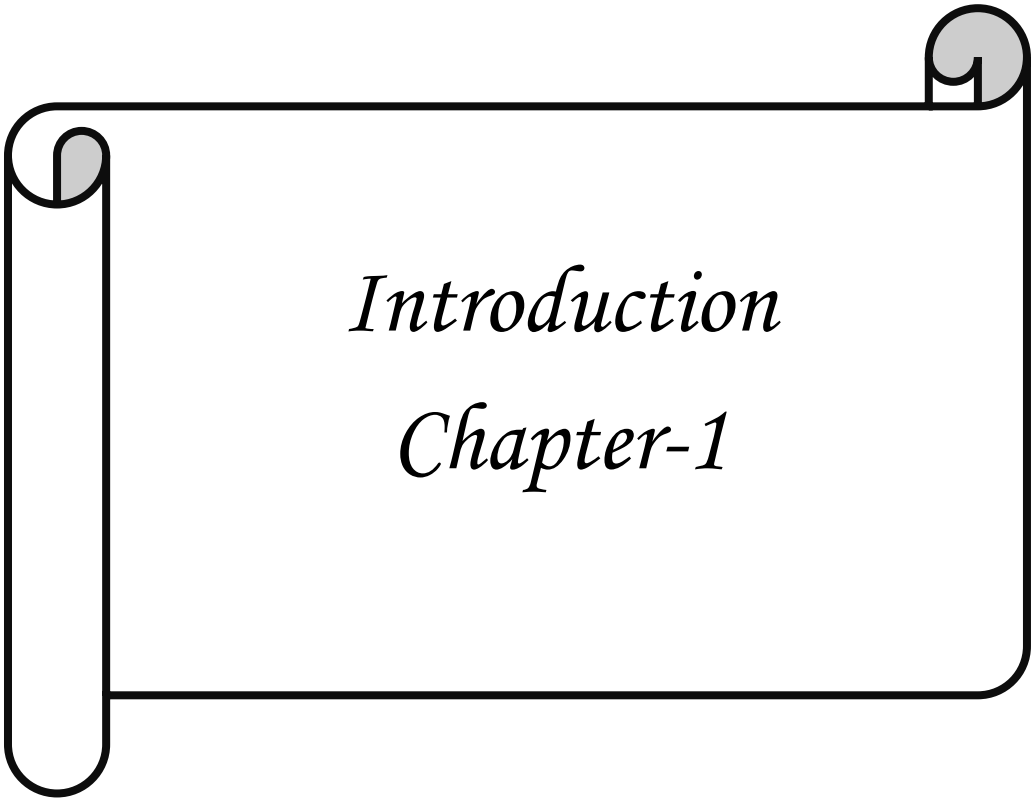
Introduction
Chapter-1

CHAPTER 1

INTRODUCTION

Now a day, sustainable energy and safe water are the two major challenges faced by humanity. According to the report of the world health organization (WHO), the world's 700 million population (11% of the total population) are facing energy and safe water problem (Bos et al., 2008; Dziegielowski et al., 2020). In recent years rapid industrialization has created a lot of positive and negative impacts on the environment. They directly or indirectly contaminate our groundwater system, lake, rivers, and they also disrupt our ecological balance. These industries produce a large amount of colored wastewater which is not aesthetically acceptable only but also has a serious threat to the environment. The color which appears in wastewater is mainly due to the presence of synthetic dyes, minerals, and other organic matter such as humic and fulvic acid and natural tannins (Padmanaban et al., 2018; Rajeswari et al., 2016; Zhao et al., 2020). In India, textile industries contribute a very important role in the economic growth of the nation, but it also has another dark side of polluting the environment (Aiyer et al., 2020; Khalik et al., 2018).

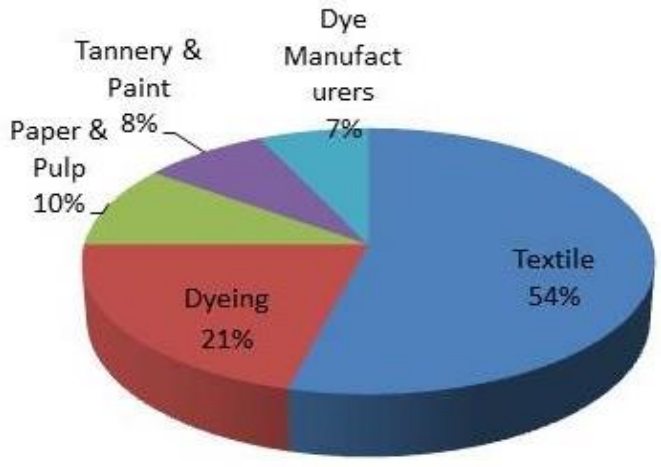

Figure1.1 Sources of dye wastewater(Chowdhury et al., 2020)

These colored wastewaters are not only produced by textile industries but also produced by many industries such as tanning, pulp & paper, paint, food, cosmetics, and pharmaceutical industries(Rajeswari et al., 2016). Approximately 54% of dye wastewater comes from the textile industry and the remaining from the dyeing, pulp & paper, tannery, and paint industries(Chowdhury et al., 2020). They cannot be used for irrigation purposes because they may disrupt the fertility of the soil, inhibit plant growth, or may enter into our food chain. If they are discharged without proper treatment in rivers, lakes, or ponds, it becomes toxic to aquatic life. Since the colored wastewater is dark and therefore it will hinder light penetration through the surface of the water which results in a decrease in photosynthetic activities and diffusion of oxygen inside the water. It ultimately results in a decrease in dissolved oxygen (DO), alteration in pH, increases in biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and bioaccumulation which is life-threatening to the water bodies (El-Kassas and Mohamed, 2014; Tyagi, 2020; Wu et al., 2020). These dyes are reported to be very toxic, carcinogenic, mutagenic, recalcitrant to degradation, stable to oxidizing agents, thermostable, and very toxic to humans(Liu et al., 2020; Long et al., 2019). Dyes acute and long-term chronic exposure results in dermatitis and respiratory disease, irritation to eyes and skin and mucous membrane, cancer, and gene mutation (Ghodake et al., 2011; Katheresan et al., 2018; Shobana and hangam, 2012).

Dyes are generally colored compounds and are characterized by their light-absorbing property invisible (400-700 nm) regions. Commercially 10,000 different synthetic dyes are produced and 80% of the dye produced belongs to the Azo (-N=N-) group. They are produced annually at a rate of 7×10^{5} tons of dye per year and a large amount of (approximately 15 %) dyes are washed off during their manufacturing and applications. Among the variety of dye classes available for coloring cellulosic fiber, the

Azochromophores based reactive dyes are the most utilized ones due to their bright colors, water-solubility, ease of application, and cost (Fang et al., 2017; Muniyasamy et al., 2020).

Figure1.2 Wastewater discharge into the river (www.civildigital.com)

Due to its recalcitrant nature, dye decolorization in wastewater is a major challenge for the different treatment processes. Dye decolorization can be achieved with physical, chemical, or biological methods or a combination of any above three. Adsorption, reverse osmosis, flocculation, oxidation, precipitation, sedimentation, ultrafiltration, color irradiation, ozonation, and coagulation are some physical and chemical method which completely or partially decolorizes dyes present in wastewater (Chowdhury et al., 2020; Daud et al., 2019; Muniyasamy et al., 2020; Saini, 2017; Sarkar et al., 2017). Although all these methods are very efficient for dye decolorization. They only have the disadvantage that these methods are chemical or energy-intensive processes and they produce secondary waste which requires additional treatment, thereby increasing the

overall cost of the treatment. Taking these disadvantages into considerations, here biological methods seek attention (Fang et al., 2018; Fernando et al., 2014a; Sharma and Kaur, 2018). Dye decolorization using biological methods can be achieved with the help of fungus, algae, yeast, and bacteria in different bioreactors. These methods are less energy-intensive, environmentally safe, less costly than physical and chemical methods and they partially or completely biodegrade these dyes to stable and non-toxic compounds.

The biological method decolorizes dyes with these basic mechanisms either by biosorption (adsorption) or bioaccumulation (absorption) or microbial degradation (bioremediation) or a combination of any (Katheresan et al., 2018; Parmar and Shukla, 2018; Zuraida et al., 2013). Asgher et al.(Asgher et al., 2013) found Solar Brilliant Red 80 decolorization (84.4%) using white-rot fungus in 7 days, Syafiuddin et al.(Syafiuddin and Fulazzaky, 2020) have reported Remazol Brilliant Blue R dye decolorization (87%) in 14 days by *Pestalotiopsis species*. Similarly, a very high decolorization (%) was reported by many researchers using yeast and algae for different dyes (El-sheekh et al., 2009; Mahmoud, 2016;Sameera and Padma, 2014). Unnikrishnan et al. (Unnikrishnan et al., 2018) have reported the decolorization (96%) of Reactive Red 198 using calcium alginate bead encapsulated bacterial cells in 72 h. Roy et al.(Roy et al., 2018) found decolorization (81%) of Crystal Violet by *Enterobacter sp.* HSL69 in 72 h. All these methods are cost-effective and alternative to physical and chemical methods and have very low secondary waste generation. Decolorization of dyes using the bacterial method rather than yeast, fungus and algae are more preferable because (i) bacterial decolorization is faster, (ii) can be easily cultivated and have rapid growth than yeast, fungus, and algae, (iii) ease of adaptability of bacteria in the extreme condition of salinity and temperature.

Since the dyes have a complex nature which makes them recalcitrant to biodegradation which in turn decreases overall decolorization efficiency. Generally, dyes degraded easily in an anaerobic environment but they lead to the formation of aromatic amines, therefore further aerobic treatment was recommended for complete mineralization of amines into non -toxic compounds. Several combinations of anaerobic and aerobic biological treatments have been suggested for enhanced dye degradation. Generally, Azo dyes are degraded under anaerobic conditions. This biological process for dye degradation is cost-effective, but the only disadvantage associated with these processes is that they are slow in nature and methane-rich gas is produced, and also, they require co-substrate for creating a reduced environment. Aromatic amines that resulted from reductive cleavage of Azo dyes have high redox potential which is difficult to degrade in an anaerobic environment (Dai et al., 2020; Ilamathi and Jayapriya, 2017; Pe et al., 2015). Sometimes bacterial method alone cannot be used for dye removal. Developments of the toxic intermediates during anaerobic degradation and lower decolorization are some of the operational limitations of biological methods. Hence the study is being diverted in search of new technology that increases the overall biological process efficiency.

The Microbial fuel cell (MFC) technology has been already well established for electricity production, but a very small amount of work for dye degradation with electricity production has been reported to date (Ilamathi and Jayapriya, 2017; Solanki et al., 2013). Here, MFC has emerged as new green, sustainable and clean technology that serves both as a non-combustion based energy producer as well as waste remediation, which overall increases the efficiency of the process (Bose et al., 2019; Li and Yu, 2015; Vaez et al., 2015). However, literature available to date suggests that electricity produced with MFC technology is very low. So there is a need for combining this technology of electricity production with waste remediation so that the overall process efficiency can

be maximized. This MFC technology generates low biomass comparing to other biological processes because most of the waste is utilized for electricity production rather than the growth of microorganisms(Franks and Nevin, 2010; Logan, 2009; Tao et al., 2020; Yang et al., 2017). Many designs of MFC have been suggested like single-chambered, dual-chambered, tubular, and multi-stack MFC and each design have their advantages and disadvantages. Mostly dual-chambered MFC is popular due to its ease of design and maintenance(Rossi et al., 2019; Vilas Boas et al., 2015).

Dual chambered MFC consists of an anode and cathode chamber separated by the proton exchange membrane (PEM). On the anode side, waste material is anaerobically oxidized and produces electrons and H^+ ions. These H^+ ions are transported to the cathode side via a proton exchange membrane (PEM) while the electron is transported to the cathode side via an external circuit. On the cathode side, the external supply of O_2 is maintained (in most cases if H_2O is desired as a byproduct) which gets combine with H^+ and electrons and produces water (Ardakani and Badalians Gholikandi, 2020; Dessie et al., 2020; Kisieliute et al., 2019).

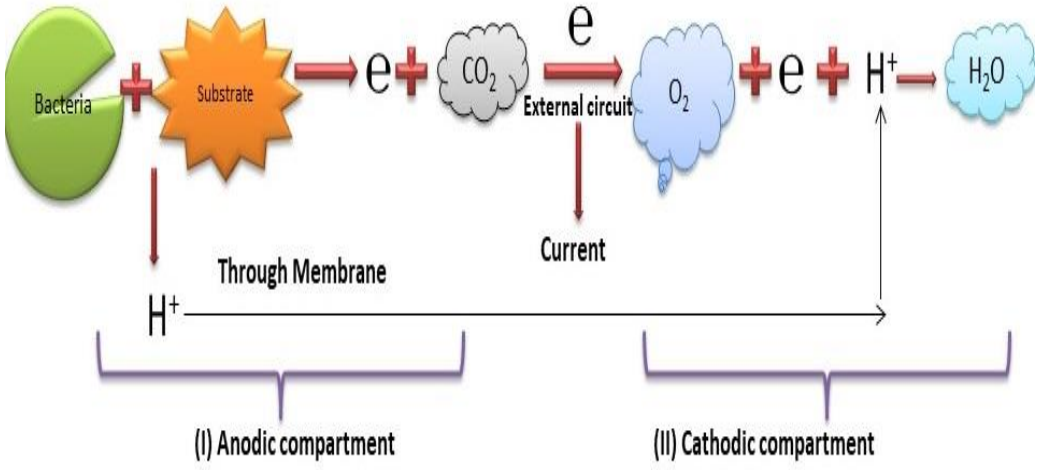

Figure 1.3 Mechanism of waste remediation and electricity production

Equation 1.1 and 1.2 shows the typical reactions that occur at the anode and cathode side in MFC using acetate as a substrate at the anode chamber.

$$\textbf{Anode side: } CH_3COO^- + 2H_2O \longrightarrow \text{Biomass} + 2CO_2 + 7H^+ + 8e^- \quad \textbf{(1.1)}$$

$$\textbf{Cathode side: } 2O_2 + 8e^- + 8H^+ \rightarrow 4H_2O \quad \textbf{(1.2)}$$

Combining Eq.1.1 and Eq. 1.2, we have the breakdown of the waste which results in carbon dioxide and water as byproducts. Based on the above redox reaction, an MFC have the capability of producing electricity using the electron flow from the anode side to the cathode side via an external circuit by direct consumption of waste using microbes(Liu et al., 2005; Rossi et al., 2019).

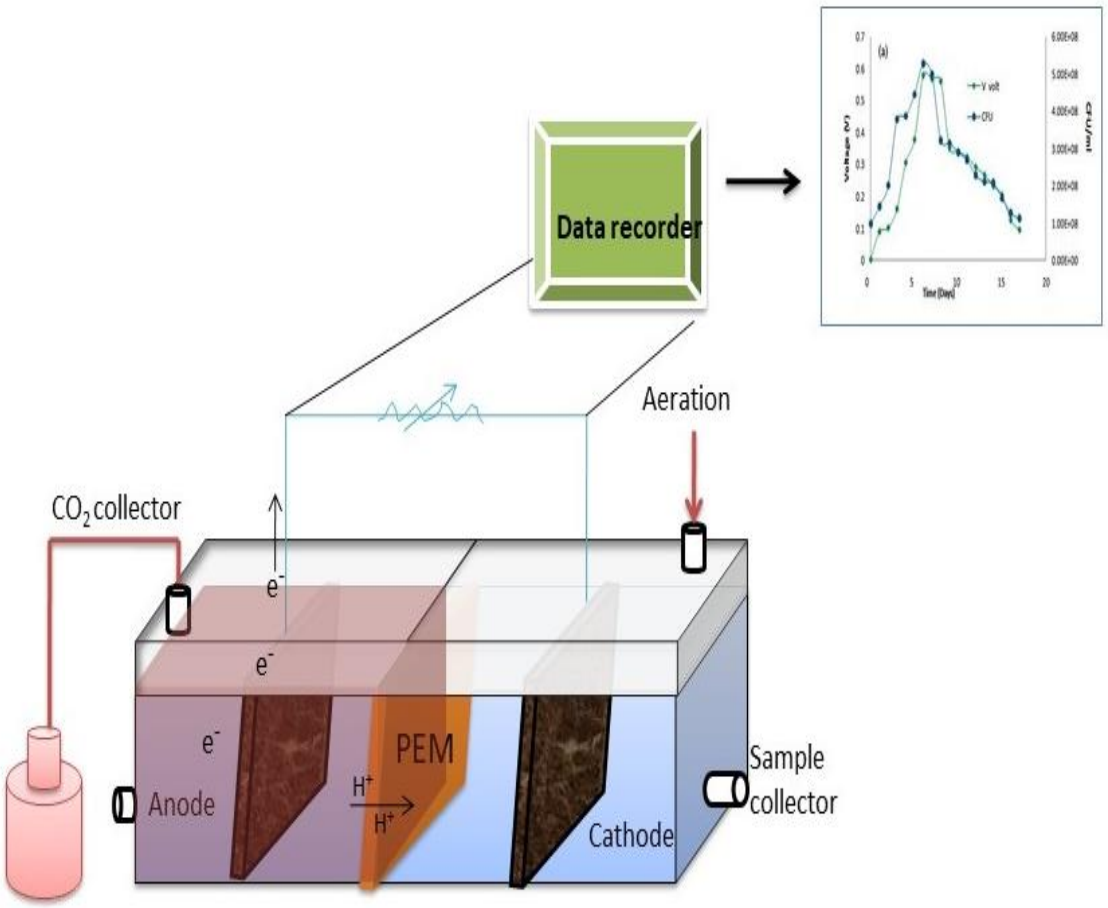

Figure 1.4 Dye remediation in MFC

Several microorganisms like *Geobacter rsulfurreducens, Rhodoferax ferrireducens, pseudomonas sp., Shewanella sp., Methanobacteriumpalustre, Methanobrevibacterarboriphilus, and Methanocorpusculum parvum, Enterococcus faecalis B101, Acinetobacter calcoaceticus C1, Desulfuromonas* have been reported by many researchers which catalyzes organic waste present in MFC and simultaneously produces electricity(Holmes et al., 2015; Jiang et al., 2014; F. Liu et al., 2020; Liu et al., 2019). Mostly exoelectrogens bacteria are used which can directly convert waste into electricity.

Performance of MFC is mainly affected by the material used for anode and cathode, proton exchange membrane, size of the reactor, type of microbes used and substrate (waste material), and external environments such as pH, temperature, and time. Generally, carbon-based material is used for anode due to its biocompatibility and chemical and thermal stability. PEM used in MFC should possess low resistance to H^+ transport and also limits O_2 and substrate diffusion (M. Li et al., 2018; Wang et al., 2016; Xu et al., 2017). Mostly Nafion as PEM has been reported by many researchers due to low resistance to H^+ transport and high power density(Çetinkaya et al., 2015; Ghasemi et al., 2015; Shahi et al., 2017). Research on several modifications for anode and PEM is also going on in search of cost-effective material to increase the power output (Angioni et al., 2017; Gao et al., 2013; Jiang et al., 2010; Yee et al., 2012).

MFC has been reported for the treatment of many different wastes such as brewery, food, pharmaceutical, domestic sewage, distillery, paint and paper and dairy industry(Das and Mangwani, 2010; Jayashee et al., 2016; Li et al., 2019; Zhang et al., 2015). Recently, this technology has been used for few years in the field of the biosensor for real-time environmental monitoring and measuring the concentration of many compounds during biodegradation, monitoring COD/BOD of the pond and for hydrogen

production(Adekunle et al., 2019; Hou et al., 2017; H. Sun et al., 2019; Velasquez-Orta et al., 2017). Recently, it shows its efficiency towards the decolorization of textile waste such as Acid Orange 7, Congo Red, Methyl Orange, Reactive black, Methylene Blue, And Brilliant Red dyes(Ahmadpour et al., 2020; Cao et al., 2017; Hou et al., 2017; S. Liu et al., 2020; Oon et al., 2020; Pereira et al., 2014). Unlike other anaerobic systems, MFC utilizes oxygen as the terminal electron acceptor which has high redox potential, which results in an increase in the rate of microbial metabolism than any other anaerobic system, and ultimately a faster rate could be expected for Azo bond reduction for dyes. As a result, enhanced dye decolorizes kinetics can be achieved in MFC than any other biological process. The only by-product of MFC technology is CO_2 gas and water. The anaerobic environment of the MFC is the best suitable environment for Azo dye degradation(Ahmadpour et al., 2020; Aiyer et al., 2020; L. C. Wu et al., 2020). However, due to the recalcitrant nature and higher toxicity of these dyes, their removal efficiency in MFC varies. Many researchers have combined the MFC technology with other technology to increase dye removal efficiency. Zou et al.(Zou and Wang, 2017) had used MFC coupled electrolysis cells for enhanced Methyl Red degradation and achieved 89.3% removal efficiency. Fang et al. (Fang et al., 2017) had used MFC coupled constructed wetlands for long time treatment of dyes (ABRX3) in wastewater and a very high concentration of 271.53 mg/L of dye was successfully removed. Cao et al.(Cao et al., 2017) found enhanced degradation of Reactive Brilliant Red X-3B dye when they coupled MFC with biofilm electrode reactor. MFC removal efficiency can be increased either by changing the design or coupling MFC with other technology. Many researchers also observed that bacteria isolated from previously acclimatized MFC have enhanced power output than acclimatized directly from any other source. This is a well-known fact that gene modification of bacterial strain or changes in bacterial respiration rate could

increase metabolic rate which results in enhanced dye degradation and increased power output in MFC (Angelaalincy et al., 2018; Y. Cao et al., 2019; Franks and Nevin, 2010; Yi et al., 2009).

In summing up, rapid industrialization results in high energy demands, and also there is a need for more efficient and less time-consuming treatment technology for the treatment of waste produced from these industries. The MFC method is a promising way of treating these wastes along with electricity production in the form of sustainable energy. Dye removal in MFC with single and mixed cultures is studied recently. However, few pieces of literature are available on dye removal using previously acclimatized MFC. Hence, identification of more efficient microorganisms for enhanced dye degradation and study of the optimized condition like pH, time and concentration, and kinetic study is necessary for establishing the bioremediation phenomenon of dyes in MFC.

1.1 Microbial fuel cell for Azo dyes treatment

As discussed earlier, biodegradation of Azo dye is a slow process and they require electron donors for creating a reductive environment. And a very high concentration of dye cannot be treated only using the bioremediation process (Neifar et al., 2019; Zee and Villaverde, 2005). Considering all these disadvantages, a microbial fuel cell can be novel alternative technology for dye remediation along with electricity production. MFC has proven its utility in many fields like treating wastewater, in the field of the biosensor, quality monitoring, and H_2 production (Chouler et al., 2017; Du et al., 2007; Jiang et al., 2018; L. Wang et al., 2019). In MFC, an anaerobic environment is present which is suitable for Azo dye reduction. Successful degradation of dyes and electricity production has been observed by many researchers (Chen et al., 2016; Thung et al., 2015). Unlike other anaerobic systems, MFCs utilize oxygen which has high redox potential as an

electron acceptor. Hence, dye degradation in MFC could be expected higher than other anaerobic biological treatment methods(Ilamathi and Jayapriya, 2017; Yaqoob et al., 2020). **Table 1.1** shows a short review of work done by many researchers in the field of dye degradation using MFCs.

Table 1.1 Electricity production using Dyes

Used	**Design**	**Electrical parameters**	**Dyes Decolorization**	**References**
Congo red (200 mg/L)	Single-chamber air cathod MFC	0.02 W/m^2	88% in 36 h	(Dai et al., 2020)
Congo red (50 mg/L)	Double chambered	988.1±5.2 mW/m^2	89.7±2.4% within 24 h	(Liu et al., 2020)
Reactive blue 19 (50 mg/L)	Double chambered	0.02 W/m^2	89% in 48 h	(Wang et al., 2019)
Acid Orange 7(50 mg/L)	Single chambered up-flow membrane-less	167.4 ± 11.6 mV	80.6% in 24 h	(Thung et al., 2018)
Reactive Black 5 (200 mg/ L)	Tube double-chambered MFC	700 mV	90% in 3 h 45 min.	(Saba et al., 2018)
Methyl Red (50 mg/L)	Electrolysis cell combines with MFC	0.56 V	93 % in 48 h	(Zou and Wang, 2017)
Methyl Orange (25 mg/L)	Photoelectron catalytic double-chambered	0.12 W/m^2	84.5% in 36 h	(Han et al., 2017)
Acid Orange 7 (500 mg/L) ethyl acetate was added as a co-substrate.	Air-cathode cylindrical chamber	0.21 W/m^2	96.7% in 24 h	(Lai et al., 2017)
New coccine (0.0463 ± 0.004 mg/L h)	double-chambered	0.02 W/m^2	73 % in 72 h	(Oon et al., 2017)

1.2 Dye degradation mechanism in microbial fuel cell

Dye degradation in MFC (**Figure 1.5**) follows the same mechanism as followed by anaerobic degradation in batch reactor and similar degradation products like aromatic amines are produced as intermediates. Anaerobic degradation of Azo dyes by bacteria generally takes place by azoreductase enzyme which plays a major role in cleaving the Azo bond present in dye and thereby producing aromatic amines. Equations 1.3 and 1.4 show the Azo bond cleavage in dyes (Khan et al., 2014).

$$\mathbf{Ar\text{-}N{=}N\text{-}Ar' + 4e^- + 4H^+ \longrightarrow Ar\text{-}NH_2 + Ar'\text{-}NH_2} \quad \textbf{(1.3)}\ \text{(anaerobically)}$$

$$\mathbf{Ar\text{-}NH_2 + Ar'\text{-}NH_2 \longrightarrow \text{mineralized salts}} \quad \textbf{(1.4)}\ \text{(aerobically)}$$

Under long-term retention time in anaerobic conditions, Azo dyes metabolites produced from reductive cleavage could get mineralized (Işik and Sponza, 2007). The color removal of Azo dyes occurs due to the reductive cleavage of the Azo bond and not by any biosorption. But the only difference is that these aromatics are further degraded to smaller compounds to enter into the Tricarboxylic Acid Cycle (TCA) cycle where it is degraded completely or mineralized into salts. In an anode chamber, degradation takes place basically by the co-metabolism of anodic microbes. Here reaction rate is faster than the batch anaerobic reactor because O_2 acts as a terminal electron acceptor due to which a faster reaction rate for decolorization could be expected in MFC.

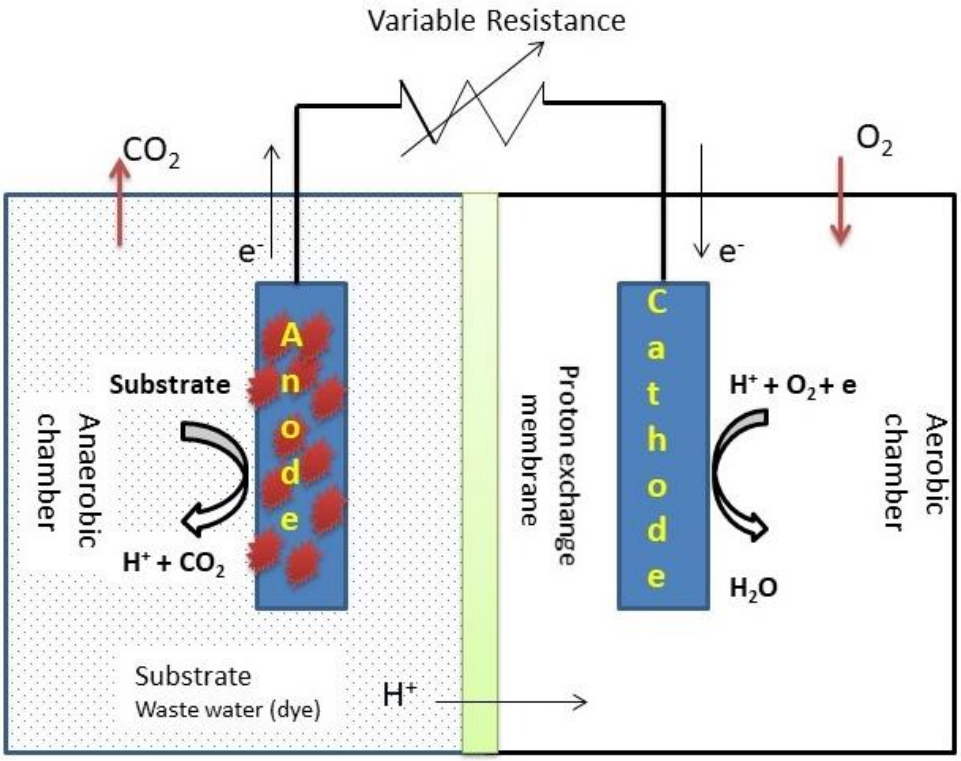

Figure 1.5 Mechanism of waste degradation in MFC

1.3 Effects of operational parameters on dye removal efficiency in MFC

- **Effect of Dye Concentration**

With an increased concentration of dye, degradation increases but overall removal efficiency decreases i.e. decolorization decreases. A small concentration can be treated efficiently. Ahmadpour et al. (Ahmadpour et al., 2020) investigated the effect of reactive black 5 (RB5) dye decolorization in a photo-electrocatalytic microbial fuel cell. Their study cleared that, decolorization rate decrease with increasing RB5 concentration. At a low concentration of dye, electrical results remain unaffected but with increased concentration, power output also gets decreases due to the toxicity of dyes inside MFC. Similar findings were also reported by Wang et al. (Wang et al., 2019) for decolorization of Reactive Blue 19 in a dual-chambered microbial fuel cell. At 50 mg/L of Reactive Blue

19 concentration, 95% color removal was obtained and 80 mg/L initial dye concentration color removal was found to be 75%.

- **Effect of Dye Structure**

Dye structure greatly affects its decolorization. Monoazo dyes are easy to decolorize compared to poly Azo dyes (Saha and Rao, 2019; Sameera and Padma, 2014). Reductive cleavage of the Azo bond directly gets affected by the electron-withdrawing group position. Reductive cleavage of Azo follows the order as para>ortho>meta because ortho and para position involves in resonance stability of the compound due to which electron-withdrawing group, withdraws electron easily at this position thereby making Azo bond more electrophilic thereby they get reduced easily. Comparing to ortho and para, para is more susceptible to decolorization because of a steric hindrance at the ortho position(Oon et al., 2017).

- **Effect of Co-Substrate**

From the literature, it can be concluded that different bacterial *sp.* requires different co-substrate for better dye decolorization. Hou et al. (Hou et al., 2017) have observed accelerated Azo dye degradation and concurrent hydrogen production when he used acetate as co-substrate in a single-chambered MFC. Cao et al.(Cao et al., 2010) investigated the effect of different co-substrates during decolorization of Congo red and on power density in a single-chambered MFC. He found a maximum power density of 103 mW/m^2 for glucose as co-substrate which is much higher when sodium acetate (85.9 mW/m^2) and ethanol (63.2 mW/m^2) were used as co-substrate for degradation of Congo red dye. Therefore, the co-substrate selection is an important parameter for maximizing the power output of MFC.

- **Effect of External Resistance**

MFC external resistance directly affects the anode potential and therefore the activity of exoelectrogens gets affected. Penetration of Azo dyes into the cellular interior of microbes is difficult due to the large size of the dye molecule. Therefore, the transfer of reducing equivalents to the Azo dyes occurs extracellularly. Menicucci et al. (Menicucci et al., 2006) found that external resistance affects the current and power generation in MFCs. Fernando et al (Fernando et al., 2014b) also studied the external resistance as a potential tool for influencing Azo dye reductive decolorization kinetics in microbial fuel cells. Their finding concluded that external resistance greatly influences the extracellular electron transfer mechanism of microbes to the Azo dyes for their reductive cleavage kinetics. It was observed that, with high external resistance, more reducing equivalents will be produced which is responsible for Azodye reduction and the rate could be expected faster. But along with more reducing equivalents, high external resistance also supports the growth of fermentative and methanogenic microbes in MFCs and thus the environment inside MFC becomes unfavorable for exoelectrogens (Jung and Regan, 2011). Oon et al. (Y. Oon et al., 2018) studied the different resistance 100, 1000, 10000 Ω and found that lower resistance produces more output power because at low resistance electrons can easily be transported via an external circuit. These findings suggest that external resistance is an important parameter for reductive decolorization kinetics of Azo dye and power output in MFCs.

- **Effect of Hydraulic Retention Time (HRT)**

It is an important parameter for Azo dye degradation. Its behavior is the same as batch biodegradation. As HRT increases, dye degradation increases. Haavisto et al. (Haavisto et al., 2017) studied the effect of HRT on continuous electricity production from xylose

in up-flow microbial fuel cells and found that with the increased HRT, xylose degradation increases. Similar findings were reported by Oon et al. (Y. Oon et al., 2018) and he found that with increasing HRT from 1 day to 2-day color removal increase by 15 %.

- **Effect of MFC Elements**

The selection of anode and cathode material also affects dye decolorization greatly. Ilamathi et al. (Ilamathi and Jayapriya, 2017) have reviewed many anode and cathode material and found that carbon-based material is mostly used such as carbon paper, carbon felt, etc. Cao et al. (Cao et al., 2017) has used a graphite rod as anode and stainless steel ring with carbon fiber as the cathode and investigated the degradation of Reactive Brilliant Red X-3B dye successfully in stacked MFC and observed enhanced decolorization of 29 % than a single unit. With the increased surface area of the anode, dye degradation increases as it provides more surface for the anodic reactions.

1.4 PROBLEM STATEMENT

Dependency on non-renewable fossil fuels is the main cause of the energy crisis and global warming and environmental pollution to a very high level. Therefore, in recent years demand for sustainable energy such as solar and wind energy has increased tremendously(Ahmadpour et al., 2020; Jaiswal et al., 2020). MFC has emerged as a green technology that can serve both as a sustainable energy source and waste treatment technology. However, these technologies have limitations such as high internal resistance, energy losses, cost of construction, and difficulty in scaling up (Khater et al., 2018; Logan, 2009). If MFC is combined with dye treatment then the overall efficiency of the process can be increased. Dye treatment using chemical or physical methods produces a very large amount of secondary waste which ultimately increases the overall cost of the process. Biological methods for dye treatment are more popular because it is

economical and ecofriendly. But these biological processes have low removal efficiency for dye treatment, therefore when it is combined with MFC technology, overall dye removal efficiency, and hence economical viability of the MFC process may be increased.

1.5 SIGNIFICANCE OF THE RESEARCH

The energy demand of the world is increasing continuously and most of the conventional processes used for the production of energy are also responsible for the deterioration of the environment. Therefore, research is continuously going on to search for an eco-friendly alternative to conventional energy sources and economical waste abatement technologies. MFCs in the future may be an alternative solution to sustainable and green energy from waste utilization. Therefore, MFCs are not only providing a green source of energy but also treat the waste with very good efficacy in a very economical manner without the generation of secondary pollutants.

In the present study, an attempt has been made to degrade dye waste in Microbial Fuel Cell (MFCs) along with the production of electricity.