

Third-Generation Biofuels: Production and Applicability

Amanda Adne Gama Moreira¹; América Ellen Teodozio Souza^{1*}; Juliana Lima Pinto¹; Luane Silva Lima¹; Lucas Santos Pereira¹, Wadson Leite Barbosa², Jársia de Melo²

¹ Universidade Senai Cimatec, ² Department of Petrochemistry, Salvador, Bahia, Brazil

*Ameria.souza@ba.estudante.senai.br

The growing demand for decarbonization and energy security has driven the search for renewable alternatives to fossil fuels. In this context, biodiesel produced from microalgae emerges as a promising solution due to its high productivity and CO₂ fixation capacity. This study analyzes the applicability of biodiesel derived from *Phaeodactylum tricornutum* and its integration into biorefineries through a qualitative and explanatory literature review. Publications from SciELO and Google Scholar were consulted, addressing microalgae cultivation, technological routes for lipid extraction and conversion, and environmental and regulatory aspects. The results indicate that microalgal biodiesel meets physicochemical parameters compatible with ASTM standards; transesterification catalyzed by NaOH proved effective in converting biomass lipids into esters, achieving high ester content and an adequate cetane number. Despite challenges related to cost and scalability, biodiesel from *P. tricornutum* represents a technically and environmentally viable alternative for diversifying the energy matrix, especially when integrated into circular economy and biorefinery strategies.

Keywords: Biofuels. Biodiesel. Microalgae. *Phaeodactylum tricornutum*.

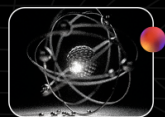
1. INTRODUCTION

With the advent of industrialization, the intensive use of fossil fuels as an energy source has driven economic and technological development. However, this process has led to a significant increase in the concentration of greenhouse gases (GHGs) in the atmosphere, particularly carbon dioxide (CO₂). These emissions have caused substantial changes in ecosystem biodiversity and have impacted future outlooks on a global scale. The growing need to decarbonize the atmosphere, aiming for the development of sustainable energy security over the years, has become a globally relevant topic. In this context, alternatives related to the petroleum sector, such as the development of biofuels, are emerging as promising strategies, offering positive results in mitigating environmental impacts and promoting economic applicability.

According to the Brazilian National Agency of Petroleum, Natural Gas and Biofuels [1], third-generation biofuels have been gaining prominence in the current energy landscape, with

microalgae-based biodiesel being one of its main representatives. Produced through the transesterification process, which uses oils extracted from the oleaginous biomass of microalgae, this biofuel plays a significant role in environmental sustainability by converting carbon dioxide (CO₂) into biomass during photosynthesis, directly contributing to the mitigation of industrial emissions and the reduction of global warming.

Although Brazil is considered a promising market for microalgae biomass, there are still challenges to be overcome in transforming scientific advances into biomass applications in industrial processes. Given the context presented, this article Therefore, some objectives are established: to explain the potential of microalgae in the production of third-generation biodiesel, to compare the physicochemical characteristics of microalgae biodiesel with conventional diesel, and to analyze the advantages and disadvantages of this biofuel in relation to fossil fuels. Thus, this



article aims to demonstrate the technical feasibility of biodiesel production from microalgae cultivation, highlighting its high lipid content and high productivity.

1.1. Biofuels

Biofuels can be classified into three generations according to the type of biomass used in their production process. First-generation biofuels are obtained from crops such as soybean, corn, wheat, sugarcane, palm, among others. Second-generation biofuels are produced from biomass sources such as cellulose, fibers, and agro-industrial residues, for example. Finally, third-generation biofuels are obtained from microalgae, a highly efficient source of biomass and lipids.

Figure 1. Classification of biodiesel production generations



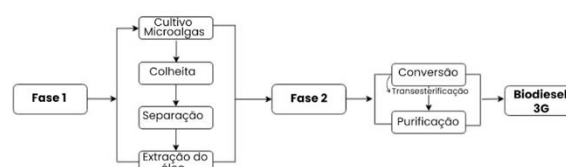
Source: Adapted from Oliveira, 2024.

1.2. Third-Generation Biodiesel Production

In the biodiesel production process from microalgae, it is noteworthy that this biomass presents a high lipid content, fast cultivation, and does not compete with agricultural land intended for food production. In addition, the application of appropriate technologies allows the production of biodiesel with properties compatible with international standards, reinforcing its potential as a sustainable and scalable alternative to fossil fuels [1].

According to the methodology used by [8], the production process of third-generation biofuels is subdivided into two sequential phases, as illustrated in Figure 2.

Figure 2. Classification of biodiesel production generations

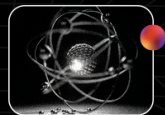


Source: Adapted from Rektenvald, 2022.

The first phase corresponds to biomass processing, encompassing everything from microalgae cultivation to oil extraction. Next, in the second phase, the extracted oil is converted into biodiesel through base-catalyzed transesterification.

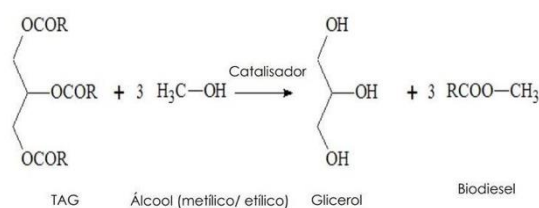
Microalgae can be cultivated through various metabolic pathways, with the photoautotrophic pathway (oxygenic photosynthesis) being the main one, where light converts CO₂ into organic compounds while releasing oxygen [6]. This cultivation can take place in open systems, such as raceway ponds, or in closed systems, such as photobioreactors, with the choice depending on the species' characteristics, costs, and available space [3].

Among the diversity of microalgae species, *Phaeodactylum tricornutum* was selected as the study subject due to its favorable characteristics for the process and the availability of literature references. This species shows good oil yield and greater ease in cultivation and pretreatment processes [3].



The stages of harvesting and processing microalgae vary according to the cultivation and the species, being essential for productive efficiency. Techniques such as centrifugation, microfiltration, and flocculation are used, influencing yield and costs [2]. Lipid extraction occurs through chemical, mechanical, or biological methods, with chemical methods, via a solid-liquid process, being the most common to transfer the oil to a solvent [9]. Finally, in phase 2 of the process, it encompasses the reaction process for conversion into biodiesel through transesterification, as shown in figure 3.

Figure 3. Biodiesel transesterification reaction



Source: Adapted from Cruz, 2022.

This stage occurs through reversible and sequential steps. Initially, the triglycerides present in oils or lipids are converted into diglycerides, which, in turn, are transformed into monoglycerides. In the final stage, the monoglycerides react with an alcohol (usually methanol or ethanol), resulting in the formation of esters, that is, biodiesel, and the generation of glycerol as a byproduct.

2. METHODOLOGY

This study adopts a qualitative and descriptive approach, aiming to analyze the processes of using microalgae biomass for the production of third-generation biofuels. The research was structured as a systematic literature review, using

scientific and academic journals as primary sources.

Data collection was conducted from February 19 to May 21 in electronic databases such as ScienceDirect, Periódicos Capes, SciELO, and Google Scholar. Key words such as "Biofuels," "Microalgae," "Biodiesel," "Transesterification," and "Phaeodactylum tricornutum" were used as selection criteria. The resulting papers yielded 10 articles, covering the following main topics: Third-generation biofuel production process (4); Applicability and challenges (3); Biodiesel transesterification process (3).

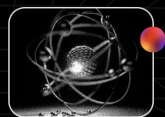
The selection of materials included studies addressing the production process of third-generation biofuels, reflecting the scientific interest in improving the efficiency and sustainability of these technologies. Next, discussions on applicability and challenges are highlighted, reinforcing the importance of evaluating the practical feasibility of large-scale implementation. Finally, studies on biodiesel transesterification demonstrate that, despite being an established technology, there is still room for innovation.

3. RESULTS AND DISCUSSION

The presentation of the obtained results will follow, accompanied by discussions aimed at a better understanding of the article.

3.1. Production Process

The production process of 3rd-generation biodiesel consists of two phases, the first being focused on the development of algal biomass, involving the stages of cultivation, biomass separation, and oil extraction. The second phase

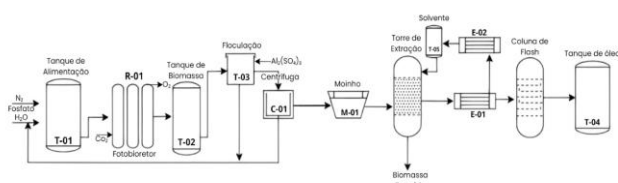


consists of the chemical conversion of the extracted biocompounds through a transesterification reaction with ethanol using basic catalysis.

3.1.1 Phase 1

Phase 1 of the process is related to the cultivation, harvesting, and oil extraction of the microalga *Phaeodactylum tricornutum*. As described in the flowchart (Figure 4), the first step refers to the production of microalgal biomass in the photobioreactor, since it allows greater control of the parameters and cultivation conditions such as pH, temperature, and light intensity.

Figure 4. Flowchart of Phase 1 of the 3rd-Generation Biodiesel Production Process



Source: Adapted from Zardo, 2011.

Preceding the production stage in the reactors (R-01), the culture medium is prepared in a tank containing saltwater, as this provides the essential ions for microalgae development. Additionally, nitrogen and phosphate sources must be added. The load from the culture tank will be continuously fed into the reactor during the daylight period. Along with this, there will be continuous injection of CO₂ at the bottom of the column (the main nutrient for microalgae growth) and the release of O₂ at the top. Finally, the generated biomass will be directed to a centrifuge to separate part of the water.

Moreover, frequent analyses of the culture medium are necessary, including measurement of

temperature, pH, nutritional composition, cell concentration, and CO₂ and O₂ levels, with the aim of making adjustments whenever necessary. The product from the reactors is directed to the tank (T-02), then forwarded to the flocculation process to separate the aqueous phase from the algal biomass. In this step, aluminum sulfate (Al₂(SO₄)₃) is used as a flocculant agent. At the end of the campaign period, flocs with higher sedimentation capacity are formed, facilitating the separation of microalgae from the liquid phase.

The biomass paste proceeds to the centrifugation step to remove part of the water. Subsequently, the biomass is directed to the oil extraction stage, which will be performed via a wet method using hexane as the solvent, as it has high efficiency in this process and does not allow solubilization of other components [10].

Before the extraction step, the microalgal biomass must undergo a pretreatment process to promote cell disruption and increase the accessibility of intracellular lipids, facilitating their subsequent extraction. Among the evaluated methods, the mechanical technique demonstrated low operational cost and lower risk of denaturation of enzymes present in plant cells. After grinding in the ball mill (M-01) is completed, the ground biomass proceeds to the solid-liquid extractor. At this stage, the hexane solvent, coming from the recycle tank (T-05), is incorporated to separate the oil from its contaminants. At the end of the extraction process, three distinct phases are formed. The bottom stream, denser, is composed of a solid



phase (defatted biomass), considered a process residue, and a supernatant phase, composed of water. The top stream, on the other hand, consists of the miscella, a mixture formed by hexane and the extracted oil.

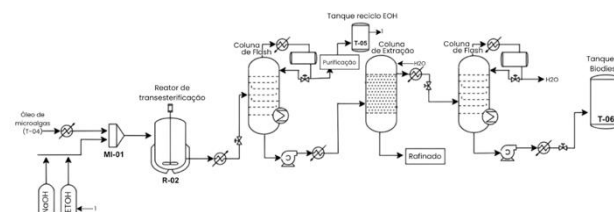
Next, this light stream is fed into the evaporator (E-01) for separation, since the solvent, being lighter, will evaporate and be recovered in the condenser (E-02), and stored in the tank (T-05). Meanwhile, the non-evaporated part contains oil and water. This mixture will be sent to a flash column to remove moisture up to 3%. Finally, the obtained product will consist of oil that will be sent to the storage tank (T-04) to be used in the transesterification process for biodiesel production.

The oil extracted from microalgae presents important environmental and productive advantages. Its photosynthetic capacity contributes to the reduction of atmospheric CO₂, while its cultivation can be done in non-arable areas, without competing with agriculture. For these reasons, microalgae stand out as a strategic and sustainable alternative in the field of bioenergy.

3.1.2 Phase 2

With the completion of Phase 1 of the production process, Phase 2 begins. This stage consists of the transesterification of the oil extracted from the microalga, reacting with a transesterifying agent, ethanol, under the action of sodium hydroxide (NaOH) as a catalyst. Phase 2 promotes the conversion of triglycerides present in the oil into fatty acid esters (biodiesel), being considered the central stage of chemical conversion.

Figure 9. Flowchart of Phase 2 of the 3rd-Generation Biodiesel Production Process

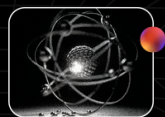


Source: Adapted from Rektenvald, 2022

To initiate the process, the feed stream from tank (T-04), composed of microalgal oil, is heated until reaching the optimal reaction temperature. Subsequently, this stream is directed to mixer M-01, where the incorporation of two other fundamental streams occurs: the first containing the process catalyst (NaOH), and the second composed of anhydrous alcohol, in this case, ethanol. After complete homogenization of the three streams in the mixer, the reactive fluid is sent to the main reactor of the process, R-02, where the transesterification reaction takes place. This reaction results in the formation of biodiesel, as well as the byproduct glycerol.

To ensure a high conversion rate, ranging from 94% to 97%, strict control of process variables is essential. One such variable is the molar ratio between oil and alcohol in the transesterification reaction, which should be 1:6. This proportion favors the shift of the reaction equilibrium toward the products, promoting the complete consumption of triglycerides present in the oil extracted from microalgae and allowing efficient separation of glycerol as a byproduct [5].

Furthermore, another critical variable for the efficiency of the transesterification reaction is the water content in the system, which significantly



affects the catalyst's action since it favors undesirable reactions such as saponification.

Upon completion of the transesterification reaction, both the desired product (biodiesel) and its byproduct (glycerol) remain contaminated with residues of catalyst, alcohol, and unreacted oil during this stage. Therefore, the reactive mixture is directed to the purification stage, encompassing ethanol recovery, wet washing, and removal of the water used as solvent in this process.

In the ethanol recovery step, the reactive stream from reactor R-02 undergoes preheating to reach the appropriate temperature for separation in the primary flash column. After separation, ethanol is withdrawn from the top of the column and then sent to a complementary purification stage. This step aims to eliminate impurities and contaminants still present in the stream, ensuring the necessary quality for its reuse in the process.

To purify the plant product, ethyl esters (biodiesel) undergo a new separation stage to remove residual compounds still present in the stream, such as residual ethanol, catalyst (NaOH), unreacted oil, and primarily glycerol. After the initial removal of ethanol, the bottom stream of the primary flash column is sent to the liquid-liquid extraction column, where water is used as a solvent to promote selective removal of these impurities.

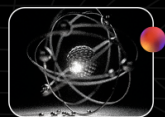
Finally, the stream called the "extract," predominantly composed of biodiesel and solvent, is directed to the removal of the used solvent, water. For this purpose, the stream is heated and subjected to pressure reduction, then

directed to the secondary flash column. After complete separation of water, the purified biodiesel is adjusted to ambient temperature and pressure conditions (25 °C and 101.3 kPa) to ensure greater accuracy in determining its composition.

3.2 Physicochemical Properties

In view of this, as a result of Phase 2 of the process, the purified biodiesel obtained from the oil of the microalga *Phaeodactylum tricornutum* exhibited physicochemical properties compatible with the requirements for application as a biofuel. Studies such as [7] demonstrate that this biomass represents a sustainable and technically viable source, presenting a high ester content (99.9% w/w) and a cetane number of 53.7, a value considered ideal for performance in diesel engines.

Furthermore, the biodiesel's performance is also evidenced by the comparison between its main physicochemical parameters and those of conventional diesel oil, based on the specifications of the American Society for Testing and Materials (ASTM). As shown in Table 2, microalgal biodiesel satisfactorily meets the limits established by the standard, standing out for its density ($0.864 \text{ kg}\cdot\text{L}^{-1}$), which falls within the normative range, and its flash point of 115 °C, contributing to greater operational safety and exceeding the minimum required by technical standards. The acidity index ($0.374 \text{ mg KOH}\cdot\text{g}^{-1}$) remains within established limits, indicating good product stability, while the



calorific value of $41 \text{ MJ} \cdot \text{kg}^{-1}$ reinforces its energetic potential.

These results confirm that microalgae-derived biodiesel exhibits properties compatible with currently used fuels, consolidating its viability as a renewable and environmentally sustainable alternative.

Table 1. Physicochemical Characteristics of Fossil Diesel and Microalgae Biodiesel

Properties	Microalgae Biodiesel	Diesel Oil	ASTM Standards
Density (kg L^{-1})	0.864	0.838	0.84 – 0.90
Viscosity ($\text{mm}^2 \text{s}^{-1}$, 40 °C)	5.2	1.9 – 4.1	3.5 – 5.0
Flash point (°C)	115	75	min 100
Freezing point (°C)	-12	-50 – 10	--
Acidity (mg KOH g^{-1})	0.374	max 0.5	max 0.5
Calorific value (J kg^{-1})	41	40 – 45	--

Source: Pereira, 2012.

3.3. Catalyzed Transesterification

Regarding the conversion of lipids extracted from *Phaeodactylum tricornutum* into biodiesel, the catalyzed transesterification route has proven effective. Studies indicate that the lipid composition of the microalga, rich in unsaturated fatty acids, favors the production of biodiesel with good properties [5]. The use of alkaline catalysts, such as sodium hydroxide, has shown good results in converting the triacylglycerols present in algal oil into methyl esters (biodiesel) and glycerol.

However, in the context of the production of the biofuel under analysis, despite showing good conversion rates, the transesterification step emerges as a key point for improving economic competitiveness [9]. The analyses conducted indicate that the production cost of microalgae biodiesel is generally higher than that of fossil diesel.

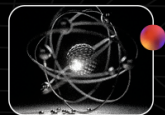
4. FINAL CONSIDERATIONS

The conducted study highlights the potential of the microalga *Phaeodactylum tricornutum*, cultivated in closed bioreactors and mechanically extracted, as a promising raw material for biodiesel production. The research reinforces that biofuels derived from microalgae are sustainable alternatives to conventional diesel. Although challenges remain, such as production costs and process efficiency, technological advances suggest a positive future for the development of this type of biofuel.

It was concluded that third-generation biodiesel, derived from microalgae, demonstrates remarkable potential to integrate into the renewable energy matrix. Its quality, when validated by technical standards such as ASTM, positions it as a sustainable and innovative technological solution in the energy sector, significantly contributing to the diversification of energy production and Brazil's transition to cleaner sources, reducing dependence on fossil fuels.

REFERENCES

- [1] ANP – National Agency of Petroleum, Natural Gas and Biofuels. Biodiesel [Internet]. 2024 [cited 2025 Jun 26]. Available from: <https://www.gov.br/anp/pt-br/assuntos/producao-e-fornecimento-de-biocombustiveis/biodies>
- [2] Cruz DCP, Oliveira P, Santos M, Almeida L, Rodrigues R. Evaluation of physicochemical properties and performance of three generations of biodiesel through transesterification: a review. Research, Society and Development [Internet]. 2022. Available from: <https://rsdjournal.org/index.php/rsd/article/view/27234>
- [3] Fortes MM. Photobioreactors for microalgae cultivation aimed at biodiesel production [thesis]. Rio de Janeiro:



Federal University of Rio de Janeiro, School of Chemistry;
2015.

[4] Lima LO, Carvalho ADP, Zuber A, Rektenvald JH.
Biodiesel production from microalgae: a technical-
financial analysis. Rev e-TECH Tecnol Compet Ind
[Internet]. 2023;16(2). Available from:
[https://etech.sc.senai.br/revista-
cientifica/article/view/1269](https://etech.sc.senai.br/revista-cientifica/article/view/1269)

[5] Mancio AA, Costa R, Ferreira L, Almeida D.
Simulation of biodiesel production and purification process
from microalgae oil [dissertation]. Belém: Federal
University of Pará; 2011 [cited 2025 Jun 26].

[6] Oliveira ACV, Souza R, Martins A, Lima G, Rocha L.
Circular Bioeconomy and biorefinery: concepts,
opportunities and challenges considering the use of
microalgal biomass in Brazil. Rev Bras Planej Desenvol
[Internet]. 2024;. Available from:
<https://revistas.utfpr.edu.br/rbpd/article/view/16978>

[7] Pereira F, Lima P, Gomes R, Andrade M. Economic
analysis of microalgae biodiesel production in bioreactors
of different configurations. Eng Custos. 2019;.

[8] Rektenvald JH. Production of biodiesel from
microalgae: a technical-financial analysis [undergraduate
thesis]. Francisco Beltrão: Federal Technological
University of Paraná; 2022. Available from:
<https://riut.utfpr.edu.br/jspui/handle/1/29543>

[9] Souza P, Martins T. Development of low-cost
bioreactors for large-scale production of microalgae for
biofuel. Inov Tecnol Energ. 2024.

[10] Zardo I. Economic feasibility analysis of biodiesel
production from microalgae [undergraduate thesis]. Porto
Alegre: Federal University of Rio Grande do Sul; 2011.
Available from: <http://hdl.handle.net/10183/38387>