

## Review Paper:

# Unveiling Future Advancements in Azo Dye Degradation and Enhanced Bioelectricity Production using Microbial Fuel Cells

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## Abstract

Around 20 % of industrial water pollution is contributed by the textile dyeing and finishing of fabric. The dyes used in textiles pose environmental hazards and adversely affect human health. Thus, researchers are on a stride to find out an energy-efficient and eco-friendly process to remediate dyes. One such process is Microbial Fuel Cells (MFC) that has gained immense popularity in the past decade for their ability to produce electricity from wastewater. In this review, we focus on the remediation of Azo dye and the simultaneous generation of bioelectricity using MFC. We have discussed the mechanism of using azo dye at the anode as a co-substrate and at the cathode as an electron acceptor.

We have also discussed the factors affecting the performance of an MFC system, such as substrate and dye concentration. Finally, we have provided a comprehensive overview of the current research trends and potential advancements of MFC technology. MFC is a challenging yet promising technology and its performance can be improved through further research.

**Keywords:** Microbial Fuel Cells, Azo Dye, Bioelectricity, Co-substrate, Factors, Future advancements.

## Introduction

The trajectory of a nation's development is shaped by the rapid pace of its industrialization. However, accompanying these advancements and progress are some significant repercussions. The increase in industries leads to higher levels of emissions including toxic and hazardous chemicals, waste generation and resource consumption. Consequently, this leads to alarming levels of environmental pollutants, thus compelling researchers to focus on remediating the pollutants.

Bioremediation refers to “real-world environmental cleanup” which implies to use of microorganisms to remediate polluted soil and water (“Bioremediation”). It is based on the microbial degradation of the pollutants and enhancing its natural biodegradable fate (“Bioremediation”). Various bioremediation techniques have been practiced and improved over time. But no one technique can be used

solely. Based on the site of remediation, the techniques can be broadly divided into 2 categories *in situ*, for example, bioslurping, biosparging, phytoremediation and *ex situ*, for example, biopile, bioreactor, land farming etc.<sup>3</sup>

Nonetheless, bioremediation and other conventional physical and chemical methods have their disadvantages leading to the introduction of bioelectrochemical systems (BESs). BESs are capable of converting chemical energy into electrical energy from the oxidation of waste using electrogenic biocatalysts. Types of BES include microbial fuel cells (MFCs), anaerobic microbial fuel cells (ANMFCs), thermophilic microbial fuel cells (TMFCs) and so on<sup>49</sup>. MFCs have experienced a significant surge in popularity over the past decade, this is mainly due to three reasons: they can use wastewater as substrate, simultaneously produce electricity and carbon neutrality<sup>1</sup>. Other than wastewater management, MFCs are widely used in microbial studies, biosensors, biohydrogen production and bioelectricity production<sup>1</sup>.

However, in this review, we mainly focus on industrial wastewater management and specifically textile wastewater i.e. to remediate Azo dyes. Industrial wastewater mostly includes microorganisms, organic matter, metals, dyes and radioactive substances. MFCs can remediate each of these components as seen in various studies<sup>18,23,42</sup>.

In India, the textile industry ranks as the second-largest and is a rapidly evolving sector among various industries. Unfortunately, this progress results in the generation of a considerable volume of waste during dyeing, printing and finishing<sup>51</sup>.

The artificial dyes used do not bind strongly to the textile fabric and get discharged as effluent into the water bodies. Without any pretreatment, the continuous release of the effluent can have a severe impact on the aquatic organisms and can even enter the food chain<sup>2</sup>.

## Azo Dyes

Dyes are being used in all kinds of industries including textiles, foods, cosmetics, papers, leather etc. In general, a dye can be defined as a chemical with a chromophore group that imparts colour. At present, more than 10,000 different dyes are produced on an industrial scale with an annual production rate of 600 crore kg worldwide<sup>59</sup>. Synthetic dyes are categorised based on their chemical structure - azo, anthraquinone, sulphur, phthalocyanine and triaryl methane.

**Table 1**  
**Classification of Azo dyes**

Azo dye	Characteristics	CI no.	Examples
Monoazo <sup>5</sup>	Z-N=N-W Z and W the benzene/ heterocyclic derivatives/ naphthalene	11000–19999	Chrysoidine, Orange IV, Mordant black 17
Diazo <sup>5</sup>	two groups –N=N- Primary and secondary diazo dyes.	20000–29999	Brown dye, blue direct dye, orange direct dye
TriAzo <sup>5</sup>	three groups –N=N-	30000–34999	
PolyAzo <sup>5</sup>	Repetition of the azo group from three or more times in the same molecule.	35000–36999	direct red dye
Azoic <sup>5,13</sup>	Also known as ice colours and magic colours	37000–39999	

Out of these, Azo dye is the most important because around 70 % of all the dyes used in industries are azo dyes<sup>5</sup>. These are synthesized by diazotization and coupling reactions. They are mainly characterized by the presence of a -N=N- functional group called the azo linkage.

Further, based on the number of azo linkages present in the dye, they are divided into monoazo, disazo, trisazo, polyazo and azoic. These dyes are also given a number according to the Colour Index (CI) corresponding to their chemical structure<sup>5,24,25</sup> (Table 1). The main problem in using these dyes is the release of 10–15% of the total dyestuff into water bodies<sup>51</sup>. Dyes are synthetically stable in water and are non-biodegradable, thus, making them one of the major water pollutants. Untreated release of dye effluents blocks sunlight penetration into water, disrupts oxygen levels, increases Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) and as an extension affects animals including humans. Dyes can also be mutagenic or carcinogenic and teratogenic<sup>44</sup>.

Many physio-chemical methods have been used to remove these synthetic dyes from wastewater, but they have certain limitations including high cost, low efficiency, interference with other wastewater components and waste management<sup>51</sup>. Thus, more research interests grew towards bioremediation. And now specifically use MFC to remediate dye and simultaneously generate electricity. The degradation of dye using MFC is usually measured as the decolorization efficiency (%) which is calculated by

$$DE (\%) = (A - B) / A * 100\%$$

where A is the initial absorbance and B is the observed absorbance due to presence of dye<sup>17</sup>.

**Bioelectricity:** Microorganisms play a pivotal role in the burgeoning field of bioelectricity production, contributing significantly through substrate metabolism and the release of electrons. MFCs stand out as instrumental tools in harnessing bioelectricity where various pollutants such as heavy metals, organic wastes and dyes from wastewater are ingeniously repurposed as substrates for electricity

generation<sup>56</sup>. Microorganisms, functioning as catalysts within their intricate systems, effectively convert chemical energy present in these substrates into electrical energy. Notably, the manipulation of external resistance emerges as a critical factor in influencing current production with lower external resistance fostering the growth of electrogens. Electrogenic are microbes that act as an integral part of MFC as they are capable of extracellular electron transfer<sup>50</sup>.

Electrochemical tests involve noting the voltage difference between anode and cathode across a resistor every 10 min using a multimeter. Polarization curves are obtained by varying external cell resistance from 50 to 5000 ohm and measuring the resulting cell voltage<sup>54</sup>. The voltage is then converted to power density.

Current densities (I) and power densities (P) are normalized by anode projected area (A). Formulas used are:

$$I = V/RA \text{ (A/m}^2\text{)}$$

$$P = V^2 / RA \text{ (W/m}^3\text{)}$$

or by using current output (I') and power output (P')

$$I' = V / R \text{ (A)}$$

$$P' = V^2 / R \text{ (W/m}^2\text{)}$$

where V is voltage, R is resistance and I is current.

To quantify the efficiency of this bioelectricity production, researchers employ the concept of columbic efficiency. This metric, measured by calculating the ratio of electrons derived from microbial oxidation to those consumed in generating an electric current, provides a crucial benchmark for assessing the effectiveness of MFCs. As an illustrative example, the Columbic efficiency for azo dye decolourization is computed by comparing the theoretical current estimation based on the full azo bond reduction of the dye at the cathode with the actual current flowing across the bioelectrochemical systems (BESs)<sup>39</sup>. Bioelectricity production has recently gained momentum and there is research going on in the development of microbial strains and electrodes to increase the efficiency of power generation.

### MFC for simultaneous Azo dye degradation and Bioelectricity production

**Microbial Fuel Cells:** The development of Microbial Fuel cells (MFC) is an important step towards sustainable energy. MFC works on a microorganism-catalysed reaction which converts stored energy in chemical bonds of organic matter into electrical energy by a redox reaction<sup>32</sup>. An MFC setup includes a cathode and anode chamber separated by a proton exchange membrane (PEM). The bacterial degradation/oxidation of the organic matter happens at the anode generating CO<sub>2</sub>, protons and electrons. Proton diffuses through PEM and electrons travel through an external circuit and both reach the cathode. At the cathode, O<sub>2</sub> the most widely used electron acceptor is present which combines with proton and electron to commonly give H<sub>2</sub>O and electricity (Fig. 1 and 2)<sup>32,56,58</sup>.

A wide variety of substrates can be used at the anode to generate harvestable energy, including glucose, lactate, acetate and various sources of wastewater<sup>38</sup>. Each component of the MFC as mentioned above can be the basis of classification for the types of MFCs. For instance, electron acceptors could be inorganic like O<sub>2</sub>, nitrogen compounds and metal ions or organic compounds like azo dyes and chlorophenols<sup>27</sup>. Next, the design of an MFC is of great importance, because for a particular application a specific, setup is required to increase the efficiency of the reaction. There are two basic setups of MFC i.e. Two chambered- MFC and Single chambered- MFC. The former configuration features a proton exchange membrane that separates the anaerobic anode chamber from the aerobic cathode chamber<sup>36</sup>.

Mostly graphite plates, serving as non-catalysed electrodes, are employed in both chambers. In the anodic chamber, a peristaltic pump facilitates the recirculation of wastewater,

while the cathodic chamber receives aeration from the bottom<sup>54</sup>. On the other hand, the single-chamber MFC presents a more simplified and cost-effective alternative. It features a microfiltration membrane air cathode in the anode chamber, allowing the exposure of air on the inside and water on the outside. Both designs demonstrate their unique advantages in addressing azo dye decolorization and bioelectricity generation. However, more modifications are made to the setup based on the requirements of the experiments like Up-flow membrane-less MFC, or they could be coupled with wetlands, bioreactors, etc. as mentioned in table 2 to increase their efficiency<sup>20,41</sup>.

These MFCs have gained immense attention in past decades, owing to its potential to recover energy from wastewater treatment. MFC treatment has been successful in removing organic compounds, heavy metals and even dyes from the wastewater<sup>8</sup>.

As discussed above, azo dye is ubiquitous and needs proper treatment before being released into the water bodies. With the advancements in understanding MFC, researchers have tried to use azo dye as a substrate and simultaneously harness the bioelectricity produced. Interestingly azo dye can be used as a substrate at the anode and undergo anaerobic degradation, but also it can be used as an electron acceptor at the cathode<sup>58</sup>.

**Azo dye at Cathode:** A good electron acceptor is one that has a high redox potential and is economically valuable, sustainable and easily available<sup>27</sup>. Azo dye has the characteristic azo linkage (-N=N- bond) which is an electron-withdrawing bond that makes it electrophilic<sup>43</sup>. Therefore, it can be used as an electron acceptor at the cathode.

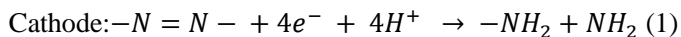
**Table 2**  
**Overview of current research trends.**

S.N.	Dye	No. of Chambers	Anode	Cathode	Location of azo dye	Decolouration efficiency	Maximum Power Density or power output (W/m <sup>3</sup> or W/m <sup>2</sup> )
1.	Congo Red <sup>29</sup>	Single-chamber MFC	RM modified graphite felt	Carbon paper plated with platinum catalyst	Anode	70%	0.193 ± 0.0023 W/m <sup>3</sup>
2.	Azo dye <sup>42</sup>	Double-chamber MFC	Activated Carbon Fiber (ACF) in stainless steel mesh	Activated Carbon Fiber (ACF) in stainless steel mesh	Anode	88%	10.83 ± 1.21 W/m <sup>3</sup>
3.	Reactive Brilliant Red X-3B <sup>10</sup>	Stacked MFC-Biofilm electrode	Granular Activated Carbon (GAC)	Air-cathode	Anode	97.77%	0.257 W/m <sup>3</sup>

		reactor (BER) Coupled System		layer of GAC			
4.	Congo Red <sup>54</sup>	Aerobic biocathode Two-chamber MFC	Carbon-based	Carbon-based	Anode	Decolorization rate constant $k=0.0501\text{h}^{-1}$	29 mW/m <sup>2</sup>
5.	Acid Orange 7 (AO7) <sup>57</sup>	Up-flow Membrane-less MFC	Carbon felt	Carbon plate	Cathode	75.70%	$7.07 \pm 1.4$ mW/m <sup>2</sup>
6	Acid Orange-7 <sup>22</sup>	Integrated Single chamber MFC-aerobic reactor	Carbon fabric	Carbon fabric	Anode	>90%	$51.9 \pm 4$ mW/m <sup>2</sup>
7.	Reactive Brilliant Red X-3B <sup>19</sup>	Planted microbial fuel cell and constructed wetland coupled (planted CW-MFC)	Granular activated carbon (GAC)	Stainless steel mesh coupled with GAC	Anode	91.24%	0.302 W/m <sup>3</sup>
8.	Congo Red <sup>61</sup>	Air-cathode Single chamber MFC	Graphite felt	40% Pt/C	Anode	95%	359.72 mW/m <sup>2</sup>
9.	Active Brilliant Red X-3 <sup>19</sup>	Microbial fuel cell coupled constructed wetland (CW-MFC)	Activated carbon and stainless-steel mesh	Activated carbon and stainless steel mesh	Anode	92.83%	0.0619 W/m <sup>3</sup>
10.	Active Brilliant Red X-3B <sup>55</sup>	Air-cathode Single-chamber MFC	Porous carbon papers	Porous carbon papers	Anode	86%	150 mW/m <sup>2</sup>
11.	Congo Red <sup>34</sup>	Anerobic-aerobic sequential reactor and MFC coupled system (A/O MFC)	Carbon felt	Graphite-granule	Anode	~40%	256.33 mW/m <sup>2</sup>
12.	New Coccine <sup>42</sup>	Aerobic Biocathode Single chamber MFC	Activated carbon flakes	Activated carbon flakes with Platinum catalyst	Anode	90%	$10.83 \pm 1.21$ W/m <sup>3</sup>
13.	Wastewater containing a mixture of 18 azo dyes <sup>22</sup>	H type MFC	Carbon cloth	Carbon cloth with Pt catalyst	Anode	Decolourization Rate constant $k=0.27 \pm 0.029\text{h}^{-1}$	$25.6 \pm 2.27$ mW/m <sup>2</sup>
14.	Acid Red 18 (AR18) <sup>41</sup>	Up-flow Constructed wetland-MFC	Carbon felt	Carbon felt	Anode	91%	8.67 mW/m <sup>2</sup>
15.	Brilliant red X-3B (ABRX3) <sup>20</sup>	Up-Flow Coupled Wetland MFC	Granular Activated Carbon	Granular Activated Carbon	Anode	92.7%	0.117 W/m <sup>3</sup>
16.	Congo red <sup>54</sup>	Aerobic Biocathode Two chambered MFC	Carbon based	Carbon based	Cathode	Decolourization rate constant $k=0.0375\text{h}^{-1}$	29 mW/m <sup>2</sup>
17.	Orange I, Orange II and Methyl Orange (MO) <sup>35</sup>	Two Chamber MFC	Carbon felt	Carbon felt	Cathode	-	34.77 mW/m <sup>2</sup>
18.	Acid Orange 7 (AO7) <sup>39</sup>	Single Chambers MFC	Granular Graphite	Granular Graphite	Cathode	$78.7 \pm 0.7\%$	$0.31 \pm 0.03$ W/m <sup>3</sup>



The microbial organic oxidation takes place at the anode which produces electrons and protons which are transferred to the cathode. At the cathode, the azo dye is reduced to amines by the consumption of 4 electrons and 4 protons resulting in dye decolourization and formation of intermediates (Fig. 1)<sup>35</sup>.



Oon et al<sup>43</sup> used monoazo and diazo dyes as a terminal electron acceptor in their MFC setup. The study proved that the chemical structure of the dye affects the power generated and the decolourization efficiency. The presence of an electron-withdrawing group substituted at the para position with respect to the azo bond; makes the dye even more electrophilic and favourable for its reduction<sup>43</sup>.

In another study, Mani et al<sup>37</sup> compared two different MFC setups; one MFC with acid orange 7 dye at the anode and

one MFC with the same dye at the cathode in the presence of laccase. The power output was higher in the MFC Dye Cathode with  $50 \pm 4 \text{ mW m}^{-2}$  compared to the MFC Dye Anode with  $42.5 \pm 2.6 \text{ mW m}^{-2}$ . Even the decolourization was  $>80\%$  in 24 hours in the MFC dye cathode, while only 20 % was in the MFC dye anode. This study concluded that laccase-based MFC-dye decolourization systems were a better setup for the decolourization and detoxification of azo dyes while simultaneously producing good power output (Mani et al.). Other studies also proved that an additional biocatalyst like laccase could lead to increased decolourization of dye and higher power density<sup>4</sup>.

**Azo dye at the Anode:** As discussed above, azo dye is used as an electron acceptor at the cathode. Azo dye along with a co-substrate at the anode produces a synergistic effect of co-metabolism and amplification of the decolorization process at the anode.

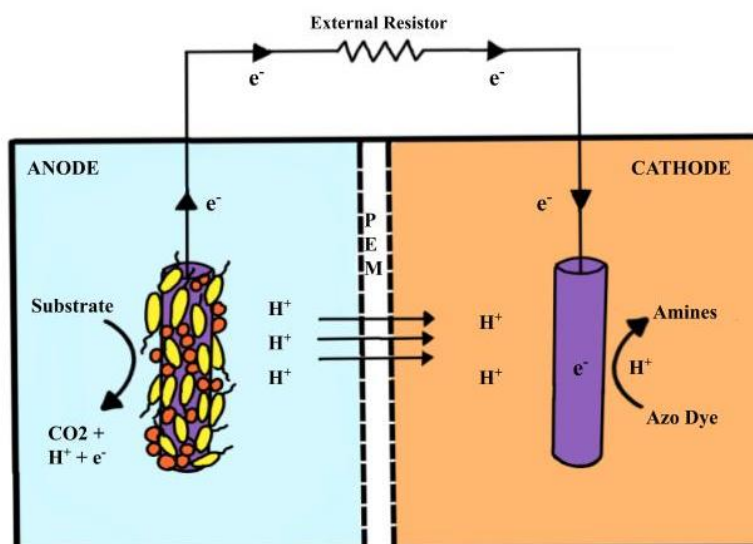


Figure 1: MFC setup with azo dye at cathode.

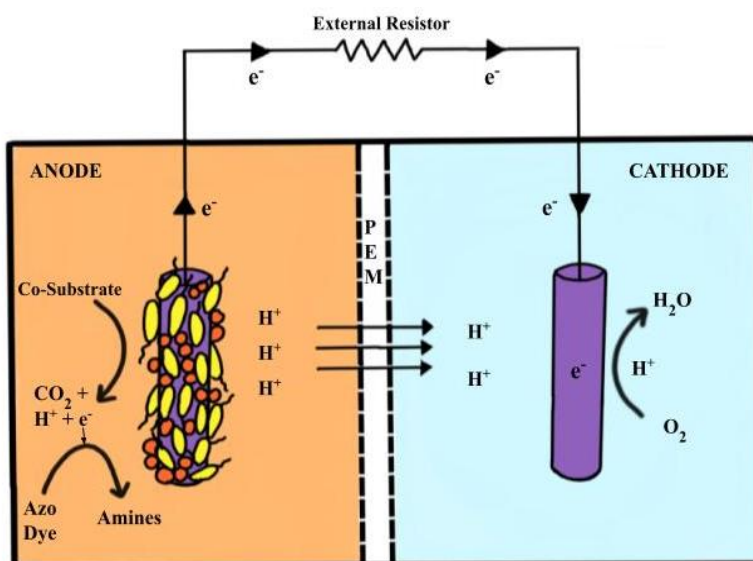
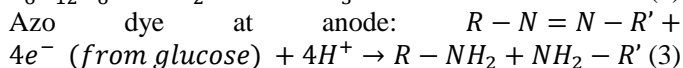
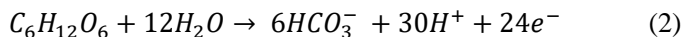


Figure 2: MFC setup with azo dye at anode.

The underlying mechanism involves the oxidation of co-substrate (glucose) at the anode, releasing electrons that flow towards the azo dye, progressively decolorizing it. At the anode, several reactions take place, shaping the electrochemical landscape (Fig. 2).

Glucose at anode:



Three distinct mechanisms have been elucidated to explain this intricate process. First, the involvement of anaerobic electrogens at the anode has been identified as a pivotal factor in accelerating azo dye reduction. Electroogens such as *Geobacter sulfurreducens* play a crucial role in extracellular electron transfer, using cytochrome c to facilitate the decolorization of azo dyes. The diverse array of electroogens exhibits varying electron transfer techniques, contributing to the ongoing exploration of their potential applications<sup>54</sup>.

Secondly, non-electrogenic anaerobic bacteria, equipped with enzymes like azo reductase and nitrate reductase, contribute significantly to effective azo dye decolorization at the anode. Reduced coenzymes, including FADH<sub>2</sub> and FMNH<sub>2</sub>, are instrumental in the reduction of azo dyes, with electron shuttles such as flavin facilitating the transfer of electrons between azo reductase and the dye<sup>14</sup>.

Lastly, the incorporation of redox mediators at the anode has emerged as a strategy to enhance decolorization efficiency. The addition of redox mediators, such as humic acid and riboflavin, has been shown to increase the maximum power density of bioelectricity production. This enhancement is attributed to the reduction of anode charge transfer resistance and the promotion of electron transfer to the dye. Moreover, redox mediators have been found to foster the growth of specific microbial species, further influencing the overall performance of MFCs in azo dye decolorization<sup>46</sup>.

### Factors affecting MFC productivity

MFC efficiency is directly influenced by certain factors and optimization of these factors plays an important role in increasing the decolorization efficiency and power production. First and foremost is the microorganism's ability to tolerate the dye and degrade it. According to the studies by Sun et al<sup>54</sup>, the influence of anodic inoculum on output voltage in Microbial Fuel Cells is evident in the comparison between two scenarios: MFC M, housing bacteria that required time to adapt to the anodic environment of Congo red and MFC I where bacteria demonstrated a greater tolerance to Congo red. Remarkably, MFC M exhibited a 3.22 times higher power density than MFC I. This superior performance was attributed to the abundant presence of electrogenic bacteria, sourced from a dye-bearing sewage treatment plant, highlighting the crucial role of the microbial community in determining MFC efficiency<sup>54</sup>. In addition to the microbial composition, substrate concentrations play a

pivotal role in bioelectricity production and Chemical Oxygen Demand (COD) degradation.

As observed by Hong et al<sup>28</sup>, the introduction of a new medium to the MFC resulted in a significant spike in output voltage, underlining the sensitivity of the system to changes in substrate composition. Assessing COD removal efficiency for varying concentrations of (LB), concentrations of 0.1x, 0.2x, 0.3x and 0.4x yielded removal rates of 38.17%, 39.46%, 56.03% and 61.41%, respectively. Correspondingly, the output voltage increased proportionally, with values of 66.62 mV for 0.1x, 76.42 mV for 0.2x, 87.75 mV for 0.3x and 96.87 mV for 0.4x. The initial decolorization rate exhibited a similar trend, escalating with an increase in LB concentration<sup>28</sup>.

Furthermore, the impact of dye concentration on the MFC's performance was explored by Hong et al<sup>28</sup> during a 100-hour operation for Methylene Blue (MB) treatment. The COD removal efficiency decreased with higher concentrations of dye (0, 200, 400, 600, 800 mg), registering values of 36.52%, 31.87%, 25.12%, 22.88% and 17.54%, respectively. This decline is attributed to the heightened inhibitory potency of microbial cells at elevated dye concentrations, revealing a complex interplay between substrate concentrations and microbial activity in MFCs.

Lastly, as the dye concentration increases (200 mg/L New Coccine) the decolorization efficiency also increases by over 90% while simultaneously decreasing the power density (10.83 1.21W/ m<sup>3</sup>) as per studies done by Oon et al<sup>42</sup>. This is because both anode and azo dye molecules compete for electrons and if the organic load is increased, more electrons are utilized for decolorization of azo dye and fewer are available for bioelectricity production due to which power density drops<sup>42</sup>.

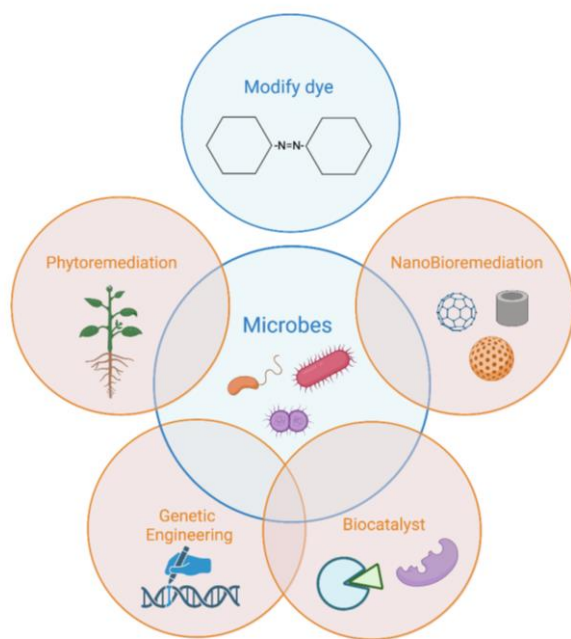
Other than this, effective current production relies on a multifaceted interplay of factors, including the generation of redox mediators, activation of the electron transport chain, expression of membrane-bound proteins, the involvement of cytochromes in immobilized cells and the formation of conductive pili<sup>26</sup>. To optimize this electrochemical process, it is crucial to employ stable electroactive microorganisms, ensuring the maximization of current output. Simultaneously, efforts should be directed towards minimizing internal resistance, thereby enhancing the efficiency of the overall electroactive system<sup>15</sup>.

### Potential Advancements in Microbe based Remediation

Till now in this review, we have discussed how azo dye is an omnipotent pollutant and can be remediated using MFC while simultaneously generating electricity. Some of these advancements are shown in fig. 3.

**Nano Bioremediation:** Nanoparticles (NPs) are substances that lie in a size range between 1 and 100 nm. As we go from bulk material to nanomaterial, there is a drastic change in

their physiochemical properties. For instance, nanomaterials have a greater net surface area and higher reactivity compared to their counter bulk material<sup>62</sup>. When biological particles such as DNA, protein and enzymes are used to create nanoparticles, they are called bio-nanoparticles and using them to remediate environmental pollutants is termed as nano bioremediation<sup>51,62</sup>. The high absorptivity, reduced waste production and low toxicity of oxide nanoparticles make them a good alternative for remediation of textile dyes<sup>48</sup>. Sha et al used hollow cobalt nanoparticles for rapid degradation of methyl orange azo dye due to which degradation efficiency increased to 99% within 4 mins<sup>48</sup>.



**Figure 3: Advancements for bioremediation of textile dyes.**

Microbes, plants or algae alone cannot remediate the high amount of pollutants, thus the need to conjugate microbes and nanoparticles increases the surface area for interaction with the dyes. One of the techniques is immobilizing microorganisms and enzymes using nanoparticles, membranes and supports with great selectivity towards the pollutants<sup>11</sup>. For instance, Qiu et al<sup>45</sup> observed that lacasse immobilization on chitosan NPs has higher removal efficiency (100%) for 2,4-dichlorophenol in water at 25 °C due to the enzymatic properties<sup>45</sup>.

**Modifying Dye structure:** Owing to the recalcitrant nature of dyes, inappropriate or incomplete treatment of dyes can produce toxic intermediates/metabolites. Reduction of azo dye leads to the formation of aromatic amines which are often more toxic than the dye itself<sup>9</sup>. The decolourization efficiency is an important indicator of the efficiency of the azo dye-reducing system. The higher is the decolourization efficiency, the more efficient the system is in reducing the azo dye. It is important to assess the product or metabolites produced after the process of remediation using tools like

Fourier transform infrared spectroscopy (FTIR), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS) and so on<sup>9</sup>.

To overcome this issue, we can develop more effective dyes, which are easier to break down and produce less toxic intermediates<sup>12</sup>. Studies have shown that the nature of substituents on the aromatic rings has an impact on their reduction and the products formed<sup>30</sup>. Thus, synthetic dyes with strategic modifications could lead to a better and effective remediation process.

**Combinatorial approach:** To maximize the effectiveness of microbe-based remediation, it is important to use a mixed culture of bacteria or a microbe consortium or even use bacteria, fungi and algae in different combinations. This increases the stability and enhances the adaptability of microbes to remediate the recalcitrant dyes<sup>60</sup>.

Even after using mixed consortia, just a single treatment cannot work to remediate all the components of wastewater. Thus, there is a need for combining the different techniques. It could be an integrated process in which one or more treatments are done after the other, or it could be a hybrid treatment in which two treatments are fused into a single process<sup>41</sup>. Such examples are also seen in table 2. Studies done by Fang et al<sup>19</sup> have used Constructed wetland (CW) coupled MFCs for azo dye degradation and simultaneous bioelectricity production<sup>19</sup>. CW uses natural substances like wetland vegetation, sand media and its associated microbial genera for the mineralization and removal of textile dyes and wastewater<sup>47</sup>. So, these integrated approaches can make the process of bioremediation more specific and efficient.

**Genetic Engineering:** One of the most obvious solutions we can think of, is to genetically alter the microorganism so that its efficiency in remediating pollutants increases. For instance, we could either isolate new halotolerant and/or thermophilic microbes or modify the existing ones to increase their adaptability<sup>53</sup>. Genetic engineering and more specifically metabolic engineering is done in the microorganism to increase or change the specificity and affinity of an enzyme towards a particular pollutant. It can also increase the growth rate and can control the regulatory pathways of the microbe<sup>51</sup>, thus creating exceedingly adaptive and superior bioremediation techniques for highly recalcitrant dyes. For instance, Varjani et al constructed *E.coli* JM109 (pGEX-AZR) for decolourization of direct blue 71 dye<sup>59</sup>. Not only microbes but plants can also be genetically modified by introducing specific genes for enhanced dye reduction enzymes and better adaptation in stressful environments.

**Phytoremediation:** Phytoremediation is an advanced technique that uses plants for the degradation and mineralization of pollutants<sup>51</sup>. Few plants have shown great ability to use various hazardous organic and inorganic



recalcitrant pollutants as the sole source of carbon and nitrogen while some plants use their enzymatic mechanisms to remove and detoxify the hazardous pollutants, such as dyestuff, salts, heavy metals etc.<sup>47</sup>

There are many mechanisms of phytoremediation: phyto-extraction, rhizo-filtration, phyto-transformation using plant enzymes, phyto-volatilization, rhizo-remediation or phyto-stabilisation<sup>51</sup>.

Using plants for remediating textile dyes could be considered an eco-friendly, effective and economical alternative for the future. *Glandularia pulchella* plants are studied for effective and faster decolourization using enzymes such as lignin peroxidase, veratryl alcohol oxidase, tyrosinase and dichlorophenolindophenol reductase in studies performed by Kabra et al<sup>51</sup>. It is also possible to tap into the alliance between plant and microbe which means microbe-assisted phytoremediation. This could lead to increased degradation efficiency and the formation of lesser toxic products<sup>16</sup>.

**Biocatalyst:** Microbes produce intra-/extra-cellular enzymes that cause the reduction and degradation of the dyes<sup>47</sup>. But in case, these enzymes are used directly, it can drastically reduce the time required for the growth of microbe and therefore the production of enzymes. Furthermore, using techniques of immobilization can increase the enzyme's thermal and chemical stability, absorption surface area, recovery and reusability<sup>60</sup>. Some important enzymes used in bioremediation are laccases, azoreductase, manganese peroxidase, lignin peroxidase and hydroxylases. Out of these, azoreductase and laccase are well known for their ability to reduce azo dyes<sup>16</sup>.

Other biocatalysts like redox mediators such as quinones are studied to increase the reduction rate. Role of redox mediators is confirmed by the studies conducted by Šafaříková et al<sup>46</sup> in which cells were autoclaved to prevent growth of microbes. Dye degradation was observed even in absence of microbial activity further concluding the presence of some reducing factors also called as redox mediators and their role in dye degradation.

In theory, all the above-discussed developments can improve bioremediation drastically. But there are still many problems with the practical implementation of bioremediation on a larger scale. The first is the recalcitrant nature of dyes making them tough to completely remediate. For microbe-based remediation, maintaining an optimum pH and temperature for microbial growth is tough to calibrate on a large scale<sup>60</sup>.

Also, most studies use synthetic dye in their experiments, so there is very little information about the decolourization of real textile dyes<sup>56</sup>. Thus, it is very essential to do more extensive research, especially at the site of dye disposal to test the efficiency of microbe-based remediation along with all advancements on real textile dyes.

## Conclusion

In conclusion, both environmental and energy challenges can be addressed by the burgeoning field of Microbial Fuel Cells. Calculated metrics such as power density and decolorization efficiency showcase MFCs promising for textile dye degradation. MFC's can be used as a sustainable and multifaceted solution. With further technological advancements and ongoing research, MFCs can revolutionize the energy landscape. This review underscores the use and functioning of MFCs optimistically and their potential for both economic and environmental benefits.

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