# Research paper

# Agronomic characterization of Taiwan grass [*Cenchrus purpureus* (Schumach.) Morrone] and evaluation of its potential to produce bioethanol in the warm sub-humid climate of Mexico

Caracterización agronómica del pasto Taiwán [Cenchrus purpureus (Schumach.) Morrone] y evaluación de su potencial para la producción de bioetanol en clima cálido subhúmedo de México

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# Abstract

The objective of this study was to evaluate the biomass production, chemical composition, proximate analysis, calorific value and theoretical yield of bioethanol of Taiwan grass under 6 cutting frequencies. The highest production of biomass (33 t DM/ha), cellulose content (41.3%), calorific value (17.5 MJ/kg DM) and potential bioethanol yield (7,936 L/ha) were recorded at a cutting frequency of 180 days. The highest moisture content of the dehydrated samples and ash and crude protein concentrations were observed at a harvest frequency of 30 days with 9.2, 12.1 and 10.5%, respectively. The highest concentrations of extractives were obtained at harvest frequencies of 60 and 120 days (13.9 and 13.7%, respectively), while lignin concentrations were greatest at harvest frequencies of 150 and 180 days (21.1 and 20.9%, respectively). The highest concentration of fixed carbon was observed at a harvest frequency of 90 days (18.5%), while the lowest concentration of volatile matter occurred at a harvest frequency of 30 days. The data indicate that Taiwan grass has significant potential for use to produce bioethanol but assessment of the carbon footprint, life cycle analysis, energy yield (energy produced:energy consumed) of the entire production process is needed to ensure there are positive effects on climate change and greenhouse gas emissions before this process is adopted.

Keywords: Biofuel, calorific value, chemical composition, cutting frequencies, Pennisetum.

# Resumen

El objetivo de este estudio fue evaluar la producción de biomasa, composición química, análisis proximal, valor calorífico y rendimiento teórico de bioetanol del pasto Taiwán (*Cenchrus purpureus* Schum.) Morrone a seis frecuencias de corte. La producción más alta de biomasa, contenido de celulosa, valor calorífico y bioetanol se registró en el corte de 180 días con 33 Mg DM/ha, 41.3%, 17.5 MJ/kg DM, and 7936.2 L/ha, respectivamente. El contenido mayor de humedad, cenizas y proteína cruda se observó a la frecuencia de corte de 30 días con 9.2, 12.1 and 10.5%, respectivamente. La concentración mayor de extractivos fue obtenida en la frecuencia de corte de 60 y 120 días (13.9 y 13.7%), y la lignina las frecuencias de corte de 150 y 180 días mostraron los mayores valores (21.1 y 20.9%). La concentración más alta de carbono fijado se observó a los 90 días (18.5%), mientras que la concentración más baja fue en la frecuencia de corte de 30 días. De acuerdo con los resultados

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obtenidos el pasto Taiwán tiene potencial para ser usado para producir bioetanol, pero se necesita evaluar la huella de carbono, el análisis de ciclo de vida, el rendimiento de energía (energía producida;energía consumida) para asegurar que hay efectos positivos sobre el cambio climático y las emisiones de gases de efecto invernadero para que se adopte este proceso.

Palabras clave: Biocombustible, composición química, frecuencias de corte, Pennisetum, valor calorífico.

# Introduction

The depletion of oil reserves and the increase of greenhouse gas emissions have caused a rising interest in the search for alternatives to liquid fuels from lignocellulosic biomass. Biofuels from biomass can be a valuable substitute and a complement to fossil fuels. In addition, they are environmentally friendly, due to the benefit of reducing greenhouse gases (Rio Andrade et al. 2012). The polysaccharides in the grasses can be used as raw material to produce biofuels, once they have been pretreated and decomposed into simple sugars for efficient fermentation. However, the biochemistry of the lignin attached to cellulose hinders the efficiency of hydrolysis and fermentation processes (Ladisch et al. 2010). Cellulose linked to lignin requires greater amounts of enzymes to hydrolyze it, because of its complex structure (Fu et al. 2011). However, in comparison with woody biomass, grass biomass contains lower lignin concentrations, which makes it less recalcitrant to the action of enzymes and leads to simpler pretreatment conditions (Mohapatra et al. 2017). Grasses are considered as dedicated energy crops due to their high yield per hectare, ready availability, utilization of the whole plant, high concentration of carbohydrates and lower lignin concentration than woody species (Ventura et al. 2015). On average, grass biomass contains 25-46% cellulose, 19-46% hemicellulose and 13-30% lignin (Ramos et al. 2013; Godin et al. 2013; Ventura et al. 2015). About 30-35 grass species and varieties are documented to be potentially sustainable feedstocks for cellulosic ethanol production (Mohapatra et al. 2017).

Lignocellulosic biomass from C4 grasses is readily available in the tropical zones of Mexico, where varieties of *Cenchrus purpureus* (Schumach.) Morrone (syn. *Pennisetum purpureum* Schumach.; also known as Elephant and/or Napier grass) have been introduced in the past decades for use in animal feeding. Previous studies indicate that they have a great potential for growth and biomass production, ranging from 37 to 46 t DM/ha (Ramos et al. 2013; Calzada et al. 2014).

However, there are few studies showing the optimum harvesting age for highest production and chemical composition of the biomass to produce bioethanol, although it is reported that age of grass is the factor that most influences the chemical composition of cell walls (Rowell et al. 2012).

While *Cenchrus* grasses have been studied intensively, most evaluations have focused on the production of forage, nutritional value and animal performance (Grajales et al. 2018); fewer studies have evaluated cultivars of this species for bioethanol production (Ventura et al. 2015; Mohapatra et al. 2017). In Mexico, evaluation of the potential of grass biomass to produce cellulosic ethanol is limited. We consider that *Cenchrus* grasses have significant potential to provide biomass for bioethanol production, so designed this study to evaluate the biomass yield, chemical composition, heating value, proximate analysis and theoretical ethanol yield of Taiwan grass (*C. purpureus* cv. Taiwan) harvested at different cutting intervals to determine its potential as a bioenergy crop.

# **Materials and Methods**

# Experimental site and sampling

The experiment was carried out at the "Papaloapan" Experimental Site of INIFAP (18°06' N, 95°31' W; 65 masl) in Cd. Isla, Veracruz, Mexico, with an Awo climate and mean annual temperature of 25.7 °C (García 2004). The soil type is a sandy-loam orthic Acrisol, with a pH from 4 to 4.7 and is poor in organic matter, nitrogen, calcium and potassium and medium to high in phosphorus and magnesium (Enríquez and Romero 1999). The average rainfall recorded during the study is presented in Table 1, with data from the Meteorological Station of the Papaloapan Experimental Site. The experiment started on 22 July 2013, when vegetative material (stems) of Taiwan grass (C. purpureus cv. Taiwan) was planted in plots 5 m wide by 16 m long, with 3 replications, and finished on 17 July 2014. Stems were sown in rows with a continuous cord with 4.33 germination points (plants) per linear meter and inter-row spacing of 0.5 m, giving a density of 87,033 plants per hectare. A fertilizer dose of 120:36:0 kg/ha of N:P:K was applied in 2 equal applications (at 43 and 112 days after planting). Six cutting frequencies (30, 60, 90, 120, 150 and 180 days) were compared with 3 replications arranged in a complete randomized block design with split-plots, where the major plot was the grass and the minor plot was cutting frequency. The study continued for 360 days except for the 150-day harvest interval where harvests ceased after only 300 days.

2013					2014				Total					
Month	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	
Precipitation (mm)	380	460	110	320	90	40	60	70	20	10	20	136	232	1,948

Table 1. Average rainfall during the study in Cd. Isla, Veracruz.

#### Biomass production

At each harvest a central area of  $2 \times 3$  m was harvested (6 m<sup>2</sup>) from each plot at 20 cm above ground level. The harvested biomass was weighed on a precision scale (Ohaus, Mod. GT-4000) before a representative sample (15% of the total biomass) was taken, weighed and dried in an oven (Felisa, Mod. FE-243A) at 55 °C until constant weight to determine dry matter yield. Dried samples were ground in a Thomas-Wiley<sup>®</sup> mill (Arthur H. Thomas Co., Philadelphia, PA, USA) and sieved to pass through a No. 40 mesh (0.42–1.00 mm) and retained on a No. 60 mesh (0.25–0.42 mm). This sieved material was used to perform the chemical and calorific determinations. Following sampling the remaining grass on each plot was cut and removed.

#### Proximate analysis

Moisture content, volatile matter (VM) and ash (on a dry matter basis) were determined according to ASTM E871, ASTM E872 (<u>ASTM 2012</u>) and ASTM D 1102-84 (<u>ASTM 2009</u>) standards, respectively. Fixed carbon (FC) was computed by subtracting the concentrations of ash and volatile matter from the oven-dry sample mass [FC = 100-(VM+Ash)]. The moisture content of the samples was determined on an Ohaus MB45<sup>®</sup> scale with 3 samples per plot, giving 9 determinations for each cutting frequency.

#### Higher heating value

Higher heating value (HHV) was determined using an adiabatic bomb calorimeter (Isoperibol, Parr 1266) following ASTM E711 (<u>ASTM 1996</u>) standard at  $30\pm0.5$  °C, with pellets weighing 1 gram. Five determinations were performed per plot with a total of 15 samples per cutting frequency. Energy production was calculated by multiplying the biomass yield per hectare by HHV.

#### Chemical composition

Extractive release was carried out by following the TAPPI T-264 standard, including lipids (galactolipids,

triglycerides and phospholipids), waxes, fat-soluble vitamins, pigments and steroids (Barbosa et al. 2017). Holocellulose concentration was determined by the acid chlorite method and ASTM D1104 (ASTM 1977) standard was used for cellulose determination. Hemicellulose was calculated as the subtraction of cellulose from holocellulose. Lignin was determined according to TAPPI T-222 standard and nitrogen concentration by the semi-micro Kjeldahl procedure (AOAC 1990). Two samples per plot were determined giving a total of 6 determinations per cutting frequency.

#### Theoretical ethanol yield (TEY)

The TEY of grass biomass for each cutting frequency was estimated as follows (<u>Badger 2002</u>):

TEY = (B + B1), where: for cellulose: B = C × RE × E × GFE; and for hemicellulose: B1 = H × RE × E × XFE; B = kg of bioethanol/tonne of dry biomass; B1 = kg of bioethanol/tonne of dry biomass; C = kg of cellulose/tonne of dry biomass; H = kg of hemicellulose/tonne of dry biomass; RE = Recovery efficiency (0.76 for cellulose; 0.90 for hemicellulose); E= Ethanol stoichiometric yield (0.51); GFE= Glucose fermentation efficiency (0.75); and XFE= Xylose fermentation efficiency (0.50).

The unit of bioethanol yield, calculated with this formula, is kg/ha/yr. The density of ethanol (0.789 kg/L) was used to show the results in L/ha/yr.

# Statistical analysis

The experimental design was a randomized complete block design with: whole plot being genotype and subplot cutting frequency (30, 60, 90, 120, 150 and 180 d), with 3 replications. An analysis of variance (ANOVA) was carried out to investigate the effects of study factors on response variables by using the SAS/GLM procedure and treatment means were compared with the Tukey test (P $\leq$ 0.05). The data were analyzed to estimate the effect of cutting frequency using SAS for Windows version 9 (SAS 2011).

# Results

# Biomass production

As harvest interval increased, biomass yields (Table 2) increased from 10.2 t DM/ha/year with harvesting every 30 days to 38.4 t DM/ha/year with harvesting every 180 days (increase of 278%).

# Proximate analysis

The results for concentrations of moisture, ash, fixed carbon, volatile matter and higher heating value and energy production are presented in Table 2.

Moisture content of dehydrated grass. As harvest interval increased, moisture content decreased (P<0.05) since plants advanced in physiological development, i.e. from 9.2% at 30-day harvests to 7.0% at harvest intervals greater than 120 days (Table 2).

Ash. Ash concentration decreased (P<0.05) as plants progressed in physiological development from 12.2% at 30-day harvests to 4.5% at 120-day and longer harvest intervals (Table 2).

*Fixed carbon.* There was no consistent effect of harvest interval on concentration of fixed carbon in the grass with highest value of 18.6% at 90-day harvest intervals and a mean of 16.4% for the remainder (P<0.05) (Table 2).

*Volatile matter.* The concentration of volatile matter in the grass was similar for harvest intervals of 120, 150 and 180 days (mean 79.3%), which was higher than for the other cutting frequencies, with the lowest value for 30-day harvests (71.3%) (Table 2).

# Higher heating value and energy production

Energy concentration in the harvested grass increased from 15.6 MJ/kg at 30-day harvests to 17.2 MJ/kg at

90-day harvests and then plateaued (P<0.05). Energy production increased progressively with harvest interval from 158.5 GJ/ha at 30-day harvests to 675.7 GJ/ha at 180-day harvests (P<0.05) (Table 2).

# Chemical composition

*Extractives.* There was little consistency in the concentrations of extractives in the harvested grass with those from 90- and 180-day harvests being lowest at 7.2 and 7.8% (P>0.05), while remaining treatments varied from 10.2 to 14.0% (P>0.05) (Table 3).

*Holocellulose*. Holocellulose concentrations did not vary between treatments (P>0.05) with an overall mean of 72.3% (Table 3).

*Cellulose*. Cellulose is the main feedstock to produce ethanol, since it is a glucose polymer; its concentration increased from 38.3% at 30-day harvests to 42.8% at 90-day harvests (P<0.05) and then plateaued (Table 3).

*Hemicellulose*. Concentration of hemicellulose in the grass declined as harvest interval increased to 90 days but then plateaued (P<0.05) (Table 3).

*Lignin*. Lignin concentration increased linearly as plants advanced in physiological development from 17.7% at 30-day harvests to 21.0% at 150- and 180-day harvests (P<0.05) (Table 3).

*Crude protein.* Protein concentration decreased as plants advanced in physiological development from 10.5% at 30-day harvests to 2.7% at 150-day harvests (P<0.05) (Table 3).

# Bioethanol yield

Theoretical ethanol yields that can be produced from the grass biomass for the various treatments are presented in Table 4. Bioethanol yield per hectare increased progressively (P<0.05) as harvest

Table 2. Average biomass yield (DM), proximate analysis and calorific power of Taiwan grass at 6 cutting frequ	encies
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Cutting frequency	Yield (t/ha/yr)		Proximate	Higher heating value	Energy production		
(days)	(0 110/91) =	Moisture <sup>1</sup>	Ash	Fixed carbon	Volatile matter	(MJ/kg)	(GJ/ha)
30	$10.2\pm2.60d$	$9.2 \pm 0.26a$	$12.2\pm0.87a$	$16.5\pm1.42b$	$71.3\pm2.0c$	$15.6\pm0.43c$	$158.5\pm45.09d$
60	$10.5\pm2.03 cd$	$8.3\pm0.28b$	$8.1\pm0.65b$	$17.1\pm0.74ab$	$74.8\pm0.53b$	$16.2\pm0.25 bc$	$170.2\pm34.64d$
90	$14.0 \pm 1.22 c$	$7.4 \pm 0.26c$	$6.6\pm0.6\ c$	$18.6\pm0.66a$	$74.8 \pm 1.03 b$	$17.2 \pm 1.22a$	$239.8 \pm 10.92 \texttt{c}$
120	$18.8\pm3.40b$	$6.6\pm0.19\text{d}$	$4.7\pm0.30d$	$16.4\pm1.94b$	$78.8 \pm 1.89 a$	$17.0\pm0.32ab$	$319.4\pm 61.39b$
150	$18.2\pm2.42b^{\scriptscriptstyle 2}$	$6.9\pm0.26d$	$4.0\pm0.12d$	$15.7\pm1.98b$	$80.3\pm2.07a$	$17.2 \pm 0.78a$	$313.2\pm45.03b$
180	$38.5 \pm \mathbf{6.80a}$	$7.5\pm0.57c$	$4.7\pm0.21\text{d}$	$16.4\pm0.91b$	$78.9\pm0.95a$	$17.6\pm0.66a$	$675.7 \pm 56.12a$

Means within a given column followed by different letters are significantly different by Tukey's test (P $\leq$ 0.05). <sup>1</sup>Moisture of dried biomass. <sup>2</sup>Two harvests at 150-d intervals, i.e. only 300-days production.

Cutting			Compo	onent (%)			(GJ/ha)
frequency (days)	Extractives	Holocellulose <sup>1</sup>	Cellulose <sup>1</sup>	Hemicellulose <sup>1</sup>	Lignin <sup>1</sup>	Crude protein	-
30	$10.2\pm2.09 abc$	$71.4 \pm 3.51a$	$38.3 \pm 0.91 b$	$33.1 \pm 4.22ab$	$17.7\pm0.94c$	$10.5\pm0.71a$	$158.5\pm45.09d$
60	$14.0\pm3.45a$	$72.2 \pm 1.62a$	$37.7\pm0.78b$	$34.6 \pm 1.68 a$	$16.8\pm0.36c$	$6.4 \pm 1.09 b$	$170.2\pm34.64d$
90	$7.2 \pm 1.41c$	$71.9 \pm 1.49a$	$42.8\pm0.90a$	$29.1 \pm 1.54 c$	$19.6\pm0.18b$	$4.6\pm0.18c$	$239.8 \pm 10.92 \text{c}$
120	$13.8 \pm 1.38a$	$74.4\pm0.62a$	$42.7\pm0.57a$	$31.7\pm0.60 abc$	$19.8\pm0.41b$	$3.5\pm0.52cd$	$319.4\pm 61.39b$
150	$11.6\pm0.95 ab$	$72.2\pm0.56a$	$43.2\pm0.37a$	$29.0\pm0.39c$	$21.1\pm0.23a$	$2.7\pm0.45d$	$313.2\pm45.03b$
180	$7.8 \pm 1.82 bc$	$71.4\pm0.60a$	$41.4\pm1.96a$	$30.0\pm1.54bc$	$21.0\pm0.91a$	$3.5\pm0.51c$	$675.7 \pm 56.12a$

Table 3. Average chemical composition (%) of Taiwan grass at 6 cutting frequencies.

Means within a given column followed by different letters are different by Tukey's test ( $P \le 0.05$ ). <sup>1</sup>Values based on moisture and extractive free weight.

Table 4. Theoretical annual bioethanol yield from Taiwan grass at 6 cutting frequencies.

Cutting		L/ha/yr		
frequency (days)	Glucose	Xylose	Total	
30	$1,138 \pm 317.6c$	$758 \pm 161.5c$	$1,896 \pm 469.9d$	$2,400 \pm 594.8d$
60	$1,146 \pm 212.4c$	$829\pm146.8c$	$1,975 \pm 353.4d$	$2,499 \pm 447.4d$
90	$1,739 \pm 168.8 bc$	$926 \pm 41.0c$	$2,665 \pm 203.6$ cd	$3,373 \pm 257.7$ cd
120	$2,341 \pm 450.2b$	$1,365 \pm 237.9b$	$3,706 \pm 687.5b$	$4,692 \pm 870.3b$
150	$2,291 \pm 314.7b^{1}$	$1,214 \pm 159.9b^{1}$	$3,504 \pm 473.9 bc^{1}$	$4,436 \pm 599.9 bc^{1}$
180	$4,010 \pm 1,025.1a$	$2,\!259\pm366.2a$	$6,270 \pm 1,385a$	$7,936 \pm 1,754a$

Means within columns followed by different letters are significantly different by Tukey's test (P $\leq$ 0.05). <sup>1</sup>Two harvests at 150-day intervals, i.e. 300-days production.

interval increased with maximum yields from the 180day harvest of 4,010 and 2,259 kg/ha/yr from cellulose (glucose) and hemicellulose (xylose) sources, respectively. Maximum total theoretical bioethanol yield was 6,270 kg/ha/yr or 7,936 L/ha/yr.

#### Discussion

This study has demonstrated that Taiwan grass has considerable potential for biomass production, which can then be utilized to produce bioethanol. It is obvious that the longer the intervals between harvests the greater the biomass production per annum up to 180-day intervals, as longer intervals were not studied in this work. While bioethanol production also increased as interval between harvests increased, the increase in production was not as great as for biomass yields.

# Biomass yield

The maximum biomass yield obtained of 38.5 t DM/ ha at 180-day harvest intervals was somewhat less than the 46.3–58.4 t DM/ha/yr obtained by Ramos et al. (2013) with 3 cultivars of *Cenchrus* but slightly greater than the 11-25 t DM/ha/yr reported for other *Cenchrus purpureus* cultivars (<u>Habte et al. 2020</u>). These differences are not surprising as biomass yield is affected by genotype, soil properties including fertilization, age of the plant, agronomic management and amount and distribution of rainfall (Liu et al. 2014; <u>Ventura et al. 2015</u>). The increases in biomass yield as harvest interval increased are similar to those reported by Calzada et al. (2014).

# Proximate analysis

Fixed carbon. Crude fiber (CF) in the grass from 90day harvest interval contained a higher concentration of fixed carbon (FC; 18.6%) than CF in most other treatments (mean 16.4%) (Table 2). FC concentrations of 18.6 and 18% have been reported for Sudan grass (Sorghum × drummondii) (Parikh et al. 2005) and barley straw (McKendry 2002), respectively, while the CF of rice straw showed a FC value of 16.2% (Parikh et al. 2005). FC is the residue from the release of volatile compounds excluding moisture and ash in the pyrolysis process (Basu 2018). According to Santiago et al. (2016) high concentrations of FC limit the calorific value of grass. Since the FC represents the solid carbon in the biomass that remains in the char in the pyrolysis process, this fraction cannot be used for the purpose of producing bioethanol. For this reason, the ideal biomass for producing biofuel should contain the least amount of FC.

*Volatile matter.* The concentrations of volatile matter obtained for the different cutting frequencies were lower than the values reported for 2 cultivars of *Cenchrus*  (77.0-85.3%) (Braga et al. 2014; Mohammed et al. 2015). In all combustion processes, volatiles such as CO, nH<sub>2</sub>O, CO<sub>2</sub>, H<sub>2</sub>, carbohydrates and tars are released in an exothermic process and the greater the presence of volatiles the greater the reactivity of the biomass. However, bioenergetic studies have shown that the higher the concentration of volatiles the lower the calorific value of the material (Lewandowski and Kicherer 1997).

#### Calorific value

Higher heating value, also known as the gross calorific value or gross energy calorific value, is directly related to the potential of material for production of bioethanol and is an important characteristic for evaluating materials (Ramírez et al. 2012). It is the amount of heat released during the combustion of one gram of fuel to produce  $CO_2$  and  $H_2O$  at its initial temperature and is usually used to define the energy content of fuels and thereby their efficiency (Godin et al. 2013). The higher heating values (14.9–16.5 MJ/kg DM) reported for 9 cultivars of *C. purpureus* (Ramos et al. 2013; Mohammed et al. 2015) were similar to the energy values obtained in this work (15.6–17.6 MJ/kg DM). In contrast, an energy value of 18 MJ/kg DM has been reported for Switchgrass (*Panicum virgatum*) (Ram and Salam 2012).

#### Chemical composition

The composition of biomass produced is important in considering a plant's potential for bioethanol production as it influences the heating value and combustion processes (Brosse et al. 2012). For example, hemicellulose is a polymer with units of glucose, xylose, galactose, mannose and glucuronic acid. Some wild type microorganisms have the metabolic capacity to use xylose and galactose to produce ethanol, while the glucose from hemicellulose can also be used to produce bioethanol. On the other hand, lignin is a complex polymer formed by units of phenyl propane (p-coumaryl, coniferyl and synapyl alcohol) and presents a problem in ethanol production as it can prevent the release of cellulose and hemicellulose during the production process.

*Extractives*. Extractives are non-structural compounds of grass biomass (waxes, fats, oils, resins, free sugars, chlorophyll, organic acids, alditols, and polyphenolics), easily extractable with water or solvents that can interfere with carbohydrate and lignin characterization in plants (Sannigrahi et al. 2010). They function as metabolic intermediaries and energy reserves and are

responsible for the color, smell and resistance to wilting of grasses (Olanders and Steenari 1995). However, they cannot be converted to ethanol, so lignocellulosic biomass with a higher concentration of extractives will produce a lower yield of ethanol (Santiago et al. 2016). Gomes et al. (2015) suggest that biomass extractives present problems because they cause difficulties in the operation of industrial equipment through stickiness. In previous studies, Cardona et al. (2013) reported 16.9% for elephant grass (C. purpureus), while Mateus et al. (2012) reported 10.7% for Maralfalfa (C. purpureus), which is similar to the average obtained in the present study. The absence of any consistent relationship between level of extractives in the grass and harvest interval suggests that this parameter will not be affected significantly by duration between harvests.

Holocellulose. Holocellulose is formed by cellulose and hemicellulose (Jacobsen and Wyman 2000) and a higher concentration of holocellulose will produce higher amounts of bioethanol. However, pretreatments, hydrolysis and fermentation will directly determine the bioconversion of glucose and xylose to bioethanol (Victor et al. 2015). The values recorded in this study (71.4–74.4%) are similar to the values reported for Panicum maximum (now: Megathyrsus maximus) (69.9%) and Brachiaria brizantha (now: Urochloa brizantha) (71.7%) by Lima et al. (2014), as well as for elephant grass (C. purpureus) (72%) and C. purpureus cv. Enano (71.2%) by Wongwatanapaiboon et al. (2012). Since holocellulose concentrations in the biomass produced in our study were not related to harvest interval, frequency of harvests is unlikely to affect this parameter for the grass.

*Cellulose*. The distribution of cellulose in grasses is commonly 10% in leaves and 20–40% in stalks (Cafall and Mohnen 2009), so it was not surprising that cellulose concentration in biomass was higher at harvest intervals of 90 days than at 30- and 60-day intervals as stem percentage increases as grasses mature. In previous studies, Santiago et al. (2016) and Lima et al. (2014) reported 42.6% for Taiwan grass (at 270 days of age) and 43.4% for *U. brizantha* (at 180 days of age), which are similar to values obtained for the longer harvest intervals in our study. On the other hand, Rueda et al. (2016) registered concentrations of 37.7 and 36.7% for *C. purpureus* cv. Muaklek at 90 days of age, which is similar to concentrations for the 30- and 60-day harvests.

*Hemicellulose*. Hemicellulose is a complex carbohydrate polymer that constitutes 25–50% of the biomass in Gramineae (Ebringerová et al. 2005). The concentrations

we recorded are towards the bottom of this range and generally below the 37.6% recorded for elephant grass by Wongwatanapaiboon et al. (2012) but similar to the 31.0% reported for King grass (*Cenchrus* hybrid) by the same authors. Similarly, Lima et al. (2014) reported an average of 28% for C. *purpureus*. To produce bioethanol, hemicellulose concentration must be low because not all ethanol-producing microorganisms can metabolize xylose and galactose (hemicellulose-forming units). Increasing the harvest interval increased biomass production and reduced hemicellulose concentration, making the biomass obtained more suitable for bioethanol production.

*Lignin*. Lignin is a complex polymer constituted by units of phenyl propane (p-coumaryl, coniferyl and synapyl alcohol) and represents 10–30% of the total biomass in Gramineae (Limayem and Ricke 2012). While concentrations of lignin in biomass from our study increased as age at harvest increased as indicated by McCan and Carpita (2008), even at the longest harvest interval the concentration was lower than the 24% reported by Lima et al. (2014) for *C. purpureus* cultivars but greater than the 16.3% reported for Maralfalfa (*C. purpureus*) by Mateus et al. (2012). In terms of bioenergetics evaluations, high concentrations of lignin are undesirable, as the architecture and biochemistry of its bonds makes the hydrolysis of cellulose and hemicellulose difficult.

*Crude protein.* It is important to evaluate the concentration of CP in biomass because it can interfere with lignin quantification and change the chemical composition of biomass (<u>Du et al. 2020</u>). High concentrations of nitrogen limit the bioconversion of total sugars to ethanol (<u>Santiago et al. 2016</u>). The reduction in CP concentration, as stage of development at harvest increased, would favor bioethanol production from the material produced.

# Bioethanol yield

The polysaccharides from cell walls can be used as raw matter to produce bioethanol and other biofuels once they have been pretreated and hydrolyzed into simple sugars for efficient fermentation (<u>Rio Andrade et al.</u> 2012). In the current study, potential yields of bioethanol were estimated according to the procedure of Badger (2002) and strongly favored the longest harvest interval, largely as a reflection of higher DM yields at this harvest frequency, despite increasing lignin concentration in the more mature material. Wongwatanapaiboon et al. (2012)

reported bioethanol yields of 6,331 L/ha/yr for Mott grass (*C. purpureus*) and 6,717 L/ha/yr for guinea grass (*M. maximus*), both from rather aged biomass, which were 20 and 15% lower than the 7,936 L/ha/yr obtained in our study at 180-day harvest intervals.

It seems that Taiwan grass is a suitable source of biomass for production of second-generation bioethanol, with the potential to produce 8,000 L/ha/yr. It could compete with other first-generation primary sources like sugarcane juice and corn (grain) that can produce 6,900 L/ha and 2,900 L/ha, respectively (Somerville et al. 2010). Another reason for using C4 grasses for bioenergy is that they are more efficient in the use of water than C3 grasses (Weijde et al. 2013). These authors suggest that it is necessary to evaluate structural and non-structural components of the cell wall in order to produce bioethanol profitably and sustainably. Ethanolproducing microorganisms cannot convert 100% of fermentable sugars to ethanol, because they need to use part of these sugars to perform some other vital metabolic functions. Therefore, the theoretical yield based on 100 g of glucose that would produce 51.4 g ethanol and 48.8 g CO<sub>2</sub> would not be possible (<u>Badger 2002</u>).

Current research is being undertaken by the United States Department of Energy with the objective of accelerating the conversion process from lignocellulosic biomass to liquid bioethanol. It is expected that by 2030 around 30% of the gasoline currently consumed worldwide will be replaced by bioethanol from plant material. A significant benefit would be that the use of bioethanol from cellulosic biomass could reduce the greenhouse gas emissions about 86% (Wang et al. 2007). Nevertheless, there is controversy about the environmental and economic benefits of biofuels. Science-based information will help to guide decisions about the crop, cultivation strategies, age of harvest and the bioethanol production process. Then, the environmental and economic impacts of biofuel production will become clearer. Furthermore, in terms of the balance of energy consumed:energy produced, cellulosic ethanol is less efficient than ethanol from starch and other traditional sugar crops. For example, for switchgrass and Miscanthus the ratios are 10.8-11.3:1 and 22:1, respectively. By comparison, the ratios for traditional sugar crops and corn are 8.1-10:1 and 1.4-2.3:1, respectively (Byrt et al. 2011). Obviously, starchy products are a much more efficient source of energy production than cellulosic materials. From an ethical point of view, it is not advisable to obtain fuel ethanol from raw materials considered as food for

humans. For the production of ethanol from biomass to be economically and technically viable, the production process must be improved.

# Conclusions

Climatic conditions, the type of soil in the Gulf of Mexico and the agronomic management developed in this study favor the growth of Taiwan grass. The highest yield of biomass was 38 t DM/ha/yr with a production of 675 GJ energy/ha at a harvest interval of 180 days. The plasticity, regrowth speed and resistance to pests and diseases make the grass an appropriate raw material to produce liquid biofuel in Mexico. Nevertheless, it is necessary to study the carbon footprint, life cycle analysis, energy yield (energy produced:energy consumed) of the entire production process to ensure there are positive effects on climate change and greenhouse gas emissions before this process is adopted.

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# References

#### (Note of the editors: All hyperlinks were verified 15 December 2021).

- AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis. 15th Edn. Association of Official Analytical Chemists, Washington, DC, USA.
- ASTM (American Society for Testing and Materials). 1977. Method of test for Holocellulose in wood. ASTM D1104– 56. In: Annual Book of ASTM Standards. Part 22: Wood; Adhesives. ASTM, West Conshohocken, PA, USA. p. 346–348.
- ASTM (American Society for Testing and Materials). 1996. Standard test method for gross calorific value of refuse– derived fuel by the bomb calorimeter. ASTM E711. In: Annual Book of ASTM Standards. Waste management. ASTM, West Conshohocken, PA, USA. p. 265–271.
- ASTM (American Society for Testing and Materials). 2009. Standard test method for ash in wood. ASTM D1102. In: Annual Book of ASTM Standards. Volume 04.10. ASTM, West Conshohocken, PA, USA. p. 174–175.
- ASTM (American Society for Testing and Materials). 2012. Biological effects and environmental fate; biotechnology. ASTM E870, ASTM E871, ASTM E872. In: Annual Book of ASTM Standards. Volume 11.16. ASTM, West Conshohocken, PA, USA.

Badger CP. 2002. Ethanol from cellulose: A general review.

In: Janick J; Whipkey A, eds. Trends in new crops and new uses. ASHS Press, Alexandria, VA, USA. <u>stanford.io/</u> <u>33mXM8c</u>

- Barbosa MM; Detmann E; Valadares Filho SC; Detmann KSC; Franco MO; Batista ED; Rocha GC. 2017. Evaluation of methods for the quantification of ether extract contents in forage and cattle feces. Anais da Academia Brasileira de Ciências 89: 1295–1303. doi: 10.1590/0001-3765201720160708
- Basu P. 2018. Biomass gasification, pyrolysis and torrefaction. 3rd Edn. Academic Press, London, UK. doi: <u>10.1016/</u> <u>C2016-0-04056-1</u>
- Braga, RM; Costa TR; Freitas JCO; Barros JMF; Melo DMA; Melo MAF. 2014. Pyrolysis kinetics of elephant grass pretreated biomasses. Journal of Thermal Analysis and Calorimetry. 117:1341-1348. doi: 10.1007/s10973-014-3884-2
- Brosse N; Dufour A; Meng X; Sung Q; Ragauskas A. 2012. Miscanthus: a fast-growing crop for biofuels and chemicals production. Biofuels, Bioproducts and Biorefining 6:580– 598. doi: 10.1002/bbb.1353
- Byrt CS; Grof CPL; Furbank RT. 2011. C<sub>4</sub> Plants as biofuel feedstocks: optimizing biomass production and feedstock quality from a lignocellulosic perspective. Journal of Integrative Plant Biology 53:120–135. doi: <u>10.1111/j.1744-7909.2010.01023.x</u>
- Cafall KH; Mohnen D. 2009. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydrate Research 344:1879–1900. doi: <u>10.1016/j.</u> carres.2009.05.021
- Calzada JM; Enríquez JF; Hernández A; Ortega E; Mendoza SI. 2014. Growth analysis of maralfalfa Grass (*Pennisetum* sp.) in a warm humid climate. Revista Mexicana de Ciencias Pecuarias 5(2):247–260. (In Spanish). bit.ly/3IRD9RV
- Cardona EM; Ríos JA; Peña JD; Rios LA. 2013. Alkaline pretreatment of Elephant Grass (*Pennisetum Sp*) and King Grass (*Pennisetum hybridum*) cultured in Colombia for ethanol production. Información Tecnológica 24:69–80. (In Spanish). doi: <u>10.4067/S0718-07642013000500009</u>
- Du L; Arauzo PJ; Zavala MFM; Cao Z; Olszewski MP; Kruse A. 2020. Towards the properties of different biomass-derived proteins via various extraction methods. Molecules. 25:488 doi: <u>10.3390/molecules25030488</u>
- Ebringerová A; Hromádková Z; Heinze T. 2005. Hemicellulose. In: Heinze T, ed. Polysaccharides I. Advances in Polymer Science. Springer, Berlin, Germany. p. 1–67. doi: <u>10.1007/b136816</u>
- Enríquez JF; Romero J. 1999. Seasonal growth rate at different regrowth ages of 16 *Brachiaria* spp. ecotypes in Isla, Veracruz. Agrociencia 33:141–148. (In Spanish). <u>bit.ly/3221c24</u>
- Fu Ch; Mielenz JR; Xiao X; Ge Y; Hamilton Ch; Rodriguez M; Chen F; Foston M; Ragauskas A; Bouton J; Dixon RA; Wang ZY. 2011. Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. Proceedings of the National Academy of

Sciences 9:3803-3808. doi: 10.1073/pnas.1100310108

- García E. 2004. Modificaciones al Sistema de Clasificación Climática de Köppen (para adaptarlo a las condiciones de la República Mexicana). Instituto de Geografía, Universidad Nacional Autónoma de México, Mexico. <u>bit.</u> <u>ly/3dRhElU</u>
- Gomes FJB; Colodette JL; Burnet A; Batalha LAR; Santos FA; Demuner LF. 2015. Thorough characterization of Brazilian new generation of eucalypt clones and grass for pulp production. International Journal of Forestry Research 2015:814071. doi: 10.1155/2015/814071
- Godin B; Lamaudière S; Agneessens R; Schmit T; Goffart JP; Stilmant D; Gerin PA; Delcarte J. 2013. Chemical characteristics and biofuels potentials of various plant biomasses: influence of the harvesting date. Journal of the Science of Food and Agriculture 93:3216–3224. doi: <u>10.1002/jsfa.6159</u>
- Grajales Zepeda R; Lazo JA; Ocampo Sánchez EM; Hernández García B; Jiménez Sánchez YJ; Aguilar Hernández JO; Pérez Moreno LI; Aguilar Sánchez E; Tuero Martínez R. 2018. Evaluation of *Cenchrus purpureus* cultivars for forage production. Livestock Research for Rural Development 30: Article#26. (In Spanish). <u>lrrd.org/ lrrd30/2/jale30026.html</u>
- Habte E; Muktar MS; Abdena A; Hanson J: Sartie AM; Negawo AT; Machado JC; Ledo FJS; Jones CS. 2020. Forage performance and detection of marker trait associations with potential for napier grass (*Cenchrus purpureus*) improvement. Agronomy 10:542. doi: <u>10.3390/</u> <u>agronomy10040542</u>
- Jacobsen S; Wyman CE. 2000. Cellulose and hemicellulose hydrolysis models for application to current and novel pretreatment processes. Applied Biochemistry and Biotechnology 84:81–96. doi: 10.1385/ABAB:84-86:1-9:81
- Ladisch MR; Mosier NS; Kim Y; Ximenes E; Hogsett D. 2010. Converting cellulose to biofuels. Chemistry Engineering Progress 106:56–63. <u>bit.ly/332bQ7d</u>
- Lewandowski I; Kicherer A. 1997. Combustion quality of biomass: practical relevance and experiments to modify the biomass quality of *Miscanthus x giganteus*. European Journal of Agronomy 6:163–177. doi: <u>10.1016/S1161-</u> 0301(96)02044-8
- Lima MA; Gomez DL; Steele-King GC; Simister R; Bernardinelli OD; Carvalho MA; Rezende AC; Labate CA; de Azevedo ER; McQueen-Mason SJ; Polikarpov I. 2014. Evaluating the composition and processing potential of novel sources of Brazilian biomass for sustainable biorenewables production. Biotechnology for Biofuels and Bioproducts 7:10. doi: 10.1186/1754-6834-7-10
- Limayem A; Ricke SC. 2012. Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects. Progress in Energy and Combustion Science 38:449–467. doi: 10.1016/j.pecs.2012.03.002
- Liu M; Liu G; Gong L; Wang D; Sun J. 2014. Relationships of biomass with environmental factors in the grassland area

of Hulunbuir, China. PLoS One 9:e102344. doi: <u>10.1371/</u>journal.pone.0102344

- Mateus L; Hernández O; Velázquez M; Diaz JJ. 2012. Dilute sulfuric acid pretreatment of goliath grass (*Pennisetum glaucum* x *Pennisetum purpureum*) for ethanol cellulosic. Revista Colombiana de Biotecnología 14:146–156. (In Spanish). <u>scielo.org.co/pdf/biote/v14n1/v14n1a13.pdf</u>
- McCan MC; Carpita CN. 2008. Designing the deconstruction of plant cell walls. Current Opinion in Plant Biology 11:314–320. doi: <u>10.1016/j.pbi.2008.04.001</u>
- McKendry P. 2002. Energy production from biomass (part 1): overview of biomass. Bioresource Technology 83:37–46. doi: 10.1016/S0960-8524(01)00118-3
- Mohammed IY; Abakr YA; Kazi FK; Yusup S; Alshareef I; Chin SA. 2015. Comprehensive characterization of Napier grass as a feedstock for thermochemical conversion. Energies 8:3403–3417. doi: <u>10.3390/en8053403</u>
- Mohapatra S; Mishra C; Behera SS; Thatoi H. 2017. Application of pretreatment, fermentation and molecular techniques for enhancing bioethanol production from grass biomass A review. Renewable and Sustainable Energy Reviews 78:1007–1032. doi: 10.1016/j.rser.2017.05.026
- Olanders B; Steenari BM. 1995. Characterization of ashes from wood and straw. Biomass and Bioenergy 8:105–115. doi: 10.1016/0961-9534(95)00004-Q
- Parikh J; Channiwala SA; Ghosal GK. 2005. A correlation for calculating HHV from proximate analysis of solid fuels. Fuel 84:487–494. doi: 10.1016/j.fuel.2004.10.010
- Ram D; Salam PA. 2012. Estimation of higher heating value of biomass from proximate analysis: A new approach. Fuel 99:55–63. doi: <u>10.1016/j.fuel.2012.04.015</u>
- Ramírez LF; Rodríguez JE; Jaramillo AR. 2012. Predicting cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition. Fuel 91:102–111. doi: 10.1016/j.fuel.2011.06.070
- Ramos TO; Canul SJR; Vera FDJ. 2013. Production of three varieties of *Pennisetum purpureum* fertilized with two different sources of nitrogen in Yucatan, Mexico. Revista Bio Ciencias 2:60–68. (In Spanish). <u>bit.ly/30VMksp</u>
- Rio JC; Prinsen P; Rencoret J; Nieto L; Jiménez J; Ralph J; Martínez AT; Gutiérrez A. 2012. Structural characterization of the lignin in the cortex and pith of Elephant grass (*Pennisetum purpureum*) stems. Journal of Agricultural and Food Chemistry 60:3619–3634. doi: 10.1021/jf300099g
- Rowell M; Pettersen R; Tshabalala MA. 2012. Cell wall chemistry. In: Rowell R, ed. Handbook of wood chemistry and wood composites. 2nd Edn. CRC Press, Boca Raton, FL, USA. p. 33–72. <u>fs.usda.gov/treesearch/pubs/42245</u>
- Rueda JA; Jimenez EO; Hernández-Garay A; Enríquez-Quiroz JF; Guerrero-Rodríguez JD; Quero-Carrillo AR. 2016. Growth, yield, fiber content and lodging resistance in eight varieties of *Cenchrus purpureus* (Schumach.) Morrone intended as energy crop. Biomass and Bioenergy. 88:59-65. doi: 10.1016/j.biombioe.2016.03.007

- Sannigrahi P; Ragauskas AJ; Tuskan GA. 2010, Poplar as a feedstock for biofuels: a review of compositional characteristics. Biofuels, Bioproducts and Biorefining 4:209-226. doi:<u>10.1002/bbb.206</u>
- Santiago MA; Honorato JA; Quero AR; Hernández A; López C; López I. 2016. Biomass of Urochloa brizantha cv. Toledo as raw material for bioethanol production. Agrociencia 50:711–726. <u>bit.ly/3IPJ6yF</u>
- SAS Institute. 2011. SAS/STAT User's Guide. Release 9.3. SAS Institute Inc., Cary, NC.
- Somerville C; Youngs H; Taylor C; Davis SC; Long SP. 2010. Feedstocks for lignocellulosic biofuels. Science 329:790– 792. doi: 10.1126/science.1189268
- Ventura RJ; Honorato SJA; Hernández GA; Reyes-García VI. 2015. Producción y calidad del pasto Maralfalfa (*Pennisetum glaucum x Pennisetum purpureum*) a diferentes frecuencias de corte. Abstract 385. XXIV Congreso de la Asociación Latinoamericana de Producción Animal y XL Congreso de la Sociedad Chilena de Producción Animal, SOCHIPA. A.G. Puerto

Varas, Chile, 9-13 November 2015. p. 9-13.

- Victor A; Neel I; Gedanken A. 2015. Assessment of holocellulose for the production of bioethanol by conserving *Pinus radiata* cones as renewable feedstock. Journal of Environmental Management 162:215–220. doi: <u>10.1016/j.jenvman.2015.07.038</u>
- Wang M; Wu M; Huo H. 2007. Life-cycle energy and greenhouse gas emission impacts of different corn ethanol plant types. Environmental Research Letters 2:024001. doi: 10.1088/1748-9326/2/2/024001
- Weijde TVD; Alvim Kamei CL; Torres AF; Vermerris W; Dolstra O; Visser RGF; Trindade LM. 2013. The potential of C4 grasses for cellulosic biofuel production. Frontiers in Plant Science 4:107. doi: 10.3389/fpls.2013.00107
- Wongwatanapaiboon J; Kangvansaichol K; Burapatana V; Inochanon R; Winayanuwattikun P; Yongvanich T; Chulalaksananukul W. 2012. The potential of cellulosic ethanol production from grasses in Thailand. Journal of Biomedicine and Biotechnology 2012:303748. doi: 10.1155/2012/303748

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