Literature Reviews

Chapter-2

CHAPTER 2

LITERATURE REVIEW

2.1 Dyes and their history

Dyes are the substance that imparts color and is used by many industries such as textile, pulp and paper, leather, distillery, food, cosmetic, and pharmaceutical industries. The practice of using dyes began thousands of years ago. Before the discovery of synthetic dyes, most of the dyes derived from a vegetable or animal source were used for coloring purposes. The dyes derived from natural sources are known as organic/natural dyes. They are ecofriendly and biodegradable but they cannot fulfill the huge demand by different coloring industries. Their production has also been reduced significantly due to the overexploitation of natural sources and high cost as compared to synthetic dyes. The use of synthetic dyes began in the late 19th century and at present covered most of the market of coloring industries by replacing natural dyes (Fernando, 2014; Natarajan et al., 2017; Saini, 2017). Synthetic dyes are artificially produced by industries on a large scale. Synthetics dyes are more popular than organic dyes due to their easy availability, low cost, and bright sheds (Ji et al., 2020; Oliveira et al., 2020; H. Wang et al., 2019). Dyes bind to the fabric, paper, etc either by a covalent bond or by physical adsorption. Therefore, it must be completely or partially soluble in the medium before application on fabrics. Generally, dyes are color compounds and characterized by their property of absorbing light in the visible region (400-700 nm), with at least one chromophore group (the group responsible for color), structure with conjugate system i.e. double or single bond, and with resonance structure (gives stability to organic compound)(Carreon-Valencia et al., 2010)(Carreon-Valencia et al., 2010). Chromophore group is the unsaturated group (acceptor of electrons)like keto (>C=O), Azo (-N=N-), nitro ($>NO_2$), ethylene group (=C=C=), nitroso (R-N=O), etc which shows characteristics absorption of light in the visible range and thus gives colors to the dyes. Along with chromophore dyes consists of auxochromes which alone cannot produce color but when present alongwith chromophore intensifies the color of dye by intensifying the light-absorbing property of chromophore.These auxochromesare electron donor group consists of a functional groups such as carboxyl group (–COOH), sulfonate (SO_3H), amine ($-NH_2$), and hydroxyl groups (-OH)which is responsible for their binding property to the substrate on which it is applied(Fang et al., 2018; Misal and Gawai, 2018; Saini, 2017).

2.1.1 Classification of dyes

Dyes are classified based on origin, application, and chemical structure. (Chowdhury et al., 2020; Ghodake et al., 2011; Ji et al., 2020).

- **Based on origin**

- **Natural Dyes**

These are derived from vegetable or animal sources such as bark, leaves, roots wood, and biological sources such as fungi and lichens, shellfish, mineral origin, etc. These are costly due to their limited source, non-availability in ready-to-use form, and their non-reproducible shades. These dyes are generally used by the food and pharmaceutical industries. Example are:

Plant origin dyes: Indigo (Blue Dye, Very Popular), Alizarin/ Madder (Red Dye), Safflower (Red), Turmeric (Yellow), Saffron (Yellow), Marigold (Bright Yellow), Flame Of Forest (Orange)

Animal origin dyes: Tyrian Purple (Violet), Cochineal (Red), Kermes (Crimson Red)Mineral Origin: Cinnabar, Red Ocher, Yellow Ocher, Malachite, Ultramarine Blue, Gypsum are responsible for mineral origin dyes.

Microbial and Fungi origin dyes: There are some bacteria which produces secondary metabolites as coloring substance. These dyes have a complex chemical structure and are classified based on their chemical structure such as Indigoid, Anthraquinone, Napthoquinone, Benzoquinone, Flavanoid, Carotenoid, and Tannin based dyes.

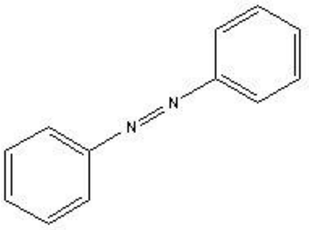
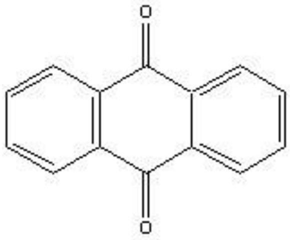
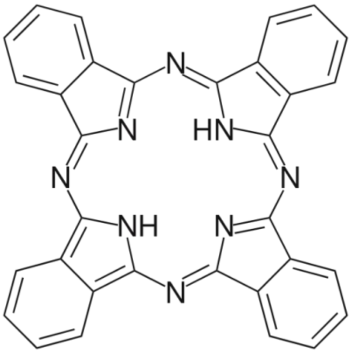
- **Synthetic Dyes:**

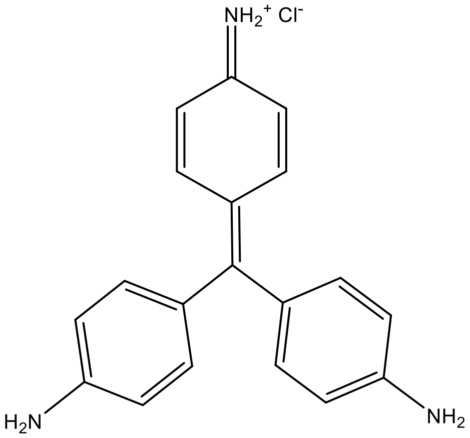
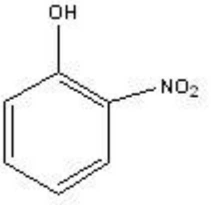
Almost all the color we see in our day-to-day life corresponds to synthetic dyes. There are 10,000 different synthetic dyes available commercially with a very high production rate of 7×10^{5} tons of dye per year. Examples of such dyes are acid dye, basic dye, vat dyes, etc. which we can see everywhere from dying fabric to paper or wood or any other day-to-day use material (García-Martínez et al., 2015; Natarajan et al., 2017; Tantak and Chaudhari, 2006). Unlike natural dyes, synthetic dyes are manmade compounds, and therefore, not easily biodegradable when inter in the environment through different routes. For their proper treatment, it is necessary to know the type of dye in the waste stream so that they can be remediated based on their chemical and physical properties. Classification of synthetic dyes is as follows:

❖ Based on Structure of Chromophore

Dyes are classified based on their chromophores group like keto (>C=O), Azo (-N=N-), Nitro ($>NO_2$), Ethylene group (=C=C=), Nitroso (R-N=O), Sulfur (SO_3H), etc. which are responsible for color and stability of dyes. Among all these dyes, 80 % of dyes belong to the Azo class (Dai et al., 2020b; Misal and Gawai, 2018; H. Wang et al., 2019). In Azo dye, one or more than one Azo bond (–N=N–) is present and is generally attached with the aromatic ring. After the Azo dye, the next class of dye which is abundantly used is Anthraquinone (Disperse Red 60), Phthalocyanine dyes, and Triarylmethane(Lucas et al., 2007; Ramya et al., 2010; Srikantan et al., 2018). **Table 2.1** shows examples of different dyes.

Table 2.1 Classification of dyes based on chromophore group

Class	**Chromophore**	**Examples of dyes**
Azo		Methyl Orange Disperse Orange 1 Congo Red
Anthraquinone		Disperse Red 9 Acid Green 25 Ramazol Blue
Polythyocynanin		Direct Blue 86

Triaryl methane		Basic Blue 1
Nitro		Disperse Yellow 14 Acid Yellow 1

❖ Based on affinity towards material

Based on the charge present on the materials, these dyes are further classified as cationic, anionic, and nonionic.

- **Cationic dyes**

These dyes break down in the aqueous solutions into positively charged ions and are also called basic dyes. They usually interact with a negative group of the fiber to form salt and thus get attached to the fiber firmly and produce color to the fiber(Saini, 2017; Sivashankar et al., 2014). Examples are Basic Red 29, Basic Yellow11, Brilliant Red B, Cationic Black L, etc. Generally, these dyes contain a sulphonic group that has a strong affinity for water, and hence these dyes are strongly water-soluble. They are mostly used for dyeing polyester and acrylic-type fibers, wood, paper, leather, and silk(Buthelezi et al., 2012; Saini, 2017).

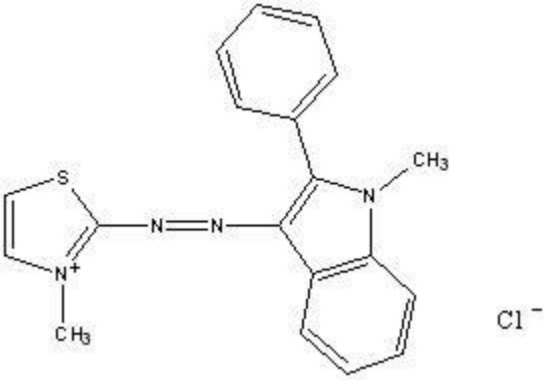
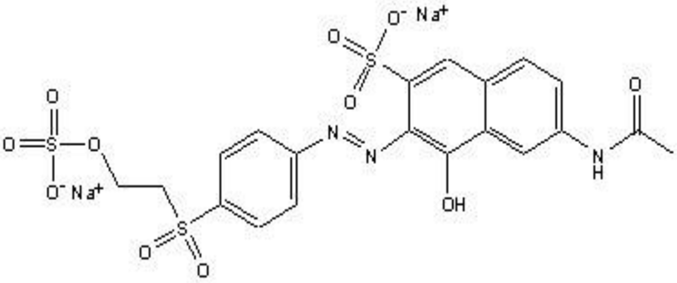
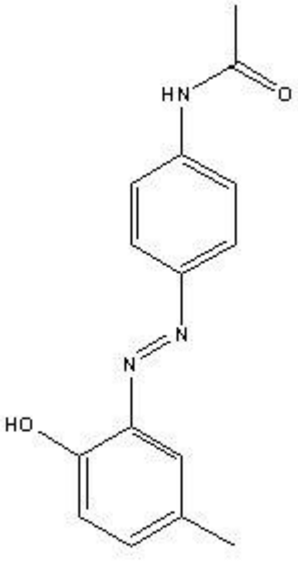
- **Anionic dyes**

Acidic, reactive, and direct dyes fall into the category of anionic dyes and interact with a positive group of fiber. Applications of anionic dyes are mainly in the printing, textile, pharmaceutical, and food industries. Examples are Methyl Orange, Reactive Orange 16, Reactive Red 120, etc.(Liu et al., 2015; Svobodová et al., 2007).

- **Nonionic dyes**

The dyes used to impart color to hydrophobic fibers such as nylon, polyester, acetate, and plastic are known as nonionic or disperse dyes. Due to their hydrophobic nature, they have low solubility in water generally less than 1 ppm. A dispersing agent is used for making the solution of these dyes. They get attached to the fiber using Van der Waals force or dipole forces. They generally contain hydroxyalkyl, $–NO_2$, and –CN group-based chromophores. Examples are Yellow3, Yellow54, Orange25, Red1, And Red4, etc. (http://www.pburch.net/dyeing/disperse_dyes.shtml).

Table 2.2 Examples of dyes based on affinity towards material

Dye	Name	Structure
Cationic dyes	**Basic Red 29**	
Anionic dyes	**Reactive Orange 16**	
Nonionic dyes	**Yellow 3**	

❖ Based on application methods

Another important classification of dyes is based on the technology of the application on a substrate rather than their method of synthesis and chemistry. These are mostly classified as reactive, vat, disperse, acid, basic, direct, azoic (naphthol), sulfur, and premetallized dyes(Gregory, 1990).

- **Reactive dyes**

As the name suggests, these dyes have a reactive group that reacts with the fiber by covalent linkage. Reactive dyes are anionic in nature and water-soluble. These are very popular due to their good fastness, brilliant colors, ease of application, and low cost. They are generally used for dyeing cellulose fibers including wool. Examples of reactive dyes are Reactive Orange 16, Reactive Red 135, Reactive Blue 15, etc. (Martinez et al., 2017).

- **Vat dyes**

They are water-insoluble dyes and they have to be dissolved in water before applying to cellulose fiber. The dissolving process takes place in a large container in presence of NaOH and sodium hydrosulfite salt at 50ºC. Once they get solubilize, behave as direct dyes for dying cotton fibers. Generally, these dyes are the class of indigo and anthraquinone groups. Examples are Vat Red 10, Vat Violet 13, And Vat Orange 1 (Roessler and Crettenand, 2004).

- **Disperse Dyes**

These are the nonionic dyes (free from the ionizing group) and slightly water-soluble which are used for dyeing polyester and acetate fibers (hydrophobic fibers). They exist in water in dispersed form using a dispersing agent. The molecular size of these dyes is the smallest among all dyes. Generally, these are derivatives of Azo, Anthraquinone, Nitro, and Quinone groups. Examples are Dispersed Red 1, Disperse Orange 37, etc. (https://textilelearner.blogspot.com).

- **Acid dyes**

Acid dyes are highly water-soluble and anionic. These dyes contain sulphonic acid groups usually present as sodium sulphonate salts and are responsible for their high solubility in water. After dissolving in water, acid dyes with the positively charged fiber molecule with an ionic bond, H-bond, and Van der Waals bonds. For this, the dyeing process takes place in an acidic environment to protonate fiber. Dyeing of silk, wool, nylon, and modified acrylics takes place with these dyes. Anthraquinone, Azo, and Triphenylmethane are the main class of these types of dyes. Acid Yellow 17, Acid Blue 25, etc. are sodium salt of organic bases

(https://textilelearner.blogspot.com/2012/01/acid-dyes-properties-of-acid-dyes_21.html).

- **Basic dyes**

Basic dyes are water-soluble cationic dyes in presence of alcohol or methylated spirit and are mainly used to dye acrylic fibers. In water, basic dye acquired a positive charge and react with negatively charged fibers. Chromophore in basic dye molecules contains a positive charge. Examples of basic dyes are Basic Red 29, Basic Green 1, etc.

- **Direct Dyes**

They are alkaline dyes and are applied to the fabric in neutral or alkaline conditions. These dyes are attached to the fiber with H-bond due to which they have poor fastness and discharge into the water in a large amount. They are Azo dyes and are used to dye cotton, wool, and silk, etc. The Wash fastness of these dyes is poor but it may be improved by after-treatment. Examples are Direct Red 81. Congo Red (Direct Red 28).

- **Azoic (Naphthol) dye**

These are Azo group (-N=N-) dyes insoluble in water due to which they have very good wash fastness. They are not found in readymade form. They are produced by a reaction between two components namely Naphthol and diazo compound or diazo base or diazo salt during the dying process in water. These dyes are less popular due to difficulty in application and the ability to impart limited sheds. These are generally used for dying natural cellulosic fibers.

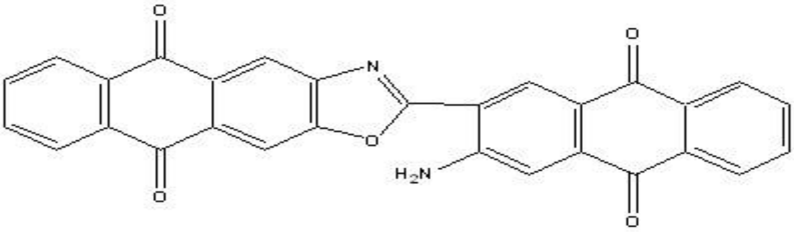
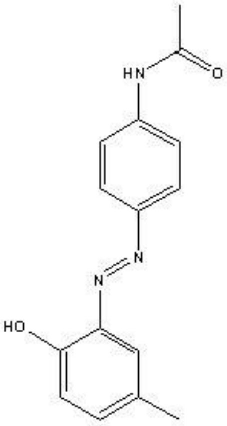
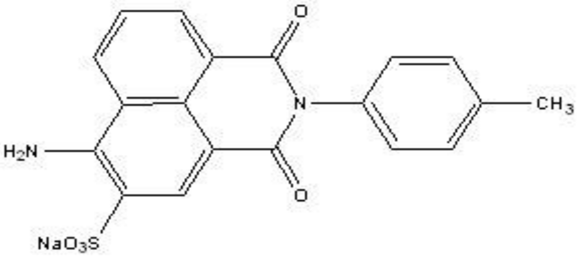
- **Sulfur dye**

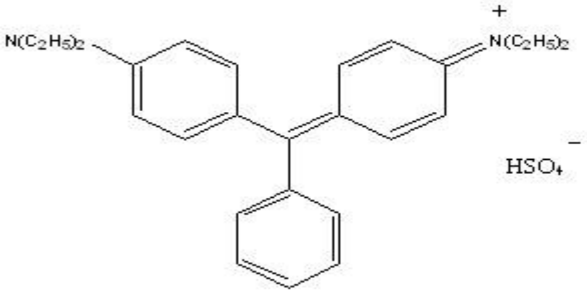
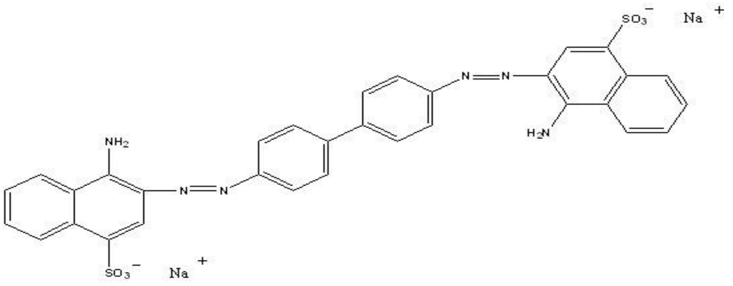
Sulfur dyes are insoluble in water but upon treatment with alkali, they form soluble compounds. These dyes are used for coloring cotton, jute, and flex upon solubilization. Limited sheds are available.

- **Premetallized dye**

Metal complex dyes are known as premetallized dyes. These dyes are treated with the metal salts of copper and chromium on the surface of the fabric to increase the washing fastness. The role of metal is to form a complex with dye already present on the surface of the fabric.

Table 2.3 Examples Based on application methods

Dye	Name	Structure
Reactive dyes	Reactive Orange 16	
Vat dyes	Vat red 10	
Disperse Dyes	Yellow 3	
Acid dyes	Acid Yellow 7	

Basic dyes	Basic Green 1	
Direct Dyes	Congo Red	

2.1.2 Potential threats of Azo dyes to the environment and their treatment technology

The release of a variety of dye-containing wastewater from different industries is a serious concern because they are widely used and deteriorate our environment. The waste stream from various coloring and dye manufacturing units may contain many organic and inorganic materials. Even a very small amount of dye (< 1 mg/L) can be recognized by the naked eye and so they are not aesthetically acceptable. This wastewater is characterized by high chemical oxygen demand (COD), biochemical oxygen demand (BOD), a large number of suspended solids, unbound colorants, and dye impurities(Cao et al., 2017; Chouler and Di Lorenzo, 2015; Paul et al., 2011; Tizaoui and Grima, 2011).

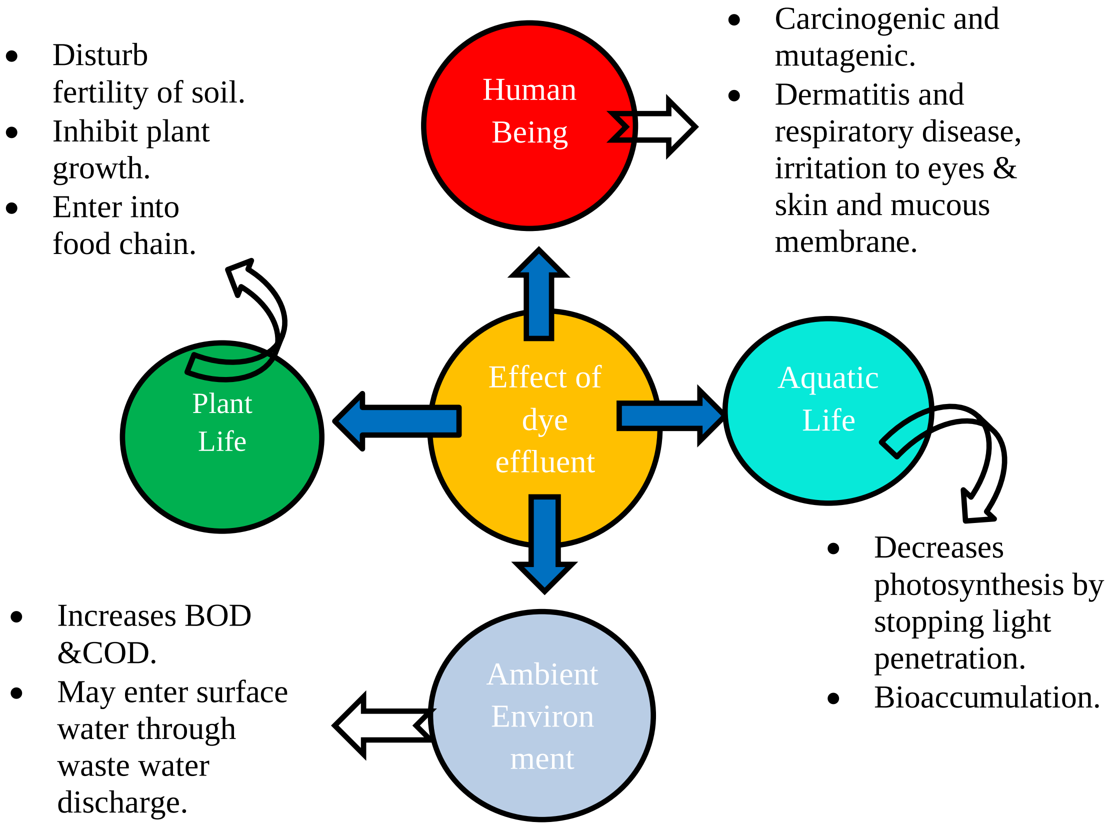


Figure 2.1 Effects of dye wastewater

Out of total dye release in the waste stream, 80 % of dyes belong to the Azo group (-N=N-) (Fang et al., 2017; Muniyasamy et al., 2020). Hence, they are the largest group responsible for the waste generated from different industries such as textile, printing, pulp, and paper, tanning, etc. During the dying process in various industries, fixation on colors is usually incomplete, resulting in the generation of a large amount of dye wastewater (Carvalho et al., 2020; Zhu et al., 2020). According to reported data, approximately 0.7 million tons of dyes are produced each year of which 10% to 15% are directly released into the wastewater (Guo et al., 2020; Misha et al., 2020; Ramya et al., 2010). **Table 2.4** shows the typical data for the dye effluent from the textile industry and **Table 2.5** shows the data of effluent to be discharged to the environment.

Table 2.4 Characteristics of textile industry effluent(Bhatia et al., 2017)

S.No.	**Characteristics of textile industrial effluent**	**WHO range for inland surface water body**	**Common effluent treatment plant (Ministry of Environment and Forests, Government of India**
1	pH	5.5-9.0	5.5-9.0
2	Temperature	Not exceed 5°C above the temperature of receiving water body	Not exceed 40°C in any part of the receiving water body within 15 meters downstream from the outlet of effluent
3	Total dissolved solids (mg/L)	2000	2100
4	Total suspended solids (mg/L)	100	100
5	Biological oxygen demand (mg/L)	30	30
6	Chemical oxygen demand (mg/L)	250	250
Trace metals (mg/L)			
7	Iron (Fe)	3	3
8	Zinc (Zn)	5	5
9	Copper (Cu)	3	3
10	Arsenic (As)	0.2	0.2
11	Nickel (Ni)	3	3
12	Fluoride (F)	2	2
13	Manganese (Mn)	2	2
14	Vanadium (V)	-	0.2

15	Cadmium (Cd)	2	1
16	Mercury (Hg)	0.01	0.01
17	Total residual chlorine (Cl)	1	1
18	Cyanide (CN)	0.2	0.2
19	Total Chomium (Cr)	2	2
20	Oil and grease	10	10
21	Phenolic compounds	1	1

Table 2.5 International standard of dye effluent discharge into the environment (Katheresan et al., 2018)

Factors	**Standard allowed**
BOD	<30 ppm
COD	<50 ppm
Color	< 1 ppm
pH	6-9
Suspended solids	<20 ppm
Temperature	<42
Toxic pollutants	Not allowed

If dye-containing wastewater is discharged without proper treatment, they surely contaminate our landfills, groundwater, and aquatic system, which not only affect human being but also animal, plant, and aquatic life by entering into our food chain (Buthelezi et al., 2012; Kardi et al., 2017; Misha et al., 2020). Dyes have an acute and chronic effect on living beings. Dye effluents when entering into natural water sources cause foul odor and eye soreness, skin irritation, nausea, vomiting, breathing difficulty, and some of them are reported to be carcinogens also. When they enter into natural water sources, they increase the turbidity of water by forming a visible layer on the surface because of their lower density of 0.8 kg/m^3 compared to water1kg/m^3(Katheresan et al., 2018). The presence of untreated dyes in the water body hinders the photosynthesis process by blocking the light penetration and decreasing the solubility of oxygen. Azo double bonds in Azo dyes are linked to aromatic amines which are reported as a carcinogen for humans and animals and also aesthetically unacceptable for day-to-day use purposes such as washing, drinking and cleaning (Oliveira et al., 2020; Quan et al., 2018). Therefore, decolorization of dye wastewater is necessary before its discharge into an open environment. A successful and ideal dye removal technique should remove a large amount of dye from waste without generating hazardous secondary waste.

2.1.3 Current treatment technology for dye degradation

A large number of conventional dye removal methods based on physical, chemical, biological processes are reported in the literature. All these methods have their advantages and disadvantages associated with them. Dye wastewater treatment technology is mostly studied worldwide because a single specific treatment is not enough for treating a broad variety of dye effluent coming from industries. Continuously research is ongoing on different treatment technology in the modification of physical, chemical, and biological methods for enhanced dye degradation.

Chemical and physical processes are highly efficient but they require the addition of some chemicals or energy for dye degradation and leads to the generation of secondary waste (Fang et al., 2018; Fernando et al., 2014a; Sharma and Kaur, 2018). The secondary waste requires additional treatment which increases the overall cost of the process. Chemicals that are added during chemical treatment require regeneration if possible, which increases the time and cost of the process. The biological methods have several advantages over physical and chemical methods and may be an alternative to them. The important advantages of biological processes are their cost-effectiveness, eco-friendly nature, and no generation of secondary pollutants during treatment. However, biological methods also have the following limitations which need to be addressed by the scientific community before its wide-scale application for dye remediation

- In biological methods, a live organism is used; they have their tolerance limit for dye, so a very high concentration removal of dye with biological is a problem.
- The biological processes are not as reliable as physical and chemical processes due to the use of living organisms.
- The slow nature of biological processes for dye remediation is also a challenge for researchers.

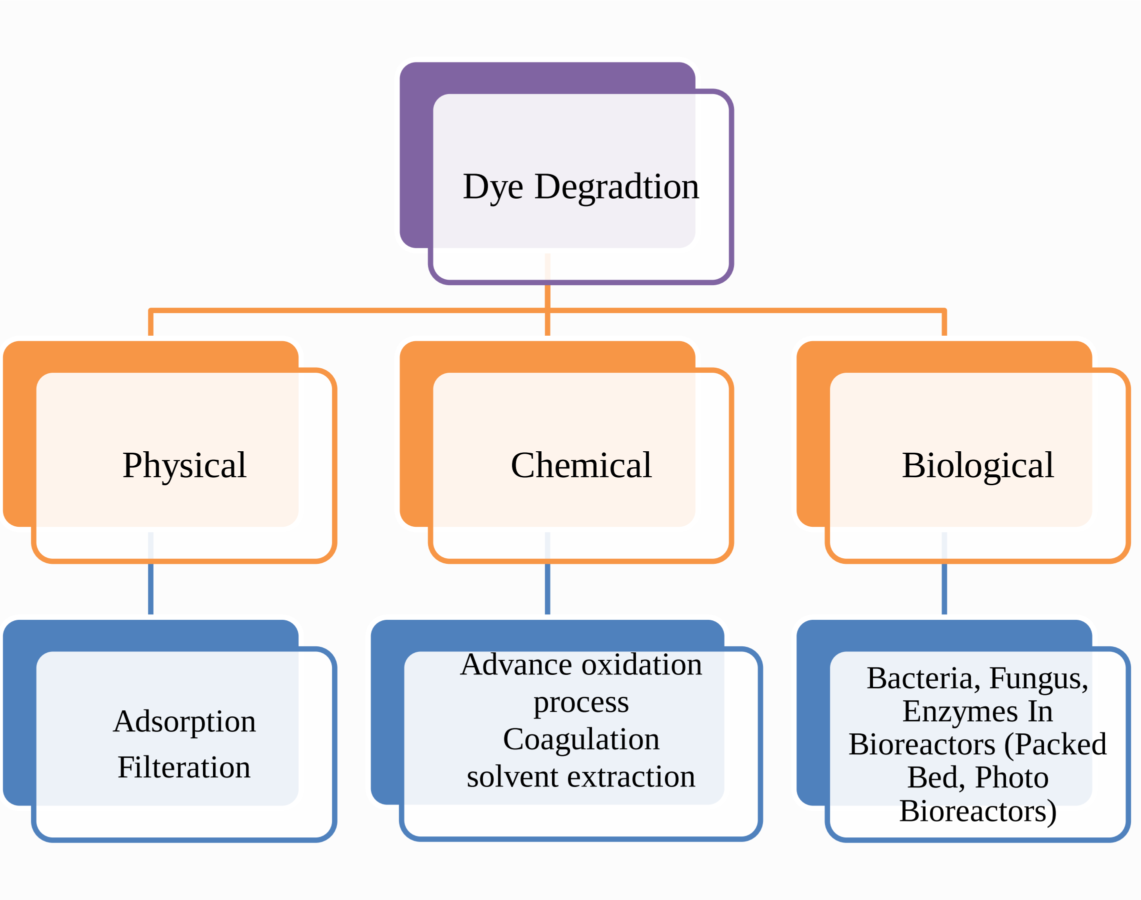


Figure. 2.2 Dye treatment technology

- **Physical method**

- **Adsorption**

It is a physiochemical method of treatment of dye that is popular due to its simplicity and availability of low-cost adsorbent. Various natural and man-made adsorbents are used for dye removal. Activated carbon is the most commonly used adsorbent but the generation of a large amount of sludge and their low efficiency towards some ionic dyes have limited their use for dye removal (Sharma and Kaur, 2018). Daud et al. (Daud et al., 2019) have reported new modified adsorbent hybrids of layered double hydroxide (LDH) for the removal of toxic dyes and this adsorbent is a non-toxic low cost, and readily available. Biswas et al. (Biswas et al., 2020) have successfully removed methylene blue dye using sugarcane biogas biochar and alginate composite adsorbent. Priya et al. (Priya et al., 2020) have used a hybrid technique of photocatalytic degradation along with natural adsorbent for the enhanced degradation of Crystal Violet dye. Many different adsorbents such as metal hydroxide sludge, fly ash, coal ash, amide-functionalized cellulose-based porous adsorbent, activated carbon obtained from natural sources, Activated carbon pellet, activated carbon from a bamboo shoot, etc have been reported by many researchers as efficient adsorbents for removal of varieties of dyes(Hou et al., 2020; Hui et al., 2020; J. Liu et al., 2020; Tyagi, 2020). The method of adsorption has a demerit that it has a low capacity of adsorption. For the high removal capacity, the ion-exchange method was found very effective which removes a high concentration of dye at a very low dosage of ion exchange resin. Dyes that are not removable through activated carbon-like adsorbents particularly ionic dyes are removed with the ion-exchange method. There are several published works on the application of the ion-exchange method for the removal of dyes. Wu et al.(J. Wu et al., 2020) has used magnetic resin as an advanced adsorbent material for the removal of acid dyes. Shabaan et al. (Shabaan et al., 2020) has used a multi-wall carbon nanotube for the removal of cationic and anionic dyes and found that 98 % of dyes (10 ppm) removed successfully. But the cost of the ion exchange adsorbent is very high which limits their use for wide applications (Anantha et al., 2020; Priya et al., 2020; Yagub et al., 2014).

- **Filtration**

Some researchers have developed a semi-permeable membrane by adding some adsorbent to increase the separative property of the membrane for dye removal from wastewater and successfully used to filtrate dye from wastewater(Buyuktuncel and Bektas, 2001; Han et al., 2007; Y. Wu et al., 2020). For this dye, wastewater should be pre-treated to remove suspended particles. Ultrafiltration, nanofiltration, and reverse osmosis are some techniques that are employed for the treatment of wastewater(Abdi et al., 2018; Ma et al., 2016; Sahinkaya et al., 2018)**.** This method is costly because high pressure is used for filtration and the cost of membrane preparation is also high(Abdi et al., 2018; Li et al., 2019)**.**

❖ Chemical methods

- **Advanced oxidation process**

This is a traditional method for the removal of dyes. In this, a strong oxidizing agent such as H_2O_2 Fenton’s reagent (H_2O_2 + Fe^{2+}), Cl_2 is used to generate hydroxyl radicals which oxidized dyes into CO_2, H_2O, and inorganic salts by attacking the chromophore group of the dye. These radicals are produced using the oxidizing agent in the presence of a catalyst or UV radiation, due to which they are classified as non-photochemical (only H_2O_2, Ozone, Cl_2) and photochemical (UV/H_2O_2, O_3/UV, $UV/O_3/H_2O_2$, $Fe^{3+}/UV/H_2O_2$). This method is less costly than other methods but has a disadvantage of the generation of sludge or the use of high electricity for O_3 generation in ozonation. In case of incomplete oxidation, the process may also produce toxic intermediates. Ozone is a much powerful oxidizing agent than H_2O_2 and Cl_2, therefore, the Ozonation method was reported by many researchers for the removal of different dyes (Atalay and Ersöz, 2015; Jegan Durai et al., 2020; Nidheesh et al., 2018; Sharma and Kaur, 2018).

- **Coagulation**

This method is very popular due to its low cost and less possibility of the generation of toxic intermediates. In this Al^{3+} or Fe^{3+} based salt such as Aluminum sulfate, aluminum chloride, ferric chloride, ferric sulfate, calcium oxide, and magnesium oxide are used. These are very low-cost chemicals and flocs are formed by the neutralization of cation (Al^{3+} or Fe^{3+}) and anionic dyes. The only disadvantage associated with these methods is that they cannot be used for low molecular wt, high soluble and cationic dyes, also sludge produced due to floc is very high(Moghaddam et al., 2010; Pavithra et al., 2019; Shi et al., 2007).

- **Solvent extraction**

It is a new technique that is used for the removal of dye from textile industries where high purity is required. It can be solid-liquid or liquid-liquid extraction, and for this, a solvent is used which is immiscible in the wastewater. The principle involved in this process is the distribution coefficient of dye should be high for solvent than water. A different solvent such as benzene, xylene, toluene, chloroform, and hexane has been reported by many researchers(Muthuraman et al., 2009; Pandit and Basu, 2004; Sharma and Kaur, 2018; Sonawane et al., 2017). Sonawane et al. (Sonawane et al., 2017) had successfully removed crystal violet using Tributyl phosphate (TBP) as an extractant and 1-Dodecanol as a diluent from textile water. Similarly, Muthuraman et al. (Muthuraman et al., 2009) had reported the removal of Methylene Blue using benzoic acid as a solvent. Unlike dye degradation, this method recovers dye from wastewater. This method results in high purity dye and ease of operation which are the main highlighted advantages. The only disadvantage associated with this method is the separation of dyes from the solvent which required the addition of energy. So, it is an energy-intensive process. **Table 2.6** shows a review of most of the chemical and physical processes used for dye degradation along with their advantages and disadvantages.

Table 2.6 Advantages and disadvantages of various physical and chemical methods (Chowdhury et al., 2020; Parmar and Shukla, 2018; Priya et al., 2020)

S. No.	Dye degradation method	Different stages of treatment	Advantages	Disadvantages
1	Adsorption	Pre/post-treatment	High removal efficiency for a wide range of dyes	Costly, regeneration is required, large sludge production
2	Irradiation	Post-treatment	High removal of a wide variety of dyes	High Dissolved oxygen is required, Ineffective for light-resistant dyes
3	Coagulation and precipitation	Pre/main treatment	Fast and low capital cost	The cost of coagulant is high, dewatering and sludge management are problematic.
4	Membrane filtration	Main treatment	removal of a wide variety of dyes	Cost of the membrane, membrane fouling, low volume of treatment
5	Electrochemical	Pre-treatment	The non-toxic compound is produced during degradation	The high cost of electricity

6	Advanced oxidation	Pre/ main treatment	Degrade both soluble and non-soluble dyes	Large sludge is produced
7	Ozonation	Main treatment	Degrade most of the dyes	The high cost of electricity, not target specific

❖ Biological methods

The biological method of dye treatment is an attractive, eco-friendly, and cost-effective method, and also sludge production is less compared to a chemical and physical process. In biological techniques mainly microbial species are used for the degradation of dyes. These methods are versatile because of the genetic diversity of microbial species due to which the biological techniques can be developed to treat a different variety of dyes. They partially or completely degrade dye to stable and non-toxic compounds. Complete degradation leads to the formation of water, CO_2, and salts (inorganic in nature) which are non-toxic. The advantage with microbial-based techniques is that the microbial species can acclimatize and change themselves according to the nature of pollutants and resultant may new and more efficient strains that degrade toxic dyes to non-toxic compounds. Dye remediation can be achieved through different mechanisms such as microbial degradation, biosorption, and bioaccumulation using living or dead biomass using different microorganisms such as fungus, algae, yeast, and bacteria(Parmar and Shukla, 2018; Pavithra et al., 2019; Zuraida et al., 2013).

• Biosorption and Bioaccumulation

When a pollutant (dye) gets concentrate or bind on the cellular surface of dead biomass then the process is called biosorption (adsorption) and when the pollutant gets bind and accumulated inside the cell of a living microorganism then it is called bioaccumulation (absorption). Biomass could be bacteria, fungi, yeast, algae, seaweeds, and even industrial or agricultural wastes (Chojnacka, 2010; Daneshvar et al., 2012; Waghmode et al., 2019). This technology is most preferred when the dye is non-biodegradable. This technology is low-cost to physical adsorption, precipitation, and reverse osmosis(Arica and Bayramoglu, 2007; Buthelezi et al., 2012; Solís et al., 2012). Biosorption is more practiced and popular than bioaccumulation because dead biomass used in biosorption does not need any nutrient for their growth and maintenance and rate of removal is also high, whereas living organism in bioaccumulation requires nutrient, and growth needs time, so this is a slower process than biosorption. The toxicity of the dye for living microorganisms is another factor in bioaccumulation, which makes biosorption a popular method for the removal of non-biodegradable dyes. In biosorption, only biomass cell surface is available whereas in the case of bioaccumulation pollutant gets firstly adsorbed and then transported inside the cell structure, hence the overall binding site for a pollutant is more in case of bioaccumulation than biosorption. The biosorption capacity depends on many factors such as pH, temperature, type of biomass adsorbate, and presence of other competing pollutants (Chojnacka, 2010; Das and Charumathi, 2012; Solís et al., 2012). Mona et al. (Mona et al., 2011) had successfully removed 94 % Reactive Red 198(100 ppm) dyes using cyanobacterium, Nostoclinckia HA 46 with an uptake capacity of 93.5 mg/g. Daneshvar et al. (Daneshvar et al., 2012) have reported biosorption of Acid Blue 25 (AB25), Acid Orange7(AO7), and Acid Black1 using Brown macroalgae *Stoechospermummarginatum* with an adsorption capacity of 22.2, 6.73, and 6.57 mg/g respectively.

- **Microbial degradation (bioremediation)**

In the context of the present research, bioremediation is important because dye degradation in microbial fuel cells takes place using the bioremediation process.

Bioremediation is a process in which the natural capability of live microorganisms is utilized for the degradation of waste into less toxic or non-toxic components. They are mainly of two types aerobic and anaerobic. In context to the Azo dye degradation, degradation takes place by the breakdown of the Azo bond to a less toxic compound than the parent dye. Azo dyes are electron-deficient compounds due to the presence of (−N=N−) chromophore along with many electron-withdrawing groups which may interrupt or decreases the degradation process by other methods (Parmar and Shukla, 2018; Sarkar et al., 2017; Yien et al., 2019). Since the single bacterial species can produce different enzymes in different metabolic pathways which makes the bacterial process is highly efficient for Azo dye degradation. Since the Azo dye has complex nature, so degradation of these dyes takes place in two steps : **(i)** breaking of Azo bond anaerobically to colorless aromatic amines **(ii)** further these aromatic amines are catabolized aerobically to non-toxic compounds(Bhatia et al., 2017; Parmar and Shukla, 2018)**.** Therefore, the combination of aerobic and anaerobic biological treatment has shown a promising way for the complete mineralization of dyes(Katheresan et al., 2018; Sarkar et al., 2017). Microbial degradation involves a wide range of microorganisms from bacterial culture, yeast, or fungal culture to any consortia.

Bacteria can degrade dye aerobically or anaerobically to a significant level. Anaerobic or facultative microbes are more efficient and fast for Azo dye degradation. Azo dyes are degraded through reduction to colorless aromatic amines and then these amines are metabolically oxidized to electrophilic species which get bind to DNA through covalent bonds irreversibly(Fernando et al., 2014a; Oliveira et al., 2020; Sarkar et al., 2017). Kiayi et al. (Kiayi et al., 2019) have reported degradation of Carmoisine dye (50 ppm) using *Saccharomyces cerevisiae* ATCC 9763 in 7h of operation under anaerobic shaking conditions. Enzymatic degradation was also popular because a small quantity of enzymes is required for degradation. High potential for Enzymatic azo bond breakdown through a well-known azo reductase, laccase has been reported by many researchers. Peroxidase and oxidase enzymes have been reported for dye degradation up to a certain extent (Ghodake and Jadhav, 2011; Ramya et al., 2010; Solís et al., 2012). Razo-Flores et al. (Razo-Flores et al., 1997) found complete mineralization of Azo dye under methanogenic conditions and claimed that Azo dye was first reduced to aromatic amines and then under anaerobic condition completely mineralized. Sarayu and Sandhya (Sarayu and Sandhya, 2010) had observed that in aerobic conditions aerobic azo reductase firstly cleaved the Azo bond and then break down products are further degraded through mono-oxygenase or di-oxygenase enzymes catalysis which is responsible for the incorporation of O_2 in the aromatic ring structure of dye before ring fission. Roy et al. (Roy et al., 2018) have observed successful complete degradation of crystal violet (150 ppm) dye using *Enterobacter sp*. CV–S1 in 72 h in aerobic conditions. Oliveira et al. (Oliveira et al., 2020) had used facultative bacteria *Klebsiella* strains a promising bacteria for complete mineralization of Direct Black 22. Many fungal and yeast species such as Saccharomyces cerevisiae, *Candida tropicalis* TL-F1, White rot fungus *Irpexlacteus* have been reported which have the capability of dye in aerobic conditions(Mahmoud, 2016; Neifar et al., 2019; Sameera and Padma, 2014; Svobodová et al., 2007; Tan et al., 2013).

The main disadvantage associated with the bioremediation of Azo dyes is that these dyes are reduced under anaerobic conditions results in the formation of aromatic amines which is a slow process and requires an electron donor (co-substrate) for its reduction. The addition of co-substrate could increase cost and unnecessarily methane production(Oon et al., 2017; Zee and Villaverde, 2005). There is a scope for further research to make the anaerobic degradation of dyes more efficient and viable from a cost point of view. In the present work, we have explored dye degradation in MFCs because of its several advantages over pain anaerobic degradation in bioreactors.

2.2 Microbial fuel cell (MFC) and its history

About a century ago in 1910, the first microbial fuel cell was introduced by Potter et al. (Potter, 1911). Due to very low power output, this finding was not well appreciated until the 1980s, when the idea of electron mediators came into existence which substantially improved the output power of MFCs (Hernández-Fernández et al., 2015b; Zhou et al., 2011). However, his work provided scope for new interdisciplinary research which includes the study of material of construction, design, biology, electrochemistry, electronics, and catalysis for MFC in the field of sustainable alternative energy from waste using microbes(Tommasi and Lombardelli, 2017; Yaqoob et al., 2020). A real breakthrough was made when some researchers found exoelectrogens (Cárcer and Ha, 2011; Dai et al., 2020) a special type of bacteria that can transfer electrons directly to the anode. Application of MFC for wastewater treatment increase greatly during the 1990s, especially when Logan et al.(Logan and Regan, 2006) and other researchers were successfully developed new MFCs for the treatment of municipal or industrial wastewater(Ahn and Logan, 2012; Liu et al., 2005; Logan et al., 2006; Logan and Regan, 2006). Theoretically, MFC technology exhibits the possibility of reaching conversion efficiency up to 50%. This technology is becoming popular as an alternative energy source and wastes remediation over conventional wastewater treatment methods due to its mitigated environmental impacts related to greenhouse gas emission and low sludge production(Ardakani and Badalians Gholikandi, 2020; Sotres et al., 2016). Low output power and long startup time is taken by bacteria for acclimatization and material cost are still drawbacks for the practical application of MFCs(H. Liu et al., 2020; Sotres et al., 2016). So there is a good scope for further research to improve the overall process efficiency of MFCs in terms of electricity production as well as waste remediation.

A Microbial Fuel cell (MFC) is a **bio-electrochemical** system where anode respiring bacteria have the capability of direct converting waste material into electricity. Microorganism plays a vital role in MFC because they metabolize waste and transfer electrons to the anode by an extracellular electron transfer mechanism (Dai et al., 2020; Suzuki et al., 2018). Different microorganisms like *Geobacter sp., Burkholderia, Desulfovibrion Pseudomonas sp., Desulfuromonas and Pseudoxanthomonas, Stenotrophomonas, Shinella, Petrimonas,* etc. have been reported good extracellular electron transfer capability in MFCs (Cárcer and Ha, 2011; Dai et al., 2020; Liu et al., 2005; Sotres et al., 2016; Zhang et al., 2019). Gandu et al.(Gandu et al., 2020) have used *Geobacter sulfurreducens* encapsulated in alginate beads and found the highest current density of 11.52 A/m^2 at a potential of 0.20 V in single-chambered MFC of working volume 80 ml. **Table 2.7** shows a review of the work done by other researchers in the area of MFC.

Table 2.7 Work done by other researchers in MFC

S. No.	References	Substrate	Microorganism	Proton exchange membrane	Electrode	Dimension of MFC	Results
1.	(Ahmadpour et al., 2020)	Reactive Black 5 (RB5) dye	Anerobic sludge	Nafion (29 cm^2)	AgBr/CuO hybrid photocathode 3× 3 × 0.5 cm	Photo-MFC dual-chamber 220 ml.	28.33 mW /m^2

2.	(Liu et al., 2020)	Acid Orange 7 (AO-7)	White rot fungus	Hydrogel (PVA) 99 cm^2.	Carbide porous ceramic 61.4 cm^2	Tubular mfc (1044.8 ml)	223mW/m^2
3.	(Gandu et al., 2020)	Acetate	Geobactersulfurreducens	-	Carbon-cloth (2.5 cm × 2.5 cm Platinum coated	Single chambered MFC (100 ml)	Highest current density 11.52 A/m^2 with 0.20 V
4.	(Aiyer et al., 2020)	Domestic water containing (acetate glucose, fructose, sucrose)	domestic waste water	Cation exchange membrane (7 cm^2)	Carbon cloth electrode (area 12 cm ^{2} cathodes) (0.50 cm^2 anode)	Multi-chambered mfc consisted of a central cathode (500 ml) chamber four anode (200 ml)	70.28 mW/m^2 with acetate
5.	(S. H. Liu et al., 2020)	Ethyl acetate	Activated sludge in	Polyvinyl alcohol hydrogen (25 cm^2)	Porous ceramic ring (pcr) (size c.1.3 cm dopped in molasses	Tubular microbial fuel cells (tube-mfcs)10 ×10 ×10 cm	186.5 mV
6.	(Khajeh et al., 2020)	Milk + Acid Orange 7	Activated sludge	Nafion N966 membrane	CuO/ZnO nanoparticle deposited graphite electrodes	Hybrid two-chamber MFCs(9 cm × 8 cm × 10 cm) (700 ml)	51.84 mW/m^2 at a current density of 144 mA/m^2
7.	(Gao et al., 2020)	Quinolone+ waste water	Sewage sludge	Nafion	Carbon cloth	Hybrid MFC single-chamber (500 ml)	1.0 V

8.	(Gajda et al., 2020)	Urine	Anaerobic activated sludge mixed with urine	Terracotta	Modified carbon fiber (22.5 cm^2)	Multistack MFC (cylindrical 50 mm, 22 mm inside and 30 mm outside) Total volume 5L	21.1 W /m^3
9.	(Kumar et al., 2020)	The sodium chloride concentration of 6 g/L	Sulfate-reducing bacteria	Nafion117 (6 × 6 cm^2)	Graphite sheets (81.9 cm^2)	dual-chamber Microbial fuel cell (7 × 7 × 7 cm^3)	1188 mW/m^3 was
10.	(Yousefi et al., 2020)	Raw wastewater from the septic tank	Waste water	Chitosan/Montmorillonite (CHI/MMT) nanocomposites deposited over ceramic	Carbon cloth and stainless steel (SS)	Double chambered	Power and current densities of 229.12 ± 18.5 mW/m^2 and 1422.22 ± 41.2 mA/m^2
11.	(H. Liu et al., 2020)	Congo red + Acetate	Anaerobic sludge	Nafion	Polydopamine decorated reduced oxide graphene on carbon cloth	Double chambered	988.1±5.2 mW/m^2

12	(Li et al., 2019)	Diesel	Microbes isolated from previous MFC	Nafion 9 cm^2	Carboncloth (6 cm×7 cm)	Dual chambered (600 ml)	Current density (61.76 mA/m^2 and power density16.02 mW/m^2 50% diesel (3.26 g/L) in maximum during 8 days
13	(Martinez and Di Lorenzo, 2019)	Food waste	Microbes present in food waste	-	Graphite felt (5×5 cm^2)	Multi stack Floating MFC (membrane less air cathode)	51 ± 2 mW/m^3
14	(X. Zhang et al., 2019)	Pretreated sludge supernatant	Sludge	Nafion 117 49 cm^2	Carbon felt 5 cm×2.5 cm	Hybrid MFC Working volume 500 mL	the power density of 8.15 w/m^3 at a current density of 18.05 A/m^3
15	(Bose et al., 2019)	Sewage wastewater	Waste water	Nafion 117	Anodeisa plain carboncloth (28.26 cm^2),activatedcarbon cathodeis Biomass-derived	Double-chambered	Current density and power density of 0.40 mA/m^2 and 110 ± 6.58 mW/m^2

16	(G. Sun et al., 2019)	Pyroligneous liquor	Anaerobic sludge	Cation exchange membrane (CMI-7000) 120 cm^2	Carbon felt for anode (3 cm × 8 cm) Carbon cloth (Platinum coated) for cathode	Two-chamber, air cathode reactors	Current density (1.94 A/m^2)
17	(Xie et al., 2018)	Synthetic wastewater	Activated sludge	-	Graphite	Single chamber membrane-less air cathode MFC coupled constructed wetland	0.59 W/m^3
18	(Mohanakrishna et al., 2018)	Petroleum refinery wastewater (PRW)	Sewage water	-	Graphite (75 mm height) (34 mm dia cathode & 75 mm height and 62 mm dia for the anode)	Cylindrical microbial fuel cell (MFC)	28.27 W/m^3
19	(Chen et al., 2018)	Sodium acetate	Inoculum from Previously acclimatized MFC	Anion-exchange membrane (AMI-7001)	Carbonized waste tires	Double chambered (45 ml)	A current density of23.1±1.4 A/m^2

20	(Xu et al., 2017)	Orange II (kept at cathode side)	Activated sludge	Nafion 117	carbon paper as anode and peptide nanotubes modified cathode	two-chamber MFC 27 ml (3cm × 3cm × 3cm)	15.5 mW/m^2
21	(Shen et al., 2017)	Dairy manure	Microbes present in manure	Salt bridge potassium chloride	Graphite rods	Two-compartment MFC (1100 ml)	212 mV The power density of 29.9 mW/m^2
22	(Daries Bella et al., 2016)	Glucose	Pseudomonas aeruginosa	Nafion (1.5 cm×1.5 cm)	Carbon felt of dimension 2.5cm×2.5cm was used as anode and cathode.	Dual chambered (3cm×3cm×4.5 cm) Working volume = 27 ml	The maximum power density of 7.97 W/m^3 at 1.92 A/m^3.
23	(Hassan et al., 2016)	Dichlorophenol	Bacillus subtilis	Gel polystyrene cross-linked with divinylbenzene membrane	carbon cloth (3.0cm ×3.0cm)	Dual-chambered (250 mL)	The current density of 64.0 mA/m^2
24	(Jayashree et al., 2016)	Seafood processing wastewater	Sludge from pre-acclimatized	Nafion	Activated carbon fiber felt	Tubular upflow (L=19 cm, b=15 cm, d=6 cm	Tubular upflow (L=19 cm, b=15 cm, d=6 cm

			MFC that				
25	Guo et al. 2016	Petroleum refinery	Activated sludge	Nafion	carbon rod (0.6 cm ×18 cm)	Dual – chambered 400 mL	(12.23 W/m^2)
26	Boas et al., 2015	Dairy industry effluent	Lactobacillus	Nafion	Carbon paper	Dual chambered(1000 ml)	The maximum power dencity, 8.09 mW/m^2
27	Flores et al., 2015	Sucrose	Sulfate-reducing inoculum	Nafion	Carbon-cloth containing 0.5 mg/cm^2 platinum catalyst	The MFCs consisted of horizontal cylinders (80 mm length,57 mm I.D)	9000 mW/m^3

2.2.1 Working Principle of MFC

MFC is similar to any battery or fuel cell having anode and cathode as electrodes connected through an external circuit. Like other batteries, electrons are produced from oxidation/reduction reactions. In MFC, energy stored in organic material is converted to electrical energy using microbes(Dalvi et al., 2011; Liu et al., 2005; Logan and Regan, 2006). The waste material (substrate) in the anode chamber is oxidized by microbes for their maintenance and growth through their respiration under anaerobic conditions, and they produce electrons and protons, and CO_2. The electrons which are produced due to microbial metabolism are transferred to the surface of the anode using redox-active compounds present on the outer surface of the bacterial cell or it may be produced by bacteria (Franks and Nevin, 2010; Kumar et al., 2016). **Figure 2.3** and **Figure2.4** show the mechanism of electron transport to the anode surface.

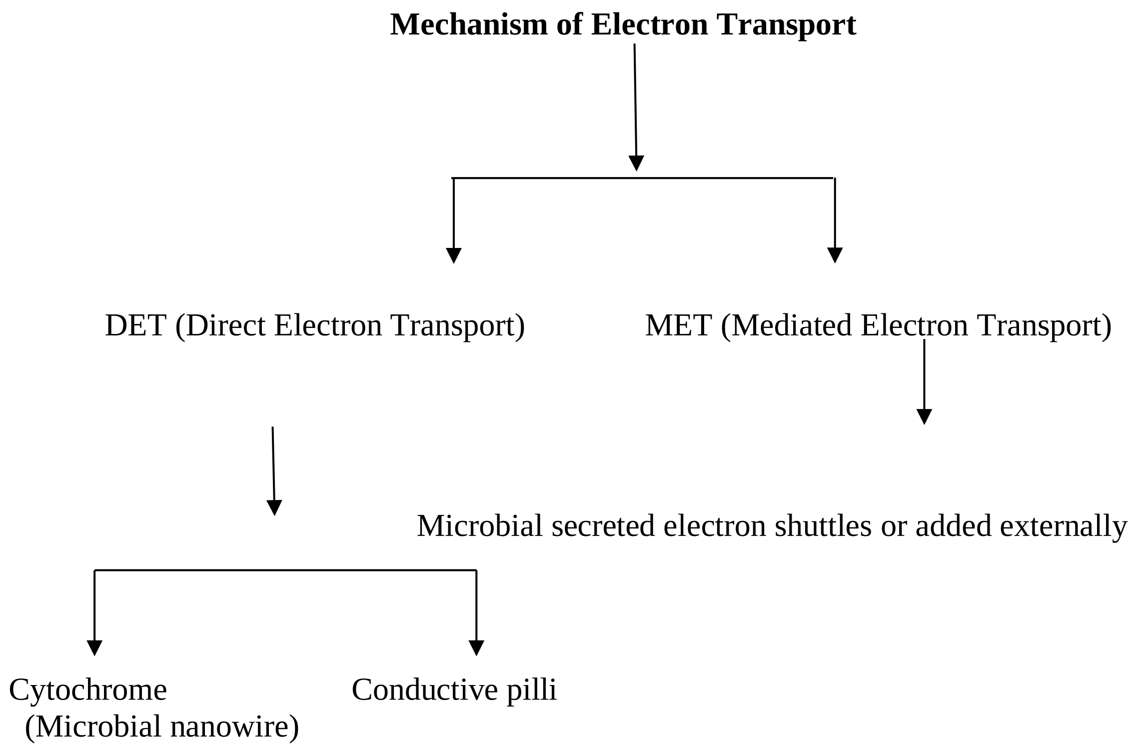


Figure 2.3 Mechanism of electron transport to the anode surface

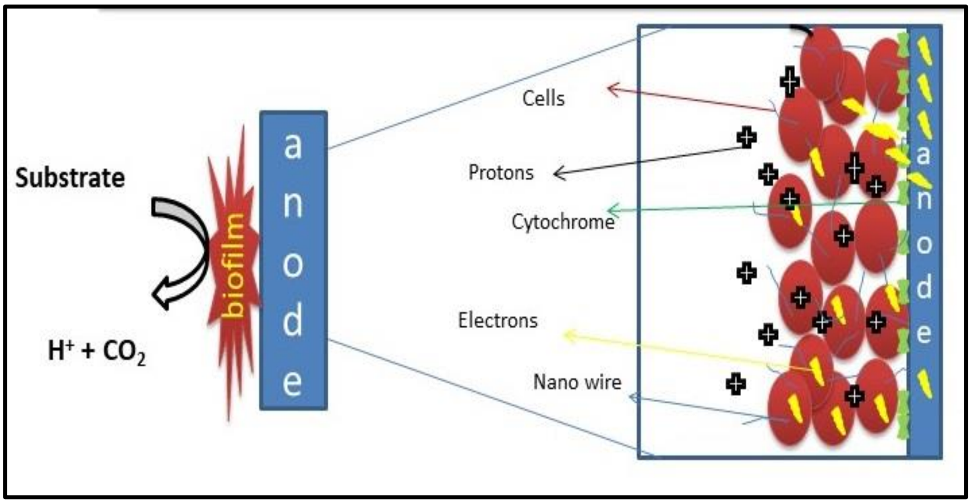


Figure 2.4 Electron transport mechanism from bacterial metabolism to the surface of the anode

- ***DET* (Direct electron transfer)**

Some species of bacteria such as *Geobacter* and *Shewanella sp.* have been reported to transfer the electrons to an anode surface without any mediator. They transfer electrons via active-redox proteins such as cytochrome present on the outer surface of a bacterial cell or conductive pilli (microbial nanowires). Conductive pilli play their role when multi-layer biofilm is formed i.e when direct contact ofc-cytochrome is difficult. For monolayer biofilm, c type cytochrome acts as electron carrier(Kiely et al., 2011; Malvankar et al., 2014; Nevin et al., 2008).

- ***MET* (Mediated electron transfer)**

In this, electrons are transported using microbial secreted soluble electron shuttles such as flavins, pyocyanin or via present naturally such as humic substances or artificially added such as certain dyes (Methylene Blue, Neutral Red, etc.), quinones, heme, iron, sulfur, or copper ions(Malvankar and Lovley, 2012; Shen et al., 2014).

After these electrons are transferred to the anode surface, they are transported to the cathode side via an external circuit. The H^+ ion produced at the anode side is transported from the anode to the cathode side through the proton exchange membrane (PEM). At cathode reduction reaction occurs where H^+ ion, electrons, and oxygen (aeration is maintained externally) get combined and produce H_2O (Hernández-Fernández et al., 2015b; Liu et al., 2004; Zhou et al., 2011). O_2 is used in most cases as an electron acceptor due to its high redox potential and also it is low cost (freely available in the air). However, till literature available shows that the aeration to the cathode compartment is an energy-intensive process (Thung et al., 2015; Ucar et al., 2017).

2.2.2 Metabolism in MFC

Based on anode potential, different metabolic pathways in MFC have been suggested due to which different microbes such as facultative and anaerobic have been reported to date. Rabaey et al. (Rabaey and Verstraete, 2005) had suggested metabolic pathways for MFC as follows:

- High redox oxidative metabolism
- Medium to low redox oxidative metabolism
- Fermentation

At low external resistance, MFC has high anode potential (low MFC cell voltage) during biomass growth the facultative aerobes and anaerobes dominate. Bacteria can use the respiratory chain in oxidative metabolism. Electrons and protons can be transported through the NADH dehydrogenase, ubiquinone, coenzyme Q, or cytochrome(Rabaey and Verstraete, 2005). During this, MFC energy efficiency was found to be up to 65 %. Bacteria involved are *Pseudomonas aeruginosa, Enterococcus faecium,* and *Rhodoferax ferrireducens* (Chaudhuri and Lovley, 2003; Rabaey et al., 2004).

If electron acceptors are present other than NADH, coenzyme Q, or cytochrome such as sulfate, then electrons get deposited onto these components. Kim et al. (Kim et al., 2004) found methane production when the inoculum was anaerobic sludge and the electron acceptor is a sulfate. If none of the electron acceptors are present such as sulfate, nitrate then fermentation will dominate in MFC(Logan and Regan, 2006; Rabaey and Verstraete, 2005)**.**

At moderate anode potential and high current when the biomass grows sufficiently, these conditions favor facultative anaerobes, however, strict anaerobes will still be restricted due to the redox potential of anode and may be due to fusion of oxygen through the membrane (Liu and Logan, 2004). At high resistance, the potential of the anode becomes low which supports low redox facultative anaerobes and strict anaerobes(Khater et al., 2018; H. Li et al., 2018; Logan, 2009).

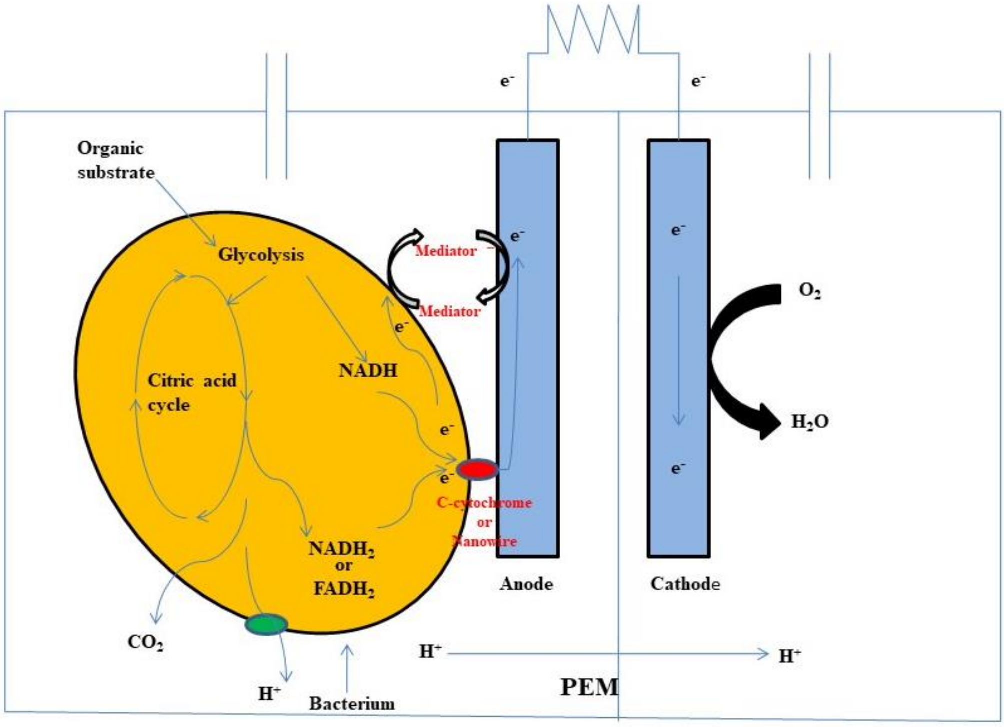


Figure 2.5 Metabolism of a substrate (waste) in MFC (Kumar et al., 2017; Logan et al., 2006)

2.2.3 Current status of research on MFCs

The researchers are continuously working to enhance the overall efficiency of MFCs by novel MFC design, searching efficient microorganisms, improving the proton exchange membrane, and optimizing the process parameters in the MFCs. By improving above mentioned factors it’s possible to use the MFCs for remediation of most of the organic waste along with a good amount of electricity generation.

- **Improvement in Reactor design**

Many different designs have been proposed like single-chambered, double-chambered, multi-stack, and tubular, membrane-less, sediment MFC, by different researchers (Chouler et al., 2016; Hernández-Fernández et al., 2015b; Wang et al., 2017). In most cases, double-chambered MFC is considered due to ease of design and maintenance. Double chambered MFC consist of an anodic chamber with bacteria and waste, and a cathode chamber separated by a proton exchange membrane and with an external supply of air (Liu and Logan, 2004). In a typical double-chambered MFCs, water at the cathode side is used where it is bubbled externally with air to maintain dissolved oxygen for the reaction at the cathode side.

- **Modification of electrodes/current collectors**
    - **Anode**

Anode plays a very important role in MFC as they facilitate bio-film growth and electrochemical reactions on their surface. Ideal anode material used for MFC should be highly conducting, cost-efficient, possess a large surface area, stable to change in an environment like pH & temperature, and biocompatible. Carbon-based material such as carbon cloth, graphite rod, carbon brush, graphite felt is more popular for MFC electrodes due to its high conductivity, biocompatibility, and chemical stability(Jiang et al., 2010; Passos et al., 2016; Takeuchi et al., 2017; Yaqoob et al., 2020). Research for finding a suitable cost-effective and biocompatible anode material is going on. Use of composite materials, heat treatment, increasing functionality with acid treatment, metal oxide and metal treatment, coating with conductive polymers are some common anode modification method which is employed continuously to increase anode surface reaction and electron transport so that overall power could get an increase(Feng et al., 2011; Hou et al., 2015; Li et al., 2011; Yuan et al., 2011; Zhou et al., 2011). Gandu et al.(Gandu et al., 2020) used a carbon cloth platinum-coated electrode and each working electrode (carbon-cloth) was pretreated by cold low-pressure nitrogen plasma and observed the highest current density of 11.52 A/m^2 at a voltage of 0.20 V.

❖ Cathode

Cathode material acts as a current collector as it accepts electrons from the external circuit and here O_2 is reduced. The anode materials mentioned above can be used as a cathode. Sometimes functionality is improved by coating different materials such as platinum to increase the rate of O_2 reduction(Chouler et al., 2016; M. Li et al., 2018; Milner et al., 2016). Khajehe et al. (Khajeh et al., 2020) has improved the property of graphite electrode by electrophoretic deposition of CuO/ZnO and found a maximum voltage of 363.5 mV by the modified graphite electrode under the irradiation after 47 h which was 32.22% higher than that of the bare graphite.

Recently, a new study has begun in search of different electron acceptors because of the (i) high redox potential, (ii) needs for efficient cathode reaction to increases overall power output (iii) for expansion of MFC based technologies in different areas(Oon et al., 2017; Thung et al., 2015; Wu et al., 2017). Other electron acceptors such as potassium ferricyanide, sodium bromate, sodium hypochlorite, potassium persulfate, potassium per magnet, some dyes such as methylene blue, hexavalent chromium, ferric chloride have high oxidation-reduction potential (ORP) than O_2, have been reported by many researchers which increases the power output 1.5- 9 times. But it will increase the cost (Dai et al., 2016; Kim et al., 2017; S. S. Kumar et al., 2017; Oon et al., 2017). Ucar et al. (Ucar et al., 2017) had reviewed many-electron acceptors and mediators used in MFC that they are also pollutants of the aquatic system and increases the cost but on the other hand, simultaneously they increase the power output of MFC many-fold higher when the only O_2 is used as the electron acceptor.

❖ Improvement in Proton exchange membrane (PEM) / Separator

PEM plays an important role because it facilitates the transfer of H^+ proton from anode to cathode side and prevents the transport of substrate and O_2(Ghasemi et al., 2015; Oliot et al., 2016). PEM selection should be done based on high proton conductivity, good chemical, thermal and mechanical stability, and slow fouling properties. Many researchers have used Nafion117 as PEM but Ultrex CMI 7000 membrane is also used(Angioni et al., 2017; Çetinkaya et al., 2015; Gajendra and Panda, 2018; Hernández-Fernández et al., 2015b). Research on Some cost-effective membranes such as sulfonated polyether ketone membrane, bipolar membrane, and forward osmosis membrane, anion exchange membrane is ongoing to overall decrease the cost of MFC(Chouler et al., 2017; Hernández-Fernández et al., 2015a; Li et al., 2017; Ping et al., 2016). Yousefi et al.(Yousefi et al., 2020) developed chitosan/montmorillonite nanocomposite deposited over ceramic and serve as low-cost membrane and observed decreases in ohmic resistance by 73.2% along with power and current densities of 229.12 ± 18.50 mW/m^2 and 1422.22 ± 41.20 mA/m^2.

2.2.4 Types of MFC

MFCs are classified based on the nature of electron transfer from bacteria to the surface of the anode, design of MFCs, type of flow in the MFCs, stacking of MFCs, etc. A brief discussion of different classifications of MFCs are as follows:

- **Based on the mediator**

MFC are classified based on how electrons are transferred from bacteria to the surface of the anode. They are as follow:

- **Mediator less MFC**

When microbes (electrochemically active) used in MFC can transfer electrons direct to anode surface then these MFC are known as Mediator less MFC.

- **Mediator MFC**

When microbes (electrochemically inactive) used in MFC are not able to transfer electrons direct to anode surface they require some electron shuttles known as mediators such as methylene blue, neutral red, metal and metal oxides, etc. however these mediators may be toxic to microbes and they increase the process cost. This type of MFC is called Mediator MFC.

- **Based on the design of the reactor and mode of operation**(Gajendra and Panda, 2018; Hernández-Fernández et al., 2015b; R. Kumar et al., 2017)

- **Single chambered**

  As the name suggests, it contains only one chamber which is an anode chamber, and the cathode is exposed to air (**Figure 2.6**). It is also known as air cathode MFC. Sometimes membrane is used to separate anode and cathode. Some researchers also attempted to coat the cathode inside with suitable material which provides ion exchange efficiently and consequently enhances the overall efficiency of the MFC.

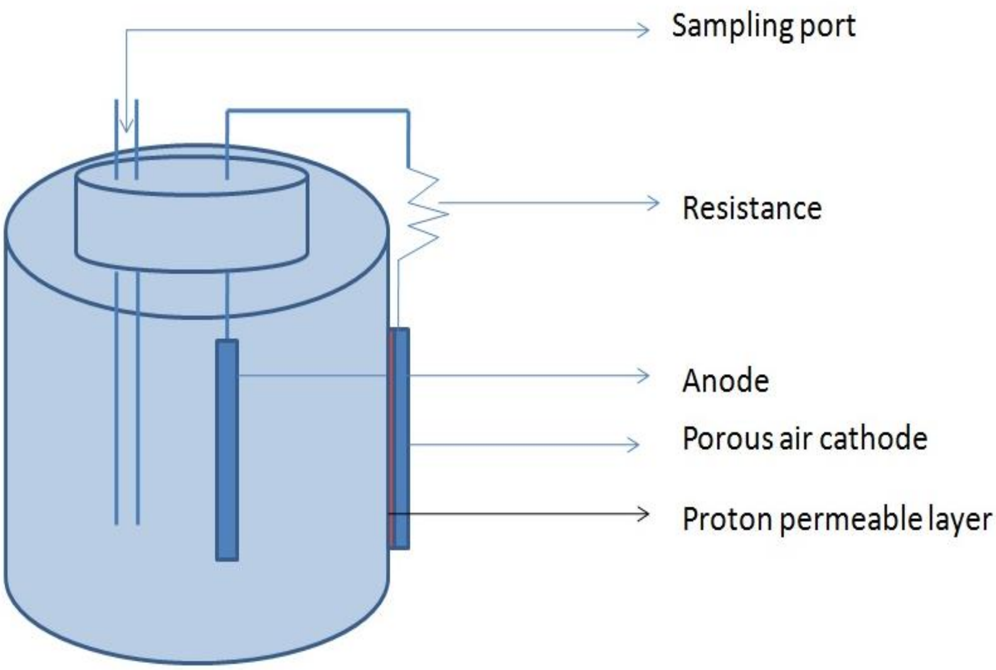


Figure 2.6 Schematics of single-chambered MFC

In the single-chambered MFC (**Figure 2.6**) aeration from outside is not required because the cathode is in direct contact with the atmospheric air. This design is simple and the cost is also less than the double-chamber. Single-chambered MFCs have shown very good power output even better than double-chambered MFCs (Cheng and Logan, 2011). Liu and Logan et al. (Liu and Logan, 2004) evaluated the performance of single-chambered MFC with and without PEM air cathode in order to decrease the cost and observed that air cathode MFC without PEM produces the highest power output. But the disadvantage associated with design is that bacterial adulteration may occur also the back diffusion of O_2 from the cathode could decrease overall power output (R. Kumar et al., 2017; Liu and Logan, 2004).

- **Double-chambered**

Double chambered design is very simple in design, easy to maintain, and most popular (Borah et al., 2013; Wen et al., 2010; Zhou et al., 2018). The schematic diagram of the Double Chambered MFC is shown in **Figure 2.7**. Details of double-chambered design have already been discussed in section 2.2.3.

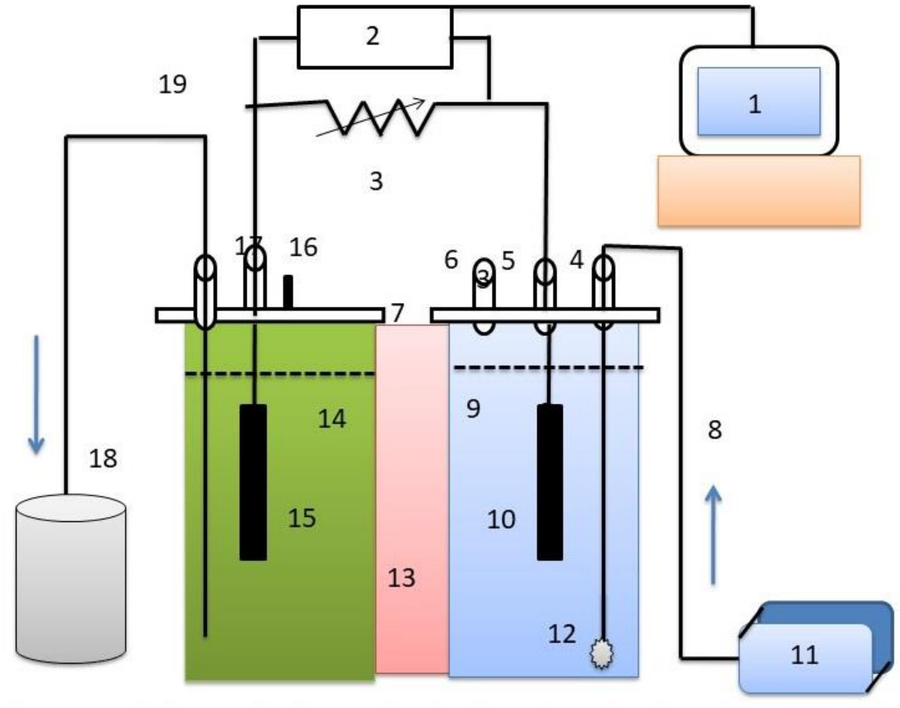


Figure 2.7 Schematics of double-chambered MFC

1.computer,2.multimeter,3.variable external resistance,4.bore for air sparging,5.bore for passing electrode,6.bore for excess air exit,7.compartment lid,8.plastic tube for air sparging,9.cathode compartment,10.cathode,11.air pump,12.spherical ceramic,13.salt bridge,14.anode compartment,15.anode,16.sampling bore,17.bore for passing electrode,18.bore for N_2 sparging,19.cuper electrical wire

- **Up-flow MFC**

This MFC consists of a cathode chamber at the top and an anode at the bottom and is separated by PEM or glass wool and glass bead layers. The wastewater (substrate) is forced through a peristaltic pump from bottom to top. A gradient is formed between anode and cathode which favors the action of MFC. In this design, there are no distinct anolyte and catholyte. The major disadvantage associated with this is the energy cost of the pump (Koroglu et al., 2016; Tamilarasan et al., 2017).

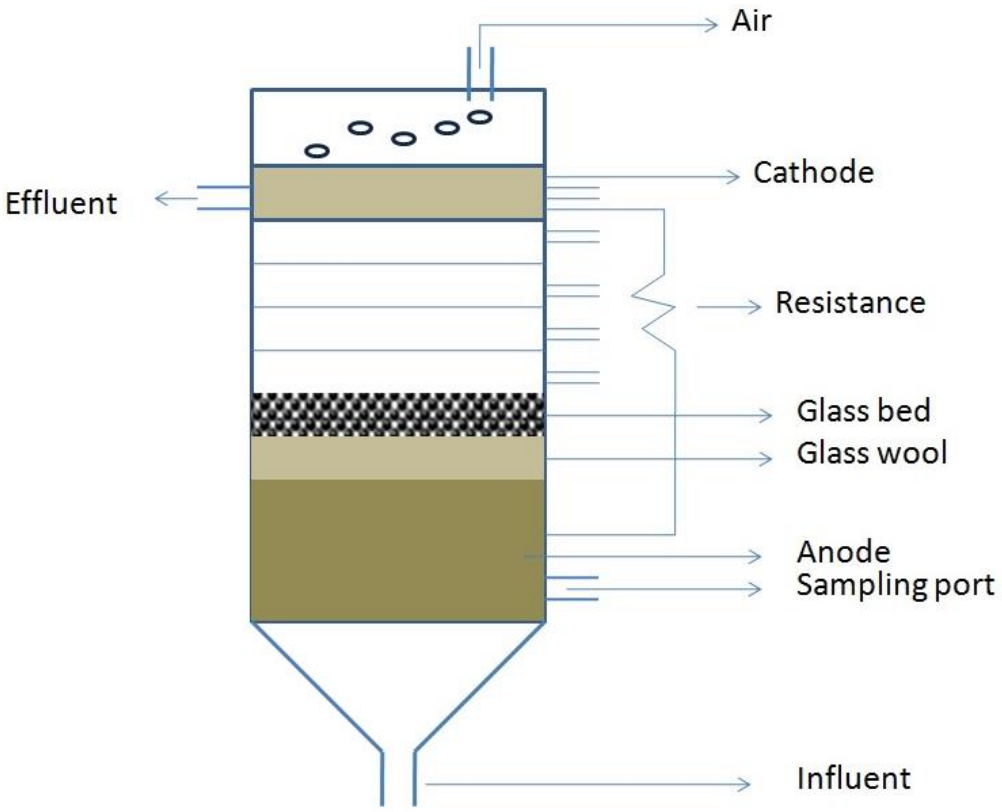


Figure2.8 Schematics of Up-flow MFC

- **Stacked MFC**

When multiple small units of MFCs are combined in a single large unit then this configuration is called stacked MFC. It is done to increase the overall power output of the system because the power of a single MFC unit is very low (Hassan et al., 2014; S. Zhang et al., 2017).

2.2.5 Parameters affecting the performance of MFC

MFC performance depends on both biological and electrochemical factors. Principally, the power output is affected by substrate degradation rate, electron transfer rate from the bacteria to the anode surface, total internal resistance (circuit resistance, resistance offered by PEM to H^+ transport and, resistance offered by electrolyte and external operating conditions) (Kang et al., 2017; Rossi et al., 2019; Xu et al., 2018)**.**

- **Microorganism**

Bacteria in MFC that are used for the degradation of waste have the capability of transferring electrons outside the cell to an electron acceptor present at anodic chambers. Some time mediators such as some dyes, iron, and other metals are used to collect the electron, or the electrode itself acts as an electron (current) collector. These special types of bacteria are also known as exoelectrogens / anodophiles / electrogenic microorganisms/anode respiring bacteria (Mei et al., 2017; Zhang et al., 2020). *Geobacter metallireducens, Aeromonas hydrophila, Rhodoferax ferrireducens, and Shewanella putrefaciens*, are reported exoelectrogens for their higher power output than any other species along with these species may bacterial sp. have been reported to possess exoelectrogenic properties (Christwardana and Kwon, 2017; Logan, 2009; Logan and Rabaey, 2014).

- **Electrodes**

Electrodes also play important role in MFCs operation. As the material of electrodes gets changes, their physical and chemical property (conductivity, surface area, chemical stability, and biocompatibility) also changes which ultimately affects the rate of electron transfer, attachment of biofilm, and rate of surface reaction(Jiang et al., 2017; Li and Yu, 2015).

❖ Substrate degradation rate

The substrate degradation rate is affected by the population density of bacterial species which is generally represented by colony-forming unit (CFU/ml). The other important factors which affect the substrate degradation are the rate of mass transfer of substrate on the surface of the electrode, the bacterial kinetics which is expressed in terms of the maximum specific growth rate (μmax), and half-saturation constant (Ks), and inhibition constant (Ki), the loading rate (g substrate/g biomass present/day), resistance offered by the proton exchange membrane for H^+ ions, electron transfer rate and external condition (time and temperature) (Min et al., 2005; Rabaey and Verstraete, 2005; Yang et al., 2016).

❖ Total internal resistance (Apparent resistance)

This is the most important parameter of MFC because it affects the power output of the cell. As it decreases, power output increases due to low energy loss. The total internal resistance consists of ohmic resistance and non-ohmic resistance. Non-ohmic resistance consists of charge transfer resistance and mass transfer resistance. Charge transfer resistance is caused by the reaction energy barrier required for the electrochemical reaction, while mass transfer resistance by mass transfer inhibition (Guo et al., 2016; Kang et al., 2017; Rossi et al., 2019)**.**

❖ Operational Conditions

The important operational parameters include pH, temperature, organic loading, feed rate, and shear stress. The performance of most of the MFC technologies are evaluated at the laboratory level and their performance was found to be very low as compared to the maximum possible performance obtained theoretically. Therefore, it is highly beneficial to optimize the operating conditions to improve the overall performance of the MFCs (R. Kumar et al., 2017; Logan et al., 2006)**.**

- **pH**

pH maintenance in the anode chamber is important for maintaining bacterial growth. After a long time operation of MFCs, the pH at the anode side was reduced due to proton accumulation which is a result of the slow or restricted flow of protons via PEM. Since most of the bacteria grow at neutral pH so acidic environment will lead to a decrease in voltage. Similarly, the reduction of O_2 at the cathode side requires low pH, but due to the restricted flow of protons, pH will increase and results in a reduction of O_2 and a decrease in power output.

- **Temperature**

Most of the MFC is operated at room temperature 25 ° C to 30 ° C. But it was found that with an increase in temperature from 30 to 45 ° C, power output increases significantly. This may be attributed to an increase in bacterial activity as most of the bacteria work most efficiently in a range of 30 to 45 ° C. Temperature also play important role in increasing the surface reaction rate on the anode.

- **Feed rate**

MFC can be operated in batch or continuous mode. Batch mode is mostly preferred because microbes get a larger time for the oxidation of the waste (substrate) which resulted in higher power output. In continuous mode, the flow of substrate may cause shear stress due to which attachment of biofilm may get affected. Due to the movement of fluid, the thickness of bio-film gets decrease and it decreases power output. Also, a higher flow rate decreases cod removal efficiency and columbic efficiency.

2.2.6 Limitations of MFCs

The power density obtained from MFC is lower than other chemical fuel cells due to high internal resistance, substrate toxicity, the selectivity of microbial species for different types of substrates, unreliable nature of microbial species, different metabolic pathways which may result in different biofilm kinetics, and fouling of the PEM (Logan and Rabaey, 2014). Although MFC is a green technology due to the high cost of operation the practical application is still very limited. Research is still ongoing to make MFCs a viable option for real-time applications.

2.2.7 Research Gap

Based on the literature review following research possibilities were identified to improve overall efficiency and making it more viable from an economic point of view

- The high cost of material used in the fabrication of MFCs, current instability, high internal resistance, low power output limits the use of MFCs only for specific areas.
- Availability of very limited work on remediation of industrial waste such as dye-containing waste along with power generation in MFCs.
- There is scope for identification of efficient microbial species with the aim to achieve more dye degradation along with high energy production
- There are very few reported works on the optimization of important process parameters to improve the overall efficiency of MFCs. The synergistic effect of optimum parameters on the process of bio-decolorization also needs to be studied.

Keeping the above facts in consideration, the present study has been planned to explore the dye biodegradation in various types of MFCs under different operating conditions to improve the overall performance of MFCs.

2.2.8 Research objectives

The overall aim of this research is to investigate the technical feasibility of MFC for the treatment of Azo dye-containing wastewater. To achieve the above aim the research was directed through the following specific objectives.

- ☐ To fabricate experimental setup and evaluate its performance in terms of electricity production.
- ☐ To investigate the dye decolorization using mixed culture and its kinetics study in MFC.
- ☐ To isolate exoelectrogens from previously acclimatized MFC for enhanced dye decolorization under optimum process conditions.