Life Cycle Impacts of Enzyme Production: Xylanase Production Case Study via Solid-state Fermentation and Suspended Culture Methods

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Research Article

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Abstract

Xylanase enzyme has the potential to be used in bread production, as addition of small amounts of xylanase can significantly increase the volume of the bread, making it more appealing to the consumer. In this study, the environmental life cycle assessment of producing xylanase via suspended culture and solid-state fermentation methods has been realized by using CCaLC software with Ecoinvent 2 database and CML2001 method and the following impacts were calculated: carbon footprint, acidification potential, eutrophication potential, ozone layer depletion potential, photochemical smog potential, and human toxicity potential. Raw material acquisition, production, and transportation stages were taken into account. Results show that solid-state fermentation method has much higher environmental impact than the suspended culture method, mostly because of the higher yield of the latter. Energy consumption in the bioreactor stage, followed by high amounts of water use in separation and cleaning processes emerged as the main hotspots. The results showed consistency with earlier literature for high-purity enzyme production. An analysis of the potential implications of the nation-wide use of xylanase in bread production in Turkey showed that xylanase can increase the carbon footprint of bread production by 20% on average (of the two methods).

Highlights

- Environmental impact analysis of xylanase production was performed.
- Solid-state fermentation and suspended culture methods were compared.
- Suspended culture method turned out to be much more environmentally friendly.
- Energy consumption in the bioreactor was found to be the main hotspot.
- Xylanase use in bread production can increase the carbon footprint of bread by approximately 20%.

1. Introduction

Climatic variables such as temperature, precipitation and cloud cover have been displaying significant signs of change recently, mostly due to global warming caused by the emission of greenhouse gases (GHG) as a result of industrial activities. The impacts of these changes could be serious such as loss biodiversity, loss of habitable land due to increased sea levels, drop in cultivable fields, drought, etc. (Yu, et al., 2019). Electricity generation, building construction and management, transportation, and food supply have emerged as the main contributors to global GHG emissions (Fekete, et al., 2021). Thanks to international efforts such as the Paris Agreement in 2016, countries are trying to develop and implement solutions in order to mitigate their GHG emissions. A globally popular approach in order to achieve this goal is to make use of biotechnology. Biotechnology presents great opportunities for the development of products and processes that are environmentally sustainable. Some examples of the sustainable use of biotechnology include but are not limited to biocatalysis, use of enzymes, metabolic engineering and synthetic biology in biorefineries (Aguilar, et al., 2019). These applications combine the utilization of renewable raw materials with sustainable and efficient production methods, which leads to reduced environmental impacts when compared to conventional chemical production of the same products (Choudhury, 2020). Amongst these applications, enzyme utilization emerges as a high-potential market, with an expected annual growth rate of 7.1% from 2020 to 2027 (grandviewresearch.com, 2020). Enzymes, which can be defined as catalysts in biological systems, are used in the production of various products such as food and beverages, detergents, animal feed, biofuels, textiles, pulp and paper, nutraceutical products, personal care and cosmetic products as well as wastewater treatment. Enzymes are beneficial in the sense that they guarantee the quality and stability of the products, the production of whom they are used for, with increased production efficiency. They also help provide environment-friendly products to consumers thanks to using less energy, water and raw materials and generating less waste (Amfep.com, 2020). According to a report by the World Wild Fund, the use of enzymes can save up to 139 $MtCO_2$eq. in the food industry and up to 65 $MtCO_2$eq. in traditional industries like detergents, textiles, pulp and paper (World Wild Fund (WWF), 2009). However, in order to be able to accurately claim that enzymes are indeed environmentally-friendly, the environmental impacts associated with their production should also be calculated by using a life-cycle approach and considering different stages such as raw material acquisition, production, and use.

As far as the existing literature on the environmental impact analysis of enzyme production is concerned, Catalan et al. performed the attributional life cycle assessment of cellulase production from coffee husks by solid-state fermentation (Catalan, et al., 2019). Functional unit was selected as 1 kg of dry cellulase produced and the following life-cycle stages were taken into account: fermentation, extraction, purification. ReCiPe 2016 midpoint methodology was used. The results showed that electricity consumption, mostly associated with the lyophilisation process, is responsible for most of the impacts. In another study focusing on cellulase enzyme, Gilpin and Andrae studied the comparative attributional life cycle assessment of European cellulase enzyme production for use in second-generation lignocellulosic bioethanol production (Gilpin & Andrae, 2017). They considered three scenarios, which differed from one another in terms of the source of glucose. Their functional unit was 1 kg of wet cellulase produced and the following stages were included in the model: media preparation, seed train, aerobic cultivation. Pretreated softwood was found to be the most environmentally friendly source of glucose compared to sugar cane molasses or glucose. It was also found that the results could be highly sensitiye to parameters such as carbon source origin, applied allocation, market changes, process efficiency and electricity supply. Harding and Harrison also focused on cellulase, and investigated the use of generic flowsheeting approach to obtain material and energy data for lifecycle assessment of cellulase production (Harding & Harrison, 2020). Their functional unit was 1 kg of cellulase and they preferred CML 2 Baseline 2000 v2.03 methodology. Their main finding was that energy and electrical inputs account for the majority of the impacts and should be a critical part of early-stage modelling. Feijoo et al. investigated the environmental life cycle assessment of β-Galactosidase production at an industrial scale with a cradle-to-gate perspective (Feijoo, et al., 2017). Functional unit was selected as 1 kg of β-Galactosidase produced. Their results suggested that the use of specific chemicals, most notably HNO_3, during the production processes was responsible for more than 75% of the overall impacts. In fact, replacing HNO_3 with H_2SO_4 alone was reported to have the potential to reduce certain impacts by up to 70%. Kim et al. performed the environmental life cycle assessment of enzymes used for pharmaceutical processes (Kim, et al., 2009). The functional unit was 1 kg of an enzyme cocktail containing an aldolase, a carbamoylase, and a hydantoinase enzyme. Media preparation, fermentation, separation, cell disruption, immobilization, sanitization and waste management stages were

considered. The main conclusions were that enzyme production is a highly energy-intensive process, and immobilization and media preparation subprocesses were found to be the primary local environmental impact sources, especially for acidification, eutrophication, and photochemical smog formation impacts. Finally in a comprehensive review study by Jegannathan and Nielsen (Jegannathan & Nielsen, 2013), more than 25 industrial processes during which enzymes are used have been analyzed in terms of their environmental impacts, and the contribution of the enzymes to these processes have been mentioned. However, in these studies the functional units were not defined in terms of the quantity of the enzyme, but rather in terms of the output for which the enzyme has been used.

In this study, the carbon footprint of xylanase production via solid-state fermentation and suspended culture methods has been calculated by employing a life-cycle approach. Xylanase is a commonly used enzyme with annual market of approximately USD 500 million. Xylanase can be used in animal feed production, food production, paper and pulp production and bioconversion of lignocellulosics (Chadha, et al., 2019). One particular type of food product for the production of whom xylanase can be used is bread. Significant increase in the bread volume was noted upon the use of xylanase in various concentrations (Cunha, et al., 2018). This is especially noteworthy for Turkey, where this particular study was conducted, as Turkey is the leading country in the world in terms of bread consumption per capita (Quilez & Salas-Salvado, 2012), and with a population exceeding 80 million, Turkey is one of the largest producers of bread globally. Therefore, the subject of the case study for the life cycle assessment of enzyme production was selected as xylanase, however it should be noted that the main purpose of this paper is to compare the environmental impacts of different enzyme production methods, that is, suspended culture and solid-state fermentation; and xylanase was studied a case-study. Furthermore, unlike other enzymes such as cellulase whose environmental impacts have been extensively studied earlier, to the best of the authors' knowledge there is only one other study in the literature concerned with the environmental life cycle assessment of xylanase. Nielsen et al. investigated the life cycle environmental impacts of using xylanase for improving the protein digestibility of pig and poultry feed (Nielsen, et al., 2008). However, in that particular study xylanase was an input to the process and the functional unit was not defined as a certain amount of xylanase. Therefore, rather than providing information on the product only, this study also provides an insight on the environmental impacts of different enzyme production methods and therefore can be of significant use to researches and industrial enzyme manufacturers alike.

2. Methodology

2.1. Goal and scope definition

The goal of this study is to conduct the environmental life cycle impact assessment of xylanase production in Turkey, by using two different methods: solid-state fermentation and suspended culture. The functional unit was chosen as one 1-kg of xylanase in powder form. This is an attributional LCA study with two main purposes: performing the environmental performance of these two particular enzyme production methods as well as comparing the environmental performance of xylanase with other commercial enzymes.

The following stages have been modelled in detail by employing a cradle-to-gate approach:

1. raw material supply
2. production processes
3. transportation to the point of use

"Use" and "end-of-life treatment" stages were not taken into account. The use of xylanase in bread-making increases the volume of bread per unit mass, and thereby makes it more appealing to the buyer. Hence, bakeries would want to use xylanase during bread-making (Chen, 2018). However, the addition of xylanase does not create any non-economic benefits or burdens, as it does not change any mass and/or energy inputs or emissions regarding bread production. Therefore, the use stage was not considered as part of the life cycle model. Once xylanase is added into the bread dough, it cannot be disposed or retrieved in any manner, therefore end-of-life treatment is not applicable, either. Finally, no by-products were present in the model; thus, allocation was not required.

2.2. Life cycle assessment methodology

Life cycle assessment (LCA) is a useful method to evaluate the environmental burden of a product or service by quantifying the impacts of all inputs and outputs associated with corresponding production and supply processes (Omahne, et al., 2020; Heimersson, et al., 2014; Cremonez, et al., 2015). The methodological guidelines of LCA are defined in the ISO 14040 and ISO 14044 standards (ISO, 2006a; ISO, 2006b). In this study, CCaLC2 LCA software was used to model the system and estimate the impacts according to the CML 2001 method. Ecoinvent 2 database was used, except for the Turkish electricity dataset which has been created manually and added into the database by the authors (please see Tables 2 and 3 below). The following impacts can be estimated in CCaLC2 and were considered in this study: global warming potential (GWP), acidification potential (AP), eutrophication potential (EP), ozone layer depletion potential (ODP), photochemical smog potential (PSP) and human toxicity potential (HTP). Further midpoint impact calculation was unfortunately not possible as CCaLC is limited to these six particular impacts.

2.3. Life cycle inventory

The stages associated with suspended culture and solid-state fermentation-based production of xylanase can be found below in Fig. 1; followed by a detailed, step-by-step explanation of the procedure where the main differences between suspended culture and solid-state fermentation methods are highlighted. The life cycle inventories (materials and energy inputs) required for the production of 1 kg of xylanase by suspended culture and solid-state fermentation methods are presented in Tables 2 and 3, respectively. Preceding Table 2 and Table 3, Table 1 provides a pedigree matrix which explains the data quality analysis approach employed in this study. The rightmost columns in Tables 2 and 3 show the data quality indicator scores for each of the inputs, according to the

order of indicators listed in Table 1. In both Table 2 and Table 3, inputs are shown with regular font whereas emissions are shown with italic and underlined font. All the data presented in Tables 2 and 3 were obtained at pilot-plant scale.

In both methods the first step is the preparation of polydextrose agar (PDA) by dissolving the PDA in powder form in distilled water and then heating in microwave oven for 30 minutes, followed by autoclave sterilization for 1 hour. Afterwards inoculation takes place in a laminar cabinet for 30 minutes. The culture used for inoculation had been being developed in an incubator for one week prior to inoculation. After the spore inoculation, the PDA growth media are heated in the autoclave for 1.5 hours to ensure that all the microorganisms would be eliminated before disposal. Following inoculation comes the enzyme production stage in a continuously-stirred bioreactor kept at 30°C for a period of one week. After the contents inside the bioreactor is removed, the bioreactor is cleaned first with water, and then with superheated steam at 135°C treatment for one hour. After being taken out of the bioreactor, the contents first go through raw filtering, followed by centrifugation for 2.5 hours. The waste obtained at the end of these separation stages are then autoclaved for 2.5 hours. Then, the enzyme undergoes ultrafiltration for 10 hours, followed by another centrifugation stage of 1 hour to ensure effective precipitation. Then comes the diafiltration stage of 15 hours in order to remove ammonium sulfate from the medium. The final stage is lyophilization, at the end of which xylanase enzyme in powder form is obtained and this stage lasts 48 hours. Both methods produce xylanase with 99+% purity. The main difference between the methods is that in solid-state fermentation, water with a ratio of 5:1 (to the amount of enzyme mixture) is used during the roughing filtration stage whereas in suspended culture method, no additional water use is required (Malarvizhi, et al., 2003; Deschamps & Huet, 1985). Furthermore, two methods have different yields. Thus, the amounts of material and energy inputs in Table 2 and Table 3 for the same processes are mostly different.

Since commercial bread production is carried out in every single town in Turkey, it was not possible to come up with a certain transportation distance for the final product. Therefore, three different scenarios were included in the study, and in these scenarios the transportation distances were considered as 100, 500, and 1000 km, respectively. The reason behind this approach is to analyze if the model is sensitive to the transportation stage or not.

As far as the quality of the data presented in Tables 2 and 3 is concerned, the average scores for reliability, completeness, temporal correlation, geographical correlation and further technological correlation are 1.24 (excellent), 3.18 (good), 1.33 (excellent), 2.45 (very good), and 2.09 (very good), respectively. Assuming equal weights for all the indicators would lead to an overall data quality of 2.06 (very good), meaning that this particular study is highly satisfactory regarding its data quality.

3. Results And Discussion

In this section, data on six environmental impacts that were mentioned earlier in section 2.2 are presented for both methods, by investigating the contribution of each life cycle stage to each impact, and the results of this detailed analysis can be found in section 3.1. A sensitivity analysis was deemed unnecessary as the model entirely relies on real-life data. The results of the comparison of the environmental impacts of xylanase production and the production of other enzymes found in the literature are presented in section 3.2. Finally, in section 3.3 the life cycle impacts of the nation-wide use of xylanase were calculated by extrapolation.

3.1. Environmental impacts of xylanase production

In Fig. 2 below, the environmental impacts associated with the production of xylanase can be found for suspended culture (SC) and solid-state fermentation (SSF) methods. It should be noted that the raw material stage accounts for all the material inputs to the process whereas the production stage accounts for the energy inputs and emissions in Fig. 2.

Fig. 2 shows that suspended culture method has lower scores for 5 out of the 6 impacts analyzed in this study. The only impact which had a lower score in the case of solid-state fermentation is photochemical smog potential. On average, solid-state fermentation has 2.2 times higher impact than suspended culture method if all impacts are assigned equal weights. The difference between the environmental impacts of the two method is mainly attributed to the differences in the energy consumption for separation and purification processes, as the material yield of solid-state fermentation is much lower than that of suspended culture method.

Fig. 2 also shows that transportation has virtually no effect on any of the impact scores. For carbon footprint (CF), acidification potential (AP), eutrophication potential (EP) and ozone layer depletion potential (ODP), production stages are mainly responsible for the impacts whereas in the case of photochemical smog potential (PSP) and human toxicity potential (HTP), the main contributor turned out to be the raw material supply stage. Hotspot analysis shows that for 4 impacts out of 6 (CF, AP, EP, and ODP), enzyme production in the bioreactor stage accounts for approximately 77% of the total impact score on average. The reason for the high contribution of this stage can be explained as follows: First, enzyme production in the bioreactor is a constant temperature processes that requires continuous stirring. Although the process temperature is not high (30°C), it still requires a certain energy input, and so does continuous stirring. But the main reason for the high impact contribution of the bioreactor stage is its duration. As already indicated in section 2.3, this stage takes one week; and keeping the reactor at 30°C while being continuously stirred for such a long duration inevitably increases the energy consumption to extremely high values. To give a specific example, more than 99.9% of the carbon footprint created by the bioreactor stage is actually caused by energy consumption. Another major hotspot was the excessive water use. Water was not only used to create the required medium for microbiological activity, but also for cleaning the equipment post-production. As far as other hotspots are concerned, Tween® mixtures emerged as a significant contributor to PSP and ODP whereas ammonium sulphate was found to account for approximately 62% of the HTP.

3.2. Comparison with other enzymes

Table 4 below provides the comparison the carbon footprint scores of xylanase found in this study and those of several other enzymes. The reason for not including impacts other than carbon footprint in Table 4 is simply the lack of data in the literature in many cases, if not all. Detailed information about each

study (functional unit, system boundaries, etc.) has been given in order to be able to make an accurate comparison.

As shown in Table 4, the carbon footprint of enzyme production can vary greatly, even for the same enzyme. This is mainly attributed to the differences in the purities of the final product, as different studies on the LCA of cellulase production report CF values that vary by a factor of four digits. In fact, it has been reported that the energy intensity of enzyme production increases exponentially with respect to the purity of the final product (Catalan, et al., 2019). Furthermore, different production methods have very different yields, and the source of the biological feed for the fermentation process also plays an important role. For example, in the study whose scope is the production of B-galactosidase (Feijoo, et al., 2017), it has been reported that the volumetric yield of the process is less than 1%. The complexity of the downstream processing (where separation and purification processes are involved) is linked to the desired purity of the final product, and usually, high purity is paid with low yield (Wu, et al., 2019). A similar trend was also observed in our study, too, as evident from the input values tabulated in Tables 2 and 3. Compared to other enzymes, xylanase has a quite high carbon footprint, but there are studies in the literature in which carbon footprints of same order of magnitude have been reported (Catalan, et al., 2019; Feijoo, et al., 2017). These high scores can also be attributed to the fact that the data used in this study was obtained from a pilot-plant and not an industrial scale producer. However, it should be noted that the main purpose of this study is to compare the environmental impacts of different enzyme production methods and xylanase production is considered as a case study rather than the main focus of our work. Therefore, it is the relative impact scores of these two methods with respect to one another rather than how they compare to the literature that matters. Literature comparison was included to validate our approach.

3.3. Nation-wide environmental implications of xylanase use

As indicated earlier in section 1, xylanase can be used in bread production to increase the volume, thereby making the bread more appealing to the customer and increasing the sales. As the biggest consumer of bread per capita with an approximate figure of 200 kg/per person.year, there is great potential for the widespread use of xylanase across Turkey. Previous studies show that the optimum content of xylanase in bread is 20 ppm (Chen, 2018). Multiplying this factor by the annual bread consumption in Turkey gives us the maximum annual consumption of xylanase as 320 tons (Çimenlik, 2020). Obviously, it is not realistic to assume that all bread producers will use xylanase in their recipe. Thus, we analyzed the environmental implications of using xylanase if 10% of the bread produced in Turkey contains xylanase, leading to an annual xylanase consumption of 32 tons.

CF_{SC} = 32 tons xylanase/year × 8900 ton CO_2eq./ton xylanase = 284,800 ton CO_2eq./year (1)

CF_{SSF} = 32 tons xylanase/year × 28100 ton CO_2eq./ton xylanase = 899,200 ton CO_2eq./year (2)

where CF_{SC} and CF_{SSF} are the annual carbon footprints of using xylanase obtained by suspended culture and solid-state fermentation methods, respectively, in 10% of the breads produced in Turkey. According to the literature, the carbon footprint of producing 1.6 Mtons (10% of Turkey's annual bread consumption) of bread is approximately 2.176 MtonCO_2eq. (Chiriaco, et al., 2017). These numbers show that using xylanase would increase the carbon footprint of bread by 11.6% in the case of suspended culture method and 29.3% in the case of solid-state fermentation method. A recent study investigated the carbon footprint of nutrition in Turkey (Üçtuğ, et al., 2021). According to that study, bread consumption accounts for approximately 0.9% of the total carbon footprint of a normal diet based on traditional Turkish cuisine. When the results of this particular study and the study by Üçtuğ et al. (Üçtuğ, et al., 2021) are combined, it can be concluded that the use of xylanase in bread-making can increase the nutrition-related carbon footprint in Turkey by 0.1% to 0.23%, depending on the method of xylanase production.

4. Conclusions

In this study the cradle-to-gate life cycle assessment of producing xylanase via suspended culture and solid-state fermentation methods has been performed. The results showed that solid-state fermentation has much higher environmental impact than suspended culture method, mostly because of the lower yield of the former. Energy consumption during the bioreactor stage emerged as the main hotspot, followed by water and chemical use in the downstream processes and equipment cleaning.

This study was consistent with previous studies in the literature in the sense that the environmental impacts of enzyme production can vary dramatically depending on the purity of the final product as well as method of production. Our results clearly show that more emphasis should be given to alternative methods of enzyme production and/or cleaning of the equipment used in the production process. Improvements in the production of xylanase, or any enzyme in general, should focus on determining the ideal conditions for microbiological activity, reducing the production time, repeatable use sterile fermenter systems and less energy and water intensive downstream processes.

Declarations

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Conflict of 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.

Availability of Data and Material

The data that support the findings of this study are not openly available due to the fact that it belongs to an ongoing, externally funded project and are available from the corresponding author upon reasonable request.

Code availability

The CCaLC files used in the calculation of the environmental impacts can be shared by the corresponding author upon request (please see the "Availability of Data and Material Statement" above).

Authors' contributions

Sercan Çimenlik: Investigation, Experimentation.

Gaye Öngen Özgen: Conception, Methodology, Visualization, Review & Editing.

Fehmi Görkem Üçtuğ: Software, Writing, Review & Editing.

Ethics Approval

This article does not contain any studies with human participants or animals performed by the authors.

Consent to Participate

There are no human participants whose consent is necessary in this study.

Consent for Publication

There are no human participants whose consent is necessary in this study. Consent to submit this manuscript has been received from all co-authors and responsible authorities at the institute/organization where the work has been carried out before the work is submitted.

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Tables

Table 1. Pedigree matrix with data quality indicators [26]

Indicator score	1 Excellent	2 Very good	3 Good	4 Fair	5 Poor
Reliability	Verified data based on measurements	Verified data partly based on assumptions or non-verified data based on measurements	Non verified data partly based on assumptions	Qualified estimate	Non-qualified estimate
Completeness	Representative data from all sites relevant for the market considered, over an adequate period to even out normal fluctuations	Representative data >50% of the sites relevant for the market considered, over an adequate period to even out normal fluctuations	Representative data from only some sites (50% of sites but from shorter periods	Representative data from only one site relevant for the market considered or some sites but from	Unknown or ≥15 years of difference
Temporal correlation	≤3 years of difference to year of stud	3 to 6 years difference	5 to 10 years difference	10 to 15 years of difference	Unknown or ≥15 years of difference
Geographical correlation	Data from area under study	Average data from larger area in which the area under study is included	Data from area with similar production conditions	Data from area with slightly similar production conditions	Data from unknown area or area with very different production conditions
Further technological correlation	Data from enterprises, processes and materials under study	Data from processes and materials under study but from different enterprises	Data from processes and materials under study but from different technology	Data on related processes or materials but same technology	Data on related processes or materials but different technology

Table 2. Inventory data for 1 kg of xylanase production by suspended culture method

Stage	Inputs / *emissions*	Amount	Ecoinvent dataset	Data quality (*)
Potato dextrose agar preparation	Agar	0.68 kg	User-defined process [a]	(3, 4, 1, 3, 5)
	Deionized water	226 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Dextrose	0.91 kg	User-defined process [a]	(3, 4, 1, 3, 5)
	Potato starch	0.19 kg	Potato starch, at plant	(1, 4, 1, 3, 2)
	Electricity	367 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
Incubation	Electricity	1024 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
Spore inoculation	Deionized water	29 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Tween-80 [c]	19 g	User-defined process [c]	(3, 4, 1, 3, 5)
	Electricity	6.4 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
Enzyme production in bioreactor	Deionized water	4640 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Calcium chloride	0.15 kg	Calcium chloride, $CaCl^2$, at plant	(1, 4, 1, 3, 2)
	Magnesium sulfate	2.20 kg	Magnesium sulphate, at plant	(1, 4, 1, 3, 2)
	Malt grass	72.4 kg	Malted barley, NL	(1, 4, 1, 3, 2)
	Potassium phosphate	7.24 kg	Potassium phosphate, at plant	(1, 4, 1, 3, 2)
	Electricity	55200 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
Roughing filtration	*Filtered waste*	18.1 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Centrifugation	Electricity	720 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	45.2 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Ultrafiltration	Deionized water	452 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Sodium hydroxide	3.62 kg	Sodium hydroxide (NaOH) (concentrated)	(1, 4, 1, 3, 2)
	Tween-20 [c]	0.14 kg	User-defined process [c]	(3, 4, 1, 3, 5)
	Electricity	23.6 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	118 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Precipitation	Ammonium sulfate	66.7 kg	Ammonium sulfate, as N, at regional storehouse, Europe	(1, 4, 1, 3, 2)
	Deionized water	36.2 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Electricity	210 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	24.7 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Diafiltration	Deionized water	271 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Sodium hydroxide	0.72 kg	Sodium hydroxide (NaOH) (concentrated)	(1, 4, 1, 3, 2)
	Electricity	39.2 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	27.2 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Lyophilization	Electricity	1252 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	3.61 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)

(*) based on the order of indicators presented in Table 1

[a] Data retrieved from [27, 28]

[b] User-defined process, adopted from [29]

[c] Tween-20 and Tween-80 are trademark names for specific polysorbates [30]. LCI data was obtained from [31, 32].

Table 3. Inventory data for 1 kg of xylanase production by solid-state fermentation method

Stage	Inputs / *emissions*	Amount	Ecoinvent dataset	Data quality (*)
Potato dextrose agar preparation	Agar	0.68 kg	User-defined process [a]	(3, 4, 1, 3, 5)
	Deionized water	241 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Dextrose	0.82 kg	User-defined process [a]	(3, 4, 1, 3, 5)
	Potato starch	0.16 kg	Potato starch, at plant	(1, 4, 1, 3, 2)
	Electricity	2680 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
Incubation	Electricity	7688 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
Spore inoculation	Deionized water	13.6 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Tween-80 [c]	14 g	User-defined process [c]	(3, 4, 1, 3, 5)
	Electricity	9.84 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
Enzyme production in bioreactor	Deionized water	7150 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Calcium chloride	31 g	Calcium chloride, $CaCl^2$, at plant	(1, 4, 1, 3, 2)
	Diammonium phosphate	2.05 kg	Diammonium phosphate, as N, at regional storehouse, Europe	(1, 4, 1, 3, 2)
	Magnesium sulfate	62 g	Magnesium sulphate, at plant	(1, 4, 1, 3, 2)
	Malt grass	103 kg	Malted barley, NL	(1, 4, 1, 3, 2)
	Potassium phosphate	1.54 kg	Potassium phosphate, at plant	(1, 4, 1, 3, 2)
	Electricity	166000 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
Roughing filtration	Deionized water	2050 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Filtered waste	18.1 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Centrifugation	Electricity	2272 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	68.4 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Ultrafiltration	Deionized water	684 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Sodium hydroxide	5.47 kg	Sodium hydroxide (NaOH) (concentrated)	(1, 4, 1, 3, 2)
	Tween-20 [c]	27 g	User-defined process [c]	(3, 4, 1, 3, 5)
	Electricity	177 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	178 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Precipitation	Ammonium sulfate	101 kg	Ammonium sulfate, as N, at regional storehouse, Europe	(1, 4, 1, 3, 2)
	Deionized water	54.7 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Electricity	1584 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	37.6 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Diafiltration	Deionized water	410 kg	Deionized water, from ground water	(1, 4, 1, 3, 2)
	Sodium hydroxide	1.10 kg	Sodium hydroxide (NaOH) (concentrated)	(1, 4, 1, 3, 2)
	Electricity	296 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	41.0 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)
Lyophilization	Electricity	9468 MJ	Turkish electricity [b]	(1, 1, 2, 1, 1)
	Filtered waste	5.47 kg	Disposal, municipal solid waste, 22.9% water, to municipal incineration	(1, 4, 1, 3, 2)

(*) based on the order of indicators presented in Table 1

[a] Data retrieved from [27, 28]

[b] User-defined process, adopted from [29]

[c] Tween-20 and Tween-80 are trademark names for specific polysorbates [30]. LCI data was obtained from [31, 32].

Table 4. Comparison of the carbon footprints of different enzymes

Type of enzyme	Carbon footprint ($kg\ CO_2$ eq. per functional unit)	Functional unit	System boundaries	Reference
Xylanase, suspended culture [a]	8,900	1 kg of enzyme in powder form	Cradle-to-gate	This study
Xylanase, solid-state fermentation [a]	28,100	1 kg of enzyme in powder form	Cradle-to-gate	This study
B-galactosidase	13,524 [a]	1 kg of enzyme in powder form	Cradle-to-gate	[11]
Cellulase	13.1 [a]	1 kg of enzyme in liquid solution form	Cradle-to-gate	[33]
Cellulase	9.3 [a]	1 kg of enzyme	NA	[9]
Cellulase	425,722	1 kg of enzyme in powder form	Cradle-to-gate	[8]
Cellulase	6.98 [a]	1 kg of enzyme in liquid solution form	NA	[10]
Immobilized enzyme cocktail	20.5 [a]	1 kg of immobilized enzyme cocktail	Cradle-to-gate	[12]
Enzyme cocktail	1.87	1 kg of enzyme cocktail	Cradle-to-gate	[34]

[a] average of multiple scenarios

NA: information not available

Figures

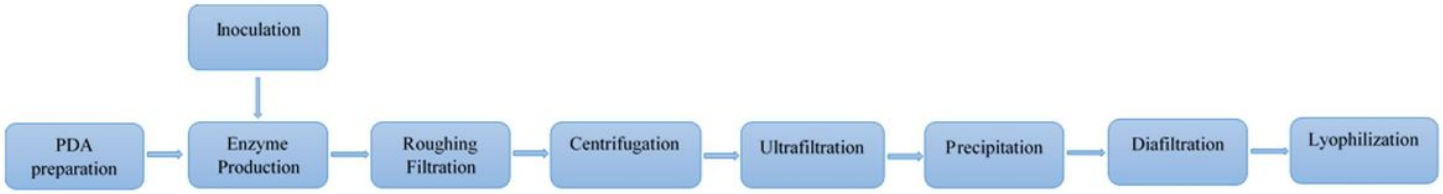

Figure 1

Flowchart for the production of xylanase by suspended culture and solid-state fermentation methods

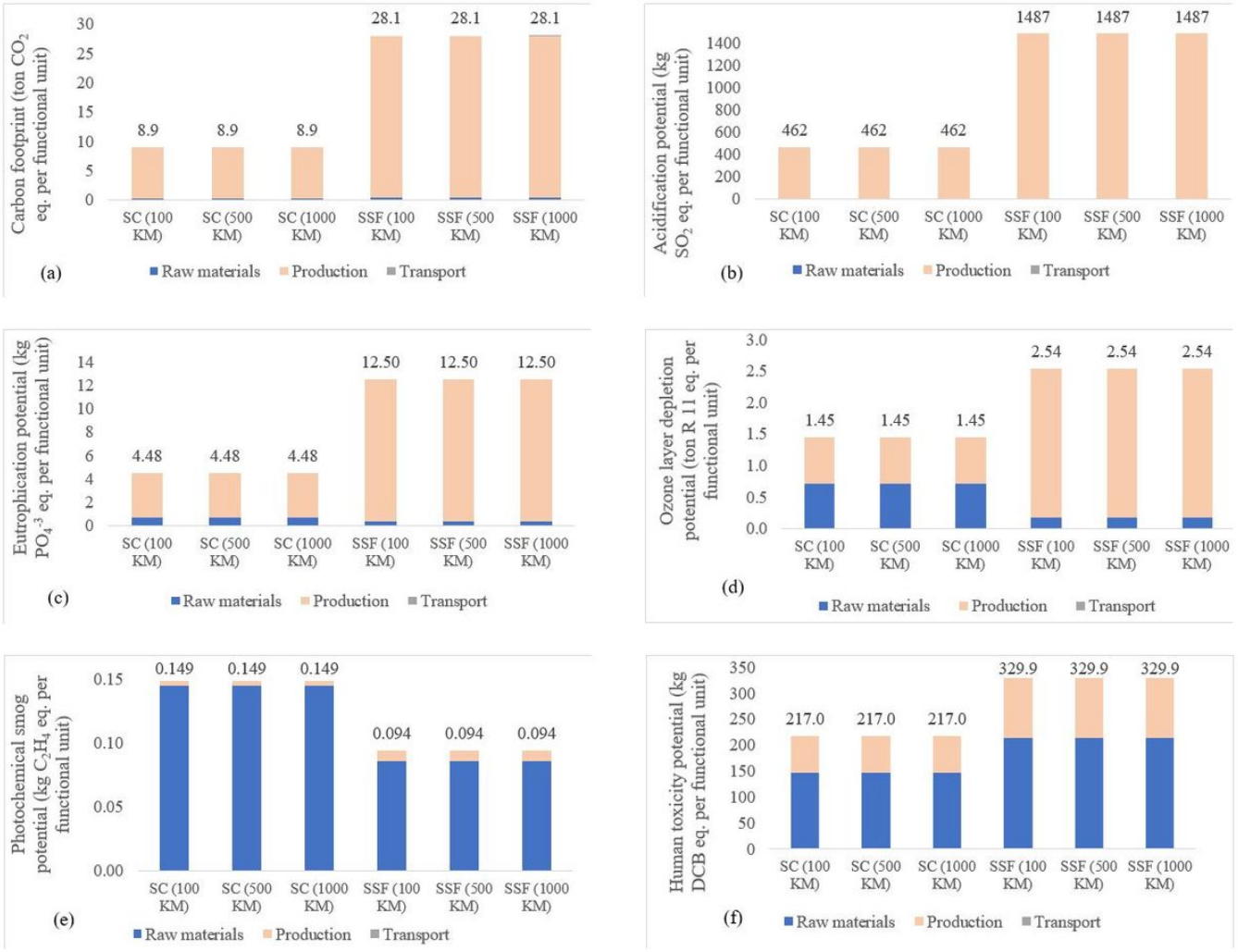

Figure 2

Environmental impacts associated with SC and SSF production of xylanase - (a) carbon footprint, (b) acidification potential, (c) eutrophication potential, (d) ozone layer depletion potential, (e) photochemical smog potential, (f) human toxicity potential

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