Chapter

Microalgae Cultivation in Photobioreactors Aiming at Biodiesel Production

Mateus S. Amaral, Carla C.A. Loures, Fabiano L. Naves, Gisella L. Samanamud, Messias B. Silva and Arnaldo M.R. Prata

Abstract

The search for a renewable source as an alternative to fossil fuels has driven the research on new sources of biomass for biofuels. An alternative source of biomass that has come to prominence is microalgae, photosynthetic micro-organisms capable of capturing atmospheric CO2 and accumulating high levels of lipids in their biomass, making them attractive as a raw material for biodiesel synthesis. Thus, various studies have been conducted in developing different types of photobioreactors for the cultivation of microalgae. Photobioreactors can be divided into two groups: open and closed. Open photobioreactors are more susceptible to contamination and bad weather, reducing biomass productivity. Closed photobioreactors allow greater control against contamination and bad weather and lead to higher rates of biomass production; they are widely used in research to improve new species and processes. Therefore, many configurations of closed photobioreactors have been developed over the years to increase productivity of microalgae biomass.

Keywords: raw material for biodiesel production, biomass, microalgae, photobioreactors, biodiesel synthesis

1. Introduction

The continuous rise in global temperature caused by the emission of greenhouse effect gases, mainly carbon dioxide (CO_2) mostly due to anthropogenic activities such as the burning of fossil fuels, has led the search for alternative and renewable fuels, such as biofuels. Biofuels present a balance between the CO_2 emitted during burning and the subsequent absorption during the formation of biomass, a sustainable cycle.

Biofuels such as bioethanol and biodiesel are alternative substitutes to gasoline and diesel, respectively and have several conventional renewable sources of raw materials. The raw materials for the production of biodiesel are based on oils and fats, which may be of vegetable origin (soybean, peanut, cotton and sunflower oils), animal origin (fish oil, beef tallow, lard and fat from chicken) or residual frying oils and fats, all sources committed to the food chain.

However, another raw material, not competitive with the food chain, has received special attention for presenting many advantages when compared to conventional sources. These are microalgae which are photosynthetic microorganisms capable of accumulating high levels of oil in their cytoplasm which, in turn, can be extracted from biomass and converted into biodiesel.

Microalgae grow at a high speed (less than a week), requiring small territorial areas to develop, presenting high photosynthetic efficiency and, consequently, good absorption of atmospheric CO_2. They may use domestic and industrial effluents to develop (not requiring clean water), thus standing out as a promising raw material for the production of biodiesel.

Microalgae cultivations generate high concentration of biomass and, therefore, can be used in the production of biodiesel. The cultivation is generally conducted in illuminated bioreactors, called photobioreactors, which can have different shapes and configurations. Some configurations include columns of bubbles, tubulars, flat plates and open tanks in the shape of "raceways", the most well-known and widely spread configuration worldwide. However, the design and development of photobioreactors with new configurations and different lighting modes still play a crucial role in optimizing cell growth and increasing biomass formation.

In this context, this chapter will present the reasons for the use of microalgae as sources of biodiesel, the main photobioreactors used for the production of microalgae biomass and a brief description of the reaction synthesis of biodiesel.

2. Biodiesel sources

The average temperature of the planet has been increasing year by year and causing serious climatic imbalances, such as changes in the rain pattern and melting of the glaciers, resulting in losses in local agriculture and rising sea levels. Much of this rise in global temperature is due to the anthropogenic emission of carbon dioxide (CO_2) into the atmosphere, mainly from the burning of fossil fuels [1].

According to IPEA [2], the anthropogenic contribution to the emission of CO_2 in the atmosphere is mainly related to energy purposes, in which transportation is responsible for 67% of the emissions as a result of carbon oxidation during the combustion of the most varied fuels.

For energy purposes, the contribution of fossil fuels is higher than 80% of the anthropogenic emission of CO_2 into the Earth's atmosphere, corresponding to approximately 35 giga tons of CO_2 emitted in 2015 [2, 3].

Although technically there is a diversified energy matrix, including hydroelectric, thermoelectric, wind, solar and nuclear energy sources, the planet is highly dependent on thermoelectric plants, which burn coal (mainly) and natural gas, fossil fuels that are of finite origin and unsustainable, basically transferring terrestrial carbon to the atmosphere in its majority in the form of CO_2 [3].

In addition to the massive presence of fossil fuels in the generation of electric energy, they also dominate the transportation sector, overloaded with vehicles powered by combustion, with exponents busses and trucks fueled by diesel, a fossil fuel that highly contributes to emissions of CO_2 [4].

Biodiesel is the biofuel that can replace diesel and can be produced from vegetable oils (soy, palm, sunflower, cotton, peanuts and others), oils from microorganisms (cyanobacteria and microalgae), animal fat and the frying oil reuse [5, 6]. The current biofuel projections are based on sources that are also food commodities and resources suitable for conventional agriculture. Thus, the biodiesel production involves the use of lands already used to food production [7].

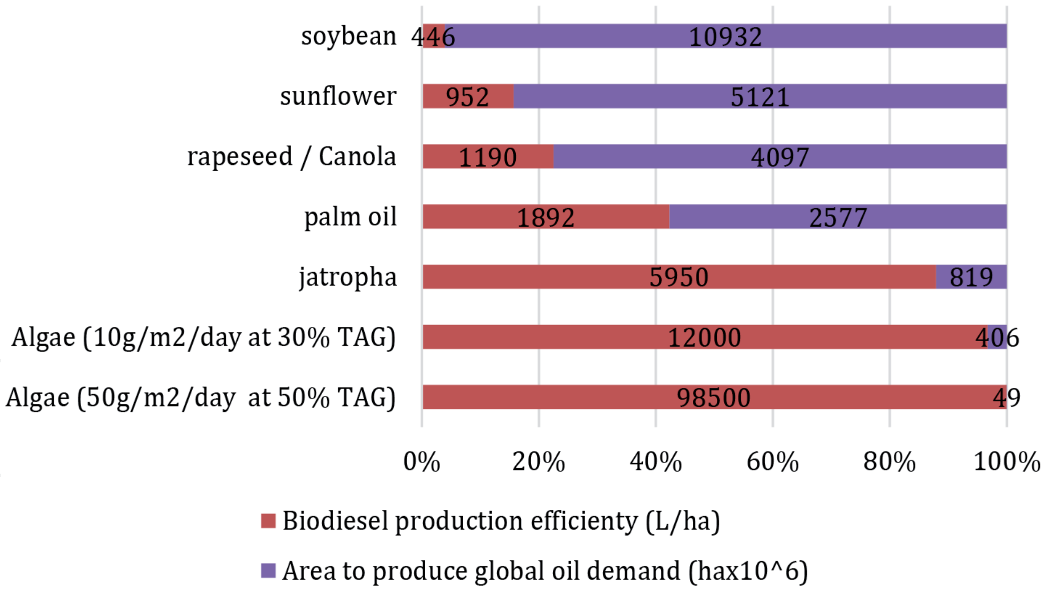

Figure 1.
Different sources of biodiesel and their respective production efficiencies and territorial demand.

In addition, the use of microalgae biomass for the production of biodiesel has been gaining prominence, as this type of microorganism has high growth rates even on smaller areas of cultivation, microalgae biomass can generate high amounts of biomass and oils extracted from its dry biomass compared to vegetable oilseeds as seen in **Figure 1**. The cultivation of microalgae promotes the biofixation of CO_2, allows the use of wastewater to treat industrial and domestic effluents and does not compete with agricultural food production [8, 9].

3. Microalgae

Microalgae are single-celled or multicellular organisms with microscopic dimensions, capable of photosynthesis due to the presence of chlorophyll [10]. Microalgae mainly inhabit aquatic environments such as: lakes, rivers and oceans, although they can live in solid humid environments such as wet soil and rocks. They have about 50% carbon in their composition, based on their dry biomass, as a result of photosynthesis [11, 12].

In the sea, microalgae constitute phytoplankton and are responsible for approximately 90% of photosynthetic activity in the oceans which can lead to an annual fixation of 45–50 Gton of carbon in the oceans [13]. It is estimated that 1.8 kg of absorbed CO_2, during photosynthesis, generates 1 kg of microalgal biomass [14, 15]. The photosynthesis reaction that represents this conversion of CO_2 into biomass can be represented in a simplified way by Eq. (1).

$$6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2 \tag{1}$$

The biological composition of microalgae varies widely between species and is strongly influenced by environmental factors such as temperature, lighting, photoperiod, pH of the culture medium, mineral nutrients, CO_2 supplement, etc. [15]. It is known that the concentration of nitrate in the microalgae culture medium significantly influences the production of lipids by the cell, especially when available in low concentrations inducing its accumulation inside the cell [16].

Microalgae synthesize lipids from the carbon source, whether inorganic such as CO_2 or organic (glucose, acetate, etc.). The components and levels of lipids in microalgal cells vary from species to species, being basically divided into neutral lipids (triglycerides and cholesterol) and polar lipids, such as phospholipids. Neutral lipids, such as triglycerides, are considered as the main material to produce biodiesel [17].

The oil content commonly found in microalgae is in the range of 15 to 50%, but these levels can be increased according to the manipulation of the crops aiming at the accumulation of lipids. In addition, microalgae of the Chlorella genus require less cultivation time and because of its high cell productivity and potential lipid extraction, microalgae can be widely used as a raw material in the production of biodiesel. The conversion to biodiesel occurs by the transesterification reaction using alcohol in the presence of acid or base as catalysts [12, 18].

Another group of substances abundant in the composition of most microalgae species are proteins. The high amount of proteins present in the cell composition in the most varied species of microalgae gives these microorganisms their recognition as an unconventional source of proteins. Proteins extracted from microalgal biomass for human nutrition are currently commercialized in various forms, such as tablets, capsules and liquids, which can be incorporated into pasta, cakes, sweets and drinks [19].

Along with proteins, the considerable composition of numerous essential vitamins in microalgae stands out: vitamin A, B1, B2, B6, B12, C, E, biotin, folic acid, among others, in levels that vary between species and according to cultivation techniques, as well as nucleic acids, which comprise from 1 to 6% of the cellular

Species	Proteins	Carbohydrates	Lipids	Nucleic acids
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	—	1.9	—
Scenedesmus dimorphus	8–18	21–52	16–40	—
Chlamydomonas rheinhardii	48	17	21	—
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	—
Chlorella minutissima	35.5	—	31	—
Spirogyra sp.	6–20	33–64	11–21	—
Dunaliella bioculata	49	4	8	—
Dunaliella salina	57	32	6	—
Euglena gracilis	39–61	14–18	14–20	—
Prymnesium parvum	28–45	25–33	22–38	1–2
Tetraselmis maculata	52	15	3	—
Porphyridium cruentum	28–39	40–57	9–14	—
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina máxima	60–71	13–16	6–7	3–4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrica	43–56	25–30	4–7	—

Fonte: [10].

Table 1.
Composition of microalgae.

composition of microalgae and play a vital role in cell growth and repair. Due to the presence of phosphorus and nitrogen in large amounts in nucleic acids, microalgal biomass can also be used as a fertilizer [10].

Carbohydrates, on the other hand, comprise the final product of photosynthesis in microalgae and can vary from 4 to 64% of their cell composition (**Table 1**) [10]. These are synthesized by fixing CO_2 in the form of glucose, disaccharides and starch as the primary source of energy for the cell [20].

In terms of microalgal biomass, carbohydrates can be extracted and converted into ethanol by the anaerobic fermentation process using yeasts (*Saccharomyces cerevisiae*, for example), or undergo degradation of organic matter in anaerobic conditions leading to the formation of methane and carbon dioxide [10].

All microalgae have one or more types of chlorophyll, among the four existing types, chlorophyll a, b, c and d, with chlorophyll being the most important pigment for photosynthesis playing a major role in the arrangement of photosystems to capture light energy. The other chlorophylls play secondary roles, contributing to increasing the total light absorbed by the microalgae. Commercially, chlorophylls are used mainly in the dye industry [20].

4. Photobioreactors

Photobioreactors are equipment designed to allow the use of light energy, whether natural or artificial, by the cells of the micro-organism present in it, with the purpose of providing the desired bioproduct. In the case of microalgae, it is desired that the photobioreactor helps promoting cell growth, and for this purpose several types of photobioreactors have been designed, such as open tanks, bubble columns, flat and tubular plates (conical or helical), among others [21].

According to Huang et al. [22], photobioreactors can be confined and the protective covered structures if built with transparent materials allow natural li lighting if the artificial lighting is not an option. They may also be available outdoors, subjected to weather conditions which randomly interfere in the development of the crop.

To minimize the interference associated with external factors, such as variations in the climate, impaired light by cloudy days or long rainy periods and possible contamination by insects, the photobioreactors that allow this direct contact of the culture medium with the external environment (called "open" photobioreactors), can be replaced by "closed" photobioreactors, in which the culture medium has no contact with the external environment, and if provided by artificial lighting they do not suffer from changes in radiance, ensuring greater control and predictability [23].

Among the open photobioreactors, we highlight the open ponds or ponds, known by the English term "Open ponds", which are cultivation containers made of plastic materials (PVC, for example), fiberglass or cement, and should contain smooth internal surfaces in order to reduce likely damages from friction and guarantee ease cleaning. In the construction of the tanks, the depth can vary from 10 to 50 cm approximately, so that it allows the diffusion of carbon dioxide from the atmosphere and the penetration of sunlight as well as allowing aeration by bubbling air in the culture medium [12, 20].

The circular ponds, or circular tanks, reach an average of 1000 m^2, 30cm deep, provided by large rotational arms that can extend up to 45 m in diameter, reaching yields that can vary from 1.5 to 16.5 g m^{-2} d^{-1} of dry biomass, while the "raceway pond" type gets its name from the format that resembles a racing circuit. It is the most used system at both pilot and commercial scale due to its ease of operation [24]. Productivities can vary from 0.19 to 23.5 g m^{-2} d^{-1} of dry biomass [10]. **Figure 2** shows open ponds photobioreactors.

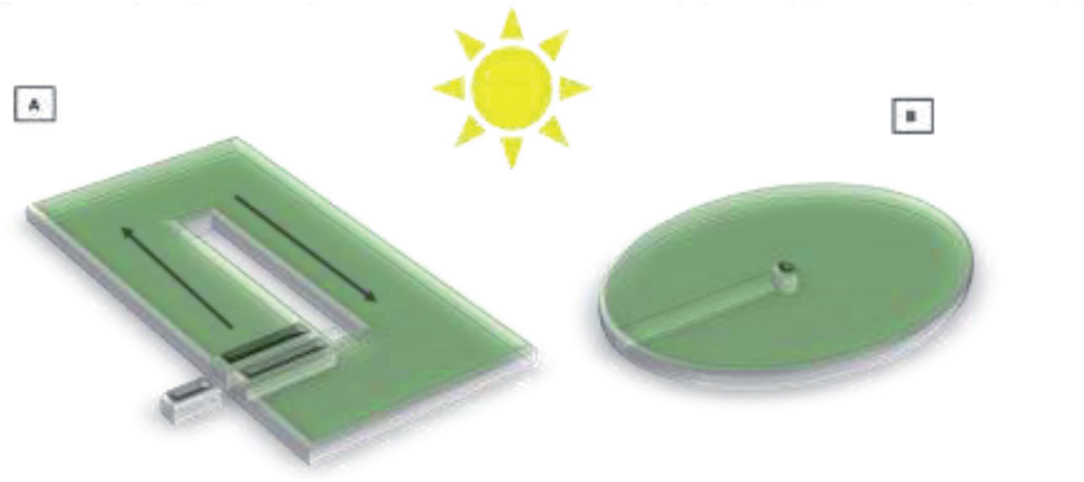

Figure 2.
Open pounds photobioreactors. Raceway pond (A); circular pond (B). Source: authors.

According to Huang [22], in the class of "closed" photobioreactors, the bubble column type is widely used by both researchers on a laboratory scale and commercial purposes due to its versatility of operation, handling and construction, as they are basically cylinders with a high height/diameter ratio and made of transparent materials such as glass and mainly polymers (acrylics and polyvinyl chlorides for example).

According to Carvalho [21], the bubble column photobioreactor has a unique configuration with an air dispersion system in its internal structure, which allows controlling the size and release of air bubbles promoting a random pneumatic agitation of the medium of cultivation.

In order to promote an oriented movement of the liquid inside the cylinder, photobioreactors of "air-lift" models can be used, which are equipped with devices called "draft tube". These devices that allow the distinction of two regions: one that it presents an upward flow of medium where air bubbles are released (Riser), and another of downward liquid flow (Downcomer) [25].

Flat plate photobioreactors are characterized by cobblestones of glass, plastic or other material that allows the passage of solar radiation. The air, enriched with carbon dioxide necessary for the growth of biomass is injected by the base, and promotes turbulence so that all cells are affected by solar radiation [26].

The arrangement of flat plate photobioreactors can vary vertically, horizontally or at an angle. They have the advantage of having a large surface area, which allows better use of the received radiation favoring the photosynthetic activity of microalgae [10]. **Figure 3** shows bubbles column, air-lift and flat plate photobioreactors.

Tubular photobioreactors are the most popular; they have a high ratio of exposed surface to volume, high efficiency of CO_2 usage and sunlight. They consist of sets of transparent tubes, usually glass or plastic, whose diameters are less than 0.1 m, since light is required in the photosynthesis process. Such photobioreactors can be designed in several formats (**Figure 3**): serpentine, inclined, spiral, coils and in parallel [10, 21, 24, 27].

The photobioreactors of the agitated tank type, on the other hand, have a structure composed of cylindrical tubes of glass or material that allows the passage of natural or artificial light and occupy small areas. The reactor may have mechanical or pneumatic agitation systems (air distributors), or even both. They are known as mixing bioreactors, responsible for more than 90% of applications in the fermentation industry [10, 21]. **Figure 4** shows tubular and agitated tank type photobioreactors.

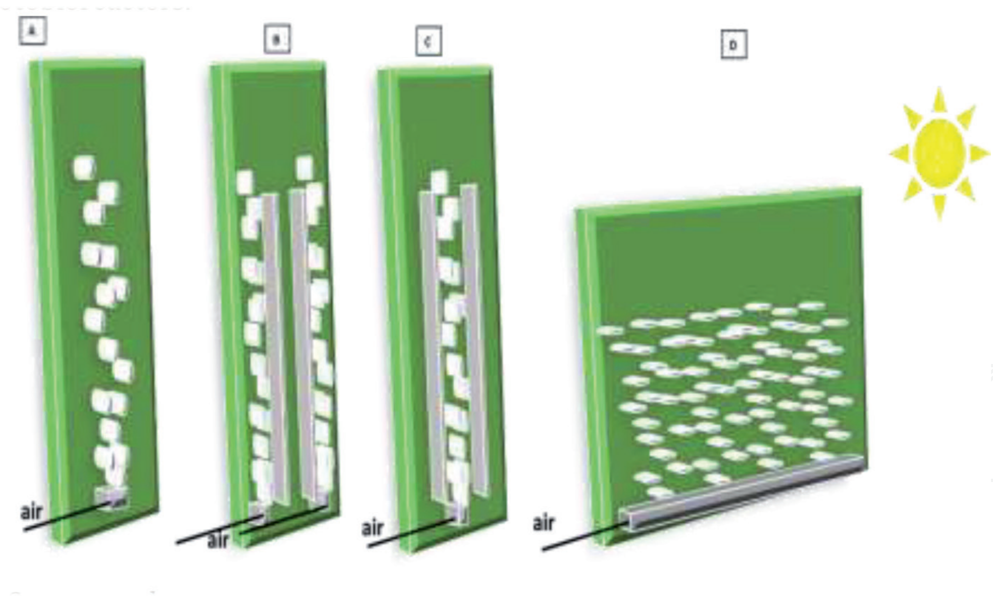

Figure 3.
Bubbles column (A), air-lift (B and C) and flat plate (D) photobioreactors. Source: authors.

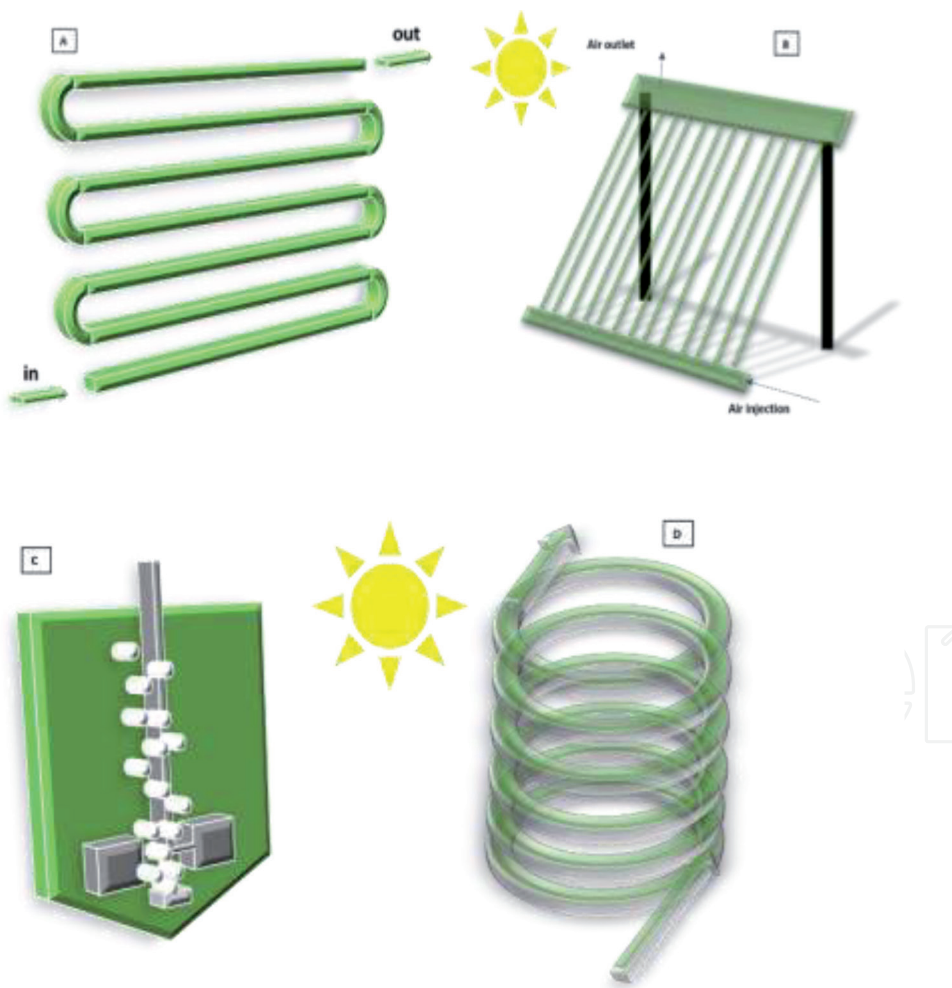

Figure 4.
Tubular photobioreactors: serpentine (A), inclined (B) and coil (D). Agitated tank photobioreactor (C). Source: authors.

In the municipality of Almeria, Spain, the microalgae *Scenedesmus almeriensis* is grown commercially in a 3000 L vertical tubular photobioreactor, owned by the CAJAMAR Foundation. The tubes are made of transparent polymeric material 90 mm in diameter and 400 m long, connected to a 3.5 m high bubble column [28].

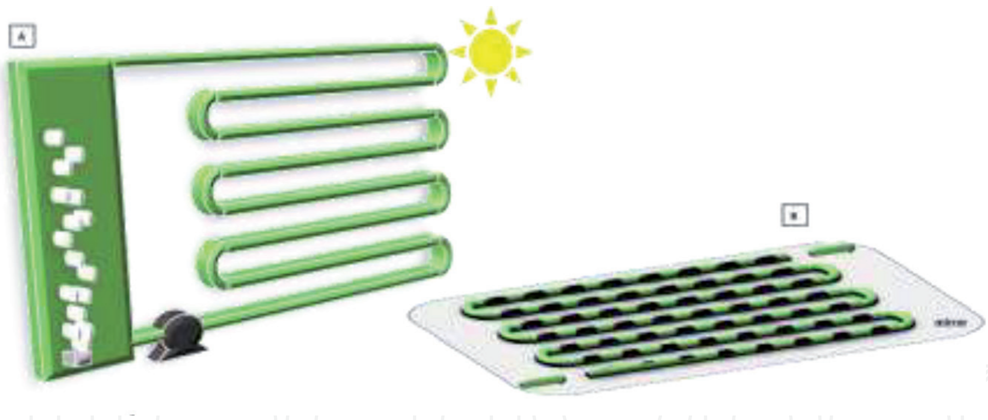

Figure 5.
Tubular arrangements of photobioreactors: (A) serpentine tubular reactor; (B) serpentine tubular reactor with mirror at its base to increase the efficiency of light capture by reflecting the incident light. Source: authors.

Many researchers have been designing and developing new configurations or strategies for the arrangement of photobioreactors to stimulate cell growth and the accumulation of metabolites in microalgae. As an example, the photobioreactor developed by Liao et al. [29] (**Figure 5A**), who horizontally disposed a serpentine tubular reactor, with regions of the tube that do not allow the passage of light, creating light and dark regions in photobioreactor to induce the accumulation of lipids during cell growth. A similar strategy was devised and developed by Ilus and Abu-Goshi [30] (**Figure 5B**), with a similar photobioreactor, plus a mirror at its base to increase the efficiency of light capture by reflecting the incident light.

An unconventional photobioreactor was developed by Huang et al. [31], named as a rotating float consisting of bottles in hexagonal arrangement and used as paddles of a raceway pond for the cultivation of microalgae *Dunaliella tertiolecta*. Another interesting photobioreactor has been proposed by Pruvost et al. [32], in which a flat plate photobioreactor is integrated into a building, with the purpose of producing microalgae biomass. This photobioreactor takes advantage of the exhaust gases from the chimney of a commercial building, thereby increasing the production of biomass during cultivation. In addition, it provides thermal comfort to the building in which it is integrated, as it reduces the internal temperature of the place due to the absorption of sunlight by microalgae while growing in the photobioreactor, which is located next to the building. **Figure 6** shows flat plate photobioreactor integrated into a building and rotating float photobioreactor.

It seems that microalgal cultures can be conducted under the most diverse configurations of photobioreactors from open to closed tanks, bubble columns, airlift, tubular

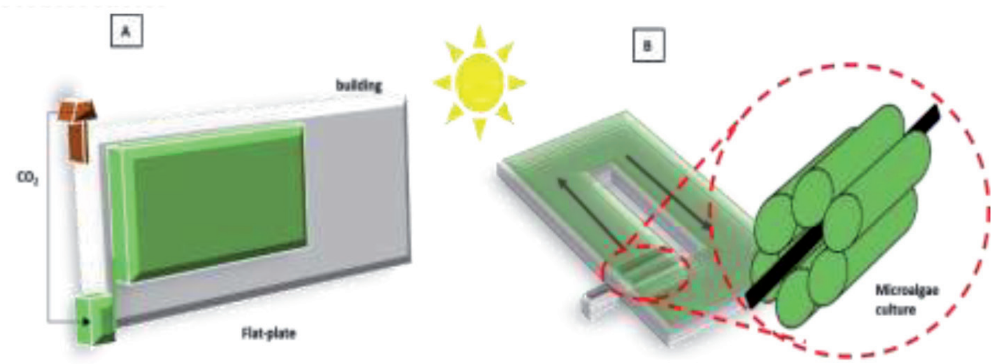

Figure 6.
Flat plate photobioreactor integrated into a building (A) and rotating float photobioreactor (B). Source: authors.

Configuration	Summary	Reference
Bubble column	The marine microalgae *Chlorella minutissima* was grown in a 20 L photobioreactor for the study of several controlled factors, such as temperature, CO2 and nutrients	Loures et al. [33]
Raceway	The cultivation of *Nannochloropsis* microalgae under nutritional stress conditions in an open tank in order to accumulate lipids to produce biodiesel.	Perrier et al. [34]
Tubular	The microalgae *Chlorella vulgaris* was cultivated in two stages as a strategy to accumulate lipids for the biodiesel production	Chen et al. [35]
Placas planas	The cultivation of the microalgae *Chlorella pyrenoidosa* was evaluated under different lighting gradients throughout the photobioreactor.	Huang et al. [36]

Table 2.
Use of traditional photobioreactors in research on microalgae cultivations.

(vertical, horizontal, inclined or spiral), combinations of more than one photobioreactor or even allied to architectural projects like the plate-type photobioreactor built next to an industrial building that rejects CO_2 in its chimney. **Table 2** summarizes some works carried out with several microalgae in the most common photobioreactors.

5. Harvesting biomass

After the microalgae cultivation period in the photobioreactors, the microalgal biomass must be harvested. This step is considered by many to be the key stage for the industrial production of biofuels from microalgae oils because they grow in very diluted cultures. The energy consumption is high due to the processing of large quantities of liquid which are needed to guarantee a feasible harvesting [37].

The main methods of harvesting microalgae are: flocculation, electroflocculation, biofloculation, gravimetric sedimentation, flotation, filtration and centrifugation [38].

The flocculation process involves the addition of chemicals capable of inducing the aggregation of microalgae cells either by neutralization, inversion of the electrical charges on the cell walls, or by the formation of bonds between the microalgae. It allows biomass recovery with lower economic costs when compared to the centrifugation process [37].

The main flocculating agents used for harvesting microalgae are salts such as $FeCl_3$, $Al2(SO_4)_3$ and $Fe_2(SO_4)_3$, polymers (chitosan) and bases such as NaOH or KOH, due to the change in pH [38].

The electrofloculation process, not widely disseminated, is based on the characteristic of microalgae to be electronegatively charged. Thus, by adapting a culture medium with the insertion of electrodes and allowing the passage of electrical current, it is possible to separate the microalgae cells of culture medium [38].

According to Barros et al. [38], in the biofloculation process, the flocculating agent is a microorganism, such as bacteria of the genus *Flavobacterium*, *Terrimonas* and *Sphingobacterium*, or fungi (*Rhizopus oryzae*, *Penicillium expansum*, *Aspergilus* and *Mucor circinelloides*), forming aggregates with the microalgal cells and favoring the microalgal cells gravimetric settling.

Biofloculation can eliminate the need for chemical flocculating agents, although for some cases it is necessary to add $CaCl_2$ to guarantee the efficiency of the process [38].

The flotation process can be defined as the "reverse" of sedimentation, where air bubbles promote the ascension of the cells of the culture by separating it from the liquid medium. However, cultures of marine microalgae are unlikely to undergo flotation, due to the high salinity of the medium [38].

The filtration method consists of passing the culture medium through a small pore membrane that retains the microalgal cells and allows only the passage of the liquid medium [37].

According to Lourenço [20], in the filtration of small volumes, a filtration apparatus such as a Kitasato flask or similar is applicable, however, on a large-scale cultivation, the separation of huge volumes of microalgae and biomass processing is only feasible if the species have large or filamentous cells, which are easily retained in the filter; a short process time.

Regarding the harvest time, gravimetric sedimentation is a slow and simple process which involves the separation of microalgae cells from the culture medium by the action of gravitational force. The downside of this process is the low efficiency [20].

On the other hand, centrifugation, cell sedimentation equipment that acts by the action of centrifugal force, is a fast process of biomass recovery, however, it requires high energy consumption. By this technique, biomass is concentrated without the addition of chemicals, preserving its original characteristics [20].

6. Extraction of lipids

Microalgae oils can be extracted similarly to other oilseed biomasses, which usually use physical extraction by adding a chemical solvent to improve the extraction process; solvents such as hexane and methanol are widely used [24].

The extraction of oil with solvent is a process of transferring soluble constituents (oil) from an inert material (biomass) to a solvent, in a purely physical process, without any chemical reaction. Solvent extraction is currently the most economical method and the use of hexane and chloroform has made it the fastest and most efficient method for lipid extraction from the most diverse biomasses. However, the presence of these chemical solvents can affect the lipid composition of the extracted oil [39].

Mechanical presses such as screw and piston, among others, can rupture the cells and release the oil, but these operation lead to low efficiency. On the other hand, when combined with the use of organic solvents, oil extraction efficiency can be reached in the order of 95% [10].

High concentrations of salts can cause a sudden change in the osmotic pressure, which can lead to the rupture of cells and the release of oil from inside; a method known as "osmotic shock" [39].

A method that has received notable attention is supercritical extraction, which uses fluids above the critical point of temperature and pressure, providing properties such as low viscosity and high diffusivity, allowing to achieve greater efficiency in the lipid extraction step. In this context, the use of CO_2 as a supercritical fluid is highly attractive due to its high selectivity, non-toxicity and short processing period. The only drawback is the high cost associated with installing adequate equipment to operate under safe conditions at high temperature and pressure [10].

In the search for environmentally friendly processes, enzymatic extraction is highly recommended, since it uses enzymes such as pectinase and cellulase, to degrade the cell wall, providing highly efficient extractions without affecting the microalgal lipid composition, unlike the method that uses organic chemical solvents [39]. On the other hand, these methods are still used only on a laboratory scale, due to the high costs involved with the acquisition of enzymes [24].

7. Synthesis of biodiesel

Biodiesel is the natural and renewable substitute fuel for petroleum diesel. It is produced from vegetable oils such as soy, palm, sunflower and jatropha and/or animal fat, such as beef tallow and chicken fat. In other words, any sources that have lipids can, in theory, be useful in the production of biodiesel [6].

First generation raw materials such as soy, palm and sunflower are not considered socially sustainable because they compete with the demand for food. In contrast, second generation raw materials such as beef tallow, residual frying oils and chicken fat, stand out as alternatives to produce biodiesel. Physic nut, which grows in semi-arid regions, where other sources of oilseeds are not able to grow and, therefore, do not compete with the food chain can also be used to produce biodiesel [6].

Microalgae, as well as cyanobacteria, belong to the group of third generation raw materials. These are a promising alternative, as they are able to accumulate high levels of lipids combined with high growth rates and biomass productivity compared to conventional oilseeds, and they do not compete with the food chain [40].

Biodiesel can be defined as a mixture of alkyl esters of fatty acids obtained by transesterification of triacylglycerides from vegetable oils or animal fat, or by the esterification reaction of free fatty acids resulting in alkyl esters of fatty acids and water. Both reactions occur in the presence of alcohols and catalyst [41].

Alcohols are considered to be transesterification agents, and may be methyl alcohol (methanol), ethyl alcohol (ethanol), propyl, butyl or amyl. Methanol is the most widely used alcohol due to its low cost and its physical–chemical properties (polarity and lower carbon chain), while ethanol has stood out for its potential for low toxicity and easy availability [37].

Transesterification is a multi-stage reaction, including three reversible stages in series, so that triglycerides are converted to diglycerides, then diglycerides are converted to monoglycerides and finally monoglycerides are converted to esters (biodiesel) and glycerol (co-product). Stoichiometrically the transesterification reaction requires 3 moles of alcohol for every mole of triacylglyceride, with an excess of alcohol being used to shift the balance towards the formation of the products, as it is a reversible reaction. The esterification reaction requires 1 mole of alcohol to 1 mole of fatty acid and requires excess alcohol to favor the direction of product formation [42].

The biodiesel synthesis reactions can be catalyzed homogeneously or heterogeneously. Homogeneous catalysts can be acidic (sulfuric, sulfonic and hydrochloric acid) or alkaline (sodium or potassium hydroxides), while heterogeneous catalysts can be enzymes or metallic compounds. In the catalyst determination, the acidity index is the main characteristic of the oil to be observed since high amounts of fatty acids do not allow alkaline catalysis (soap formation in the reaction medium). In this case, the use of acid catalysts is suggested [18].

On enzymatic catalysis, enzymes such as lipases, present in several organisms including animals, plants, fungi and bacteria have the biological function of accelerating the hydrolysis of fats and vegetable oils, releasing fatty acids, monoglycerides, diglycerides and glycerol [43].

Enzymes have some advantages when compared to the chemical catalytic process, such as high selectivity, reaction temperature in the range of 30–40°C and pH between 4 and 9. On the other hand, it requires long reaction periods to achieve high conversions and, therefore, costly [43].

Leung, Wu and Leung [44] explained that in general there will be advantages and disadvantages associated with the choice of the type of catalyst. The main

Microalgae	Catalyst	Condition	Reference
Chlorella minutissima	H_2SO_4	molar ratio (alcohol:lipid) 9:1, 90°C e 8 h	Loures et al. [45]
Chlorella protothecoides	H_2SO_4	Molar ratio (methanol:lipid) 56:1, 30 °C and 4 h	Miao, Wu [46]
Chlorella sp.	H_2SO_4	Molar ratio (alcohol:lipid) 30:1, 60°C and 4 h	Amaral et al. [47]
Source: authors.			

Table 3.
Catalysts used in biodiesel reactions for Chlorella microalgae.

advantages of alkaline catalysts are high catalytic activity, low cost and moderate operating conditions. However, it requires a low acidity index to prevent soap formation. Acid catalysts have the advantage of preventing soap formation. However, these catalysts corrode the equipment and require long reaction times. Heterogeneous catalysts (enzymes and metallic compounds) are highly selective and allow recycling, but at high costs.

The oil extracted from marine microalgae has a high acidity index, indicating acid catalysis as the most suitable for biodiesel production, as can be seen in **Table 3** which highlights the research done in the process of biodiesel synthesis via acid catalysis [45].

The synthesis of biodiesel from microalgal oils is carried out in a reactor. The mixture of alcohol and catalyst reacts with the triglyceride and/or fatty acids present in the microalgal oil. After the reaction, the mixture is transferred to a separation tank to guarantee the formation of the upper layer consisting of methyl ester, excess of alcohol and catalyst (acid or base) and the lower layer, predominantly glycerol [24].

8. Reaction of biodiesel in situ

The biodiesel production process usually involves the extraction of oils or lipids from biomass, which will later be processed to produce biodiesel; thus called a two-stage process. Currently, industries still require new technologies in the oil extraction and purification of the biodiesel, which are estimated to impact 70 to 80% on production costs [48].

Direct or “in situ” transesterification, an expression of the Latin that means “on the spot”, is the most prominent to perform lipid extraction and the transesterification reaction concurrently, in a single step of the process. Currently, the process has been called a Reactive Extraction Process. Therefore, the direct processing of biomasses seems to be an economically viable alternative as it results in a more economical use of resources in the production process. This direct use of biomass in the reactor is called Direct Transesterification or “In Situ”. The “In situ” Transesterification process emerges as a viable alternative to this problem as it makes direct use of the raw material without the need to prioritize oil extraction (**Figure 7**) [49].

The TEIS of microalgae biomass is shown as a potential alternative in reducing the costs of microalgal biodiesel production by eliminating the pre-treatment of biomass, enabling lipid extraction and purification, which has been the main factor in preventing the advancement of this industry; it minimizes the high consumption of solvents and uptime [49]. The elimination of the lipid extraction step not only reduces production steps but also results in a lower initial investment cost, equipment installation and maintenance, and energy [50].

Figure 7.
The "In situ" Transesterification process use of the raw material without the need to prioritize oil extraction.

As reported by Skorupskaite et al. [50], in situ technology can be applied to almost any raw material of plant origin or waste. However, attention should be focused on the biomass characteristics such as humidity, particle size, oil composition, acid content and reaction conditions (characteristics of reagents and catalyst, reaction time, temperature, etc.).

The reaction system for the TEIS process is suitable if the agitation of the reaction medium is effective enough to keep the biomass suspended [42]. This implies that there is a homogeneous dispersion of the liquid solvent and the solid biomass in the TEIS process [50]. that is, a perfectly agitated reactor. However, several authors have proposed different reaction systems to produce biodiesel in situ from microalgae.

9. Conclusion

The high rate of cell growth, the high content of lipids in the biomass and the non-competition in the food chain place microalgae as sources of raw material suitable in the production of biodiesel. To maximize biomass productivity, photobioreactors are key equipment in the success of a microalgal biodiesel production chain, as the photobioreactors allow an accelerated growth of microalgae, making more lipids available to be converted in biodiesel. In this sense, different configurations of photobioreactors can be used to produce microalgae biomass, with raceways and tubular photobioreactors being the most used configurations.

Acknowledgements

We thank Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for financial support to conduct this work.

Author details

Mateus S. Amaral[1*], Carla C.A. Loures[2], Fabiano L. Naves[3], Gisella L. Samanamud[4], Messias B. Silva[5,6] and Arnaldo M.R. Prata[5]

1 Laboratory of Technological and Environmental Chemistry, Department of Chemistry, Institute of Exact and Biological Sciences (ICEB), Federal University of Ouro Preto, Campus Universitário Morro do Cruzeiro, Ouro Preto, MG, Brazil

2 Centro Federal de Educação Tecnológica Celso Suckow Fonseca – CEFET-RJ, Angra dos Reis, RJ, Brasil

3 Federal University of São João del Rei – UFSJ – Campus Ouro Branco, Ouro Branco, MG, Brazil

4 Department of Chemical and Materials Engineering, University of Kentucky, Paducah Extended Campus, Paducah, KY, USA

5 Engineering School of Lorena – EEL-USP, Universidade de São Paulo - USP, Lorena, SP, Brazil

6 Universidade Estadual Paulista - UNESP, Campus Guaratinguetá, Guaratinguetá, SP, Brasil

*Address all correspondence to: mateus.amaral@ufop.edu.br

References

[1] Yeong TK, Jiao K, Zeng X, Lin L, Pan S, Danquah MK. Microalgae for biobutanol production – Technology evaluation and value proposition. Algal Research. 2018;**31**:367-376. Available from: http://www.sciencedirect.com/science/article/pii/S2211926418300080

[2] Chen HM. IEA, CO2 emissions from fuel combustion Highlights 2017, A Report by the International Energy Agency, 2017. Vol. 53, Library Technology Reports; 2017

[3] Heck V, Gerten D, Lucht W, Popp A. Biomass-based negative emissions difficult to reconcile with planetary boundaries. Nature Climate Change. 2018;**8**(2):151-155. Available from: https://doi.org/10.1038/s41558-017-0064-y

[4] Zhu L., Huo, S., Qin, L. A microalgae-based biodiesel refinery: Sustainability concerns and challenges. International Journal of Green Energy. 2015;**12**(6):595-602. Available from: https://doi.org/10.1080/15435075.2013.867406

[5] Rós P, Silva C, Silva-Stenico M, Fiore M, & Castro H. Microbial oil derived from filamentous Cyanobacterium Trichormus sp. as feedstock to yield fatty acid ethyl esters by enzymatic synthesis. Journal of Advances in Biology & Biotechnology. 2017;**12**(4):1-14. Available from: https://doi.org/10.9734/jabb/2017/33045

[6] Ahmad AL, Yasin NHM, Derek CJC, Lim JK. Microalgae as a sustainable energy source for biodiesel production: A review. Renewable and Sustainable Energy Reviews. 2011;**15**:584-593

[7] Brownbridge G, Azadi P, Smallbone A, Bhave A, Taylor B, Kraft M. The future viability of algae-derived biodiesel under economic and technical uncertainties. Bioresour Technol. 2014;**151**:166-173

[8] Tan XB, Lam MK, Uemura Y, Lim JW, Wong CY, Lee KT. Cultivation of microalgae for biodiesel production: A review on upstream and downstream processing. Chinese Journal of Chemical Engineering. 2018;**26**(1):17-30

[9] Shuba ES, Kifle D. Microalgae to biofuels: 'Promising' alternative and renewable energy, review. Renewable and Sustainable Energy Reviews. 2018;**81**(April 2016):743-755

[10] Hattab M, Ghaly A. Production of biodiesel from marine and freshwater microalgae: A review. Advances in Research. 2015;**3**(2):107-155. Available from: http://www.sciencedomain.org/abstract.php?iid=695&id=31&aid=6322

[11] Mata TM, Martins AA, Caetano Nidia S, Mata TM, Martins AA, Caetano Nidia S. Renewable & sustainable energy reviews. Renewable and Sustainable Energy Reviews. 1997;**14**:217-232. Available from: https://EconPapers.repec.org/RePEc:eee:rensus:v:14:y:2010:i:1:p:217-232#.XxCxKFy3yaA.mendeley

[12] Chisti Y. Biodiesel from microalgae. Biotechnology Advances. 2007;**25**(3):294-306. Available from: http://www.sciencedirect.com/science/article/pii/S0734975007000262

[13] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. American Journal of Bioscience and Bioengineering. 2006;**101**(2):87-96. Available from: http://www.sciencedirect.com/science/article/pii/S1389172306705497

[14] Yadav G, Sen R. Microalgal green refinery concept for biosequestration of carbon-dioxide vis-à-vis wastewater remediation and bioenergy production:

Recent technological advances in climate research. Journal of CO2 Utilization. 2017;**17**:188-206. DOI: 10.1016/j.jcou.2016.12.006

[15] Sánchez Mirón A, Cerón Garcıa MC, Garcıa Camacho F, Molina Grima E, Chisti Y. Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: Studies in fed-batch culture. Enzyme and Microbial Technology. 2002;**31**(7):1015-1023. Available from: http://www.sciencedirect.com/science/article/pii/S0141022902002296

[16] Ördög V, Stirk WA, Bálint P, Aremu AO, Okem A, Lovász C, et al. Effect of temperature and nitrogen concentration on lipid productivity and fatty acid composition in three chlorella strains. Algal Research. 2016;**16**:141-149. Available from: http://www.sciencedirect.com/science/article/pii/S221192641630073X

[17] Huang G, Chen F, Wei D, Zhang X, Chen G. Biodiesel production by microalgal biotechnology. Applied Energy. 2010;**87**(1):38-46. Available from: http://www.sciencedirect.com/science/article/pii/S0306261909002530

[18] Demirbas A. Use of algae as biofuel sources. Energy Conversion and Management. 2010;**51**(12):2738-2749. Available from: http://www.sciencedirect.com/science/article/pii/S0196890410002207

[19] Sathasivam R, Radhakrishnan R, Hashem A, Abd Allah EF. Microalgae metabolites: A rich source for food and medicine. Saudi Journal of Biological Sciences. 2019;**26**(4):709-722

[20] Lourenço SO. Cultivo de microalgas marinhas: princípios e aplicações. RiMa, editor. São Carlos; 2006. P. 588

[21] Carvalho JCM, Matsudo MC, Bezerra RP, Ferreira-Camargo LS, Sato S. Microalgae Bioreactors. In: Bajpai Rakesh K, Ales P, Zappi ME, editors. Algal Biorefineries. Dordrecht: Springer Netherlands; 2014. pp. 83-126. Available from: http://link.springer.com/10.1007/978-94-007-7494-0_4

[22] Huang Q, Jiang F, Wang L, Yang C. Design of Photobioreactors for mass cultivation of photosynthetic organisms. Engineering. 2017;**3**(3):318-329

[23] Singh RN, Sharma S. Development of suitable photobioreactor for algae production - a review. Renewable and Sustainable Energy Reviews. 2012;**16**(4):2347-2353

[24] Suali E, Sarbatly R. Conversion of microalgae to biofuel. Renewable and Sustainable Energy Reviews. 2012;**16**(6):4316-4342. Available from: http://www.sciencedirect.com/science/article/pii/S1364032112002304

[25] Mendes CE, Badino AC. Hydrodynamics of Newtonian and non-Newtonian liquids in internal-loop airlift reactors. Biochemical Engineering Journal. 2016;**109**:137-152. Available from: http://www.sciencedirect.com/science/article/pii/S1369703X16300079

[26] Arun J, Varshini P, Prithvinath PK, Priyadarshini V, Gopinath KP. Enrichment of bio-oil after hydrothermal liquefaction (HTL) of microalgae C. vulgaris grown in wastewater: Bio-char and post HTL wastewater utilization studies. Bioresource Technology. 2018;**261**(April):182-187. DOI: 10.1016/j.biortech.2018.04.029

[27] Wang B, Lan CQ, Horsman M. Closed photobioreactors for production of microalgal biomasses. Biotechnology Advances. 2012;**30**(4):904-912

[28] Solimeno A, Gabriel F, García J. Mechanistic model for design, analysis, operation and control of microalgae

cultures: Calibration and application to tubular photobioreactors. Algal Research. 2017;**21**:236-246

[29] Liao Q, Li L, Chen R, Zhu X. A novel photobioreactor generating the light/dark cycle to improve microalgae cultivation. Bioresource Technology. 2014;**161**:186-191

[30] Iluz D, Abu-Ghosh S. A novel photobioreactor creating fluctuating light from solar energy for a higher light-to-biomass conversion efficiency. Energy Conversion and Management. 2016;**126**:767-773

[31] Huang JJ, Bunjamin G, Teo ES, Ng DB, Lee YK. An enclosed rotating floating photobioreactor (RFP) powered by flowing water for mass cultivation of photosynthetic microalgae. Biotechnology for Biofuels. 2016;**9**(1):1-18

[32] Pruvost J, le Gouic B, Lepine O, Legrand J, le Borgne F. Microalgae culture in building-integrated photobioreactors: Biomass production modelling and energetic analysis. Chemical Engineering Journal. 2016;**284**:850-861

[33] Pereira FM, Loures CCA, Amaral MS, Gomes FM, Pedro GA, Machado MAG, et al. Evaluation of fatty acids production by Chlorella minutissima in batch bubble-column photobioreactor. Fuel. 2018;**230**(December 2017):155-162

[34] Perrier B, Crampon C, Guézet O, Simon C, Maire F, Lépine O, et al. Production of a methyl ester from the microalgae Nannochloropsis grown in raceways on the French west coast. Fuel. 2015;**153**:640-649. Available from: http://www.sciencedirect.com/science/article/pii/S0016236115002926

[35] Chen CY, Yeh KL, Chang HY, Chang JS. Strategies to improve oil/lipid production of microalgae in outdoor cultivation using vertical tubular-T ype photobioreactors. In: Energy Procedia. Elsevier Ltd; 2014. pp. 2755-2758

[36] Huang J, Li Y, Wan M, Yan Y, Feng F, Qu X, et al. Novel flat-plate photobioreactors for microalgae cultivation with special mixers to promote mixing along the light gradient. Bioresource Technology. 2014;**159**:8-16. Available from: http://www.sciencedirect.com/science/article/pii/S096085241400159X

[37] Rastogi RP, Pandey A, Larroche C, Madamwar D. Algal green energy – R&D and technological perspectives for biodiesel production. Renewable and Sustainable Energy Reviews. 2018;**82**(October 2017):2946-2969. DOI: 10.1016/j.rser.2017.10.038

[38] Barros AI, Gonçalves AL, Simões M, Pires JCM. Harvesting techniques applied to microalgae: A review. Renewable and Sustainable Energy Reviews. 2015;**41**:1489-1500. Available from: http://www.sciencedirect.com/science/article/pii/S1364032114008107

[39] Rashid N, Ur Rehman MS, Sadiq M, Mahmood T, Han J-I. Current status, issues and developments in microalgae derived biodiesel production. Renewable and Sustainable Energy Reviews. 2014;**40**:760-778 Available from: http://www.sciencedirect.com/science/article/pii/S1364032114005565

[40] Reis CER, De Souza Amaral M, Loures CCA, Da Rós PCM, Hu B, Filho HJI, et al. Microalgal feedstock for bioenergy: Opportunities and challenges. Biofuels in Brazil: Fundamental Aspects, Recent Developments, and Future Perspectives. 2014. p. 367-392. Springer International Publishing. Available from: https://doi.org/10.1007/978-3-319-05020-1_17

[41] Amaro HM, Guedes AC, Malcata FX. Advances and perspectives in using microalgae to produce biodiesel. Applied Energy.

2011;**88**(10):3402-3410. Available from: http://www.sciencedirect.com/science/article/pii/S0306261910005210

[42] Salam KA, Velasquez-Orta SB, Harvey AP. A sustainable integrated in situ transesterification of microalgae for biodiesel production and associated co-product-a review. Renewable and Sustainable Energy Reviews. 2016;**65**:1179-1198. Available from: http://www.sciencedirect.com/science/article/pii/S1364032116304075

[43] Vieira APDA, da Silva MAP, Langone MAP. Biodiesel production via esterification reactions catalyzed by lipase. Latin American Applied Research. 2006;**36**(4):283-288

[44] Leung DYC, Wu X, Leung MKH. A review on biodiesel production using catalyzed transesterification. Applied Energy. 2010;**87**(4):1083-1095. Available from: http://www.sciencedirect.com/science/article/pii/S0306261909004346

[45] Loures CCA, Amaral MS, da Rós PCM, Zorn SMFE, de Castro HF, Silva MB. Simultaneous esterification and transesterification of microbial oil from Chlorella minutissima by acid catalysis route: A comparison between homogeneous and heterogeneous catalysts. Fuel. 2018;**211**(September 2017):261-268

[46] Miao X, Wu Q. Biodiesel production from heterotrophic microalgal oil. Bioresource Technology. 2006;**97**(6):841-846. Available from: http://www.sciencedirect.com/science/article/pii/S0960852405002191

[47] Amaral MS, Loures CC, da Rós PCM, Machado SA, Reis CER, de Castro HF, et al. Evaluation of the cultivation conditions of marine microalgae chlorella sp. to be used as feedstock in ultrasound-assisted ethanolysis. Biofuel Research Journal. 2015;**2**(3):288-294

[48] Go AW, Sutanto S, Ong LK, Tran-Nguyen PL, Ismadji S, Ju Y-H. Developments in in-situ (trans) esterification for biodiesel production: A critical review. Renewable and Sustainable Energy Reviews. 2016;**60**:284-305. Available from: http://www.sciencedirect.com/science/article/pii/S1364032116001003

[49] Lewis T, Nichols PD, McMeekin TA. Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. Journal of Microbiological Methods. 2000;**43**(2):107-116. Available from: http://www.sciencedirect.com/science/article/pii/S0167701200002177

[50] Skorupskaite V, Makareviciene V, Gumbyte M. Opportunities for simultaneous oil extraction and transesterification during biodiesel fuel production from microalgae: A review. Fuel Processing Technology. 2016;**150**:78-87. Available from: http://www.sciencedirect.com/science/article/pii/S0378382016301904