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Biochemical Methane Potential Assay as a Tool for Selecting Elephant Grass Genotypes for Anaerobic Co-Digestion with Cattle Manure

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Abstract

The environmental impacts associated with fossil fuel use have intensified the need for more sustainable energy sources. In this context, plant biomass has emerged as a promising alternative for renewable energy generation through anaerobic digestion. This study assessed the biogas and methane production potential of different elephant grass (*Cenchrus purpureus* (Schumach.) Morrone) genotypes, a perennial species widely recognized for its high biomass productivity. A laboratory biochemical methane potential (BMP) assay was conducted using ten elephant grass genotypes. Significant variation was observed, with T_23.1 achieving the highest specific biogas yield (496.9 L $kgVS^{-1}$) and the greatest dry matter productivity under field conditions. This combination resulted in a high estimated energy yield per unit area (17,973.5 m^3 ha^{-1} yr^{-1}), highlighting its potential for large-scale bioenergy production. These findings demonstrate that genotype selection plays a critical role in optimizing biogas production. Elephant grass shows strong potential as a feedstock for renewable energy generation, particularly in anaerobic co-digestion with cattle manure.

1. Introduction

In recent decades, burning of fossil fuels for energy generation has become the main source of anthropogenic greenhouse gas (GHG) emissions, intensifying global warming and climate change. Scientific evidence indicates that these emissions are the primary cause of the increase in average global temperature since the pre-industrial period, requiring urgent mitigation actions. In this context, the transition to alternative low-emission energy sources is considered essential to reduce environmental impacts, meet the targets established by the Paris Agreement, and strengthen global energy security [1, 2].

Brazil has an energy matrix with a high share of renewable sources, which reached approximately 50% of the Domestic Energy Supply (DES) in 2024, a value significantly higher than the global average [2, 3]. In this context, biomass plays a relevant role in the national energy supply, accounting for approximately 60% of the renewable share provided in the country, highlighting its importance for energy security and diversification of the energy matrix [4]. Biological biomass conversion processes, especially anaerobic digestion aiming at biogas production, present high potential to contribute to sustainable energy generation [5–7].

The anaerobic digestion (AD) of organic substrates results in the production of biogas, a renewable fuel composed mainly of methane (CH_4) and carbon dioxide (CO_2), which can be used to generate thermal, mechanical, or electrical energy, or to produce biofuels after purification processes. In parallel, the process generates digestate, a stabilized and nutrient-rich effluent, especially in nitrogen, phosphorus, and potassium, which can be used as a biofertilizer, contributing to nutrient recycling and reducing the use of mineral fertilizers [5, 8–10].

Anaerobic co-digestion (AcoD) is a technique that allows the optimization of biogas and methane production through the combined use of substrates with complementary physicochemical properties. In this context, the selection of appropriate co-substrates is a crucial factor for successful AcoD performance. This practice makes it possible to improve the carbon-to-nitrogen (C/N) balance, achieving values close to the ideal range (20:1–30:1) to ensure high biodigestion efficiency [5, 11]. In addition, AcoD promotes system buffering and stability, as well as the supply of fermentable carbohydrates and essential nutrients for microbial activity [9, 12, 13]. As a result, the production efficiency and quality of biogas generated through AcoD tend to exceed those observed in the mono-digestion of each substrate individually [14–17].

Biogas production via AD can employ different biomass sources, including dedicated energy crops, agricultural residues, and livestock manure. Among these, energy crops stand out due to their high biomass productivity and potential to enhance biogas and methane yields in AcoD with other substrates. Several plant species are used for bioenergy production, including maize, sorghum, sugarcane, and elephant grass [18–20]. Among them, elephant grass (*Cenchrus purpureus* (Schumach.) Morrone) stands out due to its high productivity, reaching approximately 50 t of dry matter (DM) ha^{-1} yr^{-1}, and its wide adaptability to different edaphoclimatic conditions [21]. It is a perennial C_4 grass, characterized by high efficiency in atmospheric CO_2 fixation, rapid growth, and regrowth capacity, allowing multiple harvests throughout the year. These attributes favor the continuous supply of biomass for energy purposes and make elephant grass competitive compared to other crops dedicated to biogas production [21, 22].

The co-digestion of elephant grass with cattle manure is a promising strategy to increase biogas production potential, due to the complementary characteristics of these substrates. Elephant grass mainly acts as a source of structural carbohydrates with fermentable potential, whereas cattle manure contributes nutrients, especially nitrogen, improves system buffering, and also acts as an inoculum by providing a diverse microbial community derived from the rumen, adapted to the degradation of plant fibers [9, 23–26]. This microbiota favors the hydrolysis step and increases organic matter conversion efficiency, reducing system adaptation time and enhancing methane yield. Thus, AcoD stands out as a sustainable alternative for agricultural waste management, converting low value-added materials into energy and agricultural products of interest, contributing to the reduction of environmental impacts associated with improper waste management, as well as improving energy efficiency and promoting the circular economy.

Despite the recognized potential of elephant grass as an energy biomass, its wide genetic diversity implies the existence of genotypes with distinct physical, chemical, and bromatological characteristics [27–29]. Therefore, identifying the most suitable genotypes for biogas and methane production is essential to ensure greater process efficiency and optimization. In this context, the present study aimed to evaluate the use of elephant grass as a substrate for biogas and methane production, considering the use of cattle-derived inoculum, as well as to compare the performance of ten different genotypes and identify those that stand out in terms of renewable energy generation potential.

2. Materials and Methods

2.1. Experimental design

The experiment was conducted at the José Henrique Bruschi Experimental Field (CEJHB), belonging to Embrapa Dairy Cattle, located in the municipality of Coronel Pacheco, Minas Gerais state, Brazil, where elephant grass was cultivated. A randomized complete block design was adopted, with three replications. Each plot consisted of four 6.0 m-long rows (4 × 6 m), spaced 1.0 m apart. Eight elite genotypes from the Embrapa Dairy Cattle elephant grass breeding program, with promising characteristics for bioenergy production (T_12.9, T_HE10, T_23.1, T_47.1, T_25.11, T_41.2, T_44.1, and PCEC), and two reference cultivars used as controls, BRS Coronel and BRS Capiaçu, were evaluated.

2.2. Sample preparation and physicochemical analysis

Elephant grass was harvested 105 days after planting, with cutting performed at approximately 15 cm above the soil surface. The collected samples were weighed on a digital scale to determine fresh matter yield. Subsequently, a representative stalk from each elephant grass treatment was separated and pressed using pliers to extract the broth, and the total soluble solids content (°Bx, expressed as % w/w) was measured using a digital refractometer (model MA 871, Milwaukee, USA).

The samples were then chopped using a forage chopper to obtain particles of approximately 2 cm. Subsamples were pre-dried in a forced-air circulation oven at 55°C for 72 h to remove most of the moisture, following the partial DM (two-step drying) procedure commonly adopted for forages and high-moisture biomasses [30–32]. This pre-drying step reduces moisture, resulting in approximately 85–90% DM content, allowing safe comminution with minimal losses of volatile compounds and structural alterations [32, 33]. After pre-drying, the materials were milled to pass through a 1 mm screen using a Wiley-type mill (model R-TE-650/1, Tecnal, Brazil) and subsequently subjected to physicochemical analysis for biomass characterization at the Food Analysis Laboratory of Embrapa Dairy Cattle. Total DM was then determined on ground subsamples by oven drying at 105°C, according to standard gravimetric procedures [34].

Analyses of total solids (TS), volatile solids (VS), and fixed solids (FS) or ash were performed according to reference methodologies [35–37], as well as the determination of DM content and dry matter yield (DMY) [30, 31]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG) contents were determined using the Van Soest and Wine method [38]. Cellulose (CEL) and hemicellulose (HCEL) contents were calculated as the differences between ADF and LIG and between NDF and ADF, respectively [39]. Crude protein was determined using the Kjeldahl method [40].

2.3. Biochemical methane potential assay

A biochemical methane potential (BMP) assay was carried out following the VDI 4630 guideline [41], which establishes protocols for biogas production tests from organic substrates, in addition to other reference materials [42–44]. Biogas production was monitored throughout the experiment, and methane

content was measured on selected sampling days. This assay primarily emphasized biogas production, expressed as specific biogas yield (SBY), whereas methane fraction was determined only at defined time points to characterize biogas quality. SBY (L $kgVS^{-1}$) was calculated from the cumulative volume of biogas produced by the samples, subtracting the volume generated by the inoculum (blank), and dividing by the mass of VS added as substrate. All biogas volumes were normalized to normal temperature and pressure (NTP; 273 K and 1013 hPa).

The inoculum used in the experiment was prepared from cattle manure diluted with wash water from the cattle housing facilities of the CEJHB dairy system. The material was previously sieved through a 1.6 × 3.4 mm mesh to obtain a homogeneous inoculum with a VS content of approximately 2.5%, suitable for proper BMP testing [41]. Subsequently, it was acclimated for 14 days according to the methodology described by Steinmetz et al. [45]. The presence of microorganisms originating from the bovine rumen in the inoculum provided high efficiency for the degradation of lignocellulosic substrates and was an essential factor for satisfactory test performance [23].

During assay setup, a standard amount of 200 g of inoculum was added to each sample. An inoculum-to-substrate ratio (ISR) of 2:1 was adopted, on a VS basis, as recommended for degradability tests to ensure process stability and to prevent microbial inhibition due to organic overloading [41, 45–47]. Based on this proportion, the masses of substrate to be added to each sample were determined according to the VS mass contained in 200 g of inoculum.

Three experimental runs were performed. Samples of each treatment, prepared in triplicate, consisted of the calculated mass of elephant grass mixed with 200 g of inoculum, whereas blanks contained only inoculum. The incubation period was 21 days, which was necessary to reach process stabilization, defined when daily biogas production was lower than 1% of the cumulative total [41–44]. The pH of the samples was measured at the beginning and at the end of the experiment.

The assay was carried out in batch anaerobic reactors under mesophilic conditions (37°C) and constant mechanical agitation at 100 rpm, using a BMP test set (Nautilus, Anaero Technology, Cambridge) consisting of a digital water bath with capacity for up to 15 bottles. In addition, the bottles were connected by hoses to a water-filled platform equipped with 15 individual box-shaped chambers, which functioned as gas flow measurement cells, as illustrated in Fig. 1. The biogas produced by each sample was transferred to its respective chamber, which rotated upon reaching a volume of approximately 8 mL, according to prior calibration of the system. The accumulated biogas volume was automatically recorded by a computer connected to the equipment, which also enabled real-time monitoring of temperature and pressure.

Gas samples were collected on days 7, 14, and 21 of the assay to determine biogas composition by gas chromatography (7820A GC System, Agilent Technologies, USA) [48]. Biogas production data and biomass characterization results were subjected to analysis of variance (ANOVA), and means were compared using the Scott–Knott test at a 5% significance level.

3. Results and discussion

3.1. Physicochemical characteristics and biomass productivity of elephant grass genotypes

The results of the physicochemical characterization of the biomass of ten elephant grass genotypes are presented in Table 1.

Table 1
Physicochemical characterization and dry matter yield of elephant grass genotypes.

Genotype	DMY ($t\ ha^{-1}\ yr^{-1}$)	DM (%)	Ash (%)	CP (%)	Brix (°Bx)	CEL (%)	HCEL (%)	LIG (%)
T_12.9	32.6 b	22.7 c	6.0	5.7 a	5.0 a	39.2	29.0 a	7.5 b
T_HE10	36.6 a	27.0 a	5.1	4.4 b	5.9 a	41.7	27.2 b	10.5 a
T_23.1	41.1 a	20.7 c	5.4	6.4 a	6.1 a	38.9	26.5 b	7.8 b
T_47.1	38.9 a	23.7 c	6.2	4.4 b	6.2 a	39.7	26.6 b	8.4 b
T_25.11	37.1 a	22.7 c	5.6	5.8 a	6.2 a	40.3	26.5 b	7.6 b
T_41.2	33.8 b	23.0 c	5.2	4.9 b	6.1 a	38.0	26.5 b	7.3 b
T_44.1	30.1 b	23.7 c	7.1	7.1 a	6.5 a	36.6	28.4 a	6.9 b
PCEC	37.6 a	24.7 b	6.3	6.3 a	6.2 a	37.8	31.2 a	6.7 b
BRS Coronel	29.5 b	22.0 c	7.7	6.6 a	6.6 a	39.5	27.2 b	6.9 b
BRS Capiaçu	35.7 a	24.3 b	6.5	4.7 b	6.4 a	39.2	28.6 a	7.6 b

Means followed by the same letter belong to the same group according to the Scott & Knott test at 5% probability. DMY = Dry matter yield; DM = Dry matter; CP = Crude protein; CEL = Cellulose; HCEL = Hemicellulose; LIG = Lignin.

3.1.1. Lignocellulosic composition and cell wall structure

The lignocellulosic composition of elephant grass genotypes was evaluated based on CEL, HCEL and LIG fractions. The mean contents of 39.1%, 27.8%, and 7.7%, respectively (Table 1), were consistent with the results reported by Martins et al. (38.0%, 29.5%, and 6.5%) [49] and by Kullavanijaya and Chavalparit (36.8%, 26.2%, and 8.3%) [50]. Similarly, Beber et al. [51] reported comparable proportions, averaging 36.9% CEL, 30.9% HCEL, and 7.3% LIG for elephant grass cv. BRS Capiaçu harvested at 90 days of growth.

LIG is widely recognized as a factor that limits biomass degradability. Plants with higher LIG contents tend to exhibit more recalcitrant tissues, characterized by structurally more complex cell walls that are less susceptible to enzymatic hydrolysis [52]. In elephant grass, increasing plant age is directly associated with higher DM and LIG contents, contributing to greater tissue rigidity [49]. In the present study, genotype T_HE10 showed the highest DM and LIG contents, differing significantly ($p < 0.05$) from the other genotypes, which may represent a limitation in terms of substrate degradability.

CEL and HCEL constitute the main biodegradable fractions of elephant grass biomass. However, these carbohydrates are closely associated with LIG within the plant cell wall, which can hinder the access of microorganisms and hydrolytic enzymes to fermentable compounds during AD [53]. In addition to the relative proportions of these fractions, CEL structural properties — such as crystallinity and degree of polymerization — are recognized as important factors limiting enzymatic hydrolysis, particularly for long-chain and highly ordered CEL molecules [53–55]. In this context, different types of physical, chemical, and biological pretreatments have been developed to improve biomass digestibility by disrupting the cell wall structure and increasing organic fraction availability, and have shown promising results by enhancing biomethane production potential [17, 52, 54, 56].

3.1.2. Ash and crude protein

The mean ash and crude protein contents observed in this study were 6.1% and 5.6%, respectively (Table 1), values consistent with those reported by Martins et al. [49] for elephant grass harvested at similar maturity stages. Ash content reflects the inorganic fraction of the biomass and is associated with the presence of macro- and micronutrients essential for microbial metabolism in AD systems, contributing to buffering capacity and enzymatic activity. However, excessive mineral concentrations may also exert inhibitory effects depending on their nature and bioavailability. Crude protein content is related to nitrogen availability, which plays a key role in microbial growth and process stability. Martins et al. [49] reported reductions in ash and protein contents with advancing plant maturity, accompanied by lower dry matter and organic matter degradability and reduced biogas production, reinforcing the influence of maturity-related changes in biomass quality.

Nitrogen availability is a critical factor for AD performance, as nitrogen deficiency can limit microbial activity, while excess nitrogen may lead to elevated ammonia concentrations and increased pH, potentially inhibiting methanogenic microorganisms [57, 58]. In this context, the anaerobic co-digestion (AcoD) of nitrogen-rich substrates, such as cattle manure, with carbon-rich substrates, such as elephant grass, promotes a more balanced C/N ratio, enhances buffering capacity, and improves process stability. This synergistic effect contributes to higher process efficiency and increased biogas and methane production, as reported in previous studies [16, 24, 26, 57, 58].

3.1.3. pH and total soluble solids (Brix)

In addition to biomass structural characteristics, the physicochemical properties of the samples used in the BMP assay are relevant for interpreting AD performance. The initial pH of the inoculum-substrate mixture samples averaged 7.14, whereas the final pH reached 7.88, remaining within the optimal range

for microbial activity in AD processes (6.5–8.0). This behavior reinforces the role of cattle manure in providing buffering capacity and maintaining process stability throughout digestion [9, 17, 26, 46]. Total soluble solids contents (5.0–6.6 °Bx) were close to the recommended range (6.0–8.0 °Bx), favoring proper fermentation and minimizing the occurrence of undesirable secondary fermentations [59].

3.1.4. Biomass productivity and cost-related aspects

From a biomass productivity perspective, DMY and DM content of elephant grass genotypes were analyzed. DMY ranged from 29.5 to 41.1 t ha^{-1} yr^{-1}, with genotype T_23.1 standing out (Table 1). These values are consistent with literature reports indicating that elephant grass can reach DMY values of up to 50 t ha^{-1} yr^{-1} [60], confirming its high biomass productivity. These values exceed the DMY of other energy biomasses, such as sorghum (25–40 t ha^{-1} yr^{-1}), as well as eucalyptus and sugarcane bagasse, which typically present yields around 15–20 t ha^{-1} yr^{-1} [61]. Additionally, DM content ranged from 20.7% to 27.0%, values close to those reported in the literature, averaging 25.2% for elephant grass harvested at 112 days of growth [49], and 17.5% and 21.2% for elephant grass harvested at 90 and 120 days of age, respectively [62].

Pereira et al. [60] further reported that elephant grass exhibits fresh biomass productivity approximately three times higher than maize and sorghum, in addition to silage production costs up to 57% lower than maize, 42% lower than sugarcane, and 43% lower than sorghum, highlighting its superiority over other energy biomasses in terms of productive capacity and economic feasibility. The ensiling of plant crops is a widely used technique for biomass preservation, enabling the formation of stable feedstock stocks for biogas production, ensuring continuous availability, and mitigating the effects of seasonality. This high biomass productivity, combined with the feasibility of ensiling as a preservation strategy, supports the suitability of elephant grass as a consistent feedstock for biogas production, reducing the effects of seasonality.

3.2. Biogas yield of elephant grass genotypes

Figure 2 presents the SBY of the evaluated genotypes, as well as the methane (CH_4) concentration results of the samples collected on days 7, 14, and 21 of the experiment. Overall, biogas production from the different elephant grass genotypes showed a stabilization trend from the ninth day of the experiment onward, following daily volume increases during the previous days (Fig. 2a). The genotype T_23.1 maintained a notably higher productive performance during approximately two-thirds of the experimental period, with biogas production stabilizing only in the last five days (Fig. 2a), thus standing out in comparison to the others.

Overall, methane concentrations exhibited an increasing trend over the course of the experiment, reaching higher values at the end of the monitoring period, when the final chromatographic gas analysis was conducted (day 21). This pattern was not observed for genotype BRS Coronel, which exhibited a reduction in methane content on day 14 followed by an increase in the final analysis (day 21), nor for

genotype T_44.1, which presented its lowest methane concentration across the evaluated sampling points at day 21 (Fig. 2b).

The SBY of the analyzed elephant grass genotypes ranged from 139.6 to 496.9 L $kgVS^{-1}$, with genotype T_23.1 showing the best performance, reaching 496.9 L $kgVS^{-1}$ and 65% methane, whereas genotype T_12.9 also achieved 65% methane and the second highest SBY (361.5 L $kgVS^{-1}$). These two genotypes differed statistically ($p < 0.05$) from the others and stood out due to their higher SBY and methane content.

When compared with other energy crops, the results obtained in this study demonstrate the competitive and, in several cases, superior performance of elephant grass. The SBY achieved for genotype T_23.1 (496.9 L $kgVS^{-1}$) was markedly higher than that reported by Dahunsi et al. [63] for untreated sorghum stalks (295 L $kgVS^{-1}$), indicating a greater conversion efficiency of elephant grass biomass under similar AD conditions. In addition, the value obtained in this study was higher than the maximum SBY reported by Zhang et al. [64] for sorghum stem (478 L $kgVS^{-1}$).

Regarding maize-based substrates, Krzystek et al. [65] reported SBY values of maize silage ranging from 195 to 315 L $kgVS^{-1}$, which are considerably lower than those obtained in the present study for genotype T_23.1 (496.9 L $kgVS^{-1}$). Similarly, Li et al. [66] reported 417 L $kgVS^{-1}$ for corn stover, also below the maximum yield observed in this work. On the other hand, higher SBY have been reported for maize under certain conditions, such as 655 L $kgVS^{-1}$ for maize silage and 720 L $kgVS^{-1}$ for maize grains without pretreatment [67], as well as values between 613 and 678 L $kgVS^{-1}$ for maize silage [68]. These results indicate that the SBY obtained for elephant grass, particularly genotype T_23.1, falls within the upper range of values reported for maize-based substrates, exceeding several studies, although some maize materials have been reported to achieve higher maximum yields.

Nevertheless, elephant grass presents a major agronomic advantage over both maize and sorghum due to its exceptionally high biomass productivity per unit area, which is consistently reported to exceed that of these crops. This characteristic substantially enhances the overall biogas yield per hectare, compensating for differences in SBY and positioning elephant grass, particularly genotype T_23.1, as one of the most promising crops for sustainable and large-scale biogas production systems.

At the harvest performed for use in this study, elephant grass DMY averaged 35.3 t ha^{-1} yr^{-1}, with genotype T_23.1 reaching 41.1 t ha^{-1} yr^{-1} (Table 1). Thus, to estimate the real-scale biogas production potential, the area-based biogas yield (BY_{DMY}) was calculated considering the DMY and the VS content of each genotype. The BY_{DMY} results are presented in Table 2.

Table 2
Specific biogas yield of elephant grass genotypes obtained in the laboratory-scale assay (L $kgVS^{-1}$) and estimated biogas yield considering genotype DMY (m^3 ha^{-1} yr^{-1}).

Genotype	SBY (L $kgVS^{-1}$)	BY_{DMY} (m^3 ha^{-1} yr^{-1})
T_12.9	361.5 a	10,312.6 b
T_HE10	278.6 c	9,021.6 b
T_23.1	496.9 a	17,973.5 a
T_47.1	206.6 d	7,036.2 c
T_25.11	139.6 d	4,566.6 d
T_41.2	325.4 b	9,795.0 b
T_44.1	198.3 d	5,178.1 d
PCEC	328.6 b	10,840.3 b
BRS Coronel	285.9 c	7,281.2 c
BRS Capiaçu	243.6 c	7,615.6 c

Means followed by the same letter belong to the same group according to the Scott–Knott test at 5% probability. SBY = specific biogas yield; BY_{DMY} = estimated biogas yield considering dry matter yield; DMY = dry matter yield.

The maximum BY_{DMY} was estimated at 17,973.5 m^3 ha^{-1} yr^{-1} (T_23.1), highlighting the strong bioenergy production capacity of elephant grass. Genotype T_23.1 exhibited the highest performance in terms of both SBY (L $kgVS^{-1}$), obtained in the laboratory-scale assay (Table 2), and DMY (t ha^{-1} yr^{-1}) (Table 1). Consequently, it presented a BY_{DMY} considerably higher than the other genotypes, with a statistically significant difference ($p < 0.05$).

Genotype T_HE10, despite presenting a significantly lower ($p < 0.05$) SBY than T_23.1 and T_12.9, showed high DMY (Table 1) and did not differ significantly in BY_{DMY} from genotypes T_12.9, PCEC, and T_41.2 (Table 2). Genotype PCEC, in turn, although presenting a SBY of 328.6 L $kgVS^{-1}$, lower than T_12.9 (361.5 L $kgVS^{-1}$), achieved a BY_{DMY} of 10,840.3 m^3 ha^{-1} yr^{-1}, higher than that of T_12.9, due to its high DMY.

The physicochemical composition of the biomasses (Table 1) showed significant differences among elephant grass genotypes. However, no direct linear correlation was observed between the analyzed

compositional parameters and SBY (Table 2). Whereas the BMP assay clearly differentiated the energetic performance of the genotypes, this lack of direct correlation suggests that anaerobic digestion performance is influenced by substrate-related characteristics not fully captured by conventional fiber fraction analysis alone.

In lignocellulosic biomasses, factors such as CEL accessibility, crystallinity, degree of polymerization, and lignin-carbohydrate interactions play a crucial role in determining hydrolysis efficiency and biodegradation dynamics [54]. Variations in these structural properties can substantially affect microbial access and enzymatic activity, even among substrates with similar CEL, HCEL, and LIG contents. In addition to CEL, HCEL and LIG, other plant cell constituents — such as proteins, lipids, pectins, minerals, and waxes, all of which are components of lignocellulosic biomass — may also contribute to overall recalcitrance and influence biodegradation dynamics [54].

Although these structural features were not directly assessed in the present study, differences in cell wall organization among elephant grass genotypes may have contributed to the variability in SBY. In particular, restricted CEL accessibility and non-productive enzyme adsorption promoted by LIG may limit hydrolysis rates and reduce effective biogas yields. These results suggest that differences in SBY among elephant grass genotypes are more closely related to variations in cell wall arrangement and substrate accessibility than to conventional fiber composition alone.

The obtained results confirm elephant grass as one of the most promising vegetal species for biogas and methane production, due to the combined effect of high biomass productivity and favorable anaerobic digestibility. Among the evaluated genotypes, T_23.1 consistently exhibited superior performance, presenting higher SBY and DMY values, which resulted in an estimated BY_{DMY} nearly twofold higher than the average of the other genotypes. Overall, the integrated analysis of laboratory-scale biogas yields, and area-based productivity highlights that genotype selection for bioenergy purposes should consider not only biogas and methane potential, but also agronomic performance and biomass quality. In this context, genotype T_23.1 is proposed as a reference material for future AcoD studies and for the development of upscaled biogas production systems.

4. Conclusions

The evaluation of elephant grass as a substrate for biogas production demonstrates its feasibility as a renewable energy resource, particularly when applied in AcoD systems. These findings are consistent with previous studies that identify elephant grass as one of the crops with the greatest bioenergy potentials [17, 69, 70]. Among the analyzed genotypes, T_23.1 stood out, presenting a SBY of 496.9 L $kgVS^{-1}$, the highest methane content (65%) and the greatest DMY (41.1 t ha^{-1} yr^{-1}), resulting in an estimated real-scale biogas yield (BY_{DMY}) of 17,973.5 m^3 ha^{-1} yr^{-1}. Furthermore, AcoD using cattle manure as inoculum proved effective, ensuring adequate process stability and enabling satisfactory biogas production from elephant grass biomass.

Therefore, the present study contributes to the selection of elephant grass genotypes with high productive efficiency and strong potential for bioenergy generation. Further research should evaluate the application of elephant grass in AcoD systems operated under continuous and semi-continuous regimes, including the evaluation of different co-substrate proportions and organic loading rates (OLR), in order to determine optimal conditions that enhance biogas and methane productivity, improve process kinetics and support full-scale implementation. In this context, special attention should be given to high-performing genotypes, including T_23.1. Additionally, elephant grass pretreatment methods should be investigated to identify the most effective strategies for improving substrate utilization by enhancing biomass biodegradability. Finally, economic feasibility studies are required to comprehensively assess process costs and the potential benefits associated with the use of elephant grass for energy generation through AcoD.

Declarations

Conflicts of Interest:

The authors declare no conflicts of interest.

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Author Contribution

Conceptualization, F.F.C.; Methodology, F.F.C., G.H.d.S., E.H.O.D., M.H.O. and J.C.M.; Investigation, F.F.C.; Writing—original draft preparation, F.F.C.; Writing—review and editing, F.F.C., G.H.d.S., E.H.O.D., M.H.O. and J.C.M.; Supervision, G.H.d.S., E.H.O.D., M.H.O. and J.C.M. All authors have read and agreed to the published version of the manuscript.

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Figures

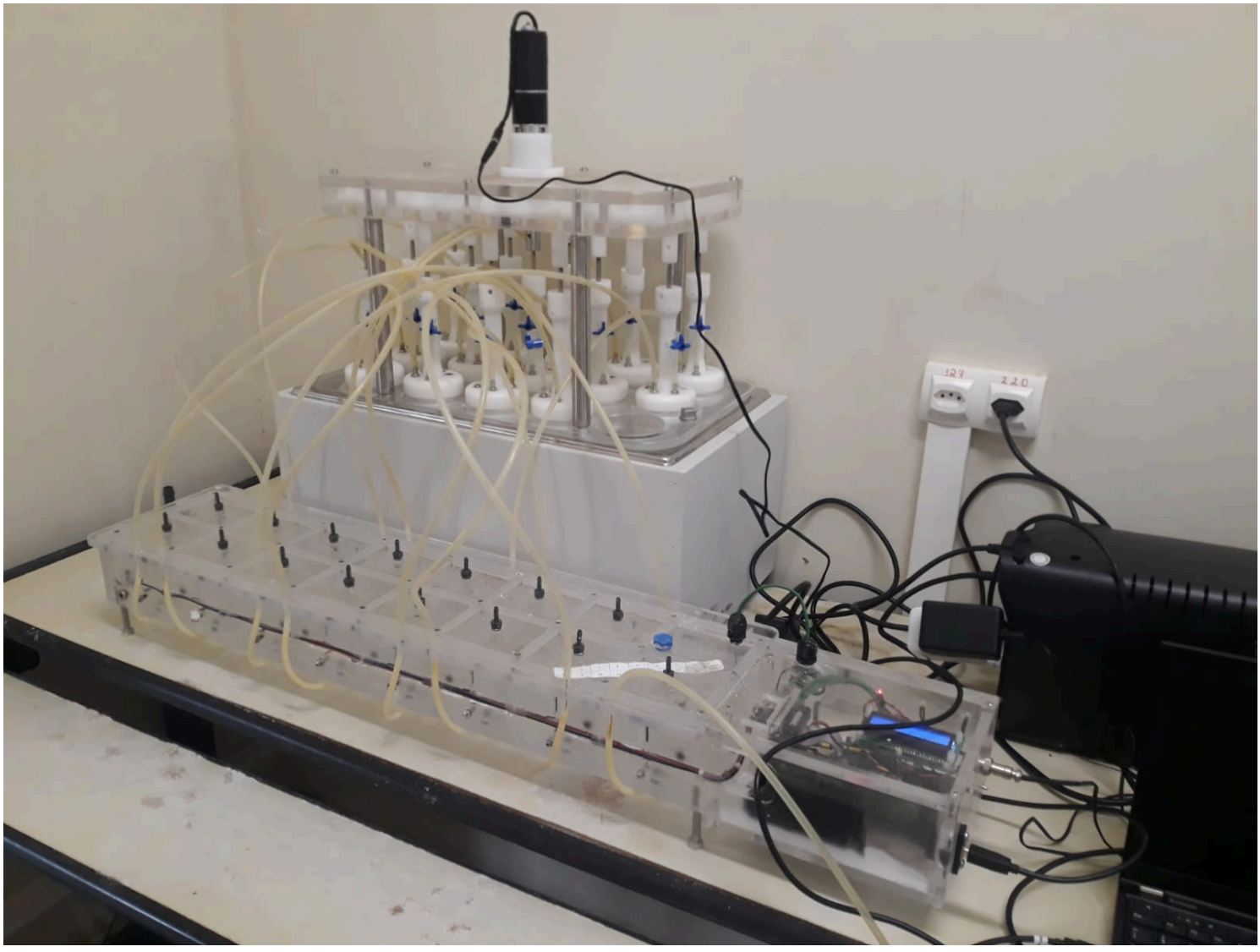


Figure 1

Nautilus equipment used in the biochemical methane potential (BMP) assay.

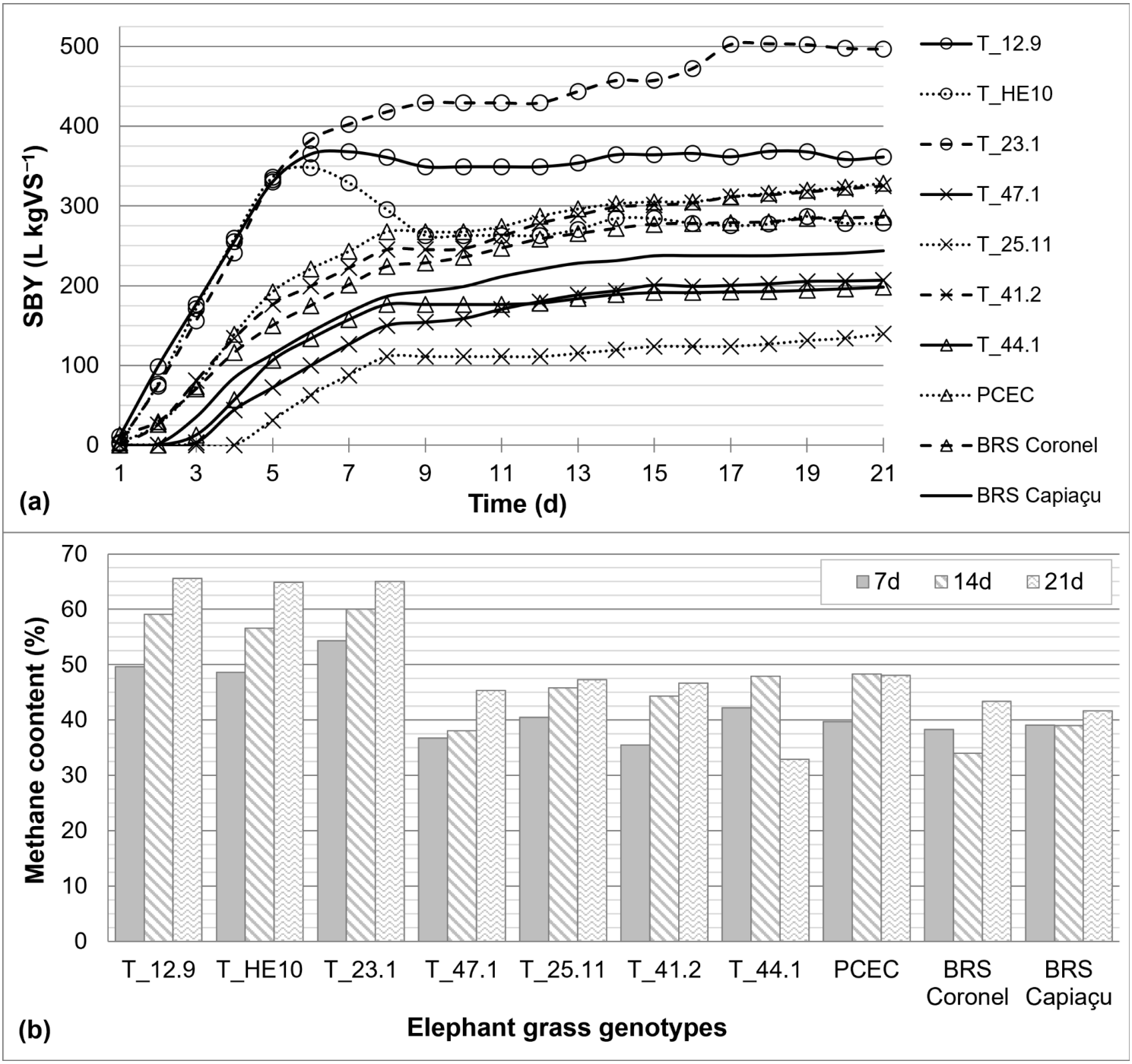


Figure 2

(**a**) Specific biogas yield (SBY) of each elephant grass genotype throughout the experimental period; (**b**) Methane contents (%) at 7, 14, and 21 days of the experiment.