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Operation of a high-rate reactor for biogas production: Integration of sugarcane vinasse and cattle manure

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Abstract

Biogas has emerged as a promising solution for the anaerobic treatment of organic waste, offering energy, economic, and environmental benefits. This study evaluated the co-digestion of sugarcane vinasse and cattle manure (5:1 v/m) in an anaerobic structured bed reactor (AnSTBR) for methane production optimization. The reactor operated under mesophilic conditions (35 °C) for 332 days across five phases with progressively increasing organic loading rates (OLR), including assessment under reduced alkalizing-agent use. At an OLR of 12.0 kg COD m^{-3} d^{-1}, methane yield reached 297.5 ± 60.2 NmL CH_4 g^{-1} COD removed, with a volumetric production rate of 4,103.5 ± 816.9 NmL CH_4 L^{-1} d^{-1}. Higher OLRs (18 kg COD m^{-3} d^{-1}) further enhanced methane production while maintaining operational stability under controlled conditions. The AnSTBR demonstrated robustness in processing agroindustrial waste at high organic loads with reduced chemical input, confirming its potential as an efficient and sustainable technology for biogas generation from vinasse-based co-digestion systems.

Key-words: anaerobic co-digestion, anaerobic structured-bed reactor, sugarcane-livestock integration, bioenergy

Highlights

- AnSTBR showed high robustness in the co-digestion of vinasse and cattle manure.
- Vinasse–manure mixtures remained stable even with reduced alkalizing use.

- Higher organic loading rates resulted in increased methane production.
- AnSTBR showed a strong capacity to process large volumes of agro-industrial waste.

1. INTRODUCTION

Biogas production has been recognized as an effective approach for the anaerobic processing of organic residues – including agro-industrial waste, animal production waste, urban and municipal organic waste, sanitary sewage and sludge, and pulp and paper industry waste – providing substantial energy, economic, and environmental advantages [1–8]. This technology enables the generation of a renewable energy source while producing a nutrient-rich byproduct, digestate, which is widely utilized as a fertilizer [9]. In addition to mitigating the environmental impacts, anaerobic digestion (AD) is a strategic process for resource recovery that transforms waste into bioenergy and value-added products. This approach aligns with the biorefinery concept, emphasizing its pivotal role in energy transitions and in advancing sustainable production chains [10, 11].

High-rate anaerobic reactors have advanced the treatment of wastewater by optimizing hydraulic retention time (HRT) and solid retention time (SRT) while maintaining high cell densities [12, 13]. Numerous reactor configurations and operational strategies enhance substrate digestibility and stabilize methane (CH_4) production [14, 15]. Bioreactor design selection is critical for process efficiency, requiring careful evaluation of parameters such as pH, temperature, C/N ratio, organic loading rate (OLR), and system stability [14, 16].

Among the various substrates used in AD, sugarcane vinasse is a key effluent generated in ethanol distilleries, with a production rate of approximately 10–15 L/L ethanol [11, 17, 18]. This byproduct is characterized by a low pH, a high organic load, and the presence of potentially toxic compounds, such as phenols, polyphenols, and heavy metals [19, 20]. The bioethanol industry often employs fertigation practices as an alternative use for sugarcane vinasse. However, despite its widespread implementation, careful management is required to prevent soil salinization and contamination of water resources [20, 21].

In addition, the beef cattle industry is also a source of organic waste; for example, cattle in feedlots produce, on a daily basis, approximately 5–6% of their body weight in manure. [22]. Oliveira et al. (2020) estimated a mean daily production of fresh cow manure of 27.8 ± 7.6 kg per animal, with an availability factor of 47.3 ± 23.0%. Although manure is commonly used as a fertilizer, its improper management can contribute to water and air pollution. Water contamination may occur through leachate overflow or surface runoff during heavy rainfall, adversely affecting aquatic ecosystems. Air pollution, in turn, is primarily associated with ammonia emissions from manure handling, storage, and management in agricultural and livestock production facilities [22]. Consequently, anaerobic digestion of cattle manure presents a promising alternative, particularly when combined with co-substrates [24], enabling efficient biogas production and energy recovery.

This study underscores the importance of anaerobic digestion as a viable process for valorizing agro-industrial and livestock waste, aligning with the principles of sustainability and the circular economy. The integration of sugarcane vinasse, a substrate with a high organic load and inherent compositional challenges, with cattle manure has demonstrated the potential to overcome operational limitations and enhance process stability and biogas production. This approach not only mitigates environmental impacts from improper waste disposal but also supports bioenergy generation. The utilization of an anaerobic structured bed reactor (AnSTBR) is essential for optimizing the process, maximizing organic matter conversion, and improving energy recovery. Accordingly, the objective of this study was to evaluate the performance of biogas

production from the integration of sugarcane and livestock residues with a focus on optimizing vinasse monodigestion and ensuring effective treatment of the resulting effluents.

2. MATERIAL AND METHODS

2.1 Substrate definition and feed characterization

The substrate utilized in this study included sugarcane vinasse, a by-product of ethanol production, which was collected during the sugarcane harvest period at Usina Ester, Cosmópolis, São Paulo State, Brazil. The co-substrate, cattle manure, was sourced from Frigorífico Nossa Senhora da Saúde, Poços de Caldas, Minas Gerais, Brazil. A mesophilic anaerobic inoculum obtained from a full-scale continuous stirred-tank reactor (CSTR) designed for biogas production from vinasse and other organic substrates was employed.

The characterization of the substrates used in this study is detailed in Table 1.

The reactor was fed with a mixture of sugarcane vinasse and cattle manure at a 5:1 (v/m) ratio. To ensure homogeneity and minimize interference from solid particles, the mixture was infused for 24h, followed by filtration, resulting in the exclusive use of the liquid fraction of the sample. This processed mixture is referred to in this study as the "liquid mix V+CM (5:1)".

Table 1. Physicochemical characteristics of sugarcane vinasse, cattle manure, a mixture of both, and inoculum

	pH	TS (g L^{-1})	FS (g L^{-1})	VS (g L^{-1})	COD (g L^{-1})
Vinasse (V)	3.80	34.47 ±0.68	7.48 ±0.21	26.99 ±0.47	41.76 ±0.69
Cattle manure (CM)	-	181.12 ±9.21	16.49 ±0.21	164.63 ±9.27	-
Mix V+CM (5:1)	4.09	35.24 ±0.10	8.57 ±0.07	26.67 ±0.14	46.00 ±0.43
Inoculum	7.90	31.96 ±0.33	13.70 ±0.12	18.26 ±0.22	-

TS, total solids; FS, fixed solids; VS, volatile solids; COD, chemical oxygen demand

The reactor feed was prepared following predefined OLRs, ensuring that the system adapted to the established operational conditions. This preparation involved initial homogenization of the liquid mixture V + CM (5:1), followed by dilution to adjust the organic concentration, thereby facilitating effective reactor operation and management. The key physicochemical characteristics of the reactor feed during operation are listed in Table 2.

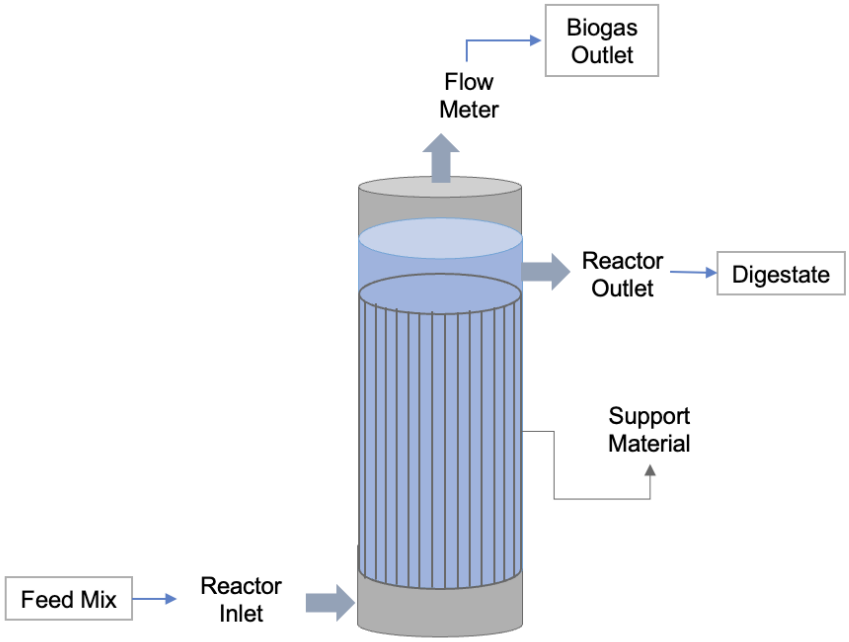
Table 2. Physicochemical characteristics of mixtures of sugarcane vinasse and cattle manure used as feed in the reactor

Mix V+CM	OLR (kg COD m^{-3} d^{-1})	pH	TS (g L^{-1})	FS (g L^{-1})	VS (g L^{-1})	COD (g L^{-1})	TOC (mg L^{-1})	TN (mg L^{-1})	C/N
Mix 1	3.5 (Step I)	4.05	7.39	2.13	5.26	8.99	2005.25	60.10	33.37
Mix 2	6 and 9 (Step II and III)	4.20	11.18	3.16	8.02	16.60	3011.50	95.35	31.58
Mix 4	12 (Step IV)	4.11	10.55	2.91	7.64	14.80	2729.00	81.45	33.51
Mix 5	18 (Step V)	4.08	11.50	3.22	8.28	16.00	3165.00	92.40	34.25

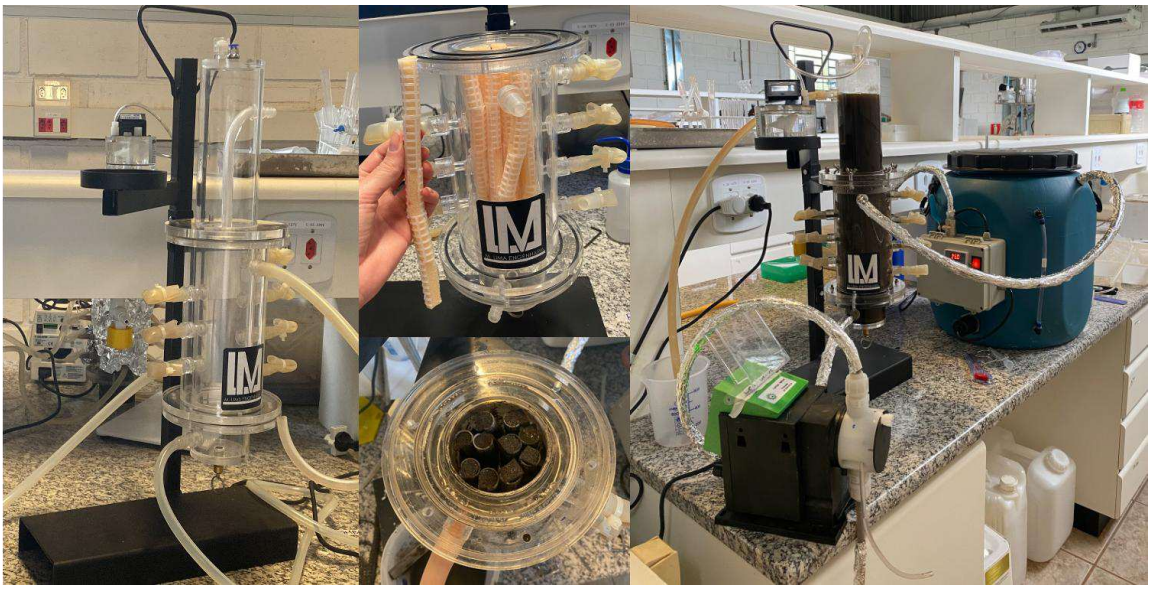
OLR: organic loading rate; TOC: total organic carbon; TC: total nitrogen; C/N: carbon/nitrogen ratio

2.2 Reactor and operating conditions

A bench-scale AnSTBR was constructed from acrylic with a total volume of 1.1 liters. The structured bed was assembled using polyurethane foam strips (1×1.5×22 cm), arranged vertically between stainless steel screens positioned at the upper and lower ends of the reactor. To stabilize the foam strips, they were extended and secured in place using bobbins distributed longitudinally along each strip, for a total of 10 foam strips. The effective volume of the structured bed is approximately 0.8 liters. The design, assembly, and operation of the AnSTBR are illustrated in Fig. 1.



(a)



(b)

Fig. 1 Anaerobic structured-bed reactor (AnSTBR): (a) schematic representation of the reactor showing main components; (b) bench-scale reactor photographs showing the assembled unit, structured packing detail, and complete experimental setup.

The reactor was inoculated with mesophilic sludge obtained from a full-scale CSTR. The inoculation process described in Zaiat et al. (1994) was followed, which involved submerging polyurethane foam strips in the inoculum for approximately

2 h to allow microbial colonization of the support medium. After this period, excess sludge was drained, and the reactor was fed with the prepared substrate mixture.

The reactor was fed using a peristaltic pump, and the substrate mixture was diluted and cooled according to the pre-established operational conditions for each phase, with the OLR taken into account. Before feeding, the pH was adjusted using sodium hydroxide (NaOH) solution. The system performance was assessed to minimize the use of alkalizing agents, thereby reducing the overall process costs.

The reactor operation was conducted over five distinct phases, as outlined in Table 3, characterized by progressively increasing the OLRs of 3.5, 6, 9, 12, and 18 kg COD m^{-3} d^{-1}. The gradual increase in OLR corresponded to a reduction in the HRT, which decreased from approximately 64.8 hours at the lowest OLR (3.5 kg COD m^{-3} d^{-1}) to 21.6 hours at the highest OLR (18 kg COD m^{-3} d^{-1}). The reactor was operated for a total of 332 days at mesophilic temperatures (35 °C).

Table 3. Operating conditions applied in the operation of the AnSTBR

Phase	Feed Mix	Operation period (day)	OLR (kg COD m^{-3} d^{-1})	Mean Input pH	COD In (kg m^{-3})	HRT (days)
I	Mix 1	1 – 42	3.5	7.68 ± 0.18	8.9	2.7
II	Mix 2	43 – 51 52 – 58	6.0	6.93 ± 0.0 5.97 ± 0.0	16.5	2.7
III	Mix 2	59 – 73 74- 121	9.0	5.02 ± 0.07 4.10 ± 0.03	16.7	1.9
IV	Mix 3	122 – 136 137 – 148 149 - 179	12.0	5.71 ± 0.28 5.03 ± 0.02 7.26 ± 0.40	14.8	1.2
V	Mix 4	180 – 231 232 – 332	18.0	7.02 ± 0.04 6.08 ± 0.07	16.0	0.9

2.3 Analytical methods

System monitoring includes pH, chemical oxygen demand (COD), total volatile acids (TVA), partial alkalinity (PA), and intermediate alkalinity (IA). Analyses of pH, COD, and VS were performed according to the procedures outlined in the Standard Methods for the Examination of Water and Wastewater [26]. PA and IA were determined using the titrimetric potentiometric method described by Ripley and Boyle (1986). The TVA concentrations were determined according to a previously established protocol by Dilallo and Albertson (1961).

The characterization of volatile fatty acids (VFAs), alcohols, and sugars was performed using high-performance liquid chromatography (HPLC) (PROMINENCE HIC-SP/HIC-NS, Shimadzu®). Concentrations of total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) were measured using a total organic carbon analyzer (TOC-L, Shimadzu®) equipped with the TNM-1 module.

Biogas flow rates were monitored using gas meters (MilliGascounter MGC-1 PMMA, Dr. Ing. Ritter Apparatebau GmbH & Co. KG, Germany) connected to the reactor headspace. The composition of the biogas, including methane (CH_4), carbon dioxide (CO_2), hydrogen sulfide (H_2S), and hydrogen (H_2), was analyzed via gas chromatography (Nexis™ GC-2030, Shimadzu®).

2.4 Response variable calculations

Performance was evaluated by: COD removal (%), biogas flow rate (NmL d^{-1}), $\%CH_4$, volumetric methane production (VMP) (NmL CH_4 L^{-1} d^{-1}), methane yield (MY) (NmL CH_4 g^{-1} COD removed), and intermediate alkalinity-to-partial alkalinity (IA/PA) ratio. All biogas values were standardized to 1 atm and 0 °C per Equations 1–4.

Biogas production response variables were standardized to normal temperature and pressure conditions (1 atm and 0 °C; 273.15 K). The calculations were performed according to Equations 1–4:

$$COD\ removal\ (\%) = \left(\frac{COD_{In} - COD_{Out}}{COD_{In}}\right) \times 100 \quad (1)$$

where COD_{In} represents the input COD of the system, expressed in mg L^{-1}, and COD_{Out} denotes the output COD of the system effluent, also expressed in mg L^{-1}.

$$Biogas\ flow = \frac{V_{Biogas\ production}}{time} \quad (2)$$

The $biogas\ flow$ is expressed in mL d^{-1} and $V_{Biogas\ production}$ refers to the total volume of biogas produced over a given time (day) period.

$$Volumetric\ methane\ production\ (VMP) = \frac{Biogas\ flow \times \%CH_4}{Useful\ volume_{reactor}} \quad (3)$$

The volumetric methane production (VMP) is expressed in mL CH_4 L^{-1} d^{-1}, $\%CH_4$ is the methane content in the biogas (percentage), and $useful\ volume_{reactor}$ is the effective volume of the reactor, given in liters.

$$Methane\ yield\ (MY) = \frac{Biogas\ flow \times \%CH_4}{Q_{In} \times (COD_{In} - COD_{Out})} \quad (4)$$

The methane yield (MY) is expressed in mL CH_4 g^{-1} COD, Q_{In} refers to the influent flow rate of the system, expressed in L d^{-1}.

2.5 Estimation of energy potential

The energy potential of the system was estimated using the model proposed in Fuess et al. (2017b). $Energy\ potential$ expressed in MJ m^{-3}, was calculated using Equation 5.

$$Energy\ potential = COD_{In} \times COD\ removal \times CH_4\ yie(ld \times LHV_{CH4} \quad (5)$$

LHV_{CH4} is the lower calorific value of CH_4, considered as 35.8 MJ m^{-3} CH_4.

3. RESULTS AND DISCUSSION

3.1 Operational stability and organic matter removal

Reactor operation commenced with an OLR of 3.5 kg COD m^{-3} d^{-1} (Phase I) and lasted 42 days. During the first 23 days, the system was undergoing an adaptation process, with an AI/AP ratio of 5.17 ± 3.96 and an effluent pH of 6.86 ± 0.65. Even so, between days 23 and 42, the effluent pH remained stable at a mean of 8.27 ± 0.19 (Fig. 2a), corroborating stable operational conditions. During this period, the system achieved a mean COD removal efficiency of 85.1 ± 6.7% (Fig. 2b). The IA/PA ratio decreased from 0.59 (day 23) to 0.29 (day 42) (Fig. 2c), indicating better conditions for the anaerobic processes. Kunz et al. (2019) suggested an optimal IA/PA ratio between 0.3 and 0.4, but emphasized that variations are

common depending on the characteristics of the reactor and substrate. Overall, Phase I demonstrates efficient organic matter conversion and stable operational conditions. Additionally, at this stage, the feed pH was adjusted to reach a mean of 7.68 ± 0.18. Notably, the initial pH of the liquid mix V + CM (5:1) was approximately 4.0, so NaOH solution was added to reach the target level.

In Phase II, the OLR was increased to 6.0 kg COD m^{-3} d^{-1} (day 43). Between days 43 and 51, the mean influent pH was 6.93. From day 52, the inlet pH was reduced to approximately 5.97 to assess system adaptability and reduce alkalizing agent consumption. The system demonstrated stability, achieving a mean effluent pH of 8.29 ± 0.18 (Fig. 2a), COD removal efficiency of 89 ± 2% (Fig. 2b), and IA/PA ratio of 0.33 ± 0.07 (Fig. 2c). These results confirm the robustness of the system in treating organic matter efficiently under low-pH feed conditions.

Phase III featured an OLR of 9.0 kg COD m^{-3} d^{-1}, with influent pH reduced to 5.02 ± 0.07 (days 59–73). Despite this condition, the system performed well, with effluent pH at 8.41 ± 0.32, COD removal efficiency at 87 ± 2%, and IA/PA ratio at 0.38 ± 0.08 (Fig. 2a–c). To test the system resilience, feeding without pH adjustment began on day 74, with a mean influent pH of 4.10 ± 0.03. Even under these adverse conditions, the system remained stable, achieving a mean effluent pH of 8.05 ± 0.21, COD removal efficiency of 87 ± 4%, and IA/PA ratio of 0.39 ± 0.16 (Fig. 2a–c). Transient instability occurred (days 77–82), with IA/PA ratios peaking at 0.71 but stabilizing by day 83. These findings highlight the adaptability and resilience of systems under challenging conditions.

In Phases I, II, and III, despite the outlet pH consistently exceeding the ideal range of 6.8–7.4 [30], the system maintained stability and efficiency, as corroborated by studies like Santi Caraça et al. (2023) who reported similar pH values exceeding 8.0 during the operation of UASB reactors treating sugarcane vinasse co-digested with diluted dairy cow manure and effluent recirculation. Despite the elevated pH levels, the process maintained operational stability and efficiency, suggesting that the ideal pH range for AD may vary with system configuration, substrate composition, and operational strategies.

In Phase IV, the OLR was increased to 12.0 kg COD m^{-3} d^{-1}. During adaptation (days 122–136), the mean influent pH was 5.71 ± 0.28, and subsequently reduced to 5.03 ± 0.02 (days 137–148). Meanwhile, the IA/PA ratio fluctuated between 0.29 and 20.0 (Fig. 2c), accompanied by decreases in effluent pH (5.72 on day 149) and COD removal efficiency (36% on day 149), suggesting that operational stability may have been compromised. Furthermore, an increase in the accumulation of total volatile acid (TVA) was observed, with concentrations ranging from a minimum of 615 mg HAc L^{-1} (day 129) to a maximum of 5,625 mg HAc L^{-1} (day 150) during Phase IV. To restore stability, the feed pH was adjusted to 7.26 ± 0.40 on day 149. By day 179, COD removal efficiency improved to 53%, IA/PA ratio decreased to 1.48, and TVA concentrations dropped to 3,360 mg HAc L^{-1} (Fig. 2b-c), demonstrating partial recovery.

In Phase V, an OLR of 18.0 kg COD m^{-3} d^{-1} was applied. Between days 180 and 231, influent pH was adjusted to 7.02 ± 0.04, resulting in a mean effluent pH of 8.12 ± 0.20 and COD removal efficiency of 65 ± 10%. The IA/PA ratio decreased progressively from 1.39 (day 182) to 0.36 (day 226), indicating enhanced stability. Reducing feed mean pH to 6.08 ± 0.07 (days 232-332) improved COD removal efficiency to 84 ± 4%, with a mean IA/PA ratio of 0.32 ± 0.07.

A key observation was the system's ability to prevent destabilization until extreme pH conditions arose, highlighting the role of the buffering capacity. As Fuess (2017) reported, methanogenic instability is often marked by a sharp drop in pH driven by the accumulation of organic acids that deplete alkalinity. The system's capacity to withstand reduced feed pH and high organic loads underscores its operational resilience, potentially offering insights into optimizing AD processes

with minimal chemical dependency. Overall, the system exhibited remarkable resilience and efficiency under varying operational conditions, with the IA/PA ratio and pH serving as key indicators of stability.

Fig. 2 presents the results obtained for the key operational parameters over time. This finding highlights the system's capacity to handle high organic loads, consistent with previous studies on the treatment of sugarcane vinasse. For instance, Fuess et al. (2017b) successfully operated a system at an OLR of 30 kg COD m^{-3} d^{-1}, while Aquino et al. (2017) reported stability at 18.0 kg COD m^{-3} d^{-1}.

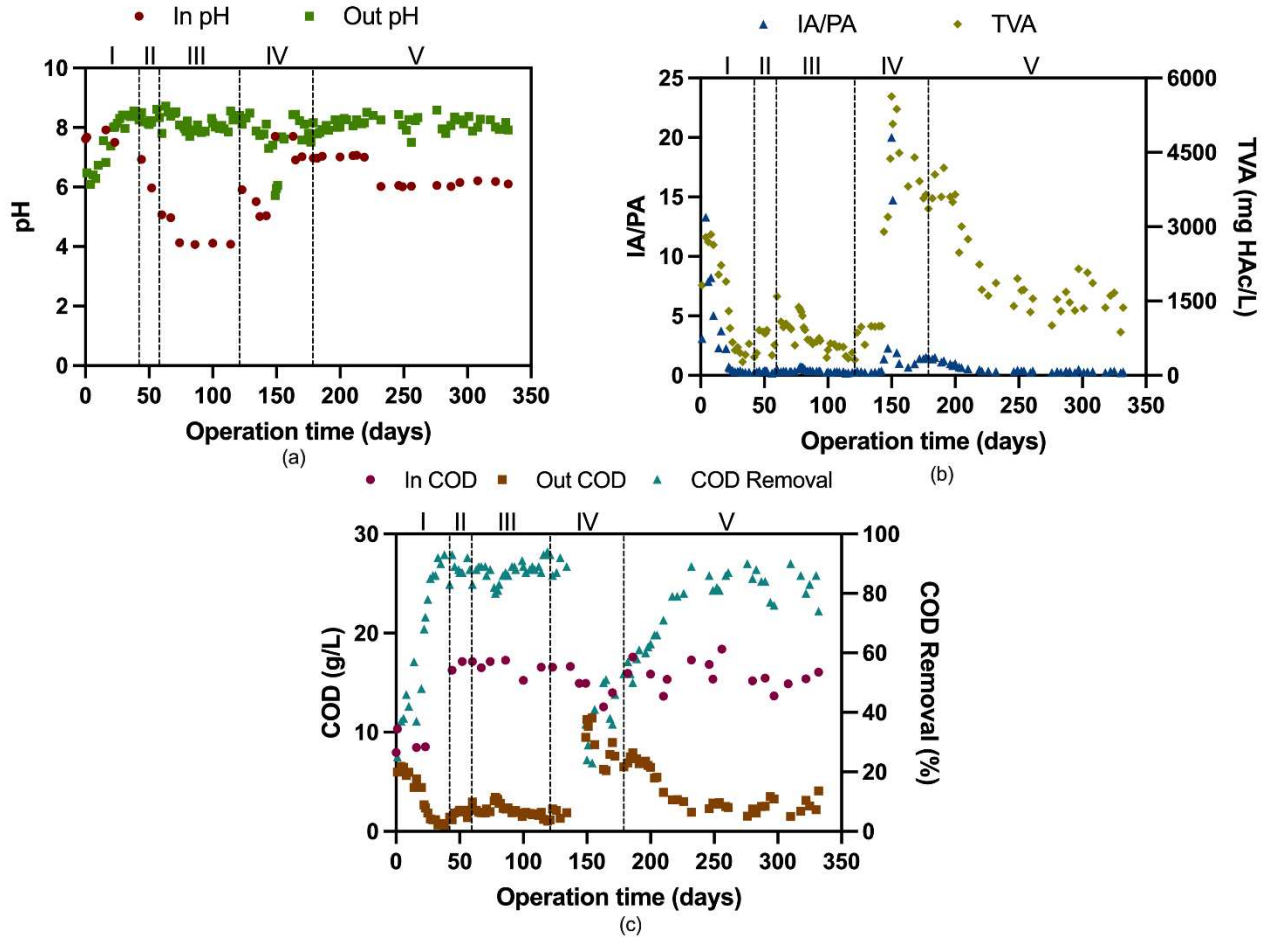


Fig. 2 Temporal profiles for (a) in pH and out pH, (b) in COD, out COD, and COD removal, and (c) IA/PA ratio and TVA concentration.
* Vertical dashed line indicates the division of operating phases.

The study demonstrated that variations in influent pH affected system performance, highlighting the importance of pH control. Operational stability was maintained at high OLRs with an influent pH close to 6.0. In contrast, influent pH values near 5.0 led to instability at an OLR of 12.0 kg COD m^{-3} d^{-1}. Moreover, operation without alkalizing agents was feasible only up to an OLR of 9.0 kg COD m^{-3} d^{-1}; above this limit, the reactor ceased to operate efficiently under non-alkalized conditions.

Santi Caraça et al. (2023) evaluated the co-digestion of sugarcane vinasse with diluted dairy cattle manure in UASB reactors operated in series with effluent recirculation. They achieved improved alkalinity and system balance without adding alkalizing agents, with a mean total COD removal efficiency of 77%. Their findings demonstrated the potential of co-digestion to enhance the nutritional profile of vinasse and stabilize AD systems.

Similarly, Qin et al. (2022) investigated the co-digestion of bovine manure (BM) and molasses vinasse (MV) at an OLR of 1.5 g SCOD L^{-1} d^{-1}. They identified an optimal BM-to-MV ratio of 1:3, achieving a soluble COD removal rate of 66.4 ± 1.2%. This study highlights the high buffering capacity of BM, which is attributed to its alkali metal content.

The present study observed similar benefits of co-digestion. The V:CM mixture (5:1 v/m) used in this study contributed to pH stability under non-alkaline conditions; cattle manure provides a strong buffering capacity, as noted by Font-Palma (2019) and Qin et al. (2022). These findings highlight the potential of co-digestion to enhance system stability and optimize anaerobic digestion performance in high-load applications.

3.2 Methane production

Biogas and methane production results were consistent with the operational stability and organic matter removal efficiency discussed in Section 3.1, thereby reinforcing the performance of the AnSTBR. Fig. 3 shows the biogas composition, MY, and VMP throughout the experimental period.

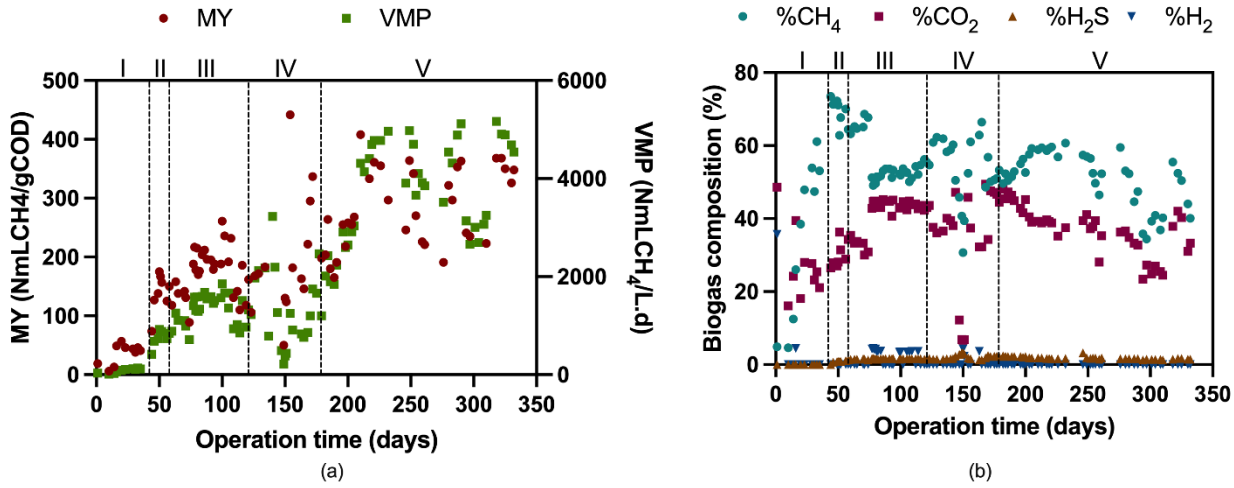


Fig. 3 Temporal profiles for (a) methane yield and volumetric methane production and (b) biogas composition

Phase I (OLR of 3.5 kg COD m^{-3} d^{-1}), considered the adaptation stage of the system, obtained a mean CH_4 concentration of 35.0 ± 21.4%, CO_2 concentration of 27.2 ± 9.9%, and H_2 concentration of 4.0 ± 11.2%, with no H_2S detected. The presence of H_2 is likely attributable to the reactor start-up phase, as hydrogen is a transient intermediate during AD aimed at biogas production. The process comprises three primary stages: the decomposition of complex organic matter into soluble compounds, the production of VFAs and hydrogen, and the consumption of VFAs and hydrogen to generate biogas. Hence, the transient H_2 accumulation observed during startup may indicate metabolic adjustments until stable operational conditions are established [35].

During Phase I, COD removal was 64.6 ± 23.2%, the MY was 35.8 ± 17.0 NmL CH_4 g^{-1} COD removed, and the VMP was 79.6 ± 44.6 NmL CH_4 L^{-1} d^{-1}. The increased COD removal observed during Phase I, combined with the low methane yield, may be attributed to the diversion of electron flow toward cellular synthesis [20].

Phase II, with an increased OLR of 6.0 kg COD m^{-3} d^{-1}, revealed an improvement in the conversion of organic matter into methane as well as improved reactor adaptation. During this phase, the reactor was tested under two inlet pH conditions: 6.93 (days 43–51) and 5.97 (days 52–58). For the first condition the mean CH_4 and CO_2 concentrations of were 70.2 ±

4.2% and 29.2 ± 4.1%, respectively, while H_2S reached 0.6 ± 0.4%, with no H_2 detected. The MY was 136.0 ± 40.0 NmL CH_4 g^{-1} COD removed, and the VMP reached 727.5 ± 200.6 NmL CH_4 L^{-1} d^{-1}. For the second condition, the mean CH_4 and CO_2 concentrations of were 67.4 ± 2.8% and 31.5 ± 2.7%, respectively, while H_2S reached 1.1 ± 0.2%, with no H_2 detected. The MY was 144.1 ± 16.9 NmL CH_4 g^{-1} COD removed, and the VMP reached 820.5 ± 75.0 NmL CH_4 L^{-1} d^{-1}.

Phase III, with an OLR of 9.0 kg COD m^{-3} d^{-1}, explored different influent pH conditions. From days 59 to 73, the mean inlet pH was 5.02 ± 0.07, resulting in a mean CH_4 concentration of 65.4 ± 2.0%, CO_2 concentration of 33.1 ± 2.0%, and H_2S concentration of 1.5 ± 0.1%, with no H_2 detected. The MY and VMP were 137.2 ± 14.6 NmL CH_4 g^{-1} COD removed and 1,068.5 ± 138.4 NmL CH_4 L^{-1} d^{-1}, respectively.

Still in Phase III, between days 74 and 121, the mean inlet pH was further reduced to 4.10 ± 0.03, without the addition of alkalizing. As a result, CH_4 concentration declined to 52.9 ± 3.7%, while CO_2 and H_2S concentrations increased to 43.2 ± 2.8% and 1.6 ± 0.1%, respectively. Additionally, H_2 was detected at a mean concentration of 2.0 ± 1.9%. The presence of H_2 suggests shifts in microbial activity but did not significantly impair methane production. Despite these challenges, the MY increased to 182.0 ± 41.0 NmL CH_4 g^{-1} COD removed, and the VMP rose to 1,389.8 ± 293.9 NmL CH_4 L^{-1} d^{-1}, indicating methane production under acidic feed conditions. This may be related to the fact that biogas production involves three main microbial groups, namely hydrolytic bacteria, fermentative bacteria, and methanogenic archaea. Fermentative bacteria operate over a wide pH range (4.0 – 8.5), with optimal pH values of 5.0 and 6.0, while methanogens are active between 5.5 and 8.5, with an optimal range of 6.5 to 8.0 [36].

At an OLR of 9.0 kg COD m^{-3} d^{-1}, fermentative bacteria initiated anaerobic digestion even under acidic conditions (pH 4.0–5.0), promoting VFA production and subsequent consumption. VFA conversion by methanogenic archaea led to bicarbonate formation, contributing to medium neutralization and maintenance of physicochemical and biological homeostasis in the reactor [37]. Previous studies have likewise reported microbial acclimation and methane production under acidic conditions, with pH ranges of 4.8–5.5 [38] and 4.0–5.3 [39].

Phase IV involved further increasing the OLR to 12.0 kg COD m^{-3} d^{-1}. Initially, with a mean influent pH of 5.71 ± 0.28 (days 122–136), the system achieved stable performance, with mean CH_4 and CO_2 concentrations of 59.9 ± 3.6% and 38.5 ± 3.5%, respectively, while H_2S was 1.6 ± 0.1%, and no H_2 was detected. The MY and VMP reached 157.0 ± 34.5 NmL CH_4 g^{-1} COD removed and 1,874.7 ± 440.4 NmL CH_4 L^{-1} d^{-1}, respectively. However, when the mean inlet pH was gradually reduced to 5.03 ± 0.02 (days 137–148), operational instability ensued, necessitating pH adjustment to 7.26 ± 0.40 (days 149–179). In this period, the CH_4 concentration fluctuated between 30.7% and 66.4%. On average, CH_4 and CO_2 concentrations were 52.1 ± 9.0% and 34.2 ± 15.3%, respectively, while H_2S and H_2 concentrations were 2.2 ± 0.6% and 0.6 ± 1.4%, respectively. The MY and VMP were 204.4 ± 112.3 NmL CH_4 g^{-1} COD removed and 1,217.0 ± 790.1 NmL CH_4 L^{-1} d^{-1}. These results indicate the system's pH sensitivity at higher OLRs and identify 9.0 kg COD m^{-3} d^{-1} as the upper limit for stable operation without alkalizing agents.

Phase V, at an OLR of 18.0 kg COD m^{-3} d^{-1}, tested two inlet pH conditions: approximately 7.0 and 6.0. With a mean influent pH of 7.02 ± 0.04, mean CH_4, CO_2, and H_2S concentrations were 55.6 ± 3.6%, 42.0 ± 3.8%, and 2.0 ± 0.3%, respectively, with no H_2 detected. The MY and VMP reached 265.2 ± 74.0 NmL CH_4 g^{-1} COD removed and 3,227.0 ± 1,098.8 NmL CH_4 L^{-1} d^{-1}, respectively. Reducing the mean influent pH to 6.08 ± 0.07 caused the CH_4 mean concentration to decline to 48.3 ± 7.9%, while CO_2 and H_2S concentrations 33.6 ± 5.8% and 1.7 ± 0.4%, respectively. Nevertheless, the MY and VMP increased to 297.5 ± 60.2 NmL CH_4 g^{-1} COD removed and 4,103.5 ± 816.9 NmL CH_4 L^{-1} d^{-1}.

Considering the maximum theoretical methane yield (350 NmL CH_4 g^{-1} COD), the observed value achieved 85% (297.5 ± 60.2 NmL CH_4 g^{-1} COD) of this theoretical maximum. These results are comparable to those of Ferraz Júnior et al. (2016); Fuess et al. (2017b); Janke et al. (2016); Qin et al. (2022); Takeda et al. (2022), as described in Table 4.

Table 4. Methane yields obtained in the literature for vinasse under different operating conditions

Substrate (s)	Type of reactor	OLR	Temperature	MY	Reference
Sugarcane vinasse	Upflow Anaerobic Sludge Blanket (UASB)	9.6 g COD L^{-1} d^{-1}	40 °C	239 NmL CH_4 g^{-1} $COD_{removed}$	Janke et al. (2016)
Sugarcane vinasse	UASB	25.0 g COD L^{-1} d^{-1}	55 °C	234.2 NmL CH_4 g^{-1} $COD_{removed}$	Ferraz Júnior et al. (2016)
Sugarcane vinasse	Methanogenic phase: Anaerobic structured-bed reactor (AnSTBR)	25 g COD L^{-1} d^{-1}	55 °C	301 ± 12 NmL CH_4 g^{-1} $COD_{removed}$	Fuess et al. (2017)
Sugarcane vinasse (V) and glycerol (G)	AnSTBR	5.0 g COD L^{-1} d^{-1}	30 °C	100%V → 250.6 ± 13.2 NmL CH_4 g^{-1} $COD_{removed}$ 50%V:50%G (COD basis) → 268.8 ± 12,5 NmL CH_4 g^{-1} $COD_{removed}$	Takeda et al. (2022)
Molasses vinasse and cattle manure - 7:1(cod basis)	Anaerobic digester reactor vessel	10.0 g $COD_{soluble}$ L^{-1} d^{-1}	36 °C	285.4 ± 9.9 mL g^{-1} $COD_{soluble}$	Qin et al. (2022)

Table 5 presents the mean values of biogas composition (%CH_4, %CO_2, %H_2S, and %H_2), MY, and VMP for each OLR applied during reactor operation.

Between Phases I and II (overall mean values), MY increased, accompanied by an enhancement in VMP. Based on the assessment of operational parameters, the system was considered to have reached an adaptation stage in Phase II. The greater availability of carbon likely stimulated microbial activity, resulting in higher organic matter conversion efficiency. As described in Section 3.1, COD removal efficiency increased from 65% to 89%, indicating improved system performance.

In Phases II, III, and IV, methane production intensified with increasing OLR. During these phases, different influent pH conditions were applied to minimize the use of alkalizing agents, establishing 9.0 kg COD m^{-3} d^{-1} as the maximum loading rate that allowed stable operation without external alkalization.

Table 5. Final mean for biogas composition, methane yield, and volumetric methane production by operation phase

Phase	OLR	Mean Input pH	%CH_4	%CO_2	%H_2S	%H_2	MY (NmL CH_4 g^{-1} COD)	VMP (NmLCH_4 L^{-1} d^{-1})
I	3.5	7.68	35.0 ± 21.4	27.2 ± 9.9	0.0 ± 0.0	4.0 ± 11.2	35.8 ± 17.0	79.6 ± 44.6
II	6.0	6.93	70.2 ± 4.2	29.2 ± 4.1	0.6 ± 0.4	0.0 ± 0.0	136.0 ± 40.0	727.5 ± 200.6

		5.97	67.4 ± 2.8	31.5 ± 2.7	1.1 ± 0.2	0.0 ± 0.0	144,1 ± 16.9	820.5 ± 75.0
III	9.0	5.02	65.4 ± 2.0	33.1 ± 2.0	1.5 ± 0.1	0.0 ± 0.0	137.2 ± 14.6	1068.5 ± 138.4
		4.10	52.9 ± 3.7	43.2 ± 2.8	1.6 ± 0.1	2.0 ± 1.9	182.0 ± 41.0	1389.8 ± 293.9
IV	12.0	5.71	59.9 ± 3.6	38.5 ± 3.5	1.6 ± 0.1	0.0 ± 0.0	157.2 ± 34.8	1874.7 ± 440.4
		5.03*	54.8 ± 6.3	35.4 ± 13.4	2.0 ± 0.5	0.0 ± 0.0	178.0 ± 30.5	1066.5 ± 587.7
		7.26	51.1 ± 9.9	33.7 ± 16.4	2.2 ± 0.6	0.8 ± 1.5	208.2 ± 111.3	1072.2 ± 621.7
V	18.0	7.02	55.6 ± 3.6	42.0 ± 3.8	2.0 ± 0.3	0.0 ± 0.0	265.2 ± 74.0	3227.0 ± 1098.8
		6.08	48.3 ± 7.9	33.6 ± 5.8	1.7 ± 0.4	0.0 ± 0.0	297.5 ± 60.2	4103.5 ± 816.9

*The mean obtained under this condition may not accurately represent the overall system behavior, as operational instability during this period led to a modification of the condition within a relatively short time frame, resulting in a limited number of representative experimental data points.

Ferraz Júnior et al. (2016) evaluated thermophilic anaerobic digestion of vinasse in a single-stage, continuously operated UASB reactor without alkalizing addition at an OLR of 15 kg COD m^{-3} d^{-1}. Insufficient partial alkalinity compromised medium stability, requiring sodium bicarbonate ($NaHCO_3$) supplementation. Nevertheless, $NaHCO_3$ dosing in high-rate anaerobic reactors has been reported to compromise process economic feasibility [12].

Furthermore, between Phases IV and V, a notable increase was observed in the final mean values of MY, which increased by 48%, from 192.6 to 284.2 NmL CH_4 g^{-1} COD removed, while VMP rose by 208%, from 1,216.8 to 3,752.9 NmL CH_4 L^{-1} d^{-1}. The increase in the methane yield suggests that the higher OLR applied in Phase V enhanced the conversion of organic matter into biogas without compromising the system stability. In contrast, Phase IV exhibited periods of operational instability, likely due to the low-pH substrate feed.

Previous studies have reported improved operational performance and higher methane productivity at elevated OLRs, including 18 kg COD m^{-3} d^{-1} [33], 25 kg COD m^{-3} d^{-1} [12, 41], and 33 kg COD m^{-3} d^{-1} [42], in AD systems treating sugarcane vinasse. These findings indicate that the system promoted biomass accumulation in the biofilm, enhancing degradation rates and process resilience [14, 33, 43].

3.3 Estimation of energetic potential

The theoretical energy potential throughout the reactor operation was based on experimentally obtained methane yield values (Table 6). Notably, the conversion of biogas into energy systems, such as generators, reciprocating engines, boilers, and turbines, was not considered; thus, the inherent losses associated with these systems were not included in the analysis.

When evaluating the energy potential of the liquid mix V + CM (5:1 v/m), a broader perspective that considers large-scale applications becomes relevant. This assessment was based on the total volume of vinasse generated during the 2023/2024 sugarcane harvest. According to UNICA data, ethanol production from sugarcane during this harvest reached approximately 27.3 billion liters [44]. Considering a vinasse generation rate of 10 to 15 liters per liter of ethanol produced [11, 17, 18], a mean value of 12.5 liters of vinasse per liter of ethanol was adopted for the calculations in this study. Based on this parameter, it is estimated that approximately 341.3 million cubic meters of vinasse will be generated during the 2023/2024 harvest.

For the evaluated mix, the estimated amount of cattle manure required was 68.3 million tons. This demand is feasible, given that Brazil has registered a cattle herd of 238.6 million heads in 2023 [45], with a mean daily fresh manure production of 27.8 kg per animal [23].

Table 6. Results obtained for energy potential during the operational phases of the reactor

Phase	OLR (kg COD m^{-3} d^{-1})	Mean input pH	Energy Potential (MJ m^{-3})
I	3.5	7.68	7.5 ± 4.4
II	6.0	6.93	69.6 ± 19.2
		5.97	78.3 ± 6.9
III	9.0	5.02	72.4 ± 9.4
		4.10	94.1 ± 20.5
IV	12.0	5.71	82.8 ± 19.5
		5.03	56.4 ± 37.0
		7.26	39.4 ± 19.7
V	18.0	7.02	100.4 ± 35.3
		6.08	140.4 ± 24.5

Based on the energy potential of the evaluated mixture obtained in Phase V (OLR = 18 kg COD m^{-3} d^{-1}) and the total vinasse volume generated during the 2023/2024 harvest, the estimated energy generation could reach approximately 16.2 million MWh. This corresponds to about 19% of the total electricity generated by Itaipu (83.9 million MWh) [35], the second-largest hydroelectric plant in the world, highlighting the significant bioenergy potential of sugarcane mill residues.

3.4 Degradation routes: Volatile fatty acids, sugars, and alcohol indications

AD occurs in four stages. During hydrolysis, organic macromolecules, such as lipids, proteins, and carbohydrates, are degraded into simpler compounds (sugars, amino acids, glycerol, and fatty acids). Subsequently, during acidogenesis, these products are fermented to produce volatile fatty acids (VFAs), alcohols, and other intermediates, among which acetate, propionate, and butyrate are direct precursors of methanogenesis [46]. However, the excessive accumulation of VFAs can compromise process stability.

During acetogenesis, VFAs are converted into acetate, CO_2, and H_2 by acetogenic bacteria. Finally, in methanogenesis, methanogenic archaea transform these compounds into methane via two main pathways: the hydrogenotrophic pathway, which converts CO_2 and H_2 into CH_4 and water, and the acetoclastic pathway, which converts acetate into CH_4 and CO_2 [46, 47].

Therefore, the acidogenic phase of AD plays a central role in determining the metabolic pathways that convert biomass into intermediate compounds. Different VFAs are formed during this stage, each of which is associated with a specific metabolic pathway. In this context, this section presents the VFA concentration profiles throughout the reactor operation based on analyses performed using HPLC. Fig. 4 shows the profiles of the organic intermediates monitored throughout reactor operation.

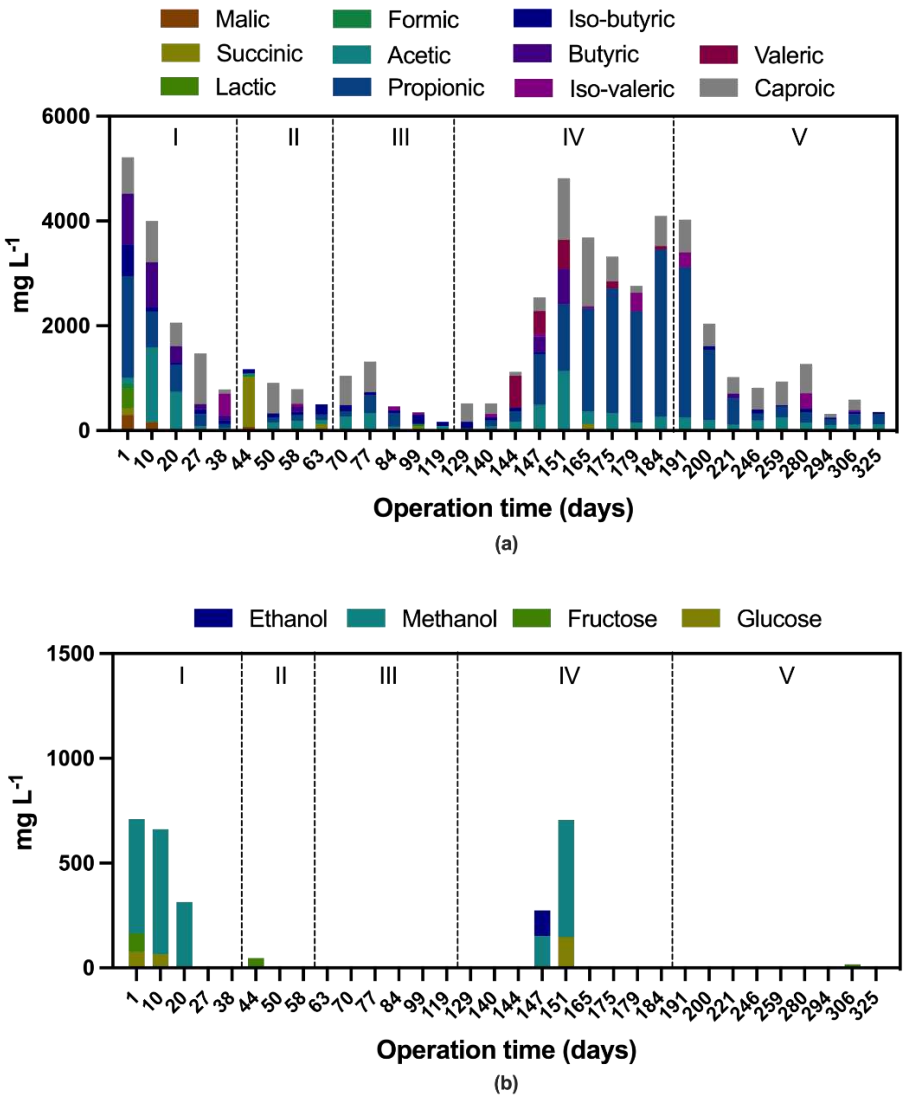


Fig. 4 Temporal profiles for the (a) volatile fatty acids production and (b) alcohols and sugars production

In Phase I, corresponding to the start-up period of the system, a high concentration of VFAs was observed, especially propionic, butyric, and caproic acids (Fig. 4a), as well as the presence of methanol and, at lower concentrations, sugars such as fructose and glucose (Fig. 4b). This accumulation is characteristic of the initial stages of microbial adaptation, during which fermentation activity predominates and the subsequent conversion of these intermediates into methane is still limited. As Phase I progressed and the microbiota adapted to the substrate and operating conditions, a progressive reduction in the concentrations of these compounds was observed, indicating a transition to a more stable environment.

In Phases II and III, no significant peaks in VFA production were detected, nor were alcohols and sugars present, indicating the greater efficiency of the acetogenic and methanogenic microbial communities in converting intermediates into final products.

In Phase IV, the increase in OLR to 12.0 kg COD m^{-3} d^{-1} and the decrease in mean influent pH from 5.71 ± 0.28 to 5.03 ± 0.02 compromised process stability, resulting in the accumulation of intermediates and a drop in the effluent pH. Under these conditions, an increase in the concentration of propionic, acetic, valeric, caproic, and butyric acids was observed,

indicating that the acetogenesis stage was impaired. This accumulation was evidenced by the increase in the TVA concentration, as discussed in Section 3.1.

Meegoda et al. (2018) reported that VFAs can vary significantly across digesters operating at different pH levels, and the accumulation of VFAs is widely recognized as a key indicator of instability and dysfunction in anaerobic digestion systems.

These results demonstrate the system's sensitivity to acidic feeds under high organic loads. As a corrective measure, the feed pH was adjusted to 7.26 ± 0.40 to restore the system. As feed pH increased, the concentrations of caproic, valeric, butyric, and acetic acids decreased, whereas the concentration of propionic acid remained high.

Propionate formation is strongly influenced by environmental factors such as pH, inoculum type, and product inhibition. pH values between 4.0 and 4.5 favor its production during acidogenesis [47, 48].

In Phase V, with the OLR increased to 18.0 kg COD m^{-3} d^{-1} and the effluent pH adjusted to 7.02 ± 0.04, the system demonstrated more consistent signs of stability. At this stage, a continuous reduction in the concentration of intermediate compounds and greater efficiency of their conversion to methane were observed, reflecting the recovery of the balance between the fermentative, acetogenic, and methanogenic stages of AD.

4. CONCLUSION

Evaluation of the AnSTBR system demonstrated that the co-digestion of vinasse and cattle manure is a promising strategy for biogas production, yielding favorable results across various OLRs. At OLRs below 9.0 kg COD m^{-3} d^{-1}, the system operated efficiently and stably, even with low-pH feedstock. This stability was attributed to the cattle manure's buffering capacity, which supports microbial growth and activity. However, at higher OLRs, the low pH of the feed leads to process instability, emphasizing the need for more stringent operational control.

The AnSTBR system was demonstrated to be a robust technology capable of tolerating high organic loads and optimizing the conversion of organic matter to biogas. Methane production increased significantly under the optimized conditions. The results indicated that operating the reactor at higher OLRs enhanced methane production, demonstrating the efficiency of this high-rate reactor in processing large volumes of waste. This efficiency not only resulted in high methane yields (297.5 ± 60.2 $NmLCH_4$ g^{-1} COD removed) but also ensured operational stability, provided that key operating parameters were properly controlled.

STATEMENTS AND DECLARATIONS

Author Contributions

Ana Beatriz Soares Aguiar: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft; and Writing – review & editing. **Renata Piacentini Rodriguez:** Conceptualization; Formal analysis; Supervision; Validation; and Writing – review & editing. **Bruna de Souza Moraes:** Conceptualization; Formal analysis; Funding acquisition; Project administration; Supervision; Validation; and Writing – review & editing.

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Declaration of interests

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

Data availability statement

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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