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Application Potential and Technical Challenges of *Agave* in Bioethanol Production

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Abstract This study explores the potential application of *Agave* species in bioethanol production and its associated technical challenges, including the assessment of bioethanol yield efficiency, *Agave*'s adaptability to various environmental conditions, and its economic feasibility as a biofuel feedstock. The study found that *Agave* species, particularly *Agave americana* and *Agave neomexicana*, show significant promise as bioethanol feedstocks due to their high carbohydrate content and low recalcitrance to enzymatic hydrolysis. Ethanol yields from *Agave* are comparable to those from traditional biofuel crops like sugarcane and corn, with *Agave neomexicana* producing (119±11) mg ethanol/g biomass. Additionally, *Agave*'s ability to grow in semi-arid and arid regions without significant water inputs makes it a sustainable option for biofuel production. The study also highlights the development of efficient enzyme cocktails, such as those produced by *Aspergillus niger*, which significantly improve the saccharification process. The findings suggest that *Agave* has substantial potential as a bioethanol feedstock, particularly in regions unsuitable for traditional crops. Its high yield, low water requirements, and adaptability to harsh climates make it a viable and sustainable option for biofuel production. However, further research and development are needed to optimize the fermentation processes and improve economic feasibility.
Keywords *Agave*; Bioethanol; Biofuel feedstock; Enzymatic hydrolysis; Sustainable energy; Semi-arid regions; *Saccharomyces cerevisiae*; *Aspergillus niger*

1 Introduction

Bioethanol, a type of biofuel, has garnered significant attention as a renewable energy source due to its potential to reduce greenhouse gas emissions and dependence on fossil fuels. Bioethanol is produced through the fermentation of sugars derived from various biomass feedstocks, including food crops, lignocellulosic materials, and algae (Rodionova et al., 2017; Bušić et al., 2018). It is considered a cleaner alternative to gasoline, emitting fewer pollutants and contributing to a reduction in overall carbon footprint (Chilakamarry et al., 2021; Karimi et al., 2021).

Globally, bioethanol production is dominated by the United States and Brazil, primarily using corn and sugarcane as feedstocks, respectively (Zhou and Yan, 2024). These traditional feedstocks, while effective, present challenges such as competition with food supply and high water usage (Bušić et al., 2018; Raud et al., 2019). The search for alternative feedstocks that do not compete with food resources and can be cultivated in less arable land has led to increased interest in lignocellulosic biomass and other non-food sources (Broda et al., 2022; Kumar and Shahi, 2023).

Traditional feedstocks like corn and sugarcane have been the backbone of bioethanol production (Hong and Huang, 2024). However, their use raises several issues, including high water and fertilizer requirements, competition with food crops, and significant land use (Bušić et al., 2018; Raud et al., 2019). These limitations necessitate the exploration of more sustainable and less resource-intensive alternatives (Rezania et al., 2020; Kumar and Ram, 2021).

Agave, a succulent plant native to arid and semi-arid regions, has emerged as a promising alternative feedstock for bioethanol production. *Agave* species are known for their high sugar content and ability to thrive in water-limited environments, making them an attractive option for sustainable bioethanol production (Yan et al., 2011; Kumar and Shahi, 2023). The use of agave could mitigate some of the environmental and resource-related challenges associated with traditional feedstocks (Kumar and Ram, 2021).

This study aims to explore the application potential and technical challenges of *Agave* as a feedstock for bioethanol production. It will provide a comprehensive overview of the current state of bioethanol production, the limitations of traditional feedstocks, and the advantages of *Agave*. Additionally, it will discuss the technological advancements and challenges in converting *Agave* biomass into bioethanol, with a focus on future prospects and the research directions required to optimize this process.

2 *Agave* as a Bioethanol Feedstock

2.1 *Agave* plant biology

Agave species, such as *Agave americana*, *Agave tequilana*, and *Agave salmiana*, have been identified as promising candidates for bioethanol production due to their high biomass yield and sugar content. These species are traditionally used in the production of alcoholic beverages, but recent research has expanded their potential to include biofuel production from the whole plant, including leaves and bagasse (Corbin et al., 2015; Mielenz et al., 2015; Flores-Gómez et al., 2018).

Agave plants possess several characteristics that make them highly suitable for bioethanol production. *Agave* species are rich in fermentable sugars such as glucose and fructose, which are essential for ethanol production (Corbin et al., 2015; Jones et al., 2020). *Agave* plants are highly drought-tolerant, allowing them to thrive in semi-arid and arid regions where other crops may fail. This characteristic reduces the need for irrigation and makes *Agave* a sustainable feedstock option (Davis et al., 2011; Jones et al., 2020). The low lignin content in *Agave* biomass facilitates easier enzymatic hydrolysis, enhancing the efficiency of sugar extraction and subsequent fermentation processes (Yang and Pan, 2012; Corbin et al., 2015).

2.2 Cultivation practices

Agave species are well-suited to grow in semi-arid and arid regions, including parts of Mexico, the southwestern United States, and other tropical and subtropical areas. These regions often have marginal agricultural lands that are not suitable for traditional food crops but can support *Agave* cultivation (Davis et al., 2011; Mielenz et al., 2015). *Agave* cultivation practices emphasize sustainability. Due to their drought resistance, *Agave* plants require significantly less water compared to other bioethanol feedstocks, making them an environmentally friendly option (Davis et al., 2011; Jones et al., 2020). *Agave* can be grown on lands that are unsuitable for other crops, thus not competing with food production and reducing the risk of land-use conflicts (Davis et al., 2011). The residues from *Agave*, such as leaves and bagasse, can be used for bioethanol production, minimizing waste and enhancing the overall sustainability of the cultivation process (Mielenz et al., 2015; Flores-Gómez et al., 2018).

2.3 Sugar composition and yield

Agave plants have a high content of fermentable sugars, primarily glucose and fructose. For instance, the juice extracted from *Agave* leaves can contain up to 48 g/L of fermentable hexose sugars. The hydrolysis of fructan oligosaccharides in *Agave* further increases the concentration of fermentable sugars, making it a highly efficient feedstock for bioethanol production (Corbin et al., 2015).

When compared to other bioethanol feedstocks such as sugarcane and corn, *Agave* demonstrates several advantages. *Agave*'s ability to grow in arid conditions with minimal water input gives it an edge over water-intensive crops like sugarcane (Jones et al., 2020). Studies have shown that *Agave* can produce substantial yields of fermentable sugars, rivaling or even surpassing those of traditional bioethanol feedstocks. For example, *Agave americana* has been found to yield greater carbohydrates from enzymatic hydrolysis than advanced bioenergy crops like Miscanthus and switchgrass (Jones et al., 2020). *Agave*'s cultivation on marginal lands and its low water requirements make it a more sustainable option compared to other feedstocks that require fertile land and significant water resources (Davis et al., 2011).

3 Technological Aspects of *Agave* Bioethanol Production

3.1 Pre-treatment processes

Pre-treatment is a crucial step in the bioethanol production process from lignocellulosic biomass, including *Agave*. Various methods such as chemical, physical, and biological pre-treatments are employed to break down the complex structure of lignocellulosic biomass, making the cellulose and hemicellulose more accessible for enzymatic hydrolysis (Figure 1). Chemical methods include acid and alkali treatments, which are effective in removing lignin and hemicellulose, thus enhancing the digestibility of cellulose (Moodley et al., 2020; Rezania et al., 2020; Das et al., 2021). Physical methods like steam explosion and ammonia fiber expansion (AFEX) are also used to disrupt the biomass structure (Flores-Gómez et al., 2018; Zhao et al., 2021). Biological methods involve the use of microorganisms or enzymes to degrade lignin and hemicellulose (Muthuvelu et al., 2019; Malik et al., 2021).

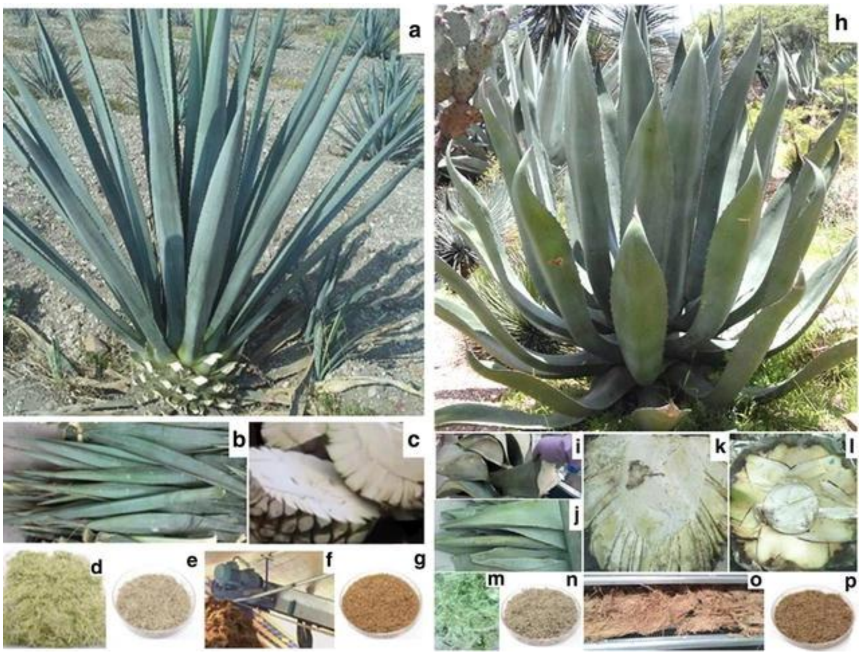

Figure 1 *Agave tequilana* and *Salmiana* plants and its main fractions (Adopted from Flores-Gómez et al., 2018)
Image caption: *A. tequilana* plant (a) and its harvested stem "piña" and leaves (a-c), leaf fiber matter (d, e), the stem-processed tequila residue "bagasse" (f, g). *A. salmiana* plant (h), *A. salmiana* leaves (h-j), stems (k, l), its leaf fiber matter (m, n), and the stem-processed Mezcal residue: *A. salmiana* "bagasse" (o, p) (Adopted from Flores-Gómez et al., 2018)

The primary challenge in pre-treating *Agave* biomass lies in its recalcitrant lignocellulosic structure, which is resistant to enzymatic breakdown. Lignin, a complex aromatic polymer, acts as a physical barrier, protecting cellulose and hemicellulose from enzymatic attack. This necessitates the use of effective pre-treatment methods to break down lignin and expose the polysaccharides (Das et al., 2021; Broda et al., 2022). Additionally, the presence of inhibitory compounds released during pre-treatment can hinder subsequent fermentation processes, posing another significant challenge (Flores-Gómez et al., 2018; Solarte-Toro et al., 2019).

3.2 Fermentation techniques

Optimizing the fermentation process is essential to maximize ethanol yield from *Agave* sugars. Factors such as pH, temperature, nutrient supplementation, and fermentation time need to be carefully controlled. The use of optimized commercial enzyme cocktails has been shown to enhance sugar conversion rates significantly (Flores-Gómez et al., 2018). Additionally, employing co-fermentation techniques, where multiple microbial strains are used, can improve the efficiency of sugar utilization and ethanol production (Malik et al., 2021).

Various microbial strains are employed in the fermentation of *Agave* biomass. *Saccharomyces cerevisiae* is the most commonly used yeast due to its high ethanol tolerance and efficient sugar conversion capabilities (Flores-Gómez et al., 2018; Malik et al., 2021). Other strains, such as *Pachysolen tannophilus*, are also used for their ability to ferment pentose sugars, which are abundant in hemicellulose (Malik et al., 2021). The selection of microbial strains is crucial for achieving high ethanol yields and efficient fermentation.

3.3 Distillation and purification

Distillation is the primary method used to separate ethanol from the fermentation broth. Techniques such as azeotropic distillation and vacuum distillation are employed to achieve high-purity ethanol (Sharma et al., 2020). Additionally, advanced purification methods like molecular sieves and membrane separation can be used to further purify ethanol, removing any remaining water and impurities (Solarte-Toro et al., 2019).

The efficiency of distillation and purification processes directly impacts the overall yield of bioethanol. High energy consumption and the need for multiple distillation steps can reduce the process's economic viability (Sharma et al., 2020). Moreover, the presence of residual inhibitors from the pre-treatment stage can affect the purity of the final product, necessitating additional purification steps (Solarte-Toro et al., 2019). Addressing these challenges is essential for improving the overall efficiency and yield of bioethanol production from *Agave*.

4 Application Potential of *Agave* Bioethanol

4.1 Economic viability

The economic viability of agave-based bioethanol production hinges on several factors, including feedstock availability, pretreatment costs, and energy consumption. *Agave* residues, such as bagasse and leaf fibers, are abundant by-products of the tequila industry, providing a low-cost feedstock option. However, the economic feasibility is influenced by the pretreatment process. For instance, the ammonia fiber expansion (AFEX) process has shown promising results in terms of sugar conversion efficiency, but the cost of ammonia and the need for optimized enzyme mixtures can add to the overall production costs (Flores-Gómez et al., 2018). A comparative study between agave juice and sugarcane molasses indicated that while agave juice has environmental benefits, its economic viability improves significantly when renewable energy sources are integrated into the production process (Figure 2) (Parascanu et al., 2021).

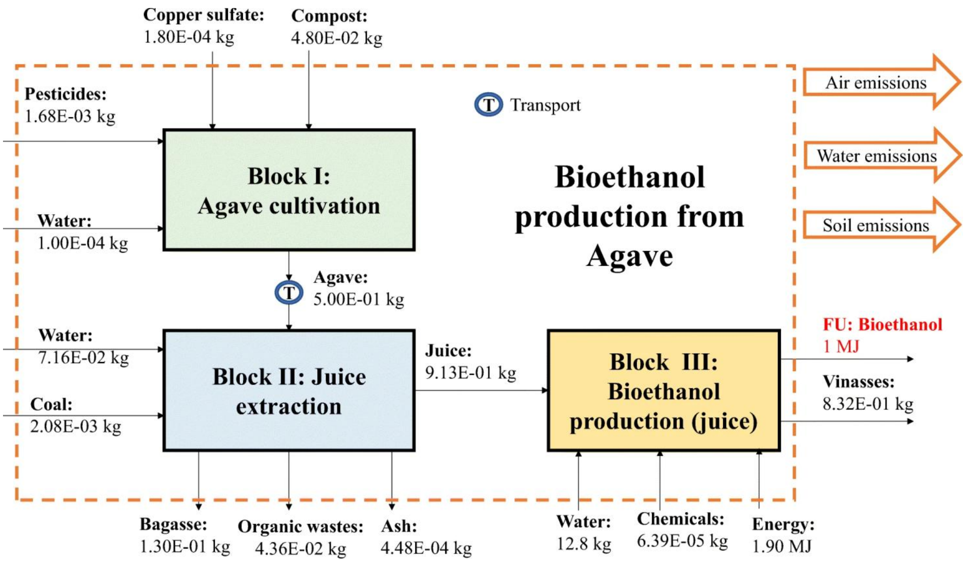

Figure 2 System boundaries for the bioethanol production, taking into account Block (I) agave cultivation, (II) agave juice extraction, and (III) agave juice fermentation (Adopted from Parascanu et al., 2021)

The market potential for agave-based bioethanol is promising, particularly in regions with established agave industries, such as Mexico. The commercialization prospects are bolstered by the high sugar conversion rates and ethanol yields achieved through optimized pretreatment and fermentation processes (Aguilar et al., 2018; Flores-Gómez et al., 2018). Additionally, the use of renewable energy sources can enhance the economic feasibility, making agave-based bioethanol a competitive alternative to traditional fossil fuels and other bioethanol sources (Parascanu et al., 2021). The integration of agave bioethanol into existing fuel markets could also benefit from government incentives and policies aimed at promoting renewable energy sources.

4.2 Environmental impact

Agave-based bioethanol has a lower carbon footprint compared to other bioethanol sources such as sugarcane molasses. This is primarily due to the lower consumption of fertilizers and pesticides in agave cultivation, as well as reduced emissions during the fermentation process. Life cycle assessments (LCA) have shown that agave bioethanol production results in fewer greenhouse gas emissions, making it a more environmentally friendly option (Parascanu et al., 2021). Furthermore, the use of lignocellulosic residues from agave plants, which are otherwise considered waste, contributes to a more sustainable bioethanol production process (Flores-Gómez et al., 2018).

The sustainability of agave-based bioethanol is underscored by its ability to utilize agro-industrial waste, thereby reducing environmental pollution and promoting waste valorization (Carrillo-Nieves et al., 2019). *Agave* plants are drought-resistant and require less water compared to other bioethanol feedstocks, which enhances their ecological benefits (Zhao et al., 2020). Additionally, the use of advanced pretreatment technologies, such as hydrothermal and AFEX processes, can further improve the sustainability of bioethanol production by maximizing sugar yields and minimizing energy consumption (Aguilar et al., 2018; Flores-Gómez et al., 2018).

4.3 Case studies

Several pilot projects have demonstrated the feasibility of using agave residues for bioethanol production. For instance, a study on the hydrothermal pretreatment of agave bagasse achieved high saccharification yields and ethanol concentrations, highlighting the potential for scaling up the process (Aguilar et al., 2018). Another project focused on the AFEX pretreatment of agave residues, which resulted in high sugar conversion rates and ethanol yields, further validating the technical viability of agave-based bioethanol production (Flores-Gómez et al., 2018).

Commercial operations utilizing agave for bioethanol production are still in the nascent stages, but there are promising developments. The tequila industry in Mexico generates substantial amounts of agave bagasse, which can be repurposed for bioethanol production, thereby creating a circular economy (Aguilar et al., 2018; Flores-Gómez et al., 2018). The integration of renewable energy sources and advanced bioprocessing techniques can enhance the commercial viability of these operations, paving the way for large-scale production and market integration (Parascanu et al., 2021).

5 Technical Challenges and Solutions

5.1 Agricultural challenges

Scaling up *Agave* cultivation presents several challenges, primarily due to the plant's specific growth requirements and the need for extensive land areas. *Agave* spp. are resilient in hot and dry conditions, making them suitable for regions with limited water resources. However, expanding cultivation to meet bioethanol production demands requires careful consideration of climate and soil conditions. Research indicates that the potential growing region for *Agave americana* could expand by 3%-5% with climate warming scenarios, suggesting that future climate conditions may favor *Agave* cultivation in new areas (Davis et al., 2021). Additionally, rock mulching techniques can further reduce irrigation needs and increase suitable cropland area by 26%~30%.

Pest and disease management, along with soil degradation, are significant concerns in large-scale *Agave* cultivation. The resilience of *Agave* spp. to pests and diseases is relatively high compared to other crops, but continuous monoculture practices can lead to increased vulnerability. Implementing integrated pest management (IPM) strategies and crop rotation can mitigate these risks. Furthermore, soil degradation due to intensive farming practices can be addressed by adopting sustainable agricultural practices, such as minimal tillage and organic amendments, to maintain soil health and productivity (Pérez-Zavala et al., 2020; Davis et al., 2021).

5.2 Process optimization

Optimizing the pre-treatment, fermentation, and distillation processes for *Agave* biomass is crucial for efficient bioethanol production. One of the main challenges is the effective breakdown of the plant's fibrous structure to release fermentable sugars. Hydrothermal pretreatment has shown promise, with studies indicating high saccharification yields of up to 99.5% at high-solids loading (Aguilar et al., 2018). However, achieving consistent results at a commercial scale remains challenging due to variations in biomass composition and processing conditions. Additionally, the fermentation process must be optimized to handle the high sugar concentrations obtained from *Agave* biomass, which can inhibit microbial activity.

Innovations in process technology are essential to overcome the challenges in *Agave* bioethanol production. Advanced pretreatment methods, such as hydrothermal and enzymatic hydrolysis, have been developed to improve sugar yields and reduce processing times (Aguilar et al., 2018). Additionally, integrating pre-saccharification and fermentation strategies can enhance ethanol yields, achieving up to 90.84% efficiency. Continuous research and development in these areas are necessary to refine these technologies and make them economically viable for large-scale production.

5.3 Supply chain and infrastructure

The logistics of transporting *Agave* biomass from cultivation sites to processing facilities pose significant challenges. The bulky nature of *Agave* biomass requires efficient transportation methods to minimize costs and environmental impact. Developing a decentralized network of processing facilities closer to cultivation areas can reduce transportation distances and associated costs. Additionally, optimizing the supply chain through better planning and coordination can ensure a steady supply of biomass to processing plants (Pérez-Zavala et al., 2020).

Establishing the necessary infrastructure for processing and distributing *Agave*-based bioethanol is critical for the industry's success. This includes building processing plants equipped with advanced pretreatment and fermentation technologies, as well as storage and distribution facilities for the final product. Investment in infrastructure is essential to support the scale-up of *Agave* bioethanol production and ensure a reliable supply chain from farm to fuel (Aguilar et al., 2018; Pérez-Zavala et al., 2020).

6 Future Prospects

6.1 Research and development directions

The future of *Agave* in bioethanol production holds significant promise, particularly in the areas of genetic engineering and improved fermentation methods. Genetic engineering can be utilized to enhance the carbohydrate content and stress resistance of *Agave* species, making them more suitable for bioethanol production (Bautista-Montes et al., 2022). Additionally, optimizing fermentation methods, such as consolidated bioprocessing and co-fermentation of hexose and pentose sugars, can significantly improve ethanol yields and process efficiency (Carrillo-Nieves et al., 2019; Zhao et al., 2020). Research into advanced pretreatment techniques, such as hydrothermal and ammonia fiber expansion (AFEX), has shown potential in increasing sugar recovery and ethanol yield from *Agave* biomass (Aguilar et al., 2018; Flores-Gómez et al., 2018).

6.2 Policy and incentives

Government policies play a crucial role in promoting the adoption of *Agave* as a feedstock for bioethanol production. Policies that support research and development, provide subsidies for bioethanol production, and set mandates for renewable energy use can drive the growth of this sector. For instance, policies that incentivize the use of renewable energy sources over grid energy can make bioethanol production from *Agave* economically viable (Parascanu et al., 2021). Additionally, regulatory frameworks that facilitate the commercialization of genetically modified crops can accelerate the development of high-yield *Agave* varieties (Bautista-Montes et al., 2022).

Incentives for farmers and producers are essential to encourage the cultivation of *Agave* for bioethanol production. Financial incentives, such as subsidies and tax breaks, can reduce the initial investment required for *Agave* cultivation and processing. Training programs and technical support can help farmers adopt best practices for *Agave* cultivation, ensuring high yields and sustainable farming practices. Moreover, creating a stable market for *Agave* bioethanol through government procurement programs can provide a reliable income stream for producers (Carrillo-Nieves et al., 2019; Parascanu et al., 2021).

6.3 Global implications

Agave bioethanol has the potential to play a significant role in the global renewable energy landscape. Due to its high carbohydrate content and drought-resistant nature, *Agave* can be cultivated in arid and semi-arid regions where other crops may not thrive, thus not competing with food crops for arable land (Jones et al., 2020). The environmental benefits of *Agave* bioethanol, such as lower fertilizer and pesticide requirements, further enhance its appeal as a sustainable biofuel source (Parascanu et al., 2021). As countries seek to reduce their greenhouse gas emissions and dependence on fossil fuels, *Agave* bioethanol can contribute to achieving these goals by providing a renewable and eco-friendly energy alternative (Flores-Gómez et al., 2018; Ramachandra and Hebbale, 2020).

7 Concluding Remarks

Agave species, particularly those grown in semi-arid regions, present a promising feedstock for bioethanol production due to their high biomass yield and adaptability to harsh climates. Research has demonstrated that various *Agave* species, including those not traditionally used for alcoholic beverages, can be effectively converted into fermentable sugars and subsequently into bioethanol. For instance, *Agave neomexicana* has shown significant ethanol yields when processed with appropriate enzymatic blends. Additionally, the use of agave bagasse, a byproduct of tequila production, has been successfully scaled up for bioethanol production, achieving high saccharification and ethanol yields. These findings underscore the potential of *Agave* as a sustainable and efficient source of bioethanol, particularly in regions unsuitable for other biofuel crops.

Despite its potential, several technical challenges must be addressed to optimize *Agave* for bioethanol production. One major challenge is the efficient pretreatment of *Agave* biomass to enhance enzymatic hydrolysis. Various pretreatment methods, such as hydrothermal, dilute acid, and ammonia fiber expansion (AFEX), have been explored to improve the digestibility of *Agave* biomass. Each method has its advantages and limitations, with AFEX showing promise in achieving high sugar conversions and ethanol yields without the need for washing steps or nutrient supplementation. Another challenge is the presence of inhibitory compounds in some *Agave* species, which can hinder fermentation at high solids loadings. Optimizing enzyme cocktails, such as incorporating hyperactive pectinase, has been shown to significantly improve saccharification efficiency. Additionally, the development of robust fermentation strains capable of co-fermenting multiple sugars and tolerating inhibitors is crucial for maximizing ethanol production.

The future of *Agave* in the bioethanol industry looks promising, particularly as research continues to address the technical challenges associated with its conversion to biofuels. The adaptability of *Agave* to grow in semi-arid and marginal lands makes it an attractive feedstock for regions facing water scarcity and land degradation. Furthermore, advancements in pretreatment technologies and enzyme optimization are likely to enhance the economic viability of *Agave*-based bioethanol production. As the demand for sustainable and renewable energy sources grows, *Agave* could play a significant role in diversifying the bioethanol feedstock portfolio and contributing to global energy security. Continued research and development, coupled with supportive policies and investment, will be essential to fully realize the potential of *Agave* in the bioethanol industry.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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