

# Bioelectricity generation and physicochemical evolution of a substrate with sheep compost in microbial fuel cells in a high Andean area

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## ABSTRACT

The recovery of organic waste, such as sheep compost, is a key strategy for energy valorization. This study evaluated its potential as a substrate in microbial fuel cells (MFCs) using zinc (anode) and copper (cathode) electrodes and analyzed the evolution of its physicochemical properties, using soil samples from a high Andean area of the Chacapampa district, Peru. Two configurations of ground-mounted MFCs in series were compared: C1 (16 reactors of 400 g) and C2 (8 reactors of 800 g), maintaining a total mass of 6.4 kg. The C2 configuration was significantly more efficient, generating a median power of 819.53  $\mu\text{W}$ , more than double the 380.92  $\mu\text{W}$  of C1 ( $p=0.002$ ). The final physicochemical analysis revealed that the process transforms the substrate, increasing electrical conductivity and phosphorus availability, although potassium decreased. It is important to note that due to the use of reactive metal electrodes, the system operates as a hybrid microbial-galvanic cell, where the zinc anode is consumed. It is concluded that sheep compost is an effective substrate and that consolidating the volume in fewer reactors optimizes electrochemical performance, although long-term environmental impacts regarding zinc accumulation must be monitored.

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## 1. INTRODUCTION

The transition to sustainable energy sources drives research into technologies that not only generate clean energy, but are also integrated into a circular economy, valorizing organic waste. In this framework, microbial fuel cells (MFCs) stand out for their ability to convert the chemical energy of organic matter directly into electricity through the catalytic activity of microorganisms [1], [2].

Studies on energy recovery from biomass waste have presented a proposal as an emerging alternative; this is evident in the articles [3]–[5]. Over the past two decades, the field has advanced from proofs of concept to systems capable of powering small sensors and contributing to wastewater treatment [6], [7]. However, the main challenge to its large-scale implementation remains its low power density compared to conventional energy technologies [8]–[10].

The state of the art focuses on optimizing components and substrates. Soil MFCs have gained interest for their simplicity and low cost, operating directly in the environment without expensive membranes [11].

Current research focuses on using local organic waste, such as manure, sludge or compost, to make technology more sustainable [12]. In this study, metal electrodes (Zinc and Copper) were selected to create a sediment battery configuration. While this introduces a galvanic component distinct from pure biological oxidation, it provides a robust and cost-effective model for low-tech terrestrial applications [13], [14].

This is where a critical first knowledge gap lies. While daisy-chaining is a critical strategy for scaling [15], the impact of the physical architecture of this connection specifically, how a total mass of substrate is distributed among individual units has been little explored. Most studies have focused on optimizing electrode materials or the distance between them within a single cell [16], [17], but not in whether a system composed of many small units or a few large units is more efficient. This question is critical, as further system fragmentation could increase internal endurance losses, drastically impacting overall performance.

There is a second important gap in literature. Most research on substrates for MFCs has focused on the degradation rate of organic matter and the efficiency of energy conversion [18], [19]. However, the resulting biogeochemical transformations in the substrate, especially from an agroecological perspective, have received less attention. It is unclear whether the power generation process irreversibly depletes the soil's nutritional capacity or if, on the contrary, it could induce beneficial changes, such as mineralization and availability of key nutrients.

Recent research shows that the efficiency of MFCs increases when parameters such as ohmic load, pH, and substrate conductivity are improved. Apollon demonstrated that properly processed manure promotes the growth of electrogenic bacteria, reaching power densities higher than those obtained with other organic substrates [20]. Another study by Navar shows that cow manure has a biochemical composition that allows for better electron transfer [21], [22]. Additionally, Syed evaluates goat manure, highlighting the performance of this livestock due to its higher content of nitrogenous and carbonaceous compounds [23], [24]. These losses include ohmic resistance (resistance to ion and electron flow) and contact resistance (resistance at the interface of connections), which can drastically impact overall performance.

Therefore, this research is important because it directly addresses these gaps. The study has a double objective: first, to validate sheep compost, an abundant organic waste, as a viable substrate for the generation of bioelectricity; and second, to analyze the evolution of the physicochemical properties of the substrate to determine if the process degrades or transforms it in a beneficial way. By comparing two series configurations (C1:16 small reactors vs. C2:8 large reactors), this work seeks to offer clear design guidelines and assess the biogeochemical impact of power generation, thus contributing to the feasibility of future practical applications of soil-based MFCs [25], [26].

## 2. METHODS

### 2.1. Substrate and conditioning

A sample composed of 20 kg of sandy loam soil enriched with sheep compost was collected from an agricultural land in Chacapampa, Huancayo, Peru. This was sifted to remove aggregates and plant debris. It was then incubated in a container at room temperature (approx. 18-22 °C) for 15 days, keeping the humidity constant to stabilize and encourage the activity of the endogenous microbial community.

### 2.2. Design and experimental assembly

Cylindrical plastic reactors (pots) were used to build the individual MFC units. A pair of electrodes were installed in each reactor: a zinc anode and a copper cathode shown in Figure 1. The geometric surface area of the electrodes was approximately 50 cm<sup>2</sup> each.

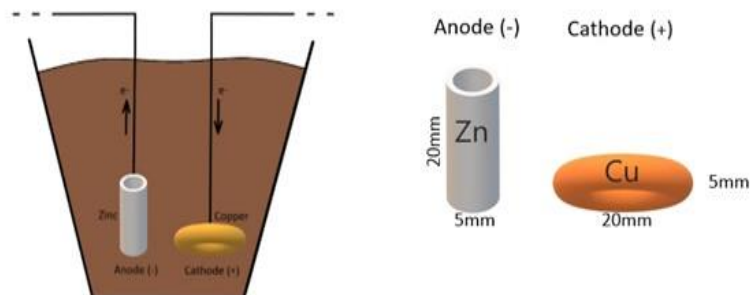


Figure 1. Schematic of the arrangement of electrodes within an individual soil microbial fuel cell reactor

a. Area

$$A = \pi(R_1^2 + R_2^2 + a(R_1 + R_2)) \quad (1)$$

b. Volume

$$V = \frac{h\pi}{3}(R_1^2 + R_2^2 + a(R_1 + R_2)) \quad (2)$$

When  $R_1$  is radius of the lower base,  $R_2$  radius of the upper base and  $a$  is height. It should be noted that sterile control was not included in this experimental design. Consequently, the current generation represents the combined effect of microbial activity and the galvanic corrosion of the zinc anode [1], [27]. Then, two experimental configurations were established, both with a total substrate mass of 6.4 kg.

Then, two experimental configurations were established, both with a total substrate mass of 6.4 kg, as illustrated in Figure 2. Figure 2(a) shows configuration 1 (C1), which was composed of 16 smaller volume reactors, each filled with 400 g of substrate. Whereas Figure 2(b) depicts configuration 2 (C2), formed by 8 reactors of greater volume, each filled with 800 g of substrate.

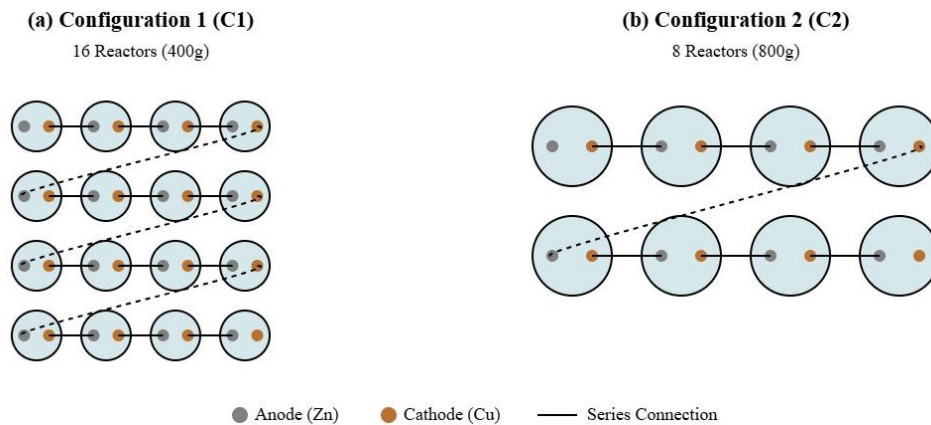


Figure 2. Final disposal of reactors (a) circuit 1 and (b) circuit 2

### 2.3. Experimental monitoring and electrical measurements

For 15 consecutive days, the electrical parameters of each system (C1 and C2) were measured three times a day (8:00, 13:00 and 18:00 h). A digital multimeter (UNI-T, UT33B+) was used to record the voltage ( $V$ ) through an external load resistor of  $330 \Omega$ . Current ( $I$ ) and power ( $P$ ) were calculated by applying Ohm's law, following standardized methodologies [28]:

a. Current (A)

$$I = V / R \quad (3)$$

b. Power (W)

$$P = V * I \quad (4)$$

### 2.4. Physicochemical analysis

Samples of the initial substrate were taken for complete physicochemical analysis before the experimental setup and at the end of the 15 days of operation. These were carried out by an accredited laboratory (INIA, Santa Ana).

### 2.5. Statistical analysis

Electrical data was tabulated and processed in OriginLab and IBM SPSS Statistics v29. A significance level of  $\alpha=0.05$  was established. The normality of the power data was assessed with the Shapiro-Wilk test. Due to the failure to comply with the assumption of normality, the non-parametric Mann-Whitney U test was applied to compare the power distributions between the two configurations.

**3. RESULTS AND DISCUSSION**

**3.1. Initial characterization of the substrate**

The initial physicochemical analysis of the substrate (soil with sheep compost) established the starting conditions for the experiment. The material had a slightly alkaline pH of 7.9, a high content of organic matter (15.2%) and an electrical conductivity of 25.7 mS/m. Key nutrient concentrations were elevated, with an available phosphorus level of 55.0 mg/kg and an especially notable available potassium content of 4,133.11 ppm shown in Figure 3.

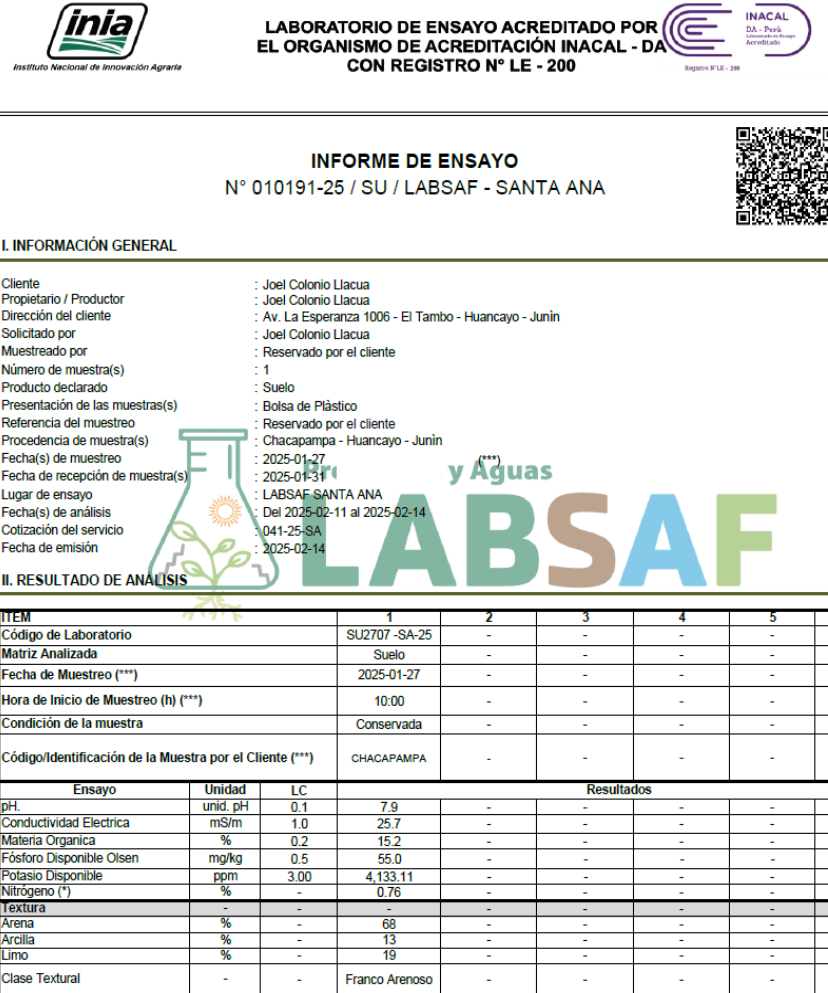


Figure 3. Initial physicochemical characterization of the substrate

**3.2. Electrical performance and configuration comparison**

Both systems generated electricity in a sustained way during the 15 days of operation. However, the performance of the two configurations was markedly different. As illustrated in the daily evolution of the parameters shown in Figure 4. Figure 4(a) shows the average daily voltages, where C2 consistently exceeds C1. Figure 4(b) illustrates the current generation, showing a similar trend of superiority for the C2 configuration. Figure 4(c) compares the average daily power, highlighting the significant gap between the 8-reactor and 16-reactor systems.

Statistical analysis of aggregate data confirmed this observation. The C2 configuration showed a significantly higher median power (Mdn=819.53 μW, Interquartile range [IQR]=383.23 μW) than the C1 configuration (Mdn=380.92 μW, IQR=10.57 μW). The Mann-Whitney U test corroborated that this difference was statistically significant (U=37.0, Z=-3.132, p=0.002). The box diagram in Figure 5 visually summarizes the superiority of the C2 configuration, showing not only a higher median, but also a greater dispersion and a higher range of values.

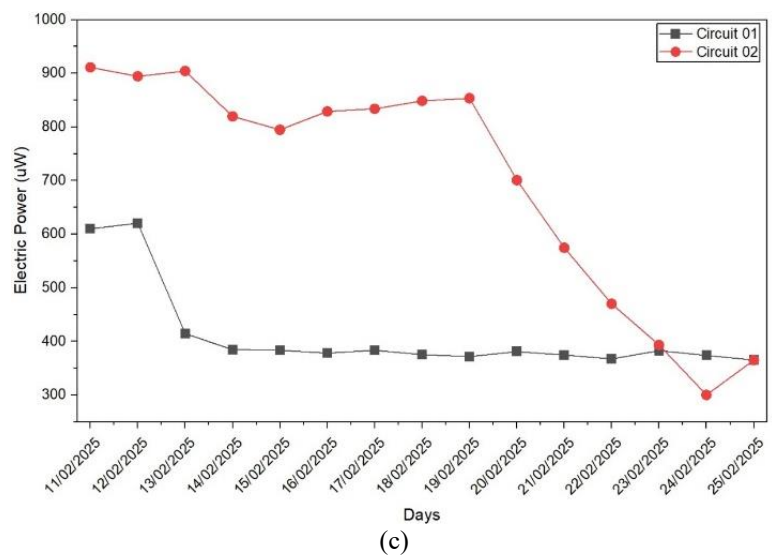
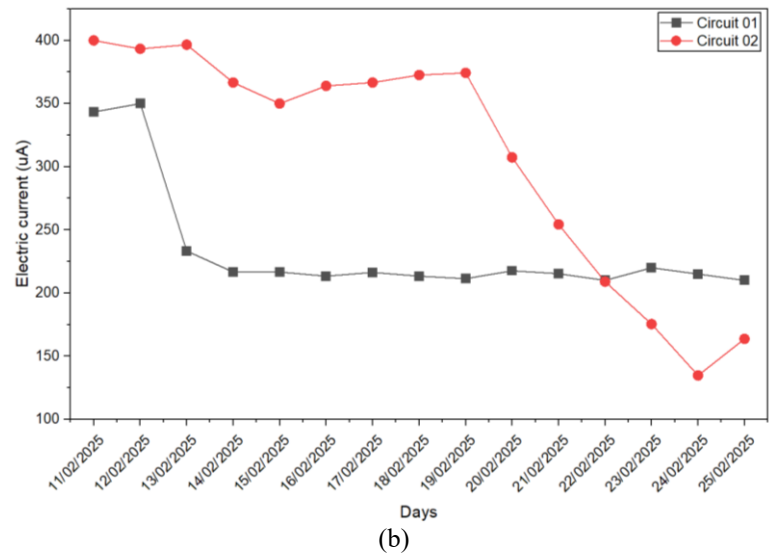
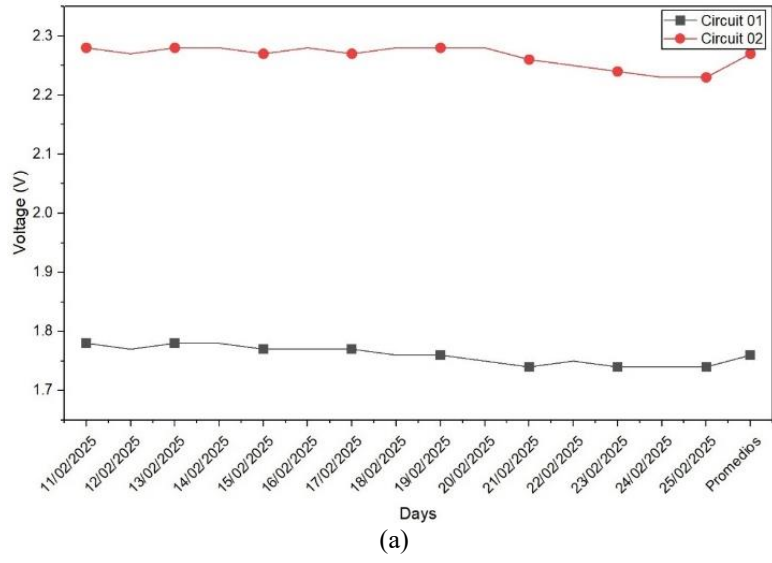


Figure 4. Daily evolution of electrical parameters for C1 and C2 configurations: (a) comparison of average daily voltages, (b) comparison of average daily currents, and (c) comparison of average daily power

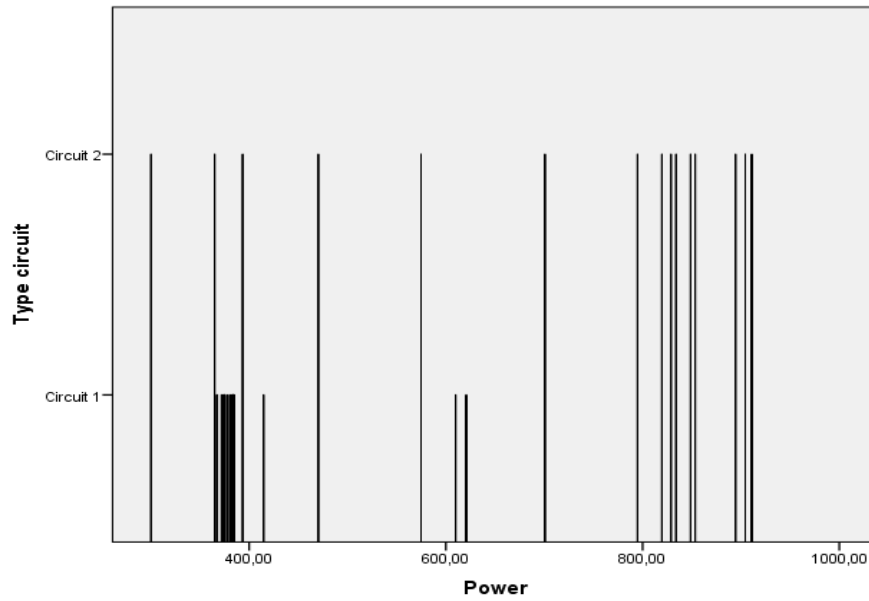


Figure 5. Box diagram comparing the power distribution (W) between circuit 1 and circuit 2

It is observed that the median, dispersion and total power range are considerably higher in Circuit 2. The system was able to generate enough voltage to power an LED diode, as seen in Figure 6, confirming the energy production.

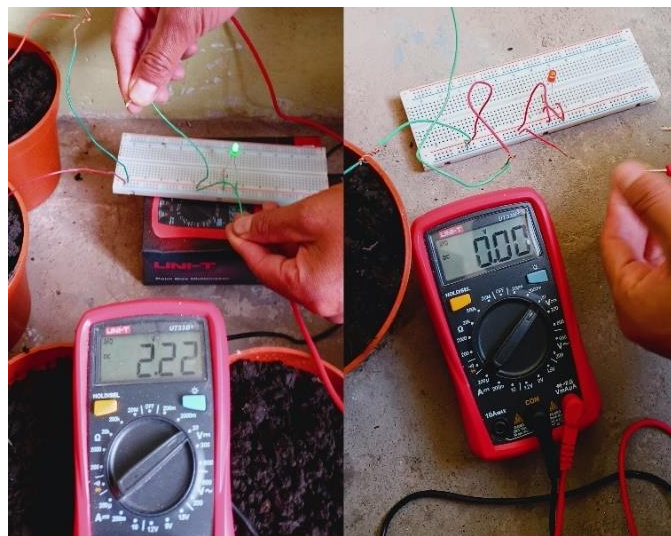


Figure 6. Lighting LED spotlights

The area and volume were calculated taking into consideration the dimensions of the eight reactors of the circuit (a).

- Total area

$$A = 0.22 \text{ m}^2 \tag{5}$$

- Total volume

$$V = 19.26 \text{ }\mu\text{m}^3 \tag{6}$$

The area and volume were calculated taking into consideration the dimensions of the sixteen reactors of the circuit (b).

- Total area

$$A = 0.33 \mu\text{m}^2 \quad (7)$$

- Total volume

$$V = 19.86 \mu\text{m}^3 \quad (8)$$

The relationship between the calculated area and the electrical parameters is presented in Table 1, which details the performance of voltage, current, and power according to the area. Similarly, Table 2 demonstrates the performance of these electrical parameters according to the reactor volume.

Table 1. The performance of voltage, current and power according to the area

Area ( $\text{m}^2$ )	Voltage (V)	Current ( $\mu\text{A}$ )	Power ( $\mu\text{W}$ )
0.22	5.74	819.12	2660.09
0.33	7.76	1116.79	3803.17
0.44	9.78	1414.37	4945.89
0.55	11.80	1711.95	6088.61
0.65	13.82	2009.53	7231.33
0.76	15.84	2307.11	8374.05
0.87	17.86	2604.68	9516.77
0.97	19.89	2902.26	10659.49
1.08	21.91	3199.84	11802.21
1.19	23.93	3497.42	12944.93
1.29	25.95	3795.00	14087.65
1.40	27.97	4092.58	15230.37
1.51	29.99	4390.16	16373.09
1.62	32.01	4687.74	17515.81
1.72	34.03	4985.31	18658.53
1.83	36.06	5282.89	19801.25
1.94	38.08	5580.47	20943.97
2.04	40.10	5878.05	22086.69
2.15	42.12	6175.63	23229.41

Table 2. The performance of voltage, current and power according to the volume

Volume ( $\mu\text{m}^3$ )	Voltage (V)	Current ( $\mu\text{A}$ )	Power ( $\mu\text{W}$ )
19.26	9.64	1393.97	4867.54
19.86	9.91	1432.97	5017.32
20.47	10.17	1472.26	5168.19
21.08	10.44	1511.55	5319.06
21.69	10.71	1550.84	5469.93
22.30	10.97	1590.13	5620.80
22.91	11.24	1629.41	5771.67
23.52	11.51	1668.70	5922.54
24.13	11.77	1707.99	6073.41
24.74	12.04	1747.28	6224.28
25.35	12.31	1786.57	6375.15
25.96	12.57	1825.86	6526.02
26.57	12.84	1865.15	6676.89

### 3.3. Physicochemical evolution of the post-operation substrate

At the end of the 15 days of power generation, the physicochemical analysis of the substrate, as detailed in Figure 7, revealed significant transformations compared to its initial state. The pH was alkalized, increasing from 7.9 to 8.3. Electrical conductivity also increased markedly, from 25.7 to 40.5 mS/m, indicating a release of soluble ions into the medium.

In terms of nutrients, opposite trends were observed. The concentration of available potassium decreased considerably from 4,133.11 to 2,883.33 ppm. In contrast, available phosphorus increased from 55.0 to 83.37 mg/kg, suggesting a process of solubilization or mineralization induced by microbial activity.



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**INFORME DE ENSAYO**  
**LABSAF SANTA ANA**  
**N° 053890-25 / SU / SA**



**I. INFORMACIÓN GENERAL**

Cliente : JOEL COLONIO LLACUA  
Propietario / Productor : JOEL COLONIO LLACUA  
Dirección del cliente : Av. La Esperanza 1006, El Tambo, Huancayo  
Solicitado por : JOEL COLONIO LLACUA  
Muestreado por : Reservado por el cliente  
Referencia del muestreo : Reservado por el cliente  
Procedencia de muestra(s) (\*\*\*) : Junín/Huancayo/Chacapampa  
Fecha(s) de muestreo (\*\*\*) : 2025-04-27  
Fecha de recepción de muestra(s) : 2025-05-05  
Lugar de ensayo : LABSAF SANTA ANA  
Fecha(s) de análisis : Del 2025-05-30 al 2025-06-04  
Cotización del servicio : 195-25-SA  
Fecha de emisión : 2025-06-04

**II. RESULTADO DE ANALISIS**

ITEM	1	2	3	4	5	6
Código de Laboratorio	SU11095 -SA-25	-	-	-	-	-
Matriz Analizada	Suelo	-	-	-	-	-
Fecha de Muestreo (***)	2025-04-27	-	-	-	-	-
Hora de Inicio de Muestreo (h) (***)	10:00	-	-	-	-	-
Código/Identificación de la Muestra por el Cliente (***)	JCL - Muestra	-	-	-	-	-
Ensayo	Unidad	LC	Resultados			
pH	unid. pH	0.1	8.3	-	-	-
Conductividad Eléctrica	mS/m	1.0	40.5	-	-	-
Fosforo Disponible	mg/kg	0.50	83.37	-	-	-
Materia Orgánica	%	0.2	16.0	-	-	-
Potasio Disponible	mg/kg	3.00	2883.33	-	-	-
Textura	-	-	-	-	-	-
Arena	%	-	81	-	-	-
Arcilla	%	-	6	-	-	-
Limo	%	-	13	-	-	-
Clase Textural	-	-	Arena Franca	-	-	-

Figure 7. Final physicochemical characterization of the substrate

#### 4. DISCUSSION

The results obtained in this study provide significant insight into the behavior of sheep compost as a substrate in bio-electrochemical and sediment battery systems, specifically comparing two scaling architectures. The discussion is addressed in three key dimensions: electrical performance, physicochemical evolution, and environmental implications.

##### 4.1. Impact of reactor configuration on energy performance

The significant superiority of the C2 configuration (8 large reactors) over C1 (16 small reactors) challenges the intuitive notion that more units in series always equal higher voltage. This phenomenon is explained through the concept of total internal resistance. Every physical connection between reactors introduces a contact resistance. System C1, having twice the number of interconnections, likely suffered from higher parasitic losses due to the Joule effect at the terminals.

Furthermore, literature suggests that in systems with many cells in series, the “weakest” cell limits the total current, and can even lead to voltage reversal, where a cell acts as a load rather than a generator [29], [30]. Consolidating the substrate into fewer, larger units (C2) minimizes these risks. Although a larger volume could theoretically increase the distance between electrodes (increasing ohmic resistance), our results indicate that reducing the number of external interfaces was the dominant factor for efficiency [15], [31]. Validation of this hypothesis using direct resistance measurements (e.g., with an ohmmeter) is recommended for future specific characterization.

##### 4.2. Mechanisms of physicochemical transformation

The evolution of the substrate reflects intense electrochemical activity. The increase in pH (from 7.9 to 8.3) is consistent with the oxygen reduction reaction at the cathode, which consumes protons or generates hydroxide ions [6], [32]. This alkalization is crucial as it can influence the solubility of soil nutrients.

Regarding conductivity, the marked increase indicates a higher concentration of free ions. In a standard biological system, this is attributed to the mineralization of organic matter by Ex electrogenic

bacteria. However, in this hybrid system, it is crucial to acknowledge the role of the zinc anode. The corrosion of zinc releases  $Zn^{2+}$  ions into the soil matrix, which directly contributes to the conductivity increase and may chemically interact with soil phosphates [33], [34].

### 3.4.3. Nutrient dynamics and environmental safety

The increase in available phosphorus (from 55.0 to 83.37 mg/kg) is a promising finding for potential agricultural reuse. This mobilization may be due to the dissolution of inorganic phosphates facilitated by local changes in pH and the chelating action of organic acids produced by microbial metabolism [14], [35]. Conversely, the decrease in potassium confirms its role as a mobile cation involved in charge balancing within the electrochemical cell [36], [37].

However, the “biofertilizer” potential of the spent substrate requires a critical caveat. Caution must be exercised regarding the “biofertilizer” label. While the enrichment of phosphorus is beneficial, the dissolution of the zinc anode implies that the soil is being enriched with zinc. While zinc is a micronutrient, excessive accumulation can be toxic to plants and microorganisms. Therefore, the spent substrate must be analyzed for heavy metal content before any agricultural application [27], [38].

## 5. CONCLUSION

This study successfully demonstrated the viability of sheep compost as a potent substrate for bioelectricity generation in high-Andean environmental conditions, utilizing a zinc-copper electrode configuration that functions as a hybrid bio-galvanic system. The comparative analysis of reactor architecture reveals a critical design principle for scaling up terrestrial MFCs: consolidation of substrate volume into fewer units (C2) is vastly superior to fragmentation (C1), primarily because minimizing the number of external interconnections significantly reduces total contact resistance and mitigates voltage reversal phenomena. Beyond energy production, the system induced a distinct physicochemical transformation in the substrate, notably increasing phosphorus availability, which suggests a potential dual utility for soil remediation; however, this agronomic benefit is counterbalanced by the depletion of potassium and the release of zinc ions from anode corrosion. Therefore, while technology offers a promising pathway for rural electrification and waste valorization, the spent substrate cannot be unconditionally classified as a safe biofertilizer without rigorous ecotoxicological screening for heavy metal accumulation. Future investigations must prioritize electrochemical impedance spectroscopy (EIS) to precisely map internal resistance components and employ molecular tools such as 16S rRNA sequencing to decouple the biological contribution from the galvanic activity, ensuring that the environmental footprint of the zinc anode is fully understood before widespread application.

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Authors state there is no funding involved.

## AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Joel Colonio	✓	✓	✓	✓	✓	✓		✓	✓	✓			✓	✓
Elvis Carmen		✓				✓			✓	✓	✓	✓		
Arlitt Lozano	✓		✓	✓			✓	✓		✓	✓		✓	✓
Alizze Colonio			✓		✓		✓	✓	✓	✓		✓		✓

C : Conceptualization

M : Methodology

So : Software

Va : Validation

Fo : Formal analysis

I : Investigation

R : Resources

D : Data Curation

O : Writing - Original Draft

E : Writing - Review & Editing

Vi : Visualization

Su : Supervision

P : Project administration

Fu : Funding acquisition

**CONFLICT OF INTEREST STATEMENT**

Authors state no conflict of interest.

**INFORMED CONSENT**

We have obtained informed consent from all individuals included in this study.

**DATA AVAILABILITY**

The data that supports the findings of this study are available from the corresponding author, JCL, upon reasonable request.




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