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Bibliometric Analysis of Research on Bioethanol Production from Algaroba (*Prosopis Juliflora*)

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This study conducts a bibliometric analysis of the last decade, focusing on the production of 2G ethanol from Algaroba as a biomass source. Using the Web of Science database, four main areas were categorized: Product (second-generation ethanol, bagasse, ethanol production, bioethanol, ethanol), Process (biomass treatment, fermentation, distillation, hydrolysis, saccharification, adsorption), Equipment (bioreactors, fermenters), and Raw Material (Algaroba, *Prosopis juliflora*). The combination of Product and Raw Material revealed significant clusters, highlighting 40 prominent articles in the areas of biotechnology, renewable energy, and biological resource technology. The predominant process involves steps such as drying, size reduction, pretreatment, hydrolysis, detoxification, fermentation, and bioethanol separation. The choice of microorganisms, especially *S. cerevisiae* and *Z. mobilis* in the last three years, reflects the trend towards thermotolerant yeasts for increased saccharification and fermentation efficiency. Co-culturing for the fermentation of sugar mixtures emerges as a promising alternative. This study contributes to understanding the research dynamics in second-generation ethanol production from Algaroba, providing crucial insights to advance toward a more sustainable energy matrix.

1. Introduction

Due to the benefits associated with the use of ethanol as an alternative or complementary fuel for the transportation sector, there is a global growth in interest in the research and development of biofuels derived from renewable sources, promoting a sustainable approach based on biomass (Hemansi et al., 2019). Cellulosic material obtained from wood, agricultural residues, municipal solid waste, and energy crops represents the most abundant global source of biomass, which are renewable resources that store solar energy in their chemical bonds (Romaní et al., 2013). Lignocellulosic biomass stands out as the most promising raw material due to its wide availability, low cost, and non-direct competition with food production (Hemansi et al., 2019). Lignocellulosic materials are rich in lignin and holocellulose, that is, cellulose and hemicellulose. Lignin needs to be separated to convert cellulose and hemicellulose into fermentable sugars. Bioethanol is produced from these sugars through fermentation. The physicochemical properties of lignin present challenges that are subject to variations based on its origin, the extraction method used, and the process by which it was derived, therefore, the applicability of lignin as a raw material for value-added products may vary considerably (Fabricia et al., 2016). The bioconversion of lignocellulosic biomass into ethanol requires four main unit operations, including pre-treatment, hydrolysis, detoxification, and fermentation (Chandel et al., 2007). In this context, Algaroba, *Prosopis juliflora* (*PJ)* emerges as a promising biomass due to its tolerance and ease of growth in arid and saline soils, with alkaline pH and extreme temperatures (Ramírez et al., 2019). Known for their high phytoremediation potential, erosion control, and soil fertility improvement, these plants are typical of arid and semi-arid regions and have been colonized in many countries, such as the Americas, Africa, and Asia. Considered an invasive species, it grows quickly and can produce up to 100 tons per hectare in 15-year rotations, being one of the trees that can produce 2.5 tons of wood per hectare per year where nothing else can grow (Felker, 2009). Invasive

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plants can be managed using them as firewood or in the biofuel industry due to their high carbohydrate content of 69.25% (in dry weight) (Naseeruddin et al., 2021), as the higher the carbon concentration in the biomass, the more sugars are available for fermentation and therefore more ethanol can be produced. According to Pasha et al. (2008), the tree can be used as a substrate for long-term sustainable bioethanol production, as it is not part of the main food or feed cycle. Therefore, this study aims to carry out a technological exploration of recent trends in the production of second-generation (2G) bioethanol from *PJ* and illustrate the advances that have emerged in this area of research in recent years.

2. Materials and Methods

In this research, a bibliometric analysis of bioethanol production from Algaroba biomass (*PJ*) was conducted. The study involved the time interval from 01/01/2013 to 23/10/2023.To assume robust information, the Web of Science (WoS) database was carefully chosen as the main reference source. This selection was based on the recognized range and diversity of scientific publications available on the platform. The initial stage involved the cataloging and organization of keywords, which were divided into thematic groups, as depicted in Table 1. Subsequently, multiple combinations among these groups were examined, as can be observed in the following Table 2.

Table 1: Elaboration of groups and variants for search.

Variants	Product (Group 01)	Process (Group 02)	Equipment (Group 03)	Raw material (Group 04)
01	2nd generation ethanol	Pretreatment of biomass	Bioreactor	Algaroba
02	Bagasse	Fermentation	Fermentor	*Prosopis juliflora*
03	Ethanol production	Distillation	-	-
04	Bioethanol	Hydrolysis	-	-
05	Ethanol	Saccharification	-	-
06	-	Adsorption	-	-

The objective was to extend the analysis beyond disciplinary limits, allowing a deep understanding of the relationships between the different aspects explored about the PJ biomass work. Narrowing the search criteria to titles, author keywords, and keywords allowed for a more accurate and relevant selection of results. As for the types of documents, the focus was limited to articles, covering research, conferences, and reviews, found in WoS. The analysis of the results was carried out using the VOSviewer software, which facilitated the understanding of the segmentation of thematic groups and the visualization of the clusters involved.

Table 2: Strategy adopted for bibliometric analysis.

Filter	Combinations	Description of the research
01	#1 AND #4	("2nd generation ethanol" OR "bagasse" OR "ethanol production" OR "Bioethanol" OR "Ethanol") AND ("Algaroba" OR "*Prosopis juliflora*")
02	#2 AND #4	("pretreatment of biomass" OR "Fermentation" OR "Distilation" OR "hydrolysis" OR "saccharification" OR "adsorption") AND ("Algaroba" OR "*Prosopis juliflora*")
03	#1 AND #2 AND #4	("2nd generation ethanol" OR "bagasse" OR "ethanol production" OR "Bioethanol" OR "Ethanol") AND ("pretreatment of biomass" OR "Fermentation" OR "Distilation" OR "hydrolysis" OR "saccharification" OR "adsorption") AND ("Algaroba" OR "*Prosopis juliflora*")

Using polynomial regression, the data were adjusted to obtain the curve. This choice was motivated by the presence of a visible curvature in the data, which could not be better captured by a linear or quadratic trend line. The 'trend' function in Excel software, which calculates polynomial coefficients, was used and adjusted to third order, which allowed for more flexible modeling, providing a more accurate representation of the trend in the underlying data. It is worth mentioning that the choice of the order of the polynomial was based on the behavior observed in the data and was evaluated in terms of quality of fit and visual interpretation.

3. Results and discussions

The research explored various combinations of thematic groups, identifying a total of 165 publications through the WoS database for the period from 2013 to 2023. The analysis reveals that the year 2013 marked a modest beginning with 2 publications, followed by a turning point in 2015 when there was only one publication. From 2016 onwards, however, there was a noticeable acceleration, reaching a peak of 9 publications in 2022. The low quantity of scientific contributions points to the use of Algaroba (PJ) for 2G ethanol production as an innovative technology. As of now, there are no recorded academic contributions for the year 2023 regarding 2G ethanol from *PJ* biomass (Figure 1). Assumptions about this gap may include the anticipation of results from ongoing experiments or the possibility of a strategic reflection period in the academic community regarding the next steps in research on 2G ethanol from Algaroba.

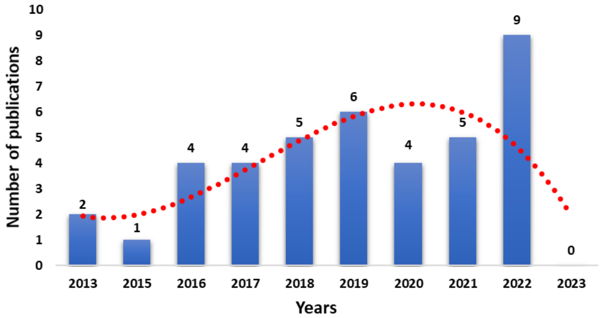

Figure 1: Quantitative distribution of publications for 2G ethanol from Algaroba, 2013–2023.
"Note: The data depicted in this figure for the year 2023 spans from January 1st to October 23rd."

To analyze the main research themes related to "*PJ*" linked to "ethanol production," the most occurring keywords among the retrieved documents were extracted and linked. The analysis of keywords related to "*PJ*" and terms related to "ethanol production" (Filter 1) resulted in the co-occurrence of 320 keywords. Among them, only 31 (9.6 %) reached the threshold of at least three co-occurrences in the same document. The keyword network is shown in Figure 2. The keywords obtained during the search process were classified into three clusters. The terms with the highest co-occurrence in the networks were "*PJ*" (n = 14), "biomass" (n = 10), and "fermentation" (n = 8). These words are prominently featured and are crucial to the theme, having connections with all the words in the network. Analyzing the clusters, it is observed that in cluster 1, the main term is *PJ* (n = 14), and it is contextualized with other terms such as biomass (n = 10), sugarcane bagasse (n = 6), performance (n = 5), cellulose (n = 4), combustion (n = 3). In cluster 2, the terms are related to fermentation (n = 8) and with other components of the same, such as bioethanol (n = 7), pretreatment (n = 6), substrate (n = 5), and simultaneous saccharification (n = 3). Finally, in cluster 3, the emphasis is on terms related to the most worked microorganism, *Saccharomyces cerevisiae* (n = 6), detoxification (n = 5), acid hydrolysis (n = 4), and delignification (n = 3). Table 3 presents the top 5 most cited articles between 2013 and 2023 on the WoS using Filter 1. Although the number of citations does not necessarily constitute an indicator of the quality of an article, it is considered a reliable indicator of its impact and visibility. The top five articles on this list presented content on the main topics involving 2G ethanol production and *PJ*.

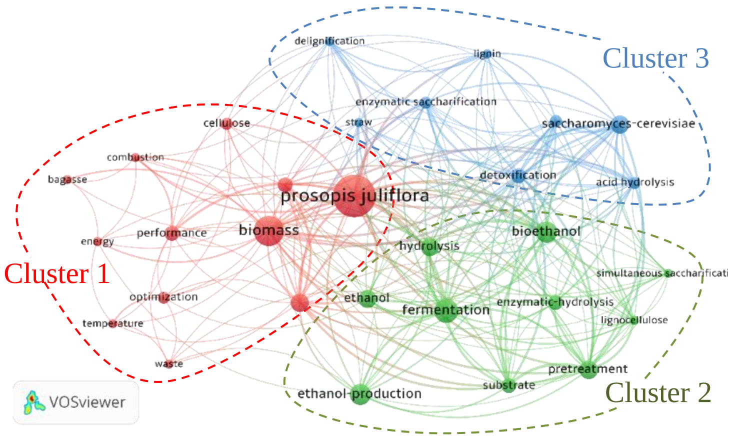

Figure 2: Keyword network using Filter 1 ("2nd generation ethanol" OR "bagasse" OR "ethanol production" OR "Bioethanol" OR "Ethanol") AND ("Algaroba" OR "*Prosopis juliflora*").

Table 3: The most cited articles between 2013 and 2023 on the Web of Science using Filter 1.

Title	Journal	Citations	Author
Selection of the best chemical pretreatment for lignocellulosic substrate *Prosopis juliflora*	Bioresource Technology	44	Naseeruddin et al., 2013
Bioethanol production from woody stem *Prosopis juliflora* using thermo tolerant yeast *Kluyveromyces marxianus* and its kinetics studies	Bioresource Technology	33	Sivarathnakumar et al., 2019
Process optimization of microwave-assisted alkali pretreatment for enhanced delignification of *Prosopis juliflora* biomass	Environmental Progress & Sustainable Energy	23	Alexander et al., 2020
Ethanol production from lignocellulosic substrate *Prosopis juliflora*	Renewable Energy	20	Naseeruddin et al., 2017
Co-culture of *Saccharomyces cerevisiae* (VS3) and *Pichia stipitis* (NCIM 3498) enhances bioethanol yield from concentrated *Prosopis juliflora* hydrolysate	3 Biotech	6	Naseeruddin et al., 2021

In the process of bioethanol production from Algaroba, the main steps follow a general pattern common to bioethanol production from other biomass sources (Figure 3). However, the differences are in the specific sub-steps within each phase of the process. Substrate drying, size reduction, pretreatment, hydrolysis, detoxification, fermentation, and ethanol separation constitute the fundamental structure of the bioethanol production process.

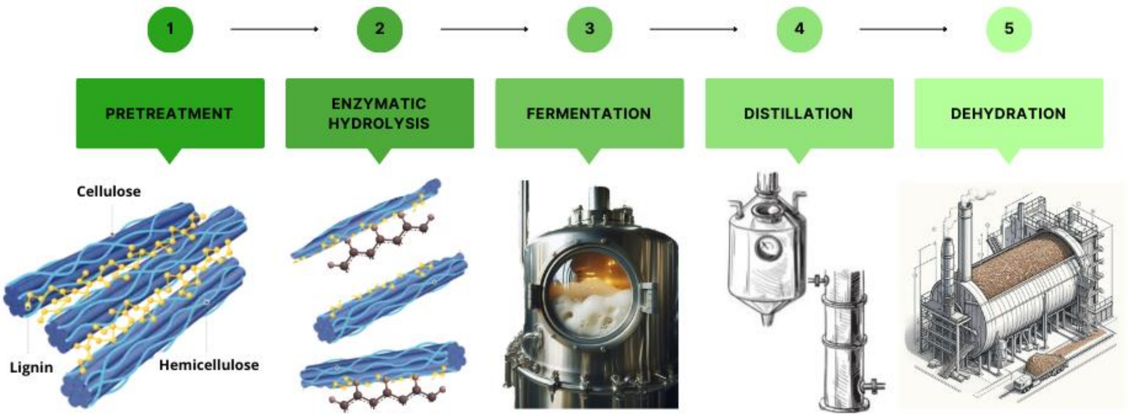

Figure 3: Technological route to produce bioethanol from lignocellulosic biomass.

However, the techniques, conditions, and methods used at each stage are adapted to the specific characteristics of the Algaroba biomass. This adaptation is crucial to optimize the efficiency and quality of bioethanol obtained from this specific resource. During the research, filter 2 presented results far from the research focus, such as adsorption processes, charcoal production, and phytotherapeutic applications using Pj. It was also observed that the documents found by Filter 3 were present in the results of Filter 1. Among the 40 documents retrieved in Filter 1 (Table 2), the most cited article (44 citations) was authored by (Naseeruddin et al., 2013). They adopted a chemical pre-treatment using alkalis (NaOH, KOH, and NH_3), reducing agents ($Na_2S_2O_4$, Na_2SO_3), and $NaClO_2$ at different concentration ranges at room temperature (30 ± 2 °C) to remove the maximum lignin with minimal sugar loss, followed by bifunctional acid saccharification. The second most cited study, with 32 citations, was an article published by (Sivarathnakumar et al., 2019). This article stands out for studying cell growth, substrate utilization, and product formation through simultaneous saccharification and fermentation (SSF), using the thermotolerant yeast *Kluyveromyces marxianus* (MTCC 1389). In the third most cited article, (Alexander et al., 2020), conducted a study on the delignification of *PJ* biomass through alkaline pre-treatment assisted by microwave. According to the study, the researchers also employed Response Surface Methodology (RSM) to optimize process parameters such as microwave irradiation power, microwave irradiation time, alkali concentration, and solid-liquid ratio for effective delignification of *PJ* biomass. The hotspot created on the surface of the pretreated biomass facilitated the degradation of the woody matrix, visible through scanning electron microscopy, and the alkaline solution promoted swelling and increased porosity of the external surface, resulting in the breaking of bonds between lignin and carbohydrates.

The fourth most cited article resulting from this search, (Naseeruddin et al., 2017), represents an extension of the first article by (Naseeruddin et al., 2013), where sodium dithionite ($Na_2S_2O_4$) was selected as the suitable chemical based on the amount of hydrolyzed holocellulose and inhibitors released in dilute bifunctional acid hydrolysis when compared to other pretreatment methods already available for *PJ*. The authors explored different co-culture combinations and discussed ethanol production using hydrolysates obtained from *PJ*, selecting *P. stipitis* and *S. cerevisiae* VS3 as promising for the maximum conversion of sugars.

Finally, the fifth most cited article, (Naseeruddin et al., 2021), takes an approach to using concentrated hydrolysate from *PJ* pretreated with diluted acid through different co-culture combinations: (a) *P. stipitis* (NCIM3498) with *S. cerevisiae* (VS3) and (b) *P. stipitis* (NCIM3498) with *S. cerevisiae* (NCIM3455). The maximum ethanol yield was achieved after 36 hours of fermentation regardless of the co-culture configurations. The strategy used in the article is an extension of the work by (Naseeruddin et al., 2013), for substrate pretreatment and the acid and enzymatic hydrolysates prepared as described by (Naseeruddin et al., 2017). Combination (b), where *S. cerevisiae* (VS3) is added to the medium after 24h of *P. stipitis* (NCIM3498) addition, could be successfully used as a substrate through a mixture of concentrated acid hydrolysate and enzymatic hydrolysate taken in a 1:1 ratio and supplemented with nutrients.

The other articles, with fewer citations, brought relevant technical discussions, such as the development of an efficient method for reducing microbial inhibitors on a pilot scale, where activated carbon adsorption was considered the most effective and cost-effective method for inhibitor reduction, making it industrially competitive. The potential use of PJ pods and their solid byproduct as fuel after hydrolysis. The use of the enzyme *Zymomonas mobilis* for cellulose ethanol production from the woody stem of *PJ*. And the study of *S. cerevisiae* strains regarding different stress tolerances for bioethanol production using *PJ.*

With an average calorific value of 18 MJ/Kg, after hydrolysis, it enables the integration of *PJ* 2G ethanol production with energy production, reducing the use of firewood, charcoal, agricultural waste, and fossil fuels as an energy source, which has several reported environmental consequences. The average total carbon content of 48% (wood), and 42% (pods), favors applications in biomass biorefineries. Low average proportions of lignin 28% (wood), and 5% (pods), make *PJ* suitable for the fermentative production of biofuels since lignin is one of the first barriers present in plant cells. By exploiting the potential of mesquite, we can not only diversify renewable energy sources but also contribute to sustainable development goals 07 and 12 (mostly), identified through analysis of results in WoS.

4. Conclusions

This study provides an overview of the main themes related to bioethanol production from Algaroba, also known as *PJ*, that have been researched in recent years. The trend had been indicating a promising growth in annual publications related to 2G PJ, however, in 2023, there was a sharp decline to zero academic contributions. Assumptions about this gap may include the anticipation of results from ongoing experiments or the possibility of a strategic reflection period in the academic community regarding the next steps in research on 2G ethanol from Algaroba. This decline highlights significant challenges in the technological route, such as the low yield of produced ethanol and the utilization of yeast that converts hexoses and pentoses more efficiently. Based on the analysis of the most cited keywords, it was identified that raw material pre-treatment is one of the main studied themes. Researchers are focused on finding a balance between lignin removal and maintaining sugars present; optimizing parameters of this process, such as the pentose-hexose ratio and substrate load, is crucial for achieving high ethanol yields. New strategies are being developed, including the use of different chemical agents, such as sodium dithionite, to achieve ideal delignification, increasing substrate porosity, and making it more accessible for saccharification. The use of alkaline pre-treatment assisted by microwaves stands out in effectively removing lignin from biomass. Activated carbon and ion exchange resins have been investigated as effective methods for inhibitor removal and increasing bioethanol yield. Additionally, the co-fermentation of microorganisms such as *S. cerevisiae* and *P. stipitis* has shown promising results in ethanol production from lignocellulosic residues of *PJ*. Based on the results presented, it is clear that mesquite has significant potential as a source of biomass for the production of 2G ethanol. The ability to integrate 2G ethanol production with power generation represents a promising opportunity to reduce dependence on electrical energy sources and mitigate associated environmental impacts. Furthermore, its chemical composition favors its application in biomass biorefineries, facilitating the fermentative production of biofuels. By exploiting this potential, we not only diversify renewable energy sources but also contribute to achieving sustainable development goals.

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