

Article

Assessing the Feasibility of Enzymatic Biodiesel Production Using the 5W2H Framework: A Brazilian Case Study with Distiller's Corn Oil

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Abstract

This study adopts the 5W2H management tool to investigate the opportunities and challenges of enzymatic biodiesel production from residual oils. The methodological approach enables a structured evaluation of technical, economic, environmental, and governance aspects, clarifying critical conditions for feasibility and scalability. To illustrate this framework, the research applies it to the Brazilian biofuel sector, focusing on the valorization of Distillers Corn Oil (DCO), a by-product of corn ethanol production not intended for human consumption. Results show that enzymatic conversion of DCO can reduce feedstock costs by more than 50% and energy demand by up to 86.8% compared with conventional chemical processes. Nevertheless, the scalability of this technology faces critical barriers, such as enzyme costs, reaction times, and regulatory uncertainties. The study concluded that public–private partnerships and targeted policies, such as those under Brazil's National Biodiesel Program (PNPB), are essential to overcoming these challenges and bridging the “valley of death” toward commercialization. By combining technical, economic, and governance perspectives, the study demonstrates that DCO-based enzymatic biodiesel has the potential to reinforce Brazil's role in the global biofuels market while promoting a circular and sustainable energy transition.

Keywords: advanced biodiesel; enzymatic process; residual oil; distiller's corn oil; Brazil



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1. Introduction

In addition to the environmental and social impacts caused by the intense consumption of fossil fuels, concerns about the depletion of energy resources continue to increase, leading many countries to shift their focus toward renewable energy sources [1]. Among these, biodiesel has consolidated itself as a relevant option [2–4], not only because it is biodegradable and less polluting [5], but also because it represents a strategic alternative to meet growing energy demand in a climate-constrained world [3].

There are several types of biofuels that can be classified considering the raw material used or by the technological readiness level of the production pathway, resulting in lower average emissions in their life cycle. If the raw material is considered as classification system, the biofuels can be of first, second or third generation. First-generation biofuels consist of edible feedstock as main raw material [6], while second generation relies on non-edible feedstock or residues, such as waste or forest residues [7]. Third-generation biofuels have algae as raw material [8]. According to Abdullah et al. [9], when genetic modification is conducted in algae used as raw material, the biofuel can be classified as fourth generation.

Based on technological maturity, biofuels can be classified as either conventional or advanced. Conventional biofuel technologies involve well-established, commercial-scale production processes. Conventional biofuels are commonly known as first-generation and include sugar- and starch-based ethanol, biodiesel from oilseed crops, pure vegetable oil, and biogas from anaerobic digestion. Typical feedstocks for these processes include sugarcane, sugar beets, starch-rich grains like corn and wheat, and oilseed crops such as rapeseed (canola), soy, and palm [10].

In contrast, advanced biofuel technologies are still in the research and development (R&D), pilot, or demonstration phase, often referred to as second or third generation [10,11]. The advanced biofuels are derived from non-food feedstocks or residues and waste, which implies a significant reduction in greenhouse gas (GHG) emissions if compared to fossil fuels [11].

From a technical perspective, biodiesel has long been recognized for its compatibility with diesel engines, allowing its use without requiring significant adaptations [12]. Another important aspect is the wide range of possible feedstocks, such as vegetable oils, animal fats, microbial and algae sources [13], which in principle enables the supply chain to adapt to regional specificities and resource availability [14]. However, the historical dependence on edible oils such as soybean, corn, and sunflower remains problematic, since it intensifies the food vs. fuel debate [1], increases agricultural pressure, and exposes biodiesel costs to volatility in commodity markets.

For this reason, the search for alternative raw materials that do not compete with the food chain is a priority [14]. In recent years, attention has turned to waste oils generated in large volumes by agro-industrial and food sectors [1]. These residues not only reduce environmental liabilities but also improve the economics of biodiesel production, since as highlighted by Canesin et al. [15], feedstocks alone can account for approximately 70% of total biodiesel production costs, which undermines its competitiveness against fossil fuels.

Nevertheless, residual oils are characterized by a high acidity level, which results in soap formation in chemical catalyst reactions. However, when the conversion is carried out via the enzymatic route, new challenges arise. Despite advantages such as higher product purity, operation under milder conditions, and the possibility of reusing biocatalysts, enzymatic biodiesel still faces scalability bottlenecks, including high enzyme costs, the need for optimized immobilization techniques of enzymes for high-FFA feedstocks conversion into biodiesel, and limited integration with industrial-scale processes. Overcoming these barriers requires coordinated action that combines technological development with supportive institutional frameworks and effective engagement of stakeholders across government, industry, agriculture, research institutions, and civil society. Require not only technological advances but also a coordinated effort among different stakeholders.

Against this backdrop, this paper investigates the strategic role of stakeholders in enabling enzymatic biodiesel production, applying the 5W2H management tool (What, Who, Why, Where, When, How, and How Much) as an analytical framework. Unlike generic discussions of biodiesel, the use of 5W2H allows a structured and multidimen-

sional assessment of the opportunities and challenges related to this technological route, bridging technical, economic, and governance aspects. Specifically, the Brazilian case study focuses on biodiesel production from Distillers Corn Oil (DCO), a low-value by-product of corn ethanol plants not destined for human consumption. The valorization of DCO as a biodiesel feedstock avoids food competition and contributes to the efficient use of agroenergy residues. To ground the analysis, the Brazilian biofuel sector is adopted as a case study, combining literature review and stakeholder insights to validate theoretical findings and to highlight pathways for advancing sustainable second-generation biodiesel.

2. Materials and Methods

To guide the proposed research, the 5W2H management tool proves to be an effective methodological strategy, allowing for the logical and objective organization of the main elements that comprise the analysis. Through the seven key questions—What, Who, Why, Where, When, How, and How Much—it is possible to clearly structure the objectives, justification, scope, and methods of the study, clarifying the research problem through a systematic approach.

2.1. Systematic and Documentary Review Protocol

Thus, this study proposes to investigate, through a systematic review of the literature, the feasibility of enzymatic biodiesel produced from waste oil, with the support of the 5W2H tool. The objective is to provide a comprehensive and structured analysis, contributing to the understanding of the potential and challenges involved in the application of advanced biodiesel. All studies included in the database were selected using careful inclusion and qualification filters (quality and applicability), as shown in Table 1.

Table 1. Inclusion and qualification criteria for selected studies.

Criterion	Description
Thematic relevance	Preference for studies that directly address the use of waste oil in the production of enzymatic biodiesel, although studies of other routes that may support the rationale are not highlighted due to the limited number of studies that specifically address the enzymatic route.
Geographical context	Studies developed internationally and nationally on the subject.
Type of study	Scientific articles from indexed databases, dissertations, theses, and technical publications with theoretical and/or experimental basis.
Publication period	Preference for publications from the last 10 years, to ensure the data and technologies analyzed are up to date.
Language	Studies published in English, Portuguese, or Spanish.
Access to full content	Only works with full text available for systematic analysis.
Methodological quality	Studies with clear, replicable methodology and well-founded results.
Practical applicability	Works that present data or analyses applicable to the productive and/or environmental reality.
Adherence to 5W2H	Studies that allow the extraction of information compatible with the elements of the 5W2H tool (What, Why, Where, When, Who, How, How much).

In order to obtain a representative sample of studies related to the topic, we initially opted to use the Web of Science and Scopus databases, which are recognized for their broad scope and satisfactory coverage. For the search, we used English-language variations in terms related to biodiesel or biofuel production—“Biodiesel production” or “Biofuel production”, “Biodiesel processing” or “Biofuel processing”, and “Biodiesel synthesis” or

“Biofuel synthesis”—, in addition to the expression “Residual oil” and the term associated with the enzymatic route, represented using the keyword “Enzymatic”, as shown in Table 2. It should be noted that, initially, it was considered pertinent to use the terms “Enzymatic route” and “Enzymatic pathway”; however, as the search did not return any results, it was decided to make the criteria less restrictive, using only the term “Enzymatic.” In addition, it was decided to also consider a combination with the terms “Acidic oil” and “Lipase”.

Table 2. Determination of search processes.

Criteria	Description
Topics	Web of Science—TS = (“Biodiesel production” AND “Residual oil” AND “Enzymatic”) OR TS = (“Biodiesel processing” AND “Residual oil” AND “Enzymatic”) OR TS = (“Biodiesel synthesis” AND “Residual oil” AND “Enzymatic”) OR TS = (“Biofuel production” AND “Acidic oil” AND “Enzymatic”) OR TS = (“Biofuel processing” AND “Acidic oil” AND “Enzymatic”) OR TS = (“Biofuel synthesis” AND “Acidic oil” AND “Enzymatic”) OR TS = (“Biodiesel production” AND “Residual oil” AND “Lipase”) OR TS = (“Biodiesel processing” AND “Residual oil” AND “Lipase”) OR TS = (“Biodiesel synthesis” AND “Residual oil” AND “Lipase”) OR TS = (“Biofuel production” AND “Acidic oil” AND “Lipase”) OR TS = (“Biofuel processing” AND “Acidic oil” AND “Lipase”) OR TS = (“Biofuel synthesis” AND “Acidic oil” AND “Lipase”) Scopus—TITLE-ABS-KEY (“Biodiesel production” AND “Residual oil” AND “Enzymatic”) OR TITLE-ABS-KEY (“Biodiesel processing” AND “Residual oil” AND “Enzymatic”) OR TITLE-ABS-KEY (“Biodiesel synthesis” AND “Residual oil” AND “Enzymatic”) OR TITLE-ABS-KEY (“Biofuel production” AND “Acidic oil” AND “Enzymatic”) OR TITLE-ABS-KEY (“Biofuel processing” AND “Acidic oil” AND “Enzymatic”) OR TITLE-ABS-KEY (“Biofuel synthesis” AND “Acidic oil” AND “Enzymatic”) OR TITLE-ABS-KEY (“Biodiesel production” AND “Residual oil” AND “Lipase”) OR TITLE-ABS-KEY (“Biodiesel processing” AND “Residual oil” AND “Lipase”) OR TITLE-ABS-KEY (“Biodiesel synthesis” AND “Residual oil” AND “Lipase”) OR TITLE-ABS-KEY (“Biofuel production” AND “Acidic oil” AND “Lipase”) OR TITLE-ABS-KEY (“Biofuel processing” AND “Acidic oil” AND “Lipase”) OR TITLE-ABS-KEY (“Biofuel synthesis” AND “Acidic oil” AND “Lipase”)
Database	Web of Science and Scopus
Indexes	All indexes from both databases
Date of demand	1 June 2025, at 08:00 p.m.

The keywords and their combinations used in the search strategies were defined through a structured brainstorming session carried out by the authors. This process began after preliminary search attempts with narrower terms had returned few or no results, which highlighted the need to broaden the scope of the search strategy. During the session, all terms and expressions that came to mind were recorded without immediate judgment, including synonyms, variations, and related concepts from the literature. Subsequently, the list was refined by excluding terms considered unrealistic or too ambiguous to produce relevant results within the context of enzymatic biodiesel production.

The brainstorming followed a semi-structured approach, combining the experience of the researchers with prior evidence gathered from exploratory searches. Restrictions were introduced at the refinement stage: for example, overly generic terms (e.g., “bioenergy” alone) were discarded, while highly specific combinations that produced zero results in preliminary tests were also omitted. The final list of keywords therefore reflects both the inclusiveness of the brainstorming process and the pragmatic adjustments required to ensure compatibility with database search algorithms. This dual approach—open idea

generation followed by critical refinement—helped maximize coverage while maintaining methodological rigor and relevance.

It should be noted that the keywords and their combinations used in the search strategies were defined during a brainstorming session between the authors of this study. This collaborative process aimed to maximize the scope and relevance of the results obtained from the databases, ensuring that the selected terms accurately reflected the main thematic aspects of the research. During the meeting, different terminological variations, synonyms, and related expressions in English were considered, based on previous literature and the experience of the researchers involved. In addition, the specificity of the topic—the production of enzymatic biodiesel from waste oil—and the need to adapt the terms to ensure compatibility with the search algorithms of the Web of Science and Scopus databases were taken into account.

Keywords related to the Brazilian context could not be included, as they returned no results in the databases searched. This limitation highlights the scarcity of internationally indexed publications on the topic focusing on Brazil. In addition, it was also not possible to add keywords related to stakeholders, because this strategy also reduced the sample to zero results.

It is important to note that, due to the small number of studies obtained from the Web of Science (only 15 studies) and Scopus (only 11 studies, of which 8 were duplicates) databases, it was considered necessary to expand the initial sample, which contained only 12 studies (direct database searches). To this end, we decided to conduct a complementary literature search with the aim of enriching and diversifying the available data, including reports from important international organizations. As a result of this complementary strategy, 50 more relevant studies were identified and added to the final research repository. This brings the total to 62 studies (direct searches combined with document searches), providing a more robust and representative basis for future analysis and consideration.

2.2. Methodological Approach for Applying the 5W2H Tool

This subsection aims to present, in detail, the methodological approach adopted for the application of the 5W2H tool, based on a systematic review of the literature combined with a complementary documentary search. The methodological strategy aims to ensure the necessary scope and depth for the identification, selection, analysis, and organization of information relevant to the topic, ensuring the robustness of the data supporting the tool's development and use.

In this context, the *What* corresponds to the clear definition of the central object of study: the production of biodiesel through the enzymatic route using waste oil as raw material. This approach represents an innovative and sustainable alternative in the field of second-generation biofuels, as it allows the reuse of industrial waste with high energy potential, while reducing environmental impacts and costs related to raw materials.

The actors involved (*Who*) cover a broad spectrum of stakeholders, including waste oil suppliers, academic researchers, investors, government agents, public policy makers, and civil society representatives. Each group exerts direct or indirect influence on the biodiesel value chain, and it is essential to understand their motivations, interests, and challenges.

The rationale for the study (*Why*) lies in the urgent need to diversify the energy matrix and the search for environmentally responsible solutions. Understanding the role and coordination of stakeholders in this process is essential to identify viable paths for consolidating second-generation biofuel production, promoting a more efficient, clean, and integrated energy transition.

The spatial scope of the analysis (*Where*) focuses on countries that have favorable technical, economic, and institutional conditions for the production of biodiesel from waste

oil through the enzymatic route. Among these, countries with consolidated agro-industrial chains, high oil waste generation, and public policies aimed at promoting renewable energy stand out. Brazil, for example, stands out as one of the most promising contexts due to its rising production of corn ethanol [16], which consequently ensures a DCO supply for biodiesel production. This is supported by a long-standing history of regulatory frameworks that encourage the use of biofuels, such as RenovaBio Program [17], a policy that seeks to expand the use of biofuels while ensuring stability of supply, as will be discussed in Section 4.

With regard to the time frame (When), the research considers the current scenario, in which there is a growing demand for renewable energy sources and national and international pressure for production practices with low environmental impact. The analysis, therefore, is inserted in the contemporary context of energy transition and the sustainable development agenda.

As for the opportunities and challenges for enabling the production of biodiesel from waste oil (How), this study consists of a systematic literature review based on the selection, analysis, and synthesis of academic studies that address this topic. The objective is to map different raw-material, enzymes, reaction conditions, time reaction, conversion rates and co-products, highlighting the differences in enzymatic and conventional chemical biodiesel production and highlighting the opportunities and challenges related to the application of the enzymatic route.

Regarding financial aspects (How Much), although this study does not involve laboratory experiments or direct operating costs, an exploratory analysis of case studies and related projects will be carried out in order to estimate the typical investments required to make similar initiatives viable, thus providing a more comprehensive view of the economic feasibility of the technology under investigation.

2.3. Results Validation Process Application of the 5W2H Tool to a National Project

To validate the results obtained through systematic and documentary review, this study applies the 5W2H tool to a representative project in the Brazilian context, with the aim of verifying its adherence to the national reality of the biofuel production chain. The practical application allows testing the consistency of the elements surveyed and identifying specific gaps, opportunities, and challenges related to the production of enzymatic biodiesel from corn ethanol waste oil, i.e., DCO.

The simulated project involves the structuring of an enzymatic biodiesel production plant using DCO as raw material, based on technical-economic and regulatory parameters obtained from official sources, such as the National Agency of Petroleum, Natural Gas and Biofuels (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis—ANP, in Portuguese), the Energy Research Company (Empresa de Pesquisa Energética—EPE, in Portuguese), and EMBRAPA. This simulation serves as a case study for the application of the seven axes of the 5W2H tool, allowing for an integrated analysis of the technical, economic, and institutional factors that influence its feasibility.

In the What dimension, the project focuses on converting a by-product of the ethanol industry into a second-generation biofuel, contributing to waste utilization and reducing environmental impacts.

The Who dimension identifies the main stakeholders involved: ethanol producers, biodiesel plants, suppliers of biotechnological inputs (enzymes), regulatory agencies, research centers, and financing entities. Each actor plays a strategic role in the viability and scalability of the enzymatic route.

The justification (Why) lies in the search for sustainable biomass energy sources that do not compete with the food chain, in addition to the need to add value to existing industrial waste.

The Where axis locates the initiative in Brazilian regions with the highest concentration of ethanol plants and infrastructure favorable to logistics and the distribution of biofuels, such as the Midwest and Southeast.

In terms of When, the project fits into the current energy transition scenario, marked by the urgency for low-carbon solutions and the existence of national goals and policies that encourage the production and consumption of biofuels.

As for How, the proposed model adopts existing technologies of enzymatic process for biodiesel production in two steps, as described by Pasha et al. [Citation], including remarks on needs of pretreatment of DCO, application of free enzyme and immobilized, needs of biodiesel purification process, compared to the results described in the technical literature (Section 3). The study also considers operational barriers, such as the need to optimize production processes.

Finally, in the How much dimension, the study conducts an exploratory cost analysis based on similar projects and studies, including actual diesel vs. biodiesel price, enzyme price in the Brazilian market, as well as prospective analysis of DCO supply and biodiesel demand in the transport sector.

This application of 5W2H reinforces the potential of the methodology as a strategic analysis tool, by articulating multiple dimensions of the technical, economic, and institutional feasibility of enzymatic biodiesel production from DCO, offering practical support for the development of sustainable solutions in the Brazilian energy sector.

3. Results and Discussion

This section brings together the main results of bibliometric and systematic analyses related to biodiesel production. It should be noted that, for the application of the 5W2H tool, studies that contribute to strengthening the theoretical basis of the work will also be considered.

3.1. Bibliometric Results of the Literature Review

This subsection presents the main bibliometric findings directly related to biodiesel production via enzymatic routes, considering the 62 studies identified in the databases with direct application to the topic.

Figure 1 illustrates the temporal evolution of publications on biodiesel production from waste oils between 2003 and 2025. Although initial records date back to 2003, research activity remained sparse and irregular until 2016, when alkaline-catalyzed transesterification still prevailed as the dominant industrial process due to its high conversion efficiency and rapid kinetics [5]. By contrast, enzymatic transesterification—despite offering advantages such as higher product purity, simplified glycerol separation, and milder reaction conditions—faced prohibitive costs associated with enzyme production and reuse, limiting its industrial appeal [3].

From 2017 onwards, a noticeable upward trend emerges, with a sharp rise between 2019 and 2021. This shift coincides with global debates on waste valorization, circular economy, and greenhouse gas mitigation, all of which stimulated academic attention toward sustainable feedstocks and alternative catalytic pathways. The peak observed in 2025, particularly in the expanded database (orange bars), underscores that biodiesel production from waste oils via enzymatic routes is no longer a marginal topic but rather a maturing and strategically relevant research field.

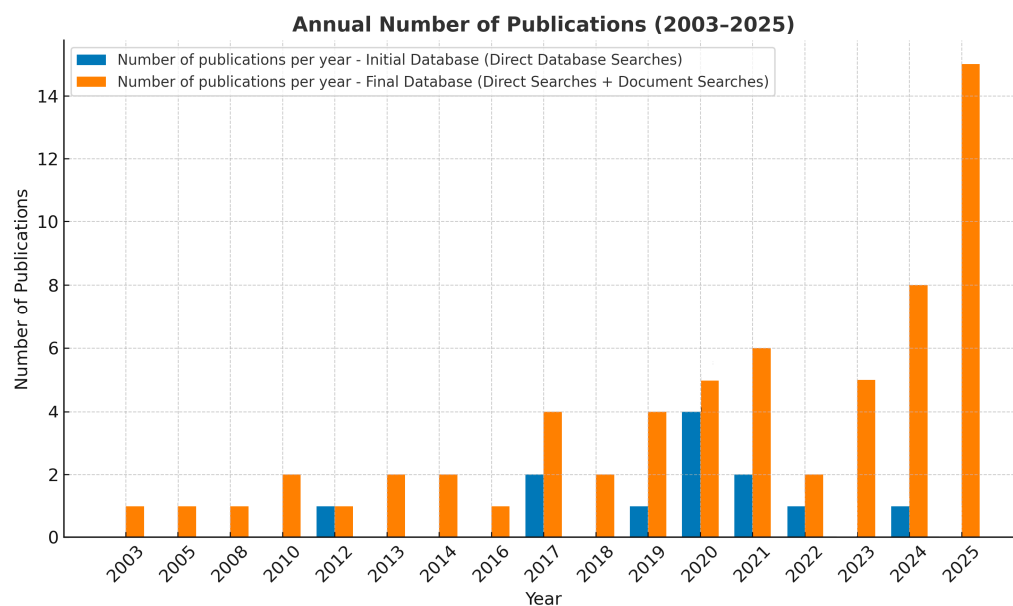


Figure 1. Number of publications per year.

The contrast between the initial and expanded databases also highlights a methodological point: without document-level searches, a considerable share of relevant studies remains invisible. This methodological discrepancy emphasizes the importance of adopting comprehensive bibliometric strategies to avoid biased interpretations of research evolution.

Figure 2 displays the distribution of studies by research area. Unsurprisingly, Energy & Fuels dominates (33%), followed by Engineering (22%) and Chemistry (19%). These areas collectively reflect the dual technical challenge of (i) designing scalable processes for enzymatic catalysis and (ii) managing the chemical complexity of heterogeneous waste oils, often with high free fatty acid (FFA) content. However, smaller yet relevant shares in Food Science & Technology (11%), Materials Science, and Biotechnology (4% each) reveal a growing interdisciplinary engagement.

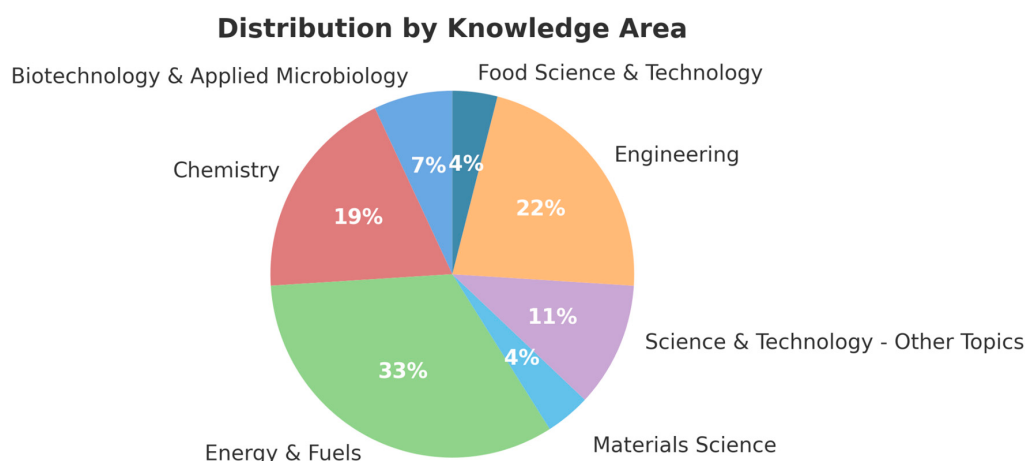


Figure 2. Division of studies by area of application.

These contributions include studies on enzyme immobilization supports, valorization of by-products, and the safe use of waste streams—all of which are critical for industrial feasibility. Nonetheless, the relatively limited presence of biotechnology-oriented work suggests that opportunities remain for advances in enzyme engineering, carrier development, and biocatalyst stabilization.

Figure 3 depicts a VOSviewer (Version 1.6.16.) heat map of keyword co-occurrence. Central terms such as “biodiesel”, “lipase”, “immobilized lipase”, “cooking oil”, and “acid oil” reinforce the prominence of enzymatic approaches and the practical focus on high-FFA and degraded oils. Other frequent terms are “fatty acids”, “esters”, and “bioethanol production” that highlight cross-cutting interests in valorizing side-streams and diversifying bio-based energy products. Furthermore, terms such as “catalyst selectivity”, “bioconversion”, and “adsorption” suggest an increasing concern with process efficiency and integration with broader biorefinery concepts.

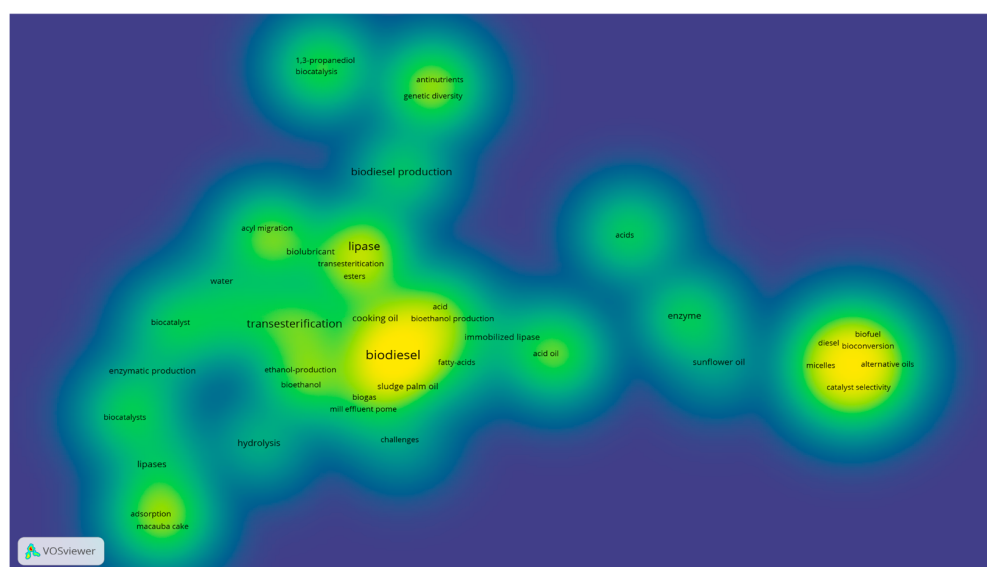


Figure 3. Heat map of the main occurrences of keywords.

Taken together, the bibliometric results not only confirm the growing maturity of the field but also help expose critical research gaps that this study directly addresses:

- Optimization of enzyme immobilization for easier recovery and reuse strategies in high-FFA oils conversion—Although “immobilized lipase” is a recurrent keyword, few studies provide systematic comparisons of immobilization techniques tailored to acidic and degraded feedstocks. This gap directly hinders cost reduction and long-term enzyme stability.
- Development of region-specific techno-economic models—Despite abundant technical studies, bibliometric mapping shows a scarcity of economic analyses adapted to local realities (feedstock availability, waste-management policies, energy markets). Such models are essential to evaluate the feasibility of enzymatic biodiesel production beyond laboratory scales.
- Integration into circular economy and waste valorization frameworks—Although circularity is an implicit theme, relatively few studies quantitatively assess life cycle impacts or explore synergies with other waste-to-energy pathways (e.g., co-production of biogas, bioethanol, or biochemicals).
- Process intensification and hybrid catalytic systems—The coexistence of terms such as “acid oil”, “adsorption”, and “catalyst selectivity” indicates ongoing interest in hybrid or sequential catalytic approaches, yet the field lacks consolidated methodologies to integrate enzymatic and non-enzymatic steps efficiently.

Policy and scalability assessments—The bibliometric record shows limited engagement with policy instruments, incentive mechanisms, or industrial-scale case studies—factors that ultimately determine whether enzymatic biodiesel can transition from academic promise to commercial deployment.

Figure 4 complements the previous analysis by presenting a co-occurrence network of terms extracted from the literature on biodiesel, structured by interconnected thematic clusters. Each color represents a community of semantically related terms, highlighting the main research focuses on the field. At the center of the network, the terms “biodiesel” and “transesterification” occupy a strategic position connecting the different groups, indicating their role as structuring axes of scientific discussions.

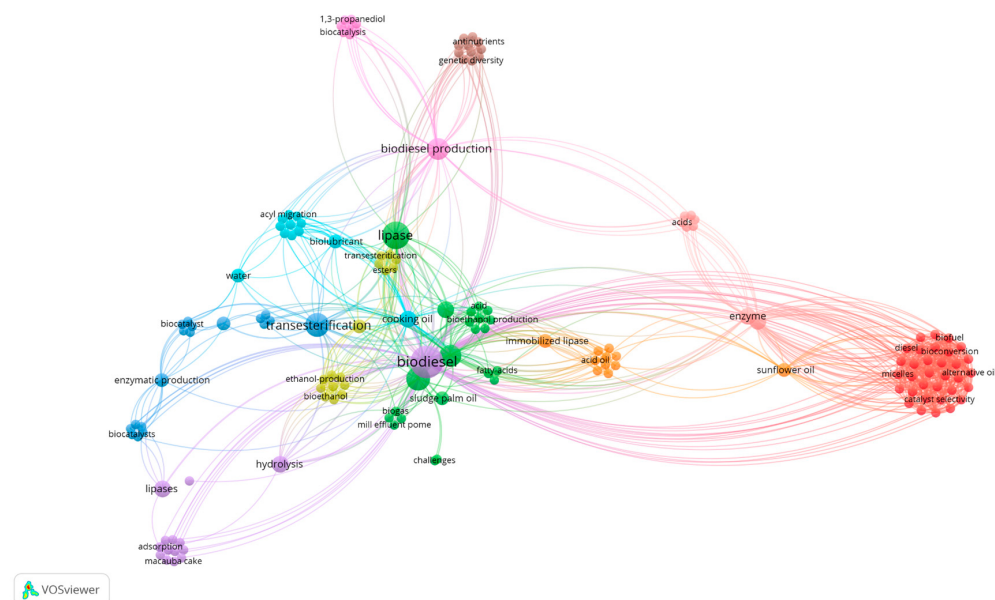


Figure 4. Interconnection network between keywords.

The red cluster stands out, focusing on alternative oils and catalytic selectivity, with a high density of terms such as “biofuel”, “bioconversion”, “alternative oils”, and “catalyst selectivity”, suggesting a line of research focused on catalyst performance and raw material diversification. The green cluster, on the other hand, focuses on studies related to the action of lipases, “immobilized lipase”, “acid oil”, and “fatty acids”, reinforcing the emphasis on enzymatic routes, which are widely used in the conversion of waste oils such as DCO.

The blue group relates to technological and reaction aspects, such as “biocatalyst”, “enzymatic production”, “acyl migration”, and “water”, comprising the field of research focused on the efficiency and conditions of the transesterification process. The purple cluster includes terms such as “macauba cake”, “adsorption”, and “hydrolysis”, suggesting the study of emerging raw materials and pretreatment techniques also applicable to DCO. Finally, the presence of the pink cluster, centered on “biodiesel production” and terms such as “genetic diversity” and “antnutrients”, points to an interface with areas such as microbiology and bioengineering, which can contribute to the biotechnological use of waste.

The heat map (Figure 3) highlights the centrality of the term “biodiesel” and its strong connection with expressions such as “transesterification”, “lipase”, and “immobilized lipase”, which are key elements in the enzymatic conversion of waste oils—exactly the methodological focus of this work. The concentration of terms related to waste, such as “cooking oil”, “acid oil”, and “fatty acids”, reinforces the relevance of waste oil as a strategic raw material for a sustainable technological route. These findings are consistent with the analysis proposed by 5W2H, especially in the following areas: (i) “What”, defined by which product is being produced, (ii) “How”, i.e., how the production process occurs, and (iii) “Why” this alternative is relevant, strengthening the technical justification of the proposal.

Figure 4 complements the analysis by demonstrating the relational structure between recurring themes in the literature, organized into interconnected communities. The network configuration shows that enzymatic biodiesel production involves multiple dimensions—catalysts, alternative raw materials, operating conditions, and applications—which directly corresponds to the “Who” field of the 5W2H tool. The diversity of terms related to technological innovation and biocatalysis indicates that the viability of the process is strongly conditioned by cooperation between research institutions, the productive sector, and regulatory agents, in line with SDG 17, which proposes strengthening partnerships for sustainable development. This can be observed through public policies designed to promote biofuels, such as the RenovaBio program, which considers the entire biofuel life cycle and now includes small-scale raw material producers [17]. Furthermore, this support is complemented by financial incentives, such as grants from government agencies like FINEP and BNDES for new ethanol facilities [18] and investments in technological development, which are directed to both companies [19] and collaborative projects with scientific institutions, such as universities and scientific institutions, such as universities [20]. In addition, the presence of topics related to catalytic selectivity, bioconversion, and the use of enzymes highlights the importance of investments in research and innovation, aspects that relate to the items “How Much” the costs and effort required and “When”, i.e., at what stage in time and technology the route is of the 5W2H tool. This reinforces the need for strategic planning and interinstitutional alignment to enable the production of enzymatic biodiesel on an industrial scale.

3.2. Application of the 5W2H Tool

This subsection presents and defines the fundamental concepts associated with each element of the 5W2H tool. In doing so, it establishes the analytical framework that guides the interpretation of the study. The purpose is to clarify what the central focus of the investigation is and how it is framed within the broader research problem, as well as to identify who the key actors, stakeholders, and beneficiaries are involved in the process.

In addition, it seeks to explain why the study is relevant, highlighting its practical, scientific, or societal significance. It also delineates where the study is situated or applicable, whether in a specific geographical context, an industrial sector, or an institutional environment, and determines when the study was or will be implemented, situating it temporally within a project or policy cycle. These questions provide not only descriptive clarity but also strategic orientation, ensuring that the research aligns with real-world conditions and demands.

The subsection describes how methodologies, strategies, and practices are applied in the development of projects and case analyses, and assesses how much the initiatives cost, addressing the financial dimension and its implications for feasibility. Beyond merely answering these guiding questions, the application of the 5W2H methodology is essential because it fosters a structured, systematic, and transparent approach to problem-solving. It ensures that no critical dimension is overlooked, facilitates communication among diverse stakeholders, and enhances decision-making by integrating technical, economic, and contextual perspectives. By articulating each of these dimensions, the subsection not only contextualizes the object of study but also underscores the methodological rigor and robustness of the research design.

3.2.1. What?

The selection of residual oil, such as waste cooking oil (WCO), as a feedstock for biodiesel production aligns with the principles of sustainability, circular economy, and bioeconomy, as it enables the transformation of an environmental liability into a valuable energy resource [21]. Unlike conventional feedstocks such as edible vegetable oils

(e.g., soybean, canola, sunflower), waste and non-edible oils—such as *Jatropha curcas*, *Ricinus communis* (castor oil), *Carapa guianensis* (andiroba), acid soybean oil and DCO—do not compete with food production and do not exert pressure on agricultural land intended for food security [14,22,23].

Moreover, these alternative feedstocks present significant economic and logistical advantages: they are abundant, low-cost, and widely available, particularly in urban centers and densely populated regions. Used cooking oil, for instance, is generated on a large scale by households, restaurants, and food industries, creating substantial potential for collection and reuse [24,25].

It is worth noting that although several technological routes have been developed for biodiesel production, the enzymatic route has attracted increasing attention in recent research [3]. The enzymatic conversion of waste oils into biodiesel stands out due to its technical and environmental efficiency [26]. Unlike conventional processes that employ chemical catalysts (acidic or alkaline), enzymatic catalysis enables a cleaner and more selective reaction with lower waste generation, operating under milder temperature and pressure conditions. These features reduce the need for intensive purification steps, decrease energy consumption, and minimize the corrosiveness of reagents—making the process more sustainable and better suited for small- and medium-scale production.

Therefore, the analysis proposed in this study is based on the understanding that enzymatic biodiesel production from waste oil offers strategic advantages from environmental, economic, and technological perspectives. It simultaneously promotes the valorization of urban waste, contributes to the diversification of the energy matrix, and strengthens local renewable energy supply chains. In this regard, the choice of this route as the central focus of analysis is justified by its relevance in addressing the contemporary challenges of energy transition and sustainability.

3.2.2. Who?

Stakeholders play a pivotal role in determining the technical, economic, social, and environmental feasibility of producing biodiesel from residual oil derived from corn ethanol using the enzymatic route as an innovative approach and sustainable alternative [27]. Companies that collect residual oil, for instance, provide the essential feedstock for biodiesel production, and their active participation is critical to maintaining the continuity of the production flow.

Equally important are biotechnology companies, which supply the enzymes required for the enzymatic process and are therefore essential for ensuring both the technical and economic efficiency of production. Investors and financial institutions also play a decisive role, as their willingness to provide funding depends largely on their assessment of the project's risks and returns, as well as their confidence that other actors are committed and willing to collaborate.

Another key stakeholder is the academic and research community, which, through Research and Development (R&D) projects, significantly contributes to technological advancements, process validation, and the generation of technical-scientific knowledge that supports strategic decision-making. Furthermore, the involvement of research institutions promotes the training of qualified professionals, which is vital for the consolidation and evolution of the biodiesel value chain. The effective articulation among these various stakeholders is, therefore, essential to the success and sustainability of the project, requiring an integrated and collaborative approach from the initial concept to the operational phase of enzymatic biodiesel production. Although partnerships and cross-sector alignment are important, investors typically base their decisions on clear economic incentives, expected returns, and risk mitigation strategies.

Public opinion and proactive industry measures can exert on the direction of biodiesel development worldwide. Previous studies have suggested that public acceptance of biofuels is shaped not only by perceived risk–benefit assessments but also by broader considerations regarding economic and environmental impacts [2].

In this regard, Winarno et al. [28] examined the challenges faced by the biodiesel industry in Indonesia amidst global market dynamics and evolving international regulations. Their study emphasizes that the engagement of multiple actors—including government agencies, the private sector, farmers, and non-governmental organizations—is fundamental to the sector’s sustainable development. Environmental concerns, economic uncertainties, and technical barriers in production and distribution have been identified as factors that undermine Indonesia’s competitiveness in this market. Based on a qualitative approach drawing on relevant secondary sources, the study investigates stakeholder involvement patterns and the obstacles that hinder the industry’s progress. The findings indicate that the sector’s sustainability relies heavily on cross-sector cooperation and the capacity to adapt to international standards. Favorable regulations, supply chain transparency, and technological innovation emerge as key elements to enhance production and distribution efficiency. With the right strategies, Indonesia could strengthen its global market position while advancing a more sustainable energy transition.

As summarized in Table 3, identifying and engaging stakeholders directly contributes to safer and better-informed decision-making, ensuring that the feasibility study addresses not only technical and economic aspects but also social, legal, and environmental variables that may influence the project’s success. This comprehensive perspective enhances the quality of strategic planning, increases the project’s attractiveness to investors and partners, and facilitates the alignment of public policies and incentives aimed at fostering innovation and sustainability.

It is also worth noting that the active involvement of stakeholders strengthens the project’s legitimacy and expands opportunities for strategic partnerships, technology transfers, and institutional collaborations. In the case of enzymatic biodiesel production, this is a sustainable innovation with great potential for positive impact, whose consolidation depends directly on the coordination of all actors in the value chain. Therefore, understanding the profile, perceptions, and priorities of these actors is not only relevant but also crucial to the success of the project.

Thus, the study by MENDES et al. [29] provides a valuable reference by investigating the main factors and alternatives related to the reference conditions for the biodiesel production chain within the scope of the Brazilian Biodiesel Production and Use Program. Using multicriteria mapping of stakeholder preferences, the authors conducted 20 interviews with experts from three key sectors: academia, industry, and regulatory agencies. Based on this survey, they structured the reference conditions for the chain, organizing its links, critical factors, and respective priorities as identified by the interviewees. The results show that six parameters account for 55.5% of the total priorities: increasing land production capacity, avoiding deforestation, production inputs, access to land, engine problems, and land use for food production. These aspects stand out as the most critical for ensuring the sustainability of the biodiesel chain in Brazil.

Table 3. Stakeholders in a Feasibility Study for Biodiesel Production via the Enzymatic Route.

Stakeholder	Type	Level of Influence	Action	Interests/Desires	Challenges /Risks	Engagement Strategies
Federal Government/Environmental Agencies	Public	High	Regulation, oversight, incentives through public policies	Stimulating bioenergy, social inclusion, regulatory compliance, energy security	Conflict between innovation and inclusion, bureaucracy, discontinuation of public partnerships due to political changes	Continuous dialogue, impact studies, and the Social Fuel Seal
Biotechnology companies/production units	Private	High	Enzyme and technology supply	Market expansion, technical efficiency, and profitability	High enzyme costs, industrial adaptation, immobilized enzyme production by a few companies	Technical-commercial partnerships, collaborative R&D, long-term supply contracts
Waste Oil Receiving Companies	Private	Medium	Supply of residual raw material	Waste valorization, increased revenue/reduced input costs	Reverse logistics, quality and regularity of residue	Agreements with distilleries, tax incentives for the circular economy
Investors/Banks/Green Funds	Private	High	Financing, capital contribution	Financial return, risk mitigation, ESG compliance	Regulatory uncertainty, operational risks	Clear business models, guarantees, sustainability and risk indicators
Family Farmers	Civil Society	Medium	Supplementary supply, regional support	Productive insertion, stable income, government support for carbon credits	Low access to technology, risk of exclusion through the enzymatic route	Technical training, adaptation of PNPB policies, cooperatives
NGOs and Community Associations	Civil Society	Medium	Social and environmental oversight, local engagement	Sustainability, social justice, positive local impact	Lack of transparency, projects that disregard the community context	Public consultations, participation in councils, accessible impact reports
Local Community	Civil Society	Low	Population directly affected by operations and logistics	Employment, quality of life, environmental safety	Environmental impact, water/soil consumption, health risks	Public hearings, socio-environmental compensation, ongoing transparency
Universities and Research Centers	Academic	Medium	R&D, training, technical support	Scientific innovation, publication, practical application of knowledge	Restricted funding, project discontinuation	Collaborative projects, scholarships, participation in technical forums
Biodiesel Industry Sector	Private	High	Plant operation, integration of production chains	Cost reduction, productivity, source diversification	Adaptation to Enzymatic route, quality requirements, and regularity of raw materials	Incentives for innovation, certifications, industrial clustering
Consumers/Public Opinion	Civil Society	Low	Final Product Destination—Use of Biodiesel in the Market	Fair Price, Sustainability, Vehicle Performance	Misinformation, Resistance to the New Fuel	Educational Campaigns, Promotion of Environmental and Social Benefits

Source: Own elaboration.

3.2.3. Why?

Biodiesel derived from residual oils (frying oils, DCO), non-edible oils (jatropha, castor oil, castor bean, andiroba) and other vegetable oils (soybean acid oil) are renewable substitutes for fossil fuels, such as diesel oil, which can be used without controversy, as they do not compete with raw materials intended for food [14,22,23]. Furthermore, it can be emphasized that, because they are inedible and subject to disposal, these sources of raw material have a lower cost than refined oils and are available in abundance, which makes them attractive for biodiesel production [24,25].

Consequently, the interest in biodiesel production using immobilized lipases has been growing steadily due to the advantages these enzymes offer compared to conventional processes that use chemical catalysts, namely, milder reaction conditions, lower energy consumption, compatibility with raw materials of different qualities, and the possibility of biocatalyst recovery [30].

Furthermore, the use of enzymes as biocatalysts in industrial processes, such as biodiesel production, has proven to be an attractive strategy because it avoids the complex steps involved in the design and synthesis of artificial catalysts, and reduces environmental impacts such as waste generation, lower energy consumption, and reduced greenhouse gas (GHG) emissions [14]. In the specific context of the use of lipases for biodiesel production, it can be said that enzymatic catalysis enables high yields of this biofuel [9]. However, it is important to consider the energy inputs and environmental costs associated with enzyme production when evaluating the overall sustainability of such processes.

With regard to enzymes, liquid formulations of free lipases are a highly competitive alternative, making the enzymatic route particularly suitable for the use of low-quality raw materials, given that the biocatalyst has high specificity for substrates and high tolerance to high levels of acidity and water content [31]. Although some processes involving the enzymatic route are not widely developed commercially, studies, articles, and patents have been produced that corroborate the use of the technology [32]. Table 4 shows the main benefits of biodiesel production from waste oils in environmental, social, and economic pillars.

3.2.4. Where

The analysis of energy supply policies and patterns presented by BARRAGÁN-OCAÑA et al. [33] shows that countries such as Brazil, Argentina, the United States (USA), and members of the European Union, such as Germany and France, have favorable conditions for the production of biodiesel from waste oils. These countries stand out for their combination of consolidated agro-industrial chains, high generation of oily waste, and regulatory frameworks aimed at promoting renewable energy. In the case of Brazil, for example, there is regional leadership in the use of sources such as biomass and waste, reflecting robust public policies in the sector, positioning the country as one of the main players on the world stage for energy transition, alongside other countries such as China, the USA, and Russia [34].

State support through subsidies in countries such as the European Union and the United States promotes the achievement of emission reduction targets and stimulates technological innovation, driving the development of sustainable biofuel routes, including the enzymatic route. These elements position these nations as leaders in the advancement of energy solutions based on waste recovery [33].

The production of biodiesel from waste oils using enzymatic routes (especially lipases) is an active area of research with some industrial application worldwide. South Asian countries, such as Indonesia and Thailand, for example, can be a reference in biodiesel production from palm waste oil, as they are major palm oil producers, with Indonesia being the world's largest producer, generating a significant amount of this waste oil [22,24,35,36]. According to the study by LOH et al. [25], palm oil is the most produced vegetable oil in the

world, and in 2020, approximately 72.3 million tons of palm oil were produced in Southeast Asia, Africa, and Central and South America.

Table 4. Importance of the enzymatic route for biodiesel production from waste oils for environmental, social, and economic pillars.

Pillars	Benefits	Description
Environmental	Waste Recovery	By using waste oils, the process avoids competition for edible oil.
		It avoids improper disposal and promotes the reuse of lipid waste in biodiesel production.
		During the reaction, alcohol (methanol/ethanol) can be recovered and reused, as well as the immobilized enzyme, which can be reused between 10 and 15 cycles without losing its activity.
	Reduction in GHG emissions	Enzyme production operates under milder temperature conditions, which avoids the use of aggressive chemical catalysts, positively impacting energy consumption and potentially reducing GHG emissions.
	Reducing the environmental impact of pretreatment	The use of immobilized lipase allows for the direct conversion of free fatty acids from waste oils, eliminating the need for chemical pretreatment steps, which typically involve toxic or corrosive reagents, contributing to a cleaner process.
Social	High efficiency with less waste generation	The high level of conversion into esters reduces the formation of by-products, contributing to more efficient production and less waste of unused materials.
	To promote food security	By using non-edible raw materials (such as waste oils and animal fat), the process avoids competition with food production.
	Green job creation	The application of enzymatic methods with the conversion of more accessible inputs and the use of local waste oils, such as cooking oil, suggests potential for the development of small industries and regional services, from collection to production, which could benefit local communities. Furthermore, the jobs generated by the biofuel value chain are considered entirely green.
	Potential for adding value to socio-biodiversity products	The use of residual oil such as babassu, typical of extractive regions in countries such as Brazil, can be an opportunity for regional economic development through the use of waste from socio-biodiversity production chains, promoting productive inclusion and strengthening traditional communities.
Economical	Diversification of the energy matrix	The expansion of renewable energy sources, using different types of waste oils and fats as feedstock for biodiesel, promotes energy resilience by reducing dependence on fossil fuels or monocultures.
	Economic additionality in the industry value chain	The biodiesel production system can be integrated into the circular economy and the biofuel chain by utilizing industrial by-products, which adds value to agro-industrial waste.

Source: Own elaboration, based on [8,13,14,19,21–23].

Thailand could also be a leader in terms of biodiesel production from coconut husk waste oil, since the country is the sixth largest producer of coconuts in the world, and 80% of the fruit is discarded, constituting significant generation of waste [28]. Brazil stands out for its research into macauba waste oils, due to the widespread use of macauba as

a raw material in the national biodiesel production industry, with a potential yield of 1500–5000 kg of oil per hectare, which is only lower than palm oil production, as well as biodiesel from used cooking oil [37], residual chicken fat oil [20], and residual babassu oil [30]. Argentina stands out for its production and export of soybean oil and sunflower oil [14].

3.2.5. When?

In recent decades, enzymes have emerged as a novel class of catalysts within the field of modern synthetic chemistry [14]. It is noteworthy that environmental concerns have increasingly driven the pursuit of technologies characterized by low greenhouse gas (GHG) emissions throughout their life cycle.

Biofuels play a critical role in the energy transition and the decarbonization of the transportation sector, particularly in hard-to-abate transport modes, such as aviation, maritime shipping, and heavy-duty road transport [38]. Their relevance stems from the fact that they can be integrated into existing infrastructure and do not require substantial modifications to current vehicle fleets, thereby facilitating their adoption [39]. Consequently, the enzymatic production of biodiesel from residual feedstocks offers a pathway to reduce net CO₂ emissions in sectors where electrification remains impractical, with the added advantage of compatibility with fossil diesel blends.

Several international institutions have adopted net-zero emission targets for 2050, reflecting a global commitment to climate mitigation. Among these, the International Maritime Organization (IMO) has established specific goals for the decarbonization of maritime transport [40]. In light of these commitments, it becomes imperative to investigate alternative production pathways capable of generating advanced biodiesel.

Furthermore, the European Union, particularly France and Germany that historically lead the production of biodiesel, relied predominantly on palm oil as a feedstock. However, the use of palm oil has faced increasing restrictions following the implementation of renewable energy targets under the Renewable Energy Directive II (REDII), which limits the use of first-generation biofuels associated with indirect land use change, including palm oil [39].

The development of advanced biodiesel from residual oils contributes to the fulfillment of these regulatory goals and, from an economic standpoint, opens new market opportunities for advanced biofuels. The enzymatic route could present a solution due to its potential to convert residual oils under environmentally favorable conditions. Amongst the feedstocks identified in the literature review, residual palm oil was the most frequently utilized, accounting for approximately 42% of the studies analyzed in Section 3.2.6.

This need reflects a growing interest in scaling up the production of advanced biofuels. For the enzymatic route to achieve a high Technology Readiness Level (TRL) and become both technically and economically viable for commercial-scale production, substantial investment in research is required to optimize the process. This route enables the conversion of residual oils without the need for pretreatment, in contrast to conventional chemical transesterification [41]. Accordingly, the specialized literature reveals a concerted effort to develop enabling technologies that support the technical, economic and environmental feasibility of the enzymatic pathway in recent years, as illustrated in Figure 1.

3.2.6. How?

The residual oils are characterized by a high acidity which implies difficulties in conversion by conventional chemical route (alkaline catalysis) due to soap formation, an issue that is avoided in enzymatic conversion of this type of oil.

Literature review on enzymatic biodiesel production revealed that more than half of studies employed enzymatic transesterification, while the others utilized direct esterification of free fatty acids (FFAs). The route selection was directly influenced by feedstock lipid composition, wherein triglyceride-rich oils favored transesterification, yielding biodiesel and glycerol as byproducts [31,32,35], whereas high-FFA feedstocks like palm acid oil (PAO) from crude palm oil (CPO) refining were converted via esterification, producing biodiesel and water [23,30].

This distinction critically impacts purification stages and technical feasibility. Transesterification requires glycerol separation through decantation, centrifugation, or solvent extraction, plus neutralization washes. In contrast, enzymatic esterification generates only water as a byproduct, simplifying purification [23]. Notably, 67% of studies used non-edible residual feedstocks, avoiding food competition and land use impacts. These included palm oil mill effluent [22,25,35], coconut oil [42], chicken fat [20], crude *Jatropha curcas* oil [43], and babassu oil (*Orbignya* sp.) [30]. Studies by Moreira et al. [30], Muanruksa and Kaewkanetra [24], and Rachmadona et al. [23] specifically selected direct esterification due to the absence of triglycerides or their removal via pretreatment.

Microbial lipases dominated enzyme choices, particularly *Thermomyces lanuginosus* used in commercial formulation of Eversa Transform 2.0[®] [22,31] and *Candida rugosa* lipases [42]. Enzymatic conversion in presence of methanol was used in 64% of studies due to its high reactivity and low cost [24,25,31], while 44% used renewable-sourced ethanol [23]. Reaction conditions consistently employed moderate temperatures, predominantly 35–40 °C, to maintain enzyme stability and reduce energy demand [35,36]. Agitation ranged 200–300 rpm, with optimal alcohol/oil molar ratios between 3:1 and 9:1. Approximately 27% of studies implemented stepwise alcohol addition to prevent enzyme inhibition [22,25,31].

Immobilized lipases were employed in five studies using supports like activated carbon, plant biomass, or magnetic nanoparticles [23,24,30,32,43]. Enzyme reuse was reported in 36% of works, with five up to 15 cycles achieved without significant activity loss [23,24,30,32], highlighting industrial sustainability potential. Reaction times varied between 4 and 24 h for ethanol-based systems [22,23,30] and between 4 and 72 h for methanol tests, (typically 12–36 h). Average conversions exceeded 88%, with peak values near 98 wt%, meeting EN 14,214 and ASTM D6751 standards (>96.5%).

Geographical analysis reveals enzymatic biodiesel research aligns with regions abundant in residual oils [33] and key studies originated countries with robust agroindustrial waste streams and supportive policies such as Brazil [30,31], Indonesia [22,23], and Thailand [24,35,36,42]. Indonesian and Thailand studies focused on palm/coconut oil residues [36,42], while Brazilian research exploited regional resources like chicken fat and babassu oil [19,20]. Argentina's focus on soybean/sunflower oils [8] reflects its role as a vegetable oil export economy. This spatial distribution confirms that enzymatic biodiesel advancement depends on synergies between agroindustrial waste availability and sustainable energy policies.

3.2.7. How Much?

While several studies identified in the literature review qualitatively address cost factors, only two provide detailed quantitative economic analyses. Rachmadona et al. [23] and Pasha et al. [44] present explicit cost comparisons between enzymatic and chemical routes, including capital and operational expenditures. Other studies indicate trends, such as high enzyme costs or the economic advantages of residual oils, without systematic financial evaluations. This gap underscores the need for more comprehensive economic models to validate such claims.

Biodiesel production costs are heavily influenced by the feedstock used. Conventional biodiesel production via alkaline-catalyzed transesterification requires feedstocks with low free fatty acid (FFA) content ($<0.5\%$) to avoid soap formation [44]. However, residual oils typically exhibit high acidity, limiting conventional chemical routes due to yield reduction and increased waste generation, necessitating costly pretreatment [44]. Commercial lipases enable high conversions of acidic oil without pretreatment, potentially reducing operational costs and improving the economic viability of second-generation biodiesel [31]. Conversely, studies by Dias et al. [45] and Gusniah et al. [46] note that the enzymatic route's slower reaction rates may increase energy consumption.

Pasha et al. [44] demonstrate that these limitations can be overcome through two-stage enzymatic technology, which tolerates high feedstock acidity. This approach efficiently converts low-quality oils (e.g., waste cooking oil) without compromising biodiesel quality, offering economic and environmental advantages over conventional chemical routes, including high-purity biodiesel, reduced effluent generation, milder reaction conditions (lower energy demand), and lower capital investment [47,48].

Rachmadona et al. [23] emphasize that refined oils increase costs, with enzymatic biodiesel priced at \$0.63/L compared to \$1.08/L for the chemical route. Residual oils like palm acid oil (PAO) may reduce costs by 50% due to their high FFA content, eliminating pretreatment needs. The low cost of widely available residual feedstocks could enhance enzymatic viability, as feedstock costs represent up to 80% of total biodiesel production costs for refined oils [30,31,44,49].

Enzyme costs remain a critical barrier to commercial-scale production [32,44–47], driving research into cost-effective alternatives [44]. Muanruksa and Kaewkannetra [24] argue that immobilized enzymes combined with residual oils could mitigate total costs through reuse. Pasha et al. [44] show that immobilized lipases (\$145–290/kg) reduce energy demand by ~86%, lowering the minimum selling price to \$0.66/kg (residual oil) versus \$0.82/kg (soybean oil). Although enzymes are costlier than chemical catalysts, optimization strategies (e.g., low-dosage liquid enzymes or cheaper commercial lipases) could improve competitiveness [25]. Suwanno et al. [35] compared commercial lipase (\$406.30/g) with partially purified crude palm-derived lipase (\$0.01/g) to reduce cost impacts.

Infrastructure and energy requirements also affect costs. The chemical route incurs 33% higher expenses due to corrosion-resistant equipment and neutralization waste [44], while enzymatic methods simplify infrastructure [23].

4. Project Developed in Brazil Focused on Biodiesel Production from Residual Oil of Corn Ethanol Using the Enzymatic Route

The Research and Characterization Center for Crude Oil and Fuels (Coppecomb) and the Freight Transport Laboratory (LTC) of the Alberto Luiz Coimbra Institute for Graduate Studies and Research in Engineering (COPPE), through CNPq Process 405875/2022-3 linked to the CNPq/MCTI/FNDCT Call No. 18/2022, are conducting the “Preliminary Economic-Financial and Environmental Feasibility Study for Scaling Up Biodiesel Production from Residual Oil of Corn Ethanol via the Enzymatic Route.”

Since 2002, the team has been conducting laboratory-scale research on the enzymatic route and recently carried out initial technical feasibility studies on using residual organic load (DCO) via this enzymatic pathway for biodiesel production in a pilot project. However, further studies are required to support the scale-up of production. Consequently, the currently proposed method consists of four phases: (1) a pilot project to optimize biodiesel production; (2) collection, analysis, and processing of primary and secondary data; (3) a preliminary economic-financial and environmental feasibility study to ensure the

competitiveness of this sustainable biofuel in the Brazilian market; and (4) communication of the study results.

The project aims to conduct a preliminary economic-financial and environmental feasibility study to adapt an existing plant with new enzymatic biodiesel production technology, utilizing part of its installed capacity to process residual oil from corn ethanol production. The study focuses on a conventional biodiesel plant (chemical route) owned by Cesbra, part of the Sumatex group, operating since 2006 and located in the state of Rio de Janeiro. The objective is to determine the production cost of biodiesel under these conditions and assess its competitiveness in the national market, thereby expanding the supply of this biofuel to meet the gradual increase in the mandated blending rate of biodiesel in mineral diesel. Regarding the application of the 5W2H Tool described in Sections 2 and 3, the following subsections present a summary of its application within the context of this project.

4.1. What?

DCO is established as a strategic raw material for biodiesel production, especially when combined with the enzymatic route. Derived as a byproduct from the fermentation and distillation process of ethanol at bioenergy plants, DCO is obtained after the separation of solids and liquids present in the final stage of corn ethanol production. This lipid residue, previously underutilized, has gained value as an energy input due to its chemical composition rich in free fatty acids (FFA) and triglycerides.

From a technical standpoint, DCO exhibits properties that make it highly suitable for biodiesel conversion. Its high concentration of fatty acids, particularly linoleic acid and oleic acid, favors conversion into methyl esters through transesterification, especially when enzymatic catalysis is employed in presence of methanol. While conventional chemical processes face limitations due to the high acidity of DCO, requiring a pretreatment step, Enzymatic catalysis, due to its selectivity and tolerance to impurities, allows direct conversion of acidic oil with higher yield and lower generation of undesired byproducts such as soaps.

Beyond technical and operational feasibility, the use of DCO offers important environmental and economic advantages. As a residue from the established industrial process of corn ethanol production, it is available in large volumes, especially in the United States and countries that adopt corn as a base for their bioenergy matrix. This gives DCO the character of an abundant, low-cost byproduct with a logistics chain integrated into the biofuel sector, reducing transportation and storage costs.

Another relevant factor is that being non-edible, DCO avoids conflicts with food security and contributes to the consolidation of a second-generation biofuel chain. Its use as a feedstock for biodiesel promotes the reuse of agroindustrial residues and reduces pressure on agricultural crops intended for edible vegetable oil production, favoring more sustainable and multifunctional agricultural practices.

Thus, the choice of DCO as a focus for biodiesel production via the enzymatic route is justified not only by its technical and economic viability but also aligns with contemporary demands for clean, circular energy solutions integrated into the agroindustrial chain. Its use strengthens synergy between sectors—ethanol and biodiesel—promoting systemic efficiency gains and contributing to the energy transition on a more sustainable basis.

4.2. Who?

Stakeholder mapping enables the identification of the wide range of actors involved in biodiesel production via the enzymatic route, allowing anticipation of risks, detection of potential conflicts of interest, and development of effective mitigation strategies [27]. The key stakeholders encompass a broad spectrum of participants, including ethanol

producers, biodiesel plants, suppliers of biotechnological inputs such as enzymes, waste oil suppliers, academic researchers, investors, government agents, public policy makers, and representatives from civil society. Each group plays a strategic role, exerting either direct or indirect influence over the biodiesel value chain. Understanding their diverse motivations, interests, and challenges is essential to ensuring the viability, scalability, and sustainability of the enzymatic biodiesel production process.

Among these, local communities represent a vital stakeholder group due to their exposure to potential impacts from changes in land use, natural resource consumption, and waste generation. A transparent and participatory approach involving these communities is critical to securing social acceptance and minimizing opposition to biodiesel projects.

Regulatory agencies, such as the National Agency of Petroleum, Natural Gas and Bio-fuels (ANP), alongside environmental authorities, are key players responsible for licensing and ensuring compliance with environmental and operational standards. It is important to recognize that the production capacity and actual output correspond to biodiesel plants authorized by the ANP, whose numbers fluctuate annually [50].

Operating within this regulatory framework, the National Program for the Production and Use of Biodiesel (PNPB), established in 2003, and initiated in 2005 [51], aims to promote the sustainable production and use of biodiesel in Brazil's energy matrix, with a strong focus on social inclusion and regional development. Recognized as the foundational regulatory framework for biodiesel in Brazil, the PNPB actively engages multiple stakeholders to fulfill its objectives [50]. Its key directives include (i) implementing a technically and economically viable program that fosters job creation and income generation; (ii) ensuring competitive prices, quality, and reliable biodiesel supply through government mechanisms and controls; and (iii) encouraging the cultivation of diverse oilseeds across Brazil's regions for biodiesel feedstock [52].

To strengthen social inclusion and regional growth, the Social Fuel Seal (*Selo Combustível Social*—SFS) was introduced in 2005 and is actually regulated by Decree n° 10.527/2020. This certification consolidates social policies by incentivizing the use of regionally suitable oilseeds, securing raw material supply for biodiesel production, guaranteeing product quality for consumers, and enhancing the competitiveness of biodiesel compared to conventional diesel [52].

In the Brazilian context, CARDOSO, ASSIS SHIKIDA & FINCO [50] investigated stakeholder perceptions regarding the PNPB's guidelines, considered the sector's main regulatory milestone. Their survey-based analysis revealed a trade-off between advancing production technologies and achieving the social inclusion goals of the program. Specifically, the sector shows reluctance toward adopting more advanced technologies, as this could impede the inclusion of family farmers, a core stakeholder group. This highlights the need for revising PNPB policies should technological development become a primary focus, in order to maintain smallholder participation in the national economy.

Supporting this view, MATOS & SILVESTRE [27] explored how companies such as Petrobras and Eletrobras have successfully managed stakeholder conflicts by embedding sustainability in their business models. Their findings emphasize that active engagement of diverse stakeholders, promotion of local capacity building, and a shift from singular economic objectives to integrated economic, social, and environmental goals are fundamental to implementing sustainable business practices in complex environments.

In summary, the actors involved in enzymatic biodiesel production represent a complex and interconnected network, spanning ethanol producers, biodiesel manufacturers, enzyme suppliers, waste oil suppliers, academic researchers, investors, government officials, public policymakers, and civil society representatives. Each group's motivations, interests, and challenges must be comprehensively understood and integrated into pol-

icy and operational decisions. Only through this holistic stakeholder approach can the enzymatic biodiesel value chain achieve sustainable growth that balances technological innovation with social inclusion and environmental responsibility. Furthermore, it is expected that future policies will increasingly recognize the value of waste and residue streams alongside traditional raw materials, promoting a more circular and efficient biofuel economy [50].

4.3. Why?

DCO is a by-product of corn ethanol production, obtained together with Distillers Dried Grains with Solubles (DDGS), which are used for animal feed. However, removing the DCO from the grain increases the value of the feed, which becomes more palatable for animal consumption. Thus, DCO is a low-cost raw material that is already available on the market. Due to its prominent role in ethanol production in Brazil, the sector sought to produce ethanol from other raw materials, such as corn. Brazil is the third largest producer of corn in the world and the second largest exporter of the grain [53], ensuring the continuity of ethanol production from this source and, consequently, of DCO.

For the use of DCO, the enzymatic route is a viable technological alternative on a small scale, promoting lower production costs, as it does not require pretreatment of the waste oil, in addition to allowing the recovery and reuse of the enzyme used in the process. Furthermore, it generates less waste in the process, produces higher quality glycerin with greater added value, and generates less effluent.

However, to reach commercial scale, innovative technologies must overcome the so-called Death Valley, in which pilot projects play an important role [54]. To scale up the production of an innovation, two phases are necessary: the pilot phase, which concerns the innovation itself, and the scaling phase, which concerns its dissemination [55]. The implementation of pilot projects to enable commercial scale has a major role to play in diversifying the energy matrix and promoting environmentally responsible solutions, as well as providing social and economic benefits, as shown in Table 5.

4.4. Where

It is important to understand how the market, represented by the potential supply of raw materials (DCO), and demand, characterized by biodiesel production, behaves in the Brazilian scenario. In order to identify the challenges and bottlenecks generated by the proximity between supply and demand, especially when it comes to complying with the SDGs, mainly those related to decarbonization, and to analyze transportation costs.

4.4.1. Supply of Raw Materials

With regard to the location of raw material production in Brazil, it can be observed that over eleven consecutive harvests, there has been an increase in corn ethanol production, from 37 million liters in 2011/2012 to 6.27 billion liters in 2023/2024. In the 2023/2024 harvest, corn ethanol accounted for 18.7% of total ethanol produced, compared to 17% in the previous harvest (2022/2023). Production is highly concentrated in the states of Mato Grosso (72%), Mato Grosso do Sul (16%), and Goiás (11%), reflecting the consolidation of this biofuel in regions where its viability was previously considered limited [60].

According to the União Nacional do Etanol de Milho [52], Brazilian biorefineries producing ethanol from corn, described in Table 6, are divided into biorefineries in operation, authorized by Agência Nacional do Petróleo, Gás Natural e Biocombustíveis (ANP) and industrial plant projects. Regarding the 24 operational plants of corn ethanol, in 2024, 13 plants were full-cycle units and 11 were flex-fuel units.

Table 5. Importance of using DCO for environmental, social, and economic pillars.

Pillars	Benefits	Description
Environmental	Waste Recovery	By using waste oil, the process avoids competition for edible oil.
		It avoids improper disposal and promotes the reuse of lipid waste in biodiesel production.
		During the reaction, alcohol (methanol/ethanol) can be recovered and reused, as well as the immobilized enzyme, which can be reused between 10 and 15 cycles without losing its activity.
	Reduction in GHG emissions	Enzyme production operates under milder temperature conditions, which avoids the use of aggressive chemical catalysts, positively impacting energy consumption and potentially reducing GHG emissions.
	Reducing the environmental impact of pretreatment	The use of immobilized lipase allows for the direct conversion of free fatty acids from waste oils, eliminating the need for chemical pretreatment steps, which typically involve toxic or corrosive reagents, contributing to a cleaner process.
Social	High efficiency with less waste generation	The high level of conversion into esters reduces the formation of by-products, contributing to more efficient production and less waste of unused materials.
	To promote food security	By using non-edible raw materials (such as waste oils and animal fat), the process avoids competition with food production.
	Green job creation	The application of enzymatic methods with the conversion of more accessible inputs and the use of local waste oils, such as cooking oil, suggests potential for the development of small industries and regional services, from collection to production, which could benefit local communities. Furthermore, the jobs generated by the biofuel value chain are considered entirely green.
	Potential for adding value to socio-biodiversity products	The use of residual oil such as babassu, typical of extractive regions in countries such as Brazil, can be an opportunity for regional economic development through the use of waste from socio-biodiversity production chains, promoting productive inclusion and strengthening traditional communities.
Economical	Diversification of the energy matrix	The expansion of renewable energy sources, using different types of waste oils and fats as feedstock for biodiesel, promotes energy resilience by reducing dependence on fossil fuels or monocultures.
	Economic additionality in the industry value chain	The biodiesel production system can be integrated into the circular economy and the biofuel chain by utilizing industrial by-products, which adds value to agro-industrial waste.

Source: Own elaboration, based on [26,30,32,56–59].

Table 6 underlines the current strength of the Midwest region as a producer, with around 84% of the country's biorefineries in operation, around 44% of authorized biorefineries, and approximately 62% of planned biorefineries.

4.4.2. Biodiesel Production Plants

According to ANP [62] in 2024, approximately 9 million m³ of biodiesel were produced and distributed across 59 facilities, with the southern region (41%) and central-western region (40%) accounting for the majority of production. The states of Mato Grosso

(2 million m³) and Goiás (1 million m³) located in the Midwest region, as well as the states of Rio Grande do Sul (2 million m³) and Paraná (1 million m³) located in the South region, accounted for about 60% of national production, while the state of Rio de Janeiro, located in the Southeast region, produced only 147,000 m³ of biodiesel, just 2% of national production.

Table 6. Corn ethanol biorefineries in Brazil.

Situation	Units	Local	
In operation	25	2 in the Northeast region	1 in Alagoas
			1 in Maranhão
		21 in the Center-West region	7 in Goiás
			11 in Mato Grosso
			3 in Mato Grosso do Sul
		1 in the Southeast region	1 in São Paulo
		1 in the South region	1 in Paraná
Authorized	16	2 in the North region	1 in Rondônia
			1 in Tocantins
		2 in the Northeast region	2 in Bahia
		7 in the Center-West region	7 in Mato Grosso
		5 in the southern region	1 in Paraná
			1 in Santa Catarina
			3 in Rio Grande do Sul
Projects	16	3 in the North region	2 in Pará
			1 in Tocantins
		3 in the Northeast region	2 in Bahia
			1 in Piauí
		10 in the Center-West region	2 in Goiás
		8 in Mato Grosso	

Source: Own elaboration by UNEM [61].

The use of soybean oil as raw material stood out in 2024, accounting for about 72% of production, while other raw material sources, such as animal fat (chicken, pork), cottonseed oil, canola oil, used cooking oil, and corn oil, accounted for only about 6% of production. The remaining 22% was represented by beef fat, other fatty materials, palm oil, and sunflower oil [62]. In terms of biodiesel production from corn ethanol by-products, we can highlight the Neomille plants operating in two states in the central-west region, Goiás and Mato Grosso do Sul [63], the planned investment in corn ethanol production and, consequently, biodiesel from by-products by the Potencial Group located in Paraná in the southern region [64]. In the state of Rio de Janeiro, in the southeastern region, the CESBRA study for the production of biodiesel from by-products, more specifically DCO, stands out.

4.4.3. Proximity Between Supply and Demand—Challenges

The location of the biodiesel production plant plays an important role in reducing the costs of supplying vehicles for transporting raw materials and GHG emissions [65], which favors biodiesel production from the use of DCO, mainly in the central-western region of Brazil, which has the largest number of corn ethanol production units.

This study identifies a challenge associated with the proximity between the supply of raw material (DCO) and the production unit, to be overcome by the project, since the state of Rio de Janeiro does not produce corn ethanol, with the nearest units located in other states such as São Paulo and Paraná, which creates a need for raw material storage infrastructure and uncertainty regarding the efficiency of large-scale production [66].

4.5. When?

Brazilian Nationally Determined Contributions (NDC), aligned with the Paris Agreement, aim to mitigate GHG emissions to prevent global rise in average temperature. Brazil commits to reducing emissions by 59–67% by 2035 and achieve net zero emissions in 2050. Given Brazil's historical reliance on biofuels in the transport sector, its energy transition is tied to this industry, particularly ethanol and biodiesel.

Da Costa et al. [39] highlight the potential of biofuels in hard-to-abate transport modes, such as maritime and road transport, to achieve Brazil emissions reduction targets in the medium and long term. Guerra et al. [34] concludes that Brazil has significant green energy potential as a key player in the global energy transition scenario.

In this context, investing in advanced biofuels, such as enzymatic biodiesel production in symbiosis with corn-ethanol by-product, could not only pave the way toward achieving emission targets but also offer a more sustainable alternative to diesel while strengthening export market supply, including Europe Union market in alignment with REDII regulations [39].

4.6. How?

The study of enzymatic biodiesel conversion process has been optimized in laboratory scale to achieve better conversion rates and biodiesel characterization under the ANP parameters norms and regulation. In this context, the enzymatic transesterification of DCO into biodiesel in the presence of methanol has been conducted in two steps, based on the approach proposed by Pasha et al. [44]. The first step employs free enzymes and the second utilizes immobilized enzymes.

Experimental reactions using 0.5 L of DCO have been performed with varying proportions of methanol and different types of enzymes, aiming to assess their impact on conversion efficiency and reaction time. The free and immobilized enzymes tested thus far include liquid lipases and Habio Lipozyme produced and kindly donated by Tsinghua University. The reactions were carried out under mild conditions, consistent with those reported in the literature for enzymatic conversion, at a temperature of 40 °C.

During the first phase, DCO was blended with free lipase at concentrations ranging from 1% to 8% *v/v*. Methanol was gradually added at a molar ratio of 7:1. The highest yields were obtained with lipase concentrations between 2% and 6% *v/v*, reaching biodiesel conversion rates of 96.7% and 97.2%, respectively.

In the second phase, the concentration of immobilized lipase varied from 1% to 5.5% wt, based on the mass of the oil substrate. The best result was achieved with 1 wt% of immobilized lipase, yielding 90% biodiesel. Reaction times ranged from 20 to 30 h, with the most optimized conditions resulting in completion around 23 h.

The experimental procedures are still ongoing, with the objective of optimizing biodiesel production via enzymatic transesterification using DCO as the feedstock. Current efforts focus on refining reaction parameters such as enzyme concentration, methanol-to-oil molar ratio, and reaction time, in order to enhance conversion efficiency, ensure compliance with ANP biodiesel specification [67], and process scalability. The review of existing literature could support the theoretical framework of this study, allowing for the identification of best practices, benchmarking of results, and contextualization of findings within the

broader field of biofuel research. By systematically evaluating different combinations of free and immobilized lipases under mild reaction conditions, the study in laboratorial scale using DCO aims to establish a robust and economically viable route for sustainable biodiesel synthesis from waste oil sources.

In this context, two-step enzymatic routes may differ from those identified in the literature. The second step aims to meet the parameters established by ANP in Brazil, with focus on reducing water content and acidity level while increasing conversion efficiency and ester content. Low conversion rates could be observed in tests in laboratory scale, inferior to those of literature. Compared to the literature studies analyzed, the two-step enzymatic route presented a higher reaction time, enhancing the need of optimization.

4.7. How Much?

This study, conducted within the scope of the project, aims to assess, among other analyses, the economic feasibility of enzymatic biodiesel production from DCO. Currently, this route could be classified as Technology Readiness Level (TRL) 3–4, as it involves laboratory-scale testing to evaluate technical feasibility [39].

The preliminary financial analysis will be based on the reaction mass balance, which will be used to scale up enzymatic biodiesel production to an industrial level at CESBRA's facility, complementing conventional chemical route biodiesel production. Capital Expenditures (CAPEX) and Operational Expenditures (OPEX) will be estimated, enabling the calculation of the enzymatic biodiesel production cost and an assessment of its competitiveness in the national market.

Although Pasha et al. [44] investigated the costs of a similar two-step enzymatic route, a direct economic comparison with the Brazilian project is challenging. The costs of key inputs for biodiesel production are expected to differ significantly due to regional market variations, a key example being the lack of domestic enzyme production in the Brazilian market.

Since this involves an adaptation of existing production infrastructure, capital costs will be reduced, requiring only the acquisition of the reactor developed and described by Pasha et al. [44] and the necessary equipment for its integration into the plant. Additionally, an economic analysis of feedstock supply and demand in the Brazilian market will be analyzed, considering biodiesel production from DCO and the increasing biodiesel demand due to the mandatory blending policy in Brazil, which is expected to reach a blend of 20% biodiesel in mineral diesel (B20) by 2030 [38].

In 2023, the processing of 13.3 million tons of corn generated 5.8 billion liters of corn ethanol [38] and approximately 200 million liters of DCO [68]. Projections indicate that corn ethanol production will reach 15.5 billion liters by 2034 [38], corresponding to an estimated DCO supply of approximately 389 thousand tons by 2034.

For biodiesel, considering only the mandatory blending mandate and its inclusion in marine bunker fuel for export, demand is expected to reach 12.5 billion liters by 2034. The PNPB advocates for the diversification of regional feedstocks, emphasizing the need for investments in this input mix [38]. In this context, as discussed in Section 4.4, the Central-West region of Brazil is a suitable location for DCO-based biodiesel production, adding value to the corn ethanol supply chain while mitigating high logistics costs.

Furthermore, special consideration will be given to the supply of enzymes used in the process, which may lead to increased raw-material acquisition costs and potentially hinder commercial scale-up. The most commonly used commercial enzymes found in the literature were the immobilized enzyme Novozymes 435, now marketed as Lipozyme 435[®] (lipase from *Candida antarctica*, activity > 2 U/mg), and the liquid enzyme Eversa Transform 2.0[®]. Both are produced by Novonesis [69] and commercialized in Brazil by average cost of

R\$1388.00 per gram [70] and R\$5.892,00 per liter [71], respectively, confirming the high costs of enzymes in the production process.

Methanol is a key input in biodiesel production. The United States dominates global methanol production due to its access to low-cost natural gas, the primary feedstock. In 2023, Brazil imported 767.1 thousand tons of methanol to support its biodiesel industry. As a commodity, methanol prices are determined by global supply and demand, making it a strategic variable in national energy planning. This dependence underscores the need to evaluate domestic production alternatives to ensure long-term supply security and price stability. In the first half of 2025, the average price of methanol was approximately \$870.00 per ton [72].

In 2025, the average refinery-gate price of biodiesel (B100) was R\$5.60 per liter, while mineral diesel was priced at R\$2.80 per liter, increasing to approximately R\$3.80 per liter after biodiesel blending [73,74]. Enzymatic biodiesel may face market entry challenges if its production cost raises the final price of the biodiesel-diesel blend.

5. Conclusions

This study examined the feasibility of enzymatic biodiesel production from waste oils, with particular attention to distiller's corn oil (DCO). The results indicate that Brazil holds significant structural advantages, including a growing supply of residual oils and a strong agro-industrial base. However, critical barriers persist, such as high enzyme costs, limited process optimization, and uncertainties in regulatory frameworks, which hinder the transition from pilot-scale initiatives to industrial-scale deployment.

Cost comparisons show that enzymatic routes can achieve competitive levels relative to conventional chemical processes, particularly when residual oils are used, as they represent the majority of production costs. This reinforces the potential of enzymatic biodiesel as an economically viable pathway, provided that technical challenges—such as enzyme stability, reaction efficiency, and scalability—are effectively addressed.

Another central finding is the importance of governance and institutional support. The evidence suggests that technological progress alone will not be sufficient to consolidate enzymatic biodiesel as a competitive alternative. Regulatory modernization, investment incentives, and coordinated stakeholder collaboration, including public–private partnerships, are essential to bridge the “valley of death” between laboratory innovation and market adoption. Furthermore, international dynamics play a strategic role. Restrictions on conventional feedstocks, such as the European Union's limitations on palm oil, highlight the opportunities for advanced biofuels. In this context, Brazil's competitiveness will depend not only on its technological capacity but also on policy alignment with global sustainability standards.

Enzymatic biodiesel production presents promising opportunities to diversify renewable energy sources, valorize waste oils, and contribute to decarbonization targets. Its successful deployment will rely on the convergence of three dimensions: (i) technological innovation to enhance efficiency and reduce costs, (ii) institutional and regulatory frameworks that provide stability and incentives, and (iii) effective stakeholder engagement that integrates academia, industry, and government.

6. Future Research Directions

Future studies should prioritize advancing technological innovations to optimize enzymatic biodiesel production. Reducing enzyme costs remains a critical challenge, which may be addressed through strategies such as immobilization on low-cost supports, development of recombinant and engineered lipases, and adoption of hybrid catalytic systems that combine enzymatic and chemical processes. Pilot-scale experiments and process

simulations will be essential to validate laboratory results under real-world conditions, identify technical bottlenecks, and optimize parameters such as enzyme concentration, alcohol-to-oil ratios, and reaction times.

Further research should also explore system integration. For example, coupling enzymatic biodiesel plants with existing ethanol refineries could enhance circular production chains, particularly when using distillers corn oil (DCO) as a feedstock. Such approaches could create systemic synergies, improving both economic and environmental performance.

In parallel, comprehensive economic and environmental assessments are necessary to strengthen the business case for enzymatic biodiesel. Detailed techno-economic analyses and sensitivity studies should evaluate the impacts of feedstock variability, enzyme reuse, energy requirements, and logistics. Moreover, the application of life cycle assessment (LCA) and social life cycle assessment (S-LCA) would enable a more complete comparison between enzymatic and conventional routes, incorporating indicators such as greenhouse gas (GHG) emissions, energy balance, water use, job creation, and local development opportunities.

Finally, institutional, regulatory, and governance aspects deserve closer attention. Future research should investigate how national programs such as RenovaBio and the PNPB can be adapted to foster advanced biofuels while maintaining social inclusion mechanisms like the Social Fuel Seal. Comparative analyses of international regulatory frameworks, including those in the European Union, United States, and Southeast Asia, could provide valuable lessons for Brazil to strengthen its competitiveness. Additionally, studies on financing models, green investment instruments, and public–private partnerships will be fundamental to overcoming the financing gap that often prevents promising pilot projects from reaching commercial maturity.

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