

**ISOLATION AND CHARACTERIZATION OF EXO-ELECTROGENIC
BACTERIA FROM WASTEWATER USING MICROBIAL FUEL CELL**

BY

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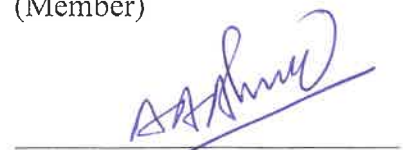
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To my family, who supported me during my journey and shaped me to become who I am,
their support and guidance are what motivated me to continue on with my life

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LIST OF ABBREVIATIONS

MFC	Microbial Fuel Cell
BHM	Bushnell Haas Media
CO ₂	Carbon Dioxide
PIM	polymer inclusion membrane
PEM	Proton Exchange Membrane
PCE	Porous Clay Earthworm
COD	Chemical Oxygen Demand
STWW	Septic Tank WasteWater
PBS	Phosphate Buffer Solution
SEM	Scanning Electron Microscopy
NA	Nutrient Agar
UV	Ultraviolet Light

BLAST	Basic Local Alignment Search Tool
NCBI	National Center for Biotechnology Information
OD	Optical Density
CFU	Colony Forming Units

ABSTRACT

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Increasing population is a factor in the rising demand for power which is further enhanced by urbanization, and economic development. The current primary sources of electricity, such as fossil fuels and nuclear power, are non-renewable, implying environmental concerns such as greenhouse gas emissions, pollution, and waste disposal. Therefore, there is a growing necessity for new sources of electricity to address the increasing demand for sustainability. Thus, microbial fuel cells have emerged as a viable candidate as an alternative source of electricity due to its advantages. In this study we tested the wastewater from the sewage company in Dhahran to investigate exo-electrogenic bacteria using a microbial fuel cell which have never been reported in the literature We have isolated three bacteria (*Pseudomonas extremaustralis*, *Pseudomonas veronii*, and *Bacillus circulans*) from the wastewater sludge. The exo-electrogenic capacity of each strain varied e.g., *Pseudomonas extremaustralis*, *Pseudomonas veronii* and *Bacillus circulans* showed highest potential of 120, 80 and 53 mV respectively. Hence, *Pseudomonas extremaustralis* can be considered as a potential strain to act as a biocatalyst in MFC This study highlights the potential of MFC as a promising energy source that can help alleviate the growing need of energy but also provides insights on new strains that have been tested in microbial fuel cell.

ملخص الرسالة

الاسم الكامل: الحسن مصطفى

عنوان الرسالة: عزل وتوصيف البكتيريا المنتجة للكهرباء الخارجية باستخدام خلية الوقود الحيوي

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تزايد السكان هو عامل في الطلب المتزايد على الطاقة، والذي يُعزّز بشكل إضافي بواسطة التحضر والتنمية الاقتصادية. المصادر الحالية الرئيسية للكهرباء، مثل الوقود الأحفوري والطاقة النووية، هي غير قابلة للتجديد وتأتي مع مخاوف بيئية مثل انبعاثات الغازات الدفينة والتلوث والتخلص من النفايات. لذلك، هناك حاجة متزايدة إلى مصادر جديدة للكهرباء للتعامل مع الطلب المتزايد على الاستدامة وبالتالي، فإن البحث عن مصدر متجدد للطاقة الذي يمكن أن يلبي الاحتياجات المتزايدة للسكان يعتبر ذا أهمية بالغة. ظهرت الخلية الوقود الجرثومية كمرشح قابل للتحقيق كمصدر للكهرباء بسبب فوائدها العديدة، ولذا قمنا في هذه الدراسة بفحص مياه الصرف الصحي من شركة الصرف الصحي في الظهران للبحث عن وجود بكتيريا خارجية الكترولوجينية باستخدام خلية وقود جرثومية حيث إنه مصدر لم يتم الإبلاغ عنه من قبل. وظهرت نتائج الدراسة بأننا نجحنا في فصل ثلاثة فصائل بكتيرية وهم: *pseudomonas veronii*, *pseudomonas extremaustralis* و *bacillus circulans*. ثم تم قياس قدرة البكتيريا لإنتاج الكهرباء ووجدنا تفاوت في القدرة الإلكترولوجينية لكل فصيلة حيث وجدنا أن *pseudomonas extremaustralis* لديها أعلى إمكانيه كهربائية حيث أنها تستطيع إنتاج 120mv تليها سلالة *pseudomonas veronii* حيث أظهرت النتائج بأنها تستطيع إنتاج 80mv وأخيرا كانت السلالة *bacillus circulans* حيث وجد أن قدرتها علي إنتاج الكهرباء هي 53mv. ومن خلال هذه النتائج توضح أن *pseudomonas extremaustralis* هي السلالة التي لديها أفضل الإمكانيات لإستخدامها في الخلية الوقود الجرثومية لإنتاج الكهرباء ويليه *pseudomonas veronii* مع أنها ليست فعالة بنفس قدر *pseudomonas extremaustralis* لكنها لا تزال تحمل إمكانيه جيده للعمل كمحفز حيوي في الخليه البكتيريّه إلا أن *bacillus circulans* أظهرت النتائج أنه ذات تأثير إلكترولوجيني خارجي منخفض في سياق

الخلية الوقود البكتيري. في ختام الدراسة، لا تبرز دراستنا فقط إمكانية الخلية الوقود الجرثومية كمصدر واعد للطاقة يمكن أن يساعد في التخفيف من الحاجة المتزايدة إلى المزيد من الطاقة في مواجهة تزايد السكان، ولكنها توفر أيضًا رؤى حول سلالات جديدة لم تختبر في سياق خلية الوقود البكتيري.

CHAPTER 1

INTRODUCTION

The rise of cities and factories has led to a surge in the global energy needs that it has now reached a level that is considered as a global crisis, the main reason for this crisis is due to inadequate supplies of different fossil fuels such as gas and oil, moreover, huge amount of waste is released into the environment due to the anthropogenic activities, these waste include heavy metals, dyes, chemical fertilizers, Polycyclic aromatic hydrocarbon and others which can be a source of various kinds of pollution that leads to various effect on both the environment in the guise of global warming and on the human health [1-3]. Thus, in response to these various impacts, researchers have resorted to different methods in order to explore a carbon-free, renewable, and alternative source of energy. So, bioenergy which is both sustainable and environmentally friendly is a promising solution to the growing predicament of global warming. In addition, there is a huge driving force that pushes researchers to pave the way for new power generation processes that are characterized as a renewable type of energy and have zero net carbon dioxide emissions [4-6].

1.1 Biomass as an energy source

Currently, organic wastes, especially biomass has emerged as a viable candidate for replacing traditional energy sources. When discussing organic wastes, we have to highlight that biomass is both eco-friendly and cost-effective as a means of sustainable

energy [7]. The energy generated from biomass has the potential to generate a wide range of fuels that including hydrogen fuel cells, bio-diesel, ethanol, and microbial fuel cells (MFCs). Thus, biomass can be considered as one of the effective wastes to energy strategy which is a green process that can produce energy from different types of wastes [8,9]. This strategy has a vital role in the reduction of greenhouse gases emission [10,11].

1.2 Microbial fuel cell

Among the different options of biomass, MFCs are considered as an ecologically attentive and resilient technique that utilizes different microorganisms to produce electricity, that makes them a clean and efficient energy source. Moreover, microbial fuel cells capitalize on the huge amounts of microbes that can degrade a variety of substrates which exists in various locations like sludge, wastewater, and sea sediments where the conditions to flourish microorganism are available [12-15]. Although multiple studies have researched the possibility of utilizing microorganisms as a biocatalyst in fuel cells since the beginning of the 1970s, it was only recently when microbial fuel cells have garnered attention, and it was developed with increased energy production.

At present, Researchers have discovered that numerous types of electricity can be produced by microbes via microbial fuel cells. In addition, it was also found that there is a class of microorganisms when using a self-sustainable microbial fuel cell can change different organic compounds into electric energy which can be applied on a large scale, these microorganisms are called exo-electrogenic microorganisms which are characterized by the capacity to totally oxidize organic substances, which makes the microbial fuel cell viable for a variety of applications with the right development [16-19]. During the last few decades, microbial fuel cells have been employed to power different electronic devices

such as cars and household generators. Additionally, MFCs have shown great potential and development when they were tested for waste-to-energy conversion and bioremediation of polluted areas, that are considered as hot topics in the scientific community. Nonetheless, these ideas and theories are currently not possible, this is because MFCs only generate electricity that is sufficient only for powering small devices and for only a short period of time. That is why even though there is extensive and ongoing research on MFCs to enhance their power generation there are multiple challenges and limitations that are first needed to be addressed before this technology can see the light [20-22].

1.3 Principle of microbial fuel cell

To understand the principle of MFCs, we have to know its essential constituents which include a cathode, proton exchange membrane, and anode. Various materials can be used in constructing microbial fuel cells such as glass, plexiglass, polycarbonate, or plastic bottles to hold the electrodes. [23,24]. MFCs operate on an oxidation-reduction mechanism, where a biocatalyst contains microorganisms that have the ability to convert organic substances and toxic pollutants present in a variety of sources into non-toxic by-products e.g. biofuel and electricity. Therefore, MFCs can be considered as an alternative approach for wastewater treatment and production of sustainable energy [25-27]. However, the effectiveness of microbial fuel cells relies on several parameters including the type of electrode substrate, microorganisms PH, and temperature of the MFC system [28]. Figure 1 shows different sources of biowastes used in microbial fuel cells for the production of bio-electricity.

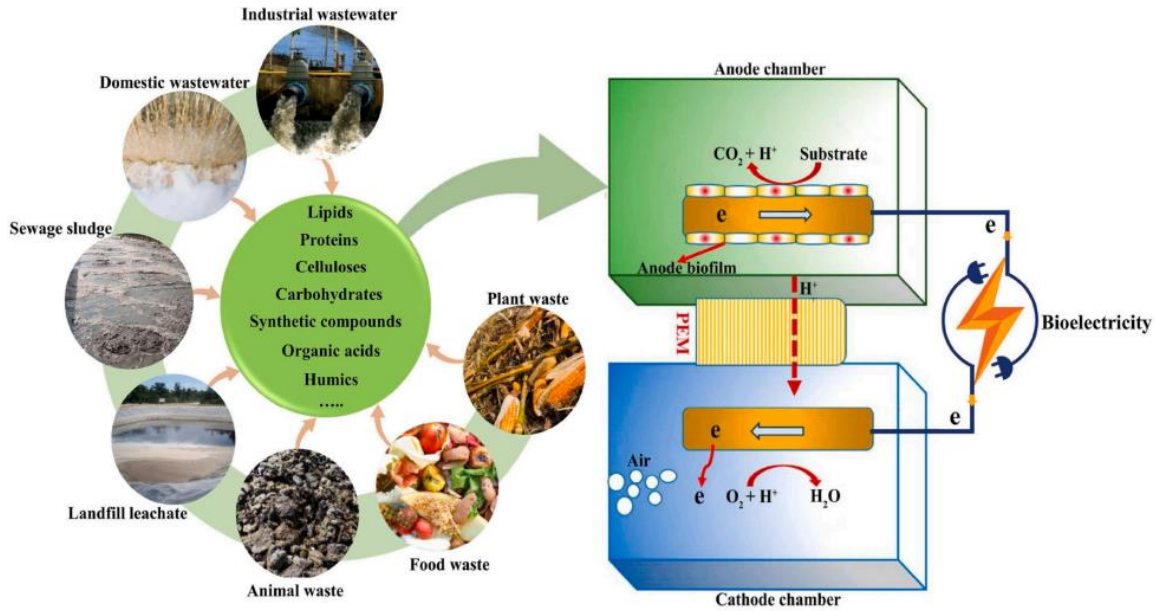


Figure 1: Microbial fuel cell for producing electricity from different waste sources [29]

1.4 Advantage of microbial fuel cell

Renewable sources of electricity such as solar, wind, hydro, geothermal, and biomass have been developed to varying degrees but are still not widely adopted. due to challenges such as intermittent availability, high upfront costs, and geographical limitations. Furthermore, there is a need for new sources of electricity that can provide power in remote or off-grid areas where traditional sources of energy are not feasible or practical. This is particularly important for developing countries where access to electricity is limited, and new sources of energy can contribute to their social and economic development. Considering these limitations microbial fuel cells (MFCs) can be adopted as a substitute paradigm to generate electricity. Compared to traditional sources of electricity, MFCs have several advantages. First, they can operate using a variety of organic matter sources, including wastewater, agricultural waste, and food waste, which are abundant and readily available. Second, MFCs continually generate electricity if there is an organic substrate available, unlike

renewable energy sources such as solar and wind power which can be intermittent. Third, MFCs are scalable and can be utilized in both small and large applications, making them versatile in meeting diverse energy needs. The utilization of sewage as a fuel source for MFC provides several advantages, including decreasing the amount of waste material that needs to be disposed of while generating sustainable energy. Additionally, wastewater can provide a cost-effective and abundant source of fuel for MFCs, particularly in regions where access to traditional energy sources is limited.

CHAPTER 2: OBJECTIVES

The objectives of this study were the following.

- 1. Selection of the exo-electrogenic bacterial consortium from wastewater.**
- 2. Isolation and characterization of exo-electrogenic bacterial consortia from wastewater.**
- 3. Assessment of the individual bacterial strain for electricity generation.**
- 4. Identification of bacterial species by using 16S rRNA sequencing.**

CHAPTER 3: LITERATURE REVIEW

3.1 *Microbial fuel cell characteristics*

The structure of MFC contains a proton exchange membrane that separates the anode and cathode from one another. Fig 2 describes the different components and materials needed to make MFC.

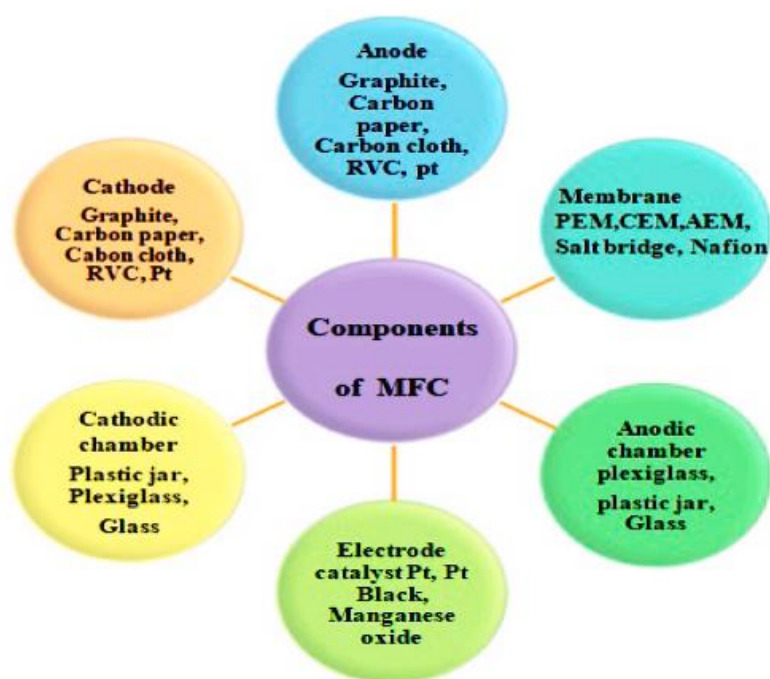


Fig 2: Components of MFCs.

3.1.1 Anode

One of the vital components of MFCs is the anode chamber. composed of microorganisms, mediators and substrates. When the anode is exposed to anaerobic conditions, the microorganisms act as a catalyst breaking down organic substrates into simpler molecules, creating an electron-proton pair. To get to the cathode chamber, the proton is transferred

through the proton exchange membrane [30]. Meanwhile, the electrons travel externally, generating a current in the conductor wire [31].

Materials used in anode MFCs must have certain characteristics such as high conductivity, favorable biocompatibility, and exceptional chemical stability [32]. However, materials like copper are not an optimal choice with regards to the anode though its ability to conduct electricity is considered as a benchmark, this is because copper has antibacterial properties. Therefore, a more appropriate alternative must be considered like a non-corrosive stainless-steel mesh that is less harmful than copper. Another candidate can also be considered that is carbon contain variety of features that separates from other materials such as high adaptability and versatility and can be designed into multiple forms like compressed graphite granules, plates, rods, glassy or fiber carbon [33].

3.1.2. Cathode

There are multiple factors affecting the cathode chamber performance in MFCs includes concentration and configuration of electrons, prescience of proton, and activity of catalyst [34]. The cathode chamber is an anaerobic chamber, due to the existence of oxygen as an electron acceptor, capable of attracting and consuming electrons before interacting with the hydrogen ions from the separating membrane [35]. This process will result in the production of water, which is a positive environmental benefit MFCs [36]. Depending on the material's availability and oxidation capacity, several cathode materials have been utilized. Furthermore, for a steady MFC performance, the chosen material's and its oxygenated counterparts must be nontoxic.

3.1.3 Membrane

In a typical MFC, a cationic membrane made of clayware or ceramic material isolates the anode and cathode chambers. [37]. The generation of bio-electricity in MFCs is driven by the bio-potentiality of the microorganisms inside the cathode chamber caused by their physiological and metabolic processes. In MFCs frequently the Nafion and Ultrex CMI-7000 membrane, with CMI-7000 being more cost effective have been used. Furthermore, in MFC a polymer inclusion membrane based on ionic liquids (PIMs) can be used [38-39]. It has been observed that materials made of porous clay, such as polymer electrolyte membranes (PEM) and innovative porous clay earthenware (NCE), can produce more energy when used as separators [40]. The amount of immobilized ionic liquid membrane is therefore shown to be associated with the overall energy production of MFCs. This finding shows that the ionic liquid can play a helpful role in aiding the transportation of activated protons through the membrane.

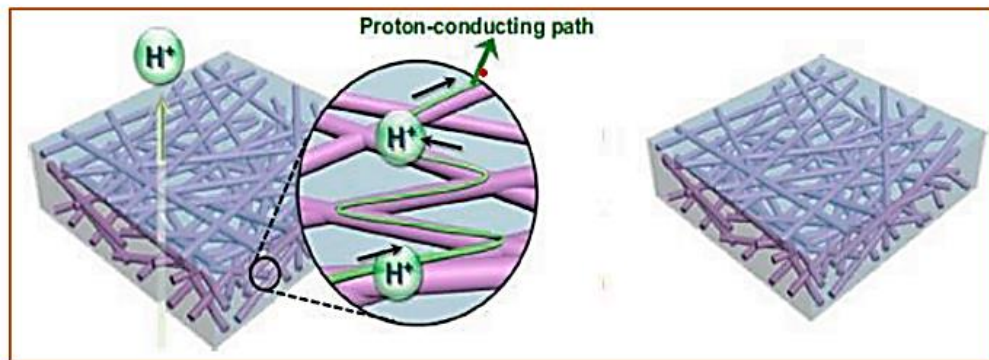


Fig 3: Structure of the PEM that transfers hydrogen ions.

3.1.4. Microorganisms

Microorganisms in MFC are responsible for decomposing the organic material and produce electricity by protons and electrons which are released as the organic material degrades. Moreover, the type of microorganism used, and the quality of the organic matter utilized in MFCs have an enormous effect on how well the MFC can remove pollutants [41]. There are multiple pathways that a microorganism can use in order to transform organic material into protons and electrons which depend on the conditions that the microorganism is subjected to such as the presence of oxygen [42]. These generated subatomic particles then travel to a suitable terminal electron acceptor through various routes [43]. There are some microbes such as *Geothrix Fermentans* and *Shewanella* bacteria, can make their own electron carriers which can help in providing more electron transport in a more efficient manner when they are properly aligned [44,45]. However, *Shewanella* species are not a very good choice when using organic waste since they are incapable of generating electricity from it. Since these types of bacteria can only partially degrade and oxidize lactate to form acetate [46], which leads to a less output of electricity [47]. This is because they decompose organic matter anaerobically and the intermediates are not located near the cell surface.

There are some microbes called *Geobacter sulfurreducens*, which have started to garner interest worldwide due to having unique capacity to produce a current density that is relatively high [48]. These species of *Geobacter* also give an obvious advantage when it comes to MFCs because they are capable of transferring electrons directly to the electrode without competing for the limited surface area of the anode which is present in sediment microbial fuel cells [49]. Also, numerous studies have confirmed *G. sulfurreducens* great

capacity to supply electrons in MFCs for power generation because their genetic code and entire genomic sequencing are available. [50-52]. With the overall rate being determined by the direct extracellular electro transfer, the mechanism that generates electricity calls for the use of membrane-bound redox proteins to transfer electrons from the cathode to the anode. *G. sulfurreducens* and *Shewanella oneidensis* strains can also transfer electrons between themselves (i.e., as bacteria) and extracellular electron acceptors like iron, magnesium, and uranium, making them dissimilatory metal-reducing microorganisms [47].

The scientific community are now centered on the behavior of the various bacterial communities and how that affected the MFC's power output, in addition to the research on how microbial fuel cells can induce the formation of biofilms and identify their growth kinetics. There are several bacteria that can transfer electrons from organic materials after it is oxidized to both the anode and the cathode electrode without the need for a mediator [47]. The exchange of electrons can happen through a variety of methods that includes self-assembled mediators, bacterial nanowires and bacterial cell surfaces. There are a few strains of bacteria that can form pilus or nanowire on the outer film which can promote the transfer of electrons [53]. There are many factors that can influence the selection of microorganism species for utilization in MFC such as the capability of the microorganism to transport electrons to the anode from the organic substrate. As demonstrated in Table 1, mixed cultures can include a variety of bacteria from soil sediment and marine sources to obtain improved biological stability. Several mixed cultures containing anodophilic and metal-reducing bacteria have been employed in earlier investigations. Conversely, some bacteria, including *Shewanella putrefaciens*, cannot break down complex chemical

molecules because they require unfavorable circumstances for electron-donating [54]. The primary electron transport chain in these species (mostly cytochrome c-type) consists of redox proteins joined by redox chains. To address the current technological issue, more studies are needed to comprehend the primary channel of electron transfer and pinpoint the function of proteins. The mechanism of electron transport can be further understood thanks to the development of molecular biology techniques, which also demonstrate how created bacteria can use complicated fuels to power electricity.

Table 1: Microorganisms applied in MFCs.

Microbial community	Energy produced (mW/m ²)	Mechanism followed	reference
<i>Candida melibiosica</i> 2491	260 ± 8	Mediated Electron Transfer/Direct Electron Transfer	55-56
<i>Shewanella oneidensis</i> MR1	142 (using anode1) 601(using anode 2) 203(using anode 3)	Outer membrane cytochrome proteins and excreted redox mediators (riboflavin)	57
<i>Acinetobacter johnsonii</i> NIUx72	46.35	Mediated Electron Transfer	58
<i>P. aeruginosa</i>	1530	Endogenousphenazine mediator	59
<i>E. coli</i>	1624	-	60

Table 1 (Continued)

<i>R. ferrireducens</i>	0.57mA _{m2} / 620 mV (electrode 1) 74mA _{m2} / 445 mV (electrode 2)	Direct contact mediated nanowires	61
<i>Clostridium butyricum</i>	1.3	Mediated Electron Transfer	62
<i>Cupriavidus basilensis</i>	902	Mediated Electron Transfer	63
<i>S. oneidensis</i>	1060	-	64
<i>E. coli</i> K12	9.8	Mediated Electron Transfer	65
<i>Exiguobacterium acetylicum</i> NIU-K4	28.3	Mediated Electron Transfer	66
<i>G. sulfurreducens</i>	7	Direct electron transfer	67
<i>Proteus hauseri</i> ZMd44	43.09	Mediated Electron Transfer	68
<i>Saccharomyces cerevisiae</i>	-	Direct electron transfer	56
<i>Bacillus subtilis</i>	1.066	Mediated Electron Transfer	69
<i>Desulfuromonas acetoxidans</i>	-	Direct Electron Transfer	70
<i>Geobacter metallireducens</i>	-	-	70

Table 1 (Continued)

<i>Hasenula anomala</i>	2.9W/m ³	Direct Electron Transfer	71
<i>Deltaproteo bacterium</i>	14	-	72
<i>Klebsiella pneumoniae</i>	2.9W/m ³	Direct Electron Transfer	73
Beta proteobacteria	40 ± 2		74

3.2 Construction and design of MFC

The basic principle of an MFC is to convert microbial energy into electrical. Having a single or a dual chambers system. In both cases an external circuit joins an anode and cathode divided by a semi-permeable membrane, that enables movement of H⁺ ions from the anode to the cathode [75-78]. The anode chamber receives wastewater or solid waste as input with microorganisms attached to electrode that break down complex substrates and produce CO₂, other metabolites, electrons and protons. The circuit and membrane are used to transport protons and electrons to cathode chamber [79,80].

Anode electron transfer is possible by microbes either by using proteins on their outer membrane or by using a mediator in the chamber. The membrane and circuit are then used to transport electrons and protons to the cathode chamber. Electricity is produced by a potential difference caused by the movement of electrons. The cathode chamber has oxygen (aerobic) which reacts with electrons and protons to produce water and bioelectricity. This process continues until all the organic substrates are oxidized and the

electricity is collected and stored. The amount of electricity depends on how well the microorganisms oxidizes the substrates. MFCs can treat wastewater by using waste residues as input instead of pure substrates. The reactions in the anode and cathode are shown below:

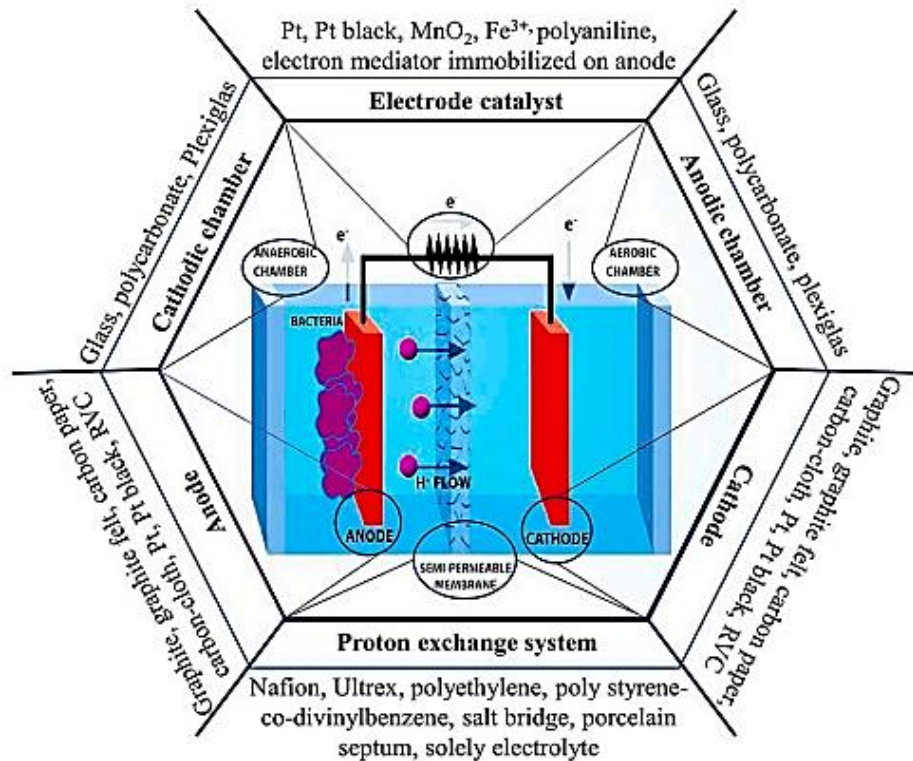
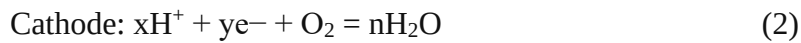


Fig 4: Used material for designing and configuration of MFCs.

3.3 Factors influencing MFC performance.

MFC is a potential tool for the treatment of wastewater and the production of sustainable energy. However, its performance still needs improvement for practical applications. Therefore, understanding and maximizing the variables affecting MFCs' power output and efficiency is crucial.

3.3.1. Electrode

The electrodes can be improved to increase the power output of an MFC that has specific features such as low cost and good electron transfer mechanisms. Moreover, they should be more stable to support increased current voltage if they have a large surface area. The materials of electrodes have a significant effect on the cost and performance of MFC [81]. Current research seeks to find substitutes for platinum electrodes because they are expensive and have many other uses as catalysts, electronics, and electrodes [82]. However, platinum is not a suitable material for electrodes because it has antimicrobial properties that hinder *E. coli* growth by releasing products during electrolysis. To address this issue non-corrosive and carbon-based materials are employed as the foundation for MFC electrodes. Therefore, in an MFC setup, the choice and use of electrodes have a significant impact on the power output [83].

3.3.2. Substrate

Using simple or complex substrates plays an important role in MFC performance [84]. Wastewater can be used as a substrate to generate electricity in MFC. In a study, vegetable waste composites were employed as substrates in single-chamber MFCs without mediators [85]. A power density of 57 mW/m² was obtained with an elimination of 62% of COD.

The common substrates used to gauge MFC effectiveness include acetate. Ferricyanide serving as catholyte has been used to achieve the maximum power densities of 286 and 212 mW/m², respectively [86]. In another study, a maximum power density of 90 and 66 W/m³ has been obtained in MFCs [87].

Different substrates have been tested which affect MFC systems in various ways, such as COD, electricity output, and columbic efficiency [88]. Agro-food materials have been used to improve MFC performance. However, mixed substrates made things more complicated and interlinked with different types of bacteria [89]. Methanogenesis occurs in anode chamber because of oxygen causing unwanted reactions [90]. Highly fermented substrates have lowered columbic efficiency and increased methanogenesis [91]. Currently, researchers have tried new ways to make synthetic wastewater with different nutrient levels for the better performance of MFC [92].

3.3.3. Microorganisms

Many microorganisms like *Geobacter*, *Shewanella*, *Pseudomonas*, and *Clostridium* species have been used to convert organic wastes into electricity [93]. These microorganisms move electrons by three different mechanisms: direct electron transfer, the redox-active protein on the cell's outer membrane, and indirect electron transfer using facilitated electron shuttle molecules [88]. The mediators can take the electrons from the respiratory chain and deliver them to the electrodes in a reduced state. The main sources of microorganisms for MFC are freshwater sediment, agro-product wastewater, marine sediment, and activated sludge because they have high organic content [94]. According to

earlier research, there are two steps to choosing the right microbes to boost power production: screening and identifying microbes that produce energy and building chromosomal libraries for those found bacteria [95,96].

Bacteria in organic materials can move electrons to the anode in MFCs. They use different ways to do this depending on what electron acceptors are present. Some bacteria can do this directly without needing any helpers, while others need helpers such as Fe^{3+} , Mn^{4+} , neutral red, etc. Bacteria that can do this directly are good for making renewable energy from carbon-based materials. One example is *G. sulfurreducens*, which has been well-researched for this purpose. However, using a mix of different bacteria can make more electricity because they can use more kinds of materials in wastewater and other organic wastes. They can also change their ways to fit new situations. Another way to make MFCs better and clean up the environment is to use biocathodes with bacteria [97-101].

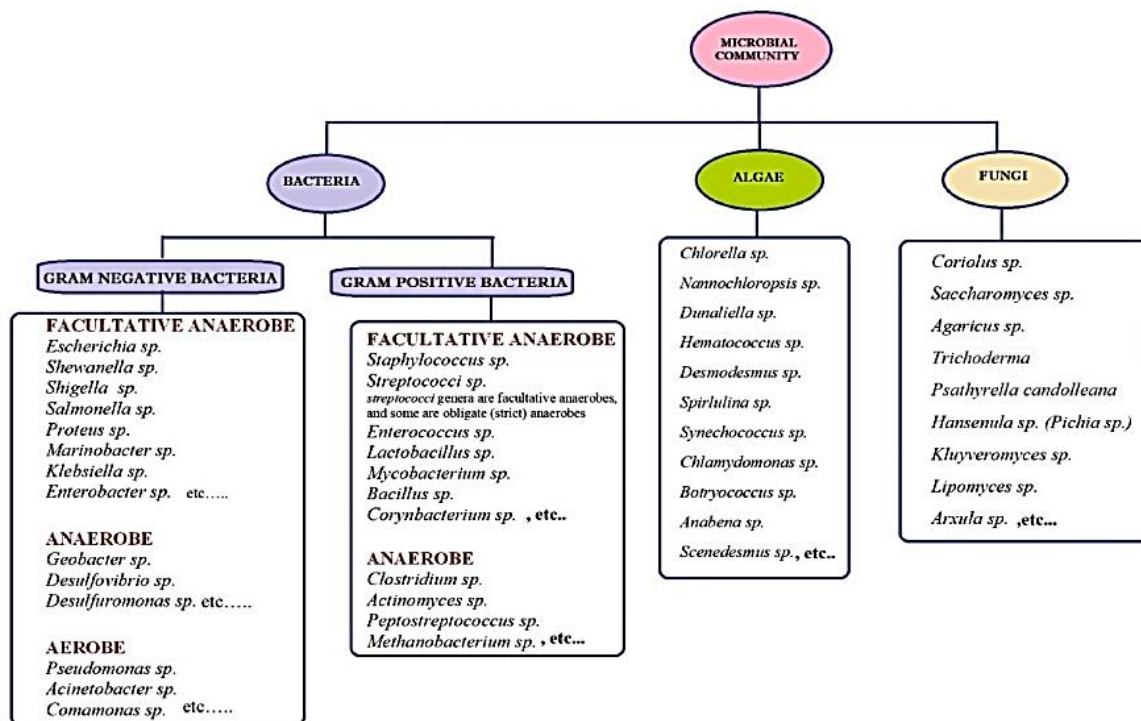


Fig 5: MFCs based on classification of the organisms.

3.3.4. pH and temperature

The oxidation reaction catalysis, bacterial kinetics, and the proton mass transfer rate are all influenced by temperature, which affects the performance of MFC [102-103]. The best temperature for MFC performance is around 20-35 °C or higher, which is similar to room temperature. MFCs require more time to begin operating at low temperatures of 4 to 30 °C. Even after a month, MFCs are unable to produce power at temperatures lower than 15 °C. MFCs, however, may create power at temperatures as low as 4 °C and 10 °C provided they are started at a high temperature of about 30 °C [104]. It has been reported that the power density is twice as high at 40 °C than at 30 °C [106]. But the power density drops by four times when the temperature reaches 50 °C.

A neutral pH favors bacterial growth and substrate pH, such as wastewater, affects the MFC's effectiveness. Low pH wastewater negatively impacts the MFC's effectiveness such as using rice mill effluent with a pH range of 3.0-4.0 [105, 107]. At a pH of about ~ 7.5, increased power production and organic matter clearance have been observed. Compared to other chemical fuel cells, the internal resistance is larger because the proton concentration is comparatively low at neutral pH [102,103]. One can lessen internal resistance without affecting the pH of the solution by increasing its conductivity. The conductivity of the solution might rise as the ionic strength increases. The majority of wastewater generated globally is saltwater, which can enhance MFC performance and the production of energy. Increased salinity promotes proton transport and conductivity, which reduces internal resistance. [108].

3.4 Recent studies done on Microbial Fuel cells.

Table 2: Recent studies on MFCs.

Substrate type	anode	cathode	Microbial community	Energy output	reference
wastewater	Carbon felt (20 ml of POME)	Carbon felt (MnO ₄)	Co-culture of Klebsiella variicola and Pseudomonas aeruginosa	Power density: 12.21 mW/m ³	109
Waste water	Carbon paper	Carbon paper coated with pt	Anaerobic sludge	Power density: 58.19 mW/m ²	111
wastewater	rGO and MnO ₂ coated on carbon felt	Carbon brush	Mixed bacterial culture	2065 mW/m ²	110
Domesticated sludge	PDDA modified carbon felt	Carbon felt	Mixed bacterial culture	537.8 mW/m ²	112
Escherichia coli	Graphite powder	Graphite powder	P.Aeruginosa	1575 μ W/m ²	113
Spent caustic	graphite	graphite	Mixed	82.1 mV	114

Table 2 (Continued)

Septic tank wastewater	Plain graphite plate	Plain graphite plate	Cronobacter sakazakii and Pseudomonas otitidis	280 mW/m ²	116
Biological treatment	Carbon felt	Carbon felt	anaerobic sludge(mixed community)	822 mW/m ²	115
Active sludge	Carbon paper	Carbon felt	<i>Pseudomonas</i>	0.32 mW	119
Organic acid fermentation	Nickel-coated sponge	Carbon paper	<i>Saprospiraceae</i> and <i>Caldilineaceae</i>	543.75 mW/m ²	117
Yam waste	Graphite rods	Graphite rods	<i>Mixed community</i>	0.0744 mW/m ²	117
Anaerobic sludge	Carbon felt	Carbon brush	<i>Mixed community</i>	14.04 W/m ³	118

Table 2: Continued

Industrial acid mine + municipal	Graphite rods	Graphite rods	<i>Mixed community</i>	1188 mW/m ²	120
Anaerobic sludge	Carbon cloth	Carbon fiber	<i>Mixed community</i>	237.23 mW/m ²	121

CHAPTER 4: EXPERIMENTAL METHODS

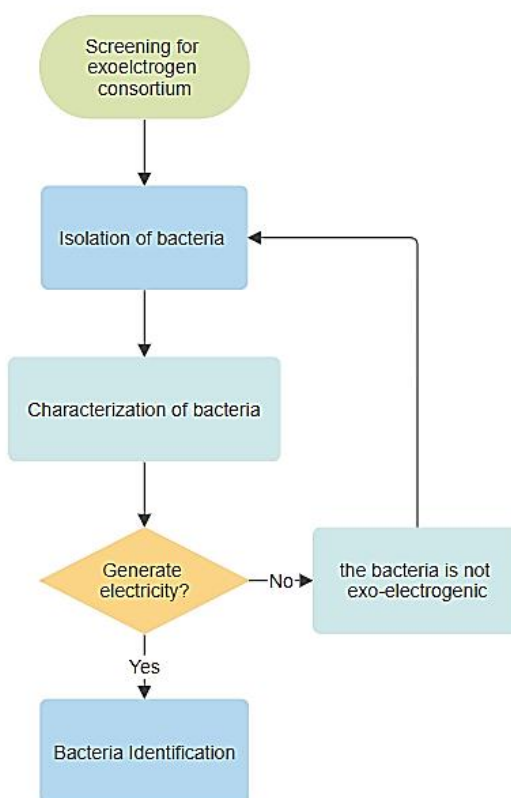


Figure 6: Scheme showing the methodology used in this study.

4.1 Medium and cell culture

The anaerobic sludge was obtained from the pond in front of the research institute at KFUPM, this sludge was transferred aseptically into a sterile plastic bottle which was preserved at 4 °C in a fridge. Then, a sample of about 1 ml was taken from the sludge and placed into Bushnell Haas agar medium (BHM) that is containing (0.2 g/L of magnesium sulfate (MgSO_4), and 0.02 g/L of calcium chloride (CaCl_2), Monopotassium phosphate (KH_2PO_4) - 1.0 g/L, Dipotassium phosphate (K_2HPO_4) - 1.0 g/L, Ammonium nitrate (NH_4NO_3) - 1.0 g/L, Ferric chloride (FeCl_3) - 0.05 g/L, Agar - 20.0 g/L). Bushnell media was used because it does not contain any carbon source and can be added separately

depending on the type of microorganism. Moreover, Petri dishes and an anaerobic chamber that was flushed with Nitrogen were prepared for incubation and isolation of a single bacterial strain from the anaerobic sludge.

4.2 MFC design and configuration

A twin-chamber microbial fuel cell (bio-electrochemical system) that converts chemical energy into electrical power by employing microorganisms as bio-catalysts, was used in this study. The layout of a double chambered MFC is typically composed of a cation exchange membrane separating the two chambers of an electrode: the anode and the cathode. The substrate and the bacteria that oxidize it are housed in the anode chamber, while the cathode chamber contains the electron acceptor (usually oxygen). The dimensions of the double-chambered microbial fuel cell are 4*4*2CM 1.76ML, ultra-thin fuel cell. The anode and cathode used as lead electrodes and the proton exchange membrane (Nafion-117) showed good thermal stability and resistance to high temperature and electrochemical oxidation, low corrosion and high chemical stability, better mechanical properties, and long life, enhanced conductivity, high output power. The parameters of the membrane are Brand: Dupont, Model: N-117 or N117 or Nafion 117, Thickness: 183um, Density: 360g / m², Conductivity: 0.083S / cm, Exchange capacity: 0.89 meq / g. The pretreatment method utilized is soaking with deionized water for half an hour. Then it is immersed in 5% dilute sulfuric acid (mass ratio) for 1 hour. Finally, soak with deionized water for half an hour.

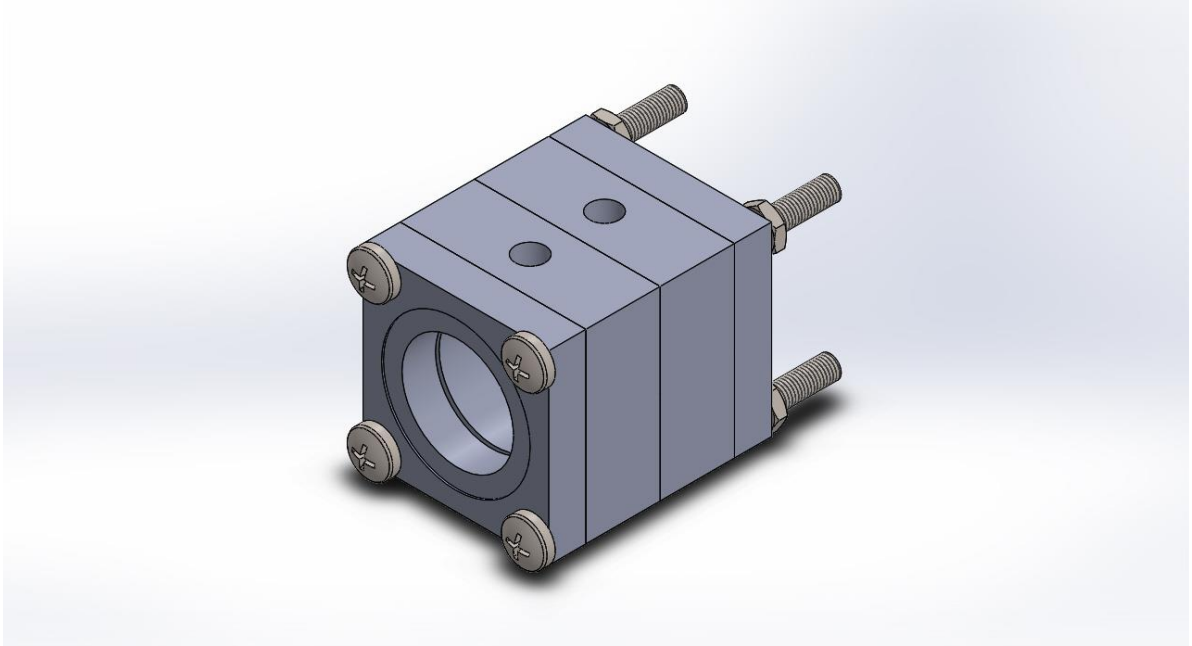


Figure 7: Microbial Fuel Cell design used in the study.

4.3 Selection of Functional Consortium

To select a sludge that has the potential to generate enough electricity, we incorporated different samples of the sludge into 15ml tubes containing Bushnell medium mixed with glucose as a carbon source for the growth of bacteria. These sludge samples were randomly collected to ensure the isolation of different bacteria and transferred to MFC. In addition, peptone was added to the mixture which can compensate for any potential mineral deficiencies and ensure that the growth conditions are optimal for all bacteria present in the mixture.

The MFC was incubated at room temperature for 5 days, voltage measurements were recorded by a multimeter at 8-hour intervals. The aim was to identify a sludge sample that can maintain a stable voltage output of at least 120 millivolts over 8 hours or more. Moreover, the sludge samples that failed these criteria were not disposed of instead they were preserved in a refrigerator at a constant temperature of -4°C.

4.4 Isolation of bacteria

To isolate a single bacterium requires a series of serial dilutions using sterile 0.9% saline water (10^{-1} to 10^{-4}). The samples were then inoculated into a Bushnell medium (BH) plate and Nutrient Agar plates for bacterial growth. The inoculation was done through the spread plate technique. After 48 hours, the incubated plates unveiled a mosaic of mixed bacterial colonies. The streak plate technique was used to attain a pure culture for the subsequent analysis.

4.5 Morphological characterization

Individual bacterial strains were characterized using light microscopy to assess their morphological characteristics such as shape, color, form, and margin.

4.6 Gram Staining

Gram staining is a technique in which the bacteria undergo color changes in one of two distinct color sets from pink to red or purple to blue when the bacteria are exposed to a specific sequence of stains. This process will categorize the bacteria as either “gram-negative” or “gram-positive”. The mechanism behind Gram staining relies on distinguishing bacteria based on the unique chemical and physical properties of their cell walls.

The Gram staining steps are as follows:

1. Loop Sterilization: Begin by sterilizing an inoculating loop in a flame until it turns red-hot, followed by a 30-second cooling period. This critical step prevents excessive heat from damaging bacterial cells during sample collection.

2. Sample Transfer: For broth cultures, transfer a loopful of culture to the center of a clean slide. When working with bacteria from agar plates, create a suspension by mixing a loopful of bacteria with a drop of water. Use a second slide to place a drop of water for sampling. Exercise caution, not to add an excessive number of bacteria, as it can result in overly thick smears.

3. Sample Spreading: Using the sterile inoculating loop, spread the bacterial sample evenly on the slide, creating a thin layer. Allow the smear to air dry. Avoid adding excessive liquid to prevent extended drying times.

4. Heat-Fixation: Gently pass the slide, smear side up, through a Bunsen burner flame 2 to 3 times while holding it with a clothespin or clamp. Overheating should be avoided to prevent damage to bacterial cell walls and morphology.

5. Crystal Violet Staining: Cover the heat-fixed smear with crystal violet solution for 30 seconds. Ensure the entire slide's top is evenly covered.

6. Rinse with Water: Rinse the slide with water until the runoff is clear, typically taking 5 to 10 seconds. Allow excess water to drip off the slide without disturbing the smear.

7. Gram's Iodine Solution: Apply Gram's iodine solution to the smear and allow it to act for 1 minute.

8. Rinse Again: Rinse the slide with water until the runoff is clear.

9. Decolorization: If using 95% ethanol, gently tilt the slide to allow the decolorizing solution to run across the smear until the runoff becomes colorless, usually within 20 seconds. Proper decolorization is essential to avoid false positive results.

10. Final Rinse: Rinse the slide with water until the runoff is clear.

11. Safranin Counterstain: Apply safranin counterstain for 1 minute, ensuring even coverage by adding the stain dropwise.

12. Rinse Once More: Rinse the slide with water until the runoff is clear.

13. Blot Dry: Gently blot the slide dry with bibulous paper or paper towels without disturbing the smear. Ensure the slide's bottom is clean.

4.7 Scanning Electron Microscope (SEM)

The scanning electron Microscopy centered on the emission of secondary electron particles. The SEM is the best tool to study the morphological features and growth of bacteria, viruses, or any unicellular organisms until the depth of the nanometers.

Step 1: Culturing Bacteria

To begin preparing the sample for SEM, we have to culture the bacterial cells by introducing them into a nutrient-rich broth solution in a sterile tube. This culture will then be placed in an incubator at 37°C so that the bacteria can multiply over 24 hours.

Step 2: Centrifugation

After incubation, the bacteria are subjected to centrifugation, so that we can separate it from the liquid nutrient broth. The resulting precipitate that contains the desired bacteria will be used for further analysis.

Step 3: Sample Rinsing

The rinsing step is vital in SEM sample preparation because the presence of contaminants or residuals from the nutrient broth can interfere with the quality of the image. And so, we use saline water to rinse the samples. This rinsing step will be repeated 3 times to ensure the removal of any residual substances.

Step 4: Fixation

After removing the contaminants, next, we must preserve the structural integrity of the bacteria by using a fixating agent. So, we used glutaraldehyde, which is a commonly used fixative, in the samples to immobilize the cellular components and ensure that the cells maintain their natural form for examination.

Step 5: Additional Rinsing

After fixing the sample, a thorough rinsing is important to remove excess fixative which can interfere with the next steps, and so this rinsing lasts typically between 5 to 10 minutes.

Step 6: Dehydration

To prepare the sample for SEM, it must be dehydrated to remove all moisture content. This is achieved through a series of ethanol washes. The sample is first exposed to a 10 percent ethanol solution for 10 minutes, followed by 25 percent ethanol, 70 percent ethanol, 95 percent ethanol, and finally 100 percent ethanol, each for 10 minutes. This gradual process ensures that water is replaced with ethanol, preventing structural damage to the cells during drying.

Step 7: Mounting on a Stub

Once the sample is adequately dehydrated, it is mounted on a stub. The stub serves as a holder, securing the sample in place for microscopy. Proper mounting ensures that the sample remains stable during analysis.

Step 8: Coating with Gold or Carbon (SEM-specific)

For SEM analysis, an additional step is required. To enhance the sample's conductivity and improve imaging quality, it is coated with a thin layer of gold or carbon. This coating

process ensures that the electron beam used in SEM interacts effectively with the sample's surface, producing high-resolution images.

4.8 Assessing the electrogenic capacity of individual strain.

To Assess the potential electricity that can be generated from each bacterium that we have isolated from the sludge, we design an experimental protocol that involves various essential steps, including membrane pretreatment, sterilization, media preparation, fuel cell assembly, voltage measurement, and optical density monitoring, which are as follows:

1. Membrane pretreatment:

In this step, we begin by performing the membrane pretreatment procedure that is provided by the manufacturer. This is done to ensure that the membranes are appropriately prepared for their application in the MFC system and to reduce the risk of contamination.

2. Sterilization:

It is crucial to maintain aseptic conditions by sterilizing the entire MFC setup. The sterilization is done by treating the system with ethanol followed by exposing it to UV light for 5 minutes to eliminate potential contaminants.

3. Media preparation:

The next step is to prepare the growth media, which typically includes a substrate like glucose as described above. Ensure the media is sterile by autoclaving it to prevent any microbial contamination.

4. Fuel cell assembly:

Then we assemble the MFC system, by incorporating the anode and cathode compartments, the pretreated membrane, and the necessary connections.

5. Inoculation of bacteria:

In the anode chamber, introduce 15 mL of sterile media along with the selected bacterial strain. These bacteria will serve as the electrogenic microorganisms responsible for electricity generation.

6. Cathode setup:

In the cathode chamber, add 15 mL of distilled water. Create a small opening to allow oxygen to pass through, as oxygen functions as the terminal electron acceptor.

7. Anaerobic condition formation:

To facilitate anaerobic conditions in the anode chamber, flush it with nitrogen gas for 20 minutes. This step is crucial to create an environment conducive to efficient microbial growth.

8. Voltage measurement:

Allow the bacteria to grow and carry out their metabolic processes in the MFC system. Continuously monitor the voltage generated by the MFC over a designated observation period, typically lasting 4 hours. Record voltage measurements at specified intervals during this time.

Calculate the average voltage generated by the MFC over the observation period.

9. Optical Density Measurement:

To gain insight into bacterial growth, measure the optical density (OD) of the bacterial culture daily. This will provide a quantitative assessment of bacterial population density over time.

4.9 Bacteria identification

4.9.1 16S rRNA sequencing

To identify the bacterial species a pre-culture of bacterial cells within a 100 mL volume of nutrient-rich LB medium was prepared. Subsequently, these cells underwent separation via centrifugation at 5000× g for 5 minutes at room temperature. Following this separation step, the cells were subjected to lysis, and their DNA was then extracted and purified utilizing the Qiagen Powerfecal Kit, a product manufactured by Qiagen and headquartered in Hilden, Germany.

After the DNA extraction, we initiated a process involving the amplification of the 16S rRNA gene, which was followed by sequencing, as previously detailed in documented procedures. In brief, the primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (CGGTTACCTTGTACGACTT) were employed to generate an amplification product of approximately 1400 base pairs (bp) from the 16S rRNA genes. This amplification product was subsequently subjected to sequencing procedures using the Big Dye terminator cycle sequencing kit, a product of Applied Biosystems, which operates under the Thermo Fisher Scientific umbrella and is in Waltham, MA, USA. The sequencing process was carried out utilizing the primers 518F (CCAGCAGCCGCGGTAATACG) and 518R (GTATTACCGCGGCTGCTGG) and was facilitated by an automated DNA sequencer, specifically the model 3730XL by Applied Biosystems.

To achieve the identification and confirmation of the bacterial species, we employed the Basic Local Alignment Search Tool (BLAST), a bioinformatics tool accessible through the US National Center for Biotechnology Information (NCBI) databases. In addition to

BLAST, we utilized EzBioCloud, a recently established comprehensive database of 16S rRNA gene sequences, for species identification and confirmation.

4.9.2 Bioinformatic and phylogeny analysis

We obtained the 16s rRNA gene sequence of the novel strain and deposited it in GenBank, a worldwide repository of genes. We used the BLASTn Software from the NCBI to compare our sequences with other strains in the database. We selected the sequences that had the highest dissimilarity percentage and the lowest E-value, following the criteria in [122]. We then aligned these sequences with the MEGA 11 bioinformatics tool [123]. We viewed the alignment in Jal View and downloaded it in FASTA format [124].

To perform phylogeny analysis, we applied MEGA XI software [125]. We generated initial trees for heuristic search by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances, computed using the Maximum Composite Likelihood model. We chose the topology with the highest log likelihood value. We also included a discrete Gamma distribution in this analysis.

CHAPTER 5: RESULTS

5.1 Screening for *exo-electrogenic consortium*

Distinctive patterns in electricity generation have been observed and monitored after subjecting various sludge samples to the MFC. A subset of these samples demonstrated remarkable stability in voltage output, consistently maintaining levels exceeding 120 millivolts over extended periods as shown in Figure 1(a, b). These findings signify the presence of a consortium of bacteria within specific sludge samples with exceptional electrogenic capabilities.

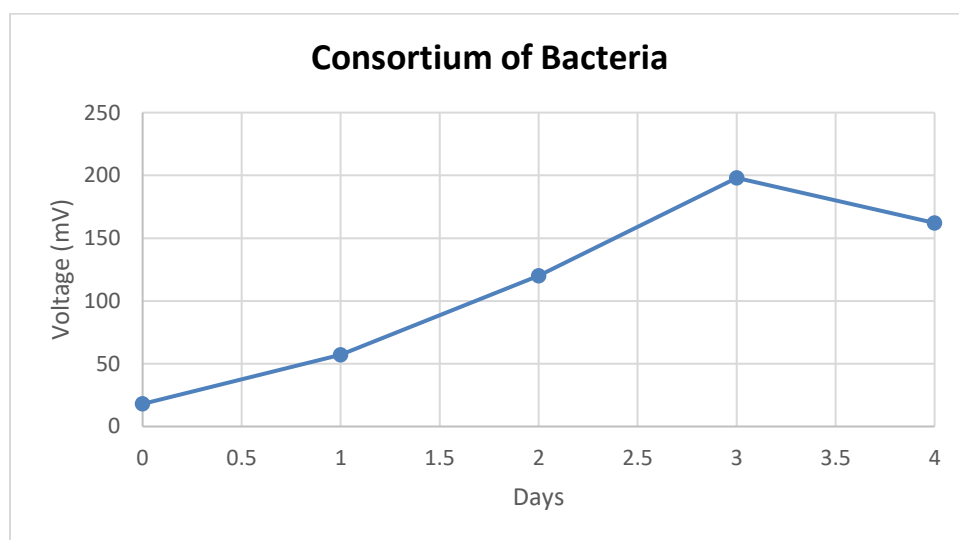


Figure 8a: Electricity generation of a sludge sample 1.

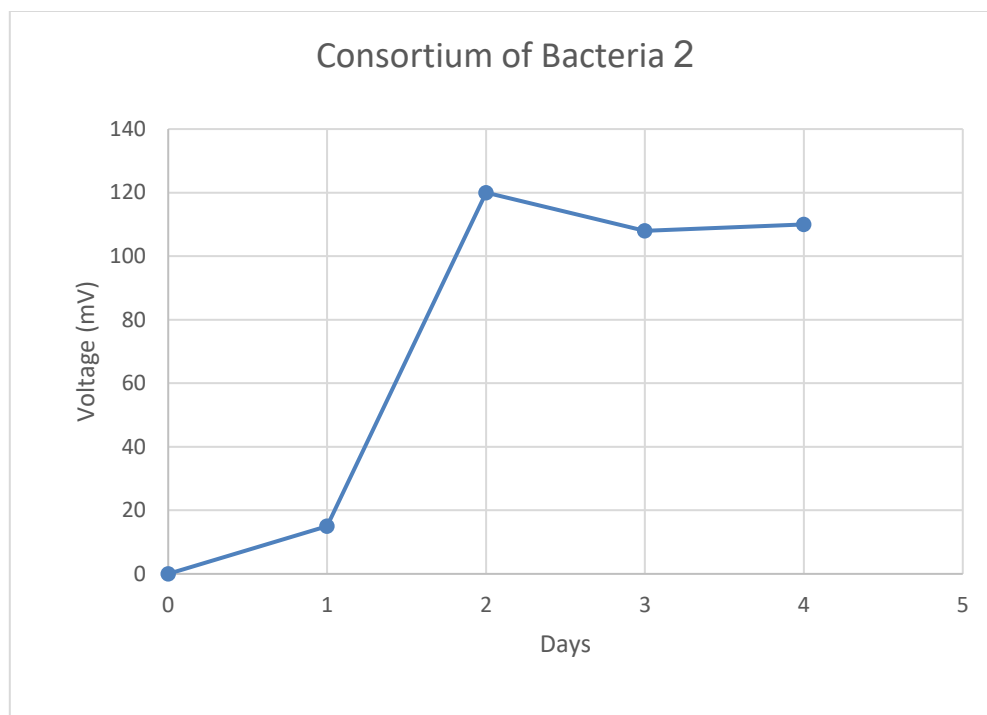


Figure 8b: Electricity generation of a sludge sample 2.

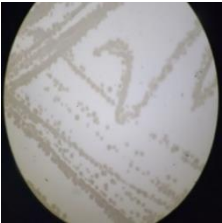
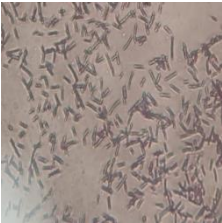
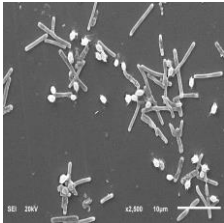
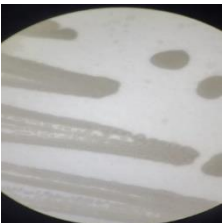
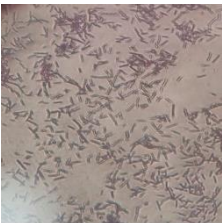
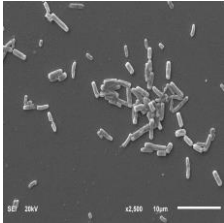
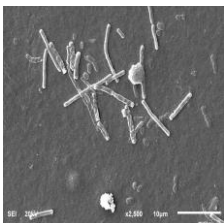
5.2 Isolation and characterization of bacteria

After obtaining the sludge containing a consortium of exo-electrogenic bacteria the next step was to isolate individual strains of these microorganisms for further analysis. Microbial community residing within the wastewater, comprising a diverse array of bacteria at varying concentrations. Individual bacterial species have been isolated successfully by serial dilution method, ensuring the purity of each growing colony on the plates.

After the isolation process, bacteria were subsequently characterized. It was accomplished through several key techniques, including morphological characterization with an optical microscope, gram staining, and-scanning electron microscope (SEM).

Morphological characterization enables the identification of visual characteristics of the bacteria, such as the color and shape of individual cell colonies. Meanwhile, gram staining aids in classifying bacteria into two distinct groups: gram-positive and gram-negative.

Table 3: Optical microscope and Gram staining of the isolated strains.

Strain	Isolated Plates	Optical Microscope	Gram Staining	SEM Images
MFC-1 A				
MFC-2 B				
MFC-3 C				

5.3 Assessment of the electricity generation of individual strain

The isolated strains have been investigated for electricity generation. We have checked the electrical performance of each strain under standard conditions (pH, Temperature, etc.) We used voltage to measure the exo-electrogenic ability of each strain and utilized optical density to understand the bacterial growth inside MFC by using a spectrophotometer.

Table 4: Electricity generation capacity of the strains.

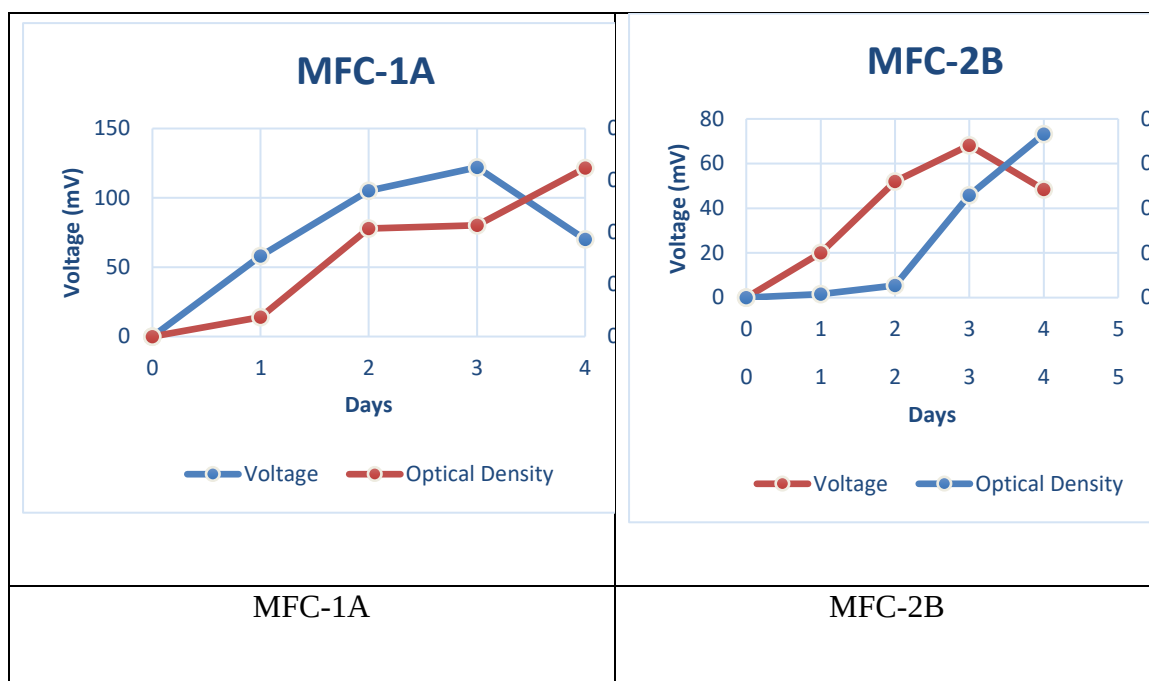
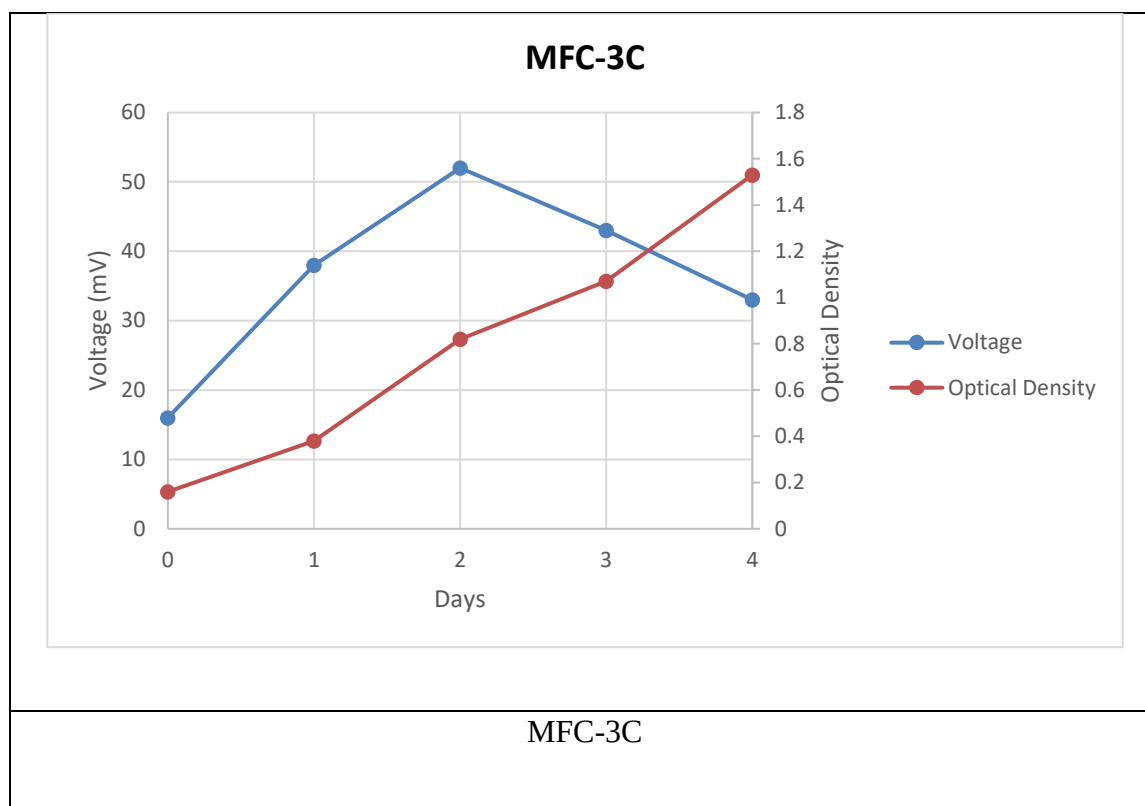


Table 4 (Continued)



5.4 Bacteria Identification

5.4.1 16S rRNA Sequencing

The genetic material the isolates have been analyzed through amplification and sequencing of their 16S rRNA gene, coupled with bioinformatics tools (BLAST and EzBioCloud).

Table 5: Sequencing results of the strains.

	Description	Scientific Name	Max Score	Total Score	Query Cover
✓	Pseudomonas extremaustralis strain 4BR4AA5 16S ribosomal RNA gene, partial sequence	Pseudomonas extremaustralis	1423	1423	100%
✓	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 3MP-B-2AC-56	uncultured bacterium	1423	1423	100%
✓	Uncultured Pseudomonas sp. clone P7_C 16S ribosomal RNA gene, partial sequence	uncultured Pseudomonas sp.	1421	1421	100%
✓	Uncultured Pseudomonas sp. clone B57_C 16S ribosomal RNA gene, partial sequence	uncultured Pseudomonas sp.	1421	1421	100%
✓	Pseudomonas sp. strain NAS15 16S ribosomal RNA gene, partial sequence	Pseudomonas sp.	1421	1421	100%
✓	Pseudomonas fluorescens strain ESR25 16S ribosomal RNA gene, partial sequence	Pseudomonas fluorescens	1421	1421	100%
✓	Pseudomonas sp. strain G6 16S ribosomal RNA gene, partial sequence	Pseudomonas sp.	1421	1421	100%
✓	Uncultured Pseudomonas sp. clone 16S_4 16S ribosomal RNA gene, partial sequence	uncultured Pseudomonas sp.	1421	1421	100%
✓	Uncultured Lactobacillus sp. clone 16S_3 16S ribosomal RNA gene, partial sequence	uncultured Lactobacillus sp.	1421	1421	100%
✓	Pseudomonas extremaustralis strain Y39-6 16S ribosomal RNA gene, partial sequence	Pseudomonas extremaustralis	1421	1421	100%
Sequence of MFC-1A Strain					
	Description	Scientific Name	Max Score	Total Score	Query Cover
✓	Pseudomonas extremaustralis strain 4BR4AA5 16S ribosomal RNA gene, partial sequence	Pseudomonas extremaustralis	1423	1423	100%
✓	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 3MP-B-2AC-56	uncultured bacterium	1423	1423	100%
✓	Uncultured Pseudomonas sp. clone P7_C 16S ribosomal RNA gene, partial sequence	uncultured Pseudomonas sp.	1421	1421	100%
✓	Uncultured Pseudomonas sp. clone B57_C 16S ribosomal RNA gene, partial sequence	uncultured Pseudomonas sp.	1421	1421	100%
✓	Pseudomonas sp. strain NAS15 16S ribosomal RNA gene, partial sequence	Pseudomonas sp.	1421	1421	100%
✓	Pseudomonas fluorescens strain ESR25 16S ribosomal RNA gene, partial sequence	Pseudomonas fluorescens	1421	1421	100%
✓	Pseudomonas sp. strain G6 16S ribosomal RNA gene, partial sequence	Pseudomonas sp.	1421	1421	100%
✓	Uncultured Pseudomonas sp. clone 16S_4 16S ribosomal RNA gene, partial sequence	uncultured Pseudomonas sp.	1421	1421	100%
✓	Uncultured Lactobacillus sp. clone 16S_3 16S ribosomal RNA gene, partial sequence	uncultured Lactobacillus sp.	1421	1421	100%
✓	Pseudomonas extremaustralis strain Y39-6 16S ribosomal RNA gene, partial sequence	Pseudomonas extremaustralis	1421	1421	100%
Sequence of MFC-2B Strain					

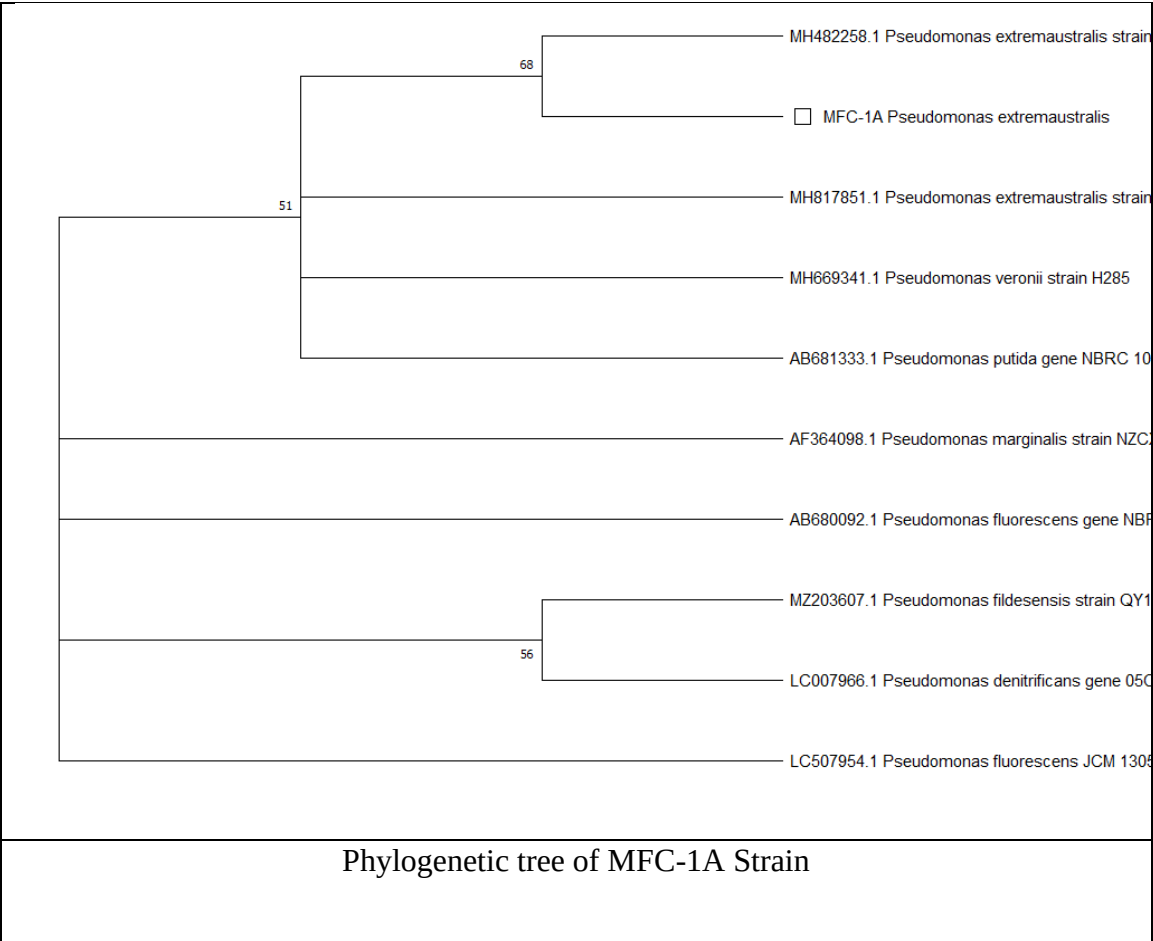
Table 5 (Continued)

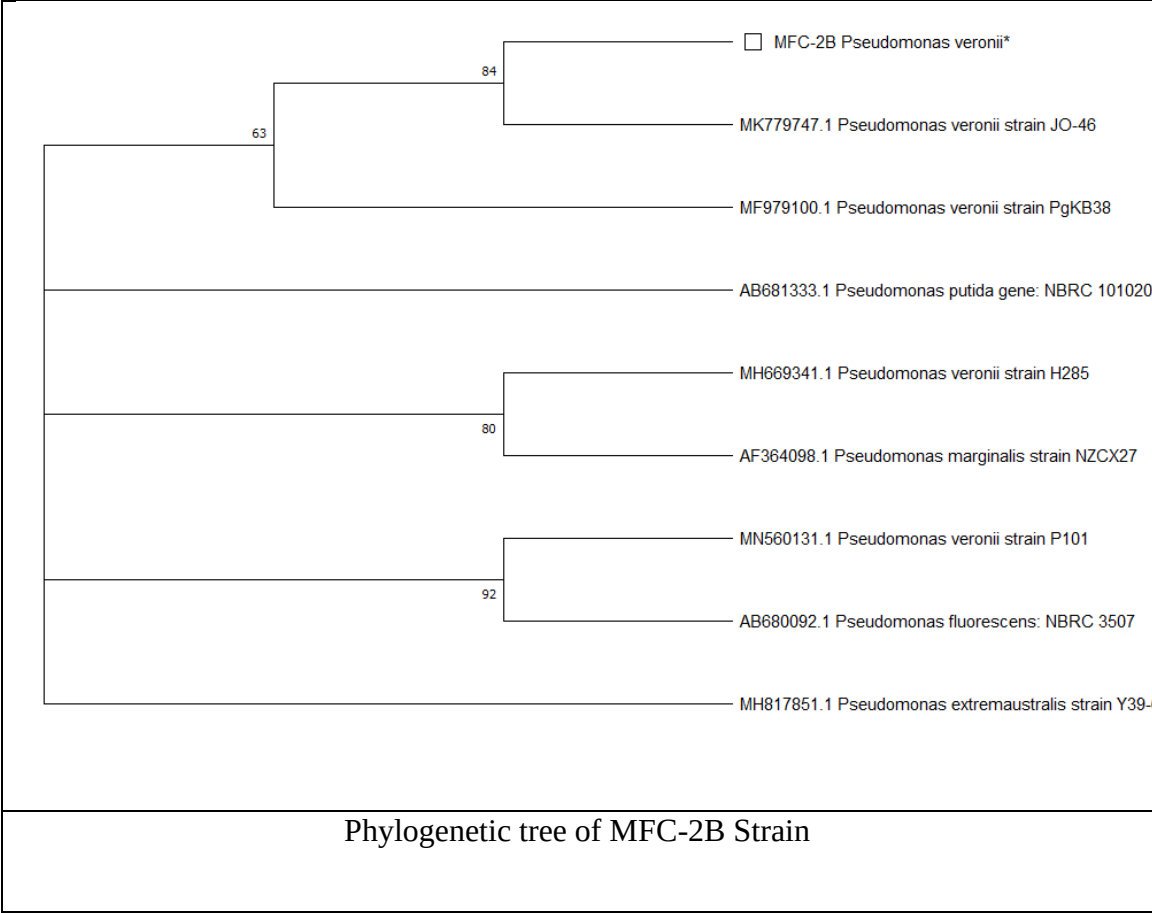
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value
✓	Bacillus circulans strain 1910ICU214 16S ribosomal RNA gene, partial sequence	Niallia circulans	1844	1844	100%	0.
✓	Bacillus sp. (in: Bacteria) strain C4P019a 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	1844	1844	100%	0.
✓	Bacterium strain BMVC15 16S ribosomal RNA gene, partial sequence	bacterium	1844	1844	100%	0.
✓	Niallia alba strain UniB3 16S ribosomal RNA gene, partial sequence	Niallia alba	1844	1844	100%	0.
✓	Bacillus sp. (in: firmicutes) strain CDRSL-7D 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	1844	1844	100%	0.
✓	Bacillus sp. (in: firmicutes) strain CDRS-2D 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	1844	1844	100%	0.
✓	Bacillus nealsonii partial 16S rRNA gene, strain Marseille-P2085	Niallia nealsonii	1844	2347	100%	0.
✓	Bacillus circulans strain MD1 16S ribosomal RNA gene, partial sequence	Niallia circulans	1844	1844	100%	0.
✓	Niallia nealsonii strain TIE02 16S ribosomal RNA gene, partial sequence	Niallia nealsonii	1844	1844	100%	0.
✓	Niallia alba strain UniB3 16S ribosomal RNA, partial sequence	Niallia alba	1844	1844	100%	0.
Sequence of MFC-3C Strain						

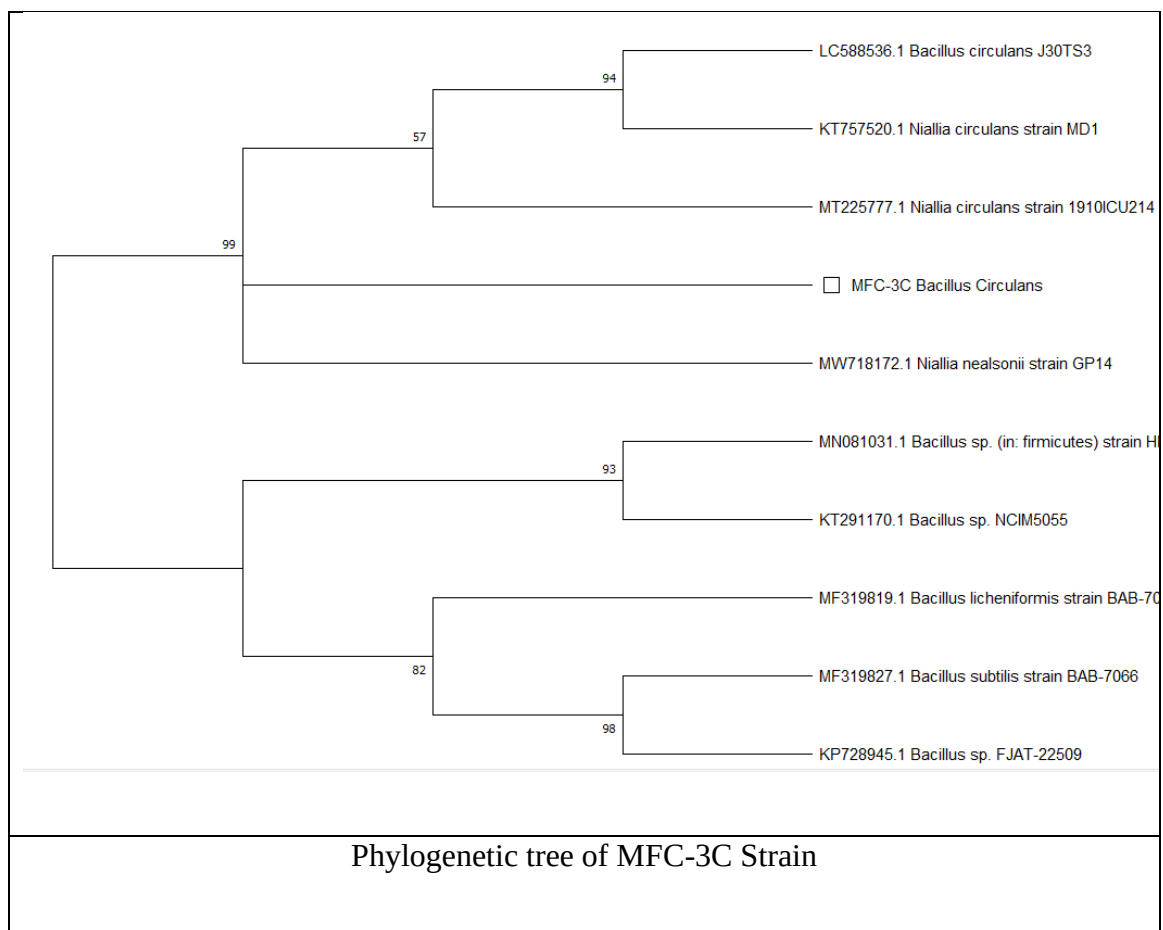
5.4.2 Phylogenetic tree

A phylogenetic tree of each strain has been generated that represents the evolutionary history of the targeted strain. Mega 11 software has been used to align the sequences of different strains (existing in database) compared with our isolated strains. A phylogenetic tree using the Neighbor-Joining method and the bootstrap consensus tree was generated from 10,000 replicates (Table 6).

Table 6: Evolutionary relationships of the taxa.







CHAPTER 6: DISCUSSION

Currently researchers have focused on MFC due to its have become a hot research topic for scientists to investigate its potential to overcome the energy crisis. In this study, we investigated the sludge wastewater of Dhahran sewage company as a source to isolated indigenous bacteria. Samples have been collected from various depths of wastewater randomly to check out bacterial composition of the sludge and to find out which part of the wastewater contains exo-electrogen bacteria. We have found 2 sludge samples that have the capability to generate steady electricity with voltage above 120 mV for more than 8 hours. Next step was to isolate bacteria from these samples that showed promising exo-electrogen capability. Serial dilution and streaking of the isolated bacterium in the diluted petri-dish have been done and three strains (MFC-1A, MFC-2B, and MFC-3C) have been isolated (Table 3 Further characterization of the isolated have been done by using optical microscope, gram staining, and scanning electron microscope.

The first bacteria (MFC-1A) were green colored, with a flat elevation, an entire margin, and a circular shape. While MFC-2B has a creamy color, with a convex elevation, with a lobate margin, and an irregular form. Similarly, MFC-3C strain has a cloudy color, with a raised elevation, a punciform shape, and an entire margin. Both MFC-1A and MFC-2B are gram negative with a rod-shaped cell, while MFC-3C was a gram positive with a bacilli shaped cell. Then the electricity generation ability of the strains was investigated with the goal to find the maximum electromotive force as by determining the maximum E emf, we can estimate the maximum voltage production in MFC, which can be used as a ceiling for the producible cell voltage. This was done by placing each strain in MFC and the optical

density was studied using spectrophotometer at a wavelength of 600nm which can give an idea of the growth trends of each bacterium. MFC-1A showed a promising potential difference that reached about 120 mV at its peak on the 3rd day from the start of the MFC experiment (Table 4). Similarly, MFC-2B showed a promising exo-electrogenic capacity, however, it was not as good as MFC-1A as it showed a peak potential difference of about 73 mV on the 4th day (Table 4). The MFC-3C strain was the strain with the least potential for generating an electromotive force with a peak potential difference of about 53mV on the 2nd day (Table 4). MFC-1A showed the best potential with 120 mV while MFC-3C showed the lowest potential with an electric difference of 53mV. Therefore, both strains were intermittently tested, to check out MFC as a biobattery.

The isolated strains have been and using NCBI database, we found that our strains (MFC-1A, 2B and 3C) have been identified as (*Pseudomonas extremaustralis*, *Pseudomonas veronii* and *Bacillus circulans* or now called *Nalia circulans*) respectively. Then a phylogenetic tree composed of 9 different strains which contains at least 3 strains that have the same genus name as our strain into the MEGA 11 software which has the ability to align the nucleotides of the different strains and generate a phylogenetic tree through the neighboring-joining method and through the bootstrap method we can generate a bootstrap consensus tree, and the results showed that the sequence results are accurate and so we can say that we have managed to isolate 3 strains of bacteria and applied them in the context of microbial fuel cell application for the first time.

After identifying the isolated strains, we did a background check on each of them, and discovered that *pseudomonas extremaustralis* are a type of extremophiles that have several distinct characteristics that prove it as a promising candidate for microbial fuel cell such as

it can use different metabolic pathways for energy production under low oxygen tension, such as arginine and pyruvate fermentation that allows the bacterium to produce electrons that can be transferred to the anode of the MFC, generating a current, another characteristics that can make it as a promising substitute is their ability to produce alginate, a type of exopolysaccharide, that can enhance its attachment to hydrocarbons and the anode surface, which can improve the biofilm formation and the electron transfer efficiency in the MFC, this characteristic is also present in *Pseudomonas veronii* which may be the reason why they are capable of generating a good voltage, however, even though *Bacillus circulans* can degrade some organic hydrocarbon, their electricity generation was found to be low. Nevertheless, we cannot determine whether they are efficient in generating electricity since this depends on multiple factors such as the design of the cell, the type of electrode used, the biocatalyst or the bacteria used and most importantly the charge transfer efficiency from the bacteria to the electrode.

Nonetheless, from our results we can conclude that *Pseudomonas extremaustralis* is a very promising candidate as a biocatalyst in the cell, and while *pseudomonas veronii* is not as promising as *pseudomonas extremaustralis*, it is still can be considered as a promising candidate as the electrode used was lead which is not the best type of electrode, and so it requires further studies and using of better electrodes such as carbon cloth or graphite before we can conclude whether *pseudomonas veronii* can be considered as a strong exoelectrogen candidate or not. And finally, it can be concluded that *Bacillus* or *Nalia circulans* is not a very promising candidate since even if we improve the conditions by using a bigger cell or better electrode, I believe at most it can be considered as a weak exoelectrogen.

Overall, this research has opened a new direction for *pseudomonas extremaustralis* and *pseudomonas veronii* as a potential biocatalyst candidate in bacterial-based fuel cell.

CHAPTER 7: CONCLUSION AND FUTURE PROSPECTS

This study demonstrates the significant potential of wastewater as a source of exoelectrogenic bacteria and we have isolated exoelectrogenic strains of bacteria (*Pseudomonas Extremaustralis*, *Pseudomonas veronii*, and *Bacillus Circulans*). To the best of our knowledge, no prior reports are available on these strains in the context of electricity generation in MFC.

Both *Pseudomonas Extremaustralis* and *Pseudomonas veronii* exhibited a promising potential as primary biocatalysts in MFC, while *Bacillus Circulans* does not appear to be a favorable candidate for electricity generation. Additionally, we conducted comprehensive morphological and microscopic characterizations of these strains.

MFCs come in various designs, and their efficiency can be influenced by multiple factors. To determine the highest possible voltage generated by each strain, it is advisable to use standard electrodes like carbon cloth or graphite.

The results from this study support the idea that these strains can be employed for electricity generation, akin to a biobattery, in a batch mode. However, for industrial-scale applications, it is necessary to scale up the system to volumes such as 100ml or 500ml. Additionally, the strains should be evaluated in a continuous mode MFC, where we control the flow rate of the medium through inlet and outlet, ensuring that the bacteria remain in an exponential growth state and continuously produce electricity.

Furthermore, MFCs have diverse applications, including bioremediation and hydrogen production. Therefore, we can explore how well these strains perform in these specific applications to determine their suitability.

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Publications	Research Articles 1. Abdelnaby, Mahmoud M., Mansur Aliyu, Medhat A. Nemitallah, Ahmed M. Alloush, El-Hassan M. Mahmoud, Khaled M. Ossoss, Mostafa Zeama, and Moataz Dowaidar. 2023. "Design and Synthesis of N-Doped Porous Carbons for the Selective Carbon Dioxide Capture under Humid Flue Gas Conditions" Polymers 15, no. 11: 2475. https://doi.org/10.3390/polym15112475. Book Chapters 1. F.Kanwal, R.Deabel, A. Alqahtanil, E.M. Mahmoud, M. Naeem, M. Ovais, I. Ahmad, "Evolution of Genome Editing Technologies" In: CRISPRized Horticultural Crops, (Ed. K.A. Abd-Elsalam, S. Fiaz, A. Ahmad), Elsevier (2024) 2. Alqahtanil, E.M. Mahmoud, R. Deabel, F. Kanwal, Q. Ahmad, M. Naeem, I. Ahmad, "CRISPR/Cas: Effectors, Mechanism and Classifications" In: CRISPRized Horticultural Crops, (Ed. K.A. Abd-Elsalam, S. Fiaz, A. Ahmad), Elsevier (2024).