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Influence of Ensiling Time and Elephant Grass Silage Alkaline Pretreatment in Anaerobic Co-digestion with Vinasse for Methane Production

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Abstract

This study investigated the anaerobic co-digestion of sugarcane vinasse (V) and elephant grass silage (S) to produce methane. Box-Behnken experimental design was applied to verify the statistical effects of the elephant grass ensiling time (40, 80 and 120 days), alkaline pretreatment of elephant grass silage (0.5, 2.25 and 4.00% w/v NaOH) and S:V mixture ratio (25:75, 50:50 and 75:25) on the methane yield. The results showed that the ensiling process resulted in the low degradation of lignocellulosic substances, emphasizing the need for pretreatment using more efficient techniques, such as thermo-alkaline, to improve the breakdown of elephant grass fibres. COD removals varied between 35 and 85%, and carbohydrate consumptions ranged from 63 to 72%, with the higher efficiencies for both parameters occurring in the reactors with lower percentages of silage. Cumulative methane yield ranged from 190.77 $mLCH_4/gVS$ (in the reactor with S:V of 75:25, 0.50% w/v NaOH and 80 ensiling days) to 1729.80 $mLCH_4/gVS$ (in the reactor with S:V of 25:75, 2.25% w/v NaOH and 120 ensiling days). According to ANOVA, S:V ratio was the only variable with a significant effect ($p < 0.05$) on cumulative methane yield. Therefore, the findings indicate that the relative composition of substrates within the mixture exerted the most significant influence on the process, underscoring the critical role of vinasse as a co-substrate in enhancing methane production despite silage pretreatments.

Keywords Batch reactors · Lignocellulosic biomass · Sodium hydroxide solution · Box-Behnken experimental design · Statistical analysis

Highlights

- The mixing ratio between vinasse and elephant grass silage was the most significant variable for the process.
- Higher concentrations of lignocellulosic material are unfavourable for methane production.
- Co-digestion of vinasse and elephant grass silage promoted higher methane yields compared to isolated substrates.

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Introduction

Energy demand supply will undergo structural changes in the twenty-first century with the challenge posed by the global climate emergency state, mainly associated with the indiscriminate use of fossil fuels. In this scenario, anaerobic digestion is notable as a practical and efficient approach for producing biofuels and meeting renewable energy demands. The digestive process consists of several steps that use microorganisms to degrade substrates rich in organic matter, transforming them into biogas (mainly methane and CO_2) and other products with high added value [1]. Biogas stands out among clean energy sources and can derive from different substrates, such as energy crops, agro-industrial waste, sanitary sewage and organic fractions of solid wastes [2].

Sugarcane vinasse, one of the critical agro-industrial residues produced in Brazil, can be used as a substrate for biogas generation through anaerobic digestion. Vinasse is a liquid

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effluent from ethanol production that is highly polluting due to its high organic matter content, requiring adequate treatment and final disposal [3]. Cremonez et al. [4] compiled results from studies on the methanogenic potential of this residue, citing values for methane yield ranging from 249 to 302 $mLCH_4/gCOD$ [5, 6]. Nevertheless, recalcitrant and toxic elements found in vinasse may hinder the progress of anaerobic digestion. Yet, implementing treatments like physical–chemical or enzymatic processes can effectively address this concern and optimize the performance of anaerobic reactors [7, 8].

Another agricultural residue suitable to produce biogas is elephant grass, a species of tropical forage grass that stands out as an energy crop because of characteristics such as high biomass production potential and elevated concentration of components with high calorific value [9, 10]. Previous studies on methanogenic yield from using this substrate resulted in productions varying between 190 and 372 $mLCH_4/gVS$ [11–14].

Elephant grass contains approximately 46% of cellulose, 34% of hemicellulose and 21% of lignin [15] as a percentage of dry matter. However, these values can vary according to species variants and cultivation conditions [16]. Molecules of this type of biomass have a high structural complexity and, consequently, reduce the efficiency of hydrolytic bacteria that act in the first stage of anaerobic digestion. Thus, the decomposition of these substrates requires a pretreatment phase using physical, chemical or biological processes.

While physical treatments use mechanical or radiation energy to change the biomass structure, facilitating its digestibility, and one of its advantages is the use of fewer chemical substances [17], biological pretreatments include the addition of enzymes and the use of pure cultures or consortia of microorganisms capable of decomposing lignocellulosic substances [18]. Both methods also involve factors that can affect their large-scale application, such as the high energy consumption involved in the process or the sensitivity of microorganisms.

The thermal pretreatments employ maximum temperatures of 220 °C to heat substrates under pressure, causing more breakdown of the fibres and increasing methanogenic production [19]. Chemical treatments such as alkaline, acid and oxidative are widely used to increase the digestibility of lignocellulosic biomass.

Particularly, alkaline pretreatment promotes better lignin solubilization besides breaking acetate groups present in hemicelluloses. This treatment can be carried out by adding substances such as sodium hydroxide (NaOH), calcium hydroxide [$Ca(OH)_2$] and potassium hydroxide (KOH) [20]. When studying hydrogen production via anaerobic fermentation using grasses, Cui and Shen [21] observed that the substrate treated with an alkaline solution, compared to the pure substrate, resulted in four times greater gas production. However, more severe acid and alkaline pretreatments can generate high operational costs and inhibitory by-products [18]. Therefore, the conditions of their application must be studied and optimized.

In this context, ensilage — a storage method traditionally used in agriculture — has been investigated for its potential as a biological pretreatment since it promotes acidic conditions in the environment, possibly increasing the digestibility of lignocellulosic substrates [22]. Zhang et al. [23] evaluated the effect of ensilage as a pretreatment of elephant grass, considering different cutting heights and plant parts, obtaining the value of 361 $mLCH_4/gVS$ as the maximum methanogenic production. Pardang et al. [24] investigated the impact of harvest age, temperature and ensilage on biogas production using hybrid elephant grass species as substrates. Under mesophilic conditions, samples that underwent ensiling showed superior performance, reaching a specific methane yield of 154 $mLCH_4/gVS$.

The present study associated the ensiling process with thermal-alkaline treatment, which presents the advantages of greater efficiency in breaking down lignocellulosic substances, lower energy consumption compared to physical alternatives and a simple and easily adaptable methodology.

Nevertheless, in addition to the type of substrate and its pretreatment (when necessary), a set of factors also influences the biogas production, such as the inoculum source, temperature, pH and co-digestion, which can contribute to enhancing the digestive process, as it promotes the mixing and dilution of toxic compounds, nutrient and pH balance, as well as improving synergistic effects between existing microorganisms [4].

Previous studies performed the co-digestion of agro-industrial by-products intending to produce biogas. Sousa et al. [25] achieved high productivity and methane yield when co-digesting vinasse with cheese whey compared to mono-digestions. González et al. [26] effectively tested the co-fermentation of vinasse and sugarcane cake. However, research on co-digestion using vinasse and elephant grass silage is still scarce in the literature, as is the effect of ensiling time on methanogenic production.

Therefore, this work aimed to improve methane production from the anaerobic co-digestion of vinasse and elephant grass silage, seeking a balance between the high organic load of vinasse and the elevated content of lignocellulosic substances in elephant grass. For this purpose, a Box-Behnken experimental design allowed the evaluation of the influence of elephant grass ensiling time, mixing ratio between substrates and alkaline pretreatment in elephant grass silage as factors of interest in methanogenic production.

Materials and Methods

Elephant Grass Pretreatment

Brazilian Agricultural Research Agency (EMBRAPA), which cultivates several varieties of this species in a research execution unit located in Rio Largo, Alagoas, Brazil, provided the elephant grass specimen (*Cenchrus purpureus*). The samples were acquired from the complete crushing of twelve plants (stems and leaves) harvested in October 2020, having a cutting age of 6 months. The average plant height was 4.03 m, with a spacing of 1 m between crop rows. The elephant grass samples were homogenized, dried in an oven and subsequently underwent processing in a forage crusher, resulting in particles ranging between 1 and 2 cm in size.

Afterwards, the samples were ensiled in PVC mini silos, each with a diameter of 200 mm and a height of 45 cm, and no chemical or biological additives were introduced during the ensiling process. The mini silos were hermetically sealed using a lid and silicone glue to prevent air from entering and stored at room temperature, around 30 °C. After 40 days of ensilage, the pH values of the samples were approximately 3.50.

The EMBRAPA carried out bromatological and elemental analyses of the crude elephant grass and ensilage samples (after ensiling periods of 40, 80 and 120 days [27]), evaluating the neutral detergent fibre (NDF) and acid detergent fibre (ADF) content. This analysis aims to identify cellulose, hemicellulose and lignin fibres present in the biomass, investigating the effect of ensilage on breakdown lignocellulosic substances. The fibre content variation was calculated based on the total dry matter (DM) percentage for each sample of elephant grass silage [28].

After ensiling, elephant grass samples were subjected to thermal-alkaline pretreatment [21] aiming to ensure a higher breakdown of lignocellulosic fibres. A mixing ratio of 1.0 g of elephant grass silage was adopted for 20 mL of aqueous sodium hydroxide solution (NaOH) at different concentrations (0.50, 2.25 and 4.00% w/v). Each mixture was heated at 90 °C for 10 min, then filtered and diluted solutions of HCl 15% v/v and NaOH 4% m/v were lastly added to neutralize pH to 7.

Substrates and Inoculum

Elephant grass silage samples after pretreatment showed the following physicochemical characteristics: COD of 11.84–23.63 g/L, carbohydrate concentration of 8.66–12.93 g/L, volatile solids of 8.60–13.58 g/L and pH of 7.00–7.05.

Sugarcane vinasse, collected at a local sugar and alcohol distillery plant, presented a COD of 39.93 g/L, carbohydrate concentration of 10.23 g/L, volatile solids of 23.90 g/L, total nitrogen Kjeldahl of 364 mg and pH of 3.98. As lignocellulosic substances that are difficult to degrade are absent from the vinasse composition, unlike elephant grass biomass, this substrate does not require a pretreatment step. Furthermore, the formation of inhibitory substances in the process was also not identified.

Vinasse and grass silages were diluted in water to adjust the COD concentration to 5 g/L. The COD of the substrates was adjusted to standardize this parameter in all reactors, avoiding its influence on the process. Furthermore, the raw substrates exhibited high COD values, which could potentially overload the reactors.

The inoculum obtained in a UASB reactor from a sewage treatment system at room temperature (ranging between 27 and 35 °C) presented 24 gCOD/L, total solids of 30 g/L, total suspended solids of 21 g/L, volatile suspended solids of 17 g/L and pH of 6.38.

Experimental Design

The present study followed a Box-Behnken experimental design to investigate the methane production from the anaerobic co-digestion of the proposed substrates. The factors analysed in the experimental design were the ensiling time of elephant grass (*T*), the proportion between substrates (*P*) (silage:vinasse — S:V) and the concentration of NaOH solution used as pretreatment of grass silage (*C*), with three levels of variation (− 1, 0, + 1) (Table 1).

In the thermal-alkaline pretreatment of elephant grass silage, all samples were firstly heated at a temperature of 90 °C for 10 min and then cooled, ensuring a consistent thermal treatment. Therefore, the experimental design did not consider the effect of temperature. The study also included the control reactors: C1, C2 and C3, with elephant

Table 1 Experimental design

Reactor (R)	T (days)	P	C (%)
R1 (40, 25:75, 2.25)	40 (− 1)	25:75 (− 1)	2.25 (0)
R2 (120, 25:75, 2.25)	120 (+ 1)	25:75 (− 1)	2.25 (0)
R3 (40, 75:25, 2.25)	40 (− 1)	75:25 (+ 1)	2.25 (0)
R4 (120, 75:25, 2.25)	120 (+ 1)	75:25 (+ 1)	2.25 (0)
R5 (40, 50:50, 0.50)	40 (− 1)	50:50 (0)	0.50 (− 1)
R6 (120, 50:50, 0.50)	120 (+ 1)	50:50 (0)	0.50 (− 1)
R7 (40, 50:50, 4.00)	40 (− 1)	50:50 (0)	4.00 (+ 1)
R8 (120, 50:50, 4.00)	120 (+ 1)	50:50 (0)	4.00 (+ 1)
R9 (80, 25:75, 0.50)	80 (0)	25:75 (− 1)	0.50 (− 1)
R10 (80, 75:25, 0.50)	80 (0)	75:25 (+ 1)	0.50 (− 1)
R11 (80, 25:75, 4.00)	80 (0)	25:75 (− 1)	4.00 (+ 1)
R12 (80, 75:25, 4.00)	80 (0)	75:25 (+ 1)	4.00 (+ 1)
R13 (80, 50:50, 2.25)	80 (0)	50:50 (0)	2.25 (0)
R14 (80, 50:50, 2.25)	80 (0)	50:50 (0)	2.25 (0)
R15 (80, 50:50, 2.25)	80 (0)	50:50 (0)	2.25 (0)

T ensilage time (days), *P* proportion between substrates (silage:vinasse), *C* NaOH solution (% w/v) concentration of the pretreatment grass treated with 2.25% NaOH and ensiled for 40, 80 and 120 days, respectively; C4 containing only vinasse; and C5, inoculum.

Reactor Operation

The reactors were glass flasks of 120 mL with useful volume of 60 mL and a headspace of 60 mL. The inoculum was added at a concentration of 10% in all reactors. Sodium bicarbonate ($NaHCO_3$) at a proportion of 0.5 $gNaHCO_3$/gCOD was applied to supplement alkalinity in the system and buffer the pH [29].

Buffering was carried out to avoid pH fluctuations and ensure that the microorganisms present in the reactors followed the methanogenic production route. The ideal pH range for methanogenesis is between 6.6 and 7.4, with stability around 6.0 and 8.0. For this reason, a decrease in pH can lead to the accumulation of volatile acids (acidification) in the reactors, causing a reduction in the rate of methanogenic growth [30].

After adding the substrates, the pH was adjusted to 7 ± 0.05, and reactors were closed with crimp-type seals coupled to valves for gas collection. The reactors also received nitrogen flow to remove oxygen in the medium. Then, the reactors were capped and placed in a stirring chamber, maintaining the operating temperature in the mesophilic range of 35 °C and rotation of approximately 100 rpm.

The reactors were maintained under these conditions and frequently monitored to assess biogas production. The experiment lasted 59 days, allowing the reactors to reach a steady-state condition determined based on a constant CH_4 production rate with a variation within 5–10% over 5–10 consecutive days [31]. The experiment also included control reactors containing only the isolated substrates or inoculum.

The reactors were operated for two months, totalizing approximately 1400 h of experiment duration, with frequent monitoring of methane production through gas chromatography analyses.

Analysis of Biogas Production

Biogas production was periodically monitored using gas chromatography by manually injecting 0.3 mL samples of the gaseous phase produced in the reactors into a gas chromatograph. The equipment used was a Shimadzu GC-2010 model chromatograph equipped with a thermal conductivity detector (TCD), Supelco Carboxen 1010 PLOT column (30 m × 0.53 mm) and operated with a carrier gas flow of 21.9 cm/s; the injector, detector and column temperatures were 30, 200 and 230 °C, respectively. The gas chromatography allowed the indirect quantification of methane (in moles and millilitres) used to plot the curves of accumulated methane production as a function of elapsed time for each reactor.

Statistical Analysis

Accumulated methanogen production data were fitted to the modified sigmoid model of Gompertz shown in Eq. 1 [32]:

$$M(t) = P \times \exp\left\{ -\exp\left[\frac{R_m \times \exp(1)}{P}(\lambda - t) + 1 \right] \right\} \quad (1)$$

where:

$M(t)$ cumulative methane production after time t (mL);

P methanogen production potential (mL);

R_m methane production rate (mL/h);

λ lag phase, that is, minimum time for biogas production (h);

t incubation time of the reactors (h).

The effects of the factors on the response variable, standard errors, standardized effects, p-values and regression model coefficients were calculated using the software Statistica 8.0 from StatSoft, considering a significance level of 5%. Analysis of variance (ANOVA) was also performed to verify the statistical significance of the factors of interest and to evaluate the fit of the proposed model to the experimental data.

Results and Discussion

Ensilage Performance as Pretreatment

Initially, the fibre content was evaluated to verify the efficiency of ensiling as a pretreatment. The crude elephant grass presented 28.31% of hemicellulose, 40.18% of cellulose and 8.45% lignin based on dry matter. After 40, 80 and 120 ensiling days, respectively, hemicellulose decreased to 26.79, 25.60 and 25.89%; cellulose content achieved 40.15, 41.35 and 40.93%; and lignin fibres augmented to 9.36, 9.69 and 10.64% of DM.

The ensiling process has been reported to lead to the gradual disintegration of lignocellulosic structures, primarily hemicelluloses, through the effects of weak acid-driven hydrolysis under conditions of pH lower than 4.0 [33]. The outcomes of the present study show that hemicelluloses had a higher degree of breakdown. Nonetheless, the ensiling process alone did not cause a significant variation (less than 3% of DM) in the fibre content of the biomass, reinforcing the need for a more efficient pretreatment.

These results corroborate the conclusion of Ambye-Jensen et al. [34], which verified the low breakdown of cellulose after the ensiling, proposing using enzymatic additives to improve the process. Since physicochemical pretreatments are more efficient in enhancing the lignocellulosic biodigestibility of substrates used in anaerobic digestion, the present study associated elephant grass ensilage with the thermal-alkaline technique [33].

Variation of Physical–Chemical Parameters

COD Removal

Figure 1 presents COD removals (the supplementary material shows the data for initial and final COD concentrations). In general, reactors were efficient in consuming organic matter [35], achieving COD removals greater than 60%, except for R3 (40, 75:25, 2.25), R8 (120, 50:50, 4.00) and R10 (80, 75:25, 0.50), which removed 57, 44 and 35% of COD, respectively.

The lowest COD removal observed in reactor R10 (80, 75:25, 0.50) may be associated with the higher amount of elephant grass silage in the mixture (75%), pretreated using the alkaline solution with the lower concentration of NaOH (0.50% m/v). In this case, the improper degradation of lignocellulosic material in elephant grass silage may have hindered the release of simpler molecules, such as oligosaccharides or monosaccharides, necessary for consumption through anaerobic digestion [36].

Meanwhile, reactors with smaller quantities of elephant grass silage (≤ 50%) and pretreatment using higher concentrations of NaOH (≥ 2.25% NaOH m/v) had COD removals greater than 80%, such as R2 (120, 25:75, 2.25) (84%), R11 (80, 25:75, 4.00) (85%), R13 (80, 50:50, 2.25) (84%), R14 (80, 50:50, 2.25) (83%) and R15 (80, 50:50, 2.25) (85%). The higher amounts of vinasse in these reactors may have favoured COD consumption since the organic matter present in its composition is easily accessible for the action of hydrolytic bacteria that initiate the process of anaerobic digestion [37].

Carbohydrate Conversion

Figure 1 exhibits the percentages of carbohydrate conversion (the supplementary material discloses the data for initial and final carbohydrate concentration). All reactors achieved conversions above 60%, indicating a satisfactory consumption of carbohydrates present in the medium. In contrast to the COD removal values, which presented a wide range of variation (35–85%), the carbohydrate conversion range varied less, between 63 and 72%.

Reactors R10 (80, 75:25, 0.50) and R11 (80, 25:75, 4.00), with the lowest removal efficiency (63%), and R9 (80, 25:75, 0.50) and R12 (80, 75:25, 4.00), with highest carbohydrate consumption (72%), were composed of different combinations of experimental design levels. Therefore, no patterns were identified that justify the differentiated performance.

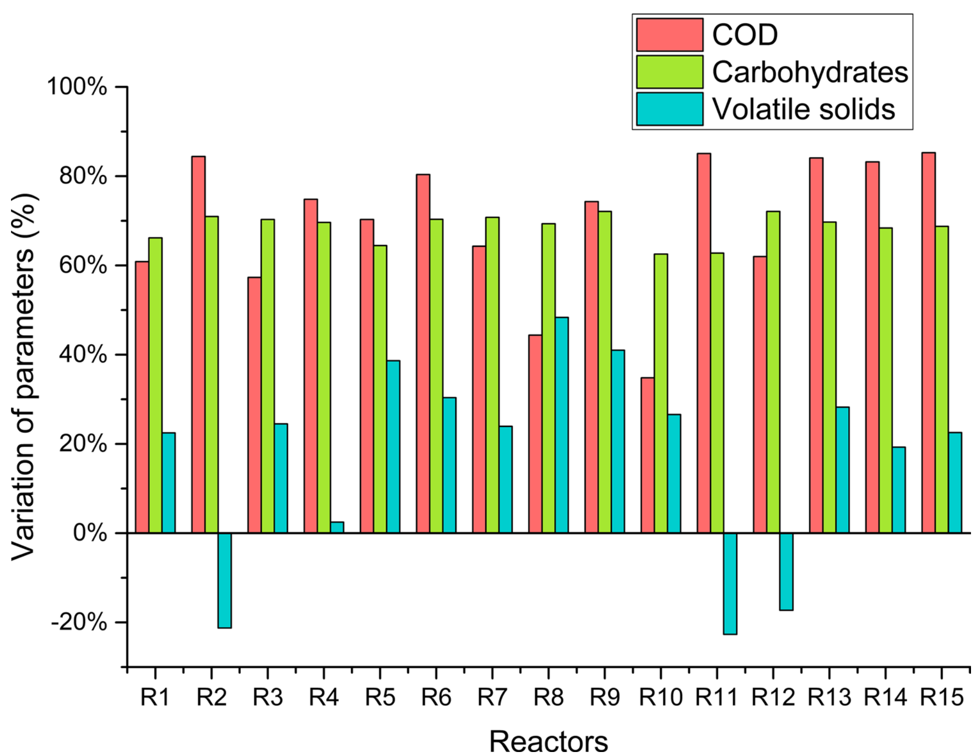

Fig. 1 Percentage of removal of COD, carbohydrates, and volatile solids in the reactors

Volatile Solid Removal

Figure 1 presents the percentage variations of volatile solids (VS), with negative values indicating an increase in solids content (the supplementary material shows the data for initial and final VS). The minimum VS removal occurred in R4 (120, 75:25, 2.25) (2%) and the maximum in R8 (120, 50:50, 4.00) (48%). Most reactors presented removal percentages higher than 20%, except for R4 (120, 75:25, 2.25), R2 (120, 25:75, 2.25) (− 21%), R11 (80, 25:75, 4.00) (− 23%), R12 (80, 75:25, 4.00) (− 17%) and R14 (80, 50:50, 2.25) (19%).

The typical curve of microorganisms' growth can clarify the increase in solids in R2, R11 and R12 (which presented negative percentages). Besides the dry matter from the substrates, the solid content indicates the presence of material coming from the cells of microorganisms involved in anaerobic digestion [38, 39]. Therefore, these reactors may have suffered a deactivation during the multiplication of microorganisms, presenting an augmentation in VS concentration. In contrast, reactors that showed higher VS removals probably underwent the microbiological exponential growth phase and consumed part of the available substrate.

Reactors R5 (40, 50:50, 0.50), R8 (120, 50:50, 4.00) and R9 (80, 25:75, 0.50) achieved higher VS removals of 39, 48 and 41%, respectively, similar to values reported in the literature. Carvalho et al. [40] observed a VS removal of 48% in the co-digestion of elephant grass hydrolysate and sewage sludge. Ojediran et al. [41] reported a decrease of approximately 40% in VS after co-digestion of elephant grass with piggery manure.

The three reactors with the highest VS removal presented S:V ratios of 25:75 and 50:50, indicating the volume of vinasse was equal to or greater than that of elephant grass silage. Tena et al. [37] also found higher VS removals with an increase in the percentage of vinasse in the reaction medium. The authors evaluated the co-digestion of sewage sludge (SS) with wine vinasse (WS) in SS:WS ratios of 100:0, 75:25, 50:50, 25:75 and 0:100, achieving maximum removals of 37 and 49% at mixing ratios of 25:75 and 0:100, respectively.

Therefore, this result suggests that the use of elephant grass in amounts greater than 50% causes an imbalance in co-digestion, affecting the organic matter consumption, as similarly observed in the COD removal. Thus, in principle, the S:V ratio was the most relevant factor for VS removal, while the effects of ensiling time and alkaline pretreatment were negligible.

Methane Production

Figure 2 exposes the accumulated methane production (mL) and the adjustment curves of the experimental data to the modified Gompertz model described in Eq. 1. R^2 values varied between 0.9909 and 0.9981, indicating that the model adequately describes the data obtained in this study. Reactors R9 (80, 25:75, 0.50), R5 (40, 50:50, 0.50), R14 (80, 50:50, 2.25) and R1 (40, 25:75, 2.25) performed better, with accumulated methane production superior to 300 mL at the end of 1400 h.

Table 2 presents the kinetic parameters obtained from the adjustment of experimental data of accumulated production to the modified Gompertz model. The maximum methanogenic production potential (P) ranged from 59.73 mL in R4 (120, 75:25, 2.25) to 440.40 mL in R9 (80, 25:75, 0.50) with the estimated maximum methane production rate (R_m) of 0.04 and 0.38 mL/h, respectively. The lag phase time (λ) varied between 52.58 h in R4 (120, 75:25, 2.25) and 580.54 h in R7 (40, 50:50, 4.00). Reactors with the highest amount of elephant grass silage, R3 (40, 75:25, 2.25), R4 (120, 75:25, 2.25), R10 (80, 75:25, 0.50) and R12 (80, 75:25, 4.00), presented the lowest accumulated yields (< 300 $mLCH_4/gVS$) and shorter phase lag.

Regarding control reactors, those that contained only silage as substrate (C1, C2 and C3) showed accumulated methane yield between 295.79 and 302.32 $mLCH_4/gVS$, similar to values found in previous studies. Gunaseelan [38] obtained methane yield values between 372 and 342 $mLCH_4/gVS$ when applying different parts of elephant grass as substrate. Zhang et al. [23] used different cutting heights and parts of the plant, obtaining the value of 361 $mLCH_4/gVS$ as the maximum yield of methanogenic production.

The use of elephant grass silage as the predominant substrate in the mixture (100 and 75% of silage) resulted in lower methane yields (190.77–302.32 $mLCH_4/gVS$) regardless of the other factors involved (ensilage time and NaOH concentration). On the other hand, methane yield increased in experimental conditions with the addition of minor amounts (25 and 50%) of elephant grass silage, reaching the maximum of 1729.80 $mLCH_4/gVS$ in R2 (120, 25:75, 2.25).

Therefore, despite the pretreatments applied, lignocellulosic material probably hindered the microbial digestion of elephant grass fibres. In this type of feedstock, the hydrolysis rate is slow and limits methane production [15, 39]. Furthermore, the relative deficiency of nutrients such as nitrogen and phosphorus in elephant grass can hinder microbial growth and inhibit fermentation [16]. Thus, these factors highlight the need to use a more biodegradable substrate, such as vinasse, to improve fermentation.

Increasing the proportion of vinasse in the mixture caused an increase in methane yield for any ensiling time and NaOH concentration. Besides that, control reactor containing only vinasse (C5) presented a methane yield of 918.53 $mLCH_4/gVS$. Tena et al. [37] also reported the positive influence of increasing the fraction of vinasse in co-digestion systems. Aiming to produce hydrogen from

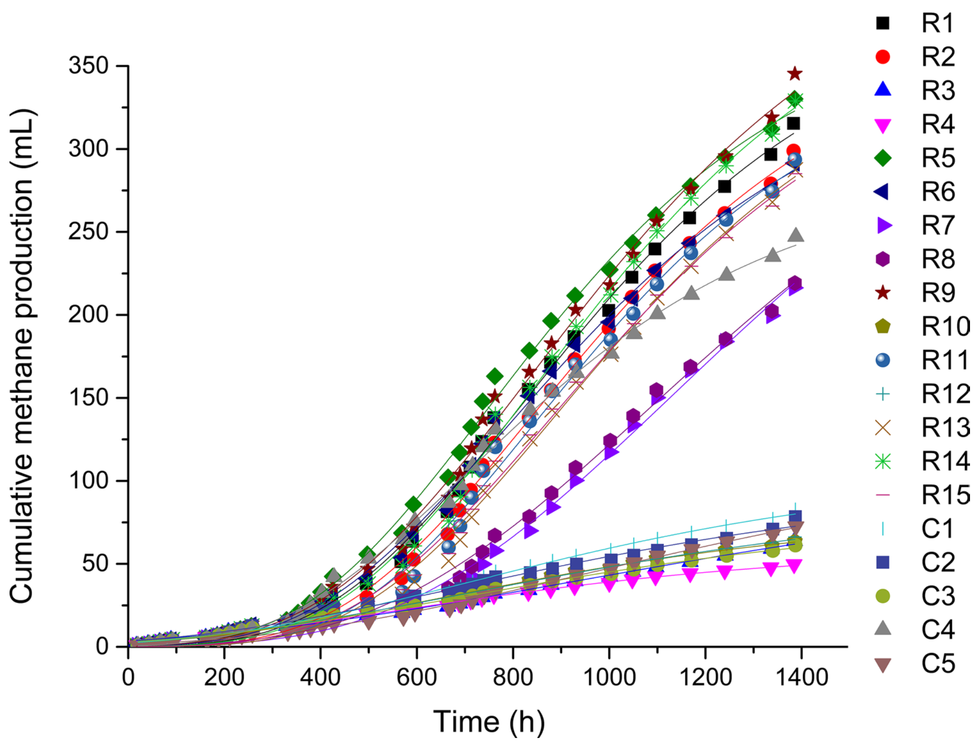

Fig. 2 Cumulative methane production (mL) and respective fitting curves to the modified Gompertz sigmoid model

Table 2 Parameters of the modified Gompertz model for fitting curves and accumulated methane yield of reactors

Reactor	*P* — methanogenic production potential (mL)	R_m — methane production rate (mL/h)	*λ* — lag phase (h)	R^2	Accumulated methane yield (mL_{CH4}/gVS)
R1 (40, 25:75, 2.25)	392.83	0.36	417.21	1.00	1219.23
R2 (120, 25:75, 2.25)	377.75	0.35	446.24	1.00	1729.80
R3 (40, 75:25, 2.25)	106.60	0.05	164.11	0.99	214.82
R4 (120, 75:25, 2.25)	59.73	0.04	52.58	1.00	196.98
R5 (40, 50:50, 0.50)	387.98	0.38	367.67	1.00	1002.66
R6 (120, 50:50, 0.50)	358.54	0.33	386.33	1.00	1018.77
R7 (40, 50:50, 4.00)	413.54	0.28	580.54	0.99	678.69
R8 (120, 50:50, 4.00)	392.97	0.26	538.76	0.99	719.24
R9 (80, 25:75, 0.50)	440.40	0.38	404.73	1.00	1081.83
R10 (80, 75:25, 0.50)	79.98	0.06	138.09	1.00	190.77
R11 (80, 25:75, 4.00)	363.77	0.36	467.38	0.99	1633.73
R12 (80, 75:25, 4.00)	88.84	0.06	128.88	1.00	295.83
R13 (80, 50:50, 2.25)	378.56	0.35	485.27	0.99	1086.85
R14 (80, 50:50, 2.25)	419.49	0.38	437.09	1.00	1157.57
R15 (80, 50:50, 2.25)	375.93	0.34	469.31	1.00	1011.08

vinasse and sewage sludge, the authors observed that mixtures using 50 and 75% vinasse resulted in yields 13 to 14 times superior to those obtained by mono-fermentation of sewage. The improvement in process performance was attributed to the organic matter present in the vinasse that is easily digestible by microorganisms. Besides that, the non-lignocellulosic character and higher amount of organic matter in vinasse facilitated the digestive process [37].

In general, the values for methane yield obtained in previous studies were lower than the maximum result achieved in this study (1729.80 $mLCH_4$/gVS). Carvalho et al. [40] analysed the anaerobic co-digestion of elephant grass hydrolysate with sewage sludge in a continuous anaerobic reactor, reaching a maximum yield of 0.21 LCH_4/gVS. The authors identified that the yields in co-digestion were 23 to 38% higher than the results obtained in mono-digestion of sewage sludge. González et al. [26] verified the anaerobic co-digestion of sugarcane press mud with vinasse. The maximum methane yield (248 $mLCH_4/gCOD_{fed}$) resulted from the mixture containing 50% of each substrate. This value was 13% higher than that obtained using only pressure mud.

Haryanto et al. [41] produced methane from a 1:1 mixture (25 kg:25 kg) of cow dung and elephant grass (without pretreatment) under various dilution rates in water (P1 = 50 L, P2 = 75 L and P3 = 100 L). The 25:25:100 (cow dung:grass:water) composition resulted in 39.3 $mLCH_4/gTS_{removal}$. An improvement in yield was observed with increasing dilution and decreasing total solid content. The authors suggested applying mechanical pretreatment to elephant grass to improve process performance.

Himanshu et al. [42] verified the prevalence of synergistic effects in co-digesting perennial ryegrass silage (grass harvested at two growth stages — PRS1 and PRS2) or red clover silage (clover harvested at two growth stages — RCS1 and RCS2) with cattle slurry. Various substrate proportions were tested on a volatile solid basis (forage silage:cattle slurry — 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1). Digestions isolated from forage silage yielded 317.5 (PRS1), 285.8 (PRS2), 286.7 (RCS1), 255.0 (RCS2) and 281.9 (cattle slurry) LCH_4/kgVS. The highest yields were obtained in the mixtures 0.25:0.75 for PRS1 with cattle slurry (330.5 LCH_4/kgVS) and 0.5:0.5 for RCS1 with cattle slurry (294.8 LCH_4/kgVS).

Lovato et al. [43] evaluated the methane production by co-digesting vinasse and cheese whey. The increase in the percentage of whey from 0 to 100% caused an augmentation in methane yield. An amount of 7.8 $molCH_4/kgCOD_{fed}$ was obtained in the mono-digestion of vinasse and 10.9 $molCH_4/kgCOD_{fed}$ with the association between 25% vinasse and 75% whey.

Ojediran et al. [44] analysed the co-digestion of treated and untreated piggery manure and elephant grass. The addition of piggery manure improved the methane production capacity of elephant grass. An amount of 409.5 m^3CH_4/kgVS was obtained from co-digestion with treated elephant grass and 306.2 m^3CH_4/kgVS with untreated elephant grass.

Volpi et al. [45] investigated the generation of methane through the application of lignocellulosic residues, vinasse and other effluents from ethanol production, concluding that anaerobic co-digestion enabled increases of almost 40% in the biochemical potential of methane (BMP), compared to isolated substrates. An amount of 605 $mLCH_4$/gVS was reached with the association between vinasse and liquor deacetylation as co-substrates.

Statistical Analysis

The work analysed the effects of ensiling time, proportion between substrates and NaOH solution concentration of the pretreatment (independent variables) on accumulated methane yield (dependent variable) (Table 2).

Table 3 presents standard errors, standardized effects, *p*-values and coefficients of each factor of the polynomial regression model at a 95% confidence level. The linear effect of proportion between substrates (*P*) presented a *p*-value lower than 0.05, which means that this factor was statistically significant for methane yield. The effects of ensiling time and alkaline pretreatment were non-significant because *p*-values were higher than 0.05. However, all factors studied were maintained in the analysis, aiming to obtain a model that best fits the experimental data.

Table 4 presents the analysis of variance (ANOVA) of the experiment. *F*-value, a parameter used to assess the

Table 3 Effects, statistical significance and model coefficients for the independent variables

	Effect	Standard error	Standardized effects, *t*(2)	***p***-value	Model coefficient
Overall average	831.86	21.15	39.33	0.00	1085.17
(1) T (L)	137.35	51.80	2.65	0.12	68.67
(1) T (Q)	95.33	38.13	2.50	0.13	−95.33
(2) P (L)	−1191.55	51.80	−23.00	0.00	−595.77
(2) P (Q)	149.63	38.13	3.92	0.06	−149.63
(3) C (L)	8.36	51.80	0.16	0.89	4.18
(3) C (Q)	135.00	38.13	3.54	0.07	−135.00
T×P	−264.21	73.26	−3.61	0.07	−132.10
T×C	12.22	73.26	0.17	0.88	6.11
P×L	−223.42	73.26	−3.05	0.09	−111.71

Statistical significance: $p < 0.05$

T ensilage time, *P* proportion between substrates, *C* alkaline pretreatment concentration, *L* linear effect, *Q* quadratic effect statistical significance of regression, is calculated from the sums and square means. F_{cal} of the regression (6.98) was higher than the tabulated $F_{9.5}$ (4.77), indicating that the proposed model is valid and adequately describes the experimental data. The coefficient of determination (R^2) was 0.93, confirming that it adequately describes the behaviour of the process under study at a confidence level of 95%. Furthermore, the F_{cal} (14.94) of the lack-of-fit was lower than the $F_{3.2}$ tabled (19.16), showing no lack-of-fit in the model. Therefore, the model adjusted to the experimental data is presented in Eq. 2.

$$R = 1085.16 + 68.67T - 95.33T^2 - 595.77P - 149.62P^2 + 4.18C - 134.99C^2 - 132.10TP + 6.11TC - 111.71PC \quad (2)$$

where:

R methane yield ($mLCH_4/g_{VS}$);

T ensiling time (coded values between − 1 and 1);

P proportion between substrates (coded values between − 1 and 1); and.

C solution concentration of pretreatment (coded values between − 1 and 1).

Coefficients in Eq. 2 display the majority and negative linear effect of the substrate proportion factor (P = S:V) on the methane yield (− 595.77), indicating that the addition of elephant grass silage (S) is linked to lower R values (methane yield). As previously explained, this behaviour probably occurs because of an increase in the lignocellulosic substances present in elephant grass, which is a substrate of greater digestive difficulty for part of microorganisms, reducing the rate of hydrolysis and can delay or inhibit methane production [15, 39].

As for the other linear effects, although they proved to be statistically non − significant, it is observed that the increase in factors T (coefficient + 68.67) and C (coefficient + 4.18) contributes positively to the methane yield. Furthermore, except for the positive effect of the TC interaction (coefficient + 6.11), the other second-order interactions (coefficients − 132.1 and − 111.71) and quadratic interactions (coefficients − 95.33, − 149.62 and − 134.99) have negative coefficients and, therefore, contribute to the reduction of R.

Surface and contour graphs showing the effect of the factors and their interactions on the accumulated methane yield were generated based on the model proposed in Eq. 2, using Minitab 21 software. These graphs offer a visual display of variations in planning, factors affecting the response of the experiment and possible strategies for optimizing these results, revealing which experimental conditions lead to the most desirable response.

Figure 3 shows the surface and contour graphs for the proportion between substrates (P) and ensiling time (T). The surface presents curvature in the axis corresponding to the P factor, with the level S:V = 75:25 resulting in lower methane yields and level S:V = 25:75 presenting significantly higher values for the response variable. Thus, within the limits of the present study, it was evident that combining vinasse and elephant grass silage in anaerobic digestion yielded more methane when the silage volume was approximately 25% of the reactor's useful volume. Since lignocellulosic biomasses are usually characterized by high C/N ratios, which must be maintained in the range of 20–30:1 for adequate operation of anaerobic reactors [22], it is possible to infer that the ratio between silage and vinasse equivalent to 25:75 provided a more adequate C/N value and a better nutritional balance in the reactors.

Furthermore, it is also possible to argue that elevated quantities of silage mean an overload of fibres in the reactor, negatively affecting biogas production if these fibres have not been efficiently degraded in the pretreatment processes. In this sense, the observation of the axis referring to the T factor in the response surface in Fig. 3 shows that the yield was higher for the level equivalent to 120 days of ensiling. Wellinger et al. [46] explain that the extended ensilage process may promote the breakdown of lignocellulosic

Table 4 Analysis of variance (ANOVA) for methane yield

Source of variation	*SQ*	d.f	*MQ*	F_{cal}	F_{tab}	R^2
Regression	3,157,609	9	350,845.41	6.98	4.77	0.93
Residues	251,334	5	50,266.87			
Lack of fit	240,600	3	80,200	14.94	19.16	
Pure error	10,734	2	5367			
Total	3,408,943	14				

Significance level: 5%

SQ sum squared, *d.f.* degrees of freedom, *MQ* mean square, F_{cal} calculated *F*, $F_{tab:}$ tabulated *F*, R^2 coefficient of determination

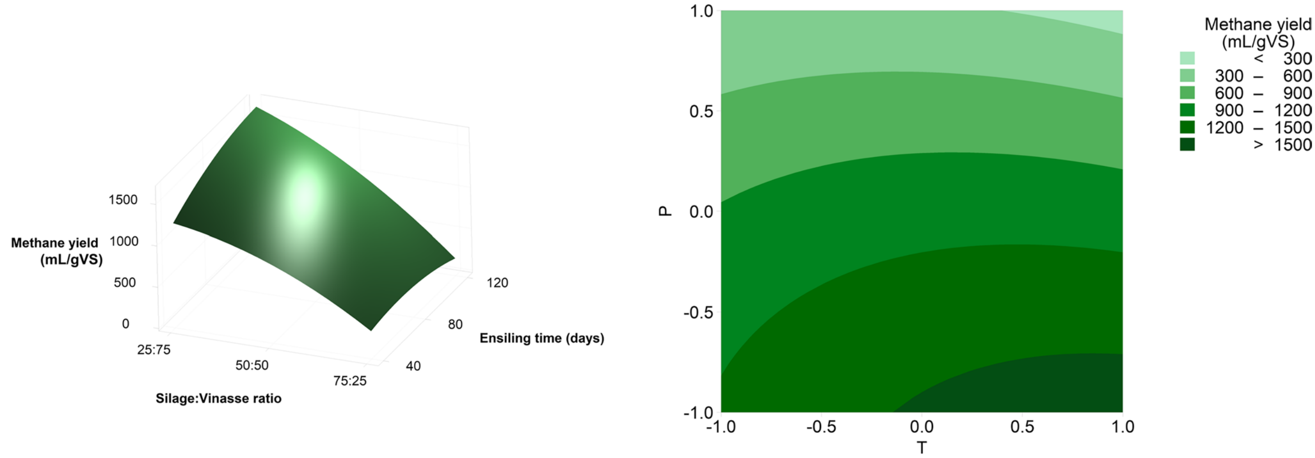

Fig. 3 Surface and contour plots for methane yield (mL/gVS) due to the interaction between factors: proportion between substrates (silage:vinasse) and ensilage time (days)

compounds in elephant grass, leading to improved hydrolysis during anaerobic digestion. Thus, together with other factors, the lengthier ensiling time can contribute to a more efficient consumption of organic matter from the grass.

Figure 4 shows the surface and contour graphs for the NaOH solution used in pretreatment (C) and the ensiling time (T). The interaction range of the average levels of factor C and factor T, using a pretreatment solution of 2.25% and an ensiling time of 80 days, resulted in the best response. These findings suggest that, when only the pretreatments of elephant grass are taken into account and the influence of P is ignored, satisfactory methanogenic production is not affected by the most extreme conditions (4% concentration and 120 days of silage).

Figure 5 presents the surface and contour plots for the pretreatment solution concentration (C) and substrate ratio (P). In this case, the optimum point is located in the interaction between C equal to 4% m/v and P equal to 25:75, reinforcing the analysis of the effect of alkaline treatment and substrate balance on methane yield. The surface curvature on the axis of factor C is not highly pronounced in the range of 2.25–4.00, suggesting that the reactors' performance did not undergo substantial enhancements when transitioning from the intermediate to the maximum level.

Conclusions

The isolated ensiling process was not enough to degrade the elephant grass fibres, requiring the combination of another pretreatment method to enhance methane

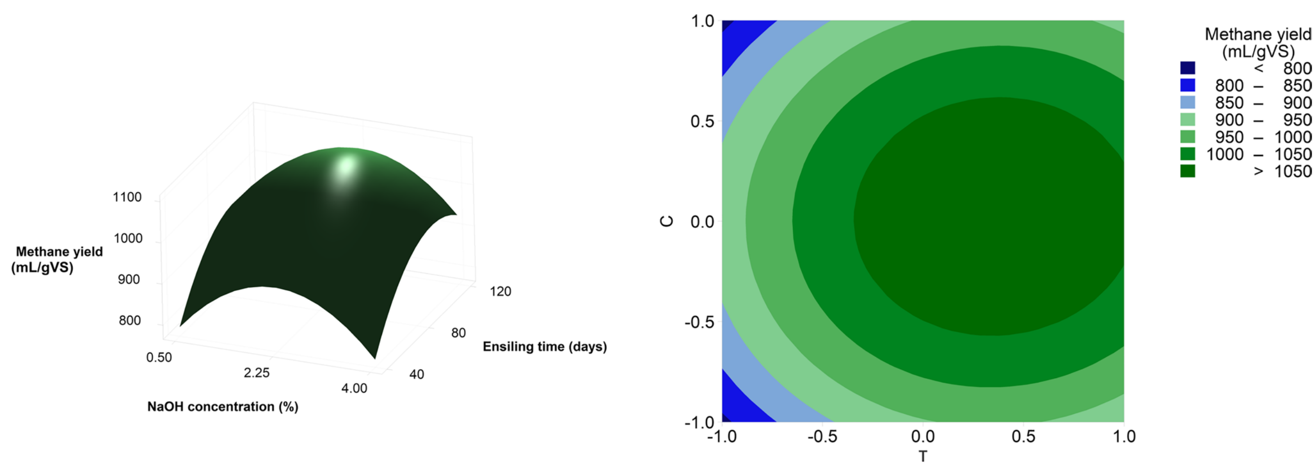

Fig. 4 Surface and contour plots for methane yield (mL/gVS) due to the interaction between factors: NaOH concentration (% w/v) and ensilage time (days)

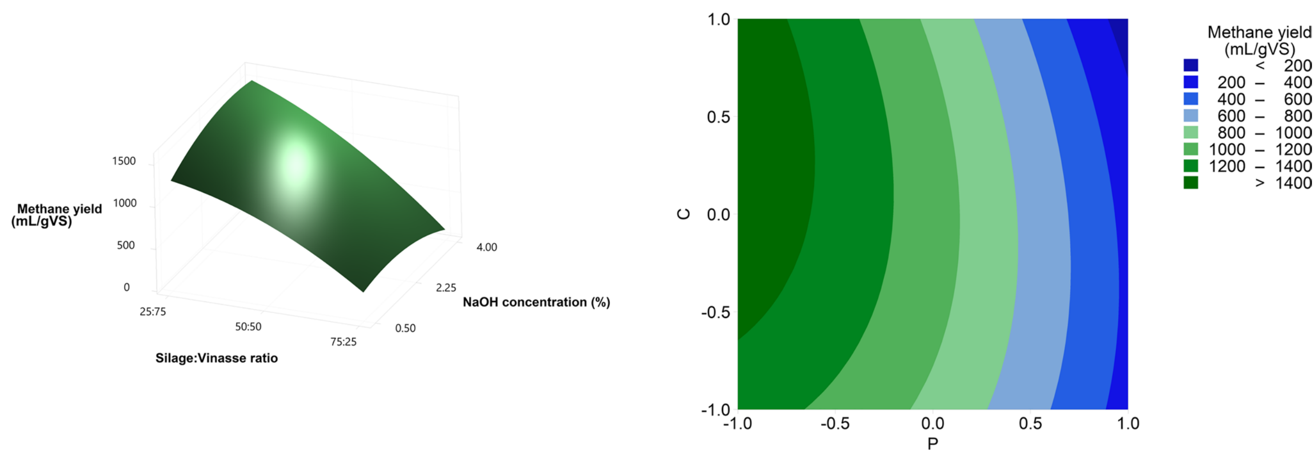

Fig. 5 Surface and contour plots for methane yield (mL/gVS) due to the interaction between factors: proportion between substrates (silage:vinasse) and NaOH concentration (% w/v)

production. Expressive organic matter removals indicate a satisfactory treatment of substrates used. The mixing ratio of elephant grass silage at 25% and vinasse at 75% promoted the highest methane yields, indicating that this configuration should be extensively investigated before increasing the scale of the process. For future works, it is interesting to apply more analyses on the efficiency of ensilage in methanogenic production.

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Author Contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Heloisa Vital Domingos, Thayse Farias de Barros and Dayana de Gusmão Coêlho. Anderson Carlos Marafon and Eduardo Lucena Cavalcante de Amorim are responsible for the laboratories where the study was conducted and for collecting the materials used. The first draft of the manuscript was written by Fernanda Santana Peiter and Taciana Carneiro Chaves, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Archana K, Visckram AS, Senthil Kumar P et al (2024) A review on recent technological breakthroughs in anaerobic digestion of organic biowaste for biogas generation: challenges towards sustainable development goals. Fuel 358:130298. https://doi.org/10.1016/J.FUEL.2023.130298
2. Vasco-Correa J, Khanal S, Manandhar A, Shah A (2018) Anaerobic digestion for bioenergy production: global status, environmental and techno-economic implications, and government policies. Bioresour Technol 247:1015–1026. https://doi.org/10.1016/J.BIORTECH.2017.09.004
3. Moraes BS, Zaiat M, Bonomi A (2015) Anaerobic digestion of vinasse from sugarcane ethanol production in Brazil: challenges and perspectives. Renew Sustain Energy Rev 44:888–903. https://doi.org/10.1016/j.rser.2015.01.023
4. Cremonez PA, Teleken JG, Weiser Meier TR, Alves HJ (2021) Two-Stage anaerobic digestion in agroindustrial waste treatment: a review. J Environ Manage 281:111854. https://doi.org/10.1016/j.jenvman.2020.111854
5. Fuess LT, Kiyuna LSM, Ferraz ADN et al (2017) Thermophilic two-phase anaerobic digestion using an innovative fixed-bed reactor for enhanced organic matter removal and bioenergy recovery from sugarcane vinasse. Appl Energy 189:480–491. https://doi.org/10.1016/J.APENERGY.2016.12.071
6. Almeida WA, Ratusznei SM, Zaiat M, Rodrigues JAD (2017) AnSBBR applied to biomethane production for vinasse treatment: effects of organic loading, feed strategy and temperature. Braz J Chem Eng 34:759–773. https://doi.org/10.1590/0104-6632.20170343s20150584
7. Ziemiński K, Kowalska-Wentel M (2015) Effect of enzymatic pretreatment on anaerobic co-digestion of sugar beet pulp silage and vinasse. Bioresour Technol 180:274–280. https://doi.org/10.1016/J.BIORTECH.2014.12.035
8. Rego GC, Ferreira TB, Ramos LR et al (2022) Bioconversion of pretreated sugarcane vinasse into hydrogen: new perspectives to solve one of the greatest issues of the sugarcane biorefinery. Biomass Convers Biorefin 12:5527–5541. https://doi.org/10.1007/S13399-020-00984-8/METRICS

9. Paterlini EM, Arantes MDC, Gonçalves FG et al (2013) Evaluation of elephant grass for energy use. J Biotechnol Biodivers 4:126–133. https://doi.org/10.20873/jbb.uft.cemaf.v4n2.paterlini
10. de Morais RF, Quesada DM, Reis VM et al (2012) Contribution of biological nitrogen fixation to elephant grass (Pennisetum purpureum Schum.). Plant Soil 356:23–34. https://doi.org/10.1007/S11104-011-0944-2/METRICS
11. Chynoweth DP, Turick CE, Owens JM et al (1993) Biochemical methane potential of biomass and waste feedstocks. Biomass Bioenergy 5:95–111. https://doi.org/10.1016/0961-9534(93)90010-2
12. Gunaseelan VN (2004) Biochemical methane potential of fruits and vegetable solid waste feedstocks. Biomass Bioenergy 26:389–399. https://doi.org/10.1016/J.BIOMBIOE.2003.08.006
13. Tong X, Smith LH, McCarty PL (1990) Methane fermentation of selected lignocellulosic materials. Biomass 21:239–255. https://doi.org/10.1016/0144-4565(90)90075-U
14. Raposo F, De La Rubia MA, Fernández-Cegrí V, Borja R (2012) Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. Renew Sustain Energy Rev 16:861–877. https://doi.org/10.1016/J.RSER.2011.09.008
15. Sawatdeenarunat C, Surendra KC, Takara D et al (2015) Anaerobic digestion of lignocellulosic biomass: challenges and opportunities. Bioresour Technol 178:178–186. https://doi.org/10.1016/J.BIORTECH.2014.09.103
16. Kommula VP, Reddy KO, Shukla M et al (2013) Physico-chemical, tensile, and thermal characterization of Napier grass (Native African) fiber strands. Int J Polym Anal Charact 18:303–314. https://doi.org/10.1080/1023666X.2013.784935
17. Gallego-García M, Moreno AD, Manzanares P et al (2023) Recent advances on physical technologies for the pretreatment of food waste and lignocellulosic residues. Bioresour Technol 369:128397. https://doi.org/10.1016/J.BIORTECH.2022.128397
18. Wen B, Yuan X, Li QX et al (2015) Comparison and evaluation of concurrent saccharification and anaerobic digestion of Napier grass after pretreatment by three microbial consortia. Bioresour Technol 175:102–111. https://doi.org/10.1016/J.BIORTECH.2014.10.043
19. Bhatia P, Fujiwara M, Toda T (2024) Anaerobic digestion of an invasive aquatic plant Ludwigia grandiflora by thermal hydrolysis pretreatment: Correlations between methane kinetics and substrate composition. Fuel 359:130223. https://doi.org/10.1016/J.FUEL.2023.130223
20. Doran PM (2013) Bioprocess engineering principles, 2nd edn. Academic Press, Oxford
21. Cui M, Shen J (2012) Effects of acid and alkaline pretreatments on the biohydrogen production from grass by anaerobic dark fermentation. Int J Hydrogen Energy 37:1120–1124. https://doi.org/10.1016/j.ijhydene.2011.02.078
22. Abraham A, Mathew AK, Park H et al (2020) Pretreatment strategies for enhanced biogas production from lignocellulosic biomass. Bioresour Technol 301:122725. https://doi.org/10.1016/J.BIORTECH.2019.122725
23. Zhang Y, Li L, Kang X et al (2019) Improving methane production from Pennisetum hybrid by monitoring plant height and ensiling pretreatment. Renew Energy 141:57–63. https://doi.org/10.1016/J.RENENE.2019.03.084
24. Pardang P, Sonwai A, Pholchan P et al (2020) Potential of lignin-rich grass, Pennisetum purpureum × Pennisetum typhoideum, as a feedstock for biogas production. J Environ Eng 146:04020074. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001741
25. Sousa SP, Lovato G, Albanez R et al (2019) Improvement of sugarcane stillage (vinasse) anaerobic digestion with cheese whey as its co-substrate: achieving high methane productivity and yield. Appl Biochem Biotechnol 189:987–1006. https://doi.org/10.1007/S12010-019-03056-4/METRICS
26. López González LM, Pereda Reyes I, Romero Romero O (2017) Anaerobic co-digestion of sugarcane press mud with vinasse on methane yield. Waste Manage 68:139–145. https://doi.org/10.1016/J.WASMAN.2017.07.016
27. Muck RE, Nadeau EMG, McAllister TA et al (2018) Silage review: Recent advances and future uses of silage additives. J Dairy Sci 101:3980–4000. https://doi.org/10.3168/JDS.2017-13839
28. Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https://doi.org/10.3168/JDS.S0022-0302(91)78551-2
29. Vuitik GA, Fuess LT, Del Nery V et al (2019) Effects of recirculation in anaerobic baffled reactors. J Water Process Eng 28:36–44. https://doi.org/10.1016/J.JWPE.2018.12.013
30. Wu D, Li L, Zhao X et al (2019) Anaerobic digestion: a review on process monitoring. Renew Sustain Energy Rev 103:1–12. https://doi.org/10.1016/J.RSER.2018.12.039
31. Chaves TC, Gois GNSB, Peiter FS et al (2021) Biohydrogen production in an AFBR using sugarcane molasses. Bioprocess Biosyst Eng 44:307–316. https://doi.org/10.1007/s00449-020-02443-0
32. Jyi-Lay J, Li-You Y, Noike T (1996) Effect of moisture content and chemical nature on methane fermentation characteristics of municipal solid wastes. Doboku Gakkai Ronbunshu 1996:101–108. https://doi.org/10.2208/JSCEJ.1996.552_101
33. Sun H, Cui X, Li R et al (2021) Ensiling process for efficient biogas production from lignocellulosic substrates: methods, mechanisms, and measures. Bioresour Technol 342:125928. https://doi.org/10.1016/J.BIORTECH.2021.125928
34. Ambye-Jensen M, Johansen KS, Didion T et al (2013) Ensiling as biological pretreatment of grass (Festulolium Hykor): the effect of composition, dry matter, and inocula on cellulose convertibility. Biomass Bioenergy 58:303–312. https://doi.org/10.1016/J.BIOMBIOE.2013.08.015
35. Kainthola J, Kalamdhad AS, Goud VV (2019) Enhanced methane production from anaerobic co-digestion of rice straw and Hydrilla verticillata and its kinetic analysis. Biomass Bioenergy 125:8–16. https://doi.org/10.1016/J.BIOMBIOE.2019.04.011
36. Shrestha S, Fonoll X, Khanal SK, Raskin L (2017) Biological strategies for enhanced hydrolysis of lignocellulosic biomass during anaerobic digestion: current status and future perspectives. Bioresour Technol 245:1245–1257. https://doi.org/10.1016/J.BIORTECH.2017.08.089
37. Tena M, Luque B, Perez M, Solera R (2020) Enhanced hydrogen production from sewage sludge by cofermentation with wine vinasse. Int J Hydrogen Energy 45:15977–15984. https://doi.org/10.1016/J.IJHYDENE.2020.04.075
38. Gunaseelan VN (2007) Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition. Bioresour Technol 98:1270–1277. https://doi.org/10.1016/J.BIORTECH.2006.05.014
39. Reddy KO, Maheswari CU, Shukla M, Rajulu AV (2012) Chemical composition and structural characterization of Napier grass fibers. Mater Lett 67:35–38. https://doi.org/10.1016/J.MATLET.2011.09.027
40. Carvalho AR, Fragoso R, Gominho J et al (2016) Water-energy nexus: anaerobic co-digestion with elephant grass hydrolyzate. J Environ Manage 181:48–53. https://doi.org/10.1016/J.JENVMAN.2016.06.012
41. Haryanto A, Hasanudin U, Afrian C, Zulkarnaen I (2018) Biogas production from anaerobic codigestion of cowdung and elephant grass (Pennisetum Purpureum) using batch digester. IOP Conf Ser Earth Environ Sci 141: https://doi.org/10.1088/1755-1315/141/1/012011
42. Himanshu H, Murphy JD, Grant J, O'Kiely P (2018) Synergies from co-digesting grass or clover silages with cattle slurry in

in vitro batch anaerobic digestion. Renew Energy 127:474–480. https://doi.org/10.1016/J.RENENE.2018.04.086
43. Lovato G, Albanez R, Triveloni M et al (2019) Methane production by co-digesting vinasse and whey in an AnSBBR: effect of mixture ratio and feed strategy. Appl Biochem Biotechnol 187:28–46. https://doi.org/10.1007/S12010-018-2802-7/FIGURES/3
44. Ojediran OJ, Dahunsi SO, Aderibigbe V et al (2021) Valorization of Pennisetum purpureum (elephant grass) and piggery manure for energy generation. Fuel 302:121209. https://doi.org/10.1016/J.FUEL.2021.121209
45. Volpi MPC, Brenelli LB, Mockaitis G, et al (2021) Use of lignocellulosic residue from 2G ethanol production to enhance methane production through co-digestion. bioRxiv 2021.02.19.432018. https://doi.org/10.1101/2021.02.19.432018
46. Wellinger A, Murphy J, Baxter D (2013) The biogas handbook: science, production and applications. The Biogas Handbook: Science, Production and Applications 1–476. https://doi.org/10.1533/9780857097415