JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2027, 28(1), 285–298
https://doi.org/10.12911/22998993/240149
ISSN 2299–8993, License CC-BY 4.0

Received: 2026.08.27
Accepted: 2026.11.16
Published: 2026.12.01

Effect of food waste composition from a high-complexity hospital on anaerobic digestion by-products

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ABSTRACT

Food waste (FW) represents the largest fraction of municipal solid waste (MSW) and hospital generated waste, and its conventional disposal leads to landfill overuse, greenhouse gas emissions, and leachate generation. Due to its high organic content, FW is particularly suitable for valorization through anaerobic digestion (AD). This study evaluated the effect of substrate physicochemical composition on methane production and digestate quality under mesophilic conditions (35 °C) using hermetically sealed batch reactors over 32 days. Two substrates were assessed: fruit waste (FrW) and a mixture of fruit waste with potato peel residues (FW). Methane yields reached 211 mL CH_4/g VS for FrW and 305 mL CH_4/g VS for FW. The digestate derived from FW exhibited higher nutrient content. Germination index (GI) assays showed that a 25% digestate concentration produced the greatest stimulatory effect on radish seeds. The results demonstrate that substrate composition plays a key role in AD performance and the agronomic quality of the resulting digestate. Optimized substrate selection enhances methane yields while improving nutrient availability, thereby supporting environmental impact mitigation, renewable energy recovery, and the sustainable production of organic fertilizers for agricultural applications.

Keywords: anaerobic digestion, fruit waste, food waste, biogas, digestate.

INTRODUCTION

Inadequate management of municipal solid waste (MSW) leads to water, soil, and air contamination, generating environmental and public health concerns (Colvero et al., 2025). It is estimated that global MSW generation could reach 3.4 billion tons by 2050, representing a substantial increase compared to the 2 billion tons generated in 2023 globally (Olatunji et al., 2024). Of this total, food waste (FW) accounts for approximately 40–70% of total MSW generated (Rodríguez-Jiménez et al., 2022). In Latin America, waste management faces significant challenges compared to other regions globally, with an es-timated annual generation of 127 million tons of FW. In Colombia alone, approximately 9.7 million tons of FW are produced each year (Yepes and Soto, 2021).

Although most studies on organic waste have focused on domestic or municipal sources, the hospital sector also represents a significant source of organic waste. In healthcare settings, approximately 75–90% of solid waste (SW) is comparable to MSW, while 10–25% corresponds to infectious or hazardous waste (Rodríguez-Miranda et al., 2016). Of the SW generated in hospitals, roughly 50% consists of FW, equivalent to approximately 281.4 thousand tons annually

(Hassan et al., 2024). Hospital FW includes both inedible organic material (peels and vegetable residues) and food still suitable for human consumption (plate leftovers) (Cook et al., 2023). It has been reported that patients may leave up to 30% of the food served, influenced by clinical conditions, menu design, portion size, and environmental factors (Baygut and Bilici, 2025). In Colombia, hospitals classified as levels I, II, and III are estimated to generate approximately 5560 tons of hospital and related waste annually, which includes FW (Rodríguez-Miranda et al., 2016).

Landfilling remains the most widely used final disposal method worldwide for MSW, including FW (Olatunji et al., 2024). However, it is not the most sustainable strategy, as it requires extensive land areas and strict operational control to prevent environmental impacts associated with leachate generation and the emission of methane (CH_4) and carbon dioxide (CO_2) (Pradeshwaran et al., 2024). Landfills account for approximately 18% of global anthropogenic CH_4 emissions and 3.8% of total greenhouse gas (GHG) emissions (Scharff et al., 2024).

Given this scenario, identifying sustainable alternatives for waste management becomes essential. AD emerges as a promising technology capable of treating FW, generating renewable energy, producing organic fertilizers, and reducing environmental pollution (Olatunji et al., 2024). Technologies such as AD are aligned with the Sustainable Development Goals and the Global Green and Healthy Hospitals Agenda (Goal 3: Waste Management; Goal 5: Energy and Climate Change; Goal 7: Sustainable Food Systems), positioning AD as a strategy that contributes to a resilient and energy-sustainable future (Salud sin Daño, 2025; Üçok and Yang, 2025).

AD is a biochemical process involving the decomposition of organic matter through the synergistic action of microorganisms operating under oxygen-free conditions (Hassan et al., 2024). During this process, the organic fraction is primarily converted into biogas (90%), composed mainly of CH_4 (50–70%), the component of greatest interest due to its fuel potential, and CO_2 (30–50%) (Rabinovich et al., 2024), along with minor fractions of other gases such as hydrogen sulfide (H_2S), hydrogen (H_2), and nitrogenous species such as ammonia (NH_3)(Olatunji et al., 2024).

The remaining fraction (10%) consists of a semisolid material known as digestate, which can be separated into solid and liquid fractions. The solid digestate (SD) is commonly composted to produce biofertilizer, whereas the liquid digestate (LD) contains substantial proportions of carbon (30–40%), nitrogen (65–75%), phosphorus (35–45%), and potassium (70–80%), enabling its use as a liquid fertilizer (Yan et al., 2023).

Several studies have demonstrated that substrate selection in AD significantly influences methane yield. For instance, Chubur et al. (2024) evaluated fruit-based substrates and reported methane productions of 148.9, 178.7, and 117.2 L·kg·VS using citrus residues, jujube, and dates, respectively. Similarly, Rabinovich et al. (2024) reported a methane yield of 284 L·kg·VS from banana and pineapple peels, whereas Mu et al. (2017) achieved 295 L·kg·VS using a mixture of cabbage and potato peels as substrate. Collectively, these findings indicate that carbohydrate-rich substrates can enhance methane production; however, the ultimate yield depends on the specific chemical composition and biodegradability of each material.

The biological stability of digestate is closely associated with the amount of residual organic matter remaining after AD (Mei et al., 2020). Depending on the degree of degradation achieved during the process, either unstabilized sludge with high organic matter content or stabilized sludge (biosolids) may be produced. Stabilized materials exhibit reduced vector attraction, lower odor and pathogen levels, and enhanced agronomic potential due to their nutrient content (Koukoulakis et al., 2025). However, their agricultural utilization also depends on biological stability and quality parameters, including heavy metal concentrations, pathogen presence, and phytotoxic compounds, which may limit their application to soils (Bertanza et al., 2024).

Therefore, the objective of this study was to evaluate the influence of substrate composition on the quality of biogas and digestate quality generated during AD through biochemical methane potential (BMP) assays and digestate phytotoxicity tests. To this end, two substrate types obtained from a high-complexity hospital were used to prepare a food waste (FW) mixture composed primarily of carbohydrates and fruits.

MATERIALS AND METHODS

The methodology comprised three experimental phases: compositional and physicochemical characterization of food waste and inoculum;

biochemical methane potential (BMP) assays and digestate Stability and Maturity digestate stability assessment. To achieve the proposed objectives, the methodological procedures described below were followed.

Compositional and physicochemical characterization of food waste and inoculum samples

This phase included the determination of the physical composition and physicochemical characterization of fruit waste (FrW) and food waste (FW) samples obtained from a high-complexity hospital located in Santiago de Cali, Valle del Cauca, Colombia. The institution implements source separation and selective collection programs for MSW, which are considered fundamental principles for MSW valorization (Casallas-Ojeda et al., 2020).

To determine the physical composition of the two substrates, sampling was conducted over seven consecutive days. The collected samples were transported to the university laboratory assigned to the project for further analysis. Substrate weighing was performed using an electronic balance (model BBG-S115).

The physical composition of the first substrate, corresponding to FrW, consisted of mandarin peels (8.6%), pineapple peels (30.8%), papaya peels (21.0%), and watermelon peels (39.6%). The second substrate, corresponding to FW, comprised mandarin peels (4.3%), pineapple peels (15.4%), papaya peels (10.5%), and watermelon peels (19.8%), in addition to carbohydrate-rich residues, primarily potato peels (50.0%).

Subsequently, each substrate was manually cut into fragments of approximately 12 mm (Zhang and Banks, 2013). The residues were thoroughly mixed to form a homogeneous pile, and the quartering method was applied to obtain a representative sample. Finally, the material was ground using an industrial grinder (model CB15, Warning Comercial) operating at 15800 rpm until a particle size (PS) below 10 mm was achieved (Filer et al., 2019; Wang et al., 2015). This procedure was performed independently for each substrate.

For physicochemical characterization of the substrates, the following parameters were determined: pH (NTC 5167), moisture content (SM 2540 B), total alkalinity (TA) (SM 2320 B), bicarbonate alkalinity (BA) (Ripley et al., 1986), alkalinity associated with volatile fatty acids (VFAs), total solids (TS) (SM 2540 B), total volatile solids (TVS) (SM 2540 D), and chemical oxygen demand (COD) (SM 5220 C) (APHA et al., 2017)

The inoculum was obtained from an anaerobic domestic wastewater treatment system. Physicochemical characterization was conducted following the recommendations of (Angelidaki et al., 2009; Filer et al., 2019, APHA et al., 2017). The following parameters were determined: pH (SM 4500-H^+ B), total solids (TS) (SM 2540 B), total volatile solids (TVS) (SM 2540 E), total alkalinity (TA) (SM 2320 B), bicarbonate alkalinity (BA) (SM 2320 B), and VFAs (SM 5560 B).

Biochemical methane potential (BMP) assays

BMP assays were conducted following the guidelines established by the International Water Association (IWA), which are widely accepted internationally (Angelidaki et al., 2009) and have also been applied in national studies (Cleves et al., 2016). Experiments were performed in amber glass reactors with a total volume of 250 mL. The working volume was 125 mL (liquid and solid phases), leaving a 125 mL headspace (50% of total volume) for biogas accumulation (Odedina et al., 2017). Reactors were hermetically sealed using aluminum crimp caps to ensure complete confinement of the produced biogas within the headspace.

Biogas production was monitored using IoT-based pressure sensors individually integrated into each of the 12 reactors and previously calibrated. This system enabled continuous online data acquisition, providing frequent and precise pressure measurements in every bottle, and ensuring independent and comprehensive monitoring of biogas production across all treatments (Pérez-Vidal et al., 2025). BMP assays were conducted under mesophilic conditions (35 °C), with temperature maintained using a 216 L BOD incubator (Piedrahita-Rodríguez et al., 2022).

Four experimental treatments were established for AD, each performed in triplicate, resulting in a total of 12 reactors. Two treatments consisted of substrate mixtures (FrW or FW) combined with inoculum. Additionally, a positive control using cellulose as a standard substrate was included to verify the methanogenic activity of the inoculum, and a negative control containing only inoculum and distilled water was used to quantify endogenous biogas production

(Angelidaki et al., 2009; González-Sánchez et al., 2015). The experimental design is presented in Figure 1, and the experimental trial was conducted over a total period of 32 days.

To initiate the process, a substrate-to-inoculum ratio of 0.20 g $VS_{substrate}/g_{VSinoculum}$ was established, following the recommendation of (Filer et al., 2019). The inoculum concentration was 19 g VS/L, while the substrate concentration in each reactor was 3.8 g VS/L (Filer et al., 2019; Holliger et al., 2016).

To determine the amount of substrate added to each reactor, the substrate volume (V_s) was calculated. This calculation considered the working volume of the reactor ($V_{reactor}$, L), the volatile solids present in the reactor ($VS_{reactor}$, g VS/L), and the volatile solids concentration of the substrates (VS_R, g VS/L).

$$Vs = \frac{Vreactor \times VSreactor}{VSR} \quad (1)$$

The nutrient solution proposed by (Aquino et al., 2007) was used. Total alkalinity (TA) of the substrates was determined on the raw material prior to any pH correction. Due to the predominantly acidic nature of FW (Parra-Orobio et al., 2021), pH was subsequently adjusted to near-neutral values (7.0) using $NaHCO_3$ following Raposo et al. (2012), to minimize the risk of acidification during the initial microbial adaptation phase.

After completion of the assays, BMP was calculated from the measured CH_4 pressure data using the equations described by (Aquino et al., 2007), (*VDI.,* 2016), and (Hafner et al., 2020) (Table 1).

Digestate stability and maturity

Digestate stability was evaluated following the methodology proposed by Tigini et al. (2016) by determining TS, VS, moisture content, pH, and alkalinity. Digestate maturity was assessed through phytotoxicity evaluation using germination tests (Siles-Castellano et al., 2020). Two types of digestate were analyzed: one obtained from the AD of FrW (D-FrW) and another from the AD of FW (D-FW).

Four treatments were established based on digestate and distilled water mixtures at the following volumetric ratios: 100:0, 75:25, 50:50, and 25:75, along with a control treatment (0:100) (Venegas et al., 2019). Dilutions were homogenized for one hour prior to testing. For the germination assay, 10 radish seeds (*Raphanus sativus* L.) were placed in Petri dishes, and 5 mL of the corresponding treatment was added to each dish. All treatments were performed in triplicate (Parra-Orobio et al., 2021).

Petri dishes were sealed and maintained at 25–27 °C throughout the experimental period to prevent moisture loss and promote germination. Samples were incubated for 144 h (Cárdenas–Talero et al., 2025). An intermediate evaluation was conducted at 72 h to record germinated seeds and monitor growth progression.

After 144 h, the total number of germinated seeds in each treatment was recorded and compared to the control (distilled water). Root length was carefully measured following the procedure described by Venegas et al. (2019). The

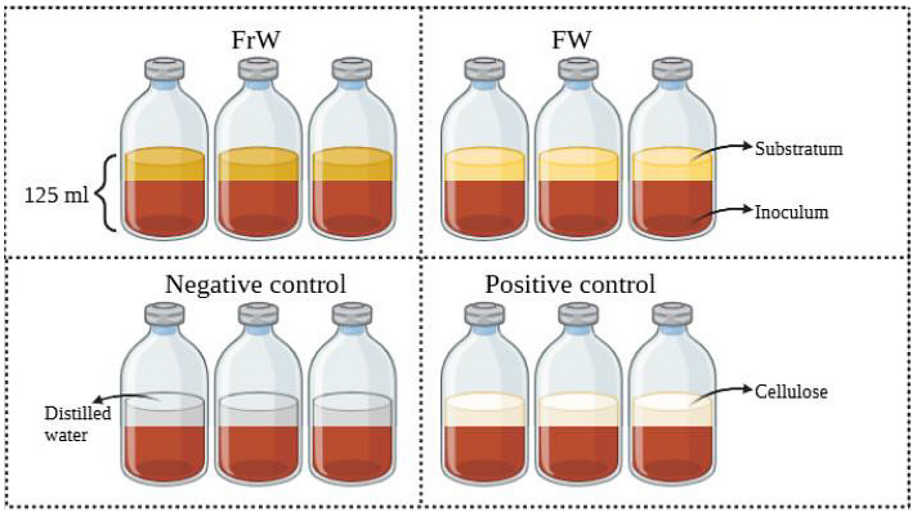

Figure 1. Schematic representation of the experimental design for AD. Created in BioRender. Ipiales, A. (2026) https://BioRender.com/urczrq5

Table 1. Equations used for BMP calculation

No.	Equations	Parameters
(2)	$V_{Biogas-CE} = Vl * \frac{(P - P_{H2O})}{P_{CE}} \cdot \frac{T_{CE}}{T_E + T_{CE}}$	$V_{Biogas-CE}$: Biogas volume at standard conditions (L) V_l: Headspace volume (L) P: Pressure increase measured in the manometer (kPa) P_{H2O}: Water vapor pressure Te: Experimental temperature (°C) Tce: Temperature at standard conditions (°C) Pce: Pressure at standard conditions (kPa) (101.325 kPa) C_{CH4}: Methane content $C_{CH4\ measured\ in\ the\ gas}$: CH_4 content measured by gas chromatography $P_{Measured}$: Gas phase pressure at the time of measurement $V_{CH4\ dissolved}$: Dissolved CH_4 volume (must be calculated for treatments, positive control, and negative control) $V_{CH4\ dissolved,\ negative\ control}$: CH_4 volume originating from negative controls $M_{CH4\ dissolved}$: Molar concentration of dissolved CH_4 (mol L^{-1}) m: Inoculum volatile solids (g VS) $n_{CH4\ dissolved}$: Moles of dissolved CH_4 (mol) BMP: Biochemical methane potential (L / g VS) $BMP_{dissolved}$: Dissolved biochemical methane potential (L / g VS) g VS: Initial volatile solids of the substrate (g VS)
(3)	$P_{H2O} = 0.61094e^{\left(\frac{17.625Te}{243.04+Te}\right)}$	
(4)	$C_{CH4\ \%} = C_{CH4\ measured\ in\ the\ gas} \cdot \frac{Pmeasured}{Pmeasured - P_{H2O}}$	
(5)	$C_{CH4} = V_{CH4} \cdot Vbiogas - CE$	
(6)	$V_{Total\ CH4} : V_{CH4RA} - V_{CH4\ negative\ control} \cdot m$	
(7)	$BMP = \frac{V_{CH4}}{g\ VS\ FW}$	

Note: (Aquino et al., 2007; Hafner et al., 2020).

germination index (GI) was calculated based on the relative seed germination (RSG) and relative root elongation (RRE), determined according to the following equations.

$$RRE = \left(\frac{Number\ of\ germinated\ seeds\ in\ the\ treatment}{Number\ of\ seeds\ in\ the\ control}\right) \cdot 100 \quad (8)$$

$$RRE = \left(\frac{Root\ length\ in\ the\ treatment}{Root\ length\ in\ the\ control}\right) \cdot 100 \quad (9)$$

$$GI = \frac{RSG \cdot RRE}{100} \quad (10)$$

During phytotoxicity assessment, results were interpreted according to criteria reported in the literature. GI values ≤ 50% indicate high phytotoxicity; values between 50% and 80% reflect moderate phytotoxicity; whereas values ≥ 80% suggest low or no phytotoxicity, indicating a stable and mature digestate (Siles-Castellano et al., 2020). Furthermore, GI values above 120% are considered indicative of a phytonutrient or phytostimulant effect on plant growth (Cárdenas–Talero et al., 2025; Parra-Orobio et al., 2021).

RESULTS AND DISCUSSION

Physicochemical characterization of food waste generated in the hospital and the inoculum

This section presents and discusses the results of the initial characterization of FrW and FW, and the inoculum used in AD assays. Table 2 summarizes the values of the analyzed parameters, determined according to the procedures described in the methodology. Additionally, comparisons with previous studies reporting similar physicochemical

Table 2. Physicochemical characterization results of food waste samples and inoculum

Parameters	Unit	FrW	FW	Inoculum
pH		5.58	5.17	7.11
TA	mg/L $CaCO_3$	560	5600	3440
BA	mg/L $CaCO_3$	0	0	1420
Moisture	%	84.69	82.29	91.44
TS	%	18.09	21.52	9.35
VS	%	13.27	15.49	4.18
(VS / TS)	%	73.35	71.98	44.70
COD	mg/L	71715	263792	-

compositions are included to provide a comparative assessment of their properties.

The pH of FrW and FW was 5.58 and 5.17, respectively, indicating notable acidity in both substrates. Chubur et al. (2024) reported pH values ranging from 5.3 to 5.6 for different substrates (citrus, dates, and jujube) and stated that low pH may constitute a limiting factor in AD, as it can inhibit methanogenic activity if not properly controlled. Methanogenic microorganisms require an optimal pH range of 7.0–7.2 for stable performance (Rabinovich et al., 2024).

The TA was 560 mg/L $CaCO_3$ for FrW and 5600 mg/L $CaCO_3$ for FW. The BA was not detected in either substrate, suggesting low buffering capacity and increased susceptibility to acidification. (Cleves et al., 2016) indicated that BA plays a critical role in AD stability, since low levels may promote the accumulation of volatile fatty acids (VFAs), which can inhibit methanogenic bacteria.

The moisture content of FrW and FW was 84.69% and 82.29%, respectively. These values are consistent with those reported by Rabinovich et al. (2024), who obtained a moisture content of 85.4%. According to Paul et al. (2018), moisture levels above 60% are suitable for AD. In this context, the high moisture content observed in both substrates promotes gradual biomolecule degradation, enhances mass transfer, and facilitates microbial activity, potentially leading to increased biogas production (Muenmee and Prasertboonyai, 2021).

The TS were 18.09% for FrW and 21.52% for FW, while the VS reached 13.27% and 15.49%, respectively. Rabinovich et al. (2024) reported maximum values of 18.6% for TS and 16.0% for VS, which are primarily comparable to the FrW substrate. Similarly, Mu et al. (2017) obtained a TS content of 18.2% in potato peel, a substrate comparable to FW. Although slightly lower than the value observed in the present study, this difference may be associated with the incorporation of fruit residues in the mixture analyzed here. The VS/TS ratio was 73.35% for FrW and 71.98% for FW, indicating a high biodegradable organic matter content. This parameter is particularly relevant, as higher volatile solid fractions are associated with greater CH_4 production potential during AD (Avena et al., 2023).

The COD reached 71715 mg/L for FrW and 263792 mg/L for FW, indicating a high organic load, especially in FW. These values suggest considerable energy potential, since COD represents the organic fraction susceptible to conversion into biogas. In comparison, Santos et al. (2020) reported a COD of 18508 mg/L in orange bagasse, a substantially lower value, likely reflecting differences in substrate composition and concentration. Overall, the physicochemical parameters indicate that both substrates present favorable conditions for AD and substantial potential for biogas generation.

The inoculum presented a pH of 7.11, which falls within the recommended range (7.0–8.5) to ensure proper microbial activity, particularly methanogenic activity. Maintaining pH within this interval promotes stability process and minimizes the accumulation of VFAs that could inhibit AD, as reported by Holliger et al. (2016).

The TA reached 3440 mg/L as $CaCO_3$, with BA of 1420 mg/L $CaCO_3$ and VFA-associated alkalinity of 2020 mg/L $CaCO_3$. These values demonstrate adequate buffering capacity to neutralize acids produced during AD (Ramírez et al., 2024). Furthermore, the recorded TA is significantly higher than the recommended minimum threshold (>1500 mg/L $CaCO_3$), ensuring sufficient buffering capacity to prevent medium acidification and maintain optimal conditions for methanogenic activity (Holliger et al., 2016). The inoculum exhibited a moisture content of 91.44%, which is typical of fresh biological materials and facilitates organic matter degradation and mass transfer during the process. The TS and VS were 9.35% and 4.18%, respectively, with a VS/TS ratio of 44.7%.

Biochemical methane potential assays

Figure 2 illustrates the cumulative methane production, expressed as BMP, as a function of time for the FrW and FW substrates. During the first day of the assay, both substrates exhibited similar behavior, characterized by a gradual increase in BMP from 0 to approximately 50 mL CH_4/g VS. This pattern reflects an initial microbial adaptation phase followed by the rapid degradation of readily biodegradable compounds.

Subsequently, differences in CH_4 accumulation profiles became evident. Up to day 25, the FW substrate showed higher cumulative methane production compared to FrW, reaching values close to 300 mL CH_4/g VS, whereas FrW achieved approximately 219 mL CH_4/g VS within the same period. This disparity suggests a greater availability of biodegradable organic matter in FW. The higher COD values measured in FW indicate a larger amount of organic matter available

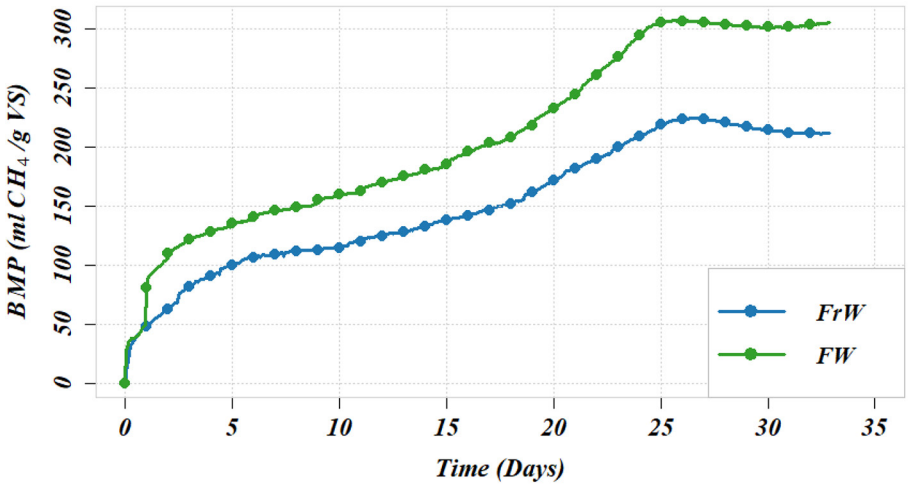

Figure 2. Cumulative methane production from the AD of FrW and FW originating from the hospital

for microbial degradation, whereas only minor differences were observed in TS and VS contents. These findings suggest that organic matter availability played a more important role in methane production than solids content alone.

During the final seven days of the experiment, CH_4 production stabilized for both treatments, indicating substrate depletion and the completion of the active digestion phase. The final cumulative CH_4 yields were 211 mL CH_4/g VS for FrW and 305 mL CH_4/g VS for FW. The higher CH_4 production observed for FW was likely associated with its composition and physicochemical characteristics. FW contained 50% potato peel residues, which are reported in the literature to be rich in starch and readily biodegradable carbohydrates, a composition that is consistent with the higher COD measured for this substrate (Table 2) and could plausibly promote methane generation during AD (Liang and McDonald, 2015; Mu et al., 2017). However, starch and total carbohydrate content were not directly quantified in the present study, and this explanation should therefore be regarded as a plausible mechanism supported by indirect evidence (COD) rather than a demonstrated cause. Future studies should include direct carbohydrate quantification to confirm this relationship.

This interpretation is further supported by the higher organic load measured in FW, which provided more substrate for microbial conversion into methane. Furthermore, the considerably higher alkalinity of FW may have enhanced process stability by providing greater buffering capacity against pH fluctuations caused by volatile fatty acid accumulation (Ripley et al., 1986; Santos et al., 2020). Therefore, the superior methane yield obtained for FW was likely associated with the combined effect of a higher organic load, readily de-gradable carbon sources, and improved buffering capacity.

The CH_4 yields obtained for FrW were compared with studies employing similar substrate compositions and experimental conditions. Santos et al. (2020) evaluated a mixture of fruit residues, specifically orange bagasse, and reported a maximum methane yield of 128 mL CH_4/g VS. Similarly, Chubur et al. (2024) investigated a citrus fruit mixture under a substrate-to-inoculum ratio comparable to that used in the present study and obtained a maximum methane production of 148.9 mL CH_4/g VS. Both studies emphasized the importance of pH and alkalinity control as critical factors for maximizing biogas production.

In comparison, the methane yield obtained for FrW (211 mL CH_4/g VS) was substantially higher, suggesting a greater availability of biodegradable organic matter and favorable digestion conditions. Although FrW was composed exclusively of fruit residues, its methane yield exceeded those reported in previous studies, indicating efficient conversion of the organic fraction under the operational conditions applied in this work.

For FW, the results were compared with those reported by (Liang and McDonald, 2015), who evaluated potato waste and obtained 239 mL CH_4/g VS. Likewise, Mu et al. (2017) reported that a 1:1 mixture of potato peel and cabbage improved methane yield to 295 mL CH_4/g VS. In the present study, FW achieved 305 mL CH_4/g VS, slightly exceeding values previously reported for similar substrates. This enhanced performance is consistent with the higher organic load and alkalinity

measured for FW, as well as the incorporation of potato peel residues, which provide readily biodegradable carbon sources for methane generation.

Overall, for both FrW and FW, the BMP values obtained in this study were higher than those reported in previous investigations using comparable residues. These differences may be attributed to variations in substrate composition, organic matter availability, alkalinity, experimental conditions, operational temperature, inoculum characteristics, and reactor configuration.

Nevertheless, the consistency in methane production trends validates the applied methodology and underscores the decisive role of substrate composition in anaerobic digestion performance. Collectively, these findings support the viability of AD as an effective technology for the energy recovery of organic waste.

Digestate stability and maturity

The characterization results of the digestate derived from the AD of FrW and FW are presented in Table 3. The data showed no statistically significant differences in moisture content, VS, TS, or ammoniacal nitrogen content. However, parameters such as VFAs, total phosphorus, total potassium, and TOC exhibited statistically significant differences; interestingly, the highest values were observed in the digestate derived from FW. In addition, the heavy metal concentrations were below 0.05 mg/kg, except for Pb, which presented a value of 0.15 mg/kg. Finally, the operating conditions of the AD process were not sufficient to completely eliminate pathogens such as *Salmonella* spp and *Escherichia coli*.

Table 3. Results of the measured parameters in the digestate

Parameters	Unit	D-FrW	D-FW	Reference Value
Moisture - FM	%	75.42	78.74	
Volatile solid (VS)	%	11.66	11.99	
Total solid (TS)	%	25.91	26.95	
VS/TS	%	44.99%	44.50%	< 65%*
Volatile Fatty Acids (VFAs) - FM	mg/L	80.93	101.63	
Total Kjeldahl Nitrogen (TKN) - DM	g N/kg	48.02	36.93	
Ammoniacal nitrogen – DM	g N/kg	0.06	0.06	
Total phosphorus – DM	g P/kg	27.52	36.80	1
Total potassium - DM	g K /kg	4.18	8.24	
Total Organic Carbon (TOC) - DM	%	8.20	9.13	Mín. 5%**
Fecal Coliforms – DM	CFU/g ST	1.0E+02	5.6E+02	Absent**
Viable Helminth Eggs – DM	HHV/4g ST	0.00	0.00	Max 1 in 4 g of sample**
Salmonella spp – DM	MPN/4g	<0.006473	<0.006473	Absent in 25 g**
Escherichia coli – DM	MPN/g	1.4E+02	5.1E+02	Absent**
Total arsenic – DM	mg/kg	<0.05	<0.05	Max 41**
Total cadmium – DM	mg/kg	0.05	0.05	Max 39**, 1.5***
Total copper – DM	mg/kg	0.09	0.06	Max 200**, 200***
Total chromium – DM	mg/kg	<0.01	<0.01	Max 1200**, 100***
Total mercury – DM	mg/kg	<0.01	<0.01	Max 17**, 1***
Total molybdenum – DM	mg/kg	<0.06	<0.06	
Total nickel – DM	mg/kg	<0.01	<0.01	Max 420**, 50***
Total lead – DM	mg/kg	0.15	0.10	Max 20**
Total selenium – DM	mg/kg	<0.01	<0.01	
Total zinc – DM	mg/kg	0.43	0.37	Max 400***

Note: FM: fresh mass; DM: dry mass. *CONAMA (2020) applies to biosolids; **NTC 5167 (2022) applies to solid and liquid organic fertilizers, soil amendments, and conditioners; and ***BSI (2010) applies to whole digestate, separated liquor, and separated fibre. BSI reference values are expressed on a dry-matter basis. Since the digestate evaluated in this study was not separated into solid and liquid fractions, the applicable NTC 5167 reference values were considered according to each parameter.

In terms of stability, the TS values were 25.91% for D-FrW and 26.95% for D-FW. According to Koukoulakis et al. (2025), these values fall within the organic matter range (10–30%) associated with improved water retention capacity, soil aeration, infiltration, and erosion control, supporting the potential agricultural application of the digestate. Cárdenas–Talero et al. (2025) in a study using dewatered and non-dewatered sludge, reported VS/TS ratios ranging from 30% to 50%. Although derived from a different substrate than that evaluated in the present study, the comparable values indicate that, following AD, the biodegradable organic fraction is substantially reduced due to its conversion into biogas.

Furthermore, the VS/TS ratio for both digestates remained below the maximum recommended threshold (65%) established by Brazilian regulations (CONAMA, 2020), indicating an adequate level of stabilization. Finally, the VFAs concentrations were 80.93 mg/L for D-FrW and 101.63 mg/L for D-FW. According to Mei et al. (2020), VFA concentrations below 300 mg/L are indicative of a well-stabilized digestate. In this context, the obtained values confirm a high degree of maturity and stability of the produced digestate.

In terms of nutrient composition, both digestates contained nitrogen (N), phosphorus (P), potassium (K), and TOC, supporting their potential use as organic fertilizers. These nutrients are essential for plant development, as they enhance germination rates, plant growth, and chlorophyll content, thereby contributing to agricultural productivity (Zapata-Morales and Moreno-Andrade, 2025).

Differences in nutrient concentrations were observed between the digestates, reflecting the composition of the original feedstocks used during AD. Although organic matter is progressively degraded during the process, mineral nutrients are largely retained and concentrated in the digestate. Consequently, the final nutrient profile is influenced by the characteristics of the substrates. The higher phosphorus and potassium contents measured in D-FW may be associated with the incorporation of potato peel residues, whereas the higher nitrogen content observed in D-FrW could be related to the predominance of fruit residues in the substrate mixture.

Additionally, the TOC content of both digestates exceeded the minimum percentage established by Colombian technical standards for organic fertilizers (ICONTEC, 2022), supporting their agronomic suitability.

Regarding sanitary quality, digestate hygienization is assessed based on the presence of indicator microorganisms such as fecal coliforms, *Escherichia coli,* helminth eggs, and *Salmonella* spp. In the present study, fecal coliforms and *Escherichia coli*. levels exceeded the limits established by Colombian standards (ICONTEC, 2022), whereas helminth eggs and *Salmonella* spp. complied with regulatory requirements. A higher bacterial load was observed in D-FW compared to D-FrW.

Therefore, although the digestates demonstrate promising agronomic potential, an additional post-treatment step is recommended to reduce microbial contamination and ensure safe application as organic fertilizer. Regarding heavy metal content, elements such as As, Cr, Hg, Mo, and Ni were below the analytical detection limits in both digestates. For Cd, Pb, Zn, Cu, and Se, measured concentrations were within the maximum permissible values established by Colombian regulations (ICONTEC, 2022) and within the limits defined by European standards (BSI, 2010).

It is noteworthy that Zn and Cu, although classified as trace metals, function as essential micronutrients that support normal plant growth and development (Koukoulakis et al., 2025). From a heavy metal perspective, the obtained results indicate compliance with regulatory criteria for agricultural application and suggest no significant risk of metal contamination under the evaluated conditions.

Although nutrient content, sanitary quality, and heavy metal concentrations indicate favorable characteristics for agricultural use, digestate suitability must also be assessed through phytotoxicity tests. Therefore, a germination assay was conducted to evaluate the effects of the produced digestates on seed germination and early plant development (Panuccio et al., 2021).

Phytotoxicity assessment

Figure 3 shows that the control treatment (0:100) exhibited GI values between 80–120%, indicating the absence of phytotoxic effects, as expected due to the lack of digestate in the mixture. In contrast, the 100:0 treatment (undiluted digestate) resulted in a significant reduction in GI for both digestates. D-FrW and D-FW predominantly showed values within the 40–80% range, suggesting the presence of phytotoxic compounds or elevated salt concentrations when digestate is

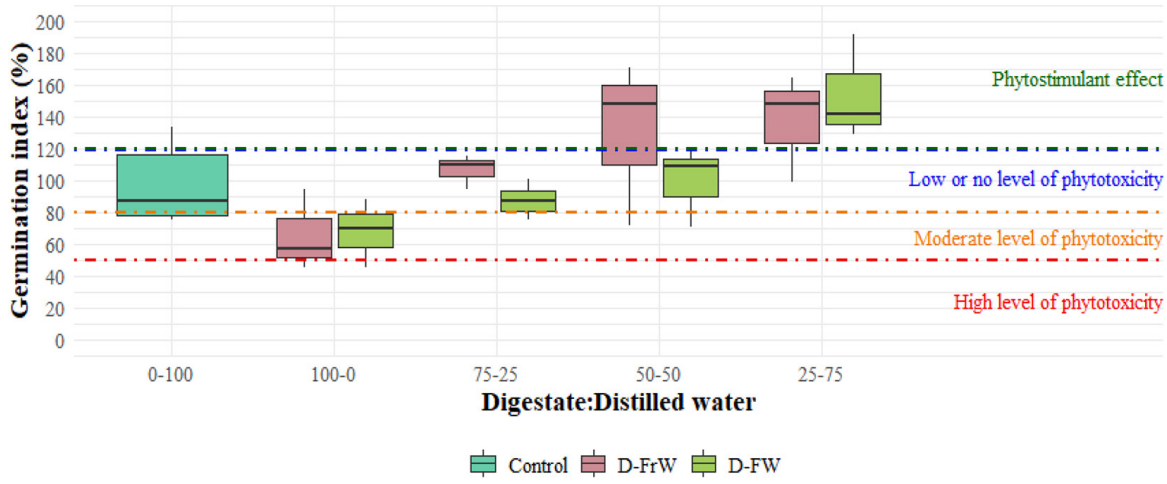

Figure 3. Germination index of the digestate from the AD of FrW and FW originating from the hospital

applied without dilution, negatively affecting early physiological processes such as seed imbibition and root elongation, although this could not be confirmed directly, as electrical conductivity was not measured.

As the proportion of distilled water increased (75:25 and 50:50), a progressive increase in GI was observed. For D-FrW, maximum values of 115% and 170% were recorded, indicating absence of phytotoxicity and a potential stimulatory effect on germination. In contrast, D-FW exhibited GI values between 100–120%, indicating no phytotoxic effects but a comparatively lower stimulatory response than D-FrW. In the 25:75 mixture, both digestates exceeded 100% GI, with averages above 150% for D-FW and close to 160% for D-FrW, demonstrating a significant positive effect on germination relative to the control.

The observed differences between digestates may be attributed to variations in their physicochemical and microbiological composition. The phytotoxic response observed in the undiluted treatments cannot be attributed to digestate instability, since both materials exhibited low VFAs concentrations and VS/TS ratios consistent with a stabilized digestate (Cárdenas-Talero et al., 2025; CONAMA, 2020; Mei et al., 2020). Instead, the inhibitory effect is more likely associated with the high concentration of soluble compounds, including ammoniacal nitrogen and mineral salts, which may increase osmotic stress and negatively affect seed germination and root development, consistent with mechanisms widely reported in the literature for digestate and compost extracts. However, electrical conductivity (EC) was not measured in this study, and therefore this explanation should be regarded as a hypothesis consistent with the broader literature rather than a directly demonstrated cause. Future assessments of digestate phytotoxicity should incorporate EC measurements alongside ammoniacal nitrogen, given that recent evidence indicates these parameters act synergistically rather than independently in determining phytotoxic thresholds. As dilution increased, these inhibitory effects were progressively reduced, allowing the beneficial contribution of nutrients such as nitrogen, phosphorus, and potassium to become more evident, highlighting the importance of proper characterization prior to agricultural application.

Overall, the results demonstrate a concentration-dependent effect of digestate on radish seed germination. While the undiluted material exhibits inhibitory effects, intermediate and higher dilutions enhance germination performance, suggesting its potential as a bio-stimulant when applied under controlled conditions.

Similar findings were reported by Panuccio et al. (2021), who evaluated liquid and solid digestate fractions in tomato cultivation and observed beneficial effects up to 20% concentration for the liquid fraction and 50% for the solid fraction. In agreement with those findings, the present study indicates that dilution represents a key strategy to maximize agronomic benefits while minimizing potential initial phytotoxic effects.

CONCLUSIONS

This study analyzed the influence of substrate composition on the by-products of an-aerobic

digestion. Biochemical methane potential (BMP) results demonstrated that substrates with readily degradable structures promote higher methane production. Carbohydrates, due to their rapid biodegradability, provide an accessible carbon source for micro-organisms during the initial stage of digestion, preventing delays in subsequent phases and reducing the accumulation of volatile fatty acids (VFA) that may inhibit the process.

This enhanced degradation contributes to digestate stability, defined as the residual fraction of the substrate after digestion. The substantial reduction in remaining organic load decreases the risk of undesirable microbial proliferation following soil application, supporting its use as an organic amendment in agricultural systems. However, future re-search should evaluate the effects of digestate applications on different crops and assess long-term soil fertility dynamics under continued use. Although conducted at laboratory scale, the methodology supported by the literature and the results obtained indicate the potential for process scalability, contributing to improved waste management strategies and value generation within a circular economy framework.

Acknowledgements

The authors would like to thank Universidad Santiago de Cali for its financial support. They also acknowledge the support of the Ministerio de Ciencia, Tecnología e Innovación – Colombia (MinCiencias) for funding doctoral student Lina Mariana Rodríguez-Jiménez through the national doctoral fellowship program [Call Doc Nal - BB 2019 01]. The authors used ChatGPT (OpenAI) only for English language editing and translation. All content was reviewed and verified by the authors, who take full responsibility for the manuscript.

This research has been funded by Dirección General de Investigaciones of Universidad Santiago de Cali under calls No. DGI-02-2025, No. DGI-01-2026 and Project No. 820-621124-327.

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