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Strategies for enhancing methane production from coffee pulp via co-digestion, urea supplementation, and biochar-assisted process stabilisation

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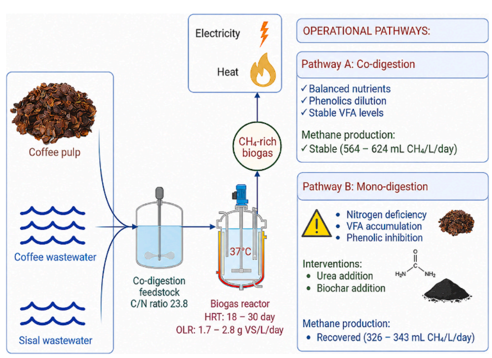
HIGHLIGHTS

- Coffee pulp co-digestion with agro-industrial wastewaters improved stability.
- Semi-continuous CSTRs achieved > 560 mL CH_4/L/day methane production.
- Nitrogen deficiency constrained coffee pulp mono-digestion performance.
- Urea supplementation restored nutrient balance and reactor stability.
- Co-digestion enabled sustainable valorisation of coffee processing residues.

GRAPHICAL ABSTRACT



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ABSTRACT

Coffee pulp (CP), a lignocellulosic agro-industrial residue rich in organic matter, contains phenolic compounds and other constituents that can limit anaerobic digestion (AD) performance. This study evaluated semi-continuous AD of CP in continuously stirred tank reactors under mesophilic conditions (37 °C), comparing co-digestion with coffee wastewater (CWW) and sisal wastewater (SWW) against CP mono-digestion. Co-digestion achieved stable reactor performance, with methane production rates of 564 ± 89 and 624 ± 92 mL CH_4/L/day and methane contents of 56–59%. This improved performance was associated with enhanced nutrient balance (C/N = 23.8), dilution of potential inhibitory compounds, and low VFA concentrations (<1 g/L). In contrast, CP mono-digestion resulted in process instability, characterised by reduced methane production, VFA accumulation (>1.5 g/L), pH decline, and nitrogen limitation (TAN = 56.0–64.8 mg/L). Urea supplementation restored nutrient availability and reactor stability, resulting in methane production of 343 ± 18 mL CH_4/L/day. Although combined urea and biochar supplementation supported recovery (326 ± 26 mL CH_4/L/day), the independent effect of biochar could not be isolated, and biochar did not prevent instability at increased organic

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loading rates. Batch assays further showed that inoculum adaptation enhanced initial methane production kinetics, whereas cattle manure inoculum achieved the highest final methane yield (328 ± 8 mL CH_4/g VS). Overall, co-digestion with agro-industrial wastewaters represents an effective strategy for improving CP digestion, while maintaining adequate nitrogen availability is critical for stable CP mono-digestion.

1. Introduction

Coffee pulp (CP) and coffee wastewater (CWW) are generated during the wet processing of coffee cherries, whereas sisal wastewater (SWW) is produced during the decortication of sisal leaves. CP is the primary solid residue from coffee processing and accounts for approximately 40–50% of the fresh fruit weight (Murthy and Naidu, 2012; Pandey et al., 2000). It contains substantial organic matter (Král et al., 2024), including carbohydrates (45–89%), proteins (7–13%), lipids (1–3%), and bioactive compounds such as phenolic compounds, predominantly chlorogenic acids (approximately 2–6% dry mass), caffeine (1–4%), tannins (1–9%), and pectin (~1%) on a dry mass basis (Franca and Oliveira, 2009; Murthy and Naidu, 2012). However, CP composition varies considerably depending on coffee species, geographical origin, processing methods, and post-harvest handling conditions (Esquivel and Jimenez, 2012; Murthy and Naidu, 2012). Due to its high moisture and readily biodegradable organic content, CP is susceptible to uncontrolled microbial degradation, which can generate acidic leachates and contribute to environmental pollution if improperly managed (Murthy and Naidu, 2012; Pandey et al., 2000). Therefore, sustainable valorisation of CP is essential for improving waste management and recovering valuable resources.

Anaerobic digestion (AD) is an established biological process that converts organic waste into biogas, mainly methane and carbon dioxide, together with nutrient-rich digestate under oxygen-free conditions (Li et al., 2011; Ward et al., 2008). The process consists of four sequential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Bułkowska and Zielińska, 2025; Menzel et al., 2020). Although CP contains considerable biodegradable organic matter and has high methane potential, its lignocellulosic structure restricts hydrolysis and limits microbial accessibility to structural carbohydrates (Nava-Valente et al., 2023; Saritha et al., 2012). Furthermore, bioactive compounds such as polyphenols, caffeine, and tannins may inhibit microbial activity, particularly methanogenesis, reducing AD efficiency (Juliastuti et al., 2018; Selvankumar et al., 2017).

Stable AD generally requires a balanced carbon-to-nitrogen (C/N) ratio, commonly reported within the range of 20–30 (Nwokolo et al., 2020; Shahbaz et al., 2019). However, CP commonly exhibits relatively high C/N ratios (approximately 28–38), which may result in nitrogen limitation during AD (Hirpa, 2020; Sánchez et al., 1999). In addition, CP is naturally acidic (pH 4.5–5.5), associated with organic acids such as chlorogenic, malic, and citric acids (Esquivel and Jimenez, 2012; Murthy and Naidu, 2012). The release and accumulation of these acids may decrease reactor pH and inhibit methanogenic activity, which generally occurs optimally under near-neutral conditions (pH 6.8–8.5) (Liu and Whitman, 2008).

Various pretreatment approaches, including physical, chemical, biological, and hybrid methods, have been investigated to improve CP biodegradability and methane production. These strategies enhance hydrolysis by disrupting lignocellulosic structures and reducing inhibitory compounds. For example, alkaline and hydrogen peroxide pretreatments can reduce lignin and phenolic compounds (Pudjiastuti et al., 2019), whereas acidic and thermal treatments can improve substrate solubilisation (Nava-Valente et al., 2023). Biological approaches using fungi or microbial consortia have also been explored (Chen et al., 2010; Juliastuti et al., 2018; Wan and Li, 2012), and hybrid treatments have demonstrated further improvements in methane production (Getachew et al., 2023; Phuong and Nguyen, 2024). However, pretreatment increases process complexity, energy requirements, and operational costs. Moreover, severe thermal and chemical treatments may generate inhibitory compounds such as 5-hydroxymethylfurfural (Brodeur et al., 2011; Kim and Karthikeyan, 2021), while biological pretreatments generally require extended treatment periods (Chen et al., 2010; Wan and Li, 2012).

Co-digestion has emerged as an alternative strategy to overcome the limitations of CP mono-digestion by improving nutrient balance, diluting inhibitory compounds, and enhancing microbial activity. Previous studies have reported improved methane production and reactor stability through co-digestion of CP with nutrient-rich substrates, including animal manure, coffee-processing residues, and other organic wastes (Juliastuti et al., 2017; Karki et al., 2025; Rodrigues et al., 2023; Sumardiono et al., 2021; Widjaja et al., 2017). However, most studies investigating CP AD have been performed under batch conditions, with comparatively fewer studies evaluating long-term semi-continuous operation (Boopathy, 1987; Corro et al., 2014; Corro et al., 2013; Getachew et al., 2023; Juliastuti et al., 2018; Montoya et al., 2020a; Montoya et al., 2019; Montoya et al., 2020b; Nava-Valente et al., 2023; Rodrigues et al., 2023; Selvankumar et al., 2017; Sumardiono et al., 2021; Widjaja et al., 2017). Semi-continuous systems provide a more realistic assessment of long-term reactor stability, process adaptation, and operational performance compared with batch assays (Li et al., 2011; Mata-Alvarez et al., 2011). Furthermore, the limited semi-continuous studies available have mainly focused on pretreatment, nutrient supplementation, or co-digestion with a restricted range of co-substrates (Chala et al., 2018; Karki et al., 2025; Nava-Valente et al., 2023; Rojas-Sossa et al., 2017; Widjaja et al., 2019).

However, the potential of locally available agro-industrial effluents, such as SWW and CWW, as co-substrates for long-term CP AD remains insufficiently explored. These liquid residues contain soluble organic matter and nutrients (Ijanu et al., 2020; Muthangya et al., 2013), which may improve AD performance by diluting potential inhibitors, supplying nutrients and trace elements, and reducing freshwater requirements. Their integration into CP digestion systems may therefore provide a sustainable approach for simultaneous waste treatment and renewable energy recovery.

This study evaluated the semi-continuous co-digestion of CP with CWW and SWW compared with CP mono-digestion under mesophilic conditions. Urea supplementation and exploratory biochar addition were investigated as recovery strategies to improve nutrient balance and process stability during CP mono-digestion. In addition, microbial adaptation to CP degradation was evaluated using inocula obtained from co-digestion and mono-digestion reactors.

2. Materials and methods

2.1. Substrates and feedstock preparation

2.1.1. Coffee pulp

Fresh CP was collected during the 2023 coffee processing season from a wet-processing facility in Jimma, Ethiopia (7° 40′N, 36° 50′E). The material contained 186.3 ± 1.4 g total solids (TS)/kg. After collection, the CP was sun-dried for four consecutive days, sealed in airtight polyethylene bags, transported to Denmark, and stored at − 20 °C until use in the AD experiments (Arega et al., 2026).

2.1.2. Coffee wastewater

Fresh CWW was collected during the 2023 coffee processing season from the post-fermentation washing stage of a wet-processing facility operated by Burka Coffee Estate Limited in Arusha, Tanzania (3°22′S, 36°41′E). To ensure representative sampling, the effluent was homogenised and collected at the outlet of the primary fermenter. Samples were transferred into airtight 5-L plastic containers (approximately 80% filled), frozen at − 20 °C, transported in insulated boxes to Denmark, and stored at − 20 °C until use in the AD experiments.

2.1.3. Sisal wastewater

Fresh SWW, the primary effluent generated during the dry decortication of mature sisal leaves, was collected in 2023 from a factory operated by Sisalana Tanzania Company Limited near Mombo, Korogwe District, Tanga, Tanzania (5°01′S, 38°22′E). To ensure representative sampling, the effluent was homogenised and collected at the outlet of the primary decorticator. Samples were transferred into airtight 5-L plastic containers (approximately 80% filled), frozen at − 20 °C, transported in insulated boxes to Denmark, and stored at − 20 °C until use in the AD experiments.

2.1.4. Substrate pretreatment and characterisation

Sun-dried CP was crushed to a particle size of approximately 2 mm prior to the AD experiments to enhance hydrolytic accessibility and facilitate semi-continuous reactor operation by preventing tube blockage (Kothari et al., 2010). The physicochemical properties of the sun-dried CP, homogenised CWW, and homogenised SWW were subsequently characterised and are summarised in Table S1 (Supplementary Material).

2.1.5. Preparation of feedstock for coffee pulp mono-digestion

Crushed CP was diluted with tap water to prepare a slurry containing 50 g volatile solids (VS)/L for semi-continuous mono-digestion. The slurry contained 53.4 g TS/L, 76.5 mg total ammonia nitrogen (TAN)/L, 2.1 g total volatile fatty acids (VFAs)/L, 972.9 mg gallic acid equivalents (GAE)/L of total phenolics, and 722.8 mg catechin equivalents (CE)/L of total flavonoids. The slurry had a pH of 4.5 ± 0.0 and a C/N ratio of 30.9. The feedstock was prepared weekly and stored at 4°C until use in the AD experiments.

2.1.6. Preparation of feedstock for co-digestion of coffee pulp, coffee wastewater, and sisal wastewater

Crushed CP, homogenised CWW, and homogenised SWW were mixed to prepare a co-digestion feedstock containing 50 g VS/L, with VS contributions of 60%, 13%, and 27%, respectively. The feedstock contained 56.2 g TS/L, 1440 ± 4 mg TAN/L, and 1.4 g total VFAs/L. The feedstock had a pH of 4.6 ± 0.0 and a C/N ratio of 23.8. It was prepared weekly and stored at 4 °C until use in the AD experiments.

2.2. Inoculum

Inoculum was obtained from digestate collected at the mesophilic Hashøj full-scale biogas plant near Dalmose, Denmark (55.30°N, 11.35°E), which primarily treats cattle manure. Prior to use, the inoculum was pre-incubated at 37 °C for 10 days to reduce residual biodegradable material and minimise background methane production (Angelidaki et al., 2009; Raposo et al., 2012). The degassed inoculum contained 34.2 ± 0.2 g TS/L, 23.4 ± 0.1 g VS/L, 115.7 ± 0.0 mg total VFAs/L, and had a pH of 7.8 ± 0.1 and an electrical conductivity of 15.0 ± 0.0 mS/cm.

2.3. Chemicals and other materials

The following chemicals and materials were used in this study: Avicel PH-101 (microcrystalline cellulose, ≥99% purity; Sigma-Aldrich, CAS No. 9004–34-6), cyclohexane analytical standard (assay ≥ 99.7%; Sigma-Aldrich, CAS No. 110–82-7), urea (CH_4N_2O, ≥99.5% purity; Sigma-Aldrich, CAS No. 57–13-6), and biochar powder (produced at CIRAD from woody green waste collected in winter 2024 by pyrolysis at 950 °C for 30 min at the Marmagne site, Bourges, France). The biochar had an ash content of 7% (dry basis), volatile matter content of 3% (dry basis), fixed carbon content of 89% (dry basis), and a specific surface area of 462 m^2/g.

2.4. Reactor operation

2.4.1. Reactor configuration

Two identical borosilicate glass continuously stirred tank reactors (CSTRs), designated Reactor 1 (R1) and Reactor 2 (R2), were operated with a working volume of 1.8 L each (Fig. 1). Substrates, with or without additives, were fed every 24 h using calibrated peristaltic pumps, with occasional interruptions when required to maintain reactor stability.

The reactors were maintained at 37 °C and continuously mixed at 80 rpm using magnetic stirrers to ensure homogenisation and prevent settling of fibrous particles. Each reactor was equipped with a gas-tight sampling port for liquid sampling. Effluent outlets were connected via gas-tight tubing to 1-L conical flasks for digestate collection and gas–liquid separation. Biogas volume was measured continuously using calibrated automatic water-displacement gas meters, while butyl rubber septa installed along the gas lines enabled periodic biogas sampling for composition analysis.

The reactors were operated simultaneously in three successive phases: (i) co-digestion of CP with CWW and SWW, (ii) CP mono-digestion without additives, and (iii) CP mono-digestion with urea supplementation in both reactors and additional biochar supplementation in R2. Reactor pH and VFA concentrations were monitored before each feeding cycle, and feeding rates were adjusted or temporarily suspended when required to maintain process stability.

2.5. Reactor start-up and fed-batch operation

2.5.1. Coffee pulp-coffee wastewater-sisal wastewater co-digestion

The reactors were initially operated in fed-batch mode using the co-digestion feedstock described in Section 2.1.6. Each reactor received 360 mL inoculum (20% of the working volume; Section 2.2) and 180 mL tap water, resulting in an initial liquid volume of 540 mL. The headspace was purged with nitrogen for 15 min to establish anaerobic conditions.

The reactors were fed daily for four days, increasing the liquid volume to 740 mL. Feeding was suspended for the following four days to allow microbial acclimatisation. Reactor contents were subsequently combined, homogenised, and redistributed equally (700 mL per reactor) to ensure identical starting conditions. After a further 15 min nitrogen purge, inoculum (Section 2.2) was added to achieve a working volume of

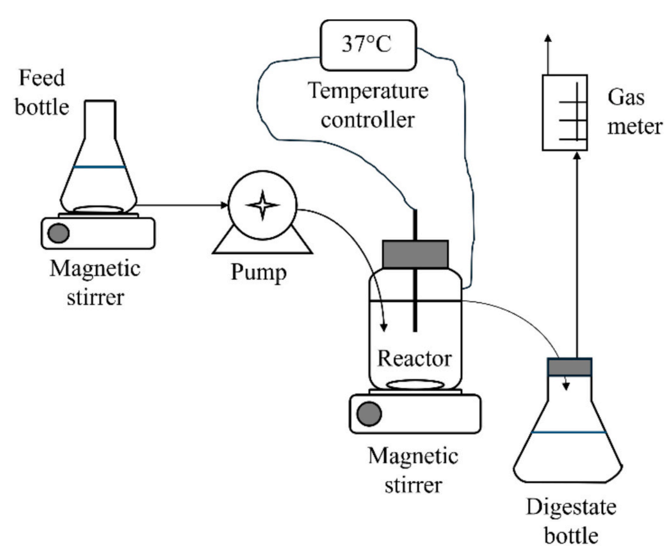

Fig. 1. Reactor setup.

1.0 L. This time point was designated as day 0, from which pH, VFA concentration, biogas production, and methane content were monitored.

Following a three-day acclimation period without feeding, semi-continuous operation commenced. The working volume was gradually increased to 1.8 L, while the organic loading rate (OLR) increased from 2.0 to 2.8 g VS/L/day and the hydraulic retention time (HRT) decreased from 25 to 18 days. This start-up strategy facilitated microbial acclimatisation and stable reactor performance before steady-state operation.

2.6. Semi-continuous reactor operation

2.6.1. Co-digestion of coffee pulp, coffee wastewater, and sisal wastewater

After reaching the full working volume (1.8 L), both reactors were operated semi-continuously from day 22 to day 85 using the co-digestion feedstock at an HRT of 18 days (Table S2). This phase was used to evaluate steady-state reactor performance, process stability, and methane production.

2.6.2. Coffee pulp mono-digestion without additives

From day 85 to day 114, the feed was switched to CP slurry (Section 2.1.5) at an HRT of 18 days (OLR: 2.8 g VS/L/day). Process instability during this phase necessitated operational interventions, including temporary feeding suspensions (in R1 or both reactors) and partial cross-inoculation. By the end of this phase (day 113), R1 contained 249 mg PO_4^{3-}/L, 467.5 mg K^+/L, and 64.8 mg TAN/L, while R2 contained 245 mg PO_4^{3-}/L, 425.0 mg K^+/L, and 56.0 mg TAN/L.

2.6.3. Coffee pulp mono-digestion with additives

To improve reactor performance, recovery strategies were initiated on day 114 (Table S2). Reactor 1 received staged urea supplementation over two consecutive days, with urea added together with the feed on day 114 and without feeding on day 115, resulting in a total nitrogen addition of 940 mg N/L. Concurrently, Reactor 2 received staged biochar supplementation under the same regime, reaching a final concentration of 5 g/L. Both reactors were subsequently operated without feeding until day 126.

From day 127 to day 131, R1 was operated at an HRT of 25 days (OLR: 2.0 g VS/L/day), followed by operation at an HRT of 30 days (OLR: 1.7 g VS/L/day) from day 148 to day 166, while maintaining urea supplementation at 940 mg N/L. In contrast, R2 remained unfed from day 115 to day 158 but received additional urea supplementation on days 127 and 128 to achieve the same nitrogen concentration (940 mg N/L).

Feeding in R2 resumed from day 159 to day 166 at an HRT of 30 days, with continued urea (940 mg N/L) and biochar (5 g/L) supplementation. Both reactors were subsequently operated under these conditions from day 166 to day 189 at the same HRT. Finally, to evaluate reactor performance under an increased OLR, the HRT was reduced to 25 days in both reactors from day 189 to day 205.

2.7. Evaluation of microbial adaptation

Microbial adaptation to CP following semi-continuous operation was evaluated using three inocula: (i) cattle manure inoculum (CMI), representing the original microbial consortium without prior CP adaptation; (ii) co-digestion inoculum (CDI), collected from the CP–CWW–SWW CSTRs during stable operation; and (iii) mono-digestion inoculum (MDI), collected from the CP mono-digestion CSTRs on day 103, prior to liquid cross-inoculation and additive supplementation. Before use, the digestates were pre-incubated at 37 °C for 10 days to minimise background methane production (Angelidaki et al., 2009; Raposo et al., 2012). The physicochemical properties of CMI are described in Section 2.2, whereas CDI contained 18.8 ± 0.4 g TS/L and 13.7 ± 0.6 g VS/L, with a pH of 7.3 ± 0.0. MDI contained 31.5 ± 0.2 g TS/L and 21.6 ± 1.2 g VS/L, with a pH of 6.8 ± 0.1.

The assays were conducted in 250-mL serum bottles with a working volume of 100 mL at an initial substrate loading of 2 g VS/L. The inoculum-to-substrate ratio (ISR) was 4.1 for CMI and 2.8 for CDI and MDI, which were within the recommended range to avoid substrate overloading (Raposo et al., 2011). Inoculum accounted for 40% of the working volume, with tap water added to achieve the final volume. Two assay series were performed: CP digestion to evaluate microbial adaptation and Avicel microcrystalline cellulose digestion as a reference substrate. Blank controls containing only inoculum were included for each inoculum type to correct for background methane production.

2.8. Determination of substrate methane potential

Batch digestion tests were conducted to determine the biochemical methane potential (BMP) of CP, CWW, and SWW. Assays were performed in 118-mL serum bottles with a working volume of 50 mL at an initial substrate loading of 2 g VS/L (ISR ≈ 4.1) using cattle manure inoculum (Section 2.2). The inoculum accounted for 40% of the working volume to provide sufficient microbial density and buffering capacity, with tap water added to achieve the final volume. Blank assays were included to correct for background methane production, while Avicel microcrystalline cellulose was used as a positive control to verify inoculum activity.

All assays were conducted in triplicate following established BMP protocols (Angelidaki et al., 2009; Holliger et al., 2016), with minor substrate-specific modifications. Serum bottles were sealed, purged with nitrogen for 15 min, and incubated at 37 °C and 110 rpm. Methane production was monitored until daily methane production remained below 1% of cumulative methane production for three consecutive days (Holliger et al., 2016).

2.9. Analytical methods

CP was milled to approximately 2 mm using a Willey knife mill. Total suspended solids (TSS), volatile suspended solids (VSS), TS, and VS were determined in triplicate according to APHA standard methods (APHA, 2017). Total Kjeldahl Nitrogen was analysed using the macro-Kjeldahl method (Bremner, 1996), while TAN was determined using the modified Kjeldahl method without digestion (APHA, 2017). Soluble chemical oxygen demand (COD) was measured using LCK1014 kits and a Hach Lange spectrophotometer (Hach, Germany).

pH and electrical conductivity were measured using calibrated Mettler Toledo FiveEasy Plus meters (FP20 and FP30). Solid samples were analysed as 1:10 (w/v) suspensions. Orthophosphate and potassium concentrations were determined using LCK350 and LCK328 kits, respectively.

Biogas composition (CH_4, CO_2, N_2) was analysed by GC-TCD (TRACE 1310, Thermo Fisher Scientific) using helium as the carrier gas and certified gas standards for calibration. Gas samples (0.1 mL) were analysed in duplicate. VFAs were quantified using GC-FID (Agilent 7890A) after centrifugation and filtration of liquid samples and 1:10 (w/v) solid suspensions. Biogas pressure was measured using a digital manometer (HD2124.2, Delta OHM, Italy) prior to volume standardisation.

Samples were weighed with a precision of ± 0.1 mg. Lipid content was determined by Soxhlet extraction (Thiex et al., 2003), structural carbohydrates and lignin according to NREL methods (Sluiter et al., 2008), and elemental composition by combustion analysis (Vario EL Cube, Elementar Analysensysteme GmbH, Germany), with oxygen calculated by difference.

2.10. Data analysis and calculations

Experimental BMP yields were reported at standard temperature and pressure (STP; 0°C and 1 atm) (Angelidaki et al., 2009; Holliger et al., 2016). Batch experimental data are presented as the mean ± standard deviation (SD) of triplicate measurements, and error bars in figures represent SD. Methane production kinetics were described using the modified Gompertz model (Ahmed et al., 2019; Kafle and Kim, 2013). Theoretical methane yields were estimated from substrate CHNS composition using the modified Buswell equation (Buswell and Mueller, 1952). The biodegradability index was calculated as the ratio of experimental BMP to theoretical methane yield, expressed as a percentage.

3. Results and discussion

3.1. Substrate characterisation

Substrate composition strongly influences AD performance. Fresh CP (Section 2.1.1) exhibited the highest TS content (186.3 g/kg), reflecting its relatively concentrated lignocellulosic structure, whereas fresh CWW and SWW were considerably more dilute (13.2 and 41.6 g/kg, respectively; Table S1). All substrates contained a high organic fraction, as indicated by VS/TS ratios of 93.6%, 87.1%, and 81.5% for CP, CWW, and SWW, respectively, indicating substantial biodegradable organic matter. Among the liquid substrates, CWW exhibited lower TSS and a higher VSS/TSS ratio than SWW, indicating a greater proportion of suspended organic matter available for microbial degradation.

All substrates were acidic (pH 4.5–4.9), below the optimal range for methanogenic activity (Liu and Whitman, 2008). Nevertheless, the high soluble COD concentrations measured in CWW and SWW (Table S1) indicate a considerable load of soluble organic compounds that could contribute to methane production after appropriate pH adjustment and microbial conversion.

Carbohydrates represented the dominant organic fraction, particularly in CP (50.0% DM), which contained 36.7% DM cellulose and 16.8% DM hemicellulose. However, the relatively high lignin content (31.8% DM) may restrict enzymatic accessibility and hydrolysis of structural carbohydrates, thereby limiting biodegradability (Corro et al., 2014; Nava-Valente et al., 2023). Lipid concentrations were low across all substrates (1–4% DM), reducing the likelihood of long-chain fatty acid accumulation and associated inhibition during AD (Cirne et al., 2007; Silva et al., 2016).

The C/N ratios of CP (30.9), CWW (12.8), and SWW (24.5) indicate that co-digestion could improve nutrient balance by combining carbon-rich CP with nitrogen-containing liquid substrates (Hailu et al., 2020; Mutegoa et al., 2020). The relatively low sulphur contents reduce the potential risk of H_2S-related inhibition. SWW contained elevated orthophosphate and potassium concentrations, which may provide essential nutrients for microbial growth but could contribute to ionic stress at excessive concentrations (Carliell-Marquet and Wheatley, 2002). CP also contained a relatively high VFA concentration (34.7 g/kg), which may promote acidification during AD, highlighting the importance of sufficient buffering capacity, nutrient balance, and substrate dilution.

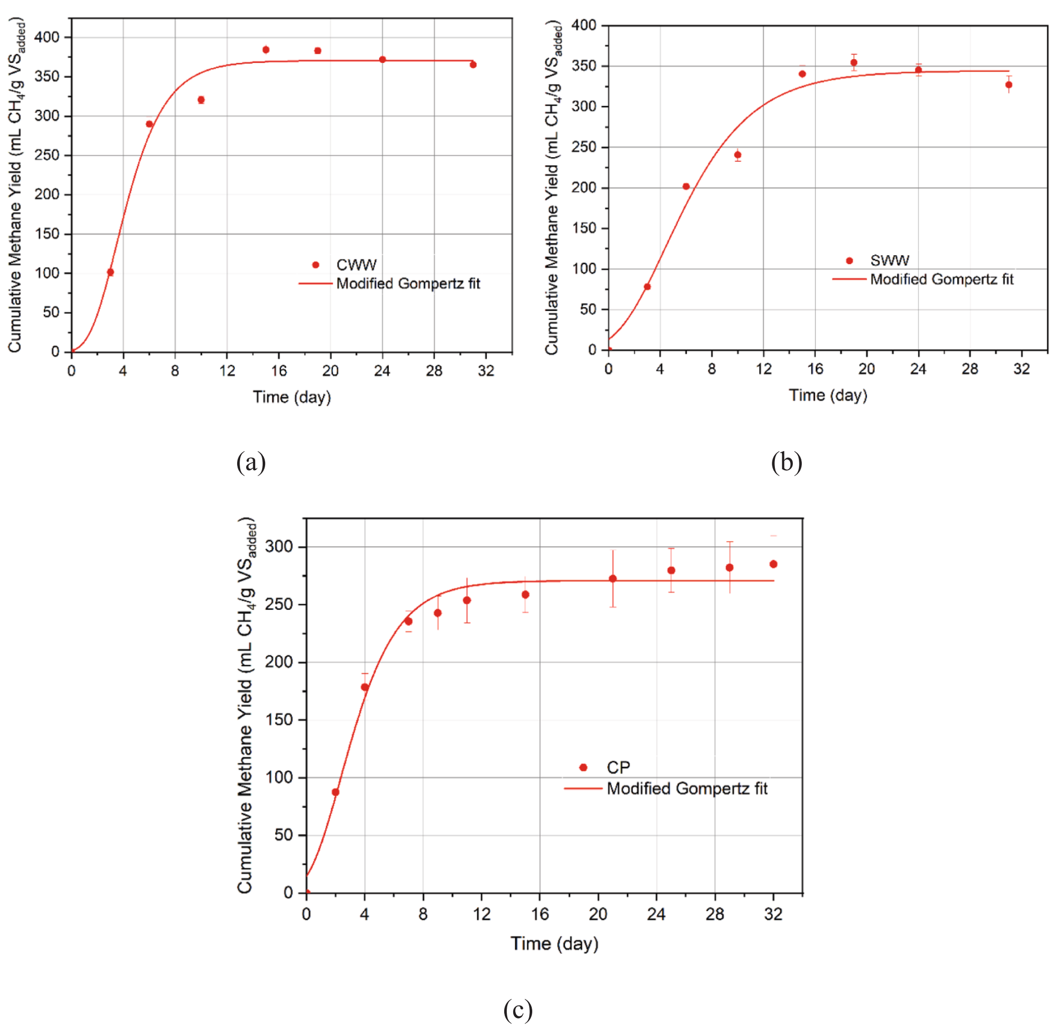

Fig. 2. Biochemical methane potential (blank subtracted) of (a) coffee wastewater (CWW), (b) sisal wastewater (SWW), and (c) coffee pulp (CP).

3.2. Methane potential and biodegradability of substrates

3.2.1. Methane potential and biodegradability

BMP varied substantially among the substrates (Fig. 2), reflecting differences in substrate composition, organic matter accessibility, and biodegradability. CWW achieved the highest methane yield (371 ± 9 mL CH_4/g VS; Fig. 2a), followed by SWW (344 ± 14 mL CH_4/g VS; Fig. 2b) and CP (271 ± 5 mL CH_4/g VS; Fig. 2c). In contrast, methane biodegradability indices showed an inverse trend, with CP exhibiting the highest biodegradability (~73%), followed by CWW (~66%) and SWW (~54%).

The difference between methane yield and biodegradability reflects the distinction between absolute methane production and the fraction of theoretical methane potential converted to methane. Although CWW produced the highest methane yield, its higher theoretical methane potential resulted in a lower biodegradability index. Conversely, CP achieved the greatest proportion of its theoretical methane potential despite its lower methane yield, indicating efficient conversion of its accessible biodegradable organic fraction. The lower biodegradability of SWW was likely associated with its higher proportion of recalcitrant lignocellulosic components, which limited hydrolysis and subsequent conversion of structural carbohydrates.

3.2.2. Methane production kinetics

Methane production kinetics further highlighted differences in substrate biodegradability (Fig. 2). CWW exhibited the highest methane production rate (66 ± 12 mL CH_4/g VS/day) but also the longest lag phase (1.4 ± 0.5 days), suggesting that the methanogenic community required additional adaptation time for the conversion of soluble fermentation intermediates. CP showed a lower methane production rate (46 ± 6 mL CH_4/g VS/day) and a negligible lag phase (0.2 ± 0.4 days), indicating rapid utilisation of its readily accessible biodegradable fraction. SWW exhibited the lowest methane production rate (34 ± 6 mL CH_4/g VS/day) and a moderate lag phase (0.7 ± 0.9 days), consistent with slower hydrolysis due to its higher content of recalcitrant lignocellulosic material.

Overall, all substrates demonstrated suitability for AD, although their conversion characteristics differed. The relatively high methane yield of CWW indicates its potential as an energy-rich co-substrate, whereas the high biodegradability of CP suggests efficient utilisation of its biodegradable fraction despite structural recalcitrance. The lower biodegradability and methane production rate of SWW indicate that pretreatment or co-digestion strategies may be beneficial to improve substrate conversion and process performance.

3.3. Semi-continuous reactor performance

3.3.1. Steady-state co-digestion of coffee pulp, coffee wastewater, and sisal wastewater (Days 22–85)

Following start-up and fed-batch operation, both reactors reached the full working volume (1.8 L) on day 22 and were subsequently operated semi-continuously at an HRT of 18 days (OLR: 2.8 g VS/L/day) until day 85. Mean methane production rates were 564 ± 89 and 624 ± 92 mL CH_4/L/day for R1 and R2, respectively (Fig. 3). Methane production was numerically higher in R2 (approximately 11% difference); however, this difference is reported descriptively because the reactors were operated as parallel systems rather than replicated treatment groups. Methane content remained stable throughout this period, averaging 56 ± 2% in R1 and 59 ± 2% in R2 (Fig. 6).

Although methane content remained within the moderate range (56–59%), these values fall within the range commonly reported for wet AD of agricultural and agro-industrial residues, where biogas typically contains 50–70% CH_4 (Aworanti et al., 2023; Shahzad et al., 2024). The moderate methane concentration likely reflects the carbohydrate-rich and low-lipid composition of the feedstock mixture, which favours conversion pathways associated with relatively high CO_2 production during AD. In addition, the relatively high TAN concentration in the co-digestion feed (1440 ± 4 mg/L) may have contributed to bicarbonate formation and enhanced buffering capacity through increased dissolved inorganic carbon. As no biogas upgrading was applied, the measured methane concentration represents the composition of raw biogas.

Process stability was demonstrated by low VFA concentrations (<1 g/L; Fig. 4) and stable pH values (7.4 ± 0.2 in both reactors; Fig. 5). These values remained within ranges favourable for methanogenic activity, indicating efficient conversion of fermentation intermediates and sufficient buffering capacity.

The favourable performance was attributed to improved nutrient balance and dilution of potentially inhibitory compounds present in CP. The feedstock C/N ratio (23.8) was within the optimal range commonly reported for AD (20–30) (Nwokolo et al., 2020; Shahbaz et al., 2019), while TAN concentrations (1440 ± 4 mg/L) were sufficient to support microbial growth without reaching concentrations commonly

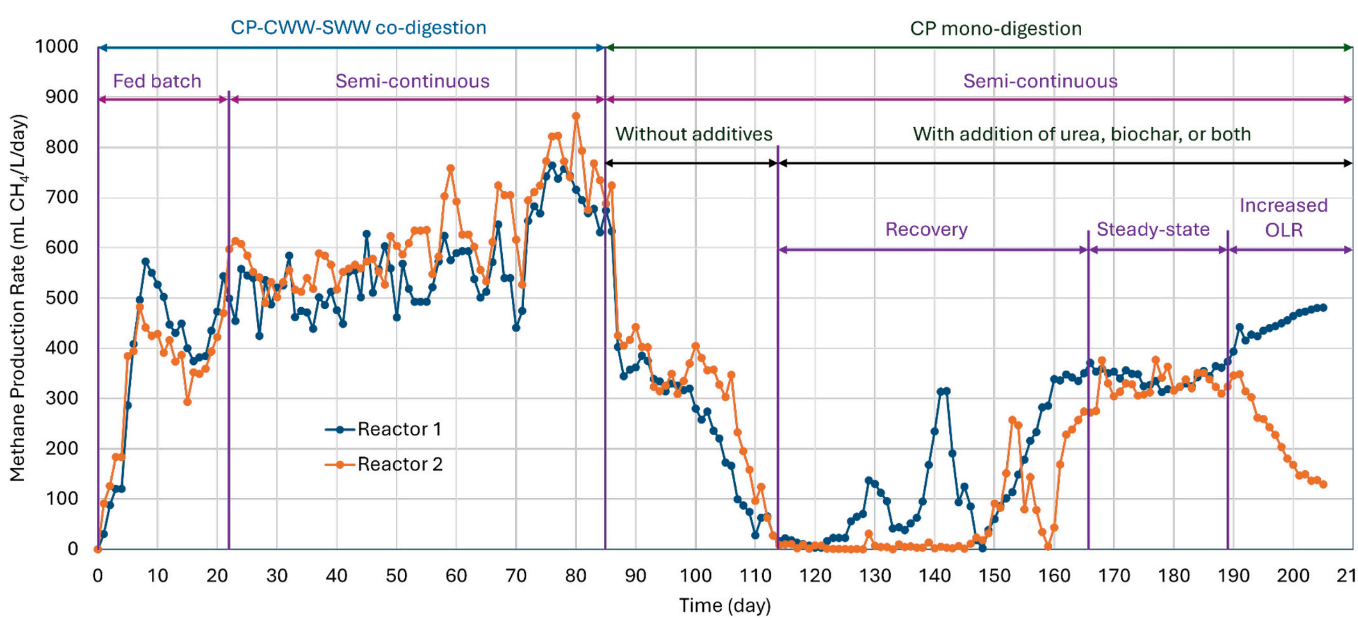

Fig. 3. Methane production profiles during reactor operation. CP, coffee pulp; CWW, coffee wastewater; SWW, sisal wastewater; OLR, organic loading rate.

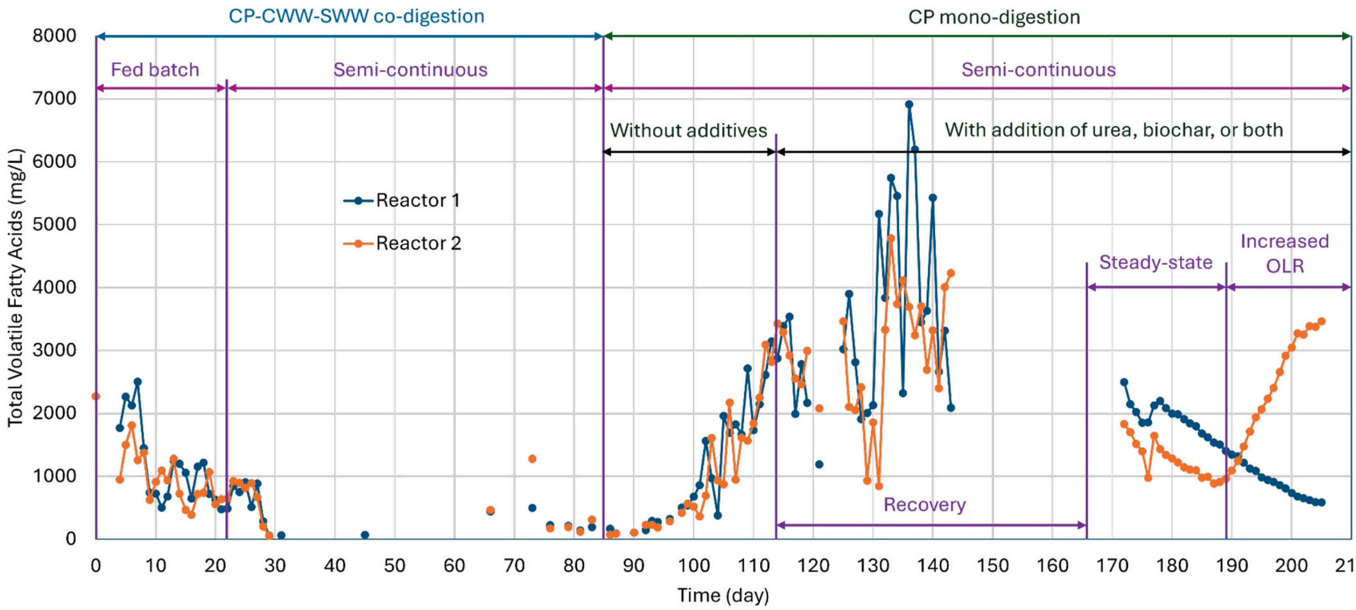

Fig. 4. Total volatile fatty acid concentration profiles during reactor operation: CP, coffee pulp; CWW, coffee wastewater; SWW, sisal wastewater; OLR, organic loading rate.

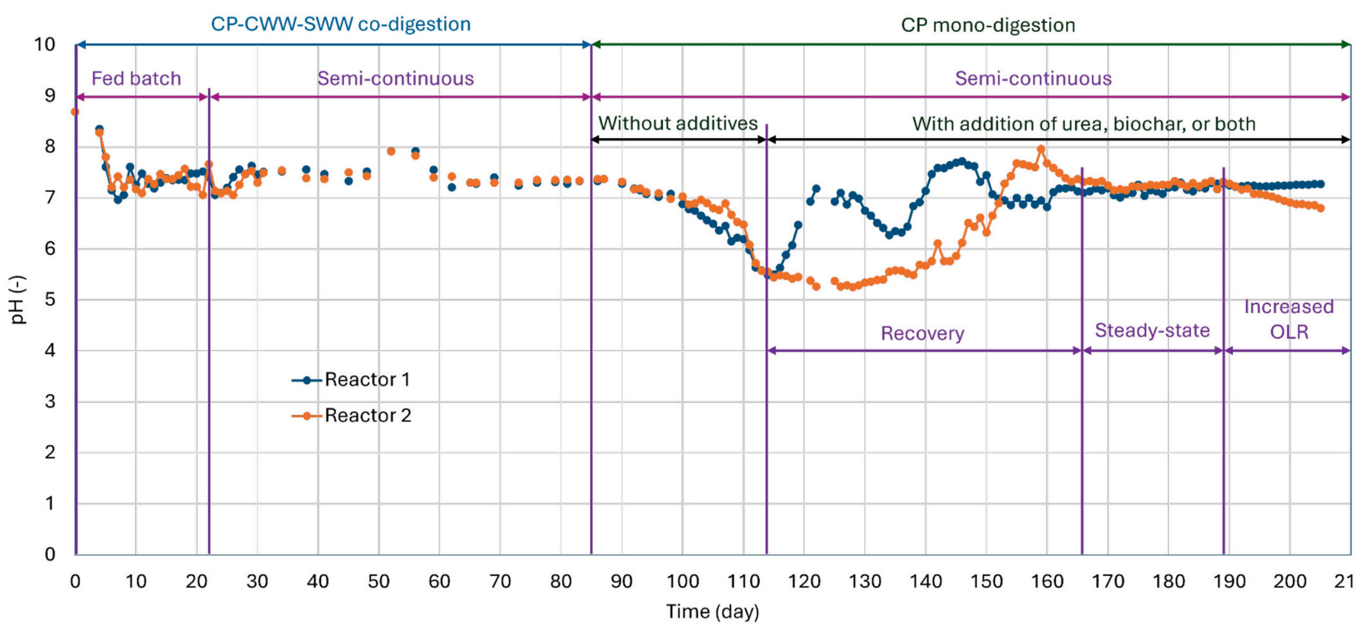

Fig. 5. Ph profiles during reactor operation: cp, coffee pulp: cww, coffee wastewater; sww, sisal wastewater; olr, organic loading rate.

associated with ammonia inhibition (<1500–3000 mg/L) (Chen et al., 2008; Rajagopal et al., 2013). Furthermore, CWW and SWW supplied additional water and readily biodegradable organic matter, reducing the requirement for freshwater dilution.

Overall, co-digestion of CP with CWW and SWW enabled stable reactor operation, favourable nutrient conditions, and sustained methane production under semi-continuous conditions.

3.3.2. *Coffee pulp mono-digestion without additives (days 85–114)*

Following stable co-digestion operation, both reactors were switched to CP slurry (Section 2.1.5) as the sole substrate while maintaining the same HRT (18 days) and OLR (2.8 g VS/L/day).

Methane production declined immediately after the substrate transition. Between days 86 and 103, methane production decreased from 633 to 236 mL CH_4/L/day in R1 and from 724 to 357 mL CH_4/L/day in R2 (Fig. 3). Methane content also decreased, reaching approximately 44% in R1 and 52% in R2 (Fig. 6).

The decline in performance coincided with substantial VFA accumulation, increasing from <200 mg/L to approximately 1564 mg/L in R1 and 1611 mg/L in R2 (Fig. 4), This indicated that acidogenic production exceeded the methanogenic conversion capacity of the reactors. Concurrently, pH declined to approximately 6.7 in R1 and 7.0 in R2 (Fig. 5), reflecting reduced buffering capacity and increasing process imbalance.

Several factors likely contributed to reactor deterioration. First, the CP feed contained a high concentration of phenolic compounds (972.9 mg GAE/L). Phenolic compounds are recognised as potential inhibitors of anaerobic digestion and have been associated with reduced

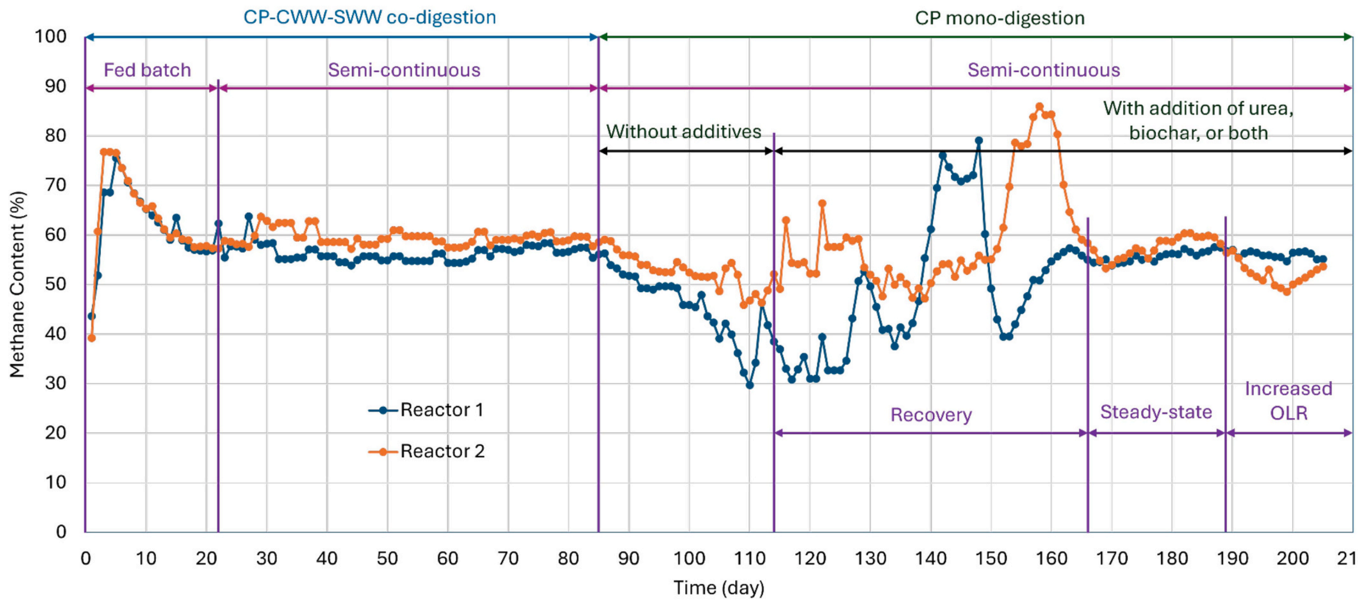

Fig. 6. Methane content profiles during reactor operation: CP, coffee pulp: CWW, coffee wastewater; SWW, sisal wastewater; OLR, organic loading rate. Methane concentrations observed during periods of very low gas production should be interpreted cautiously because analytical uncertainty may increase when gas volumes are limited.

methanogenic activity in phenolic-rich agro-industrial waste streams (Li et al., 2018; Wang et al., 1989; Zema et al., 2018). However, inhibitory thresholds vary considerably depending on substrate composition, microbial acclimation, and operating conditions. Therefore, the specific contribution of phenolic compounds to inhibition in the present study cannot be quantified directly.

Second, nitrogen availability appeared insufficient, as indicated by the elevated C/N ratio (30.9) and low TAN concentration (76.5 mg/L). Third, the CP feed contained a relatively high VFA concentration (2.1 g/L), which may have accelerated acid accumulation and contributed to imbalance during mono-digestion.

Several operational interventions were implemented to restore reactor performance, including temporary feeding suspensions and partial cross-inoculation involving approximately 22% exchange of reactor contents. However, these interventions did not result in stable recovery. By day 113, TAN concentrations remained low (56.0–64.8 mg/L), below concentrations commonly reported as sufficient to support methanogenic activity (~200–1500 mg/L) (Yenigün and Demirel, 2013). In contrast, phosphorus (245–249 mg/L) and potassium (425.0–467.5 mg/L) concentrations remained adequate to support microbial growth (Chen et al., 2008; Hussain et al., 2015).

Overall, the results indicate that nitrogen deficiency was a major limiting factor contributing to CP mono-digestion instability. This interpretation is supported by the subsequent recovery observed following urea supplementation, which improved methane production and reduced VFA accumulation. Elevated phenolic concentrations may also have contributed to inhibition; however, their relative contribution could not be separated from the effects of nutrient imbalance and process acidification. VFA accumulation likely resulted from an imbalance between acidogenic activity and methanogenic conversion capacity rather than representing the initial cause of process failure. These findings highlight the importance of adequate nitrogen availability while considering the potential contribution of phenolic compounds during CP mono-digestion.

3.4. *Coffee pulp mono-digestion with additives: recovery and stabilisation*

3.4.1. *Recovery phase (days 114–147)*

Recovery interventions commenced on day 114 (Table S2). R1 received staged urea supplementation totalling 940 mg N/L, applied with feed on day 114 and without feeding on day 115. R2 received staged biochar supplementation under the same schedule, reaching a final concentration of 5 g/L. Both reactors were subsequently operated without feeding until day 126.

During the starvation period (days 116–126), methane production remained low (Fig. 3), while VFA concentrations remained elevated (>2.0–3.5 g/L; Fig. 4). However, pH in R1 gradually increased towards neutrality (6.9–7.1; Fig. 5), whereas R2 remained acidic (5.3–5.6), indicating that nutrient limitation and accumulated organic acids continued to constrain microbial activity.

From days 127 to 131, R1 was operated at an HRT of 25 days (OLR: 2.0 g VS/L/day) with continued urea supplementation. Methane production increased to approximately 137 mL CH_4/L/day, accompanied by a reduction in VFA concentration to approximately 2.0 g/L. In contrast, R2 remained unfed but received additional urea supplementation on days 127 and 128 to achieve the same nitrogen concentration as R1 (940 mg N/L). Despite nitrogen addition, R2 exhibited limited recovery, with persistent acidification (pH 5.3–5.4) and negligible methane production.

Continued starvation from days 132 to 147 promoted gradual recovery in both reactors. Recovery was more pronounced in R1, as indicated by declining VFA concentrations, increasing pH (up to 7.7), and methane content approaching 72% (Fig. 6). In comparison, R2 recovered more slowly, reaching pH 6.5 and approximately 54% methane content by day 147. The slower recovery of R2 may have been associated with its prolonged unfed period and more severe acidification prior to recovery.

3.4.2. *Stabilisation under reduced organic loading rate (days 148–166)*

From days 148 to 166, R1 was operated at an HRT of 30 days (OLR: 1.7 g VS/L/day) with continued nitrogen supplementation. Reactor performance improved progressively, with methane production exceeding 280 mL CH_4/L/day (Fig. 3) and pH stabilising near neutrality (Fig. 5).

Following prolonged starvation, R2 was re-fed from days 159 to 166 under comparable operating conditions, with continued nitrogen supplementation and biochar addition. During this period, methane production remained lower than that of R1; however, methane content increased to approximately 86% (Fig. 6). This apparent methane enrichment should be interpreted cautiously because total biogas production remained low. Under such conditions, small variations in CO_2 concentration can substantially influence calculated methane percentages, and the observed increase may partly reflect analytical variability rather than a true change in gas composition.

3.4.3. Steady-state operation with additives (days 166–189)

From days 166 to 189, both reactors were operated at an HRT of 30 days with nitrogen supplementation (940 mg N/L), while R2 additionally received biochar (5 g/L). Stable operation was achieved in both reactors. R1 maintained a pH of 7.2 ± 0.1 (Fig. 5) and produced 343 ± 18 mL CH_4/L/day (Fig. 3), with methane content of approximately 56% (Fig. 6). Similarly, R2 maintained a pH of 7.3 ± 0.1 and produced 326 ± 26 mL CH_4/L/day with methane content of approximately 58%. VFA concentrations declined to 1406 mg/L in R1 and 963 mg/L in R2 (Fig. 4).

These results demonstrate successful restoration of reactor performance following nutrient supplementation, reduction of organic loading, and operational recovery strategies. Although R2 achieved performance comparable to R1, the reactors experienced different operational histories, including different starvation durations and recovery pathways. Therefore, the independent contribution of biochar to process recovery cannot be isolated from the effects of nitrogen supplementation and operational adjustments.

3.4.4. Performance under elevated organic loading rate (days 190–205)

When the OLR was increased to 2.0 g VS/L/day (HRT: 25 days), R1 showed further improvement, with methane production increasing to 481 mL CH_4/L/day (Fig. 3) and VFA concentrations decreasing to 588 mg/L (Fig. 4). The stable pH (7.2 ± 0.0; Fig. 5) indicated sustained methanogenic activity under the increased loading condition.

In contrast, R2 exhibited reduced process stability. Methane production decreased to 130 mL CH_4/L/day, while VFA concentrations increased to 3.5 g/L and pH declined to 6.8. Under the conditions tested, biochar supplementation was insufficient to maintain process stability at the increased organic loading rate.

3.4.5. Factors governing process recovery

The recovery observed between days 114 and 205 was most consistently associated with nitrogen supplementation, which corrected nutrient imbalance and supported restoration of methanogenic activity. The biochar used in this study possessed properties commonly associated with beneficial effects during AD, including high fixed-carbon content (89% dry basis) and specific surface area (462 m^2/g), which may support adsorption of inhibitory compounds and microbial attachment. However, these mechanisms were not directly quantified in the present study and therefore cannot be separated from the effects of nutrient supplementation and operational changes.

3.4.6. Practical implications

Co-digestion with CWW and SWW improved nutrient balance, diluted inhibitory compounds, reduced freshwater requirements, and enhanced reactor stability. In contrast, CP mono-digestion resulted in process instability characterised by nitrogen deficiency, VFA accumulation, and reduced methane production.

Urea supplementation effectively restored nutrient balance and reactor performance, demonstrating its potential as a practical nutrient correction strategy for CP digestion. Biochar may provide complementary benefits through adsorption, microbial support, buffering effects, and mechanisms potentially associated with direct interspecies electron transfer (DIET). However, its independent contribution and economic feasibility require further evaluation.

Gradual increases in OLR were essential for maintaining stability during recovery. Overall, co-digestion provides a practical waste-to-energy strategy for coffee- and sisal-producing regions, while nutrient supplementation represents a relatively simple approach for improving CP mono-digestion performance. Further techno-economic assessment is required to determine the feasibility of integrating biochar into large-scale systems.

3.5. Effects of microbial adaptation

3.5.1. Methane production from coffee pulp

Cumulative methane production profiles differed among inocula (Fig. S1a). CDI, derived from steady-state CP–CWW–SWW co-digestion CSTRs, and MDI, obtained from CP mono-digestion CSTRs, exhibited faster initial methane production than CMI, suggesting that prior exposure to CP-containing substrates enhanced early-stage microbial acclimatisation. Similar effects have been reported for adapted microbial communities, which often exhibit shorter lag phases and improved initial degradation kinetics (Hu et al., 2024; Shrestha et al., 2022).

Despite differences in initial methane production rates, cumulative methane yields converged by day 13 for CMI and CDI, whereas MDI remained slightly lower. Methane production approached a plateau between days 30 and 35, and by day 45, CMI achieved the highest final methane yield, followed by CDI and MDI. These results indicate that prior adaptation improved initial degradation kinetics but did not enhance the ultimate methane conversion potential of CP.

Low residual VFA concentrations (45 ± 13 mg/L in CMI and undetectable in CDI and MDI) and near-neutral pH values in all assays (Fig. S1b) confirmed efficient conversion of fermentation intermediates to methane. The higher methane yield obtained with CMI may be related to the established activity of manure-derived microbial consortia, which are widely used in BMP assays due to their stable methanogenic performance. Differences in functional potential, prior exposure to inhibitory compounds, and reactor history may have contributed to the lower yields observed with CDI and MDI.

The inocula were collected under different reactor conditions, which should be considered when interpreting adaptation effects. CDI originated from stable steady-state co-digestion, whereas MDI was collected on day 103 during CP mono-digestion, after methane production had declined and VFA accumulation had occurred. Therefore, the lower methane yield of MDI may reflect residual physiological stress and reduced methanogenic activity associated with its reactor history, rather than adaptation effects alone.

3.5.2. Methane production from Avicel microcrystalline cellulose

Avicel microcrystalline cellulose digestion exhibited typical sigmoidal methane production profiles for all inocula (Fig. S1c). Methane production remained low during the initial phase, with the slightly negative value observed for MDI likely resulting from measurement variability following blank correction.

Methane production subsequently increased, with cumulative methane yields for CDI and MDI approximately 65% and 40% higher, respectively, than CMI by day 13. Thereafter, CMI exhibited rapid methane production, increasing its cumulative methane yield by approximately 124% between days 13 and 18. By the end of the assay, CMI achieved the highest methane yield, followed by CDI and MDI. The methane yield obtained with CMI approached the theoretical methane potential (415 mL CH_4/g VS; Fig. S1d), indicating efficient conversion of cellulose-derived substrates to methane.

Residual VFA concentrations remained low (38 ± 10 mg/L in CMI and undetectable in CDI and MDI), while pH remained near neutral in all inocula (Fig. S1d), confirming stable digestion conditions. The lower methane yields obtained with CDI and MDI may reflect differences in the capacity of the respective microbial communities to hydrolyse and convert cellulose-derived substrates compared with the manure-derived inoculum.

4. Practical implications

Adapted inocula enhanced initial methane production kinetics but did not improve final methane yields from either CP or Avicel. This suggests that adaptation primarily influenced early-stage substrate conversion rather than the overall methane conversion potential. The results highlight the importance of maintaining active and functionally robust microbial consortia, such as those associated with manure-based inocula or stable co-digestion systems, for efficient methane production from CP.

5. Study limitations

A limitation of this study is that CP, CWW, and SWW were stored at −20 °C prior to experimentation. Although freezing is widely applied for substrate preservation, storage and freeze–thaw cycles may alter substrate characteristics by affecting cell structure, organic matter accessibility, and phenolic compound stability. Therefore, the measured methane yields and inhibition characteristics may not fully represent freshly generated substrates. Nevertheless, all substrates were characterised after storage and treated consistently throughout the study, enabling valid comparisons among treatments.

Only one reactor was operated under each recovery strategy; therefore, statistical comparisons between R1 and R2 were not performed. Differences in reactor performance should consequently be interpreted descriptively rather than as evidence of treatment effects. This limitation is particularly relevant during the recovery phase, where different operational histories, including feeding interruptions, cross-inoculation, and staged recovery interventions, prevent definitive attribution of observed differences specifically to biochar addition.

Finally, microbial community composition was not analysed. Therefore, interpretations regarding microbial adaptation, community structure, and functional potential remain inferential and require confirmation through future molecular investigations.

6. Conclusion

This study evaluated semi-continuous AD of CP through co-digestion with CWW and SWW, as well as mono-digestion enhancement strategies involving urea supplementation and biochar addition under mesophilic conditions.

Co-digestion achieved stable reactor performance, with methane production exceeding 560 mL CH_4/L/day. This performance was associated with improved nutrient balance, dilution of potentially inhibitory compounds, near-neutral pH, and low VFA concentrations. In addition, CWW and SWW provided process water and additional biodegradable organic matter, reducing the requirement for freshwater addition and improving overall process sustainability.

In contrast, CP mono-digestion without additives resulted in reduced methane production, VFA accumulation, and pH decline. Recovery following urea supplementation indicates that nitrogen deficiency associated with the elevated C/N ratio appeared to be a major constraint, although phenolic compounds may have contributed to the observed process imbalance. Feeding interruptions and cross-inoculation alone were insufficient to restore stable operation.

Biochar addition provided complementary benefits during CP mono-digestion recovery; however, its independent contribution could not be separated from the effects of nitrogen supplementation and operational changes. Furthermore, biochar addition did not prevent process deterioration when the OLR was increased under the conditions evaluated.

Microbial adaptation enhanced initial methane production kinetics but did not improve final methane yields, whereas the manure-derived inoculum achieved the highest ultimate methane production. These results suggest that inoculum history influences early substrate conversion dynamics but may not determine final methane recovery under the tested conditions.

Overall, co-digestion of CP with CWW and SWW represents an effective strategy for improving methane production and process stability, while maintaining adequate nitrogen availability is essential for successful CP digestion. Future studies using replicated reactors, controlled biochar comparisons, and microbial community analyses are required to elucidate the independent contributions of biochar and microbial adaptation.

CRediT authorship contribution statement

Seleman Mahamoud Said: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Parisa Ghofrani-Isfahani:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Revocatus Lazaro Machunda:** Writing – review & editing, Supervision, Resources, Conceptualization. **Anthony Manoni Mshandete:** Writing – review & editing, Supervision, Resources, Conceptualization. **Amelia Kajumulo Kivaisi:** Writing – review & editing, Supervision, Conceptualization. **Irini Angelidaki:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Seleman Mahamoud Said reports financial support was provided by Ministry of Foreign Affairs of Denmark. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article include Figure S1, showing methane production and pH during anaerobic digestion of coffee pulp and Avicel using different inocula, Table S1 summarising the physicochemical characteristics of the substrates used in this study, and Table S2 detailing the feeding schedule, cross-inoculation events, and additive supplementation during semi-continuous reactor operation. Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2026.135520.

Data availability

Data will be made available on request.

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