

Enzyme-driven pathways for FDCA production: Environmental insights into a scalable and sustainable bioprocess

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ABSTRACT

The growing demand for sustainable alternatives to petrochemical-based plastics has positioned 2,5-furandicarboxylic acid (FDCA) as a promising monomer for next-generation bioplastics like polyethylene furanoate (PEF). Among the available routes, enzymatic oxidation of 5-hydroxymethylfurfural (HMF) using 5-hydroxymethylfurfural oxidase (HMFO) stands out for its selectivity and mild operational conditions. This study aims to assess the sustainability potential of FDCA production via HMFO (including also the analysis of the enzyme production), integrating both techno-economic analysis (TEA) and life cycle assessment (LCA) methodology. Four fermentation volumes were evaluated for the HMFO production (5 L–1 m³), showing that the environmental loads decrease with scale-up, being the 1 m³ scenario the most promising one under an environmental perspective. Regarding the main contributors of the environmental profiles, glucose and cellulose are the process inputs that stand out, while renewable energy and reduced solvent input showed significant improvements in lowering burdens. Regarding the FDCA production, it was modelled both at lab and scale-up levels, including ethanol recycling as a mitigation strategy. In this case, HMF was identified as the primary hotspot across all scenarios and impact categories. On the other hand, the TEA revealed that a minimum selling price of \$33.56/kg is needed to ensure that the process is economically viable, with enzyme reuse offering significant potential for cost reduction. Even though the sustainability potential has been demonstrated, both environmentally and economically, further research is needed in terms of enzyme reuse and recovery.

1. Introduction

The large use of petroleum-based plastics represents a growing environmental and economic threat, linked to the depletion of fossil resources, the generation of waste and greenhouse gas emissions. In response, initiatives such as the European Union's Circular Economy Action Plan promote a transition towards more sustainable materials, encouraging innovation in bioplastics, recyclability and products derived from renewable sources. In this context, bio-based plastics emerge as a promising alternative, which could help reduce the dependence on fossil resources and improving the environmental profile of plastic products (Di Bartolo et al., 2021). Although their market

penetration is still limited, their development is favored by current and coming plastics regulations, but further research is still needed in order to overcome the technical and economic challenges associated with their scale-up (Parveen et al., 2024).

Among next-generation bioplastics, polyethylene furanoate (PEF) is considered as a promising renewable alternative to polyethylene terephthalate (PET). (Brizga et al., 2020; X. Zhao et al., 2023), due to its 100 % bio-based origin and its superior properties (Heo et al., 2023; Pandey et al., 2021). Derived from 2,5-furandicarboxylic acid (FDCA), PEF not only offers technical advantages (Getahun et al., 2024), but also a lower environmental impact in key categories such fossil resource scarcity (Zuiderveen et al., 2021) and global warming potential

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(Papageorgiou et al., 2021). Unlike PET, which is synthesized from fossil-based terephthalic acid, PEF can be integrated into a circular economy model if it is produced from second-generation sugars and through waste-to-product conversion routes (Mujtaba et al., 2023). Furthermore, life cycle assessment (LCA) studies have confirmed that, under certain production conditions, PEF can achieve net carbon footprint reductions of up to 50 % compared to its fossil-based counterpart (Stegmann et al., 2023).

For the production of PEF, several synthetic routes have been assessed, considering FDCA as a monomer, ranging from chemical oxidations to biotechnological processes (Blanco-Cejas et al., 2023; Yang et al., 2022). However, technical limitations persist, including the instability of intermediates, by-products, formation and specific reaction conditions (Cajko et al., 2020; Prasad et al., 2023).

The production of FDCA is primarily based on the oxidation of 5-hydroxymethylfurfural (HMF), a platform compound that can be obtained from sugars derived from lignocellulosic biomass (Herlina et al., 2024; Shen et al., 2024; Wang et al., 2024). The conversion of HMF into FDCA requires the sequential oxidation of both functional groups present in the molecule: the aldehyde group and the hydroxyl group (Kumar et al., 2024; Padligur et al., 2025). Initially, either the aldehyde group or the hydroxyl group may be oxidized, depending on the catalyst or enzyme employed and the reaction conditions. One common pathway involves the oxidation of the aldehyde group to a carboxylic acid, forming 5-hydroxymethyl-2-furancarboxylic acid (HMFA) (Prasad et al., 2023). Subsequently, the hydroxyl group is oxidized to a second aldehyde group, generating 5-formyl-2-furancarboxylic acid (FFCA), which is finally oxidized to FDCA (Zhao et al., 2020). This transformation can be carried out through chemical or biocatalytic pathways, the latter being particularly attractive due to their operation under mild conditions, without the need for heavy metals or toxic solvents, which significantly improves the environmental profile of the process.

In this regard, bioprocesses could be considered as potential alternatives for PEF production, aiming at enhancing its sustainability potential. Biocatalytic processes offers a more selective alternative under mild conditions, which significantly increases the productivity of the process and reduces the energy requirements (Prasad et al., 2023c). In this regard, Dijkman and Fraaije (2014) the enzyme 5-hydroxymethylfurfural oxidase (HMFO) from *Methylovorus* sp. as an effective catalyst for the conversion of HMF to FDCA in cascade enzymatic systems, with reduced by-product formation. Likewise, the review by Milić et al. (2023) highlights that these routes can lower the environmental impacts associated with FDCA production, although technical challenges remain, such as biocatalyst stability.

In parallel, several studies have evaluated the environmental performance of FDCA and PEF production routes, mainly for chemo catalytic pathways from sugar or biomass derived HMF (Davidson et al., 2021; Bello et al., 2018). Other works have examined the techno-economic feasibility of these processes using process simulation combined with cost analysis (Kim et al., 2020; Patel et al., 2022), while more recent contributions have focused on PEF as a biobased alternative to PET (Sanders et al., 2024). Overall, these studies combine LCA and, in some cases, TEA, but they predominantly address chemically catalysed routes; to date, no study has reported an integrated TEA–LCA of FDCA production via HMFO explicitly including the enzyme production stage and the effect of scale.

In this context, this article aims to develop comprehensive sustainability assessment, considering both environmental and techno-economic ones, of the biotechnological production of FDCA from HMF, focusing not only on the conversion process but also on the environmental implications of producing the key biocatalyst, the enzyme HMFO. Sustainability assessments of biotechnological production processes are essential to ensure that alternatives based on renewable resources represent true progress compared to conventional fossil-derived routes (Arias et al., 2023). In this regard, the goal is not only to identify environmental hotspots, but also to assess the effect of

production scale, to analyse the economic viability, and to evaluate critical process variables for sustainable optimization of both the HMFO and FDCA production processes.

2. Methodology

2.1. Life cycle assessment (LCA)

LCA is a recognized and well-established methodology that aims to quantify and analyse the environmental burdens associated with a product or system throughout its life cycle (Curran, 2013; Hauschild et al., 2017). The application of LCA is governed by the ISO 14000 series of standards, specifically ISO 14040:2006 – Principles and Framework, and ISO 14044:2006 – Requirements and Guidelines (ISO, 2006)

2.1.1. Objective, functional unit and system boundaries

The primary objective of this assessment is to determine the environmental profile of the biotechnological production of the HMFO enzyme, followed by the enzymatic synthesis of 2,5-furandicarboxylic acid (FDCA). Several scale-up scenarios are investigated for HMFO production, considering different fermenter capacities to evaluate the impact of scaling up on the environmental performance. For FDCA production, both laboratory-scale synthesis (5 L) and a larger scale scenario targeting the production of 1 kg of FDCA per batch were considered.

The defined system boundaries (Fig. 1) encompass all relevant stages of the processes. In the case of HMFO production, these include pre-inoculum preparation, inoculum development, fermentation, and downstream processing. For FDCA production, the boundaries cover the enzymatic oxidation of HMF and all associated purification steps. The cradle-to-gate assessment considers all stages, from the extraction and supply of raw materials such as chemicals and energy to the production of the final product, whether it is the immobilized enzyme or purified FDCA. The selected functional unit (FU) is 1 kg of FDCA produced, providing a consistent basis for comparison across different process and production scales. Detailed process flow diagrams, developed using SuperPro Designer, are provided in Figs. 2 and 3.

2.1.2. Stage 1: HMFO enzyme production

The HMFO enzyme production process consists of four principal stages: pre-inoculum, inoculum, fermentation, and downstream processing. Each stage is simulated using dedicated equipment, with the process evaluated across four different scales: 5 L, 25 L, 50 L, and 1 m³. Fig. 2 depicts the simulation flowsheet of the upstream bioprocess to produce the immobilised HMFO enzyme.

During the pre-inoculum and inoculum stages, cultivation is performed in Erlenmeyer flasks, represented by units P-1/SFR-101 and P-2/SBR-101, respectively. These stages are carried out under identical conditions (37 °C with continuous shaking at 150 rpm). Regarding batch times, 4 h are required for pre-inoculum stage, while 12 h for the inoculum one. The culture medium consisted of macronutrients, salts, and trace elements, which are detailed in Table 1. The fermentation process, carried out in unit P-3/FR-101, proceeds through three sequential phases: a 16.5-hour batch phase until glucose depletion; a pre-induction fed-batch phase (2–4 h) with controlled feeding; and an induced fed-batch phase (6–8 h) operated at 25–30 °C to promote enzyme expression. The difference between the two induction phases lies in their respective objectives: while the pre-induction phase prepares the cellular system through growth under controlled nutrient conditions, the induction phase initiates and sustains the production of the target enzyme.

Following fermentation, the downstream processing begins with centrifugation (P-6/DS-101), followed by cell disruption using high-pressure homogenization (P-7/HG-101). A second centrifugation step (P-8/BC-101) yields the crude lysate. The soluble enzyme is purified and immobilized in one-step, using cellulose at a ratio of 4 enzymatic units

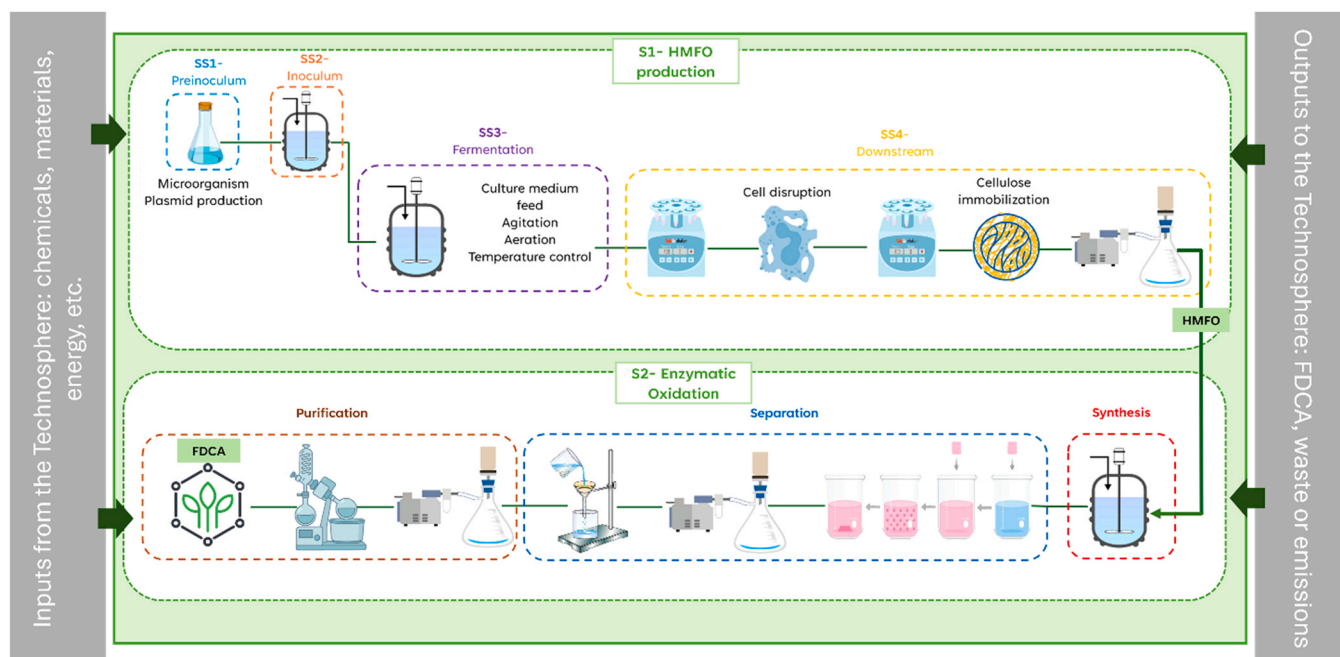


Fig. 1. Overview of process stages included in the assessment for enzymatic FDCA production and defined system boundaries.

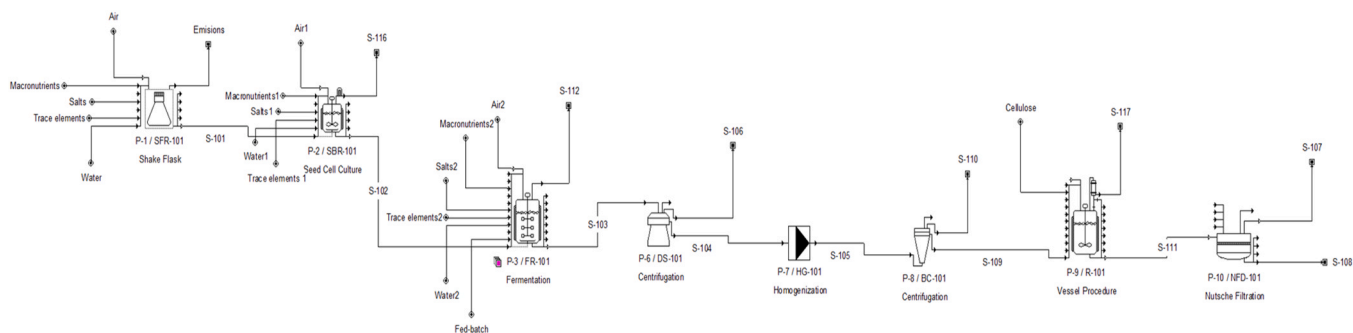


Fig. 2. Simulation of the upstream bioprocess to produce the immobilised HMFO enzyme.

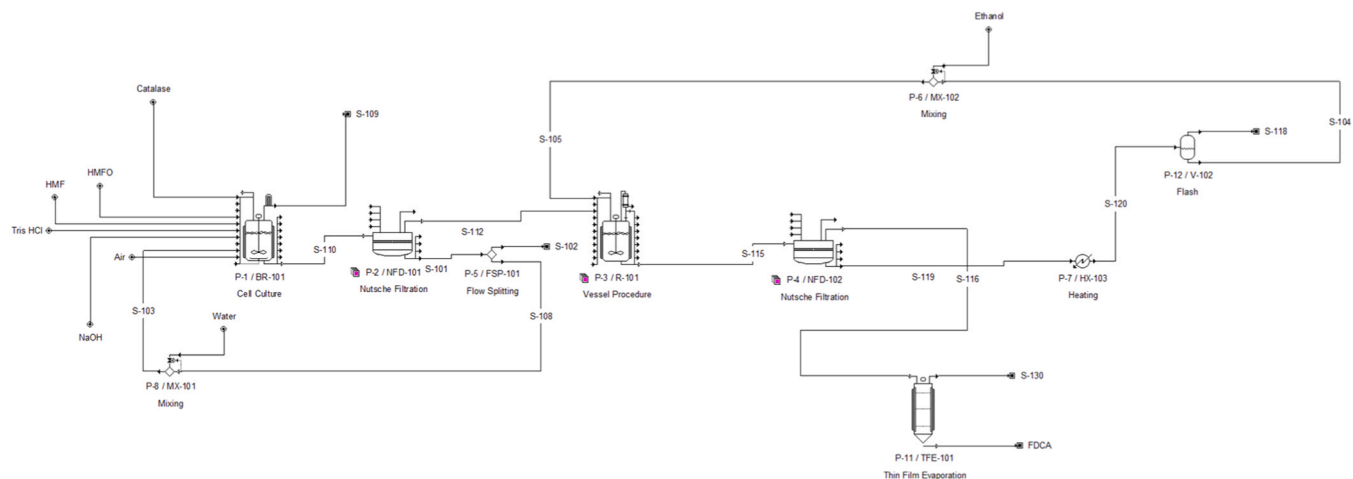


Fig. 3. Simulated process of FDCA synthesis via enzymatic oxidation of HMF.

per gram of cellulose (process taking place in the P-9/R-101 reactor), achieving an immobilization yield of 80 %. Final purification is conducted using filtration and washing (P-10/NFD-101) to remove residual

cellulose and non-immobilized proteins, resulting in a highly purified product with a 20-fold purification factor.

Table 1
Life Cycle Inventory S1 – 5 L. Functional unit: 1 batch.

Inputs from Technosphere					
Compound	Amount	Unit	Compound	Amount	Unit
Glucose	0.47	g	AlCl ₃ · 6 H ₂ O	0.01	mg
K ₂ HPO ₄	0.62	g	ZnSO ₄ · 7 H ₂ O	0.13	mg
KH ₂ PO ₄	0.13	g	CoCl ₂ · 6 H ₂ O	0.02	mg
NaCl	0.06	g	CuSO ₄	0.23	mg
(NH ₄) ₂ SO ₄	0.19	g	H ₃ BO ₃	0.00	mg
MgSO ₄ · 7 H ₂ O	0.02	g	MnCl ₂ · 4 H ₂ O	0.21	mg
FeCl ₃	1.2 · 10 ⁻²	g	NiCl ₂ · 6 H ₂ O	0.00	mg
Citrate	1.55	mg	Na ₂ MoO ₄ · 2 H ₂ O	0.00	mg
CaCl ₂ · 2 H ₂ O	0.21	mg	Thiamine	4.7	mg
Energy	Amount	Unit	H ₂ O	50	mL
Electricity	0.03	kWh			
Outputs to Inoculum stage			Compound	Amount	Unit
			Pre-inoculum	50	mL
Inputs from Technosphere and previous stage					
Compound	Amount	Unit	Compound	Amount	Unit
Glucose	1.88	g	AlCl ₃ · 6 H ₂ O	0.02	mg
K ₂ HPO ₄	2.43	g	ZnSO ₄ · 7 H ₂ O	0.52	mg
KH ₂ PO ₄	0.5	g	CoCl ₂ · 6 H ₂ O	0.09	mg
NaCl	0.24	g	CuSO ₄	0.9	mg
(NH ₄) ₂ SO ₄	0.76	g	H ₃ BO ₃	0.00	mg
MgSO ₄ · 7 H ₂ O	0.92	g	MnCl ₂ · 4 H ₂ O	0.84	mg
FeCl ₃	0.48	g	NiCl ₂ · 6 H ₂ O	0.00	mg
Citrate	6.2	mg	Na ₂ MoO ₄ · 2 H ₂ O	0.01	mg
CaCl ₂ · 2 H ₂ O	0.86	mg	Thiamine	0.01	g
Energy	Amount	Unit	H ₂ O	190	mL
Electricity	0.05	kWh	Pre-inoculum	10	mL
Outputs to Fermentation stage			Compound	Amount	Unit
			Inoculum	200	mL
Inputs from Technosphere and previous stage					
Compound	Amount	Unit	Compound	Amount	Unit
Glucose	645	g	AlCl ₃ · 6 H ₂ O	0.27	mg
K ₂ HPO ₄	50.67	g	ZnSO ₄ · 7 H ₂ O	5.5	mg
KH ₂ PO ₄	10.14	g	CoCl ₂ · 6 H ₂ O	0.1	mg
NaCl	3.64	g	CuSO ₄	9.7	mg
(NH ₄) ₂ SO ₄	7.38	g	H ₃ BO ₃	0.06	mg
MgSO ₄ · 7 H ₂ O	18.3	g	MnCl ₂ · 4 H ₂ O	8.9	mg
FeCl ₃	0.94	g	NiCl ₂ · 6 H ₂ O	0.04	mg
Citrate	0.6	g	Na ₂ MoO ₄ · 2 H ₂ O	0.13	mg
CaCl ₂ · 2 H ₂ O	8.2	g	Thiamine	0.8	g
Energy	Amount	Unit	H ₂ O	3.3	L
Electricity	0.74	kWh	Inoculum	200	mL
Outputs to Downstream stage			Compound	Amount	Unit
			Enzyme	270	g
				1350	UI
Inputs from Technosphere and previous stage					
Compound	Amount	Unit	Compound	Amount	Unit
Tris HCl	0.64	g	Cellulose	0.34	kg
Glutaraldehyde	0.24	mg	Energy	Amount	Unit
H ₂ O	0.37	kg	Electricity	0.85	kWh
Outputs to Technosphere			Compound	Amount	Unit
			Enzyme	1350	UI
			Waste to treatment		
			Wastewater	270	mL
			Biomass	0.49	kg

2.1.3. Stage 2: FDCA production

The immobilised HMFO enzyme is used as a biocatalyst for the selective oxidation of HMF in aqueous solution to synthesize FDCA. To enhance the reaction and prevent hydrogen peroxide accumulation, catalase is added as an auxiliary enzyme. The bioconversion is performed in batch mode within the P-1/BR-101 bioreactor, under controlled conditions: 30 °C, 0.6 vvm aeration, 700 rpm agitation, and pH 8.0 (automatically regulated using 1.5 M NaOH). The total reaction time is 81 h. The integration of this biocatalytic step into the overall process flow is shown in Fig. 3.

Upon completion of the reaction, FDCA is precipitated by lowering the pH to 2 using HCl. The precipitate is recovered through vacuum filtration (P-2/NFD-101) and washed with acidified water. For further purification, the solid is dissolved in a 3:1 ethanol-to-solute ratio using the P-5/MX-101 mixer and the P-3/R-101 reactor. On the other hand,

the filtration with unit P-4/NFD-102 removes any insoluble materials. The aqueous filtrate from the initial vacuum filtration is recycled for use in subsequent batches, achieving an approximate 80 % water recovery rate.

Finally, the ethanol-FDCA solution undergoes vacuum evaporation using the P-7/HX-103 heating unit and the P-12/V-102 flash separator to remove ethanol at 40 °C under vacuum conditions. The resulting purified FDCA is obtained as a dry solid. The recovered ethanol is reintegrated into the process via a return line to P-5/MX-101, thus closing the solvent loop.

It should be mentioned that two operational scenarios are evaluated for this stage: one in which ethanol is recovered and reused as previously described, and the other in which ethanol is not recovered and is discarded. This comparison allows for a comprehensive assessment of the environmental and economic implications of solvent recovery in the FDCA production process.

2.1.4. Description of scenarios and scope

To evaluate the technical and environmental performance of the process under varying operational conditions, multiple scenarios are established, informed by experimental data and projected scale-up models. The laboratory-scale scenario serves as the reference baseline, from which scale-up configurations are considered for both enzyme and FDCA production. This approach facilitates the analysis of environmental burdens and helps to estimate the scalability of the process.

In the case of HMFO production, the following scenarios are considered:

- Scenario 1 (S1): HMFO production in a 5 L fermenter (laboratory scale).
- Scenario 2 (S2): HMFO production in a 25 L fermenter.
- Scenario 3 (S3): HMFO production in a 50 L fermenter (pilot scale).
- Scenario 4 (S4): HMFO production in a 1 m³ fermenter, approaching industrial scale.

These intermediate scales make it possible to assess potential non-linear changes in energy demand, auxiliary service consumption, and material use before reaching pre-industrial operation. The 1 m³ case was selected as a small industrial-scale fermenter, which is large enough to approximate early industrial implementation while still remaining compatible with the process design and assumptions used in this study.

Each of these scenarios encompasses four sub-stages, corresponding to the main phases of enzyme production: pre-inoculum (SS1), inoculum (SS2), fermentation (SS3), and downstream processing (SS4).

For the enzymatic production of FDCA, the following scenarios were analysed: Scenario 5 (S5): production of 1 kg of FDCA per batch, including ethanol recovery and reuse. To evaluate the influence of ethanol recycling, two sub-scenarios are defined at the 5 L reactor scale: Sub-scenario SS5: Without ethanol recovery and Sub-scenario SS6: With ethanol recovery implemented.

2.1.5. Life cycle inventory (LCI)

The compilation of all input and output data—including raw materials (chemicals, water, energy), products, co-products, emissions, and waste—is essential for constructing the LCI of the assessed scenarios. This step is critical, as the reliability and accuracy of impact assessment results directly depend on the quality of the underlying LCI data.

The LCIs corresponding to the main scenarios are presented in Tables 1–3: Table 1 represents the fermentation stage for HMFO production in a 5 L fermenter, Table 2 corresponds to the FDCA production at the 1 kg batch scale and Table 3 shows the FDCA production at the 5 L scale, including sub-scenarios with and without ethanol recovery. The LCIs for the 25 L, 50 L, and 1 m³ HMFO production scenarios are provided in the Table SM1–3 of the Supplementary Material.

For the larger fermentation volumes (25 L, 50 L, and 1 m³), the inventories were obtained by scaling up the 5 L experimental data using

Table 2

Life Cycle Inventory S5 – FDCA production with HMFO – 1 kg of FDCA with solvent (ethanol) recovery.

Inputs from technosphere		
Compound	Amount	Unit
HMF	770.78	g
HMFO	28.12	g
Catalase	29.54	g
Tris-HCl buffer	909.24	g
Ethanol	9.81	kg
Cellulose	738.47	g
NaOH	418.85	g
Water	135.88	L
Energy inputs		
Electricity	1.26	kWh
Cooling water	1.21	kg/batch
Steam	105.84	kg/batch
Outputs to technosphere		
Compound	Amount	Unit
FDCA	1	kg
Liquid effluents	24.13	L

Table 3

Life Cycle Inventory (SS5-SS6) – FDCA production with HMFO – 5 L enzymatic reactor with and without solvent (ethanol) recovery.

Inputs from technosphere			
Compound	Amount (with ethanol recovery)	Amount (without ethanol recovery)	Unit
HMF	33.41	33.41	g
HMFO	1.22	1.22	g
Catalase	1.28	1.28	g
Tris-HCl buffer	39.41	39.41	g
Ethanol	0.40	9.87	kg
Cellulose	32	32	g
NaOH	18.30	18.30	g
Water	0.96	0.96	kg
Energy inputs			
Electricity	0.05	0.05	kWh
Cooling water	5.34	5.34	kg/batch
Steam	4.57	0.01	kg/batch
Outputs to technosphere			
Compound	Amount	Amount	Unit
FDCA	36	36	g
Liquid effluents	2,39	10.86	L

SuperPro Designer®, which provided estimates of energy requirements (electricity) and wastewater/effluent streams for each scale. The LCIs for these scaled HMFO production scenarios are reported in Supplementary Tables SM1–SM3. All mass and energy flows reported in **Tables 1–3** and **SM1–SM3** were normalized to the functional unit and implemented as input–output flows in the LCA model for each scenario, thus constituting the quantitative basis for the environmental impact assessment presented in this work. These LCI datasets were subsequently imported into SimaPro® where the process systems for HMFO production and FDCA synthesis were modelled and linked to the corresponding background datasets. Environmental impacts were calculated by applying the characterization factors of the selected LCIA method to each elementary flow, as described in **Section 2.1.6**.

All electricity consumption values reported in **Tables 1–3** and **SM1–SM3** (kWh per batch) were entered as foreground inputs in SimaPro and modelled using the Ecoinvent dataset “Electricity, medium voltage {RER} | market group for | Cut-off, U”, representing the European medium-voltage grid mix. These energy flows were subsequently characterized together with the other inventory data using the selected LCIA method.

2.1.6. Methods

The ReCiPe 2016 Midpoint (H) v1.07 / World (2010) method was used to assess the environmental impacts associated with HMFO and FDCA production at various scales. ReCiPe is a widely recognized methodology, particularly at the European level, due to its methodological robustness and its capacity to provide detailed characterization across multiple environmental impact categories (Huijbregts et al., 2017).

In this study, the ReCiPe midpoint has been selected, as it enables a more targeted analysis of specific environmental indicators such as climate change, eutrophication, or ecotoxicity, facilitating a clearer interpretation of the results within the context of biotechnological processes. On the other hand, the hierarchical (H) perspective of the ReCiPe method is adopted due to its appropriate balance between impact severity and time horizon (Huijbregts et al., 2017). This perspective helps to ensure consistent and comparable results, an essential requirement when evaluating systems at different production scales.

While ReCiPe Midpoint includes a total of 18 impact categories, this analysis focuses only on those most relevant to biotechnological processes, as summarized in **Table 4**. This targeted selection allows the study to concentrate on the most environmentally significant aspects aligned with the defined goal and scope. The selected impact categories include the following: Global Warming (GW), associated with greenhouse gas emissions; Terrestrial Acidification (TA), which indicates the potential of certain compounds to degrade soil quality; and Eutrophication, encompassing both Freshwater (FE) and Marine (ME) impacts, according to the contribution of phosphorus or nitrogen releases, respectively. Toxicity in environmental and human environments was also considered, expressed in 1,4-dichlorobenzene equivalents, covering Terrestrial, Freshwater and Marine ecotoxicity (TET, FET, MET), as well as Carcinogenic and Non-Carcinogenic human toxicity (HCT, HNCT). Finally, Fossil Resource Scarcity (FRS) represents the depletion of non-renewable resources and its implications for long-term process sustainability.

2.2. Techno-economic evaluation (TEA)

To determine the economic feasibility of the FDCA production process, a techno-economic analysis (TEA) was conducted, according to a discounted cash flow (DCF) approach. This evaluation was performed under the assumption of a production scale of 1000 kg of FDCA per batch, representative of a pilot plant or small-scale commercial operation. The analysis focuses on key economic performance indicators, including the Net Present Value (NPV), which measures the profitability of the investment over time and the payback period, defined as the time required to recover the initial capital outlay. Additionally, the Minimum Selling Price (MSP) of FDCA was calculated, indicating the lowest price at which the product must be sold to ensure the process is economically viable.

The first stage of the TEA involved estimating the equipment

Table 4

Impact categories under assessment.

Acronym	Impact category	Unit
GW	Global Warming	kg CO ₂ eq
TA	Terrestrial Acidification	kg SO ₂ eq
FE	Freshwater Eutrophication	kg P eq
ME	Marine Eutrophication	kg N eq
TET	Terrestrial Ecotoxicity	kg 1.4-DCB ¹
FET	Freshwater Ecotoxicity	kg 1.4-DCB
MET	Marine Ecotoxicity	kg 1.4-DCB
HCT	Human Carcinogenic Toxicity	kg 1.4-DCB
HNCT	Human Non-Carcinogenic Toxicity	kg 1.4-DCB
FRS	Fossil Resource Scarcity	kg oil eq

Note: ¹1.4 dichlorobenzene

purchase costs, which were calculated based on the technical specifications derived from process modelling performed using SuperPro Designer. Equipment sizing, particularly volumes and throughput capacities, was determined in accordance with simulation data. Cost estimation was performed using cost correlation equations proposed by Smith, (2005) and Towler et al., (2008). These cost values were subsequently updated to reflect 2025 monetary values using the Chemical Engineering Plant Cost Index (CEPCI), with base years of 2000 (CEPCI = 394.1) and 2006 (CEPCI = 478.6) applied as reference points.

Following the calculation of equipment costs, the Fixed Capital Investment (FCI) was estimated by applying a Lang factor of 5, which lies within the typical range of 3–6. This factor accounts for the indirect costs associated with plant construction, installation, piping, instrumentation, and other engineering expenses. Additionally, working capital was estimated at 5 % of the FCI, consistent with established methodologies (Humbird et al., 2002).

The annual operating costs were then assessed, with particular attention given to “raw materials and chemicals” category, which include essential inputs such as the HMFO enzyme, ethanol, sodium hydroxide (NaOH), hydrochloric acid (HCl), and phosphate buffers; “utility costs”, which were estimated using standard rates for electricity, steam, cooling water, and process water and “cost of labor” (COL), considering that the facility operates with 5 technicians per shift, across three daily shifts (each of 8 h), with an hourly wage of \$12. All cost parameters, unit prices, and methodological assumptions are detailed in Table 5, which provides a comprehensive overview of the economic inputs used in the TEA.

The plant is assumed to be operative for 11 months per year, using 1 month for maintenance, with a project lifetime of 15 years, a construction period of 6 months, and a start-up phase of 4 months. The financial assumptions include an interest rate of 10 %, linear depreciation over 7 years, inflation rate of 2.2 %, and a 25 % tax rate on profits.

3. Results

This section presents the results of the integrated techno-economic and environmental assessment of the FDCA production process. It begins with the techno-economic analysis (TEA), which evaluates the financial viability of the system using key performance indicators such as the minimum selling price (MSP), payback period, and return on investment (ROI). Subsequently, the environmental performance is analyzed, with a focus on the life cycle impacts associated with HMFO enzyme and FDCA production, complemented by sensitivity analyses aimed at identifying opportunities for sustainability improvements.

Table 5
Values used for each parameter.

Materials cost		
	Unit cost (\$)	Source
Bulk material		
Catalase	3.2	(de Lima et al., 2022)
Ethyl Alcohol	0.75	SuperPro Designer database (2025)
HMFO	0.94	(Kazi et al., 2011)
HMFO	258.6	Calculated by modelling 1 m ³ -scale enzyme production scenario (Colacicco et al., 2024)
NaOH	0.68	SuperPro Designer database (2025)
Tris-HCl	0.13	SuperPro Designer database (2025)
Utilities cost		
Utility	Unit cost (\$)	Source
Std power	0.10	SuperPro Designer database (2025)
Steam	32	
Cooling water	0.10	
Chilled water	0.50	
Hot water	0.40	

The environmental assessment is structured in two main sections. First, the baseline scenario for HMFO production at 5 L laboratory scale is presented and disaggregated into four sub-stages—pre-inoculum, inoculum, fermentation, and downstream processing (Sub-scenarios SS1–SS4). Then, a comparative evaluation of scale-up scenarios (25 L, 50 L, and 1 m³) is conducted to examine how environmental impacts evolve with increasing process scale. Finally, a sensitivity analysis is introduced to identify key drivers of environmental burden and assess alternative configurations.

For FDCA production, the reference scenario (S5) models the enzymatic synthesis of 1 kg of FDCA per batch using immobilized HMFO. Additional sub-scenarios (SS5 and SS6) are evaluated at the 5 L scale, comparing configurations with and without ethanol recovery, to determine their influence on the environmental profile.

3.1. Techno-economic analysis

A techno-economic evaluation is based on a reference case scenario with an annual production capacity of approximately 106,788 kg of FDCA, representative of a medium-scale bioprocess implementation, and serves as a benchmark for assessing scalability. Table 6 lists the major processing equipment used, along with their operational capacities and associated capital costs. This information is fundamental for estimating infrastructure requirements and the total investment needed for process implementation.

The total capital investment (TCI) was estimated at \$37.99 million, encompassing direct and indirect capital costs, contractor fees, and start-up expenditures. Annual operating costs (AOC) were calculated at \$1.98 million, with raw materials (76.6 %) and utilities (12.6 %) representing the primary cost components. The two most cost-intensive inputs identified were the HMFO enzyme and ethanol.

The unit production cost of FDCA was calculated at \$18.56/kg. A market minimum selling price (MSP) of \$33.56/kg was considered in the financial model, which corresponds to the breakeven point yielding a net present value (NPV) of zero at a 6 % discount rate. At this price, the production process achieves a gross margin of 44.71 %, translating into an annual gross profit of \$1.60 million and a net profit of \$1.20 million after accounting for taxes and depreciation.

From a financial perspective, the project yields a return on investment (ROI) of 3.15 %, with a payback period of 18.5 years and an internal rate of return (IRR) of 6.00 % post-tax. These figures suggest modest profitability, with high sensitivity to cost factors such as enzyme usage and raw material prices.

It is worth noting that the model assumes the use of fresh enzyme in each production cycle, which significantly increases the overall operating cost. However, given that the enzyme is immobilized, it is technically feasible to reuse it over multiple cycles. Implementing enzyme recycling strategies could significantly reduce operating costs and, consequently, the MSP, thereby improving the process's economic performance.

The fixed capital investment (FCI) was determined using a bottom-up costing approach with 2025-adjusted prices. Table 7 provides a detailed breakdown of the fixed capital estimate, which includes all major cost components required for the construction and commissioning of the

Table 6
Main equipment and operating volumes used for FDCA production process.

Equipment ID	Equipment description	Operating volume/Area	Cost (\$)
R-101	Stirred reactor (x2)	20,852.54 L	8024,000
BR-101	Bioreactor	176.51 m ³	2804,000
NFD-101	Nutsche filter (x3)	5.40 m ³	1017,000
NFD-102	Nutsche filter (x3)	4.41 m ³	888,000
TFE-101	Thin film evaporator	0.77 m ³	110,000
MX-103	Heat exchanger	12.23 m ³	41,000
V-102	Flash Drum	1986.64 L	12,000
	Unlisted equipment		3224,000

Table 7

Fixed capital estimate.

Total plant direct cost (TCPD)	Cost (\$)
Equipment purchase cost	16,121,000
Installation	5862,000
Process Piping	806,000
Instrumentation	645,000
Insulation	484,000
Electrical	645,000
Buildings	484,000
Yard Improvement	806,000
Auxiliary facilities	1612,000
TPCD	27,463,000
Total plant Indirect cost (TPIC)	
Engineering	1922,000
Construction	1922,000
TPIC	3845,000
Total plant cost (TPC)	
TCP	31,308,000
Contractor's Free & Contingency (CFC)	
Contractor's Free	1565,000
Contingency	3131,000
CFC	4696,000
Direct fixed capital (DFC)	
DFC	36,004,000

production facility.

As shown in Table 7, the total direct plant cost (TPDC) amounts to \$27.46 million, with the equipment purchase representing the largest expenditure. Indirect costs, including engineering and construction, contribute an additional \$3.85 million, bringing the total plant cost (TPC) to \$31.31 million. When contractor fees and contingency allowances are included, the direct fixed capital cost (DFC) reaches **\$36.00 million**. This investment is a critical component in evaluating the financial viability of the FDCA production process.

3.2. Environmental profiles

3.2.1. HMFO enzyme production

The environmental performance of the HMFO enzyme production process at laboratory scale (S1–5 L) was evaluated by analyzing its four main sub-stages: pre-inoculum, inoculum, fermentation, and downstream processing.

The environmental profile of the pre-inoculum stage (Fig. 4A) shows that the main environmental hotspots are associated with electricity consumption, required for agitation and heating to 37 °C, and the use of dipotassium phosphate (K_2HPO_4), dosed at 12.3 g/L. Additionally, glucose (9.4 g/L) shows a notable contribution to marine eutrophication (ME), consistent with findings by Dunn et al. (2012). Similarly, in the inoculum stage (Fig. 4B), the same inventory elements imply the higher contributors: energy, K_2HPO_4 and glucose.

The fermentation stage (Fig. 4C) demonstrates a substantially higher impact, primarily driven by the intensive use of glucose—measured at 22.5 g/L pre-fed batch and 400 g/L during the fed-batch phase. K_2HPO_4 , used at similarly high concentrations, is a significant contributor, particularly in ecotoxicity-related categories (TET, FET, MET, HCT, HNCT). Interestingly, glucose presents a negative contribution in the HNCT category, indicating a marginal environmental benefit in that impact dimension.

The downstream processing stage (Fig. 4D) reflects the cumulative burden of previous stages while introducing new hotspots. Major impacts are evidenced in ME (80 %), TA (70 %), and GW (60 %), with additional burdens in FE (48 %), HCT (52 %), and FRS (54 %). The main contributor is cellulose, used as the support for enzyme immobilization, sourced from post-consumer paper waste (according to EcoInvent), at a ratio of 1 g per 3.2 enzyme units, based on a 4 U/g loading and 80 % immobilization efficiency.

Although some micronutrients such as $FeCl_3$, thiamine or $CaCl_2$ show

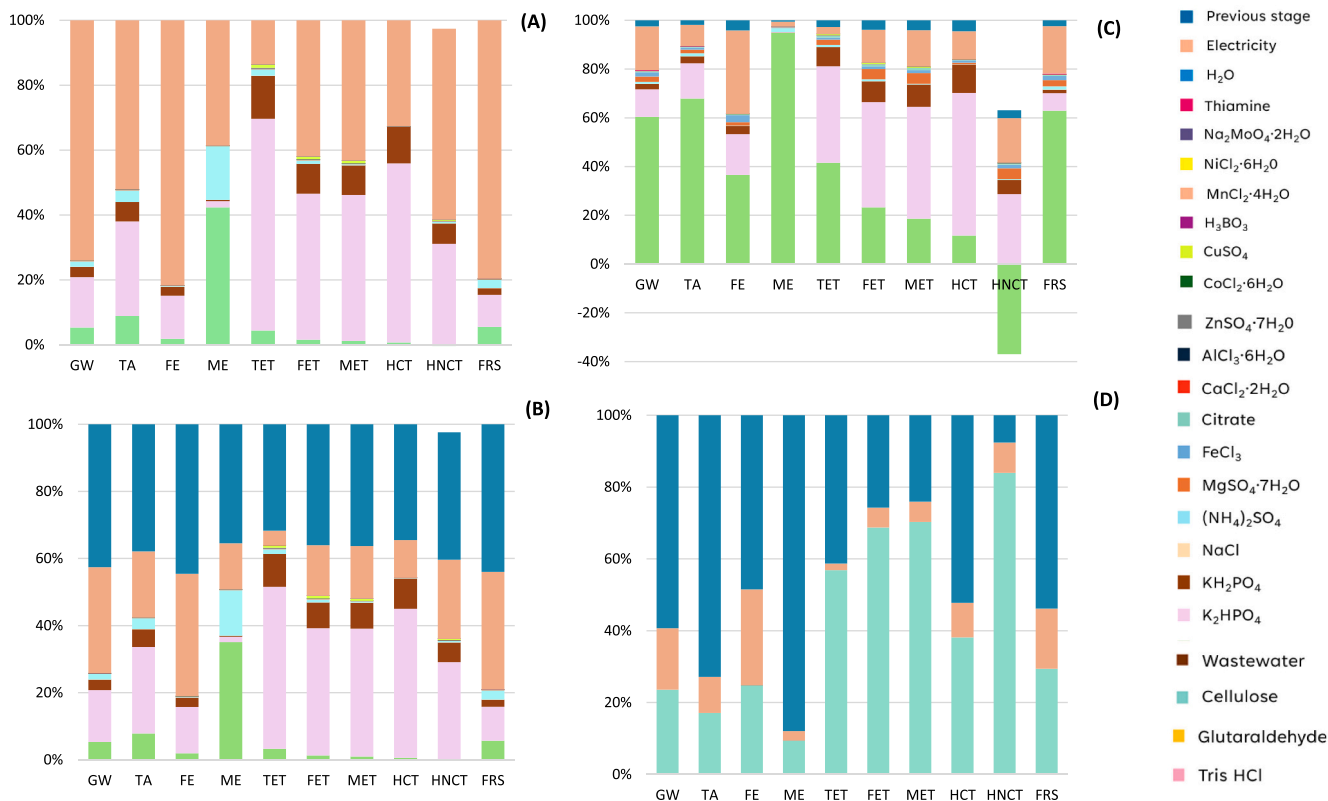


Fig. 4. Environmental profiles of the enzymatic production process of HMFO (5 L), divided by stage: (A) S1 – Pre-inoculum, (B) S2 – Inoculum, (C) S3 – Fermentation, and (D) S4 – Downstream.

large changes in absolute mass when extrapolating from laboratory to pilot and pre-industrial scales, these variations reflect the proportional increase in working volume, as the medium composition per liter was kept constant across all scenarios. In the impact assessment, their contribution to the overall environmental profiles is negligible compared to major hotspots such as glucose, cellulose and HMF.

Wastewater and residual biomass flows reported in the supplementary LCIs were also included in the LCA model and treated as municipal wastewater and non-hazardous biomass waste, respectively. However, their contribution to the overall environmental profile is minor compared with the main hotspots (HMF, glucose, cellulose and electricity) and does not affect the conclusions of the hotspot analysis.

A holistic comparison of stage contributions is provided in Fig. 5A, highlighting that the fermentation stage is the primary impact driver across six out of nine categories, while the downstream stage dominates the remaining four. The disparity is particularly pronounced in TA and ME (due to glucose) and in FET/MET (due to cellulose). In contrast, the pre-inoculum and inoculum stages contribute minimally, which is attributed to their shorter duration (12 h versus 32.5 h for fermentation) and lower material consumption. Finally, Table 8 compiles the quantitative environmental impact data per category and per sub-stage, offering critical insights into process hotspots and identifying key improvements for optimisation.

Fig. 5B presents a comprehensive comparison of the environmental performance of the HMFO enzyme production process across different production scales, using the laboratory-scale scenario (S1 – 5 L) as the reference. All impact values are expressed as relative figures, normalized to the S1 baseline (value = 1.00). As the scale increases to 25 L (S2), 50 L (S3), and 1 m³ (S4), a consistent trend of decreasing environmental impacts is observed across all evaluated categories.

The most significant reductions occur in the Freshwater Eutrophication (FE) category, with impact reductions reaching up to 47.1 % at the largest production scale. Notable decreases are also observed in Global Warming (GW), where the impact decreases from 1.00 kg CO₂ eq at the 5 L scale to 0.86 kg at 25 L, 0.80 kg at 50 L, and 0.62 kg CO₂ eq at 1 m³, representing an overall reduction of 38.0 %. Similar downward trends are noted in Terrestrial Acidification (TA), with a reduction of up to 33.5 % at the largest scale.

Toxicity-related categories also show considerable improvements. Human Carcinogenic Toxicity (HCT) decreases by 18.1 % from S1 to S4, while Human Non-Carcinogenic Toxicity (HNCT) is reduced by 7.95 %. Although the ecotoxicity-related categories—Terrestrial (TET),

Freshwater (FET), and Marine Ecotoxicity (MET)—exhibit more moderate reductions (ranging from 2 % to 6.4 %), the overall trend remains positive. Among these, TET shows the smallest improvement, with a reduction of only 2 % at the 1 m³ scale, suggesting that certain inputs or process parameters continue to present a dominant influence on this category regardless of scale.

The Fossil Resource Scarcity (FRS) category displays a substantial reduction of 32.9 % from S1 to S4, reflecting the improved efficiency in energy and solvent use associated with process scale-up. Collectively, this analysis confirms that increasing the production scale of HMFO enzyme yields significant environmental benefits, with the 1 m³ configuration (S4) consistently outperforming smaller scales across almost all impact categories.

Following the identification of key environmental hotspots, a set of sensitivity analyses was carried out to evaluate potential strategies for improving the sustainability of the HMFO production process. These strategies directly address the most impactful parameters identified in the baseline laboratory-scale scenario (S1). The sensitivity scenarios examined were:

- SA1: 10 % reduction in K₂HPO₄ dose during the pre-inoculum, inoculum, and fermentation stages.
- SA2: 10 % reduction in glucose concentration during fermentation.
- SA3: 20 % reduction in overall energy requirements through process optimization.
- SA4: substitution of fossil-based electricity with renewable energy sources.
- SA5: enhancement of enzyme immobilization efficiency on cellulose to 100 % yield, enabling a 20 % reduction in cellulose use.

The individual and combined results of these scenarios are presented in Fig. 6 for the S1 baseline. Among the single-parameter strategies, SA1, SA2, and SA3 achieved modest average reductions of 1.12 %, 2.66 %, and 1.41 %, respectively. In contrast, SA4 and SA5 yielded significantly greater benefits, with average reductions of 16.79 % and 8.45 %. The substitution of fossil-based electricity with renewable sources (SA4) showed the greatest potential for impact reduction.

When all five measures were implemented simultaneously (GA), the overall environmental burden was reduced by 28.80 %, demonstrating strong synergistic effects between process optimizations. Category-specific analysis showed marked reductions in FE in the scenarios SA3, SA4, and GA; HCT in SA1; HNCT in SA5; and ME in the case of SA2.

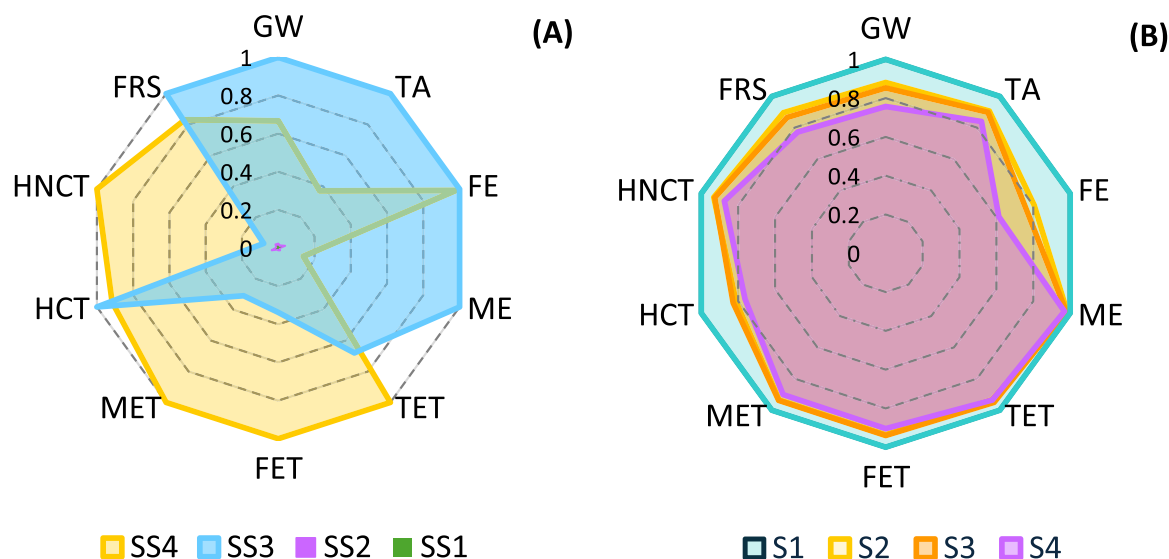
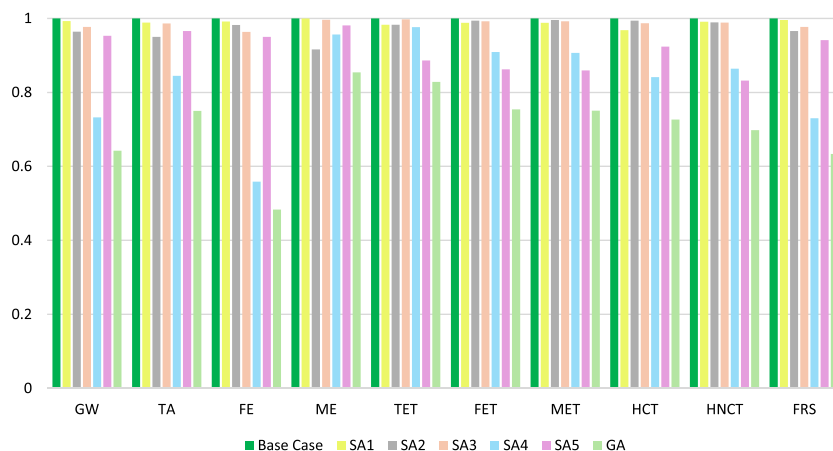


Fig. 5. Environmental impact comparison for enzyme production: (A) Contribution of each sub-stage in the base case scenarios (S1–5 L); (B) Global environmental performance of scaled-up scenarios (S1, S2, S3 and S4).

Table 8

Impact values for S1-laboratory scale- 5 L fermentation volume. Functional unit: 1 batch process.

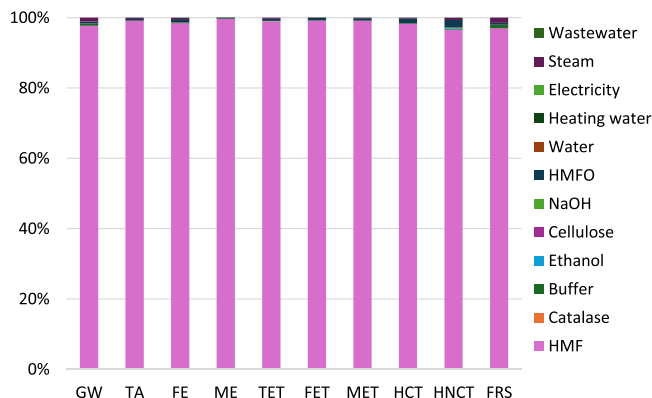
Impact category	Pre-inoculum	Inoculum	Fermentation	Downstream	Global	Unit
GW	$1 \cdot 10^{-2}$	$3 \cdot 10^{-2}$	1.19	0.68	1.92	kg CO ₂ eq
TA	$6.61 \cdot 10^{-5}$	$1.70 \cdot 10^{-4}$	$8.95 \cdot 10^{-3}$	$2.78 \cdot 10^{-3}$	$1.19 \cdot 10^{-2}$	kg SO ₂ eq
FE	$1.32 \cdot 10^{-5}$	$2.61 \cdot 10^{-5}$	$6.50 \cdot 10^{-4}$	$5.74 \cdot 10^{-4}$	$1.26 \cdot 10^{-3}$	kg P eq
ME	$1.65 \cdot 10^{-6}$	$4.77 \cdot 10^{-6}$	$8.24 \cdot 10^{-4}$	$9.19 \cdot 10^{-5}$	$9.23 \cdot 10^{-4}$	kg N eq
TET	$3.43 \cdot 10^{-2}$	0.12	4.37	5.06	9.60	kg 1,4-DCB
FET	$5.67 \cdot 10^{-4}$	$1.60 \cdot 10^{-3}$	$3.94 \cdot 10^{-2}$	0.11	0.13	kg 1,4-DCB
MET	$7.68 \cdot 10^{-4}$	$2.10 \cdot 10^{-3}$	$4.98 \cdot 10^{-2}$	0.12	0.18	kg 1,4-DCB
HCT	$1.42 \cdot 10^{-3}$	$4.22 \cdot 10^{-3}$	$9.29 \cdot 10^{-2}$	$7.20 \cdot 10^{-2}$	0.17	kg 1,4-DCB
HNCT	$1.92 \cdot 10^{-2}$	$4.63 \cdot 10^{-2}$	0.33	3.50	3.89	kg 1,4-DCB
FRS	$3.56 \cdot 10^{-3}$	$7.31 \cdot 10^{-3}$	0.29	0.21	0.52	kg oil eq

**Fig. 6.** Sensitivity assessment for the S1 scenario (5 L).

These results indicate that targeted actions can yield differentiated benefits depending on the environmental category, offering guidance for priority areas of improvement. Overall, the implementation of renewable energy sources (SA4) emerges as the most effective strategy for reducing environmental impacts across all scenarios, followed closely by the reduction of cellulose inputs (SA5), particularly at higher production scales.

3.2.2. Environmental performance of FDCA production

The environmental analysis of the large-scale scenario for enzymatic FDCA production was conducted using a detailed process simulation model developed in SuperPro Designer, assuming a batch production capacity of 1 kg of FDCA. The resulting life cycle impact profile is illustrated in Fig. 7, providing insights into the relative contributions of each input across a comprehensive set of impact categories.

**Fig. 7.** Environmental profile of the production of 1 kg of FDCA-S5.

A dominant finding from the analysis is the excessively high contribution of 5-hydroxymethylfurfural (HMF), which accounts for between 95 % and 98 % of the total environmental burden across all evaluated categories. This overwhelming influence identifies HMF as the primary environmental hotspot in the FDCA production process, corroborating trends previously observed in laboratory-scale evaluations.

The high impact of HMF is principally attributable to its petrochemical origin and the energy-intensive nature of its synthesis and purification processes (Davidson et al., 2021). These factors result in substantial emissions and resource consumption, particularly in categories such as global warming, human toxicity, and fossil resource scarcity. Unless more sustainable, bio-based HMF production pathways are commercialized at scale, the environmental footprint of FDCA production will continue to be constrained by the impacts associated with this precursor. In contrast, all other inputs demonstrate relatively minor environmental contributions.

The HMFO enzyme, central to the catalytic transformation of HMF to FDCA, contributes marginally, typically 1–2 % of the overall environmental impact in most categories. This low contribution reflects the relatively small quantity of enzyme required and its high catalytic efficiency. Similarly, steam consumption exerts a limited influence, primarily affecting categories associated with HCT and TET. Other auxiliary inputs, including ethanol and buffering agents used for pH regulation, make only negligible contributions, with marginal relevance observed in isolated categories.

The environmental performance of enzymatic FDCA production at laboratory scale was assessed by comparing two sub-scenarios: SS5, without ethanol recovery, and SS6, which includes ethanol recovery by distillation. The comparative results, presented in Table 9, highlight both absolute impact values and the percentage variations across all impact categories.

Table 9

Environmental loads. Comparison between SS5 and SS6.

Impact category	GW	TA	FE	ME	TET	FET	MET	HCT	HCNT	FRS
SS- No solvent recovery	127.8	0.96	0.05	0.11	696	7.79	9.52	7.59	54.61	32.85
S6- Solvent recovery	118.1	0.80	0.05	0.09	657	7.18	8.99	7.11	37.44	31.00
% impact reduction	7.59	16.67	-	18.18	5.60	7.83	5.57	6.32	31.44	5.63

The most substantial difference between the two configurations arises from the management of ethanol, a key solvent used during the purification stage. In SS5, where ethanol is discarded after use, it represents a significant contributor to the overall environmental burden, accounting for up to 30 % of the impact in certain categories such as HNCT and ME. This is due to both its high consumption volume and the emissions associated with its upstream production, whether fossil- or biomass-derived.

In contrast, the implementation of ethanol recovery in SS6 (Fig. 8) results in a dramatic reduction of 90–95 % in ethanol-related environmental impacts across most categories. Consequently, the overall environmental footprint is reduced by an average of 10.48 %, with particularly notable improvements observed in HNCT (−31.4 %), ME (−18.2 %), and TA (−16.7 %). Additionally, the environmental burden in S6 becomes more relevant in the primary raw material, HMF.

These findings provide compelling evidence that ethanol recovery represents an effective and implementable strategy to reduce the environmental burden of FDCA production at the laboratory scale. Beyond the quantifiable reductions in environmental impacts, ethanol recovery enhances resource efficiency, promotes circularity, and aligns the process with broader green chemistry principles.

4. Discussion

The environmental assessment presented in this study highlights the pivotal role of enzyme production within the broader sustainability framework of biocatalytic processes, particularly in the context of FDCA synthesis. In alignment with previous studies (Bello et al., 2020b), our findings reinforce that upstream enzyme production can represent a

significant proportion of the overall environmental burden, largely due to energy-intensive fermentation and purification operations. However, by applying a combined strategy of scale-up, energy decarbonization (SA4), and improved immobilization efficiency (SA5), our results demonstrate a substantial mitigation of environmental impacts across nearly all evaluated categories. Specifically, for the 25 L production scale, reductions of up to 91 % in GWP, 89 % in TA, 81 % in TET, 86 % in HNCT, and 91 % in FRS were achieved relative to previous benchmarks. These figures underscore the effectiveness of process optimization, especially when complemented by the use of renewable energy sources and efficient biocatalyst design.

Overall, these results support the conclusion that a sustainable production of FDCA through biocatalytic pathways relies not only on conversion efficiency, but also on an integrated approach that encompasses biocatalyst production, energy optimization, and downstream efficiency. Despite the progress achieved, several limitations remain, opening new avenues for research. For instance, while HMFO immobilization was modeled as an environmental improvement strategy, the actual number of reuse cycles without significant loss of activity has yet to be evaluated—an essential factor for accurately assessing its industrial-scale impact. Similarly, the use of cellulose as a support, although environmentally preferable to synthetic polymers, could potentially be replaced with materials of lower impact or higher durability. Another critical area is the sourcing of HMF, as its origin and production method can significantly influence the overall environmental footprint of the process. Finally, the standardization of functional units in enzyme LCA studies, along with improved availability of enzyme-specific inventories, will enable more consistent and robust comparisons across alternative technologies, strengthening the path toward industrially viable and environmentally responsible bioprocesses.

A critical methodological advancement in this work lies in the adoption of enzyme units as the functional unit rather than mass-based metrics (e.g., kg of enzyme). This approach enhances the alignment between the environmental assessment and the actual catalytic performance of the enzyme in its operational context, thus enabling more meaningful evaluations of process efficiency. However, this choice also introduces challenges when comparing with conventional enzyme LCAs that default to mass-based units, highlighting the urgent need for harmonization of functional units across the literature to enable robust cross-study comparisons.

Another central aspect concerns the potential for enzyme recovery and reuse, which remains a largely untapped opportunity in the current process configuration. Although the immobilization of HMFO was included as an optimization strategy, the system still assumes the addition of fresh enzyme per batch, without incorporating reuse cycles. This represents a conservative assumption. Literature evidence, such as that from Saha et al. (2017), indicates that even two reuse cycles can yield reductions of up to 50 % in environmental impact, and stabilized HMFO variants (e.g., 8BxHMFO) have demonstrated enhanced stability and retained activity under operational conditions, reinforcing the feasibility of enzymatic recycling in industrial applications (Wu et al., 2020). Thus, future research should prioritize the empirical evaluation of HMFO immobilization stability, to validate its contribution to long-term environmental performance.

Beyond enzyme production, the sustainability of FDCA synthesis is also conditioned by downstream processing, particularly product purification stages. Bello et al. (2020a) compared multiple FDCA separation

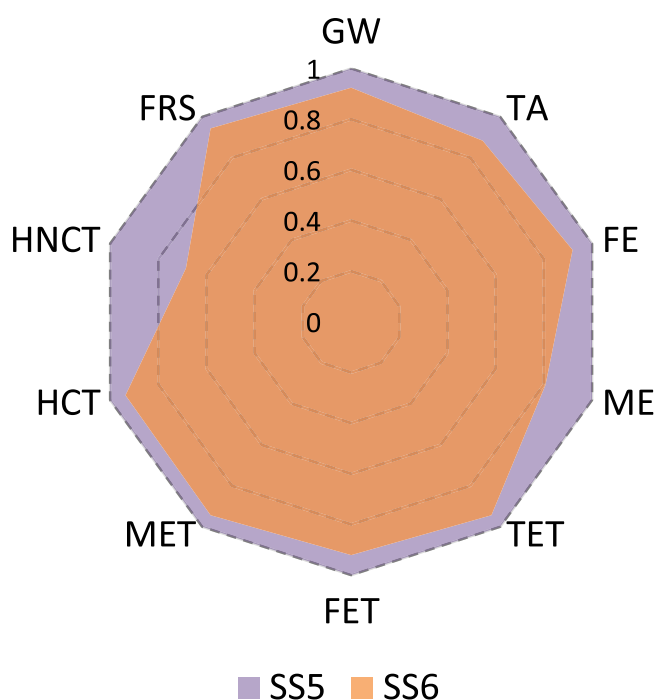


Fig. 8. Environmental impact comparison for enzymatic FDCA production without (SS5) and with (SS6) solvent recovery.

methods and found that different routes (e.g., direct crystallization vs. extraction with trioctylamine) can result in up to 18 % variation in GWP. While the focus of the present study was on the enzymatic oxidation of HMF, the downstream ethanol-based purification step proved to be a non-negligible contributor to the environmental footprint, particularly in categories such as HNCT and ME. The incorporation of an ethanol recovery system (SS6) significantly alleviated these impacts—achieving an average reduction of 10.5 % in total environmental load—thus highlighting solvent recovery as a practical and impactful mitigation strategy.

In terms of process integration, the results resonate with broader findings in literature. For instance, Kim et al. (2022) demonstrated that thermal integration strategies such as heat recovery can reduce environmental impacts by over 30 % in FDCA biorefineries. Although our system does not incorporate active heat integration, the use of mild reaction conditions and renewable electricity led to a substantial 16.8 % reduction in the enzyme production footprint, supporting the notion that early-stage energy optimization can offer meaningful environmental benefits without complex infrastructural upgrades.

Collectively, these results confirm that achieving sustainable FDCA production via biocatalytic routes requires more than efficient catalytic conversion. It demands a systemic and integrated perspective, addressing not only enzyme synthesis and energy use but also downstream processing, material sourcing, and process circularity. However, despite the improvements achieved, several limitations and research opportunities remain:

- Reuse potential of immobilized HMFO must be empirically quantified to validate its long-term environmental and operational viability.
- Cellulose supports, though advantageous over synthetic carriers, could be replaced with alternative materials offering superior reusability, lower impact, or improved mechanical properties.
- The source and production route of HMF continues to dominate the impact profile of FDCA synthesis. Transitioning to bio-based HMF or low-impact synthesis methods is crucial to unlocking the full sustainability potential of the bioprocess.
- Standardizing functional units and improving access to enzyme-specific LCI data would greatly enhance the robustness and comparability of enzyme-based LCA studies.

In conclusion, this study demonstrates that the environmental performance of biocatalytic FDCA production can be significantly enhanced through scale-up, energy transition, enzyme immobilization, and solvent recovery. However, further efforts are needed to close remaining sustainability gaps—particularly in biocatalyst reuse and upstream raw material sourcing—if enzymatic pathways are to become truly competitive and environmentally viable alternatives to petrochemical routes.

CRedit authorship contribution statement

Aroa Carballido: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Ana Arias:** Writing – review & editing, Visualization, Investigation. **Maria Teresa Moreira:** Writing – review & editing, Validation, Supervision, Conceptualization. **Gumersindo Feijoo:** Writing – review & editing, Validation. **Ángel T. Martínez:** Writing – review & editing. **Marina Guillen:** Writing – review & editing. **Juan Carro:** Writing – review & editing. **Francisco Javier Ruiz-Dueñas:** Writing – review & editing. **Gloria Caminal:** Data curation. **Kirian Bonet:** Data curation. **Gloria González:** Resources. **Oscar Romero:** Resources. **Garazi Ortiz-Orruño:** Data curation. **Darly Concha:** Resources.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.indcrop.2026.122625](https://doi.org/10.1016/j.indcrop.2026.122625).

Data availability

Data will be made available on request.

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