

Review of Pretreatment Strategies for *Agave* Biomass Toward Ethanol Production

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Abstract: With the growing interest in the potential of *Agave* lignocellulosic bagasse for biofuel production, it is essential to evaluate pretreatment strategies that effectively disrupt the lignocellulosic structure, enhance glucose release from structural cellulose, and enable its efficient conversion into ethanol. These advancements contribute to the sustainable utilization of resources within the global energy matrix. This review discusses chemical, physical, physicochemical, and biological pretreatment methods applied to *Agave* biomass, highlighting the most promising approaches for second-generation ethanol production.

Keywords: *Agave* biomass, Pretreatment, ethanol production.

1. INTRODUCTION

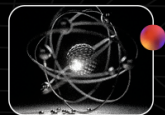
Second-generation ethanol is produced from the lignocellulosic fraction of plant biomass that remains after the extraction of juice rich in fermentable sugars, which is typically used for first-generation (1G) ethanol production [1]. The residual bagasse from this initial stage contains a complex lignocellulosic matrix that, after appropriate pretreatment and hydrolysis, can be converted into additional ethanol [2].

The increasing demand for sustainable energy sources from plant-derived residues has drawn significant industrial interest toward expanding biofuel production lines [3]. However, efficient conversion of lignocellulosic biomass requires the disruption of tightly bound structural components, particularly lignin — a complex aromatic polymer that plays a key role in protecting plant integrity and resisting microbial degradation [4]. According to Medeiros (2019) [5], lignocellulosic biomass is mainly composed of cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%), along with minor amounts of extractives and minerals. The considerable fermentable sugar

content of this material reinforces its potential contribution to diversifying the global energy matrix and reducing reliance on fossil-based fuels [6]. Recent studies have focused on *Agave* species as promising feedstocks for second generation ethanol production [7]. For this to be achieved, the structural polymers in the biomass must be depolymerized into fermentable sugars, which requires an effective pretreatment step to increase cellulose accessibility and improve subsequent enzymatic hydrolysis [3]. Pretreatment methods — including physical, chemical, physicochemical, and biological approaches — influence not only lignin and hemicellulose removal but also sugar yield and the formation of fermentation inhibitors [8].

Considering these aspects, the following sections review the main pretreatment strategies applied to *Agave* biomass, discussing their mechanisms, advantages, limitations, and potential to enhance ethanol production.

2. METHODOLOGY

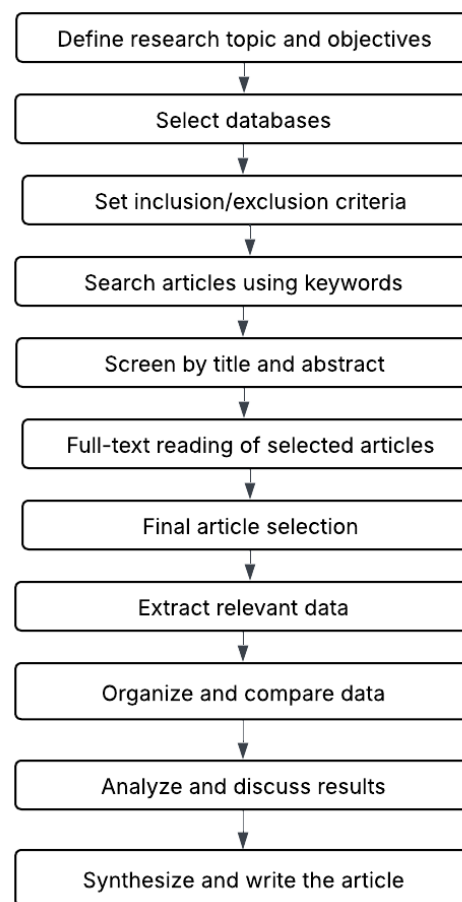


This article presents a systematic literature review aimed at understanding the main pretreatment methods and evaluating their feasibility for producing second-generation ethanol from *Agave* lignocellulosic residues. The literature search was conducted across academic databases including Scopus, Web of Science, ScienceDirect, and Google Scholar, targeting publications from 2014 to 2025.

Search terms included combinations of “*Agave* biomass,” “lignocellulosic pretreatment,” “second-generation ethanol,” “bioethanol production,” and “fermentable sugars.” Selected studies were those providing detailed information on pretreatment techniques applied to *Agave* biomass or closely related lignocellulosic materials, with emphasis on process parameters, sugar yields, enzymatic hydrolysis outcomes, and ethanol production efficiencies [1–21].

The methodology followed the flowchart illustrated in Figure 1, which describes the stages of this systematic review: definition of the research scope and objectives, database selection, keyword-based article search, screening by title and abstract, full-text analysis for inclusion, data extraction, and qualitative synthesis.

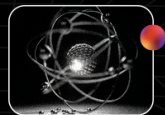
Figure 1. Flowchart of the systematic review methodology for *Agave* biomass pretreatment in ethanol production.



Data extracted from the literature were organized to compare physical, chemical, physicochemical, and biological pretreatment methods, focusing on operational conditions, sugar release efficiency, lignin removal, inhibitor formation, environmental impact, and scalability. This systematic approach allowed the identification of promising pretreatment strategies and knowledge gaps in the field.

3. PRETREATMENT OF BIOMASS

The main criteria analyzed in pretreatment processes is their efficiency in terms of cellulose and hemicellulose availability [9]. The functionality of this process may depend on the characteristics of the biomass, in addition to the method that will be applied for bioethanol



production [10] [11]. The main pretreatments applied to *Agave* are chemical, physical, physicochemical, and biological; each category may contribute to the characterization stage, such as the production of fermentable sugars [12].

Therefore, through the collection of scientific articles on the types of pretreatment used in the lignocellulosic composition of *Agave* bagasse, this work will present the pretreatments carried out in the processing of *Agave* biomass for the production of 2G ethanol, in order to evaluate the efficiency in relation to exposure to sugars, execution cost, environmental impacts, in addition to other factors that may affect the scaling of the process to the industrial level of the bioenergy sector.

3.1. Physical-Chemical Pretreatment

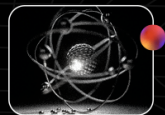
Physical pretreatment plays a fundamental role in the subsequent steps. Drying ensures biomass stability and prevents moisture interference in subsequent processes [13]. Grinding reduces particle size, increasing the specific surface area and facilitating enzyme access to the cellulose [14]. Finally, sieving allows for the standardization of biomass particle size, ensuring greater uniformity in enzymatic attack and improved reactor performance [10].

In the study conducted by Alencar (2023) [15], the efficiency of a continuous physical pretreatment process using a twin-screw extruder applied to *Agave tequilana* biomass was evaluated. Extrusion was performed with

varying operating parameters, such as temperature, biomass moisture content, and screw rotation speed, to promote the disruption of the lignocellulosic matrix. This type of equipment allows the simultaneous application of mechanical and thermal forces, resulting in cell wall rupture and modification of the plant fiber structure.

After pretreatment, the samples were subjected to enzymatic hydrolysis, and the released fermentable sugars were quantified. The results indicated a glucose yield of over 60%, in addition to offering advantages such as reduced energy consumption and compatibility with integrated biorefinery systems, demonstrating the potential of extrusion as viable technology for industrial-scale application.

During dilute acid pretreatment of *Agave lechuguilla* biomass [16], performed an experimental design combined with response surface methodology was used to optimize the acid pretreatment of *Agave* by varying temperature (160–200 °C) and acid concentration (0.5–1.5% w/v). Optimal conditions were found to be 180 °C and 1.24% (w/v) H₂SO₄ with 10% biomass loading, resulting in 87% recovery of hemicellulosic sugars and 68 g glucose per 100 g glucose in raw *Agave*. The resulting slurry was enzymatically saccharified and co-fermented by the ethanologenic *Escherichia coli* MM160, enabling complete sugar fermentation in a single step and achieving an ethanol yield of 73.3%.



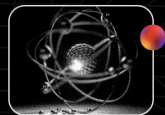
The research conducted by Avila-Lara et al. (2015) [17] aimed to optimize the alkaline and dilute acid pretreatments applied to *Agave* bagasse. The biomass was subjected to alkaline (NaOH) and dilute acid (H₂SO₄) pretreatments under varying conditions, according to the experimental design. Subsequently, enzymatic hydrolysis was performed with Cellic® CTec2 and HTec2 complexes, and total reducing sugars (TRS) were quantified using the DNS method.

The results indicated that the alkaline pretreatment produced total sugar up to 533 mg/g of biomass under the optimal conditions of 1.87% NaOH, 50.3 min, and 13.1% solids. In the other hand, acid pretreatment, while also effective, had a maximum yield of 457 mg/g under 2.1% H₂SO₄, 33.8 min, and 8.5% solids. Compared to raw biomass (135 mg/g), both methods yielded significant gains.

Furthermore, alkaline pretreatment promoted greater lignin removal, resulting in increased porosity and cellulose accessibility, while dilute acid favored hemicellulose solubilization. Porosimetry revealed an increase in specific surface area, especially in the treated samples, which may have favored subsequent enzymatic action. During dilute acid pretreatment of *Agave lechuguilla* biomass, performed in a stirred reactor with 10% solids and different severity conditions, significant variations in the composition of the solid and liquid fractions were observed. Solids recovery after pretreatment ranged from 37.8% to 54%, being lowest under the most severe

conditions (CS = 2.38). Water-insoluble solids remained between 38% and 46%, with no notable differences between the CS extremes. However, total recovery of this fraction ranged from 73.3% to 100%, with complete recovery achieved under milder conditions (CS = -0.75). Hemicellulose solubilization was strongly influenced by the severity of the pretreatment, ranging from 23% to 95%. Under intermediate conditions, hemicellulose sugar recovery averaged 90%. However, under severe CS, degradation of these sugars was observed, with the HSR dropping to only 55.3%.

Taking into account the results reported by Pérez-Pimienta et al. [18], *Agave* bagasse was subjected to two pretreatment methods to evaluate its potential for ethanol production. In the first, the ionic liquid [C₂C₁Im][OAc] was used, where 50 g of dry biomass was mixed with 450 g of the solvent and heated at 120°C for 3 hours. After treatment, a wash with 9,000 g of deionized water was performed to remove the residual ionic liquid. In the second method, the biomass was treated with a solution of water (74.5%), ethanol (25%), and sulfuric acid (0.5%) at 160°C for 10 minutes, followed by washing with ethanol and water under similar conditions. Both pretreatments allowed significant lignin removal (~45%) and presented different mass recoveries: 71.2% for the ionic liquid (IL) method and 53.8% for the organic solvent (OV) method, the latter due to the greater xylan removal (86%). In the enzymatic saccharification step, the IL-treated bagasse generated 20.9 kg of glucose and 8.3 kg

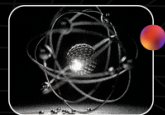


of xylose per 100 kg of biomass, while the OV method resulted in 26.8 kg of glucose and 2.5 kg of xylose. The total fermentable sugar production was similar in both cases. In the subsequent fermentation, ethanol yields were 12.1 kg (IL) and 12.7 kg (OV) per 100 kg of biomass, representing a significant increase compared to the untreated biomass (5.7 kg). The remaining solid residues, composed of lignin and unconsumed sugars, can be used to generate energy or synthesize value-added products. Furthermore, the liquid streams generated in the process contain valuable compounds such as xylan and lignin, which can be converted into new ionic liquids, completing the cycle of a sustainable biorefinery.

Autohydrolysis is one of the *Agave* pretreatment processes that does not involve the use of chemicals, only water at a high temperature and pressure determined by the Severity Factor. This method depolymerizes hemicellulose into oligomers and converts it to ethanol. In the experiment proposed by Rios-Gonzalez et al. (2017) [19], experiments were conducted based on different Severity Factor (SF) levels, which relate temperature and reaction time. The results indicated that the higher the SF, the greater the solubilization of xylan, a more easily degraded hemicellulose. Glucan, the main component of cellulose, was partially preserved but suffered losses in tests with higher SF. The SF, corresponding to 190°C for 30 minutes, presented the best balance between glucan preservation in the solids and low formation of toxic byproducts

such as acetic acid, furfural, and HMF. Another, more severe test resulted in the highest glucose yield after hydrolysis (7.97 g/L), but with greater glucan degradation and an increase in the production of undesirable byproducts. Lignin, in turn, was not significantly removed due to the relatively low pH of the treatment, which also influenced the final composition of the solid fractions. The study concludes that higher SFs eliminate xylan, which acts as a physical barrier to enzyme action, and this improves glucan hydrolysis. However, there is a necessary balance between severity and cellulose preservation to avoid losses and generate fewer inhibitory compounds. Therefore, a severity factor of 190°C for 30 minutes is considered the most efficient for maximizing glucan content with less interference from byproducts.

Taking into account Ammonia fiber expansion (AFEX) pretreatment, the study by Flores-Gómez et al. (2018) [20] aimed to disrupt the lignocellulosic structure of the bagasse and leaf biomass of *Agave tequilana* and *Agave salmiana* species. Initially, the researchers optimized the pretreatment conditions for *Agave tequilana* bagasse using statistical design, varying temperature, humidity, residence time, and the ammonia-to-biomass ratio (NH₃/biomass). The best results were obtained under intermediate-severity conditions in the AFEX process: a temperature of 120°C, NH₃/biomass above 1.75 g/g dry matter (DM), humidity of 0.4 g H₂O/g DM, and a residence time of approximately 38 minutes.



Leaf biomass showed slightly higher sugar conversion than bagasse, attributed to its lower lignin content. In the next step, the pretreatment conditions were tested on other residues: *A. tequilana* leaf fiber, bagasse, and *A. salmiana* leaves. For *A. salmiana* bagasse, a total of 30 experiments were conducted. It was observed that moisture and ammonia load, as well as their interaction, strongly influence the conversion of glucan and xylan. The combination of high ammonia load and low moisture content favored the formation of a more reactive cellulose, facilitating enzymatic action, which is consistent with studies on corn stover. Next, the conditions for *A. tequilana* and *A. salmiana* leaf fibers were optimized by varying the moisture content and NH_3 /biomass, at a constant temperature and for 30 minutes. A strong interaction between these two factors was observed, with better conversions (~93% glucan) obtained with 3g NH_3 /g DM and 0.2g H_2O /g DM at moderate temperatures. This can be attributed to the lower lignin content in the leaves and the combination of high ammonia load and low moisture content. Despite the high sugar conversions, the researchers observed that these conditions are not necessarily the most economical. For the following tests, intermediate NH_3 /biomass ratios were adopted for *A. tequilana* bagasse and *A. salmiana* leaves, and for *A. tequilana* leaves and *A. salmiana* bagasse. These choices aim to maintain the economic viability of the process without compromising the efficiency of converting biomass into fermentable sugars.

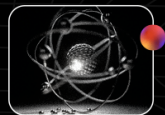
3.2 Biological Pretreatment

The study investigated the biological pretreatment of *Agave lechuguilla* biomass using the fungus *Phanerochaete chrysosporium* H-298 [21]. After universal physical pretreatment, the bagasse was sterilized and incubated with the fungus in modified Kirk's medium, with controlled humidity at 60% and a temperature of 30°C. The treatment was carried out for periods of up to 20 days and then extended to up to 60 days with different concentrations of ammonium tartrate (nitrogen source).

In the initial tests, a maximum lignin degradation of 15.9% was observed after 15 days, with preservation of cellulose and hemicellulose, demonstrating the fungus's selectivity. In the optimization stage, with a concentration of 1M ammonium tartrate and 60 days of incubation, lignin removal reached 36.15%. Higher concentrations inhibited the action of ligninolytic enzymes, resulting in low efficiency. These results indicate that treatment time and adequate nitrogen quantity are key factors for efficient lignin degradation, allowing greater biomass digestibility and maintaining its structural sugars for later conversion into bioethanol.

4. CONCLUSION

The reviewed pretreatment strategies demonstrate varied strengths and challenges. Twin-screw extrusion offers operational viability for mechanical disruption. Acid and



autohydrolysis methods efficiently solubilize hemicellulose but necessitate careful control of inhibitors. Ionic liquid and organosolv approaches provide selective lignin removal compatible with circular biorefineries. Alkaline and AFEX pretreatments enhance cellulose accessibility and reduce toxic compounds, albeit with operational complexities. Biological pretreatment presents a low-impact option, demanding further optimization.

Despite these advances, a lack of standardized comparative analyses limits comprehensive understanding of critical factors such as glucose yield, inhibitor formation, and energy consumption. Future research should adopt systemic evaluations integrating technical, economic, and environmental aspects to guide scalable *Agave* biomass pretreatment for ethanol production.

Different pretreatment methods for *Agave* biomass have distinct pros and cons. Physical methods like grinding and extrusion improve accessibility but can be energy-intensive and may not remove lignin effectively. Chemical pretreatments, such as dilute acid and alkaline in combination, efficiently solubilize hemicellulose and lignin, increasing sugar yields, but acid methods may produce inhibitors, and alkaline processes can be costly and require longer times. Ionic liquids and organosolv treatments selectively remove lignin and maintain sugar yields but face challenges in cost and solvent recovery. Physicochemical methods like autohydrolysis and AFEX are greener alternatives but need

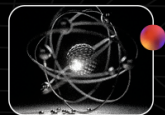
careful optimization to avoid sugar loss. Biological pretreatments using fungi are environmentally friendly and selective but are slow and require controlled conditions. Choosing the best method depends on balancing efficiency, cost, environmental impact, and scalability.

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