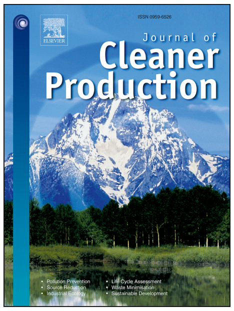

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Environmental Assessment of Industrial Production of Microalgal Biodiesel in Central-South Chile

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ABSTRACT

Biofuels from microalgae have the potential to replace fossil fuels, without competing with other products derived from crops. This study aims to perform a cradle-to-gate Life Cycle Assessment (LCA) of the industrial production of microalgal biodiesel, using an autochthonous Chilean *Phaeodactylum tricornutum* strain, considering 1 MJ of biodiesel as the functional unit. For the Life Cycle Inventory (LCI) analysis, real experimental data were obtained from the pilot-scale cultivation in a PBR module located in the city of Concepción, in Chile. The scale-up to the industrial plant considers that PBR modules are of the same size as those used in the pilot-scale. The Life Cycle Impacts Analysis (LCIA) considered the ReCiPe 2016 Endpoint (H) V1.00 method. Results show that the whole process contributes to a total of 5.74 kgCO_2eq per MJ of biodiesel produced. PBR construction materials and energy consumption are the main contributors to the life cycle environmental impacts. The sensitivity analysis showed that energy consumption, water reuse and transportation distance of seawater from ocean to the industrial plant are the critical parameters that most affect the overall environmental performance of the system. The rate of water reuse is particularly critical to the Global Warming Potential (GWP). Results also show that the valorization of co-products is an important aspect to improve the environmental performance of microalgal biodiesel production. Therefore, this study supports the decision-making process in biofuel production to promote the development of sustainable pilot and large-scale algae-based industry.

KEYWORDS: Biodiesel; Chilean conditions; Life Cycle Assessment; Microalgae; PBR cultivation; *Phaeodactylum tricornutum*.

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1. INTRODUCTION

Population growth and uncontrolled anthropogenic activity have triggered the demand for energy, as well as the environmental impacts resulting from greenhouse gas (GHG) emissions due to the burning of fossil fuels. The International Energy Agency estimates a 55% rise in the world's primary energy demand from 2005 to 2030, in which fossil fuels remain the dominant source of primary energy (Nakicenovic, 2007). Therefore, there is the challenge of finding alternative sources of renewable energy to control pollution and to contribute to the energy security of countries (Caetano et al., 2017). Several alternatives have been studied with different degrees of success and implementation phases, such as solar, thermal or photovoltaic, geothermal, wind, hydroelectric and biofuels (Martins et al., 2018b). Each has its pros and cons, depending on the area of application, but all have a common goal of reducing GHG emissions and replacing fossil fuels with renewable energy sources (Mata et al., 2013b). Biodiesel and bioethanol can replace diesel and gasoline, respectively, with few or no modifications in the engines of vehicles. In particular, biodiesel can be produced from oils or fats by esterification and/or transesterification reactions (Mata et al., 2013a).

On the other hand, the sustainability of biofuels has been debated due to the dilemma between bioenergy production and food security, since the traditional crops for biofuels compete for arable land (Mata et al., 2011). To overcome this issue, the production of biodiesel can be done using inedible oils, such as waste oils, animal fats, and greases (Mata et al., 2014; Mata et al., 2010). However, the availability and quantities of these inedible oils and fat sources are not enough to meet the current demand for biodiesel. In this context, microalgae are an interesting alternative for biodiesel production, as they do not directly interfere with food production and do not require arable land for cultivation. In addition to being a source of lipids for biodiesel production, microalgae also contain proteins, sugars, essential fatty acids, carotenoids, and vitamins, which can be used as food additives, supplements for aquaculture and animal feed, feedstock for cosmetic or pharmaceutical industries (Mata et al., 2010).

Microalgae can grow in almost all ecosystems and, depending on the species, can be cultivated in salty, fresh or brackish water. Microalgae are microscopic organisms, prokaryotic or eukaryotic, multicellular or unicellular. Some species can grow autotrophically, producing biomass and oxygen, using sunlight as a source of energy for photosynthesis, and carbon dioxide (CO_2) as a source of carbon and inorganic salts. Other species can grow in the dark, heterotrophically, using organic nutrients as a source of energy and carbon. Microalgae can also be cultivated mixotrophically, using sunlight as an energy source, CO_2 and organic compounds as carbon sources (Mata et al., 2016).

Different techniques and methods are possible for microalgae cultivation, harvesting, lipid extraction and biomass processing for biodiesel production, which are well described in the scientific literature (Mata et al., 2012). The lipid content of microalgae can reach more than 50% of biomass dry weight (DW), with variable biomass and lipid yields (Patel et al., 2017). However, the microalgae lipid content varies, depending on growth conditions, nutrient availability or environmental factors such as temperature, pH, heavy metals and salinity (Martins et al., 2016). The fatty acids profile of microalgae directly influences the quality of biodiesel (Williams and Laurens, 2010).

Among microalgae, marine diatoms are one of the most productive and environmentally adaptable microalgae, highly abundant, diversified and with a cell wall made of nanostructured silica. In particular, this work focuses on biodiesel production from *Phaeodactylum tricornutum*, one of the most exhaustively studied diatom species (Branco-Vieira et al., 2018a). *Phaeodactylum tricornutum* is a marine diatom that can also grow in freshwater. It is highly productive and environmentally adaptable and can be used not only for biodiesel production (Branco-Vieira et al., 2018c, 2017) but also for other applications through a biorefinery process, due to its interesting biochemical composition and ease of cultivation, even in outdoor conditions (Branco-Vieira et al., 2018b, 2018a).

There is no single solution for the industrial production of biodiesel from microalgae. There are many process variables to be considered, depending on the microalga species, local climate conditions and nutrient availability, which in turn will affect the total amount of biomass and lipids available and used for biodiesel, as well as the efficiency of the downstream processes. Besides, there are choices to be made for biomass processing (e.g. harvesting, pretreatment, and lipid extraction methods). Therefore, different results will be obtained in different locations and depending on the choices made.

It is of fundamental importance to know the environmental performance of the whole process in order to support decision making, which requires performing a Life Cycle Assessment (LCA) of the products and processes involved. Currently, few LCA studies are available, in the scientific literature, for the *P. tricornutum* species used in this work and especially based on real process data. Furthermore, existing LCA studies for microalgal biodiesel are not easily comparable and many are non-reproducible. Most studies are based on secondary information from literature or from process simulations performed. Other studies do not identify the microalga species or the production technologies or deal with just specific environmental indicators to compare substitute products or process alternatives.

For example, Batan et al. (2010) evaluated the net energy-ratio and net GHG emissions of microalgal biodiesel considering the life cycle steps from microalgae growth to biodiesel distribution to consumer pumping stations, using LCI data from the Argonne National Laboratory’s GREET® model (Wang et al., 2007). Baliga and Powers (2010) developed an LCA of biodiesel production from *P. tricornutum* in cold climates, aiming to determine the most suitable operating conditions for minimizing energy consumption and environmental impacts. To estimate actual yields for algae biomass production, energy consumption and emissions, these authors considered a model of a hypothetical tubular closed photobioreactor (PBR), utilizing solar data and biological growth rate information, and for the other life cycle steps the authors used information from the GREET® model (Wang et al., 2007). Furthermore, Frank et al. (2012) evaluated the energy use and GHG emissions of microalgal biodiesel, based on secondary data from literature and the GREET® model (Wang et al., 2007). Wu et al. (2018) estimated the life-cycle GHG emissions of producing microalgal biodiesel and ethanol simultaneously in a process simulated in Aspen Plus® (Aspen Technology - Inc, 2013), based on literature data necessary to perform the process simulations. The authors analyzed different scenarios and possible combinations of lipid percentages, cultivation and pretreatment processes, to maximize total revenue with minimum environmental impact.

Campbell et al. (2011) analyzed the life cycle GHG of microalgal biodiesel, based on literature data from algae cultivation in ponds in another location and adjusted for Australian conditions. Brentner et al. (2011) compared various pathways for algal biodiesel production to inform process design of full-scale production, based on literature information and combinatorial LCA. Khoo et al. (2011) carried out energy and CO_2 balances for a hypothetical integrated PBR-raceway microalgae-to-biodiesel, based on literature data adjusted to production in Singapore. Also based on literature information for the inventory analysis, Clarens et al. (2011) estimated the life cycle GHG emissions, energy, and water uses of microalgal biodiesel production, comparing different scenarios. Lardon et al. (2009) considered a virtual facility to perform a comparative LCA study of microalgae biomass production for biodiesel combustion, using literature data about similar technologies applied to other feedstocks (e.g. soybeans) for the Life Cycle Inventory (LCI) compilation.

Hou et al. (2011) developed an LCA study for microalgal biodiesel comparing it with other biodiesel feedstocks, using secondary data from the scientific literature, but they did not identify the microalgae species and the process technologies considered in the life cycle steps. Yang et al. (2011) focused on the water and nutrient usage for microalgal biodiesel production, based on literature data for the LCI analysis. Adesanya et al. (2014) performed an LCA of a hypothetical plant for biodiesel production from *Chlorella vulgaris*, based on literature information about microalgae cultivation in a hybrid system consisting of airlift tubular reactors and raceway ponds. Collet et al. (2014a) performed an LCA study of biodiesel production from *Nannochloropsis occulata* cultivated in open raceways under greenhouses. Based on literature information to perform the inventory analysis, these authors considered for microalgae cultivation the available technologies used by companies that produce high added value microalgal co-products. Furthermore, in the case of lipid extraction from biomass, they considered laboratory results of similar processes applied to other feedstock as a proxy, because mature technologies do not yet exist for large-scale microalgae oil extraction.

Morales et al. (2019) carried out an LCA focusing on energy performance and environmental impact of biodiesel from microalgae *Chlorococcum* sp. and *Desmodesmus* sp. Their study considers a virtual facility for biodiesel production over 145 ha, for which biomass yield and electricity production results were estimated and used as input for an LCI implemented into SimaPro 8 software. The microalgae yield was simulated using mathematical modeling considering cultivation in raceways located in a solar greenhouse, with variable percentages of photovoltaic panel coverage, under meteorological conditions in the south of France.

Finally, a few studies have used real process data or extrapolate them to an industrial facility to perform LCA. In this regard, Siqueira et al. (2018) performed a prospective LCA of biodiesel produced from the heterotrophic cultivation of *Phormidium autumnale*, for which the LCI was based on experimental data from lab-scale cultivation and lipid extraction, complemented with literature information. Sarat Chandra et al. (2018) developed a comparative LCA of the carbon capture potential of *Scenedesmus dimorphus*, during autotrophic growth in closed airlift PBR and open raceway pond under Indian conditions. The analysis was based on primary data obtained from the pilot-scale cultivation of this microalga in both types of cultivation systems, and these data were extrapolated to hypothetical large-scale cultivation. Mediboyina et al. (2020) developed a comparative LCA of autotrophic cultivation of *Scenedesmus dimorphus* in raceway pond coupled to biodiesel and biogas production. The analysis was based on primary data obtained from algal cultivation in a pilot-scale raceway pond and on secondary data of the downstream processes involved in biodiesel production, such as drying, reaction, and purification.

Commonly, the assumptions, scenarios, and scope of LCA studies may vary, leading to different LCA results. Besides, LCA studies are region-specific, since there are differences in energy grids, feedstock, transportation modes, and available technologies; specifically, for microalgae-based products, the intrinsic climate conditions for algae growth vary. This implies that environmental impacts for similar products manufactured in two distinct regions may differ significantly.

The process for microalgal biodiesel production still has immature technology that is not available commercially. Consequently, it is essential to explore all different aspects of this approach, considering the local characteristics, different technologies and microalgae growth conditions. This manuscript explores the industrial cultivation of microalgae for biodiesel production in a Chilean region that represents an interesting site for an industrial flow of products and raw materials due to its central location, in which the existing industrial conglomerate can promote the industrial symbiosis and a circular economy. Thus, this study aims to analyze, for the first time, the environmental life-cycle impacts of the industrial production of biodiesel from an autochthonous strain of *P. tricornutum*, using real experimental data from this microalga cultivation in a pilot-scale PBR plant located in the city of Concepción, in Chile.

2. MATERIALS AND METHODS

An LCA study is used to evaluate the potential environmental impacts of a product, process or activity, considering its life cycle inputs (e.g. materials and energy) and outputs (e.g. waste and emissions to air, water, and soil). LCA is standardized by ISO 14040:2006 (ISO, 2006) and it is conducted over four phases: (i) goal and scope definition, (ii) inventory analysis, (iii) impact assessment and (iv) interpretation of results.

2.1. Goal and scope definition

The goal of this study is to conduct an LCA study, considering an attributional approach, of biodiesel produced from *P. tricornutum* cultivated in a microalgae industrial plant facility located at Concepción, Chile. The functional unit (FU) of this LCA is defined as 1 MJ of biodiesel, calculated based on the Lower Heating Value (LHV) of biodiesel, equivalent to 37.80 MJ kg^{-1} (Lardon et al., 2009). The system boundary for this study considers a cradle-to-gate perspective and is represented in Figure 1.

--Figure 1 here--

To deal with the multifunctionality of the process, a system expansion approach is adopted to avoid allocation, considering glycerol and the residual biomass as avoided products.

The process for producing microalgal biomass and biodiesel consists of the following steps: (i) pumping of seawater to the reservoir; (ii) mixing of the seawater with the nutrients required for *P. tricornutum* growth; (iii) feeding the culture medium to the PBR; (iv) pumping of microalgae culture to the tank reservoir after microalgae cultivation; (v) harvesting through centrifugation; (vi) filtering of the remaining culture water medium, after biomass centrifugation, and returning it to the seawater tank to be reused in another culture batch; (vii) extraction of lipids from the recovered biomass (containing 15% of dry matter) and; (viii) biodiesel production. The entire production system of biodiesel and co-products can be summarized into upstream and downstream processes. The upstream processes include microalgae cultivation and biomass harvesting. The downstream processes include biomass drying, cell disruption, lipid extraction, oil refining, and transesterification of lipids to biodiesel production. Besides biodiesel, glycerol and residual biomass are also produced as co-products of this process.

2.2. Life Cycle Inventory

The source of foreground data for the compilation of the LCI was a pilot-plant facility for the microalgae cultivation in outdoor PBR (Branco-Vieira et al., 2020a) and the biochemical characterization of the microalgae biomass (Branco-Vieira et al., 2020b). To conduct the assessment, the scale-up of the process to an industrial plant was used, considering the experimental results obtained from the PBR pilot-plant (Branco-Vieira et al., 2018c) and the methodology proposed by Spruijt et al (2015). The industrial scaling up considers 10,000 modules of vertical bubble column PBR, totalizing 8,000 m^3 of cultivation medium. The model considers a 24/7 (hours/days) time of operation and 4 periods per year for the cleaning of reactors, which corresponds to 14 unavailable days for biomass production.

According to this model, a total of 181 tons of algal biomass and 15 tons of biodiesel can be produced per year. Consequently, to produce 1 kg of biodiesel it is necessary to have about 12 kg of dried algal biomass, considering 9.08% of lipids in its intracellular constitution. Proteins and glycerol are co-products of the process. Each 1 kg of biodiesel generates about 10-fold more of protein-rich residual biomass and 10% of glycerol.

The main sources of background data were the Ecoinvent database, version 3.3 (Wernet et al., 2016), the European Reference Life Cycle Database (ELCD) version 3.2, and the Agri-footprint database version 3.0 (Durlinger et al., 2017). The SimaPro version 9 software was used to compile the LCI. In the following sections, the plant infrastructure, the upstream and downstream processes for biodiesel production, and the main assumptions taken in the compilation of the LCI are all described. The mass flow of LCI is presented in terms of kg of mass material per kg of biodiesel.

2.3. Infrastructure

The modeled system considers an industrial facility that occupies a total area of 2.51 ha. This area is divided as follows: 1.35 ha to install the outdoor PBR, 1.08 ha of workspace between the PBR, and 796 m^2 of construction dedicated to the downstream processes. The lifespan considered for the industrial plant infrastructure is 30 years. For the PBR construction the use of polymethyl methacrylate (PMMA) was considered as the main construction material of the PBR bubble columns, whose dimensions were measured *in loco*. Inventory data for infrastructure are shown in Table 1.

--Table 1 here--

2.3.1. Upstream processes

The upstream processes are represented by the cultivation of microalgae and biomass harvesting through centrifugation. The microalgal biomass production was performed using an autochthonous Chilean *P. tricornutum* strain cultivated in natural seawater. The initial lab cultures were scaled-up from laboratory culture collection to 20 liters for the PBR inoculations.

The outdoor microalgal cultivation system was performed in a PBR described by Branco-Vieira et al. (2018c) under natural conditions at Concepción, Chile, during summer-autumn. For the PBR culture, a commercial modified Guillard f/2 formulation with silicate was used (Guillard and Ryther, 1962). The supply of atmospheric CO_2 was provided by an air pump. Since data on some of the chemicals used in microalgae cultivation are not available, some replacements were made: $ZnCl_2$ was replaced by $ZnSO_4$, $MnCl_2.2H_2O$ was replaced by $MnSO_4.H_2O$ and $FeCl_3.6H_2O$ was replaced by $FeCl_3$, assuming that there was a similar inventory for raw material extraction, processing, manufacturing, and distribution. In the case of $CoCl_2.6H_2O$, vitamin B12, vitamin B1, vitamin H and $(NH_4)_6Mo_7O_{24}.4H_2O$, no replacements could be identified and, thus, they were not considered in the LCI. Furthermore, all of the materials or processes whose contribution to the total environmental impacts in any category is less than 1% may be excluded.

The highest biomass concentration obtained was 0.96 kg_{DW} m^{-3}, the maximum volumetric yield was 0.13 kg_{DW} m^{-3} d^{-1} and the photosynthetic efficiency calculated was 5%. The biochemical characterization of *P. tricornutum* revealed an amount of 9.08% of lipids, 7.85% of carbohydrates and 38.40% of proteins (Branco-Vieira et al., 2020b). The total biomass concentration calculated per year is 181 tons. Two cultivation batches per month were considered. The modeling parameters considered one-year of biomass production and were measured *in situ*. The environmental parameters are presented in Table 2.

--Table 2 here--

The biomass produced monthly is harvested by centrifugation. The equipment considered for centrifugation is Spiral Plate Technology from Evodos (Evodos Dynamic Settler, Raamsdonksveer, The Netherlands), which produces an algal concentration of 150 kg_{DW} m^{-3} of culture. After biomass harvesting, it was considered that 90% of the water returns to the PBR and is reused in another batch culture. The resulting algal paste contains about 15% of dry biomass after centrifugation. The inventory data for the upstream processes are presented in

Table 3.

--Table 3 here—

2.3.2. Downstream processes and avoided products

Downstream processes include biomass processing for biodiesel production, which is performed by first recovering the intracellular lipids and then converting them into Fatty Acid Methyl Esters (FAME) or biodiesel, via the transesterification reaction. Thus, the process steps in this phase include biomass drying, cell disruption, lipid extraction, oil refining, and transesterification reaction. All these process steps were modeled based on the calculations published by Spruijt et al (2015), using the total amount of biomass produced per one year of operation. In this phase, only data on electricity consumption and chemical materials of the LCI were considered.

Briefly, after biomass harvesting, the algal paste is dried in freeze-drier technology with an efficiency of 80%. The drying process was adopted in this model to enhance lipid extraction using a CO_2 supercritical extraction (CO_2-SC). For the calculation of total electricity consumption for biomass drying, the energy needs for removing water from the algal paste were considered. This step accounted for the total biomass losses of the process, represented by the culture crashes and all the remaining biomass inside the processing equipment. The biomass lost in this phase is treated by composting the bio-waste scenario.

The cells of dried biomass are milled in a ball mill machine to enhance the extraction of the intracellular components, for which a cell disruption efficiency of 95% is assumed. The ball mill machine has a capacity to process 12.5 tons of algal biomass in 8,000 operational hours. The electricity consumption for ball milling is assumed to be 1.87 kWh kg^{-1} of dry algal biomass (Balasundaram et al., 2012).

Lipid extraction is performed using a CO_2-SC process in which it is assumed that only triglycerides are obtained from the biomass, with an efficiency of 95% of extraction. This process was modeled through the base case for using 10 tons of algal biomass, a vessel size of 10 L and 8 kg of biomass per batch. The co-product of this process is protein-rich residual biomass.

Before the transesterification process, refining is usually necessary to separate cellular debris, membrane lipids (e.g. phospholipids), and pigments from the triglycerides. This step is required to reduce the contaminants present in the future FAME mixture to acceptable levels for international biodiesel standards. For the refining process, the addition of 4% (w/w lipid) of water, 85% of phosphoric acid at 0.25%, 0.05% of citric acid and caustic soda as a neutralizing agent is considered. The refining step is carried out at 65 ºC in a mixer-settler tank and the electricity used is the sum of the energy needed to heat up the oil to 65 °C and the energy to mix the material in the tank.

The transesterification process is based on the alkali-catalyzed reaction, using potassium hydroxide (KOH) as the catalyst agent, an amount of 1% (w/w) of the lipids entering in the process. Methanol was used as a reagent for the transesterification reaction. The molar ratio of methanol to lipid is assumed to be 6:1 (v/v), and 98% of triglycerides conversion to FAME in 1 hour of reaction time is considered. Furthermore, a final step is included after the transesterification reaction, to remove the catalyst, by adding sulfuric acid (H_2SO_4) to neutralize it, followed by water washing, also to remove any remaining glycerol and salts from the biodiesel. The density of biodiesel considered in this study is 880 kg m^{-3}. The inventory data for the downstream processes are shown in Table 4.

--Table 4 here—

2.4. Life Cycle Impact assessment

The Life Cycle Impact Assessment (LCIA) phase is conducted considering ReCiPe 2016 (H) V1.00 (Huijbregts et al., 2016). The hierarchist perspective is chosen as it relies on scientific consensus regarding the time frame and plausibility of impact mechanisms (Huijbregts et al., 2016). Since water use (both fresh and marine) is critical for the production of biodiesel from the microalga strain used in this study, the ReCiPe midpoint was selected over other available methods since it considers, among its impact assessment categories, water consumption, freshwater ecotoxicity, and marine ecotoxicity.

2.5. Sensitivity analysis

A sensitivity analysis was conducted to assess the robustness of the results due to the methodological choices and initial assumptions of the model. To assess the sensitivity of results to selected parameters, a perturbation analysis was performed. The perturbation analysis is based on a variation of ±10% on each of the selected parameters (Heijungs and Kleijn, 2001). To that end, a sensitivity ratio (SR) was used to calculate the rate between two relative changes (Equation 1).

$$SR = \frac{\Delta result / initial\ result}{\Delta parameter / initial\ parameter} \qquad (1)$$

Where $\Delta result$ is the variation obtained in the result, $\Delta parameter$ is the variation of ±10% on the selected parameters, $initial\ result$ is the result initial value and $initial\ parameter$ is the parameter initial value.

For the evaluation to be more realistic and representative, only those analyses with a SR = ±1% of change in total results are considered to significantly influence the total results. This cut-off criterion was selected as they reflect that a 10% change in the assessed parameter generates a 0.1% variation in the results.

The sensitivity analysis considered the following parameters: (i) energy use of the whole process; (ii) the total amount of nitrogen (N content) and (iii) phosphorus (P content) used in the cultivation system; (v) water reuse factor in the cultivation system; (iv) the amount of CO_2 used in the lipid extraction process; (vi) methanol recovered and reused in the transesterification process (vii) transport distances of seawater from ocean to the industrial plant. Energy use is selected as it is known to be a relevant aspect of the environmental impacts of the production of biodiesel from microalgae (Collet et al., 2014). The other variables are considered as they reflect critical aspects for microalgae cultivation (N and P are critical for biomass growth), for reflecting best practices (water reuse), for lipid extraction from the biomass (CO_2 use in CO_2-SC technology), for biodiesel production (methanol recovery is critical for the transesterification process due to its toxicity), and to assess strategic aspects of the production (transport distances of seawater from ocean to the industrial plant, which affect the plant location).

3. RESULTS

3.1. Contribution analysis of microalgae-based biodiesel production

The contribution analysis of the involved processes throughout the microalgae-based biodiesel production system was conducted based on each impact category of the characterization method based on the obtained results (Figure 2). It consists of assessing processes that contribute the most to the potential environmental impacts of the production system. The absolute values of the results per impact category are presented in the Supplementary Material (Table S1).

--Figure 2 here--

The microalgae cultivation represents a considerable influence on the environmental impacts in almost all impact categories, which are more thoroughly discussed in the next sub-sections. Other major contributors to the impacts are the downstream processes, infrastructure and harvesting. Downstream processes are the second-largest process stage contributing to each impact category. Since they consist of several sub-processes, Figure 3 presents its disaggregated contributions to the total impacts, which shows drying and cell disruption as the greatest contributor to all impact categories.

--Figure 3 here--

Regarding the positive impacts of the assessed system, they occur as proteins and glycerol are considered as avoided products in the LCA modeling. In terms of mass flow, each 1 kg of biodiesel generates about 10 times more protein-rich residual biomass and 10% of glycerol. The protein-rich biomass can be considered for animal feed, and glycerol can be used for several applications in the pharmaceutical, medical, food and personal care industries.

3.1.1. Global Warming Potential (GWP)

Concerning GWP, the impacts of the system are well distributed across the production phases. The total amount of CO_2 equivalents (CO_{2eq}) emitted during the entire process is 5.74

$kgCO_{2eq}$ per 1 MJ of biodiesel. The infrastructure is the largest contributor to this impact category, summing up 1.85 $kgCO_{2eq}$. The GWP of infrastructure in this sub-process is mostly due to the use of PMMA in the construction of the PBR, which accounts for 97.8% of total carbon emissions. Cultivation is the second largest contributor to carbon emissions, and it is responsible for emitting about 1.51 $kgCO_{2eq}$. The high rate of carbon emissions in this phase is noted by the transportation of seawater, by truck, from ocean to the industrial facility, which represents 100% of carbon emissions in this process. Otherwise, the CO_2 consumption from microalgae during their growth is associated with the reduction of 0.373 $kgCO_{2eq}$, added to the negative contributions, linked to the avoided production of glycerol and biomass-rich protein, which totalize an emission reduction of 0.385 $kgCO_{2eq}$ by these co-products.

The total contribution of the downstream processes to the carbon emissions is about 1.4 $kgCO_{2eq}$, followed by the harvesting process (contribution of 1.37 $kgCO_{2eq}$). Regarding the downstream processes, the main contributor to the carbon emissions is biomass drying (48.4%), which is responsible for 0.677 $kgCO_{2eq}$, followed by cell disruption (0.365 $kgCO_{2eq}$) and lipid extraction (0.330 $kgCO_{2eq}$). Drying is the largest contributor since it is the most energy-intensive process, accounting for 44.27% of the total energy requirements, considering only the primary energy demand from the grid. The electricity consumption in the drying process accounts for almost 98% of the total carbon emission in this step (0.663 $kgCO_{2eq}$). The lipid extraction sub-process is the third-largest contributor to the carbon emission of the downstream processes for biodiesel production (Figure 3). The transesterification reaction for biodiesel production represents the smallest contributor to the GWP among all the downstream processes.

3.1.2. Stratospheric Ozone Depletion (SOD)

Impacts on SOD are mostly related to the cultivation step. In this phase, the cultivation contributes about 68.1% to other sub-processes. Particularly, the use of chemicals in the cultivation system contributes 79.9% to this impact category. The use of sodium nitrate, within the use of chemicals, has a relative contribution of 98.3%. Regarding the downstream processes, biomass drying showed a contribution of 57.7%, represented mostly by energy consumption (77.4%) and composting of the biomass residues, modeled in this step to represent all the biomass lost in the whole process (22.6%) due to culture crashes and the biomass residues that remain inside the equipment. The biomass composting has a significant contribution only to this impact category, totaling 22.6%.

3.1.3. Ionizing Radiation (IOR); Ozone Formation, Human Health (OFH); Ozone Formation, Terrestrial Ecosystem (OFT)

Contributions to IOR are mostly explained by emissions related to the energy use in cultivation, harvesting and downstream phases, and the harvesting process is the main contributor to this category, representing 50.1% of the total. The impacts on OFH and OFT categories are partly mitigated due to the avoidance of the production of rich-protein biomass (modeled later as fishmeal), addressed to the feed market. The contribution of the cultivation system to both categories represents about 59% of the total impacts, which is related to the use of fossil fuels to transport seawater to the industrial plant. Subsequently, in the downstream process, biomass drying contributes 55% to these impact categories summed together.

3.1.4. Fine Particulate Matter Formation (FPF)

Concerning the FPF, the downstream processes contribute 72.5% to this impact category. The assessment of FPF shows that biomass drying is responsible for 59.9% of this impact, mainly due to the dispersion of particulates with granulometry below 25 µm and sulfur composites associated with Chilean electricity matrix emissions.

3.1.5. Terrestrial Acidification (TAC)

Regarding the TAC, there is a similar contribution from the infrastructure (29.9%), cultivation (29.5%) and downstream processing (27.0%). The contributions to this category are mainly due to the emission of sulfur and nitrogen composites. For infrastructure, the emission of these compounds is related to the PBR construction, while in the cultivation phase they are linked to fossil fuel use during the transport of seawater to the plant, and finally, in the downstream processes they are related to the electricity use for biomass drying.

3.1.6. Freshwater Eutrophication (FWE)

The main contributor to FWE is the downstream processes (45.3%). This contribution is mostly explained by the electricity consumption in biomass drying and its phosphorus emission, even in low proportions, by the Chilean electricity grid.

3.1.7. Terrestrial Ecotoxicity (TEC)

Regarding the Terrestrial Ecotoxicity (TEC), 74.1% contribution to this impact category is associated with the cultivation process, mainly related to the seawater transport to the plant and due to the necessary raw materials for truck construction, especially copper extraction. The latter is represented by the Ecoinvent database input and it is reflected in this assessment due to the choice of using this kind of transport modality.

3.1.8. Freshwater Ecotoxicity (FEC); Marine Ecotoxicity (MEC); Human Carcinogenic Toxicity (HCT); and Human Non-carcinogenic Toxicity (HNT)

FEC, MEC, HCT and HNT all have contributions from the cultivation process, followed by the downstream processes, more specifically, due to the emissions of Zinc and Chromium VI, substances that are highly toxic to human health and the aquatic environment.

3.1.9. Land Use (LUS)

Regarding the LUS category, the downstream processes represent the highest contribution (43.4%), which is greater than that of the site infrastructure that includes the impacts of direct land use for the industrial plant. The infrastructure contributes 19.2%, followed by cultivation (25.2%) to land use. Since this category considers the damage to ecosystems due to the effects of occupation and transformation of land, the downstream processes highly influence land occupation impacts, mainly those related to the use of raw materials. By analyzing the downstream process, it is possible to observe that the drying process contributes to 54.2% of total impacts inside this category, due to the electricity consumption from the grid. This could be explained by the fact that the Chilean electricity grid mix is composed of 31% of hydropower and 38% of mineral carbon as a primary energy supply in 2018 (CNE, 2018).

3.1.10. Mineral Resource Scarcity and Fossil Resource Scarcity (FRS)

FRS is mostly explained due to the consumption of fossil fuels (mineral diesel). As mentioned above, terrestrial transportation is considered for the transport of seawater to the water treatment facility to be used in the cultivation process, and to the transportation of raw material to the industrial plant during the construction phase.

3.1.11. Water consumption (WAC)

About 90% of the WAC impact is related to the cultivation step, since seawater is collected as a medium for the microalgae cultivation in the PBR. Water consumption in this step is obtained by summing both sea and freshwater. Although seawater is used for the culture medium preparation, freshwater is used for the culture thermoregulation in the cooling equipment, which is particularly important during the summer season.

This study considered a water reuse model for the WAC, a practice that is still not so common in the microalgae industry. The reuse model considered that 90% of the water, after the harvesting process, is returned to the PBR, to feed another microalgae cultivation batch. This practice considerably reduces the impacts on water use. The harvesting process shows negative impact values, i.e. a positive outcome on the environmental performance of the process. This positive effect can be explained because the culture medium is almost totally returned to the reactor (about 90%), after microalgae harvesting by centrifugation, and thus, the generation of residual water that is conducted for treatment is minimal. Due to the composition of this wastewater, it can be sent to a typical domestic treatment wastewater facility.

The other contributions to this WAC impact category are related to the use of water in the downstream processes, especially by the drying process, because the model accounts for the percentage of microalgal biomass humidity that will evaporate and, thus, could not be reused.

Furthermore, the waste scenario of the remaining biomass for composting generates a positive effect, represented by negative values on the total impact factor for this process, owing to the possible substitution of fertilizers.

3.2. Sensitivity analysis

The perturbation analysis (described in Section 2.5) was based on an increase and decrease of 10% of seven different parameters. The results of the sensitivity analysis are shown in Table 5, in which the parameters that change the results by more than 1% are highlighted. The sensitivity analysis presents a strong correlation between the LCIA results.

--Table 5 here--

Based on the results achieved in the sensitivity analysis, it is seen that the energy consumption and transport distance are the parameters that most affect the environmental performance of the evaluated system. The energy consumption and transport distances affect all impact categories, except WAC.

Water reuse is a very critical parameter, with the highest sensitivity of all scenarios in its contribution to GWP. A change of 10% in this parameter generates a change of 16% in results. This occurs because water use contributes 74.8% to the GWP, considering biomass harvesting upstream process. Water reuse also presents high sensitivity for GWP, SOD, and TAC, and contributes very little to IOR, FPF, and FRS. Since water reuse is directly related to the uptake of seawater from the ocean and its transportation to the industrial plant, this parameter shows a huge contribution to the GWP and in a lower proportion to the other categories, because of the use of mineral fossil fuels by trucks that do the transportation. On the other hand, water reuse is also related to the amount of wastewater generated by the harvesting process that needs to be sent to treatment. Therefore, the greater the water reuse, the lower the total impacts generated in the process.

The CO_2 applied to the downstream process does not have sensitivity effects on the analyzed system, since the values achieved for all categories are below ± 1%. Additionally, the use of fertilizer in this study also has no sensitivity in relation to the impact categories, although the nitrogen use contributes to the SOD. Similarly, the P content and the percentage of methanol produced represent almost no contribution to the impact categories when varied.

4. DISCUSSION

The coast of continental Chile is about 4,200 km long and extends from 18°S to about 56°S, which makes marine resources of great importance for the country's economic development. The most economically important trading ports are located in Central Chile, in the Bío-Bío Region, the third most populated zone of the country (Aguilera et al., 2019). The Concepción commune is located in this region and it can be seen as an interesting place for microalgae cultivation because of its strategic localization, which facilitates the transportation of materials and products, besides the presence of an industrial conglomerate that can supply CO_2 for microalgae cultivation. Moreover, this region has favorable climate conditions and proximity to the coast, which can provide the water for the culture medium and other sea resources. Some studies on microalgae cultivation in Chile have focused on the North of the country (Bravo-Fritz et al., 2015), mainly in the arid region, whose economic characteristics and climate conditions are substantially different from those of the Central-South. Thus, this study faced the challenge of promoting the discussion about implementing an industrial microalgae facility in the city of Concepción in central-south Chile.

The use of biofuels is an import strategy to achieve the decarbonization of transportation systems and the reduction in the energy dependency of countries. For Chile, these issues are of major concern as the country is a large importer of energy resources, whose high prices have increased the marginal costs of power generation and the price of electricity (FAO, 2013). The country has one of the highest electricity prices in Latin America and above the average charged in other OECD countries (FAO, 2013). The Chilean Strategy Energy Report 2012-2030 (Chile, 2012) declared the requirement of clean and renewable energy in their electric grid. In this way, the Government will aim to accelerate the incorporation of non-conventional renewable energies and reduce the participation of thermoelectric generation.

Currently, the country has no commercial production of biofuels. There is low availability of land in the country for the production of biofuels, which means that degraded marginal lands need to be used as an alternative for the production of biofuels (Román-Figueroa and Paneque, 2015). In this context, the production of third-generation biofuels from microalgae could become a solution, as they do not require arable land. Biodiesel production from microalgae is still an emerging technology and, to date, there is no commercial production of biofuel from algae anywhere in the world. This translates in uncertainties about its economic and environmental feasibility when compared to other biofuels. Further studies are necessary to understand the economic feasibility of this approach, and other strategies could be analyzed, such as the application of industrial symbiosis in this region to promote a circular economy.

Chile's 2050 energy policy (Ministerio de Energía, 2014) envisions a reliable, sustainable, inclusive and competitive energy sector based on four pillars: Security and Quality of Supply, Energy as an Engine for Development, Compatibility with the Environment and Efficiency, and Energy Education. The development of biodiesel from microalgae may be centered on the proposed pillars, since specific policies target boosting industries, such as support and investments in R&D; fiscal incentives to develop national technology; incentives to use less energy-intensive technologies in the production chain; incentives to promote the implementation and use of off-grid energy sources at the production facility; financial support to promote the circular economy and industrial symbiosis to reduce feedstock cost; tax credits or benefits to the microalgae-based biodiesel producers; and, after ensuring the sufficient annual production of biodiesel, implementing the mandatory blend of biofuels into fossil transportation fuels. If the studies are consistent with local contexts, the government will be able to implement more targeted and real public incentive policies.

Studies assessing the sustainability of third-generation biofuels focusing on the local situation are important to establish a competitive biofuel market in the Chilean context. To this end, LCA studies play an important role in supporting the development of Governmental policies, because they help identify the processes’ bottlenecks. Getting results linked to the specific Chilean conditions is particularly relevant, as it is hard to compare different LCA studies since each microalga strain has intrinsic growth characteristics and a biochemical composition that leads to a varied level of oil production. Each cultivation site presents its particular climate conditions that require a diverse technology portfolio to establish the cultivation system and downstream processes.

It was found that the material used for PBR construction contributes greatly to environmental impacts, mainly due to the energy use for PMMA manufacturing. The results also showed that energy consumption and transportation distance are the parameters that most affect the results. Strategies to reduce impacts on the production of biofuels from microalgae regarding these two parameters may consider the reduction of energy use from the grid, the implementation of off-grid renewable energy solutions in the industrial plant, the adoption of alternative fuels for use in transport, and finally, planning the localization of the industrial plant closer to the ocean to supply the process of seawater. This study has demonstrated that cultivation and downstream processes (particularly biomass drying) are the most critical processes in the production of biodiesel from *P. tricornutum*.

The avoided products improve the environmental performance of the entire process because a considerable amount of energy content can be found as organic matter in the microalgal biomass, after oil extraction (Collet et al., 2014), meeting the requirements of European Directive on Renewable Energy (European Union, 2009). Almost 90% of the environmental impacts of microalgal biomass production, in this study, are allocated to residual biomass and glycerol, and only 10% to biodiesel production. This fact implies that a large quantity of energy in the residual microalgae biomass can be exploited as co-products of the process. Further discussion is shown in sections 4.1 to 4.3, focused on the most critical impact categories of the assessment.

4.1. Energy consumption

Different studies related to microalgae-based products and biofuels have discussed the impacts on energy consumption as the process bottleneck. Examples include the study of Collet et al (2011), which performed an LCA study to analyze biogas production from *Chlorella vulgaris* and the study of Pérez-López et al. (2014), which performed an LCA to evaluate the production of eicosapentaenoic acid (EPA) from *P. tricornutum*. Both studies have concluded that electricity consumption is the main contributor to environmental impacts. These findings imply that the energy mix in the country of production is a critical aspect to consider in the production of biodiesel from microalgae.

In this study, the energy consumption of all stages and sub-processes was demonstrated to be the highest contributor to the impacts on most of the analyzed categories. The most energy-intensive step in microalgal biodiesel production is biomass drying, totaling 1.08 kWh per MJ of biodiesel and the total energy use of 2.47 kWh per MJ of biodiesel. This result is in line with those obtained by Papadaki et al. (2017), in which an LCA was conducted to assesses the impacts of extraction techniques for recovery of phycocyanin from *Arthrospira platensis*, showing that the drying step is the main contributor to the environmental impacts due to its energy intensity.

Another reason that cultivation system operation seemed to be less energy-intensive is that no cultivation heating system was adopted during the winter. This is another advantage of cultivating this autochthonous microalga strain in Concepción, Chile, even with the reduction of biomass production in this period (Branco-Vieira et al., 2020b). Studies considering temperature regulation data from real pilot plants operated in summer, fall and winter have shown that energy consumption is the main environmental burden (Pérez-López et al., 2017). In a Mediterranean climate, closed systems are more energy-intensive, due to artificial solar irradiation and indoor temperature control both in open and closed PBR (Foteinis et al., 2018).

Future studies must focus on the development of new technologies for drying processes that are less energy-intensive or that develop approaches for using wet biomass for lipid extraction. In this context, Schneider et al. (2018) performed an LCA of the production of microalgae *Desmodesmus subspicatus*, using electroflotation instead of flocculation with NaOH for harvesting the biomass, followed by centrifugation and drying, causing fewer environmental impacts. Furthermore, Sills et al. (2013) investigated the uncertainty analysis of diverse LCA studies for algal-based biofuels and found that using wet lipid extraction is crucial to increase the environmental performance of this approach. In this study, the assessment performed was based on the scale-up proposed by Spruijt et al. (2015), which does not disclose the variability of estimates. The scale-up model was constructed and validated with real process data to minimize or eliminate most of the uncertainties related to the lack of available data or available but wrong or ambiguous data.

4.2. Global warming potential

Regarding GWP, a total of 5.74 kgCO_2eq per 1 MJ of biodiesel was emitted by the entire assessed process, 1.85 kgCO_2eq per 1 MJ of biodiesel by infrastructure, 1.51 kgCO_2eq per 1 MJ of biodiesel by cultivation system, 1.37 kgCO_2eq per 1 MJ of biodiesel by biomass harvesting process and 1.40 kgCO_2eq per 1 MJ of biodiesel by downstream processes. These results are rather high compared to related studies in the literature, particularly because most of them have performed their modeling using theoretical assumptions and best scenarios for a hypothetical biodiesel production process. The microalga strain, microalgal biomass yields, cultivation system, biomass oil content, climate conditions, and the source and percentage of CO_2 supply differ considerably between studies, which usually adopt an optimistic scenario to perform prediction LCA studies. Consequently, direct comparison of existing LCA results in the literature is hard enough, due to the differences in system boundaries, functional units, LCA methodology used and inherent technological uncertainties (Uctug et al., 2017). According to Cuéllar-Franca and Azapagic (2015), the total GWP varies significantly between studies, ranging from 0.019 to

0.534 $kgCO_2eq$ per 1 MJ of biodiesel, mainly due to the large difference of the method assumed for disposal of waste biomass generated from microalgae during the production of fuel.

On the other hand, Brentner et al. (2011) analyzed the LCA of biodiesel from microalgae, cultivating a strain of *Scenedesmus dimorphus*, supplied with 15% of CO_2, considering several technology options, selected yielding a best-case scenario, comprised of a flat panel enclosed photobioreactor and direct transesterification of algal cells with supercritical methanol. The authors achieved a value of 0.08 $kgCO_2eq$ per 1 MJ of biodiesel for the best case, compared with 0.534 $kgCO_2eq$ per 1 MJ of biodiesel for the base case. The base case was characterized by using paddle wheel cultivation system, a solvent process for oil extraction and esterification. However, this work does not consider the electricity and upstream process for CO_2 supply, and the material used for PBR construction was less energy-intensive than those used in this study.

Furthermore, Stephenson et al. (2010) discussed the production of biodiesel from *C. vulgaris* using air-lift tubular bioreactors and raceways, supplied with 12.5% of CO_2, considering 40% of lipids in the microalgal biomass and producing 40 ton year^{-1} of biomass. These authors obtained an amount of 0.32 $kgCO_2eq$ per 1 MJ and 0.02 $kgCO_2eq$ per 1 MJ of biodiesel for PBR and raceways systems, respectively. Therefore, different values of GWP have been found, when the production was down or up-scaled, obtaining an amount of 1.38 $kgCO_2eq$ per 1 MJ of biodiesel for microalgae cultivation using PBR, when the production is down-scaled to 10 ton ha^{-1} year^{-1}, which represents 431% more GWP. These results suggest that the GWP reduction is sensitive to the scalability of the production. Therefore, it would be interesting to analyze how the scale of the operation and lipid percentage of the strain affects GWP performance.

Similarly, Somers and Quinn (2019) performed an LCA to examine the implications of various CO_2 delivery methods to a production-scale algal biorefinery, showing that only uncompressed, pure sources of gaseous CO_2 with pipeline transportation of 40 km or less, and compressed, supercritical CO_2 from pure sources for pipeline transportation up to 100 km contributed less than 0.02 $kgCO_2eq$ per 1 MJ of fuel to the overall system. In this study, the model was based on the use of SC-CO_2 for lipid extraction using a compressed, supercritical CO_2 from pure sources, without considering pipeline transportation, which accounted for 0.237 $kgCO_2eq$ per 1 MJ of biodiesel added to the contribution analysis of the system. This fact could be corroborated in the sensitivity analysis whose parameters (CO_2 and methanol) do not have sensitivity effects on the analyzed system. Although the GWP achieved in this study for algal biodiesel does not meet the mandatory thresholds in the international standards (European Union, 2009; Sissine, 2010), which is 0.045 $kgCO_2eq\ MJ^{-1}$, it would be possible to meet the requirement by replacing technology by lower impact options in the processes.

4.3. Water consumption

For water consumption, the results showed that although microalgae cultivation is a water-intensive step, not only for the preparation of the culture medium but also for its thermoregulation, this water consumption is minimized by 90% of wastewater reuse. Currently, this approach is considered a best practice in this sector, since the business as usual scenario is not reusing wastewater in the industry, generating high water consumption and increasing the environmental impacts of the system. Martins et al. (2018a) evaluated the water footprint of microalgae production in a closed pilot-scale PBR on a gate-to-gate approach, showing that the total water footprint lies in the range of 2.4-6.8 m^3 per kg of dry biomass. In this study, water consumption of 17.6 m^3 per 1 MJ of biodiesel was achieved, which corresponds to 0.47 m^3 of water per kg of dry microalgal biomass.

Results showed that the use of N and P for the microalgae culture medium was not a major contributor to the environmental impacts of the system, and had no sensitivity regarding the impact categories. Some studies have reported that the use of wastewater as a culture medium could minimize the environmental impacts of microalgae culturing. Schneider et al. (2018) performed an LCA of the production of microalgae *Desmodesmus subspicatus* in raceway ponds, 4 tanks of 2,000 L, considering its cultivation in wastewater or NPK medium and different configurations of biomass separation. Results showed that using wastewater to cultivate microalgae, instead of NPK, causes fewer environmental impacts. In this study, the low impact of fertilizer use can be explained mostly by the reuse of water after the harvesting process, which decreases the amount of effluent generated and sent to wastewater treatment.

5. CONCLUSION

Biofuel production is paramount in moving towards the decarbonization of transportation systems. Microalgae-based biodiesel production is strategic for this purpose as microalgae cultivation contributes to lower land use impacts when compared to other feedstock and does not compete with food production. This study conducted an LCA of microalgae-based biodiesel production from an autochthonous strain of *P. tricornutum* in Central-South Chile to achieve a better understanding of its environmental impacts. The study considered a scaled-up facility using real experimental data obtained from a pilot plant located in Concepción, Chile.

The LCI compiled in this study considered site-based process data and environmental parameters. Several potential environmental impact categories were assessed, allowing for the identification of cultivation and downstream processes as the main contributors to the total environmental burdens. The results show that PBR construction materials and energy use are the main contributors to environmental impacts. Finding possible ways to reduce the energy intensity of biodiesel production, for example using more renewable energy sources, and developing more sustainable alternative materials for PBR construction, would decrease the life cycle's environmental impacts.

The interest in biofuels from microalgae has steadily increased over the years. However, it is not feasible to make a comparison across studies, assessing environmental impacts, due to the differences in the cultivation process and the gap that exists between theoretical studies and those using real process data. Therefore, this study brings the opportunity for Governmental agencies and decision-makers to subsidize the implementation of public policies to foster the research and development of non-conventional renewable energies in Chile. However, future studies are under preparation to investigate the economic feasibility of this approach under the assessed scenario.

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Table 1. Inventory of mass flow for the infrastructure, considering one year of operation. All values are reported in terms of kg of mass material per kg of biodiesel.

Parameter	**Inputs**	**Value**	**Unit.kg^{-1}**
	Infrastructure		
Capital goods	Land	1.66E-04	ha
	PMMA	0.218	m^3

Table 2. Monthly parameters used to calculate biomass production.

Parameter[1]	**Months**											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
T (°C)	18.4	17.1	15.2	12.3	12.9	8.60	9.30	10.4	12.1	12.6	15.1	16.3
RH (%)	71.7	70.2	77.2	81.2	90.0	85.8	87.7	85.5	77.2	80.1	72.2	71.7
PREC (mm)	1.8	0.0	14.2	67.2	117.6	3.4	189.4	54.0	49.8	71.8	14.4	32.4
GRAD (MJ$\cdot$kg^{-1})	735	775	663	501	320	449	308	416	598	604	740	621

[1] T= Temperature (Chile, 2018); RH = Relative humidity (DGAC, 2017); PREC = Precipitation (DGAC, 2017); GRAD = Monthly global radiation (calculated).

Table 3. Inventory of flow mass for upstream processes, considering one year of operation. All values are reported in terms of kg of mass material per kg of biodiesel.

Feedstock	Inputs	Value	Unit.kg^{-1}	Outputs	Value	Unit kg^{-1}
Cultivation						
	$ZnCl_2$	2.62E-07	kg	--	--	--
	$CoCl_2$.6H2O	2.50E-07	kg	--	--	--
	$(NH4)_6Mo_7O_{24}.4H_2O$	1.12E-07	kg	--	--	--
	$CuSO_4.5H_2O$	2.50E-07	kg	--	--	--
	B12 Vitamin	1.25E-08	kg	--	--	--
	B1 Vitamin	1.25E-08	kg	--	--	--
	H Vitamin (Biotin)	2.50E-08	kg	--	--	--
Chemicals	$FeCl_3.6H_2O$	1.62E-02	kg	--	--	--
	$MnCl_2.2H_2O$	4.50E-03	kg	--	--	--
	H_3BO_3	0.42	kg	--	--	--
	EDTA	0.56	kg	--	--	--
	$NaH_2PO_4.2H_2O$	0.25	kg	--	--	--
	$NaNO_3$	1.25	kg	--	--	--
	Na_2SiO_3	0.21	kg			
	CO_2	-24.06	kg	--	--	--
Energy	Electricity	1.07	kWh	--	--	--
Water	Freshwater	585.31	m^3	--	--	--
	Seawater	13.07	m^3	Water reuse	-11.77	m^3
Biomass	--	--	--	Culture	12.50	m^3
Harvesting						
	--	--	--	Wastewater	1.31	m^3
Water	--	--	--	Phosphorus-lost	0.22	kg
	--	--	--	Nitrogen-lost	0.51	kg

Energy	Electricity	20.25	kWh	--	--	--
Biomass	Culture	12.50	m^3	Biomass Paste	79.97	kg

Table 4. Inventory of mass flows for the downstream processes, considering one year of operation. All values are reported in terms of kg of mass material per kg of biodiesel.

Process stage	**Inputs**	**Value**	**Unit kg^{-1}**	**Value**	**Unit**	**Unit kg^{-1}**
Drying						
Water	Freshwater	67.97	m^3	Water vapor	64.97	kg
Energy	Electricity from the grid	40.79	kWh	--	--	--
Biomass	Paste Biomass	79.97	kg	Dried biomass	12.00	kg
	--	--	--	Lost biomass	2.17	kg
Cell disruption						
Energy	Electricity from the grid	22.43	kWh	--	--	--
Biomass	Dried biomass	12.00	kg	Processed biomass	12.00	kg
Lipid extraction						
Energy	Electricity from the grid	7.50	kWh	--	--	--
Biomass	Processed biomass	12.00	m^3	Residual biomass	10.97	kg
Gas	CO_2	9.57	kg	--	--	--
Oil	--	--		Lipid	1.03	kg
Refining						
Chemicals	H_3PO_4.	1.05E-04	kg	--	--	--
	NaOH	3.16E-04	kg	--	--	--
	$C_6H_8O_7$	6.62E-05	kg	--	--	--

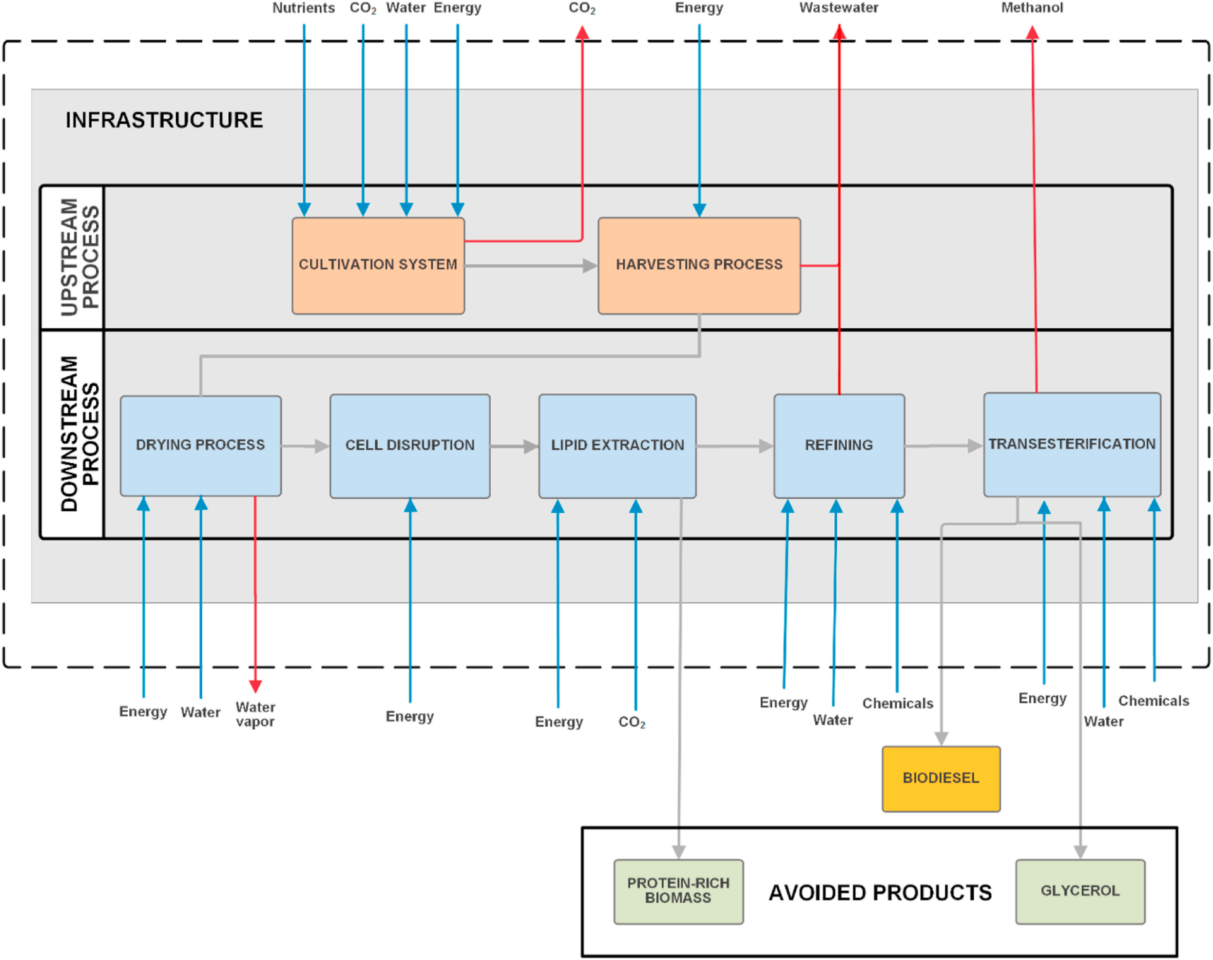
Water	Wash water	4.10E-02	m^3	Wastewater	0.05	m3
Energy	Electricity from the grid	3.17E-02	kWh	--	--	--
Oil	Lipid	1.03	kg	Refined oil	1.02	kg
			Transesterification			
Chemicals	KOH	0.01	kg	--	--	--
	CH_3OH	0.22	kg	CH_3OH	0.11	kg
	--	--	--	CH_3OH recovered	0.10	kg
	H_2SO_4	8.87E-03	kg	--	--	--
Water	Wash water	0.25	m^3	--	--	--
Energy	Electricity from the grid	0.06	kWh	--	--	--
Products	Refined oil	1.02	kg	Biodiesel	1.00	kg
	--	--	--	Glycerol	0.10	kg
	--	--	--	Residual biomass	10.97	kg

Table 5. Sensitivity analysis scenarios obtained by variations of ±10 % in each input parameter. The output is indicated for all impact categories.

Impact category*	**Energy consumption (%)**	**N content (%)**	**P content (%)**	**CO_2 (%)**	**Water Reuse (%)**	**Transportation distance (%)**	**Methanol (%)**
GWP	±2.65	±0.29	±0.04	±0.35	±16.04	±3.25	±0.02
SOD	±1.73	±5.51	±0.01	±0.12	±9.72	±1.39	±0.01
IOR	±0.71	±0.54	±0.09	±2.68	±1.83	±4.23	±0.01
OFH	±3.96	±0.17	±0.03	±0.11	±0.04	±6.37	±0.00
FPF	±7.83	±0.07	±0.02	±0.07	±1.25	±0.81	±0.00
ODF	±3.87	±0.16	±0.03	±0.11	±0.06	±6.29	±0.01
TAC	±3.43	±0.21	±0.06	±0.16	±5.86	±2.63	±0.01
FWE	±5.33	±0.19	±0.10	±0.44	±0.01	±1.17	±0.02
TEC	±1.23	±0.24	±0.04	±1.17	±0.22	±7.00	±0.00
FEC	±4.01	±0.32	±0.14	±0.65	±0.01	±4.24	±0.02
MEC	±3.76	±0.32	±0.14	±0.61	±0.03	±4.56	±0.02
HCT	±3.98	±0.21	±0.52	±0.41	±0.04	±3.98	±0.05
HNT	±3.33	±0.34	±0.11	±0.63	±0.04	±5.00	±0.01
LUS	±5.69	±0.09	±0.13	±0.23	±0.00	±2.32	±0.00
MRS	±0.92	±0.48	±0.18	±0.83	±0.17	±6.31	±0.01
FRS	±2.48	±0.15	±0.03	±0.19	±3.03	±3.62	±0.01
WAC	±0.00	±0.00	±0.00	±0.00	±0.16	±0.00	±0.00

* GWP - Global Warming Potential (kg CO_2 eq); SOD - Stratospheric Ozone Depletion (kg CFC11 eq); IOR - Ionizing Radiation (kBq Co-60 eq); OFH - Ozone Formation, Human Health (kg NOx eq); FPF - Fine particulate matter Formation (kg PM2.5 eq); OFT - Ozone formation, Terrestrial ecosystems (kg NOx eq); TAC - Terrestrial Acidification (kg SO_2 eq); FEW - Freshwater Eutrophication (kg P eq); TEC - Terrestrial Ecotoxicity (kg 1,4-DCB eq); FEC - Freshwater Ecotoxicity (kg 1,4-DCB eq); MEC - Marine Ecotoxicity (kg 1,4-DCB eq); HCT - Human Carcinogenic Toxicity (kg 1,4-DCB eq); HNT - Human Non-carcinogenic Toxicity

(kg 1,4-DCB eq); LUS - Land Use (m^2a crop eq); MRS - Mineral Resource Scarcity (kg Cu eq); FRS - Fossil Resource Scarcity (kg oil eq); WAC - Water Consumption (m^3).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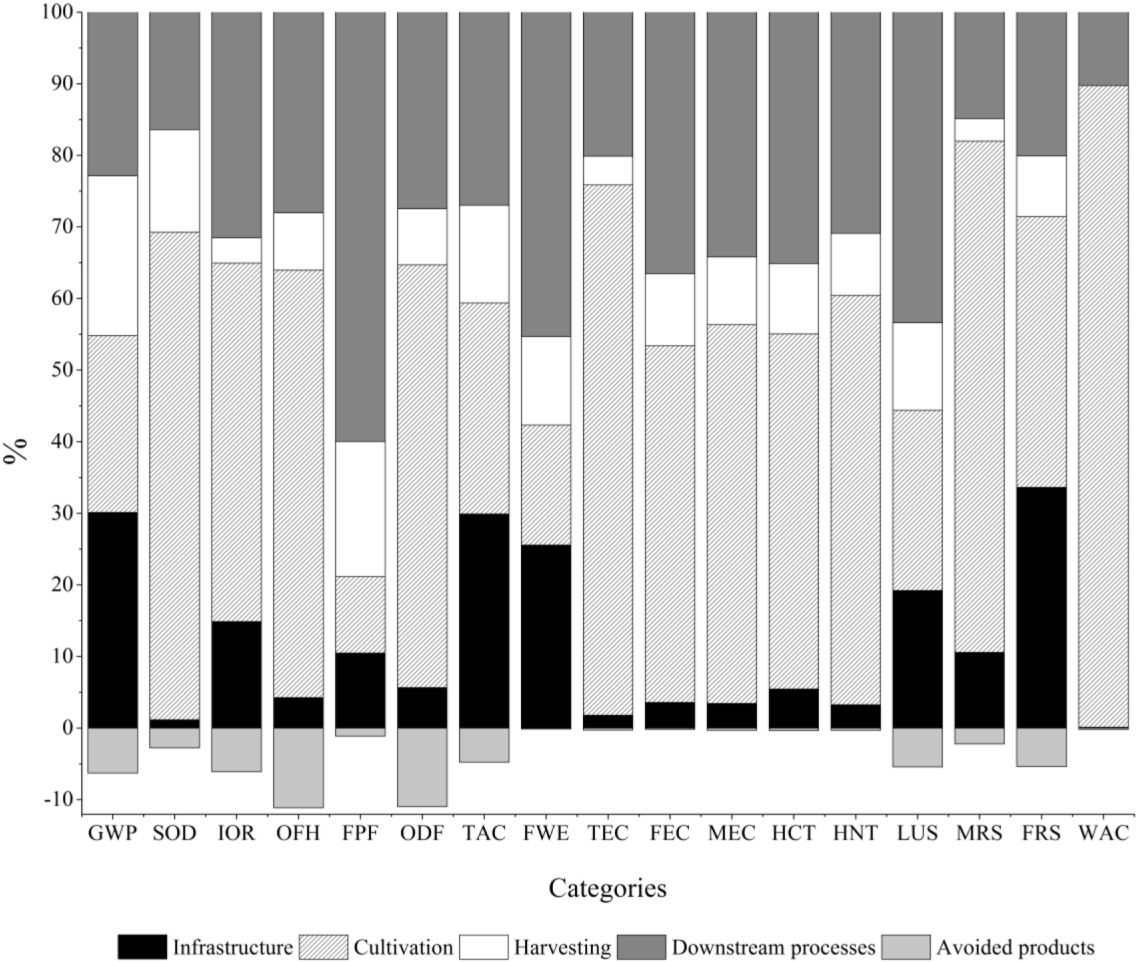
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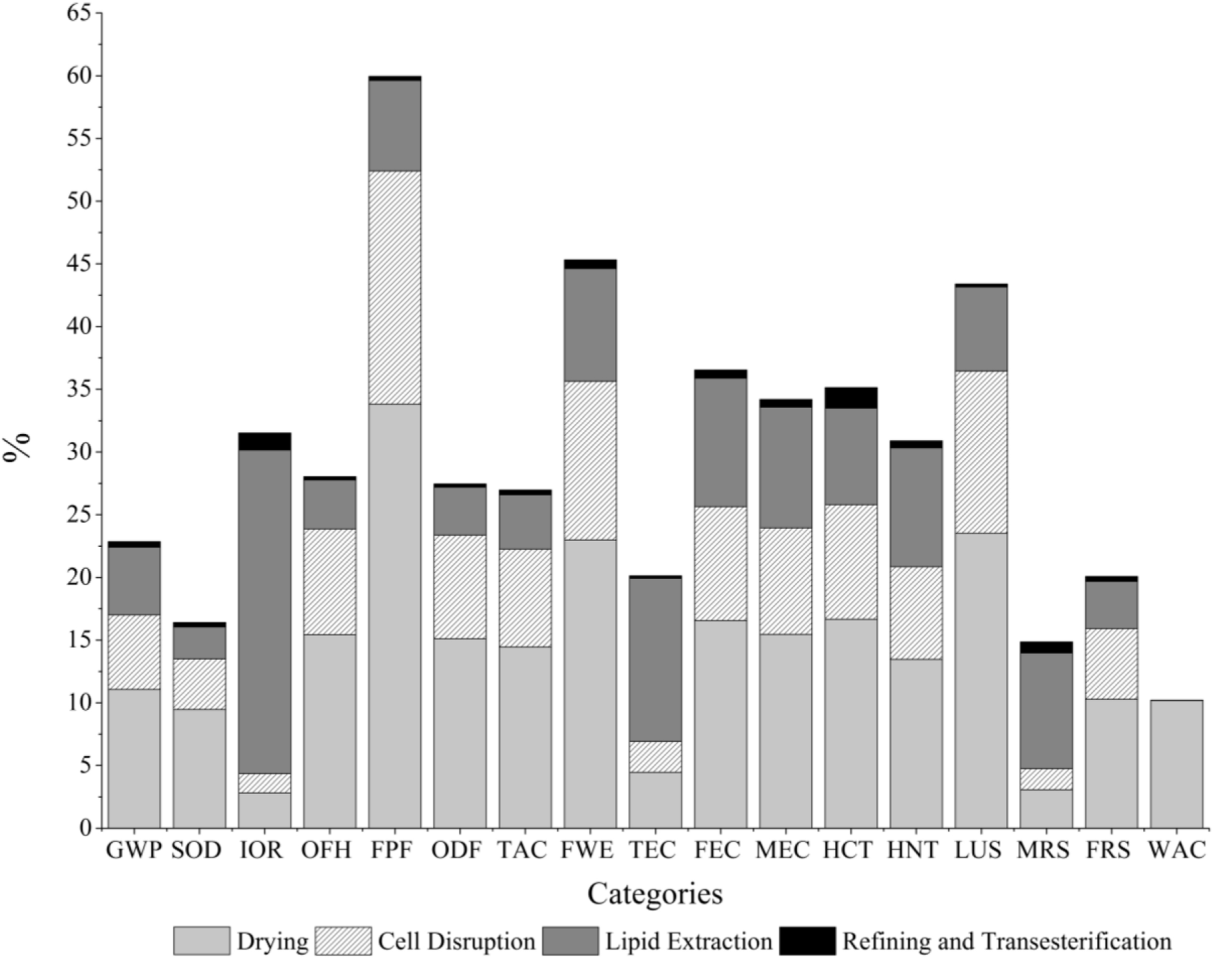

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LUS
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FRS
WAC
Categories
Drying
Cell Disruption
Lipid Extraction
Refining and Transesterification

- Life cycle assessment of microalgal biodiesel was conducted for a Chilean scenario.
- Inventory data of microalgae cultivation was obtained from real pilot-scale PBR.
- An autochthonous strain of *Phaeodactylum tricornutum* was cultivated.
- PBR construction materials and energy consumption are critical aspects of the system.
- A total of 5.74 kgCO_2 equivalent is emitted per 1 MJ of microalgal biodiesel.

Declarations of interest

Declarations of interest: none.