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ORIGINAL PAPER

Effect of Cosubstrate Ratio and Temperature on Sewage Sludge and Agro-industrial Fruit and Vegetable Waste Anaerobic Co-digestion

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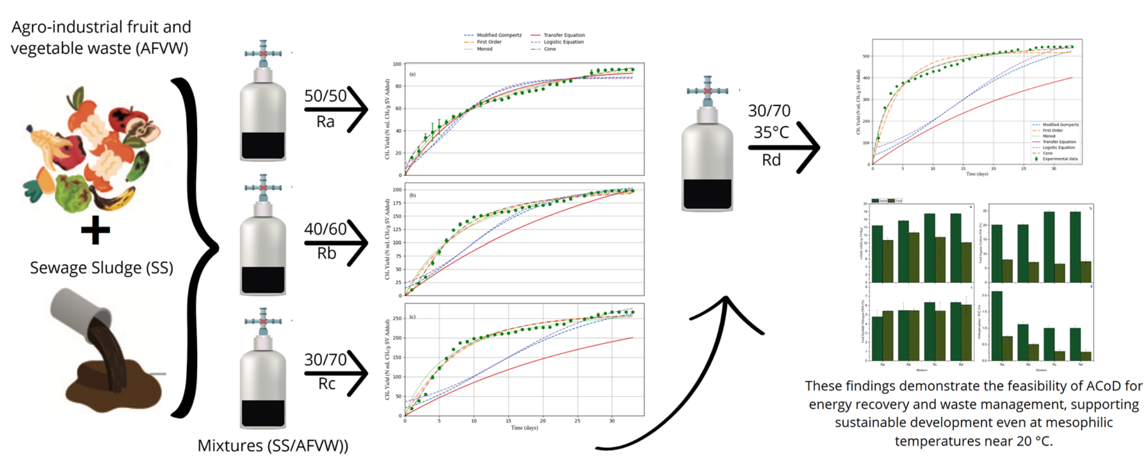
Abstract

The continuous increase in the generation of organic waste and sewage sludge, coupled with rising global energy demands, calls for sustainable and integrated waste-to-energy strategies. Anaerobic co-digestion (ACoD) emerges as a promising solution, enhancing energy recovery while ensuring environmental protection. This study investigates the ACoD of sewage sludge (SS) and the agro-industrial fruit and vegetable waste (AFVW) under different substrate ratios (50/50, 60/40, and 70/30) and temperature conditions (ambient and mesophilic at 35 °C). The objective was to optimize methane yield, organic matter degradation, and pathogen removal. The highest methane production (542.88 mL CH_4/g VS) occurred in the 70/30 mix under mesophilic conditions, associated with improved biodegradability and a favorable C/N ratio. Volatile solids and total organic carbon removal were enhanced without signs of ammonia inhibition. Importantly, significant reductions in Escherichia coli, Salmonella spp., and helminth eggs were achieved, meeting safety thresholds for agricultural biosolids reuse. Among six kinetic models, the Cone model best fitted the experimental data (R2 > 0.98), validating its predictive potential for ACoD performance. The 70/30 mix under ambient conditions also exhibited competitive results, reinforcing the feasibility of low-energy-input systems in decentralized contexts. Overall, this work highlights the potential of ACoD to simultaneously address organic waste management, renewable energy generation, and pathogen control, supporting the transition toward a circular economy and the achievement of Sustainable Development Goals.

Graphical Abstract



Keywords Anaerobic co-digestion · Sewage sludge · Fruit and vegetable waste · Pathogen inactivation

Extended author information available on the last page of the article

Introduction

After water is utilised in various human activities, it undergoes physical, chemical, and biological transformations, leading to water resource contamination that must be removed at wastewater treatment plants for the removal of suspended and dissolved particles via primary sedimentation and biomass growth during organic matter degradation in biological processes, solid and semi-solid residues known as SS are generated [1].

Numerous studies highlight that SS generation represents substantial volumes in wastewater treatment plants. Larney & Angers [2] reported a generation rate of 1.400 tonnes of dry sludge per day in Canada, while Zhang et al. [3] noted that China produces approximately 30 million tonnes of wet sludge annually. These authors also stated that the global annual production of dry sludge reaches 45 million tonnes. Additionally, Myo et al. [4] projected a 66% increase in sludge generation over the next 30 years in Southeast Asian Nations (ASEAN), a trend attributed to the access for over 2.1 billion people to potable water and basic sanitation since 2009, according to the UN.

The generation of waste, alongside human population growth, rapid urbanization, economic expansion, and changing consumption patterns, has become one of the most significant drivers of social, economic, and environmental impacts [5, 6]. According to Kaza et al. [7] two billion tonnes of waste were generated in the referenced year, the majority of which was disposed of in landfills, contributing to greenhouse gas emissions [8] and other detrimental effects.

Both SS and the AFVW can be treated using physicochemical processes such as gasification, carbonization, pyrolysis, and direct combustion [9] or through biological processes like anaerobic digestion, which requires lower economic investment and is easier to implement [10]. Anaerobic processes consist of multiple stages involving microbial communities: hydrolysis of carbohydrates, proteins, and lipids into sugars, amino acids, and alcohols; conversion of these intermediates into products such as propionate, butyrate, ethanol, and volatile fatty acids (VFAs) via fermentation; and finally, methanogenesis, which produces acetic acid and methane [11] Particularly ACoD combines two or more substrates to generate synergies, enhancing methane production and organic matter degradation [12]. The co-substrate ratio plays a critical role in regulating key parameters, such as the carbon-to-nitrogen (C/N) ratio, which is a limiting factor for microbial metabolism [13] due to the accumulation of ammoniacal nitrogen [14]. Xie et al. [15] determined that a C/N ratio between 20 and 25 optimises biogas production and digestion efficiency.

Temperature is another critical parameter in ACoD. According to Athanasoulia et al. [16], although methane production occurs across a wide temperature range, anaerobic digestion performs optimally under mesophilic conditions (30–40 °C), promoting rapid microbial growth and enhanced organic matter consumption [17]. While thermophilic methanogens (near 50 °C) are more efficient in methane production, slight temperature fluctuations of 1–2 °C can destabilise the process [17]. Consequently, thermal control via high-energy-consuming boilers may make anaerobic digestion technically and economically unfeasible, particularly in regions without seasonal variations where boiler implementation is unnecessary [18].

Regarding the ACoD of SS and AFVW, studies such as that of Morero et al. [19] have demonstrated the potential to integrate the treatment of SS and AFVW, increasing methane generation and minimizing environmental impacts. Tong et al. [20] conducted a life-cycle assessment comparing two scenarios: mono-anaerobic digestion and co-digestion of SS and AFVW. They found that biogas production increased by up to 30% in the co-digestion scenario, alongside reductions in associated environmental impacts. Additionally, ACoD can yield nutrient-rich digestates with low risks to human and environmental health [21], enabling the closure of biogeochemical cycles, reducing the need for agrochemicals [19], and lowering operational costs [22]. For instance, Nicoletto et al. [23] highlighted that digestates exhibit characteristics comparable to, or even superior, to commercial agrochemicals. Similarly, Di et al. [24]reported low phytotoxicity and germination indices exceeding 77%.

The objective of this study is to evaluate the influence of co-substrate ratios and temperature on ACoD at a laboratory scale, assessing not only methane production but also the efficiency of organic matter mineralisation and pathogen inactivation, aiming to achieve energy and material recovery from SS and AFVW.

Materials and Methods

Co-substrates and Inoculum

For this study, SS was obtained from the sludge thickener at the San Fernando wastewater treatment plant, located in the southern region of the Aburrá Valley Metropolitan Area. Anaerobic sludge from the same plant's anaerobic digesters was used as the inoculum. This wastewater treatment plant that treats domestic wastewater has been operational for over 20 years, ensuring that the inoculum is well-suited to adapt to the new substrate.

The AFVW was collected from the "La Minorista" market, the second-largest marketplace in the Aburrá Valley Metropolitan Area. This fraction primarily consisted of fruit and vegetable scraps and peels. Following the recommendations of Holliger et al. [12], the inoculum was incubated at ambient temperature for eight days. Additionally, as per Krause et al. [18], the AFVW was shredded to achieve particle sizes of 2 mm or smaller, and it was refrigerated at 4 ± 1 °C until the experimental setup.

Experimental Setup and Start-Up of Anaerobic Reactors

This study was conducted in two stages with the aim of optimizing the ACoD process of agro-industrial fruit and vegetable waste (AFVW) and sewage sludge (SS). In the first stage, three co-substrate ratios were evaluated in terms of volatile solids (VS), based on the findings of [25], who reported that a higher proportion of AFVW enhances methane production. The mixtures studied included AFVW:SS ratios of 50:50 (Ra), 60:40 (Rb), and 70:30 (Rc), all processed at ambient temperature. This decision was intentional and methodologically relevant, as it allowed the evaluation of anaerobic digestion feasibility under naturally occurring mesophilic-like temperatures (~ 25 °C), which are typical in many tropical and subtropical regions. Demonstrating satisfactory performance at room temperature is critical for assessing the potential applicability of ACoD in decentralized or low-resource settings, where reducing operational costs—particularly those associated with reactor heating—can significantly enhance the economic viability of the technology.

Although one of the main objectives of this study was to evaluate the feasibility of anaerobic digestion under ambient temperature conditions—relevant for decentralized applications and low-energy systems—it was considered essential to conduct a complementary evaluation under controlled mesophilic conditions (35 ± 2 °C) using the mixture that showed the best performance in the first stage. This comparison allowed for the analysis of temperature effects on key parameters such as methane production, organic matter degradation, and process stability, in order to determine whether this factor significantly influences system performance. This approach provides critical information for assessing the trade-offs between the energy input required for reactor heating and the potential benefits in terms of efficiency and energy yield.

All experiments, in both stages, were conducted in triplicate using 1 L glass batch reactors, each containing 300 mL of the respective mixture. The reactors were hermetically sealed with rubber stoppers fitted with a three-way valve system, which allowed biogas sampling and an initial nitrogen purge for 2 min. This procedure ensured strictly anaerobic conditions and enabled verification of system airtightness.

Monitoring of Parameters and Analytical Methods

The process monitoring included the evaluation of physical, chemical, and microbiological parameters. Methane measurement was conducted every 24 h by connecting each reactor to the three-way valve system and subsequently to a WIKA CPG1500 manometer, where the accumulated gas pressure was recorded during the period. A fraction of the biogas was characterised using a GFC Multi-Gas Detector G640 to determine methane concentration.

Using the recorded pressure values, the ideal gas equation was applied to calculate the moles of biogas present, and the measured concentration was used to determine the volumetric fraction of methane in the samples and the negative control (inoculum). Methane production attributed to the substrate was calculated as the difference between the samples and the negative blank. Subsequently, the data were standardised to reference conditions (0 °C and 1 atm), and the cumulative methane volume produced per gram of volatile solids added was calculated, enabling a precise characterisation of process performance.

Chemical and microbiological parameters were determined at the beginning and end of the experiment. The pH was measured using a potentiometric method, while alkalinity, total solids (TS), and volatile solids (VS) were analysed following Standard Methods 2023, 2540B, and 2540E, respectively. Additionally, total organic carbon (TOC), Kjeldahl nitrogen (KTN), phosphate (PO_4^{3-}), Salmonella, Escherichia coli, and helminth eggs were determined according to protocols established in Colombian Technical Standards (NTC) 5167, 370, 5021, and 5167, respectively. These analyses allowed for an assessment of the process's stability and efficiency in chemical and microbiological terms, aiming at the production of renewable energy and digestate.

Kinetic and Statistical Analysis

Methane production was analysed using time series, and six mathematical models, described in Table 1, were applied to identify the model that best represented the ACoD process. The remaining parameters were assessed by comparing initial and final concentrations, allowing for the determination of each treatment's efficiency in organic matter degradation and pathogen inhibition.

Results and Discussion

Characteristics of the Inoculum, Substrates, and Mixture

The characterization of substrates (SS and AFVW), inoculum, and mixtures (50/50, 60/40, and 70/30), as presented

Table 1 Kinetic models

Kinetic Model	Equation	References
Modified Gompertz	$B = B_0\left\{exp\left(-exp\left(\frac{R_m*e}{B_0}[\lambda - t] + 1\right)\right)\right\}$	[26]
First Order	$B = B_0 * \left(1 - e^{-kt}\right)$	[27]
Monod	$B = B_0 * \left(\frac{k*t}{1+(k*t)}\right)$	[28]
Transfer Equation	$B = B_0 * \left\{1 - exp\left(-\frac{R_m*(t-\lambda)}{B_0}\right)\right\}$	
Logistic Equation	$B = \frac{B_0}{1+exp\left\{4R_m*\frac{(\lambda-t)}{B_0}+2\right\}}$	
Cone	$B = \frac{B_0}{1+(k*t)^{-n}}$	[26]

Where: B: Accumulated methane (mL CH_4/gVS), B0: Maximum production potential (mL CH_4/gVS), Rm: Maximum production rate (mL CH_4/gVS*day), λ: Latency phase (d), t:Time (d), k: Rate constant (d^{-1}), n: Dimensionless factor, e: Euler's number

in Table 2, provides critical information on the influence of these elements on the ACoD process. The TS and VS content reveals significantly higher values for AFVW (TS: 119.2 g/kg; VS: 104.96 g/kg) compared to SS (TS: 11.48 g/kg; VS: 8.62 g/kg). Consequently, the mixtures exhibit an increase in TS and VS values as the proportion of AFVW rises, ranging from 65.34 g/kg in the 50/50 mixture to 86.88 g/kg in the 70/30 mixture. Moreover, the VS/TS ratio, an indicator of biodegradable organic content, also increases with the proportion of AFVW, reaching a value of 0.84 in the 70/30 mixture. This suggests that a higher inclusion of AFVW enhances biodegradable content, potentially boosting methane production [29]. Similarly, TOC values increased progressively from 32.28 g/kg in the 50/50 mixture to 36.42 g/kg in the 70/30 mixture, consistent with the higher organic content of AFVW.

Additionally, the carbon-to-nitrogen (C/N) ratio also increased from 23.96 in the 50/50 mixture to 27.49 in the 70/30 mixture, falling within the optimal range for anaerobic digestion [30], thereby reducing the risk of inhibition due to excess nitrogen. Conversely, $PO_4 3^-$ content decreased in the mixtures as the AFVW proportion increased, from 0.73 g/L in the 50/50 mixture to 0.51 g/L in the 70/30 mixture. Although phosphate is an essential nutrient for microbial growth, its adequate availability across all mixtures suggests that significant nutrient limitations are unlikely [31].

Regarding alkalinity, the mixtures exhibited significantly low levels, with total alkalinity values decreasing from 0.70 g $CaCO_3$/L in the 50/50 mixture to 0.04 g $CaCO_3$/L in the 70/30 mixture. This reduction in buffering capacity as the AFVW proportion increases suggests greater susceptibility to acidification [32], particularly in the 70/30 mixture. However, this issue can be mitigated by combining the mixtures with the inoculum [33], which in this study exhibited partial alkalinity of 3.44 g $CaCO_3$/L, intermediate alkalinity of 0.78 g $CaCO_3$/L, and total alkalinity of 4.23 g $CaCO_3$/L, indicating a high buffering capacity.

Methane Gas Production and Kinetic Models

The effect of co-substrate proportions on methane production using AFVW and SS mixtures at 50/50, 60/40, and 70/30 ratios, as well as the effect of temperature by analysing the 70/30 mixture at 35 °C, was studied in the present research. Each mixture was monitored following the procedures outlined in the methodology, with a monitoring period of 34 days, during which regular measurements were taken until the methane production trendline exhibited asymptotic behaviour. The results obtained, presented below, provide insights into the impact of co-substrate proportions and temperature on the ACoD process (Fig. 1).

Effect of Substrate Ratio

The Fig. 2 presents the experimental data (green dots) for cumulative methane (CH_4) production and the fitted curves corresponding to six models applied to three AFVW and

Table 2 Characteristics of the Inoculum, Substrates, and Mixtures

Parameters	SS	AFVW	Inoculum	Mixtures		
				50/50	60/40	70/30
TS (g/Kg)	11.48 ± 0.07	119.2 ± 0.06	27.34 ± 0.06	65.34 ± 0.11	76.11 ± 0.04	86.88 ± 0.05
VS (g/Kg)	8.62 ± 0.02	104.96 ± 1.69	12.38 ± 0.10	56.79 ± 0.06	66.42 ± 0.19	76.05 ± 0.06
VS/TS	0.75 ± 0.00	0.88 ± 0.002	0.45 ± 0.00	0.81 ± 0.002	0.82 ± 0.00	0.84 ± 0.00
PA (gCaCO3/L)	0.03 ± 0.00	–	3.44 ± 0.03	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.0
IA (gCaCO3/L)	0.10 ± 0.01	–	0.78 ± 0.02	0.053 ± 0.00	0.04 ± 0.01	0.03 ± 0.01
TA (gCaCO3/L)	0.14 ± 0.01	–	4.23 ± 0.06	0.70 ± 0.00	0.05 ± 0.01	0.04 ± 0.0
TOC	21.94 ± 2.59	42.63 ± 1.59	10.75 ± 1.24	32.28 ± 0.24	34.35 ± 0.30	36.42 ± 0.53
KTN	1.45 ± 0.96	1.3 ± 0.10	6.19 ± 0.94	1.37 ± 0.72	1.33 ± 0.82	1.34 ± 0.96
C/N	15.13 ± 0.58	32.79 ± 0.00	1.73 ± 0.54	23.96 ± 0.52	25.72 ± 0.60	27.49 ± 0.75
PO_4^3	1.29 ± 0.10	0.18 ± 0.10	2.56 ± 0.19	0.73 ± 0.23	0.62 ± 0.08	0.51 ± 0.07

PA Partial Alkalinity, *IA* Intermediate alkalinity, *TA* total alkalinity

Fig. 1 Experimental setup

SS mixtures in 50/50 (a), 60/40 (b), and 70/30 (c) ratios. Methane production is shown as a function of time (days) and highlights significant differences in methane yield attributable to variations in the mixture composition. In the 50/50 mixture (a), cumulative methane production reached approximately 95 mL CH_4/g VS at the end of the monitoring period. In contrast, the 60/40 mixture (b) exhibited a substantial increase, reaching approximately 198 mL CH_4/g VS, which represents more than double the yield compared to the 50/50 mixture. This result indicates the positive influence of a higher proportion of AFVW, which provides increased bioavailable carbon and improves the C/N ratio [20] thereby enhancing the metabolic activity of methanogenic microorganisms [34].

Finally, the 70/30 mixture (c) achieved the highest cumulative methane production, reaching approximately 260 mL CH_4/g VS. This value represents a 38.9% increase compared to the 60/40 mixture and a threefold increase compared to the 50/50 mixture. The higher proportion of AFVW in this mixture, along with the consequent improvement in the C/N ratio, contributed to higher biodegradable organic matter content and favourable conditions for microbial activity [35], significantly boosting methanogenic performance. However, it is important to note that system stability in mixtures with high AFVW proportions could be compromised by the accumulation of volatile fatty acids [36]. Nonetheless, this potential inhibitory effect appears to have been mitigated by the alkalinity provided by the inoculum used.

The results observed in Fig. 2 suggest that the ACoD of AFVW and SS present a viable option for the energy recovery of these wastes, even under ambient temperature conditions [37]. This finding is particularly relevant for communities and regions where environmental temperature is relatively stable, as it enables the implementation of this technology without the need for costly boiler-based heating systems, which often constitute a barrier to adopting such solutions. The feasibility of operating without heating not only improves economic accessibility but also extends the reach of the technology to resource-limited regions, promoting more sustainable organic waste management [38].

Table 3 presents the kinetic parameter values (B_0, R_m, λ, k, and n) obtained by applying six mathematical models (Modified Gompertz, First Order, Monod, Transfer Equation, Logistic Equation, and Cone Model) for three AFVW and SS mixtures at 50/50, 60/40, and 70/30 ratios under ambient temperature conditions. Model fitting was evaluated using determination coefficients (R2) and root mean square errors (RMSE), allowing for the identification of the model that best described the behaviour of each mixture.

For the 50/50 mixture, the Cone Model emerged as the most accurate, with an R2 of 0.99 and an RMSE of 1.67 mL CH_4/g VS, consistent with previous findings by Pan et al. [39] and Elif Gulsen Akbay [28]. This model outperformed others, accurately describing the phases of methane production. The Monod Model also exhibited notable performance, with an R2 of 0.98 and an RMSE of 2.67 mL CH_4/g VS, ranking as the second-best model for this mixture. In contrast, the Modified Gompertz Model displayed significantly lower accuracy, with an R2 of 0.92 and an RMSE of 6.58 mL CH_4/g VS, indicating its inability to adequately capture the process phases. Similar values were reported by Alshikhradhi et al. [40], who found that Gompertz was the best-fitting model among those studied but achieved only an R2 of 0.94. In the 60/40 mixture, the Cone Model again provided the best fit, with an R2 of 0.98 and an RMSE of 6.30 mL CH_4/g VS. This model accurately described both the maximum methane production ($B_0 = 204.78$ mL CH_4/g VS) and associated kinetic parameters, demonstrating high fidelity in fitting. The First-Order Model also performed well, with an R2 of 0.97 and an RMSE of 8.53 mL CH_4/g VS, representing a valid alternative for this mixture. Conversely, the Modified Gompertz Model showed significantly poorer performance, with an R2 of 0.82 and an RMSE of 24.11 mL CH_4/g VS, making it unsuitable for describing this mixture's behaviour. Finally, for the 70/30 mixture, the Cone Model remained the most suitable, with an R2 of 0.98 and an RMSE of 9.14 mL CH_4/g VS, confirming its capability to describe systems with a high AFVW proportion. This model precisely captured the maximum methane production ($B_0 = 276.02$ mL CH_4/g VS) and other relevant kinetic parameters. The First-Order Model also showed acceptable performance, with an R2 of 0.97 and an RMSE of 11.31 mL CH_4/g VS. However, models such as Modified Gompertz and the Transfer Equation demonstrated significantly inferior performance, with R2 values of 0.42 and 0.28, respectively, and high RMSE values (55.50 and 82.80 mL CH_4/g VS), evidencing their inability to capture the dynamics of this mixture.

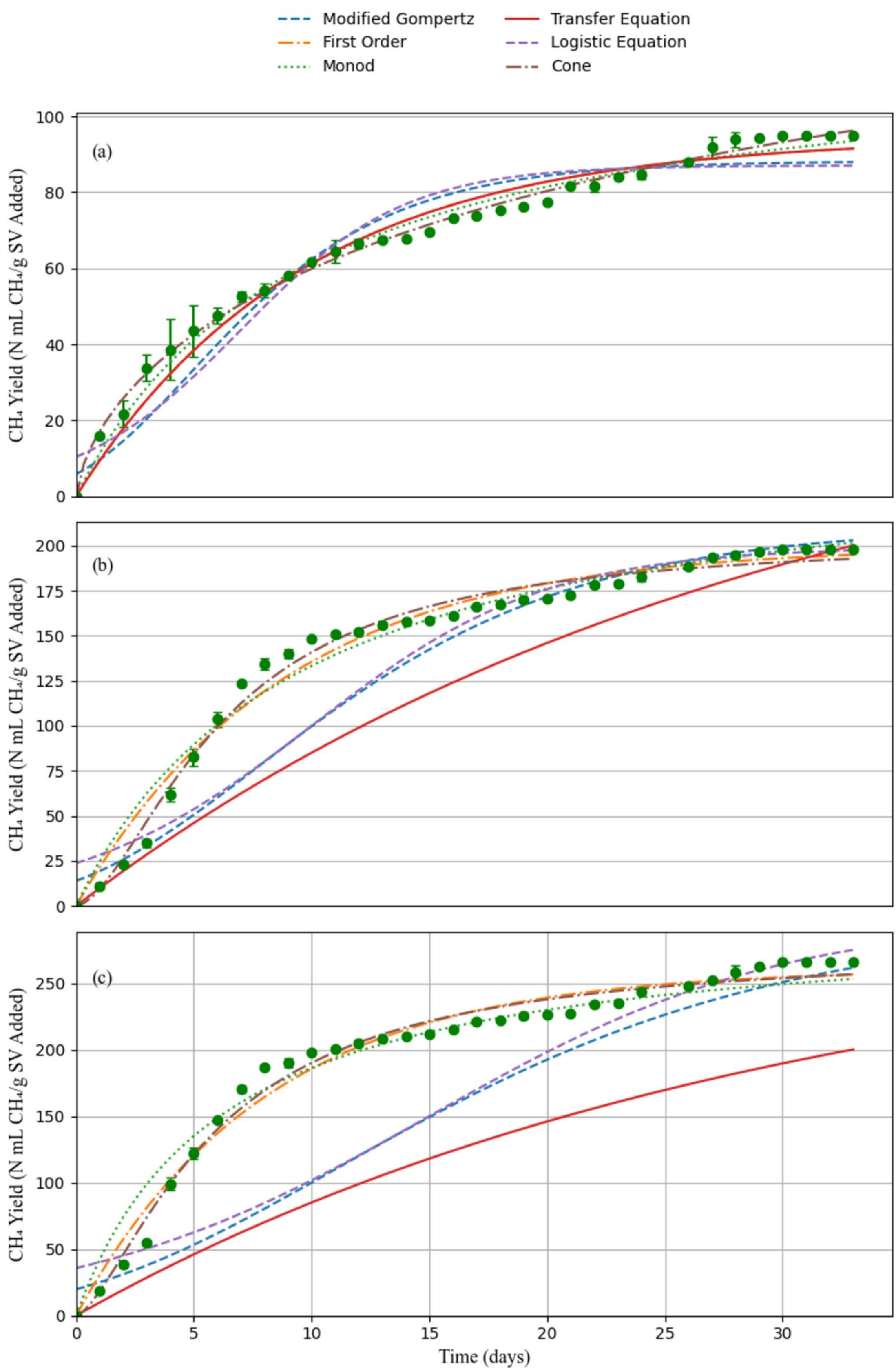


Fig. 2 Methane gas yield for the different mixtures: **a** 50/50 mixture, **b** 60/40 mixture, and **c** 70/30 mixture

Table 3 Kinetic parameters for anaerobic co-digestion mixtures

Model	Parameters	Ra (50/50)	Rb (60/40)	Rc (70/30)	Rd (70/30–35 °C)
Gompertz	B_0 (mL CH_4/gVS)	88.22	211.65	300.00	600.00
	R_m (mL CH_4/gVS*days)	6.68	10.00	10.00	20.00
	λ (d)	0.00	0.00	0.00	0.00
	R^2	0.92	0.82	0.42	0.89
	RMSE	6.58	24.11	55.50	167.16
First Order	B_0 (mL CH_4/gVS)	94.55	199.65	260.74	516.05
	k (d^{-1})	0.10	0.13	0.12	0.23
	R^2	0.97	0.97	0.97	0.94
	RMSE	4.01	8.53	11.31	29.26
Monod	B_0 (mL CH_4/gVS)	120.91	259.59	300.00	586.88
	k (d^{-1})	0.10	0.10	0.16	0.37
	R^2	0.98	0.97	0.94	0.98
	RMSE	2.67	9.39	14.00	15.46
Transfer Equation	B_0 (mL CH_4/gVS)	94.55	300.00	300.00	600.00
	R_m (mL CH_4/gVS*days)	9.86	10.00	10.00	20.00
	λ (d)	0.00	0.00	0.00	0.00
	R^2	0.97	0.62	0.28	0.23
	RMSE	4.01	34.96	82.80	220.76
Logistic Equation	B_0 (mL CH_4/g VS)	87.06	199.29	300.00	600.00
	R_m (mL CH_4/g VS*day)	6.25	10.00	10.00	20.00
	λ (d)	0.00	00.00	0.00	0.00
	R^2	0.90	0.82	0.48	0.68
	RMSE	1.67	23.74	52.71	157.56
Cone	B_0 (mL CH_4/g VS)	193.28	204.78	276.02	
	k (d^{-1})	2.99	0.16	0.17	600.00
	n	6.64	0.16	1.49	0.32
	R^2	0.99	0.98	0.98	0.98
	RMSE	1.67	6.30	9.14	14.53

Overall, the Cone Model proved to be the most robust and accurate for describing cumulative methane production in the three mixtures evaluated under ambient temperature conditions. This model stands out for its ability to capture the different phases of the process, including latency, exponential growth, and production stabilisation, positioning it as a useful tool for simulating anaerobic digestion processes with varying co-substrate proportions. This performance has also been corroborated by other authors, such as Polastri et al. [41]. On the other hand, while models such as Monod and First Order provided reasonable fits for certain mixtures, their performance was less consistent compared to the Cone Model.

Effect of Temperature

Figure 3 illustrates methane production data for the 70/30 mixture operated at 35 °C. In this case, cumulative methane production reached approximately 542.88 mL CH_4/g VS, representing a significantly higher yield compared to the same mixture at ambient temperature. This behaviour is attributed to the optimal activation of microbial communities under mesophilic conditions, where enzymatic activity and metabolic efficiency reach their maximum potential [18]. The graph shows a very short initial lag phase, followed by rapid exponential growth and subsequent stabilisation. This accelerated increase in methane production is reflected in the close alignment of kinetic models (particularly Cone and First-Order) with the experimental data during the acceleration and stabilisation phases.

Compared to Fig. 2c (70/30 mixture at ambient temperature), where cumulative methane production reached a lower value of approximately 266.34 mL CH_4/g VS, metabolic limitations imposed by lower temperatures are evident, affecting the activity of methanogenic microorganisms, particularly archaea. Additionally, the lag phase is longer than in the mixture operated at 35 °C, indicating a slower process startup rate. When comparing both conditions, it is evident that operation at 35 °C not only enhances maximum methane yield but also accelerates the time required to reach stabilisation. This underscores that temperature is a key factor in optimising the anaerobic

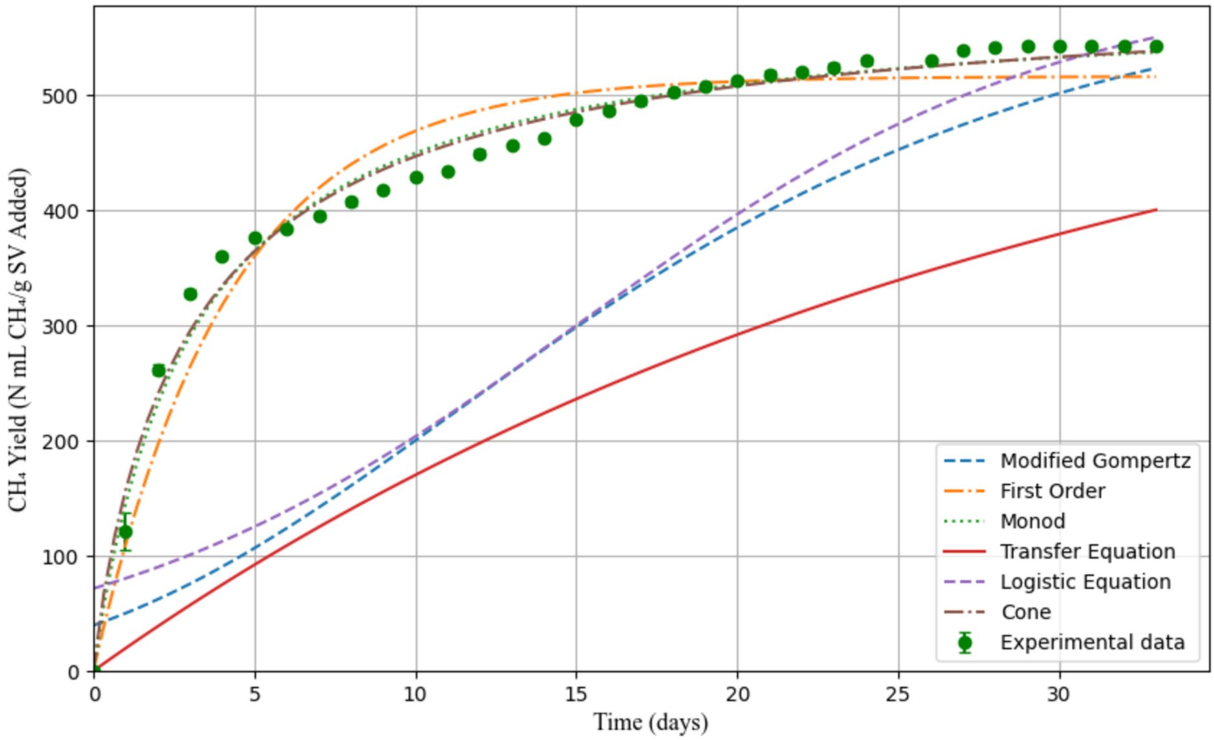


Fig. 3 Methane gas yield for 70/30 mixture at 35 °C

digestion process, especially for mixtures with a high proportion of AFVW.

Table 3 presents the kinetic parameters of the six models applied to cumulative methane production for the 70/30 mixture operated at 35 °C. Results showed that the Cone Model provided the best fit, with an R2 of 0.986 and an RMSE of 14.53 mL CH_4/g VS. This model proved to be the most robust in representing the process kinetics, accurately capturing the exponential growth and stabilisation phases. The Monod Model also exhibited outstanding performance, with an R2 of 0.984, ranking as the second-best model under this condition. In contrast, models such as Modified Gompertz (R2 = 0.89 and RMSE = 167.16 mL CH_4/g VS), the Transfer Equation (R2 = 0.23 and RMSE = 220.76 mL CH_4/g VS), and the Logistic Equation (R2 = 0.687 and RMSE = 157.57 mL CH_4/g VS) failed to adequately describe the kinetic behaviour of the process.

Organic Matter Degradation

Figure 4 a shows the initial and final pH behaviour for the four experimental ACoD treatments: Ra (50/50), Rb (60/40), Rc (70/30 at ambient temperature), and Rd (70/30 at 35 °C). Results indicate that in all cases, the initial pH remained between 7.45 and 7.63, values close to neutrality, which are optimal for the methanogenic phase of the process [42]. This pH range is characteristic of well-balanced anaerobic systems [43], where the alkalinity provided by the initial inoculum offers the buffering capacity needed to counteract potential increases in VFAs during the early stages of digestion [40]. Although a slight decrease in final pH was observed across all treatments, it remained within the neutral range (7.2–7.6), suggesting that the co-digestion process was stable and free of significant disturbances [44]. The slight pH reduction may be attributed to the temporary production and accumulation of VFAs during the acidogenic phase, a common phenomenon in systems with a high load of biodegradable organic matter [45]. However, the system's buffering capacity, provided by alkalinity generated during protein and carbohydrate decomposition, was sufficient to prevent critical acidification that could affect methanogenic archaeal activity. The stability of the final pH, particularly in mixtures Rc (70/30 at ambient temperature) and Rd (70/30 at 35 °C), correlates with the high biogas production reported in Figs. 2 and 3. The higher methane generation indicates efficient VFA consumption during methanogenesis, which prevents excessive acid accumulation and maintains the pH within neutral values [44].

Regarding initial alkalinity (Fig. 4 b), all mixtures exhibited values exceeding 2 g $CaCO_3$/L, characteristic of systems with significant inoculum presence, capable of providing an initial buffering capacity. Similar values have been reported by Sedighi et al. [44]. However, as the AFVW proportion in the mixture increased, the initial alkalinity progressively

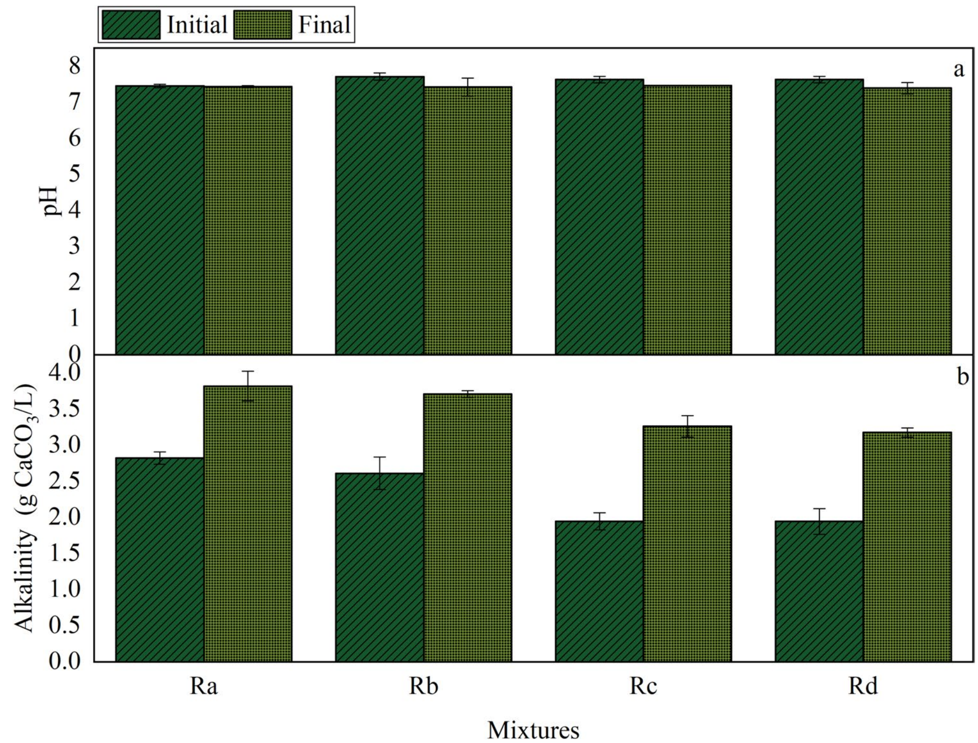


Fig. 4 Evolution of pH and Alkalinity during the Anaerobic Co-Digestion of SS and AFVW: **a** pH, **b** Alkalinity

decreased. For instance, while the 50/50 mixture recorded a value of 2.8 ± 0.083 g $CaCO_3$/L, the 70/30 mixture presented 1.99 ± 0.17 g $CaCO_3$/L. This decline can be attributed to the higher content of readily biodegradable organic matter in AFVW, causing a temporary reduction. This phenomenon aligns with previous studies that highlight the inverse relationship between carbonaceous matter content and the system's initial alkalinity [46]. In terms of final alkalinity, the results show a significant increase of approximately 1 g $CaCO_3$/L across all treatments, reflecting a progressive system stabilisation [47]. This increase is attributable to the production of bicarbonates (HCO_3^-) and carbonates resulting from VFA consumption during the methanogenic phase [48]. It is noteworthy that the 70/30 mixtures exhibited the highest alkalinity increases, slightly higher in the ambient temperature treatment, closely followed by the 35 °C mixture. This behaviour suggests that while mesophilic conditions (35 °C) promote higher metabolic efficiency of acidogenic and methanogenic bacteria, ambient temperature conditions also successfully maintain adequate alkaline balance due to the sustained decomposition of carbohydrates in AFVW and their conversion into buffering compounds. The relationship between final alkalinity and methane production further reinforces the hypothesis that mixtures with higher AFVW proportions (70/30) exhibit greater biochemical potential [25]. Elevated methane production in these treatments is directly related to the efficient VFA consumption during intermediate process stages [32]. This consumption not only prevents system acidification but also fosters bicarbonate formation, contributing to increased alkalinity and ensuring anaerobic process stability. In contrast, the 50/50 and 60/40 mixtures, although showing final alkalinity increases, provide less biodegradable organic matter, limiting both methane production and the formation of alkaline compounds compared to the 70/30 mixtures.

The Fig. 5 illustrates the evolution of key parameters during the ACoD of SS and AFVW in the four experimental treatments: Ra (50/50), Rb (60/40), Rc (70/30 at ambient temperature), and Rd (70/30 at 35 °C). The behaviour observed in volatile solids (VS), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), and orthophosphates (PO_43^-) reflects the process efficiency in organic matter degradation and his correlation with biogas production. Initial volatile solids (VS) values ranged between 12 and 17 g VS/kg across all treatments, with a significant reduction at the end of the process. This decrease was particularly pronounced in mixtures with higher AFVW proportions (Rc and Rd), where final values showed a reduction of approximately 5 and 6 g VS/kg, representing a degradation percentage of around 35%. This reduction indicates efficient degradation of the biodegradable organic fraction, facilitated by the high carbohydrate content and easily hydrolysable compounds in AFVW [49, 50]. Mesophilic conditions at 35 °C in Rd optimise the metabolic activity of hydrolytic and methanogenic bacteria, enabling a faster conversion of VS into volatile fatty acids (VFAs) and subsequently into methane [51].

Similarly, total organic carbon (TOC) levels showed a sharp decrease across all treatments, dropping from initial

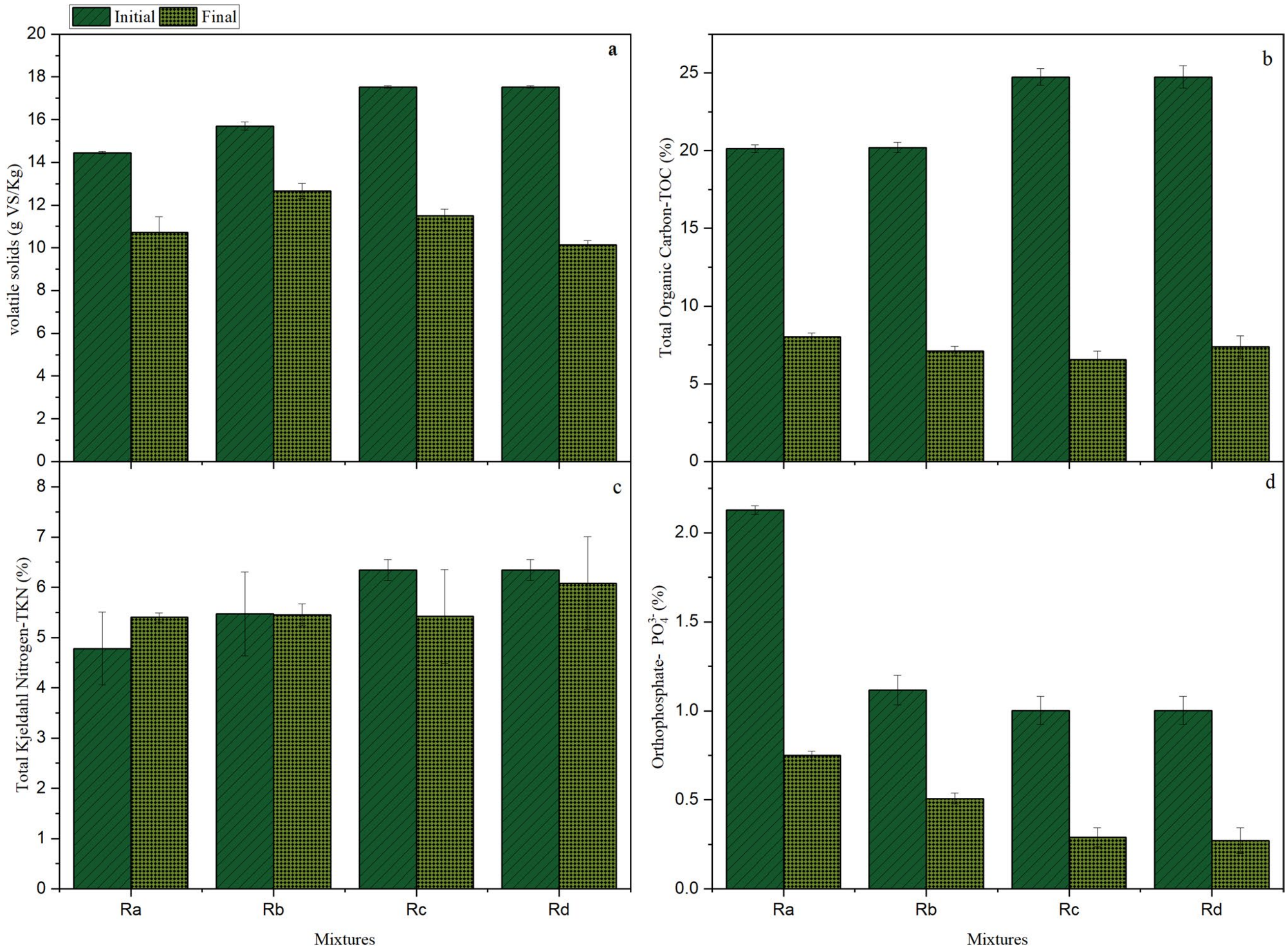


Fig. 5 Behavior of Physicochemical Parameters during the ACoD of SS and AFVW: **a** Volatile Solids, **b** Total Organic Carbon, **c** Total Kjeldahl Nitrogen and **d** Orthophosphates

values between 20 and 25% to final levels close to 5–7%. This behaviour was particularly evident in Rc and Rd mixtures, where the greater availability of biodegradable organic matter and mesophilic conditions allowed a more efficient transformation of carbon into biogas [52]. The correlation between TOC reduction and methane production confirms the system's ability to consume carbonaceous substrates and convert them into gaseous end products, mainly CH_4 and CO_2 [24]. On the other hand, total Kjeldahl nitrogen (TKN) remained relatively constant, with initial and final values oscillating between 5 and 7%. The stability of TKN suggests that the degradation of proteins and other nitrogenous compounds during hydrolysis released ammonium (NH_4^+) [53, 54], which did not accumulate at inhibitory levels for methanogenic archaea [55, 56]. This behaviour can be attributed to the system's buffering capacity, where the generated ammonium did not negatively affect pH or alkalinity [57]. Additionally, the assimilation of some nitrogen by microbial biomass in anabolic processes contributed to maintaining system stability. The Rd treatment at 35 °C exhibited a slight trend toward higher values, potentially indicating greater protein degradation facilitated by mesophilic conditions, without disrupting system equilibrium.

Finally, orthophosphates (PO_43^-) showed a significant reduction at the end of the process, with initial values close to 2% in Ra and final values ranging between 0.5 and 0.7% in treatments with higher AFVW content. This reduction can be explained by the release of phosphorus during the degradation of phosphorus-containing compounds and its subsequent assimilation by microbial biomass or chemical precipitation as insoluble phosphates [58]. The lower final orthophosphate content in Rc and Rd suggests more efficient nutrient consumption in these treatments, promoting biomass synthesis and the metabolic activity of microbial communities responsible for methane production [59].

Overall, the results reflect an efficient and stable anaerobic co-digestion process, where the higher degradation of volatile solids and total organic carbon in Rc (70/30 at ambient temperature) and Rd (70/30 at 35 °C) treatments is directly linked to the higher biogas production observed. The stability of TKN levels and the reduction of orthophosphates reinforce the notion of adequate metabolic balance, where microbial activity remains uninhibited, and released nutrients are efficiently utilized in catabolic and anabolic processes. Mesophilic conditions in the Rd treatment optimize overall process efficiency, although the robustness of the Rc system at ambient temperature highlights the viability of co-digestion in operational scenarios with less thermal control.

Inhibition of Pathogenic Microorganisms

The graph presents the initial and final concentrations of pathogenic microorganisms during the ACoD of SS and AFVW in the four experimental treatments: Ra (50/50), Rb (60/40), Rc (70/30 at ambient temperature), and Rd (70/30 at 35 °C). The evaluated parameters include the concentration of *Escherichia coli* (CFU/mL) (a), the presence of *Salmonella spp.* (b), and the number of helminth eggs (c), complemented with representative images of helminth specimens (d.1–d.4). These results enable the assessment of the anaerobic process's efficacy in pathogen reduction, a critical criterion for the sanitary safety and valorisation of digestates as agricultural amendments.

In Figure a, corresponding to *E. coli*, a considerable reduction is observed in all treatments at the end of the process. Initial concentrations vary around 1.25×10^4 CFU/mL, highlighting the elevated microbiological load of the initial system. At the end of the process, concentrations decrease significantly, with values nearing zero in Rc (70/30 at ambient temperature) and Rd (70/30 at 35 °C). This reduction reflects the anaerobic process's efficiency in *E. coli* inactivation, facilitated by system-specific conditions such as VFA generation. The combination of the higher organic load in Rc and mesophilic conditions in Rd enhances microbial activity, increasing pathogen inactivation [60, 61]. Regarding *Salmonella spp.* presence (Figure b), measured by presence or absence, the results show the presence of *Salmonella spp.* in all initial samples. Total inhibition of *Salmonella spp.* was achieved in treatments Ra, Rb, and Rd. In contrast, Rc (70/30 at ambient temperature) exhibited significant reduction, with two of the three reactors showing absence, while one reactor evidenced presence. The inhibition efficiency of *Salmonella spp.* has been linked to inhibitory agents such as increased ammonium or VFA concentrations [62].

Figure c illustrates the behaviour of helminth eggs, a key indicator of digestate sanitary stability. Initial concentrations are elevated across all treatments, with values exceeding 25 eggs/4 g of sample. By the end of the process, a drastic reduction is observed in all cases, with final values between 2 and 3 eggs/4 g, meeting the accepted limits for Type B biosolid reuse. Rd displays the lowest values, reinforcing the process's effectiveness under high organic load and mesophilic conditions. Helminth reduction can be explained by the combination of thermal, biochemical, and retention time effects, which cause membrane and cellular structure destruction in these organisms [63]. The representative images of helminth eggs (d.1–d.4) show the presence of pathogens such as *Ascaris lumbricoides* (corticated and decorticated), *Trichuris trichiura*, and *Hymenolepis diminuta*, which are associated with intestinal diseases.

The results demonstrate that the anaerobic co-digestion process is effective in reducing pathogenic microorganisms such as *E. coli*, *Salmonella spp.*, and helminth eggs, particularly in treatments with higher organic loads (70/30) and mesophilic conditions (35 °C). Pathogen inactivation is closely related to VFA production, which contributes to a hostile environment for pathogen survival. The observed reductions in these sanitary indicators confirm the digestate's potential as a safe product for agricultural amendment applications, meeting the required quality and sanitary safety standards (Fig. 6).

Conclusions

This study confirms the technical and environmental feasibility of anaerobic co-digestion (ACoD) of sewage sludge and AFVW, particularly at a 70:30 ratio under mesophilic conditions, achieving high methane yields (542.88 mL CH_4/g VS), enhanced organic matter removal, and stable performance without ammonia inhibition. The favourable C/N ratio promoted microbial activity, accelerating hydrolysis and methanogenesis. Moreover, the process ensured effective hygienisation, complying with international standards through the complete inactivation of *Escherichia coli*, *Salmonella* spp., and significant reduction of helminth eggs, thus enabling safe agricultural reuse of biosolids. The Cone model proved most accurate for predicting methane production (R2 > 0.98), reinforcing its relevance for process optimisation. Notably, the robust performance observed at ambient temperature underscores the applicability of ACoD in low-energy or decentralised settings. Altogether, these findings establish ACoD as a resilient, scalable strategy for the simultaneous valorisation of two high-impact waste streams, advancing circular economy goals through integrated waste management and renewable energy generation.

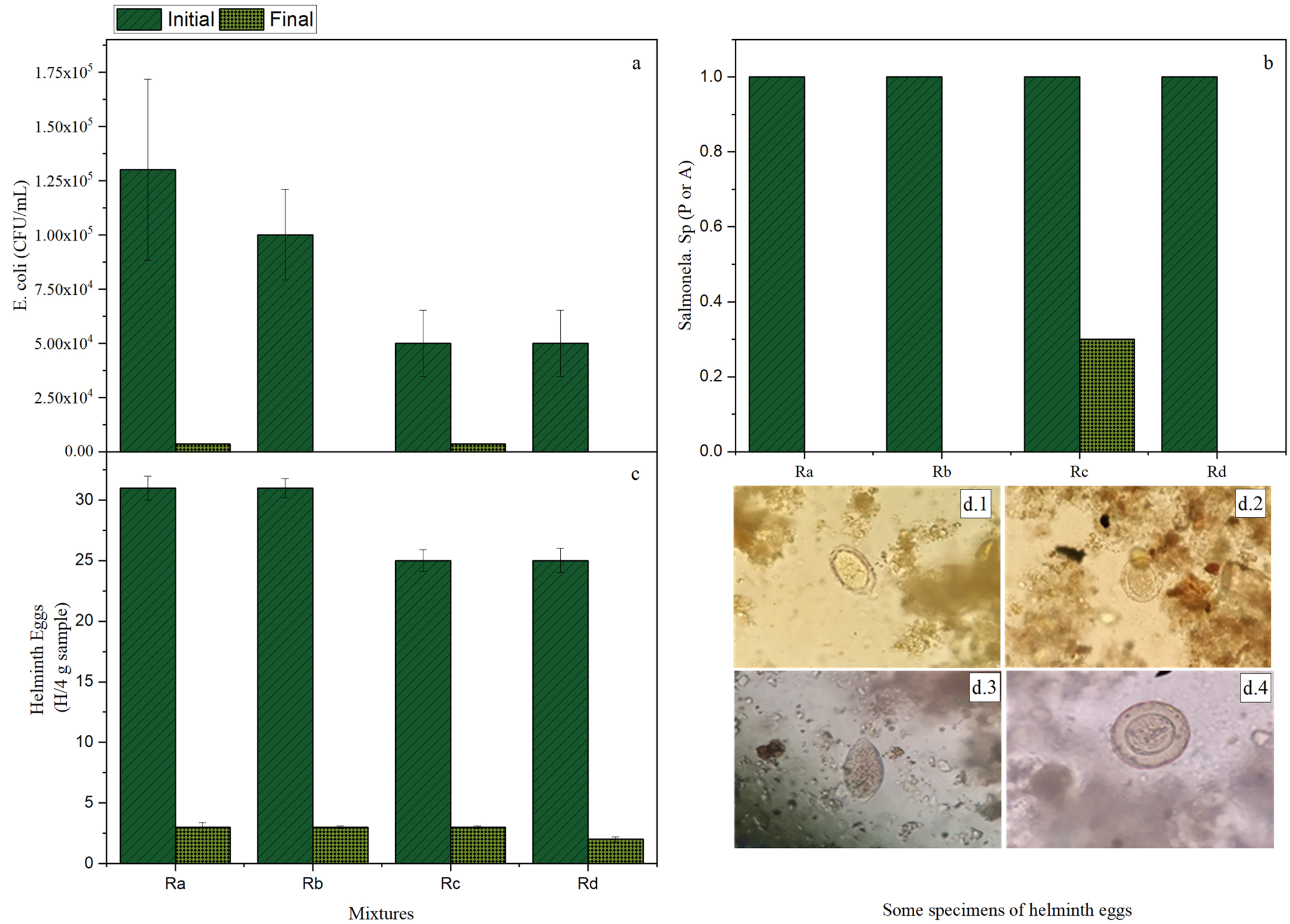


Fig. 6 Behavior of Pathogenic Microorganisms **a** E. coli, **b** Salmonella sp., **c** Helminth Eggs and **d** Specific Specimens of Helminth Eggs

Author Contributions Esteven Pulgarin-Muñoz: Conceptualization, Experimental setup and monitoring, Data analysis, Manuscript writing, Project administration, Research. Julio César Saldarriga-Molina: Conceptualization, Project administration, Supervision, Manuscript writing review. Mauricio Andrés Correa-Ochoa: Conceptualization, Project administration, Manuscript writing review. Johan Camilo Castro-Jimenez: Data analysis.

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Data Availability Data will be made available on request.

Declarations

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.

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