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RESEARCH ARTICLE

Towards a sustainable energy future: Evaluating *Arundo donax* L. in continuous anaerobic digestion for biogas production

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Abstract

In response to the EU's REPowerEU initiative (COM (2022) 108) which encourages an increase in biogas production by 20% in member states by 2030 to boost energy independence, it has become essential to identify sustainable alternatives to traditional feedstocks for biogas production, especially in the EU Member states where there is still high dependence on corn silage as the main raw material in biogas plants. While corn silage, predominantly used in the European biogas plants today, serves primarily for the livestock sector, alternative sources need to be explored. Therefore, this study aimed to evaluate the potential of *Arundo donax*, a perennial energy crop, as an alternative feedstock in a continuous anaerobic process. The biogas yield and its quality, characterized by CH_4, CO_2, H_2S and O_2 content, were determined during a continuous process with *A. donax*, compared with two mixed feedstocks of *A. donax* and corn silage over a 5-month period in a continuous anaerobic digestion process. The results revealed that *A. donax* exhibits a biogas yield and methane content comparable to corn silage, indicating its potential as a viable and sustainable alternative feedstock for biogas production.

KEYWORDS

Arundo donax L., biogas quality, continuous anaerobic digestion, corn silage, perennial energy crops, sustainable biogas production

1 | INTRODUCTION

The potential for agricultural biomass as a renewable and sustainable energy source was proposed as an energy-generation method back in the 2000s, as it represents a long-term renewable and CO_2-neutral source (Johnson et al., 2007). As the International Energy Agency (IEA, 2021) forecasts continued growth in the deployment of renewable energy in the coming years, driven by the challenges and opportunities of the current global energy crisis, the European Commission (2022) responded with the REPowerEU document (COM (2022) 108) in May 2022, setting an ambitious target to increase biogas production in the EU member states by 20% by 2030.

Biogas is generated through the process of anaerobic digestion, which involves the microbial decomposition of

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biodegradable organic materials in the absence of oxygen, resulting in the production of gas (Bhatia, 2014). Biogas is primarily composed of methane (CH_4) which is a major component of energy production. Other elements that can be found in biogas are carbon dioxide (CO_2), hydrogen sulphide (H_2S) in small amounts, oxygen (O_2) and other trace elements (Teng et al., 2014). The CO_2 content of biogas can also be high (20%–50%), and its presence can reduce the energy content of the gas. So, it needs to be removed or reduced to increase the energy content of the biogas. The presence of H_2S in biogas can cause corrosion and damage to equipment. Therefore, if a certain raw material produces biogas with a high content of H_2S, it is desirable to remove it. Oxygen content in biogas can typically be found in traces, as it is injected during the anaerobic digestion process to lower the H_2S content (Tippayawong & Thanompongchart, 2010).

The exact amount and quality of biogas can vary depending on the feedstock used and the conditions of the anaerobic digestion process (Bharathiraja et al., 2018). Currently, in the European Union, biogas production primarily uses corn silage as a raw material (Sobczak et al., 2022). Corn silage is used in biogas production because it provides exceptionally high green mass yields and, proportionally, a large amount of energy in the process of biogas production (Amon et al., 2007; Fuksa et al., 2020). However, one of the major downsides of its utilization is the direct competition of corn silage use for biogas production with food production, as corn silage is primarily used as animal feed (Kiesel & Lewandowski, 2014). This has significantly affected the price of corn silage, leading to questionable economic viability of biogas production. In response, the Bioenergy Task 37 working group (IEA, 2021) provided guidelines for ensuring sustainable biogas production by using alternatives to currently used corn silage. Additionally, the RED III directive will require biogas plants to certify a sustainable production. Therefore, it is necessary to rapidly start using alternative crops in biogas production that will not have these negative impacts, and with the overall aim of meeting the biogas production goals set in the current EU regulations. To reduce the negative impact of using annual conventional crops in biogas production, sustainability could be achieved by applying perennial energy crops as a raw material (Whittaker et al., 2016). Considering these challenges, it is necessary to identify crops that can replace corn in sustainable energy production. Therefore, the present study aims to evaluate the potential of *Arundo donax*, a perennial energy crop, as an alternative feedstock for biogas production. This could pave the way for more sustainable practices in the biogas sector, ultimately leading to the reduction of the sector's carbon footprint and its overall impact on the environment.

A. donax, commonly known as giant reed, is a perennial rhizomatous grass native to the Mediterranean Basin and Middle East. This crop has gained considerable interest due to its high biomass yield, broad adaptability to different climatic and soil conditions, and low input requirements (Angelini et al., 2009; Corno et al., 2014.). Its robust growth and high productivity make it an attractive feedstock for bioenergy production. Moreover, *A. donax* is not used as a food crop, which eliminates the competition with food production and can contribute to the sustainability of the bioenergy sector (Lewandowski et al., 2003; Manenti et al., 2016). Recent studies such as Ceotto et al. (2021) and Ragaglini et al. (2014) deal with the optimization of *A. donax* for biomethane production. They investigated double harvesting strategies, discovering the possibility of surpassing the methane yield of traditional feedstocks such as corn silage. However, exploring the full potential of *A. donax* in biogas production requires further research, highlighting the need for additional studies to reveal its potential for sustainable bioenergy production (Mantineo et al., 2009).

A. donax used in this study, was harvested during the late summer season and directly utilized as feedstock in a continuous process of anaerobic digestion. The primary aim of this study was to investigate the biogas yield and quality from *A. donax*, comparing it to the conventionally used corn silage. The objective was to evaluate the yield of methane (CH_4), carbon dioxide (CO_2) and hydrogen sulphide (H_2S) as the key performance indicators in the biogas production process. Furthermore, this study aimed to understand the potential impact of using *A. donax* as an alternative feedstock on the overall sustainability and economic viability of biogas production.

2 | MATERIALS AND METHODS

Samples of *A. donax* biomass were collected in September 2021 on the island of Pag (Croatia, 44°29′48″ N 14°57′33″ E), in its natural habitat. After collection, the samples were stored at −18°C. Before the anaerobic digestion (AD) process, the samples were milled to a maximum particle size of <10 mm. For AD setup, the digestate (inoculum) from the nearest biogas plant was taken, as well as the corn silage and cow manure samples. Corn silage and cow manure samples were also stored properly at −18°C. Following the gathering of all samples, the AD process started according to Table 1. Before use, part of the samples was defrosted and prepared into a paste which was stored at 4°C for daily use. In this way, the samples were in optimal condition for AD process.

Continuous AD was performed in a 30-L bioreactor (CROTEH d.o.o., Croatia), at the mesophilic temperatures

TABLE 1 Experimental setup of anaerobic digestion process for each sample.

Sample	Days of AD	Manure (g)	*Arundo donax* (g)	Corn silage (g)
Corn silage, 100%	0–52	75	0	75
A. donax:corn silage (ratio 1:2)	53–202	75	25	50
A. donax:corn silage (ratio 2:1)	203–352	75	50	25
A. donax, 100%	353–502	75	75	0

Abbreviation: AD, anaerobic digestion.

TABLE 2 Substrate to inoculum ratio during AD process.

Sample	Min (g/g)	Max (g/g)	Average (g/g)
Arundo donax:corn silage (ratio 1:2)	0.020	0.065	0.042 ± 0.010
A. donax:corn silage (ratio 2:1)	0.020	0.041	0.032 ± 0.005
A. donax, 100%	0.023	0.061	0.038 ± 0.006

Abbreviation: AD, anaerobic digestion.

of 38 ± 2.0°C for 502 days. The process began with 100% corn silage and cow manure and it lasted for 52 days. In that period only biogas, methane and CO_2 production were monitored. AD process then continued with the substitution of corn silage with chopped *A. donax* samples to achieve the (*A. donax*:corn silage) ratio 1:2 (for 150 days); the process proceeded to the (*A. donax*:corn silage) ratio 2:1 (150 days) until a 100% *A. donax* was reached (for 150 days). The production of biogas and methane (NL day^{-1}) was monitored and the quality of biogas from each setup was compared. The bioreactor was fed every 24 ± 2 h to maintain a consistent organic loading rate (OLR) with initial analysis of dry matter (%), volatile solids (%) and chemical oxygen demand (COD) (mg kg^{-1} DM) performed before adding the substrate paste. This preparation ensured accurate tracking and optimization of the AD process. Sampling of the digestate and substrate was also conducted to monitor the reactor's performance and to adjust the set-up of AD process if needed. The Hydraulic Retention Time was maintained at 50 ± 2 days, depending on substrate concentration and recirculating digestate, with the substrate-to-inoculum ratio managed throughout the AD process to ensure optimal microbial activity and biogas production provided in Table 2. For OLR calculation next equation was used:

$$\text{OLR} = \left(\text{VS(kg/day)} / \text{reactor volume}\left(\text{m}^3\right)\right). \quad (1)$$

In the study, biogas concentrations were measured using a portable multi-gas detector Dräger X-am 7000 (Dräger, Germany). Detector was equipped with a range of four sensors, each specifically designed to detect a particular gas. For the purpose of this research, the device was calibrated and utilized to precisely measure the amount of CH_4 (methane), CO_2 (carbon dioxide) and concentrations of O_2 (oxygen) and H_2S (hydrogen sulphide). The biogas volume was determined by a water displacement measurement device, normalised to standard (normalised) conditions (273 K, 1013 mbar).

In order to evaluate the anaerobic digestion process, calculations such as biogas production rate (BPR) (2), specific biogas production (SBP) (3) and specific methane production (SMP) (4) were employed using provided equations:

$$\text{BPR} = (\text{biogas (L)} / \text{reactor volume (L)} / \text{day}), \quad (2)$$

$$\text{SBP} = (\text{biogas (L)} / \text{mass of VS added (kg)}), \quad (3)$$

$$\text{SMP} = (\text{methane (L)} / \text{mass of VS added (kg)}). \quad (4)$$

All data were collected and statistically analysed. The data has been grouped by month, with each month representing a period of 30 days, to make it easier to interpret. Statistical analysis was performed by using TIBCO STATISTICA 13.3.0 software (StatSoft TIBCO Software Inc., Palo Alto, CA, USA). For the purpose of understanding the accumulated data, several statistical techniques were used including the computation of mean values, standard deviations, Tukey's HSD test ($p < 0.05$) to compare means, correlation analysis, univariate analysis and principal component analysis (PCA).

3 | RESULTS

The inoculum used in the anaerobic digestion process was analysed for several key parameters like dry matter content which was determined to be 8.557 ± 0.084% as shown in Table 3. It contained a high percentage of organic matter and organic carbon, measured at 97.62 ± 0.014%

and 46.785 ± 0.629%. Total nitrogen was determined at 0.405 ± 0.007% and ammonia nitrogen at 0.23 ± 0.001% with the presence of total phosphorus at 0.15 ± 0.001%. Lastly, FOS/TAC ratio was 0.3 ± 0.006 with FOS (fatty organic substances) 4548 ± 132,936 mg kg^{-1} and TAC (total alkalinity) 15,147 ± 140,007 mg kg^{-1}.

Table 4 shows the input feedstock data analyzed for dry matter (DM), volatile solids (VS) and COD as indicators of substrate quality for potential biogas production. The samples DM content remained relatively stable across the 5 months, ranging from a minimum of 0.06% to a maximum of 0.12%, indicating a marginal fluctuation in the DM percentage. The VS analysis, which represents the organic fraction of the DM, showed a slightly broader range, from 0.85% to 0.93%, suggesting a modest variability in the organic matter content within the biomass. Chemical oxygen demand was observed to vary considerably between 50,846.25 and 73,811.25 mg kg^{-1} DM.

Table 5 presents the output feedstock parameters from the biogas production process. The DM content varied minimally, ranging from 0.03% to 0.06%, indicating a low variability in the dry matter across the samples and throughout the months. The VS content, representing showed a slightly higher range of 0.69% to 0.80%. The pH values, which are crucial for maintaining microbial health and efficacy in biogas systems, stayed within a narrow optimal range for anaerobic digestion, fluctuating between 7.18 and 7.71. This stability in pH is suitable for biogas production processes. COD exhibited a broader range of 27,890–49,801 mg kg^{-1} DM.

Figure 1 shows OLR trends during AD process for used substrates determined using Equation (1). From Figure 2, it can be observed that the OLR values for 100% *A. donax* show fluctuations, ranging from approximately 1.43 to 2.15 kg m^{-3} day^{-1}, with a noticeable peak towards the latter part of the AD period. The 2:1 *A. donax* and corn silage mixture shows a relatively lower and more stable OLR, maintaining levels generally below 1.5 kg m^{-3} day^{-1}, with only

TABLE 3 Initial analysis of digestate (inoculum).

	Inoculum
DM (%)	8.557 ± 0.08
Organic matter (%/DM)	97.62 ± 0.01
Organic carbon (%/DM)	46.785 ± 0.63
Total nitrogen (%)	0.405 ± 0.01
Ammonia nitrogen (%)	0.23 ± 0.01
Total phosphorus (%/DM)	0.15 ± 0.01
FOS (mg kg^{-1})	4548 ± 132.94
TAC (mg kg^{-1})	15,147 ± 140.01
FOS/TAC	0.3 ± 0.01

Abbreviations: DM, dry matter; FOS, fatty organic substances; TAC, total alkalinity.

TABLE 4 Initial substrate analysis in AD process.

Sample	Month	DM (%)	VS (%)	COD (mg kg^{-1} DM)
Arundo donax:corn silage (ratio 1:2)	1	0.07 ± 0.01^{cba}	0.92 ± 0.02^{cb}	$55,233.75 \pm 3419.52^{cba}$
	2	0.07 ± 0.01^{cba}	0.91 ± 0.03^{cb}	$52,703.75 \pm 5258.65^{ba}$
	3	0.06 ± 0.01^{a}	0.92 ± 0.04^{c}	$59,132.22 \pm 3474.51^{edcba}$
	4	0.07 ± 0.01^{cba}	0.9 ± 0.02^{cb}	$57,598.75 \pm 4237.17^{dcba}$
	5	0.08 ± 0.02^{cba}	0.85 ± 0.05^{a}	$55,551.25 \pm 3907.81^{cba}$
A. donax:corn silage (ratio 2:1)	1	0.06 ± 0.01^{ba}	0.91 ± 0.02^{cb}	$58,946.25 \pm 3342.36^{edcba}$
	2	0.08 ± 0.01^{dcb}	0.9 ± 0.02^{cb}	$65,547.14 \pm 3554.72^{hgfed}$
	3	0.07 ± 0.01^{cba}	0.93 ± 0.02^{c}	$67,521.11 \pm 5643.82^{hgfe}$
	4	0.08 ± 0.02^{dcb}	0.89 ± 0.02^{cba}	$68,855 \pm 10,596.40^{hgf}$
	5	0.09 ± 0.01^{edc}	0.87 ± 0.04^{ba}	$50,846.25 \pm 4818.67^{a}$
A. donax 100%	1	0.11 ± 0.01^{fe}	0.93 ± 0.02^{c}	$70,808.75 \pm 3524.03^{hg}$
	2	0.09 ± 0.01^{dc}	0.93 ± 0.02^{c}	$71,616.67 \pm 2036.1^{hg}$
	3	0.12 ± 0.01^{f}	0.91 ± 0.02^{cb}	$73,811.25 \pm 5176.34^{h}$
	4	0.11 ± 0.01^{fe}	0.89 ± 0.04^{cba}	$63,291.25 \pm 5019.10^{gfedc}$
	5	$0.1 \pm 0.02d^{fe}$	0.9 ± 0.02^{cb}	$60,983.75 \pm 7047.31^{fedcb}$
Minimum		0.06	0.85	50,846.25
Maximum		0.12	0.93	73,811.25
Statistical significance		*	*	*

Note: Different letters in the same column represent the difference between the observed values according to the Tukey HSD post hoc test ($*p < 0.05$).

Abbreviations: AD, anaerobic digestion; COD, chemical oxygen demand; DM, dry matter; VS, volatile solids.

TABLE 5 Output substrate analysis of AD process.

Sample	Month	DM (%)	VS (%)	pH value	COD (mgO kg^{-1} DM)
Arundo donax:corn silage (ratio 1:2)	1	0.05 ± 0^{hg}	0.71 ± 0.01^{ba}	7.4 ± 0.17^{dcb}	$46{,}302.5 \pm 2747.08^{fe}$
	2	0.05 ± 0^{gfed}	0.75 ± 0.04^{ba}	7.46 ± 0.07^{edc}	$42{,}666.25 \pm 3021.18^{fed}$
	3	0.04 ± 0^{ba}	0.8 ± 0.04^{b}	7.28 ± 0.08^{cba}	$39{,}707.78 \pm 3069.6^{edc}$
	4	0.03 ± 0.01^{a}	0.76 ± 0.09^{ba}	7.2 ± 0.06^{a}	$28{,}440 \pm 6073.2^{a}$
	5	0.04 ± 0.01^{cba}	0.7 ± 0.1^{ba}	7.32 ± 0.13^{cba}	$27{,}890 \pm 3653.53^{a}$
A. donax:corn silage (ratio 2:1)	1	0.05 ± 0^{h}	0.7 ± 0.02^{ba}	7.65 ± 0.27^{fe}	$49{,}761.25 \pm 3506.09^{f}$
	2	0.05 ± 0^{hgfed}	0.75 ± 0.01^{ba}	7.56 ± 0.11^{fed}	$44{,}697.14 \pm 3843.88^{fed}$
	3	0.04 ± 0^{dcb}	0.78 ± 0.03^{ba}	7.31 ± 0.1^{cba}	$49{,}801.11 \pm 2916.46^{f}$
	4	0.04 ± 0.01^{fedc}	0.77 ± 0.03^{ba}	7.2 ± 0.05^{a}	$39{,}713.75 \pm 11{,}694.43^{edc}$
	5	0.04 ± 0^{edc}	0.72 ± 0.05^{ba}	7.2 ± 0.09^{ab}	$30{,}591.25 \pm 7098.11^{ba}$
A. donax 100%	1	0.05 ± 0^{hgfe}	0.75 ± 0.03^{ba}	7.71 ± 0.17^{f}	$48{,}382.22 \pm 3011.05^{f}$
	2	0.05 ± 0^{gfedc}	0.76 ± 0.03^{ba}	7.31 ± 0.15^{cba}	$48{,}586.67 \pm 5517.18^{f}$
	3	0.05 ± 0.01^{hgf}	0.69 ± 0.18^{a}	7.25 ± 0.06^{ba}	$37{,}040 \pm 2271.62^{dcb}$
	4	0.05 ± 0.01^{hgfed}	0.78 ± 0.06^{ba}	7.19 ± 0.08^{a}	$33{,}185 \pm 1669.83^{cba}$
	5	0.06 ± 0^{h}	0.78 ± 0.02^{ba}	7.18 ± 0.06^{a}	$33{,}108.75 \pm 1239.73^{cba}$
Minimum		0.03	0.69	7.18	27,890.00
Maximum		0.06	0.80	33.31	49,801.11
Statistical significance		*	*	*	*

Note: Different letters in the same column represent the difference between the observed values according to the Tukey HSD post hoc test ($*p < 0.05$).
Abbreviations: AD, anaerobic digestion; COD, chemical oxygen demand; DM, dry matter; VS, volatile solids.

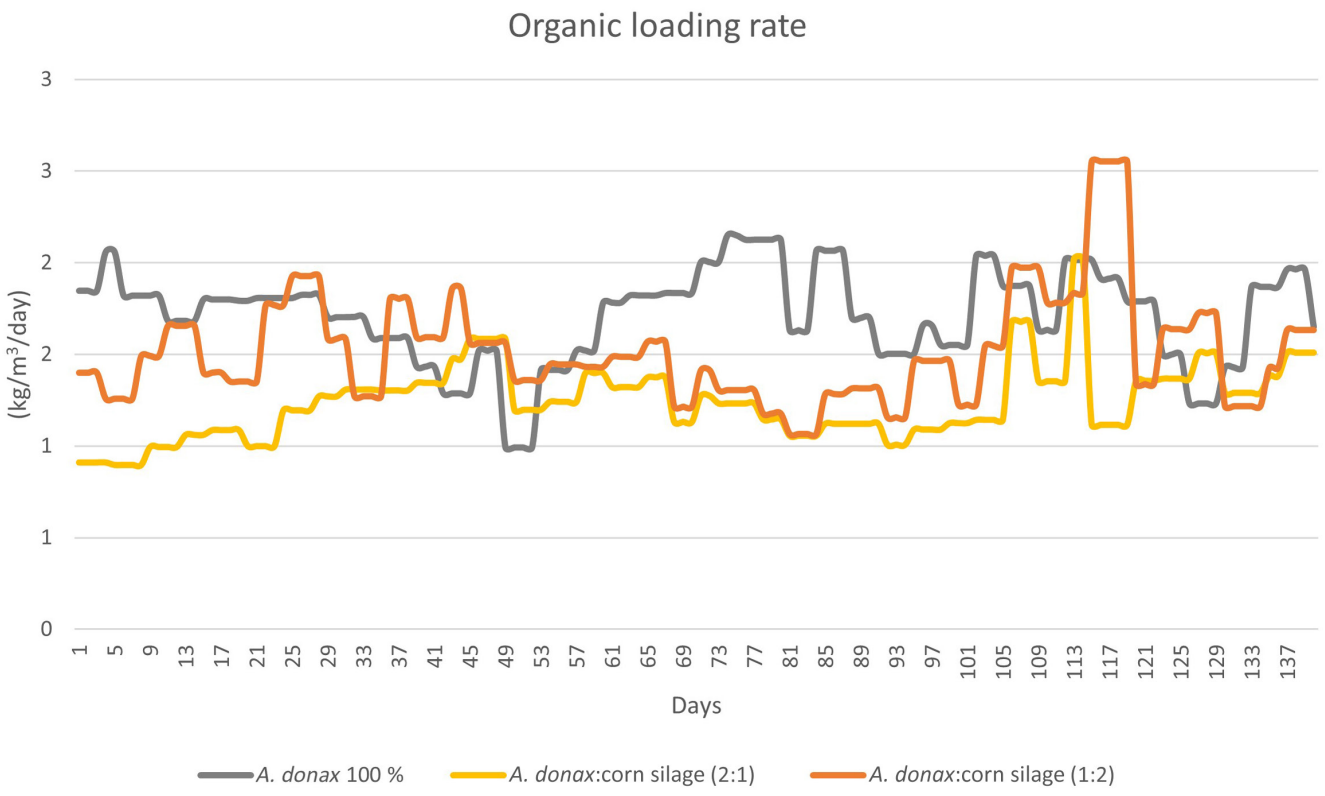


FIGURE 1 Organic loading rate during anaerobic digestion process.

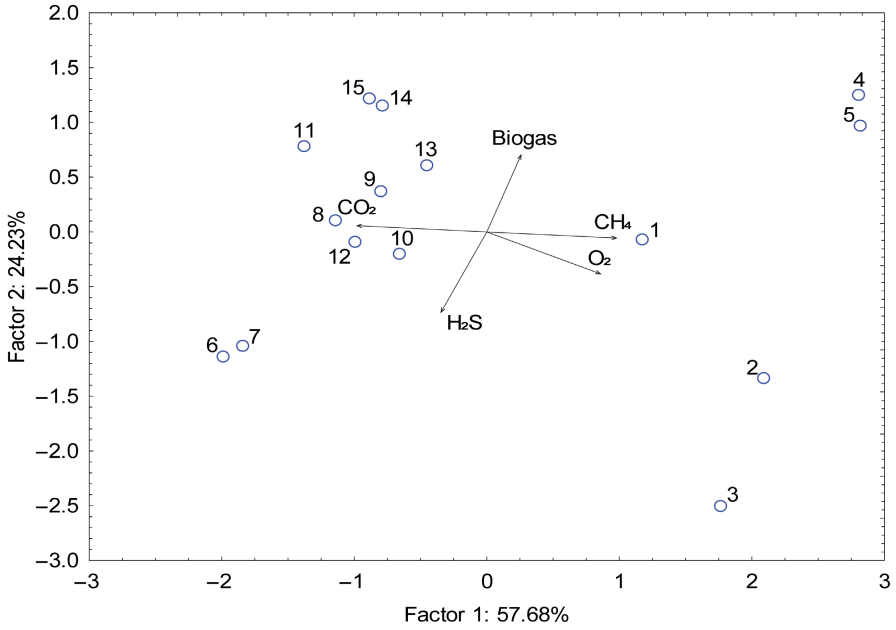


FIGURE 2 Principal component analysis of observed values.

TABLE 6 Mean values of oxygen, hydrogen sulphide, carbon dioxide, methane and biogas of investigated samples.

Sample		Month	O_2 (ppm)	H_2S (ppm)	CO_2 (%)	CH_4 (%)	Biogas (NL day^{-1})
1	*Arundo donax*:corn silage (ratio 1:2)	1	0.99 ± 0.62^{ba}	$225.80 \pm 148.13^{gfecd}$	41.77 ± 2.87^{ba}	58.23 ± 2.87^{cb}	22.76 ± 4.13^{fed}
2		2	1.40 ± 1.16^{b}	292.90 ± 162.92^{gfe}	40.23 ± 4.23^{a}	59.77 ± 4.23^{c}	21.69 ± 3.29^{dcb}
3		3	1.41 ± 0.72^{b}	321.87 ± 142.21^{gf}	40.40 ± 4.86^{a}	59.6 ± 4.86^{c}	17.02 ± 3.08^{a}
4		4	1.10 ± 0.51^{ba}	101.37 ± 116.12^{ba}	37.98 ± 2.39^{a}	62.02 ± 2.39^{c}	25.39 ± 6.10^{fe}
5		5	1.09 ± 0.47^{ba}	75.90 ± 70.49^{a}	37.76 ± 4.29^{a}	62.25 ± 4.29^{c}	22.96 ± 3.56^{dfe}
	Mean		1207 ± 0.766^{b}	$213{,}607 \pm 164{,}635^{a}$	$39{,}759 \pm 4043^{a}$	$60{,}242 \pm 4043^{b}$	$21{,}893 \pm 5037^{b}$
6	*A. donax*:corn silage (ratio 2:1)	1	0.51 ± 1.09^{a}	331.40 ± 130.96^{g}	48.01 ± 2.95^{c}	51.99 ± 2.95^{a}	18.19 ± 5.05^{ba}
7		2	0.64 ± 0.85^{a}	258.53 ± 135.76^{gfed}	48.53 ± 3.52^{c}	51.47 ± 3.52^{a}	16.21 ± 6.53^{a}
8		3	0.71 ± 0.69^{a}	168.67 ± 113.94^{dcba}	47.75 ± 8.34^{c}	52.25 ± 8.34^{a}	18.83 ± 3.92^{cba}
9		4	0.46 ± 0.40^{a}	116.47 ± 97.05^{cba}	45.50 ± 3.41^{cb}	54.50 ± 3.41^{ba}	16.6 ± 3.20^{a}
10		5	0.56 ± 0.70^{a}	283.25 ± 99.15^{gfed}	44.84 ± 2.41^{cb}	55.16 ± 2.41^{ba}	21.64 ± 3.00^{edcba}
	Mean		0.576 ± 0.78^{a}	$229{,}507 \pm 141{,}05^{a}$	$47{,}076 \pm 4913^{b}$	$52{,}925 \pm 4913^{a}$	$18{,}056 \pm 4896^{a}$
11	*A. donax*, 100%	1	0.65 ± 0.85^{a}	265.27 ± 115.77^{gfed}	48.50 ± 8.22^{c}	51.50 ± 8.22^{a}	25.90 ± 2.65^{f}
12		2	0.61 ± 0.60^{a}	279.17 ± 134.62^{gfe}	46.34 ± 3.59^{c}	53.66 ± 3.59^{a}	22.12 ± 4.68^{edc}
13		3	0.72 ± 0.81^{a}	202.07 ± 144.76^{edcb}	45.91 ± 3.41^{c}	54.09 ± 3.41^{a}	23.12 ± 2.80^{fed}
14		4	0.57 ± 0.59^{a}	218.90 ± 108.27^{fedc}	46.45 ± 2.89^{c}	53.55 ± 2.89^{a}	25.69 ± 1.13^{f}
15		5	0.65 ± 0.97^{a}	151.15 ± 83.94^{dcba}	47.43 ± 3.08^{c}	52.57 ± 3.08^{a}	23.43 ± 3.64^{fed}
	Mean		0.638 ± 0.753^{a}	$231{,}014 \pm 128{,}416^{a}$	$46{,}891 \pm 4813^{b}$	53.11 ± 4813^{a}	$24{,}097 \pm 3479^{c}$
Min			0.46	75.90	37.76	51.47	16.21
Max			1.41	331.40	48.53	62.25	25.90
Average			0.80	219.51	44.49	55.51	21.43
Statistical significance			*	*	*	*	*

Note: Means values with standard deviation and statistical significance *$p \leq 0.01$ according to Tukey's HSD test showed as lowercase superscripts.

a few instances exceeding this mark. In contrast, the 1:2 mixture exhibits a broader fluctuation in OLR, with values oscillating between roughly 1.25 and 2.55 kg m^{-3} day^{-1}, indicating a more dynamic response to the feeding protocol.

Table 6 shows the differences in the composition of biogas. The biogas volume was expressed in NL day^{-1}. The feedstocks used in the analysis include (i) a mixture of *A. donax* and corn silage at a 1:2 ratio, (ii) a mixture of *A.*

donax and corn silage at a 2:1 ratio and (iii) 100% *A. donax*. While examining the biogas production across various samples, several observations were found.

The volume of biogas generated demonstrated a substantial variability depending on the sample and month of observation. In the first sample (*A. donax* 100%), and during the 1st month, a volume of 25.9 ± 2.65 NL was recorded, which depicted one of the highest biogas yields among the subsequent months. This volume slightly decreased in the 2nd month to 22.12 ± 4.68 NL day^{-1} and fluctuated over the next few months, with the 4th month almost matching the initial volume at 25.69 ± 1.13 NL day^{-1}.

For the second sample (a 2:1 mixture of *A. donax* and corn silage), the volume of biogas produced was lower than that produced from the *A. donax* 100% sample, with the volumes ranging from 16.21 ± 6.53 NL day^{-1} in the 2nd month to 21.64 ± 3.00 NL day^{-1} in the 5th month of sample digestion. Furthermore, the third sample (a 1:2 mixture of *A. donax* and corn silage) exhibited varied biogas production volumes among the three sample types. The highest volume in this sample was recorded in the 4th month at 25.39 ± 6.10 NL day^{-1}. However, the 3rd month showed a decrease to 17.02 ± 3.08 NL day^{-1}. To summarize, the average biogas volume across all samples was 21.43 NL day^{-1}, with the minimum and maximum volumes being 16.21 and 25.90 NL day^{-1}, respectively.

Oxygen content, a typically low component in anaerobic environments, remained consistent with this expectation across all samples. With a mean value of 0.80 ppm, it fluctuated between the narrow margins of 0.46 and 1.41 ppm. Although these variations exist, they highlight the predominantly AD conditions maintained throughout the biogas production process. Hydrogen sulphide (H_2S) exhibited a significant variability across samples, ranging from a minimal 75.90 ± 70.49 ppm to a considerable 331.40 ± 130.96 ppm in the blend of *A. donax* and corn silage at the 2:1 ratio. Carbon dioxide (CO_2) levels hovered between 37.76% and 48.53%, achieving an average concentration of 44.49%. Given that CO_2 is a typical by-product of AD, it maintained a relatively stable range. The primary biogas component, methane, exhibited an average concentration for all samples of 55.51%, with a peak concentration of 62.25% observed in the mixture at a 1:2 ratio of *A. donax* to corn silage. In contrast, the 2:1 mixture ratio had the lowest methane concentration at 51.47%.

Table 7 presents the correlation coefficients among different variables pertaining to biogas composition and production. Oxygen (O_2) displays a slight negative relationship with H_2S at −0.24, a moderate negative correlation with carbon dioxide (CO_2) at −0.44, a moderate positive link with methane (CH_4) at 0.44, and a positive association, but not significant with biogas volume at 0.71. Hydrogen sulphide (H_2S) reveals a slight positive relationship with CO_2 at 0.17, a minor negative association with CH_4 and biogas volume at −0.17. Carbon dioxide (CO_2) and methane (CH_4) have a perfectly inverse relationship at −1.00. As CO_2 levels increase, CH_4 equivalently decreases and vice versa. Lastly, CO_2 shows almost no correlation and significance with the biogas volume, evident with a value of −0.06, while methane (CH_4) exhibits a negligible positive correlation with biogas volume at 0.06, but also not significant.

Table 8 outlines a univariate ANOVA's findings, assessing the impact of different samples (df = 2) and months (df = 4) on gas variables (O_2, H_2S, CO_2, CH_4 and biogas volume). The interaction between the samples and months was also considered (Sample × Month, df = 8). Significantly, "Sample" and "Month," both independently and in the interaction, contribute to variances in O_2, CO_2, CH_4 and the overall biogas volume. Particularly for H_2S, its variability is more influenced by "Month" and the interaction term rather than the "Sample" alone.

Principal component analysis in Figure 2. Shows complex connections between various samples and their relationship to specific gas variables. The proximity of sample numbers to specific gas labels provides insight into their respective concentrations. Samples 9, 11, 13, 14 and 15 seem to be positively associated with the biogas, suggesting higher biogas concentrations in these samples. Conversely, samples 2 and 3 lean towards the O_2. Samples 1, 4 and 5 are closely related to CH_4, indicating a potential higher concentration of methane in these samples. Moreover, samples 6, 7, 8, 12 and 10 exhibit a strong association with CO_2, suggesting they have

TABLE 7 Correlation of the observed variables.

Variable	O_2	H_2S	CO_2	CH_4	Biogas
O_2	1.00	−0.24*	−0.44*	0.44*	0.71
H_2S	−0.23*	1.00	0.17*	−0.17*	−0.17*
CO_2	−0.44*	0.18*	1.00	−1.00*	−0.06
CH_4	0.44*	−0.18*	−1.00*	1.00	0.06
Biogas	0.71	0.17	−0.06	0.06	1.00

Note: Statistical significance *$p < 0.01$.

TABLE 8 Univariate analysis of individual gas volumes across samples and months.

		SoS				
Parameter	**df**	**O_2**	**H_2S**	**CO_2**	**CH_4**	**Biogas**
Intercept	1	264.66*	19,712,497.00*	809,862.60*	1,260,442.00*	188,001.20*
Sample	2	32.18*	60,385.00	4846.30*	4846.00*	2316.9.90*
Month	4	4.05	1,262,222.00*	462.40*	462.00*	735.60*
Sample × Month	8	1.95	1,202,533.00*	257.70	258.00	1160.00*
Error	405	238.92	6,360,391.00	8127.50	8128.00	6645.90

Note: Statistical significance *$p < 0.01$.

Abbreviations: df, degrees of freedom; SoS, sum of squares.

TABLE 9 Biogas production rate for each sample and month.

	Sample		
Month	***Arundo donax*, 100%**	***A. donax*:corn silage (ratio 2:1)**	***A. donax*:corn silage (ratio 1:2)**
1	0.86 ± 0.09^{f}	0.61 ± 0.17^{ab}	0.76 ± 0.14^{def}
2	0.74 ± 0.16^{cde}	0.54 ± 0.22^{a}	0.72 ± 0.11^{bcd}
3	0.77 ± 0.09^{def}	0.63 ± 0.13^{abc}	0.57 ± 0.10^{a}
4	0.86 ± 0.04^{f}	0.55 ± 0.11^{a}	0.85 ± 0.20^{ef}
5	0.78 ± 0.12^{def}	0.72 ± 0.10^{bcde}	0.77 ± 0.12^{def}

Note: Means values with standard deviation and statistical significance *$p \leq 0.01$ according to Tukey's HSD test showed as lowercase superscripts.

elevated levels of carbon dioxide. Interestingly, while H_2S is graphically represented, its influence across the given samples seems minimal. This suggests that hydrogen sulphide might not significantly contribute to the overall variability in these samples. From the PCA, Factor 1 accounts for 57.76% of the variance, whereas Factor 2 accounts for 24.14%. Collectively, these two factors explain 81.9% of the total variability in the data. This leaves a mere 18.1% of the variance unexplained by these two principal components.

Methane (CH_4) and carbon dioxide (CO_2) emerge as the predominant components, which not only dictate the energy content of the biogas but also its combustion characteristics and environmental impact. Typically expressed in volume percentages (%), the ratio of CH_4 to CO_2 can significantly influence the calorific value of the gas, its combustion efficiency and its greenhouse gas emissions profile.

Table 9 presents the BPRs according to Equation (2). For the sample of *A. donax* (100%), the BPR was highest in the 1st month, with a mean of 0.86 ± 0.09 NL L^{-1} day^{-1} and remained relatively consistent over the 5-month period, ranging from 0.74 ± 0.16 NL L^{-1} day^{-1} in the 2nd month to 0.86 ± 0.04 NL L^{-1} day^{-1} in the 4th month. The 2:1 mixture of *A. donax* and corn silage exhibited its highest BPR in the 5th month at 0.72 ± 0.10 NL L^{-1} day^{-1}. However, the lowest production rate for this mixture was observed in the 2nd month, with a value of 0.54 ± 0.22 NL L^{-1} day^{-1}. For the 1:2 mixture of *A. donax* and corn silage, the 4th month recorded the highest production rate of 0.85 ± 0.2 NL L^{-1} day^{-1}. The 3rd month displayed the lowest rate at 0.57 ± 0.10 NL L^{-1} day^{-1} for this sample mixture.

The results in Table 10 demonstrate that SBP and SMP obtained using Equations (3) and (4) across different feedstock ratios show variability. For the 100% *A. donax* feedstock, SBP values ranged from 219.39 ± 32.10 L kg^{-1} VS in the 3rd month to 281.01 ± 78.63 L kg^{-1} VS in the 2nd month, with corresponding SMP values ranging from 408.31 ± 71.80 L kg^{-1} VS to 527.87 ± 148.67 L kg^{-1} VS. In the 2:1 *A. donax* to corn silage mixture, SBP values showed a minimum of 208.25 ± 91.71 L kg^{-1} VS in the 2nd month and a maximum of 304.45 ± 90.72 L kg^{-1} VS in the 1st month, with SMP values observing a similar trend. The 1:2 mixture of *A. donax* to corn silage exhibited the highest SBP and SMP values across all samples and months, with peak SBP at 385.13 ± 57.15 L kg^{-1} VS and peak SMP at 618.84 ± 83.26 L kg^{-1} VS in the 5th month.

Figure 3 reports the biogas composition using 100% of *A. donax*. The experiment yielded a wide range of biogas production volumes, spanning from a minimum of 4.46 NL day^{-1} to a maximum of 31.57 NL day^{-1}. CO_2 content in the biogas ranged from 28.74% to 64.68%, while CH_4 content varied between 35.93% and 71.25%. These results

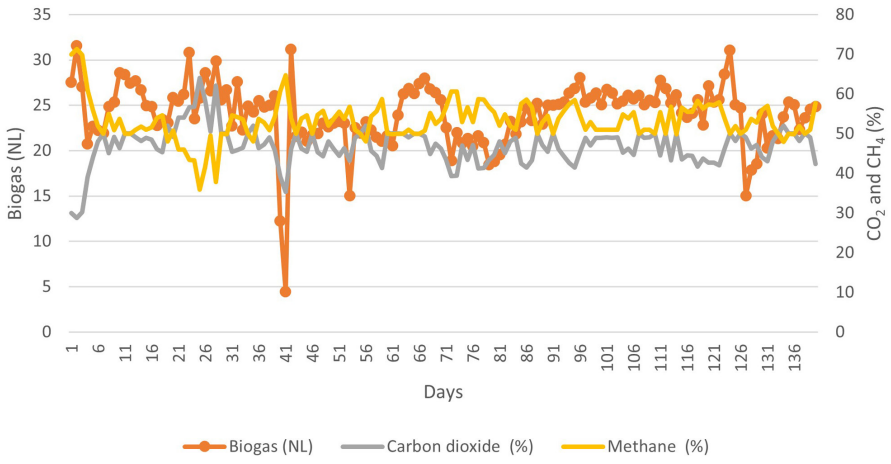
TABLE 10 Specific biogas and methane production expressed in volatile solids.

Sample	Month	SBP (L kg^{-1} VS)	SMP (L kg^{-1} VS)
Arundo donax:corn silage (ratio 1:2)	1	597.93 ± 114.2^{gf}	349.39 ± 76.09^{fe}
	2	576.18 ± 89.13^{gfed}	345.35 ± 64.77^{fe}
	3	$524.43 \pm 96.4g^{fedc}$	313.78 ± 68.44^{ed}
	4	605.05 ± 105.94^{g}	375.21 ± 66.96^{f}
	5	618.84 ± 83.26^{g}	385.13 ± 57.15^{f}
Average		582.03 ± 103.41^{b}	351.53 ± 71.01^{b}
A. donax:corn silage (ratio 2:1)	1	585.63 ± 170.64^{gfe}	304.45 ± 90.72^{edc}
	2	401.78 ± 169.05^{a}	208.25 ± 91.71^{a}
	3	$525.03 \pm 106.41^{gfedc}$	275.78 ± 77.49^{dcb}
	4	458.12 ± 128.49^{cba}	249.1 ± 69.93^{cba}
	5	$518.01 \pm 78.67^{gfedcb}$	286.21 ± 49.22^{edc}
Average		496.26 ± 151.33^{a}	263.23 ± 85.13^{a}
A. donax 100%	1	479.99 ± 59.18^{dcba}	246.69 ± 49.83^{cba}
	2	$527.87 \pm 148.67^{gfedc}$	281.01 ± 78.63^{dc}
	3	408.3 ± 71.8^{ba}	219.39 ± 32.1^{ba}
	4	492.27 ± 64.87^{edcba}	263.59 ± 37.88^{dcba}
	5	$491.87 \pm 90.56^{fedcba}$	258.85 ± 50.08^{dcba}
Average		479.22 ± 100.36^{a}	253.55 ± 55.82^{a}
Minimum		401.78	208.25
Maximum		618.84	385.13
Statistical significance		*	*

Note: Different letters in the same column represent the difference between the observed values according to the Tukey HSD post hoc test (*$p < 0.05$).

Abbreviations: SBP, specific biogas production; SMP, specific methane production.

FIGURE 3 Biogas quality of *Arundo donax* sample.



indicate the fluctuations in the gas composition, suggesting potential variations in the balance of the AD process. Among the readings, the highest CH_4 content was observed at 71.25%, highlighting the suitability of *A. donax* for methane production.

The results detailed in Figure 4 delineates the results from an experimental assessment of biogas production using a mixture of *A. donax* and corn silage at a 2:1 ratio. The volume of biogas obtained from the *A. donax* and corn silage mixture fluctuates considerably, with readings as low as 0.07 NL day^{-1} to as high as 31.57 NL day^{-1}. This variance potentially indicates an inconsistency in the fermentation process or other external factors impacting the biogas production. The collected biogas predominantly comprises CH_4 and CO_2. The CO_2 volume percentage varies between approx. 31% and 64%, while the CH_4 volume percentage inversely spans from approx. 35% to 68%. It is evident that the methane content, essential for the energy

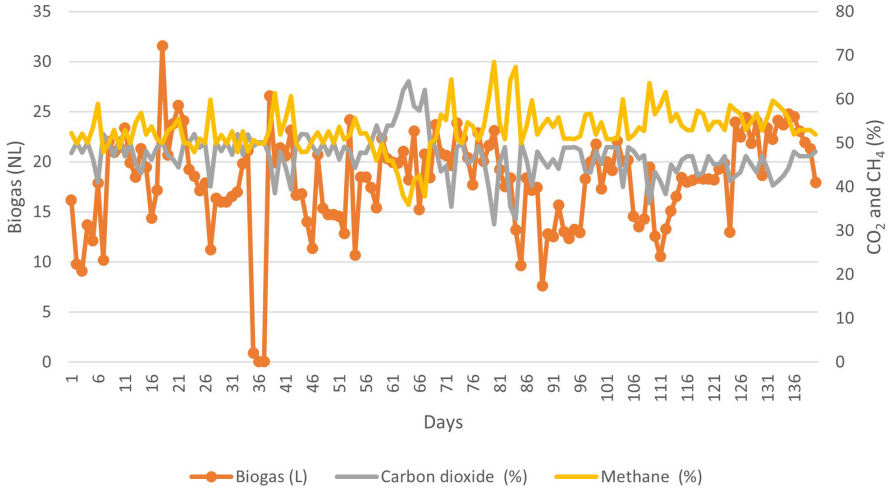


FIGURE 4 Biogas quality of *Arundo donax* and corn silage in ratio 2:1.

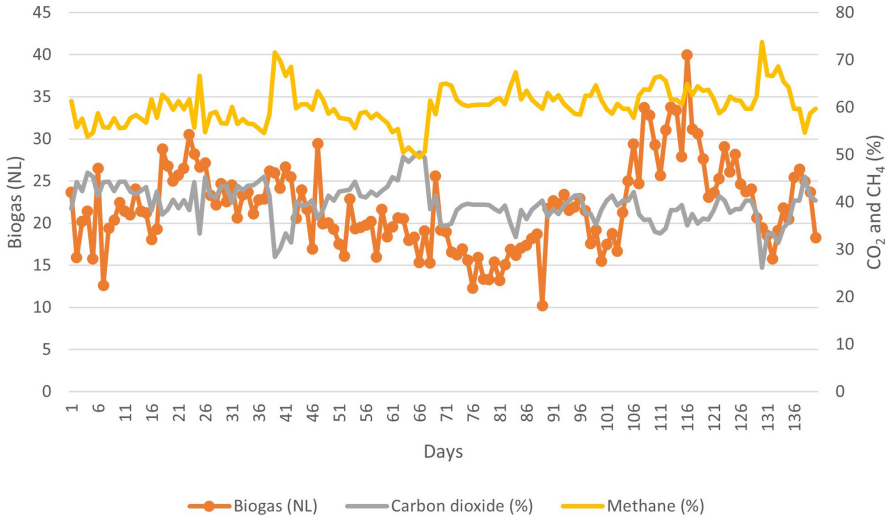


FIGURE 5 Biogas quality of *Arundo donax* and corn silage in ratio 1:2.

production, varies substantially across all investigated samples.

The data presented in Figure 5 show the lowest observed daily output was found to be 10.21 NL day^{-1}, whilst the highest recorded output reached 39.95 NL day^{-1}. Over the course of the experiment, the daily biogas output exhibited considerable variations. These fluctuations were not distinctly progressive, displaying both peaks and troughs throughout the AD. Regarding the composition of the biogas, the methane content also exhibited a wide range of values, with the highest and lowest percentages standing at 73.97% and 49.48% respectively. As with biogas production, the CH_4 percentages also did not follow a clear trend, varying greatly over the experimental period. The CO_2 composition in the biogas mirrored the variability seen in the methane content, however, in a reverse trend. The lowest CO_2 percentage recorded was 26.21%, with the maximum reaching 50.52%. As the methane percentage in biogas decreased, the CO_2 percentage conversely increased, and vice versa which was expected.

The data in Figure 6 describe the daily production of biogas from corn silage during the first period of 52 days of reactor set-up. The measurements also include the corresponding shares of methane (CH_4) and carbon dioxide (CO_2) within the produced biogas. Over the observed period, there was a noticeable variability in the volume of biogas produced daily. The minimum biogas output observed was 5.12 NL day^{-1} on Day 3, while the maximum output was 24.30 NL day^{-1} recorded on Day 36. As a general trend, an increase in biogas production was observed towards the later part of the experimental period. The methane content in the biogas also exhibited considerable fluctuations over time. At the onset of the experiment, methane content was relatively high, peaking at 74.76% on Day 1. However, the subsequent trend generally indicated a decline over time, interspersed with occasional rises. Contrarily, the percentage of CO_2 in the biogas displayed an opposite trend. Although this too was punctuated with periodic dips, the general trend showed an increase over time.

FIGURE 6 Biogas quality of corn silage.

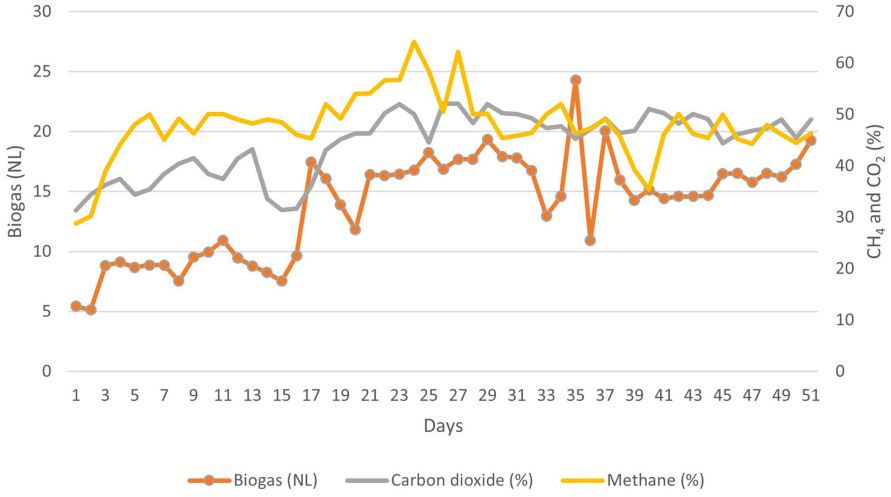
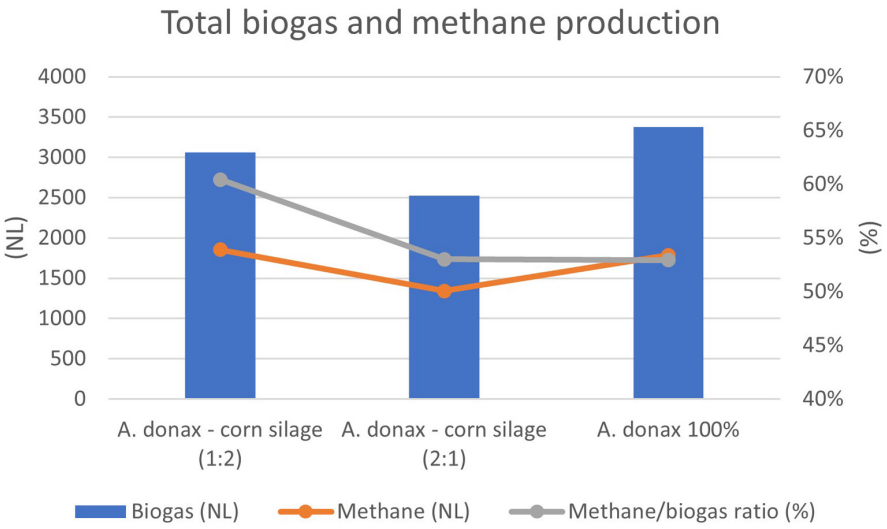


FIGURE 7 Overall biogas and methane production for all samples during a 5-month continuous anaerobic digestion.



The data presented in Figure 7 shows the overall biogas and methane production measured from three different feedstock and their mixtures during a 5-month continuous AD for each sample. From Figure 7, it can be observed that a 100% *A. donax* sample yielded the highest volume of biogas as already mentioned. The lowest biogas yield was found to be with the 2:1 mixture of *A. donax* and corn silage. While the 100% *A. donax* sample had the highest biogas volume, its methane to biogas ratio was much lower compared to the 1:2 which had the highest methane to biogas ratio overall 5-month continuous AD process.

4 | DISCUSSION

The research on various RES options is crucial to meet growing energy demands and reduce the environmental impact associated with fossil fuel use; studies on biogas production from feedstocks such as *A. donax* as an alternative to corn silage are important from the sustainability point of view as well. This study aimed to understand the biogas potential and quality from different mixtures of *A. donax* and corn silage. *A. donax* has been previously explored as a substrate for biogas production (Corno et al., 2016), showcasing its potential as an alternative to corn silage. While it exhibits satisfactory biogas production capabilities (Ragaglini et al., 2014), there is a need to determine the quality of the produced gas (Corno et al., 2015), primarily by analysing the proportions of CH_4, CO_2, H_2S and O_2. In this study, it was found that the composition of gases was predominantly constituted of CH_4 and CO_2 as expected for biogas production, followed by low volumes of H_2S and O_2.

Upon analysing the obtained results, primarily focusing on biogas production, significant disparities among the investigated feedstocks are discerned. Intriguingly, the 100% feed of *A. donax* yielded the highest volume of biogas, amounting to 25.90 NL day^{-1} in the 1st month of production. This sample maintained relatively consistent production values throughout the anaerobic digestion

process. From Figure 7, it is apparent that the cumulative biogas production over a span of 5 months is the highest for 100% *A. donax* and the lowest when utilizing a 2:1 mixture. Giertl et al. (2022) reported that the average cumulative biogas produced amounted to 641 dm^3 for corn silage, and 351.5 dm^3 for *A. donax* during 600 h of anaerobic digestion process. This highlights that corn silage generated a larger cumulative quantity of biogas. However, contrasting with this observation, the current study exhibited that corn silage did not result in an increase in biogas volume production (NL day^{-1}); on the contrary, it led to a reduction. The initially high biogas yield from *A. donax* showcases its substantial potential as a standalone feedstock for biogas production. On the contrary, the notable decline in biogas volume production with the addition of corn silage, especially at a ratio of 2:1, presents an unexpected outcome, which contradicts the assumptions based on previous research. Such a reduction prompts a deeper examination of the synergies of different feedstocks within the anaerobic digestion environment and optimal feedstock ratios.

Although the initial volume-based (NL day^{-1}) analysis suggested that 100% *A. donax* feedstock yielded the highest biogas production, when considering the production quantities expressed relative to volatile solids (SBP and SMP), a different pattern emerges. The average SMP and SBP values reveal that the mixture of *A. donax* and corn silage at a ratio of 1:2 not only achieved the highest methane production but also the highest biogas production per unit of volatile solids. This finding suggests that, while the total volume of biogas and methane produced is an important metric, it doesn't provide a complete picture of feedstock efficiency.

The research determined a methane proportion ranging from 51.47% to a maximum of 62.25% using a mixture at a ratio of 1:2. Utilizing *A. donax* (100%) as a substrate showed a methane proportion ranging from 51.50% to 54.09%, which is lower compared to the findings by Corno et al. (2015), who obtained a methane proportion of 64.1 ± 20% with *A. donax* as the feedstock. Interestingly, the same authors indicate that the methane proportion when using corn silage was lesser compared to *A. donax*, specifically amounting to 61.3 ± 20%. However, from the conducted research, it can be observed that the highest methane production was achieved using a mixture at a ratio of 1:2 (*A. donax*:corn silage), implying that an increase in methane production occurred with the addition of a higher quantity of corn silage which is also confirmed by SMP average values. Consistent with these findings there are several authors that highlighted similar SMP that ranged from 144 to 234 mL g^{-1} VS (Baldini et al., 2017; Di Girolamo et al., 2014; Jiang et al., 2016; Shilpi et al., 2019).

The concentration of carbon dioxide also displayed noticeable variations across different samples and feedstock mixtures. As illustrated in the research results, the proportion of CO_2 ranged from 37.76% to 48.53% across all examined feedstocks, representing a relatively high share. The data presented in Table 2 also demonstrates that the highest proportions of CO_2 were observed in feedstocks with a larger share of *A. donax* whilst the lowest values were detected in the mixture with a ratio of 1:2 (*A. donax*:corn silage). According to the study by Giertl et al. (2022), the average proportion of CO_2 was 44%, which is lower compared to the results obtained in this investigation, where the sample of *A. donax* 100% generated biogas with an average CO_2 content of 46.93%. An exceedingly low proportion of CO_2 was reported in the study by Sciuto et al. (2023), where the concentration amounted to merely 18.54 ± 7.91%, indicating a significantly low presence of CO_2 in biogas. This variation in CO_2 proportions underscores the significance of feedstock composition in influencing the gas composition, which in turn impacts the quality and potential applications of the produced biogas.

High H_2S concentrations could potentially indicate challenges, given its corrosive nature; thus, understanding and managing its levels are pivotal in biogas applications. Based on the previous research (Aita et al., 2016; Moreno-Andrade et al., 2020), the permissible concentration of H_2S in biogas for utilization in thermal and electrical energy conversion is determined at 500 ppm. Biogas with higher concentrations of H_2S necessitates undergoing a purification process. In the present investigation, the highest value of H_2S concentration recorded was 331.40 ppm, observed in the mixture of *A. donax* and corn silage at a ratio of 2:1, whereas the lowest average concentration of H_2S was noted in the mixture at a ratio of 1:2 (*A. donax*:corn silage). The results presented diverge from the results of Giertl et al. (2022), who report a concentration of H_2S of 730 ppm using *A. donax* as a feedstock in biogas production. This value significantly exceeds the permissible level. However, considerably lower mean concentrations of H_2S were observed in the study by Sciuto et al. (2023), where the H_2S concentration amounted to 112.10 ppm, which is similar to the results given in this research. An intriguing aspect concerning the concentration of H_2S can also be discerned from the research findings of Corno et al. (2015), where the difference in biogas quality between the feedstocks *A. donax* and corn silage was investigated in batch reactor. The authors indicate that the concentration of H_2S in the biogas derived from *A. donax* was lower than that obtained from corn silage. Specifically, the biogas from corn silage exhibited an H_2S content of 0.20%, equivalent to 2000 ppm, whereas the sample of *A. donax* yielded a lower H_2S concentration, amounting to 0.10% or 1000 ppm. According to the findings of Corno et al. (2015), the H_2S share is higher in corn silage, while the results of this study show that an

increase in the addition of *A. donax*, both as a 100% feedstock and in a 2:1 ratio (*A. donax*:corn silage), led to an increase in the concentration of H_2S. This presents a contrasting outcome that may be attributable to variations in experimental conditions or other intervening factors, warranting further investigation to elucidate the underlying causes of such discrepancies. Accordingly, the feedstocks utilized in this study satisfy the previously mentioned requirements about H_2S concentration, indicating that it is not necessary to additionally purify biogas via a desulphurization procedure.

Monitoring oxygen levels is crucial as excessive oxygen can disrupt the AD process, negatively impacting biogas yields. Oxygen, as a component, should be present in minimal quantities during the biogas production process due to the anaerobic digestion process involved. Primarily, the O_2 content in biogas should not exceed a proportion of 2%, or 2000 ppm, as this indicates an aberrant anaerobic digestion process, signifying that entirely favourable conditions for biogas production have not been achieved (Giertl et al., 2022). The same authors also investigated the oxygen content in biogas produced from *A. donax*, yet their findings did not reveal any detectable presence of oxygen. As mentioned in the results, the average accessible concentration of O_2 stands at 0.8 ppm, while relatively higher oxygen values were attained with a larger addition of corn silage, specifically, in the mixture of *A. donax* and corn silage (1:2). Nonetheless, in the current study, exceedingly low concentrations of oxygen were observed, corroborating that the anaerobic digestion process was conducted appropriate, facilitating proper biogas production.

5 | CONCLUSION

This study evaluated the biogas production potential of *A. donax* and a mixture of corn silage and *A. donax* in two mixtures (1:2 and 2:1) through the process of anaerobic digestion. During this process, the comparative analysis accentuates the viability of both, *A. donax* and corn silage, as well as their mixtures as potent feedstocks for biogas production. It was observed that a pure *A. donax* substrate exhibited a superior biogas yield initially, but detailed analysis based on SBP and SMP normalized to volatile solids revealed a different trend. When adjusted for VS, the mixtures, particularly the 1:2 ratio of *A. donax* to corn silage, demonstrated the highest efficiency in biogas and methane production. Notably, the levels of H_2S were within permissible limits, eliminating the need for further desulphurization procedures, which is important. Further studies are necessary to identify the optimal mixture ratios of corn silage and *A. donax* that maximize biogas and methane production. Future research should also explore the feasibility of storing the crop, considering the continuous process of anaerobic digestion and the seasonality of feedstock, to ensure more consistent and efficient biogas production. The findings of this research could provide valuable insights into the bioenergy sector and support the development of sustainable and economically viable biogas production strategies.

AUTHOR CONTRIBUTIONS

Karlo Špelić: Conceptualization; writing – original draft. **Mario Panjičko:** Methodology; supervision. **Gregor Drago Zupančić:** Investigation; writing – review and editing. **Anamarija Lončar:** Investigation; visualization. **Ivan Brandić:** Data curation. **Ivana Tomić:** Writing – review and editing. **Ana Matin:** Visualization. **Tajana Krička:** Methodology; resources. **Vanja Jurišić:** Methodology; resources; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare having no conflicting or competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at http://doi.org/10.5061/dryad.2ngf1vhx7.

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