Bio-anode assisted removal of mercury at the cathode of microbial fuel cells

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Certificate of Examination

This is to certify that the dissertation titled “Bio-anode assisted removal of mercury in the cathode of microbial fuel cells (MFC)” submitted by Mr. Rohit Kumar (MS13133) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Dated: April 24,2019

Declaration

The work presented in this dissertation has been carried out by me under the supervision of Dr. Sunil A. Patil at the Department of Environmental Sciences, Indian Institute of Science Education and Research (IISER) Mohali. This work has not been submitted in part or full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bona fide record of original work done by me and all sources listed within have been detailed in the bibliography.

Date Rohit Kumar

In my capacity as supervisor of the candidate's thesis work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Sunil A. Patil
(Supervisor)

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Abstract

Due to the rapid increase in industrialization and modern life standards, water bodies are getting polluted with several pollutants such as pharmaceutical waste, heavy metals, pathogens leading to toxic effects on the ecosystem and human health. Among these pollutants, heavy metals are considered to be the most hazardous due to their density greater than 4000 kg/m^3. These heavy metals are emitted from various anthropogenic and natural activities and invade the food chains of various life forms including humans. Mercury is one of the major concerns among all the heavy metals due to its high toxicity at lower concentrations and unique bioaccumulation and biomagnification behavior. There are several methods for the removal of heavy metals from the water bodies, but most are not cost-effective and environmentally friendly. The use of Bio-electrochemical systems is an emerging approach for the removal of different types of pollutants including heavy metals. In this study, bio-anode assisted removal of Hg(II) ions at the cathode of microbial fuel cells (MFCs) was tested. An electroactive biofilm (EAB) was developed at the anode of MFCs using chronoamperometry technique at an applied potential of 200 mV. Bioeletcroctalytic current generation and scanning electron microscopy (SEM) imaging of the bioanode confirmed the EAB formation. The maximum power density of 32.5 mW/m^2 and 35 mW/m^2 was obtained with oxygen and Hg(II) electron acceptors, respectively thereby suggesting mercury as the efficient reductant at the cathode of MFCs. In bioanode-assisted mercury removal tests, 98% removal in Hg(II) ions at the cathode was achieved within 24 h. This study thus validates the application of the low-cost bioelectrochemcial approach for the removal of mercury from contaminated environments such as groundwater and freshwater reservoirs.

Chapter 1

Introduction

Water is a basic need for life. On the one hand, the available freshwater sources are rapidly dwindling, and on the other, the water reservoirs such as groundwater and rivers are getting polluted because of various anthropogenic and natural sources or activities. Due to increase in industrialization and modern life standards, different types of hazardous pollutants such as heavy metals and micropollutants (e.g., pesticides, disinfectants, personal care products, and pharmaceuticals) are causing toxic effects to ecology, environment, and human health. Among these heavy metals are considered to be the most hazardous due to their density greater than 4000 kg/m^3 (*Jobin & Namour, 2017*). Most heavy metals are poisonous even in trace amounts of ng/L or µg/L. Such metals as As, Hg, Pb, Cr, Ni, Cd, and Sr are considered to be the major concern to the environments as per the environmental protection agency (*Tchounwou, Heavy Metal Toxicity and the Environment. Molecular, Clinical and Environmental Toxicology, 2012*). These are introduced to different environments through anthropogenic sources such as industries, and thermal power plants (Table 1) (*Sahni, hazardous metals and minerals pollution in india: sources, toxicity and management, 2011*).

Table 1: Major heavy metal contaminants in the environment and their sources

Metal	Sources of emission
Chromium (Cr)	Mining, Industrial coolants, chromium salt manufacturing
Lead (Pb)	Lead acid batteries, coal based thermal power plants, E-waste, ceramics
Mercury (Hg)	Chlor-alkali plants, thermal power plants, hospital waste, thermometers, barometers, electrical appliances

Arsenic (As)	Geogenic/Natural processes, fuel burning, thermal power plant
Copper (Cu)	Mining, Electroplating and Smelting operations
Vanadium (V)	Sulfuric acid plant, Spent catalyst
Nickel (Ni)	Thermal power plants, Smelting operations, battery industry,
Cadmium (Cd)	Zinc smelting, waste batteries, paint sludge, incinerations and fuel combustions
Molybdenum (Mo)	Spent catalyst
Zinc (Zn)	Smelting and Electroplating

Most heavy metals enter into the food chain through bioaccumulation process, which eventually can lead to serious health issues. So, it is necessary to treat the industrial effluents before discharging them into the environment. The maximum contamination level (MCL) of a few toxic heavy metals as per the US environmental protection agency (USEPA), and Indian standards is presented in table 2.

Table 2 : Maximum contamination levels (MCL) of the major heavy metal contaminants as per the US environmental protection agency (USEPA) and Indian standards.

Heavy Metal	MCL(mg/L)(USEPA)	Indian Standards(mg/L)
Arsenic	0.050	0.05
Cadmium	0.01	0.01
Chromium	0.05	0.05
Nickel	0.20	0.05
Lead	0.006	0.1
Mercury	0.00003	0.001

Table 2 suggests that the subtle amount (µg/L to mg/L) of these heavy metals can lead to

a serious threat to living beings. Therefore, it is highly desirable to remove heavy metals from contaminated wastewater, groundwater or any other sites to diminish their effects to various life forms and the environment (*Tchounwou, Yedjou, Patlolla, & Sutton, 2013*). Various approaches and technologies based on physical, chemical and biological processes have been explored and tested for the remediation of heavy metal contaminated environments (*Liu, Li, Song, & Guo, 2018*). An overview of different methods along with their advantages and limitations is presented in Table 3.

Table 3 : Advantages and disadvantages of the techniques that are used for the heavy metal removal from the soil, wastewaters and groundwater environments.

Technology/Method	**Advantages**	**Disadvantages**
Physical - Mechanical Seperation - Electrokinetic remediation	Can remove different types of metals	- Only applicable for homogenous distribution of pollutants
Chemical - Soil washing - Soil flushing	Very efficient in removing heavy metals	-This method is very costly and there are chances of production of more toxic compounds as a waste while removing heavy metals

Biological	This process is environmental friendly as microorganisms are used for the immobilization or removal of heavy metals	- It is time consuming and construction of special installations (Growth media, pH,temperaturare required. - Large amount of waste is generated. - Long term monitoring required - Affected by the availability of nutirents and electron donor/acceptor conditions.
Phytoremediation	- Environmental friendly - Low cost - No side effect	- Contaminants are not removed from the soil, only immobilized. - Long term monitoring
Nanotechnological	Very efficient for removing various metals	- Costly - Still under development?*?
Electrochemical	Very efficient for removing several active forms of metals	- Use of additional chemicals - External power source is required - Costly

Because of a few critical disadvantages associated with each technique, it is of prime importance to develop an alternative environment-friendly, cost-effective and sustainable approach for the removal of heavy metals from contaminated environments such as groundwater.

1.1. Mercury: sources, chemistry, toxicity and cycling

According to Table 2, it is clear that mercury has a very low permissible limit of about 1 µg/L for discharge into the environment (Indian standards). The agency for Toxic Substances and Disease Registry (ATSDR) has designated mercury as one of the "priority hazardous substances" as it is the most toxic metal, having very high mobility

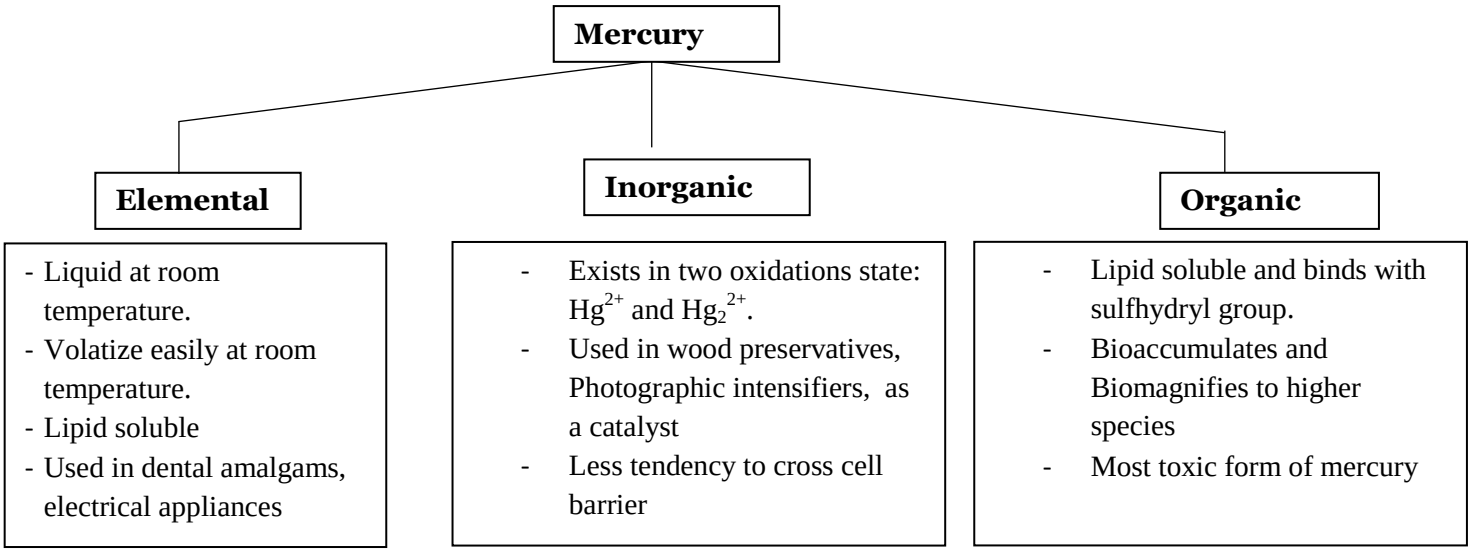
and the more persistency in the environment (Rai, 2008a). Mercury occurs naturally in the environment, and due to several anthropogenic sources, such as fossil fuels combustion, mining it gets invaded into the atmosphere and risks human life. The main sources of mercury emissions are thermal power plants, chloralkali industries, hospital wastes, thermometers, and barometers industries. Table 4 summarizes the different sources and the amount of mercury usage in tonnes. The Chlor-alkali industries are one of the main sources of mercury emissions in the atmosphere.

Table 4 : Different sources that uses mercury and their mercury usage quantity

Source/Industry	Mercury usage (tonnes)
Chlor-alkali Industry	10
Thermometers	7.2
Mercury zinc	25
Fluorescent lamps	7.89
Thermostat switches	18.23
Alarm clocks	0.96
Hearing aids	0.04

In nature, mercury exists in its three different oxidation states as shown in figure 1, out of which organic mercury is considered to be the most toxic form.

Figure 1: Three different forms of mercury present in environment.



Exposure to methyl mercury ($[CH_3Hg]^+$) affects the immune system, alters genetic and enzyme systems, and damages the nervous system, including coordination and the senses of touch, taste, and sight *(Rice, Walker, Wu, Gillette, & Blough, 2014*). Short-term exposures to elemental mercury can lead to anxiety, sleeping problems, eye irritation, and increase in blood pressure, anorexia and many more health issues (*www.who.int/news-room/fact-sheets/detail/mercury-and-health*).

The biogeochemical cycling of mercury in the environment is illustrated in figure 2 (*Barkay, Kroer, & Poulain, 2011*). Elemental mercury gets oxidised to its highly oxidized form Hg(II) due to photochemical reaction and reaches to the earth surface through dust particles and rain. It is also the most active form and central to the mercury cycle. In aquatic systems, presence of anaerobic sulphate reducing and iron reducing bacteria can methylate oxidized form of mercury to methyl mercury. One strain of sulphate-reducing bacteria, *Desulfovibrio desulphuricans* ND132, and iron reducing bacterium, *Geobacter* can also methylate elemental mercury*(Rani, Rockne, Drummond, Al-Hinai, & Ranjan, 2015*). There are certain anaerobic microorganisms that methylate Hg(II) to neurotoxic methylmercury, $[CH3Hg]^+$, but the fundamental mechanisms involved in this process remains poorly understood. To our present knowledge, sulphate- or iron-reducing *Deltaproteobacteria* are known to methylate Hg(II) to methyl mercury. Recently, an organism outside the *Deltaproteobacteria* has been predicted to generate $[CH3Hg]^+$ *(Kerin et al., 2006*).

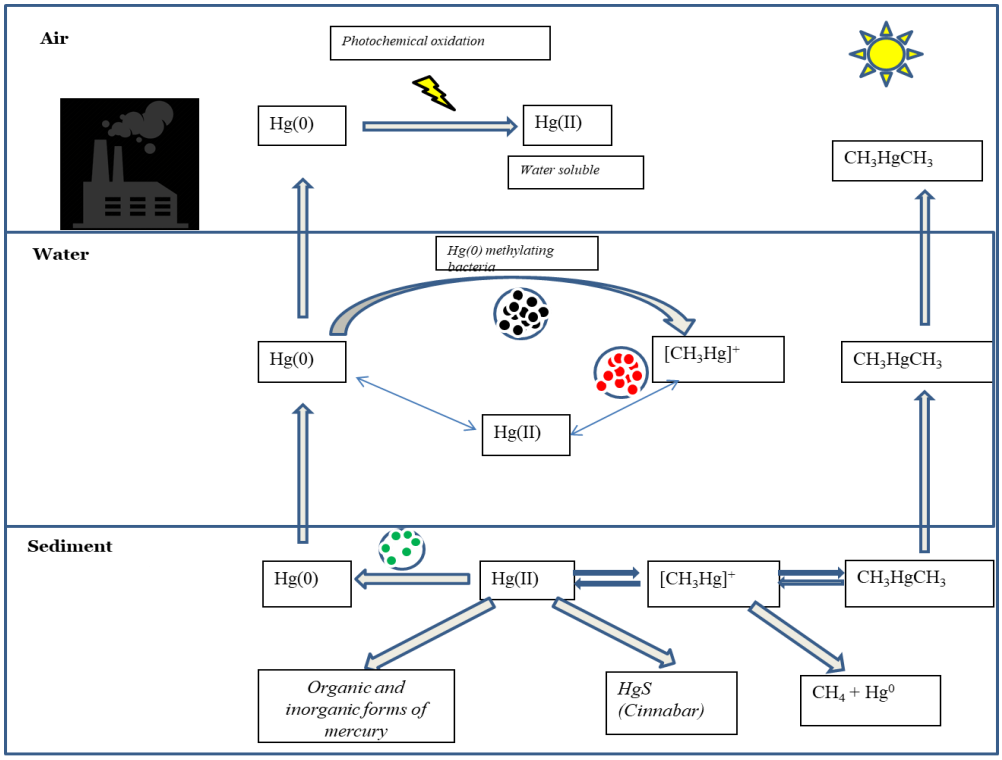


Figure 2: Bio-geochemical cycling of mercury in the environment.

Through water and air, this cycle of mercury continues in the environment and enters the food chain of humans leading to various serious threats. There are many sites in India that are affected by mercury pollution. In Haryana, for instance, the most polluted sites include nearby regions of Panipat thermal power plant (Jind Road, Village Assan, Panipat, Haryana 132105) with 268 µg/L and Singrauli (UP) with 182 µg/L mercury concentration (*Mukherjee et al., 2009*). The high contamination of mercury in the groundwater of villages nearby Panipat thermal power plant has led to the many health issues, such as cancer. Hence, many villages had been vacated because of the groundwater contamination by mercury (*https://www.tribuneindia.com/2011/20111121/haryana.htm#6*).

1.2 Mercury removal technologies

Mercury is a metal with chemical similarities to zinc and cadmium. The metal is liquid at

room temperature, with a freezing point at –31°C (http://www.pollutionissues.com/Li-Na/Mercury.html) and is not soluble in water at room temperature; however, it is soluble in acids at high temperature. Its solubility dependents on the pH, temperature and the oxidation state in which it is present. All these properties of mercury make it hard to remove from wastes or water at neutral conditions. Researchers have developed several mercury remediation technologies such as solidification and stabilization, soil washing and acid extraction, thermal treatment, precipitation, and membrane processes(USEPA, Treatment Technologies For Mercury in Soil, Waste, and Water, 2007). These processes are well studied and efficient in removing mercury from the soil, water, and sludge. However, there are several pros and cons associated with these technologies. The main disadvantage of all the above-mentioned techniques is that they are not environment-friendly and also not cost effective. There is the usage of additional chemicals for solubilisation, precipitation, and extraction of mercury from waste since it is insoluble in water. Biological treatment of mercury-contaminated waste is an environment-friendly process as microorganisms play a vital role in the removal of different types of organic environmental pollutants. Some strains of Pseudomonas bacteria are capable of converting Hg (II) to elemental mercury (Gu et al., Nature Geoscience, 2013). The main disadvantage with this process is that an optimum pH and growth conditions should be maintained to enhance the microbial activity. The high concentration of mercury in the waste can also inhibit microbial activity. There are several other bench-scale technologies such as nano-technology, in-situ thermal desorption, and phytoremediation, which are cost-effective and reliable alternatives for mercury treatment. Nanotechnology approach uses a synthesized thiol-based absorbent which can bind to the ionic species of mercury and can enhance the removal of mercury from aqueous medium. However, the synthesis of this absorbent is an energy-intensive process (USEPA, Treatment Technologies For Mercury in Soil, Waste, and Water, 2007). Phytoremediation can be considered as an environment friendly approach for remediation of heavy metals. The problem associated with plants is that they are immobile and mercury removal can occur within specific and limited sites. Apart from being a slower process, the removal of immobilized metals via

phytoremediation is also costly. Therefore, it is imperative to develop a cost-effective, sustainable and environment friendly alternative approach for the remediation of mercury-contaminated environments.

Bio-electrochemical systems (BES) are one of the emerging technologies for remediation of heavy metals from wastewater. Bio-electrochemical systems can be defined as the integration of microbial processes with electrochemical systems (*Rabaey, Bioelectrochemical systems: from extracellular electron transfer to the biotechnological application, 2010).* If we consider a basic electrochemical cell, it consists of anode and cathode chambers. At anode oxidation of substrate takes place and at cathode reduction of metals takes place. In BES, either of or both the anodic and cathodic reactions can be catalysed by microbial activities. BES can be operated in two different modes: either in a microbial fuel cell (MFC) or in a microbial electrolysis cell (MEC) mode. MFCs produce energy whereas MECs consume energy to facilitate the desired reaction. An MFC can be defined as a system in which microorganisms acts as catalysts to convert chemical energy into electrical energy. A general schematic of an MFC setup is shown in figure 3.

The MFC technology has gained widespread attention for the treatment of organic matter containing wastewaters as well as for the removal of other pollutants (*Franks & Nevin, 2010*). Oxygen reduction is the most common reaction at the cathode of MFCs. It also offers the possibility to link the substrate oxidation reaction at the anode to the reduction of metals at the cathode. The microorganisms present at the anode will oxidize the substrate such as acetate and produce CO_2, $H^{+,}$ and electrons (*Mathuriya and Yakhmi, 2014*). These exoelectrogens present at the anode forms an electroactive biofilm on the anode and uses extracellular electron transfer mechanisms for transfer of electrons to the anode (*Kumar et al., 2016, Nancharaiah et al., 2015).* These electrons then move to the cathode of MFC where metals can act as the sole electron acceptor.

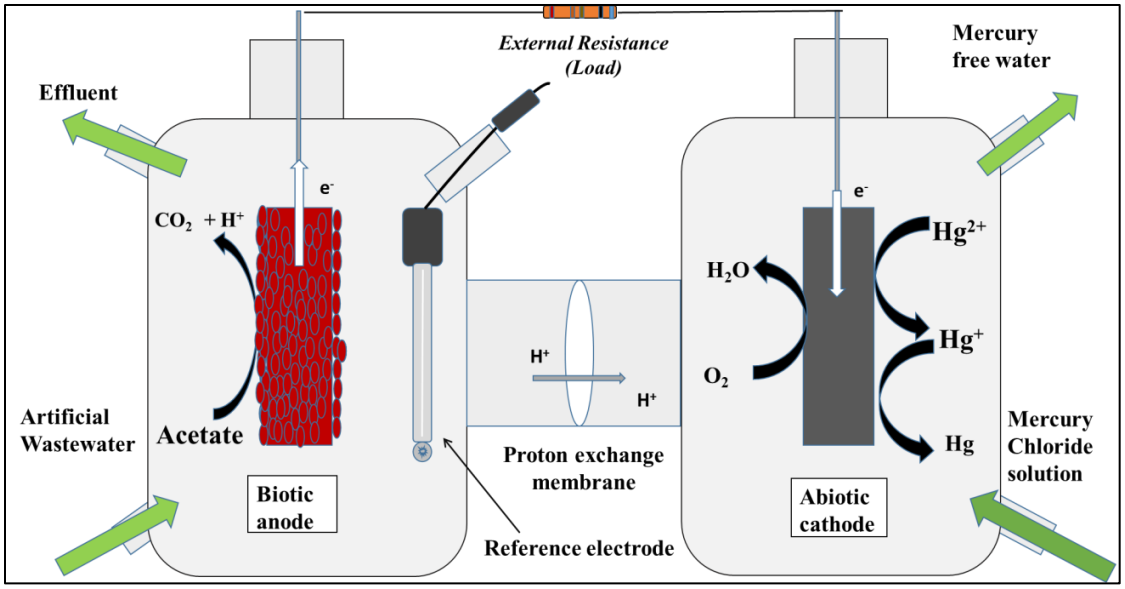


Figure 3: Schematic representation of a microbial fuel cell. Acetate oxidtaion recation at anode is linked to two different exemplary cathodic reactions Viz., O_2 and metal reduction.

Representative reactions that can occur at the anode and cathode of MFCs are described below.

Anode: $CH_3COONa + H_2O \longrightarrow CO_2 + 7H^+ + 8e^-$ $E^0_{ox} = -0.280$ V vs SHE*

Cathode: $O_2 + 2H^+ + 2e^- \longrightarrow H_2O$ $E^0_{cell} = 0.82$ V vs SHE

$M^{n+} + re^- \longrightarrow M^{(n-r)+}$ (The reduction potential is varied for different metals)

*(SHE: Standard hydrogen electrode)

These type of systems are being explored for remediating different types of heavy metals such as Arsenic, Chromium, Selenium, Lead, and vanadium (*Mathuriya & Yakhmi, 2014*). For instance, hexavalent chromium reduction to trivalent chromium with a maximum power density of 150 mW/m^2 and removal percentage of 80 % was achieved using MFC (*Wang et al. 2008*). In the case of silver, recovery efficiency of 99.91 % was achieved with initial concentrations varying from 50-200 ppm and a maximum power

density of 4.25 W/m^2 (*Choi and Cui, 2012*). There are studies available on the removal of different types of heavy metals using bioelectrochemical systems(*Nancharaiah, Venkata Mohan, & Lens, 2015).* However, despite its high toxicity, mercury removal has not been tested extensively. The reasons may be the reduction potential of Hg(II) to elemental mercury is +0.85 V vs. SHE which is very close to the water oxidation potential of about +0.82 V vs. SHE. Only one study on mercury removal using MFCs has been reported. It demonstrated removal of 100 mg/L Hg(II) at pH 2 with a maximum power density of 433.1 mW/m^2. In this study, the authors reported the removal of Hg(II) ions at different pH (2, 3, 5) conditions and found pH 2 as the best condition. No experimental data was shown for the redox potential of Hg(II) reduction at the cathode as well as the possible mechanisms involved in mercury removal (*Wang, Lim, & Choi, 2011).* In this work, we tested the bio-anode assisted removal of mercury at the cathode of MFCs to understand the mechanisms of mercury removal and to validate the bioelectrochemical approach.

Chapter 2

Materials and Methods

2.1 Materials

All chemicals (such as H2SO4, Chloroform, Acetic acid, Dithizone, Trace metals components, Vitamins components, M9 buffer media components) were purchased from Sigma-Aldrich (Now Merck). Media preparation and all experiments were conducted using anaerobic culturing techniques. Replenishment of media was performed under N2 conditions. The tubing and connections were assembled in a manner to avoid oxygen intrusion, and that allowed easy medium replenishments (*Patil, Arends, Vanwonterghem, & Meerbergen, n.d.*).

The experiemnts were conducted in three steps as elaborated below.

2.2 Development of the Electroactive biofilm at the anode of BES

2.2.1 BES configuration and operation

A three-electrode configuration in two-chambered (500 mL each) H-shape BES reactors were used for the development of microbial electroactive biofilm at the anode. Graphite electrodes with a projected surface area 38 cm^2 were used as the cathode (counter electrode), an anode (working electrode) and a Reference electrode in BESs as shown in figure 4. In order to monitor the potential of individual electrodes, a reference electrode (Ag/AgCl, 3.5 M KCl) was placed in the anode chamber of BES. Two chambers were separated with a proton exchange membrane (Nafion 117 membrane, Sigma- Aldrich).

Growth medium containing trace metals, vitamins and modified M9 buffer (pH-7) was used in the anode chamber of the BES reactor. The composition of the growth medium is described in the tables 2.1-2.3(*Patil, Harnisch, Kapadnis, & Schröder, 2010*). Sodium acetate (10 mM) was used as the electron and carbon source for the microbes. Only buffer medium without any trace metals, vitamins and substrate was used as the catholyte.

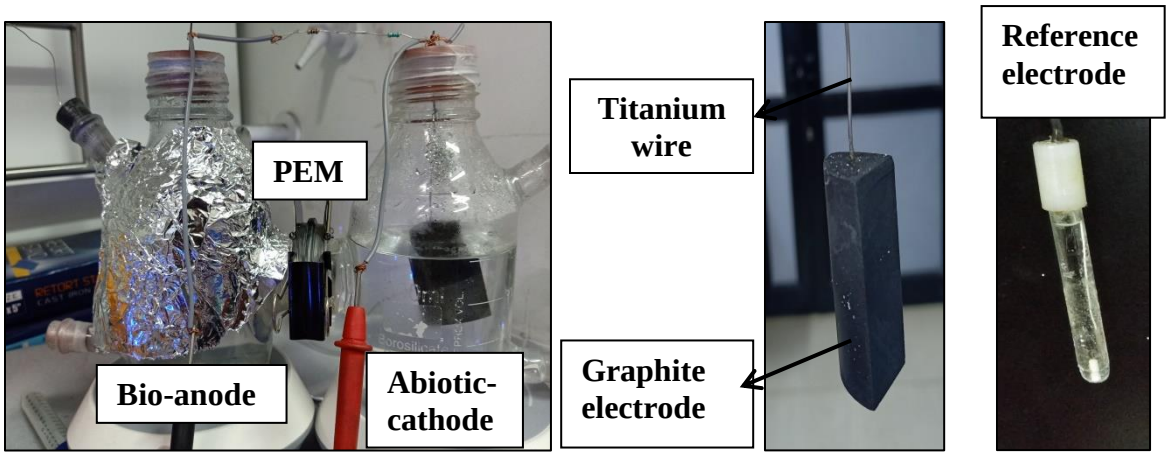


Figure 4: Images of H-shaped BES reactor along with the graphite and Ag/AgCl reference electrodes

All microbial growth experiments were performed under the anaerobic conditions. In order to develop or grow the electroactive biofilm at the anode, an external potential of +200 mV (vs. Ag/AgCl) was applied at the working electrode using chronoamperometry (CA) technique. CA is a powerful technique to observe the rate of change of current concerning time. This also allows monitoring the biofilm growth at anode based on the substrate oxidation current by the microbial activity. The iron-oxidizing reactions in nature occur at the redox potential of 189 mV (vs. SHE) under standard conditions(*Weber, Achenbach, & Coates, 2006*). Therefore, in order to mimic the indigenous environment for the microbes 200 mV potential was set at the working electrode. The electroactive microorganisms use extracellular electron transfer mechanism to interact with the final terminal electron acceptor which is a graphite electrode in this case in the anode chamber (Patil et al., 2012). The bioelectrochemical experiments were conducted in a fed-batch mode by replenishing the spent medium with the fresh growth medium after each batch cycle. Triplicate reactors (hereafter referred to as R1, R2, and R3) were set up for the development of the anodic electroactive biofilms.

Table 5 : Trace elements used in M9 medium

Trace elements	Concentration (g/L)
Nitriloacetic acid	1.5
$FeCl_2$	0.1
$CoCl_2$	0.1
$ZnCl_2$	0.1
$CaCl_2.2H_2O$	0.1
NaCl	1
$MgCl_2.6H_2O$	3
$Na_2MoO_4.6H_2O$	0.01

Table 6 : Vitamins used in M9 medium

Vitamins components	Concentration (mg/L)
Biotin	10
Folic acid	4
Pyridoxine HCl	20
B_{12}	10
Riboflavin	10
Pentomenate	10
p-amino benzoic acid	10
Cyano cobalamine	0.2
Lipoic acid	10
Nicotnic acid	0.5

Table 7 : The composition of modified M9 buffer media components

Modified M9 buffer media components	Concentration (g/L)
NH_4Cl	0.31
KCl	0.13
Na_2HPO_4	4.33
NaH_2PO_4	2.69
Trcae elements	10 mL
Vitamins	10 mL

2.3 Polarization and power density curves of MFC

After the development of electroactive biofilm at anode through several CA cycles, BESs were operated in the MFC mode.

2.3.1 Polarization and power density curves with O_2 as the electron acceptor at the cathode

Polarization test is a powerful tool for the analysis and characterization of microbial fuel cells and is used to determine the cell design point. Polarization curve is expressed in terms of voltage as a function of current density. In order to determine the maximum performance of MFCs, the polarization tests were conducted initially with O2 as the electron acceptor at the cathode. Initially, the open circuit voltage (OCV) of the MFC was monitored to estimate the maximum voltage output of the system at an initial zero current flow. To increase the cell voltage, the electrolyte in the cathode chamber was continuously aerated. The polarization tests were conducted by the varying resistance method. The external resistances varying from 100 kΩ to 10 Ω were used. The potential

of anode and cathode was monitored using a Ag/AgCl reference electrode. The current and potential values were recorded only after achieving the steady-state conditions which took several minutes to hours depending on the type of resistance connected. The pseudo-steady-state conditions were temporary and were due to depletion of the substrate at the anode. Polarization curves are generally divided into three zones: (i) starting from the OCV at zero current, there is an initial steep decrease of the voltage: in this zone the activation losses are dominant; (ii) the voltage then falls more slowly and the voltage drop is fairly linear with current: in this zone the ohmic losses are dominant; (iii) there is a rapid fall of the voltage at higher currents: in this zone the concentration losses (mass transport effects) are dominant (*Logan et al., 2006*).

Power curves represent power density as a function of current density that can be calculated from the polarization curve. There was no current flowing through the system when the MFC was operated in an open circuit mode. Since power is directly proportional to the current of the system, so no current means no power. As the external resistances varied from high (100 KΩ) to low (10 KΩ), power also varied accordingly and reached to a maximum value known as a maximum power point (MPP) at a particular external resistance. Beyond this MPP there was a drop in the power due to increasing ohmic losses and electrode overpotentials*(Wales, 2016*). Ohmic losses play an important role in attaining the maximum power point as the ionic conductivity of substrate solution changes with time.

With the help of the polarization curve, we can calculate the internal resistance of the system. The slope of the polarization curve represents the internal resistance of the MFC setup.

$$R_{int} = - dE/dI$$

Where R_{int} is the internal resistance of the system, E represents electric potential and I represent electric current in the system.

2.3.2 Polarization and power density curve with Hg (II) as the electron acceptor at the cathode

The polarization and power density curves of MFCs were then obtained with Hg (II) as the electron acceptor at the cathode. It was used at a concentration of 10 mg/L prepared in NaCl electrolyte. In order to rule out the possibility of oxygen as an electron acceptor in the cathode, anaerobic conditions were maintained in the cathode chamber of MFCs. The MFCs with Hg(II) were first operated in the open circuit voltage (OCV) mode to determine its performance in comparison to the MFCs with O2 electron acceptor After achieving the pseudo-steady state conditions, the polarization test with varying external resistors (100 KΩ to 10 Ω) was conducted. The cell voltage was measured using a multimeter (179 true RMS). The potential of individual electrodes was measured using 179 true RMS multimeter with respect to Ag/AgCl reference electrode. The voltage readings were noted after attaining the pseudo-steady state conditions.

2.4 Hg(II) removal tests in MFC

After successfully developing the electroactive biofilm at the anode, and conducting the polarization tests of MFCs Hg (II) removal tests in the cathode chamber of MFC were conducted. The external circuit was closed with the external resistance of 560 Ω (It was determined through the polarization tests). For these tests, non-limiting supply of substrate (20 mM sodium acetate) along with vitamins and trace metals was ensured to the electroactive biofilm grown at the anode and $HgCl_2$ solution with Hg(II) concentration of 10 mg/L and 1.5 g/L NaCl as an electrolyte was used in the cathode chamber of MFC. The initial catholyte pH was recorded around 2.25. With each MFC reactor, the mercury removal tests were conducted for at least three times to check the reproducibility and validate the performance of these systems. As a prelude to our main experiments, two control experiments were conducted as discussed below.

2.4.1 Control experiment 1 (Unconnected & biotic)

In this control experiment, the possibility of Hg (II) reduction in an open circuit mode (no external resistance connected) was checked. The anode chamber was kept biotic and anaerobic while the cathode chamber was kept anaerobic with 10 mg/L $HgCl_2$ and NaCl

electrolyte as shown in figure 5. Since we have kept our MFC setup unconnected. So, the electron transfer path is closed leading to no current generation in our system. This experiment was performed to elucidate how much Hg(II) ions are removed due to physical adsorption at the graphite cathode.

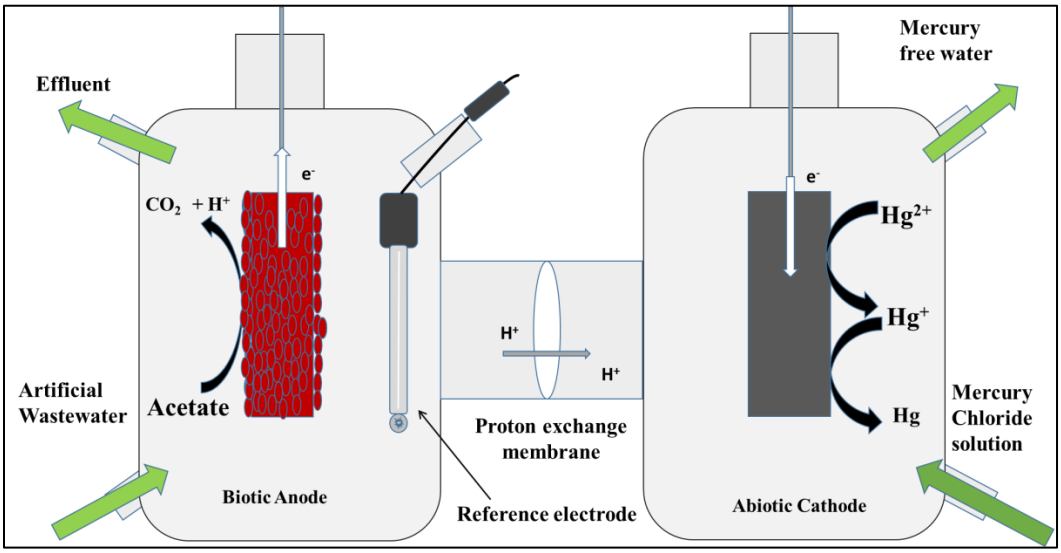


Figure 5: A Schematic of control experiment 1

2.4.2 Control experiment 2 (Connected & abiotic)

Through this control experiment 2, we have investigated the role of biofilm produced reducing power or electrons in reducing Hg(II) at the cathode of MFC. In this case, both anode and cathode were abiotic but connected externally with resistance as shown in figure 6. These two control experiments ruled out the possibility of the factors that can reduce Hg (II) except for the microbial bioanode-assisted electrochemical reduction process. In the main experiment, MFCs with the biotic anode and abiotic cathode were operated.

In all cases, cell voltage, anodic potential, cathodic potential, pH and Hg (II) concentrations were monitored after a defined interval of 4 hours. To measure pH, Oakton PC 2700 pH/conductivity meter was used.

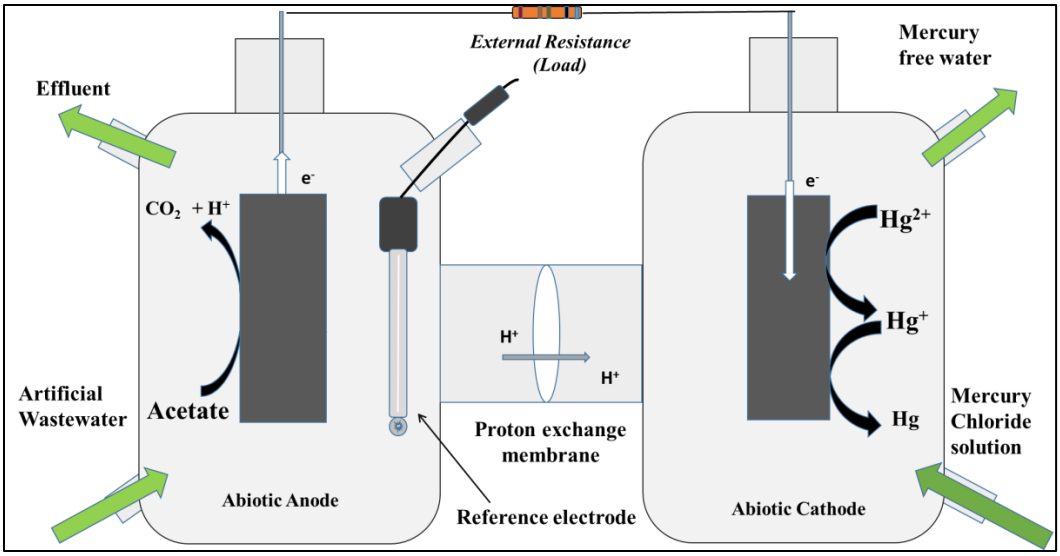


Figure 6: **A schematic of control experiment 2**

2.5 Analysis

2.5.1 Diphenylthiocarbazone (Dithizone) a spectrophotometric detection method for Hg (II) and Hg (I) ions in environmental samples

In order to analyze the Hg (II) concentration in the cathode after a defined interval of 4 hours, we used dithizone, which is a spectrophotometric method for detection of ionic species of Hg (*Elly et al., Water Pollution Control Federation, 1973*). The UV-Vis spectrophotometer was used to measure the absorbance of Hg (II)-Dz orange colored complex at 500 nm.

1,5-diphenylthiocarbazone (dithizone) is one of the most widely used photometric reagent complexes. Mercury dithizonate complex is a photochromic compound and is stable form. Since metal–dithizone complexes are water-insoluble, therefore, their determination requires a prior solvent extraction step that was carried out by using chloroform (CCl4), followed by spectrophotometric measurements. The dithizone-Hg (II) complex is orange colored, and the chemical bonding between these compounds is shown in figure 7.

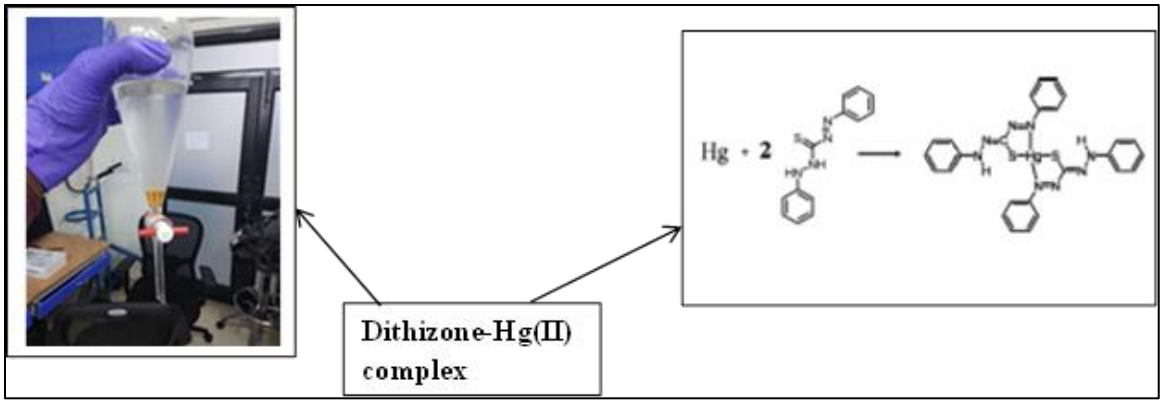


Figure 7: Dithizone-Hg (II) complex formation in the lab and the chemical reaction

Dithizone-Hg (II) complex can exist in two different photoisomers, one is an orange form which is stable form, and the other one is photoactivated and blue as shown in figure 8. There is equilibrium between these two forms, and they can be interconverted in the presence of light. The chemical bonding of dithizone to Hg (II) changes as blue form converts into the orange one and vice versa. Since sulphur forms a strong covalent bonding with mercury which makes the orange form more stable. The stoichiometry of the Hg (II)–dithizone complex or metal to ligand ratio was found to be 1: 2 using mole ratio and continuous variation method (Delaire et al., Photochem. Photobiol. Sci., 2003).

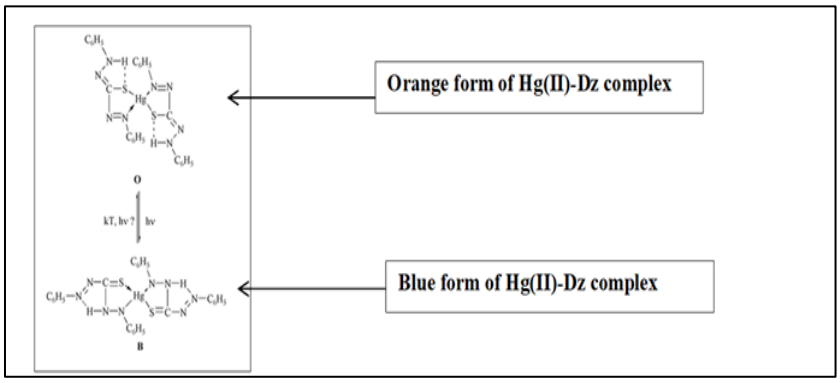


Figure 8: Dithizone-Hg (II) complex and its two different photoisomers.

2.5.2 Scanning Electron Microscopy (SEM) for analysing anodic biofilm

A scanning electron microscope (SEM) is a technique that uses electrons beam instead of light on the surface, and the emitted electrons are captured to develop an image. These emitted electrons interact with the sample, producing various signals which give us the information about the surface topography and composition of the specimen. In order to capture the morphology of the anodic electroactive biofilm, we have used SEM technique (*Cho et al., Sustainable Chemistry and Engineering, 2013*).

2.5.2.1 Pre-treatment of EAB for SEM

In order to fix the enriched biofilm, first, it was recovered from the reactor under completely anaerobic conditions (Don Whitley A37 Anaerobic workstation). The biofilm was fixed using a mixture of 2.5 % (v/v) glutaraldehyde and 2 % (v/v) paraformaldehyde. This mixture was kept undisturbed overnight at 4^{o}C. Post-fixation of the EAB was performed using osmium tetroxide solution (containing 3 mL of 0.2 M sodium phosphate buffer, 3 mL of 2 % OsO_4 and 3 mL of distilled water) and this solution was kept undisturbed for 90 minutes. Further, the EAB was dehydrated and washed using ethanol with different concentrations (50 %, 70 %, 80 %, and 90 %) with successive 10 minutes incubation. The incubation time was increased to 20 minutes for 20 % for 95 % and 100 % ethanol concentration. After dehydration, the EAB samples were dried overnight. To increase the conductivity of EAB surface, gold (Au) sputtering was done at 20 mA current for 45 seconds using JEOL-JEC-1600 Autofine coater (*Cho et al., Sustainable Chemistry and Engineering, 2013*).

2.5.2.2 SEM imaging

After pre-treatment of EAB, it was examined using JSM 6610LV at 10 KV with a magnification of 2 µm, and the SEM images were captured.

Chapter 3

Results and Discussion

3.1 Development of electroactive biofilm (EAB) on anode

EAB was developed using chronoamperometry (CA) at an applied potential of 200 mV. The CA cycles were run for 26 days, and the media was replenished after every 5-6 days.

3.1.1 Chronoamperometry (CA) cycle

Figure 9 represents the CA cycles for EAB development. Initially, there was an increase in the current because of the substrate (acetate) combustion by the microorganisms which is known as a turnover condition. When the substrate combustion was at the maximum and maximum no. of electron production was taking place and known as the maximum turnover state. The drop in the current after maximum turnover condition arises because of the lack of availability of acetate to the microbes. We have replenished the media with a fresh one before current falls to zero and then increase in the current is shown in the CA cycles. Such cycles were continued until the maximum current remained at the same level into subsequent cycles. The bioelectrocatalytic current density was (0.25 ± 0.031) mA/cm^2, (0.20 ± 0.0138) mA/cm^2 and (0.22 ± 0.058) mA/cm^2 for reactor 1, 2 and 3 respectively. Replenishment of media was performed under the complete anaerobic conditions (by purging N_2).

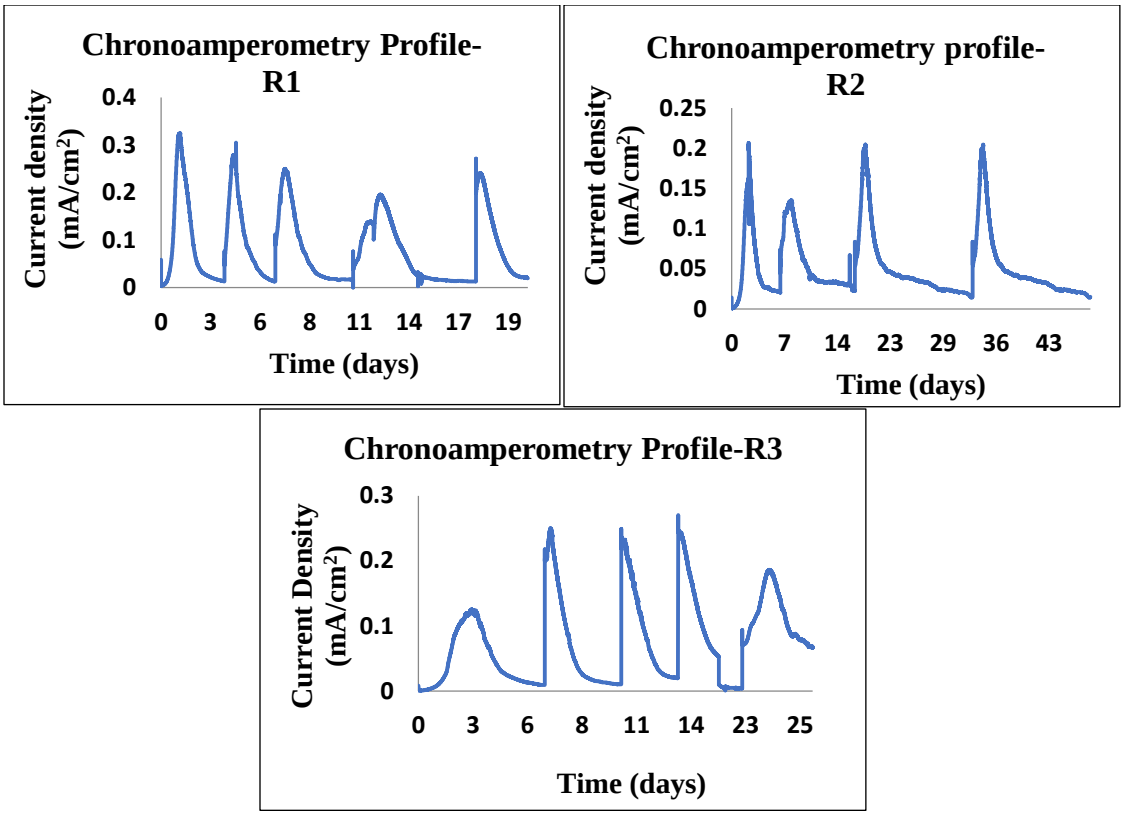


Figure 9: Chronoamperometry profiles showing the bioelectrocatalytic current generation by the EAB growing at the anode of three reactors (R1 to R3).

3.1.2 SEM Imaging

SEM imaging of the anode surfaces showed growth of EAB with clear rod-shaped bacteria

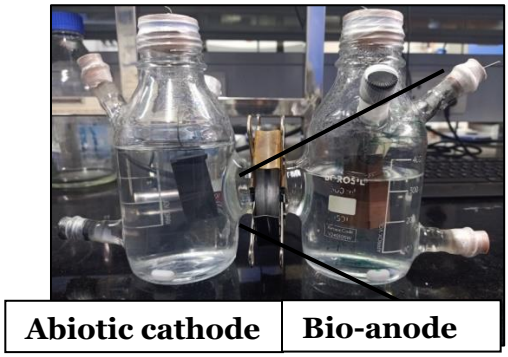


Figure10: **Brownish EAB at anode of MFC**

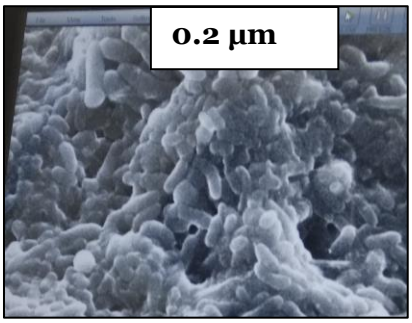


Figure 11: SEM imaging of the EAB

The reactor setup clearly showed the reddish-brown biofilm at the anode. Figures 10 & 11 shows EAB at the anode and SEM imaging of the biofilm, respectively.

Most likely the enriched biofilm is dominated by *Geobacter sp.* under the used experimental conditions in this study (*Harnisch et al., Energy and Environmental Science, 2011*), *(Chen et al., 2011*)**.**

3.2 Polarization and power density curves

3.2.1 Open circuit curve (OCV) with and without Hg (II)

The OCV monitoring suggests the maximum voltage attained by the MFC setup in an open circuit mode. Figure 12 represents the OCV plot with O2 at the cathode of R1 MFC. Initially, no artificial aeration was provided to the system. So, the maximum OCV attained was 300 mV. However, after artificial aeration (using air pumps) system showed an increase in the maximum voltage because of the increase in the electron acceptor (O2) conditions. The maximum voltage reached 572 mV after aeration thereby highlighting the importance of the availability of electron acceptor at the cathode of MFCs.

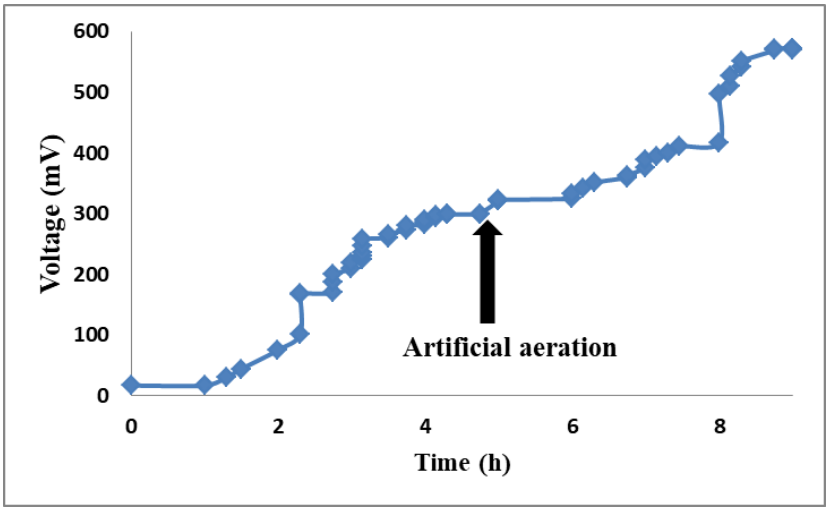


Figure 12: OCV for the MFC with O_2 as an electron acceptor at cathode.

Figure 13 represents the OCV plot for MFC with Hg (II) as an electron acceptor. The maximum voltage of 760 mV was attained in this case. It suggests that mercury ions

acted as better electron acceptor as compared to O_2.

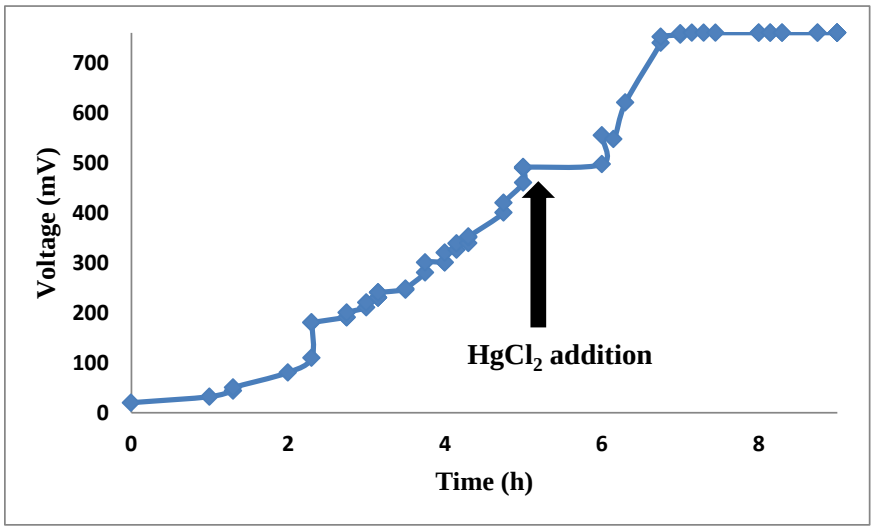


Figure 13: OCV plot for the MFC with Hg (II) as an electron acceptor at cathode.

3.3 Polarization and power density curves with O_2 and Hg (II) conditions

The polarization and power density curves for the MFC setup with O_2 as an electron acceptor in the cathode of MFC with NaCl electrolyte are shown in Figure 14.

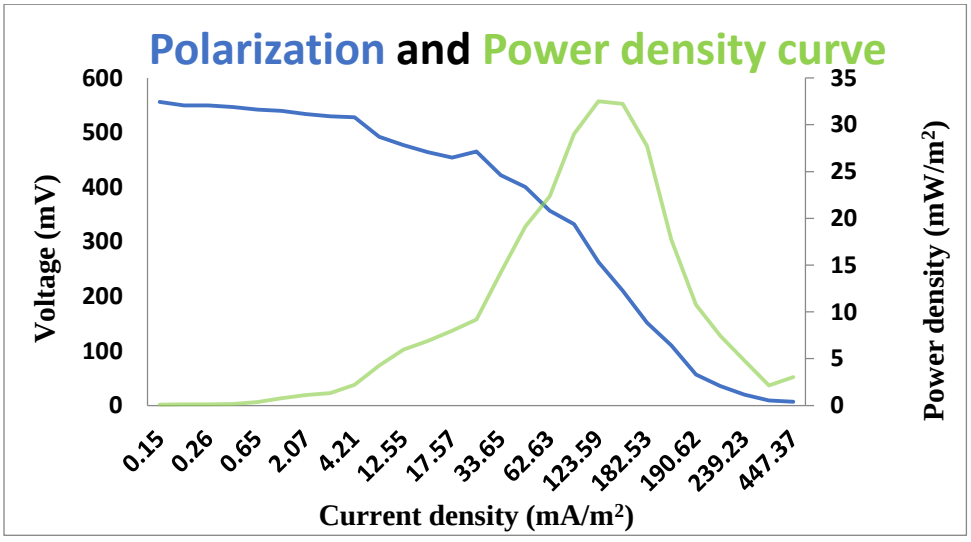


Figure 14: Polarization and power density curve of MFC with O_2 at cathode.

Typical polarization and power density curves were obtained.Similarly, we have generated polarization and power density curve for the MFC setup with 10 mg/L $HgCl_2$ and NaCl (1.5g/L) in the cathode of MFC as shown in figure 15.

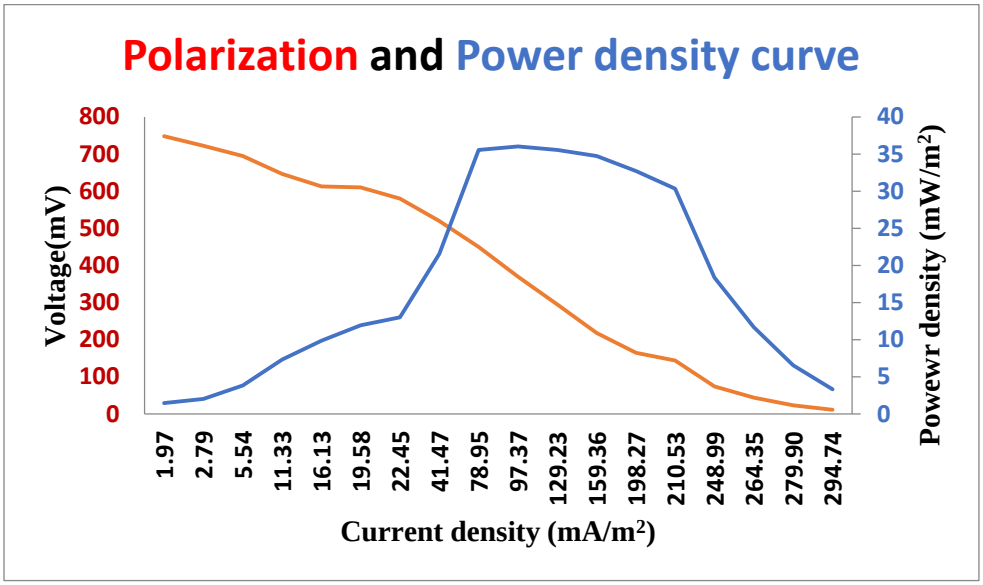


Figure 15: Power density and polarization curve of MFC with Hg (II) at cathode.

The internal resistance was calculated from the slope of the polarization curve and found to be 560 Ω. The maximum power density obtained in this case was found to be 35 mW/m^2.

3.4 Hg (II) ions removal tests in MFC

The removal of mercuric ions in the cathode of R1 MFC was studied. HgCl2 (with an initial concentration of 10 mg/L) and NaCl (with a concentration of 1.5 g/L) was used as the electrolyte in the cathode chamber or catholyte. External resistance of 560 Ω was inserted to close the MFC circuit, and the catholyte samples were analysed after every 4 hours. Figure 3.8 shows the Hg (II) removal over 24 h duration. The removal efficiency of 98 % for the Hg (II) ions was observed after 24 h (Figure 16). To validate the results, this experiment was performed for three times represented as H1, H2, and H3 under similar conditions. No Hg(I) was detected in the catholyte, thereby suggesting the

reduction of Hg(II) to into Hg^0. However, this needs to be confirmed through X-ray Photoelectron Spectroscopy (XPS) analyses. The pH of the cathode chamber was increased from 2.2 to 3.3 after 24 h.

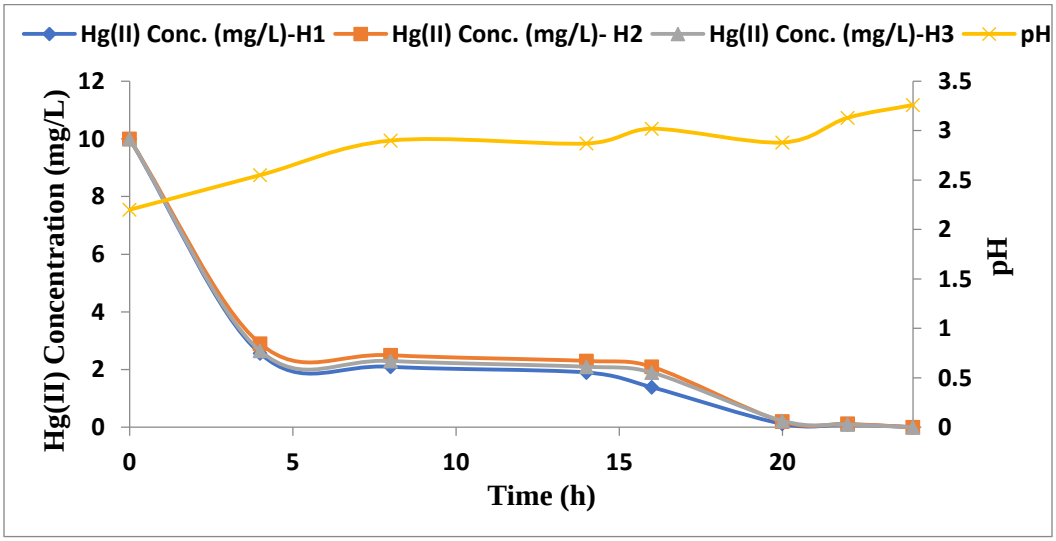


Figure 16: Hg (II) removal tests (H1 to H3) as a function of time.

During 24 h MFC tests, white coloured deposits occurred at the cathode (Figure 17). These deposits will be analyzed through XPS.

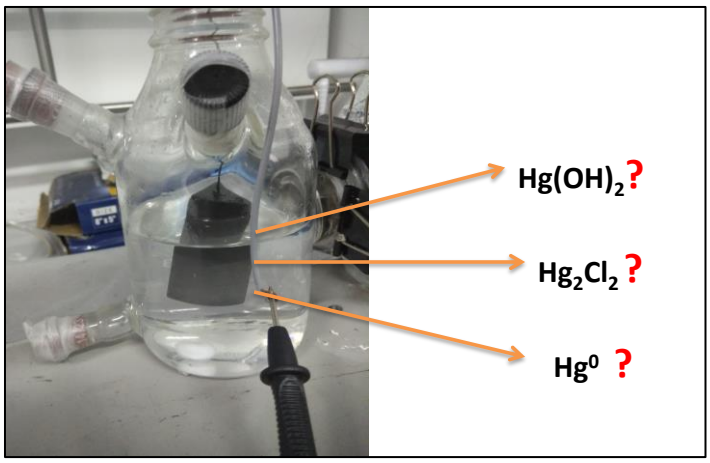


Figure 17: White precipitates at the cathode of MFC

The white precipitate can be due to the formation of hydroxide compound of Hg(II), the formation of mercurous chloride (Hg_2Cl_2) or elemental mercury which get adsorbed at the cathode. Two control experiments were performed to understand the role of anodic biofilm-assisted Hg(II) removal and physical Hg(II) removal.

3.4.1 Control experiment 1

This experiment was run in open circuit mode. We expect that the Hg(II) concentration should remain the same as there is no source of electrons in the cathode of MFC. The Hg(II) concentration decreased from 10 mg/L to 9.82 mg/L after running MFC for 24 hours. This indicates that 1.8 % of Hg(II) ions were removed from the cathode and this can be due to physical adsorption of Hg(II) ions on the graphite electrode. Figure 18 represents the removal of Hg(II) ions due to physical adsorption at the electrode.

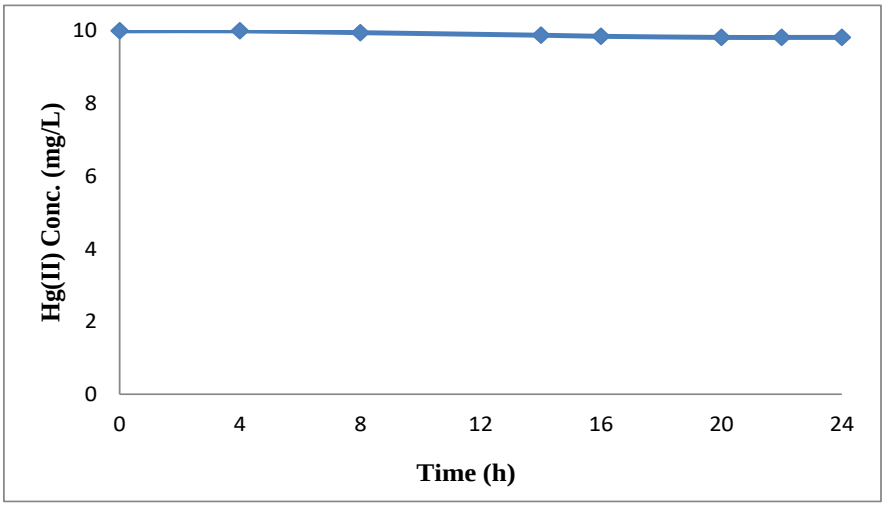


Figure 18: **Hg(II) removal test in control experiment 1 (C1).**

3.4.2 Control experiment 2

This experiment was run in a closed-circuit mode and with the abiotic anode. The Hg(II) ions concentration was almost similar after 24 hours of running the MFC as shown in figure 19. Since there is no Electroactive biofilm available at the anode there is no

substrate oxidation and thus no electron generation. It means that under such condition there is reducing power transferring to the cathode for the reduction of Hg(II) ions. The Hg(II) concentration decreases to 2 % from an initial concentration of 10 mg/L. Both control experiments confirm the role of anodic electroactive biofilm in providing the electrons for the Hg(II) reduction reaction via cathode.

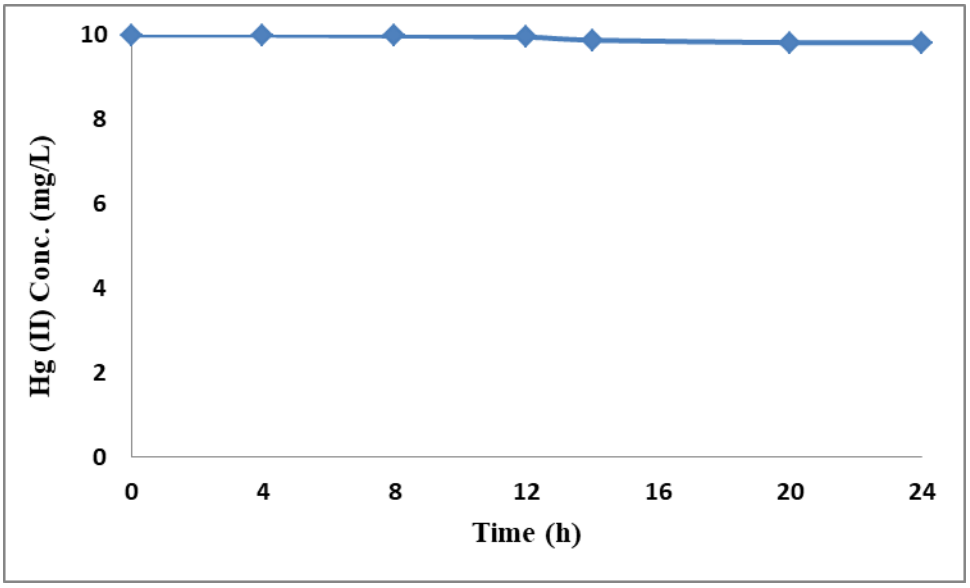


Figure 19: Hg(II) removal test in Control experiment 2 (C2)

Chapter-4

Conclusions and Future perspectives

An electroactive biofilm of *Geobacter sp.* was successfully developed at the anode of MFCs. MFC with Hg(II) ions as the electron acceptor at the cathode showed better OCV and power density than the MFCs with O_2 as the electron acceptor. The removal efficiency of 98 % for Hg(II) ions was achieved in the cathode of MFC. The biotic anode unconnected and an abiotic anode connected control experiments showed a decrease of only about 1.8 % and 2 % in the Hg(II) concentration. It is mainly attributed to the physical adsorption process at the graphite cathode. These control experiments also confirmed the role of bioanode in assisting the electrochemical reduction of Hg(II) ions at the cathode.

Bioelectrochemical removal of heavy metals is an emerging approach, and our study showed an efficient removal of Hg(II) ions from the cathode of MFC. Our study focussed on the removal of Hg(II) ions without paying much attention at pH. This study can be tried with different pH conditions and with different types of electrolytes. In this study, NaCl was used as an electrolyte which has a very high dissociation constant. Other electrolytes such as Na_2SO_4, $MgSO_4$ can also be used to explore the possibilities of Hg(II) removal. Since mercury can form amalgams with other metals, this possibility can also be explored to amalgamate mercury with metals such as Fe, Zn in the cathode of MFC.

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