

# Microbial Degradation of Azo Dyes From Textile Industry - Review

Devarakonda Pavan Srivatsav<sup>\*1</sup>

<sup>\*1</sup>M. Tech Biotechnology,  
Dept. of Chemical Engineering, AUCE(A),  
Andhra University, Visakhapatnam,  
Andhra Pradesh, India

Dr. Veluru Sri Devi<sup>2</sup>

<sup>2</sup>Professor,  
Dept. of Chemical Engineering,  
AUCE(A), Andhra University, Visakhapatnam,  
Andhra Pradesh, India

Padala Sai Vikas Reddy<sup>3</sup>

<sup>3</sup>B. Tech, Chemical Engineering,  
AUCE(A), Andhra University College of Engineering,  
Visakhapatnam, Andhra Pradesh, India

Marupudi Sree Venkat<sup>4</sup>

<sup>4</sup>B. Tech, Chemical Engineering,  
AUCE(A), Andhra University College of Engineering,  
Visakhapatnam, Andhra Pradesh, India

Husam Talib Al Shammari<sup>5</sup>

<sup>5</sup>M. Tech, Chemical Engineering,  
AUCE(A), Andhra University College of Engineering,  
Visakhapatnam, Andhra Pradesh, India

**Abstract:-** Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries such as textile, food, cosmetics and paper printing. They are generally recalcitrant to biodegradation due to their xenobiotic nature. Azo dyes account for the majority of all dyestuffs, produced because they are extensively used in the textile, paper, food, leather, cosmetics and pharmaceutical industries. However, microorganisms, being highly versatile, have developed enzyme systems for the decolorization and mineralization of azo dyes under certain environmental conditions. A variety of synthetic dyestuffs released by the textile industry pose a threat to environmental safety. This review provides an overview of classification, structure and degradation of azo dyes from the wastewater effluents of textile industry. This review mainly focuses on the different mechanisms of decolorization and discusses the effect of various physicochemical parameters on the dye removal efficiency of different bacteria.

**Keywords:** Azo Dye, Textile Industry, Microbial Dehydration Mechanism

## I. INTRODUCTION

Dyes are synthetic and natural compounds that make the world look brighter through colored products. Textile dyes represent a category of organic compounds, generally considered pollutants, discharged into wastewaters resulting mainly from processes of chemical textile finishing [1].

Textile industry is one of the largest generators of contaminated effluents, hence the environmental problems created by the textile industry have received increased attention for several decade [2].

During the coloration process, dyes do not bind to the fabric and a large percentage is lost to the wastewater stream. Approximately 10-15% dyes are released into the environment during dyeing process making the effluent highly colored and aesthetically unpleasant.

Wastewater resulting from these processes has adverse impacts in terms of Total Organic Carbon (TOC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD),

suspended solids, salinity, color, a wide range of pH (5–12) and the recalcitrance of organic compounds, such as azo dyes [3].

More than ten thousand synthetic dyes were developed and used in manufacturing by the end of the 19th century [4].

Moreover, the growth of the worldwide textile industry in the years since then had seen a commensurate increase in the use of such synthetic dyes, and this has been accompanied by a rise in pollution due to wastewater contaminated with dyestuff [5].

The chemical dyes employed more frequently on industrial scale are the azo, anthraquinone, Sulphur, indigoid, triphenylmethyl (trityl), and phthalocyanine derivatives. It must be emphasized that majority of synthetic dyes currently used in the industry are azo derivatives. The azoketo hydrazone equilibria can be a vital factor in the easy breakdown of many of the azo dye system.

Since 1856, over 105 different dyes have been produced worldwide with an annual production of over  $7 \times 10^5$  metric tons [6].

Paper and pulp mills, textiles and dyestuff industries, distilleries, and tanneries are some of the industries, which release highly colored wastewaters [7].

All dyes do not bind to the fabric; depending on the class of the dye, its loss in wastewaters could vary from 2% for basic dyes to as high as 50% for reactive dyes, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries [8].

Generally, the azo dye degradation by bacteria proceeds in two stages. The first stage involves reductive cleavage of the dyes' azo linkages, resulting in the formation of generally colorless but potentially hazardous aromatic amines and the second stage involves degradation of the aromatic amines. Bacterial biodegradation of aromatic amines is an almost exclusively aerobic process whereas, Azo dye reduction usually requires anaerobic conditions.

The interest in publication of research and review articles dealing with the remediation of environmental pollution caused by synthetic dyes is an indication and proof of the global concern over this issue. During the last two decades, the scientific community in the whole world and especially in India and China has been active in research on the problems caused by this source of environmental pollution and its effective remediation. This review paper deals with the biodegradation of synthetic dyes and various factor that influence the degradation process.

### A. Classification of Dyes

The major structure element responsible for light absorption in dye molecules is the chromophore group, i.e., a delocalized electron system with conjugated double bonds. Chromophores frequently contain heteroatoms as N, O, and S, with non-bonding electrons. Common classes of dyes, based on the chromophore present, are shown in the following (fig. 1) [9].

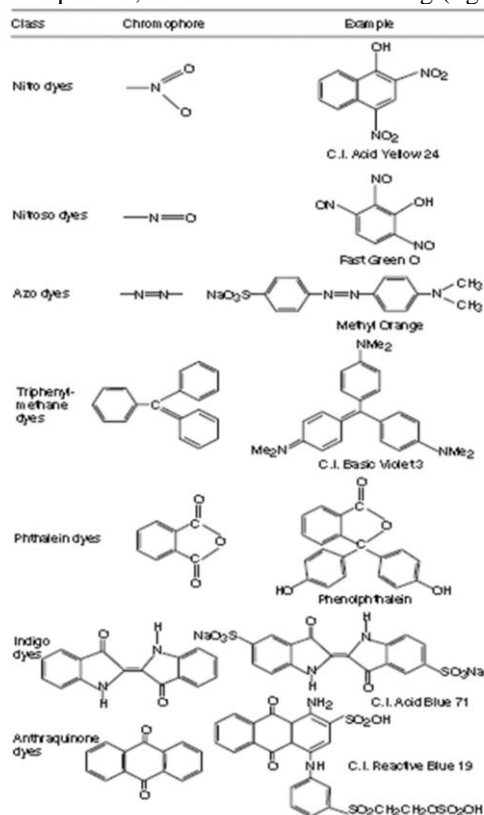


Fig. 1. Classification of dyes. (based on chromophore present)

### B. Structure of Dyes

Synthetic dyes exhibit considerable structural diversity (fig. 2). The chemical classes of dyes employed more frequently on industrial scale are the azo, anthraquinone, sulfur, indigoid, triphenylmethane (trityl), and phthalocyanine derivatives. It should be noted that azoketohydrazone equilibria can be a vital factor in the easy breakdown of many of the azo dye system. Some dyes quoted in the review have only a marginal importance from the point of view of industrial application.

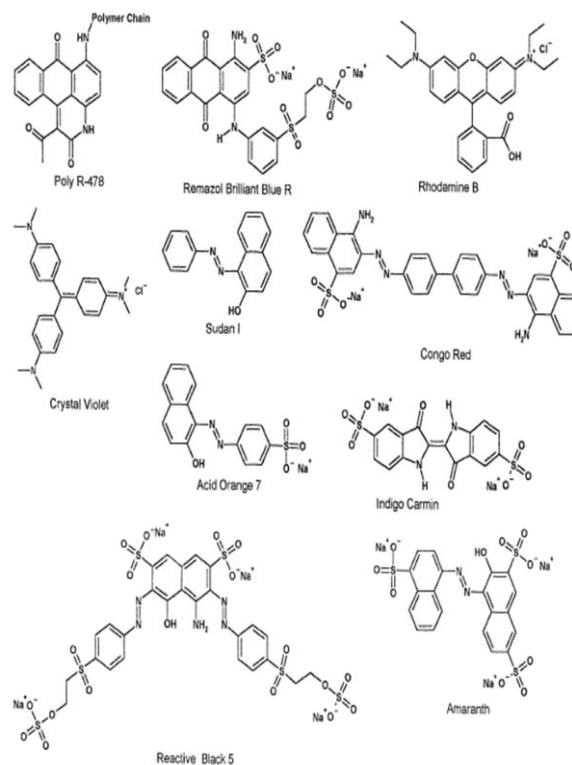


Fig. 2. The chemical structure of synthetic dyes. (most frequently studied in degradation experiments)

## II. DIFFERENT METHODS FOR THE REMOVAL OF DYES

There are different methods for the treatment of textile waste effluents. Various physicochemical methods have been used for the removal of dyes from wastewater effluent. However, implementation of physical/chemical methods have the inherent drawbacks of being economically unfeasible (as they require more energy and chemicals), being unable to completely remove the recalcitrant azo dyes and their organic metabolites, generating a significant amount of sludge that may cause secondary pollution problems, and involving complicated procedures [10, 11].

However, microbial or enzymatic decolorization and degradation is an eco-friendly cost-competitive alternative to chemical decomposition process that could help reduce water consumption compared to physicochemical treatment methods [12].

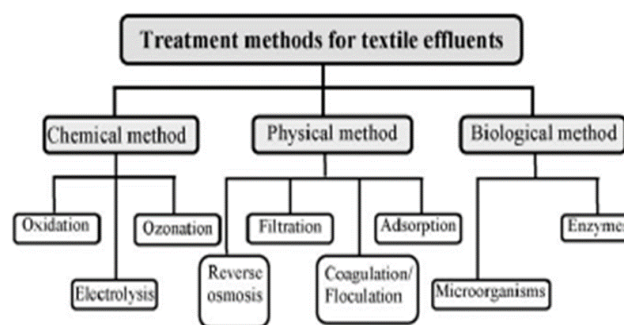


Fig. 3. Treatment methods for the removal of dyes (from wastewater effluents)

### **A. Importance of biological treatment relative to physicochemical methods**

Dye-house effluent typically contains only 0.6–0.8 g L<sup>-1</sup> dye, but the pollution it causes is mainly due to durability of the dyes in the wastewater [13].

Hence it is necessary to search for and develop effective treatments and technologies for the decolorization of dyes in such effluents. Various physical/chemical methods, such as adsorption, chemical precipitation, photolysis, chemical oxidation and reduction, electrochemical treatment, have been used for the removal of dyes from wastewater (fig. 3). Moreover, there are many reports on the use of physicochemical methods for the color removal from dye containing effluents [14].

### **B. Physical & Chemical Methods**

Physical methods based on coagulation–flocculation of dyes is effective for the removal of mainly Sulphur and disperse dyes but show very low coagulation–flocculation capacity for acid, direct, reactive and vat dyes. In addition, the low color removal efficiency and large amount of sludge produced limits the application of these techniques [15].

Adsorption methods have attracted considerable interest due to their higher efficiency for the removal of a wide range of dyes. The selection of an adsorbent is based on characteristics such as high affinity, capacity for target compounds and the possibility of adsorbent regeneration [16].

Although activated carbon (AC) is a very effective adsorbent for various types of dyes, it is not often used due to its high cost [17].

Some researchers use low cost adsorbent materials like peat, bentonite clay, fly ash, polymeric resins, ion exchangers and many biological materials such as, corn/maize cobs, maize stalks, and wheat straw for the color removal of dye wastewater to make the process more economically feasible [18].

However, the practical application of these adsorbents has been limited by problems associated with their regeneration or disposal, high sludge production, low effectiveness with regard to wide range of dyes and high cost [19].

Filtration methods such as nano-filtration, reverse osmosis and ultra-filtration have been used for water reuse and chemical recovery. In the textile industry, the use of membranes provides interesting possibilities for the separation of hydrolyzed dyestuffs and dyeing auxiliaries that simultaneously reduce the color, BOD and COD of wastewater. With this approach, the selection of the type and porosity of the filter depends upon the chemical composition of the wastewater and the specific temperature required for the process [20].

However, membranes have some significant drawbacks, including high investment costs, potential membrane fouling and the production of secondary waste streams which need further treatment [21].

Chemical oxidation methods enable the destruction or decomposition of dye molecules, and such approaches use various oxidizing agents, such as ozone (O<sub>3</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and permanganate (MnO<sub>4</sub>). Modification in the chemical composition of a compound or a group of compounds takes place in the presence of these oxidizing

agents, and thus the dye molecules become susceptible to degradation [22].

Ozonation has been found to be effective due to its high reactivity with many azo dyes, the lack of alteration of the reaction volume due to its gaseous state, and good color removal efficiencies [23].

However, its short lifetime, ineffectiveness towards dispersed dyes and those insoluble in water, low COD removal capacity, as well as the high cost of ozone, limits the practical application of this technique [24].

In advanced oxidation processes (AOP) (photochemical and photocatalytic), oxidizing agents such as O<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> or heterogeneous photocatalysts are used with catalysts, such as TiO<sub>2</sub>, ZnO<sub>2</sub>, Mn and Fe, in the presence or absence of an irradiation source which generates (OH) radicals for the destruction of hazardous dye pollutants [25].

### **C. Biological Methods**

The initial step of the bacterial degradation of azo dyes is reductive cleavage of the –N=N– bond. Decolorization of azo dyes occurs under anaerobic (methanogenic), anoxic and aerobic conditions by different trophic groups of bacteria. Decolorization of azo dyes under these different conditions is briefly discussed in subsequent sections.

## **III. AZO DYE DECOLORATION**

### **A. Under anaerobic conditions**

Methanogenesis from complex organic compounds requires the coordinated participation of many different trophic groups of bacteria, including acidogenic, acetogenic and methanogenic bacteria [26].

Dye decolorization under these conditions requires an organic carbon source or an energy source. Substrates like acetate, starch, glucose, ethanol and more complex ones, such as whey, have been used for dye decolorization under methanogenic conditions [27, 28].

Extensive studies have been carried out to determine the role of the diverse groups of bacteria in the decolorization of azo dyes [29].

Use of molecular methods to characterize the microbial populations in anaerobic-baffled reactors treating industrial dye waste showed that members of the  $\gamma$ -proteobacteria, together with sulfate reducing bacteria (SRB), were prominent members of mixed bacterial populations. Along with these, a methanogenic population dominated by *Methanosaeta* species and *Methanomethylovorans hollandica* contributed to the treatment of industrial wastewater [30].

Yoo et al. (2001) showed that decolorization of Orange 96 was not significantly affected by 2-bromoethanesulfonic acid (BES), an inhibitor specific to methanogens. This suggests that methanogens took no part in the decolorization, and it contradicts the findings of many other investigators. On the other hand, in the presence of acetate and sulfate, molybdate, an inhibitor specific to SRB, caused a significant decrease in the decolorization rate [31].

Reduction under anaerobic conditions appears to be nonspecific, as most of a varied group of azo compounds are decolorized, although the rate of decolorization is dependent



on the added organic carbon source, as well as the dye structure [32].

### **B. Under anoxic conditions**

Anoxic decolorization of various azo dyes by mixed aerobic and facultative anaerobic microbial consortia has been reported [33, 34].

Many of these cultures were able to grow aerobically but their decolorization was achieved only under anaerobic conditions. Pure bacterial strains, such as *Pseudomonas luteola*, *Aeromonas hydrophila*, *Bacillus subtilis*, *Pseudomonas* sp. and *Proteus mirabilis*, decolorized azo dyes under anoxic conditions [35].

Azo dye decolorization by mixed, as well as pure, cultures generally required complex organic sources, such as yeast extract, peptone, or a combination of complex organic source and carbohydrate [36].

The preferred substrate in anaerobic dye decolorization under methanogenic conditions is glucose. Depending on the bacterial culture, the suitability for anoxic dye decolorization by facultative anaerobes and fermenting bacteria seems to vary. Glucose enhanced the decolorization of Mordant Yellow 3 by *Sphingomonas xenophaga* Strain BN6, whereas a significant decrease in azo dye decolorization in its presence was reported for *P. luteola*, *Aeromonas* sp. and few other mixed cultures [37, 38].

### **C. Under aerobic conditions**

During the past few years several bacterial strains that can aerobically decolorize azo dyes have been isolated. Many of these strains require organic carbon sources, as they cannot utilize dye as the growth substrate [39].

*P. aeruginosa*, an organism that decolorized a commercial tannery and textile dye, Navitan Fast blue S5R, in the presence of glucose under aerobic conditions. This organism was also able to decolorize various other azo dyes [40].

Very few bacteria were able to grow on azo compounds as the sole carbon source. These bacteria utilize amines as the source of carbon and energy for their growth by cleaving the  $-N=N-$  bonds. Such organisms are specific towards their substrate. Examples of these kind of bacterial strains are *Xenophilus azovorans* (previously *Pseudomonas* sp. KF46) and *Pigmentiphaga kullae* (previously *Pseudomonas* sp. K24), which can grow aerobically on carboxy-orange I and carboxy-orange II, respectively [41]. These organisms could not grow on acid orange 20 (Orange I), structurally analogous sulfonated dyes and AO7.

## **IV. FACTORS AFFECTING DYE DEGRADATION BY BIOLOGICAL METHODS**

Operational parameters greatly influence the efficiency of biological treatment systems. The level of aeration, temperature, pH and redox potential of the system must be optimized to produce the maximum rate of dye reduction. The concentrations of the electron donor and the redox mediator must be balanced with the amount of biomass in the system and the quantity of dye present in the wastewater.

The ability of the bacterial cells to reduce dyes from a range of dye classes (acid, basic, direct, disperse, metal-complex, reactive, Sulphur and vat) must be tested to determine the types

of wastewater that can be treated by the system. The composition of textile wastewater is varied and can include organics, nutrients, salts, Sulphur compounds and toxicants as well as the color [42].

These compounds might have an inhibitory effect on the dye reduction process. The effect of these factors must be investigated before the biological system can be used to treat industrial wastewater in the color removal process.

### **A. Oxygen**

The effect of oxygen on cell growth and dye reduction is the most important factor to be considered. As mentioned previously, during the cell growth stage, oxygen will have a significant effect on the physiological characteristics of the cells. During the dye reduction stage, if the extra cellular environment is aerobic, the high-redox-potential electron acceptor, oxygen, may inhibit the dye reduction mechanism. This is because the electrons liberated from the oxidation of electron donors by the cells are preferentially used to reduce oxygen rather than the azo dye, and the reduction product, water, is not a reductant [43].

The postulated intermediates of the dye reduction reaction include hydrazine form of the dye and the azo anion free radical form of the dye, tend to be reoxidised by molecular oxygen [44].

### **B. Temperature**

In many systems, the rate of color removal increases with increasing temperature, within a defined range that depends on the system. The temperature required to produce the maximum rate of color removal tends to correspond with the optimum cell culture growth temperature of 35–45 °C. The decline in color removal activity at higher temperatures can be attributed to the loss of cell viability or to the denaturation of the azo reductase enzyme [45].

The microenvironment inside the support offers protection for the cells. Therefore, Immobilization of the cell culture in a support medium results in a shift in the optimum color removal temperature towards high values.

### **C. pH**

The optimum pH for color removal is often at a neutral pH value or a slightly alkaline pH value and the rate of color removal tends to decrease rapidly at strongly acid or strongly alkaline pH values. As a result, the colored wastewater is often buffered to enhance the color removal performance of the cell culture. Biological reduction of the azo bond can result in an increase in the pH due to the formation of aromatic amine metabolites, which are more basic than the original azo compound [46].

### **D. Dye concentration**

Wuhrmann et al. observed that, after an initially rapid reduction of color, the rate of color removal decreased more rapidly than would be predicted by a first order reaction [47].

This effect was caused by the toxicity of the metabolites that were formed during dye reduction. The higher the dye concentration, the longer the time required to remove the color. Sani et al found that dyes with concentrations of 1–10 mM were easily decolorized, but when the dye concentration was increased to 30 mM, color removal was reduced [48].

### E. Dye structure

Some azo dyes are more resistant to removal by bacterial cells [49]. Dyes with simple structures and low molecular weights exhibit higher rates of color removal, whereas color removal is more difficult with highly substituted, high molecular weight dyes [48].

Cultures adapted to produce azoreductase enzymes had very high specificity towards particular dye structures. One such enzyme, Orange I azoreductase, exclusively reduced the azo groups of Orange I and its derivatives with their hydroxy group in the para position. Another enzyme, Orange II azoreductase, was specific for Orange II-type compounds with their hydroxy group in the ortho position. It was also shown that sulphonated dyes were reduced faster than carboxylated dyes due to the higher electronegativity of the sulpho group, which renders the azo group more accessible to electrons [50].

Hydrogen bonding, in addition to the electron density in the region of the azo bond, has a significant effect on the rate of reduction [51].

The nature of substituents and their position on the dye molecule influence the azo-hydrazone tautomerism of hydroxyazo compounds. The hydroxy proton of phenylazo-naphthol derivatives is labile and can bond with a nitrogen atom of the azo group, causing a rapidly formed tautomeric equilibrium between the azo and hydrozone forms. This equilibrium is influenced by both the nature of the medium surrounding the molecule and by the structural factors within the molecule.

## V. MECHANISM OF AZO DEGRADATION

The major step in the bacterial degradation of azo dyes, in either aerobic or anaerobic conditions, is the reduction of the  $-N=N-$  bond. This reduction may involve different mechanisms, such as enzymes, low molecular weight redox mediators, chemical reduction by biogenic reductants like sulfide, or a combination of these (fig. 4). Additionally, the location of the reactions can be either intracellular or extracellular.

### A. Direct enzymatic azo dye reduction

The mechanism in the direct enzymatic azo dye reduction involves enzyme-mediated transfer of reducing equivalents, generated from the oxidation of the substrate to azo dyes. These enzymes can be specific, catalyzing only azo dye reduction, or nonspecific. The latter enzymes catalyze the reduction of a wide range of substrates. Due to their nonspecific nature, these enzymes gratuitously reduce azo dyes.

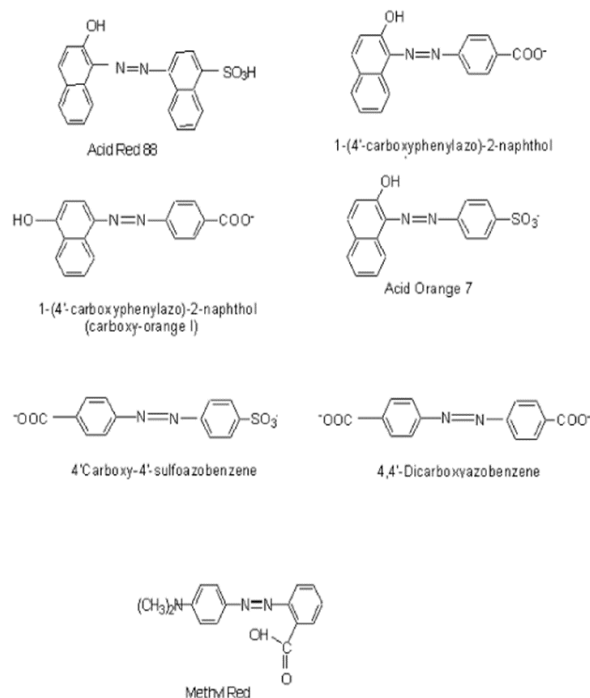


Fig. 4. Structures of a few azo dyes degraded (under aerobic conditions)

### B. Under anaerobic conditions

The presence of azoreductases in anaerobic bacteria that decolorized sulfonated azo dyes during growth on solid or complex media was first reported by Rafii et al. [52].

These strains belonged mainly to the genera Eubacterium and clostridium. Azoreductases from these strains were produced constitutively and released extracellularly. Later investigations made with *C. perfringens* showed that azo dye reduction is catalyzed by an enzyme presumed to be flavin adenine dinucleotide dehydrogenase, which can also reduce nitro aromatic compounds [53].

## VI. CONCLUSION

Accumulation of dyestuff and dye wastewater creates environmental pollution as well as medical and aesthetic problems. As regulations are becoming even more stringent, there is an urgent need for technically feasible and cost-effective treatment methods. Microbial and enzymatic decolorization and degradation of azo dyes have significant potential to address this problem due to their environmentally friendly, inexpensive nature, and also because they do not produce large quantities of sludge. Bacteria have many other advantages such as a fast growth rate and high hydraulic retention time, and thus they could be efficient in treating high-strength organic wastewaters.

It is essential to know whether the azoreductase enzyme system in bacteria is constitutive, inducible, or repressible. A better understating of azoreductase will lead scientist to search for new azoreductases with broad substrate specificity and high specific activity. Although a number of microorganisms capable of azo dye reduction have been identified, there are only a relatively small portion of bacteria have been screened and studied in detail for azo dye degradation so far. Therefore, it could be important to isolate new bacteria with high

capabilities to degrade azo dyes. The biochemical pathways of azo dye mineralization are still poorly understood in bacteria. The complexity of the total degradation of azo dyes indicates the need for more research.

This literature review discusses that anaerobic-aerobic biological methods may be appropriate for the treatment of dye-containing wastewaters. However, the extent of mineralization of aromatic amines need to be assessed, as amines can undergo autooxidation, leading to the formation of soluble recalcitrant polymers, which may be toxic.

Microorganisms that use the dyes as a sole source of carbon, nitrogen, and energy are of special interest and significance because they consume the dye for their growth and activities while at the same time eliminate the pollutant in a real sense. Such microorganisms are a valuable gift from Nature. Over the past two decades, much attention has been focused on the biodegradation of synthetic dyes using different groups of microbes. The science of bioremediation is emerging as a unique tool to deal with the removal of synthetic dyes and other xenobiotics from the environment.

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## AUTHORS PROFILE



**D Pavan Srivatsav** is a graduate in B.Tech (Bio Technology) from GITAM University, Visakhapatnam. He is currently pursuing M. Tech (Bio Technology) from the Department of Chemical Engineering, Andhra University, Visakhapatnam. Pavan Srivatsav is extremely fascinated by the increasing growth in the field of Bio Technology. With an unceasing enthusiasm, he grabbed the opportunity to work as a summer internship trainee in water management department of Visakhapatnam steel plant. Further, he has been a graduate trainee in Center for Cellular and Molecular Biology (CCMB), Hyderabad for a duration of three months and conducted a research study on DNA bar-coding of some common weed plants of India. He is highly-driven towards piloting more research studies in future and the current research paper is one of the indexes of his inquisitiveness.



**Dr. V. Sridevi** is Professor having an experience of 21 years in teaching and more than 10 years of Research experience from the Department of Chemical Engineering, Andhra University College of Engineering (A), Andhra University, Visakhapatnam. Dr. V. Sridevi has significantly contributed in the field of Industrial Pollution and Control Engg. She has worked on Biodegradation, Bio reactor design and Industrial Pollution & its control. She has successfully guided 40 M. Tech students and 13 Research scholars were pursuing PhD (03 Awarded & 02 submitted) under her guidance. She has published more than 150 papers in National & International Journals. She is serving as Quality Management System Internal auditor for ISO 9001:2015 certification. She is also serving as Departmental Research Committee member & a member of departmental development, Examinations committee and Industrial Training & student activities.