DESIGN AND DEVELOPMENT OF A SOPHISTICATED BIOSENSING PLATFORM FOR BACTERIAL SENSING VIA AMPLIFIED EXTRACELLULAR ELECTRON TRANSFER PROFICIENCY

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CERTIFICATE

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DEDICATION

With profound reverence,

We dedicate this project to our **beloved parents** and **respected teachers** whose guidance and unflinching support helped us throughout the process.

ACKNOWLEDGEMENTS

Praise be to Almighty Allah who made us capable to do our best and get through this project.

For the successful completion and execution of our final year project, we sincerely acknowledge all the advice and support we have received from our Supervisor Dr. Usman Liaqat. Without his diligence, we would not have been able to project our true capabilities for this strenuous task.

Furthermore, all the guidance our Co-Supervisor Dr. Waheed Miran provided us with, helped us achieve a better understanding and problem-solving approach. We thank him for the provision of all the required material samples.

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We pay our sincerest regards to all the staff and faculty members of the Materials Engineering department for guiding and helping us at various stages throughout the process.

ABSTRACT

This study focuses on the development of a biosensing system suitable for bacterial detection. Bacterial diseases are a major concern worldwide and therefore constant research is going on to develop methods for rapid their rapid and timely detection.

Recent research shows that pathogens can undergo extracellular electron transfer (EET) in anaerobic conditions. Bacteria can transfer an electron across their cell wall to an available insoluble solid electron acceptor and produce a current signal if connected to an external circuit. This current can be used for bacterial detection but has a low magnitude.

A new sensing material has been synthesized which is a porous metal-organic framework (MOF) known as Fe-MIL-88B-NH_2 with Carbon Nanotubes (CNT) and Copper (Cu) nanoparticles attached to its surface. The MOF provides a relatively large surface area, whereas the attached Cu and CNT particles increase the material's conductivity. Two chemical mediators, riboflavin and 2-hydroxy-1,4-naphthoquinone (HNQ), were also used to enhance the current production.

This material Fe-Mil-88B-NH_2 has never been used for bacterial sensing before. Its composite with Cu @ CNT nanoparticles has also not been synthesized before.

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INTRODUCTION

1.1 Major Health Issues

The world is at a high risk of epidemics caused by infectious illnesses due to overpopulation, unsafe drinking water, inadequate sanitation, poor socioeconomic situations, low health awareness, and inadequate vaccination coverage. The spread of different diseases has caused the mortality rate to grow significantly.

Different organizations are working to lower mortality, but the number of diseases is increasing with time. The diseases include Tuberculosis, Cancer, Heart disease, stroke, respiratory failure, and neurological disorders, just a few of the health ailments that fall under this umbrella term.

1.1.1 Bacterial Diseases

Bacteria are single celled (unicellular) organisms and are an integral part of planet's ecosystem. Bacteria has different shapes such as spherical, rod and spiral amongst other types. Bacterial cells have dimensions on the micron scale and are inherently found everywhere, especially inside human bodies. Most types of bacteria do not cause any harm to humans. However, pathogenic bacteria can cause harm to humans as they are responsible for causing several infectious diseases.

Pathogenic bacteria have the tendency to multiply rapidly inside the human body and cause infections. This is because pathogenic bacteria have the capability to overcome the natural defense of the body against invasion of foreign species and travel through the tissues present in the human body. Some commonly known diseases caused by pathogenic bacteria include pneumonia, typhoid fever, syphilis amongst others. An analysis done for the Global Burden of Disease Study in 2019 showed that approximately 7.7 million deaths were caused by 33 identified

bacterial pathogens [1]. Staggering statistics such as these are an indication that bacterial infections are one of the leading causes of mortality globally [2].

1.1.2 Antimicrobial Resistance

With the passing of time, bacteria have the potential to change form or adapt to the human body's natural defense mechanisms and stop responding to regularly used medicines to counter a certain type of bacteria known as an antibiotic. Each antibiotic is specific to a different type of bacteria that is responsible for a particular disease. Initially, pathogenic bacteria are isolated from body samples such as saliva and are grown in cultures for identification. Once the bacteria type is identified, a list of antibiotics that may be successful in treating a particular bacterial infection is generated.

When patients are prescribed with a particular antibiotic drug, a course of treatment is designed for a particular number of days depending on the severity of the infection. In many cases, patients fail to complete their prescribed course which makes the bacteria still present in the bacteria more adaptive to the body's defense mechanism. Eventually, the bacterial strain becomes stronger and more resistant and stops responding to the initially prescribed antibiotics. A similar reaction is also observed in the case that an antibiotic drug is misused, used persistently to treat even minor infections without consulting doctors which eventually leads to bacteria becoming resistant to the misused antibiotic drug. Once bacteria develop a resistance towards antibiotic drugs, the drugs are no longer effective in treating infections which eventually leads to death in severe cases. According to the World Health Organization (WHO), it is estimated that approximately 4.9 million deaths annually are associated with antimicrobial resistance (AMR) [3]. Deaths caused by antimicrobial resistance (AMR) are more prevalent in developing regions which calls for designing methods for improving antibiotic drug development and testing to effectively treat bacterial infections.

1.2 Respiration

"Life is nothing but an electron looking for a place to rest," these words were spoken by Dr. Albert Szent-Gyorgyi, who was a renowned biochemist and known for his discovery of vitamin C [4]. This statement describes the significance of

electron transfer for the survival and sustenance of any living organism since all living cells generate energy for growth and maintenance by the process of electron transfer.

The food that we consume contains carbon in the form of different molecules or compounds, these carbon containing molecules act as electron donors. Our body metabolizes these carbon rich molecules such as glucose to produce electrons alongside hydrogen ions. The Oxygen that we inhale is the final electron acceptor due to its strong oxidizing nature. Oxygen is thus being utilized by the cells to generate the necessary energy to carry out different activities [5]. CO_2 and H_2O are produced as the by-products of this redox reaction occurring during the process of aerobic respiration (in the presence of oxygen). A similar phenomenon is also seen in bacteria under aerobic conditions as shown by Figure 1. The produced energy is used by the bacteria for their growth and sustenance.

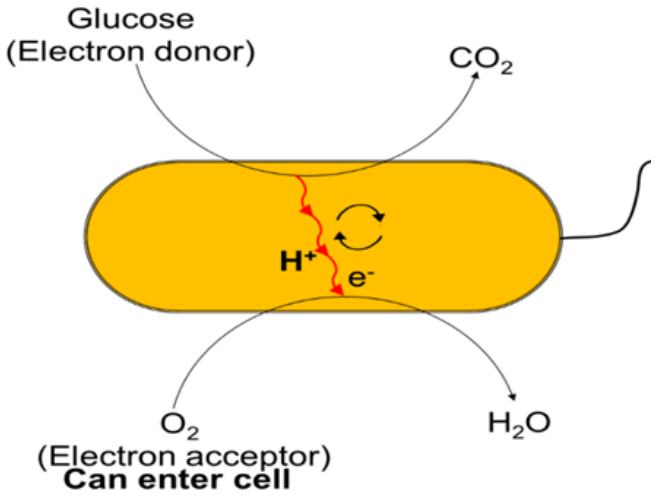

Figure 1: Bacterial respiration in aerobic conditions

1.2.1 Bacterial Respiration in Anaerobic Conditions: Extracellular Electron Transfer (EET)

Under anaerobic conditions humans cannot survive due to the absence of an electron acceptor, however, there exist some bacteria that have the tendency to survive in anerobic conditions as well by means of anaerobic respiration. Under anaerobic conditions when no soluble electron acceptor is present, the bacteria

transfer electrons across their cell wall, this phenomenon is termed as "Extracellular Electron Transfer." If an insoluble electron acceptor(electrode) is connected to a circuit, an electrical signal will be produced.

Multiple mechanisms have been proposed for EET; however, the most widely accepted mechanisms are Direct Electron Transfer (DET) and Mediated Electron Transfer (MET) as shown by Figure 2. When the bacteria can directly transfer its electron to an electrode, the process is termed DET [6]. However, in some cases, bacteria are incapable of DET, in such cases certain chemical compounds called mediators assist in the process of EET. These mediators act as electron shuttles, they enter the bacteria in the oxidized form, accept the electron and come out in reduced form, the electron is then lost to the electrode. This process is termed Mediated Electron Transfer [7].

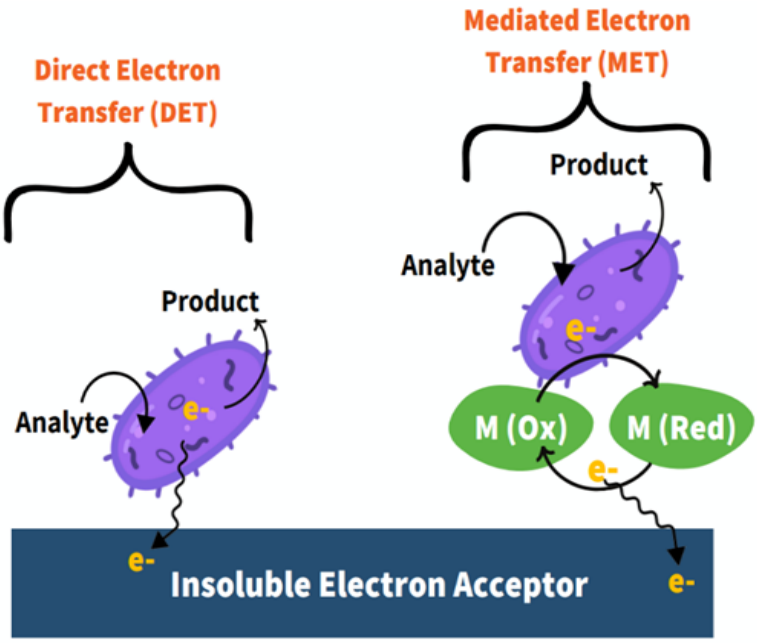

Figure 2: Mechanisms of Extracellular Electron Transfer

1.3 Problem Statement

Our project identifies bacterial diseases as a major health concern with millions of deaths reported worldwide annually due to diseases caused by bacteria. Several medications known as antimicrobials exist for the treatment of these bacterial infections. However, there has been an alarming increase in antimicrobial resistance in recent years. Antimicrobial Resistance (AMR) is a condition in which bacteria, viruses, fungi, and parasites evolve over time and cease to respond to

antibiotics, making infections more difficult to cure and raising the risk of disease transmission, life-threatening sickness, and death.

Drug resistance makes it harder or impossible to treat illnesses and renders antibiotics and other antimicrobial medications useless [8]. It is estimated that approximately 4.5 million deaths are reported annually due to antimicrobial resistance related diseases worldwide [9].

A revolutionary bacterial sensing technology that combines quick detection with the capacity to handle the problems brought on by antibiotic resistance is therefore urgently needed. This method should make it possible to quickly identify and characterize bacterial infections, allowing for rapid and effective treatment actions. This project seeks to create a cutting-edge platform that can identify bacteria in a reliable and timely manner by utilizing modern sensing technologies.

1.4 Objectives

The primary goals of this study are to increase the sensitivity of bacterial detection and to identify extracellular electron transfer (EET) in bacteria. The first goal entails finding EET in bacteria, which is the method by which bacteria can transmit electrons to an electrode. The behavior and metabolic activities of bacteria can be better understood and detected by recognizing this phenomenon.

The second goal aims to increase bacterial detection's sensitivity. Two strategies are used to do this. The first technique involves amplifying electrical signals by including mediating agents. These substances serve as a bridge between the bacteria and the electrode, aiding the flow of electrons and boosting signal intensity. To further increase the electrical signals produced by the bacterial interaction with the electrodes, the electrochemical system is modified.

In this endeavor, material synthesis plays an equally important role for signal amplification. To boost the current for bacterial detection, a composite material based on metal-organic frameworks (MOFs) is being synthesized. With its distinctive qualities like high porosity and surface area, MOFs can improve the interaction between bacteria and electrodes, increasing current output and enhancing detection sensitivity.

The design of an appropriate reactor for bacteria detection is next discussed. This entails designing a specialized system that offers the best conditions for bacterial detection while considering variables like the electrode configuration. The reactor is designed to maintain experimental control and reproducibility while ensuring accurate and reliable bacterial identification.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The major focus of this chapter is elaboration on Metal Organic Framework (MOF), Composition of Fe-Mil-88B-NH_2, metallic nanoparticles and Copper oxide (CuO) nanoparticles, Cu @ CNT nanoparticles, the synthesis methods that are currently available for synthesizing Fe-Mil-88B-NH_2 and CuO nanoparticles. The elaboration of the biosensors i.e., the electrochemical biosensors and the limitations in detecting current produced by bacteria will also be done.

2.2 Metal Organic Framework (MOF)

Metal Organic Framework (MOF) represents a class of porous materials that are composed of metal centers that are bonded using organic ligands. Metal Organic Frameworks (MOFs) have a high relative surface area due to the large volume of pores which make up approximately 90% of the crystalline volume of the structure [10]. Metal Organic Frameworks (MOFs) have high thermal stability due to the presence of strong organic bonds comprised of carbon (C-C), carbon and hydrogen (C-H), carbon and oxygen (C-O) and metal and oxygen (M-O). The structural characteristics of Metal Organic Frameworks (MOFs) mainly depend on the large number of coordination geometries taken up by the metal ions and the flexibility of the organic ligands used to bond the metal centers [11]. For this reason, transition metal ions, lanthanides and alkaline earth metals are used for making Metal Organic Frameworks (MOFs) as they display a variety of coordination numbers, geometries, and oxidation states [12].

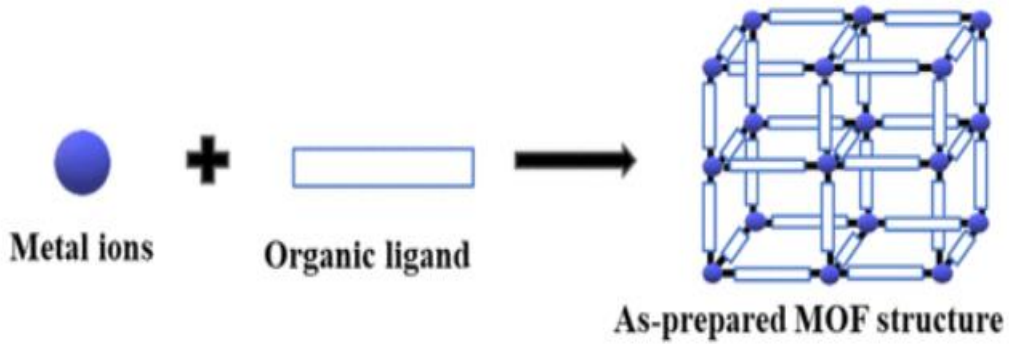

Figure 3: MOF synthesis

2.2.1 Classification of Metal Organic Frameworks

Metal Organic Frameworks (MOFs) can be combined with different materials such as polymers, quantum dots and other molecules and enzymes to create Metal Organic Framework (MOF) nanocomposites. By doing this, you can obtain diverse properties making these nanocomposites suitable for tailored applications. Depending on the component unit, Metal Organic Frameworks (MOFs) are put into specific categories such as: Isoreticular Metal Organic Frameworks (MOFs), Zeolitic Imidazolate Frameworks (ZIFs), Porous Coordination Networks (PNCs), Material Institute Lavoisier (MIL), etc. [13]

2.2.2 Material Institute Lavoisier (MIL)

Among the categories discussed above for the classification of Metal Organic Frameworks (MOFs), the Material Institute Lavoisier (MIL) type is of particular importance for our project. These types of Metal Organic Frameworks (MOFs) are prepared using an organic compound that contains two carboxylic functional groups and elements that contain valence electrons. Material Institute Lavoisier (MIL) materials include MIL-101, MIL-100, MIL-53, MIL-88, MIL-125 amongst others [14]. Material Institute Lavoisier (MIL) materials can be used particularly for biosensing applications because of their high relative surface area, permanent porosity and uniform pore size with the ability to immobilize proteins or other biomolecules.

2.2.3 Composition of Fe-Mil-88B-NH_2

The Metal Organic Framework (MOF) that was synthesized for our project was the Fe-Mil-88B-NH_2. Iron (Fe) is the metal center for the Metal Organic Framework (MOF) and is derived from the use of $FeCl_3.6H_2O$ (Iron Chloride), Mil-88 is its identity and NH_2BDC (2-Aminoterephthalic acid) is used as an organic ligand to connect the metal centers.

2.2.4 Synthesis Routes of Metal Organic Frameworks (MOF)

The synthesis of Metal Organic Frameworks (MOFs) can be done by using various methods; few of which are mentioned in the details below.

1. **Microwave Assisted Approach**

   With this approach, you can synthesize small metal and oxide particles [15]. In this process, you add a substrate mixture with a compatible solvent in a vessel which is sealed and placed in a microwave at a set temperature and for a particular time duration. The electromagnetic energy of the microwave is converted to thermal energy wherein the permanent dipole of the molecules interacts with an applied electric field that causes the molecules to collide and enhance the temperature of the system. Microwave assisted approach is feasible for rapid crystallization and formation of nanoscale products that have improved purity such as that required for synthesizing polymorphs [16].

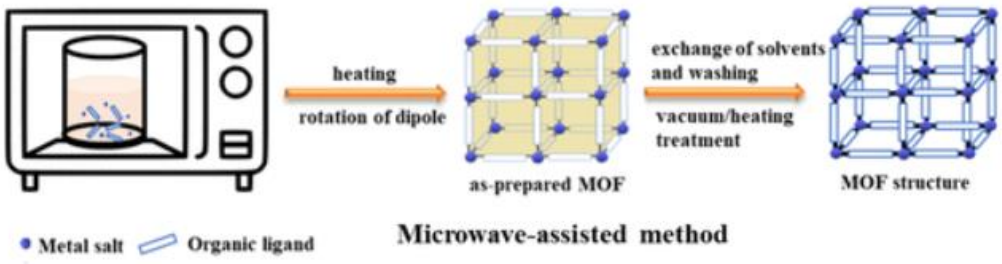

Figure 4: Schematic diagram of Microwave Assisted MOF synthesis

2. **Electrochemical Method**

   In this method, the source of metal ions are electrons. These electrons are passed through the reaction mixture that contains the ligand (organic

linker) and an electrolyte through an anodic dissolution process as a metal source instead of metal salts [17]. An appropriate voltage is applied to the electrode placed in a solution of PBU (Tributyl phosphine) and the electrolyte which causes the metal to dissolve. The metal ions formed through this process instantly interact with the ligand molecules in the solution forming a Metal Organic Framework (MOF) near the electrode surface. This method is feasible for acquiring a higher solid content than the ordinary batch reaction [18].

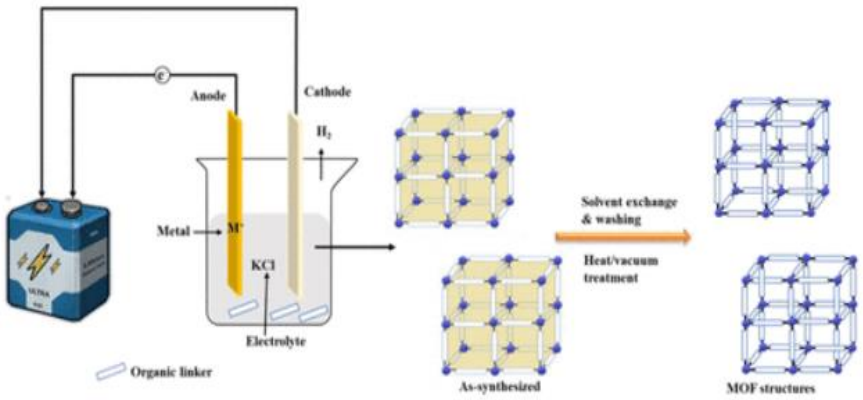

Figure 5: Schematic diagram of Electrochemical Method for synthesizing MOF

3. Solvothermal Method:

This approach is widely used for synthesizing Metal Organic Frameworks (MOFs) because it's a relatively easy and simple process to undertake. In the solvothermal method, the selected metal salts and organic ligands are stirred in protic or aprotic organic solvents which have formamide functionality [19]. Protic organic solvents include alcohols such as methanol and ethanol. Whereas aprotic organic solvents include DMF (Dimethyl formamide), DMA (Dimethylacetamide) amongst others. The mixture is placed in a closed vessel at autogenous pressure and temperature above the solvent's boiling point for many hours. During this reaction, the salt melts as the solvent is heated above its boiling point.

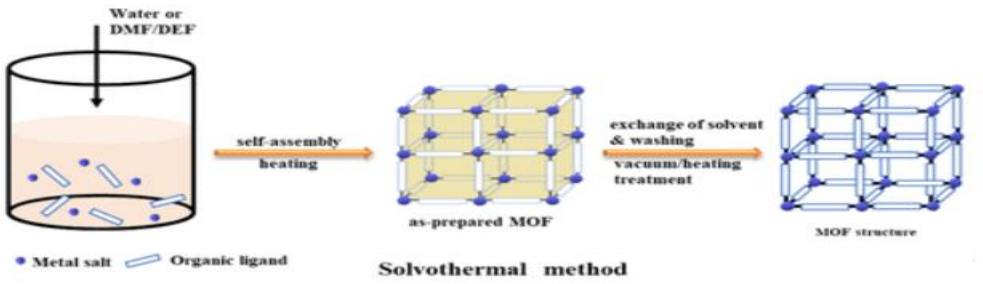

Figure 6: Schematic diagram of Solvothermal Method to synthesize MOF

2.3 Nanoparticles

Nanoparticles are a subject of interest because of their enhanced properties compared to bulk materials. Nanoparticles are materials that have one dimension less than 100 nm. The properties of nanomaterials keep changing as their size decreases.

Nanomaterials can be synthesized to have outstanding magnetic, electrical, optical, mechanical, and catalytic properties in comparison to their bulk counterparts [20]. These properties can be controlled by altering the synthesis route taken which would allow selected size reduction to take place and obtain nanoparticles with a desired shape for a particular application. The unique properties which make nanoparticles a viable candidate for biosensing applications include their biological activity, changing chemical reactivity due to the high relative surface area.

Nanoparticles are 0D (zero dimension) nanomaterials. Nanoparticles are composed of three layers which include: i) The functionalized surface layer that is compatible with specific molecules, polymers, or metallic ions, ii) The shell layer that has a varying chemical composition from the core, iii) The core at the center of the nanoparticle, also known as the nanoparticle, itself [21].

2.3.1 Types of Nanoparticles

Nanoparticles can be made from a variety of materials. Two types of nanoparticles with relevance to the project are discussed below:

2.3.1.1 Carbon Based Nanoparticles

Carbon-based nanoparticles are classified in two ways: carbon-based nanoparticles which contain fullerenes, and the other class which contains carbon

nanotubes. Fullerenes (bucky balls) based nanomaterials have closed hollow cages with sp2 hybridized carbon atoms. Fullerene based carbon nanoparticles have excellent electrical conductivity and high strength [22]. Carbon nanotubes are 1D nanomaterials which have one dimension greater than 100nm. Carbon nanotubes are synthesized by using graphene sheets. A single sheet of graphene is rolled to form a hollow tubular structure. Carbon nanotubes have a high surface area, excellent strength and stiffness which allows them to be used for various applications such as manufacturing sports equipment, body armor and vehicle parts [23].

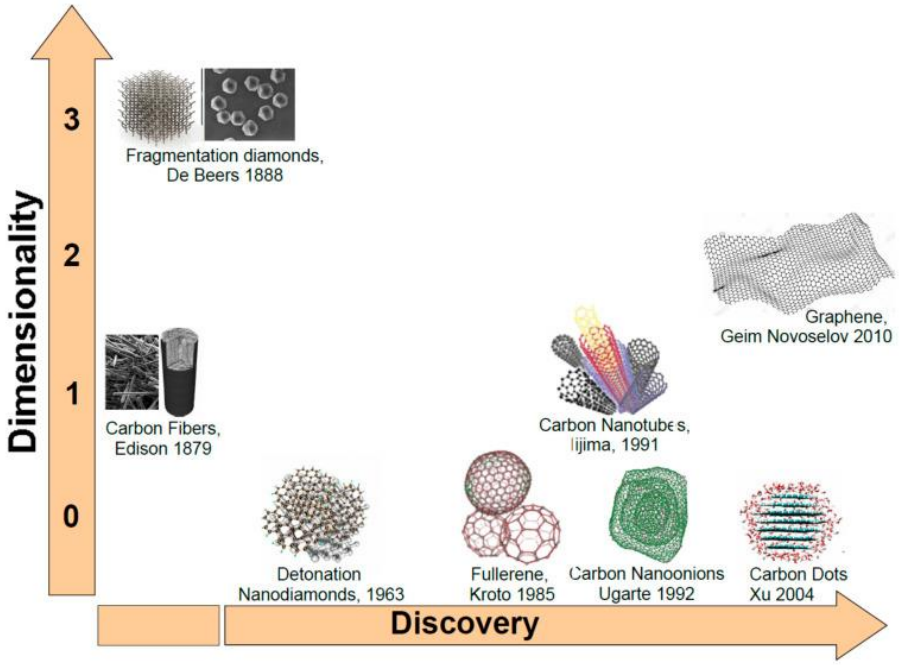

Figure 7: Schematic diagram of different carbon-based nanomaterials

2.3.1.2 Metal Nanoparticles

These types of nanoparticles are comprised of only one element – a metal. Metallic nanoparticles are flexible in nature as their shape, size and even optical properties can be controlled. Metallic nanoparticles have excellent optical properties, very high surface energies and experience phenomena such as plasmon excitation and quantum confinement [24]. Metal nanoparticles are used for various applications such as in solar cells, imaging sensors and in biomedical applications such as tumor treatments and drug delivery because of their unique properties [25].

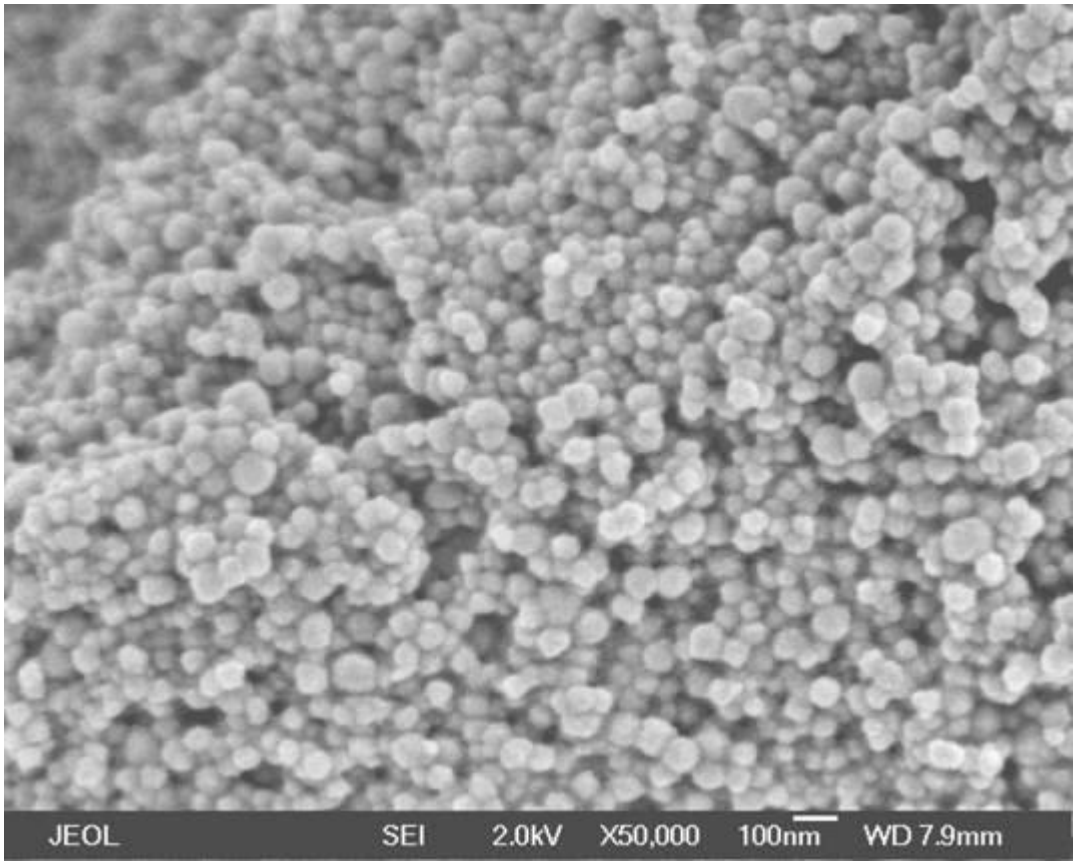

Figure 8: SEM image of Silver Nanoparticles

2.3.2 Synthesis of Nanoparticles

There are two types of approaches that can be used to synthesize nanoparticles. The synthesis route taken affects the morphology, uniformity, and size of the nanoparticles. The two approaches that can be used include:

- Top-down approach
- Bottom-up approach

2.3.2.1 Top-Down Approach

In the top-down approach, a bulk material is taken and broken down into smaller particles up until the nano-sized regime is reached. The top-down approach includes several techniques like mechanical milling, etching and laser ablation which will be discussed below in detail.

2.3.2.1.1 Mechanical Milling

This is the most cost-effective technique for producing nanomaterials and can be used to synthesize blends and nanocomposites as well. The bulk materials are

broken down through mechanical actions such as abrasion with the balls present in the ball mill. Upon striking the bulk material with the balls, the material is broken down and the size is reduced until the particles are nanosized. As the milling time is increased, the size reduction of the bulk material also increases, smaller particles can be obtained.

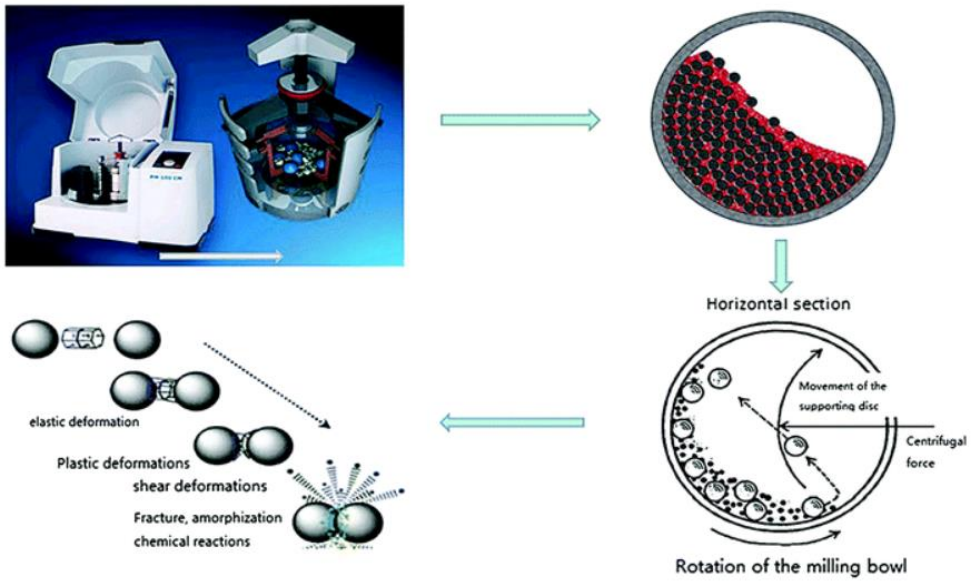

Figure 9: Principal of Ball Milling

2.3.2.1.2 Lithography

In this approach, a focused beam of light or electrons are used to create nano-sized features on your desired surface. There are two main types of lithography: masked and maskless lithography [26]. In masked lithography, a template or mask is placed over your surface.] Light or electron beam is directed onto that template which allows nanopatterns to be deposited on your surface. In maskless lithography, the nanopatterns are generated without using a template or mask. Instead, focused ion beam implantation on the desired surface followed by wet chemical etching using KOH (potassium hydroxide) is used to generate nanopatterns.

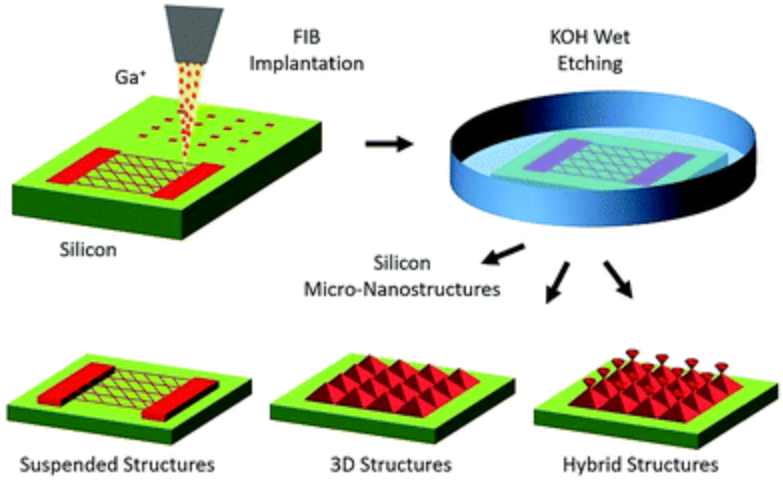

Figure 10: Schematic of Maskless Lithography

2.3.2.1.3 Laser Ablation

In this technique, a power laser beam hits the target material. When the beam hits the target material, the precursor material vaporizes because of the high energy of the laser irradiation [27] which results in the production of nanoparticles. This technique can be used to produce a variety of nanoparticles such as those made of metals, particularly noble metals, ceramics, and oxide composites [28].

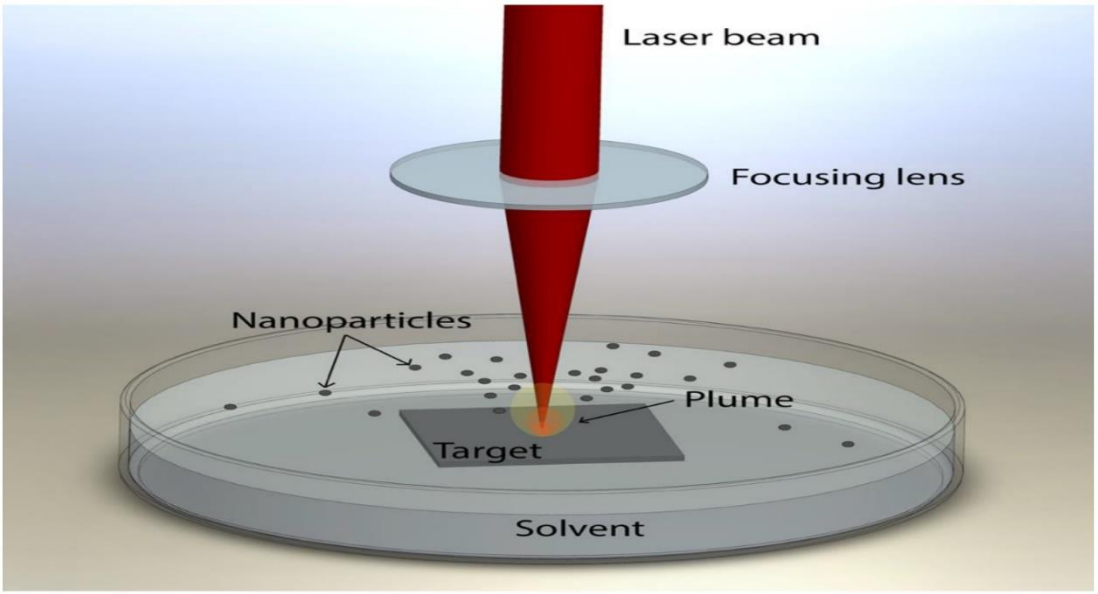

Figure 11: Schematic of Laser Ablation

2.3.2.2 Bottom-Up Approach

In the second type of approach, smaller atoms are brought together or bind to each other to synthesize nanoparticles or until your desired size for the particles is

achieved. There are several techniques that fall under the bottom-up approach which will be discussed below.

2.3.2.2.1 Chemical Vapor Deposition (CVD)

The Chemical Vapor Deposition (CVD) technique is used for depositing thin films of your desired material on a substrate. A gas containing a precursor is flown into a vacuum chamber and heated. Upon heating, the gas decomposes to release atoms of the precursor which get deposited onto the surface of the substrate. Different catalysts can be used for the chemical vapor deposition process (CVD) which can help control the size and morphology of the nanoparticles obtained [29].

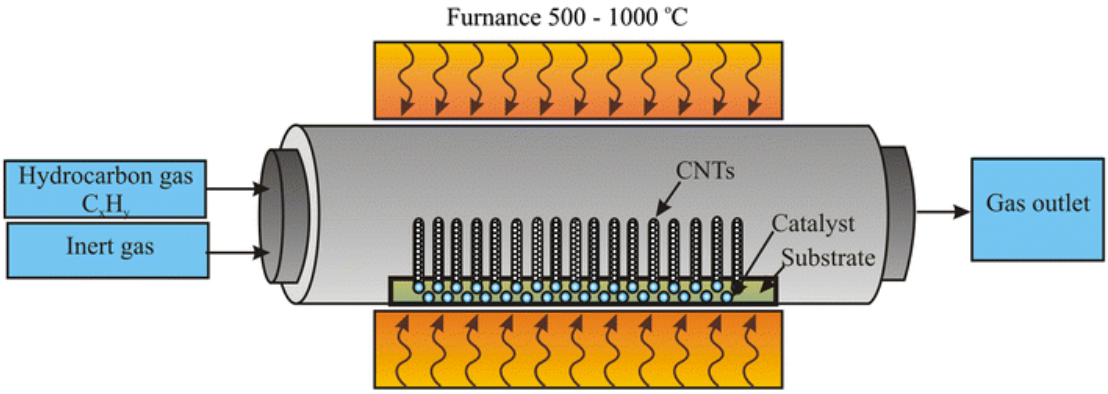

Figure 12: Schematic for Chemical Vapor Deposition of CNTs

2.3.2.2.2 Sol-gel Process

The sol-gel technique is a wet-chemical technique that uses metal alkoxides as precursors to produce nanomaterials. Initially, the precursor is hydrolyzed using water or another solvent such as alcohol to produce the “sol”. The sol is then dried or undergoes a condensation reaction to form a gel. This semi-rigid gel structure is then calcinated to obtain nanoparticles [30].

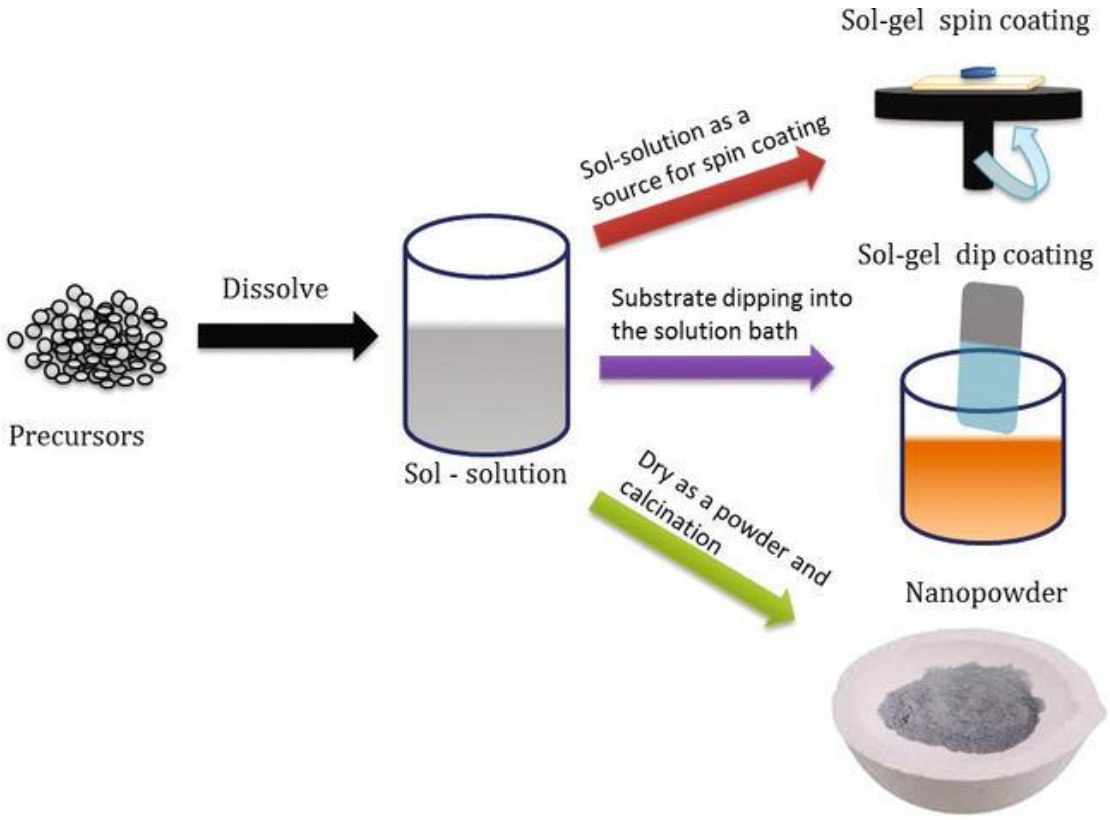

Figure 13: Schematic for Sol Gel Process

2.3.2.2.3 Templating Methods

Templating methods are also a type of bottom-up approach used to synthesize nanoparticles. There are two types of templating methods: soft and hard. In the soft templating method, soft templates like flexible organic molecules and other materials like block copolymers are used to create nano-porous materials [31].

In hard templating, a specifically designed solid material with pores is used to synthesize nanoparticles. This approach is also known as nano-casting and the pores present in the template are filled with precursors. Once the precursor dries, the template is removed to obtain the final nanostructure.

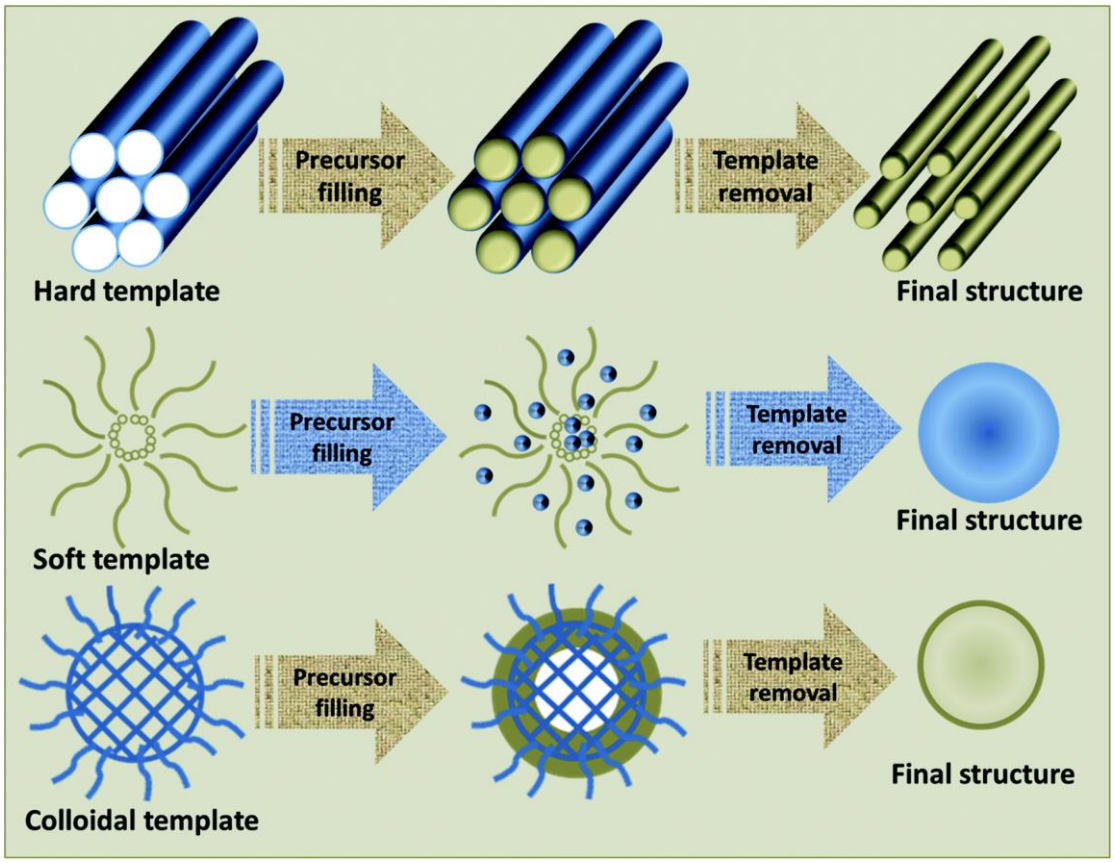

Figure 14: Schematic for Templating Methods

2.4 Biosensors

Biosensors are devices that can measure a certain reaction that is biological or chemical in nature depending on the amount of analyte present in a particular reaction [32]. Typically, biosensors are used to determine different pollutants or measure the concentration or presence of certain biological markers in the blood that would indicate the presence of a certain disease or infection.

There are several components that work together to make a biosensor. These components include the analyte which is the substance that will be detected. The second component is the bioreceptor which is a molecule that only recognizes the analyte. Once the analyte is recognized by the bioreceptor, a signal such as a change in temperature, light or charge is generated in response. This signal is then converted into another energy form which is a computer readable signal using a third component called the transducer. The electronics component then displays this measured signal by converting it from analogue to digital form and is ultimately displayed on a computer screen for analysis by the observer.

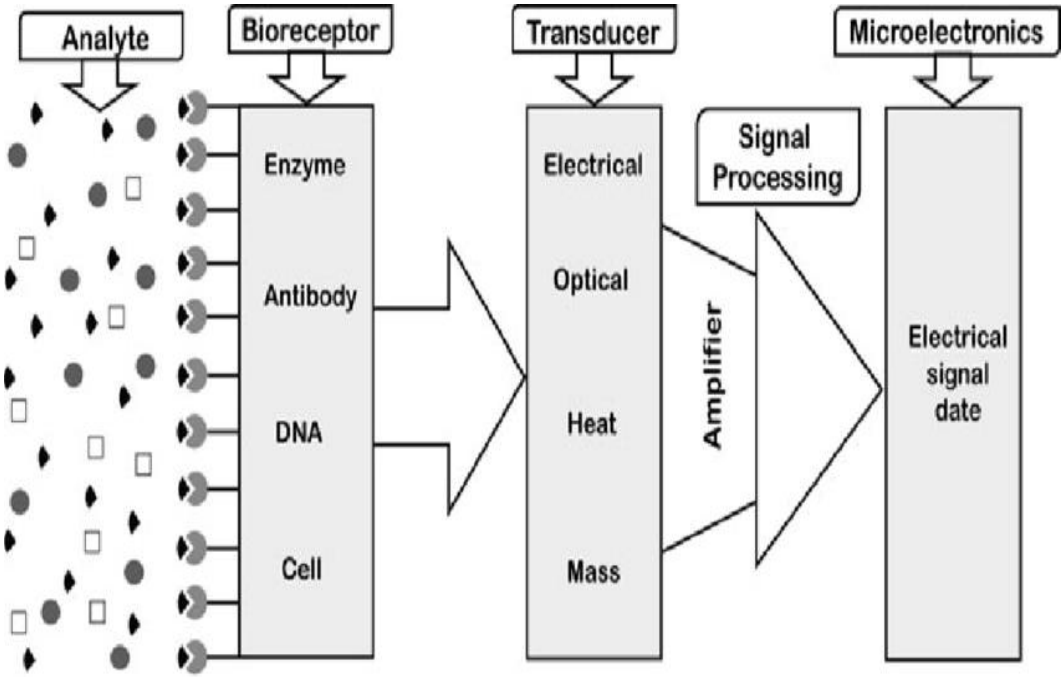

Figure 15: Schematic for working of a biosensor

2.4.1 Types of Biosensors

The classification for biosensors is done based on two distinctions: the biological element used, or the sensor device incorporated. The first category refers to the use of biological elements or biorecognition elements such as enzymes, antibodies, proteins amongst others.

The second group of biosensors depends on the type of sensing device used and the transduction method being incorporated. The transduction method used can allow us to classify the biosensors into three further categories: optical, electrochemical, and mass-based sensors. These sensors can further be divided into more subcategories and different measurements can be obtained from each biosensor type. In reference to our project, the most relevant biosensor – an electrochemical biosensor will be discussed in detail.

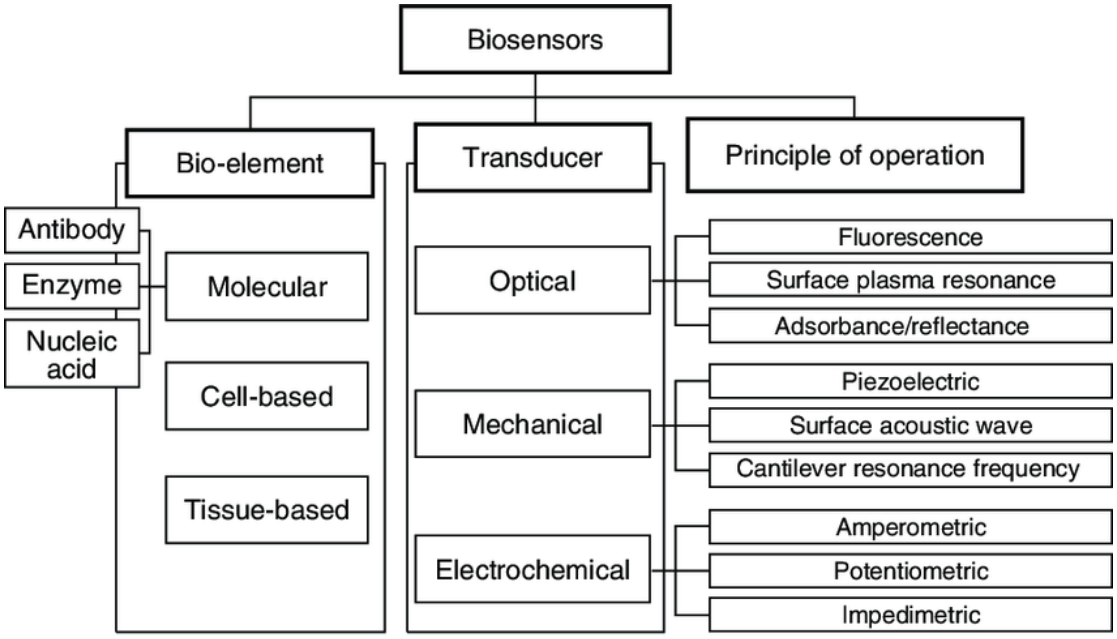

Figure 16: Classification of biosensors

2.4.1.1 Electrochemical Biosensors

In electrochemical biosensors, primarily any chemical reactions that are occurring such as an enzyme catalysis reaction for example, will consume or generate ions or electrons. This will cause a change in the electric properties of the solution being used and the change in these properties can be reflected in changing values of current or voltage which can be measured.

Some biological components such as glucose and urea are not electroactive, however upon undergoing certain reactions, an electroactive or measurable element can be produced. This element can reflect changes in a parameter like current density to determine the exact concentration of the analyte.

Electrochemical biosensors use the three-electrode system which includes: a working/ measuring electrode, reference electrode and counter electrode. The reaction between the electrode itself and the analyte occurs at the working electrode.

Such electrochemical biosensors can be used to determine the activity of electroactive bacteria. Electrochemical biosensors can be further classified into three types: amperometric, potentiometric and conductimetric biosensors.

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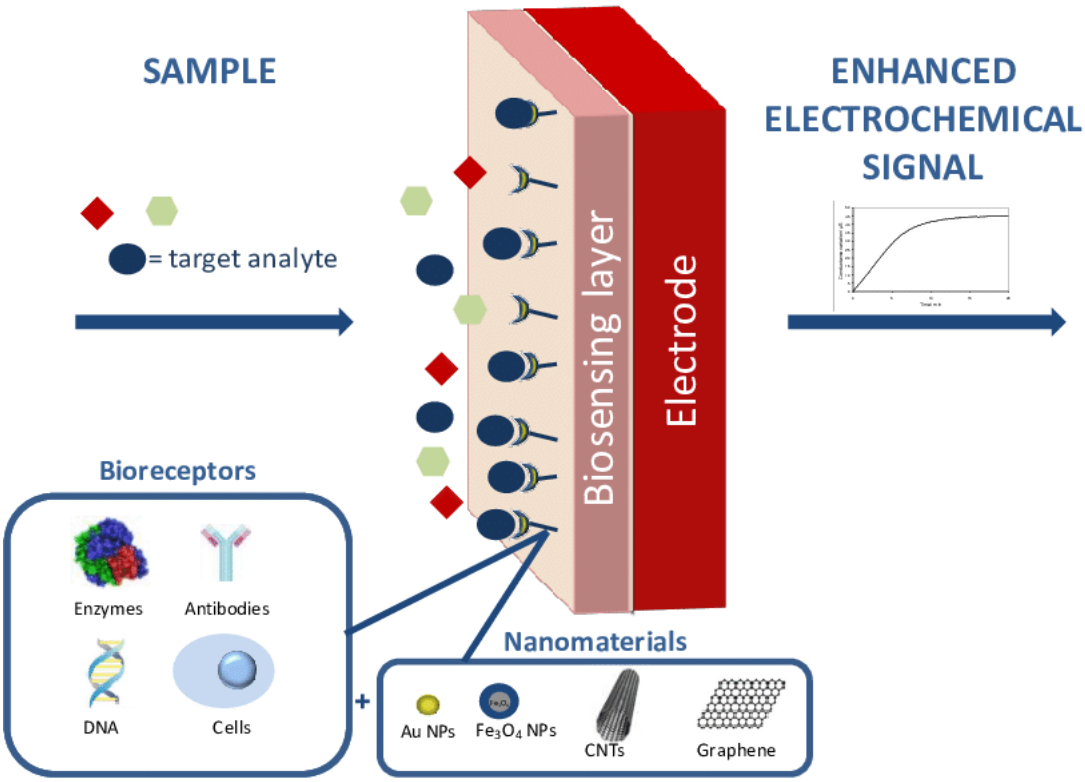


Figure 17: Schematic of Electrochemical Biosensor

Amperometric biosensors measure the amount of current being produced in response to a bio electrochemical reaction. The change in current being produced reflects the amount of analyte or substrate present. Potentiometric biosensors measure the change in potential across an ion selective membrane [33]. Potentiometric biosensors have shown promising results in detection of a cancer biomarker called hPRL-3 [34]. Conductimetric biosensors work by measuring electrical conductance of the solution [35]. The measurement of electrical conductance has a relatively lower sensitivity. Conductimetric biosensors use AC (sinusoidal) voltage source which prevents phenomena such as concentration polarization or double layer charging to occur which would directly affect the conductivity of the solution.

2.5 Limitations to Bacterial Sensing Using Conventional Electrochemical Systems

Organism	Source	Electron donor	Electron acceptor	Applied potential (mV vs SHE)	Current (μA/cm²)	Reference
Klebsiella pneumonia	Human Gut	Glucose	ITO Electrode	+ 400	0.13	(Naradasu et al., 2018)
Pseudomonas aeruginosa	Human Lungs	Glucose	Graphite Rod	+ 400	2.00	(Rabaey et al., 2005)
S. loihica	Marine	Lactate	ITO Electrode	+ 400	0.60	(Nakamura et al., 2009)
Listeria monocytogenes	Human Gut	Glucose	Graphite Felt	+ 600	23	(Light et al., 2018)
Capnocytophaga ochracea	Human Oral	Glucose	ITO Electrode	+ 400	0.06	(Zhang et al., 2020b)
Clostridium cochlearium	Mouse Gut	Glucose	Graphite Rod	+ 700	530.00	(Schwab et al., 2019

Figure 18: Current Production of different bacterial strains

The table above shows the results of different experiments conducted to determine the amount of current produced by electroactive bacteria. It is important to note that highly conductive electrodes such as graphite rod, ITO electrode and graphite felt are used in the electrochemical biosensing system but the currents being produced by the bacteria are very small and lay in the micro-ampere range. This limited current production makes it difficult to detect bacteria and increases the need for using sophisticated electrochemical biosensing systems that can detect very small currents with high sensitivity, but such systems are highly expensive.

To counter this, a method to amplify the current signals being produced by the bacteria is required. In addition to amplification of current signals, focus on synthesizing a material that improves the capability to sense bacteria is also required to draw comparisons between how current production changes when such changes are made to the electrochemical biosensing system.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter focuses on the experimentation that was carried out for the synthesis of Fe-Mil-88B-NH_2 (MOF), copper @ carbon nanotubes nanoparticles, and the composite after the successful synthesis of the constituents.

3.2 Synthesis of Fe-Mil-88B-NH_2

The synthesis of Fe-Mil-88B-NH_2 was carried out through a room temperature solvothermal process [36]. The apparatus, chemicals, and process being used have been discussed in this section.

3.2.1 Chemicals and materials used:

- Ferrous chloride hexahydrate ($FeCl_3.6H_2O$)
- 2-Aminoterephthalic acid (NH_2BDC)
- Dimethylformamide (DMF)
- Deionized water (DI water)

Deionized water and Dimethylformamide are used as solvents, NH_2BDC was used as the ligand whereas Ferrous chloride was the precursor.

3.2.2 Apparatus Used:

The following apparatus was used to carry out the synthesis:

- Drying Oven

- 50 ml measuring cylinder
- Pipettes of 10 ml
- Petri dishes
- Hot Plate
- Magnetic stirrer
- Spatula
- Weighing balance
- Aluminum foil
- Centrifuge Machine
- Sonicator
- Mortar and pestle
- 100ml beakers
- Falcon tubes

3.2.3 Procedure

As mentioned above, a room temperature method has been used for the synthesis of Fe-Mil-88B-NH_2. The procedure is as follows:

1. First, the solution containing ferrous chloride is prepared.
    a. 2.16g of $FeCl_3.6H_2O$ is measured using a weighing balance and transferred to the 100ml beaker.
    b. 7.6ml of DI Water is measured using as pipette and transferred into the beaker.
    c. 12.4ml of DMF is measured using a measuring cylinder and transferred into the beaker.
    d. Stirring is carried out using a magnetic stirrer on a hot plate until a uniform solution is obtained.
2. The solution containing NH_2BDC is now prepared.
    a. 0.72g of NH_2BDC Is measured using a weighing balance and transferred into a separate beaker.

b. 20ml of DMF is then measured using a measuring cylinder and transferred into the beaker.
c. Sonication is carried out to break the clumps of ligand and form a uniform solution.

3. The NH_2BDC solution is then poured into the beaker containing the ferrous chloride.
4. The beaker is covered using an aluminum foil and stirring is carried out for 24 hours.
5. Once the reaction is complete, the solution is centrifuged at 3000rpm for 15mins.
6. The solute is then washed twice with DI water followed by washing with ethanol.
7. After the washing is completed, the solute is dissolved in ethanol and emptied in a petri dish.
8. The sample is dried at 60°C in a drying oven for 3 hours.
9. The dried sample is then grinded using a mortar and pestle to obtain a fine powder.
10. The powder is again transferred to a petri dish and placed in a vacuum oven at 60°C for 24 hours.
11. The sample was then characterized using different techniques.

3.3 Synthesis of Multiwalled Carbon Nanotubes @ Copper Nanoparticles

A low temperature chemical reduction method was used for the synthesis of the Cu @ MWCNT composite nanoparticles [37]. The apparatus, chemicals, and process being used have been discussed in this section.

3.3.1 Chemicals and Materials

- Deionized Water (DI Water)
- Copper Sulphate Pentahydrate ($CuSO_4.5H_2O$)

- Multiwalled Carbon Nanotubes (MWCNT)
- Sodium Hydroxide (NaOH)
- Sodium Borohydride ($NaBH_4$)
- Acetone

$NaBH_4$ was used as the reducing agent whereas copper sulphate was the precursor, NaOH was used to maintain the pH and DI water was used as a solvent [37].

3.3.2 Apparatus used

The following apparatus was used for the synthesis:

- Magnetic stirrer
- Hot plate
- Weighing balance
- 100 ml media bottle
- Aluminum foil
- Spatula
- Measuring cylinder
- 100 ml beakers
- Sonicator
- Drying oven
- Centrifuge machine
- 15ml falcon tubes
- Mortar and pestle

3.3.3 Procedure

The procedure for the synthesis of carbon nanotubes @ copper nanoparticles was the reduction as mentioned above [37]. So, the procedure is as follows:

1. First, a suspension of Carbon Nanotubes is prepared in acetone.

   a. 0.2 g of CNTs are measured using a weighing balance and transferred into a media bottle.
   b. A pipette is used to transfer 20ml of acetone into the media bottle.
   c. The medium is sonicated for 1 hour.
2. A solution of 1% copper sulphate is prepared.
   a. 0.1 g of $CuSO_4.5H_2O$ is measured using a weighing balance and transferred into a beaker.
   b. 10 ml DI water is transferred into the beaker using a pipette.
   c. Stirring is carried out using a magnetic stirrer on a hot plate until a uniform solution is obtained.
3. A solution of 0.1 M $NaBH_4$ is prepared.
   a. 0.0378 g of $NaBH_4$ is measured using a weighing balance and transferred into a beaker.
   b. 10 ml DI water is transferred into the beaker using a pipette.
   c. Stirring is carried out using a magnetic stirrer on a hot plate until a uniform solution is obtained.
4. A solution of 1 M NaOH is prepared.
   a. 0.400 g of NaOH is measured using a weighing balance and transferred into a beaker.
   b. 10 ml DI water is transferred into the beaker using a pipette.
   c. Stirring is carried out using a magnetic stirrer on a hot plate until a uniform solution is obtained.
5. The media bottle is placed on a hot plate and stirring is turned on.
6. The copper sulphate solution is slowly added into the media bottle.
7. The sodium borohydride solution is then added very slowly to the media bottle.
8. In the end, sodium hydroxide is added slowly into the media bottle.
9. Media bottle is sealed and stirring is carried out for 24 hours.
10. The solution is transferred to falcon tubes and centrifuged at 4000 rpm for 10 mins.
11. Subsequent washing is carried out thrice with DI water and twice with ethanol.
12. The nanoparticles are dissolved in ethanol and poured into a petri dish.

13. The petri dish is placed into a drying oven for a few hours.
14. After drying, the resultant product is grinded using a mortar and pestle.
15. The prepared sample was then characterized using different techniques.

3.4 Synthesis of Composite

After the successful synthesis of Fe-Mil-88B-NH_2 and Cu @ CNT nanoparticles, the composite was prepared by combining them. In-situ synthesis of composite was carried out. The Cu @ CNT nanoparticles were added during the synthesis of Fe-Mil-88B-NH_2 [36]. The desired composite was achieved upon the completion of the reaction. The chemicals & materials, and apparatus which were used are as follows:

3.4.1 Chemicals and Materials

The chemicals and materials used for this process are mentioned as follows.

- Ferrous chloride hexahydrate ($FeCl_3.6H_2O$)
- 2-Aminoterephthalic acid (NH_2BDC)
- Dimethylformamide (DMF)
- Deionized water (DI water)
- Copper @ Carbon Nanotubes (Cu @ CNT) nanoparticles

3.4.2 Apparatus used

The following apparatus was used to carry out the synthesis of composite.

- Magnetic stirrer
- Hot plate
- Weighing balance
- Aluminum foil
- Spatula

- 10ml pipette
- 100ml beakers
- Sonicator
- Drying oven
- Vacuum oven
- Centrifuge machine
- Falcon tubes
- 10ml measuring cylinder
- Mortar and pestle

3.4.3 Procedure

The procedure for the synthesis of the composite is as follows:

1. First, the solution of ferrous chloride is prepared.
    a. 2.16g of $FeCl_3.6H_2O$ is measured using a weighing balance and transferred to the 100ml beaker.
    b. 7.6ml of DI Water is measured using as pipette and transferred into the beaker.
    c. 12.4ml of DMF is measured using a measuring cylinder and transferred into the beaker.
    d. Stirring is carried out using a magnetic stirrer on a hot plate until a uniform solution is obtained.
2. The solution consisting of NH_2BDC, and Cu @ CNT nanoparticles is now prepared.
    a. 0.72g of NH_2BDC is measured using a weighing balance and transferred into a separate beaker.
    b. 20ml of DMF is then measured using a measuring cylinder and transferred into the beaker.
    c. Sonication is carried out to break the clumps of ligand and form a uniform solution.
    d. 216 mg (0.216 g) of CNT @ Cu nanoparticles are measured using a weighing balance and transferred into the beaker.
    e. Sonication is again carried out to disperse the nanoparticles.

3. The prepared solutions are then transferred to a single beaker.
4. The beaker is covered using aluminum foil and stirring is carried out for 24 hours.
5. The solution is then equally transferred (by mass) into two falcon tubes and weighed to ensure equal mass distribution.
6. The solution is then centrifuged at 4000 rpm for 10 mins.
7. Subsequent washing is carried out thrice using DI water and then twice using ethanol.
8. The resultant product is dissolved in ethanol and poured into a petri dish.
9. The samples are dried at 60°C in a drying oven for 3 hours.
10. The dried samples are then grinded using a mortar and pestle to obtain a fine powder.
11. The powders are again transferred to petri dishes with aluminum foil at the bottom and placed in a vacuum oven at 60°C for 24 hours.
12. The samples are now ready for characterization and stored in a vial.

3.5 Bacterial Culture of Mixed Colony

The mixed bacterial colony was obtained from a sample of sludge collected from the NUST Membrane Filtration Plant. The bacterial culture of mixed colony was carried out in Minimal Defined (MD) medium [38]. The chemicals & materials, apparatus, and procedure used are discussed below.

3.5.1 Chemicals and Materials

The chemicals and materials that were used for this process are as follows.

- Glucose ($C_6H_{12}O_6$)
- Deionized Water (DI Water)
- Ammonium Chloride (NH_4Cl)
- Calcium Chloride ($CaCl_2$)
- Sodium Bicarbonate ($NaHCO_3$)
- Sodium Chloride (NaCl)
- Magnesium Chloride ($MgCl_2$)
- 70% Ethanol

3.5.2 Apparatus used

- 15ml Falcon Tubes
- Magnetic Stirrer
- Hot Plate
- Weighing Balance
- Aluminum Foil
- Spatula
- 500ml Media Bottle
- Syringes
- Incubator
- 500ml Beaker
- Filter Paper

3.5.3 Procedure

The Minimal Defined medium was first prepared followed by the culturing of bacteria according to the mentioned procedure [38]. However, glucose was used instead of yeast as the carbon (nutrient) source. All work related to bacterial cultivation was carried out in a clean environment inside a laminar flow hood.

1. The minimum defined medium is first prepared for the growth of the bacteria.
    a. 500ml of DI Water is measured into a beaker.
    b. The salts are first weighted using a weighing balance in the following proportions:

Chemical	**Mass (g)**
Ammonium Chloride (NH_3Cl)	0.10
Calcium Chloride ($CaCl_2$)	0.04
Sodium Bicarbonate ($NaHCO_3$)	1.25
Sodium Chloride (NaCl)	5.00
Magnesium Chloride ($MgCl_2$)	0.10

    c. The salts are transferred into the beaker containing the DI water.

d. The glucose concentration was kept as 20 mM in the medium. 100 g of glucose was added to the beaker.
e. Stirring is carried out using a magnetic stirrer on a hot plate until a uniform solution is formed.
f. The magnetic stirrer is extracted, and the salt solution is transferred to a media bottle which is sealed.
g. The medium is autoclaved and stored.

2. UV light is turned on inside the workbench to kill any bacteria present.
3. After 15 minutes, the UV lamp is turned off and the ethanol burner is lighted. The workbench is now ready for carrying out bacterial culture.
4. Using a pipette, 10 ml of the MD medium is transferred into a falcon tube.
5. The fresh bacterial culture is filtered using a filter paper to remove the sludge particles.
6. The remaining volume of the falcon tube is filled with the filtered bacterial solution using a syringe.
7. The falcon tube is then sealed and placed inside the shaking incubator, a temperature of 37°C is maintained.

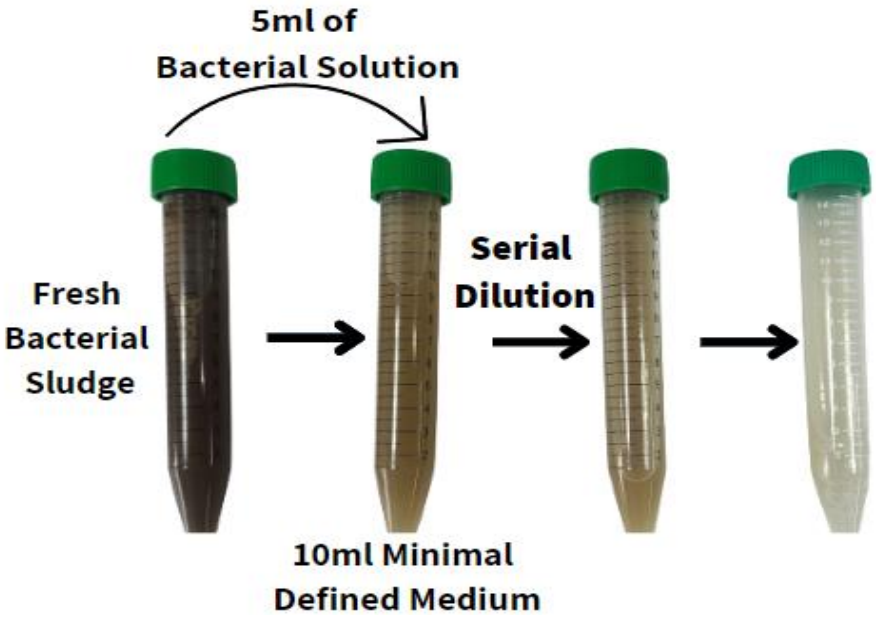


Figure 19: Bacterial culture of mixed colony

8. The workstation is cleaned using an ethanol spray.

9. The apparatus is first washed with water and then rinsed thoroughly with ethanol.
10. The apparatus is then autoclaved to kill any remaining bacteria present on the apparatus. Subsequent cultures can be prepared using the prepared culture and repeating the same procedure.

3.6 Bacterial Culture of Pure Strain

Pure strain of Shewanella xiamenensis was obtained from the Institute of Environmental Sciences and Engineering (IESE) in the form of a frozen stock tube. The bacterial strain was grown in the Luria-Bertani (LB) broth. The cultured bacterial strain is then preserved in glycerol medium at -20 °C for future use.

3.6.1 Chemicals and Materials

The chemicals and materials that were used for bacterial culture are discussed below.

- Distilled water
- LB medium powder
- Bacterial Strains
- Ethanol Spray
- 50% Glycerol

3.6.2 Apparatus used

- 500 ml Media Bottle
- Cryogenic Vials
- Pipette
- Laminar Flow Hood
- Freezer
- Falcon Tubes
- Spatula
- Incubator

- 1000 µL Micro-pipette
- Media Bottle
- Weighing Balance
- Autoclave
- Sonicator
- Ethanol Burner

3.6.3 Procedure

1. The LB broth is first prepared in distilled water.
    a. The media bottle is autoclaved at 120 °C for 30 mins.
    b. 12.5 g of LB medium powder was weighed using a weighing balance and transferred to the media bottle.
    c. A measuring cylinder was used to measure 500 ml of distilled water and transferred to the media bottle.
    d. The medium was sonicated to break any clumps.
    e. Once a suspension was formed, the solution was autoclaved at 120 °C for 30 mins.
2. The UV lamp is turned on to sterilize the work bench.
3. All equipment entering the work bench is first sterilized using an ethanol spray.
4. An ethanol burner is lit inside the work bench.
5. 10 ml of LB broth media is transferred to each falcon tube using a pipette.
6. Since the strains were obtained in form of frozen stocks, a spatula is used to extract them and inoculate them in the growth media.
7. The falcon tubes are sealed and placed inside the incubator at 37 °C. The rotation is also turned on at 80 rpm.
8. The work bench is sealed carefully, and the UV lamp is left on for at least 15 mins.
9. After 24 hours, the falcon tubes are checked. If the solution has become muddy, it means that the bacteria has been cultured.

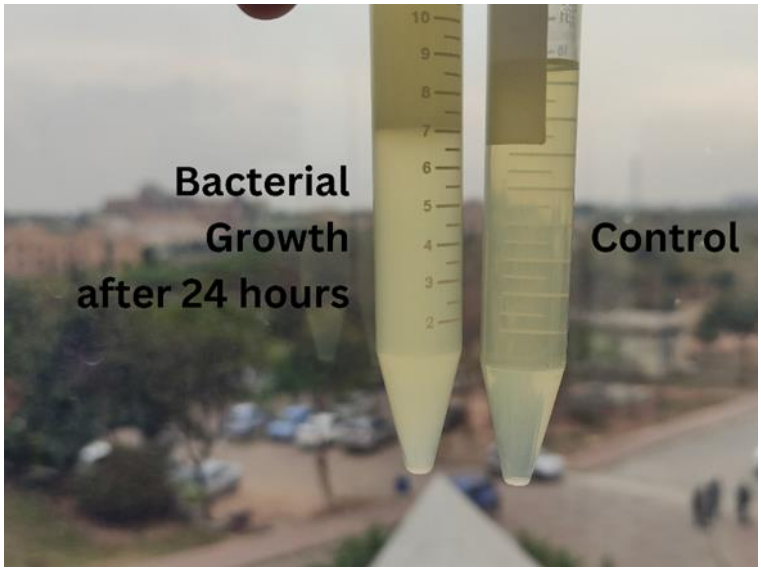


Figure 20: Growth of Shewanella Xiamenensis after 24 hours

10. The same protocol is followed to sterilize the work bench.
11. A micropipette is used to transfer 0.5 ml of 50% glycerol and 0.5 ml of cultured bacterial solution into a cryogenic vial using a micropipette.
12. The vial is then preserved at low temperatures inside a freezer.
13. All equipment is washed, rinsed with ethanol, and then autoclaved.

3.7 Electrolyte Preparation

The electrolyte used for electrochemical testing was composed of the minimal defined medium and the cultured bacterial cells.

3.7.1 Chemicals and Materials

The chemicals and materials that were used are discussed below.

- Minimal Defined Media
- Cultured Bacterial Solution (shewanella xiamenensis)
- Ethanol Spray

3.7.2 Apparatus used

- Pipette
- Laminar Flow Hood
- Falcon Tubes
- 1000 µL Micro-pipette

- Weighing Balance
- Autoclave
- Ethanol Burner
- Centrifuge Machine

3.7.3 Procedure

1. The falcon tubes consisting of the cultured bacteria are centrifuged at 4000 rpm for 10 minutes, the cultured bacteria settle the bottom of the tube.
2. The LB broth is then removed, and washing is carried out twice with the minimal defined medium.
3. The UV lamp is turned on to sterilize the work bench.
4. All equipment entering the work bench is first sterilized using an ethanol spray.
5. The ethanol burner is lighted.
6. Using a pipette, 4.5 ml of the minimal defined medium is transferred to the falcon tube and the culture is dissolved in it.
7. The resultant solution is then transferred to the reactor using a micropipette.
8. The electrolyte is now ready for electrochemical testing.
9. The work bench is sealed carefully, and the UV lamp is left on for at least 15 mins.
10. All equipment is washed, rinsed with ethanol, and then autoclaved.

Chapter 4

CHARACTERIZATION TECHNIQUES

4.1 Introduction

To identify the composition, morphology, structure, and performance of our synthesized material, several characterization techniques were used. Electrochemical studies were also carried out to determine if our material is suitable for bacterial detection. The techniques we used are briefly explained below.

4.2 Scanning Electron Microscopy (SEM)

A conductive sample surface's composition and topography can be determined using this highly sensitive imaging technique. It makes use of an incoming electron beam that interacts with the sample's surface to create backscattered and secondary electrons. The imaging software then uses these electrons to generate images consisting of vital information.

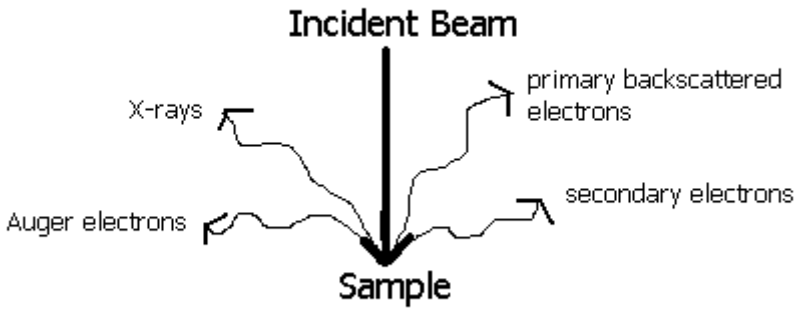


Figure 21: Interaction of incident electron beam with the sample

The electron gun, detector, focusing lenses, and sample chamber make up the SEM apparatus. High energy electrons are released by an electron gun which strikes the sample's surface. This electron beam is focused onto the sample by the focusing lenses, and the detector picks up the emitted secondary

electrons [39]. To stop the air molecules from obstructing the electron beam, vacuum is created inside the chamber using a vacuum pump.

4.3 Energy Dispersive Spectroscopy/ Energy Dispersive X-Ray analysis

EDX analysis is used to determine the sample's composition. It is a very sensitive method of compositional analysis that operates on the concept of distinctive x-ray emission. Electrons are knocked off when incident electrons strike the atoms on the surface of the sample. The atoms are ionized because of this and upon deexcitation they emit x-rays that are unique to the orbital energy at which the electron transition took place. As a result, the typical x-ray energy of each element is different. These emitted x-rays are detected using a semiconductor detector.

A major advantage of EDX is the ability to perform rapid elemental analysis and wide range of elemental coverage [40]. The position of each peak in the resulting spectrum identifies the element present, and the area beneath each peak indicates the relative number of atoms of the element in the examined region [41]. The element is then located, and the findings are provided by software. Similar apparatus assembly as SEM is used, however, additional amplifiers and detectors are also present.

4.4 X-Ray Diffraction

X- Ray Diffraction is a non-destructive technique which serves as a powerful tool for research. An x-ray beam is directed towards the sample; most X-ray beam interactions with the normal three-dimensional arrangements of atoms in a crystal result in destructive interference and cancel out one another, but in some specific orientations, the X-ray beam interactions result in constructive interference [42]. Hence, these diffracted x-ray beams create sharp peaks in the final pattern representing the crystal structure of the material.

While XRD is frequently used for qualitative and quantitative analyses of the crystalline phases in materials, much more information can be obtained by

carefully examining the diffraction patterns. These include the characterization of solid solutions, crystallite size and shape, crystal orientation, internal elastic strains/stresses at different levels, and effect of temperature on the sample [43].

4.5 Chronoamperometry

A common technique for tracking the generation of microbial current via electron transfer over time is chronoamperometry (CA). During CA experiments, the working electrode is poised to a fixed potential using a potenstiostat and the current is then measured against time. A three-electrode system is required because the reference electrode helps poise the working electrode at a fixed potential whereas the counter electrode accounts for the change transfer at the working electrode [44].

In simple terms, chronoamperometry counts the number of electrons transferring from the bacterial cell to the working electrode with respect to time or the rate of change transfer. Choosing the right ranges for the potential is crucial in achieving high accuracy, as too negative or positive potentials can lead to the oxidation or reduction of the electrolyte or the electrode. Most of the chronoamperometry measurements with various pathogens are carried out at +400 to +600 mV against the standard hydrogen electrode (SHE) [45].

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Introduction

Several characterization techniques were used to identify the proper composition and structure of our produced samples. This chapter investigates the results obtained for all the samples that were worked on and the discussion of the results is also present in this chapter.

5.2 Characterization of Fe-Mil-88B-NH_2

XRD, SEM, and EDX were performed to characterize our sample.

5.2.1 XRD of Fe-Mil-88B-NH_2

XRD was carried out to determine whether the synthesized Fe-Mil-88B-NH_2 had the required crystal structure. The results are shown in Figure 22. 3 sharp peaks can be observed between $9<2\theta<12$ which represent the (101), (002), and (102) planes. These peaks confirm the presence of a hexagonal space group which represents the structure of Fe-MIL-88B-NH_2 [46].

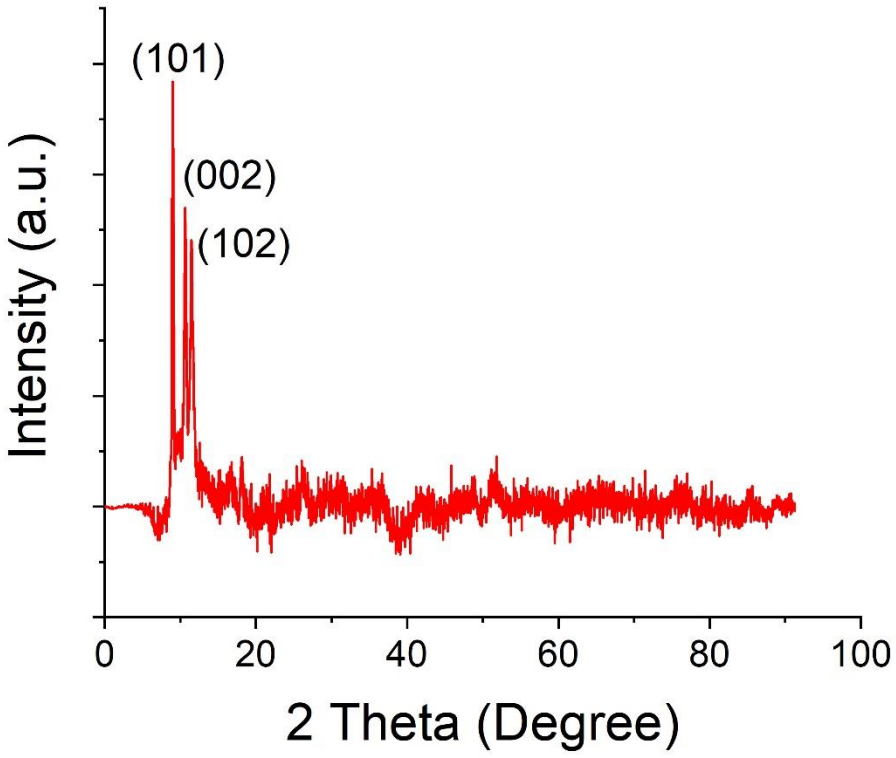


Figure 22: XRD results of Fe-Mil-88B-NH_2

5.2.2 SEM Analysis of Fe-Mil-88B-NH_2

SEM analysis was carried out to analyze the microstructure and morphology of the Fe-Mil-88B-NH_2. The SEM results in Figure 23 clearly show that microparticles of Fe-MIL-88B-NH_2 have been formed, it can also be seen that these particles have a bipyramidal hexagonal structure [47].

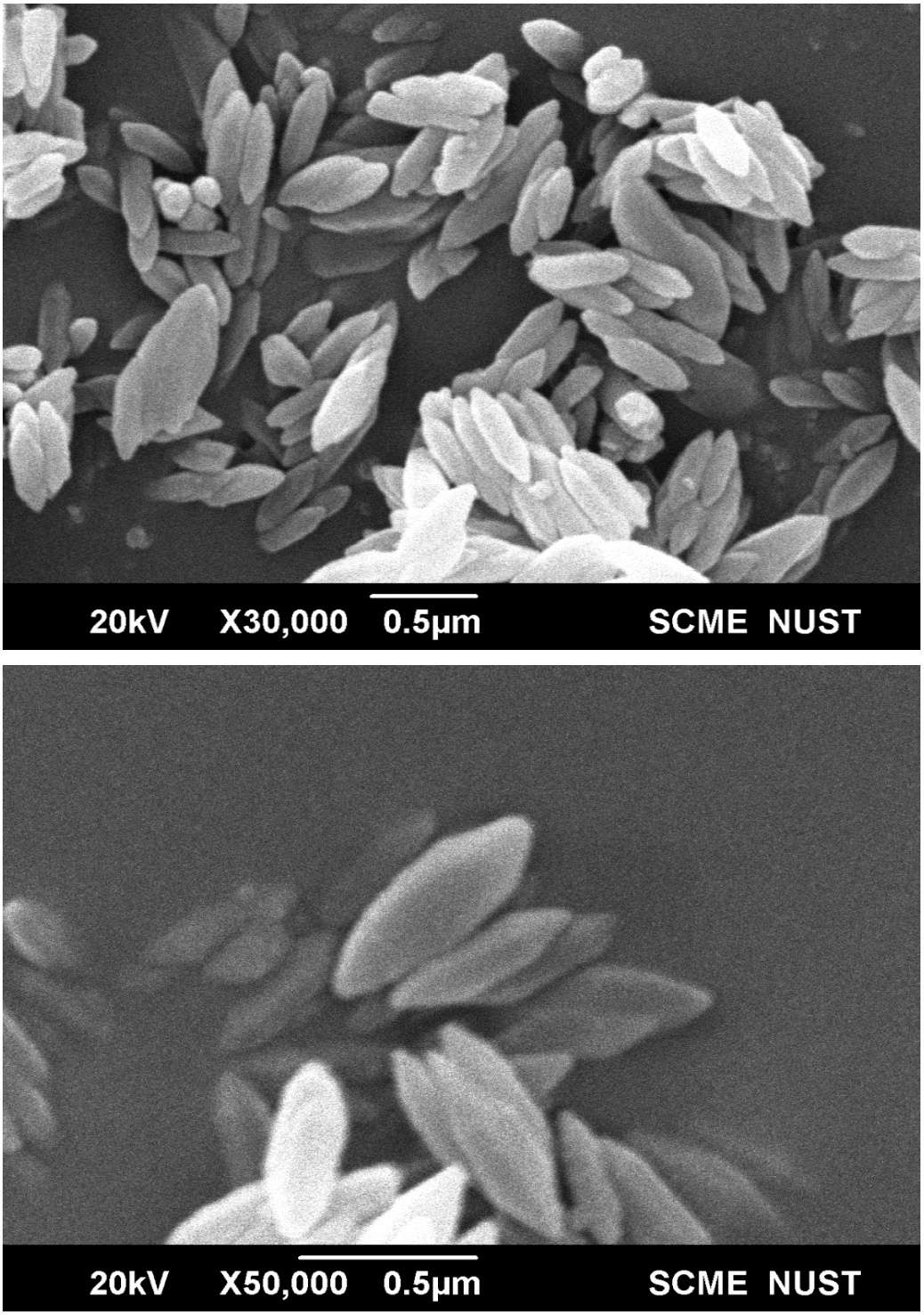
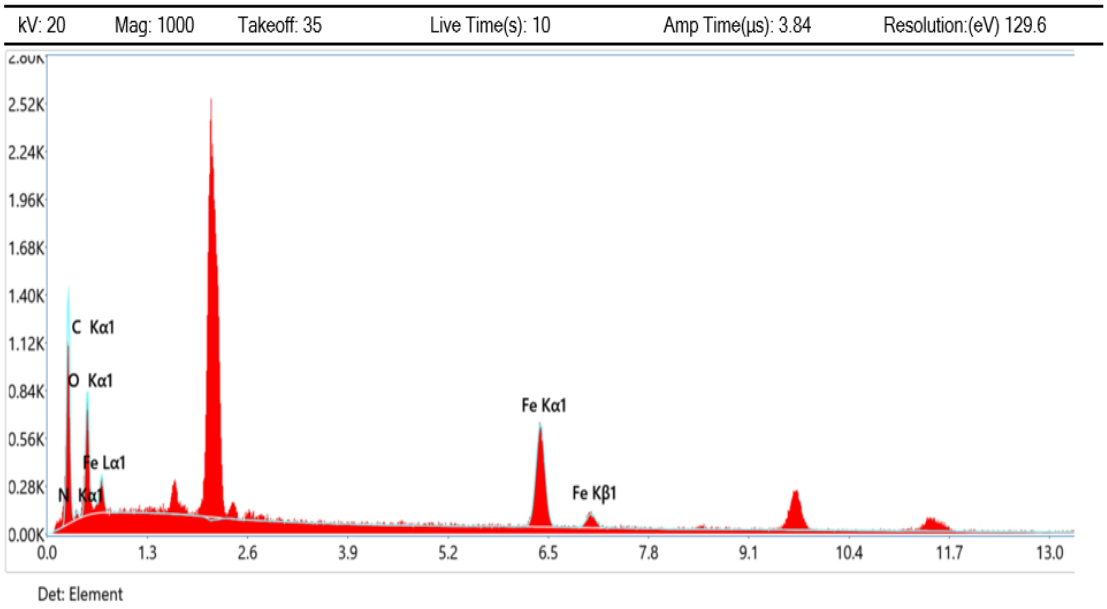


Figure 23: SEM micrographs of Fe-Mil-88B-NH_2

5.2.3 EDX of Fe-Mil-88B-NH_2

EDX was also carried out on the Fe-Mil-88B-NH_2 to identify the elements present and their relative percentages. Figure 24 shows the results of EDX performed. The EDS results confirm the presence of Iron, Carbon, Nitrogen and Oxygen as their peaks are clearly visible. Furthermore, it can be seen that 26.1 % of Iron is present.



Element	Weight %	MDL	Atomic %	Error %
C K	44.5	0.73	61.3	13.0
N K	4.1	2.50	4.9	66.9
O K	25.2	1.03	26.1	14.9
Fe K	26.1	0.76	7.7	5.4

Figure 24: EDX of Fe-Mil-88B-NH_2

5.3 Characterization of Copper @ Carbon nanotubes nanoparticles

To characterize and identify the presence of copper in our copper @ carbon nanotubes nanoparticles XRD, SEM, and EDX were carried out.

5.3.1 XRD of Copper @ Carbon nanotubes Nanoparticles

The presence of copper nanoparticles along with carbon nanotubes was confirmed by carrying out XRD analysis. Figure 25 shows the XRD results. A sharp peak was observed at 43° which indicates the presence of a crystalline material. The peak corresponds to the (111) plane of copper and indicates a cubic structure of copper. This was also confirmed by the standard diffraction card, file no: 00-001-1241.

On the other hand, the peak at 25° and 79° correspond to the (002) and (110) planes of carbon nanotubes respectively. The carbon nanotubes have a hexagonal lattice symmetry. This was confirmed by the standard diffraction card, file no: 00-058-1638. The peak corresponding to (002) plane is broad due to contributions from the intratube and intertube structure in multiwalled carbon nanotubes [48].

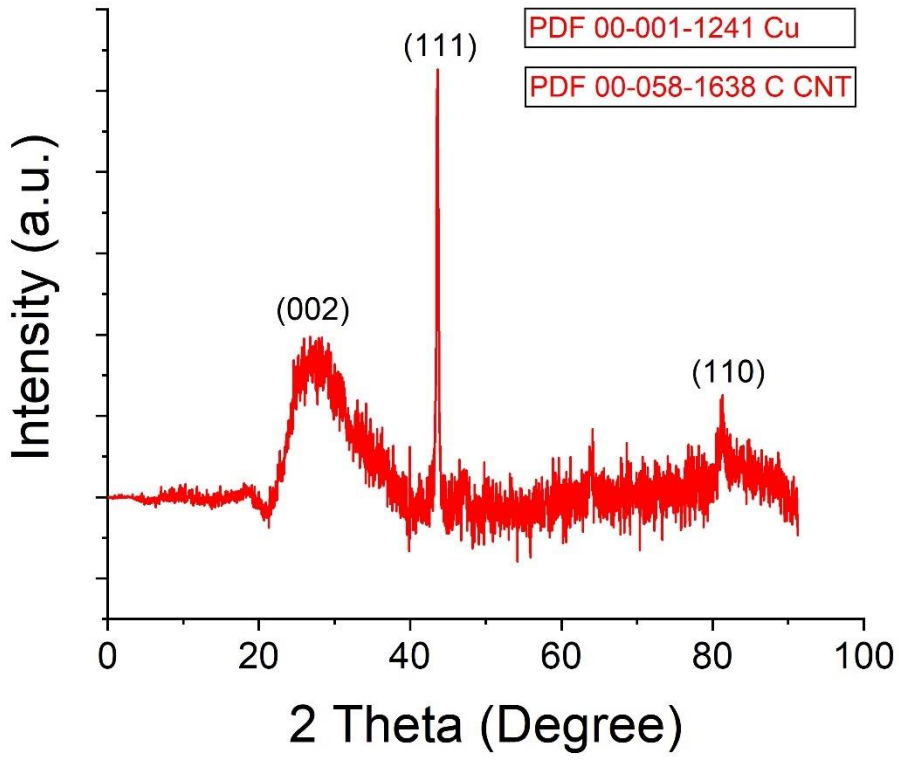
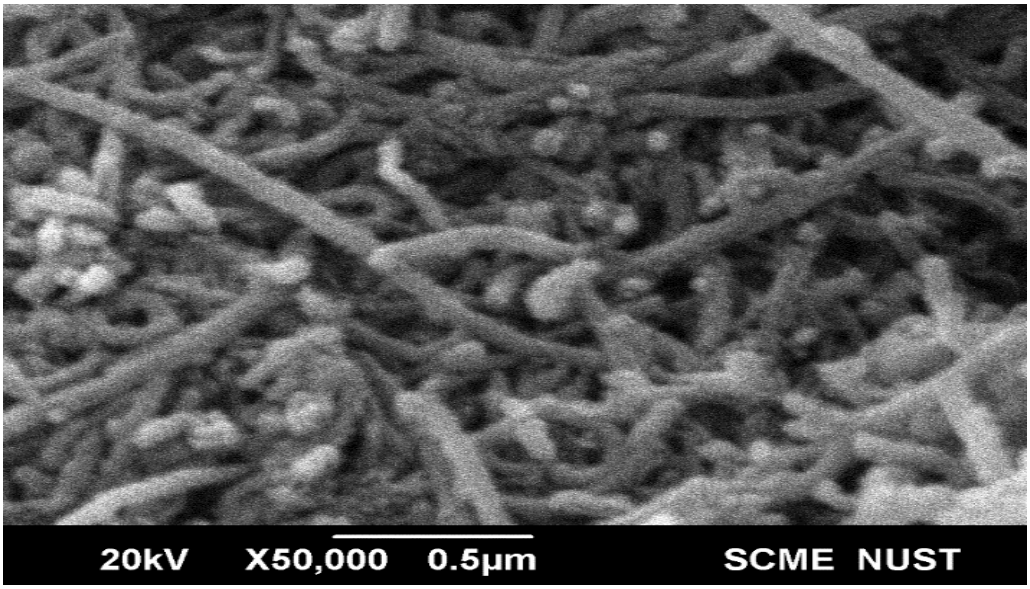


Figure 25: XRD of Cu @ CNT nanoparticles

5.3.2 SEM of Copper @ Carbon Nanotubes Nanoparticles

The results of SEM analysis can be seen in Figure 26 below. Rod like structures of carbon nanotubes can be easily observed in these images. The boundaries of the structures are easily distinguishable. Copper nanoparticles can also be seen on the surface of the carbon nanotubes.

The SEM images confirm the attachment of copper nanoparticles on the surface of the carbon nanotubes with the desired morphology.



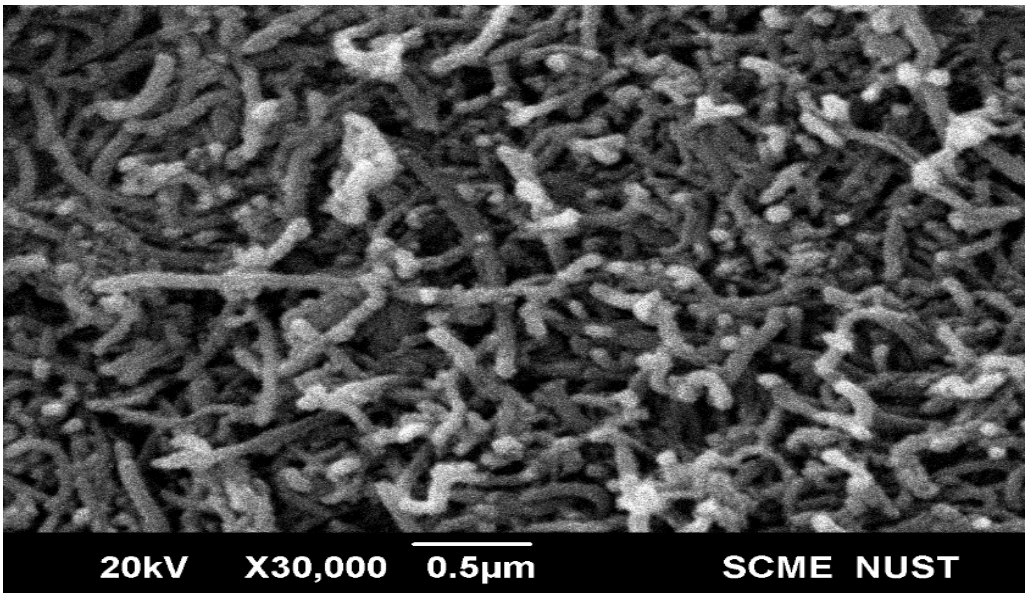
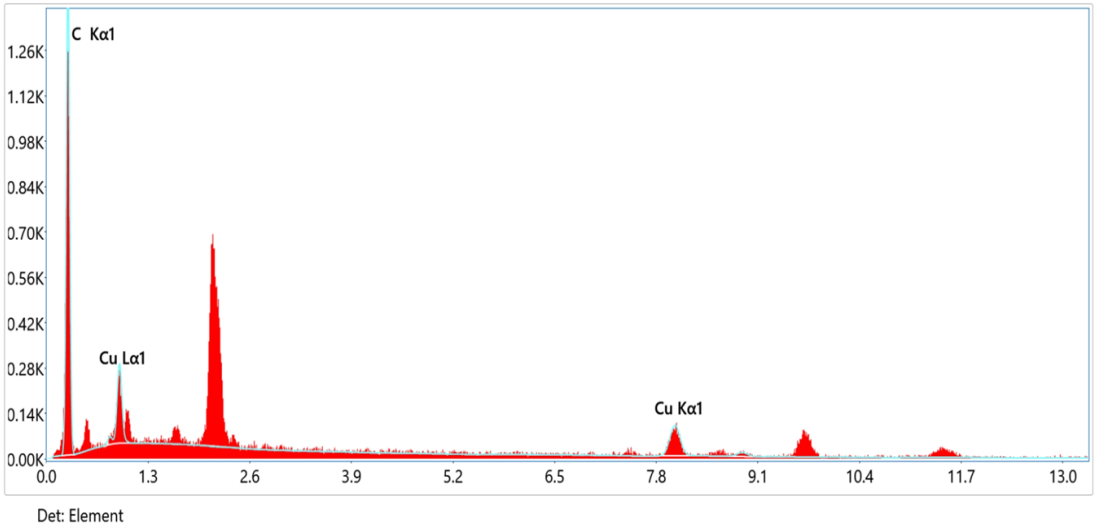


Figure 26: SEM results of Cu @ CNT nanoparticles

5.3.3 EDX of Copper @ Carbon Nanotubes Nanoparticles

The EDX results shown in Figure 27 further confirm the presence of copper and carbon as their peaks can be clearly seen. 83.9% carbon by weight whereas 16.1% copper is present.



Element	Weight %	MDL	Atomic %
C K	83.9	0.58	96.5
Cu K	16.1	1.46	3.5

Figure 27: EDX of Cu @ CNT nanoparticles

The obtained characterization results confirm the formation of both the Fe-Mil-88B-NH_2 and Cu @ CNT nanoparticles. The desired morphology has also been obtained.

5.4 Characterization of Composite Fe-Mil-88B-NH_2 @ Copper @ Carbon nanotubes

Once the structures of Fe-Mil-88B-NH2 and copper @ carbon nanotubes nanoparticles were separately analyzed and the required structures were obtained, the composite was analyzed using XRD, SEM and EDX analysis.

5.4.1 XRD of the Composite

Figure 28 shows the results of the XRD analysis of the composite. 3 sharp peaks can be observed between $9°<2\theta<12°$ which represent the (101), (002), and (102) planes. These planes confirm the presence of a hexagonal space group which represents the structure of Fe-MIL-88B-NH_2 [46].

The peak at 25° corresponds to the (002) plane of carbon nanotubes whereas the peak at 43° corresponds to the (111) plane of copper. The following XRD results

confirm the incorporation of copper nanoparticles and carbon nanotubes into the Fe-MOF composite.

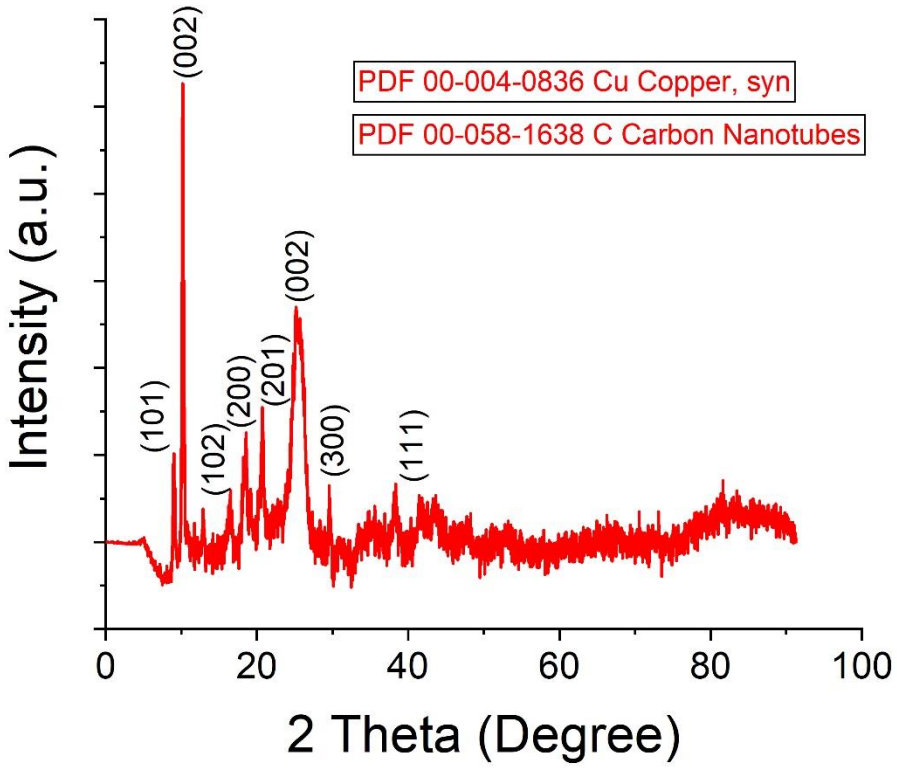


Figure 28: XRD of Composite

5.4.2 SEM Analysis of Composite

The SEM images in Figure 29 show a bipyramidal hexagonal morphology of Fe-MOF micro-crystals, hence the morphology of Fe-Mil-88B-NH_2 [47]. The Cu @ CNT nanoparticles should be decorated on the surface of Fe-Mil-88B-NH_2. The attachment of carbon nanotubes can be confirmed from these SEM images. However, copper nanoparticles are not visible due to their small size.

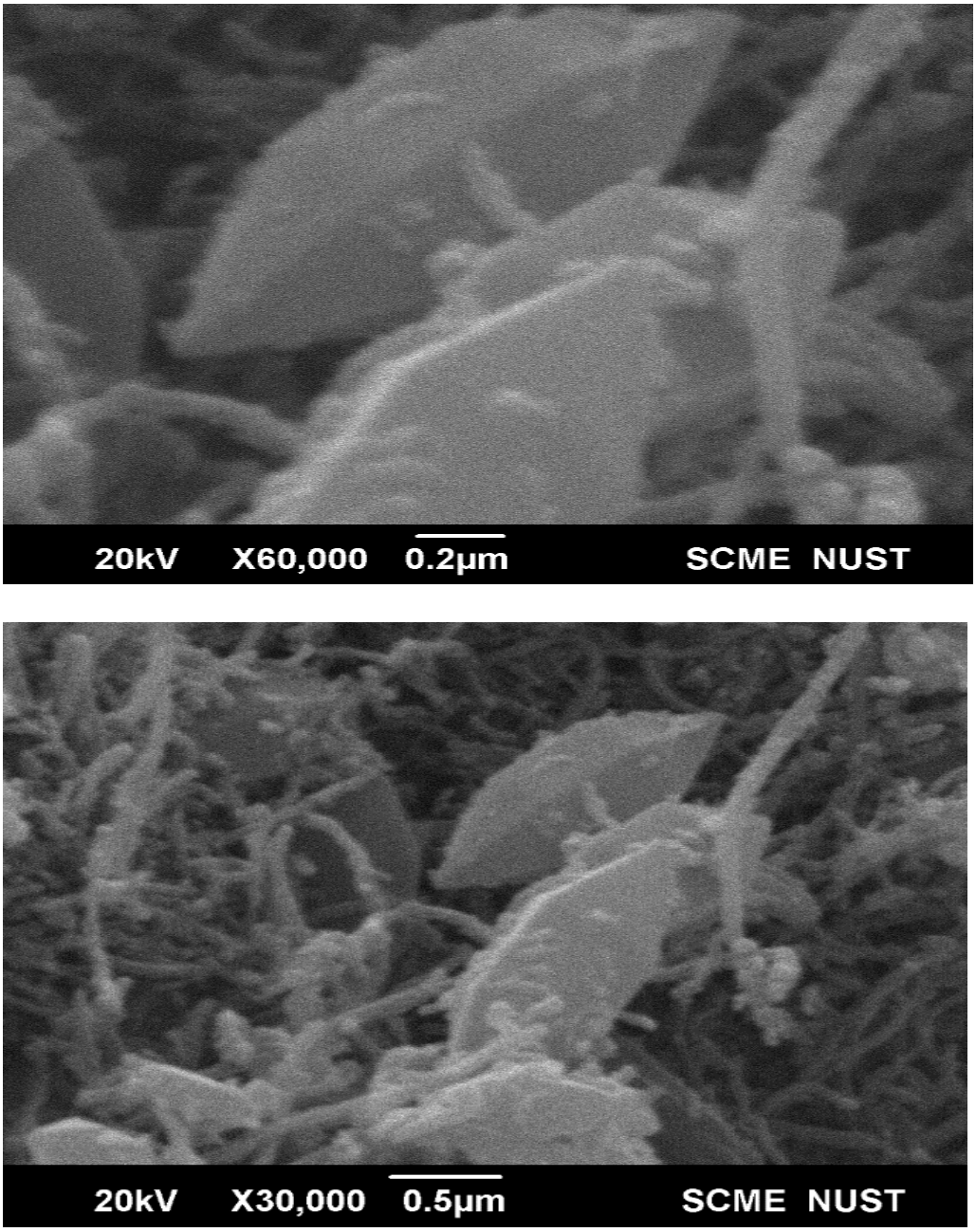
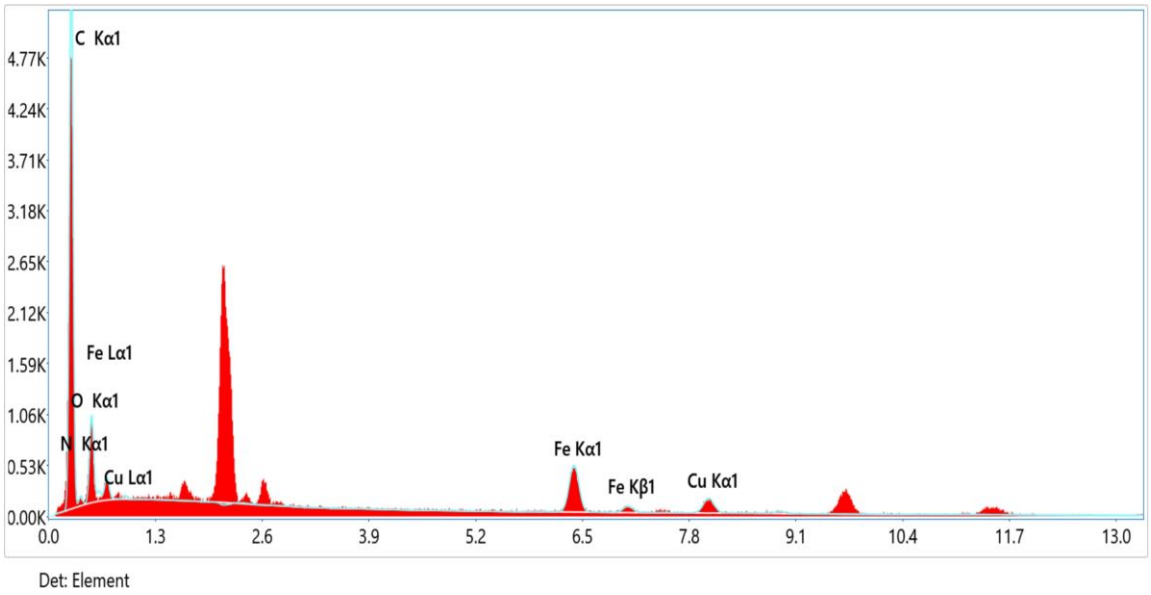


Figure 29: SEM results of composite

Although copper nanoparticles are not visible in the SEM image, the presence of copper can be confirmed by EDX analysis.

5.4.3 EDX Analysis of the Composite



Element	Weight %	MDL	Atomic %
C K	63.7	0.28	75.8
N K	4.7	1.69	4.7
O K	18.1	0.70	16.2
Fe K	8.6	0.33	2.2
Cu K	4.9	0.52	1.1

Figure 30: EDX of composite

The EDX results shown in Figure 30, confirm the presence of carbon, nitrogen, iron, copper, and oxygen. Nitrogen, oxygen, iron and carbon are present in Fe-Mil-88B-NH_2 whereas carbon is also present in the form of nanotubes. 4.9 wt% copper is present which confirms the formation of the composite.

5.5 Electrochemical Study

For the electrochemical testing, BioLogic potentiostat was used to perform chronoamperometry. Chronoamperometry was carried out to monitor the current production by bacteria and the effect on current production upon the addition of mediators and the composite. An optically transparent Fluorine doped tin oxide

(FTO) glass was used as the working electrode for all electrochemical testing that were carried out.

5.5.1 Reactor Design

Anaerobic conditions are fundamental for the bacteria to carry out extracellular electron transfer as in the presence of Oxygen, the bacteria will transfer its electron to Oxygen due to its strong oxidizing capability. Hence, the reactor used for EET studies should not allow any ingress of air and should be in the form of an airtight chamber. Moreover, the working electrode should be situated at the bottom of the reactor as bacteria have the tendency to settle at the bottom. If conventional hanging electrodes are used the bacterial attachment obtained would hence be reduced.

To meet the requirements of the reactor, we designed our own reactor. This reactor has a sealed chamber which provides anaerobic conditions for bacterial respiration. The working electrode is situated at the bottom in the form of a glass slide, whereas the reference and counter electrodes are inserted from the top and sealed using rubber stoppers. The walls of the reactor were made from acrylic and rubber gaskets were placed between the top wall and the bottom wall to prevent any leakages. The configuration of the reactor was held in place using steel screws, which also allowed for easy assembly and disassembly of the reactor.

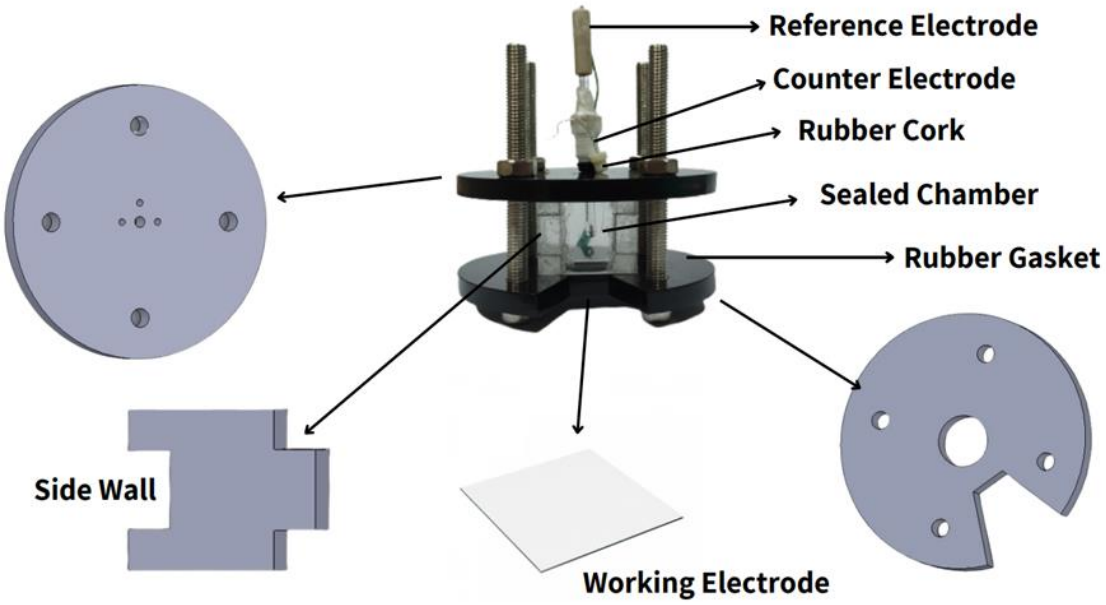


Figure 31: Reactor design

5.5.2 Setup for Electrochemical Testing

The electrochemical testing was carried out in the reactor that we designed as mentioned in the previous section. FTO glass was used as the working electrode, platinum wire as the reference electrode whereas Ag/AgCl was used as the reference electrode.

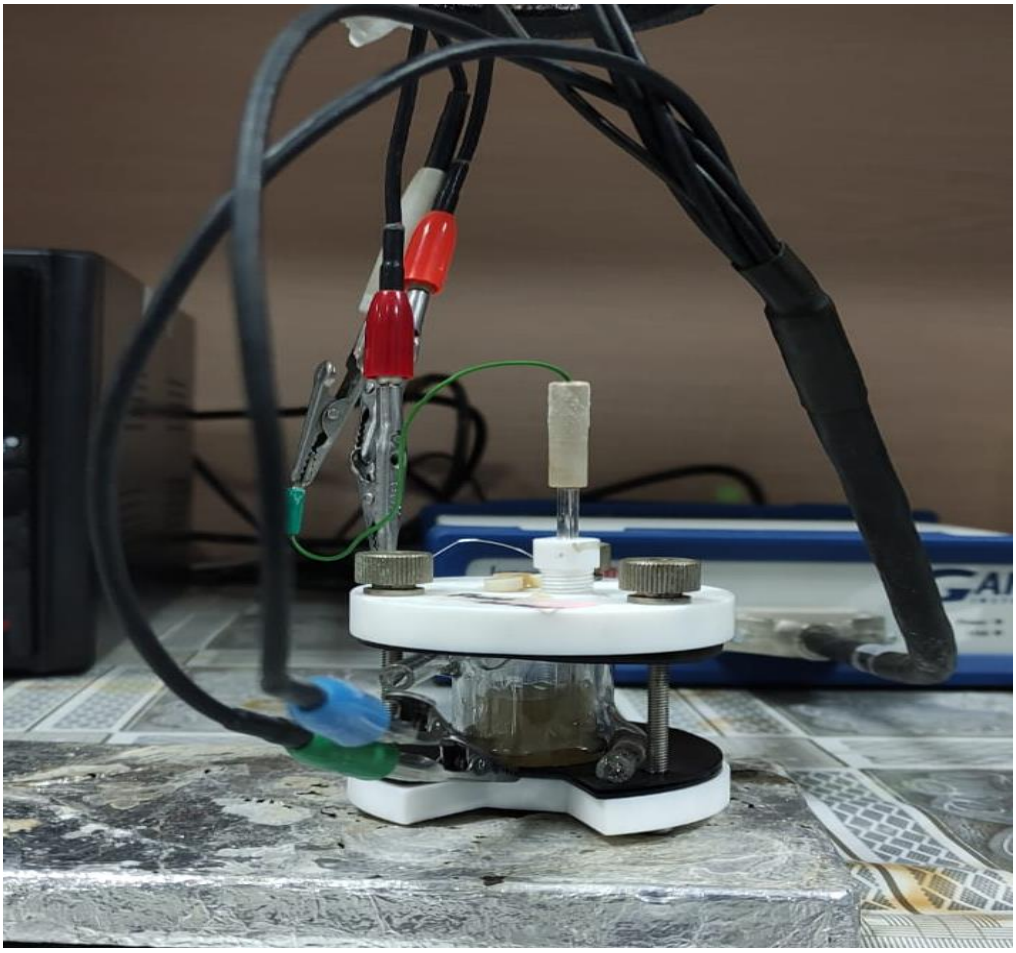


Figure 32: Electrochemical testing setup

5.5.3 Chronoamperometry of Mixed Bacterial Culture

Chronoamperometry was first carried out on a mixed culture of bacteria to obtain the proof of concept for extracellular electron transfer. Initially no current production was observed as confirmed by the graph obtained as shown in Figure 33, this is due to the presence of Oxygen as a soluble electron acceptor. After some time, an increase in the current is seen, this is because of oxygen scarcity and there is a point reached where there is no oxygen present – anerobic conditions are

created. Bacteria anaerobically respire and transfer the produced electrons directly to insoluble conductive solid (electrode). This phenomenon is known as Direct Electron Transfer (DET). Fluctuations in the current produced are also seen and there are multiple reasons that can be associated with it. Some of these reasons are: bacteria are capable of producing their own natural mediators, fluctuations in the amount of oxygen present and the difference in concentrations of oxygen in different layers of formed biofilm. The internal layers of a biofilm may have less concentration of oxygen which would promote anaerobic conditions.

As time passes, the amount of current produced starts to decrease because bacteria's activity decreases and the amount of nutrients available reduces. At this point, we add our mediator: Riboflavin after which we note an instantaneous spike in the current being produced. This is because bacteria that could not previously undergo DET can now transfer their electron by Mediated Electron Transfer (MET). An approximate increase of 91% in the current production is noted after the addition of riboflavin.

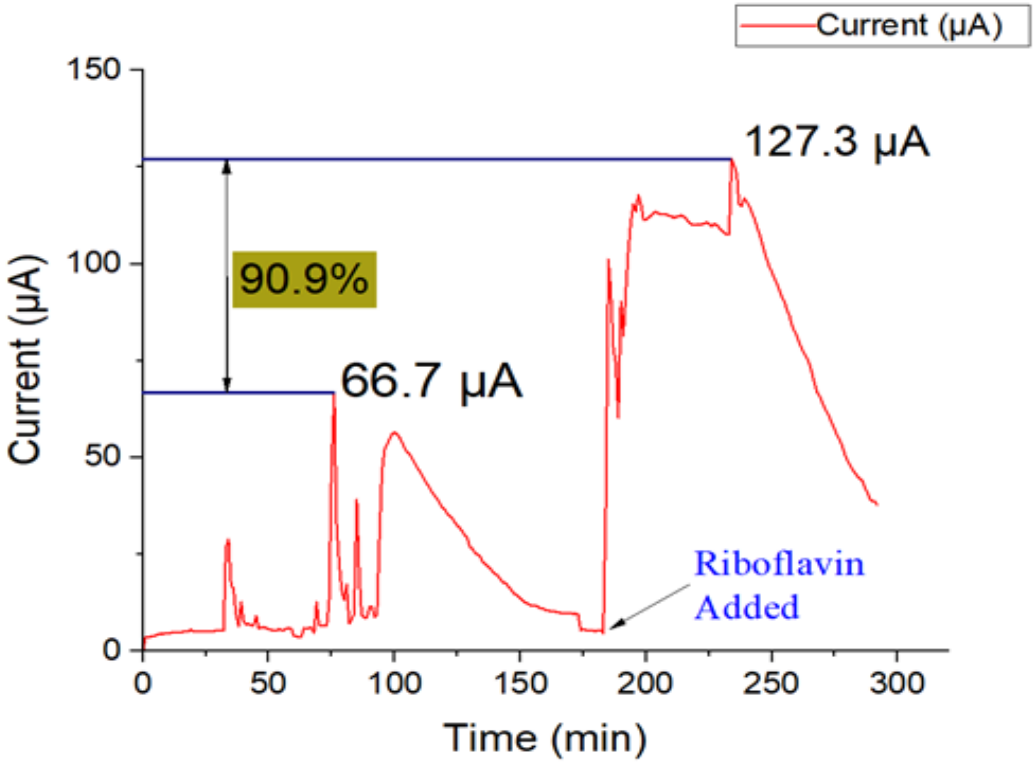


Figure 33: Chronoamperometry of mixed bacterial culture

5.5.4 Electrochemical Study of Mediators

The pure strain of shewanella xiamenensis was used to test the efficiency of the mediators. Riboflavin and 2-hydroxy-1,4-naphthoquinone (HNQ) were the mediators that were tested by chronoamperometry. The mediators were added into the reactor once the current production had stabilized during the chronoamperometry test. A concentration of 100 µM of mediator was added into the reactor.

Figure 34 shows the chronoamperometry graph obtained when HNQ was added to the electrolyte containing shewaenalla xiamenensis cells. Initially no significant current production was observed, however, the current started to increase immediately after the addition of HNQ. After one hour, an increase of 42.1% was seen in the current.

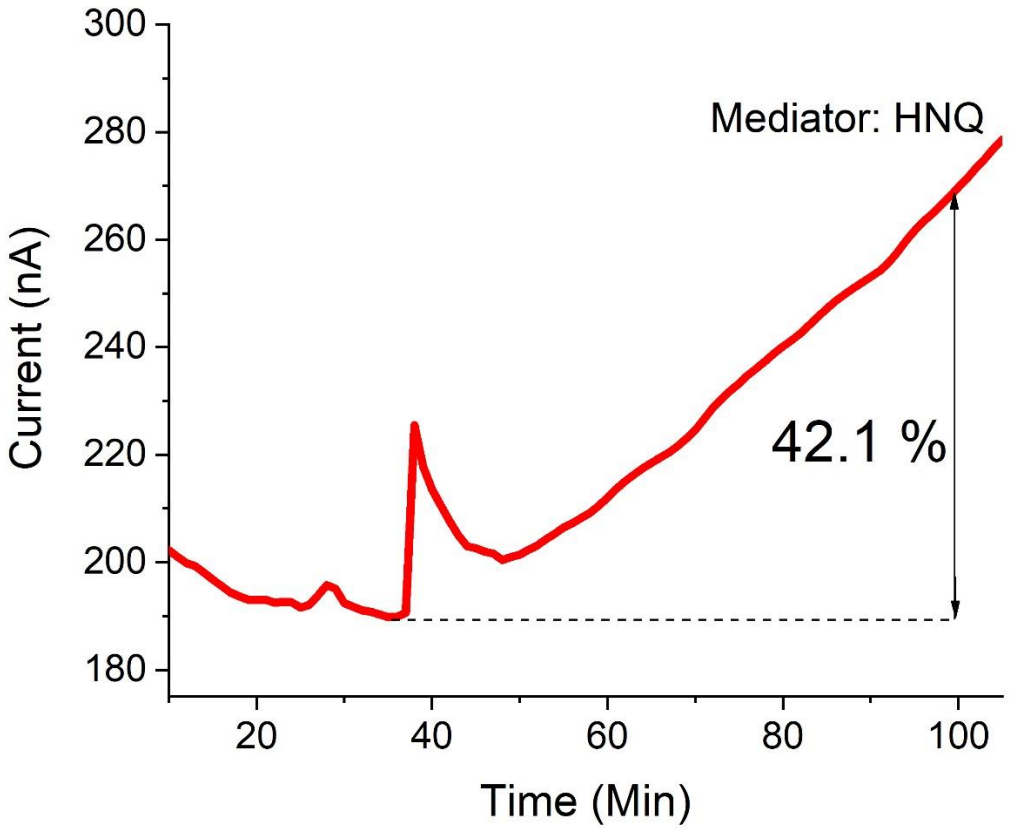


Figure 34: Chronoamperometry of shewanella xiamenensis with HNQ

The chronoamperometry experiment was then repeated and Riboflavin was added as the mediator. The Figure 35 shows the chronoamperometry results of shewanella xiamenensis with the addition of Riboflavin. Similar to chronoamperometry results obtained with HNQ, no current production was seen initially. However, a very rapid increase in current was seen upon the addition of riboflavin. After one hour an increase of 336.7% was seen in the current.

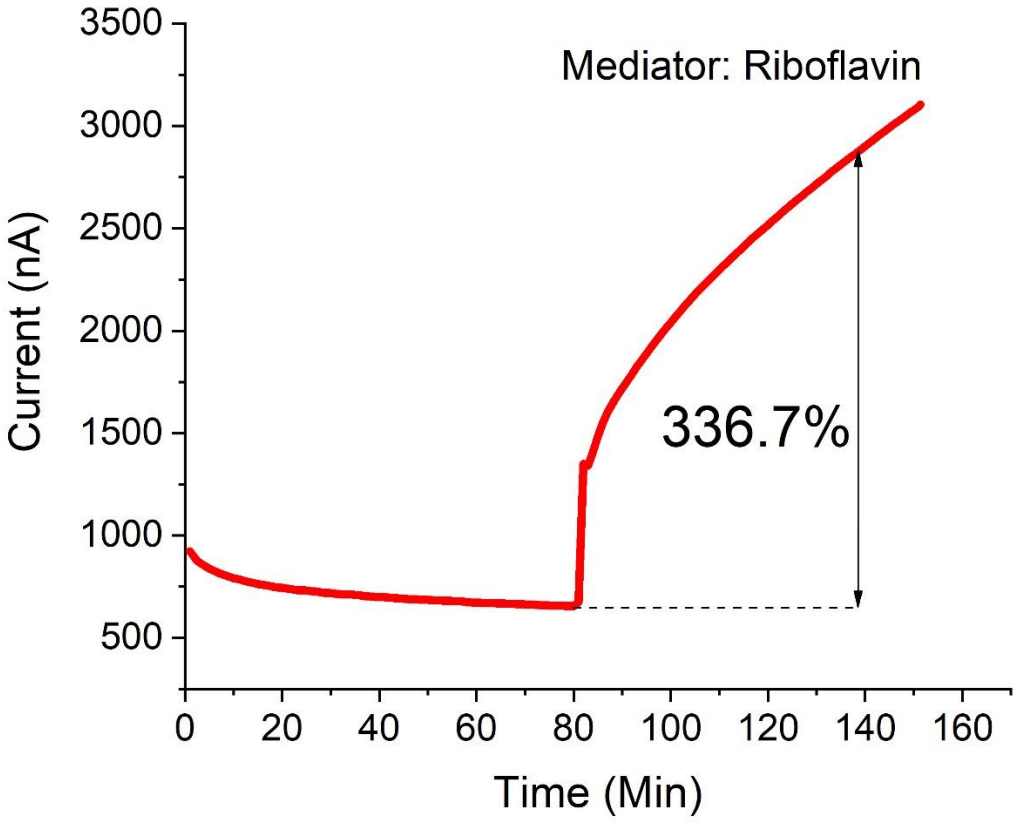


Figure 35: Chronoamperometry of shewanella xiamenensis with Riboflavin

Riboflavin proved to be more effective as it showed a much more rapid increase in current. The current increase observed one hour after the addition of riboflavin was much greater than the current increase after the addition of HNQ. A higher current increase is observed by riboflavin because most microorganisms secrete the flavin compound [49]. Hence, the riboflavin molecule can easily travel across the bacterial cell wall.

5.5.5 Electrochemical Study of Composite

The composites capability to enhance the current generation was observed by once again carrying out chronoamperometry. 5 mg composite was added in 1 ml DI water and sonicated. 0.5 ml of the prepared solution was added into the reactor once the current had stabilized. Initially, very low values of current are observed as only bacteria directly attached to the working electrode are generating current. Bacteria that are not attached to the electrode surface are electrically insulated from the electrode, hence they cannot carry out extracellular electron transfer [50]. Figure 36 shows the mechanism of long rage extracellular electron transfer in bacteria.

We hypothesized that the high surface area of our composite would result in increased attachment of the bacteria on the composite surface. The conductive nature of the composite would then allow for the bacteria to arrange themselves in a conductive inside the reactor. This would allow these bacterial cells to carry out long range extracellular electron transfer [50, 51]. The obtained results are shown in Figure 37. Upon the addition of composite to the reactor, a sudden spike in the current generation was seen. The current kept increasing even after one hour and an increase of 171.3% was seen after one hour. The highest increase in current was recorded as 331.2% immediately after the addition of the composite.

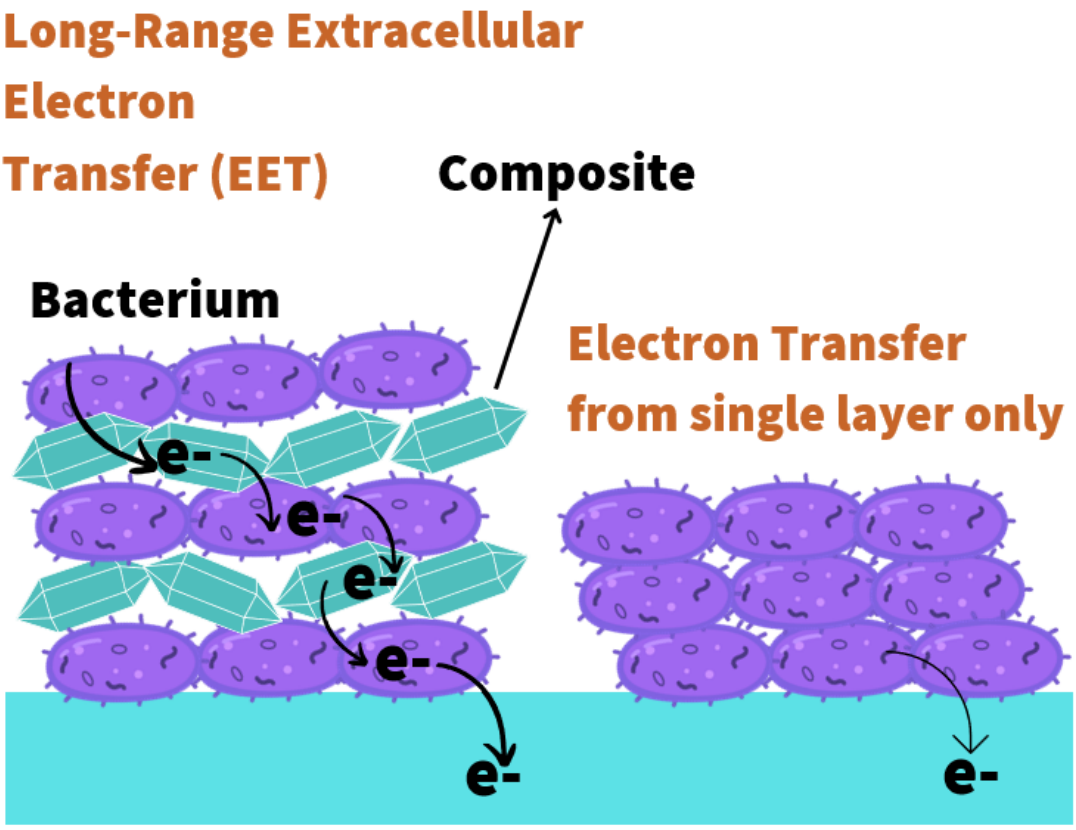


Figure 36: Mechanism of Long-Range Extracellular Electron Transfer

When the same experiment was repeated without bacterial cells, no change in current was seen upon the addition of the composite. This control experiment confirmed that the increase in current was observed because the addition of the composite enabled the bacterial cells to carry out long range extracellular electron transfer.

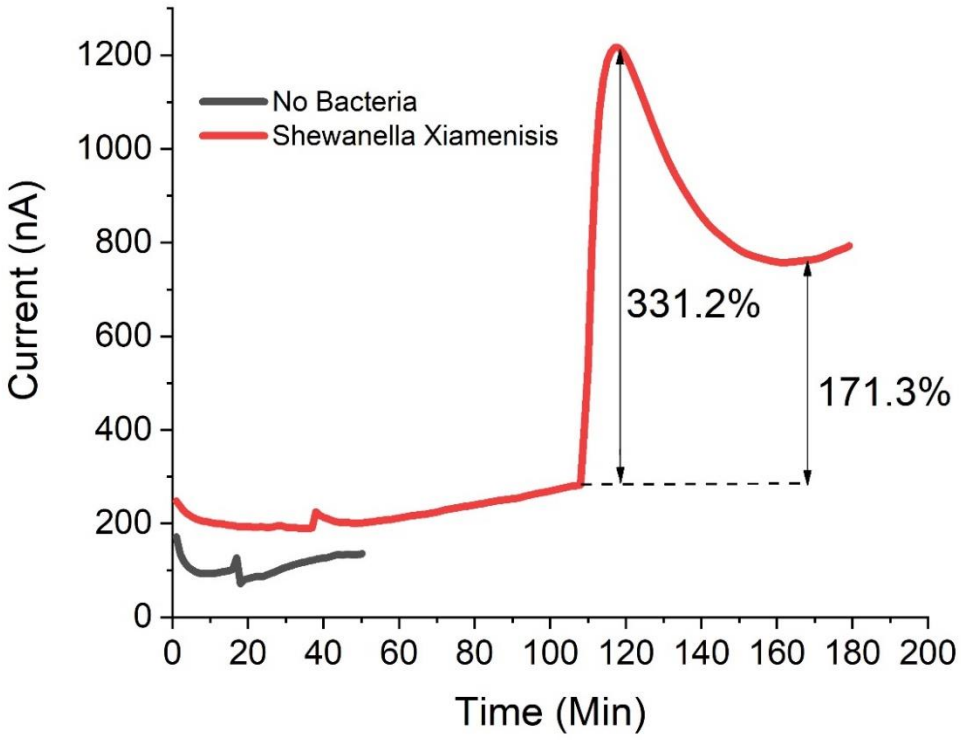


Figure 37: Chronoamperometry of shewanella xiamenensis with composite

CHAPTER 6

CONCLUSION AND FUTURE PROSPECTS

6.1 Conclusion

The results obtained show that our material can be used for rapid sensing of bacteria. The successful synthesis of a composite material which can amplify the current produced by the bacteria has been achieved. Additionally, amongst the mediators tested, Riboflavin has proven to be a sensitive and versatile mediator making it suitable for detecting various bacterial strains.

6.2 Future Prospects

Some further studies may be carried out on the same material to test its sensitivity and selectivity. Further chronoamperometry experiments with varying concentrations should be carried out to determine the efficiency of the synthesized material. The material should also be tested for longer periods of up to 24 hours along with cyclic voltammetry, which would provide a better understanding of the mechanism by which the current is enhanced.

Once such results are obtained, the material may be considered for more improvement and use in further applications.

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