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BIBLIOMETRIC ANALYSIS OF TRENDS AND TECHNOLOGIES IN PRE-TREATMENT FOR ETHANOL PRODUCTION FROM *AGAVE* FOR 1G AND 2G

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ABSTRACT

This bibliometric analysis delves into the latest trends and state-of-the-art technologies within pre-treatment processes for obtaining both 1G and 2G ethanol from Agave sp. The study comprehensively explores a diverse array of academic literature and patents, scrutinizing advancements, and innovations in the field. The primary focus encompasses optimization techniques, technological breakthroughs, and emerging methodologies associated with ethanol production from *Agave*. The analysis not only highlights existing developments but also identifies technological gaps that need attention to address the evolving landscape of pre-treatment strategies. This information is invaluable for researchers, professionals, and policymakers in the biofuels industry. In the face of escalating demand for sustainable energy sources, a nuanced understanding of the bibliometric landscape surrounding *Agave*-based ethanol production is essential to effectively steer future research and development endeavors.

Keywords: Ethanol Production *Agave tequilana*. Pre-treatment. Bibliometric Analysis. Biofuel.

1 INTRODUCTION

The search for sustainable and renewable energy sources has become crucial in addressing global challenges related to climate change and energy security. Among promising alternatives, ethanol derived from *Agave sp*. stands out as a potential biofuel resource due to its inherent advantages, such as high biomass yield and resilience in diverse environments.

Various pre-treatments can be applied to produce 1G and 2G ethanol. 1G ethanol synthesized from *Agave's* juice can be extracted through various methods, including milling, pressing, heating, among others. Thus, the pre-treatment for 1G ethanol production is closely tied to the method of extracting juice from biomass, i.e., extracting the liquid fraction from the plant for fermentation use. Additionally, *Agave* juice contains furans, which, due to their polymeric nature, may not be assimilated by a wide range of microorganisms. Consequently, the liquid fraction of *Agave* may need to be hydrolyzed beforehand to become a fermentable material[1].

On the other hand, 2G ethanol is produced from the solid residual material, or bagasse, obtained from sap extraction. Due to the complex structures of the plant cell wall in these lignocellulosic materials (LCM), various pre-treatment methods, whether physical, chemical, or biological, can be applied. Physico-chemical methods include steam explosion, ammonium fiber expansion, pre-treatments with diluted acid or alkali, organic solvents, enzymes, among others. These pre-treatments have proven capable of reducing biomass recalcitrance for subsequent biological methods, such as enzymatic saccharification for biofuel production or high-value-added products[2]. In this context, pre-treatment technologies are constantly evolving to enhance the technical and economic utilization of lignocellulose in biorefineries for ethanol production. These pre-treatment technologies differ in their mode of action and their effects on different lignocellulosic materials.

Therefore, the aim of this bibliometric analysis is to provide an overview of current trends and technologies in pre-treatment processes specifically tailored for obtaining 1G and 2G ethanol from *Agave*. As the global demand for sustainable energy continues to rise, understanding the bibliometric landscape of *Agave*-based ethanol production becomes imperative to guide and inform future research and development efforts.

2 MATERIAL & METHODS

The methodology is structured into three phases for evaluation and systematic data extraction. First, a thorough search was carried out in the Lens.org database, focusing on defining search criteria and refining records collected from 2014 to 2024. Subsequently, data visualization was obtained by exporting documents to the software *VOSviewer* for bibliometric analysis. In order to obtain reliable and accurate details based on selected keywords included in the publications, authors, countries, institutions, journals, and patents (actives), they were taken into consideration in the analysis by restricting the search. Additionally, titles and abstracts of academic papers and patents found were included in the analysis to ensure comprehensiveness and coherence.. The third phase involved data analysis, identifying key themes *Agave tequilana* and Pre-treatment, exploring use in Ethanol production and application in 1G and 2G ethanol, and considering protection of the technology through patent examination. Manually deleting irrelevant terms ensured a focused analysis. Furthermore, the grouping of keywords was described as follows: ("*agave tequilana*") AND ("chemical treatment" OR "physical treatment" OR "biological treatment" OR "hydrolysis") AND ("biofuel" OR "ethanol") facilitated the identification of relevant themes based on the keyword connections in each cluster.

3 RESULTS & DISCUSSION

The production of ethanol from *Agave sp*. involves a series of processes, such as: Receipt and standardization of raw materials; Extraction of agave juice (separation of juice and biomass); Hydrolysis or pre-treatments (when necessary, in different steps of process); Fermentation; Distillation. Therefore, defining the best conditions for each of these processes guarantees a product that meets the established quality requirements[3]. Patents play a crucial role in safeguarding innovations, promoting technological advancement, and providing incentives for research and development. Regarding patents, 591 active patents were identified, 232 of which were for applications in ethanol and biofuels. Figure 1 (a) illustrates the International Patent Classification and the type of document, showing application in Ethanol and techniques for obtaining fermentable sugars from lignocellulosic material and other sources. However, Figure 1 (b) shows the main depositors, generally companies that work with microorganisms and enzymes for different applications. There are also beverage, food, and pharmaceutical companies, as sugars and other bioactive compounds can be removed through pre-treatments.

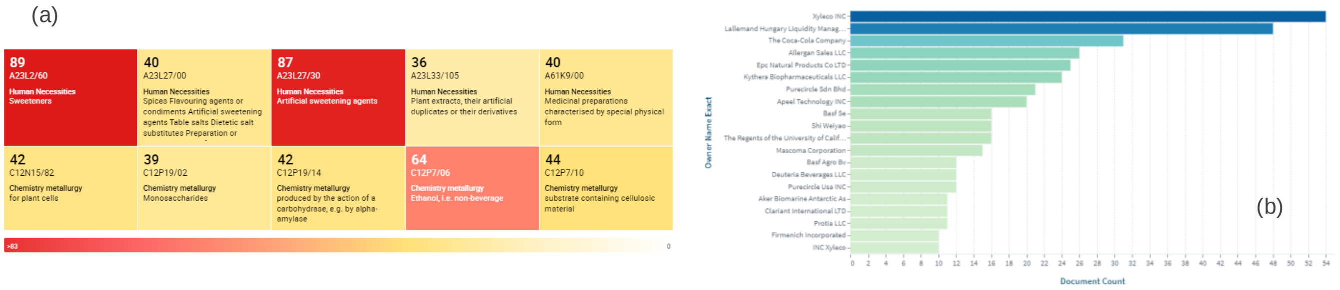

Figure 1 (a) Patent classification heat map and (b) Top owners.

The Table 1 summarizes some examples of pre-treatments used to treat agave biomass and demonstrates the importance of pre-treatment to release fermentable sugars. There is also a preference for *Agave tequilana*, most studies are focused on this species. Due to its established market for Tequila production. It is worth mentioning that in the search of 234 articles found, only 150 articles matched the search keywords.

Table 1 Studies containing different *Agave* extraction and pre-treatment (hydrolysis) processes.

Raw Material (References)	Juice extraction	Solid Waste Pre-Treatment	Results
***A. tequilana* Bagasse**[4]	Cut and cook the plant at 90°C in a steam oven. Further crushing to separate juice from bagasse	Auto-hydrolysis: Bagasse (dry basis) loaded into a reactor with distilled water (ratio 1:6 m/v) stirred at 200rpm at a temperature of 190 °C between 15-60 min. Followed by enzymatic hydrolysis with cellulases at 50 °C stirred at 200 rpm for 72 h	Auto Hydrolysis: Glucans: 64.7% and Xylans: 6.02%. Enzymatic Hydrolysis: Glucose: 131 g/L with a yield of 81.5%
***A. tequilana* Bagasse**[5]	Milling	Acid and enzymatic hydrolysis	Acidic: 24.9 g/L of fermentable sugars and 36.1% yield. Enzymatic: 41.0 g/L of fermentable sugars and 73.6% yield
***A. tequilana* leaves powder**[6]	Dry mill at 100 °C	Enzymatic hydrolysis with Inulinase or Cellulase	Inulinase: 36.2% yield. Cellulase: 16.8% yield.
***A. tequilana* Bagasse**[7]	Dried at 80 °C for 24 hours in an electric oven and then partially reduced in size in a blade mill.	Acid hydrolysis (H_2SO_4) and steam explosion	71.11 g/L of reducible sugars in the supernatant (59.29% glucose, 29.05% xylose and 11.66% fructose) and unconverted organic matter from enzymatic hydrolysis bagasse (35.4% α-cellulose, 7.33% hemicellulose, 49.91% lignin and 7.31% ash)
***A. tequilana* leaves - 2 years**[8]	Crushing, drying at 45°C and grinding	Use of dilute acid by soaking the ground biomass overnight in a solution of 5.0 kg.m^3 H_2SO_4 at a ratio of 0.009 m^3 $H2SO_4$ per kg dry weight of biomass	133.5 g.kg $^{-1}$ soluble free sugar
		No hydrolysis treatment	95.8 g.kg $^{-1}$ soluble free sugar
***A. tequilana* Bagasse**[9]	Sun drying and shredding	Alkaline hydrogen peroxide (AHP) followed by enzymatic saccharification with cellulase (Celluclast 1.5 L) and hemicellulase (Viscozyme L)	97% delignification and recovery of 88% cellulose and hemicellulose after only 1.5 h of treatment
		Enzymatic hydrolysis with cellulase	4.72 ± 0.2 g/L total sugars
		Treatment with AHP and cellulase	10.3 ± 0.9 g/L total sugars
		Enzymatic hydrolysis with hemicellulase	9.3 ± 0.1 g/L total sugars
		Enzymatic hydrolysis with a mixture of cellulase and hemicellulase	24.9 ± 0.1 g/L total sugars
		CH Sequential Enzymatic Hydrolysis	19.0 ± 0.3 g/L total sugars
		HC Sequential Enzymatic Hydrolysis	26.6 ± 0.4 g/L total sugars

***Agave tequilana* leaves**[10]	Dried at 100 °C, particle size ≤ 0.3 mm	Dilute acid (H_2SO_4 or HCl) at different concentrations (0.5% - 2.0% v/v) and enzymatic hydrolysis was performed using four commercial enzymes: Viscozyme, Cellic HTec, Celluclast and Cellic CTec-2 separately.	Best result: H2SO4 (0.5% v/v) and enzymatic hydrolysis with Cellic CTec-2 for 18 h. Release of sugars and fructans with a yield of 97%, obtaining 68 g of reducing sugars per 100 g of agave powder.
***A. tequilana* Bagasse**[11]	Grinding in a knife mill with particle size between 2-0.3 mm	Autohydrolysis (160, 170 and 180°C for 30 min) and enzymatic hydrolysis with Cellic® CTec2	The maximum concentration reached was at 180 °C in isothermal regime (30 min) with 12.42 g/L of glucose and saccharification yield of 99%.

The different types of hydrolysis applicable to the process and their respective results inherent to each one were observed. Therefore, as a way of analyzing the most used processes in scientific research, bibliometric research was carried out, carried out in the Lens.org database according to the methodology's topic descriptors and analyzed by the *VOSViewer* software, in total 145 documents were found, among these documents, the focus is generally on 2G Ethanol and it is clear that 1G is still little explored. In general, the main pre-treatments applied to improve the profile of fermentable sugars of *Agave* are acid and enzymatic treatments, as shown in Figure 2 (a), in Figure 2 (b) the main fields of research relevant to the topic are presented.

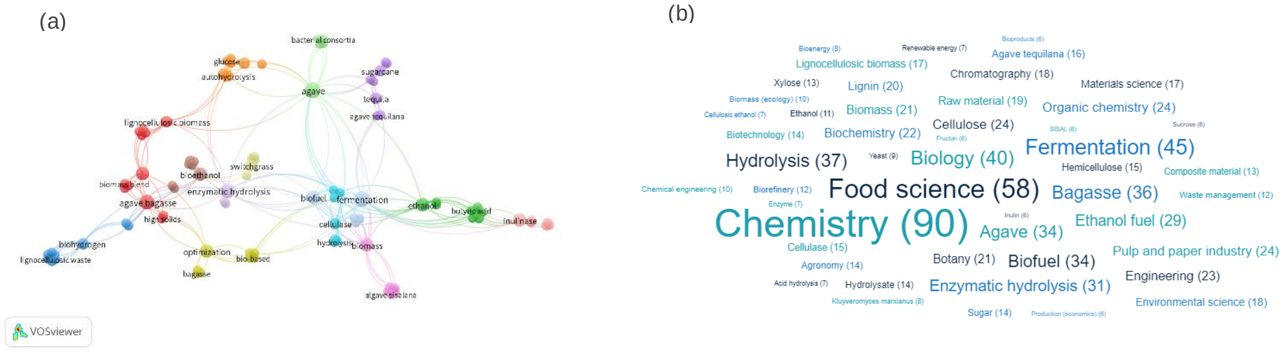

Figure 2 (a) Network analysis with keywords (*VOSviewer* software) and (b) Fields of research relevant

Therefore, this detailed search is important to clarify the main technological gaps for ethanol production considering this raw material.

4 CONCLUSION

The ethanol production from *Agave* it involves complex processes such as standardization of raw materials, juice extraction and pre-treatments such as hydrolysis, which are crucial to producing high-quality ethanol. Patents play a key role in driving technological advances in ethanol production, with a significant focus on ethanol and biofuel applications. Acid and enzymatic treatments emerge as predominant strategies in releasing fermentable sugars from Agave biomass. Although considerable attention has been given to 2G ethanol, exploration of 1G ethanol remains limited. Interdisciplinary collaboration and continuous innovation are essential to optimize ethanol production processes from *Agave tequilana*. and realize its potential as a sustainable source of biofuels.

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