Deanship of Graduate Studies

Al-Quds University

Microbial Fuel Cell with Carbon supported metal nanoclusters as electrodes

Lena Abedalkader Ali Qawasmi

M.Sc. Thesis

Jerusalem – Palestine

1435 / 2014

Microbial Fuel Cell with Carbon supported metal nanoclusters as electrodes

Prepared by:

Lena Abdelkader Ali Qawasmi

B.Sc. Biology/medical technology. Al-Quds University-Palestine

Supervisor: Prof. Mukhles Sowwan

Co- Supervisor: Dr Larisa Kiseleva

A thesis Submitted in Partial fulfillment of requirements for the degree of Master of Applied &Industrial Technology, Al- Quds University.

1435 / 2014

Al-Quds University

Deanship of Graduate Studies

Applied and Industrial Technology Program

Thesis Approval

Microbial Fuel Cell with Carbon supported metal nanoclusters as electrodes

Prepared by: Lena Abdelkader Ali Qawasmi

Registration No.: 21212636

Supervisor: Dr. Mukhles Sowwan

Co- Supervisor: Dr Larisa Kiseleva

Master thesis submitted and accepted, Date:

The names and signatures of the examining committee members are as follows:

1-Head of Committee: Prof. Mukhles Sowwan Signature _____

2-Internal Examiner: Dr. Wadie Sultan Signature _____

3-External Examiner: Dr. Jamal ghabboun Signarure _____

Jerusalem – Palestine

1430 / 2009

Dedication

My humble efforts I dedicate to my family and many friends. A special feeling of graduated to my loving parents

ABEDALKADER & IBTESAM

Whose affection, love, encouragement and Prays of day and night make me able to get such success honor.

Lena Qawasmi

Declaration

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:

Lena Abedalkader Ali Qawasmi

Date:

Acknowledgement

I would like to express my sincere appreciation to my supervisor Dr. Mukhles Sowwan not only for the support he gave me during my research, but most of all for providing the ability to work in a stimulating environment, eager of discovery and continuous learning. I sincerely thank him.

I also sincerely appreciate Dr. Larisa Kiseleva for being an extraordinary Co-supervisor who helped to get me started on the path and reach this degree.

I gratefully acknowledge Dr. Cathal Cassidy and David Simpson for their hard work. They were always available for my questions and they were positive and gave their time and vast knowledge generously.

I'm also grateful to Dr. Jeong-Hwan Kim, Dr. Alexander Porkovich, Dr. Kengo Aranishi, Murtaza Bohra and Vidya Singh at OIST Graduate University for helping me out in this research and for providing me many details and valuable comments.

I must also thank my colleagues in the Nanotechnology Research Laboratory for all the help they've provided me, especially Ms. Maryam Faroun and Eng. Riham Muzaffar.

Special thanks to my dear friends Eng. Neveen Qawasmi, Rawan A.Ghieth and Siham Jubran, May Baker, Jehan A. Sbaieh, Alaa Qawasmi, Abeer Karmi, Basma Halbya, Amani Maswadeh, and Ruba Qawasmi for the incorporeal support to me and to be with me in achieving my dream of obtaining a master degree.

I convey special acknowledgement to my sister in low Dr. Rawan Nassar and for Ms. Areej Ashhab for special help and support.

Finally I never forget to thank my caring family, Dr. Ayman, Dr. Feras, Eng.Tareq, Eng. Hani, Eng. Akram, Dr. Kareem, Nora, Dr. Rawan, Runa, Shahd, Anya, Judy, Yahya and my best friend Abood. Thank you all.

Abstract:

Microbial Fuel Cells (MFCs) are considered a source of green and sustainable energy for electricity generation. They are capable of converting chemical energy available in organic materials to electrical energy using living microorganisms as biocatalysts. In the present study, Au and Pd nanoparticles (NPs) decorated graphite electrodes by using DC magnetron sputtering *w*ere developed and evaluated in newly designed MFCs. Scanning Electron Microscope (SEM) was used to examine the morphologies of the electrodes before and after deposition of NPs. Metagenomic analysis was performed to identify the microbial species which detected highly diverse taxonomic composition of microorganisms embracing 28 bacterial phyla. It's found that decorated graphite electrode with high coverage of Pd NPs produced power less than that produced by plain graphite cathode while graphite cathode with low coverage gave same amount of power produced by the plain one. However, the cathode with medium coverage gave power enhancement 25-50% more. Moreover, MFC with Au NPs modified graphite electrode was developed. This modification of the anodes or cathodes promoted the electricity generation up to 9-15 folds higher than control. These results demonstrated that nano-decoration could greatly enhance the performance of microbial electrodes.

الملخص

خلايا الوقود الميكروبية وتدعيم أقطاب الكربون بحزئيات نانونية

خلايا الوقود الميكروبية هي أحد أنواع الطاقة الخضراء التي تقوم بتحويل المواد العضوية الى كهرباء بتحفيز من الميكروبات. في هذه الدراسة, جزيئات نانوية من البلاديوم والذهب تم ترسيبها على سطح اقراص الجرافيت الخاص بالخلايا بإستخدام DC magnetron sputtering لدراسة تأثير الجزيئات في انتاج الطاقه. كما قمنا بدراسة كل سطوح الجرافيت قبل و بعد تعديلها بالجزيئات النانونية من خلال المجهر الإلكتروني الماسح (SEM) لتقدم تحليل واضح للنتائج التي ظهرت. ولمعرفة المجتمع الميكروبي الموجود في خلايا الوقود الميكروبيةالتي تعمل بمثابة الحافز الحيوي في الخلايا تم استخدام التحاليل الميتاجينومية (metagenomic analysis), ووجد انواع كثيرة من الكائنات الدقيقة الموجودة في الخلايا التي تم استخدامها حيث وصل عددها الى 28 عائلة من البكتريا. أظهرت النتائج أن توليد الطاقة عند ترسيب جزيئات البلاديوم لمدة دقيقة كان مماثل لعمل الخلايا قبل تعديل اقراص الجرافيت بالجزيئات , وعند ترسيب الجزيئات لمدة أربع دقائق كانت الطاقة الناتجة أقل , لكن عند ترسيب لمدة دقيقتين و ثلاث كانت الطاقة 25 50%. و تم الحصول على نتائج مذهله لجزيئات الذهب حيث وصلت الطاقة المنتجة من الاقراص المعدلة أكثر بخمسة عشر ضعفا. فمن هذه النتائج يظهر لنا أن أستخدام الجزيئات النانونية ممكن أن تؤثر بشكل واضح على عمل خلايا الوقود الميكروبي.

Table of Contents

List of Tables

List of Figures

Abbreviations

MFC	Microbial Fuel Cell
NPs	Nanoparticles
SEM	Scanning Electron Microscope
EDX or EDS	Energy-dispersive X-ray spectroscopy
PEM	Proton-exchange membrane
MEC	Microbial Electrolysis Cell
Au	Gold
DNA	Deoxyribonucleic Acid
Pd	Palladium

Chapter ONE

Introduction

Chapter ONE

Introduction

The drawbacks of rapid industrial development include (i) excess use of fossil fuels and (ii) production of large quantities of waste products (Surana, 2006). These disadvantages can be balanced by exploring alternative renewable energy sources, and development of efficient waste-treatment systems.

It is evident that to protect and maintain healthy environment, the industrial, agricultural and municipal wastes should be treated before they are discharged into the waterways. Recently, biological processes – based systems, such as Microbial Fuel Cell (MFC) and Microbial electrolysis Cell (MEC) are the focus of intensive research and development in the field of renewable energy.

The main idea of MFC innovation consists in harvesting the electrons that particular microbial species release in the environment by utilizing organic compounds, and turning them into electrical current (Lovley, 2006). Thus, the ecological and economical benefits of MFC include removal of unwanted organics coupled with energy production. Microbial production of electricity may become an important form of bioenergy because microbial fuel cells offer the possibility of extracting current from a wide range of complex organic waste and renewable biomass (Lovley, 2008). Although the power output in such systems is rather

modest, the electricity produced during anaerobic respiration would be enough to support this wastewater cleaning system operation.

MFCs have operational and functional advantages over the technologies currently used for generating energy from organic matter. First, the direct conversion of substrate energy to electricity enables high conversion efficiency. Second, MFCs operate efficiently at ambient temperature. Third, an MFC does not require gas treatment because the off-gases of MFCs are enriched in carbon dioxide and normally have no useful energy content. Fourth, MFCs have potential widespread application in locations lacking electrical infrastructures and can also operate with diverse fuels to satisfy our energy requirements (Lui et al., 2007).

Basically, the MFCs can have a double-chamber (Figure 1.1A) or a single-chamber design (Figure 1.1B), depending on a source of oxygen for cathode reactions. For the present study the double-chamber MFC was designed. The most important operational issue in this configuration is choosing a separator that one hand allows protons pass through the chambers but on the other hand does not allow the substrate or electron acceptor pass in the cathode chamber (Logan et al., 2006). Regardless of the design, the principle of MFC operation is the same: microorganisms degrade organic substrates in the anodic chamber producing and releasing electrons and protons; electrons released by microorganisms captured by the anode and travel to the cathode through the external circuit; protons released by microorganisms travel to the cathode directly or diffusively through proton-exchange membrane (PEM). Electrons, protons and oxygen combine and form water in the catalytic reaction on cathode. Thus, given biodegradable waste as input, MFC produces water and electricity. The electrode reactions can be described by the following equations (Du et al., 2007):

(Anode) $CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$

(Cathode) $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$

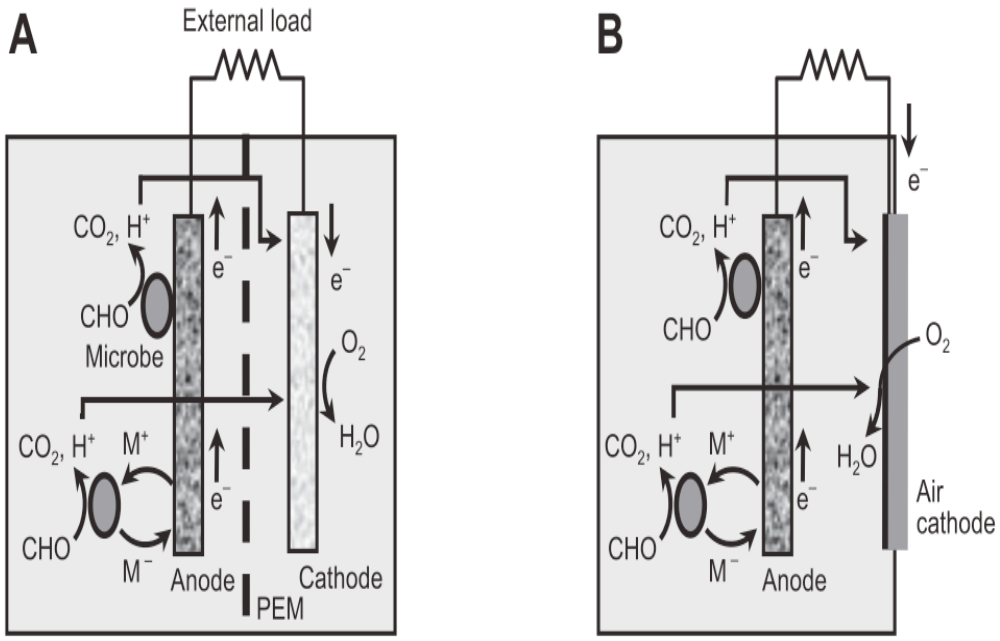

Figure 1.1: Typical microbial fuel cell configurations. (A) A double-chamber MFC, in which a cathode chamber is bubbled with air to supply oxygen for the cathode reaction. (B) A single-chamber MFC equipped with an oxygen-diffusion membrane cathode.

Cathode reactions may vary depending on the type of electron acceptor and catalyst. In the double-chamber MFC design, the catholyte consists of an electron acceptor solution. Although air oxygen is the most commonly used electron acceptor, other oxidants, such as ferricyanide or permanganate, can also take this role (Logan et al., 2006). High-energy aqueous catholytes

including ferricyanide are particularly useful in the small-scale MFCs for fast test experiments, as no cathode catalyst required, but not practical for industrial-scale systems since they must be chemically regenerated or replaced. In this study, ferricyanide was used as the oxidant in the cathode chamber. In any case, the MFC must be designed to keep the microbes separated from the catholyte, because additional electron acceptors in the anodic area will inhibit electricity generation.

The analyte consists of a substrate and the electrochemically active microbial community. In this study, the anolyte consisted of anaerobic sludge obtained from operating MFC bioreactors that treat wastewater from Okinawa local distillery, so it contained the microbial consortium proven to produce electricity and wastewater as a substrate for microbial activity. Another study was conducted by Mathuriya (2009) after using different substrate in MFC. He found out that wastewater from sugar industry had the best performance due to the availability of waste sugar in water which is rapidly oxidized and can generate good current in less time.

The main components of the MFC are the electrodes (anode and cathode) and if present, the proton exchange membrane to separate between them. The electrodes must be made of highly conductive but non-corrosive material, have high specific surface area, easily manufactured and scaled to larger sizes (Wei et al., 2012). Nowadays carbon- and graphite-based electrodes in form of sheets, cloth, rods, plates, foams or granules are commonly used in the MFCs. These materials have high conductivity and appear to be well suited for bacterial growth (Logan, 2007). In this study, two different types of nanoparticles were deposited on graphite electrodes, and the effect of nanoparticles distribution on power production was studied in the double-chamber MFCs. In previous studies they used different nanoparticles such as Fe, which

produced current up to 5.89-fold higher than plain graphite anodes (Xu et al., 2011). In another one they used Pd nanoparticles, which produced current 50-150% higher than the control (Fan et al., 2010).

The proton exchange membrane provides a physical and chemical barrier between the anolyte and catholyte. Protons selectively migrate from the anolyte to catholyte through proton exchange membrane to maintain charge balance during power generation (Franks et al., 2010). In this study, the nafion membrane separated anode and cathode chambers.

The microbial community inhabiting anodic compartment of the MFC plays a key role in both current generation and biodegradation (Logan, 2009). The system's electrical power output depends on the ability of bacteria to transfer electrons to solid extracellular electron acceptors. Usually, this ability is associated with dissimilatory metal reducing bacteria that conserve energy through extracellular electron transfer to solid conductive surfaces. In the natural environment these bacteria reduce metals such as Fe (III) and Mn (IV) to Fe (II) and Mn (0), respectively. In the absence or low concentrations of soluble electron acceptors, bacteria may transfer electrons to solid electron acceptors, i.e. anodes. Due to the capacity of extracellular electron transfer to chemicals or materials, directly or indirectly, the current-generating bacteria have been called exoelectrogens.

The diversity of bacteria capable of exoelectrogenic activity is just beginning to be discovered. Among already known exoelectrogens, *Shewanella* and *Geobacter* are the most studied bacterial genera in the field of extracellular electron transfer mechanisms. Based on extensive research of these model organisms, three types of extracellular electron transfer were described: (i) direct electron transfer via outer membrane c-type cytochromes; (ii) direct

electron transfer via nanowires; and (iii) indirect electron transfer via endogenously secreted flavins.

As it was mentioned, microorganisms release electrons for power generation; they also break down organic substances inflowing in the anodic compartment. The range of components that can be utilized in the MFC depends on metabolic potential of microbial community. Usually, more complex and diverse communities have greater metabolic capacities to treat given wastes. In this study, we identified biodiversity and structure of microbial community from successfully operating MFC bioreactor that was used for inoculation our experiment MFCs.

Most of the studies of MFC microbial communities have used DNA fingerprinting techniques such as terminal restriction fragment length polymorphism (TRFLP), denaturing gradient gel electrophoresis (DGGE), and clone libraries. All these methods are based on PCR amplification of a target gene, usually 16S rRNA, and until recently were the standard in microbial identification. However, rapid development of high-throughput sequencing techniques, often called next-generation sequencing, allowed much deeper analysis of environmental microbial consortia and non-culturable microorganisms. Compared to traditional approaches, shotgun sequencing provides more direct, quantitative and accurate picture of microbial community composition. In present work, whole community genomic DNA shotgun sequencing method was applied to identify microbial diversity and relative ratio of bacteria from MFC anolyte.

In order to improve efficiency of MFC two main aspects of this system should be considered: materials selected for the electrodes and microbial community serving as

biocatalyst. Therefore, in this work we studied both the properties of electrodes modified by different materials nanoparticles and the structure of microbial community of the MFC.

Research objectives:

(i) To assemble and run functional double-chamber microbial fuel cell in laboratory conditions;

(ii) To coat the graphite electrodes with Au and Pd catalysts with a range of deposition conditions;

(iii) To compare the impact of catalyst deposition on the generation of electrical power by the MFCs operated in identical conditions;

(iv) To observe anodic biofilm morphology and distribution in the examined MFCs by scanning electron microscopy;

(v) To identify biodiversity and structure of MFC microbial community with next-generation sequencing technique and metagenome analysis.

Thesis organization

The thesis consists of four chapters, with references listed at the end of the last chapter. Chapter 1 introduces the concept, advantage and application of MFCs, and the research objectives established for this work. Chapter 2 describes the materials, analytical methods, and computational data analysis applied in this research. Chapter 3 presents and discusses the results of comparison of MFCs with catalyst-modified electrodes with respect to electricity production, and the results of microbial community analysis. Chapter 4 includes the principle conclusions and provides a set of recommendations for future work.

Chapter TWO
Methods and Materials

Chapter TWO

Methods and Materials

2.1 Microbial Fuel Cells construction

In this study, double-chamber MFCs of 20 ml volume each were organized in arrays, each containing three cells. Table 2.1 lists the materials required for one MFC array, Figure 2.1 shows a schematic view of one MFC array construction, and Figure 2.2 shows the materials prepared in the laboratory for MFC assembling. The MFCs were assembled at the OIST Graduate University following the manufacturer manual (MPowerWorld LLC, Russian Federation).

Before assembling, the integrity of the membrane and the thickness of carbon plates were checked (membrane should be solid, with no holes, cracks, etc., carbon plates should be installed firmly in the groove, the upper part should not extend above the level of the cell's panel). Also, all of the materials were sterilized and cleaned before use: the panels were submerged in the sodium oxide and rinsed with distilled water; screws were treated with 70% ethanol for 1 h; the graphite plates were immersed into isopropanol, sonicated for 1 h and baked at 180^{o}C for 24 h to evaporate isopropanol.

The MFC assembling was performed in the following order: a carbon plate was inserted into the panel, plates and panel were covered with a membrane and the next layer of the carbon

plates was placed on the membrane. Then second panel with grooves covered the panel down. After installing the components, the bolting was done on the perimeter and between sections of the cell, and then screws were set for the current output from carbon plate. Then, the neutral sealant was applied on the joint of the panels and screws to seal the cell and prevent leakage. The assembled cells were left to dry for 2 days.

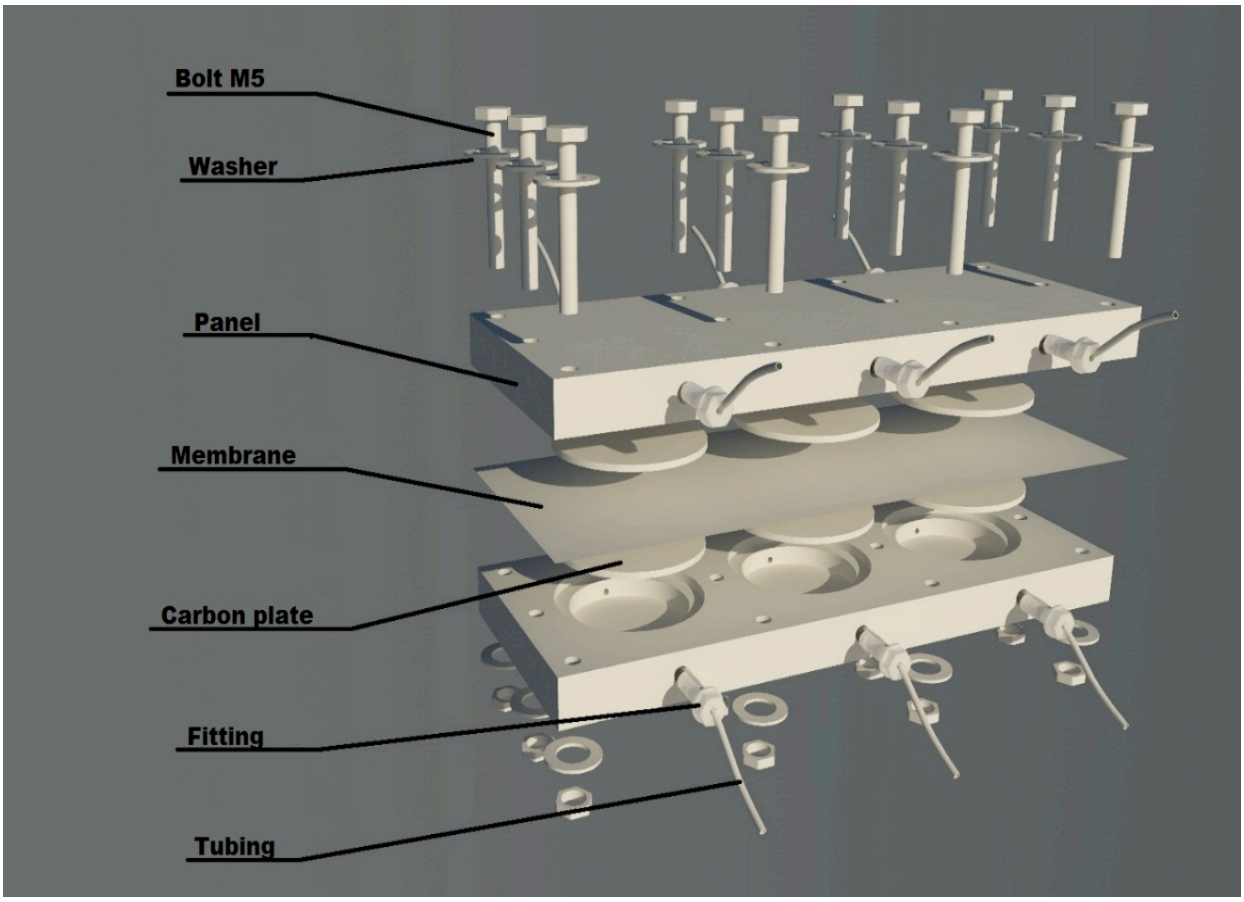

Figure 2.1: Schematic view of the MFC construction.

Table 2.1: Microbial Fuel Cell components.

1	Bolt DIN 933 Stainless. Steel A2 M5X60	4 pcs.
2	Bolt DIN 933 Stainless. Steel A2 M5X30	8 pcs.
3	Nut with locking ring DIN 985 Stainless. Steel A2-70 M5	12 pcs.
4	Enlarged washer DIN 9021 Stainless. Steel A2 M5 (D5,3 mm)	24 pcs.
5	Screw DIN 963 Stainless. Steel M4X 25	6 pcs.
6	Fitting FESTI CK-M5-PL-3	24 pcs.
7	Tubing FESTO PUN-H-4X0, 75-NT	0,5 m
8	Membrane Nafion ® N117-175 microns 875X 225 mm	1 pcs.
9	Carbon plate D 49,5 mm, thickness 3 mm	6 pcs.

Figure 2.2: The materials prepared for MFC assembling in laboratory setting.

Nafion membranes. Are proton-conductive polymer films, Nafion is a copolymer of tetrafluoroethylene (Teflon) and perfluoro-3,6-dioxa-4-methyl-7-octene-sulfonic acid. Figure 2.3 shows the model of hydrated Nafions, it has received a massive amount of interest as a proton conductor for proton exchange because of its excellent thermal and mechanical stability, the pores in the membrane allow movement of cations but the membrane do not conduct anions or electrons.

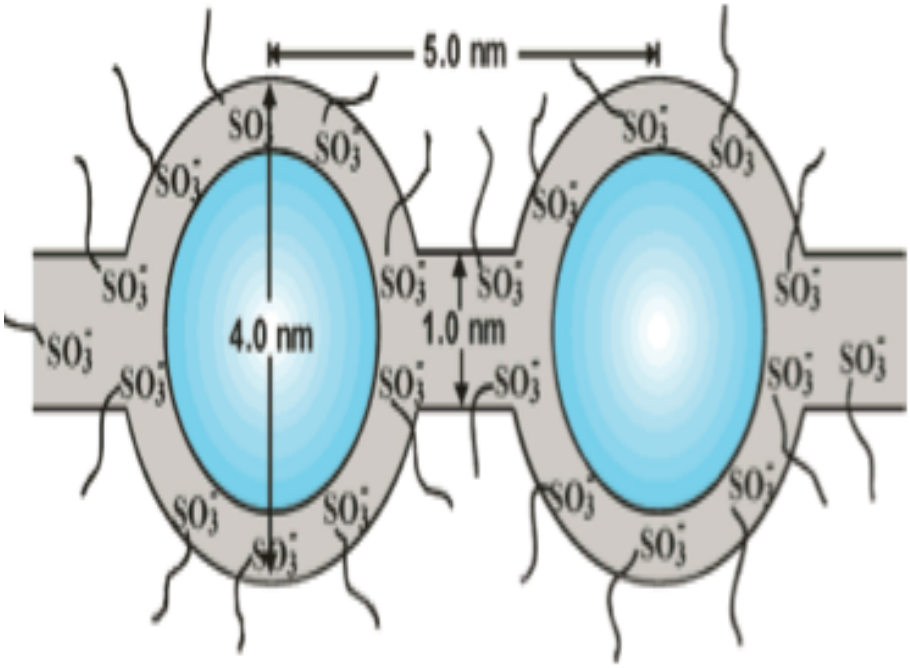

Figure 2.3: Cluster-network model for the morphology of hydrated Nafion (Mauritz, 2004).

Graphite electrode. The mineral graphite is an allotrope of carbon; it is the most stable form of carbon under standard conditions.

Normal photograph of graphite electrode shown in Figure 2.4 is an indispensable material; it has an excellent electrical conductivity and remarkable anti-thermal shock capability. Graphite has the benefits of high temperature strength, good thermal and electrical conductivity, low thermal expansion and appropriate machinability. These features should be present in the anode side (Guo et al., 2012), therefore we found the graphite electrodes most suitable material to be use in our experimental MFCs for both anode and cathode.

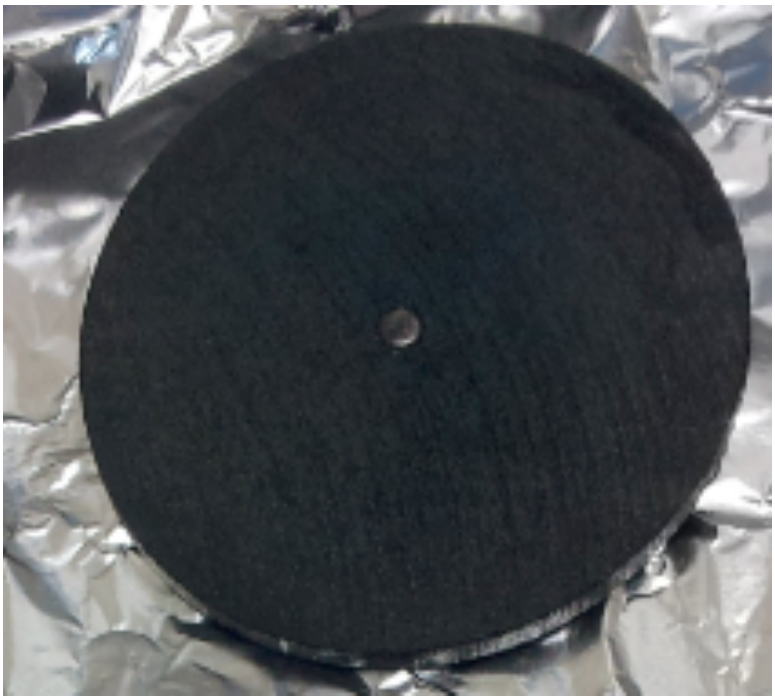

Figure 2.4: Normal photograph of the graphite electrodes.

2.2 Nanoparticles deposition

2.2.1. Gold and Palladium catalysts:

To have an efficient electrode material, palladium (Pd) and gold (Au) were deposited on the anode and cathode. It was predicted that this would enhance the efficiency of the MFCs performance due to catalytic enhancement of the reaction.

The methodology of experiment:

1- 20 ml syringe was used to fill the internal volume of the cathode area (back section) with prepared solution of potassium ferricyanide; the pH was adjusted to be 8 using 0.5 M NaOH solutions (Silicone tubing was used to connect capillaries in and out).
2- 8.5 grams of sludge was taken, and filled in internal volume of the anode area

(front section).

3- Similarly to the first step the anode chambers were filled with substrate providing the bacteria with enough chemical energy for the duration of the experiments. The figure shows MFC with complete assembling.

2.2.2. Graphite electrodes decoration with catalysts:

Prior to decoration with Pd or Au, all graphite electrodes were thoroughly cleaned, in the same way that was used for control electrodes.

Pd was deposited using four deposition times (and thus various surface coverage and thicknesses): one, two, three and four minutes, by using DC sputtering (UHV Mantis Deposition system) which is shown in figure 2.5 in the laboratory of Prof. Sowwan at OIST Graduate University. Depositions were performed using 15 W powers and flowing 40 sccm of Argon gas.

The magnetron head is positioned at a distance of 12 cm from the substrate, with an oblique angle of 45°. The substrate was rotated at 2 rpm, constantly during deposition, to ensure homogeneity and avoid shadowing effects. All the depositions were on the cathode side to understand the effect of deposition time on the power enhancement.

Au was deposited using two different deposition times: one and three minutes, using a low vacuum Au sputter coater. Depositions were separately performed on both anode and cathode sides, to explore the dependence on the electrode type. For Au sputtering, the power was 4 W, and used oxygen/nitrogen plasma. The sputter deposition was executed at normal incidence, with a distance of 15 cm between target and substrate.

2.2.3. SEM characterization for decorated and plain electrodes:

After deposition, the metal coating was examined by studying the topography of decorated graphite by scanning electron microscopy (SEM, FEI Quanta) and Energy Dispersive X-ray spectrometry (EDX, EDAX Octane Pro, with detection area of 10 mm^2). For SEM/EDX studies, a small piece was cut and cleaned via the same process. After that substrates were annealed under vacuum for about 48 hours at 200^oC, 10^{-2} mbr.

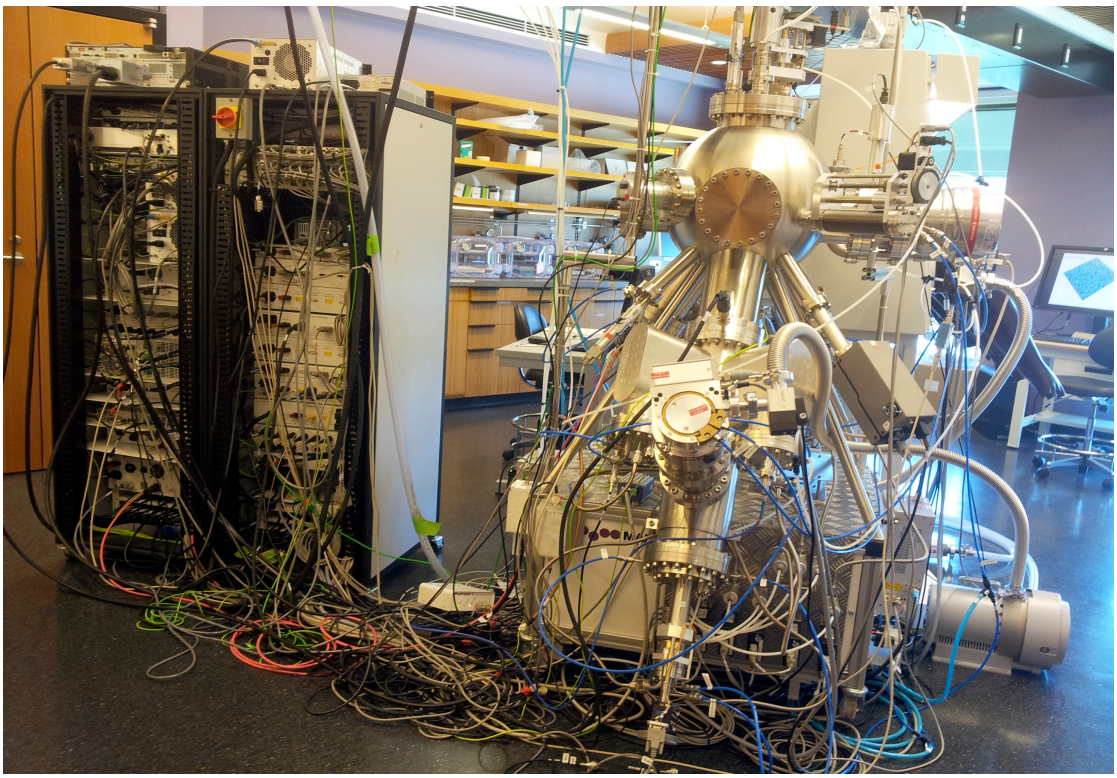

Figure 2.5: UHV Mantis Deposition system. (Nanoparticles by Design Unit, OIST Graduate University).