Microbial Electro-Fenton Driven Process Employing Graphite Electrode in MFC for Acid Red 114 Dye Degradation

QUAID-I-AZAM UNIVERSITY

ISLAMABAD

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In

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بسم الله الرحمن الرحيم

Dedication

Every challenging work needs self-effort as well as guidance of elders especially

those who are very close to our heart.

My humble effort I dedicate to my sweet and loving

Father, Mother

Whose affection, love, encouragement and prayers of day and night make me able

to get such success and honor.

Along all the hardworking and respected

Teachers.

Declaration

The material and information contained in this thesis is my original work. I have not previously presented any part of this work elsewhere for any other degree.
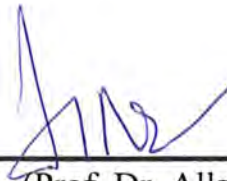
Rafia Shahid

Certificate

This thesis submitted by *Rafia Shahid (02052111010)* is accepted in its present formby the Department of Microbiology, Quaid-i-Azam University Islamabad,and Pakistan; as satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

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Abstract

Rapid advancements in the textile industry have led to massive dye discharge in fresh water. This calls for unconventional and highly efficient treatment technology. Since the last decade bio elefctrofenton system have gained a lot of consideration owing to their low operating cost and feasible applicability. The current study has been designed to treat Acid Red 114 (AR114) in microbial Fenton system (MFS). The aim was to run a self-assembled lab scale dual chambered Microbial Fuel Cell (MFC). For this purpose, graphite powder (GP) electrode was fabricated using epoxy binder on a stainless-steel mesh (SSM). The electrode was integrated in Bioelectrofenton system to be used as cathode. The MFS was run in batch mode with GP cathode and Carbon Felt (CF) bioanode. Dye solution containing 20ppm AR 114 mimicking as industrial effluent was treated in cathode chamber by generation of Fenton Reagent (FR). System performance was analyzed for H2O2 production, current, voltage, Fourier Transform Infrared Microscopy (FTIR), TDS and EC. 85% degradation was achieved with 6805µl/ml H_2O_2 in 8h at optimized conditions, pH 3, 0.5mM $FeSO_4$, 0.75mM Na_2SO_4, continuous oxygen supply. FTIR data confirmed removal of Azo bond. Maximum voltage and current of 0.2 V and 55µA were measured across 100Ω resistor. The performance of GP cathode in MFS appeared to be a promising self-sustainable, eco-friendly and cost-effective method for the removal of AR 114.

1. Introduction

Research on sustainable development has recently taken on a new perspective owing to the ongoing exploration of biotechnology for the health of ecosystems, the reduction of pollution, the production of biodegradable materials, and the creation of eco-friendly production and disposal techniques. Effectively addressing the problems of waste management and climate change is essential for a sustainable future [1]. The worldwide search for new, sustainable water sources is still being driven due to the increasing requirement for clean water supplies. For years, it has been essential to prevent the water environment from being contaminated with toxic chemicals via the removal of poisonous and bio refractory organic substances from wastewater and groundwater. [2]. The discharge of untreated wastewater from diverse sources including home, agricultural, and industrial establishments directly into the freshwater supplies is the primary cause of major environmental implications, such as hypoxia, eutrophication of surface waters, and algal blooms degrading potential drinking water supplies [3]. To prevent water problems and environmental degradation, wastewater treatment and utilization are important [4]. The use of conventional techniques such as coagulation, adsorption, filtering, sedimentation, and filtering has been proven to be technically feasible but due to the potential creation of various secondary wastes that require additional treatment, these systems could be challenging and unsustainable [2].

Large volumes of water and chemicals are used in the textile industry. Textile processing wastewater has effects on high BOD, COD, TOC, turbidity, color, pH that ranges from 5 to 12, temperature, suspended particles, and harmful organic compounds. Discharging even a tiny amount (about 1mg L^{-1}) of dye is unacceptable because it could lead to the production of hazardous chemicals at the end of the treatment procedure [5]. The most widely used synthetic dyes are azo dyes, which have ringed structure containing double Nitrogen bonds [6] Azodyes are an important component of different industries like cosmetics, food, paper printing, and textile dyeing [7]. These dyes possess electron scavenging groups that cause electron

deficiency, making them xenobiotic molecules that can withstand degradation [8].

The discharge of azodyes in wastewater has profound impact on surrounding ecosystems. It is estimated that around 10 lakh tons of azodyes are manufactured yearly around the world, in which most of them are structurally diverse from one another [7]. Acid dyes are an anionic class of water-soluble pigments. They are part of the textile industry as they work well on cloth fibers such as silk, wool, nylon, and acrylics. These dyes adhere to the fibers by hydrogen, ionic and Van Der Waals bonding. AR114, AB 78, AB 36, and AY 35 are a few examples of Acid dyes based on their chromophores. Effluent-containing acid dye coming from industries creates serious environmental pollution that affects water transparency. It causes many serious health issues like asthma, nasal and respiratory symptoms, dermatitis and rhinitis, allergies, genotoxicity, mutagenicity, and carcinogenicity [9].

Treatment methods employed in wastewater treatment plants are based on separation of pollutants from water and degradation of pollutants either chemically or physically. Separative processes are energy intensive, laborious, have carcinogenic byproducts, and constitute many steps. Among chemical degradation methods Among advanced oxidation processes (AOPs) are of great intrest, Because of their efficiency in the efficient breakdown of dyes. It involves the production of hydroxyl radicals (OH$^-$), which have a significant oxidizing potential of E (OH/H$_2$O) 1/4 2.8 V/SHE, making them extremely reactive oxidants [10]. When produced insitu Hydroxyl radicals scavenge electrons from organic compounds to produce organic radicals through redox process. The intermediates thus produced mineralize the organic pollutants due to their extremely strong and non-selective oxidation abilities until complete decolorization [11] (eq.1). Electro-Fenton process is a type of indirect AOPs, this method effectively removes persistent organic contaminants from water. The quick removal of organic compounds, high rate of mineralization of persisting pollutants, Eco friendliness of the EF process make it a desirable process for pollutant degradation in treatment plants. The principle of Electro Fenton's reaction is the insitu generation of Fenton's Reagent

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electrochemically, which is achieved by adding Ferrous salts as a Fe^{-2} source. FR is a mixture of H_2O_2 and Fe^{-2}, which react to form OH^- ions (eq2) [10]. Electro-Fenton Process provides greater benefits than the chemical Fenton process due to the high utilization efficiency of Fenton's reagents (such as H_2O_2) and cost-effectiveness by minimizing the transportation and storage of chemicals. Creating an energy intensive EFS should be very appealing and challenging [12].

Recently, bio-electrochemical technologies for wastewater treatment, including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), are developed [13]. Numerous studies on the microbial fuel cell (MFC) demonstrated that the organics present in wastewater can supply electrons on a continual basis. The use of these bio electrons generated by microbial metabolism can make the bioelectro-Fenton (Bio-E-Fenton) concept practicable. Setting up an MFC reactor, comprising of two chambers, anode chamber is filled with biodegradable organic substrates, cathode chamber containing target contaminant separated by CEM. The bioreaction at the anode releases the electrons, which are then moved with the help of an external load circuit to the cathode [12]. The organic compounds are degraded in the anaerobic anode by electrogenic microorganisms generating electrons, for driving the Fenton reaction in cathode containing pollutant, iron source, and oxygen supply [13]. The cathode is aerated to produce H_2O_2 by reduction of O_2, thus achieving complete decolorization in a matter of hours [14]. Numerous azo dyes including Methylene Blue, Reactive Black, Orange 2, Acid Blue 113, Methyl Orange, and Methylene Blue have been degraded in the BEF reactor system, achieving decolorization efficiency of 98%, 89%, 100%, 71%, 87%, and 95% respectively [14- 19].

The decisive factor in assessing the cost and efficacy of MFC is the electrode. The greatest hurdle to making MFCs an affordable and scalable technology is the designing of the electrodes. A variety of electrode designs and materials have been explored and developed to enhance MFC performance and reduce material costs recently. Studies for MFCs have continuously grown more interested in the electrode material and its

configuration in recent times. A number of electodes have been thoroughly investigated for MFCs over the past ten years. All electrode types must typically have base materials with strong mechanical strength, low cost, good chemical stability, and good conductivity. Currently, the most widely used base materials include carbon compounds and non-corrosive metals [22]. The most common electrode types used today are based on carbonaceous or metallic material. The following carbonaceous materials are used as electrode materials that are available commercially: carbon cloth, carbon rod, carbon mesh carbon brush, granular activated carbon, carbon felt, carbon paper, and granular graphite [23]. The properties of Granular Graphite are identical to that of Activated carbon characteristics. In addition, Graphite is also known for its good electrical conductivity [23]. All these characteristics make both a potential electrode fabrication material. The scope of the present study is to determine the degradation of textile dyes through the production of H_2O_2 in MFC driven Bio-electro Fenton system. The experimental setup was designed to evaluate the degradation of Acid Red 114 dye via Microbial Electro-Fenton driven process in MFC. For the Bio-electro Fenton system, Carbon felt was used as a bioanode while the electrode for the cathode was fabricated using Graphite Powder [20]. The bio-electrochemical performance of the reactor was evaluated via cyclic voltammetry (CV), and the performance assessment was carried out by estimating the power density, cell potential and COD removal rate in MFC. Percentage decolorization and degraded metabolites were examined spectrophotometrically and through FTIR analysis. Scanning electron microscopy (SEM) was also employed to study the surface morphologies of electrodes from the MFC system. The aim of this study is the application of MFC driven Bio-electro Fenton system for the degradation of Acid red 114 dye, an azo dye used in the textile industry, and a potential threat to the environment due to its presence in the textile and wastewater effluents. The outcomes of the study undertaken for bio-electrochemical degradation of pollutants will contribute to the development of cutting-edge technology for large-scale wastewater treatment plants.

Aim and Objectives

The aim of the present study is **the treatment of Acid Red 114 in MFS with modified graphite powder stainless steel mesh cathode.**

OBJECTIVES

1. To Fabricate Electrodes through
* Modification of the stainless-steel mesh electrode with graphite powder.
* Characterization Of Electrodes.
2. To Develop dual chamber MFS (Microbial Fenton System) with a bioanode and abiotic cathode for the degradation of Acid Red 114.
3. To study MFS performance under varying conditions,
* Dye concentration, pH, Iron concentration, catholyte concentration, different catholytes.
4. To determine the effect of optimized parameters on the degradation of AR114.
5. To assess the AR 114 degradation using analytical technique such as FTIR and SEM

2. Literature Review
2.1 Textile dyes chemistry and Toxicology

Textile industry being the major contributor of Pakistan's growth and development, it generates 65% of country's export. Karachi and Punjab constitute approximately 670 textile units which are major contributors of growing GDP, this sector alone contributes 8.5% of total GDP. Dyes are the second most important raw material in the textile industry after the fabric itself. Although Pakistan is ranked 3rd worldwide cotton production but we need to import dyes from other countries to meet the requirements of our growing industry. They contribute color to the fabric of choice permanently. It is reported that annually 10,000 different dyes are produced globally to meet the demands of the industrial world [25]. Most of the dyes used in industry belong to azo, anthraquinone and pathocynine. The process of dying is water intensive, so it generates a lot of dye dissolved wastewater, altering physiochemical characteristics of water bodies.

2.1.1 Molecular Chemistry of Dyes

The definitive structural characteristics of dye molecule is the benzene ring backbone with alternate C double bonds having a delocalized electron pair, this arrangement helps in transmission and reflection of white light resulting in various color [26]. For use in industry dyes are have a strong integral structure and are water soluble to ensure wide range of application. Nearly 40% of dyes produced have an organic bonded chlorine [27]. Extensive use and discharge of dyes from dye bath is a major cause of presence of dye precursors molecule dioxin or related intermediates in the fresh water as persistent organic pollutants. Most lethal form of dioxins are the ones containing halogen analogues, these are difficult to remove from water sources. Benzoic structure which imparts recalcitrance and structural strength also makes their use and discharge highly objectionable as it avoids natural process of mineralization in oxygenic state [28]. Such substance makes it difficult to release the majority of their elements in the in environment leading to a more persistent problem of low biotic output. The presences of functional groups like sulpho, choro and nitro results in highly reactive dye molecules.

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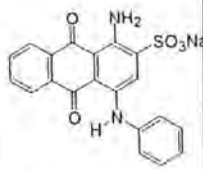
About 700,000 dye pigments are manufactured worldwide having more than 100,000 types. The primary structure of the dyes and related compounds comprises of a characteristic chromophore which can be ethylene group (=C=C=), azo group (-N=N), carbonyl group (=C=O, methine group (-CH=), carbon-Sulphur (=C=S; ≡CS-S-C≡), nitro (-NO2; -NO-OH),), carbon-nitrogen (=C=NH; -CH=N-), nitrozo (-N=O; =N-OH) etc. Auchochrome group renders the binding capability to any type of fabric or onto solid surfaces. This group gets ionized to bind the dye with fabric. The typical auxochrome groups are: -COOH (carboxyl), -NH2 (amino), -OH (hydroxyl) and -SO3H (sulphonate) [29]. 65-75% of dyes are either Azo or anthraquinone, and about two thirds of them are azoic in nature containing Carbon Carbon double bond. The parent compound of most dyes are Cohloranilines, aniline, Benzidines, methylamines, Napthylamines, Phenylenediamines and others the presences of these groups make dye molecules toxic and mutagenic to a variable degree at different levels of trophic. They may also escape biological catalysts like enzymes and bacteria [27][30].
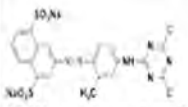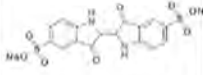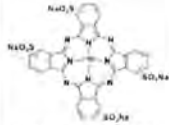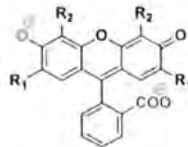
The characterized reactive groups of azodyes bonds covalently with HS-, HO- or HN groups of fibers such as wool, silk, cotton, nylon etc. [31]. Azodyes are commonly used for red, yellow, and orange colors. They are generally non-volatile, and the existence of an amino group is the reason for a higher water solubility, higher boiling point and a lower Henry's law constant in comparison with hydrocarbons [32]. The delocalized orbital system reacts with lone pair on the N atom, as a results if which Azo dyes receive protons. The acceptor substitute at the aromatic ring like -NO2 or Cl, minimize the basicity of aminic groups. The basicity of aromatic aminic groups increases when functional groups like –OR or –CH3 (at meta and para positions) are used. Donor substituents in the ortho position, on the other hand, can sterically obstruct protonation, lowering the basicity of aminic groups. Amphoteric azo dyes have an extra acidic group in their molecules (hydroxyl, carboxyl or sulfoxyl). They can be non-ionic in normal conditions, cationic when protonated at the amino group and anionic when deprotonated at the acidic group that can be depended on pH [27]. The aromatic structure and number of Carbon atoms, the quantity and type of substitute also the molecular structure of

backbone all play a role in dye environmental placement [27].

2.1.2 Classification of dyes

Dyes are aromatic compounds which are used extensively in industries and because of this they have a variety of shades and hues which differ in positioning of single functional groups. Pigments and dyes are often classified on the basis of structure and technique of application onto different fabrics. on their chemical structure or their technique of application to various substrate items. On the basis of chemical structure dyes are classified in 8 categories. The defining characteristic of dye molecule is the chromophore group like ethylene (=C=C=), azo (-N=N), carbonyl (=C=O, methine (-CH=), carbon-Sulphur (=C=S; ≡CS-S-C≡), nitro (-NO2; -NO-OH),), carbon-nitrogen (=C=NH; -CH=N-), nitrozo (-N=O; =N-OH) etc. Auxochrome group renders the binding capability to any type of fabric or onto solid surfaces. This group gets ionized to bind the dye with fabric. The typical auxochrome groups are: -COOH (carboxyl), -NH2 (amino), -OH (hydroxyl) and -SO3H (sulphonate) [29]. 65-75% of dyes are either Azo or anthraquinone, and about two thirds of them are azoic in nature containing Carbon Carbon double bond. The parent compound of most dyes are Cohloranilines, aniline, Benzidines, methylamines, Napthylamines, Phenylenediamines and others [28]. There are 10 major classes of dyes categorized into two major classes water insoluble and soluble. The redox ability of dyes depends on structure and functional groups that also relates to their dyeing ability. Fixation of dyes depend on the structure of molecule their solubility and affinity with different fibers. Some basic details of dyes classification is mentioned below:

Class	Chromophore	Examples	Structure	Reference
Anthraquinone	C=O. and =C= C≡ forming an anthraquinone complex (quinone	acid blue 62, reactive blue 19	Acid blue	33

	nucleus			
Azo	-N=N-	Yellow reactive 4, black reactive 5	 Yellow reactive 4 $C_{20}H_{12}C_{12}N_6Na_2O_6S$	34
Indigo	C=O, NH$_2$, C = C, C–O, C=N, C–C	blue acid 74, indigo blue	 Blue acid 74 $C_{16}H_8N_2Na_2O_8S_2$	35
Phthalocyanine	Phthalocyanine nucleus	pigment blue 15/3, nickel (II) tetrasulfonic acid	 Nickel (II) tetrasulfonic aci $C_{32}H_{12}N_8Na_4NiO_{12}S_4$	36
Xanthene	Xanthylium or di-benzo-g-pyran nucleus	fluorescein, eosin, erythrosine	 Erythrosine $C_{20}H_6I_4Na_2O_5$	37
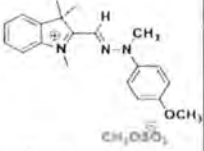
Polymethinic	=HC–HC= CH–CH	basic yellow 28, polymethine dye 2630	Basic yellow 28 $C_{21}H_{27}N_3O_5S$	38

Table 2.1: Major Classes of Dyes based on Chromophore
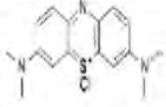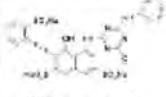
H2O soluble dyes					
Dye	Type	Chromophore	% Discharge	Example	Reference
Anionic/Acidic	Azo, anthraquinone or triaryl	SO_3^-, NH_2, OH	7-20	Red acid 27 $C_{20}H_{13}N_4Na_3O_8S$	53
Cationic/Basic	Diaryl, triaryl, anthraquinone, azo	NH_2	2-3	Basic blue 9 $C_{16}H_{18}CIN_3S_2H_2O$	54
Reactive	Azo, phthalocyanine	NH_2	10-50	Red cinscrom 3 $C_{27}H_{17}CIN_6O_{14}S_4$	55
Metalliferous	Azo, phthalocyanine	SO3	2-10	32	
Direct	Azo, phthalocyanine	NH_2, SO_3, OH	5-30		8

Table 2.2 : Water Soluble Dyes

2.1.3 Acid dyes

Acid dyes are water soluble class of dyes applied to wool, nylon and silk in acidic medium in which pH is maintained around 3-7. Acid dyes are brightly colored dyes, their Lightfastness usually falls in between blue-scale ranges of 5.0 and 6.0, and their wet-fastness ranges from moderate to good. The size of the dye molecule directly affects the level of interaction and, consequently, the level of color fastness [39].

Acetic or formic acids are used to create acidic medium in which dyes are applied, level of acidity depend on dye properties. Acid dyes vary in structures that contain metal complex Sulphonated groups, that contribute to water solubility, are the group's distinctive property. The interaction of sulphonate groups and the ammonium groups on fiber surface contribute to their adherence to fiber. Van der Waals forces additional bonding contact [40].

Azo, anthraquinone, copper phthalocyanine and triphenylmethane dyes are made water soluble by addition of one to four sulphonate groups.

Acid red 114 is one of the dyes in Acid class of dye. Chemically acid red is an Azo dye, which means that it has Nitrogen double bond. Acid red 114 has two double nitrogen bonds. In powdered form at room temperature, it is a deep maroon powder. The powder is soluble in water and slightly soluble in Ethanol. When applied on fabric such as nylon, silk and leather it gives a deep red color. Like another textile dyes they are also released untreated [41]. The structure of acid red 114 is given below.
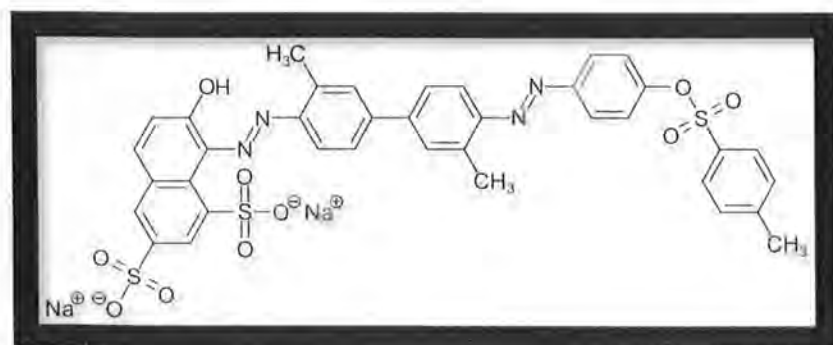
Commercial name	Acid Red 114
Molecular formula	$C_{37}H_{28}N_4O_{10}S_3Na_2$
Purity	50%
Chromophore	Diazo
Molecular weight	830
λ_{Max} (nm)	522 nm
Appearance	Dark red powder
Chemical name (IUPAC)	Disodium 8-(3,3'-dimethyl-4'-((4-((4-methyl phenyl) sulphonyl) oxy) phenyl) azo)-(1,1'-biphenyl)-4-yl) azo)-7-hydroxy-1,3-naphthalenedisulphonate

Figure 2.1: Acid Red 114 structure and characteristics

2.2 Environmental Fate of Textile Dyes

2.2.1 Textile dyes in Wastewater

Multiple industries like paper, pulp, leather tanning, textile dye stuff manufacturing, pharmaceutical and karat bleach units are reported as major source of dyes discharge in water bodies. [27]. Around two hundred-thousand-ton salt is released from textile units [27, 42]. Almost 10-60% of dyes and pigments employed are released from dye bath, roughly about 280,000 tons per annum [26]. Table: shows the amount of different dye discharge from units. The release untreated or partially treated effluents results in addition of significant amount of TOC, phosphate, nitrates and heavy metal ions like iron, zinc mercury cadmium, copper and cobalt. Contents of water raise in terms of TDS, TSS and COD/BOD causing eutrophication [43]. Textile dye have an important impact on the water ecology for this purpose they have been studied a lot [44]. Due to their immense production, use and discharge, azo dyes aye studied most as model dyes. More than 250ppm dye molecules from leather units are discharged leading COD increase Upton about 7000ppm, making water acidic. [45]. One of the major concerns regarding dye molecules is presence of dioxins and Cohloranilines, which are precursor

molecules for anthraquinone, which are released during manufacture and treatment by UV or photolysis [46].

It was reported by the Blacksmith Institute and Green cross, Switzerland that 125 million inhabitants living in the developing and underdeveloped countries were at high risk of industrial wastes/ pollutants. However, due to inadequate facilities besides keeping the cost of end products in control the industries typically in the developing countries are avoiding pretreatment of dyes effluent. There's a lack of domestic or industrial wastewater treatment facilities in the developing countries. Even if there are they are very basic or ineffective for persistent organic pollutants [47]. Limiting factors like power outrage and high cost of treatment at facilities further reduce the pretreatment of the dye water. In light of all these cons all of the effluent are discharged untreated after only primary treatment from settlement tanks into water bodies where they persist for time that is related to their half-life causing issues in fresh water ecology as they are gradually broken down into more potent intermediates Table2 shows the efficacy of in terms of percentage fixation and percentage loss into hydrosphere.

Class Of Dyes	Fiber	Percentage Fixation	Effluent Loss
Acid Dyes	Polymeric amide	80-95 %	5-20%
Basic Dyes	Acrylic	95-100%	0-5%
Direct Dyes	Cellulose	70-95%	5-30%
Indirect Dyes	Polyester	90-100%	0-10%
Dispersed Dyes	wool	50-90%	2-10%
Metal Complex Dyes	Cellulose	10-50%	10-50%
Sulphur Dyes	Cellulose	60-90%	10-40%
Vat Dyes	Cellulose	80-95%	5-20%

Table 2.3: The Amount of Different Dye Discharge from Units

2.2.2 Toxicological Impacts on Aquatic Life

The release of dyes from dye bath is a root cause of a major problem they become toxic

to everything around them [48]. They alter the physical characteristics of water by changing color of water from blue, green or brown to more unnatural colors like red, purple raising concerns [27]. This makes water aesthetically objectionable also presence of Color prevent penetration of sunlight in the water limiting the ecological productivity in the aquatic life. When dyes mix in the water in huge amount, the bacteria in water cannot utilize them as organic source creating a stagnant aquatic environment in aquatic ecosystems. These chemicals have an impact on the water chemistry and associated quality to raise in the pollution indicators such as COD, BOD, and TDS. Oxygen depletion leads to oxygenic stress which limits a significant amount of respiratory physiology in aqueous life forms. While high TDS result in high toxicity and increase in electrical conductivity. All these sun up create conditions that cause aquatic plants and animals to die and decompose [49]. On the basis of solubility, dyes can be categorized as cationic nonionic or anionic [50]. Acid, Reactive and Direct dyes are anionic dyes, among them reactive dyes are hard to remove from environment as they impart bright color and are water soluble [27]. Disperse dyes which are nonionic in nature don't ionize in the aqueous system, whereas azoic, anthraquinone are cationic dyes.

Some of dispersed dyes get bio accumulated in the environment. Their Azo and nitro groups get reduced in the soil to release toxic amines [27]. Dyes with longer half-life from 2-13 years accumulate in the sediments of water which are abiotic in nature like dead or fishes or other things [51] [52]. Even the dyes which are soluble in water get collected in suspended matter and bio accumulate on the all-life forms limiting their physiological processes. Bioaccumulation is unlikely to occur when dyes are present 2000mg/L. Adsorption varies from high to medium in disperse depending on the degree of sulphonation or the ease with which they get hydrolyzed. Group one Dyes are highly sulphonated so they are highly water soluble and get adsorbed on biomass. Group 2 Dyes are also sulphonated but they set adsorbed on sludge. Azo dyes are poorly adsorbed whereas diazo, anthraquinone are better adsorbed than monoaos.

Dyes and aromatic products have the ability of transforming biomolecules in living

organisms at very low to substantial levels due to their highly reactive nature. In living biomass or aquatic sedimentation, such modifications to the living biomolecular structure negatively affect the physiological status of organisms that deteriorate under hypoxic conditions [53]. Acute toxicity of textile dyes results from ingesting and inhalation and can cause bladder cancer [54], dermatitis, neurological problems [52], decreased enzymatic activity [53], and skin and eye irritations. Reactive dye workers may develop allergic conjunctivitis, rhinitis, occupational asthma, contact dermatitis, and other conditions.

The reactivity of dyes has also been closely connected with their persistence. It rises in proportion to the amount and type of electron-donor substituents, which are typically halogen, alkyl, nitro, and sulphonic groups [27]. Additionally, substituents at the ortho and para positions increase the likelihood of cancer development. Aminic group protonation resulted in a decrease in toxicity. Azodyes can cause DNA damage in extreme circumstances, which can lead to mutations, malignant development, and death. At 209 g/g, there was a significant drop in tadpole survival, whereas at the two highest concentrations measured in sediment, there was a significant rise in deformities. Especially with Azo dyes, both the dye and its metabolites have the potential to cause cancer [54].

Several anilines (such as 4, 4'-dimethylendianiline, and 2-nitroaniline, etc.), nitrosamines, dimethylamines, benzidine and its derivatives and various azo dyes (such as benzidine precursor or 4-phenylenediamine, etc.), are known to cause cancer in both humans and animals. Genes involved in necrotic cell death, mRNA processing and chromosomal condensation, were affected by high concentrations of dye (DY7) in sediment at genotoxic levels, which also enhanced the transcription of genes related to cellular stress. Chromosomal aberrations can also be caused by dyes [54]. Duplex RNA and helical DNA were discovered to be impacted by dyes like Azure B [56,57]. While at cytotoxic levels, they block the monoamine oxidase A and MAO-A enzymes, which are connected to the neurological system and affect human behavior. Similar to this, it has been observed that the dye Disperse Red 1 has mutagenic potential on human

lymphocytes, hepatocyte imitating cells, and hepatoma (HepG2) cells, producing the appearance of micronuclei, which indicates mutagenicity at the chromosomal level [58] Additionally, it resulted in DNA adducts, a significant Salmonella spp. cancer-causing event. Disperse Orange 1 also showed mutagenicity, inducing frameshift mutations and base pair substitutions in Salmonella spp. Additionally, HepG2 cells experienced cytotoxicity and death as a result. Although it's prohibited, Sudan I (Solvent Yellow 14), another dietary additive found in paprika, has been enzyme-transformed by the gut microbiota into carcinogenic aromatic amines. Neoplastic liver nodules in rats provide evidence of Sudan I dye [57]. Basic Red 9, a dye that is frequently used in the leather, paper, textile and ink industries, has been linked to environmental and human carcinogens after partially degrading into carcinogenic aromatic amines under anaerobic circumstances. In aquatic ecology, the disposal of such substances revealed the potential for mutations, allergic reactions, skin irritation, cancer, etc [55]. Additionally, they include sarcomas and local tumors in the mammary glands, liver, bladder, hematological system, and bladder. A cationic dye called Crystal Violet (triphenylmethane group) disrupted the chromosomes in Chinese hamster ovules and induced mitotic malfunction and suspension at metaphases. The promotion of ovarian atrophy and calcified gland adenoma in rats as well as hepatocarcinoma, reticular cell sarcoma in the uterus, vagina, bladder and ovary have also been linked to crystal violet. Additionally, it resulted in respiratory/renal failure, cystitis, and skin/digestive tract irritation [59].

2.3 Degradation of Dye Molecules

2.3.1 Physical Methods of Dye Removal

Multiple processes such as photolysis, sonolysis, membrane filtration, adsorption, coagulation, filtration, wet land treatment, electro coagulation, filtration and biodegradation are employed to treat synthetic dyes from aqueous solution. Depending on the type of principal mechanism these processes are divided into two types separative methods such as adsorption, filtration and degradative which can be chemical

and biological. Among the abovementioned methods commonly applied methods are separative, but the discharge of sludge containing dye, absorbents saturated with dye are cons of this method which limits its application, concentrated dye solutions and dye adsorbed on absorbents after final treatment. To say that these methods still leave us with the problem of proper disposal of dyes even after their removal from waste waters. When compared to chemical degradation methods, dye molecules undergo a series of oxidation steps to produce safe chemical compounds. Complete mineralization of dyes is achieved by advanced oxidation processes, dye molecules are reduced to simpler molecules like water, CO2 and inorganic compounds which are then safe to release in water [10].

2.3.2 Advanced oxidation process

A great deal of attention has been given to Advanced oxidation processes (AOPs) as an efficient means for degradation of organic pollutants. It involves the production of hydroxyl radicals (OH⁻), which have a significant potential for oxidation about E (OH/H_2O) ¼ 2.8 V/SHE, making them extremely reactive oxidants [10]. When generated insitu they reduce target contaminant to produce organic radicals through a redox process. The intermediates thus mineralize the organic pollutants due to their extremely strong and non-selective oxidation abilities until complete decolorization [11] The reactive OH species follow one of the three different reactions to attack the organic pollutants: (1) electron transfer, (2) electrophilic addition to p system, (3) H atom abstraction [61,62] which are capable of producing reactive organic radicals starting chain reaction involving organic radicals and reactive oxygen molecules, the generated oxidizing agents OH, HO_2 and H_2O_2 react with the intermediates to complete the mineralization of organic pollutants.

$$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$$
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + HO\cdot$$
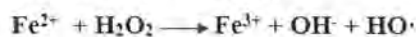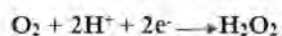
The key reactions involved in AOPs are redox reaction via electron transfer, dehydrogenation (removal of H) and Hydroxylation which is addition to π system. All

of these reactions lead to production of radicals of organic compounds which start reaction with O2 to form reactive oxidizing agents like H2O2, HO2 and OH. It is these radicals which attain complete mineralization of targeted pollutants [10]

2.3.3 Electrochemical Advanced Oxidation (EAO)

EAO processes are types of AOPs, which have been developed in recent decades, they have shown promising results in synthetic dye removal. OH˙ is produced from electrolyte and is used for complete degradation of pollutants. Depending on the way OH medicals are produced based on the involvement of catalyst there are two types of EAOPs direct and indirect. In direct EAOPs hydroxyl radicals are produced directly via oxidation of H_2O on the anode. The production of OH ions depend on the anode material's catalytic activity, the rate at which pollutants diffuse at the active site of the anode and the current density that is applied in the reaction [63,64]. One of the major advantages of the process is that no external reagent needs to be added to aid the production of OH ions. Whereas in indirect EAOPs, Fenton's reagent must be added to aid the production of OH radicals, which can be H_2O_2 of ferrous ions. When H_2O_2 is added externally or produced insitu through the reaction between the catholyte, it reacts with anodically produced or externally added ferrous ions. For insitu production of H_2O_2 a suitable electrode material should be used. A very good example of such an indirect reaction is the Electro Fenton process where ferrous ions added externally react with H_2O_2 which is generated internally react together to produce OH ions.

The advantages EAOPs have over conventional physical treatments methods employed for removal of organic pollutants is that this process is environment friendly [65]. The main requirement to drive the reaction are electrons and OH ions which can be easily generated insitu. These processes have been reported to have high efficiency of pollutant removal, safe operational conditions and to some extent they can be automated [62].

2.3.4 Electro-Fenton (EF) Process

In recent years electro-Fenton system have been studied greatly, it is an environment friendly and economically feasible process, which efficiently removes recalcitrant organic pollutants from water. It was first reported by Outran and Brillas in the year 2000. As a degradation process this process received a lot of recognition due to its efficient and fast removal rate [61]

Electro-Fenton process is a type of indirect AOPs, this method effectively removes persistent organic contaminants from water. The immense degradation efficiency of persisting contaminant and Eco friendliness make it a desirable process for pollutant degradation in treatment plants. The principle of Electro Fenton's reaction is production of Fenton's Reagent (FR) electrochemically which is achieved by adding Ferrous salts as a Fe-2 source. FR is a mixture of H2O2 and Fe-2, that react to form OH- ions (eq2) [10]. The Electro-Fenton Process provides greater benefits than the chemical Fenton process due to the high utilization efficiency of Fenton's reagents (such as H2O2) and cost-effectiveness by minimizing the storage and transportation of chemicals. The electrochemical regeneration of ferrous ions throughout the process is an advantage it has over other Fenton processes [61]. One of the major operational conditions that must be maintained is pH, for an efficient Fenton reaction pH must be maintained in a range of 2-3[66], this is because ferrous ions are regenerated in the cathode. Fe (OH) undergoes $F+^3$ regeneration at low pH [67] Fe ions are regenerated.

The form of Fe added is also one of the major decisive factors. To achieve high efficiency of treatment Fe+2 is added as Fenton catalyst for OH radicals' generation in most of the reported literature, other forms of iron such as Fe0, which has zero valance is also added as iron source, Fe+3 is also added as iron source, but it is only ferrous ion that undergoes regeneration. Highest removal efficiency is achieved by addition ferric ions, followed by ferrous ions and then zero valent Fe 0 ions [10]. Creating an energy-saving E-Fenton System should be very appealing and challenging [12]. Recently, bio-electrochemical technologies for wastewater treatment, including microbial Electrolysis cells (MECs) and microbial fuel cells (MFCs), have been created [13]. Numerous

studies on the microbial fuel cell (MFC) demonstrated that the organics present in wastewater can supply electrons on a continual basis. The use of these Bioelectron's generated by microbial metabolism can make the bioelectro-Fenton (Bio-E-Fenton) concept practicable. [68]

2.3.5 BioElectrofenton System

It is a hybrid system in which Microbial fuel cell setup is used to generate hydroxyl radicals in cathode by the external addition of iron source. The BioElecrtoFenton degradation of organic contaminants have shown promising results and are an effective replacement for H_2O_2 production. The concept of MFC driven Fenton reaction involves degradation of pollutants by Fenton reaction in which, Fenton reagent is created insitu by electrons donated from microbes. The reactor setup involves bio anode, abiotic cathode compartments separated by anion or cation exchange membrane. In the anode compartment, which constitutes wastewater and sludge, organic matter is broken down by anaerobic bacteria through biochemical reaction, which develops an active biofilm on anode. In anode bacteria act as biological catalyst, which oxidize the organic compound or electron donors present in wastewater [68]. This breaks down generates electrons and protons. Electrons are carried from bacterial cells to bio anode which then follow external circuit to enter cathode and are collected at cathode. Protons on the other hand pass through the cation exchange membrane and enter the cathode. Protons and electrons react with the oxygen in the cathode to form H_2O_2, along with electricity production. H_2O_2 reacts with Fenton reagent to generate reactive hydroxyl ions that react with contaminants, producing intermediate which are degraded to CO_2 and water with the passage of time.
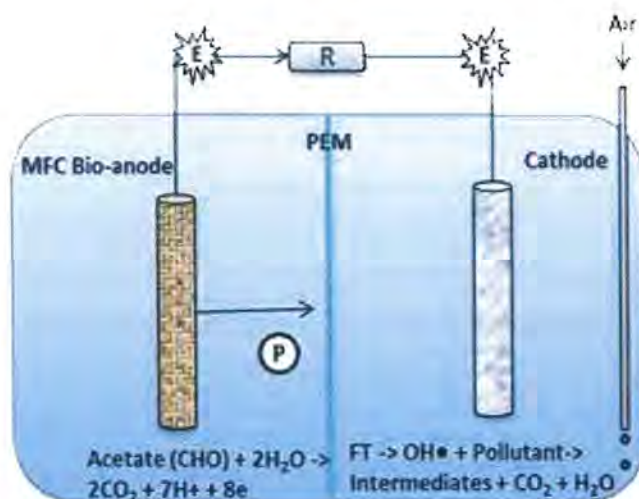
Figure 2.2 : Microbial Fenton Cell

2.3.5.1 BEF For Treatment of Waste

BEF is capable of complete reduction of wastewater and simultaneously achieves complete oxidation of organic pollutants. The process has been studied and developed since last decade. The first study was conducted in the year 2009, Ni and Zahu reported complete degradation of P-nitro phenol within 12 hrs. of time, 85% of Toc removal was achieved in 96hrs [69]. This laid the basis of pollutant removal through MFC coupled electro Fenton system. A variety of contaminants are targeted for removal through this process while conserving energy and chemical inputs. A variety of cathode and anode materials are being utilized to acive maximum amount of pollutant removal. These contaminants range from antibiotics, drugs, medicinal herbs which are a threat to water ecology to coal gasification, and landfills. Xu P et all targeted coal gasification present in wastewater as pollutant and achieved removal of 85.7% phenols, in a double chambered BEF system with a proton exchange membrane separating the two, with carbon felt as anode as well as cathode with $FeVO_4$ modification. With the production of 0.05mmol⁻L 849.7mWm⁻3 power density was achieved [70]. In a study conducted by [71] Treated wastewater contains Triphenyltin choloride in BEF system. They used graphite felt anode and granular graphite modified with Fe_2O_3, approximately 78% of

pollutant was removed, 57.25mWm-2 power density was achieved with 135.96µmol·L of H2O2 was achieved [71]. Zhang L, Yin X and Li SFY targeted the removal of paracetamol from wastewater systems using graphite felt as anode and graphite plate as cathode, to achieve 70% removal and generated power of 217.27mWm-2 [73].

Previously [74] achieved 79% of COD removal and 935mWm-2 power density along with 181mgL - H2O2 production, when they treated landfill leachate using carbon clothe as anode and activated carbon modified through treatment with glucose and zero valiant iron, proton exchange membrane separating cathode and anode [74]

2.3.5.2 Treatment of Dye Solutions

Textile effluents are one of the major causes of public safety concern, as dye molecules are carcinogenic as well as recalcitrant so their treatment before discharge into waterbodies is essential.

Textile discharge owes visible effects of water pollution, specifically red and orange colored dye molecules that are not normally found in water bodies. Most of the dyes are anthraquinone, azo, Indigo and xanthene derivatives, these dyes are difficult to degrade and pose a high risk of health hazards. Azo dyes, being most used, are studied and worked upon by scientists for efficient removal. Several studies have been reported of dye removal through Bioelectro Fenton reaction which are conducted in double Chambered MFCs, to minimize their effect on public health. When oxidized in Fenton driven MFC several operational parameters should be maintained, which are pH, iron conc, catholyte conc, air flow rate and applied voltage, these parameters affect the dye degradation considerably [14].

In the year 2010 [75] demonstrated 95% decolorization of 15mg-L Rhodamine Blue Dye in cathode, and 95% of TOC removal within 12hrs keeping the other operating conditions such as 0.2g -L Fe2O3, pH at 3 and aeration rate of 300mL min-1. They operated without external energy input but efficient mineralization at cathode is highly dependent on the current densities that are generated under short circuit conditions. Their study also showed that anodic wastewater can also be reduced to simpler forms [75]. Orange 2 was 100% decolorized at pH 7 in cathode

chamber within 14 hours by a study conducted by [76] in 2010. They compared the decolorization data with MFCs without external addition of Fenton's reagent, which showed 8-10% of decolorization in 14 hrs., this study also proved the Importance of addition of iron source to drive Fenton reaction [76]. [77] ran an anodic Fenton reaction in which 85% Acid orange 7 was degraded in anodic chamber within 30 min. Sacrificial iron source was used as Fe source, H2O2 was added externally. The energy to drive Fenton reaction in anode was driven from coupling with MFC based double chambered air cathodes, which influenced dye degradation, demonstrating that this could be an energy saving and cost-effective method for dye removal. The study also proved that degradation rate increased with increased oxygen concentration [77].

[78] reported decolourization of 5 dyes Crystal Violet, indigo Carmine, Reactive blue 5, Lissamine green B and Poly R-478 in MFC coupled Fenton reaction. The dye degradation percentages were reported were 96.2%,98.2%,88%,97% and 19.1%. H2O2 was externally added into the system, TOC removal was 82% after 9h. The study conducted also confirmed that higher decolourization efficacy can be achieved by integrating MFC with Fenton reaction [78].

In a study conducted by [80] compared Fenton Based MFC's performance with controls, having 1) no Fe 2) open circuit and no aeration at cathode. They reported 97% decolorization of Methylene Blue dye along with wastewater treatment. Reactors operated as controls showed no visually detectable decolourization, confirming that external circuit connection, aeration and Fe are major driving factors in Methylene Blue decolourization. Zhang et al also reported decrease degradation and H2O2 production with increase in external load resistance from 0-200 ohms [80].

Ling et al contributed to literature by degrading Methyl orange via bio-electro Fenton system, using Activated Carbon Felt modified with Ferrous oxide, electrodes which were treated with nitric acid to increase Fe ion deposition on the electrodes. On average 88.63mol/L of H2O2 was produced throughout the retention time, 86% degradation was achieved in 72 h. They verified that electrode modification led to increased H2O2 production, when conditions were 2mMol FeSO4, aeration rate of 750/min, pH 2 and

external resistance, 100 Ω. This work laid the foundation of developing biofriendly electro Fenton system [81].

Li et al reported degradation of Orange G in a Fenton driven Microbial Reverse Electrochemical cell, 100mg\L of dye was degraded up to 88% in 3 hours' period and to 100% in 5 hours. No significant degradation was observed in controls operated on open circuit condition, anodes without microorganisms, without Fe. Negligible amount degradation in controls proved that degradation in Fenton based reactor is based on Fe concentration, presence of electrogens in anode and oxygenation. Hung et al reported in the year 2018 that increasing the concentration of ferrous ions also directly affected the degradation of Ethyl orange in MFC driven Fenton reaction. The production of a higher amount of H2O2 in cathode resulted in increased concentration of OH ions. But with increase in Ethyll Orange concentration with constant Fe concentration dye degradation decreased in cathode [82].

Zou et al optimized operational conditions for degradation of Methylene Blue, in a small-scale benthic microorganisms-based fuel cell, which was driven by Fenton reaction in continuous mode. The best conditions observed for dye degradation reported were pH 2, FeSO4 was 0.02mMolar, 50mMolar of Na2SO4, 0.04V external current and 350 mL\min oxygen supply proved best for dye degradation. When provided such conditions in a scaled up 2 Liter reactors efficient dye removal was observed in time of 28h, 90% dye was degraded in first 12h and increased to 99% in next few hours [83].

2.4 Electrode Materials

Electrodes used in MFC cells is of great importance, electrodes characteristic such as material, reactivity, conductivity and surface area. They play vital role in biofilm development in case of bioelectrodes so their surface characteristics such as pore size, thickness, porosity matters before even running an MFC. Sometimes cathode and anode are both used of same material but sometimes they are if different material. Commercial based electrodes can be modified to improve their conductance it they can be fabricated in labs from raw materials. In case of Fenton reaction anode is biotic and cathode is

cathode is abiotic. So, the anode used in this scenario must highly support biofilm formation as the formation of Fenton's reagent is highly dependent on the electron coming from electrogen grown in anode through external circuit. Cathode in the Fenton based cell is the working electrode, this is the electrode which will electrochemically and directly take part in generating OH^- ions. Cathode's ability to react with O_2 and low capability to degrade hydrogen peroxide electrochemically is the reason carbon-based electrodes are preferred to be used to aid Fenton's reaction [10]. Several materials are preferred to be used as anode and cathode we'll discuss them below.

2.4.1 Anode materials

Preferably carbon material electrodes are used to develop biofilms efficiently. They effectively support the life forms, they receive electrons from cell and transfer them to their respective destination therefore before deciding which material to be used we must consider certain characteristics of material such as good bacterial adhesion, efficient conductivity of electrons, biofilm ability, availability, porosity, surface area and above all they should not be costly [84]. Carbon based electrodes fit most of these criteria, so they are highly sought after. There is a huge variety of carbon materials to choose from, some are commercially available, some are modified and other are fabricated from scratch. Carbon brush, felt,paper,cloth and mesh are mostly preferred anode materials owning to their biocompatibility and low cost. Graphite is one of the mostly used anode materials as it is highly conductive [84]. They are available as rods, felts brushes and powder form. Their application in MFC were reported in several studies Logan et al reported power density of 26mWm-2, when they used graphite rod as anode. Maximum Dye removal percentage of 80 percent was achieved, where pollutants in industrial wastewater provided electrons essential to drive the reaction [85]. Use of modified Graphite was reported by Pico et al. Used aryldiazonium salts to modify electrode to enhance their biophilic property by increasing surface charge and hydrophobicity [86]. Carbon material provide higher energy output hence MFCs with higher efficacy are reported in literature. Activated Carbon powder is known to reported to increase interaction of electrode surface and the microorganisms growing on them. Studies

reported on this topic confirm better biofilm development and increased power density [84] such as Leripoulos et al used PTFE to coat carbon black, cloth to attain the power output of 60.7 mWm-2 higher than unmodified ones [84]. A carbon-based electrode with high surface area, excellent mechanical properties, highly conductivity and stability are Carbon Nano Tubes, they are also used to modify metal-based electrodes [84]. Composite electrodes which comprise of metal oxides, are also one of the choices available to be used as bio anodes. Metal oxides are used with other materials that are highly conductive .3D anodes have now grabbed attention because of their efficacy, they have proven to be efficient in COD removal [87] increased current density. When they are modified using phosphate, ammonia and trace metals they become more capable of supporting biofilm growth.

2.4.2 Cathode Materials

Cathode is the working electrode in Fenton based MFC, they accept electrons from anode compartment and then transfer them to catholyte for reactions to occur. Carbon material are 3 dimensional materials, with high porosity, which come in use for adsorption of dissolved oxygen on their surface. This adsorption capability of carbon-based electrodes aids in production of higher amount of H_2O_2. Carbon based cathodes are preferred as they are stable, highly conductive and cheap. Various materials are used as cathode for efficient pollutant removal these include carbon felt, carbon black, activated carbon, carbon brushes, graphite, Carbon Nano Tubes and stain Steel mesh. Graphite is used as it provides good current conductivity. Nidheesh and Gandhimathi used graphite electrodes for removal of Rhodamine B, they reported increase in efficiency of electrode functionality as they were immersed in the cells. This was due the increased contact between oxygen bubbles and surface of cathode. Authors reported that using electrodes which are lengthy attain higher percentages of dye degradation [10] Among various forms of Graphite that are available commercially Graphite Felt is the one most used for its large surface area. Yu et al.used PTFE and carbon black modified graphite felt, to mineralised methyl orange. The efficiency of modified

cathode was 4times higher than unmodified ones, also in terms of H_2O_2 production they proved to be more effective, 10.7 times higher amount of H_2O_2 was produced. Stainless is also used as cathode as it is an excellent current collector, a study was conducted by Almomani et al. In which they used SSM cathode in a double chamber cell, in which various dyes like Congo Red, Methylene blue and yellow dirmaren were 100% degraded. Wang et al. Modified SSM by generating CNTs, and iron phthalocyanine was added as catalyst, an increase in current density was reported by 2595 times, using these modified electrodes they reported dye degradation upto 84.6% in only 12 hours [69] Activated carbon powder when made pasted onto SSM or stainless steel, gets transformed into an electrode with highly surface area for H_2O_2 production, these electrodes are efficient and cheap. These carbon-based electrodes are cheaper than metal-based electrode and much easier to modify which make them a feasible option to choose from. Another significant property of AC is it can be prepared from organic raw material in a study reported by Huang et al. Granular powder of Activated carbon was produced from bamboo as raw material, the powder was then filled in the cathode chamber completely. The study aimed to treat methyl orange dye and attained more than 80% decolourization. Electro Fenton cell with Granular activated carbon powder attained higher voltage and power output. Maximum being 39.73mE/m [88].
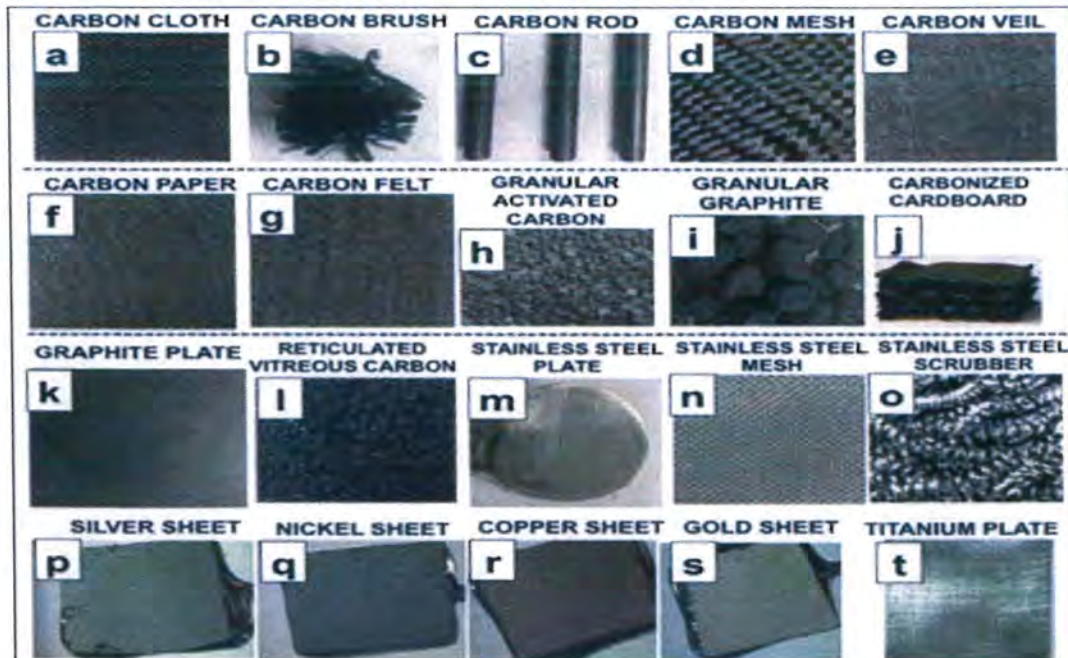
Figure 2.3 : Carbon Based Electrode Materials used commonly [69]

2.5 Operational Models of MEF System

Despite being newly developed concept, it has a considerable solution for environmental waste management. Though idea is still under development, but the studies reported have used this concept in different configurations, with different microbial communities, different membranes separating the anode and cathode compartments, different pollutants. As MEF is and amalgam of Microbial fuel cell and Microbial Electrolysis Cell so different reactor setup, in batch, continuous flow and sequential manner have been introduced to achieve higher rate of dye removal, quick and efficient treatment by changing the retention time. As per based on configuration the cells can have single compartment, double compartments or can be hybrid. We'll discuss the different types of MEF systems [69].

2.5.1 Batch Mode

As the name suggests catholytes and anolytes are added for a specific time for treatment

and then they are changed. Mostly by scientist's batch mode is preferred as it gives the operator tight control on the cell and of course is easy to handle. The cathode is continuously aerated and stirred by magnetic stirrer. Retention time varies from hours today depending on the efficiency of degradation that is required to treat the specified waste. Simpler organic compounds take less time where complex ones take longer. The limitations of batch mode include limited number of organic components for bacteria to utilize, this means slower mass transfer. With passage of time nutrient also get depleted and efficiency of reactor is also reduced [90].

2.5.1.2 One step batch treatment

In one step batch treatment only one cell is used for treatment of pollutants and generation of electronic potential. The types of reactor configuration are given below.

2.5.1.3 Microbial Fuel Cell based Electro Fenton System

It is a two chambered reactor setup for complete removal of organic compounds through Insitu generation of Fenton reagent. The pollutants treated are mostly dyes and pesticides. This is a cost-effective system for pollutants removal as energy is generated rather than being supplied. This is self-sufficient system as OH radicals generated are enough to effectively remove
pollutant.

2.5.1.4 Automated Microbial Electro-Fenton system

It is a recently developed reactor setup, which includes carbon adsorbed on FeO cathode for Fenton reagent generation and similar anode connected by an external circuit. The fabricated electrodes have a large surface area owning to their macro and micro porous. It is an automatically operated system which is functionally similar to the process of transpiration in plants. These systems are used for contaminants degradation of soil and sludge [69].

2.5.1.5 MFC-3D Electro-Fenton system

It is a granular Activated Carbon filled simple fuel cells reactor. GAC is either filled in both chamber or in one of them as electrode. The filled chamber shows increases

voltage and power output as the GAC has a three-dimensional structure, so it has more contact points with catholyte or analyte depending on the chamber it is present in, achieving dye degradation efficiency of 84% [69]

2.5.1.5. Microbial Reverse Electro-dialysis Cell

MREC is a hybrid system in which Insitu electricity is generated by microbes which use organic compounds and the slope which is between various fresh and salt channels that does not require power input. A RED stack separates anode and cathode chamber, so the reaction going on in them are undisturbed by continuous supply of waste water. The efficiency of MREC is high when compared to MEC and MFC together in terms of dye removal which in case of MREC is 99% and cost of treatment which is 45% lower than both [69].

3. Material and methods

The following research was designed to study the degradation of azo dye (acid red 114) in Microbial Electro-Fenton driven Microbial Fuel Cell (MFC) through the production of hydrogen peroxide. All the research was carried out in the Department of Microbiology's Microbial Biotechnology and Bio-Engineering Lab at Quaid-i-Azam University in Islamabad, Pakistan.

3.1 Electrode Fabrication

The electrodes were fabricated using Stainless steel mesh of area 4 cm^2 as a support material as well as a current collector. Firstly, the stainless-steel mesh was washed with distilled water to remove any impurities and then was weaved through using copper wire (0.8mm width). A thin layer of conductive epoxy was applied and allowed to dry in a completely sterile environment for a brief period.

3.1.1 Procedure

Graphite powder (30μm) electrodes were fabricated by a simple fabrication procedure. Magic Epoxy was used as a binder and finely grounded graphite powder was used as an active material. 2.4g of depoxy was mixed with 0.32 g of activated graphite powder forming a paste. This paste was applied on both sides of the wired stainless-steel mesh. After evenly applying the GP/binder mixture, reinforcing was carried out by quickly adding additional GP to both sides of the electrode and repeatedly compressing it between two flat metallic surfaces (SS). The fabrication of the electrode was marked by the achievement of a nearly homogenous surface. Manual pressing was carried out until the AC atoms began to cross-link. The purpose was to improve the conductance for increased electron transport and to reduce the resistance of electrodes. The electrodes were dried overnight and then washed again with distilled water to remove the unbound graphite. [94]

Figure 3.1 : Graphite powder Electrodes

3.1.2 Electrode characterization

Redox potential/ conductivity test

CV (Cyclic Voltammetry)

Cyclic voltammetry (CV) was used to determine the electrochemical response of electrodes under applied potential. It is used to determine the variation in redox potential in different electrolytes. CV was done at turnover conditions over a potential range of (-2.5V to 2.5V) at a scan rate of 20 mV^{-1} sec. All the electrochemical measurements were done using Graphite as the working and counter electrode while Ag/AgCl was used as a reference electrode.

The conductivity of the electrodes was assessed using a potentiostat, connected to a laptop computer, and voltammograms were generated using EC lab software. The performance of electrodes in the electrolyte solution was measured. Fabricated AC/graphite electrodes were used as working electrodes and an AgCl electrode was used as a reference electrode. The conductivity of the electrodes was assessed in various

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electrolytes 15,30 ppm red dye, 15,30 ppm pesticide,0.5M H_2SO_4, and NaOH respectively.

● Thickness of electrodes

The thickness of AC/graphite electrodes was measured using Vernier callipers, zero was corrected and it was compared to that of plain stainless-steel mesh.

● Resistance

Resistance of electrodes was measured using (prescion) multimeter in AR114 solution.

● Voltage

A precision multimeter was used to measure the voltage production of the electrodes in the PBS solution

● FTIR Analysis

The surface functional groups of bare stainless-steel mesh and Activated carbon- modified electrodes were analysed by FTIR. FTIR analysis was performed on a Cory 630 FTIR spectrophotometer (Agilent), to investigate the changes in the bare and activated carbon modified electrode. The transmittance spectra were recorded in the range of 4000-515 cm^{-1}.

● SEM

A Quanta 200 field emission scanning electron microscope from the COMSATS University Islamabad was used to examine the micromorphology of bare stainless steel mesh and AC modified stainless steel mesh electrodes samples, with an accelerating voltage of 3000 V, a working current of 10 μA, and a working distance of 2.0~2.5 mm.

3.2 Scaled Up Electrode

16cm^2 electrodes were fabricated by doubling the amount of depoxy (4.8g) and AC (0.64g) respectively, after confirming the electrode's redox potential. After fabrication, they were again tested on various parameters.

3.3 Architecture and Operation of Microbial fuel cell

Microbial fuel achieves a higher percentage of degradation. In this study, H_2O_2 production was measured over the course of changing conditions (parameters) Different concentrations of H_2O_2 produced throughout the experiment were monitored along with degradation within 24 hrs. Different dye concentrations were degraded via the Fenton reaction in dual chamber MFCs in this work, and the current response, COD elimination, and Energy production were examined over time. The overall reaction is as follows:
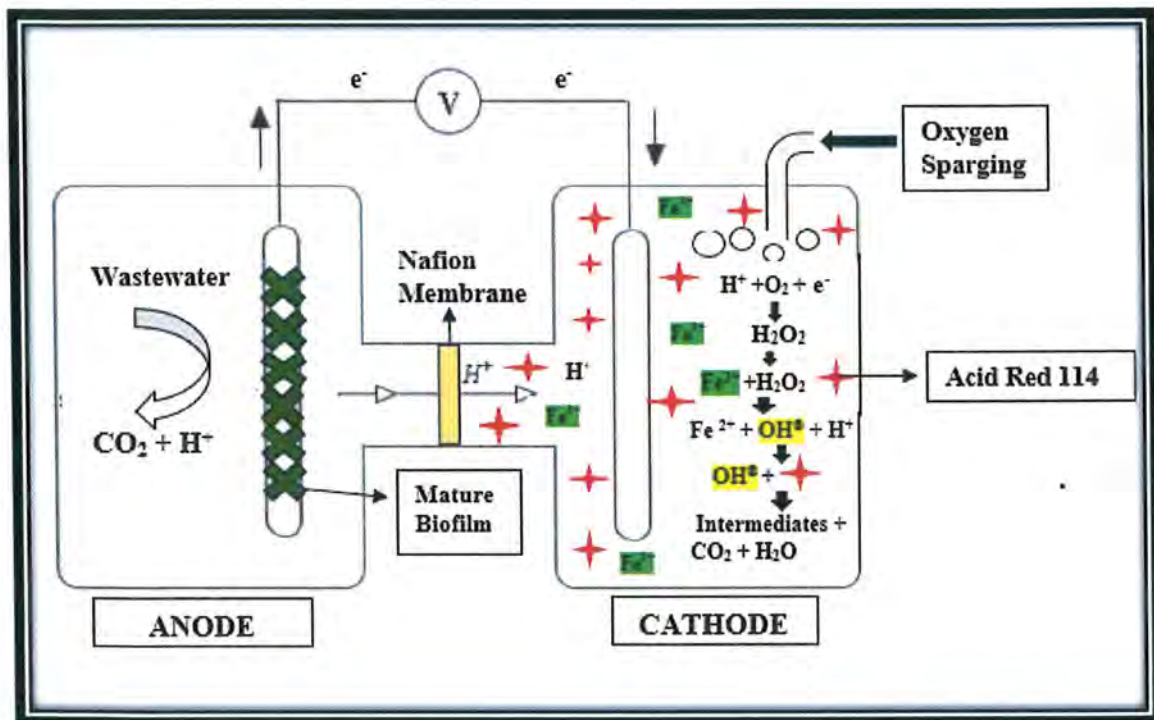
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2{}^{\bullet} + H^+ \qquad [100]$$

$$OH^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + OH^- \qquad [101]$$

$$OH^{\bullet} + H_2O_2 \rightarrow H_2O + HO_2 \qquad [102]$$

$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2 \qquad [103]$$

$$Fe^{2+} + HO_2{}^{\bullet} \rightarrow Fe^{3+} + HO^{2-} \qquad [104]$$

$$Fe^{3+} + HO_2{}^{\bullet} \rightarrow Fe^{2+} + O_2 + 2H^+ \qquad [105]$$

$$HO_2{}^{\bullet} + H_2O_2 \rightarrow H_2O + OH^{\bullet} + O_2 \qquad [107]$$

$$HO_2{}^{\bullet} + HO_2{}^{\bullet} \rightarrow H_2O + O_2 \qquad [100]$$

$$O_2{}^{\bullet-} + Fe^{3+} \rightarrow Fe^{2+} + O_2 \qquad [106]$$

$$O_2{}^{\bullet-} + Fe^{2+} + 2H^- \rightarrow Fe^{3+} + H_2O_2 \qquad [107]$$

$$HO_2{}^{\bullet} + O_2{}^{\bullet-} + H_2O \rightarrow H_2O_2 + O_2 + OH^- \qquad [108]$$

$$OH^{\bullet} + HO_2{}^{\bullet} \rightarrow H_2O + O_2 \qquad [109]$$
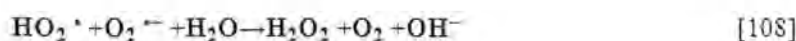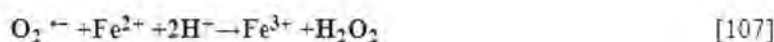
Fig 3.2 Schematic design of double-chambered MFC-based Bio-Electrofenton System

The following steps were involved in the Architecture and Operation of the dual chamber microbial fuel cell figure.

3.4 Fabrication of Cells

A double chamber MFC was constructed in a conventional "H" shape design where the anode and cathode chambers were sample collection bottles (110ml) joined by a tube (3.5 cm long) holding a slice of proton exchange membrane (4×4cm Nafion 115, Gas Hub Pte Ltd, Du Pont Company, USA). Silicone sealant was used to attach the Nafion membrane to the mouth of one tube. Salt bridge 2.5cm long was attached to nafion and glass slab. Two Glass slab held salt bridge and nafion firmly pressed in between them. Silicon sealant was used to prevent leakage through the slabs. Slabs were tightened by using screws. The salt bridge was then inserted into the plastic bottles. And the

connection was ensured by applying epoxy along all sides of the bottles and slabs. As for the caps of the bottles, small holes for sample collection, oxygenation, and electrode wire were made into them.
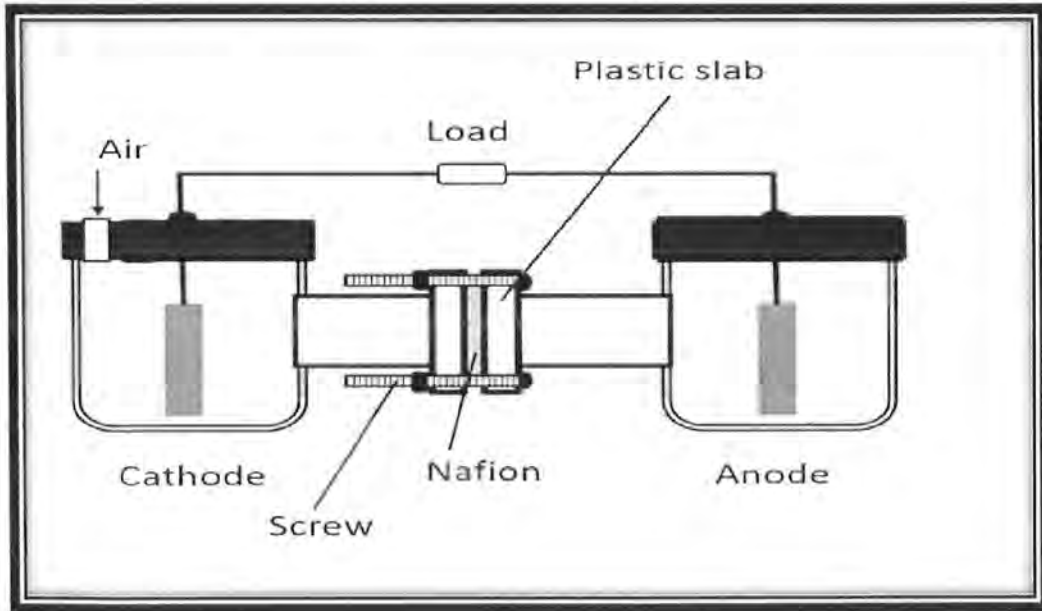
Figure 3.3 : Diagrammatic Assembly of Dual Chamber Reactor

3.5 Operation of MFC

3.5.1 Anodic electrode fabrication

Carbon-felt electrodes were used as bioanode in the anode chamber because their large surface area enables biofilm growth better than other carbon-based electrodes. 4×4cm electrodes were cut and soaked in distilled water for 2-3 hrs then it was placed in 40% nitric acid for etching, at 80°C for 4 hrs. It was then taken out placed in a clean Petri dish and dried in a hot air oven at 80°C overnight. The prepared electrode was then wired using naked copper wire, the uncovered part of the wire outside the electrodes was coved with depoxy to prevent the loss of electrons in the anolyte.

3.5.2 Cathodic Electrode fabrication

Electrodes were fabricated in the size 4×4 corresponding to the diameter of the salt bridge 2.4g of depoxy was mixed with 0.32g of AC/graphite powder applied on both sides of the washed and wired stainless steel mesh. After that, both sides were rolled and pressed manually. The uncovered part of the wire was covered with depoxy to prevent the loss of electrons in the catholyte.

3.5.3 Sample collection

Samples of wastewater and sludge were collected from the Quaid-i-Azam university type D colony. Domestic wastewater and anaerobic sludge were collected in sterile bottles with the help of a sterile spatula. The sterile bottles were submerged in a wastewater collection tank for the sampling of domestic wastewater as well as for anaerobic sludge which was collected from an anaerobic digester. The sample container was tightly closed and was transported back to the lab and stored in a cool and dry place until further use.

3.5.4 Anode chamber preparation

The anode in this study was highly anaerobic, the contents of the anode chamber were wastewater, anaerobic sludge, and 5ml of 50mM sodium acetate. Before using the fabricated clues were sterilized under UV light, working volume of the anode was 110 ml, and wastewater and sludge were mixed in a ratio of 50:6 i.e., for 50 ml of wastewater 6 ml of sludge was poured into the mixture, and the volume of the mixture was increased up to 110ml and then poured into the anode. The fabricated carbon felt electrode was already fixated on the cap through a hole made into it using depoxy. 5 ml sodium acetate was mixed into the anolyte. The anode was quickly caped and then placed in anaerobic camber for nitrogen sparging to remove any oxygen in the anode. 99.99 percent pure nitrogen gas was sparged into a caped anode through one of the holes in the cap, the anode was sparged for 20 min as reported in [95] After 20 min cells were taken out and sealed completely using silicon sealant. The cells were then incubated in an anaerobic chamber for 14 days to develop biofilm on the Carbon felt electrode. On the fourteenth day, the current was measured at the anode to monitor the

growing biofilm using a multimeter the experiment was started after biofilm development.

3.5.5 Cathode chamber preparation

The cathode was abiotic and aerobic ac/graphite electrodes were fixed in the cap so it could be suspended in a cell facing the salt bridge. Catholyte consisted of Na_2SO_4, $FeSO_4$, and Acid Red 114 solution. pH 3 was maintained in the cathode chamber for providing acidic conditions. The oxygen supply was provided via oxygen motors and pipes were inserted through caps. Catholyte was repealed every day for every new parameter for the time cells were running. Samples for H_2O_2 detection were taken from the sampling pit. The cathode chamber was cleaned thoroughly every day using methylated spirit swabs.
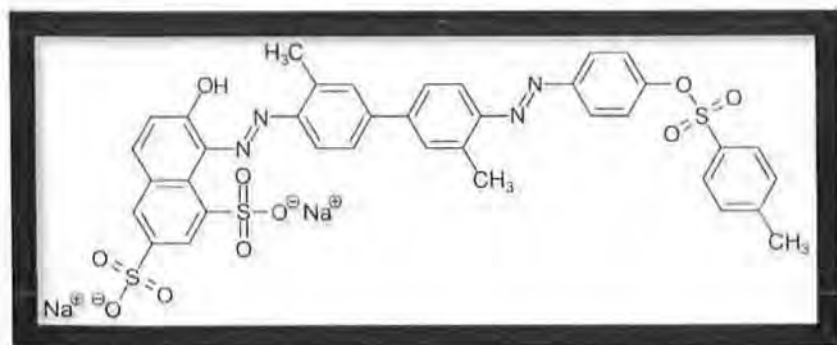
3.5.6 Structure of AR 114

Figure 3.4 : structure of Acid Red 114

3.6 H2O2 Standard Curve

H$_2$O$_2$ production is the main reaction in the Fenton where it combines with oxygen to degrade pollutants and its monitoring was one of the main focus of the study. The H$_2$O$_2$ standard curve was made before starting the optimization reaction. Serial dilution of 35% pure H2O2 was made in deionized water and absorbance was measured at 351nm. All the glassware used was washed with autoclaved deionized water.
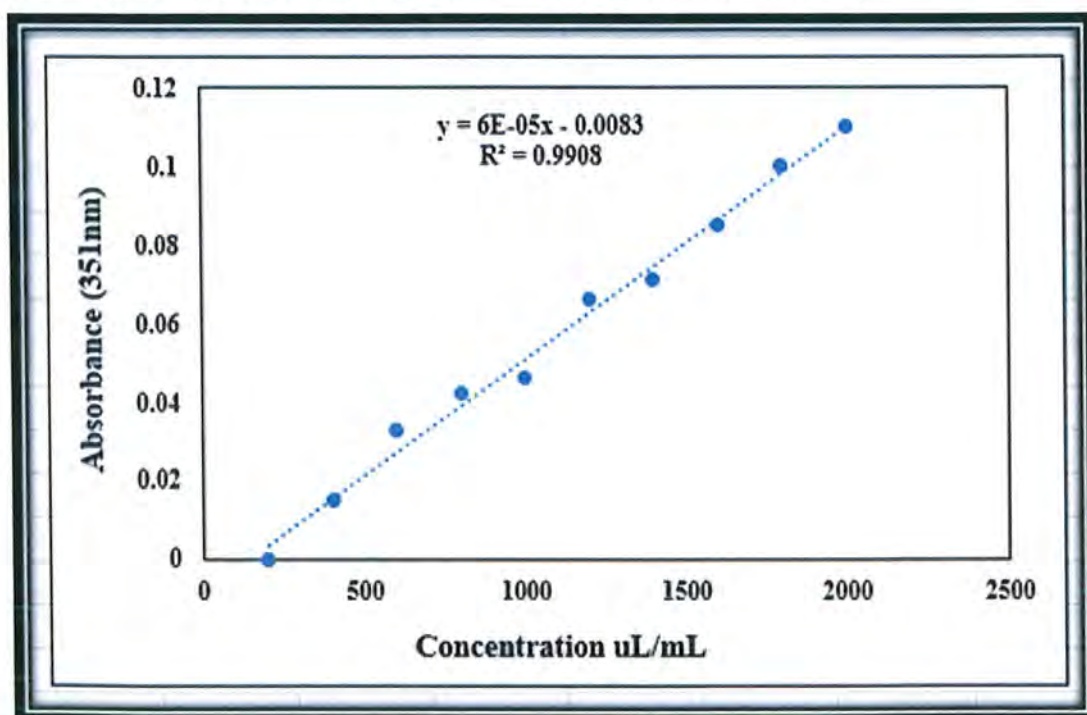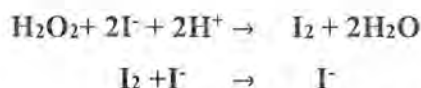

Fig 3.5 : Standard Curve of Hydrogen Peroxide

3.6.1 H_2O_2 Measurement Protocol

Hydrogen Peroxide was measured using the standard Iodide Method based on the spectrophotometric analysis of I^{-3} produced with the addition of the H_2O_2 into the concentrated solution of I^-. The reaction takes place according to the following equation:

$$H_2O_2 + 2I^- + 2H^+ \rightarrow I_2 + 2H_2O$$

$$I_2 + I^- \rightarrow I^-$$

The basis of the Iodide method is marked by the estimation of I^{-3} via spectrophotometric analysis. Two solutions were used in this method. Their composition and method are given below.

Solutions

- **Solution A**

Solution A was made by dissolving 33g of KI, 1g of NaOH, and 0.1g of Ammonium molybdate tetrahydrate in 500 ml of distilled water. The pH of the solution was maintained at 12.8.

- **Solution B**

Solution B was an aqueous buffer of KHP in 500 ml of distilled water. The pH of the solution was maintained at 4.03.

Procedure

Equal volumes of Solution A and B were added into the test tube with the help of a pipette followed by the addition of H_2O_2 solution. The absorbance of the final solution was observed at 351nm in the cuvette. The concentration of the hydrogen peroxide was determined by subtracting the blank absorbance from the absorbance of the final solution. The blank consisted of an equal volume of Solution A and B [96].

3.7 Operational Properties of Cell

Biofilm was fully developed in the anode chamber after 14 days of incubation. The cell was then run on a working bench with no changes in the anode whereas the catholyte was daily changed and monitored.

Optimization Reaction

The experiment was conducted to study the effect of 5 different operating factors including pH, temperature, catalyst concentration, catholyte composition, and different catholytes on dye degradation and H_2O_2 production. All these parameters were changed over time along with the functional cells. The varying concentrations of dye, $FeSO_4$, Na_2SO_4, pH, and different electrolytes were altered and the change in H_2O_2 production was measured.

1. Varying initial pH

H_2O_2 production and dye degradation was checked by varying five consecutive pH values 2,3,4,5,7 keeping other conditions the same (20ppm dye, 5mM Na_2SO_4, 2mM $FeSO_4$, oxygen supply). The reaction was run for 24 hrs and H_2O_2 production was measured at 5,20,40,60,80, and 100 min at 351nm.

2. Varying initial FeSO4 Concentration

As H2O2 production is directly related to FeSO4 concentration so it was one of the most important parameters. Five FeSO4 concentrations of 0,25,50,75,100 mM were varied keeping other conditions the same (pH 3, 5mM Na2SO4, 2mM FeSO4,20ppm dye, oxygen supply). The reaction was run for 24 hrs and H2O2 production was measured at 5,20,40,60,80,100, and 120 min at 351nm. Dye degradation was measured initially and after 24 hrs.

3. Varying Catholyte

The effect of different catholyte on H_2O_2 production and dye degradation was monitored. 50mM KCl, NaCl, Na_2SO_4, $KHPO_4$, and NaNO3 solution were used as catholyte keeping other conditions the same (pH 3, 20ppm dye, 2mM $FeSO_4$, oxygen

supply). The reaction was run for 24 hrs and H_2O_2 production was measured at 5,20,40,60,80, and 100 min at 351nm. Dye degradation was measured initially and after 24 hrs.

4.Varying initial Catholyte (Na_2SO_4) Concentration

H_2O_2 production and dye degradation at five different catholyte concentrations 0,25,50,75, and 100mM were monitored keeping other conditions the same (pH 3, 20ppm dye, 2mM $FeSO_4$, oxygen supply). The reaction was run for 24 hrs and H_2O_2 production was measured at 5,20,40,60,80, and 100 min at 351nm. Dye degradation was measured initially and after 24 hrs.

4. Varying Dye Concentration

Acid red 114 dye concentrations were changed consecutively for 5 days in increasing order keeping all other conditions the same (pH 3, 5mM Na_2SO_4, 2mM $FeSO_4$, oxygen supply). Initially, 15ppm concentration of dye was added to the catholyte mixture and the reaction was run for 24 hrs. Then 20, 25, 30, and 40 ppm dye concentrations were added to the Na_2SO_4 solution. H_2O_2 samples were measured at 5,20,40,60,80, and 100 min intervals at 351nm. Dye degradation was measured initially and after 24 hrs.

3.7.1 Dye Decolorization Assay

The dye decolorization assay was analyzed by UV- spectrophotometer in terms of percentages. The equation used for calculating the decolorization percentage was [109]:

$$\text{Percentage decolorization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

3.7.2 Operational Properties of MFC Under Optimal Conditions

The performance of MFC based Bio- Fenton was observed under optimal parameters. Anode chamber was inoculated with activated sludge and domestic wastewater collected from type D Colony Quaid-i-Azam University Islamabad where the ratio of activated sludge to wastewater was 6 to 50. The total volume of the cell was 110ml. The experiment was performed in fed-batch mode. The anode in this study was highly

anaerobic, the contents of the anode chamber were wastewater, anaerobic sludge, and 5ml of 50mM sodium acetate. 99.99 percent pure nitrogen gas was sparged into the sealed anode for 20 min through one of the holes in the cap. The cathode was abiotic and aerobic. Catholyte consisted of Na_2SO_4, $FeSO_4$, and Acid Red dye 114 solution. pH 3 was maintained in the cathode chamber for providing acidic conditions. The oxygen supply was provided via oxygen motors and pipes were inserted through caps. The cathode and anode electrodes with a surface area of $16cm^2$ were electrically connected to the resistor by an electric circuit and the voltage across the resistor was measured by using a precision multimeter (model: UT33B; UNI-T). Catholyte was changed every day for every new parameter for the time cells were running. Samples for H_2O_2 detection and dye degradation were taken from the sampling pit after 0, 20, 40-, 60-, 80-, and 100-min intervals. The final and initial pH, TDS, EC of the catholyte was also measured.

3.7.3 Analysis of Treated Samples

Several analyses of treated samples were performed to confirm reduction of AR 114, Including

FTIR

Treated and untreated samples were compared. FTIR analysis was performed on a Cory 630 FTIR spectrophotometer (Agilent), to investigate the changes in the AR 114 pure samples and treated ones. The transmittance spectra were recorded in the range of 4000-515 cm-1.

TDS and EC

Total dissolved solids and Electricity conductivity of treated dye solution were performed using TDS meter and EC meter.

4. Results

The research was carried out to evaluate the potential of microbial EF driven MFC for the effective degradation of Acid Red 114 and the production of H_2O_2. The experimental study was divided into certain aspects including Fabrication and characterization of Electrode. Incorporation of electrode into BEF system as cathode to treat AR 114. The study evaluated the ongoing prowess in terms of H_2O_2 production, dye degradation, TDS, EC, ORP and FTIR of final degraded sample.

4.1 Characterization of Graphite Electrode

4.1.1 SEM

Bare electrode shows the gaps present in the stainless-steel mesh while the modified electrode shows the successful adsorption of Graphite onto the stainless-steel mesh. The rough patches displayed in the SEM contribute to the increased surface area of the electrode.
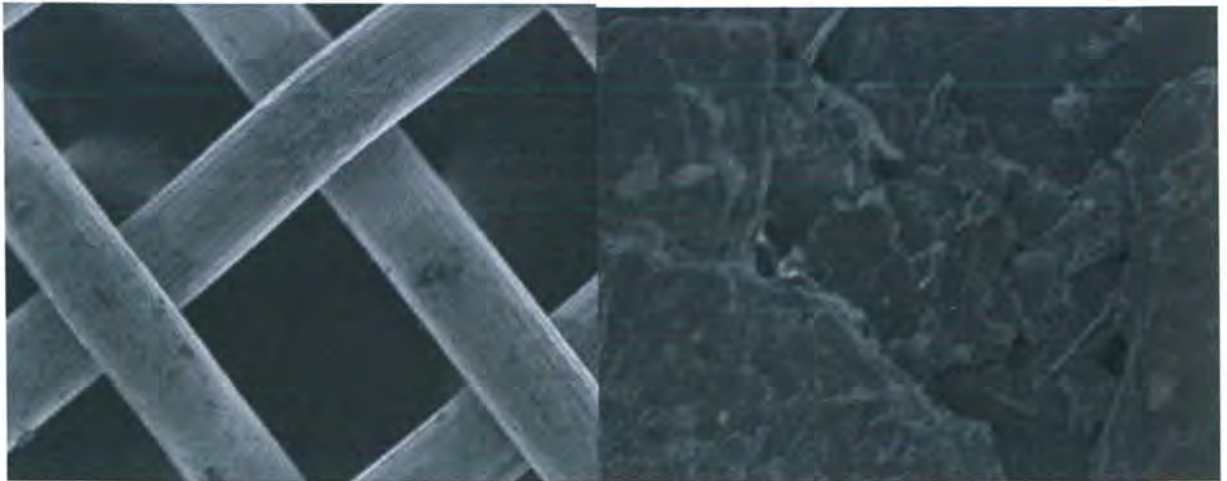
Figure 4.1SEM images of bare stainless-steel mesh and Graphite Stainless- steel mesh electrode (200μm)

4.1.2 Cyclic Voltammetry

CV was done at turnover conditions over a potential range of (-2.5V to 2.5V) at a scan rate of 20 mV $^{-1}$ sec. All the electrochemical measurements were done using powdered graphite electrode as working and counter, reference electrode was Ag/AgCl. The conductivity of the electrodes was assessed in various electrolytes 15 ppm AR 114, 0.5M H_2SO_4, and NaOH. Oxidation and reduction peaks were observed at 1.8, 0.4 and 0.8mA/cm 2 ^{f}or H2SO4, AR114 and NaOH.

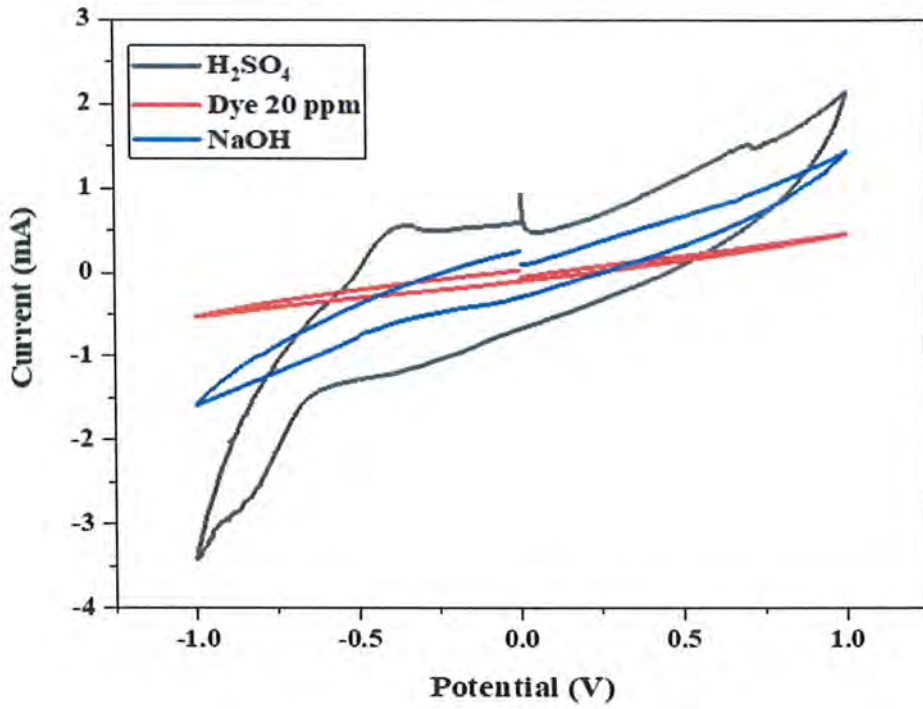Figure 4.1: Voltamogram of graphite particle electrode

4.1.2 Thickness of electrode

The thickness of bare SSM was 0.035mm after the graphite epoxy paste was applied it was 0.328mm.

4.1.3 Voltage, Current and Resistance

Voltage	Current	Resistance
53.5mV	3.5μA	0.0165Ω

Table 4: voltage current and resistance of Gp electrode in AR 114 solution

4.1.4 FTIR Analysis of the Electrode

The IR spectrum of bare stainless steel mesh electrodes were compared to electrodes modified with Graphite. It was observed that the height of peaks in the range of 700 cm^{-1} to 550 cm^{-1} showed variations. The change in Absorbance and shift in the peak frequencies revealed that the graphite paste has crosslinked firmly onto the SSM.When the spectrum from bare electrode was compared with graphite modified electrode, new peaks were observed at 1581 cm^{-1} which is characteristic carbon C=C stretch & 1211.9 cm^{-1} C-O indicating the adsorption light by graphite atoms pasted onto SSM.
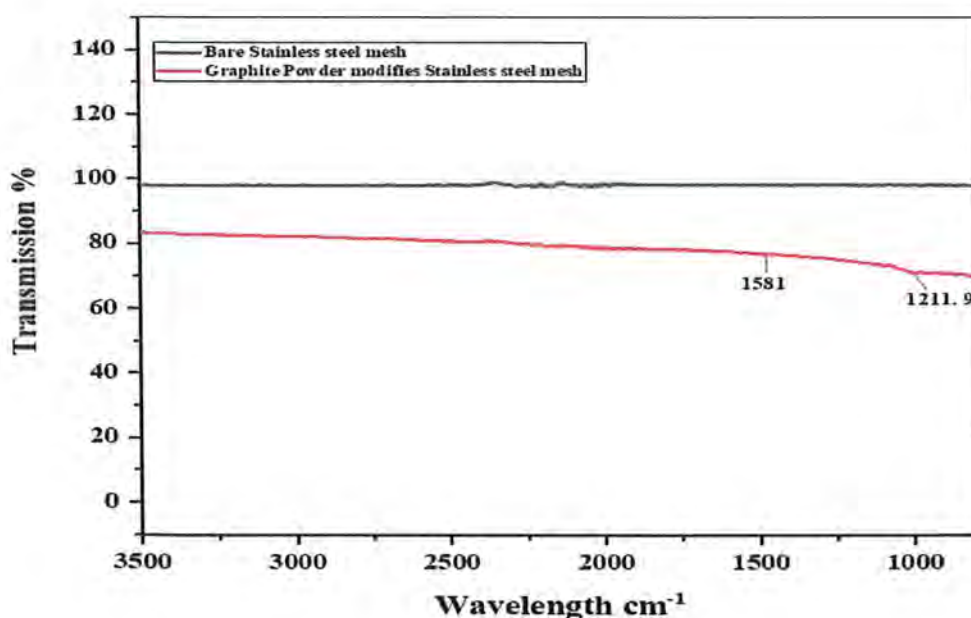

Fig 4.2 : FTIR Spectrum of (a)Stainless Steel mesh (Bare) (b) Graphite Powder modified Stainless steel mesh

4.1.2   Optimization of Different Parameters

The effects of different operational factors including dye concentration, pH, $FeSO_4$ concentration, catholyte concentration, and different catholytes were evaluated for the optimum fed-batch performance of microbial electro-Fenton driven MFC intended for the effective degradation of Acid Red 114 and the production of H_2O_2. The detailed results are as under:

1. Effect of initial of pH

Different pH conditions effect both the production of hydrogen peroxide and dye degradation (pH 2, 3, 4, 5, and 7). The different pH conditions were maintained in the dual chamber MFC for 24 hours with a working capacity of 110 ml. Since the H_2O_2 formed gets consumed as soon as it is formed, the experiment to measure the maximum concentration of H_2O_2 produced was carried out for about 1.5 hours. Samples were obtained at intervals of 5, 20, 40, 60, 80, and 100 minutes. The samples were spectrophotometrically examined at 351nm. Dye degradation was assessed initially and at termination of reaction. A significant decrease in the dye degradation and production of H_2O_2 was observed with the increasing pH. The maximum H_2O_2 production peak was obtained at 4088 ug/ml H_2O_2 was measured at pH 7 in reaction, and the overall best dye degradation obtained was 80% degradation of dye was achieved at pH 3 within 16 hrs at pH 3. Based on the results pH 3 was considered optimum for MFC operation. Maximum H_2O_2 production was directly linked to the Acid Red 114 dye degradation.
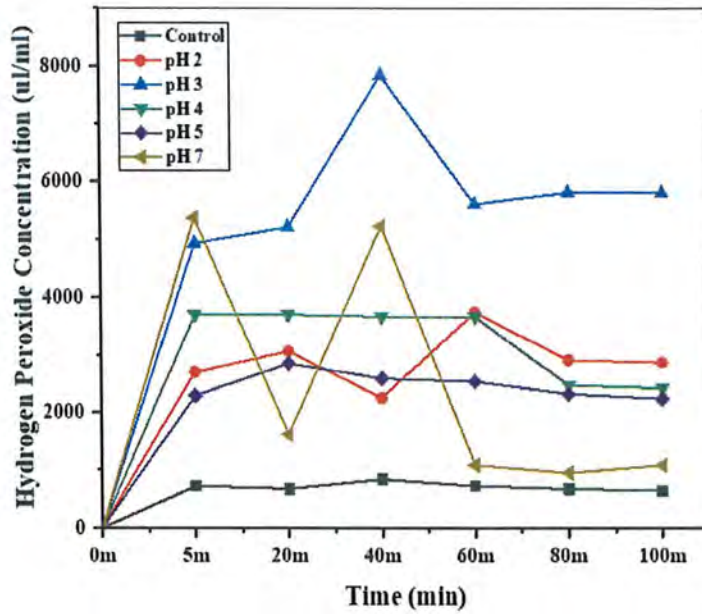
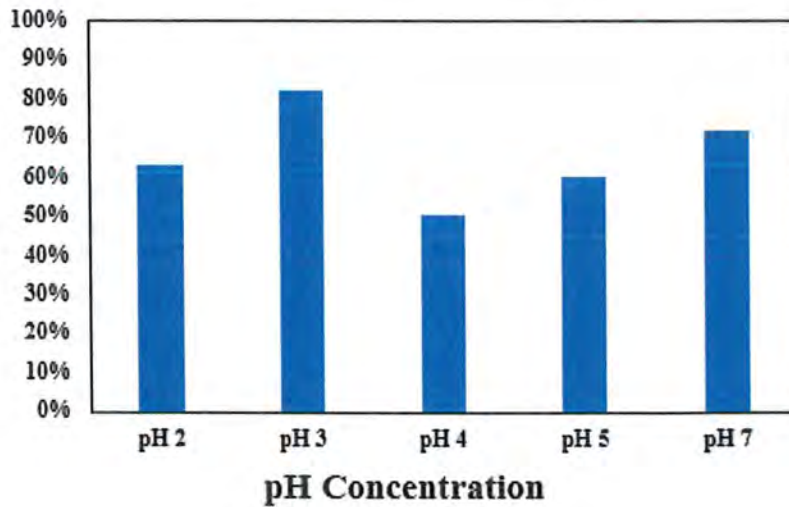Fig 4.4: Effect of initial pH (2, 3, 4, 5, and 7) on H2O2 Production (ul/ml)

Fig 4.5 : Effect of initial pH (2, 3, 4, 5, and 7) on Acid Red 114 Degradation

2. Effect of initial FeSO₄ (catalyst) Concentrations

FeSO$_4$ was used as an iron source in this experiment. FeSO$_4$ is important to produce OH⁻ and has a great influence on the functioning of MFC. 5 different FeSO$_4$ concentrations were added and their effect on H$_2$O$_2$ production and Dye degradation was examined. The sampling for H$_2$O$_2$ was performed after 5, 20, 40, 60, 80, and 100 min respectively. The samples were spectrophotometrically examined at 351nm. Dye degradation was assessed initially and at termination of reaction. The maximum peak for the production of H$_2$O$_2$ was 10130ug/ml. Maximum H$_2$O$_2$ was produced at 1mM FESO4 but 90% degradation was achieved at 0.5mM in less than 16 hr. So, 0.5mM was considered optimum for MFC operation. Concentrations greater than 0.5mM lead to decreased production of H$_2$O$_2$ along with decreased dye degradation which can be explained by the fact that increased concentration of Fe may lead to excessive formation of OH⁻ which reacts with H$_2$O$_2$ produced. This simultaneous reaction results in the consumption of H$_2$O$_2$ and OH⁻ produced. affecting the Acid Red 114 dye degradation. 0.5mM FeSO$_4$ was chosen for the MFC operation depending on the results obtained.

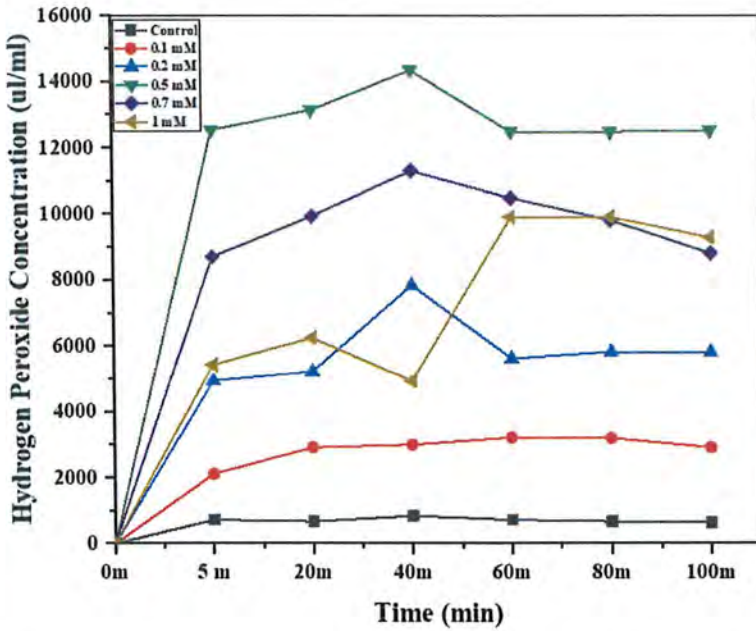**Fig 4.6: Effect of initial FeSO4 Concentrations (0, 0.2, 0.50, 0.75, and 1 mM)**
on H_2O_2 Production (ul/ml)
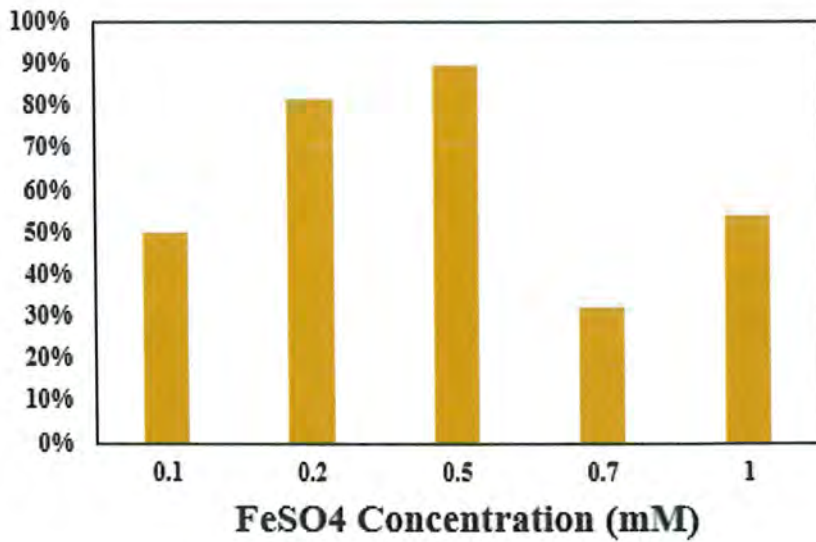
Fig 4.7: Effect of initial FeSO$_4$ Concentrations (0.1, 0.2, 0.5, 0.7, and 1 mM) on
Acid Red 114 Degradation

3. Effect of Different Catholytes

Electrolyte is necessary for the conductivity and smooth flow of electrical current. Different catholytes are used in Advance Oxidation processes. 5 different electrolytes were assessed for their performance in MFC including Na_2SO_4, $NaNO_3$, $NaCl$, KCl, and $KHPO_4$. The sampling for H_2O_2 was performed after 5, 20, 40, 60, 80, and 100 min respectively. The samples were spectrophotometrically examined at 351nm. Dye degradation was assessed initially and at termination of reaction. The maximum H_2O_2 production was 4188 ul/ml reported in Na_2SO_4. The degradation efficiency was observed in the order $Na_2SO_4 > KCl > NaCl > NaNO_3 > KHPO_4$. Based on the results Na_2SO_4 was chosen as the catholyte for the optimum MFC operation.

Fig 4.8: Effect of different Cathode Electrolytes on H₂O₂ Production (ul/ml)
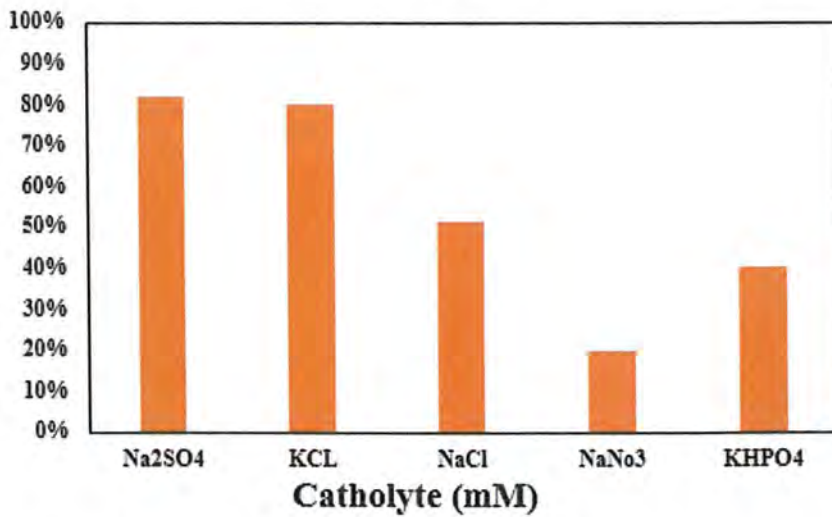
Fig 4.9: Effect of different Cathode Electrolytes on Acid Red 114 Degradation

4. Effect of initial Na_2SO_4 Concentrations

Na_2SO_4 was used as cathode electrolyte and various concentrations including 0, 25, 50, 75, and 100mM were tested for their maximum H_2O_2 production and dye degradation ability., the sampling for H_2O_2 was carried out after 5, 20, 40, 60, 80, and 100 minutes, respectively. Spectrophotometric analysis of the materials was performed at 351 nm. Samples of dye degradation were taken before and after the experiment. The highest peak for H_2O_2 generation and the highest dye degradation were obtained at 75 mM Na_2SO_4 concentration reported to be 4905 ul/ml and 97% respectively. Results showed the production of H2O2 at different Na_2SO_4 concentrations. Maximum H_2O_2 peak was obtained at 75mM concentration reported to be 4905 ul/ml and the maximum dye degradation was reported to be 90% respectively. Further increase in the concentration leads to decreased production of H_2O_2 and subsequent decrease in dye degradation. This can be explained by the fact that the excess of $SO4^{-2}$ could be absorbed on the surface of electrode blocking sites for O2 attachment and minimizing the H_2O_2 production along with the decrease in the production of OH^- affecting the dye degradation respectively.

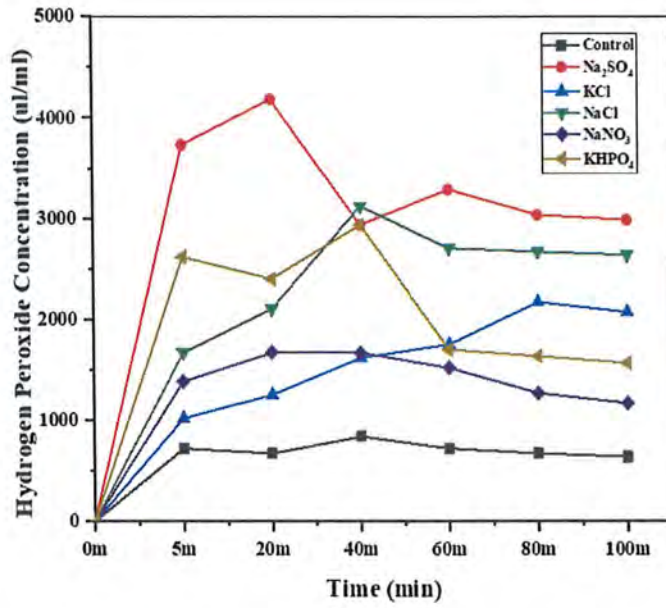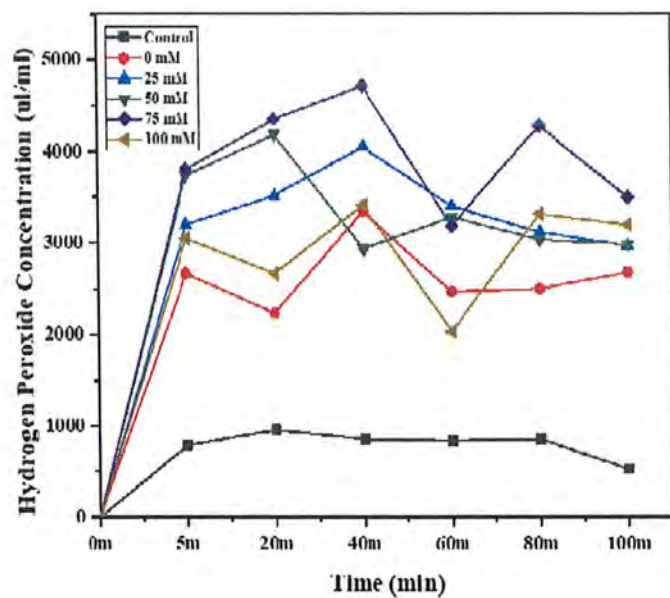**Fig 4.10: Effect of initial FeSO4 Concentrations (0.1, 0.2, 0.5, 0.7, and 1 mM) on H$_2$O$_2$ Production (ul/ml)**
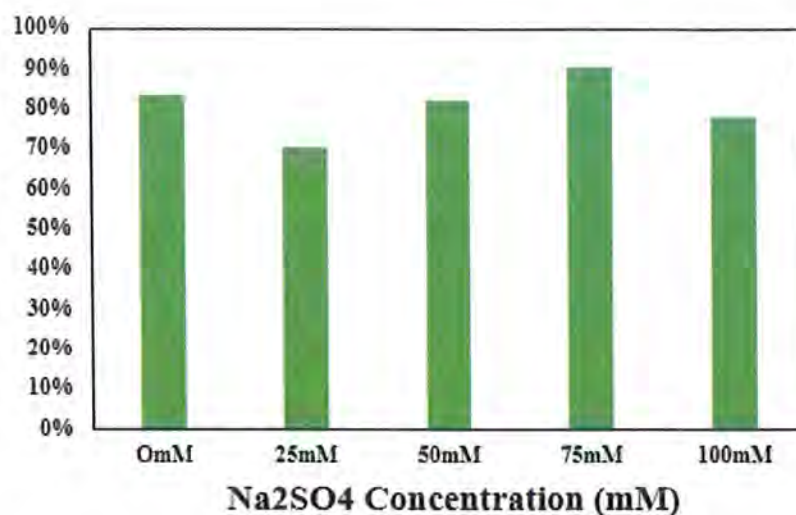
Fig 4.11: Effect of initial Na2SO4 Concentrations (0, 25, 50, 75, and 100 mM) on
Acid Red 114 Degradation

5. Effect of initial Dye concentrations

The overall production of Hydrogen peroxide and dye degradation was affected by the increasing dye concentrations (15, 20, 25, 30, and 40 ppm). The dual chamber MFC with a working volume of 110 ml was operated for 16 hours with each dye concentration. The experiment for measuring the maximum concentration of H_2O_2 produced was conducted for approximately 1.5h. The samples were taken at 5, 20, 40, 60, 80, and 100 min intervals respectively. The samples were analyzed spectrophotometrically at 351nm. Dye degradation was assessed initially and at termination of reaction. Fig. 1 illustrates the production of H_2O_2 against each dye concentration. The increase in the production of H_2O_2 can be seen with the increasing dye concentration which shows that the increase in H_2O_2 production is directly related to the increase in dye concentration. Maximum H_2O_2 production was 5638.33 ul/ml observed at 40 ppm. Fig. shows the degradation of Acid Red 114 at different concentrations (ppm). The dye degradation showed a different pattern, the increase in dye concentration was inversely proportional to the increase in degradation. The 30 and

40 ppm dye concentrations showed less degradation in 16 hrs. Maximum degradation was observed at 20 ppm dye concentration reported to be 80% within 16 hrs. The greater the dye concentration, the more time is required for its degradation. Based on the results obtained, 20 ppm was considered the optimum dye concentration for the functioning of MFC.
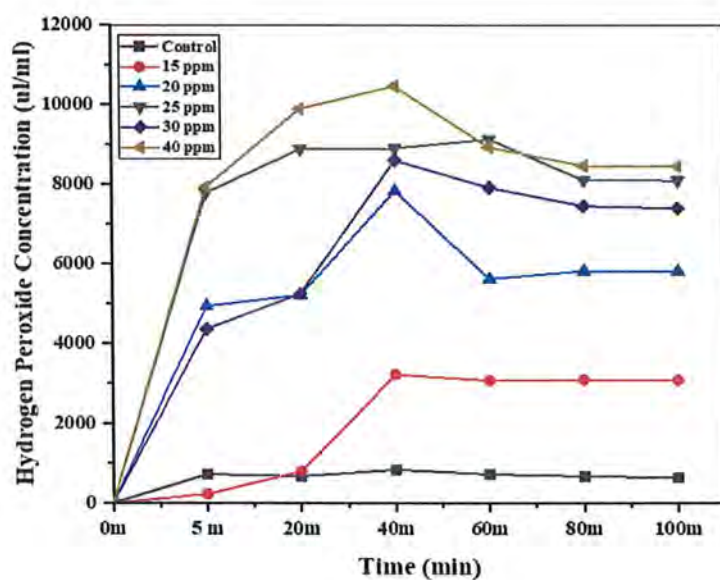
Fig 4.12: Effect of different initial dye concentrations (15, 20, 25, 30, and 40 ppm) on H₂O₂ Production (ul/ml)
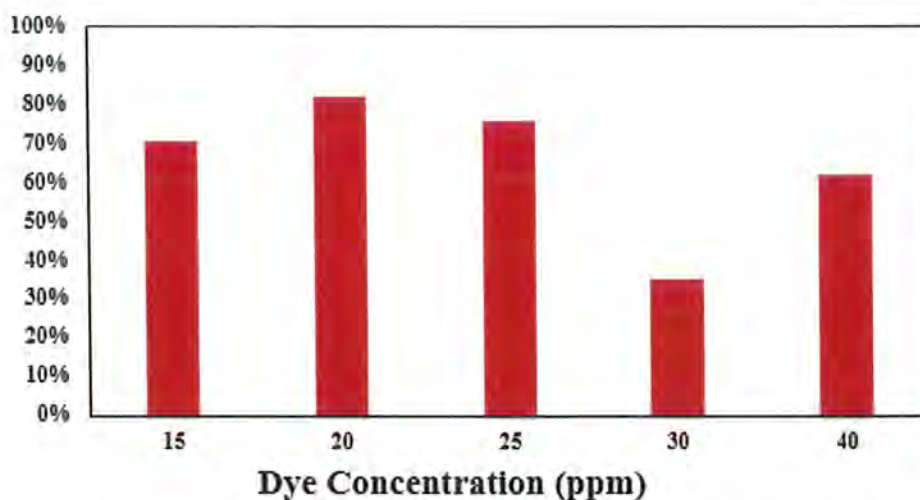
Fig 4.13: Effect of different initial dye concentrations (15, 20, 25, 30, and 40 ppm) on Acid Red 114 Dye Degradation

4.2 Operation of BEF system under optimized conditions

The selected parameters (20ppm dye, 0.5mM $FeSO_4$, 75mM Na_2SO_4, pH 3) were employed to MFC and their effectiveness on the production of H_2O_2 and Acid red 114 degradation was assessed. The total working volume of the experiment was 110 ml. The samples for H_2O_2 were taken at 5, 20, 40, 60, 80, and 100 min respectively. Dye degradation was assessed initially and at termination of reaction. The maximum H_2O_2 production obtained under optimal working conditions was 6805 ul/ml and the dye degradation obtained within 15 hours was reported to be 85%.The critical time or high time of dye removal was reported to be 5h with 80% degradation. The calculated P value of 0.02 signifies the correlation between H_2O_2 production and dye degradation. The maximum power density of the cell calculated was 8958.5mW cm^{-2} showing the energy efficiency of the electrodes fabricated.
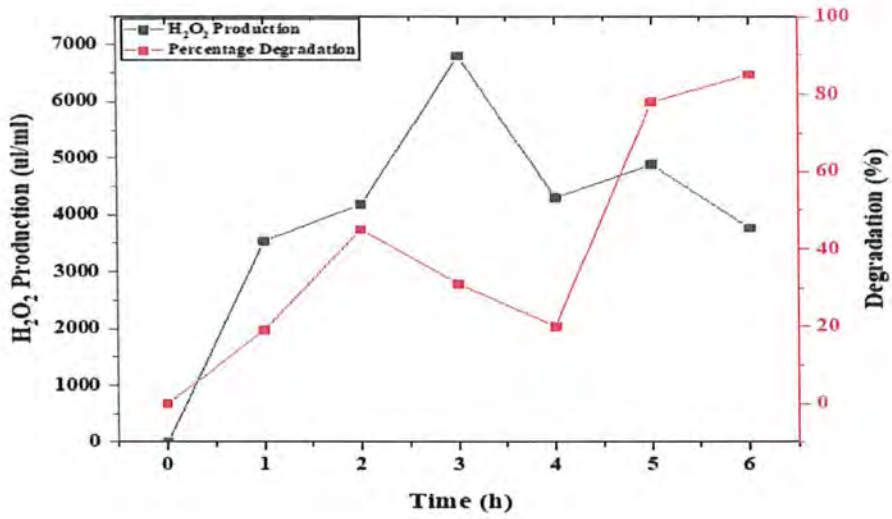
Fig 4.14: Hourly degradation and H₂O₂ production (ul/ml)
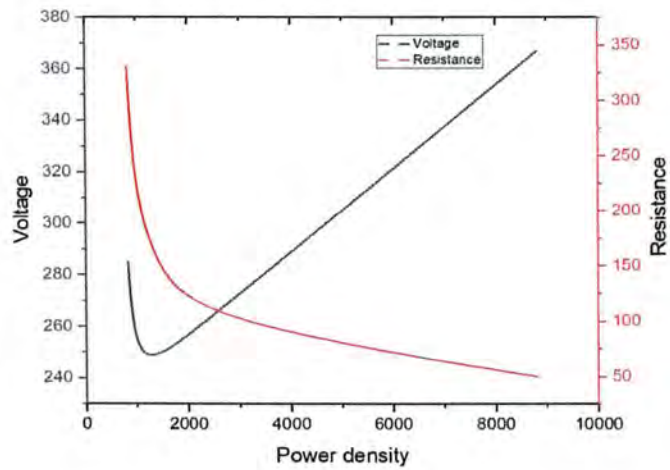
Figure 4.15 power Density of Final Cell

4.5 Characterization of Degraded Acid Red 114

UV-VIS

The UV-Vis spectroscopy was used to analyze the degradation of the Acid Red 114. The sample for degradation assessment were taken at the start and end of the experiment. The experiment was conducted for 24 hours under optimized conditions and the degradation of 92% was obtained within 12 hours. The sample was taken in the cuvette and its absorption was observed at 535nm. The degradation was obtained in terms of percentages. The equation used for calculating the decolorization percentage was [109]:
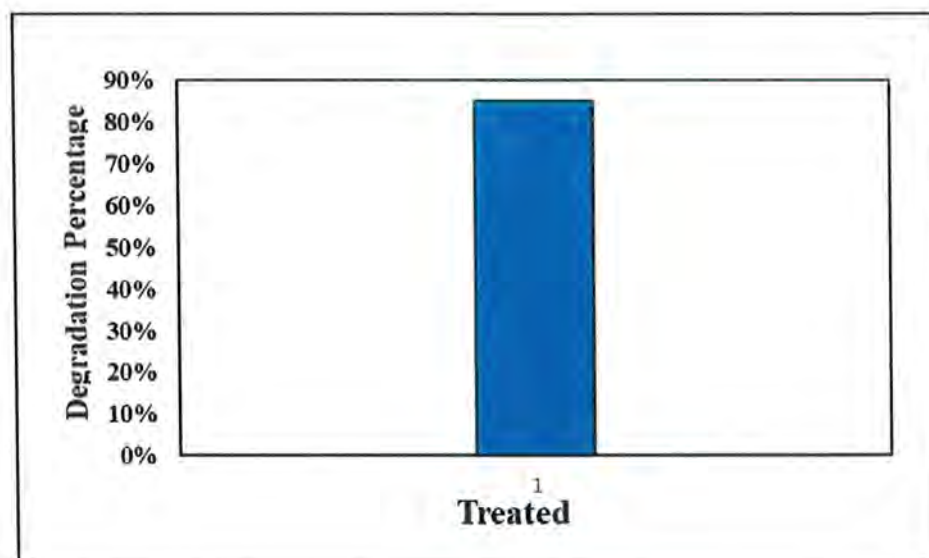
Fig 4.15: Effect of Optimized Conditions on the Degradation of Acid Red 114

4.5.1 FTIR of Acid Red Dye 114 Degraded Sample

The structural alterations of Acid Red 114 were analyzed by FTIR spectrophotometer. FTIR spectra showed significant variation among the treated and the untreated sample. Figure shows the FTIR spectra of treated and untreated Acid red 114 dye samples. There is a clear difference in the spectra, suggesting dye degradation. The untreated AR 114 sample spectra reveals prominent peak at $3419.23 cm^{-1}$ indicating an OH stretching of asymmetric intramolecular hydrogen bonded single bridge alcoholic or phenolic compound. The peaks at 2923, 2163, 1599 and 1176 cm^{-1} show CH_3 stretch, N=N stretch and SO stretch, respectively. Dominant peak was detected in the FTIR spectra of Acid red 114 degraded sample at 3253 (N–H stretch) indicating the presence of primary amine, The loss of peak at 1596 cm−1 (N=N) was observed. The new peaks appeared at 2189, 2040, 1637 and 1100 cm^{-1} along with the removal of 3419, 2923, 2163 peaks of the untreated Acid red 114 FTIR spectra. The difference in the spectrum of treated and untreated Acid red 114 samples indicate that Acid red 114 dye has been degraded [97].
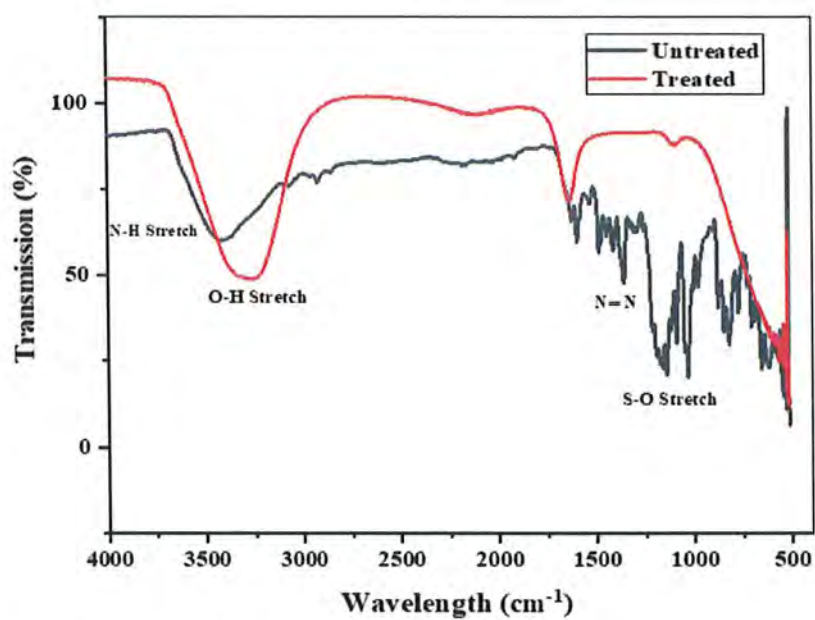
Figure 4.16: FTIR Analysis of Treated and Untreated Sample of AR 114

Other Parameters

Different analytical parameters including TDS, EC, ORP and pH were analyzed. The TDS, EC and ORP values increased with the degradation of Acid Red 114.

Parameters	Initial Range	Final Range
Total Dissolve Solid (ppm)	6828	7101
Electrical Conductivity(us/cm)	1329	1370
Oxygen Reduction Potential (mV)	256	390
pH	3	4

Table 5. Performance of the MFC Driven Bio-Electro Fenton during the treatment of dye in cathode chamber.

5. Discussion

Dyes are the basic and the most important raw material of textile industry, when discharged into water bodies they release aromatic amine and become carcinogenic. Efficient removal of them is very crucial, and it must be economically and environmentally feasible. Acid Red 114 is an Azo dye having double N bonds, it requires acidic environment to impart deep maroon color [26]. Like other 50% of dyes AR114 is also released untreated and is barely reduced to nontoxic forms. Since the last decade Microbial fuel cell has been considered as a robust technology for generation of electricity along with pollutant removal. Recent advances in technology include microbial driven Fenton reaction, which is generation of OH radicals from electroactive bacteria rather than electrochemically. This Less expensive use of biochemical redox reaction has been employed in biodegradation of several dyes like Methyl orange [81], Acid blue 113 [5], orange G [36], orange 2 [12] and reactive black 5 [43] using several BEF systems like Reverse MFC, Benthic MFC and dual chamber MFC. Previously phytodegradation of AR114 has been reported [41]. So far, the degradation of AR114 has not been reported in the Fenton system. The current study has proposed an environmentally friendly and cost-effective method for treatment of AR 114 in a self-assembled double chambered MFS along with graphite powder electrode fabricated on a stainless-steel mesh current collector. The cathode material is the most important component in the BEF system [10]. It has been reported by [91] carbon cathode has high affinity for O_2 to bind at it active sites and enhance H_2O_2 production. Keeping this in mind graphite powder was chosen to be conducting material. Graphite is known to be highly conductive, is relatively inert and has a wide voltage range [92]. After reviewing the previous literature available graphite powder paste was prepared and pasted on SSM. Previously [93] prepared GP electrode using PTFE as binder and employed it as cathode in double chamber MFC to treat wastewater via FR generation [94] modified GP electrode by loading iron oxide in the conductive material to increase its activity and use ferric oxide as internal iron source. As the purpose of the study was to limit cost, so epoxy binder was used contrary to PTFE.

FTIR analysis of the electrode confirmed adherence of GP onto SSM as the transmittance decreased when compared to bare SSM indicating absorbance of light by carbon molecules.

Fenton systems are highly dependent on operation conditions to obtain maximum decolorization optimum conditions should be maintained. For this purpose, in the current study different operational conditions were applied on the reactor. The results obtained in terms of H_2O_2 production were linked to AR114 degradation. The amount of H_2O_2 produced directly relates to amount of OH^- radicals produced, which attack the aromatic ring structure in AR114 dye structure, to initiate cascade of intermediates. The first parameter optimized is pH as degradation is highly dependent upon pH as reported by previous studies. In a study reported [14] system performance and methylene blue degradation was highly dependent on pH, optimum pH 2 was reported for maximum degradation of 99% of MB among pH range 2-8. A similar study conducted by ling et al reported that when pH was increased from 2-7 system performance decreased in terms of degradation of Orange G, the highest percentage of degradation obtained was 100%. Contrastingly in the present study optimum pH for decolorization of AR114 was 3. This correlates to a study reported [99] in which AR 73 was treated in Fenton driven reactor where optimal pH reported was 3 report ho conc. This confirms that for acid red class optimal pH is 3. As per studies conducted [14][81][99] with increase in pH insitu generation of H_2O_2 lead to decomposition of H_2O_2 to oxygen and water [98]. Moreover, with increase in pH Fe+3 ion concentration lowers due to formation of Fe $(OH)_3$ precipitates [100]. Iron concentration is one of the most influential parameters impacting the rate of oxidation of organic compounds. 89.9% degradation efficiency was obtained with 0.5mMolar $FeSO_4$. Previously different concentrations have been reported for different pollutants [14] reported 0.2 mM Fe^{+3} conc to obtain 99% decolorization in 28h. [81] added 10Mm of divalent iron to attain 100% degradation in 6h. evidently low concentration of iron 0.1Mm, 0.2mM are insufficient for adequate OH- production and high amount of Fe^{+3} 0.75mM and 1M leads to production of excessive amount of Fe^{+3}, and abundant amount OH^- Surplus hydroxyl ions react with H_2O_2 depleting both hydrogen

peroxide and hydroxyl ions for AR114 removal [14]. Catholytes employed in electro Fenton process are of prime importance not only because they contribute to conductance of current through the cathode but also provide strong oxidizing agents to aid the degradation. In the present study maximum degradation of 82% was achieved when 0.5 M Na_2SO_4 was used. Na_2SO_4 is the most widely used catholyte in the electro Fenton system, [14] reported the efficacy of catholytes in the following order Na_2SO_4>NaCL>KCL>$NaNO_3$>$NaCLO_4$. Similar trend was observed in present study, Na_2SO_4 was the efficient followed by NaCL. The effect of different initial concentration of Na_2SO_4 was analyzed for its effect on degradation, 0.75mM Na_2SO_4 was found to be effective for degradation of AR114 90.7% degradation was achieved which was higher than other concentrations, this finding was contrasting to those reported before [14][81] where the preferred concentration was 0.5mM, this could be because gp cathode had high internal resistance so more SO_4^- ions were required by the system for efficient conductivity of current for dye removal. A control system was also run with no Fe ion source, no aeration and open circuit conditions, negligible dye removal, minimal current and voltage validated that formation of Fenton reagent totally dependent on Fe^{+3}, dissolved oxygen and closed circuit. The last parameter that was optimized was dye concentration. When dye concentrations were increased keeping the other conditions constant, a decrease in dye degradation efficacy was observed. This lined up with the studies reported by [14][81] where dye concentration and degradation rates were in directly proportional the study also reported that more time was required to degrade the dyes in same conditions. This is because the rate of diffusion of Fe^{+3} and H_2O_2 gets lowered and dye molecules limit their mass transport from electrodes lowering the hydroxyl ions produced.

The performance of final cell was observed as per optimized conditions i.e., 20ppm AR114, pH 3, $FeSO_4$ 0.5mm, 0.75mm Na_2SO_4 as Catholyte. 85% degradation was achived. Optimal conditions are indeed effective due to following reasons, (1) degradation of AR114 in final cell increased from 82%-85% (2) it took only 8h to achieve maximum degradation(3) maximum H_2O_2 in final cell was 7000µl/L, which was higher than H_2O_2 produced during optimizing reactions , the efficacy of BEF system also improved in terms

of voltage output initially voltage across 100Ω resistor was only 53mV but when reaction started a stable voltage around 140mV and 30µA current was measured, thus validating our speculations about effectiveness of parameters.

FTIR analysis of treated and untreated sample was compared, visible peak shift was observed for Azo -N=N-, -SO_3 bond and other significant bonds confirming effective dye removal. Moreover, TDS and EC also increased as compared to initial sample indicating dye reduction to intermediates that are colorless and get dissolved in water.

6. Conclusion

The study undertaken aimed to explore ways to run an efficient, self-reliable and cost-effective microbial Fenton system. To achieve the desired properties a carbon-based electrode was fabricated. The raw materials for electrode were easily available at reasonable prices. Graphite powder paste made in epoxy binder, pasted on stainless steel mesh, served the purpose of a cathode that aided in production of H_2O_2 in large quantities. The redox potential of electrode under applied voltage were found to be 1.8,0.4 and 0.8 mA/cm^2. BEF proved to be very efficient in removal of 20ppm dye at pH 3, 0.5mM $FeSO_4$, 75mM Na_2SO_4 and continuous aeration within 8h. 85% of initial dye concentration was removed within 8h, results were confirmed by spectrophotometer and validated by FTIR. Open circuit voltage and current of reactor was 289mv and 40μA. Voltage across the resistor decreased when external resistance was increased, maximum voltage and current measured across 100Ω resistor were 251mV and 55μA. Maximum power and current calculated across 100 Ω was 8958.5 mW cm^{-2}. In final cell initial dye concentration was increased to 30 and 50ppm, reactor was operated on conditions optimized for 20ppm although H_2O_2 concentration increased but only 53-65% degradation was achieved confirming that these conditions are best for only 20ppm and different dye concentration require optimized conditions for efficient removal. The efficiency of the cell in terms degradation percentage and batch retention time increased from 82-85% in only 8h. The cell was operated for more than 50 days, validating the sustainable use of electrodesand the reactor which was fabricated from scratch. Inferring from results it can be said that epoxy is a suitable binder for fabricating eco-friendly and high-performance electrodes as compared to other binders.

Future prospects:

Microbial electro Fenton technology has been recognized as a cutting edge, energy efficient and multifaced technology. As an emerging technology it has attracted of researchers. It's widespread use and real-life application is limited by number of restraints. Although the performance of constructed setup fulfilled all of the requirements of being a reliable efficient and cost effective. However, we can improve its performance in terms of current generation and quick degradation.

- First of all, we can improve graphite powder cathode activity by using a binder that is more conductive than epoxy but is at same time less expensive. Graphite powder can be treated by acid to make it more conductive. Further investigations are needed to go for binder free system as they release toxic byproducts in the system.

- To improve current density, we can apply external voltage of about 0.1-0.3mV.

- Iron catalyst limit the effectiveness because of their toxic effects, there is a possibility for catalyst free system that could replace expensive chemical by biological ones.

- The use and efficiency of bioelectro Fenton system can be improved by combining it with conventional wastewater treatment methods for producing synergistic effects.

- Through study is needed for full scale employment of BEF system for their performance under actual conditions.

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