



Life cycle assessment of protein extraction from microalgae biomass using ohmic heating

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ABSTRACT

Life Cycle Assessment (LCA) is an effective tool for assessing the environmental impacts associated with the implementation of innovative processing technologies in biotechnology. This study focuses on the life cycle environmental impacts of thermal protein extraction from microalgae biomass using ohmic heating. *Coelastrella* was selected as the model organism due to its double cell wall, which presents the worst-case extraction scenario. The environmental impact assessment was conducted using the ReCiPe 2016 method from an equalitarian perspective, encompassing eighteen environmental impact categories. The results demonstrate that applying an electric field of 217 V/cm during protein extraction significantly enhances extraction efficiency while simultaneously promoting a more environmentally sustainable performance. This is achieved through a rapid heating pulse that reaches a maximum temperature of around 100 °C, with a holding time of <1 s. In addition, an alternative energy scenario utilizing entirely renewable energy was evaluated for both the worst and best treatments identified in the LCA study, compared to the reference treatment. The results indicate improvements in at least fifteen of the eighteen indicators assessed under this renewable energy scenario.

1. Introduction

The exponential growth of the population presents a multifaceted challenge with widespread implications, placing significant pressure on resources and the environment. It is essential to find sustainable solutions that align population growth with the planet's capacity. In this regard, adopting efficient and sustainable practices is fundamental, particularly those that adhere to the principles of the (bio)circular economy. This approach emphasizes the reuse, recycling, and regeneration of resources, thereby optimizing resource utilization and minimizing waste generation.

The search for alternative protein sources is gaining momentum, with a notable shift towards more sustainable options, ranging from plant-based to insect-based proteins. One promising alternative is microalgae, which are nutrient-rich and require minimal soil, water and fertilizers. They can grow rapidly in various aquatic environments,

positioning them as a promising alternative for sustainable food production [1]. Microalgae are photosynthetic organisms that thrive in freshwater, seawater, and even wastewater [2]. They accumulate a variety of metabolites of industrial interest [3] and have the potential to produce higher biomass per unit area than traditional crops. Furthermore, their cultivation demands less water and land compared to conventional agriculture and can effectively utilise wastewater and carbon dioxide as nutrient sources [4,5].

Microalgae are capable of synthesising proteins as primary metabolites, positioning these microorganisms as an excellent alternative for human nutrition [6,7]. Certain species such as *Arthrospira maxima*, *Chlorella vulgaris*, or *Euglena gracilis*, can accumulate over 50 % of their dry weight in proteins [6]. Utilizing microalgae biomass as a protein source offers several advantages over traditional sources. Notably, microalgae can thrive in non-potable water sources, including saline, hypersaline, and wastewater, thereby alleviating competition for

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freshwater resources. This adaptability, combined with their minimal reliance on arable land, makes microalgae a resilient and sustainable option [5].

However, the presence of a cell wall poses a significant challenge, as it hinders the bioaccessibility of microalgae macronutrients and complicates their efficient extraction [6]. To address this, further research and development are essential to enhance the entire production process, from cultivation to extraction and recovery of microalgae compounds. Gaining this knowledge will not only improve the economic viability of microalgal protein production but also ensure scalability while maintaining nutritional value and safety. Key aspects such as selective and efficient release of bioproducts, alongside high recovery rates, are critical for optimizing cell extraction and disruption processes [5].

In this context, Ohmic heating (OH), also referred to as Joule heating or resistive heating, involves passing an electric current through a conductor, typically a liquid or solid matrix. As the electric current encounters resistance within the material, it generates heat. This technology enables rapid and uniform heating of products while allowing for precise control over both electrical and thermal effects [5,8,9]. One of the significant advantages of OH is its efficiency and ability to precisely control the heating process, potentially leading to reduced processing times and lower energy consumption. As an emerging technology, OH offers a more precise and energy-efficient alternative to traditional heating methods, aligning with the broader goal of sustainable and environmentally conscious practices. Furthermore, the electro-heating process facilitated by OH presents a promising opportunity for controlled cell disruption with minimal energy consumption, thereby enhancing the bioaccessibility of intracellular compounds [5,8].

Ohmic heating has proven effective for extracting proteins and pigments from *Coelastrella* sp., significantly reducing energy consumption compared to conventional heating (CH). For instance, CH at 70 °C for 10 min consumes 1678 kWh·kg_{DW}⁻¹, whereas OH at the same temperature and duration requires only 99.9 kWh·kg_{DW}⁻¹, as reported by Sousa et al. [8]. Recent advancements further indicate that combining OH using moderate electric fields with subcritical water extraction enhances environmental performance, making this approach promising for sustainable agar production [10]. This synergy underscores the potential of electric fields and OH technology to optimize resource use while improving extraction efficiency, aligning with the increasing demand for sustainable processing technologies in food and bioproduct industries. However, it is important to recognize that the overall environmental impact of OH is influenced by various factors, including the specific application, the energy source employed, and the life cycle assessment of the processing equipment. Ideally, the electricity utilized for OH should be derived from renewable sources to minimize its environmental impacts.

The contemporary business paradigm, known as the triple bottom line, emphasizes not only profit but also social and sustainability, often referred to as the 3 P's: People, Planet and Profit [11–13]. To effectively implement sustainable strategies, companies require quantitative assessment tools to measure the impact of technological changes on existing products or development of new ones. In this context, Life Cycle Assessment (LCA), serves as an internationally standardized methodology that identifies and compares the potential environmental impacts of various products, processes, or services [14]. Additionally, LCA helps pinpoint the life cycle phases that contribute most significantly to environmental impacts (hotspots), enabling the formulation of targeted environmental improvements for specific products, services, or processes [15].

Few LCA studies in the literature have focused on evaluating the production of proteins from microalgae cultivation, as most have concentrated on of biofuel production or lipid extraction (omega-3 or omega-6) [16–20]. To the best of the authors' knowledge, this study is the first to analyze the environmental impacts of extracting proteins from microalgae biomass through OH treatments, following the LCA methodology from microalgae cultivation to the extraction. In this

research, *Coelastrella* sp. LRF1 selected as the subject of study due to its double cell wall, which presents a significant challenge for the extraction process. Consequently, this LCA was conducted under one of the worst-case scenarios.

2. Materials and methods

This LCA study follows the methodology outlined in the ISO 14040 [21] and 14044 [22] standards, encompassing four iterative phases: (1) Goal and scope definition, which establishes the system considerations essential for conducting the LCA; (2) Life Cycle Inventory (LCI) analysis, which identifies and quantifies all inputs and outputs associated with the system; (3) Life Cycle Impact Assessment (LCIA), which translates these inputs and outputs into their corresponding environmental impacts; and (4) Interpretation, which summarizes the findings by evaluating the results of the LCI and LCIA in relation to the study's goal and scope.

The iterative nature of this methodology allows for the refinement of inventory data and the adjustment of system boundaries as additional information is gathered, leading to increased certainty in the results. This process ultimately enhances the quality of interpretations, conclusions, and recommendations.

2.1. Goal and scope definition

The primary objective of this study is to assess the environmental impacts associated with protein extraction from *Coelastrella* sp. LRF1 biomass, adopting a “cradle-to-gate” approach that encompasses all life cycle phases, from microalgae cultivation to the protein extraction process. This analysis compares OH and CH for protein extraction (details can be found in Table S1 of the supplementary information). Additionally, the study identifies critical points within the extraction process and proposes potential improvement strategies.

In this study, the functional unit (FU) is defined as the extraction of 1 g of protein from microalgae biomass.

This LCA study is of an attributive nature, as it aims to quantify and evaluate the environmental impacts of a product across its entire life cycle. In this context, “attributive” refers to the process of assigning environmental impacts to specific stages or components within the product life cycle.

The results of this study aim to inform stakeholders about the environmental impacts linked to protein extraction from microalgae biomass. This information could be particularly valuable to companies in the food sector that cultivate microalgae and process proteins under similar geographical conditions in Portugal.

The system boundary for this study encompasses the entire process from raw material extraction and microalgae cultivation to protein extraction from the harvested biomass, as illustrated in Fig. 1. The technologies and processes used were defined based on the laboratory research detailed in Sousa et al [8].

The key differences between the technologies compared for protein extraction from biomass are as follows:

- Conventional heating (CH): Conducted at 60 °C and 70 °C, denoted as CH60 and CH70, respectively.
- Ohmic heating (OH): Performed at 60 °C and 70 °C with an electric field of 35 V/cm, designated as OH60 and OH70, respectively.
- Ohmic Heating Pulses (OHP): Conducted at 100 °C using electric fields of 133 V/cm and 217 V/cm, referred to as OHP100 and OHP100*, respectively.
- OHP with NaOH: Executed at 100 °C using an electric field of 217 V/cm in the presence of 5 mM NaOH, indicated as OHP100**.

2.2. Life cycle inventory analysis

As previously noted, the inventory analysis seeks to identify and

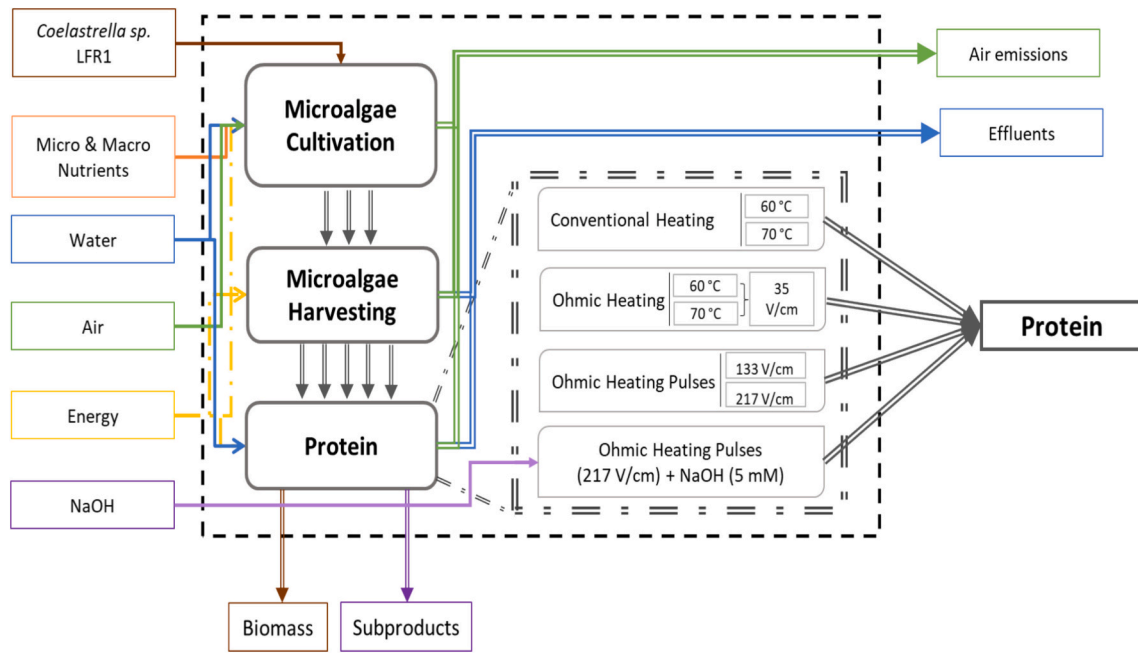


Fig. 1. – Definition of the system boundary (indicated by the dashed rectangle) for the LCA study, following a “cradle-to-gate” approach, encompassing the entire process from microalgae cultivation to protein extraction.

quantify all material and energy flows entering and leaving the system. In this study, experimental data from Sousa et al. [8] was utilized to inform the analysis. This experimental data was normalized per functional unit (FU) as detailed in the supplementary information (Table S2), and, when necessary, supplemented with information from the EcoInvent (version 3.9.1) database using SimaPro (version 9.5.0) software.

2.2.1. Material component

The material inputs of the system include the macro and micro-nutrients, along with water used for microalgae cultivation and for the conventional heating and refrigeration processes during the extraction stage (amounting to approximately 9 L). Additionally, auxiliary materials, specifically sodium hydroxide (NaOH) at a concentration of 1 mol/L, were incorporated during the extraction phase. The composition of the microalgae growth medium included the following components: $\text{CH}_4\text{N}_2\text{O}$ (1.100 g/L), KH_2PO_4 (0.238 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.204 g/L), EDTA NaFe (0.040 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.116 g/L), H_3BO_3 (0.416 g/L), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.473 g/L), $\text{Cl}_2\text{Mn} \cdot 4\text{H}_2\text{O}$ (1.647 g/L), $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ (0.308 g/L), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (1.339 g/L), $(\text{NH}_4)_8\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (0.086 g/L) and NH_4VO_3 (0.007 g/L). The total volume of the medium used was 3 L. Additionally, the growth was conducted under autotrophic conditions over a 15-day period, with an air flow rate of 1.8 vvm and a CO_2 concentration of 3 %.

To account for transportation of the components, it was assumed that most of them are sourced from within Portugal. Accordingly, an average transportation distance (d_m) of 200 km, was considered, with the components being transported via 32-ton trucks. The transportation component, denoted as TC_j was calculated for each trip and expressed in tonne-kilometres (tkm) using Eq. (1):

$$\text{TC} = m_t \times d_m \quad (1)$$

where m_t is the components total mass (expressed in tonnes, t).

During the various stages of the process, liquid effluents are treated according to the end-of-life (EoL) treatment procedure, which is incorporated into the system's inventory and overall environmental impact assessment. Additionally, the EoL is solely dependent on the volume of water utilized in the system, measured in cubic meters (m^3).

2.2.2. Energy component

The energy values (E) associated with the equipment represent electricity consumption and are expressed in kilowatt-hours (kWh), as detailed in the supplementary information (Table S3). These values were derived using Eq. (2). For most equipment, the power rating (P) was provided in the specifications. In cases where this information was unavailable, P was calculated using Eq. (3), given that both the electric current (I) and voltage (V) were specified. Additionally, Eq. (4) was employed to determine the energy consumption for lighting (E_{il}), as the power specification was provided in watts per meter (W/m).

$$E = P \times t \quad (2)$$

$$P = I \times V \quad (3)$$

$$E_{il} = P \times t \times d \quad (4)$$

where t represents the duration of equipment usage in hours, and d denotes the length of the lighting wire in meters.

Most of the information regarding equipment usage times was sourced from Sousa et al. [8]. However, certain equipment, such as the heating circulator, required additional time to reach temperatures of 60 °C (CH60) or 70 °C (CH70). Consequently, extra time was factored in for specific devices, including the computer, waveform generator, signal amplifier, and heating/refrigerated circulator (detailed in Table S4 of the supplementary information). The Portuguese electricity mix from the EcoInvent database was employed to analyze electrical energy consumption).

Although some equipment incorporated energy efficiency features, the power ratings provided by the manufacturers were used in all energy calculations. This conservative approach ensures a worst-case scenario for energy consumption.

2.3. Life cycle impact assessment

2.3.1. Environmental impact assessment method and categories

Following a comprehensive literature review [23–26], the ReCiPe 2016 method was selected for this study, utilizing the Equalitarian (E) perspective, which prioritizes a precautionary approach regarding the

temporal prevalence of impacts. The LCIA was conducted using SimaPro software v 9.5.0, incorporating inventory databases such as EcoInvent v3.9.1.

Based on the system description and its key inventory components (primarily energy and electricity consumption), 18 impact categories from the ReCiPe method were analysed, as detailed in Table S5 [27] of the supplementary information. The combustion of fossil resources directly contributes to the global warming potential (GWP) through CO₂ emissions and indirectly through the consumption of non-renewable resources, leading to specific categories related to mineral and fossil resource scarcity (SOP and FFP, respectively).

Additionally, combustion generates other pollutants, such as NO_x, which contribute to eutrophication in freshwater (FEP) and marine environments (MEP), as well as acidification and ozone depletion (ODP). Sulfur oxides (SO_x) contribute to terrestrial acidification (TAP). These pollutants, alongside particulate matter (PMFP) and hydrocarbons, lead to photochemical smog, adversely impacting human health (both carcinogenic, HTc, and non-carcinogenic, HTnc effects) and ecosystems (OFte). Furthermore, the cultivation of microalgae necessitates land use (LOP) and is highly dependent on water resources, warranting a specific environmental impact category for water use (WCP). Additional categories considered include ionizing radiation (IRP) and ecotoxicities for terrestrial (TETP), freshwater (FETP), and marine (METP) environments.

2.3.2. Component modelling

Due to the lack of data on certain components—namely ammonium molybdate, ammonium metavanadate, EDTA iron (III) sodium salt, manganese (II) chloride, and monopotassium phosphate - in EcoInvent V3.5, it was necessary to create custom models for these substances. To accomplish this, the “life cycle tree” model was employed, which considers the reactants required for the production of these compounds [28]. Consequently, detailed models for each of the following compounds can be found in the supplementary information (Figs. S1 to S5): ammonium molybdate, ammonium metavanadate, EDTA iron (III) sodium salt, manganese (II) chloride, and monopotassium phosphate.

2.3.3. Characterisation factors

To determine the impact categories (g), the classification factors (FC_i) were first calculated for each component, i, and extraction process, j, using Eq. 5. These values were then combined and aggregated within each category to derive the characterisation factors ($FC_{i,j}$), also known as midpoint factors, as shown in Eq. 6.

$$FC_{i,j,g} = I_{g,i} \times va_{i,j} \quad (5)$$

$$FC_{j,g} = \sum_{i,j,g} FC_{i,j,g} \quad (6)$$

where $I_{g,i}$ corresponds to the impact category indicator per standard unit for the component i, and $va_{i,j}$ is the value of component i per functional unit, of the extraction process j.

2.3.4. Uncertainty estimation

To evaluate the uncertainty associated with the environmental impacts of the protein extraction process, multiple replicates were conducted to calculate three impact values for each process and impact category. These values were derived by averaging the protein mass measurements and incorporating their standard deviation (SD). The variation in the range of impact values was used as a measure of uncertainty, while other potential sources of uncertainty could not be estimated due to a lack of sufficient information.

Statistical analysis was performed using GraphPad Prism software (version 8.0.2, by Dotmatics). One-Way ANOVA, Two-Way ANOVA, and Tukey post-hoc tests were employed to identify significant differences in the mean values between the various extraction processes and electricity

sources. The results are presented as mean $\pm$ SD, with statistical significance indicated by distinct superscript letters at a significance level of $p < 0.05$. Results marked with different superscript letters denote statistically significant differences among the groups.

3. Results

3.1. Environmental impact assessment of extraction technologies

This LCA study was performed to compare the environmental impacts of various protein extraction methodologies, namely CH, OH, and OHP. Fig. 2 illustrates the potential environmental impacts associated with these extraction technologies and their treatment variants (i.e., CH, OH, OHP100, OHP100* and OHP100**) for obtaining protein-rich fractions from microalgae biomass.

The results indicate that CH60 is the extraction treatment with the worst environmental performance in all the 18 impact categories evaluated, while OHP100** showed the best environmental performance. Generally, there were no statistical differences in the impact values of CH70 and OH70. However, CH70 technology performed better than OH60. The OHP100 and OHP100* technologies showed no statistical difference in the impacts compared to OH and CH70.

3.2. Contribution of inventory classes to environmental impacts

To assess the contribution of various inventory items to the 18 environmental impact categories, these items were grouped into the following six inventory classes:

- i). Micro & Macro – includes the production of micro- and macro-nutrients used in microalgae cultivation,
- ii). Energy - accounts for the production and utilization of energy (heat and electricity) during the extraction process,
- iii). Water - encompasses the water used in microalgae cultivation and in the conventional heating and cooling stages of protein extraction,
- iv). Auxiliary components - includes the production of the chemicals used during the extraction process,
- v). Transportation - involves the transport of micro- and macronutrients, as well as auxiliary materials,
- vi). EoL (End-of-Life) – includes the treatment of wastewater and biowaste.

For the majority of impact categories (Fig. 3), electricity consumption was the dominant contribution, with energy usage contributing most significantly in 16 of the 18 impact categories analysed.

In fact, this proportion can exceed 85 % in nine of the impact categories analysed: ODP, IRP, OFPhh, PMFP, OFPte, TAP, HTPC, LOP and FFP.

Conversely, EoL inventory class accounts for the largest relative contribution in the MEP impact category, representing over 57 %. However, in the other impact categories, the contribution of this class is minimal, accounting for <1 % in 15 categories.

The Micro & Macro inventory class is the primary contributor to the SOP impact category, contributing 74 %.

The inventory classes of Water, Auxiliary Components, and Transportation had a negligible contribution of <0.1 % across the majority of impacts, except for the GWP category, where Auxiliary Components contributed 32 %.

3.3. Process steps contribution to the environmental impacts

This section analyses the contribution of each life cycle stage to the environmental impacts. Adopting a “cradle-to-gate” approach, all life cycle stages were considered, from the growth of microalgae to the extraction of protein from the biomass. Fig. 4 illustrates the relative

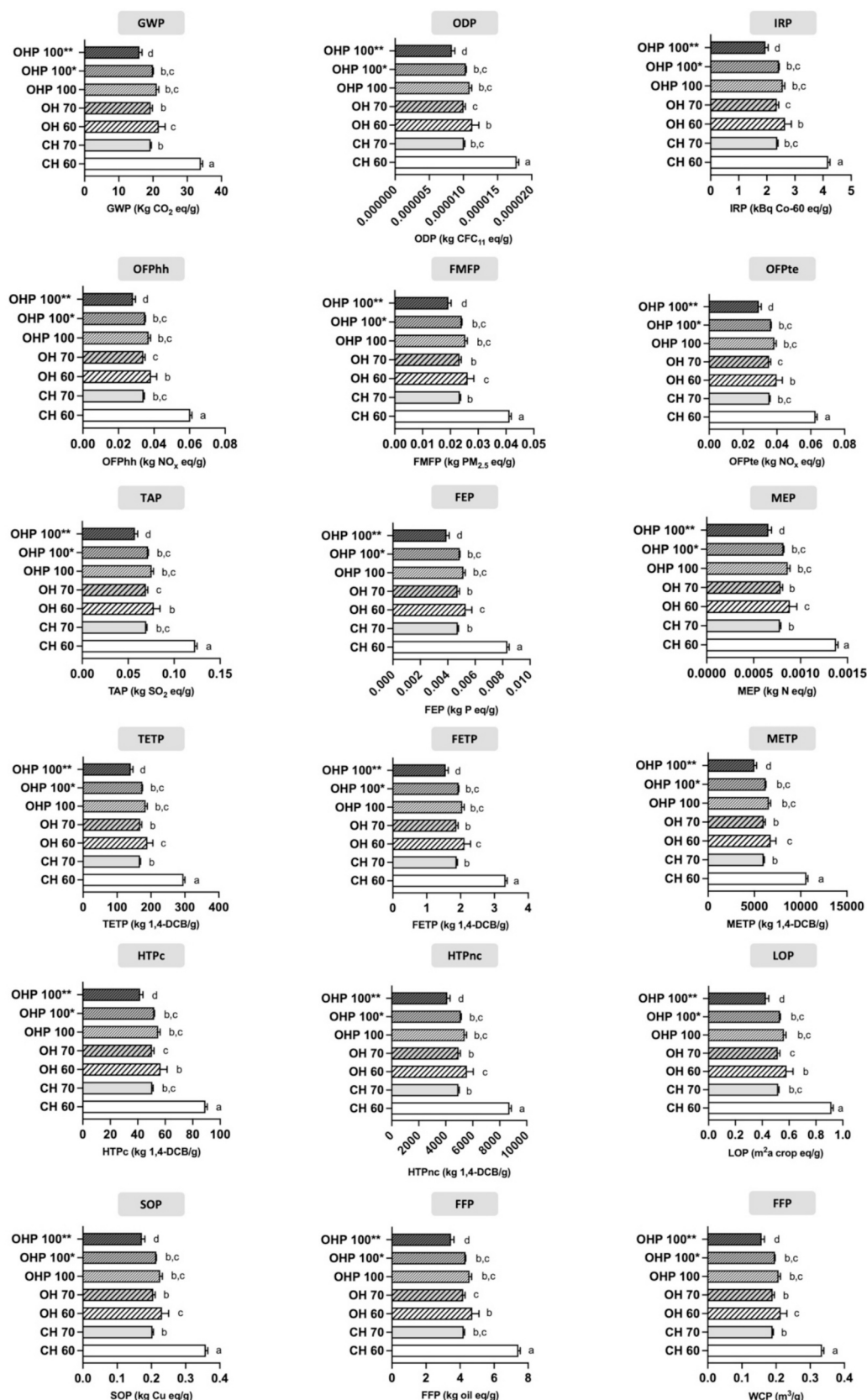


Fig. 2. – Life cycle environmental impacts of protein production from microalgae, comparing different extraction technologies: conventional heating (CH) with ohmic heating (OH) at 60 and 70 °C; ohmic heating pulses (OHP) at 133 V/cm (OHP100) and 217 V/cm (OHP100*); and OHP at 217 V/cm with 5 mM NaOH (OHP100**).

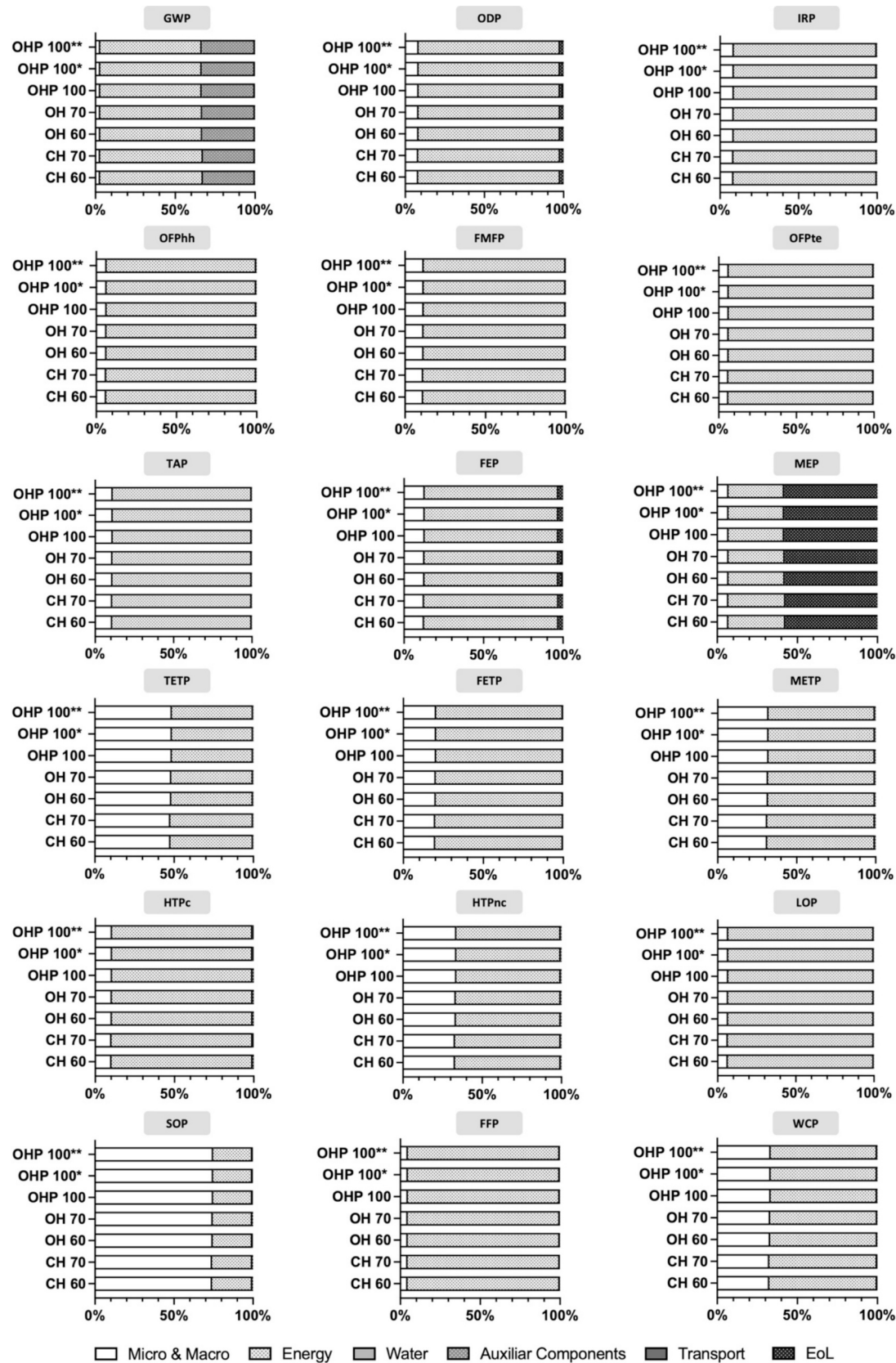


Fig. 3. Relative contribution of each inventory class to the environmental impacts per 1 g of protein extracted (functional unit, FU) from *Coelastrella* sp. LRF1.

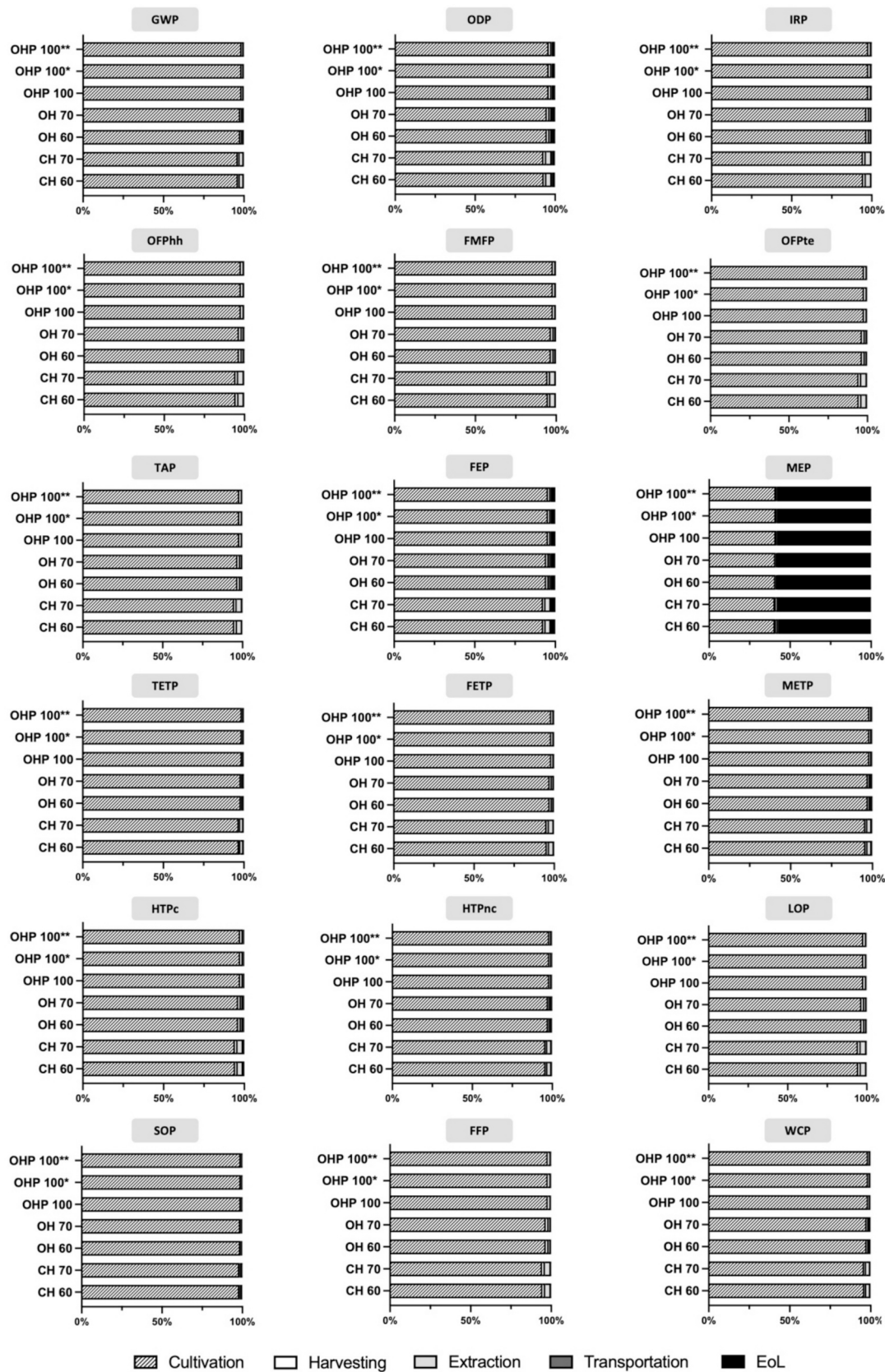


Fig. 4. - Relative contribution of each process step (cultivation, harvesting, extraction, transportation, and end-of-life (EoL)) to the environmental impacts associated with the extraction of 1 g of protein from *Coelastrella* sp. LRF1, comparing the different extraction technologies under investigation.

contribution of each process step (cultivation, harvesting, extraction, transportation, and EoL) to the impact categories, comparing the different technologies analysed for the extraction of 1 g of protein from *Coelastrella* sp. LRF1.

Based on the results presented in Fig. 4, the stage with the highest relative contribution to environmental impacts is cultivation, which accounts for over 92 % in 17 of the 18 impact categories analysed. The only exception is the MEP category, where the contribution from cultivation is lower, with EoL making the largest contribution. Nonetheless, cultivation still represents over 40 % of this impact category, compared to 57 % from EoL.

Significant differences were also observed among the various extraction technologies within each assessed impact category. However, these differences were largely overshadowed by the substantial influence of the cultivation phase on the overall process.

3.4. Deep analysis of the extraction process

Although this study employed a “cradle-to-gate” system, its primary aim was to compare various technologies for extracting proteins from microalgal biomass. To gain deeper insights into the environmental performance of protein extraction, this stage was analysed independently from the other life cycle phases. Consequently, Fig. 5 illustrates the potential environmental impacts associated with protein extraction from microalgal biomass, highlighting the differences among the extraction technologies under study.

The results presented in Fig. 5 indicate that, across all impact categories, CH 60 exhibits the worst environmental performance, followed by CH 70, OH 60 and OH 70. In contrast, OHP demonstrated the best environmental performance, with no statistically significant differences among the three treatment variations analysed.

Moreover, transitioning from conventional heating to ohmic heating resulted in statistically significant reductions in environmental impacts across all categories. This underscores that utilizing ohmic heating is a more environmentally advantageous method for protein extraction from *Coelastrella* sp. LRF1. Additionally, statistically significant differences were noted within both conventional and ohmic heating processes based on treatment temperatures. Increasing the treatment temperature effectively reduced environmental impacts, a trend that was particularly pronounced with conventional heating. In summary, the use of OHP for protein extraction emerged as the extraction technology with the lowest environmental impact.

When comparing OHP100** with the other extraction technologies (detailed in supplementary Table S6), it is observed that OHP100** has environmental impacts that are at least 61, 36, 15, and 14 times lower than those of CH60, CH70, OH60, and OH70, respectively, across 17 of the 18 impact categories analysed. Notably, when focusing solely on the three OHP extraction technologies, no statistically significant differences were detected.

The environmental performance of OH is influenced not only by equipment factors, such as power supply, but also by product-specific properties, including electrical conductivity, which is inherent to the composition and structure of the microalgae biomass. Additionally, the efficiency of the process, operational temperature, and the material and configuration of the electrodes play critical roles, as these parameters affect energy consumption and the durability of the system.

Variations in product viscosity and phase composition can also impact the performance of OH, influencing heat transfer rates and power demands. Consequently, optimizing OH setups tailored to different products is essential for maximizing efficiency and minimizing environmental impacts across a range of applications.

3.5. Analysis of energy source scenarios

The alternative energy scenario utilizing photovoltaics as a fully renewable energy source was compared with the Portuguese electricity

mix (Fig. 6). Renewable energy comes from resources that naturally replenish on a human timescale, including sunlight, wind, rain, tides, waves, and geothermal heat. These resources are consistently available or can be rapidly replenished through natural processes, providing a virtually inexhaustible supply when managed effectively. This comparison focused solely on the extraction technologies that exhibited the best and worst performance: OHP100** and CH60, respectively. The results indicate that implementing photovoltaics improves 15 of the 18 indicators for OHP100** and 16 of the 18 indicators for CH60.

Interestingly, the improvements observed in the renewable energy scenario exhibited a nearly linear trend between the two treatment conditions analysed. However, for the FETP impact category, a statistically significant difference was found in CH60 between the two scenarios, while no such difference was noted for OHP100**. In percentage terms, the CH60 treatment condition demonstrated a more pronounced improvement when comparing the baseline scenario to the enhanced one. Despite these observations, the OHP100** treatment consistently outperformed CH60 across all evaluated scenarios.

4. Discussion

Addressing the initial research question, it is clear that the OHP100** technology consistently demonstrated the lowest environmental impact values. This can be attributed to OHP100**'s superior extraction capacity (2.94 ± 0.14 wt%), in contrast to CH60, which exhibited the worse extraction efficiency (1.42 ± 0.02 wt%) [8], resulting in the highest impact values.

The application of OH, whether in continuous or pulsed form, proved advantageous compared to CH. The higher extraction rates observed at the same treatment temperature with OH are attributed to the combined effects of moderate electric fields and temperature. This suggests the occurrence of non-thermal putative effects, such as electroporation phenomena or electrical damage to cellular structures, which likely enhance environmental performance.

Furthermore, the energy consumption associated with extracting compounds from *Coelastrella* sp. LRF1 biomass using CH is significantly higher (1678 kWh/kg_{DW} at CH70) than that of OH at the same treatment temperature (99.9 ± 8.5 kWh/kg_{DW}). The disparity becomes even more pronounced when comparing to OHP treatments; with an electric field of 217 V/cm, the energy consumption drops to just 8.0 ± 4.6 kWh/kg_{DW} [8].

The OHP100** treatment presents a win-win scenario by combining enhanced extraction efficiency with superior environmental sustainability.

These results demonstrate that using OHP instead of CH significantly reduces the environmental impacts associated with protein extraction from *Coelastrella* sp. LRF1 biomass. Moreover, these emerging patterns in the data prompted a more in-depth analysis of the relative contributions of the inventory classes (Fig. 2) and the various process phases (Fig. 3), aimed at identifying the dominant factors influencing these outcomes.

Based on the data presented in Figs. 2 and 3, the dominant factor (hotspot) influencing the environmental impacts is the energy consumption during the microalgae cultivation stage, which is the primary contributor in 16 of the 18 impact categories analysed. The exceptions to this trend are the MEP and SOP impact categories, where the main contributors are the EoL processes and the Micro & Macronutrients, respectively.

During the cultivation stage, the contribution of energy primarily stems from electricity consumption required to sustain artificial illumination throughout the fourteen days of cultivation. Depending on the extraction technology employed, energy consumption during the cultivation phase accounts for between 94.3 % and 98.0 % of the system's total electricity consumption. This finding aligns with the results reported by Pechisiri et al [20], who investigated the environmental impacts of various bioreactor types for microalgae cultivation on a pilot

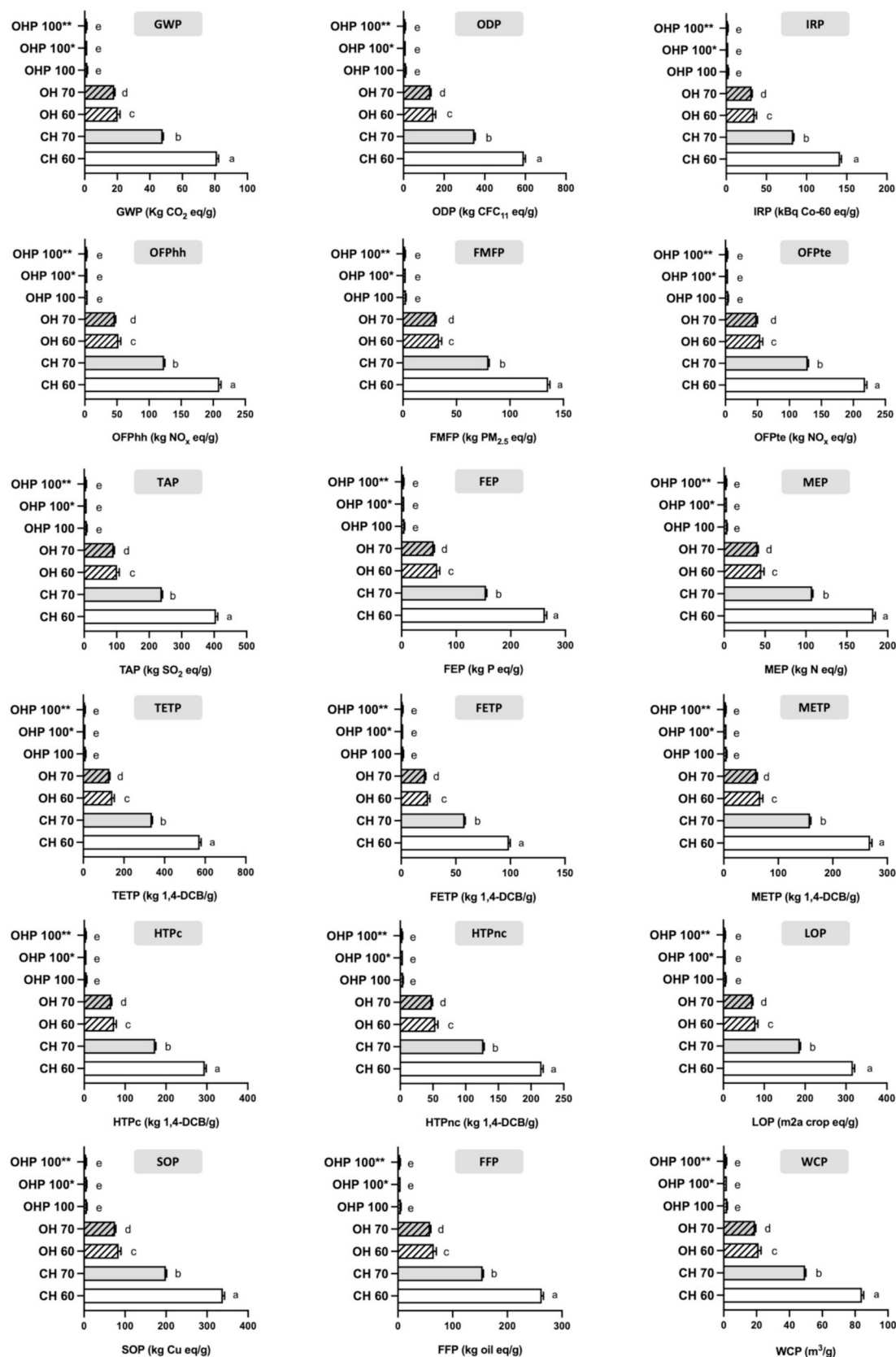


Fig. 5. Potential environmental impacts per 1 g of protein (functional unit, FU) during the protein extraction life cycle stage from *Coelastrella* sp. LRF1, comparing the various extraction technologies analysed in this study.

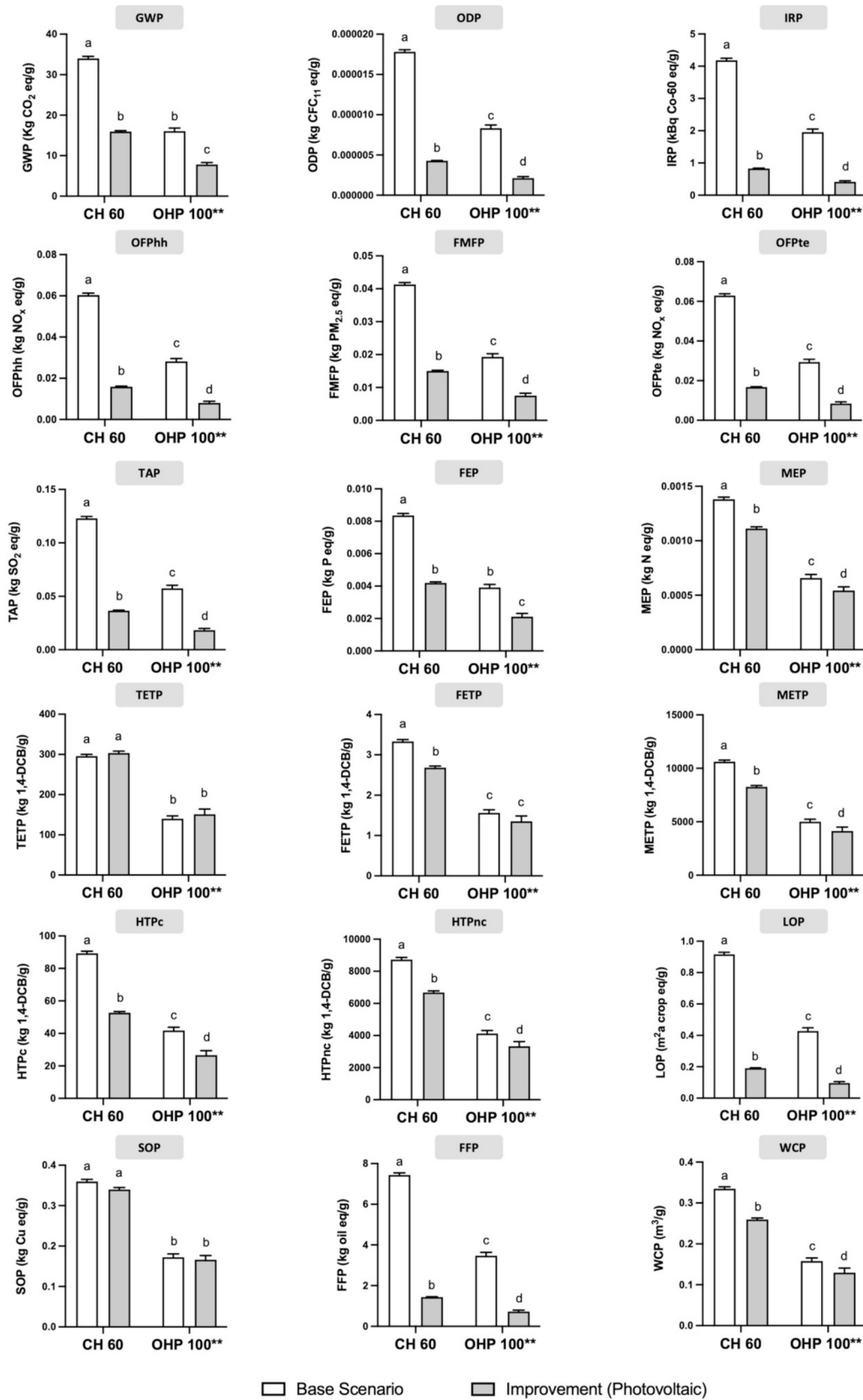


Fig. 6. Comparison between the case study scenario utilizing the Portuguese electricity mix and the photovoltaic energy scenario.

scale and concluded that energy consumption for illumination is the dominant impact factor, representing approximately 41 % of the total.

Several alternatives exist for reducing energy consumption associated with lighting in microalgae cultivation. One viable solution is outdoor cultivation, whether in open or closed systems, which leverages natural sunlight and minimizes reliance on artificial lighting. However, open cultivation systems face challenges such as increased exposure to contaminants and dependence on regional climatic conditions. Another approach is heterotrophic cultivation, known for its higher biomass growth rates. However, research has indicated that protein productivity tends to be lower in heterotrophic systems [29,30]. In contrast, employing photoperiods under autotrophic and/or mixotrophic conditions has been shown to enhance the protein productivity of microalgae [31,32].

Additional strategies to reduce energy consumption in microalgae cultivation include: (i) Integration of solar panels to generate electricity for the lighting system can significantly decrease reliance on conventional energy sources [33]; (ii) The use of photobioreactors that are specifically engineered to distribute light efficiently throughout the cultivation system can enhance overall light utilization [34]; (iii) The implementation of smart lighting systems that automatically adjust the intensity and duration of light based on the specific needs of microalgae at various growth stages can improve energy efficiency; (iv) Adopting energy-efficient LED lights tailored to the wavelengths essential for photosynthesis can reduce electricity consumption while providing a longer lifespan compared to traditional lighting sources; (v) Adaptation of the light spectrum to meet the specific requirements of microalgae not only enhances growth rates but also contributes to lower overall energy consumption; (vi) Implementation of sensors and monitoring systems to track the performance of the lighting setup allows for continuous optimization of lighting conditions, ensuring greater energy efficiency [35,36].

In the EoL stage, wastewater treatment is the primary contributor (98 %) to the MEP impact category. Following treatment, this wastewater is discharged into natural water bodies in compliance with regulations lead to marine eutrophication [37,38]. One alternative to conventional wastewater treatment is the reuse of this water for microalgae cultivation. However, this approach poses the risk of potential contamination. Therefore, some form of pre-treatment may be necessary before the water can be reused, which may include processes such as filtration, sedimentation, and/or chemical treatments.

In the Micro & Macronutrients inventory class, cobalt (II) sulphate (CoSO_4) is the primary contributor (63.5 %) to the SOP category.

Cobalt is typically sourced as a by-product of nickel and copper mining, a process that involves several steps. Initially, the ores are blasted to fragment them into smaller pieces, which are then subjected to various processing methods, including crushing, grinding, and electrolysis, to extract and refine the target metals. These extraction processes require significant heat and electricity, further contributing to the SOP category [39,40]. As an alternative, magnesium sulphate could be considered, as it has a lower classification factor in this category. The extraction of magnesium minerals involves less intensive mining processes compared to cobalt, making it a potentially more sustainable option.

While this study did not explore the potential for reducing environmental impacts through the use of bioreactors or alternative cultivation methods - primarily due to a lack of experimental data and other necessary information - its main objective was to evaluate the environmental performance of extraction technologies. Notably, the cultivation phase emerged as the dominant factor influencing environmental impacts, with 99.9 % of the impacts attributed to electricity consumption during the extraction stage.

The results comparing CH60 and CH70 indicate that the higher energy consumption required to achieve elevated treatment temperatures is offset by the enhanced extraction efficiency that this approach yields. Conversely, CH70 (2.51 ± 0.03 wt%), OH60 (2.20 ± 0.02 wt%) and

OH70 (2.47 ± 0.07 wt%) exhibit similar protein extraction efficiencies. However, the impact values for the OH technologies are more than halved compared to those for CH, owing to the superior energy efficiency of the OH processes, which require less energy to reach the desired operating conditions. The impact values for protein extraction using the OHP technologies are ten times lower than those of OH, as they achieve comparable extraction and energy efficiencies while necessitating significantly shorter operating times. Although the results show no statistically significant differences between OHP100* and OHP100**, the average impact values for OHP100** are 1.2 times lower. This reduction is attributed to the use of NaOH, which enhances protein extraction (OHP100*, 2.36 ± 0.01 wt%; OHP100**, 2.94 ± 0.14 wt%) without increasing the associated impacts.

In the improved scenario, the integration of photovoltaic panels to meet energy requirements fully led to a reduction in contributions across 16 of the 18 impact categories. Unlike traditional energy sources such as fossil fuels, solar energy harnesses the sun's energy without relying on hydrocarbon combustion, offering numerous environmental benefits. However, the SOP, TETP, and FETP categories (the latter only for OHP100**) did not show statistical differences, primarily due to the mining of copper, chromium, cadmium, tellurium, and gold used in the production of photovoltaic (PV) panels and electrical installations [41–43]. Current photovoltaic panel modules play a crucial role in mitigating the potential environmental impact of these energy-intensive biotechnologies. However, with efficiencies ranging from 10 % to 30% [44–46], there remains significant room for improvement, which could enhance the competitiveness of these alternatives.

5. Conclusions

This study evaluated the environmental performance of protein extraction technologies from *Coelastrella* sp. LRF1. The results indicated that OH technology, utilizing electric pulses at 217 V/cm with 5 mM NaOH (OHP100**), provided the best environmental performance alongside higher extraction efficiency. The primary hotspot was identified in the cultivation phase, primarily due to high energy consumption from artificial illumination. Additionally, wastewater treatment and cobalt sulphate emerged as the main contributors to MEP and SOP impact categories, respectively. Although alternative improvement strategies were proposed, further exploration is constrained by limited experimental data. OH technology demonstrated energy efficiency and reduced environmental impacts during the extraction stage. Furthermore, substituting the Portuguese electricity mix with photovoltaic panels resulted in decreased environmental impacts across most energy-related environmental impact categories.

CRedit authorship contribution statement

Vitor Sousa: Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Roberto Novais:** Writing – original draft, Visualization, Methodology, Investigation. **Teresa M. Mata:** Writing – review & editing, Software, Methodology, Conceptualization. **António A. Martins:** Writing – review & editing, Software, Methodology, Conceptualization. **Ricardo N. Pereira:** Writing – review & editing, Supervision, Methodology, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.algal.2025.103962>.

Data availability

Data will be made available on request.

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