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Waste biorefinery towards a sustainable biotechnological production of pediocin: Synergy between process simulation and environmental assessment

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ABSTRACT

The development of biotechnological processes through which high added value products can be obtained, using non-valuable industrial streams, has become a primary objective in the scope of sustainability and circular economy strategies. It is in this context where this research report is framed, in which a biorefinery approach has been developed using mussel processing wastes (MPW) as a resource, thus avoiding the environmental impacts involved in their management. Through an enzymatic pretreatment and a batch fermentation process, this waste stream is valorized, and three high added value products are obtained: pediocin, lactic acid and crude protein. To develop comparative scenarios, a detailed analysis of the environmental impacts associated with the production of pediocin at two purity levels (2.5 % and 95% w/w), using the MPW and a commercial fermentation medium (MRS) has been performed by applying the life cycle assessment (LCA) methodology. The results obtained show the great potentiality of MPW as C-source, both from an environmental, economic and technological point of view since, when compared with MRS, the carbon footprint is reduced from 24.02 to 15.87 and from 23.85 to 15.66 kg CO_2 eq/kg of products for a purity of 2.5% and 95% w/w of pediocin, respectively. Besides, analogous impact reduction values are observed for the rest of the impact categories evaluated. However, certain hot spots have been identified, which future improvement studies should focus: energy and calorific requirements, mainly, as a contribution higher than 50% is observed for most of the impact categories.

1. Introduction

Bacteriocins are traditionally considered as biologically active peptides with bactericidal properties against pathogens (Benítez-Chao et al., 2021). The antimicrobial activity of bacteriocins represents a great potential for the food industry since they can be used as biological preservatives in substitution of chemical ones with the advantage that, being proteins, secondary compounds with potential toxicity are not produced (Lianou et al., 2016). The prevention of the occurrence of diseases associated with spoiled food has motivated the industrial and scientific world to develop production processes with lower economic cost and environmental impact in order to favor the introduction of natural preservatives in the market and give confidence to consumers with a high added value bioactive compound (Lorenzo et al., 2018; Mesías et al., 2021).

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Lactic acid bacteria (LAB) are not only interesting in the food industry for inducing the development of desirable organoleptic properties in foods, but also for inhibiting the development of harmful, pathogenic microorganisms thanks to the antimicrobial substances they produce. In particular, bacteriocins are the most interesting technologically, since, due to their protein nature, they inactivate the proteolytic enzymes of the gastrointestinal tract and are non-toxic and non-immunogenic, which makes them suitable candidates for food preservatives (Aka-Gbezo et al., 2014; Iseppi et al., 2021; Silva et al., 2018). Among the different bacteriocins produced by LAB such as *Pediococcus acidilactici* and *Pediococcus pentosaceus* (Yusuf, 2018) pediocin, a Class IIa group bacteriocin with high activity against Gram-positive bacteria such as Listeria, stands out (Delves-Broughton, 2012; Espitia et al., 2016; Mogoşanu et al., 2017; Villalobos-Delgado et al., 2019). In general, microbial cultures of *P. acidilactici* and *P. pentosaceus* take place in aerobic media, leading to a higher yield of pediocin production (Papagianni and Anastasiadou, 2009).

It is well known that the use of a rich fermentation medium, such as MRS broth, will result in a higher level of pediocin titer as it fosters adequate biomass growth. However, the MRS medium is very rich in protein substances which would hinder the purification of antimicrobial protein substances (Pratiwi et al., 2020). In addition, it should be noted that the formulation of commercial fermentation media involves a significant process cost that can amount to 25%–40% of the total cost (Zhang et al., 2020). That is why it is imperative to search for new sources of C from industrial process waste streams, characterized by their high content of fermentable sugars, nutrients or other components that may allow the production of pediocin at reduced cost.

In particular, it is worth noting as an additional advantage the fact that *Pediococcus* strains can utilize different sugar sources (i.e., glucose, galactose, fructose, cellobiose, arabinose, ribose, mannose), thus raising the possibility of taking advantage of broad-spectrum waste streams in the formulation of culture media. Such is the case of mussel processing waste (MPW), which has a significant glycogen content: 5.33 g/L (Guerra et al., 2005), which could be converted into glucose after enzymatic hydrolysis. The main producers of MPW are China (43%), Chile (15%) and Spain (12%) (Avdelas et al., 2021), the latter accounting for 40%–45% of European mussel aquaculture production (European Commission (EUMOFA), 2020).

The mussel processing industry comprises a series of stages in which liquid effluents are produced with varying levels of organic load: High organic load (HOL) effluents, corresponding to the mussel cooking and dehydration stages and Low organic load (LOL) effluents from mussel washing and cleaning of the cans and facilities, classified according to chemical oxygen demand (COD) and total suspended solids (TSS): 84.51 g/L and 1.63 g/L for HOL, and 1.90 g/L and 1.24 g/L for LOL, respectively (Barros et al., 2009). It should be noted that the large water consumption in the process, which implies that the process wastewater flow can reach levels of 300 L/ton of raw mussel processed (Murado et al., 1994; Prieto et al., 2015).

Taking into account both the flow and composition of the waste streams and the environmental problems encountered, it is important to search for more sustainable alternatives that promote their valorization. In this study, the option of valorizing these waste streams as a C source for the biotechnological production of pediocin is proposed. In this work, the conceptual design of the process is carried out based on the mass and energy balances of the process, as well as the design of the equipment using SuperPro Designer® as a simulation tool. From these data, it will be possible to obtain the necessary process inventories for the Life Cycle Assessment (LCA) study, in order to carry out the environmental evaluation of the production process. In addition, in order to identify the advantages or weaknesses of this process alternative versus the process based on MRS as a synthetic medium, the simulation of the conventional pediocin production process will be developed.

To this end, the aims of this research article were (i) large-scale design of the valorization process of mussel processing residues using the SuperPro Designer tool, (ii) application of the Life Cycle Assessment methodology for the environmental evaluation of the pediocin production process, (iii) identification of the main hotspots of the process and (iv) comparative analysis between the use of mussel processing waste and MRS as culture media for the fermentation process.

2. Materials and methods

2.1. Process description

Four pediocin production scenarios were considered depending on the purity of the final product (95% w/w for pharmaceutical use and 2.5% w/w as a food preservative) produced with a standard lactic acid bacteria (MRS) culture medium or with a mussel processing waste (MPW) stream (Fig. 1) (Jones et al., 2005; Firouz et al., 2021). In the case of low purity pediocin, a composition similar to commercial bacteriocins of the Nisaplin and Novasin type (Trademarks: ALTA 2341/2351 and Fargo 23) was considered (Deegan et al., 2006; Jozala et al., 2015; López-Cuellar et al., 2016; Martinez et al., 2016). It should be noted that in addition to pediocin, lactic acid bacteria produce two co-products in significant proportions: lactic acid and crude protein. Lactic acid will be marketed at a concentration of 76% w/w after a vacuum evaporation process (Lee et al., 2017). Crude protein provides a valuable nutritional product due to its high amino acid content (Erickson et al., 2012; Murado et al., 1994; Templeton and Laurens, 2015)

Pediocin production presents five main stages or subsystems (Fig. 2). Subsystem 1 (SS1) includes the inoculum train so that the starter culture after reaching an optimal microbial density is fed to the main fermenter (SS3). The preparation of the culture medium (MRS or MPW) fed to the inoculum train and to the main fermenter is performed in subsystem SS2.

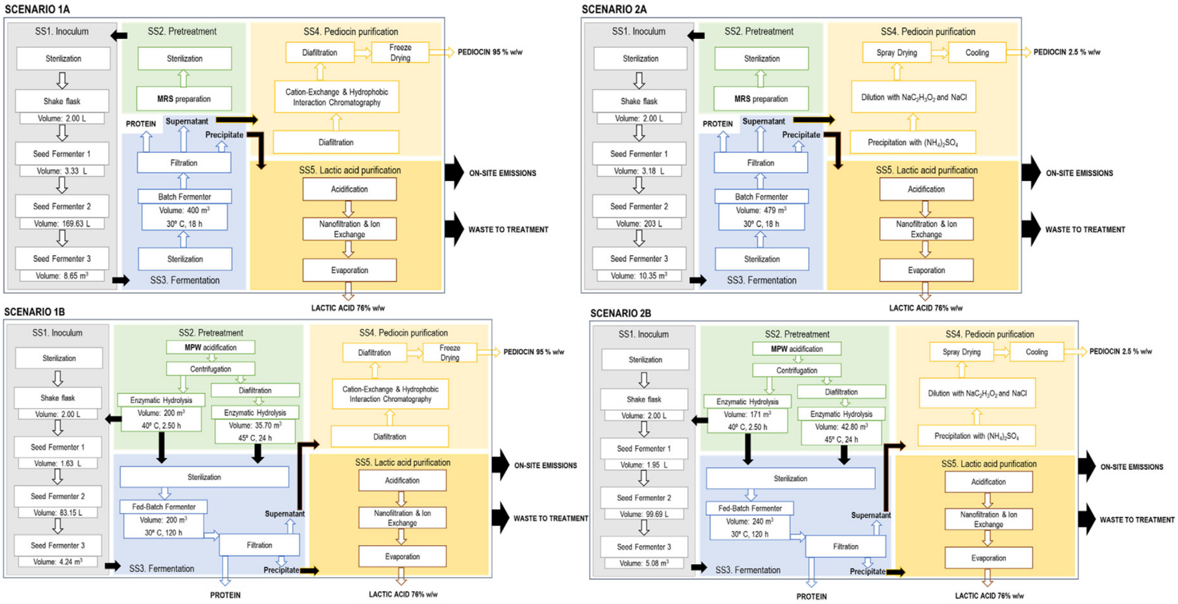

Fig. 1. Scenarios considered for the environmental assessment of pediocin production.

In order to valorize MPW as culture medium, it is necessary to include additional steps of acidification, centrifugation, diafiltration and enzymatic hydrolysis for the separation of solids and release of fermentable sugars at a temperature between 40–45 °C (Amado and Vázquez, 2015).

The fermentation strategy is different depending on the composition of the culture medium. Recognizing that certain specific components of the culture medium can interfere with pediocin production, it also plays a significant role if the microbial growth rate and the possibility of extending the productive period in a fed-batch operation. It has been found that in the case of MRS, a batch procedure lasting 18 h allows high pediocin activity values, whereas when using MPW, fed-batch operation is considered for the purpose of improving yield, which implies a process time of 120 h with periodic MPW pulses (Guerra et al., 2005). After fermentation, a filtration stage is carried out, through which 3 streams are obtained: the crude protein stream, which will be used as animal feed, impure pediocin and lactic acid, which will be purified in SS4 and SS5, respectively.

Considering the degree of purity, the pediocin purification scheme is different. When 95% w/w purity is required, the following steps are performed: filtration, cation exchange chromatography (CEC) and hydrophobic chromatography (HPC), diafiltration and lyophilization. For CEC, sodium chloride solution is used as eluent and ammonium acetate as buffer. The selected operating parameters are those leading to 100% binding of pediocin with a yield of 0.9 (Beaulieu et al., 2006). On the other hand, in HPC, the eluted CEC stream is treated with ammonium acetate, acetonitrile and sodium chloride, obtaining an eluent with a composition of approximately 40% acetonitrile, 9% pediocin and 51% water (Beaulieu et al., 2006). Purification of pediocin requires the removal of acetonitrile and water; consequently, diafiltration and lyophilization steps are carried out (Li et al., 2019).

Alternatively, if a lower purity product is desired, ammonium sulfate precipitation of the supernatant stream is performed, a step that requires a residence time of 24 h at 4 °C to achieve 64% precipitation of the initial pediocin (Vijay Simha et al., 2012). Precipitation is followed by a dilution step with NaCl and spray drying to remove acetic acid and excess water to achieve the target of 2.5% pediocin packaging (Uteng et al., 2002; Vijay Simha et al., 2012). As for the lactic acid stream, four different stages are needed to obtain the concentrated lactic acid stream (76%): an acidification with HCl, nanofiltration, ion exchange and evaporation (Lee et al., 2017).

2.2. Environmental assessment

2.2.1. Description of the methodology

The Life Cycle Assessment (LCA) methodology has been selected to quantify the environmental impact associated with the production of pediocin, considering two alternatives of culture medium: commercial MRS or a mussel processing waste stream (MPW). Taking as an initial premise the attributional LCA methodology to determine the environmental impacts attributed to pediocin production, the functional unit (FU) of 1 kg of products has been defined, as well as the scope of the analysis, the system boundaries and the impact assessment methodology. Taking into account the cradle-to-gate approach, the system boundaries include all the processes involved from the extraction of raw materials to the production of three

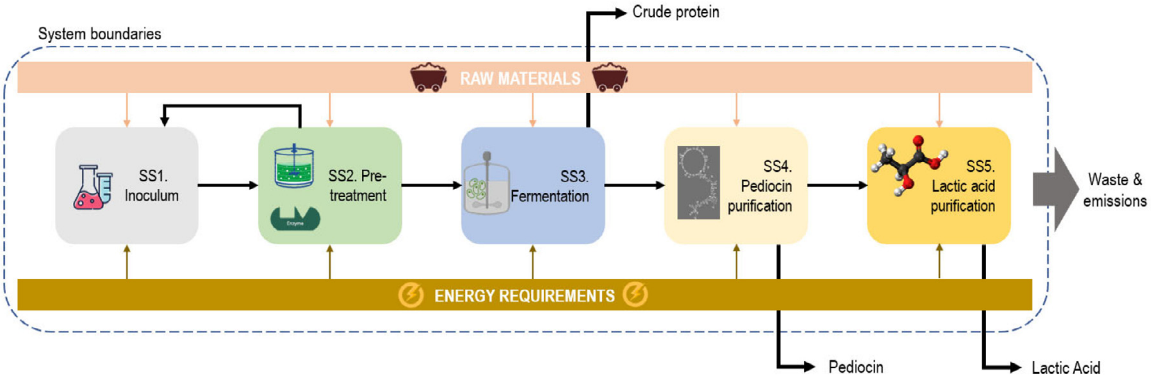

Fig. 2. System boundaries considered for applying the LCA methodology.

products: pediocin, lactic acid and crude protein, including emissions and waste streams, leaving out of the system the activities of transportation and maintenance of the facilities (Fig. 2). Regarding the LCA methodology, two approaches have been used: ReCipe 2016 hierarchist MidPoint method V1.03 World (2010) according to the environmental impacts presented in Table 1SM, as well as the ReCiPe 2016 hierarchist EndPoint method V1.03 World (2010) H/H (Huijbregts et al., 2017), providing a single score that considers the relevance of three damage categories: Human Health (HH), Ecosystem Quality (EQ) and Resource Scarcity (RS).

2.2.2. Life cycle inventories

Conducting life cycle inventories involves the collection of all process data, both input and output streams, including materials, energy requirements, products, on-site emissions and waste streams. The compilation of these data requires the resolution of mass and energy balances for all process flows. Therefore, a SuperPro Designer® simulation tool was used, considering the five subsystems defined in Section 2.1 Process description. The summary of the data corresponding to the four full-scale pediocin production scenarios is presented in Tables 2–5SM.

2.3. Mass and economic allocation

Since three main products (crude protein, pediocin and lactic acid) are obtained, a mass and economic allocation has been performed in order to report the environmental loads of each product and identify which one accounts for the highest impact. The values considered for mass and economic allocation are shown in Table 6SM.

3. Results and discussion

Taking the conceptual design of the biotechnological production of pediocin as a starting point, it is necessary to highlight the specificities of the process with respect to the microbial growth and metabolism of *Pediococcus* based on different compositions in the formulation of the culture medium. While the MRS culture medium is certainly specific for lactic acid bacteria but complex as it includes glucose, peptone, meat and yeast extract, sodium acetate, polysorbate 80, dipotassium phosphate, ammonium citrate, magnesium sulfate and manganese, the MPW requires the development of enzymatic glycogen hydrolysis procedures as a source of fermentable sugars.

3.1. Scenario 1A: High purity pediocin and MRS culture medium

As shown in Fig. 3, three main critical points could be identified in the environmental profile of high purity pediocin produced using the standard medium: energy consumption associated with steam and electricity requirements and the MRS medium itself. In relation to the subsystems that integrate the overall process (Fig. 3, top figure), the contribution of the fermentation process (SS3), and the purification of lactic acid (SS5) stand out. In particular, SS3 has a leading role with the highest contributions in most of the impact categories, except GW, FRS, HT and TET, where the contribution of SS5 is remarkable.

Performing a more exhaustive analysis of the process subsystems, the critical points identified in each process subsystem are analogous to those of the global profile (Fig. 3, bottom figure). The contribution of MRS stands out in subsystems SS1 and SS3, while it is steam that gives rise to the greatest impact in subsystems SS2 and SS5. The results obtained were to be expected, based on the importance of the culture medium in subsystems SS1 and SS3. On the other hand, the process requires significant energy consumption in the preparation and sterilization of the MRS medium and the purification of lactic acid in the acetonitrile distillation unit.

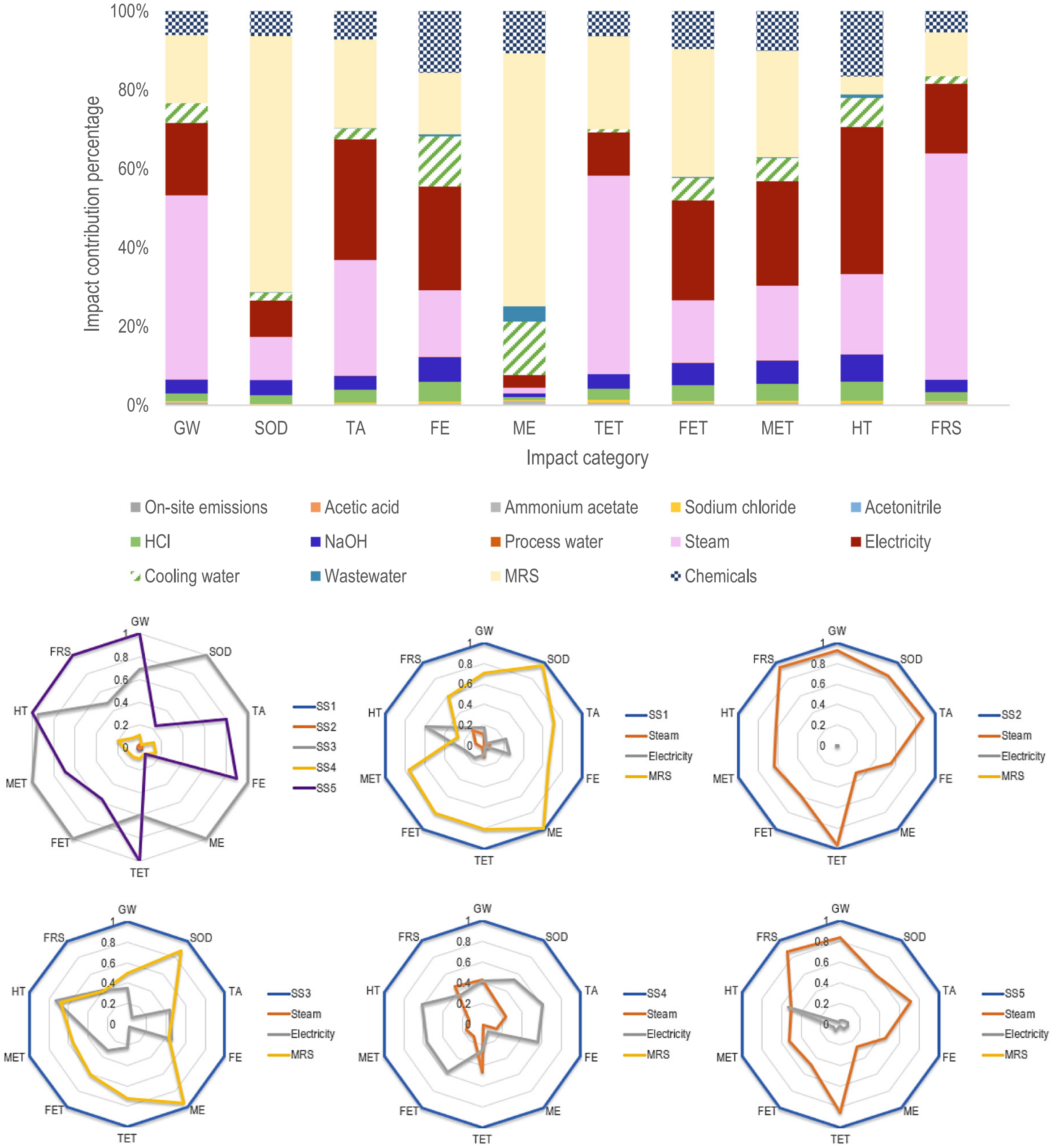

Fig. 3. Environmental profile of Scenario 1A: pediocin with 95% w/w purity using MRS culture medium (top figure) and contribution to the impact of the main hotspots per process subsystem (bottom figure).

With respect to electricity requirements, its environmental contribution is not very significant in subsystems SS2 and SS5, while in the other three subsystems its influence is clear, especially in the fermentation process (SS3) and pediocin purification (SS4) subsystems. In SS3, energy consumption is associated with the agitation necessary to maintain a homogeneous medium during the fermentation process. On the other hand, since this is an aerobic process, the use of the compressor to supply air to the culture broth is noticeable. With respect to SS5, the filtration equipment is the most energy demanding, specifically the first stage of diafiltration of the pediocin stream from SS3 and the reverse osmosis filtration used for the concentration and purification of wastewater streams, just prior to their further processing as waste output.

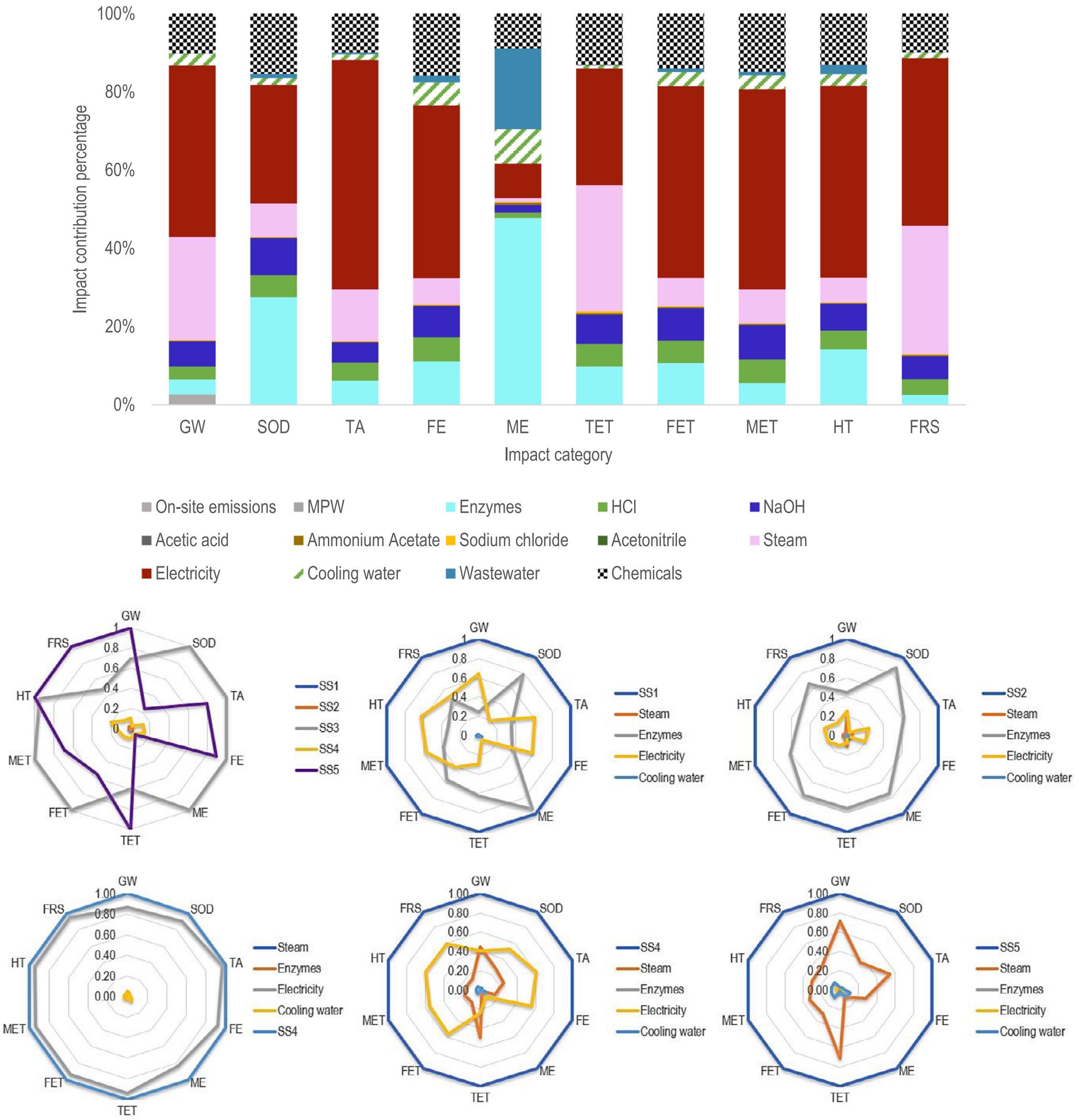

Fig. 4. Environmental profile of Scenario 1B: pediocin with 95% w/w purity using MPW culture medium (top figure) and contribution of the impact of the main hotspots per process subsystem (bottom figure).

3.2. Scenario 1B: High purity pediocin and MPW culture medium

The electricity demand is a critical point in Scenario 1B (Fig. 4, top figure). The environmental contribution of enzymes, mainly in the ME impact category, is associated with energy requirements and inputs for the formulation of the culture medium for enzyme production. In addition, a significant contribution of wastewater is also observed in the eutrophication category associated with the organic load and nutrients present in the wastewater.

Another important difference between this environmental profile and the one obtained previously (Scenario 1A) is observed in the GW impact category. In this case, a certain contribution from on-site emissions is noted, due to the discharge of CO_2 from the fermentation taking place, which is significantly higher compared to that obtained when using MRS culture medium: 98.33 g/kg of products in Scenario 1A versus 473.13 g/kg of products in Scenario 1B.

Detailed evaluation of the subsystems shows that electricity is the main contributor of impact on the SS3 subsystem (Fig. 4, bottom figure), mainly attributed to the duration of the fermentation process when MPW is used as the C source.

Table 1
Characterization values of pediocin production scenarios considering ReCiPe MidPoint methodology, using as functional unit 1 kg of products.

Impact category	Unit	Scenario 1A	Scenario 1B	Scenario 2A	Scenario 2B
GW	kg CO_2 eq	24.02	15.87	23.85	15.66
SOD	mg CFC-11 eq	24.95	11.26	22.87	10.64
TA	g SO_2 eq	84.71	71.79	80.60	68.65
FE	g P eq	5.30	5.04	4.95	4.76
ME	g N eq	3.92	2.19	3.36	1.99
TET	kg 1.4-DCB	31.57	18.59	33.13	19.95
FET	kg 1.4-DCB	0.21	0.18	0.26	0.21
MET	kg 1.4-DCB	0.28	0.23	0.35	0.28
HT	kg 1.4-DCB	8.07	9.98	9.47	10.65
FRS	kg oil eq	6.88	4.42	6.93	4.41

With respect to steam, both purification stages, SS4 and SS5, are those in which its contribution is higher. Regarding the impact of enzymes, the use of MPW entails an important environmental benefit, since the use of enzymes did not contribute as much as MRS does.

3.3. Scenario 2A: Low purity pediocin with MRS culture medium

Scenario 2A is the one referred to the production of 2.5% w/w pediocin using MRS as C-source. The contribution of the items of Scenario 2A (Fig. 5, top figure) is analogous to that of Scenario 1A, except for the impact caused by the use of ammonium sulfate, which in this scenario is significant and, in fact, can be considered as a hotspot in some impact categories such as ecotoxicity (TET, FET, MET) and human toxicity (HT). With respect to the other critical points of the environmental profile, as in Scenario 1A, three are identified: MRS broth, electricity and steam.

A detailed analysis by subsystems (Fig. 5, bottom figure) shows that subsystems SS3 and SS5 are the most significant contributors to the environmental impact of the overall production process. As for the analysis considering the identified hotspots, it is determined that, in the case of MRS, its environmental contribution is observed in subsystems SS1 and SS3, while, in the case of steam, it is relevant for sterilization (SS2) and lactic acid purification (SS5). Finally, electricity is especially relevant in the SS3, given the need of agitation.

3.4. Scenario 2B: Low purity pediocin with MPW culture medium

Scenario 2B shows an environmental profile similar to that of Scenario 1B, but there is a difference in the environmental contribution of ammonium sulfate (Fig. 6, top figure). The main hot spots that could be identified are the following: electricity, steam and enzymes. Analyzing each of the subsystems separately (Fig. 5, bottom figure), steam is the main hotspot identified for both SS4 and SS5, the subsystems linked to products purification. On the other hand, the environmental contribution of the enzymes is reduced to subsystems SS1 and SS2, corresponding to inoculum preparation and MPW pretreatment. In addition, it is important to mention that the electrical requirements of the fermentation process are high due to the agitation and longer operation period of the fed-batch operation. This translates into a practically total contribution of electricity on the environmental profile of SS3 stage.

3.5. Comparison between scenarios using ReCiPe MidPoint characterization values

To quantify the environmental burdens in different impact categories, the characterization values of the ReCiPe MidPoint methodology were used. Table 1 shows the impact values obtained for low and high purity pediocin and the differences are not significant in any of the impact categories. The maximum differences between scenario 1A and 2A are identified in the categories of SOD (+2.08 mg CFC-11 eq for Scenario 1A), TA (+4.11 g SO_2 eq for Scenario 1A), TET (+1.56 kg 1.4-DCB for Scenario 2A) and HT (+1.4-DCB for Scenario 2A). When it comes to the scenarios based on MPW, the maximum differences are observed in the categories of HT (+3.14 g SO_2 eq for Scenario 1B) and TET (+1.36 kg 1.4-DCB for Scenario 2B). In the remaining categories the difference between the environmental impact considering the production of pediocin at low or high purity is less than 1. Therefore, it can be established that the values obtained are promising, since while the production of high purity pediocin does not imply a higher environmental impact. Approached from an economic point of view, the production of high purity pediocin entails a greater economic benefit due to its higher price.

On the other hand, if a comparison is made between MRS and MPW as culture medium, the use of MPW results in a lower environmental impact in all the impact categories studied, except in the HT category, where the impact value is 1.91 times higher for the high purity pediocin production scenario, and 1.18 times higher for the low purity pediocin scenario.

Fig. 5. Environmental profile of Scenario 2A: pediocin with 2.5% w/w purity using MRS culture medium (top figure) and contribution to the impact of the main hotspots per process subsystem (bottom figure).

3.6. Comparison of scenarios using ReCiPe EndPoint values

In order to make a comparison between scenarios, all impact categories have been quantified in a single indicator comprising three damage categories: human health, ecosystem quality, and resource scarcity. As can be seen in Table 2, the damage results for Scenarios 1B and 2B, (those using MPW as the culture medium) are significantly lower compared to Scenarios 1A and 2A, based on the MRS medium. The most notable difference is derived from the damage value in the human health category (Fig. 1SM), where the use of MRS broth results in a 100-fold higher damage value. In the other two

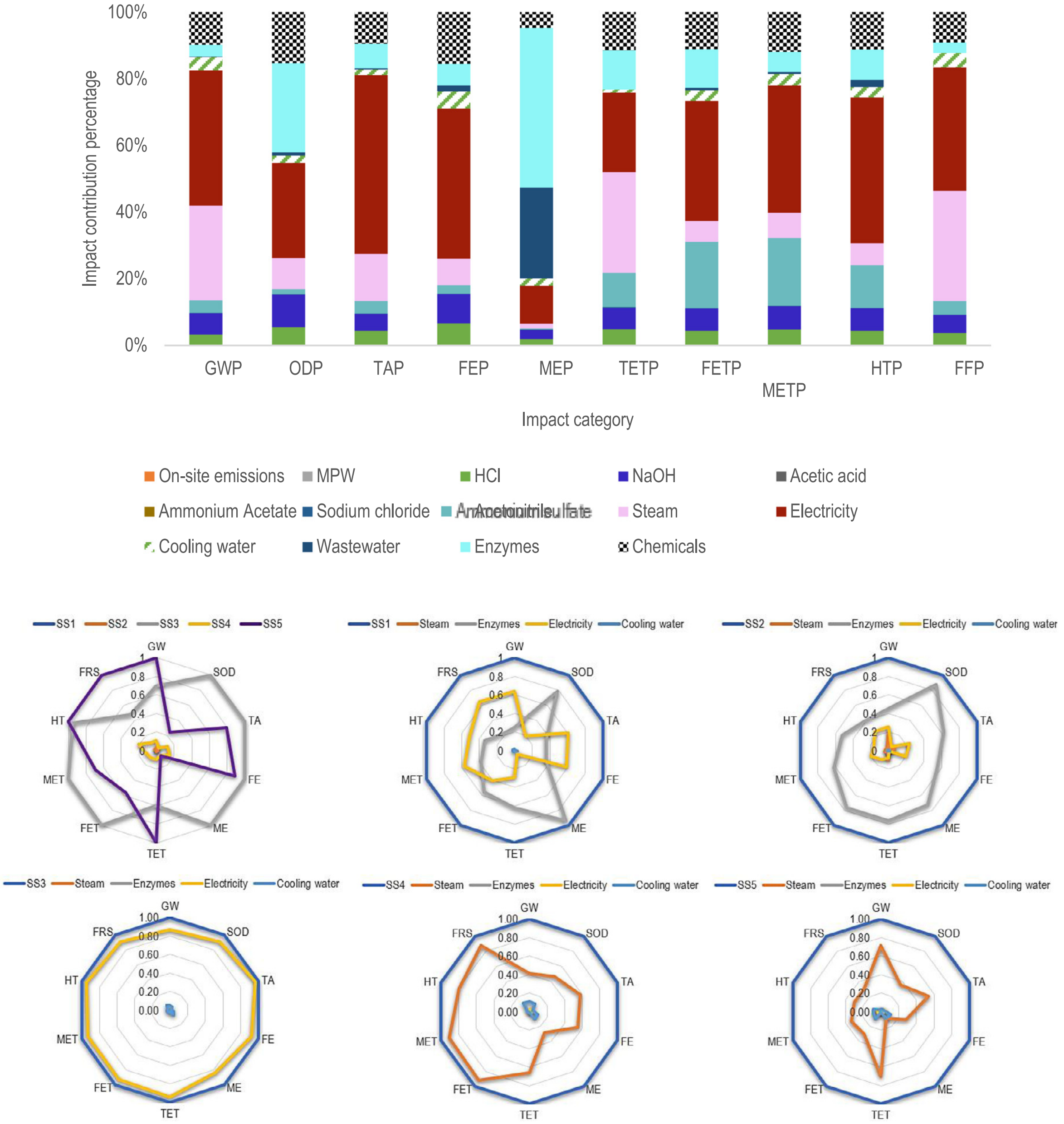

Fig. 6. Environmental profile of Scenario 2B: pediocin with 2.5% w/w purity using MPW as culture medium (top figure) contribution to the impact of the main hotspots per process subsystem (bottom figure).

damage categories, although the use of MRS broth also results in a higher damage value, the difference is not as significant, being approximately 26 points higher in the ecosystem quality category and 10 times higher in the resource scarcity category. On the other hand, considering a comparison between the scenarios of high pediocin production (Scenarios 1A and 1B) or low purity (Scenarios 2A and 2B), the difference is practically null, which reinforces the statement mentioned in the previous section.

3.7. Mass and economic allocation

The pediocin production process, whether low or high purity, results in the co-production of three products, crude protein, which can be used for animal feed, pediocin itself, which will be used as a food preservative (in the case of low

Table 2
Single score values obtained for each scenario by applying the ReCiPe EndPoint calculation methodology.

Damage category	Scenario 1A	Scenario 1B	Scenario 2A	Scenario 2B
Human health (mPt)	330.09	231.64	330.68	230.63
Ecosystems Quality (mPt)	54.66	38.47	53.50	37.56
Resources Scarcity (mPt)	23.18	12.96	23.63	13.20
Single Score (mPt)	***407.93***	***281.39***	***407.82***	***283.08***

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

		Scenario 1A			Scenario 1B		
		MA	EA	Total	MA	EA	Total
GW	kg CO_2 eq	$9.52 \cdot 10^{-2}$	12.03	**24.02**	$4.58 \cdot 10^{-2}$	6.94	**15.87**
SOD	mg CFC-11 eq	$9.89 \cdot 10^{-2}$	12.50	**24.95**	$3.25 \cdot 10^{-2}$	4.92	**11.26**
TA	g SO_2 eq	0.34	42.44	**84.71**	0.21	31.38	**71.79**
FE	g P eq	$2.10 \cdot 10^{-2}$	2.66	**5.30**	$1.46 \cdot 10^{-2}$	2.21	**5.04**
ME	g N eq	$1.56 \cdot 10^{-2}$	1.97	**3.92**	$6.31 \cdot 10^{-3}$	0.96	**2.19**
TET	kg 1.4-DCB	$1.25 \cdot 10^{-1}$	15.82	**31.57**	$5.37 \cdot 10^{-2}$	8.13	**18.59**
FET	kg 1.4-DCB	$8.26 \cdot 10^{-4}$	0.10	**0.21**	$5.09 \cdot 10^{-4}$	0.08	**0.18**
MET	kg 1.4-DCB	$1.11 \cdot 10^{-3}$	0.14	**0.28**	$6.68 \cdot 10^{-4}$	0.10	**0.23**
HT	kg 1.4-DCB	$3.20 \cdot 10^{-2}$	4.04	**8.07**	$2.88 \cdot 10^{-2}$	4.36	**9.98**
FRS	kg oil eq	$2.73 \cdot 10^{-2}$	3.45	**6.88**	$1.28 \cdot 10^{-2}$	1.93	**4.42**
		Scenario 2A			Scenario 2B		
		MA	EA	Total	MA	EA	Total
GW	kg CO_2 eq	2.68	20.95	**23.85**	1.32	13.28	**15.66**
SOD	mg CFC-11 eq	2.57	20.09	**22.87**	0.90	9.02	**10.64**
TA	g SO_2 eq	9.06	70.80	**80.60**	5.79	58.22	**68.65**
FE	g P eq	$5.56 \cdot 10^{-1}$	4.35	**4.95**	$4.01 \cdot 10^{-1}$	4.04	**4.76**
ME	g N eq	$3.77 \cdot 10^{-1}$	2.95	**3.36**	$1.68 \cdot 10^{-1}$	1.69	**1.99**
TET	kg 1.4-DCB	3.72	29.10	**33.13**	1.68	16.92	**19.95**
FET	kg 1.4-DCB	$2.88 \cdot 10^{-2}$	0.23	**0.26**	$1.75 \cdot 10^{-2}$	0.18	**0.21**
MET	kg 1.4-DCB	$3.90 \cdot 10^{-2}$	0.30	**0.35**	$2.32 \cdot 10^{-2}$	0.23	**0.28**
HT	kg 1.4-DCB	1.06	8.32	**9.47**	$8.98 \cdot 10^{-1}$	9.03	**10.65**
FRS	kg oil eq	$7.79 \cdot 10^{-1}$	6.09	**6.93**	$3.71 \cdot 10^{-1}$	3.74	**4.41**

Acronyms: MA (Mass Allocation) and EA (Economic Allocation).

purity pediocin) or for pharmaceutical uses (high purity pediocin), and lactic acid, which can be used as an input for the production of polylactic acid (PLA), of high added value in the market due to its properties as a bioplastic.

According to the mass and economic allocation values shown in Table 6M, the environmental loads considering pediocin as the allocated co-product are included in Table 3. As seen in the inventory tables, the amount of pediocin produced is relatively low compared to the amounts of crude protein and lactic acid. This fact will likely change the values of the environmental loads values obtained when considering an allocation assessment. When a mass allocation is performed, the contribution of pediocin, compared to the other co-products, in the impact value obtained is not very significant. However, the environmental load in economic terms is the one that stands out the most, reaching practically the total impact value. The reason for this contribution is simple: the monetary market value of pediocin is significantly higher than that of lactic acid and crude protein. On the other hand, it is observed that this contribution is even higher when it comes to high purity pediocin (Scenarios 1A and 2A), as this gives a higher productive benefit compared to low purity pediocin, whose economic value is already very high compared to the other two co-products, but lower than that of pediocin at 95% w/w purity.

4. Practical applications and future research prospects

The increase in world population, together with the concern for the preservation of environmental quality, has led to the need to implement new industrial process alternatives, in which biorefineries and circular economy strategies have a strong potential. In addition, another outstanding aspect is the demand of consumers for natural and minimally processed foods. Within this context, this manuscript addresses the approach of circular economy for the valorization of mussel processing residues as a carbon source for the biotechnological production of pediocin, an antimicrobial additive with high potential to replace synthetic preservatives. The environmental assessment of the process provides valuable information for potential large-scale development in the search for a sustainable and optimized production process.

5. Conclusions

According to the LCA results, it can be concluded that the use of MRS broth as culture medium contributes more significantly to environmental impacts, while the use of MPW leads to economic and environmental benefit. In fact, a triple benefit is achieved, firstly, the use of a waste stream to obtain high-added value products, secondly, the reduction of costs associated with the pediocin production process, since the use of MRS broth is avoided and a zero cost stream is used, and finally, the prevention of the emission of MPW into the environment, which has a high organic load and can result in significant environmental impacts, whether it is disposed of in a landfill or otherwise managed. It has also been found that there is no significant environmental impacts difference between high or low purity pediocin production, which is a great economic advantage, since higher profits will be possible.

CRediT authorship contribution statement

Ana Arias: Methodology, Formal analysis, Investigation, Writing – original draft. **Daniel Barreiro:** Methodology, Formal analysis, Investigation. **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 interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2022.102306.

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