A microbial fuel cell (MFC) or biological fuel cell is a bio-electrochemical system that drives a current by using bacteria and mimicking bacterial interactions found in nature. A microbial fuel cell is a promising bio-electrochemical system capable of converting organic compounds (e.g. organic wastes) into useful electricity energy by the catalytic reaction of microorganisms. It consists of two half cells the anode and the cathode which are typically separated by an ion selective membrane. The anode electrode is immersed in a solution containing organic compounds and the bacteria, growing on the anode surface, oxidize the organic substances through their metabolic processes. At the cathode, oxygen is normally used as the final electron acceptor. Microbes in the anode compartment oxidize a soluble electron donor (e.g., glucose, acetate) generating electrons and protons. Electrons are then transferred to the anode surface and from there to the cathodic compartment through the electrical circuit, while the protons migrate through the electrolyte and then through the cationic membrane. Electrons and protons produced from redox reactions are transported to cathode from different paths and electrochemically react with oxygen as electron acceptor to produce water. This way, electrical energy is generated while unwanted organic matters are removed. Pure organic compound, real wastewater, and biomass have been successfully used as fuel for power generation in MFCs.

Current efforts in ongoing MFC research are directed towards improving the efficiency of this process to make it economically and commercially feasible.

The maximum current that can be produced by a MFC depends on the actual rate of substrate biodegradation, whereas maximum theoretical cell voltage (also called electromotive force or emf) depends on Gibbs free energy of the overall reaction and can
be calculated as the difference between the standard reduction potentials of the cathodic oxidant (oxygen) and the chosen anodic substrate.

1.2 History of Microbial fuel cell

The idea of using microbial cells in an attempt to produce electricity was first conceived in the early twentieth century. M. Potter was the first to start work on the subject in 1911 (Potter 1911). A professor of botany at the University of Durham, Potter managed to generate electricity from E. coli, but the work did not receive any major coverage. In 1931, however, Barnet Cohen drew more attention to the area when he created a number of microbial half fuel cells that, when connected in series, were capable of producing over 35 volts, though only with a current of 2 milliamps (Cohen, 1931).

Elaborate study on the subject was made by DelDuca et al. who used hydrogen produced by the fermentation of glucose by Clostridium butyricum as the reactant at the anode of a hydrogen and air fuel cell. Though the cell functioned, it was found to be unreliable owing to the unstable nature of hydrogen production by the micro-organisms (DelDuca et al., 1963). This issue was later resolved by Suzuki et al. in 1976 (Karube et al., 1977). A year later the current design concept of an MFC came into existence a year later initiated by Suzuki (Karube et al., 1977). In the late 1970’s, when Suzuki undertook and worked on that, little was understood about how microbial fuel cells functioned; however, the idea was picked up and studied later in more detail first by MJ Allen and then later by H. Peter Bennetto both from King's College London. People saw the fuel cell as a possible method for the generation of electricity in developing countries. His work, which started in the early 1980s, helped to build an understanding of how fuel cells operate. He was seen as the foremost authority on the subject until his retirement. It is now known that electricity can be produced directly from the degradation of organic matter in a microbial fuel cell. Like a normal fuel cell, an MFC has both an anode and a
cathode chamber. The anoxic anode chamber is connected internally to the cathode chamber via an ion exchange membrane with the circuit connected by an external wire. In May 2007, the University of Queensland, Australia completed its prototype MFC with the cooperative effort of Foster's Brewing. The prototype, a 10 L design, converts brewery wastewater into carbon dioxide, clean water and electricity. If the prototype is proved to be successful, there are plans to produce a 660 gallon version from the brewery, which is estimated to produce 2 kilowatts of power. Though the power generated amount is less, the production of clean water is of utmost importance to Australia, as drought is a constant threat to them.

1.3. Microbial Fuel Cells

Production of electrical energy using microorganisms through microbial fuel cells (MFC) is one such renewable and sustainable technology that is considered to be one of the most efficient (HaoYu et al., 2007; Salgado, 2009) and carbon neutral energy sources (Lovley, 2006). MFCs are fuel cells that are capable of converting chemical energy available in organic substrates into electrical energy using bacteria as a biocatalyst to oxidize the biodegradable substrates (www.microbialfuelcell.org). The fact that bacteria can oxidize the substrates to produce electricity makes MFCs an ideal solution for wastewater treatment and domestic energy production (Schwartz, 2007). Logan (2010) reported that MFCs can generate power densities as much as 1kW/m3 of reactor volume. MFCs as a source of bioenergy production has accelerated the research worldwide and the technical aspects of MFCs have been reviewed extensively (Pant D, 2010).

Definition:

A microbial fuel cell is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms (Allen and Bennetto, 1993). A typical
microbial fuel cell consists of anode and cathode compartments separated by a cation (positively charged ion) specific membrane. In the anode compartment, fuel is oxidized by microorganisms, generating CO$_2$, electrons and protons. Electrons are transferred to the cathode compartment through an external electric circuit, while protons are transferred to the cathode compartment through the membrane. Electrons and protons are consumed in the cathode compartment, combining with oxygen to form water.

The basic MFC design consists of an anode, a cathode, a proton exchange membrane (PEM) and an electrical circuit, as shown in Fig.1 (Logan, 2008). In an MFC, bacterial community present in the anode compartment uses organic substrates as fuels to produce electrons and protons through biological processes (Rabaey and Verstraete, 2005) (www.microbialfuelcell.org). These electrons are accepted by nicotinamide adenine dinucleotide (NADH) in the electron transport chain and subsequently transferred to terminal electron acceptors such as nitrate, sulphate and oxygen and then reaches the outer membrane proteins (Logan and Regan, 2006; Salgado, 2009). Bacteria then transfer these electrons to anode from where electrons reach the cathode via an external electrical circuit, thus producing electric current, which is measured by a voltmeter or ammeter connected to the device (Salgado, 2009). The protons generated are diffused through the PEM to the cathode and subsequently combine the electrons and oxygen to form water. The anode compartment is typically maintained under anaerobic conditions as oxygen inhibits electricity generation whereas the cathode is exposed to oxygen (Logan, 2008; Rahimnejad Mostafa, 2009). Du et al., 2007 reported that the electrode reaction is the breakdown of the biodegradable substrate to carbon dioxide and water along with production of electricity using acetate as a substrate.

Anode reaction: $\text{CH}_3\text{COO}^− + 2\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + 7\text{H}^+ + 8\text{e}^−$  \hspace{1cm} (1)

Cathode reaction: $\text{O}_2 + 4\text{e}^− + 4\text{H} \rightarrow 2\text{H}_2\text{O}$  \hspace{1cm} (2)
1.4. Types of Microbial fuel cell

Microbial fuel cell is broadly, classified into two types of microbial fuel cell:

1. Mediator microbial fuel cells and
2. Mediator-less microbial fuel cells.

The anode electrode, which contains a matrix for the attachment of the microorganisms, is an important factor of the MFC design. Modifying the electrode surface by the binding of mediators such as neutral red and metals such as iron and manganese has resulted in the increase of current generation (Park and Zeikus, 2002).

Several types of MFCs have been developed in order to examine the effects of different architectures of performance, including two-chamber (Min and Logan 2004), single-chamber (Liu and Logan 2004), flat plate (Aelterman et al. 2006), upflow and stacked reactors (Dekker et al. 2009). The main performance goals are increasing power densities, the recovery of electrons as current [columbic efficiency (CE)] and energy recovery while using simple designs and low cost materials (Logan et al. 2006).
A double-chamber MFC is composed of two compartments separated by an Ion Exchange Membrane (IEM) which can be either a proton or a cation exchange membrane. The anodic compartment contains the organic substrate and the bacteria, whereas the cathodic compartment contains an electrolyte rich in electron acceptor (e.g. oxygen).

A single-chamber MFC is composed of an anodic compartment alone (Figure 4). The cathode is merged with the reactor so that it interfaces the internal electrolyte and the external air. This type of membrane is called air-cathode. A membrane can be placed on the inner side of the cathode to limit the diffusion of oxygen within the reactor. The main advantage of this type of MFC is that it does not require any aeration of the cathodic electrolyte, reducing thus the hydraulic flow.

1.4.1. Mediator microbial fuel cell

Most of the microbial cells are electrochemically inactive. The electron transfer from microbial cells to the electrode is facilitated by mediators such as thionine, methylviologen, methyl blue, humic acid, and neutral red (Delaney et al., 1993; Lithgow et al., 1986). Most of the mediators available are expensive and toxic.

1.4.2. Mediator-free microbial fuel cell

Mediator-free microbial fuel cells do not require a mediator but use electrochemically active bacteria to transfer electrons to the electrode (electrons are carried directly from the bacterial respiratory enzyme to the electrode). Among the electrochemically active bacteria are, Shewanella putrefaciens,(Kim et al., 1999a) Aeromonas hydrophila (Pham et al., 2003) and others. Some bacteria, which have pili on their external membrane, are able to transfer their electron production via these pili. Mediator-less MFCs are a more recent area of research and, due to this, factors that affect optimum efficiency, such as the strain on bacteria used in the system, type
of ion-exchange membrane, and system conditions (temperature, pH, etc.) are not particularly well understood.

1.4.2.1. Plant microbial fuel cell (PMFC)

![Plant microbial fuel cell](https://commons.wikipedia.org/wiki/file:plant_Microbial_FuelCell.png)

Mediator-less microbial fuel cells can, besides running on wastewater, also derive energy directly from certain plants. This configuration is known as a plant microbial fuel cell. Possible plants include reed sweet grass, cord grass, rice, tomatoes, lupines, and algae. Given that the power is thus derived from living plants (in situ-energy production), this variant can provide additional ecological advantages.

1.4.2.2. Microbial electrolysis cell

A variation of the mediator-less MFC is the microbial electrolysis cells (MEC). Whilst MFC's produce electric current by the bacterial decomposition of organic compounds in water, MECs partially reverse the process to generate hydrogen or methane.
by applying a voltage to bacteria to supplement the voltage generated by the microbial decomposition of organics lead to the electrolysis of water or the production of methane. A complete reversal of the MFC principle is found in microbial electro-synthesis, in which carbon dioxide is reduced by bacteria using an external electric current to form multi-carbon organic compounds.

1.4.2.3. Soil-based microbial fuel cell

Soil-based microbial fuel cells adhere to the same basic MFC principles as described above, whereby soil acts as the nutrient-rich anodic media, the inoculum, and the proton exchange membrane (PEM). The anode is placed at a certain depth within the soil, while the cathode rests on top of the soil and is exposed to the oxygen in the air above it.

Soils are naturally teeming with a diverse consortium of microbes, including the electrogenic microbes needed for MFCs, and are full of complex sugars and other nutrients that were derived from the decayed plant and animal kingdom which have accumulated over millions of years. Moreover, the aerobic (oxygen consuming) microbes present in the soil act as an oxygen filter, much like the expensive PEM materials used in laboratory MFC systems, which cause the redox potential of the soil to decrease with greater depth. Soil-based MFCs are becoming popular educational tools to teach science in classrooms.
Sediment microbial fuel cells (SMFCs) application for wastewater treatment is a relatively new field. Most SMFCs used for wastewater treatment contain plants to mimic constructed wetlands. Both synthetic and real wastewaters have been used as substrates in SMFCs that achieved satisfactory performance in organic removal.

In 2015 researchers announced an SMFC application that extracts energy and charges a battery. Salts found in the waste, dissociates into positively and negatively charged ions in water and move and adhere to the respective negative and positive electrodes, charging the battery and making it possible to remove the salt effecting microbial capacitive desalination. The microbes produce more energy than is required for the desalination process (Clark, Helen, 2015).
1.4.2.4. Phototrophic biofilm microbial fuel cell

Phototrophic biofilm MFCs (PBMFCs) are the ones that make use of anode with a phototrophic biofilm containing photosynthetic microorganism like chlorophyta, cyanophyta etc., since they could carry out photosynthesis, they act as both producers of organic metabolites and also as electron donors (Elizabeth, Elmy, 2012).

A study conducted by Strik reveals that PBMFCs yield one of the highest power densities and, therefore, show promise in practical applications. Researchers face difficulties in increasing their power density and long-term performance so as to obtain a cost-effective MFC (Strik et al., 2011). The sub-category of phototrophic microbial fuel cells, use purely oxygenic photosynthetic material at the anode and are sometimes called biological photovoltaic systems (Bombelli, 2011).

1.4.2.5. Nano-porous membrane microbial fuel cells

The United States Naval Research Laboratory (NRL) developed the nanoporous membrane microbial fuel cells which operate the same as most MFCs, but use a non-PEM to generate passive diffusion within the cell. The membrane used instead is a nonporous polymer filter (nylon, cellulose, or polycarbonate) which generates comparable power densities as Nafion (a well-known PEM) which remains more durable than Nafion. Porous membranes allow passive diffusion thereby reducing the necessary power supplied to the MFC in order to keep the PEM active and increasing the total output energy from the cell (Biffinger et al., 2007). MFCs that do not use a membrane can deploy anaerobic bacteria in aerobic environments however, membrane-less MFCs will experience cathode contamination by the indigenous bacteria and the power-supplying microbe. The novel passive diffusion of nano-porous membranes can achieve the benefits of a membrane-less MFC without worry of cathode contamination. Nano porous membranes are also ten times cheaper than Nafion.
1.5. Electrode materials

The choice of electrode material affects the performance of MFCs. Various materials have been investigated with electrodes to increase the performance and power output of the MFCs. For anode, carbon cloth, carbon felt, graphite felt, carbon mesh and graphite fiber brush are frequently used due to their stability, high electric conductivity and large surface area (Logan, 2010; Logan and Regan, 2006). For cathodes, platinum (Pt), platinum black, activated carbon (AC), graphite based cathodes and bio-cathodes are used (Chen et al., 2008; Du et al., 2007). Platinum coated electrodes are more efficient and superior in power production due to its higher catalytic activity with oxygen than other electrodes. They are not cost effective too (Logan, 2010; Oh et al., 2004). Alternate catalysts for platinum include ferric iron, manganese oxides, iron and cobalt based compounds. Ferric cyanide (K3Fe(CN)₆) is frequently used as an electron acceptor in the MFCs due to its good performance and low over potential (Logan and Regan, 2006). Bio cathodes increases the power by decreasing the over potential (Huang et al., 2011). Alternately, the cathode can contain oxygen and is preferred because it simplifies the operation of the cell and is the most commonly used electron acceptor in MFC. The power output depends on proton transfer from anode to cathode. Transfer of protons to the cathode is a slow process that causes high internal resistance (Kazuya, 2008; Osman et al., 2010). Most of the MFCs require a salt bridge or PEM to separate the anode and cathode compartments. The PEM is commonly made from polymers like Nafion and Ultrex (Schwartz, 2007). Membrane-less, single chamber MFCs are reported to produce higher power density, whereas the absence of membrane would increase oxygen to the anode and thus lowers the columbic efficiency and bio electro catalytic activity of the microbes (Logan, 2010; Wen et al., 2010).
1.6. Electrical Generation Process

When micro-organisms consume a substance 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, as described below (Cheng et al)

\[ \text{C}_{12}\text{H}_{22}\text{O}_{11} + 13\text{H}_2\text{O} \rightarrow 12\text{CO}_2 + 48\text{H}^+ + 48\text{e}^- \] (3)

Microbial fuel cells use inorganic mediators to tap into the electron transport chain of cells and as a result channel electrons are produced. Initially the mediator crosses the outer cell lipid membranes and bacterial outer membrane, then it begins to liberate electrons from the electron transport chain that would normally be taken up by oxygen or other intermediates.

The reduced mediator exits the cell laden with electrons, then it transfers to an electrode where it gets deposited them. This electrode becomes the electro-generic anode (negatively charged electrode). The release of the electrons means that the mediator returns to its original oxidised state ready to repeat the process. It is important to note that this can happen only under anaerobic conditions, if oxygen is present, it will collect all the electrons, as it has a greater electronegativity than mediators.

In a microbial fuel cell operation, the anode is the terminal electron acceptor recognized by bacteria in the anodic chamber. Therefore, the microbial activity is strongly dependent on the redox potential of the anode.

This is the principle behind generating a flow of electrons from most micro-organisms (the organisms capable of producing an electric current are termed exoelectrogens). In order to turn this into a usable supply of electricity, this process has to be accommodated in a fuel cell. In order to generate a useful current it is necessary to create a complete circuit. The mediator and micro-organism, in this case, yeast are mixed together in a solution to which is added a suitable substrate such as glucose. This mixture
is placed in a sealed chamber forcing the micro-organism to use anaerobic respiration. An electrode is placed in the solution that will act as the anode as mentioned earlier. In the second chamber of the MFC is another solution and electrode. This electrode is called the cathode and gets positively charged and is the equivalent of the oxygen sink at the end of the electron transport chain. Now it is external to the biological cell. The solution is an oxidizing agent that picks up the electrons at the cathode. As with the electron chain in the yeast cell, there could be a number of molecules such as oxygen, electrons and protons. However, 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. Connecting two electrodes through wire (or other electrically conductive path) and completing the circuit and connecting the two chambers is a salt bridge or ion-exchange membrane. This last feature allows the protons produced, as described in Eqt. 3 to pass from the anode chamber to the cathode chamber. The reduced mediator carries electrons from the cell to the electrode. Here the mediator is oxidized where the electrons gets deposited. These then flow across the wire to the second electrode, which acts as an electron sink. From here they pass to an oxidising material.

1.7. Design

There are several types of MFCs, such as double chamber MFC’s (Antonopoulo et al., 2010; Chae et al., 2010), single chamber MFCs (Cheng and Logan, 2011), tubular MFCs (Kim et al., 2010), up flow MFC (Zuo et al., 2010), and multi-anode/cathode MFCs (Jiang et al., 2010a) have been developed. However, single chamber microbial fuel cells (SCMFCs) are believed to be superior because of their simple design, flexibility, low internal resistance, and relatively low cost (Du et al., 2007).
There are many types of reactors but they all share the same operating principles. Different configurations of MFCs are being developed using a variety of materials. They are operated under different conditions to increase the performance, power output and reduce the overall cost.

**1.8. Types of Reactor:**

There are various types of MFC reactors designed in accordance with the chamber design.

**1.8.1. Single chamber MFC**

This design has only one compartment that contains both the anode and the cathode as shown in Fig. 4.

![Fig.4. Single chamber MFC](Figure drawn to illustrate a photo in Park and Zeikus, 2003)
The anode is either placed away or close to the cathode separated by PEM (Liang et al. 2007). It is proved that when the anode is closer to the cathode, it reduces internal ohmic resistance by avoiding the use of catholyte and combining two chambers results in the increase of power density. Compared to two chamber MFC, it offers simple, cost effective design and produces power in a more efficient way (Du et al., 2007). However, in the membrane-less configuration, microbial contamination and back diffusion of oxygen from cathode to anode without PEM are the major drawbacks (Kim 2008).

The MFC reactors used in Single chamber MFC are,

1. Air cathode without membrane

   Advantages: Air cathode MFC’S are simple in structure, compact size and scalable.
   Disadvantages: Low Columbic Efficiency.

2. Air cathode with membrane

   Advantages: Air cathode MFC’S are simple in structure and compact size.
   Disadvantages: The membrane used are expensive.

3. Up flow tubular

   Advantages: Up flow tubular MFC’s are scalable and continuous operation
   Disadvantages: Low Columbic Efficiency and toxicity.

4. Up flow cylindrical

   Advantages: Up flow cylindrical MFC’s utilizes glass wool and bead as separator.
   Disadvantages: High internal resistances.

5. Up flow rectangular

   Advantages: Up flow rectangular MFC’s are fully mixing and with dissolved oxygen gradient.
   Disadvantages: Low Columbic Efficiency and H₂O₂ consumption.
6. Submersible MFC

Advantages: simple in design and in situ applicable.

Disadvantages: Aeration and has high internal resistance.

1.8.2. Two chamber MFC

This is the most widely used design consisting of two chambers with the anode and cathode compartments separated by an ion exchange membrane (Fig3a). This design is generally used in basic research and literature suggests that the power output from these systems are generally low due to their complex design, high internal resistance and electrode based losses (Du et al., 2007; Logan and Regan, 2006; Nwogu, 2007).

![Double chamber MFC](image)

**Fig.5. Double chamber MFC**

(Figure to illustrate a photo in Zhuwei et al., 2007 and Lavanya et al., 2014)

The MFC reactors used in Double chamber MFC are,

1. Salt bridge

   Advantages: Cheaper materials.

   Disadvantages: High internal resistances.
2. H – shape
   Advantages: Basic of research and are stable.
   Disadvantages: Has large electrode spacing and low power density.

3. Rectangular
   Advantages: Utilize short electrode distance.
   Disadvantages: The membrane used are expensive.

4. Flat miniature
   Advantages: Flat miniature MFC’s are portable power source.
   Disadvantages: Has low power output and its fabrication cost is high.

5. Up flow tubular
   Advantages: Up flow tubular MFC’s are scalable and continuous operation.
   Disadvantages: The membrane used are expensive and fluid recirculation is required.

6. Sediment MFC
   Advantages: Simple in structure, compact size and scalable.
   Disadvantages: Low Power generation.

The MFC reactors used in four chamber MFC is,

1. CEM bridged stack
   Advantages: The cations transfer rate is higher and has low internal resistance.
   Disadvantages: The membrane used are expensive.

The MFC reactors used in Five chamber MFC is,

1. Bipolar plate stack
   Advantages: The voltage drop is minimized.
   Disadvantages: Voltage reversal problem occurs.
1.8.3. Stacked MFC

In this design, several single cell MFCs are connected together in series or in parallel to achieve high current output (Du et al., 2007). Due to higher electrochemical reaction rate, a parallel connection can generate more energy than a series connection when operated at the same volumetric flow but is prone to higher short circuiting compared to a series connection (Fig.5) (Aelterman et al., 2006; Schwartz, 2007).

Fig.6. Stacked MFC (Figure drawn to illustrate a photo in Aelterman et al., 2006 and Zhuwei Du et al., 2007)
1.8.4. Up flow MFC

Up-flow MFC: The cylinder shaped MFC consists of the anode (bottom) and the cathode (top) partitioned by glass wool and glass beads layers. The feed is supplied from the bottom of the anode passes upward of the cathode and exits at the top (Fig. 7). The diffusion barrier among the electrodes provides a gradient for proper operation of the MFCs (Du et al., 2007; Kim 2008; Schwartz, 2007). This design has no physical separation and so there are no proton transfer associated problems and is attractive for wastewater treatment (Kim 2008).

![Diagram of Up flow MFC]

Fig.7. Up flow MFC (Figure drawn with modifications after Jang et al., 2004, Tartakovsky and Guiot, 2006, respectively)

1.9. Applications

The emergence of a new technology will be aided by finding the most immediate and useful niche applications. For MFCs, these are probably in wastewater treatment and as power sources for environmental sensors, but opportunities for other applications also exists (Bruce. E. Logan and John. M. Regan, 2006). Although MFCs have been studied as
an alternative energy source, their application is presently limited to certain niche areas. With further improvements in design, cost effectiveness and performance efficiency based on these near-term applications, it would be possible to scale-up and use MFCs as a renewable energy resource (S. Vishwanathan and S. Siva Sankara Sai, Sri Sathya Sai University).

1.9.1. Education

Soil-based microbial fuel cells are popular educational tools, as they employ a range of scientific disciplines (microbiology, geochemistry, electrical engineering, etc.) and can be made using commonly available materials, such as soils and items from the refrigerator. There are also kits available for classrooms and hobbyists, and research-grade kits for scientific laboratories and corporations.

1.9.2. Biosensor

Since the current generated from a microbial fuel cell is directly proportional to the energy content of wastewater used as the fuel, an MFC can be used to measure the solute concentration of wastewater (i.e., as a biosensor system) (Kim et al., 2003). When BOD values are used as a real-time control parameter, 5 days' incubation is too long.

An MFC-type BOD sensor can be used to measure real-time BOD values. Oxygen and nitrate are preferred electron acceptors over the electrode reducing current generation from an MFC. MFC-type BOD sensors underestimate BOD values in the presence of these electron acceptors. This can be avoided by inhibiting aerobic and nitrate respirations in the MFC using terminal oxidase inhibitors such as cyanide and azide (Chang et al., 2005). The United States Navy is looking into microbial fuel cells particularly for environmental sensors. The use of microbial fuel cells to power environmental sensors would be beneficial because they would be able to sustain power for a longer amount of
time and enable the collection and retrieval of undersea data without using a wire infrastructure. The energy created by these fuel cells was enough to sustain sensors after an initial startup time in research to demonstrate the effectiveness of the fuel cell as a power source for such sensors (Gong et al., 2011). Due to undersea conditions (high salt concentrations, fluctuating temperatures, and limited nutrient supply), the U.S. Navy is looking to deploy their MFCs with a mixture of salt-tolerant microorganisms. A mixture would also allow for a more complete utilization of available nutrients to be converted into electricity. Currently, *Shewanella oneidensis* is their primary microorganism for electrical generation, (Biffinger et al., 2011). This alternative energy form will be more helpful as it is being improved continuously.

1.9.3. Bio-recovery

In 2010, A. ter Heijne et al 2010, constructed a device capable of producing electricity and reduce the ion Cu (II) to copper metal. Microbial electrolysis cells have been demonstrated to produce hydrogen (Zhang et al., 2013).

1.9.4. Water treatment

Microbial Fuel Cells are being used in the water treatment process to harvest energy utilizing anaerobic digestion (a method used in the microbial fuel cell to collect bioenergy from wastewater). The process is well developed and can handle a high volume of wastewater and reduce pathogens. However, the process requires high temperature (upwards of 30 degrees Celsius) and requires an extra step in order to convert biogas to electricity. Spiral spacers may also be used to increase electricity generation by creating a helical flow in the microbial fuel cells. The challenge is that it is difficult to scale up the MFCs for practical wastewater treatment because of the power output challenges of a larger surface area MFC (Zhang et al., 2013).
1.9.5. Current Research Practices

Some researchers (Menicucci et al., 2005) point out some undesirable practices, such as recording the maximum current obtained by the cell when connecting it to a resistance as an indication of its performance, but of the steady-state current is often a degree of lower magnitude. Often the data about the values of the used resistance is minimal, or even non-existent, making the data non-comparable across all studies. This makes extrapolation from standardized procedures to difficult or not impossible at all.

1.9.6. Commercial Applications

A number of companies have emerged to commercialize microbial fuel cells. These companies have attempted to tap into both the remediation and electricity generating aspects of the technologies. Some of these companies are mentioned here (Pant et al., 2011).

Microbial fuel cells have a number of potential uses. It is mostly used for harvesting electricity produced and used use as a power source (Chua et al., 2014; 2013). The use of MFCs is attractive for applications that requires low power but to replace batteries may be time-consuming and expensive with wireless sensor networks (Subhas et al., 2013). Virtually any organic material could be used to feed the fuel cell, which includes coupling cells to wastewater treatment plants. Bacteria would consume waste material from the water and produce supplementary power for the plant. What we gain out of that MFCs enable a very clean and efficient method of energy production. Chemical processing wastewater (Venkata Mohan et al., 2008a; 2008b) and designed synthetic wastewater (Venkata Mohan et al., 2007; 2008) have been used to produce bioelectricity in dual- and single-chamber mediator-less MFCs (non-coated graphite electrodes) apart from wastewater treatment. Higher power production was observed with biofilm covered anode (graphite) (Venkata Mohan et al., 2008a; 2008b). A fuel cell’s
emissions are well below regulations Choi et al., 2000). MFCs also use energy much more efficiently than standard combustion engines, which are limited by the Carnot Cycle. According to this theory, an MFC is capable of energy efficiency far beyond 50% (Yue & Lowther, 1986). As per the new research conducted by René Rozendal, using the new microbial fuel cells, conversion of the energy to hydrogen is 8 times as high as conventional hydrogen production technologies. However, MFCs do not have to be used on a large scale, as the electrodes in some cases need only 7 μm thick by 2 cm long (chen et al., 2001). The advantages of using an MFC in this situation as opposed to a normal battery is that it uses a renewable form of energy and would not need to be recharged like a standard battery would. In addition to this, they could operate well in mild conditions, 20 °C to 40 °C and also at pH of around 7 (Bullen et al., 2015). Although they are more powerful than metal catalysts, they are currently very unstable for long-term medical applications such as in pacemakers (Biotech/Life Sciences Portal). Besides wastewater power plants, as mentioned earlier, energy can also be derived directly from crops. This allows the set-up of power stations based on algae platforms or other plants incorporating a large field of aquatic plants. According to Bert Hamelers, the fields are best set-up in synergy with existing renewable plants (e.g., off shore wind turbines). This reduces the cost as the microbial fuel cell plant can make use of the same electricity lines as the wind turbines.

1.9.7. Other Applications

MFCs can also be applied to other than wastewater treatment and renewable energy. By placing the anode electrode in marine sediments and emplacing the cathode in the overlying water, it is possible to generate electricity from the bacterial decomposition of the organic matter in the sediment. The electricity generated is not sufficient to make it
economically feasible as a source of renewable energy, but it could be sufficient for powering devices in remote marine and estuarine locations.

1.10. Bacterial Metabolism

Biofilms are microbial consortia physically aggregated from a competitive advantage in forming a community function. A biofilm can be formed from a single species, but it is more commonly comprised of many, often distantly related, microorganisms that utilize specific metabolic pathways (Kim, G.T et al., 2006). Biofilms on the electron source (i.e. anode) have been demonstrated to increase the current due to the direct electron transfer between the microbes and the surface of the anode (Picioreanu et al.,2007). Only specific groups of microbes can be used to power a microbial fuel cell, due to their adaptation to donate electrons externally during respiration, and, so, researching the physiology of these organisms can improve the efficiency of the cell (Kim, G.T et al., 2006). The formation of a biofilm is initiated by an attachment and growth of a cell on a surface. Attachment is aided by expression of exo polymers that adhere to a surface (Gottenbos, B et al., 1999). As the biofilm grows it spreads along a surface and grows in thickness. The growth along the surface is due to the dispersion of microbial cells that detach from the layer of biofilm and adheres to another part of the surface (Gottenbos, B et al., 1999). (Davies, D.G., et al., 1998). This spreading property is especially useful for MFC efficiency because it means that the number of electrons transferred to the anode is directly proportional to the anode's surface area given minimal loss of electrons due to close proximity between the biofilm and the anode (Picioreanu, C., et al., 2007). Also, a thicker biofilm that does not decrease efficiency of the MFC is advantageous because it ensures that a dormant layer of bacteria is present to replace any dead or removed cells (Mah, T.C., and O'Toole, G.A et al., 2001).
The evolutionary advantage of a biofilm found in nature is that multiple microbes, such as Archaea and Bacteria can form a community in which the product of one species are the substrates for another (a.k.a. Intermediary community metabolism) (Hall-Stoodley, L., et al., 2004). A detailed study on the MFC biofilm composition by Kim et al. (2006) suggests that diverse species present on the anode allow multiple candidates for MFC operation given at different environmental conditions. Hence a mixed biofilm of Pelotomaculum thermopropionicum, Methano thermobacter, thermautotrophicus and Geobacteraceae could produce a greater potential difference than a film of just Geobacteraceae colonies.

1.10.1. Electron transfer mechanisms

In MFCs, the bacterial transfer of electrons from the substrates to electrodes is mainly through two ways (Fig.8). The mechanism of electron transfer may be through either direct transfer (mediatorless) or indirect electron transfer (mediator MFC) (Yan-ping, 2008). *Shewanella putrefaciens, Geobacter sulfurreducens, G. metallireducens* and *Rhodoferax ferrireducens* that transfer electrons from inside the cell to extracellular acceptors via c-type cytochromes, biofilms and highly conductive pili (nanowires) (Derek R, 2008). These microorganisms have high Columbic efficiency and can form biofilms on the anode surface that act as electron acceptors and transfer electrons directly to the anode resulting in the production of more energy (Chaudhuri and Lovley, 2003; Kim et al., 2002).
1.10.2. Electron transfer by own/artificial mediators

In this mechanism, electrons from microbial carriers are transported onto the electrode surface either by a microorganism’s (Shewanella oneidensis, Geothrix fermentans) own Mediator which in turn facilitate extracellular electron transfer or by added mediators. The MFCs that use mediators as electron shuttles are called mediator MFCs. Mediators provide a platform for the microorganisms to generate electrochemically active reduced products. The reduced form of the mediator is cell permeable, accept electrons from the electron carrier and transfer them onto the electrode surface (Lovley, 2006). Usually neutral red, thionine, methylene blue, anthraquinone-2, 6-disulfonate, phenazines and iron chelates are added to the reactor as redox mediators (Du et al., 2007). Mediators are required in MFCs that use Proteus vulgaris, Escherichia coli, Streptococcus lactis, and Pseudomonas species as these bacteria cannot transfer electrons outside the cell. To be effective, the mediator should be able to penetrate the cell membranes easily, able to grab the electrons from the electron carriers of the electron transport chains. It should increase electron transfer from the metabolite, stable for longer periods of redox cycling and non-toxic to microbes (Du et al., 2007; Ieropoulos et al., 2005; Osman et al., 2010).
1.10.3. Microbes in MFC

A wide variety of bacterial communities are found to have the ability to oxidize organic compounds and transfer electrons to the anode. MFCs could make use of both the mixed cultures and pure bacterial cultures (Cheng et al., 2005, Rabaey et al., 2005) reported that the mixed cultures have high resistance for process disturbances, substrate consumption and higher power output. The electrochemically active bacteria in MFCs may be aerobes or facultative anaerobes and the reaction temperature in MFCs depend on the bacterial tolerance to temperature (mesophilic/thermophilic) (Logan, 2008; Rabaey and Verstraete, 2005). Not only the electrochemically active, iron-reducing bacteria (Shewanella and Geobacter) but also other group of bacteria (Klebsiella pneumonia, Rhodopseudomonas palustris, Desulfobulbus propionicus) that are isolated from the wastewater showed great potential to be used in MFCs (Sharma and Kundu, 2010). A number of recent reports reviewed screening, identification of microbes, their ability to generate electric current and power densities in detail (Logan, 2009; Logan et al., 2005).

In MFCs, microbes play crucial roles in energy output and organic contaminants removal (Pant D, et al., 2012). The ability of microbes to transfer electrons in the anode can significantly affect the performance of MFCs. (Zjie Wang et al., 2014).

1.10.4. Substrate in MFC

Substrate provides not only energy for the bacterial cells to grow in the MFCs but also influences the economic viability and overall performance such as power density and cumblic efficiency of MFCs. The composition, concentration and type of the substrate also affect the microbial community and power production (Cheng and Logan, 2011; Pant D, 2010). Many organic substrates including carbohydrates, proteins, volatile acids, cellulose and wastewater have been used as feed in MFC studies. It can range from simple, pure, low molecular sugars to complex organic matter containing waste water to
generate electricity. In most of the MFCs, acetate is commonly used as a substrate due to its inertness towards alternative microbial conversions (fermentations and methanogenesis) that lead to high columbic efficiency and power output (Pant D, 2010). Power generated with acetate found to be higher when compared with other substrate (Chae et al., 2009; Liu et al., 2005). Different substrate and their columbic efficiency and power output have been reviewed by many authors (Lee et al., 2008; Niessen et al., 2004; Pant D, 2010; Zuo et al., 2006). However, the economics of substrate is not known.

Table 1: List of substrates in MFC studies

<table>
<thead>
<tr>
<th>Substrate type</th>
<th>Concentration</th>
<th>Current density (mA/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>lg/L</td>
<td>0.8</td>
</tr>
<tr>
<td>Lactate</td>
<td>18mM</td>
<td>0.005</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.7mM</td>
<td>0.7</td>
</tr>
<tr>
<td>Sucrose</td>
<td>2674mg/L</td>
<td>0.19</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>6.7mM</td>
<td>1.18</td>
</tr>
<tr>
<td>Phenol</td>
<td>400mg/L</td>
<td>0.1</td>
</tr>
<tr>
<td>Sodium fumarate</td>
<td>25mM</td>
<td>2.05</td>
</tr>
<tr>
<td>Starch</td>
<td>10g/L</td>
<td>1.3</td>
</tr>
<tr>
<td>Cellulose particles</td>
<td>4g/L</td>
<td>0.02</td>
</tr>
<tr>
<td>Xylose</td>
<td>6.7mM</td>
<td>0.74</td>
</tr>
<tr>
<td>Domestic waste water</td>
<td>600mg/L</td>
<td>0.06</td>
</tr>
<tr>
<td>Brewery wastewater</td>
<td>2240mg/L</td>
<td>0.2</td>
</tr>
</tbody>
</table>

1.11. Current maturity of the technology

MFC is an active research field and scientific research has advanced rapidly increasing power density from a few Wm-2 to over 1kWm⁻³ of reactor volume under ideal conditions (Logan, 2010). The use of MFCs in wastewater treatment and remote power generation is being tested as a pilot scale project. Schwartz (2007) reported that biosensors powered by MFCs are closer to market readiness. Recently MFC designs have
advances new types electrode materials and significant progress in optimizing other
types electrode materials and significant progress in optimizing other
types electrode materials and significant progress in optimizing other
parameters and a few pioneering demonstrations were held at Queensland, Australia,
University of Connecticut and Cambrian Innovation, MA indicate that this technology
can be deployed for sustainable energy production and other applications in a few years
from now (Logan, 2010; Viscarolasaga, 2008) Currently, different MFC designs (single
chamber, tubular, series) are used with enhanced electrode materials and various
substrates to enhance the MFC power output (Min et al., 2005). Aelterman et al., (2006)
reported that power output can be increased when the MFC cells are connected in series.
However, getting a higher power output from MFC still remains a technically challenging
task and requires further increase in power generation for market readiness. Also, the cost
of new materials and their performance for long-term use need to be evaluated (Schwartz,
2007). Further, bacteria are evolved to oxidize different substrates (Table 1). It is essential
to understand that the metabolic pathway and gene regulatory systems of bacteria are
needed to achieve enhanced electricity production. It is also important to map the
transcriptional and metabolic pathways in different species of bacteria to enhance the
power output with higher efficiency. Continued research on scientific advancements will
lead to cost effective materials and designs that would accelerate commercialization of
this technology in the next few years.

1.12. Key Findings

Identification of bacterial species such as Clostridium butyricum and
Pseudomonas aeruginosa that produces their own mediators reduced the addition of
artificial chemical mediators to MFC for electron transport from bacteria to the electrode
(Osman et al., 2010). The direct communication of exoelectrogens like Geobacter species
that are capable of oxidizing organic compounds and their efficiency in transferring
electrons to electrodes via highly conductive filaments were considered remarkable in
MFC research (Derek R, 2008). Mixed bacterial cultures can produce power densities equal to pure cultures (Liu et al., 2004) and gradual increase in power densities (Rabaey et al. 2003) which accelerated the interest to research MFCs.

1.12.1. Challenges

To date, MFCs have emerged as a promising, yet challenging technology to extract energy from different sources and turn them into electricity. Despite the rapid progress, there are some areas in which further research needs to be done to overcome the constraints associated with MFC.

1.12.2. Low power

The major challenge in the application of MFCs is its low power density. The voltage generated by MFC is so low that it can only be used in limited applications and the actual current densities that can be generated are not yet known. Saldago (2009) reported that the current generation is only 14mA, which could power only small devices. Kim et al. (2007) reported that even using similar biocatalyst and substrate showed differences in the power density. Abhije et al. (2009) reported that the power obtained from MFCs is about 300 Wm⁻³ which is low for commercial applications.

1.12.3. Microbe/electrode interaction

Though the electron transfer mechanism is understood in some bacteria, further research is needed to create genetically engineered strains to generate more current (Lovely 2008). Current production by bacteria in MFC is a complex process that is regulated by more than few genes and requires further insight into the process of electron transfer (Franks and Nevin, 2010). Cheng et al. (2006) reported that bio-fouling of cathode affect MFC performance. As the electrode properties affect microorganism wiring and MFC performance, there is a need to develop higher catalytic material with
superior performance to avoid bio-fouling, corrosion and other degradation mechanisms of electrodes (Huang et al., 2011).

1.12.4. Large scale

The main challenge in implementing MFC on a large scale is in maintaining low costs, minimizing hazards while maximizing power generation (Schwartz, 2007). The performance of the MFCs is influenced by current, power density, fuel oxidation rate, loading rate and columbic efficiency (Balat, 2009; Kim et al., 2007). The power density is affected by high internal resistance or over potential related ohmic, activation and mass transfer losses (Logan and Regan, 2006) whereas the fuel oxidation rate is influenced by anode catalytic activity, fuel diffusion, proton and electron diffusion and consumption (Balat, 2009). Min et al (2005) described that diffusion of oxygen into the anode chamber lowers the columbic efficiency by more than half (55% to 19%) and reduces the power output. It has also been suggested that columbic efficiency and maximum theoretical amount of energy depend on complete oxidation of substrate to CO₂ (Franks and Nevin, 2010). The internal resistance can be minimized by reducing the electrode spacing, increasing the electrode surface area, using highly selective proton membrane and increasing catalyst activity (Oh and Logan, 2006). Liu et al. (2005) showed that closer electrode spacing, increased the power density by 68%. Chaudhuri and Lovely (2003) described threefold increase in current with larger surface area of electrode material. The performance is also affected by factors such as pH, temperature, substrate, microbial activity, resistance of circuit and electrode material. Yong Yuan et al. (2011) found that alkaline conditions (pH 9) favor electricity generation by enhancing electron transfer efficiency. However, Gil et al. (2003) reported that the highest current was obtained in the pH 7 –pH 8 range but not at pH 9. The power density decreases as the system size increases and further improvements are needed to construct highly efficient reactors with
reduced internal resistance and electrode over-potential to maximize power in large scale systems (Cheng and Logan, 2011).

1.12.5. Other factors

- Polarization resistance of anode and slow rate of proton movement from the biofilm to cathode and accumulation within the biofilm inhibits power production (Franks and Nevin, 2010; Wen et al., 2010).

- Cathode is an important factor for better performance of MFCs but oxygen reduction at the cathode occurs at a very slow pace that leads to high over potential, which is a limiting factor in obtaining high current density (Kim 2008).

- Optimizing MFC conditions and its performance needs to be evaluated over time to identify the variations such as change in fuel composition, build-up of metabolites and electrode fouling that affect the performance in large scale applications (Osman et al., 2010).

- Better understanding of fluid flow, ion migration and its concentration, proton mass transfer and bio-chemical pathway used by the exo-electricigens for higher metabolic rate and transfer of electrons to acceptors outside the cell need further investigation.

- MFC can be applied to some wastewater types that are not suitable for biogas processes, including low-strength wastewater, wastewater whose major components are volatile fatty acids, and those containing high concentrations of nitrogen and/or sulfur. Kazuya Watanabe (2008), Rittmann, B. E(2008).

1.13. Waste water treatment

Many kinds of wastewaters have been tested in recent years, including synthetic, domestic and industrial wastewaters (He et al., 2005; Huang and Logan, 2008; Ahn and Logan, 2009). Rapid start-up of any biological process used for wastewater treatment is desirable to avoid discharge of untreated wastewater. Liu and Logan (2004) demonstrated
that the single-chamber, air-cathode MFC could produce a consistent maximum voltage after 140 h (0.32 V, 4 cycles) when inoculated with the effluent of a primary sedimentation tank, but a stacked MFC using a ferric cyanide catholyte required 103 days following inoculation with a mixture of anaerobic and aerobic sludge (Aelterman et al., 2006). A cassette-electrode MFC with air-cathodes took 15 days before stable performance was achieved with a synthetic wastewater (Shimoyama et al., 2008). One of the most efficient methods for starting up a new MFC is to use the effluent from an existing reactor treating the same type of substrate (Jung and Regan, 2007; Chae et al., 2009; Kim et al., 2007). However, a large volume of pre-acclimated exoelectrogens may not be available for starting-up larger scale reactors. For example, a 1000 L pilot-scale microbial electrolysis cell (MEC) had to be inoculated with domestic wastewater to treat winery wastewater, and it required 60 days for start up (Cusick et al., 2011). In the case of domestic wastewater treatment, it is not known if startup can be accelerated or subsequent performance can be improved through creating different conditions in the wastewater during the start up phase of operation. Several approaches can be used to improve startup. First, additional substrates can be added since the wastewater strength is typically lower than that is used in laboratory systems (1 g/L chemical oxygen demand, COD). Second, the addition of specific alternate electron acceptors can be used to encourage the growth of known exoelectrogenic bacteria such as various Geobacter or Shewanella species. Third, the conductivity of domestic wastewater is low (~1 mS/cm) compared to buffered laboratory solutions (~7 mS/cm for 50 mM phosphate buffer).

1.14. Single-chamber air cathode Microbial fuel cell

The single-chambered air cathode offers several advantages over two chambered systems such as; (a) the cathode does not need to be aerated, oxygen in the air can directly react at the electrode, (b) recycling or chemical regeneration of the catholyte is not required and (c) higher volumetric power density is easily achievable due to smaller cell
volume (Fan et al., 2007; Liu and Logan, 2004; Liu et al., 2004). Air-cathode MFCs have shown to produce higher power output when operated without PEM (membrane-less MFC), which has been attributed to higher cathode potential (Liu and Logan, 2004; Liu et al., 2004). However, the major drawback of a membrane-less air cathode MFC is oxygen diffuses into the anode, which promotes the consumption of acetate by aerobic bacteria and thus results in lower Columbic efficiency (CE) (Cheng et al., 2006a; Liu et al., 2004). Oxygen diffusion to anode can be controlled by applying cathode coatings, such as polytetrafluoroethylene (PTFE), which would form hydrophobic gas diffusion layer on the air side of cathode (Cheng et al., 2006a). PTFE coating on cathode improves the power density/Coulombic efficiency and has been used as a diffusion layer in most of the air cathode studies (Cheng et al., 2006a; Nimje et al., 2011; Pant et al., 2010; Wang et al., 2010). Because of their high power output, simple structure and low cost, single-chamber air-cathode MFCs hold great promise for large scale applications in waste water treatment (Logan et al., 2006; Logan, 2004) (Shaoan Cheng et al., 2014). The single-chambered air cathode MFC has the ability to use passive air for oxygen reduction compared to earlier two-chamber design (Logan et al., 2007).

1.15. Future outlook

MFC is a promising technology for bioelectricity generation and waste water treatment. Recent research and development and analysis of literature review show that higher power densities can be obtained from improved MFC designs with the use of cost effective materials. Intensive research on this topic significantly reduced the complexity of rate-limiting steps which in turn has enhanced higher current output. Some companies (mfc tech, Opencel) have emerged to use MFC technology for fuel and other potential applications including remote power, bioremediation and biosensors (Caspermeyer, 2011; MFC tech) proving that this technology could have greater impact in development of clean energy within a few years.
MFCs are presently under serious considerations as devices for the treatment of industrial, agriculture and municipal waste water through microorganisms that oxidize organic compounds present in waste water, electrons are released yielding a steady source of electrical current (Akuma Oji et al., 2013; Kaufmann F. and Lovely R. 2001; Angenent L.T., et al., 2004)

1.16. Players and Research

In recent years, there are many research projects worldwide exploring MFC as a new source of energy. As a result of rapid advances in MFC research, several research publications have been reported in peer reviewed journals. Logan (2010) reported that the citation on the topic MFC have increased from 2,415 to 10,700 within a few years (2002-2009). A number of recently established startups and academic groups have collaborated to explore the commercial applications of MFC. Cambridge based IntAct’s lab (Cambrian Innovation) obtained funding from National Science Foundation (NSF) and the U.S. Department of Agriculture for developing MFCs for wastewater treatment projects. It has plans to startup a pilot plant for wastewater remediation. Similarly, Lebone, which was founded in 2007, obtained $200,000 grant from the World Bank and launched a pilot program in Tanzania and Namibia using MFC technology to provide power to small equipments like cell phone chargers and LEDs (Craven, 2010). The University of Glamorgan, UK had been awarded one million dollars for microbial fuel cell research to develop sustainable power (Lane, 2010). Emefcy, an Israeli biotechnology company had developed MFCs for electricity generation from wastewater and had plans for commercial implementation of MFCs by 2012 (Clary, 2011). Bruce Logan’s group at Penn state university was funded by ARPA-E for development of fuel using Rhodobacter (Logan) and have collaborations with National Renewable Energy Laboratory (NREL) and the Department of Energy (DOE) for fuel cell development. As noted earlier there are several groups working on MFC, this table (table2) is a non-comprehensive list of researchers and their research.

35
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Research</th>
</tr>
</thead>
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<td>Biodesign Institute, Arizona state University</td>
<td>Anode electrochemistry</td>
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<tr>
<td>Largus Angenent</td>
<td>Cornell University, NY</td>
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<tr>
<td>Bruce Logan</td>
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<td>Reactor design and scaling up power generation</td>
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<td>Harold May</td>
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<td>University of Colorado, Denver</td>
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<td>Modeling of MFC, PEM</td>
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