



## RESEARCH ARTICLE

# Life Cycle Assessment (LCA) of Third-Generation Biodiesel Produced Heterotrophically by *Phormidium Autumnale*

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### Abstract:

#### Objectives:

The aim of this work was to perform a prospective life cycle assessment of the third-generation biodiesel (3G) produced from the heterotrophic cultivation of *Phormidium autumnale*, using sucrose as the carbon source.

#### Materials and Methods:

The study focused on the optimization of the process parameters, in the life cycle assessment and in the biofuel quality analysis in diverse microalgae-based scenarios.

#### Results:

In the best scenario, the production of microalgal biodiesel has positive energy production (50.59 MJ/kg) associated with low consumption of water (28.38 m<sup>3</sup>/kg) and low CO<sub>2</sub> emissions (9.18 kg CO<sub>2</sub>-eq/kg). In terms of composition, this oil was predominantly saturated (45.20%), monounsaturated (34.70%), and polyunsaturated (19.90%), resulting in a biodiesel that complies with U.S., European, and Brazilian standards.

#### Conclusion:

The high potential capacity for lipid production obtained is interesting for the generation of quality biodiesel that meets or surpasses the most stringent U.S., European, and Brazilian fuel standard requirements.

**Keywords:** LCA, Microalgae/cyanobacteria, Heterotrophic cultivation, Sucrose, Biodiesel (3G), *Phormidium autumnale*.

## 1. INTRODUCTION

According to the International Energy Agency (IEA), more than 80% of the world's primary energy supply derives from fossil fuels [1]. Expanding energy demand is associated with the growth of developing economies, which represents a considerable increase in the consumption of transport fuels. This is driving a shift towards biofuels. As a result of this, biofuel research and development has progressed through several stages globally and within Brazil [2]. The microalgal oil industry, though presently in its infancy, could provide future liquid transportation fuels that can improve world energy security.

In addition, microalgae-based fuels are third-generation biofuels and may offer a promising biofuel option. Their high growth rates and lipid content, after extraction, are trans-esterified to obtain biodiesel, thus turning them into one of the most promising feedstocks [3, 4]. Some microalgae can grow heterotrophically in stainless steel fermenters on organic substances (e.g., sugars, organic acids) used as the only source of carbon and energy. In the heterotrophic

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culture of microalgae, glucose is the most commonly used source of organic carbon [5]. However, the high cost of glucose, which sometimes reaches about 80% of the total cost of the medium, makes the heterotrophic cultivation of microalgae economically infeasible [6]. An alternative to lowering the cost of production by up to 40% is to replace certain sources of organic carbon with low-cost substrates, such as sucrose [7]. Therefore, it is important to know the lifecycle performance of microalgae biodiesel production systems in order to establish the environmental benefits over conventional products.

Currently, numerous research has focused on the Life Cycle Assessment (LCA) of microalgal biofuels, but little work has focused on heterotrophy. In addition, published LCA data are based on already existing data in the literature, often presenting extrapolated results that generate doubts about the production process. Every activity involved in the production of biofuels from microalgae is energy-intensive and produces greenhouse gases, besides having a high consumption of water; thus, it is, of course, essential to evaluate the energy balance, CO<sub>2</sub> emissions, and water footprint of microalgal fuels [8]. Many life cycle assessments, based on viability studies that use the net energy balance or net energy ratio as viability indicators, have already been attempted for the production of liquid fuels from microalgae, but most studies have shown very low or negative values for the energy balance [9 - 11].

Life cycle assessment is divided into three sections: energy balance, water footprint, and an analysis of greenhouse gases. Worldwide, Life Cycle Assessment (LCA) is recognized as a standardized and structured method for evaluating the environmental impacts arising throughout the entire life cycle of a product, process, or activity [12]. The biggest challenge in crafting an LCA is that it must address these impact categories during early-stage processes while ultimately accounting for the whole scope of product development. Additionally, LCA is extremely helpful in determining the energy products and co-products, which can demonstrate economic viability and performance [13].

In this sense, the aim of this work was to perform a prospective life cycle assessment of the third-generation biodiesel produced from the heterotrophic cultivation of *Phormidium autumnale*, using sucrose as the carbon source. The study focused on the optimization of the process parameters, in the life cycle assessment and in the biofuel quality analysis in diverse microalgae-based scenarios.

## 2. MATERIAL AND METHODS

### 2.1. Goal and Scope Definition

The technical framework for the LCA methodology, according to the International Organization for Standardization (ISO) 14000 series [14], consists of four phases: (1) goal and scope definition, (2) inventory analysis, (3) impact assessment, and (4) interpretation. Data were obtained from experiments in a laboratory, where the necessary requirements for the process and for the inventory were selected. Subsequently, the data were normalized for a functional unit of 1 kg biodiesel. This methodology was used to estimate the energy balance, CO<sub>2</sub> emissions, and the water footprint.

Since microalgae biofuel industry is still a recent process, data on large scale microalgae production is lacking. In the present study, laboratory observations combined with published data of known industrial processes have been used and extrapolated [9, 15, 16]. Furthermore, the calculation experimental is based on promising technologies which might be commercialized in the near future to determine the development potential.

As shown in Fig. (1), the proposed process of microalgal biodiesel production can be divided into nine sections.

### 2.2. Microalgae Biomass Cultivation and Harvesting

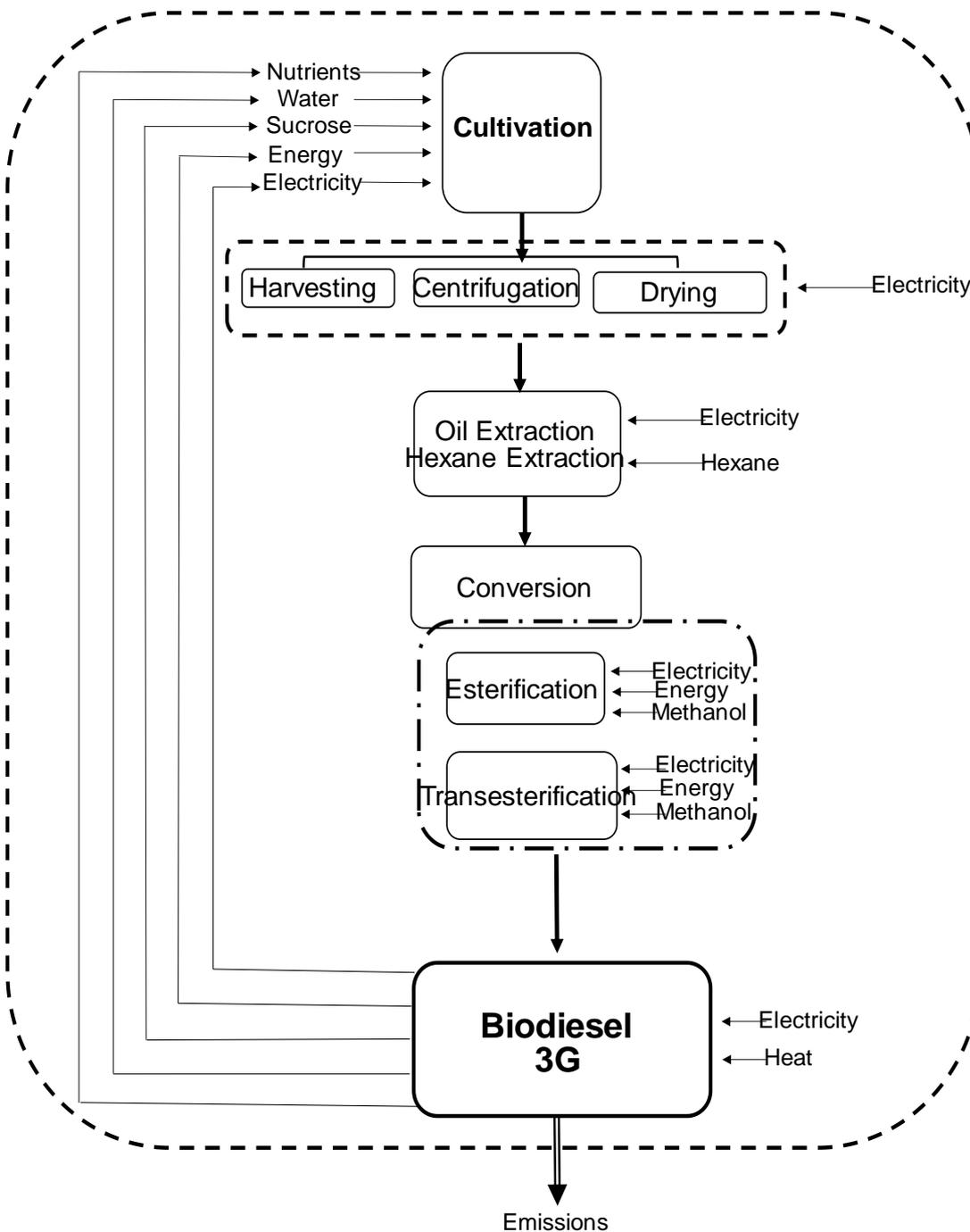
Axenic cultures of *Phormidium autumnale* were originally isolated from the Cuatro Ciénegas desert (26°59'N, 102°03'W-Mexico). Stock cultures were propagated and maintained in solidified agar-agar (20 g/L) containing synthetic BG11 medium [17]. The incubation conditions used were temperature of 25 °C, a photon flux density of 15 μmol/m<sup>2</sup>/s and a photoperiod of 12:12 h (light: dark). To obtain the inoculums in a liquid form, 1 mL of sterile synthetic medium was transferred to slants, the colonies were scraped and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

### 2.3. Process Description

Single-cell oil production was made in a bubble column bioreactor. The reactor specifications were followed according to Francisco *et al.* [18].

Experiments were performed in a bioreactor operating in batch mode, fed with 2.0 L of culture medium. The experimental conditions were as follows: Initial concentration of inoculum 100 mg/L, temperature 30°C, pH adjusted to 7.6, aeration of 1 VVM (volume of air per volume of culture per minute), and absence of light. The culture medium consisted of BG11 synthetic medium modified and supplemented with different concentrations of sucrose to obtain carbon/nitrogen ratios of 20 (C20), 30 (C30), 35 (C35), 40 (C40), 50 (50), 60 (C60), 70 (C70), and 80 (C80). The concentration of sucrose was adjusted stoichiometrically according to the methodology proposed by Francisco *et al.* [19].

The experiments were each performed twice and in duplicate. Therefore, kinetic data refer to the mean value of four repetitions.



**Fig. (1).** Flow diagram of the microalgae biodiesel process.



(Table 1) *contd....*

Stages	In/out	Utilities/Materials	Base Case	Amount (by scenarios)								
				C20	C30	C35	C40	C50	C60	C70	C80	
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	Desolventizer-Toaster-Dryer-Cooler (DTDC) (kWh)	0.35	-	-	-	-	-	-	-	-	-
-	-	Electricity (kWh)	0.13	-	-	-	-	-	-	-	-	-
-	-	Heat (MJ)	41	-	-	-	-	-	-	-	-	-
-	Output	Biodiesel (MJ)	-	22.14	45.11	44.31	50.59	43.70	23.78	15.58	13.77	

### 2.6.1. Energy Balance

The Net Energy Ratio (NER) of a system is defined as the ratio of the total energy produced (energy content of the residual biomass) over the energy required for all plant operations [10]. Energy is reported in terms of Megajoules (MJ) and is calculated according to Eq. (1):

$$NER = \frac{\sum E_{out}}{\sum E_{in}} \quad (1)$$

where  $E_{out}$  is the renewable energy output, and  $E_{in}$  is the fossil fuel energy input.

Energy Balance (EB) considered all forms of energy of the system, calculated according to Eq. (2):

$$EB = \sum \text{inputs} - \sum \text{outputs} \quad (2)$$

Output energy was quantified by multiplying the energy potential of microalgal biodiesel, expressed in MJ/kg [7].

### 2.6.2. Water Footprint

The Water Footprint (WF) was determined as the sum of the water used in the system during all stages of the process of microalgal biodiesel production, expressed in  $m^3/kg$  biodiesel.

#### 2.6.2.1. Blue Water Footprint

Blue water footprint (WF) blue refers to the amount of water incorporated in the product. This is determined by the evaporation rate, according to Eq. (3):

$$WF_{blue} = \text{Blue water evaporation} + \text{incorporation} + \text{return flow} \quad (3)$$

#### 2.6.2.2. Green Water Footprint

Green WF refers to the volume of water consumed in the production process, plus the water incorporated into the harvested crop, calculated according to Eq. (4):

$$WF_{green} = \text{Green water evaporation} + \text{incorporation} + \text{return flow} \quad (4)$$

### 2.6.3. Evaporation Rate

The evaporation rate of the process was calculated according to Eq. (5), where the loss of mass ( $m$ ) is obtained per unit time at a given temperature ( $^{\circ}C$ ):

$$ER = \frac{\Delta m}{\Delta t} \quad (5)$$

In this study, the water footprint associated with the culture process is caused by evaporation, therefore, it will not be considered the gray water footprint, since microalgae have the removal capacity of pollution load of the effluent that will be thrown in the watershed, thus, it will be considered only the green and blue water footprint.

### 2.6.4. CO<sub>2</sub> Emissions

The emission of CO<sub>2</sub> gas is calculated according to Eq. (6):

$$E = \sum_i M_i \cdot P_i \quad (6)$$

where  $M_i$  is the mass of substance  $i$  which contributes to impact  $E$ , and  $P_i$  is the characterization factor of substance  $i$ , expressed in kg CO<sub>2</sub>-eq.

## 2.7. Statistical Analyses

Analysis of variance (one-way ANOVA) and Tukey's test ( $p < 0.05$ ) were used to test differences between scenarios. The analyses were performed with Statistica 7.0 software (StatSoft, Tulsa, OK, USA).

## 3. RESULTS AND DISCUSSION

### 3.1. Energy Balance

Assessing the sustainability of a biofuel production system requires energy input analysis over the life cycle. In this sense, biomass production, oil production, and the energy balance of 3G biodiesel production from heterotrophic cultivation by *Phormidium autumnale* were examined. The results are summarized in Table 2.

**Table 2. Production of biomass and single-cell oil, and analysis of the net energy ratio (NER) for production of 1kg of biodiesel.**

Scenario	Balance Energy					
	Biomass (kg/m <sup>3</sup> )	Oil (kg/m <sup>3</sup> )	Fossil Energy Input* (MJ)	Produced Energy Output (MJ)	Energy Ratio	Energy Balance (MJ)
C20	2.7 <sup>a</sup> ± 0.03	0.54 <sup>a</sup> ± 0.01	27.21 <sup>a</sup>	22.14 <sup>a</sup> ± 0.01	0.81 <sup>a</sup> ± 0.01	5.07 <sup>a</sup> ± 0.01
C30	5.26 <sup>b</sup> ± 0.03	1.05 <sup>b</sup> ± 0.01	27.21 <sup>a</sup>	45.11 <sup>b</sup> ± 0.01	1.65 <sup>b</sup> ± 0.01	-17.90 <sup>b</sup> ± 0.01
C35	5.6 <sup>c</sup> ± 0.03	1.12 <sup>c</sup> ± 0.01	27.21 <sup>a</sup>	44.31 <sup>c</sup> ± 0.02	1.62 <sup>c</sup> ± 0.01	-17.10 <sup>c</sup> ± 0.01
C40	6.17 <sup>d</sup> ± 0.03	1.23 <sup>d</sup> ± 0.01	27.21 <sup>a</sup>	50.59 <sup>d</sup> ± 0.01	1.85 <sup>d</sup> ± 0.01	-32.38 <sup>d</sup> ± 0.01
C50	5.33 <sup>b</sup> ± 0.03	1.06 <sup>b</sup> ± 0.02	27.21 <sup>a</sup>	43.70 <sup>e</sup> ± 0.01	1.60 <sup>e</sup> ± 0.01	-16.49 <sup>e</sup> ± 0.01
C60	2.9 <sup>e</sup> ± 0.03	0.58 <sup>f</sup> ± 0.01	27.21 <sup>a</sup>	23.78 <sup>f</sup> ± 0.01	0.87 <sup>e</sup> ± 0.01	3.43 <sup>f</sup> ± 0.01
C70	1.9 <sup>f</sup> ± 0.03	0.38 <sup>g</sup> ± 0.02	27.21 <sup>a</sup>	15.58 <sup>g</sup> ± 0.02	0.57 <sup>f</sup> ± 0.01	11.63 <sup>g</sup> ± 0.01
C80	1.68 <sup>g</sup> ± 0.03	0.33 <sup>h</sup> ± 0.01	27.21 <sup>a</sup>	13.77 <sup>h</sup> ± 0.02	0.50 <sup>g</sup> ± 0.01	13.44 <sup>h</sup> ± 0.01

Within the same column, means with different superscripts are significantly different ( $p < 0.05$ ) by Tukey's test.

\*The energy input was considered the equal for all experiments.

The best performance of the process was achieved in between scenarios C30 and C50, with maximum biomass production and oil in scenario C40. In this condition, average biomass production was 6.17 kg/m<sup>3</sup>, resulting in oil production of 1.23 kg/m<sup>3</sup>. These results show that in heterotrophic metabolism, the production of intracellular oils by *Phormidium autumnale* is critically affected by the carbon/nitrogen ratio in the culture medium.

As shown in Table 2, the energy balance is a function of the oil production. The condition that presented the best performance was the scenario C40, with higher values fossil energy input (27.21 MJ), energy output (50.50 MJ), energy ratio (1.85 MJ) and energy balance (-32.38 MJ), followed by the scenarios C30, C35 and C50. These results show that primary energy contained in the biomass is greater than the primary energy input in all the system ( $NER > 1$ ), demonstrating that these processes have a great potential for energetic exploitation.

In the other scenarios (C20, C60, C70, and C80) the energy balance was unfavorable. There is a linear relationship between biomass and oil production and energy ratio. This is associated with the metabolism of microalgae; these microorganisms possess structurally specific mechanisms for the active transport of sucrose into the cell membrane. Heterotrophic microalgae have an inducible active carbohydrate symport system responsible for the uptake of these molecules from the culture medium. Induction of this transport is achieved by specific sugars. If the concentration and type of sugar are adequate in the culture, the symport system is induced to promote greater cell growth and, consequently, higher yields of biomass and oil production [23].

By comparison, soybean is one of the most-used oil crops for the production of biodiesel. According to Fore *et al.* [24], the production of biodiesel from soybean requires energy input of 4588 MJ, generating fossil energy output of

21.401 MJ. The soybean crop needs a larger area for cultivation, overcoming traditional crops such as sugarcane. The higher energy expenditure at the entrance of the system is related to the higher consumption of fertilizers [25]. According to Pradhan *et al.* [25], these values reach an energy expenditure of approximately 319 MJ/kg for the use of herbicides and 325 MJ/kg for the use of insecticides.

Compared to other biofuels of organic origin, such as soybean biodiesel and bioethanol from sugar cane, microalgae do not compete for arable land [26]. Thus, with biodiesel 3G was possible to get on the best scenarios required an energy input of 27.21 MJ to generate fossil energy output of 50.50 MJ. The value of the energy produced by microalgae is twice as large as that of the biodiesel produced by soybean.

On the other hand, the biodiesel 3G may be produced by microalgae that use sucrose as the substrate, making it a more profitable alternative than bioethanol from sugar cane. In sugarcane biorefinery, the bioethanol production requires a fossil energy input of 1 MJ per kilogram of sugar cane, which generates 0.05 MJ of output energy per kilogram of bioethanol produced. These values reached an unfavorable energy of 0.05 MJ [27]. Moreover, it was considered that the total energy yield (bioethanol and bagasse) is 2.185 MJ per kg of cane, if consider only 11% of the total energy of bioethanol production using the microalgae, it is possible to achieve 3.52 MJ per kg of the sugar-cane. This value is larger compared to the total of energy (bioethanol) produced by sugarcane [28]. The microalgae biodiesel demonstrated a high potential of energetic exploration since the final yield of the process exceeded the demand for energy in the system input, promoting the sustainability of this technological route.

### 3.2. CO<sub>2</sub> Emissions

The environmental impact of converting sucrose to oil is analyzed based on emissions over the complete life cycle. It is important to consider the CO<sub>2</sub> emission per unit of energy output from products, since the principal objective of any biodiesel system is to produce an energy carrier. Table 3 shows the CO<sub>2</sub> emissions under the eight scenarios calculated per unit of mass and energy output (MJ/kg).

**Table 3. Comparison of CO<sub>2</sub> emissions from the scenarios C20, C30, C35, C40, C50, C60, C70, and C80 calculated per unit of energy from the biodiesel.**

Scenario	Emissions of CO <sub>2</sub> (kg CO <sub>2</sub> -eq/kg biodiesel)
C20	6.58 <sup>a</sup> ± 0.01
C30	8.23 <sup>b</sup> ± 0.01
C35	27.12 <sup>c</sup> ± 0.01
C40	18.09 <sup>d</sup> ± 0.01
C50	7.71 <sup>e</sup> ± 0.01
C60	10.51 <sup>f</sup> ± 0.01
C70	10.62 <sup>e</sup> ± 0.01
C80	16.47 <sup>h</sup> ± 0.01

Within the same column, means with different superscripts are significantly different ( $p < 0.05$ ) by Tukey's test.

As shown in Table 3, CO<sub>2</sub> emissions per kg of biodiesel were the lowest in scenario C20, followed by C50. The highest emissions of CO<sub>2</sub> were found in scenario C35, followed by C60 and C80. While scenario C20 produced the fewest emissions of CO<sub>2</sub>, it also produced less energy. These results suggest that adding less sucrose to the culture requires less energy for the microalgae to convert the carbon. Thus, respiration releases less CO<sub>2</sub>, since carbohydrates in heterotrophic cultivation serve as the sole source of energy [5]. Furthermore, Posada *et al.* [29] reported that the energy demanded to supply the input of a lower emission of CO<sub>2</sub> is higher than that required to emit a higher amount of CO<sub>2</sub>. Under these conditions, then, the C40 scenario presents the best result in combining energy balance and CO<sub>2</sub> emissions. These results agree with Khoo *et al.* [30], who observed that higher emissions of CO<sub>2</sub> will produce more energy by leading to a greater final yield of microalgal biomass.

Comparatively, emissions of CO<sub>2</sub> from soybean biodiesel can reach 51 kg CO<sub>2</sub>-eq/kg, considering both CO<sub>2</sub> emissions during the extraction of soybean oil and in the transesterification to obtain biodiesel [31]. Additionally, if CO<sub>2</sub> emissions from combustion are included, such emissions are about 70 kg CO<sub>2</sub>-eq/kg and may represent about 70% of total lifecycle greenhouse gas emissions [32]. The highest CO<sub>2</sub> emissions from microalgal biodiesel, observed in scenario C35 (27.12 kg CO<sub>2</sub>-eq/kg), were still much lower than for soybean biodiesel.

On the other hand, bioethanol utilization in Brazil must consider the whole agro-industrial system. Essentially, a portion of the consumed fossil fuel is used to yield bioethanol for external use as a biofuel in addition to a proportion of the bagasse used for sugar production [26]. According to Khatiwada *et al.* [2], the CO<sub>2</sub> emissions were estimated at 19.1 kg CO<sub>2</sub>-eq/kg of bioethanol. These data were also supported by Souza and Sansone [33], who conducted an LCA of an integrated Brazilian sugarcane biorefinery with a similar framework, estimating CO<sub>2</sub> emissions between 20 kg CO<sub>2</sub>-eq/kg and 37 kg CO<sub>2</sub>-eq/kg. These values of CO<sub>2</sub> emissions are still higher than the favorable microalgal scenarios under study.

### 3.3. Water Footprint

In additional, the water footprint includes the inventories of the process water consumed, the water consumption associated with process energetic, of material inputs for each stage of the fuel cycle, and the water credits associated with the coproducts. In this sense, were calculated the green and blue water footprint, and the evaporation rate of the whole production process of biodiesel from microalgae and the results are summarized in Table 4.

**Table 4. Life cycle of the Water Footprint (WF), and the Total Evaporation (TE) of the biodiesel production from microalgae.**

Scenarios	TE	Green WF	Blue WF	Total WF
-	m <sup>3</sup> per kg of Biodiesel			
C20	0.1442 <sup>a</sup> ± 0.01	14.19 <sup>a</sup> ± 0.01	3.95×10 <sup>-5a</sup> ± 0.01	14.19 <sup>a</sup> ± 0.01
C30	0.2163 <sup>b</sup> ± 0.01	21.28 <sup>b</sup> ± 0.01	7.68×10 <sup>-5b</sup> ± 0.01	21.28 <sup>b</sup> ± 0.01
C35	0.2523 <sup>c</sup> ± 0.01	24.82 <sup>c</sup> ± 0.02	8.19×10 <sup>-5b</sup> ± 0.01	24.82 <sup>c</sup> ± 0.01
C40	0.2885 <sup>d</sup> ± 0.01	28.38 <sup>d</sup> ± 0.01	9×10 <sup>-5c</sup> ± 0.01	28.38 <sup>d</sup> ± 0.01
C50	0.3607 <sup>e</sup> ± 0.01	35.48 <sup>e</sup> ± 0.01	7.75×10 <sup>-5b</sup> ± 0.01	35.48 <sup>e</sup> ± 0.01
C60	0.4328 <sup>f</sup> ± 0.01	42.57 <sup>f</sup> ± 0.01	4.24×10 <sup>-5a</sup> ± 0.01	42.57 <sup>f</sup> ± 0.01
C70	0.5050 <sup>g</sup> ± 0.01	49.68 <sup>g</sup> ± 0.01	2.7×10 <sup>-5d</sup> ± 0.01	49.68 <sup>g</sup> ± 0.01
C80	0.5721 <sup>h</sup> ± 0.01	56.28 <sup>h</sup> ± 0.01	2.41×10 <sup>-5d</sup> ± 0.01	56.28 <sup>h</sup> ± 0.01

Within the same column, means with different superscripts are significantly different ( $p < 0.05$ ) by Tukey's test.

The blue WF represents the local water requirements for the microalgae-to-biofuel process. Blue WF varies between the scenarios; the lowest consumption of blue water occurred in the C80, C70, and C20 scenarios, with 2.41×10<sup>-5</sup> m<sup>3</sup>/kg, 2.7×10<sup>-5</sup> m<sup>3</sup>/kg, and 3.95×10<sup>-5</sup> m<sup>3</sup>/kg consumption, respectively. Concerning the balance of green water, the scenarios that demonstrated the least water consumption were C20 (14.19 m<sup>3</sup>/kg), C30 (21.28 m<sup>3</sup>/kg), and C35 (24.82 m<sup>3</sup>/kg), followed by C40 (28.38 m<sup>3</sup>/kg). According to Handler [34], as heterotrophic bioreactors are closed systems, they only require the volume of water necessary for biomass metabolism. This volume is on average 7.5×10<sup>-5</sup> m<sup>3</sup>/kg, making the blue water consumption low in all scenarios.

By contrast, the total WF is the sum of the blue and green WFs, representing the balance between them. In all the scenarios, total water footprint and evaporation rate increased with increasing sucrose concentration, as expected. The scenario that presented the lowest total water consumption was C20 (3.95×10<sup>-5</sup> m<sup>3</sup>/kg), followed by C30, C35, and C40. However, the C40 scenario offered the best combination of energy balance and CO<sub>2</sub> emissions. Considered as a long-term process, the C40 scenario becomes highly sustainable, with low water consumption, positive energy balance, and low CO<sub>2</sub> emissions.

We compared the WF of microalgal biodiesel to the WF of bioethanol. According to Pereira *et al.* [35], for bioethanol, the total WF of bioethanol produced from sugarcane is 7700 m<sup>3</sup>/kg, or 4600 m<sup>3</sup>/kg of green water, 2500 m<sup>3</sup>/kg blue water, considering irrigation needs, and 500 m<sup>3</sup>/kg of gray water.

Additionally, Papong *et al.* [36] evaluated the water balance of bioethanol from sugar cane molasses. The water footprint for this process had a total consumption of 702.79 m<sup>3</sup>/kg of molasses, being 516 m<sup>3</sup>/kg for the green water footprint and 186.37 m<sup>3</sup>/kg for the blue water footprint. In this study the gray water footprint was not considered. Microalgal biodiesel has a small water footprint compared to bioethanol, because microalgae have no gray WF due to their heterotrophic metabolism and because these microorganisms can simultaneously convert organic pollutants present in wastewater.

On the other hand, the water footprint of biodiesel produced from soybean is also much larger than microalgal biodiesel, because it requires relatively large amounts of irrigation combined with smaller biodiesel yields per unit of crop [37]. The WF for biodiesel from soybean is about  $42 \times 10^5 \text{ m}^3$  per kg of biodiesel produced, much higher than the best microalgal scenario, C40 ( $28.38 \text{ m}^3/\text{kg}$ ). Thus, microalgal biodiesel is a competitive, alternative source of biodiesel, using less water to produce 3G biodiesel compared to conventional feedstock.

### 3.4. Oil Composition and Biodiesel Properties

Finally, besides the environmental issues established by the life cycle analysis, the quality of the produced biodiesel will determine its applicability. Thus, we evaluated the properties of biodiesel produced from microalgae. To ensure a final product of high quality, biodiesel must meet the EN 14214, ASTM 6751, or ANP 255 specifications in Europe, the United States, and Brazil, respectively [38 - 40]. The fatty acid profile, properties of quality, and properties of combustion of the best-performing C40 biodiesel are presented in Tables 5 and 6.

**Table 5. Fatty acid composition of biodiesel microalgal in the C40 scenario.**

Fatty Acid Profile	Methyl esters (%)
Lauric (C12:0)	4.9 ± 0.1
Myristic (C14:0)	7.3 ± 0.1
Palmitic (C16:0)	22.5 ± 0.5
Palmitoleic (C16:1)	8.5 ± 0.1
Stearic (C18:0)	10.5 ± 0.2
Oleic (C18:1n9c)	26.2 ± 0.8
Linoleic (C18:2n6c)	17.8 ± 0.5
$\gamma$ -Linolenic (C18:3n6)	2.10 ± 0.03
$\Sigma$ Saturated	45.2
$\Sigma$ Monounsaturated	34.7
$\Sigma$ Polyunsaturated	19.9

**Table 6. Properties of microalgal biodiesel in the scenario C40 and its comparison with soybean and the standards used in the US (ASTM 6751), Europe (EN 14214) and Brazil (ANP 255).**

Properties	Microalgae	Soybean <sup>a</sup>	ANP 255	ASTM 6751	EN 14214
EC (%)	99.8	96.9	-	-	min 96.5
CN	56.31	49.0	min 45	min 47	min 51
IV ( $\text{g}_2 \text{ 100g}^{-1}$ )	70.04	128	-	-	max 120
DU (%)	74.5	143.8	-	-	-
SV	211.8	-	-	-	-
LCSF (%)	7.5	1.6	-	-	-
CFPP (°C)	7.09	-5.0	max 19	-	-
CP (°C)	6.87	-	-	-	-
APE	66.0	-	-	-	-
BAPE	22.0	-	-	-	-
OS (h)	8.52	1.3	-	min 3	min 6
HVV	39.5	-	-	-	-
$\mu$ ( $\text{mm}^2 \text{ s}^{-1}$ )	4.68	4.2	-	1.9-6.0	3.5-5.0
$\rho$ ( $\text{g cm}^{-3}$ )	0.87	-	-	-	-

EC: Ester Content; CN: Cetane Number; IV: Iodine Value; DU: Degree of Unsaturation; SV: Saponification Value; LCSF: Long-Chain Saturated Factor; CFPP: Cold Filter Plugging Point; CP: Cloud Point; APE: Allylic Position Equivalents; BAPE: Bis-Allylic Position Equivalents; OS: Oxidation Stability; HVV: Higher Heating Value;  $\mu$ : kinematic viscosity;  $\rho$ : kinematic density.

Regarding composition (Table 5), this microalgal oil contains eight different compounds, primarily oleic acid (26.2%). The oil was predominantly saturated (45.20%), followed by monounsaturated (34.70%) and polyunsaturated (19.90%). This profile demonstrates the potential application of this type of biomass as an input for biodiesel production, since oils with predominantly saturated and monounsaturated composition are the most suitable for synthesizing biodiesel [41].

The biodiesel produced from microalgal oil has the following fuel properties (Table 6): ester content 99.8%, cetane number 46.31, iodine value 70.04  $\text{gI}_2/100\text{g}^{-1}$ , degree of unsaturation 74.5%, saponification value 211.8, long-chain saturated factor 7.5%, cold filter plugging point 7.09°C, cloud point 6.87°C, allylic position equivalents 66.0, bis-allylic position equivalents 22.0, oxidation stability 8.52 hr, higher heating value 39.5, kinematic viscosity 1.28  $\text{mm}^2\text{s}^{-1}$ , and kinematic density 0.87  $\text{g cm}^{-3}$ . All these parameters comply with the limits established by U.S., European, and Brazilian regulators [38 - 40] and are comparable to soybean biodiesel [41]. These results indicate the potential to use microalgal biomass as a suitable lipid input for manufacturing biodiesel.

## CONCLUSION

Under the various tested scenarios, the best scenario for producing microalgal biodiesel was a ratio of C/N 40, which values for biomass and oil production respectively of 6.17  $\text{kg/m}^3$  and 1.12  $\text{kg/m}^3$ . A life cycle assessment of the third-generation biodiesel produced from the heterotrophic cultivation of *Phormidium autumnale* in the C40 scenario showed positive energy production (50.59 MJ/kg) associated with a low water footprint (28.38  $\text{m}^3/\text{kg}$ ) and low  $\text{CO}_2$  emissions (18.09  $\text{CO}_2\text{-eq/kg}$ ). The high potential lipid production obtained is interesting for generating quality biodiesel that meets or surpasses the most stringent U.S., European, and Brazilian requirements.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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