

Ethanol Production from Residual Glycerol Generated during Biodiesel Processing **Camille Rios^{1*}, Leticia Assis², Nalanda Santos³, Vanessa Santos⁴, Jársia De Melo⁵, Wadson Barbosa⁶**

Universidade Senai Cimatec, Salvador, Bahia, Brazil

camille.rios@ba.estudante.senai.br, leticia.assis@ba.estudante.senai.br, nalanda.s@ba.estudante.senai.br,
vanessa.n.santos7@ba.estudante.senai.br, wadson.barbosa@ba.docente.senai.br,
jarsia.melo@ba.docente.senai.br

In the pursuit of alternative and sustainable fuels, biodiesel production from biomass generates residual glycerol as a byproduct, which holds significant potential for reuse in the petroleum industry. This study aims to investigate the process of converting glycerol into second-generation ethanol through microbial fermentation using the yeast *Pichia membranifaciens*. The research was conducted through a qualitative literature review, covering topics ranging from glycerol purification to the factors that influence fermentation, such as pH, temperature, and medium composition. The results show that the use of purified glycerol enhances ethanol production efficiency due to the reduced interference of impurities in microbial growth. The yeast *Pichia membranifaciens* stood out for its ability to metabolize glycerol in low- or zero-oxygen environments, making it a viable alternative. It is concluded that the valorization of glycerol as a raw material for ethanol production contributes significantly to sustainability by reducing waste and adding value to the biodiesel production chain, promoting practices aligned with the circular economy and the development of more efficient and environmentally friendly biofuels.

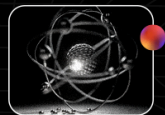
Keywords: Biodiesel, Glycerol, Second-Generation Ethanol, Microbial Fermentation.

The search for clean and sustainable energy sources has encouraged the exploration of alternatives to fossil fuels. In this context, biodiesel stands out as a viable option, being produced from vegetable oils or animal fats through the transesterification process, which generates glycerol as a byproduct.

According to Bueno et al. (2009), improper management of glycerol can cause serious environmental problems, especially regarding the pollution of water bodies. Therefore, converting glycerol into ethanol—a key input as a renewable and sustainable fuel, as well as an ally in environmental preservation—not only adds economic value to the byproduct but also benefits the environment by reducing the negative impacts caused by its improper disposal. Furthermore, the use of

biomass-based raw materials in the production of second-generation (2G) ethanol may represent a viable solution to the current energy crisis and the growing global demand for sustainable energy sources.

In this scenario, the development of integrated processes that allow maximum utilization of generated residues becomes essential. The valorization of glycerol not only promotes environmental sustainability but can also economically boost the biodiesel production chain by providing new applications for a byproduct once considered to have low commercial value. This perspective reinforces the importance of scientific and technological research in identifying more efficient conversion routes, including the use of microorganisms with biotechnological potential capable of transforming waste into high value-added



products.

Biomass, due to its high oil content and the possibility of utilizing its residues in biodiesel production, emerges as a promising raw material in this context. The integration of processes that use biomass for biodiesel production and the conversion of its residual glycerol into ethanol can result in a more efficient and sustainable production chain, aligned with the principles of the circular economy.

Given this, several questions arise: How can residual glycerol from biodiesel be efficiently converted into ethanol, and what are the technical and economic challenges of this process? What are the environmental benefits associated with ethanol production from residual glycerol? How can microbial fermentation, especially using yeasts such as *Pichia membranifaciens*, optimize this conversion?

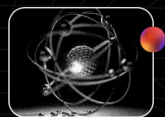
This study aims to present a proposal for the process of ethanol production from residual glycerol, a byproduct of biodiesel production using biomass as feedstock. The specific objectives of this study include: outlining the stages of the production process, highlighting the chemical processes involved; analyzing the physicochemical properties of glycerin, which will serve as the raw material for ethanol production; and finally, emphasizing the advantages of the fermentation process used in production.

Theoretical Framework

This project is based on bibliographic research, integrating the knowledge of renowned authors on the production of ethanol from residual glycerin derived from biodiesel. Among the main authors consulted are Kovalski (2015), Vidal (2020), Duarte et al. (2022), and Pironti (2024). Thus, a detailed analysis of the available studies reveals the ethanol production process using microbial fermentation of glycerol and its purification, characterizing this residue obtained from biodiesel production through analyses of its physicochemical properties, and comparing it to alternative fossil fuels and other biofuels.

Among the references used, the significant contribution of Kovalski (2015) stands out, providing a solid theoretical foundation for our bibliographic research. The author addresses ethanol production from glycerol fermentation through microbial conversion, presenting this pathway as a sustainable alternative to traditional biofuel production. By using glycerol—an abundant byproduct of the biodiesel industry—as a raw material, the study proposes a strategy for the utilization of industrial waste, combining energy efficiency with the valorization of renewable resources.

Based on the author's contribution, this study proves relevant in the context of discussions on sustainability and the reuse of industrial waste. By addressing glycerol

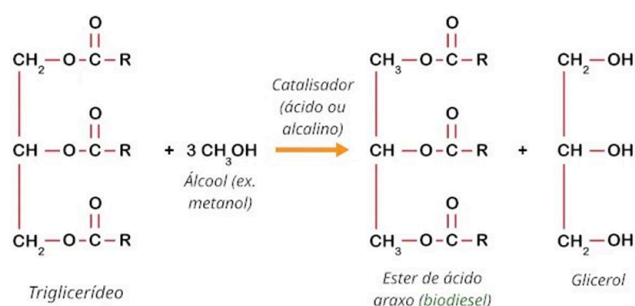


fermentation via microbial conversion as an alternative for ethanol production, it expands innovation opportunities within the industry while contributing to the reduction of byproduct accumulation from biodiesel production. The study not only provides the theoretical foundation for this research but also points to viable pathways for the development of cleaner, more affordable biofuels aligned with the principles of the circular economy.

According to Duarte et al. (2022), biodiesel has emerged as one of the most promising biofuels, being widely adopted in Brazil. However, the high production cost still represents a significant challenge to the expansion of renewable energy consumption in developing countries. It is observed that the valorization of biodiesel industry coproducts is crucial to promoting sustainability. Among these coproducts, some stand out as promising raw materials for the production of biofuels, playing an important role in the sector's economic and environmental feasibility.

Due to the production of biodiesel (Figure 1), which is carried out through the transesterification reaction, glycerin—commonly known as glycerol—is obtained as its main residue.

Figure 1. Biodiesel production from the transesterification of vegetable oils



Reference: FRANÇA, A. L. F. (2021), adapted.

As shown in Figure 1, under the action of a basic catalyst and in the presence of methanol or ethanol, the oil undergoes transesterification, forming three molecules of methyl or ethyl esters of fatty acids—which essentially constitute biodiesel—and releasing one molecule of glycerol (glycerin).

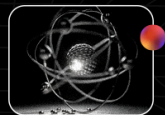
METHODOLOGY

The research involved the analysis of eight documents, including scientific articles, dissertations, and technical reports, which were organized according to their predominant themes. Most of the materials (50%) addressed the conversion of glycerol into ethanol and the processes of microbial fermentation, the central theme of this study.

Subsequently, 25% of the texts dealt with biodiesel production and the transesterification process, contributing to the theoretical framework. The ethanol scenario in Brazil was covered in 12.5% of the materials, while the remaining 12.5% consisted of technical and agricultural data to assess the feasibility of

To achieve an even higher degree of purity, the clarified Distillate I is subjected

Quantum Technologies: The information revolution that will change the future - 2025



to a second distillation, this time under vacuum conditions in order to prevent the thermal decomposition of glycerol. The system includes condensers and coolers, which allow for the efficient recovery and condensation of the generated vapors. The final product obtained, referred to as Distillate II, is a high-purity glycerin.

The characteristics of glycerol to be used as a raw material for ethanol production present specific parameters. When comparing crude glycerin with pure glycerin, it becomes evident that the purification process previously described plays a fundamental role, since the impurities present in the crude form can compromise efficiency and yield, while the purified form ensures better performance.

Pure glycerin is a more stable and efficient substrate for ethanol conversion, unlike crude glycerin, whose impurities can inhibit microbial growth and reduce fermentation efficiency. Properties such as acidity, density, moisture content, pH, glycerol content, viscosity, flash point, and energy value directly influence the fermentation process. Acidity affects enzyme activity; density and viscosity influence the solubility and transfer of nutrients and oxygen; moisture is essential for cellular metabolism; and the pH must be suitable for the microorganism used (such as *Pichia membranifaciens*).

Inadequate glycerol levels, whether too low

or too high, can compromise cell growth.

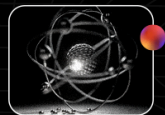
A high flash point increases the safety of glycerin use. Likewise, a high energy value favors ethanol production and sustainability. The higher the glycerol purity, the greater the efficiency in biofuel production

In ethanol production as a biofuel, the fermentation process stands out for obtaining the final product. This process is mainly carried out with the yeast *Saccharomyces cerevisiae*, which converts biomass into fuel. The use of *S. cerevisiae* in biorefineries is due to its high conversion and production rates, as well as its strong resistance to osmotic stress and to the ethanol it produces.

However, in the production process using glycerol, this yeast does not prove to be as effective. A promising yeast in this regard is *Pichia membranifaciens*, which has the ability to metabolize glycerol under both anaerobic and microaerobic conditions, converting it into ethanol.

The conversion of glycerol into ethanol, through a pathway known as the metabolic route, takes place in two distinct regions of the cell: the cytosol and the mitochondria. The initial steps occur in the cytosol, while the final reactions, when under aerobic conditions, take place in the mitochondria.

Initially, glycerol is converted into dihydroxyacetone (DHA) through an oxidation



reaction that occurs in the cytosol. This step involves the participation of NAD^+ , an electron carrier molecule, which acts by removing electrons from glycerol, facilitating its transformation into DHA. Next, DHA is converted into glyceraldehyde-3-phosphate (G3P) through the addition of a phosphate group. This reaction also occurs in the cytosol and enables the molecule to enter the glycolytic pathway.

Next, G3P is metabolized through glycolysis, still in the cytosol, producing pyruvate. During this phase, the G3P molecule is broken down into smaller compounds, generating energy in the form of ATP and yielding pyruvate as the final product.

From pyruvate, the process can follow two distinct pathways, depending on the availability of oxygen. Under aerobic conditions, pyruvate is directed to the mitochondria, where it is metabolized in a process that generates large amounts of energy but without ethanol production. On the other hand, in environments with low oxygen availability (anaerobic or microaerobic conditions), pyruvate remains in the cytosol, where it is converted into acetaldehyde through decarboxylation, releasing a molecule of CO_2 .

Finally, the last step of the process takes place in the cytosol, where acetaldehyde is

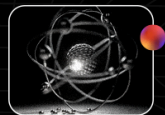
reduced to ethanol. This reaction is catalyzed by a reductase enzyme and once again involves NADH as the electron donor. This completes the metabolic pathway from glycerol to ethanol, highlighting the central role of the cytosol in the initial and final stages of the conversion, and of the mitochondria only when there is a significant presence of oxygen.

Brazil is the second-largest producer of first-generation ethanol in the world, obtained mainly from the fermentation of sugarcane juice by the yeast *Saccharomyces cerevisiae*.

Studies indicate that second-generation ethanol, obtained from residues such as sugarcane bagasse and glycerol, is a more sustainable alternative compared to fossil fuels and other biofuels. This technology can significantly reduce environmental impacts and increase productivity, while promoting more efficient use of natural resources and reducing air and soil pollution.

The production of second-generation (2G) ethanol is important as it offers a more sustainable alternative by reducing waste, in addition to lowering CO_2 emissions and providing greater energy efficiency.

The advancement of 2G ethanol is not only a technological matter but also a strategic one, positively impacting the political and economic landscape. It demonstrates that there is a global movement in favor of biofuels, which



reinforces the importance of investing in this area.

FINAL CONSIDERATIONS

This study aimed to investigate and optimize the process of converting residual glycerol from biomass-based biodiesel production into ethanol through microbial fermentation. The research was based on a systematic literature review, addressing the main scientific advances related to the topic, as well as the technical and economic challenges associated with the feasibility of this conversion.

The analysis showed that the utilization of residual glycerol represents a promising strategy not only for adding value to by-products from the biodiesel production chain but also for reducing the environmental impacts associated with the improper disposal of this compound.

The conversion of glycerol into ethanol, a second-generation biofuel, aligns with the principles of the circular economy and sustainable development, promoting energy efficiency and contributing to the mitigation of climate change effects.

Among the aspects investigated, the role of microbial fermentation using yeasts such as *Pichia membranifaciens* stands out, as they show potential in the bioconversion of glycerol into ethanol, although challenges related to process

efficiency, substrate purity, and industrial scalability still need to be overcome.

In addition, the physicochemical characteristics of glycerol and the factors that influence its use as a raw material in fermentation processes were considered.

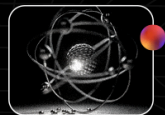
During the development of the research, several limitations were identified, such as the scarcity of consistent experimental data, the diversity in the composition of residual glycerol, and the variability in the operational conditions of fermentation processes. These factors add complexity to the implementation of efficient and economically viable industrial solutions.

In summary, the results obtained indicate that the conversion of glycerol into ethanol holds great application potential, provided that technological improvements in the processes are made and experimental studies are expanded. The adoption of this approach could represent a significant advancement for the biofuel sector, promoting greater environmental, economic, and energy sustainability.

BIBLIOGRAPHIC REFERENCES

BUENO, O. C.; ESPERANCINI, M. S. T.; TAKITANE, I. C. Biodiesel Production in Brazil: Socioeconomic and Environmental Aspects. *Ceres*, v. 56, n. 4, p. 507-512, 2009.

BUENO, P. H. S.; OLIVEIRA, J. E.; SOUZA, M. M. Glycerol Valorization for the Synthesis of



Carbon-Based Nanostructured Materials Using Zeolites: A Review. *Perspectiva Journal*, v. 43, n. 160, p. 45-58, 2009.

inhibitors or in combination. 2024. Available at:
. Accessed: Mar. 22, 2025.

DUARTE, L. G. et al. Biofuels: A Review of the Historical Overview, Production, and Applications of Biodiesel. *Meio Ambiente (Brazil)*, v. 1, n. 2, p. 1-19, 2022.

PIRONTI, Lucas Antoszczesen. Second-Generation Ethanol Production by *Pichia membranifaciens* in the Presence of Isolated or Combined Inhibitors. 2024.

VIDAL, Fátima. Perspectives for Brazilian Ethanol. Rio de Janeiro: BNDES, 2020.

KOVALSKI, Gláucia. Ethanol production from glycerol fermentation and paper industry residues. Guarapuava, PR, 2015. [s.l: s.n.]. Available at:
<https://www.unicentro.br/posgraduacao/mestrado/bioenergia/dissertacoes/09.pdf>. Accessed: Apr. 10, 2025.

GÊNICA, E. de C. 1st and 2nd generation ethanol: understand the differences. Available at:
<https://agro.genica.com.br/2024/03/08/etanol/>. Accessed: Apr. 19, 2025.

PIRONTI, Lucas Antoszczesen. Second-generation ethanol production by *Pichia membranifaciens* in the presence of isolated