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Life cycle assessment of diatom frustule production: a multitask bio-material

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ABSTRACT

Diatom frustules have gained increasing interest for their potential use as multitask biomaterials, due to their highly intricate mesoporous siliceous structure that is an attractive scaffold for a variety of special applications. To be used, the organic part must be eliminated from frustules, while preserving the peculiar structure. Four techniques are proposed in the literature, namely: i) oxidative washing with H_2O_2, ii) chemical treatments with oxidants and acids iii) thermal treatment and iv) plasma treatment. Only techniques (i) and (ii) can be considered of general use, as the siliceous structure is sensitive to the application of (iii), and (iv) is not suitable for some specific applications of the materials. In this paper, Life Cycle Assessment methodology was applied to assess a) the production of frustules by $KMnO_4$-acid treatment or oxidation with H_2O_2, the two least aggressive techniques and potentially applicable to a larger type of frustules; b) the deep cleaning of so-obtained frustules by thermal treatment, reaching zero-residual-carbon. LCA shows that $KMnO_4$-acid treatment is 10 times less burdening than the H_2O_2-based oxidation method and that the production of diatoms generates an impact higher than the cleaning on Global warming and on all other categories. The overall production of clean frustules at lab scale results in an impact of 12 kg CO_2 eq./g of frustule, a value expected to decrease substantially with the implementation of upscaled cultivation methods. Biorefinery approach, that allows the extraction of several added-value products from diatoms, would produce an economic advantage coupled to the reduction of the impact.

1. Introduction

Diatoms are abundant and diverse primary producers accounting for roughly 20 % of the total primary production on Earth, sustaining the oceanic food web. Being surrounded by silica shells called frustules, they are responsible for the production of 255 Tmol of biogenic silica annually [1]. Beside the key role in ocean ecosystems, diatoms are deeply investigated for several biotechnological applications. In general, microalgae have been considered for their potential for the cheap production of important metabolites exploited as: cosmetic ingredients [2], food or feed supplements [3–6], fertilizers [7,8], accumulators for the bioremediation of aquatic environments [9,10]. Recently, they have been even assessed for the production of biofuels [11–13]. In particular, the natural reserve of biosilica in diatoms is considered with much attention for potential innovative applications [14–16]. A quite new application has been discovered within the ongoing European Project DESIRED (Horizon n. 101083355) where their ability to act as template for the growth of specific facets of semiconductors has been demonstrated [17].

Frustules are a highly intricate ornamented and porous structure, with a species-specific pattern. Beside radial or bilateral symmetry, all frustules present an hypotheca inserted in a bigger epitheca connected with systems of girdle bands, like a Petri-dish, surrounding the living cell: the two valves may show different porous layers. Frustules are basically composed by hydrated amorphous silica ($SiO_2{\cdot}nH_20$) with Si-OH (silanol) and Si—H (silane) groups on the surface [18]. FTIR spectroscopy has allowed to identify organic residues in the silica matrix (mainly C—H bonds and sulphur compounds) [19,20]. Frustules are surrounded by extracellular polymers (organic coating) involved in sessile adhesion, gliding, formation of biofilms and colonies, protection

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against drying and prevention of silica dissolution [21]. Although sensitive to basic environments that may dissolve silica, specific studies have shown that frustule-scaffolds possess quite unique mechanical properties with an incredible strength, much higher than other natural biomaterials, [22] with hardness values ranging from 0.5 to 36 GPa, and elasticity ranging from 1 to more than 20 GPa [18,21,22]. In addition to their mechanical properties, frustules are also ascribable to photonic crystals, due to their periodic architecture and distribution of refractive index which can block the propagation of light of specific wavelength. The interaction with light consists in three documented phenomena: light confinement [23], selective transmission [23,24], photoluminescence (they emit blue radiations if irradiated with UVB light) [18]. These interesting properties and the high surface area of frustules make them exploitable in several fields: (i) biomedical application, (ii) micro-lenses, (iii) bio-inspired solar-cells and UV-filters, (iv) optical sensors and biosensors, (v) lasers, and (vi) photocatalysis, filling the global demand for cheap nano-structures [14–16]. Biosilica obtained from diatoms can be considered an attractive alternative as compared to synthetic materials, because of the reduced requirements for high temperatures and aggressive chemicals to gain a small, hard, elastic, with high degree of complexity material [20,25]. Moreover, the variable surface structure and its flatness may represent a quite interesting and different alternative with respect to channelled inorganic materials such as zeolites, as catalytic centres supported on them would result more accessible. Given the potentiality of frustules as silica-based biomaterials and their added-value exploitation, it looked to us interesting to assess which way to obtain frustules is more sustainable and viable, also in view of a large-scale production. To obtain the bio-silica scaffold from diatoms, the organic matter that adhered on their surface has to be removed, preserving the frustule shape, and avoiding its erosion [16,26,27]. The ways used to obtain frustules can be categorized as follows (i) Oxidative washing using H_2O_2 solutions, (ii) Chemical treatments with oxidants and acids, (iii) Baking frustules at high temperatures, [16,26,28–31] and, quite recently, iv) Oxygen-plasma asher (reaction with charged oxygen ions to remove organic components) [27]. It must be emphasized that not all the methods are suitable for all the kind of frustules as they can impact on the structure quality [26,27]. The thermal treatment, for example, even if is the simplest and less expensive method, can result aggressive on some species and deeply alter the frustule architecture [16,26,28–31]. Moreover, some treatments can affect the final use of the frustule: in particular, oxygen plasma treatment, even though is very effective in cleaning frustules without perturbing the ultrastructure, may not be suitable for subsequent frustule-surface modification, crucial step for targeted drug delivery applications [32]. Therefore, the most commonly used methods for producing frustules at laboratory scale, are the H_2O_2-oxidative washing and the $KMnO_4$-acid treatment, that can be used with a large number of frustules and for a wide range of applications [32]. Noteworthy, both such methods leave a residual amount of organic matter [26]: for many purposes, such residues would not influence the application of frustules (e.g. adsorption of contaminants and drug delivery) [33,34], while if the final use of the bio-material is to act as support of chemo-catalysts, such residual organic matter can interfere with the chemical process and must be eliminated most likely using a thermal treatment [17,35].

Frustules may represent an alternative to conventional materials such as zeolites (natural and synthetic) and other supports, playing the unique card of their particular structure; however, their production must be sustainable and economically viable. Despite microalgal cultivation on a large scale to produce biodiesel and other high-value products is already in the spotlight and numerous efforts have been made to investigate its sustainability [36–42], literature is scant in studies addressing the sustainability of frustule production.

The purpose of this paper is to fill this lack in knowledge, assessing using the LCA methodology which of the two most-widely used techniques of production of frustules is the most sustainable from the environmental point of view, even including a final controlled thermal treatment for eliminating residual-carbon and producing refined frustules. LCA is a methodological framework for estimating and assessing the environmental impacts (in different impact categories) of products, processes and services, considering their whole life cycle. The analysis was carried out in agreement with LCA ISO standard 14,040:2006 (*Environmental management — Life cycle assessment — Principles and framework* International Organization for Standardization, Geneva, Switzerland, 2006) and 14,044:2006 (*Environmental management — Life cycle assessment — Requirements and guidelines* International Organization for Standardization, Geneva, Switzerland, 2006) and it includes the four main steps of: goal and scope definition, life cycle inventory analysis, life cycle impact assessment, and life cycle interpretation.

LCA analysis in this case is not applied to a large-scale production of the material analysed, since still no implementation worldwide occurred. Despite numerous research projects in the field are financed due to the promising use of this versatile biomaterial (https://www.bioinnovation.se/en/projekt/diatom-frustules-a-biobased-and-sustainable-carrier-structure-for-controlled-release-in-multiple-applications-step-1/), the production of diatom biomass to obtain frustules is mostly under investigation. For this reason, LCA serves as a valuable guide for the future implementation of the technology. A comprehensive comparison of all production stages at small scale was conducted in this study, identifying the most critical phase that requires improvement for a sustainable scale-up. Furthermore, a detailed sensitivity analysis allowed us to examine the impact of electricity variation on the overall process, considering the use of industrial equipment in the context of process scale-up. To this end, we explored various algal cultivation configurations. The maximization of the economic value of diatoms requires the recovery of value-added products before implementing cleaning techniques for organic elimination and the production of C-free frustules, in alignment with Biorefinery principles. The costs and profits of this configuration was here preliminary estimated using data taken from literature.

2. Material and methods

2.1. Algal growth

Diatom species selected for testing the cleaning procedures were *Chaetoceros muelleri* (CCAP 1010/3, https://www.ccap.ac.uk/), *Conticribra weissflogii* (DCG 0320, https://bccm.belspo.be/about-us/bccm-dcg), *Navicula* sp. (DCG 1034), and *Seminavis robusta* (DCG 0105). The first two species were established in 3 L Erlenmayer flasks filled with 1.5 L of F/2 + Si [43], while cultures of *Navicula sp.* and *S. robusta* were established in plastic cell flasks with 300 cm^2 of growth area (200 mL working volume, vented cap). After 10 days, cells were collected and washed to obtain cleaned frustules using the protocol described in Session 2.2. Species with both different ultrastructures and cultivation modes were selected to broaden the applicability of the cleaning techniques and ensure their wider usability.

To obtain data for LCA analysis, several replicates of *Navicula* sp. cultures were established to obtain 1 g of cleaned but not refined frustules (corresponding to almost 30 L of growth medium).

2.2. Cleaning procedures and their functionality

Cells were treated using two different techniques to obtain cleaned frustules.

A) The first method was based on the oxidation of the organic matter using H_2O_2 (modified from BCCM/DCG protocol). The salts of the culture medium were washed out from the cells with deionized water followed by centrifugation at 3000 rpm for 10 min (three cycles), then 30 % H_2O_2 was added to the cell suspension in water to reach a final concentration of about 15 %. So obtained samples were heated in a closed vessel at 60 °C for 3 days in an oven. Finally, the solid material was separated by centrifugation, washed four times with deionized water to carefully remove residual H_2O_2 and dried.

B) The second method was a chemical treatment with $KMNO_4$ and acids (modified from [44]). Cells were washed in deionized water and centrifuged at 3000 rpm for 10 min. The procedure was repeated three times. The resulting pellet was resuspended in 1.5 mL of deionized water, charged with 1.5 mL of a saturated solution of $KMnO_4$ and left at ambient temperature for 24 h. Subsequently, 3 mL of HCl (37 %) were added to solution containing diatoms and $KMnO_4$ and the resulting suspension was heated in boiling water until it became colourless (1 h approximately). Frustules were recovered by centrifugation and washed four times with deionized water to carefully remove the acid.

Samples of the cleaned material obtained with the two methods above were mounted on stubs and analysed by SEM (High Resolution ZEISS – SUPRA 40) to verify that the cleaning procedures removed the organic matter without affecting the frustules ultrastructure [45]. A further thermal treatment of the cleaned frustules to completely remove the organic matter was carried out on the strongest frustules (*C. weissflogii* and *Navicula sp.*). Heating for 30 min at 400 °C was followed by a rise of temperature up to 700 °C in additional 30 min. Samples of the materials were analysed by SEM to exclude eventual changes of the ultrastructure.

2.3. Goal and scope of the LCA analysis of the cleaning procedures

Life Cycle Assessment (LCA) was applied to quantify the potential environmental impacts of the cleaning process for producing frustules across its entire life cycle, from raw material extraction to production, using inventory data and impact assessment methods as defined by ISO 14040/44. The analysis was conducted in two steps. First, two selected cleaning procedures were compared by assessing their impacts across sixteen categories (Table S4) to identify the more sustainable option for obtaining cleaned frustules from living diatom cells. Second, the overall environmental impact of frustule production was evaluated to determine the most critical phases that should be carefully addressed in view of potential scale-up.

In Fig. 1 the system boundaries of the first step are reported, that include: algal washing, removal of the organic matter using the two different techniques, frustule washing. Since the main objective of the first phase is to compare the effectiveness of different cleaning procedures used to remove organic matter from diatom biomass, the processes common to both procedures related to algal cultivation and harvesting were excluded from the system boundaries.

Similarly, the final thermal treatment commonly applied to eliminate residual organic carbon was also excluded. This process is not universally required but rather depends on the specific end-use of the frustules (e.g., applications demanding highly purified silica structures). To maintain clarity and comparability across the different cleaning approaches, and to avoid introducing application-specific biases, the present phase of the study is restricted to evaluating the methods for organic matter removal only. In particular, Scenario 1 is relevant to the oxidation method based on H_2O_2 for removing the organic matter from frustules, while Scenario 2 targets the $KMnO_4$-acid treatment procedure (details of the methods are described in 2.2). The functional unit is 1 g of non-refined frustules, and the analysis has assessed the impact connected to the consumption of energy and raw materials. Table 1 summarizes the considered input and output flows involved in the two Scenarios.

In the second phase of the study, however, the system boundaries were expanded to include upstream processes such as algal cultivation and downstream treatments like thermal removal of residual carbon, in order to provide a more comprehensive evaluation of frustule production and potential applications. Fig. 2 represents this expanded system boundary, which covers algal cultivation and the final thermal treatment required to achieve zero organic carbon in the production of 1 g of non-refined frustules, applied to the most sustainable cleaning technique, thereby enabling an overall assessment of the end biomaterial production process. Data were obtained from laboratory scale cultivation of diatom *Navicula* sp. as source of 1 g non - refined frustules. A climatic chamber and enriched seawater (F/2 + Si medium) were used to grow algae. Since the functions of this biomaterial can be multiple (as already presented in the introduction, from support for chemical catalysis to drug delivery), it was chosen to use a declared product unit that includes all its possible functions.

Table 2 resumes the considered input and output flows of the frustule production process at laboratory scale. Such expansion allows to compare the energy and materials demand and relevant impacts of the three distinct modular phases: i) production of diatoms; ii) production of cleaned frustules; iii) production of refined frustules (zero residual carbon).

While laboratory-scale data provide valuable insights for the preliminary evaluation of processes and can serve as a basis for guiding scale-up strategies, their representativeness is intrinsically limited. Results obtained under laboratory conditions are often strongly influenced by specific experimental settings, equipment, and operational parameters, which may not accurately reflect larger-scale or industrial scenarios. To address this limitation and reduce potential bias, a sensitivity analysis was implemented, enabling a more robust interpretation of the results within the current boundaries. Nevertheless, future studies based on pilot- or industrial-scale data will be essential to validate these findings and to support a more comprehensive life cycle and techno-economic assessment of frustule production.

The LCA for Expert software v. 10.9.1.10 integrated with My Professional Database v. 2024.1 was used for the production of energy and raw materials and the assessment of the environmental footprint related to the two Scenarios. The impact of $KMnO_4$ was obtained from Ecoinvent database 3.10 (integrated within the software SimaPro 9.6.0.1). The method selected for the analysis, which included classification, characterization, normalization and weighting steps, was Environmental Footprint (EF) 3.0, including all environmental categories (Table S4), recommended models at midpoint, together with their indicators, units and sources. As energy source, photovoltaic was preferred to the standard grid to comply with the principles of green energy[1]. The technologies included in the European photovoltaic energy considered for the analysis are mono-silicon (39.1 %), multi-Silicon (54.6 %), Cadmium-Telluride (CdTe, 4.5 %) and Copper-Indium-Gallium-Diselenide (1.8 %).

All technologies are modelled according to the national or region-specific situation. The robustness of the included processes was evaluated by the LCA Software, a Pedigree matrix which assesses reliability, completeness, temporal correlation, geographical correlation and technological correlation giving a qualitative score from 1 (the best one) and 5 (the worst one) [46]. All the considered processes have a score between 1 and 2, and they are considered reliable. Possible variability due to the impact categories is not considered relevant for the comparison among the scenarios since they were assessed by the same method (EF 3.0). The impact allocation was not necessary since the process does not produce by-products.

3. Results and discussion

3.1. Functionality of the two methods

Fig. 3 present SEM images of frustules obtained with oxidation cleaning procedure using H_2O_2 (black border) and through $KMnO_4$-acid

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[1] https://www.ellenmacarthurfoundation.org/the-circular-economy-in-detail-deep-dive

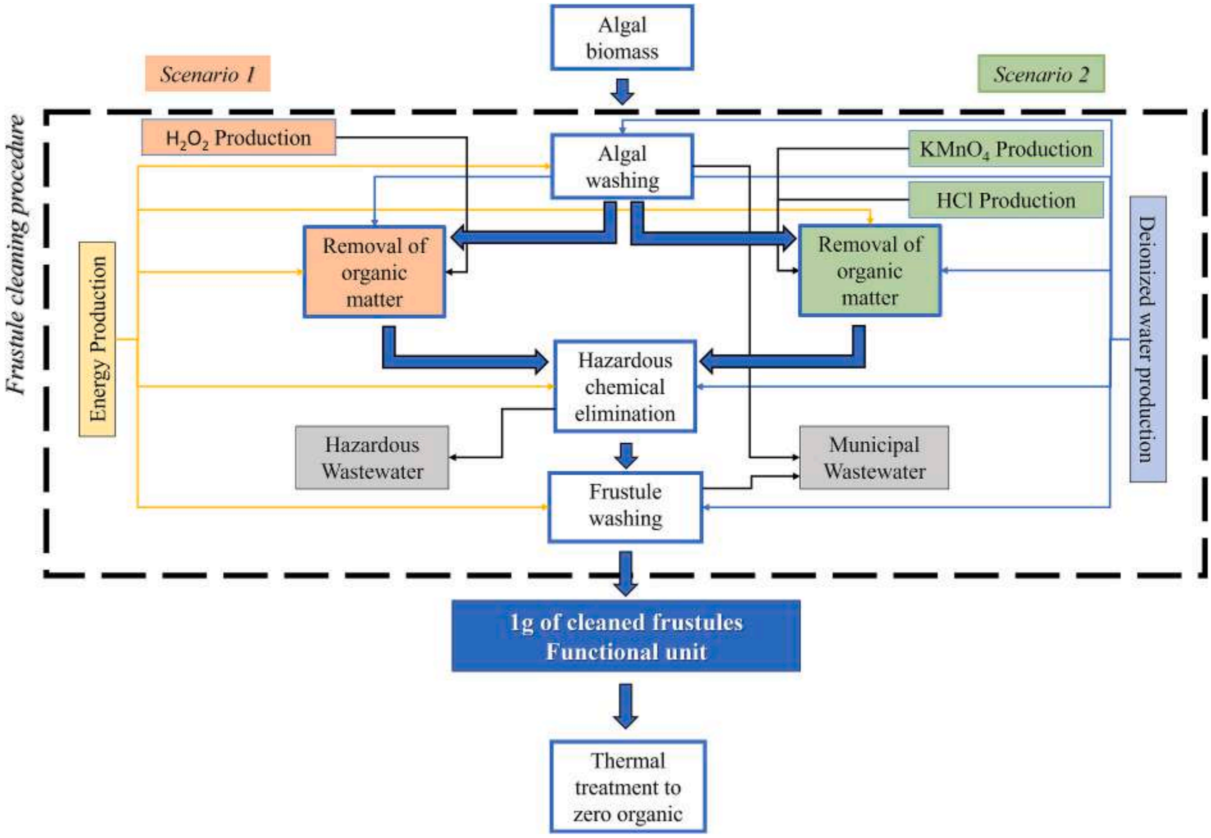

Fig. 1. System boundaries considered for the environmental footprint assessment – comparison between two cleaning procedures. The algal cultivation and the thermal treatment were excluded from the boundaries because they are common to both scenarios.

Table 1
Input and output flows considered for the environmental footprint assessment of the 2 Scenarios (Functional unit: 1 g of non-refined frustules).

	Input Flow		Output Flow	
Scenario 1	Electricity	67 kWh	Municipal wastewater	195 L
	Deionized water	190.5 L	Hazardous wastewater	30 L
	H_2O_2	4.5 kg		
	Seawater	30 L		
Scenario 2	Electricity	15.1 kWh	Municipal wastewater	125.4 L
	Deionized water	98.67 L	Hazardous wastewater	3.6 L
	HCl	0.33 L		
	$KMnO_4$	0.028 kg		
	Seawater	30 L		

treatment (modified from [44], red border). Images highlight that both methods lead to the removal of the organic matter coating frustule surface without damaging the ultrastructure of frustules (evident in the detail figures of nanopores, Fig. 3 G, H, K, L). Both procedures can be used to obtain the biomaterial from the tested species; two with a radial geometry, *C. muelleri* and *C. weissflogii* (Fig. 3 A, B, C and D), and two with bilateral geometry, *S. robusta* and *Navicula* sp., (Fig. 3 E, F, G, H, I, J, K; L). The number of diatom species found in nature may reach values up to 25,000 [47]. More recently evolved species may present a reduced degree of silicification [45,48,49], that makes the relevant frustules more sensitive to aggressive treatments that can destroy their functional structure [16,27]. For this reason, the study focuses on the resistance of species differing in geometry (centric and pennate) and mode of life (planktonic and benthic) to various treatments, thereby confirming the broader usability of the selected cleaning procedures. However, beside the selection of suitable cleaning procedures, for a successful implementation of frustules as biomaterial, it is essential to balance frustule yield, mechanical robustness, and the suitability of pore patterns for the intended application. Smaller species, such as *C. muelleri*, exhibit faster growth rates (data not shown), but their frustules are more fragile and yield less material compared to larger species. This makes them better suited for applications requiring only small amounts of material, such as drug delivery. In contrast, applications that demand larger quantities of frustules, such as their use as catalyst supports [17], benefit from larger species, which offer higher yields and thereby reduce production costs. For catalytic applications, both centric and pennate species proved suitable. However, the specific pore structures strongly influenced the performance of the resulting catalysts [17]. This indicates that, beyond sustainability and cost considerations, careful species selection is critical to optimize the technology.

Furthermore, in the case of chemo catalysts support, it is essential that the frustule ultrastructure is completely free of organic residues. Elemental analysis (Energy-Dispersive X-ray Spectroscopy, EDX and X-ray Photoelectron Spectroscopy, XPS, [17]) revealed that samples prepared with the method described here still contained residual organic matter, necessitating an additional thermal treatment (Fig. 4A). Such treatment, as already mentioned, is not suited for all the species: the peculiar ultrastructure of the fragile frustule of *C. muelleri* was lost after thermal treatment at 700 °C. Nonetheless, the tough frustule of *C. weissflogii* (Fig. 4B) resisted such conditions and the organic matter was completely removed. Fig. 4A highlights that 1 h of thermal treatment is enough to completely remove any trace of organic matter from the frustule structure (*C. weissflogii* is shown, but the treatment can also be applied to *Navicula* sp. without damaging its fine structure).

3.2. *Assessment of the environmental impact of the frustule-cleaning procedures*

Fig. 5 presents the impact assessment on the “*climate change*” category, expressed in $kgCO_2$ eq. released to produce 1 g of non-refined

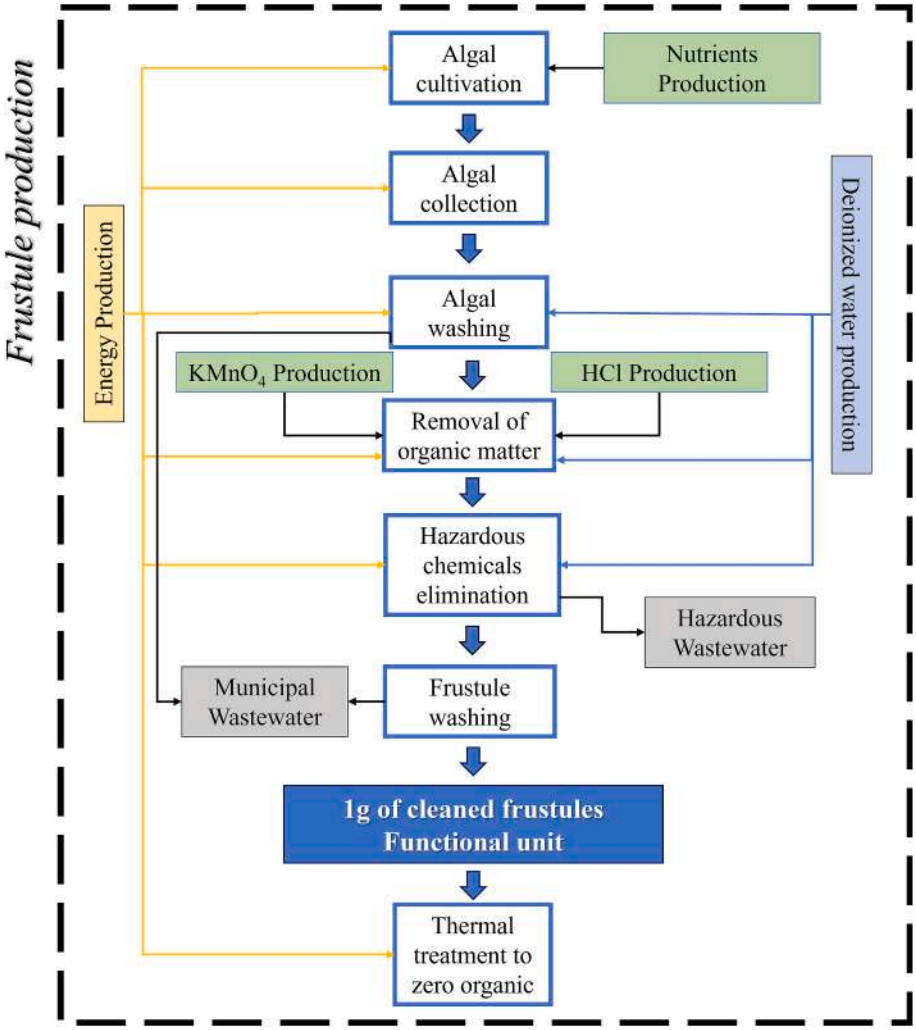

Fig. 2. System boundaries considered for the environmental footprint assessment for frustule production. In this inventory diatom cultivation at lab scale was included and the most sustainable technique for frustule production was implemented. At the end of the process, a thermal treatment to completely remove the organic matter was added.

Table 2
Input and output flows for the environmental footprint assessment of frustules production (Functional unit: 1 g of cleaned frustules).

	Input Flow		Output Flow	
Biomass production	Electricity	339 kWh	Diatom biomass	3 g
	Seawater	30 L		
	$Na_2SiO_3·9H_2O$	$9·10^{-4}$ kg		
	NaNO3	$2.25·10^{-3}$ kg		
	$NaH_2PO_4·2H_2O$	$1.7·10^{-4}$ kg		
	Na_2EDTA	$1.25·10^{-4}$ kg		
	$FeCl_3·6H_2O$	$9.45·10^{-5}$ kg		
	$CuSO_4·5H_2O$	$3.0·10^{-7}$ kg		
	$ZnSO_4·7H_2O$	$6.6·10^{-7}$ kg		
	$CoCl_2·6H_2O$	$3.0·10^{-7}$ kg		
	$MnCl_2·4H_2O$	$5.4·10^{-6}$ kg		
	$Na_2MoO_4·2H_2O$	$1.8·10^{-7}$ kg		
Frustule cleaning procedure	Electricity	15 kWh	Municipal wastewater	125.73 L
	Deionized Water	99 L	Hazardous wastewater	3.6 L
	HCl	0.33 L		
	$KMnO_4$	0.029 kg		

frustules from diatom biomass, an aspect under particular scrutiny these days. Data presented in this section are referred to the inventory shown in Fig. 1, thus the algal cultivation and the final thermal treatment were excluded because common in both the Scenarios. Scenario 1 has a greater impact (10 $kgCO_2$ eq.) as compared to Scenario 2 (0.97 $kgCO_2$ eq.). In detail, in Scenario 2 not only eco-friendlier reagents were employed (0.21 $kgCO_2$ eq. for HCl and $KMnO_4$ while 7.7 $kgCO_2$ eq. for H_2O_2), the use of water and energy was reduced and a lower environmental burden due to hazardous waste was produced (0.03 $kgCO_2$ eq. in Scenario 2 while 0.27 $kgCO_2$ eq. in Scenario 1, Fig. 5). The electricity consumption is another key-issue identified in the process: Scenario 1 requires the prolonged use of the oven (72 h, as reported in 2.2) for removing the organic matter in a gently way (responsible for almost 2 $kgCO_2$ eq. burdened), while Scenario 2 just requires boiling water for a shorter time (with 0.5 $kgCO_2$ eq. burdened). The integration of renewable energy supply is fully justified, thus, considered that in this way the impact on climate change is reduced to only 0.03 $kgCO_2$ eq. per consumed kWh. Noteworthy, if the biomaterial must be organic-matter-free, the value reported above should be equally added of 0.008 $kgCO_2$ eq. due to the thermal treatment at 700 °C.

The process of production of H_2O_2, namely the anthraquinone process considered in this study, is responsible of the high contribution of impact on climate change (Fig. 5) for Scenario 1 as compared to Scenario 2. Such process is based on cyclic oxidation-catalytic (back-)reduction of

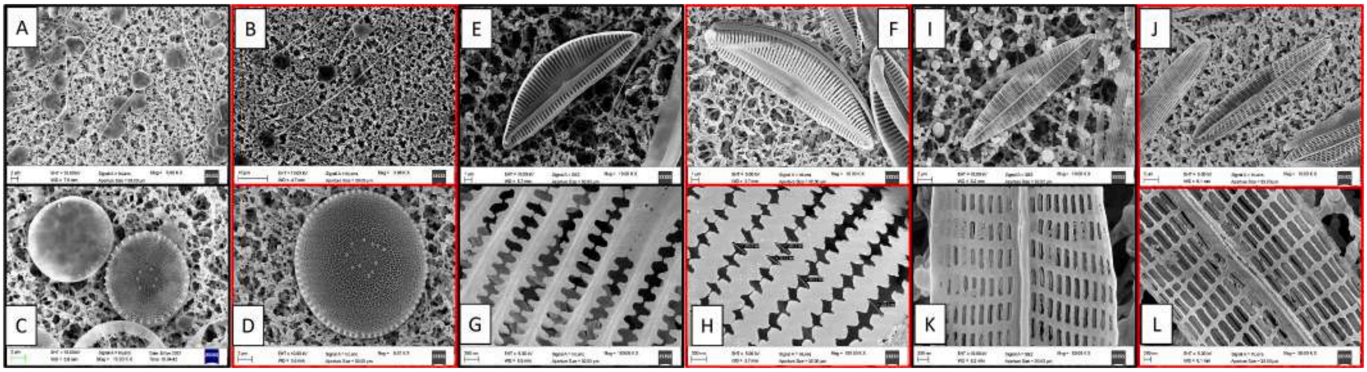

Fig. 3. SEM images of frustules obtained through oxidation method. (A) *C. muelleri* treated with H_2O_2; (B) *C. muelleri* treated with $KMnO_4$-acid acid; (C) *C. weissflogii* treated with H_2O_2; (D) *C. weissflogii* treated with $KMnO_4$-acid; (E) *S.robusta* treated with H_2O_2; (F) *S.robusta* treated with $KMnO_4$-acid; (G) details of *S. robusta* pores treated with H_2O_2; (H) details of *S. robusta* pores treated with $KMnO_4$-acid; (I) *Navicula* sp. treated with H_2O_2; (J) *Navicula* sp. treated with $KMnO_4$-acid; (K) details of *Navicula* sp. pores treated with H_2O_2; (L) details of *Navicula* sp. pores treated with $KMnO_4$-acid.

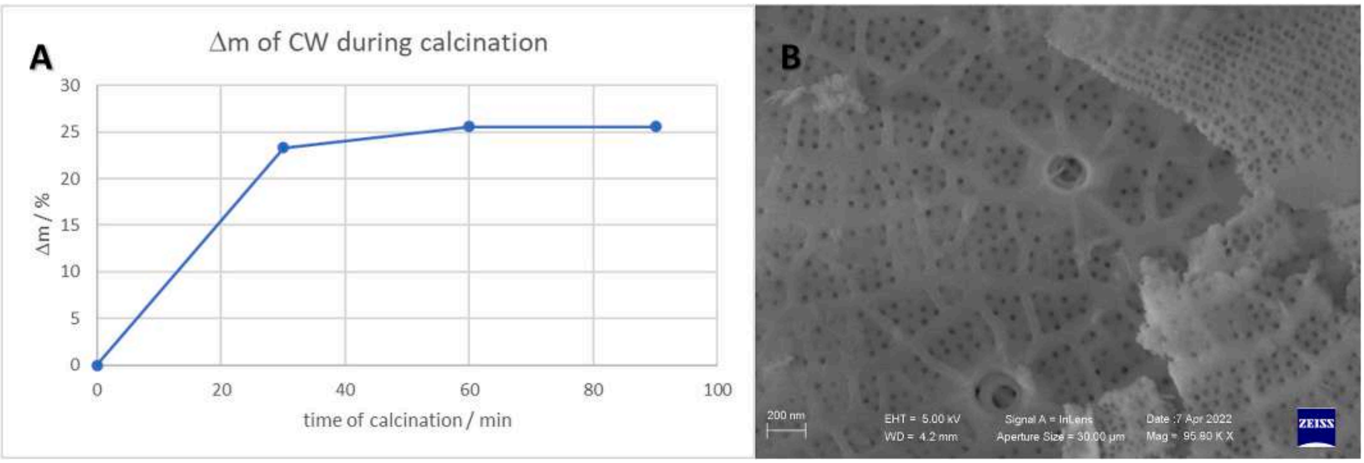

Fig. 4. (A) Mass/time variation curve of thermal treatment carried out on frustules: 30 min at 400 °C and then up to 700 °C in further 30 mins (B) SEM images of *C. weissflogii* frustule after thermal treatment: the fine structure is well preserved.

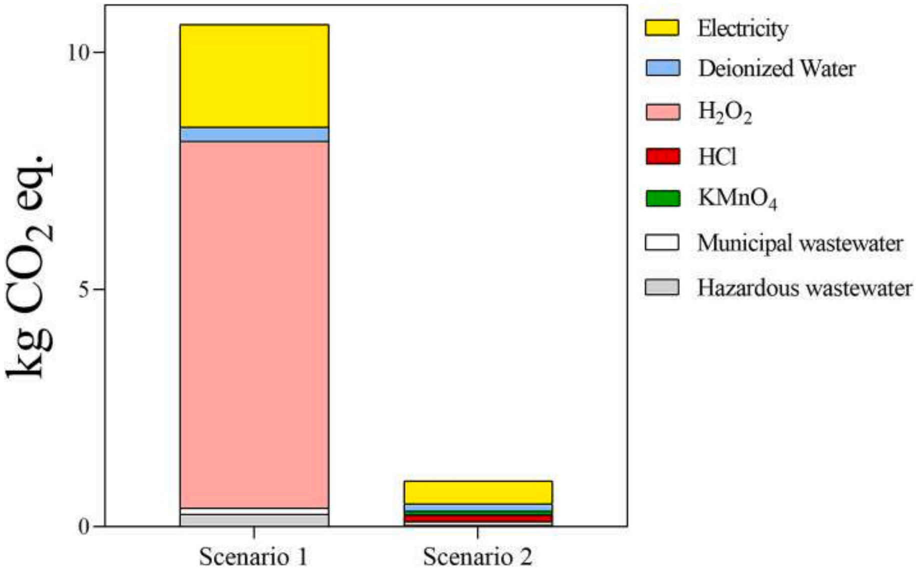

Fig. 5. Evaluation of the impacts on climate change of the two considered Scenarios for cleaning frustules: (1) oxidation with H_2O_2 (2) $KMnO_4$-acid treatment.

2-ethyl-anthraquinone; it has been validated in the software database for the period 2023–2026 and its high environmental impact has already been emphasized [50]. More ecofriendly catalytic methods are being developed, but they are not largely applied at the industrial scale, and the relevant emissions are not validated. The recent application of the Fenton and sono–Fenton processes to remove organic matter from frustule structure [51] appears quite interesting in the perspective of the decarbonization process. Nevertheless, the presence of hydrogen peroxide in the Fenton reagent suggests that the environmental impact of this method needs to be carefully assessed. The analysis of impact has been extended to all the sixteen categories (Table S4, Fig. S1), and the classification and characterization have identified Scenario 2 (based on $KMnO_4$-HCl) as the most sustainable.

The normalization and weighting steps allowed the identification of the most affected categories in both the considered Scenarios (Fig. 6). In this regard, it is evident that in Scenario 2, the percentage of impact is majorly given by freshwater ecotoxicity and resource use categories. The entire process of cleaning highlights that the production of wastewater has the largest impact (Fig. 7). Hazardous waste is composed of the strong acids and oxidizing agents used for removing the organic matter from the silica scaffold; their release into the environment must be preceded by neutralization and dilution, it is therefore very important to select less impactful cleaning reagents to increase the sustainability of the entire process. Scenario 2 is more eco-friendly than Scenario 1 even for the use of fossil resources that in Scenario 2 prevails on other categories (Fig. 6), once again because of the production of H_2O_2 (Fig. 7).

LCA analysis, thus, clearly confirms that the treatment with $KMnO_4$-acid is the most sustainable option between the two taken into consideration, without perturbing the frustule ultrastructure (Fig. 3). This treatment is more advantageous due to its lower requirement of energy; indeed, the oxidation process is shorter (one hour in boiling water instead of three days in the oven), thus, more suitable for industrial application. The lower requirement of chemical reagents is further translated into the lowest production of out-flows that require treatment for the inertization of hazardous waste.

3.3. Assessment of the environmental impact of diatom production

Although this paper deals with the comparison of the two cleaning techniques, we also report some data relevant to diatom production in order to have an idea of the incidence of the cleaning procedure on the whole cycle of production of diatoms-cleaning of frustules. The study reveals that the impact of diatoms cultivation at laboratory scale followed by the removal of organic matter through $KMnO_4$-acid treatment cause an overall emission of 12 $kgCO_2$ eq. (Fig. 8A), much higher than the amount (1.3–2.4 $kgCO_2$ per kg of dried microalgae biomass) of CO_2 captured for photosynthetic metabolism [52–54]. It is also clear that the burden related to algal cultivation is higher than that produced by the frustule cleaning procedure (expressed in $kgCO_2$ eq.) (Fig. 8A). The energy used for algal cultivation in chambers is the major responsible for the emission of CO_2. (Fig. 8B). As already highlighted in previous studies [39], the comparison among LCA analyses is strongly influenced by both the specific productivity of the considered species and the intended end-use of the biomass. Nevertheless, a common finding across these studies is that algal biomass cultivation consistently represents the most impactful stage. In our case, lab-scale cultivation markedly increased the burden in terms of kg CO_2eq. However, it should be emphasized that, in a commercial perspective, diatoms would be cultivated in photobioreactors or open ponds rather than in indoor chambers. For instance, the cultivation of the diatom *P. tricornutum* in photobioreactors has been reported to generate an impact of 257 kg CO_2eq. per kg of biomass [39], which is considerably lower than the values obtained in our study, although it refers to a species with different productivity characteristics. Even lower burdens can be achieved with open raceway pond systems, where an impact of 5.34 kg CO_2eq. for 12 kg of dry biomass has been reported [37]. Outdoor raceway ponds would be preferred because they exploit solar energy, even requiring less capital investment, involving lower operational costs, utilizing wasteland or barren land, and being easy to maintain [55]. However, diatom cultivation outdoor is still poorly documented, because it is site- and species-specific, thus detailed studies are required for achieving efficient production processes and setting reference parameters [56,57].

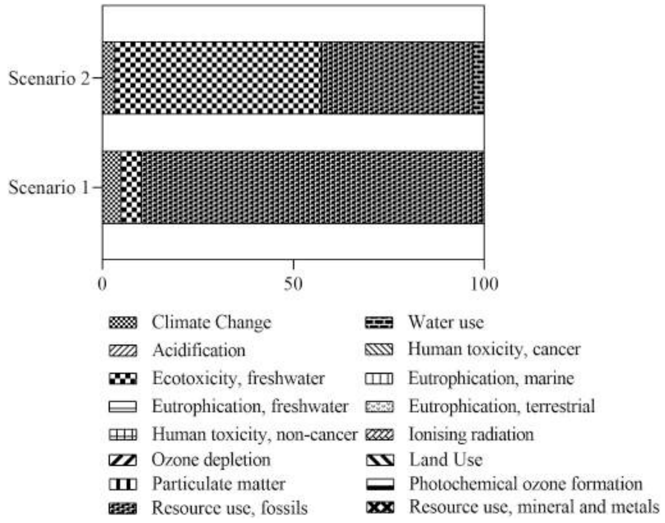

Fig. 6. Normalization and weighing results. Effect of the different input and output flows on the considered impact categories.

On the other hand, nutrients used to grow algae in optimal conditions have a very low impact on the whole process (Fig. 8A); noteworthy, the one with the highest impact is nitrogen delivered as sodium nitrate (Fig. 8C). Interestingly, a strategy to reduce the impact of nutrients for algal growth is the use of unconventional growth media which exploit the presence of nutrients deriving from waste products; numerous efforts are being done to assess the tolerance and the performance of diatoms in such kind of media with promising results [58,59]. The ability of microalgae to use N- or P-compounds contained in wastewater (municipal- and selected-industrial-waters) for growing can be considered an environmentally friendly and economical method to purify wastewater, while producing valuable products [60,61].

Normalization and weighting steps have been performed to assess the relevance of the environmental issues also on the whole process (Fig. 9). For frustule cleaning procedure, the major impact is given by freshwater ecotoxicity (Fig. 9A) with an important role played by reagents-water used and waste generated (Fig. 9B). For biomass production, the highest impact is given by resource-use, climate change and freshwater ecotoxicity. Noteworthy, today the chemicals production implies the use of fossil-C.

Frustules can be classified as high-value silica-based mesoporous materials, usable, thus, as catalysts-support, for metal adsorption and drug delivery. Mesoporous MCM-41 and SBA-15, hexagonal mesoporous silica (HMS), mesoporous COK12 bioinspired silica [62] and mesoporous silica nanoparticles (MSNs) have recently been studied for several applications, from food industry [63,64] to drug delivery [65,66]. To date, the literature reports scanty assessment studies on the environmental impacts of the production of the above synthetic materials [67]. Recently, [68] reported that a non-scaled up synthesis of 1 kg of mesoporous silica material emits 2500 $kgCO_2$ eq. Brambila and coauthors in 2022 [62] stated that high-value silicas perform poorly in the newly used Green Chemistry Evaluator (DOZN 2.0), highlighting that a more sustainable synthesis of the high-values silica required for emerging markets is necessary. Frustules, as demonstrated by the data reported above, can stand the synthetic materials and can perform much better from the environmental point of view. Concerning their cost of production, much must be done, and some considerations are reported

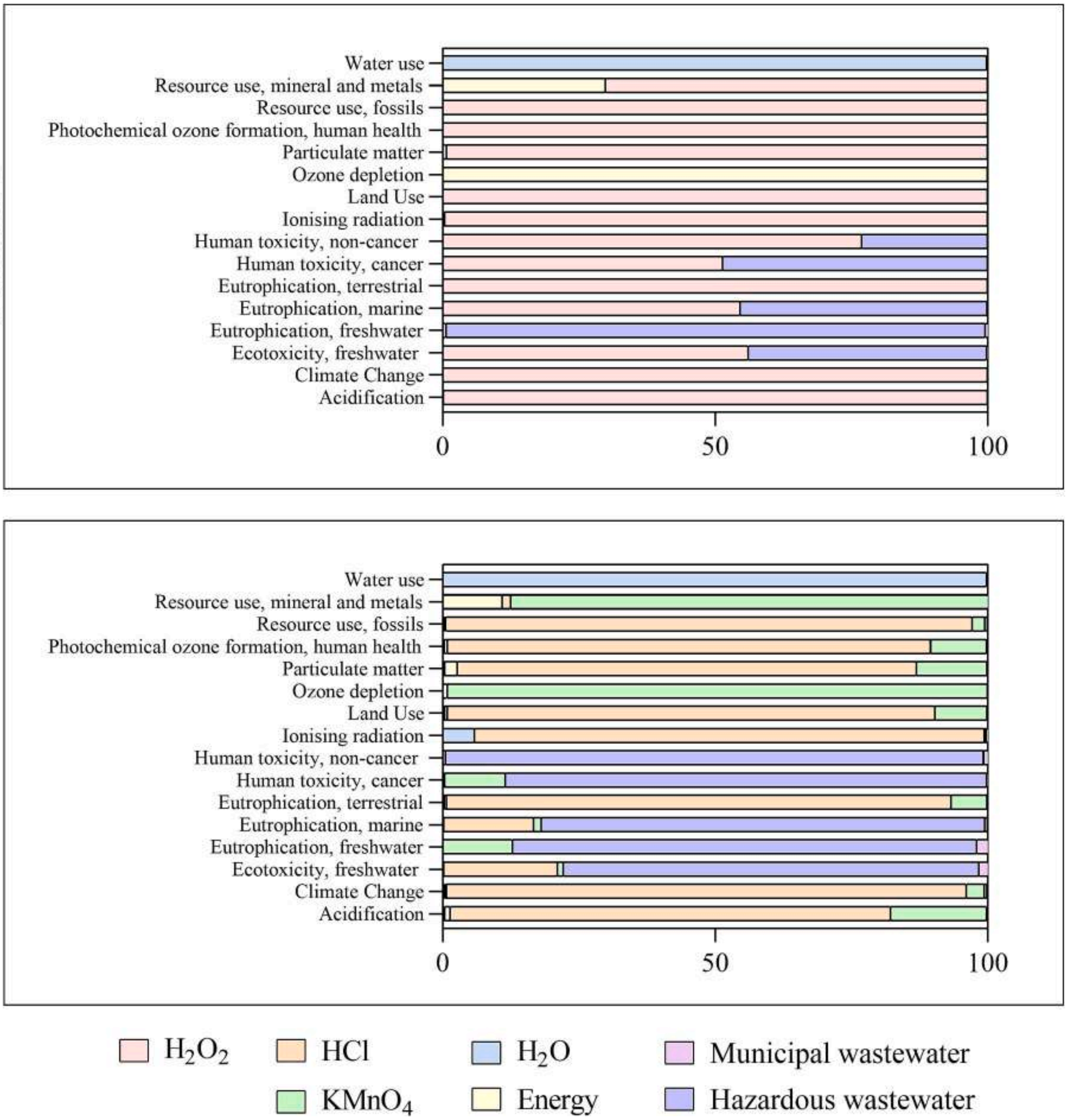

Fig. 7. Normalization and weighing results. Effect of the different input and output flows on the considered impact categories.

in 3.4.

3.4. Sensitivity analysis

What emerged from laboratory data was that the energy for algal cultivation has the major impact in terms of kg of CO_2 equivalent (Fig. 8). In case the cultivation process is implemented at larger scale, it can be calculated that for the production of 3 g of diatom biomass, the energy demand would decrease from 355 kWh (laboratory conditions) to 7.95 kWh (photobioreactors) or 0.267 kWh (open ponds) [69–71]. In a scaled-up cultivation mode, emissions were estimated at 2.6 kg CO_2eq. for photobioreactors and 0.1 kg CO_2eq. for open ponds when using a standard grid mix. These values decrease to 0.3 kg CO_2 eq. and 0.01 kg CO_2eq., respectively, when powered by photovoltaic electricity, demonstrating that green energy can reduce the environmental burden by approximately 88–90 %. Moreover, the benefit of an open pond configuration is shown in Fig. 10 which proves a possible impact decrease of more than 95 %, compared to the photobioreactor. Considering the decrease in electricity demand due to the use of industrial scale equipment, the contribution of cleaning step is highlighted at a larger scale, confirming the effectiveness of LCA as a tool to drive toward the most sustainable choice. Overall, the combination of the open pond configuration and the frustule cleaning by HCl-$KMnO_4$, reduced the Climate change burden of more than 95 %, compared to the initial lab scale scenario (Fig. 8). However, these data highlighted that the cleaning procedure at higher scale has a stronger impact as compared to algal cultivation, suggesting the need to find a sustainable solution for the future upscale of the technology.

In the case of diatoms, a strategy that can be considered for increasing their economic value and, most likely, reducing the environmental impact of the production of frustule, is the extraction of added-value chemicals before applying the cleaning procedure to afford non-refined frustules. [72,73] A Biorefinery approach, that may integrate the production of high-value co-products and biosilica, may result beneficial for valorising the diatom biomass. Very recently Jain and coauthors (2024) [73] proposed a sustainable way for biorefining diatom biomass to recover both organic and inorganic fraction, but still a lot of work needs to be done in this field to implement this technology

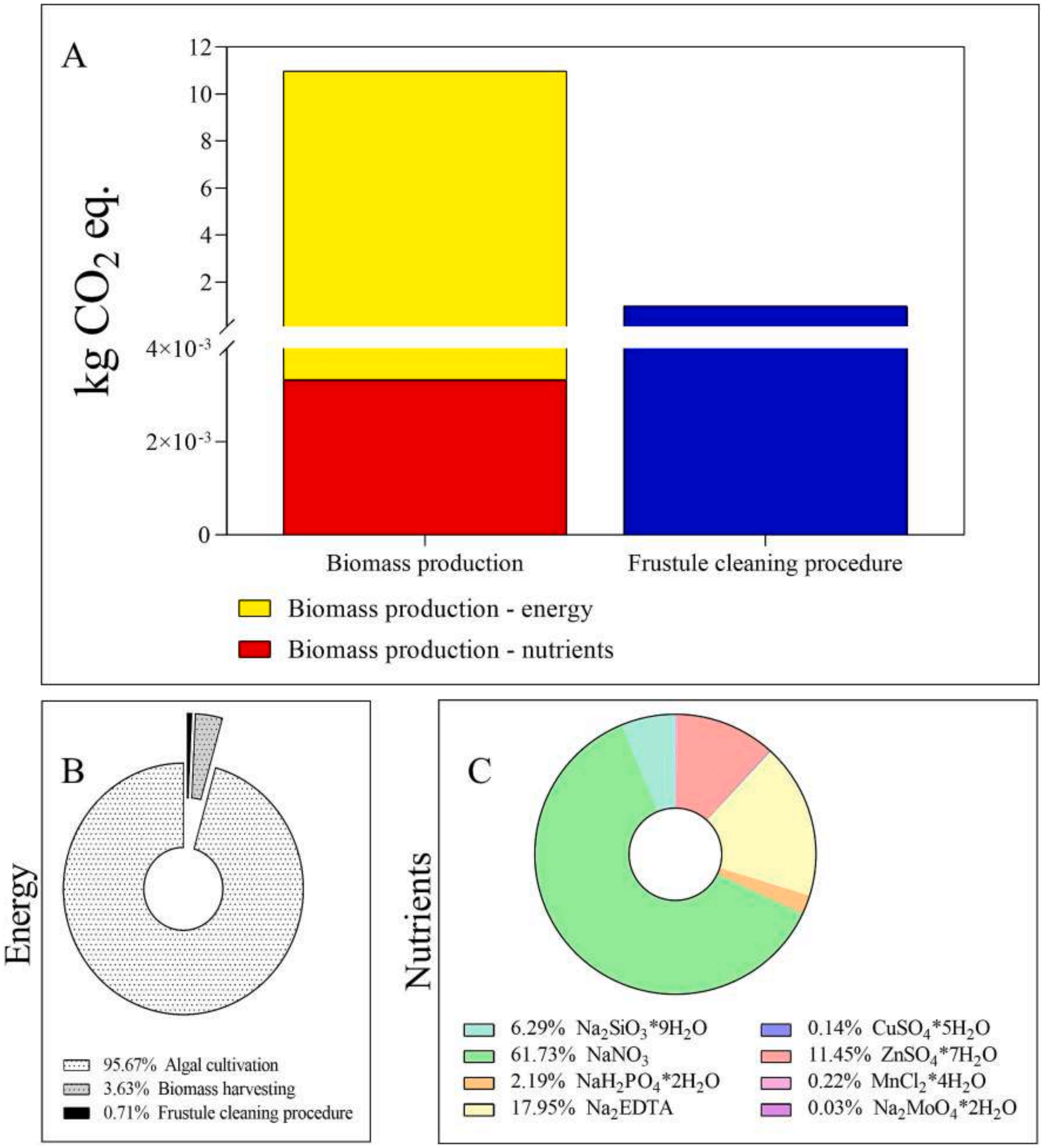

Fig. 8. Evaluation of the impacts on climate change of the entire frustule production. (A) kg CO_2 eq. burden for frustule production. (B) % of kg CO_2 eq. of the energy requirement distributed on different steps of the process. (C) % of kg CO_2 eq. of the nutrients required by diatoms to grow.

at higher scale. In the present work, to add nuances to the topic, we shortly addressed in the following preliminary considerations the putative costs and profits of a diatom biorefinery. These data are functional to what discussed above, but demand a complete study, ongoing in our Laboratory, for demonstrating their validity.

Data taken from a multi-objective optimization framework for designing a microalgal biorefinery with multiple target products [71] were used to calculate the impact of the production of biosilica from diatoms coupled to the extraction of added-value chemicals. Among more than 720 feasible routes, the present model predicts that the optimal way to set up the biorefinery is to cultivate diatom in a medium with urea as N-source and in a high concentration open pond; algae would be collected by centrifugation, washed, and 2-butanol would be used for the first extraction of organic products. In this way, which does not foresee cell disruption, the high value carbohydrate laminarin would be extracted with polar EPA from the organic phase. The second step of extraction would be made on the residual biomass separated by filtration. Cell would be disrupted through bead milling; cyclopentyl methyl ether used as solvent to extract fucoxanthin, a valued pigment which has anti-inflammatory, anti-cancer, anti-obesity, anti-diabetic, and hepatoprotective properties [74]. At this point, the biomass would be cleaned of the residual organics through thermal treatment, as discussed above. In this way a biosilica material ready for multitask application would be available.

Based on this model, the impact of diatom cultivation at large scale with the subsequent extraction of added-value products should emit almost 186 $kgCO_2$ eq. per 0.5 kg of biomass, a reduction compared to the values obtained at laboratory scale. Beyond lowering environmental impacts, the biorefinery approach also provides an economic advantage through the sale of co-products, as preliminarily estimated and reported in Table 3. Average operational consumption, extracted chemical yields, and market prices derived from the biorefinery model [71] were used to calculate the output from 3 g of *Navicula* sp. biomass (corresponding, according to experimental data in this study, to 1 g of non-refined

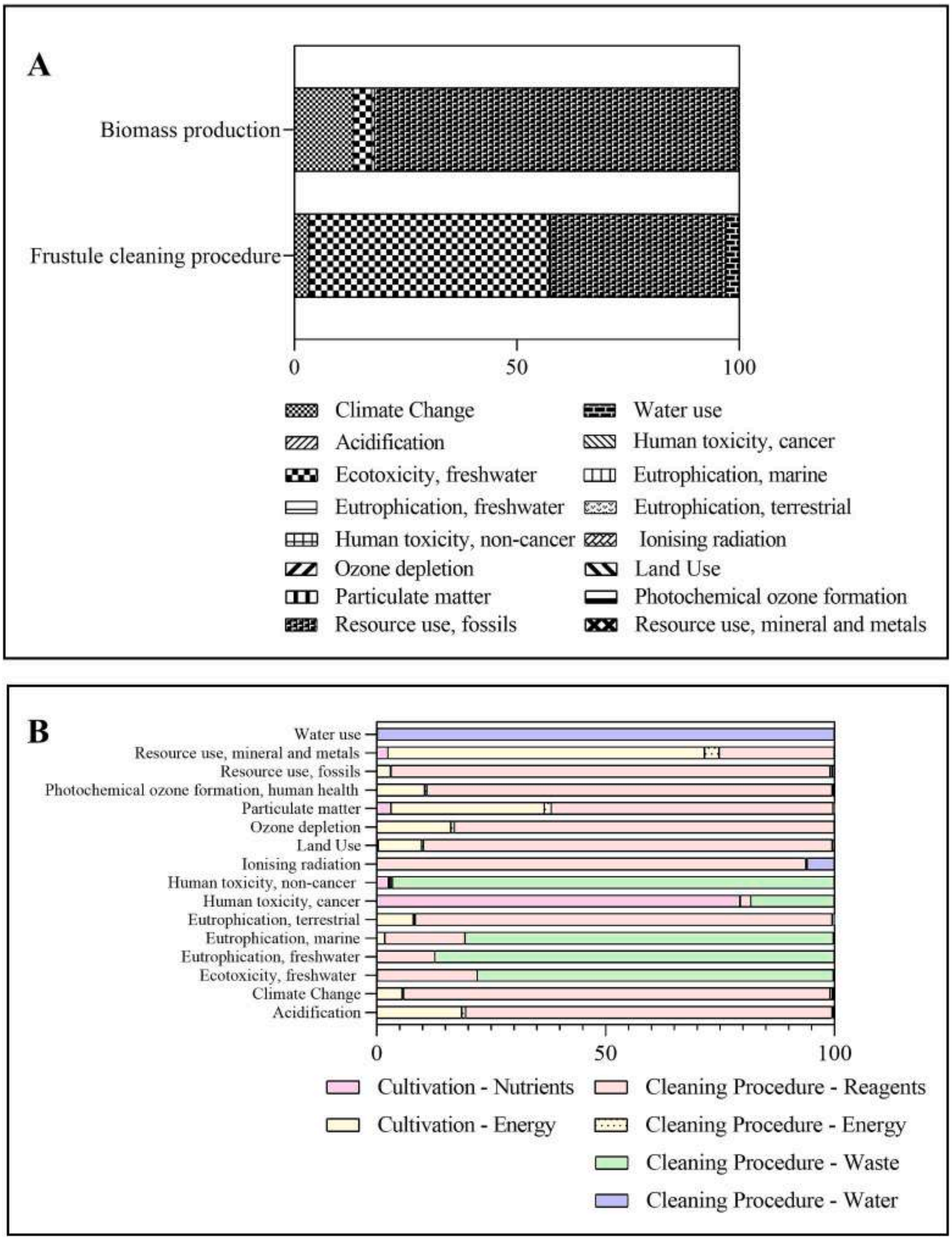

Fig. 9. Normalization and weighing results. (A) Percentage distribution of impacts derived from input and output flows in process. (B) Effect of the different steps on the considered impact categories.

frustules). This preliminary assessment indicated a production cost of €0.11 for 3 g of biomass (excluding capital investment for the production plant) and an income of €0.15 from end-products, highlighting the potential profitability of a biorefinery setup for diatom production of both frustules and other valuable molecules. These first results can serve as a reference for future scale-up, demonstrating both the environmental and economic advantages of the technology.

4. Conclusion

Biomaterials are gaining momentum in several applications in sectors such as bio-medical and chemical applications. The LCA of diatom frustule production presented in this study revealed that two standard laboratory-scale cleaning procedures for removing organic matter from the silica scaffold (washing with H_2O_2 or with $KMnO_4$ in inorganic acid) are applicable to a wide range of species differing in geometry and mode of life, although the latter is more environmentally friendly. The impact of the entire process for diatoms-frustule production was estimated at 12 kgCO_2 eq. per 1 g of non-refined frustules; this high value primarily reflects the reliance on laboratory-scale data and the adoption of a mass-based functional unit, suggesting that it is expected to be significantly reduced when upscaling diatom cultivation systems. In a view of industrial scale up, the cleaning procedure step and the overall energy requirement need to be improved to make frustules a valuable and sustainable option with respect to conventional silica-based materials. To verify whether the extraction from the algal biomass of valuable organic compounds (lipids, carbohydrates and pigments) can give an economic and environmental advantage to the process, due to the sale of the added-value products and reducing the need for an aggressive cleaning procedure of the biomass, a diatom-biorefinery was evaluated.

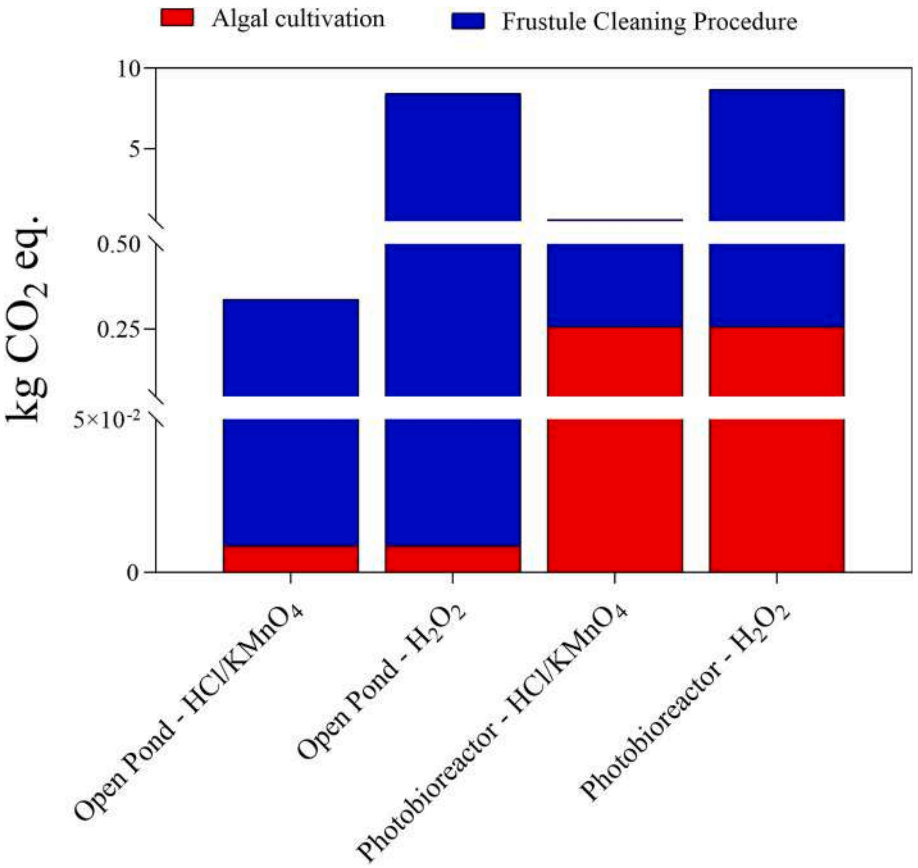

Fig. 10. Impact of a putative upscaled process expressed in terms of kg CO_2 eq. Electricity for frustule cleaning procedure was estimated using industrial scale equipment (Table S2) while data for algal cultivation are obtained from literature data [69–71].

Table 3

Preliminary economical evaluation of the biorefinery process for 3 g of *Navicula* sp. biomass.

	COSTS					
	Operation phase	Mean consumption	Source	Consumption/1 g non refined frustule	Price	Finale price (EUR)
Energy	Open pond cultivation	0.009 kWh/kg med	[70]	0.3 kWh	0.1 EUR/kWh	0.03
	Centrifuge	0.003 kWh/kg med	[70]	0.1 kWh		0.01
	Bread milling	0.1 kWh/kg DW	[70]	0.0004 kWh		0.00004
	Industrial Oven	100 kWh/1440 kg DW	Table S2	7 10^{-5} kWh		7 10^{-6}
Reagents	2-Butanol	25 kg/kg DW	[70]	75 g	0.9 EUR/kg	0.07
	CMPE	60 kg/ kg DW	[70]	179 g	5.5 EUR/ton	0.001
					Estimated total costs	***0.11***

	PROFITS					
	Chemical content in *Navicula* sp.	Source	Extracted chemical/3 g DW	Purity of the extracted chemical	Price	Final price (EUR)
Laminarin	3.40 %	[75]	33.6 mg	33 %[a]	40 EUR/kg[a]	0.001
EPA	18.2 % lipids, among them 8.7 % EPA	[76]	9.8 mg	20.8 %[a]	1.99 EUR/kg[a]	0.00002
Fucoxanthin	0.54 %	[77]	1.16 mg	7.3 %[a]	1.68 EUR/kg[a]	0.0000019
Silica Biomaterial	33.3 %	Present work	500 mg	50 %[c]	276.35 EUR/kg[b]	0.15
					Estimated total profits	***0.15***

[a] [70].

[b] Price referred to zeolite Y (usually used as catalysis support in chemical reactions, Zeolyst international, https://www.zeolyst.com/).

[c] Present work.

Addressing this option will provide a sustainable way to obtain an extraction of added-value products prior to cleaning of diatoms, following the principles of Biorefinery. Preliminary calculations of such approach show that the integration would produce the reduction of the impact coupled to an economic advantage, due to the overall reduction of use of aggressive chemicals. Even though the study presents some limitations, the LCA was confirmed as an effective tool to guide the process scale-up toward sustainability.

CRediT authorship contribution statement

Alessandra Petrucciani: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Alessia Amato:** Writing – review & editing, Validation, Supervision, Investigation, Formal analysis, Conceptualization. **Alessandra Norici:** Visualization, Supervision. **Angela Dibenedetto:** Project administration, Funding acquisition. **Michele Aresta:** Writing – review & editing, Validation, Supervision, Funding acquisition.

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.2025.104353.

Data availability

Data will be made available on request.

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