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CUTTING FREQUENCY IN CAYMAN GRASS (*Urochloa* HYBRID) ON THE CALORIFIC POWER OF THE MEXICAN WET TROPIC

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ABSTRACT

The objective of this study was to evaluate the effect of cutting frequency in Cayman grass (*Urochloa* HYBRID) on biomass yield, moisture, ash, ethereal extract, neutral detergent fiber (FDN), acid detergent fiber (FDA), acid detergent lignin (LDA), crude protein (PC), calorific value, and theoretical bioethanol yield. Four cutting frequencies were established as treatments: 30, 60, 90, and 120 d, arranged in a completely randomized block design with three replications. Data were analyzed with GLM (SAS), and means were compared with the Tukey test ($p \leq 0.05$). The highest biomass production (11.9 Mg ha^{-1} year^{-1}), calorific value (15.1 MJ kg^{-1}), and LDA (5.7 %) were obtained at the 120 d cutting frequency. The concentration of FDN (61.8 %), FDA (43.6 %), cellulose (38.1 %), and theoretical bioethanol production (218.4 L Mg^{-1} MS) were statistically different at the cutting frequency of 90 d. The values of hemicellulose (18. 7 %) and ethereal extract (1.8 %) were statistically different at the 60 d-cutting frequency; while PC (9.7 %) and ash (11.8 %) showed significant differences at the 30-d cutting frequency. Based on the biomass yield and calorific value of Cayman grass, it can be considered as a potential plant material for cellulosic ethanol production.

Keywords: biofuel, energy crop, cutting frequency, grass.

INTRODUCTION

In recent decades, the imminent depletion of petroleum, the steady increase in the price of fossil fuels, and their unequivocal contribution to global warming has spurred research into the production of biofuels using fodder as raw material.

A biofuel is defined as a high-energy chemical generated from biological processes derived from biomass or living organisms (Jhang *et al*., 2020); therefore, they are considered renewable and environmentally friendly. In addition, they can contribute to mitigate the problem of global warming and climate change.

The most common liquid bioenergies are biodiesel and bioethanol (Zhao *et al*., 2009), which can replace fossil fuels or be used as additives in hydrocarbon composite

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products (Sun *et al*., 2021). In the particular case of bioethanol, one of the most discussed aspects is the raw material from which it will be obtained. Thus, the so-called first-generation bioethanol is obtained from food products such as corn, sorghum, and sugarcane; however, this entails the ethical dilemma of using food to generate energy. Second generation bioethanol is produced from lignocellulosic materials, avoiding the use of foodstuffs.

Energy crops are considered feedstock for second generation bioethanol and among these we find grasses and C_4 plants, which are highly efficient at converting sunlight into biomass (Ventura-Ríos *et al*., 2022).

Calorific value is the key parameter for assessing fuel quality and available energy per unit mass (Ram and Salam, 2012), and in grasses it can reach up to 20 kJ kg^{-1} (Sun *et al*., 2021). Grass biomass, according to Mohammed *et al*. (2015), is chemically composed of cellulose (34 %), hemicellulose (20 %), lignin (24 %), protein (11.3 %), silica (1 %), and other minerals, and also includes FDN (75 %) and FDA (45 %; Maia *et al*., 2014). The components in the cell wall and cell content can vary according to the geographic area, phenological stage of the plant, and cell tissue analyzed. For example, Bernal-Flores *et al.* (2017) report variability in lignin concentration by studying various tissues of 30 genotypes of Banderilla grass [*Bouteloua curtipendula* (Michx.) Torr.] in Mexico.

It is also important to evaluate holocellulose (Santiago-Ortega *et al*., 2016), fixed carbon, volatile material, moisture, and extractives (Mohammed *et al*., 2015) to know the physical and chemical properties of grasses. Given the complexity of the cell wall of grasses, the biomass must be pretreated to break the lignin bonds and favor the saccharification process by up to 95 % (Sun *et al*., 2021), allowing us to obtain 311.5 L of bioethanol Mg^{-1} from biomass or 2967 L ha^{-1} (Zhao *et al*., 2009; Lima *et al*., 2014).

Varieties of the genus *Urochloa* (C_4), used in animal feed, have been introduced in tropical Mexico. In 2011, the company Papalotla, S. A. de C. V., based in Mexico, released the hybrid Cayman with very particular qualities to improve animal production (Hare *et al*., 2015). However, research on plant physiology, pasture behavior, and chemical composition of this grass is limited, and for a better utilization, it is necessary to determine the optimum age of regrowth, where it reaches the highest biomass production and chemical composition.

In Mexico, different cultivars of the genera *Cenchrus* (Ventura *et al*., 2021a), *Megathyrsus* (Ventura-Ríos *et al*., 2021b) and *Urochloa* (Santiago *et al*., 2016) have recently been evaluated to determine their agronomic performance, calorific value, immediate analysis, and production of fermentable sugars to obtain cellulosic ethanol. Therefore, the hypothesis of this research was to test if regrowth age influences the energy concentration of Cayman grass. The objective of the present study was to evaluate at what age of regrowth of four cutting frequencies (30, 60, 90, and 120 d), the highest forage quantity and quality is obtained and its influence on the calorific value, biomass production, chemical composition, and theoretical bioethanol yield of Cayman grass (*Urochloa* hybrid cv. CIAT BR02/1752), under rainfed and fertilized conditions in the Mexican humid tropics.

MATERIALS AND METHODS

Study area

The research was conducted at the "Papaloapan" Experimental Field of the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), located at 18° 06′ N and 95° 31′ W, at 65 m altitude, in the municipality of Isla, Veracruz, Mexico.

The climate is classified as Aw, hot sub-humid with 80 % of the seasonal rainfall in summer; the 40-year average annual precipitation and temperature were 1142 mm and 25.8 °C, respectively (García, 2004). The soils are vertisols with a pH of 5.4, clayey-loam texture and organic matter is 2.6 % (Enríquez and Romero, 1999).

Pasture management and treatments

Cayman grass (*Urochloa* hybrid cv. CIAT BR02/1752) was sown on July 22, 2017 at a rate of 10 kg ha^{-1} of pure viable seed, which was provided by Papalotla S. A. de C. V. de México. The soil was prepared with fallow and two passes of harrow, and furrowed 0.50 m apart. The experimental plots were established at 5 m x 16 m, with three replications. Granular fertilizer [120-80-00 kg ha^{-1}; N-P_2O_5-K_2O] was used at 43 and 112 d after sowing by hand. Prior to sampling, a uniformity cut was made with machetes at a height of 15 cm above ground level. Sampling and fertilization were done in the morning throughout the experiment. The experiment was concluded on July 24, 2018. Four cutting frequencies were established as treatments: 30, 60, 90, and 120 d interval, carried out for 365 d.

Variables evaluated

Dry matter (MS) determinations were carried out at the Lignocellulosic Materials Laboratory of INIFAP in San Martinito, Puebla, Mexico; with the exception of nitrogen (N) concentration, which was carried out at the Soil Fertility and Environmental Chemistry Laboratory of the Colegio de Postgraduados Campus Montecillo, in Montecillo, State of Mexico, Mexico.

The variables evaluated were biomass yield, dry matter, nitrogen (N), neutral detergent fiber (FDN), acid detergent fiber (FDA), cellulose, hemicellulose, acid detergent lignin (LDA), ethereal extract, ash, calorific value, moisture, and theoretical bioethanol yield.

Biomass yield

Biomass production was determined at 30, 60, 90, and 120 d intervals after uniformity cutting for one year. In each plot, on each sampling date, a 1 m^2 metal frame was thrown randomly on five occasions in each experimental unit, and cut at 20 cm residual forage height.

Green harvested biomass was weighed on a precision, pre-calibrated Ohaus balance with a capacity of 6.2 kg±0.1 g (GT-4000®; TEquipment, Parsippany, NJ, USA); then a subsample was weighed and immediately placed in pre-labeled paper bags in a forced convection oven (FE-243A; Felisa; Guadalajara, Mexico) at 55 °C until constant weight, and then weighed.

Preparation of samples for analysis

The dry samples were pulverized in a Wiley® mill (Arthur H. Tomas, Philadelphia, PA, USA) and passed through No. 40 (0.42 - 1.00 mm) and No. 60 (0.25 - 0.42 mm) sieves.

Chemical analysis

Samples were incinerated for 2 h at 600 °C in a muffle (Thermo Scientific, BF 51842, Fowlerville, MI, USA) to obtain the concentration of organic matter and ash according to ASTM D 1102-84. The crude protein (PC) concentration was determined by the semi-micro Kjeldahl method (N x 6.25), while the ethereal extract was determined in a Soxhlet extractor (PYREX® Corning Life Sciences, 3840-L, Corning, NY, USA). Neutral detergent fiber, acid detergent fiber, and acid detergent lignin concentrations were obtained sequentially on the ANKOM200® fiber analyzer (Ankom Technology, Fairport, NY, USA), using Ankom® F57 filter bags with a pore size of 30 μm. The FDN was determined with thermostable α-amylase (ANKOM Technology200®) to solubilize sugars, starch and pectins, and sodium sulfite (Na2SO3) to remove starch and nitrogen (Van Soest *et al*., 1991). Cellulose (CL) and hemicellulose (HC) were calculated with the following formula: CL = FDA – LDA, while HC = FDN - FDA, respectively.

Calorific value

The calorific value was determined in an adiabatic bomb calorimeter (Isoperibol, Parr 1266, Parr Instrument Company; Moline, IL, USA) according to ASTM standard (E711) and calorimeter operating instructions (Parr, 1999) at 30 ± 0.5 °C, with compressed pellets of 1 g maximum.

At the same time, moisture content was determined in a previously calibrated Ohaus thermobalance (MB45®; TEquipment; Parsippany, NJ, USA). To determine this variable, ground samples passed through No. 60 sieves (0.25 - 0.42 mm) were used, with five determinations for each sample, and 15 replicates for each FC.

Theoretical bioethanol yield

The theoretical bioethanol yield (RTB) (equation 3) was determined using the formulas of Badger (2002) and Dien (2010). The formulas include hydrolysis, chemical, and stoichiometric reactions of cellulose (equation 1) and hemicellulose (equation 2) transformation into ethanol.

Cellulose

$$RTBC - C * C\ g/c * Ecc * Ret * Efg * Det \quad (1)$$

Hemicellulose

$$RTBH - H * H\ x/h * Ech * Ret * Efx * Det \quad (2)$$

Total

$$RTB - RTBC + RTBH \quad (3)$$

where *RTB*: L Mg^{-1} MS, C: kg $_{cellulose}$ Mg^{-1} $_{biomass}$; H: kg $_{hemicellulose}$ Mg^{-1} $_{biomass}$; $C_{g/c}$: glucose concentration 1.111 (kg $_{glucose}$/kg $_{cellulose}$); $H_{x/h}$: xylose concentration 1.136 ($kgxy_{lose}$/$kg_{hemicellulose}$); Ecc: cellulose conversion efficiency (0.76); Ech: hemicellulose conversion efficiency (0.90); Ret: stoichiometric ethanol yield (0.511 $kg_{ethanol}$/$kg_{glucose}$; 0.511 $kg_{ethanol}$/kg_{xylose}); E_{fg}: glucose fermentation efficiency (0.75); E_{fx}: xylose fermentation efficiency (0.50); D_{et}: ethanol density (0.78 Mg m^{-3}). In addition, the theoretical annual amount of bioethanol (L) produced by one ha of Cayman grass was calculated for each FC. The procedure consisted of multiplying the theoretical bioethanol yield per unit of biomass by the annual biomass yield according to the methodology by Badger (2002) and Dien (2010).

Experimental design and statistical analysis

Data were analyzed as a completely randomized design, where Cayman grass cutting frequency was considered as treatments (30, 60, 90, and 120 d) with three replications per treatment.

An analysis of variance (Anova) was performed to identify the effect of cutting frequency on response variables using the GLM/SAS procedure, and treatment means were compared with the Tukey test ($p \leq 0.05$) using SAS for Windows version 9.3 (SAS, 2011).

RESULTS AND DISCUSSION

Biomass production

Annual biomass production increased as FC increased (Table 1; $p \leq 0.05$), where the highest production was observed every 120 d (11.9 Mg MS ha^{-1} $year^{-1}$). The cut at every 120 d was 20, 40, and 67 % higher than the FC at 90, 60, and 30 d, respectively. Therefore, in Toledo grass (*Urochloa brizantha*) Santiago *et al*. (2016), reported 11.1 Mg ha^{-1} $year^{-1}$ every 30 d, which exceeded by 4 Mg ha^{-1} $year^{-1}$ that was found in the present study. Likewise, Rojas-García *et al*. (2018) conducted a study in tropical Mexico during

Table 1. Biomass and energy production of Cayman grass (Urochloa hybrid cv. CIAT BR02/1752) at four cutting frequencies in the municipality of Isla, Veracruz, Mexico.

Cut frequency (days)	Yield (Mg ha^{-1} $year^{-1}$)	Calorific value (MJ kg^{-1})	Energy (GJ ha^{-1} $year^{-1}$)	Moisture (%)
30	7.1d	14.9b	105.6d	8.4a
60	8.4c	14.8b	126.1c	8.4a
90	9.9b	14.6b	145.2b	8.7a
120	11.9a	15.1a	180.6a	8.0b
Media	9.35	14.9	139.4	8.4
EE	1.03	0.10	15.9	0.14

EE: Standard error. Different letters show differences among cuts (Tukey, $p \leq 0.05$).

the dry season under rainfed conditions and without fertilization, where the result obtained for Cobra grass (*Urochloa* hybrid BR02/1794) was 2.5 Mg ha^{-1}, which was lower than that obtained in the present research. Therefore, the conditions in the municipality of Isla, Veracruz, are better for crop development, together with higher rainfall (> 300 mm).

There are other varieties of tropical grasses that exceed 12 Mg of biomass, such as Taiwan grass (*Cenchrus purpureus*; Ventura-Ríos *et al*., 2021a). However, the chemical composition of grasses presents changes in the cell wall and cell content as they advance in physiological maturity. Therefore, it is important to report the energy content and its potential as biofuel as a function of the cutting period.

Calorific value and moisture content

The FC every 120 d showed higher calorific value ($p \leq 0.05$; Table 1) over the other FCs because the plant at that FC had lower moisture content ($p \leq 0.05$); the FC of 30, 60, and 90 d were similar ($p \leq 0.05$). The highest energy production per ha^{-1} year^{-1} corresponded to the 120 d FC (180.6 GJ ha^{-1} year^{-1}) which was different ($p \leq 0.05$) from the other FCs, exceeding the 30 d FC by 71 % (equivalent to 75 GJ ha^{-1} year^{-1}); 43 % (equivalent to 54.5 GJ ha^{-1} year^{-1}) to the 60 d FC, and 24 % (equivalent to 35.4 GJ ha^{-1} year^{-1}) to the 90 d FC (Table 1).

Grass biomass is of great interest due to its carbon neutrality, its thermal potential for energy production, and its composition and chemical structure favor the concentration of energy per unit of biomass (Wang *et al*., 2021). It is important to identify the calorific value of grass biomass to evaluate its potential as a feedstock for bioenergy production, and also determine the moisture content of the biomass, because it decreases the energy expression within the biomass (Majumder *et al*., 2008). Moisture content was similar at 30, 60, and 90 d ($p > 0.05$; Table 1) and difference was only observed at 120 d ($p \leq 0.05$), which showed the lowest moisture content. The calorific value in the present experiment ranged from 14.9 to 15.1 MJ kg^{-1}, which was lower than that reported by Said *et al*. (2019) who found 16.7 MJ kg^{-1} in Napier grass (*C. purpureus*). Likewise, Karampinis *et al*. (2012) reported 18.7 MJ kg^{-1} in miscanthus (*Miscanthus giganteus*).

The average obtained in this experiment was 14.9 MJ kg^{-1}, which is lower by 1.6 and 3.4 MJ kg^{-1} than the average reported by Santiago *et al*. (2016) and Ventura-Ríos *et al*. (2021a) who found 16.5 and 18.3 MJ kg^{-1} in Toledo (*U. brizantha*) and Maralfalfalfa (*C. purpureus*) grasses, respectively, under warm sub-humid climate conditions in the municipality of Isla, Veracruz, Mexico, by fertilization with the following formula: 120-80-00 kg ha^{-1}. On the other hand, grasses of the genus *Urochloa* (C_4) have a high efficiency to convert sunlight into biomass compared to trees, which allows reaching biomass yields exceeding 28 Mg ha^{-1} year^{-1} under fertilization systems (Santiago *et al.*, 2016). In addition, its calorific value can exceed 20 kJ kg^{-1} (Sun *et al*., 2021), which is a favorable quality to consider biomass as a suitable raw material for renewable energy production, compared to coniferous and broadleaved wood, whose calorific value can be 20.5 and 20.2 MJ kg^{-1}, respectively (Ram and Salam, 2012).

Crude protein

The highest crude protein concentration was found at 30 d, ($p \leq 0.05$; Table 2). As FC increases, plant N concentration decreases, as it happened in this study, when going from 30 to 90 d the protein concentration decreased by 44.3 % and from 30 to 120 d by 55 %. In other studies, conducted in tropical areas of Goiás, Brazil, with the addition of fertilizers based on: 200 kg ha^{-1} P_2O_5, 30 kg ha^{-1} N, 60 kg ha^{-1} K_2O, 2 kg ha^{-1} boron, 0.4 kg ha^{-1} molybdenum, using as sources: simple superphosphate, potassium chloride, boric acid, and sodium molybdate, Maia *et al*. (2014) reported values of 15 % in *U. brizanta* cv. Piata and Xaraés, and 7.4 % in cv. MG4. Normally, the concentration of N in the plant tends to decrease as physiological maturity advances; however, this element is highly related to the addition of fertilizers, precipitation, and solar radiation. High protein concentration is not desirable in biomass for bioenergy purposes, so it is important to quantify it. The higher the amount of nitrogen, the lower the bioconversion of total sugars (holocellulose) to bioethanol, because the nitrogen present in the cell wall has the capacity to form bridges with ferulic acid and increase the resistance and insolubility of the whole structure (Bidlack *et al*., 1992).

Table 2. Chemical composition of Cayman grass (*Urochloa* hybrid cv. CIAT BR02/1752) at four cutting frequencies in the municipality of Isla Veracruz, Mexico.

Cut frequency (days)	Component (%)					
	PC	FDN	FDA	LDA	Ext. E.	Ash
30	9.7a	52.9d	35.2d	4.4d	1.4c	11.8a
60	6.4b	55.7c	37.0c	4.9c	1.8a	9.6b
90	5.4c	61.8a	43.6a	5.4b	1.2d	8.6c
120	4.3d	61.5b	43.2b	5.7a	1.5b	7.5d
Mean	6.5	58.0	39.7	5.1	1.5	9.4
EE	1.16	2.19	2.14	0.28	0.12	0.91

C: crude protein, FDN: neutral detergent fiber, FDA: acid detergent fiber, LDA: acid detergent lignin. Ext. E.: Ethereal extract, EE: standard error. Different letters per column show differences among cuts (Tukey, $p \leq 0.05$).

The International Energy Agency (IEA) incorporated the term biorefinery, where it defines biomass as a compound of biological origin to produce from this bioenergy and, on the other hand, take advantage of the content of crude fiber, crude protein, and lignin to obtain food, chemicals, and materials (Amore *et al*., 2016).

Neutral Detergent Fiber (FDN) and Acid Detergent Fiber (FDA)

The FDN concentration was higher at 90 d ($p \leq 0.05$; Table 2) and exceeded by 17, 11, and 0.5 % the FC of 30, 60, and 120 d, respectively. The highest FDA concentration was found at 90 d and was different from the other cutting frequencies ($p \leq 0.05$), exceeding by 24, 18, and 1 % the FC of 30, 60, and 120 d, respectively. Therefore, FDN concentration increased by 17 % from 30 to 90 d cut; the same occurred with Maralfalfa grass (*C. purpureus*), where a 12 % increase was observed as FC increased from 30 to 120 d of harvest (Ventura-Ríos *et al*., 2019). Furthermore, Maia *et al*. (2014) reported FDN values of 63.7 and 75.2 % in *U. brizantha* cv. Piata and MG4, respectively, indicating that FDN concentration increases as physiological maturity in the plant advances, given that FDN evaluates the concentration of cellulose, hemicellulose, and lignin. On the other hand, when evaluating the growth curve of Cobra grass (*Urochloa hybrid* BR02/1794), Rojas-García *et al*. (2018) reported at 63 d of regrowth concentrations of 79 and 44.7 % for FDN and FDA, respectively, which are similar to what was found in the present experiment. There is little information on the use of fiber for bioenergy purposes, its usefulness is closely related to the paper industry or directly in combustion issues replacing coal (Samson *et al*., 2005) or for animal feed (Maia *et al*., 2014).

Lignin acid detergent

As plant age increased, lignin concentration also increased, and was higher at 120 d, which exceeded by 29, 16, and 5.5 % the FC at 30, 60, and 90 d, respectively ($p \leq 0.05$; Table 2). In other studies, values very similar to those obtained in the present experiment have been found. For example, Wongwatanapaiboon *et al*. (2012) reported concentrations of 5.8, 5.0, and 4.7 % for Atratum (*Paspalum atratum*), Pangola (*Digitaria decumbens*) and Ruzi (*Urochloa ruziziensis*) grasses, respectively.

High concentrations of LDA in biomass as biofuel feedstock are undesirable. Lignin is a highly complex polymer formed by phenyl-propane units (*p*-coumaryl, coniferyl and sinapyl alcohols), and is found within cell wall matrices in grasses. The biosynthesis of lignin as the plant progresses through its phenological stage, changes in its concentration, structure, and its hexose (glucose) and pentose (hemicelluloses) linked bonds (Li and Chapple, 2010).

Lignin, being a hydrophobic material, the water contained in the cell wall matrix is expelled, allowing the plant flexibility, permeability and modifying its rigidity, so that grasses during their growth and development, depending on the climate and environmental factors where they develop, tend to modify the concentration of this compound in their structure (Hatfield *et al*., 2017). Therefore, it is important to account for this compound in the biomass, since a higher concentration of lignin will limit the efficiency of the saccharification process, also obtaining fermentable sugars (glucose, fructose, sucrose, mannose, xylose, etc.), thus limiting the quality of the forage biomass (Van Soest, 1994). For this reason, it is desirable to obtain raw materials with low lignin content (Mohammed *et al*., 2015; Vogel *et al*., 2017).

Ethereal extract

The highest ethereal extract concentration was found at 60 d, which was different ($p \leq 0.05$) and exceeded the FC at 90, 30, and 120 d by 50, 28, and 20 %, respectively (Table 2). In previous research, Ventura-Ríos *et al.* (2019) reported 1.8 % ethereal extract in Maralfalfa grass (*C. purpureus*), when cut at 90 d. However, the concentration of extracts can be higher than 10 % (Mohammed *et al*., 2015; Ventura-Ríos *et al*., 2021a).

The ethereal extract in grasses has a heterogeneous composition, basically of lipids (galactolipids, triglycerides, and phospholipids) and other non-polar compounds that can be extracted by solvents, such as: phosphatides, steroids, pigments, liposoluble vitamins, and waxes (Van Soest, 1994).

The compounds that form the ethereal extract cannot be transformed to ethanol, so lignocellulosic biomass with a higher concentration of extractives will cause a lower ethanol yield. Quantification of total extracts is relevant in physical and chemical analyses, since this compound has the ability to limit bioenergetic expression and alter biomass chemical results.

Ash

The highest ash concentration was found at 30 d, which was different from the other FCs ($p \leq 0.05$; Table 2), exceeding by 23, 37, and 57 % the FCs of 60, 90, and 120 d, respectively. The concentration of ash in the plant was higher in early stages; however, inorganic materials are not desirable in bioenergy evaluations because they interfere with combustion processes, so it is necessary to optimize operating processes and reduce the costs associated with waste production in boilers or heaters.

Values reported in other evaluations with grasses are similar to the present study, e.g., 7 % in *U. brizantha* (Santiago *et al*., 2016), 8.5 % in *U. decumbens*, 9.5 % in *Chloris gayana*, and 10.6 % in *U. brizantha* cv. Xaraés (Nguku *et al*., 2016).

Inorganic material in pastures is variable in ash concentration among species; normally up to 12% or more can be found in herbaceous plants and agricultural residues in early stages of growth. However, this compound does not provide any energy value and can otherwise limit the energy expression of biomass (Ram and Salam, 2012).

Cellulose

Cellulose concentration was higher at 90 d, which was different ($p \leq 0.05$; Table 3), and was 24, 18, and 2 % higher than the FC at 30, 60, and 120 d, respectively. In recent

Table 3. Structural carbohydrates of Cayman grass (*Urochloa* hybrid cv. CIAT BR02/1752) at four cuttng frequencies in the municipality of Isla, Veracruz, Mexico.

Cut frequency (days)	Component			
	Cellulose		Hemicellulose	
	(%)	(Mg ha^{-1} year^{-1})	(%)	(Mg ha^{-1} year^{-1})
30	30.8d	2.1d	17.7d	1.2d
60	32.1c	2.7c	18.7a	1.5c
90	38.1a	3.7b	18.1c	1.7b
120	37.4b	4.4a	18.2b	2.1a
Media	34.6	3.2	18.2	1.7
EE	1.84	0.5	0.20	0.19

EE: Standard error. Different letters show differences among cuts (Tukey, $p \leq 0.05$).

research, Santiago *et al*. (2016) in *U. brizantha* grass reported on average 42.1 % cellulose, which is 21 % higher than that obtained in this experiment at the 90 d FC. However, Wongwatanapaiboon *et al*. (2012) in Pangola grass (*D. decumbens*) reported values of 33 %, in Ruzi (*U. ruziziensis*) 33.6 %, and in Elephant grass (*Pennisetum purpureum*) 35.2 %, which was similar to the values found in our study at 60 d FC.

The chemical composition of grasses changes with increasing plant age; however, it is necessary to know the sugar content at different plant ages to maximize the glucose and xylose content per gram of sample and per unit area. Considering biomass yields per ha^{-1} $year^{-1}$, calculations indicated that cuttings at every 120 d (4.4 Mg ha^{-1} $year^{-1}$) produced higher cellulose concentration ($p \leq 0.05$), exceeding by 109, 63, and 19 % the FC at 30, 60, and 90 d, respectively (Table 3).

To produce bioethanol by fermentation, fermentable sugars (mainly monosaccharides) are required as a carbon source for consumption by the microorganisms involved in this bioprocess. Second generation bioethanol uses agro-industrial wastes or energy crops as raw material. Fermentable sugars in these raw materials are obtained from a process that has as a first step a delignification (chemical, physical, mechanical, biological pretreatments or combinations of these) to then make an enzymatic hydrolysis of cellulose and hemicellulose. Hence the importance of having raw materials with a high content of these polymers (Barrera *et al*., 2016).

Hemicellulose

The highest hemicellulose concentration was found at 60 d and was 5.6, 3.3, and 3 % higher than the FC at 30, 90, and 120 d, respectively ($p \leq 0.05$; Table 3). In grasses, hemicellulose constitutes 11 to 34 % of biomass, and concentrations similar to the present experiment have been reported in previous work, for example, 18.7 % in Napier grass (*P. purpureum*; Mohammed *et al*., 2015) and concentrations of 15 to 23 % in Mombaza grass (*P. maximum*) (Ventura-Ríos *et al*., 2021b). Hemicellulose is made up of six- and five-carbon sugars, hexoses, and pentoses, respectively. However, the microorganisms commonly used to produce ethanol do not have the metabolic capacity to assimilate five-carbon sugars, xylose, and arabinose in the case of hemicellulose (Barrera *et al*., 2016). For this reason, although monosaccharides or disaccharides can be obtained from hemicellulose, the use of these to produce ethanol will depend on the microorganism used.

Theoretical bioethanol yield

Based on the calculations proposed by Badger (2002) and Dien (2010), the highest theoretical bioethanol yield per Mg MS, calculated based on glucose and xylose concentrations, was found at 90 d and exceeded by 17, 12, and 1.2 % the FCs at 30, 60, and 120 d, respectively ($p \leq 0.05$; Table 4). Annual bioethanol yield is directly related to harvested biomass production, and in this experiment the highest calculated theoretical yield was found at 120 d ($p \leq 0.05$), which was higher than the FC of 30, 60, and 90 d by 94, 55, and 18.7 %, respectively.

Table 4. Theoretical bioethanol yield of Cayman grass (*Urochloa* hybrid cv. CIAT BR02/1752) at four cut frequencies in the municipality of Isla, Veracruz, Mexico.

Cut frequencies (days)	Theoretical bioethanol production	
	(L Mg^{-1} MS)	(L ha^{-1} $year^{-1}$)
30	186.8d	1326.7d
60	195.5c	1660.9c
90	218.4a	2166.1b
120	215.8b	2572.5a
Mean	204.1	1931.5
EE	7.7	274.6

EE: Standard error. Different letters show differences among cuts (Tukey, $p \leq 0.05$).

In studies conducted in Mexico and other parts of the world under tropical conditions, the different values have been reported for bioethanol yield. For example, in grasses of the genus *U. brizantha* Santiago *et al*. (2016) reported 262.6 to 281.3 L Mg^{-1} MS, while Lima *et al.* (2014) reported 311 L Mg^{-1} MS, which exceed those found in the present experiment. On the other hand, Ventura-Ríos *et al*. (2021c) reported 227.2 L Mg^{-1} MS in Humidicola grass (*Urochloa humidicola*), which is similar to that found in the present study. In other tree evaluation studies, the following values have been reported, for example, Piccolo and Bezzo (2009) reported 284 L Mg^{-1} MS in broadleaf trees, which is 39 % higher (equivalent to 80 L Mg^{-1} MS) than the average obtained in this experiment; however, comparing coniferous and broadleaf wood sources, tropical grasses (C_4) are more efficient in transforming sunlight into biomass and provide more sugars per unit area and time (Zhao *et al.*, 2009; Ventura-Ríos *et al*., 2022). On the other hand, it is important to consider that there are several factors that influence bioethanol production performance. For example, the type of pretreatment and its conditions, the presence of inhibitors that affect the growth and metabolic capacity of the microorganisms, the enzymatic saccharification yield, and the fermentative capacity of the microorganism to produce bioethanol (Souza-Rodrigues *et al*., 2021). Furthermore, the raw material used to obtain bioethanol is of utmost importance, since its origin, chemical composition, and even morphology will determine, to a large extent, the efficiency of pretreatment and saccharification, and therefore of fermentation (Brito *et al.*, 2003).

The difference in the theoretical yield of second-generation bioethanol basically depends on the content of total sugars and other chemical compounds, including furfural and hydroxymethylfurfural, which alter the conversion efficiency of total sugars into fermentable sugars (glucose, fructose, sucrose, mannose, and xylose). It is also important to consider that lignocellulosic biomass undergoes changes in its structure depending on the cutting season, physiological stage, and plant varieties (Barrera *et al*., 2016). In this regard, Wongwatanapaiboon *et al*. (2012) reported 2561 L ha^{-1} $year^{-1}$ in elephant grass (*P. purpureum*), which is 11 L ha^{-1} $year^{-1}$ lower than that found every 120 d.

In other studies conducted in tropical Mexico, Ventura-Ríos *et al*. (2022) reported yields from 2400 to 7936 L ha^{-1} $year^{-1}$ in Taiwan grass (*Cenchrus purpureus*), and was higher than in this study by 55.2 to 208.5 % respectively. This is due to the higher biomass production of these *Cenchrus* grasses and the chemical composition of their cellular structure.

Finally, under tropical conditions, C_4 grasses produce biomass yields exceeding 10 Mg ha^{-1}, and are acceptable yields of cellulosic bioethanol to partially replace hydrocarbon-based composite products. On the other hand, tropical grasses are an alternative in the production of second-generation bioethanol due to their regrowth capacity, their sugar concentration in their different physiological stages, and the financial investment for their establishment and management.

CONCLUSIONS

Biomass production of Cayman grass (*Urochloa* hybrid cv. CIAT BR02/1752) increases with increasing plant age. The concentration of lignin and structural carbohydrates in the plant changes with the physiological maturity of the plant and the days of cutting, as indicated by the results of this study where the highest concentrations of lignin, cellulose, and hemicellulose were found at 120, 90, and 60 d, respectively.

The maximum biomass and caloric power values are reached with the 120-day FC, which has a positive impact on bioethanol production per hectare per year. The results of dry matter yield and biomass chemical characteristics indicate that Cayman grass is a feedstock with potential for second generation bioethanol production under tropical conditions.

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