Energy Nexus 14 (2024) 100303

Contents lists available at ScienceDirect

Energy Nexus

journal homepage: www.elsevier.com/locate/nexus

Methane production from sugarcane vinasse: The alkalinizing potential of fermentative-sulfidogenic processes in two-stage anaerobic digestion

Renan Coghi Rogeri [a], Lucas Tadeu Fuess [a], Matheus Neves de Araujo [a,*], Felipe Eng [a], André do Vale Borges [a], Márcia Helena Rissato Zamariolli Damianovic [a], Ariovaldo José da Silva [b]

[a] Biological Processes Laboratory, São Carlos School of Engineering, University of São Paulo (LPB/EESC/USP), 1100 João Dagnone Ave, Santa Angelina, São Carlos 13563-120, SP, Brazil

[b] School of Agricultural Engineering (FEAGRI), University of Campinas (Unicamp), Cândido Rondon Ave, 501, Barão Geraldo, Campinas 13083-875, SP, Brazil

ARTICLE INFO

Keywords:
Energy assessment
Methane-rich biogas
Sugarcane biorefinery
Sulfate-free methanogenesis
Vinasse management

ABSTRACT

The two-stage anaerobic digestion (2st-AD) of sugarcane vinasse is widely studied and well-known for improving the energy recovery potential in sugarcane biorefineries. Maintaining enhanced substrate acidification in a separate (first stage) reactor directly improves the performance of methanogenesis (second stage). However, problems derived from the presence of sulfate (SO_4^{2-}) and the subsequent sulfide formation in the second stage are not prevented in conventional 2st-AD systems. In addition, high costs related to reactor alkalinization still represent significant drawbacks in that configuration. The energy recovery potential via methanogenesis was assessed from acidified sugarcane vinasse samples collected from different dark fermentative systems, namely: V1 (subjected to NaOH+$NaHCO_3$ dosing), V2 (subjected to NaOH dosing) and V3 (subjected to no pH control). Despite the harmfulness of sulfide, the enhanced production of acetate from the incomplete oxidation of organic matter in sulfidogenesis can benefit methanogens. The highest methane yield (296.3 NmL-CH_4 g-COD^{-1}) and global energy recovery potential (354,603 GWh per season) were obtained from the lactate and SO_4^{2-}-rich vinasse (V2). Nevertheless, from a technological perspective, the methanogenesis of vinasses subjected to the fermentative-sulfidogenic process (V1) provided a higher quality biogas due to a higher calorific power (26.4-27.0 MJ Nm^{-3}) and decreased H_2S content in the biogas. Finally, the fermentative-sulfidogenic process as an alkalinizing strategy was demonstrated to be the best economic approach for scaling up the 2st-AD of sugarcane vinasse, overcoming the main economic drawback of this configuration.

1. Introduction

Anaerobic digestion (AD) has great potential to reduce some of the most relevant environmental impacts triggered by fertirrigation, i. e. the direct land application of vinasse in sugarcane crops [46]. Using AD decreases the polluting organic load, removes the sulfur compounds (sulfate and sulfide) and preserves the nutritional (mainly the potassium content) characteristics of vinasse. Simultaneously, the produced biogas is rich in methane (CH_4) and, therefore, can be used as a complementary sugarcane-derived biofuel [44–46].

However, the occurrence of sulfate (SO_4^{2-}) exerts some implications in AD, resulting in the reduction of biogas production or increasing the costs of its utilization as a biofuel. Sulfate-reducing bacteria (SRB), the microbial group mediating sulfate reduction, compete with methanogenic *Archaea* (MA) for the same substrates, which means the diversion of the electron flow from methanogenesis to sulfidogenesis, i.e., CH_4

Abbreviations: 2st-AD, two-stage anaerobic digestion; AD, Anaerobic digestion; ANOVA, analysis of variance; AnSTBR, anaerobic structured-bed reactor; bioH_2, biohydrogen; CH, total carbohydrates; CH_4, methane; CO_2, carbon dioxide; CODs, soluble chemical oxygen demand; CODt, total chemical oxygen demand; ER, (general nomenclature) removal efficiency; F/M, food-to-microorganism ratio; GERP, global energy recovery potential; HAc, acetic acid; HBu, butyric acid; HLa, lactic acid; H_2S, hydrogen sulfide; H_2SO_4, sulfuric acid; LCV, lower calorific value; MA, methanogenic archaea; MY, methane yield; NaOH, sodium hydroxide; $NaHCO_3$, sodium bicarbonate; PA, partial alkalinity; SO_4^{2-}, sulfate; SRB, sulfate reducing bacteria; SOB, sulfur oxidizing bacteria; TDS, total dissolved sulfide; TVS, total volatile solids; UV, unit value; V_{CH4}, cumulative methane production; VFA, volatile fatty acids.

* Corresponding author.
E-mail address: matheusnevesaraujo@usp.br (M.N. Araujo).

https://doi.org/10.1016/j.nexus.2024.100303
Received 25 October 2023; Received in revised form 13 April 2024; Accepted 26 April 2024
Available online 30 April 2024

production is impaired [33]. Biogas production can also be impaired by the hydrogen sulfide (H_2S) generated as an end product of SO_4^{2-} reduction, which can cause microbial inhibition due to its highly toxic nature [43,64]. A COD/SO_4^{2-} ratio of 10 is considered critical in terms of soluble H_2S generation which can potentially compromise the activity of MA [37]. In addition, the gas phase H_2S needs to be removed prior to the energetic exploitation of biogas due to its corrosive character, otherwise the useful life of prime movers is severely decreased [11,32,65]. Furthermore, the desulfurization process is pointed out as the main cost of biogas upgrading in Brazilian sugarcane biorefineries [36].

The two-stage anaerobic digestion (2st-AD) of sugarcane vinasse has been highlighted as a great strategy to improve methane production [15,22,54] because optimal operational and environmental conditions can be provided for distinct microbial groups (hydrolytic/fermentative and methanogenic populations). 2st-AD was conceived to overcome the limitations observed in the anaerobic processing of organic-rich wastewater [52]. Therefore, the metabolic imbalance between fast-growing fermenters and slow-growing methanogens caused by organic overloads and toxic compounds can be minimized, providing more stability to the whole system [17].

Diversifying the production of bioenergy can also be achieved through stimulating the production of hydrogen-rich biogas during dark fermentation. Numerous studies have demonstrated sugarcane vinasse as a great feedstock to produce biohydrogen ($bioH_2$) and soluble metabolites in dark fermentative systems [12–14,21,26,23,49,54,55,59,61–63]. Nevertheless, qualitative aspects of vinasses can negatively affect the energy recovery potential of 2st-AD, despite the benefits derived from the production of $bioH_2$ and CH_4, specifically when high SO_4^{2-} concentrations (characterized by a COD/SO_4^{2-} ratio of 10 or less) occur [21,25]. The COD/SO_4^{2-} ratio of fermented vinasse is more critical than that of fresh vinasse because 10–15 % of the organic matter (COD) is removed during fermentation, while no SO_4^{2-} reduction is observed when optimal conditions for $bioH_2$ production (pH = 5.0–5.5) are applied [26,59].

Sulfidogenesis can be successfully established in fermentative reactors processing sugarcane vinasse through applying suitable pH control (pH > 6) [18,26,50,51,59]. In this case, the SO_4^{2-} is reduced and H_2S is formed as an end-product which is stripped to the gas phase along with the biogas biologically produced. The removal of sulfur compounds (i.e., SO_4^{2-} and H_2S) in the first stage (now named "fermentative-sulfidogenic stage") prevents the occurrence of problems related to microbial competition (SRB *vs.* MA) and inhibition in the second stage [42,56], because fermenters are less susceptible to sulfide inhibition than MA and SRB [8,27,43]. The compositional characteristics of biogas from the methanogenic reactor is also improved by both the decrease in H_2S concentrations and the increase in CH_4 content (higher lower calorific value) [19,25,56]. Furthermore, studies with sugarcane vinasse have demonstrated the low contribution of $bioH_2$ for the energy recovery potential of 2st-AD (usually less than 5 %) [15,21,26,22]. Therefore, the optimization of CH_4 production due to the increase in biodegradability of fermented vinasse must be considered the main premise to improve the energy recovery potential of the 2st-AD, necessarily involving the removal of SO_4^{2-} prior to methanogenesis.

Apart from the compositional requirements, using cost-competitive alkalinizing compounds in AD systems represents another relevant strategy to achieve economic feasibility in CH_4 production. Techno-economic studies have demonstrated the alkalinization of methanogenic reactors as the main economic limitation for the scale-up of the 2st-AD of sugarcane vinasse [20,24]. Interestingly, fermentative-sulfidogenic processes can also act as a solution for this limitation by naturally buffering fermented vinasse. SO_4^{2-} reduction coupled with the complete oxidation of organic carbon (e.g. acetate and ethanol) generates bicarbonate alkalinity (i.e., inorganic carbon) [68] as one of the end-products of the reaction, which has the potential to minimize or eliminate the requirements of alkalizing compounds in methanogenesis. Furthermore, sulfide itself acts as a neutralizing agent by consuming the H^+ produced during fermentation [28].

This study aimed to assess the methanogenic potential of fermented sugarcane vinasses with different compositional characteristics resulting from applying different pH control strategies during dark fermentation: NaOH + $NaHCO_3$ dosing (target: SO_4^{2-} reduction; V1); NaOH dosing (target: $bioH_2$ production; V2); and no chemical dosing (target: soluble metabolite production; V3). Because compositional differences have the potential to induce distinct metabolic pathways in anaerobic processes, it is imperative to understand the response of methanogenesis in terms of both the process kinetics and the energy extraction from the substrate (assessed by the methane yield; MY). This study also innovatively evaluates the fermentative-sulfidogenic process as an alkalinizing strategy for methanogenesis. Finally, an energetic and economic analysis of the different alkalinizing strategies used in 2st-AD was carried out to understand the impacts on the global energy recovery potential (GERP) (TWh/season) and the unit value (UV) (US$/GWh) of the CH_4-rich biogas.

2. Material and methods

2.1. Compositional characteristics of fermented sugarcane vinasse

Fermented vinasse samples (V1, V2 and V3) were collected from three thermophilic (55°C) anaerobic structured-bed reactor (AnSTBR) systems subjected to different pH control/alkalinization strategies [59]: V1 was obtained after dosing both sodium hydroxide (NaOH, 50 % m/V) to achieve a pH of 6.5 and sodium bicarbonate ($NaHCO_3$) in a ratio of 0.05-0.15 g-$NaHCO_3$ g-COD^{-1} to prevent enhanced pH drops; V2 was obtained after dosing NaOH (50 % m/V) to maintain an influent pH of 6.5–8.5; and no external pH control/adjustment was carried out in the reactor from which V3 was collected. These strategies aimed to [i] stimulate sulfidogenesis by maintaining a fermentation pH > 6.0 (V1), [ii] optimize $bioH_2$ production reaching a fermentation pH within 5.0–5.5 (V2) and [iii] achieve enhanced lactic acid (HLa) production (V3). The fermented vinasse samples were collected from the outlet of each AnSTBR on day 22 of the operation [59] and were frozen (-20ºC) to preserve the compositional characteristics until the assembly of the batch tests. After unfrozen, each fermented vinasse sample was centrifuged (9000 rpm for 5 min) to remove the excess solids (fermentative biomass). Details of the performance of each reactor and vinasse sources can be found elsewhere [59]. The compositional characteristics of the fermented vinasses are presented in Table 1.

2.2. Experimental setup and monitoring

The inoculum used in this study presented 10.36 % of total volatile solids (TVS) and was obtained from a methanogenic pilot-scale hybrid anaerobic reactor treating sugarcane vinasse under mesophilic conditions. Batch experiments were carried out in Erlenmeyer flasks (BOECO, Hamburg, Germany; 250 mL of working volume) and filled with 80 mL of diluted fermented vinasse to obtain a total chemical oxygen demand (CODt) of approximately 10 g L^{-1}. A food-to-microorganism (F/M) ratio of 0.5 g-CODt g-TVS^{-1} was adopted, as proposed elsewhere [4,60].

Four experimental conditions were assessed to identify the impacts of the different compositional aspects of fermented vinasse on CH_4 production and evaluate the alkalinizing potential of the fermentative-sulfidogenic process: R1, R1-0, R2 and R3. R1 and R1-0 were fed with fermented vinasse V1, whilst V2 and V3 were used to feed R2 and R3, respectively. $NaHCO_3$ was added to the reactors referring to conditions R1, R2 and R3 at a dose of 0.3 g-$NaHCO_3$ g-$CODt^{-1}$ [3,9]. Meanwhile, no alkalinization strategy was carried out in condition R1-0, in which the alkalinizing potential of the fermentative-sulfidogenic process was assessed. The operating parameters of these conditions are summarized in Fig. 1. The reactors were fluxed with nitrogen (N_2) prior to the sealing (with rubber stoppers and plastic caps) to maintain anaerobic conditions. The flasks were placed in a shaker (model Multitron PRO

Table 1
Compositional characterization of the fermented sugarcane vinasses (after centrifugation at 9,000 rpm at 5 min) used as substrate in this study.

AnSTBR [59]		V1	V2	V3
pH control/alkalinization strategy		NaOH + $NaHCO_3$	NaOH	none
Parameters	pH	7.21	4.80	4.07
	CODt [A]	26,967	26,875	29,283
	CODs [A]	26,083	26,800	28,450
	SO_4^{2-} [A]	832	2305	2305
	Total carbohydrates [A]	2217 (9.0%) [B]	2567 (10.2%) [B]	4400 (16.4%) [B]
	Lactic Acid [A]	130 (0.5%) [B]	5008 (19.9%) [B]	4227 (15.9%) [B]
	Acetic Acid [A]	949 (3.9%) [B]	839 (3.3%) [B]	487 (1.8%) [B]
	Propionic Acid [A]	487 (2.8%) [B]	76 (0.4%) [B]	10 (0.05%) [B]
	Butyric Acid [A]	2390 (16.7%) [B]	639 (4.3%) [B]	13 (0.1%) [B]
	Ethanol [A]	1183 (9.5%) [B]	1324 (10.3%) [B]	1136 (8.3%) [B]
	Methanol [A]	174 (1%) [B]	209 (1.2%) [B]	0
	Total Phenols [A]	2353 (12.3%) [B]	2353 (12.3%) [B]	2353 (12.3%) [B]
	$CODt/SO_4^{2-}$	36	11.66	12.7
	$CODs/SO_4^{2-}$	31.3	11.63	12.34

Parameters: CODt = total chemical oxygen demand; CODs = soluble chemical oxygen demand; SO_4^{2-} = sulfate.
Legend:
[A] values in mg L^{-1}
[B] fraction relative to the CODs.

Incubator Shaker e Infors HT, Infors AG, Bottmingen-Basel, Switzerland) with constant agitation (100 rpm) and under mesophilic temperature (37°C). The outlined operating conditions were assessed in triplicate by temporal monitoring of the liquid and gas phases.

The monitoring period (approximately 10 days or 240 hours) was defined based on the stabilization of CH_4 production, characterized by a variation coefficient lower than 5 % for at least 3 points consecutively measured. The biogas production and composition were monitored 3 to 1 time per day based on the response of the system: overall, 3–2 times in the first 48 hours and afterward 1 sample per day. The monitoring of biogas (pressure in the headspace) and calculations of CH_4 production followed the protocol reported elsewhere [60]. Liquid phase monitoring was initially based on the daily collection of a 1 mL-sample until the sixth day of incubation (144h), after which only one additional sample was obtained on day 8 (192h). With this approach, no more than 10 % of the initial liquid volume was taken during the incubation period.

2.3. Analytical methods and response variables

The monitoring of the liquid phase during the experimental runs was carried out exclusively by measuring the soluble chemical oxygen demand (CODs) [2], which was further used in the kinetic assessment. The samples were centrifuged and filtered in 0.45 μm filters (Chromafil GF/PET, Macherey-Nagel GmbH & Co. KG, Düren, Germany) prior to CODs determination. Additionally, the following parameters were assessed at the end of the experimental runs: partial alkalinity (PA) [57], volatile fatty acids (VFA) [31], total phenols [7], pH, SO_4^{2-} and total dissolved sulfide (TDS), the last three parameters based on procedures described in Standard Methods for the Examination of Water and Wastewater [2]. Complementary parameters used to characterize the fermented sugarcane vinasse samples (Table 1) included: total chemical oxygen demand (CODt) [2], total carbohydrates (CH) [10], lactic acid (HLa) [66] and soluble metabolites (VFA + solvents) distribution [1]. Biogas composition was monitored by gas chromatography using a thermal conductivity detector (GC/TCD) and hydrogen as the carrier gas [35].

The main response variables used to assess reactor performance herein included: the removal efficiency of CODs (ER_{CODs}; %) and sulfate (ER_{SO4}; %), the cumulative methane production (V_{CH4}; NmL) and the methane yield (MY; NmL-CH_4 g-$CODs^{-1}$). Kinetic parameters were obtained by fitting selected models to temporal profiles of methane production and organic matter (CODs) consumption. The modified Gompertz model (Eq. (1) [69]) was fitted to methane evolution profiles to obtain the potential methane production (P_{CH4}; NmL), the maximum methane production rate (R_{CH4}; NmL h^{-1}) and the lag phase (λ; h). In Eq. (1), the terms V_{CH4} (t) and *e* are the cumulative methane production as a function of incubation time (t) and the Euler's number, respectively. Regarding the kinetics of organic matter removal, the first order decay model (Eq. (2) [58]) was fitted to the CODs profiles to obtain the first order kinetic constant (k_1; h^{-1}), the initial (C_0^{CODs}) and the residual (C_R^{CODs}) organic matter concentrations. The term C^{CODs} (t) (Eq. (2)) corresponds to the CODs calculated as a function of the incubation period. Model fitting was carried out using the software OriginPro 8 (OriginLab Corporation, USA) through the Levenberg-Marquardt algorithm.

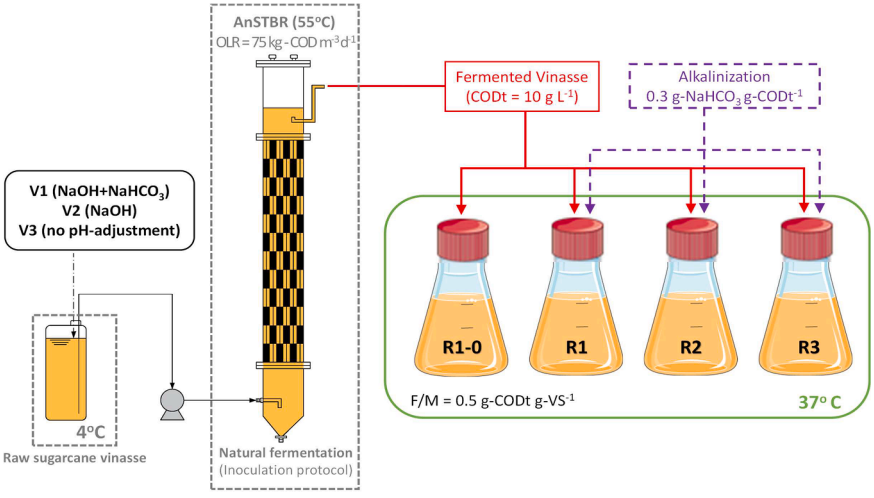

Fig. 1. Basic experimental arrangement and incubation used in the methane-producing tests. Legend: V1, V2 and V3 = types of fermented vinasses, AnSTBR = anaerobic structured-bed reactor, OLR = organic loading rate, R1-0, R1, R2 and R3 = nomenclature of experimental conditions, F/M = food-to-microorganism.

$$V_{CH4}(t) = P_{CH4}\cdot exp\left\{ - \ exp\left[\frac{R_{CH4}\cdot e}{P_{CH4}}(\lambda - 1) + 1\right]\right\} \quad (1)$$

$$C^{CODs}(t) = C_R^{CODs} + \left(C_0^{CODs} - C_R^{CODs}\right)\cdot exp\left(- k1^{CODs}\cdot t\right) \quad (2)$$

2.4. Energy recovery and economic assessment of alkalinization

The volume of biogas produced from vinasse (V_B; Nm^3) in full scale applications of the processes studied herein was calculated according to Eq. (3). The volume of vinasse ($V_{AnVin} = 1837.5\times10^3\ m^3$) generated was determined considering an annexed sugarcane biorefinery producing ca. 205.5×10^3 tons of sugar and $214.5\times10^3\ m^3$ of ethanol per season (200 days) [24]. The remaining parameters used in Eq. (3) were obtained experimentally, including the COD_S in vinasse, the CODs removal efficiency (ER_{CODs}), the methane yield (MY; NmL-CH_4 g-$CODs^{-1}$) and the methane fraction in biogas (F_{CH4}; %). In the particular case of F_{CH4}, the median value observed during the incubation period was used. V_B values were further used to estimate the GERP (thermal + electrical) according to Eq. (4). The lower calorific value (LCV) of biogas (LCV_B; MJ Nm^{-3}) was calculated by multiplying the LCV of methane (35.82 MJ Nm^{-3} [34]) by F_{CH4}. The global energy efficiency of an internal combustion engine (η_{el+th} = 94.5%) (GE Jenbacher, model JMS 620 GS-F12) was adopted, considering electrical (η_{el}) and thermal (η_{th}) conversions of 43.0 % and 51.5%, respectively.

$$V_B\left(Nm^3\right) = \frac{V_{AnVin}\cdot COD_S\cdot ER_{CODs}\cdot MY}{F_{CH4}} \quad (3)$$

$$GERP\ (GWh) = V_B\cdot\eta_{el+th}\cdot LCV_B\cdot10^{-3} \quad (4)$$

The consumption of alkalinizing compounds in vinasse 2st-AD ($NaHCO_3$ and/or NaOH) was calculated based on experimental dose values used in both the fermentative [59] and methanogenic (this study) stages. The market values of 0.32 US$ kg^{-1}NaOH and 0.87 US$ $kg^{-1}NaHCO_3$ were considered, assuming the exchange rate of 0.18 US$ per Brazilian real (R$) observed in May 2020 (Química e [53]). Therefore, total costs related to reactor alkalinization (per season) were obtained, and the unit value (UV; US$ GWh^{-1}) of biogas was calculated for each experimental condition.

2.5. Statistical analysis

The data obtained for V_{CH4}, MY, TDS, F_{CH4}, H_2S fraction in biogas (F_{H2S}) and GERP (dependent variables) were subjected to mean comparison tests to check for statistically significant differences between the different experimental conditions outlined, i.e., R1-0, R1, R2 and R3 (independent variables). This evaluation was based on the procedure presented elsewhere [5]. Initially, tests of normality and homoscedasticity were performed to guarantee the fundamental assumptions for the application of the One-way ANOVA test. Data normality was assessed by the Shapiro-Wilk test or Kolmogorov-Smirnov test (depending on the sample size), while the assumption of data homoscedasticity was assessed by the Levene test. The Tukey HSD test allowed verifying significant differences between the reactors for the dependent variables that satisfied the assumptions of normality (Gaussian probability distribution) and homoscedasticity (variance homogeneity). Bootstrapping procedures (1000 re-samplings; 95 % confidence interval (CI) BCa) were performed to correct deviations from normality in the probability distribution of samples that presented a non-Gaussian probability distribution, as well as to present a CI of 95 % for the differences between the averages to obtain greater statistic reliability [30]. The samples that showed heterogeneity of variance were submitted to Welch correction and multiple comparisons for further evaluation of significant differences by the Games-Howell technique [16].

3. Results and discussion

3.1. Overall performance: kinetics, substrate consumption and methane yield

The temporal profiles for the cumulative CH_4 production and the CODs decay are depicted in Fig. 2. The kinetic parameters of CH_4 production are shown in Table 2, whilst details of the liquid phase performance (including the kinetic parameters obtained for the CODs decay) are presented in Table 3. The main biochemical reactions and respective Gibbs free energy (ΔG^0; kJ mol^{-1}) observed in acetogenesis, sulfidogenesis and methanogenesis are shown in Table 4 for a better understanding of the methane evolution patterns. The methane production (V_{CH4}; terminal values) during the incubation period (Fig. 2a) was statistically different (see Supplementary material) as a direct result of the different compositional aspects. These compositional differences, such as the high butyric acid (HBu; 2390 mg L^{-1}) and lower SO_4^{2-} (832 mg L^{-1}) concentrations in V1, as well as the high HLa (5008–4227 mg L^{-1}) and high SO_4^{2-} (2305 mg L^{-1}) concentrations in V2 and V3 (Table 1) also impacted the kinetics of methane production (Fig. 2a and Table 2). Finally, $NaHCO_3$ dosing before the incubation (R1 *vs.* R1-0) positively impacted the cumulative CH_4 production from sulfate-poor vinasse, also triggering a remarkable difference in the CODs decay during the initial incubation period (0–96h; Fig. 2b).

The CH_4 evolution (statistically equivalent; p-value = 0.214; Supplementary material) and the CODs decay in reactors fed with lactate- and sulfate-rich vinasse (R2 and R3) followed similar kinetic patterns (Fig. 2), with some differences observed in the performance parameters (Table 2 and 3) caused by higher or lower levels of acidification in the dark fermentation. A marked exponential CH_4 production was observed in R2 and R3 up to 48 h (R_{CH4} = 1.86–1.58 NmL h^{-1}; Table 2), followed by a decrease in the production rate towards the end of the incubation (0.84–0.72 NmL h^{-1}; 48–240 h; Table 2). This pattern can be attributed to the activity of incompletely oxidizing SRB through the mediation of HLa oxidation to acetate (HAc) (Reaction 9; Table 4), which is thermodynamically favorable ($\Delta G^0 = -160.1$ kJ.$mol^{-1} < 0$). This promptly supplied substrate for acetoclastic MA (Reaction 19; Table 4), directly enhancing the initial CH_4 evolution. Comparing the methanogenesis of fresh and fermented vinasses with different sulfate levels, Fuess et al. [25] reported the same pattern and highlighted the positive impact of sulfidogenesis to provide HAc for acetoclastic methanogenesis in the biodigestion of sulfate-rich vinasses. The kinetics of CODs consumption for both R2 and R3 presented one single decay pattern (Fig. 2b), showing similar values for k_1 ($1.5–1.7\times10^{-2}\ h^{-1}$; Table 3). However, the slightly higher k_1 value ($1.7\times10^{-2}\ h^{-1}$) observed for the less acidified vinasse condition (R3), characterized by a higher CH content relative to V2 (16.4 *vs.* 10.2 %; Table 1), most likely resulted from a higher fermentative activity, with some diversion of the electron flow towards cell synthesis.

Conversely, the kinetics of CH_4 evolution in the case of sulfate-poor fermented vinasse (V1) was characterized by a higher dependence on the acetogenic activity to convert intermediate metabolites (mainly HBu, which accounted for 16.7 % of the CODs; Table 1) to HAc. Compared to R2 and R3, lower CH_4 production rates (roughly 2-fold lower) were observed in R1 and R1-0 in the initial incubation period (0–48 h: R_{CH4} = 0.95–0.99 NmL h^{-1}; Table 2). Meanwhile, increasing production rates were observed towards the end of the incubation in R1 and R1-0 (72–240 h; R_{CH4} = 1.15–1.53 NmL h^{-1}; Table 2), up to 2-fold higher than in R2 and R3 in the same period (Table 2). The oxidation of HBu to HAc (Reaction 2; Table 4) is thermodynamically unfavorable (ΔG^0 = +48.0 kJ $mol^{-1} > 0$), depending, therefore, on an efficient consumption of HAc (as well as hydrogen) by MA to support an equally efficient CH_4 evolution. It is worth highlighting that methanogenic activity highly depends on an efficient HAc availability to be oxidated to CH_4 through the acetoclastic pathway (Eq. 19; Table 4). Therefore, the higher CH_4 production rates achieved after 72 h of incubation in R1 and

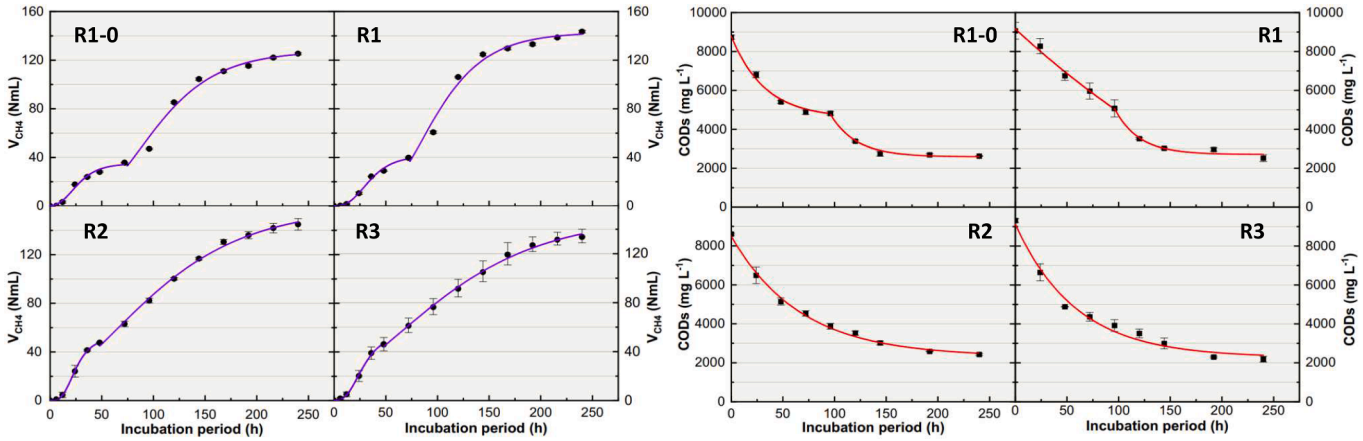

Fig. 2. Temporal profiles experimentally obtained and fitted models for the CH_4 production (V_{CH4}) and CODs decay. Legend: ●, ■ = experimental values, — = fitted models.

Table 2
Kinetic parameters obtained from fitting the modified Gompertz model to cumulative methane production profiles.

Condition	Period (h)	P_{CH4} (NmL-CH_4)	R_{CH4} (NmL-CH_4 h^{-1})	λ (h)	R^2
R1-0	0-72	34.77 ± 1.22	0.95 ± 0.08	8.16 ± 1.37	0.9846
	72-240	127.71 ± 2.8	1.15 ± 0.08	0	0.9779
R1	0-72	41.21 ± 1.27	0.99 ± 0.06	13.12 ± 1.08	0.9928
	72-240	142.82 ± 2.33	1.53 ± 0.1	0	0.9807
R2	0-48	51.17 ± 2.01	1.86 ± 0.14	10.85 ± 0.98	0.9909
	48-240	159.05 ± 3.15	0.84 ± 0.03	0	0.9927
R3	0-48	53.3 ± 5.23	1.58 ± 0.19	10.63 ± 1.8	0.9718
	48-240	152.17 ± 7.75	0.72 ± 0.06	0	0.9653

Kinetic parameters: P_{CH4} = potential methane production (NmL-CH_4); R_{CH4} = maximum methane production rate (NmL-CH_4 h^{-1}); λ = lag phase period (hour). Notes: R1-0 and R1 (sulfate-poor fermented vinasse; V1; NaOH + $NaHCO_3$ dosing in fermentation); R2 (sulfate-rich fermented vinasse; V2; NaOH dosing in fermentation); R3 (sulfate-rich fermented vinasse; V3; no pH adjustment in fermentation). Except for R1-0, $NaHCO_3$ was dosed in all methanogenic reactors.

Table 3
Performance of the liquid phase and kinetic parameters obtained for the CODs decay.

Parameter	Period (h)	Conditions			
		R1-0	R1	R2	R3
C_0^{CODs} (mg L^{-1})	0-96h	8773 ± 98	10,202 ± 892 [A]	8499 ± 106 [B]	9129 ± 181 [B]
	96-240h	4828 ± 56	5072 ± 136		
Cr^{CODs} (mg L^{-1})	0-96h	4385 ± 159	3291 ± 2018 [A]	2317 ± 110 [B]	2275 ± 161 [B]
	96-240h	2617 ± 41	2613 ± 121		
k_1 (h^{-1})× 10^{-2}	0-96h	2.76 ± 0.28	1.38 ± 0.89 [A]	1.50 ± 0.08 [B]	1.70 ± 0.13 [B]
	96-240h	4.71 ± 0.38	4.24 ± 0.70		
R^2	0-96h	0.9894	0.9309 [A]	0.9903 [B]	0.9783 [B]
	96-240h	0.9889	0.9451		
ER-CODs (%)	-	69.9 ± 0.3	72.1 ± 2.8	71.8 ± 0.6	76.5 ± 1.8
pH (-)	Influent	7.87 ± 0.01 [C]	8.11 ± 0.02 [C]	7.50 ± 0.05 [C]	7.16 ± 0.05 [C]
	Effluent	7.29 ± 0.01 [C]	7.31 ± 0.02 [C]	7.20 ± 0.02 [C]	7.07 ± 0.01 [C]
PA (mg-$CaCO_3$ L^{-1})	Influent	282 [D]	931 [D]	483 [D]	308 [D]
	Effluent	1810 ± 73	2152 ± 57	1924 ± 52	1681 ± 37
ER_{SO4} (%)	-	100	100	90 ± 1	89 ± 1
TDS (mg L^{-1})	Effluent	64 ± 1	69 ± 3	121 ± 12	103 ± 8

Kinetic parameters: C_0^{CODs} = initial organic matter concentration (mg-CODs L^{-1}); Cr^{CODs} = residual organic matter concentration (mg-CODs L^{-1}); k_1 = first-order kinetic constant ($h^{-1}\times10^{-2}$); Performance assessment: ER-CODs = CODs removal efficiency (%); PA = partial alkalinity (mg-$CaCO_3$ L^{-1}); ER_{SO4} = SO_4^{2-} removal efficiency (%); TDS = total dissolved sulfide (mg L^{-1}).
Notes:
[A] Assessed for the period between 24 and 96 h
[B] Related to the overall period (0-240h)
[C] Fermented vinasse + anaerobic sludge
[D] Diluted and alkalinized fermented vinasse prior to incubation.

R1-0 (Fig. 2a) most likely resulted from the favoring of HBu oxidation, considering the enhancement of HAc uptake by more active populations of acetoclastic MA.

Despite using the same source of fermented vinasse (V1), the condition R1 presented statistically higher CH_4 production than R1-0 (p-value = 0.00; Supplementary material) in addition to lower organic matter conversion rates, possibly resulting from a higher inorganic carbon availability derived from $NaHCO_3$ dosing. The higher amount of inorganic carbon in R1 (evidenced by the PA, i.e., 931 *vs.* 282 mg-$CaCO_3$ L^{-1}; Table 3) possibly stimulated the occurrence of reductive pathways by providing CO_2, such as hydrogenotrophic methanogenesis (Reaction 20; Table 4) and homoacetogenesis (Reaction 5; Table 4) simultaneously to the oxidative ones i.e., consuming organic carbon sources. Kinetic parameters obtained for the Gompertz model (Table 2) showed a 19 % higher P_{CH4} in the initial incubation period (0–72h; 41.21 *vs.* 34.77 NmL-CH_4) and 33 % higher R_{CH4} at the last one (72–240h; 1.53 *vs.* 1.15 NmL-CH_4 h^{-1}). The differences observed in the CODs decay in the initial incubation period of R1 and R1-0 (Fig. 2b) most likely resulted from the higher availability of inorganic carbon in R1. In this condition, a nearly linear CODs decay was observed, whilst an exponential decay occurred in R1-0. For comparison purposes, the k_1 value estimated for R1-0 was ca. 2-fold higher than that of R1 (2.76 *vs.* 1.38×$10^{-2}$$h^{-1}$; Table 3) in the first 96 h of incubation and slightly higher towards the interruption of the experimental run with 240 h (4.71 *vs.* 4.24×10^{-2}-h^{-1}; Table 3). Hence, despite the similar CH_4 evolution patterns (Fig. 2), hydrogenotrophic methanogenesis played a more significant role in R1 compared to R1-0, evidenced by the lower substrate conversion rates in addition to higher CH_4 production and potential. Because hydrogenotrophic methanogenesis (ΔG^0 = −135.6 kJ mol^{-1}; Table 4) is thermodynamically more favorable than the acetoclastic pathway (ΔG^0

Table 4
Acetogenic, sulfidogenic and methanogenic reactions of interest in the biodigestion of sugarcane vinasse.

Reaction		ΔG^0 (kJ mol^{-1})
Acetogenesis		
1)	$HPr + 3H_2O \rightarrow HAc + H^+ + HCO_3^-$	+76.1 [a,b]
2)	$HBu + 2H_2O \rightarrow 2\ HAc + H^+$	+48.3 [a,b]
3)	$EtOH + H_2O \rightarrow HAc + H^+ + 2H_2$	+9.6 [b]
4)	$HLa + 2H_2O \rightarrow HAc + HCO_3^- + H^+ + 2H_2$	−4.2 [a,b]
Homoacetogenesis		
5)	$2\ HCO_3^- + 4\ H_2 + H^+ \rightarrow HAc + H_2O$	−104.6 [a,b]
Sulfidogenesis (incompletely oxidizing SRB)		
6)	$3\ SO_4^{2-} + 4\ HPr \rightarrow 3HS^- + 4\ HCO_3^- + H^+ + 4\ HAc$	−150.6 [c]
7)	$SO_4^{2-} + 2\ HBu \rightarrow HS^- + H^+ + 4\ HAc$	−55.5 [c]
8)	$SO_4^{2-} + 2\ EtOH \rightarrow HS^- + 2\ H_2O + H^+ + 2\ HAc$	−132.7 [c]
9)	$SO_4^{2-} + 2\ HLa \rightarrow HS^- + 2\ HCO_3^- + H^+ + 2\ HAc$	−160.1 [c]
10)	$SO_4^{2-} + CH \rightarrow HS^- + 2\ HCO_3^- + 3H^+ + 2\ HAc$	−358.0 [c]
Sulfidogenesis (completely oxidizing SRB)		
11)	$SO_4^{2-} + HAc \rightarrow HS^- + 2\ HCO_3^-$	−47.3 [c]
12)	$7\ SO_4^{2-} + 4\ HPr \rightarrow 7\ HS^- + 12\ HCO_3^- + H^+$	−341.0 [c]
13)	$10\ SO_4^{2-} + 4\ HBu \rightarrow 10\ HS^- + 16\ HCO_3^- + 2H^+$	−492.0 [c]
14)	$3\ SO_4^{2-} + 2\ EtOH \rightarrow 3\ HS^- + 4\ HCO_3^- + H^+ + 2\ H_2O$	−227.3 [c]
15)	$3\ SO_4^{2-} + 2\ HLa \rightarrow 3\ HS^- + 6\ HCO_3^- + H^+$	−255.3 [c]
16)	$3\ SO_4^{2-} + CH \rightarrow 3\ HS^- + 6\ HCO_3^- + 3\ H^+$	−452.5 [c]
17)	$3\ SO_4^{2-} + 4\ MeOH \rightarrow 3\ HS^- + 4\ HCO_3^- + 4\ H_2O + 4\ H^+$	−361.7 [c]
Sulfidogenesis (hydrogenotrophic SRB)		
18)	$SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$	−151.9 [b]
Acetoclastic methanogenesis		
19)	$HAc + H_2O \rightarrow HCO_3^- + CH_4$	−31.0 [a,b]
Hydrogenotrophic methanogenesis		
20)	$HCO_3^- + 4\ H_2 + H^+ \rightarrow CH_4 + 3H_2O$	−135.6 [a,b]

References:
[a] Muyzer and Stams [48]
[b] Harper and Pohland [29]
[c] Zhou and Xing [68].
Notes: CH-carbohydrates; EtOH-ethanol; HAc-Acetate; HBu-butyrate; HLa-lactate; HPr-propionate; MeOH-methanol

= −31 kJ mol^{-1}; Table 4), microbial groups capable of using both pathways, such as *Methanosarcina* [6], tend to use the first one whenever possible. In future studies, molecular biology analyses can be applied to confirm this hypothesis of the influence of the inorganic carbon dosing, i.e., $NaHCO_3$, on the methanogenic microbial community activity and resulting metabolic pathways.

It is worth highlighting the greater capacity to produce alkalinity *"in loco"* showed by condition R1-0 (no $NaHCO_3$ dosing) most likely due to the favoring of acetoclastic methanogenesis. The influent PA of conditions R1-0 and R1 represented the two extreme values among all experimental conditions, i.e., 282 and 931 mg-$CaCO_3$ L^{-1}, respectively (Table 3), which resulted in marked differences in the influent pH (7.82 *vs.* 8.11; Table 3). Interestingly, the effluent pH measured in both conditions reached equivalent values at the end of the incubation period (ca. 7.3; Table 3), whilst the production of PA (effluent PA – influent PA) was 25 % (307 mg-$CaCO_3$ L^{-1}) higher in R1-0 compared to R1 (1528 *vs.* 1221 mg-$CaCO_3$ L^{-1}; Table 3). This finding corroborates the hypothesis describing the prevalence of acetoclastic methanogenesis over the hydrogenotrophic pathway in R1-0: bicarbonate (HCO_3^-) is produced in the first (Reaction 19; Table 4) and consumed in the latter (Reaction 20; Table 4). From a practical perspective, this finding can be very useful for future studies on the 2st-AD of sugarcane vinasse, showing the potential to reduce costs with alkalinization by recycling the biologically produced HCO_3^-. Biodigested vinasse can, therefore, be re-introduced in both the methanogenic and fermentative-sulfidogenic units.

The MY (Fig. 3a) also showed statistical differences (Supplementary material) in the different experimental conditions assessed; therefore, it was influenced by the level of acidification and $NaHCO_3$ dosing. Despite the compositional similarities observed in V2 and V3 (high HLa and SO_4^{2-} concentrations; Table 1 and Fig. 3c), the highest (296 NmL-CH_4 g-COD^{-1}; R2) and the lowest (239 NmL-CH_4 g-COD^{-1}; R3) MY values were observed in these conditions, respectively (Fig. 3a), which were statistically different (p-value = 0.004; Supplementary material). The higher content of CH in V3 (16.4 % of the CODs; Table 1 and Fig. 3c) may have favored the establishment of fast-growing acidogenic groups once the reactors were incubated, diverting electrons from methanogenesis to cell synthesis. The MY profile was also impacted by the $NaHCO_3$ dosing, as observed when comparing conditions R1 and R1-0 (statistical equivalents; p-value = 0.386; Supplementary material). In the initial incubation period (0–96 h), R1 showed lower CODs removal efficiency (Fig. 3b) and higher MY (Fig. 3a) compared to R1-0, both patterns associated with the favoring of hydrogenotrophic methanogenesis. This provided just a 7%-higher terminal MY in R1 (279 *vs.* 260 NmL-CH_4 g-CODs^{-1}; Fig. 3a). Nonetheless, the lower hydrogenotrophic activity influenced negatively the MY of R1-0 when compared with R2 (statistically lower; p-value = 0.043; Supplementary material), which was statistically equivalent to the condition with $NaHCO_3$ dosing (R1; p-value = 0.421; Supplementary material). The expected benefits of a lower sulfate concentration in V1 on the methanogenic activity were not high enough to offset the benefits of the enhanced acetate generation by SRB in the beginning of the incubation of V2, which explains the statistical equivalence of the MY in R1 and R2.

Although using sulfate-poor fermented vinasse (V1) did not directly favor the production of CH_4, the concentrations of sulfide in the liquid phase (TDS = 64–69 mg L^{-1}; Table 3) were roughly 2-fold lower (and therefore statistically different; p-value = 0.000 – 0.002; Supplementary material) in conditions R1 and R1-0 compared to conditions R2 and R3 (TDS = 121–103 mg L^{-1}; Table 3). TDS values measured in R2 and R3 coincided with the lower limit of the inhibition range (100–800 mg L^{-1}) reported by Chen et al. [8]. However, it is worth stressing that the real TDS concentrations should be approximately 3-fold higher than the measured values (Table 3), because of the lower sulfate inputs resulting from vinasse dilution. Therefore, even the use of sulfate-poor vinasses would produce TDS levels within the inhibition range (363–309 *vs.* 200 mg L^{-1}; in R2/R3 and R1/R1-0, respectively). In this context, it is imperative to improve the sulfidogenic activity in the fermentation stage to prevent as much as possible the inhibition potential of sulfide during methanogenesis. Meanwhile, it is also crucial to stimulate the production of HAc in the fermentative-sulfidogenic processing of sugarcane vinasse to boost CH_4 production and not to strictly depend on acetogenesis as the main HAc provider.

3.2. Energetic and economic aspects

The energetic potential of the CH_4-rich biogas and the economic impact of each strategy used to control the pH of vinasse was assessed, considering both the fermentative [59] and the methanogenic (this study) stages in the latter. The global energy recovery potential (GERP; TWh) and the unit values (UV; US$ GWh^{-1}) related to the alkalinization strategies used strictly in the methanogenic stage and the 2st-AD process (fermentation + methanogenesis) were evaluated considering a period equivalent to the sugarcane season. Complementarily, a box plot analysis showing the compositional characteristics of biogas is depicted in Fig. 4. The level of substrate acidification and the establishment of sulfidogenesis during the fermentation directly impacted the LCV of biogas (as evidenced by variations in the CH_4 proportion; Fig. 4 and Table 5), consequently affecting the GERP.

The removal of sulfate during the fermentation step and the relatively low availability of inorganic carbon during methanogenesis increased the CH_4 content and consequently the LCV of the biogas. The use of sulfate-poor vinasse (V1) resulted in statistically equivalent LCV for both derived conditions (R1-0 and R1; p-value = 0.691; Supplementary material). These values were higher (p-value = 0.000; Supplementary material) than the one observed when using vinasse subjected to no pH adjustment in fermentation (V3). Conversely, only condition R1-0 provided an LCV higher (p-value = 0.003; Supplementary material) than R2, in which sulfate- and VFA-rich was biodigested (V2). Hence, dosing $NaHCO_3$ in methanogenesis (R1 *vs.* R1-0) was

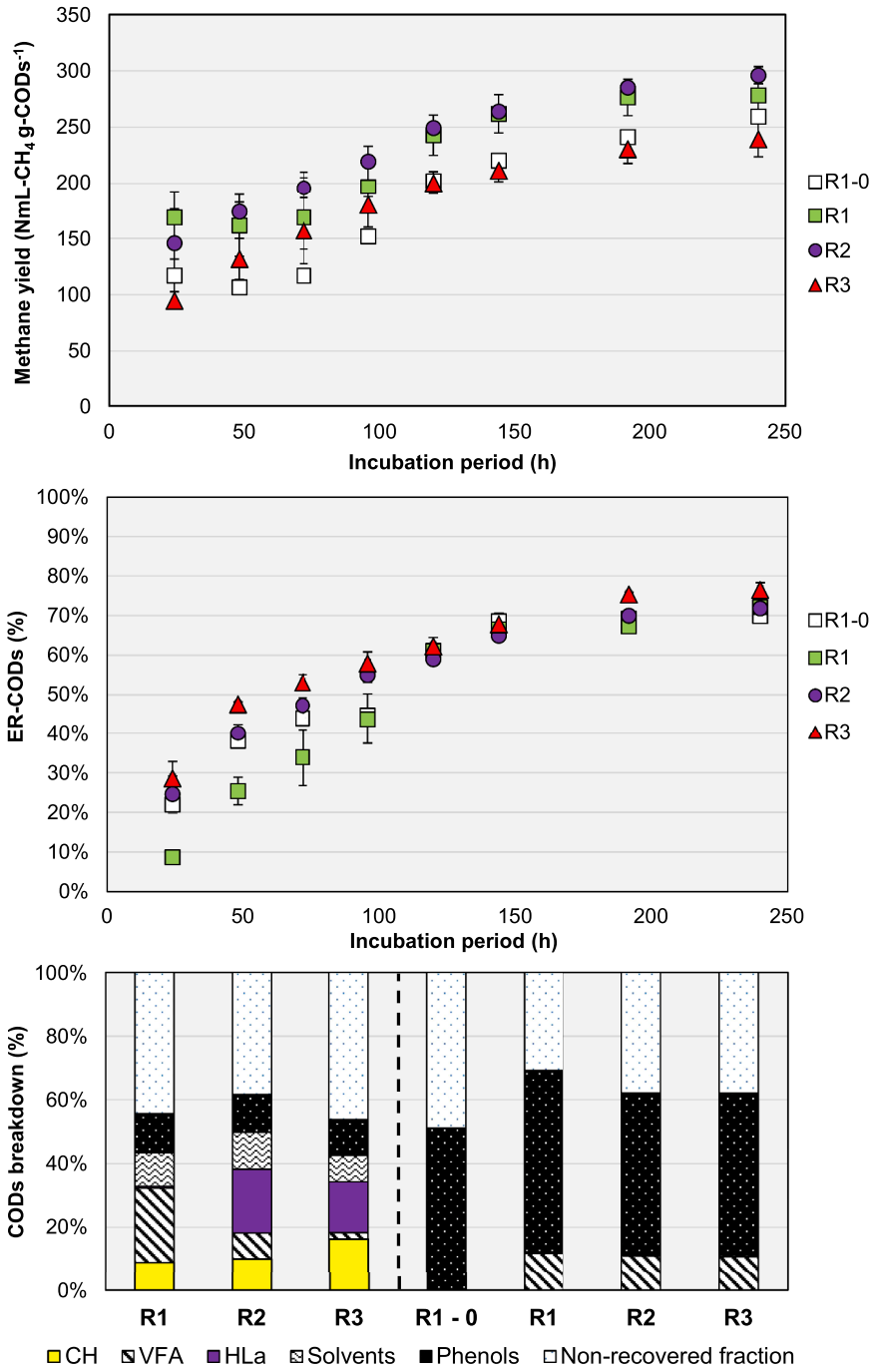

Fig. 3. Dynamics of methane generation and organic matter consumption: (a) methane yield, (b) CODs removal efficiency and (c) breakdown of the CODs in fermented and biodigested vinasse.

unfavorable possibly due to the higher presence of inorganic carbon (HCO_3^-), whose dissociation releases the inert, i.e., non-energetic, carbon dioxide and dilutes the calorific power of methane [51,59]. Nevertheless, the removal of sulfur compounds prior to methanogenesis needs to be considered the first premise to improve biogas quality. Besides decreasing the inputs of sulfur compounds into the methanogenic stage, the establishment of SRB in the fermentation [59] improved the acidification level of vinasse (as evidenced by a lower CH content compared to R2 and R3; Fig. 3c). The combination of both factors explains the high CH_4 content observed in conditions R1 and R1-0 (>70 %; Fig. 4 and Table 5). Similarly, Fuess et al. [25] reported CH_4 contents within the ranges of 70–75 % and 80–90 % in the biogas produced from sulfate-rich and sulfate-poor fermented vinasses, respectively.

The harmfulness of biogas caused by the occurrence of H_2S (Fig. 4) was also decreased when removing sulfate during dark fermentation. The statistical analysis (Supplementary material) indicated that conditions using V1 (R1 and R1-0) produced gas phase H_2S contents equivalent (p-value = 0.559; Supplementary material) and lower than conditions using V2 and V3 (p-value = 0.000-0.007; Supplementary material), both sulfate-rich vinasses. However, further effort is required to produce a sulfur-free (including the removal of both sulfate and dissolved sulfide) fermented substrate, potentially eliminating desulfurization units when planning the energy recovery from CH_4-rich biogas in sugarcane biorefineries. The relatively low value observed in R1-0 and R1 (up to 3.2 %) compared to R2 and R3 (up to 3.7 %) would still require the installation of a desulfurization process prior to the utilization of the

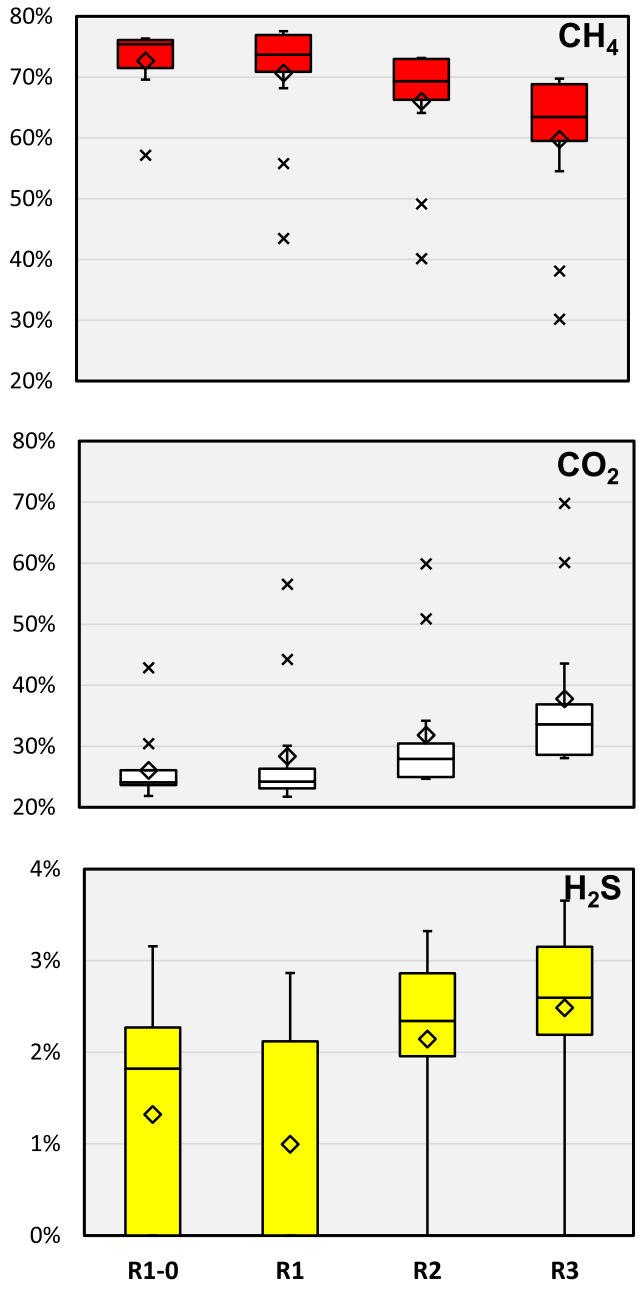

Fig. 4. Compositional characteristics of the biogas (CH_4, CO_2 and H_2S) in the different incubation conditions.

biogas as a biofuel in both cases. Tolerated H_2S concentrations for gas turbines varied in the range of 200–1000 ppm, i.e., 0.02–0.1 % [32,65]. The occurrence of H_2S in biogas releases sulfuric acid (H_2SO_4) when sulfide reacts with water vapor on the surface of heat exchangers, which can cause the corrosion of equipment components and the most likely failure of the prime mover [11]. The desulfurization of the biogas derived from vinasse in the Brazilian sugarcane biorefineries is considered a relevant economic drawback for energy recovery systems. The lack of local technologies available for the desulfurization process implies high costs directly dependent on importation taxes and exchange rates [36].

The strategies used to control the pH and/or alkalinize the reactors ($NaHCO_3$ dosing) in both stages of 2st-AD affected biogas production on both quantitative and qualitative bases, leading to different energetic performances according to the experimental conditions. The highest GERP was estimated for condition R2 (a consequence of the highest MY; Fig. 3a), reaching 354,603 GWh/season from the conversion of 15.1 million Nm^3 of biogas (Table 5). Interestingly, for an equivalent volume of biogas, the energy production in R2 was ca. 9.5 % higher than that in R3 (324,030 GWh; Table 5). The GERP calculated for R1 was also slightly higher than in R3 (325,603 GWh; Table 5), despite the lower biogas production (13 *vs.* 15 million Nm^3; Table 5). These findings resulted from the positive impacts of producing biogas with higher CH_4 content (and consequently, higher LCV) due to partial removal of sulfate during the fermentation. It is worth stressing that gas engines and turbines achieve higher conversion efficiencies (η) when the CH_4 content in the biogas is increased [65]. However, despite considering a constant conversion efficiency (η_{el+th}, as preconized by the manufacturer) regardless of the biogas quality, this operational parameter would vary (increase) as a consequence of the higher LCV (R1-0 and R1), increasing, thus, the differences in the energetic performances of the evaluated conditions. The lowest GERP was observed in condition R1-0 (294,411 GWh; Table 5), reaching a value ca. 10 % lower than R1, despite the use of the same source of fermented vinasse. The enhancement of the hydrogenotrophic methane-producing pathway (triggered by the higher availability of inorganic carbon) in R1 explains this difference.

Nevertheless, the analysis of the unit costs related to the pH control strategies ($NaHCO_3$ and/or NaOH) in the 2st-AD plant (UV; US$ GWh^{-1}; Table 5) associated the best economic performance with condition R1-0 (Table 5). On one hand, a 17%-difference was observed between the highest (354,603 GWh, R2) and the lowest (294,411 GWh, R1-0) GERP values (Table 5), characterizing a disadvantage for the non-externally alkalinized fermentative-sulfidogenic process. On the other hand, the unit value estimated for R1-0 (9.4 US$ GWh^{-1}) was 76.5 % lower than that for R2 (40.1 US$ GWh^{-1}) (Table 5), characterizing a highly promising technological approach. Assuming an energy production in R2 equivalent to that of R1-0 (294,411 GWh; Table 5), savings of ca. 9.1 US$ million would be achieved, resulting from a 13.2 ton-reduction in the seasonal consumption of $NaHCO_3$.

Finally, it is worth noting positive impacts of dosing NaOH in the fermentative reactor on the economic performance of the 2st-AD process. Dosing NaOH in the fermentation (R2) improved the compositional characteristics (higher CH_4 content) of the biogas (LCV = 24.8 *vs.* 22.7 MJ Nm^{-3}, MY= 296 *vs.* 239 NmL-CH_4 g-$CODs^{-1}$) and, consequently, the GERP (354,603 *vs.* 324,030 GWh) (Table 5) relative to R3 (no chemical dosing in fermentation), requiring only a marginal increase of 0.5 % in the total costs with chemicals, including the use of $NaHCO_3$ in methanogenesis (78,792 US$ *vs.* 14.1 million US$; Table 5). Consequently, an 8.2%-lower unit biogas cost was estimated for R2 compared to R3 (40.1 *vs.* 43.7 US$ GWh^{-1}; Table 5).

3.3. *A new application for fermentative-sulfidogenic process*

The benefits of using fermentative-sulfidogenic processes when managing sulfate-rich wastewaters in biodigestion plants are widely known, including the minimization/elimination of the competition between SRB and MA, the prevention of MA inhibition by sulfides and the production of a less harmful CH_4-rich biogas [27,38–41,42,56,67]. However, this study innovatively tested an additional benefit of this operating strategy by demonstrating its use as an alkalinization strategy in biodigestion plants, contributing to reducing the operational costs incurred with chemicals. This finding can be extremely valuable for the field, mainly in the context of sugarcane vinasse biodigestion, in which alkalinizing methanogenic reactors is considered one of the main operational challenges when working with high organic loading rates. The dosing of chemicals (e.g. $NaHCO_3$ or NaOH) coupled or not to the recirculation of the liquid phase defines the economic feasibility of using biogas as an energy source [20,24].

Hence, using the fermentative-sulfidogenic process as a strategy to both neutralize (using HS^- as a H^+ consumer; [28]) and alkalinize anaerobic systems (as a source of biologically produced bicarbonate;

Table 5
– Global energy recovery potential and unit cost values calculated for biogas according to the different experimental conditions.

Parameter	Unit	Conditions			
		R1-0	R1	R2	R3
MY	Nm^3 kg-COD^{-1}	259.69	278.44	296.36	239.43
CODs	kg m^{-3}	26.1	26.1	26.8	28.5
ER-CODs	%	69.9	72.1	71.8	76.5
F_{CH4} [(B)]	%	75.4	73.7	69.3	63.4
V_B	Nm^3 $season^{-1}$ [(A)]	11,538,409	13,055,223	15,120,736	15,102,901
LCV	MJ Nm^{-3}	27.0	26.4	24.8	22.7
GERP [(C)]	GWh [(A)]	294,411	325,603	354,603	324,030
NaOH	kg [(A)]	246,225	246,225	246,225	-
	US$ [(A)]	78,792	78,792	78,792	-
$NaHCO_3$	kg [(A)]	3,077,813	19,339,688	16,261,875	16,261,875
	US$ [(A)]	2,677,697	16,825,528	14,147,831	14,147,831
UV	US$ GWh^{-1}	9.4	51.9	40.1	43.7

Parameter: MY = methane yield; F_{CH4} = CH_4 fraction in biogas; V_B = biogas produced; LCV = lower calorific value; GERP = global energy recovery potential; UV = unit value.
Notes:
[(A)] Value related to a 200 day-season
[(B)] Median value from incubation period
[(C)] Electric + thermal energy recovery potential.

Table 4) can directly improve the economic performance of the 2st-AD process, further encouraging its scalability. In parallel, the improvements in biogas quality resulting from increasing the CH_4 content and decreasing or eliminating H_2S from the biogas evolved from the second-stage reactor are also beneficial on an economic basis [25,56]. Low CH_4 concentrations in biogas (low LCV) are considered one of the main technological drawbacks for energy generators [32,65]. In addition, low quantities of H_2S (200–1,000 ppm or 0.02–0.1 %) can cause serious corrosion problems in heat exchangers, as previously described [11,32,65]. Nevertheless, it is highly important to stress that the sulfide-containing biogas evolved from fermentation still requires proper handling [25], in a way that the implementation of desulfurization units in vinasse-fed biodigestion plants may still be obligate. Alternative approaches, such as coupling the production of elemental sulfur by sulfur-oxidizing bacteria (SOB) in the desulfurization unit can be a strategy to minimize costs [47].

In practical aspects, some efforts are still required to optimize the fermentative-sulfidogenic processing of sugarcane vinasse, aiming to simultaneously obtain an acetate-rich and sulfur-free fermented substrate. Recent reports on the application thermophilic fermentation of sugarcane vinasse in sulfate-reducing environments systematically indicate butyrate as the main soluble phase metabolite [18,26,51,59], even when sulfate removal reaches values higher than 90 % [18]. Results reported herein, as well as those presented elsewhere [25], identified some kinetic limitations when the conversion of butyrate to acetate is the main pathway supplying methanogens with acetate (in the absence or low availability of SO_4^{2-}). Therefore, it is imperative to define operational parameters that stimulate the acetogenic activity during the fermentation, such as favoring the establishment of incompletely oxidizing SRB and/or offering a suitable environment for homoacetogens. The operation of the fermentative systems under mesophilic conditions could be an alternative to favor the homoacetogenic activity, as recently observed elsewhere [18]. In addition, the monitoring of continuous reactors (aiming to define suitable operating parameters, mainly for methanogenic units) and more robust techno-economic assessments are still required to effectively optimize the whole 2st-AD process, considering: [i] the maximization of both the sulfate removal and the acetate production in fermentation, [ii] the maximization of methane production under high organic loading rates and [iii] the definition of cost-competitive approaches to both manage sulfide and use the CH_4-rich biogas.

4. Conclusions

The level of acidification, prevailing fermentation-derived metabolites, alkalinization strategy and sulfidogenic activity were demonstrated to directly impact the kinetics of methane production and substrate conversion in the second stage (methanogenesis) of sugarcane vinasse processing. The biodigestion of sulfate- and lactate-rich vinasses presented similar patterns so the incomplete oxidation of lactate to acetate by SRB was essential to promptly establish methane production. The removal of sulfate prior to methanogenesis improved the compositional characteristics of the biogas by increasing the lower calorific value (>70 % of CH_4) and decreasing the hydrogen sulfide content. Finally, maintaining a sulfate-reducing fermentative unit prior to methanogenesis has the potential to positively impact the economics of sugarcane vinasse biodigestion in high-rate reactors, once the use of chemicals can be suppressed through utilizing the bicarbonate derived from SRB activity. In practical terms, these findings support the encouragement of scaling up the fermentative-sulfidogenic process as a strategic alternative to overcome techno-economic drawbacks in 2st-AD systems processing sugarcane vinasse. Furthermore, this strategic alternative can also provide greater compactness and enhanced bioenergy recovery when compared to the usual low-rate lagoon systems implemented in biogas plants in sugarcane biorefineries worldwide.

CRediT authorship contribution statement

Renan Coghi Rogeri: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lucas Tadeu Fuess:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Matheus Neves de Araujo:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Felipe Eng:** Writing – review & editing. **André do Vale Borges:** Writing – review & editing. **Márcia Helena Rissato Zamariolli Damianovic:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Ariovaldo José da Silva:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

The authors warmly thank Maria Angela Tallarico Adorno and Carolina Aparecida Sabatini Mirandola for their laboratory support during the investigations. The authors are grateful to the Coordination for the Improvement of Higher Education Personnel (CAPES, grant number 88887.339567/2019-00) and to the São Paulo Research Foundation (FAPESP, grant number 2015/06246-7) for the financial support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nexus.2024.100303.

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