Effects of Open Circuit Potential and Characterization of Electro-Active Biofilm for Microbial Fuel Cells using Compost Leachate

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Abstract- A microbial fuel cell produces electric energy and clean up effluents thanks to bio-electrochemical reactions in which electro-active bacteria are used as catalysts, it is considered a sustainable technique. This paper focuses on the effect of different durations of open circuit potential on the current density produced by compost leachate. The obtained results demonstrate that an electro-active biofilm was developed using compost leachate, with a potential (-0.2V/SCE). Maximum current density (458.3 µA/cm²) was reached after only 3 hours of polarization after an open circuit duration of 28 days. Scanning electron microscopy has been used to characterize the biofilm formed on carbon graphite electrodes at different phases.

Keywords: Microbial fuel cell, Electro-active biofilm, Platinum, Oxygen reduction, Scanning Electron Microscopy, Depollution, Carbone.

Nomenclature:

C	Concentration (mmol.L^{-1})
CA	Chronoamperometry
CV	Cyclic Voltammetry
E	Potential (V)
EA	Electro-active
SCE	Saturated Calomel Electrode
J	Current density (µA/cm²)
MFC	Microbial fuel cell
SEM	Scanning Electron Microscopy

S Electrode Surface

1. Introduction

The consumption of fossil fuels such as petroleum, coal, and natural gas is very high and growing, and their reserves are limited. It is estimated that humanity has about 200 years to use up these reserves, which took millions of years to produce. Renewable energies, which are generated from natural periodic phenomena, on the other hand, are almost unlimited as long as they are produced at a rate higher than their consumption. They are also environmentally friendly, producing little or no pollutants [1]. Fuel cells, and specially the microbial fuel cell (MFC), are hopeful innovative technologies within the realm with renewable energies, if the fuel used is renewable and obtained naturally. MFCs generate electrical energy by allowing bacteria to consume organic compounds present in wastewater [2]. These bio-electrochemical systems convert the organic matters chemical energy into electricity through the bacteria's metabolism by transferring electrons with the anode. Wastewater can be used as a substrate for MFCs because it is a rich source of organic materials that store large amounts of energy, which makes MFCs a sustainable technology for electricity production and for the treatment of wastewater without generating any pollution [3]. The discovery of the concept of the production of electricity by bacterial metabolism in 1910 is attributed to Michael Cresse Potter, who was a professor at the University of Durham in the United Kingdom. It was not until the early 2000s that the development of MFCs reached a upland, thanks to the team of Derek Lovley from the University of Massachusetts, who discovered that some types of microorganisms could transfer electrons to an electrode by electro-microbial catalysis by forming biofilms [4].

Recently, there has been increased attention given to the use of MFC systems, particularly in wastewater treatment. In fact, MFCs have been shown to be highly effective in treating evaporative water, reaching a pollutant removal efficiency of 70.05% [5]. To diminish electrode costs, researchers have utilized readily available materials rather than precious metals. Electrodes made from arachidic shells mixed with metals (copper, cobalt, etc.) have been tested in fuel cells on a micro-billion scale. Exploratory testing yielded a very high-power density of 205.4 mW/m^2 [6]. The addition of an iron-carbon filler for a voltage of 300.16 mV has been shown to improve both water quality treatment and power generation [7]. An innovative approach to bioenergy involves production of green hydrogen from ammonia-rich wastewater, using a system of MFCs coupled with ammonia electrolysers. Despite relatively low carrying capacities, this approach has many advantages: low price, accessibility of substrate and well-matched with agriculture [8]. Another innovative system is the plant-microbial fuel cell (PMFC) which can produced 0.2 mmol of hydrogen per day per cubic meter of roots [9]. MFC systems produce energy and valuable compounds from organic waste in presence and in absence of inorganic matters, through the metabolic activity of microorganisms. This system can be considered as a clean chemical energy storage, if it coupled to a water electrolyser producing hydrogen or syngas. The hydrogen produced could be used to capture carbon dioxide for synthesize methane gas [10].

The aim of this study is to investigate how the duration of electrode immersion affects the current density in the formation of bio-anodes using garden compost leachate. The microbial anodes were produced through delayed polarization at a voltage of -0.2 V, and their electrochemical behavior was analyzed via cyclic voltammetry. Furthermore, the morphology of the anodes were examined using scanning electron microscopy.

2. State of the Art

2.1. Configuration of microbial fuel cells

Microbial fuel cell reactors can vary in their design, with factors such as the shape of the reactor, the type of electrode materials used, the spacing between them, and the presence or absence of a membrane all playing a role. Additionally, the number of chambers in the reactor (single or double) should also be considered. One major benefit of the dual-chamber design is that the anode and cathode liquids are kept separate by a membrane, whereas in a single-chamber design, the anode and cathode are located on opposite sides within a single chamber [11].

Various MFC designs have been developed over time, each serving specific purposes, as depicted in Figure 1. The goal of creating new MFC systems has always been to address the shortcomings of conventional systems, which include high membrane and electrode costs, a scarcity of cathodic oxygen, and limitations in system design.

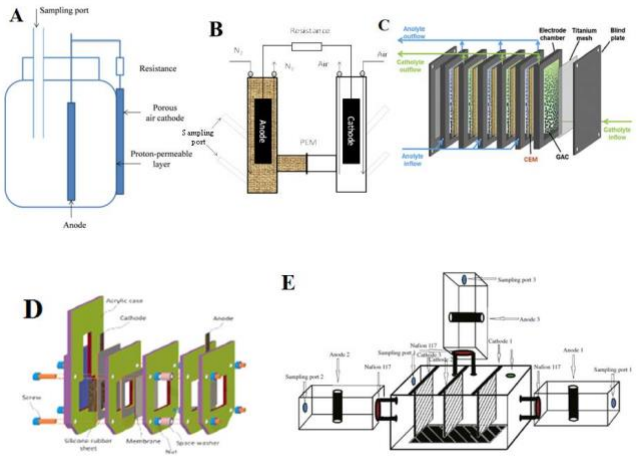

Fig. 1. Different microbial fuel cell designs: A) Single-chamber MFC [12], B) dual chamber [13], C) stacked MFC (SMFC) [14], D) plugged type soil microbial fuel cell (PSMFC) [15], E) multiple anode chamber and single cathode chamber microbial fuel cell (MAC-MFC) [16].

2.2. Role of Anode Material in Enhancing Electrochemical Interactions with Biofilms in Various Systems

The choice of anode material is crucial in enhancing the electronic exchanges in the interfaces between the biofilm and the electrode material in various electrochemical systems. An anode material is an electrode through which current flows out of the system, and it should possess certain characteristics to ensure optimal performance [17].

Firstly, the anode material should be electrically conductive to allow for the efficient flow of electrons. This feature enables the system to produce a stable electrical current for the desired electrochemical reaction to occur. In addition, the material should also be corrosion-resistant to withstand harsh conditions and resist degradation over time.

Secondly, the anode material should have high mechanical resistance to maintain its structural integrity under high pressure or stress. This feature ensures the longevity of the material and helps prevent damage to the system.

Thirdly, a developed surface area is important as it provides more surface area for the biofilm to adhere and grow on. This feature increases the efficiency of the electrochemical system and enhances the performance of the anode material.

Fourthly, the anode material should be biocompatible to ensure that it is not harmful to the biofilm and other microorganisms present in the system. This feature is particularly important in biological wastewater treatment systems, where the biofilm plays a crucial role in treating wastewater.

Fifthly, the anode material should be environmentally friendly to minimize any negative impact on the environment. This feature is increasingly important in today's world as sustainability concerns become more prominent.

Lastly, the anode material should be cost-effective to make it accessible and affordable for widespread use in various electrochemical systems. The main types of electrode materials used are substrates based on carbon and metals. Carbon-based materials can be fabrics, fibers, cylinders, mesh, veils, papers, felts, powders, carbon graphite or glassy carbon [18].

2.3. Factors Affecting the Efficiency of Electrochemical Reactions at the Cathode

The efficiency of electrochemical reactions occurring at the cathode is affected by various factors. One of the critical factors is the concentration of the electron acceptor, which is usually a species that accepts electrons from the electrode surface. In most cases, the electron acceptor is present in the electrolyte, and its concentration can influence the rate of the reaction.

Another critical factor that affects the efficiency of reactions at the cathode is the availability of protons that come from the electrolyte. Protons play an essential role in most electrochemical reactions occurring at the cathode, and their availability can significantly influence the reaction rate.

The overall performance of the catalyst used in the electrochemical reaction also plays a significant role in the efficiency of reactions at the cathode. A good catalyst can significantly enhance the reaction rate, while a poor catalyst can hinder the reaction rate.

The structure of the electrode is also an essential factor that affects the efficiency of reactions at the cathode. The electrode structure can affect the surface area available for the reaction, the diffusion of the reactants, and the transport of electrons.

Similar to anodes, carbon and graphite-based materials are commonly used as cathodes due to their charge and performance. However, these materials need to be enriched with noble metals such as platinum, which act as catalysts in the electrochemical reaction. The noble metals enhance the efficiency of the reactions occurring at the cathode by facilitating the transfer of electrons and protons between the reactants and the electrode surface [19].

2.4. Design Considerations for Membranes in Microbial Fuel Cells with Air Cathodes

Microbial fuel cells (MFCs) are devices that use bacteria to convert organic matter into electrical energy. In MFCs with air cathodes, a specific type of membrane must be used as a separator between the anode and the cathode.

This membrane must meet certain criteria to ensure optimal performance of the MFC. Firstly, it should be electrically non-conductive, which means that it does not allow the flow of electrons between the anode and cathode, as this would short-circuit the device and prevent the production of electrical energy.

In addition, the membrane should have high ion permeability, which allows positively charged particles (protons) to pass through it easily. This is important because protons are involved in the process of electricity generation in MFCs, and the ability of protons to pass through the membrane is critical to the efficiency of the device.

However, the membrane should also have low oxygen permeability, which means that it does not allow oxygen to pass through it easily. This is because the presence of oxygen at the cathode can compete with the process of proton reduction, reducing the overall efficiency of the MFC.

Proton exchange membranes (PEMs) can be used in MFCs, which are similar to the membranes used in chemical gas fuel cells. However, the use of PEMs can also create additional barriers to the improvement of MFCs, such as limited durability and high cost. Therefore, the choice of membrane material is an important consideration in the design of MFCs with air cathodes [20].

2.5. Microorganism sources for electroactive biofilm formation

In the microbial fuel cell, electro-active biofilm formation is based on two types of inoculums, either complex or simple. The formation of electro-active biofilms from inocula complexes can be obtained from various natural environments such as sediments, soils, wastewater, urban effluents and garden soil.

Table 1 displays the various types of natural complex cultures that were utilized in the microbial fuel cell to generate electricity.

Table 1. Efficacy of microbial fuel cells using natural, intricate inocula.

Inoculum	Anode	Cathode	Membrane	Type of MFC	j (mA/m^2)	Ref.
Waste water	Aluminium and carbon	Copper	Proton exchange membrane	Dual chamber	9	[21]
Cashew apple juice	Carbon	Pt/c	Nafion®	Dual chamber	350	[22]
Sediment	Copper	Zinc	-	Single chamber	3.491	[23]
Digestate	Graphite felt	Graphite felt	-	Mfc air cathode without membrane	104.76	[4]
Avocado waste	Zinc	Copper	Proton exchange membrane	Dual chamber	3.7326	[24]
Synthetic waste water	Graphite plates	Graphite plates	Salt bridge	Double chamber type "h	101.85	[25]
Anaerobic granular sludge	Stainless steel/poly-pyrrole	Air cathode	-	Single chamber	1366.4	[26]
Domestic waste water	Graphite rod	Graphite rod	Nafion®	Single chamber	125	[27]

3. Materials and Methods

3.1. Formation and characterization of electroactive biofilm through delayed polarization

The delayed polarization process plays a crucial role in the development of both electroactive (EA) and non-electroactive (non-EA) bacteria. While the full polarization method exclusively promotes the growth of EA bacteria through selective pressure polarization, delayed polarization allows for the formation of both types. EA bacteria, in particular, exhibit the ability to utilize the electrode as a final electron acceptor [28]. These bacteria demonstrate rapid growth, leading to the formation of a dense biofilm with efficient electron transfer. However, this process typically requires a significant operational timeframe of approximately 15 days of continuous polarization [29]. On the other hand, the delayed polarization technique offers the advantage of achieving a promising current density in a shorter period, typically around 3 days [30].

Therefore, this study aims to investigate the formation and characterization of electroactive biofilms obtained through the implementation of delayed polarization.

3.2. Inoculum source preparation

Garden compost was used for the elaboration of biofilm. Its principal characteristics are resumed in table 2.

Table 2. Proprieties of garden compost used for biofilm formation.

Norm	NF U 44-551*
Composition	Sphagnum peat moss Vegetable compost
Analysis	Dry matter (by mass of raw product): 60% Organic matter (by weight of dry product): 35% pH: 6.1 Conductivity: 120 mS/cm Water retention capacity: 680 ml/l

*AFNOR standard NF U44-051 governs the conditions for the marketing of compost produced from organic waste in France.

The evaluation of microbial electrical activity was performed by chronoamperometry.

A mixture of 1L of the garden compost with 2L of distilled water containing 10 mM NaCl was prepared. The mixture was stirred for 24 hours under room temperature then filtered. To promote EA bacteria growth, a 10mM solution of sodium acetate was added.

During the 24-hour stirring period, the mixture of garden compost and distilled water allowed for the extraction of essential nutrients and organic matter from the compost, facilitating the formation of a nutrient-rich leachate. Filtration was performed to remove any solid particles or debris, ensuring a homogeneous and clear solution.

The addition of a 10 mM solution of sodium acetate served as a carbon source, providing a readily available substrate for the growth of electroactive bacteria. This carbon source acts as an electron donor, fueling the microbial metabolism and facilitating their ability to transfer electrons to the biofilm on the carbon graphite electrodes.

The pH value of 6.64 obtained in the soil leachate indicated a slightly acidic environment, which is favorable for many microorganisms involved in bioelectrochemical processes. The conductivity of 2.46 mS/cm indicated the presence of ions and dissolved substances in the leachate, which can contribute to the electrical conductivity and overall performance of the biofilm.

The delayed polarization technique was chosen to analyze the effect of open circuit time on the current density. By allowing the system to reach a steady state and equilibrate over a certain period, it becomes possible to observe the evolution of microbial activity and electron transfer within the biofilm. This technique provides valuable insights into the bioelectrochemical behavior of the system over time.

By conducting experiments for different open circuit times (0, 8, 14, 28, and 35 days), a comprehensive understanding of the temporal dynamics of the microbial activity and current density can be gained. These experiments enable the observation of any changes in the electrochemical performance of the biofilm and help identify the optimal open circuit time for maximum current generation.

3.3. Electrochemical set-up

Electrochemical tests were conducted using a standard three-electrode setup. The setup included an auxiliary electrode made of platinum wire and a reference electrode of saturated calomel. The working electrode used was a cylindrical carbon graphite electrode with a surface area of S=1.03 cm^2.

To perform the required tests, a potentiostat/galvanostat type PGZ100 was employed, which was controlled using Voltamaster 4 software. This equipment allowed for precise control and monitoring of the electrochemical processes.

For chronoamperometry measurements, the electrode was polarized at -0.2V/SCE for a duration of 3 hours. This technique helped in studying the electrochemical behavior and stability of the electrode over an extended period.

Cyclic voltammetry spectra were acquired in the potential range from -700 to 200 mV versus the reference electrode, with a scan rate of 1 mV/s. By sweeping the potential, this method provided valuable information about the redox processes occurring at the electrode surface.

The decision to work with negative potentials was motivated by the research conducted by Torres et al. [31]. Their findings demonstrated that when a biofilm is developed under negative polarization, it exhibits a more rapid increase in current density and reaches higher values. By selecting negative potentials for the experiments, the study aimed to gain insights into the biofilm development and its influence on the electrochemical behavior of the system.

To ensure accurate measurements, all electrochemical tests were performed under controlled conditions, including a temperature-controlled environment. The temperature was maintained at a constant value throughout the experiments to minimize the impact of temperature variations on the electrochemical responses.

Before each measurement, the electrodes were thoroughly cleaned and polished to ensure a clean and uniform electrode surface. This step is crucial for obtaining reliable and reproducible electrochemical data.

4. Results and Discussion

4.1. Exploring the Kinetics of Electrochemical Reactions with Chronoamperometry: Impact of Open Circuit Time on Current Density

Chronoamperometry is a powerful electroanalytical technique used to investigate the kinetics of electrochemical reactions. This method involves the application of a constant potential, typically a potential sufficiently positive or negative to oxidize or reduce a redox entity, and the recording of the resulting current as a function of time.

One of the main advantages of chronoamperometry is its ability to probe rapid electron transfer events on a sub-millisecond timescale. This makes it particularly useful for investigating the kinetics of fast electron transfer reactions, as well as for studying the behavior of electroactive species that are difficult to characterize using other electroanalytical techniques [32] .

Figure 2 illustrates the effect of open circuit time on the current density.

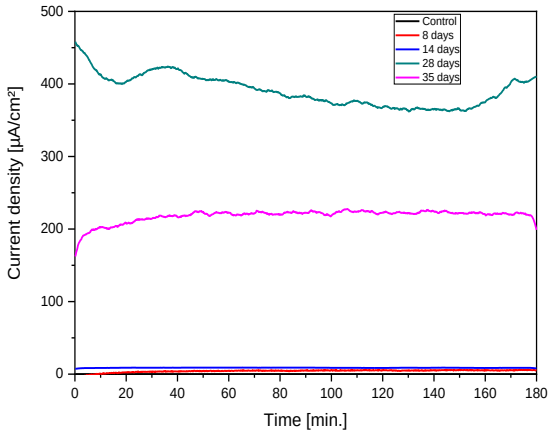

Fig. 2. Current densities (j) obtained during 3h of polarization at -0.2V on biofilms at 8, 14, 28 and 35 days.

It is clear from Figure 2 that the open circuit time significantly influences the current density. The absence of current during the 3-hour polarization period on the control electrode, which was directly polarized after being submerged in the compost leachate, emphasizes the impact of open circuit time. However, for the experimental case, the current density showed a notable increase with immersion time. After only 8 days of immersion, the current density was recorded at 6.4 µA/cm^2, but it significantly rose to a maximum value of 458.3 µA/cm^2 after 28 days of immersion. This substantial increase suggests the formation of an electro-active biofilm on the surface of the electrode, facilitating the oxidation of leachate components.

Interestingly, after 35 days of immersion, a decline in current density becomes evident. This observation indicates a progressive decrease in the concentration of electro-active species near the working electrode. The diminishing current density highlights the gradual depletion of these species, likely due to their consumption during the oxidation process.

4.2. Cyclic voltammetry

Cyclic voltammetry is a widely used electrochemical technique that involves the controlled variation of the electrode potential within predefined limits. The potential is varied linearly between two fixed boundaries, typically set as -700 mV/SCE as the lower limit and +200 mV/SCE as the upper limit. By measuring the resulting current densities generated by the redox reactions occurring at the electrode, valuable information about the electrochemical system can be obtained.

The acquired current density values are then plotted against the corresponding electrode potential (E), creating characteristic curves known as voltammograms [33]. These voltammograms serve as a visual representation of the electrochemical processes taking place during the cyclic voltammetry experiment. By analyzing the shape, magnitude, and position of peaks or other features within the voltammograms, important insights into the kinetics, mechanisms, and thermodynamics of the redox reactions can be gained.

Figures 3-7 represent the cyclic voltammetry analysis of the different samples.

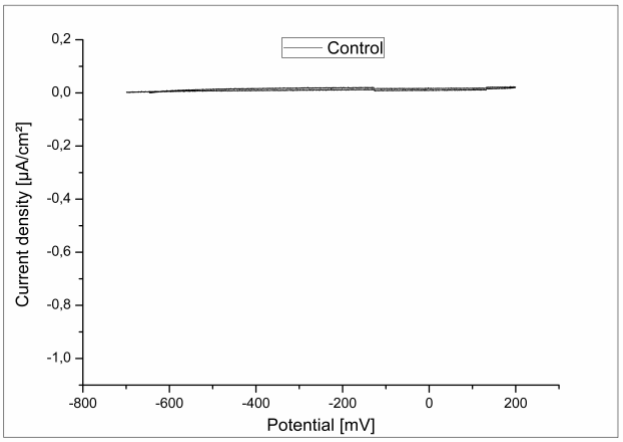

Fig. 3. CVs after 3 hours of polarisation at -0.2 V vs SCE of initial electrode with scanning speed of 1 mV/s.

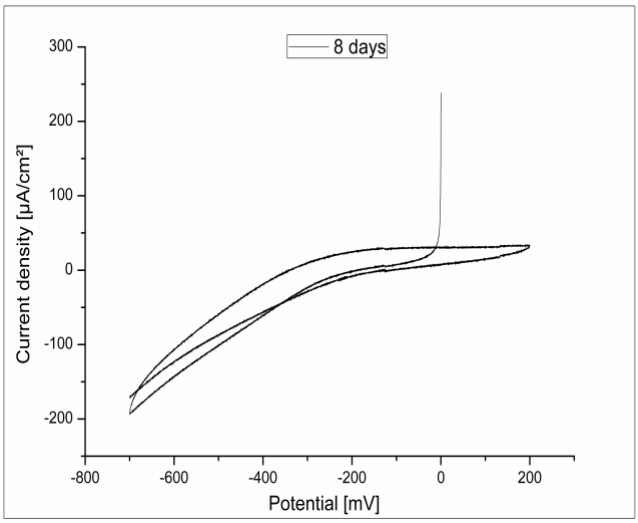

Fig. 4. CVs after 3 hours of polarisation at -0.2 V vs SCE of electrode (open circuit time = 8 days) with scanning speed of 1 mV/s.

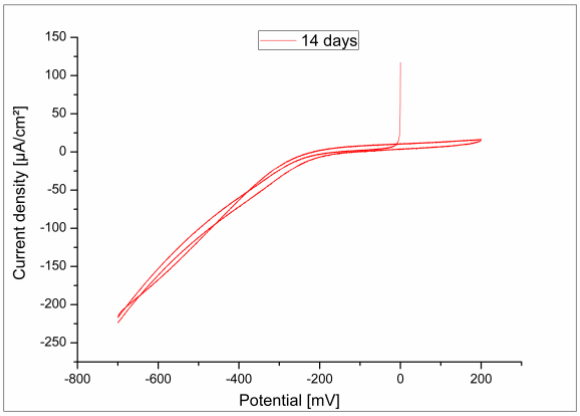

Fig. 5. CVs after 3 hours of polarisation at -0.2 V vs SCE of electrode (open circuit time = 14 days) with scanning speed of 1 mV/s.

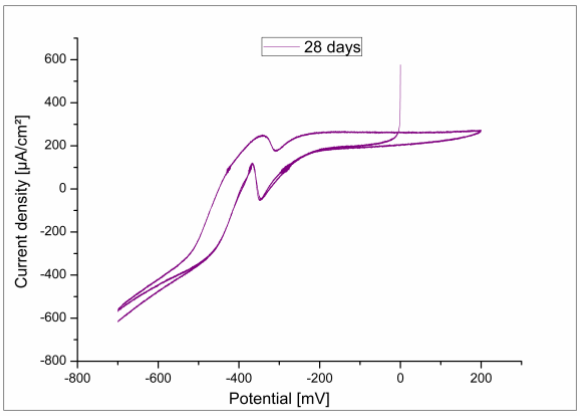

Fig. 6. CVs after 3 hours of polarisation at -0.2 V vs SCE of electrodes (open circuit time = 28 days) with scanning speed of 1 mV/s.

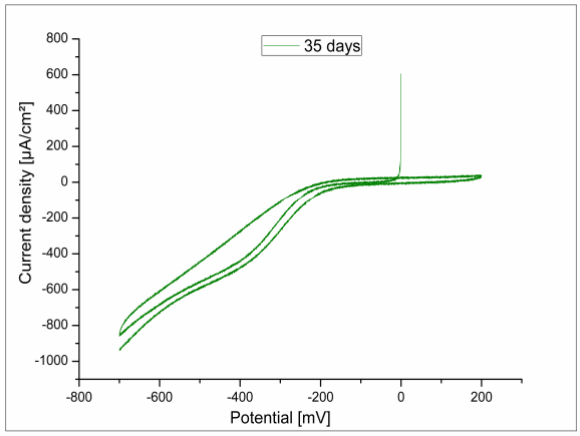

Fig. 7. CVs after 3 hours of polarisation at -0.2 V vs SCE of electrodes (open circuit time = 35 days) with scanning speed of 1 mV/s.

According to Figures 3-7, the CV curves detected a variety of electrochemical characterizations, providing valuable insights into the biofilm formation and its electrochemical behavior. At the control electrode, a classical sigmoidal shape was observed, indicating that the biofilm had not yet had sufficient time to develop. This suggests that the observed CV curves at this stage primarily reflect the baseline electrochemical response of the system.

However, at 28 days, the CV curves exhibited remarkable peaks in comparison to the biofilms obtained at 8, 14, and 35 days. These prominent peaks can be attributed to the presence of an efficient electron transfer mechanism within the biofilm. This finding strongly suggests that the biofilm has matured and is exhibiting enhanced electrochemical activity, potentially due to the development of a more complex microbial community or the accumulation of active metabolic byproducts.

Moreover, the observed cyclic voltammetry peaks not only signify the biological activity of the biofilm on the electrode but also indicate the oxidation of the leachate components. The biofilm's ability to facilitate electron transfer and the subsequent oxidation of leachate constituents contribute to the overall electrochemical performance observed in the CV curves. This correlation between the biofilm's activity and the oxidation process is in line with the results obtained from Chronoamperometry analysis, further validating the findings.

By combining the information from CV curves and Chronoamperometry analysis, a comprehensive understanding of the biofilm's electrochemical behavior and its impact on leachate oxidation can be achieved.

4.3. Scanning electron microscopy (SEM)

The surface structure and morphology of the bio-anode were comprehensively analyzed using scanning electron microscopy (SEM) at various stages of development to observe the bacteria attached to the electrode surface (figure 8). In order to prepare the electrode for analysis, a rigorous pre-treatment procedure was employed. Firstly, the electrode was immersed in a solution containing 50% glutaraldehyde at 4% concentration, along with a 0.4M phosphate buffer solution with a pH of 7.5, and left in contact for a duration of 20 minutes. Subsequently, the sample underwent a thorough rinsing process with a solution of 0.4M sucrose in distilled water to remove any residual impurities.

To facilitate further analysis, the sample was dehydrated using a mixture of actéon/distilled water with increasing concentrations. The dehydration process involved immersing the sample in a 50% actéon/distilled water mixture for 5 minutes, followed by a 70% mixture for an additional 5 minutes, and finally using 100% actéon for a duration of 30 minutes. This step ensured the removal of excess moisture from the sample, which is crucial for achieving high-quality SEM images.

To enhance the conductivity and stability of the sample during microscopy, a gold nano-layer coating was applied. The coating process involved uniformly depositing a thin layer of gold onto the surface of the sample. This gold nano-layer not only provided enhanced electrical conductivity but also served as a protective barrier, preventing any potential damage to the sample during the SEM analysis.

Overall, these rigorous sample preparation techniques and imaging procedures allowed for detailed observation and analysis of the bio-anode surface, elucidating the attachment of bacteria to the electrode and providing valuable insights into the bioelectrochemical processes at play.

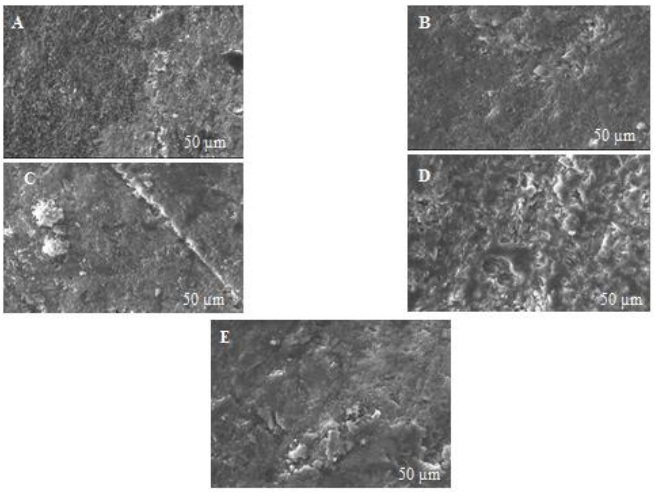

Fig. 8. Scanning electron microscopy of biofilms obtained after 3 hours polarization of biofilms: A control, B 8 days, C 14 days, D 28 days, E 35 days.

Figure 8-D depicts a significant colonization of microorganisms on the surface of the electrode, serving as compelling evidence for the augmented current density observed during this specific timeframe. This microbial colonization is indicative of a thriving microbial community that actively participates in the electrochemical reactions taking place. Moreover, the presence of diverse microorganisms on the electrode surface suggests the establishment of a complex microbial ecosystem, which further supports the notion that microbial activity is intricately linked to the enhanced current density observed in this study.

Furthermore, the visualization provided by Figure 4-D enables a deeper understanding of the spatial distribution and organization of these microorganisms on the electrode surface. The presence of biofilms or microbial aggregates can be observed, suggesting the formation of intricate microbial structures that contribute to the overall electrochemical performance. These biofilms act as conductive networks, facilitating the transfer of electrons, nutrients, and metabolic byproducts, thereby promoting a more efficient electron flow and ultimately leading to the observed increase in current density.

5. Conclusion

In conclusion, this study has successfully demonstrated the potential of compost leachate as a substrate for the development of an electro-active biofilm in microbial fuel cells. The findings of this research indicate that optimizing the duration of open circuit potential can significantly enhance the efficiency of microbial fuel cells by promoting the growth of electro-active bacteria. This exciting development holds great promise in the field of sustainable energy production and effluent treatment, offering a potential solution to pressing environmental challenges.

Furthermore, the utilization of scanning electron microscopy for characterizing the biofilm formed on carbon graphite electrodes has provided valuable insights into the intricate mechanisms underlying the bio-electrochemical process. This analytical technique has allowed researchers to visualize and analyze the structural and morphological features of the biofilm, enabling a deeper understanding of its formation and behavior within the microbial fuel cell system.

The outcomes of this study significantly contribute to the expanding body of knowledge surrounding microbial fuel cells, highlighting their potential applications in renewable energy production and environmental remediation. The successful utilization of compost leachate as a substrate and the optimization of open circuit potential duration represents key advancements in the field. These findings not only offer a sustainable and eco-friendly approach to energy generation but also emphasize the importance of bio-electrochemical reactions in addressing environmental concerns.

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