Chem Eng 40:1093–104
Chen T, Zhang W, Yang F, Bai Y, Wong Y (2006) Mixotrophic culture of high selenium-enriched Spirulina platensis on acetate and the enhanced production of photosynthetic pigments. Enzym Microb Technol 39:103–107
Chen CY, Saratale GD, Lee CM, Chen PC, Chang JS (2008) Phototrophichydrogen production in photobioreactors coupled with solar-energy-excitedoptical fibers. Int J Hydrog Energy 33:6878–6885
Chisti Y (2006) Microalgae as sustainable cell factories. Environ Eng Manag J 5:261–274
Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306

Choi SL, Suh IS, Lee CG (2003) Lumostatic operation of bubble column photobioreactors for Haematococcus pluvialis cultures using a specific light uptake rate as a control parameter. Enzym Microb Technol 33:403–409

Converti A, Lodi A, Del Borghi A, Solisio C (2006) Cultivation of Spirulina platensis in a combined airlift-tubular reactor system. Biochemical Eng J 32:13–18

Cornet JF, Dussap CG, Gros JB (1998) Kinects and energetics of photosynthetic micro-organisms in photobioreactors. Application to Spirulina growth. Adv Biochem Eng/Biotechnol 59:155–194

Cuaresma M, Janssen M, End EJVD, Vílchez C, Wijffels RH (2011) Luminostat operation: a tool to maximize microalgae photosynthetic efficiency in photobioreactors during the daily light cycle? Bioresour Technol 102:7871–7878

Danesi EDG, Rangel-Yagui CO, Carvalho JCM, Sato S (2002) An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by Spirulina platensis. Biomass and Bioenergy 23:261–269

Das P, Aziz SS, Obbard JP (2011) Two phase microalgae growth in the open system for enhanced lipid productivity. Renew Energy 36:2524–2528

De Beer D, Stoodley P, Lewandowski Z (1994) Liquid flow in heterogeneous biofilms. Biotechnol Bioeng 44:636–641

Degen J, Uebele A, Retze A, Schmid-Staiger U, Trösch W (2001) A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. J Biotechnol 92:89–94

Del Campo JA, García-González M, Guerrero MG (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Appl Microbiol Biotechnol 74:1163–1174

Demain A, Davies J (1999) Manual of industrial microbiology and biotechnology. Washington. DC., ASM

Demirbas A, Demirbas MF (2010) Algae energy—algae as a new source of biodiesel. Springer-Verlag, London

Doucha J, Livanský K (2006) Productivity, CO_2/O_2 exchange and hydraulics in outdoor open high density microalgal (Chlorella sp.) photobioreactors operated in a Middle and Southern European climate. J Appl Phycol 18:811–826

Doucha J, Livanský K (2009) Outdoor open thin-layer microalgal photobioreactor: potential productivity. J Appl Phycol 21:111–117

Dyer DL, Richardson DE (1962) Materials of construction in algal culture. Appl Microb 10:129–131

Fernández FGA, Sevilla JMF, Pérez JAS, Grima EM, Chisti Y (2001) Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. Chem Eng Sci 56:2721–2732

Ferreira LS, Rodrigues MS, Converti A (2010) A new approach to ammonium sulphate feeding for fed-batch Arthrospira (Spirulina) platensis cultivation in tubular photobioreactor. Biotechnol Prog 26:1271–1277

Ferreira LS, Rodrigues MS, Converti A, Sato S, Carvalho JCM (2012a) Kinetic and growth parameters of Arthrospira (Spirulina) platensis cultivated in tubular photobioreactor under different cell circulation systems. Biotechnol Bioeng 109:444–450

Ferreira LS, Rodrigues MS, Converti A, Sato S, Carvalho JCM (2012b) Arthrospira (Spirulina) platensis cultivation in tubular photobioreactor: use of no-cost CO_2 from ethanol fermentation. Applied Energy 92:379–385

Fontes AG, Moreno J, Vargas MA (1989) Analysis of the biomass quality and photosynthetic efficiency of a nitrogen-fixing cyanobacterium grown outdoors with two agitation systems. Biotechnol Bioeng 34:819–824

García-Malea MC, Acién FG, Fernández JM, Cerón MC, Molina E (2006) Continuous production of green cells of Haematococcus pluvialis: modeling of the irradiance effect. Enzyme Microb Technol 38:981–989

Garcia-Malea Lopez MC, Del Rio Sanchez E, Casas Lopez JL, Acien Fernandez FG, Fernandez Sevilla JM, Rivas J, Guerrero MG, Molina-Grima E (2006) Comparative analysis of the

outdoor culture of Haematococcus pluvialis in tubular and bubble column photobioreactors. J Biotechnol 123:329–342
Grobbelaar JU, Nedbal L, Tichý V (1996) Influence of high frequency light/dark fluctuations on photosynthetic characteristics of microalgae photoacclimated to different light intensities and implications for mass algal cultivation. J Appl Phycol 8:335–343
Gudin C, Chaumont D (1991) Cell fragility—the key problem of microalgae mass production in closed photobioreactor. Bioresour Technol 38:145–151
Haque MW, Nigam KDP, Joshi JB (1986) Optimum gas sparger design for bubble columns with a low height to diameter ratio. Chem Eng J 33:63–69
Hattori A, Myers J (1966) Reduction of nitrate and nitrite by subcellular preparations of Anabaena cylindrica. I. Reduction of nitrite to ammonia. J Plant Physiol 41:1031
Hsieh C-H, Wu W-T (2009) A novel photobioreactor with transparent rectangular chambers for cultivation of microalgae. Biochem Eng J 46:300–305
Hu Q, Guterman H, Richmond A (1996) A flat inclined modular photobioreactor for outdoor mass cultivation of photoautotrophs. Biotechnol Bioeng 51:51–60
Hu Q, Kuran N, Kawachi M, Iwasaki I, Miyachi A (1998) Ultrahigh-cell-density culture of a marine alga Chlorococcum littorale in a flat-plate photobioreactor. Appl Microbiol Biotechnol 49:655–662
Janssen M, Slenders P, Tramper J, Mur LR, Wijffels RH (2001) Photosynthetic efficiency of Dunaliella tertiolecta under short light/dark cycles. Enzyme and Microb Technol 29:298–305
Janssen FJJ, Van IJzendoorn LJ, Schoo HFM, Sturm JM, Andersson GG, Denier van der Gon AW, Brongersma HH, de Voigt MJA (2002) Degradation effects in poly para-phenylene vinylene derivatives due to controlled oxygen exposure. Synthetic Metals 131(1–3):167–174
Kaewpintong K, Shotipruk A, Powtongsook S, Pavasant P (2007) Photoautotrophic high-density cultivation of vegetative cells of Haematococcus pluvialis in airlift bioreactor. Bioresour Technol 98:288–295
Kaidi F, Rihani R, Ounnar A, Benhabyles L, Naceur MW (2012) Photobioreactor Design for Hydrogen Production. Procedia Eng 33:492–498
Kunjapur AM, Eldridge RB (2010) Photobioreactor design for commercial biofuel production from microalgae. Industrial Eng Chemistry Research 49:3516–3526
Lau R, Mo R, Sim WSB (2010) Bubble Characteristics in shallow bubble column reactors. Chem Eng Res Des 88:197–203
Lee YK (2001) Microalgal mass culture systems and methods: Their limitation and potential. J Appl Phycol 13:307–315
Lee YK, Low CS (1992) Productivity of outdoor algal cultures in enclosed tubular photobioreactor. Biotechnol Bioeng 40:1119–1122
Li Y, Horsman M, Wang B, Wu N, Lan CQ (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. Appl Microbiol Biotechnol 81:629–636
Lodi A, Binaghi L, De Faveri D, Carvalho JCM, Converti A, Del Borghi M (2005) Fed-batch mixotrophic cultivation of Arthrospira (Spirulina) platensis (Cyanophyceae) with carbon source pulse feeding. Annals of Microbiol 55:181–185
Lopez-Elias JA, Voltolina D, Enríquez-Ocaná F, Gallegos-Simental G (2005) Indoor and outdoor mass production of the diatom Chaetoceros muelleri in a mexican commercial hatchery. Aquacultural Eng 33:181–191
Loubiere K, Pruvost J, Aloui F, Legrand J (2011) Investigations in an external-loop airlift photobioreactor with annular light chambers and swirling flow. Chem Eng Res Des 89:164–171
Markl H, Bronnenmeier R, Wittek B (1991) The resistance of microorganisms to hydrodynamic stress. Int Chem Eng 31:185–197
Márquez FJ, Sasaki K, Kakizono T, Nishio N, Nagai S (1993) Growth characteristics of Spirulina platensis in mixotrophic and heterotrophic conditions. J Fermentation Bioeng 76:408–410
Marxen K, Vanselow KH, Lippemeier S, Hintze R, Ruser A, Hansen UP (2005) A photobioreactor system for computer controlled cultivation of microalgae. J Appl Phycol 17:535–549

Masojídek j, Kopecký J, Giannelli L, Torzillo G (2011) Productivity correlated to photobiochemical performance of Chlorella mass cultures grown outdoors in thin-layer cascades. J Industrial Microbiol Biotechnol 38:307–317

Masojídek J, Papáček Š, Sergejevová M, Jirka V, Červený J, Kunc J, Korečko J, Verbovikova O, Kopecký J, Štys D, Torzillo G (2003) A closed solar photobioreactor for cultivation of microalgae under supra-high irradiance: basic design and performance. J Appl Phycol 15:239–248

Matsudo MC, Bezerra RP, Sato S, Perego P, Converti A, Carvalho JCM (2009) Repeated fed-batch cultivation of Arthrospira (Spirulina) platensis using urea as nitrogen source. Biochem Eng J 43:52–57

Matsudo MC, Bezerra RP, Converti A, Sato S, Carvalho JCM (2011) Use of CO_2 from alcoholic fermentation for continuous cultivation of Arthrospira (Spirulina) platensis in tubular photobioreactor using urea as nitrogen source. Biotechnol Prog 27:650–656

Matsudo MC, Bezerra RP, Sato S, Converti A, Carvalho JCM (2012) Photosynthetic efficiency and rate of CO_2 assimilation by Arthrospira (Spirulina) platensis continuously cultivated in tubular photobioreactor. Biotechnol J 7:1412–1417

Maxon WD (1954) Continuous fermentation. a discussion of its principles and application. Appl Microbiol 3:110–122

Merchuk JC, Ronen M, Giris S, Arad S (1998) Light-dark cycles in the growth of the red microalga Porphyridium sp. Biotechnol Bioeng 59:705–713

Miyamoto K, Wable O, Benemann JR (1988) Vertical tubular reactor for microalgae cultivation. Biotechnol Lett 10:703–708

Molina GE (1999) Microalgae, mass culture methods. In: Flickinger MC, Drew SW (eds) Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation, vol. 3. Wiley, New York

Molina Grima E, Acién FFG, García Camacho F, Chisti Y (1999) Photobioreactors: light regime, mass transfer, and scaleup. J Biotechnol 70:231–247

Molina E, Fernández J, Acién FG, Chisti Y (2001) Tubular photobioreactor design for algal cultures. J Biotechnol 92:113–131

Morita M, Watanabe Y, Saiki H (2000) Investigation of photobioreactor design for enhancing the photosynthetic productivity of microalgae. Biotechnol Bioeng 69:693–698

Morocho-Jácome AL, Converti A, Sato S, Carvalho JCM (2012) Kinetic and thermodynamic investigation of Arthrospira (Spirulina) platensis fed-batch cultivation in a tubular photobioreactor using urea as nitrogen source. J Chem Technol Biotechnol 87:1574–1583

Ogbonna JC, Tanaka H (1997) Industrial-size photobioreactors. Chemtech 27:43–49

Ogbonna JC, Toshihiki S, Tanaka H (1999) An integrated solar and artificial light system for internal illumination of photobioreactors. J Biotechnol 70:289–297

Olguín E, Galicia S, Mercado G, Pérez T (2003) Annual productivity of Spirulina (Arthrospira) and nutrient removal in a pig wastewater recycling process under tropical conditions. J Appl Phycol 15:249–257

Pamboukian CRD (2003) Utilização de processos contínuos para a produção do antitumaral retamicina por Sreptomyces olindensis. In: XIV SINAFERM: Anais. Florianópolis

Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y (2011) Heterotrophic cultures of microalgae: metabolism and potential products. Water Res 45:11–36

Perner-Nochta I, Posten C (2007) Simulations of light intensity variation in photobioreactors. J Biotechnol 131:276–285

Posten C (2009) Design principles of photo-bioreactors for cultivation of microalgae. Eng Life Sci 9:165–177

Pulz O, Scheibenbogen K (1998) Photobioreactors: design and performance with respect to light energy input. Adv in Biochem Eng/Biotechnol 59:124–154

Pulz O (2001) Photobioreactors: production systems for phototrophic microorganisms. Appl Microbiol and Biotechnol 57:287–293

Quesnel LB (1987) Sterilization and Sterility. In: Bu'Lock J, Kristiansen B (eds) Basic Biotechnology, chapter 7. Academic Press Ltd, London, pp 197–215

Raehtz KD (2009) Challenges and advances in making microalgae biomass a cost efficient source of biodiesel. Basic Biotechnology eJournal 5(1). http://ejournal.vudat.msu.edu/index.php/mmg445/rt/printerFriendly/378/360

Reyna-Velarde R, Cristiani-Urbina E, Hernández-Melchora DJ, Thalassoa F, Cañizares-Villanuevaa RO (2010) Hydrodynamic and mass transfer characterization of a flat-panel airlift photobioreactor with high light path. Chem Eng Process Process Intensif 49:97–103

Richmond A (1986) Outdoor mass cultures of microalgae. In: Richmond A (ed) Handbook of microalgal mass culture. CRC Press, Boca Raton, p 285

Richmond A, Becker EW (1986) Technological aspects of mass cultivation—A general outline. In: Richmond A (ed) Handbook of microalgal mass culture. CRC Press, Boca Raton, p 245–264

Richmond A, Grobbelaar JU (1986) Factors affecting the output rate of Spirulina platensis with reference to mass cultivation. Biomass 10:253–264

Richmond A (2000) Microalgal biotechnology at the turn of the millennium: a personal view. J Appl Phycol 12:441–451

Rodrigues MS, Ferreira LS, Converti A, Sato S, Carvalho JCM (2010) Fed-batch cultivation of Arthrospira (Spirulina) platensis: potassium nitrate and ammonium chloride as simultaneous nitrogen sources. Bioresour Technol 101:4491–4498

Rodrigues MS, Ferreira LS, Converti A, Sato S, Carvalho JCM (2011) Influence of ammonium sulphate feeding time on fed-batch Arthrospira (Spirulina) platensis cultivation and biomass composition with and without pH control. Bioresour Technol 102:6587–6592

Rodríguez-Maroto JM, Jiménez C, Aguilera J, Niell FX (2005) Air bubbling results in carbon loss during microalgal cultivation in bicarbonate-enriched media: experimental data and process modeling. Aquacultural Eng 32:493–508

Rosa APC, Carvalho LF, Goldbeck L, Costa JAV (2011) Carbon dioxide fixation by microalgae cultivated in open bioreactors. Energy Convers Manag 52:3071–3073

Sanchez Miron A, Ceron Garcia M, Garcia Camacho F, Molina Grima E, Chisti Y (2002) Growth and characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture. Enzyme Microb Technol 31:1015–1023

Sánchez Mirón A, Contreras Gómez A, García Camacho F, Molina Grima E, Chisti Y (1999) Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. J Biotechnol 70:249–270

Sánchez Pérez JA, Rodríguez Porcel EM, Casas López JL, Fernández Sevilla JM, Chisti Y (2006) Shear rate in stirred tank and bubble column bioreactors. Chem Eng J 124:1–5

Sánchez-Luna LD, Converti A, Tonini GC, Sato S, Carvalho JCM (2004) Continuous and pulse feedings of urea as a nitrogen source in fed-batch cultivation of Spirulina platensis. Aquac Eng 31:237–245

Sánchez-Luna LD, Bezerra RP, Matsudo MC, Sato S, Converti A, Carvalho JCM (2007) Influence of pH, temperature and urea molar flowrate on Arthrospira platensis fed-batch cultivation. A kinetic and thermodynamic approach. Biotechnol Bioeng 9:702–711

Santos AM, Janssen M, Lamers PP, Evers WAC, Wijffels RH (2012) Growth of oil accumulating microalga Neochloris oleoabundans under alkaline-saline conditions. Bioresour Technol 104:593–599

Sassano CEN, Carvalho JCM, Gioielli LA, Sato S, Torre P, Converti A (2004) Kinetics and bioenergetics of Spirulina platensis cultivation by fed-batch addition of urea as nitrogen source. Appl Biochem Biotechnol 112:143–150

Sassano CEN, Gioielli LA, Almeida KA, Sato S, Perego P, Converti A, Carvalho JCM (2007) Cultivation of Spirulina platensis by continuous process using ammonium chloride as nitrogen source. Biomass Bioener 31:593–598

Sassano CEN, Gioielli LA, Ferreira LS, Rodrigues MS, Sato S, Converti A, Carvalho JCM (2010) Evaluation of the composition of continuously-cultivated Arthrospira (Spirulina) platensis using ammonium chloride as nitrogen source. Biomass Bioener 34:1732–1738

Schenk PM, Thomas-Hall SR, Stephens E, Marx U, Mussgnug JH, Posten C, Kurse O, Hankamer B (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioener Res 1:20–42

Schlösser UG (1982) Sammlung von Algenkulturen. Berlin Deutsche Botanische Gesellschaft. 95:181–276
Schneider D (2006) Grow your own?: would the widespread adoption of biomass-derived transportation fuels really help the environment. Am Sci 94:408–409
Scoma A, Giannelli L, Faraloni C, Torzillo G (2012) Outdoor H_2 production in a 50-L tubular photobioreactor by means of a sulfur-deprived culture of the microalga Chlamydomonas reinhardtii. J Biotechnol 157:620–627
Shah YT, Kelka BG, Godbole SP, Deckwer W-D (1982) Design parameters estimations for bubble column reactors. Am Inst Chem Eng J 28:353–379
Shimamatsu H (2004) Mass production of Spirulina, an edible microalga. Hydrobiologia 512:39–44
Siegel MH, Robinson CW (1992) Applications of airlift gas-liquid-solid reactors in biotechnology. Chem Eng Sci 47(13/14):3215–3229
Silva HJ, Cortifas T, Ertola RJ (1987) Effect of hydrodynamic stress on Dunaliella growth. J Chem Technol Biotechnol 40:40–49
Silva PC, Basson PW, Moe RL (1996) Catalogue of the Benthic Marine Algae of the Indian Ocean, vol 79. University of California Publications in Botany, Berkeley, p 1259
Singh RN, Sharma S (2012) Development of suitable photobioreactor for algae production—a review. Renew Sustain Energy Rev 16:2347–2353
Sobczuk TM, Camacho FG, Rubio FC, Fernández FG, Grima EM (2000) Carbon dioxide uptake efficiency by outdoor microalgal cultures in tubular airlift photobioreactors. Biotechnol Bioeng 67:465–475
Soletto D, Binaghi L, Ferrari L, Lodi A, Carvalho JCM, Zilli M, Converti A (2008) Effects of carbon dioxide feeding rate and light intensity on the fed-batch pulse-feeding cultivation of Spirulina platensis in helical photobioreactor. Biochem Eng J 39:369–375
Sonnleitner B (1999) Instrumentation of small scale bioreactors. In: Demain AL, Davies JE (eds) Manual of Industrial Microbiology and Biotechnology. ASM Press. DC, Washington, p 221–235
Torre P, Sassano CEN, Sato S et al (2003) Fed-batch addition of urea for Spirulina platensis cultivation. Thermodynamics and material and energy balances. Enzyme Microbiol Technol 33:698–707
Torzillo G (1997) Tubular bioreactors. In: Vonshak A (ed) Spirulina platensis (Arthrospira): phisiology, cell-biology and biotechnology. Taylor and Francis, London, p 101–115
Tredici MR (2004) Mass production of microalgae: photobioreactors. In: Richmond A (ed) Handbook of microalgal culture. Biotechnology and applied phycology. Blackwell Publishing, Oxford, p 178–214
Tredici MR, Zittelli GC (1998) Efficiency of sunlight utilization: tubular versus flat photobioreactors. Biotechnol Bioeng 57:187–197
Tredici MR, Carlozzi P, Chini G, Materassi R (1991) A vertical alveolar panel for outdoor mass cultivation of microalgae and cyanobacteria. Bioresour Technol 38:153–159
Ugwu CU, Ogbonna JC, Tanaka H (2002) Improvement of mass transfer characteristics of inclined tubular photobioreactors by installation of internal static mixers. Appl Microbiol Biotechnol 58:600–607
Ugwu CU, Ogbonna JC, Tanaka H (2003) Design of static mixers for inclined tubular photobioreactors. J Appl Phycol 15:217–223
Ugwu CU, Aoyagi H, Uchiyama H (2008) Photobioreactors for mass cultivation of algae. Bioresour Technol 99:4021–4028
Valderrama G, Cardenas A, Markovits A (1987) On the economics of Spirulina production in Chile with details on drag-board mixing in shallow ponds. Hydrobiologia 151/152:71–74
Vasumathi KK, Premalatha M, Subramanian P (2012) Parameters influencing the design of photobioreactor for the growth of microalgae. Renew Sustain Energy Rev 16:5443–5450
Vega-Estrada J, Montes-Horcasitas MC, Dominigues-Bocanegra AR, Canizares-Villanueva RO (2005) Haematococcus pluvialis cultivation in split-cylinder internal-loop airlift photobioreactor under aeration conditions avoiding cell damage. Appl Microbiol Biotechnol 68:31–35

Vieira DCM, Matsudo MC, Sato S, Converti A, Carvalho JCM (2012) Simultaneous use of urea and potassium nitrate for Arthrospira (Spirulina) platensis cultivation. Biotechnol J 7:649–655

Vonshak A (1997a) Spirulina: growth, physiology and biochemistry. In: Vonshak A (ed) Spirulina platensis (Arthrospira): physiology, cell-biology and biotechnology. Taylor and Francis, London, p 43–66

Vonshak A (1997b) Outdoor Mass Production of Spirulina: the basic concept. In: Vonshak A (ed) Spirulina platensis (Arthrospira): physiology, cell-biology and biotechnology. Taylor and Francis, London, p 79–99

Vonshak A, Cheung SM, Chen F (2000) Mixotrophic growth modifies the response of Spirulina (Arthrospira) platensis (Cyanobacteria) cells to light. J Phycol 36:675–679

Watanabe Y, Hall DO (1995) Photosynthetic CO_2 fixation technologies using a helical tubular bioreactor incorporating the filamentous cyanobacterium Spirulina platensis. Energy Convers Manag 36:721–724

West JA (2005) Long-term microalgal culture maintenance. In: Andersen RA (ed) Algal Culturing Techniques. Elsevier Academic Press, London, p 157–165

Xu L, Weathers PJ, Xiong XR, Liu CZ (2009) Microalgal bioreactors: Challenges and opportunities. Eng in. Life Sci 9:178–189

Xu L, Wang F, Guo C, Liu CZ (2012) Improved algal oil production from Brotryococcus braunii by feeding nitrate and phosphate in an airlift bioreactor. Eng Life Sci 12:171–177

Yamane T, Shimizu S (1984) Fed-batch techniques in microbial processes. Adv Biochem Eng/Biotechnol 30:148–194

Yoo C, Jun SY, Lee JY, Ahn CY, oh HM (2010) Selection of microalgae for lipid production under high levels carbon dioxide. Bioresour Technol 101:S71–S74

Zhang K, Kurano N, Miyachi S (2002) Optimized aeration by carbon dioxide gas for microalgal production and mass transfer characterization in a vertical flat-plate photobioreactor. Bioprocess Biosyst Eng 25:97–101

Zimmerman WB, Zandi M, Bandulasena HCH, Tesař V, Gilmour DJ, Ying K (2011) Design of an airlift loop bioreactor and pilot scales studies with fluidic oscillator induced microbubbles for growth of a microalgae Dunaliella salina. Appl Energy 88:3357–3369