

Review - Agriculture, Agribusiness and Biotechnology

# Advances in Microalgal Research in Brazil

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Editor-in-Chief: Paulo Vitor Farago

Associate Editor: Paulo Vitor Farago

Received: 2020.08.19; Accepted: 2021.02.24.

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## HIGHLIGHTS

- Microalgae science is a very active area of research in Brazil.
- Brazilian government has been funding projects in algal biotechnology.
- Brazilian institutes have gathered a deep knowledge in algal research.
- Researchers have been focused on bioproducts and biofuels from microalgae.

**Abstract:** Microalgae research has attracted interest worldwide and in order to advance algal biotechnology in Brazil, government has been funding several projects. In the last 10 years, two main funds were provided by the National Council of Scientific and Technological Development (CNPq) agency to researchers in Brazil, who study the potential uses of microalgae for biomass, bioproducts and biofuels production. These funded projects addressed aspects of algal strain identification, development of algal cultivation techniques, designing photobioreactors and raceway ponds, modeling harvesting and dewatering process, maximizing biomass and oil productivities, characterizing chemical composition with different extractions systems and determining physiochemical properties of biodiesel. This review presents the state of art of algal research conducted by Brazilian institutions. Special attention is given to the recent progress on microalgal cultivation, high-value products extracted from microalgae and potential biofuels production. This review may serve as a policy instrument for planning next steps for algal research in Brazil as well as for attracting attention from international researchers who work with microalgae and would like to pursue a future partnership on algal research with Brazilian research institutions.

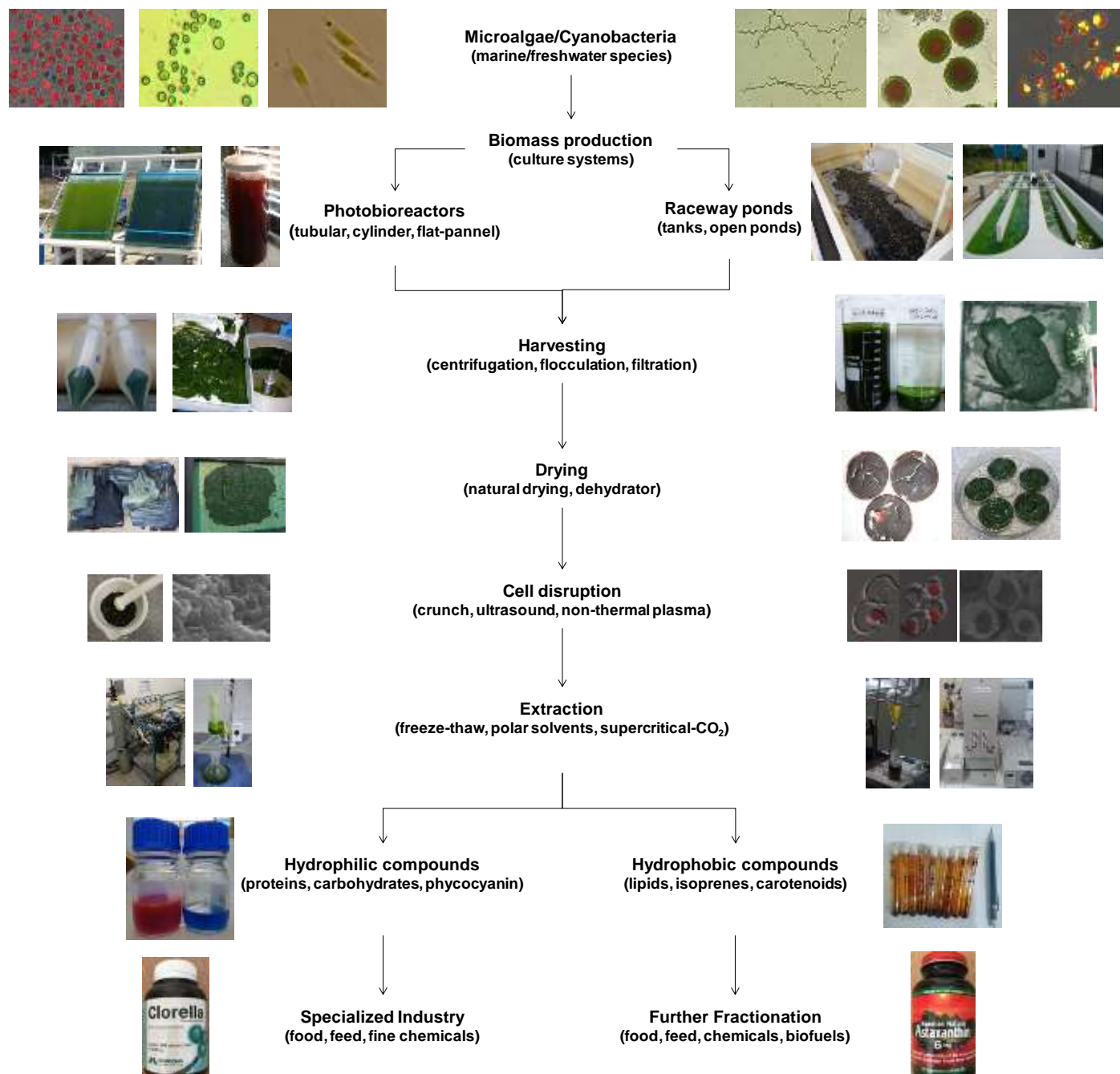
**Keywords:** microalgae culturing; algal biotechnology; photobioreactor; bioprocess; bioproducts; biofuels.

## INTRODUCTION

Microalgae are unicellular photosynthetic microorganisms typically found in freshwater and marine systems that convert sunlight, water and carbon dioxide to algal biomass. With an outstanding ability to synthesize several chemical compounds, their biomass is rich in compounds like proteins [1], carbohydrates and sugars [2], lipids and fatty acids [3], pigments [4], polymers [5], enzymes [6], peptides [7] and sterols [8]. The biodiversity of microalgae is enormous, representing an almost untapped resource for food manufacturing [9], fine chemical synthesis [10], biofertilizers [11] and biofuels production [12,13].

Cultivation of microalgae at large-scale have been conducted for over 50 years [14]. *Chlorella* cultivation was the first microalgae species produced at commercial interest in Japan in early 1960, [15]. In early 1970,

the cyanobacteria *Spirulina* was mass scale cultivated in Mexico, South America [16]. Since 1980, the salt-tolerant microalgae *Dunaliella salina* has been cultivated in large scale in Australia and Israel for  $\beta$ -carotene production [17]. The main reason for the successful growth of these microalgae species is the particular ability to grow at high selective environments, *i.e.*, *Spirulina* thrives well at high bicarbonate concentration ( $16.8 \text{ g L}^{-1}$  of  $\text{NaHCO}_3$ ) and elevated pH (10.0-11.0) [18], *Dunaliella salina* grows well at high salinity (10% to 35% (w/v) NaCl) [19], while *Chlorella* has the capability to tolerate high source of nutrients, notably nitrogen and phosphorus (1:4 to 1:40 N:P ratio), usually found in agricultural wastewater, such as digested abattoir effluent [20].



**Figure 1.** Microalgal biorefinery plant concept [21,22].

In the late 1990s, commercial production of the freshwater green alga *Haematococcus pluvialis* as a source of the carotenoid astaxanthin started in Hawaii, USA [23]. The culture system consists of a combination of closed photobioreactors and raceway ponds. Closed photobioreactors are mainly used for producing of high-value products such as pigments (phycocyanin and carotenoids), single cell protein and single cell oils composed by eicosapentaenoic, docosahexaenoic and arachidonic acids (EPA + DHA + AA) [24]. Photobioreactors have the distinct advantage of preventing water evaporation, reducing the risks of contaminants, limiting the CO<sub>2</sub> losses and flexibility in technical design. The main disadvantages of closed

systems are the high costs of construction and its technical operation such as pumping, cooling, cleaning and sterilization. Raceway ponds are the most usual setting for large-scale outdoor microalgae cultivation, which are considered low cost and easier to build and operate than closed photobioreactors [25].

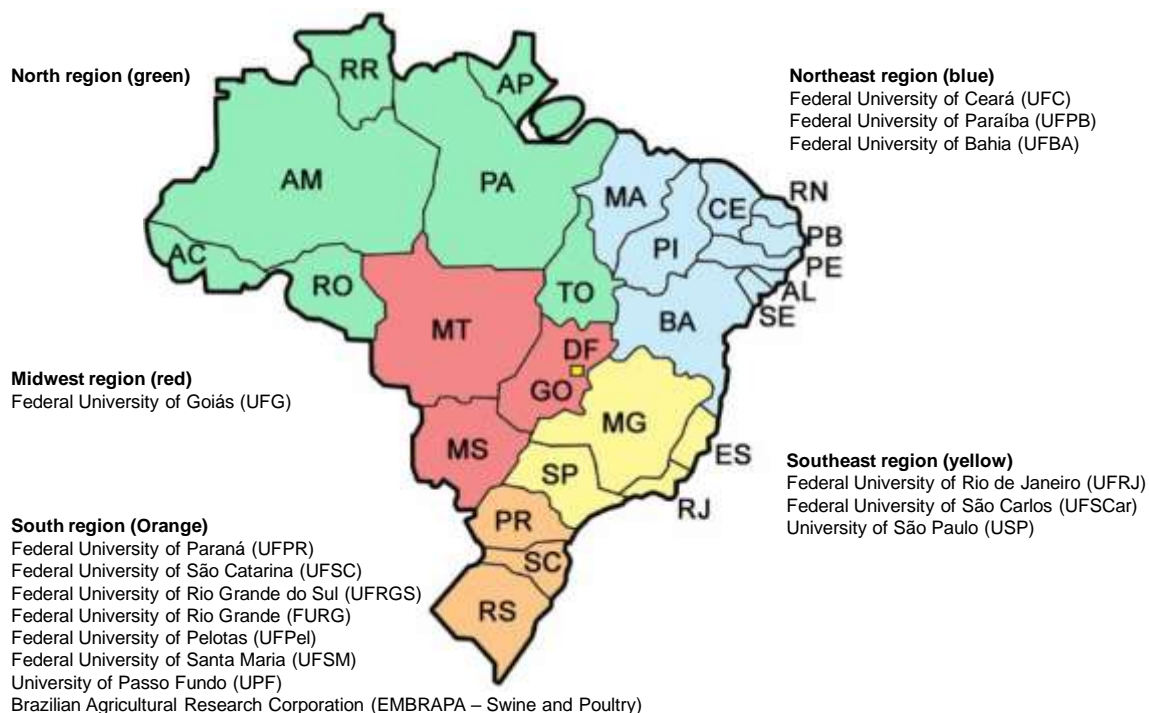
Conventional microalgae production system consists of growth and cultivation of microalgae either in photobioreactors or raceway ponds, biomass recovery or harvesting, followed by further processing steps such as dewatering, drying, cell disruption, extraction and product purification (Figure 1).

### Investment on algal research in Brazil

The exploitation of algal biomass for commercial purposes has prompted several research groups in different countries to seek the most appropriate algal production model associated to greater biomass productivity, environmental sustainability and economic viability [26-29]. Several nations, including Brazil, have invested in this development by funding researchers to advance our knowledge in algal biotechnology.

In 2008, the Ministry of Science, Technology and Innovation (Ministério da Ciência, Tecnologia e Inovação - MCTI) in partnership with the Secretary of Aquaculture and Fisheries (Secretaria de Aquicultura e Pesca) through the National Council of Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq), called for algal research proposals (MCTI/CNPq/MPA Nº 26/2008), funding R\$ 4.5 million (US\$ ~1 million dollar) with the main purpose to support projects focused on producing biodiesel from microalgae. According to Franco et al. [30], 63 project proposals were submitted and the top eleven projects were selected. These funded projects addressed aspects of algal biology, development of algal cultivation techniques, designing photobioreactors and raceway ponds, modeling harvesting and dewatering process, maximizing biomass and oil productivities, characterizing chemical composition with different extractions systems, determining physiochemical properties of biodiesel, and evaluating techno-economic and life-cycle assessment of using algae as main source of biomass.

In 2013, CNPq (MCTI/CNPq Nº 56/2013) called again for proposals for R&D on biofuels and bioproducts from microalgae with a gross funding of R\$ 11,230.000 (US\$ ~ 2.5 million dollars), divided in two categories: *i*) energy potential of microalgae for biodiesel production, and *ii*) biotechnological potential of microalgae within biorefineries. Seventeen top researchers with broad expertise from different Brazilian institutions were selected by CNPq to conduct further studies. The majority of the funding was awarded to researchers and institutions located in the south region of Brazil, as indicated in Figure 2. Except for the University of Passo Fundo (Universidade de Passo Fundo – UPF), all other institutions are public.



**Figure 2.** Institutions awarded financial support by the National Council of Scientific and Technological Development (CNPq) to advance projects in algal research.

To track advances in algal biotechnology by Brazilian institutions, the Curriculum Lattes Platform (<http://lattes.cnpq.br/>), a virtual system created by CNPq, which integrates the curricular databases, research groups and institutions into a single information system, was used to screen the main articles on algal research published in the literature and results of this search are discussed below.

### Microalgae cultivation and production systems in Brazil

In Brazil, microalgae cultivation has been performed either in photobioreactors or in open culture systems. An outstanding photobioreactor system with a volume capacity of 12,000 L was developed and patented by Federal University of Paraná (UFPR) researchers [31]. Another notable patented system, developed by Federal University of Santa Catarina (UFSC) researchers, includes a tubular photobioreactor combined with thin-layer cascades [32]. Both systems were designed for wastewater bioremediation through microalgal cultivation. Further examples about microalgal cultivation systems are shown in Table 1, together with the main microalgae species, culture medium and harvesting process. In addition, Jorquera et al. [26] analyzed and compared the energy life-cycle of oil-rich microalgae *Nannochloropsis* sp. for maximal biomass production cultivated in raceway open ponds (0.35 g L<sup>-1</sup>), in tubular (1.02 g L<sup>-1</sup>) and in flat-plate photobioreactor (2.70 g L<sup>-1</sup>) systems.

Several Brazilian researchers are studying the feasibility of using microalgae for wastewater treatment, which include, *i*) growth of *Scenedesmus* sp. using different concentrations of cattle manure effluent [33,34], *ii*) growth of *Amphora* sp. cultured in shrimp farm wastewater [35], *iii*) growth of *Chlorella vulgaris*, *Spirulina platensis* and *Nannochloropsis gaditana* in cultures mixed with different concentrations of desalination concentrate [36], *iv*) growth of *Spirulina platensis* in cultures with the addition of residues from the ultra and nanofiltration of whey protein [37], and *v*) growth of *Arthrospira maxima* OF15 using sugarcane vinasse for potential biological new peptides production [38]. These studies have been carried out from the analysis of laboratory-based and pilot-scale cultures, and most of the research are focused on evaluating the ability of microalgae to remove abundant organic and inorganic nutrients from wastewater associated with biomass production.

Harvesting is still a major hurdle in the microalgae production chain, representing for up to 30% of the total productions costs. It is believed that a two-stage process is required to concentrate microalgal biomass, such as flocculation followed by centrifugation. In reality, some studies as shown in Table 1, suggest prior flocculation of microalgae with cationic polymers, because these polymers neutralize the negative charge of microalgae before the centrifugation step [31,39,40]. Filtration techniques are another option that is applied in microalgae separation, especially for *Spirulina* species [41].

After dewatering, the microalgae biomass can be used directly as a source of animal feed or human food. In addition, many intracellular compounds can be extracted from microalgae and several studies on the extraction of high-value compounds by a wide range of analytical techniques have been conducted in Brazil (Table 2).

**Table 1.** Microalgae cultivation developed in Brazil using different systems from closed photobioreactors to open raceway ponds.

Cultivation system	Culture volume	Microalgae	Culture medium	Harvesting and dewatering	Reference
Tubular photobioreactor*	10 m <sup>2</sup> / 12,000 L	<i>Scenedesmus</i> sp.	Biodigested swine wastewater diluted (0.025 L/L)	Tanfloc (210 mg/L) at pH 7.8	[31]
Tubular photobioreactor combined with thin-layer cascades**	15.7 L	<i>Scenedesmus obliquus</i>	Provasoli medium	Centrifugation	[32]
Plexiglass bubble column photobioreactors	30 L	<i>Chlorella vulgaris</i> and <i>Nannochloropsis oculata</i>	Wright's cryptophyte medium	Twenty-five cationic polymers tested. Tanfloc (5 mg/L) most efficient and economical option	[39]

**Cont. Table 1**

Glass fiber cylinder photobioreactor, open tank	180 L and 500 L	<i>Nannochloropsis oculata</i> , <i>Phaeodactylum tricornutum</i> and <i>Porphyridium cruentum</i>	Conway media	Continuous centrifugation at 12,000 rpm	[4]
Fiber cylinder photobioreactor	100 L	<i>Chlorella vulgaris</i> , <i>Spirulina platensis</i> , <i>Nannochloropsis gaditana</i>	Bold Basal medium, Paoletti Synthetic medium, F/2 medium	Continuous centrifugation at 4000 rpm for ~1 hour	[42]
Raceway tanks	250 L	<i>Arthrospira maxima</i> OF15	Sugarcane vinasse	Filtration with filter press	[38]
Raceway tanks	240 L	<i>Spirulina</i> sp. LEB-18	Zarrouk medium	Centrifugation (15,000 g x 15 min)	[41]
Outdoor raceway tank	2000 L	<i>Scenedesmus obliquus</i>	Bold Basal medium	Two steps: Flocculation (Flopam 10 mg/L) and centrifugation (12,000 rpm)	[40]
Open raceway ponds	4000 L	<i>Chlorella vulgaris</i>	BBM + Desalination concentrate (25%)	Two steps: flocculation (1.0 g/L CaCl <sub>2</sub> and pH = 10.5-12.0) and centrifugation (3600 rpm continuously for 3 h)	[43]
Raceway ponds	4000 L	<i>Spirulina platensis</i>	PSM + Desalination concentrate (60%)	Filtration (sieve 10 µm diameter)	[44]
Open circular tank system	5000 L	<i>Scenedesmus obliquus</i>	Bold Basal medium	Centrifuged (Sharples S16 operating at 6000 times g)	[45]

\* Patented process INPI Brazil (BR1020130263958 – Tubular photobioreactor for treatment of integrated liquid effluent and emissions)

\*\* Patented process INPI Brazil (BR102016013102 – Device for effluent treatment and biomass production through aquatic organism cultivation)

### High-value products from microalgae in Brazil

Recently Brazilian researchers have characterized intracellular compounds from microalgae (Table 2), and analyzed the proximate composition of moisture, ash, fiber, carbohydrate, protein, lipids and fatty acids in freshwater and marine microalgae species [41,42,46].

Some microalgae, including the genus *Chlorella* are capable of accumulating intracellular starch in their cell walls, in addition to the presence of structural polysaccharides [47-49]. A study conducted by researchers from the Federal University of Rio de Janeiro (UFRJ) determined neutral sugars (glucose, galactose, rhamnose, arabinose and mannose), using an alternative assay based on one-step diluted sulfuric acid hydrolysis with reduced sample size, followed by high performance anion exchange chromatography (HPAEC) [50]. This one-step method was also successfully applied to another green microalga, *i.e.* *Mychonastes homosphaera*, indicating its applicability within the Chlorophyta phylum.

Chlorophyll and carotenoids are lipid-soluble pigments found in microalgae [51,52]. For instance, three carotenoid esters in *Aphanotece microscopica* and *Phormidium autumnale* were studied by the Federal University of Santa Maria (UFSM) researchers [53]. The authors emphasized that esterified carotenoids are more stable and possess higher antioxidant and bioavailability than non-esterified carotenoids, enabling to be incorporated in new food formulae, including functional foods. Feller et al. [4] from the UFSC evaluated the total carotenoids and antioxidant activity using DPPH free radical scavengers of three marine microalgal (*Nannochloropsis oculata*, *Phaeodactylum tricornutum* and *Porphyridium cruentum*) extracts after utilization of supercritical CO<sub>2</sub> and subcritical *n*-butane apparatus extraction. Results indicated that *Porphyridium cruentum* extract by subcritical *n*-butane technique had potent scavenger activity mainly due to the

antioxidant compounds, *i.e.*, tocopherols, carotenoids, phycoerythrin and sulfated polysaccharides, found in this microalgae species.

Special studies have been carried out by researchers from the Federal University of Rio Grande (FURG) and the University of Passo Fundo (UPF) on several compounds from *Spirulina* sp. LEB-18. For example, they investigated *i)* the techno-functional and physicochemical properties of protein concentrate as food enrichment [54]; *ii)* the purification of C-phycoerythrin pigment by ultrafiltration for food, pharmaceutical and medical applications [55]; and *iii)* the extraction of polyhydroxyalkanoates (PHBs), to be used as polymer substitutes in water-based paint formulations. The blue colour of phycoerythrin pigment makes it suitable as paint coloration while the phenolic compounds have antimicrobial properties due to their biocide effect [56].

Squalene and phytoosterols are a class of secondary metabolites that can be obtained from microalgae and have applications in medicine, food and the cosmetic industry [8,9]. Within this context, researchers from the UFSM characterized the compounds squalene and sterols (stigmasterol, cholesterol and  $\beta$ -sitosterol) in *Phormidium autumnale*, which was cultivated in a heterotrophic system after analysis of the biosynthesis pathways through lipidomics technique [8]. Squalene is traditionally extracted from the shark liver oil, but the biotechnological route of squalene production from microalgae is a way forward to avoid this resource that conflicts with marine wildlife preservation [57].

In summary, researchers from Brazilian institutions have advanced knowledge and expertise in extracting high-value products from a diverse microalgae species, using a wide range of analytical methodologies (Table 2). These above examples highlighted the exceptional qualities of microalgae to synthesize high-end products with potential market to be explored in Brazil.

**Table 2.** Chemical compounds and high-value products extracted from microalgae based on studies conducted by Brazilian institutions.

Metabolite analyzed	Main methodologies applied	Microalgae	Reference
Moisture, mineral, dietary fiber, protein, carbohydrate, lipid, fatty acids	Drying and heating samples, enzymatic hydrolysis, Kjeldahl method, Soxhlet, gas chromatography	<i>Chlorella vulgaris</i> , <i>Spirulina platensis</i> , <i>Nannochloropsis gaditana</i> , <i>Nannochloropsis oculata</i> , <i>Phaeodactylum tricornutum</i> , <i>Porphyridium cruentum</i>	[42]
Elemental composition, protein, amino acids, sugars, lipids, fatty acids, carotenoids	Bligh and Dyer method, Kjeldahl method, thermogravimetric analysis, HPLC, GC, infrared spectrometry, total organic carbon analysis	<i>Chlorella</i> sp.	[46]
Minerals determination	Inductively coupled plasma optical emission spectrometry (ICP-OES)	Marine microalgae (36 algal species)	[58]
Lipids, fatty acids, total carotenoids	Supercritical CO <sub>2</sub> , subcritical <i>n</i> -butane, UV-Vis spectrophotometer	<i>Nannochloropsis oculata</i> , <i>Phaeodactylum tricornutum</i> , <i>Porphyridium cruentum</i>	[4]
Lipids, fatty acids	Soxhlet (hexane, ethanol, chloroform:methanol), compressed fluids (propane), supercritical (CO <sub>2</sub> , ethanol, hexane)	<i>Choricystis minor</i> var. <i>minor</i>	[59]
Lipids, fatty acids	Soxhlet (hexane), compressed fluids (propane), supercritical CO <sub>2</sub> + EtOH	<i>Muriella decolor</i>	[60]
Carotenoids (violaxanthin, zeaxanthin, $\beta$ -carotene)	High performance liquid chromatography (HPLC)	<i>Aphanothece microscopica</i> , <i>Phormidium autumnale</i>	[53]
Protein, chlorophyll, lipids, amino acids, organic acids	Kjeldahl method, UV-Vis spectrophotometer, Soxhlet, HPLC, GC	<i>Scenedesmus obliquus</i> , CPC05	[61]



Cont. Table 2

Polyhydroxyalkanoates (PHBs)	Six different PHBs methods studied, property analysis (fourier transform infrared spectroscopy, molecular mass, X-ray diffraction, monomeric composition)	<i>Spirulina</i> sp. LEB-18	[62]
Protein (techno-functional and physicochemical properties)	Protein solubility, water absorption capacity, foaming and emulsifying properties, rheological behaviour	<i>Spirulina</i> sp. LEB-18	[54]
Protein, lipids, peptides (nanoemulsions)	Enzymatic hydrolysis, degree protein hydrolysis, nanoemulsions preparation (ultra turrax), stability of nanoemulsions, DPPH assay	<i>Spirulina</i> sp. LEB-18	[63]
Proximate composition, nutritional content, physical properties (snack enriched with <i>Spirulina</i> )	Extrusion, flavoring, <i>in vitro</i> protein digestibility, expansion index, bulk density, hardness, water absorption index and water solubility index, color analysis, scanning electron microscopy (SEM), microbiological, sensory and stability analyses	<i>Spirulina</i> sp. LEB-18	[52]
Phenolic compounds, encapsulation (liposome), antifungal activity	HPLC, multilamellar large vesicles liposome preparation, fourier transform infrared spectroscopy, nuclear magnetic resonance, differential scanning calorimetry	<i>Spirulina</i> sp. LEB-18	[64]
Poly(3-hydroxybutyrate), C-phycoerythrin, phenolic compounds (latex paint formulations)	Paint characterization (density, solid content, pigment and hiding power, abrasion resistance and adhesion, drying time, resistance of paints to fungal growth, reflectance and emissivity)	<i>Spirulina</i> sp. LEB-18	[56]
Phycocyanin (food grade)	Freeze-thaw extraction, microfiltration and ultrafiltration purification	<i>Arthrospira platensis</i>	[55]
Squalene, sterols (cholesterol, $\beta$ -sitosterol, stigmasterol)	Sterol extraction by direct saponification, gas chromatography identification	<i>Phormidium autumnale</i>	[8]
Fatty acids, free amino acids, organic compounds (succinic, malic and citric acids)	Bligh and Dyer method, gas chromatography identification	<i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> , <i>Phormidium</i>	[65]
Sugars and carbohydrates (arabinose, rhamnose, galactose, glucose, xylose, mannose, cellobiose)	One-step dilute sulphuric acid hydrolysis followed by high performance anion exchange chromatography (HPAEC)	<i>Chlorella sorokiniana</i> , <i>Mychonastes homosphaera</i>	[50]

### Biofuels from microalgae in Brazil

Due to the increasing interest in renewable energy, microalgae biomass is in the spotlight as a new source of biofuels such as biodiesel, bioethanol, biomethane and biohydrogen production (Table 3) [48,66,67]. Studies by researchers from the UFRJ, in partnership with Petrobras (Brazilian Petroleum Corporation), have particularly focused on this renewable route in the field of algal biofuels [68,69]. For example, Viêgas et al. [70] studied the synthesis of biodiesel and fuel properties from lipid-rich *Chlorella* species after *in situ* transesterification of biomass with later biodiesel deoxygenation to increase the oxidative stability of algal biodiesel.

Researchers from the Federal University of Bahia (UFBA) conducted a multivariate analysis of the potential of *Botryococcus braunii* for the biorefinery. It is one of the most scrutinized microalgal lipid producers and is rich in monounsaturated fatty acids. The data indicated that the biodiesel properties from this microalgae are in accordance with international quality standards (EU, USA and Brazil) [27].

A partnership of researchers from the Federal University of Paraíba (UFPB) and the Federal University of Goiás (UFG) have studied the influence of chemical microelements (e.g., Zn, V, Tl, Ti, Sr, Sn, Pb, P, Ni, Na, Mo, Mn, Mg, Li, Fe, Cu, Cr, Co, Cd, Ca, Bi, Be, Ba, B, Al) in twenty-six marine microalgae species, for potential biodiesel production [58]. Such information is essential as these microelements can negatively affect biodiesel properties, such as storage durability, corrosion and damaging of fuel container, formation of gum and clogging in the engine, ash exhaust emissions, among others.

In addition to biodiesel production, the residual biomass also contains proteins, sugars and carbohydrates that can be used towards the production of bioethanol and biomethane [71-73]. In this context, the production of bioethanol by hydrolysis of the *Spirulina* sp. LEB-18 carbohydrate and corn starch, with subsequent alcoholic fermentation and distillation, was studied [71]. In a next step, the wastes of the enzymatic saccharification and alcoholic fermentation of *Spirulina* sp. LEB-18 residues were successfully utilized to produce biomethane through anaerobic digestion in a pilot-plant located at Embrapa Swine and Poultry (Concórdia, Santa Catarina, Brazil) [73]. This collaborative process represents a promising alternative to utilize the entire fraction of the microalgal biomass for generation of two combined biofuels products.

Biohydrogen production from microalgae has been demonstrated only at the laboratory scale, as highlighted by Federal University of Paraná (UFPR) researchers [74,75]. Their recent study evaluated biohydrogen production from *Scenedesmus obliquus* microalgae, cultivated in a 11 L airlift photobioreactor fed with hazardous diesel engine emissions [74]. Vargas et al. [75] proposed a mathematical model for hydrogen production in genetically modified *Chlamydomonas reinhardtii*, considered a model organism, and suggested that genetic improvements of the chloroplast H<sub>2</sub> evolution activity would result in higher H<sub>2</sub> production.

**Table 3.** Biofuels production from microalgae based on studies conducted by Brazilian researcher's institutions.

Type of biofuel	Methodology	Microalgae	Reference
Biodiesel	Lipid and fatty acid quantification, biodiesel calculated using empirical equations (multivariate analysis)	<i>Botryococcus braunii</i> (IBL-C116)	[27]
Biodiesel	Lipid and fatty acid determination, biodiesel predicated based on empirical equations	Twelve freshwater algal species	[28]
Biodiesel	Lipid and fatty acid determination, cell disruption by non-thermal plasma, biodiesel estimations	<i>Nannochloropsis gaditana</i> (BMAK 130)	[76]
Biodiesel	Synthesis via <i>in situ</i> transesterification of algal oil through catalytic deoxygenation	<i>Chlorella</i> sp.	[70]
Bioethanol	Simultaneous saccharification and fermentation of algal biomass + corn starch	<i>Spirulina</i> sp. LEB-18	[71]
Biomethane	Fermentation assays through anaerobic degradation of algal biomass harvested from swine digestate treatment	<i>Scenedesmus</i> spp.	[72]
Bioethanol and Biomethane	Saccharification of algal biomass, alcoholic fermentation, methane production through anaerobic digestion	<i>Spirulina platensis</i>	[73]
Biohydrogen	Biophotolysis hydrogen generation analyzed through numerical simulations	<i>Scenedesmus obliquus</i>	[74]
Biohydrogen	Modelling hydrogen production via genetic modification	<i>Chlamydomonas reinhardtii</i>	[75]

### Studies on microalgae strain development in Brazil

With growing interest in green microalgae, especially those microorganisms isolated from the natural environment, for biotechnological applications, the identification of green microalgae (phylum Chlorophyta) can be a difficult task and often requires careful microscopic examination of live cultured cells by a trained specialist. To advance the use of native strains from the local environment, fifty-one freshwater green



microalgae isolated from natural freshwater bodies within the Amazon rainforest, the Cerrado savanna and the Pantanal flooded grasslands, were analyzed by DNA barcoding to identify corresponding species and deposited in the Collection of Microorganisms and Microalgae Applied to Agroenergy and Biorefineries at Embrapa (Brasília, Distrito Federal, Brazil) [77]. To establish a microalgae culture collection for the long-term, protocols for cryopreservation were optimized and validated for fifteen chlorophyte microalgae, based on intrinsic biological factors (*i.e.*, cell morphology and phylogenetic origin) and cryoprotectant agent type [78].

Another method to identify microalgae utilizes the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique. It was used by Federal University of São Carlos (UFSCar) researchers to differentiate freshwater coccoid green microalgae of the family *Selenastraceae* at the species and strain levels [79]. This robust technique discriminates green microalgae species through chemotaxonomy analysis after revealing a mass range from 0.4 to 2.0 kDa, making it a suitable and alternative method for application in algal taxonomic studies.

Some efforts have been channeled towards developing computational support for taxonomical classification of green microalgae species, especially in the *Selenastraceae* family [80]. In this context, Borges et al. [81] described a specialized methodology for segmenting 2D microscopy digital images of freshwater green microalgae. According to the authors, microalgae cell structures, such as mucilage and concavities are fundamental to recognize specific algae species, and these structures were segmented after incorporating specific smoothing and contrast enhancement steps based on descriptions of Gaussian distributions. Their proposed method for taxonomical classification achieved high segmentation accuracy when compared with ground-truth segmentations provided by an expert biologist.

The extensive biological diversity of algae can be leveraged to produce a wealthy of valuable bioproducts via genetic manipulation. Emerging technologies such as synthetic biology, molecular engineering, high-throughput phenomics and the application of internet of things (IoT) to algal technology can advance the understanding of algal biology at molecular level [82]. Implementation of high-tech engineering and molecular genetics approaches has effectively improved the productivity, cost-effectiveness and environmental impact of agricultural crops such as soy, corn, wheat and rice [83], which can be also applied to algal research [84]. *Nannochloropsis* species, for example, have been shown to outperform other industrial production candidates such as *Chlorella* or *Tetraselmis* species in terms of lipid productivity [85]. Researchers from the Synthetic Genomics Inc. (La Jolla, California, USA) have engineered a lipid-rich *Nannochloropsis gaditana* strain through CRISPR-Cas9 reverse-genetic modification that enabled to insert 18 transcriptional mutagenesis factors in different metabolic pathways, resulting in overproducing synthesis of triacylglycerols (TAG) [86]. In Australia, researchers investigated via genetic modification of marine diatom *Phaeodactylum tricorutum*, the extrachromosomal expression for the heterologous biosynthesis of monoterpenoid (geraniol), which is used as feedstock chemicals (fragrances, flavourings and cleaning agents) for a variety of polymers [87]. In Brazil, however, few researchers and institutions are working in this field and much effort and investments in research, training and education in algal molecular biology are needed to push forward the development of genetic manipulation of microalgae in Brazilian laboratories.

## Prospects of algal production in Brazil

Brazil has approximately 12% of the world's freshwater supply, large tropical coastal area (10,959 km in total) and receives an average insolation level of 8-22 MJ/m<sup>2</sup>/day. According to Brasil, Silva and Siqueira [66], there are over 40 laboratories and institutions in Brazil where algae cultures (microalgae, macroalgae and cyanobacteria) are kept, and about 3496 algae catalogued species, which makes it an excellent country to explore the biotechnological potential of both marine and freshwater microalgae species [88]. In addition, the northeast region of Brazil, known for its semiarid climate, ample solar irradiance, low seasonal variation, extensive arid lands and massive brackish groundwater has exceptional qualities to produce algae in large scale [89,90]. To date, however, few Brazilian companies are working in this field, for instance, two specific startups such as Séston Biotecnologia (Florianópolis, Santa Catarina) and Algae Biotecnologia (Holambra, São Paulo) have been cultivating microalgae in open raceways ponds, while the Brazilian company – Olson Nutrição, located in Camaquã (Rio Grande do Sul, Brazil) has been producing and manufacturing a native *Arthrospira platensis* species cultivated in greenhouse-covered raceway ponds with biomass production estimated to be around 400 Kg/dry biomass in the year of 2020. In 2014, Bunge's global food oil sales started marketing AlgaWise ultra omega-9 fatty acid algae oil [91]. Manufacturing is taking place in a modest industrial plant with capacity to produce 100 Mt of renewable oils per year. The industrial operation is located in São Paulo, Brazil that is adjacent to Bunge's Moema sugarcane mill [92]. The process utilizes sugarcane

juice as carbon source for genetically modified microalgae cultivated in a closed heterotrophic system to produce oil for renewable chemicals (*i.e.*, lubricants and cosmetics) [66].

An option to expand the cultivation of microalgae in Brazil is the integration of microalgae production to the sugarcane-ethanol industry [93,94]. Brazil is the leader producer of ethanol from sugarcane [95], and there are currently 400 sugarcane-ethanol production facilities in Brazil, mostly concentrated in Northeastern and Southeastern regions of the country [96]. Microalgae production and processing can be feasible coupled to sugarcane biorefineries as exemplified by Brasil et al. [66]. The same authors mentioned that renewable oils for industrial use and personal care can be produced from starch-rich microalgae grown on sucrose, vinasse and CO<sub>2</sub>, which are sub-products of ethanol industry [97]. Furthermore, these microalgae strains can be genetically modified to synthesize recombinant proteins such as cellulosic enzymes [6,98].

The Petrobras company has been also making investments on microalgae production and processing in the petrochemical sector [99,100]. Petrobras currently explores oil in off shore and on shore platforms, giving rise to significant amounts of effluents, such as produced water and CO<sub>2</sub>. Microalgae can be cultivated using both of these effluents to generate oil-rich biomass [101]. Algae extracted oil or algae biocrude, which is generated by the hydrothermal treatment can be used as feedstock for the production of bio-based chemicals, biomaterials and biofuels [66]. In 2012, the company settled in the Northeastern costal region (Rio Grande do Norte state) of Brazil, due to its favorable hot climate and sunny days year-round, to start a pre-commercial operation of a native *Nannochloropsis oculata* strain cultivated in open raceway ponds [102].

## CONCLUSION

This review elucidated the research conducted by Brazilian research institutions that have gathered a deep knowledge of microalgae biotechnology and have produced increasingly high quality research. It is noted that the researchers in Brazilian institutions are working in partnership and are bridging the gaps in microalgae science through interdisciplinary communications.

Several Brazilian institutions are developing projects on microalgae cultivation either in photobioreactors (tubular, cylinder, tanks) or raceway (open ponds) using an ample variety of microalgae species, including marine (*e.g.*, *Nannochloropsis oculata*, *Phaeodactylum tricornutum*, *Porphyridium cruentum*), freshwater (*e.g.*, *Chlorella sp.*, *Scenedemus sp.*, *Chlamydomonas reinhardtii*, *Botryococcus braunii*) and cyanobacteria (*e.g.*, *Spirulina platensis*, *Phormidium autumnale*, *Aphanothece microscopica*). In addition, researches are also exploring and evaluating the bioremediation of wastewater through microalgae cultivation. Some efforts have been done on determination of chemical composition and extraction of high-value products (*e.g.*, phenolic compounds, polyhydroxyalkanoates, phycocyanin, carotenoids,  $\omega$ -3 fatty acids, amino acids, sterols, squalene, peptides) from these microalgae, using a wide range of analytical techniques. Other researchers are interested in investigating the biofuel production from microalgae such as biodiesel, bioethanol, biomethane and biohydrogen, and few works are focused on strain development and genetic engineering of microalgae.

With the recently growing number of research groups focusing on different trends within algal biotechnology across Brazil, a next step would be creating a forum between academic and industrial sectors and establish a platform for scientific discussion and network communication, encouraging new collaborations across all disciplines of algal research. Such as forum may simulate the connection between fundamental algal research and industrial applications and facilitate the transfer of academic results to commercial engagement.

**Acknowledgments:** the author would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for funding Prof. Dr. Ernani S. Sant'Anna to the project 'Microalgae: an alternative source for bioproducts and biodiesel production in the semiarid region of Brazil' under contract number 574.716/2008-4.

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