CHAPTER 1

INTRODUCTION

The constantly growing population in our country demands a proportionate availability of basic goods such as water and electricity. The reliance on current energy production methods including oil, natural gas, and coal cannot be continued into the future. The dependence on fossil fuels has led to a tremendous increase in the atmospheric CO₂ levels as the stored carbon is released. By 2100, it is estimated that CO₂ concentrations will reach anywhere in the range of 540 to 970 ppmv. The increasing CO₂ levels may lead to intensified environmental and global climate change. The biggest environmental challenge is to solve energy crisis at the same time decreasing CO₂ release (Logan 2008). Renewable energy sources such as solar energy, wind energy, biomass, hydropower etc. are promising as alternate energy sources because of their carbon neutraliy. Biomass is the mostly used renewable energy source and is expected to stay as the most paramount primary source of renewable energy for the decades to come. It is of paramount importance to convert biomass efficiently and unblemished into useful energy. Therefore, simple and cost-effective technologies are needed for efficient conversion of biomass into modern energy in developing countries.

Bioelectrochemical systems have been emerging as a promising technology for efficient conversion of biomass into electricity because of the potential advantages such as efficient conversion in a single step, operation at ambient temperatures and environmental sustainability. Microbial
Desalination Cells (MDC) are the latest among the bioelectrochemical systems possessing additional advantage of desalination which can present a solution for the challenging issue of water scarcity. The biodegradation taking place in a MDC can be advantageously exploited for bioremediation of toxic pollutants.

1.1 BIOELECTROCHEMICAL SYSTEMS (BES)/ MICROBIAL ELECTROCHEMICAL SYSTEMS (MES)

Bioelectrochemical systems (BES) or Microbial electrochemical systems (MES) are one of the most rapid technologies that deal with water availability and energy. MES utilize microorganism to convert the chemical energy stored in biodegradable materials to direct electric current and chemicals. This emerging technology offers a new and transformative solution for integrated waste treatment, energy and resource recovery. These offer a flexible platform for redox reaction oriented processes. MES work on the principle of oxidation of biodegradable waste materials to electricity. This technology has opened up a new interdisciplinary field for research and development integrating microbiology, electrochemistry, materials science, chemical engineering, and many other related areas. Few researchers (Wang & Ren 2013) have discovered diversified functionalities of MES through the multiple utilization of electrons on the cathode side. Based on these diverse functions, different terminologies have been articulated to MES technology like MXCs, where X stands for different applications. The following are some examples for MXCs.

1. **Microbial fuel cells, MFC** - The current produced in the anode chamber is captured directly for electricity generation (Fornero et al. 2010; Liu & Logan 2004; Ren et al. 2007).
2. **Microbial electrolysis cells, MEC** - The current produced are used to produce H₂ and other value added chemicals with the help of an external power supply (Cheng et al. 2009; Liu et al. 2010; Logan et al. 2008).

3. **Microbial electrosynthesis, MES** - The electrons generated in the anode chamber is used in the cathode chamber to synthesize organic compounds using an external power supply and cathode biofilm. In microbial electrosynthesis, microorganisms accept electrons from a cathode for the reduction of carbon dioxide to organic compounds and are excreted from the cell (Nevin et al. 2011; Lovley & Nevin 2011; Cheng et al. 2009; Liu et al. 2005; Rabaey & Rozendal 2010; Rozendal et al. 2009; Rabaey & Rozendal 2010).

4. **Microbial remediation cells, MRC** – The electrons produced by the microbes at the anode are used to remediate contaminants (Vater et al. 2008; Butler et al. 2010; Gregory & Lovley 2009; Lovley & Nevin 2011).

5. **Microbial desalination cells, MDC** - The potential across the electrodes is used to drive desalination (Cao et al. 2009a; Jacobson et al. 2011b; Luo et al. 2011; Luo et al. 2012a; Mehanna et al. 2010b).

The basic types of MXC are depicted in Figure 1.1.
1.2 HISTORY OF BES

In 1911, Michael C Potter demonstrated the electric current production through microbial oxidation of non-electrolytes eg. organic compounds (Potter 1911). In 1931, Barnett Cohen reconfirmed Potter’s findings by producing 0.2 mA current by poising a half cell at +0.5 V using a potentiostat (Cohen 1931). The first experimental evidence on bioelectricity was reported by Luigi Galvani by recording the electric response obtained from connecting frog legs to a metallic conductor (Piccolino 1998). In 1960’s, NASA proposed a novel idea to use MFCs for producing electricity from human waste during space flights (Canfield et al. 1963) which made the MFC
technology popular. In 1980s, the MFC research was revived when H. Peter Bennetto demonstrated the use of artificial mediators to enhance electric power in MFC (Bennetto et al. 1984).

Past decade has attracted the scientific community to MFC research due to a spectacular discovery on the extracellular electron transport (EET) mechanism in microbes that allows them to use insoluble solid surfaces as a terminal electron acceptor. These microbes can transport electrons outside their cell wall in order to breathe and survive in anaerobic environments that lack fermentable substrates (e.g. glucose) and conventional, terminal electron acceptors (e.g. dissolved oxygen and nitrate) (El-Naggar et al. 2008; El-Naggar et al. 2010). The concept of microbial desalination cell (MDC) was demonstrated in 2009, to facilitate ionic transport in MFC with an additional chamber containing salt water (between anode and cathode chambers) to achieve removal of salts (Cao et al. 2009a).

1.3 MECHANISM OF BIOELECTRICITY GENERATION USING A MICROBIAL FUEL CELL – ELECTROGENESIS

Microbial fuel cell (MFC) is the newest energy generating approach producing bioelectricity from biomass using bacteria. The breakthrough in MFC research occurred in 1999 when it was recognized that it was not necessary to add mediators to the anodic chamber of MFC (Kim et al. 1999). In an MFC, the organic matter is degraded (oxidized) by microorganisms producing electrons that travel through a series of respiratory enzymes located on cell membrane and make energy for the cell in the form of ATP. The electrons are then released to a terminal electron acceptor (TEA) which accepts the electrons and becomes reduced. For example, oxygen can be reduced to water through a catalyzed reaction of the electrons with protons.
The following equations show the reactions at anode and cathode (Saeed et al. 2015):

At anode:

\[(\text{CH}_2\text{O})_n + n\text{H}_2\text{O} \rightarrow n\text{CO}_2 + 4n\text{e}^- + 4n\text{H}^+\]

At cathode:

\[\text{O}_2 + 4n\text{e}^- + 4n\text{H}^+ \rightarrow 2\text{H}_2\text{O}\]

Many TEAS such as oxygen, nitrate, sulfate, and others readily diffuse into the cell where they accept electrons forming products that can diffuse out of the cell. Exoelectrogens are bacteria that can exogenously (i.e., outside the cell) transfer electrons to a TEA that may be a metal oxide. The exoelectrogenic property of such bacteria is exploited by MFC to produce power. The method of electron-generating process is named as electrogenesis, with the bacteria exoelectrogens and the reactor a microbial fuel cell (MFC) (Logan 2008).

The anode and cathode chambers in an MFC are separated by a proton exchange membrane (PEM) which permits the charge transfer between the chambers as shown in Figure 1.2. The bacteria grow on the anode under anaerobic condition, forming a biofilm over there and oxidize the organic matter. The electrons produced are released to the anode and protons to the solution. The cathode is sparged with air to provide dissolved oxygen for the reactions of electrons, protons and oxygen at the cathode, the PEM prevents this air to enter the anode. Both the electrodes are connected by a wire containing a load or a resistor completing the circuit and producing power. The protons that are produced at the anode can permeate through the membrane and migrate to the cathode where they combine with electrons and oxygen to form water. The current produced by MFC is calculated by
monitoring the voltage drop across the resistor by a voltmeter (intermittent sampling) or a multimeter or a potentiostat hooked up to a computer for essentially continuous data acquisition.

Figure 1.2 Schematic diagram of MFC

1.4 ELECTRODE BIOFILMS - ELECTROCHEMICALLY ACTIVE BACTERIA (EAB)

Electrochemically active biofilms can be defined as biofilms that can exchange electrons with conductive surfaces-electrodes. Recognizing the role of electrodes as reactants in these bioelectrochemical processes, biofilms that deliver electrons to the biofilm electrode are called as anodic/electrode-reducing biofilms, and biofilms that accept electrons from the biofilm electrode are called as cathodic/electrode-oxidizing biofilms (Babauta et al. 2012).

A series of electron transfer reactions are involved in microbial respiration facilitated by a group of energetic substances. In general, dissolved substances act as the donors and acceptors of electrons, however, some
Microorganisms can use solid substances such as minerals and metals as electron donors and/or electron acceptors, in respiration (Nealson & Finkel 2011). EABs are also known under several other names such as electricigens (Lovely 2006), electrochemically active microbes (Chang et al. 2006), exoelectrogenic bacteria (Logan & Regan 2006), and anode-respiring (Torres et al. 2007) or anodophilic (Park & Zeikus 2003, Marsili et al. 2010) species. The mechanism of such electron transfers is not clearly elucidated. There are different hypotheses put forward to explain this electron transfer.

1. The recently proposed hypothesis says that electrons are transferred by conduction through extracellular materials or appendages called nanowires (Reguera et al. 2005; El-Naggar et al. 2010; Malvankar et al. 2011). Examples for bacterial species possessing nanowires include *Geobacter sulfurreducens* and *Shewanella oneidensis*.

2. Sophisticated spectroelectrochemical studies support the hypothesis that the cells of *G. sulfurreducens* in direct contact with the anode surface transfer electrons to the anode via c-type cytochromes displayed on the outer cell surface (Busalmen et al. 2008).

3. The more traditional point of view, that the electrons are transferred using redox mediators or soluble electron shuttles (Coursolle et al. 2010; Velasquez-Orta et al. 2010).

*Shewanella oneidensis* releases riboflavin which plays the role of electron shuttles. This was most clearly apparent from the electrochemical analyses that gave a response for *S. oneidensis* that was different significantly from the response given by *G. sulfurreducens* which transfers electron directly to the anode (Marsili et al. 2008). Phenazines are redox mediators endogenously produced by *P. aeruginosa* biofilms and allow for the normally
aerobic microorganism to survive under anoxic conditions (Pierson & Pierson 2010).

One or more of the above mechanisms may exist in a single species of bacterium to make it electrochemically active. The microorganisms in a biofilm attach to an electrode surface that acts as a scaffold. It also works as a breathing apparatus, providing a continuous sink for electrons and influences structural and functional gradients within the biofilm matrix (Semenec & Franks 2015). Also, the extracellular electron transport (EET) components of exoelectrogens, such as their pili/nanowires, c-type cytochromes or endogenous electron mediators, determine the structure and conductivity of anode biofilms.

(Source: Lovely 2008)

**Figure 1.3 Different Mechanisms for electron transfer to the anode in microbial fuel cells**

Dots represent outer surface cytochromes, lines indicate nanowires and the clouds represent the possible extracellular marix which contains c-type cytochromes giving conductivity.
Within the biofilm, c-type cytochromes (heme-containing proteins) get reduced during substrate oxidation by EABs and are oxidized as they pass electrons to an electron acceptor (pili, electron shuttles or an external electron acceptor, such as metals/electrodes) (Richter et al. 2009; Shi et al. 2009). On the other hand, nanowires of some species may act as a direct redox interface between substrate and electrode in the case of long distance EET (Malvankar & Lovely 2012). Different mechanisms for electron transfer are illustrated in Figure 1.3. The microscopic view of nanowires present in *Shewanella oneidensis* MR-1 growing on an electrode is shown in Figure 1.4.

(Source: Logan 2008)

**Figure 1.4 Nanowires produced by *Shewanella oneidensis* MR-1**

Generally anodic biofilms are used in MFCs, the utilization of biocathodes (comprising of electrotrophic microorganisms that receive electrons from an electrode) has been emerging as a promising technology for biofuel and commodity production and for bioremediation of wastewater contaminants. Biocathodes replace the need for precious metal catalysts on cathodes. They are useful in sediment MFC to drive oxygen reduction reactions (Babauta et al. 2014). It is also used in MES to produce synthetic
commercial compounds from carbon dioxide such as ethanol, methane etc. (Lovely & Nevin 2013; Nevin et al. 2011).

There are key differences between anode and cathode biofilms including the microbial populations, components involved in EET to/from the electrodes and their structural and chemical propensities. Cathode biofilm electrotrophs are classified into aerobic and anaerobic depending on the final electron acceptors they utilize. Anaerobic biocathodes include bacteria that can use hydrogen, nitrate, fumarate, carbon dioxide or sulfate as electron acceptors (He & Angenent 2006). Hydrogen generating ability of cathode biofilms depends on the expression of cytochromes and hydrogenases (Rosenbaum et al. 2011). Examples for cathodic biofilm electrotrophs include \textit{G.sulfurreducens}, \textit{G.metallireducens}, \textit{G.lovleyi}, \textit{P.aeruginosa}, \textit{p.fluorescens}, \textit{shewanella putrefaciens}, \textit{E.coli} etc. Table 1.1 summarizes electron transfer mechanisms of various electrogenic microbes.

**Table 1.1** Electron transfer mechanisms of various electrogenic microbes in MFC

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In anodic biofilm</strong></td>
<td>DET</td>
</tr>
<tr>
<td>\textit{Aeromonas hydrophila}</td>
<td>DET</td>
</tr>
<tr>
<td>\textit{Geobacter metallireducens}</td>
<td>DET</td>
</tr>
<tr>
<td>\textit{Rhodoferax ferrireducens}</td>
<td>DET</td>
</tr>
<tr>
<td>\textit{Shewanella putrefaciens}</td>
<td>DET</td>
</tr>
<tr>
<td>\textit{Actinobacillus succinogenes}</td>
<td>MET</td>
</tr>
<tr>
<td>\textit{Alcaligenes faecalis}</td>
<td>MET</td>
</tr>
<tr>
<td>\textit{Enterococcus gallinarum}</td>
<td>MET</td>
</tr>
<tr>
<td>\textit{Proteus vulgaris}</td>
<td>MET</td>
</tr>
<tr>
<td>\textit{Shewanella oneidensis}</td>
<td>MET</td>
</tr>
</tbody>
</table>
Table 1.1 (Continued)

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In cathodic biofilm</strong></td>
<td></td>
</tr>
<tr>
<td>Geobacter sulfurreducens DL1</td>
<td>DET</td>
</tr>
<tr>
<td>Geobacter sulfurreducens</td>
<td>DET</td>
</tr>
<tr>
<td>Acidithiobacillus ferrooxidans</td>
<td>DET</td>
</tr>
<tr>
<td>Shewanella putrefaciens</td>
<td>DET</td>
</tr>
<tr>
<td>Desulfovibrio vulgaris</td>
<td>DET</td>
</tr>
<tr>
<td>D. vulgaris</td>
<td>DET</td>
</tr>
<tr>
<td>Clostridium beijerinckii</td>
<td>MET</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>MET</td>
</tr>
<tr>
<td>Shewanella oneidensis</td>
<td>MET</td>
</tr>
<tr>
<td>Acinetobacter calcoaceticus</td>
<td>MET</td>
</tr>
</tbody>
</table>

(Source: Zhou et al. 2013)

DET-Direct Electron transfer, MET-Mediated Electron Transfer

### 1.5 MEDIATORS

A mediator is a chemical compound which transfers electrons produced from the redox species in microbial metabolism to the anode. These electron shuttles tap electrons from electron transport chain, get reduced in the process and carry these electrons through the lipid membrane and the outer cell membrane (Kim et al. 2011). Mediators get oxidized by transferring these electrons to anode. These electrons are then carried to the cathode through an external electric circuit where the electrons are released to oxygen. Even after undergoing all the redox reactions, the mediator remains unchanged at the end of each cycle (Logan et al. 2006). There are two types of mediators- 1) Exogenous mediators which are added externally to the cell suspension and 2) Endogenous mediators which are self produced by the bacteria.
Examples of exogenous mediators include neutral red (Park et al. 1999), anthraquinone-2-6, disulfonate (AQDS), thionin, potassium ferricyanide (Bond et al. 2002), methyl blue, methyl viologen, humic acid etc (Logan 2004; Rabaey & Verstraete 2005). Depending on whether the external mediators are added or not, the MFC processes are divided into mediated and non-mediated MFC. Examples for endogenous chemical mediators include phenazines such as pyocyanins and pyorubins (produced by *P. aeruginosa*) (Rabaey et al. 2004), riboflavins (*S.oneidensis*) and Flavin Mono Nucleotides (*S.putrefaciens*) (von Canstein et al. 2008).

A good mediator has to possess the following properties:

1. The mediator should not have any intervention with other cellular metabolic processes (Rozendal et al. 2008).
2. All the oxidation states of the mediator is to be chemically stable in electrolyte solution to adsorb well onto electrode surface and the bacterial cells (Logan et al. 2008).
3. The mediator should not get reduced or decomposed by the cellular reactions (Bergel et al. 2005).
4. The reduced as well as the oxidized states of the mediator should pass freely through the cell membrane to reach the respective oxidized or reductive species inside/outside the bacteria (Scholz et al. 2003, Strik et al. 2008).
5. The oxidized state of the mediator must be able to easily access the membrane-bound redox active complexes to be reduced and
6. The oxidation reaction by the mediator should be fast and electrochemically reversible (Logan et al. 2008, Kim et al. 2011).
1.6 APPLICATIONS OF MFC

The major applications of MFC technology include bioenergy production, pollution remediation, production of hydrogen and other bioproducts, biosensors etc. which are summarized below.

1.6.1 Bioenergy Production

Recovery of bioenergy from low-grade substrates such as wastewater is one of the potential applications of microbial fuel cell (MFC) technology. In MFC, the chemical energy stored in the chemical compounds is converted to electrical energy with the aid of microorganisms. Because chemical energy from the oxidization of fuel molecules is converted directly into electricity instead of heat, the Carnot cycle with a limited thermal efficiency is avoided. Hence, a much higher conversion efficiency can be achieved (>70%) theoretically in MFC just like conventional chemical fuel cells.

Electricity generations with an electron yield as high as 80% (Chaudhury & Lovley 2003), 89% (Rabaey et al. 2003) and 97% (Rosenbaum et al. 2006) were already reported. Ge etal (2013) have summarised the average power production in continuous MFCs till date in terms of normalized energy recovery (NER). The maximum NER achievable in an MFC is affected by power output, organic loading rate, removal efficiency and wastewater flow rate which can be expressed in one of the following ways:

NER = (power x time t) / [wastewater volume (treated within time t)]
    = Power/waste water flow rate (expressed as kWh/m³) or
NER = (power x time t)/ [COD (removed within time t)]
    = (power)/ (wastewater flow rate x ΔCOD) as (kWh/kg)
The theoretical energy content in organic compounds is 3.86 kWh/kg of COD and on average MFCs could recover <2.0 kWh/kg of COD. It was observed that the power density decreased with increasing MFC size, but there were no significant differences in NER between the MFCs with different sizes. Pure substrates showed better performance in both power and energy recovery. Low-strength substrates were preferred in MFCs to extract more energy (kilowatt hour per kilogram of COD). The membranes used as separators in MFC had no significant effect on NER (Ge et al. 2013).

MFC has been used as a power source in small electronic devices. An example for such an application for MFC technology is powering implanted medical devices using glucose and oxygen from blood. Such an implanted MFC could supply indefinite power and there was no need of surgery to replace batteries. This abiotic fuel cell was based on noble metal catalysts and activated carbon to produce energy from blood glucose in vitro and in vivo (Kerzenmacher et al. 2008).

1.6.2 Waste Water Treatment and Pollution Remediation

One of the greatest advantages of MFC is the broad spectrum of substrates that can be used to generate power by microorganisms. It was observed that any compound degradable by bacteria could be transformed into electricity. This makes the MFCs applicable in treating different kind of wastewaters and this may be more valuable than production of electricity itself in case of in situ environmental cleanup. MFCs could produce power using minimum COD concentrations of 21.8 mg/l (Jang et al. 2006) and maximum as high as 127,500 mg/l (Zhang et al. 2009) with considerable COD removal efficiencies.

The range of substrates include, but not restricted to, glucose (Kim et al. 2000), acetate (Bond & Lovely 2003; Nevin et al. 2008), starch(Lu et al.
2009), cellulose (Ren et al. 2008), wheat straw (Zhang et al. 2009), pyridine (Zhang et al. 2009), phenol (Luo et al. 2009), p-nitrophenol (Zhu & Ni 2009) and complex substrates such as domestic waste water (Liu & Logan 2004; You et al. 2006), brewery waste (Feng et al. 2008), land fill leachate (Gálvez et al. 2009), chocolate industry waste (Patil et al. 2009), mixed fatty acids (Freguia et al. 2009) and petroleum contaminants (Morris et al. 2008). The biomass production in these systems is lesser when compared to equivalent aerobic treatments and there is no need for an energy intensive aeration process.

Another promising application of MFC is using the electrodes to serve as inexhaustible electron acceptors (anode) or donors (cathode) for underground contaminant remediation (Morris & Jin 2008; Yuan et al. 2010). Such systems are called as Microbial remediation cells (MRC). MRC used in groundwater or soil remediation may include a single or an array of electrodes without using enclosed containers. Such bioelectrochemically enhanced approach can stimulate microbes to concurrently degrade underground pollutants such as petroleum contaminants (Morris et al., 2009) and produce additional electricity. The process is sustainable because there is no need to inject expensive chemicals and reduces energy cost for operation as compared to other technologies.

1.6.3 Production of Hydrogen and other Bioproducts

MFC can be modified slightly as an MEC to derive value-added products such as H₂, methane, and H₂O₂. External power supply is required in such a process where electrons and protons produced in anodic chamber are combined to form Hydrogen at cathode. Hydrogen gas generation in the cathodic chamber of microbial electrolysis cells has two pathways—H₂O reduction and proton reduction. When a membrane-less MEC is used, methanogenesis is a side reaction by methanogens in an anodic biofilm.
community. For methanogenesis, these microbes oxidize $H_2$ escaped from cathode to anode, which compromises hydrogen productivity (Zhou et al. 2013).

1.6.4 Biosensors

MFC with replaceable anaerobic consortium could be used as biosensor for online monitoring of organic matter. Even though different methods for determining organic contents in waste water based on biological oxygen demand (BOD) are available, most of them are not suitable for online monitoring and control of waste water treatment processes. A linear correlation between coulombic yield of MFC and strength of waste water makes MFC as a biosensor for determining the organic content of a specific waste water sample (Chang et al. 2004).

1.6.5 Hybrid Technologies - Sediment MFC/Benthic MFC

A hybrid technology means merging two or more techniques to form a new process, which combines the respective merits of the individual technologies. A number of promising hybrid or modified MFC-based wastewater treatment technologies have been emerged recently with some of them exhibiting great potential for future application. An example is Benthic MFCs/Sediment MFCs where power is produced through the microbial oxidation of organic substrates in anoxic marine sediments coupled to reduction of oxygen in the overlying water column. Electrons are harvested from the metabolism of the naturally occurring microorganism in the marine sediments hence do not require the addition of any exogenous microorganisms or electron shuttles (Bond et al. 2002; Tender et al. 2002; Reimers et al. 2001). Benthic MFCs were used to power meteorological buoys employed for measuring air temperature, relative humidity, pressure,
water temperature etc. and transferring data via real-time line of sight radio frequency telemetry (Tender et al. 2008).

1.6.6 Microbial Solar Cells (MSC)

Microbial solar cells is the term collectively used for different MES that integrate the photosynthetic reaction with microbial electricity (or chemical) production using synergistic relationships between EAB and photosynthetic organisms (Strik et al. 2011). While EAB are generally the same bacterial groups as in other MES, the organisms that are responsible for converting solar energy to organic matter may include higher plants, photoautotrophic bacteria and algae. Depending on this MSC can be categorized as - plant MSC, phototrophic MSC, and algae MSC.

A variety of names and systems related to MSC have been reported, such as microbial photoelectrochemical solar cell (Malik et al. 2009), photomicrobial fuel cell (p-MFC) (Thorne et al. 2011), solar-powered microbial fuel cell (Strik et al. 2010), photobioelectrochemical fuel cell (Rosenbaum et al. 2005), photosynthetic microbial fuel cell (PMFC) (Zou et al. 2009) etc. The basic principle of MSC in all these different designs include 4 steps (i) photosynthesis of organic matter (ii) transport of organic matter to the anode compartment (iii) anodic oxidation of organic matter by EAB and (iv) cathodic reduction of oxygen or other electron acceptors (Strik et al. 2011).

1.6.7 Microbial Electrolysis Cells (MEC)

MEC oxidize organic matters electrochemically using microbial biofilms in an anodic chamber to yield protons and electrons, which are subsequently used in a reduction reaction to produce value added products such as hydrogen and methane. The electrogenic biofilm on the anode acts as
a biocatalyst to push the anodic reaction forward. Electrons donated by the anodic biofilm to the anode reach the cathode via an external electrical circuit, where they reduce H₂O and proton to produce OH⁻ and H₂, which is released from the cathode compartment. An externally supplied voltage is required because the coupled redox reaction is thermodynamically unfavorable. Less power is needed for the process than in water electrolysis because degradation of organic carbon in an MEC supplies part of the needed energy. In addition to biohydrogen, other products such as methane can also be produced if something other than proton or water is reduced on the cathode (Zhou et al. 2013).

1.7 STATUS OF DESALINATION PROCESSES

Due to the rapid increase in human population and industrialization, the demand for pure drinking water has become the primary demand of human beings. Eventhough water being the abundant natural resource on the planet, seawater comprises about 97% of the total available water which cannot be used for drinking as such. Hence desalination of the available sea water or brackish water is the only way to meet the global water demand. Table 1.2 includes a summary of the major desalination processes employed currently. The chief limitation of all the desalination technologies listed here are the high energy demand in the form of power required for aeration, thermally driven distillation or high pressure membrane operation (Sevda et al. 2015).

Desalination of typical seawater (35 g/L of total dissolved solids) requires a theoretical minimum energy of ~1.0 kWh/m³, assuming the process to be thermodynamically reversible at 50% water recovery. Extra energy may be required for pumping feed water to the system, water pre-treatment etc. Including all these, the overall energy consumption may be increased to 3 to 4 kWh/m³ in typical reverse osmosis (RO) plants for desalination. Further
advances in RO systems will not be able to reduce the overall energy consumption below 3kWh/m$^3$ (Elimelech & Phillip 2011; Semiat 2008). Therefore, it has become a need of the hour to find out an energy efficient desalination technology and MDC has been emerged as an energy-neutral or positive technology.

**Table 1.2 Different Desalination processes**

<table>
<thead>
<tr>
<th>Desalination Technology</th>
<th>Key Features</th>
<th>Limitations/ Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Membrane Based Desalination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse Osmosis</td>
<td>Suitable for treating brackish water. Energy consumption is low compared to thermal process. High recovery rate. Start-up and shutdown of the process is quick. Commercially available.</td>
<td>Membrane fouling. High maintenance cost.</td>
</tr>
<tr>
<td><strong>Microbial Desalination Cell</strong></td>
<td>Desalination is driven by energy from treating wastewater. Wastewater treatment, desalination and power production in one system. No external power supply needed.</td>
<td>Commercially not available. Longer start-up time. Still at lab scale. High capital cost.</td>
</tr>
<tr>
<td><strong>Thermal Based Desalination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multistage flash distillation</td>
<td>Most commonly used. Commercially available. Economical for large volumes.</td>
<td>High energy demand.</td>
</tr>
<tr>
<td>Multiple effect distillation</td>
<td>Suitable for removing high salt concentration.</td>
<td>Not suitable for low volumes.</td>
</tr>
<tr>
<td>Mechanical vapor compression</td>
<td>Suitable where waste heat source is available or cost of energy is low.</td>
<td>High capital cost. High maintenance cost.</td>
</tr>
</tbody>
</table>

(Source: Sevda et al. 2015)
1.8 MICROBIAL DESALINATION CELL (MDC)

Microbial Desalination Cell (MDC) can reduce or eliminate the electrical energy requirement for desalination. The key feature of the MDC is that electrochemically active microorganisms produce electrical potential by degrading organic matter, which is used for desalination of water by driving ion transport via ion-exchange membranes (IEMs). When wastewater is used as organic source, the MDC can accomplish triple goals of desalination, energy production, and wastewater treatment. The typical energy stored in domestic wastewater is in the range of 1.8 to 2.1 kWh/m$^3$ which is equivalent to the minimum energy required for practical desalination of seawater (1.8–2.2 kWh/m$^3$). In addition to this, domestic wastewater generally possesses a relatively low ionic strength (0.8 g/L as total dissolved solids) when compared to sea water (35 g/L of TDS). This creates an additional energy from the salinity gradient between the seawater and wastewater (Kim & Logan 2013).

The MDC shares the principle of bioelectrochemical reactions in MFC. The MDC couples ionic current in the electrolyte with electric current at the electrodes, via bioelectrochemical oxidation of organic matter at the anode, and electrochemical reduction at the cathode respectively.

1.8.1 Working Principle of MDC

A typical lab scale MDC reactor is comprised of anodic chamber containing anode, electrogens and organic matter, desalination chamber containing salt water and the cathodic chamber containing a cathode and the final electron acceptor (Figure 1.5). In the anodic chamber, the breakdown of organic matter is catalysed by microbes, into protons, electrons, CO$_2$ and other byproducts. The electrons released from the metabolic oxidation of
organics are transferred to the anode and discharged by way of current collectors into the cathode which can be extracted as DC current by placing an external load in the electric circuit. The electron acceptors (e.g. oxygen) in the cathode provide a permanent sink for both electric and ionic currents (Gude et al. 2013).

A desalination chamber is packed in between the anodic and cathodic compartments, and the anolyte and catholyte are separated with anion exchange membrane (AEM) and cation exchange membrane (CEM) respectively as in Figure 1.5. The desalination of salt water is possible by the transfer of anions and cations across AEM and CEM respectively. Due to the discharge of electric current from the oxidation of organic matter in the anode, the negatively charged ions from the desalination compartment (e.g. Cl\(^-\), NO\(_3^-\) and SO\(_4^{2-}\)) migrate to the anode to maintain the electroneutrality condition. Similarly, in the cathode, the terminal electron acceptors receive electrons and get reduced, and in turn, positively charged ions from the desalination chamber (e.g. Na\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\)) travel through the cation exchange membrane to the cathode chamber (Cao et al. 2009a). Finally simultaneous desalination and power generation are achieved by bioelectrochemical oxidation of organic compounds.

The movement of anions from the desalination chamber into the anodic chamber increases ionic strength and conductivity of the anolyte (i.e. wastewater). This can also increase wastewater alkalinity which in turn will enhance treatment efficiency and power generation.

On the other hand, the desalination process is getting improved by the increased potential output due to better anolyte property. MDC can serve a potent role in desalination systems by acting as a pretreatment for subsequent reverse osmosis (RO) processes and considerably reduce the energy cost and membrane fouling (Luo et al. 2012b). Furthermore, MDC provides a
distinctive prospect to improve the energy production efficiency during wastewater treatment by incorporating desalination into the same MFC reactor.

Figure 1.5 Schematic diagram of MDC

1.8.2 Types of MDC Configurations

Table 1.3 List of MDC configurations

<table>
<thead>
<tr>
<th>MDC Configuration</th>
<th>Key Features</th>
<th>Main Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Cathode MDC</td>
<td>Oxygen used as a terminal electron acceptor.</td>
<td>High reduction potential.</td>
</tr>
<tr>
<td></td>
<td>Approximately 63% salt reduction.</td>
<td></td>
</tr>
<tr>
<td>Biocathode MDC</td>
<td>Catalyzes reduction reactions through use of microbes.</td>
<td>Self generating and sustainable.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhanced water desalination.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced start-up time.</td>
</tr>
<tr>
<td>Upflow MDC</td>
<td>Tubular reactor containing two compartments separated by IEM.</td>
<td>Efficient fluid mixing within the chambers.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Easier to scale up.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased power density.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Improved desalination efficiency.</td>
</tr>
<tr>
<td>MDC Configuration</td>
<td>Key Features</td>
<td>Main Advantages</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Recirculation MDC</td>
<td>Catholyte and anolyte solutions sequentially re-circulated through cell. Low concentration buffers should be used.</td>
<td>Increased power density. Increased desalination efficiency.</td>
</tr>
<tr>
<td>Stack structure MDC</td>
<td>Alternating AEMs and CEMs. Total desalination rate approximately 1.4 times that of a typical MDC unit.</td>
<td>Improves charge transfer efficiency. Increased separation of ion pairs from salt water. Increased energy recovery.</td>
</tr>
<tr>
<td>Osmotic MDC</td>
<td>AEM replaced with FO membranes. Dilute saltwater by increasing water flux. Potassium ferricyanide usually used as catalyst.</td>
<td>Improves overall desalination performance. Enhances organic matter removal from wastewater. Cost of FO membrane lower than cost of AEM.</td>
</tr>
<tr>
<td>Capacitive MDC</td>
<td>Incorporates the concept of capacitive deionization. Double layer capacitor formed on the surface electrodes. Salts should be continually removed from electrodes.</td>
<td>Reduced salt contamination in anode and cathode chambers. Resolving ion migration problems.</td>
</tr>
<tr>
<td>Microbial electrolysis desalination and chemical production cell</td>
<td>Formed by introducing an acid-production chamber and a bipolar membrane in an MEDC.</td>
<td>Reduced pH fluctuations. Increased desalination rate.</td>
</tr>
<tr>
<td>Bipolar membrane MDC</td>
<td>Anion and cation selective layers laminated together to make BPM. BPM replaced next to the anode chamber, making a four-chamber MDC.</td>
<td>High permselectivity. Low water splitting voltage drop and electrical resistance. Long-life duration of BPM. Increased desalination efficiency. Maintaining desired pH in the anode chamber.</td>
</tr>
<tr>
<td>MDC Configuration</td>
<td>Key Features</td>
<td>Main Advantages</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Ion exchange resin coupled MDC</td>
<td>Desalination chamber packed with mixed anion- and cation- exchange resins.</td>
<td>Stabilized ohmic resistance. Reduced energy consumption. Increased charge transfer efficiency and desalination rate.</td>
</tr>
<tr>
<td>Submerged microbial desalination-denitrification cell</td>
<td>Integrates the denitrification system into an MDC. Nitrates ions removed from groundwater and used in electricity generation.</td>
<td>No additional treatment for nitrate required. No risk of bacteria leakage into groundwater.</td>
</tr>
<tr>
<td>Decoupled MDC</td>
<td>Anode and cathode units placed directly in salt solution. Stainless steel mesh wrapped with carbon cloth used as an electrode</td>
<td>Easy to control and vary the liquid volume ratios. Ease in repairing or replacement of any damaged parts. Easy to scale up.</td>
</tr>
</tbody>
</table>

(Source: Saeed et al. 2015)

After the first proof of concept study of MDC by Cao et al. (2009a), there emerged different designs and configurations of MDC in addition to the basic three chambered MDC. A literature review of various MDC configurations are given below and the Table 1.3 summarizes major MDC configurations.
1.8.2.1 Air cathode MDC

In MDC, ferricyanide was used as the electron acceptor for the initial studies. Despite its fast reduction kinetics and ability to produce high cathode potentials, ferricyanide cannot be used for large scale application due to its toxicity and high cost. Thus, an air cathode MDC was introduced by Mehanna et al. (2010b) where oxygen is used as a final electron acceptor (TEA) due to its high reduction potential and cost effectiveness. 63% of desalination was achieved in a single cycle using carbon cloth electrode with platinum as catalyst in an air-cathode MDC.

![Diagram of Air-cathode MDC](Source: Mehanna et al. 2010b)

**Figure 1.6 Air-cathode MDC**

One of the disadvantages of an air cathode MDC is the need for expensive catalytic materials on the cathode because of slow redox kinetics in ambient conditions. Another problem is the high energy required to maintain the dissolved oxygen concentration in the cathode. These problems can be eliminated by either exposing the cathode to the atmosphere, or using passive methods for air circulation, or increasing surface area of carbon electrodes to
increase oxygen reduction levels without the use of expensive catalysts (Gude et al. 2013).

1.8.2.2 Biocathode MDC

The use of chemical catholytes or chemical catalysts in air cathode suffers from the problems of high cost and requires continuous replacement. Hence biocathode MDC was used (Croese et al. 2011) which uses the microbial populations present on its surface or in the catholyte as biocatalysts to catalyze the reduction reactions. It has the advantages of self-regenerating and sustainability. Biocathode MDC was reported to produce more power and enhanced desalination (Wen et al. 2012).

(Source: Wen et al. 2012)

Figure 1.7 Biocathode MDC

1.8.2.3 Stacked MDC

Stack structure of MDC was used to increase the desalination performance of a MDC where multiple pairs of ion-exchange membranes
(IEMs) were inserted between the anode and cathode chambers, to enhance the charge transfer efficiency by allowing the saline water to flow through a series of MDCs. The stack shown in Figure 1.8 includes alternating AEMs and CEMs which makes alternating pairs of desalting and concentrating cells in the desalination chamber, thus increasing the separation of ionic groups from the saltwater for every electron passing through the circuit (Kim & Logan 2013). The stacked MDCs are cost-effective as they can recover more energy compared to other MDC configurations (Shehab et al. 2013). Choi and Ahn (2013) have treated domestic waste water using five different types of stacked connections and showed that the parallel electrode connection in series flow mode resulted in 15 to 20% more electricity production and 80 to 85% higher coulombic efficiency compared to the case of series connection.

(Source: Kim & Logan 2013)

**Figure 1.8 Stacked MDC**
1.8.2.4 Recirculation MDC

A major disadvantage of conventional MDC was pH imbalance which decreased both power generation and desalination. pH was found to be decreasing in anodic chamber and increasing in cathodic chamber as the desalination progresses. The pH imbalances can be minimized by increasing the volume of the anolyte or by adding acids and bases, or by using buffers, but this may increase both energy and cost. Hence recirculation MDC (rMDC) was suggested as a pH control system in which the catholyte and anolyte solutions are sequentially recirculated through the cell to neutralize the pH in the cell (Saeed et al. 2015) and it is illustrated in Figure 1.9. Luo et al. (2011) have operated rMDC with 25 mM of PBS and produced 53% increase in power density and 48% increase in desalination performance.

(Source: Kim & Logan 2013)

Figure 1.9 Recirculation MDC
1.8.2.5 **Microbial electrolysis desalination and chemical-production cell**

A combination of the electrodialysis and microbial electrolysis cell (MEC) has resulted in the development of microbial electrolysis desalination cell (MEDC) (Chen et al. 2012a; Mehanna et al. 2010a). When an acid-production chamber and a bipolar membrane (BPM) were inserted into MEDC, microbial electrolysis desalination and chemical-production cell (MEDCC) was formed, which could simultaneously desalinate seawater, produce hydrochloric acid and generate sodium hydroxide (Chen et al. 2013). In MEDCC, OH\(^{-}\) was produced at the anode chamber which resolved the problems of pH fluctuations and Cl\(^{-}\) buildup in a conventional MDC (Chen et al. 2013). The desalination rate of the stacked MDC and MEDCC is typically around 1.4 times the rate of a conventional cell using a single desalination chamber. According to Chen et al. (2012b), MEDCC desalination demonstrated to perform better than MEDC and the electrolysis desalination and chemical-production cell (EDCC). The schematic structure of a MEDCC is illustrated in Figure 1.10.

![Schematic of MEDCC](Source: Chen et al. 2012)

**Figure 1.10 Microbial electrolysis desalination and chemical-production cell**
1.8.2.6  Capacitive microbial desalination cell

The salt accumulation in the anodic and cathodic chambers by the ions removed from the saline water, results in rise in the ionic concentrations of the anolyte and catholyte in a conventional MDC. This hinders the reuse of anolyte and catholyte and may cause concerns for disposal, where the total dissolved solids (TDS) is regulated. To avoid this problem, a capacitive MDC (cMDC) system was developed which removed the salt by incorporating the concept of capacitive deionization (Yuan et al. 2012; Forrestal et al. 2012). A double-layer capacitor was formed on the high surface electrodes and the ions were adsorbed when a saline solution was flown between the two charged electrodes. The ions were allowed to flow back into the liquid when the potential gradient was removed. Thus, the saltwater was deionized by “electrochemical salt adsorption” on the electrodes thereby preventing the salt contamination of anode and cathode chambers.

(Source: Zhang et al. 2012)

Figure 1.11 Capacitive MDC
1.8.2.7 Upflow microbial desalination cell

Jacobson et al. (2011a) have designed an upflow MDC (UMDC), as a tubular unit with two concentric compartments separated by IEMs where an increased surface area is provided for desalination to progress. The inner compartment served as the anode chamber filled with graphite granules. Two graphite rods were immersed into the graphite granules as current collectors that formed a part of external circuit. The anode chamber was enclosed with an anion-exchange membrane tube. The outer compartment served as the desalination chamber containing the saline water which was sealed with a cation-exchange membrane tube. In the UMDC, the inlet of saline water is from the bottom, while the outlet for desalinated water is positioned at the top of the anodic chamber.

(Source: Jacobson et al. 2011a)

Figure 1.12 Upflow MDC
The upflow MDC was found to be a feasible option having advantages such as mixing of fluids within the chambers achieved without agitation, and 100% of the water was recovered by osmosis (He 2011). This allowed the anodic cultures to remain in suspension and to effectively oxidize the organic matter. This configuration has achieved 99% salt removal from NaCl solution with an original concentration of 30 g/L over a 4-d desalination cycle (Jacobson et al. 2011a).

1.8.2.8 Osmotic microbial desalination cell

Forward Osmosis membranes were integrated into MDC and MFC replacing the ion exchange membranes, to form osmotic MDC or MFC respectively (Zhang & He 2013). Water is passed through the FO membrane, whereas the transport of ions from the middle chamber to both electrode chambers is reduced considerably. Here the salts are not removed, but are somewhat concentrated. The osmotic MDC is shown in Figure 1.13, which was able to treat waste water, generating electricity and diluting the saline water (Werner et al. 2013).

In contrast to a typical osmotic unit where the concentration gradient of salts is the driving force, the driving force in an osmotic MDC is the difference in electrical potential (Cath et al. 2006). Zhang et al. (2012a) have coupled osmotic MFC with conventional MDC. An osmotic MFC consists of two chambers-the anodic chamber where the wastewater is fed and the cathodic chamber which is fed with saline water. The saline water gets diluted by the water flux from the wastewater. The diluted saline water was fed to the desalination chamber of a conventional MDC to reduce the salt content and the wastewater was fed into the MDC anode (Cath et al. 2006; Zhang & He 2012b). The coupled system was able to improve the overall desalination performance and wastewater degradation. Zhang and He (2013) have operated an osmotic MDC and found that high-power operation led to
considerable desalination performance of ~95.9% and energy production of 0.16 kWh/m$^3$ of treated saline water.

(Source: Werner et al. 2013)

**Figure 1.13 Osmotic MDC**

They have achieved 85% reduction in conductivity with a salt solution having 10-50 g/L of NaCl. One of the disadvantages with osmotic MDC is the reduced current efficiency due to the use of FO membrane which decreases the potential of ionic separation in the middle chamber.

### 1.8.2.9 Bipolar membrane microbial desalination cell

Another modification of the MDC is the bipolar membrane MDC which is illustrated in Figure 1.14. The bipolar membrane includes an anion and a cation exchange membrane heat-pressed or glued together as a single membrane unit. The bipolar membrane is normally positioned next to the anode chamber of the MDC, thus creating a four-chamber MDC (Kim &
Water is passed through the membrane which gets splitted into protons and hydroxyl ions at the interface of the bipolar membrane and a high potential gradient is created. As the oxidation of organic matter takes place in the anode chamber, the hydroxide ions are flown from the bipolar membrane into the anode chamber. The hydrogen ions are released into the fourth chamber forming hydrochloric acid. At the same time, salt is removed from the seawater in the desalination chamber and sodium hydroxide is produced in the cathode chamber. Thus, by including a bipolar membrane into the MDC system, the pH of the anode chamber was maintained.

An external power supply is required in such a system for the bipolar membranes to split water into hydroxyl ions in the anodic chamber and hydrogen ions in the cathodic chamber (Ping & He 2013). However, the high cost of this type of MDC can be compensated as the chemicals produced (HCl and NaOH) in this process are economically profitable.

(Source: Zhang et al. 2013)

Figure 1.14 Bipolar membrane MDC
1.8.2.10 Decoupled microbial desalination cell

In case of conventional MDC, problems are faced when it is required to change the liquid volume in one of the compartments or for maintenance. This may require complete dismantling of the unit. To solve this issue, a decoupled MDC system was developed where the anode and cathode units were kept in a salt solution instead of placing in different chambers. Here, the anode unit, has a plate configuration with AEM on either side and the cathode unit contains CEM on both sides. The electrode in this system consisted of stainless steel mesh wrapped with carbon cloth which served as a current collector and as a supporting structure.

The main feature of decoupled MDC is that it is easy to control and vary the liquid volume ratios, and also to repair any damaged parts in the system (Chen et al. 2012c). Hence it is more flexible compared to a typical MDC and suitable for scaling up purposes as well.

1.8.2.11 Separator coupled stacked circulation microbial desalination cell

A c-SMDC-S scheme was also developed to reduce the pH imbalances across the MDC chambers. Here, a piece of glass fiber attached to the water-facing side of the cathode in the MDC acted as a separator (Morel et al. 2012). The separator helps in preventing biofouling of the cathode and improves the coulombic efficiency (CE) of the system. The configuration of a c-SMDC-S is given in Figure 1.15. Similar to conventional MDC, it consisted of anode, cathode and desalination chambers having axial cylindrical cavity separated by AEMs and CEMs. Activated carbon particles in the anode chamber allowed electrogenic bacterial growth in the chamber.
A graphite rod was inserted in the anodic chamber to act as current collector which transferred electrons across the circuit. During the operation, the buffer-free electrolyte circulated between the anode and cathode chambers and thus solved the pH imbalance problems. Chen et al. (2012c), observed that the separator coupled circulation SMDC could attain desalination rates of up to 65% higher than that of a regular SMDC.

(Source: Morel et al. 2012)

Figure 1.15 Separator coupled stacked circulation MDC

1.8.2.12 Ion-exchange resin coupled microbial desalination cell

With the progress of desalination in MDC, the ohmic resistance of the cell increases due to the reduction in the salt concentration and conductivity of the salt water. This will limit the power production and the desalination rate (Cao et al. 2009a). The ohmic resistance should be reduced for improving the MDC performance, particularly when low salinity waters, such as brackish waters or other effluents are treated.
An ion-exchange resin coupled MDC (R-MDC) can be used where the desalination chamber is packed with mixed anion- and cation-exchange resin. The scheme of this system is illustrated in Figure 1.16. These ion-exchange resins having high conductivities, act as ionic conductors in low conductivity water and reduce the ohmic resistance and thus reducing energy consumption in the desalination unit.

But in practical applications, certain bottlenecks limit the efficiency of R-MDC, such as scaling due to deposition of calcium and magnesium ions on the resin surfaces which in turn increases the internal resistance of the cell and prevent ion migration (He 2011). Morel et al. (2012) have conducted experiments in ion-exchange resin MDC and observed that the desalination rate increased by 1.5–8 times with low concentration influents (2–10 g/L NaCl). They have also reported that the ohmic resistances of the R-MDC were 3.0–4.7Ω compared to that of the conventional MDC (5.5 to 12.7Ω). Hence, the R-MDC showed improved performance in terms of both desalination rate and charge efficiency of the cell.

(Source: Morel et al. 2012)

Figure 1.16 Ion exchange resin packed MDC
1.8.3 Applications of MDC

In addition to bioenergy production, MDC has been utilized for other applications as well. A summary of currently identified functions of MDC are given below.

1.8.3.1 Desalination of sea water

Because of the energy saving mechanism, desalination of seawater using MDC has been of great interest. According to lab scale studies, up to 99% of salts could be removed using MDC depending on hydraulic retention time (HRT) (Sevda et al. 2015). When artificial seawater and real seawater were used, the desalination efficiency was found to be lower compared to the pure NaCl solution due to the complexity of ionic composition (Luo et al. 2012a). Luo et al. (2012b) have carried out an eight-month study with artificial seawater containing 100 mM NaCl and 100 mM NaHCO₃ and showed that 66% of the salt was removed at the beginning. But the desalination efficiency got reduced by 27% at the end of a 5000-h operation with a 47% decline in the current density. This was due to AEM fouling and CEM scaling. Jacobson et al. (2011a) used an upflow microbial desalination cell (UMDC) to desalinate artificial seawater and demonstrated that 74% of the salt was removed, but desalination efficiency was lower than that with NaCl at the same concentration. The occurrence of membrane scaling was more likely when using MDC to treat real seawater and hence measures need to be taken to address this during the long-term use of MDC technology.

When an Osmotic MFC was coupled to an MDC, the desalination efficiency was improved by reducing 85% chemical oxygen demand (COD) and 96.60±0.40% salts (Zhang & He 2013). Because of the slow desalination processes in MDC, it could be used as a pre-desalination process integrated to conventional desalination technologies such as electrodialysis (ED) or reverse
Zhang & He (2012b) have observed this experimentally using a 1.90-L UMDC as pre-desalination unit before an ED unit. They have observed that the MDC reduced the desalination time by 25% and energy consumption by 45.30%, compared with the ED desalination alone. It was also observed that the MDC effluent could be desalinated by ED using the bioenergy generated in the MDC.

When a microbial electrolysis desalination and chemical-production cell (MEDCC) was fed with a mixed solution of NaCl, MgCl₂, KCl and CaCl₂ as saltwater, valuable by-products such as alkali, acid and magnesium were also able to be recovered (Chen et al. 2013). As magnesium and calcium ions were removed, membrane scaling was also controlled, leading to a high desalination efficiency of 19% with artificial seawater. The MEDCC configuration was thus found to hold great promise to move MDCs towards practical applications.

### 1.8.3.2 Desalination of brackish water

Brackish water possessing total dissolved solids (TDS) less than 10 g/L (usually 1–5 g/L) is available in plenty as groundwater or in estuaries and can be treated efficiently in MDC systems to meet fresh water demand (Luo et al. 2012a). The main concern while desalinating low-salinity water is that the low conductivity can increase the internal resistance, which in turn decreases electricity generation and desalination efficiency. Ion exchange resins were added to desalination chamber of MDCs to improve the conductivity, and enhanced desalination rate was observed (1.5–8 times), while the ohmic resistance reduced by 55–272% compared to conventional MDC (Morel et al. 2012).
1.8.3.3 Water softening

Different methods such as lime soda softening, ion exchange softening, nanofiltration, reverse osmosis, carbon nanotube, ED, and distillation are employed for removing hardness from groundwater. All these methods are either energy intensive, costly or produce large quantity of waste products which need further treatment (Brastad & He 2013). To overcome these disadvantages, Arugula et al. (2012) have developed an enzymatic-style MDC in which glucose oxidation was catalyzed by a dehydrogenase enzyme to generate electricity, which removed 46% of hardness when fed with 0.80 g/L CaCO\(_3\) and 76% hardness removed with the corresponding feed CaCO\(_3\) concentration of 0.20 g/L.

The enzymatic fuel cells possessed limitations such as high capital cost and short lifetime. Therefore, conventional MDCs were later applied for water softening. A bench-scale MDC was used by Brastad & He (2013) to soften the hard water and more than 90% of hardness was removed. Also, several heavy metals such as mercury (99%), copper (97%), nickel (95%) and arsenic (89%) were all removed in this system (Brastad & He 2013).

1.8.3.4 Nutrient removal and recovery

The waste waters contain many nutrients which can be recovered using modified forms of MDC. These include nitrogen species such as ammonium, and phosphorus in the form of orthophosphate. Based on the charge of the nutrient species, the internal electric field of a bioelectrochemical cell drives directional ion transport enabling nitrogen and phosphorus removal, which is difficult to attain in conventional waste water treatment processes. Zhang et al. (2014) had developed a R\(^2\)-BES system as shown in Figure 1.17A for this purpose. In this system, ammonium ions from wastewater were transported to the cathode driven by the bioelectric field,
while phosphate ions in wastewater were removed by substitution with hydroxide ions formed by the cathodic reaction. An external power supply was required for this system.

Source: Zhang et al. 2014, Chen et al. 2015

Figure 1.17 A) R²-BES B)Microbial Nutrient Recovery Cell
As a modification of the MDC concept, a microbial nutrient recovery cell (MNRC) was constructed by Chen et al. (2015) where the relative positions of the AEM and CEM were exchanged as shown in Figure 1.17B. This configuration integrated the removal of both COD and nutrients from wastewater and achieved simultaneous nitrogen and phosphorus recovery (Chen et al. 2015). As the wastewater was circulated between the anode and cathode chambers, electrochemically active bacteria oxidized the organic materials to generate power. Using this power, the ammonium and phosphate ions were driven into the recovery chamber from the anode and cathode sides, respectively.

As a result of this technique, a concentrated solution containing nitrogen and phosphorus without biological contamination was recovered which could be used to produce struvite (a phosphate mineral) or other useful substances. The MNRC achieved about 82% COD reduction, 96% ammonia removal and 64% phosphate removal.

1.8.3.5 Hydrogen and chemical production

MDC can be modified for simultaneous desalination and hydrogen production as in Microbial electrolysis cells, by applying an additional voltage to reduce protons in the cathode. This device is named microbial electrodialysis cell (MEDC) (Mehanna et al. 2010a; Logan et al. 2008). Luo et al. (2011) had developed a reactor called as “microbial electrolysis and desalination cell” for hydrogen production, desalination and waste water treatment. By applying an external voltage of 0.8 V, this system attained a maximum H$_2$ production rate of 1.5 m$^3$/m$^3$/d (1.6 mL/h). The recirculation of anodic wastewater improved hydrogen production rate by 30%. An MEDCC was proposed by adding more chambers, for producing valuable chemicals (acid and alkali) in addition to H$_2$ production (Chen et al. 2012a).
chemical production and desalination efficiency were improved by using two different types of stack structures.

### 1.8.3.6 Ground water remediation

Groundwater forms two-third of the fresh water reserves of the world. The growing use of fertilizers and the discharge of untreated wastewater have led to the contamination of groundwater with high level of nitrates (Tong & He 2013). Intensive energy and chemicals are required for ex situ and in situ methods for nitrate removal. Zhang & Angelidaki (2013) have developed a novel design of MDC as shown in Figure 1.18, for the purpose of in situ nitrate removal from groundwater with simultaneous wastewater treatment, called as submerged microbial desalination–denitrification cell (SMDDC). Here, groundwater served as the desalination chamber into which anode and cathode chambers were submerged. Nitrate from the ground water got transferred in the anodic chamber through the anion exchange membrane; this nitrate containing anode effluent was then introduced into the cathode and reduced to nitrogen. Zhang & Angelidaki (2013) had observed that nitrate concentration was decreased from 20 to 1.90 mg-N/L with 90.50% removal efficiency and a power density of 101mW/m² was achieved.

The SMDDC possessed several key advantages like there was no need of energy input, nitrate was converted to nitrogen gas and thus no further treatment needed, microbes were not mixed with ground water and hence it was safe to use without any treatment.
1.8.4 Practical Availability and Scale-up of MDC Systems

The MDC system can be applied either as a pre-desalination method as it can appreciably reduce the salt concentration, or it can be used as a stand-alone process for decentralized wastewater treatment (Wang et al. 2011). Inspite of all the advantages that MDC can own when incorporated into a waste water treatment plant, the practical implementation of a desalination system powered by the energy generated from MDC by degrading municipal wastewater still encounters a number of technical difficulties. Requirement of high volume of anolytes and pH imbalance are a few among them (Saeed et al. 2015). Hence further investigations are needed for the scale-up of the system. Research works are currently going on worldwide for developing commercial MDC applications and to resolve the issue of scale-up, so that the MDC will be successfully implemented very soon.
1.9 GLOBAL POLLUTANTS

One of the major environmental issues in the current world is the increasing amount of organic pollutants discharged from various sources on a global level. Industrial effluents, chemical fertilizers, pesticides, dyes, oil refinery and automobiles are a few among the sources of pollutants. Large quantities of toxic industrial pollutants are thrown directly or indirectly into the environment without even primary treatment, which may be harmful to human health and the whole ecosystem (Paisio et al. 2009).

1.9.1 Strategies to Clean up the Pollutants

Different strategies including physical, chemical and biological techniques are widely employed for minimizing and/or removing the pollutants. Solvent extraction, adsorption, chemical oxidation, and electrocatalytic degradation etc. are examples of non-biological methods (Ahmadun et al. 2009). Non-biological methods of pollutant removal have serious drawbacks such as high cost and production of hazardous byproducts, hence biological treatment methods are generally preferred for pollutant remediation. It is the best method among available techniques because it is safe, eco-friendly, cheaper and more efficient than chemical and physical treatment methods. Also there is a possibility for beneficial use of degradation products from biological treatments.

1.9.2 Biodegradation

Biodegradation is a natural way of breaking down organic matter into nutrients that can be used by the organisms. There are two generalized categories of biodegradation namely mineralization and biotransformation. Mineralization involves the complete degradation of the organic matter. In biotransformation, the organic matter is not degraded completely. Only a part
of it is degraded and remaining parts are converted into other smaller chain organic compounds. The use of microbes or their enzymes as catalysts in the biodegradation of organic compounds (bioaugmentation) has advanced significantly during the past three decades. The biodegradation efficiency of organic compounds depends on several factors such as type of organic pollutant, nature of the organism, enzyme involved, mechanism of degradation, nature of the influencing factors and aerobic and anaerobic conditions (Idris & Saed 2002, Das & Chandran 2011).

Biostimulation is a process that involves application of favourable conditions such as aeration or selected micronutrients and biostimulants such as phosphorus, nitrogen or biosurfactants to a contaminated environment to stimulate and enhance the growth of the microorganisms that break down the contaminants (Kim et al. 2005). The effectiveness of biostimulation depends on the number of indigenous microbial population present in the contaminated site. When biostimulated, the native microbial population can grow rapidly, potentially increasing the rate of biodegradation.

1.9.3 Bioremediation

The term "Bioremediation" can be used to describe a broad range of processes, which are dependent on accelerating natural decomposition and breakdown of organic waste materials by consumers (degraders) under controlled conditions. Bioremediation is defined as the process of biological degradation of organic wastes under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities. Bioremediation includes the use of microorganisms such as bacteria or fungi (mycoremediation) or plants (Phytoremediation) to break down harmful environmental contaminants to restore the environment (soil and ground water) to a healthier state.
The bacteria involved in bioremediation are simply gluttonous microbes. These indigenous microbes when being in contact with the contaminants immediately begin to start breaking down the organic contaminant. This "breaking down" process consists of breaking the carbon chains, which constitute all organic molecules, until the contaminant is eliminated or its toxicity is abolished. At the end of this process, carbon dioxide and water are left behind as by-products, with traces of other harmless by-products (Alexander 1999).

1.9.4 Automobile Waste Oil

Oil is found to be the most widely distributed source of energy in the world and hence the largest global environmental pollutant. Petroleum and its refined products (gasoline, diesel, kerosene, jet fuel, engine oil etc.) are major energy resources used in industrial and automobile sectors all over the world. The lubricating oils characterize approximately 2% of the petroleum oil derivatives and are used for automotive and industrial purposes. These lubricating/motor/engine oils consist of a base oil part and as per their applications, they receive additives (organic and / or inorganic-eg. VI improvers, PPT depressant, antioxidant, detergent, dispersant etc) in their formulation in order to meet the requirements of the different intended applications (Adeyemo 2013). The composition of car engine base oil is given in Table 1.4.

During the uses, motor oil carries up a number of additional components including heavy metals, such as lead, cadmium, chromium and other materials like chlorinated hydrocarbons, naphthalene, sulphur etc. from engine wear and tear. After a period of time, the oil viscosity alters, necessitating the replacement of the oil with fresh one.
Table 1.4 Chemical composition of car engine base oil

<table>
<thead>
<tr>
<th>Component</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fraction</td>
<td>90.9</td>
</tr>
<tr>
<td>Normal paraffin</td>
<td>15.5</td>
</tr>
<tr>
<td>Cyclic paraffin</td>
<td>75.4</td>
</tr>
<tr>
<td>Aromatic fraction</td>
<td>9.1</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>1.7</td>
</tr>
<tr>
<td>Fluorene</td>
<td>1.2</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.1</td>
</tr>
<tr>
<td>Dibenzofuran</td>
<td>1.0</td>
</tr>
<tr>
<td>Dinaphthenebenzene</td>
<td>0.8</td>
</tr>
<tr>
<td>Dibenzanthracene</td>
<td>0.6</td>
</tr>
<tr>
<td>Naphtho benzothiophene</td>
<td>0.3</td>
</tr>
<tr>
<td>Perylene</td>
<td>0.2</td>
</tr>
<tr>
<td>Benzothiophene</td>
<td>0.2</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.9</td>
</tr>
</tbody>
</table>

(Source: Koma et al. 2001)

The oil that becomes unsuitable after use due to contamination, making it unfit for its original purpose, is described as used motor oil (U.M.O) and is required to be suitably disposed of. Therefore, any disposal of such used oil in soils, surface water and groundwater, sewer systems or any form of elimination that causes air pollution exceeding the level set by the environmental protection legislation is forbidden (Adeyemo 2013).

Contamination of soil by oil derived hydrocarbons especially of diesel and engine oils is prevalent in oil producing and industrialized
countries of the world. This may pose a great threat to the environment and the living forms (Mandri & Lin, 2007). Prolonged exposure to high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increased risk of cancer (Mishra et al. 2001; Propst et al. 1999; Lloyd & Cackette 2001).

The base oil consists of C16-C36 long chain hydrocarbons, and more than 75% c-alkanes. These c-alkanes in the base oil consist of 1 to 3 rings and any ring contains 5-6 members. Most of the c-alkanes in the base oil contain long alkyl side chains (Koma et al. 2003). The main feature of the motor oil for lubricative use is its viscosity expressed in terms of VI (Viscosity Index). Fresh motor oil contains more percentage of volatile and water soluble hydrocarbons that can cause acute toxicity to organisms (Boonchan et al. 2000) where as used motor oil contains metals and heavy polycyclic aromatic hydrocarbons (PAHs) that may lead to chronic hazards including mutagenicity and carcinogenicity when exposed to it (Boonchan et al. 2000). PAHs occur widely in different ecosystems that contribute to the persistence of these compounds in the environment (Van Hamme et al. 2003). The illegal dumping of used motor oil is an environmental hazard with global consequences (Blodgett, 2001). Used oils can be re-refined and recycled if safely and properly collected but in most cases it is poured into common drainages or put into the garbages where it can contaminate the subsurface soil and ground water.

It is estimated that about 4–5 L of used oil is produced after a single oil change in vehicles. In India, the bulk of U.M.O produced (about 0.4 million tons (1 ton = 0.907 t) annually) is generally used for adverse applications and a very small part (10 tons annually) is rerefined (IIP 1997). Used oils from automobile engines are drained into roadside garages and left unattended. According to the reports of Indian Institute of Petroleum (1997),
Meethi River at Kurla (Mumbai) and the Mundka and Rohtak road area near New Delhi were reported as major oil-contaminated spots in India.

1.9.4.1 Chemistry of petroleum hydrocarbons

Petroleum is defined as a mixture of natural gas, crude oil and condensate. Crude oil is a heterogeneous liquid of hydrocarbons comprising mostly of hydrogen and carbon elements in 2:1 ratio (Okoh, 2006). Other elements such as N, S and O all of which constitute less than 3% (v/v) of crude oil. Also trace constituents P and heavy metals such as V and Ni, comprises less than 1% (v/v) of crude oil. Crude oils are classified as paraffins, naphthenes or aromatics based on their respective distillation residues. Crude oils can also be classified as light, medium or heavy depending on the relative proportions of the heavy molecular weight constituents. Almost 85% composition of all crude oil can be classified as either asphalt base, paraffin base or mixed base. Asphalt base contain little paraffin wax and an asphaltic residue (Atlas 1981) with high contents of S, O and N contents. Paraffin base crudes contain little or no asphaltic materials and mixed crude oil contains high amount of oxides of N and asphalt (Adeyemo 2013).

1.9.4.2 Biodegradation of oil

Because of the critical environmental and health defects caused by oil pollution, increasing attention has been paid for developing and implementing innovative technology for cleaning up oil contaminants. Bioremediation methods are currently encouraged as promising environmental friendly and cost effective treatment technologies for hydrocarbon remediation (Sathishkumar et al. 2008). Microbial degradation of oil occurs by initial attack on aliphatic or light aromatic fractions of the oil,
with high-molecular-weight aromatics, resins, and asphaltenes found to be recalcitrant or exhibiting very low rates of biodegradation (Thenmozhi 2009).

1.9.4.3 Aerobic and anaerobic degradation

Biodegradation of Petroleum hydrocarbon by bacteria can occur under both oxic and anoxic conditions (Zengler et al. 1999), though by the action of different consortia of organisms. The aerobic pathway for hydrocarbon degradation is faster and most efficient, as aerobic reactions require less free energy for initiation and give in more energy per reaction. The hydrocarbons are broken down by a chain of enzyme-catalyzed reactions. The initial enzymatic attack occupies a group of enzymes called monooxygenases. Here, oxygen being an external electron acceptor and an organic component of the contaminating oil functions as the electron donor and energy source.

The general degradation mechanism for alkanes involves sequential formation of an alcohol, an aldehyde and a fatty acid. The fatty acid is cleaved by beta-oxidation, releasing carbon dioxide and forming a new fatty acid that is two carbon units shorter than the original one. The general mechanism for aromatic hydrocarbons involves the action of dioxygenase enzymes for cis-hydroxylation of the ring structure forming a diol (e.g. catechol). The ring is oxidatively cleaved by dioxygenases, forming a dicarboxylic acid (e.g. muconic acid). Oxidation of substituted aromatics involves initial beta-oxidation of the side chain, and then the cleavage of the ring structure. The degradative pathway for a highly branched compound, such as pristane or phytane, may proceed by omega oxidation forming a dicarboxylic acid (Hamme et al. 2003). Eventhough aerobic biodegradation processes are very effective, they are often expensive (e.g. hydrogen peroxide). Hence anaerobic biodegradation is adopted as a cost-effective method for in situ
bioremediation for the decontamination of soil, sediment, and ground water contaminated with petroleum hydrocarbons.

In oxygen limited environments such as subsurface, deep sediments, flooded soils, eutrophic lagoons, stagnant fresh and ocean waters and in oil reservoirs, the hydrocarbon biodegradation takes place under anoxic/anaerobic conditions although the exact mechanism of such reactions are not fully understood. N-alkanes, branched alkanes, cycloalkanes and a number of alkenes have been reported to be degraded under anoxic conditions. For example, cyclopentanes, unsubstituted, methyl- and ethyl-substituted cyclopentenes, and cyclohexanes were reported to be degraded readily under anaerobic conditions in the presence of sulfate but somewhat less effectively under methanogenic conditions. Also cyclohexanes and dimethyl-substituted cyclopentanes were found to be biodegraded only in the presence of sulfate (Widdel & Rabus 2001).

Numerous lab-scale and field-scale studies conducted on biodegradation had clearly established the biodegradation of BTEX (benzene, toluene, ethylbenzene and xylene isomers) compounds. The biodegradation of these individual compounds depends on the terminal electron acceptor (Johnson et al. 2003). Hydrocarbons such as alkylbenzenes including m-, o-, and p-xylene, trimethylbenzenes, naphthalene and phenanthrene can also be metabolized under anaerobic conditions. These reactions may take place under Fe (III)-reducing, denitrifying and sulfate reducing conditions and the bacteria responsible for these metabolisms may grow in syntrophic cocultures with other anaerobes (Boopathy 2004). The inherent biodegradability of the individual components of oil is a reflection of their chemical structure, but is also strongly influenced by the physical state and toxicity of the compounds. For example, the structural group, n-alkanes are the most biodegradable among petroleum hydrocarbons, but the C5 – C10 homologues are shown to
be inhibitory to most hydrocarbon degraders. These homologues exist as solvents and tend to disrupt the microbial lipid membrane structures.

As the usage of petroleum hydrocarbon products increase, soil contamination with engine oils is becoming one of the major environmental problems. Petroleum spilled on soil is chiefly adsorbed to particulate matter which decreases its toxicity but contributing to its persistence. Mechanical methods are used to reduce hydrocarbon pollution, but most of them are expensive and time consuming. These methods generally involve excavating the polluted soil, treating in separate area or in better treatment facilities. These treatments consist of incineration or burial in protected landfills (Thenmozhi 2009). The treatments are effective but after burning, this soil loses its fertility and structure. Landfill methods do not remove the contamination but only relocate it. Bioremediation provides an effective and efficient strategy to speed up the clean-up processes for remediating soils contaminated with oils (Mandri & Lin 2007). Microbial transformation of organic contaminants normally occurs because the organisms can use the contaminants for their own energy needs, growth and reproduction.

Biodegradation rates greatly depend on temperature. Ecosystems having extremely low temperatures degrade hydrocarbons very slowly. Oxygen, moisture, nutrient concentrations and pH are other important factors in determining biodegradation rates in soil. Hydrocarbon degradation by microbes depends on the composition of the community and their adaptive responses to the available hydrocarbons. Bacteria and fungi constitute the major hydrocarbon degraders while some yeasts and algae are also found to have this property. Although hydrocarbon-degrading communities are ubiquitous, they constitute less than 1% of the total microbial community. When oil pollutants are present, their populations increase, typically to 10% of the community (Alexander 1999).
Adapted microbial communities, i.e., those which have been previously exposed to the contaminant, display higher biodegradation potential than those with no prior exposure. Rates of natural degradation usually have been found to be low which are limited by environmental factors like, the contaminant, nutrient (N and P) bioavailability, physical conditions (e.g. temperature, salinity, pH) or microbial competition (Atlas & Atlas 1991). When few or no indigenous degradative organisms exist in a contaminated area and practically does not allow time for the natural enrichment of suitable population, inoculation with suitable communities, called as bioaugmentation may be a convenient alternative (Thenmozhi 2009). Hence it is a successful option to isolate such potential hydrocarbon degraders and seeding them into petroleum contaminated water or soils, with the use of chemostats or fermentors to control the conditions and reduce competition from indigenous microflora.

1.9.4.4 Hydrocarbon biodegradation in MFC

Morris et al. (2009) studied the prospective of microbial fuel cells in biodegradation of diesel range organics demonstrating the application potential of MFC in enhanced subsurface bioremediation of contaminants such as petroleum hydrocarbons, thus, eliminating the need to amend terminal electron acceptors such as oxygen. It was observed in their study that 82% removal of diesel range organics were achieved when compared to the anaerobically incubated control cell (31% removal) over a period of 21 days. Later Morris & Jin (2012) developed sediment MFC with an anode buried in sediments containing TPH concentrations at approximately 16,000 mg kg−1. It was observed that after about 66 days, the TPH degradation rates were 2% and 24% in the open-circuit control sediment MFC and active sediment MFC, respectively signifying that applying MFC technology to contaminated sediments enhances natural biodegradation by nearly 12 fold.
1.9.4.5  Biosurfactants

Hydrophobic pollutants such as petroleum hydrocarbons require solubilization before being degraded by microbial cells. Mineralization is directed by desorption of hydrocarbons from soil. Surfactants constitute a group of amphiphilic compounds having both hydrophobic (non-polar) and hydrophilic (polar) moieties that possess ability to accumulate between fluid phases such as oil/water or air/water by reducing the surface and interfacial tensions and forming emulsions. Surfactants are able to increase the water solubility of hydrocarbons in soil and water environment by increasing the surface area of these hydrophobic materials. Hence, microbial degradation of pollutants can be enhanced by the presence of surfactants. Biosurfactants are a group of surface-active molecules synthesized by microorganisms which differs structurally from each other. Biosurfactants reduce the surface and interfacial tension with low toxicity, high specificity and high biodegradability making them attractive microbial products as alternatives to chemical surfactants (Thenmozhi 2009). They exist structurally as glycolipids, lipoprotein, lipopeptides, neutral lipids, fatty acids, phospholipids, polymeric and particulate biosurfactants.

Biosurfactants are more eco-friendly, specific in action, and robust under diverse environmental conditions. They are produced by different classes of microorganisms. Table 1.4 gives a list of biosurfactants along with their producers. Chemical surfactants differ from biosurfactants in the aspect that the associated products from the reactions by chemical surfactants may lead to the production of a more harmful product, which might have a higher persistence in the environment. Biosurfactants are extremely suitable for enhanced oil recovery operations.

Biosurfactants have a lot more applications than environmental protection practices. This includes their role as cleansers, emulsifiers,
solubilizers, foaming and wetting agents, cosmetics etc. They are also used in the food industry as food formulation ingredients and antiadhesive agents. They find application in the medical industry as antimicrobial agents (antibacterial, antifungal, antiviral), immunomodulatory molecules, adhesive agents and in gene therapy. Due to the diverse applications of biosurfactants, there has been growing interest and research taking place towards the large scale production of biosurfactants, in an economically viable way.

**Table 1.4 Examples for Biosurfactants and their producers**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Biosurfactants</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Cyclic lipopeptides</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em></td>
<td>Cyclic lipopeptides</td>
</tr>
<tr>
<td><em>Pseudomonas sp.</em></td>
<td>Rhamnolipids</td>
</tr>
<tr>
<td><em>Rhodococcus sp.</em></td>
<td>Trehalose lipids</td>
</tr>
<tr>
<td><em>Torulopsis sp.</em></td>
<td>Sophorolipids</td>
</tr>
<tr>
<td><em>Acinetobacter sp.</em></td>
<td>Fatty acids, triglycerides and emulsans</td>
</tr>
<tr>
<td><em>Corynebacterium hydrocarboclastus</em></td>
<td>Proteo-lipid -carbohydrate complex</td>
</tr>
<tr>
<td><em>Corynebacterium lepus</em></td>
<td>Corynemycolic acids</td>
</tr>
<tr>
<td><em>Candida sp.</em></td>
<td>Mannosyl erythritol lipids</td>
</tr>
<tr>
<td><em>Candida bogoriensis</em></td>
<td>Sophorolipid</td>
</tr>
<tr>
<td><em>Candida petrophilum</em></td>
<td>Peptidolipid</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>Mannan-fatty acid complex</td>
</tr>
<tr>
<td><em>Rhodococcus erythropolis</em></td>
<td>Trehalose lipids and mycolates</td>
</tr>
<tr>
<td></td>
<td>Sucrose and fructose lipids</td>
</tr>
</tbody>
</table>
1.9.5 Dyes

The colored substances used in textile, leather and other industries are called as dyes. Dyes constitute aromatic compounds which can absorb light in the visible spectrum of 350 to 700 nm. All the dyes have at least one chromophore (colour-bearing group) and a conjugated system, i.e. a structure with alternating double and single bonds. Dyes display resonance of electrons, which is a stabilizing force in organic compounds. The examples for chromophores are –C=C-, -C=N-, -NO$_2$, -C=O, -N=N- and quinoid rings. In addition to chromophores, most dyes also contain auxochrome (colour helpers) groups, examples of which are carboxylic acid, sulfonic acid, amino and hydroxyl groups. These auxochromes are not responsible for colour, but their presence can shift the colour of a dye and they can influence dye solubility.

1.9.5.1 Classification of dyes

Dyes may be classified in different ways, based on their origin, chemical structure or application.

1.9.5.2 Classification of dyes based on structure

The best scientific classification of dyes adopted by researchers and dye manufacturers is on the basis of their chemical structure. According to this classification, dyes may be grouped as nitrozo, nitro, azo, thiazole diphenyl methane, triphenylmethane, xanthene, acrilidine, quinoline, methane etc. as given in Table 1.5.
Table 1.5 Classification of dyes based on chemical structure

<table>
<thead>
<tr>
<th>Chromophore</th>
<th>Dye Class</th>
<th>Chromophore</th>
<th>Dye Class</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="nitro.png" alt="image" /></td>
<td>Nitro</td>
<td><img src="xanthene.png" alt="image" /></td>
<td>Xanthenes</td>
</tr>
<tr>
<td><img src="nitrozo.png" alt="image" /></td>
<td>Nitrozo</td>
<td><img src="acridine.png" alt="image" /></td>
<td>Acridines</td>
</tr>
<tr>
<td><img src="azo.png" alt="image" /></td>
<td>Azo</td>
<td><img src="quinonemin.png" alt="image" /></td>
<td>Quinoneminines</td>
</tr>
<tr>
<td><img src="anthraquino.png" alt="image" /></td>
<td>Anthraquinones</td>
<td><img src="indigoid.png" alt="image" /></td>
<td>Indigoids</td>
</tr>
<tr>
<td><img src="triphenyl.png" alt="image" /></td>
<td>Triphenylmethanes</td>
<td><img src="cyanide.png" alt="image" /></td>
<td>Cyanides</td>
</tr>
</tbody>
</table>

1.9.5.3 Classification based on the application

Table 1.6 Classification of dyes based on application

<table>
<thead>
<tr>
<th>Dyeing method</th>
<th>Preferred substrate / typical application</th>
<th>Chemical classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct dyes</td>
<td>Cotton, regenerated cellulose</td>
<td>Anionic, poly-azo</td>
</tr>
<tr>
<td>Reactive dyes</td>
<td>Cotton</td>
<td>Azo, metallised azo, phthalocyanine, anthraquinone</td>
</tr>
<tr>
<td>Disperse dyes</td>
<td>Polyester, electronic photography</td>
<td>Non- ionic</td>
</tr>
<tr>
<td>Vat dyes</td>
<td>Cellulose fibres</td>
<td>Anthraquinone, indigoids</td>
</tr>
<tr>
<td>Sulphur dyes</td>
<td>Cotton</td>
<td>Sulphur dyes</td>
</tr>
<tr>
<td>Acid dyes</td>
<td>Nylon, wool, silk, leather, paper, ink-jets</td>
<td>Anthraquinone, azo, trimethyl amine</td>
</tr>
<tr>
<td>Solvent dyes</td>
<td>Plastics, gasoline, oils, waxes</td>
<td>Azo, anthraquinone</td>
</tr>
<tr>
<td>Cationic or basic dyes</td>
<td>Paper, polyacrylonitrile, polysters</td>
<td>Triarylmethane</td>
</tr>
</tbody>
</table>
Based on their intended applications, dyes may be categorized as acidic, basic, mordant, reactive, direct, azoic, disperse, vat, and sulfur dyes when used in textile and tanning industries. Major classes along with their target substrate and main features are listed in Table 1.6.

1.9.5.4 Pollution and Toxicity problems caused by dyes

Dyes can cause serious adverse effects on the environment when disposed carelessly. The Industrial dyes are basically xenobiotic compounds, foreign to the biosphere causing environmental damages. Hence, they accumulate in the environment contaminating shallow aquifers, ground waters and sediments. Many dyes are perceptible to human eyes at concentration as low as 0.005 mg/l in clear water. The textile processing industries release 10 to 200 mg/l of dye in their effluents. Such highly colored discharges cause aesthetic problems and can affect the photosynthetic activities of the organisms living in the receiving water bodies because of reduced light penetration. Several dyes and their degradation products have been found to be toxic to the life forms. Numerous synthetic dyes are found to be mutagenic and carcinogenic. Aromatic amines are carcinogenic compounds causing greatest health hazards to humans. They are formed either as byproducts of dye manufacturing or as metabolic intermediates during reductive cleavage of azo bonds in dyes.

Malachite green (MG) is a type of triphenylmethane dyes and is extensively used in both textile industry and fish farming industry due to its relatively low cost, ready availability, and high efficacy against fish microbial pathogens. Despite its high value, MG is highly toxic to mammalian cells, and has been banned by the US Food and Drug Administration. The recalcitrance and cytotoxicity of MG present difficulties for biodegradation. However, several microbial strains have been reported capable of decolorizing MG. These microorganisms include the white rot fungus *Phanerochaete*...
chrysosporium, bacterial strains such as Bacillus cereus DC11, Aeromonas hydrophila DN322 etc. Sunset Yellow FCF (also known as Orange Yellow or food yellow) is a petroleum-derived orange azo dye with a structure disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate. It is used widely in textiles, foods, cosmetics and drugs.

The dyestuffs generally cause low acute toxicity. Dye concentrations below 1 mg/l generally do not inhibit algal growth (photosynthesis). Cationic dyes are the most toxic dye molecules, especially those with a tri phenyl methane structure. The chance of human mortality by acute toxicity with dyes is generally very low. But, an acute sensitization reaction often occurs to humans after exposure to dyestuffs. Some disperse dyes have been observed to be responsible for allergic reactions such as eczema or contact dermatitis in humans (Mathur et al. 2005).

Some azo compounds are used as food colourants. Azo dyes in purified form are generally not directly mutagenic or carcinogenic, but some azo dyes with free amino groups have shown some degree of carcinogenicity. Though, aromatic amines are formed by reduction of azo dyes or cleavage of the azo linkage(s) in the dye molecules, most of them are known mutagens and carcinogens. Aromatic amines may induce bladder cancer in humans exposed to them (Xu et al. 2010). The aromatic amines may lead to the formation of acyloxy amines through N-hydroxylation and N-acetylation of the aromatic amines followed by O-acylation. These acyloxy amines may be converted to nitrenium and carbonium ions which can bind to DNA and RNA to induce mutations and tumor formation (Pielesz et al. 2002).

1.9.5.5 Decolourization methods

A variety of physicochemical methods exists to remove colour from dye containing effluents. The most extensively used methods are coagulation
and flocculation processes (Gogate & Pandit 2004). These methods require significant quantities of chemicals and produce considerable amounts of sludge, necessitating further handling and disposal. Adsorption and membrane filtration techniques may create secondary waste streams which may also need further treatment and/or disposal. Advanced oxidation processes (AOPs, such as ozonation, UV/H, Fenton, etc.) and membrane filtration methods are rather effective for colour removal but their applications are limited by the requirement for high energy and cost. Enzymatic decolourisation is a popular method for the decolourisation of dyes, yet having disadvantages such as cost of enzymes, enzyme stability and product inhibition (Husain, 2010). There are several factors which determine the technical and economic feasibility of each single dye removal technique. These factors include the type of dye molecules, composition of effluents, dose and costs of required chemicals, operation costs (energy and material) and environmental fate and handling costs of generated waste products.

Biological processes present a cheap and efficient alternative for simultaneous colour and organic matter removal. However, it is difficult to achieve complete degradation only using aerobic biological processes (Méndez-Paz et al. 2005). Anaerobic treatment may be a feasible alternative for treatment of dye wastewater containing (Solanki et al. 2013). Biological techniques range from sorption of dyes by algae and fungal biomass to biodecolorization and degradation by using fungi and bacteria in aerobic, anaerobic, anoxic or combined anaerobic/aerobic treatment processes. In reality, each treatment method has its own limitations. The use of a single technique often may not be sufficient to achieve complete decolourisation.

1.9.5.6 Dye removal using bioelectrochemical systems

Microbial fuel cells have been used for decolourisation of different dye molecules along with bioelectricity generation. Niu et al. (2012)
constructed a microbial fuel cell (MFC) using Fe (II)-EDTA catalyzed persulfate as the cathode solutions to decolorize Orange G (OG) and harvest electricity simultaneously. Azo dye decolourisation was observed in a single chamber MFC by Han et al. (2011) using Reactive blue 160 and achieved good decolourisation using mixed bacterial cultures. Bakhshian et al. (2011) investigated the enzymatic decolourisation of reactive blue 221 (RB221) using laccase in a dual-chamber microbial fuel cell (MFC). Here the enzyme in suspended form was used in the cathode chamber in the absence of any mediators in order to decolorize RB221. Molasses was utilized as low cost and high strength energy source in the anode chamber. 87% decolorization efficiency and 84% COD removal were achieved.

1.9.5.7 Mechanism of dye decolourization

The dye reduction mechanisms in conventional biological reactors include different processes involving enzymes and low molecular weight redox mediators or chemical reduction by biogenic reductants like sulfide or a combination of these (Pandey et al. 2007). MFCs employ anaerobic mechanisms for anodic degradation of the dye, which is similar to conventional mechanisms, except that there is an additional mode of electron and proton transfer to the dye, through the external circuit and the membrane, respectively. The products of biodegradation were seem to be similar, majorly amines and sulfanilic acids. The color removal was primarily achieved by biodegradation rather than biosorption by living cells (Sun et al. 2009).

Mu et al. (2009) explained the decolourisation mechanism of AO7. Substrate is oxidized by bacteria to produce protons and electrons at the anode, which are transferred to the cathode through membrane and external circuit respectively. The azo bonds of dye are broken using proton and electron at the cathode resulting in the formation of toxic intermediates. The biological dye decolourisation takes place in the anode chamber under
anaerobic conditions by breakdown of the azo bond of Congo red and abiotically in the cathode chamber for complete degradation of intermediates (Li et al. 2010).

The role of MFC in bioremediation has been elucidated, but to our knowledge, MDC has not yet been applied for toxicant biodegradation studies. The use of MDC instead of MFC for biodegradation of toxic pollutants increases the efficiency of biodegradation and power production along with an added advantage of saltwater desalination. The selection of bacteria possessing both the properties of electrogenicity as well as biodegradability is necessary to be used for biodegradation in microbial desalination cells.

1.9.6 Kinetic Study of Oil Biodegradation

In biodegradation reactions, kinetic studies give a measure of how effectively the microbial system is functioning (Agarry et al. 2008). Knowledge of such kinetics will help improve the process control and removal efficiency (Monteiro et al. 2000). A number of microbial growth and biodegradation kinetic models have been developed and used by many researchers for different toxic compounds. These models help prediction of amount of pollutants present at a certain time, calculation of the time required to reduce the pollutant to certain concentration and predicting the amount of biomass production achievable at a given time. A fundamental knowledge on biokinetics is essential for a successful bioremediation process design and optimization of operational conditions when conducted in large scale. Bhattacharya in 2015 has reviewed that the poor solubility of oil has limited the studies on mathematical modelling of biodegradation of industrial wastewaters containing particulate matters such as oil ($10^{-4} - 10^{-10}$ mg/L for n-alkanes with carbon number from C10 to C24).
The kinetic biodegradation process can be described from two different perspectives - microbial growth models and substrate utilization models. Growth models such as Monod’s model were based on mechanistic approach and could not describe the phenomenon of biodegradation. Hence substrate utilization models or degradation models were preferred. Some of the mathematical equations which have been proposed to describe the kinetics of the substrate degradation process are based on curve fitting of the experimental substrate consumption or CO₂ production during biodegradation.

The simplest approximation used by the majority of authors is a curve fitting of the decrease of the substrate concentration using a first order equation. While first order kinetic biodegradation equations are applicable to laboratory experiments, the reliable use of such rate constants for the environment is doubtful, for there is also evidence that discrete alterations of some chemicals in surface water samples are highly dependent on population size, making "k" a second or pseudo first order constant. Quiroga et al. (1999) have described second order kinetic model to describe the biodegradation of surfactants in sea water.

Although there were a few reports on kinetic modelling of insoluble substrates, most of the studies investigating the microbial utilization of such materials usually reported their findings using first-order decay rates with respect to the total substrate (Morgenroth et al. 2002). Kinetic modelling of degradation of petroleum hydrocarbons by the Monod model has been reported. The Haldane model, a further development of the Monod model to include substrate inhibition, was also reported in literature for kinetic modelling of degradation of high oil and grease rendering wastewater (Nakhla et al. 2006). Greene et al. (2000) has stated that when individual microbial species are considered for hydrocarbon degradation, simple competition for
the growth substrate is the only interaction involved. Hence the first-order kinetic was able to describe the rate of total oil reduction. Most of the compounds obeyed first-order kinetics for hydrocarbon removal (Bonaventura and Johnson 1996). Shankar et al. (2014) has described the biodegradation of spilled oil using first order kinetic model.

1.10 OBJECTIVES OF THE STUDY

The main objectives of the present study are as follows,

1) Optimization of MDC for maximum biodegradation, bioelectricity production and desalination

2) Screening, isolation and identification of elite bacteria for biodegradation of pollutants

3) Optimization for maximum growth of bacteria

4) Application of Microbial desalination cells for
   - Biodegradation of automobile waste oil
   - Biodegradation of dye house effluents

5) Extraction and characterization of biosurfactants from hydrocarbon degrading bacteria

6) Kinetic modelling of biodegradation in MDC.