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Exploring the production of bio-succinic acid from apple pomace using an environmental approach

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HIGHLIGHTS

- Based on lab data, the LCA of BioSA at industrial scale is modelled.
- LCA was applied to assess the environmental profile.
- The results highlight hotspots in the production process.
- Distillation unit to solvents recovery plays a key role.
- Recommendations are given to improve the environmental profile and production process.

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ABSTRACT

Fermentation-derived bio-succinic acid (BioSA) is a valuable intermediate; it is used as a chemical building block, and has multiple industrial applications as an alternative to petroleum counterparts. The aim of this study was to develop a full-scale plant to produce BioSA from apple pomace, a low-cost solid waste from the cider- and juice-making industry, based on a biorefinery concept, and to determine its environmental profile using a cradle-to-factory-gate, scaled-up LCA approach. Foreground data used in this LCA were based on mass and energy flows, modelled in detail. The production process was divided into three stages: i) reconditioning and storage; ii) fermentation with *Actinobacillus succinogenes*; and iii) purification. The results indicate that the use of enzymes is responsible for the highest environmental burdens, due to their highly energy-intensive background production processes. When these were excluded from the analysis (following other studies available in the literature), the purification stage played an environmentally significant role, due to the extraction and distillation units involved. The electricity use and the requirements for organic solvents in these operations make up the largest environmental burdens. Thus, approaches with the highest potential for improvement must involve both operations. Alternatives for improvement are proposed that offer interesting potential reductions in the environmental profile, especially at the purification stage.

1. Introduction

Today, both society and industry are facing important challenges regarding the use of biomass and the production of bio-based materials, in relation to social responsibility and environmental concerns. Hence, the substitution of petroleum-based materials by their bio-based counterparts is a key factor in the battle against climate change [1].

Bio-based products have been promoted as part of sustainable consumption strategies, and are obtained from the integration of eco-innovation approaches aimed at reducing greenhouse gas (GHG) emissions and combating the depletion of fossil sources [2]. New environmental regulations and economic considerations also support this interest in renewable sources [3]. Bio-based materials (e.g. wood, paper and textile products) and synthetic ones produced from fossil feedstocks account for 14% and 7%, respectively, of the global production of bulk materials [4]. Thus, the interest in substituting biomass sources for fossil feedstocks within the production of synthetic materials has increased in recent years, with the aim of guaranteeing the security of the supply of industrial feedstocks.

Biomass is an available resource abundant in the nature, and is diverse and recyclable; it has multiple applications either as a clean source of renewable energy or as a raw material for the production of biomaterials and biochemicals [3,5]. Concerns regarding competition with the food and feed sectors at a global level have encouraged the

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utilisation of biomass waste as a potential feedstock. In view of this, the valorisation of biomass residues is receiving attention, and its use is expected to increase in the future, mainly in emerging technologies in the production of second generation biofuels and in the recovery of high-added-value products [6].

The general assumption that bio-based materials are environmentally superior to fossil-based ones requires detailed analysis. In order to guarantee improvements in the environmental profile of bio-based products, it is mandatory to perform a life cycle assessment (LCA)-based study, since the term “bio-based” is not always synonymous with “environmentally friendly” [2], particularly in terms of less well-known impact categories such as eutrophication, acidification, water depletion [4,5] and land use. Hence, if dedicated biomass is valorised, the cultivation activities related with biomaterial feedstock production play an important role, especially in areas of significant social value [7]. A sustainability study is therefore required to identify situations in which the use of bioresources over petrochemical ones is environmentally feasible.

The introduction of the biorefinery concept and the challenge of integrating bio-based chemicals are key issues generating attention to the valorisation of waste in the industrial sector. In 2004, the US Department of Energy identified the most important chemicals that could be obtained from biorefinery carbohydrates [8]. One of these chemicals was succinic acid ($C_4H_6O_4$) (or butanedioic acid), which is a promising renewable platform chemical, mostly due to its functionality and the value of its derivatives [9]. There is extensive recent literature focused on its production and use as chemical building block [5,10–12]. Succinic acid is a precursor of several well-known petrochemical products such as 1,4-butanediol, tetrahydrofuran, γ-butyrolactone and polybutylene succinates, among others. Moreover, succinic acid has multiple industrial applications in biodegradable polymers (polyesters, polyamides and polyesteramides), foods (e.g. as an acidulant, flavorant and sweetener), fine chemicals and pharmaceuticals [13–14]. However, it has been commonly considered a niche product, primarily due to its high price [14]. Currently, it is mainly produced from n-butane/butadiene by a chemical process via maleic anhydride, using the C4 fraction of naphtha [14]. The global market has been predicted to grow by around 19% annually between the years 2011 and 2016 [5]. However, the price fluctuations of petroleum-based counterparts and environmental concerns have motivated an interest in the production of BioSA [5,13,14]. It can be obtained from the biological transformation of biorefinery sugars (via the bacterial fermentation of carbohydrates), from a variety of feedstocks and using multiple microorganisms [15]. Moreover, carbon dioxide is needed by these microorganisms for BioSA production, as carbon dioxide fixation is involved in the reductive TCA cycle, and this can provide environmental benefits such as the reduction of greenhouse gas emissions [13,14]. Several companies (e.g. BioAmber and Mitsui & Co) are therefore working on the commercialisation of BioSA [5]. Currently, this represents less than 5% of total succinic acid production [5].

As previously indicated, multiple types of biomass sources can be used for the production of BioSA through microbial fermentation [12]. The most frequently used carbon sources in industrial fermentation are purified sugars and glucose syrup from corn [13]. However, the use of agricultural and food residues and industrial side streams have interesting results, primarily from a sustainability perspective. Of these, apple pomace is a potential feedstock; this is the main solid waste produced in cider and apple juice factories [16], and can add up to as much as 35% of the total processed raw material. Apple pomace is a term for the solid residues, which consist of a mixture of skin, pulp and seeds derived from the production of concentrated apple juice, jam and sweets [17]. Since they are highly biodegradable, the disposal of these wastes represents an interesting environmental problem involving several challenges. Although apple pomace is used as a feed component (a low added value use) and in pectin production, this use requires only 20% of the total production, and the remaining 80% is sent to landfill [17]. Thus, numerous studies have been performed with the aim of identifying other potential applications [16]. The production of high added value products such as lactic acid, oligosaccharides [18], citric acid, antioxidants, dietary fibers and even biopolymers (chitosan and xanthan gum) have received particular attention [17].

In this study, an assessment is performed of the environmental impacts arising from the valorisation of apple pomace from the cider industry into BioSA by microbial fermentation; this follows the LCA methodology and uses a cradle-to-factory-gate approach. To our knowledge, there are only two peer-review studies that analyse the environmental impacts of BioSA [5,11], and these examine alternative feedstocks (such as glucose from corn or sorghum). In the following, a large-scale system for BioSA is described in detail, and particular attention is paid to the design process.

2. Materials and methods

The LCA is a widely used and standardised tool for the systematic evaluation of the environmental aspects of a product or production system throughout all stages of its life cycle [19]. It is also considered to be an ideal instrument for evaluating the environmental dimension of sustainability. Although the initial applications of the LCA involved consumer products [20], this tool has been used in the environmental analysis of industrial and chemical processes at various scales in recent years [5,11,21–23], and its applicability in this area has therefore been demonstrated.

2.1. Definitions of goal and scope

The goal of this LCA study is to provide data on the environmental impact of the BioSA production process at a commercial scale, with the aim of offering insight to researchers, industry and wider society regarding the development of this green platform chemical intermediate for bio-based polymers. To achieve this, a full-scale process is modelled based on data at the laboratory scale [24,25]. An attributional cradle-to-gate approach is used in this case study, using the apple pomace as the main raw material. The functional unit used to report the environmental profile is 1 kg of apple pomace-based BioSA (white crystalline solid form, industrial grade, ≥99.5% wt), since no application is specified in this study and since this product has a diverse range of uses. A detailed description of the BioSA crystals produced is given in Table 1.

A contribution analysis of the different stages or production steps is also performed, with the aim of identifying the environmental hotspots in the production system. The identification of these allows us to mitigate the overall environmental profile by proposing actions for improvement to be taken into consideration in further research and development activities. Energy and mass balances (including the growth of the bacteria *Actinobacillus succinogenes*) are therefore performed in the modeling of the full-scale BioSA plant, with the aim of gathering all the required data for the life cycle inventory stage.

With the aim of developing a biorefinery approach, and due to the recent interest in joint ethanol fermentation/succinate production [15], the BioSA plant has been designed as part of a promising biorefinery

Table 1
BioSA characteristics and physical properties [5]

	Property					
	Melting Point	Boiling Point	Moisture	Other Organic Acids	Appearance	Purity
BioSA	185 °C–190 °C	235 °C	≤0.5%	0.1%	White Crystalline solid	≥99.5%

platform for achieving BioSA production from residual CO_2 from a existing ethanol refinery.

In the fermentation step, the fermentative route depends on the host selected for the production. Succinate producers include microorganisms such as *Escherichia coli* or *Saccharomyces cerevisiae*, although naturally occurring bacteria (*Anaerobiospirillum succiniproducens*) and fungi (e.g. from the *Penicillium* genus) are also potential hosts [11,12]. In the factory designed in this study, *Actinobacillus succinogenes*, a wild-type bacterial strain isolated from bovine rumen [13], is chosen as the fermentative host. This bacterium is one of the most promising strains and most efficient natural producers of industrial BioSA, since it can ferment a huge range of carbon sources [10].

2.2. Description of the full-scale BioSA production facility

Several approaches have been proposed in the literature for the production of biotechnological succinic acid from biomass. These processes include separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and consolidated bioprocessing (CBP) [26–28]. SHF involves two steps: the hydrolysis of the cellulosic biomass, using enzyme cocktails to obtain hydrolysates rich in sugar, and a subsequent step involving fermentation of these sugars to succinic acid. In the case of SSF, both processes are carried out simultaneously, and this is therefore considered the most promising strategy for obtaining bioproducts through the fermentation of sugars derived from agro-food residues. The CBP strategy combines the production of a saccharolytic enzyme, the hydrolysis of biomass and fermentation into the desired products in a single step. In view of the wide variety of literature about the production of succinic acid using the SSF strategy, it was decided to use this approach in the present work for the design of the plant.

The separation and purification of succinate from the fermentation broth is another key stage in the development of a competitive biotechnological process. Several alternatives have been proposed for succinic acid recovery, including extraction with solvents and/or amines, direct crystallisation, membrane separation and ion exchange [15].

Fig. 1 displays a simplified system boundary for the production of apple pomace-based BioSA evaluated here. The production process is divided into three main stages, which reflect the activities required in the laboratory. At each stage, the various processes involved are identified and designed in detail.

In this way, the appropriate reactor, machinery or equipment is chosen and designed, resulting in a simple plant flow diagram, as shown in Fig. 2. A detailed description of each stage and the corresponding steps involved is given below.

Stage 1: Reconditioning and storage of apple pomace. At this stage, the raw material is received directly from the cider factory located nearby, and is warehoused in hoppers. It is important to note that this feedstock is a seasonal material, produced between September and December. Next, the raw material is dried at atmospheric pressure in a tray drier, with the aim of reducing its moisture content and increasing its lifespan. This drying step is performed at 60 °C for 225 min. Finally, the dried apple pomace is warehoused in silos at 20 °C and atmospheric pressure, in order to guarantee the conservation of the raw material and to avoid the proliferation of plagues.

Section 2: Fermentation. There are two main processes at this stage: the preparation of the inoculums, and the SSF process. It is important to bear in mind that all the nutrients, the apple pomace used in both processes and the equipment used (such as the pre-fermenter to produce the inoculum and the fermenter to carry out the fermentation) must be sterilised by steam injection to avoid possible contamination. For the preparation of the inoculum, cells of *A. succinogenes* (DSM 22257) are grown in a medium containing 10 g glucose/L and nutrients (10 g yeast extract/L, 5 corn steep liquor/l, 15.4 g $NaH_2PO_4{\cdot}H_2O$/l, 1 g NaCl/l, 6.4 g K_2HPO_4/l, and 0.05 g $MgCO_3$/l). After growth, cells are recovered by centrifugation, resuspended in a phosphate buffer solution (0.5*g* bacteria/l) and inoculated in the fermenter to start the fermentation. The SSF medium was prepared by mixing the desired amounts of apple pomace, water (at a liquid-to-solid ratio of 12 w/w) and enzymes (cellulase (Celluclast 1.5 L) at a ratio of 1 FPU per g of dry solid, and β-glucosidase (Novozymes 188) at a ratio of 0.25 IU[1] per FPU[2] of cellulase), and nutrients. Both the inoculum preparation and SSF were performed under the same conditions: pH (6.7 ± 0.1), temperature (37 °C), stirring (150 rpm), and CO_2 flow (0.3 vvm) to maintain anaerobic conditions.

At this stage, particular attention must be paid to the use of carbon dioxide. *A. succinogenes* is an anaerobic succinate producer, which can use both glucose and CO_2 as its carbon source, and CO_2 is required for conversion into succinate during the glucose fermentation process. According to the literature [10,13], this strain has CO_2 consumption rates that are much higher than for other species (e.g. *Chorella* sp.). One of the main purposes of this study is therefore to devise an efficient BioSA production system to capture and use the vast amounts of CO_2 released by existing bioethanol refineries.

Section 3: BioSA purification. In this stage, multiple downstream activities are performed in order to obtain the final product, that is, industrial grade BioSA (pure A-grade, i.e. ≥99.5% wt). In this step, the fermenter output, which includes succinate salt, formic acid, acetic acid, succinic acid, biomass and residual salts and proteins, is sent for purification. This stream is a solid-liquid suspension, and is first fed to a centrifuge to separate the fractions. The solid fraction is recovered as a residue for storage and is finally sent to the corresponding waste management agent. The liquid fraction is derived from the ultrafiltration membrane unit (see Fig. 2). The permeate stream is rich in succinate salt, formic acid, acetic acid and succinic acid. The remaining constituents, such as biomass, proteins and other salts, are retained in the membranes. Subsequently, the permeate stream is mixed with sulphuric acid to complete the recovery of succinic acid from the succinate salt. Following this, a reactive extraction is carried out in a mixing tank using tri-n-octilamine (TOA) in 1-octanol as dilute. After this reaction, decantation takes place, allowing the separation of the aqueous phase from the organic one. Succinic, acetic and formic acids are present in the organic phase, which is then pumped to the vacuum distillation unit to obtain the desired stream of pure succinic acid. Finally, this stream, which is rich in succinic acid (≥99.5% purity), is crystallised and stored for subsequent use.

In addition to its use in the recovery of succinic acid, the distillation unit is important because the organic solvents (TOA and 1-octanol) used in large amounts in the reactive extraction unit are recovered for recycling in the process. For this type of organic chemical, it is assumed that the recycling rate is 95% [29].

Further activities involved in the management of liquid and solid waste have been included as ancillary stages within the system boundaries. Liquid and solid wastes produced in the factory are sent to a wastewater treatment plant (WWTP) and to sanitary landfill (MSW), respectively. Both of these are expected to be located near to the factory.

2.3. Life cycle inventory data and sources

A reliable environmental assessment requires the collection of high quality inventory data. In addition to the feedstock (apple pomace) and the main product (i.e. BioSA), biochemical and biorefinery conversion plants exchange a wide range of material and energy with the technosphere and the environment, and mass and energy flows therefore need to be estimated. Hence, the mass and energy flows corresponding to the

[1] IU: This is defined as the amount of enzyme catalysing the formation of 1 μmol of D-galacturonic acid per minute at 37 °C and pH 5.

[2] FPU - Filter Paper Activity

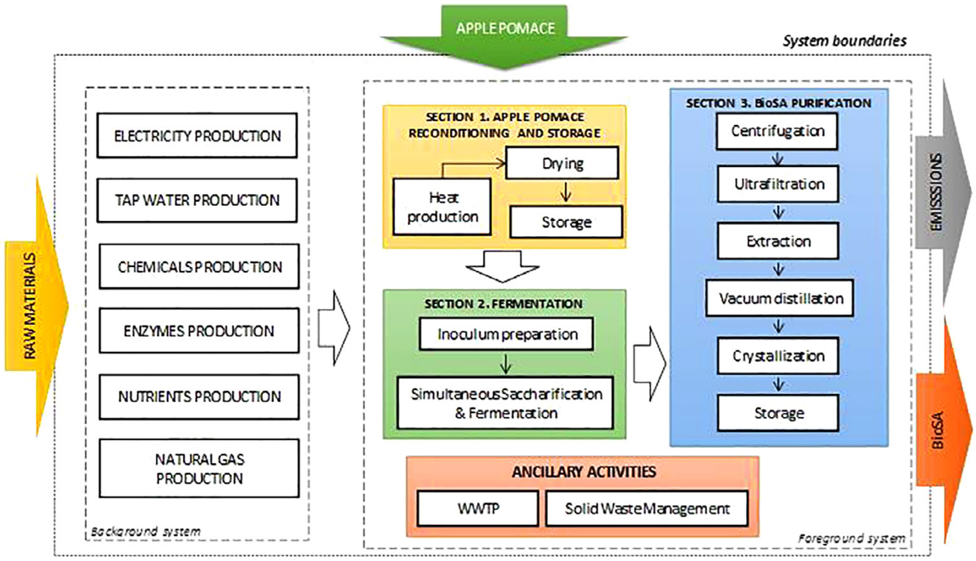

Fig. 1. System boundaries of the exanimated BioSA production process at full-scale.

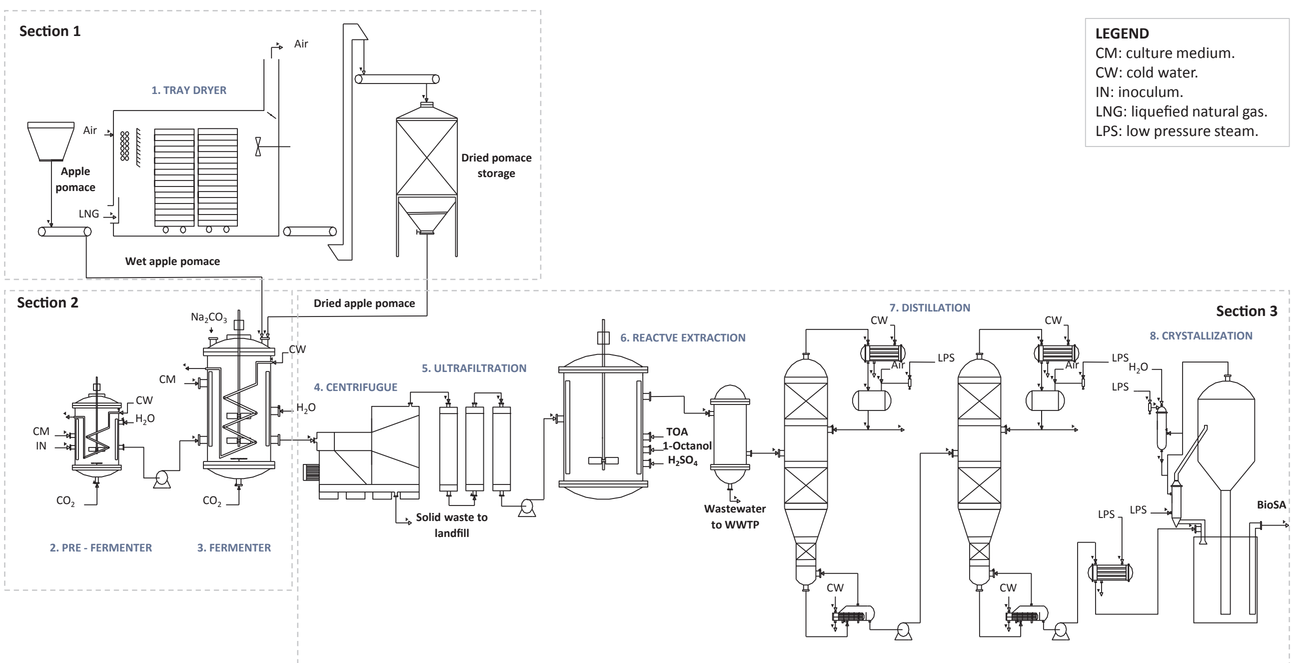

Fig. 2. Plant flow chart of the apple pomace based BioSA production process.

foreground system (Fig. 1) have been modelled in detail, and all of these have been identified for each stage. Modelling of the full-scale facility requires the scaling up of the production process. Countless studies have been carried out involving production in the laboratory, and the optimised production of BioSA at the laboratory scale is used as a starting point that provides useful information regarding a procedure for how to design a facility at a large scale. Numerous publications have considered different raw materials, hosts and even production routes. Certain selected studies [25,26] supply useful information regarding the steps and quantities required at the laboratory scale. It is important to bear in mind that at laboratory scale, the different processes involved are not usually connected, and the types of equipment (tanks, vessels, reactors, pumps or columns) are not comparable to those used at full scale. Hence, inventory data from the small scale cannot be directly extrapolated to a larger scale. The scaling-up sequence proposed by Piccinno et al. [29] was therefore followed. Information from the laboratory was used to design the plant flow diagram (Fig. 2), which included all stages and processes and the relevant equipment. Next, calculation procedures, equations [30] and ASPEN simulations were used in the specific design of the equipment. It should be borne in mind that each single process is linked through the transfer of the reaction mixtures and the inter-process heat and energy recovery. The estimated energy and mass flows are managed as foreground inventory data. Moreover, following the recommendations established by Piccinno et al. [29,31], stoichiometric amounts of each reactant (including enzymes) based on laboratory protocols were computed in the inventory data. Regarding the recovery of organic solvents in the distillation unit, the recycling rate was taken from the abovementioned studies [29,31]. In addition, a default relative solvent reduction of 20% for the scale-up was used, in order to allow a more efficient use of solvents. A summary of the inventory data corresponding to the foreground system is shown in Table 2.

Table 2
Global Life Cycle Inventory data corresponding to the foreground system for the production of BioSA. Data are reported per production batch.

INPUTS from TECHNOSPHERE	
Section 1 (S1)	
Apple pomace (75% moisture)	8444 kg
Liquefied natural gas	1457 kg
Electricity	308 kWh
Section 2 (S2)	
Water	107,081 kg
CO_2	58,539 kg
$C_6H_{12}O_6$ (to inoculum preparation)	277 kg
Na_2CO_3	12,522 kg
Yeast extract (nutrient)	1825 kg
Corn Steep Liquor (nutrient)	69 kg
$NaH_2PO_4{\cdot}H_2O$ (nutrient)	248 kg
K_2HPO_4 (nutrient)	372 kg
$MgCO_3$ (nutrient)	272 kg
NaCl	117 kg
Cellulase	59.5 kg
β-glucosidase	3.66 kg
Electricity	18,770 kWh
Section 3 (S3)	
H_2SO_4	3878 kg
1-Octanol	200,060 kg
Tri-n-octilamine	19,404 kg
Electricity	40,700 kWh
OUTPUTS to TECHNOSPHERE	
BioSA	4669
Waste to treatment	
Wastewater to WWTP from SS3	112,693
Solid waste to sanitary landfill from SS3	4131
OUTPUTS to ENVIRONMENT	
Emissions into air	
CH_4	166 g
CO	2.49 kg
CH_3COOH	12.5 g
N_2O	41.5 g
Particulates (less than2.5 μm)	8.31 g
NO_x	1.66 kg
SO_2	45.7 g

Although primary data should be used whenever possible, it is sometimes necessary to turn to secondary data. Inventory data corresponding to the background system, which involves the production of utilities (electricity, steam) and other inputs to the foreground system (chemicals, water and nutrients), were taken from pre-existing databases and the literature. The Ecoinvent® database version 3.2 [32] was used as the main source of secondary data. NREL [33] was used for the collection of inventory data regarding corn steep liquor production process. The electricity mix used in the analysis considers the update of the database in Dones et al. [34], using current data for the average electricity generation and import/export data from Spain from 2017 [35]. Data for enzyme production and the corresponding estimation of impacts were taken from Gilpin et al. [36].

Ancillary activities such as wastewater and solid waste treatment have been also included within the system boundaries, in order to compute the environmental impacts due to the various waste management treatments, as displayed in Fig. 1. Inventory data corresponding to wastewater treatment activities were taken from Doka [37]. The solid waste (such as solids from ultrafiltration, see Fig. 2) is sent to sanitary landfill [37].

Detailed information on the data sources used for the different background processes included in this study is summarised in Table 3.

2.4. Life cycle impact assessment: Methodology

Of the steps defined within the life cycle impact assessment (LCIA) of the standardised LCA tool [19], the classification and characterisation processes were followed in this study in an analysis of the production of BioSA using an environmental approach. The characterisation factors reported by the Centre of Environmental Science of Leiden University (CML 2001 method v2.05 [38]) were used in this study for the analysis. The following impact categories were evaluated: global warming potential (GWP), acidification potential (AP), eutrophication potential (EP), ozone layer depletion potential (ODP) and photochemical oxidation potential (POP). In addition, the cumulative energy demand, measured in MJ, was determined using methods developed by Frischknecht et al. [39]. The choice of these impact categories was made to give a complete and comprehensive synopsis of the environmental effects related to the production process under evaluation. SimaPro v8.2 [40] software was used for the computational implementation of the life cycle inventory data [41].

Table 3
Description of the main Ecoinvent ® database version 3.2 processes [32] and other literature sources considered in this study for the background processes.

Input	Process
Electricity	Electricity, medium voltage {ES}| market for | Alloc Rec, U
Heat	Heat, central or small-scale, natural gas {RER}| market group for | Alloc Def, U
Water	Tap water {Europe without Switzerland}| market for | Alloc Rec, U
Inorganic chemicals	Chemical, inorganic {GLO}| market for chemicals, inorganic | Alloc Rec, U
Organic chemicals	Chemical, organic {GLO}| market for | Alloc Rec, U
Sodium chloride	Sodium chloride, brine solution {GLO}| market for | Alloc Def, S
Sulfuric acid	Sulfuric acid {GLO}| market for | Alloc Def, U
Sodium carbonate	Sodium carbonate from ammonium chloride production, at plant/GLO U
Yeast starch	Yeast paste, from whey, at fermentation/CH U
Corn steep liquor	Corn steep liquor/kg/RNA (NREL), [33]
Wastewater treatment	Wastewater, average {CH}| treatment of, capacity 1E9l/year | Alloc Rec, U
Solid waste management	Municipal solid waste {CH}| treatment of, sanitary landfill | Alloc Def, U
Enzymes	Gilpin et al. [36]

3. Results and discussion

Table 4 summarises the results for the LCIA in the present study, based on a cradle-to-factory-gate perspective for the functional unit (that is, 1 kg of BioSA from apple pomace) and the inclusion or otherwise of enzyme production within the system boundaries. This distinction was made in order to enable a further comparison of the results with those of other relevant studies. The production of enzymes is highly energy- and steam-intensive [36], and specifically in the aeration and fermentation operations involved in the production system. Prior studies that focus on the production of BioSA [5,11] have not considered the production of microorganisms within the system boundaries. Thus, the inclusion of enzyme production increases the corresponding carbon footprint and CED by factors of up to 95 and 16, respectively.

Differences between the results for GWP and CED and those in the literature [5,11] can be identified when enzyme production is excluded from the analysis. The rationale behind these differences is linked to the production system itself, and this is discussed in more detail below. As previously mentioned, several production strategies could be considered for the design of the BioSA plant (i.e. for alternative solvents, extraction procedures and the recovery succinic acid). The production route and operations involved have a significant effect on the material and energy flows, and thus on the environmental profile. Hence, an alternative scenario is used for comparison purposes with the present one, in order to identify potential environmental improvements.

Table 4
Characterization results per kg of BioSA production from apple pomace.

Impact category	Acronym	Unit	Including enzymes production	Excluding enzymes production
Global Warming Potential	GWP	kg CO_2 eq	504	5.30
Eutrophication Potential	EP	g PO_4^{-3} eq	718	168
Acidification Potential	AP	kg SO_2 eq	2.73	0.73
Ozone Layer Depletion Potential	ODP	mg CFC-11 eq	26	13.6
Photochemical oxidation Potential	POP	g C_2H_4 eq	174	48
Cumulative Energy Demand	CED	MJ	3538	227

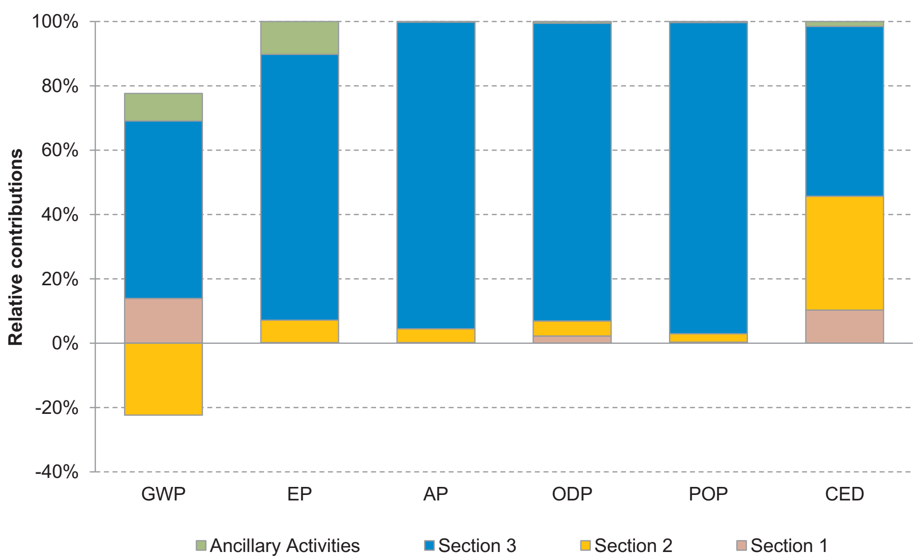

Fig. 3. Distribution of contributions to the environmental profile per sections (Sections 1, 2 and 3) and ancillary activities involved within the BioSA production plant from apple pomace. Production of enzymes has been excluded from the results. Acronyms: GWP - global warming potential, AP - acidification potential, EP- eutrophication potential, ODP - ozone layer depletion potential, POP - photochemical oxidation potential and CED – cumulative energy demand.

3.1. Global environmental results

Fig. 3 illustrates the distribution of environmental burdens and cumulative demand for energy between the stages of the production process and the ancillary activities. Based on the results, the operations involved in Stage 3, BioSA purification, form an environmental hotspot, with contributing ratios ranging from 53% to 97% depending on the category. Stage 2 also involves significant contributions in terms of CED, and these are mostly due to the production of chemicals and nutrients (upstream burdens) required in fermentation-related activities. A negative value (environmental credit) from Stage 2 to GWP can be identified; this is due to the CO_2 uptake during the sugar fermentation process that produces the succinate. Stage 1 involves significant contributions only to GWP (25%) and CED (10%), and these are mostly due to the heat requirements for reconditioning of the apple pomace, since the feedstock must be dried (from an initial average moisture content of 75%) to enable its storage. Ancillary activities related to liquid and solid waste management are important contributors in terms of GWP and EP, mostly due to GHG emissions from energy requirements (GWP) and nutrient enrichment of the aquatic environment (EP).

3.2. Identification of hotspots

One of the key issues arising from this study is the identification of environmental hotspots, i.e. the operations involved in the production process that are responsible for the highest environmental burdens and energy requirements.

Stage 1 involves apple pomace reconditioning and storage once the raw material has been received in the factory. As shown in Fig. 1, this stage includes feedstock drying and subsequent storage. Fig. 4a shows that the combustion of natural gas to produce the heat required in the dryer and the electricity used in the equipment (i.e. conveyor belts, freight elevators and air vents) are the two main contributors to the impacts arising from stage 1.

Stage 2 includes operations related to the production of succinate from the apple pomace. As previously mentioned, this section requires the preparation of the inoculums, which are subsequently fed into the fermenter. At this stage, large amounts of chemicals and electricity are required, due to the sterilisation and cooling processes. Fig. 4b illustrates the impacts from the operations involved in Stage 2. As shown in the figure, the electricity requirements for the equipment (conveyor belts, freight elevators, pre-fermenter, fermenter, pumps, refrigeration) form an environmental hotspot. Of these contributions, the sterilisation and cooling processes are responsible for 90% of the total electricity requirements at this stage. The production of Na_2CO_3, required as a buffer to regulate the pH in the SSF unit, also makes a significant contribution to the environmental burdens arising from this stage, mostly in terms of EP and ODP. Nutrients are required both in the preparation of the inoculum and the SSF unit, and can be classified as salts (i.e. $NaH_2PO_4{\cdot}H_2O$, K_2HPO_4 and $MgCO_3$) or protein sources (yeast extract and corn steep liquor). Their effect on the environmental profile is significant in terms of GWP, ODP and CED (Fig. 4b), and the salt sources are responsible for 41%–63% of the total impact. Finally, it is important to bear in mind the environmental credit associated with the carbon dioxide uptake in the SSF unit. *A. succinogenes* requires the consumption of carbon dioxide in the reductive TCA cycle to produce the succinate. The CO_2 flow is supplied from a bioethanol refinery, which should be located in the surrounding area, as previously discussed.

Stage 3 involves BioSA purification, and requires numerous activities such as centrifugation, ultrafiltration, extraction with organic solvents, distillation to recover the solvents (first the octanol and then the TOA) and separation of the succinic acid, crystallisation to produce the crystals, and finally storage. Of these operations, distillation forms the environmental hotspot, due to the very high consumption of solvents (95% of both the TOA and 1-octanol is finally recycled, so only

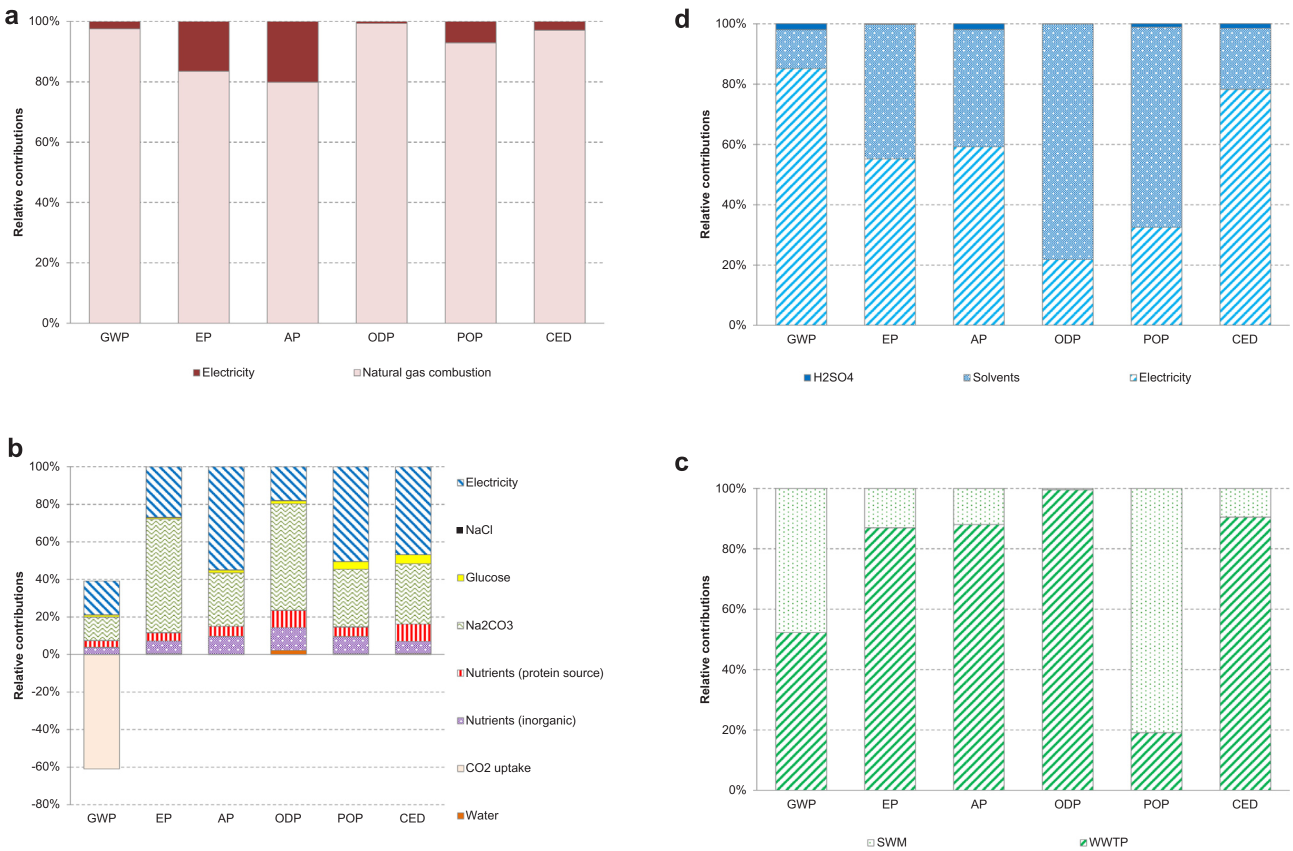

Fig. 4. Distribution of contributions per section per operations involved to identify hotspots. Production of enzymes has been excluded from the results. (a) Section 1; (b) Section 2; (c) Section 3; (d) Ancillary activities. Acronyms: GWP - global warming potential, AP - acidification potential, EP- eutrophication potential, ODP - ozone layer depletion potential, POP - photochemical oxidation potential and CED – cumulative energy demand.

5% of the total solvent required in the extraction unit is spent per batch) and demand for electricity (the distillation unit is a highly intensive equipment) [42]. As illustrated in Fig. 4c, the production of solvents and electricity plays a key role in the categories considered for analysis, and forms 98%–100% of the total burden and energy demand at Stage 3. Based on a separate assessment of the electricity contributions, the use of electricity at Stage 3 is linked to both the distillation unit and the equipment required in the remaining operations (i.e. centrifuging, pumping, heating and mixing in the extraction). The distillation unit represents 99% of the total electricity requirement at this stage. Contributions from electricity production to the global profile are significant in terms of GWP, EP, AP and CED. All electricity requirements are directly taken from the national grid, which is mainly powered by fossil fuels. The production of solvents is responsible for 78% and 66% of the total contributions to ODP and POP at this stage, respectively. These are organic solvents, and TOA has the highest contribution to these burdens (around 10 times higher than contributions from 1-octanol) since it is also consumed in larger amounts. The extraction unit also requires H_2SO_4 to convert the succinate into succinic acid. However, this stage makes negligible contributions to the overall impacts, as shown in Fig. 4c.

Finally, the ancillary activities required in the treatment of the wastewater streams (e.g. from crystallisation) and the solid waste (e.g. waste biomass and other residual organic streams) produced in the BioSA production process were computed within the system boundaries. Their effect on the global environmental profile is not large, except in terms of GWP and EP, as illustrated in Fig. 3. The WWTP is mainly responsible for the contributions to EP, due to nitrogen- and phosphorous-based emissions into the aquatic environment. Both management operations make similar contributions to GWP (around 50% for each), as shown in Fig. 4d, due to the energy requirements of the WWTP and the GHG emissions derived from landfilling activities.

3.3. Sensitivity analysis: Alternative scenarios

As previously indicated, the production of succinic acid can be carried out by means of alternative production sequences. In the designed plant, the purification stage was based on two main operations: the extraction of succinic acid with organic solvents (TOA and 1-octanol), and the recovery of succinic acid and the solvents (the latter by recycling) in the distillation unit. However, these two operations form environmental hotposts due to the large energy requirements and the very high consumption of organic chemicals. Other studies [5] propose a combination of ion exchange columns, nanofiltration and evaporation to recover the succinic acid before the crystallisation stage. This strategy has low energy requirements and is less chemically intensive than the design used in our study. To analyse this environmental behavior, this alternative strategy was therefore modelled in our plant in place of the extraction and distillation steps. The new strategy reduces the energy requirements (ion exchange columns require less electricity than distillation columns) and the amounts of chemicals (HCl and NaOH) needed for the cleaning and regeneration operations. Fig. 5a illustrates comparative environmental profiles for the plant under study and the alternative plant. The outcomes of this comparative assessment show that the alternative proposal (shown as Plant B in Fig. 5a) for the purification stage (Stage 3) yields the lowest environmental burden and energy demand. The impacts can be reduced by between 82% and 97%, depending on the category, and energy demand can be reduced by up to 52% in comparison with the plant designed for assessment (Plant A1 in Fig. 5a). It should be noted that the same BioSA production yield has

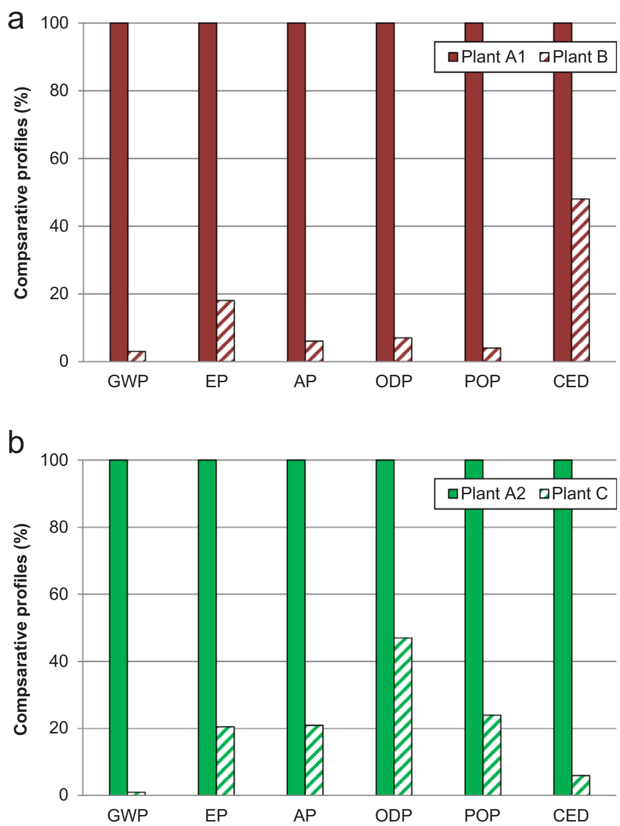

Fig. 5. Comparative profiles between alternative scenarios. (a) Plant A1 – Base plant excluding enzymes production; Plant B – Alternative plant with modifications in the purification section; (b) Plant A2 – Base plant including enzymes production; Plant C – Alternative plant with modifications in the fermentation section and consequent changes; Acronyms: GWP - global warming potential, AP - acidification potential, EP- eutrophication potential, ODP - ozone layer depletion potential, POP - photochemical oxidation potential and CED – cumulative energy demand.

been assumed in this estimation due to a lack of recovery yields for the alternative purification strategy.

Another aspect that needs to be highlighted is the potential use of apple pomace as a source of sugars and other compounds for fermentation. The use of low amounts of enzymes in the SSF unit (or even the removal of enzyme use) could derive on important amounts of succinic acid since apple pomace contains a high level of soluble sugars [16]. The use of enzymes allows us to perform enzymatic hydrolysis, thereby facilitating the availability of cellulose as a carbon source for succinate production. However, according to the literature [16], the BioSA yield will be reduced by around 35% if no enzymes are used in Stage 2, since the cellulose will not be hydrolysed. In addition, the solid waste stream will be increased due to the unspent cellulose. This scheme was assessed in order to identify the potential improvements from the use of an environmental approach. In this comparison, the environmental burdens arising from enzyme production are considered within the system boundaries In terms of their difference from the previous results, with the aim of highlighting the effect of their use (and corresponding production activities). Thus, Fig. 5b shows comparative profiles for the current scenario (Plant A2) and the alternative without the use of enzymes and a decreased BioSA production yield (Plant C). The outcome of the comparative assessment shows that the alternative proposal for the fermentation stage (stage 2) gives rise to lower environmental burdens and energy demands. Impacts are reduced by amounts ranging from 53% (for ODP) to 99% (for GWP). CED is reduced by up to 94% in comparison with the plant designed for assessment (Plant A2). Thus, in spite of reducing BioSA yield by 35%, the lack of use of enzymes should

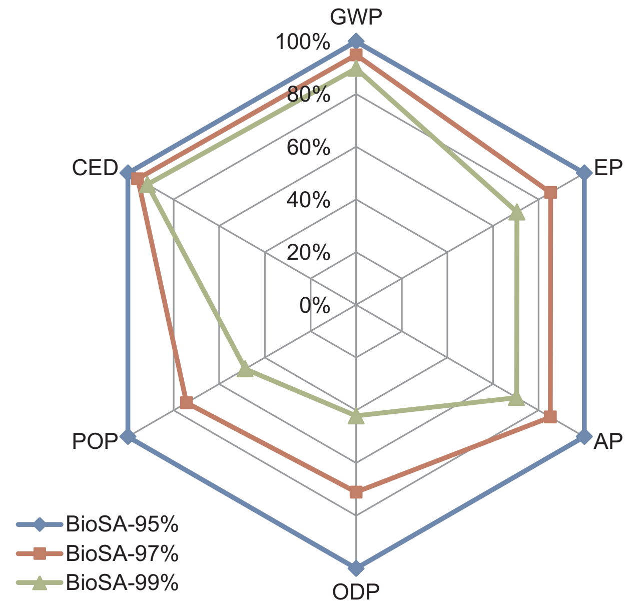

Fig. 6. Comparison of the LCA impact categories for BioSA production with different recycling rates for solvents (TOA and 1-octanol). Acronyms: GWP - global warming potential, AP - acidification potential, EP- eutrophication potential, ODP - ozone layer depletion potential, POP - photochemical oxidation potential and CED – cumulative energy demand.

be considered an interesting biorefinery choice, since enzyme production is a highly energy-intensive process that gives rise to important environmental consequences.

As mentioned above, the distillation unit has a double objective: recovery of both the BioSA and the organic solvents used in the reactive extraction unit, which are subsequently recycled in the process. Based on the literature [29], a recycling rate of 95% has been assumed. However, further research should be conducted in this vein to increase the recycling rate and thus to optimise the requirements for chemicals [29]. Alternative recycling rates are therefore proposed in order to study their effects on the environmental profiles, mostly due to the key role that Stage 3, and thus the organic solvents, plays in the overall environmental burden (see Figs. 3 and 4c). Solvent recycling ratios of 97% and 99% are used for analysis, and Fig. 6 displays comparative profiles for these. The outcome of the sensitivity analysis shows that research activities should focus on optimising the distillation unit, since global environmental improvements can be achieved in these categories, in which Stage 3 plays a key role (EP, AP, POP and ODP).

3.4. Comparison with the literature

Enzymes are considered potential biocatalysts for many applications for several reasons, such as their high selectivity and specificity and the fact that their use generates lower demands for energy and chemicals [43]. However, in view of other results, there are some discrepancies regarding the drive towards cleaner industrial systems, since the production of enzymes is highly energy-intensive [36].

Two studies can be found in the literature regarding the production of BioSA: one considers its production from sorghum grains (a dedicated energy crop), while the second uses corn and sugarcane [5,11]. Smidt et al. [11] have determined the environmental profile of fossil-fuel-based succinic acid. Inherent limitations on performing an LCA comparison of succinic acid production systems involve the use of different characterisation methods (e.g. CML, ReCiPe) [44,45], data sources, system boundaries and approaches to system expansion. In addition, the inherent characteristics associated with the carbon sources must be taken into consideration (e.g. corn needs to be dried and hydrolysed to release sugar, whereas sugarcane is an easier source

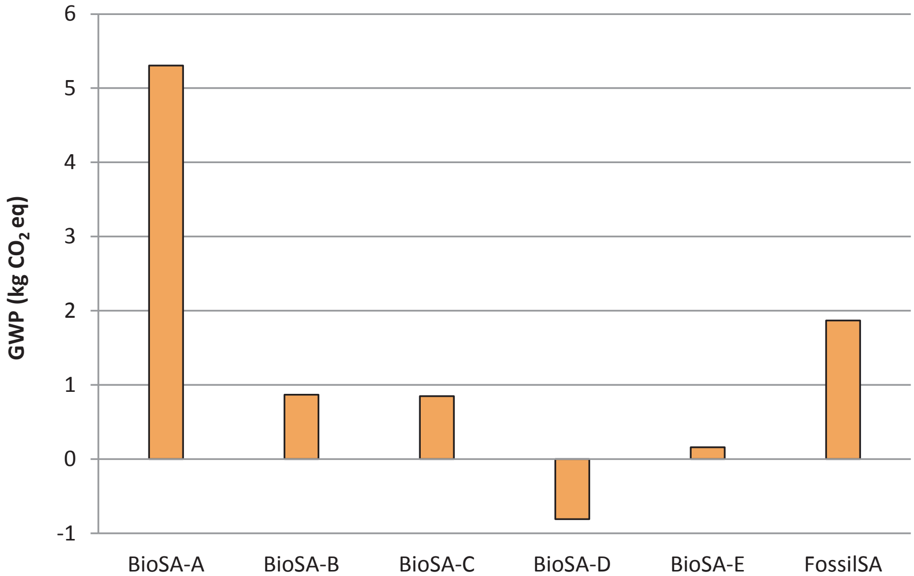

Fig. 7. Comparison in $kgCO_2eq$ per kg of succinic acid among the scenario under assessment (BioSA-A), the scenarios based on sorghum, corn and sugarcane (BioSA-B, BioSA-C and BioSA-D, respectively), the alternative scenario modifying the purification unit (BioSA-E) and the fossil scenario (FossilSA).

of sugar requiring little energy and few chemicals), as should the production process itself (e.g. large amounts of steam are generated in the surgarcane-based scenario). A comparison of our results with those found in the literature was performed in terms of GWP, excluding the production of the required enzymes from the system boundaries. Fig. 7 displays the comparative profiles, and notable differences can be identified in the corresponding carbon footprints. The best profile is shown for BioSA-D, in which sugarcane is used as a raw material. An environmental credit related to the production of a large amount of steam is behind this result. A similar carbon footprint has been reported in the literature for the other two scenarios, BioSA-B and BioSA-C. The characteristics of the production system in terms of the purification stage (in both scenarios) and the co-production of ammonium sulphate, a potential fertiliser, in the sorghum-based scenario are behind these values. The alternative scenario discussed above, in which the purification section is modified with ion exchange columns, nanofiltration and evaporation (BioSA-E), represents the second best BioSA production choice. Based on these results, interesting methods for improvement can be proposed for the plant designed in this paper, to reduce its environmental burdens.

3.5. Future outlook

Concerns about the environmental sustainability and security of fossil-based products and advances in biochemical technology have generated an interest in the use of agro-industrial wastes as potential raw materials, mostly from a biorefinery perspective.

Pretreatment activities are among the most expensive stages in any biorefinery process. In view of this, several novel approaches, including physicochemical processes such as microwaves and ultrasound, are being studied to improve the efficiency of pretreatments and increase the yield of fermentable sugars [46]. In addition, efforts are being made toward the development of genetically engineered bacterial strains (e.g. *E. coli*) [13] which can increase the productivity of BioSA, reducing downstream separations and purification.

4. Conclusions

BioSA can be obtained from the biological transformation of biorefinery sugars from a variety of feedstocks and using multiple microorganisms. In this case, the large-scale production of BioSA from apple pomace has been environmentally assessed to identify environmental hotspots and propose strategies for improvement. The production strategy used in this study gave rise to an important environmental burden, with the purification section being identified as an environmental hotspot. The use of organic solvents to extract the BioSA and the subsequent use of a distillation unit to recover both the solvents (at one site) and the pure BioSA (at the other site) lie behind these significant environmental burdens. Heat recovery from the distillation unit may be possible, and this is interesting not only from an environmental perspective but also from an economic one. However, this would be complex to implement, since the cooling water is constantly renewed and flowing through the column. Environmental credits could be allocated to this recovered heat. An optimisation of the distillation unit in terms of its electricity requirements should be borne in mind to improve the environmental profile. Alternative organic solvents to TOA and 1-octanol for extracting the BioSA should be researched, since although 95% of the total requirement is recycled from the distillation unit, significant environmental impacts are still incurred. The potential of apple pomace as a source of sugars should be highlighted, since It contains a high content of free glucose, fructose and polysaccharides that can be easily enzymatically hydrolysed. Even without the addition of enzymes, high yields of BioSA can be obtained due to this characteristic.

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