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SCIENTIFIC PAPER

UDC

TECHNO-ECONOMIC AND ENVIRONMENTAL ASSESSMENT OF ETHYL ESTER BIODIESEL PRODUCTION

Highlights

- Evaluation of strategies for improving enzymatic biodiesel feasibility.
- Economic unfeasibility of biodiesel production in the absence of enzyme reuse.
- Waste oil yields the best economic and environmental results.

The environmental impact of enzymes in biodiesel production should not be overlooked.

Abstract

Biodiesel is a key fuel for a zero-carbon future. Enzymatic synthesis using renewable materials can make it even more environmentally friendly. However, high enzyme costs and limited reuse hinder its economic feasibility. This study assessed the techno-economic and environmental performance of different processes for ethyl ester biodiesel production. The scenarios evaluated include: transesterification of soybean degummed oil using free and immobilized Eversa Transform 2.0, chemical alkaline catalysis of soybean oil, and transesterification of waste oil using ET. The main metrics were net present value and global warming potential. Results showed that the free enzyme outperformed the immobilized enzyme economically. However, chemical catalysis had an NPV nearly double that of the best free enzyme option. Sensitivity analysis revealed that enzyme cost and reuse rate were critical to net present value. Transesterification of waste oil with enzyme reuse had the lowest GWP (4.21 g CO_{2eq}*/MJ), making it the most environmentally favorable scenario. While life cycle assessment indicated lower global warming potential for enzymatic catalysis, further study is needed on emissions from enzymes. Depending on the enzyme and reuse rate, chemical catalysis might result in lower overall emissions. Integration with the biorefinery makes large-scale enzymatic biodiesel production economically viable and with low* CO_{2eq} *emissions.*

Keywords: Biorefinery, Biodiesel production, Lipase Reuse, LCA, Economic Evaluation, Sensitivity Analysis.

INTRODUCTION

Anthropogenic climate change is affecting the lives of billions of people worldwide. These effects are expected to intensify in the coming years if CO_{2eq} emissions are not drastically reduced [1]. Reducing CO_{2eq} emissions from the transportation sector is essential for achieving a low-carbon economy, as this sector is the most dependent on fossil fuels, accounting for 37% of the CO_{2eq} emissions from the final use sectors [2]. Biodiesel emerges as a promising alternative in this context. It is a renewable biofuel composed of mono-alkyl esters of long-chain fatty acids, produced from vegetable oils or animal fats, with or without

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the use of a catalyst [3,4]. Biodegradable and of low toxicity, biodiesel can replace fossil diesel in internal combustion engines without requiring major modifications [4]. It produces significantly lower emissions of sulfur compounds and hydrocarbons, and its overall carbon footprint is reduced when the full production and use cycle is considered [3,4]. Furthermore, blending biodiesel with diesel is also advantageous, as it facilitates the gradual adoption of renewable fuels within existing infrastructures, improves fuel sustainability, and contributes to the reduction of environmental impacts while maintaining the physical and performance characteristics of the fuel [5]. In light of these advantages, several countries are implementing policies to encourage the use of biofuels, such as the Renewable Fuel Standard (RFS) in the United States, the Low Carbon Fuel Standard (LCFS) in California, the Renewable Energy Directive (RED) in the European Union, and RenovaBio in Brazil, among others [6].

In the Brazilian context, RenovaBio stimulates the decarbonization of the transport sector by increasing biofuels, primarily bioethanol and biodiesel [7]. It regulates the issuance of CBios (Decarbonization Credits), which are tradable financial assets in the stock market, based on the Energetic-Environmental Efficiency Score (EEES). The biofuel EEES is determined through a life cycle assessment (LCA) of the production unit, which evaluates the global warming potential (GWP) impact of the fuel production from cradle-to-wheel, that is, from the raw material production to final use [8].

The transesterification of vegetable oils with methanol or ethanol using alkaline catalysis (NaOH/KOH) is the most common method for industrial biodiesel production [9]. However, LCA studies have indicated a potentially lower footprint when enzymatic catalysis is applied, compared to homogeneous alkaline catalysis [10–12]. Enzymatic catalysis offers simpler downstream steps in biodiesel production, as there are fewer contaminants to be removed at the end of the reaction. This is due to the high selectivity of enzymes, which minimizes side reactions [13]. Consequently, the purification of enzymatic glycerol requires fewer steps to achieve high purity, further contributing to reduced purification costs [14]. Despite these advantages, enzymatic biodiesel still incurs higher manufacturing costs compared to biodiesel via alkaline catalysis [15,16]. The primary reason for this is the cost of the enzyme, along with the longer reaction time required to achieve high conversion rates [16,17].

Due to these economic drawbacks, new production strategies are being explored to enable the production of enzymatic biodiesel. These strategies include: the use of free enzymes, which are less expensive than immobilized ones [18]; the reuse of both free and immobilized enzymes [19], the use of vegetable-based enzymes or cheaper immobilization techniques [20,21]; and the utilization of low-cost raw materials, such as waste frying oil or soybean oil deodorizer distillate, a by-product of soybean oil production [22,23]. Although biodiesel produced through enzymatic catalysis is generally considered to have a lower environmental impact, few studies adequately account for the environmental effects associated with the enzyme itself. Many studies provide limited details or overlook the impact burden of this biocomponent [10-12]. A detailed analysis of this contribution is crucial to ensure the environmental benefits of this catalytic process.

Another strategy to improve the economic feasibility of biodiesel production is its integration into an oilseed biorefinery. Barreiros *et al.* [24] studied the integration of soybean oil extraction and refining with methyl biodiesel production via alkaline homogeneous catalysis. Their study demonstrated an increase in the plant's net present value (NPV), attributed to the profits generated from marketing by-products. Similarly, Granjo *et al.* [25] integrated conventional production of biodiesel into a soybean biorefinery and observed an 18% decrease in production costs compared to the stand-alone process.

Motivated by the promising potential of using biocatalysts to reduce the environmental impacts of biodiesel, we propose, in the present study, to evaluate different previously discussed strategies, such as biorefinery integration, the use of low-cost oils, and the reuse of both free and immobilized enzymes, to enable biodiesel production from an eco-friendly perspective. Additionally, a detailed assessment of the environmental impact associated with enzyme use in the process is carried out. Four different scenarios were proposed and assessed through both techno-economic and environmental analyses. Three scenarios were based on the transesterification of degummed soybean oil integrated into a soybean biorefinery, employing different catalysts: alkaline, free enzyme (ET), and cross-linked aggregates (CLEAs) of ET. The alkaline route served as the base case. The final scenario involved the transesterification of waste frying oil, catalyzed by ET lipases, which served as a stand-alone, low-environmental-impact base case. The study also analyzed the economic and environmental implications of reusing free enzymes and provided a detailed assessment of the environmental impacts associated with enzyme utilization, an aspect often overlooked in enzymatic biodiesel research. Finally, a sensitivity analysis identified key variables that require optimization to enhance the economic viability of these processes.

MATERIALS AND METHODS

Process simulator

The soybean extraction and biodiesel production processes were simulated using the EMSO® simulator. EMSO (environment for modelling, simulation and optimization) is an equation-oriented simulator, which means it does not impose a specific order for solving the system of algebraic, differential, or algebraic-differential equations involved in the simulation [26]. This approach provides greater flexibility in modeling complex chemical systems and enables the simultaneous optimization of multiple operational and design parameters. As a result, it facilitates the identification of optimal process conditions, which improves performance, enhances efficiency, and supports informed decision-making during process development and analysis [27,28]. Economic feasibility and life cycle assessments are obtained simultaneously alongside mass and energy balances, as well as thermodynamic calculations.

Thermodynamic properties

Soybean oil is represented using pseudo-components that consist of a blend of triglycerides, diglycerides, monoglycerides, and free fatty acids (FFA), as reported by Potrich *et al.* [29]. The same methodology was applied to model biodiesel. In the simulation, the vapor phases were assumed to behave as ideal gases, while the liquid phases followed the NRTL (non-random two-liquid) model to account for non-idealities. For more detailed information, please refer to item A of the Supplementary Material.

Oil extraction

The biodiesel production process was modeled as integrated with soybean oil production, following the framework established by Potrich *et al.* [29]. The soybean biorefinery processes the soybeans to extract soybean oil using *n*-hexane as the solvent, while also producing soybean meal, lecithin, and soybean hulls. The degummed oil was subsequently utilized for biodiesel production, and various biodiesel production technologies were assessed.

A cogeneration system powered by sugarcane bagasse supplies both electricity and steam to the biorefinery. Sugarcane bagasse was selected as the boiler fuel due to the proximity of soybean oil, sugar, and ethanol facilities in various locations across Brazil. This setup facilitates the integration of these plants into a single biorefinery, producing ethanol, sugar, degummed oil, biodiesel, and other products. Detailed information about the cogeneration system can be found in item B of the Supplementary Material.

In the context of an oilseed biorefinery, the authors conducted a techno-economic-environmental analysis of different extraction solvents: *n*-hexane, anhydrous ethanol, and hydrated ethanol [29]. Although *n*-hexane is a petrochemical solvent, its impact on CO_{2eq} emissions during the extraction process was found to be the smallest compared to other stages, as it is almost fully recovered and recycled. The transportation and cultivation stages were identified as the primary contributors to environmental impacts. While the study indicated that replacing *n*-hexane with ethanol as a solvent reduced GWP by approximately 10%, this reduction was largely attributed to the increased electric energy produced from sugarcane bagasse to meet the higher steam demand of the process [29]. Conversely, the extraction process using *n*-hexane demonstrated better economic performance compared to other solvents. As a result, the conventional extraction method with *n*-hexane was selected for integration into the biorefinery. Detailed information about the oil extraction process can be found in Potrich *et al.* [29].

Technology options for biodiesel production

Four strategies for biodiesel production were modeled: three scenarios integrated with a soybean biorefinery and an additional scenario using waste oil. The DAT scenario refers to biodiesel production from degummed soybean oil via homogeneous alkaline transesterification. The DFT scenario corresponds to biodiesel production from degummed soybean oil via free enzyme transesterification. In the DIT scenario, biodiesel is produced from degummed soybean oil via immobilized enzyme Transesterification, using CLEA enzyme. Finally, the WET scenario describes the biodiesel production from waste oil via enzymatic transesterification.

Scenarios DAT, DFT, and DIT utilize degummed oil extracted within the biorefinery, while scenario WET focuses on producing biodiesel from waste frying oil in a stand-alone facility. In all scenarios, the biodiesel production was conducted in batch reactors in parallel, ensuring a continuous feed for the downstream section of the process. The following sections provide an overall description of the simulated processes. Additional information about the equipment's operational conditions can be found in Supplementary Material D.

Biodiesel production from degummed soybean oil via homogeneous alkaline transesterification (DAT scenario)

This process is based on the transesterification of neutral soybean oil with anhydrous ethanol using alkaline catalysis. This technology is widely employed in the biodiesel industry and serves as the base case for comparison with other technologies in the study.

Before the transesterification reaction, the degummed soybean oil undergoes a neutralization process. Sodium hydroxide (NaOH) was added to the oil (M101, Fig. 1) to remove FFA. The NaOH partially neutralized the acidic components, forming salts. This neutralization step is crucial for reducing the acidity of the oil and improving the efficiency of the transesterification reaction. Following the neutralization process, the oil passes through a filter press (P101) to remove any sludge that forms as a result of the reaction between NaOH and the acidic components in the oil.

In the biodiesel production process, the reaction occurred in a batch reactor (R101), and the transesterification of neutral oil with anhydrous ethanol (oil/ethanol molar ratio of 1:6) was simulated. Sodium hydroxide (NaOH) was used as a catalyst, with an oil/NaOH mass ratio of 1:0.008, at a temperature of 60 °C and 1 atm for 1 hour [30].

Following the reaction, the resulting mixture was transferred to a decanter (D101), where two phases are formed during 10 h of settling: a glycerol-rich phase and a biodiesel-rich phase. Both phases were then directed to flash tanks (F101 and F102) for ethanol recovery, operating at 130 °C and 0.75 atm, and 122 °C and 0.65 atm, respectively. The ethanol was recovered from the mixture, cooled to a saturated liquid state (C103), and sent to an extractive distillation column (T101).

In the extractive distillation column, glycerol was separated and recycled, while ethanol was purified for reuse in the process. The glycerol used in the extractive distillation column was produced within the biorefinery itself. A fraction of this glycerol, comprising 10%, underwent prior neutralization and is specifically allocated for the purification of anhydrous ethanol. Before its use in ethanol purification, this glycerol undergoes a purification process in the T102 water/glycerol separation column, which is not represented in the flowchart. After the neutralization of the biodiesel process, a centrifuge (C101) was employed to remove any remaining water from the biodiesel. The biodiesel was then subjected to two hot water washes at 90 °C (M103) to eliminate any traces of glycerol, alcohol, acid, base, or soap that might still be present. Following the washing steps, excess water was removed using a centrifuge (C102), and the biodiesel was dried further in a flash evaporator at 117 °C and 0.2 atm (F103). Similarly, the glycerol was also neutralized, and any excess water was removed in a flash evaporator at 122 °C and 0.65 atm (F104) to obtain a purified blond glycerol product. Process effluents are treated before disposal.

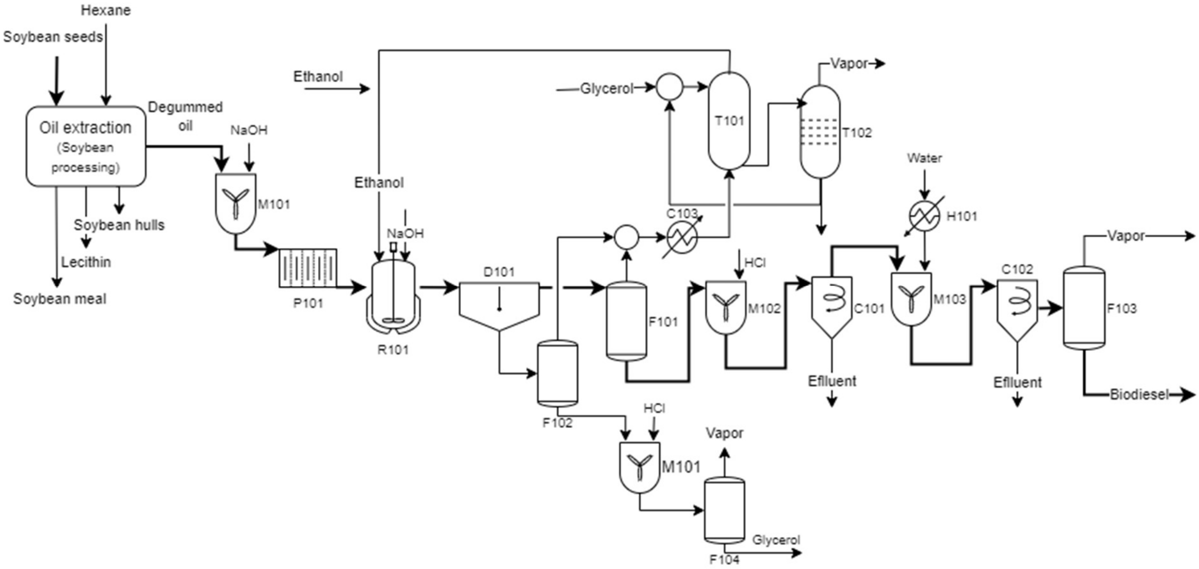

Figure 1. Biodiesel produced by transesterification through homogeneous alkaline catalysis of degummed oil (DAT scenario) in a soybean biorefinery.

Biodiesel production from degummed soybean oil via free enzyme transesterification (DFT scenario)

In the biodiesel production process from degummed soybean oil via free enzyme transesterification with ET, a batch reactor (R101, Fig. 2) was utilized at a temperature of 40 °C and 1 atm. The reaction required an excess of hydrous ethanol, with an oil/ethanol molar ratio of 1:6 and an enzyme load of 5.5% of the oil mass. The reaction time was 12 hours, and both transesterification and hydrolysis of soybean oil were simulated based on the experimental data from Miranda *et al.* [31]. Hydrous ethanol was used because water concentration impacts the performance of the free enzyme reaction, with optimal results observed at a water content of 6.5% (w/w) [31]. After the reaction, the biodiesel was separated for 10 hours in a decanter (D101). Both biodiesel-rich and glycerol-rich phases were directed to flash tanks F101 and F102, operated at 130 °C and 0.75 atm, and 122 °C and 0.65 atm, respectively, for ethanol recovery. The recovered ethanol was cooled to a saturated liquid state and sent to a distillation column (T103) for purification and recycling. The biodiesel underwent further purification through distillation (T101). The bottom stream of the biodiesel purification column was fed to a second column to separate the free fatty acids (T102). The bottom stream of this second column was recycled to the reactor R101, containing most of the unreacted oil. The glycerol phase underwent a second centrifugation step (C101), which concentrated free enzyme in the heavier phase [32]. Approximately 50% of the glycerol was returned to the reactor, enabling the reuse of the enzyme. This practice is facilitated by the post-centrifugation concentration of the enzyme, thereby preventing the accumulation of glycerol in the reactor during reuse. The remaining 50% of the glycerol was sent to a flash drum for ethanol recovery.

The ET enzyme demonstrated the ability to be reused up to three times without a significant loss in activity, achieving a fatty acid ethyl ester yield of over 70% in the ethanolysis of semen abutili seed oil [33]. Although this was a theoretical study, the reuse of the free enzyme was considered for the biodiesel production process. To maintain constant enzyme activity throughout the batches, 10% of fresh enzyme was added to each batch. In this study, the free enzyme was reused ten times. After ten cycles, glycerol was no longer recycled, and the entire reactor contents were processed. The enzyme was not included as a component of the simulation; its volume/mass was accounted for as water. To calculate the enzyme's contribution to economic and environmental viability, 10% of fresh enzyme was added to the total enzyme from the first batch at each reuse. This total was then divided by the number of reuses to calculate the amount used in each batch. Since 10 reuses were performed, the total amount per batch corresponded to 19% of the initial enzyme quantity.

Biodiesel production from degummed soybean oil via immobilized enzyme Transesterification (DIT scenario)

The transesterification of degummed soybean oil with the ET enzyme in the form of CLEAs was conducted in a batch reactor (R101, Fig. 3) at 40 °C and 1 atm, using an excess of hydrous ethanol (oil/ethanol molar ratio of 1:6) and an enzyme load of 11.2% by weight of oil [31]. After the reaction, a magnetic field was applied to retain the CLEA catalyst in the reactor while the reaction mixture was transferred to the decanter (D101). The retained CLEA was washed with tert-butanol at room temperature and remained in the reactor (R101) for subsequent reuse.

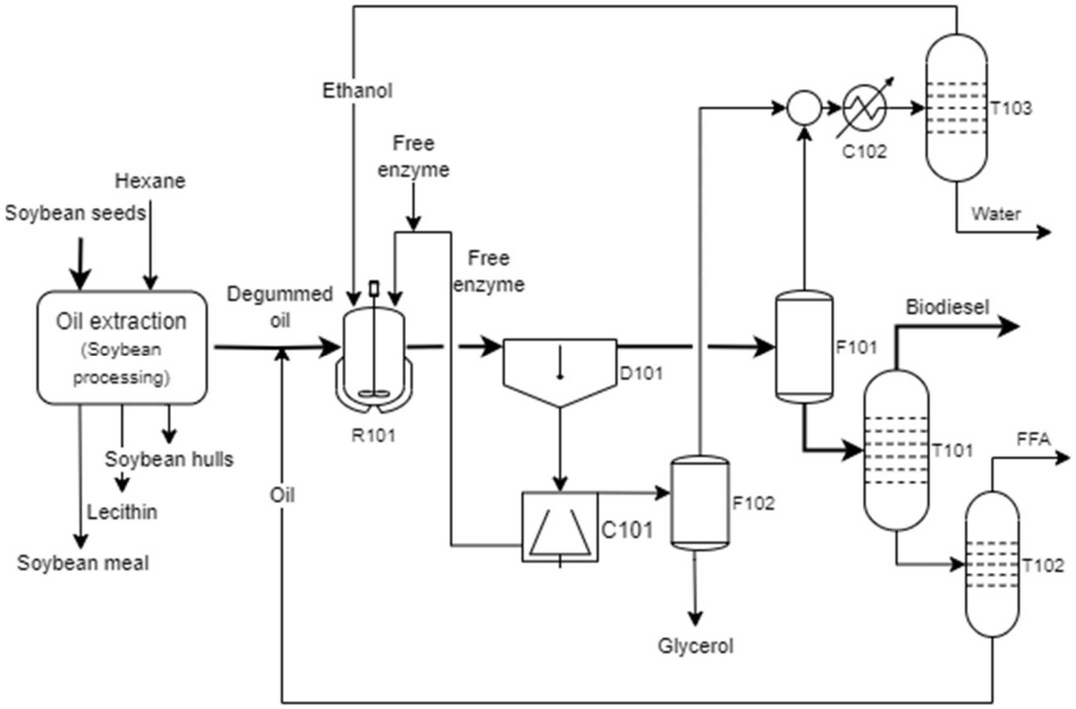

Figure 2. Biodiesel produced by transesterification of degummed oil using free Eversa Transform 2.0 lipase (DFT scenario) in a soybean biorefinery.

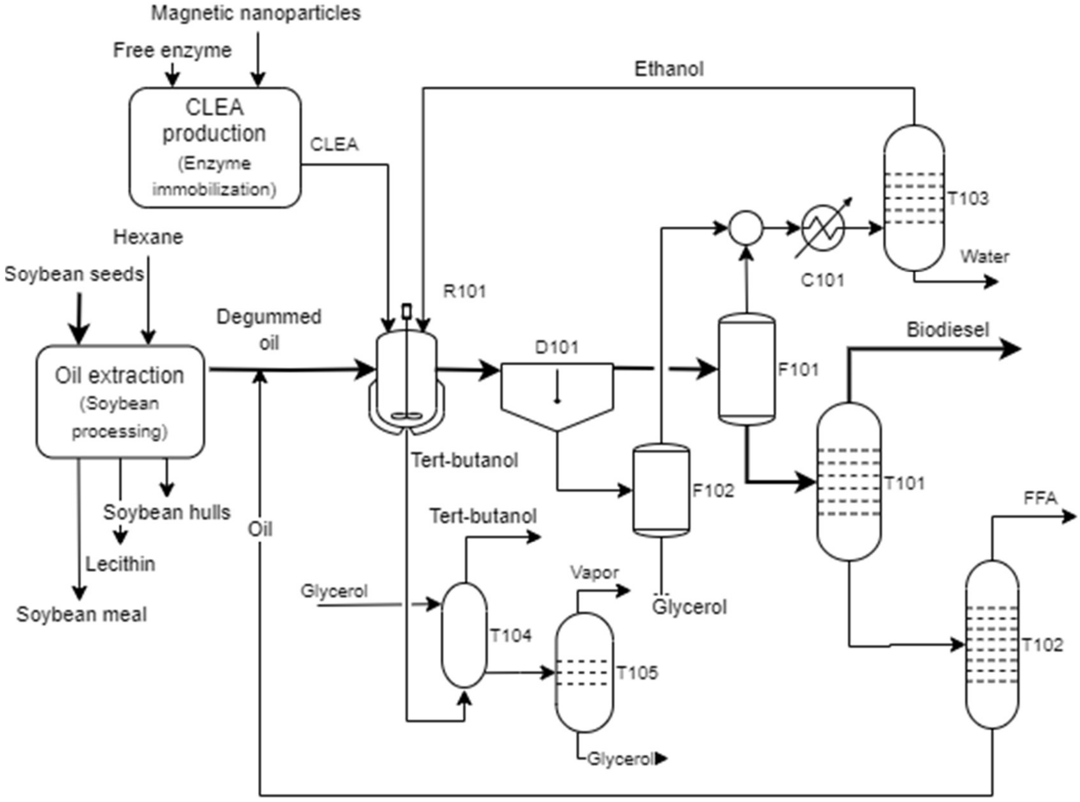

Figure 3. Biodiesel produced by transesterification with Eversa Transform 2.0 in the form of CLEA catalysis of degummed oil (DIT scenario) in a soybean biorefinery.

The enzyme was reused five times in this study [31].

The purification process of biodiesel followed the same procedure described in the DFT scenario, with the exception that all glycerol was directed to the flash (F102, 122 °C, and 0.65 atm) instead of being returned to the reactor. The ethanol and tert-butanol used in the transesterification reaction and in the washing of CLEAs were collected, purified through distillation columns (T103 for ethanol, T104, and T105 for tert-butanol), and recycled. Tert-butanol is in a loop, continuously being purified and returned to the process with a makeup stream to replace what was lost.

Biodiesel production from waste oil via enzymatic transesterification (WET scenario)

In the standalone production of biodiesel, the raw material is waste frying oil. The process closely resembles the DFT process, with the primary difference being the initial step, where waste oil undergoes filtration to remove solid particles. The enzyme reuse strategy, which involves recirculating 50% of glycerol and adding 10% of fresh enzyme load, as well as the purification steps for biodiesel, remains consistent with the DFT process, as shown in Fig. 4.

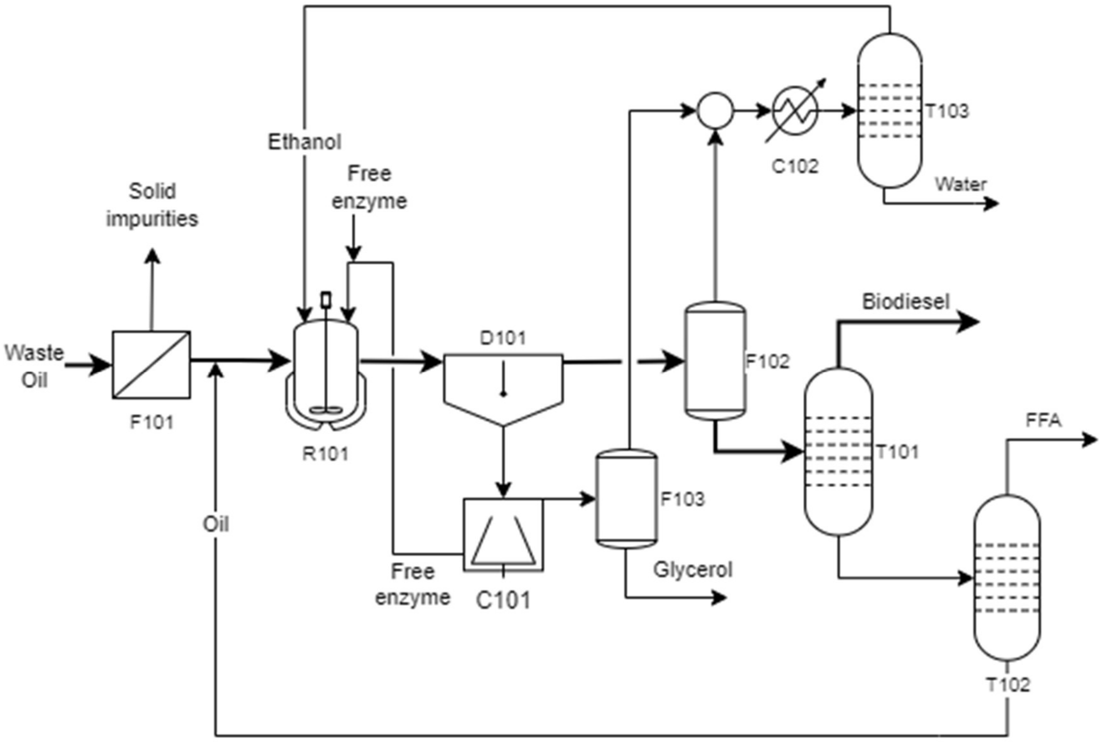

Figure 4. Biodiesel produced by transesterification of waste oil catalyzed by ET (WET scenario).

Unfortunately, there is no specific data available for the transesterification of waste oil with ethanol catalyzed by ET. However, two published studies were referenced for the simulations. Remonatto *et al.* [34] achieved 97% yield in the transesterification of waste oil using methanol and Eversa Transform lipase as a catalyst. The reaction conditions included 2.5 wt% water, 1.5 equivalents of methanol, and 1 wt% of ET enzyme, with a reaction duration of 16 hours. It is important to note that methanol is known to reduce lipase activity and stability, so a higher yield is expected when using ethanol.

In another study, Sun *et al.* [33] produced ethyl biodiesel from semen abutili seed crude oil (SASO), which shares similar characteristics with waste oil as an acidic oil. The transesterification process was conducted at 37 °C, with an oil/ethanol molar ratio of 1:7, an enzyme content of 6% (wt.), and a water mass fraction of 20%. The reaction duration was 11 hours, resulting in a biodiesel yield of 94%. Andrade *et al.* [32] provide insights into the potential performance and conditions for the transesterification of waste oil with ethanol, considering the nature of the oil and the effects of different reaction parameters.

CLEA production

The ET-CLEA was co-aggregated with functionalized magnetic nanoparticles that have amino groups. Its production, as described by Miranda *et al.* [31], was incorporated into the DIT scenario. Consequently, the CLEA production process was integrated into the biorefinery framework. The demands for reagents, energy, and utilities for enzyme immobilization, as well as the sizing of the process equipment, were estimated based on the number of biocatalyst reuses. The economic and environmental feasibility analysis included the production of CLEA. More details on the production of CLEA can be found in the Supplementary Material, section C.

Kriging metamodel

Metamodels, also known as surrogate models, are simpler mathematical relationships that describe the relationship between input and output data of a more complex model [35]. In this work, universal Kriging models (Eq. 1) were employed.

$$\gamma(x) = \mu(x) + z(x) \quad (1)$$

where γ(x) represents the Kriging prediction at point x, μ(x) is the response from the regression model, and z(x) is a zero-mean stochastic stationary random function [35,36]. The methodology proposed by Carpio *et al.* [37], with adaptations, was employed for fitting and validating the metamodels. Metamodels were defined for the oil extraction process and for replacing rigorous thermodynamic equilibrium models. To generate the input data for the metamodel fitting process, a Latin hypercube sampling experimental design was used. The MATLAB DACE toolbox, designed for fitting and validating Kriging models, was utilized in this study [36].

The simulation of the thermodynamic equilibrium models, including the decanters and distillation columns, was conducted using Aspen Plus® software V10. In this study, the liquid phase non-ideality was modeled using the NRTL (non-random two-liquid) model. The NRTL binary parameters used in the simulations can be found in Supplementary Material A. These simulations provided the data for training the Kriging metamodels.

EMSO can communicate with external software through the CAPE-OPEN (computer-aided process engineering - open) communication protocol [38]. This link was used to acquire the data for constructing the metamodels for the oil extraction process, which was modeled in EMSO. A Python program facilitated data acquisition. The regression models μ(x) were a zero-order polynomial for the distillation columns, a first-order polynomial for the decanter, and a second-order polynomial for the oil extraction simulation. All metamodels employed an exponential correlation model for the random function z(x) [38] and were directly implemented in EMSO. More information is available in Supplementary Materials D and E.

Economic feasibility analysis

The equations for evaluating economic feasibility were incorporated into the EMSO simulations and solved concurrently with mass and energy balances, Kriging metamodels, and other equipment-specific equations. The cost estimation for implementing the degummed oil extraction plant was derived from the work of Cheng and Rosentrater (2017) [39]. For CLEA and biodiesel production, equipment costs were sourced from Peters *et al.* [40] and Davis *et al.* [41]. The adjustment of equipment costs based on capacity was conducted using the six-tenths rule, while the impact of temporal price changes (inflation) was accounted for using the chemical engineering plant cost index (CEPCI) [42]. A location factor was applied to correct the cost of equipment for the implementation of a plant in Brazil. Eq. 2 shows the calculation of the location factor.

$$Plant\ cost\ A = Plant\ cost\ \mathrm{B}\ \mathrm{x} \left(\frac{F_{LocA}}{F_{LocB}}\right) \quad (2)$$

where F_{Loc} represents the location factor for the country where the plant is constructed, and the *Plant cost*, the cost of the plant at the specific location. A location factor of 1.14 was used for Brazil, while a factor of 1.0 was used for the Gulf Coast in the United States [43]. The total plant cost was calculated following the methodology described by Peters *et al.* [40]. The economic feasibility analysis was based on the NPV and internal rate of return (IRR). Operational costs for soybean oil degumming and biodiesel production, based on the economic assumptions outlined, are available in Supplementary Material F. The operating costs for oil extraction were estimated using the assumptions and data specified in the extraction sector simulation; further details are also included in the same supplementary material.

Life cycle assessment

Life cycle assessment followed the guidelines of ISO 14040 (2006) /Amd1 (2020) [44] and ISO 14044 (2006)/ Amd2 (2020) [45], as well as the instructions from the technical note of the RenovaBio program [8].

LCA Goal

The goal of the LCA was to compare the impacts of the various biodiesel production scenarios studied. This analysis also facilitates the calculation of the amount of CBios generated within the RenovaBio framework, which influences the economic feasibility of the process. Additional details on the CBios calculation can be found in Supplementary Material G. The intended audience of the study is policymakers, researchers, and technical experts in biodiesel production.

LCA Scope

The system under study includes the agricultural phase of soybean production, soybean oil extraction, and three scenarios of biodiesel production from degummed soybean oil, biodiesel transportation, and use. Or also, biodiesel production from waste soybean oil, transportation, and use.

The environmental metric used was the global warming potential over a 100-year horizon (GWP 100), as defined by the Intergovernmental Panel on Climate Change [46]. The overall methodology adhered to RenovaCalc [8], incorporating a “cradle-to-wheel” scope, attributional methodology, energy-based allocation, and characterization factors based on EcoInvent 3.1 [47]. The functional unit was defined as 1 kg of biodiesel. The characterization factors used are shown in Table H.4 in the Supplementary Material.

Data Sources

The inventory for biodiesel production was constructed using literature sources and derived from mass and energy balances in the simulation. Data for the agricultural phase, soybean oil extraction, and ethanol production data were obtained from Potrich *et al.* [29], who also employed the RenovaCalc tool. Details on the inventory for the lipase enzyme and CLEA are presented in the section “Impact of Lipase and CLEA Production” and further detailed in Supplementary Material H.

For the biodiesel production phase, the life cycle inventory (LCI) was based on the inputs and outputs from the biorefinery simulation. In the biodiesel distribution phase, a transportation distance of 700 km from the production site to the fuel station was considered, as per RenovaBio standards. Some adjustments were made to the RenovaBio methodology, as the current version does not account for the impacts of the enzyme used in biodiesel production. Additionally, FFAs were considered a by-product, with a corresponding energy allocation of part of the emissions to this product. The calorific values of the components are detailed in Supplementary Material I.

In the LCI for biodiesel derived from waste oil, it was assumed that these triglyceride sources do not contribute to CO_{2eq} emissions. These process streams were regarded as waste, with no emissions allocated to them – only to the main products. Emissions associated with the collection and transportation of waste oil (30 km, 9.72×10^{-4} g CO_{2eq}/kg waste oil) were included in the inventory, as well as those from bagasse combustion in the cogeneration system. However, biogenic CO_2 emissions from bagasse combustion were excluded, in accordance with the IPCC (2006) methodology.

Impact of Lipase and CLEA Production

The LCA of the proposed biodiesel production process must account for the environmental impact of lipase in both free and immobilized forms. Due to a lack of available information on the environmental impacts specifically related to lipase, production processes were developed for both forms to provide a basis for these calculations. While these proposed processes may not perfectly represent commercial lipase production, they offer a more comprehensive approach than ignoring enzyme impacts entirely. The LCI inputs were derived from the study of Raman *et al.* [11], with adjustments made to replace palm oil and corn protein with soybean oil and soybean protein, respectively, to better align with the Brazilian context. The methodology for enzyme immobilization follows the process detailed in section "CLEA production". For further insights into the inputs and outputs involved in calculating CO_{2eq} emissions during biocatalyst production, please refer to the Supplementary Material, Section H.

Sensitivity Analysis of NPV Results for Enzymatic Biodiesel Processes

The first-order sensitivity indices of the NPV results for various enzymatic biodiesel process scenarios were calculated using the random balance designs, the Fourier amplitude sensitivity test (RDB-FAST) method [48]. This method is a variance-based global sensitivity analysis that offers easier implementation and lower computational demand compared to Sobol's method, making it suitable when only first-order sensitivity indices are of interest [49]. These ranges were derived from experimental data for ET in both free and immobilized forms, focusing on reaction conditions that promote economic feasibility, as documented in previous studies. For instance, the standard cost of ET used in this work was 15.00 USD/kg. However, considering that alpha-amylase, a significant commercial enzyme, is priced at 6.4 USD/kg [50], this amount was established as the lower boundary for enzyme pricing. Reaction conditions for other lipases were selected from a survey of recent publications by Cavalcante *et al.* [13], which highlighted a maximum conversion of 95.1% achieved within 2 hours, while the minimum conversion noted was 85.6% for the base case using free ET. The range of selectivity observed in experimental data for the ET enzyme informed the defined conversion interval for the hydrolysis reaction that occurs concurrently with transesterification. The minimum time required to reach 80% oil conversion is noted as 4 hours, the maximum is 12h. The total reactor volume was estimated based on reaction time, along with filling, emptying, and cleaning durations. Since reactor volume influences the capital costs of the process, reaction time has an indirect effect on these costs as well. The temperature range considered for achieving over 80% yield within 12 hours was established between 30 and 55 °C. The lowest oil/alcohol molar ratio that resulted in more than 80% oil conversion within 12 hours was set at 1:4, while the maximum ratio was aligned with conditions studied in the base case (alkaline catalysis). The maximum reuse of the free enzyme was based on the experimental data, indicating that ET lipase could be reused up to twelve times [51], while for CLEA, a reuse limit of 50 cycles was selected. The sensitivity analysis was performed in Python using the SALib package, with a total of 9000 data points. More details are available in Supplementary Materials J.

RESULTS AND DISCUSSION

Technical Results

Process inputs and outputs are shown in Table 1. The simulations indicate that all scenarios yield fuel-quality biodiesel conforming to international standards, with all enzymatic processes generating FFA as a byproduct. Enzymatic catalysis produces glycerol with 96 wt% purity, compared to 80% for alkaline catalysis, requiring fewer purification steps. In the DAT, DFT, and DIT scenarios, biodiesel production was integrated with soybean oil extraction in a biorefinery, with degummed soybean oil directed toward biodiesel production. In the WET scenario, the amount of waste frying oil in São Paulo City, Brazil, was used as a reference, estimated at 12.1 t/h [52].

Regarding the volume of biodiesel, FFA, and glycerol, there has been little variation in performance among the options. Table 1 shows the relative values of the three main products (biodiesel, glycerol, and fatty acid) for all scenarios. The DFT, DIT, and WET scenarios achieved the highest volume of biodiesel produced per kilogram of oil, despite having a lower conversion rate compared to alkaline catalysis (96%). In these scenarios, the unreacted oil after biodiesel distillation is recycled back to the reactor, reducing process losses and improving overall biodiesel production. Among the scenarios, DIT has the highest glycerol production, followed by DFT and WET. Conversely, the DAT scenario uses water for glycerol neutralization and washing, resulting in a higher loss of glycerol during the purification process. Although the mass fraction of glycerol dissolved in water is low, the large volume of water used negatively impacts the glycerol yield per kilogram of processed oil.

In the biorefinery scenarios (DAT, DFT, and DIT), surplus energy generated during the cogeneration process can be sold to the grid. The DIT scenario demonstrated the highest energy production due to its higher steam demand. The use of immobilized enzymes in the enzymatic processes contributes to this increased energy and utility demand, primarily due to the CLEA production unit and the tert-butanol recovery step used for CLEA washing. This heightened demand for utilities results in increased biomass burning and, consequently, greater production of bioelectricity. In contrast, the DAT scenario exhibited the lowest energy production, as it requires fewer utilities due to its less energy-intensive biodiesel washing purification method.

Table 1. Main biorefinery streams of inputs and products, along with the specific production of biodiesel, glycerol, and free fatty acid per kilogram of oil processed in the different scenarios.

		DAT	DLT	DIT	WET
Input	Soybean (kg/h)	125000.0	125000.0	125000.0	0.0
	n-Hexane (kg/h)	122.8	122.8	122.8	0.0
	Oil (kg/h)	23470.4	23470.4	23470.4	12100.0
	Ethanol (kg/h)	3543.8	3883.5	3885.4	1945.0
	Water (kg/h)	21681.5	1669.1	116874.5	324.4
	Sodium hydroxide (NaOH) (kg/h)	189.9	0.0	0.0	0.0
	Hydrochloric acid (HCl) (kg/h)	1080.1	0.0	0.0	0.0
	Enzyme (kg/h)	0.0	37.2	2661.5	10.6
	Sugarcane bagasse (kg/h)	25011.2	25113.6	50147.4	0.0
	Energy (kW/h)	3830.9	4141.4	4059.2	408.7
	Cold Utilities (kW/h)	-10759.9	-44054.7	-57565.6	-17650.5
	Hot Utilities (kW/h)	29027.3	29469.3	52664.6	6403.0
Product	Meal (kg/h)	91546.9	91546.9	91546.9	0.0
	Oil (kg/h)	0.0	0.0	0.0	0.0
	Lecithin (kg/h)	1530.4	1530.4	1530.4	0.0
	Hulls (kg/h)	8337.5	8337.5	8337.5	0.0
	Biodiesel (kg/h)	22071.4	24994.7	24040.2	12267.8
	Glycerol (kg/h)	1541.6	2146.5	2207.2	1059.3
	Fatty acid (kg/h)	0.0	368.9	575.8	589.8
	Electricity (kW/h)	3519.4	1951.4	7890.3	0.0
Specific	Biodiesel (kg/kg)	0.940	1.065	1.024	1.014
	Glycerol (kg/kg)	0.066	0.091	0.094	0.088
	Fatty acid (kg/kg)	0.000	0.016	0.025	0.049

Economic Results

Among the different scenarios for biodiesel production, the main economic results are presented in Table 2. The DIT process is associated with the highest total capital cost, followed by the DFT. The production of biodiesel from waste oil (WET) requires less investment compared to other processes, as the volume of processed oil is only 51.5% of that used in the degummed oil process. However, when considering the specific total capital investment cost per kilogram of biodiesel, the CLEA case (DIT) has the highest value at 4601 USD/kg. The DFT process has a specific total capital investment cost of 3124 USD/kg, while the WET process has the lowest value at 864.7 USD/kg.

When comparing DAT, DFT, and DIT, which use the same source of triglycerides (degummed oil), the implementation cost of enzymatic catalysis is higher than that of alkaline catalysis. This increased cost is primarily due to the longer reaction times associated with enzymatic processes, which require larger reactor volumes and, consequently, higher reactor costs [17]. Additionally, the costs associated with the immobilized enzyme process are influenced by the enzyme preparation unit and the tert-butanol recovery unit. Granjo *et al.* [25] estimated an investment cost of 38 million dollars for a soybean biorefinery that produces soybean meal, $NaOCH_3$, glycerol

(92% purity), soybean oil deodorizer distillate, filtrate cake, NaCl, soybean lecithin, and methyl biodiesel catalyzed by $NaOCH_3$. This investment cost was adjusted for a production of 190 million tons per year of biodiesel, and the values were updated using the chemical engineering plant cost index (CEPCI), resulting in an updated cost of 43 million dollars, which is 26% lower than the DAT scenario. The cogeneration system accounts for 13–40% of the equipment costs in the biorefinery proposed in this work; however, it also generates surplus bioelectricity that can be sold to the grid.

Karmee *et al.* [16] estimated an investment cost of 2.7 million dollars for biodiesel production from waste oil via enzymatic catalysis of the transesterification reaction using an immobilized enzyme. This value was corrected for a production capacity of 87 million tons per year of biodiesel, and the values were updated via the CEPCI, resulting in 12.278 million dollars, which is 15% higher than the cost presented in the WET scenario.

The production costs reflect a similar trend to the total investment of the plant, with the highest costs associated with the DIT scenario, followed by DFT, and the lowest costs with WET. In biorefineries focused on biodiesel production, the low production cost for the DAT scenario allows it to achieve the highest NPV, as shown in Table 2. Although DAT has a lower total production cost, the costs associated with DAT and DFT are similar when calculating the cost per kilogram of biodiesel produced. Specifically, DAT is only USD 0.18 more expensive, given that the DFT scenario produces a higher volume of biodiesel. However, the lower revenue for DAT is justified by the reduced volume of products generated in this process compared to the other cases, as there is no FFA production.

The NPV of the DIT scenario is negative due to the high cost of producing the CLEA, as illustrated in Table 2. The DIT case study considers five reuses of CLEA based on the experimental data. For comparison, to achieve a positive NPV, this number would need to increase to at least 123 reuses. Given the low added value of biofuels and the high cost of immobilized enzymes, studies have shown that even with a high number of enzyme reuses, this catalysis technology is not economically competitive compared to other catalysts [15,16]. Techno-economic assessments of processes using Novozymes 435 enzyme, which offers higher conversions in the transesterification reaction of oils and the potential for 100 reuses, still do not demonstrate economic feasibility [16].

The WET scenario presented the highest IRR, followed by DAT, as shown in Table 2. Although WET does not have the largest NPV, it represents the best investment for biodiesel production. This is because the CAPEX for a waste oil biodiesel plant is only 14% of that required for the DFT biorefinery, while still achieving an NPV that is nearly half of that for the DAT scenario. The low cost of raw material, combined with enzymatic catalysis that simplifies purification due to the higher purity of the resulting product, ensures favorable economic performance for biodiesel produced from waste oil. Furthermore, the NPV of scenarios utilizing enzymatic catalysis can increase significantly if the enzyme can be reused multiple times. However, a conservative estimate of enzyme reuses was assumed in this analysis. If the enzyme proves to be even more effective and can be reused even more times, the NPV for scenarios employing enzymatic catalysis would rise further.

According to the Brazilian Association of Vegetable Oil Industry (Abiove), the consumption of edible vegetable oil in Brazil reached 8.7 million tons in 2023 [53]. Of all the oil

Table 2. Economic performance results.

	DAT	DFT	DIT	WET
Total equipment cost (MUSD)	32.62	43.46	61.97	5.50
Total capital investment cost (MUSD)	58.48	78.08	110.62	10.61
Total Production cost (MUSD)	493.35	521.45	1435.19	87.45
Total Ran Material (MUSD)	472.43	489.20	1200.64	64.44
Enzyme cost (MUSD)	0.00	34.41	741.68	16.04
Utilities cost (MUSD)	20.92	32.24	234.55	23.01
Total Revenue (MUSD)	506.91	537.94	535.79	91.10
Biodiesel Revenue (MUSD)	133.34	150.99	145.23	74.12
Glycerol Revenue (MUSD)	3.55	12.29	12.64	6.06
Meal Revenue (MUSD)	324.95	324.95	324.95	0.00
Lecithin Revenue (MUSD)	11.03	11.03	11.03	0.00
Hull Revenue (MUSD)	26.61	26.61	26.61	0.00
Fatty acid Revenue (MUSD)	0.00	4.16	6.49	6.65
Electricity Revenue (MUSD)	1.01	0.56	2.26	0.00
CBios Revenue (MUSD)	6.43	7.35	6.51	4.27
Net Present Value at 11% (MUSD)	13.50	10.95	-6071.22	7.65
Internal Rate of Return (IRR)	13.96%	12.74%	-	19.38%

used in food preparation, only 25% becomes waste, as many cooking techniques do not produce residual product [54]. Therefore, Brazil generates approximately 2.18 million tons of residual oil. If all this waste oil were transformed into biodiesel, it could account for 32% of the country's biodiesel production (6.8 million tons in 2023) [55]. However, as of November 2023, only 1.32% of Brazilian biodiesel was derived from used frying oil [55].

Life Cycle Assessment

Surveying the environmental impacts of all the inputs in the studied process is essential to conducting a reliable LCA. However, some studies overlook the impacts of enzymes in the LCA of biodiesel [9,11], often due to their classification as biocatalysts and the potential for reusing immobilized enzymes. In the following section, the impacts resulting from the production of free lipase and immobilized lipase in the form of CLEA will be calculated. These values will be utilized for the LCA of biodiesel.

Life Cycle Assessment of Microbial Lipase and CLEA

The LCA of free lipase was based on the study presented by Raman *et al.* [11] and detailed in the DFT scenario. The LCI for the enzyme immobilization process was derived from the simulation described in section CLEA production. The free enzyme has a GWP100 of 2.273 kg CO_{2eq}/kg of lipase, while immobilized lipase emits 26.578 kg CO_{2eq}/kg of CLEA. The ability to reuse the immobilized biocatalyst results in a reduction in GWP100 per batch. For the base case in the DIT scenario, with five reuses, the GWP100 was 5.315 kg CO_{2eq}/kg of enzyme. In contrast, using the free enzyme for 10 reuses would reduce the GWP100 to 0.528 kg CO_{2eq}/kg, emphasizing the need for fresh enzyme among the resources. More details regarding the LCA of free and immobilized enzymes can be found in Supplementary Material H.

Life Cycle Analysis of Biodiesel Production

The LCA of biodiesel production reveals that the largest contribution to GHG emissions is associated with soybean production, as shown in Table 3. The GWP100 values are 17.628 gCO_{2eq}/MJ for DAT, 17.013 gCO_{2eq}/MJ for DFT, 22.483 gCO_{2eq}/MJ for DIT, and 4.207 gCO_{2eq}/MJ for WET. Previous studies indicate that enzymatic biodiesel production generally results in lower GHG emissions compared to the alkaline homogeneous catalysis [9-11]. However, many of these studies did not account for the impacts of the enzyme itself. As Table 3 demonstrates, the biocatalysts contribute significantly to CO_{2eq} emissions in the process.

Furthermore, the alkaline homogeneous catalyst (DAT) not only has lower emissions (512.5 g CO_{2eq} /kg NaOH) compared to free lipase, DFT (2273 g CO_{2eq} /kg enzyme), but it also requires a smaller volume of catalyst per kilogram of biodiesel produced. In comparison, the LCAs of the different processes using degummed oil, scenarios DAT, DFT, and DIT, show that the lower CO_{2eq} emissions in the DFT scenario result from the downstream process employed for biofuel produced via enzymatic catalysis. The distillation method for purifying biodiesel showed a lower GWP100 due to its fewer processing steps, which do not involve chemicals or large volumes of water for washing the biodiesel.

The worst performing scenario in terms of environmental impact is DIT, primarily due to the use of immobilized enzymes with few reuses. However, increasing the number of reuses would reduce this impact. Among all scenarios, the WET scenario exhibits the lowest GWP100, as waste oil does not incur a CO_{2eq} input associated with its production. The CO_{2eq} impact mainly arises from the transportation of the raw material.

Cavalett and Ortega [56] and Fernandez *et al.* [10] have also studied the environmental impacts of biodiesel production in the Brazilian context. Their findings indicated GWP100 values of 977 g CO_{2eq}/kg for ethyl biodiesel and 1775 g CO_{2eq}/kg for methyl biodiesel. According to Fernandez *et al.* [10], discrepancies in GWP100 values between studies can be attributed to differences in inputs and outputs during the transesterification phase, including data from U.S. biodiesel plants, transportation factors, and energy requirements.

The lower GWP100 values presented in this study, compared to others, can be partly attributed to the use of ethanol instead of methanol. Methanol has a GWP100 of 560 g CO_{2eq} /kg, while ethanol's value is 547.1 g CO_{2eq} /kg, according to the EcoInvent 3.1 database. Additionally, Cavalett and Ortega [56] used diesel as input for the crushing process and biodiesel production. In this biorefinery context, our study employs sugarcane bagasse as fuel for the cogeneration of steam and bioelectricity. This choice significantly lowers the impact of meeting the process's energy demand. The surplus electric energy produced, which is another product of the system, minimally influences the overall results, with its impact accounting for less than 0.1% of the total impact. Table 3 reports the annual quantity of CBios within the Brazilian framework. The commercialization of CBios provides an additional financial contribution. In the scenarios evaluated, the revenue derived from carbon credits under the RenovaBio program accounted for approximately 1-4% of the total revenue.

Sensitivity Analysis

The sensitivity analysis was conducted exclusively for the enzyme-based scenarios (DFT, DIT, and WET), since the aim is to improve these processes. The RDB-Fast method provides only first-order sensitivity indices, which are sufficient to identify the main variables influencing the NPV. If the sum of the first-order indices approaches one, the combined effects of higher-order indices (second, third, etc.) can be considered negligible. For the three biodiesel production technologies analyzed, at least 96% of the total sensitivity can be attributed to first-order effects.

Previous studies have shown that the primary barrier to enhancing the profitability of enzyme-based processes is the operational cost of enzymatic biodiesel, with the enzyme itself representing a significant portion of these costs, as illustrated in Table 2. Both a decrease in enzyme price and an increase in the number of reuses positively contribute to the NPV, highlighting these as the two most impactful factors.

The sensitivity analysis results for the DFT and WET scenarios are quite similar, as their biodiesel production and purification configurations are alike (see Table 4). The main differences lie in the reaction conditions for each scenario. While transesterification conversion also influences these scenarios, its impact is less significant than that of reaction time. In contrast, the DIT scenario is dominated by a single first-order effect variable: enzyme reuse, which accounts for 98% of the output variance. Due to the high costs associated with CLEA production, the influence of enzyme pricing is minimal in the DIT process. Thus, CLEA reuse emerges as the only variable with a significant first-order effect capable of reducing biodiesel production costs using this technology. Therefore, enhancing enzyme stability - and consequently increasing the number of reuses - should be a primary focus for future studies on biodiesel production employing immobilized enzymes.

Table 3. GWP100 of the production of biodiesel for the case studies. All impacts were calculated in kilograms of biodiesel.

		DAT		DFT		DIT		WET	
Description	Unit	Input Unit/ kg Biodiesel	g $CO_{2eq.}$	Input Unit/ kg Biodiesel	g $CO_{2eq.}$	Input Unit/ kg Biodiesel	g $CO_{2eq.}$	Input Unit/ kg Biodiesel	g $CO_{2eq.}$
Inputs									
Soybean	kg	5.663	1740.8	5.001	1537	5.200	1598.2	0.000	0.000
Waste Oil*	kg	0.000	0.000	0.000	0.000	0.000	0.000	0.986	0.959
n-Hexane	kg	0.006	1.718	0.005	1.517	0.005	1.577	0.000	0.000
Ethanol	kg	0.154	73.96	0.157	75.16	0.164	78.36	0.158	75.48
Water	kg	0.982	0.009	0.067	0.001	4.862	0.043	0.026	0.000
Enzyme	kg	0.000	0.000	0.011	25.93	0.111	588.5	0.011	24.63
HCl	kg	0.003	2.133	0.000	0.000	0.000	0.000	0.000	0.000
NaOH	kg	0.009	4.410	0.000	0.000	0.000	0.000	0.000	0.000
Effluent treatment	kg	2.512	0.402	0.138	0.022	0.298	0.048	0.076	0.079
Cooling water	kg	1.082	0.027	3.911	0.097	5.296	0.131	3.193	0.079
Emissions									
CO_2eq from bagasse burning**	kg	1.133	7.026	1.005	6.229	2.086	12.93	0.000	0.000
Total	kg		1830.4		1646.1		2279.8		100.2
Outputs Energetics									
Electricity produced	MJ	1.20E+06		8.78E+05		1.79E+06		0.000	
Electricity to be Sold	MJ	5.74E+05	9.52E-06	2.81E+05	4.49E-06	1.18E+06	2.53E-05	0.000	0.000
Soybean Meal	kg	4.148	1059.5	3.663	900.9	3.808	1257	0.000	0.000
Lecithin	kg	0.060	94.61	0.053	80.45	0.056	112.3	0.000	0.000
Soybean hulls	kg	0.378	31.99	0.334	27.20	0.347	37.97	0.000	0.000
Glycerol	kg	0.063	16.89	0.086	22.22	0.092	31.89	0.086	3.467
Free fatty acid	kg	0.000	0.000	0.015	0.000	0.024	0.000	0.048	0.000
Biodiesel	kg	1.000	625.0	1.000	601.8	1.000	808.0	1.000	93.39
Biodiesel after transport and burning in gCO_{2eq}/kg	kg	1.000	664.6	1.000	641.4	1.000	847.6	1.000	158.9
Cbio/kg Biodiesel		2.592E-03		2.618E-03		2.435-03		3.101E-03	

CONLUSIONS

Four ethyl biodiesel production scenarios were examined, three via enzymatic catalysis and one with alkaline catalysis, serving as the reference (DAT). The conventional biodiesel process outperformed immobilized enzyme methods economically and environmentally, yielding a negative NPV for enzymatic routes due to high costs. The biorefinery using alkaline catalysis (DAT) achieved the highest NPV, while the WET process exhibited the highest IRR. The best environmental performance was observed in the waste oil scenario,

Table 4. First-order sensitivity indices of NPV results for different enzymatic biodiesel scenarios were calculated using the RDB -FAST method.

	DFT	DIT	WET
Variable	Values	Values	Values
Ethanol molar ratio	0.00	0.00	0.00
Enzyme Price	0.38	0.00	0.38
Reaction time	0.06	0.00	0.05
Reaction Temperature	0.00	0.00	0.00
Transesterification conversion	0.01	0.00	0.02
Hydrolysis conversion	0.00	0.00	0.01
Reuse	0.51	0.98	0.51

attributed to the low impact of the raw material, which is considered a waste. Enzymatic catalysis offers a promising alternative for low-impact biodiesel production, facilitating a purification strategy with minimal environmental effects and utilizing cost-effective raw materials. However, enzyme type and reuse frequency can sometimes make enzymatic routes more impactful than chemical methods. Thus, assessing the impact of the enzyme is crucial for accurately evaluating the environmental burden of these processes. The sensitivity analysis confirmed that the economic viability of enzymatic biodiesel production is primarily influenced by enzyme costs and reuse number. While longer reaction times and larger equipment increase costs, their impacts are smaller. Ultimately, integrating the biodiesel process into a biorefinery has the potential to enhance the economic feasibility of large-scale enzyme-based biodiesel production by promoting greater process integration and lowering production costs.

The results presented suggest promising strategies for implementing enzyme-catalyzed biodiesel production with lower environmental impact and favorable economic performance. These findings may serve as a guide for new strategies aimed at implementing enzymatic biodiesel, while also clarifying the main challenges associated with the adoption of this technology. Additionally, the assumptions made in this study could be the subject of further investigation at smaller scales, potentially strengthening the findings, along with the expansion of the biorefinery concept. Integrating additional products within a single biorefinery can lead to a reduction in the environmental impacts attributed to each product, due to the allocation of impacts across a greater number of outputs. In the Brazilian context, the construction of an integrated biorefinery producing sugarcane ethanol and soybean oil biodiesel represents a compelling alternative, given that these are the main biofuel feedstocks in the country. Ideally, by-products such as glycerol and the lignocellulosic fraction of biomass should be valorized by the production of other high-value molecules, such as microbial oil-based biodiesel, nanocellulose, biosurfactants, among others.

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NAUČNI RAD

TEHNO-EKONOMSKA I EKOLOŠKA PROCENA PROIZVODNJE BIODIZELA SA ETIL ESTERIMA

Biodizel je ključno gorivo za budućnost sa nultom emisijom ugljenika. Enzimska sinteza korišćenjem obnovljivih materijala može je učiniti još ekološki prihvatljivijom. Međutim, visoki troškovi enzima i ograničena ponovna upotreba ometaju njenu ekonomsku isplativost. Ova studija procenjuje tehno-ekonomske i ekološke performanse različitih procesa za proizvodnju biodizela sa etil estarima. Procenjeni scenariji uključuju: transesterifikaciju sojinog degumiranog ulja korišćenjem slobodnog i imobilizovane lipaze Eversa Transform 2.0 (ET), hemijsku alkalnu katalizu sojinog ulja i transesterifikaciju otpadnog ulja korišćenjem ET. Glavne metrike bile su neto sadašnja vrednost i potencijal globalnog zagrevanja. Rezultati su pokazali da je slobodni enzim ekonomski nadmašio imobilizovani enzim. Međutim, hemijska kataliza je imala neto sadašnju vrednost skoro dvostruko veću od najbolje opcije sa slobodnim enzimom. Analiza osetljivosti je pokazala da su troškovi enzima i ponovne upotrebe bili ključni za neto sadašnju vrednost. Transesterifikacija otpadnog ulja sa ponovnom upotrebom enzima imala je najniži потенцијал глобалног загревања (4,21 g CO_{2eq}/MJ), što je čini ekološki najpovoljnijim scenarijem. Iako je procena životnog ciklusa pokazala niži potencijal globalnog zagrevanja za enzimsku katalizu, potrebna su dalja istraživanja emisija iz ovog procesa. U zavisnosti od enzima i ponovne upotrebe, hemijska kataliza može rezultirati nižim ukupnim emisijama. Integracija sa biorafinerijom čini proizvodnju biodizela enzimskom katalizom velikih razmera ekonomski isplativom i sa niskim emisijama CO_{2ekv}.

Ključne reči: biorafinerija, proizvodnja biodizela, ponovna upotreba lipaze, LCA, ekonomska evaluacija, analiza osetljivosti.