BIOELECTRICITY AND WASTE MANAGEMENT

A DEMONSTRATIVE STUDY OF MICROBIAL FUEL CELL FOR THE SUSTAINABLE PRODUCTION OF ELECTRICITY

PROJECT-B

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ABSTRACT

Globally, there are staggering amounts of bio-waste, which could actually be considered as stored energy. This waste in liquid form could allow bacteria to convert it to electricity. A microbial fuel cell employs the use of these microbes to metabolise waste and generate ions. This won't only reduce the environmental stress but also reduce the operational cost for various industries.

The microorganisms involved aerobically metabolise the hydrocarbons in the biological waste by means of the Kreb's cycle, releasing ions into the chamber. The ions travel from the anode to the cathode chamber via a salt bridge, creating a flow of ions, or a current. This current travels through an external circuit and is thus a viable source of electric power. This creates an infinite loop of ion transfer, hence generating a stable electrical connection.

Bacterial strains *Shewanella oneidensis* and *Geobacter sulfurreducens* are the major contributors to this process in this project.

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CHAPTER 1

INTRODUCTION

1.1 Microbial Fuel Cell

It has been known for almost one hundred years that bacteria could generate electricity but only in the past few years has this capability become more than a laboratory novelty. The microbial fuel cell (MFC) is a new form of renewable energy technology that can generate electricity from what would otherwise be considered waste. The reasons for this recent interest in using bacteria to generate electricity are a combination of the need for new sources of energy, discoveries about microbial physiology related to electron transport, and advancement of fuel cell technologies.

Microbial fuel cells produce electricity from organic matters. Unlike conventional fuel cells, MFCs have certain advantages like high energy-conversion efficiency and mild reaction conditions. In addition, a fuel cell's emissions are well below regulations. MFCs also use energy much more efficiently than standard combustion engines which are limited by the Carnot Cycle. In theory an MFC is capable of energy efficiency far beyond 50%. In fact, using the new microbial fuel cells, conversion of the energy to hydrogen is 8 times as high as conventional hydrogen production technologies. In a MFC, bacteria are separated from a terminal electron acceptor at the cathode so that the only means for respiration is to transfer electrons to the anode. MFC is thus a bio electrochemical system that derives electricity by mimicking bacterial interactions found in nature. Microorganisms catabolize compounds such as glucose, acetate or wastewater. It is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms.

A typical microbial fuel cell consists of anode and cathode compartments. In the anode compartment, fuel is oxidized by microorganisms, generating electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, and the protons are transferred to the cathode compartment through a separator. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water. In general, there can be two types of microbial fuel cells: cells with mediator and cells without mediator. Such biological fuel cells take glucose and methanol from food scraps and convert it into hydrogen and food for the bacteria. The electrons gained from this oxidation are transferred to an anode, where they depart through an electrical circuit before reaching the cathode. Here they are transferred to a high potential electron acceptor such as oxygen. As current now flows over a potential difference, power is generated directly from microbial fuel by the catalytic activity of bacteria. The microorganisms have the ability to produce electrochemically active substances that may

be either metabolic intermediaries or final products of anaerobic respiration. When microorganisms consume a substrate such as sugar in aerobic conditions they produce carbon dioxide and water. However, when oxygen is not present, they produce carbon dioxide, protons and electrons.

$$C_{12}H_{22}O_{11} + 13H_2O \text{ ---> } 12CO_2 + 48H^+ + 48e^-$$

The cells thereafter are made to use inorganic mediators to tap into the electron transport chain of cells and to accept the electrons that are produced. The mediator crosses the outer cell lipid membranes and plasma wall; it then begins to liberate electrons from the electron transport chain that would normally be taken up by oxygen and other intermediates. The reduced mediator exits the cell laden with electrons that it carries to an electrode where it deposits them. This electrode becomes the electrogeneric anode or negatively charged electrode. The release of the electrons means that the mediator returns to its original oxidized state ready to repeat the process. It is important to note that this can only happen under anaerobic conditions. If oxygen is present, it will collect all the electrons as it has a greater electronegativity than the mediator. A number of mediators have been suggested for use in microbial fuel cells. These include natural red, methylene blue, thionine or resorfuin etc.

This is the principle behind generating a flow of electrons from micro-organisms. In order to turn this into a usable supply of electricity, this process has to be accommodated in a fuel cell. To generate a useful current, it is then necessary to create a complete circuit. The mediator and the micro-organism are mixed together in a solution to which is added a suitable substrate, glucose for example. This mixture is placed in a sealed chamber to stop the entry of oxygen, forcing the micro-organism to use anaerobic respiration thereby. An electrode is placed in the solution, which would then act as the anode. In the second chamber of the MFC, there is placed another solution and an electrode. This electrode, the cathode, is positively charged and is the equivalent of the oxygen sink at the end of the electron transport chain. It is however external to the biological cell. The solution is an oxidizing agent that picks up the electrons at the cathode. Incidentally, this is not particularly practical as it would require large volumes of circulating gas. A more convenient option is to use a solution of a solid oxidizing agent.

1.2 Project Objectives

- To construct microbial fuel cells using cow dung, drain water, rice washing water, and slurry collected from biogas plant.
- To derive mathematical models to express voltage generated from the cells as a continuous function of time, so as to have an idea practically how long a cell remains functional.
- To evaluate the rate of change of the generated voltage with respect to time.
- To extrapolate how long the microbial fuel cells stay functioning.
- To observe whether a mixture of bio-waste does actually result in higher voltages.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The first report that bacteria can generate electricity appeared almost a hundred years ago. However, it did not gain any major coverage at that time. It is only in recent years that this ability of microbes has been rediscovered. The reason for this renewed interest, as mentioned above, is the need for new resources of energy and better understanding of the microbial system in relation to the electron transport and eventually, the development of Microbial Fuel Cells. [1] A Microbial Fuel Cell (MFC) is capable of generating electricity directly from a large variety of organic or inorganic compounds, using a microbe as a catalyst. Conventionally, fuel cells convert chemical energy to electrical energy, by consumption of a fuel at the anode and an oxidant at the cathode. The electrons and protons released travel through an external circuit, producing electricity (See equation below). In MFCs, the anode and cathode are separated by an ion exchange membrane, and a solution consisting of organic matter and microbes is used as fuel (Fig. 2.1) [4].

$$\text{Anode: } C_6H_{12}O_6 + 6H_2O \longrightarrow 6C0_2 + 24H^+ + 24e^-$$
$$\text{Cathode: } 60_2 + 24H^+ + 24e^- \longrightarrow 12H_2O$$
$$C_6H_{12}O_6 + 60_2 \longrightarrow 6C0_2 + 6H_2O + \text{Electrical Energy} \quad [1]$$

Microbial fuel cells thus have the property of consuming almost any type of organic waste, and generating energy at the same time. Most of the substrates they use, like sugar and starch, are readily available, easy to store, dose and are greener than, for e.g., methanol. This twin set of extraordinary uses, if perfected, may well resolve the energy crisis and waste disposal problems that the world currently faces. Moreover, MFCs have many distinct advantages over the conventional fuel cells. For one, they have higher efficiency, and produce little pollution.

In the near future, MFCs may be developed to such a stage that they can give a reasonable and usable power output per unit the MFC volume. In such a viable scenario, a larger battery size could well be overlooked, provided the maintenance is easy and has a green and safe label [1]. This eco-friendly fuel

cell will then lead to several ground-breaking applications. As the amount of low-power devices implanted in the human body increases, the long term, stable power source used may well be the MFC.

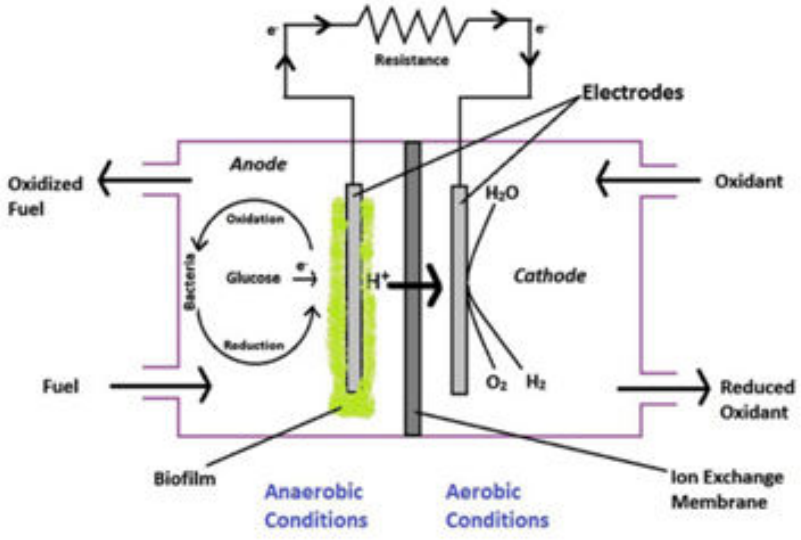

Figure 2.1 A schematic representation of MFC [Ref. 4]

Biomass has an energetic value, whether it is considered as foodstuff, energy crop or waste (in which case the value is generally negative). On average, 1 kg of sugar, as a model component, contains 4.41 kWh of energy or potentially 13106 Coulombs of charge. This 1 kg of sugar also represents 1.06 kg chemical oxygen demand (COD). Out of 1 kg carbohydrates, one can currently produce 0.5 L ethanol, 1.2 m3 H_2 gas, 0.36 m3 CH_4 gas or 0.5 m3 biogas. On average, these processes yield w1 kWh of useful energy. In the EU, 1 kWh is worth up to V0.16. Because the production of this 1 kg of sugar costs about V0.25 and the market value approximates V1, using sugar to drive batteries is not a process feasible at large scale. However, much biomass is available on the market for low or negative prices. Although the intrinsic quality of this 'waste-biomass' is lower, the energy yield might still be sufficient to allow energy recovery by means of the MFC process [2].

Water based organic matters that can be used in an MFC can be simple carbohydrates, acetate and butyrate, and complex organic compounds, domestic wastewater, and manure sludge. It is noteworthy that anaerobic digestion is basically goes on inside the MFC. Anaerobic digestion is typically applied in

sewage sludge treatment due to its advantages over aerobic systems, such as lower energy consumption, smaller amounts of solids generated, lower nutrient requirement and potential energy recovery from the produced biogas. Sewage sludge is stabilized during anaerobic digestion by converting most organic matter into biogas.

Microbial fuel cells use biocatalysts for the conversion of chemical energy to electrical energy. As most organic substrates undergo combustion with the evolution of energy, the biocatalyzed oxidation of organic substances, by oxygen or other oxidizers, provides a means for the conversion of chemical to electrical energy. Biocatalysts take part in the process of producing electricity in either of the two following ways: the biocatalysts can generate the fuel substrates for the cell by biocatalytic transformations or metabolic processes, or the biocatalysts may participate in the electron transfer chain between the fuel substrates and the electrode surfaces. A variety of electron mediators are used for the electrical contacting of the biocatalyst and the electrode.

Microorganisms have the ability to produce electrochemically active substances that may be either the metabolic intermediaries, or the final products of anaerobic respiration. For the purpose of energy generation, these fuel substances can be produced in one place and transported to a microbial fuel cell to be used as fuel. The biocatalytic microbial reactor produces the microbial fuel. The biological part of the device is however not directly integrated with the electrochemical part. It allows the electrochemical part to operate under conditions that are not compatible with the biological part of the device.

In another approach, the microbiological fermentation process proceeds directly in the anodic compartment of the fuel cell, supplying the anode with the fermentation products. In this case, the operational conditions in the anodic compartment are governed by the biological system, and therefore they are significantly different from those in conventional fuel cells. This would be a real microbial fuel cell which is not a simple combination of a bioreactor with a conventional fuel cell. This configuration is also often based on the biological production of hydrogen gas, but the electrochemical oxidation of H_2 is performed in presence of the biological components under mild conditions. Yet a third approach involves the application of artificial electron transfer relays that can send electrons between the microbial biocatalytic system and the electrode back and forth. The mediator molecules take electrons from the biological electron transport chain of the microorganisms and transport them to the anode of the microbial fuel cell. In this case, the biocatalytic process performed in the microorganisms becomes different from the natural one, because the electron flow goes to the anode and not to a natural acceptor of electrons. Since the natural electron acceptor is expectedly more efficient, it can compete with the desired scheme, and hence it is usually removed from the system. In most of the cases, the microbiological system operates under anaerobic conditions, allowing electron transfer to the artificial electron relays and, finally to the anode.

Various bacteria and algae have been found to be active in hydrogen production under anaerobic conditions. The most effective hydrogen production is observed upon fermentation of glucose in the presence of Clostridium butyricum. This conversion of carbohydrate to hydrogen is achieved by a multienzyme system. Immobilization of the hydrogen-producing bacteria, Clostridium butyricum is very important because it stabilizes the unstable hydrogenase system. In order to stabilize the biocatalytic performance, bacteria were in fact introduced into agar gel and filter paper. There are many microorganisms producing metabolically reduced sulfur containing compounds such as sulfides and sulfites. Sulfate reducing bacteria form a specialized group of anaerobic microbes that use sulfate as a terminal electron acceptor for their respiration. These microorganisms yield S2- while using a substrate, lactate for example, as a source of electrons. This microbiological oxidation of lactate with the formation of sulfide has also been used to drive an anodic process in microbial fuel cells [3].

Accumulation of sulfides in the medium results in the inhibition of the metabolic bacteria because they interact with iron containing proteins, blocking the electron transport systems. To prevent the toxic effect of H_2S, the anode should effectively oxidize it. However, many metallic electrodes are corrupted by sulfides because of their strong and irreversible adsorption. Therefore, porous graphite electrodes have also been used for the purpose. Many different organic and organometallic compounds have been tested in combination with bacteria to test the efficiency of mediated electron transfer from the internal bacterial metabolites to the anode of the microbial fuel cells. Thionine has been used extensively as a mediator of electron transport from Escherichia coli. Ferric complexes have also been successfully used for oxidizing glucose. Since thionine has frequently been used as a mediator in microbial fuel cells, mono and disulfonated derivatives of thionine have been applied to determine the effect of hydrophilic substituents on the mediation of electron transfer [2].

Engineering of the electrochemical cell provides a means of enhancing the electrical contact between a biocatalytic system and an anode, and hence to improve the cell output. The interfacial contact has been found to increase while using a three dimensional packed bed anode. It has been shown that the performance of a microbial fuel cell depends heavily on the primary substrate used in the process of fermentation. The metabolic process in the bacteria is very complex. It involves many enzymes. It may proceed by many different routes. It has been shown that a mixture of nutritional substrates can result even in higher extractable current than any single component [1].

Microbial fuel cells require continuous fermentation of living cells performing numerous physiological processes thus dictating stringent working conditions. In order to overcome this constraint, redox enzymes responsible for the desired processes may be separated and purified from living organisms and applied as biocatalysts in microbial fuel cells. Enzymes are expensive chemicals. Special ways for their utilization still remains to be established. Methods have however been suggested to electrically

contact redox enzymes and electrode supports. The methods of biocatalytic electrodes for oxidation of potential fuel substrates that act as biocatalytic anodes, and reduction of oxidizers that act as biocatalytic cathodes can be of three types. Such electrodes can then be integrated into microbial fuel cell elements. The methods are: anodes for microbial fuel cells based on enzyme catalyzed oxidative reactions, cathodes for microbial fuel cells based on enzyme catalyzed reductive reactions, and microbial fuel cells based on layered enzyme electrodes [3].

In the first method, in which anodes for microbial fuel cells based on enzyme catalyzed oxidative reactions are used, the electrochemical oxidation of fuels can be biocatalyzed by enzymes communicating electrically with electrodes. Different classes of oxidative enzymes, oxidases, and dehydrogenases, for example, require the application of different molecular tools to establish this electrical process. The mediator molecules can be adsorbed directly onto electrodes, incorporated into polymer layers, or covalently linked to functional groups on electrode surfaces. The electrical contacting of redox enzymes that defy direct electrical communication with electrodes can alternatively be established by using synthetic or biologically active charge carriers as intermediates between the redox center and the electrode. The overall electrical efficiency of an enzyme modified electrode depends not only on the electron transport properties of the mediator, but also on the steps of transfer occurring in the assembly. Diffusional electron relays have been utilized to make electrons shuttle between oxidative enzymes and anodes of microbial fuel cells, providing the bioelectrocatalyzed oxidation of organic fuels, methanol for example [2].

In the second method, cathodes for microbial fuel cells based on enzyme catalyzed reductive reactions are used. The biocatalytic reduction of oxidizers has attracted much less attention than the biocatalytic oxidation of fuels. Nonetheless, in order to construct a microbial fuel cell element, it is essential to design a functional cathode for the reduction of the oxidizer that is coupled with the anode, and allows the electrically balanced current flow. Conventional oxygen reducing cathodes used in fuel cells are usually not compatible with biocatalytic anodes since high temperature and pressure are applied for their operation. Thus, biocatalytic reductive processes at the cathode should be considered as a strategy to design all biomaterial-based functional fuel cells [2].

It has been reported that bioelectrocatalyzed reduction of H_2O_2 has been accomplished in presence of various peroxidases such as horseradish peroxidase. The biocatalytic reduction of oxidizers in no aqueous solutions immiscible with water is important because it can be coupled to biocatalytic oxidative processes through liquid liquid interfaces. Some enzymes, particularly peroxidases can function in no aqueous solutions. Horseradish peroxidase electrodes have been tested for biocatalytic reduction of organic peroxides in no aqueous solvents. The biocatalytic activity of enzymes, however, is usually lower in organic solvents than in water. Biocatalytic systems composed of enzymes and their respective

electron transfer mediators such as bilirubin oxidase, or fungal lactase are able to biocatalyze the electroreduction of O_2 to H_2O significantly decreasing the potential. These systems, however, are composed of dissolved enzymes and mediators operating through a diffusion path that is not acceptable for technological applications [2].

Rotating disk electrode experiments have been performed to estimate the electron transfer rate for the overall bioelectrocatalytic process corresponding to the reduction of O_2. Physical characterization of the systems is essential to optimize the electrode performance. For each of the functional electrodes, the complex sequence of reactions must be resolved kinetically in order to determine the rate limiting step. Once the rate limiting step is identified, biomaterial engineering on the respective redox protein could be undertaken to optimize its electron transfer functionality [4].

The third method is to use microbial fuel cells based on layered enzyme electrodes. The bioelectrocatalyzed reduction of H_2O_2 and the oxidation of glucose allow us to design microbial fuel cells using H_2O_2 and glucose as cathodic and anodic substrates respectively. The potentials of the electrodes are negatively shifted and positively shifted when the concentrations of the glucose and H_2O_2 are elevated. The microbial fuel cell voltage and current outputs are oxygen insensitive. This oxygen insensitivity of the bioelectrocatalytic process at the anode originates from the effective electrical contact of the surface with the electrode support, as a result of its alignment. The stability of the microbial fuel cell was examined at an optimal loading resistance as a function of time. The power decreases by about 50% after 3 hours of cell operation. This loss could originate from depletion of the fuel substrate, leakage of the fuel or the oxidizer into the wrong compartment, or degradation of the biocatalysts. Since the cell voltage appears to be stable, the current produced also decreases by the same factor. Recharging the cell with the fuel substrate and oxidizer could compensate for this component of the decrease in the current output. Charge transfer processes across the interface between two immiscible electrolyte solutions can provide an additional potential difference between cathodic and anodic reactions due to the potential difference at the liquid-liquid interface. Many different interfacial liquid-liquid systems have been studied using numerous experimental approaches. The reduction of peroxide in dichloromethane, and the oxidation of glucose in aqueous solution, bioelectrocatalyzed by the electrode, enables us to design a liquid-liquid interface microbial fuel cell using peroxide and glucose as cathodic and anodic substrates respectively [2].

2.2 Metabolism of Microbes in MFCs [3]

To assess bacterial electricity generation, metabolic pathways governing microbial electron and proton flows must be determined. In addition to the influence of the substrate the potential of the anode

will also determine the bacterial metabolism. Increasing MFC current will decrease the potential of the anode, forcing the bacteria to deliver the electrons through more-reduced complexes. The potential of the anode will therefore determine the redox potential of the final bacterial electron shuttle, and therefore, the metabolism. Several different metabolism routes can be distinguished based on the anode potential: high redox oxidative metabolism; medium to low redox oxidative metabolism; and fermentation. Hence, the organisms reported to date in MFCs vary from aerobes and facultative anaerobes towards strict anaerobes.

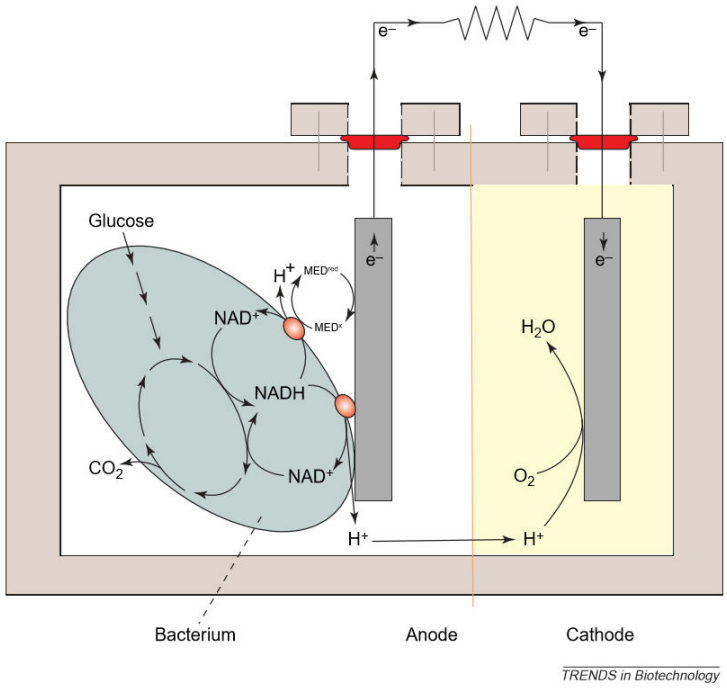

Figure 2.2. The working principle of a microbial fuel cell [Ref. 4]

At high anodic potentials, bacteria can use the respiratory chain in an oxidative metabolism. Electrons and, concomitantly, protons can be transported through the NADH dehydrogenase, ubiquinone, coenzyme Q or cytochrome. The use of this pathway was investigated by Kim et al. (2004). They observed that the generation of electrical current from an MFC was inhibited by various inhibitors of the respiratory chain. The electron transport system in their MFC used NADH dehydrogenase, Fe/S (iron/sulphur) proteins and quinones as electron carriers, but does not use site 2 of the electron transport chain or the terminal oxidase. Processes using oxidative phosphorylation have regularly been observed in MFCs, yielding high energy efficiencies of up to 65%. Examples are consortia containing Pseudomonas aeruginosa, Enterococcus faecium and Rhodoferax ferrireducens. If the anode potential decreases in the

presence of alternative electron acceptors such as sulphate, the electrons are likely to be deposited onto these components.

Methane production has repeatedly been observed when the inoculum was anaerobic sludge, indicating that the bacteria do not use the anode. If no sulphate, nitrate or other electron acceptors are present, fermentation will be themain process when the anode potential remains low. As repeatedly observed, this metabolic type can imply a high acetate or butyrate production. Several organisms that are known to produce fermentation products and belong to the genus Clostridium, Alcaligenes, Enterococcus, have been isolated from MFCs. This pathway is further substantiated by the significant hydrogen production observed when MFC enriched cultures are incubated anaerobically in a separate fermentation test.

Fermentation products such as acetate can be oxidized at low anode potential by anaerobic bacteria such as Geobacter species, which is capable of withdrawing electrons from acetate in MFC conditions.

2.3 Types and Natures of electrodes [4]

2.3.1 Anode

Anodic materials must be conductive, biocompatible, and chemically stable in the reactor solution. Metal anodes consisting of non-corrosive stainless steel mesh can be utilized (44), but copper is not useful due to the toxicity of even trace copper ions to bacteria. The most versatile electrode material is carbon, available as compact graphite plates, rods, or granules, as fibrous material (felt, cloth, paper, fibres, foam), and as glassy carbon. There are numerous carbon suppliers worldwide, for example E_TEK and Electrosynthesis Co. Inc. (USA), GEE Graphite Limited, Dewsbury (UK), Morgan, Grimbergen (Belgium), and Alfa-Aesar (Germany). The simplest materials for anode electrodes are graphite plates or rods as they are relatively inexpensive, easy to handle, and have a defined surface area. Much larger surface areas are achieved with graphite felt electrodes which have high surface areas. However, not all the indicated surface area will necessarily be available to bacteria.

Carbon fibre, paper, foam, and cloth (Toray) have been extensively used as electrodes. It has been shown that current increases with over all internal surface area in the order carbon felt > carbon foam > graphite. Substantially higher surface areas are achieved either by using a compact material like reticulated vitreous carbon (RVC; ERG Materials and Aerospace Corp., Oakland, CA) which is available with different pore sizes, or by using layers of packed carbon granules (Le Carbone, Grimbergen, Belgium) or beads. In both cases maintaining high porosity is important to prevent clogging. The long term effect of biofilm growth or particles in the flow on any of the above surfaces has not been adequately examined.

To increase the anode performance, different chemical and physical strategies have been followed. Park et al. incorporated Mn(IV) and Fe(III) and used covalently linked neutral red to mediate the electron transfer to the anode. Electrocatalytic materials such as polyanilins/Pt composites have also been shown to improve the current generation through assisting the direct oxidation of microbial metabolites. Directing the water flow through the anode material can be used to increase power. Cheng et al. found that flow directed through carbon cloth toward the anode, and decreasing electrode spacing from 2 to 1 cm, increased power densities (normalized to the cathode projected surface area) from 811 to 1540 mW/m^2 in an air-cathode MFC. The increase was thought to be due to restricted oxygen diffusion into the anode chamber, although the advective flow could have helped with proton transport toward the cathode as well. Increased power densities have been achieved using RVC in an up flow UASB type MFC or in a granular anode reactor with ferricyanide cathodes. Flow through an anode has also been used in reactors using exogenous mediators.

2.3.2 Cathode

Due to its good performance, ferricyanide ($K_3[Fe(CN)_6]$) is very popular as an experimental electron acceptor in microbial fuel cells. The greatest advantage of ferricyanide is the low overpotential using a plain carbon cathode, resulting in a cathode working potential close to its open circuit potential. The greatest disadvantage, however, is then sufficient re-oxidation by oxygen, which requires the catholyte to be regularly replaced. In addition, the long term performance of the system can be affected by diffusion of ferricyanide across the CEM and into the anode chamber. Oxygen is the most suitable electron acceptor for an MFC due to its high oxidation potential, availability, low cost (it is free), sustainability, and the lack of a chemical waste product (water is formed as the only end product).

The choice of the cathode material greatly affects performance, and is varied based on application. For sediment fuel cells, plain graphite disk electrodes immersed in the sea water above the sediment have been used. Due to the very slow kinetics of the oxygen reduction at plain carbon, and the resulting large overpotential, the use of such cathodes restricts the use of this non-catalyzed material to systems that can tolerate low performance. In seawater, oxygen reduction on carbon cathodes has been shown to be microbially supported. Such microbially assisted reduction has also been observed for stainless steel cathodes which rapidly reduces oxygen when aided by a bacterial biofilm. To increase the rate of oxygen reduction, Pt catalysts are usually used for dissolved oxygen or open-air (gas diffusion) cathodes. To decrease the costs for the MFC the Pt load can be kept as low as 0.1 mg cm^{-2}. The long term stability of Pt needs to be more fully investigated, and there remains a need for new types of inexpensive catalysts. Recently, noble-metal free catalysts that use pyrolyzed iron (II) phthalocyanine or CoTMPP have been proposed as MFC cathodes.

2.4 Fundamentals of Voltage Generation in MFCs [3]

Thermodynamics and the Electromotive Force. Electricity is generated in an MFC only if the overall reaction is thermodynamically favourable. The reaction can be evaluated in terms of Gibbs free energy expressed in units of Joules(J), which is a measure of the maximal work that can be derived from the reaction, calculated as

$$\Delta G_r = \Delta G_r^0 + RT\ln(\Pi) \qquad (1)$$

where ΔGr (J) is the Gibbs free energy for the specific conditions, ΔGr0 (J) is the Gibbs free energy under standard conditions usually defined as 298.15 K, 1 bar pressure, and 1 M concentration for all species, R (8.31447 J mol-1 K-1) is the universal gas constant, T (K) is the absolute temperature, and Π (unitless) is the reaction quotient calculated as the activities of the products divided by those of the reactants. The standard reaction Gibbs free energy is calculated from tabulated energies of formation for organic compounds in water. For MFC calculations, it is more convenient to evaluate the reaction in terms of the overall cell electromotive force (emf), Eemf (V), defined as the potential difference between the cathode and anode. This is related to the work, W (J), produced by the cell, or

$$W = E_{emf}\, Q = -\Delta G_r \qquad (2)$$

where Q) nF is the charge transferred in the reaction, expressed in Coulomb (C), which is determined by the number of electrons exchanged in the reaction, n is the number of electrons per reaction mol, and F is Faraday's constant (9.64853 × 104 C/mol). Combining these two equations, we have

$$E_{emf} = -\frac{\Delta G_r}{nF} \qquad (3)$$

If all reactions are evaluated at standard conditions, Π = 1, then

$$E^0_{emf} = -\frac{\Delta G^0_r}{nF} \qquad (4)$$

where E^0_{emf} (V) is the standard cell electromotive force. We can therefore use the above equations to express the overall reaction in terms of the potentials as

$$E_{emf} = E^0_{emf} - \frac{RT}{nF}\ln(\Pi) \qquad (5)$$

The advantage of equation 5 is that it is positive for a favourable reaction, and directly produces a value of the emf for the reaction. This calculated emf provides an upper limit for the cell voltage; the actual potential derived from the MFC will be lower due to various potential losses. Standard Electrode Potentials. The reactions occurring in the MFC can be analysed in terms of the half cell reactions, or the separate reactions occurring at the anode and the cathode. According to the IUPAC convention, standard potentials (at 298 K, 1 bar, 1 M) are reported as a reduction potential, i.e., the reaction is written as consuming electrons. For example, if acetate is oxidized by bacteria at the anode we write the reaction as

$$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O \qquad (6)$$

The standard potentials are reported relative to the normal hydrogen electrode (NHE), which has a potential of zero at standard conditions (298 K, pH2) 1 bar, [H+]) 1 M). To obtain the theoretical anode potential, EAn, under specific conditions, we use eq 5, with the activities of the different species assumed to be equal to their concentrations. For acetate oxidation, we therefore have

$$E_{An} = E^0_{An} - \frac{RT}{8F}\ln\left(\frac{[CH_3COO^-]}{[HCO_3^-]^2[H^+]^9}\right) \qquad (7)$$

For the theoretical cathode potential, Ecat, if we consider the case where oxygen is used as the electron acceptor for the reaction, we can write

$$O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O \qquad (8)$$

$$E_{cat} = E^0_{cat} - \frac{RT}{4F}\ln\left(\frac{1}{pO_2[H^+]^4}\right) \qquad (9)$$

A variety of catholytes has been used, and for each of these the cell voltage varies. For example, manganese oxide and ferricyanide have been used as alternatives to oxygen. The pH of the cathode solution can also vary, affecting the overall cathode potential. Using eq 9 and tabulated standard potentials available for inorganic compounds(57) for several different conditions, it can be seen that the theoretical cathode potential for these different catholytes range from 0.361 to 0.805 V. The cell emf is calculated as

$$E_{emf} = E_{cat} - E_{an} \qquad (10)$$

Where the minus sign is a result of the definition of the anode potential as reduction reaction (although an oxidation reaction is occurring). Equation 10 demonstrates that using the same anode in a system with different cathode conditions would produce significantly different cell voltages, and thus different levels of power output. The power produced by an MFC therefore depends on the choice of the cathode, and this should be taken into account when comparing power densities achieved by different MFCs. Open Circuit Voltage (OCV). The cell emf is a thermodynamic value that does not take into account internal losses. The open circuit voltage (OCV) is the cell voltage that can be measured after some time in the absence of current. Theoretically, the OCV should approach the cell emf. In practice, however, the OCV is substantially lower than the cell emf, due to various potential losses. For example, a typical measured potential of a cathode using oxygen at pH 7 is about 0.2 V. This is clearly lower than the expected value of 0.805 V, indicating the large energy loss occurring at the cathode. This energy loss is often referred to as over potential, or the difference between the potential under equilibrium conditions and the actual potential, which for this case is 0.605 V (0.805 V - 0.2 V). This illustrates that the main application of thermodynamic calculations is to identify the size and nature of energy losses.

2.5 Basic MFC Designs [8]

Many different configurations are possible for MFCs. A widely used and inexpensive design is a two chamber MFC built in a traditional "H" shape, consisting usually of two bottles connected by a tube containing a separator which is usually a cation exchange membrane (CEM) such as Nafion or Ultrex, or a plain salt bridge. The key to this design is to choose a membrane that allows protons to pass between the chambers (the CEM is also called a proton exchange membrane (PEM), but optimally not the substrate or electron acceptor in the cathode chamber (typically oxygen). In the H-configuration, the membrane is clamped in the middle of the tubes connecting the bottle. However, the tube itself is not needed. As long as the two chambers are kept separated, they can be pressed up onto either side of the membrane and clamped together to form a large surface. An inexpensive way to join the bottles is to use a glass tube that is heated and bent into a U-shape, filled with agar and salt (to serve the same function as a cation exchange membrane), and inserted through the lid of each bottle. The salt bridge MFC, however, produces little power due the high internal resistance observed. H-shape systems are acceptable for basic parameter research, such as examining power production using new materials, or types of microbial communities that arise during the degradation of specific compounds, but they typically produce low power densities. The amount of power that is generated in these systems is affected by the surface area of the cathode relative to that of the anode and the surface of the membrane. The power density (P) produced by these systems is typically limited by high internal resistance and electrode-based losses. When comparing power produced by these systems, it makes the most sense to compare them on the basis of equally sized anodes, cathodes, and membranes.

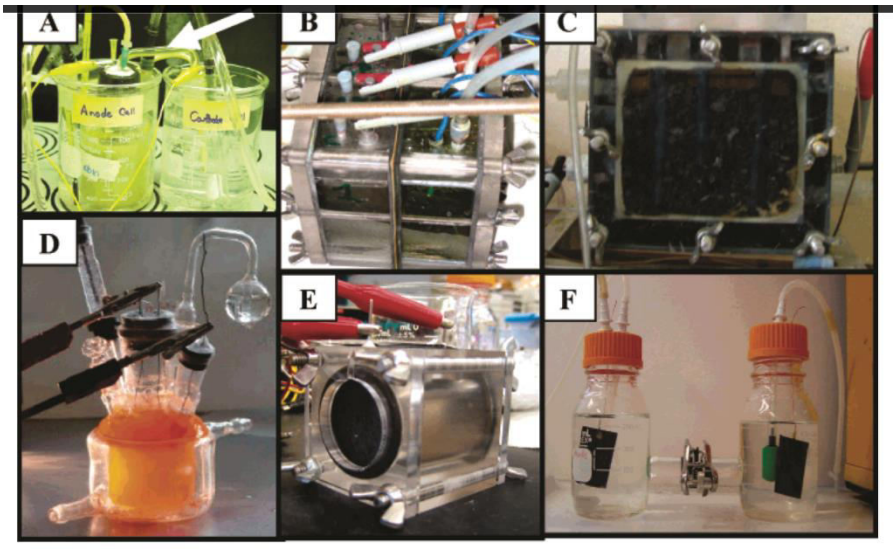

Figure 2.3 Types of MFCs used in studies [Ref. 3]

Using ferricyanide as the electron acceptor in the cathode chamber increases the power density due to the availability of a good electron acceptor at high concentrations. Ferricyanide increased power by 1.5 to 1.8 times compared to a Pt-catalyst cathode and dissolved oxygen (H-design reactor with a Nafion CEM). The highest power densities so far reported for MFC systems have been low internal resistance systems with ferricyanide at the cathode. While ferricyanide is an excellent catholyte in terms of system performance, it must be chemically regenerated and its use is not sustainable in practice. Thus, the use of ferricyanide is restricted to fundamental laboratory studies.

It is not essential to place the cathode in water or in a separate chamber when using oxygen at the cathode. The cathode can be placed in direct contact with air, either in the presence or absence of a membrane. In one system a kaolin clay-based separator and graphite cathode were joined to form a combined separator-cathode structure. Much larger power densities have been achieved using oxygen as the electron acceptor when aqueous-cathodes are replaced with air-cathodes. In the simplest configuration, the anode and cathode are placed on either side of a tube, with the anode sealed against a flat plate and the cathode exposed to air on one side, and water on the other. When a membrane is used in this air-cathode system, it serves primarily to keep water from leaking through the cathode, although it also reduces oxygen diffusion into the anode chamber.

The utilization of oxygen by bacteria in the anode chamber can result in a lower Coulombic efficiency (defined as the fraction of electrons recovered as current versus the maximum possible recovery) (30). Hydrostatic pressure on the cathode will make it leak water, but that can be minimized by applying coatings, such as polytetrafloroethylene (PTFE), to the outside of the cathode that permit oxygen diffusion but limit bulk water loss. Several variations on these basic designs have emerged in an effort to increase power density or provide for continuous flow through the anode chamber (in contrast to the above systems which were all operated in batch mode).

Systems have been designed with an outer cylindrical reactor with a concentric inner tube that is the cathode and with an inner cylindrical reactor (anode consisting of granular media) with the cathode on the outside. Another variation is to design the system like an upflow fixed-bed biofilm reactor, with the fluid flowing continuously through porous anodes toward a membrane separating the anode from the cathode chamber.

Systems have been designed to resemble hydrogen fuel cells, where a CEM is sandwiched between the anode and cathode. To increase the overall system voltage, MFCs can be stacked with the systems shaped as a series of flat plates or linked together in series. Sediment MFCs. By placing one electrode into a marine sediment rich in organic matter and sulfides, and the other in the overlying oxic water, electricity can be generated at sufficient levels to power some marine devices. Protons conducted by the seawater can produce a power density of up to 28 mW/m^2. Graphite disks can be used for the

electrodes, although platinum mesh electrodes have also been used. “Bottle brush” cathodes used for sea water batteries may hold the most promise for long-term operation of unattended systems as these electrodes provide a high surface area and are made of noncorrosive materials. Sediments have also been placed into H-tube configured two-chamber systems to allow investigation of the bacterial community.

2.6 Modifications for Hydrogen Production [3]

By “assisting” the potential generated by the bacteria at the anode with a small potential by an external power source (> 0.25 V), it is possible to generate hydrogen at the cathode. These reactors, called bioelectrochemically assisted microbial reactors (BEAMRs) or biocatalyzed electrolysis systems, are not true fuel cells, however, as they are operated to produce hydrogen, not electricity. Through modifications of the MFC designs described above (to contain a second chamber for capturing the hydrogen gas), it should be possible to develop many new systems for hydrogen production.

CHAPTER 3
MATERIALS AND METHOD

3.1 Materials

Material Requirements:

- 18 gauge Copper Wires
- Digital Multimeter
- Poly Vinyl Chloride Pipe
- Jaggery
- Aerator
- Polyvinyl Chloride Containers
- pH meter
- BOD Oxygen Meter
- COD Oxygen Meter
- Copper Rods

Chemical Requirements:

- Distilled Water
- Sodium Chloride (NaCl)
- Type I Agar

Biological Requirements:

- Biomass (Activated Sludge, Waste water and Paper Mill Waste)
- Bacterial Strains (*Shewanella oneidensis* and *Geobacter sulfurreducens)*
- Cow Dung

3.2 Methodology

Salt Bridge preparation

1. Agar was digested in three litres of distilled water, making a 1.5% solution.
2. A super saturated salt solution (40%) was made with NaCl.
3. Both solutions were mixed and stirred vigorously until uniform.
4. The solution was poured into the PVC pipe.
5. The salt-agar solution was allowed to cool in the pipe and set.

Anode preparation

1. A fixed amount of biomass was added to the PVC chamber.
2. An additional 500 grams of cow dung was added to increase microbial content.
3. 450 grams of sucrose solution (using jiggery) was added as a carbon source.
4. An aerator was attached for constant mixing.
5. A copper rod was added as conducting electrode.

Experiment Number	Weight of Biomass (grams)
1	500
2	600
3	700
4	800
5	900
6	1000

Table 3.1: Weight of Biomass taken

Cathode Preparation

1. A fixed amount of distilled water was added to the PVC chamber.
2. 100 grams of Potassium Ferricyanide ($K_3[Fe(CN)_6]$) was added to it to improve ion exchange.
3. 150 grams of Sodium Chloride (NaCl) was added to improve ionisation in the cathode.
4. A copper rod was added as the conducting electrode.

Experiment Number	Weight of Water (grams)
1	350
2	450
3	550
4	650
5	750
6	850

Table 3.2: Weight of distilled water taken

Circuit Preparation

1. Both electrode chambers were connected with the help of the set salt bridge.
2. The copper rods were connected with the help of the copper wires to form a closed circuit.
3. A galvanometer is attached to the circuit and a multimeter was used to measure potential difference.

Fig. 3.1: Pilot project

CHAPTER 4

RESULTS AND DISCUSSION

Experiment Number	Weight of Biomass (grams)	Activated Sludge (Volts)	Paper Mill Waste (Volts)	Waste Water (Volts)
1	500	0.86	0.52	0.76
2	600	0.87	0.65	0.82
3	700	0.89	0.67	0.64
4	800	0.91	0.41	0.51
5	900	0.93	0.32	0.59
6	1000	0.90	0.11	0.74

Table 4.1: Weight of Biomass and Potential Difference

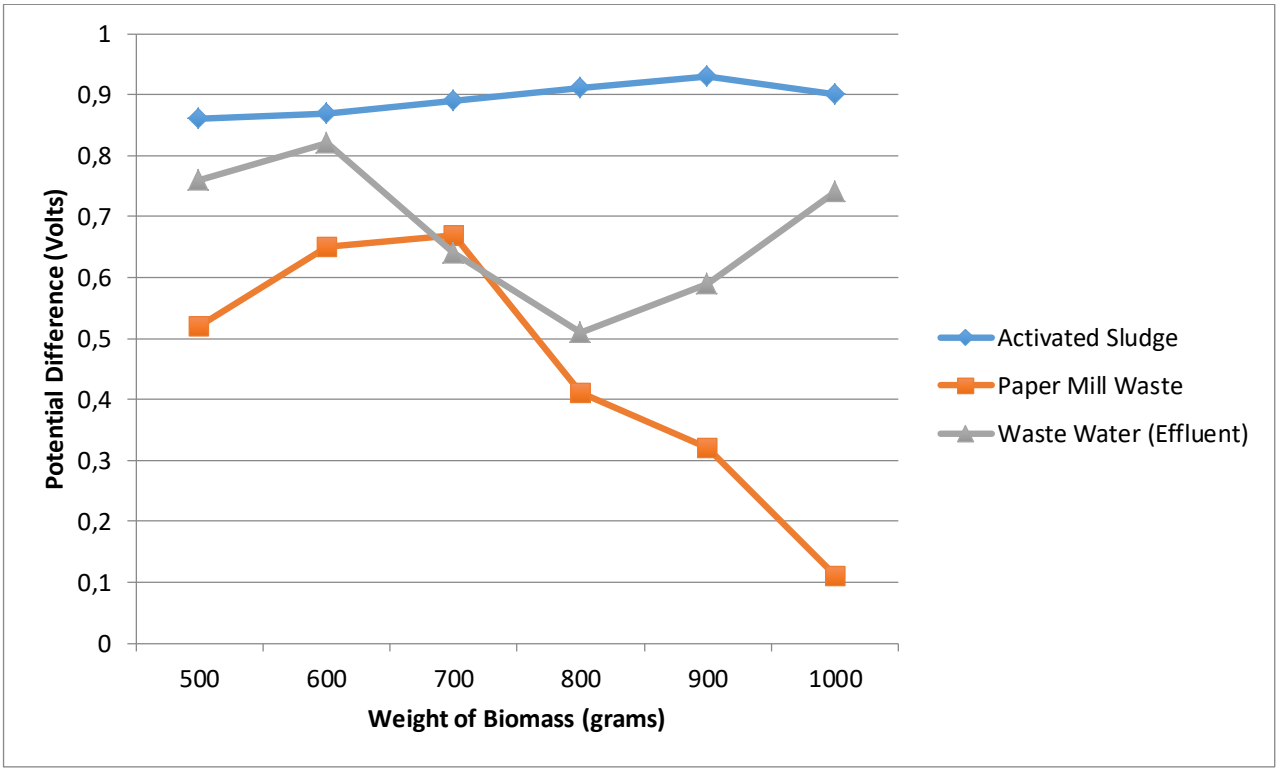

Figure 4.1: Potential Difference versus Weight of Biomass

The experiment utilised three types of sludge; Activated Sludge, Paper Mill Waste, and Waste Water in varied concentrations. After a series of experimentations, it was concluded that Activated Sludge provides a stable as well as the greatest potential difference compared to the other samples.

Experiment Number	Temperature (◦C)	Activated Sludge (Volts)	Paper Mill Waste (Volts)	Waste Water (Volts)
1	10	0.15	0.23	0.11
2	20	0.36	0.22	0.19
3	30	0.69	0.44	0.32
4	35	0.87	0.46	0.51
5	40	0.91	0.49	0.69
6	45	0.79	0.31	0.59

Table 4.2: Temperature of Biomass and Potential Difference

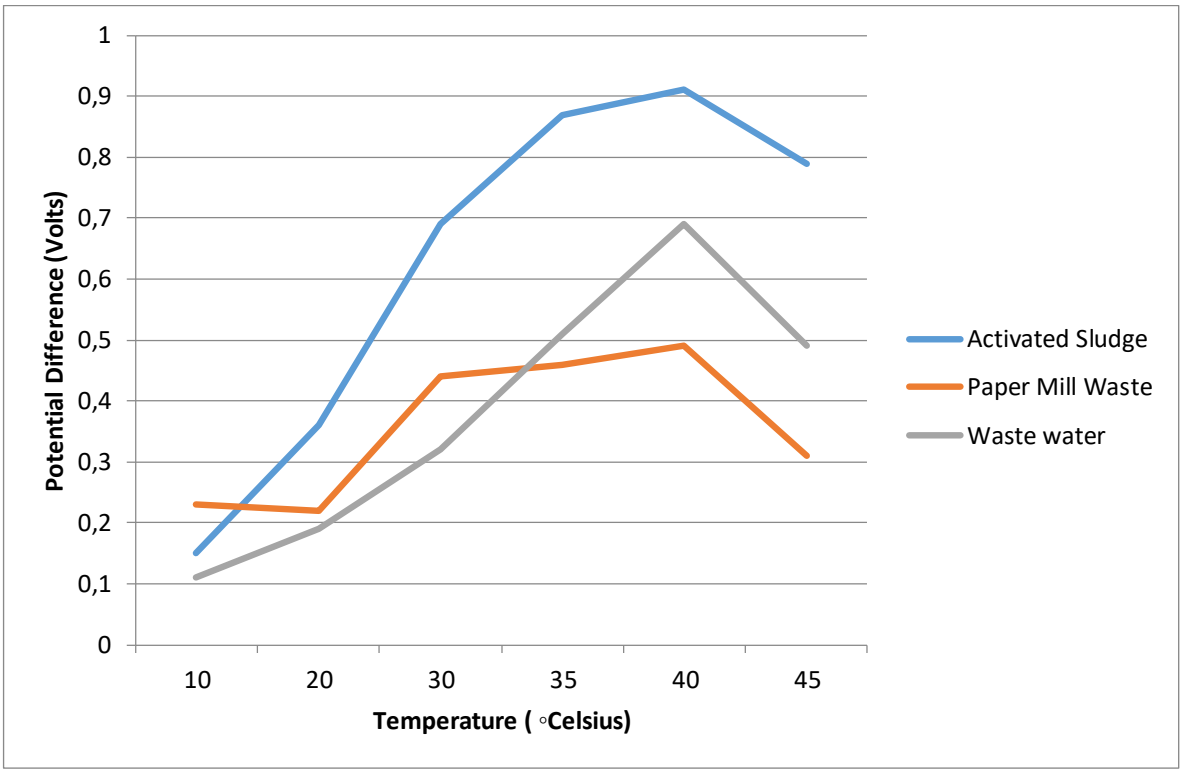

Figure 4.2: Potential Difference versus Temperature

Biomass, as a sample was exposed to various types of stress including temperature. All the samples were heated under controlled environment. This experiment proved that 35-40 ◦C was the most efficient temperature range for generation of electricity. 37.5 ◦C is the optimum temperature for cell metabolism, hence proving the result received.

Experiment Number	Time (Minutes)	Activated Sludge (Volts)	Paper Mill Waste (Volts)	Waste Water (Volts)
1	5	0.22	0.19	0.02
2	10	0.27	0.23	0.54
3	15	0.39	0.28	0.47
4	20	0.47	0.31	0.23
5	25	0.68	0.39	0.46
6	30	0.81	0.42	0.78
7	40	0.84	0.54	0.44
8	50	0.83	0.59	0.55
9	60	0.79	0.62	0.69
10	75	0.75	0.69	0.81
11	90	0.71	0.71	0.69
12	120	0.62	0.65	0.72

Table 4.3: Timeand Potential Difference

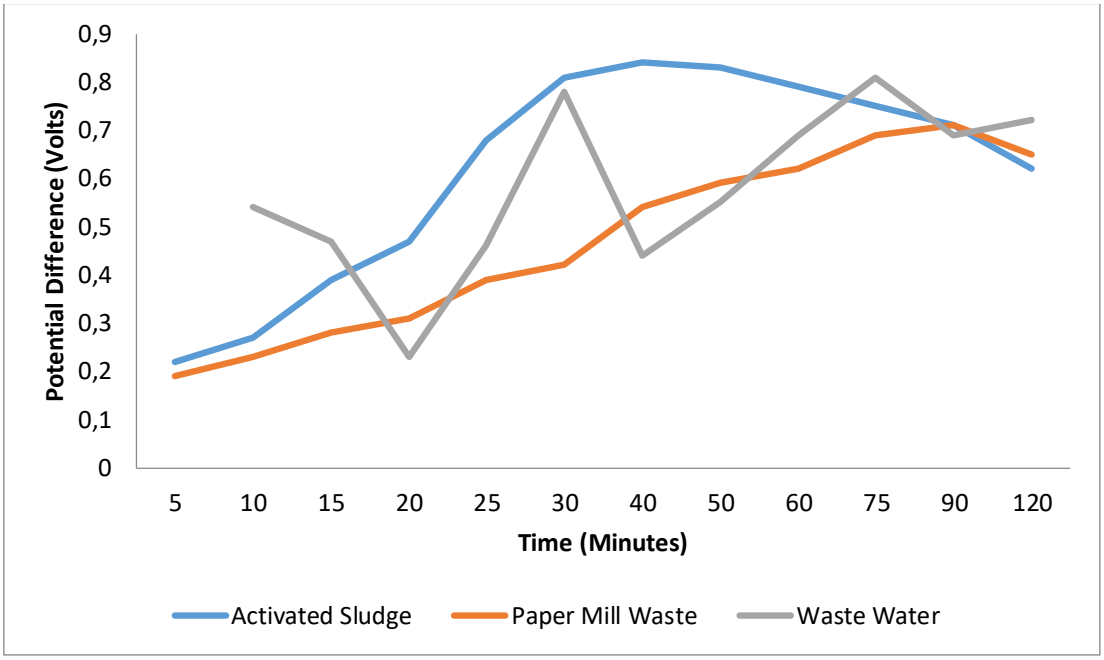

Figure 4.3: Potential Difference versus Time

The change in potential difference with respect to time was also monitored. This experiment proved that activated sludge requires 20-25 minutes of acclimation and it provides with the maximum electrical output.

Figure 4.4: Voltage and Current

Conclusion

Activated Sludge proved to be the most efficient source of energy. It is due to the presence higher microbial content compared to other sources of energy.

Activated Sludge produced the maximum potential difference along with the most stable and long lasting output.

CHAPTER 5
FUTURE PROSPECTS

The impending energy crisis and global warming warrant the need for eco-friendly sources of energy. At this juncture, when the atmosphere is laden with green-house gases, we can least afford to release stored carbon. Additionally, clean disposal of organic waste is also an environmental concern.

Any form of organic liquid waste can be used to generate electricity. As there is no need of any primary purification, a MFC can be incorporated in any industry. Hence reducing the operational cost, as a part of the total energy requirement can be supplied with the help of electricity generated. Moreover, the purification cost of effluent can also be decreased as MFC reduces organic waste, hence making it biodegradable.

Although the applications of MFCs as a viable source of alternate energy are as yet limited, with further improvements in design and efficiency, it would be possible to scale-up and use MFCs as a renewable resource in various fields.

Wastewater treatment can be carried out as MFCs perform the dual function of degrading effluents and generating power. This not only reduces the operational cost of a plant or an agricultural plant, powering of underwater monitoring devices and remote sensors can also be carried out. Another potential application is BOD sensing, i.e. using the MFC as a sensor for pollutant analysis and *in situ* process monitoring. Furthermore, hydrogen production by modified (anaerobic) MFCs for more efficient energy production. Such MFCs are termed bio-electrochemically assisted microbial reactors or BEAMRs.

If MFCs are used, it will not only solve the global energy crisis but also help in keeping the environment clean, as the waste generated is biodegradable.

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APPENDIX

Experiment Number	Weight of Biomass (grams)	Activated Sludge (Volts)	Paper Mill Waste (Volts)	Waste Water (Volts)
1	500	0.86	0.52	0.76
2	600	0.87	0.65	0.82
3	700	0.89	0.67	0.64
4	800	0.91	0.41	0.51
5	900	0.93	0.32	0.59
6	1000	0.90	0.11	0.74

TABLE A.1: WEIGHT OF BIOMASS AND POTENTIAL DIFFERENCE

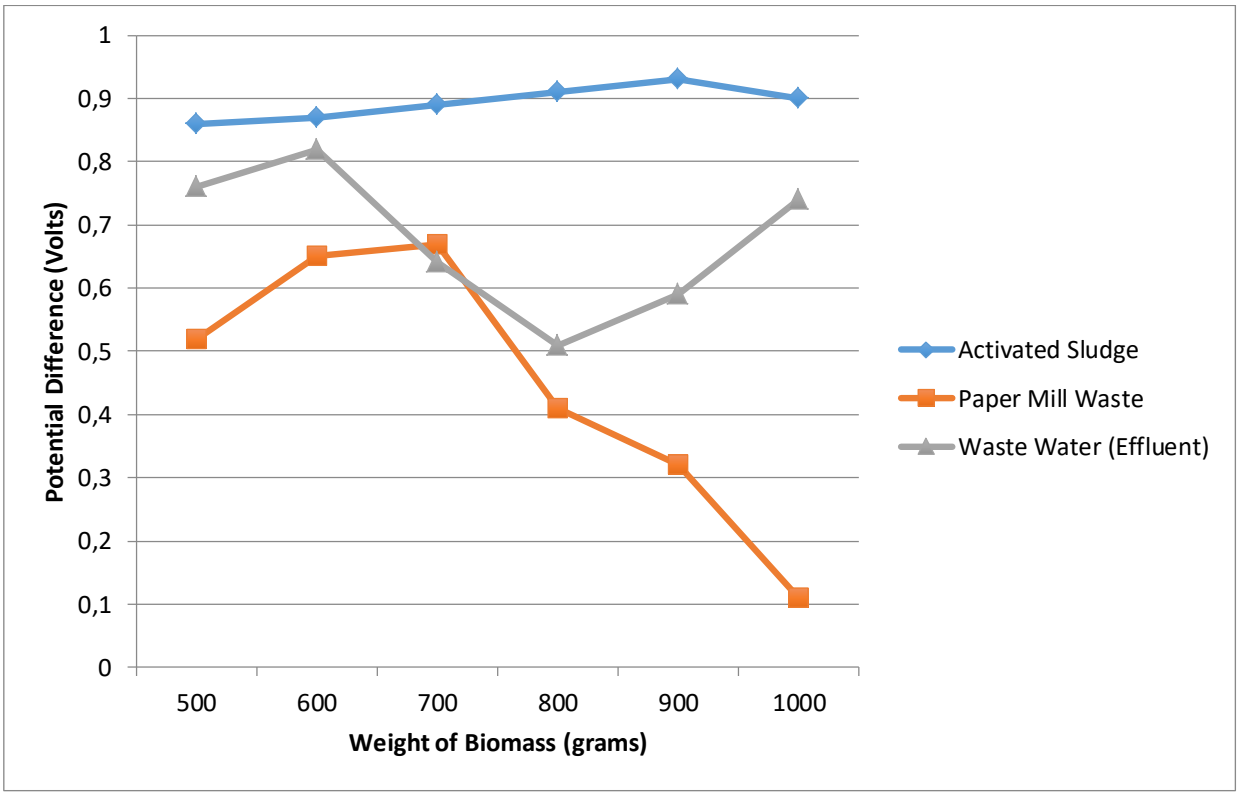


FIG. A.1: POTENTIAL DIFFERENCE vs BIOMASS WEIGHT

Experiment Number	Temperature (◦C)	Activated Sludge (Volts)	Paper Mill Waste (Volts)	Waste Water (Volts)
1	10	0.15	0.23	0.11
2	20	0.36	0.22	0.19
3	30	0.69	0.44	0.32
4	35	0.87	0.46	0.51
5	40	0.91	0.49	0.69
6	45	0.79	0.31	0.59

TABLE A.2: TEMPERATURE OF BIOMASS AND POTENTIAL DIFFERENCE

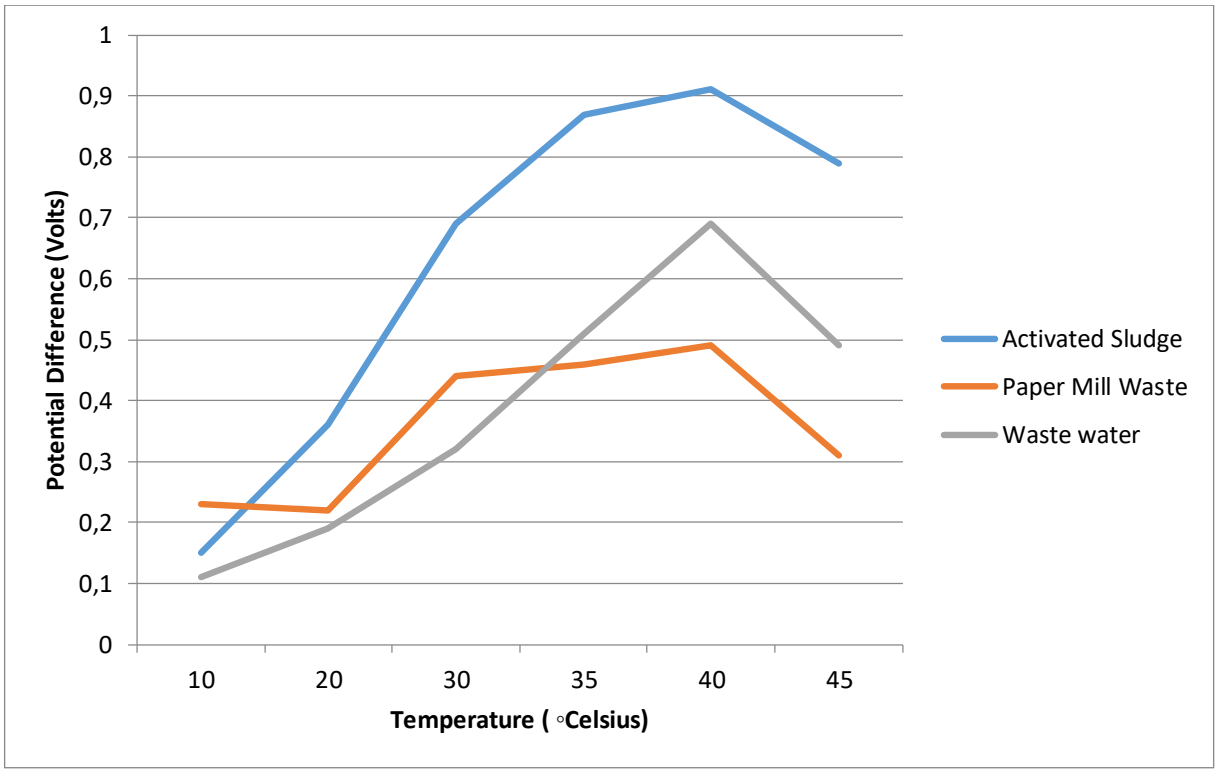


FIG. A.2: POTENTIAL DIFFERENCE vs TEMPERATURE

Experiment Number	Time (Minutes)	Activated Sludge (Volts)	Paper Mill Waste (Volts)	Waste Water (Volts)
1	5	0.22	0.19	0.02
2	10	0.27	0.23	0.54
3	15	0.39	0.28	0.47
4	20	0.47	0.31	0.23
5	25	0.68	0.39	0.46
6	30	0.81	0.42	0.78
7	40	0.84	0.54	0.44
8	50	0.83	0.59	0.55
9	60	0.79	0.62	0.69
10	75	0.75	0.69	0.81
11	90	0.71	0.71	0.69
12	120	0.62	0.65	0.72

TABLE A.3: TIME AND POTENTIAL DIFFERENCE

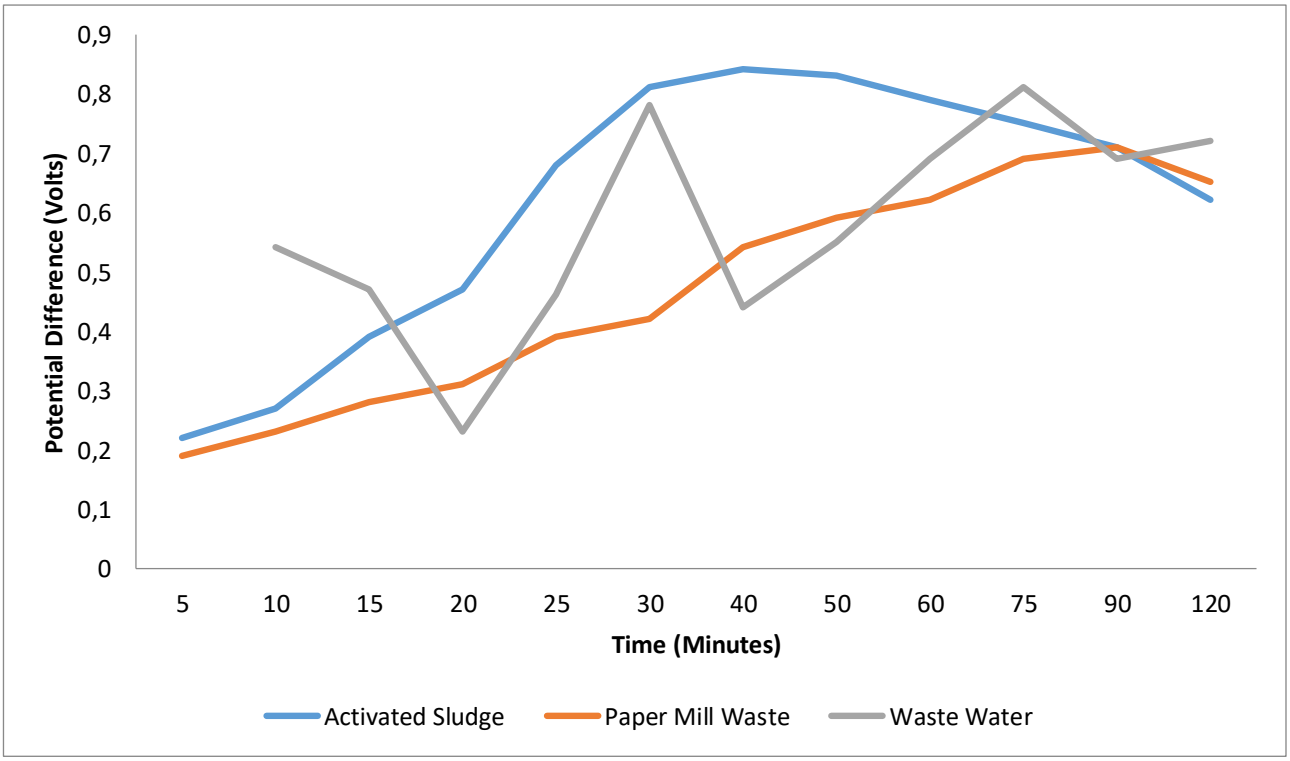
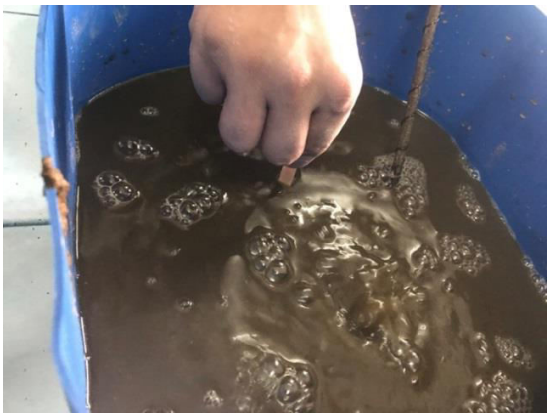


FIG. A.3: POTENTIAL DIFFERENCE vs TIME

A.4: ACTIVATED SLUDGE USED



A.5: ANODE CONTAINING ACTIVATED SLUDGE

A.6: PRIMARY TTEEATMENT OF EFFLUENT

A.7; A.8: GOMATI PRIMARY EFFLUENT TREATMENT PLANT (TOP & BOTTOM)

A.9: MFC SET-UP

DISTILLED WATER (CATHODE) AND ACTIVATED SLUDGE (ANODE)

A.10 GOMATI PLANT