
Cultivation Systems of Microalgae for the Production of Biofuels

Yordanka Reyes Cruz, Donato A.G. Aranda,
Peter R. Seidl, Gisel C. Diaz, Rene G. Carliz,
Mariana M. Fortes, Deusa A.M.P. da Ponte and
Rosa C.V. de Paula

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<http://dx.doi.org/10.5772/intechopen.74957>

Abstract

As reported in the study, the high-oil/ha-year productivity of microalgae has raised a lot of interest in their use as a source of raw materials for biofuels. However, the high costs of production and maintenance of closed culture systems (photobioreactor type) and the problems of contamination that lead to lower productivity of open systems (of the "open-pond" type) have become important limitations in evaluating the sustainability of producing biofuels from microalgae. In the view of the favorable prospects of employing microalgae as an economically viable source of raw materials for the production of biofuels, this chapter outlines the different ways microalgae are cultivated, the required nutritional conditions and the main procedures used for increasing their scale. Additionally, those more commonly used on a large scale are described and their advantages and disadvantages are pointed out. This analysis results in a proposal of a new type of photobioreactor, of the cylindrical container type, constructed of polyethylene, a non-transparent material that is cheaper and more durable than the ones that are commonly used (polycarbonate, glass or polymethyl methacrylate (PMMA)). Internal illumination of the photobioreactor is provided by a beam from plastic optical fibers that receive sunlight focused at the extremity of the beam.

Keywords: cultivation systems, microalgae, biofuels, photobioreactor

1. Introduction

The progressive exhaustion of fossil-based fuels, the uncertainty in their respective prices and the growing control of their emissions in large cities have led to the generation of energy from renewable sources that reduce the dependence on petroleum and the problems associated with environmental pollution. In Brazil, 45% of the energy and 18% of the fuels are renewable while in the rest of the world 86% is produced from non-sustainable sources. A world leader in the use of biofuels, Brazil, has reached a position that is sought by many countries that try to develop renewable sources of energy as strategic alternatives to petroleum. Bioethanol is used as an automotive fuel since the early twentieth century and all new cars are “flex-fuel” (they run on either ethanol or a combination of around 25% of anhydrous ethanol in gasoline). In the case of biodiesel, it has been added to diesel fuel since 2005, starting with 2% in 2005 and reaching 8% in March 2017 [1].

From this experience, it was possible to determine that 80% of the final cost of the production of biodiesel can be attributed to raw materials. In general, the investigation of alternative and economically viable sources has been the main goal of research on the subject. Thus, ideal sources of biofuels are determined mainly by their availability and costs.

Biofuels can be produced from many different sources. For biodiesel, for example, the most common are soy, palm, sunflower and cotton and among other sources of vegetable oils, animal fats and residues from food preparation. However these sources are not limited to conventional raw materials, they also apply to microalgae. Recent studies have confirmed that the conditions in which they grow, their high productivity in terms of oil/ha/year, viability of genetic manipulation of metabolic pathways, multiplication of yields of biomass in short periods of time and the possibility of controlling these conditions give microalgae a very large advantage in the evaluation of alternative sources of biofuels. Here, we emphasize the cultivation systems that are employed in the production of biomass from microalgae [2, 3].

2. Microalgae in the production of biofuels

Oils found in microalgae have physic-chemical characteristics similar to those of vegetable oils [4, 5] and thus are considered potential raw materials for the production of biofuels. In conventional production systems, microalgae have a higher productivity of oil by hectare than palm, the commercial source of oil with the highest productivity (**Table 1**). Recent reports confirm that microalgae are capable of meeting global demands for combustible oils [6, 7].

Microalgae can be considered a very good alternative source of lipids since they have content between 15 and 75% of their dry weight depending on the type and conditions under which they are cultivated (**Table 2**) [3]. In some cases, when this content reaches 75% of their weight relative to the dry mass, a reduction in cellular growth occurs as is the case of *Botryococcus braunii*, for example. For other microalgae, with levels of oils between 20 and 50%, such as *Chlorella* sp., *Dunaliella* sp., *Isochrysis* sp., *Nannochloris* sp., *Nannochloropsis* sp. and *Tetraselmis* sp., higher growths are reported [8].

Culture	Yield of oil (L/ha)
Microalgae	58.700
Palm	5.950
Coconut	2.689
Canola	1.190
Soy	446
Corn	172

Table 1. Yields in oil/ha from selected sources of biomass.

Microalgae	Lipid content (% dry weight)
<i>Schizochytrium</i> sp.	50-77
<i>Botryococcus braunii</i>	25-75
<i>Nannochloropsis</i> sp.	31-68
<i>Neochloris oleoabundans</i>	35-54
<i>Nitzschia</i> sp.	45-47
<i>Cylindrotheca</i> sp.	16-37
<i>Nannochloris</i> sp.	20-35
<i>Isochrysis</i> sp.	25-33
<i>Chlorella</i> sp.	28-32
<i>Phaeodactylum tricornutum</i>	20-30
<i>Tetraselmis sueica</i>	15-23
<i>Dunaliella primolecta</i>	23
<i>Monallanthus salina</i>	>20
<i>Cryptocodinium cohnii</i>	20

Table 2. Lipid content of selected microalgae.

3. Cultivation of microalgae

The growth characteristics and composition of microalgae are significantly dependent on the type of cultivation—phototrophic, heterotrophic, mixotrophic and photoheterotrophic being the principal types used [9, 10]. Phototrophic cultures occur when the microalgae use light, for

example sunlight, as a source of energy, and CO₂ as a source of inorganic carbon to produce chemical energy by photosynthesis [11].

This type of cultivation has an environmental advantage. Since atmospheric carbon dioxide, the principal contribution to the greenhouse effect, may be used in the production of microalgal biomass for biofuels, it results in a favorable energy balance. Mainly because of this, the phototrophic cultivation is most commonly used for the growth of microalgae [12].

Mata et al. [8] report that the lipid content of microalgae can vary from 5 to 60%, depending on the specie, when cultivated phototrophically. However, light intensity and the insufficient supply of CO₂ are always problematic questions for this type of cultivation. In open cultivation systems the limitation is mostly relative to the photoperiod. Besides, the irregular distribution of light intensity affects the productivity in terms of biomass. Final concentrations of biomass of over 2 g.L⁻¹ of Na are rarely reported in the study [13, 14].

Some authors suggest that supplementation with CO₂ could increase productivities of biomass and lipids, since its concentration in the atmosphere is low [15, 16]. However, it is important to construct systems that prevent the loss of excess CO₂. Still, it must be taken into consideration that the accumulation of oxygen, formed in the process of photosynthesis, increases the production of O₂ that acts as an inhibitor of hydrogenases, the enzymes responsible for the production of hydrogen necessary for the production of lipids. With this, the increase in CO₂ could result in a reduction in the production of lipids [17].

Some species of microalgae can not only grow under phototrophic conditions but can also use organic carbon in the absence of light. In this case, in which algae use organic carbon both as a source of energy and carbon, the cultivation is referred to as heterotrophic [18, 19].

Heterotrophic cultivation avoids problems associated with the limitation of light and has led to relevant results in the production of microalgal biomass, the yields being significantly superior to those from phototrophic cultivation [11].

Xu et al. [20] observed an increase of 40% in the lipid content when they altered the type of culture from phototrophic to heterotrophic for *Chlorella protothecoides*.

The choice of heterotrophic metabolism is questioned in the sense that it is necessary to add a source of organic carbon and may lead to high costs if it must be purchased, making the production of biofuels from microalgae unviable [21, 22].

In mixotrophic cultivation, the microalgae are submitted to photosynthesis and uses organic and inorganic (CO₂) compounds as a source of carbon for growth. Thus, microalgae are capable of living under both phototrophic as well as heterotrophic conditions. As they use organic compounds, microalgae release CO₂ via respiration, being absorbed and utilized under phototrophic cultivation [8].

In photoheterotrophic cultivation, microalgae require light when they use organic compounds as a source of carbon. The principle difference between the mixotrophic and photoheterotrophic is that, for the former, only light is used as a source of energy. Besides, in photoheterotrophic systems, light and other organic sources are necessary at the same time. Although the production of some metabolites regulated by the intensity of light could be increased in

	Phototrophic growth	Heterotrophic growth	Mixotrophic growth	Photoheterotrophic growth
Energy source	Light	Organic	Luz e orgánica Light and Organic	Light
Carbon source	Inorganic - Carbon dioxide	Organic - External carbon source	Organic and inorganic carbon source simultaneously	Organic External carbon source
Cell density	Low	High	Medium	Medium
Growing systems	Open pond or Photobioreactor	Conventional fermenter	Photobioreactor	Photobioreactor
Cost	Low	Medium	High	High
Main disadvantages	Low cell density. Contamination of open pond algae cultivation systems	High cost of substrate. Risk of contamination	High cost of substrate and Photobioreactor	High cost of substrate and Photobioreactor

Table 3. Cultivation methods.

photoheterotrophic cultivation, the application of this type of cultivation for production of biodiesel is very rare, as is the case with mixotrophic cultivation. Both types of cultivation are limited by the risk of contamination and the presence of light may require a special large-scale photobioreactor, resulting in high costs of operation [17].

Table 3 summarizes the main characteristics of each type of cultivation.

4. Nutritional conditions for the cultivation of microalgae

The production of lipids and the concentrations of different fatty acids in microalgae are also influenced by the composition of culture media. Frequently the increase in the accumulation of fatty acids is described as a consequence of the effects of the limitation of nutrients and the time of cultivation [8].

Under growth limiting conditions a drop in cellular division is verified in the photosynthetic rate of protein synthesis. The photosynthetic energy is deviated from the cellular division to the accumulation of carbohydrates and synthesis of lipids, also resulting in an increase in the synthesis of enzymes that are specific for the absorption of nutrients [23, 24].

On the other hand, Huerlimann et al. [18] verified an increase in the content of some lipid classes in the exponential phase of *Rhodomonas* sp. and *Isochrysis* sp. cultivated, in K⁺ medium and under 250 μ moles photons.m⁻².s⁻¹. Usually, microalgae present a small production of lipids during the exponential phase, generally of polars polyunsaturates, with an increase in the synthesis when cultures reach the stationary phase of growth, the apolars predominating [25, 26].

In the composition of microalgae, besides carbon (C), at least 19 chemical elements are present. Some are necessary in concentrations in the order of milligrams per liter, such as H, N, O, P, S, K, Na, Ca and Mg. Others can be detected as trace elements or micronutrients and normally are required in concentrations of nanograms to micrograms per liter, such as Si, Fe, Mn, Mo, Cu, Co, Zn, B and Va. These micronutrients are incorporated in essential organic molecules as a variety of coenzymes (CoA, cobamamide, etc.) that participate in reactions that are primordial to the life of the cells [27].

The macronutrients form the structural constituents of biomolecules, in the cytoplasmic membranes of the intracellular medium, and still take part in the energetic and metabolic regulation processes. The absence or insufficiency of these micronutrients can cause damage affecting some of the vital functions of these microorganisms [28].

Among the most important nutrients are phosphorus (P) and nitrogen (N) that exist in the aquatic environment in diverse forms. They may be dissolved, as particulates or in biotic form. However, only the dissolved form is directly available for growth of microalgae. Several species still require minute quantities of organic compounds for their growth, as is the case with vitamins [28].

Phosphorus is an important limiting factor for the growth of microalgae, since it is essential for cellular processes such as the transfer of energy (ATP) and the biosynthesis of nucleic acids, phospholipids, DNA, and so on, influencing the composition of biomass. Inorganic orthophosphate (PO_4^{3-}) is the ionic form of phosphorus preferred by microalgae and its absorption depends on energy. Thus, this is the source of phosphorus most commonly used in culture media. Other sources of inorganic phosphorus exist that could be absorbed by microalgae, such as dyadic phosphate or dihydrogen phosphate (H_2PO_4^-) which are species obtained from orthophosphoric acid (H_3PO_4) [29].

Vitamins are essential organic compounds for the functioning of the metabolism and many can be found as cofactors of enzymes, carrying out the functions of coenzymes that have vital roles for the viability and growth as well as the accumulation of biomolecules in the cell. Among them, biotin stands out as the coenzyme that catalyzes activation and transfer of CO_2 reactions, cobalamine (B12), a coenzyme that catalyzes de-isomerization and transfer of methyl group reactions, and thymine (B1), the coenzyme that catalyzes activation and transfer of aldehyde reactions [30].

Some biotechnological processes with microalgae aim for high yields in biomass and, for this, must choose the adequate nutrients and physic-chemical parameters, taking into consideration the natural habitat of the species in order to determine the basic necessities for their growth. On the other hand, some biotechnological applications are directed to stress conditions to optimize the biosynthesis of specific bio-compounds, such as fatty acids. The most widely studied stress factors are the concentrations of certain nutrients, light intensity, temperature, salinity and pH. The limitation of nutrients in the cultures affects, in large proportions, the chemical composition of the algae, as well as their rate of growth [31].

In studies run with microalgae cultivated under low concentrations of nitrogen, Piorreck et al. [25] observed an increase in the lipid content of these microalgae without, however, altering the lipid and fatty acid profile. In *Chlorella*, cultures in which the cellular division ceased because of the lack of nitrogen in the culture medium, the lipid content of the cells increased from 28 to 70%, coinciding with a decrease in the protein content from 30 to 8% [32]. Most of the culture media used to facilitate accumulation of lipids in the microalgae are modified according to those that are known such as BG-11 [33] and BOLD 3 N [34].

5. Advantages of using microalgae for biofuels

The cultivation of microalgae for the production of biofuels can be considered highly promising mainly because of the diverse advantages already mentioned in the study [2, 6, 35–39].

- They do not require arable land and can be cultivated in desert regions and on degraded soils since the demand for land is only utilized as a support for the cultivation system.
- They do not compete with agriculture.
- Even growing in aqueous media, they consume less water than terrestrial plants and, depending on the process utilized in concentration of biomass, the residual water may be reutilized in the process, reducing global consumption of fresh water.
- They are produced all year round and do not depend on seasons and crops.
- They have high productivity in biomass and rapidly accumulate lipids, between 15 and 50% in dry mass in many species.
- They can produce more than half the oxygen in nature.
- Their cultivation does not require the application of herbicides or pesticides.
- The nutrients for their cultivation can be obtained from residual waters and agroindustrial wastes.
- They efficiently fix atmospheric carbon, or even residues from industrial process, through photosynthesis (each ton of biomass produced consumes 1.7 tons of CO₂, 10–20 times more than is absorbed by cultures of oilseeds).
- They can produce a series of other valuable products besides lipids, such as proteins, carotenoids and carbohydrates that can be utilized as foods or fertilizers, fermented to produce ethanol or other products with high added value.

Besides the innumerable advantages previously cited, the production of oil from microalgae of approximately 60,000 liters/ha/year can surpass that of palm oil (5000 liters/ha/year) and of soya (450 liters/ha/year). More optimistic financial analyses affirm that oil from microalgae can be produced at a cost of US\$ 0.50/L [2, 3].

6. Cultivation of microalgae on a lab scale

The cultivation of microalgae requires a climatized space, with stable temperature, so that the thermal amplitude allows the activities that are necessary to the cell. The atmosphere must have controlled access to reduce the heat exchange and contamination. As the temperature affects the metabolic rate of the organisms, it must be chosen according to species that is studied and what the cultivation is for. The constancy of the temperature and the low variability ($< 0.5^{\circ}\text{C}$) provide stability and predictability to the cultivation. Tropical species can be cultivated under temperatures between 20 and 25°C , such as, for example, *Spirulina*, *Scenedesmus*, *Ankistrodermus*, *Monoraphidium*, *Chlorella* and *Chlamydomonas*, among others. Generally, the choice is for a temperature of 23°C that is tolerated, although it may not favor optimum growth. Light intensity, its duration and wavelength influence growth of phytoplankton. Incandescent lamps better simulate the amplitude of wavelengths between 350 and 700 nm, necessary for photosynthesis but could heat the cultivation. Fluorescent lamps do not heat up since the wavelengths in the red region are not emitted, but they could lead to unsatisfactory growth. Sunlight, not in excess, could stimulate growth. The efficiency of solar collectors in the production of microalgae also is being studied [40]. Lamps of 40 and 20 W are more frequently utilized, a distance of 25–30 cm from the cultivation is being recommended to minimize the heat. The adequate photoperiod is important, the use of 12:12 hours (dark light) for maintenance of cultivations and continuous light for 18:6 hours being common for commercial purposes [28, 41].

In the cultivation rooms the inoculums of microalgae are normally preserved in sterile glass tubes (**Figure 1A**) utilizing an incubator (**Figure 1B**). This preservation is conducted under controlled conditions (temperature between 20 and 25°C , light intensities of $40 \mu \text{ moles}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, photoperiod of 12 h) and with manual agitation three times a week in order to not allow the cells to decant for too long.

6.1. Scaling up the cultivation of microalgae from the lab

The cultivation of microalgae from the lab is scaled up by successive transfers of algal cultures of systems of cultivation from smaller to larger followed by the addition of culture media. In most cases four transfers are made, namely culture from the preservation tube to the cultivation system of 250 mL; activation culture, from the activation culture to the system of 1 L, a pre-inoculum culture; from the pre-inoculum culture to a system of 20 L, to obtain the bottle cultivation; followed by the propagation from the bottle to various systems of 20 L, called propagation cultivations to obtain the volume of culture to be inoculated in the photo-bioreactors reaching, in this step, the cultivation of microalgae on a pilot scale (**Figure 2**). The four transfers, from the preservation step until the cultivation propagation, are run under sterile atmospheres and also utilize sterile materials.

The growth of cultures regarding microalgae, in each step of cultivation previously described, follows the growth phases given in **Figure 3**—*Lag or adaptation* (induction of growth), *Log* (exponential growth), *Transition* (reduction of growth), *stationary* and *decline/death*. The period of duration of each phase depends on the specie and the conditions of cultivation. For the construction of

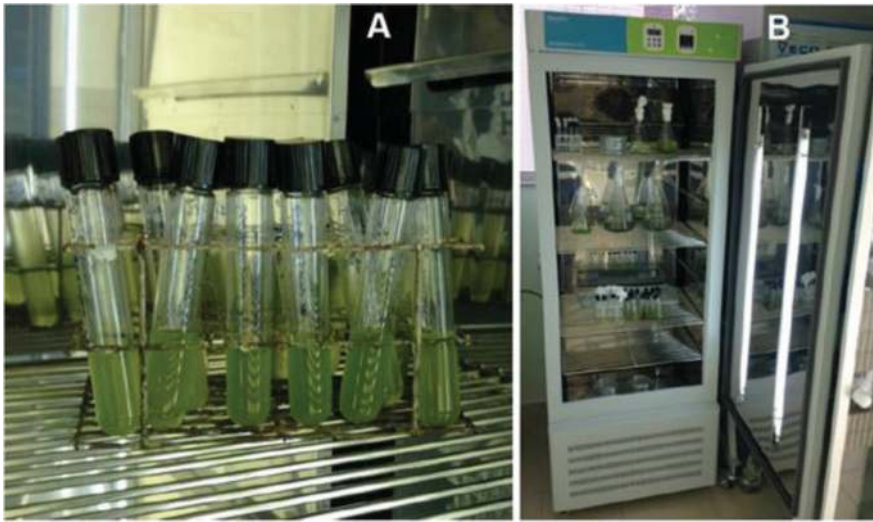


Figure 1. *Scenedesmus* sp. inoculum, conserved (A) and incubator (B).

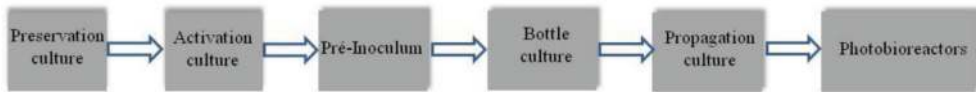


Figure 2. Flowchart of scale up for the cultivation of microalgae used by GreenTech.

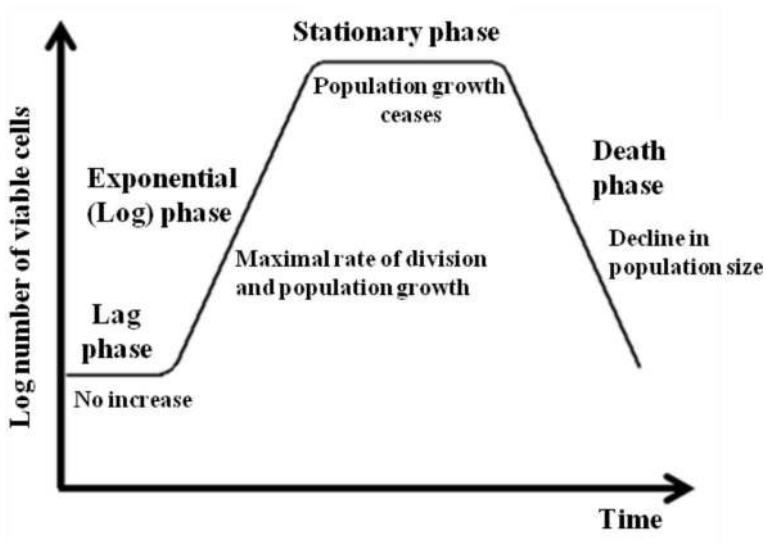


Figure 3. Microbial growth curve.

kinetic profiles of each culture, necessary to monitor the biomass produced, samples are collected every day, and their analyses of cell count, dry weight and turbidity are run.

7. Principal cultivation systems of microalgae on a large scale

The large-scale systems of cultivation of microalgae were developed during the first decades of the twentieth century. These organisms can be cultivated in diverse systems of production. The systems of cultivation on a large scale commonly employ types like “open ponds”, generally called “raceways”, where tanks of varied sizes are kept in the open air, exposed to natural conditions of illumination, temperature, evaporation and contamination. These tanks generally are shallow, constructed in concrete, fiberglass or polycarbonate, with an earth bottom or coated with plastic material, where the cultures are kept in constant circulation. The other well-known open system of cultivation is the cascade model or descending film, developed in the Czech Republic around the 1970s. Here, the culture is exposed to sunlight in a thin film, of about 1 cm, that promotes a cellular density of up to 10 g L^{-1} . This system has a circuit with a base originally in glass, which made it very expensive, but with the evolution of models, the use of cheaper materials, plastics, cement or metals made the costs decrease. The system has some advantages, namely a thin film of culture leading to a high concentration of cells, an efficient transfer of gas liquids favoring the exposure of the culture to sunlight even more, a low cost compared to other closed reactors and the necessity of less area for its installation, when compared to the *raceway*. Although it is a system with good productivity, the possibility of contamination remains analogous to systems of *raceways*. There are some variations of this system where the tray on which the culture is exposed to sunlight presents undulations, allowing cycles of light and dark, that result in an increase in lipid content of the cell, in spite of the decrease in productivity [42, 43].

An open photobioreactor of the descending film type was constructed in fiberglass at the Laboratory of GreenTechnologies, GreenTec/EQ/UFRJ, with the objective of producing biomass from microalgae to be utilized as a raw material in the development of technologies for the production of biofuels (**Figure 4**). Additionally, in this system of cultivation, it was possible to monitor the growth of lineages of microalgae that were cultivated and compare the results obtained from the growth of lineages in photobioreactors.

Today, 95% of the total production of microalgae is in open systems. The volume of around 20,000 tons of microalgae/year is considered incipient in terms of biofuels [44].

Other more sophisticated systems of cultivation of microalgae are the closed ones known as photobioreactors. These cultivators are usually constructed from transparent materials, glass or plastics, distributed in flat panels or in serpentines. There are diverse models of photobioreactors: bubble columns, windows, horizontal tubes, helicoidal, agitated tanks and so on [45]. In spite of the higher initial costs, photobioreactors have many advantages over open systems. In photobioreactors, it is possible to control the conditions of cultivation. This way the concentration of nutrients, temperature, light and pH can be adjusted to obtain higher yields of biomass in shorter times, reaching much higher productivities when compared to open systems.

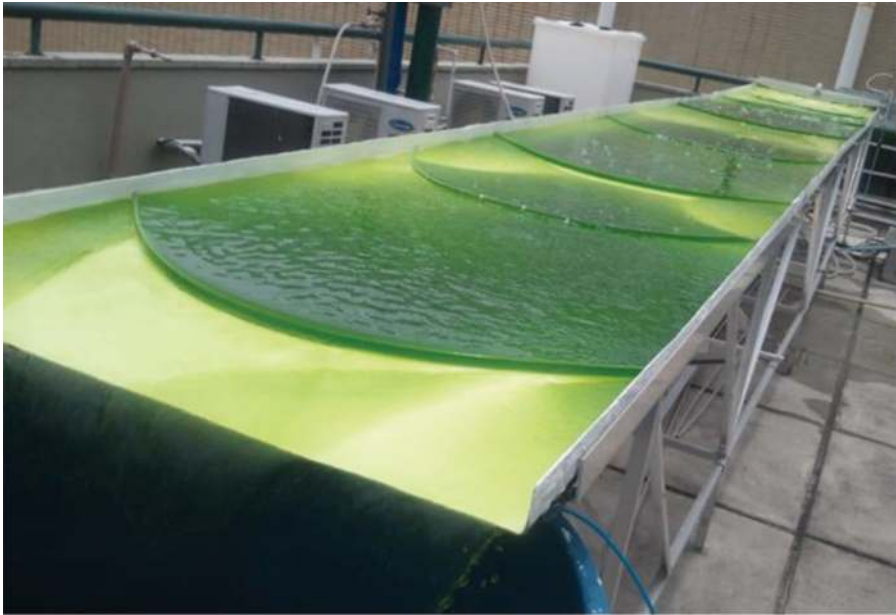


Figure 4. Thin-layer cascade cultivator installed at GreenTech.

In the GreenTec/EQ/UFRJ pilot unit 30, vertical photobioreactors of the window type are distributed in 3 series of 10 arranged in parallel as can be visualized in **Figure 5**. Each series of photobioreactors has a capacity of 1100 L of cultivation.

The series of photobioreactors of the pilot unit have the following characteristics:

- polycarbonate structure (5 mm thickness) that is 1.2 m wide, 0.8 m in height and 11 cm thickness, containing internal baffles, top lid, two lower outlets and a connections for hoses.
- three refrigeration systems composed of three iced-water generators with temperature controllers; tubes with isolation; three temperature sensors; and three stainless steel serpentine, submerged in the culture of the first photobioreactor in each series;
- an air injection system in each photobioreactor with stainless steel tubes, with outlets of bubbled air in the basal part of the reactor and valves to control the flow of air;
- a system to control the pH, composed of a control panel, three pH sensors and tubes connected to the CO₂ network through which cultures are injected when the pH limit is passed;
- recirculation of the cultivation system with tubes and pumps.

The recirculation of the culture along 10 photobioreactors of each series occurs through its passage from the first reactor to the second, from the second to the third and so successively, until reaching the tenth, from where it is pumped back to the first via tubes (**Figure 6**).



Figure 5. Photobioreactors at GreenTech.

The passage of the culture from one reactor to the other occurs by overflow through a small canal, situated between the reactors in the superior part. In each reactor, the culture follows a trajectory in a zigzag determined by the positioning of the internal baffles (Figure 7). The water utilized to make the cultures of microalgae developed in photobioreactors is treated through a hollow-fiber microfiltration system and activated pressurized charcoal that has a capacity of approximately 900 L/h.

In open systems, productions close to 180 tons per ha/year can be reached, competing naturally with the other microorganism in culture medium. The production in closed photobioreactors is more expressive, giving bigger volumes and, at times, over 1.500 tons/ha/year, since they can optimize the cultivation conditions, such as luminosity, temperature and pH that are favorable for the growth of populations of microalgae [40].

A study by Chisti [3] confirms the advantages of production of microalgae in photobioreactors in place of recirculation tanks. Taking as a basis the calculation of production of 100 tons of biomass for the two systems, with the same absorption of CO_2 , the volumetric productivity



Figure 6. Recirculation of cultivation system along the ten photobioreactors at GreenTech.

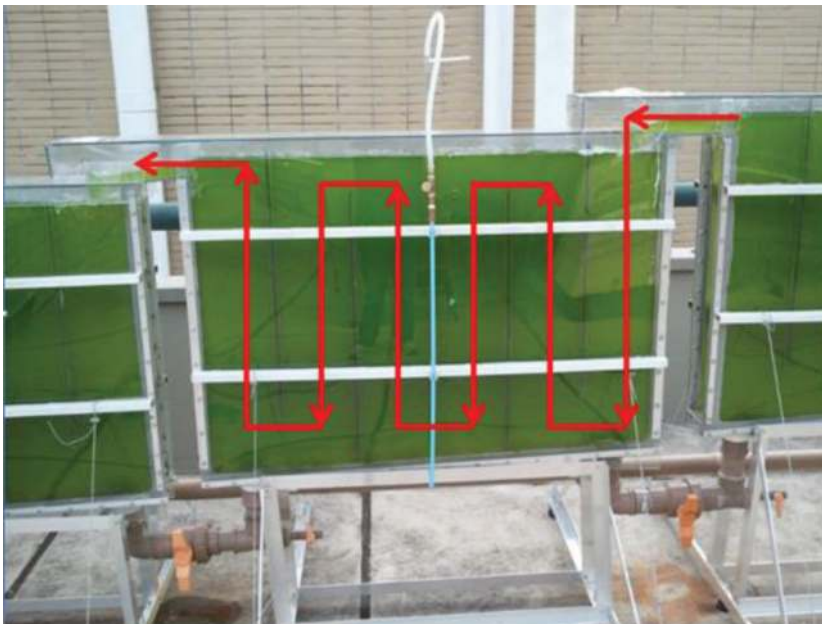


Figure 7. Trajectory of cultivation system in photobioreactors during recirculation.

of photobioreactors is 13 times larger than that of tanks. The area necessary also favors the photobioreactors, being approximately 30% inferior, assuming a similar productivity for the two systems of cultivation. The costs of separation are also an advantage of the photobioreactors, since the culture is 30 times more concentrated than in the recirculation tanks, and thus the separation of biomass from water is facilitated [46].

The estimated cost of production for each kilogram of biomass is, respectively, € 6.39 and € 3.80 for photobioreactors and recirculation tanks. These values do not take into account the costs of CO₂, which could be obtained at cost zero. If the annual capacity of production of biomass could go over 10,000 tons, the costs of production for each kilogram are reduced to US\$ 4.11 and US\$ 1.17, for photobioreactors and recirculation tanks, respectively, because of economies of scale [47].

However, photobioreactors have several disadvantages that need to be considered and validated, such as difficulties with amplification, deterioration of the transparent material utilized, high cost of construction (investments including 10 times that of an open tank) and damage to the cells because of shearing stress [8, 48].

Based on advantages and disadvantages of systems of closed cultivation (photobioreactors), at GreenTec/EQ/UFRJ, a new model of photobioreactor of the cylindrical container type, of a volume similar to that described previously but occupying a smaller area because of the relative depth, was developed. The internal illumination of the photobioreactor is made by a beam of optical plastic fibers that receive sunlight from lenses focused on the extremities of the beam. These lenses are mounted on a solar tracking system that permits a more efficient use of light.

The great differential of this system of illumination is that it is possible to use non-transparent materials in the construction of photobioreactors that are much cheaper and durable, such as polypropylene, without incurring in additional energy costs.

The proposed model will have the following advantages when compared to conventional photobioreactors: (a) systems with less entrance and exit of air, minimizing contamination; (b) occupies a small area; (c) uses materials that are not transparent, thus cheaper and more durable; (d) does not require the use of systems of heat exchangers to avoid the increase in temperature of the culture because of the use of isolation with polyurethane in the external part of the tank; (e) higher productivity of microalgae biomass relative to open systems; and (f) higher yields of lipids in the composition, fundamental aspects in the production of biodiesel.

In the pilot unit of GreenTec/EQ/UFRJ, a prototype of this new photobioreactor was mounted as can be visualized in **Figures 8 and 9** utilizing a polypropylene tank of 25 L capacity. An increase in the scale of this model is planned for a tank of similar characteristics and a volume of 1000 L.

After each cultivation is over, a culture of microalgae is directed to the concentration step. Diverse processes have been tested and continue being evaluated in this step, especially flocculation, microfiltration and centrifugation.

Two technologies flocculation followed by centrifugation and microfiltration followed by centrifugation are fundamentally utilized in the pilot unit of GreenTec/EQ/UFRJ. Biomass from microfiltration concentrated approximately 35 times is submitted to centrifugation at 10,000 rpm, 6°C, during 15 minutes, to obtain a cultivation concentrated over a 100 times (**Figure 10**).



Figure 8. Prototype: non-transparent photobioreactor with internal illumination by plastic optical fibers, coupled to solar tracking.



Figure 9. Components of the prototype.



Figure 10. Concentrated microalgal biomass.

Filtered and centrifuged residual water is treated in a system of microfiltration with activated carbon and reutilized in cultures of photobioreactors.

8. Main challenges in the production of biodiesel from microalgae

The principal challenge in the use of microalgae as raw material for biodiesel is the selection of promising species with triacylglycerides in optimum conditions of cultivation, adaptation and growth of cultures (inocula), systems on a large scale and overall reduction of production costs [26].

The production of microalgae biomass requires basic inputs such as energy, water, CO₂ and mineral nutrients. In order to assure the viability of the cultivation of microalgae on the necessary scale, industrial effluents are presently discarded into the environment and use CO₂ produced by several industries such as power plants cement factories and so on.

The refining of bio-oil extracted from microalgae is another of the current limitations in the production of international quality biodiesel. This fraction, in addition to being composed of triglycerides and fatty acids convertible into biodiesel, contains antioxidants and hydrocarbons that cannot be converted into biodiesel. The latter have polarity and degree of saturation similar to the compounds of interest for biodiesel, making it difficult to extract them.

It is also indispensable to invest in the development of processes that make full use of microalgae according to the biorefinery concept [49, 50].

However, in the last few years, the research related to this topic has been advanced, fundamentally in the cultivation stage, responsible for the higher costs of the productive process. With this objective, new and cheaper cultivation systems with higher productivity of biomass are being developed and proposed. In addition, studies are carried out to modify the composition of culture media, aiming at reducing the cost of the nutrients used as sources of nitrogen and phosphorus.

Author details

Yordanka Reyes Cruz^{1,3}, Donato A.G. Aranda^{1,2}, Peter R. Seidl^{2*}, Gisel C. Diaz^{1,2},
Rene G. Carliz^{1,2}, Mariana M. Fortes^{1,2}, Deusa A.M.P. da Ponte^{1,2} and Rosa C.V. de Paula^{1,2}

*Address all correspondence to: pseidl@eq.ufrj.br

1 GreenTechnologies, Green Tec. Rio de Janeiro, Rio de Janeiro, Brazil

2 School of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

3 Department of Organic Processes, School of Chemistry, Federal University of Rio de Janeiro, Brazil

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