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Dual Role of Sugarcane Waste in Benthic Microbial Fuel to Produce Energy with Degradation of Metals and Chemical Oxygen Demand

Ghada Mohamed Aleid [1,†], Anoud Saud Alshammari [2,†], Asma D. Alomari [3,†], Hanadi A. Almukhlifi [4], Akil Ahmad [5,*] and Asim Ali Yaqoob [6,*]

1 B.Sc. Department, Preparatory Year College, University of Ha'il, Hail 55475, Saudi Arabia; g.aleid@uoh.edu.sa
2 Department of Physics and Chemistry, Northern Border University, Rafha 76313, Saudi Arabia
3 Chemistry Department, Al-Qunfudah University College, Umm Al-Qura University, Al-Qunfudah 28821, Saudi Arabia
4 Department of Chemistry, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia
5 Department of Chemistry, College of Science and Humanities in Al-Kharj, Prince Sattam bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia
6 Université Paris-Saclay, INRAE, PROSE, 92160 Antony, France
* Correspondence: aj.ahmad@psau.edu.sa (A.A.); asimchem4@gmail.com (A.A.Y.)
† These authors contributed equally to this work.

Abstract: One of the most advanced systems of microbial fuel cells is the benthic microbial fuel cell (BMFC). Despite several developments, this strategy still has a number of significant flaws, such as instable organic substrate. Waste material (sugarcane) is used as a substrate in this work to address the organic substrate instability. The process was operated continuously for 70 days. A level of 300 mV was achieved after 33 days of operation, while the degradation efficiencies of Pb (II), Cd (II), and Cr (III) were more than 90%. More than 90% of the removed chemical oxygen demand (COD) was also recorded. The measured power density was 3.571 mW/m^2 at 1000 Ωι external resistance with 458 Ωι internal resistance. This demonstrates that electrons are effectively transported throughout the operation. The Bacillus strains are the most dominant bacterial community on the surface of the anode. This research's mechanism, which involves metal ion degradation, is also explained. Finally, parameter optimization indicated that pH 7 works efficiently. In addition to that, there are some future perspectives and concluding remarks enclosed.

Keywords: sugarcane waste; benthic microbial fuel cell; energy; organic substrate; chemical oxygen demand

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1. Introduction

Water and energy problems are the two most significant challenges that the modern world is now dealing with. Due to exceeding of the concentration limit of metal ions and organic as well as inorganic substances, industrial wastewater, agricultural wastewater, and domestic resources also present significant threats to the environment. These types of contaminants may be found in wastewater [1]. In recent times, there has been an increasing interest in the search for a solution or the removal of heavy metals from wastewater [2,3]. There will be negative effects if these metals accumulate in the body, whether from ingesting food or water, due to the inability of these metals to biodegrade and their high carcinogenicity and toxicity, even at low concentrations. These effects will be caused by the accumulation of these metals [4]. There are several standard approaches to wastewater treatment, some of which are oxidation channels, batch sequence, adsorbents, and photocatalysis, among others [5,6]. The treatment of wastewater using an external supply of power, on the other hand, is not appropriate for use on a wide scale [7]. Therefore, it is necessary to investigate whether or not a contemporary method of treating wastewater can also generate energy. Presently, microbial fuel cells, also known as MFCs, have a great deal of promise for simultaneously reducing heavy metals from wastewater and producing electricity. Microbial fuel cells (MFCs) are a kind of bio-electrochemical cell that use microbial catalysis to produce power while simultaneously reducing the amount of metal in wastewater [8–10]. Benthic microbial fuel cells (BMFCs) are the result of researchers' rising interest in MFCs, which led to the development of an advanced type of MFCs [11]. In order to cleanse wastewater while also producing electricity, BMFCs operate according to the same fundamental principles as MFCs. BMFCs are the MFC design that is the most innovative and has the maximum promise [12]. The cathode is put in the wastewater, while the anode is buried into the used plant-based waste [13,14]. BMFCs make use of waste products derived from plants, which are then oxidized by a population of bacteria, which results in the production of electrons and protons [15].

There is a serious issue with the organic substrate's poor performance and instability when exposed to bacterial populations. A suitable organic waste can provide enough power for bacteria to use in the electrogenesis process, which can help speed up the process. For the purposes of this research, organic waste from sugarcane waste was processed in this BMFC setup. According to a previous study, it showed that sugarcane waste can produce up to 453 $\pm$ 6 mV [16]. Another study showed that the sugarcane waste consists of between 94 to 98.5 % sucrose and between 1.5 to 6% additional components, such as amino acids, starch, gums, simple sugars, proteins, and several trace substances [17]. According to the findings of earlier research, sugarcane waste is a great source for nutritional uses. Few researchers have used sugarcane as a substrate with organic pollutants in the literature [18,19], where organic pollutants also serve as a carbon source. This is the first research to use sugarcane waste to generate electrons that electrochemically degrade metal ions. The scope of this investigation is restricted to the addition of Pb (II), Cd (II), and Cr (III) to domestic water. Several biological analyses and electrochemical experiments were carried out in order to determine the effectiveness of the wastewater process and the generation of energy. Finally, optimizations of pH and organic substrate were considered as well.

2. Methods and Experimentation

2.1. Chemical Materials

This study used sugarcane waste (received from a local fruit store), cadmium nitrate tetrahydrate (98%), distilled water (DI), D-(+)-Glucose (analytical standard), chromium(III) nitrate nonahydrate (99%), sulfuric acid (ACS reagent, 95.0–98.0%), sodium hydroxide ($\geq$97.0%, pellets), lead nitrate (99%) (all of these chemicals were received from Sigma-Aldrich), and tap water.

2.2. Benthic Microbial Fuel Cell Assembly and Process

After collecting the domestic wastewater, additional ions of lead (II), cadmium (II), and chromium (III) were added. The concentration of each metal was 200 mg/L within the context of this study, and domestic water (DW) is referred to as metal-supplemented pond water (MSW). The physicochemical characteristics of DW and MSW that were investigated are shown in Table 1. An apparatus was used in the process of determining the conductivity, pH, and temperature of the DW and MSW. The waste from the sugarcane was obtained from a nearby market, where it was then given a light washing and a gentle scrub before being finely chopped. After being chopped, each of the pieces had a length and breadth of exactly one centimeter. MSW (700 mL) with an initial 1g of glucose was combined with 0.5 kg of sugarcane wastes in the mixing process. This MSW and sugarcane waste was put into the setup of the BMFC. The current investigation made use of a BMFC with a single chamber, and its length and diameter were 25 cm and 10 cm, respectively. The utilized tank had a capacity of around 1200 mL. Before the operation, the inoculation source's pH was adjusted to 7 using NaOH solution, from its original value of 5.99. Since the inoculation in the BMFC performed better at a neutral pH than in either a basic or an acidic state, the inoculation source pH was adjusted to pH 7 so that it could be used in the cultures [20].

In this particular investigation, a single graphite cathode and a single graphite anode, each measuring 10 cm × 1 cm, were employed concurrently. There was a spacing of 2 cm between each anode. The anode and cathode electrodes were separated by a distance of 9 cm. A covered commercial copper wire was used to make the connections between the electrodes, and 1000 Ωι was the attached external resistance. We briefly utilized a threshold of 1000 Ωι while deciding on a certain external resistance for the first time. We observed a decrease in the voltage of the closed circuit relative to the voltage of the open circuit throughout this period. This voltage did, however, ultimately begin to rise steadily. In general terms, one should choose a greater resistance if there is no sign of a recovery and a lower resistance if there are no apparent changes. Furthermore, the experiment was conducted three times to ensure consistency of results. Figure 1 illustrates the pectoral BMFC presentation that was used in this research.

Table 1. The characteristics of the inoculation sources before and after treatment.

Physiochemical Properties	DW	MSW
pH	6.89	5.99
Temperature	Room	Room
Color	Light yellowish	Dark yellowish
Electrical conductivity	105 µS/cm	350 µS/cm
Pb (II)	0 mg/L	200 mg/L
Cd (II)	0 mg/L	200 mg/L
Cr (III)	0 mg/L	200 mg/L

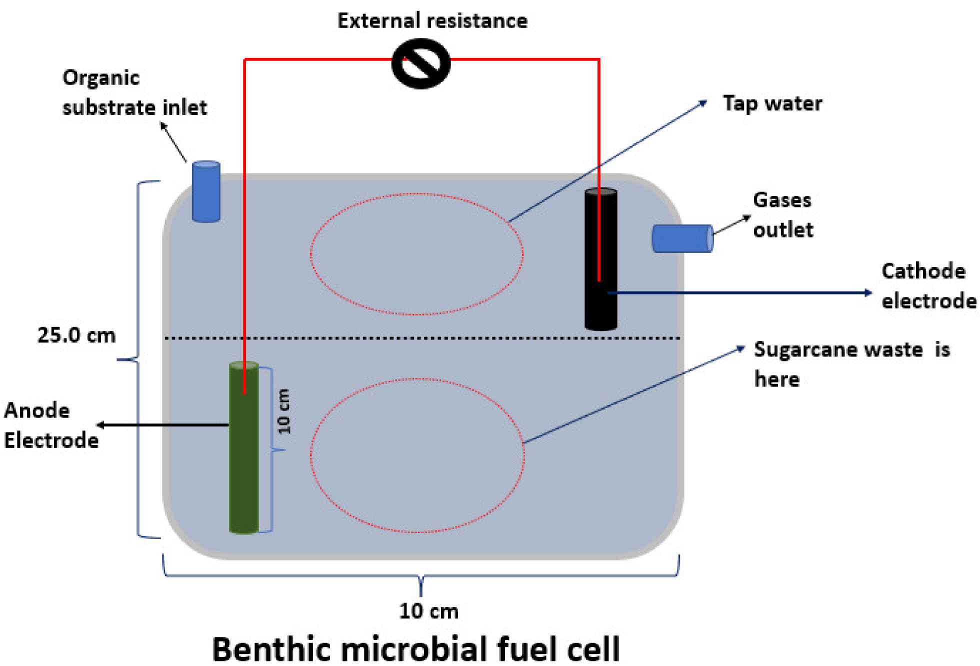

Figure 1. The conceptual setup of the BMFC in this study.

2.3. Electrochemical, Chemical, and Biological Analysis

Every day, readings of voltage potential, denoted by the letter V, were taken using a digital multimeter. Ohm's Law was followed in order to figure out the current efficiency, which included calculating the current density (CD) and the power density (PD). In order to determine the values of the internal resistance, CD, and PD, Equations (1)–(4) were used [21].

$$V = IR \quad (1)$$

$$PD = \frac{V^2}{RA} \tag{2}$$

$$CD = \frac{1}{A} \tag{3}$$

$$r = \left(\frac{E - V}{V}\right)R \tag{4}$$

where V = voltage output, I = current, R = external resistance, A = cross-sectional area, r = internal resistance, and E = electromotive force (emf). This allowed for the value to be estimated. The redox reaction that occurred while the procedure was being carried out was investigated by utilizing the cyclic voltammetry technique. A cyclic voltammogram was recorded on the different days of the reaction using a potentiostat device at a scan rate of 10 mV/s within a potential range of −0.8 V to +0.8 V. This was conducted throughout the course of 70 days. Polarization behavior was seen by using a method called "Rext-variation," which included controlling the external resistance using a variable resistance box (with values of 5000 Ωι, 4000 Ωι, 3000 Ωι, 2000 Ωι, 1000 Ωι, 500 Ωι, and 100 Ωι). After the reaction had reached a condition that was deemed to be pseudo-steady, a study of the polarization curve was carried out. When investigating the polarization behavior, a variation time of at least half an hour is required, on average.

In this research, the chemical oxygen demand (COD) investigation was carried out. The COD is an analytical indicator of the potential oxygen consumption rate in a solution by processes. The most common unit of measurement is milligrams per liter (mg/L), which is calculated by dividing the mass of oxygen utilized by the volume of solution. This would be milligrams in SI units. Using Equation (5), the total removal efficiency (RE%) of COD was obtained.

$$RE\ \% = \frac{Mi - Mf}{Mi} \times 100 \tag{5}$$

where Mf is the final concentration and Mi is the total concentration at the start. The COD concentration was calculated using the LCK514 kit (Berlin, Germany). A UV spectrophotometer was used to take the measurements.

The usage of BMFCs is a valuable technology for the degradation of pollutants found in wastewater. A device known as an atomic absorption spectrometer (AAS) was employed in order to investigate the ions of the metals. The amount of metal ions present in MSW were measured using the AAS. In all, around 2 mL of sample was taken from the BMFC's chamber at regular intervals for the purpose of analysis. The efficiency of degradation was determined by utilizing Equation (6).

$$\text{Degradation}\ \% = \frac{Ti - Tf}{Ti} \times 100 \tag{6}$$

where Ti = initial concentration of metal ions and Tf = final concentration of metal ions. The accumulation of electrode biofilm is what ultimately decides how well metal is degraded and how much energy is produced. Combining the scanning electron microscopy (SEM) and electron dispersive X-ray (EDX) techniques allowed for the investigation of the morphology as well as the chemical make-up of biofilm on electrodes.

Moreover, a biofilm overlaying the anode surface of roughly 1.00 mm was scratched off in order to separate and characterize the microbes. The biofilm was placed in distilled water for preservation after the BMFC operation. A serial dilution procedure was used to transfer the colonies from nutrient agar plates. Nutrient agar plates eventually revealed a diversity of colonies. To identify the bacterial species, they were extensively purified and examined. The sterile nutrient agar plates with the pure cultures were then stored in the refrigerator until the final measurement was taken. Bacterial 16S rRNA genes were synthesized using the polymerase chain reaction (PCR) technique. A forward primer (27F) and a reverse primer (1492R) were used to amplify the genes. The cloning kit was used to successfully clone the PCR-generated product. Bacterial 16S rRNA sequences were submitted to GenBank after DNA sequencing research.

2.4. Parameter Optimization Study

Many tests were performed in this investigation to determine the suitable factors such as pH and organic substrate. The optimized parameters were implemented to assess the performance of the BMFC and were expected to have high energy and metal degradation. Tests were also conducted at pH levels of 4, 5, 6, 7, 8, 9, and 10. After 5 days at each pH range, the voltage measurement was obtained, and the degradation efficiency was determined on day 5. H_2SO_4 (concentrated form used) and NaOH (1 g/L solution) solutions were used to adjust the pH of the inoculation source. The optimal pH range was achieved at room temperature. As an organic substrate, commercial glucose performed well to optimize the sugarcane wastes. All of the remaining parameters were the same as described in the primary operation.

3. Results and Their Significant Discussion

3.1. Electrochemical Results

In Figure 2, one can see the voltage production that took place during the metal degradation in the BMFC operation. The procedure was carried out over the course of 70 days with the 1000 Ωι resistance. On day 32, it was reported that the voltage reading had achieved its maximum level, which was measured at 300 mV. According to the data, the voltage generation began at a reasonable rate and gradually increased until it achieved its highest values on the 32nd day after the experiment began. After that, a downward trend in the voltage started to become apparent. However, after a few days it began increasing again, reaching 290 mV on day 40, and then after that it started an unstopped downward trend once again. The decline in the generation of voltage is an indication that the bacterial species are moving closer to the period of death. The observation after a few days showed that there was still a trend toward a lower voltage. This suggests that the organic substrate oxidation is continuing toward completion, since the exoelectrogens are unable to retake control of the process. This study found that day 32 was when the voltage reached its peak. Despite this, a few investigations have shown that the region with the maximum voltage is also a reliable predictor of a significant change in the metal's state [22–24].

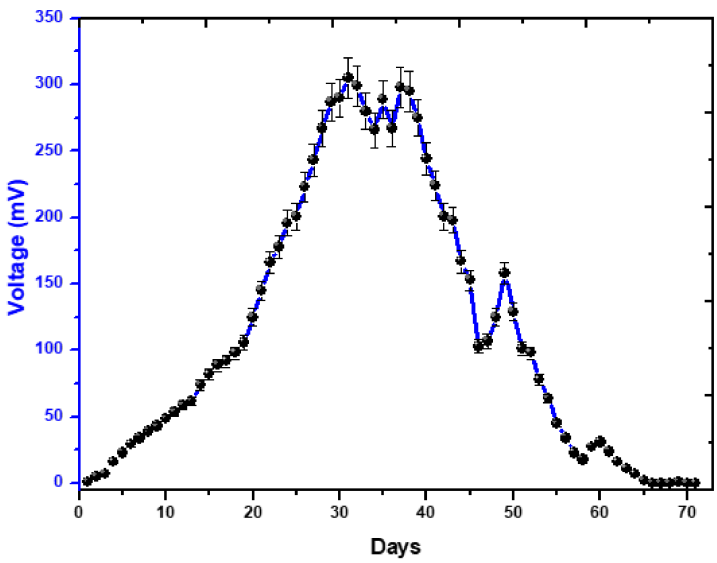

Figure 2. The voltage generation trend throughout the 70 days.

Additionally, the efficiency of polarization was examined by evaluating the CD, PD, and voltage relations utilizing different external resistances. Figure 3 has been used to demonstrate the inversely proportional connection between the voltage and CD. The PD delivered a maximum of 3.571 mW/m^2 at 1000 Ωι, but only 1.510 mW/m^2 at 5000 Ωι. Both resistances (external and internal) must be equivalent for the electron transfer to be successful. The lower electron transportation shows the maximum internal environment's resistance. The potential does not stabilize as soon as it would normally when the external resistance is low, but enough electrons are still produced and transferred. The increased electron mobility leads to voltage instability. The maximum was CD recorded was 64.51 mA/m^2. The external oxygen supply enhanced the cathodic response even more, which helped to stabilize voltage production despite the high resistance. The internal resistance was 458 Ωι. Several studies have shown electrochemical performance using this pattern [25–27].

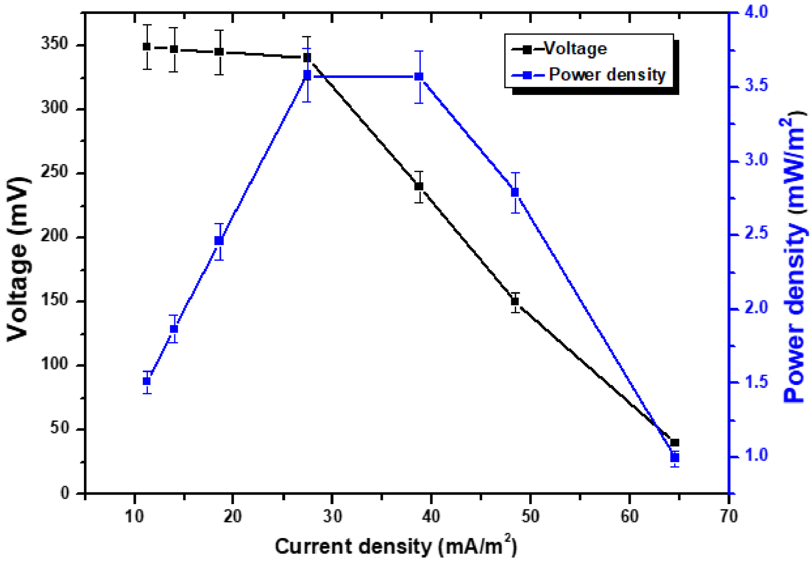

Figure 3. Polarization trend of the present study with organic feeding.

The CV study was recorded during the procedure at several periods. The graphs demonstrate the metals' tendency for redox reaction across a range of period scales. Figure 4 illustrates how the BMFC operation showed the maximum current for both scan (forward and reverse) speeds. The greatest current, measured by the forward scan, was 6 × 10^{-6} mA on cycle day 70. Similar to this, the reverse scan current was −4.2 × 10^{-4} mA on cycle day 70. The forward and backward scans on day 70 indicated that the current was at its maximum value. Forward and reverse scans show the rates at which metal ions in wastewater are oxidized with reduction. On day 70, the redox peak was maximum at 0.8V oxidation, while reduction peak was −0.8 V. This was the day when both peaks reached their dominant status. In addition, the CV study was applied to determine the Cp rates at a number of different time intervals during the procedure. The Cp values provide an indication of the rate of biofilm development as well as the stability rate when the MFC is in operation. Table 2 illustrates this point further. Due to the high Cp rates, it is clear that the biofilm formation is getting closer and closer to reaching its mature state. The Cp values were trending downward, which implies that the biofilm's development was inhibited and that the biofilm is thus not functioning as it should. The investigation's findings showed that the concentration of Cp increased from 0.00001 F/g on day 10 to 0.00004 F/g on day 70. This rise occurred throughout the course of 70 days. It was proof that the biofilm, after a certain amount of time had elapsed, had reached stability, and had formed on the anode surface without being damaged. In order to provide justification for the production and maintenance of the biofilm formation rate, Hong et al. [28] used a similar method to the one described above.

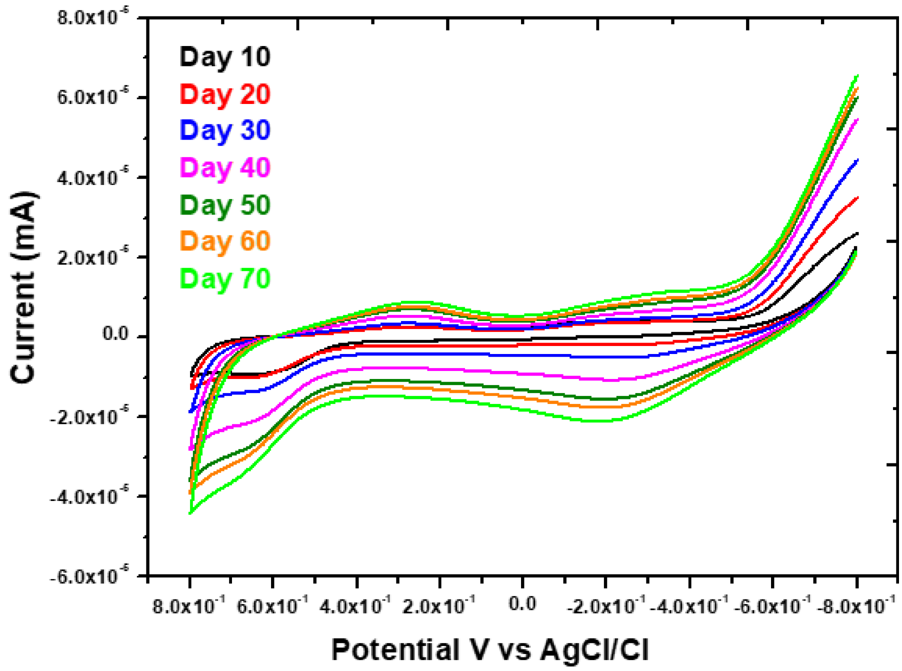

Figure 4. CV studies of this operation at different time interval.

Table 2. Cp values of the present study.

Days	Specific Capacitance (F/g)
10	0.00001
20	0.00001
30	0.00007
40	0.00007
50	0.00003
60	0.00003
70	0.04

3.2. Chemical Analysis Output of the Present Work

In order to assess the fuel cell's potential for usage as a system for wastewater treatment, the BMFC was continually monitored for waste removal (as COD). By being able to eliminate COD, a sample of wastewater can show how important it is for the bacteria living there to serve as electron donors while metabolizing the carbon source [29]. Using a sample of wastewater, this ability was shown. The results of the trials clearly show that the removing of COD and the generation of current are somewhat compatible [30]. In BMFC, there was evidence of ongoing COD eradication. The measured COD concentration at the start of the process was 500 mg/L, as shown in Figure 5. This COD value is computed using the MSW sample. According to the observation, the COD in the BMFC started to oxidize as time went on and electron generation increased; by day 70, there was only 11.3 mg/L of COD left in the chamber. Similar to this, Figure 5 depicts the elimination percentage, which on day 70 showed a value of 90.58%. It seems that the maximum COD was successfully removed by this arrangement. Zhang et al. [31] found that when electron production rises, so does the rate of COD elimination. Figure 5 shows that ongoing electron production gradually reduces the amount of COD.

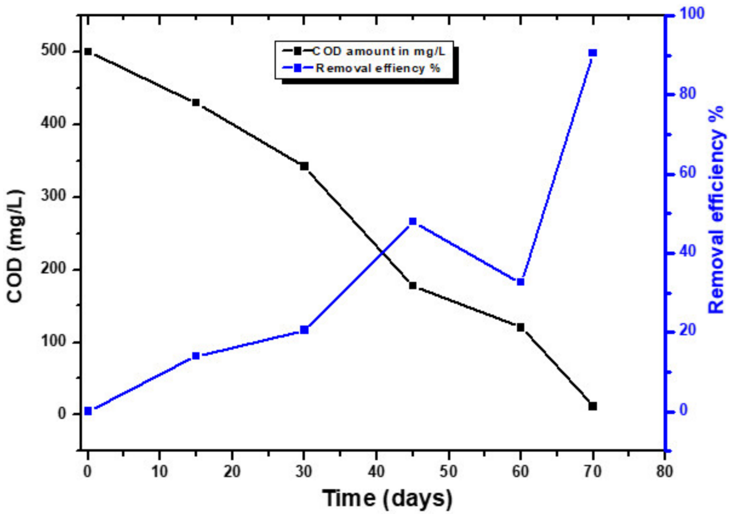

Figure 5. COD removal trend of the present study.

3.3. Biological Tests

Table 3 shows the findings of the investigation into the oxidation of metal ions. The utilization of a bio-electrochemical system to degrade metals is developing as one of the most innovative and promising techniques, particularly in connection to BMFCs. The latest study offers unique insights into how metal ions degrade in water. There is a steady increase in the effectiveness of the metal deterioration as the process nears completion. The results provided here are rather fascinating when compared to those of earlier studies. More than 90% degrading efficiency was achieved in this research attempt. The information provided here suggests that the treated synthetic water's metal ion content was decreased.

Table 3. Degradation efficiency of metals in BMFC.

Organic Substrate	Initial Concentration	Operational Days	Degradation % of Pb (II)	Degradation % of Cr (III)	Degradation % of Cd (II)
Sugarcane waste	200 mg/L	0	0	0	0
		15	30.10	29.50	29.10
		30	48.19	51.99	54.78
		45	58.30	69.25	71.40
		60	76.11	74.35	85.00
		70	88.68	87.60	90.20

The biofilm is made up of a variety of bacterial species that are accumulated on and around the anode electrode. The biofilm is in charge of controlling metal deterioration as well as energy production and transfer. The biofilm was produced naturally throughout the process without aid from outside sources. The water, extracellular polymeric substance (EPS), and bacterial species make up around 97% of biofilm [32,33]. The main biofilm component, the extracellular polymeric material, is in charge of the bacterial processes that simultaneously produce electrons and degrade the metals [34]. The extracellular polymeric material is the area of the biofilm with the maximum water content. The EPS is what establishes how long ago the biofilm was formed, and the efficacy of the EPS is dependent on how easily the biofilm can obtain an organic substrate. Further, carbohydrates, proteins, lipids, and substances other than nucleic acids are included in EPS compositions [35,36]. Biofilm strength is increased because of the good organic substrate, which improves the efficiency of the EPS. The CV data presented before suggested that the current biofilm was rather stable. Figure 6 displays scanning electron micrographs of the biofilm-covered electrode. Figure 6 also shows the untreated graphite surface, while the treated graphite (anode and cathode) indicates a clear difference. Scanning electron microscopy (SEM) images of the anodic biofilm electrode showed the expected bacterial species growth. The treated graphite shows tubes or rod shapes on the surface, which indicate the presence of biofilm, while the untreated graphite does not show anything on the surface. Additionally, the anode biofilm was inspected by SEM and was found to have a surface with a comparable form. Rod filaments in the anode electrode biofilm are characteristic of conductive pili-type bacteria, suggesting the presence of these organisms. Evidence from much research indicates that bacterial species belonging to the conductive pili type are distinguished by their rod-shaped and filamentous appendage features [27]. *Acinetobacter*, *Lysinibacillus*, *Klebsiella pneumoniae*, *Bacillus*, and *E. coli* are the most often reported bacterial species with conductive pili [33,37,38].

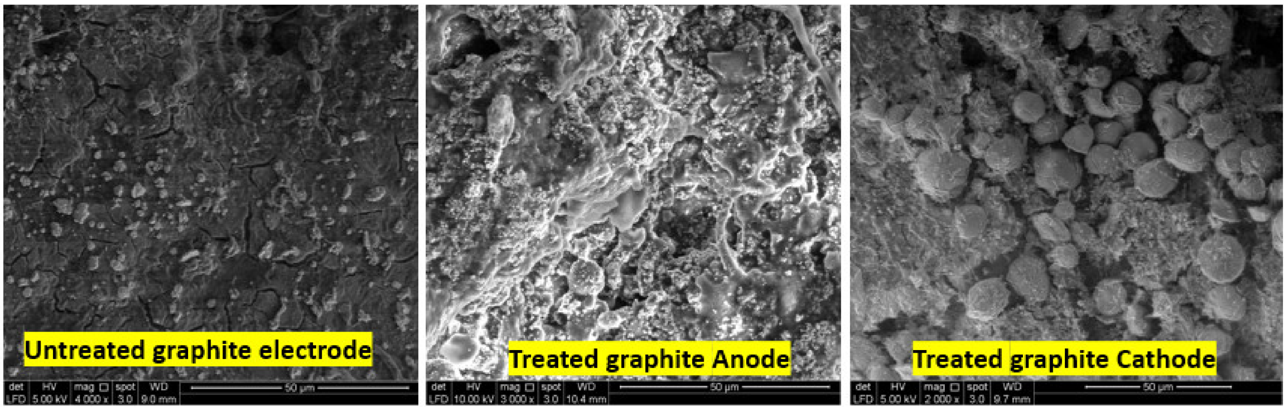

Figure 6. SEM analysis of treated anode and treated cathode after completion of BMFC operation.

After 70 days of operation, as shown in Figure 7, the treated anode with biofilm had a unique elemental composition, as seen in the EDX spectrum. According to these findings, the anode surface does not contain any hazardous substances or metal ions (biofilm). Due to this, we know that the reduced product of metal ions has no hazardous impact on the biofilm, as seen by both the EDX data and the SEM photos of the biofilm. EDX further demonstrated that the metal ions were not only adsorbed onto the electrodes' surfaces but were actually reduced by the bacteria themselves [37].

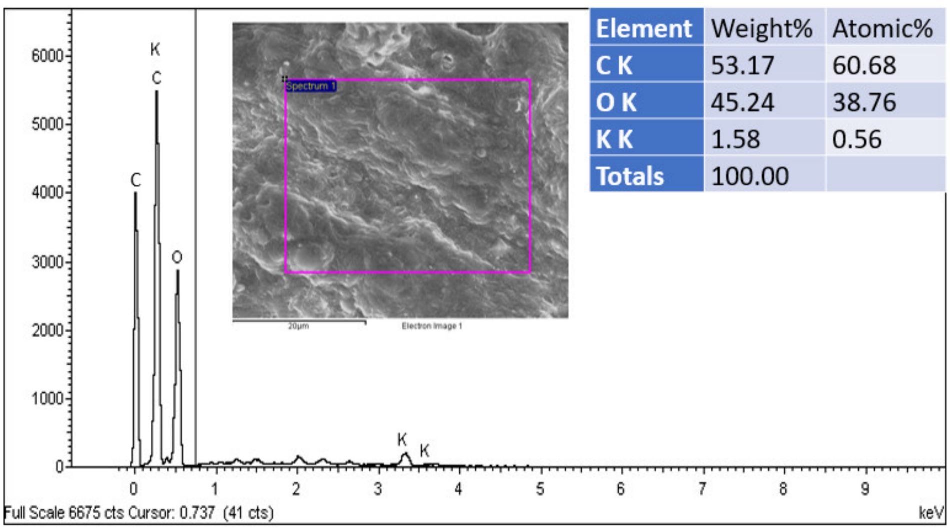

Figure 7. EDX spectra of the treated anode after completion of BMFC operation.

In the BMFC, the bacterial species are engaged in the production of electrons and protons, reducing the metal ions at the same time. For isolating and identifying the bacteria, the biofilm that had formed around the anode surface was scraped. The list of identified bacterial species found on the anode surface is shown in Table 4. They oversee producing power and the removal of metal. The detected bacterial species in the current research are well-known exoelectrogens and metal-reducing species, according to the prior literature. For example, Ayangbenro et al. [39] found that *Bacillus* species are well-known metal-reducing species, particularly for cadmium and lead ions, and that they belong to the conductive pili-type species. Nimje et al. [40] researched the *Bacillus* species as an exoelectrogens species later on, and they attained a power density of 0.000105 mW/m^2 throughout their research. Recent research has also shown that some species of *Bacillus* are exoelectrogens, which refers to well-known metal-reducing species [41–43]. On the other hand, considering the outcomes of the past as well as the results of the present, we are in a position to formulate a hypothesis suggesting that the existing species are capable of not only producing energy but also degrading the metal ions from wastewater.

Table 4. A list of the bacterial species that were found on the anode electrode's surface.

	Query Cover (%)	Identity (%)	Accession Number (16S rRNA Gene)
Bacillus thuringiensis strain	99	96.54	NR_043403.1
Bacillus sanguinis strain	99	96.54	NR_175555.1
Bacillus cereus ATCC	99	96.54	NR_074540.1
Rossellomorea marisflavi strain TF-11	99	93.54	NR_118437.1
Sutcliffiella halmapala strain	99	93.56	NR_026144.1
Priestia flexa strain	99	93.28	NR_024691.1
Peribacillus muralis strain	99	93.02	NR_042083.1
Bacillus salis strain	99	92.82	NR_179406.1
Cytobacillus kochii strain	93	94.81	NR_117050.1
Neobacillus ginsengisoli strain	95.35	92.00	NR_109068.1
Sutcliffiella cohnii strain	99	93.18	NR_113776.1
Cytobacillus purgationiresistens strain	94	95.20	NR_108492.1
Bacillus marcorestinctum strain	91	96.14	NR_117414.1

3.4. Sugarcane Waste Oxidation and Metal Reduction Mechanisms

In the present study, sugarcane waste byproducts were employed as an organic substrate for several microorganisms. In the beginning, the sugarcane waste was composed of polysaccharides, but it was broken down into glucose. Bacterial species then oxidize this glucose, producing electrons and protons in the process. This analysis revealed oxidation and reduction processes that may be stated as follows:

$$\text{Oxidation: sugarcane waste} \rightarrow C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

$$\text{Reduction: } 24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O$$

$$\text{General reaction: } C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Electricity}$$

The produced electrons and protons are then delivered to the cathode during the oxidation process. Protons in the BMFC had a clear path from the anode to the cathode since there was just one chamber between them [44]. The most studied mechanism is described in the literature and shown in Figure 8 from an earlier work [45].

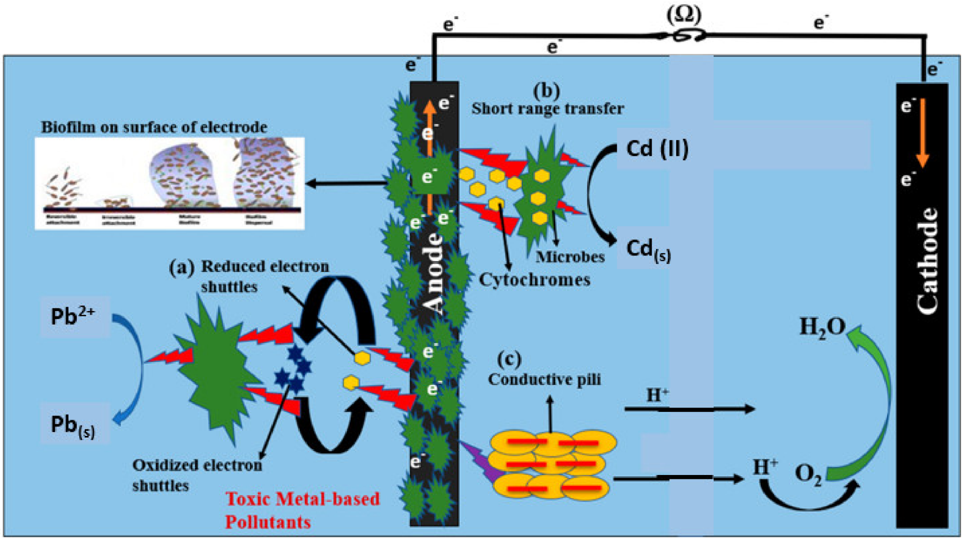

Figure 8. Mechanism of this research of BMFC (modified from reference [45], MDPI, an open-access publisher).

During a redox reaction, soluble metal ions are effectively converted to an insoluble form. As was stated before in the phrase, the insoluble condition was found to be sludge. The results of the AAS test also only showed how much metal ion was still in the water. The removed metal ions were converted to oxides, which resulted in the appearance of a sludge-like material in the BMFC. Numerous studies have shown that the extracted metal ions are converted into their oxide forms, and the resulting sludge includes the metals in their oxide forms [46,47]. A mechanism of the biological activities involved in metal reduction is as follows:

1. Reduction of Pb^{2+}

$$Pb^{2+} + 2e^{-} \rightarrow Pb_{(s)}$$

$$2Pb^{2+} + 2H_2O \rightarrow 2PbO + 4H^{+}$$

$$PbO + 2e^{-} + 2H^{+} \rightarrow Pb_{(s)} + H_2O$$

2. Reduction of Cr^{3+}

$$Cr^{3+} + 3e^{-} \rightarrow Cr_{(s)}$$

$$2Cr^{3+} + 3H_2O \rightarrow Cr_2O_3 + 6H^{+}$$

$$Cr_2O_3 + 6e^{-} + 6H^{+} \rightarrow 2Cr_{(s)} + 3H_2O$$

3. Reduction of Cd^{2+}

$$Cd^{2+} + 2e^{-} \rightarrow Cd_{(s)}$$

$$2Cd^{2+} + 2H_2O \rightarrow 2CdO + 4H^{+}$$

$$CdO + 2e^{-} + 2H^{+} \rightarrow Cd_{(s)} + H_2O$$

4. Parameter Optimizations

Figure 9 demonstrates how the pH effects the voltage trend as well as the metal degradation. It was found, based on the voltage trend, that there was a growing tendency from pH 4 all the way up to pH 10. After pH 8, a downward trend in voltage was seen due to the fact that some bacterial species are unable to live in high-alkaline environments. In the current investigation, it was discovered that 55 mV exists at pH 7. In comparison to the neutral pH, pH 4 and 23 mV demonstrated a much-reduced voltage production. In a previous study, Huang et al. [48] investigated the pH optimization and reported that pH 5.2 delivered a low voltage with acidic sludge that significantly disrupted the voltage trend, but that later at pH 7 it was regained. This was performed in order to determine whether or not the pH could be optimized. Because of the cell's unfavorable pH, the movement of bacteria might be stopped, which in turn results in a decreased rate of voltage production. On the other hand, the metal breakdown process was examined at the same pH. At a pH of 7, the RE of all metal ions was found to be at its highest, whereas RE was poor at pH 4 and 10. This was determined using BMFC. Hence, the most optimal operating temperature for the BMFC is at a pH of 7. To properly regulate bacterial activity in environments with high levels of alkaline or acidic conditions, additional attention is required. According to the findings of a number of prior research studies, a pH level of 7 is optimal for voltage production and metal degradation performance [49–51]. Recently, Yaqoob et al. [42] investigated the influence of the pH on the formation of energy and the degradation of metals, and they suggested that a pH of 7 is ideal. In spite of this, the current observation about energy production and degradation is determined to be maximum compared to the findings that they claimed. This indicates that the usage of an inoculum source also adds to the performance at a pH level of 7 when the temperature is at room temperature.

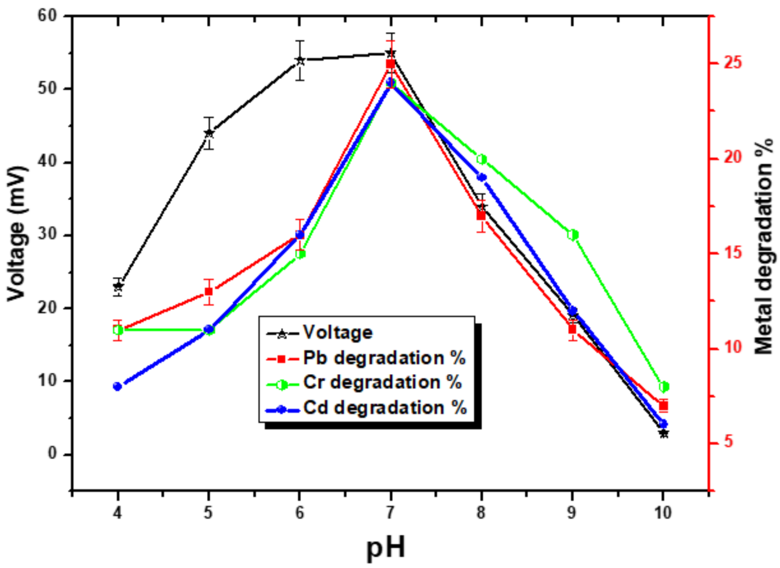

Figure 9. The pH optimization outcomes of the present study.

Some research studies have demonstrated remarkable results when applying a range of natural organic substrates, making the organic substrate an important factor to think about when boosting the performance of BMFCs [48]. Energy for bacterial oxidative respiration may be obtained from any carbohydrate source used as an organic substrate in the operation of BMFCs [52]. Nevertheless, few attempts have been made in previous research to use natural organic substrates in BMFCs, such as vegetable cellulose, biomass waste, mangroves, and brewery and cocoa effluents. Mangroves were used as an organic substrate in BMFCs by Salvin et al. [53], which improved their efficiency in producing energy. In our experiment, we compared the performance of glucose to that of an organic substrate made from sugarcane. These organic carbohydrate substrates include cellulose, starch, fiber, and simple sugar, but they provide more power nutrients for bacteria. The results of all organic substrates are summarized in Table 5, which shows that sugarcane waste performed the best. According to previous research, more than 70% of nutrients in carbohydrate waste are bacterially friendly, making it an ideal medium for bacterial growth and respiration [54].

Table 5. Sugarcane and glucose as substrate performance in BMFC.

Organic Substrate	Total Days	Voltage (mV)	Degradation % of Pb (II)	Degradation % of Cr (III)	Degradation % of Cd (II)
Sugarcane waste	10	55	25	24	24
Commercial glucose	10	28	11.20	12.99	13.00

5. Concluding Comments and Future Perspectives

The present research highlighted the use of waste from sugarcane in BMFCs for the production of electricity as well as the degradation of metal. The highest voltage that could be measured was 300 mV. According to the findings of the biological research, the fact that the surface of the anode contains several bacterial species is evidence that the metal remediation operation was effective. The high rate of efficiency with which metals are degraded is due to the beneficial bacterial activities that take place as a result of the effective oxidation of the organic substrate. The most recent electrochemical tests, on the other hand, show an energy efficiency that is more reasonable than that of the research that came before. Following in-depth investigation and analysis, a conclusion was made that, despite all of the factors that contributed, there was a reduction in energy efficiency. This was found as a result of electrochemical analysis. It might be possible that the used graphite electrode was not very effective at transporting the electrons from the anode. The literature also recommends upgrading the electrode material for BMFCs. Since graphene and its derivatives are both highly conductive and cutting-edge materials, it is recommended that electrodes be fabricated from these materials. The flow of electrons will be enhanced as a result of this step. It is necessary for the material used in the electrode to be biocompatible, chemically stable, ultra-conductive, and thermally stable for it to be effective throughout the course of a prolonged period of time. It is possible that the challenges that need to be conquered in order to bring the BMFC up to the level of commercial viability can be worked through with the collaboration of experts from a variety of different fields, such as material science, microbiology, biotechnology, environment, and electrochemistry.

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