

# Optimizing plant biomass recovery from constructed wetlands within the water-energy-food nexus: Influence of harvesting time on biogas production

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## ABSTRACT

Constructed wetlands (CWs) are increasingly recognized as multifunctional Nature-based Solutions capable of simultaneously addressing wastewater treatment and biomass valorization challenges within the Water-Energy-Food nexus. However, the seasonal variability of plant biomass composition, which is intrinsically linked to species-specific phenological stages, remains poorly investigated in relation to its impact on anaerobic digestion (AD) performance. This study evaluates the biochemical methane potential (BMP) of four common CW macrophytes (*Iris pseudacorus*, *Phragmites australis*, *Carex* spp. and *Typha latifolia*) harvested from a full-scale CW at three phenological stages (spring, summer, autumn), to assess the influence of harvesting time on biogas production potential and quality. Physico-chemical characterization revealed significant seasonal shifts in dry matter content, C/N ratio, and biochemical properties of the biomasses, which translated into marked differences in methane yields and biogas composition. Autumn-harvested feedstocks exhibited the highest cumulative methane yields (up to 357 mL CH<sub>4</sub>/gVS), attributed to a favourable balance of moisture content, moderate lignification, and optimal C/N ratios. Furthermore, autumn samples showed the highest methane enrichment (up to 61% CH<sub>4</sub>), suggesting improved process stability and microbial conversion efficiency. These findings demonstrate that the timing of CW biomass harvesting is a critical parameter in optimizing AD-based energy recovery, and supports the integration of CWs within circular bioresource management strategies aimed at enhancing system multifunctionality and resource-use efficiency.

## 1. Introduction

Climate change, exacerbated by rapid population growth, projected to reach 9 billion by 2050 (Dodson et al., 2020), is intensifying pressure on natural resources, increasing the frequency of extreme weather events, and threatening food security. Among the most critical consequences is the disruption of the water cycle, leading to extreme precipitation events, prolonged droughts, and fluctuating water availability (Ingrao et al., 2023). Anthropogenic activities contribute significantly to environmental pollution and resource degradation (Dharwal et al., 2020), while the increasing demand for energy and food continues to

rise (Huang et al., 2021). Additionally, climate change is reducing the availability of arable land suitable for food production (Akpoti et al., 2022), and coupled with food waste, this exacerbates global hunger and resource scarcity (Gage et al., 2024).

Addressing these interconnected challenges requires innovative and sustainable solutions (Raihan, 2023). It is crucial to ensure food security, maintain freshwater availability and quality (Rosinska et al., 2024), reduce environmental pollution, and meet the growing energy demand (Emodi et al., 2019). The complexity of this scenario can be effectively conceptualized through the water-energy-food (WEF) nexus, which highlights the interdependencies between these sectors and underscores

**Abbreviations:** CWs, Constructed Wetlands; WEF, Water-Energy-Food Nexus; BMP, Biochemical Methane Potential; IP, *Iris pseudacorus*; PA, *Phragmites australis*; CS, *Carex* spp; TL, *Typha latifolia*; TS, Total Solids content based on fresh weight; VS, Volatile Solids content based on dry weight; COD, Chemical Oxygen Demand; TP, Total Phosphorus; C/N, Carbon-to-Nitrogen ratio; ISR, Inoculum-to-Substrate Ratio; VFA, volatile fatty acid; AD, Anaerobic Digestion; CH<sub>4</sub>, methane; CO<sub>2</sub>, carbon dioxide; H<sub>2</sub>S, hydrogen sulphide.

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the role of renewable energy in bridging agriculture, water management, and energy production (Abdi et al., 2020; Valenti et al., 2026). Given the current reliance on non-renewable or overexploited resources such as water, fossil fuels, and soil, none of which can be considered fully renewable (FAO, 2022), decisions in one sector inevitably impact the others in terms of consumption, waste generation, and pollution (Samberger, 2022).

Within this framework, the integration of Nature-based solutions (NbS) such as CW systems, biomass harvesting, and AD represents a practical application of the WEF nexus (Fig. 1). CWs contribute to water management by treating agricultural water runoff and wastewater and enabling water reuse in agricultural irrigation (Barbagallo et al., 2014), while simultaneously generating plant biomass as a by-product of the treatment process (Chioggia et al., 2024). When properly managed, this biomass can be harvested and valorized through the AD process, producing renewable energy in the form of biogas and a nutrient-rich digestate that can be used as biofertilizer (Mancuso et al., 2026; Russo et al., 2019). The recovered energy supports the energy dimension of the nexus, whereas digestate application and irrigation water reuse contribute to agricultural production, closing resource and nutrient cycles. This integrated perspective clarifies the role of biomass harvesting as a key link between water treatment, energy recovery, and food production.

In this context, growing interest from institutional and research entities in NbS is driven by their alignment with circular economy principles. In natural ecosystems, waste does not exist; each organism thrives only if its metabolism contributes to closing biological and energy cycles, where elements serve both as resources and by-products depending on the system perspective. These solutions integrate biodiverse naturalized areas into urban, rural, marine, and coastal environments through systemic, site-specific, and economically efficient interventions.

Among NbS, CWs have gained significant attention, particularly over the last decade. CWs leverage the natural self-purification capacity of wetlands, where plants, microorganisms, and soil work synergistically to treat contaminated water (Mancuso et al., 2023). Beyond their efficiency in removing pollutants before water is discharged into natural bodies, CWs have demonstrated remarkable resilience against extreme weather events, such as floods and droughts (Ferreira et al., 2023). Their adaptability extends to handling diverse pollutant loads without requiring complex engineered infrastructure, operating with low maintenance costs and minimal technical expertise (Santos et al., 2024). Furthermore, CWs can represent even a wastewater polishing step (Mancuso et al., 2023). These attributes make CWs a viable solution for

mitigating global water scarcity while reducing the energy demand associated with conventional wastewater treatment (Saqib et al., 2022).

However, to sustain their long-term treatment capacity, periodic maintenance, such as vegetation harvesting, might be often required (Ingrao et al., 2020). Harvesting prevents excessive biomass accumulation that could disrupt treatment processes (Cui et al., 2016) and facilitates the removal of pollutants sequestered in plant tissues (Vymazal, 2020), while also supporting microbial communities essential for contaminant degradation (Zheng et al., 2015). Despite their potential, harvested plant materials are frequently considered waste. In multi-functional systems, however, by-products from one process can serve as valuable resources for another. Repurposing CW biomass as an alternative energy source, rather than treating it as waste, aligns with the WEF nexus approach and with the new wastewater directive (Directive (EU) 2024/3019, 2024).

AD represents a promising valorization strategy for CW biomass, enabling the conversion of organic material into biogas; which is a renewable gaseous biofuel (Pinho and Mateus, 2023). AD involves the microbial breakdown of organic matter under anaerobic conditions, leading to the production of an energy-rich gas mixture (typically 60% methane and 40% carbon dioxide) and digestate, a nutrient-rich by-product suitable as an organic soil amendment (Wu et al., 2021). Integrating CWs for water treatment with AD of harvested vegetation offers promising opportunities to reduce energy consumption, lower greenhouse gas emissions, and close resource loops within the WEF nexus (Del Borghi et al., 2020). Several studies have already assessed the biochemical methane potential (BMP) of CW-derived biomass, investigating key parameters such as particle size (Al-Iraqi et al., 2023; Dubrovskis and Kazulis, 2012), alkaline pre-treatment and incubation time (Al-Iraqi et al., 2023), inoculum-to-substrate ratio (ISR), vegetation typology (Czubaszek et al., 2021; Roj-Rojewski et al., 2019), plant maturity and condition (Hartung et al., 2023), and chemical composition (Vasmara et al., 2023). These works have highlighted that biomass pre-treatment and optimization of process parameters significantly improve biogas yields by enhancing lignin removal and increasing cellulose accessibility. However, to the authors knowledge, seasonal variability, which might affect biomass composition (i.e., lignin, cellulose content, C/N ratio, etc.) and thus biogas productivity, has been largely overlooked. In addition, the role of plant phenological stage and long-term CW functionality in the context of biomass harvesting strategies remains underexplored.

In this context, the present study investigates the integration of CWs

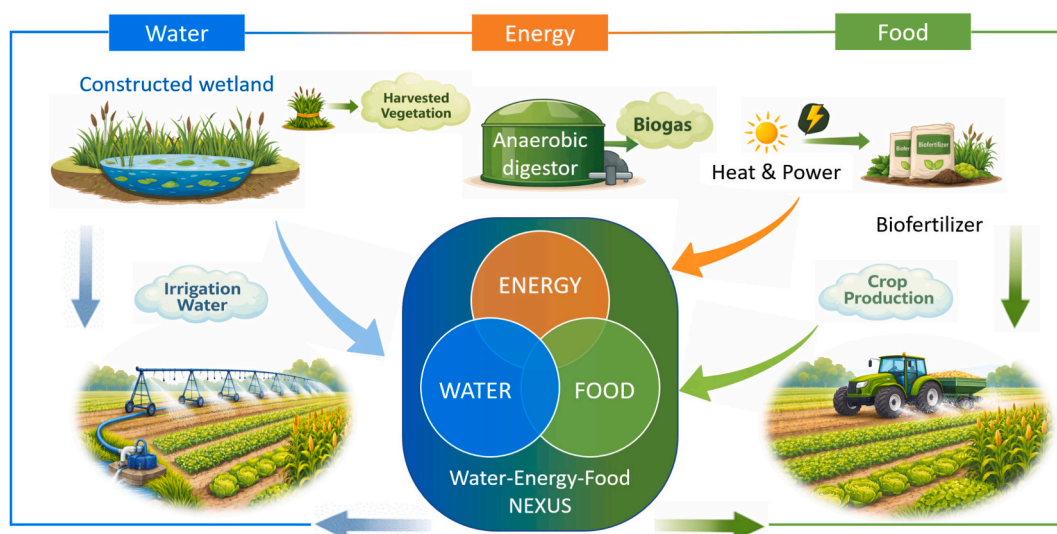


Fig. 1. Schematic overview of CW water treatment, biomass recovery, bioenergy and food production in a WEF nexus perspective.

within the WEF nexus, with particular emphasis on the valorization of plant biomass for bioenergy production. Unlike a previous study that examined the effect of storage duration (i.e., the interval between harvesting and anaerobic digestion testing) on the biogas potential of different CW plant species (Mancuso et al., 2026), this research focuses specifically on the timing of biomass harvesting itself. It evaluates how harvesting time influences AD performance, with the objective of maximizing biogas yield while ensuring the long-term ecological balance and treatment efficiency of CW systems. From this perspective, the study advances a circular and integrated approach that links the sustainable management of non-woody CW biomass with renewable energy generation, thereby contributing to the development of resilient, resource-efficient, and climate-neutral systems.

## 2. Materials and methods

### 2.1. CW biomasses and AD inoculum

Biomasses were collected from a full-scale free water surface CW system, located at the CER experimental farm (44°34'22.2"N, 11°31'45.1"E), managed by the Land Reclamation Consortium of the Emilia-Romagna region in Italy and monitored by more than 20 years (Lavrnić et al., 2020). This system treats the entire agricultural drainage water coming from the 12.5 ha experimental farm and it consists of four meanders that create a 470 m long water course with an overall surface of about 0.4 ha (Lavrnić et al., 2020). Four different plants were used as biomasses in this work, namely *Iris pseudacorus* (IP), *Phragmites australis* (PA), *Carex* spp. (CS), and *Typha latifolia* (TL). They were collected at three different periods, i.e., March 2024 ( $t_a$ ), July 2024 ( $t_b$ ) and September 2024 ( $t_c$ ), using a systematic random sampling method, from four specific locations within the CW system, as shown in Fig. 2. This approach ensured a representative and evenly distributed biomass collection across the CW system. The harvesting process involved cutting the entire aerial biomass at its base, just above the soil surface, while leaving the root systems intact to preserve the ecological balance of the wetland ecosystem.

After harvesting, the CW biomasses were chopped into small fragments of approximately 1 cm in length to facilitate the analysis of their physico-chemical properties and their use in the BMP test. To preserve their integrity, the CW biomasses were processed within ten days of collection. CW plant samples were stored under standard conditions (temperature: 20–25 °C, relative humidity: 40–60%) in a well-ventilated environment, ensuring the preservation of their chemical composition while preventing mould formation.

The AD inoculum was collected at the same three different seasonal times, from a commercial digester located in the Emilia-Romagna Region (Italy), which is fed with agro-industrial by-products. The digestate was incubated at 50 °C (14 days) for original substrate exhaustion.

Although the inoculum was collected at different seasonal times to match each biomass harvesting period, it was always sourced from the same full-scale anaerobic digester operating under stable conditions and fed with consistent agro-industrial substrates. This approach was adopted to ensure microbial activity while minimizing structural variability of the inoculum across experimental campaigns.

The CW biomasses and inoculums were characterized in terms of total solids (TS, percentage of fresh weight), volatile solids (VS; percentage of TS), carbon to nitrogen ratio (C/N) and total phosphorus (TP).

### 2.2. Biomethane potential studies

Three different experimental batteries (one for each harvesting time) were carried out to assess the BMP of the four CW-derived biomasses (IP, PA, CS and TL). Each biomass was assessed at microcosm scale, using 110 mL Pyrex bottles with a modified cap that allowed gas sampling through its septum (Fig. 3). The inoculum to substrate ratio (ISR, g of VS in the inoculum per g VS in the substrate) was >1, and the TS contents varied between 2.0 and 3.8% depending on the biomass tested as specified in Table S1 (see supplementary materials). The incubation conditions were 50 °C and 150 rpm (orbital shaker) for maintaining homogeneity. Each experimental condition was carried out in triplicate. A blank control experiment was set up (in each of the three batteries) by filling microcosms only with the inoculum, in order to determine the effective biogas production for each substrate, i.e., subtracting the amount of biogas produced by the inoculum (the control group, impossible to be perfectly exhausted) to the volume produced by each experimental group. Biogas production was measured every 1–3 days, this depending on the production stage (lag, exponential or late exponential phases) as well as weekends. Monitoring procedures were the same previously described elsewhere (Martinez et al., 2016), namely: bottles were left to achieve room temperature, biogas produced volume was determined with a glass syringe, biogas composition was analysed with the  $\mu$ -GC, the bottles were opened under nitrogen flux to keep anaerobiosis and pH was monitored. All the adopted experimental conditions were recommended by Holliger et al. (Holliger et al., 2016).

### 2.3. Analytical methods

Biogas composition (in terms of H<sub>2</sub>, O<sub>2</sub>, CH<sub>4</sub> and CO<sub>2</sub>) was determined using a gas-chromatography ( $\mu$ -GC, model 3000 A – Agilent Technologies, Milano, Italy) under the following conditions: injector temperature 90 °C; column temperature 60 °C; sampling time 20 s; injection time 50 ms; column pressure 25 psi; run time is 44 s; nitrogen as the carrier gas.

Total solids (TS) and volatile solids (VS) were measured by standard gravimetric methods (APHA, 2005), involving oven-drying at 105 °C



Fig. 2. Sampling locations of CW biomasses: 1) *Iris pseudacorus* (IP), 2) *P. australis* (PA), 3) *Carex* spp. (CS), 4) *Typha latifolia* (TL) within the CW system.

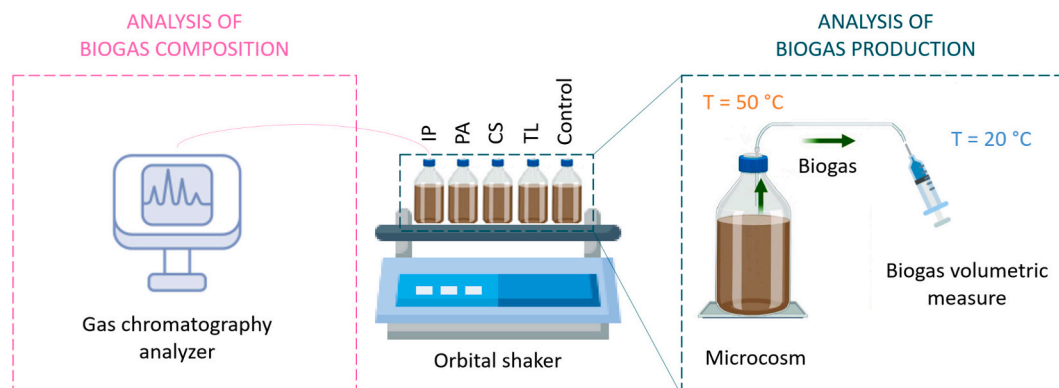


Fig. 3. Experimental setup used for the BMP test.

overnight and subsequent calcination at 600 °C for 1 h. The total carbon (C) and nitrogen (N) contents were measured in both individual CW biomasses and prepared microcosms. CW biomasses (1 cm fragments) were oven-dried and ground to a fine powder using a ball mill (MM 400, Retsch), whereas the inoculum and microcosms mixtures were freeze-dried (GFK Freezer Dryer; -40 °C, 20–2 mbar, 72 h). Elemental analysis of C and N was performed with a CHNS-O elemental analyser (Flash 2000, Thermo-Fisher Scientific). Dried and ground samples were weighed into tin capsules and subjected to flash combustion at 1000 °C in the presence of O<sub>2</sub>. Combustion gases were carried by helium (He) through an oxidation reactor containing chromium oxide and silver cobaltous oxide, ensuring complete oxidation and halogen/sulphur removal. The gas stream then passed through a reduction furnace (650 °C) containing reduced copper to convert NO<sub>x</sub> to N<sub>2</sub>. Following water removal by anhydrous magnesium perchlorate, CO<sub>2</sub> and N<sub>2</sub> were separated via gas chromatography and quantified using a thermal conductivity detector (TCD). Additionally, microcosms mixtures were analysed for chemical oxygen demand (COD) and total phosphorus (TP) with colorimetric test kits (Hach Lange and a DR3900 spectrophotometer).

### 3. Results and discussion

#### 3.1. Feedstock and inoculum characteristics

The main characteristics of each analysed feedstock (IP, PA, CS, TL)

**Table 1**  
Physico-chemical parameters of the feedstocks used in the BMP test.

Time	Parameter	IP	PA	CS	TL
(t <sub>a</sub> )	TS (%)	28.93 ± 0.53	91.14 ± 0.07	91.33 ± 0.38	91.68 ± 0.41
	VS (%)	87.19 ± 0.46	94.65 ± 0.91	93.83 ± 0.06	93.65 ± 0.95
	C/N (-)	14.12 ± 0.11	48.03 ± 1.71	19.88 ± 0.87	92.53 ± 6.78
(t <sub>b</sub> )	TS (%)	27.00 ± 1.76	53.72 ± 3.13	44.57 ± 1.60	27.07 ± 0.13
	VS (%)	89.48 ± 1.26	92.49 ± 0.49	93.36 ± 0.11	88.99 ± 0.51
	C/N (-)	39.90 ± 0.24	27.65 ± 1.41	30.39 ± 1.02	31.55 ± 0.62
(t <sub>c</sub> )	TS (%)	47.00 ± 2.75	82.96 ± 0.69	59.31 ± 1.70	60.53 ± 2.03
	VS (%)	91.82 ± 1.60	86.51 ± 0.73	92.33 ± 0.50	93.06 ± 0.18
	C/N (-)	72.57 ± 3.98	22.81 ± 1.36	63.25 ± 2.09	61.12 ± 3.17

IP = *I. pseudacorus*, PA = *P. australis*, CS = *Carex* spp., and TL = *T. latifolia*.

March 2024 (t<sub>a</sub>), July 2024 (t<sub>b</sub>) and September 2024 (t<sub>c</sub>).

Data are the average of three replicates with standard errors.

are summarized in Table 1, while Table 2 reports the properties of the inoculum.

At (t<sub>a</sub>), IP exhibited the highest moisture content among all feedstocks, as indicated by its low total solids (TS) concentration of approximately 30%. This contrasts sharply with the ~90% TS observed in the other feedstocks, highlighting IP's high-water content, which may influence microbial activity and substrate bioavailability during AD. At the same time, the carbon-to-nitrogen (C/N) ratio of PA and TL ranged between 50 and 90, whereas CS and IP showed much lower ratios, both below 20. The elevated C/N ratios in PA and TL suggest a carbon-rich composition with limited nitrogen availability, potentially leading to nitrogen deficiency and suboptimal microbial activity during AD. Conversely, the inoculum exhibited a notably low C/N ratio (<10), supporting its role as a nitrogen-rich balancing agent for carbon-heavy feedstocks (Häfner et al., 2022). Besides, the inoculum used for BMP-tests at (t<sub>a</sub>) also showed a moderate COD concentration (15.5 ± 0.3 g/L) and a TP content of 175.9 ± 3.8 mg/L, indicating the presence of readily biodegradable organic matter and available phosphorus - both critical for supporting microbial growth and metabolic activity during AD.

At (t<sub>b</sub>), the moisture content remained high for IP and TL, reflected in their TS values around 30%, while PA and CS showed higher TS values (~50%), indicating lower moisture. This variation is relevant, as moisture content influences substrate rheology mass transfer processes during AD. Regarding the C/N ratio, all feedstocks showed values between 30 and 40, falling within or closer to the optimal range for AD and suggesting improved nutrient balance for microbial degradation (Abebe, 2017). However, the inoculum continued to exhibit a C/N ratio below 10, confirming its supplementary role as a nitrogen supplier in AD batch systems. At this harvesting time, the inoculum maintained a COD concentration of 14.0 ± 0.1 g/L and a TP value of 188.6 ± 5.1 mg/L. Although slightly lower than the values observed during (t<sub>a</sub>), these levels still indicate adequate concentration of soluble organics and phosphorus to support microbial activity.

At (t<sub>c</sub>), PA emerged as the most moisture-rich feedstock, with TS slightly exceeding 80%, while the TS of other feedstocks ranged between

**Table 2**  
Physico-chemical parameters of the inoculum used in the BMP test.

Time	TS (%)	VS (%)	COD (g/L)	TP (mg/L)	C/N (-)	pH (-)
(t <sub>a</sub> )	1.99 ± 0.07	69.13 ± 1.33	15.5 ± 0.3	175.9 ± 3.8	7.11 ± 0.33	8.9 ± 0.0
	2.17 ± 0.13	65.42 ± 2.44	14.0 ± 0.1	188.6 ± 5.1	6.86 ± 0.63	8.1 ± 0.0
(t <sub>b</sub> )	3.11 ± 0.13	69.11 ± 2.12	27.5 ± 0.0	296.0 ± 5.7	10.60 ± 0.44	8.6 ± 0.0
	0.13 ± 0.07	69.11 ± 2.12	27.5 ± 0.0	296.0 ± 5.7	10.60 ± 0.44	8.6 ± 0.0

March 2024 (t<sub>a</sub>), July 2024 (t<sub>b</sub>) and September 2024 (t<sub>c</sub>).

Data are the average of three replicates with standard errors.

45% and 60%. The increased TS in PA implies a higher concentration of organic matter, potentially enhancing biogas production, showing that hydrolysis and microbial access were probably not inhibited by low water availability. The C/N ratio ( $t_c$ ) showed considerable variability across feedstocks, with many showing elevated values (up to 60–70), well beyond the optimal range for AD, indicating potential nitrogen limitations. PA, however, exhibited a lower C/N ratio ( $\sim 20$ ), making it a more balanced feedstock in terms of nutrient availability. The C/N ratio for inoculum remained consistently low also for ( $t_c$ ), confirming its secondary function as a balancing agent in AD. Interestingly, the inoculum showed a substantial increase in COD ( $27.5 \pm 0.0$  g/L) and TP ( $296.0 \pm 5.7$  mg/L), possibly due to the accumulation of soluble degradation products and nutrient release over time.

Overall, the TS content of the inoculum remained consistently below 5% across all three time-periods analysed. This consistently low TS ensures a favourable solid-liquid balance in the digester, preventing excessive viscosity and supporting efficient microbial interactions. The relatively dilute nature of the inoculum also ensures that it acts primarily as a microbial source rather than a significant contributor to the overall organic load of the system. As for the VS content, it remained relatively stable across all feedstocks throughout the study, with values around 90%, indicating a high proportion of biodegradable organic matter. In contrast, the inoculum exhibited slightly lower VS levels ( $\sim 70\%$ ), as expected for a partially digested material. This difference emphasizes the role of inoculum as a microbial carrier rather than a primary substrate.

### 3.2. Biomethane potential studies

#### 3.2.1. Biogas production with the different CW-biomasses at each harvesting time

The cumulative  $\text{CH}_4$  production trends observed at the three different harvesting times ( $t_a$ ), ( $t_b$ ) and ( $t_c$ ) are presented in Fig. 4. The BMP tests were carried out over varying durations depending on the harvesting period: 53 days for ( $t_a$ ) (Fig. 4-i), 36 days for ( $t_b$ ) (Fig. 4-ii), and 34 days for ( $t_c$ ) (Fig. 4-iii). Each test was terminated once biogas production plateaued, indicating the exhaustion of readily degradable organic matter. The highest cumulative  $\text{CH}_4$  production (up to 250 mL) was observed at ( $t_b$ ), while the lowest (up to 130 mL) was recorded at ( $t_c$ ), with intermediate values (up to 160 mL) at ( $t_a$ ). These variations can be attributed to temporal changes in the chemical composition of the CW biomasses, which are known to be strongly influenced by seasonality. Environmental factors associated with different harvesting periods can significantly affect biomass characteristics such as fibre content, lignin concentration, moisture levels, and overall biodegradability. Among the different microcosms, CS consistently exhibited the highest  $\text{CH}_4$  production. This could be attributed to a lower dry matter content and a higher proportion of readily degradable organic matter, which may have enhanced microbial activity and biogas conversion efficiency. Interestingly, CS did not exhibit the highest  $\text{CH}_4$  output during the initial stages of the BMP tests, since its early methane generation lagged behind IP and PA, but it showed a sustained and accelerated production rate during the later phases of digestion. This suggests an initial lag in microbial adaptation or a limited presence of fast-fermenting substrates.

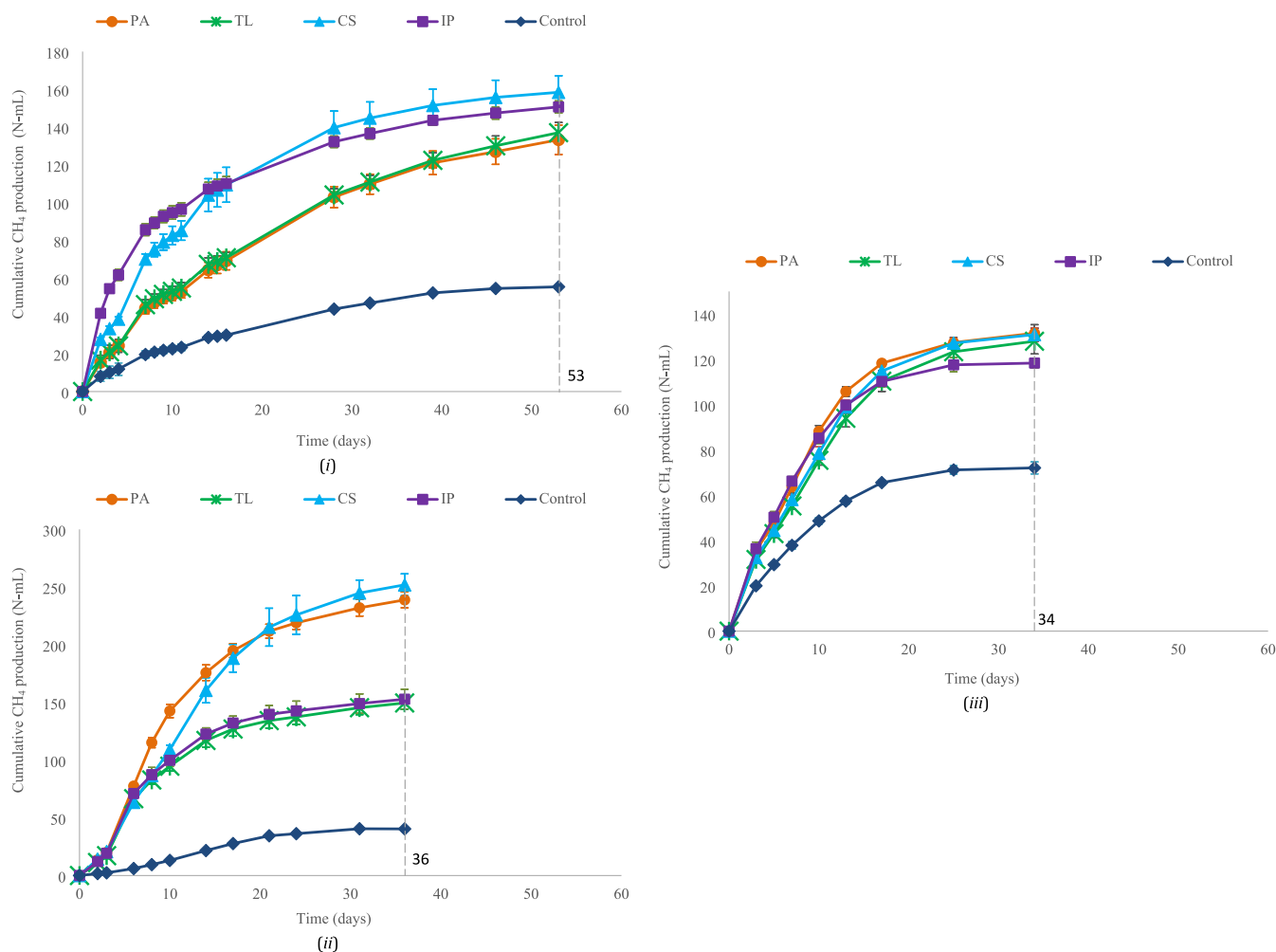


Fig. 4. Cumulative  $\text{CH}_4$  production for IP, PA, CS and TL at i) ( $t_a$ ), ii) ( $t_b$ ) and iii) ( $t_c$ ).

However, as the process progressed, composition of CS may have better supported secondary-stage microbial activity. This trend is further supported by the daily CH<sub>4</sub> production profiles at (t<sub>a</sub>), (t<sub>b</sub>) and (t<sub>c</sub>), as shown in Fig. S1, Fig. S2, and Fig. S3 (see supplementary materials). The profiles clearly indicate that all microcosms achieved peak daily CH<sub>4</sub> production during (t<sub>b</sub>), confirming this harvesting time as the most promising for maximizing methane output. Conversely, at (t<sub>a</sub>), CH<sub>4</sub> production patterns were more irregular and subdued, likely due to the presence of less accessible organic matter and suboptimal nutrient balance. At (t<sub>c</sub>), although a pronounced initial production peak was observed, this was followed by a rapid decline in daily CH<sub>4</sub> generation. This suggests that the organic content was highly biodegradable in the early stages, but became increasingly recalcitrant over time, possibly due to higher lignin content or a depletion of labile compounds. As expected, control samples without feedstock additions consistently exhibited significantly lower CH<sub>4</sub> production compared to all microcosms. This result reinforces the biogas potential of the tested CW biomasses and confirms their suitability for AD applications. The BMP results highlight the critical role of harvesting timing in determining the biomethane potential of CW-based feedstocks. The enhanced performance at (t<sub>b</sub>) suggests that a strategic harvesting schedule can significantly improve substrate quality and, consequently, biogas production efficiency. These findings support the integration of seasonality-aware planning in AD operations utilizing CW-derived biomass.

To assess the efficiency of the AD process, qualitative analyses of the biogas produced during the BMP test were performed, focusing on its CH<sub>4</sub> and CO<sub>2</sub> content. The results are reported in Fig. 5. The highest CH<sub>4</sub> concentrations across all microcosms were recorded during (t<sub>c</sub>), indicating that, despite the overall biogas productivity during (t<sub>c</sub>) was lower compared to (t<sub>a</sub>) and (t<sub>b</sub>), this period was associated with the highest biogas quality with CH<sub>4</sub> content ranging consistently between 65% and 67% across all microcosms.

This finding may initially appear unexpected, given that (t<sub>b</sub>) featured the most favourable plant characteristics for AD, including a balanced C/N ratio (approximately 20–30), moderate moisture content, and lower lignification levels, as shown in Table S1 (Supplementary Material). These features are typically associated with enhanced biodegradability and microbial activity, supporting increased CH<sub>4</sub> yields (Triolo et al., 2012).

Nevertheless, the higher CH<sub>4</sub> concentrations observed at (t<sub>c</sub>), as presented in Fig. 4, suggests that biogas quality is influenced by more than just substrate composition. Several factors may explain this phenomenon: such as improved microbial adaptation and process stability during (t<sub>c</sub>), potentially due to a longer acclimatization period of the inoculum or a shift in the microbial community toward methanogenic dominance; a possible reduction in easily fermentable substrates,

leading to slower but more consistent degradation rates, which can promote steady methanogenesis over rapid acidogenesis, resulting in a higher CH<sub>4</sub>-to-CO<sub>2</sub> ratio; and reduced variability in CH<sub>4</sub> content across all microcosms at (t<sub>c</sub>), indicating a more uniform and stable microbial response to the substrates. A potential seasonal influence of the inoculum cannot be completely excluded. All anaerobic digestion experiments were conducted using the same inoculum source; however, it is acknowledged that variations in microbial activity over time, including possible changes in viable methanogenic biomass, could introduce some degree of variability in methane production. If such an effect were significant, a progressive decrease in methane yield would be expected in the experiments performed later in time. This pattern was not observed in the present study. Instead, methane yields and biogas composition showed variations that were consistent with the harvesting period and the associated plant phenological stage. Therefore, while a minor contribution of inoculum-related variability cannot be ruled out, the results suggest that differences in feedstock characteristics were the primary factor influencing anaerobic digestion performance.

These findings reinforce the notion that biogas quality (i.e., CH<sub>4</sub> concentration) does not always correlate directly with cumulative CH<sub>4</sub> volume, but instead reflects a complex interplay between feedstock characteristics, microbial dynamics, and operational conditions.

Importantly, all microcosms demonstrated a satisfactory CH<sub>4</sub> content (>60%) throughout the BMP tests, regardless of harvesting period. This confirms the technical feasibility and reliability of CW-derived plant residues as feedstock for renewable energy production via AD. From a practical perspective, these results highlight the importance of not only maximizing biogas volume but also optimizing biogas composition, particularly when considering downstream applications such as biomethane upgrading and grid injection.

The qualitative analysis of biogas composition, summarized in Table 3, reveals key insights into CH<sub>4</sub> and CO<sub>2</sub> production dynamics across the three sampling periods and provides a more nuanced understanding of the performance of CW-derived feedstocks during AD.

At (t<sub>a</sub>), CH<sub>4</sub> represented between 52% and 59% of the biogas, with CS producing the highest CH<sub>4</sub> volume (158 ± 9 mL) and PA showing the highest methane percentage (59%). At (t<sub>b</sub>), a general increase in both total biogas volume and CH<sub>4</sub> yield was observed, particularly for CS (464 mL, 252 ± 10 mL CH<sub>4</sub>) and PA (432 mL, 239 ± 7 mL CH<sub>4</sub>), indicating more active methanogenesis. Conversely, at (t<sub>c</sub>) although the methane proportion peaked (60–61%) across all biomasses, the total volume of biogas decreased significantly, suggesting the exhaustion of the most readily degradable organic fraction. These results highlight the

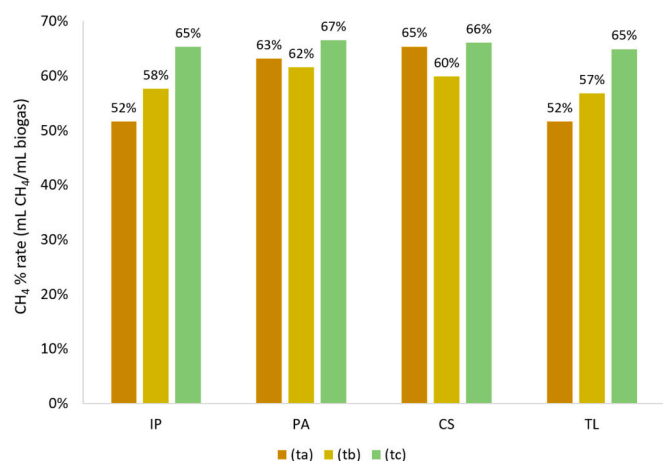


Fig. 5. CH<sub>4</sub>% rate at the end of the BMP test.

Table 3

Qualitative analysis of the biogas produced. Composition in terms of CH<sub>4</sub> and CO<sub>2</sub>.

Time	Parameter	IP	PA	CS	TL
(t <sub>a</sub> )	CH <sub>4</sub> (mL)	151 ± 3 (52%)	133 ± 8 (59%)	158 ± 9 (56%)	137 ± 5 (57%)
	CO <sub>2</sub> (mL)	137 ± 4 (48%)	93 ± 11 (41%)	124 ± 3 (44%)	102 ± 5 (43%)
	Biogas (mL)	288	226	282	239
(t <sub>b</sub> )	CH <sub>4</sub> (mL)	153 ± 9 (50%)	239 ± 7 (55%)	252 ± 10 (54%)	150 ± 6 (50%)
	CO <sub>2</sub> (mL)	152 ± 10 (50%)	193 ± 14 (45%)	212 ± 2 (46%)	151 ± 4 (50%)
	Biogas (mL)	305	432	464	301
(t <sub>c</sub> )	CH <sub>4</sub> (mL)	118 ± 2 (60%)	126 ± 8 (61%)	126 ± 9 (60%)	128 ± 6 (61%)
	CO <sub>2</sub> (mL)	79 ± 4 (40%)	81 ± 12 (39%)	84 ± 7 (40%)	82 ± 4 (39%)
	Biogas (mL)	197	207	210	210

The percentages (values in brackets) refer to the composition of CH<sub>4</sub> and CO<sub>2</sub> as a proportion of the total biogas.

dynamic behaviour of each biomass during AD, with PA and CS offering the highest methane productivity during peak fermentation.

### 3.2.2. Physico-chemical analyses of microcosms before and after the BMP test

A comparative evaluation of the physico-chemical properties of the microcosms at the beginning and at the end of the BMP test, as reported in Table 4 and Table 5, reveals notable reductions in key parameters including TS, VS, TP and COD. These decreases offer quantitative confirmation of the AD efficiency and the extent of biodegradable matter conversion into biogas.

The decline in TS and VS values across all microcosms highlights the degradation of organic material, as volatile solids are primarily composed of biodegradable compounds such as carbohydrates, proteins, and lipids. This trend confirms that methanogenic and hydrolytic microbial populations successfully utilized the feedstocks, resulting in the release of methane-rich biogas. Similarly, the reduction in COD values, which serves as an indirect indicator of the total amount of oxidizable organic matter, further validates the progression of the AD process. The consumption of COD during digestion suggests the effective transformation of soluble and particulate organic matter into methane and carbon dioxide, thus underscoring the bioconversion efficiency of the microbial consortia involved. The decrease in TP is also indicative of the mineralization and uptake processes that occurred during digestion. Although phosphorus is generally conserved in anaerobic systems, partial removal may be attributed to precipitation reactions, microbial assimilation, or conversion into less soluble forms during the AD process. In contrast, the inoculum, characterized by an initially lower COD and VS content due to its partially stabilized nature, showed minimal changes in these parameters at the beginning and at the end of the BMP test. This stability reaffirms the role of the inoculum as a microbial carrier rather than a significant source of additional organic matter, and its primary function is to catalyse and facilitate microbial degradation rather than contribute directly to biogas generation. Overall, the

**Table 4**  
Characterization of the microcosm mixtures at the beginning of the BMP tests.

Parameter	Time	IP	PA	CS	TL	Control
TS (%)	(t <sub>a</sub> )	2.87 ± 0.01	2.97 ± 0.01	2.95 ± 0.00	2.94 ± 0.01	1.99 ± 0.07
		2.98 ± 0.00	3.83 ± 0.01	3.54 ± 0.00	2.98 ± 0.00	2.17 ± 0.00
	(t <sub>b</sub> )	3.86 ± 0.25	4.57 ± 0.68	4.08 ± 0.58	4.47 ± 0.52	3.54 ± 0.11
		75.03 ± 0.04	77.71 ± 0.03	77.29 ± 0.01	77.21 ± 0.04	69.13 ± 1.33
VS (%)	(t <sub>a</sub> )	72.46 ± 0.02	77.63 ± 0.02	76.76 ± 0.02	72.33 ± 0.01	65.42 ± 0.00
		76.43 ± 1.68	77.87 ± 4.19	78.07 ± 2.23	81.01 ± 1.94	74.06 ± 2.03
	(t <sub>b</sub> )	23.2 ± 0.8	20.4 ± 0.0	20.6 ± 0.9	18.8 ± 0.9	15.5 ± 0.3
		22.7 ± 0.6	17.2 ± 0.4	22.7 ± 0.8	19.8 ± 0.9	14.0 ± 0.1
COD (g/L)	(t <sub>a</sub> )	30.9 ± 0.1	30.3 ± 0.2	31.9 ± 0.1	29.9 ± 0.0	27.5 ± 0.0
		266.0 ± 2.8	242.0 ± 2.8	296.0 ± 8.5	245.0 ± 9.9	175.9 ± 3.8
	(t <sub>b</sub> )	216.0 ± 8.5	148.0 ± 2.8	190.5 ± 4.7	199.6 ± 6.2	188.6 ± 5.1
		192.3 ± 3.0	209.1 ± 9.8	232.0 ± 0.0	210.0 ± 8.5	296.0 ± 5.7
TP (mg/L)	(t <sub>a</sub> )	8.0 ± 0.1	8.1 ± 0.1	7.9 ± 0.1	8.0 ± 0.1	8.9 ± 0.1
		8.0 ± 0.0	8.1 ± 0.0	8.1 ± 0.0	8.0 ± 0.0	8.1 ± 0.0
	(t <sub>b</sub> )	8.2 ± 0.0	8.3 ± 0.0	8.5 ± 0.0	8.4 ± 0.0	8.6 ± 0.0
		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

March 2024 (t<sub>a</sub>), July 2024 (t<sub>b</sub>) and September 2024 (t<sub>c</sub>).  
Data are the average of three replicates with standard errors.

**Table 5**  
Characterization of microcosm mixtures at the end of the BMP tests.

Parameter	Time	IP	PA	CS	TL	Control
TS (%)	(t <sub>a</sub> )	2.47 ± 0.09	2.25 ± 0.30	2.29 ± 0.51	2.17 ± 0.08	2.11 ± 0.07
		2.96 ± 0.21	2.25 ± 0.28	2.25 ± 0.31	1.88 ± 0.05	2.04 ± 0.18
	(t <sub>b</sub> )	3.45 ± 0.15	3.49 ± 0.17	3.44 ± 0.08	3.48 ± 0.11	3.48 ± 0.09
		72.61 ± 2.00	67.94 ± 0.60	71.77 ± 3.99	64.22 ± 3.46	65.12 ± 6.26
VS (%)	(t <sub>a</sub> )	55.25 ± 1.46	60.53 ± 3.09	60.63 ± 1.92	54.75 ± 3.51	60.05 ± 2.54
		73.62 ± 1.15	72.75 ± 0.80	78.42 ± 5.84	71.90 ± 0.92	74.37 ± 1.99
	(t <sub>b</sub> )	18.3 ± 0.8	18.0 ± 0.6	18.2 ± 0.2	16.7 ± 0.8	14.4 ± 0.6
		13.7 ± 0.4	11.7 ± 0.1	15.1 ± 0.1	13.9 ± 0.4	9.5 ± 0.0
COD (g/L)	(t <sub>a</sub> )	23.5 ± 0.9	25.6 ± 0.5	30.8 ± 0.5	26.9 ± 0.7	24.9 ± 0.7
		206.5 ± 7.8	187.6 ± 1.1	202.0 ± 4.5	256.8 ± 9.6	146.7 ± 1.0
	(t <sub>b</sub> )	182.4 ± 0.0	181.0 ± 3.1	193.8 ± 3.1	144.4 ± 4.5	175.4 ± 7.6
		365.2 ± 5.1	358.6 ± 5.9	374.0 ± 6.2	410.0 ± 8.5	430.2 ± 8.8
TP (mg/L)	(t <sub>a</sub> )	8.3 ± 0.0	8.0 ± 0.0	9.3 ± 0.0	8.1 ± 0.0	8.1 ± 0.0
		8.2 ± 0.0	8.2 ± 0.0	8.3 ± 0.0	8.2 ± 0.0	8.2 ± 0.1
	(t <sub>b</sub> )	9.2 ± 0.0	9.1 ± 0.0	9.2 ± 0.0	9.2 ± 0.0	9.2 ± 0.0
		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

March 2024 (t<sub>a</sub>), July 2024 (t<sub>b</sub>) and September 2024 (t<sub>c</sub>).  
Data are the average of three replicates with standard errors.

observed reductions in physico-chemical parameters across the microcosms provide compelling evidence of substrate biodegradability, microbial metabolic activity, and AD system performance. These findings support the use of CW-derived biomass as a viable and effective substrate for renewable biogas production and emphasize the importance of pre- and post-digestion analysis in assessing process efficiency and sustainability.

With particular concern to TS, the physico-chemical characteristics of the used CW might have influenced the performance of the various microcosms during the BMP test. Among all species, IP consistently exhibited the highest moisture content across all three periods. A general trend was observed across species: from spring (t<sub>a</sub>) to summer (t<sub>b</sub>), TS values decreased, reflecting active plant growth and increased water uptake. During the summer, plants typically prioritize vegetative growth over storage, resulting in higher water content and lower dry matter concentration. Conversely, from summer (t<sub>b</sub>) to autumn (t<sub>c</sub>), TS values increased due to moisture loss, structural reinforcement through lignification, and the accumulation of storage compounds as the plants matured.

The C/N ratio exhibited significant variation both within individual species across different harvesting times and between species within the same period. Notably, at period (t<sub>b</sub>), the C/N ratios for all species fell within the optimal range for AD (20–30), accompanied by moderate TS values. This balanced profile corresponded to the highest CH<sub>4</sub> yields observed, underscoring the importance of substrate composition in optimizing microbial activity and biogas production. A particularly interesting trend was identified in PA, where the C/N ratio decreased over time, an atypical deviation from the expected increase in C/N as plants mature due to lignification and carbon accumulation. This anomaly can likely be attributed to the phytoremediation setting of the study, in which CW systems treated agricultural drainage water rich in nitrogenous pollutants. PA demonstrated a remarkable nitrogen uptake and storage capacity, maintaining a relatively higher nitrogen content in its tissues than typically observed. As a result, its C/N ratio remained

elevated, likely enhancing microbial accessibility and contributing to its comparatively strong biogas potential during later harvests.

By closely examining the variations in VS reported in Fig. 6, it can be observed that the most significant variation in VS (31% for IP, 28% for PA, 27% for CS, and 32% for TL) occurred during period ( $t_b$ ). Specifically, the VS reduction at ( $t_b$ ) were 31% for IP, 28% for PA, 27% for CS, and 32% for TL. These pronounced decreases reflect a high degree of organic matter biodegradation, supporting the interpretation that CW biomasses harvested during summer ( $t_b$ ) exhibited optimal characteristics for AD, such as a favourable C/N ratio, reduced lignification, and balanced moisture content, which together enhance microbial activity and conversion efficiency. In contrast, anomalous VS trends were observed at other time points. For example, IP at ( $t_a$ ) and CS at ( $t_c$ ) showed zero or even negative variations in VS. A negative variation, indicating an increase in VS after digestion, which is atypical, as VS levels are generally expected to decrease as microbes break down organic matter during AD. Possible explanations include the incomplete degradation of recalcitrant compounds, such as lignin-bound organics, which might have remained unprocessed during initial digestion and were subsequently solubilized or chemically transformed into more detectable VS fractions during post-digestion sampling. Another contributing factor could be microbial biomass accumulation. At ( $t_a$ ), the CW biomass was characterized by high moisture content, low carbohydrate levels, and relatively higher nitrogen availability, which may have promoted excess microbial growth. The resulting biomass could contribute to a net increase in measured VS, giving the appearance of a negative degradation trend. During the late-season harvesting period ( $t_c$ ), the VS reductions were generally more modest: IP exhibited a 4% decrease, PA a 7% decrease, and TL a 13% decrease. These moderate reductions suggest a less efficient digestion process compared to ( $t_b$ ), potentially due to the higher C/N ratios, increased fibre and lignin content, or reduced microbial accessibility of the organic matter in CW biomass harvested in autumn. These compositional changes can impair hydrolysis and limit substrate availability, thus diminishing overall biogas conversion efficiency. Taken together, the data underscore the critical role of harvesting time and substrate composition in shaping the digestibility and biodegradation dynamics of CW-derived feedstocks. The clear correlation between high VS reduction and elevated methane output at ( $t_b$ ) confirms the suitability of summer-harvested CW for maximized AD performance.

### 3.2.3. Comparison of the harvesting time for each CW-biomass

The BMP-test for the four biomasses was assessed at three different harvesting times ( $t_a$ ), ( $t_b$ ) and ( $t_c$ ) with the aim of determining its influence on methane yield and identifying the biomass that produced the highest amount of CH<sub>4</sub>. The results for each biomass are presented in

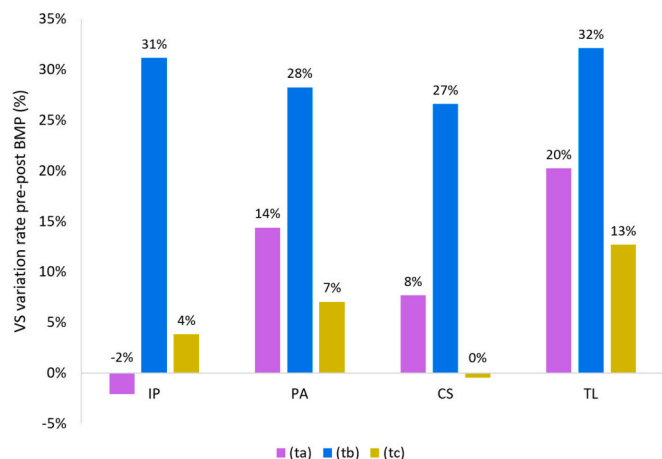


Fig. 6. VS variation rates before and after the BMP test.

Fig. 7.

CH<sub>4</sub> yields were markedly influenced by the harvesting time. In all cases, a progressive increase in CH<sub>4</sub> production was observed from the earliest harvest ( $t_a$ ) to the latest ( $t_c$ ), suggesting a strong correlation between plant maturity and biomethane potential. IP consistently outperformed the other species (Fig. 7-i), achieving a peak yield of 357 mL/gVS at ( $t_c$ ), compared to 189 mL/gVS and 233 mL/gVS at ( $t_a$ ) and ( $t_b$ ), respectively. CS also showed substantial CH<sub>4</sub> production (Fig. 7-iii), reaching 185, 254, and 327 mL/gVS across the three time points. In contrast, PA (Fig. 7-ii) and TL (Fig. 7-iv) exhibited lower yields overall, though they too followed an upward trajectory: PA increased from 137 mL/gVS ( $t_a$ ) to 251 mL/gVS ( $t_c$ ), while TL rose from 146 to 331 mL/gVS over the same interval. These results highlight not only species-specific differences in biodegradability but also emphasize the importance of harvesting time, with later harvesting seemingly enhancing substrate quality, likely due to increased lignocellulosic breakdown and accumulation of fermentable matter during plant senescence.

To better contextualize the energetic relevance of CW-derived biomasses, the methane yields observed in this study (137–357 mL CH<sub>4</sub>/gVS, depending on species and harvesting period) were compared both with values reported in the literature for both conventional agricultural residues and wetland plant species commonly used in CWs. With respect to conventional agricultural feedstocks, crop residues such as corn stover, wheat straw, and rice straw typically exhibit methane yields in the range of 241 and 281 mL CH<sub>4</sub>/gVS, with an average value of approximately 256 mL CH<sub>4</sub>/gVS, whereas manure-based substrates generally show lower biochemical methane potential values (173–250 mL CH<sub>4</sub>/gVS) (Lallement et al., 2023). Within this context, the highest-performing CW biomasses identified in the present study, such as IP harvested in autumn with methane yields up to 357 mL CH<sub>4</sub>/gVS, are comparable to or exceed those of widely used crop residues. In contrast, lower-performing cases (e.g., PA harvested in early seasons) fall within ranges typically associated with more recalcitrant agricultural substrates.

When compared with literature data for the same or closely related wetland plant species, the methane yields obtained in this study are generally consistent with, and in some cases higher than, previously reported values. For PA, biomethane yields reported in the literature typically range from approximately 140 to 260 mL CH<sub>4</sub>/gVS, depending on genotype, nutrient availability, and harvest timing (Baute et al., 2018; Eller et al., 2020). Similarly, methane yields for Typha species are commonly reported between 140 and 250 mL CH<sub>4</sub>/gVS, with higher values often associated with favourable nutrient conditions or optimized harvest strategies (Eller et al., 2020). These ranges are consistent with the values measured for e.g., PA and TL in the present study, while the highest yields obtained for selected species and harvesting periods approach or exceed the upper bounds reported for wetland biomass under comparable experimental conditions.

Fig. 8 shows the total CH<sub>4</sub>, expressed as mL CH<sub>4</sub> /gVS, for each biomass across the three harvesting periods. IP exhibits a marked increase in CH<sub>4</sub> yield during ( $t_c$ ), exceeding 350 mL CH<sub>4</sub>/gVS. This result likely reflects more favourable AD conditions during this period, potentially linked to microbial activity or greater inoculum efficiency, as suggested by the methane production trends shown in Fig. 6-i, in comparison to ( $t_a$ ) and ( $t_b$ ). PA, on the other hand, consistently recorded the lowest CH<sub>4</sub> yields across all harvesting periods, suggesting that PA may be a less suitable substrate for methane production. This could be attributed to its unfavourable chemical composition (Table S1). Both CS and TL show a similar trend, with CH<sub>4</sub> yields in ( $t_c$ ) surpassing 300 mL CH<sub>4</sub>/gVS, reaching values comparable to peak performance of IP. These results highlight that period ( $t_c$ ), corresponding to autumn harvest, was associated with the highest methanogenic potential across all biomasses evaluated. Interestingly, these findings challenge the common assumption that biodegradability declines at later phenological stages due to increased lignification and the accumulation of recalcitrant compounds. Instead, the data suggest that other factors, such as microbial

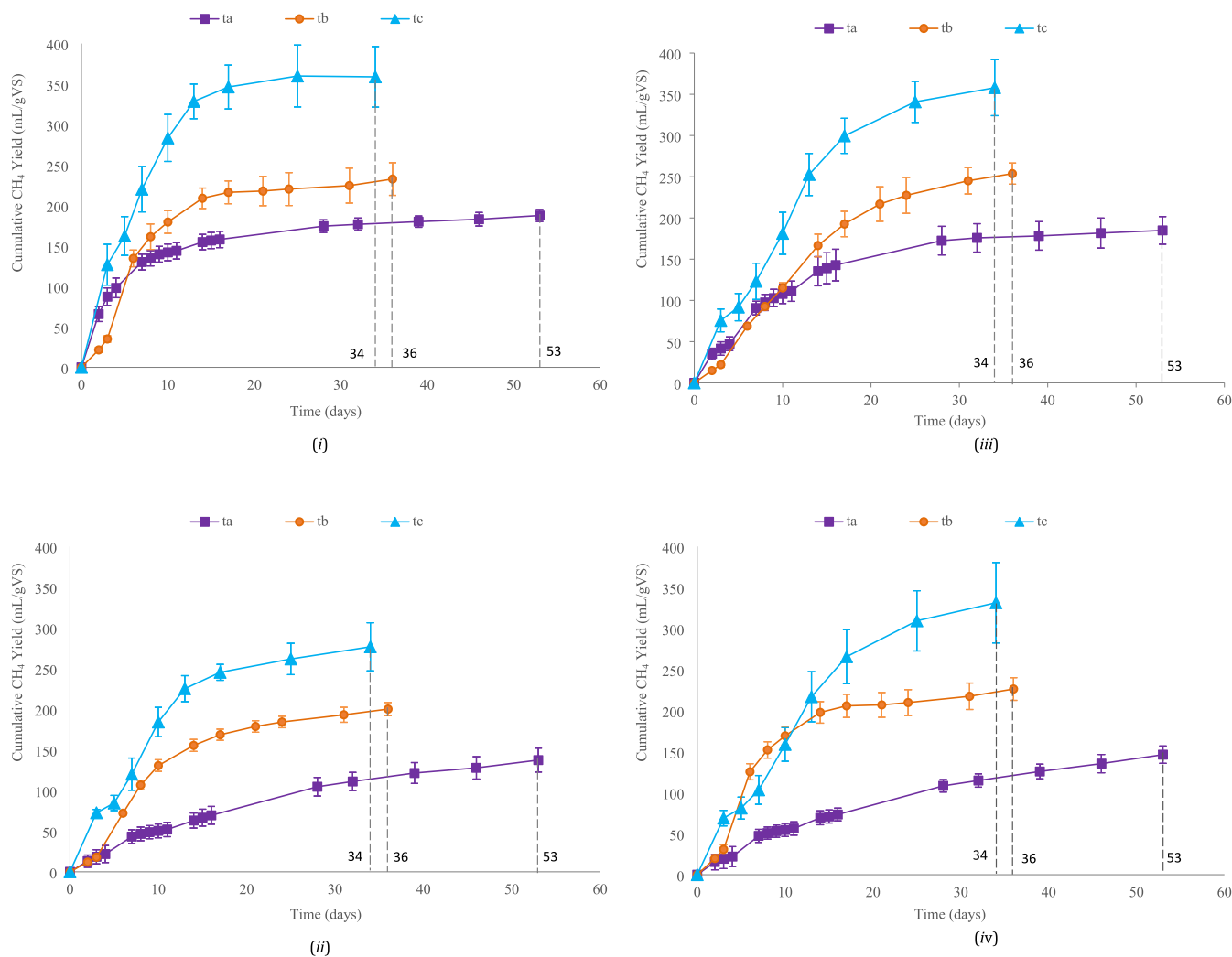


Fig. 7. CH<sub>4</sub> yields for i) IP, ii) PA, iii) CS and iv) TL at different harvesting times (t<sub>a</sub>), (t<sub>b</sub>) and (t<sub>c</sub>).

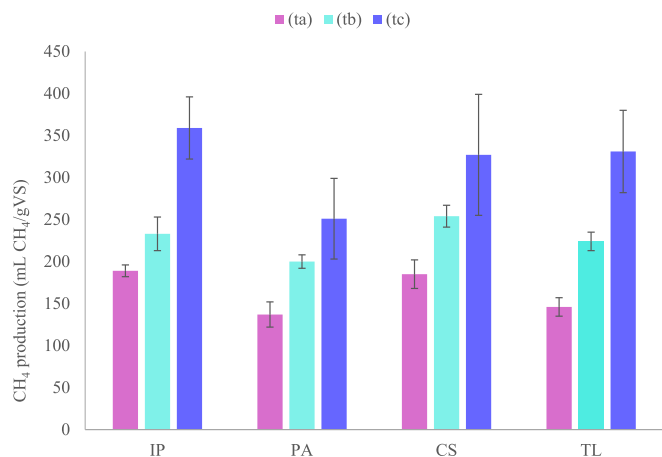


Fig. 8. Total CH<sub>4</sub> production in the BMP test.

community adaptation, substrate preconditioning, or changes in biochemical accessibility, may have contributed to enhanced biogas yield and quality during the late-season harvest.

#### 4. Conclusions

This study emphasizes the critical interactions between NbS, bio-energy production, and sustainable resource management within the broader framework of the WEF nexus. In particular, it highlights the critical influence of harvesting time on the AD performance of CW-derived biomass with direct implications for optimizing renewable energy generation. The results demonstrate that autumn-harvested biomass yielded the highest cumulative methane (CH<sub>4</sub> / gVS) production with the highest CH<sub>4</sub> concentration, reflecting superior degradability and suitable physico-chemical characteristics such as balanced C/N ratios, moderate lignification, and adequate moisture content. This suggests that seasonal shifts not only affect biomass quantity but also biogas quality, and that substrate characteristics (such as nitrogen uptake dynamics, fibre composition, and microbial accessibility) are key determinants of AD efficiency. The integration of CWs with bioenergy recovery systems offers a promising approach for aligning with the principles of the circular economy, enabling sustainable wastewater treatment, nutrient recovery, and decentralized renewable energy generation. By tailoring harvesting schedules to plant phenological stages, the multifunctionality and resource efficiency of CW systems can be significantly enhanced, supporting their role in climate-resilient, low-carbon infrastructure.

Despite these promising outcomes, the large-scale implementation of seasonal biomass harvesting in CWs may involve logistical and ecological constraints that should be carefully considered. From an operational

perspective, harvesting activities depend on site accessibility, machinery availability, labour requirements, and seasonal weather conditions, which may limit the feasibility of strictly defined harvesting windows. Moreover, intensive or poorly timed biomass removal may affect wetland biodiversity, habitat provision, and plant-mediated ecosystem functions, with potential implications for long-term treatment performance. Consequently, harvesting strategies should aim to balance bioenergy recovery with the preservation of ecological integrity and treatment efficiency, for instance through partial, rotational, or adaptive harvesting approaches.

Future research should further investigate the microbial and techno-economic aspects of CW-AD systems to improve understanding and scalability. In conclusion, this study illustrates that synchronizing CW management practices with renewable energy goals not only enhances AD performance but also reinforces the broader sustainability of NbS systems. Such alignment has the potential to unlock new synergies within the WEF nexus, contributing to the development of more adaptive, circular, and resource-efficient ecosystems in both urban and rural contexts.

### CRedit authorship contribution statement

**Giuseppe Mancuso:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Data curation. **Nico Bolognini:** Methodology, Investigation, Formal analysis, Data curation. **Gonzalo Agustin Martinez:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Data curation. **Emma Jones:** Methodology, Investigation, Formal analysis, Data curation. **Akarsh Swamilingappa Annaiah:** Methodology, Investigation, Formal analysis, Data curation. **Attilio Toscano:** Visualization, Supervision, Resources, Project administration, Funding acquisition. **Francesca Valenti:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Conceptualization.

### Declaration of competing interest

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

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecoleng.2026.107945>.

### Data availability

Data will be made available on request.

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