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Environmental assessment of the valorization of glycerol for the production of hyperthermophilic β-glucosidase under a biorefinery approach

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ABSTRACT

Bioethanol production technologies from lignocellulosic biomass are not yet optimized and do not compete economically with first-generation bioethanol production. Strategies have been investigated to produce more active, stable and temperature-tolerant enzymes to be used for biomass hydrolysis such as the hyperthermophilic β-glucosidase produced by *Yarrowia lipolytica*. The use of this strain offers an additional competitive advantage, as it can use glycerol stream from the biodiesel process as a carbon source. In this way, not only is a by-product of biofuel production used, but the enzyme could be applied in the production of lignocellulosic ethanol, increasing the value chain by closing the bioeconomy cycle. To this end, large-scale process modelling of β-glucosidase production has been developed to collect the inventory data needed for life cycle assessment methodology. The fermentation stage is the largest contributor to environmental impacts, with electricity being the main hotspot identified, contributing more than 50% in most impact categories. Residual glycerol has also been identified as a critical input, with a significant contribution in some categories. To improve the environmental profile, a sensitivity analysis has been carried out considering reductions in electricity and heat consumption, and other alternative oil-based resources for the production of biodiesel. This analysis identified that large environmental reductions could be achieved, which makes the valorization of the glycerol obtained as a side stream of biodiesel production more realistic.

1. Introduction

In the context of the transition towards the sustainability of energy production and use, the valorization of waste streams has been the main driver in the search for alternatives in biofuel production (Clauser et al., 2021; Stegmann et al., 2020a). In an effort to promote energy self-sufficiency, bioethanol ranks first as a substitute for fossil petrol. However, the feedstock functionality for first-generation bioethanol production is restricted due to the use of cereals as feedstock, which is in direct conflict with their use for food and feed, as well as overexploitation of arable land (Berndes et al., 2013; Dammer et al., 2017; Havlík et al., 2011; Tudge et al., 2021). Second-generation bioethanol production fills the gap of the first generation using non-edible feedstocks from agricultural and forestry residues (Aditiya et al., 2016; Jusakulvijit et al., 2021; Robak and Balcerek, 2018). Lignocellulosic and starchy materials can be potential sources of fermentable sugars used as a carbon source in the formulation of culture media for fermentative process (Dey

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et al., 2020; Jusakulvijit et al., 2021; Rosales-Calderon and Arantes, 2019; Saini et al., 2015a). Despite the different stages in the production of second-generation bioethanol, the initial stages of releasing fermentable sugars by enzymatic hydrolysis are of particular relevance for the conceptual design of the overall process and bioethanol yield (Lara-Serrano et al., 2018; MacRelli et al., 2012; Sharma et al., 2022; Vasić et al., 2021).

In the case of biodiesel, the production process considering the transesterification reaction is applied in most industries, generating glycerol with a degree of purity between 50 and 80% (Abdul Raman et al., 2019; Pitt et al., 2019). The presence of fatty acid methyl ethers, methanol, soap and ash impurities leads to the need for purification, mainly based on a distillation process, which it is not a viable economic option (Abdul Raman et al., 2019). To this end, this bioglycerol is a low purity value for use as a raw material in pharmaceuticals, cosmetics and food products, which reduces its marketability and applicability, thus becoming a 'waste' rather than a 'resource' (Win and Trabold, 2018). In this context, the development of an efficient and economically viable strategy would be based on the use of crude glycerol in bio-based microbial process such as enzyme production. Enzymes can act selectively and convert high molecular weight polymers present in biomass into their fermentable monomeric sugar units (Horn et al., 2012; Houfani et al., 2021; Hyeon et al., 2014; Souza et al., 2018).

Furthermore, the implementation of circular economy models in industry often involves the use of waste streams or lignocellulosic-based by-products, i.e., biomass (Dahmen et al., 2019; Devi et al., 2022; Saini et al., 2015b). Bioeconomy is considered as an integrated solution to reduce the dependence on fossil resources, use renewable resources, maximize reuse and recycling of raw materials and extend the life span of products from the design stage (Holden et al., 2022). The shift from linear production to circular process is an essential step on the way of achieving a bioeconomy manufacturing framework (Stegmann et al., 2020b; Venkatesh and Se, 2021). Resource conservation is achieved through open or closed end-of-life (EoL) strategies, whose alternatives range from mechanical, chemical or biological processes, energy recovery and/or composting (Bauer et al., 2017; Tan and Lamers, 2021).

It is in this framework that this manuscript focuses, as it considers the use of a by-product of the biodiesel production process, glycerol, to obtain the enzyme β-glucosidase, with wide applications in biotechnological processes due to its ability to selectively catalyze the conversion of cellobiose produced by enzymatic breakdown of cellulose into fermentable sugars. This hyperthermophilic enzyme could be used in the food industry for hydrolysis, for the release of aromatic compounds, to enhance the flavor of food and beverages, for the extraction of medicinal compounds by cleavage of phenolic glucosides and for the hydrolysis and breakdown of lignocellulosic biomass to produce biofuels (Ahmed et al., 2017; Singh et al., 2016; Srivastava et al., 2019; Stradwick et al., 2017; Turner et al., 2006). The crude application of this enzyme makes it an essential input for the development of biorefineries based on lignocellulosic materials. In this sense, the use of a side stream of waste from biodiesel production to obtain this enzyme, which could in fact be reused later in the same process, could be considered as a sustainable, circular and environmental-friendly strategy. To assess its advantages, large-scale process modelling has been developed that integrates the main steps in the biotechnological production of β-glucosidase. The process simulation allows the collection of inventory data necessary for the application of the Life Cycle Assessment methodology.

2. Process description

As a basis for the biotechnological conceptual design of the process, it is necessary to analyze the biochemical reaction kinetics representing microbial growth and enzyme production. The fermentation strategy is developed according to a fed-batch scheme, as it provides higher yields and productivity compared to batch operations (Abdella et al., 2020). It should be noted that the production of β-glucosidase occurs intracellularly, as higher enzyme titers are achieved, representing added value and competitive performance compared to extracellular enzyme secretion (Abdella et al., 2020; Soetaert and Vandamme, 2009). With this in mind, microbial growth in fermenters has been modelled in SuperPro Designer, with biomass being the main product obtained ($CH_{1.8}O_{0.5}N_{0.2}$), in which intracellular β-glucosidase is produced:

$$92.09\ C_3H_8O_3 + 4.59\ NH_3 + 66.76\ O_2 \rightarrow 33.15\ CH_{1.8}O_{0.5}N_{0.2} + 72.78\ CO_2 + 57.51\ H_2O$$

The culture medium was formulated based on a glycerol concentration of 40 g/L, supplemented with $NH_4H_2PO_4$, KH_2PO_4 and $MgSO_4 \cdot 7H_2O$ and the operational conditions that allow the production of beta-glucosidase correspond to a temperature of 30 °C and pH 4, as these are the most suitable conditions for the growth of *Y. lipolytica* (Chen et al., 2018; Hernández-Guzmán et al., 2016). In addition, as this is an aerobic process, filtered air is fed at a rate of 0.5 vvm through diffusers placed at the bottom of the main and seed fermenters. At the end of the fermentation, the biomass concentration obtained is 19 g/L. This value is obtained by considering the kinetic parameters of the specific growth rate, the Monod constant, as well as the reactor volume and inlet flow rate (Blanco et al., 2021).

The subsequent steps of the process correspond to a cascade strategy, in which the separation of the biomass, cell lysis to release the intracellular enzyme, as well as a sequence of purification steps have been considered. Moreover, the most commonly used format for commercialization of β-glucosidase is in solution, so a freeze-drying stage is not necessary (Ferreira et al., 2018a). The downstream process considers the use of a microfilter to concentrate the flow stream obtained just after fermentation has finished. After filtration, with a concentration of around 90 g/L (Ferreira et al., 2018a), the second step is cell disruption for the release of the intracellular enzyme using a high-pressure homogenization unit (HG-101) according to the following mass balance (Chen et al., 2018; Middelberg, 2000).

$$Biomass \rightarrow 0.43\ Cell\ Debris + 0.4\ Proteins + 0.1\ \beta - Glucosidase + 0.07\ Glycogen$$

After cell disruption, β-glucosidase is mainly in solution with salts and cellular debris. To separate the main product, a disc centrifuge followed by a dead-end filtration is used, as it provides an efficient separation of the remaining biomass and cell debris (Ferreira et al., 2018a; Heinzle et al., 2007; Soetaert and Vandamme, 2009).

Ultrafiltration is the first stage of the purification procedure, with the β-glucosidase concentrated by a factor of 2 (Hemavathi and Raghavarao, 2011). Although the ultrafiltration output stream has significantly reduced the concentration of salts, purification proceeds with ion exchange (IEX) and diafiltration steps. Considering IEX, it is based on the separation of components by different retention times on the solid support, requiring the addition of the following chemicals: HCl (5% w/w) and NaCl (0.5 M) as washing agents and NaCl (0.5 M) and NaOH (20% w/w) for the regeneration of the ion exchange resin (Al-Asheh and Aidan, 2020). The input stream to the IEX unit is mainly composed of water, glycerol, KH_2PO_4, proteins, β-glucosidase enzyme, glycogen and organic matter. To this end, the goal of the IEX unit is to separate the stream of β-glucosidase, which goes to the diafiltration stage to remove residual salts, while the rest are treated as waste. The last stage is diafiltration, to obtain a higher purified product, as it is retained in the membrane by adding the buffer solution, in this case citric acid, as a matrix to stabilize the final product (Xia et al., 2022; Zhang et al., 2017).

3. Environmental analysis using LCA methodology

LCA is a methodology for assessing the environmental profile of a product and/or a process by identifying and quantifying all mass, energy and waste streams associated. These data are compiled in what are called "Life Cycle Inventory (LCI)" which is displayed according to the functional unit selected, that is, the reference unit used for the mass, energy and waste balances. This methodology is applied according to the development of four main steps, which are described below in the framework of the manuscript.

3.1. Definition of the goal and scope of the study

The objective of the work is the analysis of the environmental burdens of the biotechnological valorization of glycerol as a carbon source for the microbial production of β-glucosidase. In terms of system boundaries, a "cradle-to-factory" approach has been selected, which includes all stages from feedstock and energy resource extraction to the biorefinery gate, i.e., the production of β-glucosidase as the main product and wastewater as side streams (Fig. 1). On the other hand, the environmental burdens of construction, decommissioning and infrastructure maintenance were excluded from the assessment, as other authors have shown that the environmental impacts of these are negligible (Arias et al., 2021, 2022; Falano et al., 2014). Finally, regarding the functional unit (FU), two were selected for evaluation. On the one hand, 1 batch/operating cycle and, on the other hand, the production of 1 kg of β-glucosidase. The selection of these two UFs has been based on obtaining an environmental profile that allows the environmental loads to be assessed globally, i.e., seeking to evaluate the sustainability of the industrial facility and compare it with other processes, and at a more individual level, to allow comparison of the efficiency and ecological alternative for the production of this enzyme.

3.2. Data collection for the LCI

Literature data were used as a basis to perform the mass and energy balances of the process, and to scale it up to an industrial manufacturing level, SuperPro Designer® has been used to model the biotechnological process. Once the process was modelled and all mass, energy and waste flows were defined and calculated, the LCI could be quantified. The background data for all LCI inputs were obtained from the Ecoinvent database. Furthermore, in order to identify the process steps with the highest contribution to environmental damage, the overall process has been divided into the main equipment used in the glycerol recovery route. With this, it has been possible to perform the appropriate sensitivity analysis to try to achieve an even better environmental profile, based on the components of the inventory data that lead to the highest environmental load, of the process.

3.3. Life cycle impact assessment (LCIA) according to MidPoint hierarchical ReCiPe 2016 methodology

For assessing the environmental loads of the process, according to the data provided in the LCI, ReCiPe 2016 hierarchist MidPoint calculation methodology V1.03 World has been used for the characterization factors of 18 midpoint impact categories, which are listed in Table 1. On the other hand, the SimaPro software has been used to develop the computational implementation of the data compiled in the LCIs.

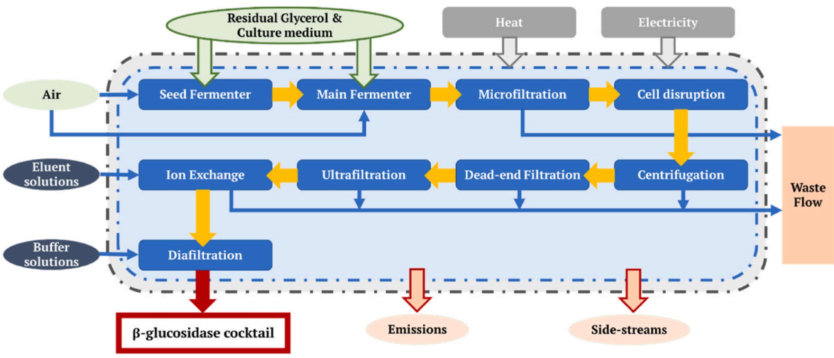

Fig. 1. System boundaries considered for assessing the environmental profile of β-glucosidase production.

3.4. Interpretation of the environmental profile and characterization values

Once the environmental loads and contributions of the input data were obtained, a sensitivity analysis was carried out, focusing on those materials that lead to a higher environmental contribution, called hotspots. For this, various process alternatives and optimization procedures were evaluated, with the aim of providing an improved environmental profile and guidance on what researchers and stakeholders should focus on in order to develop more sustainable and less environmentally damaging production systems.

4. Results and discussion

The integration of enzymatic processes for biomass valorization requires confirming whether enzyme production and use is carried out under environmental sustainability criteria. To this end, modelling the production of β-glucosidase using the simulation tool SuperPro Designer allows the collection of inventory data necessary for the application of the life cycle assessment methodology. At the same time, seeking to analyze the profile in more detail, more in-depth studies were carried out at the stages leading to the highest environmental contribution, pursuing to identify the reasons for such impact loads. With the aim of evaluating scenarios with more convincing sustainability values, sensitivity analyses were performed around the identified critical points.

4.1. Life cycle inventories

The inventory data of the biotechnological process considering as functional unit a batch is shown in Table 2, while the data corresponding to 1 kg of β-glucosidase is shown in Table 3. On the other hand, the inputs used from the Ecoinvent database are depicted on Table 4.

4.2. Modelling results

The capacity of the facility is 5200 kg of waste glycerol per batch process. The selected capacity has been defined according to other references on the production of this enzyme as well as process variables such as residual glycerol to be processed, amount of enzyme produced and the capacities of the equipment (Ferreira et al., 2018b; Klein-Marcuschamer et al., 2012; Tusé et al., 2014). The main modelling equipment of the facility is included in Table 4, with its main capacity values, together with the number of units required. The process has been divided into two main sections, those required for fermentation, both seed for inoculum preparation and main fermentation for the production of the enzyme, and the downstream stage required for purification, based on a cascade process with seven main steps, which were described in Section 2.

4.3. Environmental results

ReCiPe MidPoint calculation methodology was applied to score the environmental impacts associated with β-glucosidase production process (see Table 5). Table 6 includes the absolute impact values obtained.

In addition, in order to evaluate the overall process, to identify which of the stages leads to the highest environmental load and therefore where improvements and optimization are needed, Fig. 2 is depicted.

As can be seen, the stage leading to the highest environmental load is the main fermentation stage, as expected, followed by diafiltration, but with a much less significant contribution in comparison. The reason for the environmental load of the diafiltration stage is due to the use of citric acid in the filter (Gaber et al., 2020). To identify the reason for such a high environmental load in the main fermentation stage, Fig. 3 shows the environmental profile of this single stage, according to the LCI data provided in Tables 1 and 2

In the environmental profile of the main fermenter, three main hotspots can be identified: electricity, glycerol and steam requirements. For the impact categories certainly, as in the case of MRS and WC, the main contributors are ammonium dihydrogen phosphate and process emissions, respectively. The reason for the huge impact contribution of glycerol comes from the background activities for its production. It should be noted that, even if a valorization route is proposed, a zero-impact value cannot be assumed for this glycerol residue, as several steps are required to obtain it, as a side stream of the biofuel production process. As for electricity and steam loads, the fact that they are obtained from fossil resources, whose impact damages are well known, leads to such a large contribution. If a reduced impact value is pursued, the source could be modified by opting for renewable-based energies, whose environmental impact values are lower. These alternatives for the energy source are an aspect to be studied in the sensitivity analysis, which are presented in the following sections of this manuscript.

Table 1
ReCiPe MidPoint impact categories analysed for the environmental assessment of β-glucosidase production.

Acronym	Impact category	Unit	Acronym	Impact category	Unit
GW	Global Warming	kg CO_2 eq	TET	Terrestrial Ecotoxicity	kg 1.4-DCB
SOD	Stratospheric Ozone Depletion	kg CFC_{11} eq	FET	Freshwater Ecotoxicity	kg 1.4-DCB
IR	Ionizing radiation	kBq Co-60 eq	MET	Marine Ecotoxicity	kg 1.4-DCB
OF	Ozone Formation	kg NO_x eq	HC	Human carcinogenic	kg 1.4-DCB
FPF	Fine Particulate Formation	kg $PM_{2.5}$ eq	HNC	Human non-carcinogenic	kg 1.4-DCB
OZ	Ozone Formation	kg NO_x eq	LU	Land use	m^2a crop eq
TA	Terrestrial Acidification	kg SO_2 eq	MRS	Mineral Resources Scarcity	kg Cu eq
FE	Freshwater Eutrophication	kg P eq	FRS	Fossil Resources Scarcity	kg oil eq
ME	Marine Eutrophication	kg N eq	WC	Water Consumption	m^3

Table 2
Life Cycle Inventory of the bio-technological production of β-glucosidase from biodiesel (FU: 1 batch).

1st stage: Seed Fermenter [V = m^3]						4th stage: Cell disruption					
Inputs: Resources			Inputs: Electricity/heat			Inputs: resources			Inputs: electricity/heat		
Air	3195.3	Kg	Steam	5260.51	MJ	Water, cooling	4.88	m^3	Electricity	0.89	kWh
Water, cooling	472.63	m^3	Electricity	536.94	kWh	5th stage: Centrifugation					
Inputs: Materials			**Outputs: Emissions to air**			**Inputs: Electricity/heat**			**Outputs: Waste to treatment**		
Residual glycerol	261.72	Kg	N_2	2451.19	kg	Electricity	285.09	kWh	Wastewater	0.93	m^3
Water	4.64	m^3	O_2	626.73	kg	6th stage: Dead-end filtration					
$(NH_4)H_2PO_4$	40.97	Kg	CO_2	122.72	kg	**Outputs: Waste to treatment**					
$MgSO_4{\cdot}7H_2O$	3.28	Kg				Wastewater			0.59	m^3	
KH_2PO_4	82.57	Kg				7th stage: Ultrafiltration					
Yeast	4.97	Kg				**Inputs: Electricity/heat**			**Outputs: Waste to treatment**		
2nd stage: Main Fermenter [V = m^3]						Electricity	26.52	kWh	Wastewater	7.88	m^3
Inputs: Resources			**Inputs: Electricity/heat**			8th stage: Ion exchange					
Air	140982	Kg	Steam	83009.70	MJ	**Inputs: Materials**			**Outputs: Waste to treatment**		
Water, cooling	472.63	m^3	Electricity	45370.60	kWh	Water	9.81	m^3	Wastewater	8.30	m^3
Inputs: Materials			**Outputs: Emissions to air**			NaCl	194	kg			
Residual glycerol	3632.50	Kg	N_2	110553.20	kg	HCl	6.9	kg			
Water	14971.26	m^3	O_2	30851.60	kg	NaOH	772.07	kg			
$(NH_4)H_2PO_4$	633.51	Kg	CO_2	2954.50	kg	9th stage: Diafiltration					
$MgSO_4{\cdot}7H_2O$	53.47	Kg				**Inputs: Materials**			**Outputs: Product**		
KH_2PO_4	1210.69	Kg				Water	1.72	m^3	Enzymatic cocktail	2187.09	kg
Yeast	72.93	Kg				Citric acid	485.06	kg			
3rd stage: Microfiltration						**Inputs: Electricity/heat**			**Outputs: Waste to treatment**		
Inputs: Electricity/heat			**Outputs: Waste to treatment**			Electricity	31.28	kWh	Wastewater	0.04	m^3
Electricity	127.68	kWh	Wastewater	64.98	m^3						

Table 3
Life Cycle Inventory of the bio-technological production of β-glucosidase from biodiesel (FU: 1 kg of enzymatic cocktail).

1st stage: Seed Fermenter [V = m^3]						4th stage: Cell disruption					
Inputs: Resources			Inputs: Electricity/heat			Inputs: resources			Inputs: electricity/heat		
Air	1.461	Kg	Steam	2.41	MJ	Water, cooling	0.002	m^3	Electricity	0.407	Wh
Water, cooling	0.216	m^3	Electricity	0.25	kWh	5th stage: Centrifugation					
Inputs: Materials			**Outputs: Emissions to air**			**Inputs: Electricity/heat**			**Outputs: Waste to treatment**		
Residual glycerol	0.120	Kg	N_2	1.12	kg	Electricity	0.130	kWh	Wastewater	0.423	L
Water	0.002	m^3	O_2	0.29	kg	6th stage: Dead-end filtration					
$(NH_4)H_2PO_4$	0.019	Kg	CO_2	0.06	kg	**Outputs: Waste to treatment**					
$MgSO_4{\cdot}7H_2O$	0.002	Kg				Wastewater			0.271		L
KH_2PO_4	0.038	Kg				7th stage: Ultrafiltration					
Yeast	0.003	Kg				**Inputs: Electricity/heat**			**Outputs: Waste to treatment**		
2nd stage: Main Fermenter [V = m^3]						Electricity	0.012	kWh	Wastewater	0.004	m^3
Inputs: Resources			**Inputs: Electricity/heat**			8th stage: Ion exchange					
Air	64.46	Kg	Steam	37.95	MJ	**Inputs: Materials**			**Outputs: Waste to treatment**		
Water, cooling	0.216	m^3	Electricity	20.75	kWh	Water	0.004	m^3	Wastewater	0.004	m^3
Inputs: Materials			**Outputs: Emissions to air**			NaCl	0.089	kg			
Residual glycerol	1.661	Kg	N_2	50.55	kg	HCl	0.003	kg			
Water	6.845	m^3	O_2	14.11	kg	NaOH	0.353	kg			
$(NH_4)H_2PO_4$	0.289	Kg	CO_2	1.351	kg	9th stage: Diafiltration					
$MgSO_4{\cdot}7H_2O$	0.024	Kg				**Inputs: Materials**			**Outputs: Product**		
KH_2PO_4	0.554	Kg				Water	0.790	L	Enzymatic cocktail	1	kg
Yeast	0.033	Kg				Citric acid	0.222	kg			
3rd stage: Microfiltration						**Inputs: Electricity/heat**			**Outputs: Waste to treatment**		
Inputs: Electricity/heat			**Outputs: Waste to treatment**			Electricity	0.014	kWh	Wastewater	1.864	L
Electricity	0.058	kWh	Wastewater	0.029	m^3						

Table 4
Database used for considering the background process of the inputs required for the LCI.

Component	Database
Bioglycerol	Soy biodiesel, production, at plant/kg/RNA
Cooling water	Water, process and cooling, unspecified natural origin
Electricity	Electricity, medium voltage {Europe without Switzerland}|market group for|Cut-off, U
Wastewater	Wastewater, average {Europe without Switzerland}|market for wastewater, average| Cut-off, U
Citric acid	Citric acid {GLO}| market for | Cut-off, U
Water	Tap water {Europe without Switzerland}| market for | Cut-off, U
NaCl	Sodium chloride, brine solution {GLO}| market for | Cut-off, U
HCl	Hydrochloric acid, without water, in 30% solution state {RER}| market for | Cut-off, U
NaOH	Sodium hydroxide, without water, in 50% solution state {GLO}| market for | Cut-off, U
$(NH_4)H_2PO_4$	Monoammonium phosphate {RER}| market for monoammonium phosphate|Cut-off, U
$MgSO_4{\cdot}7H_2O$	Magnesium sulfate {RER}| production | Cut-off, U
KH_2PO_4	Potassium sulfate {RER}| market for potassium sulfate | Cut-off, U
Yeast	Yeast paste, from whey, at fermentation/CH U
Heat	Heat, from steam, in chemical industry {RER}| market for heat, from steam, in chemical industry | Cut-off, U
Steam	Steam, in chemical industry {RER}| market for steam, in chemical industry | Cut-off, U
Rapeseed oil	Glycerine {Europe without Switzerland}| esterification of rape oil | Cut-off, U
Palm oil	Glycerine {RoW}| esterification of palm oil | Cut-off, U
Soybean oil	Glycerine {BR}| esterification of soybean oil | Cut-off, U

Table 5
Main equipment of the large-scale modelling of β-glucosidase production, with the characteristic sizes and capacity values.

Equipment	Units	Size	Units	Equipment	Units	Size	Units
Air filter	5	3.75	m^3/s	Tank	1	5.27	m^3
Blending Tank	2	40.39	m^3	Homogenizer	1	5.30	m^3/h
Centrifugal Compressor	1	933.75	kW	Microfilter	2	79.65	m^2
Dead-End Filter	1	70.00	m^2	PBA Column	2	495.38	L
Diafilter	1	39.10	m^2	Seed Fermenter	1	6.24	m^3
Disk-Stack Centrifuge	2	1.99	m^3/h	Ultrafilter	1	33.15	m^2

As mentioned above, in the MRS category, the use of ammonium dihydrogen phosphate carries a significant environmental burden associated to the production of mineral fertilizers. Therefore, there is a consumption of mineral resources that has a direct impact on this category. As for the contribution of emissions from the on-site process in the WC category, it is the result of the need for water for cooling, taken from nature, which does not have a contribution in other impact categories, due to its natural origin, but does in the WC

Table 6
Absolute environmental impact values of β-glucosidase production. Functional unit: 1 kg of β-glucosidase cocktail.

Acronym	Impact category	Unit	Acronym	Impact category	Unit
GW	19.37	kg CO_2 eq	TET	29.41	kg 1.4-DCB
SOD	$4.70 \cdot 10^{-5}$	kg CFC_{11} eq	FET	0.35	kg 1,4-DCB
IR	4.77	kBq Co-60 eq	MET	0.48	kg 1,4-DCB
OF	0.03	kg NO_x eq	HC	0.59	kg 1,4-DCB
FPF	0.03	kg $PM_{2.5}$ eq	HNC	21.24	kg 1,4-DCB
OZ	0.03	kg NO_x eq	LU	8.38	m^2a crop eq
TA	0.09	kg SO_2 eq	MRS	0.03	kg Cu eq
FE	0.01	kg P eq	FRS	5.06	kg oil eq
ME	0.02	kg N eq	WC	7.48	m^3

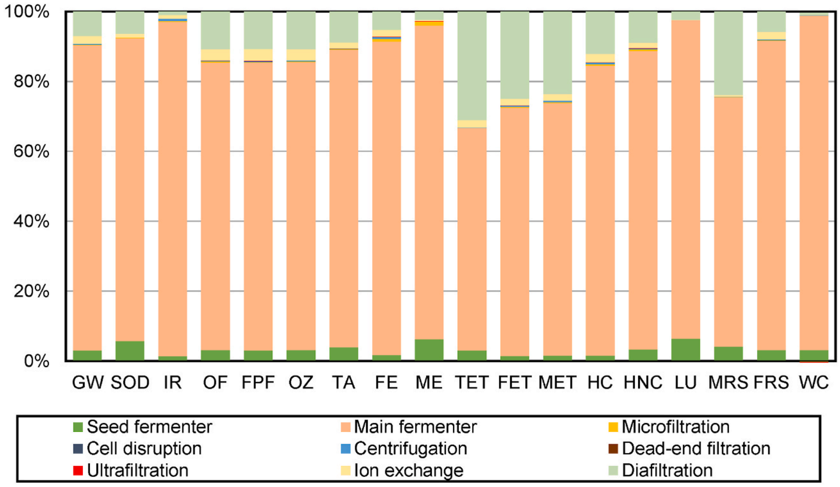

Fig. 2. Environmental profile of β-glucosidase enzyme production.

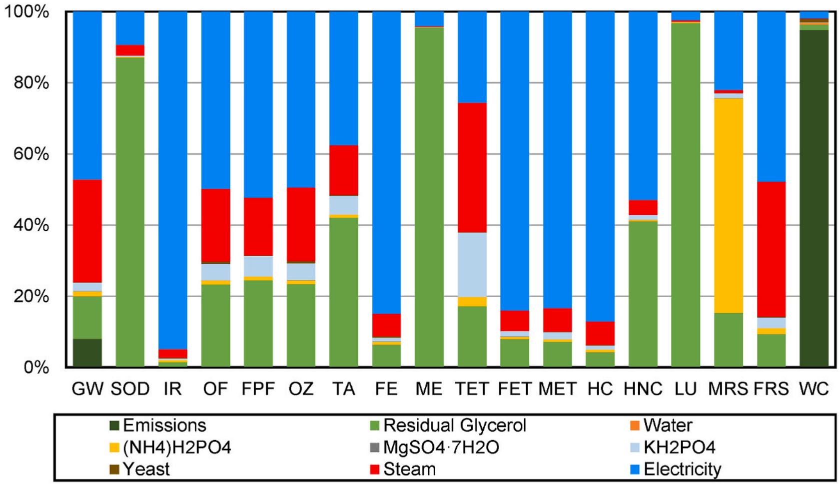

Fig. 3. Environmental profile of the main fermenter.

category as its consumption leads to a reduction of the planet's water resources.

4.4. Sensitivity analysis

The sensitivity analysis was performed according to the main hotspots of the process, that is, the main fermentation is the stage with the highest gross environmental load. The reason behind these results is based on the energy requirements, both electricity and steam, together with the bio-based glycerol. Accordingly, different modifications were considered: type of oil-based feedstock for biodiesel production (Fig. 4), reduction of electricity and type of energy source (Fig. 5) and, for steam, biomass valorization, a reduction of steam requirements and a change in the heat source (Fig. 6).

The alternative resources evaluated for glycerol production were soybean oil and palm oil. Both are common feedstocks used to produce biofuel, for example, in the case of soybean oil, according to the National Petroleum Agency, in Brazil almost 75% of all biodiesel is produced using soybean oil as a renewable source (Anastácio et al., 2014).

In the case of Europe, the most used and available vegetable oil for biodiesel production is rapeseed oil, accounting for $6.5{\cdot}10^6$ MT, followed by palm oil, $1.54{\cdot}10^6$, and soybean oil, $7.20{\cdot}10^5$ MT, according to EU Biofuels Annual 2019 Report (Flach et al., 2019). Therefore, these oilseed feedstocks have been selected for the environmental sensitivity analysis. In addition, residual glycerol has also been considered as a zero-impact stream, as it is considered a waste from biodiesel production, and the fact that it is recovered through a biotechnological process for the production of a high added value compound, avoiding its management as waste, its associated impacts and therefore reducing its impact on the environment.

The environmental results and their subsequent comparison between the proposed scenarios are shown in Fig. 4. As can be seen, the use of palm oil as a feedstock seems to be the best from the environmental point of view, as it produces the least environmental damage in all the impact categories evaluated in comparison with the other resources. In contrast, in the case of soybean oil, it is in almost all impact categories the scenario that leads to the highest environmental burdens, with the exception of four categories: SOD, TA, ME and HNC, where the use of rapeseed oil has the highest contribution on the environment. On the other hand, as expect, the fact of considering bio-glycerol as residual stream with an assigned zero impact, leads to the best environmental alternative performance, with significant reduced impacts in comparison with the base case scenario. To this end, for this first alternative sensitivity assessment, palm oil could be selected as the preferred feedstock for glycerol production and, when possible, assignation of zero impact to the residual glycerol is considered as the optimum and more desirable scenario from the environmental point of view.

When assessing the electricity requirements, a 25% reduction in energy has been considered, based on the premise that as the modelling is based on laboratory data, it is not optimized data on a large scale, so the range of improvement is quite wide, especially in terms of energy consumption. Some improvement has been achieved with this optimized scenario in mainly all impact categories, as could be seen in Fig. 5, but the results were not as pronounced as those obtained by the second optimized option. This second sensitivity assessment has been carried out by modifying the electricity source database. In the baseline scenario, the European electricity mix has been used, which includes the average mix of electricity sources used in European countries, which have a large share of fossil energy resources. In contrast, in the case of Norway, most of the electricity produced comes from hydropower, a renewable energy that leads to a significant reduction of the impact, as could be seen in Fig. 5. In fact, in impact categories such as IR and FE, the environmental load is reduced by almost 80%, and for FET, MET and HC the impact is reduced by 60%. However, as would be predicted, being a hydroelectric power plant, in the WC category, the use of this technology leads to a higher environmental load due to water use, but the difference between the other two scenarios is not significant, as it is less than 10%.

The last sensitivity analysis concerns the consumption of steam as the main heating source. The first optimized scenario is energy recovery using the biomass produced in the fermentation stage. This could be considered as a sustainable and circular economy procedure, as a "waste" stream from the process is used as a "source" for steam production. This requires anaerobic digestion coupled with a cogeneration unit. The results obtained by this valorization do not lead to a significant reduction of environmental damage, because the amount of biomass produced within the fermentation is not too high, so it does not allow to produce an abundant amount of thermal energy. Since the results obtained were not sufficiently satisfactory, the option of proposing a 25% reduction in steam consumption was considered. This alternative has been carried out based on the same precept mentioned in the sensitivity analysis of the electrical requirements: the fact that the large-scale modelling is carried out on the basis of laboratory-level data, the range of improvement is extensive. Furthermore, by performing a type of analysis, given the variety of temperatures handled throughout the process, it would be possible to use the process flows themselves as heat transfer agents, thus reducing the consumption of resources and utilities. This alternative sensitivity scenario has resulted in significant reductions in impact, as can be seen in Fig. 6, with GW, FRS and TET being the categories where the greatest decrease in environmental load has been observed. But, looking for an even more optimized scenario, the use of an alternative resource for steam production has been considered, namely a renewable bio-based resource, in this case municipal waste recovered by incineration. The impact reduction is observable in almost all impact categories, with the GW, FRS and TET impact categories achieving a huge improvement. To this end, it was thought that the evaluated sensitivity analysis scenarios for reducing the in-process environmental steam load led to reduction in most of the categories, the alternative of using heat from lignocellulosic waste seems to be the most attractive, as the impact values could be reduced by 20%–30% in some of the impact categories under study.

5. Key points to improve performance

According to the main hotspots identified on the previous sensitivity assessment, the main key points for improvement could be categorized as:

5.1. Source of bio-glycerol production

(Schmidt, 2010) has developed an environmental assessment over the impacts on the soybean and rapeseed oil cultivation. Taking into account a consistent modelling covering both oil mill and agricultural stages, soybean oil production leads to a higher contribution in most the impact categories, with the exception of stratospheric ozone depletion (SOD), terrestrial acidification (TA), marine eutrophication (ME) and land use (LU). These results are in line with the one obtained for this manuscript, as in those impact categories mentioned the use of rapeseed oil entails a higher environmental load in comparison with soybean oil.

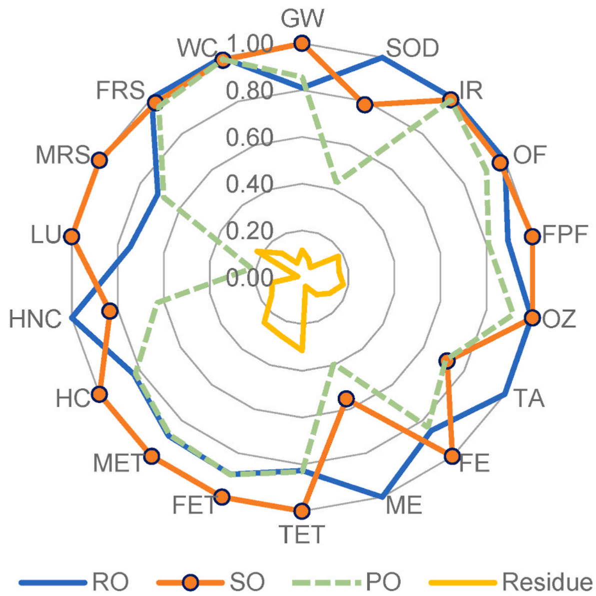

Fig. 4. Sensitivity analysis I: alternative source for glycerol. RO: Rapeseed Oil, SO: Soybean Oil, PO: Palm Oil and Residue: residual glycerol as a zero-impact input.

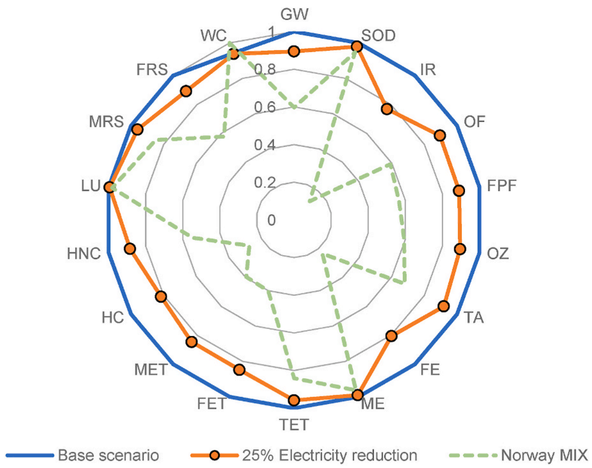

Fig. 5. Sensitivity analysis II: electricity reduction and change on electricity mix for Norway one.

On the other hand, the rationale behind the lower environmental impact of palm oil is based on the fact that it is by far the most efficient and productive vegetable oil due to its fast growth and low soil occupation. Its high crop yield makes it in one of the most sustainable lignocellulosic feedstock (Beyer et al., 2020; Oosterveer, 2020).

5.2. Energy source: moving from fossil to renewable resources

Looking for reduce the depletion of fossil resources and the environmental burdens resulting from the use of non-renewable energy, the use of renewable resources could be considered as an efficient and sustainable alternative (Šerešová et al., 2020). Several studies have focused on analysing the environmental burdens associated with the different energy sources, one of them is the ETC report (Bouman, 2020). The avoided impacts of the different bioenergy alternatives have been evaluated, concluding that an overall $1.8{\cdot}10^3$ Mt of CO_2 eq could be avoided, with the use of hydrothermal, onshore wind and solar photovoltaic energies contributing the most on the reduction of environmental loads. But, on the other hand, higher environmental loads could be obtained in toxicity impacts and land occupation, given the need of agricultural activities, fertilizers, gross infrastructures, and specialized materials for the construction of the necessary equipment (Bouman, 2020). Similar trends were concluded by (Hertwich et al., 2015) in terms of land occupation, that no significant environmental burden is obtained, when assessing hydropower and photovoltaics, certain higher impact is observed.

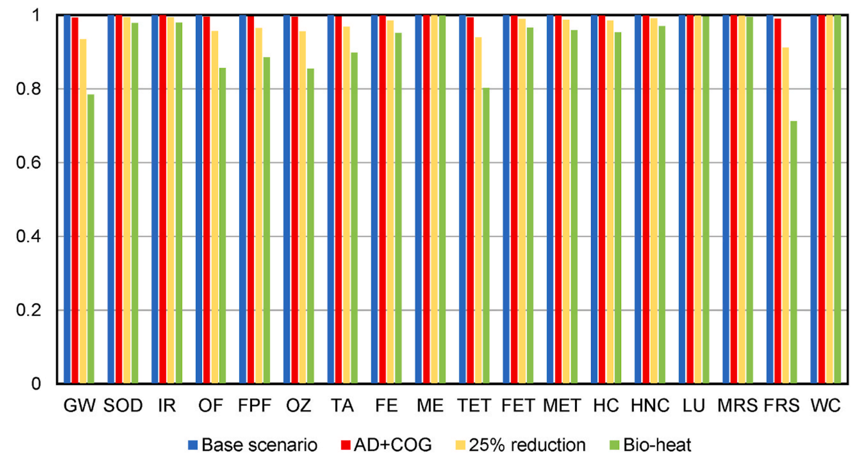

Fig. 6. Sensitivity analysis III: biomass valorization, steam reduction, change on steam source. AD + COG: Anaerobic Digestion + Cogeneration Unit and Bio-Heat: source of steam based on waste biomass.

5.3. Increasing productivity leads to reduced environmental loads

As expected, an improvement on the process yields, both in the background and foreground activities, and in productivity translates into a lower environmental impact. This statement has also been discussed in literature (Jirapornvaree et al., 2022) who considered different alternatives for agricultural production (chemical or organic), harvesting and soil preparation, concluding that eco-efficiency in the post-harvesting management and resource consumption leads to increased production and lower environmental impact. On the other hand, the use of emerging technologies, emission capture system and optimization of upstream process design could also be considered as an efficient method on the way of reducing the environmental burdens of new production process (Berg and Bendix, 2021).

6. Conclusions

This study aims to assess the environmental profile associated with the production of β-glucosidase. Despite being a bio-based process, the different stages involved could entail environmental impacts. The production scenario proposed in this study could be considered as an innovative and environmentally friendly alternative for producing enzymes following the approach of circular economy as it seeks to valorize secondary waste streams. The main critical points identified are energy requirements, both electricity and steam. In this area, the rise of biotech industry will be possible with fully optimized biotransformations, carbon-based means of waste resources, minimized use of chemicals and the application of energy integration measures.

CRediT authorship contribution statement

H. Feijoo: Methodology, Formal analysis, Investigation. A. Arias: Writing-original draft, Writing-review & editing. MT Moreira: Conceptualization, Supervision, Writing-review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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