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# A Contextual Approach to Enzymatic Hydrolysis in Ethanol Production

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***Abstract:*** *The increasing global demand for renewable energy has led to significant advancements in the production of biofuels, with ethanol being a leading candidate for reducing greenhouse gas emissions. One of the most promising approaches for improving the efficiency and sustainability of ethanol production is enzymatic hydrolysis, a process that utilizes enzymes to break down complex carbohydrates into fermentable sugars. This study explores the potential of enzymatic hydrolysis in enhancing ethanol production, focusing on the enzymatic breakdown of starch, cellulose, and hemicellulose, which are key components of biomass. The research highlights the critical role of enzymes such as α-amylase and glucoamylase in optimizing the conversion of starch into fermentable sugars, thereby increasing the efficiency of the fermentation process. Furthermore, the study examines the use of various raw materials, including agricultural residues and lignocellulosic biomass, as alternative feedstocks for ethanol production, emphasizing their potential to reduce the reliance on food-based crops and lower the environmental impact of biofuel production. Despite the promising advantages of enzymatic hydrolysis, the study also identifies several challenges, including the high cost of enzymes, the need for efficient pre-treatment processes, and the scalability of the technology for large-scale applications. The research underscores the importance of continued innovation in enzyme technology and process optimization to overcome these challenges. In addition, the integration of enzymatic hydrolysis with existing ethanol production methods is discussed as a potential pathway for enhancing the overall efficiency and sustainability of biofuels. The study concludes by suggesting that future research should focus on optimizing enzyme formulations, improving pre-treatment methods, and exploring the commercial viability of enzymatic hydrolysis in the production of second- and third-generation biofuels*

***Keywords:*** *Enzymatic Hydrolysis, Ethanol Production, Biofuels*

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## I. INTRODUCTION

The growing global demand for fuels, combined with the volatility of fossil fuel prices and the urgent need to combat climate change, has intensified the search for alternative energy sources, with biofuels emerging as a key solution. A recent and notable development in this field has been the incorporation of corn ethanol into Brazil's national biofuel matrix. This shift was made possible through diverse production models and business strategies, marking a significant milestone in the country's transition to more sustainable energy sources (Grande et al., 2024).

Since its initial implementation in 2014, ethanol production from corn has steadily increased, with the state of Mato Grosso becoming a focal point for this growth. In this region, several plants have adopted hybrid approaches, processing both sugarcane and corn, while others have focused exclusively on corn processing. The appeal of corn lies in its ability to ensure year-round production, except during scheduled maintenance, offering greater operational flexibility. Furthermore, corn can be used as boiler fuel and stored in stock, contributing to more stable production cycles (Santos et al., 2020; Costa et al., 2020).

While the production process of corn ethanol mirrors that of sugarcane ethanol—through fermentation and distillation—the distinct characteristics of these raw materials necessitate different approaches for converting starch into fermentable sugars. In sugarcane, sugars are naturally present and can be easily extracted through mechanical processes like milling and diffusion (Alcarde, 2009). However, corn and other starchy materials

require the conversion of starch into simple sugars via enzymatic hydrolysis at high temperatures, owing to the insolubility of polysaccharides (Santos et al., 2020; Vasconcellos, 2010). This enzymatic process not only improves energy efficiency but also reduces environmental impact, making it an attractive method for biofuel production. Additionally, it supports the diversification of the energy matrix by enabling the use of various types of biomass, reducing dependency on fossil fuels.

Corn ethanol production also generates valuable by-products, such as corn oil and protein-rich co-products like DDG, DDGS, WDG, and WDGS. These by-products provide additional economic opportunities for ethanol plants, enhancing the overall profitability of the process. For example, one ton of corn can yield approximately 430 liters of ethanol, 550 kg of DDG, and 15 liters of oil (Santos et al., 2020). Notably, DDGS, which has a lower water content and higher market value compared to WDGS, holds particular economic significance. Although corn oil production is constrained by an industrial yield of around 13.74 liters per ton, its potential for revenue growth within ethanol plants remains substantial (Fernandes, 2008; Ribeiro, 2023).

In contrast, the 2009 project by Galp Energia in Mozambique, aimed at developing biofuels from Jatropha curcas Linn. and palm oil, illustrates the challenges that alternative biofuel sources can face. This project, which sought to create an agro-industrial hub for biofuel production in developing countries, emphasized sustainability by using crops that do not compete with the food chain and thrive on poorer soils (Banze Junior, 2024; Parawira, 2010). However, Jatropha faced significant hurdles. Its oil required specific storage conditions, such as cool, dark spaces, which complicated its use, particularly in rural areas. Additionally, the vast quantity of seeds needed to produce a relatively small amount of biodiesel—100 kilograms of seeds for just 20 liters of biofuel—made the process economically unfeasible. These challenges ultimately rendered the use of Jatropha as a biodiesel source unsustainable on a large scale (Banze Junior, 2024; Parawira, 2010).

The contrasting experiences with corn and Jatropha underscore the importance of selecting raw materials that offer not only high energy potential but also operational feasibility and economic viability. Against this backdrop, the present study explores the crucial aspects of ethanol production, focusing on the integration of enzymatic hydrolysis with traditional methods. By leveraging innovative enzyme technologies and process optimizations, the study aims to enhance the efficiency and sustainability of biofuels.

This research also emphasizes the role of student participation, where active involvement in the development of new insights and solutions has contributed significantly to understanding biofuel production. In this context, the present study, developed through active student participation in both writing and textual construction, seeks to explore key aspects of ethanol production, emphasizing the importance of innovation in enzyme technology and process optimization for improving the efficiency and sustainability of biofuels. The research mainly focuses on how students contributed to understanding the integration of enzymatic hydrolysis with traditional ethanol production methods. Additionally, it aims to propose future research directions, including optimizing enzyme formulations, enhancing pre-treatment processes, and evaluating the commercial viability of second and third-generation biofuels. The study is grounded in the principles of experiential learning, with students actively engaged in problem-solving and the development of new insights within the context of biofuel sustainability.

## II. METHODS

This study was developed through the active participation of students, underscoring the importance of experiential learning in the academic process. In line with the theory that knowledge is generated through the transformation of experience, this research highlights the student´s role in the construction of the study's content. The accelerated production and distribution of knowledge require individuals to remain constantly informed, updated, and adaptable to uncertain scenarios, which aligns with the principles of lifelong learning. The student's involvement contributed to the research and also engaged in problem-solving and innovation, directly applying theoretical concepts in the context of corn ethanol production. This participatory approach allowed for the generation of new insights while fostering a deeper understanding of the sustainability and technological advancements in biofuel production.

## III. DEVELOPMENT

### 3.1 Enzymes.

Enzymes are biological catalysts that accelerate chemical reactions. They are produced by living organisms, typically bacteria or fungi, through fermentation. Starch hydrolysis can be carried out by five types of enzymes: amylases (endoamylases and exoamylases), isomerases, cyclodextrins, debranching enzymes, and glycosyltransferases, according to Fernandes (2008).

The active site is the exact location where the substrate binds to the enzyme, initiating the chemical reaction. This makes it the most crucial part of the enzyme’s structure (Smith et al., 2010). According to Cripwell et al. (2020), the enzymes commonly used in the hydrolysis process are α-amylases, β-amylases, and

glucoamylases. These three enzymes can achieve complete starch conversion, with 85% converted by the amylases and the remainder by the glucoamylase. Hydrolysis generally consists of three steps: gelatinization, liquefaction, and saccharification (Sampaio and de Assis, 2023).

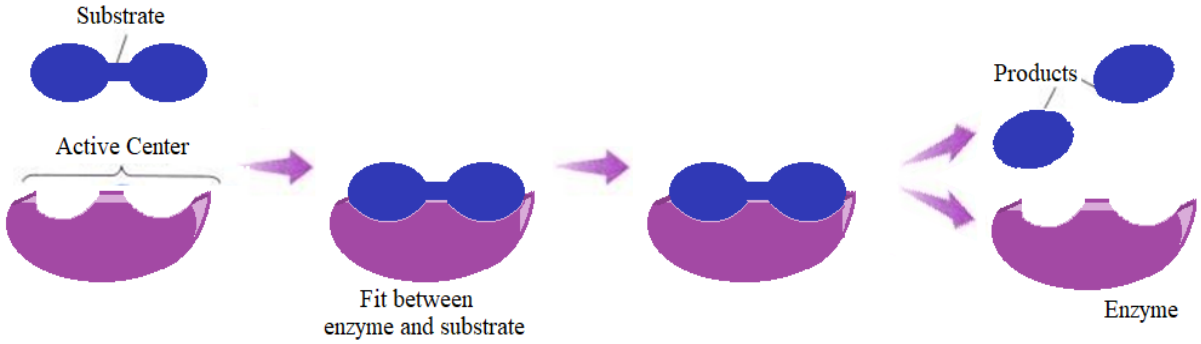
The cost of ethanol production is significantly influenced by the price of its inputs. Enzymes, being expensive and highly sensitive, have prompted researchers to explore strategies for their recovery and extended use. One well-studied solution is enzyme immobilization, which involves attaching enzymes to an inert support, facilitating their recovery and reuse. Studies by Baptista (2013) and Luchiari (2019) have reported positive results in immobilizing enzymes for corn starch hydrolysis (Ribeiro, 2023; Sampaio and de Assis, 2023).

It is essential to understand two key terms regarding enzyme functionality:

- A SUBSTRATE is the component that the enzyme breaks down.
- A PRODUCT is the substance resulting from the enzyme's action on a substrate.

The enzyme itself remains unchanged. It binds to a substrate, reduces the activation energy required for the reaction to produce the products, and is subsequently released to act on new substrates. Figure 1 illustrates a general scheme of the enzyme functionality process.

**Figure 1. Enzyme Functionality Process**




Source: Authors (2024)

Particularly, in production plants, substrate breakdown would occur without enzymes; however, enzymatic participation allows a more efficient breakdown of cellulose molecules. This results in higher biomass conversion into fermentable sugars. Consequently, less energy is required to produce the same amount of ethanol compared to traditional methods, significantly reducing process time while increasing production output (Jones et al., 2008).

Each enzyme has a specific optimal range of temperature and pH. When within this ideal range, enzymes function more efficiently and quickly. If the temperature exceeds the optimal range, the enzyme ceases to function, and if it falls below the ideal range, the breakdown of the substrate slows down. The advantages of using enzymes in technological or biological processes include enabling diverse biochemical reactions, offering high selectivity, operating under mild reaction conditions (pressure, temperature, and pH), and causing fewer environmental and toxicological issues (Brown et al., 2015).

The industrial use of enzymes is determined by their specificity, activity, storage stability, availability, and cost. Enzyme activity is influenced by factors such as enzyme concentration, substrate concentration, cofactors, inhibitors (types and concentrations), ionic potential, pH, temperature, and reaction time. The effects of these variables on enzymatic activity are studied under enzyme kinetics (Smith et al., 2010).

Enzymes are classified into six major categories based on the type of reaction they catalyze. According to IUPAC (1979), these six typical classes of industrial enzymes are oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. In the food industry, enzymes are generally categorized into four groups (Jones et al., 2008).:

- Carbohydrate hydrolases: Catalyze the hydrolysis of polysaccharides or oligosaccharides.
- Proteases: These act on peptide bonds in proteins, releasing amino acids and peptides.
- Lipases: Involved in fat metabolism and degradation processes.
- Oxidoreductases: Oxidize or reduce substrates by transferring hydrogen or electrons

### 3.2. Ethanol Production

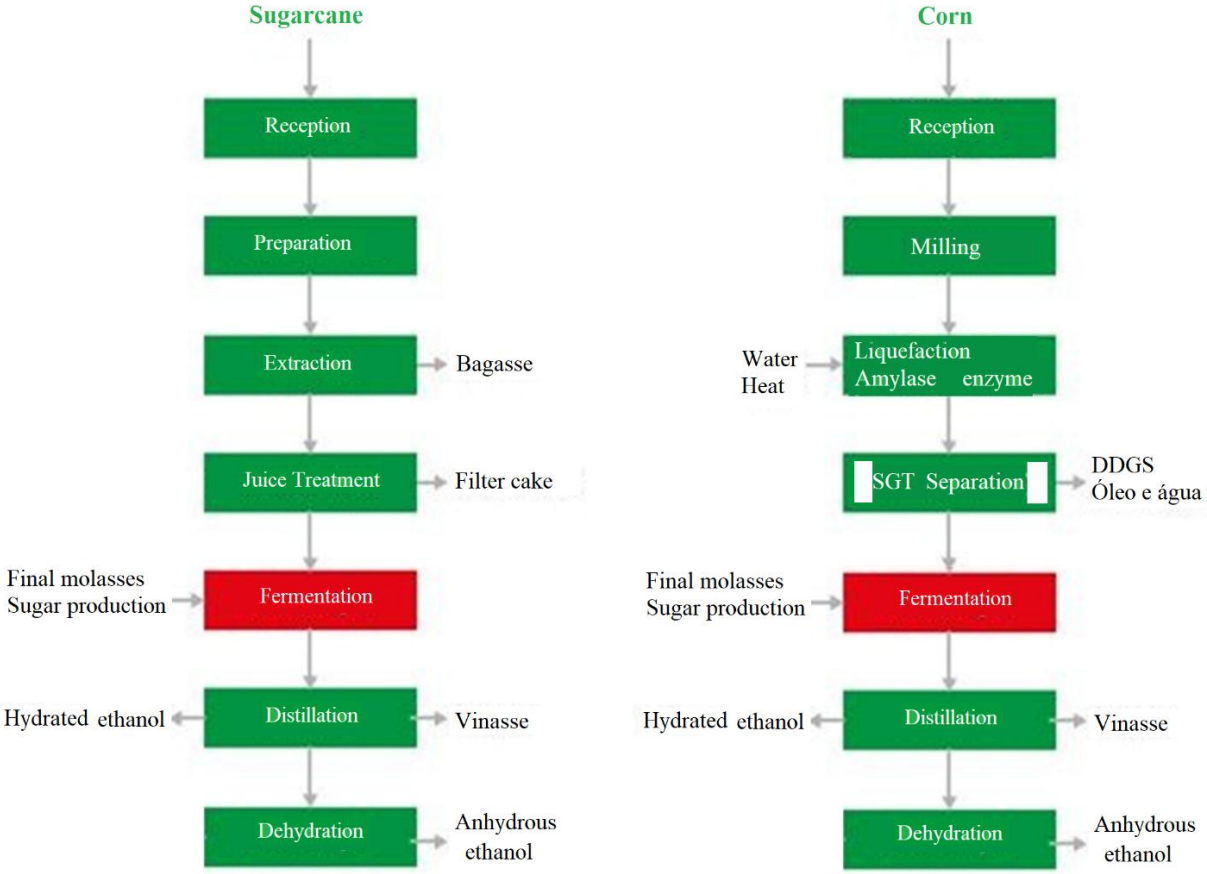
Brazil is one of the world's leading producers of biofuels, with sugarcane as the predominant raw material for ethanol production. The production of corn ethanol in Brazil, while still relatively small compared to the

United States, represented 2.8% of U.S. production and 1.5% of global ethanol production in 2019. The growth of corn ethanol production in Brazil is closely tied to the expanding productive sectors of the Central-West region (Conab, 2018; Da Silva et al., 2020; Vasconcelos, 2010; Milanez et al., 2014).

Contributing to tangible aspects of the study, among diverse plant sources for biodiesel production, it is worth highlighting that in 2019, Brazil had ten flexible ethanol plants and two standalone corn ethanol plants, with four additional facilities under construction. The growth of corn ethanol production is driven by factors such as abundant corn supply in specific regions, lower interest rates, and promising domestic ethanol consumption forecasts. While small in scale compared to sugarcane-based production, corn ethanol demonstrates integration potential with other productive sectors in regions like Brazil's Central West. Figure 2 illustrates the production routes of ethanol from corn and sugarcane, highlighting the main stages of each. The production processes for ethanol from sugarcane and corn are similar. However, the extraction of sugars differs significantly between the two raw materials. The sugars in sugarcane are readily available and can be easily extracted, while the starch in corn must be converted into sugars. Figure 3 illustrates the processing lines for sugarcane and corn, with steps in a flex plant (process (Grande et al., 2024, Conab, 2018; Da Silva et al., 2020; Vasconcelos, 2010; Milanez et al., 2014).

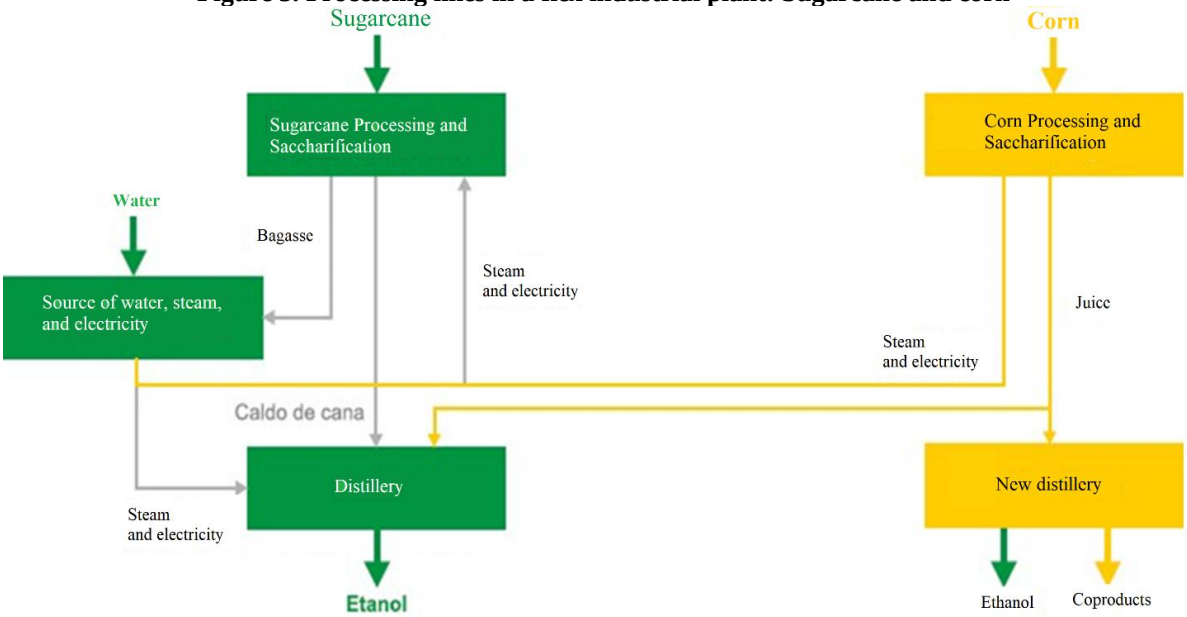
Importantly, ethanol production can be interpreted to be based on three main types of raw materials: sugar-based, starch-rich, and cellulose-based. Sugar-based raw materials, such as sugarcane, contain sugars that are used in the fermentation process, while starch-rich raw materials, such as corn, contain starch that must be converted into sugars through enzymatic processes. Cellulose-based raw materials are the most expensive and difficult to use, as they require the breakdown of cellulose into sugars using specific enzymes (Vasconcelos, 2010).

**Figure 2. Production routes of ethanol from corn and sugarcane: Main stages**




**Source:** Based onVasconcelos (2010) and Milanez et al. (2014)

**Figure 3. Processing lines in a flex industrial plant: Sugarcane and corn**




Source: Based on Vasconcelos (2010) and Milanez et al. (2014).

### 3.3 Enzymatic Hydrolysis in Ethanol Production

Hydrolysis in ethanol production can be conceptually divided into three stages: gelatinization, liquefaction, and saccharification. Gelatinization, often seen as a pre-hydrolysis step, precedes enzyme addition. During this phase, corn bran, obtained from grinding the grain, is mixed with excess water and heated until the starch granules are fully gelatinized. The gelatinization temperature of corn starch is approximately 70°C, though it can vary based on grain type, moisture content, and amylose levels. Gelatinization separates amylose and amylopectin, facilitating the action of the α-amylase enzyme during liquefaction. Starch granules, which are insoluble in cold water, absorb water and swell when heated. pH adjustment, typically through sulfuric or hydrochloric acid addition, is necessary to optimize the environment for α-amylase, which functions best at a pH of 5.0–6.0 (Ribeiro, 2023; Grande et al., 2024).

The α-amylase enzyme catalyzes endoenzymatic breakdown of starch, releasing products with more active sites that serve as substrates for glucoamylase (GMS), which enhances starch-to-glucose conversion. α-amylase is sourced from various microorganisms, with optimal temperature ranges from 50°C to 90°C and pH from 4.5 to 7.0. Glucoamylase performs best at temperatures between 50°C and 60°C, with its activity decreasing above 65°C and inactivation occurring at higher temperatures. Its optimal pH is between 4.0 and 5.0. Following gelatinization, the liquefaction stage begins.

During liquefaction, α-amylase is added to the gelatinized starch, breaking down amylose and amylopectin into dextrins and oligosaccharides. This step occurs under agitation for 30 to 60 minutes, though residence time may extend to 1.5 to 2 hours, depending on enzyme concentration. The addition of enzymes such as α-amylase, β-amylase, and glucoamylase can achieve nearly 100% conversion of starch to glucose. Saccharification, the final stage of starch hydrolysis, follows liquefaction. In this stage, the dextrins and oligosaccharides from liquefaction are further broken down into glucose by glucoamylase. The saccharification process typically lasts 45 to 90 minutes but can extend up to 6 hours. The enzyme is added at concentrations of 0.06% to 0.08% of the mass to be saccharified. After saccharification, the resulting solution, referred to as "wort," is ready for fermentation (Fernandes, 2008; Ribeiro, 2023).

In ethanol production from corn, α-amylase - specifically alpha-1,4 glycosidic bonds - plays a crucial role. Starch consists of glucose monomers linked by α-1,4 and α-1,6 glycosidic bonds. α-amylase targets the α-1,4 bonds, facilitating starch breakdown. This process leads to the creation of a mash, which then proceeds to fermentation. In the mixer, the enzyme is dosed, and the temperature is maintained between 60°C and 65°C. If the temperature is too high, pellet formation may occur, causing yield loss.

Once the enzyme completes its work, the result is a mash that moves on to fermentation. Glucoamylase is added during fermentation to catalyze the breakdown of the remaining starch and oligosaccharides into glucose. This glucose is then fermented by yeasts to produce ethanol. The final processing of ethanol is performed at the distillery, where hydrated ethanol is separated from vinasse and stored, along with by-products (Vasconcelos, 2010; Milanez et al., 2014).

Despite substantial research, the biotransformation of agricultural residues into fermentable sugars, leading to second-generation ethanol production, remains economically challenging. Technologies for the bioconversion of lignocellulosic biomass, particularly agroindustrial residues, continue to evolve to improve efficiency in industrial-scale ethanol production, with ongoing attention to reducing capital and operational costs. Second-generation ethanol (cellulosic ethanol) presents a sustainable opportunity for utilizing lignocellulosic biomass—such as agricultural residues, industrial by-products, and forestry waste—without competing for arable land. This technology focuses on improving biofuel production while addressing food security concerns (Silva et al., 2018; Da Silva et al., 2020).

The process involves four key stages: pre-treatment (to disrupt the lignocellulosic matrix for enzyme accessibility), hydrolysis (breaking down cellulose and hemicellulose into fermentable sugars), fermentation (conversion of sugars into ethanol), and distillation (separation of ethanol from fermentation broth). Despite its environmental benefits, second-generation ethanol faces hurdles like high enzyme costs and the complexity of pre-treatment Technologies. Brazil's innovative efforts in second-generation ethanol production have focused on leveraging sugarcane bagasse and straw from first-generation production. Integrated biorefineries that combine first- and second-generation technologies have become a hallmark of Brazil's ethanol sector, supported by government incentives and investments in renewable energy (Silva et al., 2018; Ribeiro, 2023).

Looking ahead, third-generation ethanol production, using algal biomass, shows potential due to algae's high productivity and minimal land use. Although still in the research phases, this method can address the limitations of earlier biofuel generations by maximizing biomass yield without competing with food production. However, scalability and cost-effective processing remain challenges (Ribeiro, 2023; Grande et al., 2024).

## IV. CONCLUSION

This study has highlighted the critical role of enzymatic hydrolysis in improving the efficiency and sustainability of ethanol production. Through the stages of gelatinization, liquefaction, and saccharification, the use of enzymes such as α-amylase and glucoamylase facilitates the conversion of starch into fermentable sugars, which are essential for the ethanol fermentation process. These enzymatic processes not only enhance the efficiency of starch conversion but also contribute to more sustainable production methods by enabling the use of a wider variety of feedstocks.

Enzymatic hydrolysis presents a promising route for advancing the biofuels industry. By improving the breakdown of lignocellulosic biomass—such as agricultural residues and other non-food feedstocks—into fermentable sugars, this technology offers the potential to reduce reliance on food-based raw materials, thereby mitigating concerns about food security. The ability to use abundant and lower-cost materials like agricultural waste and forestry by-products also helps reduce the carbon footprint of biofuel production, making it a more sustainable alternative to conventional ethanol production methods.

While the potential benefits of enzymatic hydrolysis are clear, there are still challenges to overcome. The high cost of enzymes, the need for efficient pre-treatment methods, and the optimization of enzymatic processes for large-scale industrial applications remain significant barriers. However, ongoing research and technological advancements in enzymatic hydrolysis are critical to overcoming these challenges. As enzyme efficiency improves and production processes are optimized, enzymatic hydrolysis will likely play an increasingly important role in the transition to a greener, low-carbon economy.

In addition, the integration of enzymatic hydrolysis with existing ethanol production technologies has the potential to create synergies that enhance both environmental and economic outcomes. By improving the efficiency of sugar conversion and enabling the use of diverse raw materials, this approach not only supports the development of more sustainable biofuels but also opens up new revenue streams through the valorization of co-products such as lignin and other biomass components.

Future studies could focus on several key areas to further improve the efficiency and cost-effectiveness of enzymatic hydrolysis. Research on the optimization of enzyme formulations, as well as advances in pre-treatment technologies, could help overcome some of the existing limitations, such as enzyme cost and energy input.

Additionally, the exploration of alternative enzyme sources, including microorganisms and genetically engineered enzymes, could enhance hydrolysis rates and broaden the range of feedstocks that can be effectively processed. Furthermore, the development of integrated biorefinery systems, which combine enzymatic hydrolysis with other forms of biomass processing, could maximize resource efficiency and increase the economic viability of biofuel production. Finally, exploring the scalability and commercialization of enzymatic hydrolysis for second and third-generation biofuels will be key to ensuring that this technology can meet the demands of the growing global biofuels market.

In conclusion, enzymatic hydrolysis stands out as a key innovation for the future of ethanol production. Its potential to improve efficiency, reduce costs, and increase sustainability positions it as a vital technology for

advancing the global biofuels industry. With continued research and development, enzymatic hydrolysis could become a cornerstone in the production of biofuels that contribute to a sustainable, low-carbon energy future.

**Conflict of interest**

There is no conflict to disclose.

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