Evaluation of the Potential of *Prosopis juliflora* Biomass for 2G Bioethanol Production: Chemical Characterization and Enzymatic Hydrolysis

Yuri Uriel Cerqueira Gil Braz Moreira[1]*, Geise Camila de Araujo Ribeiro[2], Lilian Lefol Nani Guarieiro[2], Carine Tondo Alves[3]

[1]Master's student in Industrial Management and Technology; PRH – ANP Master's Scholarship Holder; [2]SENAI CIMATEC University; Salvador, Bahia; [3]Federal University of Recôncavo da Bahia; Feira de Santana, Bahia, Brazil

This study aims to characterize different parts of the plant *Prosopis juliflora* (Algaroba) and to evaluate the potential of its biomass for second-generation (2G) bioethanol production. Different parts of the plant (*Prosopis juliflora in natura*) were evaluated for cellulose, hemicellulose, lignin, moisture, and ash content, in addition to the yield of reducing sugars in enzymatic hydrolysis with cellulase from *Trichoderma reesei*. The results indicated that the stem bark, rich in cellulose (38.71%) and hemicellulose (21.68%), showed the highest saccharification yield (2.74 mg/mL in 24 h). In contrast, fractions with a high lignin content, such as the pod with seed (45.91%), require pretreatments to make enzymatic conversion feasible. The strategic selection of the biomass fraction and the use of appropriate methods are crucial for optimizing the recovery of fermentable sugars, enabling the use of Algaroba in biofuel production.
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Ethanol has emerged as a viable and complementary energy option to fossil fuels in the transportation sector, stimulating research focused on improving biofuels derived from renewable sources [1]. In this context, lignocellulosic biomass stands out as a promising feedstock due to its wide availability, low cost, and the absence of direct competition with food production [2-4].

Lignocellulosic biomass is composed of cellulose (40–50%), hemicellulose (20–30%), and lignin (10–25%), in addition to smaller amounts of ash, pectin, proteins, non-structural carbohydrates, and extractives. Its chemical composition varies according to the source and cultivation conditions, making initial characterization essential to define efficient strategies for producing biofuels and bioproducts. Hydrolysis of the biomass, following pretreatment, is the second most important step in bioethanol production, as it determines the efficiency of generating the desired product.

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Address for correspondence: Yuri Uriel Cerqueira Gil Braz Moreira. Av. Orlando Gomes, 1845, Piatã, Salvador, Bahia, Brazil. Zipcode: 41650-010. E-mail: yuri-braz@hotmail.com.
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Hydrolysis converts cellulose and hemicellulose polymers into fermentable sugars through either acid or enzymatic processes [1,5,6].

The plant species *Prosopis juliflora*, commonly known as Algaroba, has garnered attention due to its characteristics: it is a small to medium-sized tree with high resistance to adverse environmental conditions, such as arid, saline, and high-pH soils, and is considered invasive in semi-arid regions [7-10].

The use of *P. juliflora* as a feedstock for bioethanol production is a promising strategy to contain its spread in ecosystems where it is invasive, combining both environmental and economic benefits. In this context, this study aims to characterize the different parts of *P. juliflora in natura* and to evaluate the properties of its hydrolysate, thereby understanding its potential for biotechnological applications and its relevance as a raw material in industrial processes.

Materials and Methods

To chemically characterize Algaroba biomass *in natura* (stem bark, woody stem, pod bark, pod with seed, pulp without seed, and seed), this procedure evaluated moisture and ash content, as well as cellulose, hemicellulose, and lignin fractions.

The determination of total solids and moisture followed the protocol described by Sluiter and colleagues [11], the sample was weighed before and after drying at 105°C. Ash content was quantified by calcination in a muffle furnace, following the method by Sluiter and colleagues [12]. Lignin quantification, adapted from Silwadi and colleagues [13] and Sluiter and colleagues [14], involved acid hydrolysis to separate the insoluble fraction (AIL) and allowed quantification of the soluble fraction (ASL) by spectrophotometry. Cellulose determination was performed as described by Asgher and colleagues [15], Bauer and Ibánez [16], Updegraff [17], and adapted from Ribeiro and Assis [18], using hydrolysis with nitric acid and acetic acid, followed by colorimetric analysis with the anthrone reagent. Hemicellulose content was estimated according to Kapoor and colleagues [19], by alkaline extraction and final precipitation induced by sulfuric acid and ethanol.

For enzymatic hydrolysis, commercial cellulase from Trichoderma reesei ATCC 26921 (Sigma-Aldrich©, Merck KGaA, Darmstadt, Germany) was used. Biomass was suspended in citrate-phosphate buffer (100 mmol/L, pH 5) at a concentration of 5% (w/v) [20-24]. The mixture was maintained at 50°C and 150 rpm for 2 hours, followed by the addition of the enzyme (10 U/g dry biomass). Two controls were used: one without substrate (enzyme only) and one without enzyme (substrate only). After 24 hours, reducing sugars were quantified using the DNS reagent, as described by Miller [25], with absorbance measured at 540 nm using a UV/Vis spectrophotometer.

Results and Discussion

The data in Table 1 show marked contrasts in the composition of different parts of *P. juliflora*, which can directly influence biotechnological utilization routes. The stem bark presented high levels of cellulose (38.71%) and hemicellulose (21.68%), totaling 60.39% holocellulose. This profile suggests potential for processes based on fermentable sugars due to the high proportion of easily hydrolyzable components.

The woody stem, although similar in cellulose content (38.24%), had lower hemicellulose content (16.46%), resulting in a slightly lower holocellulose content (54.71%). Still, both fractions show promise for biochemical conversion.

The pod bark showed a low cellulose content (6.85%) but a higher hemicellulose content (21.06%). Its high ash (5.66%) and moisture (20.87%) suggest the need for additional preprocessing steps to avoid yield losses. The pod with seed had high cellulose (39.33%) and the highest lignin (45.91%), requiring more intensive pretreatments to reduce lignin recalcitrance and enable efficient sugar release. However, this fraction also had the highest holocellulose (61.68%), indicating significant potential once lignin barriers are overcome.

The pulp without seeds, with moderate cellulose (23.56%), hemicellulose (21.13%), and lignin (20.52%), presented a balanced composition, indicating moderate potential for enzymatic hydrolysis. In contrast, the seed contained very low levels of fibrous constituents (7.12% cellulose and 10.57% hemicellulose), making it less suitable for sugar-focused bioconversion.

Comparing results with the literature, partial agreement was observed. [22,24,26,27] analyzed woody fractions of *P. juliflora* and reported cellulose contents between 45–49.4%, hemicellulose 18–25%, and lignin 18–29.1%, values close to those in this study (38.24%, 16.46%, and 22.65%, respectively). For stem bark, holocellulose (60.39%) and lignin (28.06%) aligned with Gupta, Sharma and Kuhad [21]. For the pod (or pod with seed), Gayathri and Uppuluri [28] reported 26% cellulose, 30% hemicellulose, and 4% lignin, showing partial convergence but divergence in lignin content. These differences, especially in seed and pulp fractions, reinforce the need for specific evaluations, considering lignin's barrier role in hydrolysis and holocellulose's importance for fermentation.

Table 2 presents the reducing sugar yields from the enzymatic saccharification of *P. juliflora in natura* over 24 hours and 48 hours. The stem bark showed the best performance, reaching 2.74 mg/mL (13.23%) at 24 h, and then decreasing to 2.56 mg/mL (10.77%) at 48 h, likely due to enzyme

Table 1. Chemical composition of biomass samples.

Sample	Moisture (%)	Ash (%)	Cellulose (%)	Hemicellulose (%)	Total Lignin (%)	Holocellulose (%)
Stem bark	7.76	3.83	38.71	21.68	28.06	60.39
Woody stem	2.81	0.64	38.24	16.46	22.65	54.71
Pod husk	20.87	5.66	6.85	21.06	16.87	27.92
Pod with seed	17.99	5.31	39.33	22.35	45.91	61.68
Pulp without seed	21.11	5.70	23.56	21.13	20.52	44.69
Seed	5.40	2.49	7.12	10.57	11.47	17.69

Values in % g/100 g of dry biomass.

Table 2. Enzymatic hydrolysis of raw Algaroba biomass.

Sample	Enzymatic hydrolysis after 24 h reaction (mg/mL) (%)	Enzymatic hydrolysis after 48 h reaction (mg/mL) (%)
Stem bark	2.74 (13.23%)	2.56 (10.77%)
Woody stem	0.03 (0.11%)	0.30 (1.22%)
Pod with seed	1.38 (7.43%)	1.44 (6.69%)

Values expressed in mg/mL and %.

inhibition by by-products or active site saturation. Woody stem had modest values at 24 h (0.03 mg/mL, 0.11%), increasing slightly at 48 h (0.30 mg/mL, 1.22%). The pod with seed reached 1.38 mg/mL (7.43%) at 24 h and 1.44 mg/mL (6.69%) at 48 h, indicating intermediate yields.

Other authors observed that specific pretreatments resulted in higher yields. Deswal and colleagues [20] and Naseeruddin and colleagues [29] reported 3.9 mg/mL/g sugar yields from woody *P. juliflora*. Gupta, Khasa and Kuhad [21] in 2009 achieved 18.45 mg/mL after lignin removal, and, in 2011 the same authors reported 33% yield using *T. reesei* cellulase supplemented with *A. niger* β-glucosidase (Novozyme 188) [22].

Conclusion

The results demonstrate that the composition of different *P. juliflora* fractions directly influence fermentable sugar yields. The stem bark, with higher cellulose and hemicellulose, stood out in enzymatic hydrolysis. Fractions with high lignin content, such as those with seeds, require pretreatments for efficient conversion.

Previous studies confirm the importance of lignin removal and enzyme supplementation (notably β-glucosidase) to increase hydrolysis efficiency. Thus, careful selection of biomass fraction, combined with efficient pretreatments and optimized enzyme cocktails, emerges as a decisive strategy to enhance sustainable sugar and biofuel production from *P. juliflora*.

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