



Microalgae Biomass Production for Biofuels in Brazilian Scenario: A Critical Review

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Abstract

The Brazilian environmental, economic, and social conditions for the long-term establishment of mass culture of microalgae for either biofuel production or greenhouse gases (GHG) abatement are described in detail. A brief historical introduction of the microalgal biotechnology is presented followed by a compilation of Brazilian published research works on microalgae, with special emphasis on microalgal Brazilian biodiversity and applied phycology. Several case studies on Brazilian microalgal biorefineries are presented with special emphasis on wastewater (WW) treatment. The manuscript also adds valuable new information about which regions of the country offer better growing conditions for dozens of endemic species. Favorable climatic and environmental conditions for the cultivation of several microalgae (green) and cyanobacteria species in specific regions of the country are suggested. Finally, based on realistic biomass productivities and product yields for the Brazilian context, several scenarios for biofuel production and/or carbon dioxide (CO₂) abatement have been designed, and results are presented and critically discussed. Brazilian self-sufficiency on either fuels for transportation or GHG abatement using exclusively microalgae is quite challenging but achievable accordingly with the present state of the art.

Keywords Clean energy · Sustainability · Bioresource · Bioeconomy · Tropical country

List of Abbreviations and Nomenclature

CP	Circular ponds	LSP	Large shallow ponds
DO	Dissolved oxygen	N	Nitrogen
FPPBR	Flat plate photobioreactor	P	Phosphorus
GHG	Greenhouse gases	PAR	Photosynthetically active radiation
HDP	High-density polyethylene	PBR	Photobioreactor
HRPs	High-rate ponds	POME	Palm oil mill effluent
HTL	Hydrothermal liquefaction	UASB	Upflow anaerobic sludge blanket
L/D	Light/dark	UV	Ultraviolet
		VSS	Suspended solids volatiles
		VVM	Volume of air per volume of culture per minute
		WW	Wastewater

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Introduction

Brazil is the largest and most populous country in Latin America. The country's land area is 8,514,876 km², occupying half the continent's surface and having approximately a share of 50% of South America's gross domestic product (GDP) [1]. Brazil holds an important tropical coastal length (10,959 km) and contains roughly 12% of the world's fresh water supply and receives average insolation levels of 8–22 MJ m⁻²d⁻¹ [2].

Data collected by the International Energy Agency (IEA) [1] revealed that the country is among the ten largest consumers of energy on the planet.

According to Brazilian Energy Balance [3], the energy matrix in the country is still very dependent on non-renewable sources (fossil). About 36.4% of the energy available in the country comes from oil and its derivatives. The second most available source in the country comes from biomass (17%) mainly from the ethanol produced from sugar cane. In the rest of the world, consumption of petroleum-based fuels reaches 33% and only approximately 0.9% of clean renewable sources, such as biomass [4].

In this current scenario of the Brazilian energy matrix, it is possible to observe a strong dependence on non-renewable energy sources, but with advances in terms of the use of biomass as an energy source.

Brazil has supported its expectations in the so-called pre-salt, an area that has a rocky layer of the marine subsoil with rich oil reserves [5]. Despite being a major economic and political strategy for Brazil, the discovery of pre-salt resources represents a major challenge for the country and raises several questions and uncertainties, mainly related to geological, operational, infrastructure, and environmental risks [6].

According to the Energy Research Company (EPE) [6], the pre-salt reserves can reach, by 2030, a production of around 5 million barrels (oil) d^{-1} and volumes of natural gas around 100 million $\text{m}^3 \text{d}^{-1}$. However, according to the same agency, there is a risk that the estimated reserves may have less volume than expected. In addition, for the exploitation of all this energy potential, there is a real need for considerable technological and infrastructure advances in the country. Another challenge for pre-salt exploration is the issue of CO_2 , one of the gases responsible for global warming and present in large quantities in natural gas. Still, the economic viability of natural gas is not taken for granted, depending on several factors such as the distance and transported volume, in addition to the sale price [6].

However, the country has invested in other differentiated fuel matrices. In 2018, biodiesel production in the country grew 24.7% in relation to the previous year, reaching the amount of 5,350,036 m^3 . The main raw material was the soybean oil (63%), followed by cattle tallow (12%) and other feedstocks with minor contributions such as sunflower and palm. It should also be noted that Brazil was the first country to use bioethanol in relevant volumes for supplying vehicles. This type of biofuel has been used in the country since the 1970s and continues to grow. In 2018, ethanol production rose 19.9% over the previous year, reaching an amount of 33,198 thousand m^3 [3, 5, 6]. Bearing in mind the production of sustainable energy sources, the use of biofuels has become an important alternative for the Brazilian energy matrix, since, in addition to representing a reduction in CO_2 emissions into the atmosphere, their production increases the country's energy security.

Concerning liquid biofuels, it is common to classify them as first, second, and third generation [7]. The most well-known first-generation biofuel is ethanol, extracted from the fermentation of sugar present in plants and starch contained in corn kernels, in addition to biodiesel produced from oilseed plants such as castor and soybeans. Second-generation biofuels, on the other hand, are those produced from the processing of the lignocellulosic fraction of biomass from plants [8].

On the other hand, some drawbacks about the production of biofuels from terrestrial biomass can be listed: (1) demand for large areas for cultivation; (2) deforestation to obtain arable areas; (3) environmental and social impacts associated with large-scale agribusiness, (4) use of nitrogen fertilizers that can trigger eutrophication, among others.

In this way, the number of either national or international scientific studies increases. This highlights the so-called third-generation biofuels as a promising energy alternative, with special emphasis on lipids for biodiesel extracted from microorganisms. Microalgae are considered a promising source for biodiesel extraction. Through some strains such as *Chlorella pyrenoidosa*, up to 70% of lipids can be obtained in its dry biomass [9]. With this lipid concentration, the microalgae cultivation becomes advantageous in relation to more varied terrestrial crops (Table 1).

Other potential biofuels that can be obtained via microalgae biomass are bioethanol, bio-oil, and biogas/biomethane [15].

Microalgae are microorganisms capable of converting sugars into bioethanol and are currently being studied as a source of substitution for petroleum products [16]. The *Scenedesmus*, *Chlorella*, *Dunaliella*, and *Tetraselmis* genera can accumulate between 40 and 51% of carbohydrates in their dry weight, indicating a good option for large-scale bioethanol production [17].

Another biofuel that can be obtained through microalgae is biogas/biomethane. In this context, the use of biomass in co-digestion with residues or agro-industrial wastewater, via anaerobic digestion, stands out. It is an environmentally viable

Table 1 Oil productivity: comparison between terrestrial crops and microalgae (high oil) [9–14]

Crop	Oil yield (L ha^{-1})
Corn	172
Soybean	446
Sunflower	1070
Rapeseed	1190
Jatropha	1892
Coconut	2689
Palm	5950
Microalgae (high oil \approx 70%)	136,900

option, with low production costs, when compared with the production of liquid fuels, since it eliminates the drying stage of biomass [18, 19].

Over the decades, several industrial applications of microalgal biomass have been described: bioplastics, bioinks, biofertilizers, food and animal nutrition, and also for WW bioremediation [20] (Fig. 1).

The production of energy and biodiesel/biofuels through the cultivation of microalgae overcomes the disadvantages of using first- and second-generation biofuels, since they can be cultivated in arid, saline, and contaminated lands or with low agricultural potential, reducing competition for agricultural areas and generating new economic opportunities for poor soil regions, which would not have traditionally been used for agribusiness [8].

The Brazilian estimate calculated by researchers from EMBRAPA (Brazilian Agricultural Research Corporation) suggests yields from 55 to 100 t (microalgae oil) ha⁻¹, with an average value of approximately 78 t ha⁻¹ [21]. The biomass of around 20,000 t (microalgae) year⁻¹ is considered incipient in terms of biofuel [22].

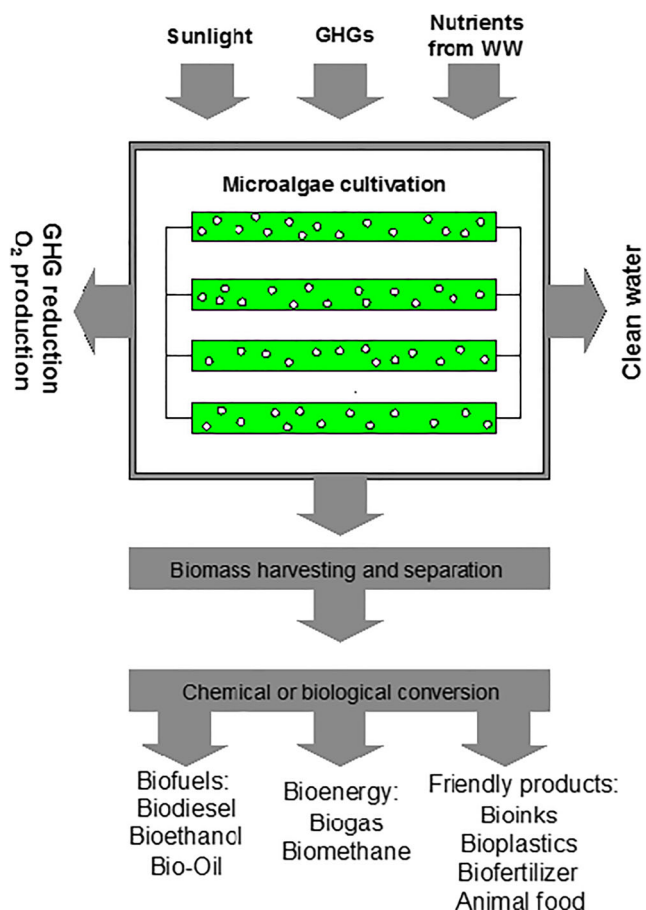


Fig. 1 Diagram block highlighting inputs and outputs of microalgal contribution with emphasis on bioenergy/biofuels/bioproducts and environmental services and benefits provided

In addition, microalgae are of great ecological importance, as they act in the capture of atmospheric CO₂ and can be used as WW treatment, due to their ability to remove nutrients, as well as organic carbon (mixotrophy) from the substrate [23]. Mixotrophy promotes synergy in the cultivation process, maximizing biomass productivity while at the same time bioremediating various types of WW [20].

Another great advantage for the dissemination of microalgae cultivation in the country is justified by the great biodiversity of cataloged species. Many of them can be valuable for the purpose of producing biodiesel and other biofuels/bioproducts. Menezes and Bicudo [24] estimate that the number of microalgae species cataloged in the Brazilian territory reaches 5614, distributed in 3689 epicontinental species (164 *Cyanophyceae*, 50 *Rhodophyceae*, 10 *Prasinophyceae*, 700 *Chlorophyceae*, 875 *Charophyceae*, 370 *Euglenophyceae*, 42 *Dinophyceae*, 20 *Cryptophyceae*, 1200 *Bacillariophyceae*, 10 *Raphidophyceae*, 2 *Prymnesiophyceae*, 14 *Chrysophyceae*, 40 *Synurophyceae*, 62 *Xanthophyceae*), and 1925 marine species (164 *Cyanophyceae*, 455 *Rhodophyceae*, 2 *Prasinophyceae*, 223 *Ulvophyceae*, 6 *Euglenophyceae*, 296 *Dinophyceae*, 653 *Bacillariophyceae*, 2 *Raphidophyceae*, 92 *Phaeophyceae*, 27 *Prymnesiophyceae*, 5 *Dictyochophyceae*).

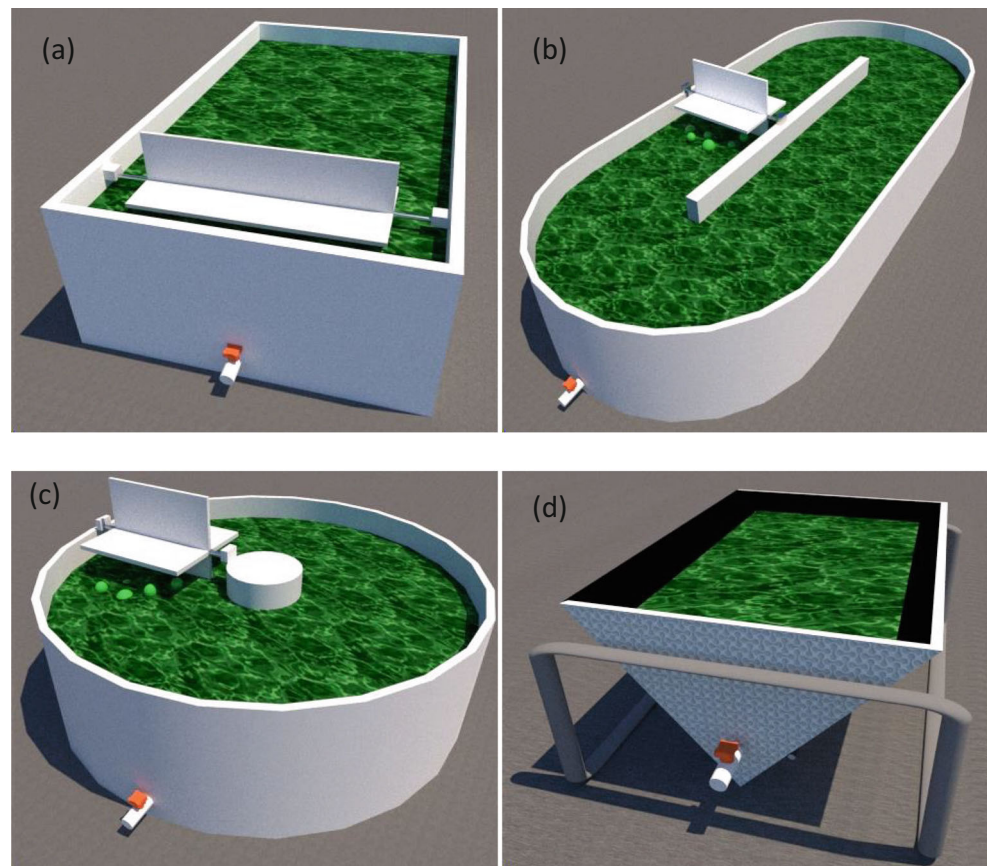
More than 40 Brazilian laboratories and institutions keep currently algae cultures (microalgae, seaweeds, and cyanobacteria) [1]. Over 150 strains are kept in only five of these facilities [1].

Thus, this study aims at highlighting the Brazilian potential for biofuel production (focus on biodiesel) from microalgal-based biomass and, based on a critical literature review, to show that the country could be among the main world producers of this feedstock, which is extremely beneficial to the environment, contributing to an increasingly greener circular bioeconomy. The manuscript also adds valuable new information about which regions of the country offer better growing conditions for dozens of endemic species.

Reactors for Microalgae Cultivation

There are currently two predominant cultivation systems, HRP (open systems) and PBR (closed systems). Open cultivation consists of ponds, which are simple and economical devices, are the most used reactors in the country. These systems can be found in different formats and sizes such as rectangular open pond with agitation, paddle wheel raceway pond, circular ponds, and V-shaped pond (Fig. 2). Closed cultivation systems present less contact with the external environment. They are called photobioreactors (PBRs). The advantages of this system in relation to open systems are the achievement of greater productivity and greater control of cultivation conditions [25]. The most common models in

Fig. 2 Open cultivation systems: (a) rectangular open pond with agitation, (b) paddle wheel raceway pond, (c) circular pond, and (d) V-shaped pond



Brazilian research are flat panel PBR, vertical tubular PBR, plastic bag PBR, and membrane PBR (Fig. 3).

Environmental (Climatic) and Nutritional Requirements to Microalgae Cultivation

Response to stimuli or change in its environment is an intrinsic characteristic of microalgae. Changes in environmental conditions may thus be defined on the basis of the response that the cell undergoes as a result of the sensed change, either a limiting- or a stress factor [26].

Just like superior plants, microalgae depend strongly on light for their development; therefore, their intensity must be considered, since there is great variation according to the depth of the culture medium and its density.

The generalized ideal condition of light intensity for the cultivation of microalgae is 2.5 klux ($\approx 34 \mu\text{mol m}^{-2} \text{s}^{-1}$) to 5.0 klux ($\approx 70 \mu\text{mol m}^{-2} \text{s}^{-1}$), and it should vary according to the volume and density of the culture [27].

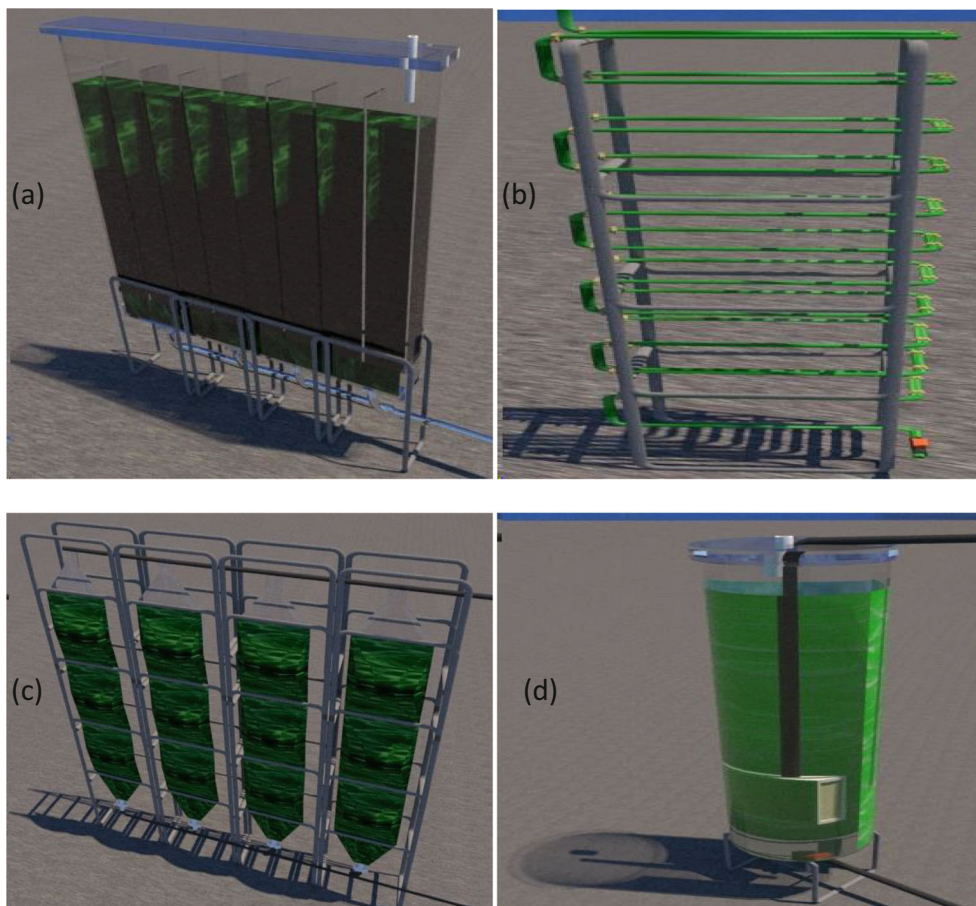
Another important climatic parameter for cultivation is temperature. According to [28], the optimal range for cultivation varies between 18 and 24 °C (green microalgae). Temperatures far below this interval can slow down metabolism/growth, while very high temperatures are lethal for a

large number of species. Thus, Fig. 4a and b represents, respectively, the temperature and radiation ranges for the entire Brazilian territory. The average temperatures in Brazil vary from 13.7 to 30.8 °C, and the radiation varies between 24.15 and 45.44 klux.

Taking into account the generalized preferential conditions of radiation and temperature, 30% of the Brazilian territory is suitable for the production of microalgae as a function of temperature. On the other hand, radiation levels in some places are high and can cause photoinhibition of microalgae if their cultivation is not properly managed in the field. In this case, at high light intensities, the shading of the culture is necessary. Shading, for example, can maximize the removal of organic matter and nutrients from substrate. Assemany et al. [30] verified this phenomenon covering 80% of an HRP. However, the same authors showed that there was no photoinhibition in an uncoated HRP subject to radiation (PAR) of approximately 30 to 250 W m^{-2} .

Interest in the study of microalgae varies according to the location and type of species (adapted to local climatic conditions). The most studied species in the USA are *Chlamydomonas* and *Nannochloropsis*; meanwhile in China, they are *Chlorella* and *Scenedesmus*; and in Spain *Phaeodactylum* and *Isochrysis* have the highest number of

Fig. 3 Close cultivation systems: (a) flat panel PBR, (b) vertical tubular PBR, (c) plastic bag PBR, and (d) membrane PBR

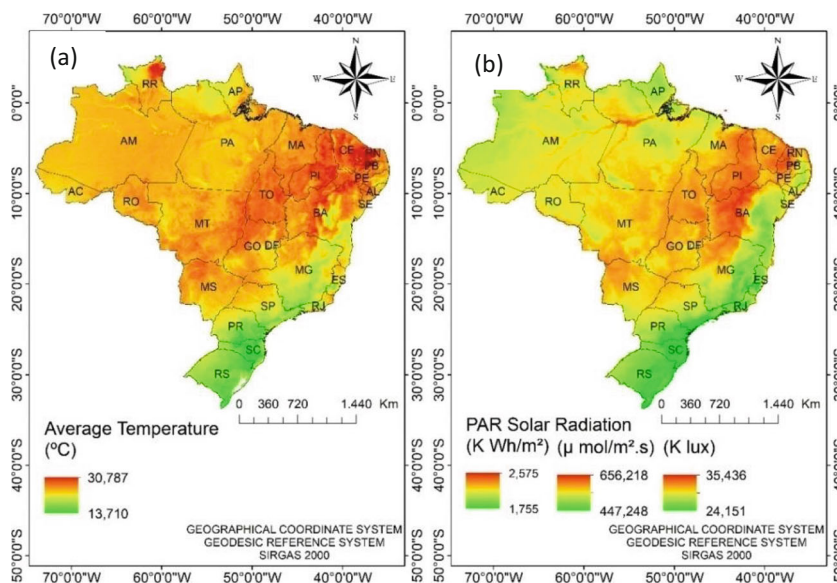


studies [31]. The seventh most widely used microalgae in the literature is the *Spirulina*, which is considered one of the most studied microalgae in Brazil [31]. However, in addition to *Spirulina*, there are several other microalgae strains being studied in Brazil for the most diverse purposes. For example, the species *Nannochloropsis gaditana* is used for water

desalination in the semiarid regions of Brazil [32] and used as animal nutritional supplementation [33].

Thus, several studies show that it is not reasonable to consider a general range of temperature and radiation for all microalgae species, due to the peculiarities of each one, and the location of their implantation.

Fig. 4 (a) Average temperature ranges in Brazil. (b) Average photosynthetic radiation ranges in Brazil. Maps produced by the authors: data from NASA, 2019 [29] - Database: 2000 to 2018



Another important factor in the microalgae cultivation is the salinity of the culture medium. The response of green microalgae and cyanobacteria to changes in the osmotic environment has attracted considerable attention since they are inhabitants of many of the biotopes characterized by high variations in salinities [26]. In general, some strains can be successfully grown in salinity ranges between 12 and 40 g L⁻¹, with the optimal range between 20 and 24 g L⁻¹ [28]. The same author points out that the reasonable pH range for cultivation, associated with good salinity ranges, is 7–9, on average, and the optimal range between 8.2 and 8.7.

Other authors note that salt concentrations between 0.035 g L⁻¹ (NaCl) can already inhibit the growth of freshwater green microalgae [34]. A study by Salama et al. [35], found that the increase in salinity from 0.43 to 25 mM increased the percentage of lipids in the biomass of *C. mexicana* and *S. obliquus* from 23 to 37% and 22 to 34%, respectively.

The supply of nutrients in ideal quantities is another important factor for cultivation. Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate; they are essential and limiting for most strains [28]. In order to obtain good productivity, the supply of CO₂ and HCO₃⁻ is imperative. A ratio of 106C:16N:1P is widely used, as a starting point, to quantify possible nutrient limitations in microalgae cultures [26]. Others besides C, N, and P are also important: S, K, Na, Fe, Mg, and Ca and trace elements such as B, Cu, Mn, Zn, Mo, Co, V, and Se.

Microalgae Biomass Productivity at Ideal Temperature Range

Singh and Singh [36] investigated the ideal temperature conditions for the cultivation of some microalgae and cyanobacteria (*Botryococcus*, *Chlamydomonas*, *Chlorella*, *Haematococcus*, *Nannochloropsis*, *Neochloris*, *Scenedesmus*, and *Spirogyra*). The authors recorded optimal ranges for the development of microalgae between 22 and 35 °C (in this case including cyanobacteria). Thus, analyzing Fig. 4a, it is possible to predict that 88% of the Brazilian territory is potentially suitable for the cultivation of microalgae genera studied by authors only based on the temperature criterion.

Ras et al. [37] and De Assis et al. [38] evaluated the preferential temperature for biomass production of 371 microalgae species, recording the interval between 15 and 30 °C. Looking at Fig. 4a, it is possible to observe that Brazil has an enormous potential for cultivating microalgae for its various purposes since the country's average temperature ranges from 13 to 30 °C [29]. If we consider the range between 15 and 30 °C, 99.88% of the Brazilian territory would be able to cultivate most of the species cultivated in the world.

Analyzing Fig. 4a, only some few areas in the states of Santa Catarina, Rio Grande do Sul, on the border of Minas Gerais with Rio de Janeiro and São Paulo, average temperature values

below the minimum suggested for the cultivation of green microalgae. These regions, with milder temperatures compared with the rest of the country, have a subtropical climate, and in the Brazilian winter, which starts in the month of June and runs until September, they tend to have lower extreme temperatures, which would lead to limit the development of cultivation of green microalgae in these regions.

In the north of the country, in contrast, some areas of the State of Bahia, Rio Grande do Norte and Ceará, have average temperature values above those considered ideals for cultivating most species of microalgae.

On the other hand, the cultivation of cyanobacteria would be more recommended for these regions of colder climate, since the temperature range for the photosynthesis of these microalgae to occur is from 0 to 20 °C in winter and from 20 to 30 °C in summer [36]. Cyanobacteria have the flexibility to adapt to different temperatures compared with green microalgae [39, 40]. In this sense, the cultivation of cyanobacteria can be an option for regions of milder climate in Brazil, identified in Fig. 4a. However, the predominant purpose of cultivation would be to obtain biomass rich in protein material—Phycobiliproteins (*Phycocyanins*, *Phycoerythrins*, and *Allophycocyanins*), soluble proteins contained above 40% of the cell mass of these organisms [41].

It should be noted that some species of cyanobacteria are cyanotoxins producers [42], such as Microcystins (released by genera: *Microcystis*, *Anabaena*, *Planktothrix*, *Nostoc*, *Hapalosiphon*, *Synechocystis*, *Aphanocapsa*, *Oscillatoria*). In these cases, these species should be strongly avoided for the purpose of feeding.

Ten different species of microalgae previously gifted from the Aquiculture Technology Center (Fortaleza - Ceará, Brazil) bank were studied [43] for either biomass and lipid (oil) productivities (*Chaetoceros gracilis*, *Chaetoceros mulleri*, *Chlorella vulgaris*, *Dunaliella* sp., *Isochrysis* sp., *Nannochloropsis oculata*, *Tetraselmis* sp., *Tetraselmis chui*, *Tetraselmis tetrahele*, and *Thalassiosira weissflogii*). All experiments have been carried out in Ceará, Brazil at 28 °C or 22 °C depending on temperature requirements for the abovementioned strains. *Tetraselmis tetrahele* yielded the highest biomass output rate (4400 g m⁻³ d⁻¹). *Chaetoceros gracilis* attained the highest recorded lipid content in biomass (60.28%) as well as lipid productivity (2100 g m⁻³ d⁻¹). According to experimental results, *C. vulgaris*, *C. gracilis*, and *T. tetrahele* proved to be the species that present the best results on large-scale oil production [43].

The genus *Spirulina* (*Arthrospira*) has the capacity to grow typically at temperatures ranging from 20 to 40 °C at temperatures ranging from 20 to 40 °C, being an ideal species for cultivation in locations with high temperatures, which were presented in Fig. 4a, representing 93.40% of the Brazilian territory. The cultivation of the species *Spirulina maxima* and *Spirulina platensis* is recommended in the country [36].

Brasil et al. [2] published a review describing the current scenario of microalgae biorefineries in the biofuels and petrochemical industries in Brazil, presenting the challenges and advances in the production.

In outdoor cultivation systems, the light intensity and room temperature will vary with time and location. In summer, the temperature in most of the Brazilian territory is high, therefore requiring temperature regulation by cooling, to create a continuous optimal temperature for the microalgae strains. The main reactor cooling mechanisms are (1) immersion in a water bath - efficient process; however, even today, its cost-benefit is very doubtful; (2) cooling by water spraying may be reliable and cost-effective in dry climates; and (3) economic considerations favor evaporative cooling over the use of heat exchangers, which may be the most suitable for Brazilian regions, except northeast (specifically where the climate is dry and arid).

Microalgae Biomass Productivity at Ideal Illumination Range

For microalgae cultivation, the source and intensity of light are factors that directly affect the performance of growth and productivity in biomass, as well as the doubling time. For outdoor cultivation, sunlight is the main source of light, as it is a free, durable, reliable, and highly efficient source for effectively exploiting the commercial potential of microalgae.

According to Gualtieri and Barsanti [44], many species of algae do not grow well under constant lighting, thus using an intermittent light/dark (L/D) cycle favoring the natural conditions of the environment. A total of 14:10 or 12:12 cycles (L/D) are generally applied, which simulate the photoperiod outdoors.

For treatments with the same temperature, but under different lighting values, higher total radiation (which usually contains from 43 to 47% of PAR) can favor the growth of microalgae both in suspension in the culture medium and in the formation of biofilms [40].

In some situations, a higher incidence of radiation, combined with a higher temperature (30–35 °C or higher) can strongly influence the growth of thermotolerant microalgae (e.g., *Desmodesmus* sp., *Coelastrella* sp., *Spirulina platensis*, *Spirulina maxima*). According to Fig. 4b, most of the country receives a significant amount of radiation, with higher concentrations in the northeastern region and lower concentrations in the south, with a tendency to decline also in the equatorial region (north of the Amazon).

In Brazil, specifically, a higher incidence of light at winter is observed. It can be explained due to the low cloudiness in this time of the year, besides of the sunnier and lighter days, compared with summer season. [30]. Thus, it is possible to once again highlight the potential of Brazil for the cultivation of microalgae, as it has high temperatures during the summer,

and high intensity of light in the winter, even when temperatures drop.

Assemany et al. [30] evaluated the composition of the phytoplankton community throughout the annual cycle in a HRP to treat domestic effluent from a upflow anaerobic sludge blanket (UASB) reactor subjected to different levels of solar radiation. Blocking 30% of the solar radiation on the HRP provided greater homogeneity and development of the phytoplankton community. The blocking of 80% of the incident radiation resulted in a reduction in biomass productivity. According to the authors, the variations in photosynthetically active radiation assessed throughout the year were slight, representing little variation with the season, in the municipality of Viçosa - Minas Gerais, southeastern Brazil.

With the increase of biomass concentration, the self-shading effect that the biomass itself creates also increases. This effect can lead to reduced productivity [45, 46]. To circumvent the effect of shading on microalgal cultures, techniques such as the operation of the reactors in semi-continuous or continuous regime can be used. The constant replacement of water and nutrients (or WW), in addition to the mixture, promotes a controlled dilution inside the reactor. This makes it possible to maintain a good harvest of microalgae and at the same time send shade from the biomass itself [26, 47, 48]. The internal agitation of reactors contributes to reduce the effects of shading. However, in order to avoid cell damage, the threshold shear rate provided by agitation must be less than 0.1 s^{-1} [49]. In general terms in FBRs, aeration at 1 VVM, and in HRP agitations at 135 rpm can be considered beneficial to optimize biomass production and at the same time reduce the self-shading effect [26].

Other authors such as Zhao et al. [40] pointed out that the *Lyngbya* genus has greater potential for growth in conditions of relatively high temperature (between 25 and 35 °C) and low PAR (between 30 and 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). *Chroococcus* grows best at low temperature (between 15 and 25 °C) and high PAR, in the ranges of 60 and 120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. *Scenedesmus* grows best under conditions with moderate temperature, 25 °C and higher PAR (in the ranges of 60 and 120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The species *Spirulina platensis* when cultivated at a temperature of 35 °C does not suffer detrimental effect in its photosynthetic activity at light intensities of 1780 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ [50]. The same authors recorded the occurrence of photoinhibition for the abovementioned light intensity when the substrate temperature reached 25 °C. In this case, the photosynthetic activity of *Spirulina platensis* was reduced to 50%. Looking at Fig. 4a and b, it can be inferred that this species (*S. platensis*) would have the relevant potential for cultivation in the whole area that ranges from the north of the State of Minas Gerais (MG), all the central-west of Brazil, northeast and north of Brazil.

Microalgae for Biofuels with Emphasis on Biodiesel

Under high salt concentration conditions, algae can considerably suffer with stress. The increase of biomass concentration or lipid content is notably greater when *C. vulgaris* is exposed to lower concentrations of desalination concentrate. At the same way, in cultures of *Chlamydomonas* sp. under saline stress, the achievement of the highest lipid content (23%) was under the lowest concentration of NaCl (0.2 M NaCl) [51]. However, significant increases in lipids have been observed in many microalgae subjected to high salinity conditions, for example *Botryococcus braunii*, *Scenedesmus*, and *Dunaliella* [52].

A literature review on microalgae was carried out analyzing the period, from 1970 to 2020, and it was found that the authors have a greater interest in studying the subjects: “biomass, biofuels, and lipids” [31]. Thus, Table 2 shows studies that aimed to analyze the production of biomass and lipid, related to different sources of substrates and microalgae species, highlighting the source of used light.

Calixto et al. [53] evaluated 12 species of microalgae. *Chlamydomonas* sp., *Monoraphidium contortum*, *Chlorella* sp., and *Synechococcus nidulans* were those that attained higher concentrations of lipids (Table 2). In addition, when evaluating biomass together with lipid, the species *Pediastrum tetra* and *Scenedesmus acuminatus* also demonstrate an adequate final product (biodiesel) quality, due to its high cetane number which indicates good ignition characteristics.

In the same work, the strains *Monoraphidium contortum* and *Synechococcus nidulans* were the only species evaluated that did not prove to be good biodiesel producers due to its lipid quality for this purpose.

Under mixotrophic cultivation with the genus *Scenedesmus* sp., Assemany et al. [57], Tango et al. [58], and De Mendonça et al. [20] record relevant biomass productivity (Table 2) using agro-industrial WW as a cultivation media. Microalgae such as *Chlorella vulgaris*, *Scenedesmus obliquus*, and *Spirulina platensis* perform mixotrophy [59], creating synergy for the removal of dissolved organic carbon, which is important for the treatment of sanitary and agro-industrial WW, as well as biomass production.

According to Araujo et al. [43], the behavior of 10 species of microalgae (*Chaetoceros mulleri*, *Dunaliella* sp., *Nannochloropsis oculata*, *Tetraselmis chui*, *Thalassiosira weissflogii*, *Chaetoceros gracilis*, *Chlorella vulgaris*, *Isochrysis* sp., *Tetraselmis* sp., and *Tetraselmis tetrahele*) in two culture media with different salinities for lipid/oil production was reported, being the extraction accomplished by the Bligh and Dyer method formerly assisted by ultrasound. Salinity is known to be a factor that affects directly and in different ways each type of microalgae in both its biomass

production and oil production capacity. *C. gracilis* exhibited the highest oil content and *C. vulgaris* presented the highest oil productivity; thus, both species provided a greater sustainability for large-scale (industrial) oil production [43].

Innovation and investment in Brazil, in terms of cultivation and production of microalgae, have grown significantly in less than a decade, as has been previously highlighted by Brasil et al. [2], giving emphasis on internationally renowned companies such as Petrobras (Petróleo Brasileiro S.A) and Embrapa (Empresa Brasileira de Pesquisa Agropecuária do Ministério da Agricultura, Pecuária e Abastecimento), which have strongly invested in the microalgae sector during the last years. Petrobras pursued the biodiesel production from marine microalgae together with WW treatment combined with produced CO₂ in its petroleum unit biofixation firm microalgae. In 2012, Petrobras started the first unit of the company for the cultivation of these marine microalgae on the Brazilian north-east coastline due to the usual advantageous climate conditions. The Program created by Embrapa aimed at the identification, isolation, and evaluation and licensing of several microalgae species in Brazil for future production of bioproducts and biofuels from this feedstock [2].

In 2014, a commercial-scale plant started to operate for the heterotrophic mass cultivation of genetically modified microalgae towards oil production directed to the final transformation into chemicals, namely lubricants and cosmetics, with a capacity of 100 Mt of oil produced per year using as substrate and carbon source the sugarcane juice from this industrial unit, primarily designed for ethanol production. Moreover, this kind of industrial production generates other types of byproducts such as vinasse (between 10 and 15 L vinasse per liter of bioethanol is generated) that can be used as a cultivation medium for microalgae together with the CO₂ produced in the various areas of the facility that can serve as a nutrient source for these microorganisms. Using genetic modification principles for microalgae, it is also possible to produce cellulase, an enzyme widely used in the bioethanol production process. This is a typical example of industrial symbiosis between sugarcane-based bioethanol production and microalgae production as the integration of several unit operations required for the whole process can be carried out sharing the same industrial plant with a concomitant decrease of production costs and increase in sustainability [2].

Brasil et al. [2] stated that Brazil holds 399 sugarcane-ethanol production plants, the majority of them spread in the Northeastern and Southeastern regions. In 2013, due to the processing of 658,820 Mt of sugarcane has yielded 37,880 Mt of sugar, together with 27.96 billion liters of ethanol as well as 8870 MW of electricity. Furthermore, two second-generation Brazilian installations started operations aiming at reaching the production capacity of 82 million and 40 million liters of cellulosic ethanol, using straw and cane bagasse, respectively, as raw material.

Table 2 Biomass and lipid production from microalgae survey for different substrates (WW), supplied illumination, and used bioreactor

Substrate	Bioreactor	Strains	Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Light source	Biomass concentration (g L^{-1})	Biomass production ($\text{g L}^{-1} \text{d}^{-1}$)	Lipid production ($\text{mg L}^{-1} \text{d}^{-1}$)	Reference
Synthetic medium	Flasks	<i>Chlorella</i> sp.	83.25 \pm 5.55 ^a	Light system	0.594	1.44 \pm 0.4	190.1 \pm 2.6	[53]
		<i>Scenedesmus acuminatus</i>			0.640	0.42 \pm 0.2	57.1 \pm 1.8	
		<i>Pediastrum tetras</i>			0.528	0.36 \pm 0.1	62.3 \pm 2.9	
		<i>Chlamydomonas</i> sp.			0.536	0.39 \pm 0.2	83.4 \pm 0.8	
		<i>Monoraphidium contortum</i>			0.296	0.15 \pm 0.1	29.8 \pm 2.1	
Swine WW	PBR	<i>Synechococcus nidulans</i>	148.5	Red light ¹	0.560	0.69 \pm 0.2	93.8 \pm 1.4	[54]
		<i>Scenedesmus</i> sp.	80	Light system	0.96	NR	16.8	[52]
		<i>Nannochloropsis gaditana</i>	58	White bulbs	3.22–3.70	0.21–0.36	62–64	[20]
		<i>Scenedesmus obliquus</i>	2500 ^b	Sunlight	NR	11.4 $\text{g m}^{-2} \text{d}^{-1}$	NR	[55]
Desalination concentrate Cattle WW WW after UASB (S1) (S1) + UV radiation Domestic sewage	HRP	(Phyla predominant)	On average 1300 ^b	Sunlight	NR	9.3 $\text{g m}^{-2} \text{d}^{-1}$	0.92 $\text{g m}^{-2} \text{d}^{-1}$	[56]
		<i>Chlorophyta, Cyanophyta and Bacillariophyta</i>	On average 1183 ^b			9.81 $\text{g m}^{-2} \text{d}^{-1}$	0.96 $\text{g m}^{-2} \text{d}^{-1}$	
			919 ^b			9.17 $\text{g m}^{-2} \text{d}^{-1}$	0.79 $\text{g m}^{-2} \text{d}^{-1}$	
			On average 910 ^b			8.05 $\text{g m}^{-2} \text{d}^{-1}$	0.94 $\text{g m}^{-2} \text{d}^{-1}$	
			On average 520 ^b			9.45 $\text{g m}^{-2} \text{d}^{-1}$	0.74 $\text{g m}^{-2} \text{d}^{-1}$	
			307 ^b			7.64 $\text{g m}^{-2} \text{d}^{-1}$	0.61 $\text{g m}^{-2} \text{d}^{-1}$	
			2,362 (max value)			7.30 $\text{g m}^{-2} \text{d}^{-1}$	10	[57]
			2,475 (max value)			NR	3.4	
			1 902 \pm 529 ^b			32.2 $\text{g m}^{-2} \text{d}^{-1}$	2.3 $\text{g m}^{-2} \text{d}^{-1}$	[58]
			1797 \pm 777 ^b			52.5 $\text{g m}^{-2} \text{d}^{-1}$	3.7 $\text{g m}^{-2} \text{d}^{-1}$	
Meat processing (primary effluent) Meat processing (secondary effluent) Meat-processing industry (primary effluent)	PBR	<i>Scenedesmus</i> sp.	2,362 (max value)	Sunlight	8.575 g L^{-1} VSS	NR	NR	
		<i>Scenedesmus staurastrum</i>	1 902 \pm 529 ^b	Sunlight	3.355 g L^{-1} VSS	32.2 $\text{g m}^{-2} \text{d}^{-1}$	2.3 $\text{g m}^{-2} \text{d}^{-1}$	
		<i>Scenedesmus</i> sp.	1797 \pm 777 ^b		NR	52.5 $\text{g m}^{-2} \text{d}^{-1}$	3.7 $\text{g m}^{-2} \text{d}^{-1}$	
Meat-processing industry (secondary effluent)	HRP		2101 \pm 342 ^b			26.5 $\text{g m}^{-2} \text{d}^{-1}$	1.8 $\text{g m}^{-2} \text{d}^{-1}$	
			2254 \pm 172 ^b			12.1 $\text{g m}^{-2} \text{d}^{-1}$	0.7 $\text{g m}^{-2} \text{d}^{-1}$	
			1269 \pm 743 ^b			11.4 $\text{g m}^{-2} \text{d}^{-1}$	0.8 $\text{g m}^{-2} \text{d}^{-1}$	
Domestic sewage	HRP		1987 \pm 578 ^b			10.5 $\text{g m}^{-2} \text{d}^{-1}$	0.3 $\text{g m}^{-2} \text{d}^{-1}$	
		<i>Chlorella</i> sp. (predominant)	259–1662	Sunlight	NR	7.2 and 7.36 kg year^{-1}	NR	[38]

NR not reported

¹ Red light emission diode light (PGL-RBC 2500, Parus) at 630 nm and 148.5 $\mu\text{mol m}^{-2} \text{s}^{-2}$

^a Original article was written in klux

^b Original article was written in $\mu\text{E m}^{-2} \text{s}^{-1}$

It is also achievable to produce microalgae from Brazilian byproducts, such as glycerol which acts as a component (C source) in the culture medium, obtained from the biodiesel production [2]. This has been previously described for either *Botryococcus terribilis* or *Chlorella vulgaris* [60]. High yields of biomass have been reported when a domestic WW-based culture medium containing 50 nM glycerol was used [60].

In 2019, the total biodiesel production in Brazil was about 5.8 billion liters, corresponding to an eight percent rise relative to the previous year, mainly based on the projected modest growth of the Brazilian economy together with an increase of the biodiesel blend to eleven percent (B11) after September 2019. The total biodiesel consumption in Brazil is estimated around 5.79 billion liters.

Assuming a productivity of biomass per area of $20 \text{ g m}^{-2} \text{ d}^{-1}$ ($7300 \text{ t km}^{-2} \text{ year}^{-1}$), a realistic 20% lipid content in biomass, 60% of saponifiable fraction, a transesterification yield of 98% a biodiesel output productivity of $858.48 \text{ t km}^{-2} \text{ year}^{-1}$ or $1031 \text{ m}^3 \text{ km}^{-2} \text{ year}^{-1}$ can be predicted. Considering the Brazilian biodiesel consumption stated above, 5614.8 km^2 of land for microalgae mass culture would be required for assuring this target [61].

On the other hand, the Brazilian consumption of diesel was $56,074,000 \text{ m}^3$ in 2018 [61]. In order to fully assure this consumption entirely by microalgae-based biodiesel, $54,377 \text{ km}^2$ of land would be required, i.e., the equivalent of Rio Grande do Norte state area.

Brazilian Microalgal Biorefineries Based in WW Treatment: Bioremediation and Biomass Production

A huge number of WWs is being produced everyday all over the world, and Brazil is not an exemption. The conventional aerobic WW treatment plants (WWTP) are the systems that have been used so far; however, they have an intense energy demand ($0.5\text{--}1.0 \text{ kWh m}^{-3}$ and represent 1–2% of the energy used by a country), and a large amount of nutrients (N, P) being released with high cost and negative impacts on the environment. There is a need to develop more efficient processes capable of achieving the discharge water quality imposed, while consuming less energy and enhancing nutrient recovery such as nitrogen and phosphorus from the WWs.

It is known that microalgae have the ability to assimilate organic compounds (mixotrophy) and mainly to efficiently nutrients [54]. This activity makes the microalgae become an important factor in the biological processes related to WW bioremediation.

In this sense, based on nutritional requirements and mechanisms, the microalgae can be classified as autotrophic, heterotrophic, or mixotrophic. Autotrophic microalgae use sunlight and atmospheric (inorganic) CO_2 as carbon sources.

Heterotrophic cultures use organic compounds and atmospheric O_2 in the absence of light, and finally, mixotrophic cultures can use both sources of organic and inorganic carbon, simultaneously, in light and darkness [62]. Thus, due to the wide availability of nutrients, greater metabolic flexibility and nutritional mechanisms and the coexistence of two complementary and cooperative nutritional processes, mixotrophy results in greater productivity of biomass and lipids, in addition to decreasing the chlorophyll content present in microalgae, improving yield in the production of biomass, biofuels, and other bio-based products [62–64]. The mixotrophic mechanisms are maximized with the gradual reduction of the concentration of nitrogen compounds in the culture medium, especially in wastewater, where the supply of soluble carbon is abundant.

It is worth noting that in both heterotrophic and mixotrophic cultivation, the reduction in chlorophyll concentrations in cells is positive when the objective is the production of biodiesel. This is because the presence of this substance in the biomass from which the lipids will be extracted impairs the transesterification process [65].

The advantage of using microalgae-based system to treat WWs takes advantage of the in situ aeration/oxygenation provided via photosynthesis by the microalgae using the WW and sludge, with costs and environmental benefits. An innovative option is the use of a thermochemical pathway (HTL) to process the whole microalgae biomass, regardless of its lipid content, improving product yield and thus performance and sustainability. This integration is expected to increase the environmental and economic performance of fuel supply, at the same time addressing to meet the current legislation to the water quality to be discharged/disposed of and to reduce the CO_2 emissions and hence, to reduce the effects of GHG.

The use of combined microalgae and bacteria for biofuel production employing WWs and anthropogenic CO_2 generated in the system, as well as from pollutant industries nearby, allows to produce up to $80 \text{ t ha}^{-1} \text{ year}^{-1}$ of rich biomass which will be converted into bioenergy through several pathways either biochemically or thermochemical or a combination of both. One attractive possibility is to process it hydrothermally into CHP (combined heat and power) (CH&P) by intermediate HTL biocrude production, bio-char (soil amendment), and also biomethane. As oxygen provided by photosynthesis replaces the one generated by mechanical stirring (the conventional approach), a significant power reduction is expected. A reduction in power consumption below 0.20 kWh m^{-3} of microalgae-based treated water versus 0.50 kWh m^{-3} of conventional WW treatment systems has been claimed [63]. On the other hand, nitrogen recoveries up to 50 g m^{-3} from WW using produced microalgae (500 g m^{-3} WW) can be expected [63] without extra energy consumption (the production of nitrogen fertilizer demands $10\text{--}15 \text{ kWh m}^{-3}$). Moreover, under the same conditions, it is possible to mitigate phosphorus

depletion by recovering it from WW up to 10 g m^{-3} [63]. Microalgae-assisted WW treatment minimizes the greenhouse gases emissions (CO_2 , NO_x , CH_4 , etc.) compared with conventional WW treatment systems, and simultaneously, can accomplish international regulations in WW treatment, get reusable water or clean enough to be discharged in the water bodies. On the other hand, the production up to $40 \text{ t ha}^{-1} \text{ year}^{-1}$ of biofertilizers (10 L per kg of microalgal biomass) [63], which eventually can be employed in agriculture to reduce the environmental burden and improve its sustainability is achievable. The list of different sources of WW that can be treated with microalgae is quite large including urban, manure, food, and feed industries (brewery, olive oil, dairy, aquaculture), paper industry, and others, widely available in Brazil.

The PBRs, in this case, promote many benefits, mainly due to the fact that pollutants are removed, and biomass is produced simultaneously [66].

Table 3 shows the results of studies carried out in Brazil in which the main objective was the cultivation of microalgae in PBRs to remove nutrients (bioremediation), from substrates such as domestic sewage, industrial, and agro-industrial WW.

Santiago et al. [55] investigated the influence of pre-disinfection by ultraviolet light to reduce bacteria that could inhibit the growth and productivity of microalgae biomass in raceways systems. The results were very similar to those registered by another research conducted by Singh and Singh [36] that demonstrated that the pre-disinfection of WW with UV allows the predominance of certain species, mainly microalgae of the class *Chlorophyceae*. In this specific case, both authors showed increased productivity of microalgae biomass. The authors also pointed out that the use of microalgae for the treatment of sanitary sewage from UASB reactors, especially in countries with tropical weather, such as Brazil, is applicable [55].

The cultivation of the species *Scenedesmus obliquus* (ACOI 204/07) was tested in bovine effluents previously digested by an UASB-AF reactor by De Mendonça et al. [20]. The results were satisfactory and removal of 99% NH_4^+ , 77.5% PO_4^{3-} , and 70% of CODs were registered in batch operation mode (12 d), with pH control close to neutrality. In continuous mode (also with controlled pH), the efficiencies in removing NH_4^+ , PO_4^{3-} , and CODs detected for the same substrate and PBRs were 94%, 70%, and 61% respectively, using a hydraulic retention time (HRT) 12 d. In this same study, when the authors replicated the experiment without controlling the pH of the solution, significant losses of ammoniacal nitrogen to the atmosphere were verified via stripping, which reduced productivity in biomass, but on the other hand, maximized productivity in lipids.

Perazzoli et al. [67] evaluated piggery effluents as substrate, with high and low concentrations of N e P. In a medium rich in nutrients, the concentration of carbohydrates, proteins, and lipids was $27.6 (\pm 3.3)$, $57.6 (\pm 0.1)$, and $3.9 (\pm 0.6\%)$,

respectively. When reducing the nutrient sources, the results were strongly changed to $54.6 (\pm 2.6)$, $24.1 (\pm 2.4)$, and $16.9 (\pm 0.8\%)$. The same phenomenon was recorded by De Mendonça et al. [20] when performed microalgae-mediated bioremediation of cattle WW. The authors noted that the greater the supply of NH_4^+ for the cultivation of *Scenedesmus obliquus*, the greater the value of protein concentration in biomass. It was also found by the authors that the PBR mode of operation also influenced the concentration of proteins and lipids in the biomass. In continuous flow, biomass tended to accumulate more proteins due to the continuous supply of N to the substrate. In continuous mode without pH control, 53% of proteins and only 13% of lipids were recorded in the biomass. When the PBRs were operated in batches without pH control, ammonia volatilized (about 70%), with increased lipid accumulation reaching 29% in dry biomass.

Due to the high concentrations of mineral salts, desalination concentrate has relevant potential as a culture medium for microalgae [52].

Matos et al. [32] mentioned that the medium of cultivation of concentrated salt was viable for *Nannochloropsis gaditana* species, increasing value for this WW. Other studies with concentrate from the desalination process have pointed out this waste as a favorable and promising alternative medium for cultivating microalgae in the semiarid regions of Brazil [68]. It can also be highlighted that this region of Brazil has exceptional qualities to produce microalgae in open channel tanks, due to the natural conditions of ample solar radiation (Fig. 4b), with low seasonal variation and abundance in brackish waters [68].

A consortium of microalgae/bacteria, grown in domestic sewage, under different intensities of solar radiation, in HRP, was evaluated, aiming to compare composition of the produced biomass [56], as well as lipid concentrations. Statistically, with 95% confidence, the total lipid concentrations did not vary under different intensities of solar radiation, presenting an average value of 9.5%. On the other hand, the results showed that blocking more than 30% of solar radiation had a negative effect on lipid productivity, where the lowest PAR ($\approx 310 \mu\text{E m}^{-2} \text{ s}^{-1}$) provided the lowest accumulation of lipids (8.8%). This lower PAR value also contributed to reduce the total biomass production, presenting $7.30 \text{ g m}^{-2} \text{ d}^{-1}$. Finally, the highest light intensity, PAR of $\approx 1300 \mu\text{E m}^{-2} \text{ s}^{-1}$ yielded the highest biomass content, $9.81 \text{ g m}^{-2} \text{ d}^{-1}$.

Authors claim that the relative abundance of phytoplankton grown in PBRs or system raceways, when subjected to CO_2 injection, tends to be greater. This is because with the addition of gas, the supply of inorganic food is enhanced, which directly reflects taxonomic changes in communities [38]. Because microalgae assimilate CO_2 from the atmosphere concomitant to its potential to extract nutrients from WW (sometimes they also extract organic carbon through the mixotrophic process),

Table 3 A survey of Brazilian microalgae-based WW treatment studies reported with emphasis on operational parameters and contaminant removal yields

Substrate	Strain	In. CODs (mg L ⁻¹)	In. NH ₄ ⁺ (mg L ⁻¹)	In. Ps (mg L ⁻¹)	RT (d)	CODs (%)	TOC (%)	NH ₄ ⁺ (%)	Ps (%)	Reference
Domestic sewage ¹	<i>Chlorella</i> sp. (predominant)	174.5	77.4	12.3	8	31.7	23.5	65.1	- 8.4	[38]
WW after UASB ¹	<i>Chlorophyceae</i>	99	40	4.1	4	30.8	24.6	65.7	- 11.3	
WW after UASB + UV radiation ¹						26	52	71	14	[55]
Cattle WW (batch) ²	<i>Scenedesmus obliquus</i>	2,422	498	23.5*	12	65–70	NR	98–99	69–77.5*	[20]
Cattle WW (cont.) ²						57–61		96–94	65–70*	
Meat processing (primary effluent) ²	<i>Scenedesmus</i> sp.	308.4	13.4	3.6	13	43.8	NR	100	47.2	[57]
Meat processing (secondary effluent) ²		56.0	40.4	0.7		NR			100	

RT, removal time; NR, not reported; NS, CODs: soluble chemical oxygen demand; TOC, total organic carbon; Ps, soluble phosphorus; Cont., continuous; In., influent

*Phosphate

¹ HRP

² PBR

these have been identified as a bioeconomic, sustainable, and circular solution for treating WW and also air atmospheric [69].

The use of waste for the production of biofuels represents today by far the most viable and most interesting option in the bioenergy sector, due to the efficient use of a waste that seeks an alternative way for its treatment. In the work of Santana et al. [70], 40 different strains of microalgae were grown in a vinasse medium from a company that processed sugarcane in order to evaluate the system in the frame of a biorefinery and its potential for biofuel and bioproduct production. The chosen strains showed higher productivity in vinasse than in the standard. The obtained biomasses were biochemically characterized, presenting high protein and carbohydrate content, (monosaccharides from 46 to 76% of the total dry weight) and a high calorific value, promising values that could contribute for the production of bioenergy and high value-added products at the industrial level. The most challenging aspect in this process is the microbial control in order to ensure that there is no contamination of the medium that can inhibit the process, especially in large-scale systems [70].

Following the same rationale of the last two studies previously presented, the work of Assemany et al. [71] was based on the cultivation of microalgae in domestic and industrial effluents (beer industry), each of which was mixed with an amount of WW from the anaerobically treated olive mill to avoid effluent pretreatment or dilution and to assess the biogas/biomethane production potential. According with the same authors, olive mill WW was an excellent substrate for microalgae production in anaerobic digestion systems. In the case of replacement of 10% of domestic sewage by olive oil WW, the best biomass conversion was obtained for a higher biogas/biomethane production. The replacement of 20% of industrial beer effluent by olive WW can be applied in anaerobic digestion systems; however, it should be considered an adaptation time for the microbiota at the anaerobic digester to the new environmental conditions [71].

In another study [72], six types of Chlorophyta strains (*Desmodesmus* sp. L02, *Chlorococcum* sp. L04, *Coccomyxa* sp. L05, *Chlorella* sp. L06, *Scenedesmus* sp. L08, and *Tetrademus* sp. L09) were taken from effluent from an anaerobic reactor that processed urban WW. These strains were cultivated in two different types of WW: non-sterile WW to verify the ability of these microorganisms to survive in this environment and sterilized WW to analyze the composition of the produced biomass and its ability to recover nutrients. The strain that achieved the longest survival time under the non-sterile environment was *Chlorella* sp. L06 (90% of capacity in ten days of culture) showing *Desmodesmus* sp. L02 (1.8%, 24-h survival) the lowest capacity. The strains presented on average 15.4% of lipids, 28.7% of proteins, and 14.8% of carbohydrates, presenting *Chlorococcum* sp. L04 the highest carbohydrate composition (29.3%). *Tetrademus* sp. L09 was

the strain that achieved the highest nitrogen and phosphorus removal, improving effluent quality and allowing for greater biomass formation which triggered increased biogas/biomethane production in anaerobic digesters [72].

According to Uebel et al. [73], the microalga *Spirulina* sp. LEB 18 was grown and physicochemically characterized as a way to evaluate its potential in the production of biofuels and bioproducts for application in an integrated microalgae-based biorefinery. This species had a minimum generation time of 5.2 d, the greatest development rate of $0.133 \text{ g L}^{-1} \text{ d}^{-1}$, and after 19 days of growth a maximum yield of 14.9 g m^{-2} , was obtained. The maximum biomass concentration (1.64 g L^{-1}) occurred past 37 days of cultivation. The biochemical composition of the species was 57% proteins, 10.6% carbohydrates, and 11.7% lipids. Considering all these results, it can be concluded that these microalgae have wide advantages to be further used in a biorefinery towards high market volume products and/or loud incorporated value materials such as biofuels and bioproducts (food and pharmaceutical sector) and lastly, in energy production (combined heat and power-CHP) [73].

On the other hand, microalgae production can also be performed using the concentrate obtained from desalination waters as culture medium [68]. The advantages offered by this medium in terms of microalgae productivity and its biochemical components like proteins, fatty acids, and lipids were evaluated. The three studied (chosen) species were *Chlorella vulgaris*, *Spirulina (Arthrospira) platensis*, and *Nannochloropsis gaditana*. Generally, they have been able to grow in this type of medium. However, each microorganism requires a specific concentration considered optimal. Under conditions of nutritional stress, microalgae tend to decrease their protein content (and simultaneously increase the production of lipids especially in the form of saturated fatty acids). *N. gaditana* had a remarkable tolerance capacity for this medium when it was highly concentrated, but higher yields have been obtained when the concentrate was blended with other medium, therefore for a beneficial mix of various nutrients [68].

In another publication [74], the *Asterarcys quadricellulare* species previously isolated from a WW system, specifically from a Water Reuse Center located in São Paulo University was evaluated concerning its concentration and lipid and carbohydrate composition. It was grown mixotrophically in 2 L PBRs at $24.2 \text{ }^\circ\text{C}$ with cyclic photoperiods of 12 h of artificial light: 12 h without light with 0.1 g L^{-1} glucose as a feedstock (substrate). A biomass concentration range of $0.463\text{--}0.567 \text{ g L}^{-1}$ was obtained with 36.6% carbohydrate and 20% lipid content. The microalga *A. quadricellulare* has potentially a high biofuel production capacity for either biodiesel ($4.705 \text{ L oil.biodiesel ha}^{-1} \text{ year}^{-1}$) or bioethanol ($7.814 \text{ L ethanol.hydrated ha}^{-1} \text{ year}^{-1}$) and could represent a viable solution for WW treatment [74].

Couto et al. [75] evaluated the thermochemical conversion of microalgae-based biomass previously produced in a

domestic sewage processing plant containing HRPBs in Minas Gerais, Brazil. The selected thermochemical pathway was hydrothermal liquefaction (HTL) in order to obtain bio-oil as an energy vector. The effect of several parameters as the process temperature, the time of the reaction, and the relation between the water and biomass on the bio-oil yield was evaluated and critically discussed. On the other hand, a nitrogen and carbon mass balance of the products (liquid phase including the bio-oil, the gas phase, and the solid residue) was performed to assess the bio-oil quality and increase the byproduct value. A time operation process of 15 min, with a temperature of $300 \text{ }^\circ\text{C}$ and a relation of 1/10 (w w^{-1}) between the biomass and water, yielded 44.4% bio-oil in dry ash free (daf) basis. Any time increment above 15 min in the reaction time did not offer important yield improvement. On the other hand, bio-oil formation yields have been improved for temperatures above $300 \text{ }^\circ\text{C}$. The need for a previous microalgae-based biomass drying step was diminished when compared with other thermochemical conversion pathways applied for bioenergy production from microalgae. In such sense is a way to reach in the HTL process a positive energy balance. Thus, the HTL is a promising alternative for the bioenergy production of very low-cost microalgae-based biomass produced when it is utilized as domestic sewage which combines environmental and economic advantages in the framework of the circular bioeconomy. High water content and low lipid content which are widely reported drawbacks for microalgae-based biodiesel products are not challenging for HTL of the whole biomass. In fact, there is no need to enhance the biomass lipid content imposing culture stress such as nitrogen starvation triggering low biomass and lipid productivities, thus, high biomass production costs. On the other hand, biomass dewatering does not seem to be crucial as the water in biomass acts under subcritical conditions as solvent and reactant for the hydrolysis of the organic matter (proteins, carbohydrates, and lipids) which takes place in the HTL reactor. For this reason, typical HTL conversion yields from biomass to bio-oil are usually higher (45–60%) compared with lipid (and biodiesel yields), which are seldom above 30%. Moreover, any organic matter regardless of its origin can be a feedstock for HTL, suggesting that any sludge from any WW treatment plant can be blended with microalgae prior the HTL conversion step. This approach extends the applicability of microalgae in the whole WW treatment value chain for Brazilian conditions.

Microalgae can be used as a biomass feedstock to produce hydrothermally biomethane and combined heat and power (CHP) at the same time recovering nutrients from WWs and mitigating CO_2 emissions.

Besides biofuel (bio-oil), other products from HTL can be considered, such as biomethane (resulting from an upgrading step of the generated biogas), biofertilizers (N and P-rich fractions from the aqueous fraction obtained during the HTL

step), soil correction agents, and filtration media for industrial applications from the bio-char produced in the HTL processing step. The smart integration of these products can have a positive effect in the viabilities on a technical, environmental, and economic levels. The realistic production of, at least, 80 t (dry microalgae-based biomass) $\text{ha}^{-1} \text{year}^{-1}$ from a WW treatment plant, abating 200 t $\text{ha}^{-1} \text{year}^{-1}$ of CO_2 is expected to yield 30 t $\text{ha}^{-1} \text{year}^{-1}$ of intermediate HTL biocrude as a feedstock for drop-in biofuel, meeting successfully the main objectives in the current energy policies, namely, in the quality of water and atmosphere.

Concerning the WW treatment technology, it is still an energy-demanding activity. According to a previous public report [76], around 2.6% of the Brazilian energy demand was related to the sanitation sector. Any attempt to combine WW treatment and energy production to be invested in the whole process will contribute to a better energy efficiency. The utilization of microalgae-mediated technology and/or biomass conversion to bioenergy, such as HTL will be strongly beneficial.

Concerning Brazilian domestic WW availability, for a reference load of 216 million population equivalent (p.e.), for a conservative share of collected urban-domestic WW treated of 35%, thus a reference load with collected treated water of 75.6 million p.e. assuming 54.75 L (gross urban WW production) $\text{p.e.}^{-1} \text{year}^{-1}$ and 1 kg of microalgae biomass as well as 0.6 kg of TSS (total suspended solids) to be produced through the conversion of 1 m^3 of raw urban WW, a prediction of $6.6 \cdot 10^5$ t (sludge) year^{-1} is generated in Brazil. For a realistic 0.45 HTL conversion yield from sludge to bio-oil and a drop-in fuel conversion from bio-oil of 0.5, $1.49 \cdot 10^5$ t of drop-in fuel gasoline-like could be produced per year. Considering a yearly Brazilian gasoline demand of $2.775 \cdot 10^7$ t, a modest share of 0.005% can be obtained from domestic WW. However, if all required biomass for HTL conversion could be produced from microalgal mass culture, 16,895 km^2 would be required, roughly equivalent to the Sergipe State area of half Rio de Janeiro state area, which is quite reachable.

Greenhouse Gases Abatement by Microalgae

As previously described, microalgae absorb the nutrients and CO_2 that allow the biomass production for further conversion into material with a high added value. The CO_2 source, quantity, and quality influence the composition of the biomass in terms of the biochemical composition, and depending on the latter, the produced microalgae biomass can be utilized for the most diverse options [38]. Table 4 shows several studies that recorded the CO_2 absorption by microalgae, using different sources of gas, different species of microalgae, and types of substrate.

As an example, the raw biogas derived from piggery WW proved to be promising, as a CO_2 source, to stimulate the production of microalgae, highlighting that there was no toxic effect of CH_4 present in biogas for biomass. In addition, the nitrogen compound removal rate was faster under the biogas presence [54]. In this sense, concomitantly with the removal of carbon dioxide from biogas, the latter became purer, increasing the concentrations of CH_4 at the PBR outlet. The results demonstrate that the microalgae-based WW treatment, together with the purification of biogas, maximizes GHG removal.

Chagas et al. [81] studied and reported the growth of *Dunaliella tertiolecta* from CO_2 produced in the fermentation stage of a beer company. The idea was based on the production of lipid and carotenoid accumulation in microalgae grown in PBRs taking advantage of the yeast fermentation and can be applied to the bioethanol industry from sugarcane, for example. By changing glucose concentrations from 10 to 60 g L^{-1} together with CO_2 from the 24-h yeast culture, maximum carotenoid and lipid microalgae production was achieved. Productivity values were much higher when compared with that of control systems that used non- CO_2 -enriched air. Therefore, systems that integrate PBRs for the formation of carotenoid-rich biomass and polyunsaturated fatty acids through CO_2 from the fermentation system presented better results than traditional (conventional) systems [81].

In 2010 and 2015, respectively, Brazil implemented challenging measures in order to guarantee its commitments related with the climate change through the COP-15 in Copenhagen and COP-21 in Paris. The country committed to minimize the GHGs emissions from domestic applications in 37% by 2025 and 43% by 2030, taking as a reference (baseline) the 2005 reported levels [61].

In order to perform the needed actions, The Brazilian Government launched in 2016 the RenovaBio Program being instituted as the “National Biofuels Policy,” which has as main objectives:

- (a) Contributing to the appropriate proportion between the efficiency of the energy and the minimization of GHG emissions, including the different actions for lifecycle assessment for biofuels,
- (b) Promoting the appropriate expansion of the generation and utilization of biofuels in the national energetic mix,
- (c) Contributing to the predictability of several biofuels in the national,
- (d) To ensure for a period (minimum) of 10 years the reduction of the annual carbon intensity goals ($\text{gCO}_2 \text{ MJ}^{-1}$),
- (e) To create the biofuels certification ensuring the efficiency concerning the reduction of GHG emissions, and
- (f) Promote decarbonization credits (CBIO). In such environment, microalgae-based bioenergy production together with anthropogenic CO_2 biofixation by microalgae in

Table 4 A survey of microalgae-based CO₂ biofixation coupled with WW treatment studies with emphasis on the WW source, bioreactor type, cultivation mode, gas type and origin, and CO₂ fixation rates

Substrate	Bioreactor	Strain	Gas type	CO ₂ fixation rate (mg L ⁻¹ d ⁻¹)	Reference
Swine WW	PBR (batch)	<i>Scenedesmus spp.</i>	Biogas (UASB) Air atmosphere	84.4 ¹ –106.8 ² 219.4 ² –126.1 ¹	[54]
Cattle WW	FPPBR (batch) FPPBR (continuous)	<i>Scenedesmus obliquus</i> ACOI 204/07	CO ₂ (99.99% purity) and air	327–547 175–247	[20]
Synthetic medium	PBR raceway (batch) PBR tubular (batch)	<i>Spirulina sp.</i>	Air - paddle wheel Air 0.3 vvm	110–128 165–183	[77]
Synthetic medium	PBR tubular (batch)	<i>Chlorella fusca</i> LEB 111	Air + CO ₂ (0.05 vvm)	171.7–257.1	[78]
Synthetic medium + nanofibers	PBR (batch)	<i>Chlorella fusca</i> LEB 111	Air + CO ₂ (2%)	216.2	[79]
Anaerobic POME	Flask and PBR (batch)	<i>Chlorella sp.</i>	Air + CO ₂ (10% v/v)	829	[80]

*Electric energy generator (Schulz S5500MG), stormed and pressurized in a compressor (3 Phase Schulz BRAVO CSL BR/100 L)

¹ Photoperiod = 12:12, mixotrophic conditions (12 h:12 h - L/D)

² Photoperiod = 24, autotrophic conditions (24-h light)

Brazil is expected to contribute significantly to achieve successfully these goals.

Reported GHGs Brazilian emissions were 1050 Mt eq CO₂ for 2018. The reported levels for 2005 (baseline) were 847 Mt eq CO₂. The target for 2025 in order to meet the COP21 commitments is 534 Mt eq CO₂. This means that a mitigation of 516 Mt eq CO₂ will be necessary to achieve this goal. Using the stoichiometric ratio stated above, a production of 2.46×10^8 t (246 Mt) of microalgae per year would assure this biofixation. Considering the same realistic areal output rate, 286,220 km² would produce the required microalgae biomass for this purpose, corresponding to São Paulo or Rio Grande do Sul area. This biomass would produce approximately 4 times the total quantities of consumed fuels (gasoline, biodiesel, and diesel) in Brazil for the transportation sector. This is a challenging but an attainable goal.

Brazil and the Bioeconomy

The world (and Brazil) cannot base on the actual economic model namely in fossil-based feedstocks. This is the right time and space for a new bio-based paradigm—the bioeconomy, bringing ecology and economy together.

The challenge is multi-dimensional and global in a world scale putting the impact of new scientific inventions in the improvement of the citizens' quality of life and planet welfare, due to limitless products and services that will be created from areas such as engineering and sciences. On the other hand, due to the opportunities that will arise from Brazilian competitive advantages, such as the greatest biodiversity of microalgae on the planet, abundant raw material mainly biomass from organic waste, great availability of land and natural resources, lower costs of biomass production, climate conditions, together with

the existence of highly qualified people in science and technology, an academic sector with international relevance, a long experience in agribusiness and advanced tropical agriculture, a pioneering implementation of biofuel policies, a natural aptitude for the bioeconomy and a public very positive about the perception of the importance of the bioeconomy in Brazil, make the country one of the main places in the world to overcome the difficulties of cultivating microalgae biomass. In Brazil, the industrial biotechnology sector is interested in the biofuel production, mainly for ethanol from sugarcane. The current biorefineries produce several fuels typologies and chemical products such as polymers, enzymes, and biofertilizers [82]. An energy production model is the production of bioenergy by microalgae. An impressive number of Brazilian institutions, companies, biotechnology joint ventures, and corporate spin-offs generate applications in different scales for these organizations, with focus on bioenergy from synthetic biological processes. Most of these Brazilian organizations do not have big-scale installations so far for microalgae (downstream) processing and cultivation [83, 84]. One example is the use of genetic engineering for the biosynthesis of bioethanol from microalgae, based on CO₂, water, and sun radiation which is, in fact, a close alternative to the typical energy production mechanisms on Earth [82]. This approach is expected to attain production levels 6-fold higher than those currently attained with the downstream processing of sugarcane and 16-fold higher than those obtained from corn [82]. Combining microalgae production together with big-scale installations to benefit the process integration and scale economy appears to be a smart and attractive attitude under a Brazilian environment, due to its dimension, biorefineries proven long experience, and scale economy. A special emphasis should be devoted to Brazilian sugarcane plants seen as infrastructures for microalgae production as in situ suppliers of very low-cost carbon, other nutrients, and

renewable electrical energy as feedstocks. Low-cost carbon in the form of CO₂ can be provided by boiler emissions, ethanol fermentation off-gas, and upgrading biogas from the anaerobic digestion of vinasse. The same authors referred the source for renewable electricity: the combustion of either sugarcane bagasse or straw. Even the development of microalgae-based pilot scale and industrial units is easier taking advantage of previous sugarcane plants. Moreover, Klein et al. [83] critically discussed how the situation of microalgae biorefineries from sugarcane can affect the land availability in Brazil.

Brazil is in the forefront of Latin-American countries concerning the development and exploitation of the bioeconomy, being clearly recognized as one of the main world players in the production of agro-industrial-based biomass with an impressive share of bioenergy production, contributing to around 24% of Brazilian energy production and 5% of worldwide energy production.

Anyway, as previously reported, Brazil does not have a devoted bioeconomy strategy [82, 85, 86], highlighting lack of the political attitude and encouragements for the articulate and maintainable development of Brazil's bioeconomy [86]. Nevertheless, several impacting sub-strategies, programs, and measures fostering bioeconomy advance have been implemented since the 70s. Everything started in 1975, during oil crisis. Brazil launched the first biofuel program (big-scale) in the world under the title "Próalcool" [82]. The main areas of the Brazilian bioenergy strategy being responsibility of the Mines and Energy Ministry highlighted a package of legislative measures, as economic support to producers, price setting, tax exemptions, blending quotas, and as consumer incentives for purchasing vehicles powered by ethanol. Moreover, the Brazilian state put money for building a network of biofuel-compatible filling station infrastructures. By 2003, Brazil implemented the "flexifuel" motor which works on ethanol, fuel, or with mixture of both, regardless the volume fraction. One year later, the Brazilian government launched the program for the expansion and utilization of biodiesel. This initiative culminated with the implementation of a compulsory mixing quota for diesel starting in 2013 as well as the launching of a social label (Selo Combustível Social) in order to stimulate the acquisition of edible oils of poorer farmers from the Brazilian northeast and north depressed regions. Currently, Brazil is widely recognized as a worldwide leader in bioenergy. Bioethanol production contributes approximately to a 25% fuel consumption share. The actual 10-year frame energy plan named "Plano Decenal de Expansão de Energia 2023" was launched in 2014 [87], after a stakeholder consultation process. This plan allows a bigger intensification in the utilization of biofuels including biomass-based electricity production fed mainly from either sugarcane bagasse or other agricultural residues [82, 85, 86].

Apart from bioenergy, the government has supported and stimulated the progress of the agro-biotechnology area, due to

the relevant global position Brazil has on the field of genetically modified crops as well as in agricultural biotechnology. By 2007, the Brazilian policy for biotechnology "Política de Biotecnologia" was launched by the government [88], aiming at the inclusive progress of biotechnology and biosciences. Anyway, the current advance of the bio-based industry is mainly private sector-driven. Four years later (2011), the Brazilian National Confederation of Industry (NCI) implemented its agenda for encouraging innovation in Brazil, with especial emphasis on life sciences, biotechnology, and biodiversity, previously selected as strategic areas. A yearly "Bioeconomy Forum" has been organized by NCI since 2012. Moreover, in 2013, the report "Bioeconomy: An Agenda for Brazil" was issued together with the Harvard Business Review. The authors stated that the terms either "bioeconomy" or "bio-based economy" were absent in the Government-issued documents, considering bioeconomy as green economy, bioenergy development, or biotechnology [82, 85].

Concerning the adoption of Brazilian biotechnology policies, under the responsibility of the Development, Industry, and Foreign Trade Ministry, a "National Committee for Biotechnology" was created involving more than twenty institutions together with all national governmental institutions (e.g., agencies and ministries) that deal with all aspects related with projects and public policies linked with biotechnology [89]. The Brazilian government is still implementing further incentives for industries, especially in what concerns business innovation financial support. Several measures created by government agencies such as the Brazilian Development Bank (BNDES) and the National Innovation Agency (FINEP), should be highlighted here, aiming at funding circular economy projects in order to minimize Brazil's dependence on foreign feedstocks and to promote rural development and income. Other initiatives comprise the National Fund for Climate Change, FINEP's Sustainable Brazil fund, and BNDES' Funtec fund. The Brazilian Government together with the Brazilian National Development Bank and the FINEP, implemented the "PAISS" program in 2010 with a new edition in 2013 supporting the development and commercialization of agri-tech innovative business mainly devoted to sugarcane-based bioenergy and chemical industries [82, 85].

The Brazilian government launched its Solid Waste Policy (Law 12,305) in 2010, paving the way for the National Policy on Solid Residues (PNRS), encouraging the multi-stakeholders discussion upon the mutual concern of product disposal and implementation of reverse logistics and waste management structures in Brazil and covering the whole generation value chain [86].

Lastly, the Brazilian Environment Ministry implemented in 2011 the Action Plan for Sustainable Production and Consumption (PPCS), disposing guidelines and coordination to further maintainable process of generation and

consumption acting as a hub for the Brazil's key development and environmental policies, mainly the National Climate Change and Solid Waste Policy and the Brazil's industrial policy—"Plano Brasil Maior" [86].

Future Perspectives

The bioeconomy is clearly recognized as the way for the re-industrialization of Brazil, encouraging the further required development and innovations of bio-based process and products that will accelerate the implementation of this norm in a global world. A long-term joint collaboration among the Brazilian government, research institutions, business, and civil society is expected for taking advantage of a new model of development, bringing ecology and economy together. A dedicated Brazilian plan for the development of the circular bioeconomy will be required where microalgal-based biorefineries are expected to play a pivotal role. For such purpose, a discussion, definition, and implementation of measures that will ensure the alignment of policies in place and long-term strategies, with the objective to Brazil fulfilling its role as a leader a global bio-based economy is urgent, involving different actors (government, business, research organizations, and civil society).

The central questions of the near future to come go beyond the climate change, the demographic explosion, and the cut in GHG emissions but how science and technology will help the economy and society to improve global sustainability and well-being. To achieve this, it is clearly believed that microalgae will play a pivotal role, not only for fulfilling bioenergy needs but also for supplying a wide range of bio-based products. The exploitation of microalgae biomass as the starting point for future biorefineries in Brazil seems to be natural and attractive, bearing in mind that there are still difficulties, challenges, and bottlenecks to solve. A huge effort in Science, Technology, and Innovation will be required in topics such as cheap, efficient, and long-lasting photobioreactors; low-cost and low energy-demanding harvesting and dewatering methods; and cheap, smart, and flexible downstream processing steps. Achieving these goals, it is believed that microalgae will play a crucial role for the Brazilian compulsory transition to a circular bioeconomy. Besides bioenergy production, a wide range of derived compounds from microalgae being used as building blocks, intermediaries of synthesis, and specialities cannot be neglected in the near future, reinforced by the wise utilization of residual biomass left from bioethanol and biodiesel production from the Brazilian territory.

Conclusions

Brazil is one of the major emitters of GHG worldwide with the main share of its emissions appearing from the land use, land

use change, and forestry (LULUCF). The Brazilian Nationally Determined Contribution has considered three main goals to cut emissions efficiently such as the intensification of the contribution of biomass to 18% in the whole primary energy production, the decrease of deforestation, and at least 45% of renewable energy in the energy mix [90]. For such purpose, it will be fundamental to broaden the share of biomass in the Brazilian bioeconomy, especially from microalgae. Microalgae-based biorefineries can take profit of unique Brazilian competitive advantages namely huge microalgal biodiversity, plentiful land, water, waste, and cheap CO₂ feedstock availability together with mild climate conditions all year round. Moreover, the undoubted long high experience in agribusiness and advanced tropical agriculture triggered the appearance of a remarkably high number of Brazilian sugarcane plants providing accessible infrastructures and manageable low-cost carbon as well as other nutrients for the potential implementation of microalgal factories. This review covers how microalgae can contribute to the Brazilian circular bioeconomy for reducing Brazilian GHG emissions as well as the external dependence of fossil-based feedstocks.

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