



Biogas Production from Anaerobic Digestion of *Phragmites australis*: Influence of Grinding Process, Harvesting Season and Co-digestion with Food Waste

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Abstract

This study investigated the effect of harvesting time and using the co-digestion strategy on the digestibility of *Phragmites australis* biomass and methane production. The *P. australis* biomass harvested from summer, autumn, and winter was digested singly (mono-digestion) and combined with synthetic food waste (co-digestion) at an inoculum to substrate ratio 1:1 based on the volatile solid content of inoculum and substrate and under mesophilic conditions (37 ± 1 °C). The results found that cumulative methane production was low during mono-digestion of summer, autumn, and winter *P. australis* (36.26 ± 4.48 , 35.59 ± 3.08 , and 34.90 ± 4.40 mL/g VS added, respectively) due to the effect of lignin on the biodegradability of *P. australis*. This issue was addressed by co-digestion of *P. australis* biomass from these three seasons with synthetic food waste, which resulted in an increase in cumulative methane production (135.66 ± 1.14 , 137.33 ± 3.21 , and 123.94 ± 1.01 mL/g VS added, respectively).

Keywords Harvesting time · Co-digesting · *Phragmites Australis* · Synthetic food waste

Introduction

In recent years, perennial herbaceous plants have gained increased attention for use as feedstock in anaerobic digestion units for production of biogas, a mixture of gases largely made up of methane (CH₄) and carbon dioxide (CO₂) that is used as a renewable energy source [1]. The main benefits of utilising herbaceous plants, such as common reeds (*Phragmites australis*), silvergrass (*Miscanthus spp.*), switchgrass (*Panicum virgatum*) and grass silages, for biogas production are their wide distribution and natural growth with high biomass yields throughout the year. These characteristics mean that energy needed for cultivation can be saved, the use of fertilisers and pesticides can be avoided, and the plants can be cultivated on non-arable and marginal

lands, so they do not compete with food and fodder crops for arable land [2–4].

Generally, the quality of herbaceous plants as a feedstock for anaerobic digestion is influenced significantly by the grass composition, which is diverse seasonally [5]. The vegetative mass of grass and their cell contents of proteins, lipids and sugars increase in the early stages of these plants' growth (often in spring to early summer), then decreases with increasing maturity (late summer to end of winter) and is offset by an increase in the percentage of cell wall components made up of cellulose, hemicellulose and lignin [6]. However, to obtain high methane production, plant biomass needs to be rich in readily degradable contents such as carbohydrates, proteins, and lipids, as well as having low lignin content [7, 8]. Hence, the effectiveness of methane production from these plants is strongly dependent on harvesting time [5].

To minimize the recalcitrance to degradation of the plants' lignocellulosic biomass during anaerobic digestion and increase methane production, the grinding process has been suggested by many researchers [9–13]. The main benefit of grinding process is the absence of the need to add external chemicals or microorganisms [14].

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Generally, grinding technique can deliver approximately 60% increases in biogas production [15, 16]. The grinding process can be carried out using different types of mills, such as knife mills, ball mills, hammer mills, disk mills, vibratory mills, colloid mills and centrifugal mills [17, 18]. The choice of milling equipment depends mainly on the type of feedstocks, required final particle size, and biomass moisture content [19]. For example, colloid mills are suitable to use with wet biomass (moisture contents 15–25%), whilst knife and hammer mills are preferred for use with dry biomass (moisture contents 10–15%) [19]. The implementation of grinding leads to a reduction in the particle size of the substrate; an increase in pore size and particle surface area – which leads to an increase in their accessibility for hydrolysis enzymes; and decreases in cellulose crystallinity and polymerisation grade by dissociation of the bonds between lignin, hemicellulose and cellulose, which leads to increased cellulose and hemicellulose availability to microorganisms, thus maximising the substrate degradation and optimising biogas production [20–22].

Moreover, biogas production from lignocellulosic biomass can be further improved by co-digesting it with other substrates [23, 24]. Co-digestion of two or more substrates can provide the appropriate nutrient composition required by microorganisms, thus enhancing anaerobic digestion performance. The co-digestion of carbon-rich lignocellulosic substrates with nitrogen-rich substrates such as food waste and animal manure helps to achieve an appropriate carbon-to-nitrogen (C: N) ratio, which is one of the critical factors that control the stability of the anaerobic digestion process [25, 26]. The low nitrogen content at a high C: N ratio negatively affects microbial growth and consumption of volatile fatty acids (VFAs), thus affecting biogas production [27–29]. In contrast, low C: N ratios lead to increased ammonia/ammonium concentrations generated from nitrogen reduction resulting in toxic effects on microorganisms and thus system inhibition or even failure [30, 31].

As far as the authors are aware, the current study uniquely explores the influence of a broad range of process parameters, including pH, alkalinity, total solids, volatile solids, VFAs, ammonia, cellulose, lignin, crude carbohydrates, crude proteins, and crude lipids, on the anaerobic digestion performance and methane production from mono-digestion and co-digestion of *P. australis* with food waste. The specific aims of the present study were to investigate the effect of (1) different harvesting times on *P. australis* cell composition and biogas production and (2) pre-treatment (grinding process) and co-digestion of *P. australis* with synthetic food waste on the organic matter removal efficiency and improving methane production. The investigation was performed through batch studies.

Materials and methods

Substrates and Inoculum

The *P. australis* samples were collected from Forrest Hills Lake, Lancaster, United Kingdom (54.007°N, 2.772°W). Sampling was conducted in three seasons: late summer (August), autumn (October), and winter (December). The *P. australis* plant samples were harvested manually using scissors, the aboveground part of the *P. australis* plants was cut at around 5–10 cm above the ground, and then the leaves and flowers were removed. The remaining stalks were packed in sealed plastic bags and taken to the laboratory. The food waste substrate used in this study was simulated and prepared in the laboratory based on the UK household food waste components data from the Waste and Resources Action Programme (WRAP) [32]. The prepared synthetic food waste (SFW) was stored in a freezer at -20 °C until use. The inoculum was obtained from a mesophilic (40 °C) farm-scale anaerobic digester (Cockerham Green Energy Limited, Lancaster, UK). The inoculum was passed through a 1 mm sieve to remove coarse particles, poured into a plastic jerrycan, and transported to the laboratory for use in the experiments. The characteristics of the inoculum, *P. australis* and synthetic food waste are displayed in Table 1.

Pre-treatment (grinding process)

The collected *P. australis* stalks from each season were manually cut into 1–3 cm pieces with scissors and then ground using a ring mill to obtain a powder with particle size < 0.4 mm. The powder was dried in an oven at 65 °C for 24 h and stored in sealed plastic bags until use.

Experiment Setup

A batch experiment was conducted using continuously stirred reactors (37 rpm) with a total capacity of 1 L and a working volume of 500 mL. Inoculum and substrates (ground *P. australis* from summer, autumn, and winter seasons, and synthetic food waste) were added into the reactors at an inoculum-to-substrate ratio (ISR) of 1:1 calculated based on the mass of volatile solid content (g VS of inoculum/g VS of substrates). For the substrate mixture, a ratio of 75:25 (synthetic food waste to pre-treated *P. australis* from each season) was used to prepare a set of co-digestion reactors. Additional reactors were prepared using synthetic food waste only and pre-treated *P. australis* from each season only, as controls. Duplicate reactors were prepared for each substrate, along with two additional blank reactors containing inoculum and deionised water only, serving as controls. After loading the inoculum and

Table 1 Inoculum, synthetic food waste and ground *P. australis* characteristics (mean±standard deviation)

Parameter	Inoculum	Synthetic food waste	Summer <i>P. australis</i>	Autumn <i>P. australis</i>	Winter <i>P. australis</i>
pH	8.61±0.00	5.46±0.00	7.00±0.02	7.00±0.01	7.00±0.01
TS (%)	6.20±0.02	18.83±0.02	97.61±0.12	98.25±0.11	98.20±0.36
VS (% dry basis)	63.11±1.22	95.87±0.04	99.54±0.17	97.63±0.01	97.59±0.43
VS (% wet basis)	3.91±0.07	18.05±0.01	97.16±0.28	95.92±0.10	95.63±0.78
Carbohydrates (% wet basis)	0.57±0.00	11.18±0.12	90.09±0.14*	87.90±0.28*	87.24±0.14*
Proteins (% wet basis)	1.60±0.03	3.20±0.02	4.49±0.07*	3.48±0.09*	2.70±0.04*
Lipids (% wet basis)	0.71±0.02	2.65±0.02	0.99±0.01*	0.90±0.01*	0.87±0.01*
Total VFAs (g/l)	0.28±0.01	3.20±0.13	ND**	ND	ND
Total ammonia-nitrogen (mg/l)	4218.50±14.43	145.44±0.43	ND	ND	ND
Partial alkalinity (as, g CaCO ₃ /l)	21.81±0.16	0.00	ND	ND	ND
Intermediate alkalinity (as, g CaCO ₃ /l)	4.63±0.10	2.05±0.07	ND	ND	ND
Total alkalinity (as, g CaCO ₃ /l)	27.07±0.18	2.94±0.06	ND	ND	ND
Cellulose (% dry basis)	9.53±0.10	3.28±0.17	48.93±0.01	48.66±0.10	48.43±0.14
Hemicellulose (% dry basis)	ND	ND	27.31±0.06	25.24±0.10	24.84±0.11
Lignin (% dry basis)	12.57±0.14	6.38±0.28	8.99±0.03	10.29±0.07	10.63±0.04
C (% dry basis)	29.33±0.25	39.20±0.14	44.62±0.75	47.16±0.08	49.44±0.06
N (% dry basis)	3.89±0.06	3.11±0.01	0.58±0.01	0.50±0.00	0.50±0.01
C/N	7.55	12.62	77.60	94.31	99.87

* % dry basis

** ND- Not determined

Table 2 Batch experimental setup - Amount of inoculum, *P. australis*, synthetic food waste and deionised water added to the reactors

Conditions	Inoculum (g wet mass)	Treated <i>P. australis</i> (g dry mass)	Synthetic food waste (g wet mass)	Deionised water (g)
Inoculum (blank)	410.97	0.0	0.0	89.03
Mono-digestion of pre-treated summer <i>P. australis</i> (control)	410.97	16.14	0.0	72.88
Mono-digestion of pre-treated autumn <i>P. australis</i> (control)	410.97	16.46	0.0	72.57
Mono-digestion of pre-treated winter <i>P. australis</i> (control)	410.97	16.47	0.0	72.56
Mono-digestion of SFW (control)	410.97	0.0	89.03	0.0
Co-digestion of pre-treated summer <i>P. australis</i> with synthetic food waste	410.97	4.04	66.77	18.22
Co-digestion of pre-treated autumn <i>P. australis</i> with synthetic food waste	410.97	4.11	66.77	18.14
Co-digestion of pre-treated winter <i>P. australis</i> with synthetic food waste	410.97	4.12	66.77	18.14

substrates into the reactors, the total volume was adjusted to 500 mL by adding deionised water (Table 2). The reactors were sealed and flushed with nitrogen gas (N₂) for two minutes to remove oxygen and establish anaerobic conditions. Finally, the reactors were placed in a water bath at 37±1 °C (mesophilic conditions) and connected to gas counter units, which were connected to 5 L gas bags and a data acquisition system (DAS).

Analytical Methods

A series of analyses was conducted on the initial and final samples of inoculum, substrates and digestate to measure pH, total solids (TS), volatile solids (VS), alkalinity, carbohydrates, proteins, lipids, total ammonia-nitrogen (TAN), carbon and nitrogen elements (C&N), volatile fatty acids (VFAs), and fibre analysis (cellulose, hemicellulose, and lignin). The pH was measured using a pH

meter (METTLER, Switzerland), TS and VS contents were determined based on standard methods [33]. The alkalinity parameters, including partial, intermediate and total alkalinity, were measured using an auto digital titrator (Orion Star T910, Thermo Scientific), following the method of Ripley et al. [34] and Wu et al. [35]. The crude carbohydrates were determined using a spectrophotometer following Dubois et al. [36]. The crude proteins were estimated following Lowry et al. [37] method. The crude lipids were measured using the Bligh and Dyer [38] method. Total ammonia-nitrogen was measured according to the method used by Gandhi et al. [39] using a SEAL Auto-analyser AA3. The C and N elements were determined using an elemental analyser (Vario EL III). VFAs C2-C8 were measured following Gandhi et al. [39] using gas chromatography (GC, Agilent Technologies 7820 A), with hydrogen as a carrier gas at a flow rate of 1.51 mL/min. The GC device was equipped with a G4567A autosampler, 30 m×0.25 mm×0.25 µm DB-Wax ultra-inert

column and a flame ionisation detector (FID). The injection port and FID detector work at operational temperatures of 280 °C. The column oven was set up at an initial temperature of 120 °C for 2 min, then ramped to 140 °C at a rate of 5 °C/min with a hold time of 3 min, followed by a ramp to 200 °C at a rate of 20 °C/min and hold time of 2 min, and then a temperature ramp to 250 °C at a rate of 20 °C/min which was then maintained for 5 min. The cellulose, hemicellulose, and lignin were determined according to the Van Soest et al. [40] method by analysis of neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) using a FOSS Fibertec™ 8000 Fibre Analyser.

The daily biogas production volume was obtained from the DAS unit that automatically recorded the gas counters' measurements. The counters were manufactured by CJC Labs Ltd., UK, and comprise of small square units filled with water to a specific level containing sensors and small tipping buckets designed to hold 6 ml of gas each. These counters are connected to the reactors, DAS unit, and gas bags, so when the biogas is released from reactors, it will enter the counters and fill their buckets, causing them to tip and release the gas when they reach their 6 ml capacity, which is then collected in the gas bags. The bucket tipping is sensed by sensors and recorded on the DAS unit screen, each single tipping count thus indicating 6 mL of biogas detected. The composition of biogas was determined at intermittent intervals (1, 3, 6, 9, and 21 days) using a gas analyser (Biogas 5000 - Geotech, England).

The cumulative biogas (methane and carbon dioxide) produced from each reactor was measured at ambient temperature and pressure and adjusted to standard temperature and pressure (273.15 K and 1 bar, respectively) using the Ideal Gas Law, and expressed in mL/g VS added (VS of inoculum + VS of substrate added). The specific methane yield (SMY) was calculated by subtracting the cumulative methane of inoculum from the cumulative methane of treatment divided by VS mass of substrate added to the treatment, and it is expressed as mL/g VS of substrate added.

The synergistic effect of co-digestion was determined based on the method used by Cheng and Zhong [41]. The synergistic effect could be represented as an extra methane yield for co-digestion substrates, exceeding the weighted average of specific methane yields obtained from each substrate. While the effect indicates antagonism when methane yield in the co-digestion substrates is lower than a weighted average of the individual substrate's specific methane yield. The weighted specific methane yield (weighted SMY) was calculated as follows [41–43]:

$$\text{weighted SMY} = (\text{SMY}_{P. australis} R_{P. australis} + \text{SMY}_{\text{SFW}} R_{\text{SFW}}) / (R_{P. australis} + R_{\text{SFW}}).$$

Where the:

- (1) weighted SMY represents the weighted average of specific methane yield for co-substrates.
- (2) $\text{SMY}_{P. australis}$ and SMY_{SFW} refer to the specific methane yields for *P. australis* and synthetic food waste.
- (3) $R_{P. australis}$ and R_{SFW} are the VS mass for *P. australis* and synthetic food waste in the mixtures of co-digestion, respectively.

Statistical Analyses

The variance between values of the treatments in terms of biogas (methane and carbon dioxide) production, specific methane yield and macromolecules degradation were analysed statistically using the One-way Analysis of Variance (ANOVA) test and Games-Howell post hoc multiple comparisons test at a confidence level of 95%, p -value < 0.05 was considered statistically significant. Moreover, an unpaired t-test for unequal variances was used to analyse differences between initial and final conditions. These analyses were conducted using IBM SPSS Statistics software V.27.

Results

Cumulative Biogas, CH₄, and CO₂ Production

After 21 days of batch digestion, the cumulative biogas, CH₄ and CO₂ production (mL/g VS added) varied significantly depending on the type of digestion and harvest season of *P. australis*. Mono-digestion (single substrate digestion) of *P. australis* produced significantly lower ($p=0.0001$) cumulative biogas than that produced from co-digestion of *P. australis* with synthetic food waste and mono-digestion of synthetic food waste (Fig. 1a, c, and e). Furthermore, the cumulative production of biogas from co-digestion of summer and autumn *P. australis* with synthetic food waste was significantly higher ($p=0.001$) than that produced from co-digestion of winter *P. australis* with synthetic food waste (Table 3). However, no significant differences ($p=1.00$) in cumulative biogas production were detected among the mono-digestions of summer, autumn, and winter *P. australis*. Generally, the mono-digestion of synthetic food waste showed the highest cumulative biogas production of all the systems.

Figure 1b, d and f showed the daily production patterns of biogas, CH₄ and CO₂ from all conditions over the 21-day of digestion period. It can be seen that the mono-digestion of synthetic food waste and the co-digestion of *P. australis* with synthetic food waste show higher biogas production rates than the mono-digestion of *P. australis* through the whole digestion period.

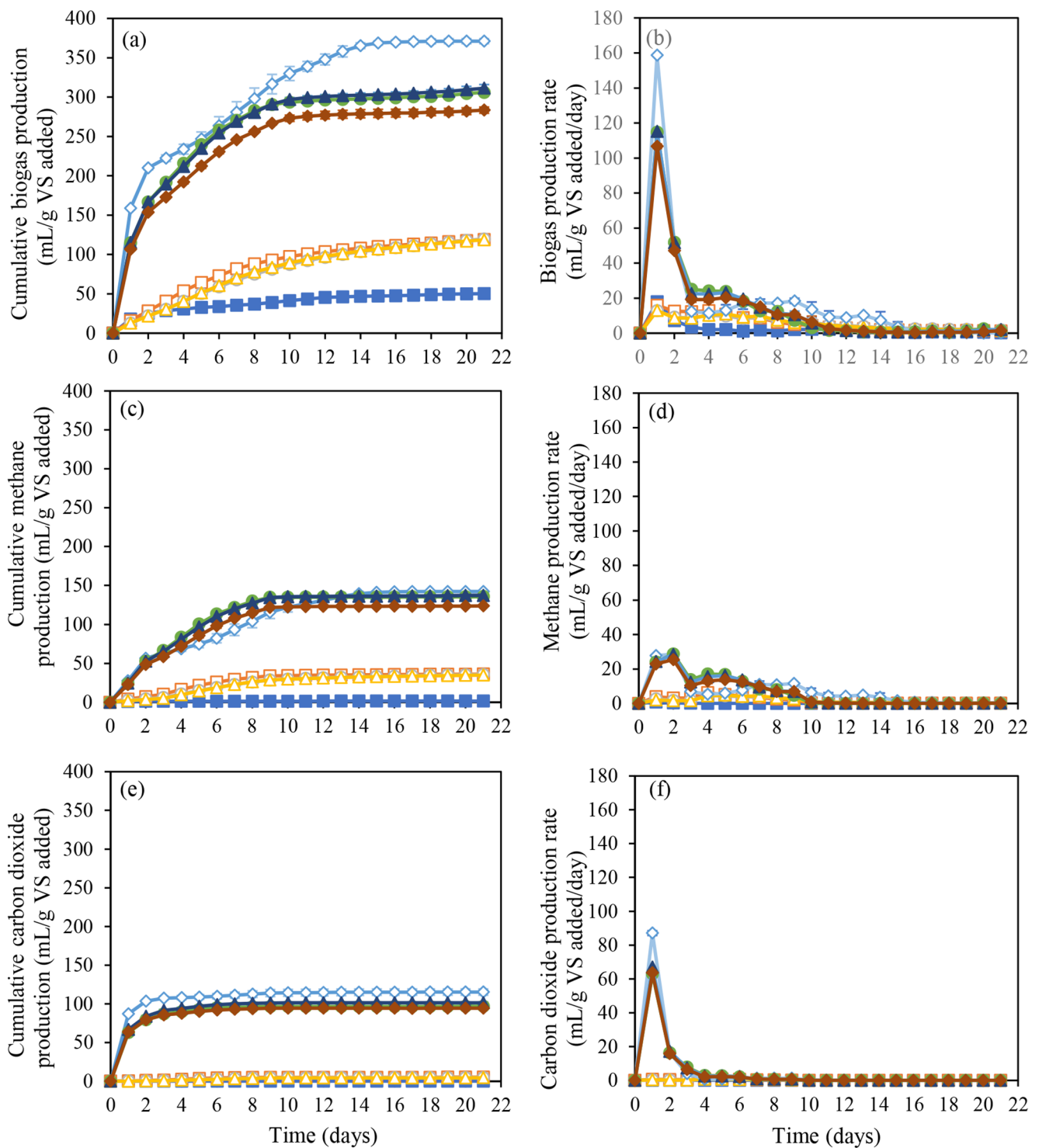


Fig. 1 Cumulative biogas (a), methane (c) and carbon dioxide (e) production (mL/g VS added), and daily production rate of biogas (b), methane (d) and carbon dioxide (f) (mL/g VS added/day) from mono-digestion of inoculum control (■), summer (□), autumn (●),

winter (▲), *P. australis*, and simulated food waste (◆) and co-digestion of summer (●), autumn (▲) and winter (◆) *P. australis* with simulated food waste over 21 days of digestion period. Data points represent mean $\pm$ standard deviation

Table 3 The total volume of cumulative biogas, methane and carbon dioxide production (mL/g VS added), peak biogas, methane and carbon dioxide production rate (mL/g VS added/day), and specific methane yield (mL/g VS of substrate added) over 21 days of the digestion period. Data represents mean $\pm$ standard deviation. The letters in parentheses represent the results of the Games-Howell pairwise comparison. Means that do not have the same letters are significantly different at the 95% confidence level ($p < 0.05$). Exact p -values for key variables are reported within the text

Conditions	Total volume of cumulative biogas production (mL/g VS added)	Peak biogas production rate (mL/g VS added/ day)	Total volume of cumulative CH ₄ production (mL/g VS added)	Peak CH ₄ production rate (mL/g VS added/ day)	Total volume of cumulative CO ₂ production (mL/g VS added)	Peak CO ₂ production rate (mL/g VS added/ day)	Specific methane yield (mL/g VS of substrate added)
Inoculum (blank)	50.22 $\pm$ 1.21 (E)	17.90 $\pm$ 0.25	1.47 $\pm$ 0.04 (E)	0.82 $\pm$ 0.02	0.16 $\pm$ 0.00 (E)	0.09 $\pm$ 0.00	–
Mono-digestion of pre-treated summer <i>P. australis</i> (control)	118.83 $\pm$ 5.17 (D)	15.91 $\pm$ 0.12	36.26 $\pm$ 4.48 (D)	6.24 $\pm$ 0.56	5.24 $\pm$ 1.43 (D)	1.02 $\pm$ 0.27	71.09 $\pm$ 1.05 (D)
Mono-digestion of pre-treated autumn <i>P. australis</i> (control)	119.70 $\pm$ 5.38 (D)	12.78 $\pm$ 0.61	35.59 $\pm$ 3.08 (D)	4.60 $\pm$ 0.05	4.73 $\pm$ 0.39 (D)	0.73 $\pm$ 0.05	69.74 $\pm$ 3.15 (D)
Mono-digestion of pre-treated winter <i>P. australis</i> (control)	118.66 $\pm$ 5.42 (D)	12.95 $\pm$ 0.12	34.90 $\pm$ 4.40 (D)	4.82 $\pm$ 0.43	4.53 $\pm$ 0.85 (D)	0.68 $\pm$ 0.12	68.37 $\pm$ 4.75 (D)
Mono-digestion of synthetic food waste (control)	371.08 $\pm$ 3.13 (A)	158.72 $\pm$ 0.25	142.16 $\pm$ 1.13 (A)	28.61 $\pm$ 0.00	115.26 $\pm$ 4.02 (A)	87.12 $\pm$ 1.40	283.00 $\pm$ 1.05 (A)
Co-digestion of pre-treated summer <i>P. australis</i> with synthetic food waste	305.98 $\pm$ 2.56 (B)	114.74 $\pm$ 0.00	135.66 $\pm$ 1.14 (B)	28.80 $\pm$ 0.00	96.74 $\pm$ 0.81 (C)	62.67 $\pm$ 0.00	270.01 $\pm$ 1.05 (B)
Co-digestion of pre-treated autumn <i>P. australis</i> with synthetic food waste	311.28 $\pm$ 4.66 (B)	115.26 $\pm$ 1.23	137.33 $\pm$ 3.21 (B)	28.41 $\pm$ 1.26	101.39 $\pm$ 1.07 (B)	67.04 $\pm$ 0.16	273.34 $\pm$ 4.14 (B)
Co-digestion of pre-treated winter <i>P. australis</i> with synthetic food waste	283.46 $\pm$ 3.93 (C)	106.66 $\pm$ 1.35	123.94 $\pm$ 1.01 (C)	25.49 $\pm$ 0.27	94.45 $\pm$ 0.81 (C)	63.62 $\pm$ 0.60	246.55 $\pm$ 3.34 (C)

Despite the absence of significant differences ($p = 1.00$) in specific methane yields (SMY) across the mono-digestion of *P. australis* from summer, autumn, and winter, the SMY from co-digestion of summer and autumn *P. australis* with

synthetic food waste were significantly higher ($p = 0.0001$) than those from co-digestion of winter *P. australis* with synthetic food waste. Moreover, the SMY produced from mono-digestion of synthetic food waste and co-digestion

of *P. australis* with synthetic food waste were significantly higher ($p = 0.0001$) than that from mono-digestion of *P. australis* (Table 3).

The analysis of the synergistic effect in co-digestion mixtures of synthetic food waste with *P. australis* biomass revealed differences between the observed SMY from co-digestion substrates and the weighted specific methane yields (WSMYs) calculated from the digestion of individual substrates (*P. australis* and synthetic food waste). The estimated WSMYs from digestion of summer, autumn, and winter *P. australis* and synthetic food waste were 229.99 ± 2.24 , 229.65 ± 1.54 , and 229.31 ± 2.20 mL/g VS of substrate added, respectively. At the same time, the observed SMYs of these co-digestion substrates were 270.01 ± 1.05 , 273.34 ± 4.14 , and 246.55 ± 3.34 mL/g VS of substrate added, respectively. This positive difference (between SMY and WSMY) in methane yields provides evidence that the co-digestion of these two substrates produces synergistic effects.

pH, Alkalinity, TAN, and VFA Profile

At the end of the digestion period, the pH values of all systems ranged between 8.50 and 8.82 (Table 3). This high pH value coincided with high alkalinity in all digesters, where the final total alkalinity values ranged between 23.95 ± 0.37 and 28.69 ± 0.04 (g CaCO_3/L). However, the ratio of intermediate alkalinity to partial alkalinity (IA/PA) in all reactors was lower than the threshold of 0.3 [44], which is used as an indicator of system stability (Table 4). Furthermore, Fig. 2 shows that the initial concentration of TAN in all reactors was increased after 21 days of digestion. However, the final TAN concentration of the mono-digestion of synthetic food waste and co-digestion of summer, autumn, and winter *P. australis* with synthetic food waste was higher than that of the mono-digestions of summer, autumn and winter *P. australis*. At the end of the digestion, only trace

amounts of VFAs, in the form of acetic acid, were found in the mono-digestions of summer, autumn and winter *P. australis* (0.029 ± 0.01 , 0.026 ± 0.00 and 0.026 ± 0.00 g/L, respectively). The mono-digestion of synthetic food waste and co-digestion of summer, autumn, and winter *P. australis* with synthetic food waste showed higher total VFA concentrations (3.17 ± 0.07 , 1.78 ± 0.02 , 1.91 ± 0.17 and 2.13 ± 0.11 g/L, respectively) with a predominance of propionic acid (Fig. 3). However, the ratio of total volatile fatty acids to total alkalinity (TVFAs/TA) in these reactors was lower than the threshold point of 0.4, which is considered as an early indicator of system stability [45, 46].

Changes and Removal of TS, VS, Carbohydrates, Proteins, and Lipids

After 21 days of digestion, all systems showed a reduction in the initial contents of TS, VS, carbohydrates, proteins, and lipids (Fig. 4a and b). The final TS and VS masses in the mono-digestion of synthetic food waste and co-digestion of *P. australis* with synthetic food waste were significantly lower ($p = 0.0001$) than in the mono-digestions of *P. australis*. Furthermore, the co-digestions of summer and autumn *P. australis* with synthetic food waste showed significantly lower ($p = 0.001$) TS and VS than the co-digestion of winter *P. australis* with synthetic food waste. No significant difference ($p = 0.9$) was detected in the final TS and VS content among the mono-digestion of summer, autumn, and winter *P. australis*. In general, this indicates that removal/degradation of TS and VS was significantly higher in the co-digestions of *P. australis* with synthetic food waste than in the mono-digestions of *P. australis*.

Figure 4b shows that carbohydrate macromolecules represented the major organic component in all reactors at the initial stage of digestion and had the highest removal compared to proteins and lipids. Furthermore, it is observed

Table 4 The values of final pH, partial, intermediate and total alkalinity, the ratio of intermediate alkalinity to partial alkalinity, and the ratio of total volatile fatty acids to total alkalinity after 21 days of digestion period. Data represents mean $\pm$ standard deviation

Conditions	pH	Partial Alkalinity (as, g CaCO_3/L)	Intermediate Alkalinity (as, g CaCO_3/L)	Total alkalinity (as, g CaCO_3/L)	IA/PA	TVFAs/TA
Inoculum (blank)	8.72 ± 0.02	18.66 ± 0.15	4.72 ± 0.17	23.95 ± 0.37	0.25	0.004
Mono-digestion of pre-treated summer <i>P. australis</i> (control)	8.56 ± 0.01	18.64 ± 0.08	4.99 ± 0.05	24.27 ± 0.03	0.27	0.001
Mono-digestion of pre-treated autumn <i>P. australis</i> (control)	8.51 ± 0.00	18.04 ± 0.03	5.13 ± 0.05	23.83 ± 0.04	0.28	0.001
Mono-digestion of pre-treated winter <i>P. australis</i> (control)	8.51 ± 0.01	18.08 ± 0.18	5.01 ± 0.11	23.69 ± 0.08	0.28	0.001
Mono-digestion of synthetic food waste (control)	8.81 ± 0.01	21.56 ± 0.08	6.30 ± 0.01	28.69 ± 0.04	0.29	0.111
Co-digestion of pre-treated summer <i>P. australis</i> with synthetic food waste	8.71 ± 0.00	20.51 ± 0.03	5.98 ± 0.00	27.22 ± 0.04	0.29	0.065
Co-digestion of pre-treated autumn <i>P. australis</i> with synthetic food waste	8.69 ± 0.02	20.66 ± 0.08	6.02 ± 0.08	27.31 ± 0.08	0.29	0.070
Co-digestion of pre-treated winter <i>P. australis</i> with synthetic food waste	8.68 ± 0.02	20.63 ± 0.02	6.02 ± 0.12	27.44 ± 0.11	0.29	0.078

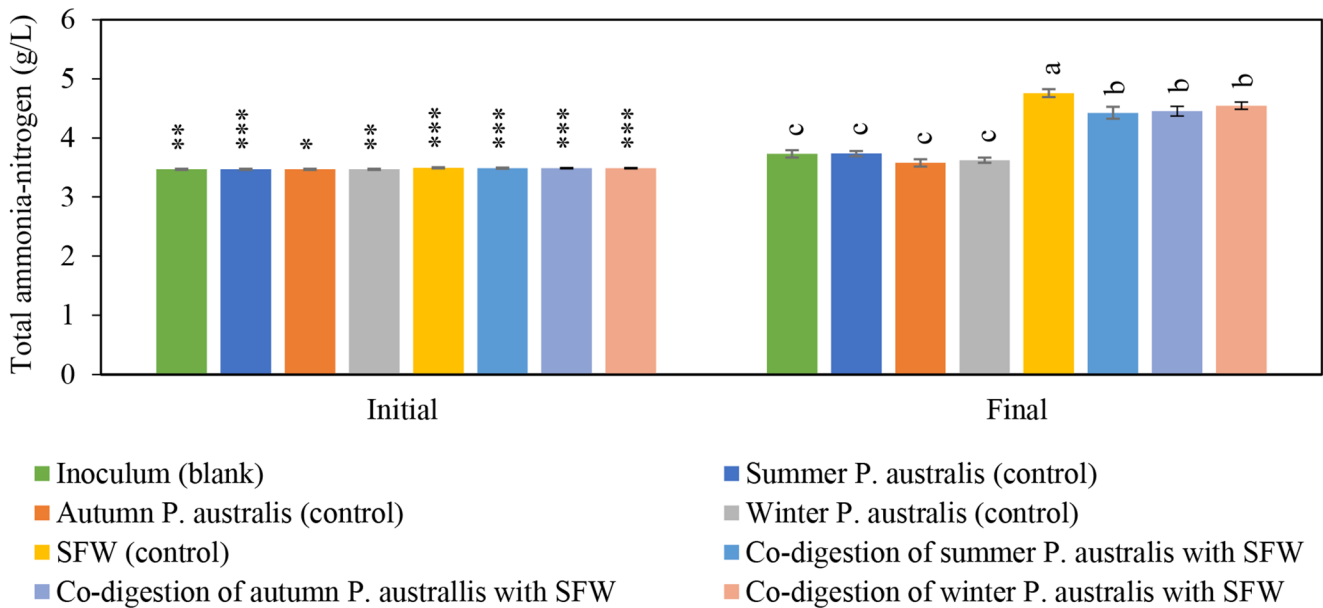


Fig. 2 Initial and final TAN (g/L) in all systems. The bars represent the mean $\pm$ standard deviation. Significant differences between initial and final quantities is represented as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The letters above the bars represent the results of a Games-Howell Pairwise Comparison. The bars that do not have the same letters are significantly different at a 95% confidence level

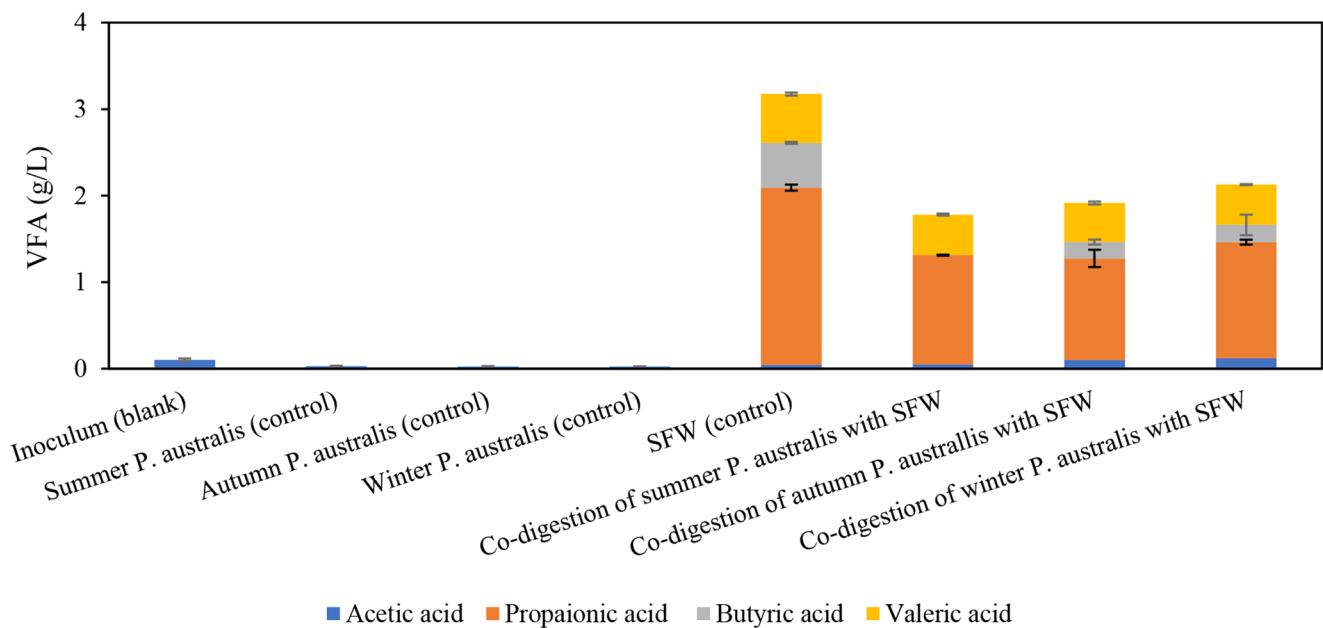


Fig. 3 Volatile fatty acid (VFA) profile after 21 days of digestion period. The bars represents mean $\pm$ standard deviation

that the degradation of carbohydrates, proteins, and lipids in the mono-digestion of synthetic food waste and the co-digestions of *P. australis* with synthetic food waste was significantly higher than those in the mono-digestions of *P. australis* (Fig. 4c). The co-digestion of summer *P. australis* with synthetic food waste showed significantly higher protein degradation than the co-digestion of winter *P. australis* with synthetic food waste. In contrast, no significant differences ($p = 1.00$) in lipid removal were observed among

the co-digestions of summer, autumn and winter *P. australis* with synthetic food waste. Moreover, no significant difference ($p = 0.7$) was observed in carbohydrate and lipid removal among the mono-digestions of summer, autumn and winter *P. australis*.

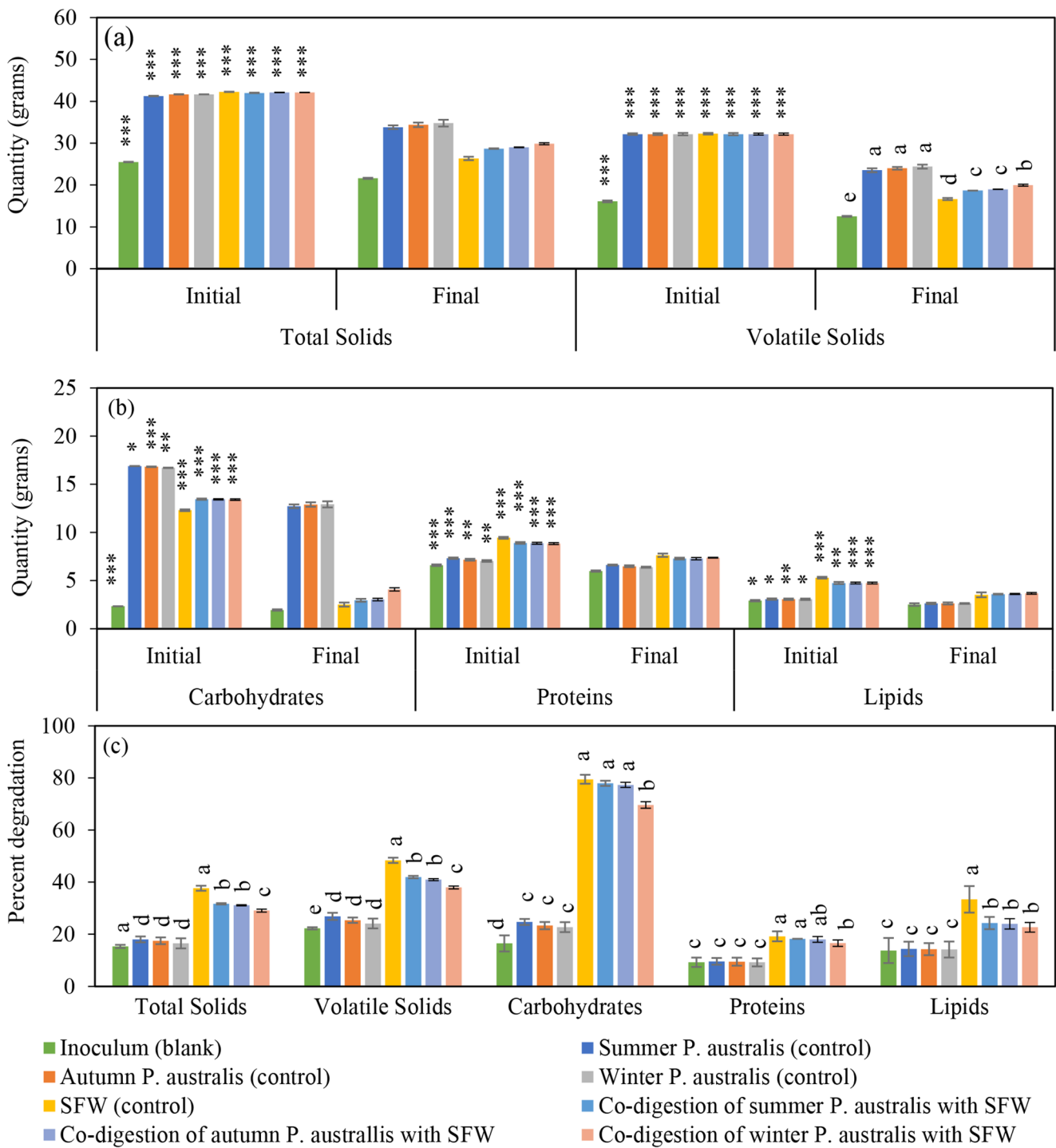


Fig. 4 The initial and final content of TS and VS (a), carbohydrates, proteins and lipids (b), and removal percentage of TS, VS, carbohydrates, proteins and lipids in digesters (c) over 21 days of digestion periods. The bars represent the mean $\pm$ standard deviation. Significant differences between initial and final quantities is represented as *

$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The letters above the bars represented the results of a Games-Howell Pairwise Comparison. The bars that do not have the same letters are significantly different at the 95% confidence level. Exact p -values for key variables are reported in the text

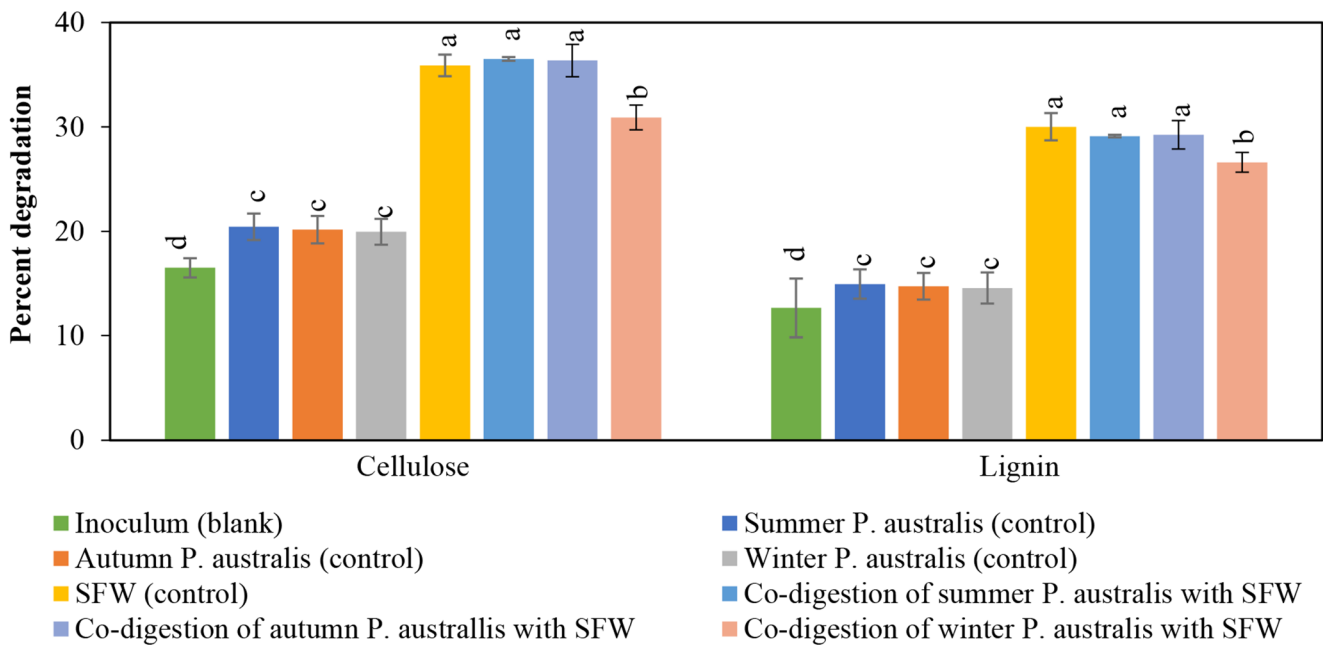


Fig. 5 Cellulose and lignin degradation (%) after 21 days of the digestion process. The bars show the mean $\pm$ standard deviation values. The letters above the bars refer to the results of a Games-Howell Pairwise

Comparison. The bars that did not share in their letters were significantly different at the 95% confidence level. Exact *p-values* for key variables are reported in the text

Degradation of Cellulose and Lignin

The degradation of cellulose and lignin was significantly higher ($p = 0.0001$) in the mono-digestion of synthetic food waste and in the co-digestions of *P. australis* with synthetic food waste compared to the mono-digestion of *P. australis* (Fig. 5). Furthermore, the co-digestions of summer and autumn *P. australis* with synthetic food waste exhibited significantly higher ($p = 0.04$) cellulose and lignin degradation than the co-digestion of winter *P. australis* with synthetic food waste. No significant difference ($p = 1.00$) was observed in cellulose and lignin degradation among the mono-digestions of summer, autumn, and winter *P. australis*.

Discussion

Effect of *P. Australis* Harvesting Time, mono-digestion and co-digestion on Biogas (methane) Production, Process Characteristics, VFAs Profile and Ammonia Production

The chemical composition of perennial grasses varies seasonally. Their nutrient content gradually increases with the onset of the growing season in spring to mid-summer. It begins to decrease in conjunction with the increase in cellulose, hemicellulose and lignin content as maturity progresses from late summer to the end of winter. These

variations could crucially control the feasibility of using these plants for anaerobic digestion and biogas production [47–50]. Contrary to expectations, this study did not show a clear effect of harvesting season on biogas production from mono-digestions of summer, autumn, and winter *P. australis*. This may be due to the high lignin content of the *P. australis* biomass from these seasons, which limited the microbial accessibility to cellulose molecules, thus reducing biodegradability and biogas production. This is consistent with previous research showing that high lignin content in *P. australis* impairs its biodegradability and leads to lower methane production. Dragoni et al. [51] demonstrated a clear negative relationship between lignin content of *P. australis* and both biogas yield and decomposition rate. Similarly, Baute et al. [52] found that autumn harvesting of reeds produced low methane amount concluding that reeds are not an economically viable feedstock for biogas production without pretreatment to improve methane production.

However, the co-digestion of *P. australis* with synthetic food waste revealed a significant influence of the harvesting season, manifested as increased biogas production from co-digestion of summer and autumn *P. australis* with synthetic food waste than from co-digestion of winter *P. australis* with synthetic food waste. This can be attributed to differences in the nutrient content (proteins and lipids) of *P. australis*, which usually decrease in winter. When sufficient synthetic food waste is available to support microbial activity, the seasonal differences in *P. australis* become substantial enough to influence biogas production [53, 54].

In general, the co-digestion of *P. australis* with synthetic food waste showed significantly higher biogas production than the mono-digestion of *P. australis*, owing to the role of compatible substrates combinations in overcoming the limitation of a single feedstock's properties. The addition of synthetic food waste compensates for the nutrient and nitrogen deficiency in *P. australis* biomass, thus assisting microbial biomass growth and reproduction [55, 56]. In return, the *P. australis* biomass acts as a source of carbon that is required by microbes' consortia for energy purposes and the building of organic acids [57].

In line with this, multiple studies have shown that co-digesting lignocellulosic materials with food waste can markedly improve both biogas and methane production compared to digesting these substrates alone. For example, Sawasdee et al. [58] found that combining rice straw with food waste led to higher methane yields than using rice straw alone, emphasizing the role of a balanced carbon-to-nitrogen ratio and the availability of easily degradable organic matter in supporting microbial activity. Similarly, Wang et al. [59] reported that co-digestion of *Phragmites australis* with food waste enhanced biogas output, primarily by improving microbial access to structural carbohydrates and reducing the inhibitory effects of lignin-rich biomass.

Taken together, these studies reinforce the idea that co-digestion can overcome the limitations of single lignocellulosic feedstocks, stabilize the anaerobic digestion process, and increase both hydrolysis efficiency and methane yield. This aligns with the seasonal trends observed in our study, where biogas production from summer and autumn-harvested reed exceeded that from winter-harvested reed, likely due to higher nutrient content in the former. When combined with food waste, this nutrient advantage further supports microbial activity and improves substrate degradation.

Furthermore, the easy biodegradable synthetic food waste substrate promotes the production of high organic acids concentrations that can serve as an acid treatment to enhance the delignification of *P. australis* biomass, thus increasing hydrolysis activity and fibre degradation of the *P. australis* substrate [60]. At the same time, the recalcitrant nature and lower degradability of *P. australis* substrate compared to synthetic food waste could prevent the excessive production of VFAs in co-digestion systems, thus maintaining the pH level of the medium and preventing inhibition of methanogens bacteria [60–64].

The mono-digestions of *P. australis* and co-digestions of *P. australis* with synthetic food waste showed a gradual increase in biogas production during the digestion period. This indicates a balance between the production and consumption of VFAs, and this was evident from the ratios of IA/PA and TVFAs/TA that were within the ideal limits below 0.3 and 0.4, respectively, which are considered essential

indicators of system stability [45, 46, 65, 66]. This may be attributed to the slow hydrolysis of *P. australis* substrate because of recalcitrant lignin, thus avoiding the accumulation of VFAs and preventing decreases in pH, thus preserving the microorganisms from inhibition. At the same time, a minor disturbance and slowing down in methane production was observed on the 2nd day in the synthetic food waste mono-digestion reactors. This may be due to the easily biodegradable nature of synthetic food waste substrates, resulting in the production of high concentrations of VFAs that exceeded the consumption ability of microorganisms, which would lead to their accumulation and cause a decrease in the pH of the medium, partially inhibiting methanogens and slowing methane production. This hypothesis is supported by the high concentrations of CO₂ that were observed on the second day of the digestion process, which was produced from the conversion of carbonates in the systems from the liquid phase into CO₂ under the influence of the low pH of the medium [39].

The mono-digestion of synthetic food waste and co-digestion of summer, autumn and winter *P. australis* with synthetic food waste resulted in higher TAN concentration compared to the mono-digestion of summer, autumn and winter *P. australis*. This could be due to the higher protein content of the synthetic food waste substrate, and its degradation leading to increasing TAN concentrations in the reactors. However, TAN concentrations in all reactors were within the inhibitory limits reported in the literature (1.7–6 g/L) [67–69]. Nonetheless, these concentrations did not lead to any inhibitory effects on the stability of the systems. This may be due to of microbial consortia that had become acclimated to the high concentrations of TAN, especially since the TAN concentration in the used inoculum was already high [70].

Degradation of TS, VS, Carbohydrates, Proteins, Lipids, Cellulose and Lignin

The type of substrates used, and their composition, the nature of the microorganisms and their activity, operating conditions, internal interactions, and reactor characteristics are among the main factors that control the degradation of VS content of substrates used in anaerobic digestion [71]. During the 21 days of digestion in this experiment, the degradation level of TS and VS content in the mono-digestion of *P. australis* were lower than TS and VS degradation in the mono-digestion of synthetic food waste and co-digestion of *P. australis* with synthetic food waste. The resistance of lignin to microbial degradation mainly contributes to the reduced bioavailability of cellulose and hemicellulose for hydrolysis enzymes. Consequently, this led to a decrease in the degradation of cellulose and carbohydrates during the

mono-digestion of *P. australis*. Furthermore, the low concentration of nutrients in mono-digestion of *P. australis* may have affected the growth and reproduction of microbes that were responsible for the degradation of particular molecules. This might in turn cause a decrease in protein and lipid degradation in those reactors compared to the reactors containing co-digestions of *P. australis* with synthetic food waste.

On the other hand, the organic acids resulting from the hydrolysis of readily degradable synthetic food waste substrates could have enhanced the breakdown of lignocellulose components of *P. australis*, thus increasing the degradation of cellulose and carbohydrate molecules in reactors containing co-digestions of *P. australis* with synthetic food waste. Zou et al. [72] reported that the high VFA concentrations resulting from the hydrolysis of food waste increased the lignocellulose components hydrolysis efficiency of corn cob by 17.3–28%. Moreover, the addition of synthetic food waste to the *P. australis* may have contributed to providing more nutrients (such as nitrogen) for microorganisms and encouraged their growth and reproduction, causing more degradation of lignin, carbohydrates, proteins, and lipids molecules in co-digestion of *P. australis* with synthetic food waste compared to mono-digestion of *P. australis* [73–76].

The observed synergistic effects between reed and food waste suggest important implications for practical biogas plant design. In particular, the enhancement of methane yield through co-digestion indicates that selecting appropriate substrate mixtures can significantly improve process stability and energy recovery. Such findings may guide operators in adjusting organic loading rates and optimizing feedstock composition, thereby supporting the scale-up of laboratory results to real biogas facilities.

It is important to note that the use of a synthetic food waste model in this study helped reduce variability and ensure controlled, reproducible experimental conditions. However, this approach does not fully reflect the heterogeneous and complex nature of real food waste streams. Therefore, the results should be viewed as a preliminary step toward understanding the underlying influences of the studied factors. It is recommended that future studies include real and diverse food waste to ensure the generalizability of the results to applied conditions in anaerobic digestion plants.

Conclusion

As it is a lignocellulosic plant, the exploitation of *P. australis* as a source for methane production is limited without implementing an effective pre-treatment process that simplifies the substrate structure and eliminates the lignin's

influence in restricting the bioavailability of cellulose. The current study shows that there was no significant difference in the biogas production during mono-digestions of *P. australis* harvested from summer, autumn, and winter seasons due to the resistance of lignin to biodegradation and its role in limiting the availability of cellulose for microorganisms, consequently led to a decrease in the production of methane.

However, the co-digestion of summer and autumn *P. australis* with synthetic food waste presented significantly higher methane production than that for co-digestion of winter *P. australis* with synthetic food waste, which could be due to the increase of lignin content with the maturity of *P. australis* biomass in winter.

The synergistic integration of *P. australis* substrate and synthetic food waste improved the digestibility of *P. australis* substrate from the three seasons during the co-digestion process. The co-digestion had a prominent role in delivering more nutrients for the microbial consortia than the mono-digestion of *P. australis*, which may encourage their reproduction and activity, thus increasing the degradation of macromolecules and the production of methane.

Moreover, the high organic acids resulting from the rapid decomposition of the synthetic food waste substrate contributed to providing an acidic treatment of the *P. australis* substrate, which may have helped in breaking down lignocellulosic components and increasing the exposure of cellulose to digestive enzymes, thus increasing methane production. Based on the results from this study, the co-digestion of synthetic food waste with *P. australis* biomass harvested in the summer and autumn seasons is considered more suitable for methane production than *P. australis* biomass harvested in the winter.

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Author Contributions Ahmed R. Al-Iraqi: Conceptualization, Methodology, Investigation, physio-chemical analysis, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. Kirk T. Semple: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Visualization, Resources, Supervision, Writing – review & editing. Andrew M. Folkard: Conceptualization, Methodology, Resources, Visualization, Supervision, Writing – review & editing. Philip A. Barker: Conceptualization, Resources, Visualization, Supervision, Writing – review & editing. Bhushan P. Gandhi: Formal analysis, Writing – review & editing.

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Declarations

Competing Interests 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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References

1. Olabi, A.G.: The 3rd international conference on sustainable energy and environmental protection SEEP 2009-Guest editor's introduction. *Energy*. **35**, 4508–4509 (2010). <https://doi.org/10.1016/J.ENERGY.2010.09.053>
2. Rinehart, L.: Switchgrass as a Bioenergy Crop. National Center for Appropriate Technology (2006)
3. Smyth, B.M., Murphy, J.D., O'Brien, C.M.: What is the energy balance of grass biomethane in Ireland and other temperate Northern European climates? *Renew. Sustain. Energy Rev.* **13**, 2349–2360 (2009). <https://doi.org/10.1016/J.RSER.2009.04.003>
4. Kang, X., Zhang, Y., Song, B., Sun, Y., Li, L., He, Y., Kong, X., Luo, X., Yuan, Z.: The effect of mechanical pretreatment on the anaerobic digestion of hybrid pennisetum. *Fuel*. **252**, 469–474 (2019). <https://doi.org/10.1016/J.FUEL.2019.04.134>
5. Rodriguez, C., Alaswad, A., Benyounis, K.Y., Olabi, A.G.: Pretreatment techniques used in biogas production from grass. *Renew. Sustain. Energy Rev.* **68**, 1193–1204 (2017). <https://doi.org/10.1016/J.RSER.2016.02.022>
6. Seppälä, M., Paavola, T., Lehtomäki, A., Rintala, J.: Biogas production from boreal herbaceous grasses – Specific methane yield and methane yield per hectare. *Bioresour Technol.* **100**, 2952–2958 (2009). <https://doi.org/10.1016/J.BIORTECH.2009.01.044>
7. Schittenhelm, S.: Chemical composition and methane yield of maize hybrids with contrasting maturity. *Eur. J. Agron.* **29**, 72–79 (2008). <https://doi.org/10.1016/J.EJA.2008.04.001>
8. Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., Gruber, L.: Biogas production from maize and dairy cattle manure—Influence of biomass composition on the methane yield. *Agric. Ecosyst. Environ.* **118**, 173–182 (2007). <https://doi.org/10.1016/J.AGEE.2006.05.007>
9. Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran K.B., Ramakrishnan, S.: Chemical and physicochemical pretreatment of lignocellulosic biomass: A review. *Res. Enzyme Res.* **17**(2011) (2011). <https://doi.org/10.4061/2011/787532>
10. Kumari, D., Singh, R.: Pretreatment of lignocellulosic wastes for biofuel production: A critical review. *Renew. Sustain. Energy Rev.* **90**, 877–891 (2018). <https://doi.org/10.1016/J.RSER.2018.03.111>
11. Zborowska, M., Waliszewska, H., Waliszewska, B., Borysiak, S., Brozdowski, J., Stachowiak-Wencek, A.: Conversion of Carbohydrates in Lignocellulosic Biomass after Chemical Pretreatment. *Energies* 2022, Vol. 15, Page 254. 15, 254 (2021). <https://doi.org/10.3390/EN15010254>
12. Pelegri, J., Holzem, R.M.: Evaluating the Impacts of Phragmites australis Pretreatment Methods on Biogas and Methane. *MJUR*. 244–259 (2017)
13. Jiang, D., Ge, X., Zhang, Q., Li, Y.: Comparison of liquid hot water and alkaline pretreatments of giant Reed for improved enzymatic digestibility and biogas energy production. *Bioresour Technol.* **216**, 60–68 (2016). <https://doi.org/10.1016/J.BIORTECH.2016.05.052>
14. Sarker, S., Lamb, J.J., Hjelme, D.R., Lien, K.M.: A Review of the Role of Critical Parameters in the Design and Operation of Biogas Production Plants. *Applied Sciences* Vol. 9, Page 1915. 9, 1915 (2019). <https://doi.org/10.3390/APP9091915>
15. Lindmark, J., Leksell, N., Schnürer, A., Thorin, E.: Effects of mechanical pre-treatment on the biogas yield from Ley crop silage. *Appl. Energy*. **97**, 498–502 (2012). <https://doi.org/10.1016/J.APENERGY.2011.12.066>
16. Hjorth, M., Gränitz, K., Adamsen, A.P.S., Möller, H.B.: Extrusion as a pretreatment to increase biogas production. *Bioresour Technol.* **102**, 4989–4994 (2011). <https://doi.org/10.1016/J.BIORTECH.2010.11.128>
17. Licari, A., Monlau, F., Solhy, A., Buche, P., Barakat, A.: Comparison of various milling modes combined to the enzymatic hydrolysis of lignocellulosic biomass for bioenergy production: Glucose yield and energy efficiency. *Energy*. **102**, 335–342 (2016). <https://doi.org/10.1016/J.ENERGY.2016.02.083>
18. Cheng, J.J., Timilsina, G.R.: Status and barriers of advanced biofuel technologies: A review. *Renew. Energy*. **36**, 3541–3549 (2011). <https://doi.org/10.1016/J.RENENE.2011.04.031>
19. Barakat, A., Mayer-Laigle, C., Solhy, A., Arancon, R.A.D., De Vries, H., Luque, R.: Mechanical pretreatments of lignocellulosic biomass: Towards facile and environmentally sound technologies for biofuels production. *RSC Adv.* **4**, 48109–48127 (2014). <https://doi.org/10.1039/C4RA07568D>
20. Olatunji, K.O., Ahmed, N.A., Ogunkunle, O.: Optimization of biogas yield from lignocellulosic materials with different pretreatment methods: A review. *Biotechnology for biofuels* 2021 14:1. 14, 1–34 (2021). <https://doi.org/10.1186/S13068-021-02012-X>
21. Mani, S., Tabil, L.G., Sokhansanj, S.: Grinding performance and physical properties of wheat and barley straws, corn Stover and Switchgrass. *Biomass Bioenergy*. **27**, 339–352 (2004). <https://doi.org/10.1016/J.BIOMBIOE.2004.03.007>
22. Taherzadeh, M.J., Karimi, K.: Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *Int. J. Mol. Sci.* 2008. **9**, 1621–1651 (2008). <https://doi.org/10.3390/IJMS9091621>
23. Yan, Y., Du, Z., Zhang, L., Feng, L., Sun, D., Dang, Y., Holmes, D.E., Smith, J.A.: Identification of parameters needed for optimal anaerobic co-digestion of chicken manure and corn Stover. *RSC Adv.* **9**, 29609–29618 (2019). <https://doi.org/10.1039/C9RA05556H>
24. Rabii, A., Aldin, S., Dahman, Y., Elbeshbishy, E.: A Review on Anaerobic Co-Digestion with a Focus on the Microbial Populations and the Effect of Multi-Stage Digester Configuration. *Energies* 2019, Vol. 12, Page 1106. 12, 1106 (2019). <https://doi.org/10.3390/EN12061106>
25. Kulichkova, G.I., Ivanova, T.S., Köttner, M., Volodko, O.I., Spivak, S.I., Tsygankov, S.P., Blume, Y.B.: Plant feedstocks and their biogas production potentials. *Open. Agric. J.* **14**, 219–234 (2020). <https://doi.org/10.2174/1874331502014010219>
26. Nguyen, D., Nitayavardhana, S., Sawatdeenarunat, C., Surendra, K.C., Khanal, S.K.: Biogas Production by Anaerobic Digestion: Status and Perspectives. *Biomass, Biofuels, Biochemicals: Biofuels: Alternative Feedstocks and Conversion Processes for the*

- Production of Liquid and Gaseous Biofuels (2nd edn). 763–778 (2019). <https://doi.org/10.1016/B978-0-12-816856-1.00031-2>
27. Rajagopal, R., Massé, D.I., Singh, G.: A critical review on Inhibition of anaerobic digestion process by excess ammonia. *Bioresour Technol.* **143**, 632–641 (2013). <https://doi.org/10.1016/J.BIORTECH.2013.06.030>
 28. Sawatdeenarunat, C., Wangnai, C., Songkasiri, W., Panichnumsin, P., Saritpongteeraka, K., Boonsawang, P., Khanal, S.K., Chaiprapat, S.: Biogas Production From Industrial Effluents. *Biomass, Biofuels, Biochemicals: Biofuels: Alternative Feedstocks and Conversion Processes for the Production of Liquid and Gaseous Biofuels* (2nd edn). 779–816 (2019). <https://doi.org/10.1016/B978-0-12-816856-1.00032-4>
 29. Abbasi, T., Tauseef, S.M., Abbasi, S.A.: *Biogas Energy*. Springer New York, Netherlands (2011)
 30. Alves, M.M., Pereira, M.A., Sousa, D.Z., Cavaleiro, A.J., Picaudet, M., Smidt, H., Stams, A.J.M.: Waste lipids to energy: How to optimize methane production from long-chain fatty acids (LCFA). *Microb. Biotechnol.* **2**, 538–550 (2009). <https://doi.org/10.1111/J.1751-7915.2009.00100.X>
 31. Yong, Z., Dong, Y., Zhang, X., Tan, T.: Anaerobic co-digestion of food waste and straw for biogas production. *Renew. Energy.* **78**, 527–530 (2015). <https://doi.org/10.1016/J.RENENE.2015.01.033>
 32. WRAP: The food we waste. WRAP: (2008)
 33. APHA: Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC, USA, 21: (2005)
 34. Ripley, L.E., Boyle, W.C., Converse, J.C.: Improved alkalimetric monitoring for anaerobic digestion of High-Strength wastes. *J. Water Pollut Control Fed.* **58**, 406–411 (1986)
 35. Wu, Y., Kovalovszki, A., Pan, J., Lin, C., Liu, H., Duan, N., Angelidaki, I.: Early warning indicators for mesophilic anaerobic digestion of corn stalk: A combined experimental and simulation approach. *Biotechnol. Biofuels.* **12**, 1–15 (2019). <https://doi.org/10.1186/S13068-019-1442-7/TABLES/5>
 36. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F.: Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**, 350–356 (1956). https://doi.org/10.1021/AC60111A017/ASSET/AC60111A017.FP.PNG_V03
 37. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: PROTEIN MEASUREMENT WITH THE FOLIN PHENOL REAGENT. *J. Biol. Chem.* **193**, 265–275 (1951). [https://doi.org/10.1016/S0021-9258\(19\)52451-6](https://doi.org/10.1016/S0021-9258(19)52451-6)
 38. Bligh, E.G., Dyer, W.J., A RAPID METHOD, OF TOTAL LIPID EXTRACTION AND PURIFICATION: *Can. J. Biochem. Physiol.* **37**, 911–917 (1959). <https://doi.org/10.1139/o59-099>
 39. Gandhi, B.P., Oteite, S.V., Fofie, E.A., Lag-Brotons, A.J., Ezeonye, L.I., Semple, K.T., Martin, A.D.: Kinetic investigations into the effect of inoculum to substrate ratio on batch anaerobic digestion of simulated food waste. *Renew. Energy.* **195**, 311–321 (2022). <https://doi.org/10.1016/J.RENENE.2022.05.134>
 40. Van Soest, P.J., Robertson, J.B., Lewis, B.A.: Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy. Sci.* **74**, 3583–3597 (1991). [https://doi.org/10.3168/JDS.S0022-0302\(91\)78551-2](https://doi.org/10.3168/JDS.S0022-0302(91)78551-2)
 41. Cheng, X.Y., Zhong, C.: Effects of feed to inoculum ratio, co-digestion, and pretreatment on biogas production from anaerobic digestion of cotton stalk. *Energy Fuels.* **28**, 3157–3166 (2014). https://doi.org/10.1021/EF402562Z/ASSET/IMAGES/LARGE/EF-2013-02562Z_0003.JPEG
 42. Li, Y., Zhang, R., Chen, C., Liu, G., He, Y., Liu, X.: Biogas production from co-digestion of corn Stover and chicken manure under anaerobic wet, hemi-solid, and solid state conditions. *Bioresour Technol.* **149**, 406–412 (2013). <https://doi.org/10.1016/J.BIORTECH.2013.09.091>
 43. Labatut, R.A., Angenent, L.T., Scott, N.R.: Biochemical methane potential and biodegradability of complex organic substrates. *Bioresour Technol.* **102**, 2255–2264 (2011). <https://doi.org/10.1016/J.BIORTECH.2010.10.035>
 44. Bernard, O., Polit, M., Hadj-Sadok, Z., Pengov, M., Dochain, D., Estaben, M., Labat, P.: Advanced monitoring and control of anaerobic wastewater treatment plants: Software sensors and controllers for an anaerobic digester. *Water Sci. Technol.* **43**, 175–182 (2001). <https://doi.org/10.2166/WST.2001.0418>
 45. Martín-González, L., Font, X., Vicent, T.: Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes. *Biochem. Eng. J.* **76**, 1–5 (2013). <https://doi.org/10.1016/J.BEJ.2013.03.016>
 46. Li, L., He, Q., Wei, Y., He, Q., Peng, X.: Early warning indicators for monitoring the process failure of anaerobic digestion system of food waste. *Bioresour Technol.* **171**, 491–494 (2014). <https://doi.org/10.1016/J.BIORTECH.2014.08.089>
 47. Kandel, T.P., Sutaryo, S., Møller, H.B., Jørgensen, U., Lærke, P.E.: Chemical composition and methane yield of Reed Canary grass as influenced by harvesting time and harvest frequency. *Bioresour Technol.* **130**, 659–666 (2013). <https://doi.org/10.1016/J.BIORTECH.2012.11.138>
 48. Ragagnini, G., Dragoni, F., Simone, M., Bonari, E.: Suitability of giant Reed (*Arundo Donax* L.) for anaerobic digestion: Effect of harvest time and frequency on the biomethane yield potential. *Bioresour Technol.* **152**, 107–115 (2014). <https://doi.org/10.1016/J.BIORTECH.2013.11.004>
 49. Wahid, R., Nielsen, S.F., Hernandez, V.M., Ward, A.J., Gislum, R., Jørgensen, U., Møller, H.B.: Methane production potential from miscanthus sp.: Effect of harvesting time, genotypes and plant fractions. *Biosyst Eng.* **133**, 71–80 (2015). <https://doi.org/10.1016/J.BIOSYSTEMS.2015.03.005>
 50. Liu, S., Ge, X., Liu, Z., Li, Y.: Effect of harvest date on *Arundo Donax* L. (giant reed) composition, ensilage performance, and enzymatic digestibility. *Bioresour Technol.* **205**, 97–103 (2016). <https://doi.org/10.1016/J.BIORTECH.2016.01.011>
 51. Dragoni, F., Giannini, V., Ragagnini, G., Bonari, E., Silvestri, N.: Effect of harvest time and frequency on biomass quality and biomethane potential of common Reed (*Phragmites australis*) under paludiculture conditions. *Bioenergy Res.* **10**, 1066–1078 (2017). <https://doi.org/10.1007/S12155-017-9866-Z/METRICS>
 52. Baute, K., Van Eerd, L.L., Robinson, D.E., Sikkema, P.H., Mushatq, M., Gilroyed, B.H.: Comparing the Biomass Yield and Biogas Potential of *Phragmites australis* with *Miscanthus x giganteus* and *Panicum virgatum* Grown in Canada. <https://doi.org/10.3390/en11092198>
 53. Gilson, E.: Biogas production potential and cost-benefit analysis of harvesting wetland plants (*Phragmites australis* and *Glyceria maxima*). (2017). <https://urn.kb.se/resolve?urn=urn:nbn:se:hh:di-va-34424>
 54. Bernal, M.P., Albuquerque, J.A., Moral, R.: Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour Technol.* **100**, 5444–5453 (2009). <https://doi.org/10.1016/J.BIORTECH.2008.11.027>
 55. Brown, D., Li, Y.: Solid state anaerobic co-digestion of yard waste and food waste for biogas production. *Bioresour Technol.* **127**, 275–280 (2013). <https://doi.org/10.1016/J.BIORTECH.2012.09.081>
 56. Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S.: A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renew. Sustain. Energy Rev.* **36**, 412–427 (2014). <https://doi.org/10.1016/J.RSER.2014.04.039>

57. Kumar, M., Ou, Y.L., Lin, J.G.: Co-composting of green waste and food waste at low C/N ratio. *Waste Manage.* **30**, 602–609 (2010). <https://doi.org/10.1016/J.WASMAN.2009.11.023>
58. Sawasdee, A., Haosagul, S., Pisutpaisal, N.: Biogas production from Co-digestion between rice straw and food waste with pilot scale. *Int. J. Environ. Res.* **17**, 1–9 (2023). <https://doi.org/10.1007/S41742-023-00523-Z/METRICS>
59. Wang, X., Zhang, L., Xi, B., Sun, W., Xia, X., Zhu, C., He, X., Li, M., Yang, T., Wang, P., Zhang, Z.: Biogas production improvement and C/N control by natural clinoptilolite addition into anaerobic co-digestion of phragmites australis, feces and kitchen waste. *Bioresour Technol.* **180**, 192–199 (2015). <https://doi.org/10.1016/J.BIORTECH.2014.12.023>
60. Ibro, M.K., Ancha, V.R., Lemma, D.B.: Impacts of anaerobic Co-Digestion on different influencing parameters. *Crit. Rev. Sustain.* **14**, 14, 9387 (2022). Page 9387 <https://doi.org/10.3390/SU14159387>
61. Xu, F., Li, Y.: Solid-state co-digestion of expired dog food and corn Stover for methane production. *Bioresour Technol.* **118**, 219–226 (2012). <https://doi.org/10.1016/J.BIORTECH.2012.04.102>
62. Pagés-Díaz, J., Pereda-Reyes, I., Taherzadeh, M.J., Sárvári-Horváth, I., Lundin, M.: Anaerobic co-digestion of solid slaughterhouse wastes with agro-residues: Synergistic and antagonistic interactions determined in batch digestion assays. *Chem. Eng. J.* **245**, 89–98 (2014). <https://doi.org/10.1016/J.CEJ.2014.02.008>
63. Lin, Y., Ge, X., Li, Y.: Solid-state anaerobic co-digestion of spent mushroom substrate with yard trimmings and wheat straw for biogas production. *Bioresour Technol.* **169**, 468–474 (2014). <https://doi.org/10.1016/J.BIORTECH.2014.07.020>
64. Hallaji, S.M., Kuroshkarim, M., Moussavi, S.P.: Enhancing methane production using anaerobic co-digestion of waste activated sludge with combined fruit waste and cheese Whey. *BMC Biotechnol.* **19**, 1–10 (2019). <https://doi.org/10.1186/S12896-019-0513-Y/FIGURES/4>
65. Van Tran, G., Unpaprom, Y., Ramaraj, R.: Evaluation of biogas production from common Reed with/without chemical pretreatment and energy analysis. *Int. J. Adv. Sci. Eng. Technol.* ISSN, 2321–9009 (2019)
66. Méndez-Acosta, H.O., Palacios-Ruiz, B., Alcaraz-González, V., González-Álvarez, V., García-Sandoval, J.P.: A robust control scheme to improve the stability of anaerobic digestion processes. *J. Process. Control.* **20**, 375–383 (2010). <https://doi.org/10.1016/J.PROCONT.2010.01.006>
67. Albertson, O.E.: Ammonia nitrogen and the anaerobic environment. *J. Water Pollut Control Fed.* **33**, 978–995 (1961)
68. Braun, R., Huber, P., Meyrath, J.: Ammonia toxicity in liquid pig-gery manure digestion. *Biotechnol. Lett.* **3**, 159–164 (1981). <https://doi.org/10.1007/BF00239655/METRICS>
69. Melbinger, N.R., Donnellon, J., Zablatzky, H.R.: Toxic effects of ammonia nitrogen in High-Rate digestion [with discussion]. *J. Water Pollut Control Fed.* **43**, 1658–1670 (1971)
70. Van Velsen, A.F.M.: Adaptation of methanogenic sludge to high ammonia-nitrogen concentrations. *Water Res.* **13**, 995–999 (1979). [https://doi.org/10.1016/0043-1354\(79\)90194-5](https://doi.org/10.1016/0043-1354(79)90194-5)
71. Leclerc, M., Delgènes, J.P., Godon, J.J.: Diversity of the archaeal community in 44 anaerobic digesters as determined by single strand conformation polymorphism analysis and 16S rDNA sequencing. *Environ. Microbiol.* **6**, 809–819 (2004). <https://doi.org/10.1111/J.1462-2920.2004.00616.X>
72. Zou, H., Jiang, Q., Zhu, R., Chen, Y., Sun, T., Li, M., Zhai, J., Shi, D., Ai, H., Gu, L., He, Q.: Enhanced hydrolysis of lignocellulose in corn cob by using food waste pretreatment to improve anaerobic digestion performance. *J. Environ. Manage.* **254**, 109830 (2020). <https://doi.org/10.1016/J.JENVMAN.2019.109830>
73. Browne, J.D., Murphy, J.D.: Assessment of the resource associated with biomethane from food waste. *Appl. Energy.* **104**, 170–177 (2013). <https://doi.org/10.1016/J.APENERGY.2012.11.017>
74. Duan, J., Huo, X., Du, W.J., Liang, J.D., Wang, D.Q., Yang, S.C.: Biodegradation of kraft lignin by a newly isolated anaerobic bacterial strain, *acetoanaerobium* sp. WJDL-Y2. *Lett. Appl. Microbiol.* **62**, 55–62 (2016). <https://doi.org/10.1111/LAM.12508>
75. Woo, H.L., Ballor, N.R., Hazen, T.C., Fortney, J.L., Simmons, B., Davenport, K.W., Goodwin, L., Ivanova, N., Kyrpides, N.C., Mavromatis, K., Woyke, T., Jansson, J., Kimbrel, J., DeAngelis, K.M.: Complete genome sequence of the lignin-degrading bacterium *Klebsiella* sp. strain BRL6-2. *Stand. Genomic Sci.* **9**, 1–9 (2014). <https://doi.org/10.1186/1944-3277-9-19/TABLES/11>
76. Billings, A.F., Fortney, J.L., Hazen, T.C., Simmons, B., Davenport, K.W., Goodwin, L., Ivanova, N., Kyrpides, N.C., Mavromatis, K., Woyke, T., DeAngelis, K.M.: Genome sequence and description of the anaerobic lignin-degrading bacterium *Tolomonas lignolytica* sp. nov. *Stand. Genomic Sci.* **10**, 1–11 (2015). <https://doi.org/10.1186/S40793-015-0100-3/FIGURES/5>

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