The Role of Enzymes in an Efficient Ethanol Production: A Review and Technological Prospection

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This work reviews the literature on the use of enzymes in ethanol production. The bibliographic survey was carried out using the Web of Science (WoS) database, where advanced search techniques were applied with relevant keywords and Boolean operators. Articles published between 2015 and 2024 were selected, focusing on "Biotechnology Applied Microbiology" and "Energy Fuels". In total, 182 articles were analyzed using the Vosviewer software to build bibliometric networks. The results highlight the importance of enzymes as biocatalysts that increase the efficiency of ethanol production and explore technologies that can be implemented in industries to increase efficiency and reduce costs.
Keywords: Bioethanol. Enzymes. Lignocellulosic Biomass. Fermentation. Technological Prospecting.

The increasing demand for sustainable energy sources and the urgent need to mitigate climate change have driven biofuel research and development [1,2]. Among these, ethanol stands out as a promising alternative to fossil fuels [1-8]. Ethanol offers several advantages, including reducing greenhouse gas (GHG) emissions and potentially decreasing dependence on fossil fuels [3,9,10]. It is key in transitioning to a cleaner and more sustainable energy matrix.

Ethanol production can be classified into first-generation (1G), using sources such as corn and sugarcane juice, and second-generation (2G), employing lignocellulosic biomass [2,4,7]. While 1G ethanol is already widely commercialized, 2G ethanol faces significant technical and economic challenges. In this context, enzymes play a fundamental role in ethanol production, acting as biocatalysts that improve the efficiency of converting biomass into fermentable sugars and subsequently into ethanol.

Enzymes, as biocatalysts, can accelerate specific chemical reactions, such as the hydrolysis of polysaccharides into fermentable sugars and the subsequent fermentation into ethanol [8]. This catalytic ability increases process efficiency by reducing time and allows using renewable and abundant raw materials, such as lignocellulosic biomass, to produce second-generation (2G) ethanol. Furthermore, developing more robust and efficient enzymes through enzyme engineering can overcome biomass resistance and byproduct inhibition challenges, resulting in a more sustainable and economically viable process [8]. The use of enzymatic cocktails in the ethanol production process is still very costly [3].

Therefore, research and enhancement of enzymes are essential to advancing the commercial viability of ethanol as a clean and renewable energy alternative.

Technological prospecting can utilize bibliometric analysis to explore and assess large datasets, identifying emerging trends, research components, and collaboration patterns in a specific scientific field. This method uses quantitative techniques to analyze publication and citation metrics, providing a robust foundation for evaluating scientific and technological development. This study aimed to conduct a bibliometric analysis of the technical and economic feasibility of using enzymes in ethanol production, investigating related research topics and highlighting recent advances and challenges in this field.

Received on 13 September 2024; revised 21 December 2024.
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J Bioeng. Tech. Health 2025;8(1):81-88

Materials and Methods

This research is a bibliographic review with a qualitative-quantitative character, as it includes a bibliometric analysis of articles published in the field of study and technological prospecting. Figure 1 provides an overview of how the research methodology for this study was established.

We used the Web of Science (WoS) academic database for this bibliographic review. This platform was chosen due to its comprehensiveness and relevance as a scientific database. The search for articles was conducted in April 2024, using the advanced search mode of WoS to achieve more terrific refinement in the research. We defined the most relevant keywords for the study topic and then included similar terms to capture more articles within the theme. Boolean operators combine the terms (AND to include all specified terms, OR to include any terms, NOT to exclude terms). In WoS, to search for terms exactly as written, they must be placed in double quotes, and to group terms and control the search order, they must be placed in parentheses. Table 1 shows how the search for articles was defined in this research.

After an initial search, we refined the study by focusing on articles from 2015 to 2024 and setting the research areas to "Biotechnology Applied Microbiology" and "Energy Fuels." This yielded 182 articles. We then began data processing, examining the relationship between years and publication numbers, identifying the most cited publications, and linking articles to the SDGs. Using Vosviewer software, we constructed bibliometric networks to analyze authors' most frequently used keywords (minimum of 5 occurrences, resulting in 25 keywords) and the most frequently occurring words in the texts (minimum of 10 occurrences, resulting in 34 keywords).

Literature Review

This section presents the most discussed concepts in publications regarding first-generation ethanol (1G), second-generation ethanol (2G), the role of enzymes in ethanol production, and emerging technologies for increasing efficiency and reducing costs in their use in industrial ethanol production processes.

First Generation Ethanol Production (1G)

First-generation bioethanol production uses food raw materials, mainly starchy materials such as corn, wheat, barley, cassava, and potatoes, as well as sucrose-containing raw materials such

Figure 1. Flowchart of method used.

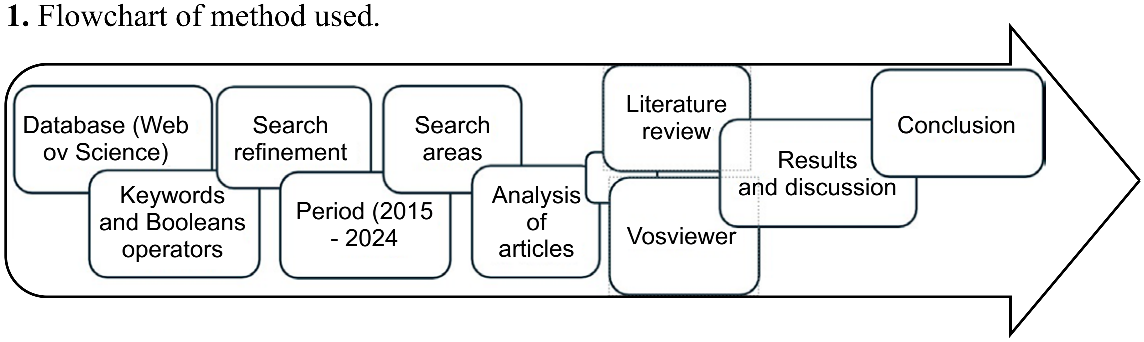


Table 1. Search parameters used in Web of Science.

(Keywords 1)	Boolean Operator	(Keywords 2)
(ethanol OR bioethanol)		(1G OR first-generation OR 2G OR second-generation)
(Keywords 3)	AND	**(Keywords 4)**
(enzymes OR enzyme)		(fermentation OR fermentacion)

as sugar cane, sugar beet, and sweet sorghum [1,3]. *Saccharomyces cerevisiae* is the most used microorganism in the bioethanol industry, having advantages such as high fermentative capacity, tolerance to ethanol, low demand for nutrients, and less formation of byproducts [9].

However, producing first-generation bioethanol from food crops, such as corn and sugarcane, raises the food *versus* fuel debate, raising concerns about food security and rising food prices [1,2]. This competition with food has brought about the need to find more sustainable ways of producing ethanol, leading to the development of second-generation (2G) ethanol.

Although 1G ethanol is well-established industrially, searching for alternatives that do not compete with food production has become essential. Using agricultural residues and lignocellulosic biomass in 2G ethanol production emerges as a promising solution, reducing the impact on food security and offering a more sustainable pathway for biofuel production.

Second Generation Ethanol Production (2G)

Second-generation (2G) bioethanol is typically produced from lignocellulosic biomass, but it is also possible to use industrial byproducts, such as whey or crude glycerol, as feedstock [1]. The conversion of lignocellulose into reducing sugars is more complex than that of starch used in first-generation (1G) ethanol production [1]. However, 2G bioethanol, produced from lignocellulosic biomass such as agricultural residues, offers an alternative that does not compete with food production and uses materials that would otherwise be wasted [5].

The yeast *Saccharomyces cerevisiae*, widely used in the ethanol industry, quickly ferments hexoses present in 1G substrates. However, it has a low capacity to ferment pentoses, which are predominant in 2G substrates [9]. Converting lignocellulosic biomass into ethanol is challenging due to the recalcitrant nature of cellulose and hemicellulose, which require pretreatment and enzymatic hydrolysis [1].

Researchers worldwide are focused on finding routes that make 2G ethanol production viable. Key areas of research include the development of efficient pretreatment methods and the engineering of microbial strains to improve pentose fermentation. These innovations are essential to overcoming the technical and economic obstacles associated with 2G ethanol production.

Enzymes and Their Applications

Enzymes are biocatalysts that directly hydrolyze polysaccharides into fermentable sugars. Compared to other industrial techniques, enzymatic hydrolysis has the main advantage of being sustainable and environmentally friendly. Unlike acids, enzymatic hydrolysis reacts only with specific substrates, generating significantly lower inhibitors [2].

The enzymatic hydrolysis of lignocellulosic biomass primarily involves cellulases and hemicellulases. Cellulases break cellulose into glucose, while hemicellulases break down hemicellulose into a mixture of pentoses and hexoses [3]. This synergistic action of the enzymes is essential for efficiently converting biomass into fermentable sugars.

The cost of enzymes is a significant component of the production cost of 2G ethanol, representing about 65% of the total cost in the short term [7]. However, these costs are expected to decrease significantly with technological advances in 2G ethanol production. Local enzyme production, genetic engineering of microorganisms to increase enzyme production, and the use of enzymatic cocktails are strategies that can potentially reduce these costs.

Technical-Economic Viability

Among the challenges in establishing efficient 2G ethanol production, the technical and economic feasibility of implementing enzymes in the process stands out. Researchers have been striving to identify efficient ways to reduce enzyme usage costs and enhance technical feasibility. Cripwell and colleagues (2020) conducted a study that concludes that integrating first—and second-generation technologies can consolidate the advantages of both by utilizing starch-rich and lignocellulosic raw materials in a single plant, significantly increasing ethanol production efficiency and reducing greenhouse gas (GHG) emissions [11].

The articles found in the literature present various processes for saccharification and fermentation of lignocellulosic substrates. Dahnum and colleagues (2015) analyze the methods of separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) [3], concluding that the SSF method has superior performance in final ethanol production since this process combines enzymatic hydrolysis and fermentation in a single reactor, increasing efficiency and reducing operational costs. This process is also corroborated by Choudhary and colleagues (2016) [6].

One way to significantly reduce enzyme costs is to produce these strains within the industry. Siqueira and colleagues (2020) [4] conducted a comparative study that demonstrates a significant cost reduction compared to the purchase and transportation of enzymes. The life cycle assessment (LCA) presented by Olofsson and colleagues (2017) reveals that integrated enzyme production can reduce greenhouse gas emissions compared to off-site enzyme production, highlighting the potential environmental benefits of integrated processes [10].

An important highlight in enzyme use is the implementation of enzymatic cocktails since each enzyme acts on a specific substrate. The use of enzymatic cocktails with different enzymes increases saccharification efficiency. Pandiyan. et al. (2019) highlight that combining cellulase and hemicellulase resulted in a 30% higher yield than isolated cellulases [5].

The studies also found a strong emphasis on genetic engineering. Choudhary and colleagues (2016) explain that developing thermotolerant yeasts through genetic and metabolic engineering can significantly improve lignocellulosic ethanol production efficiency and economic viability [6].

Rocha-Martin and colleagues (2017) show that adding additives such as PEG4000 significantly increased glucose yields by enhancing the activity of beta-glucosidase and endoglucanase without affecting their thermal stability [8]. This result demonstrates that enzymatic supplementation further enhances their hydrolytic power.

Results and Discussion

This section presents the results of the bibliometric review conducted. It explains the types of publications on the topic over the years, the most cited works, the relationship of these articles with the SDGs, the most frequently used keywords by the authors, and the most frequent keywords in the texts.

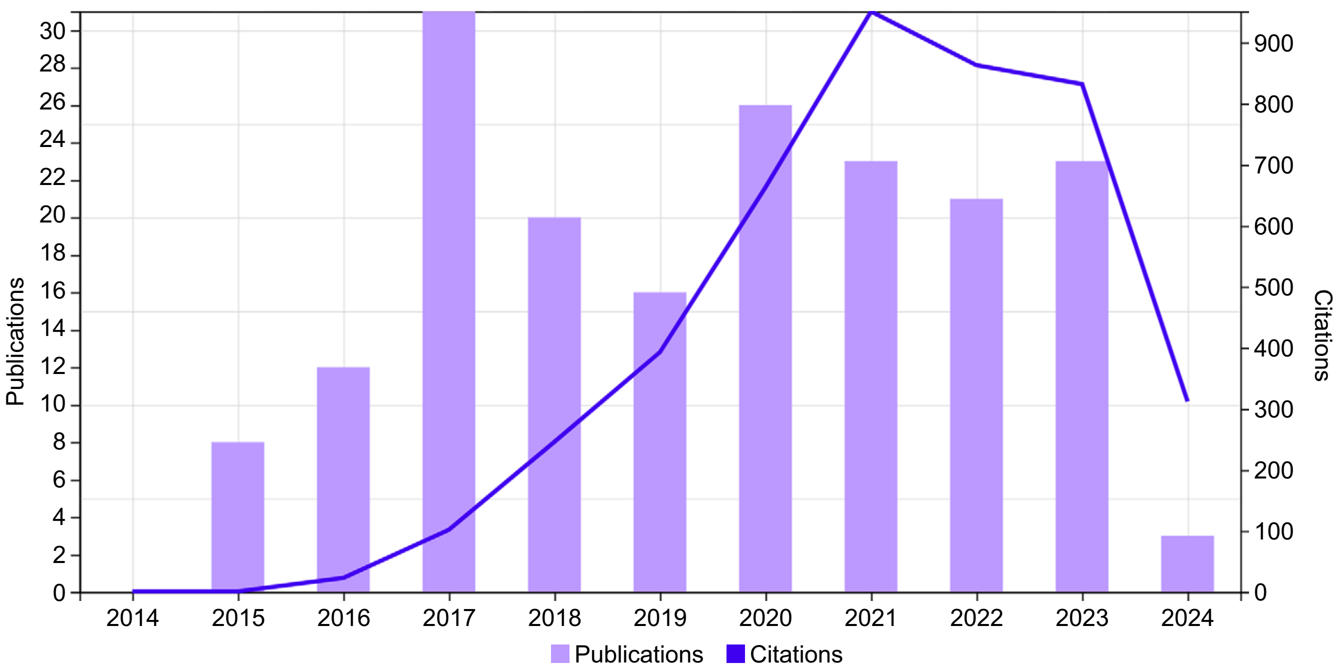
Relationship of Years with Number of Publications

Between 2015 and 2024, publications on sustainability and clean energy increased significantly. The number of publications grew exponentially from 2015 to 2017, driven by the adoption of the Sustainable Development Goals (SDGs) by United Nations member countries, peaking in 2017 as researchers rushed to present experimental results. However, there was a decline in 2019, likely due to the COVID-19 pandemic.

From 2015 to 2019, most publications were experimental with practical results. After 2019, bibliographic review articles increased, reflecting lockdown restrictions that limited laboratory access. Figure 2 shows the growth of publications and citations over the years in WoS.

In reviewing articles on the role of enzymes in ethanol production, there was a strong emphasis on the Sustainable Development Goals (SDGs). Most articles related to SDG 7 (Affordable and Clean Energy) and SDG 12 (Responsible Consumption and Production) highlight the importance of these themes for sustainability.

Figure 2. Several publications and citations *vs*. years.



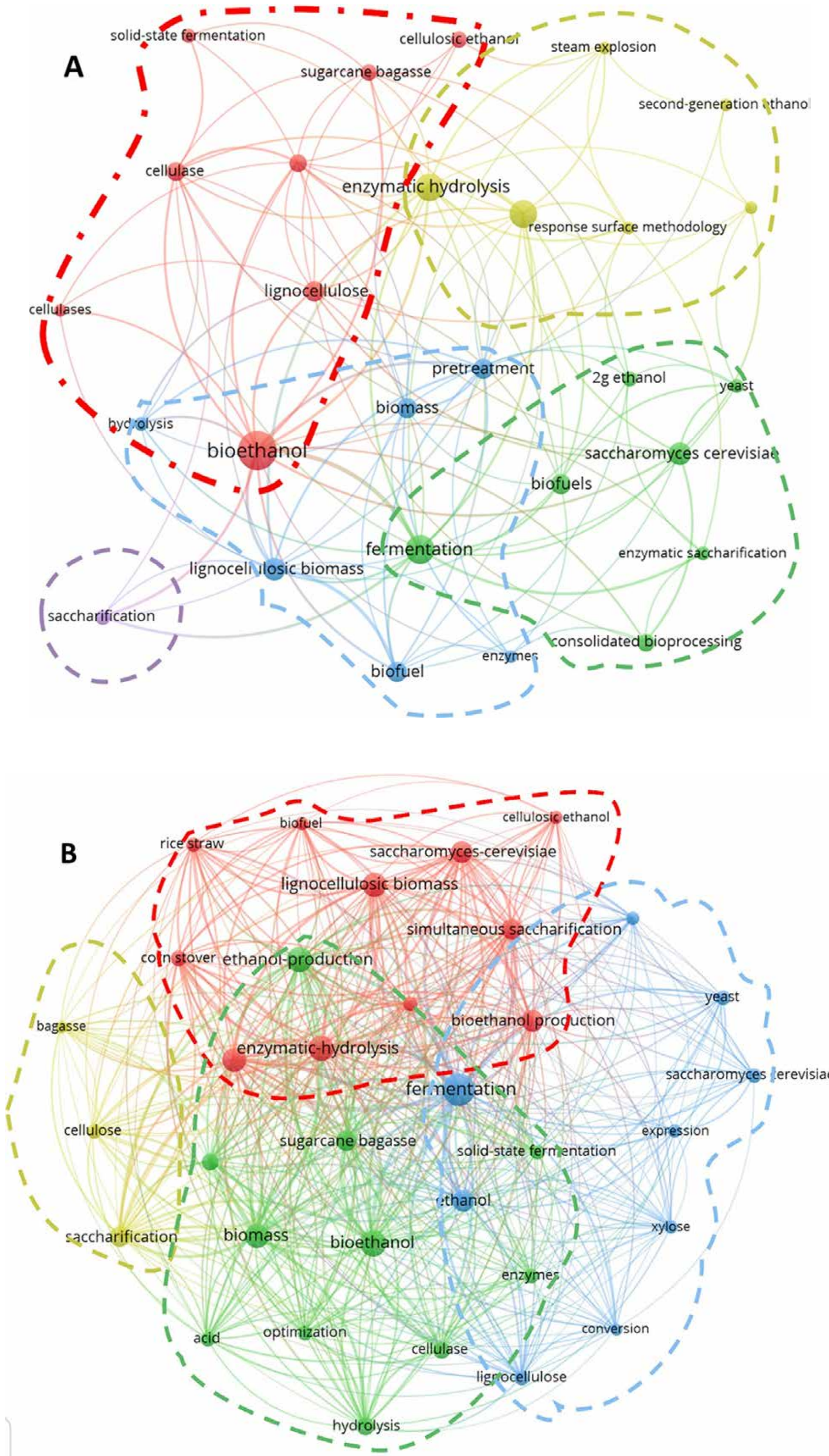
SDG 3 (Good Health and Well-being) and SDG 15 (Life on Land) were noted, emphasizing biofuels' environmental and public health benefits and their positive impact on terrestrial ecosystems. Among the countries publishing during this period, Brazil led with 53 publications, attributed to its leadership in first-generation ethanol production from sugarcane and efforts to develop second-generation ethanol from sugarcane bagasse. India ranked second with 31 publications, focusing on efficient biofuel alternatives due to food scarcity challenges. Second-generation ethanol, which doesn't compete with food production, is seen as viable. The United States was third with 16 publications, being the largest ethanol producer, mainly using corn and exploring biofuels from lignocellulosic crops for more efficient production.

Keywords Most Used by Authors and the Most Frequent in Texts

The selected publications created two bibliometric networks, as shown in Figure 3. In Figure 3A, we have the sets of the authors' most used keywords; in Figure 3B, we have the most frequently found keywords in the body of the texts. Words with the same colors indicate a higher co-occurrence in the articles, while the circle size represents the most frequent keywords. The connections between words indicate interactions among them, regardless of the group. Figure 3A, 25 high-occurrence keywords were selected and divided into five groups. Key terms include "bioethanol", "fermentation", "enzymatic hydrolysis", "ethanol", "lignocellulosic biomass" and "pretreatment". In Figure 3B, 34 high-occurrence keywords were chosen and organized into four distinct groups, identifying "fermentation," "bioethanol," "enzymatic hydrolysis," and "saccharification" as the most frequent words in each group. The analysis of bibliometric networks allows the selection of the most significant articles for reading by identifying the most relevant keywords for the search. This methodology was used to determine the references of this work among the 182 articles identified in WoS.

The reviewed studies demonstrate that implementing enzymes in the 2G ethanol production process can significantly increase process efficiency. Additionally, local enzyme

Figure 3. Bibliometric network of authors' keywords.



production can reduce production costs by up to 40%, as evidenced by Siqueira and colleagues (2020) [4]. Integrating first and second-generation technologies can increase profits by up to 25% due to economies of scale and resource optimization [11]. The life cycle assessment (LCA) presented by Olofsson and colleagues (2017) reveals that integrated enzyme production can reduce greenhouse gas emissions by up to 15% [10]. These data confirm that second-generation ethanol production using enzymatic processes is a viable and sustainable alternative with significant potential to contribute to the biofuel industry.

Conclusion

This study employed technological foresight and bibliometric analysis to thoroughly explore and evaluate large data sets, identifying emerging trends, research components, and collaboration patterns in ethanol production. The analysis highlighted the growing importance of enzymes as biocatalysts in efficient ethanol production, emphasizing their critical role in both first- and second-generation biofuels. Enzymes are essential to efficiently convert lignocellulosic biomass into fermentable sugars, reducing the presence of inhibitors. Additionally, integrating first- and second-generation technologies into a single factory can increase efficiency and reduce greenhouse gas emissions.

The study also demonstrated the superiority of the simultaneous saccharification and fermentation (SSF) process over other methods. Promising strategies to reduce enzyme-related costs include local production of enzymes, genetic engineering of microorganisms, and enzyme cocktails. These strategies make the second-generation (2G) ethanol production process more economically viable. In conclusion, this study affirms that using enzymes in the production of second-generation ethanol is a viable and sustainable alternative with significant potential to assist in the transition to a cleaner and more efficient energy matrix. Investment in research and development is essential to overcome technical and economic challenges, promote the commercial strategy for 2G ethanol, and promote scientific and technological progress in this field.

Acknowledgments

The authors would like to acknowledge Shell Brasil and ANP (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis) for the strategic support provided through regulatory incentives for Research, Development, and innovation. We also acknowledge EMBRAPII and Senai CIMATEC for the encouragement and funding.

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