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Electrochemical Characterisation of Heat-Treated Metal and Non-Metal Anodes using Mud in Microbial Fuel Cell

(Pencirian Elektrokimia bagi Logam dan Bukan-Logam Anod dengan Rawatan-Haba menggunakan Lumpur dalam Sel Fuel Mikrob)

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ABSTRACT

Microbial fuel cells (MFCs) have a high potential application for simultaneous wastewater treatment and electricity generation. However, the choice of the electrode material and its design is critical and directly affect their performance. As an electrode of MFCs, the anode material with surface modifications is an attractive strategy to improve the power output. In this study, stainless steel (SS) and carbon steel (CS) was chosen as a metal anode, while graphite felt (GF) was used as a common anode. Heat treatment was performed to convert SS, CS and GF into efficient anodes for MFCs. The maximum current density and power density of the MFC-SS were achieved up till 762.14 mA/m^2 and 827.25 mW/m^2, respectively, which were higher than MFC-CS (641.95 mA/m^2 and 260.14 mW/m^2) and MFC-GF (728.30 mA/m^2 and 307.89 mW/m^2). Electrochemical impedance spectroscopy of MFC-SS showed better catalytic activity compared to MFC-CS and MFC-GF anode, also supported by cyclic voltammetry test.

Keywords: Anode; carbon steel; graphite felt; MFC; stainless steel

ABSTRAK

Sel bahan api mikrob (MFCs) mempunyai aplikasi yang berpotensi tinggi untuk rawatan air sisa kumbahan dan penghasilan tenaga elektrik. Walau bagaimanapun, pemilihan bahan elektrod dan reka bentuknya adalah sangat penting dan secara langsung mempengaruhi prestasi mereka. Sebagai elektrod MFC, bahan anod dengan pengubahsuaian permukaan dianggap sebagai strategi yang berkesan bagi meningkatkan output kuasa. Dalam kajian ini, keluli tahan karat (SS) dan keluli karbon (CS) dipilih sebagai anod logam, manakala serat grafit (GF) digunakan sebagai anod biasa. Rawatan haba dilakukan untuk menjadikan SS, CS dan GF kepada anod yang lebih cekap untuk MFC. Ketumpatan arus maksimum dan ketumpatan kuasa MFC-SS, masing-masing telah mencapai sehingga 762.14 mA/m^2 dan 827.25 mW/m^2, iaitu lebih tinggi daripada MFC-CS (641.95 mA/m^2 dan 260.14 mW/m^2) dan MFC-GF (728.30 mA/m^2 dan 307.89 mW/m^2). Menerusi spektroskopi impedans elektrokimia (EIS), MFC-SS menunjukkan aktiviti katalitik yang lebih baik berbanding dengan MFC-CS dan MFC-GF, yang juga disokong oleh ujian kitaran voltammetri (CV).

Kata kunci: Anod; keluli karbon; keluli tahan karat; MFC; serat grafit

INTRODUCTION

Recently, various Bioelectrochemical Systems (BESs) have received great interest among researchers as a novel approach for wastewater treatment and power generation. A MFC is a BES that combines biological catalytic and electrochemical reactions. MFCs use bacteria for generation of bioelectricity through microbial metabolism by oxidation and reduction reactions (Logan 2009; Mathuriya & Yakhmi 2016; Rahimnejad et al. 2015). A general MFC consists of an anode and a cathode compartments separated by a membrane. Electrochemically active bacteria (EAB) located at the anode oxidized the organic matter into protons and electrons. These protons pass through the membrane to the cathode. Current is generated when electrons travel from the anode to the cathode using external wire as a conductive bridge. The electrons and protons finally combined at cathode to reduce oxygen and produce water as by-product (Lim et al. 2012; Logan 2009; Rahimnejad et al. 2015; Sun et al. 2016). Overall reactions occur in MFCs when acetate becomes the electron donor and oxygen as terminal electron acceptor are as follow:

$$\text{Anode:}\quad CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \tag{1}$$

$$\text{Cathode:}\quad 2O_2 + 8e^- + 8H^+ \rightarrow 4H_2O \tag{2}$$

$$\text{Overall:}\quad CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O + \text{biomass} + \text{electricity} \tag{3}$$

In essence, the performance of MFCs can be affected by several factors including microbial inoculum and electrode i.e. anode or cathode. EAB, obtained from various natural sources such as wastewater, plant waste, mud and palm oil mill effluent (Logan 2009; Zhao et al. 2017) can use different metabolic pathways for power generation. The EAB attached to the surface of the electrode, also referred to as 'biofilm' plays an important role in electrochemical processes involving anodic (ionizing or oxidation) reactions. The extracellular electron transfer (EET) mechanism by EAB can be classified into two types namely, direct electron transfer (DET) and through intermediate or mediator (MET) (Rosenbaum et al. 2011; Song et al. 2015; Uria et al. 2017). DET allows the electron from the bacterial metabolism process to be transferred directly to the anode or from the cathode to the bacterial cell through cytochromes membranes or conductive nanowires. Meanwhile, electron transfer mechanism indirectly involves mediators such as *riboflavin* and *derivative phenazine*, transporting electrons between bacterial cells and electrodes (Kato 2016; Kracke et al. 2015; Shi et al. 2016). By modifying the electrode surface the electron transfer and biofilm attachment can be enhanced (Santoro et al. 2017; Wei et al. 2011).

In MFCs, the choice of anode material should also have features such as porous and high surface area to provide more bacterial adhesion sites as well as increase the production of electrons (Mustakeem 2015; Sonawane et al. 2017); high conductivity to facilitate electron flow with lower resistance (Baudler et al. 2015; Yamashita et al. 2016); stability and durability that should be durable and stable either in acidic or alkaline conditions (Peng et al. 2016; Sonawane et al. 2017) and cost effective and readily available for commercial application (Selemboa et al. 2009).

While most previous researchers focus on the use of non-metal or carbon materials such as graphite rods, graphite brushes, graphite granules, carbon cloth, carbon rods and carbon papers, studies have found that these type of anode material has some limitations or deficiencies such as high resistivity, low mechanical strength as well as relatively difficult to apply in large scale (Baudler et al. 2015; Wei et al. 2011). Performance improvements on carbon materials through modifications are also unlikely to involve high costs (Sonawane et al. 2017). In comparison, metal electrodes such as stainless steel, mild steel, nickel, titanium, gold and copper have been considered for use as anodes due to their good corrosion resistance, great mechanical strength and conductivity as well as easily to build-up in large scale (Ledezma et al. 2015; Zheng et al. 2015; Zhu & Logan 2014). However, the major limitation in their applications is low biocompatibility thus limiting the electron transfer and current production. Surface modification with iron oxides or carbon nanoparticles (e.g. activated carbon, graphene and carbon nanotubes) has been proven to be an effective way to enhance the biocompatibility and current generation on metal anodes (Guo et al. 2015; Liu et al. 2017; Peng et al. 2016). It has been demonstrated that iron oxide layer could be *in situ* generated on metal surface through high temperature treatment or flame oxidation. In addition to the above metals, the use of stainless steel as anode in MFC increasing interest among researchers. The stainless steel is often used in various industrial applications including installation of piping, housing and hospitality systems (Sahrani et al. 2008; Sonawane et al. 2017; Wang & Ma 2016). In fact, some BES studies have found that the stainless steel with surface modification showed better performance to other metallic anodes. For example, an MFC anode material comparison conducted by Baudler et al. (2015) between oxidized stainless steel and nickel. Their results show that stainless steel is capable of producing a maximum current density of 6.74 A/m^2 compared to nickel anode (3.84 A/m^2). Considering that there are still few attempts made in comparison between different metal and non-metal electrodes in BESs.

The objective of the present work was to investigate the electrochemical performance of metal and non-metal anodes in MFCs under identical conditions. The modification through heat treatment on anode surface was chosen due to more controllable in terms of temperature, relatively low cost and compatible with large size of electrodes.

Materials and Methods

ELECTRODE PREPARATION AND INOCULUM

Stainless steel (SS) plate, carbon steel (CS) plate, and graphite felt (GF) were each cut into electrode pieces with the size of 4.5 cm × 4.5 cm (thickness of 1.5 and 2.0 mm, respectively). Before heat treatment, the SS, CS and GF electrodes were soaked in 0.1 M HCl for 2 h. Afterward, the electrodes were immersed in ethanol-acetone (50%-50%) solution for 30 min. These chemical soakings are to remove all metal ions and organic adsorbed species before it is washed with distilled water. Heat treatment was done by heating the electrodes at 600°C for 5 min in a muffle furnace and subsequently cooling down to room temperature. The anode systems without heat treatment were our control.

In this study, mud collected from the bottom of Universiti Kebangsaan Malaysia Lake was used as inoculum for microbial energy generation. The mud including the lake water was placed into clean jars with no headspace. The mud then filtered from big particles such as leaves, twigs, and pebbles before use.

ELECTROCHEMICAL CELLS SETUP AND OPERATION

MFC reactor was constructed from two machined pieces of polyacrylic plates, with a final volume of 25 mL. A cation exchange membrane (CEM; CMI-7000, Membrane International Inc.) was fixed between the two inner plates of the MFC reactor to separate the anode from the cathode. Three different anodes, i.e., SS, CS, and GF were used at the anodic compartment. Anodic medium consists of the following: 1.0 g/L CH_3COONa, 4.58 g/L Na_2HPO_4, 2.45 g/L $NaH_2PO_4 * H_2O$, 1.0 g/L yeast extract, 0.31 g/L NH_4Cl, 0.13 g/L KCl, 12.5 mL trace amount of Wolfe's mineral and

5.0 mL vitamin solution. The cathodic compartment of MFC was filled with 100 mM phosphate buffer solution (9.16 g/L Na_2HPO_4, 4.9 g/L NaH_2PO_4 * H_2O, 0.62 g/L NH_4Cl and 0.26 g/L KCl, pH = 7.0). Platinum coated GFs (4.5 cm × 4.5 cm) with a platinum loading of 0.5 mg/cm^2 was used as cathode. The anolyte received a continuous flow of nitrogen gas to maintain anaerobic condition. The MFCs operated under a fed-batch mode condition. Voltage was continuously measured every 300 s under 100 Ω resistor using a multimeter and data acquisition system (Model 2700, Keithley Instruments, Cleveland, OH, USA). pH of the anodic chamber was measured using a Benchtop pH meter (Hanna Instruments, USA).

The effluent pH of anolyte was measured at least five times for each anode. The statistical analysis of *t*-test was applied between the means and standard deviation of three different samples to confirm the same percentage or similar value of the measured anodes.

ELECTROCHEMICAL CHARACTERIZATION

Both power density (P) and current density (*i*), were calculated from the recorded voltage using Ohm's law as follows:

$$i = I/A = V/RA \quad (4)$$

$$P = I \times V/L^2 \quad (5)$$

where V (mV) is the voltage; I (mA) is the current from electrochemical tests; R (Ω) is the external resistance; A (m^2) is the projected surface area of the studied electrode; P (mW/m^2) is the power density; and L (m^2) is the length of the electrode.

Cyclic voltammograms (CVs) were performed *in-situ* using Potentiostat (Metrohm, Netherland) with a scan rate of 10 mV/s in a three-electrode configuration setting. The studied anode, the cathode and the Ag/AgCl were used as the working electrode, the counter electrode, and the reference electrode, respectively. Electrochemical impedance spectroscopy (EIS) was carried out *in-situ* in a two- electrodes configuration setting at 0.1 Hz. The two- electrodes experiment used the anode as the working electrode and the cathode as both the counter and reference electrodes.

RESULTS AND DISCUSSION

MFCS PERFORMANCE

As shown in Figure 1, approximately, a steady-state condition was achieved after day 30 of fed-batch operation, while within 70 days of MFCs operation, MFC-SS and MFC-CS bioanodes obtained the maximum current density of 762.14 and 641.95 mA/m^2, respectively. The results showed that SS performed, approximately 1.2-fold better than CS. The current of SS was higher than CS anode, which might be due to its microstructure as well as the element composition (Guo et al. 2015, 2014; Pocaznoi et al. 2012). Meanwhile, heat treatment on the electrode surface could increase the catalytic activity of microorganism and improve the formation of biofilm on the anode (Guo et al. 2015; Peng et al. 2016; Sonawane et al. 2017). The previous study demonstrated that the heat treatment increased surface iron and oxygen content due to the generation of iron oxide on the electrode surface (Guo et al. 2015, 2014).

In comparison to the carbon-based material, GF produced, approximately 1.05-fold lower current (728.30 mA/m^2) than that of SS anode. The low current could be due to the limitations of the electrocatalytic activity for the microbial reactions because of the growing biofilm clogging the pores or space of the anode (Haque et al. 2015). However, the GF showed higher in current generation compared to that of CS anode.

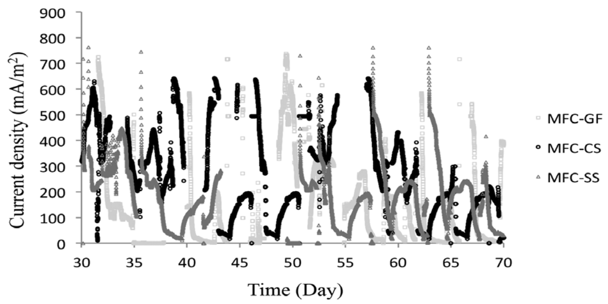

FIGURE 1. Biocatalytic current generation compared between different anode materials: stainless steel (SS), carbon steel (CS) and graphite felt (GF) as anodes

POWER OUTPUT AND POLARIZATION

Maximum power densities up to 827.25 and 260.14 mW/m^2 for the MFC-SS and MFC-CS, respectively, recorded from the metal anodes in comparison to 307.89 mW/m^2 recorded from the MFC-GF (Figure 2). The result demonstrated that the power density of the heat-treated metal of SS is significantly higher (*t*-test, $p < 0.05$) than that of the CS and GF anodes. However, as shown in Table 1, the SS metal in this study showed 1.1-fold lower power production to zinc anode studied by Haque et al. (2015), but much higher than aluminum (3.1-fold) and copper (2.1-fold). In addition, the power density obtained from GF in this study was 3-fold lower than GF anode (Friman et al. 2013) and carbon cloth (Saito et al. 2011) which was 902.0 and 910.0 mW/m^2, respectively. However, due to different reactor configurations, medium composition, microbial inoculum and operation parameters (temperature and potential), it may be not very persuasive to compare our results with the existing reports (Li et al. 2010; Sonawane et al. 2017; Wei et al. 2011). Although the performances of carbon-based reported in the literature were often higher than those with metallic anodes, the metal anode such as SS showed excellent or equivalent results in current density and power generation (Guo et al. 2014; Pocaznoi et al. 2012; Sonawane et al. 2017).

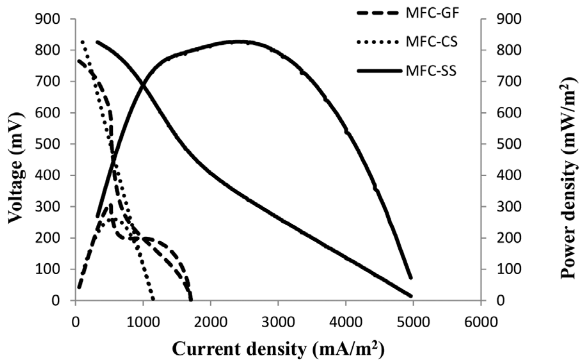

FIGURE 2. Power density and polarization curves

TABLE 1. Anode materials and their performance in MFCs

Anode materials	Inoculum	Current density (mA/m²)	Power density (mW/m² or otherwise stated)	Reference
Non-metal Carbon cloth	Anaerobic sludge	-	3.35	(Mashkour & Rahimnejad 2015)
Carbon cloth	Domestic wastewater	1130	476.0	(Hou et al. 2014)
Carbon cloth	Domestic wastewater	-	910.0	(Saito et al. 2011)
Graphite anode	*Geobacter sulfurreducens*	up to 8000	-	(Dumas et al. 2008)
Graphite anode	Anaerobic sludge	-	0.94	(Mashkour & Rahimnejad 2015)
Graphite anode	*Cupriavidus basilensis*	-	902.0	(Friman et al. 2013)
Graphite felt	Sediment MFC	255.0	127.0	(Haque et al. 2015)
Graphite felt (Heat-treated)	Mud	728.3	307.89	(This study)
Metal CS anode (Heat-treated)	Mud	641.95	260.14	(This study)
SS anode (Heat-treated)	Mud	762.14	827.25	(This study)
SS felt (Heat-treated)	Fresh anodic effluent	Up to 15000	-	(Guo et al. 2015)
SS fiber felt	Domestic wastewater	40	0.80	(Hou et al. 2014)
SS mesh	MFC effluent	-	12.0	(Zhu & Logan 2014)
Stainless steel mesh	Anaerobic sludge from septic tank	1900	-	(Behera & Ghangrekar 2009)

ELECTROCHEMICAL CHARACTERIZATION OF THE ANODE MATERIALS

High power intensity in MFCs depends on electrochemical behaviors and kinetics of the anodes (Hou et al. 2014). CV was performed to examine this behavior after replenishment of the medium. CV technique is well-known as an effective non-destructive for studying the electroactive biofilms (Pocaznoi et al. 2012; Song et al. 2015). The peak-to-peak separations ($\Delta E_p = E_p^{ox} - E_{pr}^{ed}$) calculated from Figure 3 were 0.243 V and 0.179 V for MFC-SS and 0.461 V for MFC-GF. The MFC-CS produced some electron transfer; however the current generated was too low in relative to MF-CSS and MFC-GF. The types of heterogeneous electron transfer rates: reversible, quasi-reversible and irreversible, for a system can be calculated from $\Delta E_p = 2.218RT/nF$, where R=8.3142 J/mol K, T= experimental temperature in K, n= number of electrons involved and F= 96485 sA/mol. Electrochemically reversible process with fast electron transfer will have ΔE_p of 0.057 V, with n =1 at 298 K. Although both the MFC-SS and MFC-GF inherited electrochemically irreversible process, the ΔE_p shows that the MFC-SS was 54% more electrochemically reversible with fast electron transfer than MFC-GF, n = 0.28 and 0.12, respectively. Typically, the current generated during CV is proportional to the concentration gradient at the diffusion layer of the electrode surface flows if the electron transfer is fast enough (Batchelor-Mcauley et al. 2015). The surface area of anodes and flux of the species in electrolyte could contribute to the different performance of voltammetry in this study. CVs recorded at scan rate of 10 mV/s showed that SS metal can provide large current compared to GF non-metal anodes. It may be noted that at high current density sometimes caused by the microstructure of the anode (Pocaznoi et al. 2012). Investigation through physical characterization on carbon anode done by Cui et al. (2014), they found that the power output of MFC depends on the surface morphology. However, this reason was not significant enough to conclude that microstructure may effects on the current production (Pocaznoi et al. 2012). The biofilm grown on the microstructured surface also could be affecting the current densities. Further investigation need to be done in order to determine the role of surface morphology and microbial growth against current output in this study.

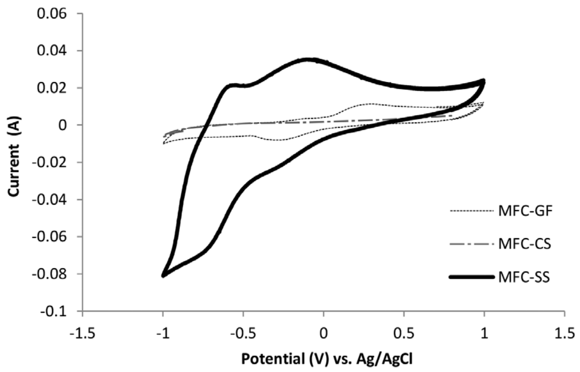

FIGURE 3. Cyclic voltammograms of MFC-CS, MFC-GF and MFC-SS as anodes in the MFCs at the scan rate of 10 mV/s. The electrolyte was 1.0 g/L acetate in 100 mM PBS

Visible oxidation peaks (E_p^{ox}):	Visible reduction peaks (E_p^{red}):
MFC-SS: -0.077 V, -0.547 V	MFC-GF: +0.221 V
MFC-SS: -0.320 V, -0.726 V	MFC-GF: -0.240 V

The electro-catalytic characterization of SS, CS, and GF is measured by EIS, which is an efficient method to explore the interfacial properties of anodes in MFCs. The inset in Figure 4 illustrated the high-frequency part of the result. The diameter of semicircle represents the charge transfer resistance (*Rct*), a straight line following the semicircle, which is affected by the kinetics of the electrode reactions (Liu et al. 2017; Manohar et al. 2008). From the EIS results, the semicircle of MFC-SS was relatively smaller compared to the MFC-GF, followed by MFC-CS, suggesting a lower *Rct* of SS (Liu et al. 2017). Here, the value of *Rct* is indicated by the diameter of the first semicircle in the Nyquist curve. As shown in Figure 4, the *Rct* of MFC-GF was approxiamately 21 Ω MFC-SS and MFC-CS are 16 Ω and 800 Ω, respectively. A smaller *Rct* brings a faster charge-transfer rate from the CE to the electrolyte to enhance the electrocatalytic activities.

The small semicircle and *Rct* observed on MFC-SS as well as MFC-GF implies that microbial community had grown and acclimatized on the anode surface and could use the anode to dispose of utilized electrons, hence faster

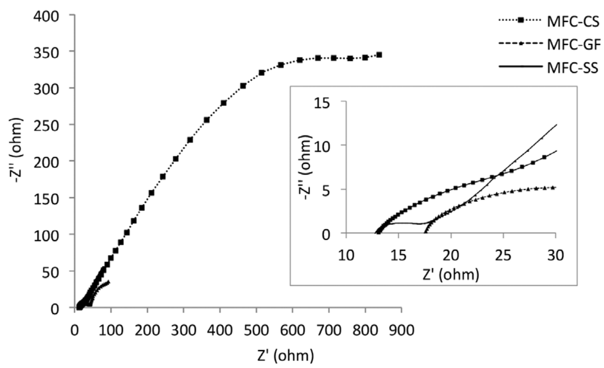

FIGURE 4. Nyquist plots of CS, GF and SS as anodes in the MFCs

reaction kinetics or enhance the electrocatalytic activities (Cheng et al. 2017; Saratale et al. 2017; Strycharz et al. 2011).

pH CHANGE

Anolyte from the reactors were taken at least 5 times for each metal and non-metal anode. As shown in Figure 5, the pH of anolyte ranges from pH7.20 to 6.50 in MFC-SS, pH6.38 to 5.46 in MFC-CS and pH7.04 to 6.40 in MFC-GF. In this study, the pH of anolyte from MFC-CS was found to decrease significantly, approximately 1.2-fold (*t*-test, $p < 0.01$) within eight days of operation, which in turn reduced the performance. pH reduction indicated that anodic environment is acidic (Haque et al. 2015; Zhang et al. 2013). In contrast, the production of high power densities in MFC- SS and MFC-GF at pH between pH6.0 and pH7.0, indicates that the bacterial culture is more resistant to high pH (Behera & Ghangrekar 2009; Mahmood et al. 2017). It was reported that most of the bacteria grow well under neutral or alkaline conditions and pH could be affected by electron transfer kinetics of anodic biofilm (Yuan et al. 2011).

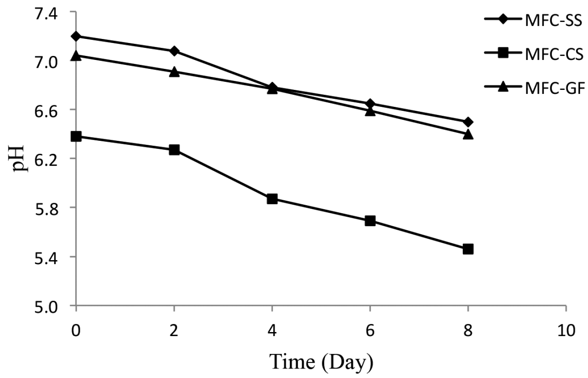

FIGURE 5. pH changes in anode chamber over time of MFC-SS, MFC-CS and MFC-GF

Conclusion

Metals from SS, CS, and non-metal from GF were used as anode materials. These materials were tested and compared to evaluate their performance in MFCs when using mud as inoculums. The MFC with SS anode showed the best electrochemical performance and the highest utilization for electricity output, followed by GF and CS anode. The maximum current density and power density of the MFC-SS were achieved up to 762.14 mA/m^2 and 827.25 mW/m^2, respectively. These electrochemical performances were higher than MFC-CS anodes (641.95 mA/m^2 and 260.14 mW/m^2) and MFC-GF (728.30 mA/m^2 and 307.89 mW/m^2). The results obtained in this work showed that metallic such as stainless steel are able to perform high power output compared to non-metal for the design of microbial bioanodes. However, further detailed on kinetics studies are needed to investigate the electron transfer between metal and carbon-based materials.

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