ACS Sustainable Chemistry & Engineering

pubs.acs.org/journal/ascecg

Research Article

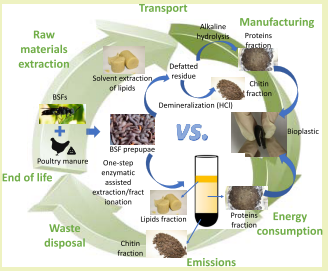
Life Cycle Assessment of Chemical vs Enzymatic-Assisted Extraction of Proteins from Black Soldier Fly Prepupae for the Preparation of Biomaterials for Potential Agricultural Use

Roberto Rosa,* Rosangela Spinelli, Paolo Neri, Martina Pini, Silvia Barbi, Monia Montorsi, Lara Maistrello, Angela Marseglia, Augusta Caligiani, and Anna Maria Ferrari

Cite This: https://dx.doi.org/10.1021/acssuschemeng.0c03795

Read Online

ABSTRACT: The cradle-to-grave life cycle assessment (LCA) was applied to the preparation of biomaterials derived from proteins, extracted from black soldier fly (BSF) prepupae, after the larvae were reared on poultry manure-based organic waste. To obtain higher value-added biomolecules, extraction represents the fundamental step. Therefore, the environmental sustainability assessments of different extraction/fractionation procedures were compared. In this way, it is possible to consider also their environmental performances in addition to the purity, yield, and integrity of the extract. A chemical method characterized by a one-step protein extraction was compared to an enzymatic-assisted protocol, employing *Bacillus licheniformis* protease. Surprisingly, the enzymatic approach resulted for the 31.87% more environmentally impacting with respect to the chemical method, despite its lack of organic solvents and reduction of alkaline and acid solutions employed. Particularly, the long time necessary for the enzymatic hydrolysis significantly contributed to the environmental impact of this protocol. Therefore, improvements such as biomass pretreatment procedures or the use of different proteolytic enzymes (e.g., operating at lower temperatures and in shorter times) are needed. Moreover, to reduce the environmental load of the protein fraction, attention should also be given to increase extraction yields of lipids and chitin biomolecules obtainable from BSF prepupae, due to the biorefinery approach under which this study was considered.



KEYWORDS: *biomass, green extraction, LCA, organic waste valorization, sustainable chemistry*

INTRODUCTION

To face the increasing production of waste materials, innovative technologies for their management and valorization are urgently needed. This is particularly true for organic waste materials, due to both their strict worldwide environmental regulations as well as the increasing global demand for renewable sources of energy, chemicals, fuels, and materials.[1,2] For these reasons, during the last few years, several strategies have been proposed for the valorization of organic wastes. They include thermochemical approaches (e.g., pyrolysis and hydrothermal treatment)[3] and the use of less conventional energy sources such as microwaves,[4] ultrasound or mechanical energy,[5] fermentation, and microbial digestion,[6,7] together with other biobased strategies. Among the latter ones, bioconversion by insects represents one of the most intriguing and promising techniques.[8] Indeed, conversion of organic waste biomasses into valuable biomolecules is a peculiar characteristic of several insects, among which *Hermetia illucens* (Linneus 1758, known as black soldier fly, BSF) represents an excellent example,[8] possessing several advantages with respect to other species.[9] Particularly, adult BSFs do not need to eat; thus, they are not vectors of diseases.[10] Moreover, BSF larvae are able to process and to develop very rapidly on different substrates, including agri-food byproducts, livestock manure, municipal solid waste, and further organic wastes,[11] thus restraining bacterial growth and bad odor development.[12−14] Despite environmental and human health issues/considerations, BSF larvae/prepupae are excellent sources of biomolecules such as lipids, proteins, and chitin, which have been exploited in the production of biodiesel,[15−17] pet food preparations,[18,19] as well as biopolymers.[20] Particularly, in this latter application field, the possibility to totally or, at least, partially replace the use of low-density polyethylene (LDPE) with biopolymers represents an actual and challenging research field with great potential for commercial implications. Indeed, LDPE represents the most employed petroleum-derived polymer in the agricultural sector. However, it presents significant environmental issues, especially related to the end of its life.[21]

Recently, some of the present authors optimized the procedure for the obtainment of a freestanding film of bioplastic from proteins extracted from BSF prepupae.[20] Due

Received: May 21, 2020
Revised: September 6, 2020
Published: September 11, 2020

to a good synergy between the plasticizer and the cross-linking agents employed together with the extracted proteins, it was possible to reach mechanical as well as moisture-related properties similar to further innovative protein-based bioplastic obtained, for example, from albumen,[22] crayfish,[23] and keratin.[24] However, further improvements are yet needed to reach tensile stresses and strains comparable with bioplastics for agricultural use already available on the market. Nevertheless, it should be highlighted how the latter ones are mostly starch-derived, thus responsible for the consumption of soils and resources, otherwise addressed to human nutrition, and characterized by high production costs.[25] Therefore, alternative biobased polymers avoiding further use of soil in their production show fundamental results and are also in need of being quantitatively assessed in terms of sustainability.

In this latter framework, the aim of this work is to present a quantitative and trustworthy environmental sustainability assessment of all of the different processes accompanying the production of a bioplastic, for potential agricultural use, from proteins extracted from BSF prepupae after the larvae were reared on poultry manure.[26]

The most important step to assure the quality of the final biomaterial is the extraction of proteins from BSF prepupae.[27] Therefore, particular emphasis is given to the comparison of the environmental performances of two different extraction procedures, which were optimized by some of the present authors[28] with the aim to separate all of the three different fractions obtainable from BSF prepupae (i.e., lipids, proteins, and chitin) at the maximum level of purity, in a cascade biorefinery approach.[29]

Particularly, a conventional chemical method characterized by a one-step protein extraction was compared to an enzyme-assisted one. In this way, it is possible to furnish also environmental performance indicators for these extraction strategies, besides the conventional metrics typically considered during the choice of a particular extraction protocol, i.e., extraction yield, time, purity, and integrity of the extract. The claimed green characteristics of enzymatic-assisted extraction[30] is investigated and quantified in a trustworthy manner for the first time to evaluate if it could fit in the green extraction procedures,[31−33] at least for the particular laboratory-scale scenario of the present case. The use of enzymes is typically associated with green chemistry practices, since they usually operate in a water medium, at low temperatures and ambient pressure. Moreover, they are produced from renewable resources and are biodegradable.[34] On the contrary, environmental criticisms of the use of enzymes in the chemistry field rely on the high energy consumptions associated with their production as well as with the product isolation from a high-boiling-point solvent. Therefore, to quantify the environmental performances of the herein proposed enzymatic method for extraction and fractionation of proteins from BSFs and to compare them with a more conventional chemical protocol, the life cycle assessment (LCA) methodology represents the sole feasible holistic approach with respect to other less comprehensive green chemistry metrics and tools.[35] The use of LCA is also recommended for the laboratory-scale chemical processes.[36] This also contributes to the development of updated LCA databases, leading to a continuous decrease in the laborious character of LCA itself.

The number of studies evaluating the bioconversion of organic waste by insects has grown during recent years. However, to the best of our knowledge, only a few of them reported the application of LCA methodology but limited to the bioconversion process.[37−39] This means that only the environmental impacts associated with the obtainment of BSF larvae were assessed and compared with more conventionally employed nutrient feed sources.

However, from a biorefinery perspective, there is the fundamental need to recover and efficiently valorize all of the three main fractions of BSFs along the same chain to gain maximum benefit from the process. Therefore, the assessment of the environmental burdens associated with the extraction and fractionation of biomolecules, as well as with their subsequent application, should be considered of paramount importance and an absolute novelty of this work.

EXPERIMENTAL SECTION

Materials and Methods. Details of the suppliers of all of the employed chemicals can be found elsewhere, together with detailed procedures for all of the different phases of the whole process;[28,40] thus, they are only summarized hereafter.

BSF Rearing. BSF rearing includes different phases. The nursery covers the BSF adult mating, oviposition, and eggs hatching into young larvae.

The adult BSFs were kept in a controlled environment at 27 °C and 70% relative humidity, with a 16:8 light/dark cycle (lit up with different LED lamps), to guarantee the optimal conditions for mating and oviposition.[41] A camera integrated to a computer (provided with dedicated algorithms) allowed extrapolating the number of BSFs and dead flies. The obtained prepupae were used both for the bioconversion phase and for the maintenance of the colony.

The optimization of the substrate composition was previously[26] reached by means of a simplex-centroid mixture experimental design based on three parameters, i.e., poultry manure (in the range of 20−45 wt %), water (in the range of 50−70 wt %), and zeolite (in the range of 5−25 wt %). Five replicate points and three additional center points were added, leading to a total of 24 experiments. The analysis of variance (ANOVA) was used to verify the statistical significance of the three studied parameters on the percentage of individuals that become prepupae.[26] The optimal substrate for the resulting larval growth was composed of 34.5 wt % poultry manure, 7.2 wt % zeolite, and 58.3 wt % water.[26] The larvae were reared on the optimized substrate and maintained at 27 °C and 60−70% relative humidity, employing a photoperiod of 16:8 light/dark cycle until reaching the prepupal stage. The prepupae were separated from the residual larval frass using a vibrating sieve. Less than 10% of the prepupae was used to maintain the production chain, allowing life cycle completion. The remaining prepupae were collected and stored at −20 °C in zip lock bags until their subsequent use. The prepupae resulted composed of 37.1 ± 0.1% of lipids, 32 ± 2% of proteins, and 9 ± 1% of chitin, as determined from triplicate analyses with respect to the dry matter, the latter being 34 ± 1% of the overall biomass.[28]

The total content of lipids and proteins in the BSF prepupae was determined using standardized procedures.[42] Particularly, the total lipid content was determined using an automatized Soxhlet extractor (SER 148/3 VELP SCIENTIFICA, Usmate Velate, Italy) and diethyl ether as the extraction solvent. Total nitrogen was determined with a Kjeldahl system (DKL heating digestor and UDK 139 semiautomatic distillation unit, VELP SCIENTIFICA). To separate the contribution of protein nitrogen from the chitin one, a nitrogen-to-protein conversion factor of 5.71 ± 0.02 was calculated from the total amino acid amount.[43] This was determined by analyzing hydrolyzed BSF prepupae samples using high-performance liquid chromatography with a fluorescence detector (HPLC/FLD, Waters Alliance 2695), according to the method described by Marseglia et al.[44]

The chitin content was first obtained by subtracting the protein contribution from the total nitrogen content. Second, the actual chitin content was also determined by quantifying glucosamine after chitin hydrolysis by means of gas chromatography-mass spectrometry (GC-MS) (Thermo Scientific Trace 1300 gas chromatograph coupled to a

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

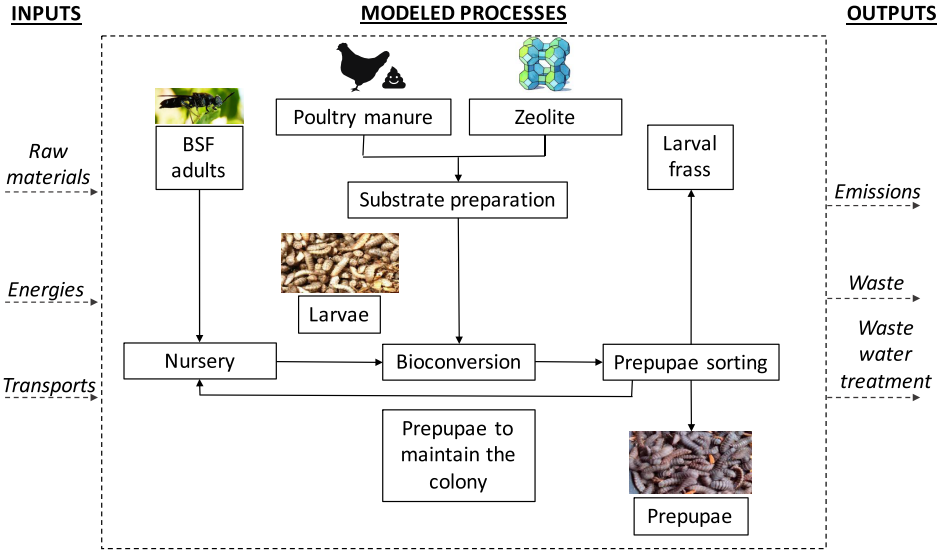


Figure 1. Flowchart showing the system boundaries considered in the LCA of the BSF larvae rearing.

Thermo Scientific Trace ISQ mass spectrometer, Thermo Scientific, Waltham), with slight modifications to the method by Flannery et al.[45]

BSF Biomolecule Extraction and Fractionation. Frozen BSF prepupae were ground and immediately subjected to one of the following two extraction and fractionation procedures: a chemical method with one-step protein extraction and an enzymatic (*Bacillus licheniformis* protease)-assisted method, as optimized by some of the present authors.[28]

Chemical Method with One-Step Protein Extraction. The lipid fraction was extracted from 375 g of BSF prepupae by vigorously mixing them with 750 mL of petroleum ether (40−60 °C boiling point fraction) for 2 h. The lipidic fraction was isolated by petroleum ether evaporation under vacuum. The defatted residue was treated with 500 ml of 1 M NaOH solution at 40 °C with stirring for 1 h.

The supernatant was neutralized and centrifuged at 3220 *g* for approximately 15 min, while the solid residue was used for the next chitin extraction step. Briefly, 40 mL of 6 N HCl solution was then added to the supernatant to precipitate the protein fraction. The solution was incubated for 12 h at −20 °C to help protein aggregation. Subsequently, it was centrifuged at 3220 *g* and 4 °C for approximately 15 min. Finally, it was dried at 90 °C for approximately 2 h, returning the protein fraction to be employed in the next bioplastic preparation phase. The recovered liquid phase was disposed of as hazardous special waste.

The solid residue, previously separated from the neutralized supernatant, was demineralized with 500 mL of 2 N HCl solution for 24 h at room temperature, then centrifuged for 30 min at 3220 *g*. The precipitate was washed twice with water and the final chitin-rich residue was dried at 90 °C for 8 h.

Enzymatic-Assisted Extraction. *B. licheniformis* protease (EC 3.4.21.62) was added together with 375 g of minced BSF prepupae (1:100 wt/wt ratio) to 3.375 L of 10 mM Na_2HPO_4 buffer solution (pH 6.5) to initiate the hydrolysis reaction, which was performed at 60 °C and prolonged for 14 h. To inactivate the enzyme, the reaction mixture was heated at 90 °C for 10 min. Subsequently, it was centrifuged at 3220 *g* and 4 °C for 30 min. This allowed obtaining three different fractions that resulted from top to bottom as follows: the lipid fraction, the supernatant protein fraction, and the chitin-based solid residue.

The lipids were easily separated from the other fractions and stored at −20 °C. The supernatant aqueous phase was separated from the decanted solid residue, obtaining the protein fraction after water evaporation.

The defatted and deproteinized chitin-rich solid residue was demineralized with 500 mL of 2 N HCl solution and washed exactly as during the previously described chemical method.

Determination of the Extraction Yields. Independent of the extraction protocol, the obtained lipid fraction was weighed and compared with the total amount of fat determined by the Soxhlet apparatus, as previously described. The extraction yield for proteins was determined by comparing the nitrogen present in the extracts and the protein nitrogen, previously determined by amino acid analysis.

The efficiency of protein from chitin separation was determined by the nitrogen content in the extracts (as determined by the Kjeldahl system). It was compared with the ratio of protein and chitin nitrogen.

Bioplastic Preparation. For the preparation of bioplastic from the extracted proteins, the experimental conditions were thoroughly investigated. Particularly, a design of experiment (DoE)-based approach was followed to optimize the obtainment of a freestanding film, due to a good synergy between the plasticizer and the cross-linking agent added in the formulation.[20] A full factorial design was selected with two replicates for each experiment for error estimation. Four center points were added with the aim to investigate the eventual presence of curvature,[20] leading to a total of 26 experiments. To point out the cause−effect relation between the component ratio and the capability to form freestanding materials, the analysis of variance (ANOVA) was performed.

The two different extraction protocols considered, although leading to proteins characterized by slightly different qualities (determined by the degree of hydrolysis, as detailed in ref 28), resulted in being irrelevant for the quality of the obtained bioplastic. On the contrary, the latter resulted in being strictly dependent upon the protein amount.

Independent of the extraction method employed, the protein fraction recovered was ground by a dry analytical mill (IKA, A10 basic) and sieved, to ensure the homogeneity of the particle size was below 40 μm, with the aim to equalize the reactive surface available during polymerization.

The optimal mixture for the preparation of the bioplastic was composed of 0.5 g of sieved proteins, distilled water (6.5 g), glycerol (0.42 g), and 1 M NaOH (0.04 g) to reach pH near 10, thus promoting protein solubilization in water. The mixture was heated at 60 °C for 30 min with stirring at 8 *g*. It was subsequently poured into an aluminum wrapper and incubated, at room temperature, under a fume hood for 24 h. The obtained protein-based thermoplastic material (after conditioning in a controlled-environment chamber at 25 °C and 50% relative humidity) was homogeneous and free-

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

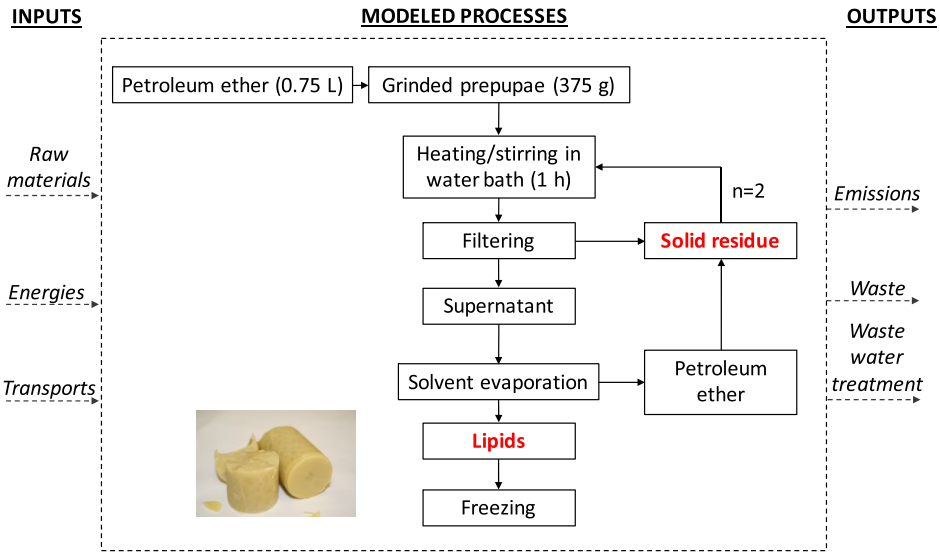


Figure 2. Flowchart showing the system boundaries considered in the LCA of the lipid fraction extraction by the chemical method.

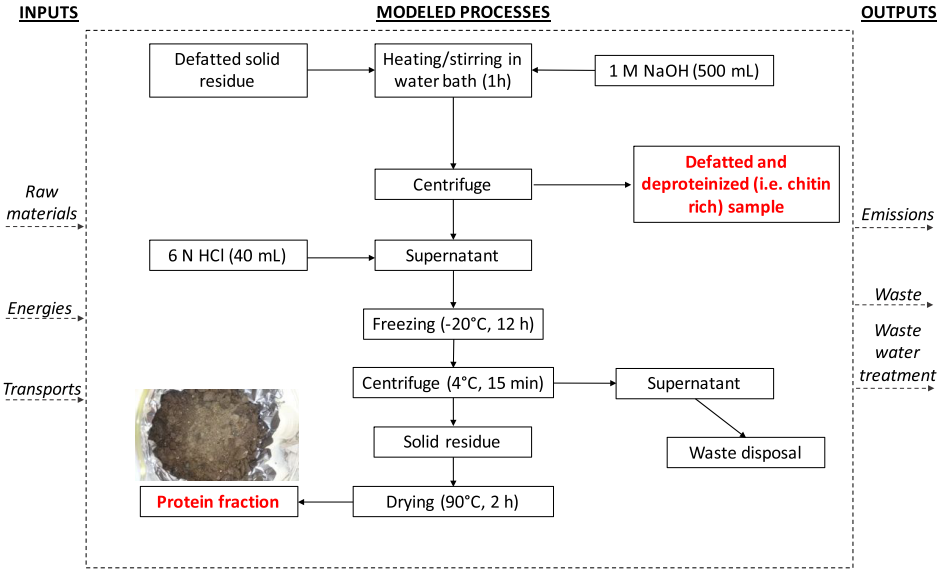


Figure 3. Flowchart showing the system boundaries considered in the LCA of the protein fraction extraction by the chemical method.

standing, possessing a thickness of 0.4 mm, a density of 1.4 g/cm^3, and an elongation at break around 30% (due to tensile stress).

LIFE CYCLE ASSESSMENT

The LCA methodology was applied according to ISO 14040[46] and 14044.[47] Its constituting phases are detailed hereafter.

Goal and Scope Definition. *Goal Definition.* The goal of the study was to assess the environmental impacts of the life cycle (from the cradle to the grave) of the process leading to a BSF protein-derived bioplastic, for potential agricultural use, to highlight the most critical phases of the whole process. Two protein extraction protocols are compared to identify the greener alternative for obtaining valuable biomolecules.

System, Functional Unit, and Function of the System. The system object of the study is the process for the preparation of a bioplastic from proteins extracted, by two alternative extraction methods, from BSF prepupae reared on poultry manure.

The functional unit was the amount of bioplastic produced from proteins extracted from BSF prepupae at a laboratory scale (i.e., 0.403 g). The amount and the quality of bioplastic were independent of the extraction method used for protein separation. The comparison between the two different extraction procedures was performed for the same amount of proteins recovered, i.e., 0.5 g. This corresponds to the amount of proteins necessary for the obtainment of the functional unit of bioplastic.

The function of the system studied was considered as the potential agricultural use (e.g., as a film of mulch) of the obtained bioplastic. However, since it has not been tested yet for biodegradability, duration, and effect on productivity, the use phase of the bioplastic was excluded from the study. Its end-of-life scenario (i.e., after use phase) was considered to be a composting one.

System Boundaries. The boundaries of the system comprise the rearing of the BSF larvae, the extraction and fractionation of biomolecules from BSF prepupae, and the final production of bioplastic from the only protein fraction, according to the experimental procedures summarized in the Experimental Section. All of the energies involved, the laboratory equipment

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

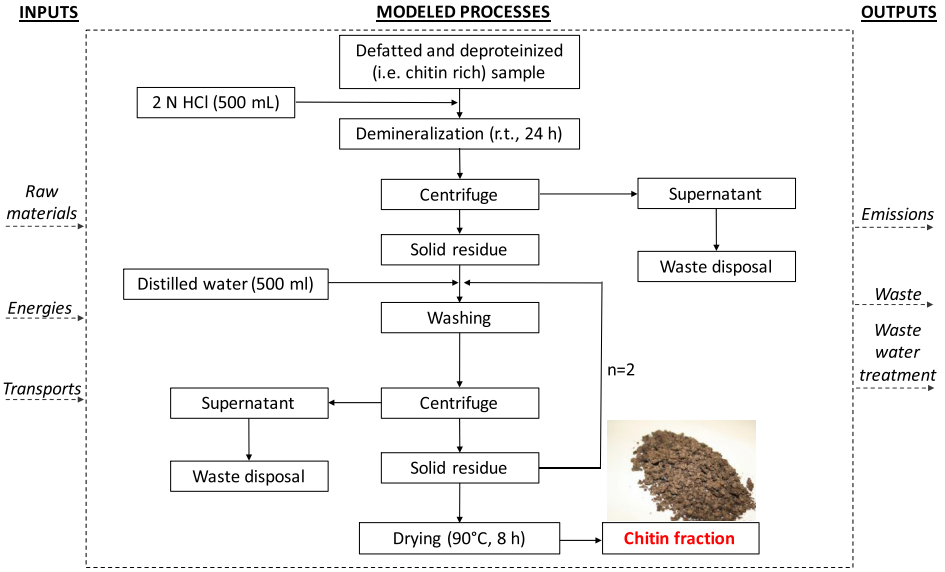


Figure 4. Flowchart showing the system boundaries considered in the LCA of the chitin fraction extraction by the chemical method.

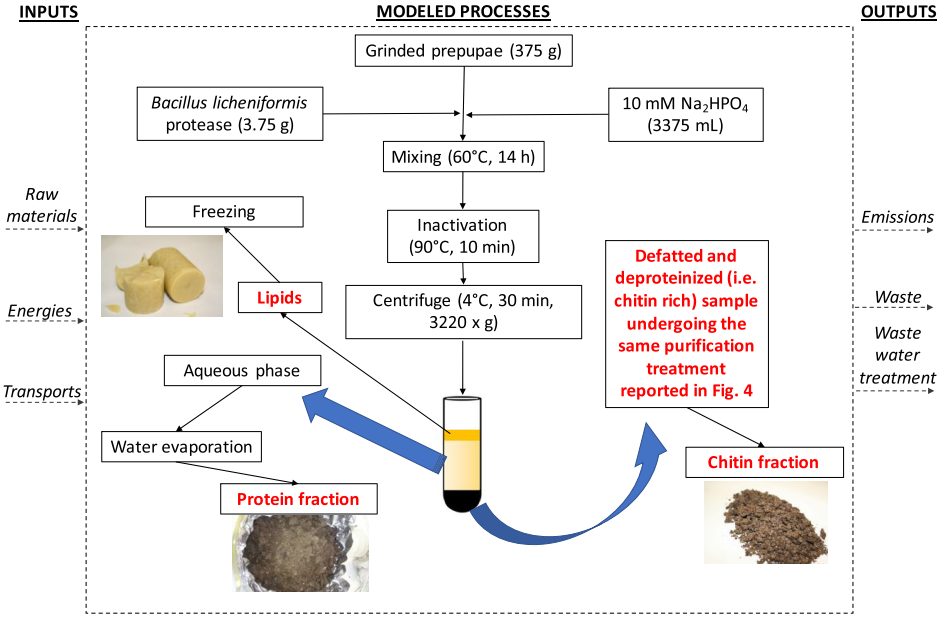


Figure 5. Flowchart showing the system boundaries considered in the LCA of the enzymatic-assisted extraction protocol, employing *B. licheniformis* protease.

employed (with their end of life), the transport contributions, together with the emissions into air and the local and the indoor emissions were considered as well. They are summarized in the flowcharts of Figure 1−6.

As reported in Figures 2−4, the chemical method comprises three separate steps, one for each of the fraction (lipids, proteins, and chitin) to be extracted from the prepupae. On the contrary, during the enzymatic-assisted extraction, enzymatic hydrolysis of the ground prepupae using protease was carried out directly, thus obtaining the three different fractions in a single step (Figure 5).

Both extraction and fractionation techniques were modeled applying a multioutput scenario, with the protein fraction as the main product and the other two fractions as co-products. This was done to not only make the comparison more reliable but to also consider the studied process from a biorefinery-approach perspective (thus considering also the potential value of lipids and chitin fractions). Particularly, a mass allocation criterion (based on the extraction yields) was adopted for both extraction protocols to allocate the environmental damages among the different obtainable biomolecules.

Life Cycle Inventory (LCI) and Life Cycle Impact Assessment (LCIA). Most of the data employed for the LCI phase were primary data, thus directly collected in the laboratory during the experimental activities. The inventory was completed with secondary data obtained from the Ecoinvent database (EID, version 3.4).[48] EID data were mainly used to model the background processes (i.e., land use, material production, fuel and electricity production, and material transport).

Particularly, the data set used for the modeling of electricity starts with medium-voltage electricity arriving at the trans-

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

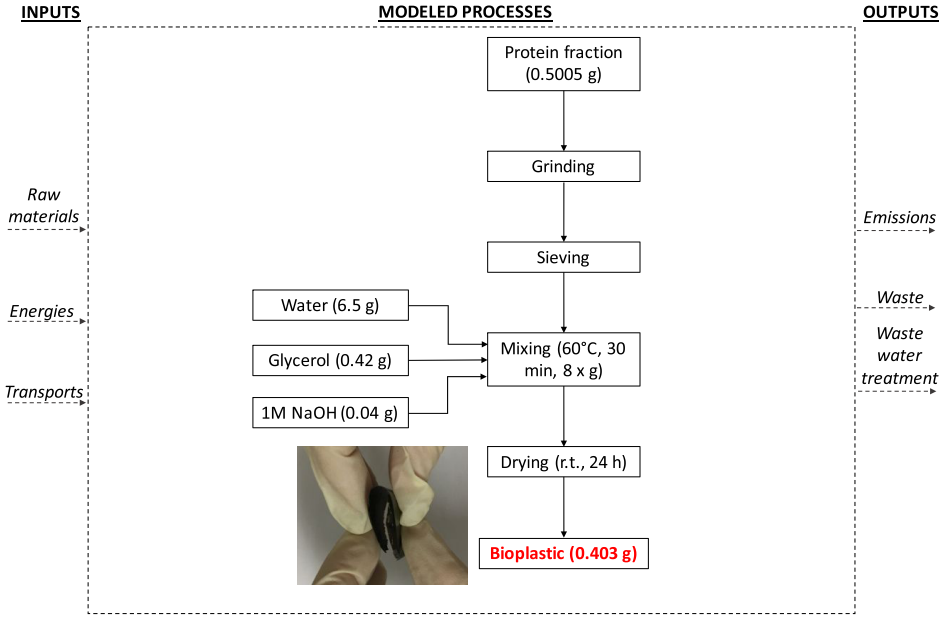


Figure 6. Flowchart showing the system boundaries considered in the LCA of the final bioplastic preparation step from the previously isolated protein fraction.

former station and ends with 1 kWh of medium-voltage electricity transformed into low voltage, including the losses during voltage transformation. Its inventory was modeled considering the Italian mix of electricity.

An average distance of 100 km was considered for the road freight transport by diesel EURO6 lorries for both materials and equipment. However, two different lorry capacities were considered, i.e., 3.5−7.5 and 16−32 metric tons for materials and equipment, respectively.

The emissions into the air were supposed to be 1% of the total mass of each precursor. Further, 99% of each emitted substance was considered to be retained by an active carbon filter. The unretained materials were considered to be released in the laboratory. The disposal in a landfill for residual materials was considered as the end-of-life scenario for the used filter.

The main contributions to the LCI for the different phases of the process are reported in Tables S1−S8 in the Supporting Information, in which the sources of data used for the considered amounts are indicated, together with those of the background processes considered. When a given substance was missing in the EID, new processes describing its preparation were modeled. This was done after performing an accurate literature search, going backward until procedures employing precursor compounds available in the EID were found. Alternatively, they were replaced by the most similar substance available in the database, as in the case of *B. licheniformis* protease. This was the material generically defined as "enzymes" in the EID, whose process assumes that they are produced from potato starch along with a bacterial strain. It is assumed that 4.16 kg of potato starch is needed to produce 1 kg of enzymes.[49] Heat and electricity inputs required for the production of the "enzymes" are considered by EID as the average values reported in ref 50 for the production of three different enzymes, i.e., α-amilase, glucoamilase, and cellulase. They belong to the enzyme class of hydrolase, exactly as the *B. licheniformis* protease experimentally employed in the enzymatic-assisted extraction process. Therefore, the approximation made was considered reasonable.

Further approximations regarded Na_2HPO_4 (necessary for the enzymatic-assisted extraction protocol) and petroleum ether (used for the separation of lipid fraction by the chemical method) chemicals. In the first case, the EID substance sodium phosphate was considered, while a 50:50 wt% mixture of pentane and hexane EID substances was used in the second case.

The inventory analysis was modeled in SimaPro 9.0.0.49.[51] The environmental impacts were calculated (LCIA phase) using the IMPACT 2002+ evaluation method,[52−54] since it accounts for more impact categories and substances compared to other methods. Moreover, it allows the environmental impact to be quantified both at a midpoint level (i.e., referring to an intermediate position along the cause−effect chain) and at an end point one (i.e., at the point at which the damage occurs). This is important since, while the former approach reduces the degree of uncertainty, it is usually less useful in decision-making with respect to the latter approach.

However, to describe the system studied in a more representative manner, the following modifications were implemented in the evaluation method:[55,56] (i) land occupation impact category was introduced using basic indicators accounting for both land occupation and transformation, (ii) additional resources from the mineral category of Eco-indicator 99[57] (i.e., silver, gravel, lithium, sand, bromine, and water in the ground) were considered to better characterize the mineral extraction impact category, and (iii) human health indoor and human health local damage categories were introduced to consider the impacts arising from the air emissions on both workers (indoor scale) and people living in the area surrounding the laboratory (local scale). To evaluate the abovementioned indoor and local emissions, the Eco-indicator 99 framework and the Gaussian Plume Modelling[58] were employed, as detailed elsewhere.[56]

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

RESULTS AND DISCUSSION

The results of the two different extraction and fractionation protocols applied to the BSF prepupae are summarized in Table 1, in terms of extraction yields.[28]

Table 1. Summary of the Results Obtained from the Two Different Extraction Methods Applied

	chemical method	enzymatic-assisted method
lipids (*Y*: %)	87	10
proteins (*Y*: %)	84	67
purity of chitin (%)	92	35

The solvent-based chemical method is characterized by higher extraction yields with respect to the enzymatic-assisted one, both in terms of lipids as well as proteins. Regarding the chitin-rich fractions, since pure chitin was not experimentally isolated by the residual solid materials but only the chitin purities of these residues were determined,[28] these values (i.e., 92% for the chemical method and 35% for the enzymatic-assisted one) were approximated in this study as the yield ones.

Environmental Impact Assessment of Bioplastic Production from Proteins Extracted by the Chemical Method from BSF Prepupae. The life cycle impact assessment (LCIA) assigns environmental loads to all of the different inputs inserted during the LCI phase. After classifying the environmental impacts into the appropriate impact categories and referring them to an intermediate position along the cause–effect chain, thus obtaining the so-called midpoint results, they are subsequently grouped into damage categories and allocated at the point at which the environmental effect occurs (i.e., end point results).

The single-score evaluation results (i.e., obtained after normalization and weighting operations internally performed by the IMPACT 2002+ evaluation method algorithm[52,59]) for the preparation of 0.403 g of bioplastic (i.e., the functional unit) from the protein fraction extracted considering the solvent-based chemical method are reported in Figure 7 and quantitatively detailed in Table 2 (the LCIA midpoint as well as end point results are instead detailed in Tables S9 and S10, respectively).

The damage associated with 0.403 g of bioplastic is 1.49×10^{-4} Pt (with Pt representing the eco-indicator points: the bigger the value, the worse the impact of that particular process on the environment). As visible in Figure 7 and inferable from the data of Table 2, the most affected damage category is the human health one. It contributes 35.19% to the whole environmental load, with Respiratory inorganics and Carcinogens impact categories contributing 24.09 and 9.82%, respectively (Table 2). The most responsible substances are hydrocarbons (for 23.38%) and particulates <2.5 μm (for 22.48%), which are mainly associated with the extraction/separation of the lipid fraction and the electricity production, respectively.

Resources is the second damage category affected by the studied process (contributing 33.66% to the entire environmental damage, Figure 7). The impact on this damage category is mainly due to the consumption of water for turbine use (for 26.53%), natural gas (for 25.51%), hard coal (for 16.05%), and crude oil (for 15.77%), accompanying electric energy as well as petroleum and natural gas production.

The climate change damage category (comprising the sole global warming impact category) is affected 24.20% (Figure 7), with the main responsible (contributing 93.87% to it) substance being CO_2, mostly (for 22.99%) generated during electricity production by hard coal.

The subprocess leading to the protein fraction is the less environmentally benign (among those considered), producing the highest contribution to all of the damage categories considered.

Therefore, to more deeply investigate the contributions to its environmental impact as well as the possible actions to be undertaken (including extraction strategies alternative to the chemical method considered here), the LCIA of this subprocess was performed. Particularly, the amount of proteins needed for the obtainment of the functional unit was considered (i.e., 0.5 g). The single-score evaluation results for the isolation of 0.5 g of protein fraction (together with the corresponding lipids as well as chitin co-products) are reported

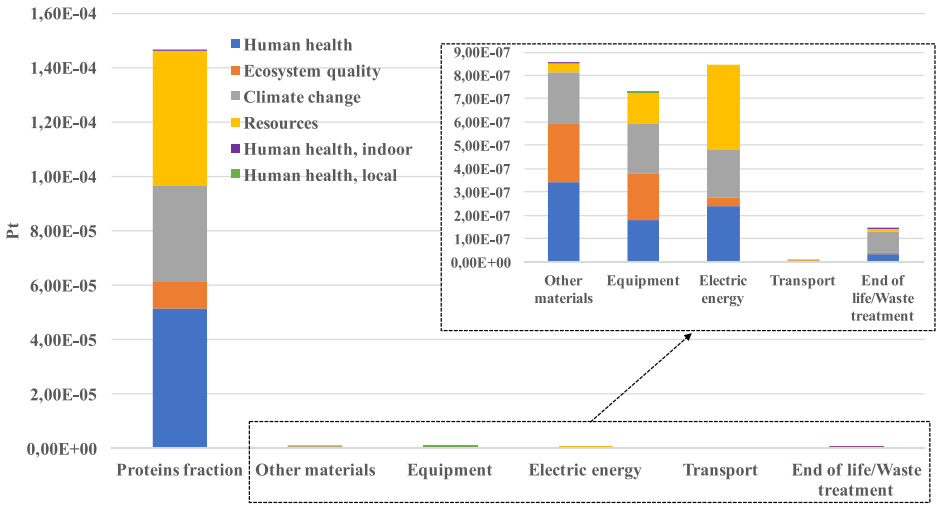


Figure 7. Single-score evaluation results for the analysis carried out for 0.403 g of bioplastic prepared by the protein fraction extracted from the BSF prepupae by the solvent-based chemical method (the total damage of the process, obtained by summing the damages of all of the contributing subprocesses indicated in the figure, is 1.49×10^{-4} Pt).

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

Table 2. Single-Score Evaluation Results for the Laboratory-Scale Production of 0.403 g of Bioplastic from the Protein Fraction Extracted from the BSF Prepupae by the Solvent-Based Chemical Method

damage category	impact category	unit	protein fraction	other materials	equipment	electric energy	transport	end of life/waste treatment
human health	carcinogens	Pt	1.45×10^{-5}	1.37×10^{-8}	3.30×10^{-8}	1.70×10^{-8}	3.16×10^{-10}	3.66×10^{-9}
	noncarcinogens	Pt	1.70×10^{-6}	4.21×10^{-9}	7.37×10^{-9}	5.63×10^{-9}	2.37×10^{-10}	7.17×10^{-9}
	respiratory inorganics	Pt	3.51×10^{-5}	3.25×10^{-7}	1.41×10^{-7}	2.16×10^{-7}	2.84×10^{-9}	2.37×10^{-8}
	ionizing radiation	Pt	1.46×10^{-7}	9.10×10^{-11}	1.74×10^{-10}	1.18×10^{-9}	7.03×10^{-12}	2.54×10^{-11}
	ozone=layer depletion	Pt	6.11×10^{-9}	9.70×10^{-12}	1.90×10^{-11}	3.93×10^{-11}	8.35×10^{-13}	3.42×10^{-12}
	respiratory organics	Pt	3.14×10^{-8}	3.97×10^{-10}	7.34×10^{-10}	1.25×10^{-10}	4.21×10^{-12}	1.19×10^{-11}
ecosystem quality	aquatic ecotoxicity	Pt	1.29×10^{-7}	-3.06×10^{-10}	4.32×10^{-10}	5.13×10^{-10}	1.13×10^{-11}	2.79×10^{-10}
	terrestrial ecotoxicity	Pt	4.40×10^{-6}	-6.06×10^{-8}	1.44×10^{-8}	2.14×10^{-8}	1.14×10^{-9}	3.16×10^{-9}
	terrestrial acid/nutri	Pt	5.44×10^{-7}	1.29×10^{-9}	8.85×10^{-10}	3.53×10^{-9}	2.14×10^{-11}	2.95×10^{-10}
	land occupation	Pt	4.72×10^{-6}	3.09×10^{-7}	1.85×10^{-7}	1.21×10^{-8}	1.19×10^{-10}	6.13×10^{-10}
	aquatic acidification	Pt	0.00	0.00	0.00	0.00	0.00	0.00
	aquatic eutrophication	Pt	0.00	0.00	0.00	0.00	0.00	0.00
climate change	global warming	Pt	3.53×10^{-5}	2.20×10^{-7}	2.11×10^{-7}	2.05×10^{-7}	3.19×10^{-9}	9.41×10^{-8}
resources	nonrenewable energy	Pt	3.47×10^{-5}	3.81×10^{-8}	1.26×10^{-7}	2.37×10^{-7}	3.35×10^{-9}	1.07×10^{-8}
	mineral extraction	Pt	1.48×10^{-5}	2.01×10^{-9}	5.03×10^{-9}	1.26×10^{-7}	5.62×10^{-11}	8.97×10^{-10}
human health, indoor	noncarcinogens, indoor	Pt	1.03×10^{-14}	0.00	2.52×10^{-16}	0.00	0.00	6.24×10^{-11}
	respiratory organics, indoor	Pt	1.21×10^{-11}	0.00	6.42×10^{-21}	0.00	0.00	2.23×10^{-18}
	respiratory inorganics, indoor	Pt	3.75×10^{-12}	7.53×10^{-13}	6.97×10^{-19}	0.00	0.00	1.04×10^{-13}
	carcinogens, indoor	Pt	5.69×10^{-8}	1.05×10^{-14}	1.85×10^{-20}	0.00	0.00	1.35×10^{-16}
human health, local	noncarcinogens, local	Pt	0.00	0.00	0.00	0.00	0.00	0.00
	carcinogens, local	Pt	3.90×10^{-15}	0.00	5.62×10^{-16}	0.00	0.00	0.00
	respiratory organics, local	Pt	0.00	0.00	0.00	0.00	0.00	0.00
	respiratory inorganics, local	Pt	1.68×10^{-12}	0.00	2.42×10^{-13}	0.00	0.00	0.00
	total	Pt	1.46×10^{-4}	8.53×10^{-7}	7.25×10^{-7}	8.45×10^{-7}	1.13×10^{-8}	1.45×10^{-7}

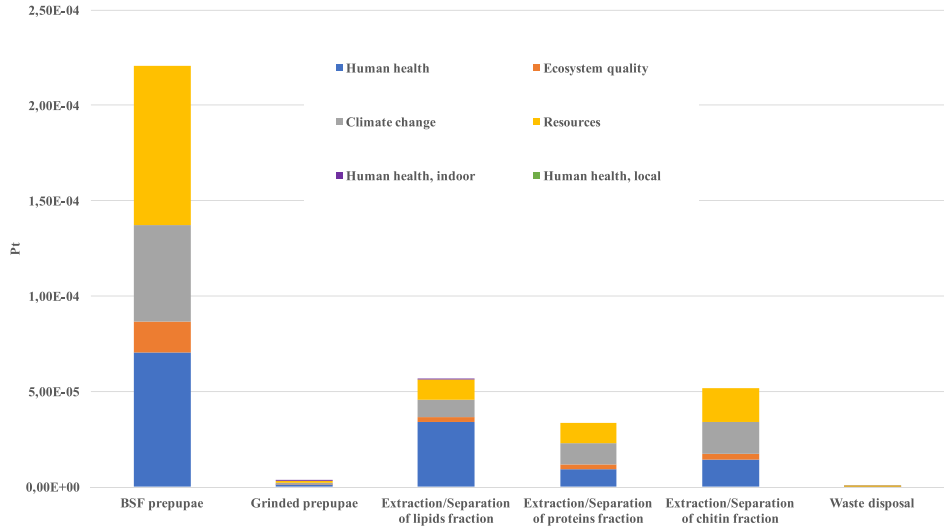


Figure 8. Single-score evaluation results for the analysis carried out for the isolation of 0.5 g of protein fraction (together with the corresponding lipids as well as chitin co-products) by the solvent-based chemical method (the total damage of the process, obtained by summing the damages of all of the contributing subprocesses indicated in the figure, is 3.65×10^{-4} Pt).

in Figure 8 (again in terms of damage categories) and more accurately detailed in Table S11 in the Supporting Information Section.

The total environmental impact, given by the sum of the impacts of all of the contributing subprocesses reported in Figure 8, is 3.65×10^{-4} Pt. However, considering the way this process was modeled in Simapro, i.e., following a multioutput approach, the abovementioned environmental damage should be allocated not entirely to the main product (i.e., the protein fraction) but also to the two co-products obtainable by this extraction/fractionation chemical method (i.e., the lipid fraction and the chitin one). Particularly, a mass-based allocation criterion was considered on the basis of the extraction yields of the three different biomolecule pools. In this way, the protein fraction must be considered to be responsible for 40.03% of the total impact of the process (i.e.,

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

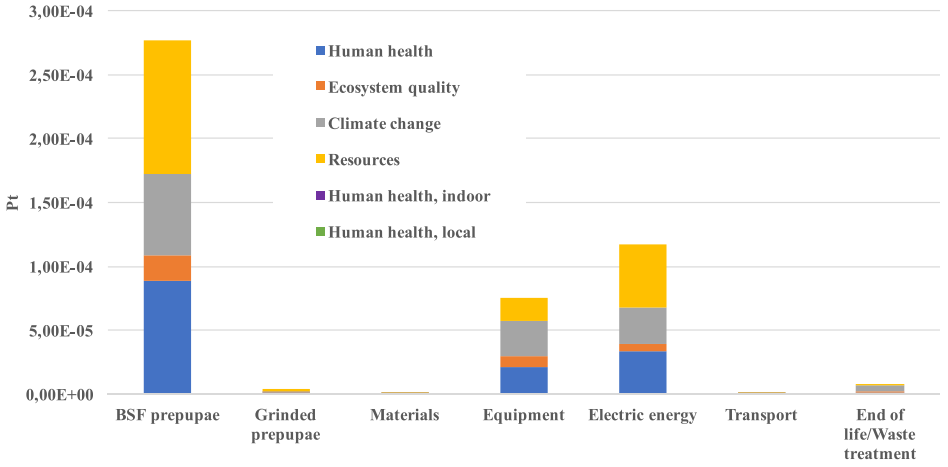


Figure 9. Single-score evaluation results for the analysis carried out for the isolation of 0.5 g of protein fraction (together with the corresponding lipid and chitin co-products) by the enzymatic-assisted method (the total damage of the process, obtained by summing the damages of all of the contributing subprocesses indicated in the figure, is 4.82×10^{-4} Pt).

1.46×10^{-4} Pt) while the lipids for 47.65% (i.e., 1.74×10^{-4} Pt) and the chitin-rich fraction for 12.33% (i.e., 4.51×10^{-5} Pt).

The modeled subprocess leading to the BSF prepupae (comprising the nursery and the bioconversion phases, as detailed in Figure 1) tremendously affects (for 60.46%, Figure 8) the whole extraction and fractionation protocol considered. However, this contribution is independent of the following steps performed on the BSF prepupae, thus by the selected extraction fractionation protocol (the latter being instead the main focus of this work). Therefore, the environmental impact details of the subprocess leading to the BSF prepupae are reported in the Supporting Information Section. Moreover, since the amount of prepupae necessary for the obtainment of the functional unit is strictly dependent upon the yields of the subsequent extraction procedures, this latter calculation was performed for a generic amount of prepupae equal to 1 g.

Particularly, Figure S1 depicts the contributions of the different phases to the environmental load associated with the obtainment of 1 g of BSF prepupae (quantitatively detailed in Table S12).

Beyond the obtainment of the BSF prepupae, the second significant contribution to the environmental load is represented by all of the processes necessary to extract and fractionate the lipid fraction (contributing 15.37%, Figure 8) by the chemical method employed, followed by those needed for chitin (contributing 14.11%, Figure 8), and finally by those associated with the proteins (contributing 9.18%, Figure 8). Particularly, the lipid fraction mainly affected (for 60.73%, Figure 8 and Table S11) the human health damage category, primarily as a consequence (for 79.20%) of hydrocarbons released during petroleum ether production/usage/manipulation/disposal.

In the case of chitin fraction, the most affected damage category is resources (affected for 34.34%, Figure 8 and Table S11). Its impact is due (for 27.41%) to depletion of natural gas associated with the EID background processes considered for the production of natural gas and polypropylene.

The operations related to the isolation of protein fraction, mainly affected (for 33.11%, Figure 8 and Table S11) the climate change damage category. Its environmental impact is due (for 95.46%) to carbon dioxide principally released (for 19.51%) during the hazardous waste incineration treatment of the spent solvent mixture.

Therefore, the possibility to investigate and environmentally evaluate alternative protocols for the extraction and fractionation of biomolecules surely represents a research field that needs to be urgently explored.

Environmental Impact Assessment of *B. licheniformis* Protease-Assisted Extraction/Fractionation of Proteins, Lipids, and Chitin from BSF Prepupae. According to the procedure detailed in the Experimental Section and depicted in Figure 5, the enzymatic-assisted protocol allowed us to obtain the three different biomolecule pools after a single hydrolysis step performed in water directly on the ground prepupae.

The single-score evaluation results of the life cycle impact assessment applied to the enzymatic-assisted extraction/fractionation protocol are reported in Figure 9, referring to the isolation of 0.5 g of proteins (together with the corresponding lipids and chitin co-products). The contributions to each impact category considered are detailed in Table S13 of the Supporting Information Section.

The enzymatic-assisted protocol considered here does not use any organic solvent. Moreover, it significantly reduces the amount of alkaline and acid solutions employed and simplifies the workup procedures necessary for the isolation of lipid and protein fractions. However, quite surprisingly, the total environmental impact for the obtainment of 0.5 g of proteins (concurrently with the lipid and chitin co-products) with this enzymatic extraction, i.e., 4.82×10^{-4} Pt, is for the 31.87% higher with respect to the chemical method one. By also considering in this case the mass allocation criterion, the total damage must be attributed to 75.79% to the protein fraction (i.e., 3.65×10^{-4} Pt), for the 13.08% to the lipids (i.e., 6.31×10^{-5} Pt), and for the 11.13% to the chitin-rich fraction (i.e., 5.37×10^{-5} Pt). This discrepancy in the distributions of the environmental loads (with respect to the case of the chemical method) is due to the different extraction yields of biomolecules during the two protocols considered. The moderately high extraction yield for proteins together with the extremely low yield for lipids typical of the studied enzymatic approach (see Table 1) are responsible for the higher allocation factor attributed to the proteins.

Again, the obtainment of BSF prepupae is the most impacting step of the whole process (Figure 9). The second

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

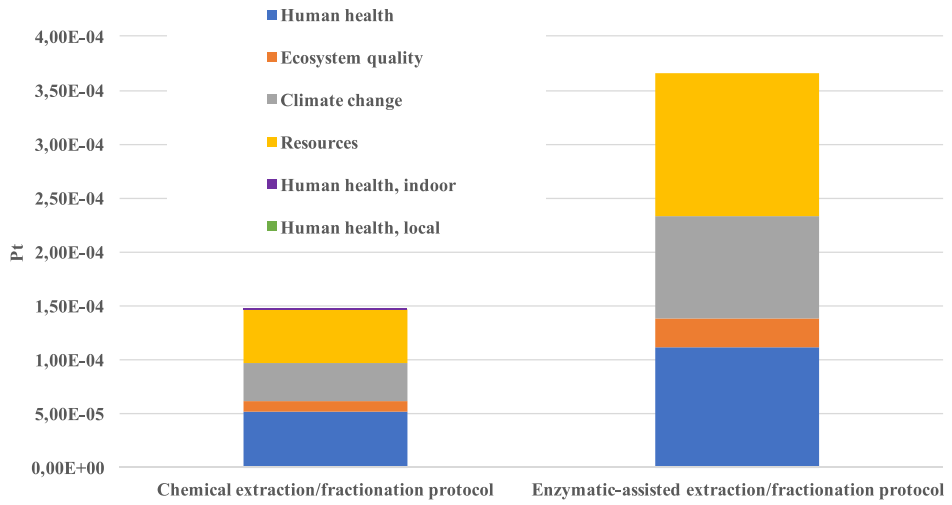


Figure 10. Comparison of the single-score evaluation results for the two extraction protocols considered (accounting for the mass allocation criterion and performed for 0.5 g of protein fraction).

main contribution is represented by the electric energy employed during the subsequent processes starting from the hydrolysis reaction in aqueous media (Figure 9). Particularly, the main contribution to the damage category Resources, excluding those related to BSF prepupae, is due to the production of the electric energy necessary for the operation of the magnetic stirrer/heater during the hydrolysis reaction (experimentally performed for 14 h). Thus, to increase the efficiency of the enzymatic hydrolysis, pretreatment of the BSF prepupae could be considered, as already demonstrated for other biomasses.[60] Moreover, the use of different proteolytic enzymes, requiring a reduced time duration of hydrolysis and working at room temperature, could also be explored.

Therefore, the as-optimized[28] enzymatic-assisted extraction protocol cannot be considered a greener alternative for the obtainment of the desired biomolecules with respect to the more conventional chemical method.

By focusing on the obtainment of the sole protein fraction by both extraction and fractionation protocols (since the designed final application is the use of these biomolecules for bioplastic production), the environmental impact comparison should account for the mass allocation criterion, as reported in Figure 10 (for 0.5 g of proteins).

The detailed single-score contributions of the two strategies to each damage category are detailed in Table 3. For the reason of completeness, the midpoint results as well as the end point ones are reported in Tables S14 and S15 in the Supporting Information Section.

As clearly visible in Figure 10, in this case, the gap between the two alternatives is even larger, further to the detriment of the enzymatic approach. The reasons at the basis of this result must not be mainly attributed to the lower extraction yield of proteins (that is, only slightly lower with respect to that of the chemical method) but rather to the extremely lower extraction yields for lipids and chitin. In this way, the whole load of the enzymatic protocol weighs almost completely on the protein fraction. On the contrary, the chemical method allows obtaining each of the three different fractions with an extraction yield higher than 80%, thus leading to an almost equal distribution of the environmental impacts.

Therefore, despite the desirable improvements discussed above in terms of hydrolysis efficiency, future research efforts should also address the possibility to concurrently increase the recovery of both lipids and chitin from the BSF prepupae by the *B. licheniformis* protease-mediated method.

Table 3. Single-Score Contributions of the Two Extraction Procedures to the Different Damage Categories by Considering the Mass Allocation Criterion (Both Calculated for the Isolation of 0.5 g of Protein Fraction)

damage category	unit	chemical extraction/ fractionation protocol	enzymatic-assisted extraction/fractionation protocol
human health	Pt	5.16×10^{-5}	1.11×10^{-4}
ecosystem quality	Pt	9.80×10^{-6}	2.67×10^{-5}
climate change	Pt	3.53×10^{-5}	9.51×10^{-5}
resources	Pt	4.95×10^{-5}	1.32×10^{-4}
human health, indoor	Pt	5.69×10^{-8}	9.03×10^{-12}
human health, local	Pt	1.68×10^{-12}	5.37×10^{-12}
total	Pt	1.46×10^{-4}	3.65×10^{-4}

CONCLUSIONS

In this work, the cradle-to-grave environmental impact assessment for the preparation of bioplastic from proteins extracted from BSF prepupae, opportunely fed on poultry manure, was performed by applying the LCA methodology. The study was modeled according to the experimental results obtained and optimized by some of the present authors.

This work allowed us to obtain quantitative and trustworthy data related to the environmental impacts associated with all of the different stages of an innovative valorization approach for organic waste residues.

The life cycle impact assessment related to the production of the bioplastic, comprising the solvent-based chemical method for isolation of the protein fraction, highlighted the tremendous contribution of the BSF larvae rearing phase to the total environmental damage of the process. The latter was mainly due to the bioconversion step.

Besides this main contribution, the extraction and fractionation of the three biomolecule pools (i.e., lipids,

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

proteins, and chitin) play a significant role, thus highlighting the importance of the study performed herein from an environmental point of view. Indeed, two different extraction and fractionation protocols were compared in detail. In this way, it is possible to assign them environmental performance indicators, in addition to more conventional ones, simply referred to as yield, purity, and integrity of the extracted biomolecules.

Quite surprisingly, the enzymatic-assisted procedure (exploiting *B. licheniformis* protease-mediated hydrolysis) resulted for 31.87% more environmentally impacting with respect to the chemical method, based on the use of petroleum ether (for the separation of lipids) and significant amounts of inorganic acid and base solutions (for the separation and purification of the other two fractions, i.e., the proteins and the chitin).

The time necessary for the completion of the enzymatic hydrolysis significantly contributed to the impact of this extraction protocol. Therefore, further optimization, involving, for example, biomass pretreatment procedures or the use of proteolytic enzymes operating at lower temperatures and in shorter times, could be in all likelihood proposed as a strategy to be pursued. Moreover, it was demonstrated that, although characterized by a lower protein-extraction yield (i.e., 67%), with respect to that of the chemical method (i.e., 87%), the detrimental factors of the environmental performances of the enzymatic extraction protocol are the significantly lower extraction yields for lipids and chitin. Thus, in view of future optimization of this protocol, particular attention should also be given to these biomolecules.

In conclusion, the as-proposed enzymatic-assisted method cannot be considered a reliable greener alternative to the conventional method based on the use of organic solvents, at least in the particular laboratory-scale scenario considered in the present work. This finding highlights once more the importance of accompanying green chemistry-related research with quantitative and trustworthy environmental performance data for the obtainment of which the LCA methodology should represent the tool of choice.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.0c03795.

LCI phase of this study (Tables S1−S8); detailed midpoint and end point results for the obtainment of the bioplastic from proteins extracted by the chemical method (Tables S9 and S10); detailed single-score results for the obtainment of 0.5 g of proteins by the chemical method (Table S11); detailed single-score results for the obtainment of 1 g of BSF prepupae (Table S12); detailed single-score results for the obtainment of 0.5 g of proteins with the enzymatic method (Table S13); detailed midpoint and end point results for the obtainment of 0.5 g of proteins by the two alternative extraction methods (Tables S14 and S15); and single-score results for the obtainment of 1 g of BSF prepupae (Figure S1) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Roberto Rosa − *Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy;* orcid.org/0000-0002-1863-1631; Phone: +390522523558; Email: roberto.rosa@unimore.it

Authors

Rosangela Spinelli − *Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy*

Paolo Neri − *Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy*

Martina Pini − *Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy*

Silvia Barbi − *Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy;* orcid.org/0000-0001-5431-6906

Monia Montorsi − *Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy*

Lara Maistrello − *Department of Life Sciences, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy*

Angela Marseglia − *Department of Veterinary Science, University of Parma, 43126 Parma, Italy*

Augusta Caligiani − *Department of Food and Drug, University of Parma, 43124 Parma, Italy*

Anna Maria Ferrari − *Department of Sciences and Methods for Engineering, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy*

Complete contact information is available at: https://pubs.acs.org/10.1021/acssuschemeng.0c03795

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to acknowledge the European Union and Emilia-Romagna Italian region for having financed the project titled "Valorization of organic waste through insects for the obtainment of biomaterials for agricultural uses" the acronym of which is "Valoribio" (http://www.valoribio.eu/en/) in the framework of the ERDF (European Regional Development Fund) of the Emilia-Romagna Regional Operational Program 2014−2020, Italy (grant number PG/2015/737518).

■ REFERENCES

(1) Arancon, R. A. D.; Lin, C. S. K.; Chan, K. M.; Kwan, T. H.; Luque, R. Advances on waste valorization: new horizons for a more sustainable society. *Energy Sci. Eng.* **2013**, *1*, 53−71.

(2) Sheldon, R. A. Green and sustainable manufacture of chemicals from biomass: state of the art. *Green Chem.* **2014**, *16*, 950−963.

(3) Liu, W.-J.; Jiang, H.; Yu, H.-Q. Thermochemical conversion of lignin for functional materials: a review and future direction. *Green Chem.* **2015**, *17*, 4888−4907.

(4) Calcio Gaudino, E.; Cravotto, G.; Manzoli, M.; Tabasso, S. From waste biomass to chemicals and energy via microwave-assisted processes. *Green Chem.* **2019**, *21*, 1202−1235.

(5) Tabasso, S.; Carnaroglio, D.; Calcio Gaudino, E.; Cravotto, G. Microwave, ultrasound and ball mill procedures for bio-waste valorisation. *Green Chem.* **2015**, *17*, 684−693.

(6) Valentino, F.; Gottardo, M.; Micolucci, F.; Pavan, P.; Bolzonella, D.; Rossetti, S.; Majone, M. Organic fraction of municipal solid waste recovery by conversion into added-value polyhydroxyalkanoates and biogas. *ACS Sustainable Chem. Eng.* **2018**, *6*, 16375−16385.

(7) Moscoviz, R.; Trably, E.; Bernet, N.; Carrère, H. The environmental biorefinery: state-of-the-art on the production of hydrogen and value-added biomolecules in mixed-culture fermentation. *Green Chem.* **2018**, *20*, 3159−3179.
(8) Diener, S.; Studt Solano, N. M.; Gutiérrez, F. R.; Zurbrügg, C.; Tockner, K. Biological treatment of municipal organic waste using black soldier fly larvae. *Waste Biomass Valorization* **2011**, *2*, 357−363.
(9) Niu, Y.; Zheng, D.; Yao, B.; Cai, Z.; Zhao, Z.; Wu, S.; Cong, P.; Yang, D. A novel bioconversion for value-added products from food waste using Musca domestica. *Waste Manage.* **2017**, *61*, 455−460.
(10) Sheppard, D. C.; Tomberlin, J. K.; Joyce, J. A.; Kiser, B. C.; Sumner, S. M. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *J. Med. Entomol.* **2002**, *39*, 695−698.
(11) Hadj Saadoun, J.; Montevecchi, G.; Zanasi, L.; Bortolini, S.; Macavei, L. I.; Masino, F.; Maistrello, L.; Antonelli, A. Lipid profile and growth of black soldier flies (Hermetia illucens, Stratiomyidae) reared on by-products from different food chains. *J. Sci. Food Agric.* **2020**, *100*, 3648−3657.
(12) Lalander, C.; Diener, S.; Zurbrügg, C.; Vinnerås, B. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (Hermetia illucens). *J. Cleaner Prod.* **2019**, *208*, 211−219.
(13) Barbi, S.; Macavei, L. I.; Fuso, A.; Luparelli, A. V.; Caligiani, A.; Ferrari, A. M.; Maistrello, L.; Montorsi, M. Valorization of seasonal agri-food leftovers through insects. *Sci. Total Environ.* **2020**, *709*, No. 136209.
(14) Gold, M.; Tomberlin, J. K.; Diener, S.; Zurbrügg, C.; Mathys, A. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: a review. *Waste Manage.* **2018**, *82*, 302−318.
(15) Wang, C.; Qian, L.; Wang, W.; Wang, T.; Deng, Z.; Yang, F.; Xiong, J.; Feng, W. Exploring the potential of lipids from black soldier fly: new paradigm for biodiesel production (I). *Renewable Energy* **2017**, *111*, 749−756.
(16) Feng, W.; Qian, L.; Wang, W.; Wang, T.; Deng, Z.; Yang, F.; Xiong, J.; Wang, C. Exploring the potential of lipids from black soldier fly: new paradigm for biodiesel production (II) − extraction kinetics and thermodynamic. *Renewable Energy* **2018**, *119*, 12−18.
(17) Li, Q.; Zheng, L.; Qiu, N.; Cai, H.; Tomberlin, J. K.; Yu, Z. Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Manage.* **2011**, *31*, 1316−1320.
(18) Bosch, G.; Zhang, S.; Oonincx, D. G. A. B.; Hendriks, W. H. Protein quality of insects as potential ingredients for dog and cat foods. *J. Nutr. Sci.* **2014**, *3*, No. e29.
(19) Patel, S.; Suleria, H. A. R.; Rauf, A. Edible insects as innovative foods: nutritional and functional assessments. *Trends Food Sci. Technol.* **2019**, *86*, 352−359.
(20) Barbi, S.; Messori, M.; Manfredini, T.; Pini, M.; Montorsi, M. Rational design and characterization of bioplastics from Hermetia illucens prepupae proteins. *Biopolymers* **2019**, *110*, No. e23250.
(21) Brodhagen, M.; Peyron, M.; Miles, C.; Inglis, D. A. Biodegradable plastic agricultural mulches and key features of microbial degradation. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 1039−1056.
(22) Félix, M.; Martín-Alfonso, J. E.; Romero, A.; Guerrero, A. Development of albumen/soy biobased plastic materials processed by injection molding. *J. Food Eng.* **2014**, *125*, 7−16.
(23) Felix, M.; Romero, A.; Cordobes, F.; Guerrero, A. Development of crayfish bio-based plastic materials processed by small-scale injection moulding. *J. Sci. Food Agric.* **2015**, *95*, 679−687.
(24) Ramakrishnan, N.; Sharma, S.; Gupta, A.; Alashwal, B. Y. Keratin based bioplastic film from chicken feathers and its characterization. *Int. J. Biol. Macromol.* **2018**, *111*, 352−358.
(25) Shogren, R.; Wood, D.; Orts, W.; Glenn, G. Plant-based materials and transitioning to a circular economy. *Sustainable Prod. Consump.* **2019**, *19*, 194−215.
(26) Bortolini, S.; Macavei, L. I.; Hadj Saadoun, J.; Foca, G.; Ulrici, A.; Bernini, F.; Malferrari, D.; Setti, L.; Ronga, D.; Maistrello, L. Hermetia illucens (L.) larvae as chicken manure management tool for circular economy. *J. Cleaner Prod.* **2020**, *262*, No. 121289.
(27) Arshadi, M.; Attard, T. M.; Lukasik, R. M.; Brncic, M.; da Costa Lopes, A. M.; Finell, M.; Geladi, P.; Gerschenson, L. M.; Gogus, F.; Herrero, M.; Hunt, A. J.; Ibanez, E.; Kamm, B.; Mateos-Aparicio, I.; Matias, A.; Mavroudis, N. E.; Montoneri, E.; Morais, A. R. C.; Nilsson, C.; Papaioannoua, E. H.; Richel, A.; Rupérez, P.; Skrbic, B.; Solarov, M. B.; Svarc-Gajic, J.; Waldron, K.; Yuste, F. Pre-treatment and extraction techniques for recovery of added value compounds from waste throughout the agri-food chain. *Green Chem.* **2016**, *18*, 6160−6204.
(28) Caligiani, A.; Marseglia, A.; Leni, G.; Baldassarre, S.; Maistrello, L.; Dossena, A.; Sforza, S. Composition of black soldier prepupae and systematic approaches for extraction and fractionation of proteins lipids and chitin. *Food Res. Int.* **2018**, *105*, 812−820.
(29) Imbimbo, P.; Bueno, M.; D'Elia, L.; Pollio, A.; Ibañez, E.; Olivieri, G.; Monti, D. M. Green compressed fluid technologies to extract antioxidants and lipids from Galdiera phlegrea in a biorefinery approach. *ACS Sustainable Chem. Eng.* **2020**, *8*, 2939−2947.
(30) Nadar, S. S.; Rao, P.; Rathod, V. K. Enzyme assisted extraction of biomolecules as an approach to novel extraction technology. *Food Res. Int.* **2018**, *108*, 309−330.
(31) Chemat, F.; Vian, M. A.; Cravotto, G. Green extraction of natural products: concept and principles. *Int. J. Mol. Sci.* **2012**, *13*, 8615−8627.
(32) Chemat, F.; Vian, M. A.; Fabiano-Tixier, A. S.; Strube, J.; Uhlenbrock, L.; Gunjevic, V.; Cravotto, G. Green extraction of natural products. Origins, current status, and future challenges. *Trends Anal. Chem.* **2019**, *118*, 248−263.
(33) Zerazion, E.; Rosa, R.; Ferrari, E.; Veronesi, P.; Leonelli, C.; Saladini, M.; Ferrari, A. M. Phytochemical compounds or their synthetic counterparts? A detailed comparison of the quantitative environmental assessment for the synthesis and extraction of curcumin. *Green Chem.* **2016**, *18*, 1807−1818.
(34) Delgove, M. A. F.; Laurent, A. B.; Woodley, J. M.; De Wildeman, S. M. A.; Bernaerts, K. V.; van der Meer, Y. A prospective life cycle assessment (LCA) of monomer synthesis: comparison of biocatalytic and oxidative chemistry. *Chem. Sus. Chem.* **2019**, *12*, 1349−1360.
(35) Pini, M.; Rosa, R.; Neri, P.; Ferrari, A. M. LCA Application to Chemical Synthesis at Laboratory Scale, In *Lyfe Cycle Assessment in the Chemical Product Chain*, Maranghi, S.; Brondi, C., Eds.; Springer: Cham, 2020; pp 101−123.
(36) Allen, D. T.; Hwang, B. J.; Licence, P.; Pradeep, T.; Subramaniam, B. Advancing the use of sustainability metrics. *ACS Sustainable Chem. Eng.* **2015**, *3*, 2359−2360.
(37) Salomone, R.; Saija, G.; Mondello, G.; Giannetto, A.; Fasulo, S.; Savastano, D. Environmental impact of food waste bioconversion by insects: application of life cycle assessment to process using Hermetia illucens. *J. Cleaner Prod.* **2017**, *140*, 890−905.
(38) Mertenat, A.; Diener, S.; Zurbrügg, C. Black soldier fly biowaste treatment − Assessment of global warming potential. *Waste Manag.* **2019**, *84*, 173−181.
(39) Bosch, G.; van Zanten, H. H. E.; Zamprogna, A.; Veenenbos, M.; Meijer, N. P.; van der Fels-Klerx, H. J.; van Loon, J. J. A. Conversion of organic resources by black soldier fly larvae: legislation, efficiency and environmental impact. *J. Cleaner Prod.* **2019**, *222*, 355−363.
(40) Spinelli, R.; Neri, P.; Pini, M.; Barbi, S.; Montorsi, M.; Ferrari, A. M.; et al. Using black soldier flies (Hermetia illucens) to bioconvert waste from the livestock production chain: a life cycle assessment case study. *WIT Trans. Ecol. Environ.* **2018**, *231*, 47−58.
(41) Macavei, L. I.; Benassi, G.; Stoian, V.; Maistrello, L. Optimization of Hermetia illucens (L.) egg laying under different nutrition and light conditions. *PLoS One* **2020**, *15*, No. e0232144.
(42) Association of Official Analytical. *Official Method of Analysis*, 16th ed.; Association of Official Analytical: Washington, DC, 2002.
(43) Tkachuk, R. Nitrogen-to-protein conversion factors for cereals and oilseed meals. *Cereal Chem.* **1969**, *46*, 419−423.

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX

(44) Marseglia, A.; Palla, G.; Caligiani, A. Presence and variation of γ-aminobutyric acid and other free amino acids in cocoa beans from different geographical origins. *Food Res. Int.* **2014**, *63*, 360−366.
(45) Flannery, M. B.; Stott, A. W.; Briggs, D. E. G.; Evershed, R. P. Chitin in the fossil record: identification and quantification of D-glucosamine. *Org. Geochem.* **2001**, *32*, 745−754.
(46) ISO 14040. Environmental management − Life cycle assessment − Principles and framework 2006.
(47) ISO 14044. Environmental management − Life cycle assessment − Requirements and guidelines 2006.
(48) Weidema, B. P.; Bauer, C.; Hischier, R.; Mutel, C.; Nemecek, T.; Reinhard, J.; Vadenbo, C. O.; Wernet, G. The Ecoinvent Database: Overview and Methodology, Data Quality Guideline for the Ecoinvent Database Version 3. http://www.ecoinvent.org (accessed Sept 10, 2020).
(49) Lages, A. C. A.; Tannenbaum, S. R. Production of glucose from Tapioca (Cassava starch) and Farinha de Mandioca (Cassava meal). *J. Food Sci.* **1978**, *3*, 1012−1014.
(50) Dunn, J. B.; Mueller, S.; Wang, M.; Han, J. Energy consumption and greenhouse gas emissions from enzyme and yeast manufacture for corn and cellulosic ethanol production. *Biotechnol. Lett.* **2012**, *34*, 2259−2263.
(51) Pré Sustainability, Stationsplein 121, 3818 LE Amersfoort, The Netherlands. https://simapro.com/contact/ (accessed Sept 10, 2020).
(52) Jolliet, O.; Margni, M.; Charles, R.; Humbert, S.; Payet, J.; Rebitzer, G.; Rosenbaum, R. IMPACT 2002+: a new life cycle impact assessment methodology. *Int. J. Life Cycle Assess.* **2003**, *8*, 324−330.
(53) Pini, M.; Rosa, R.; Neri, P.; Bondioli, F.; Ferrari, A. M. Environmental assessment of a bottom-up hydrolytic synthesis of TiO_2 nanoparticles. *Green Chem.* **2015**, *17*, 518−531.
(54) Rosa, R.; Pini, M.; Neri, P.; Corsi, M.; Bianchini, R.; Bonanni, M.; Ferrari, A. M. Environmental sustainability assessment of a new degreasing formulation for the tanning cycle within leather manufacturing. *Green Chem.* **2017**, *19*, 4571−4582.
(55) Pini, M.; Ferrari, A. M.; Gamberini, R.; Neri, P.; Rimini, B. Life cycle assessment of a large, thin ceramic tile with advantageous technological properties. *Int. J. Life Cycle Assess.* **2014**, *19*, 1567−1580.
(56) Ferrari, A. M.; Volpi, L.; Pini, M.; Siligardi, C.; Garcia-Muina, F. E.; Settembre-Blundo, D. Building a sustainability benchmarking framework of ceramic tiles based on life cycle sustainability assessment (LCSA). *Resources* **2019**, *8*, 11.
(57) Ribakov, Y.; Halperin, I.; Pushkar, S. Using Eco-indicator 99 and a two-stage nested analysis of variance test to evaluate building mitigation measures under hazard risks. *Adv. Struct. Eng.* **2016**, *19*, 860−870.
(58) Zannetti, P. Air Pollution Modeling: Theories, In *Computational Methods and Available Software*; Springer: Boston, 1990; pp 141−183.
(59) Humbert, S.; De Schryver, A.; Bengoa, X.; Margni, M.; Jolliet, O. IMPACT 2002+: User Guide. https://www.quantis-intl.com/pdf/IMPACT2002_UserGuide_for_vQ2.21.pdf (accessed Sept 10, 2020).
(60) Liu, W.; Chen, W.; Hou, Q.; Wang, S.; Liu, F. Effects of combined pretreatment of dilute acid pre-extraction and chemical-assisted mechanical refining on enzymatic hydrolysis of lignocellulosic biomass. *RSC Adv.* **2018**, *8*, 10207−10214.

https://dx.doi.org/10.1021/acssuschemeng.0c03795
ACS Sustainable Chem. Eng. XXXX, XXX, XXX−XXX