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Process modelling and environmental assessment on the valorization of lignocellulosic waste to antimicrobials

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ABSTRACT

The Sustainable Development Goals defined by the United Nations established a roadmap on the way to a sustainable society based on a circular economy in which the residual fractions of process streams emerge as raw materials for the provision of material goods and services. In this context, the approach of biorefineries as the technological scheme for processing integrally renewable resources stands out. Among the different target products, "bioactive" compounds have aroused great interest given their high added value. This work evaluates the environmental profile associated with the valorization of residual lignocellulosic waste streams: olive pruning and eucalyptus residues for the biotechnological production of nisin, an antimicrobial compound produced by *Lactococcus lactis* with application in the food preservation and biomedical sectors. Process modelling in combination with Life Cycle Analysis allowed addressing the conceptual design and the environmental assessment of a full-scale production process. As a main conclusion, the technological feasibility of the valorization of waste fractions as sources of fermentable sugars in the biotechnological process has been demonstrated. Moreover, the integral use of the raw materials can allow not only the production of nisin as an economic driver, but also of other biotechnological products such as lactic acid and energy for the self-supply of the process. The main novelty of this manuscript is based on rethinking the valorization of lignocellulosic waste beyond energy recovery to obtain bioproducts with high added value in the market with applicability in the food sector. Although energy recovery is the most widely implemented end-of-life option, the use of lignocellulosic waste as fermentable sugars has been shown to improve sustainability and circularity outcomes.

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1. Introduction

Consumers now have access to almost unlimited sources of information, which leads to a more conscious and demanding consumer profile, interested not only in the quality and characteristics of the product, but also in information on how the product was produced, its geographic origin and the

social and economic characteristics associated with the production process. In this context, the consumption of natural products with less industrial processing is an increasingly widespread demand. Beyond thermal processes and chemical additives, bacteriocins arise as powerful antimicrobial agents and offer the possibility of preserving foods and, in particular, their organoleptic quality. In particular, nisin stands out not only as a natural biopreservative (Cano-Serna et al., 2015; Özel et al., 2018) but also in the biomedical and pharmaceutical field. Nisin reduces the undesirable effects of certain antibiotics (Ellis, 2018) and is effective in the

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treatment of infections related to drug-resistant pathogens, gastrointestinal, respiratory, among others (Shin et al., 2016). The use of bacteriocins in nanomaterials has shown an interesting potential for anticancer treatment, reducing tumor cell growth (Naskar and Kim, 2021).

However, its use in the food industry is not widespread, which is mainly attributed to the economic cost associated with the formulation of commercial culture medium (Guerra et al., 2007; Özel et al., 2018). With the aim of addressing a more efficient nisin production, it is essential to tackle the coupling of biotechnology and circular economy approach through the concept of biorefineries.

Biorefineries are considered strategic sectors based on the use of renewable natural resources (Attard et al., 2020; Stegmann et al., 2020), among which lignocellulosic biomass stands out, given its wide spectrum of application and availability (Hassan et al., 2019; Lange et al., 2021). Biorefinery design strategies must take into account process stream compositions, equipment and operating conditions that ensure high efficiency and cost-effective production. Biorefineries are expected to contribute to the future development of sustainable, clean and renewable energy and products (Cherubini, 2010). To this end, best available techniques and powerful process modelling tools/software should be applied in the definition of biorefinery approaches. In terms of feedstocks for biorefineries, these could be classified into "dedicated energy crops", for those that are used entirely for energy production, and in the case of those focused on "biomass waste valorization", other high value-added co-products could be obtained (Chaturvedi et al., 2020; Kelloway & Daoutidis, 2014). Regarding the classification of biorefineries, a distinction is made between "energy-driven biorefineries" for the first category of feedstocks and "product-driven biorefineries" providing chemicals, organic acids, biopolymers, bioproducts and biofertilizers (Aristizábal-Marulanda and Cardona, 2019). Another important fact about biorefineries is that most process models are at an early stage of development, and the technology readiness level (TRL) is not high enough to guarantee market potential. Before launching a large-scale biorefinery production plan, process modelling and technical feasibility approaches need to be addressed (Xu et al., 2021; Okolie et al., 2022).

With this in mind, the microbial production of nisin from waste streams emerges as an interesting and profitable approach. In this sense, the valorization of lignocellulosic residues as sources of fermentable sugars such as olive pruning, and eucalyptus residues, would significantly reduce the costs associated with the process, in addition to providing an innovative model of circular biotechnological production, since residues from agricultural activity are being used to obtain a high value-added product such as nisin as well as lactic acid as a by-product of interest.

These materials were selected because gross quantities of these agricultural and forestry residues are produced in the Spanish country, and currently no valorization strategies are used to recover these waste streams. On the other hand, olive pruning includes both leaves and branches, together with other lignocellulosic residues obtained from the cultivation and harvesting activities of agricultural activities. The same pretext has been analyzed for eucalyptus residues. Other residues have not been considered, as the manuscript is based on residues obtained from agricultural and forestry activities, and not on waste streams obtained from the

transformation and industrial processing of these materials. On the other hand, the technologies and equipment selected for the biorefinery process have been based on bibliographical references and other research articles, going through the stages leading to higher productivity and efficiency. SuperPro modelling software has been used to evaluate the biotechnological scenario on a large scale, in order to assess its profitability in terms of environmental loads and to show the potential of the implementation of this biorefinery in the value chain of agricultural and forestry activities.

However, special care must be taken on how to integrate waste valorization into a biotechnological process. Not all waste valorization schemes are justified on the basis of technological, economic and environmental feasibility. Process schemes designed for the recovery of minor fractions usually entail high energy and chemical consumption. In order to demonstrate compliance with the sustainability of the proposed process, one of the most widespread methodologies recognized for its soundness is the Life Cycle Assessment, a tool that allows identifying the environmental impacts associated with a production process from a holistic point of view.

Therefore, in this study, the evaluation of the production scenarios has been developed, applying the Life Cycle Assessment methodology (ISO, 2006). In addition, sensitivity analyses have also been proposed, modifying those parameters that have been identified as the main contributors to the environmental profiles, thus proposing possible improvement options.

2. Methodology

2.1. Goal and scope

The main goal of this study is the environmental evaluation and comparative analysis of a biotechnological process that addresses the co-production of nisin and lactic acid from the recovery of two lignocellulosic residual streams: olive pruning and eucalyptus residues. Although the production process is analogous, three distinct processing sections are differentiated: (1) pretreatment for the release of the fermentable sugars contained in the lignocellulosic structure of the residual raw materials, (2) fermentation, in which the co-production process of nisin and lactic acid takes place, and (3) downstream, for the separation and purification of the compounds obtained. The composition of the sugars, as well as the yields of the different reactions that are encompassed throughout the production process, differ between the three raw materials considered.

Therefore, this study will develop the evaluation of the production scenarios, applying the Life Cycle Assessment methodology (ISO, 2006). In addition, sensitivity analyses will also be proposed, modifying those parameters that have been identified as the main contributors to the environmental profiles obtained, thus proposing potential improvement options. Besides, the large-scale modelling of the biotechnological process of olive and eucalyptus pruning waste valorization has been based on literature data.

2.2. Considerations for LCA methodology

The development of LCA studies involves carrying out a series of categorized and interrelated steps in a circular

perspective: definition of goal and scope, inventory analysis, impact assessment and interpretation of results. Regarding the inventory analysis, the functional unit and system boundaries must be selected, since the interpretation of the environmental profiles obtained will depend on both choices. The functional unit (FU) has been defined by the International Organization for Standardization as a quantified performance of a product system for use as a unit of reference, assuming that its value should be consistent with the objective of the assessment, must be fully defined and measurable (Bongono et al., 2020).

In this study, the production of 1 kg of products, including both nisin and lactic acid, has been considered as FU. The selection of this FU allows to conduct mass and economic allocations, in which it is possible to identify which co-product has the highest environmental and economic contribution based on the productive capacity of the process. The scope of the study has been considered within a *cradle-to-gate* approach. In this way, the LCA study is developed from the extraction of all the inputs required in the process to the moment when the product leaves the production process. Therefore, transportation activities, the use of the product by the consumer and the processes associated with its recycling, recovery or final disposal are outside the scope of the study.

It is important to mention that the available alternatives for the valorization of lignocellulosic waste resources for biotechnological processes are developed at laboratory scale (Cheng et al., 2018; Costas Malvido et al., 2017; Guerra and Castro, 2003; Khelissa et al., 2021; Vázquez et al., 2006). However, in order to evaluate the technical, environmental and economic feasibility of these new process alternatives, it is necessary to conduct process modelling, thus resembling real industrial processes and providing a more realistic view of the valorization proposals. For this purpose, the SuperPro Designer® simulation tool has been used. Section 2.3 of this study depicts the production processes developed, including a description of the necessary steps, as well as scale-up considerations. The Ecoinvent database has been selected for conducting the life-cycle inventories, as it includes basic information on the main inputs, both materials and energy requirements, and outputs, so that it provides valuable information for the quantification of the environmental assessment of the conceptual designs of the processes proposed in this study (Hesketh, 2017). The calculation methodology considered for the development of the LCA has been Recipe 2016, a methodology with a hierarchist perspective that includes a total of 21 impact indicators, divided into 18 midpoint indicators and 3 endpoint indicators (Supplementary Material includes the definition of the acronyms used for each of the impact categories used in this manuscript).

2.3. Process description

The nisin and lactic acid co-production process is developed through three process sections. The first section corresponds to the pretreatment of the lignocellulosic residues, necessary for the release of fermentable sugars, which will be used in the second section, the fermentation section, in which nisin and lactic acid, among others, are obtained as products. Finally, for purification and separation, a downstream section is completed, from which it is possible to obtain a purity nisin (above 95%), an antimicrobial that has proven useful in

the food and pharmaceutical sector, and lactic acid (at 75% w/w), which could be used for the manufacture of PLA bioplastic, a biomaterial that is in the transition phase between the research and industrial production stages.

2.3.1. Lignocellulosic residues

When considering a biotechnological process based on the valorization of agricultural and forestry residues, it is important to examine the seasonality of the crop and biomass harvest. For olive trees, pruning activities are usually carried out in autumn and winter, extending even into spring, as they must be done before flowering. Its most common application is field burning which leads to GHG emissions, as well as soil degradation (Abou Fayssal et al., 2020). The estimated annual production of pruning residue is set at around 1–5 tons/ha of crop (Suardi et al., 2020), especially in Mediterranean regions (Menéndez et al., 2018).

Finally, as for eucalyptus residues, their felling is spread throughout the year, so that forestry operations ensure a continuous flow of residues. Spain and Portugal are the European countries that stand out in the cultivation of eucalyptus, with approximately 1.4 million ha, which implies about 2.8 million tons/year of residues (Nunes et al., 2019).

This variability in harvesting operations will therefore make it possible to have agricultural residue flows throughout the annual period and, since the production process is analogous for the three residues, the adaptation of the process is simple enough to treat them in the same industrial production plant.

From the point of view of the composition of the different waste streams (Table 1), the different cellulose and hemicellulose content would make the yields in fermentable sugars different. OP have the lowest content of both hemicellulose and cellulose, resulting in higher amounts of chemicals and energy being required to obtain an equivalent amount of the sugars. But, at the same time, it has a lower amount of lignin on its composition, which facilitates the pre-treatment and molecular breakdowns. However, their year-round availability allows the development of a biotechnological process that does not depend on the seasonality of the residue, so that variations in product yield can be compensated.

Table 1 – Composition in dry weight % of OP and ER.

Source: Adapted from: Silva-Fernandes et al., 2015 Vieira et al., 2021 Alves et al., 2010 Mamaní et al., 2022 Barbanera et al., 2015

	OP	ER
Cellulose	24.5- ± 0.2	36.1 ± 0.6
Hemicellulose	18.8- ± 0.2	23.8 ± 0.3
Lignin	22.2- ± 0.2	26.7 ± 0.3
Ash	3.8 ± 0.1	2.4 ± 0.3
Protein	6.2 ± 0.1	2.8 ± 0.5
Extractives	18.6- ± 0.1	3.6 ± 0.2
Others	5.9 ± 0.0	4.6 ± 0.0

2.3.2. Pretreatment section

The pretreatment section begins with an autohydrolysis stage of the lignocellulosic biomass at 210°C, which requires high pressure steam as a heat transfer agent and continuous agitation (Silva-Fernandes et al., 2015). Conversion of cellulose around 22% and hemicellulose of 30.9% was considered. After this stage, in a flash evaporation stage, excess water is removed, which can be recirculated to the CSTR to reduce energy requirements and inputs to the autohydrolysis process. The flash output stream is separated into a solid fraction, characterized by its high cellulose content, and a liquid fraction, in which the content in hemicellulose stands out.

The solid fraction will undergo an enzymatic hydrolysis based on hydrolases at 50°C, which allows the conversion of cellulose into glucose with a yield of 93.7%, and of xylose from hemicellulose with a conversion of 38.5%. One of the bottlenecks of the process may be the reaction time required to complete the enzymatic hydrolysis process: 48 h. The liquid fraction will be processed with a chemical post-hydrolysis using sulfuric acid (72% by weight) at 121°C with a residence time of 1 h. Under these conditions, a 97% conversion is achieved for the hydrolysis of cellulose to glucose, and 22% in the hydrolysis of hemicellulose to xylose.

After both sugar conversion processes, a stream rich in fermentable sugars would be obtained, i.e., glucose and xylose, which will be used as carbon sources in the subsequent fermentation process. Of the total stream obtained, 10% is used for the seed fermenters to produce biomass for the main fermenter, assuming 97% glucose and 95% xylose conversion to yeast.

2.3.3. Fermentation section

The co-production of nisin and lactic acid corresponds to a microbial fermentation process based on the *Lactococcus lactis* strain (Guerra et al., 2007). The fermentation medium, in addition to the carbon source, in the form of glucose and xylose, requires the addition of nitrogen source as NH_4OH and phosphorus as K_2HPO_4 . The following operating conditions: 30 °C, aeration flow rate of 0.5 L/h, 200 rpm and 24 h residence time (Malvido et al., 2019), favor a higher growth of the microbial population and a higher productivity of the process. Once the fermentation is over, the outlet stream goes to the downstream section, for the separation and purification of the components.

2.3.4. Downstream section

This section of the process starts with a vacuum filter, used to separate the biomass, which can be further treated in an anaerobic digestion system (Bijarchiyan et al., 2020; Caposciutti et al., 2020). The valorization of the biogas could be used in the process itself, thus reducing the consumption of fossil resources used for the generation of the heat required in the plant.

Once the biomass has been removed, the procedure considered most effective for the recovery and purification of nisin is precipitation with ammonium sulfate: $(\text{NH}_4)_2\text{SO}_4$ (Tafreshi et al., 2020). This process starts with the acidification of the stream by HCl, and the subsequent precipitation of nisin in the presence of $(\text{NH}_4)_2\text{SO}_4$. This operation takes place at 4 °C and over a period of 24 h. To separate the precipitated nisin, the output stream from the precipitation equipment is fed into a centrifuge (12000-g, 30 min) and then dried to obtain the final product in solid form with a purity higher than 95%.

As for the recovery of the lactic acid obtained as a fermentation by-product, the process can be divided into three clearly differentiated stages. The first is based on the concentration of the stream after the centrifugation stage to reduce the water content. The second stage involves an esterification reaction of lactic acid with methanol at 80 °C to produce methyl lactate and water, with a conversion of 85% (Gil-Horán et al., 2008).

3. Results and discusión

3.1. Scenario 1. Olive Pruning valorization

The summary of the data used for the modelling of Scenario 1 on the valorization of olive pruning is included in Table 1 of the Supplementary material. It has been possible to identify three main hotspots that contribute to the environmental profile of this first stage of the production process (Fig. 1) highlighting the electricity needs, being the main contributor for most of the selected impact categories, with the exception of the GW, TET and FRS. On the other hand, some contribution derived from the use of enzymes, required in enzymatic hydrolysis, is observed on the GW, TA and FRS impact categories. In view of the results obtained in this first stage of the process, the sensitivity analysis should focus on energy optimization, in order to reduce the energy needs of the process. Regarding the second stage of the process, the distribution of the environmental loads shows that electricity requirements are the main hotspot and a higher contribution of chemicals it is also identified (Fig. 1). The main reason for the high impact caused by the energy requirements is attributed to the need of constant agitation, which would entail the use of agitation equipment with a sufficiently high power to favor satisfactory homogeneity of the medium.

As for the chemicals, the environmental load of nitrogen source in the profile is significantly higher. Therefore, in this second stage of the process, the optimization analysis should be based on reducing the amount of electricity needs and the concentration of ammonium hydroxide considered for the formulation of the culture medium.

Concerning the downstream processing, a more distributed contribution is observed in most of the impact categories, with energy requirements being the main hotspot in the environmental profile (Fig. 1). On the other hand, it is also evidenced that the use of certain chemicals, namely HCl and $(\text{NH}_4)_2\text{SO}_4$, leads to significant environmental burdens on the FET, MET and HNCT impact categories, due to the background activities associated with their production processes. With a focus on a sensitivity analysis, optimization in the use of steam and electricity requirements become the key factors in achieving a more environmentally friendly biotechnological process alternative.

Once each of the environmental profiles of the three sections of the process have been evaluated, it is important to assess which of them represents a greater contribution to the environmental profile of the process as a whole (Fig. 2). The results show that the pretreatment section, which includes the steps necessary for the release of fermentable sugars and for the preparation of the inoculum in the seed fermenters, has the highest environmental load. Its contribution is greater than 50% in all impact categories, except in the TET category, where it is the fermentation stage that

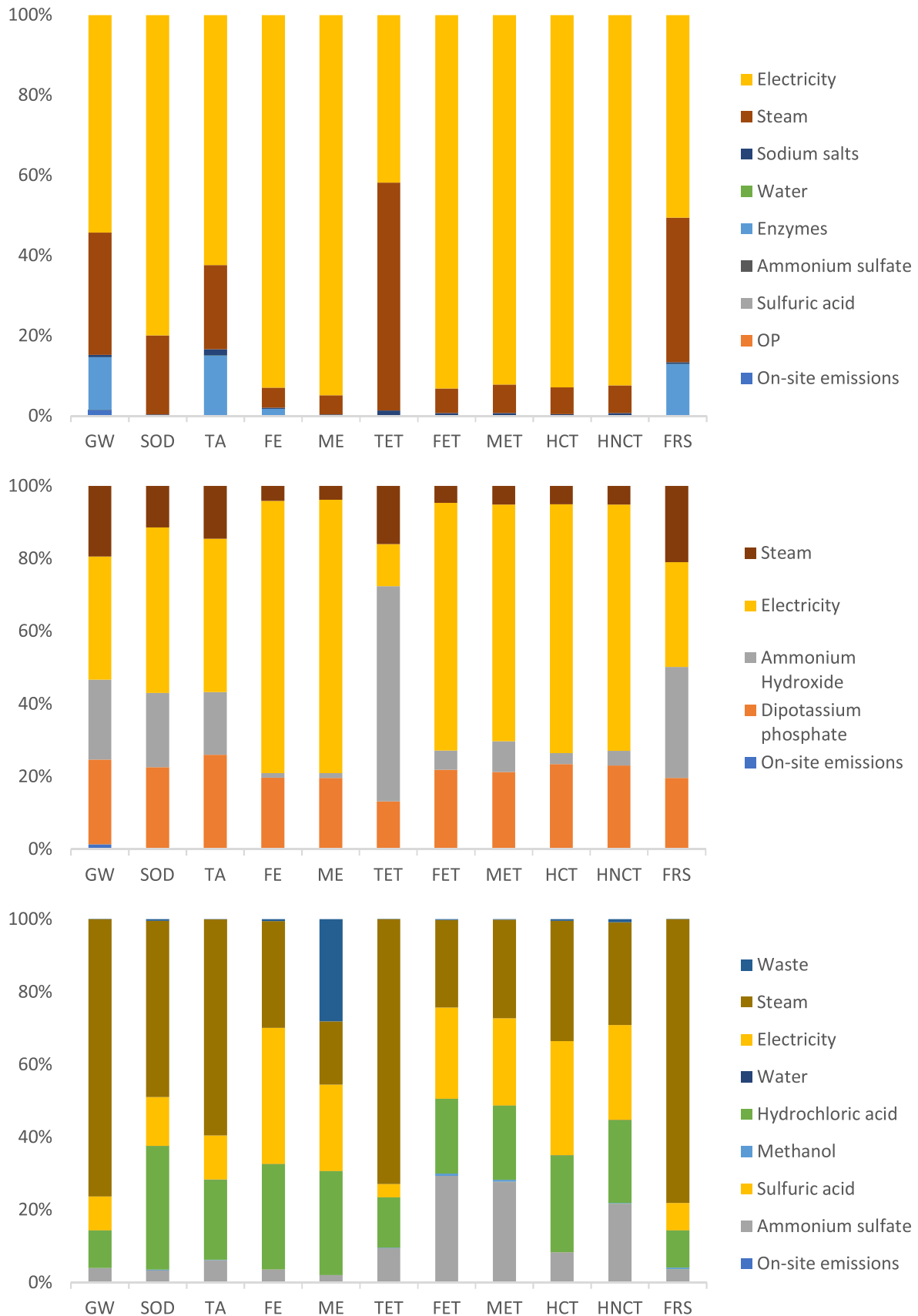


Fig. 1 – Impact assessment results for the biotechnological co-production of nisin and lactic acid from olive pruning. Pretreatment (top figure), fermentation (middle figure) and downstream (bottom figure) sections.

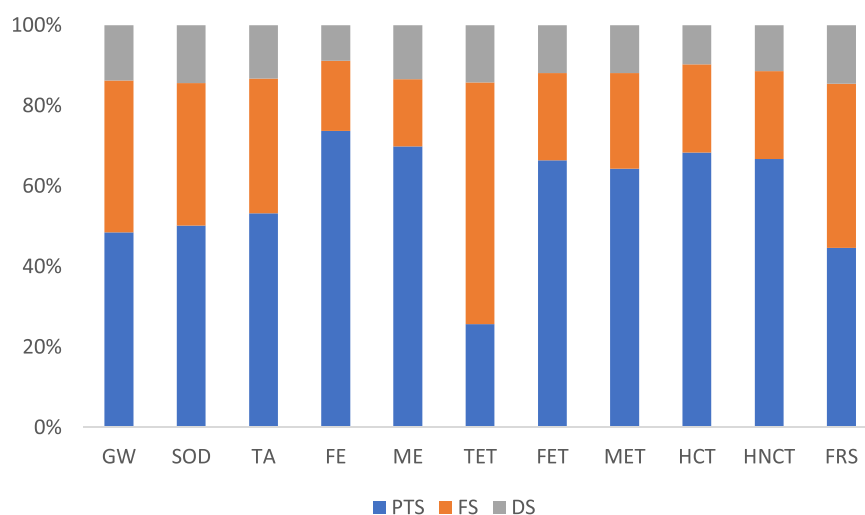


Fig. 2 – Contribution of the pretreatment (PTS), fermentation (FS) and downstream (DS) sections on the environmental profile of the biotechnological process using OP as the lignocellulosic waste input stream.

Table 2 – Sensitivity analysis % reduction results for pretreatment, fermentation and downstream sections.

Impact category	PRETREATMENT		FERMENTATION			DOWNSTREAM
	OP	ER	Energy reduction + N-reduction	Energy reduction	N-reduction	Energy reduction
	Energy reduction					
GW ¹	13.57	14.12	9.42	5.06	4.37	20.76
SOD ²	19.99	19.47	10.86	6.79	4.07	13.90
TA ³	15.60	16.53	9.73	6.30	3.43	16.74
FE ⁴	23.24	23.31	11.44	11.19	0.25	10.55
ME ⁵	23.72	23.58	11.51	11.22	0.28	5.76
TET ⁶	10.46	9.72	13.53	1.73	11.80	19.12
FET ⁶	23.29	23.11	11.25	10.18	1.07	7.89
MET ⁶	23.05	22.84	11.40	9.72	1.69	8.73
HCT ⁶	23.22	23.02	10.82	10.22	0.60	11.21
HNCT ⁶	23.10	22.91	10.91	10.12	0.79	9.27
FRS ⁷	12.64	12.95	10.38	4.31	6.07	20.95

Units: ¹kg CO₂ eq, ² kg CFC₁₁ eq, ³kg SO₂ eq, ⁴kg P eq, ⁵ kg N eq, ⁶ kg 1,4-DCB, ⁷ kg oil eq.

has the highest environmental load, partially attributed to ammonium hydroxide.

3.2. Scenario 2. *Eucalyptus* residues valorization

3.2.1. Life cycle inventory

The summary of the data used for modelling the Scenario 2 on the valorization of eucalyptus residues is included on Table 2 of the Supplementary material. The main hotspot of the pretreatment process of eucalyptus residues is the one referring to electricity requirements, together with the use of steam as heat transfer agent (Fig. 3). Seed-fermenters are those responsible for this significant energy demand, in fact, account for more than 50% of all the power required for this first section of the production process. In the case of steam, is the chemical post-hydrolysis of the stream rich in hemicelluloses the one that stands out on the consumption of this heat transfer agent, given that the temperature required to favor an adequate yield in the chemical hydrolysis operation is 121°C, thus requiring steam at high pressure.

The environmental loads of the fermentation section showed a partial contribution of the chemical agents required for the supplementation of the fermentation medium

with the sufficient amounts of nitrogen, in the form of ammonium hydroxide, and phosphorous, used as dipotassium phosphate (Fig. 3). The most significant contribution is the one observed over TET category, where ammonium hydroxide represents more than 50% of the impact caused, being the emissions associated with its production process the cause of its high contribution. Among these emissions, the release of some metal compounds to the air are those that stand out for their impact on the TET category. On the other hand, as it has occurred with the previous profile, the contribution of electricity requirements is also the main hotspot of the process.

On the environmental profile representing the last stage of the process is steam the main responsible of the environmental loads for mostly all the impact categories studied, with the exception of TET and MET, on with the chemical agents needed on the production process also leads to a significant contribution (Fig. 3). The drying equipment of this stage are the ones that represent a higher heat demand, being the spray dryer the equipment that leads to the highest demand, since it has been modeling for obtaining nisin with a high degree of purity, greater than 95% by weight. As mentioned in previous sections, the reason for obtaining this

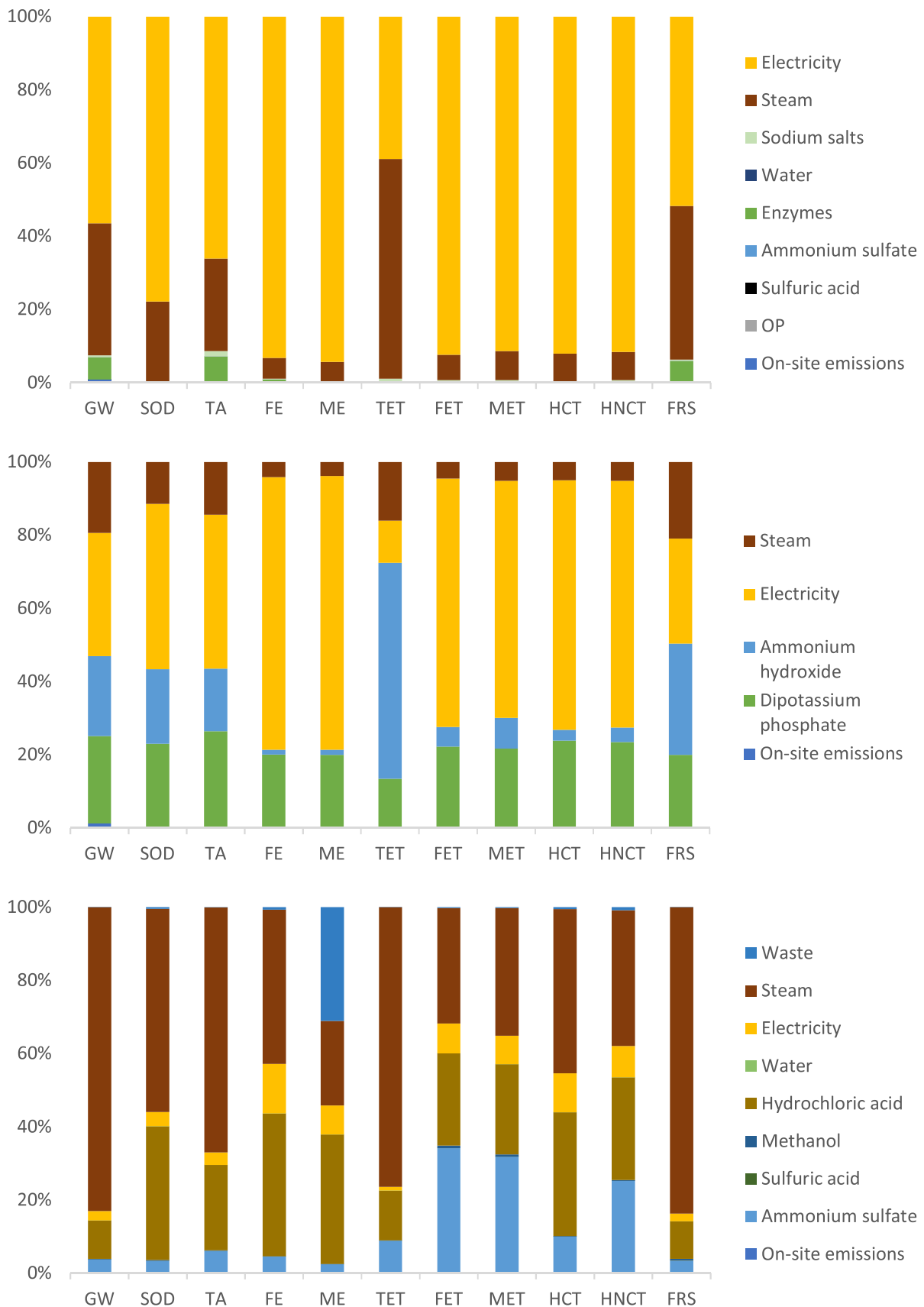


Fig. 3 – Impact assessment results for the biotechnological co-production of nisin and lactic acid from eucalyptus residues. Pretreatment (top figure), fermentation (middle figure) and downstream (bottom figure) sections.

product in such a high degree of purity is based on its possible application in the field of biomedicine and pharmacy, being required to a lesser degree if it is applied as a food preservative, which would significantly reduce the demand

for steam in the drying equipment. Therefore, the specification of the type of application of the product would be a fundamental aspect to be able to develop an environmental improvement of the proposed biotechnological process. In

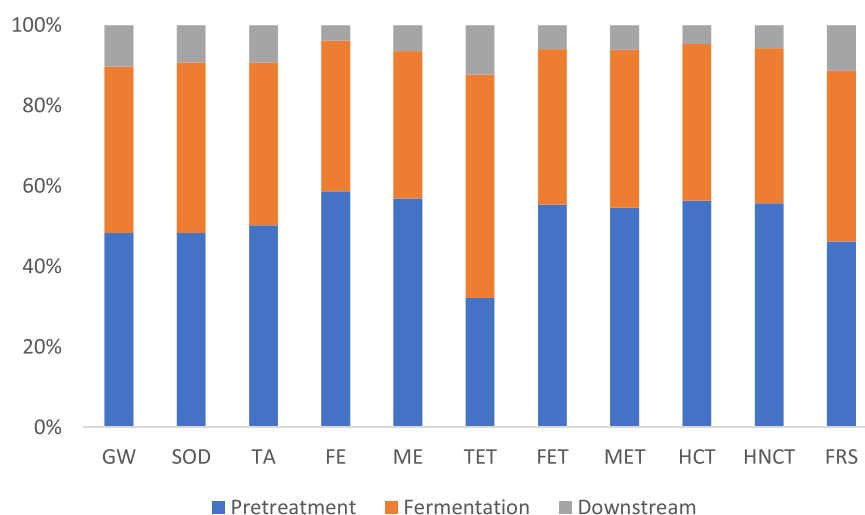


Fig. 4 – Contribution of the pretreatment (PTS), fermentation (FS) and downstream (DS) sections on the environmental profile of the biotechnological process using ER as the lignocellulosic waste input stream.

Table 3 – Mass and economic allocation considering nisin as co-product.

Impact Category	OP			ER		
	MA	EA	Total	MA	EA	Total
GW ¹	5.36	124.54	127.26	7.71	179.06	182.96
SOD ²	1.97	45.7	46.7	3.13	72.7	74.2
TA ³	0.02	0.37	0.38	2.37·10 ⁻²	0.55	0.56
FE ⁴	2.02	47.03	48.05	3.78	87.84	89.76
ME ⁵	0.15	3.45	3.52	0.27	6.35	6.49
TET ⁶	7.71	179.04	182.95	9.87	229.27	234.27
FET ⁶	0.06	1.37	1.40	0.11	2.46	2.51
MET ⁶	0.09	1.98	2.02	0.15	3.50	3.58
HCT ⁶	0.12	2.72	2.78	0.21	4.95	5.06
HNCT ⁶	2.73	63.54	64.92	4.92	114.35	116.85
FRS	1.66	38.47	39.31	2.33	54.13	55.31

Units: ¹kg CO₂ eq, ² mg CFC₁₁ eq, ³kg SO₂ eq, ⁴g P eq, ⁵ g N eq, ⁶ kg 1,4-DCB, ⁷ kg oil eq.

addition, it could also be in consideration the option of carrying out a higher degree of purification of only a part of the total nisin obtained by the batch process, thus co-producing nisin of higher and lower purity, with applications for both food and nutraceuticals industries.

To end up with the environmental study of the production process using olive pruning as residual raw material, the contribution to the environmental profile of the process as a whole, considering the three sections, is shown on Fig. 4. The pretreatment section is, once again, the one with the highest environmental load. Its contribution is greater than 50% in all impact categories, except for the TET category, in which it is the fermentation stage that has the highest. Regarding downstream section, no significant contribution is observed over the profile, so then, high efforts should be paid on both pretreatment and fermentation stages in order to acquire a more environmentally-friendly biotechnological process.

3.3. Sensitivity analysis

The proposed sensitivity analysis has been developed based on the main hotspots identified in the environmental profiles

of the three sections of the production process for the different lignocellulosic streams: OP and ER. Accordingly, in the case of the pretreatment section, a 25% reduction in electricity demand has been analyzed, which could be achieved by performing an energy optimization of the process.

The range of temperatures required in the different process equipment of this first section provides a wide margin for improvement in the energy optimization process, since it allows using the process streams as heat transfer agents (thus reducing the consumption of external heat resources). For this reason, it has been considered that a 25% reduction in demand is an adequate and realistic value.

Considering the fermentation section, two improvement options can be evaluated. First, the reduction of the electrical demand by the agitation equipment, considering a more efficient system that maintains the required homogeneity in the fermentation medium. In this case, given that the range of improvement is smaller compared to the previous case, it has been proposed to analyze the 15% reduction in energy demand. On the other hand, given the contribution of ammonium hydroxide (source of N in the fermentation) on the profile, the development of a sensitivity analysis with respect to the dose used has also been considered. In this case, the range of improvement can also be significant since, being a simulated process based on bibliographic data developed at laboratory scale, the quantities of chemicals used are larger than those that would be required in an industrial scale-up, since the performance of the fermentation process is prioritized over the technical, economic and environmental aspects, which are considered once a higher production capacity is considered. Thus, a 20% reduction in the dose of ammonium hydroxide used has been selected.

As for the last section of the process, based on the results obtained from the application of the Recipe Midpoint methodology, the sensitivity analyses focus on the optimization for the steam used in the process equipment. The variety of temperatures required to perform the different downstream operations allows a significant energy optimization of the process: 37°C for vacuum rotary filtration, 4°C for precipitation, 70°C for drying equipment and 80°C for lactic acid esterification. In this way, the streams could themselves be used as heat transfer agents, reducing the amount of

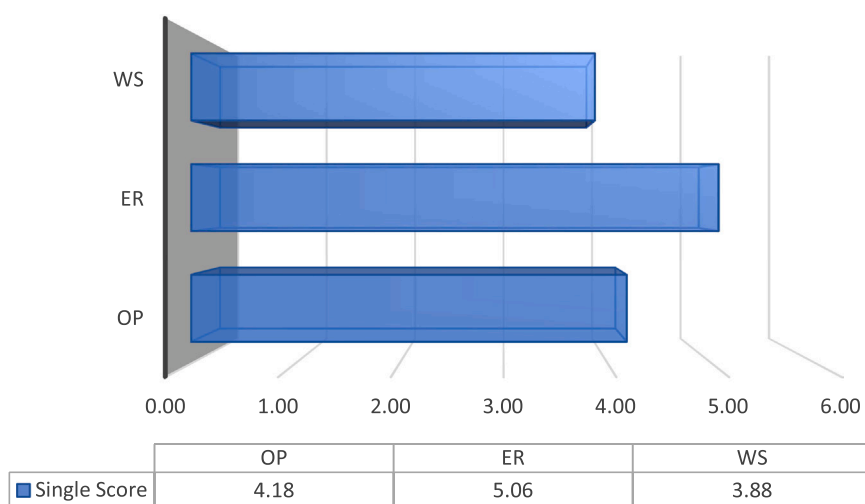


Fig. 5 – Single score values obtained for the two scenarios applying Recipe Endpoint methodology.

external heat sources. In this way, a 25% reduction in steam requirements is analyzed.

Table 2 shows the outcomes of the sensitivity analysis. The best results are obtained in the pretreatment section, where the environmental loads have been reduced by more than 20% in many of the impact categories analyzed, with the exception of the GW and FRS categories. The reason for having achieved a lower improvement of the environmental profile in those categories is based on the fact that the contribution of electricity demand in the base case profile is lower, since the contribution of steam on those categories is also significant.

As for the proposed sensitivity analysis for the fermentation section, it shows the three alternative options: considering both the two study analysis alternatives globally (energy demand and N sources), and each of them separately, in order to determine which of the options represents the greatest improvement on the environmental profile. As could be seen, the joint application of both alternatives leads to a greater improvement in the profile of the base case. In the case of having to choose between them, in most of the impact categories analyzed, it is the reduction of electricity requirements that leads to the best improvement, with the exception of the TET category, where the use of ammonium hydroxide is the main hotspot.

Finally, in the downstream section, a significant improvement is also achieved. In ME, TA, TET and FRS are the categories in which the greatest improvement of the profile has been obtained with respect to the base case.

3.4. Mass & economic allocation

Since two products are produced: nisin and lactic acid, it is pertinent to carry out an allocation study, since in this way it is possible to identify which of the two products makes a greater contribution to the environmental profiles. Although the added value of nisin is much higher compared to lactic acid, the amount obtained per batch is much lower. Thus, a mass allocation and an economic allocation have been proposed. For the development of the economic allocation, it has been considered that the selling price of nisin will be 85 €/kg, while for lactic acid, its selling value is around 1.85 €/kg. The impact values of the mass allocation (MA) and economic allocation (EA) by impact category with respect to nisin are

shown in Table 3. As expected, the contribution on the environmental profile of the nisin product is very low when applying MA, since the amount of nisin produced per batch production process is much lower compared to lactic acid, but, with the EA approach, nisin reports higher environmental burdens, since its sale price is significantly higher compared to lactic acid.

3.5. Benchmarking among scenarios

To conduct the comparison between the three proposed scenarios for the biotechnological co-production of nisin and lactic acid, the Recipe EndPoint calculation methodology has been selected, as it provides a single score value in which all the impact categories of the Recipe Midpoint are encompassed (Fig. 5). The use of olive pruning allows a better environmental profile, which implies a lower contribution to the environmental impact. Moreover, even that for olive and eucalyptus pruning residues, the process yield is similar, it is slightly higher in the case of olive pruning, which allows a reduced environmental damage due to the increase on productivity. Thus, taking into account these previous outcomes, the overall impact generated by the biotechnological process using OP as raw material is expected to be lower compared to ES scenario.

When reporting the results of the EndPoint methodology, OP gives rise to the best process alternative from the environmental point of view, since it makes a lower negative contribution to environmental sustainability. Therefore, despite the fact that the results do not show major differences between the two proposed scenarios, it is concluded that the use of OP is the most suitable alternative from an environmental point of view, although the use of ER could also be considered suitable for the development of this innovative biotechnological process.

4. Conclusions

The integration of the concept of biotechnology in industrial activities is essential to promote the use of renewable resources, as well as residual resources or whose recovery processes are not enough to generate a positive productive impact, moving away from linear processes. In accordance with these aspects, the main goal of this study was to

evaluate the biotechnological and environmental assessment of the industrial scale-up of two lignocellulosic agriculture residues for the co-production of nisin and lactic acid.

The reason for having selected those raw materials for the development of this biotechnological process is focused on three aspects, firstly, the volume of waste generated annually and its management, secondly, its cellulosic and hemicellulosic content and, thirdly, to promote the concept of circular economy and environmental sustainability. Once applying Life Cycle Assessment methodology, it could be concluded that the use of olive pruning as the input for the process is the most appropriate from the biotechnological and environmental point of view. However, given the dependence of seasonality in the availability of the inputs of the process, its similarity on its chemical composition, which allows a simple adaptation of the pretreatment stage of the process, and the results obtained, the use of both lignocellulosic residues in the same industrial biotechnological plant is considered as the most suitable alternative from a technical, economic and environmental point of view.

CRedit authorship contribution statement

Ana Arias: Methodology, Formal analysis, Investigation, Writing – original draft. **Gumersindo Feijoo:** Writing – review & editing. **Maria Teresa Moreira:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.fbp.2022.11.008](https://doi.org/10.1016/j.fbp.2022.11.008).

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