Effect of Harvesting Age and Size Reduction in the Performance of Anaerobic Digestion of Pennisetum Grass

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Keywords: particle size reduction, methane yield, biogas, harvest age, tropical climate, perennial grasses, anaerobic digestion

Abstract:

In the rural zones of Latin American and Caribbean developing countries, the poorest households rely on traditional fuels such as firewood to meet their daily cooking needs. Many of those countries are located near the equator, where they have a tropical climate and grass is one of the most common biomass crops. The aim of this study was to evaluate the effect of harvesting age (30, 44, and 57 days) in the performance of anaerobic digestion of King Grass (Pennisetum purpureum cv. King Grass) grown under tropical climate conditions. Three reduction methods of crop size were also compared. Results showed that 44-day harvesting age presented the greater specific methane yield (347.8 mLCH4 g?1VS) and area-specific methane yield (9773 m3CH4 ha?1 y?1). The machine chopped method (1?3 cm for stems and 1?10 cm for leaves) was the reduction method that maximized the methane production. From those results, the calculated area required for grass cultivation to provide the cooking energy to a typical family in the Colombian rural zones is 154 m2.

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Effect of Harvesting Age and Size Reduction in the Performance of Anaerobic Digestion of *Pennisetum* Grass

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Abstract: In the rural zones of Latin American and Caribbean developing countries, the poorest households rely on traditional fuels such as firewood to meet their daily cooking needs. Many of those countries are located near the equator, where they have a tropical climate and grass is one of the most common biomass crops. The aim of this study was to evaluate the effect of harvesting age (30, 44, and 57 days) in the performance of anaerobic digestion of King Grass (*Pennisetum purpureum cv. King Grass*) grown under tropical climate conditions. Three reduction methods of crop size were also compared. Results showed that 44-day harvesting age presented the greater specific methane yield (347.8 $mLCH_4$ $g^{-1}VS$) and area-specific methane yield (9773 m^3CH_4 ha^{-1} y^{-1}). The machine chopped method (1–3 cm for stems and 1–10 cm for leaves) was the reduction method that maximized the methane production. From those results, the calculated area required for grass cultivation to provide the cooking energy to a typical family in the Colombian rural zones is 154 m^2.

Keywords: anaerobic digestion; perennial grasses; tropical climate; harvest age; particle size reduction; methane yield; biogas

1. Introduction

About 3 billion people around the world use firewood or similar biomass to cook [1]. Firewood has a low calorific value and can cause toxicity evens if kitchens are not well ventilated and combustion is not complete. This causes around 4.3 million deaths every year [1]. Liquefied propane gas (LPG) is the main substitute of firewood in developing countries like Colombia, where is estimated that around 13 million people use it as cooking fuel and the annual consumption per housing unit is 132 kg, equivalent to approximately 6000 MJ [2]. However, its application is still limited due to its high costs and limited access of vehicle in certain areas. Biogas from anaerobic digestion (AD) of biomass in low-cost household digesters could substitute traditional biomass as firewood, which could reduce environmental impacts and improve safety and the standard of living of rural families [3].

Natural and cultivated pastures play an important role in global agriculture. They represent 67% of the world's cultivated areas and is one of the lower-cost and common biomass in rural areas [4]. For instance, grasses in Colombia, which are mainly used to feed cattle, are productive throughout the year, and their growth rates mainly depend on water availability. The *Pennisetum* sp. grasses are the most common in the region and under non-irrigated conditions or low rainfall (between 600 and 1500 mm) green Elephant genotypes are recommended [5]. Moreover, *Pennisetum* grasses are one of the energy crops with the highest agronomic yield [6] and specific methane yield worldwide, above 300 $mLCH_4\ g^{-1}VS$ [7].

The harvesting age influence of *Pennisetum* grasses on biogas production has been studied. Specific methane yields range from 104 $mLCH_4$ to 310 $mLCH_4\ g^{-1}VS$ for harvesting ages between 60 and 360 days, where young tissues produced more methane than the old tissues using the biochemical methane potential (BMP) test [8,9]. In lab continuous reactors, the literature reported yields range from 100 $mLCH_4$ to 242 $mLCH_4\ g^{-1}VS$ (4803 m^3CH_4 to 7899 $m^3CH_4\ ha^{-1}\ y^{-1}$) for harvesting ages around 60 days [10–12]. In tropical countries, the common harvesting ages are between 30 and 60 days [13,14], which could enhance the methane yields [11].

Most of the studies of biogas potential of crops have been done with crops in temperate or boreal climates, where harvest ages are much higher and agronomic yields lower. Reliable information of biogas production in Latin America and the Caribbean (LAC) is still missing, as most of the information available on pastures refers to its use as feed for livestock.

This study evaluates the effect of harvesting age in King Grass *Pennisetum purpureum cv.* King Grass)—on its specific methane yield and area-specific methane yield—evaluating three typical harvesting ages (30, 44, and 57 days) according to local practices in Colombia. Additionally, three reduction methods of particle size were compared in the same terms, with the previously selected best harvesting age. Finally, it was calculated the required area for crop cultivation to supply the cooking-energy of a rural household in LAC.

2. Materials and Methods

2.1. Crop Production and Characterization

The King Grass crop was grown at a farm located in Cali, Colombia (3°21′50.8′′ N; 76°33′45.8′′ W), with annual precipitation of 1173 mm and average temperature 23 °C (17 °C to 31 °C) [15]. The crop was fertilized following common local practices (N:P:K 325:22:42 kg $ha^{-1}\ y^{-1}$).

There were nine plots, 25 m^2 each, distributed randomly in three blocks. Grass crop was hand-harvested 5 cm above ground level [13] and refrigerated before characterization. Grass crop yield was calculated weighing 4–7 subsamples from each plot after chopped (sizes from 1–3 cm in stems and 1−10 cm in leaves).

Total solids (TS), volatile solids (VS), and pH were determined following APHA [16]; concentrations of C and N were measured according to ASTM [17] using elemental analyzer CHN 628 (LECO, St. Joseph—MI, USA); and crude protein (CP), ethereal extract (E.E.), cellulose, hemicellulose, and lignin were estimated after Van Soest [18].

A theoretical (stoichiometric) methane yield was calculated [19] to assess a possible correlation between methane yield and composition of the grass at different harvesting ages. To calculate the theoretical yield, empirical formulae for each organic component were estimated as protein ($C_5H_7O_2N$), lipid ($C_{57}H_{104}O_6$), lignin ($C_{10}H_{13}O_3$), and carbohydrates (non-lignocellulosic carbohydrates, cellulose, and hemicellulose) ($C_6H_{10}O_5$) [19]. The theoretical methane yield was estimated according to Equation (1):

$$YCH_4\left(\mathrm{mLCH_4\ g^{-1}VS}\right) = Lipid{\cdot}1.014 + Protein{\cdot}0.496 + Carbohydrate{\cdot}0.415 + Lignin{\cdot}0.727 \quad (1)$$

where YCH_4 is the theoretical methane yield of grass as mL CH_4 g^{-1}VS and 1.014 is the theoretical yield of lipid, 0.496 is the theoretical value for protein, 0.415 is the theoretical value for carbohydrate, and 0.727 is the theoretical value for lignin, all of them as g kg^{-1} VS.

2.2. Biochemical Methane Potential (BMP) Test

Methane quantification was performed in batch vials with the Oxitop® system (WTW—Xylem, Weilheim, Germany) incubated at 35.0 ± 0.5 °C in a thermostat cabinet TS 606-2 (WTW—Xylem, Weilheim, Germany). Reactors used have a working volume of 200 mL with a headspace of 50 mL. CO_2 produced was trapped in NaOH before the Measuring head, this way the gas measured was methane [20]. The composition of gas was verified weekly via gas chromatography using a GC2014 chromatograph (Shimadzu, Kyoto, Japan).

The experiments were conducted at a substrate/inoculum ratio (S/I) of 1 $gVS_{substrate} \cdot g^{-1}VS_{inoculum}$, a solution of macro and micronutrients were used, and the pH was adjusted at 7.0 [21]. The inoculum used was from a batch dry grass anaerobic digester (pH: 8.31, TS: 11.0% and VS: 75.3% dry basis).

BMP tests were carried out per triplicate. When methane production increased less than 5% between measures, the assay was stopped (90 days). The net methane production under standard conditions was calculated by subtracting the methane production of the blanks (inoculum without substrate) from the methane production of the treatment vials. Finally, methane production was fitted to the Gompertz equation (Equation (2)) [22]:

$$\boldsymbol{P} = \boldsymbol{P_m} \times \boldsymbol{exp}\left(-\boldsymbol{exp}\left[\frac{\boldsymbol{R_m} \times \boldsymbol{e}}{\boldsymbol{P_m}}\right](\boldsymbol{\lambda} - \boldsymbol{t})\right) \tag{2}$$

where $\boldsymbol{P}$ is the accumulated methane production (mLCH_4 g^{-1}VS), $\boldsymbol{P_m}$ is the specific Methane yield (mLCH_4 g^{-1}VS), $\boldsymbol{R_m}$ is the maximum methane production rate (mLCH_4 g^{-1}VS day^{-1}), and $\boldsymbol{\lambda}$ is the lag phase period of biogas production (day). The least-square sum of errors criterion was used in the fitting process and the Monte Carlo method to establish the 95% confidence interval of the estimated parameter values [23].

2.3. Experimental Design

Three harvesting ages (30, 44, and 57 days) were evaluated (n = 3), and the response variables were specific methane yield (mLCH_4 g^{-1}VS) and area-specific methane yield (m^3CH_4 ha^{-1} y^{-1}). An ANOVA followed by a post hoc Fisher's least significant difference test (LSD, $p < 0.05$) was applied to response variables using STATISTICA software, version 7.0 (StatSoft Inc., Tulsa—OK, USA).

For the harvesting age with the higher methane yield, three methods of particle reduction size were applied (n = 3). Minced (<1 cm for leaves and stems) obtained with a CB15 blender (Waring Commercial, Torrington, CT, USA) run at a speed of 15800 rpm for one minute (standard blender speed); machine chopped (1–3 cm stems and 1–10 cm leaves) obtained with a TRAPP® TR200 industrial (METALÚRGICA TRAPP LTDA, Jaraguá do Sul-SC, Brazil); and lastly, hand chopped (5–10 cm stems and 5–20 cm leaves) obtained by hand chopping using a bowie knife. The response variable was the specific methane yield (mLCH_4 g^{-1}VS) and also ANOVA followed by a post hoc Fisher's was conducted.

Finally, the biogas and area requirements to supply a cooking energy of 6000 MJ (150 m^3CH_4 equivalent), needed per housing unit per year in rural zones of Colombia, was estimated.

3. Results and Discussion

3.1. Substrate Production and Characterization

Results of the agronomic yields and substrate characterization are presented in Table 1.

Table 1. Characterization and yields of King Grass according to harvesting age (n = 3 where standard deviation is presented) (numbers of a same parameter followed by the same letter are not significantly different (p = 0.05)).

Parameter	Harvesting Age (d)		
	30	**44**	**57**
pH	7.00	6.78	6.50
Total Solids (Dry Matter) (%)	15.0	14.2	21.1
Volatile Solids (%)	86.0	86.0	85.0
N (%)	2.75	3.24	2.24
C/N Ratio	15.7	13.7	19.8
Crude Protein (g kg^{-1}VS)	127.87	145.15	90.52
Ethereal Extract (g kg^{-1}VS)	15.5	25.9	23.8
Cellulose (g kg^{-1}VS)	357.8	324.0	357.0
Hemicellulose (g kg^{-1}VS)	192.6	241.9	256.7
Lignin (g kg^{-1}VS)	26.7	20.7	19.6
Agronomic Yield (t_{DM} ha^{-1} y^{-1})	16.8 ± 2.0 a	28.1 ± 3.9 b	38.6 ± 6.6 c
Theoretical Methane Yield ($mLCH_4$ g^{-1}VS)	326.9	348.2	337.9
Specific Methane Yield ($mLCH_4$ g^{-1}VS)	241.7 ± 35.9 b	347.8 ± 27.0 a	191.2 ± 17.1 b
Area-Specific Methane Yield (m^3CH_4 ha^{-1} y^{-1})	4060.6 ± 603.1 c	9773.2 ± 758.7 a	7380.3 ± 660.1 b

Although a slight reduction in pH was observed with an increase in harvesting age, pH values for all the treatments were close to neutral. Regarding the variables related to organic matter, TS tends to increase with harvesting ages beyond 44 days, with a slight decrease in vs. (dry basis) observed. These values were similar to those obtained by Chanpla et al. [14] when growing *Pennisetum purpureum* with harvesting at ages of 35, 45, and 55 days, reporting a 18.4% and 19.2% and 20.2% of TS content respectively, with a vs. percentage between 87–89%.

The C/N ratios obtained were 15.7, 13.7, and 19.8 for harvesting ages of 30, 44, and 57 days, respectively. According to Dai et al. [24], who evaluated the co-digestion of activated sludge and ryegrass at pH 7.00, the highest specific CH_4 yields were found with C/N ratios lower than 15, while the highest methane contents in the biogas were found with a C/N of 9.

Regarding fiber composition and structural components, there were not notable differences between harvesting ages except an increase of ethereal extract from 44 days and a decrease of crude protein observed at 57 days.

As expected, agronomic yields obtained were shown to be higher when increasing the harvesting age. The values obtained for cutting ages of 30 and 44 days (16.8 ± 2.0 and 28.1 ± 8.0 t_{DM} ha^{-1} y^{-1}) are similar to those reported for King Grass of similar ages: 22.86 and 28.95 t_{MS} ha^{-1} y^{-1} for 30 and 45 days of age, respectively [13]. However, agronomic yield (38.6 ± 6.6 t_{DM} ha^{-1} y^{-1}) for a harvesting age of 57 days was higher than that obtained by Lounglawan et al. [13] of 28.93 t_{DM} ha^{-1} y^{-1} for harvesting age of 60 days in Thailand.

However, from the grass composition at different harvesting ages, the theoretical estimated methane yield showed a higher value expected for the harvesting age of 44 days.

Results show that the harvesting age influences the substrate production and characterization of King Grass. According to those characteristics, it can be foreseen that, a 44-day harvesting age would be optimal for biogas production. A 44-day harvesting age shows a better C/N ratio and CP than a 57-day age, a better C/N, CP, EE and lignin concentrations than a 30-day age and the highest theoretical methane yield.

3.2. Influence of Harvesting Age on the Methane Yield

The ANOVA of methane yields at different harvesting ages presented significant differences (p = 0.000623); the post hoc Fisher's showed that methane yield at 44-day age (347.8 ± 27.0 $mLCH_4$ g^{-1}VS) was significantly higher than the other two harvesting ages (Table 1) as expected from characterization results. Nevertheless, no significant differences were observed between 30 and 57 days. These results are similar to those reported by Chanpla et al. [14] for *Pennisetum purpureum cv. Pakchong-1*, who obtained the best specific methane yield in BMP tests for a 45-day harvesting age, followed by a 35-day and 55-day harvesting age.

Figure 1 shows the timeline of biogas production and the Gompertz equation fits. Table 2 shows the parameters of the fitting. As can be seen the fitting displayed high determination coefficients ($r^2 > 0.96$) and evidenced that the maximum methane production rate (Rm) of the 44-day harvesting age is the highest of the three evaluated ages. These results agreed with previous reports for fresh *Pennisetum purpureum* grasses between 10.64 $mLCH_4$ and 13.97 $mLCH_4$ $g^{-1}VS\ d^{-1}$ [25]. Regarding the λ parameter, very close values were observed between the treatments.

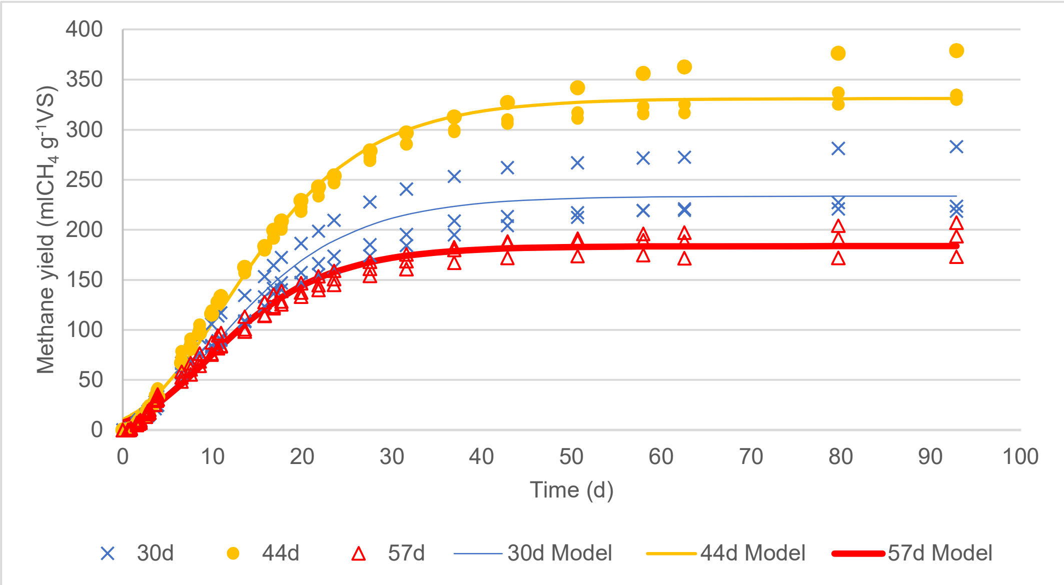

Figure 1. Specific methane yield over time for harvesting ages ($n = 3$).

Table 2. Parameters of the Gompertz equation adjustment on harvesting age biochemical methane potential (BMP) tests (*Pm*, *Rm*, and λ), confidence interval (CI with $\alpha = 0.05$) and evaluation of the coefficient of determination (r^2).

Harvesting Age (Days)	P_{max}		R_m		λ		r^2
	($mLCH_4\ gVS^{-1}$)	CI (95%)	($mLCH_4\ gVS^{-1}\ d^{-1}$)	CI (95%)	(d)	CI (95%)	
30	233.5	226.2–241.2	10.0	9.3–11.0	10.2	9.6–10.9	0.96
44	331.1	325.3–336.8	13.6	13.1–14.3	11.1	10.7–11.4	0.99
57	183.6	180.2–187.1	8.8	8.3–9.3	9.1	8.7–9.4	0.99

The statistical analysis of area-specific methane yields (Table 1) showed significant differences between all the treatments ($p < 0.05$). Results indicate the best yield was for 44-day harvesting age (9773 ± 759 $m^3CH_4\ ha^{-1}\ y^{-1}$). Although the specific methane yield obtained is within the ranges reported in the literature for digestion of non-silage grasses, the area-specific methane yields obtained for King Grass were much higher than those obtained for the same crop in subtropical climates. For instance, the study by Schank et al. [26] reported values between 5500–7500 $m^3CH_4\ ha^{-1}\ y^{-1}$.

The high agronomic yield of King Grass resulted also in a high area-specific CH_4 yield. The area-specific methane yields obtained here are higher to those reported for other climate conditions with different types of grass feedstock: 702 $m^3CH_4\ ha^{-1}\ y^{-1}$ in Denmark [27], 3500 $m^3CH_4\ ha^{-1}\ y^{-1}$ in Finland [28], and 4689 $m^3CH_4\ ha^{-1}\ y^{-1}$ in Ireland [29].

3.3. Influence of the Size Reduction Method on Specific Methane Yields

Figure 2 presents the specific methane yield over time for the three used methods of size reduction with a 44-day harvesting age. The statistical analysis evidenced significant differences with $p = 0.000130$

applying the ANOVA. The post hoc Fisher's showed that the methane yields of the hand-chopped sized method was significantly lower than those obtained with machine chopped and minced methods (Table 3). Nevertheless, there were no significant differences between machine chopped and minced.

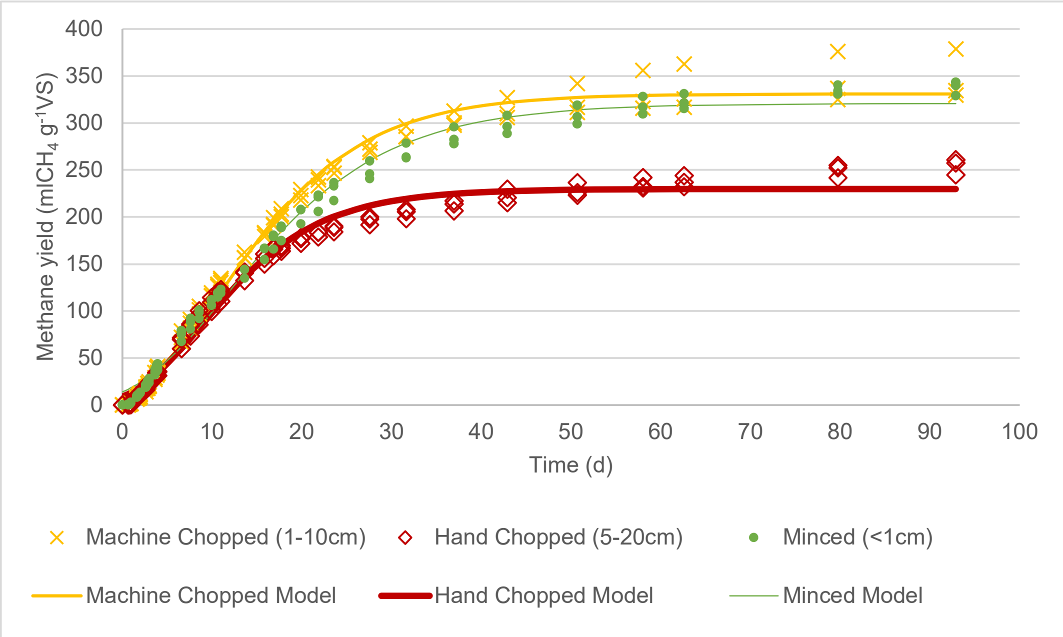

Figure 2. Specific methane yield over time for the three size reduction methods ($n = 3$).

Table 3. Yields of King Grass of 44-day age according to the size reduction method ($n = 3$ where standard deviation is presented) (numbers of a same parameter followed by the same letter are not significantly different ($p = 0.05$)).

Parameter	Size Reduction Method		
	Minced Method	Machine Chopped Method	Hand Chopped Method
Specific Methane Yield (mLCH$_4$ g^{-1}VS)	337.6 ± 7.5 a	347.8 ± 27.0 a	254.6 ± 8.4 b
Area-Specific Methane Yield (m^{3}CH$_4$ ha^{-1} y^{-1})	9486.6 ± 210.8 a	9773.2 ± 758.7 a	7154.3 ± 236.0 b

Additionally, the kinetic behavior (Table 4) analyzed through the Gompertz equation ($r^2 = 0.99$) showed that the machine chopped method presented the highest Rm. Differences in Rm applying different methods of size reduction have been reported by Tsapekos et al. [30]. These results suggest that a further reduction size of grass with a typical industrial mincer for organic wastes does not improve the kinetics of biogas production. This is in accordance with the study of Narinthorna et al. [31], who found no significant differences in methane yields of *Pennisetum* between sizes of 2–3 cm and <0.6 mm.

Table 4. Parameters of the Gompertz equation fits on the BMP tests data according the size reduction method (Pm, Rm and λ), confidence interval (CI with α = 0,05) and evaluation of the coefficient of determination (r^2).

Size Reduction Method	P_{max}		R_m		λ		r^2
	(mLCH$_4$ gVS^{-1})	CI (95%)	(mLCH$_4$ gVS^{-1} d^{-1})	CI (95%)	(d)	CI (95%)	
Minced	320.8	315.5–326.6	11.6	11.1–12.1	11.7	11.3–12.1	0.99
Machine Chopped	331.1	325.3–336.8	13.6	13.1–14.3	11.1	10.7–11.4	0.99
Hand chopped	229.8	225.6–234.3	11.4	10.7–12.0	8.8	8.4–9.1	0.99

Methane yields of 337.6 ± 7.5 and 347.8 ± 27.0 mLCH$_4$ g^{-1}VS, obtained with the minced and machine chopped method, respectively are close to values of 330 mLCH$_4$ g^{-1}VS for particle sizes of

0–1 cm of public space pastures in Japan [32] and values of 373 to 438 $mLCH_4.g^{-1}VS$ obtained from Festulolium grasses with particle sizes of 1–1.5 cm.

4. Conclusions

Under tropical climate conditions, a significant increase in the performance of the anaerobic digestion of King Grass was detected with harvesting ages bellow 60 days, that is the lowest age usually evaluated and reported by literature. An optimal harvesting age of 44 days, with a highest methane yield of 347.8 $mLCH_4\ g^{-1}VS$ and 9773 $m^3CH_4\ ha^{-1}\ y^{-1}$ was found, even when the agronomic yield increase with the harvesting age. The machine-chopped method of size reduction (1−10 cm) was the most efficient pre-treatment, although, it did not have a significant difference to the smallest size tested (<1 cm). Cooking supply energy for a typical family in rural zones of Colombia would require 154 m^2 of King Grass crop, an area that can be easily assumed by this type of housing.

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