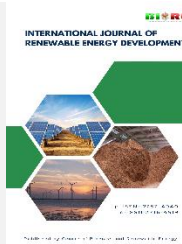




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Research Article

Assessment of tea processing waste as a potential co-substrate for biogas production through physicochemical and elemental analysis

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Abstract. Tea processing generates substantial organic residues that can be utilized for biogas production through anaerobic digestion. This study evaluates the suitability of tea processing waste (TPW) for anaerobic co-digestion with cow manure, emphasizing elemental composition and physicochemical properties. Solid wastes from sorting, withering and drying, as well as liquid waste from the cleaning channel and lagoons, were collected from Theta tea factory in Kenya. Cow manure and the inoculum were sourced from Jomo Kenyatta University of Agriculture and Technology. Standard laboratory and American Public Health Association (APHA) methods were employed to analyse total solids (TS), volatile solids (VS), pH, chemical oxygen demand (COD), ammonium nitrogen, carbon-to-nitrogen ratio (C/N ratio), oil/lipid content, and elemental composition. Fatty acid and inhibitor (polyphenols, tannins and caffeine) profiling was conducted using Gas Chromatography-Mass Spectrometry (GC-MS). Results revealed wide variability; TS ranged from 0.26 ± 0.1 to $92.63 \pm 5\%$ and VS from 0.21 ± 0.1 to $83.25 \pm 15\%$, with solid TPW showing the highest values. C/N ratios varied from 10 to 30, while COD ranged from 5333 ± 3695 to 14400 ± 1529 mg/L and ammonium nitrogen from 49 ± 8.4 to 1529 ± 500 mg/L. pH values ranged between 4.83 and 7.31, with the solid TPW being the most acidic and cow manure the most alkaline. GC-MS revealed that solid tea waste from withering (STPW1) was dominated by saturated fatty acids, suggesting greater digestion stability, while other fractions contained more than 60% (up to 90%) unsaturated fatty acids. Inhibitory compounds were also detected in both solid and liquid TPW: polyphenols (19.4 ± 4.8 to 1512.9 ± 45.3 mg GAE/100g), tannins (197.5 ± 9.0 to 9656.8 ± 214.4 mg CAE/100g) and caffeine (29.6 to 43.8 mg/L in solid waste TPW; 7.3 - 21.2 mg/L in liquid TPW). Co-digestion trials demonstrated that a 25:75 ratio of withering stage tea waste to cow manure yielded the highest methane concentration (58.3%). The findings of this study suggest that solid TPW, particularly STPW1, is a promising substrate for biogas production when co-digested with cow manure. Optimizing digestion parameters could enhance methane yield, supporting sustainable energy initiatives in Kenya's tea industry.

Keywords: Methane yield, Anaerobic Co-digestion, Inhibitor, Substrate suitability, Inhibitor profiling.



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1. Introduction

Presently, the demand for energy for industrial and domestic use is increasing rapidly worldwide, forcing developing countries to increase their use of fossil fuels (Özarslan *et al.*, 2021) that contribute substantially to climate change. However, developed countries have, in the last decade, focused on the development and use of renewable energy (Toftegaard *et al.*, 2010), which has minimal impact on global warming. Firewood and charcoal are the primary sources of energy for the poor (Naughton-Treves *et al.*, 2007) leading to deforestation, a phenomenon that has become a major threat to the environment.

The tea production process typically involves withering, rolling or cutting, fermentation, drying, grading, packing and dispatch (Aaqil *et al.*, 2023). The drying step during tea processing is an energy-intensive process that involves reducing the moisture content of the freshly harvested green leaves from about 83% to 3% wet basis (Kumar *et al.*, 2018). During the withering process, air is passed through a steam heat exchanger to raise its temperature. The leaves are then

macerated by rotor vane(s) and cut using crush, tear and curl (CTC) machines. The cut, withered tea leaves are fermented in continuous fermentation unit (CFU) machines and fed into tea driers where the moisture content is reduced to acceptable limits of 3% to 4% dry basis (Otedo *et al.*, 2024). The drier has fans for blowing air, heat exchangers, a drying chamber, cyclones, etc., which require substantial quantities of thermal and electrical energy. The final stage is grading, packaging and dispatch. These processes consume thermal or electrical energy (Van der Wal, 2008). Of all these processes, drying is known to be the most energy-intensive in tea production (Kumar *et al.*, 2023).

A report provided by GIZ (2019) and KTDA (2019) indicated that one mature tree produces 1 m³ of quality firewood and, on average, one factory consumes 11,500 trees per year translating to 11,500 m³ of firewood. Apart from the 69 Kenya Tea Development Agency (KTDA) factories, other multinational, large-scale producers also depend on wood as the major source of energy for processing black tea (Karuri, 2020). The reliance on firewood as the main source of energy for processing tea has accelerated deforestation thus reducing

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carbon sinks and exposing soil to the erosive force of water, leading to reduced soil fertility.

Tea waste produced during tea factory processing operations can be a potential source of biomass that can be converted to renewable energy through co-digestion to produce biogas. Welfle *et al.* (2020) reported that crop residue and animal waste from agricultural production in Kenya have the potential to provide energy of up to 15.6 TWh and 7.9 million tonnes of biomass, respectively, by 2050. Globally, tea is one of the oldest and most widely consumed beverages (Onduru *et al.*, 2012). During tea production, 2 to 4% of the leaves harvested are turned into waste during harvesting, transportation and factory processing (Özarslan *et al.*, 2021), which can be used as biomass feedstock for biogas production. Hakiki & Sari (2020) reported in their study that liquid tea waste contains beneficial nutrients and enhances biogas yield owing to its organic composition and is thus a potential substrate for anaerobic co-digestion.

Tea production processes in Kenya generate 190,400 tonnes of tea waste annually (Basumatary *et al.*, 2018) which is not put to beneficial use. Such processes include green leaf harvesting, transportation, receiving at the factory, withering, cutting, fermentation, drying, sorting and packaging. Tea wastes contain clipped stems, wasted tea leaves and buds and tea residues after infusion. Large-scale tea factories process over 10,000 kg of green leaf per day and generate 30% of the initial weight as waste (Ahmed & Stepp, 2013). These wastes are dumped into dumpsites together with other domestic wastes or burnt, leading to land degradation and air quality problems (Ayilara *et al.*, 2020). Sustainable management of tea wastes through anaerobic digestion can be a better solution to this problem, as it also produces clean energy by recovering useful energy from the biomass waste. Tea waste can serve as an excellent feedstock for anaerobic digestion (AD) since it produces biogas with a methane content of approximately 60 to 65% when shredded and anaerobically digested (Manyuchi *et al.*, 2018).

Tea processing is an energy-intensive process that requires reliable, affordable and efficient energy systems. The entire process of producing black tea requires 4 to 18 kWh per kg of processed tea and roughly 85% of the total energy used is thermal, with the rest being electrical (Van der Wal, 2008). The drying stage among the processing operations is the most energy-intensive step and is mainly carried out using firewood from estate plantations and natural forests. Production of biogas from tea waste through the AD process will not only supplement the energy required for industrial and domestic use but also promote environmental conservation through safe disposal of the waste. This study assesses the suitability of tea processing waste as a co-substrate for biogas production by characterizing its physicochemical and elemental attributes. By analyzing key process parameters, the research aims to provide insights into how the tea waste can be integrated into anaerobic digestion systems to enhance biogas yield and promote sustainable waste management in tea processing industries.

2. Material and methods

2.1 Sample Collection and Transportation

Tea production waste generated from withering, sorting and drying, and liquid wastes from cleaning and from lagoons, was sampled from the Theta tea factory, located at 0°57'43"S 36°46'06"E in Kiambu County, Gatundu South sub-county. The samples were transported in cooler boxes to Jomo Kenyatta University of Agriculture and Technology (JKUAT) for

laboratory analysis. The liquid wastes were sampled at the discharge channel adjacent to the factory and at the lagoon. Half a kilogram of each solid tea waste collected from the plant was randomly sampled three times (in February, March and April) from the same sampling points. Similarly, a litre of each of the liquid wastes was collected over the same period as the solid tea wastes from the same sampling points. The liquid waste was analyzed immediately to avoid changes in its composition. The sampled solid wastes were packed in zip-lock bags (Betatrad Limited, Nairobi, Kenya) and sterilized 1-litre plastic bottles (Kenpoly manufacturer Limited, Nairobi, Kenya) in the case of liquid waste and then transported to the laboratory in an 11-litre cooler box (Mumbai, India) within six hours. The co-substrate (raw cow dung) was collected from the cow shed at the JKUAT dairy unit and the inoculum from an active dairy manure biodigester running under mesophilic (37°C) conditions within the same locality. The same sampling criteria, sampling amount and interval were maintained as those used for the waste collected from the tea factory.

2.2 Characterization of Tea Production Wastes and Co-substrates to Determine their Suitability for Energy Recovery through Co-Digestion

2.2.1 Sample collection

The physicochemical characteristics of tea waste, cow manure and inoculum were determined through laboratory tests. The parameters tested included chemical oxygen demand (COD), total solids (TS), volatile solids (VS), ammonium nitrogen, C/N ratio and pH. Five samples (Figure 1) were considered for analysis and included STPW1 (solid tea waste collected from withering stage), STPW2 (solid tea waste from sorting stage), STPW3 (solid tea waste from drying stage), LTPW1 (liquid tea waste collected from the lagoon filtration stage), LTPW2 (Liquid tea waste collected at cleaning stage), CM (Cow manure) and IN (Inoculum).

2.2.2 Sample preparation

The solid and liquid tea waste samples were dried in a hot air oven (Stericox, STXL028, India) at 105±2 °C for 24 hours to remove water and isolate the actual organic solids so as to make it possible to measure the standard TS/VS. This temperature aligns with the industrial tea-drying temperature of 100-120 °C (Abhiram *et al.*, 2023). The dried solid samples were cooled in a desiccator, subjected to mechanical size reduction at the Environmental Engineering Laboratory at JKUAT using a high-speed kitchen blender (HS-208C, Gemac, China) to increase the surface area and sieved through 0.42 mm sieve to enhance substrate homogeneity. A gram of each sample was packed in 1 g vials (Famacy Glass, China) labelled in preparation for elemental analysis and the remaining stored in airtight containers with liquid tea waste refrigerated at 4 °C for further analysis. The liquid waste samples were stored in a refrigerator (Samsung RT28HAR5DWW, Samsung Electronics, South Korea) at 4°C to minimize any chemical reactions that would adversely affect the analysis. The inoculum used in this study was derived from anaerobic sludge obtained from a stable mesophilic cow manure digester.

2.2.3 Determination of COD and ammonium nitrogen

Measurement of COD was carried out using the closed-reflux titrimetric American Public Health Association (APHA) method (Amanatidou *et al.*, 2012). This procedure is applicable to COD concentrations ranging from 40 to 400 mg/L and samples

containing chloride concentrations up to 2000 mg/L. Higher COD or chloride levels can be accommodated through appropriate sample dilution. A dilution factor of 100 with distilled water was applied in this analysis to bring COD into the APHA method's valid range. A 5 mL aliquot of each sample was digested in 3 mL of potassium dichromate ($K_2Cr_2O_7$), a strong oxidizing agent, in the presence of 7 mL of sulphuric acid (H_2SO_4) whereby the 5 mL of solid sample was an aqueous extract of dried, ground solid waste. The sulphuric acid reagent was prepared by heating 500 mL of H_2SO_4 with 5 g of silver sulfate added as a catalyst according to the principle of APHA 5220B. The mixture was then placed in cultured digestion vessels fitted with outside caps and manually shaken for homogeneity, and then transferred to a block heater (HB150-S2, DLAB Scientific, China) operating at 150 ± 2 °C for 2 h. It was then left to cool at room temperature for 15 minutes. The excess dichromate was titrated with 0.1 M standard ferrous ammonium sulfate (FAS) titrant after addition of 1-2 drops of Ferroin indicator solution to the digested solution. The endpoint was marked by a blue-green to reddish-brown colour change. COD was computed using equation 1, where A is volume (mL) of ferrous ammonium sulphate (FAS) titrant used for the blank (distilled water sample), B is volume (mL) of ferrous ammonium sulphate (FAS) titrant used for the actual sample, M is molarity of the FAS titrant (mol/L), 8000 is the milliequivalent weight of oxygen $\times 1000$ mL/L, DF is the dilution factor and V_s is the volume (mL) of the sample used.

$$COD (mg/L) = \frac{(A-B) \times M \times 8000 \times DF}{V_s} \quad (1)$$

The Nessler method, a colorimetric technique, was used to measure the ammonium nitrogen concentration of the samples (Wang *et al.*, 2019). Prior to analysis, 5 g of solid sample was added to 100 mL of distilled water and stirred in a magnetic stirrer (Heidolph, Promax 2020, Germany) for 30 minutes and then filtered through a 0.45 μ m membrane filter to remove suspended solids that could interfere with absorbance readings. A volume of 25 mL of each sample was transferred into a 50 mL volumetric flask and diluted to the mark with distilled water. In each flask, 1 mL potassium sodium tartrate solution was added as a stabilizing agent, followed by 1 mL of Nessler's reagent (K_2HgI_4 in alkaline solution). The solutions were gently mixed and allowed to stand for 10 minutes for full colour development. The Nessler method involves the reaction of ammonium ions in the diluted samples with Nessler's reagent, which produces a yellow-to-brown complex colour, the intensity of which is proportional to NH_4^+ -N concentration in the sample. Absorbance was then measured at a wavelength of 420 nm using a Shimadzu UV-VIS-1800 spectrophotometer (DR 2500, Hach, USA). The NH_4^+ -N concentration of each sample was determined by reference to a calibration curve constructed from a series of standard ammonium chloride (NH_4Cl) solutions of known concentrations (0, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/L). Compared with other methods, this method is more reliable, since it has a maximum relative error of 6.91% (Wu & Cao, 2013).

2.2.4 Determination of substrate pH

pH was determined through standard laboratory tests. The pH of liquid tea waste samples was measured directly using a portable pH meter (pH211, Hanna Instruments, Romania, Europe). The slurry method was used to determine the pH of

solid samples using a mixing ratio of 1:10 (Pudipeddi *et al.*, 2008). The mixture was stirred in a magnetic stirrer (Heidolph, Promax 2020, Germany) at 130 revolutions per minute (rpm) for 30 minutes, then left to settle for 15 minutes. The pH meter was calibrated with standards (pH 4, 7 and 10) before taking pH readings.

2.2.5 Analysis of sample VS and TS

The TS and VS were determined using APHA standard method 2540B (Ebner *et al.*, 2016). The mass of clean, dry crucibles was measured using an analytical balance (AUW220D, Shimadzu Corporation, Japan) before adding any sample. Six grams of each sample was placed in an empty crucible and the total weight measured and recorded in duplicate using an analytical balance (ML204, Mettler-Toledo GmbH, Switzerland). The samples were then placed in an oven (Stericox, STXL028, India) operating at a temperature of 105 °C for 24 hours to remove free moisture and bring the sample to a constant weight. After drying, the sample was cooled in a desiccator (Kenya Chemical, Nairobi, Kenya) before reweighing using an analytical balance (AUW220D, Shimadzu Corporation, Japan). VS was obtained by igniting the oven dried sample (after 105 °C) in a muffle furnace (General muffle furnace, Science lab, Nairobi, Kenya) at 550 °C for 2 hours. The ignited samples were also placed in a silica gel desiccator (5301-0250, Thermo Fisher Scientific, USA) to cool for 30 minutes before weighing using an analytical balance (AUW220D, Shimadzu Corporation, Japan) to determine the percentage VS. The analysis was repeated in triplicate. TS was computed using equation 2, where W_{dry} is weight (g) of sample after drying at 105 °C and W_{wet} is initial wet weight of the sample (g).

$$TS (\%) = \frac{W_{dry}}{W_{wet}} \times 100 \quad (2)$$

VS was computed using equation 3. In the equation, W_{dry} is weight of sample after drying at 105 °C (g), W_{ash} is weight of residue after ashing at 550 °C (g) and W_{wet} is initial weight of the sample (g).

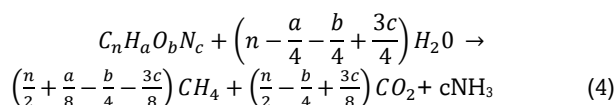
$$VS (\%) = \frac{W_{dry} - W_{ash}}{W_{wet}} \times 100 \quad (3)$$

2.2.6 Elemental composition and biomethane potential (BMP) of substrates

Dried samples that had been packed in 1 g vials and stored in zip lock bags were used for elemental analysis. Elemental (CHNS) analysis of solid tea waste, cow manure and liquid tea waste was performed using a Vario MACRO cube CHNS elemental analyzer (Elementar Analysensysteme GmbH, Langensfeld, Germany) at the Department of Biological and Chemical Engineering, Aarhus University, Denmark to determine their suitability for anaerobic co-digestion. The analysis focused on the weight percentage of carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) which are critical to anaerobic digestion. The analyzer determines the percentage of total carbon by dry combustion; hence, oxygen (O) could not be analyzed. The oxygen content was determined by difference, calculated by subtracting the sum of the measured elemental percentages and the ash content obtained from the Laboratory Analytical Procedure (LAP) from 100% (Sluiter *et al.*, 2008).

The theoretical BMP of tea waste and cow manure was estimated using the modified Buswell Equations 4 and 5, which

relate the elemental composition (C, H, N, O, S) of a substrate to methane yield in AD (Nwokolo *et al.*, 2020). Elemental percentages were converted to empirical formulas, and stoichiometric methane yields were then calculated in a sequential workflow.



$$TMBP \quad \left(\frac{mLCH_4}{gVS}\right) = \frac{22.4 \times 1000 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)}{12n + a + 16b + 14c} \quad (5)$$

where $C_nH_aO_bN_c$ is the chemical formula of the substrates derived experimentally; n , a , b , and c are the atomic masses of carbon (12), hydrogen (1), oxygen (16) and nitrogen (14), respectively and TMBP is the theoretical biogas potential at standard temperature and pressure (STP).

2.2.7 Determination of C/N ratio

The values of CHNS obtained from the elemental analysis in section 2.2.6 were used to compute the C/N ratio. C/N ratio was computed by dividing the total carbon content (% dry weight) by the total nitrogen content of the sample (% dry weight).

2.2.8 Determination of oil/lipid content

Twenty grams of each homogenized powder sample was weighed from the five collected samples (STPW1, STPW2, STPW3, LTPW1 and LTPW2) using analytical balance (ML204, Mettler-Toledo GmbH, Switzerland) at the Food Fortification Laboratory, JKUAT, Kenya. Lipids were extracted using the hexane solvent extraction method described by Zarrinmehr *et al.* (2022). Each measured sample was placed in a volumetric flask then 100 mL of n-Hexane (Merck, Darmstadt, Germany) was added. The mixture was placed in a reciprocating shaker (SHR-1 D, DAJHAN Scientific Co. Ltd) at 190 rpm for 24 hours followed by centrifugation in a 30°C refrigerated centrifuge (HERMLE Labortechnik GmbH, ZK 496, Germany) at 3500 rpm for 15 minutes for separation of the hexane phase. Siphoning was done, and the hexane extract was thereafter subjected to rotary evaporation (RE100B, Bibby Sterilin Ltd, U.K). A 2 mL volume of 0.5 M methanolic KOH was added to 50 mg of the extracted fat, which was then heated at 60°C in a water bath for 10 minutes. A 2 mL volume of 3 M methanolic HCl was then added, and the mixture was heated again under the same conditions. Thereafter, 2 mL of hexane and 5 mL saturated NaCl were added and then vortexed using a vortex mixer (IKA MS1 Minishaker, IKA Works GmbH & Co.KG, Staufen im Breisgau, Germany). The upper hexane layer was collected and dried over Na_2SO_4 then transferred to 2 mL clear borosilicate glass autosampler GC vials (12×32mm, screw cap with PTFE/silicone septum) for injection. Fatty acid methyl esters (FAMES) were analyzed using GC-MS Analyzer (Agilent 7890 GC coupled to 5975 MS) with a 40-minute run for each sample. Separation was achieved on a capillary column (HP-5MS, 30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Santa Clara, CA, USA) with helium as the carrier gas at 1 mL/min. Samples (1 µL) were injected in splitless mode at 250°C. The oven program began at 60°C (2 min hold), ramped at 10°C/min to 200°C, then at 3°C/min to 250°C, with a final hold of 10 minutes. The mass spectrometer operated in electron ionization mode (70 eV),

scanning m/z 50-550. Peak areas were integrated using GCMSD MassHunter with ChemStation software. Fatty acids were identified by comparison with the standards and quantification was based on peak area normalization. All samples were analysed in triplicate.

2.2.9 Analysis of methane concentration

Batch biochemical methane potential (BMP) tests were conducted in triplicate using 125 mL serum bottles (Science Laboratory, Nairobi, Kenya) as batch reactor digesters with a working volume of 80 mL, sealed with tight corks and Parafilm to avoid leakage. Each serum bottle was loaded with a fixed amount of substrate on a VS basis with inoculum at an inoculum-to-substrate ratio of 1:1. The substrate mixtures were prepared at five ratios of tea waste to cow manure, i.e., 100:0, 75:25, 50:50, 25:75 and 0:100. Anaerobic conditions were established by purging the headspace of each serum bottle with nitrogen gas.

Gas sampling was done using a syringe, a Luer-lock and a needle, and stored in 50 mL glass vials, then transported to the International Livestock Research Institute (ILRI), Nairobi, Kenya for compositional analysis. Methane concentrations in the collected gas samples were quantified using a Gas Chromatograph (GC) (SRI 8610C, SRI Instruments, USA) equipped with dual detectors. Sampling was performed at regular daily intervals throughout the digestion period. For each treatment, three replicate digesters were established, and one vial was collected per replicate at each sampling event. The analysis followed standard procedures for GC (Intergovernmental Panel on Climate Change, IPCC, 2006). In summary, a flame ionization detector (FID) was used for methane determination. Chromatographic separation was achieved using a HayeSep D packed column (3 m length, 1/8-inch diameter). The oven temperature was held constant at 70°C throughout the analysis which is appropriate for light gases. Nitrogen was used as the carrier gas at a controlled flow rate of 25 mL/min. For each run, a 4.5 mL gas sample was injected into the GC system. Instrument control, peak acquisition and quantification were performed using Peak Simple software. Calibration curves were generated using certified methane standards of known concentrations, and peak areas were integrated to determine sample concentrations.

2.2.10 Determination of inhibitors

Inhibitory compounds present in tea waste can significantly affect the efficiency of anaerobic digestion by suppressing microbial activity and reducing methane yields. Among the most relevant inhibitors are total phenolic compounds, caffeine and tannins, which are known to interfere with hydrolysis, acidogenesis and methanogenesis. These inhibitors were briefly determined as follows:

2.2.10.1 Total phenolic content

The concentration of total phenolic compounds in tea waste was determined using the Folin-Ciocalteu colorimetric assay with gallic acid employed as the calibration standard (ISO, 2005). After soaking 0.5 g of the samples for 72 hours, the total phenolic content (TPC) of the extracts was determined using the modified Folin-Ciocalteu method (ISO, 2005). A sample extract of 0.5 mL was mixed with 2 mL of Folin-Ciocalteu reagent (diluted 1:10 v/v) and 4 mL of sodium carbonate (0.7 M) was added, and the solution was incubated for 2 hours in the dark.

The absorbance was measured at 760 nm using a UV-Visible spectrophotometer. A gallic acid calibration curve was prepared from a 0-100 ppm concentration range. Finally, the average of the triplicates was expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g).

2.2.10.2 Caffeine

Caffeine content was quantified using high-performance liquid chromatography (HPLC) with a UV detector. Caffeine determination of the side streams was done through a solid-liquid extraction, according to Bi *et al.* (2011) and Bouhzam *et al.* (2023), and concentrations were reported in mg/g of dry sample. In summary, 2 g of each ground solid sample and 50 mL of each liquid sample were weighed into a 250 mL Erlenmeyer flask; 100 mL of distilled water was added, and the mixture was boiled for 4 min at 100 °C. After extraction, the total content of each flask was filtered using Whatman filter paper no. 1. The filtrate was then topped up to 100 mL with distilled water.

A 20 μ L aliquot of the diluted solution (prepared by mixing 5 mL of the extract with 50 mL of the mobile phase (methanol/water, 95:5)) was injected into the HPLC system for analysis. Caffeine standards were prepared using a concentration range of 10-80 ppm. Standard solutions were made by dissolving accurately weighed caffeine in the mobile phase to achieve the desired concentrations. Each standard was analyzed in triplicate to construct a calibration curve, plotting peak area against concentration. HPLC-UV analysis was conducted at a 0.6 mL/min flow rate, with a sample injection volume of 20 μ L at 36 °C and detection at a wavelength of 278 nm. From the calibration curves generated using the standards, the concentrations of samples were extrapolated using their peak areas, and the results were expressed in milligrams of the sample, calculated by multiplying the concentration (ppm) by the extract volume.

2.2.10.3 Tannins

Approximately 0.2 g and 5 g of solid and liquid tea waste samples, respectively, were weighed into clean test tubes. To each test tube, 10 mL of distilled water was added, followed by

heating in a water bath at 100 °C for 5 minutes. The mixture was shaken using an orbital shaker at 100 rpm for 10 minutes, then centrifuged using a bench-top centrifuge (Eppendorf 5810R, Germany) at 4000 rpm for 10 minutes at room temperature to obtain the supernatant. A fresh tube containing 2 mL of clarified extract was centrifuged again under the same conditions to remove particulates. The resulting supernatants were carefully combined by decanting into a clean tube, diluted with 10 mL of distilled water, reheated at 100 °C for 2 minutes, and mixed thoroughly to obtain the final extract for tannin determination.

Tannins were quantified using the Vanillin-HCl method as described by Broadhurst *et al.* (1978). A 1 mL solution of the prepared extract was mixed with 5 mL of 4% vanillin solution in methanol and 5 mL of concentrated hydrochloric acid. The mixture was incubated at 30 °C for 20 minutes to allow colour development. Absorbance was measured at 500 nm against a reagent blank using a UV-Visible spectrophotometer. Catechin standards (10-100 ppm) were prepared and analyzed in triplicate to construct a calibration curve. Tannin content was extrapolated from the calibration curve and expressed as milligrams of catechin equivalents per gram of sample (mg CE/g).

2.2.11 Data analysis

A Multivariate Analysis of Variance (MANOVA) was used to analyse the differences in mean values of different biogas parameters across the sampled tea wastes. SPSS version 27 (IBM Statistics, USA) was used for all analyses. Fisher's LSD test was used for mean separation, and all analyses were carried out at the 0.05 significance level.

3. Results and discussion

3.1 Ammonium Nitrogen Concentration

Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) values for the solid and liquid tea wastes collected from the Theta tea factory at three different times are shown in Figure 1. From Figure 1, STPW1 and STPW2 show higher ammonium nitrogen concentrations than LTPW2 and LTPW1, whereas CM shows the lowest and can therefore be used to balance the ammonium content of tea wastes with

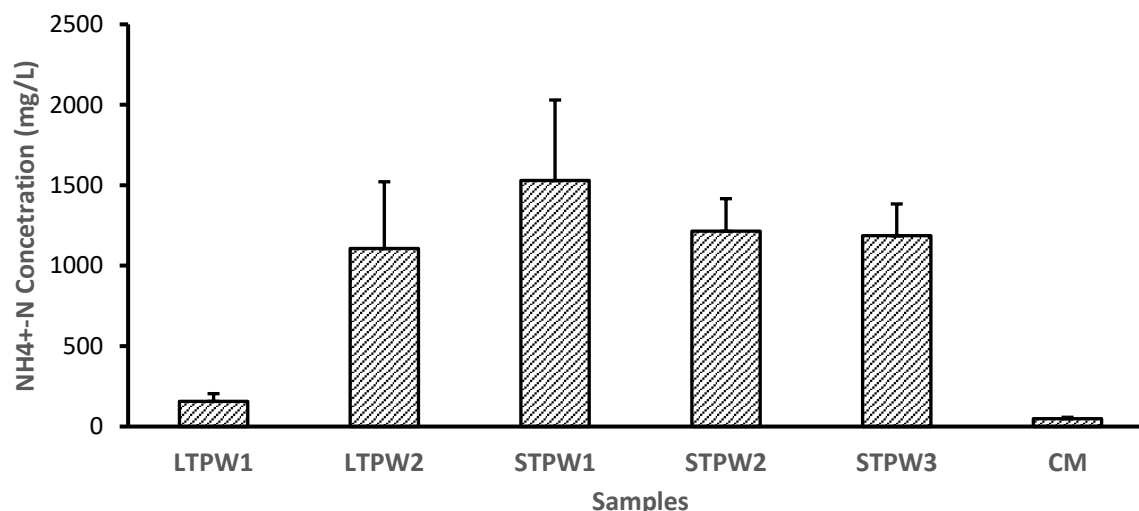


Fig 1. Ammonium nitrogen ($\text{NH}_4^+\text{-N}$) of solid tea processing waste (STPW1, STPW2 and STPW3), cow manure (CM), and the liquid tea processing waste (LTPW1 and LTPW2). All values are mean $\pm$ SD and n=3

high ammonium nitrogen concentrations. Statistical analysis of the ammonium nitrogen data showed significant ($p \leq 0.05$) differences among samples collected on different days. Similarly, the waste type was found to be a significant ($p \leq 0.05$) factor influencing the ammonium nitrogen concentration of the samples. Post hoc tests showed significantly ($p \leq 0.05$) different concentrations in ammonium nitrogen between LTPW1 and all the other samples, while STPW2 and STPW3 as well as STPW2 and LTPW2 had mean ammonium nitrogen concentrations that were not significantly different ($p > 0.05$). Ammonium nitrogen in anaerobic digestion is a process parameter that affects the methanogenic medium by increasing the buffer capacity in AD systems, thereby affecting process stability (Morozova *et al.*, 2020). According to a study by Prochazka *et al.* (2012) on the effects of ammonium nitrogen on the AD process, optimal concentrations for methanogens were reported to be 2.1, 2.6 and 3.1 g/L of ammonia nitrogen depending on inoculum origin. They also established that a high ammonia concentration (4.0 g/L) inhibited methanogenic activity, while a low concentration (less than 0.5 g/L) caused low methane yield, attributed not only to low buffer capacity but also to insufficient nitrogen, a nutrient that is important for the health of anaerobic organisms. Creamer (2010) described total ammonia nitrogen (TAN) as arising from the mineralization of organic nitrogen and the TAN already present in a substrate. Animal wastes contain readily degraded ammonia compounds such as urea and proteins. Excessive ammonia causes ammonium toxicity, resulting in the inhibition of microbial activity (Kwietniewska & Tys, 2014). Under normal operating conditions, anaerobic digesters typically contain more than 50% of their nitrogen in the form of ammonium nitrogen, as evaluated from their effluents (Creamer, 2010). Tea waste releases additional nitrogen during hydrolysis and deamination due to its protein nature (Zhang *et al.*, 2021). The accumulation of TAN beyond inhibitory thresholds can suppress acetoclastic methanogenic

activity leading to the accumulation of volatile fatty acids and instability of the digester (Jiang *et al.*, 2018).

3.2 Substrate TS, VS, C/N Ratio and COD

TS is the total mass of solids, including both organic and inorganic matter, in a waste sample measured after drying at a high temperature, typically at 105 °C, and it influences the degradation rate of substrates, bacterial growth rate and treatment efficiency of the reactor (Oduor *et al.*, 2022). Figure 2 shows the TS, VS and C/N ratio obtained from the various wastes collected from the tea factory and from the University dairy farm. From Figure 2, STPW1, STPW2, STPW3 and CM had high mean TS (>80%) and VS (>60%) values, which align with the Kenyan proximate composition data (VS, 80-85%) reported by Manganya *et al.* (2024), suggesting that they have suitable amounts of organic matter for biogas production. Similar VS fractions have been reported upon comparing thermophilic and mesophilic AD systems in India (Tiwari *et al.*, 2019; Basumatary *et al.*, 2021). Agricultural wastes require pretreatment to enhance digestibility due to their lignocellulosic content (Rahman *et al.*, 2023). According to Li *et al.* (2024) and Qu *et al.* (2024), pretreatment methods such as mechanical grinding can not only alter TS and VS percentages but also help break down complex compounds, enhancing the availability of organic matter for microbial degradation. Future research should evaluate biological approaches to selectively disrupt carbohydrate (lignin) complexity so as to unlock the full biogas potential of tea waste. Methane yield increases with an increase in the VS content, which represents the organic fraction of total solids that can be broken down by microorganisms during AD (Orhorhoro *et al.*, 2017). Angelidaki *et al.* (2009) reported TS and VS contents for fresh manure slurry of 5 and 3.5% respectively, which are lower than the values found in this study, reflecting

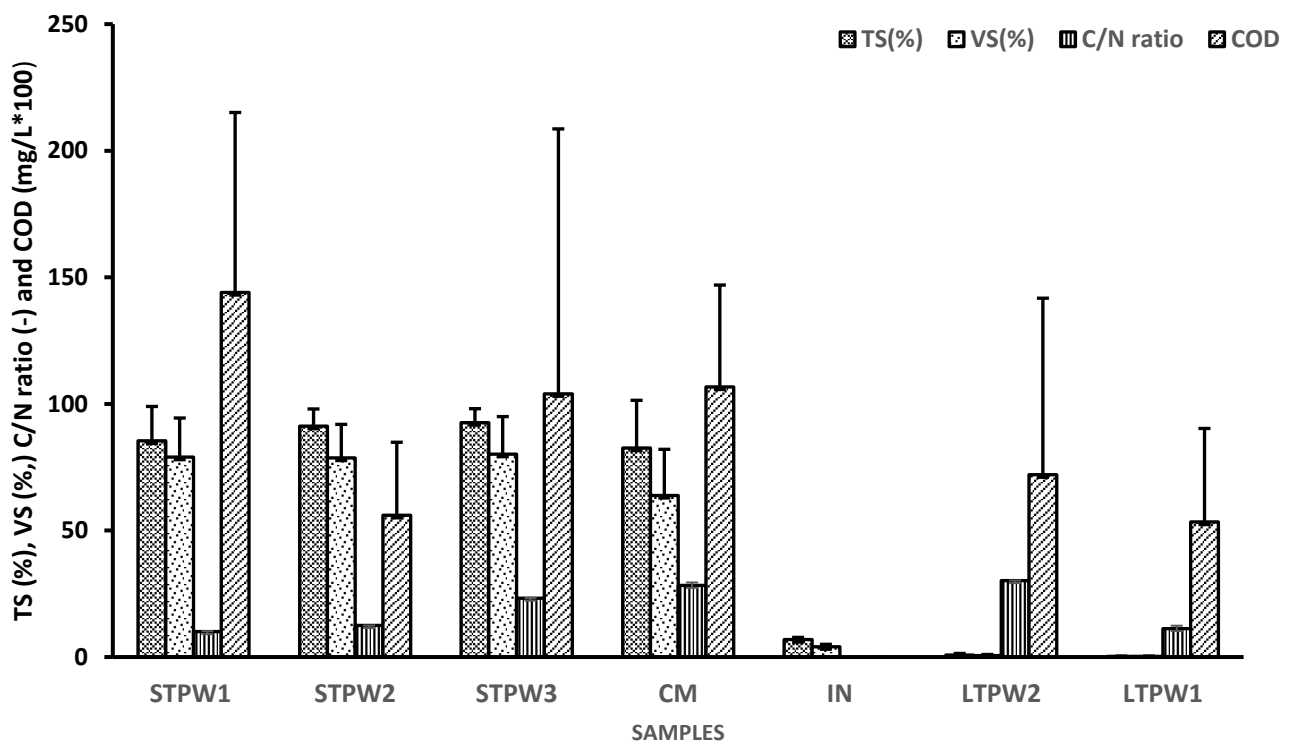


Fig 2. The TS, VS, C/N ratio and COD of the solid tea processing waste (STPW1, STPW2 and STPW3), cow manure (CM), inoculum (IN) and the liquid tea processing waste (LTPW1 and LTPW2). All values are mean $\pm$ SD and n=3

its dilute nature, with a pH of 7.2 and a volatile fatty acid (VFA) concentration <500 mg/L.

STPW3 had comparatively higher VS and TS suggesting a higher potential for methane production when used as a substrate in AD systems (Figure 2). This shows that STPW3 can be used as a standalone substrate but, owing to the presence of more solids, clogging of the digester may occur, disrupting the AD process. CM had a moderate VS content making it a good co-substrate that can help stabilize the anaerobic co-digestion process. STPW1 and STPW2 can therefore be considered once other suitable process parameters have been determined. LTPW2 and LTPW1 depicted comparatively low TS and VS. Statistical analysis of the data showed that the mean TS and VS values of LTPW1 and LTPW2 were not significantly ($p>0.05$) different. These wastes, however, had significantly lower mean VS and TS values compared to STPW1, STPW2, STPW3 and CM. It has been reported that the inoculum (IN) provides the active microorganisms responsible for the AD process and was characterized only with TS and VS fresh-weight contents of 6.9% and 4.1%, respectively, to guide the inoculum-substrate ratio during mixing (Sicchieri *et al.*, 2022) and a pH of 7.31 indicating a neutral environment suitable for methanogenic activity. These parameters therefore demonstrated that the inoculum was well-buffered, had appropriate microbial diversity for biomethane potential (BMP) assays with the capability of supporting the degradation of lignocellulosic substrates such as tea waste.

There was significant ($p\leq 0.05$) variation in the mean TS and VS concentration across the three sampling days as shown by the MANOVA, implying that the composition affects the digestion efficiency and the biogas yield. Leaf quality is affected by seasonal variations. For instance, the TS and VS differ between the rainy and dry seasons, thus causing biological heterogeneity (Seth *et al.*, 2025). The observed TS and VS values reflect seasonal heterogeneity. During the dry season, reduced rainfall and higher temperatures in the Kiambu region during sampling increase leaf dry matter, leading to elevated TS values. Conversely, rainy season conditions promote lush growth with higher moisture content, lowering TS and altering VS fractions. This aligns with Seth *et al.* (2025), who noted seasonal variation as a driver of biological heterogeneity in tea leaf composition. Environmental factors such as light intensity, temperature and moisture stress throughout a growing season alter plant biochemistry (Mohammadian *et al.*, 2014). STPW1 and CM had high SD values for TS and VS, hence the need for homogenization as a way of pretreatment. STPW1 had an SD value of 15.47%, suggesting inconsistency in the available organic matter depending on the type of tea bush from which the leaves were harvested. Among the three solid tea wastes, STPW3 had the lowest SD value suggesting a fairly consistent composition as depicted in both TS and VS percentages.

The TS of a sample mainly represents the organic and inorganic matter in a substrate (APHA, 2017; Orhorhoro *et al.*, 2017; Mrosso *et al.*, 2023). Tea waste generally has high TS values due to its fibrous nature, while CM has low TS since it contains a significant amount of water (Tutus *et al.*, 2015). For efficient AD of substrates, a balanced TS is essential because high TS can lead to clogging of the digester due to excess loading, while low TS dilutes the system and hence lowers its efficiency. The VS is a fraction of TS representing the organic matter available for microbial activity in a substrate (APHA, 2017; Orhorhoro *et al.*, 2017; Mrosso *et al.*, 2023). Solid tea waste has a high VS content implying the availability of a relatively large amount of biodegradable organic matter, but its lignin component can slow down digestion because of its complexity

(Dhull *et al.*, 2024). On the other hand, CM has a significant amount of VS but has a balanced composition suitable for a stable AD process. The optimal TS range for AD is 5-15% on wet basis depending on the type of waste, implying therefore that co-digesting tea waste with CM contributes to a more stable and efficient process (Arelli *et al.*, 2018). A VS range of 70-90%, indicating a high proportion of biodegradable matter, is ideal for the AD process (Orhorhoro *et al.*, 2017).

From Figure 2, compared with STPW1 and STPW2, STPW3 has a more balanced C/N ratio and can be used as a standalone substrate, but it will require dilution to enhance microbial degradation. STPW1 and STPW2 had low C/N ratios; hence, when digested alone, ammonia toxicity may occur, necessitating an increase in the overall C/N ratio using the high C/N ratio of CM. LTPW2 had the highest C/N ratio (Figure 2). A MANOVA of the data showed that the mean C/N ratio was significantly ($p\leq 0.05$) different across the various tea waste samples tested. Multiple comparisons showed that LTPW1 had a significantly different ($p\leq 0.05$) mean C/N ratio compared to other tea wastes. However, the mean C/N ratio of LTPW2 and STPW1 was not significantly ($p>0.05$) different.

Kwietniewska & Tys (2014) in their study reported that the optimal C/N ratio for anaerobic digestion is between 20:1 and 30:1, as these ratios ensure a balanced microbial environment that minimizes process inhibition. Kavisa *et al.* (2020) in a study where biogas was produced from tea pulp and cow manure through anaerobic co-digestion reported that the highest yield of methane (73.167 mL/g TS) was produced at a C/N ratio of 30:1. Hakiki & Sari (2020) also reported in another study involving biogas development from tea dregs and cow manure that an optimal methane yield of 77.10 % was produced at a C/N ratio of 30:1.

The C/N ratio helps maintain a suitable environment and a proper nutrient balance for the development of microorganisms during the AD process (Wang *et al.*, 2014). A low C/N ratio indicates that the substrate has a high protein content, resulting in an increase in free ammonia, which inhibits methanogenesis because of the high pH during the AD process (Algapani *et al.*, 2016). On the other hand, a high C/N ratio causes a rapid reduction in nitrogen levels and a concomitant increase in carbon-rich conditions, leading to the accumulation of VFAs and low biogas production (Wang *et al.*, 2014). These solid wastes, however, showed a low C/N ratio, which is below the optimal range of 20-30 required for stable AD. Based on experimental mass balance, a mixing ratio of TPW:CM of 1:3 on a VS basis adjusts the composite C/N ratio to approximately 23.5, which falls within the recommended range (Aksay *et al.*, 2018). This ratio thus balances the nutrient profile, provides buffering capacity, leading to improved process stability and methane yield potential as recommended in Indonesian and Chinese studies (Kavisa *et al.*, 2020). LTPW2 showed a high C/N ratio but had the lowest TS and VS contents, since much of its mass is water. The high water content in the liquid tea waste samples dilutes the concentration of solids, resulting in low TS and VS despite the presence of carbon in dissolved form.

The C/N ratio reflects the balance between available organic carbon, which acts as an energy source for the microorganisms, and the nitrogen content, which is used for protein synthesis (Van Peteghem *et al.*, 2022). According to the study by Tutus *et al.* (2015), tea waste is rich in nitrogen due to its organic composition, and its carbon content is attributed to the presence of cellulose, hemicellulose and lignin. CM has a balanced C/N ratio hence can be used to reduce the inhibitory effects of compounds such as tannins and polyphenols,

effectively acting as a buffering agent for AD systems that utilize tea waste.

STPW1 had the highest COD that was significantly ($p \leq 0.05$) higher than that of the other samples as determined using Fisher's LSD test at 0.05 significance level. STPW1 thus has more potential for use as a co-substrate, while LTPW1 had the lowest COD value of all the samples. The mean COD values were significantly different ($p \leq 0.05$) across different days of sampling with post hoc tests showing significant ($p \leq 0.05$) differences in the mean COD between samples collected on day 1 and day 2, as well as those collected on day 1 and day 3. There were, however, no significant ($p > 0.05$) differences in the mean COD of samples collected on day 2 and day 3, which may be due to seasonal weather conditions. Higher COD values indicate higher energy recovery potential from biomass sources through AD systems (Tauseef *et al.*, 2013). Although CM had a lower COD compared to STPW1, multiple comparisons showed that the mean COD of the two samples did not differ significantly ($p > 0.05$). The relatively high COD of CM indicates its suitability as a co-substrate for anaerobic co-digestion, a finding in agreement with that reported by Yang *et al.* (2019). STPW2 had the lowest SD, suggesting that the organic matter content in this sample was more consistent across the replicates and hence suitable for the AD process, while STPW3 had the highest variation, showing heterogeneity in organic matter content across the samples and hence requiring pre-processing for homogenization. Aksay *et al.* (2018) reported that co-digestion of cow manure with tea waste in the ratio of 75:25 resulted in the highest COD reduction. This ratio also yielded the highest methane content, demonstrating that cow manure stabilized the digestion environment and facilitated organic matter breakdown. Similarly, Manyuchi *et al.* (2018) in their study observed a reduction of at least 50% in COD during digestion of shredded tea waste, with a methane yield of 0.2 L/g.

3.3 Substrate pH Values

Figure 3 shows the mean pH values of cow manure, solid tea waste, liquid tea waste and the inoculum. From Figure 3, it is apparent that LTPW1 is slightly acidic but within the AD range. On the other hand, LTPW2 was more acidic, hence the need for pH adjustment. STPW1, STPW2 and STPW3 are acidic and hence cannot be used alone in AD systems. The pH values reported in the present study for both solid and liquid tea processing waste are consistent with Kenyan factory wastewater profiles (NEMA, 2006). This observation may be attributed to the abundance of organic content in tea wastes, which causes rapid acidification, a phenomenon that can inhibit methanogenesis. CM has a slightly alkaline pH and is hence good for buffering, pH stabilization and the reduction of inhibitory effects in tea wastes. CM is therefore an ideal co-substrate as it has undergone enteric fermentation in the stomach of ruminants (Macedo *et al.*, 2023). The collected inoculum is also ideal for stabilizing the microbial environment owing to its neutral to slightly alkaline pH of 7.31. Statistical analysis of the data showed significant ($p \leq 0.05$) differences in the mean pH among all the tea waste samples collected on different days. Van Peteghem *et al.* (2022) reported in their study that a pH range of 6.8-7.5 is widely acceptable for optimal methanogenic activity. Similarly, Manyuchi *et al.* (2018) demonstrated that the initial pH of shredded tea waste was mildly acidic (around 6.2) and hence required buffering to maintain optimal methanogenic conditions in batch digesters. Substrate pH affects microbial activity in AD systems. Tauseef *et al.* (2013) in their study observed that methanogens work optimally in neutral to slightly alkaline conditions (pH 6-8). Low pH (acidic conditions) can destroy microorganisms in AD systems, whereas high pH (alkaline conditions) slows down microbial activity in biogas systems. An optimum pH is therefore recommended for efficient digestion, optimal methane yield, process stability and reduced inhibitory effects.

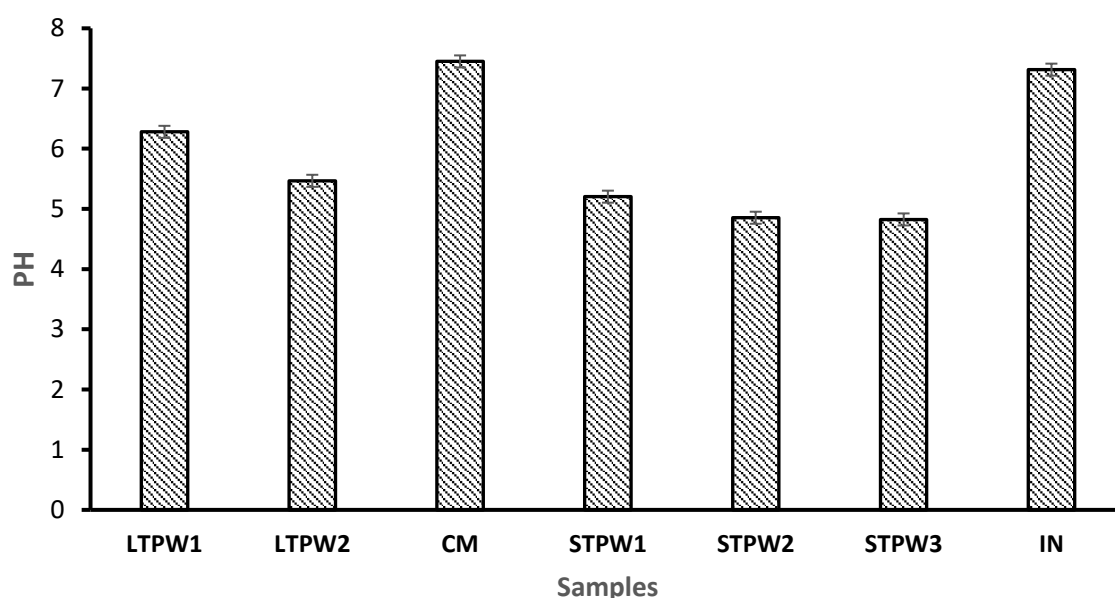


Fig 3. pH of solid tea wastes (STPW1, STPW2 and STPW3), cow manure (CM), inoculum (IN) and the liquid tea waste (LTPW1 and LTPW2). All values are mean $\pm$ SD and n=3

Table 1
Elemental composition of substrates from tea waste and cow manure

Sample	N (%)	C (%)	H (%)	S (%)
CM	1.44± 0.08 ^a	40.50± 0.62 ^d	5.67± 0.10 ^e	0.35± 0.11 ^d
STPW2	3.77± 0.01 ^b	46.85± 0.18 ^c	5.63± 0.03 ^d	0.24± 0.02 ^{bc}
STPW3	2.05±0.02 ^a	47.55± 0.13 ^f	5.81± 0.04 ^f	0.09±0.00 ^a
LTPW1	1.13±0.04 ^a	34.13±0.15 ^a	4.80± 0.04 ^a	0.10±0.03 ^a
STPW1	4.78± 0.10 ^c	47.93± 0.08 ^{cf}	5.34± 0.00 ^c	0.27 ±0.00 ^c
LTPW2	3.60±0.03 ^b	40.53± 0.38 ^b	5.07± 0.09 ^b	0.19± 0.01 ^b

All values are mean ±SD. For all measurements, n=2. STPW1 designates solid tea processing waste from withering, STPW2 for solid tea processing waste from sorting, STPW3 represents solid tea processing wastes from drying, LTPW1 designates liquid tea processing waste at lagoon stage, LTPW2 represents liquid tea processing waste from cleaning stage and CM for cow manure. Mean values within the same column marked with different letters are significantly different at 0.05 significance level.

3.4 Elemental Composition and Theoretical BMP of Substrates

Elemental composition influences the efficiency of anaerobic digestion (Szaja et al., 2023). The elemental composition of various substrates is presented in Table 1. CM exhibited moderate nitrogen and carbon content. This suggests a high C/N ratio of 28, indicating that cow manure is a carbon-rich substrate with limited nitrogen availability. Studies by Aremanda & Debretsion (2023) reported that cow manure contains approximately 1.18% nitrogen and a carbon content of 43%-50% on a dry-matter basis.

Solid tea waste (STPW1, STPW2 and STPW3) showed higher mean nitrogen levels ranging from 2.05±0.02% to 4.78 ± 0.10% implying a low C/N ratio. Higher nitrogen levels enhance microbial activity, while the higher carbon content in cow manure improves energy recovery potential (Li et al., 2021). According to Aksay et al. (2018), nitrogen levels in tea waste range from 2% to 5%, with C/N ratios between 10 and 20, which are suitable for AD processes. Liquid tea waste showed lower mean nitrogen levels ranging from 1.13±0.04% to 3.6±0.03% and moderate carbon levels (34.13±0.15 to 40.53±0.38%). This can be attributed to the fact that nitrogen in tea waste is often bound in cellular proteins, which are more prevalent in solid leaf residues than in liquid waste (Seth et al., 2025). Statistical analysis of the data showed significant ($p\leq0.05$) differences in the mean C, N, H and S values among different tea waste samples. Multiple comparisons using Tukey’s post hoc tests, however, showed that there were no significant differences ($p>0.05$) in the mean N content between LTPW1 and STPW2, as well as LTPW2 and STPW2. Similarly, the mean C content between STPW1 and STPW3 as well as STPW3 and STPW2 was not significant ($p>0.05$). All pairwise comparisons for the mean H concentration among the tea waste samples tested

were statistically significant ($p\leq0.05$). In the case of the mean S content, the mean values between LTPW1 and STPW3, LTPW2 and STPW3, as well as STPW1 and STPW2 were not statistically significant ($p>0.05$). These findings show variability in the elemental composition of the tea waste samples. The statistical analysis showed a lower degree of variation in the C content of the solid tea wastes. This was expected because of the similar origin of the samples and the relatively stable content of C in biological materials.

For optimal methane yield, STPW1 or STPW2 can be co-digested with cow manure as a mixed substrate, based on the carbon and nitrogen contents obtained from the CHNS results (Table 1), and will potentially have a balanced C/N ratio. The theoretical biomethane potential of the solid and liquid wastes, computed from the CHNS values in Table 1, is presented in Table 2. Among the solid wastes, STPW1 reached the highest theoretical BMP, while among the liquid wastes, LTPW2 showed the highest as indicated in Table 2. The elevated ash contents in LTPW1 and LTPW2 reduced the availability of volatile solids for conversion by diluting the organic matter. On the other hand, cow manure showed a lower TBMP but is more readily digestible since it has undergone enteric fermentation. Actual methane yields are constrained by the biochemical composition of tea residues (Sun et al., 2024). A methane content of 77.10 % was obtained by Aksay et al. (2018) through co-digestion of 75 % cow manure and 25 % tea waste under mesophilic conditions. Another study reported 19 % methane yield when cow manure and tea waste were co-digested in a ratio of 1:2 (Kipkemei et al., 2019). Co-digestion is therefore essential to realize the energy potential of tea waste in AD systems. Mangenya et al. (2024) reported TBMP values of approximately 350 mL CH₄/g VS for Kenyan tea processing effluents. Similarly, Unyay et al. (2025) reported TBMP values

Table 2
Theoretical biomethane potential of cow manure, solid and liquid tea waste.

Sample	Ash content	CHNS%	O%	Empirical formula	Molecular weight	mol CH ₄	TBMP (mLCH ₄ /gVS)
STPW1	13.41	58.32	28.27	C ₁₂ H ₁₆ O ₅ N ₁	253	6.136	811
STPW2	6.61	56.49	36.91	C ₁₄ H ₂₁ O ₉ N ₁	346	7.346	709
STPW3	2.57	55.49	41.94	C ₂₇ H ₄₀ O ₁₈ N ₁	665	13.640	685
CM	18.72	47.96	33.32	C ₃₃ H ₅₅ O ₂₀ N ₁	787	17.861	758
LTPW1	19.84	40.15	40	C ₃₅ H ₅₉ O ₃₁ N ₁	992	16.933	570
LTPW2	28.14	49.39	22.47	C ₁₃ H ₂₀ O ₅ N ₁	279	7.291	874

STPW1 designates solid tea processing waste from withering; STPW2 designates solid tea processing waste from sorting; STPW3 designates solid tea processing waste from drying; LTPW1 designates liquid tea processing waste at the lagoon stage; LTPW2 designates liquid tea processing waste from the cleaning stage; CM designates cow manure; and TBMP designates theoretical biomethane potential.

between 320-370 mL CH₄/g VS for black tea processing. The discrepancy noted in this study can be attributed to differences in inoculum source, substrate composition, tea variety, regional cultivation conditions and the stage of tea waste collection. Water is used extensively during tea processing, which causes nitrogen dilution and leaching (Wang *et al.*, 2024). Nitrogen levels can also vary significantly, a fact demonstrated by the variation in effluent composition depending on the processing method (Manganya *et al.*, 2024). The low nitrogen availability and variability suggest that liquid tea waste may not be a good substrate for supplementing nitrogen. Cow manure is rich in carbon, which can lead to ammonia limitation and slow microbial activity when used as the sole substrate. Solid tea waste can therefore be used to balance the C/N ratio because of its higher nitrogen content. Liquid waste can be used to improve buffer capacity and digester stability as it still contributes nitrogen despite the variability.

3.5 Lipid Content

The fatty acid profile of tea processing waste has direct implications for AD performance. Saturated fatty acids (SFAs) such as palmitic (C16:0) and stearic (C18:0) degrade slowly and do not strongly inhibit methanogens and are therefore more stable in AD systems (Lalman & Bagley, 2001; Sousa *et al.*, 2013). High concentrations of unsaturated fatty acids (UFAs), such as linoleic (C18:2), linolenic (C18:3) and oleic (C18:1), on the other hand, inhibit methanogenesis since they are prone to β -oxidation intermediates (Diamantis *et al.*, 2021). Figure 4 shows the fatty acid composition of tea processing wastes from GC-MS analysis.

From Figure 4, STPW1 recorded a higher proportion of saturated fatty acids, suggesting greater process stability. It is particularly notable that palmitic acid, a long-chain saturated fatty acid, contributes to COD and methane yield (Deaver *et al.*,

2020). STPW2 and STPW3 in contrast had high levels of unsaturated fats (>60%). While such unsaturated fatty acids theoretically enhance methane yield, they are known to inhibit methanogenesis at concentrations as low as 30 mg/L for oleic acid (C18:1) and at total long-chain fatty acid (LCFA) concentrations of 376-568 mg/L in anaerobic systems (Lalman & Bagley, 2001; Zhang *et al.*, 2011). For stable AD performance, a lipid content below 40% of total solids is generally recommended with a high ratio of saturated to unsaturated LCFAs to reduce inhibition risk (Sun *et al.*, 2024). LTPW1 and LTPW2 were characterized by oleic acid (C18:1) dominance with unsaturated fats comprising more than 90%, well above the recommended threshold, thus highlighting the need for co-digestion. Statistical analysis of the data (one-way ANOVA and post hoc tests) showed that the mean fatty acid profile was not significantly ($p>0.05$) different across all the samples.

3.6 Determination of Inhibitors

The phytochemical analysis of tea waste extracts revealed notable concentrations of phenolics, caffeine and tannins, all of which are recognized inhibitors of AD. The quantities and implications of these inhibitors for the AD process are described in the following subsections.

3.6.1 Total phenolic content

Phenolics are well-documented inhibitors of AD in polyphenol-rich effluents (Rasouli *et al.*, 2023). Their antimicrobial activity arises from disruption of microbial membranes and inhibition of enzymatic systems, leading to slower hydrolysis and acidogenesis (Serrano-Meza *et al.*, 2020). The total phenolic content of the various tea waste streams is

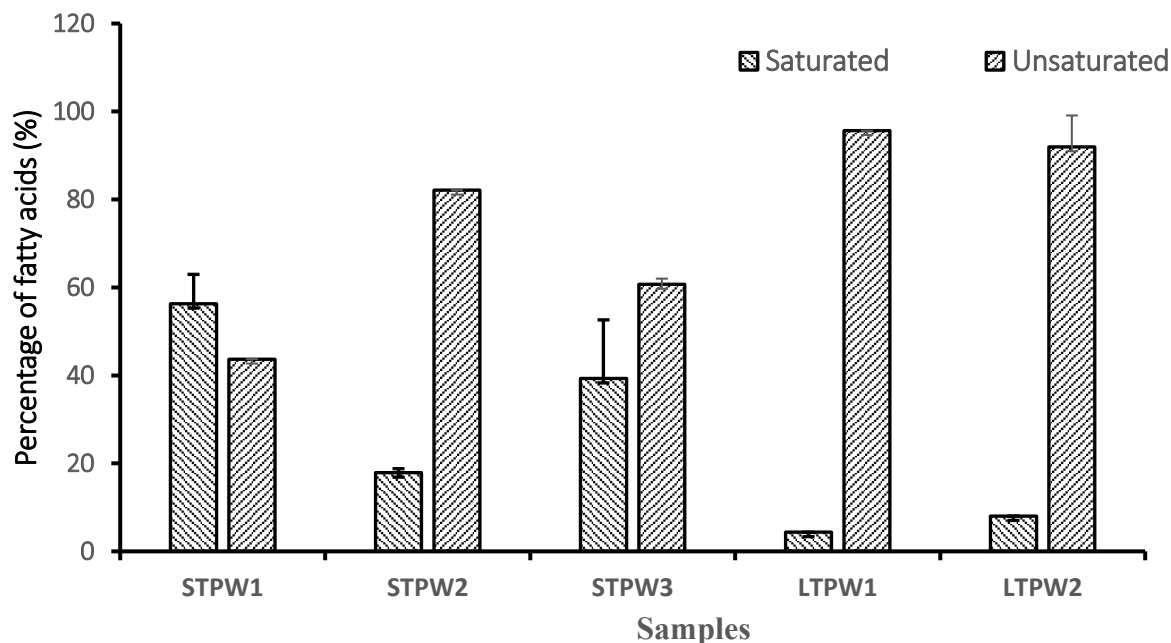


Fig 4: The quantities of saturated and unsaturated fatty acids in tea waste samples from GC-MS analysis. STPW1 designates solid tea processing waste from withering, STPW2 for solid tea processing waste from sorting, STPW3 represents solid tea processing wastes from drying, LTPW1 designates liquid tea processing waste at lagoon stage and LTPW2 is liquid tea processing waste from cleaning stage). All values are mean $\pm$ SD and n=3

Table 3
Caffeine concentration of solid and liquid tea processing wastes

Tea waste sample	Concentration (mg/L)
STPW1	30.64± 1.50
STPW2	41± 2.01
STPW3	23.12± 1.14
LTPW2	9.23± 0.00
LTPW1	15.31± 0.00

All values are mean ±SD. For all measurements, n=3. STPW1 designates solid tea processing waste from withering, STPW2 for solid tea processing waste from sorting, STPW3 represents solid tea processing wastes from drying, LTPW1 designates liquid tea processing waste at lagoon stage and LTPW2 represents liquid tea processing waste from cleaning stage

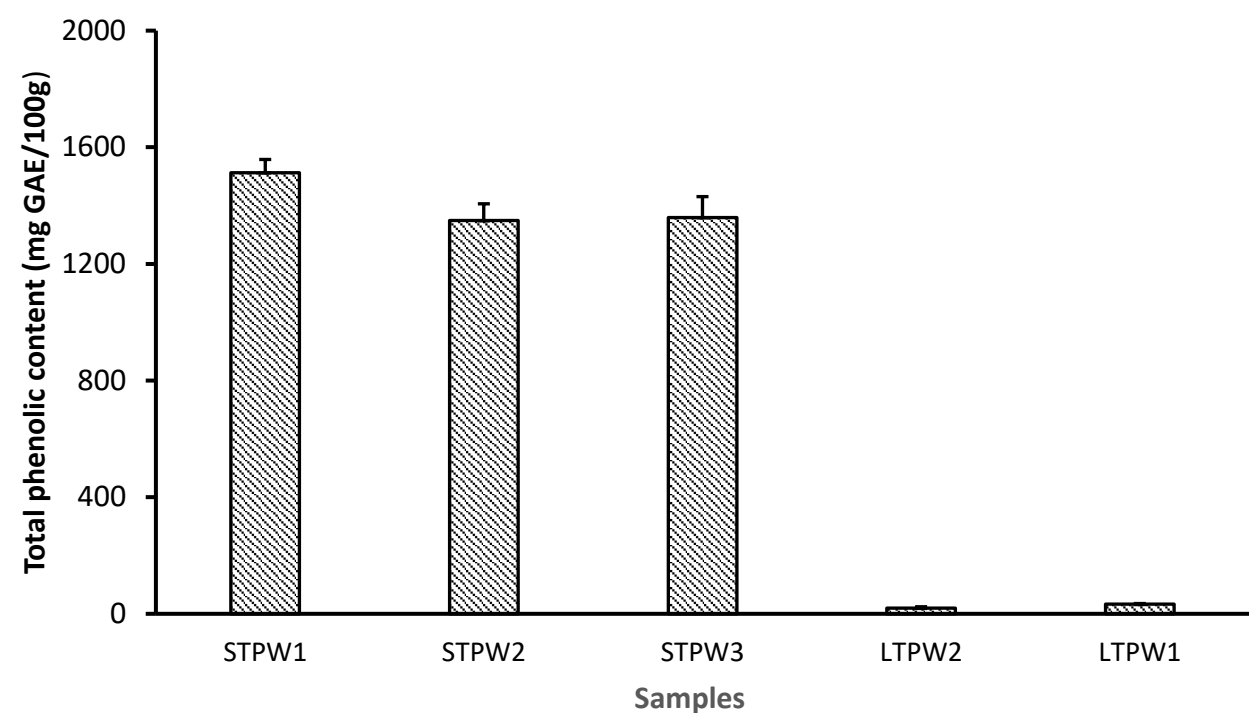


Fig 5. Total phenolic content of the tea processing wastes from different waste streams. STPW1 designates solid tea processing waste from withering, STPW2 for solid tea processing waste from sorting, STPW3 represents solid tea processing wastes from drying, LTPW1 designates liquid tea processing waste at lagoon stage and LTPW2 is liquid tea processing waste from cleaning stage. All values are mean ±SD and n=3

presented in Figure 5. From Figure 5, the total phenolic concentrations ranged from 19.4 ± 4.8 to 1512.9 ± 45.3 mg GAE/100g, with the three solid waste streams exhibiting the highest phenolic content, a finding that corroborates the fact that tea waste retains bioactive compounds even after primary processing. STPW1 recorded the highest phenolic content, since it consists largely of coarse, discarded leaf and stem material removed before the leaf is substantially macerated. For the sorting and drying stages, cell disruption is more complete and tea particles are finer, as this waste is generated after rolling and firing.

Tea waste has a high content of lignin and condensed polyphenols, which hinder enzymatic hydrolysis and inhibit methanogenic activity, constraining its direct use in AD (Sun *et al.*, 2024). Studies by Tan *et al.* (2011) and Popich *et al.* (2026) have demonstrated that tea polyphenols and tannins exhibit significant inhibition of methanogenic archaea primarily at

elevated concentrations and that the inhibitory threshold varies with inoculum adaptation and substrate dilution. Furthermore, Manyi-Loh and Lues (2023) reported that co-digestion with nitrogen-rich substrates such as animal manure substantially mitigates polyphenolic inhibition through buffering capacity and microbial community enrichment. However, untreated tea processing waste was analyzed in this study to establish baseline physicochemical and elemental properties to assess its suitability as a co-substrate and to provide estimates of biodegradability and methane yield under realistic conditions where pretreatment may not be immediately feasible. The findings highlight the inhibitory role of lignin and polyphenols, suggesting that pretreatment could enhance substrate accessibility and reduce phenolic inhibition.

According to Chen *et al.* (2008), free phenolic compounds are amongst the inhibitors most frequently implicated in digester underperformance. Batch toxicity assays

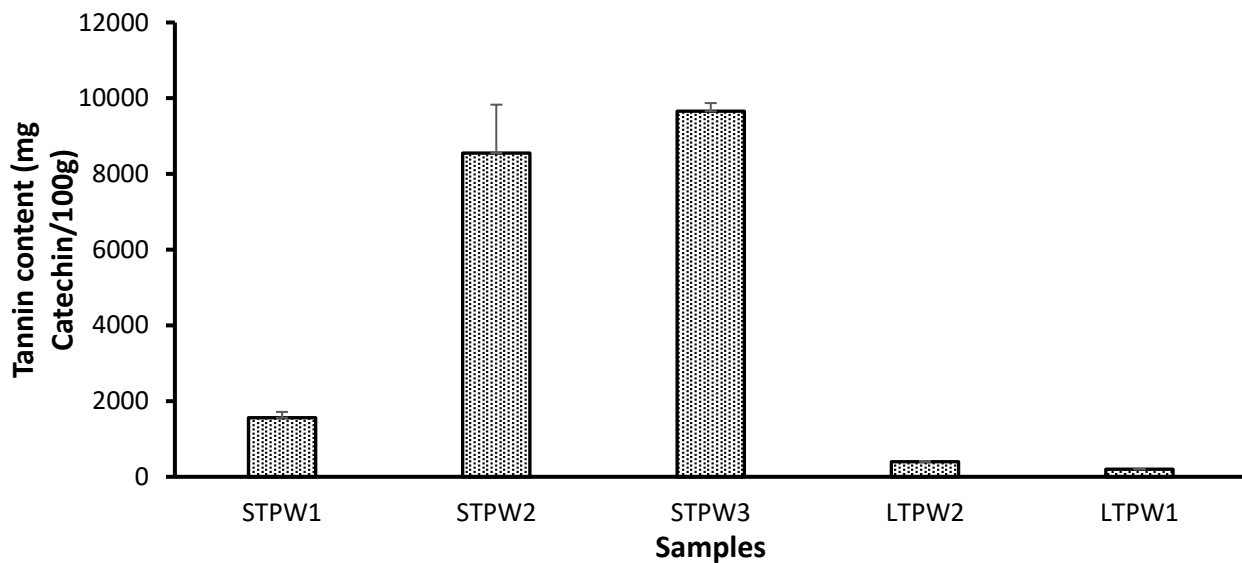


Fig 6: Total tannin content of the tea processing wastes from different waste streams. STPW1 designates solid tea processing waste from withering, STPW2 solid tea processing waste from sorting, STPW3 represents solid tea processing wastes from drying, LTPW1 designates liquid tea processing waste at lagoon stage and LTPW2 is liquid tea processing waste from cleaning stage. All values are mean $\pm$ SD and $n=3$

have shown that methanogenesis is unaffected up to approximately 1.0 g phenol/L, while progressive shifts in the methanogenic community begin from around 0.5 g/L, and that methanogenesis is completely and irreversibly inhibited above 2.0 g/L (Chapleur *et al.*, 2016). Separately, individual phenolic acids have been reported to produce measurable inhibition of biomethanation at 0.5-1.0 g/L, with complete suppression of methane production at 2.0-4.0 g/L (Chapleur *et al.*, 2016). Studies of phenolic monomers have further shown that toxicity to acetoclastic methanogens is influenced by the degree and pattern of hydroxyl substitution, indicating that not all phenolic compounds within a mixed pool will contribute equally to inhibition (Kayembe *et al.*, 2013).

3.6.2 Caffeine

Caffeine is a purine alkaloid with antimicrobial properties and is known to inhibit methanogenic archaea by interfering with enzymatic activity and energy metabolism (Mekonnen *et al.*, 2020; Mazzei *et al.*, 2019). In AD systems, methanogenic archaea are particularly sensitive to inhibitory compounds and environmental stresses, leading to reduced methane yield and accumulation of volatile fatty acids (VFAs). The caffeine content obtained from GC-MS analysis is presented in Table 3. GC-MS analysis showed caffeine concentrations ranging from 29.6-43.8 mg/L in solid tea processing waste (STPW) and 7.3-21.2 mg/L in liquid tea processing waste (LTPW). The higher caffeine content in STPW coincided with stronger inhibition of methanogenesis, consistent with reports that caffeine suppresses microbial activity above 20 mg/L (Chen *et al.*, 2018). Solid tea waste residues contain more bound caffeine, which may be attributed to adsorption onto fibrous material during processing. In contrast, LTPW exhibited lower caffeine levels, reflecting partial extraction into the liquid phase, which may result in comparatively reduced inhibition. These findings highlight the importance of substrate type in determining AD performance. While both solid and liquid tea wastes represent potential feedstocks, their caffeine content necessitates mitigation strategies such as co-digestion or pre-treatment

methods to enhance biogas recovery and minimize AD process inhibition.

3.6.3 Tannins

Tannins exert inhibitory effects in AD systems by binding proteins and inactivating microbial enzymes thereby reducing substrate availability for hydrolytic and fermentative bacteria (Zhang *et al.*, 2022). Tannin concentrations of the different tea waste streams are presented in Figure 6. Tannin concentrations determined by the Vanillin-HCl assay ranged from 197.5 ± 9.0 to 9656.8 ± 214.4 mg/100 g catechin equivalents. Among the solid tea waste streams, tannin content increased from the withering stage through the sorting and drying stages, with the sorting and drying stages carrying five- to six-fold more tannin than the withering stage. The two liquid streams on the other hand had lower tannin content since they consist predominantly of water and are hence inherently diluted. High tannin levels (>350 mg/L) have been reported to cause up to 50% inhibition of methanogenesis (Makkar, 2003). This therefore highlights the importance of incorporating pretreatment or co-digestion strategies when tannin-rich substrates are used. The processing stages that generate the solid waste streams physically disrupt cell membranes, bringing the substrate and enzymes into contact and thus increasing the extractable tannin fraction, a finding supported by results reported in this study.

Tannins inhibit microbial communities through the precipitation of extracellular hydrolytic enzymes and proteins, direct antimicrobial action, and chelation of iron and other trace metals required as microbial cofactors (Makkar, 2003). Acetoclastic methanogens are consistently reported as the functional group most sensitive to tannin and phenolic exposure within the AD consortium (Bhat *et al.*, 1998).

3.7 Methane concentration

GC analysis of biogas from co-digestion of tea waste from the withering stage (STPW1) and cow manure through batch

Table 4
Methane concentration of solid tea waste from withering stage at varied mixing ratios of tea waste to cow manure

Sampling ratio (TW:CM)	CH ₄ (ppm)	CO ₂ (ppm)	N ₂ O (ppb)	CH ₄ (%)	CO ₂ (%)
0:100	577510.95	412397.95	0.00	57.8%	41%
25:75	582923.70	369211.05	0.00	58.3%	37%
50:50	534051.25	400770.59	0.00	53.4%	40%
75:25	534313.04	379670.04	0.00	53.4%	38%
100:0	504602.47	357442.49	0.00	50.5%	36%

experiments revealed clear differences in methane yield across substrate ratios as shown in Table 4.

The highest methane concentration (58.3%) was obtained at a tea waste to cow manure ratio of 25:75, indicating that manure-dominant blends provide optimal buffering capacity and microbial support. This trend agrees with reports by Aksay & Calhan (2018) who found that 75% cow manure with 25% tea waste produced the highest methane yield (77.1% CH₄) and observed improved methane recovery in manure-rich mixtures. The difference in absolute values is attributed to the substrate type used in this study, tea processing waste from the withering stage, which has a high polyphenol content, compared with the household brewed tea residues used by Aksay & Calhan (2018). In contrast, the 100:0 ratio yielded the lowest methane percentage, consistent with the literature noting that polyphenols, tannins and caffeine in tea residues inhibit methanogenesis (Makkar, 2003).

Intermediate ratios showed a progressive and significant decline in methane concentration as tea waste loading increased. These results demonstrate that while manure alone sustains digestion since it has undergone enteric fermentation, co-digestion with tea waste at balanced ratios does not maximize methane recovery. The observed trend highlights the importance of manure as both a nutrient source and chemical buffer, diluting inhibitory compounds and stabilizing the anaerobic digestion process. Across all treatments, CO₂ concentrations ranged between 36-41%, with higher values in manure-rich blends, suggesting more complete substrate conversion and stable microbial activity. Nitrous oxide was not detected, confirming minimal nitrogen transformations under anaerobic conditions.

The methane concentrations obtained in this study (50.5-58.3%) fall within the typical range (50-70%) for stable anaerobic digestion reported by Angelidaki & Sanders (2004) and Appels *et al.* (2008). These findings confirm that co-digestion of tea waste with cow manure produced biogas of acceptable quality. They further support the widely reported observation that manure-dominant blends enhance methane yield and process stability while pure tea waste suppresses methanogenesis due to its inhibitory compounds.

4. Conclusion

This study demonstrated that tea processing waste is a potential co-substrate in anaerobic digestion that can be used to generate supplementary, sustainable energy in the tea industry. The findings clearly indicate that tea waste possesses favorable carbon and nitrogen content with a C/N ratio ranging from 10 to 23, which is recommended for AD processes, suggesting its ability to balance nutrient availability when combined with other substrates. High VS content further supports its biodegradability, making it a suitable feedstock for microbial

activity. The pH values of tea waste, cow manure and the inoculum fall within the recommended range of 6-8 for AD systems. Solid tea waste from the withering stage is the best substrate when co-digested with cow manure, owing to its high COD value. It also had low ammonium nitrogen concentrations that minimized inhibitory effects and was able to generate biogas of acceptable quality within the recommended AD range (50-70%). Polyphenols, tannins and caffeine are inhibitors of the AD process; hence, substrates rich in them require dilution and pretreatment strategies. Based on the physicochemical and elemental analysis results, optimization of process parameters should be considered to enhance biogas yield through AD, with further laboratory analysis and validation to assess the performance of tea processing waste for energy recovery in typical tea processing industries, especially in continuous AD reactors.

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