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Process and environmental simulation in the validation of the biotechnological production of nisin from waste

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ABSTRACT

Chemical and heat treatments are traditionally used to preserve the quality of food products. An alternative is based on the use of antimicrobials such as nisin to ensure food safety. Traditionally, nisin is produced by microbial fermentation in the exponential growth phase of *Lactococcus lactis*, which is a recognized starter culture in dairy products. However, its production process entails a high cost compared to its chemical-based counterparts, which reduces its competitiveness in the market. This study addresses the economic feasibility and environmental impacts of biotechnological co-production of nisin and lactic acid from three food-associated industrial waste streams: cheese whey (CW), sugar beet pulp (SBP) and corn stover (CS). To carry out the conceptual design of a process at an early stage of development, SuperPro Designer® is used as simulation tool for developing the process alternatives within an industrial approach. Life Cycle Assessment (LCA) methodology will be applied to identify the main environmental impacts associated with the production process. Based on the economic and environmental evaluation, SBP proved to be the best carbon source for the nisin production process, followed by CW. Regarding CS, this alternative should overcome the drawbacks associated with enzyme consumption and limited nisin production yield.

1. Introduction

Nowadays the fact that consumers perceive a product as natural is a decisive purchasing incentive. Thus, natural products are often minimally processed foods. It seems that "no artificial ingredients", "no additives" and "healthy" are the three most frequent associations in the consumer's mind with the word "natural". The *Realfooding®* movement is booming, which focuses on the absence of ultra-processed products in the diet. However, to avoid the presence of pathogenic microorganisms in food, it is necessary to add preservatives, most of which are chemical-based and could pose a health risk.

In this context, the use of natural bio-preservatives is an noteworthy alternative, within which bacteriocins play an important role, since they are not harmful to human health [1] and are also odorless, tasteless and colorless, so they could be added to food products without altering their main properties [2]. By avoiding the use of chemicals and heat treatments for food preservation, foods with better organoleptic and nutritional properties are obtained, which is a great advantage given the growing consumer demand for products with less industrial processing and higher nutritional quality [3].

Bacteriocins are heterogenic antimicrobial peptides released extracellularly by Gram + and Gram- bacteria and, in some cases, also by *Archaea* [1,4]. They can trigger an inhibitory activity in the presence of undesirable microorganisms, including bacteria of the same genus and unrelated pathogens, such as *Clostridium* or *Listeria* [5]. Class I bacteriocins (lantibiotics) are small peptides that undergo extensive post-translational modification to produce the active peptide. Nisin, the most studied bacteriocin, belongs to the class I bacteriocins, which are active against a broad spectrum of pathogenic bacteria. The dose of nisin required to obtain the desired effect depends on several factors, including processing and storage conditions, carbon source, chemicals, bacterial load and the formulation of the product. Due to their possible use as natural preservatives, the bacteriocins produced by lactic acid bacteria have been the subject of intense research in recent years. In particular, Nisin A has been classified as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration [6] and recognized by WHO (World Health Organization) for the food industry [7]. Although nisin is considered a high-quality preservative for food processing and preservation, the costs associated with its production process are high, up to US$770 for 25 g additive containing 2.5% of pure nisin [6,8].

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This study addresses the conceptual design and environmental impacts of the biotechnological production of nisin (co-producing lactic acid) from three waste streams from the food industry. The valorization of these waste flows offers a series of benefits, since those residual fractions have an adequate composition in sugars, nitrogen compounds and proteins to be used as a substrate. Therefore, by using those streams in a waste valorization perspective, a high value-added product would be obtained, and the costs associated with the formulation of the synthetic culture media needed for the production process could be reduced [9].

The use of a simulation tool: SuperPro Designer®, especially valid for the design of biotechnological processes, allows the definition of the process flow diagrams and the design of the equipment, with the identification of the main input and output flows of each process stage. From the identification of mass and energy flows, the methodology of Life Cycle Assessment (LCA) will be applied to identify the main environmental impacts associated with the production process.

2. Materials and methods

2.1. Raw materials

The growth of the world population and the development of the world economy have led to an increase in the demand and consumption of food products, being dairy derivatives and those obtained from agricultural crops the most demanded. However, this high consumption will lead to the need to increase crop areas and livestock farms, which will be accompanied by the generation of a large amount of waste. According to FAO data, approximately one third of food products are managed as losses or waste streams annually, at a cost amounting to billions of dollars. Thus, the development of innovative biotechnological processes for the valorization of residues from farms and food manufacturing industries would not only represent a significant improvement in waste stream management systems, but also a reduction in the pressure on the environment, since it would avoid the development of other highly polluting processes would be avoided (such as burning of residues, deposition in landfills or toxicity on aquatic environments, among others).

Lignocellulosic residual streams are produced in large quantities in the food industry: SBP represents 50% of processed SB [10] while CS, about 200 million tons are produced annually, of which a 30% is collected and the rest is discarded in the field of crop exploitation [11]. Those crops are considered as valuable renewable resources whose biotechnological conversion shows enormous potential [10]. The amount of organic matter on those resources, together with their availability and costs [12] show a high potential to be used in the development of biotechnological processes.

With respect to CW, the amount generated, in volume, is analogous to the amount of milk processed, resulting in large quantities of this industrial by-product. Its potential to cause negative effects on the environment is very high due to its high organic load. Its most common use, to date, is based on animal feed, but given the increase in the consumption of dairy products, its generation volume is so high that the development of new forms of exploitation is required to avoid the generation of large quantities of unusable surpluses. That is why its valorization as a source of fermentable sugars for the development of fermentation processes is considered a viable and appropiate option.

Within the framework of the circular economy, industrial waste streams such as cheese whey (CW), sugar beet pulp (SBP) and corn stover (CS) represent sources of fermentable sugars with potential use in biotechnological processes. The high production volume of these residual fractions represents a clear advantage in their potential valorization [13,14]. As an example, some quantitative data in the Spanish context report that around 22,251 tons of cheese whey are produced annually [15], in the case of sugar beet pulp it amounts to 2,752,710 tons [15] and corn stover is estimated at 4,184,460 tons [13,15].

In relation to the composition of each waste stream, cheese whey has an adequate composition in sugars, mainly lactose (> 73%), proteins (about 12%), lipids (approximately 1.5%) and moisture (< 5%) [16]. The high content of sugars in sugar beet pulp is also noteworthy [17]: 22–30% of cellulose, 24–32% of hemicellulose, 1–2% lignin and 38–62% of pectin, which represent up to 75–85% of the dry matter [10]. While in the case of corn stover, its chemical composition with a high content of glucan, 39.5 g/100 g of corn stover, and hemicelluloses, 19.4 g/100 g of corn stover, would allow the release of fermentable sugars after an enzymatic hydrolysis in the saccharification stage [18].

2.2. Pre-treatment of raw materials

2.2.1. Cheese whey

Before the fermentation stage it will be necessary to include a pretreatment stage that will vary depending on the composition of the residual flows. In particular, cheese whey requires the simplest pretreatment, that is the sterilization of the stream and the removal of the proteins and fats by means of a heating stage (at 121 °C for 15 min) and centrifugation (12000xg, 15 min). In this way, the interference of the protein precipitation during the acidification phase, which is attributed to the formation of lactic acid, is avoided. This allows a more accurate measurement of the amount of biomass formed during the process. The use of CW as carbon substrate source requires the supplementation with additional nutrients such as yeast extract, diammonium hydrogen citrate and bacterial peptone, resulting in 23.85 g/L of total sugars, 3.49 g/L of nitrogen and 0.50 g/L of phosphorus [5].

2.2.2. Sugar beet pulp

From a technological point of view, the use of sugar beet pulp for fermentation processes is an efficient C-source due to its high cellulose and hemicellulose content and its low lignin content. However, to ensure a higher yield of fermentable sugars, a pre-treatment process combining several stages is necessary: acidification with 2% sulfuric acid, a heat treatment at 121 °C, followed by an enzymatic hydrolysis process [10].

Since the composition of SBP is mainly cellulose and hemicellulose, it is necessary to use enzymes capable of breaking down these polysaccharides into multiple monomers of glucose. Accordingly, cocktails of cellulases and hemicellulases are required, each with a dose of 0.02 mL/g of dry matter [10]. Once the enzymatic hydrolysis is completed, the fermentation medium is formulated with an adequate balance of nutrients, added as K_2HPO_4 and NH_4OH.

2.2.3. Corn stover

This waste stream requires more severe pre-treatment stages prior to enzymatic hydrolysis. In this regard, an autohydrolysis stage is conducted at 230 °C, 150 rpm and a liquid-solid ratio of 9 g of water/g of corn stover [18]. As in the previous scenario, an enzymatic process is also required to convert the glucan and hemicelluloses present in the corn stover: 39.5% and 19.4%, respectively, into monomeric glucose units, achieving about 70% conversion [18]. The operating conditions used in this process are the following: 4% consistency of the corn stover in liquid medium, 48.5 °C, 150 rpm and an incubation time of 72 h. Regarding the amount of enzymes required at this stage, 25 cellulase FPU (Filter Paper Units)/g substrate and also 5 IU (International Units) of cellobiase are added per FPU of cellulase [18]. Once the enzymatic hydrolysis is completed, the fermentation medium is formulated with K_2HPO_4 and NH_4OH.

2.3. Fermentation procedure

The fermentation scheme for the production of nisin will undergo slight modifications depending on the raw material considered. In the case of CW, the source of sugars is in the form of lactose, as it is its main component. Regarding SBP and CS, as both are lignocellulosic raw materials, it main components are cellulose and hemicellulose, which has been converted (due to the pre-treatment procedures before the fermentation one) into glucose by an enzymatic hydrolysis process. In the case of the simulation of nisin production from cheese whey, the conceptual design of the process has been proposed based on the available reports [5]. The aerobic fermentation process is based on a batch process with a fermentation time of 24 h, 30 °C, 200 rpm and a constant aeration of 0.5 L/h. The stoichiometry of the process allows to represent the fermentative process of microbial growth and the production of nisin and lactic acid.

$$11.85\ Lactose + 0.81\ NH_4OH + 0.10\ KH_2PO_4 + 10.07\ O_2 + 4.03\ Proteins \rightarrow 0.82\ Biomass + 0.49\ Nisin + 11.18\ CO_2 + 5.23\ Lactic\ acid + 9.15\ H_2O$$

Regarding nutrients, the mass balance considers equivalent amounts of NH_4OH and KH_2PO_4 for the total concentration of nutrients present in the culture medium [19,20]. Under these conditions, a lactose conversion of 50.83% is ensured which leads to biomass and nisin concentrations of 0.82 g/L and 0.49 g/L, respectively. The formation of lactic acid as by-product (5.23 g/L) must be accounted for, so it was considered necessary to include additional separation and recovery stages for this compound in the downstream processing.

Regarding SBP and CS substrates, although the operating conditions are analogous to those of the cheese whey culture medium, the reaction model and the fermentation performance achieved are significantly different [21], as depicted in the following reaction:

$$12.39\ Glucose + 1.00\ NH_4OH + 6.0\ K_2HPO_4 + 10.07\ O_2 \rightarrow 0.44\ Biomass + 0.27\ Nisin + 11.18\ CO_2 + 6.0\ Lactic\ acid + 11.57\ H_2O$$

The glucose conversion value amounts to 91.10%, significantly higher than when using lactose as a C source, leading to a fermentation product with concentrations of 0.44 g/L of biomass and 0.27 g/L of nisin. As for nutrient supplementation, the use of K_2HPO_4 and NH_4OH was considered.

2.4. *The downstream process for the separation and purification of nisin and lactic acid*

The downstream process begins with the rotary vacuum filtration for the biomass separation. Although there are several methods for nisin purification [22], precipitation with 40% ammonium sulfate in an equipment that is based on a combination of a centrifugal extractor and a basket centrifuge. This is a suitable procedure for separating nisin from co-products, which requires a prior step of acidification with HCl from the stream entering the extractor [23]. As the equipment has a filtering medium, nisin is retained, while the rest of the components would be discharged through the bottom of the basket. Then, the nisin retained in the filter is washed with water, so that two streams are obtained, a suspension of nisin and water, and a liquid stream with lactic acid that is sent to a recovery sequence. To remove the water content from the nisin suspension, the stream is processed in a fluid bed dryer, using steam as the heating medium. In this way, nisin can be stored for later use.

The process of separation and purification of lactic acid is divided into five main stages, starting with a centrifugation stage for solids removal. In the next stage, the concentration of the lactic acid is performed in a drum drying process. The third stage of the recovery process is based on an esterification reaction, which has proven to be very effective in obtaining greater recovery and purity of the lactic acid [24]. This reaction takes place in a continuous stirred tank reactor (CSTR) at 80 °C (using steam as the heating medium), in which the reaction of lactic acid with methanol takes place to obtain methyl lactate and water with a conversion rate of 85%. Methyl lactate is separated in a distillation column at 150 °C and atmospheric pressure, while the rest of the compounds (i.e. manganese sulphate, ammonium chloride and sulfuric acid) are withdrawn through the bottom of the column and handled as process residues.

As a final stage of the lactic acid recovery process, a hydrolysis stage is required. This is carried out in a CSTR-type reactor, in which water is added, which will react with methyl lactate to produce lactic acid and methanol, which can be recycled back to the esterification stage. In order to develop the separation of the methanol and the lactic acid, a flash evaporation is carried out at 66 °C and atmospheric pressure. Within this process, 78% of pure lactic acid is obtained, and methanol is recycled to the CSTR-type reactor.

2.5. *Simulation procedure*

The nisin and lactic acid co-production process is divided into three sections (Fig. 1): the core process based on the fermentation stage and the downstream process corresponding to the separation of biomass and products: nisin and lactic acid.

Considering the fermentation stage and the processing capacity of the downstream process, the amount of nisin produced per batch is estimated at 100 kg. Applying the heuristics of the annual operating time of the facility at 330 days/year, the amount of nisin and lactic acid produced in one-year depends on the batch time of each of the production processes developed. The capacity of each production process is shown in Table 1.

Tables 2, 3 and 4 depict the global balance of components, utilities and energy requirements associated with the nisin production process using CW, SBP and CS as substrates, respectively. The data is reported on the basis of the functional unit selected: 1 kg of products.

2.6. *Environmental sssessment*

2.6.1. *Life cycle assessment methodology*

Process simulation using the SuperPro Designer® tool serves as a strategy to identify the input and output flows of each of the process units and thus compile the inventory data needed to conduct the environmental study of the impacts associated with nisin production. The Life Cycle Assessment (LCA) methodology, based on ISO 14040:2006, has been used as a tool to determine the environmental profiles of the different stages of the process. Once the environmental profiles are obtained, the main hotspots (i.e. the component (s) that give rise to the greatest contribution to the environmental impacts) can be identified and, based on these outcomes, a sensitivity analysis of certain critical variables can be carried out with the aim of improving the environmental profile of nisin production.

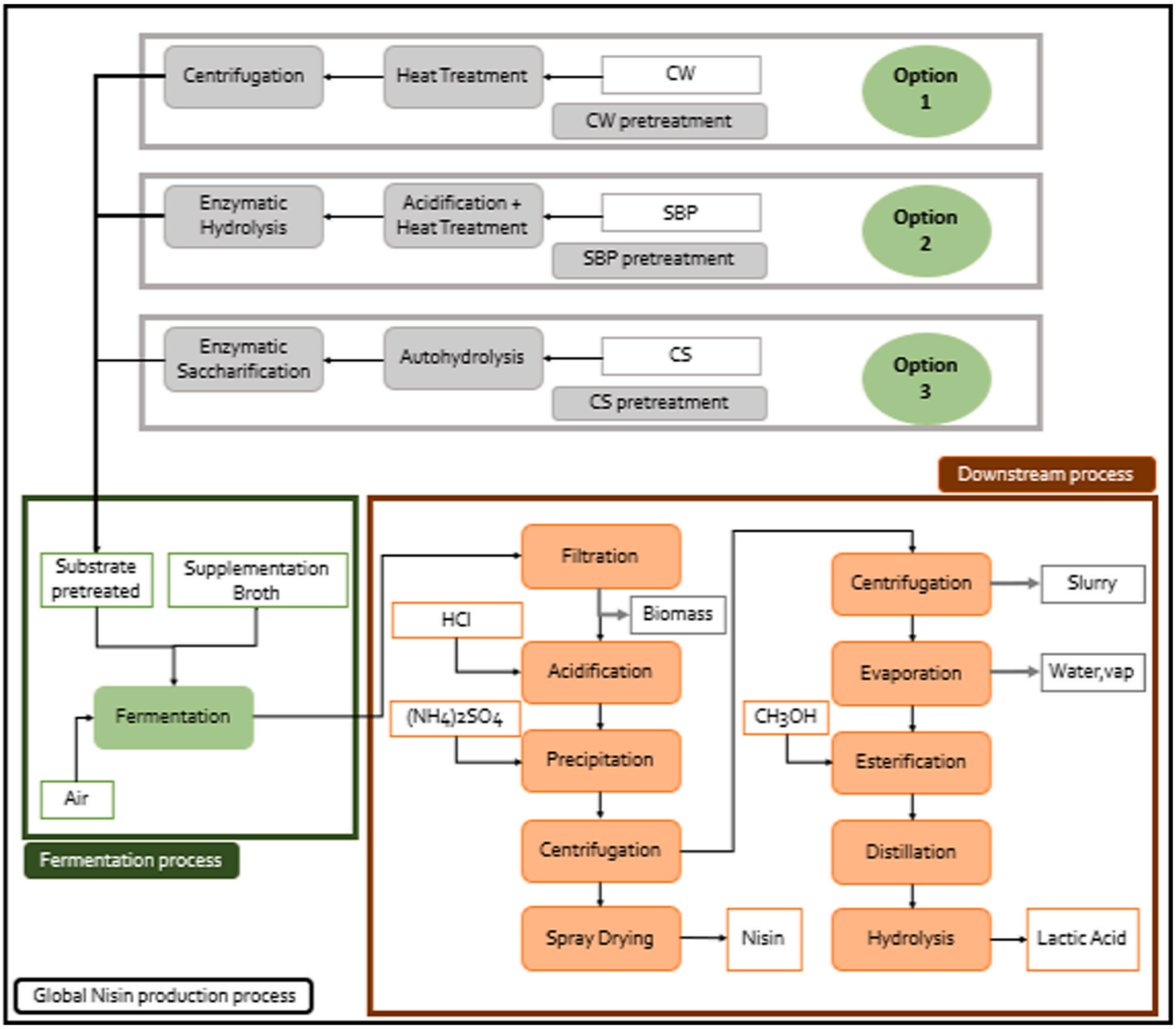

Fig. 1. Block diagram of nisin production process with lactic acid recovery using CW (Cheese Whey, Option 1), SBP (Sugar Beet Pulp, Option 2) and CS (Corn Stover, Option 3) as substrates.

Table 1

Production capacity of the different alternatives studied for nisin production.

Process	CW	SBP	CS
Batch time (h)	77	79	143.58
Cycle time (h)	30.75	25	72
Number of batches per year	256	314	109
Nisin production (kg/year)	25487.54	31640.14	10901.40
Lactic acid production (kg/year)	281216.73	765141.76	243595.91

Table 2

Overall mass balances for nisin production using Cheese Whey as substrate.

Inputs (kg/kg of products)			Outputs (kg/kg of products)		
Material	Amount	Unit	Material	Amount	Unit
Air	117.04	kg	Products	1.00	kg
Ammonium Sulfate	4.04	kg	Nisin	8.31	g
HCl	5.74	kg	Lactic acid	91.69	g
KH_2PO_4	84.34	g			
Methanol	38.29	g	Emissions to air		
CW	102.97	kg	CO_2	1.89	kg
NH_4OH	0.58	kg	Water, vapor	2.12	kg
Water	77.92	kg			
Energy & Utilities			Waste to treatment		
Material	Amount	Unit	Precipitates	0.59	kg
Cooling water	3.18	m^3	Biomass	0.23	kg
Steam	383.24	kg	Slurry	17.46	kg
Energy	13.50	kWh	Final Waste	2.12	kg

2.6.1.1. Functional unit, system boundaries and impact categories. The functional unit (FU) considered was the production of 1 kg of products. The reason for choosing a mass-based FU is the simplicity associated with information management and also the ease of comparison with other future studies on the topic [25–29]. Regarding the system boundaries, a cradle-to-gate approach was considered, from the extraction processes to the manufacturing of the final product [30]. In this study, two methodologies were selected: CML-IA Baseline V3.05 / EU25 and Recipe 2016 Endpoint (H) V1.03 World (2010) H/H. This selection is based on the recommendations of the JRC European Commission for the development of Life Cycle Assessments [31]. According to the International Reference Life Cycle Data System Handbook, the CML methodology is the one recommended to report midpoint categories and Recipe in the case of endpoint ones.

The CML impact categories considered in this study are the following: Abiotic Depletion (AD), Abiotic Depletion- Fossil Fuels (AD,

Table 3
Overall mass balances for nisin production using Sugar Beet Pulp as substrate.

Inputs (kg/kg of products)			Outputs (kg/kg of products)		
Material	Amount	Unit	Material	Amount	Unit
Air	615.43	kg	Products	1.00	kg
Ammonium Sulfate	0.41	kg	Nisin	3.94	g
Enzymes	40.49	g	Lactic acid	96.06	g
HCl	0.46	kg			
K_2HPO_4	1.58	kg			
Magnesium Sulfate	31.61	g			
Methanol	31.97	g	Emissions to air		
SBP	11.49	kg	CO_2	1.68	kg
NH_4OH	0.32	kg	Water, vapor	11.68	kg
Sulfuric Acid	0.23	kg			
Water	2.07	kg			
Energy & Utilities					
Material	Amount	Unit	Waste to treatment		
Cooling water	0.78	m^3	Biomass	0.93	kg
Steam	15.824	kg	Slurry	1.83	kg
Energy	5.984	kWh	Final Waste	0.39	kg

Table 4
Overall mass balances for nisin production using Corn Stover as substrate.

Inputs (kg/kg of products)			Outputs (kg/kg of products)		
Material	Amount	Unit	Material	Amount	Unit
Air	756.31	kg	Products	1.00	kg
Ammonium Sulfate	0.45	kg	Nisin	4.28	g
Enzymes	9.53	kg	Lactic acid	95.72	g
HCl	3.73	kg			
K_2HPO_4	1.15	kg			
Magnesium Sulfate	34.64	g			
Methanol	51.04	g	Emissions to air		
CS	10.40	kg	CO_2	1.81	kg
NH_4OH	0.35	kg	Water, vapor	97.27	kg
Sulfuric Acid	0.21	kg			
Water	95.03	kg			
Energy & Utilities					
Material	Amount	Unit	Waste to treatment		
Cooling water	22.95	m^3	Biomass	4.00	kg
Steam	123.52	kg	Slurry	17.65	kg
Energy	21.36	kWh	Final Waste	0.73	kg

FF), Global Warming Potential (GWP), Ozone Layer Depletion (ODP), Human Toxicity (HT), Freshwater Aquatic Ecotoxicity (FET), Marine Aquatic Ecotoxicity (MET), Terrestrial Ecotoxicity (TET), Photochemical Oxidation (PO), Acidification (AC) and Eutrophication (EP). In the case of Recipe Endpoint methodology, the impact categories analyzed are three: Human Health, Ecosystem Quality and Resource Scarcity.

2.6.1.2. Limitations of the study and data sources considered. Considering that the production process is proposed based on process simulation data, transport activities related to the transport of materials or products have not been considered, since, when a delocalized process is proposed, there are no fixed transport routes associated with the process. In relation to the specific data of certain chemicals and process inputs and their availability in the databases, several simplifications have been assumed. For example, KH_2PO_4 was evaluated as KOH, since the molar ratio of phosphorus in both compounds is the same. For NH_4OH added as a nitrogen source in the culture media formulation, NH_4NO_3 was evaluated as an alternative [32]. As for the database considered for the environmental assessment, the Ecoinvent® version 3.2 database has been chosen to handle the secondary data associated with the background activities of all utilities (i.e. steam, cooling water), inputs (i.e. whey, SBP, CS, ethanol) and waste streams. Database (Table 1SM) and inventories considered for applying the LCA methodology are included in Tables 2 to 4 for the overall material and energy requirements of each production process.

2.6.2. *Allocation approach*

An allocation approach is developed to analyze what contribution each co-product makes to the environmental impact values obtained. In this case, two different options were developed, the first based on a mass-allocation approach, taking into account the production of nisin and lactic acid per kg of products obtained, and the second based on an economic allocation, in which the sales prices of nisin and lactic acid are considered.

2.7. *Economic assessment*

In the case of the base scenario, the initial investment required for the installation, the income from the sales of the manufactured product and the production costs of each of the elements associated with the industrial facility were determined. As for the initial investment required for the installation, both fixed capital and working capital costs have been considered. This section determines the costs associated with the acquisition of the equipment, including indirect costs, as well as the costs associated with the purchase of the chemicals, such as ethanol, ammonium sulfate or sulfuric acid, and the utilities, i.e., cooling water, steam. As for substrates: CW, SBP and CS, as they are considered industrial processing by-product streams, no costs are allocated to their use. As for the income obtained and according to the information provided by the Belgian company Handary, the cost of nisin is 85 €/kg of nisin.

The economic calculations of the process have assumed a construction period of 9 months and 1 month for the start-up. The average life of the project is assumed to be 30 years with an income tax of 25%. It has been considered that the plant operates at 100% of its production capacity for 11 months a year, leaving 1 month for periodic maintenance. The Net Present Value (NPV) has been selected as the financial indicator that determines the viability of a project, provided that its numerical value is greater than 0. Its value has been determined considering an interest rate of 3%.

3. Results and discussion

3.1. *Outcomes of the environmental analysis*

In order to evaluate which of the alternatives proposed is the one that could be considered as the more environmentally friendly, an analysis of the impact results obtained for each manufacturing process has been carried out. Accordingly, it is possible to identify the main hotspots of each alternative to determine over which component of the system is better to work on if the reduction of the environmental impacts is required.

First, a global assessment of the impacts associated with the three production processes considered is carried out, choosing as the functional unit the production of 1 kg of products (including nisin and lactic acid). Once this first analysis has been carried out, the impact values obtained are studied taking into account a mass allocation and an economic allocation, in this way it is possible to identify the robustness and variability of the impact results based on the selection of the allocation procedure and thus, to validate the results independently of the approach considered.

3.1.1. *Environmental assessment using CW as substrate*

The environmental profile of the co-production of nisin and lactic acid using cheese whey as substrate is shown in Fig. 2.

Fig. 2 shows the contributions of the different components used for the nisin and lactic acid co-production using cheese whey as substrate. As can be seen, two main contributions can be selected according to the environmental profile: steam (produced from non-renewable energy) and whey as a carbon source for the fermentation process. For the latter, the analysis of the environmental profile of its production process is attributed to the background activities involved, especially regarding

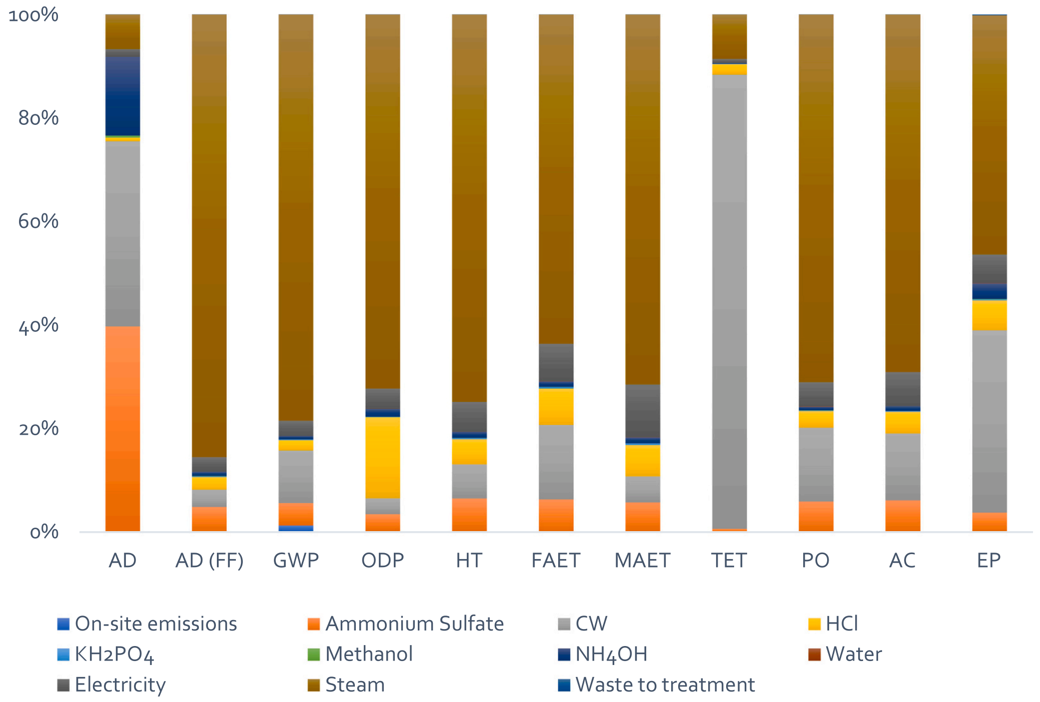

Fig. 2. Environmental profile of nisin and lactic acid co-production using CW as substrate.

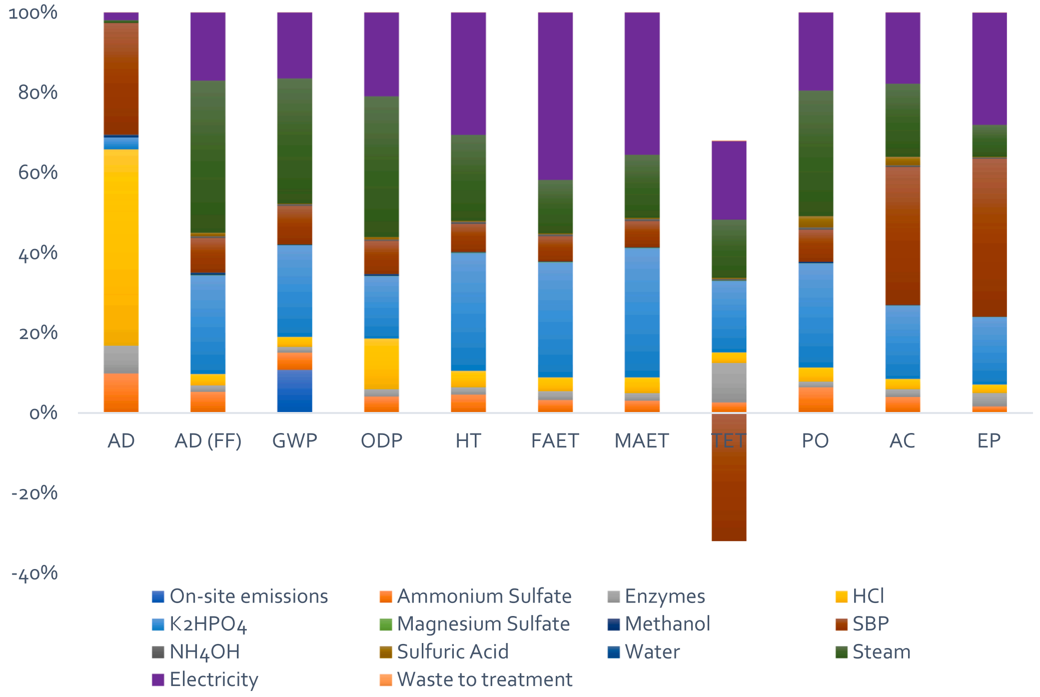

Fig. 3. Environmental profile of nisin and lactic acid co-production using SBP as substrate.

raw materials treatment processing, heat requirements and effluents processes [29]. On the other hand, the use of ammonium sulfate in the production process also implies a certain share in the environmental impacts, especially in the abiotic depletion category due to the significant requirement of energy in the production process.

3.1.2. *Environmental assessment using SBP as substrate*

Once the CML methodology is applied for the inventory considered for the co-production of nisin and lactic acid by using SBP as substrate, the environmental profile is depicted in Fig. 3.

The environmental profile of the manufacturing process using SBP as a carbon source reflects that energy needs have the greatest contribution, representing more than 50% of the impact value in almost all the categories studied. However, three exceptions could be identified, namely in the AD, AC and EP categories.

The relevance of the background activities associated with the SBP on the AC and EP impact categories show that the agricultural activities are the main contributors in the environmental profile, which is

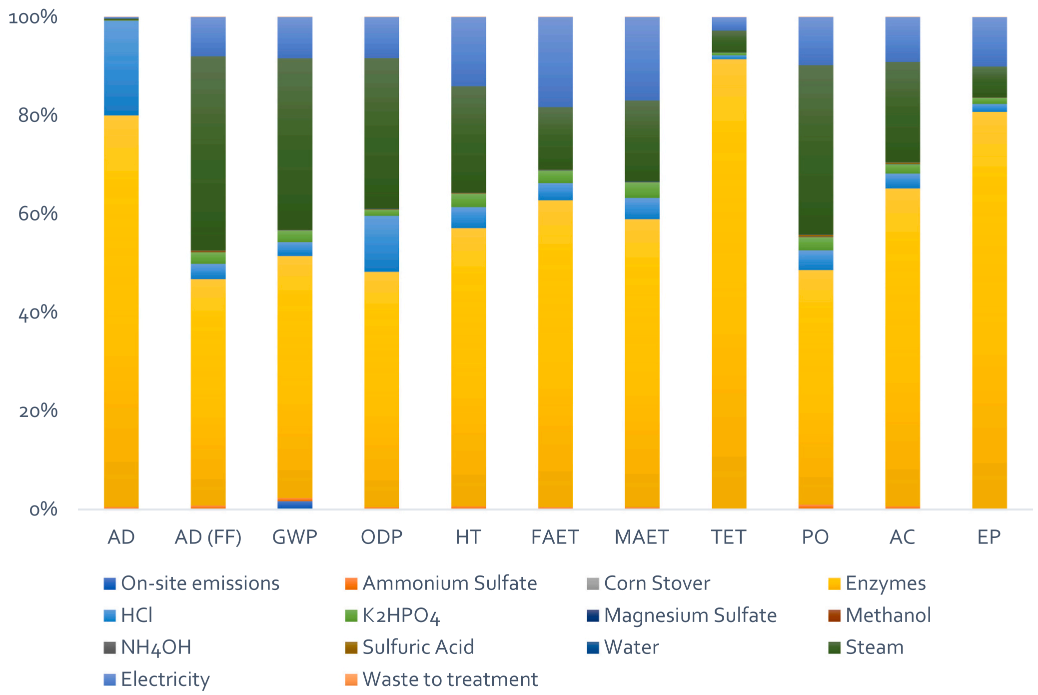

Fig. 4. Environmental profile of nisin and lactic acid co-production using CS as substrate.

attributed to field emissions resulting from the application of fertilizers and agrochemicals. On the other hand, the use of fuel and electricity needed for crop processing also has some contribution within the AC category, due to the release of acidifying substances. Finally, for the case of the AD impact category, the energy requirements for the production of HCl has been identified as the main reason for its enormous contribution.

3.1.3. *Environmental assessment using CS as substrate*

The contribution of each input associated with the production of nisin and lactic acid by using corn stover as carbon source is shown over the environmental profile obtained by applying the LCA methodology (Fig. 4).

As expected, the contribution of the use of enzymes for the saccharification process of the corn stover has a high impact on the environmental profile obtained (Fig. 4). Its contribution on the impact categories studied is equal or even higher than 50%, which makes it necessary to carry out an analytical study to identify the reason for its environmental contribution within the profile. For this purpose, the background of the activities associated to the production of enzymes has been analyzed. It has been identified that the energy requirements together with the agricultural activities necessary for the production of vegetable crops used for enzyme production are the main hot spots.

Finally, it should be mentioned that the energy requirements of the process, electricity and steam, also have some impact on the environmental profile, highlighting the categories of AD (FF), given the consumption of non-renewable fossil resources for energy production, and GWP, contribution resulting from emissions generated within the processes of steam and electricity production.

Table 5
Allocation parameters considered for performing mass and economic allocation procedures. Acronyms: MA (Mass Allocation), EA (Economic Allocation).

Allocation Parameters		Nisin	Lactic Acid
CW	MA	8.31%	91.69%
	EA	41.19%	58.81%
SBP	MA	3.94%	96.06%
	EA	24.06%	75.94%
CS	MA	4.28%	95.72%
	EA	25.70%	74.30%

Table 6
Damage potential values obtained by applying Recipe EndPoint methodology to the proposed alternatives.

Damage category	CW	SBP	CS
Human Health (Pt)	3.20	0.42	3.17
Ecosystem Quality (Pt)	0.38	0.05	0.53
Resource Scarcity (Pt)	0.15	0.01	0.09
Total (Pt)	3.73	0.48	3.79

3.1.4. *Comparison of the damage potential of each proposed alternative*

Evaluating the values obtained through the Recipe Endpoint methodology (Table 5), it is concluded that the co-production of nisin and lactic acid that results in the least damage potential is the one that uses SBP as a substrate. Another significant aspect of the results obtained is that, according to this methodology, the Human Health damage category is the most affected, since its value is almost three times higher than that obtained for the Ecosystem Quality and Resource Scarcity damage categories. The use of fewer chemicals, efficient energy use and consideration of the option of using renewable energy resources would mean a reduction in the value of the damage obtained for this impact category, reducing in turn the value of the overall damage associated with the processes. Therefore, in order to improve the overall environmental quality of the processes studied, studies based on the optimization of the production process would be required.

3.1.5. *Allocation approach*

The values considered to perform the mass and economic allocation are those included in Table 6. As it has been included in the table referring to the production capacities of the processes (Table 1), the amount of lactic acid produced annually is significantly higher than the amount of nisin generated, which will mean that when making the allocation of mass and evaluate the environmental impacts associated with nisin, these will be much lower than those corresponding to the production of lactic acid. On the other hand, the market value of nisin, given its wide potential for use in the sector of food and pharmaceuticals, is much higher compared to that of lactic acid. This fact will lead to the fact that, when conducting an environmental analysis within an economic allocation, the contribution of nisin to the environmental impacts derived from the production process will be greater to the consideration of a mass-based allocation.

But even so, an allocation percentage of more than 41.19% will not be achieved for nisin, even considering its monetary market value, since the production capacity of nisin is much lower than that of lactic acid (Table 6). Given the greater accessibility of the production of nisin from cheese whey, thanks to the fact that this substrate can be used directly in the fermentation without the need of a pre-treatment for the release of fermentable sugars (since lactose is already freely present in its composition), the value of the contribution percentage of nisin, considering both mass and economic allocations, is the highest of the three alternatives considered. This is favorable in productive and economic terms, since there is a larger production capacity of the co-product with greater potential and higher market value, but it is unfavorable in terms of the environmental impacts generated, since the nisin produced from CW will have a greater contribution in comparison with the use of SBP or CS as substrates.

Thus, globally it could be considered that the contribution of lactic acid production to the environmental impacts generated by all the productive processes studied is greater in comparison with that of nisin. This statement could be verified with the values reported in Table 7, which refer to the contribution of nisin on the environmental results for the three process alternatives for each of the impact categories of the CML methodology.

3.2. Outcomes of the economic analysis

Regarding all the considerations described on Section 2.7, the values obtained for the economic evaluation of the biotechnological co-production process of nisin and lactic acid from the different substrates are shown in Table 8.

The economic indicators showed that the proposed manufacturing processes are economically viable in the case of CW and SBP substrates, since the Net Present Values (NPV) are positive. On the contrary, in the case of the CS substrate, the negative NPV value reflects that the total revenues obtained during the life of the project considered (30 years) is not sufficient to take over all the manufacturing and investments cost associated with the process. In more detail, the item of material costs presents very different values, compared to the CW and SBP process. A percentage of 99% of the material cost of the CS process is related to the amount of enzymes needed for the saccharification stage, as can be seen in Table 4 (9.53 kg of enzymes/kg of products obtained). As the prices of the enzymes are high (around 2.47 €/kg), their contribution to the economic performance will also be significant. Thus, the economic viability of the CS process could be achieved if the amount of enzymatic materials needed for saccharification is optimized (and reduced).

Table 8
Economic parameters obtained by performing the economic evaluation of the co-production of nisin and lactic acid by using different substrates. Acronyms: CW (Cheese Whey), SBP (Sugar Beet Pulp), CS (Corn Stover).

Economic parameters	CW	SBP	CS
Total Investment [€]	33,715,000	52,222,000	27,080,000
Fixed Capital [€]	32,029,000	49,629,000	25,164,000
1. Total Plant Direct Cost [€]	27,611,000	42,784,000	21,693,000
2. Total Plant Indirect Cost [€]	4,418,000	6,845,000	3,471,000
Labor Cost [€/year]	253,808	870,286	585,914
Material Cost [€/year]	81280	129,311	6,008,225
Utilities Cost [€/year]	592,640	338,692	640,902
Annual Operation Cost [€/year]	2,222,000	3,367,000	8,271,000
Revenues [€/year]	5,259,823	11,105,971	3,577,306
1. Nisin [€/year]	2,166,441	2,689,412	926,619
2. Lactic Acid [€/year]	3,093,382	8,416,559	2,650,687
Gross Profit [€/year]	3,038,000	7,739,000	-4,694,000
Net Profit [€/year]	2,279,000	5,804,000	-4,694,000
Gross Margin [%]	57.76	69.68	-131.20
Return on Investment [%]	6.76	11.11	-17.33
Payback Time [years]	14.80	9.00	N/A
Net Present Value [€]	14,386,000	68,556,000	-116,455,000

On the other hand, regarding CW and SBP processes, the economic parameters of Return on Investment (6.67 and 11.11 respectively) and Payback time (14.80 and 9.00 respectively), reflect that the necessary investment for the development of the manufacturing processes is reasonable, leading to good alternatives for the co-production of nisin and lactic acid from waste streams, promoting in this way the circular economy concept.

3.3. Discussion

To best of our knowledge, there are no simulation studies available on the production of nisin from waste feedstocks. We would like to remark that experimental studies on the valorization of waste streams for the production of nisin can provide useful information for process modelling. The importance of the pretreatment stage for the release of fermentable sugars is evidenced as an indispensable requirement, as highlighted in the work carried out by Liu et al. [33] where the enzymatic hydrolysis of deffated soybean meal (DSM) is analyzed. In another work the influence of the pretreatment process on the amount and maximum activity of nisin produced, using soy as raw material was evaluated [34]. It should be noted that both chemical and enzymatic hydrolysis of the lignocellulosic biomass can be used for the release of fermentable sugars, although it is the enzymatic process that is the most widespread and recurrent among the reports available in the literature ([33–38], that is why it has been selected as a pretreatment step in the simulation of the proposed process for large-scale nisin production. On the other hand, regarding the fermentation medium, additional nitrogen and phosphorus are needed to formulate a balanced growth medium for L. *lactis*. Other studies available in literature have probed that the use of

Table 7
Nisin contribution over the impact categories of the CML methodology considering a mass allocation (MA) and an economic allocation (EA) procedure.

Impact category		*CW*			*SBP*			*CS*		
unit		Total	MA	EA	Total	MA	EA	Total	MA	EA
AD	mg Sb eq	**12.58**	1.05	5.18	**5.14**	0.2	1.24	**106**	4.53	27.18
AD (FF)	MJ	**1763**	146	726	**164**	6.45	39.38	**1234**	53	317
GWP	kg CO_2 eq	**150.3**	12.49	61.91	**15.57**	0.61	3.75	**109**	4.68	28.05
ODP	mg CFC-11 eq	**15.26**	1.27	6.29	**1.3**	0.05	0.31	**11.63**	0.5	2.99
HT	kg 1.4-DB eq	**25.26**	2.1	10.4	**3.64**	0.14	0.88	**28.21**	1.21	7.25
FAET	kg 1.4-DB eq	**17.97**	1.49	7.4	**3.55**	0.14	0.85	**29**	1.24	7.45
MAET	kg 1.4-DB eq	**71,302**	5925	29,368	**13,377**	527	3219	**99,896**	4279	25,669
TET	g 1.4-DB eq	**666**	55	274	**6**	0.23	1.41	**416**	17.8	107
PO	g C_2H_4 eq	**26.65**	2.22	10.98	**2.49**	0.1	0.6	**17.76**	0.76	4.56
AC	g SO_2 eq	**539**	45	222	**84**	3.32	20.26	**588**	25.17	151
EP	g PO_4^{-} eq	**145**	12	60	**35**	1.36	8.32	**345**	14.78	89

the following optimized medium [33]: 30 g/L od deffated soy meal hydrolysates, 25 g/L of KH_2PO_4, 12 g/L of sucrose, 1.5 g/L of NaCl and 0.05 g/L of $MgSO_4$ is the one with which a higher amount of nisin is produced. Thus, it is demonstrated that adding KH_2PO_4, as considered for the reaction model proposed in this manuscript, is cost-effective. Regarding nitrogen source, different compounds could be used: cysteine [33], hemin [38], peptone [36], but the use of NH_4Cl has been successfully demonstrated to be an efficient nitrogen source for the fermentation process of nisin production by L. *lactis* [39], which is the one considered for the process simulation developed in this report.

The results obtained suggest that the use of cheese whey as a carbon source in the fermentation process leads to a higher production of nisin per kg of product generated, although, on the other hand, considering the economic feasibility and the environmental impacts generated, sugar beet pulp is considered as the substrate with the greatest potential. Moreover, given that the development of these processes allows the co-production of nisin and lactic acid, it is essential to carry out an allocation approach to determine which of the products makes a greater contribution to the economic and environmental results. Since the productive capacity of the fermentation process leads to the generation of a greater amount of lactic acid, the impact of this co-product on the evaluations would be greater.

Regarding the use of corn stover as a substrate medium, the large amount of enzymes required in the enzymatic hydrolysis and saccharification process entails a large contribution in both the economic and environmental studies, so it is considered as the carbon source with the lowest potential of the three alternatives studied. In order to consider the use of this renewable resource as a potential option for the co-production of nisin and lactic acid, an optimization study of the pre-treatment processes for the release of the fermentable sugars present in its structure would be required, and given the wide availability of this substrate, it would be interesting to consider a future research study on this topic to improve the productive efficiency of the process.

4. Conclusions

This study analyzes the development of three alternatives for the co-production of nisin and lactic acid using three renewable feedstocks as carbon sources, thus favoring the concept of sustainability and circular economy, while reducing waste generation. Process simulation and life cycle analysis help to determine the feasibility of the process from a technical, economic and environmental point of view at an early stage of development.

CRediT authorship contribution statement

A. Arias: Methodology, Formal analysis, Investigation, Writing - original draft. **G. Feijoo:** Writing - review & editing. **MT 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.bej.2021.108105.

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