Decolorization of Azo Dye-Contaminated Water using Microbes: A Review

Vamshi Krishna Mukkera and Srivani Katuri

Abstract One of the heavy users of water is the textile industry, which also yields huge volumes of tainted water. The most important contaminants are azo dyes. This tainted effluent water when released into the freshwater will result in the increase of BOD, COD, TOC values of water thereby making it difficult for the aquatic beings in breathing. The toxicity of the effluent may also affect organisms when the water is ingested. Many physical and chemical techniques such as adsorption, coagulation-flocculation, oxidation, filtration, and electrochemical methods may be used for color removal from wastewater among which microbial processes for the treatment of textile wastewater have the advantage of being cost-effective and environmentally friendly. Moreover, in most cases, the final products of the physical or chemical techniques are either highly toxic than their parent toxins or produce more sludge, which is the reason microbial degradation is opted, that produce fewer toxic products and less sludge. Reductive cleavage of Azo bond, leading to the formation of aromatic amines, is the initial reaction during the biological metabolism of Azo dyes. Aromatic types of amines are mineralized under methanogenic conditions. But sulfonated aromatic amines are resistant, require aerobic microbial consortia for their clarification or degradation. Studies that used algae, fungi, bacteria and other microbial consortia along with the application of Advanced Oxidation Processes (AOPs) for the degradation of azo dyes for better results are discussed in this review. It is also discussed how the different conditions in degradation can influence the removal of azo dyes.

Keywords Azo dyes · Effluents · Decolorization · Microbes · Enzymes · Degradation · Analysis

V. K. Mukkera (✉) · S. Katuri
Department of Chemical Engineering, National Institute of Technology, Warangal, Warangal, Telangana 506004, India

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Nomenclature

AD	Azo Dye
AOP	Advanced Oxidation Process
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
MFC	Microbial Fuel Cell
NADH	Nicotinamide Adenine Dinucleotide
NADH-DCIP	Nicotinamide Adenine Dinucleotide Dichloroindophenol NADPH-Nicotinamide Adenine Dinucleotide Phosphate
TOC	Total Organic Content

1 Introduction

According to [38], intake of water and production of effluents in the textile industry relied on the processes followed in turning fibre into textile. According to [7] the stages of processing and types of dyes applied during this turning determine the characteristics of wastewater like pH, dissolved oxygen, organic and inorganic chemical content. Ubiquitously, 2.8×10^8 kg of dyes used in textile industries are freed in industrial effluents every year [25], in one of the cases of its representation is nearly 0.7 kg of NaCl, 35–65 g of dye and 75–150 L of water are required to dye 1 kg of cotton where tainted effluents produced has 25% of the applied unfixed dyes, their concentration reaching 2000 ppm [6] and its result includes the undesirable effects of azo dyes on surrounding location, such as their inhibitory effect on marine photosynthesis, their ability to lessen dissolved oxygen. They are toxic to flora, fauna. They also affect TOC, BOD, COD, suspended solids, salinity, color, values of pH. The dyes Acid Red 18 and Acid Red 27 not only cause mutations but are also carcinogenic due to the presence of the methyl group. Normally, it is significant to evaluate the toxicity of compounds formed after the process of decolorization of dyes [24]. If the ratio of BOD to COD ranges from 0.3 to 0.6, it denotes that the runoffs have a major portion of non-biologically degradable organic matter [39]. Toxicity is classified based on various methodologies and it is shown in Fig. 1.

Physically, azo dyes are branded of the azo bond whereas chemically, they have gained a property of sucking up the light when rays of the visible spectrum are projected on it [2, 13]. So as to create the diversity of azo dyes, the phenyl groups in the structure shown in Fig. 2 can be replaced with those of the naphthalene groups that also may carry various groups like methyl ($-CH_3$), chloro (–Cl), nitro ($-NO_2$), hydroxyl (–OH), amino ($-NH_2$), and carboxyl (–COOH). During routine aerobic wastewater treatment, azo dyes are not degraded [40]. The azo dyes are electron acceptors. They receive electrons from reduced flavin nucleotides. Redox mediators enhanced the reduction of azo dyes [26]. A Ds reduction results in the genesis

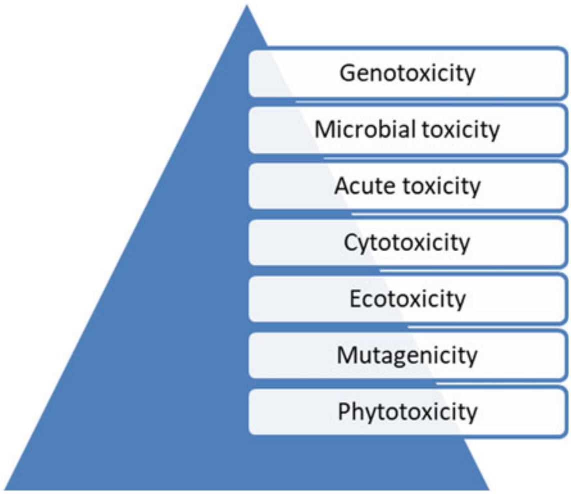

Fig. 1 Classification of toxicity

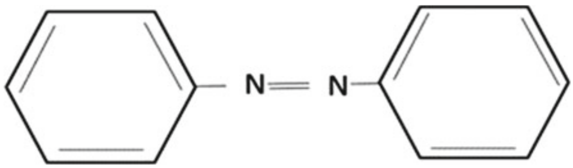

Fig. 2 Assembly of a typical azo dye

of aromatic amines with the exception of a few aromatic amines substituted with hydroxyl and carboxyl groups. They can be degraded under methanogenic or anaerobic conditions. Whereas, the exceptions can be readily degraded, aerobically [27]. Hence a combination of both the aerobic as well as anaerobic processes of degradation can be used for the reduction of azo dyes. There are a number of studies on the biological degradation of azo dyes in the effluent wastewater. Many researchers focused mainly on the usage of bacteria and fungi among microbes, out of which bacteria were adopted because of their high activity of degradation, their ability to degrade a large number of azo dyes and high flexibility of usage [15].

The present review focus on the technologies involving microbes that are used for the decolorization of azo dyes from the textile effluents. Many numbers of investigations indicated that the dyes used in textile industries and their runoffs have toxic effects on the growth rates and phytology of many plants which have vital ecological responsibilities, like setting up a natural territory for wildlife, prevention of soil from getting eroded and providing organic matter that is required for soil fertility [15]. Hence it is essential for the remediation of the effluents before being released into the environment, which can be carried out by using microbes. Usage of microbes is an appealing substitute for bioremediation processes in the treatment of textile industry effluents. Methods that involve the biotic species are ecologically friendly, yield lesser sludge than physicochemical processes and are cost-effective. The efficacy of

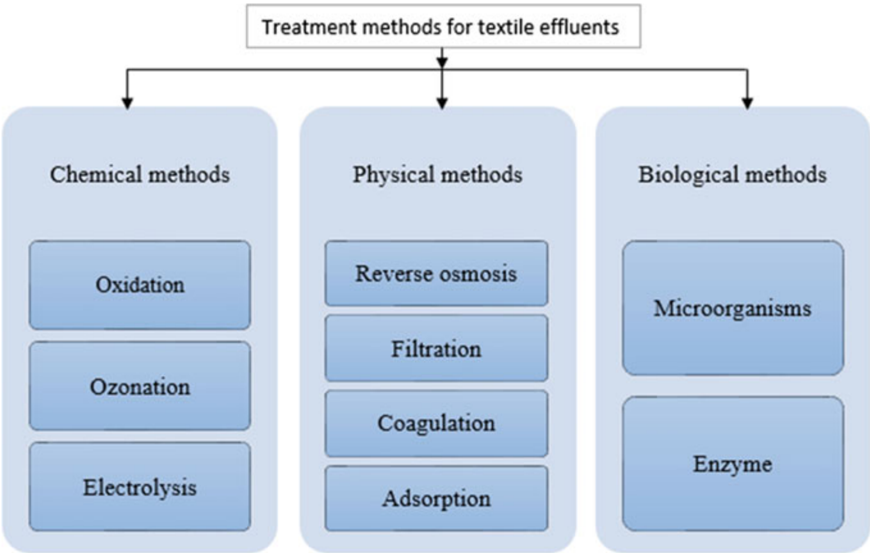

Fig. 3 Various treating methods for the degradation of dyes in tainted water effluent

different procedures in the decoloration relies on its numerous substituent groups and the physical–chemical features of runoffs, pH, temperature, and the existence of other pollutants [41].

1.1 Methods of Degradation

To remove the dyes, a number of physical and chemical methods were used. Nonetheless, their application has got many obstacles such as cost, energy. These methods cannot degrade some of the resisting azo dyes and produce serious ecological problems [17]. Azo dyes are complicated aromatic composites with significant architectural variety. Decolorization of synthetic dyes by traditional biological processes is not so effective [20]. Due to strict laws, many investigations are developed and efficient methods for handling wastewater are found, where various microbes were also adopted for developing the methods of treatment of textile effluents. Different methods of treatment are shown in Fig. 3.

1.2 Physical Azo Dye Reduction

The dyes having sulfur group can be removed by the physical methods like coagulation and flocculation but it has less effect on acid and direct dyes. Other methods like adsorption (which is used for the higher amount of removal and for large number of azo dyes), the process in which the substance used for adsorption must have a high affinity to azo dyes and the chance of adsorption to happen again should be high, i.e., for adsorbent regeneration [34]. Various adsorbents are used, one of which is powdered activated carbon (PAC) [33]. But it has a disadvantage, as it is highly expensive. Some other adsorbents like Kaolin, Bentonite, polymeric resins, peat which are less costly are also used. Some carbon-rich materials like corn, wheat, maize stalks were also used. But the application of the above materials have drawbacks with the regeneration and high amount of sludge production [3]. There are many methods of filtration like ultrafiltration, reverse osmosis. Membranes are used in textile industries for the removal of dyestuff and to reduce the color, BOD and COD. External condition like temperature is to be considered for the selection of filter. But usage of membranes has disadvantages like the high cost of investment, membrane fouling, production of secondary waste [15].

1.3 Chemical Azo Dye Reduction

Use of Advanced Oxidation Process (AOP)

In AOP hydroxyl radicals are generated so as to destroy the chemical compounds by oxidation of pollutants rapidly [30]. There are two routes to generate radicals,

1. The usage of permanganate (MnO_4), ozone (O_3), hydrogen peroxide (H_2O_2) along with the use of UV rays.
2. The usage of oxygen at moderate to high temperatures as found in Wet Air Oxidation (WAO) processes.

In Advanced Oxidation Processes (AOP), some of the catalysts like TiO_2, ZnO_2, Mn and Fe are employed along with oxidizing agents like O_3 and H_2O_2. Ozone is used because of its effect in reacting with a numerous azo dyes, and also better decolorization efficiencies [1]. Application of ozone process is limited because of its short life span, not effective to dyes which are insoluble in water, low COD reduction and its high cost [3]. Fenton's oxidation has got many advantages like high degradation efficiency, benign process, less costly materials [5]. But this process has a drawback of production of high sludge content [33]. The AOPs can either be in the homogenous phase or in heterogenous phase. Where in heterogenous phase, catalysts which are used are in solid phase and contaminants in the liquid phase [5]. Catalysts produce the OH^- to destroy the dye compounds [3]. AOPs do not produce many secondary pollutants that need additional detoxification. AOPs consume less amount of energy. But some of its drawbacks are higher operating costs, scaling up of processes to the industrial level is not [5].

1.4 Biotic Methods

The use of microbes to deal with the biodegradation is important in the treatment of wastewater. Various biotechnological methods are used to treat the azo dye-contaminated water effluents in an environmentally friendly way. Even though the dyes are very obstinate to the degradation, these methods are used for complete degradation and also have the advantages like ecologically supportive end products, less costly, generating less sludge, non-toxic end products. The following microbes can be used in degradation processes. They are Bacteria, fungi, Yeasts, Actinomycetes, Algae. The degradation products such as aromatic amines can inhibit bacterial degradation [32]. However, such inhibition action is not possible for fungi. Moreover, fungi can be used for degrading complex molecules along with some ligninolytic enzymes which include laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP) [21] and Tyrosinases [4] riboflavin reductases and NADH-DCIP reductases [42]. Fungi can also be used along with Catalysis processes.

Decolorization by Bacteria

The decolorization using bacteria proceeds in two steps. The first step is cleaving of the dye linkages reductively, producing the colorless, toxic aromatic amines whereas the second step is the degradation of the aromatic amines. Generally, Bacteria are used for degradation under aerobic conditions. If the conditions are anoxic, the strains like *Pseudomonas* sp. and *Proteus mirabilis* are used for degradation [29]. A bacterial microbe *Sphingomonas xenophaga* is used to decolorize the dye Mordant yellow 3. The effect is increased using glucose whereas, it is decreased when *Pseudomonas leuteola*, *Aeromonas hydrophila* and some additional mixed cultures were used [29]. The decolorization is analyzed using HPLC and mass spectrometry after the degradation of reactive red 22 by *Pseudomonas leuteola*, which indicated the presence of two aromatic amines. Following Table 1 gives information about the work done by some of the researchers.

Decolorization by Fungi

The fungi are present everywhere in the universe. It can be found in the soil, plants, organic wastes etc. Their endurance is based on the adaptation of their metabolism to the ever-changing content of carbon and nitrogen sources. Many of the reports have focused on the degradation using bacteria. But decolorizing of aromatic amines inhibit the action of bacteria [42]. Hence fungi are used for decolorization. Many works on the treatment of azo dye-contaminated water are concentrated on the white-rot fungi [35] among which *Phanerochaete chrysosporium* is studied widely, whereas some other white-rot fungi like *Trametes* (*Coriolus*) *versicolor*, *Bjerkandera adusta, Aspergillus ochraceus*, species of Pleurotus, Phlebia, and different other fungi [42] are also used. *Pleurotus ostreatus*, *Pichia* sp., *Penicillium* sp., and *Candida tropicalis* are used for the decolorization of azo dyes by adsorption and/or degradation [42]. However, it has some drawbacks like nitrogen limiting conditions, lengthy growth

Table 1 Degradation using bacteria

Microbe	Dye	References
Pseudomonas sp.	Reactive Blue 13	[42]
Corynebacterium glutamicum	Reactive Green 19 A	[35]
Enterobacter EC3	Reactive Black 5	[42]
Mutant Bacillus ACT2	Congo Red	[42]
Lactobacillus acidophilus and *Lactobacillus fermentum*	Water and oil soluble azo dyes	[35]
Aeromonas hydrophila	Reactive Red 141	[42]
Bacillus VUS	Navy Blue 2G	[42]
Citrobacter sp. *CK3*	Reactive Red 180	[35]
Acinetobacter calcoaceticus	Direct Brown MR	[42]
Pseudomonas sp. *SU-EBT*	Congo red	[42]
Rhizobium radiobacter	Reactive Red 141	[35]
Brevibacillus latarosporus	Golden Yellow ER	[42]

cycles. It is difficult to preserve fungi in bioreactors for longer time [8]. Following Table 2 gives information about the work done by some of the researchers.

Decolorization by Yeast

The yeasts are used since they are powerful adsorbents and can be used for a larger quantity of dye presence [35]. The treatment of azo dye-contaminated water using yeast is concerned mainly with biosorption. Yeast cultures like *Issatchenkia occidentalis* [43] are utilized generally for the degradation of ADs. Moreover, the above yeast also shows the bioaccumulation of reactive dyes like Remazol Blue, Black B and Red RB during the growth of it [9]. Moreover, some of the works also showed

Table 2 Decolorization of azo dyes using fungi

Microbe	Dye	References
Trametes versicolor	Reactive Black 5	[37]
Trametes pubiscens	Remazol Brilliant Blue	[35]
Trametes hirsute	Acid Blue, Reactive Green	[44]
Phanerochaete chrysosporium	Direct Red 80	[44]
Phanerochaete chrysosporium	Astrazon Red FBL	[35]
Aspergillus flavus	Acid Red 151	[44]
Trametes trogii	Remazol Brilliant Blue R	[44]

that yeast can act as the important dye adsorbent and can uptake high amount of dye concentration.

Decolorization by Algae

Algae, which are photosynthetic are present everywhere. A study suggests that the degradation of azo dyes by algae is possible through a form of azoreductase [11]. The color reduction of dye-contaminated wastewater effluent by algae follows the different mechanisms like complete use of chromophores for the production of algae and the adsorption of chromophores. It was concluded from the studies that nearly 30 of the azo dyes can be degraded using the algae Chlorella. The future outcomes might also intend that algae play a vital part in the degradation in stabilizing ponds, and this process can be less costly [37].

2 Conditions Affecting Decolorization

The dyes are utilized for several purposes like coloring, processing of fibre. The runoffs of water from dyeing processes are of different chemical compositions which include various organic compounds, Sulfurous compounds, nutrients, salts, toxic substances [19, 41]. Various physicochemical parameters like the level of mixing, addition or removal of oxygen, variation of temperature, variation in acidic conditions, the structure of dye, concentration of dye, supplementing different carbon and nitrogen sources affect the microbial degradation. The presence of electron donors also affects microbial degradation.

2.1 Effect of Oxygen and Mixing

The degradation of azo dye effluent occurs in anaerobic, facultatively anaerobic, aerobic conditions. It is studied that the decolorization process of azo dye-contaminated water takes place in perfectly anaerobic conditions [32]. The same results were observed when the microbial strains such as *Pseudomonas luteola*, *Proteus mirabilis*, *Pseudomonas* sp. *SUK1*, *Micrococcus glutamicus* NCIM-2168 [37] were used. It was studied that, as the mixing is augmented, the degradation of azo dyes is reduced as there is an increase in oxygen content thereby increasing DO [12]. Moreover, the impact of the presence of oxygen on the reducing process can be irreversible [9] and the intermediates obtained through decolorization of simple dyes lead to the opening of their aromatic chains [12]. It was also stated that, in the process of removal of color from Reactive Red 22 using Escherichia coli, when the agitation speed is zero, the DO content in the culture dropped to nearly zero, but as the speed is increased to 200 rpm, DO content increased to 0.5 mg per L. No notable decolorization was observed.

2.2 Effect of Carbon and Nitrogen Sources Addition/removal

The decolorization process of azo dyes is very difficult when the amount of carbon is less. AD contaminated water has less carbon content and henceforth the addition of sources of carbon or nitrogen should be done for the degradation process [14]. It was stated that anaerobic consortia, acidogenic bacteria convert the soluble substrates, such as carbohydrates, to volatile organic acids or alcohols [38]. Whereas, the addition of nitrogen sources like beef extract, peptone, urea, yeast extract generates NADH that is used as an electron donor for the effective reduction of azo dyes to amines [13]. In some reports, it was found that the presence of lignocellulose substrate increases decolorization. It was observed that the presence of lignocellulosic substrates enhances decolorization by effective production of lignolytic enzymes. Similar results are obtained when microbes like *Comamonas* sp. *UVS*, showed active decolorization of the dye Direct Blue GLL along with the generation of lignolytic enzymes.

2.3 Effect of Temperature

The activity of the microorganism is dependent on the temperature of the environment. Hence temperature plays an important role in the treatment of wastewater. A few studies are on the activation energy required for the microbial degradation [15]. It is reported that the physiological temperature changes bring about a change in activation energy [44]. It was observed that the rate of degradation was increased with increase in temperature up to a particular temperature and later it decreased [38]. But, some of the enzymes produced by the bacteria can be thermally stable even at high temperatures [31].

2.4 Effect of pH

The optimum value for the pH (since it has a large effect on degradation of azo dyes) for degradation of azo dyes is in the range of 6.5 and 10 for the microorganism *Proteus mirabilis*. The values of the pH should be neutral for the decolorization to be high whereas decolorization decreases when in highly acidic medium or highly alkaline medium [38]. Arica and Bayramoglu [4] stated that, as the value of pH was reduced, the decolorization of Reactive Red 12 dye by fungi sajor-caju was improved. In the process of degradation of azo dyes, the amines are formed which results in an increase of the pH value which showed a negative effect decolorization. But [12] obtained that the rate of reduction of dyes into amines increased 2.5 times with the increase of pH from 5 to 7 for *Pseudomonas luteola*. For the degradation of dye

Reactive Red 190 by isolated *Citrobacter* sp. *C3*, the reaction was effective at acidic conditions [44].

2.5 Effect of Dye Concentration

Many studies state that with the rise in dye concentration, there is a reduction in the decolorization rate. The reason given for it is the poisonous consequence of dyes on microbes, blocking of active sites of azoreductase by excess dye molecules with different structures [37]. Saratale et al. [36] in their study stated that azo dyes having the sulphonic group on their rings inhibited the growth of microbes. But, [37] stated that the use of cocultures of the microbes can reduce the inhibition of growth due to the dye concentrations.

2.6 Effect of Dye Structure

The structures of the dyes are very diverse because of the presence of several functional groups. It was identified that the degradation rate is higher in dyes of simpler structures than those of the ones having $–SO_3H$, $–SO_2NH_2$ in the phenyl ring [23]. The functional group of azo dyes with a higher electronic density might be not favorable to the second electron transfer forming the dianion, leading to low or no capability for decolorization [31]. Even the chemical structure of the compound may affect the decolorization rate [42].

2.7 Effect of Electron Donor

For decolorization, the azo dyes have to be reduced. But if the organic content in the effluent is low that complete decolorization process is not possible by the microbes, there will be a need for the external addition of electron donors so that reduction increases and it enhances the complete process of decolorization [19]. Hence there is a need to study how the external addition of electron donors affect the rate of degradation [22]. For the dye Reactive Orange 16 using isolated *Bacillus* sp. *ADR*. Ghodake et al. [19] reported that some of the electron contributors like sodium formate, sodium acetate, sodium pyruvate, sodium succinate, sodium citrate are added to improve the rate. Brás et al. [10] stated that the addition of glucose or acetate ions enhances the reduction. Methanol is a perfect electron donor [18]. Moreover, the addition of electron donors not only favor the reduction but also enhances enzymatic action for the decolorization process [31]. The enzymes can be either reductive or oxidative. One of the major used reductive enzymes is azoreductase and the oxidative enzyme is

Table 3 Effect of reducing enzymes

Theory	References
They can be used for the studies of environmental biotechnology	[43]
Bacillus subtilis is induced with electrophiles (catechol, diamide)	[43]
Enzymes like Xenophilus azovoranss K46F, Pigmentiphaga kulle K24, Enterococcus faecalis, Staphylococcus aureus, Esherichia colii, *Bacillus* sp. OY1 to 2, and Rhodobacter sphaeroids are studied	[43]
NADH-DCIP reductases are enzymes that are formed in the bacterial and fungi cultures and are used for the detoxification	[36]
Riboflavins role for the degradation was found out for the dyes Mordant Yellow 10 with anaerobic granulated sludge and Brilliant Blue G by a microbial conglomerate	[24]
Dye Scarlet Ruby by *P. vulgaris* and *Micrococcus glutamicus*, Dye Reactive Green 19A by M. glutamicus Dye Navy Blue Gl by isolated *Bacillus* sp.	[36]

lignin peroxidase. Table 3 gives the compilation of various works showing the effect of reducing enzymes.

The mechanism of azo dye oxidation by peroxidases such as LiP probably involves the oxidation of the phenolic group to produce a radical at the carbon bearing the azo linkages. The water attacks this phenolic carbon to cleave the molecule producing phenyldiazene, and the phenyldiazene can then be oxidized by a one-electron reaction generating N_2 [18]. Laccases are the compounds that are made of copper on the aromatic rings and hence named based on copper position. They act as the catalyst for the metabolism and degradation of various phenols and azo dyes [18]. Table 4 gives the compilation of various works showing the effect of reducing enzymes.

Table 4 Effect of oxidizing enzymes

Theory	References
LiP is used in the degradation of the multiaromatic hydrocarbons, ADs	[16]
It was detected that decontaminated enzymes have a better capability to degrade various ADs	[19]
Bacteria *Micrococcus glutamicus*, *Pseudomonas desmolyticum* can catalyze the azo dye oxidation	[36]
Enzyme laccase is used in the decolorization of dye CI reactive Orange 16	[19]
Moreover, refinement of the enzyme "veratryl alcohol oxidase" from *Commamonas* sp. *UVS* in decolorization of Red HE7B and Direct Blue GL has been described	[19]

3 Methods of Analysis

UV–vis spectroscopy is the method of analysis that gets the information of decolorization based on the appearance of peaks. The color level in the effluents of industries or the mixture of dyes can be found using American Dye Manufacturers Institute tristimulus filter method (ADMI 3WL). Another analytical procedure is “thin layer chromatography (TLC)” which is used to distinguish closely related dyes and also to find out the decolorization [28]. Another technique used for the analysis is “high performance liquid chromatography (HPLC)” which utilizes high pressure and tight packing [12]. The degradation can be found using this analysis. And the widely used analytical method is Fourier transform infrared spectroscopy (FTIR) which explains the interactions of various functional groups in ADs after degradation and also determine decolorization rate. The mass spectrometry is used for finding the spectra of the mixture, molecular weights and structural information. Based on the state of the mixture, it can be termed liquid chromatography-mass spectrometry (LC–MS) or gas chromatography-mass spectrometry (GC–MS) [37]. Moreover, nuclear magnetic resonance (NMR) analytical method is used for the study to get the structural information of the compounds. It is also possible to find out the decolorization by calculating values of BOD, COD and TOC [37].

4 Future Work

In some situations, the final products of degradation are more toxic than the parent dyes. Hence there is a need for dye mineralization after the dye degradation, a process is to be evolved for a maximum number of dyes. Moreover, sophisticated methods are to be used for the degradation. This can be obtained by combining the AOPs along with the microbial degradation. Novel Azo dyes have to be designed by physical or chemical restructuring so that more efficient degradation is possible and less effluent waste is released from the textile industries.

5 Conclusions

Since the rules and regulations in the treatment of wastewater have become strict, there should be an efficient way to degrade azo dyes. This can be achieved using microbial degradation since it is less costly and generate less sludge. The effluents when released into the freshwater, result in the increase of BOD, COD, TOC values of water thereby making it difficult for the aquatic beings in breathing and the toxicity of the effluent may also affect organisms when the water is ingested. When there is bio metabolism of azo dyes, aromatic amines are formed after the reduction of azo bonds. The bacteria are used in the degradation since they have a quick growth rate, elevated hydraulic retention stretch. Not all azo dyes are biodegradable. Hence a new type of processes of AOPs and microbial Fuel Cells combined with microbial degradation is to be established. The literature presented in this paper explained the various conditions affecting the degradation process using microbes like the presence or absence of oxygen or the oxidation or reduction processes used to degrade azo dyes. It is stated that the degradation is done by reducing the azo bonds of dyes or cleaving reactions of azo dyes. Work can be done by, (a) Choosing appropriate enzymes for biodegradation, (b) industrial effluents. Optimization studies can be done for microbial degradation of different azo dyes. From many research studies, it is evident that microbial degradation made that they are very promising to conventional treatment methods.

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