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RESEARCH ARTICLE

Valorization of different landrace and commercial sorghum (*Sorghum bicolor* (L.) Moench) straw varieties by anaerobic digestion

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Abstract

To reduce the impact on the environment and enhance the sustainability of resources, it is necessary to promote and strengthen the use of landrace cultivars that advocate regenerative agriculture. In this study, the growth and development as well as the anaerobic digestion (AD) of six different landrace cultivars, two commercial hybrids cultivars and a public genotype of *Sorghum bicolor* have been evaluated. The landrace cultivars, in general, presented greater heights, biomass yields and compactness shoots as well as similar or an improvement in grain production compare to the commercial varieties. The AD of the different sorghum straws was performed in batch mode at mesophilic temperature (35°C). The landrace cultivar Zahina (ZH) obtained the highest final methane yield (413 ± 79 NL CH_4 kg^{-1} VS, volatile solids) but the landrace cultivars Zahina gigante (ZHG) and Trigomillo (TG) were the ones that obtained the highest methane per biomass production (13.7 and 12.7 NL CH_4 shoot $unit^{-1}$, respectively). By contrast, the commercial varieties were the ones that obtained the lowest methane yields. Two mathematical models, first-order kinetics and the Transference Function model, were used to fit the experimental data with the aim of describing and simulating the anaerobic biodegradation of these *S. bicolor* straw varieties and obtaining the kinetic constants. Both models allowed for adequately fitting the experimental results of methane production with time. In particular, the fastest biomethanization occurred using the commercial variety PR88Y20 (PR88) (specific rate constant $k = 0.148 \pm 0.008\,days^{-1}$), while the slowest one was obtained from Panizo (PAN) variety ($k = 0.064 \pm 0.005\,days^{-1}$). In addition, the highest values of the maximum methane production rate, R_m, were attained for the varieties ZH and PR88, which were 87.1% and 71.3% higher than that achieved for the PAN variety, which exhibited the lowest value.

KEYWORDS
anaerobic digestion, commercial varieties, kinetic parameters, landrace cultivars, regenerative agriculture, *Sorghum bicolor*

1 | INTRODUCTION

Sorghum is among the five most produced cereals in the world, and, although the production of maize, rice and barley are forecast to decline, the stocks of sorghum are expected to increase in the following years (FAO, 2022). In the season 2021–2022, the sorghum world production reached 62.659 million tons of which 1.015 million tonnes were produced in Europe (USDA, 2022).

Sorghum bicolor (L.) Moench is a C4 plant originally from tropical, subtropical and semi-arid areas that has been recently introduced in warm regions of Europe due to new high tolerant varieties capable to adapt to a wide range of environmental and soil conditions produced by breeding techniques (Pasteris et al., 2021; Thomas et al., 2021). This crop is able to grow from clay soils to light sand, within a pH interval of 5.0–8.5, although the most suitable conditions are soils with medium/high nutritional conditions (N ≥ 260, P ≥ 12 and K ≥ 120 kg ha^{-1}), 0.6% of organic matter, 80 cm of depth, 1.4 Mg m^{-3} density and a 50% water-holding capacity (Ahmad Dar et al., 2018). The low nutrient needs of this crop allow to reduce the use of fertilizers and it has been proven to help with soil bioremediation processes enhancing the microbial communities (Carcedo et al., 2021; Pasteris et al., 2022).

Due to the efficiency of nutrient uptake, the high growth rate and the low water needs, this crop has received the attention of the agro-industrial communities specially in those areas where water and other mineral nutrients are difficult to obtain, converting *S. bicolor* into a vital food for millions of people, although, in the global north its mainly used as animal feed (Taylor et al., 2006).

In a present framework of high energy demand against the need to palliate climate change and global warming, biofuels are of great interest as an alternative to fossil fuels. Among the different biomasses studied and used for biofuels production, those with a high lignocellulosic fraction are the most effective options (Wang et al., 2019). *Sorghum bicolor* is a lignocellulosic biomass that contains both soluble and insoluble carbohydrates almost at the same ratio, which is one of the main reasons of why this crop is suitable as a substrate for biofuel production such as bioethanol and biogas, although the yield of these products is highly dependent on the cultivar, the year and the harvest period (Ahmad Dar et al., 2018).

Sorghum bicolor grows fast (around 150 days), presents a high carbohydrate content (>50% d.m.), its grains are widely used as food and feed and the produced straw is suitable for animal feed or for further manipulation into biofuels; therefore, it is one the most efficient crops in terms of a circular economy system, which is why it is considered an *Energy Crop* for several authors (Gómez-Camacho et al., 2021). Among the different technologies related to biofuel production, anaerobic digestion (AD) is presented as a viable and well-known biotechnology for converting organic substrates into biogas. In addition, the subsequent digestate fits the purposes of a circular economy model due to its recognized capacity as a biofertilizer (Bona et al., 2022; Rakascan et al., 2021). Furthermore, unlike other biofuel technologies, AD is feasible both at small scale and large scale, being of great use in developing countries and rural areas (Ahmad Dar et al., 2018).

Thus, sweet sorghum straw and silage have been studied as feedstock for AD processes (Gómez-Camacho et al., 2021; Senghor et al., 2017). Among the bibliography, the reported methane yield of *S. bicolor* straw is ranging from 270 to 400 NL CH_4 kg^{-1} volatile solids (VS), which supports the idea of a feasible process at larger scales (Ahmad Dar et al., 2018). Moreover, in a global warming scenario, where the CO_2 concentration in the atmosphere is expected to keep raising, sorghum is presented as the less affected cereal crop by elevated CO_2 concentrations (Senghor et al., 2017).

However, methane yield from sorghum biomass is highly dependent on the cultivar itself and the cultivation area. Thomas et al. (2019) reported methane yields from 200 to 259 NL CH_4 kg^{-1} total solids (TS) when assessing the biochemical methane potential (BMP) of four *S. bicolor* phenotypes cultivated at different locations and collected at the dough grain stage. While Wannasek et al. (2017) investigated the biomethane potential of the silage of five species of sorghum and reported no significant differences with an average methane yield of 338 NL CH_4 kg^{-1}, however, when the biomass yield of each specie was considered and the methane yield corrected per hectare, the differences were significant with methane yields ranging from 2300 to 6500 m^3 CH_4 ha^{-1}.

This research was carried out to assess the BMP at mesophilic temperature (35°C) of the residual straw after grain collection of 6 landrace cultivars of *S. bicolor* from different areas of the Iberian Peninsula, 2 commercial hybrids cultivars and a public genotype in controlled and optimum conditions. Two mathematical models, the first-order and Transference Function (TF) model, were tested to predict the biomethane potential of the sorghum straw varieties and to determine the kinetic parameters of the different anaerobic processes evaluated using the experimental results of methane production time. To get a better understanding of the feasibility of this technology, the methane yield has also been normalized as per shoot biomass unit in an attempt to include biomass production as a vital variable for preliminary energy sustainability analysis. Other factors such as biomass and grain production have been considered in the final conclusions. The novelty of this study relayed in the assessment of landrace and non-commercial cultivars of *S. bicolor* cultivated under controlled environmental and soil conditions. To the best of our knowledge, the methane potential of these endogenous species has not been evaluated before, furthermore, although other published studies investigated the differences between *S. bicolor* varieties, in most of those cases the environmental and soil conditions were different for each compared cultivar, thus, significant differences in methane production cannot be exclusively linked to the specific *S. bicolor* cultivar itself. Moreover, there is a general need to acquire relative data linking methane yield, biomass and grain production of the assessed crops, that is, in some cases sorghum crops are destined to grain production; thus, the valorization of the straw could be a positive process.

2 | MATERIALS AND METHODS

2.1 | Analytical methods

The different *S. bicolor* (L.) Moench cultivars and the anaerobic sludge were analysed before the start-up of the BMP assays. The subsequent digested effluents that resulted at the end of each assay were also analysed. Standard method 2540B & 2540 E (APHA, 2017) was used as reference for the determination of TS, VS and mineral solids. Total chemical oxygen demand (COD_{total}) was carried out as described by Raposo et al. (2008). Soluble chemical oxygen demand (COD_{sol}) was performed by the closed digestion and colorimetric standard method 5220D (APHA, 2017). pH and total alkalinity (TA) were carried out using a pH meter (Crison 20 basic), and TA was performed by titration to pH 4.3 and following the standard method 2320B (APHA, 2017).

Elemental analysis for the determination of C, N and H content in the different substrates was carried out by a LECO CHNS-932 Elemental Analyzer (Leco Corporation). Prior to the analysis, a representative sample of each *S. bicolor* cultivar was brought to dryness.

Analyses of soluble parameters were performed after sample centrifugation (Eppendorf, 9000×g, 10 min) and filtration (Albet, 47 mm glass fibre filter).

2.2 | Sorghum biomass

2.2.1 | Plant materials and growth conditions

Nine *S. bicolor* varieties were used in this study. PR87G57 (PR87) and PR88Y20 (PR88) are two commercial hybrid cultivars from Pioneer Hi-breed and PR898012 (P89) correspond to a public genotype. Six landrace cultivars from different Iberian regions available in the Plant Genetic Resource Centre (CRF-INIA) belonging to the Spanish Government were selected (Figure 1): Cañadú (CÑ), Milho painzo (MP), Panizo (PAN), Trigomillo (TG), Zahina (ZH) y Zahina gigante (ZHG).

Genotype	Type	Origin
Trigomillo	Landrace	Ávila (Spain)
Cañadú	Landrace	Córdoba (Spain)
Milho Painzo	Landrace	Miranda do Douro (Portugal)
Zahina	Landrace	Sevilla (Spain)
Zahina Gigante	Landrace	Cordoba (Spain)
Panizo	Landrace	Málaga (Spain)

FIGURE 1 Plant material. Geographical origin of landrace cultivars selected along Iberian Peninsula. TG (Trigomillo), CÑ (Cañadú), MP (milho Painzo), ZH (Zahina), ZHG (Zahina Gigante), PAN (Panizo).

Sorghum bicolor plants were grown in a greenhouse (CITIUS, University of Seville, Seville, Spain) with a 14/10 photoperiod at 25°C and 60% of humidity. The experiments were carried out with 16 replicates per treatment and designed as a randomized controlled trial. The seeds were germinated in the soil in 96-cell seed trays (75 ml per cell). After 2 weeks, seedlings were transferred into 5-L pots. The soil was a standard substrate made of black peat enriched with perlite and coconut fibre (Blumenerde), supplied by Gramoflor substrate. The plants were watered three times a week with tap water. The plants were harvested and analysed when the seeds were completely mature, 4–5 months after sowing.

2.2.2 | Growth and productivity parameters

Panicles were dried in an oven at 38°C for a week. Once dried, the seeds were threshed by hand and seed yield and weight were quantified. Shoot height was determined at the moment of harvesting, excluding the panicles in this measure. Shoot tissues were also dried in an oven at 65°C for a week and weighed to quantify shoot dry weight.

Finally, the sorghum straw was ground in a mortar, homogenizing the size of the fragment of between 1 and 2 mm before the AD process.

2.3 | Anaerobic inoculum

BMP assays were set up using inoculum as an anaerobic sludge collected from a full-scale up-flow sludge blanket reactor located in Seville, Spain. The anaerobic sludge was acclimated to brewery wastewaters and prior to use was degassed at 35 ± 2°C for 48 h. To determine the methanogenic activity, microcrystalline cellulose (MCC) (Avicel® PH-101, Fluka) was used as a positive control.

The main physicochemical parameters of the selected inoculum were as follows: TS, 47.3 ± 0.6 g kg^{-1}; VS, 34.4 ± 0.6 g kg^{-1}; pH, 7.14 and TA, 3334 mg $CaCO_3$ L^{-1}.

2.4 | Experimental procedure

The BMP assays were set up following the recommendations described by Holliger et al. (2016). Each assay, including blanks and positive control, was carried out in triplicate. Blanks were prepared following the same indications described below but with no substrate, and, as a positive control, MCC was used.

In total, 250 ml reactors were filled with inoculum, substrate, 20% macro and micronutrient solution (Raposo et al., 2006) and distilled water up to a working volume of 240 ml. The relation between the substrate and the inoculum was stablished at a ratio of 0.5 based on the VS. Temperature was controlled within the mesophilic range (35 ± 1°C) by a water bath and the reactors were flushed with nitrogen gas at the beginning of the experiment so the anaerobic conditions were maintained.

Methane was measured by allowing the biogas to pass through a 2 N NaOH solution. This method assumes that the liquid displacement is due to the produced methane while the generated CO_2 is trapped in the alkaline solution, as is widely accepted and reported in the literature (Casallas-Ojeda et al., 2022; Fernández-Rodríguez et al., 2021).

The experiment was finished when the accumulated volume of methane was less than 1% for three consecutive days, this period was about 31 days. The accumulated methane yield was normalized to standard temperature and pressure conditions (273.15 K and 101.325 kPa) and the endogenous production from the inoculum subtracted.

2.5 | Data analysis

2.5.1 | Biodegradability

The theoretical methane potential of each substrate was calculated as proposed by Nielfa et al. (2015), by both COD_{total} (Equation 1) and elemental composition analysis (Equation 3). Biodegradability was then calculated by comparing the theoretical values with the experimental methane yield.

$$BMP_{thCOD} = \frac{n_{CH_4} RT}{pVS_{added}} \tag{1}$$

where BMP_{thCOD} is the theoretical methane yield (NL_{CH4} kg^{-1} VS_{added}); n_{CH_4} the methane moles determined by Equation (2) (mol); R the ideal gas constant (0.082 atm L mol^{-1} K^{-1}); T the standard temperature (273 K); p the standard pressure (1 atm); VS the volatile solids of the substrate (kg)

$$n_{CH_4} = \frac{COD_{total}}{64} \tag{2}$$

where COD_{total} is the chemical oxygen demand of the substrate (g O_2 kg^{-1} TS); 64 the stoichiometric molecular weight of methane combustion (g mol^{-1}).

$$BMP_{thAtC} = \frac{22.4(n/2 + a/8 - b/4 - 3c/8)}{12n + a + 16b + 14c} \quad (3)$$

where BMP_{thAtC} is the theoretical methane yield (NL_{CH_4} kg^{-1} VS_{added}); *n*, *a*, *b* and *c* are the stoichiometric parameters of C, H, O and N, respectively, based on the atomic composition of the substrates.

2.5.2 | Kinetic models

First-order kinetic model

The kinetics behaviour and the process performance of the AD of the nine sorghum straw varieties studied were analysed by the following first-order kinetic model:

$$G = G_{max} \cdot \left[1 - \exp\left(-k \cdot t\right)\right] \quad (4)$$

where G is the cumulative specific methane production (NL CH_4 kg^{-1} VS_{added}), G_{max} the ultimate methane production (NL CH_4 kg^{-1} VS_{added}), k the specific rate constant ($days^{-1}$) and t the digestion time (days). This kinetic model is normally applied to assess the kinetics of the batch AD processes for different types of biodegradable substrates (Li et al., 2012; Scarcelli et al., 2020). This model assumes that the microbial cell mass does not affect the methane production, which is treated as only-proportional to the amount of substrate (Wang et al., 2017).

Transference function model

In addition, to the first-order model, a second mathematical model, for example, the TF model was assessed as suitable to fit the experimental data of the above-mentioned BMP results (Equation 5):

$$B = B_{max} \times \left(1 - \exp\left[-\left(R_{max}\,(t - \lambda)\right)/B_{max}\right]\right) \quad (5)$$

where B (NL CH_4 kg^{-1} VS_{added}) is the cumulative specific methane production, B_{max} (NL CH_4 kg^{-1} VS_{added}) is the ultimate methane production, R_{max} the maximum methane production rate (NL CH_4 $(kg\ VS_{added} \cdot d)^{-1}$), t (d) is the digestion time and λ (d) is the lag time.

This model assumes that the kinetics parameters of methane production are proportional to the bacterial growth rate inside the batch reactors (Wang et al., 2017), and it has been applied successfully by several authors (Donoso-Bravo et al., 2010; Fernández-Rodríguez et al., 2019; Li et al., 2012).

The goodness-of-fit and the accuracy of the results for both models have been assessed by the determination coefficients (R^2) and the standard errors of estimates (S.E.E.). The kinetic parameters for each experiment and mathematical adjustment were determined numerically from the experimental data obtained by nonlinear regression using the software Sigma-Plot (version 11).

2.5.3 | Statistical analysis

All the analyses and results were at least carried out in triplicates and values are given by means (standard deviation, SD). For single comparisons, a two-tale Student's *t*-test was carried out, while for multiple comparisons, the one-way analysis of variance (ANOVA) and the Duncan's multiple range test was the selected method. For the purposes of data discussion, a $p < 0.05$ value was accepted as statistically significant.

3 | RESULTS

3.1 | Plant biometry and biomass

Both the landrace cultivars and the public genotype cultivar were significantly taller plants than the commercial hybrid cultivars (Figure 2a). The highest cultivars were TG, PAN, MP and ZÑ (275 ± 6; 242 ± 9; 205 ± 3 and 202 ± 3 cm, respectively). Instead, the commercial hybrid cultivars presented a significantly lower height (PR87: 60 ± 3 cm and PR88: 46 ± 4 cm). The public genotype (P89) presented a height of 146 ± 5 cm. No significant differences were found between the heights obtained for the cultivars ZHG (156 ± 8 cm), P89 (146 ± 5 cm) and ZH (164 ± 4 cm; Figure 2a).

Regarding the biomass obtained (Figure 2b), the cultivars with a dry weight shoot significantly higher than the other ones studied were ZHG (53 ± 3 g) and TG (40 ± 2 g). The commercial hybrid cultivars reached a significantly lower biomass than all the other cultivars studied in this work (PR87, 15 ± 1 g; PR88, 9.5 ± 0.8 g). No significant differences were found between the public genotypes P89 and the landrace cultivars MP and ZH (23 ± 2; 23 ± 1 and 26 ± 1 g, respectively) but these data were significantly lower than those obtained for CÑ, PAN and ZH (28.8 ± 0.9; 30 ± 2 and 26 ± 1 g, respectively) (Figure 2b).

The cultivar that presented the highest compactness was ZHG (0.36 ± 0.04 g cm^{-1}), showing very tall stems and a high biomass (Figure 2c). No significant differences were found between the compactness of the 6 landrace cultivars studied (Figure 2c). ZH (0.159 ± 0.005 g cm^{-1}) did not present statically significant differences either with the public phenotype cultivar (P89, 0.157 ± 0.008 g cm^{-1}) or with the commercial hybrid cultivar PR88 (0.21 ± 0.01 g cm^{-1}). However, ZH did present significant compactness differences with the

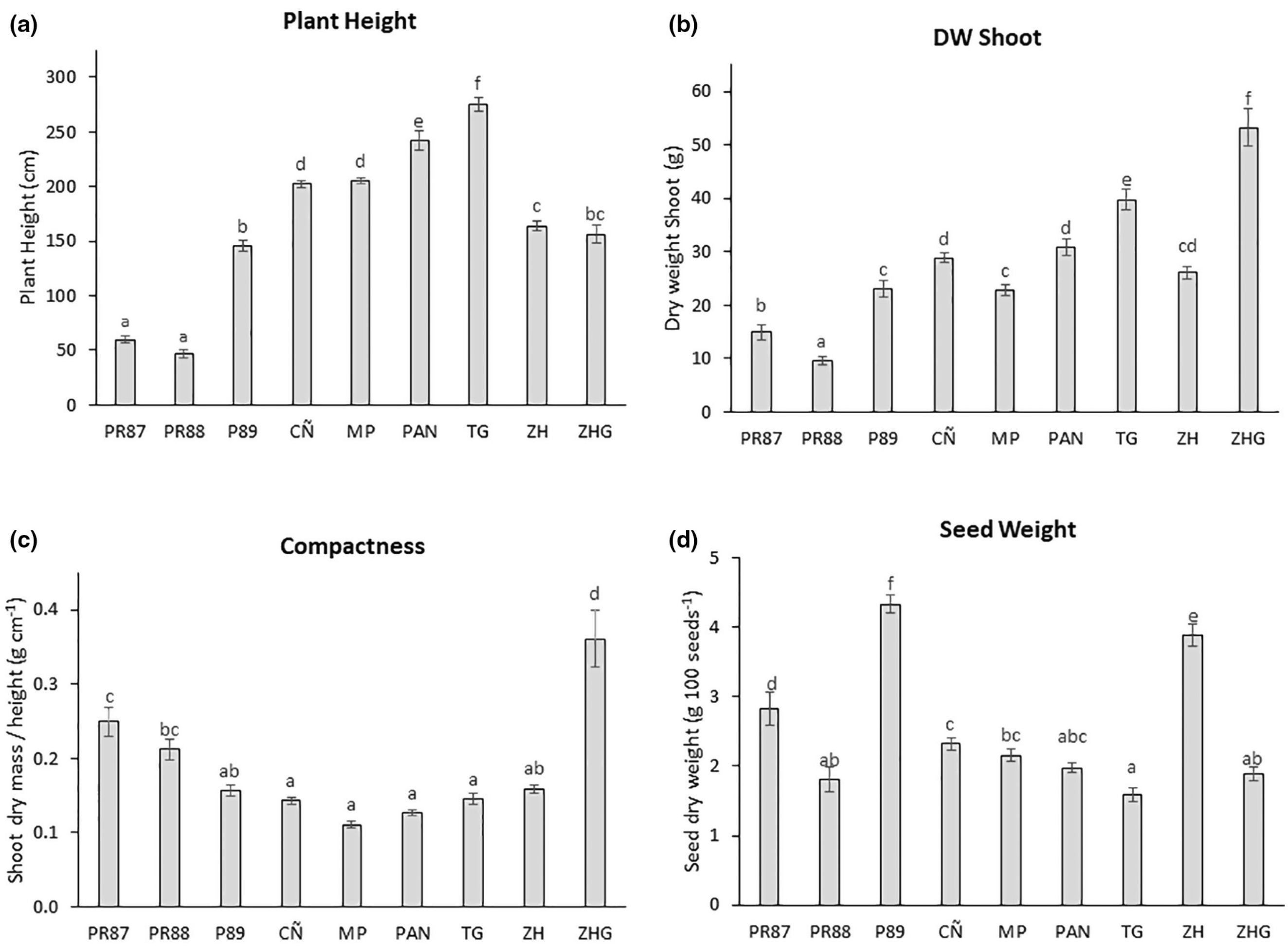

FIGURE 2 Plant height (a), dry weight shoot (b), compactness (c) and seed weight (d) of the two commercial hybrid cultivars (PR87 and PR88), the public genotype (P89) and the six landrace cultivars (CÑ, MP, PAN, TG, ZH and ZHG) of *Sorghum bicolor*. Values represent means ± standard deviation. Different letters show significant differences according to Ducan's multiple range test, $p<0.05$.

commercial hybrid cultivar PR87 ($0.25\pm0.02\,g\,cm^{-1}$). The PR87 commercial hybrid cultivar presented a greater compactness than that presented by ZH landrace cultivar (Figure 2c).

The variety with the highest seed weight was the public phenotype (Figure 2d: p89, 4.36 ± 0.1 g 100 seed $^{-1}$), followed by the landrace cultivar ZH (3.9 ± 0.1 g 100 seed $^{-1}$) and for the commercial hybrid PR87 (2.8 ± 0.2 g 100 seed $^{-1}$). Very similar seed weights were obtained from the other varieties (Figure 2d).

3.2 | Use of *Sorghum bicolor* (L.) Moench for biogas recovery

3.2.1 | *Sorghum bicolor* (L.) Moench cultivars' main characteristics

The main characteristics of the nine different varieties of *S. bicolor* studied are presented in Table 1.

The VS content of all varieties was higher than $809\pm11\,g\,kg^{-1}$. Highlighting the volatile solids content of CÑ ($862\pm5\,g\,kg^{-1}$), there being no significant differences between the VS content of the landrance cultivars PAN and TG (859 ± 6 and $852\pm5\,g\,kg^{-1}$), respectively.

The *S. bicolor* straw of the nine different varieties studied presented a high VS/TS ratio (>88%) reaching values of 94% for CÑ variety and 92% for PAN, ZHG and TG varieties. The COD_{total} values obtained for the different *S. bicolor* straws studied were higher than $980\pm21\,g\,O_2\,kg^{-1}$ (Table 1).

The nine varieties studied allowed to establish three groups in relation to their C/N contents. The first group includes the cultivars with the lowest C/N ratio (PR87, 77 ± 14; PR88, 53 ± 4) the public genotype (P89) and the landrace cultivar MP which presented C/N ratio values of 61 ± 2 and 72 ± 1, respectively. Another group would be for the TG, CÑ and ZH cultivars with C/N ratio values of 107 ± 7, 150 ± 80 and 281 ± 78, respectively. Finally, ZHG and PAN cultivar showed C/N ratios higher than 300. In

TABLE 1 Main characteristics of the nine *Sorghum bicolor* straw varieties tested

Parameters	PR87	PR88	P89	CÑ	MP	PAN	TG	ZH	ZHG
ST ($g\,kg^{-1}$)	922 ± 4	917 ± 5	924 ± 2	917 ± 4	916 ± 1	934 ± 3	927 ± 2	916 ± 5	918 ± 4
SV ($g\,kg^{-1}$)	838 ± 5	809 ± 11	817 ± 2	862 ± 5	832 ± 4	859 ± 6	852 ± 5	829 ± 8	841 ± 5
SV/ST	0.91	0.88	0.88	0.94	0.91	0.92	0.92	0.90	0.92
COD_{total} ($g\,O_2\,kg^{-1}$)	1026 ± 42	1017 ± 65	980 ± 21	981 ± 32	1062 ± 32	1092 ± 24	1079 ± 27	1018 ± 26	1702 ± 124
C/N	77 ± 14	53 ± 4	61 ± 2	150 ± 80	72 ± 1	>500	107 ± 7	281 ± 78	304 ± 95

Note: The two commercial hybrids cultivars (PR87 and PR88), the public genotype (P89) and the six landrace cultivars (CÑ, MP, PAN, TG, ZH and ZHG) of *Sorghum bicolor*. Values represent the mean ± standard deviation.

previous studies, differences in the C/N ratio content have been observed, being highly dependent on the degree of drought of the biomass under study. Drought generally causes an imbalance between carbon and nitrogen, which is reflected in an increase in the carbon:nitrogen (C:N) ratio in mature leaves (Chen et al., 2015).

3.2.2 | AD and biodegradability

The final methane yield for the nine varieties studied after 31 days of testing ranged between 413 ± 79 NL CH_4 kg^{-1} VS and 287 ± 5 NL CH_4 kg^{-1} VS (Figure 3). The landrace cultivar ZH, TG and CÑ obtained the highest final methane yield (413 ± 79, 399 ± 18 and 387 ± 34 NL CH_4 kg^{-1} VS, respectively). Instead, the lowest biogas productions were obtained with the commercial hybrid cultivar PR88, PR87 and with the public genotype P89 (287 ± 5, 316 ± 35 and 318 ± 22 NL CH_4 kg^{-1} VS, respectively).

Total COD-based biodegradability (Equation 2) ranged between 60% and 90% for genotypes PAN and ZH, respectively. Regarding the elemental analysis based on biodegradability (Equation 3), it ranged between 63% and 91% for landrace cultivars PAN and ZH, respectively.

3.3 | Estimation of the model parameters by kinetic modelling

3.3.1 | First-order kinetic model

Table 3 shows the kinetic parameters obtained when the first-order model was applied to the methane production experimental results. The low values of the SEEs and the high R^2 values confirmed the suitability of the model to describe the experimental results.

The highest specific rate constant obtained in the present research was found for the commercial hybrid cultivar PR88 (0.148 ± 0.008 $days^{-1}$). This value was 32.1% and 49.4% higher than that obtained for the landrace cultivars ZH and ZHG respectively, which presented the highest values among the different traditional varieties tested in the present research (0.112 ± 0.006 and 0.099 ± 0.004 $days^{-1}$, respectively).

On the other hand, the landrace cultivars PAN (0.064 ± 0.005 $days^{-1}$), CÑ (0.084 ± 0.004 $days^{-1}$) and TG (0.067 ± 0.003 $days^{-1}$) showed the lowest k values although these last two cultivars showed high values of the ultimate methane yield, G_{max} (409 ± 9 and 435 ± 12 NL CH_4 kg^{-1} VS, respectively).

3.3.2 | TF model

Table 4 summarizes the parameters obtained for the nine sorghum straw varieties tested in this study when the TF model was applied. The low S.E.E.s and the high R^2 confirmed the acceptable fit of the experimental results of methane production-time to the suggested model.

As can be seen in Table 4, the highest R_{max} values were found for the landrace cultivar ZH (45 ± 2 NL CH_4 (kg VS·d)$^{-1}$) and the commercial hybrid cultivar PR88 (Pioneer Hi-Breed) (41 ± 2 NL CH_4 (kg VS·d)$^{-1}$) as also occurred with the k constant from the first-order kinetic model. These R_{max} values were 87.1% and 71.3% higher, respectively, than the lowest value, which was found for the landrace cultivar PAN (24 (1) NL CH_4 (kg VS·d)$^{-1}$). It is also relevant to stand out that the *S. bicolor* landrace cultivar ZH simultaneously also showed high R_{max} and ultimate methane yield, B_{max} (402 ± 9 NL CH_4 kg^{-1} VS) values, which revealed that this landrace cultivar generates one of the highest methane yields with one of the highest rates.

3.4 | Methane production, biomass yield and methane yield per shoot

Table 2 comparatively shows the values of methane yield, biomass straw yield and methane yield per shoot.

Comparing the biogas production and the total biomass of the plants obtained (Table 2), the landrace

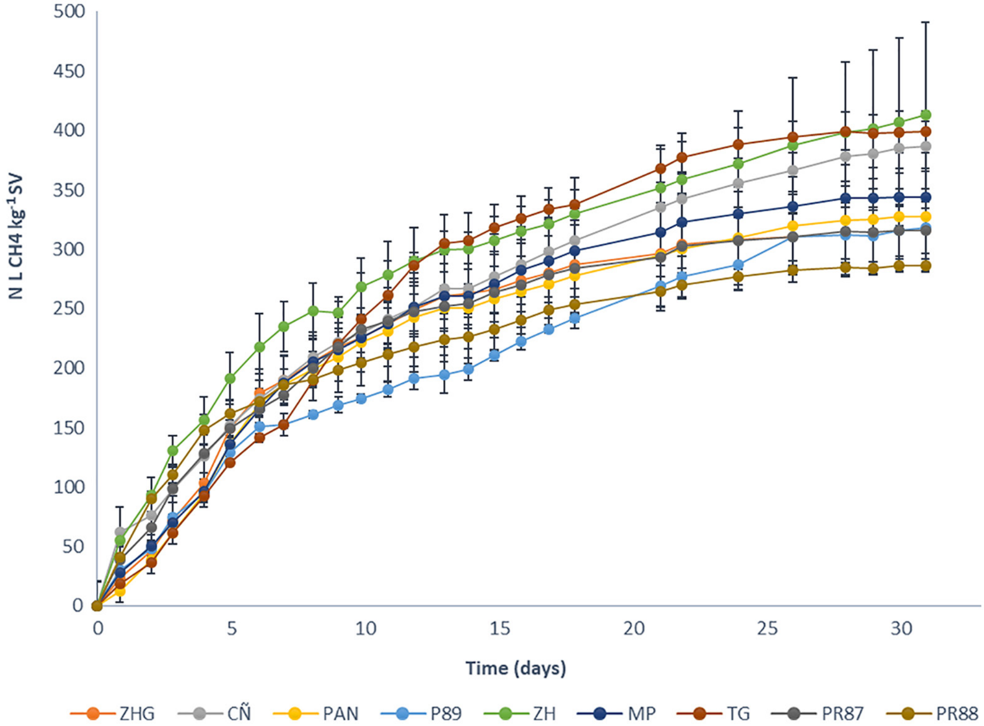

FIGURE 3 Variation in methane production versus time for the different substrates tested. The two commercial hybrids cultivars (PR87 and PR88), the public genotype (P89) and the six landrace cultivars (CÑ, MP, PAN, TG, ZH and ZHG) of *Sorghum bicolor*. Values represent means ± standard deviation.

TABLE 2 Biodegradability, methane yield, biomass straw and methane yield per shoot of the nine *Sorghum bicolor* straw varieties tested

	PR87	PR88	P89	CÑ	MP	PAN	TG	ZH	ZHG
Biodegradability COD_{total} (%)	66	64	74	80	75	60	80	90	74
Biodegradability C/N (%)	84	67	78	89	83	63	90	91	73
Methane yield (NL CH_4 kg^{-1} SV)	316 ± 35	287 ± 5	318 ± 22	387 ± 34	344 ± 22	327 ± 42	399 ± 18	413 ± 41	315 ± 14
Biomass yield (g SV shoot $unit^{-1}$)	12.5	7.7	18.8	24.8	18.9	26.4	33.8	21.6	44.8
Methane yield per shoot (NL CH_4 shoot $unit^{-1}$)	3.8	2.2	6.0	9.6	6.5	7.3	12.7	8.9	13.7

Note: The two commercial hybrids cultivars (PR87 and PR88), the public genotype (P89) and the six landrace cultivars (CÑ, MP, PAN, TG, ZH and ZHG) of *Sorghum bicolor*. Values represent the mean ± standard deviation.

cultivars ZHG and TG were the ones that obtained the highest methane per biomass production (13.7 and 12.7 NL CH_4 shoot $unit^{-1}$, respectively). The landrace cultivars CÑ and ZH obtained values of 9.6 and 8.9 NL CH_4 shoot $unit^{-1}$. By contrast, the commercial varieties again were the ones that obtained the lowest methane per biomass production (PR87, 3.8 NL CH_4 shoot $unit^{-1}$ and PR88, 2.2 NL CH_4 shoot $unit^{-1}$).

4 | DISCUSSION

Sorghum bicolor is known to be a low-demanding crop and very well adapted to marginal environments. *Sorghum bicolor* is worldwide grown for grain as well as for livestock production. When sorghum crop is used for forage production, the main drawback is that must to be harvested and processed quickly to avoid nutritional losses (Tabacco et al., 2011).

Exploring the genetic diversity of sorghum germplasm, it has been selected six landrace cultivars based on their longitudinal distribution along the Iberian Peninsula, focusing mainly in cultivars from Andalusian, in the south of Spain (Figure 1). To characterize the six landrace cultivars of *S. bicolor* (CÑ, MP, PAN, TG, ZH, ZHG) and compare with the two commercial hybrid cultivars (PR87 and PR88) and the public one (P89), the height and biomass of each cultivar were studied, as well as the weight of the grains they produced. The height values of the cultivar obtained (Figure 2a) agree with previous values reported by Mekasha et al. (2022) and are intermediate height values between those published for tall (<323 cm) and short type *S. bicolor* varieties (<182 cm) (Yosef et al., 2009). The previous studies have reported the possibility of increasing plant height by adding extra water to plants grown under drought conditions (Yosef et al., 2009).

Regarding the biomass obtained (Figure 2b), the cultivars with a dry weight shoot significantly higher than the other ones studied were ZHG and TG. The commercial hybrid cultivars reached a significantly lower biomass than all the other cultivars studied in this work. No significant differences were found between the public genotypes P89 and the landrace cultivars MP and ZH, respectively, but these data were significantly lower than those obtained for CÑ, PAN and ZH (Figure 2b).

The compactness is a useful parameter to evaluate the crop growth and development. ZHG was the cultivar that presented the highest compactness (Figure 2c). The variety with the highest seed weight was the public phenotype (Figure 2d) followed by the landrace cultivar ZH and for the commercial hybrid PR87. Since the 1960s, and later with the green revolution, the use of local varieties has been decreasing, favouring the use of commercial ones. Commercial hybrid cultivars are usually smaller size plants and good seed production, but landrace cultivars are usually better adapted to the climatic conditions of the area (Di Miceli et al., 2022). The recovery of local varieties would not only entail a wealth of genetic variety with greater ease of adaptation to both biotic and abiotic factors, but it would also provide more productive and high-quality varieties (Di Miceli et al., 2022).

In 2018, the European Parliament directive on waste (Directive (EU) 2018/851) was approved and the Spanish transposition was approved last April 2022. This law prohibits the stubble burning backing the recovery of the straw stubble as a fundamental basis. The methane production from the *S. bicolor* straw would not only avoid the process of burning it, but would also provide added value to the sowing of this cereal (Rakascan et al., 2021).

All VS/TS ratio values obtained for the biomass of *S. bicolor* straw were higher than 88% (Table 1), indicating the suitability as substrates for AD (Wang et al., 2016). COD_{total} values confirmed the high organic matter content of the studied substrates. The most important characteristic for biogas production by AD is the organic fraction of the feed since it is the degradation of short-chain organic compounds the responsible to drive the evolution of the anaerobic process (Wang et al., 2016).

The C/N ratio in all the *S. bicolor* straws studied was higher than the values established as suitable for the anaerobic process (20–30), highlighting the lignocellulosic content of the substrates (Table 1). The lignocellulosic biomass contains a high content of cellulose and hemicellulose which are ideal substrates for AD, but the low substrate nitrogen content and the complex lignocellulosic biomass framework can inhibit the anaerobic process (Paul & Dutta, 2018).

In this work, the feasibility of using the straw of different varieties of *S. bicolor* as the substrate for AD to obtain energy was studied. MCC used as a positive control achieved a final methane yield of 379 ± 16 NL CH_4 kg $^{-1}$ VS within the range required for validation. The obtained cellulose methane yield represented a 92% of the theoretical yield for this substrate and thus the high methanogenic activity of the sludge was confirmed (Holliger et al., 2016).

Despite the high C/N values observed in the different varieties of *S. bicolor*, the methanogenesis stage did not show any sign of inhibition (Figure 3).

During AD batch assay, all *S. bicolor* varieties tested showed a slight delay in methane production, which lasted approximately 1 day, due to the lack of readily biodegradable material and the difficulty of hydrolytic bacteria in degrading the lignocellulosic material (Fernández-Rodríguez et al., 2022). Then, from day 1 an almost exponential methane production growth was observed, which lasted about 15 days. Finally, during the last 5 days, the daily methane production dropped achieving values of almost zero (Figure 3).

Głąb et al. (2019) obtained methane production values comparable with those reported in the present work. No differences in final methane yield using different fertilizers were found, instead the final methane yield was affected by the cellulose, hemicellulose and lignin concentration (Głąb et al., 2019). Values of final methane yield between 232 and 427 $NmLCH_4.gVS^{-1}$ were reported for 57 genotypes of different varieties of *S. bicolor* (Thomas et al., 2021). In this study, the greatest differences in methane production were related to stem structure. They showed that both biochemical and histological factors of the *S. bicolor* stem had a decisive influence on the final methane yield (Thomas et al., 2021). Another study about the AD of *S. bicolor*, carried out with ensiled one, reported values between 231.25 and 321.31 NL CH_4 kg^{-1} VS (Pasteris et al., 2021). Pasteris et al. (2021) observed a great variability in the final methane yield depending on the *S. bicolor* cultivar and informed about the need of studying each landrace *S. bicolor* cultivars biomass to produce methane. Anaerobic biodegradability was determined based on the COD_{total} concentration and on the elemental analysis (Nielfa et al., 2015) obtaining in both cases very similar values (Table 2).

The highest specific rate constant obtained by the first-order kinetic model in the present research was found for the commercial hybrid cultivar PR88 (Table 3). It is worth

TABLE 3 Values of the kinetic constant obtained from the first-order model for the nine *Sorghum bicolor* straw varieties tested

Substrate	G_{max} (mL CH_4 g $^{-1}$ SV)	k (día^{-1})	R^2	S.E.E.	Error (%)
PR87	302 ± 3	0.110 ± 0.003	0.9977	6.04	0.2
PR88	278 ± 5	0.148 ± 0.008	0.9883	11.82	2.7
P89	354 ± 13	0.069 ± 0.005	0.9907	12.68	11.0
CÑ	409 ± 9	0.084 ± 0.004	0.9947	11.42	5.6
MP	367 ± 4	0.094 ± 0.002	0.9980	6.87	6.6
PAN	328 ± 13	0.064 ± 0.005	0.9918	11.86	18.0
TG	435 ± 13	0.067 ± 0.003	0.9952	12.11	16.0
ZH	402 ± 8	0.112 ± 0.006	0.9910	15.07	2.4
ZHG	326 ± 6	0.099 ± 0.004	0.9954	9.50	6.5

Note: The two commercial hybrids cultivars (PR87 and PR88), the public genotype (P89) and the six landrace cultivars (CÑ, MP, PAN, TG, ZH and ZHG) of *Sorghum bicolor*. Values represent the mean ± standard deviation.Abbreviations: G_{max}, ultimate methane production (ml CH_4 g^{-1} VS); k, specific rate constant or apparent kinetic constant (days^{-1}); R^2, determination coefficient; Error (%): difference (%) between the experimental and calculated ultimate methane production; S.E.E., standard error of estimate.

to highlight that the *S. bicolor* landrace cultivar ZH simultaneously showed high k (0.112 ± 0.006 days^{-1}) and G_{max} (402 ± 2 NL CH_4 g^{-1} VS) values, which revealed that this *S. bicolor* landrace cultivar generates one of the highest methane yields with one of the highest rates. In addition, these results were statistically higher ($p < 0.05$) than those attained for the other varieties. In the same way, similar specific rate constant values (0.10–0.12 days^{-1}) were found by Sambusiti et al. (2013) in the BMP tests of five varieties of sorghum collected from the south of France. This study revealed that the decrease in the lignin fraction and thereafter the solubilization of cellulose and hemicelluloses enhanced the hydrolysis stage during the AD of these varieties. In fact, the first-order constant was augmented by 40%, 61%, 64%, 54% and 40% for the varieties named biomass sorghum 133, sweet sorghum hybrid BMR sisco, forage sorghum Trudent Headless, sweet sorghum 405 and sweet sorghum 506, respectively, when these forages were subjected to an alkaline pretreatment (10 g NaOH 100 g^{-1} TS). However, alkaline treatment did not enhance the methane production obtaining values in the range 270 ± 13–303 ± 24 NL CH_4 kg^{-1} VS (Sambusiti et al., 2013). In the same way, Thomas et al. (2022) recently reported that alkaline pretreatments of sorghum biomass with NaOH and CaO (at 55°C) caused an increase in the k constant from 0.097 ± 0.010 to 0.254 ± 0.013 and 0.205 ± 0.005 days^{-1}, respectively. As can be seen, the values obtained for Thomas et al. (2022) for the kinetic constant for AD of untreated sorghum biomass (0.097 days^{-1}) were virtually identical to those achieved for the sorghum varieties ZHG (0.099 days^{-1}) and MP (0.094 days^{-1}) in the present research work. This increase in the values of the kinetic constant after alkaline pretreatments was attributed to a significant breakdown of the lignin fraction of all tissues of this sorghum biomass, followed by a large unmasking of cellulose and a reduction of crystalline cellulose (Thomas et al., 2022).

On the other hand, the landrace cultivars PAN, CÑ and TG showed the lowest k values although these last two cultivars showed high values of the ultimate methane yield, G_{max} (Table 3).

Low values of the first-order kinetic constant (0.055 ± 0.004 days^{-1}) were also found by Di Girolamo et al. (2014) in the AD of untreated fibre sorghum (*S. bicolor* (L.) Moench, Biomass 133 hybrid, Casalmorano, CR, Italy). These batch AD assays were carried out for 58 days at mesophilic temperature (35°C) (Di Girolamo et al., 2014). However, when a mild alkaline pretreatment was applied (NaOH 0.15 N at 25°C for 24 h), the AD kinetics increased and the digestion time needed to achieve 80% of the methane yield was significantly reduced, indicating the suitability of the process to be scaled-up (Di Girolamo et al., 2014). Similarly, low values of the first-order kinetic constant (0.049 days^{-1}) were also reported by Sambusiti et al. (2014) when the ensiled *Sorghum sudanense* hybrid forage (Cremona, Lombardi region, Italy) was subjected to batch AD using as anaerobic inoculum the digested sludge from cattle manure and corn silage. However, when the inoculum proceeded from an up-flow anaerobic sludge blank reactor treating wastewater from a chemical industry the kinetic constant increased up to a value of 0.093 days^{-1} (Sambusiti et al., 2014). In addition, the methane yields obtained in this study were statistically equals (258 ± 14 NL CH_4 kg^{-1} VS), and, besides, also lower than those obtained in the present research.

The lag phases obtained by the Modified Gompertz Kinetic Model were found to be almost zero in all cases, indicating that the available easy-to-biodegrade components were quickly consumed in all the AD assays investigated (Li et al., 2012). These low λ values are also in accordance

TABLE 4 Values of the parameters obtained from the transference function model for the different *Sorghum bicolor* straw varieties studied

Sustrato	B_{max} (mL CH_4 g $^{-1}$SV)	R_{max} (mLCH$_4$ g^{-1}SV·d^{-1})	λ (d)	R^2	S.E.E.	Error (%)
PR87	302 ± 3	34 ± 1	0.04 ± 0,01	0.9977	6.15	0.06
PR88	278 ± 5	41 ± 2	4.6×10^{-9}	0.9883	12.06	2.4
P89	354 ± 15	25 ± 1	6.9×10^{-9}	0.9907	12.94	10.6
CÑ	409 ± 10	34 ± 1	6.8×10^{-9}	0.9947	11.6	5.6
MP	364 ± 4	36 ± 1	0.21 ± 0.10	0.9982	6.53	5.5
TG	416 ± 8	32 ± 1	0.71 ± 0.14	0.9973	9.27	10.6
PAN	308 ± 9	24 ± 1	0.92 ± 0.18	0.9953	9.17	10.1
TG	416 ± 9	32 ± 1	0.71 ± 0.14	0.9973	9.27	10.6
ZH	402 ± 9	45 ± 2	5.5×10^{-9}	0.9910	15.38	2.4
ZHG	319 ± 4	35 ± 1	0.52 ± 0.12	0.9972	7.55	4.5

Note: The two commercial hybrids cultivars (PR87 and PR88), the public genotype (P89) and the six landrace cultivars (CÑ, MP, PAN, TG, ZH and ZHG) of *Sorghum bicolor*. Values represent the mean ± standard deviation.

Abbreviations: B_{max} is the ultimate methane production; R_{max} the maximum methane production rate; and ʎ the lag time; S.E.E., standard error of estimate; R^2, determination coefficient. Error (%): difference (%) between the experimental and calculated ultimate methane production.

with the hypothesis of a rapid microbial acclimatization to the substrate conditions and, therefore, with an efficient methanation of the organic compounds present in the evaluated biomasses (Zhang et al., 2022).

As can be seen in Table 4, the highest R_{max} values were found for the landrace cultivar ZH and the commercial hybrid cultivar PR88 as also occurred with the *k* constant from the first-order kinetic model. It is also relevant to stand out that the *S. bicolor* landrace cultivar ZH simultaneously also showed high R_{max} and ultimate methane yield, B_{max}, which revealed that this landrace cultivar generates one of the highest methane yields with one of the highest rates. On the contrary, a very low R_{max} value was found in the AD of sorghum vinegar residues (7.8 NL CH_4 (kg VS·d)$^{-1}$; Zhang et al., 2022) being this value significantly lower than those obtained in this actual study (Table 4). However, when the sorghum residue is co-digested with cattle manure a slight increase in the R_{max} up to 8.7 NL CH_4 (kg VS·d)$^{-1}$ was observed. This small increase when the sorghum residue is co-digested with the mentioned waste can be attributed to the synergy between cattle manure and vinegar residue composition and their low salt contents (i.e., NH_4^+, Na^+ and K^+) (Zhang et al., 2022).

A recent study assessed the biochar addition in batch AD tests of sweet sorghum under high loading conditions (Ma et al., 2020). Although the reported methane yields were no significantly different, the methane production began earlier when the biochar was added. An increase in biochar from 0 to 15 g L^{-1}, enhanced the R_{max} value by a 25% (from 19.9 to 25.0 NL (kg VS·d)$^{-1}$) while λ decreased by a 44% (from 2.67 to 1.49 days) (Ma et al., 2020). These reported R_m values were of the same order of magnitude as those obtained for the PAN and P89 *S. bicolor* varieties studied in the present work (Table 4) and lower than those obtained for the *S. bicolor* varieties that were degraded with the highest maximum methane production rates (ZH and PR 88).

It was also demonstrated that the degree of fragmentation of sorghum silage (1.5, 5 and 10 mm) has an influence on λ and R_{max} in BMP tests of this substrate. In this sense, the lag stage and maximum biogas production rate decreased from 6.42 to 5.77 and from 73.79 to 56.22 Nm^3 (Mg VS·d)$^{-1}$, respectively, when the degree of fragmentation diminished from 5 to 1.5 mm (Szlachata et al., 2018).

Although ZHG showed a medium-range methane yield (305 L CH_4 kg^{-1} VS) among the different varieties studied, it presented the highest biomass yield and methane yield per shoot, as well as the fourth highest *k* constants and R_{max}. The landrace cultivars showed higher biomass yield and higher methane yield per shoot than the commercial hybrids cultivars PR87 and PR88. The landrace cultivar TG showed the third methane yield value (376 NL CH_4 kg^{-1} VS) and the second value of biomass yield and methane yield per shoot.

5 | CONCLUSIONS

The landrace cultivars achieved the greater height, biomass and compactness shoot as well as a similar or an improvement in grain production compare to the commercial ones. Methane yield values in the range of 277 and 413 NL CH_4 kg^{-1} VS were observed in the batch assays of nine *S. bicolor* straw varieties during the AD process at mesophilic temperature (35°C). The landrace cultivar ZH showed the highest value (413 NL CH_4 kg^{-1} VS) but the landrace cultivars ZHG and TG were the ones that obtained the highest methane per biomass production (13.7 and 12.7 NL CH_4 shoot $unit^{-1}$, respectively). Data from AD assays of these wastes were fitted into both first-order and TF models. The highest specific rate constant obtained in the present research was found for the commercial hybrid cultivar PR88 (0.148 ± 0.008 $days^{-1}$). This value was 32.1% and 49.4% higher than that obtained for the ZH and ZHG varieties respectively, which presented the highest values among the different traditional varieties tested in the present research (0.112 ± 0.006 and 0.099 ± 0.004 $days^{-1}$, respectively). In addition, the highest maximum methane production rate (R_{max}) was found for the landrace cultivar ZH, which was 87.1% higher than the lowest value, which was found for the PAN *S. bicolor* variety (24 ± 1 NL CH_4 $(kg\ VS{\cdot}d)^{-1}$).

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Data for all analysis are available via Zenodo https://doi.org/10.5281/zenodo.7462051.

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