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Biogas Production from the Anaerobic Co-Digestion of Elephant Grass Juice and Cattle Wastewater: Batch and Semi-Continuous Performance in Anaerobic Reactors

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Abstract

Considering the limited information available on the use of elephant grass juice (EGJ) as a liquid co-substrate, this study aimed to evaluate its feasibility and biogas production potential for anaerobic co-digestion with dairy cattle wastewater (DCW), focusing on substrate ratios and reactor operating conditions. Initially, batch tests were conducted at different temperatures to evaluate the effects of varying substrate mixture ratios (EGJ/DCW). In the reactor with 20% EGJ/80% DCW at 39 °C, a biogas volume of 414.35 mL was generated, which was considerably higher than that in the test at 25 °C, where 258.7 mL was generated over the 30-day experimental period. Greater efficiency in organic matter removal and other analyzed parameters was also observed under these conditions. The best configuration was selected for the semi-continuous tests. To this end, an Upflow Anaerobic Sludge Blanket (UASB) reactor was operated with hydraulic retention times of 10, 6, 2, and 1 d and volumetric organic loading rates of 2.06, 3.39, 10.40, and 20.70 kg VS m^{-3} d^{-1}, respectively. Volatile solids were removed by 29–58%, with biogas yields ranging from 0.78 to 2.28 m^3 m^{-3} d^{-1} and a maximum CH_4 concentration of 72% (v/v). The co-digestion of the analyzed agricultural waste substrates proved to be a promising approach for bioenergy recovery. These results provide useful guidelines for optimizing co-digestion systems, improving reactor performance, and waste treatment processes.

Keywords: bioenergy; anaerobic digestion; residual energy; fermentation; lignocellulosic biomass

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1. Introduction

Anaerobic digestion (AD) is a promising technology that has been successfully applied to a variety of biomass types, such as municipal, agricultural, and industrial wastewater with high organic load and solids [1–4]. Process performance depends on the operational strategies and process parameters. Operational strategies, such as reactor type, inoculum and substrate used, hydraulic retention time (HRT), feed rate, and operating temperature, are fundamental to the proper functioning of biodigesters [5]. However, process parameters, such as pH, alkalinity, and concentrations of volatile fatty acids (VFAs), must be considered and controlled to ensure optimal fermentation and microbial metabolism [6].

AD is predominantly applied to the treatment of single substrates, also known as mono-digestion. However, anaerobic mono-digestion generally exhibits low energy productivity because of insufficient organic matter content and a low carbon-to-nitrogen (C:N) ratio [7]. These factors lead to the formation of inhibitors and toxic compounds, such as ammonia (NH_3) and hydrogen sulfide (H_2S), resulting in low biogas yields [8,9]. In addition, substrate characteristics, such as lignin content and the presence of readily degradable sugars, may affect biodegradability and process stability by promoting VFA accumulation and reactor acidification [5]. In this regard, anaerobic co-digestion is a promising approach to address the challenges related to the AD process. The main benefits of co-digestion include greater system stability and methane yield through the synergistic effects of promoting a more diverse microbial community, an appropriate C:N ratio, and the dilution of toxic compounds, including heavy metals [10,11]. Furthermore, co-digestion digestate increases the bioavailability of nutrients when used as biofertilizers or in other agricultural applications [12].

Karki et al. [7] reviewed the current status and prospects of anaerobic co-digestion. They noted that this process has great potential to improve the digestibility of various feedstocks for waste management, bioenergy generation, and other high-value products. In addition, this technology offers several advantages, including synergistic effects among microorganisms, improved nutrient balance, increased organic loading capacity, dilution of toxic substances, enhanced methane yield, and economic benefits [13]. Mehdizadeh et al. [13] further highlighted the need to develop new approaches to characterize raw materials as substrates and to better understand the dynamics of the microbial community and the pathways involved in substrate degradation. Although anaerobic co-digestion has been widely investigated, the reported results vary considerably depending on the substrate combinations and mixing ratios evaluated. Overall, biogas production has generally been enhanced through the co-digestion of different substrates; however, the optimal substrate ratio is substrate-specific. Further research is needed because many potential substrate combinations have not yet been thoroughly evaluated. In this context, two factors deserve further investigation: (i) the use and type of co-substrates and (ii) the mixing ratio for optimal reactor performance.

The use of cattle manure in bioenergy systems is a sustainable example of the transition from a linear to a circular economy [6]. Ruminant manure, especially bovine manure, is a good substrate with energy potential for biogas production because of the presence of methanogenic microorganisms as an inoculum in the fermentation phase [14]. Traditionally, animal manure has been the main substrate in anaerobic processes. However, its low carbon (C) concentration and high nitrogen (N) content can create a C deficit that is detrimental to the process in terms of the C:N ratio [15]. Therefore, the co-digestion of cattle manure with carbon-rich substrates can improve the balance of nutrients and organic matter, potentially enhancing anaerobic digestion performance [16].

Biogas production by AD from solid organic waste, such as lignocellulosic biomass, offers significant advantages from the perspective of renewable energy production and waste management [17]. In Brazil, elephant grass (*Cenchrus purpureus* (Schumach) Morrone) is found throughout the country. The species is well-adapted to the local climate and soil conditions and is traditionally used for animal feed, in addition to being considered an attractive bioresource for application in various fields, such as agriculture, the energy sector, and materials production [18,19]. The main advantages of extracting grass juice include reduced moisture content and increased calorific value of the biomass, as well as the possibility of using the juice for the production of biogas and biofertilizers [20]. The remaining grass juice is a byproduct of the bioenergy chain, derived from the thermal energy generated by burning plant biomass. Therefore, its use as a co-substrate for anaerobic digestion provides an alternative route for the valorization of this by-product, reducing its disposal as a residual stream while enabling the recovery of renewable energy and nutrients. The extraction process in mills can be highly efficient and economically and technically viable, considerably reducing the time and energy consumption of the elephant grass bagasse drying process [21].

Although several studies have investigated the anaerobic co-digestion of lignocellulosic biomass with animal manure, limited information is available on the use of EGJ as a liquid co-substrate in this process. In particular, the potential of this by-product as a value-added substrate for methane production remains largely unexplored. Therefore, this study aimed to evaluate the feasibility and biogas production potential of elephant grass juice residue as a co-substrate with dairy cattle wastewater, focusing on substrate ratios and reactor operating conditions. Batch anaerobic co-digestion of the two substrates was conducted to assess biogas production and process efficiency under different conditions. Subsequently, the study was conducted under semi-continuous feeding conditions in a UASB reactor at different HRTs. The UASB reactor was selected because both co-substrates (EGJ and DCW) were liquid, allowing operation under continuous upflow conditions without the need for mechanical mixing. This reactor configuration is well suited for the anaerobic treatment of low-solids liquid wastes [7].

2. Materials and Methods

2.1. Sampling, Preparation of Substrates and Inoculum

Samples of the BRS Capiaçu elephant grass cultivar were collected from an experimental plot at the Embrapa Gado de Leite research unit in Juiz de Fora, MG, Brazil. The grasses were cut at a height of 0.25–0.28 m above the soil surface using pruning shears 125 days after planting. After collection, the fresh weight of the plants was measured to assess biomass productivity. These harvesting conditions were selected based on previous evaluations of the BRS Capiaçu cultivar, in which this cutting stage was characterized in terms of biomass productivity, physicochemical composition, including moisture content, and energy potential for biogas production [20]. The stalks were then pressed in a semi-industrial mill with stainless steel rollers (Cana Shop Elétrica 200, Brazil, Figure 1a). EGJ was collected, and its volumetric yield was evaluated; the mass of the pressed bagasse was also measured, resulting in the juice volume-to-mass ratio (mL kg^{-1}). Subsequently, the samples were stored in a freezer (Figure 1b).

Dairy cattle wastewater (DCW), used as a co-substrate in the experiments, originated from the José Henrique Bruschi Experimental Field, belonging to Embrapa Dairy Cattle, located in Coronel Pacheco, MG, Brazil (21°33′58″ S; 43°15′21″ W and altitude of 445 m) (Figure 1c,d). The region has a tropical climate (Cwa, Köppen–Geiger classification), with a mean annual temperature of 22 °C, temperatures ranging from 18 to 35 °C, and an average annual rainfall of 1516 mm. The samples were collected and preserved in a properly sealed container and transported to the laboratory, where they were homogenized manually, and the substrate was prepared by diluting bovine feces with water used to wash the floors of the "free stall" system in the experimental station pens. EGJ and DCW samples were collected throughout the experimental period, as required for reactor feeding. The inoculum used in the experiment was prepared from the same homogenized DCW sample according to the methodology described by Steinmetz et al. [22].

Table 1 presents the results of the analyses performed on the substrates (DCW and EGJ) and the inoculum for the two tests. Tests I and II were conducted at different times, with the substrates and inoculum collected from the same sources but at different sampling times. Therefore, slight variations in their physicochemical characteristics are expected due to the inherent variability of biological materials.

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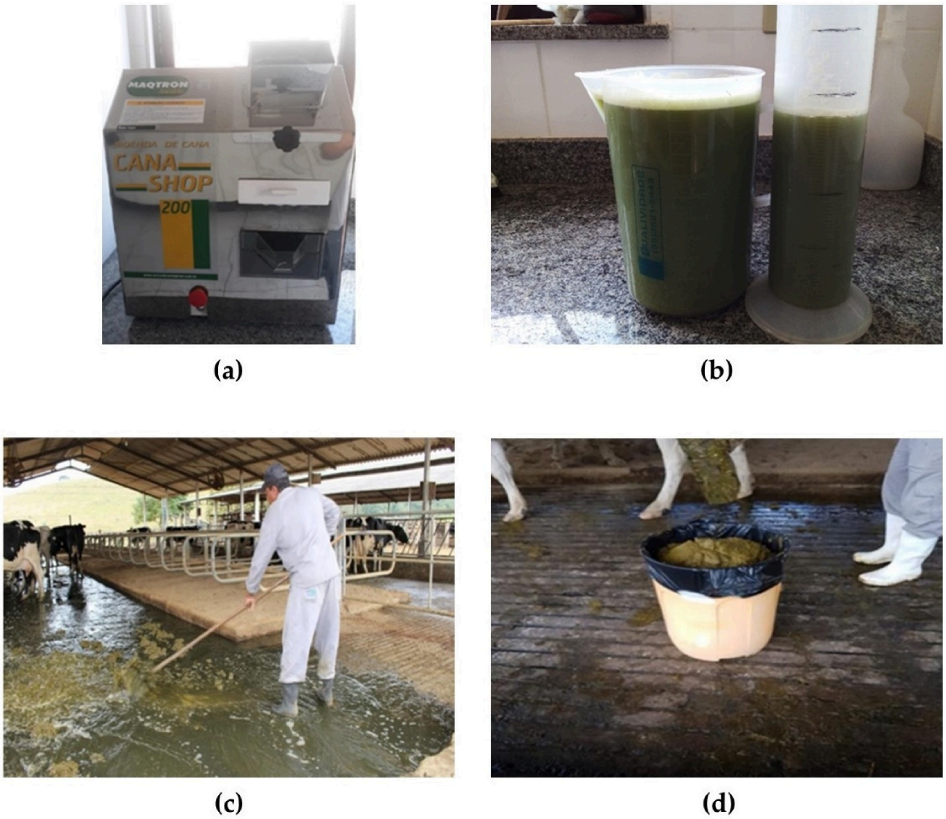

Figure 1. (**a**) semi-industrial mill with stainless steel rollers; (**b**) juice was collected; (**c**) collection of wash water from the "free stall" floors; (**d**) fresh cattle manure.

Table 1. Physicochemical characteristics of the substrates used in the experiment.

Parameter	Test I			Test II		
	DCW	**EGJ**	**Inoculum**	**DCW**	**EGJ**	**Inoculum**
pH	7.63 (0.07)	5.65 (0.01)	7.16 (0.01)	7.61 (0.02)	5.51 (0)	7.24 (0.01)
°Brix	2.45 (0.05)	4.30 (0.3)	2.20 (0.2)	2.60 (0.1)	4.25 (0.05)	2.30 (0.1)
AC (mg L^{-1} as $CaCO_3$)	320 (0.2)	860 (0.3)	440 (0.2)	360 (0.1)	1020 (0.1)	460 (0.1)
TA (mg L^{-1} as $CaCO_3$)	8280 (1.4)	2300 (0.9)	5120 (0.2)	6320 (1.4)	3220 (0.5)	5580 (1.1)
AC/TA ratio	0.04	0.37	0.09	0.06	0.32	0.08
TS (mg L^{-1})	71,876 (0.05)	43,514 (0.07)	22,620(0.04)	71,789 (0.19)	44,227 (0.02)	23,468 (0.22)
FS (mg L^{-1})	14,738 (0.01)	16,518 (0.2)	5153 (0)	14,740 (0.03)	16,717 (0.01)	5702 (0.03)
VS (mg L^{-1})	57,138 (0.06)	26,996 (0.27)	17,466 (0.04)	57,049 (0.16)	27,509 (0.30)	17,766 (0.24)
CP (mg L^{-1})	6600 (0.03)	2800 (0)	3650 (0.01)	7500 (0.05)	3200 (0.02)	3550 (0.01)
Nitrogen (mg L^{-1})	1056 (0)	448 (0)	584 (0)	1200 (0.01)	512 (0)	568 (0)
Organic Carbon (mg L^{-1})	20,955 (0.03)	6999 (0.12)	2587 (0.02)	20,914 (0.07)	7237 (0.01)	2726 (0.11)
C:N	19.8	15.6	4.4	17.4	14.1	4.8
VFA (mg L^{-1})	1768 (97)	2225 (94)	758 (9)	1574 (97)	2413 (94)	740 (9)
Acetate (mg L^{-1})	1139 (26.5)	80 (4)	239 (5)	1086 (26.5)	88 (4)	249 (5)
Propionate (mg L^{-1})	167 (0.5)	388 (27)	321 (10)	168 (0.5)	334 (27)	301 (10)
Butyrate (mg L^{-1})	462 (71)	1757 (117)	198 (4)	320 (71)	1991 (117)	190 (4)

Values in parentheses indicate standard deviation.

2.2. Batch and UASB Reactor Tests: Biogas Heating, Agitation, and Storage System

For the batch tests, the experimental conditions were divided into two distinct and sequential tests, which involved the use of EGJ and DCW in different mixing ratios. The ratios for the experiment were defined as the minimum, intermediate, and maximum amount of EGJ added for co-digestion.

The difference between the tests was the temperature used. Test I was conducted at 25 °C, which is considered representative of the ambient temperature. In Test II, the temperature was maintained at 39 °C, which was near the upper end of the mesophilic range. Experimental bench-scale digesters consisting of 100 mL glass flasks were used, with a working volume of 60 mL and a headspace of 40 mL. The working volume consisted of 54 mL of substrate mixture and 6 mL of inoculum, corresponding to a substrate-to-inoculum ratio of 90:10 (v/v). The proportions of EGJ and DCW varied according to each treatment, as summarized in Table 2. Each experimental condition was conducted in duplicate. In the study, 12 digesters were used in each test, distributed as follows: replicates of mixtures with different proportions of EGJ relative to DCW (20%/80% (A), 40%/60% (B), 80%/20% (C), 100%/0% (D), and 0%/100% (E)), and a negative control (F) containing only inoculum, which was used to correct for endogenous methane production.

Table 2. Proportion of elephant grass juice (EGJ) and dairy cattle wastewater (DCW) (%) (EGJ/DCW) for Tests I and II.

Reactors	EGJ/DCW	Total Volume (mL)	Reaction Volume (mL)	EGJ (mL)	DCW (mL)	Inoculum (mL)
A	20/80	100	60	10.8	43.2	6
B	40/60	100	60	21.6	32.4	6
C	80/20	100	60	43.2	10.8	6
D	100/0	100	60	54	0	6
E	0/100	100	60	0	54	6
F	control	100	60	0	0	6

The reactors were purged with N_2 for 2 min to create an anaerobic environment. The flasks were placed in a temperature-controlled bench shaker (Tecnal Model BTC-9090, Piracicaba, SP, Brazil) to maintain a temperature of 25 °C (Test I) and 39 °C (Test II), with agitation controlled at 100 rpm.

The incubation period for this analysis was 30 days. However, when daily methane production fell below 1.5 mL for three consecutive days, the tests were terminated [23,24]. Biogas measurement in the tests was performed using a system of communicating vessels. A 0.5–1.5 mL sample of the gas was collected using an Agilent 5190-1545 syringe (Agilent Technologies, Santa Clara, CA, USA) and then injected into the gas chromatograph.

To assess the reproducibility of the results obtained in the batch tests (Tests I and II), the best co-digestion result among the mixtures (A, B, or C) was replicated in a biodigester operated in semi-continuous mode. To this end, a UASB-type biodigester was constructed from acrylic material and equipped with an inlet valve and an effluent outlet valve. The reactor was fitted with a water jacket to aid in temperature control and had a gas outlet at the top, which was connected by a hose to the gas meter. The UASB reactor had the following dimensions: 56 cm in height, 15 cm in diameter, a total volume of 9.9 L, and a nominal capacity of 7.8 L (Figure 2a).

The heating system was maintained using a thermostat (model Ace Pet X-618, Brazil), heater set to 37 °C and a submersible pump for water circulation; both devices were installed inside the water jacket surrounding the UASB reactor. The temperature was monitored using a digital thermometer (LDT-8007A, Wenmeice, China). The biodigester was fed using a peristaltic pump with an integrated automatic dosing system (model AWM-5000-A, Provitec, Brazil) operating at a controlled flow rate. The UASB reactor was fed through a valve located at the bottom, configuring the flow as upward, which allows the sludge to remain in suspension with limited mobility within the vertical space of the reactor. The UASB reactor was operated under semi-continuous conditions, with progressively reduced HRTs to evaluate reactor performance under increasing organic loading conditions. The HRTs were selected to assess the response of the reactor to different hydraulic conditions while maintaining the same substrate mixture selected from the batch tests. The corresponding OLRs were determined from the VS concentration of the feed and the applied HRT. Reactor performance was evaluated based on biogas production, methane concentration, and physicochemical parameters, including pH and solids removal.

(a) **(b)**

Figure 2. (**a**) UASB reactor and (**b**) gasometer coupled to the reactor for biogas storage.

The UASB reactor operation was divided into six phases, comprising two start-up phases followed by four semi-continuous operation phases. The two start-up phases were used to establish anaerobic conditions and adequate methane production before continuous feeding. The four subsequent phases involved semi-continuous operation under different HRTs. During these phases, the HRT was progressively reduced from 10 to 1 day, resulting in increasing OLRs while maintaining the same feed composition. This operational strategy was adopted to evaluate the reactor response to progressively shorter HRTs and the corresponding increase in OLR.

The biogas produced in the UASB reactor was directed through a gas hose located at the top to the gasometer, which measured 40 cm in height and 10 cm in diameter and had a total volume of 3.1 L (Figure 2b). The volume of biogas produced was determined by the vertical displacement of the gasometer, which was measured using a self-adhesive ruler attached to the tank. The displacement value was multiplied by the internal cross-sectional area of the gasometer. Samples of 1.5–3 mL were collected using an aid for gas quantification in the chromatograph, and the excess volume was burned. We used the Agilent 7820 A gas chromatograph system and the EzChrom Elite interface software (version 3.3.2, Agilent Technologies, Santa Clara, CA, USA) or the determination of methane production and biogas composition, equipped with a thermal conductivity detector (TCD) and a flame ionization detector (FID) [25]. All biogas volumes were normalized to standard temperature and pressure (STP; 273 K and 1013 hPa). The biochemical biogas potential (BBP) and biochemical methane potential (BMP) were calculated after correction for the inoculum contribution using control reactors containing only inoculum. The BBP and BMP values were determined as the net biogas or methane production of the substrate by subtracting the gas produced in the control reactors from that produced in the substrate reactors and normalizing the result by the amount of volatile solids added [23,24]. Positive

BMP values indicate net methane production by the substrate after correction for the inoculum contribution, whereas negative values indicate an antagonistic (inhibitory) effect, that is, the substrate mixture produced less methane than the inoculum control.

2.3. Statistical Analysis

Batch experiments were performed in duplicate for each treatment (reactors A–F) in both Test I and Test II. Statistical analysis was performed for cumulative biogas and cumulative methane volumes. Differences among reactor means were evaluated using analysis of variance (ANOVA) followed by Tukey's test for a mean comparison at a 5% significance level (α = 0.05) using the PAST 4.03 software. Reactor F (inoculum only) was used as the control to correct the BBP and BMP values and was not included in the statistical comparison among the substrate treatments (A–E).

2.4. Analytical Methods

Physical and chemical analyses for the characterization of the EGJ, DCW, and inoculum substrates, as well as the initial and final samples from the batch tests and the UASB reactor test, were performed according to standardized methods. Table 3 presents the parameters analyzed and methodologies used throughout the experiment.

Table 3. Analyzed parameters, methods employed, and references for the analyses performed during the experiment.

Analysis	Equipment or Method	Reference
pH	Direct reading, using a Hanna Edge HI2002-02 digital potentiometer (Hanna Instruments, Woonsocket, RI, USA)	[26]
Total soluble solids (°Brix)	The °Brix value was quantified using a digital refractometer, model MA871 (Milwaukee Instruments, Rocky Mount, NC, USA)	[27]
Total solids (TS); Volatile solids (VS); Fixed solids (FS)	Gravimetric methods using oven drying at 105 °C for TS determination and muffle furnace incineration at 550 °C for FS determination. VS was calculated as the difference between TS and FS.	[26]
Acidity (AC); Total alkalinity (TA)	Acid-base titration, using the Digital Magnetic Stirrer with Heating and External Sensor (model SL-92/H, Solab, Piracicaba, Brazil)	[26]
Total nitrogen; Crude protein (CP)	Kjeldahl method	[28]
Organic carbon (OC)	Gravimetric determination of carbon based on organic matter	[29]
Gross energy	Parr Adiabatic Calorimeter (Parr Instrument Company, Moline, IL, USA)	[30]
Volatile fatty acids (VFA)	Waters e2695 HPLC with 2998 PDA detector (Waters Corporation, Milford, MA, USA)	[31–33]
Gas Chromatography	Agilent 7820A Gas Chromatograph-concentrations of CH_4 and CO_2 in biogas	[25]

3. Results and Discussion

3.1. Performance of Experimental Conditions: Test I and Test II

3.1.1. Process Stability Indicators

The physicochemical characteristics of the substrates used in the experimental conditions are presented in Table 1. The values obtained for DCW were within the ranges reported in previous studies using dairy cattle manure in anaerobic processes for biogas production [34,35]. The EGJ had a pH of 5.65 and a °Brix of 4.30, values similar to those observed in other studies [21,36,37]. These characteristics are relevant for interpreting the process stability indicators observed during the experiments.

For all the results presented in this section, reactors are identified based on the substrate composition as follows: A (20% EGJ + 80% DCW), B (40% EGJ + 60% DCW), C (80% EGJ + 20% DCW), D (100% EGJ), E (100% DCW), and F (inoculum control).

The initial and final mean values of pH, acidity, and alkalinity measured before and after the anaerobic digestion process are shown in Tables 4 and 5, respectively, for each experimental condition.

Table 4. pH, acidity, and total alkalinity in Test I at 25 °C.

Reactors	pH		AC (mg L^{-1} as $CaCO_3$)		TA (mg L^{-1} as $CaCO_3$)		AC/TA Ratio	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
A	7.14 (0.05)	7.07 (0.03)	400 (0)	580 (0.22)	6560 (0.6)	10,100 (1.85)	0.06	0.06
B	6.80 (0.01)	6.25 (0.1)	600 (0.2)	3060 (0.29)	4840 (0.6)	5480 (3.85)	0.12	0.56
C	6.58 (0.01)	4.21 (0.02)	520 (0.2)	5900 (1.61)	4020 (0.1)	N/A	0.13	N/A
D	6.32 (0)	4.13 (0.05)	860 (0.1)	6840 (4.58)	2660 (0.7)	N/A	0.32	N/A
E	7.68 (0.02)	7.03 (0.01)	440 (0)	410 (0.09)	9060 (2.7)	10,630 (4.01)	0.05	0.04
F	7.64 (0.03)	7.03 (0.03)	420 (0.1)	430 (0.09)	5480 (1.8)	5050 (1.04)	0.08	0.09

Values in parentheses indicate standard deviation.

Table 5. pH, acidity, and total alkalinity in Test II at 39 °C.

Reactors	pH		AC (mg L^{-1} as $CaCO_3$)		TA (mg L^{-1} as $CaCO_3$)		AC/TA Ratio	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
A	7.31 (0.02)	7.20 (0.05)	410 (0.05)	380 (0.22)	6480 (0.4)	6770 (2.57)	0.06	0.06
B	6.87 (0.07)	5.21 (0.03)	590 (0.15)	2090 (0.36)	4880 (0.4)	5350 (0.99)	0.12	0.4
C	6.58 (0.01)	4.30 (0.02)	580 (0.1)	5140 (3.8)	4040 (0)	N/A	0.14	N/A
D	6.38 (0.22)	4.23 (0.04)	850 (0.15)	4050 (0.68)	2690 (0.55)	N/A	0.32	N/A
E	7.63 (0.04)	7.21 (0.1)	460 (0.1)	120 (0.32)	9200 (2)	9400 (0.6)	0.05	0.02
F	7.58 (0.04)	7.27 (0.16)	400 (0)	200 (0.14)	5460 (1.7)	8680 (3.6)	0.07	0.02

Values in parentheses indicate standard deviation.

The ideal pH range for biogas production, considering the sensitivity of methanogens, is between 6.80 and 7.40 [38], indicating conditions suitable for organic matter degradation and microbial growth [39]. Most of the initial pH values of the samples in reactors (I and II) were close to neutral, except for reactors C and D, which had higher EGJ concentrations.

The anaerobic process resulted in the maintenance or slight decrease in pH in reactors A, E, and F in both tests, remaining within the optimal range for the growth of methanogenic microorganisms. However, in reactors B, C, and D, which had the highest EGJ concentrations, there was a significant decrease, which can be attributed to the accumulation of volatile acids, leading to the acidification of the reactor medium. Methanogenesis, the final stage of AD, is permanently inhibited at pH values below 5.50 [40]. Previous studies on co-digestion have reported that a pH as low as 6.60 can inhibit methanogenic archaeal growth [14].

Reactors B, C, and D exhibited higher final acidity values, which are directly related to the lower pH values at the end of the operation. This finding may be related to the effect of EGJ on reactor acidification. The increase in alkalinity in the final samples was related to the high protein content present in the initial mixtures from the cattle manure, as the decomposition of these proteins increased the alkalinity at the end of the digestion process [41]. Therefore, acidity and alkalinity are important parameters for monitoring

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anaerobic digestion stability. The AC/TA ratio is useful for identifying the potential inhibition that excessive acidification triggers in the AD process.

In addition, intermediate products, such as VFA, are important to monitor, as they are affected by the initial pH, are involved in the metabolic pathway for biogas production and can cause microbial stress at high concentrations. Temperature is crucial during acidogenic fermentation due to its direct involvement in both microbial growth and metabolism. Atasoy et al. [42] highlighted that the selection of the optimal operating temperature is directly related to the system pH, influencing VFA production and, consequently, the activity of methanogenic microorganisms in the anaerobic process. Tables 6 and 7 present the VFA values obtained. The increase in VFA accumulation in reactors B, C, and D in both tests may be due to a metabolic imbalance between acid producers and consumers, which affects acetogenic and methanogenic microorganisms [43]. The acidic microenvironment may result from an increase in VFA concentration in the reactors, which can reduce the buffering capacity. Excess VFA disrupts the balance of the digestion process, thereby inhibiting methanogens [44,45]. Generally, the inhibitory effect of VFA concentrations begins at 2000 mg L^{-1} for acetic acid or 8000 mg L^{-1} for total VFA [46].

Table 6. VFA concentrations in Test I at 25 °C.

Reactors	Initial (mg L^{-1})				Final (mg L^{-1})			
	Acet.	Prop.	But.	VFA	Acet.	Prop.	But.	VFA
A	945 (20.5)	192 (3)	446 (0.5)	1582 (23)	89 (17)	1317 (32)	112 (7)	1518 (22)
B	624 (1)	266 (1.5)	540 (5)	1430 (2.5)	2422 (4)	2022 (26)	921 (35.5)	5365 (65.5)
C	223 (2.5)	360 (5)	898 (23)	1481 (15.5)	1103 (79.5)	131 (6)	1172 (50.5)	2405 (23)
D	63 (7.5)	1107 (5.5)	953 (0.5)	2122 (13.5)	1221 (132.5)	266 (27.5)	1958 (72)	3444 (232)
E	999 (1.5)	189 (3.5)	522 (10)	1709 (15)	21 (1)	1006 (41.5)	364 (36)	1391 (6.5)
F	92 (0.5)	279 (0.5)	109 (2.5)	479 (1.5)	11 (1.5)	10 (3)	11 (0)	32 (4.5)

Acet.—Acetate; Prop.—Propionate; But.—Butyrate. Values in parentheses indicate standard deviation.

Table 7. VFA concentrations in Test II at 39 °C.

Reactors	Initial (mg L^{-1})				Final (mg L^{-1})			
	Acet.	Prop.	But.	VFA	Acet.	Prop.	But.	VFA
A	933 (32.5)	204 (8.5)	443 (3)	1579 (21)	110 (2)	659 (28)	377 (18)	1146 (48)
B	574 (51.5)	26 (6.5)	540 (5)	1374 (53)	1933 (21)	588 (30.5)	2096 (2)	4567 (49.5)
C	200 (25)	382 (16.5)	814 (61.5)	1395 (70)	2170 (109)	208 (22.5)	1917 (148)	4295 (38.5)
D	48 (22.5)	1132 (30.5)	1034 (81.5)	2213 (89.5)	2026 (63.5)	633 (20.5)	2813 (43)	5471 (0)
E	935 (62)	203 (17.5)	471 (41.5)	1608 (86)	29 (0)	41 (7)	28 (23)	98 (30)
F	77 (14.5)	296 (17.5)	118 (6.5)	490 (9.5)	18 (1.5)	2 (0)	0 (0)	20 (1.5)

Acet.—Acetate; Prop.—Propionate; But.—Butyrate. Values in parentheses indicate standard deviation.

Another factor that likely contributed to the difference in VFA reduction between the tests was the temperature. Methanogenic microorganisms operate optimally within a mesophilic temperature range of 35–40 °C, achieving high efficiency in CH_4 production [47]. In this regard, within the mesophilic range, there is active microbial enzyme activity.

The VFA values at the outlet of reactors A, E, and F in Test II ranged from 20 to 1146 mg L^{-1}, showing a range comparable to the indicative values reported by Holliger et al. [48] for well-functioning anaerobic digesters. This similarity suggests that VFA accumulation was generally controlled in these reactors, providing favorable conditions for methanogenic activity and process stability. In AD trials, Sicchieri et al. [49] reported pH values between 7.0 and 8.5, total alkalinity above 3000 mg L^{-1}, and VFA below 1000 mg L^{-1}, values similar to those found in this study in reactors A, E, and F in

Test II. The agreement in these physicochemical parameters indicates a balanced anaerobic environment in these reactors, consistent with favorable process performance.

According to Zhao et al. [50], high VFA concentrations are toxic to microorganisms in AD, resulting in low methane yield. Therefore, maintaining low VFA levels is critical for optimal process performance. In the present study, VFA concentrations in reactors B, C, and D (Tests I and II) were elevated, which may have contributed to inhibition of methanogenic activity and the lower methane production observed in these reactors. Finally, the acetic acid concentration at the start of the experiment decreased in the final samples from reactors A, E, and F (Tests I and II). Thus, VFA did not accumulate in these reactors, having been consumed and converted mainly into CH_4 and CO_2.

3.1.2. Solids Composition and Energy Content

The optimal TS concentration in the AD during batch tests should not exceed 10%, as it is important to ensure adequate mass transfer during the process [51]. The initial TS values in the reactors ranged from 2.25% to 6.55% (Test I) and 2.24% to 6.66% (Test II) (Tables 8 and 9). In the tests, an increase in removal efficiency was observed with increasing temperature. In Test I, the removal rates ranged from 7.3% to 11.8%, whereas in Test II, they ranged from 6.5% to 47.9% in the reactors, indicating a significant increase in removal under mesophilic conditions.

Table 8. Analysis of suspended solids and removal efficiency in Test I at 25 °C.

Reactors	TS (mg L^{-1})			FS (mg L^{-1})			VS (mg L^{-1})		
	Initial	Final	R (%)	Initial	Final	R (%)	Initial	Final	R (%)
A	57,504 (0.14)	52,669 (0)	7.3	12,820 (0)	12,412 (0.01)	3.2	44,684 (0.14)	40,258 (0.02)	10
B	53,061 (0.03)	47,050 (0.21)	11.3	13,043 (0.01)	12,452 (0.01)	4.5	40,019 (0.02)	34,597 (0.2)	13.5
C	43,777 (0.02)	38,891 (0.02)	11.2	14,937 (0.06)	13,902 (0)	6.9	28,839 (0.04)	24,989 (0.02)	13.3
D	40,320 (0.03)	36,163 (0.02)	9.9	14,765 (0.06)	14,095 (0.01)	4.5	25,555 (0.02)	22,069 (0.03)	13.6
E	65,521 (0.06)	57,808 (0.06)	11.8	13,495 (0.08)	12,153 (0.02)	10	52,026 (0.14)	45,655 (0.04)	12.3
F	22,520 (0.03)	20,095 (0.03)	10.8	5540 (0.01)	5154 (0.03)	7	16,980 (0.04)	14,940 (0)	12

R %—Removal. Values in parentheses indicate standard deviation.

Table 9. Analysis of suspended solids and removal efficiency in Test II at 39 °C.

Reactors	TS (mg L^{-1})			FS (mg L^{-1})			VS (mg L^{-1})		
	Initial	Final	R (%)	Initial	Final	R (%)	Initial	Final	R (%)
A	57,474 (0.02)	38,410 (0.04)	33.2	14,082 (0.05)	9782 (0)	30.5	43,392 (0.03)	28,628 (0.04)	34
B	52,146 (0.15)	43,351 (0.13)	16.8	11,780 (0.05)	10,024 (0.07)	14.9	40,366 (0.1)	33,327 (0.06)	17.4
C	44,484 (0.26)	41,578 (0.09)	6.5	15,428 (0.06)	13,483 (0.03)	12.6	29,055 (0.2)	28,095 (0.12)	3.3
D	40,431 (0.23)	37,447 (0.05)	7.4	14,746 (0.32)	13,676 (0.01)	7.2	25,685 (0.09)	23,770 (0.06)	7.5
E	66,604 (0.18)	34,696 (0.02)	47.9	14,558 (0.22)	7336 (0.01)	49.6	52,046 (0.04)	27,360 (0.01)	47.4
F	22,496 (0.06)	15,730 (0.03)	30.1	5724 (0.09)	3387 (0.05)	40.8	16,772 (0.15)	12,344 (0.02)	26.4

R %—Removal. Values in parentheses indicate standard deviation.

Thus, the temperature in the AD affects microbial growth and, consequently, impacts process stability and degradation rate [52]. At high temperatures, VS may be degraded more rapidly, potentially reducing substrate availability and affecting biogas production [53]. The thermophilic temperature range of 45–57 °C is sensitive to the process and may lead to increased ammonia inhibition, which can negatively affect microorganisms [54]. The removal of organic matter in the reactors, expressed as VS, ranged from 10.0 to 13.6% among the different treatments in Test I. However, in Test II, the removal efficiency was higher, reaching values between 3.3 and 47.4%. The lower removal efficiencies observed can be attributed to the higher proportion of EGJ used in the co-digestion, which favored acidification of the medium and, consequently, resulted in lower methane production. Therefore, the mixture ratio of 20% EGJ and 80% DCW for anaerobic co-digestion at mesophilic temperature (reactor A, Test II) may be suitable for both biogas production and organic matter removal.

The EGJ used in the co-digestion mixtures contains total soluble solids, which correspond to the sugars present in the juice, such as sucrose, glucose, and fructose, and non-sugars (organic and inorganic). Such soluble carbohydrates are highly biodegradable in AD [20,36]. In contrast, cellulose, hemicellulose, and lignin are considered insoluble components, as they have a robust structure resistant to biodegradation [55].

The ash content corresponds to the residual inorganic fraction of the substrates after AD. Therefore, higher FS removal (%) is associated with lower ash content in the final substrate, a condition more favorable for biogas production. Furthermore, high ash content reduces the calorific value of the material [56].

Gross energy analysis, or heat of combustion, can be considered an appropriate parameter for evaluating the energy potential of mixtures in anaerobic co-digestion [57]. Gross energy is defined as the total chemical energy present in the sample, obtained by its complete combustion in a bomb calorimeter. The energy balances demonstrated that all reactors showed a reduction in gross energy at the end of the process, indicating energy consumption during co-digestion, with higher conversion efficiency in Test II (7.7% to 47.0%), where the percentages of energy consumed were higher than those observed in Test I (2.0% to 15.3%). Higher energy conversion was observed in Test II, with Reactor A showing the highest energy consumption (40.9%), indicating that the EGJ and DCW ratio may enhance co-digestion performance under mesophilic conditions.

3.1.3. Effect of Co-Digestion on Biogas and Methane Production

The cumulative biogas production from mixtures of EGJ with DCW during the batch tests for each test is shown in Figure 3. Biogas production was higher in reactors with a higher proportion of DCW. The opposite trend was observed as the proportion of EGJ in the mixing ratio increased. In Test I (Figure 3a), reactors C and D had zero biogas production after 27 days.

In Test II (Figure 3b), reactor D was shut down after 16 days, and reactor C after 28 days, according to the predefined criterion of daily methane production below 1.5 mL, as described in the Materials and Methods, as they were no longer producing biogas.

The increase in EGJ content in the mixture resulted in slower biogas production during co-digestion. The proportions in reactors B and C were inefficient in the co-digestion process, and the concentration of EGJ in the mixture made biogas production unfeasible. This can be confirmed by comparing the results with reactor D in the absence of DCW. In this reactor, containing only EGJ and inoculum, biogas production was minimal in both tests, and the process was terminated before the 30-day experimental period.

Although residual EGJ is considered a byproduct of the bioenergy chain and a liquid substrate rich in carbon and minerals, its low pH and high acidity slow down the digestion process owing to increased VFA levels, making it resistant to biological processes [58]. Therefore, the inhibition of EGJ mono-digestion (reactor D) and the reduced performance of co-digestion (reactors B and C) in biogas production in both tests were due to the accumulation of VFA resulting from the breakdown of sugars into organic acids [59]. This resulted in a rapid conversion of organic matter, lowering the pH value in the system, contributing to acidification and causing process instability [60]. High levels of simple sugars can result in the rapid accumulation of VFA in the reactor, low pH, and suppression of methanogenesis.

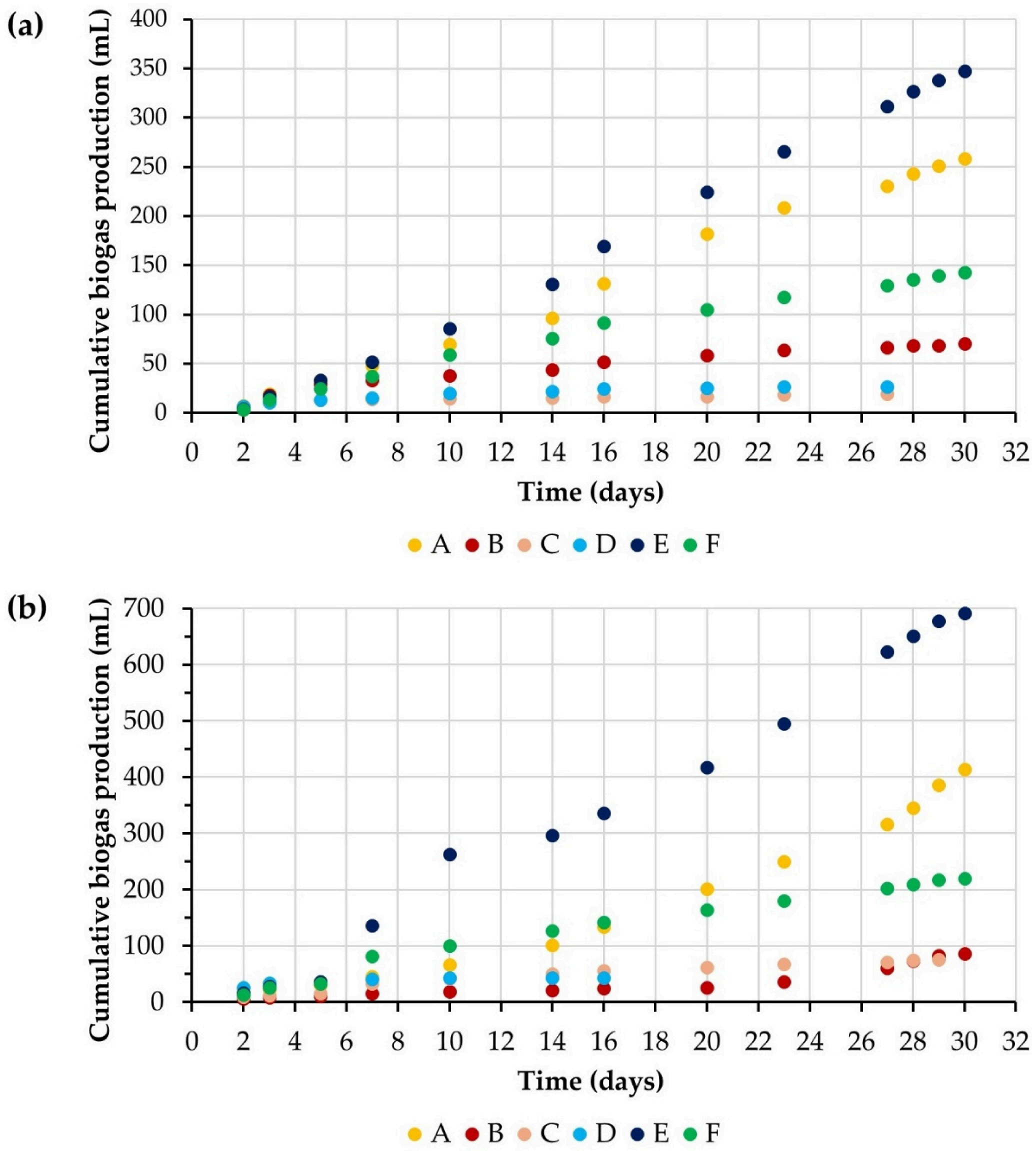

Figure 3. (**a**) Test I at 25 °C; (**b**) Test II at 39 °C. Substrate ratios in the reactors: (A)—20% EGJ/80% DCW; (B)—40% EGJ/60% DCW; (C)—80% EGJ/20% DCW; (D)—100% EGJ/0% DCW; (E)—0% EGJ/100% DCWS; (F)—Inoculum. EGJ—elephant grass juice; DCW—dairy cattle wastewater.

A challenging aspect of the co-digestion process is the ratio of the different substrates. For the balanced operation of anaerobic reactors, it is recommended to mix feedstock containing high amounts of simple carbohydrates with degradable organic waste. In this context, DCW mono-digestion has been widely reported in the literature [34,47,61]. The biogas production values obtained in this study were consistent with previously published data, especially in Test II in Reactor E under mesophilic conditions. In both co-digestion tests, Reactor A (20% EGJ/80% DCW) showed better results in terms of daily and cumulative biogas production. Regarding the co-digestion samples, we observed that increasing the DCW content resulted in faster biogas production due to the highly degradable organic components when a smaller amount of EGJ was added. In this case, the biodegradability and bioavailability at the 20% EGJ/80% DCW ratio favored delayed hydrolysis in the substrates, which ultimately allowed for the optimization of biogas production [62].

Similar trends were demonstrated by Ojediran et al. [63], who showed that elephant grass is a suitable candidate for biogas generation in co-digestion with swine manure. However, the authors used chopped elephant grass as a feedstock, assuming that the chemical and structural composition of the grass provides the nutrients and elemental composition necessary for microbial fermentation for bioenergy production. The maximum volume found by the authors for different proportions of grass and manure was 553.24 mL g^{-1} VS (50:50) and 574.28 mL g^{-1} VS (75:25), respectively.

The accumulated biogas and CH_4 volumes, together with the CH_4 content, are presented in Table 10. The accumulated values of biogas (mL) and CH_4 (mL) in reactor F in each test were used to calculate the BBP and BMP of the other reactors in co-digestion and mono-digestion, as it was considered the control, with only inoculum in the reactors.

Table 10. Biogas and methane production in the reactors.

Reactors	Cumulative Biogas Volume (mL)	Cumulative CH_4 Volume (mL)	Max. CH_4 Content (%)	BBP (mL Biogas g^{-1} VS)	BMP (mL CH_4 g^{-1} VS)
Test I					
A	258.67 $_{(12.12)}$ ^{b}	127.11 $_{(9.07)}$ ^{b}	66.35	94.85	46.14
B	70.52 $_{(3.42)}$ ^{c}	6.58 $_{(0.56)}$ ^{c}	27.3	23.23	0
C	19.36 $_{(1.99)}$ ^{d}	0.05 $_{(0.01)}$ ^{c}	0.91	3.1	0
D	26.88 $_{(1.84)}$ ^{d}	0.02 $_{(0.01)}$ ^{c}	0.7	8.78	0
E	347.65 $_{(16.33)}$ ^{a}	186.58 $_{(10.71)}$ ^{a}	61	110.39	59.1
Test II					
A	414.35 $_{(19.08)}$ ^{b}	213.38 $_{(13)}$ ^{b}	69.15	156.79	80.64
B	86.00 $_{(6.15)}$ ^{c}	8.32 $_{(1.3)}$ ^{c}	33.7	27.61	0
C	75.80 $_{(4.43)}$ ^{c}	1.33 $_{(0.09)}$ ^{c}	4.56	32.8	0
D	43.42 $_{(8.18)}$ ^{d}	0.01 $_{(0.01)}$ ^{c}	0.07	20.3	0
E	691.94 $_{(36.06)}$ ^{a}	389.40 $_{(22.62)}$ ^{a}	65.72	221.7	125.03

Values in parentheses indicate standard deviation. Mean values followed by the same letters in each column and across different tests do not differ statistically from one another according to Tukey's test at a 5% significance level.

The highest accumulated biogas volume for the co-digestion process in Test I and II was 258.67 and 414.35 mL, respectively, both in reactor A. Meanwhile, the lowest values were obtained in reactor C (19.36 mL in Test I and 75.80 mL in Test II). The accumulated volume of CH_4 in co-digestion reactors B and C in both tests was very low. In contrast, reactor A at mesophilic temperature reached a considerable value (213.38 mL). Although mesophilic conditions are generally favorable for methanogenic activity, the comparison between 25 and 39 °C was performed to evaluate the behavior of the EGJ/DCW co-digestion system under different temperature conditions and to identify the most suitable condition for the subsequent semi-continuous operation. The higher biogas production observed in the mono-digestion represented by reactor E may be associated with the greater availability of biodegradable organic matter in the substrate, which has been reported to support anaerobic microbial activity and biogas production [34]. However, although biogas production for mono-digestion was the highest, the maximum CH_4 content (%) found during the 30-day experimental period was lower (61.00%—Test I; 65.72%—Test II) when compared to the values found in Reactor A, operated in co-digestion (66.35%—Test I; 69.15%—Test II), as shown in Table 10. This result supported the selection of the EGJ/DCW co-digestion condition for the subsequent evaluation under semi-continuous operation.

The highest methane yield obtained from co-digestion in this study was higher than that reported by Lianhua et al. [64], who found CH_4 contents of 59.87% and 60.29% through the anaerobic co-digestion of *Pennisetum* hybrid and swine manure, respectively. However, Ojediran et al. [63] reported that the average CH_4 content ranged from 63% to 69%, values close to those found in our study.

Statistical analysis indicated that, at a 95% confidence level, the substrate ratio was a factor with a statistically significant effect on biogas and methane yields. One way to evaluate the role of co-digestion in methane production is to study the synergistic effect of the substrates. This effect may arise from the additional contribution of alkalinity to the medium and from nutrient balance, whereby residues with high protein content are less likely to cause a decrease in pH in BMP tests [23]. The BMP values presented in Table 10 represent the experimental methane yield obtained after correction for inoculum control. Positive BMP values indicate net methane production, as observed in reactor A (20% EGJ/80% DCW) and reactor E (100% DCW, mono-digestion). Therefore, the results suggest that reactor A (Test II) was the best-performing co-digestion treatment, as it showed the highest methane yield among all co-digestion mixtures.

3.1.4. Exponential Regression Models and Variable Interaction

To increase the efficiency of AD performance, the biogas yield under different operating conditions must be predicted to obtain stipulated responses regarding the optimal amount of substrates in the co-digestion mixture. For instance, this study aimed to determine the capacity to support the volume of EGJ material used during biodigester operation and prevent process failures. Exponential regression models were established based on the dataset (Figure 4). The application of regression models and variable interactions to the selected data resulted in two equations, with adjusted R^2 values of 0.952 and 0.938 at 25 °C and 39 °C, respectively.

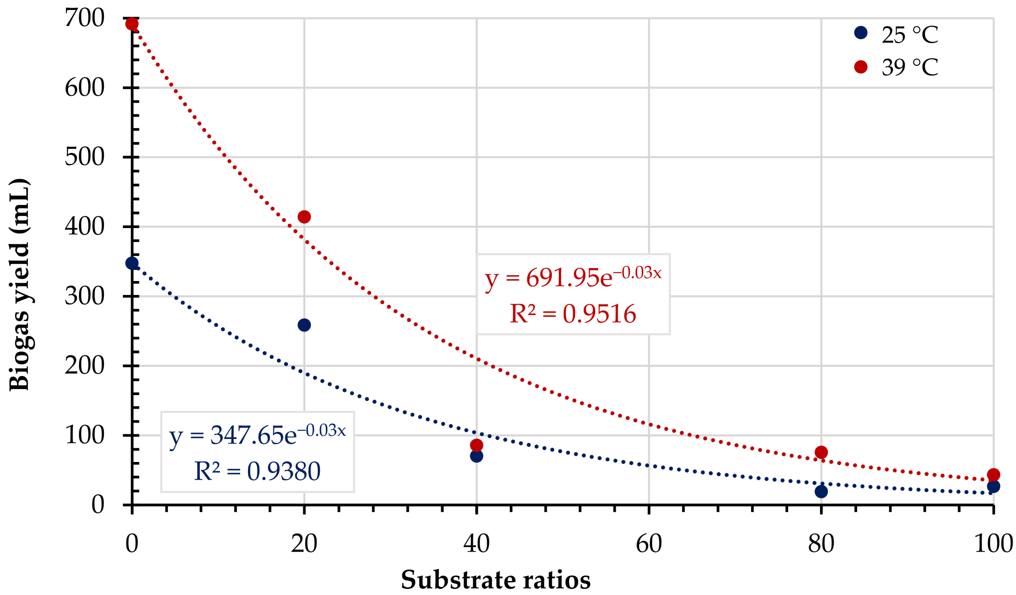

Figure 4. Exponential regression of maximum biogas production in each reactor with their respec-tive EGJ/DCW ratios at different temperatures.

As shown in Figure 4, temperature significantly impacted biogas production during co-digestion, suggesting that it is an important factor for predicting reactor yield. High biogas yields were recorded in reactors A and E at 39 °C compared to ambient temperature. However, an increase in the EGJ concentration resulted in system failure at both temperatures. This suggests the importance of interactions between temperature and substrate ratios when predicting biogas yield.

Other studies have also used regression models to estimate biogas production and other operational factors, considering substrate loading rates and temperature settings [65,66]. These models serve as decision-support tools, providing recommendations on operating conditions and estimates of biogas yield under different scenarios. However, it is noteworthy that the regression models obtained in this study were based on the results from batch studies, which represent the biogas potential of the substrates rather than actual long-term gas production.

3.2. Tests in the UASB Reactor

The substrate mixture concentration in reactor A (20% EGJ/80% DCW) test II yielded the best results in both physicochemical analyses and biogas and methane productivity. In this context, this ratio was used to prepare the mixtures for feeding the UASB reactor.

The UASB reactor was operated in six phases, as shown in Table 11. The first two phases concerned system start-up, and the other four involved semi-continuous flow operation to test different hydraulic retention times. The start-up, that is, the initiation of the anaerobic reactor, was performed first by filling it with DCW. However, after 7 days, chromatographic analysis revealed a low concentration of CH_4 (phase I), and on the same day, a new start-up process was performed by adding 1.56 L of inoculum, approximately 20% of the reactor's capacity (7.8 L). The inoculum used for reactor start-up was the same as that used in the batch assays. After 7 days of the new inoculation (phase II), a methane concentration above 60% was obtained, and the UASB reactor was subsequently fed in a semi-continuous mode (phases III to VI). No OLR was applied during the start-up phases because the reactor was not operated under continuous feeding conditions.

Table 11. Operation of the UASB anaerobic reactor.

Phase—Operation	HRT (d)	Daily Volume (L)	VS (kg m^{-3})	OLR (kg VS m^{-3} d^{-1})	pH
I—Start-up	-	-	-	-	7.62
II—Start-up	-	-	-	-	7.2
III—Semi-continuous	10	0.78	20.67	2.06	7.1
IV—Semi-continuous	6	1.3	20.38	3.39	7.12
V—Semi-continuous	2	3.9	20.8	10.4	7.12
VI—Semi-continuous	1	7.8	20.7	20.7	7.1

HRT—Hydraulic retention time; OLR—Organic loading rate.

The average biogas production and methane concentrations for each experimental phase are shown in Table 12, in addition to the solids removal. Biogas production increased gradually with increasing OLR levels, with daily volumes exceeding 1.0 m^{-3} m^{-3} d^{-1} in the last two phases, V and VI. Lianhua et al. [64] investigated the performance of different mixing ratios and organic loading rates of elephant grass with swine manure in co-digestion and found that as the organic loading rates increased in the digester, the volumetric biogas yield also increased.

Table 12. Solid removal, biogas production, CH_4 concentration.

Phase	TS		VS		Biogas Production (m^3 m^{-3} d^{-1})	CH_4 (%)
	Initial (mg L^{-1})	R (%)	Initial (mg L^{-1})	R (%)		
III	29,844 (0.11)	53	20,672 (0.02)	58	0.90 (0.05)	63 (2.12)
IV	29,030 (0.16)	42	20,383 (0.06)	46	1.22 (0.12)	68 (3.46)
V	30,167 (0.04)	36	20,794 (0.19)	45	1.96 (0.07)	69 (0.64)
VI	29,990 (0.02)	32	20,717 (0.13)	29	2.28 (0.03)	72 (0.40)

R (%)—Removal. Values in parentheses indicate the standard deviation of repeated measurements performed during each operational phase of the same UASB reactor.

Biogas production during phases III–VI ranged from 0.78 to 2.28 m^3 m^{-3} d^{-1} with average CH_4 concentrations of 63% (±2.12) to 72% (±0.40). For carbon dioxide (CO_2), concentrations ranging from 19% (±3.19) to 28% (±2.89) were recorded in semi-continuous operation (Figure 5). The values in parentheses represent the standard deviation of repeated measurements collected during each operational phase of the same UASB reactor.

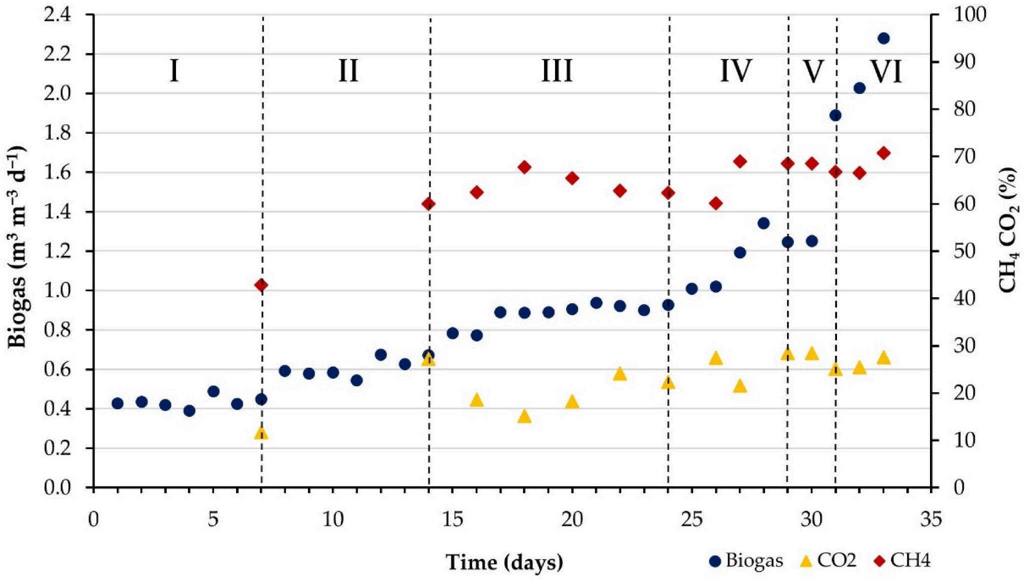

Figure 5. Biogas production and CH_4 and CO_2 concentrations during UASB reactor operation. Phases I and II correspond to reactor start-up, whereas Phases III–VI correspond to semi-continuous operation under different HRTs and OLRs.

Mendonça et al. [61] operated a single-stage UASB anaerobic reactor with cattle manure wastewater and achieved biogas productivity levels of 1.20 and 1.40 $m^3\ m^{-3}\ d^{-1}$ by applying OLR loads of 7.9 and 12.0 kg $m^{-3}\ d^{-1}$, with HRTs of 3 and 2 d, respectively. In comparison, in our co-digestion study (EGJ/DCW), the maximum biogas production was 2.28 $m^3\ m^{-3}\ d^{-1,}$ achieved in phase VI. The VS was removed by 58, 46, 45, and 29%, with corresponding methane yields of 0.46; 0.51; 0.28, and 0.27 m^3 CH_4 kg VS^{-1} removed, respectively, in phases III, IV, V, and VI.

The removal rates of TS and VS decreased with shorter HRTs and higher OLRs (Table 12). This behavior may be related to the higher OLRs and shorter HRTs, which reduced the time available for substrate degradation and may have favored the washout of anaerobic sludge and biological material with the treated effluent. VS removal peaked at 58% in phase III and then decreased successively to a minimum of 29% in phase VI, for initial HRTs of 10 d and final HRTs of 1 d (24 h), respectively. HRT plays a significant role in the treatment of waste and wastewater and in the production of valuable byproducts by microorganisms, as they require a specific amount of time to consume the substrate and synthesize nutrients [67]. Similar values were reported by Mendonça et al. [61] when using cattle manure in a UASB reactor, and by Silva et al. [14], who operated a pilot-scale biodigester (60 L) under mesophilic conditions, also fed with cattle manure.

The C:N ratio of the feed mixtures was set to 15. During anaerobic co-digestion, the average C:N ratio was 7.6, 9.5, 9.2, and 11.6 during the HRT of phases II, IV, V, and VII, respectively. The pH value was 6.97; 7.24; 7.04; and 6.52 in phases II, IV, V, and VII, respectively. The pH values changed over time, but the values remained close to the initial values. Although some decreases in pH occurred, the values remained within the optimal range for the biogas process. The average alkalinity value of the mixtures entering the reactor was 3880 mg L^{-1}, and the values found at the reactor outlet (effluent) in each phase were: 6025, 6840, 6370, and 6300 mg L^{-1}. Alkalinity is defined as the ability of an aqueous solution to neutralize acids; it is a parameter that influences AD performance, and substrates with high alkalinity can maintain an ideal pH value for methane production [67].

The total VFA concentration increased progressively during UASB reactor operation, from 938 mg L^{-1} in the feed mixture to 1553, 1632, 1913, and 2799 mg L^{-1} in Phases III, IV, V, and VI, respectively. This progressive increase may be attributed to the reduction in HRT throughout the operational phases. Despite the higher VFA concentration observed in Phase VI, biogas production and methane concentration were also the highest, likely due to the higher organic loading rate applied during this phase. VFA levels are good indicators of the metabolic state of the AD process and can help detect potential disturbances in the system [33].

The gross energy of the inlet and outlet samples was determined at each HRT to evaluate changes in the energy content of the material during anaerobic digestion. The gross energy of the inlet mixture was 506.24 cal g^{-1}. The outlet samples from the reactor at the 10, 6, 2, and 1 d HRTs were 260.13, 280.72, 377.58, and 341.33 cal g^{-1}. This parameter is considered a key factor for evaluating the energy potential of the mixtures in anaerobic co-digestion. The energy consumed in phase III with an HTR of 10 d was higher, approximately 52%. This was a result of the longer reaction time for AD within the reactor, where biogas increased gradually and occasionally resulted in a higher accumulated biogas volume during this phase. Regarding the energy conversion efficiency in phase VI, the energy consumed was 32.5%, resulting in a higher daily volume and concentration of CH_4 over 24 h.

4. Conclusions

This study investigated the potential for biogas production through the co-digestion of elephant grass juice (EGJ) with dairy cattle wastewater (DCW) in two experimental phases. In the first phase, batch experiments were evaluated in reactors at 25 and 39 °C and with different substrate ratios. Reactor A (20% EGJ/80% DCW) at 39 °C showed good results in the analyzed parameters without significantly affecting the performance of the AD system. Removal values of 33.2% for TS and 34% for VS were found in the reactor. These results indicate that there was a significant removal of organic matter present in the reactor. This reactor achieved a cumulative biogas volume of 414.35 mL with a maximum CH_4 content of approximately 70% and a BMP of 80.64 mL CH_4 g VS^{-1}. Additionally, an exponential regression analysis was applied to the experimental data on the accumulated biogas volume from the reactors at different temperatures. Two equations were obtained with adjusted R^2 values of 0.952 and 0.938, which helped predict values outside the experimental data range.

In the second phase, a 20% EGJ/80% DCW ratio was used to prepare the mixtures for feeding the 7.8 L UASB reactor at different HRTs of 10, 6, 2, and 1 d. The removal of TS and VS increased with higher HRTs. Removal values ranging from 58% to 29% were recorded in semi-continuous operation. In a co-digestion scheme, biogas production ranged from 0.78 to 2.28 m^3 m^{-3} d^{-1} and achieved CH_4 concentrations of 72%. The biogas production yield in co-digestion reinforces the adoption of AD technology for treating animal waste with other feedstocks, such as lignocellulosic residues, demonstrating the technical feasibility of this biotechnology.

The results of this study provide a better understanding of the degradation dynamics of the investigated substrates during batch and semi-continuous anaerobic digestion. However, further studies are needed to evaluate the long-term stability and performance of the process under continuous operation, as well as to assess its scale-up potential. Extended monitoring of reactor performance and additional evaluation of process and energy efficiency would also contribute to a better understanding of the feasibility of applying this co-digestion strategy at larger scales.

The results and conclusions of this study were positive for experimental data in batch and semi-continuous operations, providing an understanding of the degradation dynamics of the investigated substrates during AD and supporting future research, such as analyzing behavior in fully continuously fed reactor systems.

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