Estimating the environmental impacts of a brewery waste–based biorefinery: bio-ethanol and xylooligosaccharides joint production case study

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Abstract

In the food industry, the brewing sector holds a strategic economic position since beer is the most consumed alcoholic beverage in the world. Brewing process involves the production of a large amount of lignocellulosic residues such as barley straw from cereal cultivation and brewer'spent grains.This study was aimed at developing a full-scale biorefinery system for generating bio-ethanol and xylooligosaccharides (XOS) considering the mentioned residues as feedstock. Life Cycle Asssessment (LCA) methodology was used to investigate the environmental consequences of the biorefinery system paying special attention into mass and energy balances in each production section to gather representative inventory data. Biorefinery system was divided in five areas: i) reconditioning and storage, ii) autohydrolysis pretreatment, iii) XOS purification, iv) fermentation and v) bio-ethanol purification. LCA results identified two environmental hotspots all over the whole biorefinery chain: the production of steam required to achieve the large autohydrolysis temperature (responsible for contributions higher than 50% in categories such as acidification and global warming potential) and the production of enzymes required in the simultaneous saccharification and fermentation (>95% of contributions to terrestrial and marine aquatic ecotoxicity potentials). Since enzymes production involves high energy intensive background processes, the most straightforward improvement challenge should be focused on the production of steam. An alternative biorefinery scenario using wood chips as fuel source to produce heating requirements instead of the conventional natural gas was environmentally evaluated reporting improvements ranging from 44% to 72% in the categories directly affected by this hotspot.

Keywords: Agricultural residue; Biorefinery scheme; Hydrothermal process; LCA; Sustainability; Valorization

1. Introduction

The depletion of fossil fuels, the increasing concerns regarding climate change effects and the need of an environmental-friendly economy are forcing the interest towards the development of technologies based on renewable sources to produce bio-chemicals (e.g., plastics, foams, building blocks, polymers) and bio-energy (Sanders et al., 2007). In this sense, biomass plays a key role for the sustainable global development (Sanders et al., 2012). The main use of biomass is for food and feed, however, valorization of biomass-based waste is focusing the research and development since it could be used in other large scale applications and the no-competition with food/feed is guaranteed (Liu et al., 2012; Kolfschoten et al., 2014). Moreover, other derived achievements from biomass-based economies have been identified such as regional energy security and rural economies improvement (Liu et al., 2012). The implementation of biorefinery approach is attaining special attention not only from an environmental perspective but also because biorefineries offer unprecedented opportunities (Liu et al., 2012; Sanders et al., 2012). The biomass-based feedstocks can be deconstructed into multiple high-added value products depending on the selected strategy (Borrega et al., 2011; Horhammer et al., 2011; Liu et al., 2012; Kolfschoten et al., 2014; Vargas et al., 2015). Thus, the valorization sequence selected will considerably affects not only to the type of yielding products but also their yield and inputs/energy requirement.

Regarding potential feedstocks used in biorefineries, there is a considerable interest in straw, a lignocellulosic by-product. Cereal straw is an agricultural residue from harvesting, which has traditionally been incorporated into the soil as nutrients and carbon supplier, directly burnt for heating purposes or used as animal beeding (Soon and Lupwayi, 2012). Nevertheless, it has attracted the attention from cellulosic ethanol industry by environmental and cost-effective issues (Kumar et al., 2016; Neves et al., 2016; Vargas et al., 2016).

Barley (*Hordeum vulgare*) is an abundant cereal in the world and it is one of the ten most common crops (Krawczy et al., 2005), being Spain placed in the fifth position in terms of global production volume (Vargas et al., 2015). One of the main barley-demanding industries is the brewing industry, where the grain is the main raw material. The high starch content and the good adherence of the husks to the grain body even after malting and milling are the rationale behind barley use in beer production (Pascari et al., 2018). Beer is the most consumed alcoholic beverage in the world (Pascari et al., 2018) so, the demand for barley grain is outstanding. According to the beer production process

(Mussatto, 2014; Pascari et al., 2018), the brewer's spent grains (BSG) constituted by the husk and seed coat layers are the main residue from the brewing process (~ 20kg of wet brewer's spent grains are produced per 100L of beer produced) as well as lacks economically feasible applications (Mussatto, 2014; Rojas-Chamorro et al., 2018). Moreover, cereal cultivation stage involves the co-production of straw – up to 0.53 kg straw per kg grain (Larsen et al., 2012; Vargas et al., 2015). Both (spent grains and straw) are an important and cheap source of lignocellulosic material with high carbohydrate content, so multiple potential applications such as second generation bio-ethanol and high-added value products could be identified (Vargas et al., 2015, 2016).

Having a look into lignocellulosic structure, it is constituted by cellulose, hemicelluloses and lignin as principal components. The cellulose, through enzymatic hydrolysis and fermentation, it might be converted to liquid fuels such as bioethanol. The hemicelluloses are considered an important source of valuable compounds as xylooligosaccharides (XOS), useful in food and pharmaceutical industries. XOS (considered novel non-digestible oligosaccharides with prebiotic potential, immunostimulating effect, anti-allergy, anti-infection and anti-inflammatory properties) are made up of β-(1,4)-linked xylose units (Chung et al., 2007; Meyer et al., 2015; Reis et al., 2014). The lignin can be used for the obtaining of high added-value products, such as resin precursors, heavy metal sequestrant, antimicrobial agents, aromatic compounds, syngas products, among others (Dávila et al., 2017). Therefore, this work deals with the large scale design and optimization of an industrial process for both barley straw and BSG valorization following a biorefinery scheme. The valorization sequence chosen for analysis includes a first step of hydrothermal pretreatment, with recovery of valuable hemicellulose-derived compounds in a separate liquid stream, and other step of simultaneous saccharification and fermentation (SSF) of the solid stream to obtain high bio-ethanol concentrations. The biorefinery scheme has been assessed from an environmental following the LCA methodology and considering a cradle-to-gate approach. To our knowledge, there is no peer-review studies available in the literature that analyse the joint production of bio-ethanol and XOS from alternative feedstocks. In the following, the production process at large scale of bio-ethanol and XOS is described in detail paying special attention to the design process.

2. Methodology

2.1. Life Cycle Assessment

Life Cycle Assessment (LCA) is considered one of the most developed tools for looking holistically at the environmental consequences linked to the life cycle of production processes, products or services. In this sense, it is widely used by environmental professionals and policy makers for the systematic evaluation of the environmental dimension of sustainability. Numerous studies focused on chemical and waste management processes have been environmentally assessed following the ISO 14040 (2006) guidelines (Burgess and Brennan, 2001; Kralisch et al., 2014; Al-Salem et al., 2014; Dearsola et al., 2012). In addition, several authors have explored the implementation of LCA methodology in environmental studies of biorefineries (Mu et al., 2010; Neupane et al., 2013; Gilani and Stuart, 2015). Therefore, its applicability in this area is justified.

2.2. Goal and scope definition

The goal of this LCA study is to provide a full overview regarding the production of both bio-ethanol and XOS under a biorefinery scheme as well as to determine its environmental performance. To do so, the biorefinery process has been modelled at full-scale process based on laboratory-scale data (Vargas et al., 2015, 2016). The scale-up of chemical processes requires a certain understanding of the involved steps (Piccinno et al., 2016). Therefore, the framework proposed by Piccinno et al. (2016; 2018) for scaling-up chemical production systems for LCA studies from laboratory-scale data has been followed in detail. An attributional cradle-to-gate approach has been contemplated in this research study, considering barley straw and BSG from brewery industry as key raw materials.

Since an attributional approach has been considered, the impacts have been estimated from the processes and material/energy flows used directly in the bio-ethanol and XOS life cycle. Therefore, energy and mass balances have been performed for the modeling of the full-scale biorefinery plant with the aim of gathering all the required data for the Life Cycle Inventory stage.

As difference to laboratory processes which are often far from being optimized (mostly in terms of resource consumption and energy efficiency) as well as they do not have the benefit of economies of scale (Piccinno et al., 2018), scale up production processes give a first approach to identify bottlenecks that should need to be improved in perspective of a possible industrial production. Therefore, a contribution analysis of the different production sections has been performed with the aim of identifying the environmental hotspots.

2.3. Functional unit and allocation procedure

LCAs are often performed using a functional unit that refers to the product obtained in the production system. However, biorefineries commonly yield on multiple co-products. In biorefinery systems the choice of method for allocating environmental impacts between the co-products is a common challenge (Cherubini et al., 2011; Sandin et al., 2015) since it can considerably influence decision-making strategies. In addition, allocation problems arise when it is not feasible to split involved processes or areas between the co-products. Thus, two approaches have been considered in this study to report the environmental impacts derived from the biorefinery under study.

1. Approach avoiding allocation: the functional unit is considered as the portfolio of co-products (i.e., bio-ethanol and xylooligosaccharides) that are generated in the valorization route (Gilani and Stuart, 2015). Thus, environmental impacts are calculated for a reference flow of 74.22 tonnes of lignocellulosic stream that enters in the valorization pathway and corresponds with a production batch.
2. Approach including allocation: the environmental impacts of the biorefinery are allocated to the co-products using a partitioning method based on the economic value (market value) of co-products. This perspective is deemed reasonable since both are target products for the biorefinery. The partitioning has been applied to areas connected to both products such as raw material reconditioning and storage (area 1) and autohydrolysis pretreatment (area 2). Regarding ancillary activities (solid and liquid waste management) and on-site emissions derived from the valorization strategies, it has been possible to identify which flow correspond to each co-product and thus, partitioning has not been required. This overriding approach is acknowledged by ISO 14044 (ISO 14044, 2006).

2.4. Description of the full-scale bio-ethanol and XOS production biorefinery

The raw material considered in this biorefinery is based on the combination of barley straw from cereal cultivation stage and the BSG from the brewing process. **Figure 1** displays the simplified system boundaries for the biorefinery process considered under evaluation. The production process has been divided in five main areas according to the breakdown of a real industrial plant. Within each area, the different involved operations have been identified and designed in detail.

<Figure 1>

Thus, it has been chosen and designed the appropriate equipment required in the biorefinery process (e.g., reactors, distillation colum, ultrafiltration unit, …) as well as other secondary machinery (e.g., pumps, heat exchangers, conveyor bets, …) resulting in the simple plant flow diagram displayed in **Figure 2**. A detailed description of each area and corresponding involved operations is detailed below.

<Figure 2>

Area 1: Raw material reconditioning and storage. In this area the raw material is received directly from both a local cereal farm and a local brewery. Barley straw is milled, air-dried, homogenized and warehoused in silos at atmospheric pressure and room temperature to guarantee its conservation. Regarding the BSG, they are received with 78% of moisture content and stored in silos at 4ºC until use.

Area 2: Autohydrolysis pretreatment. Both streams (straw and spent grains) are subjected to a non-isothermal autohydrolysis with water to achieve a liquid to solid ratio of 8 g liquid per g dry material. The operation temperature in the reactor is 210ºC, conditions reported as optimal for obtaining high amounts of XOS (Vargas et al., 2015). This stage is key since it permits the selective separation of the main components of the lignocellulosic biomass to give valuable products such as oligosaccharides derived from the solubilization of the hemicelluloses and a solid fraction rich in cellulose and lignin (Dávila et al., 2016). At the end of the hydrothermal pretreatment, the reactor is cooled at 45ºC. The autohydrolysis liquors (liquid fraction) are separated from the spent solids (solid fraction) in the filtration unit (press filter).

Area 3: Xylooligosaccharides purification. The liquid fraction rich in hemicellulose-derived compounds from the filtration unit is subjected to a purification step based on ultrafiltration, evaporation and spray drying operations. A membrane ultrafiltration unit is required to removing undesired compounds (such as monosaccharides and compounds derived from extractives and lignin) generated in the autohydrolysis step and to partially concentrate the liquors (Gullón et al., 2014). The concentrated fraction rich in XOS is sent to a triple-effect evaporators train under cross-current feeding to increase the higher economy. The output-stream from the evaporation unit present an average composition of 50% (w/w) in XOS. Next, it is sent to the spray drying unit where oligosaccharides-rich stream is sprayed and the XOS power (maximum 5% moisture) is obtained as final product, which is finally stored. Natural gas is used as fuel in the drying unit.

Area 4: Fermentation. The solid fraction from the filtration unit must be sent for bio-ethanol production. The fermentative production of bio-ethanol can be performed by consecutive stages of hydrolysis and fermentation, or in a single stage of Simultaneous Saccharification and Fermentation (SSF). In the latter, enzymes and fermenting microorganisms are present in the same medium. This configuration has been chosen for designing since reports multiple advantages (Buruiana et al., 2014). Firstly, the preparation of the inoculum is carried out; for this, cells of *Saccharomyces cerevisiae* CECT1170 (Spanish Collection of Type Cultures, Valencia, Spain) are grown at 32ºC for 24 h in a medium containing 10 g glucose/L, 5 g peptone/L, 3 g malt extract/L, and 3 g yeast extract/L. After growth, cells are recovered by centrifugation, resuspended in a phosphate buffer solution and inoculated in the medium SSF. SSF media was prepared by mixing the desired amounts of barley straw and BSG with water (at a liquid to solid ratio of 8 w/w), enzymes (cellulase, Cellic Ctec2) at a ratio of 2 Filter Paper Units (FPU) per g of pretreated dry solid, and nutrients (the same as in the preparation of the inoculum but without glucose).

SSF is the second step and six fermenters are considered for this purpose. SSF is performed in fed-batch mode and substrate, enzymes and nutrients are fed in three separate loads: the first at the beginning of the fermentation, the second at 24 h and the third at 48 h (Vargas et al., 2015). The fed-batch SFF configuration (FBSSF) has been considered since allows working at high solid loading, achieving high ethanol concentrations and minimising operational problems (Vargas et al., 2015). FBSSF is performed at pH=5, 35ºC and 120 rpm. The fed-batch SSF lasts up to 120 h.

It is important to bear in mind that all the required inputs in boths steps must be carefully sterilizated as well as the equipments used (pre-fermenters to produce the inoculum and fermenters to carry out the SSF) by means of the injection of steam to avoid possible contaminations.

Area 5: Bio-ethanol purification. This stage consists on the purification of the bio-ethanol rich stream from FBSSF. Firstly, solids presented in the stream must be removed (biomass and spent solids). To do so, the stream is derived to a centrifugation unit. The liquid fraction is heated-up from 35ºC to 65ºC with the aim of transferring the dissolved CO_2 from fermentation to gas phase. Secondly, heated stream is fed into a gas-liquid separator. The gas phase rich is CO_2 is vented and the liquid phase is heated-up till 95ºC (saturated liquid temperature) before being introduced in the distillation unit. After distillation, the botton stream is mostly constituted by water together with residual sugars, enzymes and salts from the fermentation medium. Distillate is rich in ethanol (~90% in weight). After being condensed, it is sent to the dehydration unit (molecular sieves) in order to obtain fuel grade bio-ethanol (puritiy >99.5%). Finally, bio-ethanol is stored for further distribution.

As indicated in **Figure 2**, solid and liquid wastes are produced in different steps. Activities involved in these wastes management have been included within the system boundaries as ancillary stages (see **Figure 1**). Liquid and solid wastes produced in the biorefinery are sent to a wastewater treatment plant (WWTP) and to composting (SWM), respectively. It is assumed that both installations are placed in the surroundings of the biorefinery.

2.5. Life Cycle Inventory data and sources

Among the LCA stages, Life Cycle Inventory analysis is the most relevant one since all data related to the production process (relevant inputs and outputs as well as emissions) must be gathered and accounted for further steps. In addition, high quality inventory data must be managed to obtain reliable results. Data corresponding to the foreground system (i.e., the biorefinery process) have been modelled in detail and identified all of them per area. The modelling of the full-scale facility required the scale-up of the laboratory production process. The selected studies (Buriana et al., 2014; Vargas et al. 2015, 2016) supplied useful information regarding the steps and quantities required at lab scale. The scale-up sequence proposed by Piccinno et al. (2018) has been followed in detail. In addition, calculation procedures and equations have been used for the specific design of the required equipment (Sinnott and Towler, 2009). As in other industrial facilities, the single processes are linked throughout transfer of reaction mixtures and the inter-process heat and energy recovery. Therefore, the estimated energy and mass flows have been accomplished as foreground-inventory data. In addition, the stoichiometric amounts of each reactant (including enzymes) considering lab protocols have been computed in the inventory data in line with Piccinno et al. (2016, 2018). Relevant inventory data from mass and energy balances to the foreground system is summarised in **Table 1**.

<Table 1>

Whenever possible primary data must be processed to achieve representative results. Nevertheless, sometimes it is necessary to go to secondary data mainly for background processes. In this study, only secondary data have been managed for background system, which involves the production of utilities (electricity, fossil fuels) and other inputs to the foreground system (chemicals, water and nutrients). Ecoinvent® database version 3.2 (Wernet et al., 2016) has been considered as main secondary data source. The biorefinery is planned to be placed in Spain due to the large availability of raw material. Thus, current data for the average electricity generation and imports/exports from Spain in 2017 (Red Eléctrica de España, 2017) have been considered to update the electricity defined in the database (Dones et al., 2007). Regarding enzymes production process and derived environmental impacts, information has been taken from Gilpin and Andrae (2017) as well as from Nielsen and Wenzel (2007).

Ancillary activities such as wastewater treatment and solid waste management have been also included within the system boundaries to compute the environmental impacts derived. Inventory data corresponding to the wastewater treatment plant have been taken from Doka (2007). Solid residue from from the centrifugue is sent to composting and inventory data have been taken from Doka (2007). **Table 2** lists the background processes directly taken from Ecoinvent® database included in this study.

<Table 2>

2.6. Life Cycle Impact Assessment methodology

The study takes into consideration the following impact categories: acidification potential (AP) as an indicator of acid rain effect; eutrophication potential (EP) as a sign of nutrients enrichment of water and soil; global warming potential (GWP) as an indicator of greenhouse effect; ozone layer depletion potential (ODP) as a pointer of substances emission with ozone-depleting potential, photochemical oxidation potential (POP) as an indicator of photo-smog creation. In addition, toxicity-based impact categories which are linked to the exposure of toxic substances for an infinite time horizon have been included in the analysis such as human toxicity (HTP), freshwater aquatic ecotoxicity potential (FEP), marine aquatic ecotoxicity potential (MEP) and terrestrial ecotoxicity (TEP). The choice of these impact categories is because all together give a complete and comprehensive environmental profile related to the production process under evaluation. Characterization factors reported by the Centre of Environmental Science of Leiden University - CML 2001 method v2.05 (Guinée et al., 2001) have been considered in this study for the analysis. The implementation of the Life Cycle Inventory data has been performed in the SimaPro v8.2 (PRé Consultants, 2017) software (Goedkoop et al., 2013).

2.7. Statistical analysis

Statistical analysis has been carried out using the software R (version 3.4.3) due to the relevance of electricity requirements in biorefinery systems when environmental burdens are analysed (González-García et al., 2016; 2018; Gullón et al., 2018). Differences in electricity consumptions inall production areas have been tested using both one-way analysis of variance (ANOVA) and Tukey's post hoc test. Differences have been considered significant at $p < 0.05$ as reported in Table 1.

3. Results and discussion

Table 3 shows the characterisation results corresponding to the biorefinery process proposed for analysis. The results are reported per batch (i.e., for the whole production system involving the valorisation of 74,216 kg of feedstock) as well as per kg of co-product obtained that is, per kg of bio-ethanol and per kg of XOS. As indicated in section 2.3. of this manuscript, the estimation of environmental burdens between both co-products has been carried out following an allocation procedure considering the market price of both co-products (0.64 €·kg^{-1} and 0.67[1] €·kg^{-1} respectively for bio-ethanol and XOS (Joelsson et al., 2016;Alibaba, 2018).

<Table 3>

3.1. Global results

The valorisation strategy considered in the designed full-scale plant considers five production units from feedstock reconditioning till purification sections of both co-products. **Figure 3** displays the contributions from the different involved units to each impact category. According to it, the autohydrolysis pretreatment (area 2) is considered as an environmental hotspot in the whole production system with contributions ranging from 33% to 55% depending on the category, except in

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[1] The market price assumed for XOS corresponds with hemicelluloses (which is lower) due to the lack of information regarding structural characteristics of the oligosaccharides obtained

terms of MEP and TEP. Related studies of biorefinery systems also identified this section as important in terms of environmental impacts (González-García et al., 2016, 2018; Gullón et al., 2018). This outstanding effect on the environmental profile is associated with the large requirements of steam in the autohydrolysis reactor since the optimum operation temperature was fixed at 210ºC, according to lab experiments. Area 4 intended for fermentation of cellulose-rich solid fraction obtained in the filtration unit and constituted by the inoculum preparation and FBSSF plays a key role in MEP and TEP (100% and 94% of contributing ratios, respectively). Background processes involved in the production of the enzyme required to transform the cellulose into glucose is behind these remarkable ratios. The remaining areas contribute to the impact categories in a minor extent. Ancillary activities report a remarkable effect in terms of EP (around 21% of total responsible factors). This area includes the management of both wastewater in a treatment plant as well as organic solid residues under a composting scheme, being their effects distributed as 55% and 45%, respectively. Thus, improvement research activities should be carried out towards the optimization of steam requirements in the pretreatment step to obtain outstanding reduction on the global environmental profile.

<Figure 3>

3.2. Environmental assessment of XOS production

The assessment of the environmental burdens associated with the production of XOS has been addressed in detail since it allows further comparison with other alternative oligosaccharides (pectic oligosaccharides (POS) and fructooligosaccharides (FOS)) as well as with other production strategies. This analysis is also important since area 3 is specific for production XOS, so that the environmental burdens derived from it, has been entirely allocated to this product. **Figure 4a** displays the distribution of environmental burdens between the involved areas that is, area 1 (feedstock reconditioning and storage), area 2 (autohydrolysis pretreatment), area 3 (XOS purification) and ancillary activities. The latter ony includes the management of derived wastewater in a WWTP since there is not solid residues production in this valorisation route. Moreover, the characterisation results per kg of XOS are summarised in **Table 3**. According to **Figure 4a**, the pretreatment stage (area 2) plays a key role in the environmental profile being responsible for contributing ratios around ~63% in all the categories except in EP, where it is of 48%. It is important to bear in mind that the partitioning ratio corresponding to XOS is 44%, which has been estimated taking into account the market value and production yield. This partitioning ratio has been considered for the distribution of burdens from area 1 and area 2 between both co-products. Area 3 which is related with XOS recovery from the liquid fraction obtained in the filtration unit (area 2) and consequenty purification, reports also an outstanding effect on the environmental profile of the XOS production. This area is responsible for ~31% of contributing burdens in all the categories. Having a look into this area, steam is required in the evaporation unit, electricity in the ultrafiltration unit (as well as in the pieces of equipment such as conveyor belts, pumps and bucket elevators) and natural gas for heating purposes in the spray drying unit. **Figure 4b** depicts the distribution of environmental burdens linked to area 3. According to these results the production of the steam required for the evaporation unit is the responsible for more than 90% of contibutions to all the categories analysed. This can be explained because steam production requires the combustion of natural gas. Alternative renewable fuels could be considered to reduce environmental burdens from this operation. Contributions to the environmental profile from electricity requirements are negligible. Production of heat needed in the spray drying reports an outstanding effect in GWP (10% of total contributions). The rationale behind this value is the combustion of natural gas in an industrial boiler to produced heat requirements.

Finally, eutrophication potential associated with XOS production is considerably affected by the wastewater management. Activities carried out in the WWTP are responsible for 28% of total eutrophying substances.

<Figure 4>

3.3. Environmental assessment of bio-ethanol production

In line with XOS production, the environmental profile associated with the production of bio-ethanol from barley straw and BSG biorefinery has been determined. Thus, environmental hotspots can be identified and the profile can be compared with others available in the literature.

Figure 5a displays the contributions to the environmental profile from involved stages in its production. Once again, area 2 plays a key role in some environmental categories such as AP, GWP, FEP and POP with contributing ratios of 46%, 41%, 51% and 39%, respectively. As previously indicated, a partition of burdens derived from area 1 and area 2 has been considered between both co-products. In the case of bioethanol, the partitioning ratio is of 56%. Therefore, 56% of burdens from feedstock reconditioning and autohydrolysis pretreatment have been computed to bio-ethanol production. It is obvious that further improvements should be focussed on the pretreatment step to enhance the environmental profile.

However, the environmental hotspot in the profile of bio-ethanol production is associated with area 4 (SSF stage), mostly due to the use of enzymes. **Figure 5b** depicts the contributing factors responsible for burdens derived from area 4. According to it, enzymes production plays the key role in all the categories evaluated. Enzyme production an energy and steam intensive process, specifically in activities such as aeration and fermentation (Nielsen and Wenzel, 2007; Gilpin and Andrae, 2017). Further research should be carried out on the enzymes production process (i.e., nutrients, carbon source and energy requirement) as well as on the optimization of the required enzymes dose. Moreover, CO_2 emissions from fermentation are also outstanding in terms of GWP (39%). Production of nutrients (glucose and peptone) required for the preparation of inoculum and for fermentation step reports a significant effect in terms of HTP and FEP, due to their background processes.

<Figure 5>

Regarding remaining stages, area 1 (raw material reconditioning and storage) and area 5 (bio-ethanol purification) as well as ancillary activities dedicated to the management of derived waste report a different behaviour depending on the category. Contributions from area 1 are negligible in all the categories. The purification stage contribute with no-outstanding ratios in all the categories except in terms of AP (13%) and FEP (14%). The rationale behind these values is mainly associated with the production of steam required in the distillation unit (~98%). Finally, ancillary activities include wastewater treatment and solid waste management under composting. Effect from these activities is remarkable in AP and EP (19% and 18%, respectively). Composting process is responsible for 99% of acidifying emissions and 76% of eutrophying emissions.

3.4. Uncertainty regarding enzymes' effect on the results

Enzymes are required for the hydrolysis of cellulose into fermentable sugars. As previously discussed, the production of enzymes has been identified as one major contributor towards the life cycle environmental analysis of bio-ethanol production. This statement has been reported by other studies available in the literature (Wiloso et al., 2012; Sebastião et al., 2016; Gilpin and Andrae, 2017). However, it is not clear in some works which system boundaries have been taken into account (Borrion et al., 2012; Wiloso et al., 2012). In this sense, it is not evident if production of both chemicals and enzymes has been considered, which considerably difficult the environmental comparisons with studies available in the literature.

MacLean and Spatari (2009) established that 33% of greenhouse gases (GHG) emission produced all over the life cycle of bio-ethanol are attributed to enzymes and chemicals required. In our study, their contribution adds up to 20% of total in line with the findings from Sebastião et al. (2016). It is important to highlight that the enzyme activity is a key factor which directly affect the environmental profile since it is directly linked to the dose of enzyme required. In our study, around 135 kg enzyme are required per 1,000 kg of bio-ethanol produced, a value considerably higher than that reported by Daylan and Ciliz (2016) that employed 38 kg per 1,000 kg bio-ethanol. Therefore, research and development should be focused on reducing the amount of enzyme needed or increasing the enzyme productivity as well as the potential for recycling enzymes (MacLean and Spatari, 2009).

Moreover, special attention must be paid to the bio-ethanol production strategy from lignocellulosic feedstocks. Although in the designed biorefinery, FBSSF has been established for its multiple benefits such as higher bio-ethanol yields, shorter fermentation time and lower toxic effect of the medium components (Cheng, et al., 2009); in the literature can be found others approaches for effective bio-ethanol production such as separate hydrolysis and fermentation (SHF), consolidated bioprocessing (CBP) or cell recycle batch fermentation (CRBF). In SHF, enzymatic hydrolysis of pretreated biomass is made separately from ethanol fermentation (Azhar et al., 2017). In CBP, the saccharification and the fermentation are performed by one single microorganism and in one step (Hasunuma and Kondo, 2012). The CRBF is based on the recycling of the yeast cells, reducing the time and of the cost of the inoculum preparation (Matano et al., 2013).

Furthermore, the pretreatment is a key stage to improve cellulose hydrolysis and to produce a fermentable sugars stream rich in glucose; likewise, the severity of this stage has a direct effect on the amount of enzymes required. In our study, autohydrolysis pretreatment has been proposed with the aim of producting not only bio-ethanol but also XOS from hemicelluloses solubilization. In this context, an increase in the severity of the treatment leads to a greater enzymatic susceptibility of the solid fraction from the hydrothermal treatment and therefore a higher production of ethanol, however obtaining XOS could substantially be affected.

According to it, it is proved the relevance of including the impact of enzymes production in life cycle environmental studies of bio-fuels.

3.5. Production of steam requirements: Sensitivity analysis

Besides enzymes, production of steam requirements is crucial in the environmental profile of the biorefinery system under study. The rationale behind its large effect on the environmental burdens is the use of natural gas as fuel, which has been considered as proxy for the current most extended practices at industrial level. Production of steam requirements in areas 2 (to acquire the optimum temperature), 3 (in the evaporation unit) and 5 (in the distillation unit) is responsible for contributions higher than 55% in categories such as AP, GWP, ODP, POP, HTP and FEP as displayed in **Figure 6a**. Therefore, an interesting challenge to improve the environmental profile should be focused on reducing the environmental burdens from this operation. An alternative scenario has been proposed for analysis considering the production of steam from hardwood chips that is, a renewable source avoiding the use of a fossil fuel (i.e., natural gas). **Figure 6b** depicts the outcomes of the sensititivity analysis comparing the profile between base case and the alternative one. According to it, the alternative scenario yields to the lowest environmental burdens specifically in terms of GWP (reduction of 61%), AP (72%), FEP (66%) and POP (65%). Therefore, this steam production alternative should be the most convenient choice. Although background activities involved in chips production (i.e., forest system) have been computed and require the consumption of diesel in forest machines, global fossil fuels demand is lower than in the baseline. Thus, global emission parameters (e.g., PM, NO_x, SO_2) are lower in the renewable alternative.

<Figure 6>

3.6. Comparison with literature

Nowadays the interest on the biorefinery approach is capturing the industry and stakeholders' attention for multiple motives since a great fraction of energy carriers and materials come from fossil fuel refineries (Cherubini, 2010). Furthermore, European Commission is implementing strategies to "closing the loop" of product life cycles in industrial production systems from a circular economy approach (Liguori and Faraco, 2016). To the best of our knowledge, no other environmental studies have been published regarding a biorefinery producing both bio-ethanol and oligosaccharides.

Bio-ethanol from first generation technology is currently used in commercial gasoline blends. It requires the use of dedicated crops which derive on direct competition with arable land for food and feed purposes. Lignocellulosic bio-ethanol is therefore a promising energy alternative being considered a clean, low carbon and secure energy source (Borrion et al., 2012; Sebastião et al., 2016). To date, several studies are avaible regarding the environmental impact of bio-ethanol paying special attention into GHG emission (Daylan and Ciliz, 2016; Chang et al., 2017). However, the complexity of the whole bio-ethanol production chain can generate significantly different results due to differences in input data, feedstock managed, methodologies applied and assumptions, and local geographical conditions (Sebastião et al., 2016). As previously discussed, system boundaries selected for the analysis is also a critical issue since discrepancies exist regarding their definition. In this sense, the production of enzymes required in the fermentation throws up great controversy. Our study is based on a biorefinery system where not only bio-ethanol is produced but also xylooligosaccharides. Therefore, it involves specific activities (e.g., area 2) dedicated to the fractionation of the feedstock to produce both co-products. The autohydrolysis section is not common in dedicated bio-ethanol production systems playing a key role in our environmental profile. The large energy demand in the autohydrolysis reactor is behind that issue and thus, the environmental profile associated with the bio-ethanol obtained in our biorefinery is considerably worse than available studies in the literature. Reported values for second generation bio-ethanol are lower than 0.157 kg CO_2eq per MJ bio-ethanol (Sebastião et al., 2016) - which corresponds with wheat straw based bio-ethanol. In our study, the GWP adds up to 0.280 kg CO_2eq per MJ bio-ethanol assuming 26.4 $MJ \cdot kg^{-1}$ as lower caloric value[2] and being ~35% of GHG emission derived from autohydrolysis.

Regarding oligosaccharides production, González-García et al. (2016; 2018) and Gullón et al. (2018) environmentally assessed different valorization strategies at pilot scale dedicated to hemicellulosic

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[2] http://www.eubia.org/cms/wiki-biomass/biofuels-for-transport/bioethanol/ (accessed March, 2018)

oligosaccharides production (galactoglucomannas, pectioligosaccharides and xylooligosaccharides, respectively) from different feedstocks (wood chips, sugar beet pulp and vine shoots). All of them could be considered alternative oligosaccharides with interest as prebiotic functional food ingredients and biomaterials. A comparative environmental analysis in terms of GHG emission throughout the whole life cycle has been addressed with the aim of indetifying the best production strategy. The comparative profiles per kg of oligosaccharide produced are depicted in **Figure 6c**. The best result in terms of GHG emission corresponds to xylooligosaccharides production from barley straw and brewer's spent grains under a biorefinery approach together with bio-ethanol as co-product (4.21 kg $CO_2eq·kg^{-1}$). It is important to highlight that the study corresponds with a full-scale production whereas the other studies were performed at pilot scale. As previously mentioned, the production process has been modelled from laboratory data following the methodology reported by Piccinno et al. (2018) considering the benefit of economies of scale. The production of pectioligosaccharides under thermal and enzymatic treatments from sugar beet pulp (González-García et al., 2018) derived on a carbon footprint around 13 times higher. Large electricity requirements in operations such as freeze-drying are the rationale behind that result. On the other hand, the worst profiles correspond to the extraction of galactoglucomannas from residual wood waste under thermal treatment conditions (González-García et al., 2016) deriving into 189±40 kg $CO_2eq·kg^{-1}$. Purification and freeze-drying activities are the key processes responsible for these large results. According to Gullón et al. (2018), xylooligosaccharides extraction from vine shoots considering different thermal pretreatments and different valorization routes involves a GWP of 104±49 kg $CO_2eq·kg^{-1}$. Electricity requirements for freeze dryer and autoclave as well as enzymes are again environmental hotspots.

4. Conclusions and future outlook

The integration of a biorefinery approach in a production system allows the obtention of different high-added value products from renewable wastes making the process more sustainable not only from an economic but also from an environmental perspective, reducing residues production and resources consumption. In this study, wastes from brewery have been considered as potential feedstock for bio-ethanol and xylooligosaccharides production. The production factory has been modelled at full-scale considering laboratory data and environmental impacts have been determinated following the LCA methodology. The large requirement of steam, specifically in the autohydrolysis reactor, which is commonly produced from natural gas, has been identified as environmental hotspot. In addition, the production of enzymes required in the bio-ethanol production route have considerably affected the environmental profile.

The introduction of renewable sources to produce steam requirements such as wood chips can be considered as a potential improvement, deriving into outstanding environmental reductions. In addition, the enzyme specific activity is an issue that directly affect the environmental burdens. According to the outcomes, further research should be focused at large scale not only in the optimization of enzymes' dose requirement but also in the enzymes production process itself with the aim of increasing their specific activity and reducing the energy requirements as well as in the enzymes potential recycling.

Environmental sustainability has increasingly been incorporated in the design of biorefinery systems (although often reduced to GHG emission); economic dimension is often considered mostly throughout profitability and techno-economic analysis to compare biorefinery alternatives for producing a given product; however, social dimension of sustainability in contrast to economic and environmental ones, is generally omitted in design practices. Thus, future efforts must be conducted to develop an integral sustainability analysis.

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