CHAPTER 1

INTRODUCTION

1.1 GENERAL

Environmental engineering has primarily focused on waste disposal and the removal of organic and inorganic contaminants. Recently this paradigm has progressed towards considering waste as a potential resource with the advancement of proper environmental technologies (Angenent et al. 2004; Logan 2004; Pant et al. 2010; Wang and Ren 2013). The depletion of fossil fuel and strengthened environmental regulations has accelerated the development of wastewater treatment technologies which are directed to recover valuable products and energy, while concurrently achieving the objective of pollution control (Logan 2004). One of the revived bio-electrochemical concept and promising technology that address all these aspects is microbial fuel cell (MFC). MFC are bio electrochemical systems (BES) that are capable of converting the chemical energy in organic wastes or bulk biomass into electrical energy via the catalytic activity of the micro-organism (Rabaey & Verstraete 2005; Logan & Regan 2006; He et al. 2006; Pant et al. 2010; Wang & Ren 2013). In classic dual chamber MFC configuration, the anode and cathode chambers are separated by proton exchange membrane (PEM). Microbial degradation of substrates liberates electrons and protons, of which electrons are transferred from the anode chamber to the cathode chamber through external circuit for the electricity production (Kim et al. 2002; Min & Logan 2004). At the cathode, the electrons combine with oxygen provided from air and with protons diffusing...
from the anode compartment through the PEM to form water. MFC performance is influenced by four processes namely microbial catabolism, electron transfer from the microbes to the anode, reduction of the electron acceptors at the cathode and proton transfer from the anode to the cathode (Watanabe 2008).

Different reactor configuration of MFC like single chambered air cathode, dual chambered, upflow MFC and stacked MFC (series or parallel) are used in the treatment of wastewater. Carbon and graphite are predominantly used as anode and cathode electrode material. Earlier lab scale MFC studies mostly used simple and defined substrates like acetate, glucose, or other simple substrates to characterize the performance of electrode materials, reactor configurations, or microbial activities (Liu et al. 2005a; Rabaey et al. 2003). Liu et al. (2004) reported the first study employing the real wastewater as substrate in MFC. Subsequently, numerous studies have been performed with the power production from different complex substrates such as food processing wastewater (Oh and Logan 2005), chocolate industry wastewater (Patil et al. 2009), rice mill wastewater (Behera et al. 2010a), mustard tuber wastewater (Guo et al. 2013a), refinery wastewater (Zhang et al. 2014) and retting wastewater (Jayashree et al. 2015) employing various reactor configurations, electrode and membrane materials. MFC have developed tremendously in the last few years due to the progress in the reactor configurations (Logan et al. 2006), electrode and electrode surface modifications (Wei et al. 2011), microbial communities and understanding of the extracellular electron transport (EET) mechanisms (Kumar et al. 2016).

Electron liberation at the anode through microbial catalysis and subsequent electron consumption at the cathode are the defining characteristics of an MFC (Logan & Regan 2006). Electrochemically active micro-organism capable of transmitting electrons externally are called as
exoelectrogens and are the essential component for power production in MFC (Rabaey et al. 2004; Read et al. 2010). Microbial EET was first discovered in 1911 by Potter, who demonstrated that electrical energy can be generated from the cultures of *Escherichia coli* and *Saccharomyces* using Platinum electrodes. Later in 1980s, it was reported that mediators added to the microbial media could enhance electron transfer from microbial cells to the anode. Electrogens or anode respiring bacteria transmit electrons out of cell membranes to the electrode either directly through membrane-bound protein structures, such as pili, c-type cytochrome and filaments (direct electron transfer), or using electron shuttles like flavins, pyocyanin for mediated electron transfer. *Geobacter spp.* and *Shewanella spp.* have been studied widely for their EET mechanism. Both exoelectrogens exhibit direct electron transfer (DET) and mediated electron transfer (MET) mechanisms. The other exoelectrogens studied for EET mechanisms consist of *Pseudomonas aeruginosa* that produces pyocyanin and phenazine-1-carboxamide (Shen et al. 2014), *Geothrix fermentans* secretes flavins (Kolte and Bond 2012) and *Acidithiobacillus ferrooxidans* recently has shown to produce conductive nanowires (type IV pili) (Li and Li 2014). Electroactive biofilms are imperative for efficient power production in the MFC. The current generation in MFCs directly depends on biomass of the biofilm and the type of electrode surface. The positively charged and hydrophilic anode surfaces are more favorable for electroactive biofilm formation and thereby influence the performance of the MFC (Guo et al. 2013b).

MFC is a promising sustainable and cleaner technology to meet increasing energy needs, especially using wastewaters as substrates, which can generate electricity and accomplish wastewater treatment simultaneously, thus may offset the operational costs of wastewater treatment plant. Power output by MFCs has increased considerably over the last decade due to several scientific and technical advances. The advantages of using MFCs in
wastewater treatment are embedded in the economic perspective of sludge disposal and aeration energy (Oh et al. 2010; Ren, 2013; Xiao et al. 2012). Main advantage of MFC is the low biomass production. The cell yield of electrochemically active bacteria in MFC (0.07–0.16 g VSS/g COD) is much less than the activated sludge (0.35–0.45 g VSS/g COD), which in turn reduces sludge production by 50–70% (Fan et al. 2012). Treatment of the sludge alone can amount to 45–75% of plant energy costs (Pant et al. 2010). Second advantage, aeration is not required in the case of implementation of the air cathode MFC. The maximum power density achieved from the lab scale air-cathode MFC is 2.87 kW/m³ (Fan et al. 2012). The projected wastewater treatment capacity of MFCs can reach 7.1 kg chemical oxygen demand (COD)/m³ reactor volume/day, which is even higher than conventional activated sludge systems (~0.5 – 2 kg COD/m³ day) (Rozendal et al. 2008). However, there are still many challenges that need to be addressed before the MFC can be implemented in large scale. More technological advancements in terms of material, costs and substrates being used are necessary to bring these systems at a level where they can be commercially exploited. Applications of the microbe–electrode interactions have also been expanded to bio-remediation (Gregory and Lovley 2009), harvesting the energy stored in marine sediments (Donovan et al. 2011), desalination (Cao et al. 2009) and microbial solar cells (Strik et al. 2011).

1.1.1 Coir Industry and Retting Wastewater

Total world coir production is estimated as 0.5 million tonnes. India and Sri Lanka together contribute 90% of the global coir production. India has monopoly in white fibre production (1, 35,000 tonnes per annum nearly 100% against total world production). The coir industry contributes for the large employment opportunity as a traditional cottage industry in India especially for women (Shibu et al. 2013). Coir is one of the strongest natural fibres
extracted from coconut (*Cocos nucifera*) husk which is widely used for production of rope, mats, brushes and sack. The coconut husk retting effluent is considered to be one of the important sources of pollution in Cochin back waters (Ambika Devi 1993) and estuarine pollution in many river mouths of Kanyakumari district.

Coconut is grown in lowland tropical and subtropical habitats all around the world. The coconut husks comprises of the exocarp and fibrous mesocarp which is the raw material for the coir fibre. The term retting is derived from the word 'rotting' and designates the process of decomposition of tissues surrounding the vegetable fibres. Large quantities of organic substances including pectin, pentosan, fat, tannins, and toxic phenols are liberated into water due to retting. The extraction of coir fibre is performed by two methods:

1. Open retting - results in the good quality white fibre and
2. Mechanical extraction – results in production of brown fibre.

The mechanically extracted brown fibre is inferior in quality particularly in colour, length and flexibility (non – retted). The open retting involves the immersion of coconut husks in brackish water or lagoon for a period of 6 to 12 months and separation of fibre from mesocarp manually or by mechanical method. Retting is a physical process in which materials like carbohydrates, tannin and fast hydrolysable compounds from the husk get leached out into the immersion water. Microorganisms act on polysaccharides like pectin and other biodegradable compounds in the husk (Thomas et al. 2006). At the end of this process inter lamellar region of the mesocarp get loosened, pith cells get separated and fibre surface become smooth rendering good quality fibre (white fibre).
Adverse effects of retting of coconut husks are reduction in the water transparency due to formation of coloured recalcitrant humic substances and oxidative polymerization of phenolic compounds (Thomas et al. 2002). The comparative study of eukaryotic and metazoan biodiversity in retting areas with non-retting zones depicted serious deterioration, in particular higher metazoans biodiversity. Low level of dissolved oxygen has adverse role in the fish population and fishery. In developed countries, due to the extensive pollution to water bodies, this process is abandoned (Shibu et al. 2013). Anaerobic environment leads to the growth of methanogens and generates considerable emission of Green House Gas (GHG). Hydrogen sulphide and methane emission from retting area results in air pollution and related health hazards (Kumara et al. 2011). Flooding during monsoon disperses these pollutants to surrounding areas. Retting wastewater has been treated by various methods like closed retting (Shibu et al. 2013) and lab – scale UASB (upflow anaerobic sludge blanket) reactor (Neena et al. 2007).

1.1.2 Seafood Processing Wastewater

The world largest seafood consumption in the world is by Japan, followed by European Union. The global seafood market is estimated at US$ 100 billion per annum and the world demand for seafood increases by 3% each year. Sources of effluent from seafood processing includes defrosting, gutting, scaling, portioning, filleting and washing of fish, which generates enormous amount of wastewater. This effluent streams contain high organic content due to the presence of blood, flesh pieces, oils, intestinal remains and suspended solids. Effluent quality depends on the type of fish being processed and type of processing undertaken. Pollution generated from the processing of oily fish species are much higher than from white fish species (UNEP, 2000). During canning operations, effluent is discharged from the draining of cans after precooking, from the spillage of sauces, brines and oil. Odor is often the
most significant menace in the fish processing. Odor arises from the storage sites for processing waste, cooking by-products during fish meal production and fish drying processes. Deterioration causes the formation of odorous compounds such as ammonia, mercaptans, and hydrogen sulphide gas.

The effluent stream released without treatment into water resources leads to eutrophication and coastal pollution. The deterioration of the organic compounds in the wastewater leads to oxygen depletion (Scott and Hui, 2004). In addition, fish processing industries pollute nearby beaches and shores by releasing wastewater containing oils. Therefore it was decided to treat these wastewaters which are predominant pollutants of coastal water bodies using MFC. In the present study, power production and organics removal from the retting wastewater was examined using dual chamber and continuous tubular upflow MFC. Seafood processing wastewater was treated using continuous tubular upflow MFC and anodic biofilm analysis was also performed.

1.2 OBJECTIVES OF THE RESEARCH

The objectives of the study are,

- To evaluate the power production, phenol and chemical oxygen demand (COD) removal efficiency at various hydraulic retention time (HRT) of the dual chamber MFC treating retting wastewater.

- To analyze the influence of the organic loading rate (OLR) on the power production and COD consumption rate of the tubular upflow MFC treating retting wastewater.
• To identify the microbial communities from anode biofilm contributing for phenol removal in MFC through 16s rRNA sequencing.

• To investigate the effect of OLR on the performance of tubular upflow MFC treating seafood processing wastewater.

• To elucidate the influence of phosphate buffer as catholyte in power production from seafood processing wastewater.

• To identify the microbial communities in anode biofilm of the MFC constituting seafood processing wastewater through 16s rRNA sequencing.

1.3 LITERATURE REVIEW

1.3.1 Configurations of the Microbial Fuel Cell

Reactor architecture determines the power production, coulombic efficiency, and stability of the MFC during wastewater treatment. Nowadays the challenges for MFCs used in wastewater treatment are the

• Low power densities
• Low coulombic efficiencies
• Capital costs of the materials (anode, cathode and membrane)

Different reactor configuration of MFC like single chambered, dual chambered, upflow MFC and stacked MFC (series or parallel) are used. The dual chambered or H-type MFC consist of a separator like Nafion (PEM) or ultrex which separates the anode and cathode chamber which is depicted in the Figure 1.1. H - shape MFCs are suitable for basic research, like investigating power production using new substrates, or types of microbial communities that arise during the degradation of specific compounds (Logan
et al. 2006). The power production in these MFC is limited by high internal resistance and electrode based losses.

![Diagram of dual chamber MFC with working principle](source)

(Source: Logan et al. 2006)

**Figure 1.1 Schematic diagram of dual chamber MFC with working principle**

Tubular packed bed reactors are specifically designed to run in continuous flow mode. The design of first tubular reactor consists of an anode chamber and cathode chamber which is separated by glass wool and glass beads, the substrate flows from anode to the cathode (Jang et al. 2004). Rabaey et al. (2005b) designed a tubular reactor were CEM was modified in the form of tube and packed with graphite granules, and ferricyanide catholyte overflowing the outside of the reactor covered with a thick woven graphite mat. This reactor accomplished only 20 % COD removal. Two different tubular flow reactor designs were developed (He et al. 2005; He et al. 2006). The first reactor developed by He et al. (2005) comprises of an anode with
reticulated vitreous carbon (RVC), which was separated from the cathode chamber containing ferricyanide solution through CEM (Figure 1.2). This MFC recorded with an internal resistance of 84 Ω and thus lead to reduction of coulombic efficiency from 0.7 % to 8.1 %. Second upflow MFC designed by He et al. (2006) contained a CEM formed into a tube that was placed into a bed of granular activated carbon (GAC). This reactor achieved soluble COD (SCOD) removal efficiency of more than 90%. It had a total internal resistance of 17.13 Ω, which was separated into an electrolyte resistance of 8.62 Ω, a charge transfer resistance of 7.05 Ω, and a diffusion resistance of 1.46 Ω. The coulombic efficiency of this MFC ranged from 11% to 51% and more power production was achieved.

(Source: He et al. 2005)

**Figure 1.2** Tubular upflow reactor designs (A) Schematic and (B) photograph of an upflow system packed with reticulated vitreous carbon (RVC)
1.3.2 Materials Employed in the MFC

The main components in the MFC are the anode, cathode and membrane. Electrode designs are the greatest challenge in implementation of MFCs as a cost-effective and scalable technology. The cathode and membrane contributes for the largest proportion of the overall cost (an estimated 47 % and 38 % respectively) due to the use of expensive cathode catalysts (Platinum) (Rozendal et al. 2008) and expensive ion exchange membranes. Therefore it is essential to investigate cost-effective materials for anode, cathode and membrane in MFC which increases the power production and coulombic efficiency when used in wastewater treatment.

1.3.2.1 Anode materials

Anode materials should have the properties like high conductivity, chemical stability, high durability, non fouling and inexpensive. In addition, surface characteristics of anode electrode materials such as high surface area, porosity, good biocompatibility, and efficient electron transfer between bacteria and electrode surface, are essential for high bio-catalytic activity. Carbon-based electrodes are the most widely used for MFC and based on their configuration, they are divided into a

- Plane structure,
- Packed structure, and
- Brush structure
Carbon paper and cloth, carbon mesh, graphite plates or sheets belongs to the plain electrode configuration. Carbon felt, reticulated vitreous carbon, glassy carbon, granular activated carbon, activated carbon fibre felt, granular graphite and graphite discs forms the packed structure configuration (Figure 1.3). The main advantage in this configuration is high surface area which renders efficient bacterial adhesion (Min & Logan, 2004; Sun et al. 2010). Carbon or graphite granules, as three dimensional and porous materials, were reported to achieve high power densities in up-flow continuous reactor presumably due to the large surface area (Aelterman et al. 2008; You et al. 2009; Rabaey et al. 2005b). Increased internal resistance was observed in these reactors, since the carbon granules are not directly connected to each other. The graphite fibres are cut into small fragments and wound together in the form of brush. The graphite brush anode achieves high surface area, high porosity, and efficient current collection. Surface modification of anode materials by the following methods enhances the power densities obtained from the MFC.

- Ammonia,
- Heat,
- Acid treatment,
- Electrochemical oxidation and
- Carbon nanotube (CNT) - composite
Figure 1.3 Electrode materials used in MFC: (A) carbon paper; (B) graphite plate; (C) carbon cloth; (D) carbon mesh; (E) granular graphite; (F) granular activated carbon; (G) carbon felt; (H) reticulated vitrified carbon; (I) carbon brush; (J) stainless steel mesh
First, the ammonia treatment enhances the positively charged functional groups over the electrode surfaces thereby increasing the adhesion of the negatively charged micro-organism onto the anode which promotes the direct transfer of electrons to the electrodes. Second, thermal treatment of the carbon fibre brush anode exhibited a 15% increment in the power density compared to its unmodified counterpart (Feng et al. 2010b). Third, acid treatment facilitates the protonation of the functional groups on the graphite felt anode surface facilitating two fold increments in power density (Scott et al. 2007). Combined acid and heat treated anode renders high power production due to increase in specific area, higher ratio of protonated N to the total N gives more positive charge on electrode surface which favors adhesion and also reduces contaminants that interfere with charge transfer from bacteria to anode surface (Wang et al. 2009). Fourth, electrochemical oxidation favours the generation of carboxyl functional groups on anode surface which facilitates the peptide bond formation between the electrode and micro-organisms. Graphite plate modified by electrochemical oxidation resulted in 57% increase in electron transfer rates (Lowry & Tender 2008).

Surface coating materials like carbon nanotubes (CNTs), conductive polymers, mediators, metals and composites are applied to anode materials. CNTs can facilitate electron transfer from bacteria to electrode and reduces the internal resistance of the MFC. Graphene based nanomaterials (Zhang et al. 2011) and Platinum nanoparticles deposited carbon paper employed as anode material recorded with enhanced power output. Graphite anode modified with a graphite paste containing Fe$_3$O$_4$ or Fe$_3$O$_4$ and Ni$^{2+}$ possessed greater kinetic activity than plain graphite (Lowy et al. 2006). Polypyrrole-CNT composite decreases the mass transfer resistance and increased the contact between micro-organism and electrode (Zou et al. 2008). Whereas CNTs (e.g. with polyaniline or polypyrrole) as anodes in microbial fuel cells are reported to show cellular toxicity (Zou et al. 2008).
Mediators like neutral red (NR), anthraquinone-1,6-disulphonic acid (AQDS), and 1,4-naphthoquinone (NQ), were used to facilitate the shuttling of electrons from inside the cell to the electrodes. However, the drawback is that these mediators must be continually added or recycled. Iron oxide-coated anodes produced higher power densities and coulombic efficiency (CE), compared with plain anodes (Kim et al. 2005). Better understanding of the interaction of bacteria and the anode surface could further lead to efficient electron transfer and therefore higher power production.

1.3.2.2 Cathode materials

Most of the carbonaceous materials like carbon cloth, graphite plate and carbon felt used as the anodes are employed as cathode material in the MFC. Pertaining to the cathode configurations, the following are commonly employed for laboratory MFCs.

- air–cathodes and aqueous air–cathodes with catalyst,
- air–cathodes and aqueous air–cathodes without catalyst, and
- bio-cathodes

Air–cathodes are considered as more practical design for MFC cathodes because they require no aeration and generate higher power densities. Carbon cloth as a supporting material is very common for air–cathodes (Cheng et al. 2006a,b; Logan et al. 2007). Compared with carbon paper and carbon cloth, more economical and practical are MFC designs that use stainless steel. Fabrication of stainless steel as cathode material achieved a power density similar to an air–cathode based on carbon cloth (Zhang et al. 2010; You et al. 2011). Perfluorosulfonic acid (Nafion) and polytetrafluoroethylene (PTFE) are two commonly used binders in MFCs, for the binding of catalyst like Pt with the electrode surface.
Aqueous air–cathodes consist of carbon paper, carbon cloth, and platinum mesh, coated with a catalyst/binder layer (Logan et al. 2005; Scott et al. 2008b; Yu et al. 2007). To evade high oxygen fluxes and water loss through the cathode, a hydrophobic diffusion layer is applied on the air-facing side of the cathode. Four PTFE diffusion layer coatings resulted in 71% and 42% increase in the CE and power density respectively (Cheng et al. 2006a). Zhang et al. (2010) employed inexpensive coating of polydimethylsiloxane (PDMS) as a diffusion layer for stainless mesh and achieved a CE of more than 80%.

A non-catalyzed aqueous air–cathode made of granular graphite was reported by Freguia et al. (2007) and Tran et al. (2010). Activated carbon cold-pressed with a PTFE binder to form the cathode around a Ni mesh produced a higher power density than a Pt-catalyzed carbon cloth cathode (Zhang et al. 2009a). Activated carbon fiber felt (ACFF) in an upflow MFC produced more power density compared with a platinum-coated carbon paper (Deng et al. 2010). Nitric acid and thermal-activated graphite granules cathode led to a high open circuit voltage of 1050 mV, due to the increase of surface area and the emergence of nitrogen groups on electrode surface (Erable et al. 2009a). Fe$^{3+}$ graphite depicted superior performance compared with a graphite electrode, due to Fe$^{3+}$ which acts as electron mediators (Park & Zeikus 2003). The activated carbon and HNO$_3$ treated carbon powder as cathode achieved higher power production (Duteanu et al. 2010). The advancement of these inexpensive materials is mandatory for the application of MFC in wastewater treatment.

Biocathode are relatively low cost, offers good stability and favourable option for the wastewater treatment. The electrode surface area and their characteristics are the two vital criteria that affect the biomass (Huang et al. 2011b). Mostly carbonaceous materials such as graphite plate,
carbon felt, granular graphite, and graphite fiber brush, as well as stainless steel mesh are employed as biocathodes (Behera et al. 2010; Clauwaert et al. 2007a,b; Dumas et al. 2008b; Liang et al. 2009; Xie et al. 2011; You et al. 2009). MFC with stainless steel performed better than graphite biocathodes (Dumas et al. 2008b). Though packed and brush structures are superior compared with other materials since they provide larger surface areas, they are not cost effective in real time applications of the MFC.

### 1.3.2.3 Membrane

Substrate degradation at the anode chamber generates equivalent amount of protons and electrons. Electrons passage to the cathode through the circuit depends on the potential gradient and protons are transferred to the cathode by diffusion. The membrane serves to separate the anode and cathode specifically in a two chambered BES, while permitting selective transport of protons from the anode to the cathode (Logan et al. 2006). The cation or proton exchange membrane (PEM), anion exchange membrane (AEM), ultrafiltration membrane and bipolar membrane are the commonly used membranes in MFCs. Nafion 117 is an expensive PEM commonly used in MFC for proton transport from the anode to the cathode. The code 117 represents the thickness of the membrane (0.019 cm). In addition to proton ($H^+$) transfer, Nafion also conducts other positively charged species ($Na^+$, $K^+$, $Ca^{2+}$, $NH_4^+$ and $Mg^{2+}$) from the anolyte to the cathode chamber (Rozendal et al. 2006b). The competitive transport of other cations (which are present at concentration $10^5$ times higher in the anode) than protons significantly affects MFC performance. If protons cannot migrate at a sufficient rate from the anode to the cathode, the pH will decrease at the anode which sequentially affects bacterial respiration and hence power production. Rise of the pH in the cathode leads to the salt precipitation and thus inhibiting the cathode catalyst. Nafion is most permeable to the oxygen when compared with other
ultrafiltration membrane. This results in reduced coulombic efficiency due to substrate utilization by aerobic micro-organism.

Kim et al. (2007) reported higher power densities and coulombic efficiencies using an AEM compared to the use of Nafion and cation exchange membrane (CMI-7000). The better performance of the AEM was due to the use of phosphate or carbonate as proton carriers which would contribute to a better pH balance in the anode and cathode chamber. The bipolar membrane consists of an anion and cation membrane joined in series. The disadvantages of implementation of the membrane in the MFC are their high cost and increase in the internal resistance of the system. During long term usage of the membrane in MFC operation, fouling occurs. Membrane fouling can reduce the proton transfer and oxygen diffusion between the anode and cathode chamber.

1.3.3 Factors that Influence the Voltage Production in the MFC

The maximum theoretical voltage ($E_{\text{theor}}$) attained by MFC is around 1.1 V. However the OCV produced by the MFC is always less than the predicted value due to the losses that occur in the system (Logan et al. 2006). The internal losses are categorized into three different losses namely activation losses, ohmic losses and concentration losses. This loss collectively contributes to the internal resistance of the MFC. Internal resistance can be defined as the collective resistance experienced as electrons and protons travel from substrate, through the MFC to the terminal electron acceptor.

Activation Losses: This loss is characterized during startup of the MFC; when tremendous energy is needed for an oxidation/reduction of the substrates in the anode chamber. Activation losses (or activation polarization) occur during the transfer of electrons to the electrode surface. Activation losses can be minimized by increasing the electrode surface area, improving electrode
catalysis and application of preacclimatized inoculum (micro-organism) onto the anode electrode.

Ohmic Losses: The ohmic losses (or ohmic polarization) in an MFC is contributed by the two factors. First, the resistance conferred to the flow of electrons through the electrodes and interconnections (circuit). Second, the resistance to the flow of ions (H\(^+\)) through the PEM, anodic and cathodic electrolytes (Hoogers 2003; Larminie & Dicks 2000). This intermediate zone is of prime importance since it corresponds to the ‘working zone’ or ‘cell design point’ of the MFC. The ohmic losses arise due to the inherent properties of the fuel cell materials and their design. Ohmic losses can be reduced by minimizing the electrode (anode and cathode material) spacing, choosing membrane with a low resistivity, buffering capacity and increasing solution conductivity to a certain limit so that the anodic biofilm is not affected.

Concentration Losses: This occurs mainly at high current densities due to concentration gradients and transfer limitations. As the substrate diffuses toward the electrode there is a reaction rate at which the mass transport of the substrate cannot maintain with the rate at which it is being consumed at the electrode.

1.3.4 Operational Parameters for MFC

1.3.4.1 Effect of the pH

In MFC, the anode reaction produces protons that will flow to the cathode where it will react with oxygen to produce water. Continuous operation of an MFC, results in the accumulation of protons produced by the microbial oxidation of organic substrates in the anode chamber leading to reduction in pH (acidic). The acidified anode results in alterations of ion
concentration, membrane potential, decreases micro-organism activity and consequently affect the biofilm performance and stability (Zhang et al. 2011, Yuan et al. 2011, Patil et al. 2011; Jung et al. 2011). The pH in the cathode chamber increases due to slow and incomplete proton diffusion through the PEM (Biffinger et al. 2008; Jadhav & Ghangrekar 2009; Raghavulu et al. 2009; Behera & Ghangrekar 2009; Erable et al. 2009b; Zhuang et al. 2010; Winfield et al. 2011).

In dual chambered MFCs two different pH can be maintained in the anode and cathode chamber, however in air cathode MFC, its not feasible. In single chamber air cathode MFC configuration, the pH affects both anodic microbial activities and cathodic reaction. He et al. (2008) demonstrated that the anodic bacterial activity is optimal at a neutral pH, while the cathodic reaction was improved at a higher pH. Mixed culture anodophilic biofilm helps in overcoming the detrimental drifting of electrolyte pH and catalyzes the cathodic oxygen reduction in single chamber MFC. To rectify the pH gradient in MFC, acid/base dosing or addition of chemical buffer is required to sustain steady current production.

An ideal buffer maintains constant pH without interfering chemical reactions or microbial physiology, facilitate proton transfer to the electrode and increase the solution conductivity. Phosphate buffers are the most commonly used in MFC (Cheng & Logan 2007; Kim et al. 2007a; Fan et al. 2007; Min et al. 2008) and it depicts that increasing phosphate concentration within certain ranges will increase power output. Bicarbonate buffer is another cost effective buffer which can be used in MFC, especially for wastewater treatment (Fan et al. 2007). Qiang et al. (2011) found that adding a borax buffer improves the electron recovery efficiency.

Synthetic zwitterionic buffers, such as MES (2[N-morpholino] ethane sulfonate), HEPES (4(2-hydroxyethyl) 1-piperazine ethane sulfonic
acid) and PIPES (piperazine-N, N-bis [2-ethane sulfonate]) can be used as buffers in MFC. These buffers have advantages in biological studies, as their pKa values are in the range of pH 6.0 and 8.0, chemically stable and nontoxic (Nam et al. 2010a). Alternative to buffer, feeding air with carbon dioxide to the cathode of the MFC depicted an increase in power density and cell voltage (Torres et al. 2008, Fornero et al. 2010). In addition, the carbon dioxide is available as a waste gas in industrial settings making the implementation of this carbon dioxide buffered MFC system very promising due to a significant cost reduction.

### 1.3.4.2 Effect of Organic loading rate

The power density and coulombic efficiency of the MFC depend upon the substrate conversion rate, therefore the OLR applied will determine the performance of the MFC. Numerous studies have analyzed the effect of OLR and their influence on the power production and substrate degradation in MFC (Jadhav and Ghangrekar 2009; Martin et al. 2010; Wang et al. 2011). Increment in power production and substrate degradation was observed as the OLR of the substrate increases. However if the OLR was too high, power production decreases inspite of the increase in substrate degradation of the MFC (Aelterman et al. 2008; Mohan et al. 2009; Lorenzo et al. 2010; Reddy et al. 2010; Kim et al. 2010; Nam et al. 2010b; Juang et al. 2011; Goud et al. 2011; Velvizhi and Mohan 2012). The increase in the substrate degradation observed at high loading rates may be attributed to the direct anodic oxidation (DAO) mechanism. DAO of deprotonated substrate might activate the formation of oxidation species on the anode surface which leads to enhanced substrate removal rates. The higher power production can be attributed to the availability of adequate substrate concentration to sustain the metabolic activity (Mohan et al. 2009).
The internal resistance (ohmic resistance, electrolyte resistance and membrane resistance) of the cell decreases with an increase of the OLR. This is due to an improvement of the ionic strength of the anolyte, resulting from higher volatile fatty acids (VFA) concentrations, increased catalytic activity and density of the anodophilic microorganism (Martin et al. 2010; Velvizhi and Mohan 2012). With increasing OLR, the coulombic efficiency decreased, whereas the volumetric methane production rate was increased (Martin et al. 2010). High OLR, corresponding to highly saturated conditions with respect to anode surface area, may lead to competition between electrogens and other non electrogenic organisms. This leads to greater organic matter removal not related to power production which simultaneously lowers the coulombic efficiency. These studies regarding the effect of OLR on MFC depicts that with an optimized OLR the rate of the electricity production can be enhanced while the methane production can be controlled.

1.3.4.3 Effect of the flow rate and shear stress

Hydrodynamic challenges affect the MFC performance and anodic biofilm (Guillou et al. 2000; Moon et al. 2005; Rickard et al. 2004). Regarding the flow rate, an increase on the flow rate of the substrate leads to an increase in power production of the MFC, (Aaron et al. 2010; Juang et al. 2012) however very high flow rate decreases the power density (Ieropoulos et al. 2010). Hence optimal performance of MFC is obtained, after microbial community is given sufficient duration to develop and utilize the substrate. Similarly, increase in the flow rate of the feed has an opposed effect on COD removal and coulombic efficiency (Lorenzo et al. 2010; Juang et al. 2011). As the HRT decreases, consequently the time required by the microorganism to degrade the organic matter decreases.

Hydrodynamic strength is one of the vital parameters influencing microbial adhesion and biofilm formation. High shear rates result in thicker
and denser biofilms, which enhances the power output of a MFC (Alice et al. 2008; Pham et al. 2008). The development of thicker biofilms may be due to an increase of biomass production resulting from higher mass transfer. CLSM (confocal laser scanning microscopy) and SEM (scanning electron microscopy) observations reported that the bacterial density on the electrode surface for the high shear rates was significantly higher than the low shear rates. This proves that the denser and thicker anodic biofilm leads to higher electricity generation. In addition, higher shear rates decrease the diversity, therefore one species dominated the bacterial community leading to a more homogeneous biofilm (Rickard et al. 2004; Alice et al. 2008). At higher shear rate, decrease in the power production and biofilm thickness of the MFC was observed.

1.3.4.4 Effect of temperature

Changes in temperature of the MFC affect system kinetics and mass transfer, thermodynamic (free Gibbs energy and electrode potentials) and nature and distribution of the microbial community (Martin et al. 2010; Jadhav & Ghangrekar 2009; Wang et al. 2008; Hong et al. 2009; Ahn & Logan 2010). It is an essential factor in the MFC performance concerned with COD removal and electricity generation. Increase in the power output and COD removal efficiency of the MFC was coherent with the hike in the temperature (Min et al. 2008; Jadhav & Ghangrekar 2009; Wang et al. 2011; Tang et al. 2012). The increase of power density with higher temperatures may be attributed to the enhancement of the microbial metabolism and ohmic resistance reduction, due to higher conductivity of the anolyte (Qiang et al. 2011; Martin et al. 2010). Modification in the temperature barely influenced the permeability of the membrane.

Concerned with the ohmic and internal resistance, they decrease with the increase in the temperature of the MFC (Behera et al. 2011;
Martin et al. 2010; Larios et al. 2011). This could be due to the increase in the ionic conductivity of the anolyte which leads to reduction in the ohmic resistance (Guerrero et al. 2010). The exponential trend observed by the effect of temperature on MFC power output is owed to the enhancement of the microbial activity in anodic biofilm (Liu et al. 2012). Studies regarding the effect of temperature demonstrated that the initial temperature of MFC has important impact on formation of stable biofilm and MFC startup. Maximum bioelectrocatalytic activity of biofilms and MFC performance was observed at a temperature between 30°C and 45°C (Michie et al. 2011; Patil et al. 2010).

Lower and higher operation temperatures of MFC results in the slower and inactivation of metabolic activity of micro-organism respectively which in turn leads to the decrease on biofilm formation and MFC performance (Liu et al. 2011). The temperature during the initial growth phase of biofilm determines the abundance and distribution of the different microbial species in the anode electrode. This clearly shows the importance of the startup temperature on biofilm formation and that after this period the MFC can operate over a wide range of temperatures without a significant decrease on performance (Cheng et al. 2011).

1.3.5 Exoelectrogens

Micro-organisms that are capable to generate and transfer electrons to a working electrode without aid of external mediators are called as exoelectrogen (Orellana et al. 2013). They are also termed as electrochemically active bacteria, anode respiring bacteria and electricigen. G. sulfurreducens (Nevin et al. 2008) and S. oneidensis have been investigated to elucidate the different mechanisms of electron transfer from microbes to the electrodes. Exoelectrogens can transfer electrons to an electrode surface directly without mediators by (i) direct electron transfer (DET) via redox-active proteins such as cytochromes present on the outer surface of bacterial
cell membrane and conductive pili referred as microbial nanowires (ii) mediated electron transport (MET) via microbial secreted soluble electron shuttles such as flavins and pyocyanin (Figure 1.4). The other electron carrying co-factors such as quinones, heme, iron–sulfur clusters or copper ions also play a significant role in microbial electron transport.

![Figure 1.4](Image)

(Source : Logan 2009)

**Figure 1.4  Mechanism of electron transfer in the exoelectrogens**

The exoelectrogens near to the electrode surface prefer DET mechanism as their outer cell membrane with exposed c-Cytochromes remains in the direct contact with the electrode surface. *Geobacter sulfurreducens* genome contains more than 110 genes encoding putative c-type cytochromes which are expected to play an essential role in DET mechanisms (Malvanker et al. 2014). *G. sulfurreducens* near to the electrode surface utilize OM c-Cyts for the electron transfer while the cells in the biofilm distant from the electrode surface uses pili and OM c-Cyts. *Geobacter metallireducens* contains two genes imperative for Fe (III) oxide reduction.
The first gene encodes an NHL-repeat containing protein and the second gene is involved in pili glycosylation (Smith et al. 2013). The exoelectrogens in the mixed cultures can transport the electrons in between via direct interspecies electron transfer (DIET). The pilus-associated c-type cytochrome OmcS and pili have been associated to mediate DIET. Granular activated carbon (GAC) has been hypothesized to stimulate DIET between bacteria and methanogens (Rotaru et al. 2014).

Flavin mononucleotide (FMN) and riboflavin (RF) in *Shewanella oneidensis* MR-1 enhances the EET, but actually the flavins act as the cofactors for outer-membrane c-Cyts (i.e. FMN as a cofactor for MtrC and RF for OmcA) and the cytochromes also exhibits specific binding sites for these molecules. These flavin-cytochrome complexes regulate the rate of electron transfer to the surfaces which promotes MET. The electrons are transferred from the inner membrane via CymA protein to the outer membrane via MtrA and MtrB proteins. Finally, the complex of OM c-Cyts and the flavin molecules mediate the electron transfer from the microbial outer membrane to the electrode surface.

Electrochemically active bacteria such as *Comamonas testosteroni* and *Pseudomonas putida* isolated from MFC were capable of hydrocarbon degradation (Lu et al. 2014). The exoelectrogens isolated from the hydrocarbon contaminated soil expressed increased catalase activities. Other bacteria such as *S. aureus*, *T. osonensis* and *L. sphaericus* have shown the ability to act as exoelectrogens and produced significant bioelectricity in the MFC (Kumar et al. 2016). The understanding of electron transfer mechanisms and their associated proteins with the application of genetic engineering may solve the problem of low power output in the MFCs.
1.3.6 MFC applications beyond electricity generation

1.3.6.1 Microbial Electrolysis Cell (MEC)

MFC modified to produce hydrogen from the organics instead of electricity is referred to as microbial electrolysis cell (MEC). MEC also termed as biocatalyzed electrolysis, bioelectrochemically assisted microbial reactor process (BEAMR) or electrohydrogenesis (Cheng & Logan 2007a). Hydrogen generation from the anaerobic degradation of substrates by electrogens is thermodynamically unfavorable (Rozendal et al. 2006; Liu et al. 2005b). This thermodynamic barrier can be overcome by applying an external voltage, by which the protons and electrons generated at the anode migrate and combine at the cathode to form hydrogen. The required external voltage (0.14 V) is substantially lower than that needed to produce hydrogen from the direct electrolysis of water (1.23 V at pH 7). Liu et al. (2005) obtained an overall yield of 2.9 mol H$_2$/mol acetate (72.5% hydrogen recovery) in the BEAMR by supplying an external voltage of 0.85 V, when using acetate as substrate. Coulombic efficiency of this two chambered MEC separated with CEM (Nafion) varied from 60 -78 %. Hydrogen production efficiency of 53% was recorded in a biocatalyzed electrolysis system with an applied voltage of 0.5 V (Rozendal et al. 2006). Rozendal et al. (2007) developed a single chamber biocatalyzed electrolysis system by implementing a gas diffusion electrode in two configurations: with both CEM and AEM. However both configurations resulted in a lower Hydrogen recovery of only 23%, at an external voltage of 1.0 V. When domestic wastewater was employed as substrate in an MEC, the overall Hydrogen recovery was 9.8 % (Ditzig et al. 2007). This low recovery might be attributed to the low concentration of organic matter in the wastewater.

The single chamber MEC significantly increased H$_2$ generation rate, but the produced H$_2$ was probably consumed by methanogens to generate
CH₄ (Liu et al. 2010a; Logan et al. 2008). Inspite of different inhibition approaches like adding expensive methanogen inhibitors, periodically expose solution in aerobic environment, and controlling the pH and redox potentials, CH₄ contamination in MECs still remains a major hindrance (Hu et al. 2008; Liu et al. 2010a; Logan et al. 2008). Microbial reverse-electrodialysis electrolysis cells (MREC) with spontaneous H₂ production by combining together the anode organic oxidation and salinity gradient energy (Cusick et al. 2012; Kim & Logan, 2011).

1.3.6.2 Microbial Remediation Cell (MRC)

MFC employed for the successful bioremediation of the contaminated environments are referred to as Microbial Remediation Cells (Wang & Ren 2013). Electrodes can potentially serve as electron donors or acceptors for the bioremediation of contaminants such as organics or metals. *Geobacter metallireducens* oxidizes aromatic hydrocarbon contaminants with an electrode serving as the sole electron acceptor. Morris et al. (2009) reported 164% increase in degradation of diesel by MFC, compared to open circuit potential. Crude oil degradation increased by 120%, near the electrode in U-tube MFC (Wang et al. 2012). The dramatic increase in contaminant oxidation rate is hypothesized due to the faster electron transfer by more conductive electrode as compared with electron shuttles. In addition, competition between microbes to access and deliver electrons to the electrodes triggered higher metabolic activities of the organisms.

Remediation studies on ethanol, 1,2-dichloroethane and pyridine were reported (Luo et al. 2009; Pham et al. 2009; Zhang et al. 2009b). Negatively polarized electrode was employed for the dechlorination of trichloroethene (TCE) to ethane (Aulenta et al. 2008). Oxidized contaminants like perchlorate, chromium, and uranium, can be reduced using the electrode as the electron donor (Butler et al. 2010; Wang et al. 2008; Gregory and
Lovley 2009). Concerned with U (VI) reduction, horizontally distributed anodes and cathodes enabled direct correlation between acetate injection and uranium reduction and coupled with monitoring of the microbial activity (Williams et al. 2010). Optimum delivery of electron acceptors or donors to promote the desired biodegradation is the main intricacy in the bioremediation of contaminated areas.

1.3.6.3 Photosynthetic microbial fuel cell (PMFC)

Integration of the photosynthetic reaction with microbial electricity production using synergistic relationships between photosynthetic organisms and exoelectrogenic bacteria lead to the development of the photosynthetic-MFC. The working principle of the PMFC comprises (i) photosynthesis of organic matter (ii) transport of organic matter to the anode compartment (iii) anodic oxidation of organic matter by exo electrogens and (iv) cathodic reduction of oxygen or other electron acceptors (Strik et al. 2011). PMFC is also represented by other terminologies such as microbial photoelectrochemical solar cell (Malik et al. 2009), solar-powered microbial fuel cell (Strik et al. 2010), photobioelectrochemical fuel cell (Rosenbaum et al. 2005) and solar-driven microbial photoelectrochemical cell (solar MPC) (Qian et al. 2010). PMFC relies on the higher plants, phototrophic bacteria and algae for photosynthesis.

Reed mannagrass and rice plants were used first to demonstrate the syntrophic relations between rhizodeposits and exoelectrogens, with maximal power outputs of 67 mW/m² and 26 mW/m², respectively (Schampheleire et al. 2008; Strik et al. 2008). Spartina anglica was able to generate current for 119 days (Timmers et al. 2010). The phototrophic bacteria *Rhodobacter sphaeroides* can generate electricity through the in situ oxidation of photobiological hydrogen (Rosenbaum et al. 2005). The algae based PMFC is an emerging system, because the consortium can convert solar energy to
electrical energy and in addition also produces value-added chemicals, such as protein and biodiesel. Strik et al. (2010) reported a PMFC with a reversible bioelectrode, which functions as a biocathode during illumination for photosynthesis reaction and then switches to the anode in the dark for organic degradation. The advantage of PMFC is that they do not rely on external electron donors but convert inexhaustible solar energy into electrical energy. They are of immense potential provided if current challenges such as low power output are addressed.

1.3.6.4 Sediment MFC (SMFC)

Sediment MFCs (SMFC) or benthic MFC utilize the naturally occurring potential difference between the anoxic sediment and oxic seawater to produce electricity (Lovley, 2006). Microorganisms oxidize the substrates in the sediment and transfer electrons to the anode either embedded in or rested on top of the sediment. The electrons are then transferred to the cathode suspended in the overlying seawater, where dissolved oxygen is reduced to water (Donovan et al. 2011). The abundant availability of substrates in the sediment makes SMFC a very promising power source for autonomous marine sensors and underwater vehicles.

Different configurations of sediment MFC have been developed. Initial designs of SMFC comprised of the graphite plates buried in the sediment with suspended cathode in water and rendered low power output (Tender et al. 2002). Nielsen et al. (2007) developed a chamber-based SMFC with semi-enclosed anode which achieved a power production of 380 mW/m² (3.8 W/m³). First successfully demonstrated sediment MFC as a viable power source was 18 mW meteorological buoy, powered for nearly 7 months (Tender et al. 2008). Chambered SMFC powered an acoustic modem interfaced with an oceanographic sensor for over 50 days with an average power density of 44 mW/m² (Gong et al. 2011).
1.3.6.5 Microbial Desalination cell (MDC)

The basic principle of MDC is to utilize the electric potential generated across the anode and cathode to drive in situ desalination (Cao et al. 2009). Three chambered MDC configuration is obtained by inserting an anion exchange membrane (AEM) and a cation exchange membrane (CEM) in between the anode and cathode chambers for desalination. Bacteria in the anode chamber oxidize biodegradable substrates to produce electrons and protons, the anions (e.g., Cl\(^{-}\)) in the middle chamber migrate to the anode and the cations (e.g., Na\(^{+}\)) are drawn to the cathode for charge balance, thus the middle chamber solution is desalinated (Cao et al. 2009).

Higher desalination efficiency and current output can be achieved by using membrane stacks (Chen et al. 2011) and electrolyte recirculation was shown effective in stabilizing electrolyte pH (Luo et al. 2012; Qu et al. 2012). Physical and electrical adsorption of ions onto high surface area membrane electrode assemblies, such as microbial capacitive desalination cells (MCDCs) reported up to 25 times increase in salt removal efficiency (Forrestal et al. 2012). MDC setback arises due to the membrane fouling due to biofilm growth and deposition of hardness causing cations.