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Life cycle assessment of lipid production from *Pavlova gyrans*: Influence of the culture medium composition

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ABSTRACT

Microalgae have been increasingly recognized as an alternative source of lipids, especially polyunsaturated fatty acids (PUFA) such as omega 3 and 6, being essential to carefully assess their life cycle environmental impacts. Hence, this work aims to carry out a life cycle assessment (LCA) of lipid production from the microalga *Pavlova gyrans* grown in different culture media and nutrient concentrations, on a "cradle-to-gate" approach. For the life cycle inventory, primary data obtained from laboratory experiments was used for the foreground processes, complemented with secondary data from LCA databases and literature for the background processes. The functional unit chosen is 1 g of lipids extracted. For the environmental impact assessment, the ReCiPe 2016 method was used, evaluating 18 midpoint indicators from an egalitarian perspective. An uncertainty estimate was performed using experimental results and the ANOVA statistical test. Results show that the organic fertilizer medium has an overall environmental impact around 1.28 to 2 times lower than the aquaculture effluent medium. Energy is the process hotspot, contributing more than 95%, on average, to the overall potential environmental impact. Alternative renewable energy scenarios were therefore evaluated. Results show that, compared to the electricity mix, renewable energies led to significant reductions in 13 environmental impact categories, such as global warming potential, which decreased by around 70 and 90% respectively for solar and wind energy, but to significant increases in 5 impact categories, such as terrestrial ecotoxicity, which increased by around 231 and 184%, and the mineral resources scarcity, which increased by around 114 and 302% respectively for solar and wind energy. The results of this study can provide valuable information to guide research and development efforts towards more sustainable processes for obtaining lipids from microalgae.

1. Introduction

Fatty acids are integral building blocks of lipids, which are fundamental to living organisms as they provide stored energy, form the structure of cells and help in the synthesis of some active substances (Sokoła-Wysoczańska et al., 2018). Free fatty acids are classified according to the degree of saturation of their molecular chains as: (i) saturated fatty acids (SFA), if their molecule contains only single bonds between carbon atoms; (ii) monounsaturated fatty acids (MUFA), if there is only one double bond between the carbon atoms of their molecular chain, and as (iii) polyunsaturated fatty acids (PUFA), if there are two or more double bonds between the carbon atoms of their molecular chain (Mata et al., 2020). The latter is further subdivided into two groups: omega-6 (n-6 PUFA) and omega-3 (n-3 PUFA), of which linoleic acid (LA) and alpha linoleic acid (ALA) are respectively the essential fatty acids (EFA) of each group (Saini and Keum, 2018).

Unlike some algae and plant species, humans do not possess enzymes 12 and 15 desaturases, without which there is no conversion of oleic acid

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to LA and of LA to ALA, respectively (Lee et al., 2016; Cholewski et al., 2018). Therefore, humans must ingest omega-3 and omega-6 fatty acids in their diet (Minihane et al., 2016). Additionally, these n-6 and n-3 PUFA are metabolites with nutritional applications and potential benefits for human health as they have properties that protect against inflammatory and cardiovascular diseases (Calder, 2015; Lavie et al., 2009). The problem is the unbalanced ingestion between n-6 and n-3 PUFA. According to studies, in Western culture, this ratio of n-6/n-3 fatty acids ranges from 15 to 16.7/1 (Mariamenatu and Abdu, 2021). This ultimately leads to production of eicosanoids that carry out inflammatory functions, vasoconstriction and platelet aggregation (Harnack et al., 2009; Simopoulos, 2010). Therefore, there is a growing demand for omega-3 fatty acids in the form of supplements, since their ingestion through natural foods may cause problems to human health, as it is the case of some fish and shellfish species, which despite being the largest source of omega-3, expose humans, especially infants, to the neurotoxic effects of methylmercury (Antunes dos Santos et al., 2016; Syversen and Kaur, 2012). One alternative would be to produce these marine species in captivity (aquaculture). However, since they depend on the acquisition of omega-3 and omega-6 from other species at lower trophic levels, in captivity these fatty acids would have to be supplied. It is therefore necessary to obtain n-3 and n-6 PUFAs from alternative sources, such as from microalgae (Corrêa et al., 2021; Mata et al., 2010).

Microalgae belong to the first trophic level and are a rich source of carbohydrates, proteins and lipids, as they can reach a lipid content between 30 and 70 % by dry weight, 10–30 % of which are polyunsaturated fatty acids (Oliver et al., 2020; Geada et al., 2023). For example, microalgae *Nannochloropsis* and *Pavlova* sp. are rich in lipids (Rodolfi et al., 2009) and in particular, eicosapentaenoic acid (C20:5, n-3, EPA) and docosahexaenoic acid (C22:6, n-6, DHA) (Lai, 2015; Meireles et al., 2003). Additionally, it is possible to further increase this percentage through lipid induction methods, such as exposing microalgae to stress conditions during cultivation (Gorgich et al., 2020). However, this type of cultivation strategies may result in lower biomass yields (Mata et al., 2016), so they should be studied thoughtfully.

Microalgae growth regimes can be distinguished as autotrophic, heterotrophic or mixotrophic, depending on the medium's carbon source, whether it is inorganic, organic, or a combination of both (Cunha et al., 2023). Under certain cultivation conditions, microalga *Pavlova* sp. can reach a 33.22 % and 10.5 % content of EPA and DHA, respectively (Pettersen et al., 2010), but has only a biomass productivity of about 0.28 g/L/day (Patil et al., 2007). In addition, microalgae can capture atmospheric carbon dioxide, have low nutrient requirements for cultivation and, from a biochemical perspective, are capable of contributing to a range of high-value products such as proteins, pigments, vitamins, minerals and carbohydrates (Machado et al., 2022; Geada et al., 2021; Morais Junior et al., 2020). However, due to the need to assess microalgae safety before marketing, restrictive regulations may prevent/delay their market access, making authorization procedures lengthy and costly (Lafarga, 2019).

On the other hand, by integrating environmental considerations into the early stages of process development, producers can not only minimize negative environmental impacts but also identify opportunities for innovation and competitive advantage in a rapidly changing market driven by sustainability concerns. With this idea in mind, many researchers and industrialists have been adopting Life Cycle Assessment (LCA) at lower Technology Readiness Levels (TRLs) as a methodology for assessing the environmental performance of their products at an early stage of process development. This is because early assessment allows producers to identify and address potential environmental impacts before significant investments are made. This provides an opportunity to explore alternative production routes, materials, or technologies that may have lower environmental footprints.

Hence, this study aims to conduct an LCA study to compare the environmental performance of microalgae lipids production from *Pavlova gyrans* (*P. gyrans*), grown on two different culture media at different nutrients concentration. Most of the literature studies involving the LCA of microalgae are aimed at evaluating the production of biofuels (Handler et al., 2014; Branco-Vieira et al., 2020). To the best of the authors' knowledge, this is the first LCA study on lipid production from microalgae *P. gyrans*. With regard to applications other than biofuels, only two LCA studies have been published on lipid production from autotrophic or mixotrophic microalgae influenced by different culture media, but none focus on the microalgae *P. gyrans*. One is the work of Togarcheti and Padamati (2021) that evaluated the life cycle environmental impacts of PUFA production from microalgae in comparison with farmed fish. These authors (Togarcheti and Padamati, 2021) concluded that PUFA derived from microalgae could potentially replace fish oil, thus reducing the pressure on oceans. The other is the study of Gaber et al. (2022) that developed a LCA to assess the environmental sustainability of cultivating microalga *Nannochloropsis oceanica* for fatty acids production, concluding that about 60–80% of the impacts are due to the energy consumption for plant operation, infrastructure and nutrients consumption.

Although they did not evaluate the influence of different culture media, Qin et al. (2023) carried out a LCA of the production of EPA on a laboratory scale by *Phaeodactylum tricornutum*. The results indicated that metabolic engineering led to the most significant reductions (>90 %) in terms of global warming potential and energy consumption, due to the increase in biomass productivity and EPA yield. Electricity consumption is the main contributor to the associated environmental impact, followed by extraction solvents, especially chloroform. In terms of processes, the cultivation of the microalgae and the harvesting of the biomass were responsible for most of the overall environmental impact, in particular due to the long cultivation period of the microalgae and the biomass freeze-drying process.

In order to determine which conditions for microalgae cultivation contribute to lower environmental impacts, this study is based on experimental laboratory data on microalgae cultivation, thus making it possible to better control cultivation conditions (e.g., composition of the culture medium, temperature, pH, lighting, stirring and other relevant parameters).

It should be highlighted that this work does not intend to develop the industrial process, but rather to carry out an analysis based on laboratory data as part of the process to point out future paths for application on an industrial scale. In this sense, this study can provide valuable information about the environmental impacts of certain process routes, materials and energy source, as well as possible solutions or improvements to mitigate these impacts. Thus, this study analyses different environmental impact categories, pollutant emissions, consumption of natural resources, toxicity of chemical products, among others.

Based on the results of this study, industrialists and researchers can develop and test new technologies, processes or practices that have the potential to be implemented on an industrial scale in the future. It is important to note that laboratory studies generally represent an initial stage in the process of developing and implementing large-scale environmental solutions. They can help identify challenges, optimize processes and validate concepts before investments are made on a larger scale.

2. Materials and methods

The LCA methodology as described by the ISO14040 (ISO 14040, 2006) and 14044 (ISO 14044, 2006) standards, involve four interactive phases: (i) Goal and scope definition; (ii) Life cycle inventory (LCI); (iii) Life cycle impact assessment (LCIA), and (iv) Interpretation. This section presents the assumptions and considerations in each of these four phases of the LCA of lipid production from *Pavlova gyrans*, comparing the influence of the culture medium composition.

2.1. Goal of the study

The main objective of this LCA study is to compare the environmental performance of microalgae lipids production from *P. gyrans*, comparison of two agro-industry by-products used as a medium for growing microalgae: an organic fertilizer medium (OFM) and an aquaculture effluent medium (AEM) at different nutrient concentrations (as described in section 2.3).

As specific objectives, this work aims to identify the life cycle stages that contribute most to the environmental impacts and to analyze improvement scenarios in relation to the process hotspots, i.e., those with the greatest contribution to environmental impacts.

This LCA study is an attributional type, since the environmental impacts refer to a product, instead of changes on an existing system.

2.2. Scope of the study

2.2.1. Functional unit

The functional unit (FU) selected for this LCA study is 1 g of lipids derived from microalgae *P. gyrans*. This choice aligns with the primary purpose of the system, which is lipid production. Additionally, using a mass-based functional unit it is consistent with other LCA studies on lipid production from microalgae (Gaber et al., 2022). Therefore, we opted for 1 g of lipids to ensure comparability with existing studies and because of the magnitude of values obtained at the laboratory scale.

2.2.2. System boundary

From a "cradle-to-gate" perspective, this study considers the life cycle stages from microalgae cultivation, biomass harvesting, drying and lipids extraction, as shown in Fig. 1. The stages after lipid extraction, i.e. purification, packaging, use, recycling, and final disposal are outside the boundary considered for this study.

For the preparation of this work, the process and technologies implemented were based on laboratory research conducted by Madureira (2019). The cultivation and production of microalga *P. gyrans* took place in 250 mL Erlenmeyer flasks, using a medium volume of 100 mL, for 14 days. In autotrophic conditions, the Erlenmeyer flasks were put in an orbital shaker at room temperature, with 100 μmol photons $m^2 s^{-1}$ of continuous illumination. The harvesting stage was performed using a centrifuge (EBA 200, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany), during 20 min at 3000 g. The drying stage was performed in a freeze dryer (Alpha 1–4 LD Plus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) at −50 °C and 0.05 mbar for 3 days.

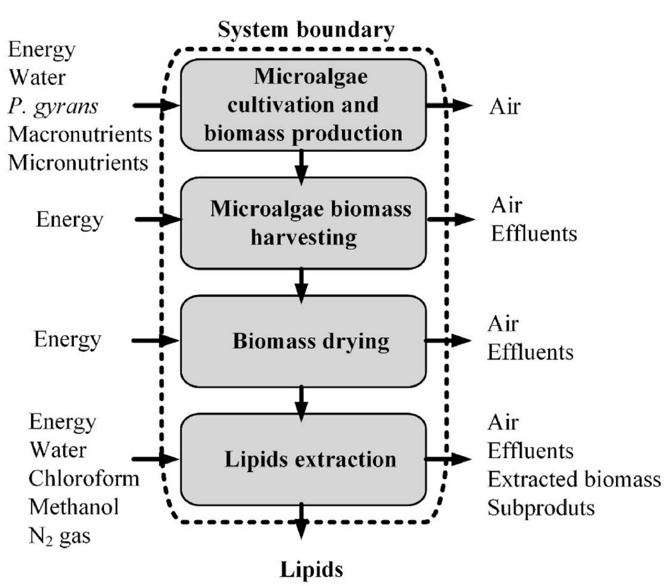

Fig. 1. System boundary definition for the LCA study, including the key process steps considered, from microalgae cultivation to lipid extraction.

The lipid extraction from microalgae biomass was performed according to a modified Bligh & Dyer method (Gorgich et al., 2020). The lipids extraction process started by adding 1 mL of a solvent mixture, chloroform/methanol (2:1, v/v) to 50 mg of microalgae powder. Then, homogenizing and incubation occurs via vortex (2 min) and heating block (30 °C, 30 min), respectively. The mixture was then centrifuged at relative centrifugal force of 2012 g for 10 min. The supernatant was poured into a pre-weighed glass tube and the biomass deposit was re-extracted seven more times, with the methods mentioned above, until no pigmentation was present. The resultant supernatant was then dried under N_2 gas. To remove any contamination, the original extract was re-dissolved in 2 mL of chloroform and 1 mL methanol, vortexed, and 0.75 mL of water were added, followed by vortex again (2 min). Finally, to promote phase separation, a centrifuge was used with the previous conditions. The organic phase was collected to a new pre-weighted tube, and the aqueous phase was re-extracted by adding 2 mL of chloroform. The collective organic phases were then dried under N_2 stream and weighted.

2.3. Life-cycle inventory: data and assumptions

The information and data used for the life cycle inventory in this work was provided in Madureira (2019) based on laboratory experiments. All the inventory data for this work is presented in Tables S1–S4 of the supplementary data. Table S5 presents the Ecoinvent V3.5 datasets considered in this work and used in the environmental impact calculations.

The calculations, estimates and assumptions for some of the inventory data are described in the following sections.

Due to the absence in the ecoinvent V3.5 database of characterization factors for the following components: ammonium molybdate, zinc chloride, manganese (II) chloride, cobalt (II) chloride, monopotassium phosphate, it was necessary to perform their modelling (Geisler et al., 2004). This was carried out according to the "life cycle tree" model (shown in the supplementary information), in which the reactants necessary for the production of these compounds were considered. The modelling of ammonium molybdate, zinc chloride, manganese (II) chloride, cobalt (II) chloride and monopotassium phosphate can be found as supplementary information in Figs. S1, S2, S3, S4 and S5, respectively.

For electricity, the Portuguese electricity mix as defined in the ecoinvent V3.5 database was considered.

2.3.1. Culture media and lipids extraction

As mentioned above, two culture media (OFM and AEM) were studied for the growth of microalgae, using different concentrations of micronutrients and macronutrients. The OFM is similar to the Conway medium and it is composed by enriched solutions of macronutrients, micronutrients and iron. The OFM has the following base composition (in mg L^{-1}): KNO_3, 100.0; Na_3PO_4, 20.00; $(NH_4)_6Mo_7O_{24}.4H_2O$, 1.8; $CuSo_4.5H_2O$, 4; $ZnCl_2$, 4.2; $MnCl_2.4H_2O$, 36.00; $FeCl_3.6H_2O$, 1.30; $CoCl_2.6H_2O$, 4.00; H_2BO_3, 33.40; $Na_2H_2EDTA.2H_2O$, 45.00. The OFM experiments were performed by adding 0.016% of the organic macronutrients (Potassium nitrate, Trisodium phosphate and Ammonium molybdate) to the enriched organic micronutrients solution at different concentrations of 0.0002% (M1), 0.002% (M2) and 0.004% (M4) (v/v) supplemented with iron and Conway vitamins solution (more information available as supplementary data, Table S1).

The synthetic AEM has the following base composition (g L^{-1}): NaCl, 27.00; $MgSO_4.7H_20$, 6.60; $CaCl_2$, 1.50; KNO_3, 1.00; KH_2PO_4, 0.07; $FeCl_3.6H_20$, 0.014; Na_2EDTA, 0.019, and 1 mL L^{-1} of a microelement solution (containing in mg L^{-1}: $ZnSO_4.7H_20$, 40.00; H_3BO_3, 600.00; $CoCl_2.6H_2O$, 1.50; $CuSO_4.5H_2O$, 40.00; $MnCl_2$, 400.00 and

$(NH_4)_6Mo_7O_{24}.4H_2O$, 370.00) (more information available as supplementary data, Table S2). For the AEM, in order to simulate the seasonal variations of water bodies, it was compared different theoretical concentrations of macronutrients: nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-}), as follows respectively.

- Phosphate intermediate effluent (PIE): 2.97, 0.048 and 0.072 mM
- Phosphate rich effluent (PRE): 3.81, 0.045 and 0.035 mM
- Phosphate limited effluent (PLE): 4.64, 0.042 and 0.002 mM

The auxiliary materials (chloroform, methanol, and nitrogen gas) and water used in the system were considered, according to the different microalgae culture media and concentrations (available as supplementary information in Table S3).

As direct emissions from the foreground processes there is only the wastewater corresponding to the spent microalgae culture medium. This corresponds to a dilute aqueous solution with some organic and nitrogen content. As they do not have toxic or hazardous components, they can be treated as urban wastewater. Therefore, in this work, they were treated as residual wastewater (referred to as "end-of-life cycle (EOL)"), based on the life cycle inventory data available in the ecoinvent 3.5 database.

Other emissions from background processes, associated with the production of the media components (micronutrients and macronutrients), consumables (nitrogen, chloroform and methanol), transportation fuels, water and electricity, were considered in the data sets obtained from the ecoinvent 3.5 database.

2.3.2. Electricity consumption and improvement scenarios

To obtain the energy consumption E (kWh) values (available as supplementary information in Table S4), Equation (1) was used. The power value, Po, was taken from the equipment used in the experiments, and considering the time, t, of the equipment usage.

$$E = Po \times t \tag{1}$$

Although some equipment has energy efficiency functions, in all energy calculations the power indicated by the instrument was taken into consideration. This resulted in an overestimation of the energy consumed, i.e., a worst-case scenario.

The energy consumed in the process is that obtained from the Portuguese electricity grid and thus, the Portuguese electricity mix was considered.

As energy consumption greatly affects the overall environmental impact of the process, it is crucial to find options with lower potential environmental impacts. Therefore, in this work energy scenarios to reduce environmental impacts are analyzed and compared with the reference scenario - the Portuguese electricity mix - focusing on changing the energy source to exclusively renewable sources: solar and wind energy.

Solar and wind energy are often considered clean and environmentally friendly sources of energy since both solar photovoltaic panels and wind turbines have no emissions during use. However, from a life cycle perspective, emissions during the manufacturing, maintenance and disposal phases of the wind turbine and photovoltaic panel must be considered, which can be reduced through the use of recycled materials.

Therefore, two alternative energy scenarios were analyzed in this work involving the replacement of the Portuguese electricity mix by the following renewable energy sources.

(1) electricity produced by silicon photovoltaic solar panels,
(2) electricity produced in wind turbines with low voltage conversion.

The flexibility of photovoltaic technology allows for low-power capacity systems, which are convenient for local-scale processes and the ability to adjust the installed power according to production capacity.

The choice of wind energy was due to its high production rate in Portugal among renewable sources. In 2020, Portugal had around 26% of its average annual electricity demand covered by wind energy (Ramos et al., 2023), and is currently at the top of the countries with the greatest contribution of wind energy to the electricity consumed. Also, in Portugal, wind energy is exclusively generated at higher voltage. To evaluate their environmental impacts at lower voltage, it was necessary to consider the impacts of conversion from higher to lower voltage as well as the transmission factor. The impacts of conversion were determined by calculating the difference between environmental impacts at high voltage and low voltage, using SimaPro software and the ecoinvent databases. Additionally, according to the Trezze's report (Itten et al., 2014) on LCI of European Electricity Mixes and Grid, the transmission losses at low voltages are around 11.5 %. Thus, the impacts of wind energy at low voltage are calculated by adding the high voltage impacts of a 3 MW onshore wind turbine with the impacts of conversion, and then multiplying the previous result by the transmission factor (of 1.115).

2.3.3. Transportation

To account for the transportation of the components used in the system under study, it was considered that they could be acquired in Portugal. Therefore, it was considered an average distance of 200 km and the transportation was performed in 32-ton trucks. All the components (nutrients and auxiliary materials used in the process, except water) for which this average transport distance was considered, are listed in Tables S1–S3 of the supplementary data.

The transportation of components, TC_j, in each way (expressed in ton kilometers, tkm) was calculated using Equation (2).

$$TC_j = m_{t_j} \times d_m \tag{2}$$

Where m_{t_j} is the total mass of components in each medium j (expressed in tonnes, t) and d_m is the average distance.

2.3.4. Carbon capture by microalgae

As stated before, the cultivation of microalga *P. gyrans* was done under autotrophic conditions, in which microalgae capture CO_2 from the atmosphere (available as supplementary information in Table S6). According to Yang and Hur (2012), *Pavlova* sp. presents four major lipids: tetradecanoic acid (C14:0, $C_{14}H_{28}O_2$); palmitic acid (C16:0, $C_{16}H_{32}O_2$); palmitoleic acid (C16:1, $C_{16}H_{30}O_2$), and eicosapentaenoic acid (C20:5n-3, EPA, $C_{20}H_{30}O_2$). This allows a simple approach to consider the carbon capture related to lipidic production for each culture medium (available as supplementary information in Equations S1 and S2).

2.4. Life cycle impact assessment

2.4.1. Environmental impacts: categories and evaluation method

In the LCIA, the foundational steps lie in the categories and method selection. This process necessitates the identification of pertinent environmental impact categories that align with the dominant aspects of our system's inventory. For example, fossil resources combustion directly contributes to the environmental impact category of "climate change", due to CO_2 emissions, and indirectly through the consumption of non-renewable resources, leading to specific categories such as "mineral and fossil resource scarcity". Combustion also generates other pollutants, such as NO_X contributing to "eutrophication", "acidification", and "ozone depletion" impact categories, and SO_X contributing to the acidification impact category. These, along with particulate matter and hydrocarbon emissions, contribute to "photochemical smog" with impacts on human health and ecosystems. Additionally, microalgae cultivation heavily relies on water (Martins et al., 2018), justifying a dedicated environmental impact category for water use.

Therefore, in this study, the ReCiPe 2016 method (Huijbregts et al., 2017) was selected, following the Equalitarian (E) perspective, as it is the method adopted by the majority of studies in this area, according to a literature review (Collotta et al., 2016) that examined the most common environmental impact categories used in 16 LCA studies of lipid production from microalgae. The Equalitarian (E) perspective was chosen since according to Goedkoop et al. (2009) this is the most precautionary perspective, compared to the individualist (I) or hierarchist (H) perspectives, as it considers the longer-term impact types that are not yet fully established but for which some indication is available.

The life cycle impact assessment was conducted with the aid of SimaPro V8.5.2 software and its life cycle inventory databases such as ecoinvent V3.5.

2.4.2. Uncertainty estimation

The uncertainty associated with the environmental impacts of the microalgae lipid production process was calculated based on the average lipid content values and their standard deviation, according to real replicates ($n = 3$) carried out during the laboratory experiment. No estimation was made for other sources of uncertainty due to a lack of information. Statistical analysis was conducted using GraphPad Prism software V8.0.2 (from Dotmatics) with a One-Way ANOVA test and Tukey post-hoc test, to identify differences in mean values between medium and composition, for each impact category and different energy sources. The results are presented as mean ± standard deviation (SD). The statistical significance is indicated by different superscript letters (a, b, c) with a significance level of $p < 0.05$. Distinct superscript letters mean that the samples are statistically different from each other.

3. Results and discussion

3.1. Environmental impact assessment

A comparison was made of the potential environmental impacts for microalgae lipids production using two microalgae culture media (OFM and AEM) at different macronutrients concentrations: M4, M1 and M2 for OFM and PIE, PRE and PLE for the AEM, as shown in the graphs of Fig. 2i and 2ii, 2iii. Each graph refers to each of the 18 environmental impact categories analyzed. The process steps and components are analyzed to identify *hot spots* of the system under study. A measure of the uncertainty associated with microalgae cultivation is represented in the form of error bars (Fig. 2i and 2ii, 2iii). The subscript letters "a" or "b" refer to the Tukey's statistical test ($\alpha = 0.05$), where having the same letter means that there is no statistical difference between the conditions analyzed, while having a different letter means that there is a statistical difference between them.

For all the 18 environmental impact categories analyzed, the OFM has on average an overall environmental impact that is about 1.28–2 times lower than the AEM, regardless of the macronutrient concentrations considered. The M4 of the OFM shows consistently the lowest environmental impacts average values for all the 18 impact categories. This is because using the M4 culture medium (with the highest concentration of micronutrients), the lipid content is the highest of the various experiments performed (15.72 ± 2.53 wt%). However, the differences between the average values of M1, M2 and M4 are small (<20%). In comparison, for the AEM the maximum lipid content (10.87 ± 2.72 wt%) is reached with PIE (as shown in Table S7 of Supplementary Information).

On the other hand, considering the uncertainty analysis for OFM and AEM with varying concentrations, given by the results of Tukey's statistical test, shown in the graphs by letters "a" and "b", it is possible to verify for which of the conditions and media analyzed there is a statistical difference between the average values of the environmental impacts. The graphs therefore show that M1, M2 and M4 of the OFM have no statistical differences between them, as they all obtained the same letter "a". Also, PIE, PRE and PLE of AEM do not present statistical differences between them, as they all obtained the same letter "b". However, PLE has consistently the highest environmental impact value in all 18 categories analyzed, as it obtained letter "b" alone, thus being

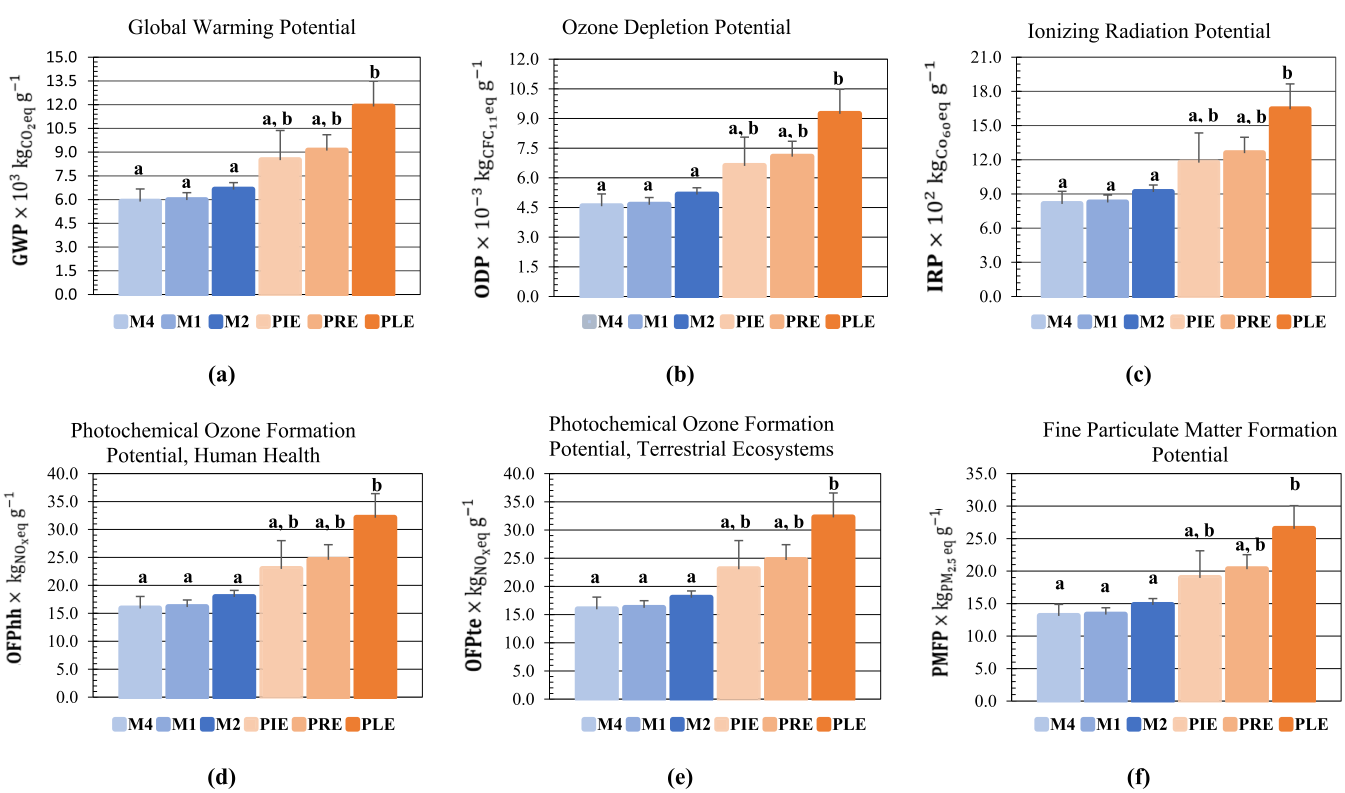

Fig. 2i. Potential environmental impacts of microalgae lipid production using two microalgae culture media (OFM and AEM) at different macronutrients concentrations: M4, M1 and M2 for OFM (blue) and PIE, PRE and PLE for the AEM (orange). The subscript letters refer to the Tukey's statistical test ($\alpha = 0.05$).

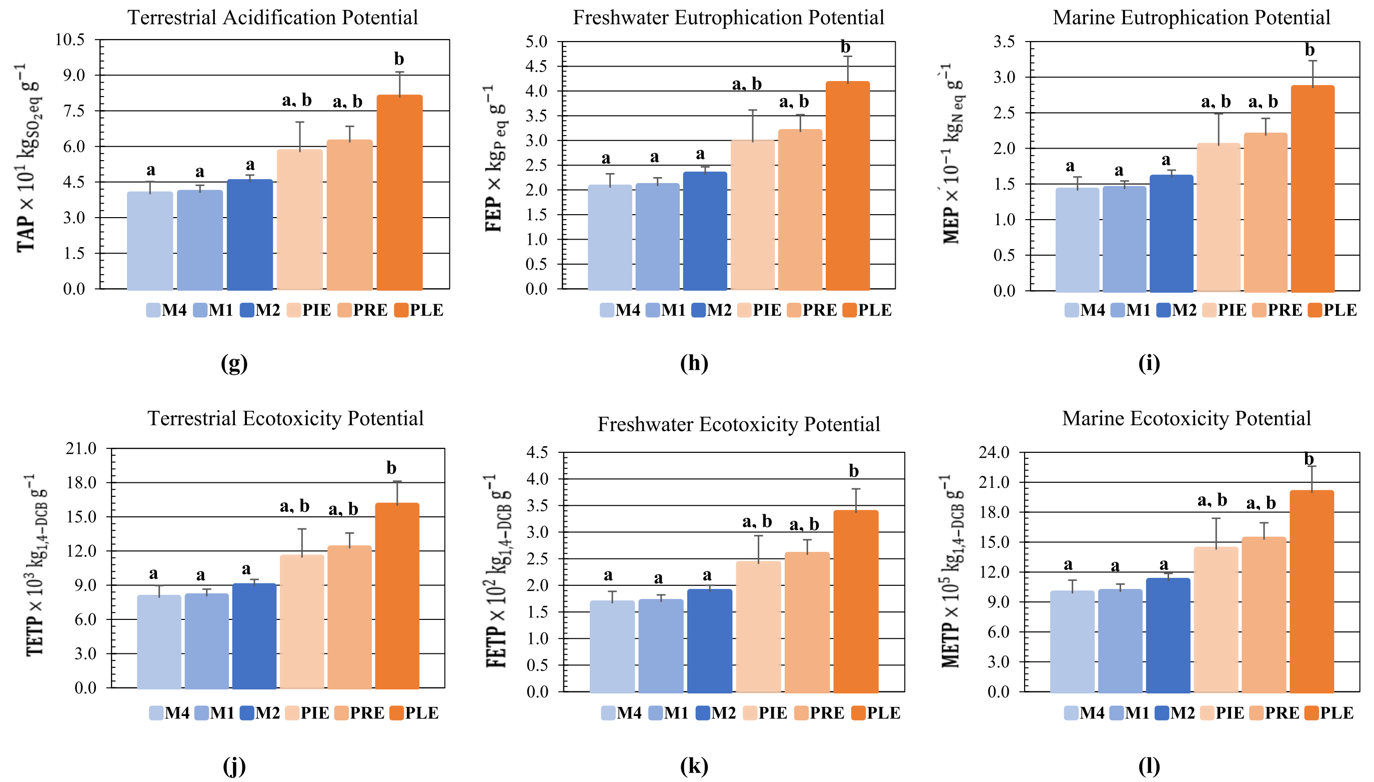

Figure 2ii. Potential environmental impacts of microalgae lipid production using two microalgae culture media (OFM and AEM) at different macronutrients concentrations: M4, M1 and M2 for OFM (blue) and PIE, PRE and PLE for the AEM (orange). The subscript letters refer to the Tukey's statistical test ($\alpha = 0.05$).

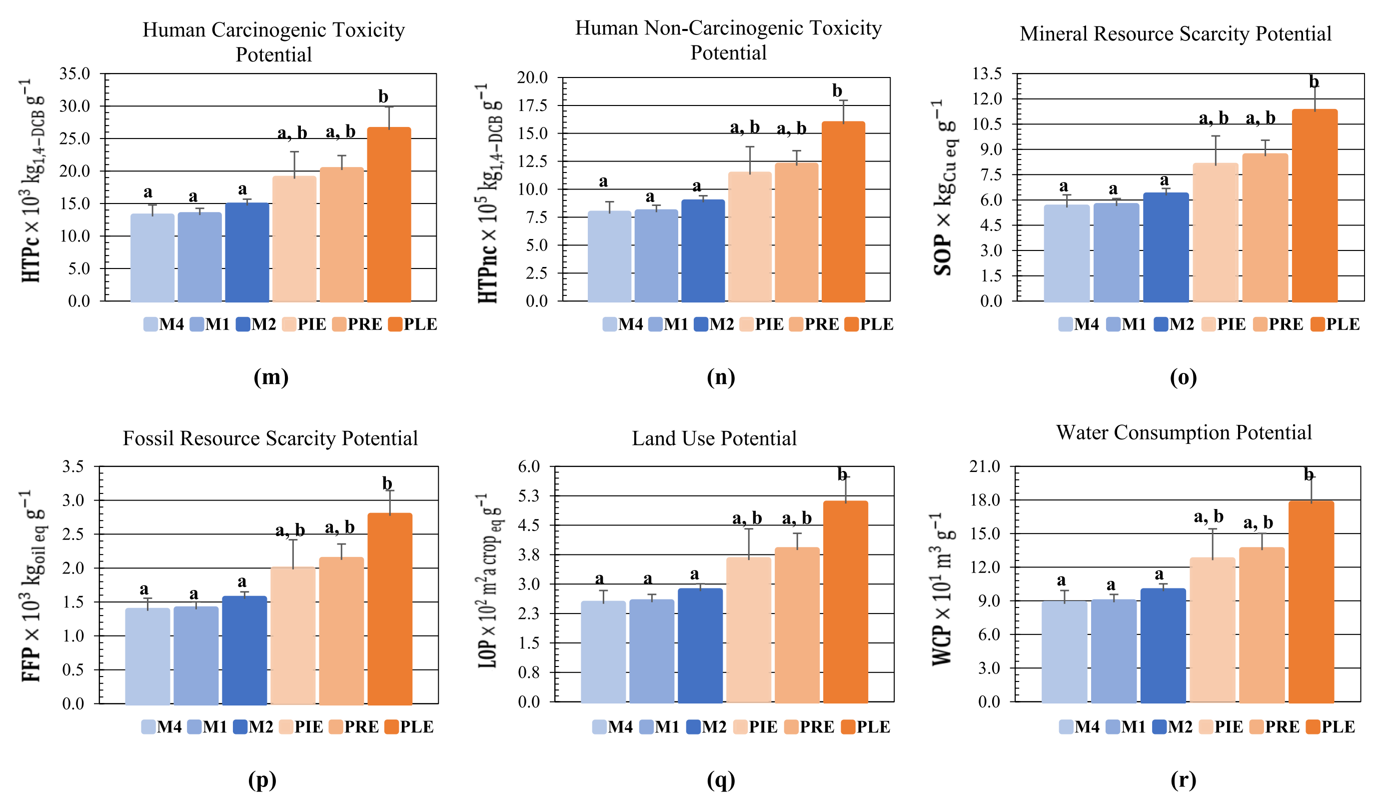

Figure 2iii. Potential environmental impacts of microalgae lipid production using two microalgae culture media (OFM and AEM) at different macronutrients concentrations: M4, M1 and M2 for OFM (blue) and PIE, PRE and PLE for the AEM (orange). The subscript letters refer to the Tukey's statistical test ($\alpha = 0.05$).

considered statistically different from M1, M2 and M4 that obtained letter "a". The same is not verified for PIE and PRE, since both obtained letters "a, b", which means that they are not statistically different from PLE (with letter "b") neither from M1, M2 and M4 (with letter "a").

Qualitatively comparing the relative values of environmental impacts of the microalgae culture media, it is possible to observe that the variation has always the same behavior independently of the environmental impact category considered. This behavior indicates the existence of some inventory term/s, or even some stage/s of the system, which control the environmental performance of the process, for both media (e.g., energy consumption was found to be a dominant factor, as it will be shown below).

To evaluate the possible dominant or controlling factors, Fig. 3 shows the relative contribution (in percentage), of each process step to the potential environmental impacts. Regardless of the culture medium and macronutrient concentration, the relative contribution of each process step to the environmental impacts follows the same behavior as shown in Fig. 3.

Fig. 3 shows that the life cycle step that contributes most to the environmental impacts is the drying of microalgae biomass (around 63%, on average), which is carried out using a freeze-dryer that is responsible for around 65% of the total energy of the microalgae lipids production system.

The second most significant step is microalgae cultivation that contributes around 34%, on average, to the environmental impacts, mainly due to electricity use in artificial lighting (about 13% of total energy), stirring plate (about 19 % of total energy) and autoclave for sterilizing laboratory equipment (about 2% of total energy) necessary to maintain the required conditions for microalgae growth. Microalgae biomass harvesting by centrifuge and solvent lipid extraction from the microalgae biomass requires less than 2% of total energy. Therefore, both microalgae cultivation and biomass drying steps, are the most energy intensive process steps, contributing around 97%, on average, to the potential environmental impacts. The remaining life cycle steps, harvesting, extraction and end-of-life, together contribute around 3%, on average, to the environmental impacts, of which the contribution of lipid extraction (around 26%) to ozone depletion (ODP) is the most significant. Similar results were obtained by Collet et al. (2014) who estimated a significant contribution (>50%) from the cultivation and production stages for most of the impacts assessed, mainly due to the use of inorganic fertilizers and electricity for the pumps and paddle wheels in the microalgae cultivation system. Moreover, these authors (Collet et al., 2014) point out that although not negligible the contribution of the transformation phase (i.e. lipid extraction and biodiesel production) is secondary to most of the impacts.

Solar drying is the most economical method, however it is slow and therefore difficult to maintain the same quality of the final product, as the wet microalgal biomass is more prone to degradation. Also, solar drying requires large surface areas, depending on the amount of biomass to be dried Therefore, spray dryers are preferable for higher value-added products and are normally chosen for use at industrial level (Mata et al., 2010). One advantage of spray dryers is that they allow a higher nutrient content to be maintained in the microalgae biomass compared to solar dryers, and the biomass is less susceptible to lipolysis than when freeze-drying is used. As a disadvantage, when using spray dryers, the biomass loses more than 10–20 % of its protein content compared to solar drying, and its carotenoids oxidize more quickly compared to freeze-drying (Chen et al., 2015). On the other hand, according to Sousa et al. (2022), lipid extraction can be achieved without the need to dry the biomass, using ohmic heating and with lower electricity consumption (10 min–5 s) compared to freeze-drying (3 days).

In summary, the drying step should be carefully chosen depending on the target components from microalgae biomass (Mata et al., 2010). Although a comparison of energy consumption for alternative drying methods would be interesting, it was not done in this work due to the lack of experimental data for the microalga *Pavlova gyrans*. On the other hand, the use of different drying methods can imply changes in the process efficiency and environmental impacts, which may or may not increase.

Fig. 4 shows the relative contribution of the main life cycle inventory items to the environmental impacts: transportation fuels, energy (electricity), water, media components (micronutrients and macronutrients), consumables (nitrogen, chloroform and methanol) and end-of-life cycle (EOL). These values are independent of the culture medium and macronutrient concentration.

As shown in Fig. 4, energy consumption is the inventory item with the most significant contribution (>95%, on average) to the potential environmental impacts of microalgal lipid production, except in the

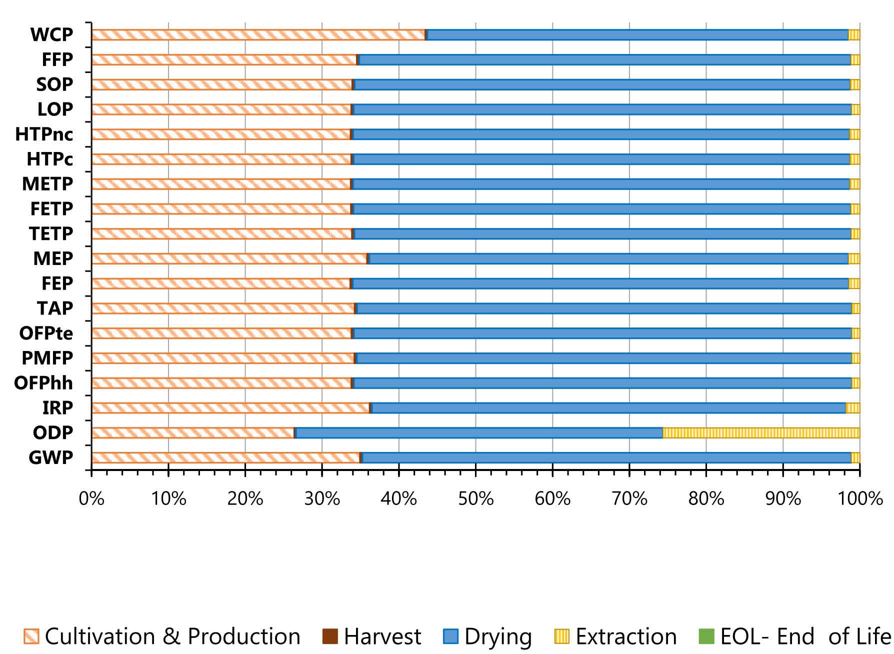

Fig. 3. Relative contribution (%) of each life cycle step to the potential environmental impacts of microalgae lipids production.

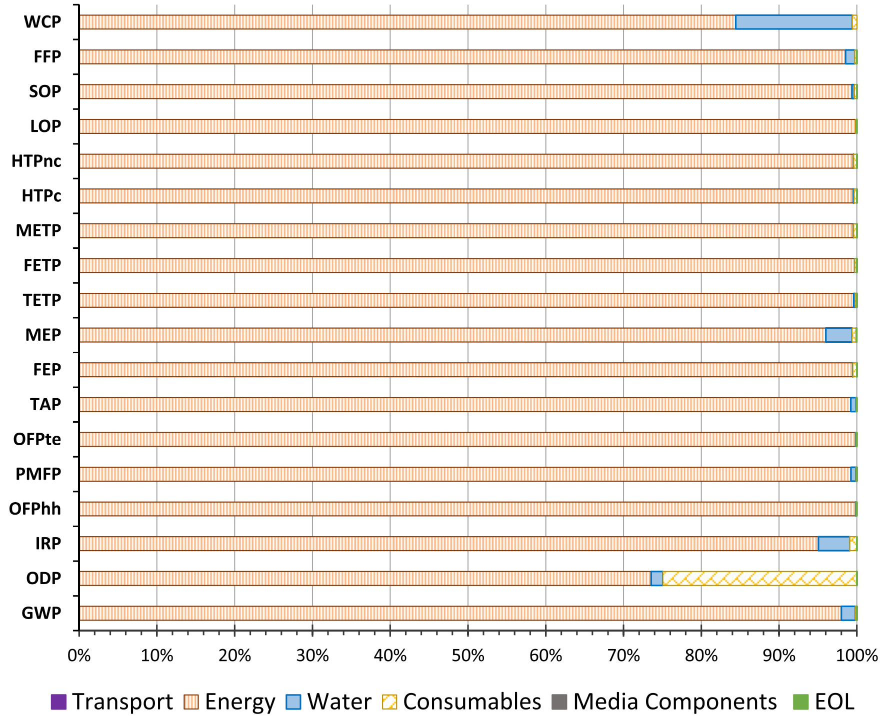

Fig. 4. Relative contribution (%) of the main life cycle inventory items to the potential environmental impacts of microalgae lipids production.

impact categories of WCP (with around 84% energy contribution) and ODP (with around 73% energy contribution).

Similarly, Porcelli et al. (2020) registered a remarkable contribution of energy consumption to the different impact categories. Gaber et al. (2022) also reported a significant contribution from energy to environmental impacts, even though infrastructure and other operational materials also make an important contribution. Therefore, transitioning to alternative energy sources with lower environmental impacts, such as renewables or those with lower carbon content, is crucial for mitigating the environmental impacts associated with microalgae lipids production.

The contribution of water use to global environmental impacts is, on average, less than 2%, of which around 15% in water consumption (WCP) and around 4% in ionizing radiation (IRP).

The contribution of consumables to global environmental impacts is, on average, less than 2%, of which around 25% for ozone depletion (ODP). In the remaining impact categories, the contribution of consumables is minimal (<1%, on average). Among the three consumables used (nitrogen, chloroform and methanol), the main contributor to ODP (around 99%) is chloroform ($CHCl_3$) used for lipids extraction from microalgae biomass, following the Bligh & Dyer method (Gorgich et al., 2020), even though it is used in much smaller quantities when compared to nitrogen (around 1800 times less). While chloroform itself may not be classified as an ozone-depleting substance under the Montreal Protocol, its production process can have indirect contributions to ozone depletion. Chloroform is typically produced through a process involving methane and chlorine in the presence of ultraviolet light. During this production process, certain by-products may be formed that are ozone-depleting substances or precursors to ozone-depleting substances. For example, chlorinated hydrocarbons and other halogenated compounds produced during chloroform manufacture can be released into the atmosphere, where they may contribute to ozone depletion indirectly by reacting with ozone or influencing other chemical processes that affect ozone concentrations. Alternative methods for microalgae lipid extraction have been reported in the literature. However, supercritical extraction requires high energy consumption due to the high pressures applied (Kumar et al., 2015). Alternatively, supercritical extraction with carbon dioxide can be faster and with lower operating costs, but requires complex and expensive equipment (Cequier-Sánchez et al., 2008). Another solution involves using alternative solvents, such as the combination of isopropanol and hexane, which have lower toxicity compared to chloroform and methanol. However, these methods have a lower lipid yield, due to the weak bonds (van der Waals) between the non-polar solvent and the neutral lipids, compared to the hydrogen bonds between the lipids and the proteins, which occur in the cytoplasm (Ansari et al., 2017). Despite the potential for reducing environmental impacts by using other technologies or solvents for lipid extraction, this comparison was not made in this work due to the lack of experimental data for *Pavlova gyrans* and the lack of other relevant information, such as process efficiency, when using the alternative methods.

3.2. Energy scenarios for improvement

Considering only M4 of the organic fertilizer medium, for which the contribution to the environmental impacts was found to be the smallest, two alternative energy scenarios were analyzed, comparing the replacement of the Portuguese electricity mix by solar or wind energy. Therefore, for M4 medium, Fig. 5 compares the potential environmental impact of using electricity produced by silicon photovoltaic solar panels and electricity produced in wind turbines with low voltage conversion, with the Portuguese electricity mix that includes fossil fuels. The potential environmental impacts have been scaled in order to allow their proper graphical visualization and comparison, and error bars indicate the uncertainty of the calculated values.

Comparing the environmental impacts of electricity consumption for the three scenarios analyzed (Portuguese electricity mix, solar energy and wind energy), it can be seen in Fig. 5 that the use of renewable energy allows reducing the impact values in 13 of the 18 categories, increasing in the remaining 5 categories.

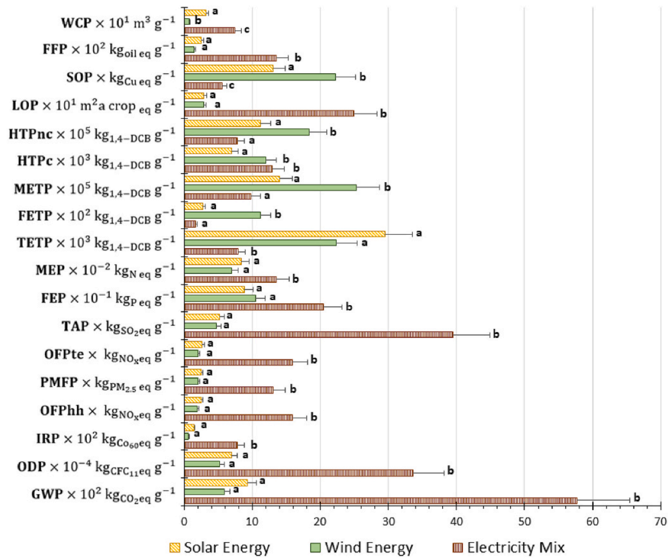

Fig. 5. Comparison of the potential environmental impacts for electricity generated by photovoltaic panels (solar energy), wind turbines (wind energy) and the 2020 Portuguese electricity mix. The subscript letters refer to Tukey's statistical test ($\alpha = 0.05$).

Compared to the electricity mix, the following impact categories values decrease for solar and wind energy respectively: GWP by around 70 and 90% for solar and wind, respectively, ODP by 67 and 85%, IRP by 69 and 92%, OFPhh by 71 and 88%, PMFP by 68 and 85%, OFPte by 70 and 87%, TAP by 73 and 88%, FEP by 47 and 49%, MEP by 32 and 48%, HTPc by 39 and 8%, LOP by 74 and 89%, FFP by around 68 and 89% and WCP by 48 and 89%.

This is explained because in comparison to electricity generation from fossil fuels such as coal, oil and natural gas that emits significant amounts of carbon dioxide (CO_2), methane (CH_4), and other greenhouse gases, solar and wind energy generation have no direct greenhouse gas emissions during operation. Also, fossil fuel combustion releases pollutants such as sulfur dioxide (SO_2), nitrogen oxides (NOx), particulate matter (PM), and volatile organic compounds (VOCs), which contribute to environmental impacts such as smog formation and human toxicity. Moreover, fossil fuel power plants, particularly coal and nuclear plants, consume large quantities of water for cooling purposes, which can lead to water scarcity. Fossil fuel extraction and power plant operations often require extensive land clearing, habitat destruction, and ecosystem fragmentation, leading to biodiversity loss and habitat degradation. Solar and wind energy installations typically occupy smaller land footprints compared to conventional power plants, especially when deployed on rooftops, brownfields, or marginal lands. Also, solar and wind energy projects can be designed to minimize land use impacts and preserve natural habitats. Additionally, renewable energy technologies continue to improve efficiency and cost-effectiveness over time, further reducing resource consumption and environmental impacts.

In the present work, it was found that, compared to the electricity mix, the following five potential environmental impact categories increase when solar and wind energy are used, respectively: TEPT increases by around 231 and 184% for solar and wind, respectively, FETP by 54 and 574%, METP by 36 and 158%, HTPnc by 36 and 136% and SOP by 114 and 302%. The increase in TEPT and SOP for renewables is statistically different ($p < 0.05$) from the electrical mix. In the case of METP, FETP and HTPnc, only for wind energy is the increase statistically different ($p < 0.05$) from the electricity mix, while for solar energy it is not.

Solar and wind energy rely on renewable resources like sunlight and wind, which are abundant and inexhaustible compared to finite fossil fuel reserves. Thus, harnessing renewable energy sources makes it possible to reduce our dependence on non-renewable resources and mitigate the environmental impacts associated with energy generation. However, from a life cycle thinking perspective, it is necessary to consider that the manufacturing of solar panels and wind turbines involves the extraction, processing, and refinement of raw materials such as metals, polymers, and rare earth elements (Carneiro et al., 2022). These processes can result in the release of pollutants and toxic substances into the environment, contributing to terrestrial (TETP), freshwater (FETP), and marine (METP) ecotoxicity. Additionally, the mining of minerals and metals may deplete finite mineral resources, leading to mineral resource scarcity (SOP). The production and use of certain materials in solar panels and wind turbines, such as semiconductor materials, coatings, and lubricants, may contain hazardous chemicals and substances. These chemicals can leach into soil, water, and ecosystems, posing risks to terrestrial and aquatic organisms and contributing to ecotoxicity potential (TETP and FETP). Improper handling and disposal of electronic waste from decommissioned solar panels and wind turbines can further exacerbate environmental contamination and human health risks (HTPnc).

Rashedi and Khanam (2020) pointed out that, in the case of solar energy, the extraction of raw materials such as quartz and metallurgical-grade silicon, for the production of monocrystalline silicon for photovoltaic panels, and the production of the copper needed for the electrical installation are the main factors contributing to the potential impacts. For wind energy, according to Schreiber et al. (2019) the manufacture and use of components, such as steel and stainless steel, for the construction of the nacelles, rotor and tower are the main factors (>80 %) contributing to the potential impacts. Similar results were found by Bonou et al. (2016) for a 3.2 MW wind turbine onshore that pointed as main contributors the construction of towers, nacelles and cables.

The production of solar panels and wind turbines requires energy for manufacturing, transportation, and installation. While solar and wind energy systems generate clean electricity during operation, the energy-intensive manufacturing processes may rely on fossil fuel-based energy sources, resulting in greenhouse gas emissions and associated environmental impacts (Carneiro et al., 2022). Additionally, energy-intensive manufacturing processes can contribute to human toxicity potential (HTPnc) by releasing hazardous chemicals and pollutants into the environment.

The end-of-life disposal of solar panels and wind turbines can lead to waste generation and environmental pollution if not managed properly (Ramos et al., 2023). Decommissioned solar panels and wind turbines contain electronic components, metals, and materials that may pose risks to human health and the environment if not recycled or disposed of responsibly (Chowdhury et al., 2020). Landfilling or incineration of electronic waste can release toxic substances and heavy metals into soil, water, and air, exacerbating environmental impacts (TETP, FETP and METP) and human toxicity potential (HTPnc).

In summary, although renewable energy technologies, such as photovoltaic panels and wind turbines, generally have a lower environmental impact than fossil fuel-based energy sources, it is essential to recognize that their environmental performance can vary depending on several factors (Ramos et al., 2023; Carneiro et al., 2022). Moreover, the decision to implement renewables should always involve careful analysis of site-specific factors to determine their practicality and effectiveness.

Fig. 6 shows the relative contribution of the various life cycle inventory items to the potential environmental impacts when using solar and wind energy. These values are independent of the culture medium or nutrients concentration considered, allowing for a general representation of the data.

Fig. 6 shows that even when using solar and wind energy, energy is the dominant inventory item in most environmental impact categories. An exception is the WCP category, when wind energy is used, where water consumption contributes around 61% and energy around 36%.

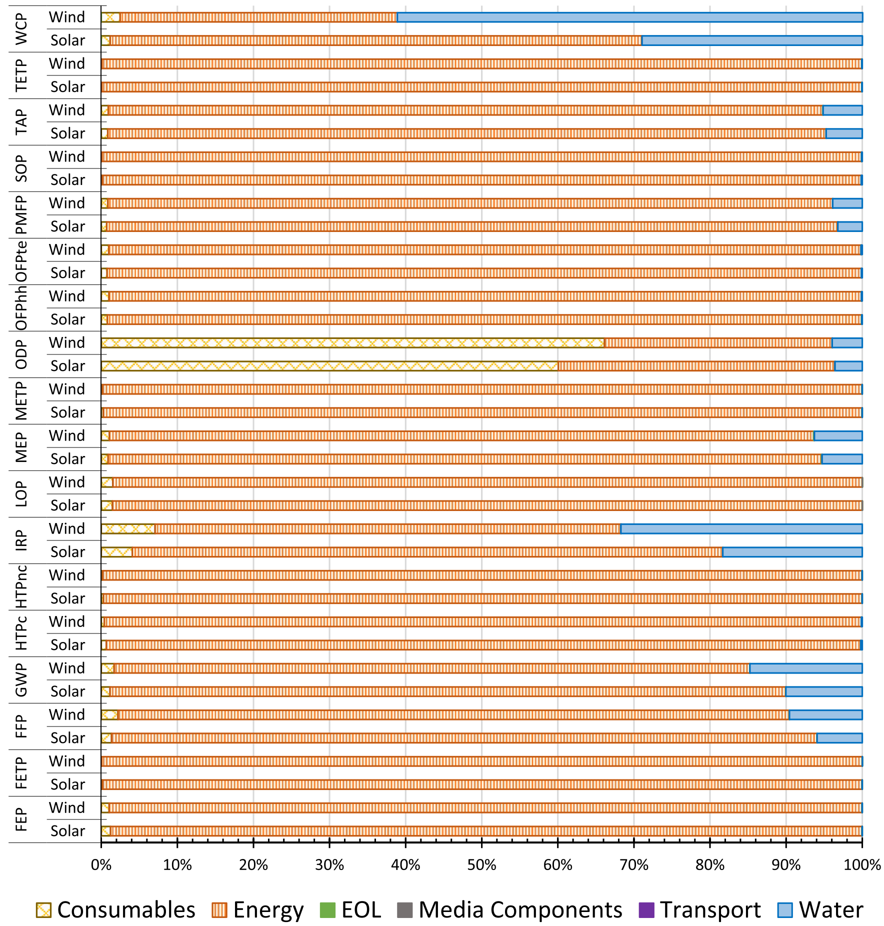

Fig. 6. Relative contribution (%) to the environmental impacts, of the main life cycle inventory items, considering the use of solar and wind energy.

Furthermore, for the ODP category, consumables are the most relevant inventory item, contributing around 66 and 60%, while energy contributes around 30 and 36%, respectively, when using wind and solar energy. It's also worth noting that in the IRP category, after energy, water consumption is the second most relevant inventory item contributing around 32 and 18%, respectively, when using wind and solar energy, while energy contributes around 61 and 78%, respectively.

4. Conclusions

This study compares the life cycle environmental impacts of lipid production from the microalga *Pavlova gyrans* grown in two different culture media. It can be concluded that, compared to the aquaculture effluent medium (AEM), the organic fertilizer medium (OFM) generally contributes to lower environmental impacts because it contributes to higher lipid production. However, according to the results of the uncertainty analysis, there is no statistical difference between the average environmental impact values for each medium, except for the PLE of AEM which consistently has the highest environmental impact value in all 18 categories analyzed, being statistically different from OFM. The life cycle steps with the highest contribution to the environmental impacts are drying (around 63%, on average), followed by biomass cultivation (around 34%, on average), together contributing an average of around 97 % for potential environmental impacts. The other life cycle steps, microalgae harvesting, lipid extraction and end-of-life, together contribute around 3%, on average, to the environmental impacts, being an exception the lipids extraction contribution (around 26 %) to ozone depletion. The electricity required to freeze-dry microalgae is about 65% of the total energy. In microalgae cultivation, electricity for artificial lighting, stirring plate and autoclave is about 34% of the total energy. The electricity required to biomass harvesting by centrifuge and solvent lipid extraction is less than 2%. Therefore, the use of alternative drying and lipid extraction methods should be analyzed in future experimental work with microalga *Pavlova gyrans* in order to conclude on the possibility of improving the environmental performance of the process. The total energy consumption is what contributes most (>95%, on average) to the potential environmental impacts, with the exception of WCP and ODP, impacts to which energy contributes approximately 84 and 73% respectively. Therefore, it is concluded that energy is the process environmental bottleneck, which is why alternative energy scenarios were analyzed. The use of renewable energy allows reducing the impact values in 13 of the 18 categories, but increased in 5 categories. For example, for solar and wind energy respectively, GWP decreases by around 70 and 90%, ODP decreases by around 67 and 85%, but TEPT increases by around 231 and 184% and SOP increases by around 114 and 302%. This study results can support decision-making processes, integrating environmental considerations in the development of microalgal lipid production processes, from the initial phase of their development. It is usually less expensive to incorporate sustainability measures into the initial design than to retrofit existing processes later on. In this way, researchers and industrialists can focus on developing technologies or processes with lower environmental impact and greater potential for commercialization. Although it is beyond the scope of this study, conducting a life cycle costing (LCC) analysis alongside LCA can provide a more comprehensive evaluation of a process's sustainability. This way decision-makers can better understand the trade-offs and synergies between environmental sustainability and financial performance, leading to more informed and holistic sustainability decisions.

CRediT authorship contribution statement

Roberto Novais: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Teresa M. Mata:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Conceptualization. **Leandro Madureira:** Methodology, Investigation, Data curation, Conceptualization. **Filipe Maciel:** Supervision, Investigation, Conceptualization. **António A. Vicente:** Validation, Supervision, Methodology, Conceptualization. **António A. Martins:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2024.143073.

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