

Vicia faba Crop Residues for Sustainable Electricity Generation Using a Sludge-based Microbial Fuel Cell



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Microbial fuel cells (MFC) simultaneously degrade organic substrates and generate electricity in a sustainable and eco-friendly way. Here, we built a 4-unit MFC and studied the efficiency of MFC at different conditions, including pH, substrate concentration of *Vicia faba* agricultural wastes with exoelectrogenic bacteria *P. aeruginosa*. The exoelectrogenic bacteria were obtained from industrial effluents and used to inoculate the MFCs. The optimized conditions in terms of yielding maximum potential of 802 mV, yielding maximum power density of 283 mW m⁻² were reported at a substrate concentration of 6 g L⁻¹ of *V. faba* waste and pH of 5.5, corresponding to a current density 1255.93 mA m⁻². Using exoelectrogenic bacteria from industrial effluents and agricultural wastes resulted in efficient MFC. Thus, the developed MFCs using *V. faba* agricultural wastes can be used in rural areas that have limited access to electricity, by reusing agricultural wastes and concomitant electricity generation.

Keywords:

V. faba agricultural wastes, *P. aeruginosa*, microbial fuel cell

Introduction

Sustainability is currently a necessity since, due to global warming, alternatives are being sought in which carbon-based fossil fuels are replaced by renewable energy sources¹. Therefore, the production of electricity or biofuels using innovative technologies and renewable sources such as biomass and agro-wastes² is a global priority in energy strategies.

Many forms of residual biomass contain large amounts of energy³ because they contain water-soluble carbohydrates, such as glucose, as well as those water-insoluble, such as pectin and cellulose⁴. In this sense, microbial fuel cells (MFCs) have emerged as a new and much more environmentally friendly energy resource than fossil fuels^{5–7}. MFC is an emerging renewable technology designed to exploit the degradation of biological substrates to produce sustainable bioenergy in the presence of active microorganisms⁸. Thus, MFCs have the simultaneous ability to produce electrical energy and degrade organic pollutants, removing them from the effluent used as substrate^{9–11}.

In MFCs, exoelectrogenic bacteria (pure or mixed cultures) catalyze organic matter degradation and transfer electrons to the anode, producing an electric current¹². Microorganisms contained in wastewater have been efficient in treatments with MFCs, this being due to the complex substrates such as carbohydrates and proteins that they consume^{13,14}. Different types of bacteria have shown exoelectrogenic activity in MFCs, some exoelectrogens, such as *Pseudomonas aeruginosa*, *Alcaligenes faecalis* were able to produce electrical energy¹⁵.

The broad bean (*Vicia faba* L.) is a legume with an economically important crop and is considered an important protein source for the human diet in the near future¹⁶. The *V. faba* is used as vegetable and staple food, and is consumed both in the fresh and dry form¹⁷. Besides being a significant source of carbohydrates, vitamins, minerals, and essential pharmaceuticals that have been proven beneficial to human health¹⁸, the broad bean shows characteristics that conform to the sustainable agriculture model¹⁹. Data provided by the Ministry of Agriculture of Peru indicated that the annual agricultural production of *V. faba* (in 2017) was 69.3 thousand metric

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tons, generating a large amount of waste from this legume. Thus, it is necessary to take full advantage of this waste to produce a good or service for local communities, such as continuous electricity supply. Generators can directly use these organic sources²⁰. To the best of our knowledge, this is the first time that *V. faba* agricultural wastes are used as a source of carbohydrates for MFC systems.

In this study, the use of *V. faba* crop residues as an economical and feasible substrate was evaluated for application in MFC after dissolving it in wastewater inoculated with an exoelectrogenic bacterial consortium containing *P. aeruginosa*. Moreover, the bioelectrochemistry process was optimized as a function of the substrate concentration, pH, and external resistance, concomitantly reducing the COD, thus resulting in environmentally friendly degradation of the *V. faba* crop residues. To the best of our knowledge, this is the first attempt to produce electricity in MFCs using *V. faba* crop residues.

Materials and methods

Substrate

Substrate utilized in the current study was sludge from wastewater collected from the Rio Seco industrial park, Arequipa, Peru. Different substrate concentrations were used with the harvest residues of *V. faba* from the agricultural fields of Cerro Pajonal, Mollebaya, Arequipa, Peru. The harvest residues were sun-dried for five days. The concentrations of *V. faba* residues utilized were 4.5 g L⁻¹, 6.0 g L⁻¹, 7.5 g L⁻¹, and 9.0 g L⁻¹. The pH was varied in the range of 5.5, 6.0, 7.0, and 8.0, and the operating temperature was 32 °C.

Growth medium

P. aeruginosa was found in various wastewater systems, including municipal wastewaters and inflow from a wastewater treatment plant²¹. Various bacterial strains were found, such as *P. aeruginosa*, *E. coli*, and *Proteus vulgaris*. The self-produced or endogenous chemical mediators, such as pyocyanin and related compounds produced by *P. aeruginosa*, can shuttle electrons to an electrode and produce electricity in an MFC²³. *P. aeruginosa* collected from the wastewater of the plant Rio Seco industrial park, Arequipa, Peru, was cultivated using 40 g L⁻¹ of Blood Agar, and the set was sterilized at 121 °C in a CASTLE autoclave CO. SPEED KEY #777 for 20 min.

P. aeruginosa bacteria, previously grown in a growth medium, were enriched in Luria-Bertani broth and incubated at 35 °C, thus increasing the bacterial population. The container with the bacterial consortium broth was stored in glass jars at 15 °C for later use.

Construction of the MFC

The MFC comprised two acrylic cubes of dimensions 7 cm × 7 cm × 9 cm. The carbon electrodes were assembled with hydrolyzed collagen having approximate dimensions of 3 cm × 2 cm × 0.3 cm at the anode, and as a cathode, a stainless steel electrode was used with the same anode dimensions.

The PEM (Proton-exchange membrane, Nafion 117[®]) was previously sterilized to eliminate all types of impurities present in the membrane. The PEM was boiled for 1 h in each of the following solutions: distilled water, 3 % solution of hydrogen peroxide, and an acid solution 0.5 mol L⁻¹ H₂SO₄. This procedure was repeated three times²⁴. The PEM plays an essential role in the MFCs, as it enables the proton exchange between half cells²⁵, and was placed in the central part of the semi-cell and used to separate the two chambers. The Nafion 117[®] is the best-known PEM, being studied in pretreatment and biofouling to produce bioelectricity and wastewater treatment in double chamber MFCs²⁴.

The anode medium was made up of 5 g of *V. faba* waste, after which wastewater from the Industrial Park of Rio Seco, Arequipa, Peru, was filled until the working volume reached 0.33 L. The cathode compartment was fed with 0.33 L of sodium chloride (J.T. Baker[®], 98.99 %) 1 mM, and operated in an aerobic environment supplied with O₂ through continuous aeration²⁶.

Fig. 1(a) displays the configuration of the home-made MFC system. In Fig. 1(b), the MFC is shown in more detail, having the following components: (1) Inoculation ports (anode), (2) Inlet ports (anode), (3) CO₂ input port (anode), (4) Outlet ports (anode), (5) Electrode port (anode), (6) Off gas port (anode), (7) Membrane Nafion 117, (8) Electrode port (cathode), (9) Inlet ports (cathode), (10) Aeration port (cathode), (11) Electrode (anode), (12) Electrode (cathode).

Measurements and analysis

The performance of the MFC was evaluated in terms of power density and current density, potential, pH, concentration of *V. faba* residues, and percentage of chemical oxygen demand (COD) degradation. Cell potential (mV) was measured each 5 h of operation using a Prasek Premium PR-75 Digital Multitester, and this was further used to calculate the electrical output as the power and current densities. All experiments were carried out at 32 °C. The external load resistances used to determine the power density of the MFC were 50, 100, 200, 300, 750, 1000, 2000, 6200, 10000, 12000, 22000, and 25000 Ω. Both the current density and power density were calculated by the following equations:

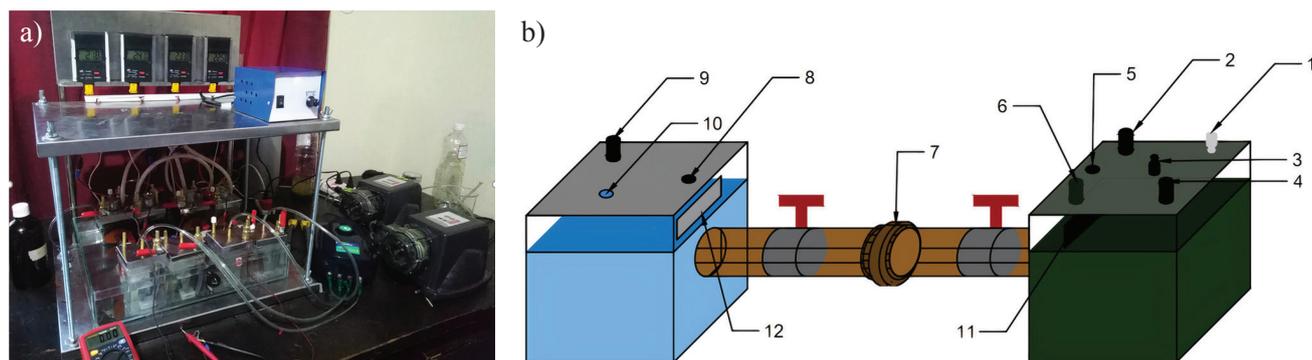


Fig. 1 – (a) Picture showing the scheme of the configuration of the developed microbial fuel cell system. (b) Scheme showing the main parts of the built microbial fuel cell system: (1) Inoculation ports (anode), (2) Inlet ports (anode), (3) CO₂ input port (anode), (4) Outlet ports (anode), (5) Electrode port (anode), (6) Off gas port (anode), (7) Membrane Nafion 117, (8) Electrode port (cathode), (9) Inlet ports (cathode), (10) Aeration port (cathode), (11) Electrode (anode), (12) Electrode (cathode)

$$\text{Power density} = \frac{VI}{A} \quad (1)$$

$$\text{Current density} = \frac{I}{A} \quad (2)$$

where I (mA) is the current, V (mV) is the voltage, and A (m²) is the projected surface area of the anode (9 cm²)²⁷.

The pH of the wastewater was monitored before and after the experiments using a Hanna Model HI98103 digital pH meter. COD values were determined using the American Water Works Association (AWWA) method. The COD removal efficiency, $n(\text{COD})\%$; was calculated using Eq. (3)²⁸:

$$n(\text{COD})\% = \frac{\text{COD}_{\text{in}} - \text{COD}_{\text{out}}}{\text{COD}_{\text{in}}} \cdot 100 \quad (3)$$

where, COD_{in} and COD_{out} are the values measured at the beginning and end of the experiments, respectively.

Results and discussion

Effect of using of *V. faba* crop residues

In MFCs, the source of carbohydrates (substrate) is a critical factor that affects the cost of production, as well as the amount of bioelectricity that can be generated²⁹. As shown in Table 1, the residues of *V. faba* from Mollebaya (100 g sample) contain a high percentage of carbohydrates, reaching 45.7 %. This value is much higher than the 4.4 % carbohydrates contained in 1 kg of dicotyledon from *V. faba* previously reported³⁰. Thus, *V. faba* residues could be efficiently used as a substrate and carbohydrate source.

Tests were carried out to determine the influence of *V. faba* crop residues on the potential generation in the MFC at a unique concentration of substrate composed of 4.5 g L⁻¹ powder *V. faba* residues at 32 °C and a pH at standard conditions of 5.5, and a volume of inoculum of *P. aeruginosa* of 2 mL.

Fig. 2 shows the potential variations generated with and without the addition of *V. faba* crop re-

Table 1 – Nutritional composition of the residues from the *V. faba* harvest

Analysis	Results in %	Test method applied	Test method concept
Humidity	8.70	Method NTP 209.085	Determination of humidity for agricultural food products in general
Ashes	4.83	Method NTP 208.005	Determination of total ash
Fat	1.07	Method NTP 209.093	Fat determination
Protein (X6,38)	13.28	Method 2.057 of AOAC	Protein determination
Fiber	26.43	Method NTP 209.047	Chemical testing methods Raw fiber determination
Carbohydrates	45.69	Method 31.043 of AOAC	Carbohydrate determination

AOAC: Association of official analytical chemists

NTP: Peruvian technical standard

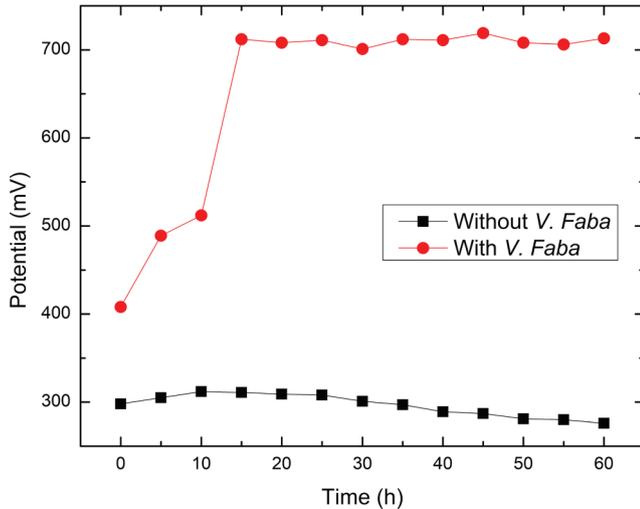


Fig. 2 – Effect of using residues of *V. faba* on the generation of potential in the MFC

sidues. The MFC without *V. faba* residues produced a maximum potential of 312 mV in 10 h. Whereas the MFC containing *V. faba* residues yielded a potential of 512 mV; however, a maximum potential of 719 mV was obtained in 45 h, indicating that this organic residue significantly influences the potential obtained.

Effect of variation of *V. faba* crop residues concentration

Here, *V. faba* residue concentrations were controlled at four values 4.5 g L^{-1} , 6 g L^{-1} , 7.5 g L^{-1} , and 9 g L^{-1} in the MFC system. Fig. 3 shows the potential variations generated by the MFC under different concentrations of bean residues in the anode chamber. It was observed that the potential output showed a similar tendency for all conditions with an initial increase until reaching the maximum values, and then a gradual decrease. Furthermore, the potential increased with concentration of bean residues to 6 g L^{-1} , but the potential tends to decrease when increasing to 7.5 g L^{-1} . For 4.5 g L^{-1} , the maximum potential of 719 mV was observed at 45 h; for 6 g L^{-1} , this was the highest (798 mV) at 15 h; for 7.5 g L^{-1} , it was 689 mV in 20 h, and 662 mV 9 g L^{-1} at 25 h.

The possible reason for this behavior is that the cathode's potential decreased substantially due to an excess of bean residues. Note that higher stability was obtained when using 6.0 g L^{-1} because it reached the maximum point, and its decrease was not notorious over time. Besides, the highest potential after 60 hours was for the 6.0 g L^{-1} concentration (i.e., 779 mV), unlike with 4.5 g L^{-1} (713 mV), with 7.5 g L^{-1} (639 mV), and finally with 9.0 g L^{-1} (612 mV). The decrease in potential for 4.5 g L^{-1} concentration occurred because the residue sub-

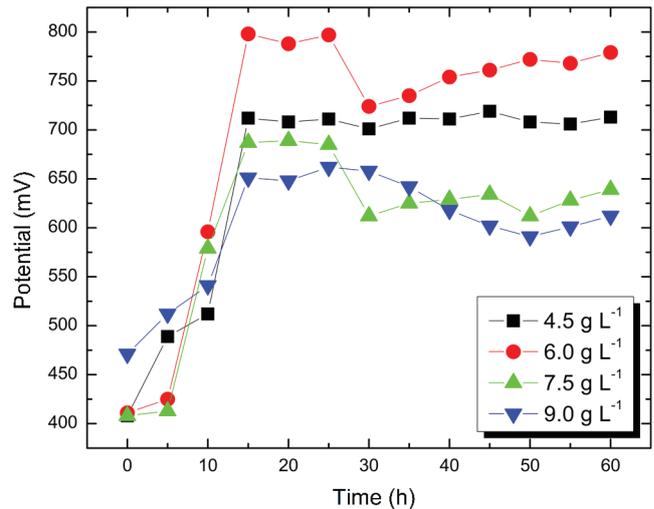


Fig. 3 – Effect of the concentration of residues of *V. faba* on the generation of potential in the MFC

strate of *V. faba* had already been consumed in its majority, and it was for this reason that the best concentration was 6.0 g L^{-1} . The results showed that, by increasing the substrate concentration over a specific range, the production of potential was inhibited at high substrate concentrations.

Similar behavior was observed by Zhang *et al.*³¹, who studied the effect of different concentrations of NO_3^- -N, using synthetic wastewater, which contained KH_2PO_4 0.02 g L^{-1} , NH_4Cl 0.04 g L^{-1} , as a substrate in an MFC. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.0056 g L^{-1} , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.30 g L^{-1} , and trace element solutions (1 mL L^{-1}).

Effect of pH variation

In order to determine the influence of pH on the generation of potential in the MFC, the initial pH value was increased with a 0.1 M sodium hydroxide solution until it reached values of 6.0, 7.0, and 8.0, keeping the initial sample under study with 5.5 pH.

Fig. 4 shows the potential changes at different pH values in the anode medium. When the sample having the original pH (5.5) was used, an increasing trend was observed; the potential increased to a maximum value of 802 mV in 30 h, and maintained a potential range of 750 V–800 mV. The same increasing behavior was observed in the anode medium with a pH of 6, giving a maximum potential of 689 mV, decreasing to 652 mV at 60 h. The result given by MFC at 5.5 pH was 16.3 % higher than MFC at pH 6, it was 83.7 % higher than MFC at pH 7, and 99.8 % higher than MFC at pH 8. The optimal pH for *P. aeruginosa* growth was 5.5³².

The effect of pH on the production of potential was because the *P. aeruginosa* could not generate electrons from the consumption of substrate at high

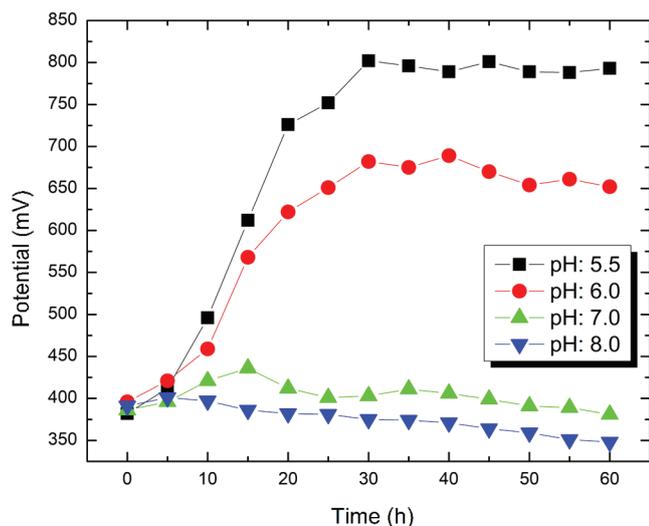


Fig. 4 – Curves showing the influence of pH on the generation of potential in the MFC

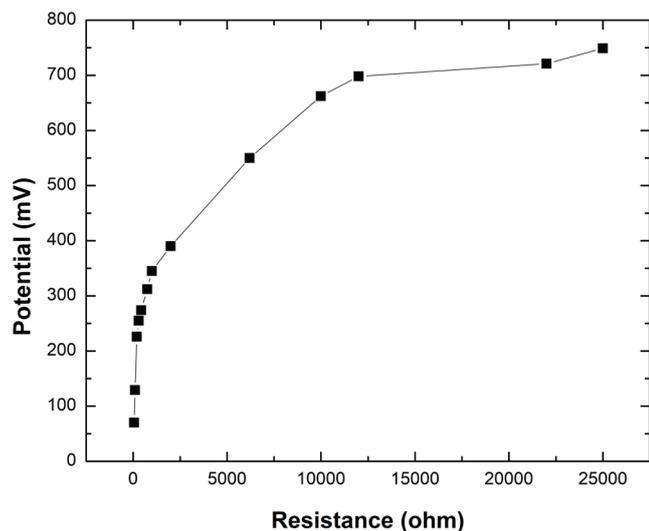


Fig. 5 – Variation of potential with external resistance

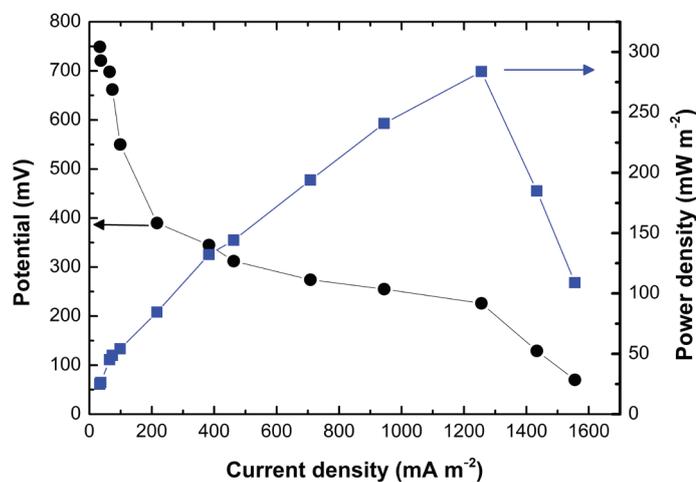


Fig. 6 – Voltage (left) and power density (right) as a function of current density

pH conditions, which led to their inability to generate new bacterial populations. However, they were much more stable, having higher oxidation under acidic conditions, confirming that the oxidation potential decreased as the pH increased³³.

Effect of current, power density, and external resistance

A maximum voltage in 26 h (749 mV) was generated and applied using different external resistances (25000 – 50 Ω) in order to evaluate the performance of the MFC. As the resistances changed, the voltage changed, thus giving information about the optimal current at a specific resistance, as seen in Fig. 5.

The potential and current values obtained from Fig. 5 were used to obtain the polarization curve and the potential density behavior at different external resistances, as displayed in Fig. 6. Low currents are obtained when the external resistances are high; likewise, the current and potential density increase when the resistance decreases. The power density was calculated using Eq. (1), while the current density was calculated using Eq. (2). A maximum potential density of 283 mW m^{-2} at 226 mV corresponds to a recorded current density of 1255.93 mA m^{-2} . Notably, a previous study reported an inoculated MFC with *P. aeruginosa* that showed a higher current density of 264 mA m^{-2} and a power density of 33.90 mW m^{-2} using a 400 Ω resistance, and an area of 83.56 cm^2 in 60 h³³. Therefore, we can highlight that the results obtained here were more efficient, probably due to the use of the adequate concentration of *V. faba* crop residues and pH stability.

The cashew apple is an attractive low-cost substrate from which bio-ethanol and other value-added products have been produced. Cashew apple juice can serve as the potential substrate for microbial fuel cells, generating an open-circuit voltage of 0.4 V, a maximum power density of 31.57 mW m^{-2} , and 350 mA m^{-2} of current density². That study showed that the potential decreased as current density increased when the MFC was inoculated with *E. faecium* Yc 201, and the maximum potential obtained was 515.7 ± 12.6 mV. Our results indicated that the voltage and power density of the MFCs were a function of the measured steady-state currents under various external resistances. The differences in MFC performance with diverse external resistances may be caused by the variations in activation losses in the MFC. These activation losses are a function of the electrochemical activity of anode-reducing microorganisms. Wu and coworkers developed an MFC system to evaluate the effects of substrates in the electrical generation of MFC. The medium used in their study included: 0.4 g L^{-1} $(\text{NH}_4)_2\text{SO}_4$, 2.1 g L^{-1} K_2HPO_4 , 1.1 g L^{-1} KH_2PO_4

and trace elements (1 L of medium contained 745 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 60 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 15 mg $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and 150 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). In their experiments, 2000 mg L^{-1} of various carbon sources were added, and the maximum power density was $121.3 \pm 4.2 \text{ mW m}^{-2}$ for the MFC at $550 \Omega^{43}$.

A recent report by Zhou *et al.*⁴⁴ displayed an MFC where the anodic electrolyte was composed of sodium acetate (1 g L^{-1}), phosphate buffer solution (0.05 mol L^{-1}), vitamins (5 mL L^{-1}), and minerals (12.5 mL L^{-1}). The electrolyte in the cathode chamber contained KCl (0.31 g L^{-1}), Na_2HPO_4 (11.36 g L^{-1}), NH_4Cl (0.13 g L^{-1}), NaH_2PO_4 (2.75 g L^{-1}), and Cr(VI) (10 mg L^{-1}). The MFC achieved a maximum power density of 535.4 mW m^{-2} . The electroactive *Pseudomonas* sp. bacteria in the biofilm on the anode surface played a crucial role in bioelectricity production and electron transfer.

Effect of COD concentration in wastewater

After optimizing the system parameters, the initial and final COD values in the anode chamber were monitored, which were 32672.33 mg L^{-1} COD and 7154.92 mg L^{-1} COD. The COD removal efficiency was 78 % after 60 h of experimentation, which led to the increase in the potential in the cathode chamber, positively impacting the degradation of organic matter in the anode chamber, which allowed its oxidation. The COD removal of 78 % after 60 h (2.5 days) is suitable compared with the literature; because the elimination efficiency found by Li *et al.*³² was 99.4 % and 98.7 % after five days of experimentation. The 78 % of COD removal found here is also similar to the 75 % of COD removal reported by Ge *et al.*³⁴ when treating municipal wastewater using an MFC. Interestingly, our experimentation reached a removal efficiency of 78 %, which would mean that the MFC had enough organic matter to continue working longer. This result is essential, since it does not require addition of more substrate and inoculum in short periods to keep the MFC working.

Effect of temperature

It is well-known that temperature influences the bioelectricity production characteristics of the MFC³⁶. The augmentative and reproductive tendencies of microbes are affected by temperature, which can change both intracellular and extracellular biochemical (or chemical) processes³⁵. Power generation was also affected by operating temperature, which is consistent with other studies^{37,38}. During the initial startup period, we observed that the power density was 70 mW m^{-2} at $30 \text{ }^\circ\text{C}$, which was 1.6 times higher than at $22 \text{ }^\circ\text{C}$ (43 mW m^{-2})³⁵. That was the reason why all experiments were carried out at $32 \text{ }^\circ\text{C}$.

Effect of biocatalyst

Several factors, such as the type of substrates, concentration of the substrates (initial COD), pH, temperature, electrode material, and biocatalysts, influence the performance of the MFC³⁹. In this regard, MFCs that use microorganisms as a biocatalyst and convert chemical energy from organics in wastewater to (bio)electricity offer a sustainable technological solution^{40,41}. For example, by applying a biocatalyst that uses acclimatized anaerobic sludge (AS) containing *Pseudomonas* spp. and *Bacillus* spp., the maximum current density of 1500 mA m^{-2} and 730 mV of potential were generated⁴². Those outcomes are comparable with our current density results: $1255.93 \text{ mA m}^{-2}$ and potential of 802 mV with anaerobic sludge (AS) that contained exoelectrogenic bacteria *P. aeruginosa* with *V. faba* bean crop residues.

Conclusions

A system composed of four MFCs was operated for the first time with *V. faba* harvest residues, and tested with different variables, such as external resistance, pH, *V. faba* residue substrate concentration: maximum potential of 802 mV, maximum power density 283 mW m^{-2} (226 mV, 200Ω), a maximum current density of $1555.56 \text{ mA m}^{-2}$ (70 mV, 50Ω), obtained using a pH of 5.5, a concentration of substrate of *V. faba* residues of 6 g L^{-1} . Furthermore, the system showed a COD removal efficiency of 78 %, which could be considered low, but it is worth considering the short time of experimentation (60 h). These results suggested that the *V. faba* bean crop residues could be activated to generate hydroxyl radicals due to the high concentration of carbohydrates. Therefore, they have great potential for energy production in wastewater treatment, thus opening up the opportunity for sustainable energy production in communities with limited access to electricity.

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