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Exploring the production of Bio-Succinic acid from Apple Pomace from an Environmental approach

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Abstract

Fermentation derived Bio-succinic acid (bioSA) is a valuable intermediate considered a chemical building block with multiple industrial applications as 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 cider and juice making industry, in a biorefinery concept as well as to determine its environmental profile using a cradle-to-factory gate scale-up LCA approach. Foreground data used in this LCA were based on mass and energy flows modelled in detail. Production process was divided in three sections: i) reconditioning and storage, ii) fermentation with *Actinobacillus succinogenes* and iii) purification. Results indicated that the use of enzymes was responsible for the highest environmental burdens due to their background high energy intensive production processes. Excluding them from analysis (in agreement with other studies available in the literature), the purification section played an environmental key role due to the extraction and distillation units. Electricity use and organic solvents requirement in these operations were behind the highest environmental burdens. Thus, the highest improvement potentials must be related with both operations. Improvement alternatives were proposed identifying interesting potential reductions on the environmental profile especially in the purification section.

Keywords: Bio-based; Biomass fermentation; Butanedioic acid; Cider factory; Life Cycle Assessment; Sustainability

1. Introduction

Nowadays, the society and industrial sectors are facing important challenges regarding the use of biomass and the production of biobased materials influenced by social responsibility and environmental consequences. Therefore, the substitution of petroleum-based materials by their bio-based counterparts is considered a key factor in the battle against climate change [1].

Bio-based products are being promoted under sustainable consumption strategies and are obtained from the integration of eco-innovation approaches aimed at reducing greenhouse gases (GHG) emission and combating fossil sources depletion [2]. Moreover, new environmental regulations and economic considerations are also supporting the 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 global bulk materials production [4]. Therefore, the interest on substituting fossil feedstocks by biomass sources in the production of synthetic materials is increasing in recent years to guarantee the security of industrial feedstocks supply.

Biomass is an available resource abundant in the nature, diverse and recyclable with multiple applications either as clean renewable energy source or as raw material for the production of biomaterials and biochemical [3,5]. Concerns regarding the competition with the food and feed sector on a global level have encouraged the utilization of biomass wastes as potential feedstocks. In this sense, valorization of biomass residues is receiving potential attention and their exploitation is speculated to increase in the future mainly by emerging technologies not only on second generation biofuels production but also on high-added value products recovery [6,7].

The general assumption that bio-based materials are environmentally superior than fossil ones requires of detailed analysis. Thus, to guarantee the 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 of environmentally friendly [2], specially in terms of not so-well-known impact categories such as eutrophication, acidification, water depletion [4,5] as well as land use. In this sense, if dedicated biomass is managed instead of residues, cultivation activities related with the biomaterial-feedstock production play an important role mainly in areas with significant social value [8]. Therefore, a sustainability study should be required so as to identify the situations in which the use of bioresources could be environmentally feasible over petrochemical.

The introduction of the biorefinery concept as well as the challenge for integrating biobased chemicals are key issues to encourage the attention into waste valorization in industrial sector. In 2004, the US Department of Energy identified the “top” chemicals that could be obtained from biorefinery carbohydrates [9,10]. One of these chemicals is the succinic acid ($C_4H_6O_4$). Succinic acid or butanedioic acid is an striking renewable platform chemical mostly due to its functionality and valuable derivatives [11]. There is an extensive recent literature focused on its production and interest as chemical building block [5,12-15]. Succinic acid is precursor of well-known petrochemical products such as 1,4-butanediol, tetrahydrofuran, γ -butyrolactone and polybutylene succinates among others. Moreover, succinic acid presents multiple industrial applications in biodegradable polymers (polyesters, polyamides and polyesteramides), foods (e.g., acidulant, flavorant and sweetener), fine chemicals and pharmaceuticals [15-20]. However, it has been commonly considered as a niche product mainly due to its high price [18,21]. Currently, it is mainly produced by chemical process from n-butane/butadiene via maleic anhydride, using the C4-fraction of naphtha [18]. The global market was predicted to grow annually around 19% in the period 2011 and 2016 [5]. However, price fluctuations of petroleum counterparts together with environmental concerns are motivating the interest on biobased succinic acid - BioSA production [5,16,18]. It can be obtained from biochemical transformation of biorefinery sugars (bacterial fermentation of carbohydrates), from a variety of feedstocks and considering multiple microorganisms [22]. Moreover, carbon dioxide is needed by microorganisms (there is a carbon dioxide fixation involved in the reductive TCA cycle) for BioSA production, providing environmental benefits such as the reduction of greenhouse gases emission [16,18]. Therefore, several companies (e.g. BioAmber and Mitsui & Co) are working on the commercialization of BioSA [5,23]. Nowadays, it represent less than 5% of total succinic acid manufacture [5].

As previously indicated, multiple types of biomass sources can be used for the production of BioSA through microbial fermentation [14]. The most used carbon sources in industrial fermentations are purified sugars and glucose syrups from corn [16]. However, the use of agricultural and food residues as well as industrial side streams turns out interesting mostly from a sustainability perspective. Within them, apple pomace is a potential feedstock. It is the main solid waste produced in cider and apple juice making factories [24,25] adding up to 35-35% of total processed raw material. Apple pomace is referred to the solid residues consisting of a mixture of skin, pulp and seeds derived from the

production of concentrate apple juice, jam and sweets [26]. Being highly biodegradable, the disposal of these wastes represents a thoughtful environmental problem, being a niche of challenges. Although apple pomace is used as a feed component (a low added value use) as well as in pectin production, it represent only 20% of total produced being the remaining 80% sent to landfill [26]. Thus, numerous research has been performed in order to identify a more potential application [24,25]. The production of high added value products such as lactic acid, oligosaccharides [27,28], citric acid, antioxidants, dietary fibers and even biopolymers (chitosan and xanthan gum) are receiving special attention [26]. In this study, the assessment of the environmental impacts derived from the valorization of apple pomace from the cider industry into BioSA by microbial fermentation has been performed following the LCA methodology and considering a cradle-to-gate approach. To our knowledge, there are only two peer-review studies that analyse the environmental impacts of BioSA [5,13] but considering alternative feedstocks (e.g. glucose from corn or sorghum). In the following, the production system at large scale of BioSA is described in detail paying special attention to the design process.

2. Materials and methods

Life Cycle Assessment (LCA) is widely used and standardised tool for the systematic evaluation of environmental aspects of a product or production system through all stages of its life cycle [29]. Moreover, it is considered an ideal instrument to evaluate the environmental dimension of sustainability. This tool has been used for the environmental analysis of chemical processes [30-32]. Thus, its applicability in this area has been demonstrated.

2.1. Goal and scope definition

The goal of this LCA study is to provide data regarding the environmental impact of BioSA production process at a commercial scale with the aim of giving insights to the researchers, industry and society regarding the development of this green platform chemical intermediate for biobased polymers. To do so, it has been modelled the full-scale process based on data at the laboratory scale [33,34]. An attributional cradle-to-gate approach has been followed in this case study, considering the apple pomace as main raw material. The functional unit considered to report the environmental profile is 1 kg of apple pomace based BioSA (white crystalline solid form, industrial grade, $\geq 99.5\%$ wt), as there is

no application included in this study and this product presents a diverse field of uses. Detailed description of BioSA crystals produced is summarized in **Table 1**.

<Table 1 around here>

Moreover, a contribution analysis of the different sections or production steps has been performed with the aim of identifying the environmental hotspots in the production system. The identification of these allows to mitigate the overall environmental profile by means of the proposal of improvement actions to take into consideration in further research and development activities. Therefore, energy and mass balances (including the growth of the bacteria *Actinobacillus succinogenes*) will be performed for 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 giving a biorefinery approach and due to the reported interest on the joint ethanol fermentation-succinate production [22,35], the BioSA plant has been designed to be part of a promising biorefinery platform for reaching BioSA production from residual CO₂ from a existing ethanol refinery.

Regarding 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*. However, naturally occurring bacteria (*Anaerobiospirillum succiniproducens*) and fungi (e.g., from *Penicillium* genus) are also potential hosts [13,14]. In the designed factory, *Actinobacillus succinogenes* – a wild-type bacterial strain isolated from bovine rumen [16], has been chosen as fermentative host since it is considered as one of the most promising strains and efficient natural producers of industrial BioSA since it can ferment a huge range of carbon sources [12,35].

2.2. Description of the full-scale BioSA production facility

Several approaches have been proposed in the literature to production of biotechnological succinic acid from biomass. These processes include separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and consolidated bioprocessing (CBP) [36-38]. The SHF involves two steps: a hydrolysis of the cellulosic biomass using enzyme cocktails to obtain hydrolysates rich in sugar and a subsequent step of fermentation of these sugars to succinic acid. In

SSF configuration, both processes are carried out simultaneously, so it is considered the most promising strategy to obtain bioproducts through fermentation of sugars derived from agro-food residues. The CBP strategy combines saccharolytic enzyme production, hydrolysis of biomass and fermentation into the desired products in one step. In basis to the wide literature about the succinic acid production using the SSF strategy, in the present work we decided to use this approach for the design of plant.

In addition, the separation and purification of succinate from the fermentation broth is other key stage for 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 [22].

Figure 1 displays the simplified system boundary for the production of apple pomace based BioSA considered under evaluation. The production process has been divided in three main sections taking into account the activities required in the laboratory. Within each section, the different involved processes have been identified and designed in detail.

<Figure 1 around here>

Thus, it has been chosen and designed the appropriate reactor, machinery or equipment resulting in a simple plant flow diagram displayed in **Figure 2**. A detailed description of each section and corresponding steps involved is detailed below.

<Figure 2 around here>

Section 1: Apple pomace reconditioning and storage. In this area the raw material is received directly from the cider factory located on the surroundings and warehoused in hoppers. It is important to remark 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. The drying step is performed at 60°C by 225 min. Finally, the dried apple pomace is warehoused in silos at 20°C and atmospheric pressure to guarantee the raw material conservation as well as to avoid the proliferation of plagues.

Section 2: Fermentation. In this area there are two main processes: the preparation of the inoculum and the SSF process. It is important to bear in mind that all the nutrients and the apple pomace used in both processes must be sterilized as well as the equipments used (pre-fermenter to produce the inoculum and fermenter to carry out the fermentation) by means of the injection of steam to avoid possible contaminations. For the inoculum preparation, 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 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ /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. SSF media was prepared by mixing the desired amounts of apple pomace and water (at a liquid to solid ratio of 12 w/w), 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 IU¹ per FPU² of cellulase), and nutrients. Both inoculum preparation and SSF were performed under the same conditions: pH (6.7 ± 0.1), temperature (37°C), stirring (150 rpm), and CO₂ flow (0.3 vvm) to maintain anaerobic conditions.

In this stage, a special attention must be paid into the carbon dioxide utilization. *A. succinogenes* is an anaerobic succinate producer which can use both glucose and CO₂ as carbon source. Thus, CO₂ is required to convert into succinate during the glucose fermentation process. According to the literature [12,16], this strain involves CO₂ consumption rates much higher than other species (e.g., *Chorella* sp.). Therefore, one of the main purposes of this study is to devise an efficient BioSA production system to capture and use the vast amounts of CO₂ released by existing bioethanol refineries.

Section 3: BioSA purification. In this area, multiple downstream activities are performed in order to obtain the final product that is, BioSA at industrial grade (pure A-grade, i.e., $\geq 99.5\%$ wt). In this section, the fermenter-output which includes succinate salt, formic acid, acetic acid and succinic acid as well as biomass and remaining salts and proteins, is sent to purification. This stream is a solid-liquid suspension which is firstly fed to a centrifuge to separate both fractions. The solid fraction is recovered as residue and storage to be finally sent to the corresponding waste management agent. The liquid fraction is derived to the ultrafiltration membranes unit (see **Figure 2**). The permeate stream is rich on 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

¹U: unit of enzymatic activity. It is defined as the amount of enzyme catalyzing the formation of 1 μmol of D-galacturonic acid per minute at 37 °C and pH5.

² FPU - Filter Paper Activity

is mixed with sulphuric acid to complete the succinic acid recovery from the succinate salt. Following, it is carried out a reactive extraction in a mixing tank using tri-n-octylamine (TOA) in 1-octanol as dilute. After the reaction, the decantation takes place allowing the separation of the aqueous phase from the organic one. Succinic acid together with acetic and formic acids are present in the organic phase, which will be lastly pumped to the vacuum distillation unit to obtain the desired pure succinic acid stream. Finally, this stream rich on succinic acid ($\geq 99.5\%$ purity) is crystallized and storage for subsequent uses.

Besides the recovery of succinic acid, the distillation unit is important since the organic solvents (TOA and 1-octanol) used in large amounts in the reactive extraction unit, are recovered to be recycled to the process. For this type of organic chemicals, it has been assumed that the recycling rates are of 95% [39].

Further activities involved in the liquid and solid wastes management have been included within the system boundaries as ancillary stages. Liquid and solid wastes produced in the factory are sent to a wastewater treatment plant (WWTP) and to sanitary landfill (MSW), respectively. It is expected that both installations should be in the surroundings of the factory.

2.3. Life Cycle Inventory data and sources

A reliable environmental assessment requires the collection of high quality inventory data. Besides the feedstock (apple pomace) and main product (i.e., BioSA), biochemical and biorefinery conversion plants present a wide range of material and energy exchanges with the technosphere and the environment. Thus, mass and energy flows need to be estimated. Therefore, the mass and energy flows corresponding to the foreground system (**Figure 1**) have been modelled in detail identified all of them per section. The modelling of the full-scale facility requires the scale-up of the production process. Countless literature has been revised for the knowledge of the production in the laboratory. To do so, the optimized BioSA production at lab scale has been considered as starting point, providing useful information regarding the procedure on how to design the facility at large scale. Multiple publications have been revised considering different raw materials, hosts and even production routes. The selected studies [34,36,40], supply useful information regarding the steps and quantities required on the laboratory scale. It is important to bear in mind that at lab scale, the different involved processes are not usually connected between them. Moreover, the type of equipment (tanks, vessels,

reactors, pumps or columns) are not comparable to the ones at full scale. Therefore, inventory data from small scale cannot be directly extrapolated to large scale. The scale-up sequence proposed by Piccinno et al. [39] has been followed. Information from the laboratory has been used to design the plant flow diagram (**Figure 2**) including all the sections and processes as well as the corresponding involved equipment. Next, calculation procedures and equations have been used for the specific design of the equipment [41]. It should be taken in mind that all the single processes are linked throughout transfer of reaction mixtures and the inter-process heat and energy recovery. The estimated energy and mass flows have been managed as foreground-inventory data. Moreover and following the recommendations established by Piccinno et al. [39,42], the stoichiometric amounts of each reactant (including enzymes) considering lab protocols have been computed in the inventory data. Regarding organic solvents recovery in the distillation unit, the amount of recycling rate has been taken from the mentioned study [39,42]. In addition, a relative solvents reduction by default by 20% for the scale-up has been considered to incorporate a more efficient use of solvents. A summary of the inventory data corresponding to the foreground system is shown in **Table 2**.

Although whenever possible primary data must be processed, sometimes it is 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) have been taken from pre-existing databases and literature. Ecoinvent[®] database version 3.2 [43] has been considered as main secondary data source. NREL [44] has been managed for the collection of inventory data regarding corn steep liquor production process. Electricity mix used in the analysis considers the update of the database [45] using current data for the average electricity generation and import/export data from Spain in 2017 [46]. Data for enzymes production and corresponding estimation of impacts have been found in Gilpin et al. [47].

Ancillary activities such as wastewater and solid waste treatment have been also included within the system boundaries in order to compute the environmental impacts from the different wastes management as displayed in **Figure 1**. Inventory data corresponding to wastewater treatment activities has been taken from Doka [48]. The solid waste (such as solids from ultrafiltration, see **Figure 2**) is sent to sanitary landfill [48].

<Table 2 around here>

A detailed information with regard to the data sources considered for the different background processes included in this study is summarized in **Table 3**.

<Table 3 around here>

2.4. Life Cycle Impact Assessment methodology

Among the steps defined within the Life Cycle Impact Assessment (LCIA) of the standardised LCA tool [29], classification and characterization steps have been followed in this study to analyse the production of BioSA from an environmental approach. The characterization factors reported by the Centre of Environmental Science of Leiden University - CML 2001 method v2.05 [49] have been considered in this study for the analysis. The following impact categories have been evaluated: global warming potential (GWP), acidification potential (AP), eutrophication potential (EP), ozone layer depletion potential (ODP) and photochemical oxidation potential (POP). In addition, cumulative energy demand, measured in MJ, has been determined using methods developed by Frischknecht et al. [50]. The choice of these impact categories is that all together give a complete and comprehensive synopsis of the environmental effects related to the production process under evaluation. The SimaPro v8.2 [51] software has been managed for the computational implementation of the Life Cycle Inventory data [52].

3. Results and discussion

Table 4 summarizes the LCIA results of the present study using a cradle-to-factory gate perspective for the functional unit that is, 1 kg of BioSA from apple pomace and considering the inclusion or not of enzymes production within the system boundaries. The rationale behind that distinction is related with the further comparison with the results from other relevant studies. The production of enzymes is high energy and steam intensive [47,53] specifically in the aeration and fermentation operations involved in the production system. Available studies focused on BioSA production [5,13] have not considered the microorganisms production within the system boundaries. Thus, the inclusion of enzymes production increases up to 95 and 16 times the corresponding carbon footprint and CED.

<Table 4 around here>

Differences on the results in terms GWP and CED have been identified with the literature [5,13], excluding enzymes production from analysis. Although it should be discussed in more detail below, the rationale behind these differences is linked to production system itself. As previously mentioned, different production strategies could be considered for the BioSA plant design (i.e., alternative solvents, extraction procedure and succinic acid recovery). The production route and involved operations have an outstanding effect on the material and energy flows and thus, on the environmental profile. Thus, an alternative scenario has been considered for comparison and discussed with the present one in order to identify potential environmental improvements.

3.1. Global environmental results

Figure 3 depicted the distribution of environmental burdens and cumulative energy demand between the sections involved in the production process as well as ancillary activities. According to the results, operations involved in Section 3 intended for BioSA purification are the environmental hotpost with contributing ratios ranging from 53% to 97%, depending on the category. Section 2 reports also outstanding contributions in terms of CED mostly due to the production of required chemicals and nutrients (upstream burdens) in the fermentation related activities. A negative value (environmental credit) from Section 2 to GWP has been identified due to the CO₂ uptake during the sugars fermentation process to produce the succinate. Concerning Section 1, it reports only remarkable contributions in GWP (25%) and CED (10%), mostly due to the heat requirements in the apple pomace reconditioning since feedstock must be dried (it is received with an average moisture of 75%) to guarantee its storage. Ancillary activities related with liquid and solid waste management are important contributors to GWP and EP, mostly due to GHG emission from energy requirements (GWP) and nutrient enrichment of the aquatic environment (EP).

<Figure 3 around here>

3.2. Identification of hotspots

One of the main key aspects derived from this study is identifying the environmental hotspots that is, the operations involved in the production process which are responsible for the highest environmental burdens and energy requirements.

Section 1 is intended for apple pomace reconditioning and storage once the raw material is received in the factory. As depicted in **Figure 1**, this section includes the feedstock drying and subsequent storage. **Figure 4a** illustrates that the combustion of natural gas to produce the heat requirement in the dryer and mostly, the electricity use in involved equipment (i.e., conveyor belts, freight elevators and air vents) are the two contributors to the impacts derived from Section 1.

Section 2 includes the operations related with the production of succinate from the apple pomace. As previously mentioned, this section requires the preparation of the inoculum which is subsequently feed into the fermenter. In this section, a huge amount of chemicals is required as well as electricity due to sterilization and cooling processes. **Figure 4b** distributes the impacts from operations involved in Section 2. According to it, electricity requirements in the pieces of equipment (conveyor belts, freight elevators, pre-fermenter, fermenter, pumps, refrigeration) can be considered as environmental hotspot. From these contributions, the sterilization and cooling processes are responsible for 90% of total electricity requirements in this section. The production of Na_2CO_3 , required as buffer to regulate the pH in the SSF unit reports also outstanding contributions to the environmental burdens derived from this section mostly in terms of EP and ODP. Nutrients are required either in the preparation of the inoculum and the SSF unit. Nutrients can be classified as salts (i.e., $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, K_2HPO_4 and MgCO_3) and proteins source (yeast extract and corn steep liquor). Their effect on the environmental profile is remarkable in terms of GWP, ODP and CED (**Figure 4b**) being the salts source responsible for 41-63% of total impacts. Finally, it is important to bear in mind the environmental credit associated to the carbon dioxide uptake in the SSF unit. *A. succinogenes* require the consumption of carbon dioxide in the reductive TCA cycle to produce the succinate. That CO_2 flow is supplied from bioethanol refinery expected to be located in the surroundings as previously justified.

Section 3 is intended for BioSA purification and requires numerous activities such as centrifugation, ultrafiltration, extraction with organic solvents, distillation to recover the solvents (firstly the octanol and next, the TOA) and separate the succinic acid, crystallization to produce the crystals and final storage. Within all these operations, distillation is considered as the environmental hotspots due to the huge

consumption of solvents (the 95% of both TOA and 1-octanol is finally recycled so only 5% of total required solvents in the extraction unit is spent per batch) and electricity demand (the distillation unit is considered a high intensive equipment) [54]. As it is depicted in **Figure 4c**, production of required solvents and electricity plays a key role in all the categories considered for analysis contributing both to 98-100% of total burdens and energy demand in Section 3. Assessing electricity contributions separately, the use of electricity in Section 3 is linked to the distillation unit as well as to the equipment required in the remaining operations (i.e., centrifuge, pumping, heating and mixing in the extraction). The distillation unit represents the 99% of total electricity requirements in this section. Contributions from electricity production to the global profile are outstanding in terms of GQP, EP, AP and CED. All electricity requirements are directly taken from the national grid, which is mainly based on fossil sources. Regarding solvents, their production is responsible for 78% and 66% of total contributions to ODP and POP in this section. These are organic solvents being TOA the most responsible for 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 in succinic acid. However, this step reports negligible contributions to the impacts as depicted in **Figure 4c**.

Finally, ancillary activities required to the treatment of both wastewater streams (e.g. from crystallization) as well as solid waste (e.g., waste biomass and other residual organic streams) produced in the BioSA production process have been computed within the system boundaries. Their effect on the global environmental profile is not so outstanding except in terms of GWP and EP as displayed in **Figure 3**. The WWTP is the main responsible of contributions to EP due to nitrogen and phosphorous based emissions into the aquatic environment. On the contrary both management operations are responsible for similar contributions to GWP (around 50% each of them) as depicted in **Figure 4d**, due to energy requirements in the WWTP and derived GHG emissions from landfilling activities.

<Figure 4 around here>

3.3. Alternative strategies

As previously indicated, the production of succinic acid can be carried out by means of alternative production sequences. In the designed plant, the purification section has been based on two main

operations. The extraction of succinic acid with organic solvents (TOA and 1-octanol) and the recovery of succinic acid and solvents (the latter to be recycled) in the distillation unit. However, both operations are considered environmental hotposts due to the large energy requirements and the huge organic chemicals consumption. However, other studies [5] propose the combination of ion exchange columns, nanofiltration and evaporation to recover the succinic acid before the crystallization unit. This designed strategy is low energy and chemicals intensive in comparison with the designed in our study. Therefore and to obtain an idea regarding the environmental behavior, this alternative strategy has been modelled in our plant in place of extraction and distillation steps. The new strategy should reduce energy requirements (ion exchange columns requires lower electricity than distillation columns) and chemicals (HCl and NaOH) for cleaning and regeneration operations. **Figure 5a** depicted the comparative environmental profiles between the plant under study and the alternative plant. The outcomes of the comparative assessment shows that the alternative proposal (in the Figure represented as Plant B) for the purification stage (Section 3) yields to lowest environmental burdens and energy demand. Impacts can be reduced in ratios ranging from 82% to 97% depending on the category and energy demand can be reduced up to 52% in comparison with the plant designed for assessment (Plant A1 in the Figure). However, it must be remarked that the same BioSA production yield has been assumed in the estimation due to the lack of recovery yields for the alternative purification strategy.

<Figure 5 around here>

Another aspect that needs to be highlighted is the potential of apple pomace as source of sugars and other compounds for fermentation. According to it, the use of low amounts of enzymes in the SSF unit (or even the removal of enzymes use) could derive on important amounts of succinic acid because the apple pomace contains a high amount of soluble sugars [24]. The use of enzymes allows performing the enzymatic hydrolysis and therefore, facilitating the availability of cellulose as carbon source for succinate production. However and according to the literature [24], the BioSA yield should be reduced of around 35% if no enzymes are considered in Section 2 since the cellulose should not hydrolysed. In addition, solid waste stream should be increased with the unspent cellulose. This consideration has been proposed for assessment in order to identify the potential improvements from an environmental

approach. In the comparison, the environmental burdens allocated to the enzymes production have been considered within the system boundaries as difference to the previous results with the aim of outstanding the effect of their use (and corresponding production activities). Thus, **Figure 5b** shows the comparative profiles between the current scenario (Plant A2) and the alternative without enzymes use and decreased production yield. The outcomes of the comparative assessment shows that the alternative proposal (Plant C) for the fermentation stage (Section 2) yields to lowest environmental burdens and energy demand. Impacts can be reduced in ratios ranging from 53% (in ODP) to 99% (in GWP). Regarding CED, it is reduced up to 94% in comparison with the plant designed for assessment (Plant A2). Thus, the no use of enzymes in spite of reducing the BioSA yield of 35% should be considered an interesting biorefinery choice. Enzymes production is a high energy intensive process which derive into important environmental consequences.

3.4. Comparison with literature

Enzymes are considered potential biocatalysts for numerous applications for multiple reasons such as their high selectivity and specificity as well as their application derives into lower demand for energy and chemicals [55]. However and in line with other results, there is some discrepancies regarding they drive into cleaner industrial systems bearing in mind the production of enzymes is high energy intensive [53,47].

Two studies have been found in the literature regarding the production of BioSA: one study considers the production from sorghum grains (a dedicated energy crop) and the second one manages corn and sugarcane [5,13]. Regarding fossil succinic acid, Smidt et al. [13] determined its environmental profile. Inherent limitations in performing a LCA comparison of succinic acid production systems are the use of different characterization methods (e.g., CML, ReCiPe) [56,57], data sources, system boundaries or even system expansion approach. In addition, it must be take into consideration inherent characteristics associated with the carbon sources (e.g., corn needs to be dried and hydrolyzed to release the sugar whereas sugarcane is an easier sugar source and requires little energy or chemicals) as well as with the production process itself (e.g., large amounts of steam are generated in the surgarcane based scenario). According to it, the comparison of our results with these found in the literature has been performed in terms of GWP and excluding the production of required enzymes from the system boundaries. **Figure 6** displays the comparative profiles and remarkable differences

can be identified in the corresponding carbon footprints. The best profile has been reported for BioSA-D where sugarcane is used as raw material. An environmental credit related with the production of huge amount of steam is behind this result. Regarding the other two scenarios BioSA-B and BioSA-C, a similar carbon footprint has been reported in the literature. The characteristics of the production system mostly in terms of purification stage (in both scenarios) as well as the co-production of ammonium sulfate – a potential fertilizer (in the sorghum based scenario) are behind these values. The alternative scenario previously discussed in which the purification section is modified with ion exchange columns, nanofiltration and evaporation (BioSA-E), represents the second best BioSA production choice. According to the depicted results, interesting improvement challenges should be proposed for the designed plant under study in this paper to reduce its environmental burdens.

<Figure 6 around here>

4. Conclusions and future outlook

The production of BioSA from apple pomace, a solid waste from cider and apple juice making factories, has been assessed from an environmental approach to identify environmental hotspots and propose improvement strategies. Moreover, the production plant has been modelled at full-scale from data at the laboratory scale. The production process considers the reconditioning and storage of the raw material, the fermentation and finally, the purification of BioSA in crystals. The production strategy considered in this study derived on important environmental burdens being considered the purification section as an environmental hotspot. The use of organic solvents to extract the BioSA and the subsequently distillation unit to recover both the solvents (on one site) and the pure BioSA (on the other site) are behind these remarkable environmental burdens. Potential heat recovery from the distillation unit could be possible and interesting not only from an environmental but also from an economic approach. However, it is complex to implement since the cooling water is constantly renewed and flowing through the column [54]. On the contrary, environmental credits could be allocated to that recovered heat. In addition, an optimization of the distillation unit in terms of electricity requirements should be taken in mind to improve the environmental profile. Alternative organic solvents to TOA and 1-octanol to extract the BioSA should be researched since although the 95% of the total requirements is recycled from the distillation unit, they report outstanding environmental

impacts. However, it must be highlighted the potential of apple pomace as source of sugars. It presents high content of free glucose and fructose (excellent carbon sources for succinic acid production) and high content of polysaccharides (such as cellulose) which can be easily enzymatically hydrolyzed. Even, due to its characteristic, without enzymes addition, high yields of succinic acid can be obtained.

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