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Production of phosphate biofertilizers as a booster for the techno-economic and environmental performance of a first-generation sugarcane ethanol and sugar biorefinery

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ABSTRACT

The intensive use of fertilizers and pesticides in agriculture has a significant economic and environmental impact worldwide. Biofertilizers (aka microbial inoculants) could be a potential alternative to decrease costs and the environmental footprint linked to the use of fertilizers while boosting productivity through biological processes. This work aimed to perform a techno-economic-environmental assessment of an industrial biofertilizer production facility integrated with a sugarcane ethanol biorefinery. To this end, systems engineering tools were employed concurrently with techno-economic-environmental analyses to assess the integration of the different processes and their feasibility. Three processes for biofertilizer production are proposed varying in terms of downstream processing and the use of single or double microorganisms. Our findings indicate that the integration of biofertilizer production can enhance the biorefinery's NPV by as much as 137% in the most favorable scenario and by a minimum of 69% in the most unfavorable scenario. Regarding environmental consequences, in general, all scenarios demonstrate an improvement over the base scenario. Global sensitivity analysis showed that the solid-state fermentation and composite formulation steps of the biofertilizer process have the most substantial influence on both economic and environmental outcomes. The uncertainty analysis further unveils that the scenarios without fungus separation exhibited greater resilience in the face of market volatility. The retro-techno-economic study defined the economically viable region. Ultimately, this study demonstrates that the integration of biofertilizers into an ethanol and sugar biorefinery is a more sustainable alternative than the isolated biorefinery regarding the environmental and techno-economic aspects of sustainability.

1. Introduction

The escalating climate crisis is driving a swift shift towards a circular, carbon-neutral economy. A crucial move in this trajectory is the enhancement of renewable energy sources by advancing technologies that harness the complete energy potential of biomass, which is estimated to be between 2 and 6 TW per year (Kim et al., 2019). Aligned with global commitments, Brazil's biofuel policy, RenovaBio, has set a goal to nearly double bioethanol production over the next decade as a substitute for gasoline (Vandenberghe et al., 2022). Despite the impact of the Covid-19 pandemic on the biofuel market, which prompted the federal government to reconsider these targets, the objective for 2030 remains ambitious: nearly 91 million decarbonization credits (CBios), or 90% of the original target (Ministério de Minas e Energia, 2022). Therefore, it is likely that Brazil will continue to use sugarcane to produce sugar, alcohol, and electricity as primary products for the foreseeable future. These incentives will open up new opportunities for the establishment of integrated sugarcane biorefineries in Brazil (Klein et al., 2019a). Sugarcane utilization is a prime example of a successful biorefinery, given its ability to produce three key marketable products: sugar, ethanol, and bioelectricity, along with a wide array of derived by-products (Vandenberghe et al., 2022).

Creating and implementing new bio-based products that promote using untreated nutrient sources like mineral rock fertilizers could mitigate the environmental issues linked to producing and applying chemical fertilizers (Favaro et al., 2022). The manufacturing process of chemical phosphate fertilizers, which involves the acid treatment of phosphate rock, is energy-intensive and results in several byproducts

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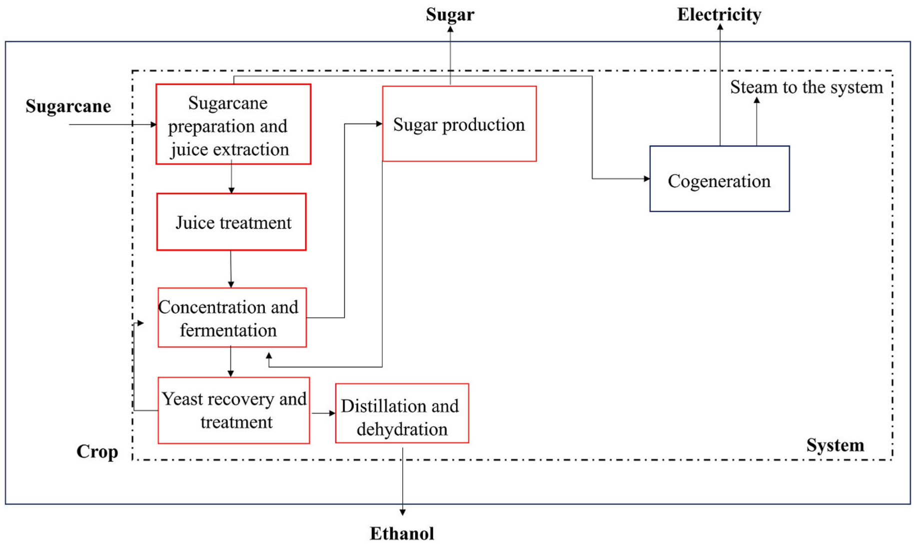

Fig. 1. Simplified diagram of the sugarcane biorefinery (base case).

that contribute to environmental pollution (Barrow, 2024). Despite their crucial role in modern farming practices, excessive use of these fertilizers can reduce soil biodiversity (Prashar and Shah, 2016). The interplay between microorganisms, soil, and plants is vital for sustainable agriculture and crop yield (Rashid et al., 2016).

Biofertilizers (also known as microbial inoculants) have been on the market since the early 1900s, but of late, they have regained the attention of both the scientific community and major corporations (Fox, 2015). A multitude of formulations based on microorganisms have been developed and applied to various crops worldwide (Lobo et al., 2019). Soybean cultivation is the primary global user of inoculants, with Brazil at the forefront. Around 78% of the soybean farmed land in Brazil, which is about 36 million hectares, is pre-inoculated. Brazil's domestic market for inoculants has seen substantial growth, with sales increasing from 10 million liquid doses in 2005 to over 45 million in 2018 (Santos et al., 2019).

Over the past decade, there has been a surge in the use of inoculants that contain various types of microorganisms, a process referred to as co-inoculation (Santos et al., 2019). The combination of different strains or species, each contributing to different microbial processes, can yield combined benefits and enhance productivity. If these microorganisms cannot be incorporated into a single product, they are produced separately but packaged together before being sold (Santos et al., 2019). Co-inoculation has proven effective under several restrictive conditions, such as in soils with low phosphate content. For crops like sugarcane, phosphorus is a crucial element as it is involved in cellular processes like photosynthesis and sugar transformation, among others (Gumiere et al., 2019).

The growth of agriculture in Brazil is significantly dependent on the use of fertilizers, particularly phosphorus. Given that Brazilian soils have a high capacity for phosphorus fixation, substantial fertilizer inputs are required to counteract the swift immobilization of inorganic phosphorus (Withers et al., 2018). The high demand for phosphorus fertilizer and the heavy reliance on imports render Brazilian agriculture particularly susceptible to potential phosphorus shortages or abrupt cost fluctuations. For instance, in 2008, the price of phosphorus soared by 800% within a span of 12–18 months (Mew, 2016). To ensure the country's agricultural sustainability, two strategies can be employed: biotechnological solubilization of phosphate rocks (RF) (Klaic et al., 2018) and the use of inoculants containing soil-dwelling phosphate-solubilizing microorganisms (Favaro et al., 2022).

Furthermore, in Brazil, most methods developed for fungal cultivation involve using cereals or pre-cooked grains, predominantly rice, as a substrate. This approach was initiated in the late 1960s and subsequently scaled up for mass production. Over the ensuing decades, modifications to the system have streamlined the process and enhanced production efficiency (Mascarin et al., 2019). Additionally, solid-state fermentation could be an effective method for the mass production of biopesticides, given that the microorganisms would grow on a substrate that resembles their natural environment (Arora et al., 2017). Recently, an article was published by EMBRAPA, a Brazilian agricultural research company, outlining the journey that has led Brazil to become the top producer and consumer of biocontrol products. The article estimates that the area in Brazil under biological control (which includes soybean, rice, sugarcane, etc.) surpasses 70 million hectares (Bettiol and de Medeiros, 2023).

This work studies the techno-economic and environmental aspects of phosphate biofertilizer production coupled with a sugarcane biorefinery. The biofertilizer is composed of microorganisms, starch and/or rice, and phosphate rock. Three cases are evaluated for producing said biofertilizer, which generates spores from solid-state fermentation (SSF) using rice as a substrate. They are as follows.

Single Microorganism with Extraction step (SM-WE): SSF is used solely to produce *T. asperelloides* fungi (a biocontrol agent). After cultivation, spores are removed from the culture medium and added to the formulation.

Single Microorganism with No Extraction Step (SM-NE): The spore/culture medium of *T. asperelloides* is used after cultivation, and the entire medium is crushed and incorporated into the biofertilizer formulation.

Double Microorganism with No Extraction Step (DM-NE): It extends SM-NE with an additional fungus (*T. asperelloides* and *A. niger*, an acidulant promoter) for spore production.

The Net Present Value (NPV) was selected as the economic metric, and Life Cycle Assessment (LCA) was chosen to estimate the environmental footprint. Uncertainty and global sensitivity analyses were employed to identify the primary variables affecting the economic and environmental aspects of the processes and their impact on the corresponding metrics. Retro-techno-economic analysis was used to delimit regions of economic viability.

The remaining parts of the manuscript are organized as follows: Section 2 presents the PSE methods and tools employed in this work. Section 3 introduces the results and discussion. Finally, Section 4 reiterates the main conclusions and highlights take-home messages.

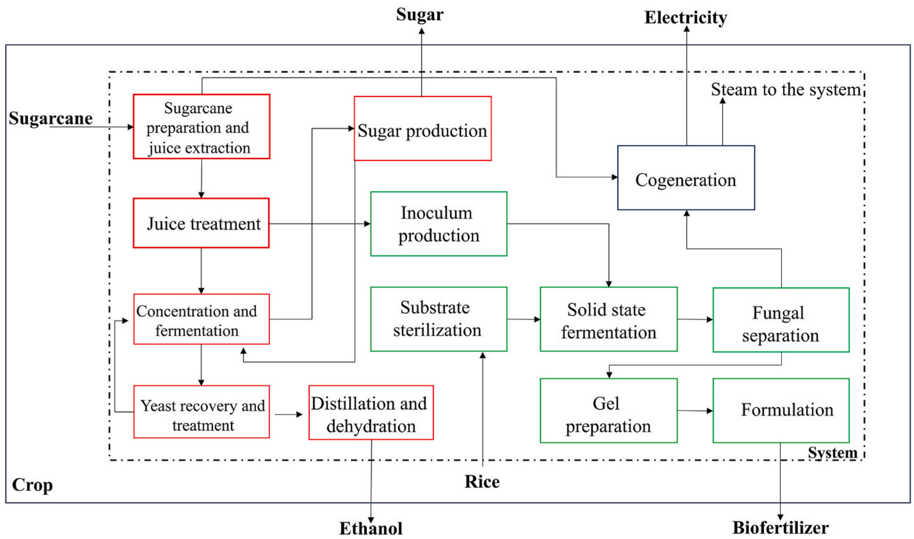

Fig. 2. Simplified diagram of biofertilizer production annexed to a sugarcane biorefinery. **Scenario SM-WE:** Single Microorganism with Extraction step.

2. Methods

2.1. Software and modeling

The EMSO software (equation-oriented and versatile process simulator) was used to develop the process models used in the base cases. EMSO offers an advantage by enabling users to inspect and extend all developed models (Soares and Secchi, 2003).

All equipment items were designed using mass and energy balance equations, considering thermodynamic and physical properties, efficiency, and process performance variables such as conversions and productivity. The equations that describe all the equipment used in this work are documented in the supplementary material. Additionally, equations describing process economic and environmental performance were added in the EMSO simulation script files to be solved alongside process equations.

EMSO can establish communication with external software using the CAPE-OPEN protocol (Soares and Secchi, 2004). The current investigation establishes the interface using EMSO as a Python function. That is, the Python routine is responsible for modifying specific process variables contained in EMSO, which returns the model output ($f(x_1, x_2, x_3, \cdots, x_k)$) for the execution of the Python routine. In this approach, the procedure employing EMSO as a Python function was used to conduct the global sensitivity analysis (GSA) through the SAlib Python package (Herman and Usher, 2017), and the uncertainty analysis (UA) was performed using the NumPy Python library. The EMSO-Python integration is necessary for running the GSA and the UA because these procedures are not suitable for equation-oriented simulators.

2.2. Process description

To assess the feasibility of producing biofertilizer as a by-product of a sugarcane biorefinery, three scenarios were compared based on different performance metrics, such as energy consumption, environmental impact, and economic viability.

2.2.1. Base case

The base case depicts a sugarcane biorefinery that produces first-generation ethanol, sugar, and electricity, as presented in Fig. 1. The facility, a state-of-the-art distillery, processes 833 tons of sugarcane hourly, retrieving straw from the field (50% of the total yield, approx. 44.79 kg of dry straw per ton of sugarcane (Junqueira et al., 2017)).

Preparing sugarcane and extracting juice involves a dry washing stage and juice extraction using mills. The juice treatment consists of chemical and physical processes that remove most impurities. The cleaned juice is split into two parts, with one portion directed towards ethanol production and the other towards sugar production, representing the production mix. The broth destined for the sugar production stage is concentrated to 60° Brix. It then undergoes downstream processing, which involves a series of cookers, centrifuges, and crystallizers to produce sugar. The molasses from the crystallization process is diluted to approximately 20° Brix using the cleaned juice and then fermented in a fed-batch process with the recirculation of yeast (*Saccharomyces cerevisiae*). The resulting alcoholic solution is distilled and dehydrated with mono-ethylene glycol, yielding anhydrous ethanol. The bagasse is divided into two parts; 95% is directed to the cogeneration unit, while the remaining 5% is stored as a backup. The cogeneration unit produces steam and electricity to fulfill the plant's energy needs, where excess electricity is sold to the grid. Additional details about the process can be found in (Elias et al., 2021). Apart from bagasse, the boiler uses straw recovered from the field as fuel. The heat requirements were reduced through a concurrent pinch analysis conducted alongside the simulations. Please refer to (Elias et al., 2019) for more information. The operating conditions and process parameter assumptions are documented in the supplementary material (Section S.2).

2.2.2. Biofertilizer production annexed to sugarcane biorefinery

In this study, the viability of producing biofertilizer within the context of a sugarcane biorefinery was evaluated by comparing three distinct scenarios. The main differences among the scenarios are based on the biofertilizer production process. However, the scenarios are identical regarding the quantity of biomass consumed, resulting in the same final composition. Of note is that these scenarios were built upon the experimental work previously conducted by our research group and presented in (Favaro et al., 2022). Furthermore, these cases have also been evaluated in a prior publication (Elias et al., 2022) in a stand-alone production plant. More details about biofertilizer production can be found in this literature reference.

The scenarios were designed to cater to approximately 1% of Brazil's biofertilizer consumption, 50,000 tons/year. These biofertilizers primarily consist of phosphate rock, maize starch, and microorganisms. SSF in rice was used for large-scale fungi growth.

The first scenario, referred to as the "Single Microorganism with Extraction step" (SM-WE), involves the separation of *T. asperelloides* spores from the cultivation medium following the SSF step (Fig. 2). The second scenario, named "Single Microorganism with No Extraction step"

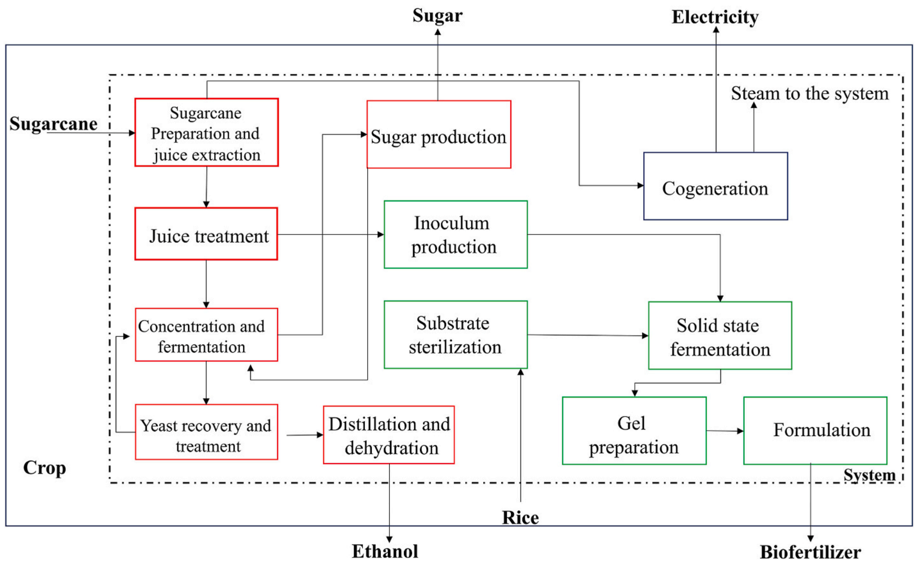

Fig. 3. Simplified diagram of biofertilizer production annexed to a sugarcane biorefinery. **Scenario SM-NE:** Single Microorganism with No Extraction Step.

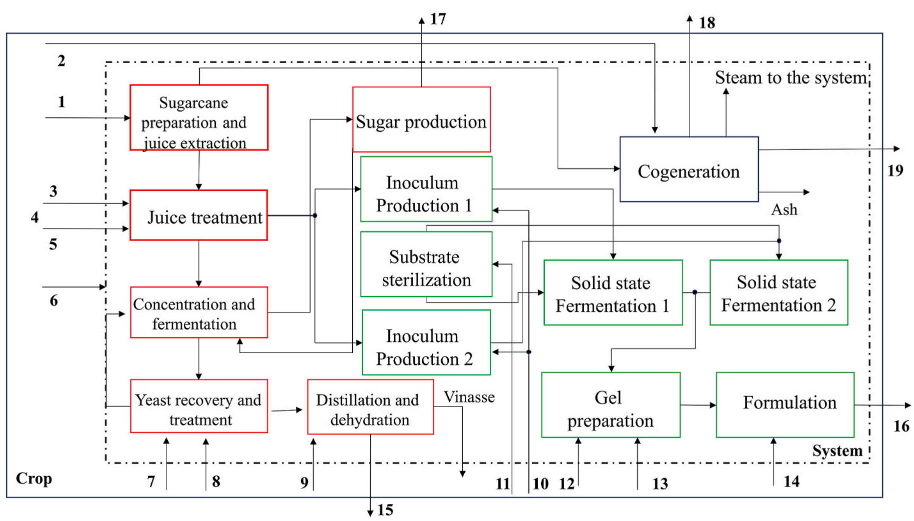

Fig. 4. Simplified diagram of biofertilizer production annexed to a sugarcane biorefinery. **Scenario DM-NE:** Double Microorganism with No Extraction Step.

(SM-NE), utilizes the entire SSF medium, which includes both the spores of *T. asperelloides* and the SSF medium in the biofertilizer formulation (Fig. 3). At last, scenario 3, here referred to as "Double Microorganism with No Extraction step" (DM-NE), employs the entire SSF medium from two distinct fungi, *A. niger* and *T. asperelloides*, which are grown separately (Fig. 4).

The process of inoculum production occurs as a submerged cultivation. The temperature regulation of the aerobic reactor changes based on the fungus type used. In all studied cases, *T. asperelloides LQC-96* was used. For the DM-NE case, *Aspergillus niger C.* was the second fungal strain. Rice, used as an SSF substrate, is sterilized and cooled at room temperature. The cultivation takes place in polypropylene bags, similar to the commercially adopted process for microorganism cultivation. In the SM-NE and DM-NE scenarios, the entire cultivation medium is crushed and forwarded to subsequent steps for composite formulation. The solid-state cultivation of the second microorganism in the DM-NE is analogous to the first fungus. The separation step between the fungus and the culture medium only exists in the SM-WE case, where the solid part residue is directed to the boiler as solid fuel. The encapsulation of the fungus is a similar process in all cases examined. The spores are encapsulated by a gel made up of starch, glycerol, and water. The encapsulation process involves three stages: gelatinization, temperature reduction, and the addition of the concentrated spore solution in the SM-WE case or the crushed growth medium in the SM-NE and DM-NE cases, all while stirring. The procedure entails combining the encapsulated material with processed phosphorus rock. This blend is then homogenized and extruded to create the final product. The operating conditions and process parameter assumption are provided in the supplementary material (Section S.2).

2.3. Economic analysis

The economic assessment considered the effects of the construction and operation of the industrial plant. The sizing of the equipment was determined by the energy and mass balances obtained from EMSO simulations. Capital expenditures (Capex) were estimated based on purchased equipment costs (Peters et al., 2002). The chemical engineering plant cost index (CEPCI) corrected the investments calculated. Operational expenditures (Opex) were estimated based on raw materials costs. The net present value (NPV) was the economic metric selected. Detailed economic assumptions are documented in the supplementary material (Section S.3).

2.4. Environmental analysis

The LCA method used a cradle-to-gate approach. Ecoinvent through the SimaPro 9.0 software provided the datasets of the main inputs. The biorefinery produces ethanol, surplus electricity, sugar, and biofertilizer. To deal with the multifunctionality, economic allocation was used. The life cycle impact assessment (LCIA) was carried out using the CML-IA baseline V3.04 (World, 2000) method. Detailed environmental assumptions are documented in the supplementary material (Section S.4).

It's important to highlight that environmental analysis directly influences economic performance, mainly through the global warming potential indicator. Brazil has implemented a carbon credit policy known as RenovaBio. The method for calculating the global warming potential indicator has been incorporated into the script, along with other equations (Matsuura et al., 2018).

2.5. Global sensitivity analysis

Global sensitivity analysis (GSA) is a technique used to assess how the variation in the output of a model can be attributed or apportioned to the variations in its input parameters or factors (Saltelli et al., 2007). GSA was performed under the factor fixing (FF) configuration, aiming to determine which factors (process variables, x_i) have a non-significant influence on the output variance, that is, the environmental and economic metrics variance. In this work, FAST-RBD was used through the SAlib Python package. The Latin Hypercube Sampling (LHS) method was applied to generate an input space with two thousand points/samples. Detailed data are documented in the supplementary material (Section S.5).

2.6. Uncertainty analysis

This study conducted an uncertainty analysis (UA), considering the uncertainties of the most influential variables identified in the GSA step. This was done for each model output, including ten environmental indicators and one economic metric. Triangular distributions were used for the process variables. To carry out the UA, data sets consisting of 2000 unique samples were collected from each distribution. Detailed data are documented in the supplementary material (Section S.6).

2.7. Retro techno-economic analysis

The traditional TEA approach demands simulating a process with predetermined operating conditions. The data obtained through this simulation is employed to estimate the economic and environmental metrics, which are typically calculated using different software environments. Retro-techno-economic analysis (RTEA) turns the conventional TEA approach upside down. The RTEA methodology unfolds in four distinct phases: (i) base case definition; (ii) integration of the TEA with the process simulation; (iii) identification of key variables via GSA; and, (iv) identification of viable operational region. The base case scenarios correspond to the processes described in Section 2.2. Economic indicators are added into the simulation as additional variables and equations, which the process simulator solves in conjunction with the models of the process units (encompassing mass and energy balances, thermodynamic and physical properties, etc.). The threshold for the economic indicator is set by an additional equation (for example, setting NPV to zero). One of the process variables is freed to maintain a solvable problem with zero degrees of freedom, meaning its value is computed to fulfill the new economic constraint. A detailed description of this methodology can be found in (Elias et al., 2021).

Table 1
Benchmarking process and economic performance obtained in this work (base case, annexed sugarcane biorefinery) against literature.

	This work	Mendes et al. (2017)	Klein et al. (2019b)	Cavalett et al. (2012)
Process performance [a]				
Anhydrous ethanol (L/TC)	47.50	53.00	54.00	
Sugar (kg/TC)	69.80	50.80	52.00	
Surplus Electricity (kWh/TC)	183.30	189.00	106.00	
Economic performance				
Capex (MM US$)	372.50			339.00 [b]

[a] TC – ton of sugarcane.
[b] Capex value was adjusted to same scale production (through the six-tenth rule) and inflation (CEPCI).

Table 2
Process and economic performances of the case studies.

	Base case	SM-WE	SM-NE	DM-NE
Process performance				
Anhydrous ethanol (L/TC) [a]	47.46	47.45	47.45	47.45
Sugar (kg/TC)	69.78	69.77	69.77	69.77
Surplus Electricity (kWh/TC)	183.34	183.01	183.07	183.07
Economic performance				
Capex (MM US$)	372.50	405.56	394.56	395.80
Opex (MM US$)	172.91	212.34	204.85	205.03
NPV (MM US$)	63.49	107.69	150.36	148.57
IRR %	13.18	14.33	15.66	15.59

[a] TC – ton of sugarcane.

3. Results and discussion

3.1. Techno-economic analysis

The annexed sugarcane biorefinery (base case) was compared with other data sources from existing literature, and the findings are displayed in Table 1.

Table 1 presents the base case performance before the integration of biofertilizer production. Notably, the production of ethanol, sugar, and electricity per ton of sugarcane is consistent across all studies used for comparison. Minor variations may occur due to the relationship between ethanol/sugar production, energy integration, and the specific process conditions used in each study. The Capex of the annexed sugarcane biorefinery was also assessed. It's common for Brazilian sugarcane biorefineries to vary their ethanol and sugar production mix based on market price and demand. The investment cost in both scenarios assumes a production mix split of 50%. The findings indicate that the investment costs are comparable in both situations.

The process and economic performances were evaluated to understand the impact of coupling biofertilizer production into the sugarcane biorefinery. Table 2 displays the results for the base case and the integration of the biofertilizer production scenarios (SM-WE, SM-NE, and DM-NE).

Table 2 shows that the process performance remains nearly unchanged after introducing biofertilizer production. This can primarily be attributed to the scale of the process. The base case processes 4 million tons of sugarcane bagasse annually, while the biofertilizer scenarios process 50 thousand tons of biofertilizer. As a result, all material and energy requirements for biofertilizer production are relatively low compared to the base case. The fraction of treated juice diverted to sugar-ethanol production has a negligible effect (less than 1% of the mass stream). The surplus electricity saw a more significant reduction (0.18%, in the SM-WE case) than other process performance indicators. This is mainly due to the electricity and thermal requirements, particularly for phosphorus rock processing and preparations, as well as the

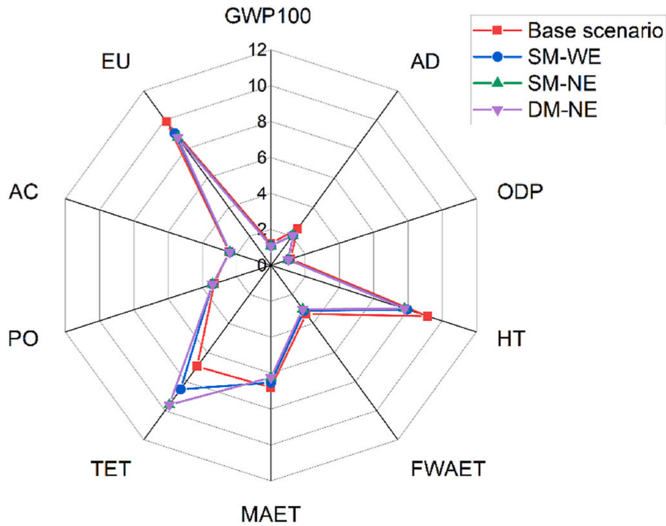

Fig. 5. Midpoint indicators for the scenarios assessed in this work with economic allocation. GWP100: Global Warming Potentials 100 years' horizon, in kg CO_2 eq./US$ ethanol; AD: Abiotic depletion, in 10^3 kg Sb eq./US$ ethanol; ODP: Ozone layer depletion, in 10^7 kg CFC-11 eq./US$ ethanol; HT: Human toxicity, in 10^1 kg 1,4DB eq./US$ ethanol; FWAET: Freshwater aquatic ecotoxicity, in 10^1 kg 1,4DB eq./US$ ethanol; MAET: Marine aquatic ecotoxicity, in 10^{-2} kg 1,4DB eq./US$ ethanol; TET: Terrestrial ecotoxicity, in 10^3 kg 1,4DB eq./US$ ethanol; PO: Photochemical oxidation, in 10^4 kg C_2H_4 eq./US$ ethanol; AC: Acidification, in 10^2 kg SO_2 eq./US$ ethanol; and EU: Eutrophication, in 10^3 kg PO_4^{-3} eq./US$ ethanol.

evaporator used for humidity control of the formulated biofertilizer.

On the contrary, adding biofertilizer production significantly impacts the economic performance, which increased the Capex and Opex by at least 5.92% and 18.47%, respectively. As can be observed, both Capex and Opex are higher than the base case. SM-WE has the highest Capex among the biofertilizer scenarios due to the fungus separation stage, as can be seen in Table 2. The Capex for SM-NE and DM-NE is similar since all the models used in the simulation primarily accomplish mass and energy balances. Therefore, the stoichiometry reaction coefficients are equal for all cases. The main differences lie in the cultivation parameters specific to each fungus, such as residence time and temperature (see supplementary material, Section S.2).

The produced biofertilizer maintains the same primary mass composition across all three cases. The solid phase consists of 70% phosphorus rock and 1.65% microorganisms, with the remainder being starch and/or SSF medium. The liquid phase contains 2% water, with the rest being glycerol. Consequently, the quantity of raw materials varies among the three cases, particularly in SM-WE, where the amount of rice and corn starch is higher than in the others. These values can be found in the supplementary material (Section S.1). As a result, the Opex is slightly higher for the three cases compared to the base case.

A value of 1.10 US$/kg was used as the biofertilizer price. This value was determined in a previous study, as this type of biofertilizer is new to the market; please refer to (Elias et al., 2022) for more details. For comparison purposes, from January to October 2021, Brazil's import of chemical fertilizers exceeded 33 million tons, with an average cost of US $ 0.3 per kg (ComexStat, 2022). In the same timeframe, the country imported over 350 thousand tons of plant growth regulators, including fungicides, herbicides, insecticides, rodenticides, and disinfectants, at an average price of US$9.29 per kg (ComexStat, 2022). This study's 1.10 US $/kg biofertilizer price might underestimate its market value. This is because, besides enhancing soil fertilization, it can also aid in controlling plant pathogens. Therefore, to avoid misinterpretation of the results, it is essential to assess the impact of these uncertainties on the techno-economic analysis.

Despite the rise in Capex and Opex, the NPV of all three scenarios is significantly higher than that of the base case. As shown in Table 2, the NPV increased by 137% in the best scenario, and in the worst scenario, it increased by 69%. As anticipated, the internal rate of return (IRR) is also higher. Even with the minimum attractiveness return rate of 11% used in the simulations (Elias et al., 2021), the positive economic performance validates the feasibility of integrating biofertilizer production into an already running sugarcane biorefinery. All main process parameters, economic assumptions, and prices are detailed in the supplementary material (Section S.3).

The deterministic assessments for the preliminary screening and design of biorefinery concepts have certain limitations. For example, the deterministic evaluation does not take into account market fluctuations and technical variabilities; this potentially leads to the misled selection of optimal process concepts. Consequently, long-term competitiveness and robustness cannot be assured due to the variability, scarcity, and uncertainty of the input information (Gargalo et al., 2016). Therefore, in this work, we investigate this by performing Global Sensitivity Analysis and Uncertainty Analysis (see Sections 3.3 and 3.4).

Notably, under the RenovaBio policy, Brazil has introduced a system of carbon credits, referred to as CBios. The sale of these CBios contributes to the increased earnings of biorefineries. Hence, the economic performance is directly tied to the environmental efficiency of the process.

3.2. Life Cycle Assessment

LCA was conducted for all scenarios to evaluate the environmental performance of biofertilizer production when integrated into a sugarcane biorefinery. The environmental impacts of all cases were estimated, and the results are presented in Fig. 5. As mentioned previously, a biorefinery is a complex multi-product system. Thus, in this work, we have employed economic allocation as the allocation rule to distribute/attribute environmental impacts among co-products based on their economic competitiveness/value (Maga et al., 2019). The primary

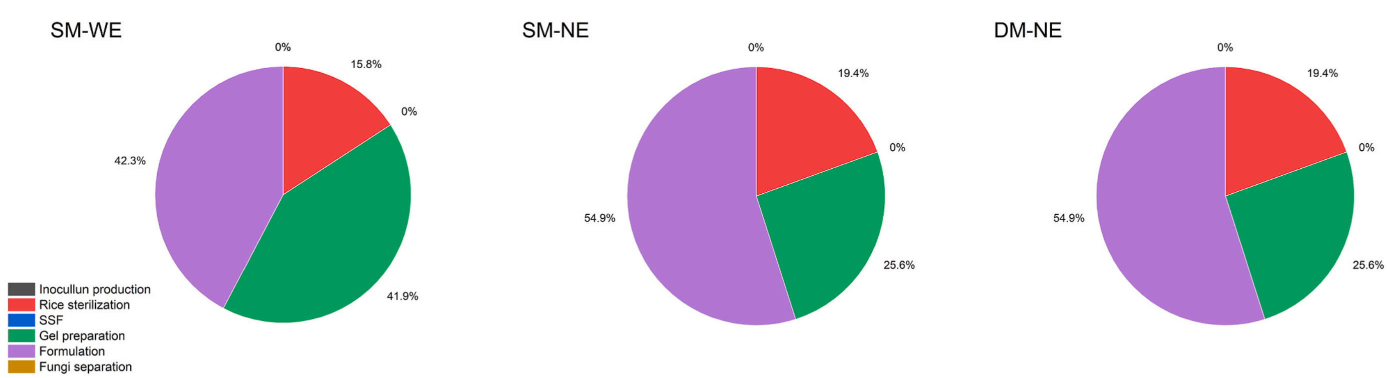

Fig. 6. Share of GWP100 emissions corresponding to each step of the biofertilizer production process.

Table 3
First-order interaction effects for SM-WE scenario.

Variable	GWP100	AD	ODP	HT	FWAET	MAET	TET	PO	AC	EU	NPV
Inoculum production											
Reactor Yx/s	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00
Reaction time in the reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum reactor volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Solid-state fermentation											
Spores proportion (spores per g of substrate)	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00
Reactor Yx/s	0.22	0.19	0.33	0.08	0.04	0.09	0.50	0.16	0.16	0.47	0.55
Reaction time in the reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum mass in each reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Reactor humidity	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.02
Fungal separation											
Efficiency of biomass separation	0.10	0.07	0.15	0.04	0.02	0.04	0.23	0.06	0.06	0.22	0.26
Vessel residence time	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum vessel volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Encapsulation											
Vessel residence time	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum vessel volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Composite formulation											
Vessel residence time	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum vessel volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phosphorus rock in granulated composite	0.63	0.02	0.47	0.87	0.94	0.87	0.16	0.00	0.00	0.22	0.01
Microorganisms in granulated composite	0.05	0.03	0.06	0.01	0.01	0.01	0.11	0.03	0.03	0.11	0.13
Granulated composite humidity	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

product of the biorefinery is anhydrous ethanol. Therefore, all environmental impacts were calculated based on this product.

Fig. 5 illustrates that environmental impacts are generally lower after integrating biofertilizers, except for Terrestrial Ecotoxicity (TET). Terrestrial ecotoxicity is the damage caused by toxic substances that are released into land-based ecosystems. In this case, the elevated TET impact is primarily attributed to the Solid-State Fermentation substrate (rice), maize starch used in gel preparation, and phosphate rock used in the formulation. This is primarily due to using fertilizers and pesticides in rice and starch production, which can have harmful environmental impacts. Additionally, phosphate rock, a crucial source of phosphorus in agriculture, can contribute to TET through the processes involved in its mining and extraction. Furthermore, the excessive use of phosphates in fertilizers and manure can lead to high levels of phosphate in the soil, making it difficult for plant roots to absorb, thereby decreasing soil fertility (Solangi et al., 2023).

As previously discussed, the incorporation of biofertilizer production has led to overall positive economic outcomes, which in turn directly affect environmental performance due to economic allocation. Among the four biorefinery products, revenue from biofertilizers has become the third most significant source of income. Consequently, a reduction in the environmental impact associated with ethanol is expected, given its decreased contribution to the total impact. The factors calculated for economic allocation are presented in the supplementary material (Section S.4).

The RenovaBio carbon credit program serves as an additional revenue stream for the biorefinery. The efficiency score, calculated based on the environmental impact of a reference fossil fuel (gasoline) and biofuel (ethanol), is used to estimate the CBios (carbon credits). The GWP100 category is used for this purpose. Therefore, it is vital to identify the primary sources of CO_2 eq. emissions in the biofertilizer production plant. Fig. 6 illustrates the share of GWP100 emissions for each step of the biofertilizer production process.

As can be observed, and as highlighted for Terrestrial Ecotoxicity (TET), the same pattern remains - rice sterilization, gel preparation, and biofertilizer formulation are the primary sources of CO_2 eq. emissions. Biofertilizer formulation accounts for at least 42% of the total emissions, primarily due to the quantity of phosphorus rock used in the formulation (70% w/w, on a dry basis). This is, as previously mentioned, due to the processes involved in its mining and extraction. Gel preparation contributes to at least 26% of the total impacts, primarily caused by using maize starch and glycerol. The use of glycerol, whether produced via the

Table 4
First-order interaction effects for SM-NE case.

Variable	GWP100	AD	ODP	HT	FWAET	MAET	TET	PO	AC	EU	NPV
Inoculum production											
Reactor Yx/s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reaction time in the reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum reactor volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Solid-state fermentation											
Spores proportion (spores per g of substrate)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reactor Yx/s	0.16	0.12	0.21	0.17	0.19	0.28	0.15	0.08	0.08	0.11	0.60
Reaction time in the reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum mass in each reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
Reactor humidity	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Encapsulation											
Vessel residence time	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum vessel volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Composite formulation											
Vessel residence time	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum vessel volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phosphorus rock in granulated composite	0.57	0.47	0.50	0.56	0.53	0.41	0.60	0.49	0.49	0.98	0.34
Microorganisms in granulated composite	0.04	0.03	0.05	0.04	0.05	0.07	0.04	0.02	0.02	0.02	0.12
Granulated composite humidity	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5
First-order interaction effects for DM-NE case.

Variable	GWP100	AD	ODP	HT	FWAET	MAET	TET	PO	AC	EU	NPV
Inoculum production											
Reactor Yx/s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reaction time in the reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum reactor volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Solid-state fermentation											
Spores proportion (spores per g of substrate)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Reactor Yx/s	0.16	0.12	0.21	0.17	0.19	0.28	0.15	0.09	0.09	0.12	0.60
Reaction time in the reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum mass in each reactor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
Reactor humidity	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Encapsulation											
Vessel residence time	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum vessel volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Composite formulation											
Vessel residence time	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum vessel volume	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phosphorus rock in granulated composite	0.58	0.50	0.50	0.56	0.53	0.41	0.60	0.52	0.52	0.98	0.34
Microorganisms in granulated composite	0.04	0.02	0.05	0.04	0.04	0.06	0.03	0.02	0.02	0.02	0.12
Granulated composite humidity	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

propylene chlorination process or as a by-product of the biodiesel process, indicates the consumption of fossil-based energy. Rice sterilization is responsible for at least 16% of CO_2 eq. emissions due to the heat consumption for the sterilization process. Furthermore, this stage includes the flow rate of rice, which is used as a substrate in the Solid-State Fermentation (SSF) stage, accounting for the emissions attributed to the rice. The other stages/steps do not seem to have a significant impact on CO_2 eq. emissions.

3.3. Global sensitivity analysis

A global sensitivity analysis was conducted across the proposed scenarios to test the robustness of the environmental and economic performances. Given that the annexed sugarcane-ethanol biorefinery is well established, only process variables specifically related to biofertilizer production were analyzed. Out of over 14,000 process variables and 600 specifications in each case, an initial ad hoc selection of variables was made to reduce the inputs covered by the Global Sensitivity Analysis (GSA). Variables related to the performance of the operating unit were chosen based on previous knowledge and experimental findings. Furthermore, to gain a deeper understanding of the system and its market-related variability, the impact of the selling price of the biofertilizer and the cost of raw materials utilized in the production of biofertilizers was also considered (Section 5, Tables S20 to S22). The GSA reveals that the biofertilizer selling price significantly impacts the NPV variance. However, since the price is not a process-controllable variable, these results will be only considered in the uncertainty analysis. Consequently, the next GSA excludes all price-related variables. As a result, a significant number of inputs were chosen for each case: 18 variables for the SM-WE case, 15 variables for the SM-NE case, and 15 variables for the DM-NE case. GSA was conducted applying RBD-FAST, using a sample input of 10,000 points created by LHS (Saltelli et al., 2007). The first-order interaction effects are shown in Table 3, 4 and 5. The parameters used in the GSA can be found in the supplementary material (Section S.5).

The GSA results indicate that most of the selected process variables can be set at the experimental design point. This outcome is linked to the impact on the variance of the outputs, which can be deemed negligible. Furthermore, the findings suggest that research and development efforts should be focused on other, more influential process variables. All scenarios are heavily influenced by variables related to the fungus, particularly the $Y_{x/s}$ of the SSF reaction (grams of microorganisms per gram of substrate). The biomass yield is directly related to substrate consumption. As observed in previous analyses, the SSF substrate impacts both economic and environmental performances, a conclusion that is corroborated by the GSA analysis.

The biofertilizer composite's phosphorus composition impacts economic and environmental performances, particularly in Scenarios 2 and 3. This is due to the quantity of rock used in the formulation. Similarly, the microorganisms in the biofertilizer composition also affect economic and environmental performances. The quantity of fungi in the final product triggers a cascade effect across all preceding stages.

The solid-state fermentation section significantly influences the economic performance of biofertilizer production. Not only does Reactor $Y_{x/s}$ play a role, but the maximum mass in each reactor and the reactor's humidity also impact the NPV variance. In this study, SSF cultivation occurs in bags, a common method used in solid-state cultivation (Mascarin et al., 2019). The mass and humidity affect the number of bags used in production. As this is a continuous production process, an additional number of bags can impact the total size of the structure used for cultivation.

The fungal separation stage is unique to the SM-WE case. As shown in Table 4, the efficiency of biomass separation significantly affects the metrics. The impact is similar to that of biomass yield, as a lower efficiency would necessitate more substrate to meet the formulation specification.

3.4. Uncertainty analysis

Both the economic and environmental deterministic analyses were built based on fixed parameter values, including process performance (conversions, productivities, etc.). These fixed parameters were used to estimate the Capex, Opex, minimum product selling prices, and environmental impacts. However, these fixed parameters are subject to significant variability and uncertainty. Therefore, assigning a range of values and a probability distribution to each uncertain parameter is crucial to assess the risk associated with each biofertilizer scenario. Uncertainty Analysis (UA) simulations consider these uncertainties and provide probability density functions for the economic and environmental metrics (outputs).

As stated previously, at least, each case has over 14,000 process variables and 600 specifications. So, conducting a GSA to identify the most influential process variables before conducting a UA is crucial. The key variables identified for each process were used as inputs to perform the UA on the outputs of interest (economic and environmental metrics). The overall impact of these key input parameters was assessed through the UA based on the information provided by GSA for each process scenario. In addition to the previously conducted GSA, a second analysis was performed. This time, it focused on assessing how raw material costs and the selling price of biofertilizers influence the NPV. The rationale

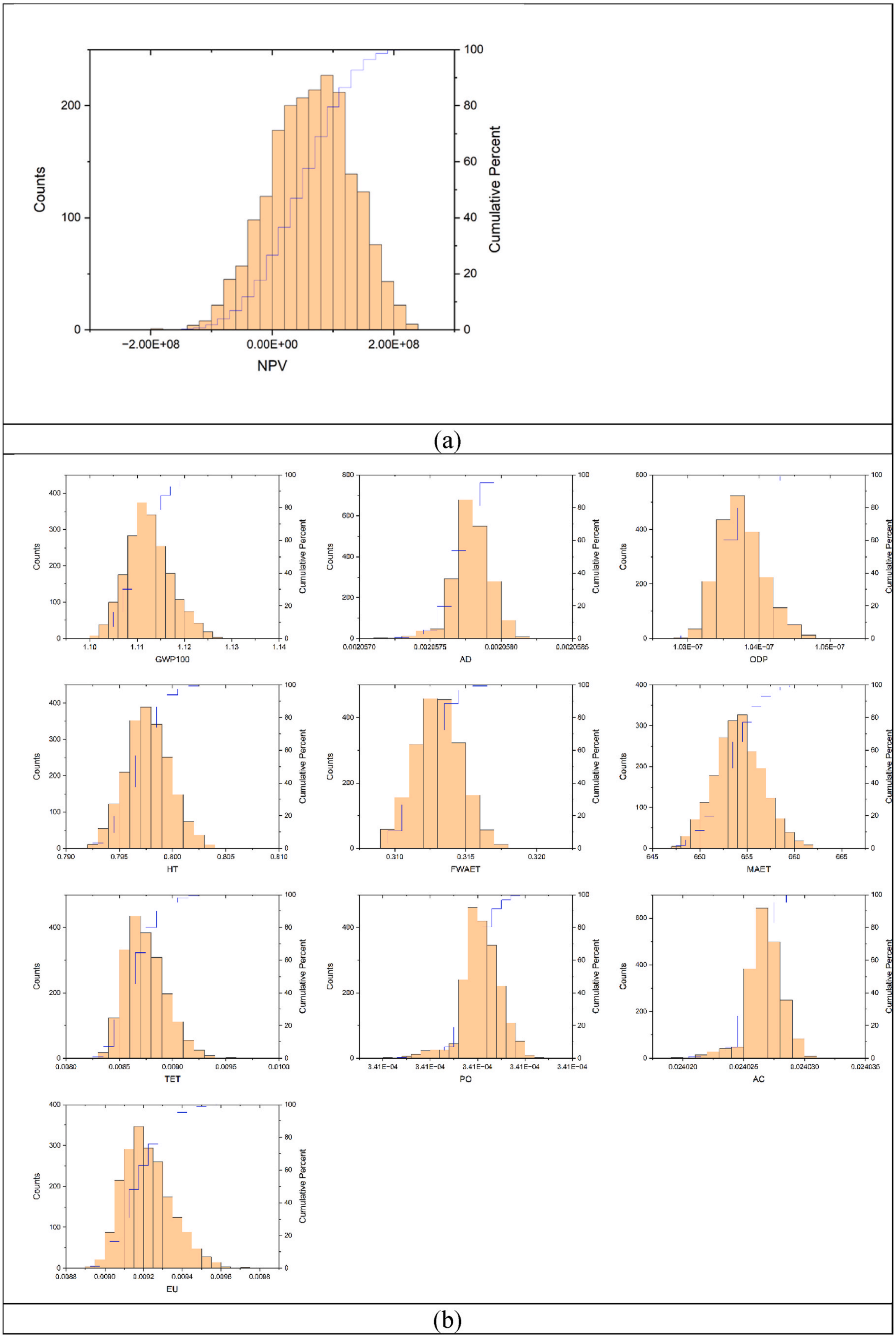

Fig. 7. Uncertainty analysis results of the SM-WE biofertilizer production scenario. The distributions are presented for the (a) NPV and (b) environmental impact categories.

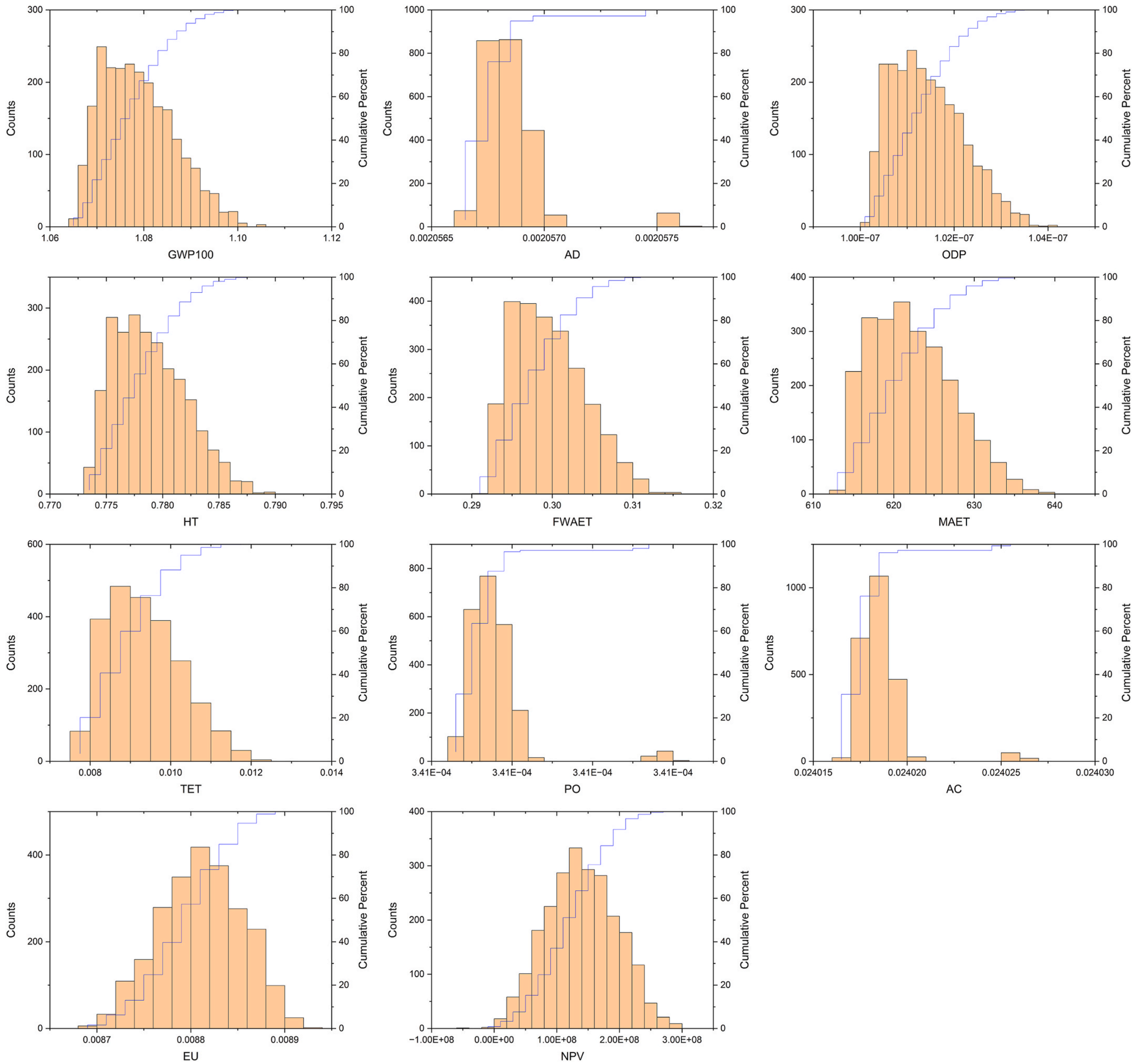

Fig. 8. Uncertainty analysis results of the SM-NE biofertilizer production scenario.

behind this approach was to account for the potential masking effect caused by the magnitude of biofertilizer prices or raw material costs, especially when compared to other variables identified in the GSA The results demonstrate that the biofertilizer's price has the highest impact on the NPV (see supplementary material, Section S.5, Tables S20–S22).

In the UA conducted on this work, process variables, raw materials costs, and biofertilizer selling price were considered. The input variables were assumed to follow triangular distributions (see supplementary material, Section S.6). The model yielded 11 outputs: one economic metric (NPV) and several environmental metrics from the Life Cycle Assessment, including Abiotic Depletion (AD), Global Warming Potential over 100 years (GWP100), Ozone Depletion Potential (ODP), Human Toxicity (HT), Freshwater Aquatic Ecotoxicity (FWAET), Marine Aquatic Ecotoxicity (MAET), Terrestrial Ecotoxicity (TET), Photochemical Oxidation (PO), Acidification (AC), and Eutrophication (EU). The results of the UA for the different biofertilizer scenarios are depicted in Figs. 7–9.

Fig. 7(a) pertains to the economic efficiency of the process, while Fig. 7(b) focuses on the environmental impacts. The NPV distribution is centralized, a characteristic attributed to the shape of the probability density function of the biofertilizer price in the SM-WE scenario (see Supplementary Material, Section S.6). The distribution of economic metric spans over a wide range, from a negative 200 MM US$ to a positive 200 MM US$. This broad range is primarily due to the influence of the biofertilizer selling price. Approximately 57.6% of the samples demonstrated a lower economic performance than the base scenario, with about 26% of the results showing an NPV less than zero. The SM-WE scenario is particularly vulnerable to market fluctuations in biofertilizer sales due to its higher Capex compared to the other scenarios analyzed.

Fig. 7(b), which analyzes the environmental performance, shows probability density functions that mirror those of the economic

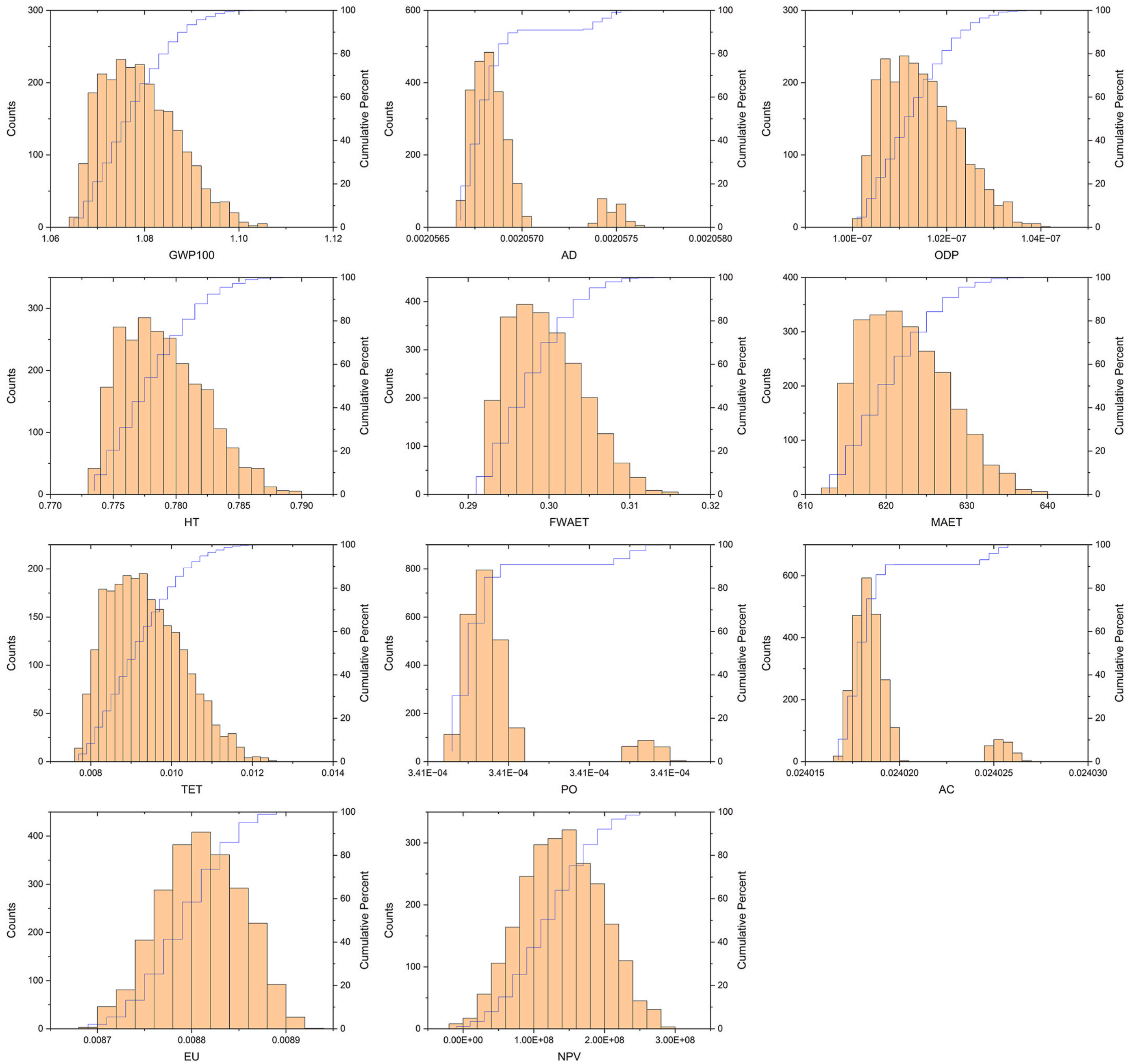

Fig. 9. Uncertainty analysis results of the DM-NE biofertilizer production scenario.

performance. This is primarily centralized due to the dispersion of variables that significantly influence the indicators, particularly the mass proportion of phosphate rock in the product and the $Y_{x/s}$ of the SSF reactor (as can be seen in Table 4).

Performing a comprehensive uncertainty analysis in conjunction with the global sensitivity analysis becomes essential to understand the UA results. For example, the median NPV is slightly skewed to the right. As previously mentioned, the biofertilizer's selling price has the most substantial impact on the NPV, followed by the rice price and the $Y_{x/s}$ of the SSF reactor. Although its influence is minor, the separation efficiency does affect the NPV distribution, as demonstrated by the shape probability density function of the fungus separation efficiencies (Fig. S5, section 6 of the supplementary material).

Figs. 8 and 9 show that the SM-NE and DM-NE scenarios display similar patterns and probability density functions. This pattern, similar to the one observed for the SM-WE scenario, is due to the distribution of the most crucial variables chosen in the GSA step (see in supplementary material, Section S.6). In both scenarios, fewer than 15% of the cases are expected to be below the base case, with less than 1% of the cases presenting an NPV of zero or lower. These two scenarios demonstrate greater resilience to price fluctuations compared to the SM-WE scenario.

In these two scenarios, there is a higher occurrence of left-skewed distributions. As stated earlier, a combined analysis with the GSA suggests that this pattern is attributed to the probability curve linked to the mass composition of the microorganism in the biofertilizer. However, as observed in Table S23 and Table S24 in the supplementary material (Section 6), the triangular distribution linked to this variable showed a centralized mode. A left-skewed distribution was observed upon examination of this variable's distribution (Fig S6 and Fig S7, supplementary material). This pattern is attributed to physical limitations and process specifications. In summary, depending on the point used, there is no convergence in the system of equations that constitute the biorefinery

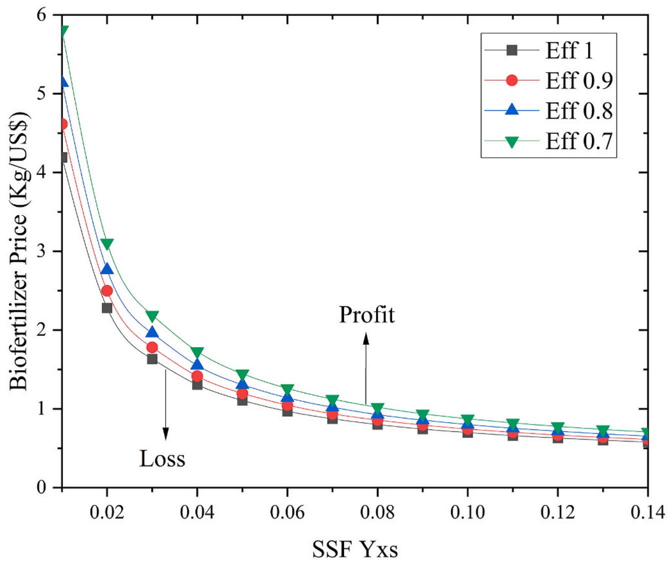

Fig. 10. The selling price of biofertilizer as a function of the SSF $Y_{x/s}$, for different fungus separation efficiencies (Eff) in the SM-WE scenario. The continuous lines represent contour plots of the isometric surface constructed based on the GSA. The profit region (where NPV >63.49 MM US$) is located above these contour plots.

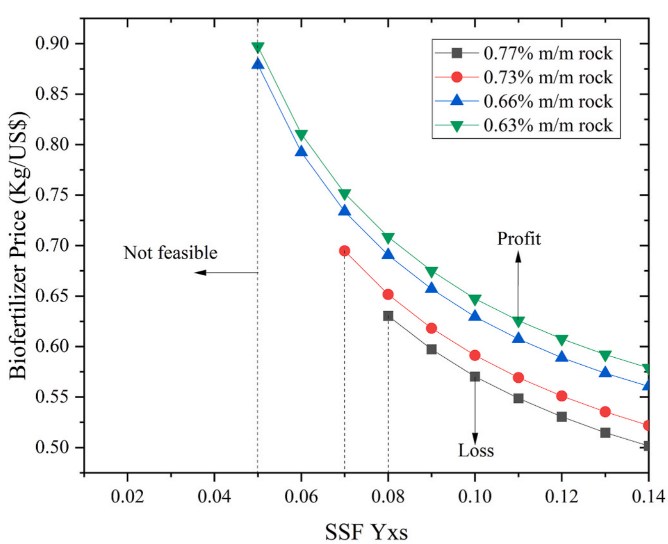

Fig. 11. The selling price of biofertilizer as a function of the SSF $Y_{x/s}$, for different percentages of phosphorus rock in the biofertilizer composition in the SM-NE scenario. The continuous lines are contour plots of the isometric surface built based on the GSA. The profitable region (where NPV >63.49 MM US$) is located above these contour plots. The area deemed unfeasible is due to physical/operational restrictions in the process.

industrial process. This pattern is also observed in the distribution displayed by the AD, PO, and AC indicators.

3.5. Retro-techno-economic analysis

Retro techno-economic analysis involves four stages: (i) setting up a base case, (ii) incorporating the TEA into the process simulation, (iii) pinpointing key variables via GSA, and (iv) delineating the feasible space (illustrated in Figs. 10–12) (Furlan et al., 2016). The equation system that depicts the economic performance is integrated into the simulation. This will be solved by the process simulator along with the process unit models (mass and energy balances, thermodynamic and

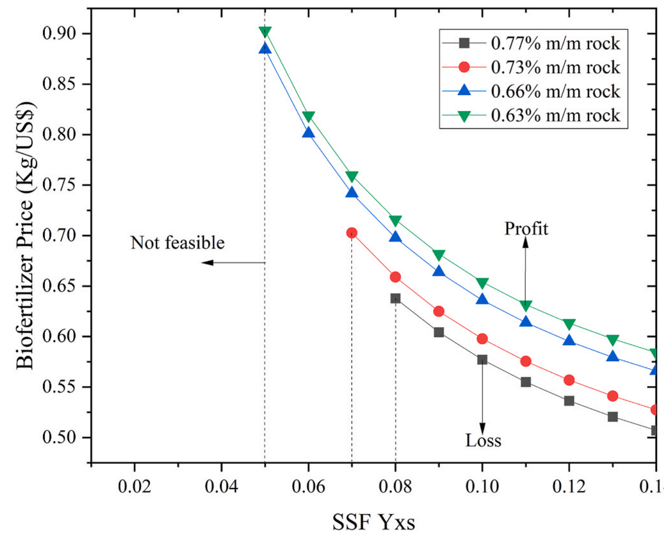

Fig. 12. The selling price of biofertilizer as a function of the SSF $Y_{x/s}$, for different percentages of phosphorus rock in the biofertilizer composition in the DM-NE scenario. The continuous lines are contour plots of the isometric surface built based on the GSA. The profitable region (where NPV >63.49 MM US$) is situated above these contour plots. The area deemed unfeasible is due to physical/operational restrictions in the process.

physical properties, etc.). A benchmark value for the economic metric (NPV) is established as an additional equation, set at 63.49 MM US$ (the same as the base case). A specified process variable must be freed to ensure a well-posed problem with zero degrees of freedom, meaning its value will be computed to satisfy the additional equation. The base case NPV was chosen to identify the region where the biofertilizer production process will not negatively affect the economic performance of the first-generation sugarcane ethanol biorefinery, i.e., for this analysis, we will solely focus on exploring the region in which the system is economically feasible.

A subsequent GSA was conducted, this time integrating the cost of raw materials and biofertilizer sale price into the sample space, as detailed in the supplementary material (Section S.5). The findings suggest that the trading price of the biofertilizer accounts for a minimum of 77% of the unconditional variance in the economic metric (NPV). As discussed previously, the selling price of biofertilizer is, by a significant margin, the most influential variable in its production. However, as price uncertainty arises from exogenous sources, it cannot be optimized by research and development teams. To overcome this challenge, the region where the process leads to an NPV higher than the base case can be obtained using RTEA, that is, calculating the biofertilizer's sales price according to the process's performance with the NPV specified by the base case. This means that the required process performance needs to be determined to ensure that the sales value of the biofertilizer does not adversely affect the NPV.

The RTEA, implemented for each scenario, used the biofertilizer price as the output, modifying the variables that exert the greatest effect on NPV as determined by the GSA. Upon revisiting the first GSA conducted, it was found that SSF $Y_{x/s}$ has the most profound influence on NPV in all cases. This is followed by the fungus separation efficiency (Eff) in the SM-WE scenario and the mass composition of phosphate rock in both SM-NE and DM-NE scenarios.

Fig. 10 illustrates that the economic viability of the process is significantly affected by the $Y_{x/s}$ of solid-state fermentation in the SM-WE scenario. To match the NPV of the base case, a $Y_{x/s}$ of 0.02 and an Eff of 0.07 needs a biofertilizer selling price higher than 3 US$/kg, which is three times the value used in this study.

Notably, a $Y_{x/s}$ greater than 0.07 has a reduced influence on the economic process performance. This is also true for the efficiency of fungus separation (Eff). When $Y_{x/s}$ exceeds 0.07, the isoeconomic curves converge, suggesting that the separation efficiency has a decreased effect on NPV under the studied conditions.

Previous analyses have shown that the SM-NE and DM-NE scenarios behave similarly, as illustrated in Figs. 11 and 12. In contrast to the SM-WE scenario, the mass composition of phosphate rock emerges as the second most influential process variable affecting the NPV variance.

By adjusting the amount of phosphate rock in the biofertilizer composition and the $Y_{x/s}$ of the solid-state fermentation stage, we identified areas where the process is not viable due to physical process limitations.

Hence, it's crucial to emphasize that the biofertilizer production process in the SM-NE and DM-NE scenarios necessitates a lower biofertilizer sales price for economic viability compared to the SM-WE scenario. This holds under identical process conditions across all three scenarios and within the feasible process regions. Therefore, it suggests that the SM-NE and DM-NE scenarios could offer greater economic benefits, particularly regarding production routes.

4. Conclusions

This work has studied three processes for large-scale biofertilizer production in the form of composite granules integrated into a first-generation sugarcane ethanol and sugar biorefinery. The main goal was to perform a techno-economic-environmental analysis of an industrial biofertilizer production facility coupled with a sugarcane ethanol biorefinery.

To this end, PSE tools (e.g., global sensitivity analysis and uncertainty analysis) were employed concurrently with techno-economic-environmental analyses to assess the integration of the different processes and their feasibility.

The economic analysis shows that adding biofertilizer production can increase the NPV of the sugarcane biorefinery by up to 137% in the best-case scenario and 69% in the worst-case scenario. All scenarios show improvement over the base case regarding the environmental impact, except for the terrestrial ecotoxicity potential (TET). The elevated TET is primarily attributed to the substrate (rice) used in the Solid-State Fermentation, maize starch used in gel preparation, and phosphate rock used in the formulation.

The global sensitivity analyses have shown that the stages with the most significant impact on economic and environmental performances are solid-state fermentation and composite formulation. Furthermore, the uncertainty analysis revealed that the single and double microorganisms' scenarios (without an extraction step, SM-NE, and DM-NE) are more stable, robust, and resilient to market price and demand fluctuations. Therefore, these insights encourage industrial biofertilizer production, demonstrating that it could be paramount in transitioning towards a sustainable, low-carbon bioeconomy.

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CRediT authorship contribution statement

Andrew M. Elias: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Carina L. Gargalo:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Cristiane S. Farinas:** Writing – review & editing. **Krist V. Gernaey:** Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2024.143400.

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