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Camalote Grass (*Paspalum fasciculatum* Willd) as a Sustainable Raw Material for the Production of Lignocellulosic Ethanol

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Abstract

The current trend of replacing a percentage of gasoline with ethanol has promoted the development of new processes for its production from lignocellulosic biomass. This work reports the production of ethanol from the Camalote grass (*Paspalum fasciculatum* Willd). The lignocellulosic biomass was subjected to acid hydrolysis at 125˚C and 15 psi with H_2SO_4 concentrations at 5%, 10%, and 20%, obtaining an average of reducing sugars (pentoses and hexoses) from the hydrolyzed juice with 12.3%, 10%, and 17% Brix, respectively. The sugars were fermented using yeast of the *Saccharomyces cerevisiae* at 30˚C for 48 hours. Finally, the ethanol was distilled at 78˚C, and the average yields were obtained through analysis of variance with a 95% confidence level. The values indicate that there is a significant difference ($p > 0.05$), the Tukey study shows that all the % v/v averages are different from each other. For H_2SO_4 concentration at 5% (10.33 ± 2), H_2SO_4 at 10% (9.33 ± 1.8), and H_2SO_4 at 20% (6.33 ± 2). The acidity analysis for the ethanol obtained from each treatment gave a value of 1.8 mg/L of acetic acid in all cases.

Keywords

Lignocellulosic Ethanol, Lignocellulosic Biomass, Camalote Grass, Acid Hydrolysis, Energy Crops

1. Introduction

Ethanol (C_2H_6O) due to its chemical composition has the characteristics to be used as a fuel and oxygenate gasoline. During the period of oil shortages between 1973 and 1979, first-generation ethanol from sugarcane juice and corn grains

was used as fuel. Since then, Brazil and the United States have used ethanol blended with gasoline at 10%, called gasohol (E10). This blend works well in conventional cars without the need to adjust the carburetor [1]. A theoretical study conducted by Castillo-Hernández (2012), it was demonstrated that CO_2 emissions decrease by 7% using gasohol with positive energy balances [2]. Other benefits of substituting ethanol for gasoline include reducing the presence of harmful aromatic compounds, replacing oxygenates such as methyl tert-butyl ether (MTBE), and reducing PM_{10} particle emissions, thereby improving air quality.

However, in Latin American countries, the use of corn and sugarcane is compromised by food security concerns, making them unsuitable as raw materials for ethanol production. Consequently, second and third-generation ethanol [3] are being produced, primarily obtained from lignocellulosic biomass (**Table 1**).

3rd Generation Ethanol

At the National Ethanol Conference 2022, grasses were considered affordable materials for ethanol production since they do not require subsidies, affect diversity, or contribute to deforestation [4]. Grasses have lignocellulosic biomass with a complex structure, where hemicellulose and lignin form a true physical barrier to cellulolytic enzyme penetration. Depending on the variety of grasses, they can contain from 5% to 18% lignin, from 25% to 43% hemicellulose, and from 29% to 50% cellulose [5], so the processes for ethanol production from grass biomass must be adjusted according to the characteristics of these components [6]. **Table 2** shows a list of different grasses used under various pretreatments for ethanol production.

Table 1. Generation feedstocks for bioethanol yield.

Bioethanol	Feedstock
1st generation	Material from agricultural sources comprising the edible parts of plants such as starch and sugar.
2nd generation	Material from agricultural and forestry residues composed mainly of cellulose, such as sugarcane bagasse and wheat straw.
3rd generation	Non-food vegetative material with rapid growth, such as perennial grasses and green algae

Table 2. Generation feedstocks for bioethanol yield.

Feedstock	Pretreatment	Bioethanol Yield	Ref
Elephant grass (*Pennisetum purpureum*)	Hydrolysis: NaOH 1 M Fermentation: *Aspergillus niger*/ *Saccharomyces cerevisiae*	30 g/L	[7]
King grass (*Pennisetum hybridum*)	Hydrolysis: Enzimática Fermentation:*Saccharomyces cerevisiae*	27.7 g/L	[8]

Continued

Kikuyo grass (*Pennisetum clandestinum*)	Hydrolysis: H_2SO_4 at 72% Fermentation: *Saccharomyces cerevisiae*	128 g/L	[9]
Timothy grass (*Phleum pratense* L.)	Hydrolysis: Enzymatic Fermentation: *Saccharomyces cerevisiae*	4 g/L	[10]
Grass lawn waste	Hydrolysis: H_2SO_4 at 10% Fermentation: Pichia stipitis	0.108 g/g	[11]
Napier grass (*Pennisetum purpureum* Schumach)	Hydrolysis: water/NH_3 Fermentation: *Saccharomyces cerevisiae*	0.174 g/g	[12]

Figure 1. Camalote grass (*Paspalum fasciculatum* Willd).

In this regard, considering the lignocellulosic biomass of grasses as a promising raw material for ethanol production, this work presents the use of lignocellulosic biomass from Camalote grass to produce ethanol. Camalote grass (**Figure 1**) belonging to the Poaceae family, is a perennial, fast-growing, and invasive grass that displaces cultivated pastures, hence considered a weed. Moreover, it is not utilized as livestock feed [13]. It is abundant in the southeast of Mexico, extending to Argentina, Uruguay, and the Antilles, thus holding great potential as a raw material for ethanol production due to its high availability, easy access, and low cost.

2. Materials and Methods

2.1. Sample Collection

1 m^2 (3.9 kg) of camalote grass (*Paspalum fasciculatum* Willd*)* was collected in the vicinity of the Universidad Popular de la Chontalpa, with coordinates 17.959 North, −93.364 West. The grass was longitudinally cut into sizes of 5 ± 1 cm to facilitate handling. Subsequently, it was air-dried for 3 days and then dried at 105˚C until reaching a constant weight.

$$\%\ moisture = (mi - mf)100 \quad (1)$$

where *mi* is the initial weight of the plant material (g) and *mf* is the final weight of the plant material (g).

2.2. Extractives Released

100 g of camalote grass were placed in a 1 L beaker and 700 mL of acetone were added. It was heated to boiling for 20 minutes with continuous stirring. Subsequently, it was allowed to cool, and the resulting plant material was filtered. It was then dried at 105˚C until reaching a constant weight.

$$\%\ extractives = \frac{mi - mf}{mi} \times 100 \quad (2)$$

where *mi* is the initial weight of the plant material (g) and *mf* is the final weight of the plant material (g).

2.3. NaClO Treatment

To 100 g of the material without extractives, 500 mL of commercial NaClO was added, and heated to boiling for 1 hour. It was then cooled and washed until neutral pH. The resulting material was air-dried for 2 days and subsequently dried at a controlled temperature of 105˚C until constant weight.

2.4. Determination of Percentage of Crystallinity

The diffraction patterns were collected using a D8 Advance Bruker with CuKα radiation (α = 1.5406 Å and energy 8.047 keV), in the range of 2θ = 7 - 60 with a 0.02 step size and an acquisition time of 5 s/step. The crystallinity percentage was calculated with Equation (2) based on the method reported by Segal (1959) [14].

$$\mathrm{CrI}(\%) = \left(1 - \frac{I_{AM}}{I_{002}}\right) \times 100 \quad (3)$$

where I_{002} is the maximum intensity of the crystalline peak at 22˚, and I_{AM} = is the minimum intensity of the crystalline peak for cellulose *I*.

2.5. Acid Hydrolysis

10 g of pretreated material were placed in a 250 mL Pyrex flask and 100 mL of a 5% H_2SO_4 solution was added. This procedure was repeated varying the concentration of H_2SO_4 to 10% and 15%. Hydrolysis was carried out at 125˚C and 15 psi for 2 hours. Subsequently, the hydrolyzed juice was separated by filtration. The % Brix measurement was performed using a portable Anpro refractometer, by placing a drop on the prism surface and reading the scale.

2.6. Fermentation of Reducing Sugars

An experimental design was conducted by varying the amount of *Saccharomyces cerevisiae* yeast (Fermentis®) to 0.5, 1, and 2 g 100 mL of hydrolyzed juice was

placed in an Erlenmeyer flask, the pH was adjusted to 5 with NaOH 0.1 N, yeast was added, and it was allowed to ferment under anaerobic conditions for 48 hours at a temperature of 30˚C ± 1˚C.

2.7. Ethanol Distillation

The fermented product was placed in a simple distillation setup and heated to 78 - 80˚C for 3 hours. The % v/v of ethanol was measured using a portable refractometer for alcohol and distilled spirits with a range of 0 - 80% alcohol, by placing a drop on the prism surface and reading the scale.

2.8. Determination of the Acidity (Acetic Acid) of Ethanol

It was carried out according to the Mexican Official Standard NOM-V-15-S-1980. 25 mL of bioethanol were placed in a porcelain dish, heated in a water bath to dryness, and then transferred to an oven at a temperature of 105˚C for 30 minutes. Subsequently, 50 mL of absolute alcohol was added to the residue left in the dish, and the resulting solution was poured over 250 mL of freshly boiled, cold water neutralized with NaOH 0.1 N and phenolphthalein as an indicator. The resulting solution was titrated with NaOH 0.1 N, using the indicator added to the water for neutralization. The acidity was expressed in mg of acetic acid per 100 mL referred to anhydrous alcohol through Equation (4).

$$\mathrm{FA} = \frac{V \times N \times 60 \times 100}{M} \times \frac{100}{D.A.R} \tag{4}$$

The fixed acidity (FA) expressed in mg of acetic acid per 100 mL referred to anhydrous alcohol; V is the volume of the NaOH 0.1 N solution spent for the titration of the sample in mL; N is the normality of NaOH; 60 represents the meq of CH_3COOH in mg; M is the volume of the alcohol sample, in mL; and *D.A.R.* is the actual alcoholic degree of the sample on the Gay-Lussac scale.

2.9. Statistic Analysis

With the experimental data of % Brix and % ethanol, the average yield of each treatment was calculated using the RM ANOVA (repeated measures ANOVA) program, using biomass quantity as the fixed factor and H_2SO_4 concentration as the random factor. The size of the arithmetic mean = 3. The average values were compared by applying Analysis of Variance with a 95% Confidence Interval for the mean, and the Tukey statistical test was applied.

3. Results and Discussion

3.1. Characterization of Camalote Grass

In the biomass's own composition, cellulose, hemicellulose, lignin are present in larger quantities, while other organic compounds know extractives are found in low concentrations in the bark, leaves, needles, exudates, branches, flowers, fruits, and seeds, contributing to the organoleptic characteristics (flavor, odor, color) of the organic material. The amount of extractives varies according to the

Table 3. Grass characterization during pretreatment.

% moisture	% lignin	% extractives
18	24	14

species, geographical location, and time of year. Among the extractables are phenolic compounds of molecular weight that are lignin precursors, as well as aromatic aldehydes and ketones. A material balance was carried out during the biomass pretreatment, and **Table 3** shows the characterization of the solid fraction of the grass. The values obtained are close to those reported for Maralfalfa grass (*Pennisetum glaucum*) at cutting frequencies of 90 to 180 days [15].

3.2. Effect of NaClO on Camalote Grass

During the NaClO treatment, lignin and hemicellulose were removed, as confirmed by X-ray analysis. In **Figure 2(a)**, the diffraction patterns for natural camalote grass are shown. The intensity of the amorphous zone I_{AM} was located at $2\theta = 10.2^{\circ}$, and the intensity of the crystalline zone I_{002} at $2\theta = 22.6^{\circ}$. In **Figure 2(b)**, the camalote grass with NaClO is shown. The intensity of the amorphous zone I_{AM} was located at $2\theta = 11.2^{\circ}$, and the intensity of the crystalline zone I_{002} at $2\theta = 22.7^{\circ}$, characteristic signals for cellulose *I*. The signal at 45.4˚ corresponds to the inorganic part of the cellulose [16]. Natural grass exhibits 42% crystallinity, and after treatment with NaClO, an increase in crystallinity to 50% is observed, attributed to the dissolution of amorphous regions (lignin and hemicellulose) in lignocellulosic biomass [17].

3.3. Effect of H_2SO_4 Concentration on % Brix

The degradation of cellulose to glucose was carried out through acid hydrolysis varying the concentration of H_2SO_4. The sugar content is commonly expressed as % Brix since there is a relationship with the sugar content. **Table 4** shows the results of % Brix for each of the treatments. Higher conversion of cellulose to reducing sugars was achieved with concentrations of 5% and 20%. The use of diluted acid concentrations minimizes the formation of hydroxymethylfurfural, which is a toxic compound for yeast during the fermentation process [18].

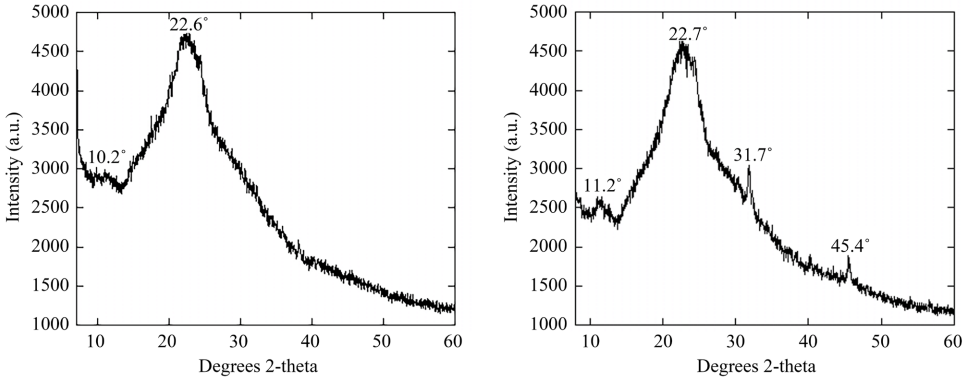

Figure 2. X-ray diffractograms. (a) Natural camalote grass, (b) Camalote grass treated with NaClO.

Table 4. Values obtained of % Brix.

Treatment		
Identification	Concentration	Average %Brix
T1	H_2SO_4 5%	12.3
T2	H_2SO_4 10%	10
T3	H_2SO_4 20%	17

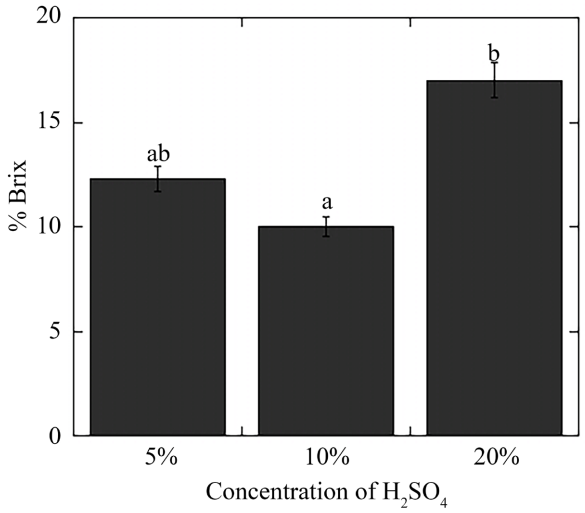

Figure 3. % Brix with respect to acid hydrolysis concentration. Data are presented as means ± SEM.

The analysis of variance showed that there are statistical differences ($p < 0.05$) among the H_2SO_4 treatments (**Figure 3**). The treatment with a 20% acid concentration presented the highest average of 17% Brix, which was statistically superior to the treatments with 5% acid concentration of 12.3% Brix and 10% acid concentration of 10% Brix. The % Brix values obtained are within recommended ranges as a high content of soluble solids increases osmotic pressure affecting the yeast *Saccharomyces cerevisiae*, resulting in low ethanol production [19].

3.4. Yield % v/v of Ethanol

Table 5 shows the ethanol yield obtained relative to the amount of fermentable sugars obtained in each treatment with sulfuric acid.

Table 5. Production of ethanol from Camalote grass.

Treatment		
Identification	% Brix	Average % Ethanol
T1	12.3	10.3
T2	10	9.3
T3	17	6.3

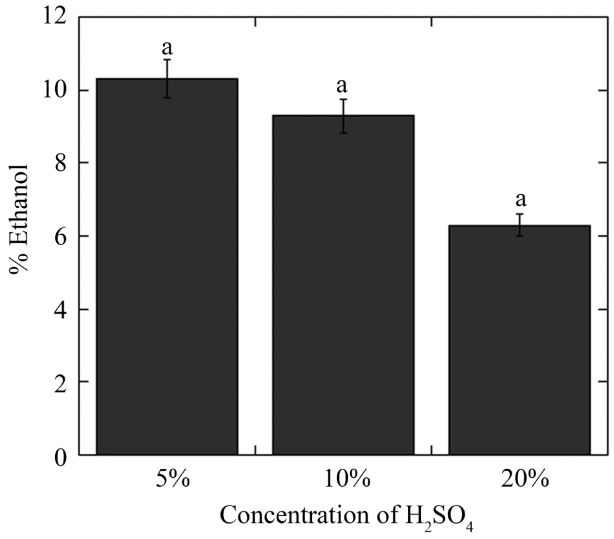

Figure 4. Ethanol with respect to acid concentration. Data are presented as means ± SEM.

Figure 4 shows that all the averages are different from each other, according to the Tukey statistical test. The treatment with a 5% of H_2SO_4 concentration produced the highest average ethanol value of 10.3%, the treatment with 10% of H_2SO_4 produced 9.3% of ethanol, and the treatment with 20% of H_2SO_4 produced 6.3% of ethanol. It is established that higher acid concentrations decrease ethanol production, as it increases the formation of toxic agents for yeast [20].

3.5. Estimation of Ethanol Production per Hectare

It is predicted for 2024 that ethanol consumption will be 134.5 billion liters, with Brazil being the largest producer, followed by the United States, the EU, and China [21]. Based on the results, the camalote grass produces 0.10 g ethanol/g of biomass, values comparable to the yields reported by Antonopoulou (2020) for lawn grass, which produced 0.108 g of ethanol/g of biomass; and by Yosuda (2013) for Napiergrass, which produced 0.174 g of ethanol/g of biomass. In 1 m^2, 3900 g of grass is obtained, therefore, 390 g ethanol/m^2 would be produced. So, ethanol production from camalote grass would contribute 3900000 liters of ethanol per hectare. This yield is economically viable considering that the cost of the raw material is zero.

$$\text{Yield ethanol}/\text{m}^2 = \left(\frac{3900\text{ g pasto}}{1\text{ m}^2}\right)\left(\frac{0.10\text{ g ethanol}}{1\text{ g pasto}}\right) = 390\text{ g Ethanol}/\text{m}^2$$

$$\text{Yield ethanol}/1\text{ h} = \left(\frac{390\text{ g ethanol}}{1\text{ m}^2}\right)\left(\frac{10000\text{ m}^2}{1\text{ h}}\right) = 3900000\text{ g Ethanol}/\text{h}$$

Thereby, grasses are promising raw materials to substitute sugar cane and corn for ethanol production, as they are not affected by climatic changes. Unlike sugar cane and corn, which only thrive in tropical climates and their crops are affected in semi-arid regions, hail, and frosts.

Table 6. Fixed acidity of ethanol obtained from Camalote grass.

H_2SO_4 concentration	FA (mg/L)
5%	1.8
10%	1.8
20%	1.8

3.6. Fixed Acidity in Ethanol from Camalote Grass

Ethanol, to be used as fuel in gasoline, must meet certain chemical properties, including fixed acidity, which is associated with corrosiveness, taking into account the material from which the car engine is made. According to the United Nations, fuel ethanol must have a maximum acidity (acetic acid) of 30 mg/L, established by ASTM D1613 standard. The ethanol obtained from Camalote grass in the three studied H_2SO_4 treatments showed a total acidity value as acetic acid of 1.8 mg/L (**Table 6**), acceptable values for the specifications set by International and Brazilian Standards [22].

4. Conclusion

In this research work, it was determined that the camalote grass (*Paspalum fasciculatum* Willd) under conditions of acid hydrolysis at low concentrations of H_2SO_4 achieves an average maximum of 17% reducing sugars and 10.3% lignocellulosic ethanol with a fixed acidity of 1.8 mg/L in 48 hours of fermentation. The results are promising to propose Camalote grass as an energy crop for lignocellulosic ethanol production, as it is economically viable, not an agricultural food crop, produced in large quantities, has zero cost, and does not require special care for its growth.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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