PRODUCTION AND OPTIMIZATION OF BIOELECTRICITY FROM WASTEWATER USING MICROBIAL FUEL CELL

By

DEBAJYOTI BOSE

SCHOOL OF ENGINEERING
(DEPARTMENT OF ELECTRICAL & ELECTRONICS)

SUBMITTED
IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE
DEGREE OF
DOCTOR OF PHILOSOPHY

TO

UNIVERSITY OF PETROLEUM & ENERGY STUDIES
DEHRADUN

OCTOBER 2019

SUPERVISOR(S)

Dr. P VIJAY

Dr. M GOPINATH

PRODUCTION AND OPTIMIZATION OF BIOELECTRICITY FROM WASTEWATER USING MICROBIAL FUEL CELL

By

DEBAJYOTI BOSE
(SAP ID 500056858)

SCHOOL OF ENGINEERING
(DEPARTMENT OF ELECTRICAL & ELECTRONICS)

SUBMITTED
IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
TO

UNIVERSITY OF PETROLEUM & ENERGY STUDIES
DEHRADUN

OCTOBER 2019

INTERNAL SUPERVISOR
Dr. P VIJAY
SENIOR ASSOCIATE PROFESSOR
UNIVERSITY OF PETROLEUM & ENERGY STUDIES

EXTERNAL SUPERVISOR
Dr. M GOPINATH
ASSOCIATE PROFESSOR
VEL TECH HIGH TECH DR. RANGARAJAN DR. SAKUNTHALA
ENGINEERING COLLEGE

AUGUST 2020
DECLARATION

I declare that the thesis entitled "**Production and Optimization of Bioelectricity from wastewater using Microbial Fuel Cell**" has been prepared by me under the guidance of Dr. P Vijay, Senior Associate Professor of Chemical Engineering, University of Petroleum & Energy Studies (UPES) and Dr. M Gopinath, Associate Professor of Department of Biotechnology, Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

Debajyoti Bose
Department of Electrical & Electronics,
University of Petroleum & Energy Studies
Energy Acres, Bidholi, PO Bidholi via Prem Nagar
Dehradun 248007, Uttarakhand, India
Date: 7th August, 2020

UPES
UNIVERSITY WITH A PURPOSE

CERTIFICATE

I certify that Debajyoti Bose has prepared his thesis entitled **"Production and Optimization of Bioelectricity from wastewater using Microbial Fuel Cell"** for the award of PhD degree of the University of Petroleum & Energy Studies, under my guidance. He has carried out the work at the Department of Chemical Engineering and the Department of Electrical & Electronics, at University of Petroleum & Energy Studies.

Internal Supervisor
Dr. P Vijay
Senior Associate Professor and Head
Department of Chemical Engineering,
University of Petroleum & Energy Studies
Energy Acres, Bidholi, PO Bidholi via Prem Nagar
Date: 7th Aug, 2020

Vel Tech High Tech
Dr.Rangarajan Dr.Sakunthala Engineering College
(Approved by AICTE, Affiliated to Anna University-Chennai,
Accredited by NAAC with "A" Grade &
NBA (Bio-Tech & Chemical) New Delhi)
#60, Avadi Vel Tech Road, Avadi, Chennai 600 062. Tamil Nadu, India.

Mobile : 73587 01998
Telefax : 044 - 26840605
E-mail : principal@velhightech.com
Website : www.velhightech.com
College : #60, Avadi-Vel Tech Road,
Vel Nagar, Avadi, Chennai - 600 062.

Dr. M. Gopinath, PhD.,
Associate Professor, Department of Biotechnology

Date: **07.08.2020**

CERTIFICATE

I certify that **Mr. Debajyoti Bose** has prepared his thesis entitled **"Production and Optimization of Bioelectricity from wastewater using Microbial Fuel Cell",** for the award of PhD degree of the University of Petroleum & Energy Studies, under my guidance. He has carried out the work at the Department of Chemical Engineering and the Department of Electrical & Electronics, at University of Petroleum & Energy Studies.

M. Gopi

External Supervisor

Dr. M. Gopinath
Associate Professor
Department of Biotechnology,
Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College Avadi,
Chennai 600 062, Tamil Nadu, India
Date: 7th Aug, 2020

ABSTRACT

The advent of industrialization and its rapid growth has contributed to the generation of large quantities of wastewater. Its treatment is energy intensive and involves, in some cases, expensive chemicals. Reducing the cost for wastewater treatment, and using it to obtain useful products, is critical from the standpoint of sustainability. Major sources of wastewater includes but are not restricted to chemical processes, bulk drugs, pharmaceuticals, pesticides, and other process industry units where such treatment is carried out. In the existing system for wastewater treatment, chemicals are employed to treat the water. If the manufacture of these expensive chemicals is considered, then fossil fuels are burnt to produce them, and this continues to increase our emission problems. Therefore, there is a continuous cycle among water, energy, and climate change, which affects the ecosystem.

Instead of treating wastewater resources with energy-intensive chemicals, these resources can be used to develop energy security by taking advantage of their rich organic content. Biotechnology can help and contribute to this and to economic security. For this to be successful, however, strategically targeted programs are needed to assess broadly the diversity and potential for commercial application of microbial reserves. In such cases, microbial fuel cells or MFCs emerge as the most promising options for complete energy recovery. They facilitate in situ conversion of energy (bioelectricity) compared to processes such as fermentation (which are plagued by issues such as low conversion and purification). With the necessary research in this field, such systems can be effectively integrated with effluent treatment plants where bioelectricity can be generated simultaneously with treatment of wastewater.

In this work, two different MFC reactors were developed, one with platinum as catalyst on the cathode in a double-chambered membrane-based system, the other being an Activated Carbon (AC) as catalyst in a single chambered brush anode air cathode system. Both systems were evaluated in terms of capacity for wastewater treatment and bioelectricity generation. These varying electrogenic reactor systems use bacteria metabolism to break down organics present in the wastewater in the absence of oxygen, and then in the process release electrons to the fuel cell circuit. Wastewater derived from sewage has been used in all the studies, and this reached an Open Circuit Voltage of around 1.45 V for platinum and 0.75 V for AC cathodes respectively, further with external load connected to the system, peak power generation for platinum based system was around 820 mW/m^2 or 0.82 W/m^2 and for AC cathode based system 460 mW/m^2 or 0.46 W/m^2. The COD removal efficiency was around 75% for platinum and around 67% with AC, Further, as the COD decreases, the BOD of the wastewater falls from an initial 520 ± 32 mg/l to 165 ± 25 mg/l for AC and 110 ± 20 mg/l for Pt-Cathode. The inoculated sludge based wastewater achieved a BOD removal efficiency of 79.26% and 63.71% for Pt and AC cathodes respectively. This shows wastewater generated at the most fundamental level (in the form of sewage) need not to follow expensive treatment processes, but can be used as a source of renewable energy recovery. This is critical in linking the wastewater infrastructure with energy and sustainability, can address issues pertaining to inadequate sanitation, need for clean water and at the same time harvesting energy from a non-fossil fuel based source.

Further, platinum based systems are usually expensive because of the catalyst itself, here the AC cathodes were indigenously prepared by a chemical activation route that has not been reported previously, to have been used in the context of MFC, Carbon black was used as a supplement, and PVDF as binder, with stainless steel mesh (SS 316L) as the current collector. System eliminated the cost for a

membrane, an aqueous catholyte, and indeed construction cost for cathode chamber. Further a novel brush was introduced as the anode, made from carbon yarn, which as preliminary evaluations found, are a good match for the size of the microbial communities that grow in these wastewater to generate electric fields. The cathode showed high stability, and can tolerate water pressure to more than a meter of height, showing the feasibility of these systems to be scaled up, while cutting the cost significantly on materials.

Further, the microbial community formed was subject to 16S rDNA sequencing, the resulting PCR product and BLAST-n analysis revealed the anaerobes, which can break down organic matter, and generate electrical power. In this work, six species of bacteria colonies were detected, which are predominantly of the *Firmicutes* phyla, along with *Proteobacteria*, and *Actinobacteria*. These include *Achromobacter xylosoxidans, Bacillus subitlis, Brevinacillus agri*, additionally the other three colony forming bacteria identified have not been reported previously in the context of symbiotic biofilm communities, namely *Microbacterium proteolyticum, Bacillus toyonensis* and *Bacillus valezensis* were also discovered. This opens up the possibility for future research to isolate these species and conduct bioremediation experiments to evaluate their individual colony capacity as well as power generation from waste. Hence, a logical deduction from such analysis is that, these bacteria can colonize on surfaces without any ecological damage, and use these colonization strategies to occupy a unique niches leading to MFC technology being a non-combustion based energy recovery process that can be an integral part of new frontiers of energy exploration, wherein a wastewater treatment plant can be converted to a power plant. Further, a cost analysis is presented with the materials used, and the future prospect of this technology is discussed, so as to how these systems can be integrated with existing wastewater treatment plants.

ACKNOWLEDGEMENTS

I would first like to thank my PhD advisors, Dr. P Vijay and Dr. M Gopinath, for the past three years at UPES. Their passion and persistence in science have constantly encouraged me during my PhD study. In my first year, both Dr. Vijay and Dr. Gopinath spent enormous efforts guiding me on research and never lost their patience when my experiment was not going well. They would seek every chance to train my paper writing and presenting skills that help build my confidence and become a qualified researcher. Without their constant support, I would never be able to accomplish my PhD study. All the knowledge and merit that they passed on me, has made me today and will continue benefiting my future life.

I am grateful to Dr. Jitendra K. Pandey and his R&D team at UPES for financial support through SEED, and further acknowledge the continuous encouragement received from Dr. Kamal Bansal and Dr. Shailey Singhal for supporting this work in all its stages. I thank Baskar Bollipalli and Dr. Veyayutham Guruviah for helping insights into MFC reactors with varying configuration, and further we collectively thank Dr. V. Ganasen and his team at ACME ProGen for providing facilities to perform 16S rDNA PCR of microbial communities. I am also grateful to the lab staff and their support at our Institute (UPES) and at the Indian Institute of Technology, Madras (IIT-M) for facilitating microscopic analysis of biofilm communities.

I would like to thank my parents for their constant support and love at every stage of my life. Completing this degree would never be possible without their love and encouragement. Finally, a gratitude to all my dear colleagues for their continuous help and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

A	magnetic vector potential
C_E	coulombic efficiency
D	diffusivity of chemical species
E	electric field
E	elemental charge
$Ø$	electric potential
F	Faraday constant
J	chemical flux into biofilm
K	Boltzmann constant
R	Gas constant
T	Temperature
∇	ionic concentration gradient
Φ	Angle for peak intensity of diffraction beams
Z	valency of the ionic specie

LIST OF ABBREVIATIONS

AC	activated carbon
AC-Cat	activated carbon catalyst
BOD	biological oxygen demand
BLAST	basic local alignment search tool
CB	carbon black
COD	chemical oxygen demand
rDNA	ribosomal deoxyribonucleic acid
MEA	membrane electrode assembly
MEGA	molecular evolutionary genetics analysis
MFC	microbial fuel cell
NCBI	National center for biotechnology information
OCV	open circuit voltage
Pt-Cat	platinum catalyst
PVDF	polyvinylidene fluoride
STP	sewage treatment plant

CHAPTER I
INTRODUCTION

1.1. Global Energy Demand

The biggest challenge which humanity is facing today, partly involves the desire for improvement in the quality of life, for people in the developing world, which primarily depends on the energy available for consumption; and the other aspect is protecting the environment and having a system that can prevent climate change. As population across the globe continues to grow exponentially, if fossil fuels are utilized to power these energy needs, climate change cannot be controlled, leading to irreversible damage to the entire ecosystem. The world energy demand saw a rise from 421 quadrillions British Thermal Units (BTUs) in 2003 and was at 563 quadrillions BTUs for 2015 and is expected to reach 722 quadrillions BTUs in 2030 [1]. There are currently over seven billion people on earth with a projection of between 9.8 billion and 12.5 billion people in 2100.

Energy is one of the main drivers of economic and social development, just a few years ago; the only uncertainty in energy was the price of oil, there is now more known resources in the world than before [2]. Peak oil remains to be a concern, and this anxiety has been replaced by myriad issues and complex challenges. For instance, while significant, the development of renewables globally, excluding hydropower has been slower than expected. By 2050, there will be a doubling of energy demand, with a share of electricity increasing faster than primary energy supply. With the present mix of renewable energy and the dominant fossil fuel

sector, GHG emissions can double or even quadruple by 2050 compared to 1990 levels, with a potential to expose our energy infrastructure to disasters.

The lack of a climate framework is one of the issues that keep global energy leaders awake at night, but energy prices and high associated volatility have become critical in the absence of a clear path for the future of CO_2 prices. Accelerated change in policies, technological innovation and consumer expectations are making energy markets increasingly complex. Current business models struggle to guarantee both competitiveness and affordable energy in the future; while political and regulatory risks threaten investment in infrastructure and innovation. There is a need to increase energy access for the world's growing population; otherwise, up to 530 million people could still lack access to even basic form of energy in 2050 [3]. This restricts life chances, opportunity and prosperity. As we look to identify sound stable policies that can address these issues and cartelize investment towards sustainable energy solutions, focus on non-combustion based energy sources should increase over the years.

Fossil fuels such as coal, oil and natural gas are continuously supporting the global economic growth and account for over 80% of total energy consumption [2]. In the course of recent years, utilization of sustainable energy sources has expanded with focus on waste streams, as they are a path for recovering resources and utilizing them in an environment friendly way. Since fossil fuel-based economy will not go on forever and that their exploration adds more to ecological damage, energy generation from such sources, which essentially originates from the non-

combustion processes, can provide reasonable energy supply with minimum damage to the ecosystem.

1.2. The Water Infrastructure

As we developed our major civilizations around big rivers, like Tigris and Euphrates, Nile, Indus and many others, the importance of water in our daily lives was noticeable. Water is the most essential element of life; it is needed for drinking and sanitation, agriculture, to produce power and cool our power plants, and to maintain our ecosystem services. With growing population, access to clean water is becoming more problematic. In a recent report by UNICEF [3], at least 2.6 billion people lack adequate sanitation, while 1.1 billion people live without clean drinking water. Water is a key component in everyday life, irrespective of where people live. Applications include energy, industry, agriculture, and livestock. While the earth is around 75% water, the problem is only 3% of that water is fresh water. Over 260 river basins are shared by two or more countries, and most of these rivers are without defined legal or institutional arrangements. In a report submitted by the US Senate Committee on Foreign Relations on water scarcity [4], it focused on the rising water tension that can destabilize Central and South Asia. According to the report, the implications of a water shortage has already caused aggravated demands for agriculture and power generation in these countries. Water without a doubt will be a huge issue, along with the use of fossil fuels, throughout this century.

Surface water resources are nearing exhaustion, there are many incidents, that have been reported over the last few years, of lakes and rivers going dry, and tapping ground water is becoming a cause for concern. Things like the Apollo Moon

Missions and other NASA programs, where recycling and re-drinking urine by the astronauts, would not help much. That is not the end of the story, because water has no respect for political boundaries, given that 148 countries share 276 international river basins [5], as water becomes scarce, there are increased chances of water conflicts.

Water conflicts, they do exists, between farmers, provinces and states. One thing imperative to remember is water conflicts are not just about water, they are connected to food, energy, economy and politics. Therefore, even if countries claim they are bargaining over water, it is beyond that, and it can essentially become a weapon to threaten neighbors. However, once the connections between water and other resources are realized, it can be seen as an opportunity for trade; water can be traded for food, energy, a better reputation at the international level. So conflicted it may seem, it provides many opportunities. This means, water can catalyze conflicts, critically it can also catalyze cooperation. Historically, cases of cooperation has been much more than conflicts on this planet.

1.3. Global Wastewater Production

The advent of industrialization and its rapid growth contributed for large quantity of wastewater generation with its treatment being energy intensive and involves in some cases expensive chemicals. Reducing the cost for wastewater treatment and in the process use that stream to obtain useful products is a critical concept in the purview of sustainability [6]. Major sources of wastewater aggregate from chemical processes, bulk drugs, pharmaceuticals, pesticides and other process industry units where such treatment is done. If we review the existing system for wastewater

treatment, it is seen that to treat the water, chemicals are being used, and if the manufacturing of these expensive chemicals are considered, then fossil fuels are burnt to produce them, and this continues to increase our emission problems. So there is a continuous cycle, between Water, Energy and Climate change which affects the ecosystem.

If we consider the wastewater sources, instead of treating them with energy intensive chemicals, the same can be used to develop energy security through its rich organic content utilization. Bioenergy systems can help develop and contribute to developing these economic securities [7]. However, for them to be successful, strategically targeted programs are needed to assess broadly the diversity and potential for commercial application of microbial reserves for a nation. In such cases, Microbial Fuel Cell or MFC, emerge as the most promising option for complete energy recovery and critically facilitates in situ conversion of energy (in the form of bioelectricity) compared to processes such as fermentation (which are plagued by issues like, low conversion, purification etc.).

Given the proper research in this field, such systems can be effectively integrated with Effluent Treatment Plants where bioelectricity can be generated simultaneously with treatment of wastewater. There exists a significant difference between the generation and treatment of wastewater especially domestic wastewater particularly across developing nations, and renewable energy recovery through these processes is possible using various tools of bioenergy. **Table 1.1** enlists the major sources of wastewater generation from process industries. These can be incorporated directly with MFCs or by inoculation with specific microbial specie. Bacteria specie that produce bioelectricity in MFC are metal reducing

bacteria such as but are not restricted to *Geobacter metallireducens* [8], *Geobacter sulfurreducens* [9], *Clostrodium butyricum* [10], *Shewanella putrefaciens* [11].

Table 1.1: List of some industries and the contaminants in its wastewater streams, along with potential avenues for reuse of the treated water.

Sources of Wastewater	**Characterization of wastewater in the discharge stream**	**Scope for Reuse of Treated Water**	**Reference**
Coal based Thermal Power Plant	Significant levels of lead, mercury, cadmium, chromium, arsenic and nitrous compounds	Primarily used in boiler feed water system	Huang et al. [12]
Food Industry	High concentration of BOD, and suspended solids	Reused in evaporators, chillers, and dust control	Xiao et al. [13]
Iron and Steel industry	Ammonia, cyanide, phenols, benzene and other organics.	Various Unit operations as a medium for process cooling	Bose et al. [14]
Paper and Pulp Industry	High suspended solids (SS), organic halides, chlorinated organic compounds, BOD, COD	Various steps of the manufacturing process, and process cooling	Sharma et al. [15]
Petrochemical Industry	Mineral oils, phenol, High COD and BOD, chromium	Primarily as cooling tower makeup water and boiler feed water	Daud et al. [16]

If domestic wastewater is considered, for instance, in India, an estimated 38,354 million Liters per Day (LPD) sewage is generated every day in the major cities with the present treatment capacity being 11, 786 million LPD, also, installed Sewage Treatment Plants (STPs) for treating this wastewater are mostly not complying with prescribed standards [3]. The reuse of this wastewater is restricted to agricultural and other industrial applications, as risks are associated with human health and the environment [17]. While considering these wastewater streams for treatment, the energy involved in running the wastewater plants and the treatment chemicals cost a lot of energy. In the United States, an analysis of organics trapped in wastewater showed that the average energy trapped inside wastewater is about 17 GW (Giga Watts) [18], A nuclear power plant is about 1 GW, so these are not trivial numbers, and given the exponential growth pattern of population density, it is expected that sewage sources will continue to rise, this represents an opportunity (for energy recovery) and a problem that has to be addressed.

1.4. Methods of Wastewater Treatment

Population growth, rapid urbanization, and industrialization are the driving forces behind the generation of wastewater. From the point of view of generation, wastewater might be characterized as waste carried by water expelled from residences, institutions, industries together with the ground water, storm water and surface water [19]. The constituents of wastewater vary with the source of its origin. Wastewater essentially includes water (90 - 98%) together with some amount of suspended and dissolved organic and inorganic solids. Starches, lignin, fats, soaps, cleansers, proteins and their disintegration are the basic organic substances comprising the sewage [20]. Different synthetic and natural chemicals from the industries are also included in this.

Wastewater treatment is used to convert wastewater into water that can be safely discharged with minimum impact on the environment or can be directly reused [21]. The treatment of residential or industrial wastewater is important to maintain the quality of water and water resources. Wastewater Treatment Plants play a crucial role to ensure that wastewater is treated suitably before the subsequent treated water is released to the environment. Wastewater treatment processes can be broadly classified based on the method used; **Table 1.2A** highlights the various physical and chemical treatment processes.

Table 1.2A: Overview of Physical and Chemical Processes for Wastewater Treatment

Type of Process	**Process Characteristics**	**Critical Parameters**	**References**
Greensand Filtration	Elimination of dissolved iron, hydrogen sulfide, and manganese from water.	Water temperature, regeneration rates	Heins et al. [22]
Sedimentation	Uses gravity to expelled suspended solids from water.	Density, Particle size, concentration	Wang et al. [2]
Flotation	Utilize techniques for air injection such that little air bubbles (<0.1mm) ought to be shaped. The production of little air bubbles can be performed chemically, electrolysis, by dissolving air in water at high pressures or by ejectors.	Reagent dosing rate, air addition rate, flotation depth, particle size distribution, feed rate	Mondal et al. [23]
Chemical Coagulation	Positively charged coagulants are introduced by chemical coagulation, which destabilize the particles, decreases the charge of particles, when the charge is reduced, aggregation of particles takes place.	Coagulant dosage, pollutant properties, pH, initial turbidity,	Subedi et al. [24]

Chemical Precipitation	Most widely recognized technique for expelling various metals which have been dissolved from the wastewater Efficiency of the process depends on the type of metal which are present in the mixture.	Reagent Concentration, solution pH, decontamination factor, particle size distribution	Mallouk et al. [25]
Chemical stabilization	The rate of bacterial growth inside the sludge gets lower by introducing an oxidant and removes odor, then from the sludge water is expelled.	Solution pH, chemical concentration	Mondal et al. [23]

1.5. Challenges in Wastewater treatment

Renovation and innovation are the two important aspects of wastewater treatment plants, as wastewater treatment plants are the heart of any utility, the water is taken from the mountains, and then it is made safe to drink, and made available to customers for consumption. Portable water that is utilized in homes, farms and other industrial complex is mostly treated using chemical processes to eliminate harmful contaminants, along with bioreactors to remove the bacteria biomass (such as in activated sludge removal process). Once treated, the water is recycled back into the system. These processes primarily involve chlorination, lime and hydrogen peroxide usage. **Table 1.2B** provides a relative understanding of the merits and demerits of some of these processes.

Table 1.2B: Merits and Demerits with Chemical treatment of Wastewater

Wastewater Treatment Method	**Merits**	**Demerits**	**References**
Typical physical/ chemical processes	Usually involves low capital costs	a. Cost of chemical consumption. b. High maintenance cost. c. Cost associated with sludge handling d. Production of non-usable treated water.	Bruggen et al. [20]
Membrane based systems	Usually involves low labor cost	a. Cost associated with cleaning agents, anti-scaling agents, biocides etc. b. High cost of concentrate handling. c. High energy and maintenance cost.	Wang et al. [2]
Vacuum Distillation/ Evaporation	Treated water is usable for common purpose	a. Very high Capital costs. b. Costs associated with high chemical consumption. c. High concentrate handling cost. d. High energy and maintenance cost.	Mondal et al. [23]

Owing to the convenience of operation, membrane separation has been effective for the treatment of these effluents, filtration processes such as Ultrafiltration (UF) and Nano-filtration (NF) along with Reverse Osmosis (RO) has gained increased importance. However, these processes can be effectively integrated with biological methods in a novel way and can contribute to environmental protection while supporting the water infrastructure.

1.6. Role of Bioenergy in Wastewater Treatment

Bioenergy is ideal for a world where consumption reflects resource availability without harming future stock. For instance, the implementation of biogas digester [26], in recent years has helped to combine effluent treatment with bioenergy generation, thus making it a practical investment for food and beverage industries, as this allows the production of on-site energy from dilute liquid residues.

The main objective of wastewater treatment is to treat the wastewater and dispose of the effluents without causing any negative impact on the environment. Generally, wastewater containing biodegradable constituents can be treated using biochemical methods with proper analysis and environmental control. The biochemical process aims to convert the dissolved biodegradable constituents to acceptable end products using the microorganisms. Before that, certain pretreatment methodologies are followed to remove toxic components present in it. The process involves the introduction of microorganism in water to be treated to reduce the BOD content and the organic matter present in these streams [27]. Major functions of biochemical treatment process include removal of nitrogen and phosphorus; nitrification which involve conversion of ammonia to nitrate and further conversion of nitrate to nitrogen and other gaseous products. Removal of microbial biomass after treatment is also important and is measured as BOD in the effluent. Since the specific gravity of biomass is greater than water, separation is achieved by gravity settling technique. A review of the existing biochemical processes based on metabolic function is shown in **Table 1.3** given in the following page.

Table 1.3: Overview of existing biochemical processes that are used for wastewater treatment

Biochemical Processes	Process Characteristics	References
Activated Sludge Process	In this method, the wastewater containing microorganisms is aerated in the aeration tank promoting microbial growth in wastewater. It is a low cost and compact process.	Logan [28]
Oxidation Pond	Bacteria, algae and organic matter which feed on organic compound interact with water in these ponds. The only demerit of this process is that it is slow and requires a large area.	Bose et al. [19]
Suspended Growth Process	Suspended growth process generally used in municipal and industrial wastewater treatment are operated with a presence of oxygen or in other words aerobic conditions are implied but suspended growth anaerobic reactors are used in high organic concentration industrial wastewater.	Mohan et al. [29]
Attached Growth Process	In this process the microorganisms responsible for purification are attached to a packing material. Attached growth process can be operated as both aerobic as well as anaerobic process. The most widely used attached growth process is the Trickling Filter.	Heins et al. [22]

1.7. Microbial Fuel Cells (MFCs)

Fuel cells are electrochemical devices, which operate on hydrogen-rich sources as long as it has a supply of the fuel to produce clean energy. This energy can be used to feed power to the grid, automobiles, and even everyday electronics that we use. As of now, delivered power modules or fuel cells join hydrogen and oxygen without

burning to create power. The oxygen can be utilized from air itself, while the hydrogen can either be delivered from existing hydrogen infrastructure or renewable energy-based systems. An overview of the same is given in **Table 1.4**, Further, new power devices are being produced that can utilize petroleum products specifically in this context.

Table 1.4: Types of Fuel Cells with power generation capacity

Fuel Cell Type	Electrolyte	Operating Temperature	Electrical Efficiency	Energy Output	References
Alkaline Fuel Cell	Potassium Hydroxide	25- 90ºC	60-70%	300W - 5kW	Bose [30]
Proton Exchange Membrane Fuel Cell	Nafion and PTFE based membranes	25- 80ºC	40-60%	1 kW	Logan [28]
Direct Methanol Fuel Cell	Nafion and PTFE based membranes	25-130ºC	20-30%	1 kW	Li et al. [31]
Phosphoric Acid Fuel Cell	Phosphoric Acid	160º- 200º C	50-55%	200 kW	Dicks et al. [32]
Molten Carbonate Fuel Cell	Molten mixture of alkali metal carbonates	620ºC- 660ºC	55-65%	2 MW- 100 MW	Oliveira et al. [33]
Solid Oxide Fuel Cell	Ceramic type membranes	800-1000ºC	60-65ºC	100kW	Bruggen et al. [20]

Another type of fuel cell is the Microbial Fuel Cell or MFCs, these are devices where bacteria can grow on one electrode, break down organic matter, while releasing electrons from the organic matter which then flows through an electrochemical circuit to complete the reaction[19]. In concept, a real simple system, having two electrodes on either side of the container, bolted together, wastewater is supplied to the system and power generation is observed.

As shown in **Fig. 1.1**, in MFCs, bacteria acts as catalyst which oxidizes the organic matter present in the wastewater and through that metabolic process generate electricity [34]. These are an older invention than the battery. In the MFC bacteria can grow on one electrode, break down organic matter present in the wastewater and release electrons from the organic matter [19, 35]. A process similar to how we get energy, we eat food, oxidize it, remove the electrons, and send these electrons to respiratory enzymes and then when we are done with them they are released to oxygen. For humans, we eat and breathe to do this, so when bacteria releases these electrons it creates a potential difference of about 0.5 V, plugging in a resistor into the system, allows us to compute current using Ohm's law (V= IR), and voltage multiplied by current is power (P= VI), and is the power generated by the system. Microbial transfer of electrons from the substrate moves to the anode, which is the negative terminal and onto the cathode, which is the positive terminal. These are linked by conductive materials; along with an external load (i.e. resistor). Electron transfer is possible by using electron mediators into the anode, usage of membrane directly for electron transfer or by use of nanowires [18]. It can be speculated that further undiscovered means can also facilitate such processes.

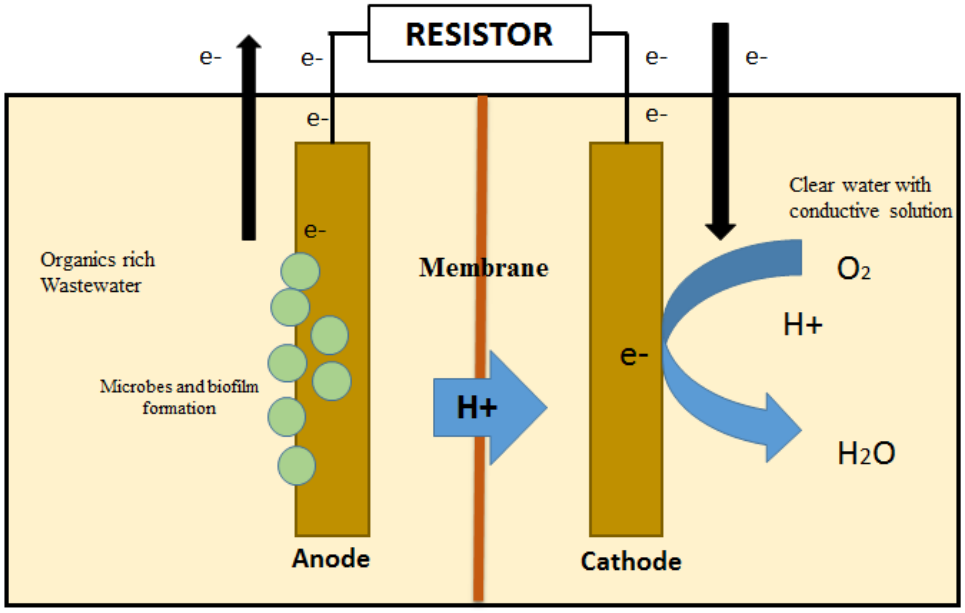


Figure 1.1: Schematics of the basic components of an MFC, wastewater is charged into the anode, on which the bacteria grows, oxidizes the organic matter present in the wastewater and release electrons to the anode [14].

1.8. Mechanisms for Microbial conversion

The microbial growth takes place on the anode, in the form of biofilms, which are capable of electron transfer by breaking down organic matter present in the wastewater, these electrons then travel the length of the circuit through load to the cathode, and the process is completed. The membrane separates the anode from the cathode and drives the ion transfer owing to different potentials of the electrodes. This aspect of bacteria decomposing organic matter is based on relatively recent discoveries, which showed that microbes can donate electrons to an electrode. The mechanism for electron transfer by the bacteria is via conductive appendages called nanowires, or directly through point of contact on the anode (as shown in **Fig. 1.2**).

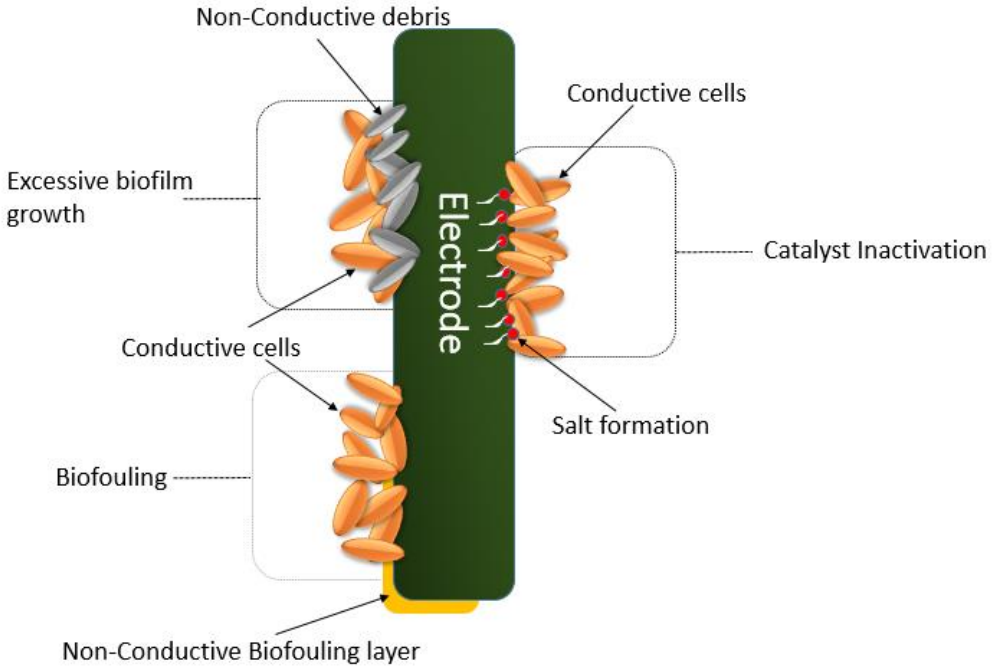


Figure 1.2: Microbial community growth on the carbon strands of the anode [16].

To date, there are no commercial applications of MFCs in wastewater treatment plants and there are only limited studies on scaling up MFCs. The main limiting factors for commercialization are the membrane cost, high cathode cost and the absence of fabrication method for cathode scale up [12]. Another issue is the low power generation from MFCs, mainly due to poor cathode performance. Platinum-based cathodes lose their activity over time with lowered power production and eventually gets fouled, and cannot be regenerated. Some of these issues has been addressed in the present work.

1.9. Objectives

This work mainly focuses on developing MFC reactors, new fabrication methods to scale up MFC cathodes, optimizing MFC performance and understanding the colonies that develop in these systems, for contaminants removal and power generation. There are five objectives as listed below:

- Reduction of organic wastes in wastewater and simultaneous Bioelectricity production using batch process
- Optimization of MFC System for Bioelectricity production using batch process
- Continuous Bioelectricity production with optimized System
- Identification of potent-microbial species responsible for Bioelectricity production
- Cost analysis for Bioelectricity production.

1.10. Scope and Outline of the Present work

In the research reported here, we begin with the advances in MFC reactor fabrication, and optimization, in the form of literature review as mentioned in **Chapter 2**, the focus is primarily on MFC reactor configurations, effect of precious metals and non-precious metals catalysts, and types of wastewaters used. The result of this survey is summarized in the paper entitled "Sustainable power generation from wastewater sources using Microbial Fuel Cell" by Debajyoti Bose, M. Gopinath and P. Vijay, the same is published in the journal *Biofuels, Bioproducts and Biorefining* by Wiley.

The following chapters addresses the various methodologies employed for creating, initially a two-chambered membrane based MFC, followed by an optimized one

chambered air cathode MFC (**Chapter 3**) and the corresponding results of each analysis (**Chapter 4**), also present are the characterization techniques for the wastewater, with contamination removal techniques and the power generation aspects. Further, the anodic biofilm was isolated to study the microbial communities that were present in it, using 16S rDNA PCR, the same was identified for six species and sub-species of bacteria, with *Firmicutes* being the dominant colony. Further, the two chambered MFC has Nafion-117 as membrane, carbon cloth as anode and Pt-Catalyst based carbon cloth as cathode. Platinum is most commonly used cathode catalyst due to its high efficiency towards oxygen reduction. The MFC system analysis was devised to show the practical aspects of such systems in real time operations, and what can be the scope of using non-precious metals as catalyst (instead of expensive platinum); which formed the basis of fabricating an air-cathode activated carbon cathode based MFC. The work was published in a series of two papers entitled "Bioelectricity generation from sewage and wastewater treatment using two-chambered microbial fuel cell" and "Sustainable Power Generation from Sewage and Energy Recovery from Wastewater with variable resistance using Microbial Fuel Cell" by the same group, the former was published by Wiley in *International Journal of Energy Research*, while the latter by Elsevier, in *Enzyme and Microbial Technology.*

One of the major issues of using platinum as catalyst is cost, while exploring optimization parameters. a new low cost indigenously prepared activated carbon (AC) based catalyst was developed with carbon black, with polyvinylidene fluoride (PVDF) phase inversion coating as cathode diffusion layer, with wastewater on one side, and air on the other. Cathodes with this new phase inversion coating has larger oxygen mass transfer coefficient than conventional platinum/carbon cloth cathode. Many studies over the years have reported the use of AC cathodes from different sources, we developed a novel indigenous cathode from a biomass source that has

not yet been reported, and a unique chemical activation route is implemented for the same, which has several advantages over the conventional process of making AC. Further, the cathode prepared lacked carboxylic acid based functional group, which means it has better stability with the stainless steel mesh (316L) current collector. Also, a novel carbon yarn based brush was implemented as anode to reduce electrode distance from the cathode, and the reactor itself was modified to a membrane less single chamber air cathode brush anode MFC, where natural air in the environment act as the catalyst. Given the construction of air cathode MFC, performance was contrasted and compared with the platinum, where the platinum based system would give peak power of around 820 mW/m^2 for the air cathode the same would be around 460 mW/m^2, with COD and BOD removal efficiencies in both cases to be higher than 60%. Further, after 14- 15 cycles, platinum would foul, forming an insoluble layer of PtO, and cannot be regenerated, while AC cathodes after similar cycles, can be regenerated with dilute acid (HCl) with more than 86% of its original activity. Further material cost for the two chambered system is around $72 and the same for AC systems is around $10, this shows systems can be optimized in terms of precious metal catalyst, reactor cost, and cathode activity, without losing significant power output and contamination removal efficiencies. This work was published the journal *Fuel,* by Elsevier, entitled "Biomass derived activated carbon cathode performance for sustainable power generation from Microbial Fuel Cells."

16S rDNA PCR revealed the electricity generating microbes present in the wastewater were predominantly of the *Firmicutes* phyla, along with *Proteobacteria,* and *Actinobacteria,* further, power generation was stable in both MFCs, these results indicate biofilm communities in mixed cultures do not go for colony competition, and in turn has a symbiosis prospect for colonization, contamination removal and bioelectricity production. Sewage derived wastewater

is one of the most interesting choice in MFC, as at the fundamental level, this is the source of wastewater which requires urgent treatment, be it in third world countries or industrialized nations[24]. The wastewater used in this study for a total of three MFCs (1 double chambered reactor and 2 single chambered) was critical for microbial growth, and the biofilm which forms on the anode for electron transfer was analyzed using 16S rDNA sequencing to retrieve PCR products which were aligned in NCBI's BLAST-n (version: BLAST+ 2.8.1, freeware) suite to identify the potent microbial species responsible for power generation, and the method adopted in this is maximum likelihood. Further, the phylogenetic tree was constructed using the freeware MEGA (version: 10.0.5). This work was published the journal *Fuel*, by Elsevier, entitled "Bioelectricity generation and biofilm analysis from sewage sources using Microbial Fuel Cell."

In **Chapter 5**, we conclude with a potential outlook on how these systems can be effectively integrated with real time wastewater treatment plants, without altering existing configurations; and the positive impact they can have on energy security, which is linked with economic stability. Treatment of industrial and domestic wastewater using the microbial reserves can contribute significantly to advancing wastewater treatment infrastructure through effective chemical and biological removal, and in the process generate value-added product in the form of bioelectricity.

CHAPTER II
LITERATURE REVIEW

2.1. Aspects of MFC Technologies

Microbial Fuel Cells (MFCs) have been depicted as bioreactors that involve energy conversion from chemical bonds of organic compounds into electrical energy through microbial activity that can be characterized as bio-catalytic under anaerobic conditions. As shown in **Fig. 2.1**, MFC innovation speaks to an imaginative approach of utilizing microorganisms for era of bioelectricity by oxidation of organic matter present in the wastewater streams.

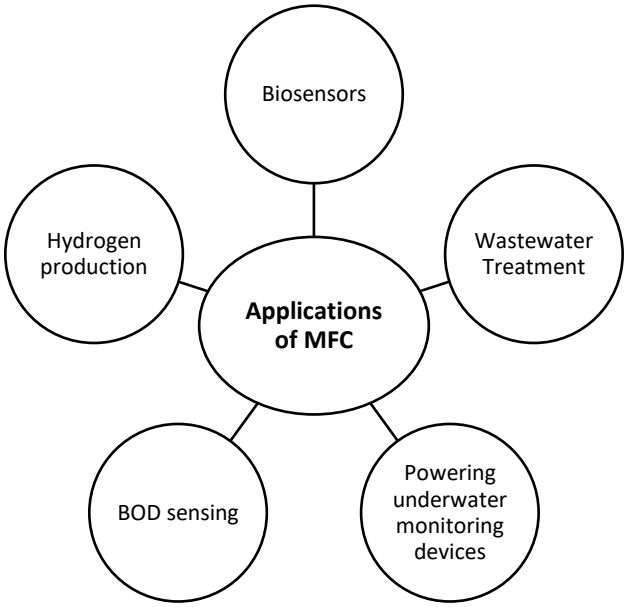


Figure 2.1: The end use application for MFCs to serve bioremediation and bioelectricity generation.

Further, as shown in **Table 2.1,** MFCs can be modified to various microbial electrochemical technologies (METs) that are currently being investigated, which

is critical to discovering new biotechnology resources, further which can promote strategically targeted program to assess and develop commercial application of a Nation's microbial reserves.

Table 2.1: Different Microbial Electrochemical Technologies which are either identical or an extension of MFCs

System Names based on Design	Mechanism	Notable Work
Microbial Electrolysis Cell (MEC)	Production of hydrogen gas at the cathode, and metal reduction	Pasupuleti et al.[36]
Microbial Electro synthesis System (MES)	Produces soluble organics such as acetate	Ross et al.[37]
Microbial Methanogenesis Cell (MMC)	Production of Methane at the Cathode	Yuan et al.[38]
Microbial Reverse Electrodialysis Fuel Cell (MRFC)	Power generation using a RED stack, yield is higher than a standard MFC	Kim et al. [39]
Sediment Microbial Fuel Cell (s-MFC)	Power generation from marine sediments, soil and mud	Schneider et al.[40]

This represents an exciting prospect to work on better understanding the bacteria that function within an electrogenic biofilm and helps us to harvest electricity from MFCs, thereby supporting the cause for sustainability for the wastewater infrastructure.

2.2. MFC modes of Operation

MFCs can be operated in batch mode, or continuous mode. In batch mode, a specific amount of wastewater is added to the anode chamber, and within a given timeframe, which can range from 24 hours to two weeks, the voltage is recorded using a data acquisition system. In continuous mode, a fixed or variable amount of wastewater is replaced and then a fresh volume is added, and parameters are measured continually. In most studies, batch mode has been reported to yield maximum power, as microbial communities require time to settle on the anode, and become sufficiently stable for electron transfer at higher rates. During the operation, COD, BOD and pH are the key contamination related parameters that are monitored, while voltage recorded (with fixed external resistance) normalized to the anode surface area gives the current density (mA/m^2) and power density (mW/m^2).

2.3. Exoelectrogenic microbial communities

Evaluating biofilm growth and community analysis using 16S rDNA sequencing techniques for PCR products reveal the bacteria specie present in the wastewater[41], and it can directly be related to colony competition or symbiosis depending on power generation curves from the MFC. Over the years, several bacterial species, through community analysis[8], have been identified individually or in mixed cultures that produce high power densities ($> 2\ W/m^2$), which are a key to bring MFCs close to the commercial platform, these metabolisms include exocellular electron transfer, via cellular respiration and cell to cell communication[42].

Over the years, analysis of microbial communities developed in MFCs do not reveal any specific trend for the biofilm growth, these are usually *Alpha-*, *Beta-* or *Gammaproteobacteria* based communities. *Proteobacteria* are mostly gram-negative bacteria that can induce nitrogen fixation, and has the capability to grow under very low level of nutrients[28]. This means for MFCs to achieve wastewater treatment through bacteria metabolism, low values of COD (≤100 mg/l) is possible while generating power. *Alphaproteobacteria* have been found consistent with MFCs with marine sediments, *Betaproteobacteria* clones have been found consistent with wastewater and anaerobic sludge derived from starch processing plants[43], and studies that have taken into consideration, activated sludge in the MFCs, have found *Gammaproteobacteria* clones dominant among other diverse anode communities[44].

One important aspect of the MFC architecture is the cathode and its effect on biofilm formation, depending on the type of system, the traditional double chambered or the single chambered, the cathode has high oxygen diffusivity, with the membrane (for double chambered system) separating the anode and the cathode is permeable to gases and other soluble organic and inorganic species[15]. Thus, for air cathodes oxygen can diffuse into the anode chamber, and similarly for membrane based double-chambered systems, the sulfate, nitrate, ammonia and other species can diffuse into the anode through the membrane[13]. This means bacteria communities have to grow using oxygen or other alternate electron acceptors, now if power generation is not affected by these parameters (which is consistent in this case); this will imply power generation from MFCs has a positive influence on diversity of the communities[9], thus paving the way for real time scale up of these MFC architectures. It must be emphasized that, in order to fully understand microbial metabolism, studies with pure culture are equally important, as the electron transfer efficiency from the microbes with pure culture, from

substrates present in wastewater is directly attributed to cell respiration[45]. The conversion rate of these substrates is hard to predict i.e. the cell yield, and with pure cultures there are estimates of achieving high COD removal and Coulombic efficiency[18] over 85%. This implies unlike other processes that convert waste into energy (such as biogas generation), the efficiency with MFCs is significantly higher, as less energy is lost because the energy conversion stages are less[46]. This makes community analysis of bacteria an exciting prospect, which can help understand the electrogenic activities of species that are known and that are yet to be discovered, and further the effect of the cathode as suitable electron acceptors for these developed biofilms over time.

2.4. MFC System Performance with Pure microbial cultures and complex cultures

Several studies have shown MFCs operating with mixed cultures achieve comparatively more power densities than those operated with pure cultures [34], [40], [47]. High power generation with pure culture is possible, but for such cases the cultures were grown externally and same device was not used in acclimatized mixed cultures [48]. Analysis of the microbial communities has revealed the sheer diversity in the processes. *Shewanella oneidensis* has been shown as an effective inoculum in a comparative study where it was inoculated with wastewater samples from agriculture, paper, food and domestic and found superior current generation as compared to mixed cultures in wastewater. The pure culture reported an Open Circuit Voltage (OCV) of 687 mV and the study also reports that only food industry wastewater accounts for maximum efficiency during operation. Most studies however found it more practical to use mixed cultures instead of pure cultures [28], [33], [49]. But it must be considered that reactor configuration is not the same, hence comparative analysis is difficult. In addition, as shown in **Table 2.2**, pure cultures tend to produce significantly less power than mixed sources.

Table 2.2: MFC studies at room temperatures (25-30 °C) with varying system configuration.

Name of the pure microbial cultures	Type of System used	Power produced	Significance related to operating conditions	References
Escherichia coli strain K-12	Double chambered PVC T-joint type	215 mW/m^2	Improvement in power density (by 25%) when manure was added as substrate.	Zheng et al. [50]
Bacillus subtilis	Single Chambered with Glass bridge	600 mW/m^2	Demonstrated the use of glycerol as a substrate, pH 7 was most suitable.	Nimje et al. [51]
Shewanella oneidensis strain 14063	Double chambered with Ultrex CMI-7000 as membrane	>40 mW/m^2 at Acid Orange (AO) 7	Selected bacteria specie showed enhanced kinetics for AO 7 decolorization and bioelectricity production	Fernando et al. [52]
Escherichia coli	Double chambered with Nafion-117 membrane	502 mW/m^2	Mediatorless system, here nutrients in potato extracts acted as biocatalyst for effective COD removal and bioelectricity generation.	Hernandez et al. [43]
Pseudomonas puti da strain 1059	Air cathode single chambered	0.005 mW/cm^2	Soluble electron shuttle from oil refinery wastewater used as a substrate.	Majumdar et al. [53]
Pseudomonas aeruginosa strain ZH1	Double chambered with Nafion-115 membrane	451.26 mW/m^2	Isolated aeruginosa strain produced more power than standard activated sludge with undefined culture.	Nor et al. [54]
Klebsiella variicola	Air cathode single chambered	1648.70 mW/m^3	At room temp. COD removal from Palm oil effluent around 74%	Islam et al. [55]
Desulfovibrio aminophilus, Adrenella kashmirensis	Double chambered with Nafion-117 membrane	7.8 W/m^3	Complete conversion of sulfate in wastewater to biogenic sulfur, exhibited good sulfur reducing property	Kumar et al. [56]

For artificial wastewater created with inoculation of *Saccharomyces cerevisiae* showed potential OCV generation close to 760 mV with varying fructose concentration [47]. The study used natural red and ferricyanide as mediators, and generated power and current density of about 33 mW/m^2 and 97 mA/m^2. However, it must be mentioned that use of ferricyanide increases power density but it needs to be chemically regenerated and running cost is high. In this case, the pollutants present in the wastewater can be used as alternative to this. One study claims the use of azo dyes as electron acceptor at the cathode, utilizing the double bond nitrogen chemistry of azo dyes [49]. More details related to performance of complex cultures is mentioned in Section 2.5.5.

2.5. Parameters affecting MFC Performance

2.5.1. Effect of Electrode Material

Research in electrode material in the form of cathode or the anode should serve some basic functionality such as, maximize power generation, improve coulombic efficiency, minimizing cost and creating scalable architecture. A critical research in developing cathode material is the need for catalyst; focus is on replacing precious metal catalysts with non-precious and other transition metals. For MFC electrodes, materials with small pore size should be avoided as they easily fill out and clog [39]. Considering the reactor designs for different operations related to wastewater treatment such as trickling filters, biofilm reactors, these are usually characterized by typical surface area of 100 m^2/ m^3 of reactor for structured plastic media [37], this avoids clogging by biofilms, avoids clogging by materials in wastewater, allowing sufficient air flow in a reactor. Electrode spacing in MFCs play a crucial role in power production capacity. One study that used cassava mills wastewater varied distance between electrodes at 18, 21 and 24 cm to see change in power density and maximum

voltage, which was around 1800 mW/m^2 and 174 mV when the spacing was at 24 cm with a feeding rate of 5 ml/min [57]. MEA was glass wool and graphite plates.

Cathode: At the cathode, protons, electrons and oxygen meet at a catalyst in a tri-phase reaction. For the cathode to work effectively, catalyst should be on a conductive surface, and all must meet at the same point. The materials mainly used for construction of the cathode has been primarily, carbon paper, carbon cloth, graphite, woven graphite, graphite granules and brushes [49]. For most of these studies, platinum has been used extensively as a catalyst and for oxygen reduction, while some studies have shown the use of Ferricyanide as an alternative to this [58], [59], but power production has been significantly lower in such cases. The future avenues for research in this area could be looking into solid phase and liquid catalysts.

For the carbon cathodes such as the ones mentioned in **Table 2.3**, all samples that were studied involved sewage wastewater and studies found that ferric cathode produced 3.8 times more power than regular graphite electrodes but was significantly lower as compared to Pt-based electrodes. Also the use of catalyst binders is important, as this allows transfer of protons, electrons and oxygen, variation to using such systems can be using air cathode MFCs with Nafion-117 membrane [60]. On the other side of the cathode facing air, a hydrophobic coating can be applied to increase system efficiency. Nafion binders can serve the same purpose. Another critical aspect of the electrode performance is the presence of a diffusion layer or a Cation Exchange Membrane (CEM), without a CEM, the coulombic efficiency (C_E) is reduced due to high flux of oxygen at cathode side. On the cathode side, some gas headspace must be maintained (for CO_2, CH_4, N_2, O_2) formation of which will depend on operating conditions.

Table 2.3: Comparison of Carbon based cathodes with and without coatings

Carbon cathode with Platinum Catalyst	**Carbon cathode with non-Platinum catalyst**	**Plain Carbon Cathodes**	**References**
Available commercially, carbon paper pre-loaded with (Platinum) Pt catalyst on one side, the side containing the catalyst faces the water also same can be fabricated in lab by applying catalyst. Example: Pt mixed along with 5% Nafion liquid solution to form a paste applied to carbon cloth at room temperature and dried for 24 hours, Pt loading varied in the study between 0.1- 2 mg/cm^2.	Materials other than Platinum are used for electrode construction such as, transition metals with complex chemical coatings, which can work at low pH environments. Example: Carbon based ferric cathodes, with composition: Ferric sulfate (3% w/w), kaolin (36% as binder), fine graphite (60%), and Nickel Chloride (1%).	Oxygen reduction reactions proceed at a reduced rate compared to catalyst based cathodes. Reduced current and power generation. Example: Carbon brush cathodes made using simple brush machines, these are non-corrosive, electrically conductive, has a high surface area to volume ratio.	Logan [28] Rabaey et al. [34]

As shown in **Table 2.4**, the use of ferricyanide in the cathode in one instance produced 1.5-1.8 times more power than a Platinum based Carbon cathode double chambered MFC with Nafion-117 membrane [58]. Ferricyanide contributes to increased power density and has good electron accepting capacity at high concentrations. However, chemical mediators such as these compounds are not very practical if the commercial application of MFCs is to be envisaged. As these are energy intensive chemicals would contribute to energy cost if becomes part of the wastewater treatment process.

Table 2.4: Solvents that can be added to the cathode of a double chambered MFC, as an alternative to using Platinum catalyst.

Aqueous Catholytes	**Limitations**	**Alternatives**	**References**
Ferricyanide	• Chemical regeneration required if not replaced regularly. • Not very practical for commercial applications. • High energy-intensive chemicals, adding more to energy cost for wastewater treatment.	• Aromatic Nitrogen based compounds • Aromatic Chlorine based compounds • Metal ions such as Chromium- VI • Azo dyes (hydrazo and amine groups)	Kim et al. [28], Logan et al. [59], Zain et al.[61]
Hexacyanoferrate			
Permanganate			
Iron			

The issue with Platinum coated metal electrodes is primarily the oxide layer (PtO) formation on platinum surface, which reduces the activity of electrode over time. Different materials such as solid Manganese (MnO_2) based electrodes, Stainless steel brushes have been studied for practical applications as underwater biosensors [28]. Studies in recent years have shown a promising new alternative in the form of activated Carbon, a study found activated carbon works as well as Pt catalyst, a Polyvinylidene fluoride (PVDF) binder is used as a coating over the activated carbon cathode [62]. Setup produced same power as Polytetra Fluoroethylene (PTFE) applied to carbon cloth or platinum. Activated carbon is a good oxygen reduction catalyst and effective with dilute acid washes to be easily regenerated. Also it works well with wastewater and wastewater with high concentrations of acetate. PVDF has a very good water pressure resistance up to 1.2 m (vs 0.2 m for PTFE). More avenues of research are expected to be explored in this field for extracting activated carbon from various carbonaceous sources such as coal and biomass.

Anode: The requirement of an anode material include being electrically conductive, non-corrosive, having high specific surface area, high porosity, doesn't foul easily, inexpensive, easily available, and should be scalable to large sizes. The microbes can transfer electrons to the anode via direct contact, chemical mediators, or nanowires. The selection of material meeting such extensive requirements is difficult, for example, while Stainless Steel meets various criteria for being a good anodic material fails to achieve quantifiable power production. This shows good conductivity is not the only criteria, the kind of chemical coatings used, the stability of biofilms on these coatings, and how these affect the rate of electron transfer from the microbes is important. Some of the most widely used materials for the MFC anode is enlisted in **Table 2.5** in the following page.

Table 2.5: Comparison of commonly used Carbon and Graphite based electrodes as the anode.

Carbon based electrodes	Graphite based electrodes	References
Carbon Paper: Good connectivity with wires, however material is stiff and brittle. Available as plain and wet proofed, plain paper is usually preferred.	**Graphite Granules:** Mostly between diameter of 1.5 – 5 mm, with good specific surface area and porosity, conductivity varies between 0.5- 1.0 Ω/ granule.	Mohan et al. [63]
Carbon Cloth: Has superior abilities than paper, flexible and has greater porosity.	**Graphite Rods:** Highly conductive but has low internal porosity.	Logan et al. [34]
Carbon Foams: Thicker than carbon cloth, allowing more active surfaces to promote microbial growth.	**Graphite Sheets:** Low porosity, facilitates proper analysis of developed microbial biofilms on the MFC anode	Hernandez et al. [43]
RVC (Reticulated Vitreous Carbon): Has the highest conductivity (200 S/cm) and porosity (97%), with different effective pore size.	**Fibers and Brushes:** Has highest specific surface area and porosity (98%), brushes have small diameter (7.2 μm) allowing high specific surface area.	Bose et al. [14]

Metal coatings of Iron Oxide (FeO) on Carbon Paper showed decreased acclimation time of reactor, and did not affect power density, however over time the coating dissolved in the water so not very practical [28]. Manganese Oxide (MnO) coatings were applied on a graphite electrode, produced around 790 mW/m^2 with complex lactate mediator [34].

2.5.2. Effect of Membrane

Membranes are primarily used for double-chambered MFCs to keep the liquid in the anode and the liquid in the cathode separate. Cathode containing ferricyanide as a aqueous catholyte or dissolved oxygen cannot be allowed to mix with the liquid of the anode[58]. The main purpose is the migration of protons from the anode to the cathode, so membrane permeability is critical for selecting. The limitation with membranes is their high cost and they can contribute to decrease in system performance when fouling starts. For instance, Nafion (Dupont Co. USA), can cost up to $\$1400/m^2$ while a simple CEM costs around $\$80/m^2$ (CMI-7000, Membrane International, Inc. USA), making it prohibitive for large scale applications[28]. More details for the same is given in **Table 2.6** in the following page.

Nafion-117 has been the most suitable of all experimentations with membrane based MFCs, having high conductivity for cations (0.2 S/cm), superior thermal and mechanical stability [34]. Nafion-117 indicates material with 1100 g equivalent weight (EW) and 0.007 inches in thickness. The EW of Nafion-117 is defined as the weight of Nafion (in terms of molecular mass) per sulfonic acid group. Having a high permeability to gases contributes to its performance limitations, and it can be degraded only by alkali metals at room temperature and pressure. Other materials that has been tried and tested for membrane replacement in MFCs include Ultrafiltration (UF) membranes and salt bridge systems, made of Agar and

saturated salts of Potassium or Sodium, but such systems show high internal resistance thereby limiting the total power production [59]. An overview of such membranes is given in **Table 2.7**, which reflects the various AEM, CEMs and BPMs used at different capacities.

Table 2.6: Overview of Membranes employed in Microbial Fuel Cell at different capacities.

Cation Exchange Membrane (CEM)	**Anion Exchange Membrane (AEM)**	**Bipolar Membrane (BPM)**	**References**
Nafion-117, Dupont Co. USA Most commonly used.	ANI 7000, Membrane International, Inc. NJ	Anion membrane and a cation membrane joined in series.	Bose et al. [64]
CMI-7000, Membrane International, Inc. NJ Thicker and stiffer than Nafion-117 (0.046 cm)	Has effective balance of pH in the MFC as compared to CEM, research is needed in membrane quality.	Ferric Ion based bipolar membrane	Nimje et al. [65]

Table 2.7: Extended list of MFCs, AEMs, CEMs, BPMs, and other novel materials

Membrane Type	Performance	References
Ultrex^R AMI-7001 (AEM), Nafion^R 117, Ultrex^R CMI-7000 (CEM), Ultrafiltration membranes (UF: 0.5 K, 1 K, 3 K)	• AEM with superior performance has the highest power density (610 mW/m^2) and CE (72%) compared with rest membranes. • Similar internal resistance for most membranes (1225 ± 749 Ω till 1300 ± 726 Ω) except UF-0.5 K (6000 ± 767 Ω). • Oxygen mass transfer coefficients (k_o = 1.3 × 10^{-4} cm/s) are highest with nafion membranes. • Both AEMs and CEMs have excellent thermal stability up to 90 °C. • AMI-7001 has exchange capacity of 1.3±0.1 meq/gm, for CMI membrane it is 1.6±0.1 meq/gm.	Kim et al. [66]
Ultrex^R AMI 7001, Nafion^R 117, Ultrex^R CM1 7000, Hyflon^R, Zirfon^R, Nylon meshes (NY 11, 20, 41, 6H), Glass fiber filter (GFAPFF, GFAP40) J-cloth, Celgard^R, SciMat^R	• Ultrex^R AMI 7001, Celgard^R, Nylon meshes (NY 20, NY 41, NY 6 H), GFAP40 and J-cloth have the lowest pH splitting extent. • Owing to its high porosity, GFAP40 has the highest ionic conductivity. • Nylon meshes (NY11, NY20 and NY41) and Hyflon^R have the lowest ionic conductivity. • Under standard testing condition at 5 psi pressure, water permeability of both AMI and CMI membranes is <3 ml/hr/ft².	Logan et al. [28]
Fumasep^R FAB (AEM), Nafion^R 117(CEM), Fumasep^R FBM (BPM), Charge mosaic membrane (CMM)	• Highest reported current density in comparison to all the membranes is with AEM. • The BPM with the highest ions transport numbers, has the lowest pH increase in cathode, and follows the trend: BPM>AEM>CMM> CEM.	Oleiveira et al. [33]
Ultrex^R AMI-7001 (AEM) and Ultrex^R CMI-7000 (CEM)	• For Cell OCV and power density, AEM has higher performance compared to CEM, with varying air cathode pressure.	Chaturvedi et al. [49]

Cation exchange membranes (CEMs) function in the same manner as PEMs, but require a less arduous pretreatment process (depending on the manufacturer). CEMs analyzed in this paper were the CMI-7000, provided by Membranes International, Inc., which have a similar structural content to that of the Nafion-117. The polymer structures of the CMI-7000 are gel polystyrene cross-linked with divinylbenzene, with a sulfonic acid functional group. CMI-7000S membranes are chemically stable in strong acidic to weak basic environments, ranging from a pH of 1.0 to a pH of 10.018. CMI-7000 membranes include a coated woven fabric18 for stability, creating a rigidity that is not seen with the Nafion-117 membranes. Manufacturers of CEMs and PEMs are very few, and this results in different membrane structures, primarily the difference includes parameters of stability durability and selective ion transfer based on practical and theoretical computations. Another important parameter is membrane life. Nafion-117 till date, is the most commonly used membrane, and has been found to be stable in most MFCs.

Owing to high conductivity for cations and superior mechanical and thermal stability as compared to other proton exchange membranes, Nafion-117 has been studied extensively in different reactor configuration and capacities for organic waste removal and simultaneous bioelectricity generation. Bioelectricity using cellulosic waste with *Clostridium acetobutylicum* and another microbial specie *Clostrodium thermohydrosulfuricum* was reported in one study [15]. The focus of this study was the difficulty involved in degrading cellulosic waste; the source of wastewater from paper industry. Membrane Electrode Assembly (MEA) consisted of Nafion-117 with carbon paper as electrodes. Varying the resistance between 0.1 Ω to 3 Ω initial current of 6.35 mA and 7.31 mA was generated, hence readily oxidizable substrates are required with other anaerobic bacteria for which the

research is needed. In a separate study the same group [67], reported the same MEA configuration with treatment of wastewater samples from brewery, dairy, municipal, and sugar industry. Current generation was up to 14.92 mA with 90.23 % COD removal efficiency, highlighting key issues such as activity loss and incomplete utilization of organic matter in the wastewater. Another study with graphite electrodes was studied in a single chamber MFC has generated peak power of 18 mW/m^2, the MEA included Nafion-117 with eight graphite electrodes as the anode and an air cathode [68]. The membrane treated with 30% H_2O_2, deionized water and 0.5 M H_2SO_4 yielded 0.32V across terminals with some uncertainty in average power production.

2.5.3. Effect of Temperature

Temperature is an important parameter for maximum COD removal and bioelectricity generation, both COD and power density increases with increase in temperature, which can further be linked with membrane permeability and the metabolism of the microbes [9]. Since different bacteria species have different appropriate temperature range, the initial growth phase of the biofilm will depend on parameters shown in **Fig. 2.2**, System kinetics will decide the rate of substrate utilization by the microbes, parameters such as activation energy, solution conductivity along with electrode potential and Gibbs free energy will come under Mass Transfer and thermodynamics, which will decide the favorability of the reactions thus contributing to microbial growth and an active biofilm. The initial growth phase of the biofilm will determine the feasibility of microbial growth within the biofilm matrix that develops on the anode.

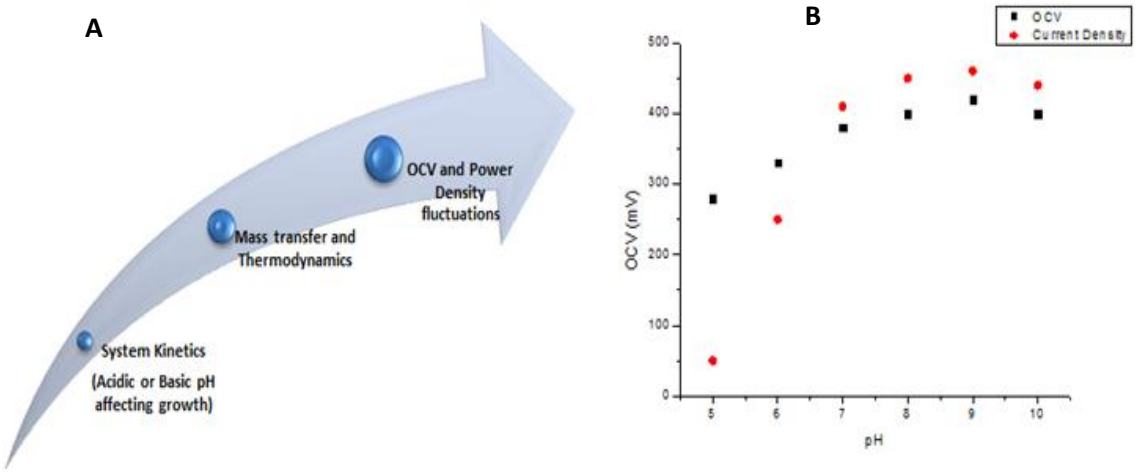


Figure 2.2: (A) Interrelated parameter that contribute to MFC system stability and active biofilm growth (B) Change in MFC voltage and current density based on sewage wastewater with varying pH, it is seen that maximum power generation happens at wastewater pH ranging between 7.5 and 8.9 respectively [34], [65], [69]. Microbial species can adjust itself to the start-up temperature thereby highlighting the critical importance of temperature for biofilm formation. As startup procedures dictate system performance, studies have suggested temperature range that vary between 30- 45°C [28], [70].

2.5.4. Effect of pH

Bacteria growth is directly affected by changes in pH, thereby further affecting parameters such as ion concentration, membrane potential, proton movement and formation of biofilm. As shown in **Fig. 2.3**, the acidification at the side of the anode can decrease bacterial activity and affect the biofilm stability and performance. Most studies have shown pH ranging from 6 – 9 is suitable for growth and operation of biofilms derived from pH neutral wastewater [33], [65], [71]. As pH values differ in both chambers of the MFC, this parameter is crucial to the output power generated from MFC.

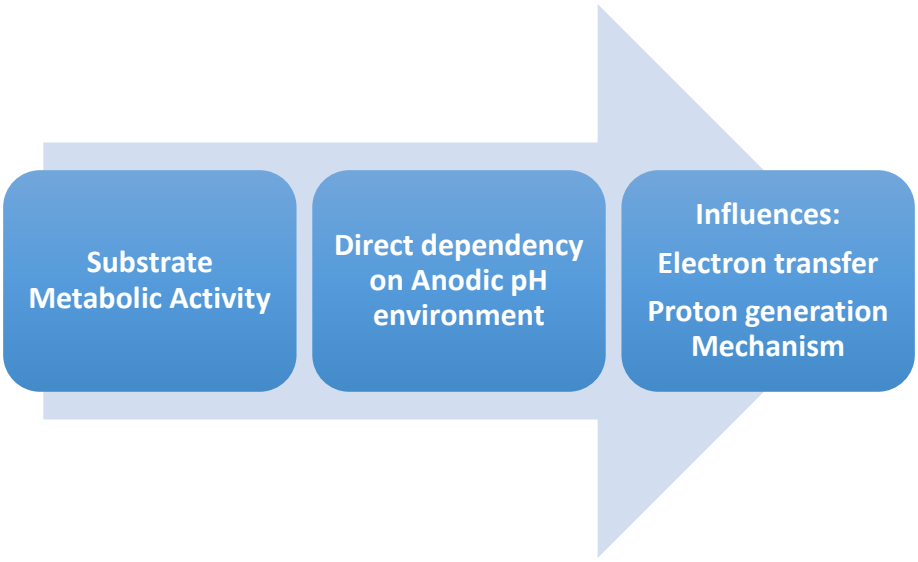


Figure 2.3: The relationship between substrate utilization rate and the pH of wastewater in the anode.

Variations in MFC system configurations to neutralize the effect of pH was done in one study [72] by inverting the polarity of the MFC continuously in the same half-cell and in the process, neutralizing the effect of pH. Addition of chemical buffers (such as bicarbonate or phosphate buffer) has shown the ability to maintain constant pH, with improved voltage stability and biofilm performance [33]. The influence of pH is however more in single chambered systems as shown in **Fig. 2.4** in the following page.

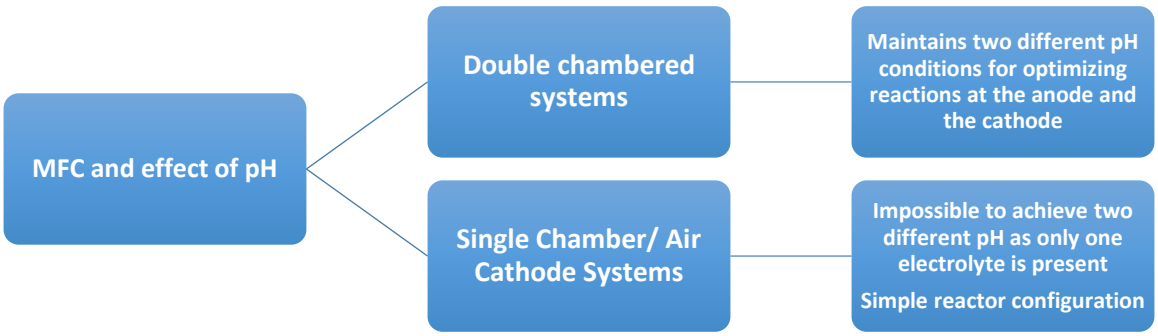


Figure 2.4: Influence of pH on different MFC configuration, the effect is more in single chambered systems, as it affects both anodic and cathode reactions, along with microbial growth.

The use of chemical dosing to maintain wastewater pH in a broader sense is not very practical as it represents substantial investment and energy input. The use of excessive phosphate can lead to eutrophication of water bodies if discharged without treatment, also the lack of cost effective phosphate recovery processes makes it even more impractical. Carbon di oxide (CO_2) can be used in the cathode as it can combine with the hydroxide ions and create a carbonate or bicarbonate buffered catholyte system, some studies have implemented this process and found an increase in power density and cell voltage with a decreased pH imbalance [71], [73].

2.5.5. Effect of Substrate Concentration

Recent advances in potential substrates include but are not restricted to the use of carbon rich sources such as xylose, ribitol, glucuronic acid, galactitol, galactose, glucose, gluconic acid, arabitol, xylitol etc.; nitrogen sources such as histidine, arginine and serine and organic acids such as lactic and acetic acid, etc. have shown improved output power performance from MFC systems. Among industrial mixed wastewater streams, brewery, cassava mill, chocolate manufacturing, sugar, biodiesel, cellulose wastewater have shown efficient capacity and potential for bioelectricity production [33], [68]. This suggests that the major metabolic fuels employed as substrate include carbohydrates, amino acids and fatty acids, which are all monomers of the organic substrate present in wastewater from process industries as well as domestic sources with varying concentration. It is however difficult to compare data in some cases, as MFC designs, operating conditions, measurement techniques vary. The data for some double chambered MFC studies with very high COD is reported in **Table 2.8**.

Microbial activity varies with pH, therefore the change in pH during MFC operation is an important factor for selection of substrate. Some studies have suggested the use of chemical mediators such as neutral red or anthraquinone 2-6 disulfonate (AQDS) to be added to the system where bacteria is unable to produce electricity using the electrode assembly [34]. If mediators are not added, direct electron transfer or mediator-less transfer is the mechanism for electricity production wherein the electron transfer attribute may not be known for such cases. For most MFC systems electrons reaching the cathode combine with proteins which have diffused from the anode via a separator and oxygen from air enables the resulting product as water. Some studies have shown the utilization of chemical oxidizers such as ferricyanide or manganese [33], [58].

Table 2.8: Double chambered MFC (with MEA) Power output and COD removal efficiency from some major industrial process wastewater sources specifically having organic loading rate in excess of 1000 mg/l with undefined microbial cultures

Wastewater	Substrate concentration (mg/l)	Working Volume of Wastewater (Liters)	COD removal efficiency (%)	Power Density	References
Cassava Mill	16, 000	30	72	1771 mW/m^2	Chiw et al. [57]
Distillery Sources	28, 400	7.27	88.38	124.03 mW/m^2	Huang et al. [74]
Chocolate Industry	1459	0.40	75	1.5 W $/m^2$	Pandey et al. [68]
Electric Power Plants	3200	0.30	82	540 mW $/m^2$	Huang et al. [75]
Rice mill	2250	0.40	96.5	2.3 W $/m^3$	Behera et al. [76]
Molasses wastewater	127, 500	1.08	53.2	1410 mW $/m^2$	Hays et al. [77]
Slaughterhouses	4850	0.12	93	578 mW $/m^2$	Katuri et al. [78]
Palm oil mill sludge	2680	0.10	3	451.26 mW/m^2	Nor et al. [54]

Utilizing kitchen waste as substrate has been reported for bioelectricity generation where shredded kitchen waste was placed in acrylic containers in a setup that connected carbon fiber electrodes separated by filter paper and reported maximum power of 682 mW/m^2 over a period of two weeks, with a reported current density of 1156 mA/m^2 [65]. Such studies need more input on surface area of electrodes, cathode composition and related parameters. The prospect of using MFC to power sensors using marine sediments has also been studied extensively with hypereutrophic lakes with low reported power densities [28]. Bioremediation purposes are served with membrane-less power production as natural oxygen gradient exists. Drop in temperature during season change is a major limiting factor for such study also steady voltage was limited to up to 0.5V. Graphite discs were used for both cathode and anode. MFCs have shown utility with domestic sewage treatment with glucose being introduced as a carbon source, with agar as a carbohydrate source [79]. Improving the power densities obtained from these processes would require influencing the rate of substrate consumption with respect to time, as discussed in the following section.

2.6. Microbial Kinetics

MFC power output can be increased by using cube reactor systems that can be stacked and connected in series to generate more power during the same time. One study used a digital control strategy where it connected four MFCs in series and got an overall voltage of 1.26 V and the system was able to reject disturbances and perturbations caused by substrate concentration, electrical loading and changes in the outside (ambient) temperature [80]. Also, when reactor performances are assessed with respect to time, Residence Time Distribution (RTD) plays an important role, as it determines the mixing characteristics in a reactor and helps understanding the elements of flow inside a reactor, as well as developing mathematical model to predict

reactor performance, an interesting avenue of research can be explored in regard to MFC performances using RTD techniques.

For introducing an equation that can relate the substrate consumption rate by the microbes with respect to time, we consider developing a biofilm based model on kinetics. For this, the first assumption has to be that the growth of bacteria is in proportion to the concentration of the bacteria, or

$$\frac{dX}{dt} = \mu X \quad (1)$$

$$\mu\, dt = X\, dX \quad (2)$$

Where $\mu\left(\frac{1}{t}\right)$ is the rate of specific growth for the microbes which is a function of the concentration of substrate, also X represents the cell concentration in mg/l (as a function of volatile solids) with respect to time (in seconds). Integrating μ over time of operation will yield the specific growth of electroactive biofilms during the time period of MFC operation with regard to cell concentration. Further, using Monod kinetics μ can be described as a function of substrate concentration and can be written as

$$\mu = \frac{\mu_{max.}\, c}{K_c + c} \quad (3)$$

Where $\mu_{max.}\left(\frac{1}{t}\right)$ is the maximum growth rate in seconds, K_c is the half saturation constant and c represents substrate concentration in mg/l. The same has been depicted in **Figure 2.5** given below.

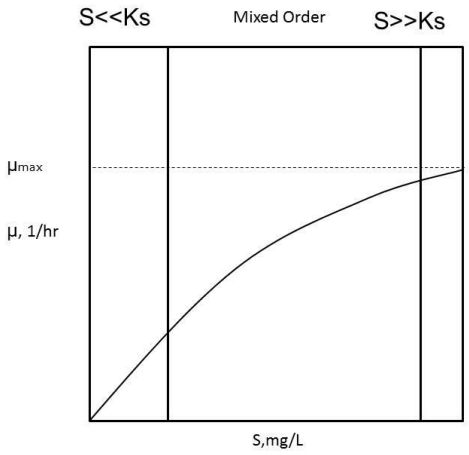


Figure 2.5: The specific growth rate (μ) as a function of substrate concentration (S) according to Monod kinetics, where K_s represent the reaction rate constant for the substrate.

Assuming substrate consumption rate by the microbes is in proportion to microbial growth, we have

$$\frac{dc}{dt} = -\frac{1}{Y_{\frac{x}{c}}}\frac{dX}{dt} \quad (4)$$

Where $Y_{\frac{x}{c}}$ is the yield constant indicating the specific mass concentration of cells grown per mass concentration of the substrate, combining above results, a rate equation for substrate concentration over time can be formed as

$$\frac{dc}{dt} = -\frac{X}{Y_{\frac{x}{c}}}\frac{\mu_{max.}\ c}{K_c + c} \quad (5)$$

This result highlights that the rate of utilization of substrate over time is not a simple function of substrate concentration as the rate varies based on the specific value of

c, further assuming constant cell density in the biofilm, it will mean that as the cells grow the biofilm will become thicker but packing density of the cells within the biofilm will not change. The two limits of the rate equation can be examined to understand biofilm kinetics further the two cases are shown in table 2.9 given below.

Table 2.9: Overview of the kinetics based model for rate of substrate concentration

Kinetic Parameters	**Concentration of the Substrate**		**References**
	Very High Substrate Concentration	**Very Low Substrate Concentration**	
Microbial Growth	Bacteria will grow at its maximum rate	Bacteria will grow at a slower rate	Bose et al. [28], Logan et al. [42], Torres et al. [81]
Substrate Concentration	c >> K_c	c << K_c	
Effect on rate equation	Rate equation becomes equation to a zero order approximation	Rate equation becomes equation to a first order approximation	
Final Equation	$\frac{dc}{dt} = -\frac{X}{Y_{\frac{x}{c}}}\mu_{max.} = -K_0$	$\frac{dc}{dt} = -\frac{X}{Y_{\frac{x}{c}}}\frac{\mu_{max.}}{K_c} = -K_1 c$	

The above-mentioned approximations in **Table 2.9** forms the limits for rate of utilization of substrate by the biofilm over changes in all growth kinetics and biofilm density over time. Keeping the above in mind, there is much room for

improving power densities from the MFCs so that it can be compete with existing processes such as anaerobic digestion. As MFCs further develop, it is expected that MFCs will be able to achieve high power densities, at least for industrial applications where microbial communities based on pure cultures can be utilized and carefully controlled [60].

2.7. Economics of MFC

Poor economic feasibility is probably the major barrier for progressing MFCs to commercial viability. Financing MFC based ventures for the most part incorporates starting capital investments and operation/maintenance costs related with energy, chemicals incorporated and utilization of sustainable materials, while the monetary income may originate from legislative financing and the expense charged from the contaminant discharge[82]. Extra income can be from the recovery of energy (e.g., bioelectricity in MFC and CH_4/H_2 in anaerobic digesters) and value added organic products (e.g., manure and compost type material).

Operation cost. The operation of MFCs can actually consume much less energy when contrasted with numerous other treatment technologies. Energy balance analysis by a study assessed that a MFC can hypothetically create a net NER of around 0.003 kW h/ kg-COD, which breaks even with an additional monetary income of about $0.0005/kg-COD given an average power cost of $0.13 kW/h [7]. In correlation, the present day treatment cost for activated sludge based Wastewater Treatment Plant (WWTP) is about $0.11 /kg-COD, expecting an energy utilization of 0.6 kW h/kg-COD and that 60% of the operation cost is utilized for energy consumption. Furthermore, the value of byproducts from the process needs to be carefully addressed. However, the achievable benefit of such procedures stays under level headed

discussion in light of the normally low product yield and the high expenses for product extraction along with purification.

Capital cost. The capital expenses of MFCs are still phenomenally high at present, because of the utilization of costly anodes (including membranes, conductivity and catalyst) and membrane materials. For instance, Ultrex films, a standout amongst the frequently utilized membranes in the bigger scale MFCs, cost roughly \$110 m^{-2}. It is evaluated that, even with moderately cheap carbon material anodes and a nonwoven fabric separator that have been exhibited in several studies [33], [34], [49], [80], for instance installing an air-cathode MFC for treatment of municipal wastewater in the United States at present reach \$3 kg^{-1}-COD (or around \$1.5 m^{-3}-metropolitan wastewater). This estimation considers a variety of factors such as recent development and design improvements of the MFC architecture, considering a treatment limit of 25 kg-COD m^3 d^{-1}, anode of \$100 m^{-2}, cathode of \$ 1500 m^{-2}, separator of \$1 m^{-2}, reactor of \$5000 m^{-3}, and a lifetime of 10 years. As contrasted with a conventional activated sludge system, the capital cost for the MFC is 30-times higher; hence a crucial parameter before its commercialization would be to reduce the capital costs of MFCs.

In retrospect, the power output of microbial fuel cells is still a long way from meeting total energy-recovery for real time operations, the ecological benefit is not direct, the performance of the processes tend to decrease over a long haul operation, and the materials of MFCs are by and large significantly high. While proceeding with change in those areas can be normal with the progressing research, it appears to be a portion of the difficulties, for example, a generally high capital cost may remain, making MFCs intensely troublesome. To meet the manageability criteria, more appropriate methodologies other than progressing MFC innovation alone ought to be looked for.

As we would like to think, integrating MFCs with different procedures may be a more feasible avenue. Some conceivable methodologies of the coordination and the reasons are talked about in the Conclusion section, under the heading of Future scope and perspectives in MFCs.

2.8. Environmental Impacts of MFC Technology

Ecological implications of treating wastewater are that the quality of the exit channel must be in line with the benchmarks of water recovery and reuse. Simultaneously, the transposition of contaminants from water bodies to the cycles in nature has to be lessened to an ecologically acceptable level.

Processes such as anaerobic digestion which has similar functionality with MFCs permit synchronous removal of pollutants and energy recuperation; by and large MFCs demonstrate a superior cleaning execution, particularly for elimination of aqueous fractious contaminants including numerous toxic contaminants. This predominant execution of MFC is likely because of the conjunction of anaerobic and vigorous biochemical processes of the microbes, which permits numerous responses that are not very effective with various anaerobic processes. Up until this point, the high capacity of MFCs and their inferred reactors (e.g., microbial electrolysis cells) has been exhibited for productive expulsion of fluid contaminants, for example, unmanageable organics, nutrients sulfur compounds and metals and to accomplish great treatment quality (COD < 20 mg/l). This component is particularly important today in light of the fact that the water quality models are getting progressively stringent while the release of human-centered chemicals (e.g., different dyes, beauty care products and medicines) is expanding.

MFC technologies are likely to have low carbon footprint, as water based processes do not have significant CO_2 emissions and capacity for CO_2 sequestration opportunities are available in a few reactors with modifications to the cathode. Also, the MFC procedure has a sludge generation which can be used as compost. Comparing the sludge yield of volatile suspended solids (VSS) in activated sludge systems which are around 0.4–0.8 g^{-1}-COD, in MFC the yield is around 0.1 g^{-1} VSS g^{-1}-COD. Thus, the auxiliary contamination dangers and additional energy utilization related with disposal of sludge can be significantly lessened.

A large portion of these ecological advantages of MFCs has not yet been demonstrated experimentally in real world facilities. A few preliminary investigations show that the acknowledgment of such advantages in a practical wastewater treatment process is difficult. For instance, the breakdown of CO_2 to organic compounds, for example, CH_4, and the autotrophic life forms that do those processes will be unable to contend with heterotrophic living beings if there should arise an occurrence of microbial contamination. Moreover, a number of the materials utilized as a part of MFCs, for example, electrodes, catalysts and membranes are petroleum derivative based and may have negative ecological effects. Henceforth, the general natural impacts of MFCs for wastewater treatment require assessments that are more thorough.

CHAPTER III
MATERIALS AND METHODS

3.1. Sample collection

The University Sewage treatment plant (STP) was the source of the wastewater; untreated water was collected from the inlet channel. As explained in the later section, for some studies, a fixed amount of sludge from sewage was inoculated with the wastewater to see the effect of substrate concentration and voltage. For all MFC reactors, wastewater operations were in batch mode with working liquid volume of 250 ml for the anode chamber, under ambient temperature conditions. Given the fact, that this channel of wastewater has some amount of sludge mixed with it, which from the perspective of MFC operation is desirable[83], as studies have shown sludge reduction is possible in MFCs in the form of COD and BOD removal from wastewater.

3.2. Chemical Characterization

The wastewater collected from inlet channel of STP was fully characterized upon arrival, cold storage or shipment facilities were not needed, as testing facilities were available at the University itself. This allowed the collected wastewater to serve as a substrate and microbial inoculum for all the experiments. Tests using sewage based wastewater ensured a better understanding of MFCs with cathodes containing a Pt catalyst[34], and at the same time, allowed the development of a better understanding of how cheaper catalyst (such as activated carbon) can perform. The effectiveness of these catalyst for achieving effecting treatment of wastewater was evaluated primarily in terms of COD removal and BOD removal, which is directly related to energy recovery.

COD removal is formulated based on the initial and final concentration of COD (in mg/l), using the COD incubator (Sonar, CDU 367) following standard titration procedures, 2.5 ml of wastewater sample is required for each test, along with Potassium Dichromate and Sulfuric acid of known concentration; post incubation ferrous ammonium sulfate with ferroin indicator is used for titration to achieve end point; given that small volumes of liquid is needed for this test, it is reasonable to hypothesize these do not alter the power generation capacity of the system significantly.

BOD removal is computed for the wastewater using a BOD Incubator (Patsio Water Solution, PWS 123) at 20 °C for 5 days, which requires around 97 ml of wastewater, and a Nitrification inhibitor (N-Allylthioharrnstoff) and NaOH (WTW) NHP 600 since bigger volumes of water is needed for this analysis, BOD computations were done only during the initial and final stages of all the MFC experiments.

3.3. Electrical measurements

Electrons generated by the microbial communities from complex substrates in MFC are exchanged to the anode (negative terminal) and stream to the cathode (positive terminal) connected by a conductive material containing a resistor, or worked under a load (i.e., creating power that runs a small electronic LED or related systems). By tradition, positive current streams from the positive to the negative terminal, inverse to that of electron stream[84]. Hence, voltage is generated by means of this transfer of electrons and protons.

Open Circuit Voltage (OCV). Cell voltage that is measured in the absence of current or resistor is known as the open circuit voltage. Ideally, the OCV should approach the cell EMF. Open Circuit voltage was computed directly by connecting the anode and cathode wires to the data acquisition system.

Current Density. For current flow, external resistors (R) were connected to the anode and cathode wires and voltage drop was measured across the arms of resistors. Using Ohm's law (V_{MFC} = I.R), current was calculated, Further, this current generation was normalized to the anode surface area (A_{anode}) to calculate current density (mA/m^2) as shown in equation 6.

$$I_{\text{Density}} = \frac{V_{MFC}}{R \times A_{anode}} \quad (6)$$

Power Density. For power generation, equation 7 was used, and similar to current density, power was also normalized to anode surface area to compute power density (mW/m^2), the purpose of this normalization is that it forms a basis to compare MFC performance on a standard scale (i.e. per m^2 basis), so that different MFC system architectures can be compared and contrasted with each other.

$$P_{\text{Density}} = \frac{V_{MFC}^2}{R \times A_{anode}} \quad (7)$$

Polarization Curves. Polarization curves refer to using varying resistors, in one full cycle of MFC operation, ideally from a high resistor (1000 **Ω**) and all the way up 50 **Ω**, this helps in understanding effectiveness of MFC systems, as they should be able to operate at varying load, to bring them a step ahead from research laboratories. It must be mentioned that starting MFC cycles with high resistors is recommended, as this puts less load on the microbial communities, in this work, resistors from 1000 **Ω** to 500, 250, 100 and 50 **Ω** were used to account for bioelectricity production.

MFCs have been proposed as a technique to treat wastewater, and along these lines it is imperative to assess the general performance using biochemical oxygen

demand (BOD), chemical oxygen demand (COD), or total organic carbon (TOC) removal. Different elements may likewise be imperative, for example, solvent versus particulate removal, and supplement or nutrient removal. The choice of treating efficiency is arbitrary but mostly used is COD removal efficiency and can be found out by the ratio between the removed and influent COD. This parameter measures the amount of the accessible "fuel" that has been changed over in the MFC, either into electrical current (by means of the Coulombic efficiency) or biomass (by means of the development yield) or through reaction with electron acceptors (e.g., oxygen, nitrate, and sulfate).

Coulombic Efficiency. The Coulombic efficiency is defined as the ratio of total Coulombs or charge which migrates to the anode on substrate degradation, to maximum possible Coulombs generated assuming complete breakdown of substrate. This is given as:

$$CE = \frac{M \int_0^t I \, dt}{F \, b \, V_{an} \, \Delta COD} \qquad (8)$$

Where M = 32 is the oxygen molecular weight; Faraday's constant is denoted as F (= 99655 C/mol); b = 4 is the number of electrons exchanged per mole of oxygen, ΔCOD is the change in COD over time (i.e. the difference between initial and final concentration) and v_{an} is the volume of liquid in the anode compartment,

3.4. Double Chambered Membrane based MFC

For the double-chambered MFC, anode chamber contained wastewater, and the cathode chamber contained a conductive PBS (phosphate buffer solution) to facilitate ion transfer[85], the anode and the cathode were sandwiched into a MEA (Membrane Electrode Assembly), with Nafion-117 membrane as the separator. The details of each components are presented in the following section.

3.4.1. Preparation of Electrodes

Plain Carbon cloth (Type CC4P, E-TEK, USA) without wet proofing electrode is used as the anode and Carbon cloth with platinum (as catalyst) coating is used as the cathode. The use of carbon based electrodes is common in MFCs as they have higher conductivity and are well suited for microbial growth [80, 86]. In the anode chamber, wastewater (from sewage) is the liquid used and for the cathode chamber, phosphate buffer is used. Phosphate buffer is prepared by dissolving potassium phosphate dibasic (Mol. Wt. 268 g/mol, molarity 0.0754 M) and potassium phosphate monobasic (Mol. Wt. 138 g/mol, molarity 0.0246 M) in demineralized water. The function of the phosphate buffer is to maintain the solution conductivity at the cathode, and to ensure ease of ion transfer from the biofilm development [29].

3.4.2. Preparation of Catalyst

For the cathode, commercially available Platinum (10 wt.% Pt/C, E-TEK) was mixed with 5% Nafion^{TM} (acting as a chemical binder) liquid solution to form a paste (8-µl-binder per mg-Pt/C catalyst) and was applied to one side of the electrode and dried at room temperature for 48 hours. The side with the catalyst loading faces the membrane.[56]

3.4.3. Fabrication of the Membrane Electrode Assembly

Membrane. Nafion-117 (Dupont, USA) was used as the membrane owing to its high permeability, with reducing unwanted substrate flux from anode to cathode (i.e. fuel crossover) and improving Coulombic efficiency.[87] Pre-treatment and activation of Nafion-117 membrane allowed removal of any impurities that were present in the film.[88] This was done by keeping it for 1 to 1.5 h in 3 % H2O2, followed by washing with deionized water, 0.5 M H2SO4 followed by again washing with deionized water. This method for pre-treatment of Nafion-117 has been modified from one study to activate and remove impurities from the membrane surface. After this, the system was bolted together, the anode and cathode chambers were filled with deionized water when the MFC was not being used to maintain the PEM's good conductivity [89].

To hold the MEA, a plexiglass system was developed (as shown in **Fig. 3.1**), with a circular housing holding the anode, membrane, and the cathode, connected to cylindrical chambers (for the anode liquid and cathode liquid, respectively). There is an inset for the anode and cathode wires from which it is connected to the respective electrodes. Gaskets are used on the outset to ensure proper system fitting of the MEA to both the anode and the cathode chamber. The anode, cathode and the membrane had an active surface area of 19.26 cm^2.

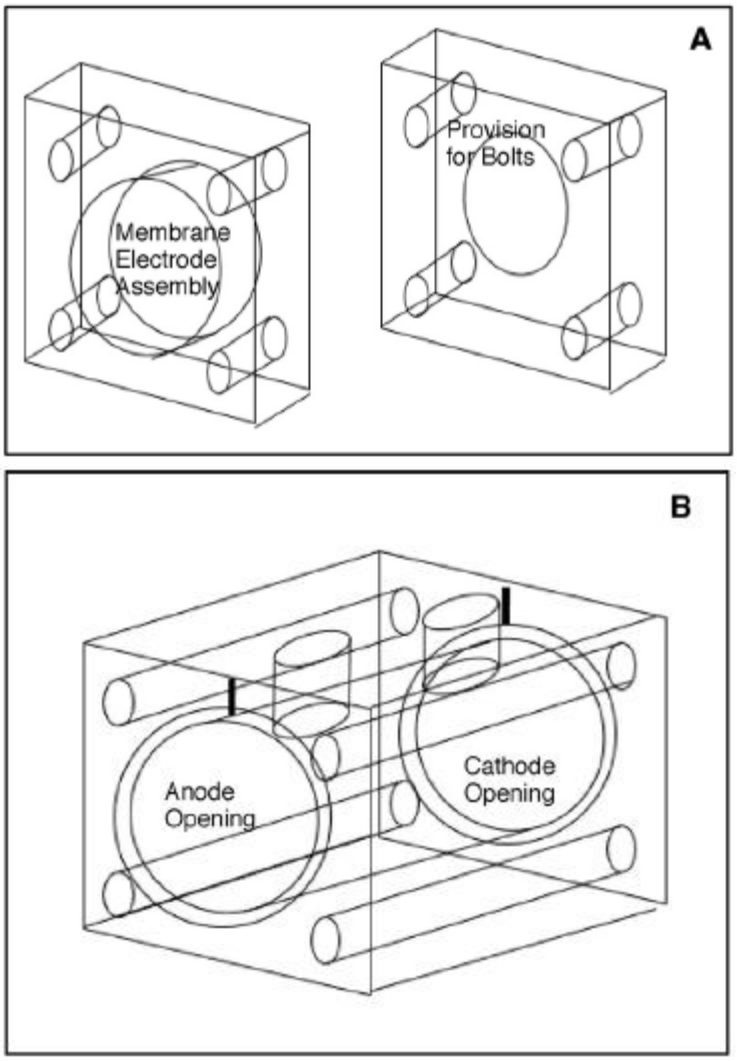


Figure 3.1: (A) CAD of the Membrane-Electrode assembly cube with provision for bolts at the corners (B) The cube is shown bolted together, with the anode and cathode on both sides, and the membrane in between them

3.4.4. Construction and Operation of Batch MFC

For the MFC, plexiglass reactor was fabricated along with the MEA connected to cylindrical chambers (for the anode liquid and cathode liquid respectively). Gaskets

are used to ensure no leakage of liquid from the system to the outside. The end plates press the gaskets towards the electrode holdings and the entire system is bolted together. Overall reactor volume for both the anode and cathode chamber was 300 ml resectively, to ensure some head space for the dissolved gases, a working liquid volume of 250 ml was kept throughout the operations.

When wastewater was charged in the MFC, for all studies, an initial assessment was done in the form of OCV, to see maximum possible voltage generation, this was followed by some studies at fixed external resistance over few cycles, and then polarization studies with varying resistance to evaluate system performance. Further, as discussed in the later sections (Section 3.6) characterization of the electrode surface in terms of Atomic Force Microscopy (AFM) imaging was done for evaluating the surface morphology of the anode and Scanning Electron Microscopy (SEM) was done to evaluate the development of nanowires on the anode as a mode of electron transfer.

3.5. Single Chambered air cathode MFC

In recent years, while evaluating feasibility of making MFCs a commercial success, a key area of exploration has been the material used for the development of the cathode.[56] Traditionally, Platinum (as a catalyst) on carbon cloth is used as the cathode, which is although an excellent catalyst, but is expensive and is subjected to fouling issues over time. Thus, finding low cost alternatives to Platinum, which can produce comparable power densities are a key area of exploration. The criteria for a new MFC cathode should include; good catalyst for oxygen reduction, can be

derived from carbon-based sources, less susceptible to fouling and can be easily regenerated [46].

While the double chambered system is the most experimented MFCs, in the last decade, a second generation reactor systems called air cathode systems have been of interest. In such systems, membrane is not needed, which brings down the cost significantly, and the cathode, faces wastewater at the side of the reactor, and air at the other side, such systems employ air as the reducing agent. However, traditional Pt on carbon cloth cannot be used as cathodes in such systems, as the cathode needs to have high water retention capacity. Using biomass as a resource, a new form of activated carbon based cathode was made using a single step phase inversion process.

3.5.1. Construction

Architecture is identical to the double-chambered system, except with few major differences. Cube single chambered reactors were made from a lexan block having an inside cylindrical chamber for the anode and an opening on the other side for the cathode.[34] Membrane is not present in such systems. As shown in **Fig. 3.2,** the side of the reactor facing the anode holds the anode liquid, and the other side, has the cathode facing the wastewater on one side, and air at the other.

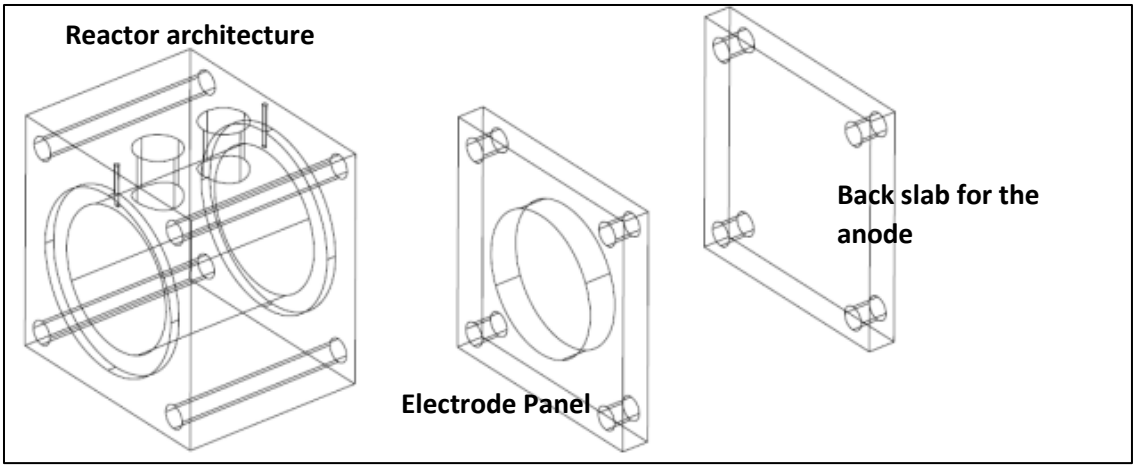


Figure 3.2: Plexiglass reactor constructed out of Lexan block, with two openings for the anode and the cathode on both sides of the system.

3.5.2. Fabrication of the Air Cathode

A novel indigenous Activated Carbon (AC) cathode is prepared with PVDF (Polyvinylidene fluoride) used as a binder on a Stainless Steel Mesh (Type 316L) through a single step phase inversion method [61]. As an alternative to expensive catalyst (such as Platinum), a comparative analysis is presented, with Activated Carbon Cathode and PVDF as binder, where the cathode itself acts as a catalyst.[58] A novel indigenous route was taken for the preparation of AC cathodes. Sugarcane refuse was collected from the local market, as these are freely available from local sugarcane juice suppliers. After collecting the same, the material was put in a dryer for moisture removal at 110 º C for three hours, this was followed by crushing the sugarcane to finer particles, followed by impregnation with 40 wt % Phosphoric acid (BDH Grade, Sigma Aldrich).This was followed by carbonization at 300, 400 and 500 ºC at one hour intervals.

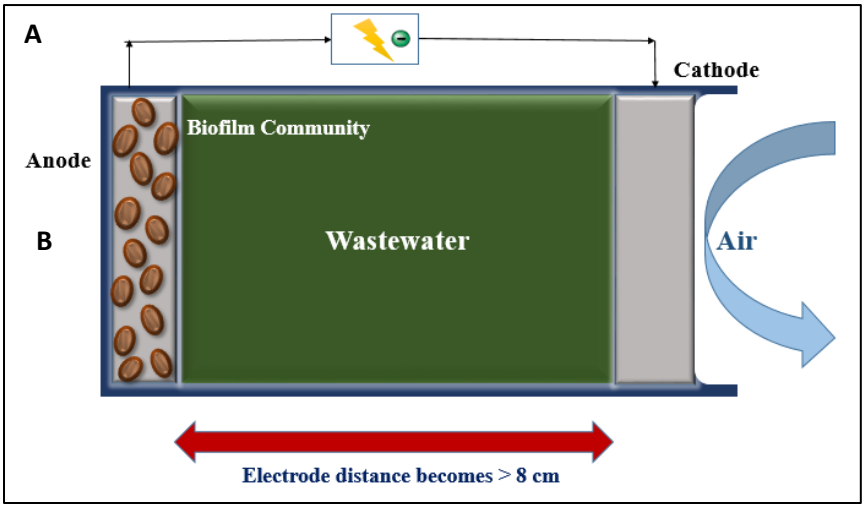
Here phosphoric acid acts as an activating agent and has several advantages such as allowing a single step process in comparison to the traditional two-stage process of carbonization and activation.[12] Further, this is achieved at room temperature and most of the acid is easily recovered in the washing of the products, and the yield is high as the burn off at higher temperature is avoided.[13] After Carbonization, the material was allowed to cool, this was followed by thorough washing of the carbon in hot water (around 90 ◦C) on filter paper (Whatman, Type 101) to adjust the pH to 7 (basic range), and then finally dried.

The Binder was prepared using a modified method from one previous study of similar nature.[61] PVDF (534 000 Da; GPC Powder, Sigma Aldrich) was prepared (10% by wt.) by mixing it in N, N-dimethyl acetamide (DMAc, 99.8%, Sigma-Aldrich) with vigorous stirring at room temperature for ten hours using magnetic stirrers (at room temp 25 ± 1◦C) until the polymer completely dissolves in it. Activated carbon and PVDF mixture was spread directly onto a stainless steel mesh (AISI 316L alloy, 40 x 40 wires/inch, 0.37 open area, Sigma Aldrich) using a spatula, the cathode was then immersed in deionized water (for 15 minutes) to induce single step phase inversion process[66], and then air dried before use.

Surface characteristics and functional group analysis for the prepared AC is done using XRD (X-Ray Diffraction) and FTIR (Fourier Transform Infrared Spectroscopy) respectively. Particle size analyser is used to evaluate the average particle size. Microscopic analysis for the surface is done using AFM (Atomic Force Microscopy) and SEM (Scanning Electron Microscopy); SEM imaging is used to see difference in biomass structure and the formed AC structure.

3.5.3. Fabrication of Anode

The air cathode reactor along with the cathode is fabricated, while anode plate is



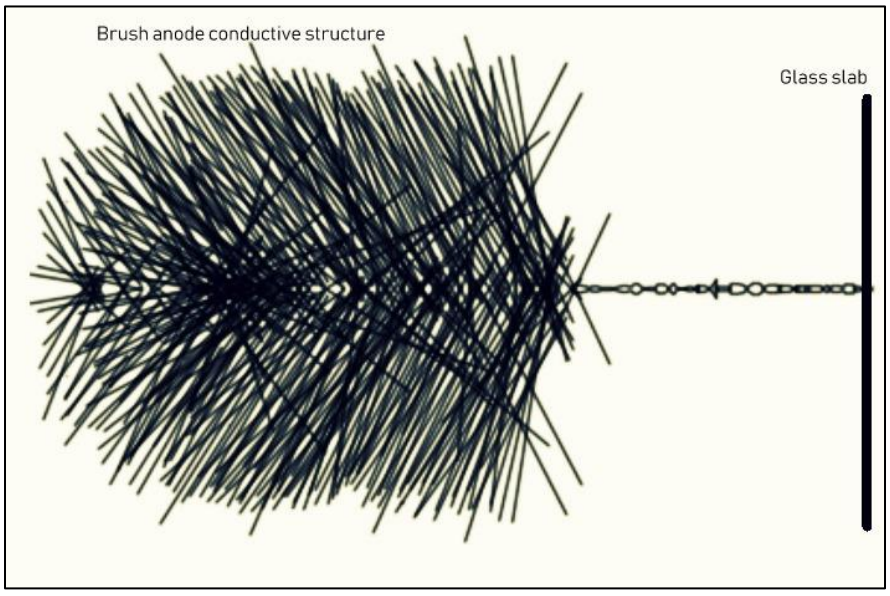


Fig 3.3: (A) Air cathode MFC with significant electrode distance, this is undesirable as it increases energy loss to background processes **(B)** Carbon yarn based brush anode attached to the anode plate using wires, and a stopper.

shut, but there is an issue with the electrode distance, which can be seen in **Fig. 3.3**, as the distance in our case exceeded over 8 cm, now in any fuel cell if the electrode distance is significant, this leads to more energy loss to background processes, as well as increased ohmic resistance, which is undesirable, one way to minimize this, is to bring the anode closer to the cathode, by fabricating a brush anode of the same active surface area (19.26 cm^2).

As shown in **Fig. 3.3B**, a brush anode was fabricated, from carbon yarn, these are a good alternative to circular anodes, as one major disadvantage of circular anodes, is as the biofilm continues to grow, the distance for electron shuttling can increase, this will not happen for brush anodes, as distribution of microbial communities will be uniform. Additionally, carbon felt is cheaper than carbon cloth, more conductive, has an open structure which is a good match for the microbial community, thus reducing energy losses.

3.5.4. Air Cathode MFC System Operation

As shown in **Fig. 3.4**, the final system bolted together with brush anode on one side, and the air cathode at the other, with electrode distance of 1.5 cm, in the interest of balance, the electrodes should not be too close to each other, as air from the outside might foul the anaerobic bacteria, as MFCs are reactors that operate in the absence of oxygen, that is undesirable. Future research (not included in this work) might include studying the critical distance for electrodes for optimal bioelectricity production, in this work the distance is chosen based on the literature available for systems with similar architecture.

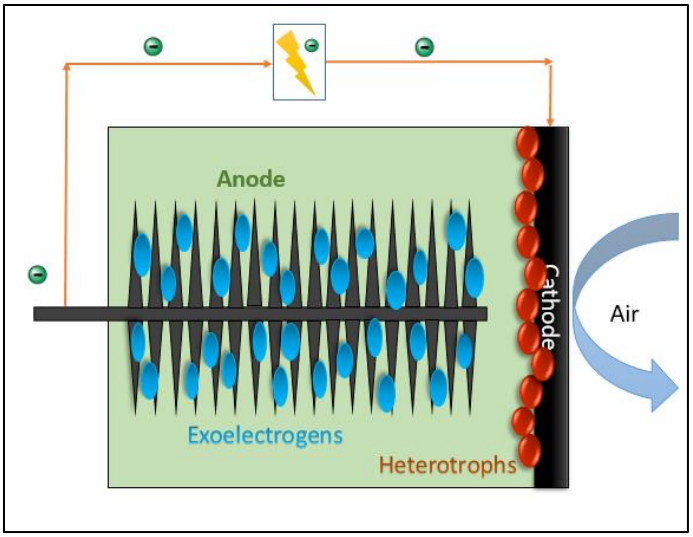


Figure 3.4: Optimized system schematics with reduced electrode distance**.**

Further, the chemical characterization for the wastewater, and the power generation from the MFC methodology remains the same, an initial OCV assessment of the wastewater in the air cathode MFC, followed by power generation curves. The chemical characterization as mentioned earlier, is done before and after the power generation, to see the overall contamination removal efficiency. Such systems eliminates the need for membrane, reduces reactor cost (for cathode chamber), further eliminates cost for aqueous catholyte and decreases internal resistance of the system, as membrane is no longer functional.

3.5.5. Comparison of MFC Reactors

Based on the contamination removal efficiency and bioelectricity generation of Pt-Cat system and the AC-Cat system, corresponding performance, stability and cost analysis are reviewed, as these findings are key to understand MFCs and its scope for commercial outlook.

3.6. Characterization of catalyst

Activated Carbon is the catalyst of interest here, as Pt-Cat systems has been extensively studied over the last two decades, however research into AC-MFC has expanded only in the last few years, and given the catalyst source (i.e. biomass) and preparation is through a novel route (of chemical activation) which has not been reported previously, based on the literature reviewed, the following characterizations were done for the same.

3.6.1. Particle Size Analysis (PSA)

As the biomass was collected from the local market, post moisture removal at 110 ^{o} C for three hours, it was crushed to finer particles. To evaluate the consistency of the particles, the particle size was analyzed using a Particle Size Analyzer (Malvern, MANO384). A probe sonicator was used to disperse some of the particles in DI water, which was then fed in a vile and into the equipment.

3.6.2. Atomic Force Microscopy (AFM)

AFM (Nanosurf, USA) was used to evaluate the activated carbon; it was performed for to study the properties at surface level. It includes a micro tip on a cantilever. The tip is in constant touch with the surface and displaces because of the interatomic forces between the surface and the tip. The cantilever is fixed to a transducer (piezoelectric) of nanometer sensitivity.[90] A laser beam is transmitted to measure the cantilever position and they were constantly recorded by feedback control. The topography of the surface was studied by analysing the feedback signals. The contact of the tip and the surfaced depends on the radius of curvature of the tip.

3.6.3. Scanning Electron Microscopy (SEM)

SEM (Quanta FEG200) was used to evaluate the activated carbon surface. For SEM, the electron beam is made to be incident on the sample at an angle of 45°. The detector detects the electrons which passes through the electrode (porous) and then deflected towards the second electron detector for further detection.[28] The observations were performed at an accelerating voltage of 5.0 kV. The magnification was ranging from 30 to 60,000. The comparison of the structures of biomass and the formed activated carbon was analyzed, in terms of chemical activation of the pores on carbon surface using phosphoric acid as an activating agent. Further, the microscopic techniques were used to evaluate anode surface post MFC operation, to see the development of microbial colonies on the anode (carbon cloth), which results for contamination removal and electron transfer.

3.6.4. Fourier Transform Infrared Spectroscopy (FTIR)

For detection of functional groups in the prepared AC, FTIR (PerkinElmer, L12) analysis was done using standard procedures (KBr pellet with AC powder).[91] The output was in the form of vibrational energies peak, using the NIST (National Institute of Standards and Technology) FTIR library[92], the functional groups were analyzed. Separate counts of FTIR has been performed, once for prepared AC catalyst, the other for the entire cathode (after adding Binder and CB). Results of which from the perspective of chemical stability were identical.

3.7. Identification of Microbial species

Evaluating biofilm growth and community analysis using 16S rDNA sequencing techniques for PCR products reveal the bacteria specie present in the wastewater[41], and it can directly be related to colony competition or symbiosis depending on power generation curves from the MFC. Over the years, several

bacterial species, through community analysis[8], have been identified individually or in mixed cultures that produce high power densities (> 2 W/m^2), which are a key to bring MFCs close to the commercial platform, these metabolisms include exocellular electron transfer, via cellular respiration and cell to cell communication[42].

3.7.1. Bacterial DNA Isolation Method

The developed biofilm on the anode was removed with a sterilized knife, and underwent serial dilution method for bacteria colonies to grow[93]. Agar gel electrophoresis was performed to separate the various DNA strands, and PCR was used for molecular photocopying which involves heating and cooling of DNA samples in the thermal cycler in the presence of oligonucleotide primers, dNTPs and heat stable enzyme called Taq Polymerase in a cyclic pattern over about 30 cycles[94]. During each cycle, a copy of target DNA sequence is generated for every molecule containing the target sequence. After about 30 cycles, a billion copies of the target region on the DNA template have been generated.

3.7.2. 16S rDNA PCR analysis

The target region of DNA generated in the above process is called the PCR product, also known as amplicon[95], was distributed in five 0.2 ml PCR tubes. The samples were mix thoroughly and divided into five reaction tubes. 0.5 µl of each DNA sample was added to the respective PCR tube. The negative control receives no DNA, the tubes are capped, and the contents are mixed by flicking and then briefly (~10 seconds) centrifuging the tubes to concentrate the reaction mix at the bottom of the tube[96]. The tubes placed in the PCR thermal cycler underwent the following process: Initial denaturation at 94ºC for 5 minutes, Cycle denaturation at 94ºC for 45 Seconds, Annealing at 55ºC for 45 Seconds, Extension at 72ºC for 1

min. This was followed denaturation for 34 cycles, then Final Extension at 72ºC for 5 minutes; retention at 22ºC and ending of the program. The program is stopped and the tubes are collected. The genetic code of each colony was generated via an automated system.

3.7.3. Phylogenetic tree

The GenBank database of the National Center for Biotechnology Information was searched using the BLAST-n (nucleotide) algorithm to analyze the 16S rDNA portion of the sequences to identify the microbial communities[97]. Further, MEGA (Molecular Evolutionary Genetics Analysis) was used to align these sequences and generate a phylogenetic tree using the MUSCLE alignment[98]. Sequences derived from the analysis were deposited in GenBank i.e. NCBI database, under accession numbers, which are discussed in the next chapter will details for each microbial specie detected and their behavioral pattern in electroactive biofilms.

Phylogenetic trees help in creating an ancestral map between different species, and in our case, help create a statistically significant correlation between power generated in the Pt-Cat and AC-Cat system, using wastewater from sewage. Not only the information pertaining to exoelectrogenic bacteria species, also their ability to go for colony competition or having a symbiosis prospect for colonization.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1. Physicochemical parameters of wastewater

The University Sewage treatment plant (STP) was the source of the wastewater; untreated water was collected from the inlet channel, and was fully characterized as shown in **Table 4.1**. As explained in the later section, for some study, the sludge from sewage was inoculated with acetate to evaluate the effect of substrate concentration on voltage.

Table 4.1: Characterization of wastewater taken from STP Inlet at University

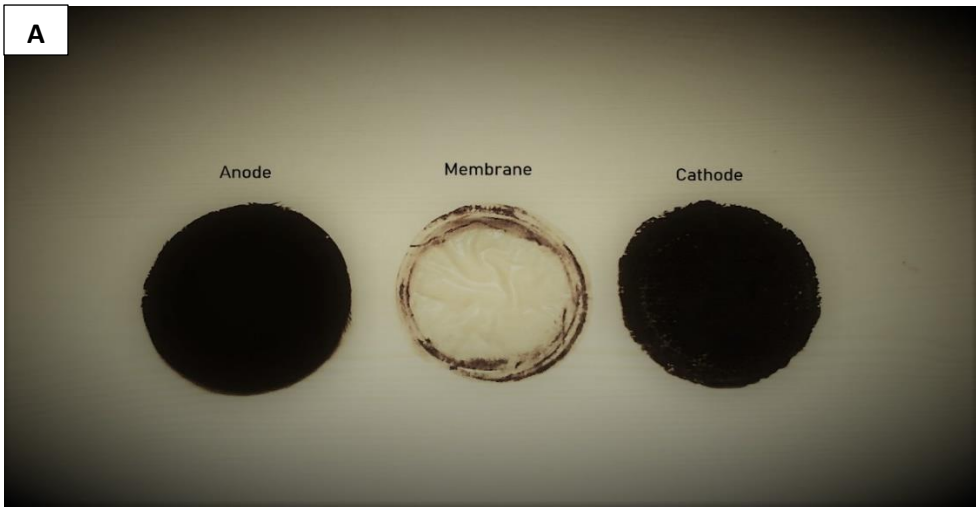
S.no	Parameters	Concentration in mg/l
1	Alkalinity as $CaCO_3$	412.0
2	Carbon di-Oxide (CO_2)	52.8
3	Total hardness (as $CaCO_3$)	176.0
4	Calcium(as Ca^{+2})	32.0
5	Magnesium (as Mg^{+2})	23.3
6	Nitrate (as $NaNO_3$)	6.7
7	Fluoride (as F^-)	0.98
8	Chlorides (as Cl)	96.0
9	Cyanide (CN)	<0.01
10	Sulphate(as SO_4)	90.0
11	TKN (as N)	<0.01
12	Ammonical Nitrogen(as NH_3-N)	98.2
13	TKN (as N)	72.2
14	Phosphate (as P)	2.18

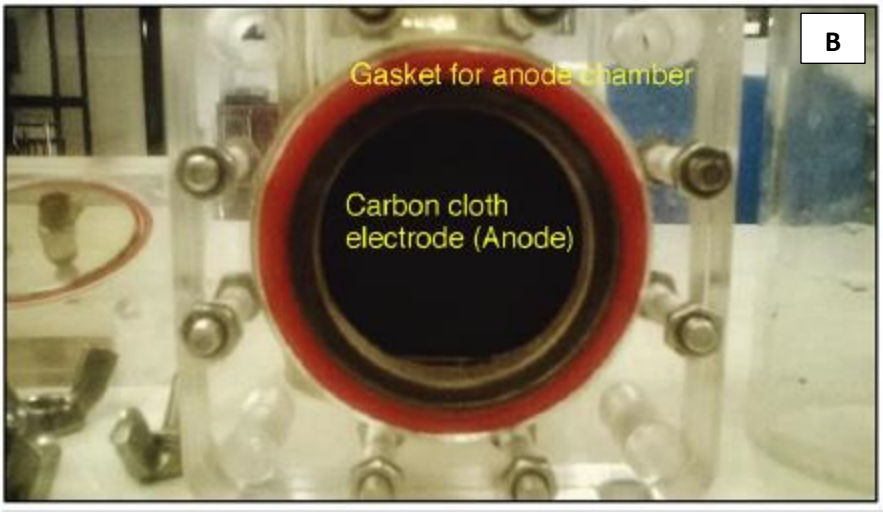
15	Alkalinity as $CaCO_3$	412.0
16	Carbon di-Oxide (CO_2)	52.8
17	Total hardness (as $CaCO_3$)	176.0
18	Calcium(as Ca^{+2})	32.0
19	Magnesium (as Mg^{+2})	23.3
20	Fluoride (as F^-)	0.98
21	Chlorides (as Cl)	96.0
22	Cyanide (CN)	<0.01
23	Sulphate(as SO_4)	90.0
24	TKN (as N)	<0.01
25	Ammonical Nitrogen(as NH_3-N)	98.2
26	TKN (as N)	72.2
27	Phosphate (as P)	2.18
28	COD	631.0
29	BOD (at 5 days)	310.0

The acceptable limit of impurities in water is primarily monitored as COD, and BOD, which globally for water discharged from treatment plants should be less than 70 mg/l (for COD) and around 30 mg/l for BOD, before it is sent back into the environment[28]. While data related to sewage may vary in terms of organic loading, the primary composition will remain the same. Given that sewage waste management is one of the major challenges in the developing nations, and the fact that MFCs are effective in removing COD and BOD content of wastewater, while generating bioelectricity, the same options are explored in this work.

4.2. Performance evaluation of Pt-Cat MFC System

In this part of the work, a two-chambered microbial fuel cell was designed and fabricated with carbon cloth electrodes and Nafion-117 membrane, having Platinum as the catalyst as shown in **Fig 4.1**. Wastewater from an organic load of around 760 ± 20 mg/l reduced to around 170 mg/l, with the change in pH from 7.65 ± 0.6 to 7. 31 ± 0.5; over the time of operation the biochemical oxygen demand from an initial 290 ± 30 mg/l reduced to 175 ± 10 mg/l. Open circuit voltage was achieved mostly between 750–850 mV, with inoculated sludge produced a peak open circuit voltage of 1.45 V between fed-batch cycles. For characterization of power generated, polarization curves are evaluated with varying resistance to examine system stability with varying resistance. The current density and power density are reported to peak at 0.54 mA/m^2 and 810 ± 10 mW/m^2 respectively. The development of stable biofilms on the anode contributes to the power generation and was evaluated using microscopic analysis[99], this shows bacteria present in wastewater are electroactive microbial species which can donate electron to an electrode using conductive appendages or nanowires, while consuming the organic matter present in the wastewater.





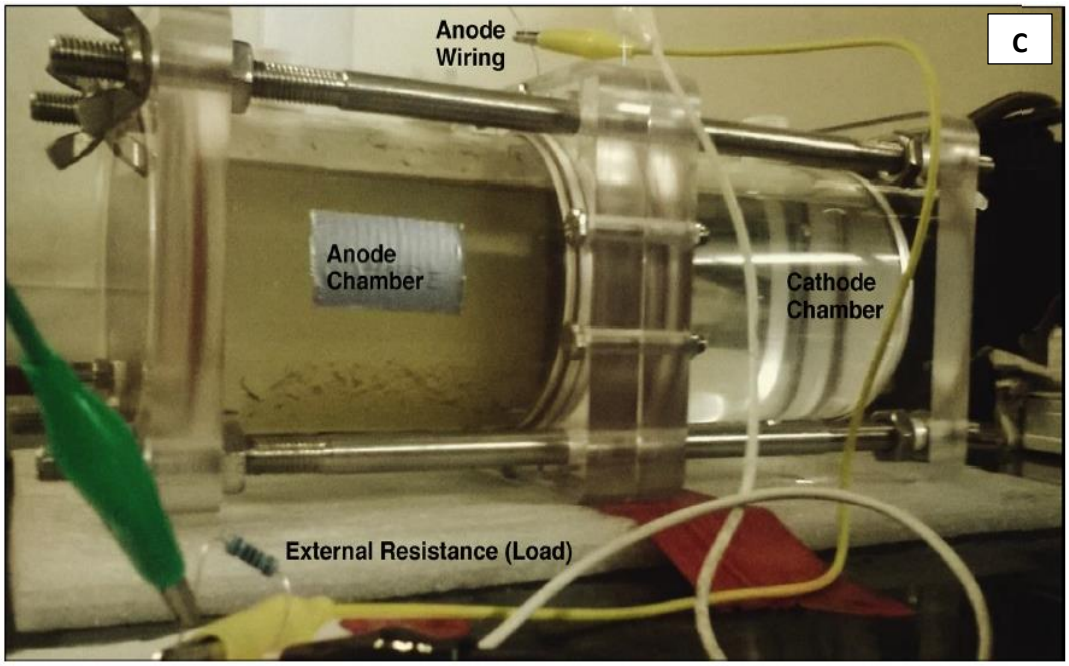


Figure 4.1: (A) The electrodes and the membrane **(B)** Actual setup with plexiglass showing the anode with the gasket provision for attaching the anode chamber **(C)** Assembled MFC setup with wastewater in the anode, and phosphate buffer in the cathode, connected to an external load.

4.2.1. Open Circuit Voltage

Under this condition, system is running at zero current, which means infinite resistance. This gives the maximum theoretical voltage that can be attained with external loads.

- Average COD for all Wastewater samples: 680 – 780 mg/l
- Average BOD: 310 – 450 mg/l

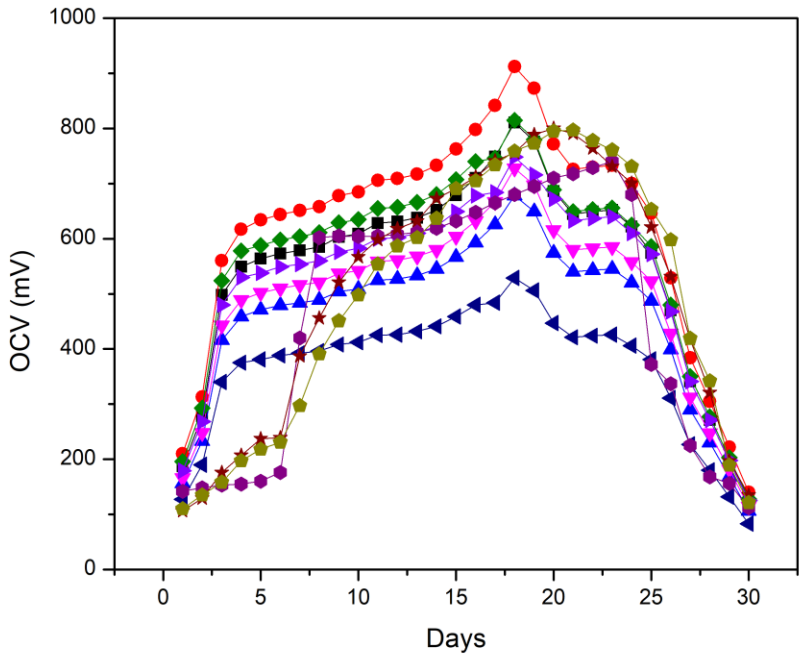


Figure 4.2: OCV shown with Sewage sources based Wastewater, with ten full runs, each of which lasted around a month, and on average generated peak voltage in the range of 0.75 V – 0.85 V.

As shown in **Fig. 4.2**, the wastewater charged in the system produced peak voltages ranging up to 0.85 V (or 850 mV) after two weeks of operation, and with other

cycles of similar nature, the generated voltage would reach a peak value between 750–850 mV, this voltage generation is measured by directly connecting the cathode and anode wire to the data acquisition system. This shows, in mixed cultures, such as wastewater streams, different bacteria can grow setting different potentials. Following peak voltage generation, a decline was observed due to depletion of organic matter. Open circuit analysis implies infinite resistance and zero current, and sets the potential for the voltage that can be achieved with wastewater streams theoretically. An important thing to remember here is that effective COD removal is only possible when system generates current i.e., using a resistor to connect the anode and the cathode[89], further this reduces the time of operation as well, thereby achieving high COD removal rates.

Further, some amount of sludge (4 gm in 250 ml water) from the STP was inoculated with acetate, to see the effect of substrate addition on MFC performance. This achieved a peak voltage of 1.45 V as shown in **Fig. 4.3**. These shows in mixed cultures, such as wastewater streams, different bacteria can grow setting different potentials. The different cycles for the same is shown in **Fig. 4.4**.

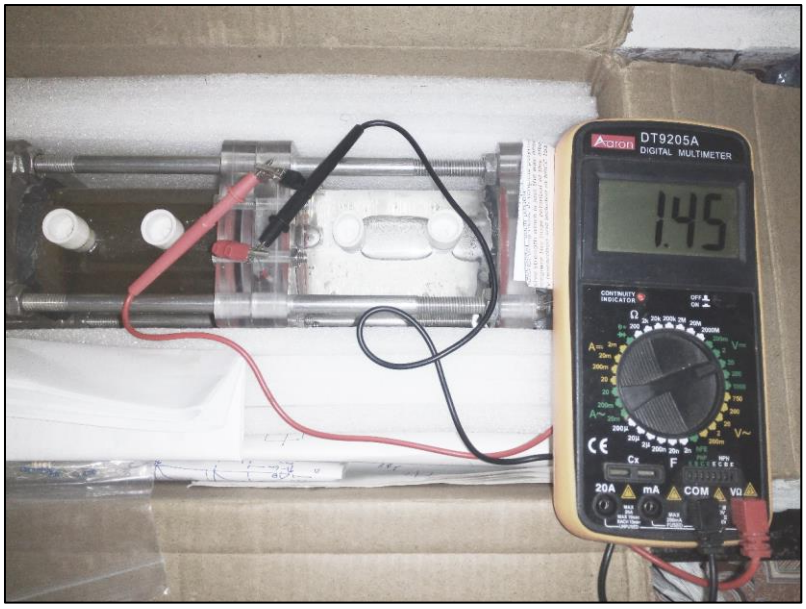


Figure 4.3: With addition of sludge into the wastewater stream, cell OCV went as high as 1.45 V, this shows MFCs can incorporate both sludge and wastewater for power generation, and opens up the possibility of even using effluents in these systems.

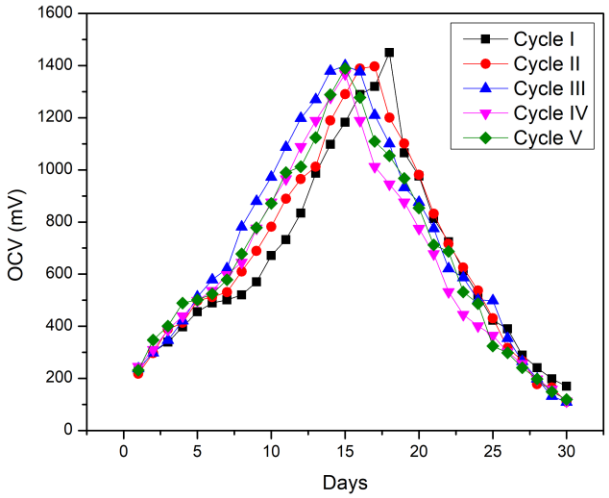


Figure 4.4: The wastewater from the Sewage Treatment Plant (STP) with inoculated sludge generated voltage in the range of 1.30–1.45 V between cycles.

This show in mixed cultures, such as wastewater streams, different bacteria can grow setting different potentials [20]. Following peak voltage generation, a decline was observed due to depletion of organic matter. Open circuit analysis implies infinite resistance and zero current, and sets the potential for the voltage that can be achieved with wastewater streams theoretically [12]. An important thing to remember here is that effective COD removal is only possible when system generates current i.e., using a resistor to connect the anode and the cathode [11], further this reduces the time of operation as well, thereby achieving high COD removal rates.

4.2.2. Water Treatment and Power Generation

The voltage generated in a MFC is far more intricate to predict or understand as compared to a chemical fuel cell [22]. In the MFC, the bacteria take time to colonize the electrode and produce enzymes or structures that are needed for electron transfer from outside its cell. In mixed cultures these electroactive microorganisms can grow [23], setting different potentials further even in pure culture. These potentials cannot be easily predicted. However, there are limits to maximum voltage generated by these systems based on thermodynamic relationship between the electron donors (i.e., the substrates) and acceptors (oxidizers).

4.2.2.1. Fixed External Resistance

An external resistance of 500 Ω was connected to the double-chambered MFC, data was observed in the form of bell curve over a period of two weeks as shown in **Fig. 4.5** on the following page. The multi-cycle method was followed for all studies with external resistor[100], where a resistor is attached to the system from beginning till the end of experiment, and then a new cycle is started with a fresh batch of wastewater, without removing the resistor. This gives stable result and efficient

system operation. As shown in **Fig. 4.6**, the current density and power density obtained using a 500 Ω resistor was consistent along all five cycles with peak values ranging around 0.54 mA/m^2 and 537 mW/m^2 respectively.

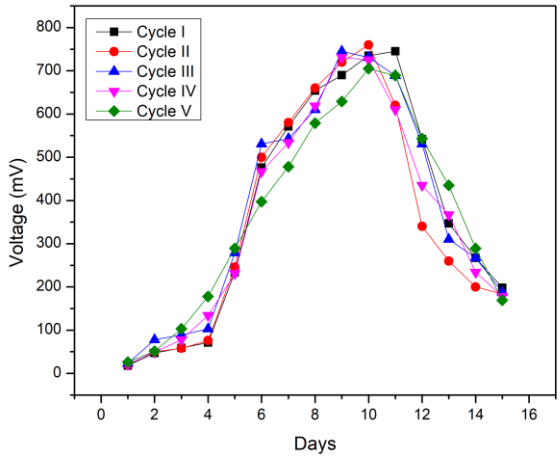


Figure 4.5: Voltage recorded across arms of 500 Ω resistor, the pattern observed is similar to OCV generation, but with external resistor, current and power values can be computed, giving better understanding of microbial potentials in MFC systems for given wastewater streams. (Peak: 0.76 V)

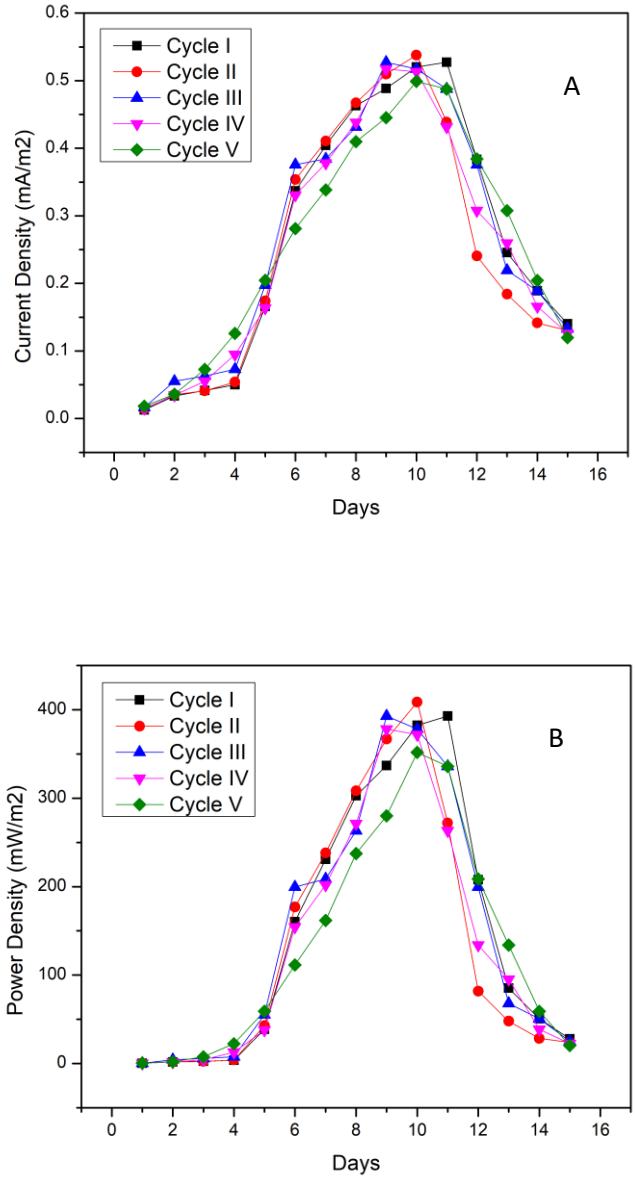


Figure 4.6: (A) Current density computed over voltages recorded for each cycle, peak current density was around 0.54 mA/m^2 for second cycle. (B) Power density computed from the different cycles, with peak at cycle II with 537 mW/m^2 or 0.53 W/m^2.

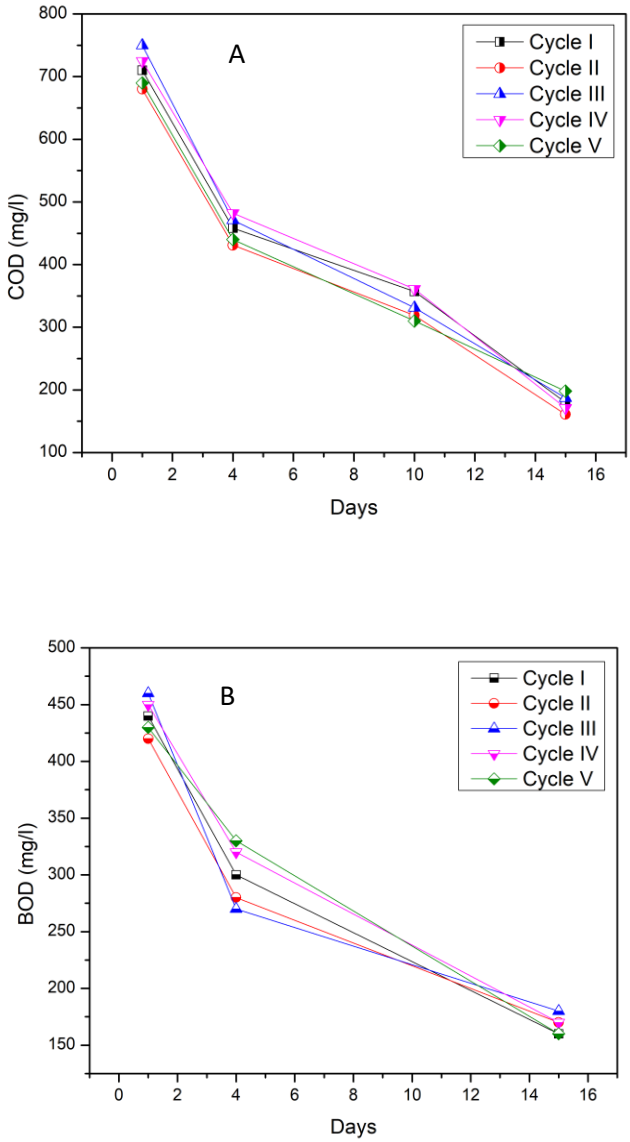
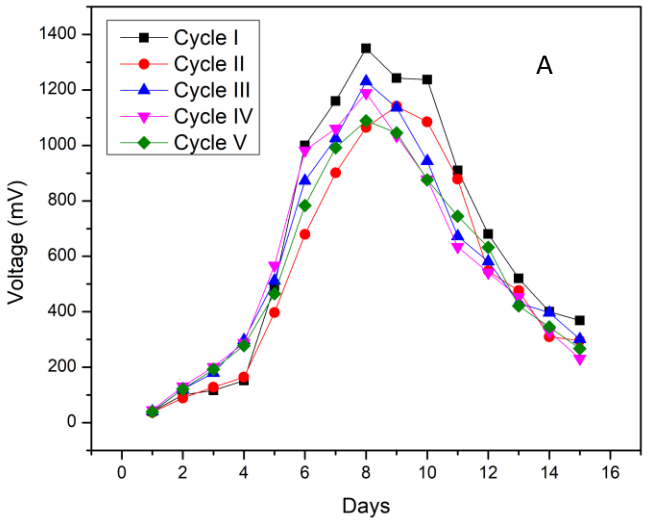


Figure 4.7: (A) The COD removal efficiency was around 75% for all cycles of power generation from an initial 710 ± 40 mg/l to around 160 ± 27 mg/l (B) BOD removal was up to 160 ± 10 mg/l from an initial 430 ± 30 mg/l with an average removal efficiency of 62%.

Further as shown in **Fig. 4.7**, the corresponding COD removal was around 75.67% with a reduction from an initial 710 ± 40 mg/l to around 160 ± 27 mg/l with BOD removal up to 160 ± 10 mg/l with an average removal efficiency of 62%. Similarly, a higher resistance of 1000 Ω was employed with the second system, for which power generation characteristics are shown in **Fig. 4.8**, which showed the peak voltage at 1.35 V between cycles, and peak power density of 820 mW/m^2 or 0.82 W/m^2. Further the chemical characterization for removal efficiency as consistent with previous results, where the COD was reduced to around 160 ± 25 mg/l with 75.23 % removal efficiency, and a higher BOD removal of around 70% as shown in **Fig. 4.9**.



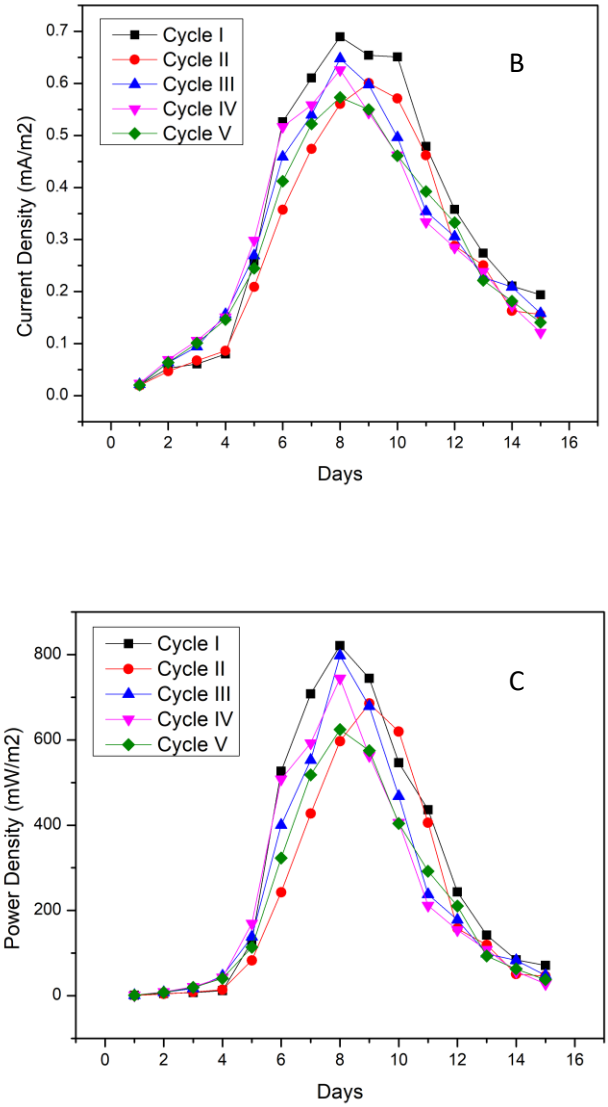
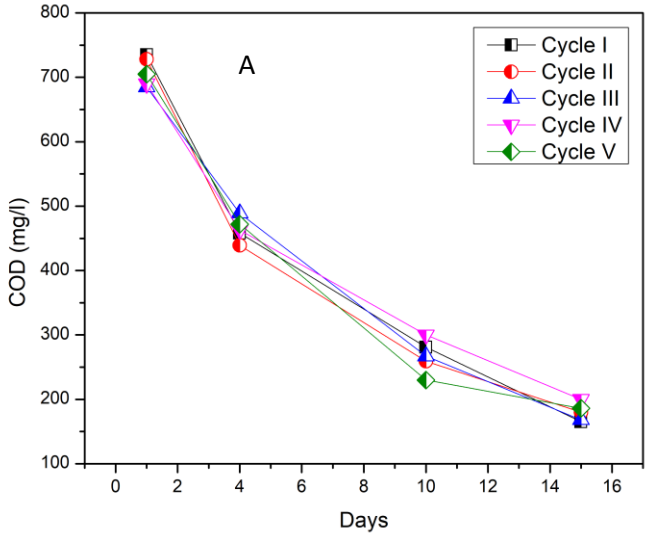


Figure 4.8: (A) Voltage curves over two weeks of operation with external resistance of 1000 ohms, peak voltage recorded from all the cycles was around 1.35 V. (B) Current Density over days of operation, achieved by normalizing the current generated over anode surface area, this peaked at 0.60 mA/m^2 which was highest among all cycles. (C) Power density values for each voltage drop recorded across the terminal with peak value at around 820 mW/m^2 or 0.82 W/m^2, among cycles average values were observed.



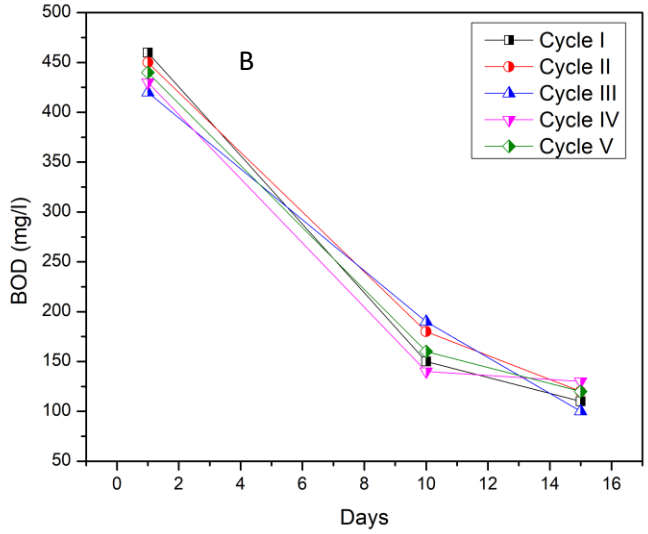


Figure 4.9: (A) Higher COD removal achieved with a higher resistor (1000 ohms as compared to 500 ohms), with an average COD removal efficiency of 75%, from an initial 690 ± 30 mg/l to around 160 ± 25 mg/l. (B) BOD removal from all the cycles achieved an overall average of 70%, with an initial ranging at 430 ± 25 mg/l to a reduced 118 ± 10 mg/l.

It is interesting to note that for the studies with external resistor, 4 gm of sludge was inoculated with all samples, and the results indicate the increase in sludge material can accelerate removal rates with higher resistance, further this opens up the scope of MFC systems to be effectively integrated with wastewater treatment plants, where significant amount of sludge can be added to the wastewater itself, and in the process, treat the waste and the sludge, while generating electricity[99]. The current density and COD removal plummets after about week of operation. When the current density plummets, this shows the system is not generating enough electrical power. For practical purposes, the electricity should be generated till the COD is completely removed [60].

4.2.2.2. Variable External Resistance

As resistances are decreased from open circuit potential, the voltage decreases. The resistors used for the polarization studies are 100 Ω, 250 Ω, 500 Ω and 1000 Ω. Each of the resistor was plugged in the system and was allowed to stabilize for a period of one hour, and voltage drop was recorded[1]. It was started with 1000 Ω, followed by 500, 200 and 100 Ω. Coulombic efficiency was calculated using the same external resistor based on changes in COD concentration.

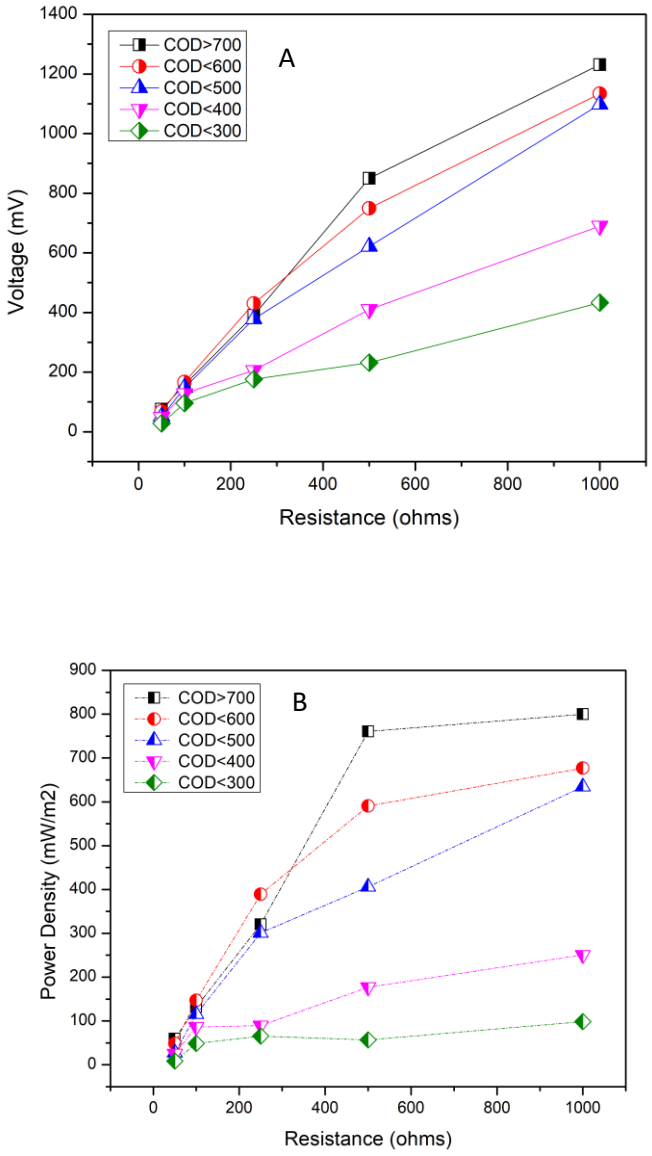


Figure 4.10: (A) Average range of voltages achieved in different cycles with different COD loadings, with varying resistors (B) Based on voltages obtained at different resistance, power density is computed with peak around 820 mW/m^2 or 0.82 W/m^2 from sewage based wastewater when COD is more than 700 mg/l.

Based on the changes in COD concentration with respect to power generation as shown in **Fig. 4.10**, Coulombic efficiency (C_E) for the existing system was evaluated to be 32%, which as studies have suggested is higher for Nafion membrane based MFCs compared to those obtained from membrane-less MFCs. At present, most literature has reported CE ranging from 5% to 59% for wastewater having complex substrates[65], [101]. Improvements for accessibility of further insoluble substrates thereby leading to better organic matter degradation and more stable anodic biofilms will be the key for achieving higher CEs.

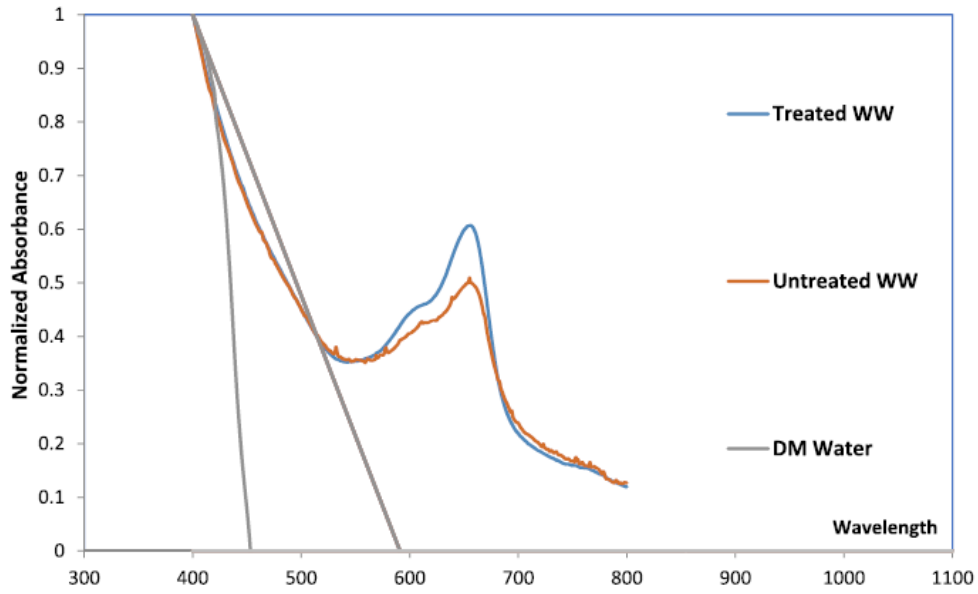


Figure 4.11: Ultraviolet–visible spectrophotometry of untreated wastewater (here shown as WW) and wastewater after 1 week of operation of MFC, the pattern shows absorbance has increased from an initial 0.5 (brown shift) to 0.6 (blue shift), thereby removing mass concentration of contaminants in the process, the ideal absorbance for a standard solution (DM water) is also shown to peak at 1.

Further **Fig. 4.11** shows how UV-Vis spectrophotometry can be used to see the difference in contamination level in the wastewater. Hence, it is seen that with most studies with the wastewater, the low resistance usually attributes to low power and related organic load removal efficiency, and the opposite is witnessed with higher resistance (like 1000 Ω). The potentials of the anode and the cathode set the limits for the maximum voltage achievable for power generation, using the potentials; substrates such as sludge from the STP can be used to accelerate these biochemical bases for power generation[102].

Bacteria capable of exo-electrogenic activity forms the background of MFC technologies. Mixed cultures present in wastewater streams, which forms electroactive biofilms in MFCs, suggest the sheer diversity of microbial communities, which so far are known to transfer electrons to anode surface via two mechanisms, namely electron shuttling through self-produced mediators and nanowires[103].

It is interesting to note that apart from conductive nanowires, electron transfer by the bacteria is also possible from surface of the cell to the anode. As shown in **Fig. 4.12**, close examination of the anode using AFM imaging reveal protrusion on the surface that are not nanowires, and certainly could be conductive points of contact. This shows mixed bacteria present in sewage have more adhesive linking with the anode under anaerobic conditions, thus allowing closer contact required for electron transfer from the cell surface even without the use of conductive appendages[64]. Although, it must be said that such information on electron transfer mechanisms are critical to describe how bacteria colonize and maintain

viable cells on the surface of the electrode, the future scope in this can be the exploration of competition among bacteria for the surface to maintain anodic potentials.

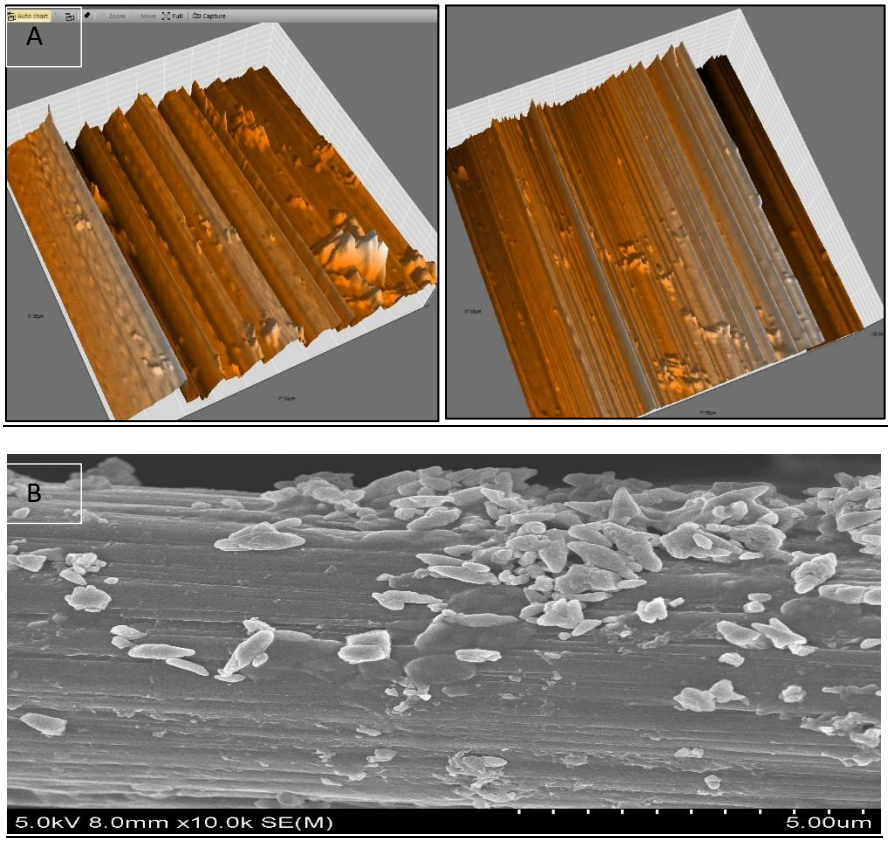


Figure 4.12: (A) AFM imaging of the anode surface at 5 μm scan, further the carbon surface shows protrusions or surface blebs, enabling cell-surface electron transfer by the bacteria. (B) 5μm SEM imaging of the anode with bacteria colony on it.

The following are the highlights from Pt-Cat MFC System:

a. Fabricated MFC reactor generated OCV of 0.75 - 0.85 mV, and increased up to 1.45 V with adding sludge as substrate, COD removal of around 75%, and BOD removal of around 70%, with peak current density of 0.67 mA/m^2 and power density of 800 ± 20 mW/m^2.

b. Nafion-117 has high permeability, reduces unwanted substrate flux from anode to cathode (i.e. fuel crossover) and improves Coulombic efficiency, Nafion-117 is composed of a perfluorosulfonic acid polymer film. When in contact with water, the hydrogen proton (H^+) detaches and hops from one sulfonic molecule (SO_3^-) to another and thus acts like an electrolyte in the presence of water. Therefore, Nafion-117 transfers H^+ across the PEM to the cathode, but does not allow electrons to cross to form water.

c. Increase in sludge material can accelerate removal rates opens up the scope of MFC systems to be effectively integrated with wastewater treatment plants, where significant amount of sludge can be added to the wastewater itself, and in the process, treat the wastewater and the sludge, while generating electricity.

d. The anode does not seem to foul over time, further the presence of positively charged compounds to naturally occurring surfaces increases the adhesion of negatively-charged bacteria due to electrostatic attraction of the cells to the surface. Biofilm communities are stable, and can continue to operate as long as contamination is available.

4.2.2.3. System Limitations and Scope

a. Membrane is clogged after twelve/ thirteen cycles, dilute acid treatment (H_2SO_4 and H_2O_2) needed for reuse, also cost associated with the membrane itself.
b. Platinum is an expensive catalyst, and fouls over time, insoluble layer of PtO is formed, regeneration not possible, ends its catalytic activity. This increases cost for the cathode.
c. Cathode chamber requires a PBS (Phosphate Buffer Solution) for maintaining ion conductivity.

Several different materials, configurations, and operational modes, are being tried and tested to produce bioelectricity from MFCs using pure and mixed cultures, but to date there is no proven design that can be considered economical for scaling up, and looking at the Pt-Cat systems, while they remain the most effective, with some studies reporting power density in excess of 4000 mW/m^2 or 4 W/m^2, but they remain a challenge when we talk about commercial outlook. However, there are some system architecture that have produced promising results using bench-scale reactors that indicate a likely scope for fitting into wastewater treatment process trains.

Second generation MFCs called air cathode MFC based on cheaper alternatives as catalyst (compared to Pt), have gained traction in the last decade, and in the next part of this work, one of the two Pt-Cat MFCs were modified into a single chambered air cathode MFC, with a novel indigenous cathode, which acts as catalyst, and a modified anode, thereby evaluating the possibility of optimizing existing system, in terms of cost, performance and stability.

4.3. Performance evaluation of AC-Cat MFC System

For understanding MFC architectures, much of the earlier studies have employed two-chambered Pt-Cat based system, however, there are other MFC architectures that can cater to the goals of such research. One such system is air cathode MFC, in such systems, membrane is not present, and the cathode itself acts as the catalyst, no additional diffusion layers are needed. Further, as these systems do not contain an MEA, the overall internal resistance is significantly less. Such systems cost almost one-fifth the Pt-Cat MFC systems are much more sustainable, hence, the same is evaluated here in terms of contamination removal efficiency, power output, Coulombic efficiency, longevity and stability. **Fig. 4.13** shows the optimized system that was configured.

For activated carbon cathode, an open circuit voltage of 630 ± 21 mV was achieved, further with sludge it reached up to 720 ± 30 mV between fed-batch cycles. Constant external load produced a peak current density and power density of 0.64 mA/m^2 and 450 ± 12 mW/m^2 respectively. Further polarization curves reveal system stability with varying resistances with a change in COD for the wastewater from 790 ± 40 mg/l to 250 ± 20 mg/l over two weeks of operation, achieving a removal efficiency of around 67%, the BOD content of the wastewater also reduced from 520 ± 20 mg/l to 165 ± 25 mg/l with an average removal rate of 62%. Activated carbon derived from biomass sources is a promising alternative to expensive platinum; further it has a low surface pH, lacks any acidic surface functional group, and can be regenerated to more than 85% of its initial performance with dilute (HCl) acid wash as compared to platinum which cannot be reused once fouled, thus implicating significant cost reduction for cathodes, with improved life and stability.

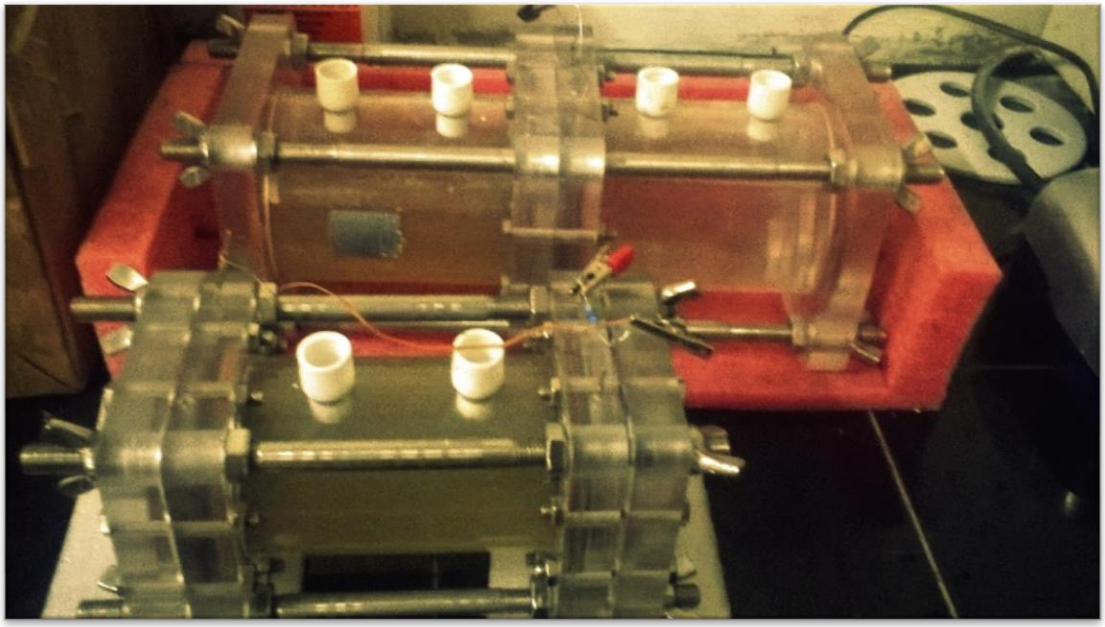
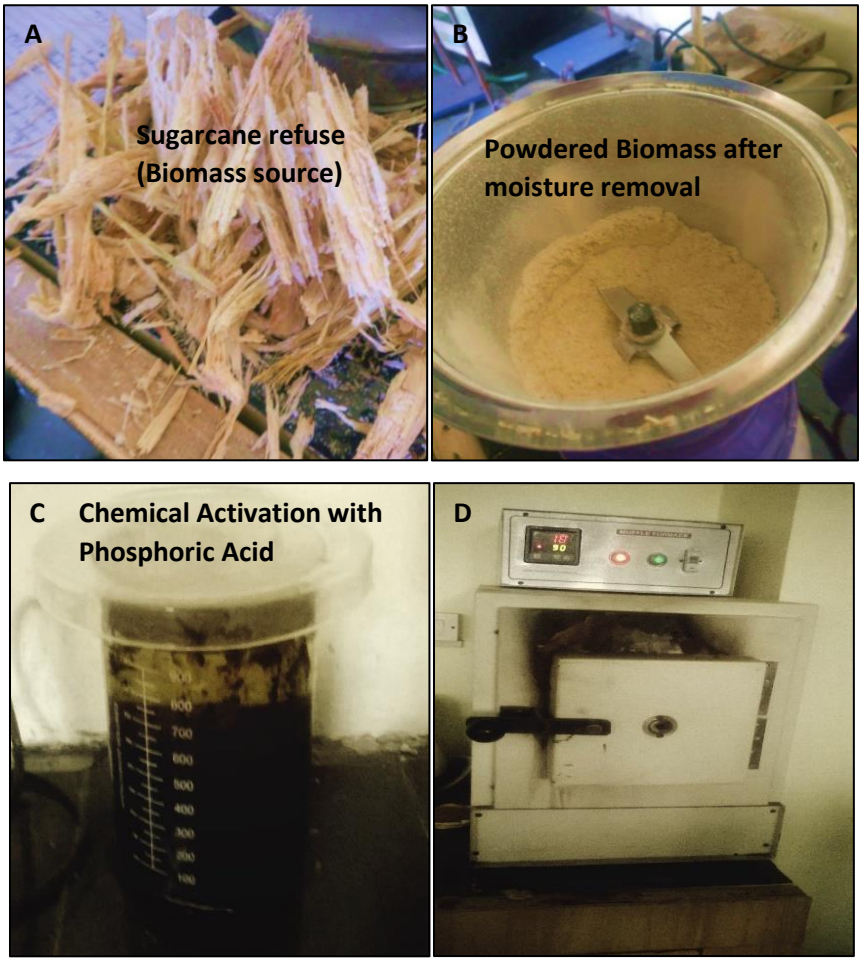
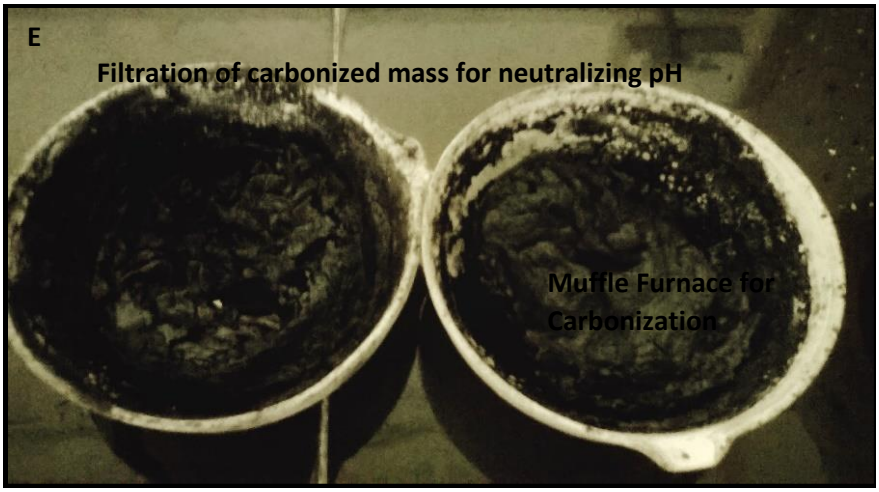


Figure 4.13: Double chambered MFC at background, in front the modified single chambered air cathode system, which forms the basis for second-generation MFC reactors.

Cathode. As an alternative to expensive catalyst (such as Platinum), a comparative analysis is presented, with Activated Carbon Cathode and PVDF as binder, where the cathode itself acts as a catalyst. A novel indigenous route was taken for the preparation of AC cathodes as discussed in the methodology section. Here phosphoric acid acts as an activating agent and has several advantages such as allowing a single step process in comparison to the traditional two-stage process of carbonization and activation. Powdered Activated Carbon with Carbon Black and PVDF mixture was spread directly onto a stainless steel mesh (AISI 316L alloy, 40×40 wires/inch, 0.37 open area, Sigma Aldrich) using a spatula, the cathode was then immersed in deionized water (for 15 min) to induce single step phase inversion

process[1], and then air dried before use. **Fig. 4.14** shows the steps involved in the process, and **Fig. 4.15** shows the cathodes that were prepared by this process.





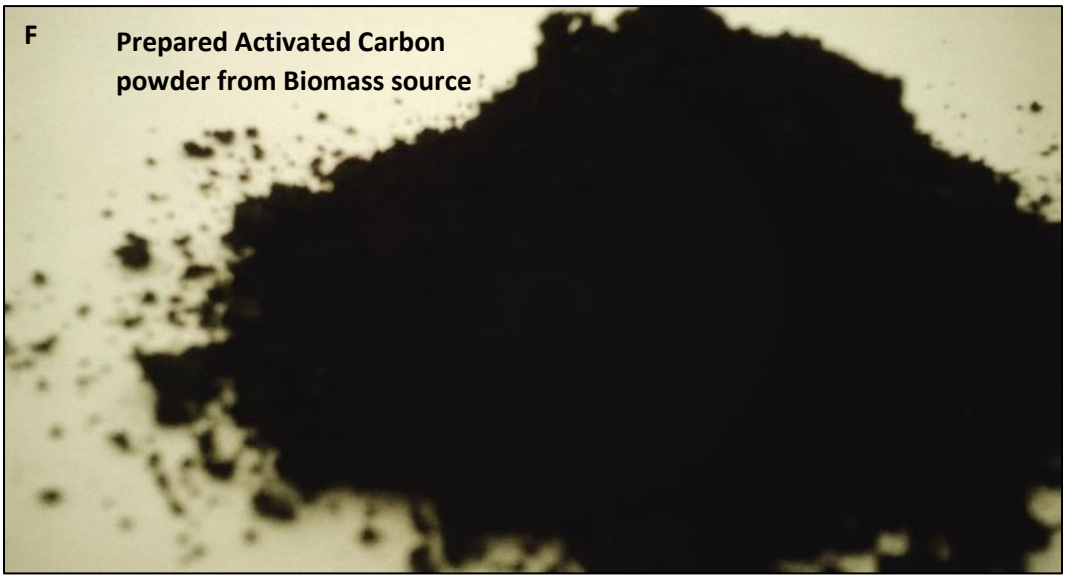


Figure 4.14: (A) Sugarcane refuse collected from market **(B)** Powdered biomass after grinding **(C)** Activation with phosphoric acid for increasing pore size **(D)** Following carbonization, the same was washed with hot water over filter papers for neutralization reaction, **(E)** This was followed by heating of the filter paper to remove the AC powder from it **(F)** Prepared Activated carbon which is used for making the MFC cathode.

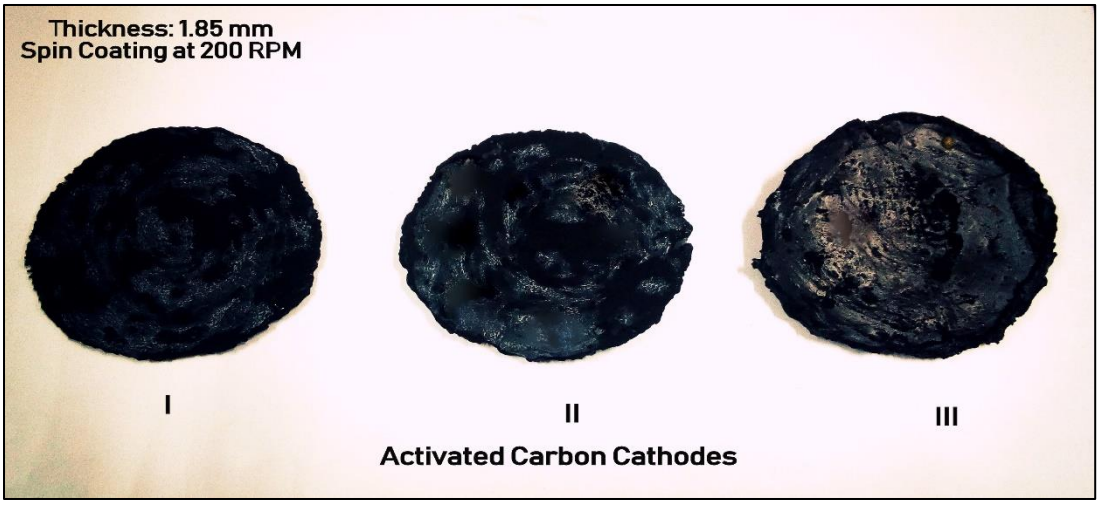


Figure 4.15: AC+CB+PVDF cathodes fabricated via single step phase inversion process.

Anode. As shown in **Fig, 3.4** previously, a novel carbon yarn brush anode was developed to increase power in the reactor, which has the potential to bring down costs significantly without compromising too much on the MFC output in terms of wastewater treatment and power generation. Based on experimentations discussed in the upcoming sections, it was observed that Carbon yarn brush anode is a good fit for microbial growth and reduces energy losses to background processes significantly.

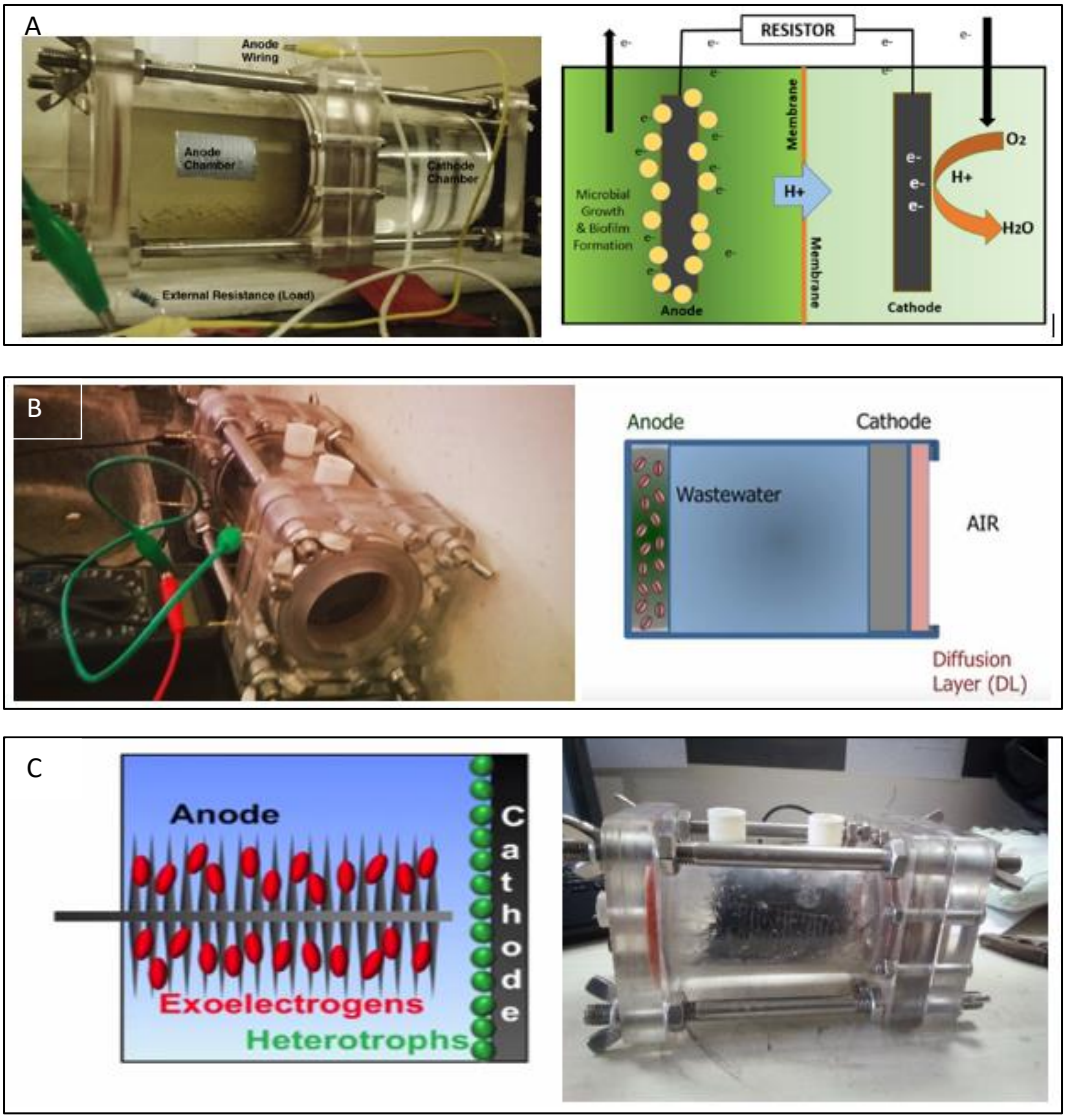


Figure 4.16: (A) Intial designed system, double chambred MFC (original setup on the left, and graphic on the right) (B) Optimizing a double chambered membrane based system, into a single chambered membrane-less system, traditionally for platinum based system a diffusion layer (DL) is needed, the same is avoided by using AC + CB + PVDF cathodes (not shown here) were able to contain the water pressure (C) Carbon Yarn woven on titanium wires (which improves rate of electron transfer), and maintained at a distance of 2 cm from the cathode.

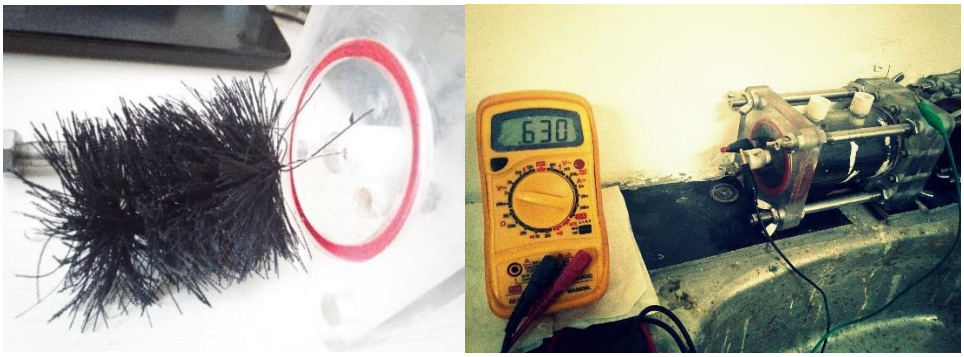


Figure 4.17: Carbon yarn based brush attached to titanium wires shown at the right, Brush anode based membrane-less air cathode MFC in operation showing OCV close to the range of Pt-Catalyst system, here it is 0.63 V or 630 mV.

An overview of how the Pt-Cat MFC was modified to an AC-Cat MFC is summarized in **Fig. 4.16**. Such system require an initial assessment, hence, post fabrication the same was charged with equivalent amount of wastewater from Sewage, and as the results show (in **Fig. 4.17**), it seems that this system architecture holds promise and formed the basis for optimizing lab scale MFCs. This shows that the optimized single chambered system performed as well as platinum, this is crucial if scale of MFCs are to be considered, as AC based systems cost around one-fifth of platinum based systems[104], further the activated carbon used in this work, the material for brush anode, which has not been reported previously (based on the literature survey) is found to be more effective than other AC based MFCs reported at different capacity[20], the same is discussed in detail in Section 4.3.3.

One reason for this is the FTIR spectra of the cathode itself which shows the complete absence of carboxylic acid groups (which is discussed in detail on Section 4.3.3 Catalyst Characterization), this makes the cathodes more stable when they are coated on SS mesh, many studies have reported that it is desirable to use AC derived

from sources that lack carboxylic acid group for improved stability, this work has certainly made significant headways in that area. System performance compared to platinum is presented in the following section.

4.3.1. Open Circuit Voltage

Three different binder loadings were evaluated, the loading was kept less as, too much PVDF can decrease the performance due to hindered proton and oxygen transfer[1]. As shown in **Fig. 4.18**, 10% PVDF gave best performance and system stability for OCV; hence, the same was used in all the cathodes. The performance of the system however did not show any significant fluctuations in power generation for the different loadings.

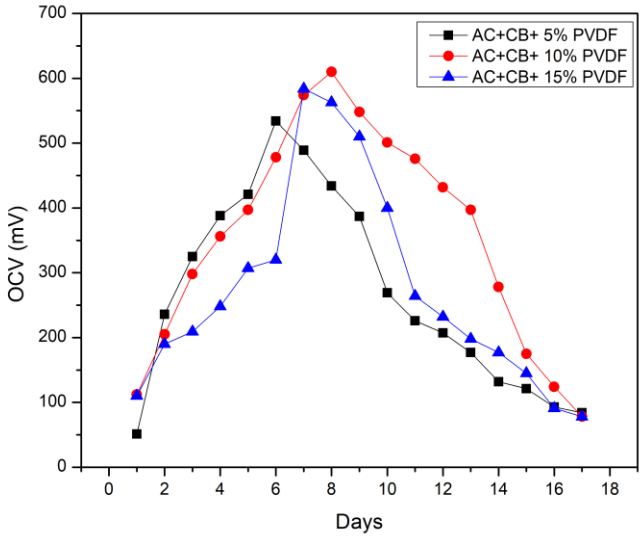


Figure 4.18: Preliminary analysis shows 10% binder gives best peak voltage (around 630 mV), based on this all further studies were done with 10% binder loading on the AC cathodes.

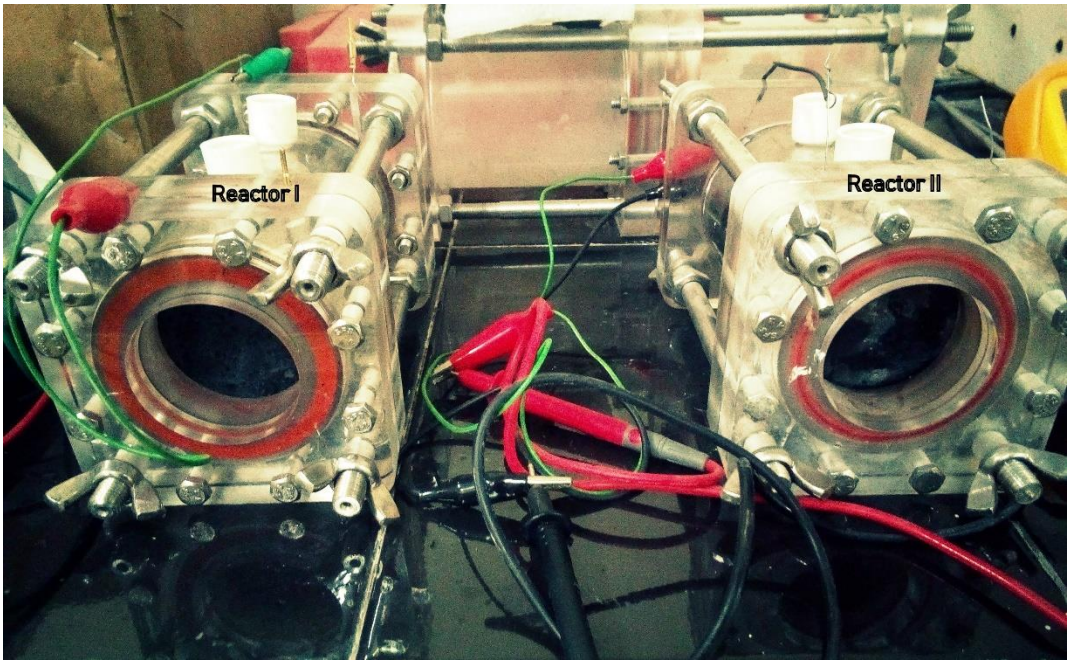


Figure 4.19: Two AC-Cat systems, in operation with wastewater from the STP.

Based on initial assessment of OCV from two reactors (**Fig. 4.19**)**,** the systems produced an Open Circuit Voltage or OCV of about 580 ± 30 mV with over two weeks of operation. For wastewater it averaged between 0.5 – 0.6 V and with increased sludge content the same increased to around 750 mV or 0.75 V. Cell OCV evaluations help form a basis for the maximum theoretical power generation from such systems, as current is zero, and resistance is maximum[105]. However, for better system evaluation current must be generated by using external resistors or load, which can help understand the efficiency of the cathodes for oxygen reduction, without losing energy to background processes. The performance of AC MFC system in terms of OCV generated is shown in **Fig. 4.20** given on the following page.

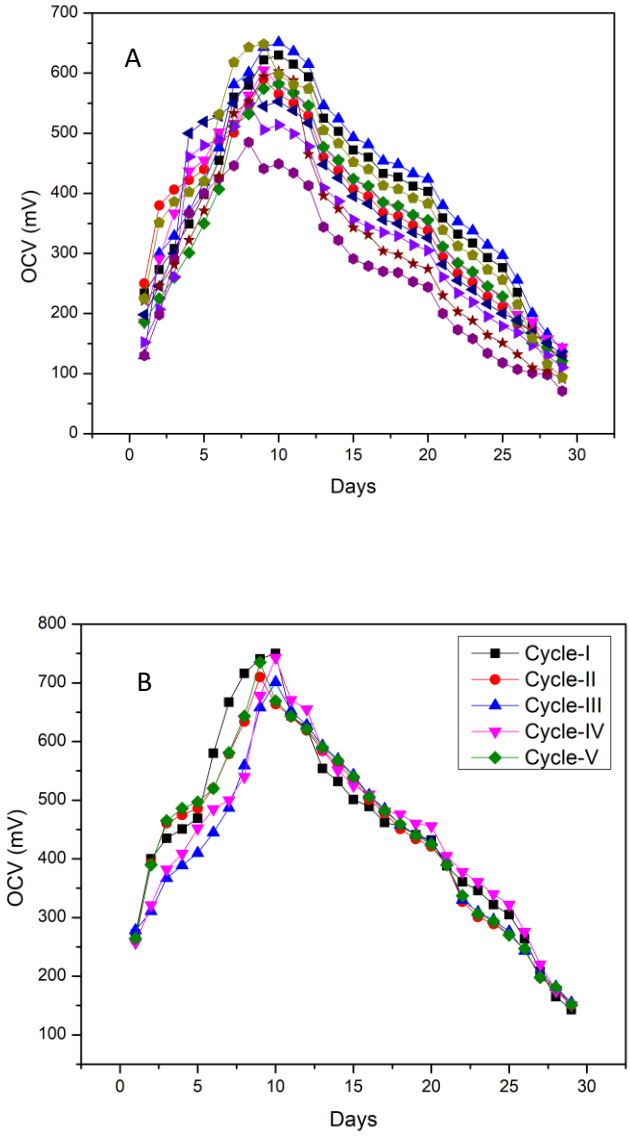


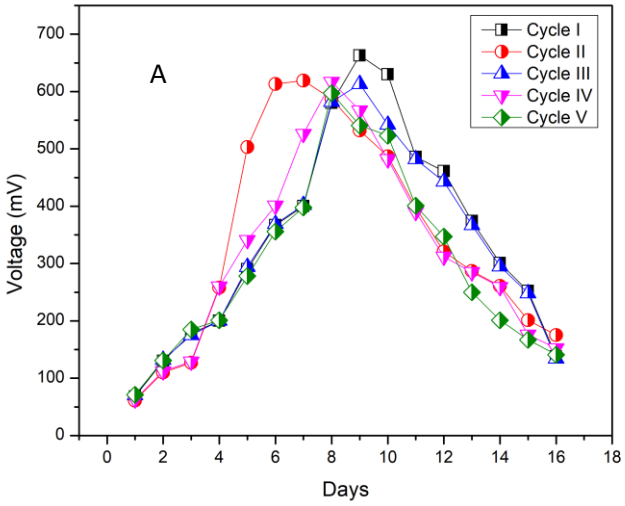
Figure 4.20: (A) Maximum possible voltage generation (in open circuit condition) averaged around 0.5 – 0.6 V for AC cathodes. (B) With addition of 10 gm sludge peak voltages recorded reached between 0.65 – 0.75 V, or 650 – 750 mV, this shows system potential can change based on concentration of substrate.

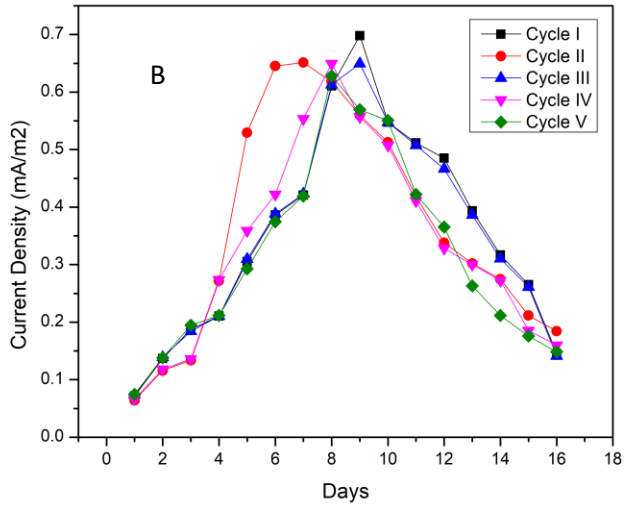
4.3.2. Water Treatment and Power Generation

As mentioned in the previous section, for power generation from wastewater, an initial multi cycle method is adopted, with different resistors, from power generation, peak and then decline[83]. The same was accompanied by polarization studies, where resistors are allowed to stabilize with the system, and then readings are taken over specified period of time.

4.3.2.1. Fixed External Resistance

Power generation initially was done using a lower resistance of 500 Ω (as compared to 1000 Ω which was used with the double chambered system) as shown in **Fig. 4.21**. The current density and power density was around 0.78 mA/m^2 and 430 ± 32 mW/m^2 respectively. The average COD removal efficiency was around 66% from an initial 830 ± 20 mg/l, and the corresponding BOD removal was 62%. A critical thing to understand here is the external resistance should be comparable to the internal resistance[47]. However, during the starting stage, the internal resistance is supposed to gradually decrease due to the development of functional microbial film on electrodes. The internal resistance depends on the surface area of the electrodes, membrane and the distances between the electrodes[74]. If the area is huge and the distance is close, a standard practice is to use 1000 Ω resistor[18], however when membrane is not present, and natural air is used as the catholyte, it is preferred to use a lower resistor (in this work 500 Ω).


A
Cycle I
Cycle II
Cycle III
Cycle IV
Cycle V
Voltage (mV)
Days
0
100
200
300
400
500
600
700
0
2
4
6
8
10
12
14
16
18


B
Cycle I
Cycle II
Cycle III
Cycle IV
Cycle V
Current Density (mA/m2)
Days
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
2
4
6
8
10
12
14
16
18

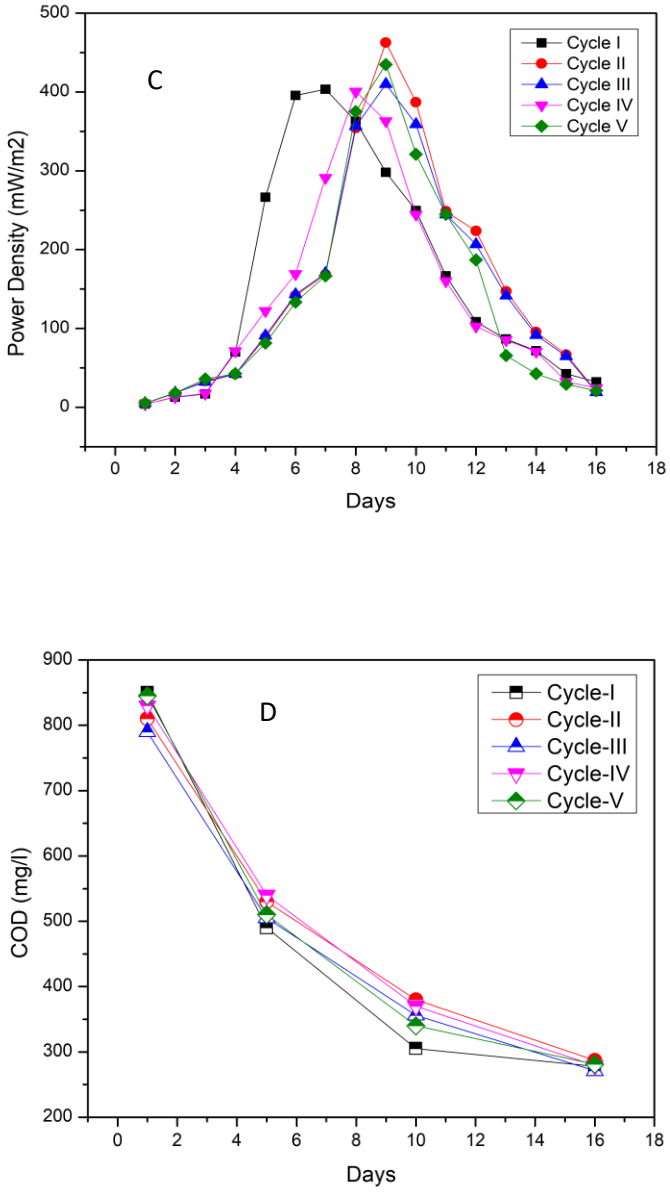
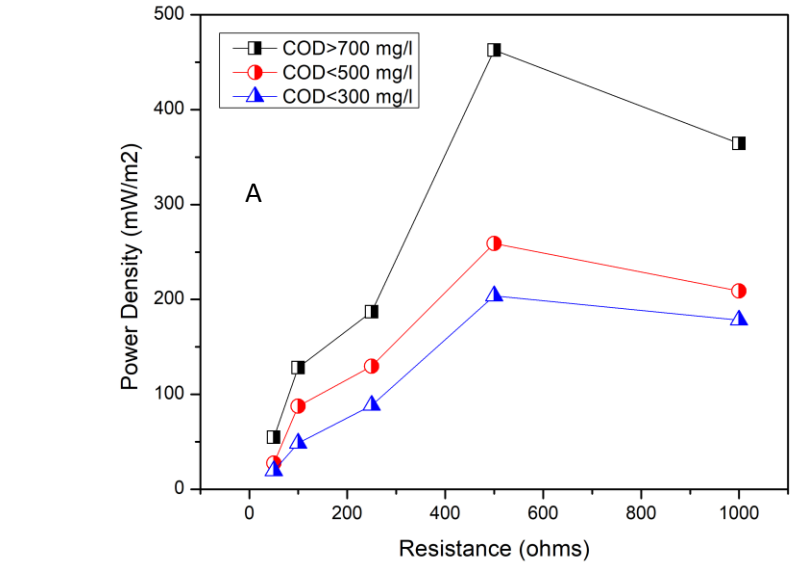


Figure 4.21: (A) Voltage generated (peak 667 mV) with 500 ohms between batch cycles **(B)** Current density peaking at around 0.69 mA/m^2 **(C)** Power Density peaked around 462 mW/m^2 or 0.46 W/m^2 between cycles **(D)** COD removal efficiencies.

Identical to the Pt-Cat system, here each “Cycle” refers to the full cycle for wastewater from being charged in the system, initial voltage, peak and decline[42]. For every operation, be it Pt-Cat or AC-Cat system, two systems were always run in parallel, and the remaining wastewater was stored in a freezer, to restrict variations in COD and BOD, and after specified period of operation, the same would be defrosted and used. There will be some changes in contamination concentration, however based on the literature reviewed it is reasonable to hypothesize these changes will be negligible in the context of overall MFC operation.

4.3.2.2. Variable External Resistance

The power density generated by the system was further evaluated through polarization method, where variable resistances are used to see the voltage output from the system[48], resistance used are from 1000 Ω through 500, 250, 100 and 50 Ω at thirty minutes interval. Polarization curves were recorded after first and second week of operation for all evaluations. The same is shown in **Fig. 4.22**, for varying COD levels present in wastewater from sewage. Further, the system was not able to sustain the maximum load (of 1000 Ω), and power generation was stable through 500 Ω and below. This shows the decreased internal resistance of the system, and through multiple cycles with varying COD loading in the wastewater.



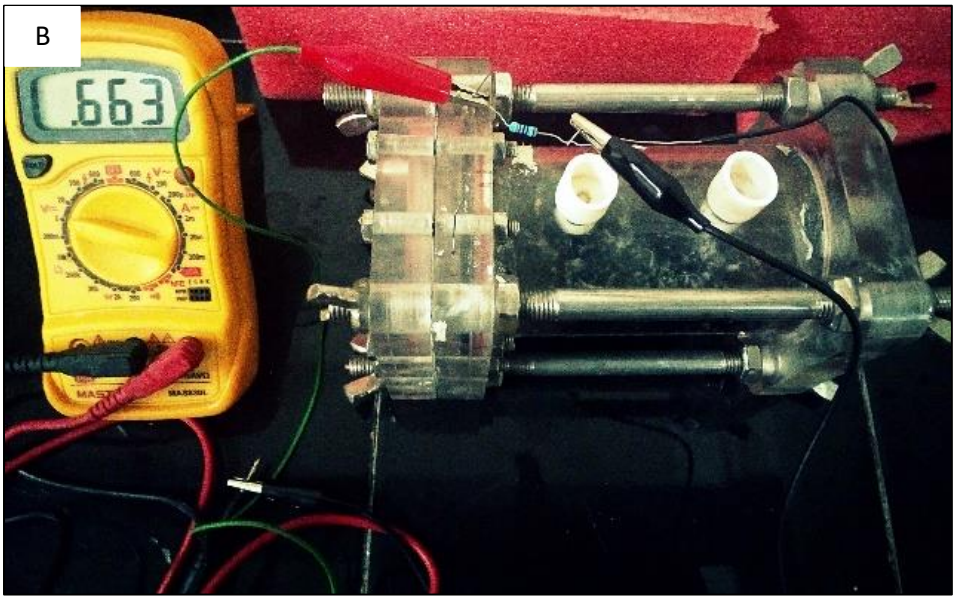


Figure 4.22: (A) Polarization curves for the AC-Cat MFC **(B)** System connected to a low external resistance, in this case achieved 0.663 V with a 500 ohms external resistor.

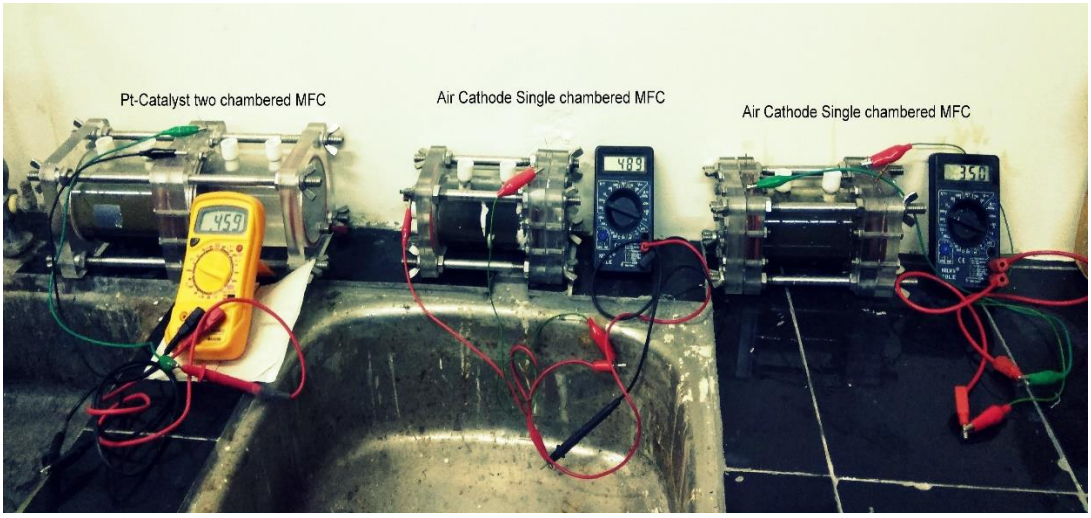


Figure 4.23: OCV achieved for Pt-Cat (0.45 V) and AC-Cat MFCs (0.48 and 0.35) during a parallel evaluation.

The AC-Cat design of MFC reactor reduces ohmic resistance in the system, brush anode is electrically conductive, non-corrosive, high surface area to volume, easy to manufacture[106]. The small size of the anode fibers and its low resistance (0.00176 Ω cm, reported by the manufacturer), along with the even distribution of yarn, makes it ideal for evaluating MFC systems, of different sizes. Bigger brushes for a variety of application will have to take into configuration the packing density, and related factors. Some areas of interest could be the effect of length, density of fiber, number of brushes and its relation with increasing power generation, while reducing internal resistance[77].

a. Size of anode is a good match for size of bacteria; flat electrodes fail the test.
b. For the Cathodes, AC based cathode is a great alternative, replaces platinum and diffusion layers (DL), both are equally expensive.

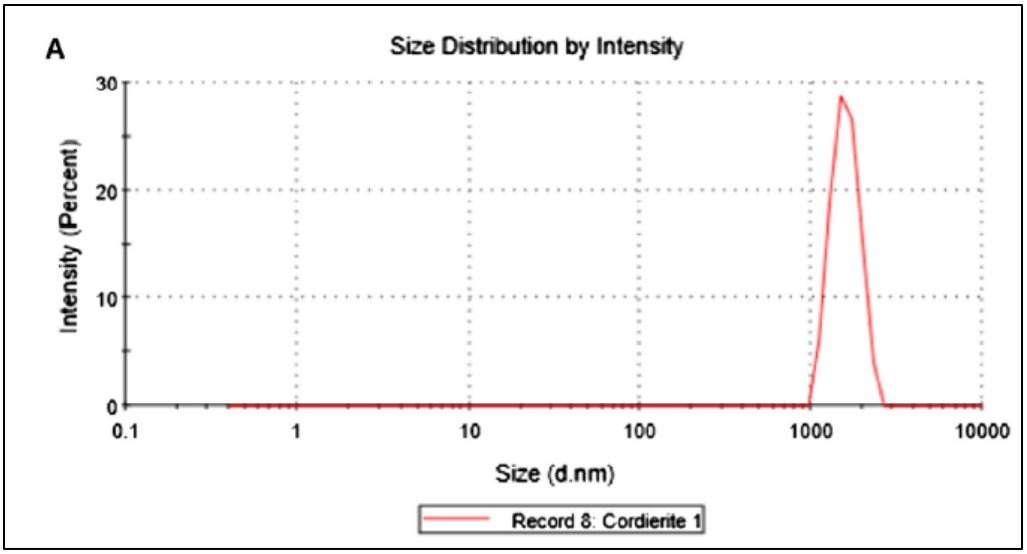
Both systems are shown together in **Fig. 4.23**, which forms the basis for energy recovery from wastewater streams, at the most fundamental level.

4.3.3. Catalyst Characterization

The main interest of characterization was the indigenously prepared Activated Carbon Cathode and its properties, additionally analysis of biofilm on the anode has also been presented in this section. Surface characteristics and functional group analysis for the prepared AC is done using XRD (X-Ray Diffraction) and FTIR (Fourier Transform Infrared Spectroscopy) respectively[42]. Particle size analyzer is used to evaluate the average particle size. Microscopic analysis for the surface is done using AFM (Atomic Force Microscopy) and SEM (Scanning Electron Microscopy); SEM imaging is used to see difference in biomass structure and the formed AC structure.

4.3.3.1. XRD analysis and Surface Morphology

The Activated Carbon powder once prepared and dried was checked for average particle size using a Particle Size Analyzer (Malvern, MANO384). A probe sonicator was used to disperse some of the particles in DI water (**Fig. 4.24A**), which was then fed in a vile and into the equipment. The average particle size was found to be 1.59 μm. Surface morphology for AC was analyzed using XRD (**Fig. 4.24B**), as shown in figure above, the diffraction pattern had an intensity peak at $2\theta=27.23$, and is attributed to hexagonal graphite i.e. the formation of small, two-dimensional graphite-like structures. As temperature increases, the intensity of amorphous peak follows a similar trend[99]. Moreover, becomes more pronounced, usually between 26 and 27°, which is consistent with graphite properties, and is associated with the processes of graphitization of the organic component and the formation of the nano-crystalline structure of the matrix.



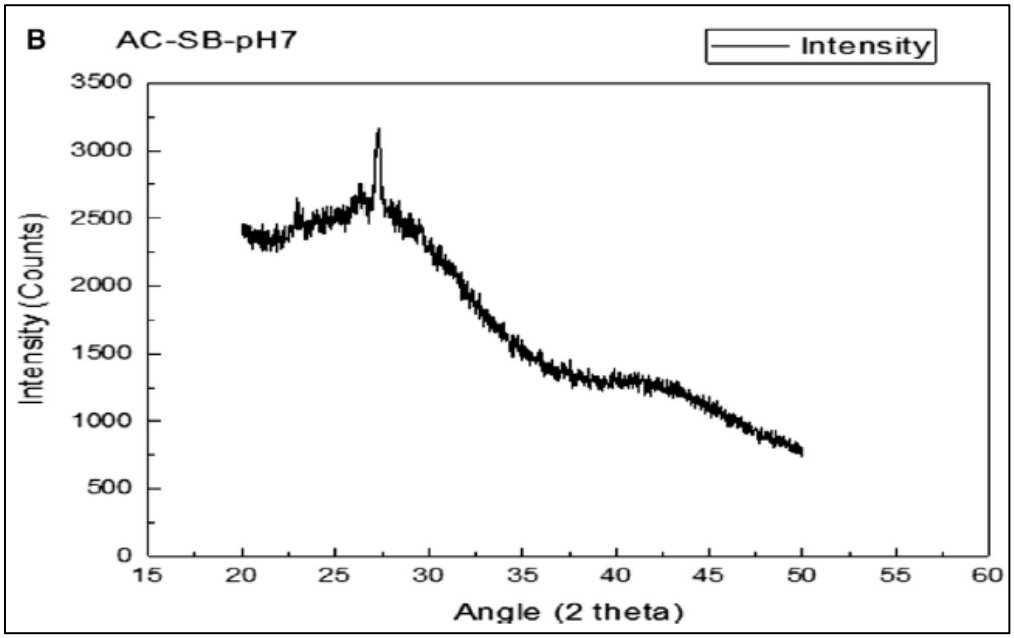


Figure 4.24: (A) Particle Size Analyzer data for average particle size of AC prepared from sugarcane bagasse, particle size was 1593 nm or 1.59 μm. (B) XRD for same AC sample at pH 7, peak intensity recorded at 27.27° at 2920, this provides surface morphology similar to that of graphite.

One way to see the difference in the initial biomass structure and the formed AC structure is by seeing the increased formation of pores on the AC surface, this can be investigated by using SEM. For SEM, the electron beam is made to be incident on the sample at an angle of 45°. The detector detects the electrons which passes through the electrode (porous) and then deflected towards the second electron detector for further detection[99]. The observations were performed at an accelerating voltage of 5.0 kV. The magnification was ranging from 30 to 60,000. The comparison of the structures is shown in **Fig. 4.25**. The biomass surface is shown in and the chemical activation of the pores on carbon surface using the novel chemical activation route.

Role of Binder in AC Cathodes. If MFCs are to be scaled up, they should be able to withstand water pressure. Further prevent the leakage of the cathode liquid into the anode. PVDF is traditionally used to make membranes inexpensively by water emersion process, and using such materials can lower the cost of electrodes to around $\$18/m^2$ hence the same was evaluated to withstand water pressure before having any significant leakage, further the same is compared to PTFE binders. For PVDF binder, depending upon the polymer loading (of around 10 mg/cm^2) went up to a meter of cathode liquid/water pressure before any leakage was detected[99]. For PTFE these values are well below 0.4 m of water pressure. Thus rendering them impossible to be used in scaled up systems[89].

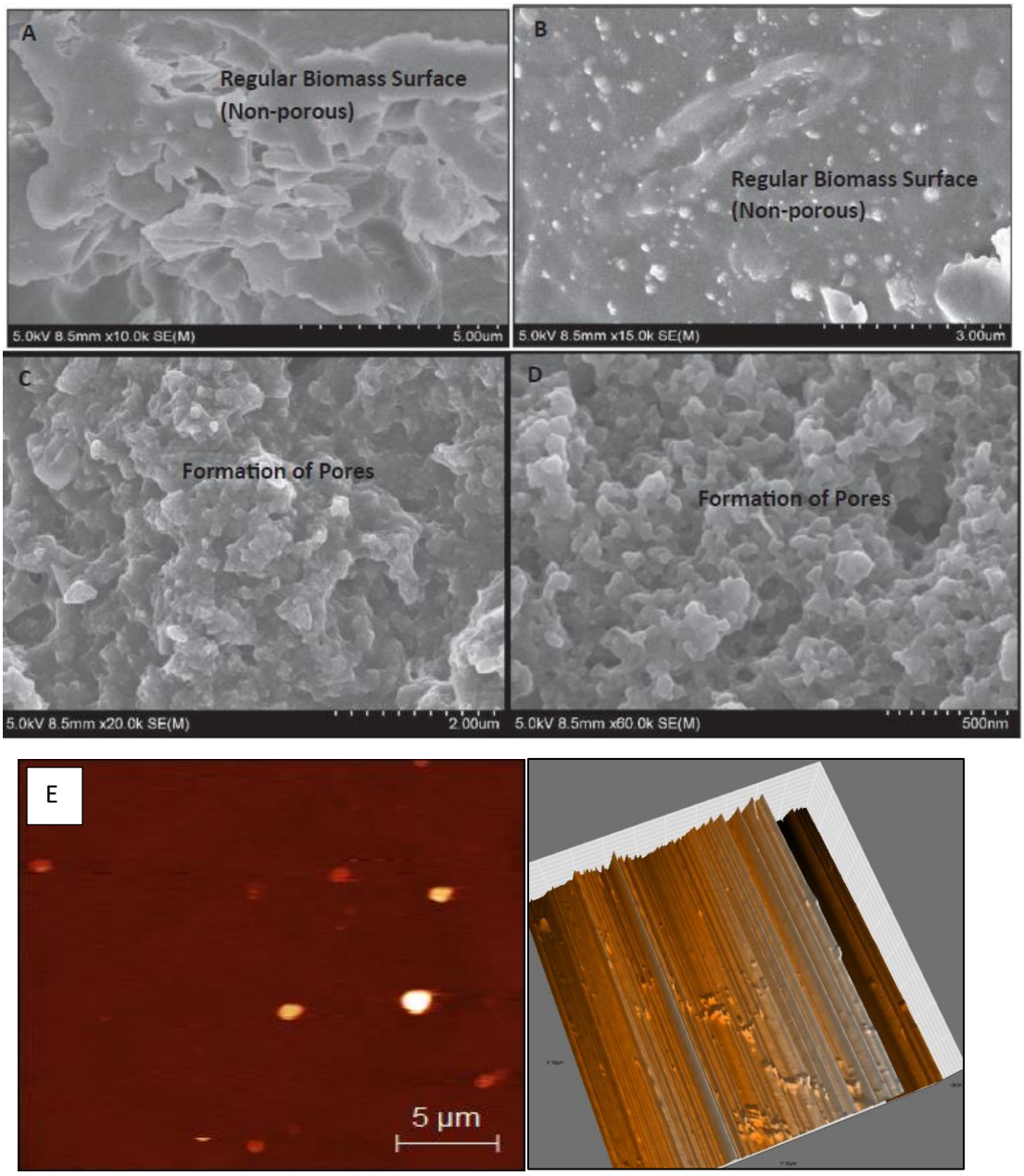


Figure 4.25: Biomass surface at (A) 5 µm scan and (B) 3 µm scan. AC surface with pores at (A) 2 µm imaging and (B) 5 µm imaging, showing the efficiency of chemical activation of carbon. (E) AFM imaging of cathode surface.

4.3.3.2. Functional Group Analysis

For detection of functional groups in the prepared AC, FTIR (PerkinElmer, L12) analysis was done using standard procedures (KBr pellet with AC powder). The vibrational energies were observed at four areas (**Fig. 4.26**), using the NIST (National Institute of Standards and Technology) FTIR library[99], the functional groups were computed, and are enlisted in the figure itself. A wide transmittance band is observed at 1900–3100 cm^{-1}. This band can be attributed to the O–H stretching activity of adsorbed water and hydroxyl groups. The position and asymmetry of the band at lower wave numbers indicate presence of strong hydrogen bonds (from phenols, carboxyl or alcohol).

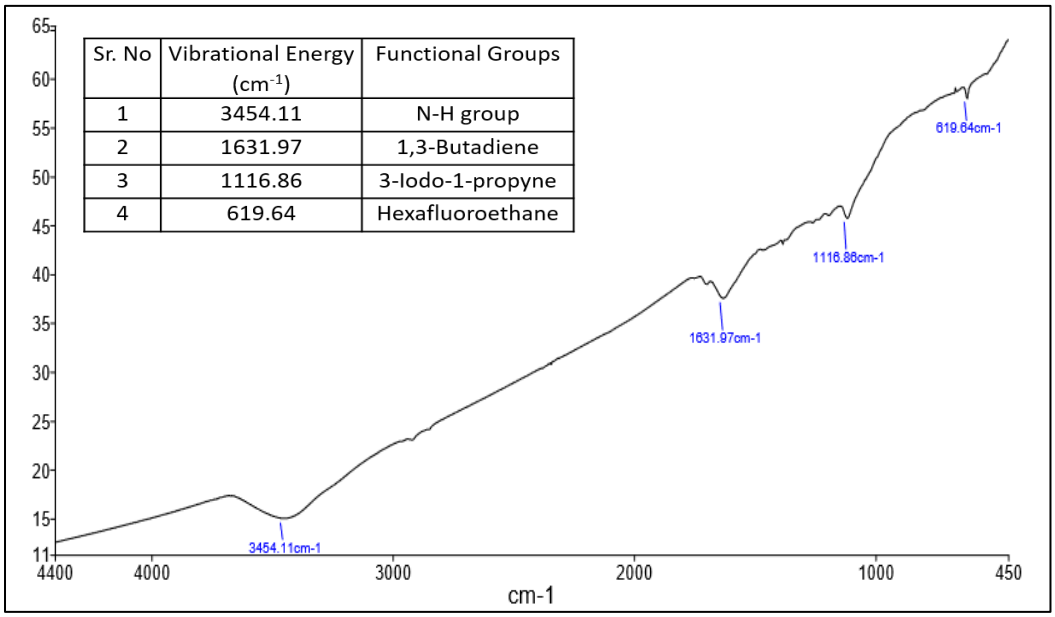


Figure 4.26: FTIR analysis of Activated Carbon, vibrational energy revealed the functional group attached to the prepared Activated Carbon

Further the FTIR analysis also shows the carbon matrix is not just restricted to carbon atoms but other heteroatoms as well, which govern the surface chemistry of activated carbon. This spectra is attributed to chemical activation by phosphoric acid, and the functional groups detected are formed through surface oxidation and attachment of other groups to the surface while developing required porosity. This shows activated carbon derived from biomass sources do not have low pH, and therefore does not cause corrosion at the interface of the carbon and the stainless steel mesh, thereby having minimum effect on the ohmic resistance of the cathode[99]. Further, such variety of functional group present on the carbon surface shows good catalytic activity for oxygen reduction[27].

As discussed previously, the optimized single chambered system performed as well as platinum, this is crucial if scale of MFCs are to be considered, as AC based systems cost one third of platinum based systems[104], further the activated carbon used in this work, the material for brush anode, which has not been reported previously (based on the literature survey) is found to be more effective than other AC based MFCs reported at different capacity[20]. One reason for this is the FTIR spectra of not just the AC powder, but the cathode itself as well (with binder and CB), which is shown in **Fig. 4.27**, which shows the complete absence of carboxylic acid groups (**Table 4.2**), this makes the cathodes more stable when they are coated on SS mesh, many studies have reported that it is desirable to use AC derived from sources that lack carboxylic acid group for improved stability, this work has certainly made significant headways in that area. System performance compared to platinum is presented in the next chapter.

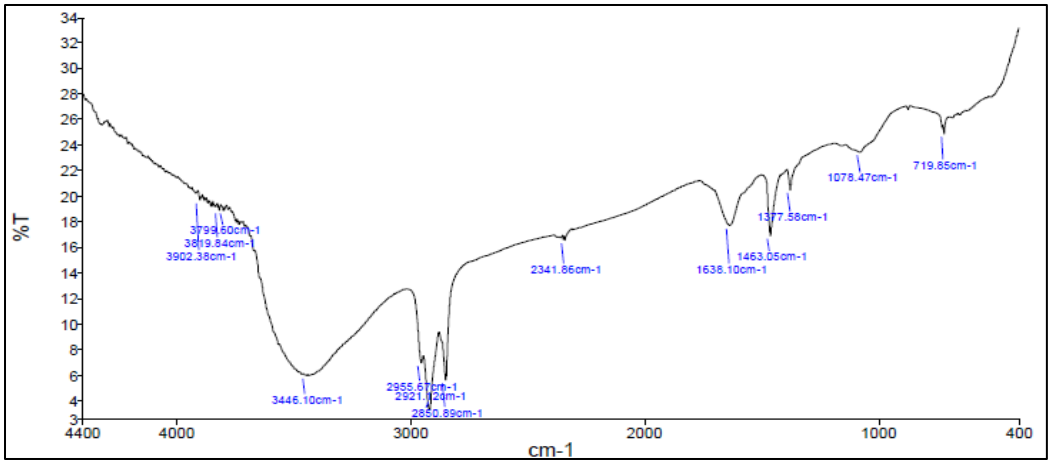


Figure 4.27: FTIR analysis of the AC+CB+PVDF cathode.

Table 4.2: FTIR spectra interpretation (from NIST Library) for functional groups detected in the AC+CB+PVDF cathodes.

Absorption Characteristics (cm^{-1})	**Functional Group**	**Inference Parameters**
3819.84, 3799.60	H_2MgO_2, H_4HfO_4	Highly reactive, supports oxidation reactions
3446.10	BH_4N, NH_3	Ionic reactivity, supports oxygen reduction
2955.67	C_3H_5N, C_2H_3FO	Good absorbent characteristics, pH control
2921.12	C_3H_9Al	Hydrogenation properties, pH control
2850.89	C_3H_7	Superior solvent characteristics
2341.86	C_3HN^+	Ionic reactivity, supports oxygen reduction
1638.10	CH_3N	Ionic reactivity, supports oxygen reduction
1463.05	C_2H_5N	Acts as adhesion product, binding affinity

1377.58	C_2H_3O	Helps maintain ionic flux within solutions
1078.47	C_3H_7N C_2H_5N	High oxygen reduction properties
719.85	N_2O_5 BF_3 C_4H_4	Allows substrates dissolution in water

Some major highlights of this section are summarized below:

a. Properties of Activated Carbon obtained from bagasse lacks carboxylic acid groups, thereby more stability with the current collector (SS 316L mesh).

b. Cathode itself is a catalyst, thereby eliminating the need for an expensive Pt (platinum) layer.

c. Cell voltage remained high, suggesting that the presence of functional groups at the surface improved electron flow to the electrode surface.

d. PVDF has high water retention capacity (around 1.2 m), thereby eliminating the need for diffusion layers (needed for Pt to stop water leaking out), this reduces cost.

e. Eliminates membrane, reduces reactor cost (for cathode chamber), further eliminates cost for aqueous catholyte and decreases internal resistance of the system, as membrane is no longer functional.

4.3.4. Performance Comparison with Platinum Catalyst

4.3.4.1. Cathode Regeneration Efficiency

While power generation from platinum remains to be high, it is interesting to know that after around 14- 15 cycles, platinum fouls, as shown in **Fig 4.28**, an insoluble layer of PtO is formed, and the cathode has to be scrapped. For practical purposes, this is not a solution if such systems were to scale up[107]. AC catalyst cathodes on the other hand after fouling was subjected to a dilute acid wash with 0.01 M HCl at room temperature and the cathodes were able to produce more than 80% of their original power.

This shows the practical feasibility of such cathodes which can be cleaned and reused again, and given the fact that these systems can generate around 0.46 W/m^2 (as compared to 0.82 W/m^2 from platinum), this shows AC cathodes as a promising approach, since the differences in COD removal (around 67% for AC cathodes, 75% for Pt), power densities are not significant, and cost analysis (as discussed in Section 4.7), shows air cathode MFCs cost one-third of Pt-based systems[1], makes them an exciting prospect for next generation of MFCs.

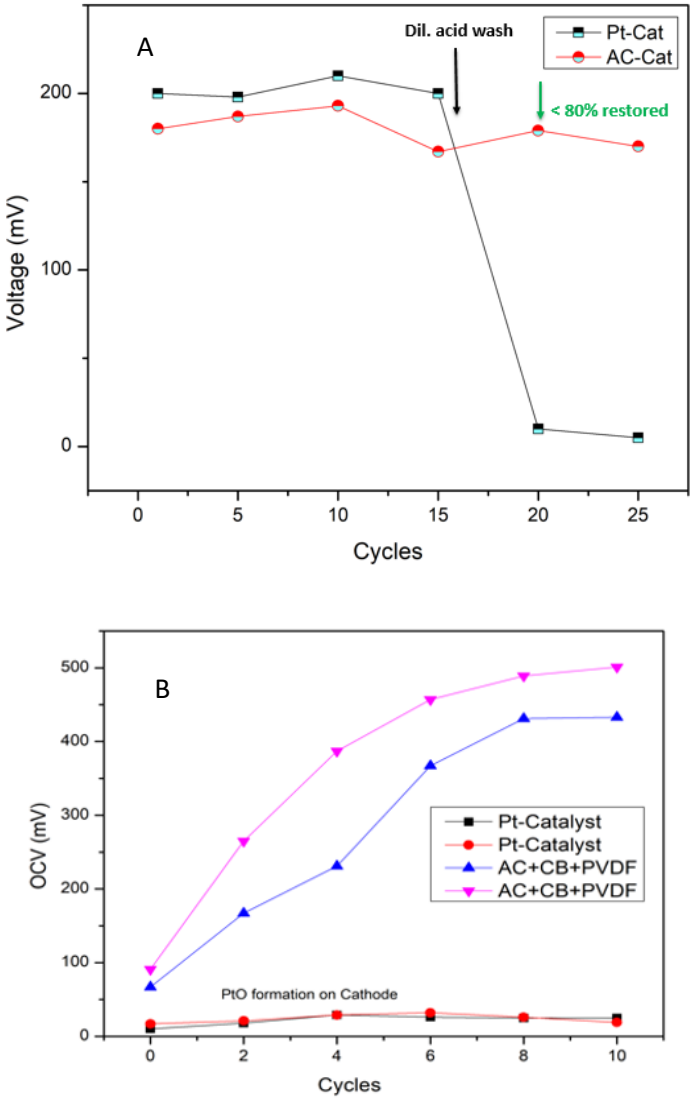


Figure 4.28: Performance of AC cathodes and Platinum catalyst based Cathode post fouling, each cycle would be on average of around two weeks, (A) after dilute acid wash AC showed more than 80% of its initial performance, and for (B) platinum there is hardly any activity left after PtO layer formation and cannot be regenerated.

To summarize, some major findings from the AC-Cat MFC evaluation include:

a. Optimized single chambered system performed as well as double-chambered Nafion based system (in terms of setting potentials in open circuit). Power generation up to 462 mW/m^2 or 0.46 W/m^2 with COD removal of 67% and BOD removal of 62%.
b. The combination of activated carbon, with carbon black and PVDF binder on SS 316L mesh is a good alternative to Platinum catalyst with diffusion layers/ binders. AC cathodes easily regenerated with dilute (HCL) acid wash, unlike Pt, which cannot be regenerated.
c. The brush-based anode are a good choice in MFCs, as they have the ease of construction and operation, and systems have low internal resistance as compared to the traditional MFC architecture.
d. Further, connecting such anode and cathode will provide new framework for investigating reactor performance at each level of scaling up.

4.4. Exoelectrogenic Activity of Microbial Colonies

The bacteria colonies that can grow and sustain itself on the anode is primarily based on the microbial ability to transfer electrons (directly from its body or through nanowires) and through interactions with other bacteria. While characterizing these developed biofilms over periods of MFC operation; it has shown *Geobacter* and among other studies *Shewanella* as the dominant community member[11], however there are several analysis that have shown a more diverse and phenotypically uncharacterized communities as well, this means diversity in the microbial communities has a positive influence on the development of biofilms, which over time are enriched through exoelectrogenic activity (i.e. the ability to donate electron from cell surface to an electrode) of the bacteria. In this section, seven colonies of

bacteria (six specie and one sub-specie) were identified from the wastewater used, further out of them, three new species has been discovered in this work i.e. not been reported previously, which can either generate bioelectricity or contribute to symbiosis for developing electroactive biofilms.

4.4.1. Microbial Community characterization

Exoelectrogenic activity means the ability to release electrons from cell surface of the bacteria, this remarkable ability makes them a uniquely potent tool for bioremediation purposes. Over the years, analysis of microbial communities developed in MFCs do not reveal any specific trend for the biofilm growth, these are usually *Alpha-, Beta-* or *Gammaproteobacteria* based communities. *Proteobacteria* are mostly gram-negative bacteria that can induce nitrogen fixation, and has the capability to grow under very low level of nutrients [108]. This means for MFCs to achieve wastewater treatment through bacteria metabolism, low values of COD (≤100 mg/l) is possible while generating power. *Alphaproteobacteria* have been found consistent with MFCs with marine sediments, *Betaproteobacteria* clones have been found consistent with wastewater and anaerobic sludge derived from starch processing plants, and studies that have taken into consideration, activated sludge in the MFCs, have found *Gammaproteobacteria* clones dominant among other diverse anode communities. Wastewater from breweries through 16S rRNA gene sequencing have found a balance between *Betaproteobacteria* (primarily *Azoarcus*, *Dechloromonus*, and *Desulfuromonas*) and *Deltaproteobacteria,* among others the dominance of *Geobacter* and *Shewanella* was not present [10]. Several studies have confirmed the presence of hydrogen producing bacteria, such as *Alcaligenes faecalis* and *Enerococcus gallinarium* with wastewater streams rich in glucose [18], [28], [78].

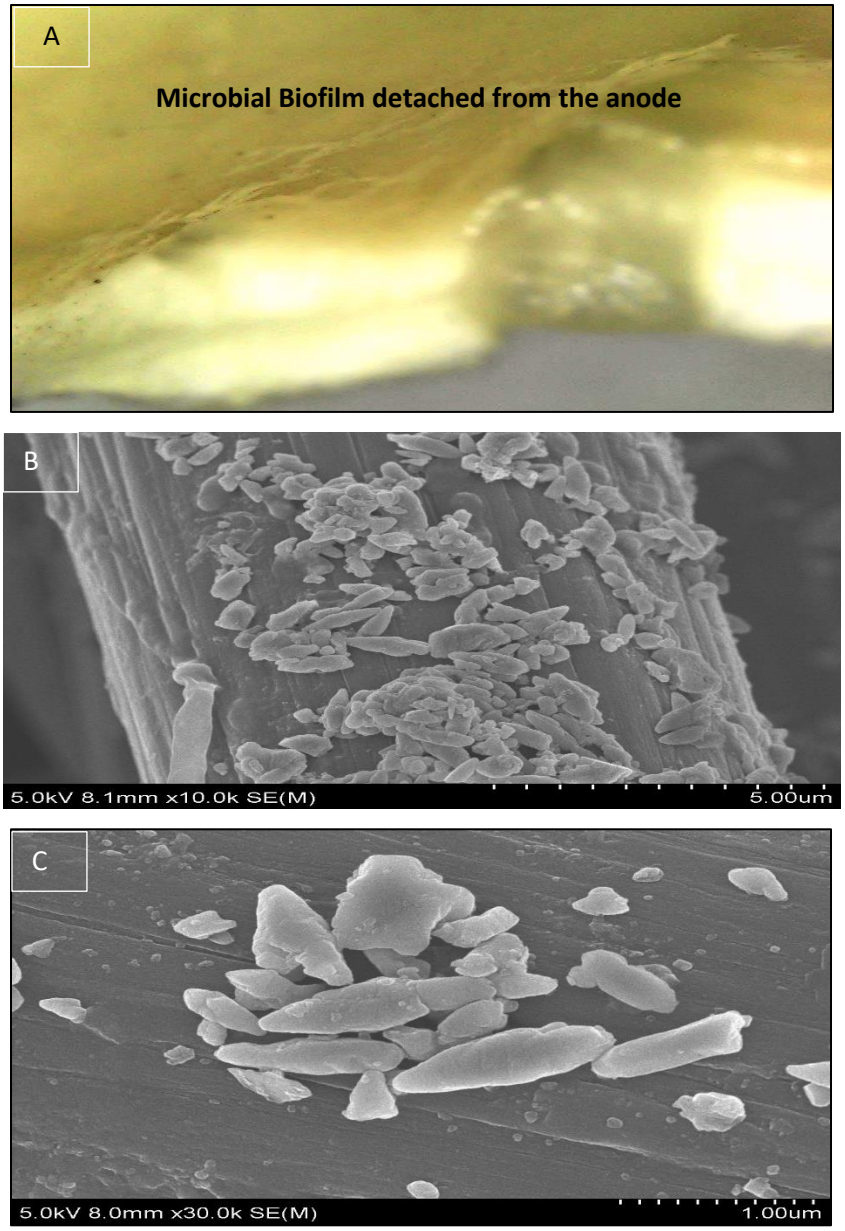


Figure 4.29: (A) Close view of biofilm removed from anode, SEM imaging for (B) anode single cloth strand showing bacteria growth (C) showing symbiotic relationship for varying microbial communities.

Further, diverse communities (that exist in biofilms, like this one as shown in **Fig. 4.29**) for some cases has shown decrease in internal resistance over time, suggesting microbial dominance and increased power generation by reducing anode potential. The electron transfer mechanisms for bacteria that we know exists is through surface via two mechanisms: development of nanowires (like Shewanella and Geobacter species) and shuttling of electrons through self-produced mediators (such as phycocyanin produced by Pseudomonas aeruginosa).

4.4.2. Genetic Code Evaluation

After the cultures were successfully grown in isolation (as shown in **Fig. 4.30**), and the 16S rDNA PCR technique was utilized to extract the genetic code of the microbes[93], which will act as an identification marker for maximum likelihood.

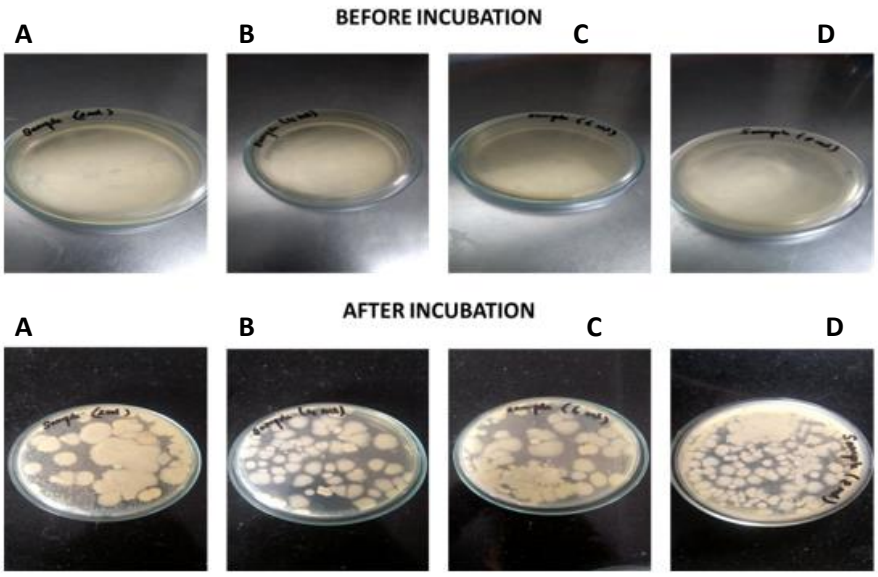
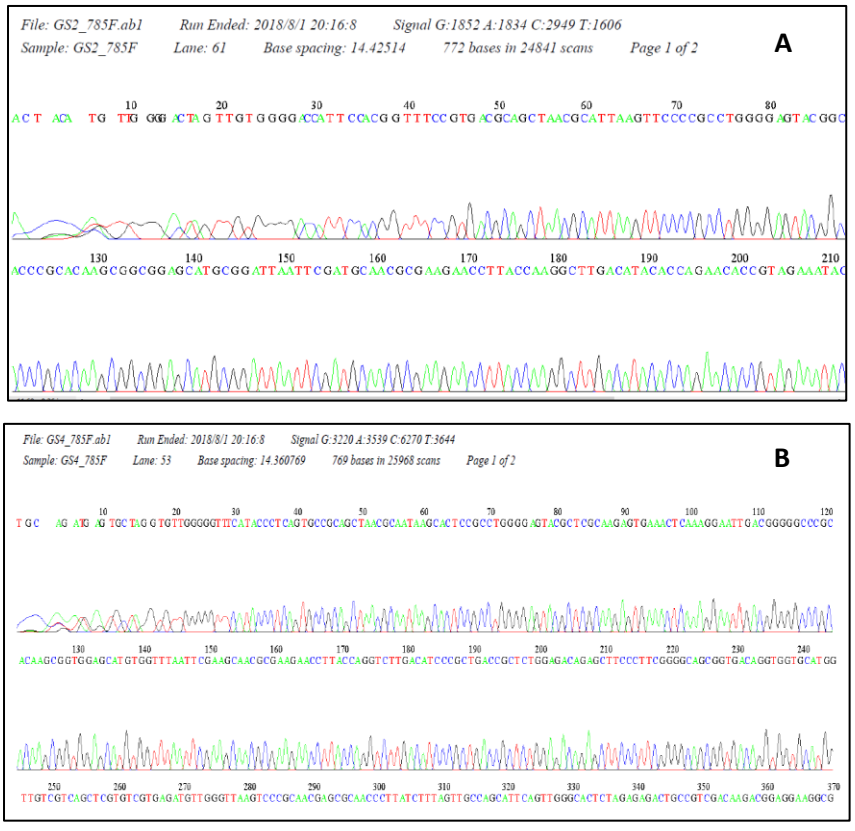


Figure 4.30: Post incubation colony growth of different bacteria can be seen in the petri dish.

Following PCR, the genetic code for each colony is generated from a digital data log system as shown in **Fig. 4.31**. The GenBank database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) was searched using the BLASTn algorithm to analyze the 16S rDNA portion of the sequences. MEGA (Molecular Evolutionary Genetics Analysis) was used to align these sequences and generate a tree using the neighbor-joining method[98]. Sequences derived from the analysis were deposited in GenBank under accession numbers MK281493, MK281514, MK281584, MK281590 MK281610, MK281612, and MK281615. Species detected and corresponding submissions (can be viewed at NCBI) are given in **Table 4.3**.



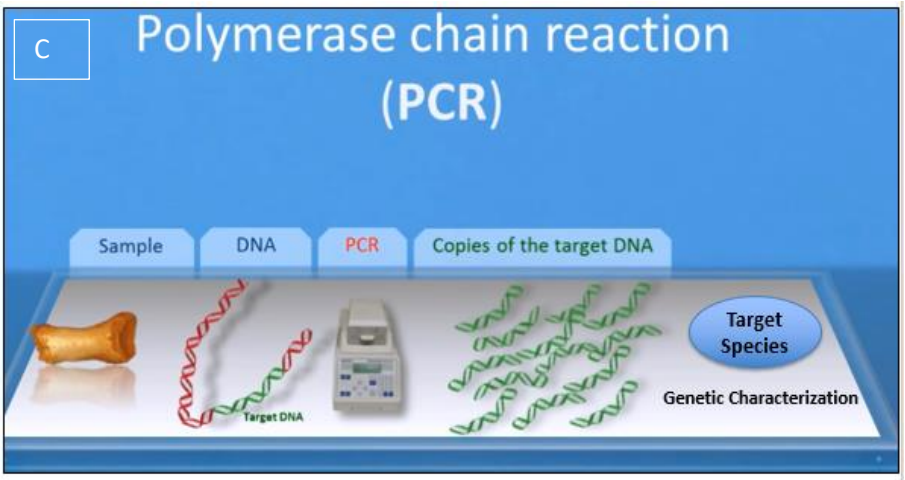


Figure 4.31: Genetic code (A) of one specie (B) another specie of bacteria (C) PCR concept for identifying microbes.

Table 4.3: Species detected from community analysis, and validated as publications in NCBI (validation link also mentioned), these serve as scientific references for microbes capable of electron transfer.

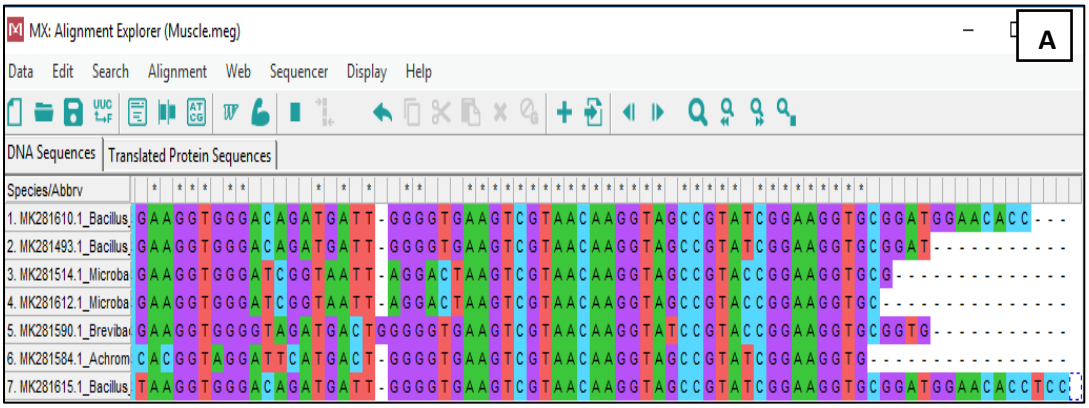
Bacteria Specie Detected (Exoelectrogens)	**GenBank Link: NCBI Database**	**Reporting Authors in NCBI**
Bacillus toyonensis	https://www.ncbi.nlm.nih.gov/nuccore/MK281615	D. Bose, M. Gopinath, P. Vijay, S. Sridharan
Microbacterium proteolyticum	https://www.ncbi.nlm.nih.gov/nuccore/MK281612	D. Bose, M. Gopinath, P. Vijay, S. Sridharan

Bacillus velezensis	https://www.ncbi.nlm.nih.gov/nuccore/MK281610	D. Bose, M. Gopinath, P. Vijay, S. Sridharan
Brevibacillus agri	https://www.ncbi.nlm.nih.gov/nuccore/MK281590	D. Bose, M. Gopinath, P. Vijay, S. Sridharan
Achromobacter xylosoxidans	https://www.ncbi.nlm.nih.gov/nuccore/MK281584	D. Bose, M. Gopinath, P. Vijay, S. Sridharan
Bacillus subtilis	https://www.ncbi.nlm.nih.gov/nuccore/MK281493	D. Bose, M. Gopinath, P. Vijay, S. Sridharan
Microbacterium proteolyticum (sub-specie)	https://www.ncbi.nlm.nih.gov/nuccore/MK281514	D. Bose, M. Gopinath, P. Vijay, S. Sridharan

4.4.3. Bootstrapping Evaluation

Before bootstrapping is done, the ancestral relation between any specie needs to be established, this is done using Phylogenetic trees, these represent evolutionary distances between different types of life forms, and in this case, we consider the microbial communities that were detected through PCR and aligned with BLAST-n using the MEGA (Molecular Evolutionary Genetics Analysis) freeware, the DNA sequences were aligned using MUSCLE alignment[98]. The same is seen in the figure below, where all the four bases, A (Adenine), T (Thymine), C (Cytosine) and G (Guanine) are aligned to construct the evolutionary tree for the exoelectrogens. All these exoelectrogens have a common ancestor, and in our case the same was found to be *Achromobacter xylosoxidans*.

Bootstrapping is a method adopted in phylogenetic trees to show the level of statistical confidence that is present in the shape of the tree, and in particular the positioning of the nodes, that contribute to the topology of the tree[97]. As shown in **Fig. 4.32**, the number above the nodes indicate bootstrap value. It represents the phylogenetic confidence of the tree topology. Normally bootstrap value above 70 (0.7) is considered a plausible score for branch validation. In such constructions, 1 represents a very high level of confidence (or statistical support) on the node or topology of the tree. Further based on the literature reviewed[90], we have found three new bacteria species that have not been reported until now, for their ability to degrade chemical contamination and generate bioelectricity, a summary of these findings is reported in **Table 4.4.** Now the power generation in both double chambered and single chambered system did not undergo any stability issue, this means these bacteria do not go for colony competition in the biofilm, and in turn has a symbiosis prospect for colonization.



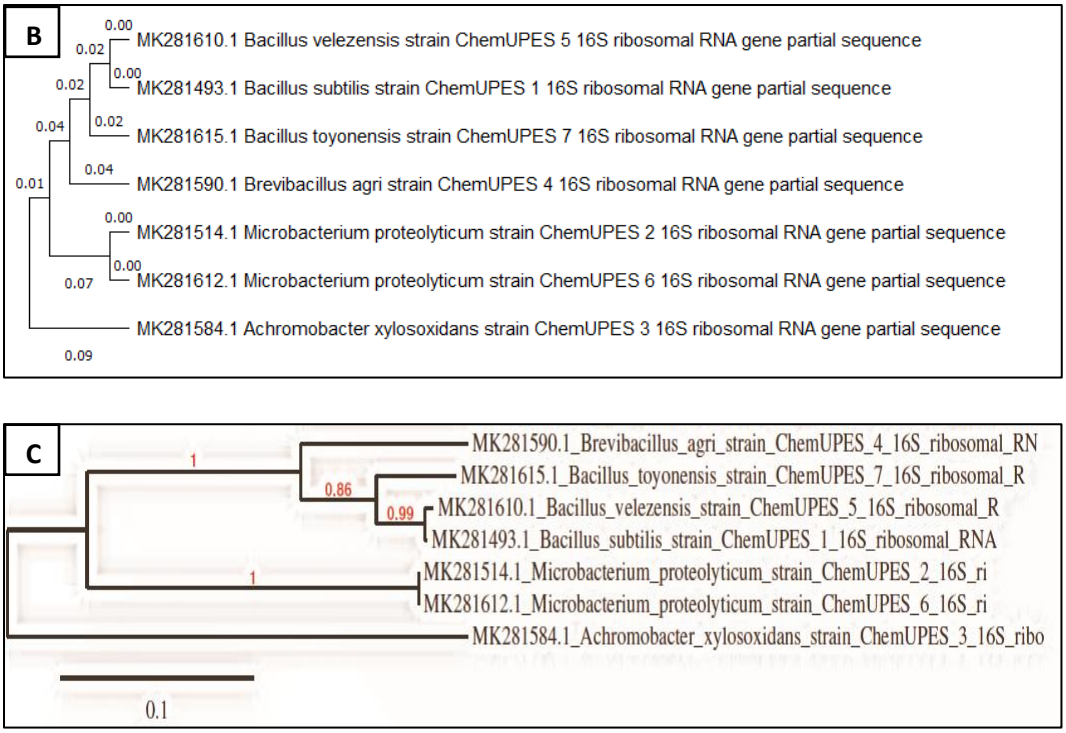


Figure 4.32: (A) Alignment of Genetic codes using MEGA (Molecular Evolutionary Genetics Analysis) for phylogenetic tree construction (B) Phylogenetic tree construction for exoelectrogens from sewage based wastewater, all species are derived from *Achromobacter xylosoxidans*, the numbers on the branches represent genetic distance. (C) Branch support values for phylogenetic tree, where 0.1 represent nucleotides per site in the alignment, this gives a measure of the scale of the genetic distance between each of the bacteria groups.

Table 4.4: Community characterization and exoelectrogenic activity of the microbes

Classification of the Bacteria	Relevance to Literature in Electrogenic Biofilms	Properties	References
Achromobacter xylosoxidans Class: *Betaproteobacteria* Phylum: *Proteobacteria*	Reported at different capacities across the spectrum for its bioelectricity generation capabilities.	Can decompose high concentration of Chromium and organic acids in wastewater.	Nor et al. [54], Zhu et al. [109]
Microbacterium proteolyticum Class: *Actinobacteria* Phylum: *Actinobacteria*	Not reported previously. Specie was discovered recently, in 2015.	Used in decomposition of cellulose and chitin. Helps in maintaining the carbon cycle.	Ren et al. [44]
Brevibacillus agri Class: *Bacilli* Phylum: *Firmicutes*	Reported at different capacities across the spectrum for its bioelectricity generation capabilities.	Prefers forming colonies with other cultures, less productive in its own colony. Can decompose toxic metals like Hg, Pb and Cd.	Kelly et al. [110]

Bacillus toyonensis Class: *Bacilli* Phylum: *Firmicutes*	Not reported previously. Discovered in 2013.	Has potential in valuable metal recovery from waste. Can break down complex organic matter.	Mohan et al. [88]
Bacillus subtilis Class: *Bacilli* Phylum: *Firmicutes*	Reported at different capacities across the spectrum for its bioelectricity generation capabilities.	Useful for Arsenic reduction in wastewater, breaks down other organics as well.	Nimje et al. [51]
Bacillus valezensis Class: *Bacilli* Phylum: *Firmicutes*	Not reported previously. Discovered in 2005.	Has a potential to be used as anti-fungal and anti-cancer extracellular lipopeptide.	Logan [28]

It is interesting to discover the presence of *Brevibacillus agri* in the wastewater, as these species or its derivatives (*other Firmicutes*) have been reported to reduce internal resistance in MFCs with wastewater, therefore loss of energy to background processes is significantly reduced, suggesting the dominance of *Firmicutes* leads to increased power generation due to a reduction in the anode overpotential [28]. Further, as seen from the phylogenetic tree, all species are derivative from *Achromobacter*, a type of *Proteobacteria*, This is consistent with several studies, where conditions for such kind of communities exists, i.e. the complete anaerobic conditions of MFCs, and the operation in batch mode[10], [67], [75]. All species detected are thermophilic in nature and thrive at room temperature

conditions. For much of the MFC biofilms which were analyzed for microbial colonies, the most common specie would usually be *Geobacter* and *Shewanella* [52]. The notable lack of *Geobacter* and *Shewanella* species in the wastewater derived from sewage, shows how diverse the microbial community is, further the effect of MFC architecture, substrate and inoculum, are needed to better understand the conditions that facilitate the microbial ecosystem to grow in these environment.

Analysis of biofilms also confirmed at different capacities the presence of *Alpha-*, *Beta-* and *Gammaproteobacteria* in other studies [28]. In one study with ethanol based wastewater, 16S rRNA gene sequencing have shown community domination by *Betaproteobacteria*, primarily *Azoarcus, Dechloromonas,* and *Desulfuromonas*; with rest of the community belonging to *Deltaproteobacteria* [66]. The range of substrates used in various studies, along with different internal resistance and Coulombic efficiencies, makes these systems intricate to analyze the factors responsible for community development inside MFCs. Further, it seems evident at this point that exo-cellular electron transfer might be common to many other species of bacteria, which have not been studied yet, this shows previous claim over Shewanella [11] being a superior competitor over MFC biofilms is no longer valid. Power generation remains the primary goal of MFC research, it also allows investigators a curiosity driven research platform for examining the microbial ecology of exoelectrogenic bacteria, as in degrading insoluble metals and other features of resources change over time and the water chemistry itself, as it becomes relativly oxidized or reduced in concentration ratio of the metal change over time. In an MFC, the electrode (i.e. the anode) is non-corrosive and allows a biofilm to develop and mature in a manner that allows researchers to analyze them using microscopic techniques [99], and in this case, 16S rDNA PCR was used to

understand the colonies that were formed from wastewater derived from sewage. Further, the biofilm that was formed was found to be stable and uniformly distributed across the surface. An interesting observation about the stability of these microbes is that the anode does not appear to found over time, which means the surface remains viable and the bacteria are able to use it continually. To summarize following were the highlights from this section:

a. All these exoelectrogens have a common origin, and in our case the same was found to be *Achromobacter xylosoxidans*, among which three new exoelectrogens were discovered in MFC biofilm community, which have not been reported previously. This opens up the possibility to isolate these species and conduct bioremediation experiments to evaluate their individual colony capacity to generate bioelectricity.
b. An interesting aspect of biofilm formation in the MFC on the anode is that it can be used continually over time. This means bacteria on the surface remains viable and able to continuously use the surface for electron transfer.
c. Hence, it is justifiable to speculate that there are mechanisms through which bacteria can colonize on surfaces without any ecological damage, leading to MFC technology being a non-combustion based energy recovery process that can be an integral part of new frontiers of energy exploration, wherein a wastewater treatment plant can be converted to a power plant.
d. The microbial ecosystem that forms inside MFCs which can shuttle electrons are a promising prospect. A future direction for this is to utilize the PCR results to study these microorganisms, and to be able to grow and study them in isolation and in mixed complex substrates.

4.5. Microbial Kinetics

When there is contamination available in the wastewater, the microbes can produce new cells, energy and reaction byproducts. In our case this was done in a batch system, so for each sample of water (250 ml) charged in the system, there is limited supply of organic load for the microorganisms. And as the substrate is exhausted, the rate of microbial growth starts decreasing. In the context of this, there are two models that are discussed here, in the first model, biofilm kinetics is explained[28], and in the second model, how electroactive biofilms in MFC respond to chemical flux in wastewater is elaborated.

4.5.1. Biofilm based Kinetic Model

As the microbial community grows and populate the biofilm (**Fig. 4.33**), the rate of substrate consumption can eventually exceed mass transfer (by diffusion) to the anode surface. Here, an assumption is made that influx of substrates into biofilm limits power generation, which means the biofilm is capable of sending the generated electrons (i.e. current) directly to the anode, such situations can arise when the current density is significantly high, to model the biofilm[28], it is assumed that these surfaces act as a catalyst layer, the flux to the biofilm can be written as:

$$J_b = k_w (c - c_{b0}) \quad (9)$$

Where J_b is the flux of the substrate in to the biofilm, k_w is the mass transfer coefficient, c is the bulk concentration of substrate at the vicinity of the anode, and c_{b0} is the substrate concentration at surface of the biofilm. The maximum rate of mass transfer is achieved when $c_{b0} = 0$, but this situation is not possible practically, as there is always some finite concentration of the substrate inside the biofilm. Assuming there is no significant mass transfer limitations, the upper limit on the

chemical flux can be computed, which happens for a biofilm that is not restricted by external mass transfer limitations[19]. If first order kinetics is considered[28], then the maximum substrate flux to a biofilm can be written as:

$$J_b = k_1 \delta_b c \frac{\tanh (B_1)}{B_1} \quad (10)$$

Here δ_b is the thickness of the biofilm, k_1 represents reaction rate constant for first order biofilm kinetics and B_1 is a dimensionless constant, which is expressed as:

$$B_1 = \left(\frac{k_1 \delta_b^2}{D_{cb}} \right)^{1/2} \quad (11)$$

Where D_{cb} is the diffusion coefficient of the substrate inside the biofilm, now if we consider the rate of reaction to be faster than the rate of diffusion[104], in that scenario B_1 becomes greater than $\tanh (B_1) \rightarrow 1$, under this condition, the above equation can be simplified and written as:

$$J_{b,1} = (k_1 D)^{1/2} c \quad (12)$$

Where $J_{b,1}$ represents the flux to the biofilm, and D is the diffusion co-efficient of the substrate to the biofilm. Depending on the substrate flux (**Fig. 4.34**), there is another possible scenario, where substrate flux completely penetrates the biofilm, and reaches the electrode surface, the biofilm flux in this case can be written as:

$$J_{b,0} = k_0 \delta_b \quad (13)$$

Here k_0 represents reaction rate constant for zero order biofilm kinetics; this solution is valid if the substrate is not exhausted before it reaches the biofilm bottom[81]. This is possible when $B_0 > 1$, where B_0 is given as:

$$B_0 = \left(\frac{2 D_{Cb} c}{k_0 \delta_b^2} \right)^{1/2} \quad (14)$$

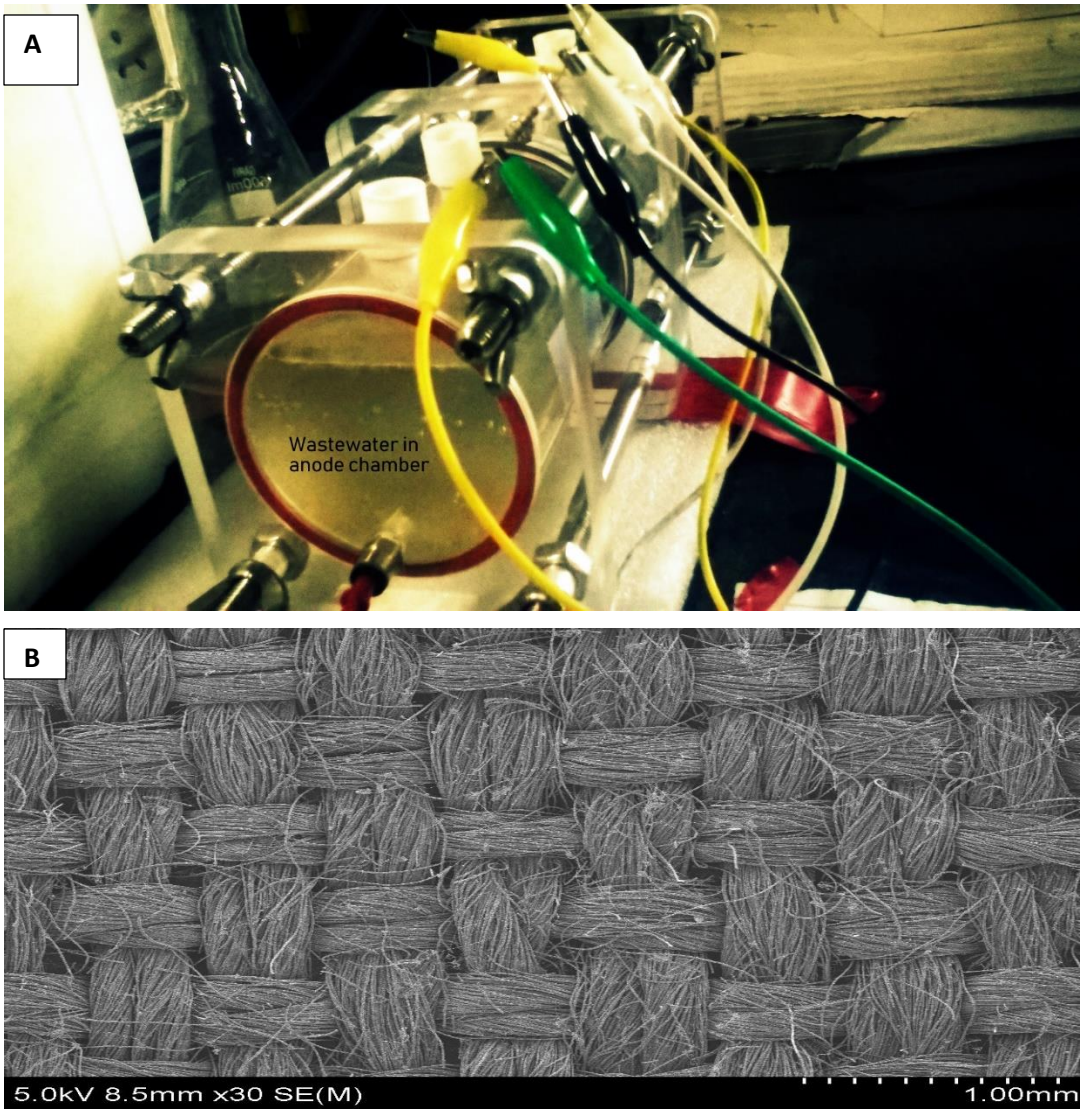


Figure 4.33: (A) Side view of the anode chamber with wastewater in Pt-Cat MFC **(B)** SEM imaging of the anode from the Pt-Cat MFC with visible microbial appendages for colony formation and electron transfer.

For the condition where the substrate is entirely used up, before it reaches the biofilm support surface on the electrode, the flux of the substrate can be written as:

$$J_{b,z} = (2k_0 D_{Cb} c)^{1/2} \quad (15)$$

While substrate flux may vary depending on the wastewater, it seems unlikely that it would reach the electrode. Further, for bacteria it is likely to conduct electron from cell surface using nanowires or self-produced mediators, and based on the biofilm thickness maximum current and power can be computed as:

$$I_{max} = k_0 \delta_b b_{es} F C_E \quad (16)$$

$$P_{max} = k_0 \delta_b b_{es} F C_E E \quad (17)$$

Here b_{es} is the total moles of electrons defined for the substrate, F is the Faraday Constant (96500 C/mole), E is the Open circuit voltage and C_E is the Coulombic efficiency which represents the total Coulombs captured in electrical current generation relative to the maximum possible assuming complete oxidation of the substrate[65]. Based on these formulations, considering the model for the MFC systems used in this work, where an recorded Open Circuit Voltage of 1 V is considered for the Pt-Cat system and 0.8 V is considered for the AC-Cat system, with uniform biofilm thickness of around 0.029 cm, having typical diffusion coefficient of 0.88×10^{-5} cm^2/sec, the maximum power density can be computed around **66 W/m^2** for the Pt-Cat system and around **50 W/m^2** for the AC-Cat system, assuming zero order kinetics region.

These evaluations show that significantly high power densities are possible before mass transfer limitations begin to affect the biofilm and limit the overall power generation[102]. It has to be mentioned that these evaluations neglect the effect of external mass transfer coefficients, but it does suggest factors other than flux of the substrate in terms of mass transport to the biofilm should be given equal importance

if power generation for any system architecture has to be improved for MFCs, which is in accordance with polarization at different external resistance[48], and infers that transport of contamination to biofilm surface is not a crucial factor that limits power generation.

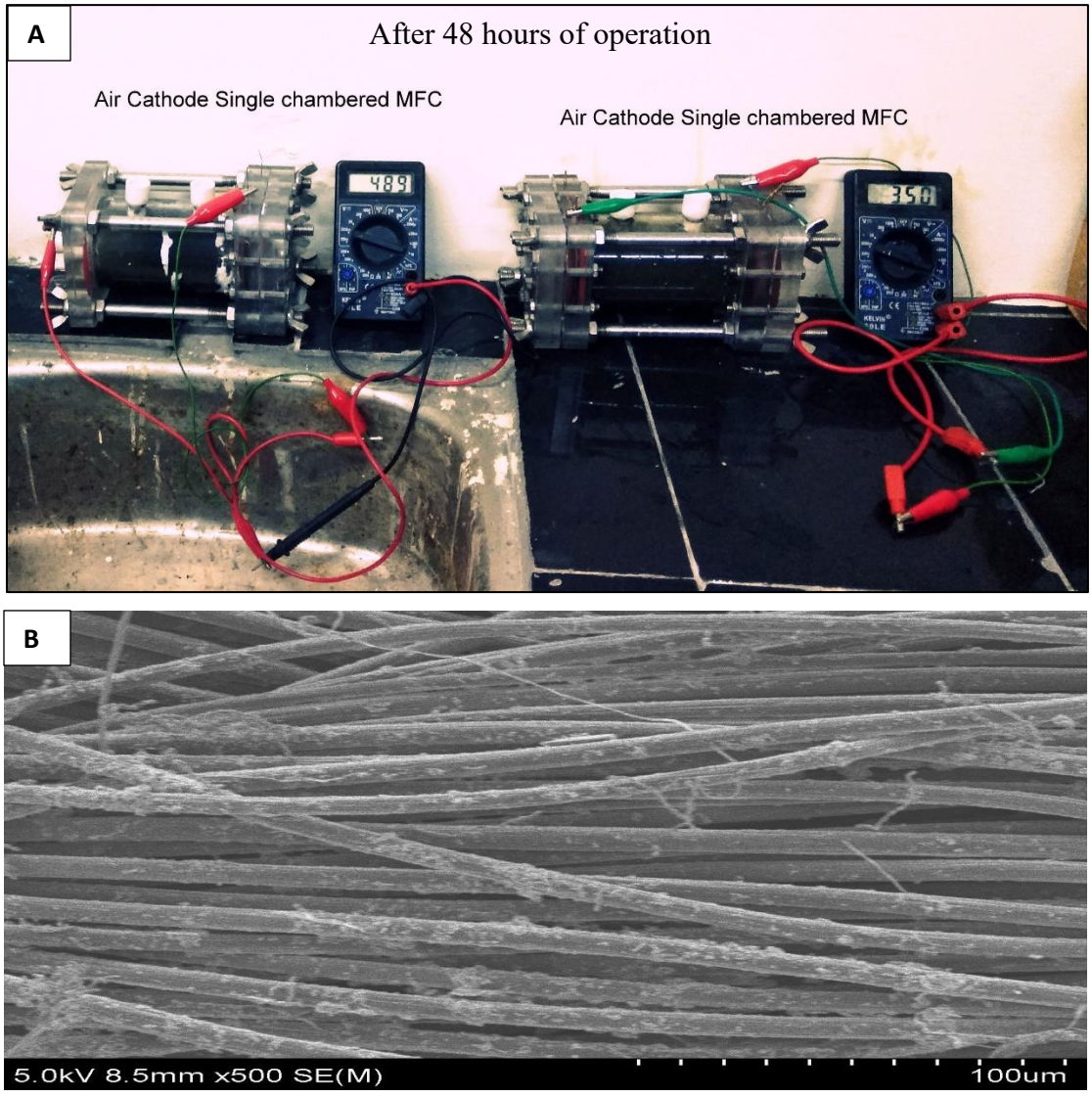


Figure 4.34: (A) Two AC-Cat Systems in operation **(B)** SEM imaging of the anode with visible microbial appendages.

4.5.2. Diffusion Flux into Anodic Biofilm

A critical thing to remember here is the energy recovery from the wastewater is essential, through microbial interactions (**Fig. 4.35**). Moreover, this can be accelerated with external resistance and by increasing the organic loading. The drop in COD level (from >600 mg/l) is attributed to the chemical flux (J) in the biofilm, as the anode and the cathode are at different potentials, this accelerates the flux of organics to the anode; mathematical simplification for the Chemical flux using Nernst-Planck equation shows a direct influence on diffusion coefficient in these electrical fields[28], so when the microbes run out of food (the mg-COD/l of the organics) the electrical field starts to collapse, as the electrical field starts to collapse, a flux is experienced by the biofilm due to shortened supply of organics and everything comes to a halt.

The chemical flux into the biofilm (J) is consistent with first order kinetics, where k_1 represents first order rate constant as a function of microbial kinetics. The flux of ions under the influence of ionic concentration gradient($\nabla . c$) and an electric field (E) is given by the Nernst-Planck equation[34] as:

$$\frac{\partial c}{\partial t} = -\nabla . J \qquad (18)$$

Where $\frac{\partial c}{\partial t}$ is the change in concentration of substrate over time, and J is the chemical flux into the biofilm[18], which can be further written as:

$$J = -[\, D\nabla c + \frac{Dze}{k_B T} c \left(\nabla \emptyset + \frac{\partial A}{\partial T}\right)] \qquad (19)$$

Where *D* is the diffusivity of the chemical specie, *c* is the change in concentration, *z* is the valence of ionic specie, *e* is the element charge, k_B is the Boltzmann constant, $\emptyset$ is the electric potential, and *A* is the magnetic vector potential.

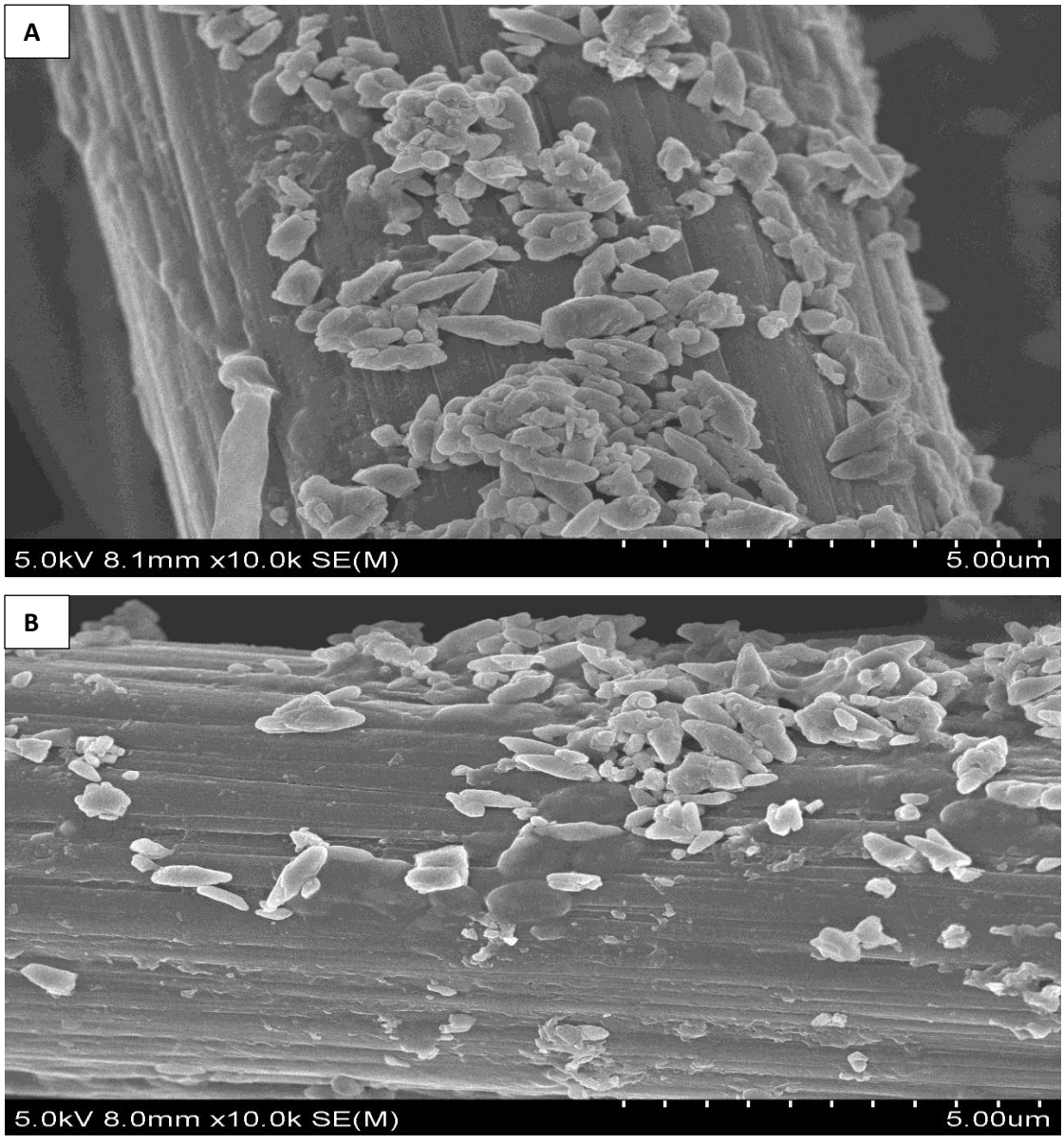


Figure 4.35: SEM imaging of the anode **(A)** front view **(B)** side view, with different microbial colonies that form within the biofilm.

Now substituting the value for chemical flux (J) into equation (iii) to simplify the Nernst-Planck equation for the diffusion coefficient (D) as a function of the electric field[39] (given by $\overline{v}\varphi$) the same can be written as:

$$J = [uc_i - Dc_i] - \frac{ziDF\nabla\emptyset}{RTc\downarrow i} \quad (20)$$

Setting time derivative to zero, and the fluid velocity to zero (with only ion specie movement):

$$J = [0 - Dc_i] - \frac{ziDF\Delta E}{RT\Delta x\ c\downarrow i} \quad (21)$$

The above can be further simplified and written as:

$$J = -\left(\frac{1+F\Delta E}{RT}\right) D \downarrow c\ 10 - \frac{\Delta c\downarrow i}{\Delta x} \quad (22)$$

Equation 22 shows the chemical flux into the biofilm *(J)* can have a factor of ten impact on the effect of diffusion coefficient *(D)* in these electrical fields [77], so when the microbes starts running out of the substrate, the electrical field starts to fall, no organic load is regenerated in batch operation to the electrodes and this brings down both the COD and the BOD content of the wastewater, thus decreasing the current density.

4.6. Cost analysis for Bioelectricity Production

As MFC system designs have improved over the years, finding suitable cathodes for oxygen reduction has also improved significantly, from values below 1 W/m^3 (of reactor volume) to around 1 kW/m^3[27]. It is expected that these power densities will improve with our understanding of the flow of nutrients such as nitrogen and phosphorus in the system causing a shift towards electrogenesis and away from methanogenesis. Instead of going for digesters that release methane (biogas), waste material can be directly converted to electricity using MFCs, thereby reducing energy losses to background processes, which happens due to thermodynamic limitations.

4.6.1. Cost analysis of Pt-Cat and AC-Cat MFCs

In this work, two systems were studied, one was the double-chambered MEA system, and then the air cathode MFC, given below is the cost analysis (in **Table 4.5**) based on materials only on meter square basis. As it can be seen significant cost reduction is possible with AC cathodes without compromising too much on the power output, this also shows how MFCs started as very expensive systems that use nafion, platinum as materials[69], and over time we have been able to eliminate these materials to employ non-precious metals to serve the same purpose.

The operation of MFCs can consume much less energy than numerous other treatment technologies. One energy balance analysis assessed that an MFC can hypothetically create a net energy recovery (NER) of around 0.004 kW h kg^{-1}-COD, which breaks even with an additional monetary income of about \$0.0005 kg^{-1}-COD given an average power cost of \$0.12 kW h^{-1}.

Table 4.5: Cost analysis between materials for a double chambered and a single chambered MFC, Cost estimations were all based on commercial price of materials in bulk quantity/ quotes from vendor.

Material Cost ($/m²) for the Two chambered MFC (Pt-Cat)		**Material Cost ($/m²) for the Single Chambered MFC (AC-Cat)**	
Membrane:	$ 1200	Anode (Yarn):	$ 20
Anode:	$ 1000	Air Cathode*:	
Cathode*:		Activated Carbon:	$ 0.4
Pt (with Carbon Cloth)	$ 1100	Carbon Black:	$ 8.0
Binder	$ 100	PVDF:	$ 5.2
…….……		SS 316L Mesh:	$ 1.4
$ 3400 / m²		……	
*Cathode is subject to fouling after 14-15 cycles, regeneration not possible. Insoluble layer of PtO is formed over the cathode.		**$ 35 / m²**	
		*Cathode is subjected to fouling after 13 – 14 cycles; regeneration to more than 86% of initial performance is possible.	

4.6.2. Cathode Cost

In comparison, the present-day treatment cost for an activated sludge-based wastewater treatment plant (WWTP) is about $0.13 /kg-COD, expecting an energy utilization of 0.5 kW h /kg-COD and about 60% of the operating cost is utilized for energy consumption. Furthermore, the value of byproducts from the process needs to be carefully addressed. However, the achievable benefit of such procedures should be viewed with caution in the light of the normally low product yield and

the high expenses for product extraction along with purification. In our work, the current collector is stainless steel mesh of grade 316L stainless steel (much cheaper than expensive chemicals), which has deposits of chromium (15%) and nickel (10%) blended to prevent corrosion.

For most studies till date with AC cathodes SS 304 has been used, in our experience 316L per forms better than these systems. A comparison of the literature with both platinum and AC catalyst is shown in **Table 4.6**, this is in terms of material used, and cost for modules per square meter.In retrospect, the power output of microbial fuel cells is still a long way from meeting total energy recovery for real-time operations: the ecological benefit is not direct, the performance of the processes tends to decrease over a long-haul operation, and the costs of the materials for MFCs are mostly significantly high.

It appears that some of the difficulties, for example the generally high capital costs, may remain, making MFCs intensely troublesome[19]. To meet manageability criteria, more appropriate methodologies, other than progressing MFC innovation, should be sought. Integrating MFCs with different procedures may be a more feasible avenue. Some conceivable methodologies are discussed in the next chapter.

Table 4.6: Cost comparison of both double and single chambered system analyzed in this work, with the literature that was reviewed, in terms of Cathode material.

Method	Catalyst Layer (g/m^2)				Diffusion Layer (g/m^2)	Current Collector ($/m^2)		Press Res. (m)	Power Density (mW/m^2)	Overall Cost ($/m^2)	Ref.
	Pt	AC	CB	Polymer	Polymer	Cloth	SS Mesh	Water			
Coating	3	-	-	60 (Nafion)	-	1000	-	-	820	1200	**This work**
Phase inversion	-	270	27	90 (PVDF)	-	-	1.4 (316L)	<1.2	430	15	**This work**
Coating	-		-	Co-based		400		<0.2	500	560	[59]
Pressing	-	270	-	30 (PTFE)	121(PDMS)	-	12 (304)	<0.18	1340	14	[33]
Phase inversion	-	265	26.5	88 (PVDF)	-	-	12 (304)	<1.26	240	12	[3]
Brushing	5		-	100 (Nafion)	555 (PTFE)	625	-	<1.2	1320	1814	[30]
Phase inversion	-	88	8.8	30 (PVDF)	-	-	12 (304)	<1.22	544	12	[34]
Rolling	-	200	-	30 (PTFE)	-	-	12 (304)	<0.18	1042	200	[21]

Some of the key highlights of this work includes:

a. For most studies till date with AC cathodes SS 304 has been used, in our experience 316L per forms better than these systems.
b. PVDF is traditionally used to make membranes inexpensively by water emersion process, and using such materials can lower the cost of electrodes to around \$14/m^2, with good water retention capacities.
c. The accomplishments in understanding how MFCs can play a key role in the climate change debate is impressive, over the years, cost has been reduced significantly by transition from expensive platinum to non-precious metals without compromising too much on power density. This work confirms the same.
d. Instead of dissolving oxygen in water (for two chambered system), air cathodes which are less expensive to operate has allowed passive oxygen transfer.
e. While cube reactors are expensive, but they allow forming the base of understanding variety of factors on power production in MFCs.
f. Sewage derived wastewater has less contaminants compared to industrial wastewater, given the fact that, in this work the developed system has been able to extract energy significantly higher than many studies, this opens the scope for future research where these reactors can be employed to treat industrial wastewater, with high efficiency for treatment and power generation.

CHAPTER V

CONCLUSIONS

In this work, five objectives were framed, as discussed in the initial sections, with Objective I: **Reduction of organic wastes in wastewater and simultaneous Bioelectricity production using batch process**. Wherein a double chambered Nafion-117 membrane based MFC with platinum catalyst was fabricated, which is consistent with most literature that achieved high power densities[49], this system achieved high power density of around 820 mW/m^2 from wastewater with contamination removal efficiency of around 75%, then based on the evaluations, it was seen that the MFC anode does not foul over time, and bacteria can use the electrode surface continually, however, the system drawbacks included fouling nature of the catalyst on the cathode, which cannot be regenerated and reactor cost. This prompted the search for alternatives to platinum as catalyst, and materials which can address the issue of cost, material performance and regeneration possibilities itself.

The same was addressed in Objective II: **Optimization of MFC System for Bioelectricity production using batch process**. A key area of MFC research has always been understanding the cathode chemistry[85], and there are studies which have shown the use of activated carbon as a suitable cathode alternative[1], in this work, a novel indigenous cathode prepared from biomass through chemical activation route using phosphoric acid, which showed more potential than much of the studies reported. While this process of activated carbon preparation itself has several advantages over the traditional physical method[111], the carbon itself lacked any acid groups, as revealed from the FTIR analysis, which showed presence

of amine groups, and other similar groups with basic properties that allow oxygen reduction at the cathode, thus, making it more stable with the mesh as current carrier. Furthermore, with the optimized cathode, the anode was modified to a carbon yarn based brush, which has higher specific surface area, resulting in greater attachment of the biofilm on the anode surface, this reduced the electrode distance, and allowed the successful optimization of the double chambered system into a single chambered membrane-less sustainable MFC reactor.

In Objective III: **Continuous Bioelectricity production with optimized System,** the system designed in Objective I was used as a control, and one of the two Pt-Cat system was modified into an air cathode single chamber membrane-less MFC (based on the optimization strategies from Objective II). As the initial results gave promising output, a similar air cathode MFC was fabricated, and several evaluations showed a peak power density of around 465 mW/m^2, with COD removal of around 67% and BOD removal of around 62%, with Coulombic efficiency higher than the Pt-Cat system (34% whereas for the Pt-Cat system it was 32%). And critically, AC-Cathodes can be regenerated post fouling to more than 85% of its original capacity, where as Pt-Cathodes have to be discarded[99], this makes the use of AC-Cathodes with suitable mediators, a perspective for future research and the commercial arena itself.

In the next section of this work, i.e. Objective IV: **Identification of potent-microbial species responsible for Bioelectricity production**. The wastewater used from the STP in these MFC reactors were analyzed using 16S rDNA techniques to identify the microbes present in these electrogenic biofilms, and six

species and one sub-species were identified, all these electrogens have a common origin, and in our case the same was found to be *Achromobacter xylosoxidans*. Overall the bacteria were from dominant from the *Firmicutes*, and the rest were *Proteobacteria* and *Actinobacteria*. The genetic code of these species were validated from NCBI and updated to the gene bank repository. The identified species included: *Achromobacter xylosoxidans, Microbacterium proteolyticum* with its sub-specie, *Brevibacillus agri, Bacillus toyonensis, Bacillus subtilis, a*nd *Bacillus velezensis*. Additionally, based on the literature reviewed, *Microbacterium proteolyticum, Bacillus toyonensis,* and Bacillus *velezensis* have not yet been reported as electrogens or in MFC biofilms so far. This is the first work to have contributed to such. Sequences derived from the analysis were deposited in GenBank under accession numbers MK281493, MK281514, MK281584 MK281590 MK281610, MK281612, and MK281615. Furthermore, some studies have reported power instability due to colony competition of the microbes within the biofilm, as in our work, power generation in both systems was stable, this means these bacteria do not go for colony competition in the biofilm, and in turn has a symbiosis prospect for colonization, wherein some are electrogenic and some help in ensuring stable pH inside the biofilm, and the chemical flux within them, further research in isolation with each of these species can yield more detail into their individual performance inside MFCs.

In Objective V: **Cost analysis for Bioelectricity production**, a contrast is presented for the traditional MFC system (based on Pt-Catalyst) which costs over $\$3000/m^2$ to the AC-Catalyst reactors which cost around $\$ 30/m^2$ and the transition which without comprising too much on the power output (i.e. more than 50%) has yield promising results in terms of overall MFC cost for materials, and regeneration

efficiencies of the cathode. This shows the transition from expensive materials to relatively cheaper alternatives which have fueled MFC research in recent years, by eliminating membranes, and using air cathodes, it is expected that aggressive research activities for MFC materials, along with refinement in gene editing techniques like CRISPR[93] which can manipulate and remove the thermodynamic limitations of the microbes, such systems can be part of a future, where energy security is from a nation's microbial reserves. Although, it has to be remembered, microbes are not responsible for low power generation; this is primarily due to thermodynamic limitations of the materials that are employed in MFCs, which are expected to be refined in the next decade or so.

5.1.1. Future Scope and Perspectives

If the operation costs for treatment of sludge at sewage treatment facilities are considered, it is observed that more than 50% of total investments are involved in this part of the treatment process [22]. Consequently, the total costs for wastewater treatment can be significantly reduced by integrating MFCs with existing systems, as the sludge often contains high levels of organics as collected from wastewater treatment plants.

Traditional Wastewater Treatment Process. A typical wastewater treatment plant treating sewage/ domestic wastewater consists of a series of unit processes, each of which serves a specific function, to facilitate the continuous monitoring and treatment of the wastewater as efficiently as possible. While various different variations are possible, a basic design should include the operations as given in the figure below. The process flow diagrams will have variations in terms of industrial

operations, owing to different chemical processes[28]. The initial stage for sewage based wastewater is removing large debris from the wastewater, consequently the flow is monitored, and passes through the Grit chamber (where chunkier particles like derivatives from coffee, etc.) are moved to protect the pumps[6]. The solids collected from the screening stage and the grit chamber is directly send to landfills.

The wastewater is then sent to the primary clarifier for physical treatment, for removing organic matter, and produce a cleaner effluent and concentrate solids. Here, the organic matter present in the wastewater is evaluated in terms of chemical contamination (as COD) and biochemical contamination (as BOD or BOD_5), COD represents a rapid assessment of the chemical contamination/ organic matter present in the wastewater, while BOD reflects what can be removed biochemically. BOD is usually reduced to around 200 mg/l. Most of the BOD_5 is reduced by collecting solids at the bottom of the primary clarifier as sludge[86], which is then sent to anaerobic digesters for further treatment.

After this wastewater from the clarifier enters a biological treatment drivetrain, which consists of two parts: a bioreactor where BOD is converted to bacteria biomass; and a secondary clarifier in the form of a settling tank where bacteria biomass is finally removed. The processes involved here are either Activated Sludge (AS) or Trickling filter[112]. The AS process involves a large aeration tank where wastewater entering is combined with solids from the secondary clarifier. Here usually the bacteria concentration is as high as 10, 000 mg/l, aeration helps bacteria to rapidly degrade the organic matter, with a retention time of 4 – 6 hours, wastewater flows into a secondary clarifer where the bacteria mass settles out, and treated wastewater has a BOD of less than 30 mg/l with TSS < 30 mg/l. This is achieved easily with modern treatment plants, making it a highly effective

process[102]. However, a large amount of oxygen is required to be sent into the clarifier to efficiently help microbes with the process, which represents the limitations of this method. This is where MFCs can play a key role, through which it can make the existing process sustainable.

Now, from the perspective of MFC, the electricity generating bacteria not only help generate power but also reduce power consumption. Considering the aeration process discussed in the previous section, the ventilator that sends in aeration to the tanks consumes an enormous amount of power, about 40% of the overall electric power (**Fig. 5.1**).

Figure 5.1: The ventilator used to provide aeration in a wastewater treatment plant in Yokohama, Japan. Based on the data sheet from the plant, the ventilator units that sends in oxygen to the aeration tank alone consumes 40% of the total energy consumption by the plant. (Source: Environmental Planning Bureau, Yokohama, Japan).

This is where a wastewater treatment system using electricity-generating bacteria can be useful, since electricity-generating bacteria do not require oxygen, there is no need to send air into the tank, this means the enormous energy used to power the ventilator becomes completely unnecessary, moreover as there is a continuous supply of organic matter, the bacteria can continue to break down the same and generate electrons, the electricity generated can then be used to power other equipment in the same facility[107]. The water then can be sent for chemical treatment in the form of chlorination (to kill bacteria), and then dechlorinated to protect the aquatic life in the receiving water body.

MFC Integration. The success of any technology depends upon how it influences the energy market and the perception of the common public since MFCs can produce electricity while removing pollutants and other organic matter from wastewater streams, it can be speculated to offer advantages such as:

a. Energy recovery from exponentially growing wastes from human activities.
b. Waste residue post bioelectricity production can be used as compost.

As shown in **Fig. 5.2** and **Fig. 5.3,** MFCs are not restricted to specific technology but has the potential to be used alongside existing technologies. Thus, MFCs represent powerful predictive tools, which will aid the design of systems exploiting bacterial capabilities. In MFC systems, chemicals are reduced at the cathode, and in some systems, it is possible to achieve chemical oxidation at the anode in situations when a high concentration of biodegradable organics is present in the wastewater[60]. For this to work, however, sufficient electron acceptors should be present at the cathode.

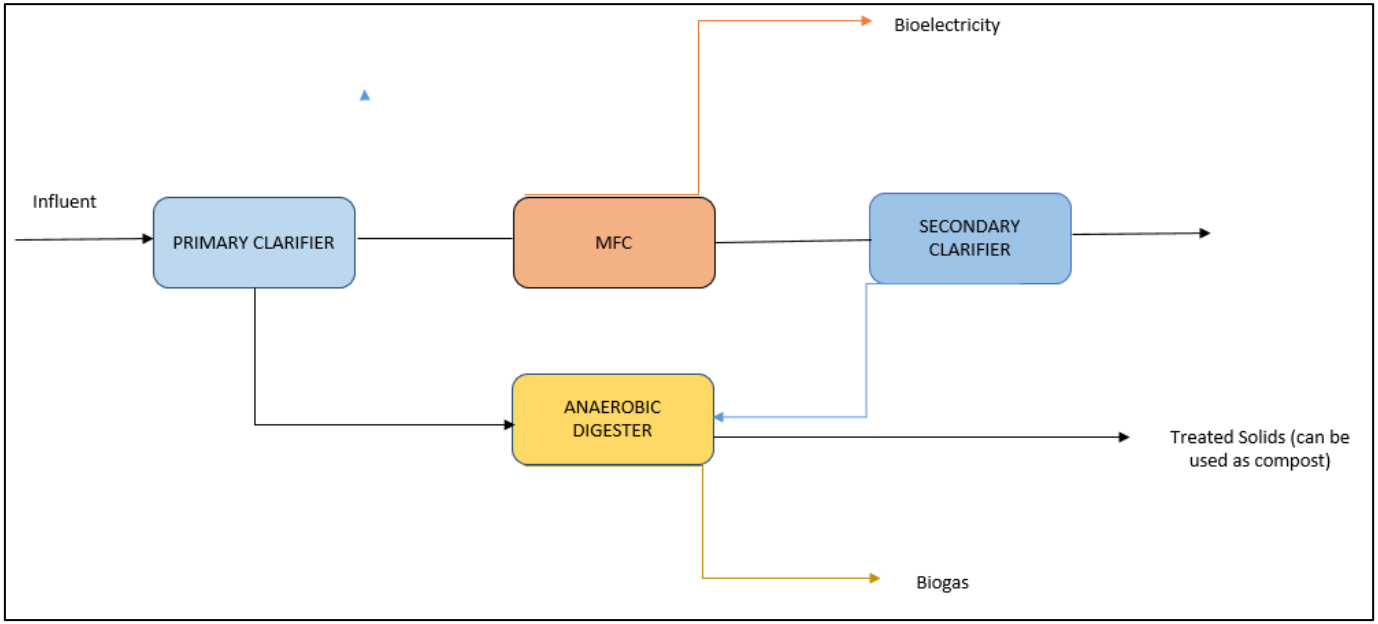


Figure 5.2: MFCs can be integrated in series with anaerobic digesters, with interconnected networks, so that biogas can be generated from the digester, and MFC can simultaneously reduce sludge content and generate electricity from the process, this represents complete energy recovery.

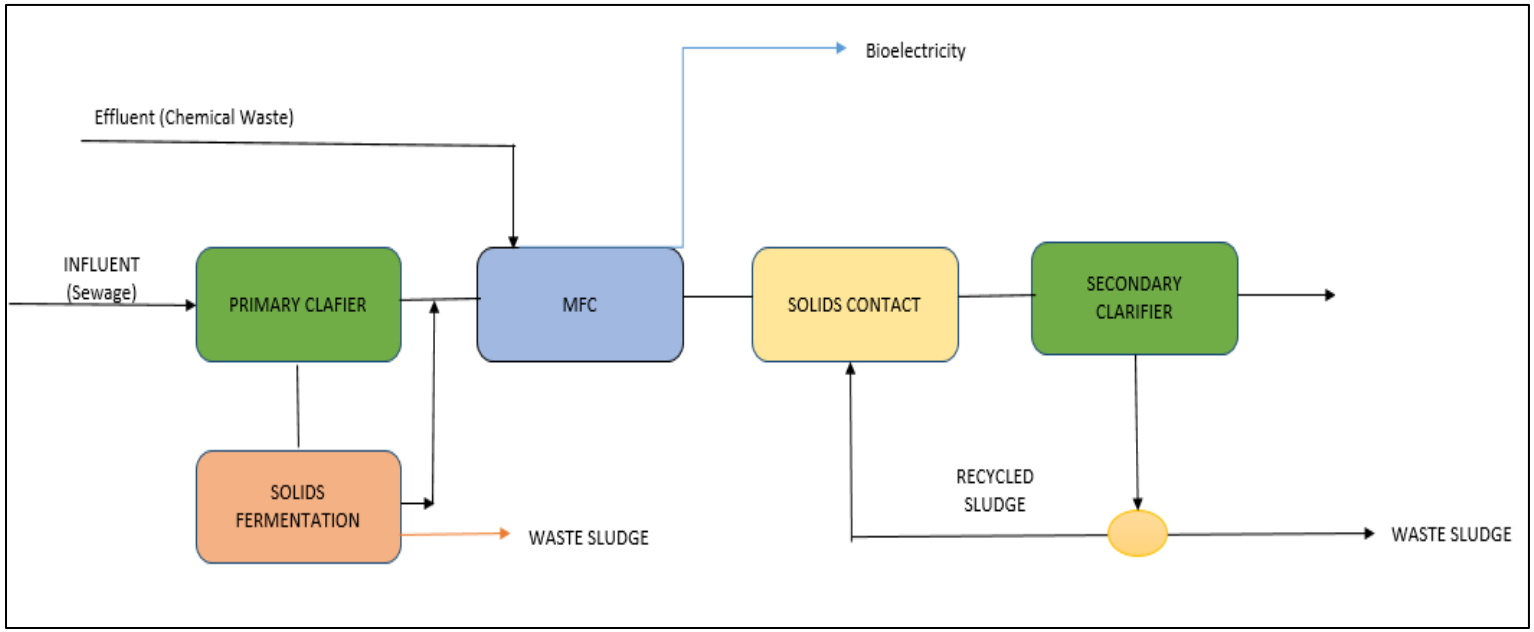


Figure 5.3: MFCs can treat both waste from sewage and industrial sources simultaneously, this means STP and ETP can be successfully integrated together, where the reduced sludge from the system can be used as compost material, bioelectricity can be produced, and wastewater itself can be treated.

The scope of MFC research is not just restricted to wastewater treatment as modified MFCs called Bio-electrochemically assisted microbial reactors or BEAMRs can be used to generate bio-hydrogen from any biodegradable matter, and such systems have shown potentials to cross the "fermentation barrier" with maximum possible conversion efficiency[108].

For MFCs, the ideal scenario will be when they can be solely used as a method of renewable energy recovery; right now, it might face challenges to grow in the shadow of large fossil fuel industries, but advances in power densities, reductions in material costs, and a global need to produce power from non-CO_2 sources will make MFCs practical for a society that is starting to tap energy from non-carbon based sources. Microbial fuel cells will have multiple applications in the near future along with wastewater treatment. For instance, by placing the system in soil sediments[113], it is possible to generate electricity from organic matter decomposition by the bacteria in the sediment. Such systems may not produce sufficient electrical power to make them economically feasible as sources of renewable energy but they could be sufficient for powering small electronics, which can act as a data transmitter. As an effective bioremediation tool, MFCs can be used to eliminate nitrates and even decompose uranium wastes (converting it from soluble U (VI) to insoluble U (IV) from water)[114].

For MFCs to achieve a successful commercial outlook, more avenues need to be explored to make it cost-competitive with existing fuel sources, one way to do this is if Carbon taxes are considered for energy generation, MFCs will clearly emerge out as the most progressive method of resource utilization from wastes by removing

contamination and generate useful power in the process. The progress of MFCs remains in the hands of researchers, who believe that MFC technologies are a part of the bright and promising future based on the foundations of a new generation of sustainable microbial reactors.

REFERENCES

[1] W. Yang, W. He, F. Zhang, M. A. Hickner, and B. E. Logan, "Single-step fabrication using a phase inversion method of poly (vinylidene fluoride)(PVDF) activated carbon air cathodes for microbial fuel cells," *Environ. Sci. Technol. Lett.*, vol. 1, no. 10, pp. 416–420, 2014.

[2] M. Wang, Z. Wang, X. Gong, and Z. Guo, "The intensification technologies to water electrolysis for hydrogen production--A review," *Renew. Sustain. Energy Rev.*, vol. 29, pp. 573–588, 2014.

[3] W. H. Organization and others, "Safely managed drinking water: thematic report on drinking water 2017," 2017.

[4] M. Mekonnen and A. Y. Hoekstra, "National water footprint accounts: the green, blue and grey water footprint of production and consumption," 2011.

[5] J. Allouche, "The governance of Central Asian waters: national interests versus regional cooperation," in *Disarmament Forum*, 2007, vol. 4, no. Central Asia at the Crossroads, pp. 46–55.

[6] G. U. Semblante *et al.*, "Fate of trace organic contaminants in oxic-settling-anoxic (OSA) process applied for biosolids reduction during wastewater treatment," *Bioresour. Technol.*, 2017.

[7] R. K. Lukman, P. Glavič, A. Carpenter, and P. Virtič, "Sustainable consumption and production–Research, experience, and development–The Europe we want," *J. Clean. Prod.*, vol. 138, pp. 139–147, 2016.

[8] S. Ishii, S. Suzuki, Y. Yamanaka, A. Wu, K. H. Nealson, and O. Bretschger, "Population dynamics of electrogenic microbial communities in microbial fuel cells started with three different inoculum sources,"

Bioelectrochemistry, 2017.

[9] C. Lopez, C. Santoro, P. Atanassov, M. D. Yates, and L. M. Tender, "Microbial Fuel Cell Anode Materials: Supporting Biofilms of Geobacter Sulfurreducens," in *Meeting Abstracts*, 2016, p. 1852.

[10] J. Huang, G. Guo, Y.Li., "Performance evaluation and bacteria analysis of AFB-MFC enriched with high-strength synthetic wastewater," *Water Sci. Technol.*, vol. 69, no. 1, pp. 9–14, 2014.

[11] Y. Qiao, X.-S. Wu, and C. M. Li, "Interfacial electron transfer of Shewanella putrefaciens enhanced by nanoflaky nickel oxide array in microbial fuel cells," *J. Power Sources*, vol. 266, pp. 226–231, 2014.

[12] L. Huang, X. Yang, X. Quan, J. Chen, and F. Yang, "Amicrobial fuel cell-electro-oxidation system for coking wastewater treatment and bioelectricity generation," *J. Chem. Technol. Biotechnol.*, vol. 85, no. 5, pp. 621–627, 2010.

[13] X. M. Li, K. Y. Cheng, A. Selvam, and J. W. C. Wong, "Bioelectricity production from acidic food waste leachate using microbial fuel cells: effect of microbial inocula," *Process Biochem.*, vol. 48, no. 2, pp. 283–288, 2013.

[14] D. Bose, M. Gopinath, and P. Vijay, "Sustainable power generation from wastewater sources using Microbial Fuel Cell," *Biofuels, Bioprod. Biorefining*, 2018.

[15] A. S. Mathuriya and V. N. Sharma, "Bioelectricity production from paper industry waste using a microbial fuel cell by Clostridium species," *J. Biochem. Technol.*, vol. 1, no. 2, pp. 49–52, 2009.

[16] N. M. Daud, S. Rozaimah, S. Abdullah, H. A. Hasan, and Z. Yaakob,

"Production of biodiesel and its wastewater treatment technologies : A review," *Process Saf. Environ. Prot.*, vol. 94, no. October, pp. 487–508, 2014.

[17] D. Cardoen, P. Joshi, L. Diels, P. M. Sarma, and D. Pant, "Agriculture biomass in India: Part 2. Post-harvest losses, cost and environmental impacts," *Resour. Conserv. Recycl.*, vol. 101, pp. 143–153, 2015.

[18] B. E. Logan and K. Rabaey, "Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies," *Science (80-.).*, vol. 337, no. 6095, pp. 686–690, 2012.

[19] D. Bose, V. Kandpal, H. Dhawan, P. Vijay, and M. Gopinath, "Energy Recovery with Microbial Fuel Cells: Bioremediation and Bioelectricity," in *Waste Bioremediation*, Springer, 2018, pp. 7–33.

[20] B. der Bruggen, C. Vandecasteele, T. Van Gestel, W. Doyen, and R. Leysen, "A review of pressure-driven membrane processes in wastewater treatment and drinking water production," *Environ. Prog. Sustain. Energy*, vol. 22, no. 1, pp. 46–56, 2003.

[21] D. Bose, A. Bose, S. Mitra, H. Jain, and P. Parashar, "Analysis of sediment-microbial fuel cell power production in series and parallel configurations," *Nat. Environ. Pollut. Technol.*, vol. 17, no. 1, 2018.

[22] W. F. Heins and others, "Is a paradigm shift in produced water treatment technology occurring at SAGD facilities?," *J. Can. Pet. Technol.*, vol. 49, no. 01, pp. 10–15, 2010.

[23] P. Mondal and A. Nandan, "Removal of fluoride from water by suitable low cost environmental friendly methods," 2014.

[24] B. Subedi, K. Balakrishna, R. K. Sinha, N. Yamashita, V. G. Balasubramanian, and K. Kannan, “Mass loading and removal of pharmaceuticals and personal care products, including psychoactive and illicit drugs and artificial sweeteners, in five sewage treatment plants in India,” *J. Environ. Chem. Eng.*, vol. 3, no. 4, pp. 2882–2891, 2015.

[25] T. E. Mallouk, “Water electrolysis: Divide and conquer,” *Nat. Chem.*, vol. 5, no. 5, pp. 362–363, 2013.

[26] P. V Pannirselvam, M. M. Cansian, M. Cardoso, a H. F. Costa, and R. S. Kempegowda, “Optimization of Integrated Clean Production of Pyrogas , Biogas , Methanol , Bioelectricity , Fertilizer and Feed from Agro Wastes with Reduced Emission,” 2011.

[27] D. Bose, M. Gopinath, and P. Vijay, “Sustainable power generation from wastewater sources using Microbial Fuel Cell,” *Biofuels, Bioprod. Biorefining*, vol. 12, no. 4, pp. 559–576, May 2018.

[28] B. E. Logan, *Microbial fuel cells*. John Wiley & Sons, 2008.

[29] S. Venkata Mohan, G. Mohanakrishna, B. P. Reddy, R. Saravanan, and P. N. Sarma, “Bioelectricity generation from chemical wastewater treatment in mediatorless (anode) microbial fuel cell (MFC) using selectively enriched hydrogen producing mixed culture under acidophilic microenvironment,” *Biochem. Eng. J.*, vol. 39, no. 1, pp. 121–130, 2008.

[30] D. Bose, “Fuel Cells: The Fuel for Tomorrow,” *J. Energy Res. Environ. Technol.*, vol. 2, no. 2, pp. 71–75, 2015.

[31] W.-W. Li, H.-Q. Yu, and Z. He, “Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies,” *Energy Environ. Sci.*,

vol. 7, no. 3, pp. 911–924, 2014.

[32] A. Dicks and D. A. J. Rand, *Fuel cell systems explained*. Wiley Online Library, 2018.

[33] V. B. Oliveira, M. Simões, L. F. Melo, and A. Pinto, "Overview on the developments of microbial fuel cells," *Biochem. Eng. J.*, vol. 73, pp. 53–64, 2013.

[34] B. E. Logan *et al.*, "Microbial fuel cells: methodology and technology," *Environ. Sci. Technol.*, vol. 40, no. 17, pp. 5181–5192, 2006.

[35] R. Uma Maheswari, C. Mohanapriya, P. Vijay, K. S. Rajmohan, and M. Gopinath, "Bioelectricity production and desalination of Halomonas sp.--the preliminary integrity approach," *Biofuels*, pp. 1–9, 2016.

[36] S. B. Pasupuleti, S. Srikanth, S. V. Mohan, and D. Pant, "Development of exoelectrogenic bioanode and study on feasibility of hydrogen production using abiotic VITO-CoRE™ and VITO-CASE™ electrodes in a single chamber microbial electrolysis cell (MEC) at low current densities," *Bioresour. Technol.*, vol. 195, pp. 131–138, 2015.

[37] D. E. Ross, C. W. Marshall, H. D. May, and R. S. Norman, "Draft genome sequence of Sulfurospirillum sp. strain MES, reconstructed from the metagenome of a microbial electrosynthesis system," *Genome Announc.*, vol. 3, no. 1, pp. e01336-14, 2015.

[38] H. Yuan, J. Li, C. Yuan, and Z. He, "Facile synthesis of MoS2@ CNT as an effective catalyst for hydrogen production in microbial electrolysis cells," *ChemElectroChem*, vol. 1, no. 11, pp. 1828–1833, 2014.

[39] Y. Kim, R. D. Cusick, and B. Logan, "Reverse electrodialysis supported

microbial fuel cells and microbial electrolysis cells." Google Patents, 2015.

[40] G. Schneider, T. Kovács, G. Rákhely, and M. Czeller, "Biosensoric potential of microbial fuel cells," *Appl. Microbiol. Biotechnol.,* vol. 100, no. 16, pp. 7001–7009, 2016.

[41] J. R. Kim, S. H. Jung, J. M. Regan, and B. E. Logan, "Electricity generation and microbial community analysis of alcohol powered microbial fuel cells," *Bioresour. Technol.,* vol. 98, no. 13, pp. 2568–2577, 2007.

[42] D. Bose, H. Dhawan, V. Kandpal, P. Vijay, and M. Gopinath, "Bioelectricity generation from sewage and wastewater treatment using two-chambered microbial fuel cell," *Int. J. Energy Res.,* 2018.

[43] E. Herrero-Hernández, T. J. Smith, and R. Akid, "Electricity generation from wastewaters with starch as carbon source using a mediatorless microbial fuel cell," *Biosens. Bioelectron.,* vol. 39, no. 1, pp. 194–198, 2013.

[44] Z. Ren, T. E. Ward, and J. M. Regan, "Electricity production from cellulose in a microbial fuel cell using a defined binary culture," *Environ. Sci. Technol.,* vol. 41, no. 13, pp. 4781–4786, 2007.

[45] S. W. Hong, H. J. Kim, Y. S. Choi, and T. H. Chung, "Field Experiments on Bioelectricity Production from Lake Sediment Using Microbial Fuel Cell Technology," *Bull. Korean Chem. Soc.,* vol. 29, no. 11, pp. 2189–2194, 2008.

[46] L. Xu, B. D. Wang, X. H. Liu, W. Z. Yu, and Y. Q. Zhao, "Maximizing the energy harvest from a microbial fuel cell embedded in a constructed wetland," *Appl. Energy,* vol. 214, pp. 83–91, 2018.

[47] T. Jafary, G. D. Najafpour, A. A. Ghoreyshi, F. Haghparast, M. Rahimnejad,

and H. Zare, “Bioelectricity power generation from organic substrate in a Microbial fuel cell using Saccharomyces cerevisiae as biocatalysts,” *World Renew. Energy Congr. 2011 Sweden*, pp. 1182–1188, 2011.

[48] S. Venkata Mohan, G. Mohanakrishna, S. Srikanth, and P. N. Sarma, “Harnessing of bioelectricity in microbial fuel cell (MFC) employing aerated cathode through anaerobic treatment of chemical wastewater using selectively enriched hydrogen producing mixed consortia,” *Fuel*, vol. 87, no. 12, pp. 2667–2676, 2008.

[49] V. Chaturvedi and P. Verma, “Microbial fuel cell: a green approach for the utilization of waste for the generation of bioelectricity,” *Bioresour. Bioprocess.*, vol. 3, no. 1, p. 38, 2016.

[50] X. Zheng and N. Nirmalakhandan, “Cattle wastes as substrates for bioelectricity production via microbial fuel cells,” *Biotechnol. Lett.*, vol. 32, no. 12, pp. 1809–1814, 2010.

[51] V. R. Nimje *et al.*, “Stable and high energy generation by a strain of Bacillus subtilis in a microbial fuel cell,” *J. Power Sources*, vol. 190, no. 2, pp. 258–263, 2009.

[52] E. Fernando, T. Keshavarz, and G. Kyazze, “Enhanced bio-decolourisation of acid orange 7 by Shewanella oneidensis through co-metabolism in a microbial fuel cell,” *Int. Biodeterior. Biodegradation*, vol. 72, pp. 1–9, 2012.

[53] D. Majumder *et al.*, “Electricity generation and wastewater treatment of oil refinery in microbial fuel cells using Pseudomonas putida,” *Int. J. Mol. Sci.*, vol. 15, no. 9, pp. 16772–16786, 2014.

[54] M. H. M. Nor, M. F. M. Mubarak, H. S. A. Elmi, N. Ibrahim, M. F. A.

Wahab, and Z. Ibrahim, “Bioelectricity generation in microbial fuel cell using natural microflora and isolated pure culture bacteria from anaerobic palm oil mill effluent sludge,” *Bioresour. Technol.*, vol. 190, pp. 458–465, 2015.

[55] M. A. Islam, M. R. Khan, A. Yousuf, W. C. Wai, and C. K. Cheng, “Electricity generation form pretreated palm oil mill effluent using Klebsiella Variicola as an inoculum in Microbial fuel cell,” in *Development in the in Renewable Energy Technology (ICDRET), 2016 4th International Conference on the*, 2016, pp. 1–4.

[56] S. S. Kumar, S. Basu, and N. R. Bishnoi, “Effect of cathode environment on bioelectricity generation using a novel consortium in anode side of a microbial fuel cell,” *Biochem. Eng. J.*, vol. 121, pp. 17–24, 2017.

[57] P. Kaewkannetra, W. Chiwes, and T. Y. Chiu, “Treatment of cassava mill wastewater and production of electricity through microbial fuel cell technology,” *Fuel*, vol. 90, no. 8, pp. 2746–2750, 2011.

[58] E. D. Penteado, C. M. Fernandez-Marchante, M. Zaiat, E. R. Gonzalez, and M. A. Rodrigo, “On the Effects of Ferricyanide as Cathodic Mediator on the Performance of Microbial Fuel Cells,” *Electrocatalysis*, vol. 8, no. 1, pp. 59–66, 2017.

[59] S. M. Zain, N. L. Ching, S. Jusoh, and S. Y. Yunus, “Different types of microbial fuel cell (MFC) systems for simultaneous electricity generation and pollutant removal,” *J. Teknol.*, vol. 74, no. 3, pp. 13–19, 2015.

[60] X. Zhang, W. He, L. Ren, J. Stager, P. J. Evans, and B. E. Logan, “COD removal characteristics in air-cathode microbial fuel cells,” *Bioresour. Technol.*, vol. 176, pp. 23–31, 2015.

[61] K.-Y. Kim, W. Yang, P. J. Evans, and B. E. Logan, “Continuous treatment of high strength wastewaters using air-cathode microbial fuel cells,” *Bioresour. Technol.*, vol. 221, pp. 96–101, 2016.

[62] Y. Li, L. Liu, and F. Yang, “Destruction of tetracycline hydrochloride antibiotics by FeOOH/TiO 2 granular activated carbon as expanded cathode in low-cost MBR/MFC coupled system,” *J. Memb. Sci.*, vol. 525, pp. 202–209, 2017.

[63] S. Venkata Mohan, R. Saravanan, S. V. Raghavulu, G. Mohanakrishna, and P. N. Sarma, “Bioelectricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: Effect of catholyte,” *Bioresour. Technol.*, vol. 99, no. 3, pp. 596–603, 2008.

[64] D. Bose, H. Dhawan, V. Kandpal, P. Vijay, and M. Gopinath, “Sustainable Power Generation from Sewage and Energy Recovery from Wastewater with variable resistance using Microbial Fuel Cell,” *Enzyme Microb. Technol.*, vol. 118, no. C, pp. 92–101, 2018.

[65] V. R. Nimje, CYChen, and HR Chen, *Comparative bioelectricity production from various wastewaters in microbial fuel cells using mixed cultures and a pure strain of Shewanella oneidensis*, vol. 104. Elsevier Ltd, 2012.

[66] J. R. Kim, S. Cheng, S.-E. Oh, and B. E. Logan, “Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells,” *Environ. Sci. Technol.*, vol. 41, no. 3, pp. 1004–1009, 2007.

[67] S. A. Mathuriya and V. N. Sharma, “Bioelectricity production from various wastewaters through microbial fuel cell technology,” *J. Biochem. Technol.*, vol. 2, no. 2009, pp. 133–137, 2010.

[68] P. Pandey, V. N. Shinde, R. L. Deopurkar, S. P. Kale, S. A. Patil, and D. Pant, “Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery,” *Appl. Energy*, vol. 168, pp. 706–723, 2016.

[69] R. K. Goud, P. S. Babu, and S. V. Mohan, “Canteen based composite food waste as potential anodic fuel for bioelectricity generation in single chambered microbial fuel cell (MFC): Bio-electrochemical evaluation under increasing substrate loading condition,” *Int. J. Hydrogen Energy*, vol. 36, no. 10, pp. 6210–6218, 2011.

[70] A. G. del Campo, J. Lobato, P. Cañizares, M. A. Rodrigo, and F. J. F. Morales, “Short-term effects of temperature and COD in a microbial fuel cell,” *Appl. Energy*, vol. 101, pp. 213–217, 2013.

[71] P. Mani, T. Keshavarz, T. S. Chandra, and G. Kyazze, “Decolourisation of Acid orange 7 in a microbial fuel cell with a laccase-based biocathode: Influence of mitigating pH changes in the cathode chamber,” *Enzyme Microb. Technol.*, vol. 96, pp. 170–176, 2017.

[72] K. Y. Cheng, G. Ho, and R. Cord-Ruwisch, “Novel methanogenic rotatable bioelectrochemical system operated with polarity inversion,” *Environ. Sci. Technol.*, vol. 45, no. 2, pp. 796–802, 2010.

[73] J. J. Fornero, M. Rosenbaum, M. A. Cotta, and L. T. Angenent, “Carbon dioxide addition to microbial fuel cell cathodes maintains sustainable catholyte pH and improves anolyte pH, alkalinity, and conductivity,” *Environ. Sci. Technol.*, vol. 44, no. 7, pp. 2728–2734, 2010.

[74] L. Huang and B. E. Logan, “Electricity production from xylose in fed-batch and continuous-flow microbial fuel cells,” *Appl. Microbiol. Biotechnol.*, vol.

80, no. 4, p. 655, 2008.

[75] L. Huang, Y. Sun, Y. Liu, and N. Wang, “Mineralization of 4-chlorophenol and analysis of bacterial community in microbial fuel cells,” *Procedia Environ. Sci.*, vol. 18, pp. 534–539, 2013.

[76] M. Behera, P. S. Jana, T. T. More, and M. M. Ghangrekar, “Rice mill wastewater treatment in microbial fuel cells fabricated using proton exchange membrane and earthen pot at different pH,” *Bioelectrochemistry*, vol. 79, no. 2, pp. 228–233, 2010.

[77] S. Hays, F. Zhang, and B. E. Logan, “Performance of two different types of anodes in membrane electrode assembly microbial fuel cells for power generation from domestic wastewater,” *J. Power Sources*, vol. 196, no. 20, pp. 8293–8300, 2011.

[78] K. P. Katuri, A.-M. Enright, V. O’Flaherty, and D. Leech, “Microbial analysis of anodic biofilm in a microbial fuel cell using slaughterhouse wastewater,” *Bioelectrochemistry*, vol. 87, pp. 164–171, 2012.

[79] A. D. Dalvi, N. Mohandas, O. a Shinde, and P. T. Kininge, “Microbial Fuel Cell for Production of Bioelectricity From Whey and Biological Waste Treatment,” *Int. J. Adv. Biotechnol. Res.*, vol. 2, no. 2, pp. 263–268, 2011.

[80] H. C. Boghani, R. M. Dinsdale, A. J. Guwy, and G. C. Premier, “Sampled-time control of a microbial fuel cell stack,” *J. Power Sources*, vol. 356, pp. 338–347, 2017.

[81] C. I. Torres, A. K. Marcus, P. Parameswaran, and B. E. Rittmann, “Kinetic experiments for evaluating the Nernst- Monod model for anode-respiring bacteria (ARB) in a biofilm anode,” *Environ. Sci. Technol.*, vol. 42, no. 17,

pp. 6593–6597, 2008.

[82] H. Liu, R. Ramnarayanan, and B. E. Logan, “Production of electricity during wastewater treatment using a single chamber microbial fuel cell,” *Environ. Sci. Technol.*, vol. 38, no. 7, pp. 2281–2285, 2004.

[83] D. Bose, M. Gopinath, P. Vijay, S. Sridharan, R. Rawat, and R. Bahuguna, “Bioelectricity generation and biofilm analysis from sewage sources using microbial fuel cell,” *Fuel*, vol. 255, p. 115815, 2019.

[84] D. Bose and A. Bose, “Electrical power generation with Himalayan mud soil using microbial fuel cell,” *Nat. Environ. Pollut. Technol.*, vol. 16, no. 2, 2017.

[85] M. Li *et al.*, “Microbial fuel cell (MFC) power performance improvement through enhanced microbial electrogenicity,” *Biotechnol. Adv.*, vol. 36, no. 4, pp. 1316–1327, 2018.

[86] R. Saravanan, A. Arun, S. Venkatamohan, Jegadeesan, T. Kandavelu, and Veeramanikandan, “Membraneless dairy wastewater-sediment interface for bioelectricity generation employing sediment microbial fuel cell (SMFC),” *African J. Microbiol. Res.*, vol. 4, no. 24, pp. 2640–2646, 2010.

[87] M. Rahimnejad, T. Jafary, F. Haghparast, G. D. Najafpour, and A. A. Ghoreyshi, “Nafion as a nanoproton conductor in microbial fuel cells,” *Turkish J. Eng. Environ. Sci.*, vol. 34, no. 4, pp. 289–291, 2010.

[88] S. Venkata Mohan, S. Veer Raghavulu, and P. N. Sarma, “Influence of anodic biofilm growth on bioelectricity production in single chambered mediatorless microbial fuel cell using mixed anaerobic consortia,” *Biosens. Bioelectron.*, vol. 24, no. 1, pp. 41–47, 2008.

[89] S. Cheng, H. Liu, and B. E. Logan, “Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells,” *Environ. Sci. Technol.*, vol. 40, no. 1, pp. 364–369, 2006.

[90] F. Qian, M. Baum, Q. Gu, and D. E. Morse, “A 1.5 μL microbial fuel cell for on-chip bioelectricity generation,” *Lab Chip*, vol. 9, no. 21, p. 3076, 2009.

[91] J. Xu, G.-P. Sheng, H.-W. Luo, W.-W. Li, L.-F. Wang, and H.-Q. Yu, “Fouling of proton exchange membrane (PEM) deteriorates the performance of microbial fuel cell,” *water Res.*, vol. 46, no. 6, pp. 1817–1824, 2012.

[92] B. R. Strazisar, R. R. Anderson, and C. M. White, “Degradation of monoethanolamine used in carbon dioxide capture from flue gas of a coal-fired electric power generating station,” *J. Energy Environ. Res.*, vol. 1, no. 1, pp. 32–39, 2001.

[93] J. P. Rooney *et al.*, “PCR based determination of mitochondrial DNA copy number in multiple species,” in *Mitochondrial Regulation*, Springer, 2015, pp. 23–38.

[94] J. A. Ream, L. K. Lewis, and K. A. Lewis, “Rapid agarose gel electrophoretic mobility shift assay for quantitating protein: RNA interactions,” *Anal. Biochem.*, vol. 511, pp. 36–41, 2016.

[95] B. Yan *et al.*, “Coverage analysis in a targeted amplicon-based next-generation sequencing panel for myeloid neoplasms,” *J. Clin. Pathol.*, vol. 69, no. 9, pp. 801–804, 2016.

[96] H. Chen, Y. Wu, Z. Chen, and N. He, “Full-automated thermal cycler in

nucleic acid testing workstation," *J. Nanosci. Nanotechnol.*, vol. 17, no. 1, pp. 568–572, 2017.

[97] K. Tamura, J. Dudley, M. Nei, and S. Kumar, "MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0," *Mol. Biol. Evol.*, vol. 24, no. 8, pp. 1596–1599, 2007.

[98] S. Kumar, K. Tamura, I. B. Jakobsen, and M. Nei, "MEGA2: molecular evolutionary genetics analysis software," *Bioinformatics*, vol. 17, no. 12, pp. 1244–1245, 2001.

[99] D. Bose, S. Sridharan, H. Dhawan, P. Vijay, and M. Gopinath, "Biomass derived activated carbon cathode performance for sustainable power generation from Microbial Fuel Cells," *Fuel*, vol. 236, pp. 325–337, 2019.

[100] W. Yang and B. E. Logan, "Immobilization of a metal--nitrogen--carbon catalyst on activated carbon with enhanced cathode performance in microbial fuel cells," *ChemSusChem*, vol. 9, no. 16, pp. 2226–2232, 2016.

[101] C. Lee, J. Chen, and Y. Cai, "Bioelectricity generation and organic removal in microbial fuel cells used for treatment of wastewater from fish-market," *J. Environ. Eng. Manag.*, vol. 20, no. 3, pp. 173–180, 2010.

[102] I. Gajda, J. Greenman, C. Melhuish, and I. A. Ieropoulos, "Electricity and disinfectant production from wastewater: Microbial Fuel Cell as a self-powered electrolyser," *Sci. Rep.*, vol. 6, p. 25571, 2016.

[103] D. Bose and A. Bose, "Graphene-based microbial fuel cell studies with starch in sub-himalayan soils," *Indones. J. Electr. Eng. Informatics*, vol. 5, no. 1, 2017.

[104] X. Zhang, D. Pant, F. Zhang, J. Liu, W. He, and B. E. Logan, "Long-term

performance of chemically and physically modified activated carbons in air cathodes of microbial fuel cells," *ChemElectroChem*, vol. 1, no. 11, pp. 1859–1866, 2014.

[105] S. Nasirahmadi and a. a. Safekordi, "Whey as a substrate for generation of bioelectricity in microbial fuel cell using E.coli," *Int. J. Environ. Sci. Technol.*, vol. 8, no. 4, pp. 823–830, 2011.

[106] C. Dumas, A. Mollica, D. Féron, R. Basséguy, L. Etcheverry, and A. Bergel, "Marine microbial fuel cell: use of stainless steel electrodes as anode and cathode materials," *Electrochim. Acta*, vol. 53, no. 2, pp. 468–473, 2007.

[107] S. Venkata Mohan, S. Veer Raghavulu, and P. N. Sarma, "Biochemical evaluation of bioelectricity production process from anaerobic wastewater treatment in a single chambered microbial fuel cell (MFC) employing glass wool membrane," *Biosens. Bioelectron.*, vol. 23, no. 9, pp. 1326–1332, 2008.

[108] S. B. Velasquez-Orta, T. P. Curtis, and B. E. Logan, "Energy from algae using microbial fuel cells," *Biotechnol. Bioeng.*, vol. 103, no. 6, pp. 1068–1076, 2009.

[109] W. Zhu, L. Chai, Z. Ma, Y. Wang, H. Xiao, and K. Zhao, "Anaerobic reduction of hexavalent chromium by bacterial cells of Achromobacter sp. strain Ch1," *Microbiol. Res.*, vol. 163, no. 6, pp. 616–623, 2008.

[110] K. C. Wrighton *et al.*, "A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells," *ISME J.*, vol. 2, no. 11, p. 1146, 2008.

[111] B. S. Girgis, L. B. Khalil, and T. A. M. Tawfik, "Activated carbon from sugar cane bagasse by carbonization in the presence of inorganic acids," *J.*

Chem. Technol. Biotechnol. Int. Res. Process. Environ. Clean Technol., vol. 61, no. 1, pp. 87–92, 1994.

[112] S. A. Patil *et al.*, "Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber," *Bioresour. Technol.*, vol. 100, no. 21, pp. 5132–5139, 2009.

[113] M. Sherafatmand and H. Y. Ng, "Using sediment microbial fuel cells (SMFCs) for bioremediation of polycyclic aromatic hydrocarbons (PAHs)," *Bioresour. Technol.*, vol. 195, pp. 122–130, 2015.

[114] F. Rezaei, T. L. Richard, R. A. Brennan, and B. E. Logan, "Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems," *Environ. Sci. Technol.*, vol. 41, no. 11, pp. 4053–4058, 2007.

APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER III

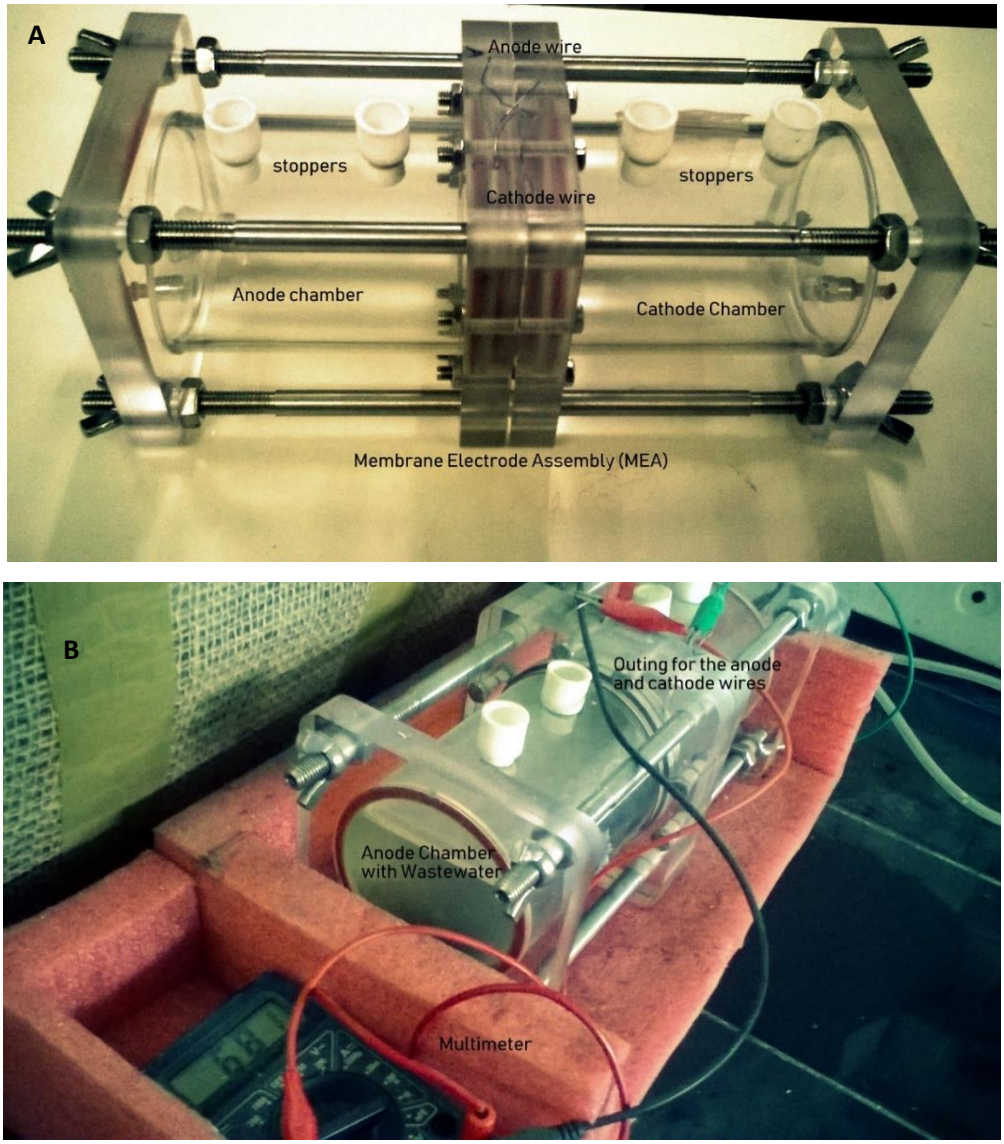


Figure S3-1: Double chambered Pt-Cat system **(A)** after fabrication **(B)** with wastewater

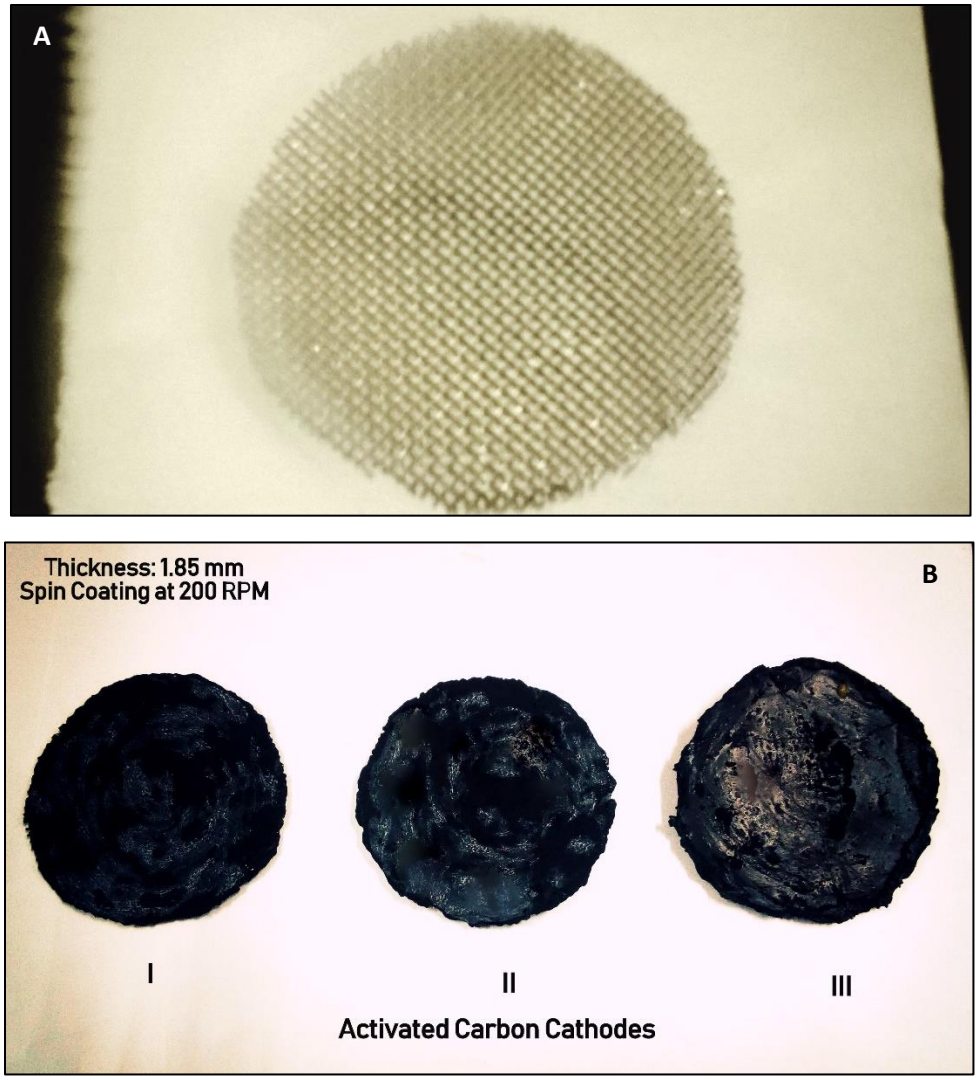


Figure S3-2: (A) SS 316L mesh (radius = 2.5 cm) on which cathode was coated (B) For even distribution of cathode material, spin coater utilized, achieved uniform thickness of 1.35 mm.

APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER IV

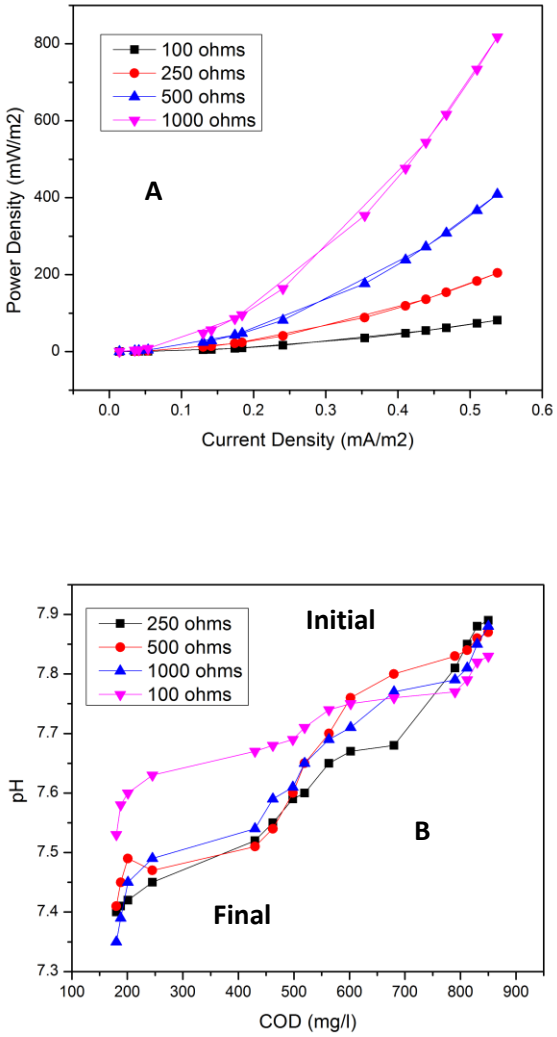


Figure S4-1: For the Pt-Cat system **(A)** Power density contrasted with current density with varying resistors **(B)** With the decrease in COD concentration pH from an initial range of 7.89 ± 0.2 came to close to clean water pH of 7.3 ± 0.15.

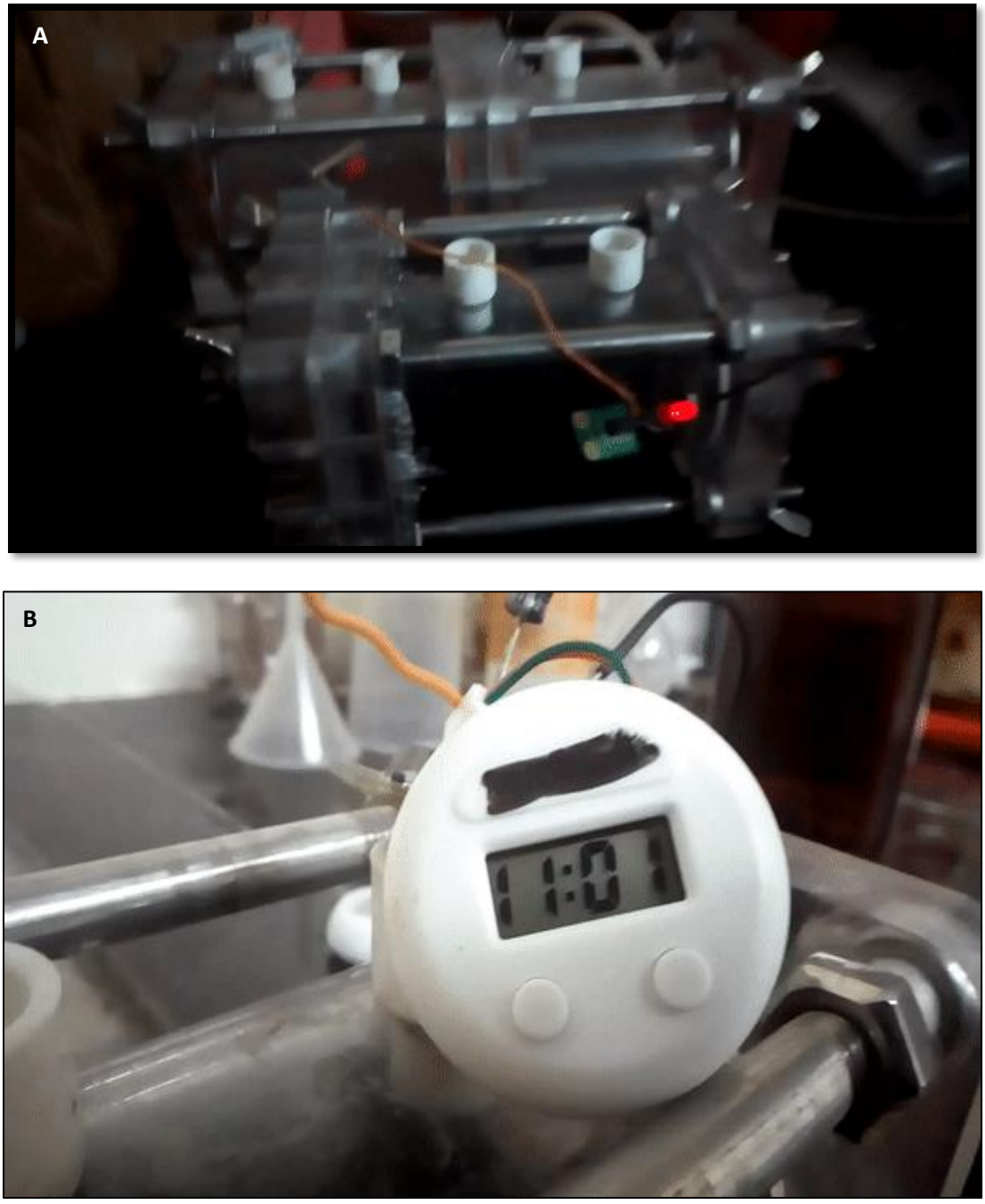


Figure S4-2: Portable electronics powered using wastewater by connecting **(A)** LED **(B)** Digital watch to the anode and the cathode wiring.

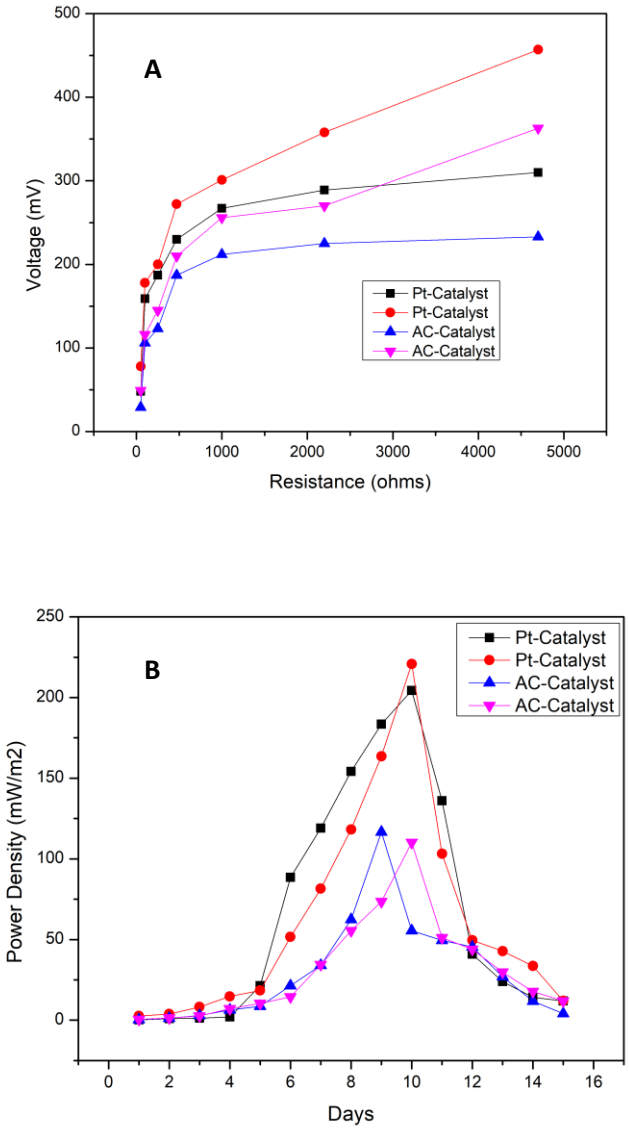


Figure S4-3: Comparative evaluation of AC-Cat and Pt-Cat MFC with **(A)** varying resistance from 50 Ω to 4700 Ω **(B)** Power density based on polarization voltage.

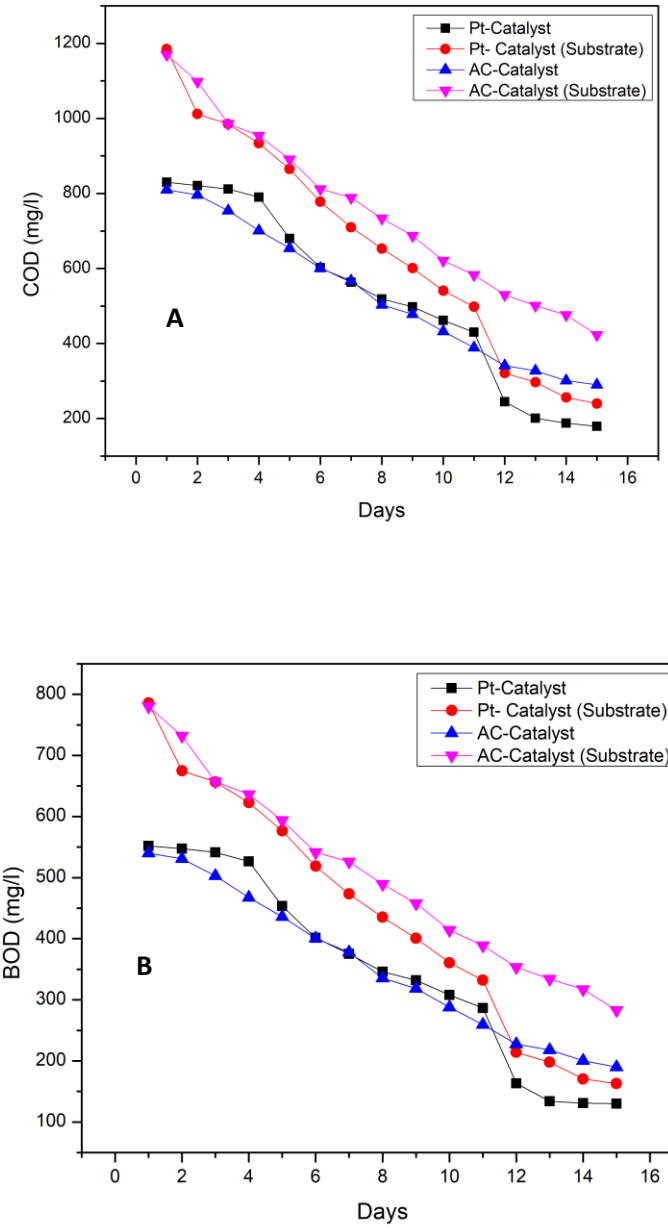


Figure S4-4: AC-Cat and Pt-Cat MFC **(A)** COD removal comparison for wastewater and substrate rich wastewater (B) BOD removal rates for wastewater with and without substrate.

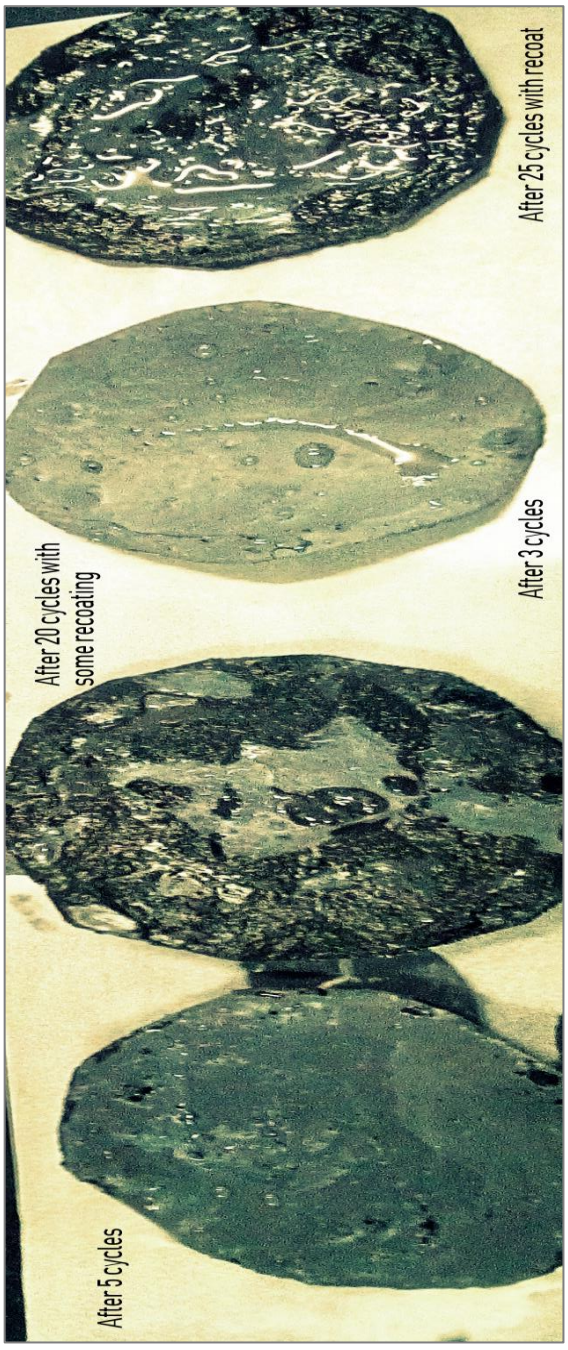


Figure S4-5: Different stages of the AC cathode after MFC operation, with recoating when needed at certain areas of the cathode.

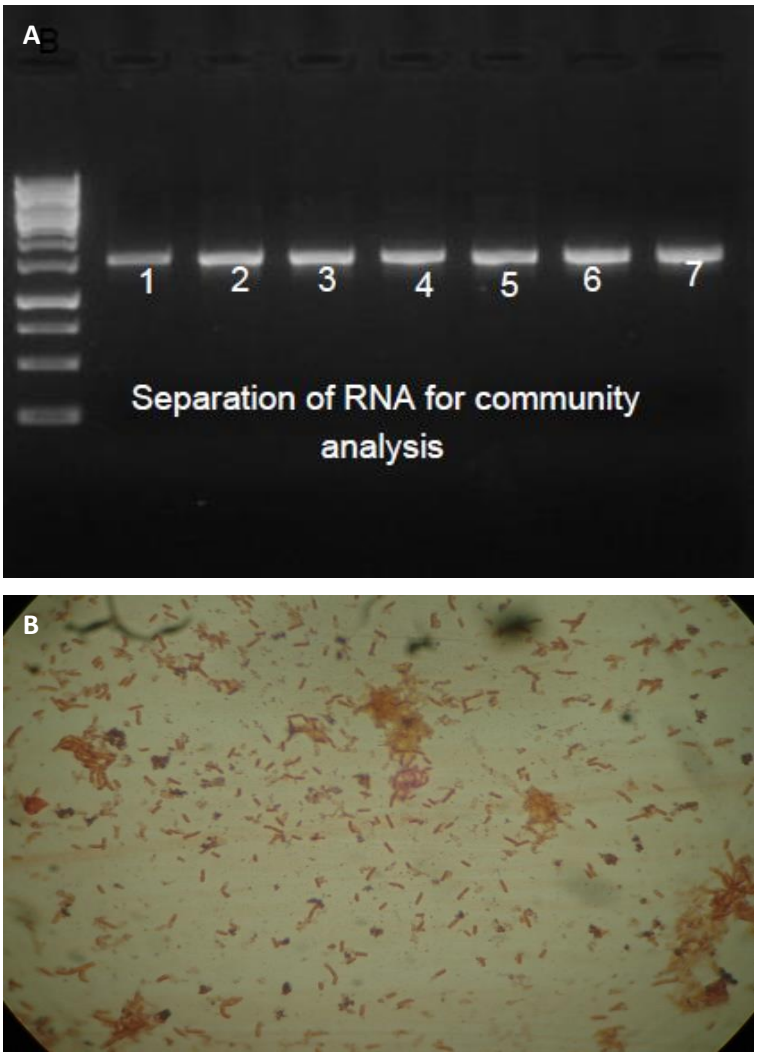


Figure S4-6: (A) Gel electrophoresis evaluation of the biofilm showing the six specie and one sub-specie of exoelectrogens identified from the biofilm **(B)** Enhanced microscopic image through spore staining of *Achromobacter xylosoxidans*.

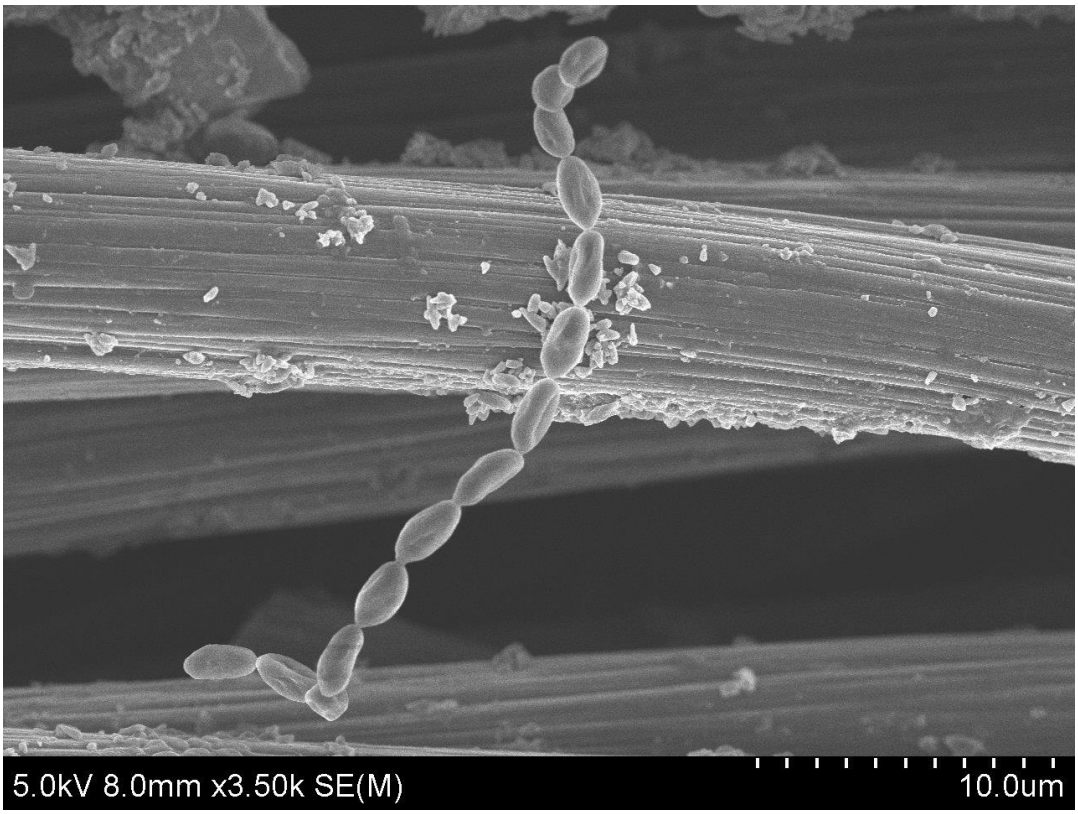


Figure S4-7: SEM image of the anode biofilm, with bacteria colonies on the fiber in front, and is visible at backdrop with other fibers of the same.

DEBAJYOTI BOSE

EDUCATION

2014-2016 **M. Tech. in Renewable Energy Engineering**, University of Petroleum & Energy Studies,

Advisor: Prof. Amarnath Bose

2010- 2014 **B. Tech. in Chemical Engineering**, West Bengal University of Technology

NOTABLE JOURNAL PUBLICATIONS

Bose, Debajyoti., Gopinath, M., Vijay, P., Sridharan, S., Rawat, R. and Bahuguna, R., 2019. Bioelectricity generation and biofilm analysis from sewage sources using microbial fuel cell. *Fuel*, **Elsevier**, *255*, p.115815.

Bose, Debajyoti, Sridharan, S., Dhawan, H., Vijay, P. and Gopinath, M., 2019. Biomass derived activated carbon cathode performance for sustainable power generation from Microbial Fuel Cells. *Fuel*, **Elsevier**, *236*, pp.325-337.

Bose Debajyoti, Dhawan H, Kandpal V, Vijay P, Gopinath M. Bioelectricity generation from sewage and wastewater treatment using two-chambered microbial fuel cell. **WILEY,** Int. J Energy Res. 2018; 42: 4335–4344.

Bose, Debajyoti, Dhawan, H., Kandpal, V., Vijay, P. & Gopinath, M. Sustainable Power Generation from Sewage and Energy Recovery from Wastewater with variable resistance using Microbial Fuel Cell. *Enzyme Microb. Technol.* **Elsevier** 118**,** 92–101 (2018).

Bose, Debajyoti, Vijay P, Gopinath M. "Sustainable power generation from wastewater sources using Microbial Fuel Cell." *Biofuels, Bioproducts and Biorefining*, **WILEY**, 12**,** 559–576 (2018).

BOOK CHAPTER

Bose, Debajyoti, Vaibhaw Kandpal, Himanshi Dhawan, P. Vijay, and M. Gopinath. "Energy Recovery with Microbial Fuel Cells: Bioremediation and Bioelectricity." In *Waste Bioremediation*, pp. 7-33. **Springer**, Singapore, 2018.

FUNDED RESEARCH

P. Vijay, Debajyoti Bose, M Gopinath, Development of novel indigenous cathode for MFC performance Optimization, UPES, SEED Project, **April 2018-June 2019**, 2.55 lacs.

PROFESSIONAL MEMBERSHIP

Society of Chemical Industry (SCI), London, United Kingdom
(Membership ID: 75566).

International Association of Engineers (IAENG), Hong Kong, China
(Membership ID: 215839).

UPES
UNIVERSITY WITH A PURPOSE

PLAGIARISM CERTIFICATE

1. We Dr. P Vijay (Internal Guide), Dr. M Gopinath (Co Guide/ External Guide) certify that the Thesis titled

Production and Optimization of Bioelectricity from Wastewater using Microbial Fuel Cell (MFC)

submitted by Scholar Mr/ Ms Mr. Debajyoti Bose having SAP ID 500056858 has been run through a Plagiarism Check Software and the Plagiarism Percentage is reported to be 8 %.

2. Plagiarism Report generated by the Plagiarism Software is attached .

P. Vijay

Signature of the Internal Guide

M. Gopinath

Signature of External Guide/ Co Guide

Debajyoti Bose

Signature of the Scholar

PhD Thesis

ORIGINALITY REPORT

8%	4%	7%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Logan. "Kinetics and Mass Transfer", Microbial Fuel Cells, 12/12/2007 Publication	1%
2	Logan. "Exoelectrogens", Microbial Fuel Cells, 12/12/2007 Publication	1%
3	Bruce E. Logan, Bert Hamelers, René Rozendal, Uwe Schröder et al. " Microbial Fuel Cells: Methodology and Technology ", Environmental Science & Technology, 2006 Publication	<1%
4	Surajbhan Sevda, Pranab Jyoti Sarma, Kaustubha Mohanty, T. R. Sreekrishnan, Deepak Pant. "Chapter 11 Microbial Fuel Cell Technology for Bioelectricity Generation from Wastewaters", Springer Science and Business Media LLC, 2018 Publication	<1%
5	"Microbial Fuel Cell", Springer Science and Business Media LLC, 2018 Publication	<1%

6	Oliveira, V.B., M. Simões, L.F. Melo, and A.M.F.R. Pinto. "Overview on the developments of microbial fuel cells", Biochemical Engineering Journal, 2013. Publication	<1%
7	Badie S. Girgis. "Activated carbon from sugar cane bagasse by carbonization in the presence of inorganic acids", Journal of Chemical Technology & Biotechnology, 09/1994 Publication	<1%
8	dbtncstcp.nic.in Internet Source	<1%
9	Li He, Peng Du, Yizhong Chen, Hongwei Lu, Xi Cheng, Bei Chang, Zheng Wang. "Advances in microbial fuel cells for wastewater treatment", Renewable and Sustainable Energy Reviews, 2017 Publication	<1%
10	Logan. "Voltage Generation", Microbial Fuel Cells, 12/12/2007 Publication	<1%
11	www.researchgate.net Internet Source	<1%
12	en.wikipedia.org Internet Source	<1%

13	Caneon Kurien, Ajay Kumar Srivastava, Niranajan Gandigudi, Karan Anand. "Soot deposition effects and microwave regeneration modelling of diesel particulate filtration system", Journal of the Energy Institute, 2020 Publication	<1%
14	Sunita J. Varjani, Edgard Gnansounou, G. Baskar, Deepak Pant, Zainul Akmar Zakaria. "Chapter 1 Introduction to Waste Bioremediation", Springer Science and Business Media LLC, 2018 Publication	<1%
15	Cheng, S.. "Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells", Electrochemistry Communications, 200703 Publication	<1%
16	slantedright2.blogspot.com Internet Source	<1%
17	journals.pan.pl Internet Source	<1%
18	www.science.gov Internet Source	<1%
19	Wulin Yang, Weihua He, Fang Zhang, Michael A. Hickner, Bruce E. Logan. "Single-Step Fabrication Using a Phase Inversion Method of	<1%

Poly(vinylidene fluoride) (PVDF) Activated Carbon Air Cathodes for Microbial Fuel Cells", Environmental Science & Technology Letters, 2014
Publication

20	upcommons.upc.edu Internet Source	<1%
21	Pandey, Prashant, Vikas N. Shinde, Rajendra L. Deopurkar, Sharad P. Kale, Sunil A. Patil, and Deepak Pant. "Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery", Applied Energy, 2016. Publication	<1%
22	University of Tennessee, Knoxville Publication	<1%
23	Samsudeen, N., T.K. Radhakrishnan, and Manickam Matheswaran. "Bioelectricity production from microbial fuel cell using mixed bacterial culture isolated from distillery wastewater", Bioresource Technology, 2015. Publication	<1%
24	repositorium.sdum.uminho.pt Internet Source	<1%
25	librarysearch.aut.ac.nz Internet Source	<1%

26	Kiely, P.D.. "Long-term cathode performance and the microbial communities that develop in microbial fuel cells fed different fermentation endproducts", Bioresource Technology, 201101 Publication	<1%
27	www.rroij.com Internet Source	<1%
28	felicitysmoak.info.tm Internet Source	<1%
29	digital.lib.washington.edu Internet Source	<1%
30	www.ncbi.nlm.nih.gov Internet Source	<1%
31	Sakine Fatemi, Ali A. Ghoreyshi, Mostafa Rahimnejad, Ghasem Najafpour Darzi, Deepak Pant. "Sulfide as an alternative electron donor to glucose for power generation in mediator-less microbial fuel cell", Journal of Environmental Science and Health, Part A, 2017 Publication	<1%
32	amsdottorato.unibo.it Internet Source	<1%
33	capenet.com Internet Source	<1%
34	Bruce Logan, Shaoan Cheng, Valerie Watson,	

	Garett Estadt. "Graphite Fiber Brush Anodes for Increased Power Production in Air-Cathode Microbial Fuel Cells", Environmental Science & Technology, 2007 Publication	<1%
35	Jeetendra Prasad, Ramesh Kumar Tripathi. "Energy harvesting from sediment microbial fuel cell to supply uninterruptible regulated power for small devices", International Journal of Energy Research, 2019 Publication	<1%
36	Bruce E. Logan, Maxwell J. Wallack, Kyoung-Yeol Kim, Weihua He, Yujie Feng, Pascal E. Saikaly. "Assessment of Microbial Fuel Cell Configurations and Power Densities", Environmental Science & Technology Letters, 2015 Publication	<1%
37	www.soci.org Internet Source	<1%
38	Wenying Li, Ruipeng Ren, Yuxiang Liu, Jianhui Li, Yongkang Lv. "Improved bioelectricity production using potassium monopersulfate as cathode electron acceptor by novel bio-electrochemical activation in microbial fuel cell", Science of The Total Environment, 2019 Publication	<1%

39	Rohit Rathour, Vidhi Kalola, Jenny Johnson, Kunal Jain, Datta Madamwar, Chirayu Desai. "Treatment of Various Types of Wastewaters Using Microbial Fuel Cell Systems", Elsevier BV, 2019 Publication	<1%
40	"Microbial Fuel Cell Technology for Bioelectricity", Springer Science and Business Media LLC, 2018 Publication	<1%
41	www.davidpublishing.org Internet Source	<1%
42	Cheng, S.. "Pre-acclimation of a wastewater inoculum to cellulose in an aqueous-cathode MEC improves power generation in air-cathode MFCs", Bioresource Technology, 201101 Publication	<1%
43	Tianshun Song. "Electricity generation from terephthalic acid using a microbial fuel cell", Journal of Chemical Technology & Biotechnology, 03/2009 Publication	<1%
44	ir.uitm.edu.my Internet Source	<1%
45	www.tdx.cat Internet Source	<1%

46	www.acsionindia.net Internet Source	<1%
47	Caneon Kurien, Ajay Kumar Srivastava, Salome Lesbats. "Experimental and computational study on the microwave energy based regeneration in diesel particulate filter for exhaust emission control", Journal of the Energy Institute, 2020 Publication	<1%
48	erefdn.org Internet Source	<1%
49	www.cheric.org Internet Source	<1%
50	digitalcommons.mtu.edu Internet Source	<1%
51	Liliana Alzate-Gaviria. "Chapter 8 Microbial Fuel Cells for Wastewater Treatment", IntechOpen, 2011 Publication	<1%
52	Cheng, Shaoan, and Weifeng Liu. "Microbial Fuel Cells and Other Bio-Electrochemical Conversion Devices", Electrochemically Enabled Sustainability, 2014. Publication	<1%
53	microbialcellfactories.biomedcentral.com Internet Source	<1%

54	Payel Choudhury, Uma Shankar Prasad Uday, Nibedita Mahata, Onkar Nath Tiwari et al. "Performance improvement of microbial fuel cells for waste water treatment along with value addition: A review on past achievements and recent perspectives", Renewable and Sustainable Energy Reviews, 2017 Publication	<1%
55	S Tiwari. "Investigation into the potential of iontophoresis facilitated delivery of ketorolac", International Journal of Pharmaceutics, 2003 Publication	<1%
56	iwaponline.com Internet Source	<1%
57	irep.ntu.ac.uk Internet Source	<1%
58	web.stanford.edu Internet Source	<1%
59	publications.waset.org Internet Source	<1%
60	isees.in Internet Source	<1%
61	cel.webofknowledge.com Internet Source	<1%
62	Hong Liu, Bruce E. Logan. "Electricity	

	Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane", Environmental Science & Technology, 2004 Publication	<1%
63	www.ijert.org Internet Source	<1%
64	gjournals.org Internet Source	<1%
65	iahr.tandfonline.com.tandf-prod.literatumonline.com Internet Source	<1%
66	hidrogeno.org.mx Internet Source	<1%
67	Advances in Water Treatment and Pollution Prevention, 2012. Publication	<1%
68	hyper.ahajournals.org Internet Source	<1%
69	Wulin Yang, Ruggero Rossi, Yushi Tian, Kyoung-Yeol Kim, Bruce E. Logan. "Mitigating external and internal cathode fouling using a polymer bonded separator in microbial fuel cells", Bioresource Technology, 2018 Publication	<1%
	ruidera.uclm.es	

70	Internet Source	<1%
71	Asimina Tremouli, Georgia Antonopoulou, Symeon Bebelis, Gerasimos Lyberatos. "Operation and characterization of a microbial fuel cell fed with pretreated cheese whey at different organic loads", Bioresource Technology, 2013 Publication	<1%
72	serdp-estcp.org Internet Source	<1%
73	Rachna Goswami, Vijay Kumar Mishra. "A review of design, operational conditions and applications of microbial fuel cells", Biofuels, 2017 Publication	<1%
74	nuicone.org Internet Source	<1%
75	Antonopoulou, G.. "Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell", Biochemical Engineering Journal, 20100615 Publication	<1%
76	T. K. Sajana, M. M. Ghangrekar, A. Mitra. "In Situ Bioremediation Using Sediment Microbial Fuel Cell", Journal of Hazardous, Toxic, and	<1%

Radioactive Waste, 2017

Publication

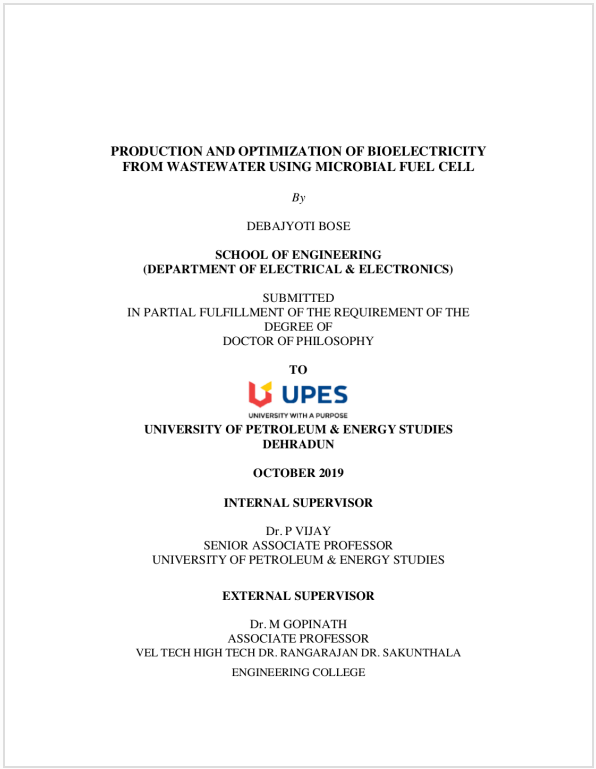
Exclude quotes	Off	Exclude matches	< 15 words
Exclude bibliography	On		

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Debajyoti Bose
Assignment title:	Plagiarism Check
Submission title:	PhD Thesis
File name:	DBose-Thesis-PlagCheck.pdf
File size:	7.82M
Page count:	200
Word count:	37,258
Character count:	197,282
Submission date:	22-Jul-2020 01:47PM (UTC+0530)
Submission ID:	1360736414



PRODUCTION AND OPTIMIZATION OF BIOELECTRICITY FROM WASTEWATER USING MICROBIAL FUEL CELL

By

DEBAJYOTI BOSE

SCHOOL OF ENGINEERING
(DEPARTMENT OF ELECTRICAL & ELECTRONICS)

SUBMITTED
IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE
DEGREE OF
DOCTOR OF PHILOSOPHY

TO

UPES
UNIVERSITY WITH A PURPOSE

UNIVERSITY OF PETROLEUM & ENERGY STUDIES
DEHRADUN

OCTOBER 2019

INTERNAL SUPERVISOR

Dr. P VIJAY
SENIOR ASSOCIATE PROFESSOR
UNIVERSITY OF PETROLEUM & ENERGY STUDIES

EXTERNAL SUPERVISOR

Dr. M GOPINATH
ASSOCIATE PROFESSOR
VEL TECH HIGH TECH DR. RANGARAJAN DR. SAKUNTHALA
ENGINEERING COLLEGE