

Article

Solid-State Fermentation (SSF) versus Submerged Fermentation (SmF) for the Recovery of Cellulases from Coffee Husks: A Life Cycle Assessment (LCA) Based Comparison

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Abstract: This article studies the environmental impacts of cellulase production by using a comparative attributional life cycle assessment (LCA) of two different scenarios of production. The first one is the commonly used submerged fermentation (SmF) using a pure substrate (cellulose powder) and a specific microorganism (*Trichoderma reesei*). The second scenario considers a novel system to produce enzymes and simultaneously treat a waste using the solid-state fermentation (SSF) process of coffee husk (CH) used as substrate. Experimental data were used in this scenario. The complete production process was studied for these two technologies including the fermentation phase and the complete downstream of cellulase. Life cycle inventory (LCI) data were collected from the database EcoInvent v3 (SimaPro 8.5) modified by data from literature and pilot scale experiments. The environmental impacts of both production systems revealed that those of SmF were higher than those of SSF. A sensitivity analysis showed that the results are highly conditioned by the energy use in the form of electricity during lyophilization, which is needed in both technologies. The results point to a possible alternative to produce the cellulase enzyme while reducing environmental impacts.

Keywords: life cycle assessment (LCA); solid-state fermentation (SSF); submerged fermentation (SmF); cellulase; coffee husk; waste

1. Introduction

Cellulase enzymes account for an important product of the global enzyme market due to their potential applications in various processes including paper pulp, textile, laundry, food and feed industry, agriculture, etc., but in recent years cellulase production has become the most important due to its use in the production of ethanol. Ethanol from biomass is a promising substitute for the petrol fuel used in transport. For this reason, the bio-based economy has promoted ethanol for the substitution of fossil fuels. This has important implications in biorefinery systems [1].

The importance of cost-effective ethanol production from biomass has been widely reported [2]. The technologies for ethanol production from organic wastes are based on using substrates containing sugars. The cost of raw materials is around 40% of the production costs [3]; in consequence, efforts are devoted to use less expensive rejected materials containing organic lignocellulosic wastes.

Lignocellulosic waste is a promising feedstock as a renewable resource for the functioning of modern industrial societies, since a considerable amount of bioproducts, especially ethanol, are produced through this type of waste [4]. In recent years, the study of ethanol production from lignocellulosic residues has gained the attention of several researchers [5,6]. This biomass is available

from agricultural residues and the waste generated from these crops. This material is better than starch in the sense that it does not compete with food resources [7]. In addition, in consonance with development in circular economy principles, the proposed strategies should take into account mechanisms to make profit from the waste generated.

Currently, cellulase is produced from lignocellulosic substrates in submerged fermentation (SmF) culture processes [8–10]. Production of commercial cellulase by SmF began in the 1970s, with cellulase produced by *Trichoderma reesei* and commercialized mainly for research [11]. Submerged fermentation is the most reported technique for cellulase production and the aerobic fungi *Trichoderma reesei* is one of the most used strains [12,13], but the relatively high cost of the enzyme production is the principal bottleneck for its application [14].

In environmental terms, lignocellulosic ethanol is intended to reduce greenhouse gas (GHG) emissions, but it is not yet in the stage of industrial development [15]. Studies from MacLean et al. [16] demonstrated enzymes production as an important contribution to greenhouse gas emissions.

In recent works, different studies [17–19] proved that a promising technology to produce enzymes using lignocellulosic biomass is solid-state fermentation (SSF). These studies present cellulase production from coffee husk (CH) through a complete solid-state fermentation process (including downstream). This technology offers potential opportunities for the use and treatment of coffee husk.

Coffee husk is a fibrous material obtained during the processing of coffee. Coffee husk is rich in lignocellulosic organic matter. Consequently, it is a good substrate for microbial processes based on solid-state fermentation for the production of cellulases [20].

However, the process has some bottlenecks that have not been studied in detail. In most of solid-state fermentation processes, downstream or purification of enzymes is neither analyzed nor considered. Since cellulase can be obtained by different processes, it is considered worthy of investigation to assess the environmental impacts of these different approaches. When obtaining products with lower environmental impacts, they must be evaluated to be advantageous, especially in sectors with strong competition [21]. This comparison among the different production techniques can be useful to clarify the advantages and disadvantages of each process. Therefore, sustainable development requires a tool to measure and compare environmental impacts. Life cycle assessment (LCA) allows calculating the potential environmental impacts of the production and purification of cellulase.

Life cycle assessment is a technique to assess and quantify environmental impacts. It can also be useful for the development and improvement of new processes focused on reducing energy and material resource requirements and emissions. Life cycle assessment is based on the guidelines provided by the International Organization for Standardization (ISO) standards in the 14040 series [22,23].

In consequence, the main aim of this work is to compare the environmental impacts associated with cellulase production via two possible technologies: submerged fermentation through a specific microorganism using a pure substrate and solid-state fermentation from coffee husk (considering the fermentation and downstream). The life cycle inventories (LCIs) of both processes are formulated in agreement with the literature review in the submerged fermentation case and from experimental data obtained from our research group in the solid-state fermentation case due to the lack of data in this field. The submerged fermentation scenario is estimated from the commercial databases of the SimaPro 8.5 LCA software [24]. To our knowledge, this comparison, in terms of environmental impacts of SmF and SSF to obtain the same product, is the first study presented in literature. A sensitivity analysis is also presented to determine the main steps regarding its environmental impacts to propose possible improvements in both technologies.

2. Materials and Methods

2.1. Goals and Scope Definition

2.1.1. Boundaries and Processes

This study has been defined using the “gate to gate” philosophy. In the case of SmF, the LCA system boundary contains the steps necessary for the production of cellulose powder, or in the case of SSF, the waste management of CH and in for both cases the environmental impact of cellulase enzyme. Both processes were analyzed as a sequential batch production.

It must be emphasized that there is little information in the literature to allow a complete LCA in all stages of cellulase production through SmF to be performed. For production through SmF or SSF, the literature survey has investigated the production of enzymes (fermentation), but not the complete downstream process to obtain the pure enzyme. However, in our research group, we performed a complete production scheme including the recovery of the enzyme and the necessary steps to present it as a commercial product [17,25]. For this reason, the same downstream process of our experiments in SSF was used for SmF too, since the products to purify the enzyme (fermentation liquid media or water extracts) are very similar.

2.1.2. Submerged Fermentation (SmF)

The SmF model for cellulase production in this study was based on previous studies (see [2,12,26–29]).

In this study, to facilitate the comparison, the authors selected the cellulase production by means of an aerobic production process by submerged cultivation using *Trichoderma reesei* which produces the extracellular enzyme.

As the bibliography shows, the main raw material was a pure cellulose powder that was used as a substrate. Figure 1 includes the SmF system boundaries considered. The boundaries include the SmF phase (which comprises the inoculum growth processes and the fermentation process itself). The information and data of this part were based on literature studies [12,26–29]. However, the information of the complete downstream and the solid waste treatment phase was insufficient, so the downstream process was considered the same as that of the SSF process for an initial approximation to study the LCA.

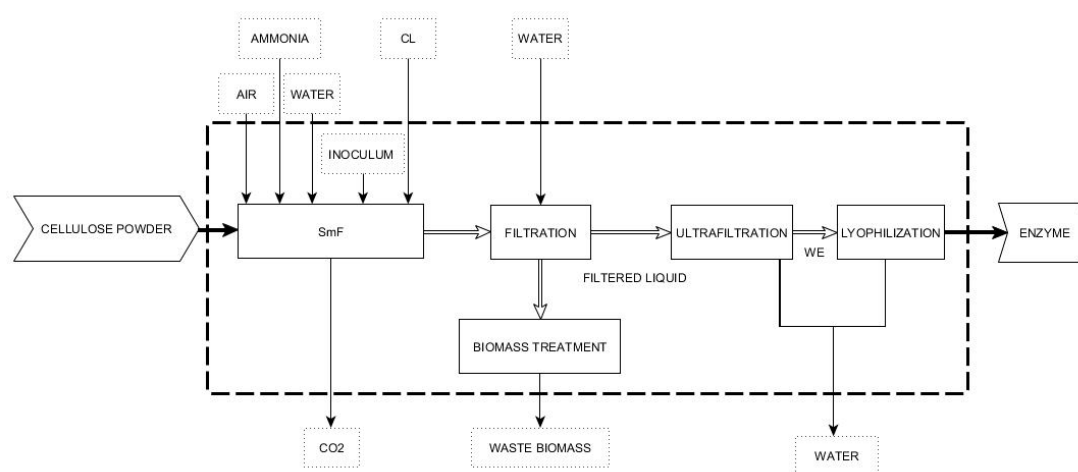


Figure 1. Flow diagram of the submerged fermentation (SmF) process. The flows of the inputs and outputs are shown with dashed lines. CL, corn liquor; WE, water extract.

The main stages of the process studied are shown below:

- (a) Inoculum: The inoculum phase was a sequence of growth seeds (3 bioreactors) to provide the necessary amount of inoculum (5% of working volume) and the preparation of the bioreactor media. The specific medium for inoculum included different substances such as ammonium sulphate, potassium phosphate or calcium dichloride, among others [28]. Three growth bioreactors provided inoculum for the production vessel (100 L). These reactors are designed to provide 5% inoculum to each submerged bioreactor to the next scale. The fermentation time of each one of the three growth bioreactors was estimated in 40 h [29].
- (b) SmF: The bioreactor working volume was 80% of the total volume and included a compressor and air filter for sterilization to provide the requirements of oxygen to perform the fermentation under optimal conditions. This bioreactor converts raw materials into the desired product: the cellulase enzymes. Ammonium was used to control pH and provide additional nitrogen to the microorganisms. Corn liquor and other trace nutrients were also added to the bioreactors as a carbon source. The bioreactors were aerated with air compressed to provide the oxygen demand and a chilled water system flowing through internal coils to control temperature. The output of fermentation is CO₂ released during cellulase production. The microorganism was fed with nutrients to form biomass, a cellulase enzyme (metabolic product), and wastewater. Flow rates were estimated from the amount of raw materials and waste.

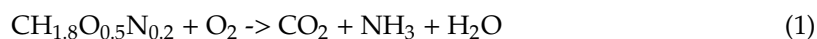
In this kind of process, some byproducts are formed, and some raw materials are not completely consumed, which implies the generation of some waste flows that need some treatment or disposal and they are considered in the LCA analysis. Although this waste is not represented in Figure 1, it was included in LCI. Therefore, a downstream process was necessary to obtain a final product.

Then, for the downstream step, three sub-phases were necessary to obtain a final product:

- (c) Filtration: The first sub-phase was filtration, which consisted of a tubular centrifugation and a 0.22 µm membrane filter where approximately 99.9% of total biomass waste was removed.
- (d) Ultrafiltration: The supernatant was ultrafiltered through tangential filtration to obtain a concentrated liquid without microorganisms. Ultrafiltration (10 kDa) was repeated until the concentration factor was 10.
- (e) Lyophilization: At the last stage, lyophilization, all the remaining water is removed, and a solid, dry enzyme is obtained as the final product.

Finally, in order to complete the zero waste strategies, the solid waste (biomass) treatment phase was considered:

- (f) Biomass treatment: its environmental impact was assessed by the oxygen consumption via the theoretical chemical oxygen demand (ThCOD) consumed in a complete oxidation reaction. The ThCOD was estimated from the approximate molecular composition of biomass. Then, the reaction considered to calculate this parameter is:



As a summary, the main raw materials of cellulose powder, medium with inoculum, ammonium, corn liquor, as well as water and energy were the inputs to LCA. Then, cellulase enzyme, residual biomass, water, and carbon dioxide were the outputs for this system.

2.1.3. Solid-State Fermentation (SSF)

The SSF data of cellulase production by means of SSF were based on experimental and recent data from our research group and can be consulted in previous works [17,25]. All data are the sum of several studies to optimize the production and downstream of cellulase at pilot scale. As main raw material, CH was used as substrate and a specialized inoculum coming from a previous fermentation from a batch fermentation was selected. This inoculum was not considered in LCA because it was not

a real inoculum. It was a fermented solid from the last batch, and it was supposed that the impact emission was attributed to that batch. Woodchips were used as a bulking agent (BA) in a ratio 1:1 (*v/v*) to increase porosity.

As shown in Figure 2, the LCA system boundaries included: cellulase production and downstream, solid waste treatment after extraction, and gaseous emissions treatment.

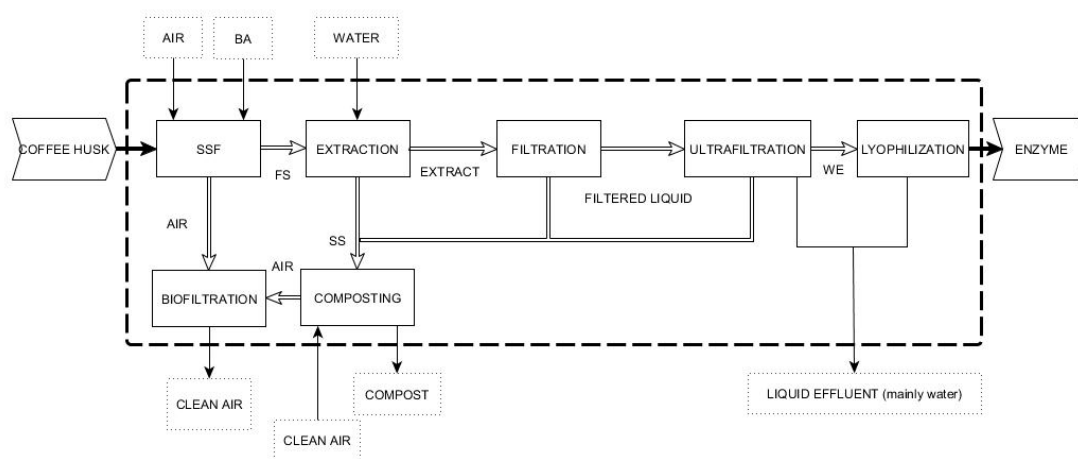


Figure 2. Flow diagram of the solid-state fermentation (SSF) process is included in the composting process to treat waste solids. The flows of the inputs and outputs are shown with dashed lines. BA, bulking agent; FS, fermented solid; SS, spend solid; WE watery extract. Reprint with permission [30]; 2020, Elsevier.

The main stages of the process studied are shown below:

- SSF: SSF was performed similarly to a composting process, in discontinuous mode in a 100 L airtight aerated reactor. In a study by Cerda and colleagues [17], the highest cellulase activity was observed after 24 h of SSF. Accordingly, residence time was fixed to this value. Approximately 10% of the fermented solid was the inoculum for the next batch [25,31,32]. Wood chips were used to provide porosity to the mixture [33]. In the same way, all fermented solid (except 10% used as inoculum) was derived to downstream stages.
- The downstream processes is composed of four phases as explained below:
- Extraction: The fermented solid was mixed with tap water in a 1:2 (*v/v*) ratio for 30 min [18]. To facilitate further filtration process, the fermented solids were mixed with water, passed through a mesh of 1 mm in the same tank with the objective of removing the biggest solid particles.
- Filtration: Then, the liquid obtained from the previous stage (extract) was centrifuged at 15,000 rpm. The resulting liquid was filtered through a 0.22 μm filter. With this operation, all the biomass and suspended solids were removed.
- Ultrafiltration: Next, this extract was filtered with an ultrafiltration device (10 kDa of cut-off) to concentrate the cellulase suspension. This step was repeated to reach a concentration ten times higher than the original one.
- Lyophilization: Finally, the enzyme concentrated was lyophilized to obtain the main product (dry and concentrated cellulase).

During all these steps, a remaining solid waste is being generated. This material is partially stabilized after fermentation and it is easily composted in a short time. The composting process is conducted in the same reactor as SSF. The process lasts 14 days to obtain a compost-like stabilized material to be used as organic amendment.

2.2. Inventory Analysis, Functional Unit, Methodology, and Assumptions

2.2.1. Functional Unit

The functional unit (FU) in this LCA must be the same for all the compared products [34]. In this study, the FU was “1 kg of produced dry cellulase” and not the waste treated since the objective is the production of cellulases [35].

2.2.2. Life Cycle Assessment (LCA) Methodology and Impact Assessment

Regarding the life cycle impact assessment (LCIA), the ReCiPe 2016 methodology was used in the software Simapro 8.5. ReCiPe 2016 is the most recent indicator available in LCIA. This LCIA transforms the life cycle inventory results into indicator categories. ReCiPe 2016 is a follow up of the ReCiPe 2008 and this is a continuation of Eco-Indicator 99 (CML methodology). This approach can be numerically expressed as: (i) midpoint indicators and (ii) endpoint indicators. One of the advantages of the ReCiPe is that it has the broadest set of midpoint impact categories. Due to significant methodological differences, the results of ReCiPe 2008 and ReCiPe 2016 cannot and should not be compared.

Each method has been created for three different perspectives. Normalization and weighing factors are not yet published; therefore, this version of ReCiPe 2016 does not include normalization and weighing. Thus, the units are expressed only in mass of a compound with respect to FU as in Table 1. Potential impacts in the future are not included, but this version assumes that these future impacts are already included in the inventory analysis, as it is different from what happens in other approaches.

Table 1. The 18 environmental impacts considered in the ReCiPe 2016 database method at the midpoint level (ML). Reprint with permission [30]; 2020, Elsevier.

Impact Category	Unit of the Results
Global warming (GWP)	kg CO _{2eq}
Stratospheric ozone depletion (ODP)	kg CFC ⁻¹¹ _{eq}
Ionizing radiation (IRP)	kBq Co-60 _{eq}
Ozone formation, human health (HOFp)	kg NO _{xeq}
Fine particulate matter formation (FPMF)	kg PM _{2.5eq}
Ozone formation, terrestrial ecosystems (EOfp)	kg NO _{xeq}
Terrestrial acidification (TAP)	kg SO ₂
Freshwater eutrophication (FEP)	kg P _{eq}
Marine eutrophication (MEP)	kg N _{eq}
Terrestrial ecotoxicity (TETP)	kg 1,4-DCB
Freshwater ecotoxicity (FETP)	kg 1,4-DCB
Marine ecotoxicity (METP)	kg 1,4-DCB
Human carcinogenic toxicity (HTPc)	kg 1,4-DCB
Human non-carcinogenic toxicity (HTPnc)	kg 1,4-DCB
Land use (LOP)	m ² year
Mineral resource scarcity (SOP)	kg Cu _{eq}
Fossil resource scarcity (FFP)	kg oil _{eq}
Water consumption (WCP)	m ³

Regarding indicators, the midpoint indicators were selected in this study. The selection between midpoint and endpoint indicators is that the first ones explain direct specific impacts on the environment, while endpoint indicators describe more general impacts. As this is the first study to compare SmF and SSF, we selected midpoint indicators as they are commonly reported in the literature. Table 1 presents the impact categories and units for ReCiPe 2016 midpoints. Normally, not all these categories are included in LCA published in literature, except the most important ones (ADP, abiotic depletion potential; AP, acidification potential; EP, eutrophication potential; GWP, global warming potential; ODP, ozone layer depletion potential; POP, photochemical oxidation potential) as reported in several studies [13,21,35]. However, all impact categories were analyzed in this work to improve the information for further research.

Attributional modeling was selected in this study. With this option, inputs are attributed to outputs using a defined relationship. Among LCA researchers [36], there is a consensus in the fact that attributional modeling is more helpful when the objective of the LCA is to present the environmental performance of a product. Therefore, according to this definition, in this work attributional modeling was the most beneficial because it defined the different ways to produce a product (enzyme).

Cellulase enzyme production through SmF is not a multi-functional process. That is, only one product is obtained, namely the enzyme. In the case of SSF, however, the cellulase and the fermented solid (to be used as organic amendment) are obtained, that is, two products. Therefore, allocation technique was used for technology. According to ISO guidelines, expansion to include co-products is based on physical and economic relationships [23]. We selected economic expansion in this study. Specifically, the allocation used the market values of the two products, with a cellulase price of 98 €/kg (cellulase powder, from *Trichoderma* sp., CAS Number: 9012-54-8 from Sigma-Aldrich, Barcelona, Spain) and 10 €/t of compost (Jorba compost plant, CIF: B-61634622 from Catalonia, Barcelona, 2019, Spain). Consequently, 90% of the environmental impacts were associated to the enzyme production.

A sensitivity analysis was also performed including new approaches and variations to the system under study. The use of energy in the form of electricity and the composting system used for the stabilization of solid exhaust material from SSF were selected for this analysis.

2.2.3. Technical Assumptions

Any LCA needs some working hypothesis and assumptions. These are the following for both technologies considered in this study.

Overall Assumptions

The first assumption considered was not to include transportation of raw materials and final products. In this work, we decided only to focus on the production process. Transportation could be a significant environmental impact for both processes. We decided not to include it in the study because the number of possible situations that could occur. For instance, carboxymethyl cellulase can be provided from different industries, with very different locations. The same happens with CH, which can be obtained from different coffee producers. If an exact location for producers and destinations of the products was set, this would need to include transportation impact but, in this case, it is out of the scope of the study. The main assumption to carry out the study was that equal masses of cellulase enzymes obtained through both technologies described above have the same specific activity indistinctly of the method of production.

The final use for cellulase enzyme has not been taken into account.

On the other hand, the conversion factor of 396 kJ/m³ [37] was used to convert air into electrical energy using electrical air compressors.

In the downstream stage, the times of the phases were: 2 h for filtration, 24 h for ultrafiltration, and 24 h for lyophilization. The energy consumption of these steps (kJ) was calculated using the equipment power and the operation time. These times were estimated according to the equipment used in the downstream of similar enzyme downstream processes [38,39].

As overall assumptions, the Ecoinvent inventory dataset and data from Spanish electrical grid were used. These energy data consider all the percentage contributions of different energy sources in Spain, which are: 21% nuclear, 19% wind, 17% gas, 16% coal, 15% hydrothermal, 5% oil, 5% solar, and 2% biofuels. These data correspond to 2017 official energy data in Spain [40].

Finally, wastewater treatment was not included. On one hand, a part of water is removed by lyophilization in the form of water vapor, not a wastewater. On the other hand, the wastewater coming from SmF medium and the extract of SSF is practically not polluted, as the biomass is used again for the sequential batch operation and the rest of the soluble compounds are in the form of very diluted salts (both for SmF and SSF).

Submerged Fermentation (SmF)

All data were obtained from the literature [2,12,28,29], where detailed operational conditions and key parameters chosen for the process model used in this case are provided.

- (a) Cellulose powder was considered with an environmental impact due its production.
- (b) The fermentation process was considered to run for 107 h in an airtight packed bed reactor, working under oxygen controlled aeration with $0.58 \text{ vvm } (L_{\text{air}} \cdot (L_{\text{reactor}} \cdot \text{min})^{-1})$ and a constant temperature of 28°C .
- (c) According to Himmel, Biwer, and Wyman [12] and Sáez [26], the principal raw material was cellulose powder. In this study, carboxymethyl cellulose was chosen as a main input (Ecoinvent database). In this case, as carboxymethyl cellulase is a commercial product used to obtain another commercial product (cellulase), its environmental burdens in the production must be considered in this process. Ecoinvent database contains the details of these environmental impacts.

Solid-State Fermentation (SSF)

Data used to perform LCA from SSF are based on the cellulase production from CH performed and published by our research group [17]. Details can be found elsewhere [25].

- (a) The main assumption was that the raw material used would not have environmental impacts because it is a waste. In this case, the environmental impacts associated with CH production are associated to the original industry (coffee production) since it is the normal procedure when dealing with waste as raw material for the production of another commercial product.
- (b) Exhaust gases emitted in SSF (and further composting) were treated in a biofilter considering that it removes 70% of emitted pollutants, as reported in the literature [41].

2.2.4. Inventory Analysis

Submerged Fermentation

Main flow inputs, outputs, and assumptions of the SmF are shown in Table 2. Main data were obtained from previous studies [2,12,28,29].

Table 2. Main input and output data of the submerged fermentation process.

	Material	Value	Comments and Assumptions
INPUTS	Cellulose powder ¹ (kg/FU)	3.62	In SimaPro the chosen input was directly carboxymethyl cellulose because it is prepared from pure cellulose [42].
	Ammonia ² (kg/FU)	0.10	Heinzle et al. [28].
	Corn liquor ³ (kg/FU)	0.75	The carbon source necessary for the microorganism growth was assumed to be corn residue, although it is typically a nitrogen source [28].
	Water (L/FU)	77.4	Heinzle et al. [28].
	Nutrients ⁴ (kg/FU)	0.55	The nutrients required during fermentation were: 32.5 g/L ammonium sulphate ((NH ₄) ₂ SO ₄), 46.5 g/L monopotassium phosphate (KH ₂ PO ₄), 7.0 g/L magnesium sulphate heptahydrate (MgSO ₄ ·7H ₂ O), 9.3 g/L calcium chloride dihydrate (CaCl ₂ ·2H ₂ O), and 4.7 g/L Tween 80 [2,29].
	Energy (kJ/FU)	1,413,935	Taking into account that the energy was the sum for each equipment power and the total air consumption. The air consumption from fermentation and the stabilization biomass was 372,701 L air/kg cellulase.
OUTPUTS	Biomass waste (kg/FU)	1.5	The required oxygen for biomass oxidation produced in 1 kg of cellulase was 102 kg. Theoretical chemical oxygen demand (ThCOD) was calculated through theory reaction of oxidation microorganism with Equation (1): CH _{1.8} O _{0.5} N _{0.2} + O ₂ + → CO ₂ + NH ₃ + H ₂ O
	Cellulase (kg/FU)	0.35	Heinzle et al. [28].
	Wastewater (L/FU)	65.2	Heinzle et al. [28].
	Corn liquor (kg/FU)	0.15	Heinzle et al. [28].
	CO ₂ (kg/FU)	0.13	This value was taken and calculated according to the study from Sáez et al. [26] where it was demonstrated that during the fermentation 18 g/L of CO ₂ in terms of bioreactor volume can be produced.

Cellulose powder¹: carboxymethyl cellulose as a cellulose input. Ammonia²: as nitrogen source. Corn liquor³: as carbon source. Nutrients⁴: as ammonium sulphate (32.5%), monopotassium phosphate (46.5%), magnesium sulphate (7.0%), calcium chloride (9.3%), and Tween 80 (4.7%). The aeration of reactors was provided by air compressors taking atmospheric air. The conversion of 396 kJ/m³ was calculated on the basis of [37]. It was used to convert air volume to electrical energy. Operational times of the different phases were: filtration 2 h, ultrafiltration 24 h, and lyophilization 24 h and the energy consumption of the different steps (kJ) was calculated by accounting for the equipment power and operation time, calculated on the basis of Sánchez et al. [38,39]. FU, functional unit.

Solid-State Fermentation

Table 3 shows the inputs, outputs, and assumptions of the SSF system. All data were obtained from experimental data [17,25]. CH and BA were the raw materials (inputs) and compost and enzyme were the products (outputs). Electricity, air, and water in the stages presented in Table 3 were also calculated.

Table 3. Main input and output data of the solid state fermentation process. Reprint with permission [30]; 2020, Elsevier.

	Materials	Value	Comments and Assumptions
INPUTS	Coffee husk ¹ (kg/FU)	84.3	Coffee husk (CH) waste coming from Marcilla S.L, Barcelona (Spain). This CH is not associated with environmental impacts as it is attributed to coffee production.
	Wood chips ² (kg/FU)	55	Cerda et al. [17].
	Water from extraction (L/FU)	678.8	Cerda et al. [17].
	Energy ³ (kJ/FU)	1,407,873	Taking into account that the energy was the sum for each equipment power and the total air consumption. The sum total (air required in SSF and the air required in composting step) of air consumption to obtain 1 kg of cellulase (FU) was 357,053 L.
OUTPUTS	Compost	186.8	Composting process was performed in the same reactor of SSF with aeration for 15 days. In this study it was assumed that the compost obtained avoided the production of fertilizer. It was considered that 50% of the total nitrogen of compost are available to plants and therefore, can replace an equivalent amount of N in the form of ammonium nitrate. This entails that 5.6 kg of fertilizer production were avoided per 1 kg cellulase (FU).
	COD ⁴ (kg/FU)	0.03	Estimated
	Wastewater (L/FU)	601.5	Cerda et al. [17].
	Methane ³ (kg/FU)	0.02	Maulini-Duran et al. [43]
	Nitrous oxide ³ (kg/FU)	0.06	Maulini-Duran et al. [43]
	VOCs ³ (kg/FU)	0.13	Maulini-Duran et al. [43]
	Ammonia ³ (kg/FU)	0.06	Maulini-Duran et al. [43]

¹Coffee husk: as substrate. ²Wood chips: as bulking agent. ³Methane, nitrous oxide, volatile organic compounds (VOCs), and ammonia: in study by Cerda and colleagues [17]. The off gases emitted during the SSF process were not measured. Therefore, for the purpose of LCA, concentration values for methane, nitrous oxide, VOCs, and ammonia were assumed according to Maulini-Duran et al. [43]. The aeration of reactors was provided by air compressors taking atmospheric air. The conversion of 396 kJ/m³ [37] was used to convert air volume to electrical energy. Operational times of the different phases were: filtration 2 h, ultrafiltration 24 h, and lyophilization 24 h and the energy consumption of the different steps (kJ) was calculated by accounting for the equipment power and operation time [38,39]. ⁴COD: chemical oxygen demand.

2.3. Sensitivity Analysis

LCA has some inherent uncertainties, so it is necessary to perform a sensitivity analysis [44]. One critical point is to determine the effect of changes in electricity consumption. Then, an alternative scenario showing the effect of the lyophilization step was considered. Specifically, under this alternative scenario, the electricity consumption of this step was removed, and it was compared to the base scenario for both technologies.

In SSF technology, we considered it important to observe the effect of the composting step in electricity consumption terms. Therefore, a specific sensitivity analysis for this technology was evaluated. In the case of composting, the use of turned windows was considered as an alternative to the initial system that considered in-vessel tunnels. In this case, it is evident that the electricity consumption decreases but diesel consumption appears as new energy impact.

3. Results and Discussion (Impact Assessment and Interpretation)

3.1. Overall Results

The summary of the results for both technologies (SSF and SmF) as well as the products obtained is included in Figure 3 as absolute values for comparison. This result was the sum of direct and indirect emissions for each technology. Although the comparison between impact categories is not possible because each value is expressed in different units, the authors considered that this figure was clear to observe the main differences between both technologies. For this reason, multipliers were used.

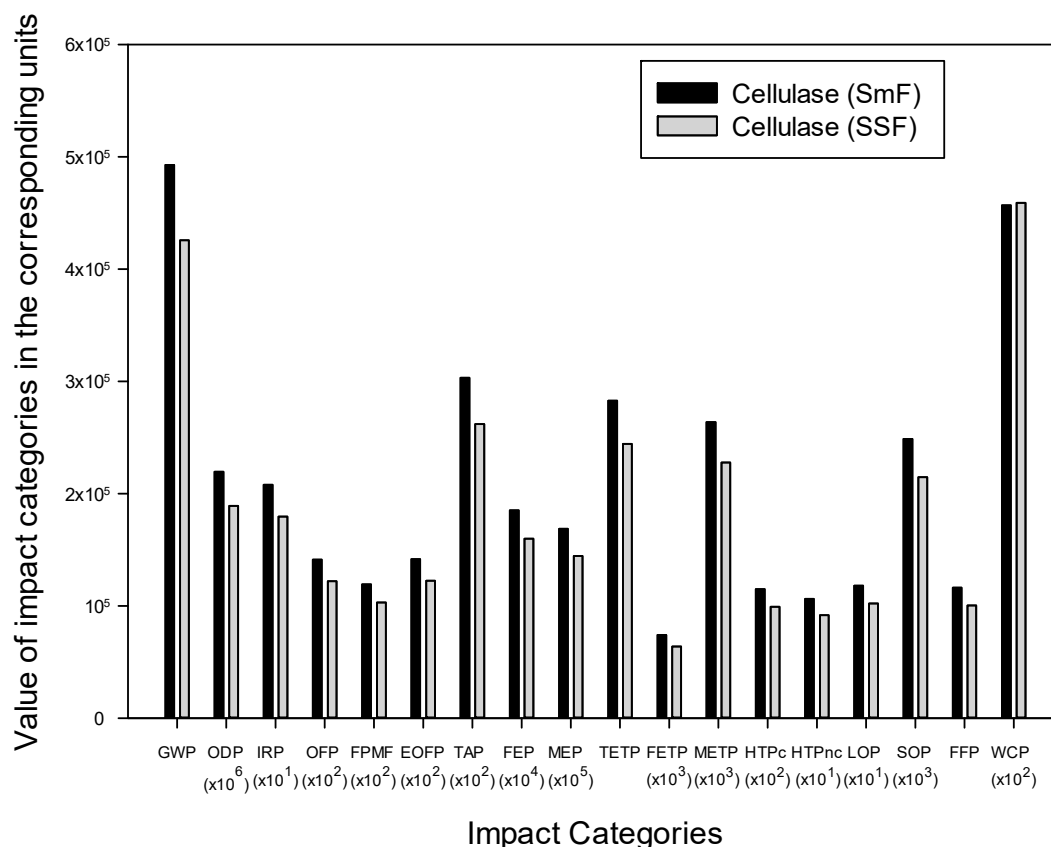


Figure 3. Comparison results to produce cellulase by SmF and SSF (absolute values). Functional unit: 1 kg cellulase produced. Results were based on the ReCiPe 2016 midpoint method. Some categories were multiplied with 10 factors for comparison. The multiplier is shown in the horizontal axis. The acronyms are defined in Table 1.

The impacts associated to the compost-like material production from SSF are not significant compared to the ones from cellulase production for both processes. For this reason, these impacts are not included in Figures 3 and 4 and Tables 4 and 5.

In all categories, the impacts of SSF technology were lower (with an average of 12.8%) than those of the corresponding impacts of the SmF technology, except in water consumption where SSF results were slightly higher (0.5%). In both technologies, global warming (492,715 kg CO_{2eq} for SmF and 425,722 kg CO_{2eq} for SSF), ionizing radiation (20,776 kBq Co-60_{eq} for SmF and 17,953 kBq Co-60_{eq} for SSF), terrestrial ecotoxicity (282,746 kg 1.4-DCB for SmF and 244,249 kg 1.4-DCB for SSF), and fossil resource scarcity (116,237 kg oil_{eq} for SmF and 100,436 kg oil_{eq} for SSF) were the category impacts with the highest value. The biggest difference between technologies was in marine eutrophication (14.4%). As observed, the impact category with major value was global warming. This result was in agreement with studies published by Nizami and Ismail [45], who suggested that the GHG emissions related to enzyme production were associated to the emissions derived from ethanol production

from lignocellulosic materials. One of the most important sources of GHG emissions is electricity production [46]. Therefore, this is an important aspect to improve in the development of alternative technologies or strategies. In addition to this, the results presented above are in agreement with other results [47,48], where this category was the main impact for biofuel production using cellulase.

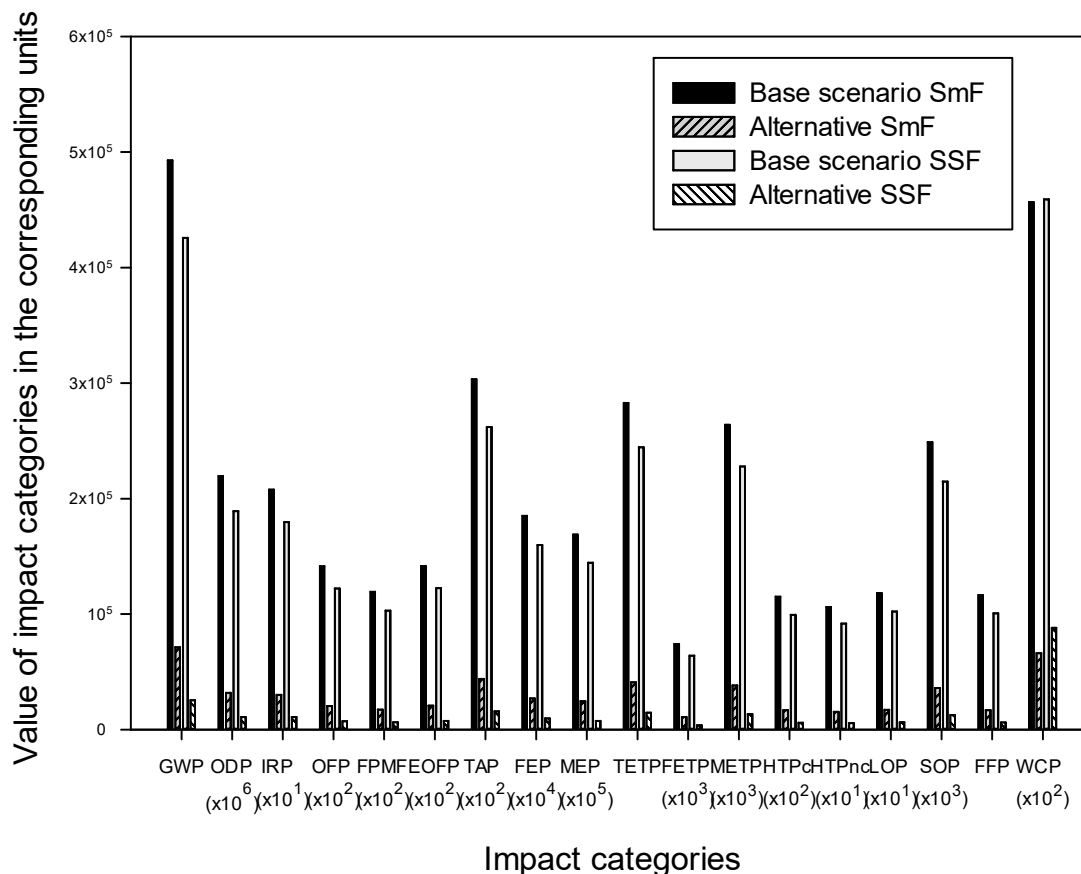


Figure 4. Sensitivity analysis: comparison results to produce cellulase by SmF and SSF without lyophilization (absolute values). Functional unit: 1 kg cellulase produced. Results were based on the ReCiPe 2016 midpoint method. Some categories were multiplied with 10 factors for comparison. The multiplier is shown in the horizontal axis. The acronyms are defined in Table 1.

Regarding other categories, environmental impact cannot be compared since, to our knowledge, no similar studies have been published in recent literature. However, their effect on the environment is commented below.

The last impact category with a significant value was fossil resource scarcity. A clear and evident relationship between fossil resource scarcity and global warming exists. CO_2 is the main GHG, and 70–75% of all CO_2 emissions are due to combustion of fossil fuels [49].

Consequently, it is important to remark the need of developing new techniques that imply a decrease in fossil fuel extraction due to reduced consumption of them. These results were in agreement with the International Energy Agency [50] who studied the effect of energy in climate scenarios.

Impact categories of stratospheric ozone depletion ($0.22 \text{ kg CFC}^{-11}$ for SmF and $0.19 \text{ kg CFC}^{-11}$ for SSF), freshwater eutrophication ($18.50 \text{ kg P}_{\text{eq}}$ for SmF and $15.98 \text{ kg P}_{\text{eq}}$ for SSF), and marine eutrophication ($1.69 \text{ kg N}_{\text{eq}}$ for SmF and $1.44 \text{ kg N}_{\text{eq}}$ for SSF) had the lowest values as the emissions to water and air were insignificant in both technologies. The difference between them could be due to the use of the raw materials (carboxymethyl cellulose or CH).

Therefore, according to the results mentioned above, this study confirms the importance of studying future technology developments on decreasing GHG emissions in enzyme production. These results are in agreement with González-García and colleagues [51] who showed that the production of enzymes for ethanol production affects the environmental impacts, especially in GHG emissions.

3.2. Specific Results

In Table 4, both direct and indirect environmental impacts for SmF technology can be observed. The results are also presented for all the sub-stages contributing to the different impact categories. Table 4 demonstrates that electricity and carboxymethyl cellulose powder used as substrate in SmF were practically the only contributors to all environmental impact categories. On the contrary, the impact of the SmF process itself, and the use of nutrients and ammonia for fermentation were insignificant. As observed in Table 4, the emissions from electricity mostly affect global warming, ionizing radiation, terrestrial ecotoxicity, and fossil resource scarcity, the same categories that presented the highest impact. Therefore, according to these results, it can be confirmed that electricity consumption is a major contributor in the impact of cellulase production by SmF. The electricity sector involves the generation, transmission, and distribution of electricity. Gases as CO₂, CH₄, and N₂O are released during the combustion of fossil fuels to produce electricity. Studies by Doric et al. [52] and Ferreira et al. [53] demonstrated that during the combustion of fossil fuels in different electricity generation technologies these kinds of GHGs were emitted in considerable amounts.

Table 4. Life cycle impact assessment (LCIA) of cellulase produced through SmF. Basis: 1 kg cellulase in the product stream. Characterization results by ReCiPe 2016 midpoint method.

Impact Category	Units	Cellulase Production	Carboxymethyl* Cellulose	Ammonia	Nutrients	Electricity
GWP	kg CO _{2eq}	1.8	16.32	0.20	1.03	492,695
ODP	kg CFC ⁻¹¹ _{eq}	0	7.50×10^{-6}	7.62×10^{-8}	3.33×10^{-7}	0.22
IRP	kBq Co-60 _{eq}	0	0.11	1.01×10^{-3}	0.01	20,775
OFP	kg NO _{xeq}	0	0.03	1.96×10^{-4}	1.85×10^{-3}	1412
FPMF	kg PM2.5 _{eq}	0	0.03	1.88×10^{-4}	1.73×10^{-3}	1191
EOFP	kg NO _{xeq}	0	0.03	2.023×10^{-4}	1.88×10^{-3}	1417
TAP	kg SO ₂	0	0.06	4.77×10^{-4}	3.73×10^{-3}	3032
FEP	kg P _{eq}	0	9.52×10^{-4}	8.67×10^{-7}	3.87×10^{-5}	18.50
MEP	kg N _{eq}	0	8.15×10^{-5}	1.39×10^{-7}	4.81×10^{-6}	1.69
TETP	kg 1.4-DCB	0	31.77	0.87	1.31	282,712
FETP	kg 1.4-DCB	1.70×10^{-17}	6.37	6.61×10^{-5}	3.01×10^{-4}	74.12
METP	kg 1.4-DCB	0.02	0.03	6.86×10^{-4}	8.14×10^{-4}	263.72
HTPc	kg 1.4-DCB	3.49×10^{-4}	0.09	3.85×10^{-4}	2.08×10^{-3}	1149
HTPnc	kg 1.4-DCB	0	1.07	7.70×10^{-3}	0.03	10,617
LOP	m ² year	0	2.61	2.04×10^{-4}	0.01	11,804
SOP	kg Cu _{eq}	0	0.03	1.00×10^{-4}	1.07×10^{-3}	248.62
FFP	kg oil _{eq}	0	4.59	0.09	0.27	116,232
WCP	m ³	0.08	0.20	5.17×10^{-3}	0.01	4566

* Impact categories are defined in Table 1.

The use of carboxymethyl cellulose as raw material in SmF indirectly affects global warming, terrestrial ecotoxicity, and fossil resource scarcity categories. This substance is derived from cellulose, which is made water-soluble by a chemical reaction. It is produced from cellulose and monochloroacetic

acid and with sodium hydroxide as the third essential reagent. The major environmental burdens are associated to electricity consumption for their production.

In addition, the use of this substance affects marine ecotoxicity, human carcinogenic and non-carcinogenic toxicity categories since wastewater usually has high COD (COD from 2100 to 6500 mg/L) and salinity (>10%), which come from organic and inorganic byproducts that appear in the production of carboxymethyl cellulose. The main byproducts included in the production are ethoxy acetic acid and sodium chloride [54].

Therefore, it could be interesting to replace this raw material for other kinds of cellulose derived products, including lignocellulosic solid waste as presented in a recent study by Wyman and co-workers [55].

Table 5 shows the difference between direct and indirect emissions, where the direct emissions are presented in the “cellulase” columns, and indirect impacts in the other columns. In Table 5, electricity consumption is the main contributor of the main impacts of cellulase production by SSF.

Table 5. Life cycle impacts assessment (LCIA) of cellulase produced through SSF. Basis: 1 kg cellulase in the product stream. Characterization results by ReCiPe 2016 midpoint method. Reprint with permission [30]; 2020, Elsevier.

Impact Category	Units	Cellulase	Wood Chips	Electricity	Nitrogen Fertilizer as N
GWP	kg CO _{2eq}	17.2	7.1	425,758	−60.5
ODP	kg CFC ^{−11} _{eq}	6.27×10^{-4}	6.20×10^{-6}	0.19	-1.26×10^{-3}
IRP	kBq Co-60 _{eq}	0	8.78×10^{-2}	17953	−0.27
HOFP	kg NO _{xeq}	0	3.08×10^{-2}	1221	−0.11
FPMF	kg PM2.5 _{eq}	0	2.18×10^{-2}	1029	-6.22×10^{-2}
EOFP	kg NO _{xeq}	0	3.29×10^{-2}	1224	−0.11
TAP	kg SO ₂	0	3.27×10^{-2}	2620	−0.26
FEP	kg P _{eq}	0	2.88×10^{-4}	16.0	-1.46×10^{-3}
MEP	kg N _{eq}	0	1.16×10^{-4}	1.46	1.35×10^{-2}
TETP	kg 1.4-DCB	0	28.1	244,303	−82.3
FETP	kg 1.4-DCB	0	6.00×10^{-3}	64.1	−0.12
METP	kg 1.4-DCB	0	2.23×10^{-2}	228	−0.15
HTPc	kg 1.4-DCB	0	0.12	993	-5.43×10^{-2}
HTPnc	kg 1.4-DCB	0	2.2	9174	−5.4
LOP	m ² year	0	19.6	10,200	−8.2
SOP	kg Cu _{eq}	0	3.18×10^{-3}	215	−0.14
FFP	kg oil _{eq}	0	2.7	100,441	−7.4
WCP	m ³	645	0.19	3946	−1.2

BA plays a main role in terrestrial ecotoxicity (28.1 kg 1.4-DCB) and land use (19.7 m²·year) categories due to deforestation [56].

The energy consumption results in high environmental burdens with regard to the following impact categories: 425,758 kg CO_{2eq} for global warming; 244,302 kg 1.4-DCB for terrestrial ecotoxicity; 100,441 kg oil_{eq} for fossil resource scarcity; 17,953 kBq Co-60_{eq} for ionizing radiation; and 10,200 m²·year for land use. Similar results were obtained by Gassara et al. [56], which is one of the few LCA studies performed for SSF and it obtained the same conclusions regarding electricity consumption. Although previous studies [15,46] have confirmed the importance of GHG emissions due to electricity consumption in cellulase production, it is not possible to compare the numeric values because the ReCiPe 2016 methodology was used to calculate these impacts. The factors in global warming differ from the 100-time horizon in the Intergovernmental Panel on Climate Change (IPCC) 2013 because

climate-carbon feedback for non-CO₂ GHGs was included. For further details, see the method's documentation [57]. Therefore, comparison with previous studies can only be done in terms of tendency and not in absolute values.

On the other hand, the production of compost-like material replaces the use of chemical fertilizers that results in the reduction of the impacts related to stratospheric ozone depletion (0.7%), marine eutrophication (0.9%), and freshwater ecotoxicity (0.2%). In these cases, the reduction is not very high, but we considered it a step forward to zero waste in the new paradigm of circular economy.

In summary, according to Tables 4 and 5, electricity consumption was the main contributor in all impact categories for both technologies, as it was observed in the energy conversion methods [58].

Therefore, according to Tables 4 and 5, it can be confirmed that the most important impact when cellulase is produced by both technologies is global warming. This is in accordance with other studies [15,47] that show that the main environmental burdens in bioethanol production are related to enzyme production.

Additionally, it is possible to confirm the conclusions of other research works in which coffee residues are presented as a substrate for obtaining bioproducts in environmental terms [59]. Thus, SSF is a promising emerging technology for producing cellulases from this waste [42].

3.3. Sensitivity Analysis

As observed in previously commented results, the electricity consumption in both processes presents a high environmental impact. In consequence, a sensitivity analysis was performed to determine if energy consumption in the downstream phase influences environmental profiles in cellulase production. The highest energy consumption was detected in the lyophilization step; consequently, two scenarios that did not include lyophilization (in both SmF and SSF) were conducted and compared to the baseline scenario that includes lyophilization.

As shown in Figure 4 (again with absolute values), when focusing on the SmF process it can be observed that the removal of lyophilization results in a significant decrease of all environmental impact categories. The average reduction was 86%.

In the case of SSF, the reductions were even more important when removing lyophilization with an average of 93% in all categories. The stage of lyophilization can be considered necessary or not depending on the use of the enzyme obtained. If the enzyme is used as a catalyst to obtain glucose for ethanol production, the separation-extraction of cellulase from the SSF process may not be necessary, since those materials are all used during ethanol fermentation [28]. However, in other applications, such as those regarding fine chemicals production, lyophilization is necessary. In any case, the need for lyophilization should be considered for each application.

Table 6 shows the results by applying an alternative to the composting system used in SSF process for the exhaust fermented solid. Therefore, a simpler composting step is proposed (turned windrows) as a composting step instead of an enclosed reactor that requires more electricity. In this simpler case, electricity consumption is very low, although diesel is necessary for turning devices. However, diesel related to the windrow turner operation is low [60].

In Table 6, it is shown that this decrease is not so important as that of the lyophilization phase, since it resulted only in a 0.5% decrease in the environmental impacts of all categories.

Table 6. Sensitivity analysis: comparison results to produce cellulase through SSF and composting using in vessel and turned windrow composting systems. Basis: 1 kg cellulase in the product stream. Characterization results by ReCiPe 2016 midpoint method.

Impact Category	Units	Cellulase Production by SSF: Base Scenario	Cellulase Production by SSF: Sub-Alternative
GWP	kg CO _{2eq}	425,722	423,714
ODP	kg CFC ⁻¹¹ _{eq}	0.19	0.19
IRP	kBq Co-60 _{eq}	17,953	17,868
HOFP	kg NO _{xeq}	1220	1215
FPMF	kg PM2.5 _{eq}	1029	1024
EOFP	kg NO _{xeq}	1224	1219
TAP	kg SO ₂	2620	2607
FEP	kg P _{eq}	16.0	15.9
MEP	kg N _{eq}	1.4	1.4
TETP	kg 1,4-DCB	244,249	243,096
FETP	kg 1,4-DCB	63.9	63.6
METP	kg 1,4-DCB	228	227
HTPc	kg 1,4-DCB	993	988
HTPnc	kg 1,4-DCB	9171	9128
LOP	m ² year	10,212	10,164
SOP	kg Cu _{eq}	215	214
FFP	kg oil _{eq}	100,436	99,963
WCP	m ³	4590	4571

3.4. Preliminary Economic Assessment

In this section, we provide an initial rough exploration on the economic impact of cellulase production process. To do that, we have taken into account the cost of production, the chemicals used in the production, and the resources demand, which is due to consumption of electricity for both technologies according to industrial prices in Spain. For product prices, we used the personal communications of plant manufacturers. In the case of cellulase from SmF, these prices were an average of different producers (Sigma Aldrich, Amano Enzyme, and Novo Nordisk); providers are able to provide several kg of cellulase powders, although manufacturers are multi-product, so the results must be analyzed carefully as the inherent error could be important. In the case of compost, we selected a well-established plant in Catalonia (Jorba, Barcelona, Spain) that has had stable prices for years; it sells different types of compost of quality coming from the source-selected organic fraction of municipal solid wastes.

In order to be able to do a preliminary economic estimation, we assumed that the SSF technology did not include the cost of the raw material, as it is a waste. In this case, the electricity consumption was relatively less the SmF. Then, with these estimations, the cost to produce 1 kg of cellulase enzyme through SSF turned out to be approximately 21% €/FU more economic (1 kg through SSF around 1100 €/FU and 1 kg through SmF around 1300 €/FU, using sale prices). These prices do not consider the benefits of the producers. Although the economic difference may not seem very high and these numbers are only an estimation, the costs are in the same order of magnitude. Moreover, one of the main objectives of the use of CH is to produce cellulase from organic waste produced on a large scale in the world, with lower environmental impact and providing a final organic amendment for agriculture.

4. Conclusions

The main conclusion of this work, when comparing the scenarios proposed, is that the SmF technology has higher environmental impacts in all impact categories when compared to the SSF technology. On average, all impact categories resulted in 13% lower impact in the SSF process than the SmF process. It was also observed that the highest environmental impacts were due to electricity consumption. Thus, there is a need to develop strategies to decrease it.

According to the sensitivity analysis, it was confirmed that the lyophilization step generated the highest impacts in both technologies. Being so high, it is important that any LCA study on enzyme production (by SmF and SSF) includes the complete downstream process. However, it is evident that further research should be necessary to consider more parameters in the sensitivity analysis, or even carrying out a complete uncertainty analysis.

Finally, when SSF processes have more relevance and experimental realistic data are available, it will be necessary to assess this technology using endpoint indicators to have data on its overall impacts.

In fact, one of the main problems when trying to perform LCA studies in biotechnology processes is the lack of realistic data to have accurate results. In this sense, it is clear that the priority is to develop new processes and obtain new products but, at this moment, environmental impacts are not one of the main issues.

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