



Influence of Elemental Composition of Geothermal Waters and Succulent Feedstocks on Cryo-mesophilic Biogas Production from Mt. Suswa, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/ajocs/2026/v16i5474>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/165727>

Original Research Article

Received: 11/07/2026

Accepted: 30/08/2026

Published: 04/09/2026

Abstract

Background: Dependence on charcoal and firewood in the Mt. Suswa region creates environmental and health concerns and supports the need to evaluate locally available feedstocks for renewable biogas production. This study investigated the elemental composition of geothermal waters and three succulent feedstocks and examined their biogas production under cryo-mesophilic conditions.

Methods: Steam-vent water, water-pan samples, and *Agave sisalana*, *Echinocactus grusonii*, and *Opuntia ficus-indica* were analysed by Microwave Plasma Atomic Emission Spectroscopy. Biogas production was monitored for 132 h in 26 laboratory-scale batch digesters operated at 20–24.5°C with different feedstock-water combinations, with or without cow dung inoculum.

Results: *Agave sisalana* contained $3,765.53 \pm 0.00$ g/L potassium, 1.23 ± 0.00 g/L cobalt, and 1.08 ± 0.03 g/L nickel. Region II steam-vent water had $50,268.93 \pm 29.19$ ppm potassium, $1,317.85 \pm 1.10$ ppm nickel, $1,184.54 \pm 5.76$ ppm cobalt, and the lowest measured arsenic concentration among the steam-vent regions.

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The highest cumulative biogas yield was 62 mL in D6, containing *A. sisalana*, water-pan sample, and cow dung. Most *O. ficus-indica* treatments yielded 12–24 mL, although D10 reached 50 mL.

Conclusion: Biogas performance varied with feedstock, water source, and inoculation. *A. sisalana*-based treatments and cow dung inoculation showed the most favourable overall performance under the tested conditions.

Keywords: *Elemental composition; anaerobic digestion; biogas production; cryo-mesophilic digestion; geothermal water; succulent feedstocks; Agave sisalana.*

1. Introduction

Biomass, comprising organic matter derived from plant and animal sources, represents a renewable energy resource that can be converted through biological or thermochemical processes into usable energy forms. Among conversion technologies, anaerobic digestion (AD) has emerged as a promising biotechnological approach for transforming organic waste into methane-rich biogas and nutrient-rich digestate. The AD process involves a consortium of symbiotic microorganisms that sequentially hydrolyze, acidify, acetogenically convert, and methanogenically transform organic matter under oxygen-free conditions (Salazar-Batres & Moreno-Andrade, 2025; Zhang *et al.*, 2024). Recent comparative work on agricultural residues has likewise shown that substrate selection and co-digestion configuration can influence biogas and methane production in anaerobic digesters (Enenebeaku *et al.*, 2026). Plant-derived biomass combined with cow dung has also been demonstrated as a practicable feedstock arrangement for biogas generation, supporting the use of animal manure alongside lignocellulosic plant material in digestion systems (Okonkwo *et al.*, 2024).

Biogas composition varies depending on substrate characteristics and process conditions, typically comprising methane (50–75%), carbon dioxide (25–50%), and trace amounts of hydrogen sulfide, ammonia, and other gases. Methane is the most desirable component due to its high calorific value (approximately 35.8 MJ/m³). The digestate byproduct can be utilised as an organic fertiliser, contributing to circular economy principles (Nwokolo *et al.*, 2020).

Trace elements play a pivotal role in AD by serving as cofactors for metalloenzymes essential for microbial metabolism and methanogenesis (Gonzalez & Stres, 2019). Nickel is required for the synthesis of coenzyme F₄₃₀, which is critical for methyl-coenzyme M reductase in methanogenic archaea (Zheng *et al.*, 2016). Cobalt is essential for vitamin B₁₂ synthesis and various enzymatic processes (Kräutler & Jaun, 2022). Iron functions in electron transport and as a component of hydrogenases (Paulo *et al.*, 2015). However, the same elements can become inhibitory at elevated concentrations, disrupting microbial cell membranes, binding to sulfhydryl groups of enzymes, and causing osmotic stress (Mudhoo & Kumar, 2013; Fang *et al.*, 2024). Recent evidence further indicates that trace-element effects in AD are concentration-dependent, because elements such as cobalt, copper, and nickel may support microbial function at appropriate levels but inhibit digestion when present in excess (Pires *et al.*, 2025).

The balance between essential and inhibitory elements is particularly critical in feedstocks derived from geothermal ecosystems, where mineral-rich waters and adapted vegetation may contain unusual elemental profiles. Geothermal waters are known to leach minerals from surrounding geological formations, resulting in elevated concentrations of elements such as sodium, potassium, calcium, and various trace metals (Mao *et al.*, 2023). Succulent plants adapted to such environments, including species of *Agave*, *Opuntia*, and *Echinocactus*, may accumulate these elements in their tissues, potentially influencing their suitability as AD feedstocks (Kumar & Ram, 2021; Lueangwattanapong *et al.*, 2020).

Mt. Suswa, located in Kenya's Great Rift Valley approximately 50 km northwest of Nairobi, is characterised by active geothermal activity, mineral-rich soils, and semi-arid vegetation dominated by succulent species. Local communities rely heavily on firewood and charcoal for domestic energy, contributing to deforestation and respiratory health issues. The region's geothermal features—including steam vents that produce condensate water and natural water pans—represent underutilised resources that could support sustainable bioenergy production. Low-temperature AD studies also indicate that reduced operating temperatures can constrain microbial activity, while process intensification may improve methane production under such conditions (Sun *et al.*, 2024).

Despite growing interest in succulent biomass for bioenergy, limited research has examined the influence of elemental composition on AD performance, particularly under cryo-mesophilic conditions (20–24.5°C) that are relevant to tropical highland regions. Furthermore, the specific elemental profiles of geothermal waters and their impact on AD have not been systematically characterised for East African geothermal systems (Myovela, 2018).

Therefore, this study aimed to: (1) characterise the elemental composition of geothermal steam-vent waters, water pan samples, and three succulent species (*Agave sisalana*, *Echinocactus grusonii*, and *Opuntia ficus-indica*) from Mt. Suswa; (2) evaluate biogas production from these feedstocks under cryo-mesophilic conditions; (3) assess the influence of cow dung inoculum on digestion efficiency; and (4) identify correlations between elemental composition and biogas yield to inform feedstock selection for sustainable bioenergy production.

2. Materials and Methods

2.1 Study Area

The study was conducted at Mt. Suswa (1°10'S, 36°20'E), an active volcanic complex in Kenya's Great Rift Valley. The area experiences a semi-arid climate with mean annual rainfall of 500–800 mm and temperatures ranging from 15–30°C. Vegetation is dominated by drought-adapted succulent species, including *Agave sisalana*, *Opuntia ficus-indica*, and various *Echinocactus* species. Geothermal activity is evidenced by steam vents, fumaroles, and hot springs, with reservoir temperatures estimated at 250–300°C.

2.2 Sample Collection

2.2.1 Water Samples

Steam-vent water samples were collected from three distinct regions within Mt. Suswa. Condensed steam was collected from plastic and metal collection pipes using acid-rinsed polyethylene bottles. Water pan samples were collected in triplicate from three points (centre, middle, and edge) using grab sampling. All water samples were acidified with concentrated nitric acid (HNO₃) to pH < 2 immediately after collection and transported to the laboratory in ice boxes.

2.2.2 Succulent Samples

Mature, healthy specimens of *Agave sisalana*, *Echinocactus grusonii*, and *Opuntia ficus-indica* were harvested using sterilised knives. Samples were collected from three regions within Mt. Suswa, labelled, stored in polythene bags, and transported to the laboratory under cool conditions.

2.3 Sample Preparation

2.3.1 Water Samples

Water samples were filtered through 0.45 µm membrane filters to remove suspended solids and microorganisms. Filtered samples were acidified with 10 mL of trace metal-grade nitric acid to achieve pH < 2.

2.3.2 Plant Samples

Thorns were removed, and samples were thoroughly cleaned with deionised water and air-dried. Approximately 100–200 g of each sample was weighed, cut into small pieces, and blended to form a homogenised aqueous solution. The resulting slurry was filtered through muslin cloth to extract sap, which was stored at 4°C until digestion.

2.4 Sample Digestion

Wet digestion was performed by accurately weighing 2 g of sap sample into digestion vessels. Twenty millilitres of concentrated nitric acid (HNO₃) was added, and the mixture was heated on a hot plate at 150°C for 3–4 hours

until the sample was nearly dry. After cooling, 1 mL of concentrated HCl was added to dissolve any precipitated salts, and the mixture was diluted with 30 mL deionised water. The digested solution was filtered through Whatman No. 42 filter paper and diluted to 100 mL with deionised water for elemental analysis.

2.5 Elemental Analysis

Elemental analysis was performed using an Agilent 4210 Microwave Plasma Atomic Emission Spectrometer (MP-AES). The instrument was calibrated using multi-element standards, and quality control measures included reagent blanks, procedural blanks, and calibration verification standards. All analyses were conducted in triplicate. Elements analysed included potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), manganese (Mn), aluminium (Al), and arsenic (As).

2.6 Biogas Production Experiments

2.6.1 Feedstock Preparation

Succulent biomass (100 g each of *A. sisalana*, *E. grusonii*, and *O. ficus-indica*) was mechanically homogenised and mixed with water sources (distilled water, water pan, steam-vent plastic, steam-vent metal) in a 1:1:1 ratio. Cow dung inoculum was added to selected treatments at a 1:1 ratio with the succulent-water mixture.

Table 1. Experimental Treatment Design

Digester	Feedstock Composition
D1	<i>A. sisalana</i> + Distilled Water
D2	<i>A. sisalana</i> + Water Pan
D3	<i>A. sisalana</i> + Steam Plastic
D4	<i>A. sisalana</i> + Steam Metal
D5	<i>A. sisalana</i> + Distilled Water + Cow Dung
D6	<i>A. sisalana</i> + Water Pan + Cow Dung
D7	<i>A. sisalana</i> + Steam Plastic + Cow Dung
D8	<i>A. sisalana</i> + Steam Metal + Cow Dung
D9	Cow Dung + <i>A. sisalana</i>
D10	Cow Dung + <i>O. ficus-indica</i>
D11	Cow Dung + <i>E. grusonii</i>
D12	<i>E. grusonii</i> + Distilled Water
D13	<i>E. grusonii</i> + Water Pan
D14	<i>E. grusonii</i> + Steam Plastic
D15	<i>E. grusonii</i> + Steam Metal
D16	<i>E. grusonii</i> + Distilled Water + Cow Dung
D17	<i>E. grusonii</i> + Water Pan + Cow Dung
D18	<i>E. grusonii</i> + Steam Plastic + Cow Dung
D19	<i>E. grusonii</i> + Steam Metal + Cow Dung
D20	<i>O. ficus-indica</i> + Water Pan
D21	<i>O. ficus-indica</i> + Steam Plastic
D22	<i>O. ficus-indica</i> + Steam Metal
D23	<i>O. ficus-indica</i> + Distilled Water + Cow Dung
D24	<i>O. ficus-indica</i> + Water Pan + Cow Dung
D25	<i>O. ficus-indica</i> + Steam Plastic + Cow Dung
D26	<i>O. ficus-indica</i> + Steam Metal + Cow Dung

2.6.2 Experimental Design

Twenty-six laboratory-scale batch digesters (1.5 L capacity) were set up with different feedstock combinations (Table 1). Digesters were placed in buckets filled with dry soil to provide thermal insulation and simulate cryo-mesophilic conditions (20–24.5°C). Biogas production was monitored using the syringe displacement method at 24, 48, 72, 108, and 132 hours.

2.7 Statistical Analysis

Data were analysed using Microsoft Excel. Mean values and standard deviations were calculated for all elemental concentrations and biogas yields. One-way analysis of variance (ANOVA) was performed to determine significant differences among treatments, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Elemental Composition of Succulent Species

Elemental analysis revealed significant differences in the elemental profiles of the three succulent species (Table 2). *Agave sisalana* exhibited the highest concentrations of potassium ($3,765.53 \pm 0.00$ g/L), cobalt (1.23 ± 0.00 g/L), and nickel (1.08 ± 0.03 g/L). *Opuntia ficus-indica* showed the highest copper concentration (177.87 ± 0.01 g/L) and elevated aluminium levels (75.14 ± 0.00 g/L). *Echinocactus grusonii* demonstrated the highest sodium concentration ($2,386.77 \pm 0.00$ g/L) and moderate levels of other elements. Iron and magnesium were below detection limits in all species, while calcium exceeded the detection range in both *A. sisalana* and *E. grusonii*.

Table 2. Elemental Composition of Succulent Species (g/L)

Element	<i>A. sisalana</i>	<i>E. grusonii</i>	<i>O. ficus-indica</i>
K	$3,765.53 \pm 0.00$	$1,144.34 \pm 0.00$	$2,496.91 \pm 0.00$
Na	$1,999.36 \pm 0.00$	$2,386.77 \pm 0.00$	$1,911.16 \pm 0.00$
Al	48.90 ± 0.00	114.57 ± 0.00	75.14 ± 0.00
Cu	27.05 ± 0.00	2.45 ± 0.01	177.87 ± 0.01
Mn	2.63 ± 0.00	4.99 ± 0.00	4.68 ± 0.00
Co	1.23 ± 0.00	0.10 ± 0.02	0.49 ± 0.01
Ni	1.08 ± 0.03	0.22 ± 0.05	0.59 ± 0.03
As	0.75 ± 0.01	1.01 ± 0.03	1.05 ± 0.01
Zn	0.15 ± 0.01	0.98 ± 0.00	0.41 ± 0.01
Fe	BDL	BDL	BDL
Mg	BDL	BDL	BDL
Ca	>DR	>DR	177.87 ± 0.03

BDL = Below detection limit; DR = Detection range exceeded

3.2 Elemental Composition of Geothermal Waters

3.2.1 Steam-Vent Water

Significant regional variations were observed in steam-vent water elemental composition (Table 3). Calcium and sodium were the dominant elements across all regions. Region II exhibited the most favourable profile for anaerobic digestion, with the highest concentrations of potassium ($50,268.93 \pm 29.19$ ppm), nickel ($1,317.85 \pm 1.10$ ppm), and cobalt ($1,184.54 \pm 5.76$ ppm), and the lowest arsenic concentration (998.16 ± 11.10 ppm). Region III showed the highest calcium ($1,788,452.58 \pm 58.62$ ppm) and sodium ($1,795,699.78 \pm 86.72$ ppm) concentrations. Aluminium was only detectable in Region III ($2,536.31 \pm 130.40$ ppm).

3.2.2 Water Pan Samples

Water pan samples contained calcium ($1,034,320.81 \pm 24.63$ ppm) and sodium ($1,082,977.59 \pm 18.97$ ppm) as the dominant elements, with moderate concentrations of potassium ($190,805.04 \pm 42.03$ ppm), copper ($23,948.45 \pm 0.25$ ppm), and iron ($1,505.75 \pm 8.71$ ppm) (Table 4).

Table 3. Elemental Composition of Steam-Vent Water by Region (ppm)

Element	Region I	Region II	Region III
Zn	92.27 ± 15.78	94.92 ± 156.36	117.30 ± 96.92
Fe	1,638.37 ± 0.41	1,541.62 ± 4.05	1,027.48 ± 102.25
Ca	926,828.52 ± 2.57	1,267,306.00 ± 59.02	1,788,452.58 ± 58.62
Cu	26,080.71 ± 0.29	26,060.22 ± 0.88	25,064.75 ± 1.57
Ni	1,166.29 ± 1.16	1,317.85 ± 1.10	1,044.19 ± 3.02
As	7,492.96 ± 1.12	998.16 ± 11.10	7,171.38 ± 1.09
Co	985.98 ± 1.11	1,184.54 ± 5.76	856.51 ± 7.53
K	31,021.63 ± 0.44	50,268.93 ± 29.19	45,955.23 ± 54.40
Mn	2,434.25 ± 0.36	2,180.75 ± 1.04	2,468.62 ± 1.61
Mg	2,258.09 ± 1.32	1,997.03 ± 45.77	1,654.07 ± 56.89
Al	ND	ND	2,536.31 ± 130.40
Na	749,607.91 ± 0.39	1,620,589.59 ± 64.98	1,795,699.78 ± 86.72

ND = Not detected

Table 4. Elemental Composition of Water Pan Samples (ppm)

Element	Concentration
Al	ND
As	6,769.78 ± 0.30
Ca	1,034,320.81 ± 24.63
Co	852.44 ± 3.12
Cu	23,948.45 ± 0.25
Fe	1,505.75 ± 8.71
K	190,805.04 ± 42.03
Mg	2,334.34 ± 7.89
Mn	3,041.67 ± 15.09
Na	1,082,977.59 ± 18.97
Ni	950.80 ± 2.79
Zn	11.35 ± 23.87

3.3 Biogas Production

3.3.1 *Agave sisalana* Treatments

Cumulative biogas production from *A. sisalana* digesters ranged from 0 to 62 mL over 132 hours (Table 5). Digesters with cow dung inoculum produced significantly higher yields ($p < 0.05$) than those without. The highest yield (62 mL) was achieved by D6 (*A. sisalana* + Water Pan + Cow Dung), followed by D5 (60 mL) and D9 (60 mL). Digesters without cow dung (D1–D4) produced minimal to no biogas.

3.3.2 *Echinocactus grusonii* Treatments

E. grusonii digesters with cow dung inoculum (D16–D18) produced 60–62 mL, comparable to *A. sisalana* treatments (Table 6). However, D19 (Steam Metal + Cow Dung) showed a significantly lower yield (24 mL), suggesting metal toxicity from the steam collection system. Digesters without cow dung produced minimal biogas (0–12 mL).

3.3.3 *Opuntia ficus-indica* Treatments

O. ficus-indica digesters produced the lowest overall biogas yields (Table 7). Even with cow dung inoculum, yields ranged from only 12–24 mL, substantially lower than those from *A. sisalana* and *E. grusonii*. Mono-digestion treatments (D20–D22) produced 12–17 mL.

Table 5. Cumulative Biogas Production from *Agave sisalana* Digesters (mL)

Digester	24 h	48 h	72 h	108 h	132 h
D1	0	0	0	0	0
D2	0	0	0	5	12
D3	0	0	0	6	15
D4	0	0	0	0	0
D5	0	15	40	60	60
D6	0	10	25	55	62
D7	0	5	12	20	35
D8	0	15	20	40	51
D9	0	10	20	42	60

Table 6. Cumulative Biogas Production from *Echinocactus grusonii* Digesters (mL)

Digester	24 h	48 h	72 h	108 h	132 h
D11	0	0	6	15	25
D12	0	0	0	0	5
D13	0	0	0	0	0
D14	0	0	0	0	12
D15	0	0	0	0	0
D16	0	10	30	45	62
D17	0	14	35	50	60
D18	0	10	30	50	60
D19	0	10	16	18	24

Table 7. Cumulative Biogas Production from *Opuntia ficus-indica* Digesters (mL)

Digester	24 h	48 h	72 h	108 h	132 h
D10	0	11.5	17	40	50
D20	0	2	5	17	17
D21	0	0	4	12	12
D22	0	4	6	15	15
D23	0	5	9	20	20
D24	0	6	10	24	24
D25	0	5	10	17	17
D26	0	2	7	12	12

3.4 Overall Performance Ranking

Based on cumulative biogas yield at 132 hours, feedstock performance ranked as follows: *Agave sisalana* > *Echinocactus grusonii* > *Opuntia ficus-indica*. Steam-vent water performance ranked Region II > Region I > Region III. Cow dung inoculation was essential for substantial biogas production across all feedstocks.

4. Discussion

4.1 Influence of Elemental Composition on Biogas Production

The results demonstrate that elemental composition is a critical determinant of anaerobic digestion performance under cryo-mesophilic conditions. The superior performance of *Agave sisalana* corresponds directly to its favourable elemental profile: high concentrations of potassium, cobalt, and nickel, combined with relatively low arsenic levels.

Potassium: Potassium plays an essential role in maintaining ionic balance, regulating osmotic pressure, and facilitating nutrient transport across microbial cell membranes (Meena *et al.*, 2016). The high potassium

concentration in *A. sisalana* (3,765.53 g/L) likely contributed to enhanced microbial stability and metabolic activity, resulting in the highest biogas yields observed.

Cobalt and nickel: Cobalt and nickel are among the most critical trace elements for methanogenesis. Cobalt is required for vitamin B₁₂ synthesis and serves as a cofactor for several enzymes involved in the methanogenic pathway (Kräutler & Jaun, 2022). Nickel is an essential component of coenzyme F₄₃₀, which catalyzes the final step of methane formation (Zheng *et al.*, 2016). The elevated concentrations of both elements in *A. sisalana* (Co: 1.23 g/L; Ni: 1.08 g/L) likely provided optimal conditions for methanogenic archaea, explaining the high biogas yields observed.

Copper: Copper exhibited the most pronounced inhibitory effect. *Opuntia ficus-indica* contained exceptionally high copper concentrations (177.87 g/L), which corresponded with the lowest biogas yields across all treatments. While copper is an essential micronutrient at low concentrations, excessive levels are toxic to anaerobic microorganisms (Mudhoo & Kumar, 2013; Wang *et al.*, 2024; Fang *et al.*, 2024). Copper ions can disrupt microbial cell walls, bind to sulfhydryl groups of enzymes, and generate reactive oxygen species that damage cellular components. The consistent low performance of *O. ficus-indica* digesters, even with cow dung supplementation, confirms that copper toxicity was the dominant limiting factor.

Sodium: Sodium demonstrated a concentration-dependent effect. While moderate sodium levels support microbial metabolism and osmotic regulation, excessive concentrations inhibit methanogenic archaea by causing osmotic stress and disrupting enzymatic activity (Zhang *et al.*, 2024; He *et al.*, 2024). *Echinocactus grusonii* exhibited the highest sodium concentration (2,386.77 g/L), which may explain its intermediate performance despite adequate levels of other nutrients.

Arsenic: Arsenic is a potent inhibitor of microbial metabolism. Sierra-Alvarez *et al.* (2004) demonstrated that arsenic compounds, particularly trivalent species, are highly inhibitory to methanogenesis with 50% inhibitory concentrations as low as 9.1–15.0 µM. The low arsenic concentration in *A. sisalana* (0.75 g/L) compared to *E. grusonii* (1.01 g/L) and *O. ficus-indica* (1.05 g/L) likely contributed to its superior performance.

Aluminium: Aluminium has no known essential function in anaerobic digestion and may inhibit microbial activity at elevated concentrations (Myszograj *et al.*, 2018). The presence of detectable aluminium in Region III steam-vent water (2,536.31 ppm) and its absence in Regions I and II may explain the lower biogas yields observed with Region III water.

Iron and magnesium: Iron and magnesium were below detection limits in all succulent samples, suggesting that these elements were not limiting factors in this study. However, their presence in geothermal waters (Fe: 1,027–1,638 ppm; Mg: 1,654–2,258 ppm) may have provided supplementary benefits to digesters using these water sources.

4.2 Role of Water Source in Anaerobic Digestion

The significant regional variations in steam-vent water composition had a substantial impact on biogas production. Region II water, characterised by the highest concentrations of potassium, nickel, and cobalt and the lowest arsenic concentration, supported the highest biogas yields. This finding aligns with the recognized importance of these trace elements in methanogenesis (Myszograj *et al.*, 2018; Salazar-Batres & Moreno-Andrade, 2025).

The contrast between plastic and metal steam collection systems was particularly instructive. In *E. grusonii* digesters, D18 (Steam Plastic + Cow Dung) produced 60 mL, while D19 (Steam Metal + Cow Dung) produced only 24 mL. This 60% reduction suggests metal ion leaching from the metal pipes, introducing toxic elements (potentially copper, zinc, or other heavy metals) that inhibited methanogenic activity (Mohammed *et al.*, 2011). This observation has practical implications for geothermal water harvesting infrastructure design.

Water pan samples, despite containing elevated arsenic (6,769.78 ppm), supported high biogas yields when combined with *A. sisalana* and cow dung (D6: 62 mL). This suggests that the beneficial effects of other nutrients (calcium, potassium, iron, magnesium) and the buffering capacity provided by cow dung inoculum may have mitigated arsenic toxicity. The high calcium concentration in water pan samples (1,034,320.81 ppm)

likely contributed to pH stabilisation and microbial aggregation, enhancing digester stability (Morais *et al.*, 2018; Toner & Catling, 2020).

4.3 Importance of Microbial Inoculation

The critical role of cow dung inoculum is evident from the stark contrast between digesters with and without supplementation. Digesters without cow dung (D1–D4, D12–D15) produced negligible biogas (0–15 mL), while those with cow dung (D5–D9, D16–D19) produced 24–62 mL. This difference reflects the essential role of microbial inoculum in providing:

1. **Methanogenic archaea** required for the final step of methane production
2. **Balanced C:N ratios** that optimise microbial metabolism
3. **Buffering capacity** that prevents pH fluctuations
4. **Enzymatic activity** for hydrolysis of complex substrates

The delayed biogas production in mono-digestion treatments (first detectable gas at 72–108 hours) compared to co-digestion treatments (gas at 48 hours) further demonstrates the importance of inoculum in overcoming the hydrolysis lag phase (Nwokolo *et al.*, 2020; Conte *et al.*, 2025).

4.4 Cryo-Mesophilic Digestion Considerations

Operating at 20–24.5°C presents challenges for anaerobic digestion, as microbial activity and metabolic rates are significantly reduced compared with mesophilic (35–40°C) or thermophilic (50–60°C) conditions. The relatively low biogas yields observed in this study (maximum 62 mL over 132 hours) are consistent with the reduced metabolic rates at these temperatures. However, the ability to achieve any measurable biogas production under these conditions is noteworthy and suggests that the elemental composition of feedstocks can partially compensate for temperature limitations.

The presence of beneficial trace elements may have enhanced enzyme stability and activity at suboptimal temperatures, enabling continued methanogenesis despite thermal constraints (Myszograj *et al.*, 2018). This finding has practical implications for biogas production in tropical highland regions where maintaining mesophilic temperatures is economically challenging.

4.5 Feedstock Suitability Ranking

Based on the comprehensive analysis of elemental composition and biogas yield, the three succulent species can be ranked for biogas production suitability as follows:

1. *Agave sisalana* (Best)

- High potassium, cobalt, and nickel
- Low arsenic
- Balanced nutrient profile
- Highest biogas yield (62 mL)

2. *Echinocactus grusonii* (Intermediate)

- Moderate nutrient levels
- Higher sodium concentration
- Susceptible to metal toxicity from collection systems
- Comparable yield with cow dung (60–62 mL)

3. *Opuntia ficus-indica* (Poorest)

- Extremely high copper (177.87 g/L)
- Elevated aluminium
- Lowest biogas yields (12–24 mL even with cow dung)

This ranking aligns with the recognized importance of trace element balance in anaerobic digestion and provides a practical framework for feedstock selection in geothermal regions (Kumar & Ram, 2021; Lueangwattanapong et al., 2020).

5. Conclusion

This study demonstrates that elemental composition significantly influences anaerobic digestion performance and biogas production from succulent feedstocks and geothermal waters under cryo-mesophilic conditions. **Elemental profiles vary substantially** among succulent species and geothermal water sources, with these variations directly impacting biogas yield.

Agave sisalana exhibits the most favourable elemental composition for anaerobic digestion, characterised by high concentrations of beneficial elements (potassium, cobalt, nickel) and low levels of inhibitory elements (arsenic, copper). This species produced the highest cumulative biogas yield (62 mL). **Steam-vent water from Region II** provides the most suitable elemental profile for anaerobic digestion, with elevated potassium, nickel, and cobalt and the lowest arsenic concentration. **Cow dung inoculation is essential** for substantial biogas production from succulent feedstocks, providing necessary microorganisms, buffering capacity, and balanced nutrition. **Copper toxicity** is the primary limiting factor for *Opuntia ficus-indica* biogas production, while sodium and arsenic contribute to reduced performance in other feedstocks. **Metal pipe infrastructure** for steam collection can introduce inhibitory elements, highlighting the importance of material selection in geothermal water harvesting systems. **Cryo-mesophilic digestion** (20–24.5°C) is feasible with appropriately balanced feedstocks, though yields are lower than those achievable at mesophilic temperatures. The findings suggest that integrated use of *Agave sisalana* biomass, Region II steam-vent water, and cow dung inoculum represents the most promising combination for sustainable biogas production in the Mt. Suswa ecosystem. This approach could provide clean energy alternatives to charcoal and firewood, reducing environmental degradation and improving community health.

6. Recommendations

Future research should focus on: (1) optimising the *A. sisalana*-water pan-cow dung ratio for maximum methane yield; (2) characterising the microbial community dynamics during cryo-mesophilic digestion; (3) evaluating the digestate quality as biofertiliser; (4) scaling up the process to pilot-scale digesters; and (5) assessing the economic viability of community-scale biogas systems in geothermal regions.

7. Limitation

This study was conducted in laboratory-scale batch digesters for only 132 h, which limits extrapolation to long-term or full-scale biogas systems. Biogas volume, rather than methane concentration or methane yield per unit of volatile solids, was reported. The experimental design also did not present direct microbial-community measurements or controlled metal-toxicity tests, so associations between individual elements and digestion performance cannot establish causation.

Acknowledgement

The authors acknowledge maaasai mara university and mung'a university for the support they gave for use of their science laboratories for experimental analysis.

I acknowledge the support given by the technical staff from murang'a university science laboratories.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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