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Modelling and Environmental Profile Associated with the Valorization of Wheat Straw as Carbon Source in the Biotechnological Production of Manganese Peroxidase

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Abstract: Interest in the development of biorefineries and biotechnological processes based on renewable resources has multiplied in recent years. This driving force is the result of the availability of lignocellulosic biomass and the range of applications that arise from its use and valorization. The approach of second-generation sugars from lignocellulosic biomass opens up the possibility of producing biotechnological products such as enzymes as a feasible alternative in the framework of biorefineries. It is in this context that this manuscript is framed, focusing on the modelling of a large-scale fermentative biotechnological process to produce the enzyme manganese peroxidase (MnP) by the fungus *Irpex lacteus* using wheat straw as a carbon source. The production scheme is based on the sequence of four stages: pretreatment of wheat straw, seed fermenters, enzyme production and downstream processes. For its environmental assessment, the Life Cycle Assessment methodology, which allows the identification and quantification of environmental impacts associated with the process, was utilized. As the main finding, the stages of the process with the highest environmental burdens are those of pretreatment and fermentation, mainly due to energy requirements. With the aim of proposing improvement scenarios, sensitivity analyses were developed around the identified hotspots. An improvement in the efficiency of steam consumption leads to a reduction of environmental damage of up to 30%.

Keywords: lignocellulosic biorefinery; manganese peroxidase; biotechnological route; life cycle assessment; environmental loads; sensitivity analysis

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1. Introduction

In the search for more sustainable production patterns, based on the use of renewable resources to avoid the depletion of fossil resources, lignocellulosic biomass plays an important role [1–3]. Its potential is mainly based on three main characteristics: its availability, its composition and its affordable cost [4–6]. Wheat straw is characterized of being composed by a high content of both cellulose and hemicellulose, converting wheat as a valuable resource of fermentable sugars [7]. It is also considered an effective C-source to produce fungal ligninolytic enzymes. This affirmation relies on the fact that some authors have assessed that in fermentations with monosaccharides, such as glucose, many of the enzymes that can be expressed by the fungus are not formed. However, if the fungus is grown on a medium based on wheat straw, the cellulose and hemicellulose naturally present in these substrate acts as an inducer to produce the full range of fungal ligninolytic enzymes [8].

However, when considering the development of biorefinery platforms, the main barrier for the use of lignocellulosic biomass lies in its complex structure and the recalcitrance of lignin, hindering the access to cellulose and hemicellulose [9–11], which implies the need for pretreatment steps to allow their conversion into biochemicals and biofuels. In this sense, this pretreatment could be considered a key step in the pathway of biomass transformation into valuable products, as the efficiency of biomass disintegration directly affects the yield of downstream stages [12,13]. Many of the pretreatment processes that are in a research and development stage must demonstrate both technological and economic feasibility as they are often energy-intensive [14–16]. At this point, given the problems of using these more conventional technologies (physical processes (such us extrusion and ultrasonication), chemical process (i.e., use of ionic liquids or deep eutectic solvents) and physico-chemical (including steam explosion and CO_2 explosion)), the option of using an enzymatic process has been investigated [17–20]. The mild reactions catalyzed by oxidative enzymes, together with their high biodegradability, are the drivers for their applications, not only for the pretreatment of lignocellulosic biomass but also for a wide range of sectors: pulp and paper, biodiesel, textiles, food and beverages, pharmaceuticals and cosmetics, among others [21–24].

In the case of ligninolytic enzymes, the manganese peroxidase (MnP) oxidoreductase shows a high oxidation potential, catalyzing the reaction from Mn^{2+} to Mn^{3+} [25], that enables its use in the degradation of lignin and xenobiotics [26–28], the decomposition of aromatic compounds [29,30] and the transformation of pollutants and dyes [31–35]. Although there is a wide range of ligninolytic fungal species, *Irpex lacteus* is considered a platform for the overexpression of oxidative enzymes: laccase and MnP from lignocellulosic waste streams, thus emerging as an alternative with potential for large-scale development [35,36]. However, research efforts should also focus on achieving high levels of enzyme activity, reducing production costs as well as the impacts associated with their production processes [37–39].

Accordingly, this research work aims to evaluate the environmental profile of a large-scale biotechnological process to produce the enzyme manganese peroxidase (MnP) by the fungus *I. lacteus* using wheat-straw waste as a carbon source. For this purpose, it is necessary to apply process modelling tools to determine the technical feasibility of the conceptual design and thus compile the necessary data to carry out the environmental assessment. The Life Cycle Assessment (LCA) methodology is considered a versatile methodology to quantify the damage potential and environmental burdens of the process and/or product(s) and to identify the main hotspots or those process steps that contribute most on the environmental profile, on which future research should be focused to increase process efficiency and enable a more sustainable process route [39–42]. Thus, the results of this assessment provide valuable information on the technological and environmental feasibility of MnP production on an industrial scale. A first stage of process modelling is proposed to address a four-step production process, with the identification and design of the main equipment, together with the microbial fermentation conditions and the yields of biomass growth and enzyme production. Since wheat straw is considered the source of C for the fermentation process, a biorefinery approach was carried out, and considering its advantages of low cost, availability and composition, it could be considered a suitable and potential route in the production of MnP.

2. Materials and Methods

This research work combines the application of two complementary methodologies to carry out the process modelling and environmental assessment of the biotechnological production of the enzyme MnP from lignocellulosic waste from wheat straw. The SuperPro Designer tool was used to carry out the large-scale modelling of the process as a mandatory stage to develop the environmental analysis, following the Life Cycle Assessment (LCA) methodology.

2.1. Process Description

The production of the MnP enzyme is carried out through four main process steps (Figure 1). The process starts with the pretreatment of wheat straw to release the fermentable sugars required in the formulation of the culture medium. Subsequently, the fermentation step takes place, obtaining a broth that must undergo different stages to remove the biomass produced and partially purify the crude enzyme with a MnP titer of 345 U/L that is equivalent to 0.31 kg enzyme/batch.

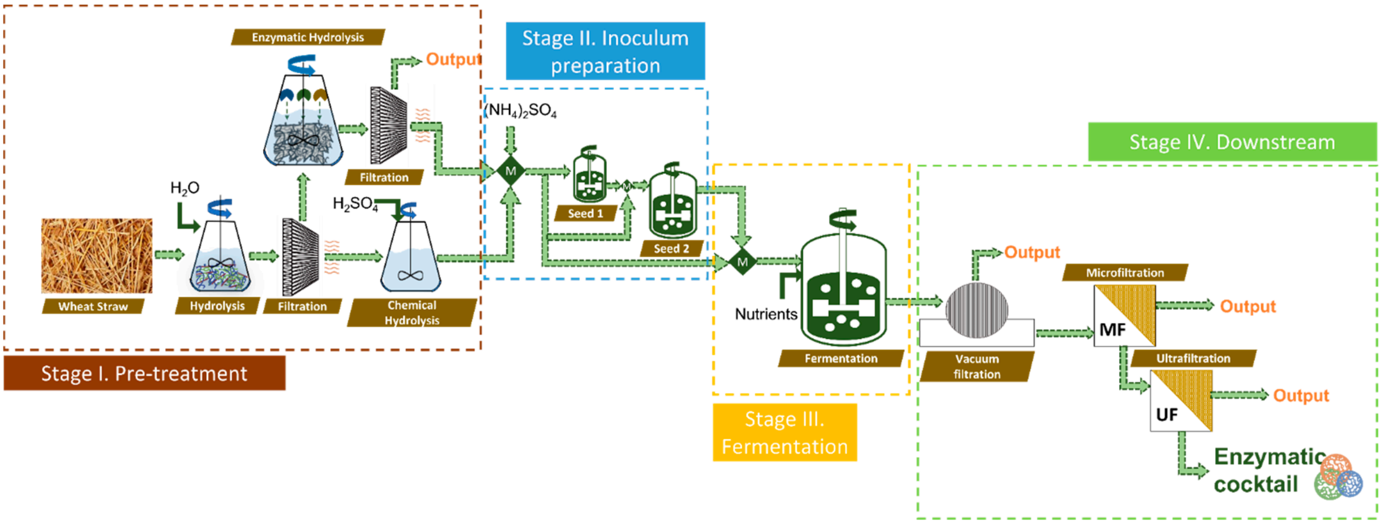

Figure 1. Process stages required for the biotechnological production of MnP enzyme by the valorization of wheat straw.

2.1.1. Pretreatment of Wheat Straw (I)

The use of lignocellulosic biomass for MnP production requires the removal of barriers that hinder access to cellulose and hemicellulose as a source of fermentable sugars, while reducing the recalcitrance of crystalline structures, in order to obtain higher reaction rates and yields. In addition, it is desirable that carbohydrates are not degraded or other products are formed that may inhibit the action of enzymes or the microorganism being grown. Fractionation of lignocellulosic materials and the use of each component separately is the current philosophy of biorefineries [43].

Among the different options of renewable raw materials that can be used as a resource for the biotechnological production of MnP, the use of wheat straw was considered for two main reasons: availability and molecular composition [44–46]. The production of wheat to meet consumer demands leads to the generation of large amounts of lignocellulosic residues, mainly wheat straw. The molecular composition of wheat straw (Table 1), given its high percentage of cellulose, hemicellulose and lignin, makes it an ideal resource for obtaining fermentable sugars, such as glucose and xylose [47,48]. For this purpose, an initial autohydrolysis was carried out at high temperature, as it favors cellulose accessibility and better digestibility of the lignocellulosic fraction in the subsequent enzymatic attack [49,50]. After autohydrolysis, a solid fraction rich in cellulose and a liquid fraction rich in hemicellulose were obtained.

Table 1. Composition in dry weight percentage of wheat straw.

Composition (% Dry Weight)			
Cellulose	30.6 ± 0.1	**Extractives**	9.8 ± 0.3
Hemicellulose	32.3 ± 0.2	Saccharides	5.3 ± 0.7
Xylan	23.5 ± 0.2	Glucose	1.2 ± 0.0
Arabinan	4.6 ± 0.3	**Protein**	5.4 ± 0.0
Lignin	16.8 ± 0.2	**Others**	0.1 ± 0.0
Ash	5.0 ± 0.0		

2.1.2. Inoculum Preparation (II)

Prior to enzyme production in the main fermenter, seed fermenters are required to ensure a sufficient amount of biomass to be used as inoculum in the main fermenter (10% *w*/*v*). The seed section comprises two fermenters, with a volume of 0.12 and 0.60 m^3, respectively, to which a glucose-rich medium is supplied to favor biomass growth under aerated conditions: 0.05 vvm and 30 °C.

2.1.3. Fermentation Section (III)

Prior to the fermentation process, the sterilization of the fermenter is required. The enzyme production is carried out in a reactor with a capacity of 1 m^3, using the fermentation medium prepared in the previous stage of the process and supplemented with a nitrogen source (ammonium sulfate) in order to ensure a correct carbon–nitrogen ratio to favor microbial growth. The fermentation conditions comprise aerobic conditions with an air supply rate of 0.33 vvm, 150 rpm, 30 °C and during a period of 3.3 days. It is worth mentioning that in the laboratory scale experiments, the pH value was kept at almost constant values throughout the fermentation; the need for pH control was not considered either. After the fermentation stage, the biomass is separated by means of vacuum filtration. The separated biomass can be used treated in an anaerobic digestor to produce biogas.

2.1.4. Downstream Section (IV)

Downstream process aims to concentrate and purify the enzyme available in the raw extract. In this section, two additional filtration steps are included to separate and concentrate the crude enzyme. It starts with microfiltration, which uses membranes with average pore diameters from 0.05 to 5 µm. In this case, a 0.2 µm membrane is used to achieve adequate filtration. This filtrate passes through another filtration step, using a 10 kDa cut-off ultrafiltration membrane. In this last step, the enzyme is retained and concentrated on the membrane, while the rest of the materials are released from the process in the filtrate.

2.2. Life Cycle Assessment Methodology

Seeking to evaluate the biotechnological route of MnP production under an environmental perspective, the Life Cycle Assessment (LCA) methodology has been recognized as a suitable tool to assess the environmental burdens associated with a production scheme [42,51–53]. The fundamentals of this methodology are based on ISO 14040:2006, which include four steps: the goal and scope of the assessment, the compilation of life cycle inventories according to the simulated process data, the impact assessment, which encompasses the selection of the appropriate calculation methodology, and the interpretation of the impact results [54,55].

2.2.1. Goal and Scope Definition

The objective of this report is to analyze the sustainability of the MnP biotechnological route using wheat straw as a carbon source. The scope of this assessment has been selected within a cradle-to-gate approach, covering all stages from the extraction activities of the process inputs to the production of the target product: the crude MnP enzyme. On the other hand, regarding maintenance and transport activities, they are left outside the scope of the assessment.

2.2.2. Life Cycle Inventories

The process is developed in four main stages, on which the inventory data are collected to assess the environmental burdens of each stage. In this way, it is possible to detect which of the stages has a greater environmental contribution. To do this, process modelling was carried out based on the data available in the literature. For this purpose, the simulation tool SuperPro Designer was used for the conceptual design. The functional unit (FU) selected for the environmental assessment was the MnP enzyme production per batch:

0.31 kg/batch. In addition, EcoInvent was selected as the database for the inventories, as it includes all information on the main inputs required for MnP production, including materials, output streams and energy requirements [56]. The global life-cycle inventory of the process considering as functional unit the production of 0.31 kg of MnP/batch is shown in Table 2.

Table 2. Global process inventory for the biotechnological production of MnP.

Global Process Inventory					
Inputs from Technosphere			**Outputs to Technosphere**		
Material	*Amount*	*Unit*	*Material*	*Amount*	*Unit*
Air	865.76	kg/batch	MnP	0.31	kg/batch
Ammonium sulfate	2.38	kg/batch			
Sulfuric acid	0.15	kg/batch			
Hydrolase	61.70	kg/batch			
Sodium azide	0.26	kg/batch	*Emissions to air*		
Sodium phthalate	15.95	kg/batch	Carbon dioxide	36.86	kg/batch
Water	1.55	m^3/batch			
Wheat straw	500	kg/batch			
Energy			*Outputs*		
Steam	1992	kg/batch	Biomass	499.29	kg/batch
Electricity	354.66	kWh/batch	Wastewater	0.55	m^3/batch
			Water, vapor	510.73	kg/batch

2.2.3. Impact Evaluation

The methodology selected to perform the environmental assessment was the ReCiPe 2016 hierarchist MidPoint method V1.03 World (2010), which includes several midpoint impact indicators, among which the following were considered: global warming (GW), stratospheric ozone depletion (SOD), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), terrestrial ecotoxicity (TET), freshwater ecotoxicity (FET), marine ecotoxicity (MET) and fossil resource scarcity (FRS).

2.2.4. Interpretation of Results

The impact values of each stage of the production process were evaluated, as well as an assessment of the biotechnological process as a whole. The environmental loads represented in the figures are expressed in terms of characterization percentages. On the other hand, sensitivity analyses were conducted on the basis of the results obtained. The usual procedure for a sensitivity analysis of the environmental impacts of the baseline scenario involves calculating the variation in these impacts according to the assumed changes in the variables contributing to the largest impacts, while keeping the other parameters constant. Accordingly, LCA becomes a circular methodology in which, once the hotspots have been identified, the values of these elements can be modified as a strategy to design the improvement process.

3. Results and Discussion

3.1. Design and Simulation Results

The process diagram simulated with the SuperPro Designer is divided into the four production stages previously considered: pretreatment of the wheat straw, which includes three reactors, the first one for autohydrolysis (R-101), followed by the enzymatic reactor (R-102) and the chemical post-hydrolysis with sulfuric acid (R-103). The inoculum preparation equipment is based on the use of two seed fermenters (SFR-101/102), the output streams of which are the input streams to the main fermenter (FR-101). Prior to the fermentation, heat sterilization of the culture medium and equipment (ST-101) is required. After the fermentation process, a rotary vacuum filtration (RVF-101) unit is required for the removal of the biomass. The downstream stage requires the combination of two filtration membranes: microfiltration (MF-101) and ultrafiltration (UF-101), from which the crude enzyme is obtained. The main unit operation blocks of the process are shown in Table 3, including its sizes or capacities and a brief description.

Table 3. Main equipment used for MnP production scheme in the SuperPro Designer simulation tool.

Label	Equipment	Size (Capacity)	Description
Pretreatment of wheat straw (Stage I)			
HX-101	Heat exchanger	2.62 m^2	Pre-heat the inlet stream to the autohydrolysis reactor
R-101	Reactor	1.58 m^3	Autohydrolysis of wheat straw, 210 °C and 10 bar.
V-101	Flash Drum	0.67 m^3	Release of high temperature water vapor
HX-102	Heat exchanger	7.45 m^2	Reduce the temperature of the outlet stream from R-101
BF-101	Belt Filtration	1.15 m	Separation of solid and liquid fraction
R-102	Reactor	0.67 m^3	Enzymatic hydrolysis of the solid fraction using hydrolases
R-103	Reactor	0.39 m^3	Chemical post-hydrolysis with sulfuric acid of the liquid fraction
BF-102	Belt Filtration	0.39 m	Biomass separation
Inoculum preparation (Stage II)			
SFR-102	Seed Fermenter	0.12 m^3	Inoculum preparation, 30 °C, 0.5 vvm
SFR-101	Seed Fermenter	0.62 m^3	Inoculum preparation, 30 °C, 0.5 vvm
Fermentation (Stage III)			
ST-101	Heat Sterilization	1.16 m^3/h	Sterilization of the inoculum, 110 °C, 45 min
G-101	Air Compressor	1.59 kW	Air compression
AF-101	Air Filtration	3 m^3/h	Filtration of the air inlet stream to the fermenter
FR-101	Fermenter	1.18 m^3	Enzyme production at 30 °C for 3.3 days.
RVF-101	Rotatory Vacuum Filtration	3.82 m^2	Separation of the biomass
Downstream (Stage IV)			
MF-101	Microfiltration	4.32 m^2	Separation of higher molecules, retained in the filter
UF-101	Ultrafiltration	2.77 m^2	Separation of the enzymatic cocktail, retained in the filter

3.2. Global Environmental Assessment

The LCA methodology was applied to the MnP biotechnology process as a whole. In this way, it is possible to assess which components of the life-cycle inventory are the most significant contributors to the environmental impacts. Thus, Figure 2 shows the environmental profile of the production process, where energy requirements, both electrical and calorific, make the greatest contribution to most of the impact categories studied, with the exception of SOD, TA and ME. As for energy requirements, their non-renewable nature is the main reason for their high impact, and they entail the emission of pollutants that have a negative impact on the different environmental ecosystems.

For the SOD category, the resulting impact is divided into two main contributors, the management of biowaste, i.e., the biomass generated after the fermentation process, and the residual feedstock itself, wheat straw. The background activities required to obtain this wheat straw include its cultivation, collection, and processing. Although it is a waste stream, it cannot be given a zero impact, as its production requires several activities that contribute to some extent to the environmental impact. This is also the reason why the ME category also shows a high impact from the use of wheat straw.

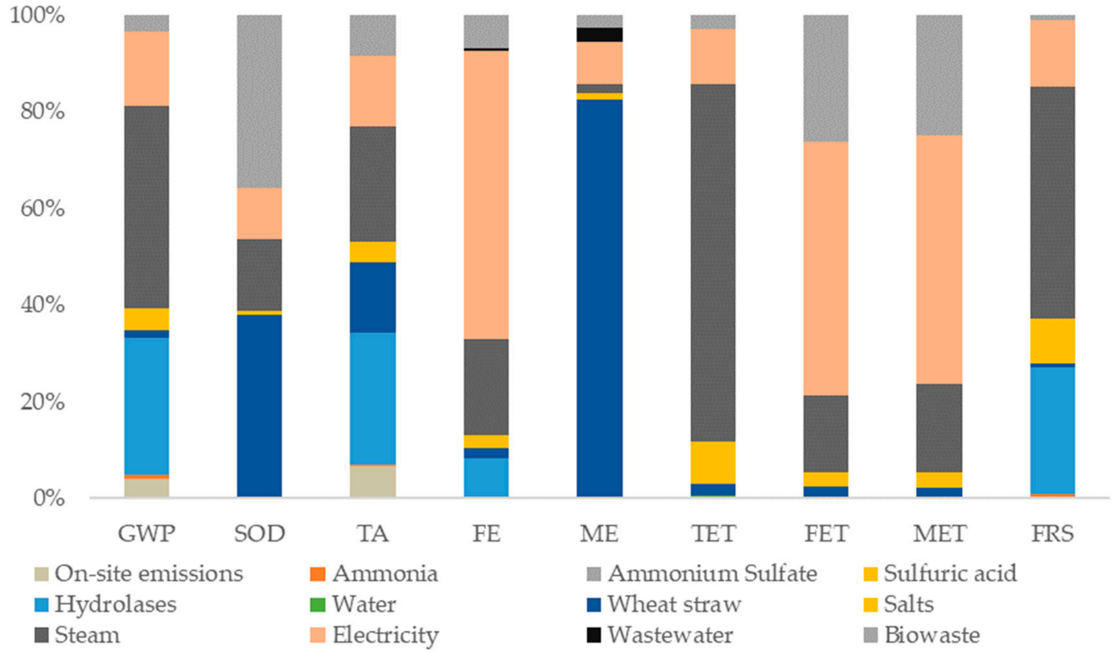

Figure 2. Environmental loads and impact contributions of the inventory data of the global biotechnological production of MnP.

In the case of the TA category, the environmental burdens of the inventory are balanced, but a slightly higher environmental contribution can be observed for the use of hydrolase enzymes. These enzymes are necessary for the enzymatic hydrolysis that takes place in the pretreatment stage of wheat straw, where the release of fermentable sugars occurs.

In order to assess which of the stages contributes most to the environmental burdens of the overall process, Figure 3 and Table 4 are shown. It could be observed that both Stages I and III have the highest environmental contribution, with Stage I being the most noticeable in all impact categories, with the exception of FE, FET and MET. In order to identify the reasons behind this contribution, the environmental profiles of Stages I and III will be analyzed separately. Furthermore, it is also at these stages that sensitivity analysis will be carried out as critical points are identified, with the aim of reducing environmental burdens.

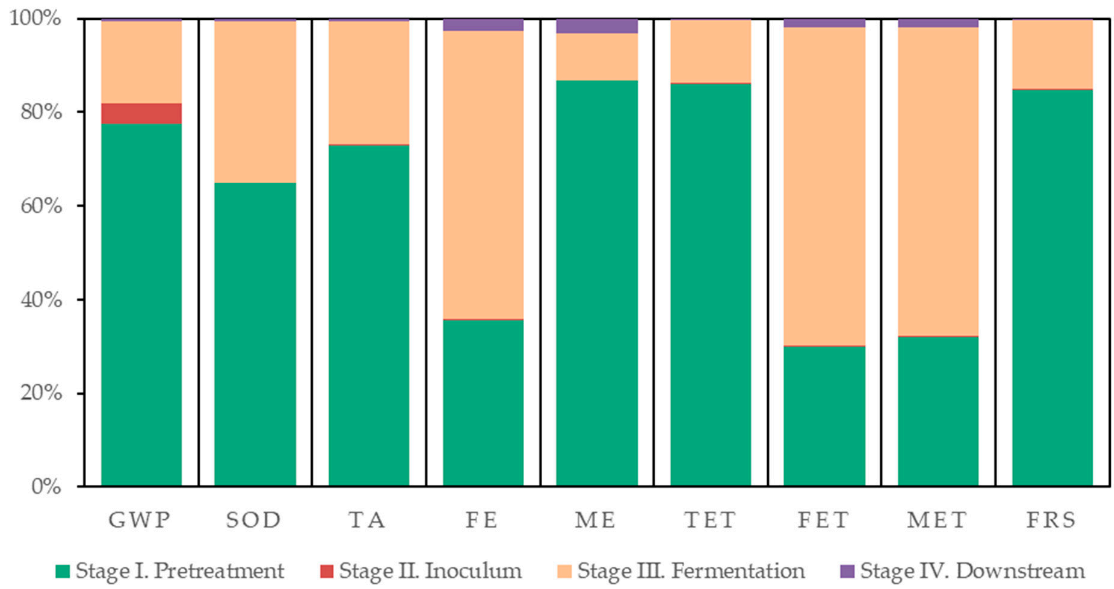

Figure 3. Midpoint impact results per process stage.

Table 4. Midpoint values per impact category and process stage.

Impact Category	Unit	Total	Stage I. Pretreatment	Stage II. Inoculum	Stage III. Fermentation	Stage IV. Downstream
GWP	kg CO_2 eq	884.27	688.89	37.91	152.59	4.88
SOD	kg CFC [1] $_{11}$ eq	6.1×10^{-4}	3.99×10^{-4}	4.98×10^{-7}	2.132×10^{-4}	3.03×10^{-6}
TA	kg SO_2 eq	3.46	2.51	4.09×10^{-3}	0.93	1.89×10^{-2}
FE	kg P eq	0.23	8.25×10^{-2}	1.04×10^{-3}	0.14	5.96×10^{-3}
ME	kg N eq	0.12	0.10	1.20×10^{-4}	1.13×10^{-2}	3.58×10^{-3}
TET	kg 1.4-DCB [1]	706.65	604.42	0.74	98.57	2.92
FET	kg 1.4-DCB [1]	6.85	2.03	2.77×10^{-2}	4.66	0.13
MET	kg 1.4-DCB [1]	9.65	3.08	3.74×10^{-2}	6.35	0.19
FRS	kg oil eq	267.83	228.55	0.29	37.69	1.30

[1] CFC: Chloro Fluoro Carbonates, DCB: DiChloro Benzene.

3.3. *Environmental Assessment for Stage I Pretreatment*

It is well known that the use of lignocellulosic materials as input streams for the development of the fermentative biorefinery process entails the need for pretreatment steps for their fractionation into sugar monomers from cellulose and hemicellulose polysaccharides. In the case of wheat straw, the main stages are an autohydrolysis stage at high temperature followed by an enzymatic hydrolysis of the solid fraction and a chemical post-hydrolysis with sulphuric acid of the liquid fraction. Steam demand is one of the main hotspots of this processing step (Figure 4). Comparing the equipment and processes required at this stage, it is autohydrolysis that contributes most notably to this thermal energy demand, a total of 3019 MT/batch. This high value is the result of the required process temperature, which amounts to 210 °C. Chemical post-hydrolysis also has some impact on the environmental loads, as it also requires a demand of 567 MT/batch.

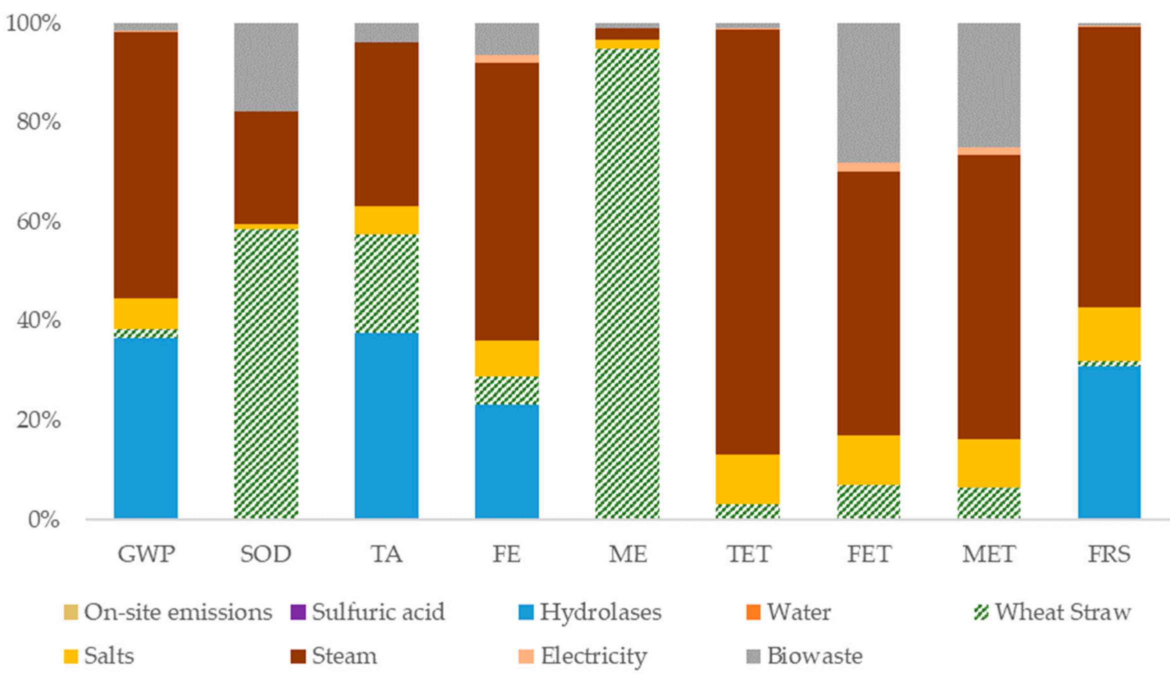

Figure 4. Environmental loads and impact contributions of the inventory data of Stage I. Pretreatment.

Wheat straw is another hotspot identified on the environmental profile of the pretreatment stage, specifically in the SOD and ME impact categories. Even if it is thought to be a renewable resource, with a significant reduced impact compared to fossil resources, its use involves background activities associated with crop production and harvesting.

On-site emissions are responsible for environmental loads; specifically, atmospheric emissions of carbon dioxide, ammonium sulfate and nitrogen oxides, emitted as a result of cultivation and harvesting activities such as fertilization, are the ones affecting the SOD impact category. On the other hand, in the case of ME, the release of heavy metals and phosphorus compounds, mainly as a result of fertilization activities, are the ones with the huge contribution.

In order to propose a more sustainable scenario, sensitivity analyses will be conducted on the reduction of steam consumption. In the case of the raw material, wheat straw, improvement activities should focus on increasing the performance of the pretreatment process and improving agricultural practices regarding the use of fertilizers. The search for a more sustainable agriculture is one of the main objectives of the EU Agenda 2030 and of the Sustainable Development Goals, specifically Goal 2, which encourages the improvement of agricultural production and productivity through the implementation of resilient practices.

3.4. Environmental Assessment for Stage III Fermentation

The fermentation stage is the main contributor in the FE, FET and MET impact categories, as seen in Figure 3. The impacts associated with the fermenter operation is significant because it is the central stage of the enzyme production process and requires an operating time of 3.3 days, a constant air flow and the largest volume of culture medium, in particular, the use of ammonium sulfate as nitrogen source. On the other hand, there is also some impact from the biomass generated after the fermentation process, which is considered an output stream of the process (Figure 5). A viable alternative to reduce the impact of this waste stream would be its valorization on a anaerobic digestion and a cogeneration unit for the production of steam and electricity, which would be used in the process itself.

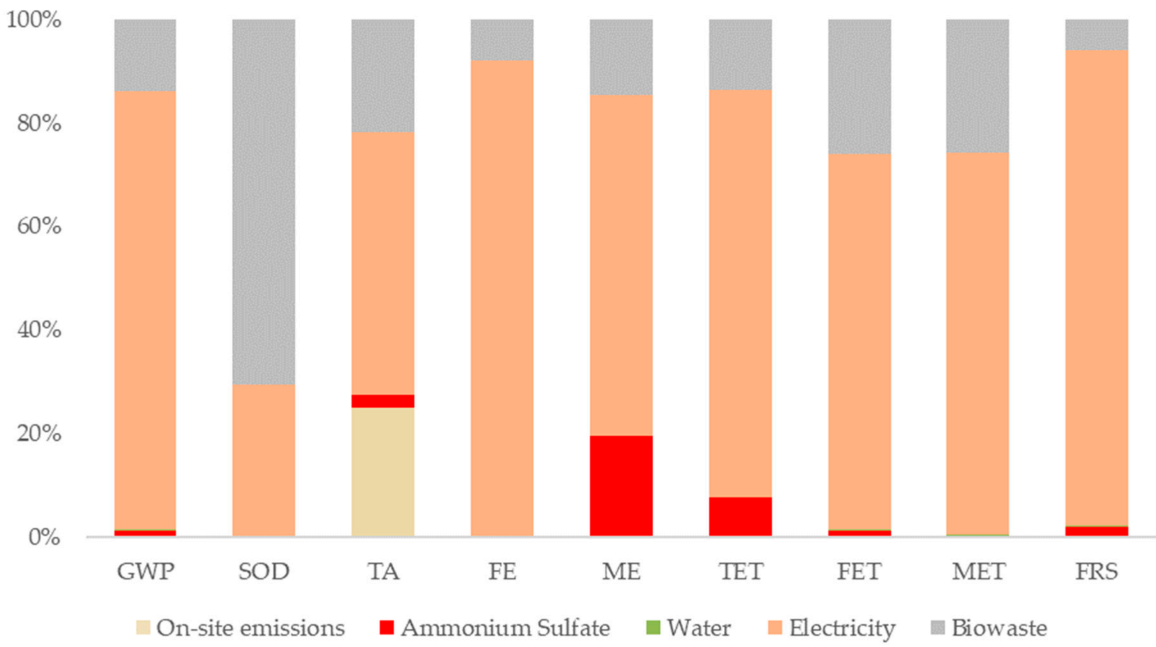

Figure 5. Environmental loads and impact contributions of the inventory data of Stage III. Fermentation.

3.5. Environmental Assessment for Stages II and IV

Although the inoculum preparation (Stage II) and downstream (Stage IV) stages do not have a significant impact on the environmental loads of the overall process, their individual profiles have also been discussed (Figure 6). Again, energy requirements are the main hotspots in most of the impact categories under assessment. However, in the case of Stage II, the contribution of ammonium sulphate in the ME and on-site emissions in the GWP impact categories is also significant. The emission of CO_2 in the seed fermenters for inoculum preparation is the reason for the impact contribution in the GWP category, while the background production activities associated with ammonium sulphate are the contributors in the ME category. For Stage IV, based on two filtration steps, a microfiltration followed by an ultrafiltration, the impact of wastewater on the ME category is also highlighted. Although this waste stream is not considered toxic, as it lacks compounds that could have a significant impact on the environment, its organic load can lead to eutrophication if directly released into the environment. However, as can be seen in Figure 3, its impact on the overall process is not significant, which is indicative of the low load and potential impact of this waste production stream.

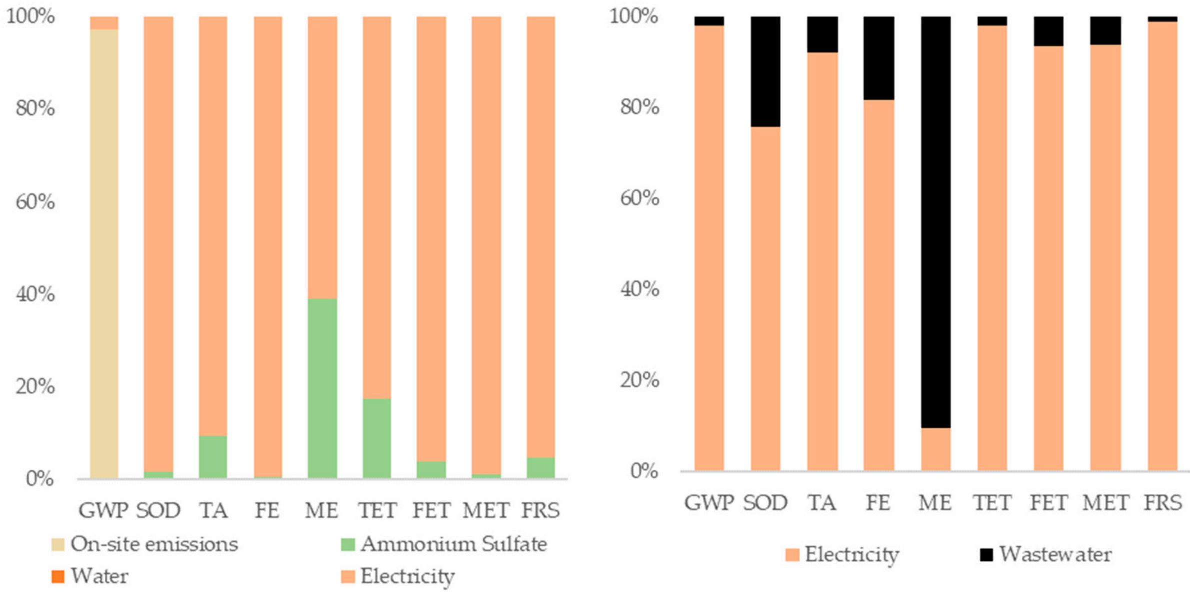

Figure 6. Environmental loads and impact contributions of the inventory data of Stage II. Inoculum preparation (**left figure**) and Stage IV. Downstream (**right figure**).

3.6. Sensitivity Analysis

Energy and steam requirements are the main critical points identified in the environmental profile of MnP production. Energy requirements could be reduced by improving process efficiency and reducing the batch-cycle time. Some authors have considered reductions of 2%, 5% and 10% in energy consumption to assess how this decrease improves environmental profiles by reducing environmental impact values [57]. Figure 7 shows the improvements in the environmental profile that could be achieved by considering energy demand reductions of 2%, 20% and 40% [58].

The energy requirements present higher sensitivities in the FE, FET and MET categories, but improvements on environmental loads are observed in all impact categories under assessment. It is in the case of FE that the largest decrease in environmental loads is achieved, amounting to a reduction of about 23% when considering a 40% reduction in electricity requirements. Similar values were obtained for the FET and MET categories, with a 20% reduction for both. In contrast, the ME and SOD categories have the lowest improvements, only achieving 3.3% and 4.1%, respectively. These values were expected, as it is in these categories where the use of waste straw as an input stream is the most notable contributor.

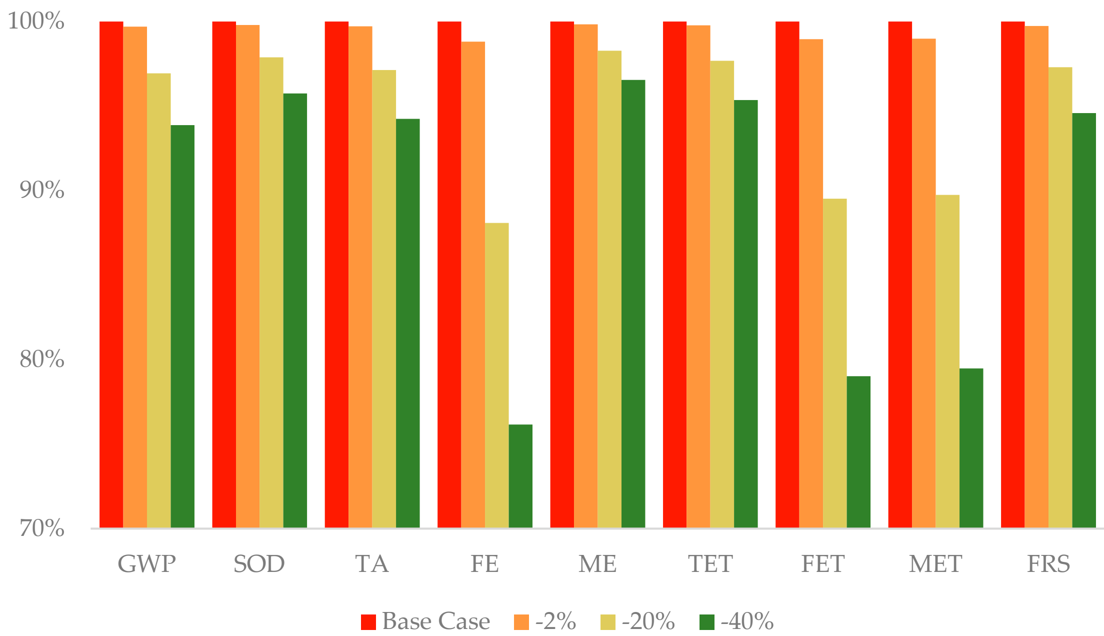

Figure 7. Values for sensitivity analysis for the effect of electricity requirements on the impact categories under study, considering reductions of 2%, 20% and 40% on electricity needs.

Two alternatives can be considered to reduce the contribution of steam to the environmental profile. As it is a full-scale process based on laboratory data, its potential for improvement in efficiency and performance is high, so significant reductions in consumption can be achieved by optimizing its use. Thus, reductions of 2%, 20% and 40% have been evaluated (Figure 8). The optimization of steam use contributes most significantly to the improvement of the environmental profile, reaching lower impact values for more impact categories, with the exception of ME, FET and MET. In the case of the ME category, the reason is identical to the one explained above, as it is in this category that the wheat straw input leads to the highest environmental load. On the other hand, for the FET and MET categories, the most significant improvement was observed when the sensitivity analysis of the electricity requirements was studied.

The highest improvements are mainly observed in three categories, with TET standing out, reaching improvement values amounting to 29.5%, when a 40% reduction in steam use is considered. The GWP and FRS categories also show a clear improvement, with impact reduction values of 17% and 19%, respectively. This direct relationship between the use of steam and its environmental load on the above-mentioned impact categories is the result of the consumption of fossil resources required for its production, which have significant environmental loads associated with them.

The second alternative would be based on the valorization of the biomass obtained after the fermentation process in a cogeneration system. In this way, after an aerobic fermentation process and energy generation equipment, this biomass would be used and a total of 61.44 MJ/batch, 36.76 kg steam/batch, would be generated, which would be used in the process itself, thus reducing the input calorific requirements by 3%. Therefore, no great improvement would be achieved by considering this alternative, as it would only reduce the steam input value by 3%; hence, its contribution to the improvement of the environmental profile is not very significant.

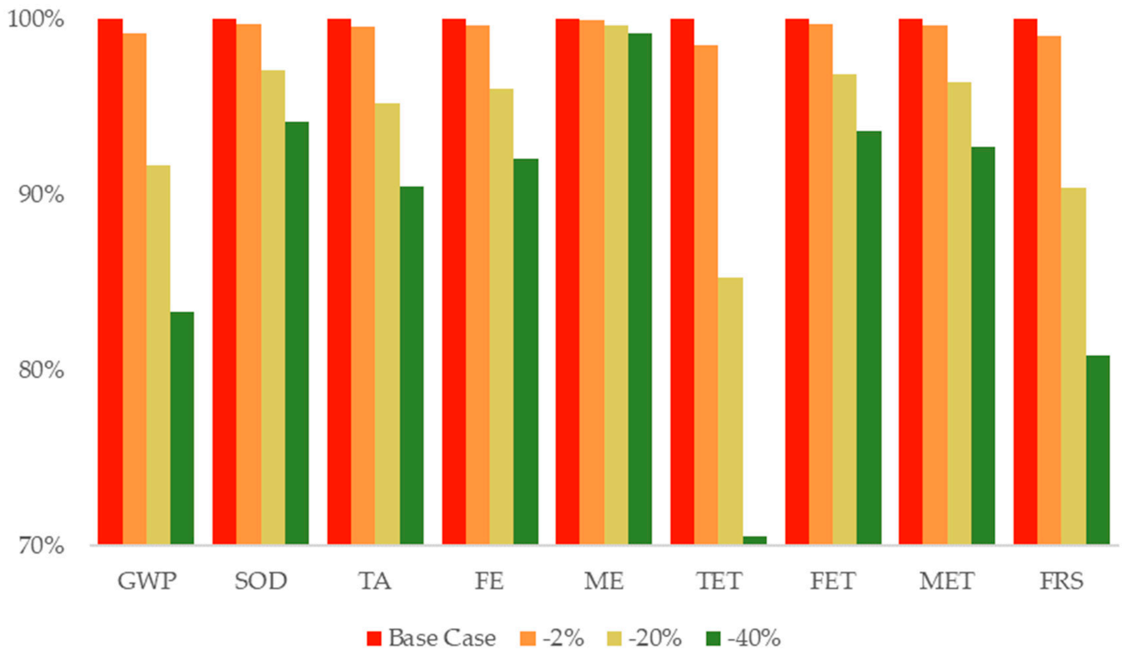

Figure 8. Values for sensitivity analysis for the effect of electricity requirements on the impact categories under study, considering reductions of 2%, 20% and 40% on steam needs.

The sensitivity analyses carried out show the potential for improving the biotechnological process to produce MnP by improving the efficiency in the use of electrical and calorific resources. However, these two alternatives were considered separately, to observe how their reduction affects the environmental profile obtained. However, it is also possible to evaluate a combination of both alternatives. The scenarios considered were the following: base case; an optimal scenario, in which energy requirements are reduced by 40%; and a conservative scenario, with an improvement in energy efficiency that achieves a 20% reduction in electricity and steam demand; and the best scenarios previously analyzed, 40% reduction in electricity and steam requirements, separately.

The values included in Figure 9 show that an optimization of steam use becomes an alternative with impact reduction values between the conservative scenario and the optimal scenario for most of the impact categories, with the exception of MET, FET and FE. Therefore, it could be concluded that more efforts should be focused on achieving thermal efficiency beyond electrical efficiency. On the other hand, as expected, a combination of 40% reduction in electricity and steam consumption, i.e., the scenario considered as optimal, results in the highest reduction of environmental loads, with reduction values up to 34% for the TET category. Furthermore, the sensitivity of the SOD and ME impact categories due to reduced steam and energy consumption is the lowest, reaching improvement values of only 10% and 4%, respectively, when considering the optimal scenario.

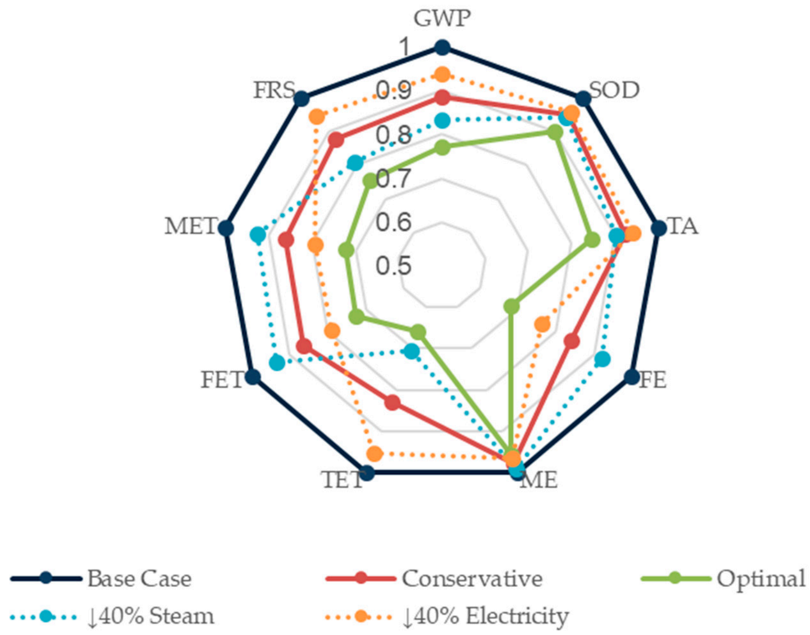

Figure 9. Results for the sensitivity analysis considering both electricity and steam requirements reductions, including the comparison with the best cases assessed previously.

4. Conclusions

This manuscript has addressed the environmental burdens of the biotechnological production of crude MnP enzyme using wheat straw as a source of fermentable sugars. An industrial scale-up simulation was developed and the environmental impacts were analyzed by applying the Life Cycle Assessment methodology. In this sense, promising results were obtained for the presented biotechnological route, considering both process yields, productivity, enzymatic activity and environmental sustainability. However, three main components of the process could be considered the main critical points: energy requirements, steam and wheat straw. Sensitivity analyses were carried out to achieve better environmental results in the case of electricity and steam requirements, concluding that reducing energy requirements could lead to significant environmental improvements, with steam having the greatest influence. Regarding the wheat straw, the contribution to the profile derives from cultivation and harvesting activities. Therefore, to reduce its environmental contribution, it would be necessary to develop more sustainable agricultural practices, which not only use less fossil resources, but also reduce the use of fertilizers, which are known to have a significant impact on the environment.

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