

MICROALGAE AS RAW MATERIAL FOR BIODIESEL PRODUCTION: PERSPECTIVES AND CHALLENGES OF THE THIRD GENERATION CHAIN

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ABSTRACT

Currently, concern about the burning of fossil fuels and the consequences for the planet has increased and become the agenda of discussions at global levels. In this sense, biofuels are an important alternative, and this article seeks to review the literature on the use of microalgae as raw material for the manufacture of biodiesel, the transesterification process as a conversion method, the technologies used to optimize this process, the characteristics of biodiesel that are required by Brazilian legislation, and the challenges for production. Brazil has a very large potential for the production of these biofuels but their production on a large scale still requires further studies so that it can be part of the country's energy matrix, including in the agro-industrial sector.

INTRODUCTION

Currently, the impacts caused by greenhouse gas (GHG) emissions are the subject of a broad discussion. Combating climate change has become one of humanity's greatest challenges, demanding global efforts. The signing of the Paris Agreement, in 2015, where the signatory countries committed to establishing and meeting targets to limit the increase in the temperature of the planet to 1.5 °C relative to the pre-industrial period is one of the most recent efforts. In 2021, the goals and objectives established in the Paris Agreement were discussed in COP26, with the reduction of fossil fuel consumption being one of the most discussed topics (Allam et al., 2022).

The burning of fossil fuels represents the main source of CO₂ emissions (Mondal et al., 2017). According to the International Energy Agency (IEA, 2021), about 1.5 Gt of CO₂ was emitted into the atmosphere in 2020 alone just by burning fuels (gas, oil, and coal). A change in the power generation sector needs to occur to allow a decrease in GHG emissions. Thus, biofuels are a valuable alternative to the use of fossil fuels.

Biofuels are produced from renewable sources (Malode et al., 2021). They are classified into generations according to the used raw material: edible and non-edible oils, biomass, lignocellulose, and residues (Athar & Zaidi, 2020; Chhandama et al., 2021; Ganesan et al., 2020).

First-generation (1G) biofuels are those that use edible oils as raw materials, such as sugarcane, soybean, and coconut (Couto et al., 2020). It is an already established technology, mainly in Brazil with sugarcane-based ethanol production, but the main discussion is the competition for arable areas with food production (de Mendonça et al., 2021). The increase in the world population has led to the need for greater food production and, therefore, this issue becomes a disadvantage of this generation. In addition, there is also the issue of environmental degradation due to the deforestation of native forests for the planting of oleaginous plants (Correa et al., 2017).

Second-generation (2G) biofuels are produced from non-edible oils or residues. However, the cultivation of crops producing non-edible lipids also requires arable lands, as required for 1G biofuels (Ullah et al., 2015). However, there is still the possibility of using residues as

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raw materials, such as used cooking oil or beef tallow, which are attractive due to their low cost (Bhuiya et al., 2016). As a disadvantage, Ganguly et al. (2021) pointed out that the conversion of these raw materials is usually less efficient compared to 1G in terms of energy and costs.

Microalgae are used as raw material for third-generation (3G) biofuels. Different biofuels can originate from microalgae, such as bio-oils, bioethanol, biodiesel, and biogas/biomethane (Chowdhury & Loganathan, 2019; de Mendonça et al., 2021). The cultivation of microalgae does not require arable lands or drinking water, as it can be grown in wastewater. In addition, microalgae have a high doubling time and a shorter cultivation time than higher plants, thus developing faster and providing higher productivity per area (58,700 to 136,900 L ha⁻¹ year⁻¹) with relevant rates of CO₂ bio-fixation of up to 1,051 mg L⁻¹ day⁻¹ (de Souza et al., 2021; de Mendonça et al., 2021). The main problem associated with these fuels is the high production costs, especially during the conversion into biofuel (Behera et al., 2020).

Considering the need for alternatives for the generation of cleaner energy and considering the high costs associated with the production of 3G biofuels, this study seeks to review alternatives found by researchers to optimize the conversion process.

Raw materials

As discussed in the previous item, many raw materials can be used in biofuel production. The sources of biofuel generation can be divided into categories: edible oils (e.g., corn, sunflower, and soybean), non-edible oils (e.g., castor, rice bran, and palm), microalgae (e.g., *Spirulina* sp., *Chlorella* sp., and other species), animal fats (e.g., beef tallow and fish oil), and residues (e.g., used cooking oil) (Athar & Zaidi, 2020).

The Brazilian territory has a variety of climate conditions that allow the cultivation of different raw materials. Palm, coconut, babassu, sunflower, castor, peanut, soybean, and cotton oils are some of the crops found in Brazil listed by Bergmann et al. (2013). However, residues can also be used for biofuel production. Lourenço et al. (2021) used rice bran to produce biodiesel. According to the authors, the biodiesel obtained complied with the specifications of the American, European, and Brazilian standards for the iodine index (IO), acidity index, saponification index, and moisture. Vieira et al., 2021) used a mixture of used soybean oil and castor oil to produce biodiesel and observed a positive influence on the oxidative stability of biodiesel, represented by the IO reduction.

Sugarcane is the main raw material used in Brazil for bioethanol production and Brazil is the world's second-largest bioethanol producer, second only to the United States (FAO, 2021). Ethanol can be used in two ways: as anhydrous ethanol (used as a gasoline blending component)

and hydrated ethanol, as commercialized fuel (ANP, 2020). However, sugarcane presents the problem of 1G biofuels, that is, competition for arable lands with food production. According to Bicalho et al. (2016), deforestation is directly and indirectly related to agro-industrial activities, including the planting of raw materials for biofuel production. In this case, most of the Brazilian territory has favorable lighting and temperature conditions for microalga cultivation (dos Santos et al., 2021).

Microalgae as a raw material for biofuels

As previously mentioned, microalgae are a promising raw material for biofuel production, as they have a higher photosynthetic rate than higher plants, have a higher growth rate, and require no arable areas to be cultivated, among other advantages. In addition, microalgae have higher oil production than terrestrial plants, producing 100,000 L oil ha⁻¹ year⁻¹, while palm and soybean produce 5,366 and 446 L oil ha⁻¹ year⁻¹, respectively (Ganesan et al., 2020; Katiyar et al., 2017).

In general, microalgae accumulate about 20 to 50% of lipids, 5 to 23% of carbohydrates, and up to 52% of proteins (Ganesan et al., 2020; Yin et al., 2020; de Souza et al., 2021; Prajapati et al., 2013). Some factors may influence the accumulation of these macromolecules, such as the microalga species, nutrient availability, lighting, pH, temperature, and operation of photobioreactors (PBR).

Nutrient availability is one of the factors that most influence lipid accumulation. Nutrient deprivation, especially nitrogen, causes stress on the biomass, as it is considered a limiting factor for the growth of these organisms. Chokshi et al. (2016) found higher percentages of lipids for *Acutodesmus dimorphus* with nitrogen deprivation, which is about 23% higher than the medium without nutrient deficiency.

PBR operation can also affect lipid accumulation. They tend to have a higher percentage of lipids when operated in batches than in continuous flow. It occurs because all the nutrients available for the biomass in the batch operation are inserted into the reactor at the beginning of the operation and the nutrients become scarcer as the microalgae grow, promoting stress on the biomass. de Mendonça et al. (2018) reported results that confirm this information when comparing *Scenedesmus obliquus* growth in PBRs operated in batch and continuous flow, with values for total lipids of 29 and 13%, respectively.

Table 1 shows studies conducted with different species of microalgae and different culture media (substrate). Different cultivation conditions and species can alter the accumulation of macromolecules. For instance, Purba et al. (2022) found 58% of lipids when growing *Desmodesmus maximus* CN06 in municipal wastewater. Cardoso et al. (2021) found 15% of lipids in the cultivation of *Spirulina* sp. LEB 18 in aquaculture wastewater.

TABLE 1. Cultivation of microalgae in different substrates and percentage of accumulated macromoleculest.

Microalgae strain	Substrate	Operating conditions	Lipids	Proteins	Carbohydrates	Ref.
<i>Arthrospira platensis</i> DHR20	ACWW	HFBR, 30°C, 265 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 24 h d ⁻¹	16,3%	45%	20%	(de Souza et al., 2021)
<i>Chlorella vulgaris</i>	SDW pre-treated by activated sludge	FBR, 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 24 h d ⁻¹ , 0.42% of CO ₂	18%	40%	38%	(de Mendonça et al., 2022)
<i>Scenedesmus obliquus</i>	SDW pre-treated by activated sludge	FBR, 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 24 h d ⁻¹ , 0.42% of CO ₂	21%	35%	39%	(de Mendonça et al., 2022)
<i>C. minutissima</i> , <i>N. muscorum</i> , <i>Spirulina sp.</i>	70% DWW + 10 g L ⁻¹ of glucose	FBR, 27°C and photoperiod of 18:6 h	14.3%	NR	NR	(Chandra et al., 2021)
<i>Nannochloropsis oculata</i>	Tannery effluent pre-treated with ozone	FBR, 25 °C, 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, photoperiod of 16 h and CO ₂ added	51%	NR	NR	(Saranya e Shanthakumar, 2021)
<i>Scenedesmus sp.</i>	Effluent from olive oil production + BG11 medium	FBR, 80±10 $\mu\text{Em}^{-2} \text{s}^{-1}$, CO ₂ e 25 °C ± 3	32%	NR	43%	(Di Caprio et al., 2015)
<i>Desmodesmus maximus</i> CN06	Wastewater	FBR, 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 24 °C	58%	NR	NR	(Purba, et al., 2022)
<i>Spirulina sp</i> LEB 18	Aquaculture wastewater	PBR, 30°C, photoperiod of 12 h, 41,6 $\mu\text{mol m}^{-2} \text{s}^{-1}$	15%	62%	12%	(Cardoso et al., 2021)

ACWW: anaerobically digested cattle wastewater; SDW: synthetic dairy wastewater; DWW: dairy wastewater; HPBR: horizontal photobioreactor; FBR: photobioreactor; NR: not reported

Energy production in agroindustry using microalgae

Agroindustry generates highly polluting residues to the environment and, therefore, their treatment is imperative. These residues can be used as raw materials for energy production. Not only biofuels can be produced from microalgae. Several studies have been developed to analyze the feasibility of the co-digestion of digestate from anaerobic digestion with microalgae (Ganesh et al., 2018; Solé-Bundó et al., 2019).

Hu et al. (2021) used *Tribonema* sp. as a co-substrate for the anaerobic digestion (AD) of pig manure and observed an increase of only over 20% in CH₄ production. The authors attributed this increase to the better C:N ratio balance, which increases AD efficiency and, consequently, methane production.

In contrast, Miyawaki et al. (2021) applied biogas to PBRs as an alternative source of CO₂ for microalgae and a way of purifying biogas. The authors concluded that there was a significant removal of CO₂ from the biogas, which led to an increase in the CH₄ concentration, providing an increase in the calorific value.

Microalgae can be used in the co-digestion of various types of substrates, sludge, animal manure, food waste, agro-industrial residues, and glycerol (Solé-Bundó et al., 2019). Most studies have sought to improve the anaerobic digestion process through the C:N ratio balance to increase biogas production. Co-digestion is a viable alternative to this issue (Karray et al., 2022). Furthermore, as proved by Miyawaki et al. (2021), microalgae can be an alternative for purifying biogas, increasing its energy potential.

Biomass conversion process into biodiesel

Transesterification

The main conversion method used to obtain biodiesel is the transesterification process. The Brazilian

National Agency of Petroleum, Natural Gas and Biofuels (ANP, 2021a) defines biodiesel as a fuel of alkyl esters of long-chain carboxylic acids produced from the transesterification and/or esterification of fatty materials and fat of animal or vegetable origin. It is a reversible reaction, which occurs when the triglyceride preferentially reacts with primary alcohol to form an ester (biodiesel) and glycerol. The reaction can still occur in the presence or absence of a catalyst.

Biodiesel needs to be purified to be commercialized (ANP, 2021b), as the presence of impurities in the final product can be noted and may lead to engine problems and even increased pollution levels (Fayyazi et al., 2021). The main advantage of the transesterification process is that the produced biodiesel has similar properties to diesel (Jayakumar et al., 2021), in addition to being possible to obtain conversions that comply with legislation, such as ANP No. 45/0214 in Brazil, which requires a minimum 96.5% conversion.

Factors that influence the transesterification process

The main factors that affect the transesterification process are temperature, the presence of free fatty acids, the alcohol-to-oil ratio, moisture, reaction time, and stirring (Freedman et al., 1984; Mathew et al., 2021; Salam et al., 2016). High concentrations of free fatty acids result in lower conversions because these acids react to form soap (saponification). Esterification can be performed to avoid this problem by converting free fatty acids into biodiesel (Athar & Zaidi, 2020).

The presence of moisture is also a factor that decreases conversion to biodiesel, as soap can be formed. Sathish et al. (2014) studied the effect of moisture on biodiesel conversion in the cultivation of *Chlorella* and *Scenedesmus* sp. in municipal wastewater. The authors observed that a moisture of 15–20% led to a decrease of 30–50% in the conversion to biodiesel when compared to

the most favorable situation.

The temperature should not exceed the boiling point of the alcohol used in the reaction, thus avoiding the loss of reagent (Koh & Mohd Ghazi, 2011). The reaction time must be such that all the oil reacts and forms biodiesel. The oil will remain raw if the reaction time is too short. On the other hand, the degradation of the final product may occur if the reaction time is longer than ideal (Mathew et al., 2021). Behera et al. (2020) observed the effect of reaction time on biodiesel conversion using 5% (m/m) biochar based on peanut shell as a catalyst, the methanol-to-oil ratio of 20:1, and a temperature of 65 °C. The reaction times varied at 2, 4, 6, and 8 h, and the highest conversion 94.91% was observed at 4 h, with the conversion decreasing after this time. The authors attributed this fact to the formation of mono- and diglycerides.

The methanol-to-oil ratio plays an important role in biodiesel production, as increasing this ratio causes the reaction equilibrium to shift to the right, increasing biodiesel production (Behera et al., 2020). However, the high amount of alcohol may mean greater difficulty in recovering glycerol (Ganesan et al., 2020) and its presence can affect the balance of the reaction, shifting it to the left, thus decreasing biodiesel production (Mathew et al., 2021). Methanol is the main alcohol used in the reaction, but it has high toxicity, unlike ethanol, which is an alternative to methanol. Furthermore, ethanol can be produced from a renewable source, which would make this process more sustainable, despite being more expensive and less reactive than methanol (Musa, 2016).

The use of catalysts is one of the factors that can optimize the transesterification process. The catalyst concentration may vary according to its type and the origin of the oil to be transesterified. Their use is discussed in the next section.

Catalysts

The transesterification reactions demand large amounts of reagents, usually methanol and ethanol, in addition to requiring a longer operating time. Therefore, the use of catalysts may be a solution to increase their speed. Some catalysts have problems in the presence of free fatty acids and hence, the conversion process can be carried out in two steps: esterification followed by transesterification (Athar & Zaidi, 2020). Basically, two types of catalysts are used: homogeneous, divided into

alkaline and acid, and heterogeneous, with alkaline, acid, and enzymatic catalysts.

Homogeneous catalysts present high conversions, but they cannot be reused. In addition, saponification occurs in the presence of free fatty acids, generating residues and increasing the cost of the process (Helmi et al., 2022; Maheswari et al., 2022). According to Lôbo et al. (2009), homogeneous alkaline catalysts have high conversion efficiency, with NaOH and KOH being the most used due to their low cost.

Sulfuric acid is the most used acid catalyst (Gebremariam & Marchetti, 2018). The main disadvantages of acid catalysis are the large amounts of reagent required, low catalytic activity, low reaction time, and high temperatures (Athar & Zaidi, 2020).

The use of heterogeneous catalysts can overcome the problems that homogeneous catalysts present, as they can be reused, be sustainable, and have a lower production cost (Changmai et al., 2020). According to Chhandama et al. (2021), the most used heterogeneous catalysts are metal oxides, mixed metal oxides, and zeolites. One of the main advantages of using a heterogeneous catalyst is the ease of separating the biodiesel at the end of the reaction, allowing its reuse.

Ahmad et al. (2020) used eggshell-based CaO as a heterogeneous catalyst for the conversion of *Chlorella pyrenoidosa* (NCIM-2738) oil and obtained conversion from 93.44 to 2.06% m/m of the catalyst, reusing the catalyst for six cycles. Singh et al. (2020) used β - Sr_2SiO_4 as a catalyst in the conversion of *Spirulina platensis* oil and found 97.88% conversion, with the reuse of the catalyst for six cycles.

Several studies have been developed in recent years to find efficient and sustainable catalysts. Table 2 shows a compilation of biodiesel production studies from microalgae, the used catalysts, and the found conversions.

The studies presented in Table 2 show the different catalysts used to obtain third-generation biodiesel. For example, Farrokheh et al. (2020) used $\text{KF/KOH-Fe}_3\text{O}_4$ as a magnetic nanocatalyst for the conversion of *Chlorella vulgaris* oil into biodiesel, obtaining a 96.8% conversion.

Most of the studies shown in Table 2 used heterogeneous catalysts in their conversion processes, except for the studies presented by Azcan & Yilmaz (2014), Jazie et al. (2020), and Kwon & Yeom (2015).

TABLE 2. Produção de biodiesel a partir de microalgas com utilização de catalisadores.

Microalgae strain	Conversion process	Reagent	Catalyst	Conditions ¹	Biodiesel production (%)	Ref.
<i>Euglena sanguinea</i>	Esterification and transesterification	Methanol	H ₂ SO ₄ (E) e White mussel shell (CaO) (T)	70 °C 80 min	98,6	(Kings et al., 2017)
<i>Chlorella vulgaris</i>	Electrolysis	Methanol	KF/KOH-Fe ₃ O ₄	25 °C 120 min	96,8	(Farrokheh et al., 2020)
<i>Chlorella protothecoides</i>	Microwave assisted transesterification	Methanol	NaOH	65 °C 5 min	96,82	(Azcan e Yilmaz, 2014)
<i>Chlorella protothecoides</i>	Transesterification	Methanol	KOH/Al ₂ O ₃	65 °C 35 min	97,79	
<i>Chlorella pyrenoidosa</i>	Microwave assisted transesterification	Methanol	Graphene oxide	90 °C 40 min	95,1	(Cheng et al., 2017)
<i>Nannochloropsis</i> sp. KMMCC 290	Transesterification	Methanol	H ₂ SO ₄ + CHCl ₃	70 °C 90 min	75,3	(Kwon e Yeom, 2015)
<i>Chlorella</i> sp.	Esterification and transesterification	Methanol	DBSA ²	100 °C 30 min	99	(Jazie et al., 2020)
<i>Anabaena</i> PCC 7120	Transesterification	Methanol	Ba ₂ TiO ₄	65 °C 180 min	98,41	(Singh et al., 2019)
<i>Chlorella</i> sp., <i>Scenedesmus</i> sp., <i>Synechocystis</i> sp., <i>Spirulina</i> sp.	Transesterification	Methanol	Biochar peanut shell	65 °C 240 min	94,91	(Behera et al., 2020)

¹temperature and reaction time; ²dodecylbenzene sulfonate

Still comparing the studies in Table 2, the two highest percentages of conversion were found by Jazie et al. (2020) and Kings et al. (2017), with 99 and 98.6%, respectively. In both studies, the authors carried out the esterification and transesterification processes. As previously mentioned, the esterification process helps to reduce the amount of free fatty acids, preventing parallel reactions such as saponification. Jazie et al. (2020) performed the esterification and transesterification processes at the same time in a fluidized-bed reactor using dodecylbenzene sulfonate (DBSA) as a catalyst. According to the authors, this combination promoted promising results under optimal conditions, with a 99% conversion rate (Table 2). Kings et al. (2017) performed the conversion into biodiesel in two steps, first the esterification with sulfuric acid as a catalyst and then the transesterification with CaO as a catalyst, and obtained 98.41% conversion.

There is also the possibility of using enzymes as catalysts. They have the advantage of converting free fatty acids into biodiesel and do not present purification, washing, saponification, and neutralization problems (Athar & Zaidi, 2020; Guldhe et al., 2015). However, the high costs of this resource are still considered one of the main disadvantages of its use (Ong et al., 2021). Moreover, it can be inhibited by alcohol (Guldhe et al., 2015). Makareviciene et al. (2019) used Lipzyme TLIM lipase as a catalyst to produce biodiesel from oil accumulated by the microalgae *Ankistrodesmus* sp., grown in BG11 synthetic medium, and reported a 97.69%

conversion. Arias-Peñaranda et al. (2013) used the microalgae *Scenedesmus incrustatus* and Novozym 435 (*C. antarctica* lipase B immobilized in acrylic resin) as a catalyst for conversion into biodiesel and found a maximum conversion of 71.7 ± 0.3% after 24 hours of reaction, with a methanol-to-oil ratio of 6:1 at 50 °C and stirring at 150 rpm.

Technologies used to assist the transesterification reaction

In addition to catalysts, other technologies can be used to assist the transesterification reaction, such as the use of ultrasound, microwaves, co-solvent, and membranes.

The introduction of microwaves promotes greater stirring between molecules, generating friction and heat, facilitating greater contact between phases, and helping to reduce operating time. Azcan & Yilmaz (2014) and Cheng et al. (2017) used this technology and obtained conversions of 97.79 and 95.1%, respectively (Table 2).

The use of ultrasound favors mass transfer and increases the pressure, temperature, and surface area of the catalyst, thus accelerating the reaction time (Karmakar & Halder, 2019). Cercado et al. (2018) used ultrasound to assist in the transesterification of *Chlorella vulgaris* oil with KOH as a catalyst and reported 85% conversion.

The immiscibility of alcohol and oil affects phase mass transfer. Co-solvents can be used to mitigate it. Therefore, it must be soluble in both alcohol and oil (Athar & Zaidi, 2020). The disappearance of the two phases

means that smaller amounts of alcohol are required for the reaction to occur. However, the addition of a co-solvent makes production more expensive, as it has to be separated from the final product in the biodiesel purification step (Kumar et al., 2011).

Membranes are physical separation mechanisms, through which alcohol, esters, glycerol, and catalyst pass, while triglycerides are retained, which increases contact with alcohol and catalyst, thus increasing conversion and reducing reaction time (Athar & Zaidi, 2020).

Supercritical transesterification is another available technology. It is an alternative to the use of catalysts and has the advantages of not generating water, facilitated separation, and reduced reaction times (Qadeer et al., 2021). CO₂ is the most commonly used fluid due to its low critical point of pressure and temperature, in addition to being highly available and non-toxic (Mohiddin et al., 2021). Tobar & Núñez (2018) performed supercritical transesterification with CO₂ as a co-solvent to convert *Spirulina platensis* oil into biodiesel. The authors tested the conversion using ethanol and methanol and obtained maximum conversions of 68 and 77%, respectively.

Challenges and prospects

As previously mentioned, biodiesel production using microalgae as raw material has many advantages, such as the lack of arable lands, CO₂ sequestration, and shorter cultivation times compared to terrestrial plants, among others.

According to Correa et al. (2017), in addition to not requiring arable lands, microalga cultivation systems require smaller areas than crops commonly used in biofuel production. The authors estimated the area required for biodiesel production to meet gasoline demand in several countries. They found that the area demanded by the microalga system was smaller comparing the area needed for microalga cultivation with that of soybean, coconut (biodiesel), and sugarcane (bioethanol) in these scenarios in Brazil, being equivalent to 3% for soybean, 24.4% for sugarcane, and 20.4% for coconut. Also, in the same area, microalgae are capable of producing raw materials for different types of biofuels, such as biodiesel and bioethanol (de Mendonça et al., 2022).

Despite these advantages, the use of microalgae as raw material still presents obstacles, mainly the conversion process into biodiesel. Several technologies can be used to optimize the transesterification reaction but the 3G biodiesel production process faces problems to be implemented in full scale even with the help of these technologies.

One of the problems that must be considered in the conversion process is the need to use high amounts of alcohol in the reaction. Methanol is the most commonly used alcohol, but it raises concerns about its toxicity. The challenge is to use ethanol in such a way that it has similar efficiency to methanol. Another point that disfavors the implementation is that catalysts can make the process more expensive, in addition to demanding additional costs in the separation at the end of the conversion. Technologies have been studied to assist the conversion, but the implementation of full-scale production should be further studied to obtain a better view of the costs and gains of large-scale biodiesel production.

In a technical-economic analysis, Tredici et al. (2016) evaluated the production of *Tetraselmis suecica* in a 1-ha plant. The analysis showed that the production of 36 t of biomass would cost € 12.4 kg⁻¹. The authors observed that the cost could be reduced by half in a location with a more favorable climate (higher temperatures, as in most regions of Brazil). Furthermore, costs would decrease to € 5.1 kg⁻¹ by increasing production to an area of 100 ha. The results of this study in the demonstrative phase were promising, as they show that increasing production can reduce the cost of production.

In the specific case of Brazil, the creation of public policies to encourage the production of this biofuel, as occurred with ethanol at the time of ProÁlcool, is necessary for this sector to grow. In 2004, the federal government created the Brazilian National Biodiesel Production Program (PNPB), which sought to introduce biodiesel into the country's energy matrix. This introduction was carried out by mixing biodiesel with diesel (Brasil, 2021). However, that mixing became mandatory only in 2008 and the percentage of mixing grew from 2 to 13% from then until 2021, with an expectation of reaching 15% by 2023 (ANP, 2021b).

Similarly, the creation of RenovaBio was an initiative of the federal government after signing the Paris Agreement and seeks to encourage the production and use of biodiesel in Brazil, as well as regulate the biofuel market (Denny, 2020; Rodrigues, 2021). Grangeia et al., (2022) recommended a review of the program's policies and objectives due to the uncertainties of the market after the Covid-19 pandemic. Despite these uncertainties, the volume of biodiesel sold in Brazil in 2020 grew 11.5% compared to 2019, contrasting with the 5.97% retraction of the national fuel market (ANP, 2021b), indicating the great potential that Brazil has to grow in this sector.

CONCLUSIONS

Microalgae are undeniably a promising source for biofuel production. They have a high capacity to adapt, which implies that they can be grown in different culture media. In addition, CO₂ of industrial origin can be added into the medium to accelerate growth, which would reduce the emission of this gas into the atmosphere. Although the transesterification reaction is easily performed, obtaining conversions that meet the standards of legislation is still a challenge, mainly on a full scale. Catalysts and auxiliary technologies offer a solution to increase conversions, but the technical-economic feasibility of producing 3G biofuels still needs to be studied. The use of catalysts can reduce process time and increase production. There is also the possibility of producing energy through co-digestion, which would fit the concepts of circular economy and bioeconomy, making the agro-industrial sector more sustainable and profitable. The challenges to be overcome so that the production of third-generation biodiesel is viable are still many. Brazil is a world reference in sugarcane-based ethanol production and also has great potential for the production of third-generation biofuels.

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