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Dog feces with glucose-feed were the most effective to produce electricity in microbial fuel cells

Heces de perro y glucosa fueron las más efectivas para producir electricidad en celdas de combustible microbianas

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Abstract

Microbial fuel cells (MFCs) generate electricity through microbial metabolism, which is an efficient, renewable, and sustainable biological alternative for domestic and potential commercial applications. Mine wastes and dog feces are common and abundant residues contributing to environmental pollution and human health concerns. This study analyzed the production of electricity by using mine waste, dog feces, and vermicompost as MFC feedstocks and tested two sources of carbon (saccharose and glucose) as co-substrates in the feeding solution. Dog feces produced the longest current output (41 days), the maximum voltage per day (0.33 V), and the highest cumulative power density (15 W $cm^{-3)}$. Dog feces with glucose generated the highest power density (5.6 to 15 W cm^{-3}), while saccharose and mine waste produced a maximum current output of 0.24 V day^{-1} and a cumulative power density of ~8 W cm^{-3}. There were changes in the mine waste-metal concentration. MFC can be a practical solution to the aged environmental concern of the disposal of wastes, especially dog feces. Moreover, it may participate in the removal of Zn present in mine wastes. It may help the worldwide energy crisis, but several aspects must be optimized to bring it to the commercial level.

Keywords: Bioelectrochemical hybrid technology, microbial electro-remediation, mine residues.

Resumen

Las celdas de combustible microbianas (CCM) generan electricidad a través del metabolismo microbiano y son una alternativa biológica renovable, sostenible y eficiente con diferentes aplicaciones. Los desechos mineros y las heces de perro son residuos comunes y abundantes que contribuyen a la contaminación ambiental y preocupan por sus efectos negativos en la salud humana. Esta investigación analizó la producción de electricidad utilizando desechos mineros, heces de perro y vermicompost como materias primas para CCM y dos fuentes de carbono (sacarosa y glucosa) como cosustratos en la solución de suplemento de celdas. Las heces de perro produjeron corriente duradera (41 días), voltaje máximo por día (0.33 V) y mayor densidad de potencia acumulada (15 W cm^{-3}). Las heces de perro con glucosa generaron la densidad de potencia más alta (5.6 a 15 W cm^{-3}). La sacarosa y los desechos mineros produjeron salida de corriente máxima de 0.24 V $día^{-1}$ y densidad de potencia acumulada de ~8 W cm^{-3}. Las CCM pueden ser solución práctica para la eliminación de residuos, especialmente las heces caninas. Lo cual auxilia en la crisis energética; pero es necesario optimizar varios aspectos para comercializarlas.

Palabras clave: Tecnología híbrida electroquímica, electro-remediación microbiana, residuos de mina.

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1 Introduction

Global electricity demand continually increases, driven by growing industrial use, air conditioning, electrification, and data centers. According to the International Energy Agency, global power consumption increased by 4.3% in 2024, equivalent to 1,080 terawatt-hours. This number is nearly double the annual average of the preceding decade. Furthermore, the power energy demand is expected to increase by 4% until 2027 (IEA, 2025b, 2025a). Meanwhile, for global fossil fuel-based energy, a 33% increase in demand is projected by 2035, which will lead to an additional 87 gigatons of CO_2 equivalent by 2050, thereby accelerating the effects of the global climate change (Ray *et al*., 2025). Most of the electricity demand over the next three years will come from emerging and developing economies (IEA, 2025b, 2025a). In this regard, fuel-based energy sources need to be gradually replaced with cleaner energy sources to keep a balance between energy demand and sustainability (Choudhury *et al*., 2017; Chouler *et al*., 2016), and the principles of the circular economy could be a vital pillar to reach this energy transition (Boloy *et al*., 2021).

Waste-to-energy is an effective component of modern waste management strategy technology, and it may be a low-cost waste treatment approach for hazardous materials while simultaneously obtaining energy (Aznar-Sánchez *et al*., 2018; Choudhury *et al*., 2017; Elhenawy *et al*., 2022; Pandit *et al*., 2021; Yousefi *et al*., 2021). Among waste-to-energy technologies, microbial fuel cells (MFCs) are gaining attention for producing bioelectricity (Logan & Rabaey, 2012; Yousefi *et al*., 2021) because of their ability to generate energy directly from biodegradable waste and operate under low-energy conditions (Apollon *et al*., 2025). Moreover, in regions characterized by limited access to electricity, the implementation of MFCs can be a viable solution (Rajesh & Kumawat, 2023).

The MFCs are devices that convert chemical energy contained within organic compounds into electricity through electrochemical reactions. These reactions are generated by the metabolism of microorganisms involved in the system (Douma *et al*., 2025). In general, in anaerobic conditions, microorganisms near the anode oxidize organic compounds by releasing protons and electrons in the anode chamber. The electrons are then transferred to the cathode by an external circuit (Foudhaili *et al*., 2019; Rajesh & Kumawat, 2023). A typical MFC comprises two compartments, an anode and a cathode, separated by a membrane (Douma et al., 2025). However, several configurations (single chamber, double chamber, up-flow, or stacked) have been explored in order to enhance the energy efficiency of these devices and scalability (Apollon *et al*., 2025).

The large-scale implementation of MFCs technology faces several challenges, including the cell configuration type and shape of electrodes (Altın & Uyar, 2025), and the substrate used as a feedstock. Among those factors, the substrate is one of the key elements for the production of electricity because it provides microorganisms, nutrients, and electron donors. For many years, sugar-based wastes and wastewater have been used as feedstock for MFCs (Apollon *et al*., 2025). Nevertheless, some research work has been conducted on the use of non-conventional substrates, such as agro-residues (Rajesh & Kumawat, 2023) or animal and human feces (Douma *et al*., 2025; Memon *et al*., 2025) to feed MFCs. Under this frame, dog feces may also be a resource for electricity production, due to 95% of feces corresponding to organic matter content, the rest is related to elements such as N, P, K, Mg, Ca, Na, S, B, Ca, Fe, Cu, Mn, and Zn (Yavor *et al*., 2020). Moreover, it is estimated that 30% of households globally have a dog, resulting in between 0.7 and 1 billion dogs (Fiães *et al*., 2025), which leads to the production of more than 5 million tons of canine feces annually (Dróżdż *et al*., 2022).

Proper management of canine feces is essential for environmental and public health reasons. Improper disposal of dog feces can lead to contamination of landfills and water sources, the spread of pathogens, and air pollution (USDA, 2005). For example, Yavor *et al*. (2020), used a life cycle impact assessment, to analyze the environmental impact of dogs which have an average lifespan of 13 years. The authors related their food and excrement (urine and feces) to fifteen impact categories. These authors concluded that dog feces significantly contribute to the eutrophication and ecotoxicity of freshwater. According to these authors, this impact is comparable to 7% of the climate change impact that a European citizen makes annually. Furthermore, with low and inadequate feces disposal control, soil contamination occurs. These wastes are a social health risk because they can produce several diseases and human health effects (Penakalapati *et al*., 2017). On the other hand, nowadays, the use of dog feces bags is another environmental issue that contributes to 0.6% of the total plastic waste generated worldwide (Dróżdż *et al*., 2022). Despite the existence of alternative options for the management of dog waste, such as incineration and composting, in many countries, there are no guidelines for the proper management of canine feces. In addition, incineration and composting have limitations. The incineration process needs the establishment and maintenance of an incinerator, and the provision of energy, most commonly derived from fossil fuels. This methodology is exclusively feasible for large-scale facilities, as composting may not be effectively eliminated some pathogens present in the dog feces. Improper management of the composting process can lead to the generation of odors, attracting flies and rodents (Fiães *et al*., 2025). Consequently, within this context, the MFC can be a potentially efficacious alternative for the treatment of canine feces.

Mining tailings, also known as mine wastes or mine residues, constitute an unconventional material that has gradually garnered interest for use as an MFC feed (Cui *et al*. 2025) because it is possible to recover energy and remove metals from low-value wastes. Mine wastes may be a feasible material for use as a final electron acceptor in MFC due to the presence of elements with a comparable or higher redox potential than oxygen (Gonzalez Olias *et al*., 2019). The sustainable management of mine waste continues to be a global concern, as well as its associated environmental and social costs (Damoah & Herat, 2022) For example, significant on-site storage problems, dust generation, and the potential release of heavy and toxic metals contained in the dust particles (Lim & Alorro, 2021). In addition, the worldwide annual generation of mine wastes is around 5-7 billion tons (Lorenzo-Tallafigo *et al*., 2022). Ideally, sustainable mine-waste management practices imply using techniques to reduce or prevent the mine waste before its generation. However, due to technical limitations and the nature of the mineral extraction process, it is almost impossible to reduce the production of mine wastes. Therefore, new approaches have been developed to address this issue, such as adding value to mine wastes through reprocessing, resource recovery, or repurposing (Calderon *et al*., 2020; Lim & Alorro, 2021). Through MFCs, the removal/recovery of some metals while generating energy is possible (Ghassemi, 2001; Mathuriya and Yakhmi 2014). However, the use of mine wastes to generate electricity is a relevant issue that has received little analysis and warrants further research.

As mentioned early, mine wastes and pet domestic waste, which pose a significant environmental and human health concern worldwide, may be suitable for producing electrical energy using MFCs and have received limited research attention. Therefore, the objectives of this research were: 1) To determine electricity production in MFC fed with non-conventional residues (mine wastes, dog feces), and one agricultural conventional transformed residue without environmental concern as vermicompost and two co-substrates functioning as extra-carbon sources (glucose and saccharose). 2) To morphologically identify the anode-bacteria of MFC by using scanning electron microscopy in the best treatments. This research addresses two alarming issues. On one hand, the mitigation of an environmental problem posed by excessive waste (from the mining industry and the most common domestic pets). On the other hand, an alternative cost-effective electricity generation is needed under an imminent energy crisis taking place in many countries.

2 Materials and methods

Mine wastes, dog feces, and vermicompost were used as feedstock materials. To start microorganisms' growth in the MFCs, glucose and saccharose were added separately, while tap water was used as a control. The treatments tested were the combination of substrates (3) and the nutrient solutions (2), plus the control (1 for each substrate involving the absence of nutrient solution). In total, nine treatments, three replicates of each treatment, were evaluated.

2.1 *Chemical characterization of feedstock substrates*

Electrical conductivity and pH of substrates were measured in a 1:2 slurry substrate: water solution using a conductimeter (Conductronic CL35), and a pH meter (Orion Research, Model 601, Beverly, MA) according to Rowell (1994) and Rhoades (1996). Organic carbon content (OC) was quantified using the method of Walkley-Black (1983); which measures oxidizable organic carbon. The chemical characterization was performed in the original substrates, and considering modifications in the MFCs, was also analyzed at the end of the experiment (42 days).

2.2 *Configuration of MFC and experimental setup*

H-type MFCs (two-chamber configuration) were constructed because they allow independent manipulation of electrodes (Rabaey *et al*., 2003). Briefly, sterile clinical bottles of 100 mL functioned as anode and cathode chambers. A saline bridge connected the anodic and cathodic chambers. The saline bridge consisted of a chlorinated polyvinyl chloride tube with a diameter of 1.27 cm and 2 cm long, containing 5 mL of 1 M KCl solution and 3% (w/v) agar-agar. The electrodes were circular pieces of aluminum (from soda cans) with a diameter of 4.6 cm (16.62 cm^2 surface area). Aluminum was chosen because it is a low-cost material that can be used as an alternative to carbon-based electrodes for oxygen reduction (Saad *et al*., 2025).

The electrodes' surface was rubbed using sandpaper to make it rough and allow biofilm adhesion. The electrodes were connected with a 13 AWG copper wire (11.5 cm long). A diagram of the configuration of MFC is provided in Fig. 1.

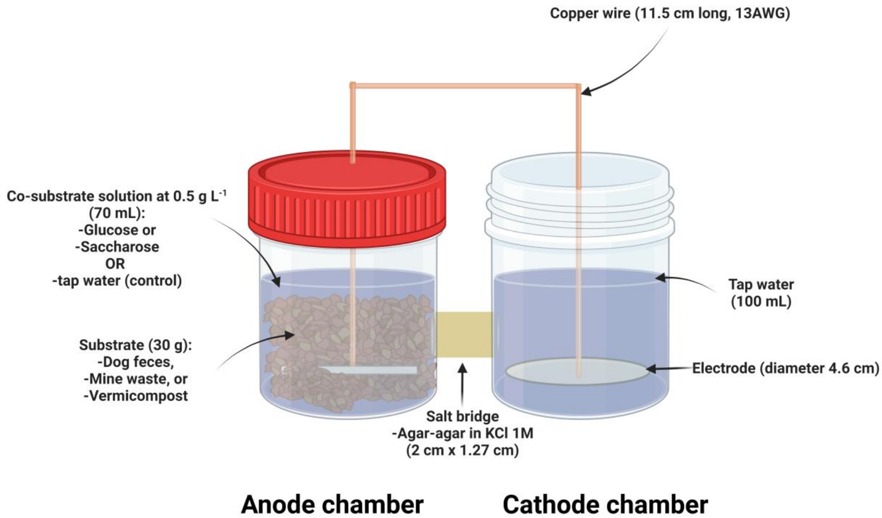

Figure 1. Design of microbial fuel cells used in this research

The anodic chamber was fed in all cases with 30 g of sieved (2 mm mesh) mine wastes (mining tailings), vermicompost, or dog feces, while the cathodic chamber was filled with 100 mL of tap water. Moreover, 70 mL of co-substrates (nutrient solutions), glucose or saccharose at 0.5 g L^{-1}, was added to the anodic chamber. In the case of the control treatment, tap water was added. The nutrient solutions or tap water were added at the beginning of the experiment establishment in a single addition. After immersing the electrodes, the anodic chamber was sealed with a plastic lid (to ensure anaerobic conditions), while the cathodic chamber was kept open. The MFCs operated for 42 days and were kept at room temperature. Room temperature was recorded daily (from 12 to 18 °C), and the voltage produced was measured daily at intervals of 24 hours using a multimeter (Steren Mul-005). The electrical resistance and the electrical power density were calculated according to Logan *et al*.(2006) and Luo *et al*. (2010). At the end of the operation the monitoring of MFCs, the electrodes, and substrates were separated for their analysis.

2.3 Calculations

The current was calculated based on the voltage and the resistance of the conducting wire used (7.55×10^{-4} Ω). The power density was calculated based on the volume of the anodic chamber and the surface area of the cathode as follows:

$$P_v = \frac{I \times V}{Volume\ of\ anodic\ chamber}$$ (Luo *et al*., 2010)

where I is the current (A), V is the voltage (V). The volume of the anodic chamber was expressed in m^3. Normalizing the power output based on volume may be helpful to compare the economic feasibility of the system in terms of size and material cost (Logan *et al*., 2006).

2.4 Scanning electron microscopy analysis

The anode-biofilm formation was examined by scanning electron microscopy. Anodes were separated from the MFC carefully, and a section of approximately 1 cm^2 was taken and fixed with 5 mL of phosphate-buffered saline solution (PBS 0.1 M pH 7.4) and 3% (v/v) glutaraldehyde. The samples were embedded for 12 hours at 4 °C with fixation solution, then washed three times with PBS solution, and kept at 4 °C until their imaging analyses. An environmental scanning electron microscope (ESEM) (Carl Zeiss EVO LS10, Jena, Germany) and an X-ray detector (EDX) (Bruker, Quantax 200, Germany) were used. The samples were placed in aluminum stubs with carbon conductive tape. A manometric pressure of 90 Pa of water vapor, an electron beam with a voltage of 30 kV, and backscattered electron detector (NTS BSD) were used. Photomicrographs were captured with the electron microscope. Elemental microanalysis of chemical elements was carried out with an EDX detector. For EDX analysis, three fields of view (each 20 mm²) were analyzed for 240 s at an acceleration voltage of 30 kV.

2.5 Metal removal and recovery

The pseudo-total concentrations were analyzed in the original substrates and after finishing the experimental stage in both anode (metal removal) and cathode (metal recovery) chambers. The pseudo-total concentration was measured following the ISO 11466 method. Briefly, 500 mg of the sample were digested with 6 mL of HNO_3-$HClO_4$ (3:1 ratio). The samples were placed in a digestion block for 8 hours at 120 °C. After cooling, the digested samples were diluted to 25 mL and filtered through Whatman No. 42. The concentrations were analyzed using an atomic absorption spectrometer (PerkinElmer, model 3110).

2.6 Statistical analysis

Data compliance with normality assumptions (Shapiro-Wilks, $\alpha = 0.05$) and variance homogeneity (Bartlett, $\alpha = 0.05$) was corroborated. Data on pseudo-total metals and organic carbon did not satisfy the assumptions and were transformed with the Box-Cox power transformation. An analysis of the variance was carried out of cumulative voltage and power density output in the microbial fuel cells. The analysis was done with the Friedman test as repeated measures existed, but with no normality compliance. Similarly, the cumulative voltage and power density output by the same substrate with different co-substrates were compared. The statistical analyses were performed using Rstudio v. 4.2.1 software for Windows.

3 Results and discussion

The production of electricity through MFCs using dog feces, vermicompost, and mine wastes as substrates was explored. The first two materials may be a potential substrate feed in MFC and a renewable source of energy due to their high OC content (Table 1), microbial populations, and different nutrients contained in them, which are required for the growth and activity of microbes for electrochemical reactions (Nandy *et al*., 2015). In contrast, the MFC is a promising technology for the recovery/immobilization of metals contained in mine wastes, because the metal ions can be reduced and deposited in the electrodes. While simultaneously obtaining bioenergy. In addition, the metals contained in the mine waste (Zn, Cd, Fe, and Cu) may serve as direct electron acceptors or donors without any external power supply (Gonzalez Olias *et al*., 2019).

Table 1. Original chemical properties of substrates used in the microbial fuel cells and their modification by electron-donor substances at the end of the experiment (41 days).

Substrate	Treatments	pH			Electrical conductivity (mS/cm)			Organic carbon (%)		
		average	sd		average	sd		average	sd	
Mine waste	Original	2.3	0.01	b	7	0.05	a	0.7	0.08	b
	Saccharose	4.3	0.17	a	2.7	0.1	c	0.51	0.05	b
	Glucose	4.1	0.03	a	3.5	0.27	b	1.79	0.13	a
	Control	4.3	0.24	a	3.3	0.3	bc	0.62	0	b
Vermicompost	Original	7.8	0.03	b	11.4	0.29	a	10.4	0.56	a
	Saccharose	8.2	0.09	a	4.6	0.14	b	9.48	0.07	a
	Glucose	8.3	0.03	a	4.1	0.17	b	7.07	0.29	b
	Control	8.4	0.09	a	4.6	0.11	b	7.21	0.58	b
Dog feces	Original	7.4	0.06	c	4.8	0.07	a	19.59	1.02	a
	Saccharose	7.7	0.16	b	4.6	0.42	a	7.93	1.06	c
	Glucose	8.5	0.16	a	4.7	0.19	a	10.02	0.11	b
	Control	8.3	0.04	a	4.4	0.38	a	8.56	0.56	bc

Average and standard deviation are showed, $n = 3$. Different lower letters refer statistical significance when comparing treatments in the same substrate tested.

3.1 Electrochemical characterization of the MFC

The voltage generation profile was different among co-substrates (Figs. 2 and 3); however, a similar shape of the voltage generation time curve was observed between the treatments vermicompost and mine waste with saccharose and the control. In both treatments, saccharose, and control (tap water) the current production was observed from day 4 until 31; however, mine waste still produced a measurable voltage by day 35 (Fig. 2a). When using glucose, the electricity production was longer than, from day 4 to 37, with saccharose and the control treatment (Fig. 2b). Dog feces produced a low but detectable voltage by the end of the experiment (41 days).

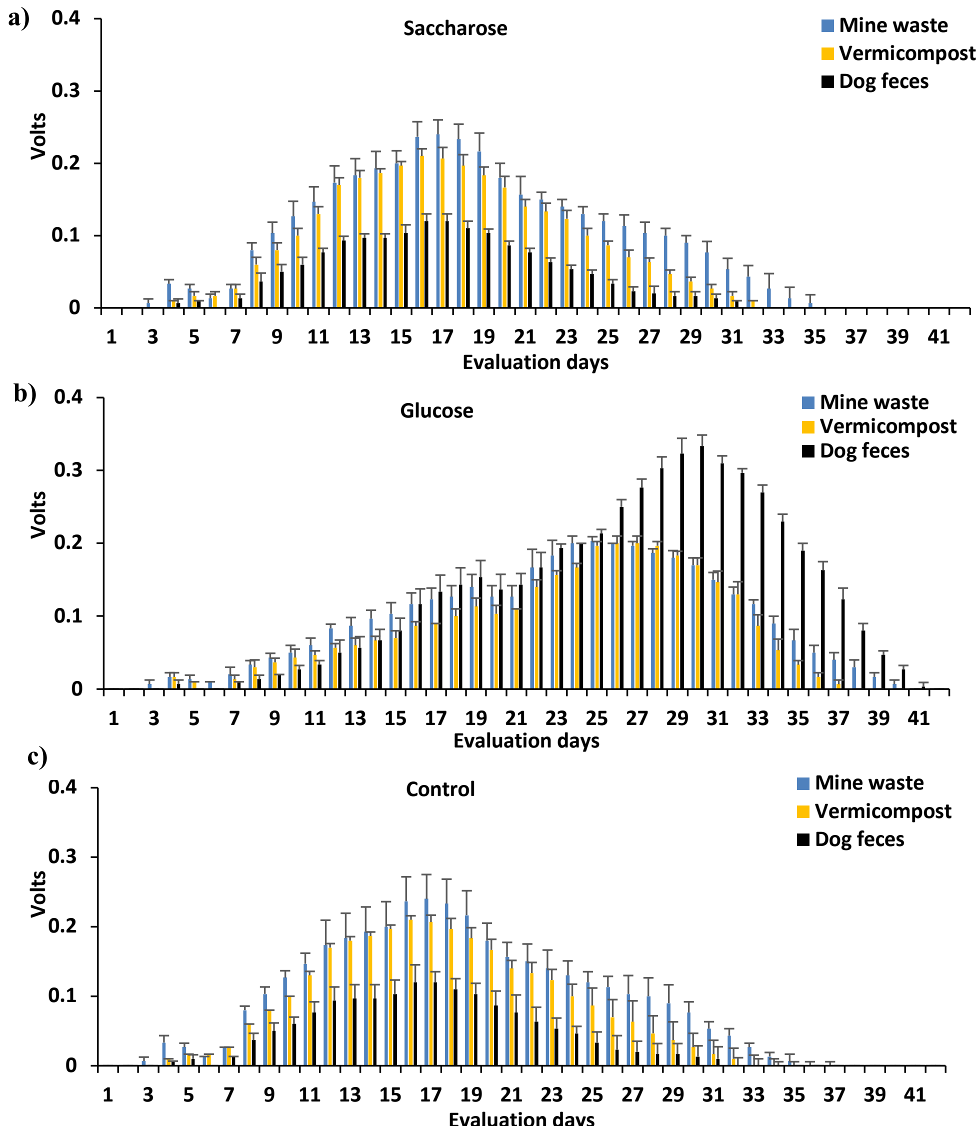

Figure 2. Kinetics of voltage output of microbial fuel cells in one operation cycle after addition of three substrates and three donor electrons sources. a) Saccharose, b) glucose, and c) tap water. Bars show average and standard deviation, n=3.

Borole *et al*. (2009) mentioned that MFC got starved before the voltage drops below 0.01 V. Aelterman *et al*. (2006) observed that the power output declined after 12 hours in MFC fed with three waste waters using hexacyanoferrate as an electron acceptor. The MFC used hexacyanoferrate as catholyte and was fed with industrial influent of an anaerobic digester from a potato-processing factory.

On the other hand, when comparing the voltage output by day, for treatments with saccharose the maximum energy production (from 0.16 to 0.24 V) was observed around days 16-17 in all substrates (Fig. 2a). In contrast, in treatments with glucose (Fig. 2b), the maximum voltage output was observed on day 30 in the case of dog feces (0.33 V), and by days 25 to 28 for the mine waste and vermicompost substrates. Logan et al. (2006) reviewed that theoretically, the maximum achievable MFC voltage in an open circuit, as used in this research, is around 0.8 V; however, during current generation, the voltages remain below 0.62 V. In the present research, using a rustic MFC, the greater voltage was half of the previously referred voltage. Therefore, making several adjustments to increase the efficiency of the MFC is needed. Also, different voltage generation profiles and the amount of energy produced can be explained by the differences in the substrate composition. Silva-Palacios *et al*. (2023) evaluated the use of mine residues as a substrate for the generation of energy through a single-chamber MFC with air cathode. After 30 days of operation of the MFC, a peak voltage value of 0.65 V was achieved. Meanwhile, Memon *et al*. (2025) developed a portable MFC feed with cow manure. The authors reported an initial open-circuit voltage of 0.49 V and a stabilization at 0.31 V after 120 hours. Elhenawy *et al*. (2022) mentioned that the type and composition of waste influence the amount of energy produced. However, the performance of an MFC is dependent upon other variables, like the operating conditions, the nature of the electrolyte, the type of microorganisms, electrode materials, operation time, and cell configuration (Foudhaili *et al*., 2019).

The cumulative electricity production also varied according to the co-substrates and substrates (Fig. 3). The lag period for current production ranged between 11 and 15 days in all the treatments. In saccharose treatment, the dog feces reached the plateau by day 26, and the lowest electricity generation was observed (Fig. 3a). In contrast, approximately the highest cumulative electricity production (5.2 V) was detected in dog feces and glucose (Fig. 3b), and the plateau production was observed by day 37. When analyzing co-substrates, glucose was the most efficient in generating electricity, while saccharose behaved similarly to the control treatment. The different co-substrates function for the carbon enrichment of substrates in the MFC; consequently, they influence the growth of the microbial consortia involved in the current output; and the MFC efficiency. Borole *et al*., (2009) tested the use of glucose and lactate in combination with several factors to encourage biofilm of electrogenic microbes, but minimize the participation of non-electrogenic ones, to reach effective power production. In the present research, we did not know the type of microorganisms involved when using different substrates and co-substrates; however, molecular analysis will help elucidate this information, including the relationship between electrogenic/non-electrogenic microorganisms and power output and electricity production.

Results of the present research show that dog feces, referred to as environmental contaminants, are useful as feedstock and may provide the opportunity to produce energy and solve an aged environmental problem. Traditionally, dog feces have been mentioned as a source of pathogenic microorganisms and pollution to soil, water, and air. Recently, Pérez-Guevara *et al*. (2021) showed evidence that feces are also a source of contamination, transport, and dispersion of microplastics such as PET and polycarbonate. These authors highlight the possible ecological and environmental consequences of the association of microplastics to feces from dogs, other animals, and human beings. These potential concerns are: longer microplastic lifespan, an increase of microplastic bioavailability to organisms, feces as a route for microplastic dispersion, and microorganisms associated with plastic/feces. The findings from the present research open the opportunity to simultaneously analyze the electricity production and possible transformation of microplastics through MFC

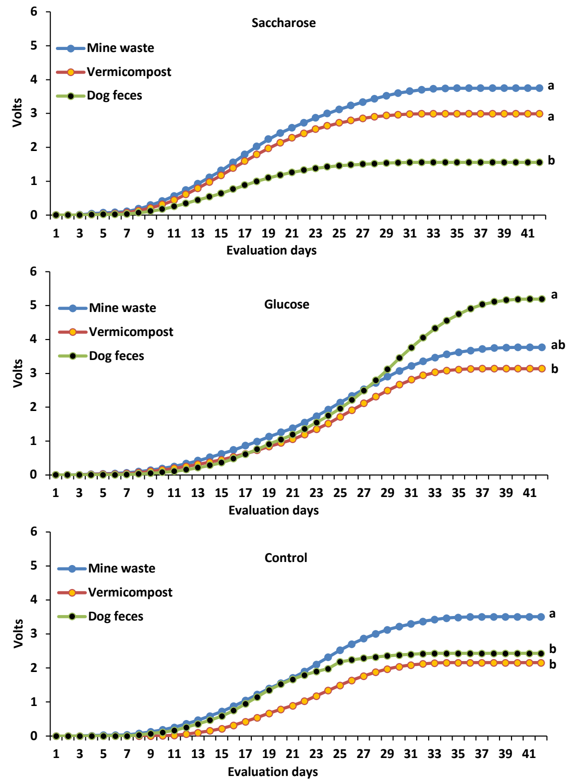

Figure 3. Cumulative voltage output in microbial fuel cells in one-operation cycle using three substrates and two donor electrons sources. Different lower letter shows statistical difference when comparing substrates in each donor electrons source (a) Saccharose, (b) glucose or (c) water.

This is a relevant and research issue to be urgently studied to give a solution to a dual environmental problem.

Although comparison of MFC devices on an equivalent basis is difficult (Logan *et al*., 2006), the power density obtained from the three substrates used in this research (Fig. 4) was detectable and comparable to some values observed in the literature. Gude (2016) reviewed the power outputs in lab-scale MFC using various wastewater sources as fuel in the anode. These authors mentioned that the power density for domestic waters was from 1.7 to 3.7 W m^{-3}, and for wastewater from hospital, it was from 8 to 14 W m^{-3}. Luo *et al*. (2010) obtained a power density from 2.1 to 3.3 W m^{-3}, within 18 hours and the operation period was up to 110 hours, in a batch-fed MFC (of an anode and a cathode, which were separated by a proton exchange membrane) using 250 mg L^{-1} of indole as the fuel. Zhang *et al*. (2009) obtained a maximum power density of 1.7 W m^{-3} from pyridine as a fuel in an MFC (two-chamber, separated by a proton exchange membrane and stocked with graphite).

In the present research, the cumulative power density by substrates is presented in Fig. 4. It was in the order dog feces > mine waste > vermicompost. By using MFC, the oxidation of OC from

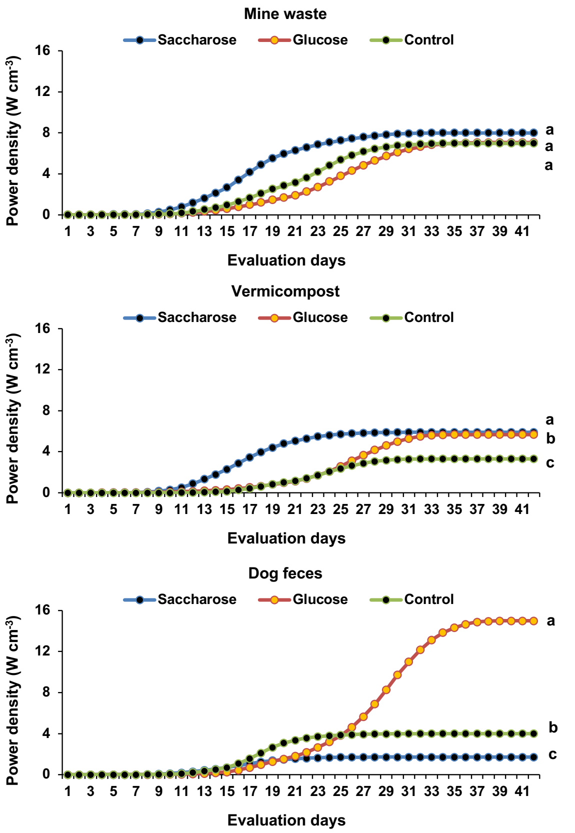

Figure 4. Cumulative power density output in microbial fuel cells in one operation cycle with the same substrate and different donor electrons sources. Different lower letters show statistical differences when comparing donor electrons in each substrate source. (a) Mine waste, (b) vermicompost, (c) dog feces.

dog feces and vermicompost was possible. Similarly, the oxidation of inorganic matter from mine wastes also produced electricity. However, the lower performance of MFC using vermicompost may be due to the higher recalcitrant OC of this source. Dunaj *et al.* (2012) observed 17 times lower performance of MFC using forest soil than agricultural. At the end of the experiment (42 days), the mine waste had a similar cumulative power density between the three co-substrates. The maximum power density was around 7.5 W cm^{-3} (Fig. 4a). Vermicompost (Fig. 4b) had the maximum accumulated power density with saccharose (5.8 W cm^{-3}), being different from this with glucose (5.5 W cm^{-3}) and this in the control (3.3 W cm^{-3}). The kinetics of cumulative power density for dog feces were different from those observed with mine waste or vermicompost (Fig. 4c). The maximum power density (15 W cm^{-3}) was nearly at the end of the experiment by glucose (day 40). In comparison, the lowest power density corresponded to saccharose as co-substrate (1.3 W cm^{-3}) and did not change from day 19 to 42. Douma *et al.* (2025) reported obtaining power densities of 0.352 W cm^{-3} by using cow dung as a feedstock for a single-chamber MFC.

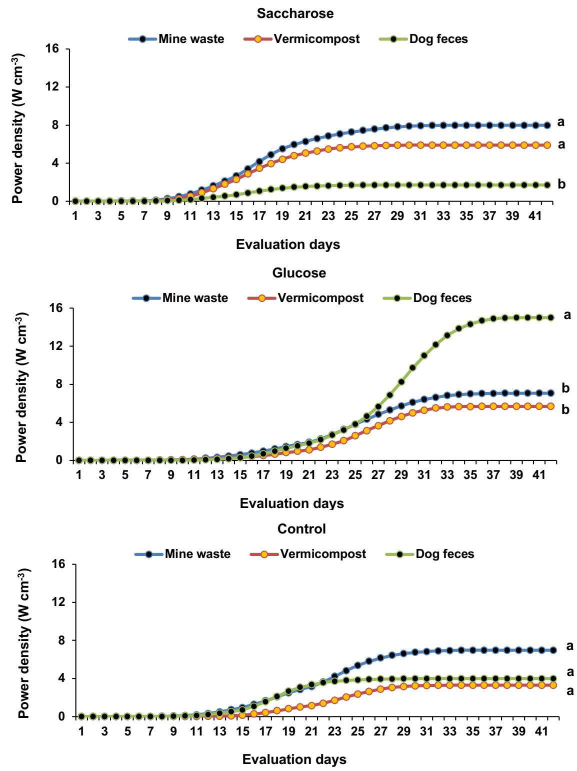

Figure 5. Cumulative power density output in microbial fuel cells in one operation cycle using three substrates and two donor electrons sources. Different lower letter show statistical difference when comparing substrates in each donor electrons source (a) saccharose, (b) glucose, (c) control.

When analyzing the effect of co-substrates on the production of power density (Fig. 5), the order of efficiency was glucose » saccharose > control treatments. Saccharose produced a power density between 1.8 and 7.9 W cm^{-3}. From day 15 to the end of the experiment propitiated a similar cumulative power density trend in the mine waste and vermicompost substrates used in the MFC (Fig. 5a). Glucose was the best promotor to produce the highest power density (5.6 to 15 W cm^{-3}) when compared to saccharose or control treatments, being dog feces the best substrate (Fig. 5b). Zhang *et al.* (2009) mentioned that higher power generation may be obtained when co-substrates are used. This is due to more electrons being generated in unit time due to the degradation of both contaminant and glucose. The maximum power density with no co-substrate was between 4 and 7 W cm^{-3} (Fig. 5c). Mine waste, even without any co-substrate, was able to produce a measurable power density at the end of the experiment without any co-substrate.

Luo *et al.* (2010) discussed that the combination of different substrates may result in differences in current output. For example, the combination of indole with glucose had stronger stimulation on bacterial activity in the electricity generation and recovered more electrons than those observed with glucose and pyridine. In the present research, the combination of dog feces and glucose propitiates higher current output and power density. This result may be related to the greater coulombic numbers when there are fuel substrate combinations than individual components, as mentioned by Zhang *et al*. (2009). In this research, coulombic numbers were not quantified; however, these should be analyzed in a future investigation to gain an understanding of the mechanism involved that leads to increased bioelectricity.

The power density and efficiency depend on the anodic microbial catalyst. Moreover, power density provides a better measure of the MFC performance and maturity of biofilm (Borole *et al*., 2009). In this experiment, the native microorganisms from each substrate were involved in the efficiency of the electrochemical reactions in the MFC. Future research should elucidate and isolate these microorganisms involved in electricity generation to identify and select them based on their superior performance. Moreover, deeper research into the nutrient composition should be followed, as it also influences microbial activity. It is recognized that the type of microorganisms and the medium´s composition impact their electrochemical activity, as mentioned by Ríos-Guzman *et al*. (2024).

3.2 Electron microscopy analysis to visualize anode-microbial biofilms

Figs. 6-8 show general observations of the anode surface at the end of the experiment from the best treatments of each substrate. In the mine waste+saccharose, stronger colonization and a very diverse biofilm (Fig. 6e-h) were observed in comparison to the respective control treatment (Fig. 6a-d). The typical bacterial morphology and abundant actinomycetes are visualized. In dog feces, the control treatment has an abundant biofilm (Fig. 7); however, the microbial morphology is different from that observed in mine wastes. In comparison, in the anodes from dog feces+glucose (Fig. 7e-h), the biofilm looks more robust and more diverse than that observed in the control treatment (Fig. 7a-d). In the vermicompost-control treatment, in general, the biofilm formed is scarce (Fig. 8a-c), although a well-formed biofilm was observed (Fig. 8d). While in the vermicompost+saccharose treatment (Fig. 8e-h) the presence of bacteria, but peculiarly fungal structures were observed (Fig. 8f, g). Dunaj *et al*. (2012) referred to the assumption that MFCs with mixed microbes have high efficiency due to microbial interactions and *in situ* electrogenic production of intermediate metabolites. The molecular identification of these anode-microbes located is being investigated by our research group for a better understanding of biofilm composition. We hypothesize that higher bacterial activity and biomass

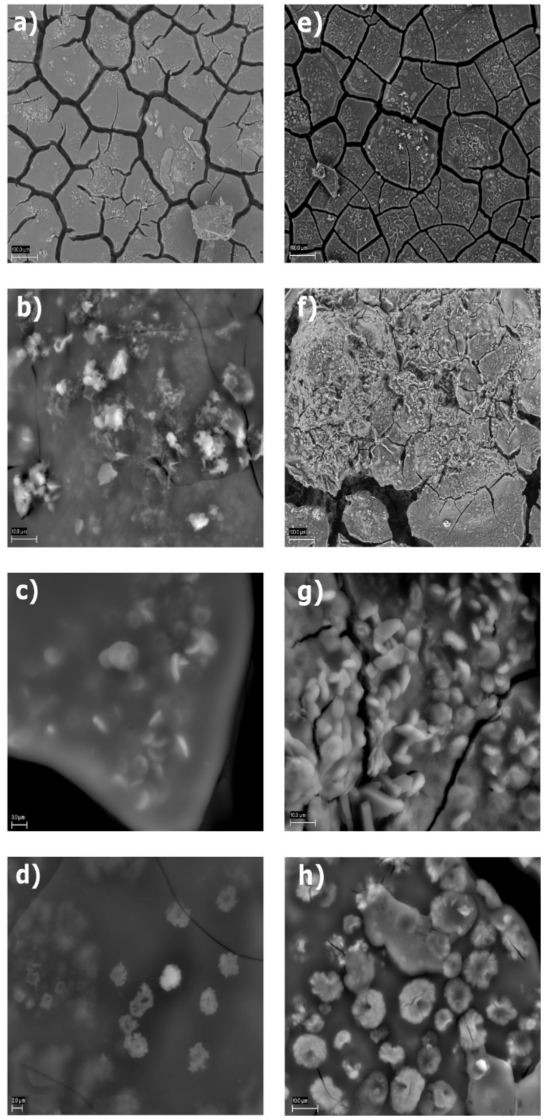

Figure 6. Scanning electron microscopy analysis of anode-surface from fuel microbial cell using mine waste as substrate. The control (a-d) and saccharose (e-h) as the best treatment producing power density are shown.

may be occurring in a diverse biofilm and then be more efficient in producing the higher power output. Dunaj *et al*. (2012) related the best MFC performance in agricultural soil (17 times more) than in forest soil to higher genomic DNA content in anodes (3-folds), which was used as a proxy for biomass. However, this hypothesis must be tested. The EDX analyses from the anode surfaces did not detect differences in elements observed (data not shown). The general elements identified were: P, S, K, and In. Interestingly, samples of anodes from mine waste treatments did not detect metals such as Pb, Cd, and others.

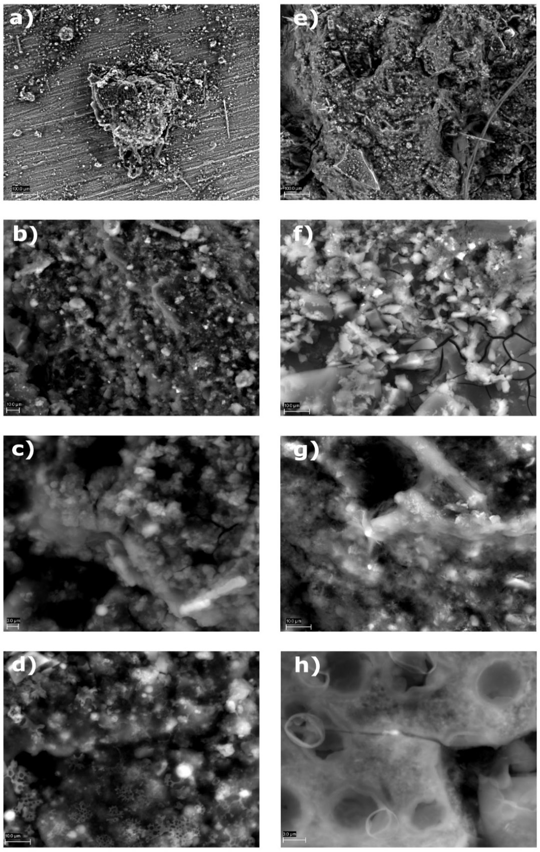

Figure 7. Morphological features observed in anodes of microbial fuel cells containing dog feces as substrate. The control (a-d) and glucose (e-h) as the best treatment producing power density are shown.

3.3 Microbial electro-remediation of hazardous wastes

In the original mine waste, Zn, Pb, and Cd concentrations were high enough and are considered an environmental risk (Table 2). In dog feces, Pb and Cd were detected; which may be related to the dogs' diet and the environmental pollution to which they are exposed. The environmental impacts of feces will depend on how they are disposed of. In another scenario where the feces are not cleaned up, they emit Pb and Cd into the environment (Yavor *et al*., 2020). Hence, the treatment and valorization of these wastes through MFC are attractive. In the case of vermicompost, the concentration of Zn was higher than the average concentration of this element in non-contaminated soils. Concerning the potential redox of Pb (-0.126 V to 1.69 V), Cd (-0.4030 V to -0.943 V), Zn (-0.76 V to -1.47 V), Mn (-1.17 V to 1.70 V), and Fe (-0.44V to 0.771 V) (Bard, 1985), these metals may act as a source of electron donors. Therefore, mine waste was more efficient in producing electricity than vermicompost.

Reduction in the pseudo-total metal concentration in the substrates after the operation time of MFC was

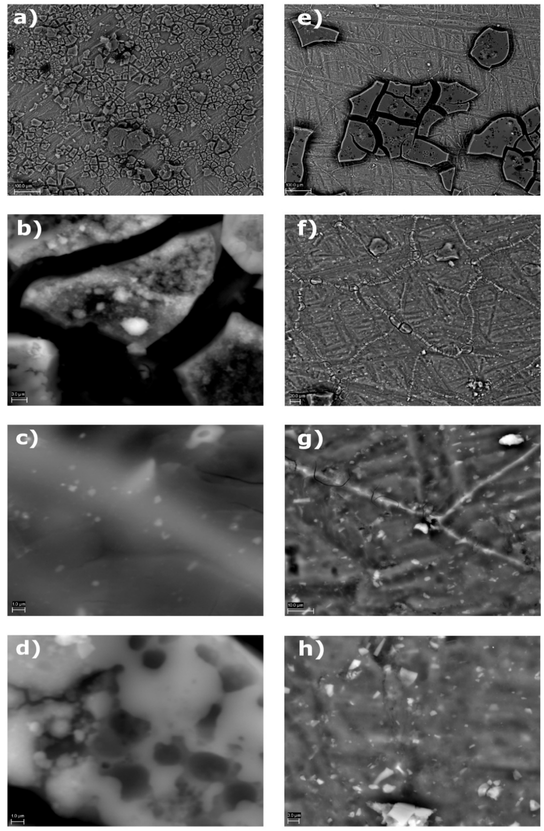

Figure 8. Scanning electron microscopy analysis of anode-surface from fuel microbial cell using vermicompost as substrate. The control (a-d) and saccharose (e-h) as the best treatment producing power density are shown.

observed, and this is referred to as metal removal, which occurred at the anode chamber. In the mine waste, the original pseudo-total concentration of Fe and Cu decreased (16% and 12%, respectively) in the same magnitude as saccharose or glucose in relation to the concentration of these metals in the control treatment (6%). In the case of the concentration of Mn, no differences were found between treatments, and the reduction was 25% in relation to the original concentration. The Zn concentration decreased similarly in both the control and glucose treatments (68%), but with saccharose, a lower concentration was observed (78%). For Pb, the concentration did not change when using saccharose as a co-substrate, but decreased by 11% with glucose. Unexpectedly, in the control treatment (tap water) there was Pb solubilization (+13%) from the mine waste. The Cd concentration in the control treatment did not change from the original concentration in the mine waste, but the reduction was obtained by saccharose (31%) and glucose (19%). (Habibul *et al*., 2016) observed 44% Pb and 31% Cd removal from spiked soil treated within an MFC (double chamber air cathode separated by a proton exchange membrane and anode compartment filled with graphite granules). In accordance with this information, in the present research, the Pb removal was lower, but a similar Cd removal was found. In the case of dog feces, relevant changes were observed. Cd concentration decreased by 46% in both the control and glucose treatments, but no modification was identified with saccharose. To explain the difference in these metal concentration changes after the incubation and electricity generation, microbial processes such as bioreduction, biosorption, bioaccumulation, and biomineralization may be suggested (Wu *et al*., 2017). The efficiency varies between metals and microbial cell composition since the standard electrode potential varies between metal ions (as referred earlier).

This wide response of differential metal concentration in the MFC makes it challenging to recover several metals from mine wastes and be successfully used in circular economy and electrochemical recycling methods, as mentioned by Pandit *et al*. (2021) and Petersen *et al*. (2021). The treatment of mine wastes and reduction of the initial Pb and Cd total concentrations by MFC represents an alternative technique to their direct disposal into the environment, which may be harmful to soil, water, air, and plants.

This primary treatment in MFC reduces the risk to ecosystems and human health by generating electricity while concurrently treating waste (Chandrasekaran *et al*., 2020). Although MFC has been proposed as an effective microbial electro-remediation method for heavy metals in groundwater, there are few reports supporting this on soil, sediments, or mine wastes. Some metallic inorganic pollutants treated in groundwater using microbial electro-remediation by MFC are Se, Cu, Cr (Pous *et al*., 2018), and Pb (Wang *et al*., 2021). Therefore, this is the first time MFC are tested for electro-remediation alternatives not only for mine wastes mainly polluted with Pb and Cd, but also for dog feces containing Cd as a contaminant. Unlike organic pollutants, heavy metals are not degraded. They are easily accumulated in different matrices. From this perspective, electrokinetic remediation is a technology that involves mobilizing metals using an electric potential generated by an external power source, resulting in excess energy costs. However, the electricity generated by MFC may achieve electrokinetic remediation effects without any additional energy cost (Wang *et al*., 2021).

Table 2. Original pseudototal metals concentration (mg kg^{-1}) in three substrates used and in the microbial cells with two electron acceptor-donor at the end of the experiment.

Treatments	Lead			Cadmium			Zinc			Copper			Manganese			Iron		
Treatments	Average	sd		Average	sd		Average	sd		Average	sd		Average	sd		Average	sd	
Mine waste																		
Original	1032	3	b	6	0.2	a	1332	33	a	386	5.9	a	245	6.7	a	128	1.3	a
Saccharose	1022	25	b	4	0.4	c	293	1	c	342	8.4	b	184	7.8	b	106	2.5	c
Glucose	914	24	c	5	0.4	bc	447	7	b	340	17.1	b	193	6.6	b	102	2.2	c
Control	1165	32	a	5	0	ab	420	11	b	385	10.4	a	191	3.4	b	119	2.2	b
SEMARNART†	22/260			37/450			400/800											
US-EPA††	400/1100			200														
† † †	10-67			0.06-1			17-25			13-24			270-525			5,000-50,000		
Vermicompost																		
Original	Nd			nd			243	16	a	72	1.2	a	357	7.3	a	15	0.1	a
Saccharose	Nd			nd			198	6	b	60	1.9	c	301	14.9	b	14	0.1	a
Glucose	Nd			nd			116	5	c	41	1.5	d	220	8.2	c	14	0.0	a
Control	Nd			nd			191	10	b	66	0.1	b	302	5.9	b	15	0.3	a
Dog feces																		
Original	12	1	a	1.3	0	a	274	30	a	35	0.9	a	353	13.6	a	13	0.4	a
Saccharose	5	0.8	c	0.7	0.3	ab	266	21	a	32	3.4	a	352	22.9	a	11	0.9	bc
Glucose	6	1	bc	0.7	0.3	b	233	17	a	36	1.6	a	335	8.2	a	12	0.5	ab
Control	9	0.6	ab	1.0	0.3	b	177	14	b	32	2.9	a	252	17.4	b	10	0.5	c

Different letter shows statistical difference when comparing treatments in each substrate analyzed (n=3) by using Tukey test ($\alpha \leq 0.05$). sd=standard deviation (n=3), nd=under detection limits. †Maximum permissible limits in soils for agricultural and industrial use established by the Mexico´s environment ministry (The Secretariat of Environment and Natural Resources SEMARNAT, 2007). ††Limits of contaminants in soil and required intervention mentioned by United States Environmental Protection Agency (US-EPA, 2001). † † †Normal concentration in non-polluted soils according to Kabata-Pendias (2010)

Conclusions

MFC may be included in eco-friendly pet waste management to avoid contamination and dispersion pathways, and consequently, reduce human health impacts. The combination of glucose with the mine wastes produced a lower power density than glucose-dog feces, but, saccharose-mine wastes increased the power density produced by mine wastes (7.9 W cm^{-3}). In the absence of glucose or saccharose, the three substrates generated a power density in the range of 4 to 7 W cm^{-3}. This power density yield may still be functional in rural areas where mine wastes are abundantly disposed of. A lower concentration of Pb and Cd in the mine waste was observed in the MFC after 41 days of electricity production, but the response was co-substrate-dependent (glucose or saccharose). The highest removal of Zn, Pb, and Cd was 78%, 31%, and 46%, respectively. Therefore, MFC represents a feasible alternative to electrochemically remediate mine wastes and, at the same time, produce electricity. These residues modify their energy and environmental value and occupy a place in the list of materials useful in waste-to-energy technology. MFC is a stand-alone power source, an electrochemical alternative, and more undesired residues should be tested under different conditions to increase the MFCs potential performance. Optimization concerning the analysis of power performance, system lifetimes, cost, and reliability is needed to afford clean energy, a healthier environment, and positive repercussions to social welfare.

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