

Analytical Study On Microbial Degradation in Textile Azo Dyes

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Abstract

Textile and other dye industry wastewater effluents include large quantities of synthetic dyes which need to be treated to avoid the pollution of groundwater. This study investigated 4 strains of azo degrading bacteria for biotechnology for the treatment of azo dyes in order to discover the effective strains and incubation time needed for coloring. He got a great deal of attention for the capacity of microbes to perform coloration. A cost-effective way to remove these contaminants from the environment is to decolorize microbiologically and to degrade the dyes. A number of microorganisms have recently been found to decolorate a variety of colors in a basic study. In this study, we have investigated and report on advances and limits on the biological decolorization of textile dyes. The FTIR and GC-MS analyses have verified the biodegradation of dye. The current approach may address major pollution issues in water systems due to textile waste disposal.

Keywords: Wastewater, Decolorization, Biodegradation, Microbial.

1. INTRODUCTION

Synthetic dyeing such azo and anthraquinone dyes, as simple to manufacture, cost-effective, stable in comparison with natural colours, is popular in the food, paper, cosmetic, textile, leather or pharmaceutical industry and are available in a variety of colours. Due to its fused aromatic structure, anthraquinone dyes are degradation resistant and survive for a longer time in the environment. Textile dye effluents comprising suspended particles, detergents and dyes, when mis-disposed into water bodies lead to sunlight penetration and, afterwards, photosynthetic activity decreased, O₂ dissolved decreased, and water quality degradation. Textile effluent water has possible adverse effects on aquatic life and leads to ecological harm. The ecological harm caused by changes in pH and elevated levels of COD, frequently producing a decoloration of water resources, into which it is discharged, is complicated by high over all organic carbon (TOC) and chemicals oxygen demand (COD).

In environmentally-based biotechnology, bioremediation of textile wastes is constantly increasing, since it is a quick and effective method for continuous color removal. Pure bacterial strains are usually unable to fully degrade the dyes and produce carcinogenic amines that are also decomposed²⁰. For wastewater treatment systems, it is important to grow and sustain large-scale pure cultures²¹. Many consortia have been explored in recent times with improved degrading capacity. The mineralization and biodegradation of synthetic dyes by the microbial consortium was considerable compared with the pure strains due to the synergistic conduct of the microbial population metabolism. By addressing various aromatic ring locations, the bacterial strains in the consortium metabolize the molecular structure of the dye and the metabolites produced were further destroyed by the surviving strains. Several researchers are presently creating an effective microbial consortium to improve the efficiency of decolorization that may remove a number of contaminants from textile effluent. In the fields of *Bacillus cereus*, *Pseudomonas putida*, BN-4, *Pseudomonas fluorescens* (BN-5) and *Stenotrophomonas acidaminiphila* several investigators have established a consortium of bacteria (BN-3). They discovered that the consortium formed exhibited an enhanced decoloration effectiveness of 78–99% with the concentration of 60 mg L⁻¹ in color within 24 hours and that the efficiency was three times better than that of the individual strain.

The most significant and biggest type of synthetic dyes used in commercial applications are azo dyes which are aromatic compounds having one (–N=N–) group or more. They are termed xenobiotic substances that are resistant for processes of biodegradation. Two-thirds of the entire dyestuff market is represented in the textile sector. Around 10-15 percent of the utilized colors are discharged in wastewater during the dyeing process. The presence of such dyes in the aquatic ecosystem is the source of severe health and environmental problems. Many techniques for the treatment of textile effluents for decolorisation are used. Physical chemical processes

including filtration, coagulation, activated carbon and chemical flocculation are included. These techniques are efficient but costly and entail the development of a concentrated loam, which presents a difficulty with secondary disposal. Dye degradation and wastewater re-use have been carried out via novel biological processes in recent years, including aerobic and an aerobic bacteria and fungus.

2. LITERATURE REVIEW

N Puvaneswari (2006) Azo dyes toxic effluents come from many sectors and severely impact the water resources, soil fertility, aquatic species and the integrity of the environment. They are harmful to aquatic creatures (fish, algae, bacteria, etc.) and mammals (lethal impact, genotoxicity, mutagenicity, carcinogenicity). In natural circumstances, they are not easily degradable and traditional waste water treatment technologies usually do not eliminate the waste water. In a number of experimental animals, benzidine-based dyes have long been identified as carcinogenic urinary bladder for human uses and tumor. Various bacteria have been discolored, transformed and even mineralized entirely. Two pseudomonas strains have a mixture culture that effectively degrades 3-chlorobenzoate (3-CBA) and phenol/cresols combination. Azoreductases of the various microorganisms are helpful for biodegradation systems because they under moderate circumstances catalyze the reductive breakage of the azo groups (-N=N-). In this review, a feasible bioremediation strategy on azo dyes removal, and harmful effects of dyeing mill effluents on plants, fish, and the environment were addressed.

M. Sudha (2014) A color color is used to provide things which are an essential element of human existence. Because azodyes are widely employed in the textile, leather, pharmaceutical and cosmetic sectors, they represent a danger to all living forms, they are responsible for the main manufactured synthetic teeth. Dyes are not efficiently removed by the physico-chemical process of industrial wastewater treatment. Azodyes have lately received more attention because to the microbial deterioration and decoloration of the environment. Aerobic and anaerobic metabolism may decolorize the colors. In addition, the effectiveness of enzymes that colour-decolorize microbially on hazardous azodyes biotransformation was addressed. This study offers a basic understanding of the microbial decoloration and degradation of azodyes with different physicochemical parameters and emphasizes the use of these treatment processes in azodye waste water treatment.

Shrabana Sarkar (2017) The primary source of water pollution leading to acute environmental and human health impacts is the release of azo teal from textile wastewater. The latest worldwide priority will be to develop any ecologically sound, economical technique that can tackle the disadvantages of physical or chemical removal of the color. The physical or chemical techniques for the pre-treatment of textile wastewater are cost-effective and highly energy efficient and create hazardous sludge in the environmental field. Thus the use of microbial techniques for the degradation of textile thinness is environmentally benign and likely a profitable alternative to physical and chemical procedures. Microbial enzymes, such as laccase and azoreductase, are affordable, simple to collect, readily processable and mobilizable downstream. Recent developments in nanoparticulate-microbial enzyme conjugates also make it extremely effective to remove azo color within minutes from textile waste. But, sadly, these techniques only stay confined to the laboratory because of the difference between academics and industry and are still a barrier for industrialisation. A collection of microbial enzymes for textile dye removal is presented in the current study.

Rajat Pratap Singh (2014) RMLRT03 bacterial strain capable of discoloring textile coloration Acid Orange coloring was recovered from Tanda, Ambedkar Nagar, Uttar Pradesh textile effluent polluted soil (India). The decoloration experiments with Acid Orange tint have been conducted in Bushnell and Haas medium (BHM). On the basis of the 16S rDNA sequencing the bacterial strain was determined to be *Staphylococcus hominis*. In static circumstances, the bacterial strain showed excellent color decoloration ability with the addition of glucose and yeast extract. *Staphylococcus hominis* strain RMLRT03 were ideal for decoloration of Acid Orange teals at pH 7.0 and 35°C at incubation speeds of 60 h. High levels of Acid Orange dye up to 600 mg l⁻¹ were tolerated by bacterial strain. The strong decoloring activity in natural environments shows that the bacterial strain is used in the treatment of wastewater containing colour.

Mohammad Reza Majid Khoshkholgh Pahlaviani (2011) Textile and other dye industry wastewater effluents include large quantities of synthetic dyes which need to be treated to avoid the pollution of groundwater. This study investigated 4 strains of azo degrading bacteria for biotechnology for the treatment of azo dyes in order to discover the effective strains and incubation time needed for coloring. The textile azo colours, Reactive Lanazol Black B (RLB), Red B Eryochrome (RN), and 1, 2 metal complexes and I. Yellow were tested in these bacteria (SGL). The results of the screening indicated that the dyes could be colored by isolates within two hours of 57 to 100%. HPLC was examined for the RLB biological degradation products produced during anaerobic and

sequential anaerobic/aerobic treatments (as a model of textile azo-dyes). Peaks were detected in the anaerobic stage and at the conclusion of anaerobic/aerobic incubation these peaks vanished entirely. This data clearly shows that the dye was catabolized and isolated. Azo dye products were less harmful than the original azo dyes to growing sorghum.

3. IMPACT OF AZO DYES

Clear and powerful colors are produced using azo dyes. The cotton, leather, esthetics and foods are utilized in the main. Azo dyes are part of the organic group. An aromatic ring binds the azo group of colors. This dye is broken down to an aromatic amine, a potentially carcinogenic arylamine, by mineralisation. Most azo dyes are water soluble and easily absorbed, leading to a risk of cancer and allergic responses, irritating to the eyes and to extremely poisonous when breathed or eaten, via skin contact and inhalation. For example, paraphenylenediamine (PPD) is an aromatic amine that is a significant part of the azo dyes, also known as 1,4-diaminobenzene or 1,4-phenylene diamine. Azo dyes that include PPDs are hazardous, causing skin irritations, contact dermatitis, chimosis, tearing, exophthalmos and continuous blindness. PPD ingestions cause oedema on the face, neck, throat, tongue and larynx and respiratory difficulty in the face.

Table 1: Relationship between light absorption and color

S.No	Color absorbed	Color observed	Absorbed radiation (nm)
1	Violet	Yellow-green	350-435
2	Blue	Yellow	435-480
3	Green-Blue	Orange	480-490
4	Blue-green	Red	490-500
5	Green	Purple	500-560
6	Yellow-Green	Violet	560-580
7	Yellow	Blue	580-595
8	Orange	Green-Blue	595-605
9	Red	Blue-Green	605-750

Dyes are the colorful substitution which, due of its high water affinity, is usually used in an aqueous solution. A chromographic group in its chemical structure contributes mostly to the color of the dye and is employed in the textile, leather or alimentary sector. In general, synthetic colors consist of oil by-products and earth minerals. Reactive dyes, azo dyes etc. are the many kinds of dyes used in the textile business. Azo dyes are the biggest category of aromatic synthetic dyes used for dyeing purposes in the textile industry and are extremely water soluble in nature. It is composed of one or more azo groups ($-N=N-$) and sulphonic groups (SO_3^-) and it has enormous economic interest. Azo dyes are typically made up of one, two, three, or more azo links, which are usually replaced for the phenyl- and naphthyl-rings by certain functional groups such as triazine amine, chloride, hydroxyl, methyl, nitrogen and sulfonate. Monoazo dyes have a bond with nitrogen ($N=N$) as well as two $N=N$ bonds with diazo dyes, three $N=N$ links with triazo dyes, and more than three $N=N$ bonds with polyazo dyes.

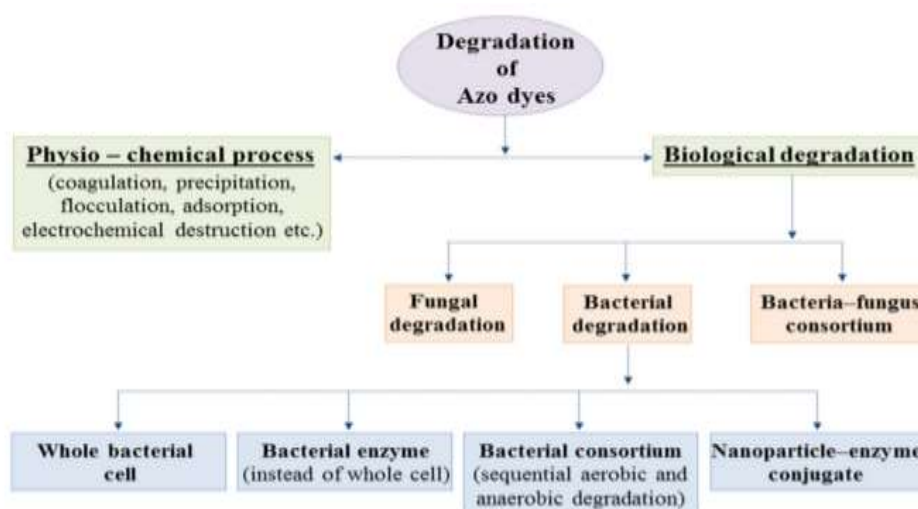


Figure 1: Different possible methods of synthetic azo dye degradation

4. BIOREMEDIATION STRATEGIES

Microbial degradation of dyes – The textile sector is a significant water consumer, from raw wool or man-made fibre through clothing manufacture. As water supplies decreased because of fast population expansion and industrial development, reuse of municipal and industrial wastewater becomes even more important after processing and removal of possible contaminants. The removal of numerous chemical and microbiological pollutants that are relevant to public health and the environment has long been conventional treatment procedures. There are own drawbacks of methods such as chemical precipitation, coagulation, adsorption and flocculation. Due to chemical precipitation technique, a large quantity of sludge is produced during effluent treatment. This sludge is hazardous to safe disposal and quite troublesome. The textile dye units have a difficulty with the detoxification and disposal of the sludge. Several bacteria have been disconnected during the past several years and converted to even mineralize azo dyes entirely. In most instances, a reductive breakage of azo bonds is the bacterial metabolism of azos that leads to amine production. The reduction of azo compounds has been investigated in aerobic microorganisms. Aerobic degradation of the sulfonate amines produced in these reactions.

Aerobic and anaerobic degradation of dyes – Aerobic decrease of azo dyes by bacteria with a different carbon source has reported many times. Aerobic bacteria destroy some aromatic amines and sulfonated amino aromas. The anaerobic cleavage of azo dyes was proposed to be used with an aerobic therapeutic approach to degrade amines. Aniline aerobic degradation has also been shown as a source of inoculum utilizing anaerobic granular sludge. In the presence of oxygen, auto oxidize aromatic amines containing one or more OH groups. The automotive process of 4-aminophenol (4-AP) and 5-amino-salicylic acid (5-ASA) has been seen in this area. Azobond cleavage produces aromatic amines which, under anaerobic conditions, cannot be degraded. Aerobic therapy may mineralize aromatic amines via hydroxylation and ring breakage through non-specific enzymes. A variety of aromatic amines are quickly biodegraded and thus are unlikely to persist for a long period in the environment. Therefore, anaerobic treatment and aerobic therapy may be utilized effectively to breakdown potential harmful and cancerous substances.

Degradation of aromatic amines – Aromatic amines are usually not destroyed and stored in anaerobic circumstances, except few hydroxyl/carboxyl grouped aromatic amines. The aerobic sludge treatment plant is more frequent to mineralize aromatic amines. Some aromatic amines, like oligomer and polymer structures, are easily autoxidised into humic. The intact molecules may be linked to cyto-receptor interactions or free radicals and arylamines generated in mammalian systems during azo reductions the toxicity and carcinogenicity of a number of azo dyes.

Immobilized cells for degradation of dyes – Whole bacteria are often used in textile dyeing waste water to reduce azo dyes. Various designs for the efficient continuous, anaerobic/ aerobic treatment of azo-dyes have been suggested during the past few years. It is a film bioreactor, a bunk bed, anaerobic/aerobic rotating biological contactors, anaerobic slurry reactor, an aerobic upstream column with aerobic slurry tank and a pulsed flux biologic⁴⁶. They are fixed film bioreactors. There are few instances of continuous bioreactors with particular color decolourisation. The discoloration takes around 10-20 days on a continual basis, and the decoloration rate remains over 80%. Several publications have observed ongoing colouration of various dyes in packed bioreactors and in high-efficiency rotating biological reactors.

Table 2: Enzymes mediated decolorization of some dyes

Substrates	Enzyme	Source of Enzyme
3-(4dimethyl amino-1 phenylazo) Benzene sulfonic acid	laccase	<i>Trametes villosa</i>
Acid Orange 6, Acid Orange 7, Methyl Orange and Methyl Red	Mixture of Bacterial Oxidoreductases	Sludge Methonogens
Direct Yellow	Horse radish peroxidases	<i>Armoracia rusticana</i>
Acid Blue	Laccase	<i>Cladosporium cladosporioides</i>
Tartrazine and Ponceau	Azoreductase	Green algae
Reactive Yellow, Reactive Black, Reactive Red and Direct Blue	Azoreductase	<i>Staphylococcus arlettae</i>

A dehydrogenase enzyme synthesis throughout a cytoplasm may be the direct enzyme reaction of Azo Reductasis.

5. RECENT PATENTING TRENDS OF MICROBIAL AZO DYE-DEGRADING ENZYMES

In recent years, newly developed enzymes that can breakdown azo color have been heavily patented. The patent pattern shows that China (1214) is the largest nation followed by Japan (377) and the USA (205), which has a much smaller number of patents, in the case of India, than all of them, with just 46 patents. The United States is at the top, followed by Japan and China, with India being the lowest in this category again, according to the rate of various kinds of efficient publications. Also, the pattern is the same as before in the form of a grant publication. From the patenting trend towards microbial degradation of textile azo colors, it is quite evident that research into microbial enzymes capable of decaying colours, is high on the international scene, while India needs to be more working in the same area to make waste water management environmentally friendly. The lacuna behind the commercialization of microbial enzymes in toxic textile water treatment should also be recognized correctly to the lacuna of the laboratory sector.

The patent trend is in direct proportion to the new research and freshly isolated microbial strains which degrade and decolorize dye, because the patented newly isolated enzymes may be readily obtained with high dye degradation efficiencies. The gap between small-scale laboratory research and the pilot industry remains enormous. As enzymes are used for one time, they may continuously be reused without significant modifications, if immobilized into an appropriate reaction matrix. Textile wastewater toxic loam is alkaline in composition and has a high temperature, creating an inappropriate setting for optimal action for most enzymes. Thus thermophilic microbial enzymes or archaeas may be a powerful remedy to typical microbial enzymes' temperature-sensitive nature since they are effective at high temperature and pH.

6. EFFECT OF PH ON DECOLORIZATION OF SCARLET RR DYE

The pH impact of Scarlet RR coloring was investigated by a percentage of decoloration. Decolorization and degradation in a broad range of pHs between 3 and 10 were accomplished. All tests and uninoculated control have been performed three times. The discoloration in both isolates follows polynomial patterns in pH. In most instances, decoloration rose above pH 5 and fell below pH 5. The most undesirable pH in dyes colouration was determined to be pH (3-6). The pH 7 and the temperature of 37°C with W2 insulates were maximum discoloration.

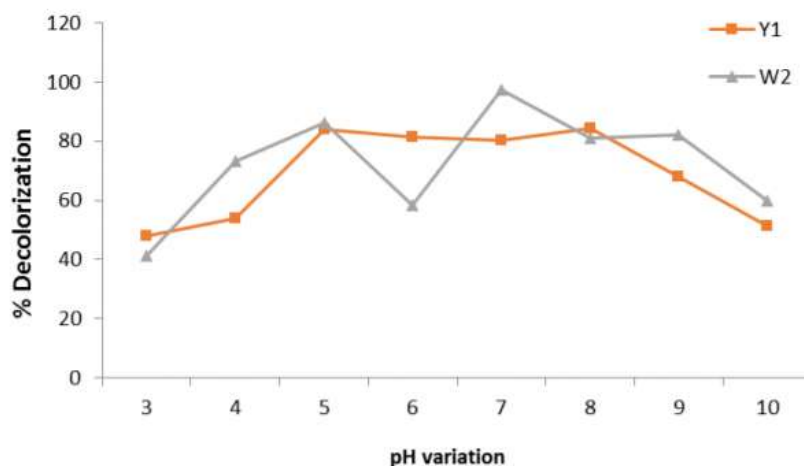


Figure 2: Effect of Ph On Decolorization by Bacterial Isolates (Y1 and W2)

7. SPECTROPHOTOMETRIC ANALYSIS

Decolorated medium has been used to measure the OD and centrifugate for twenty minutes at 5000 rpm. Scarlet RR colouration with pH 7 isolate and temperature of 37 °C using W2 was achieved at maximal colouration (Fig. 2). Marked reduction was found at λ max (470 nm) of colorant in the absorption of deteriorated samples. This may be a result of azo bond cleavage. Change in dye absorption measurements implies that the structure of the dye changes significantly.

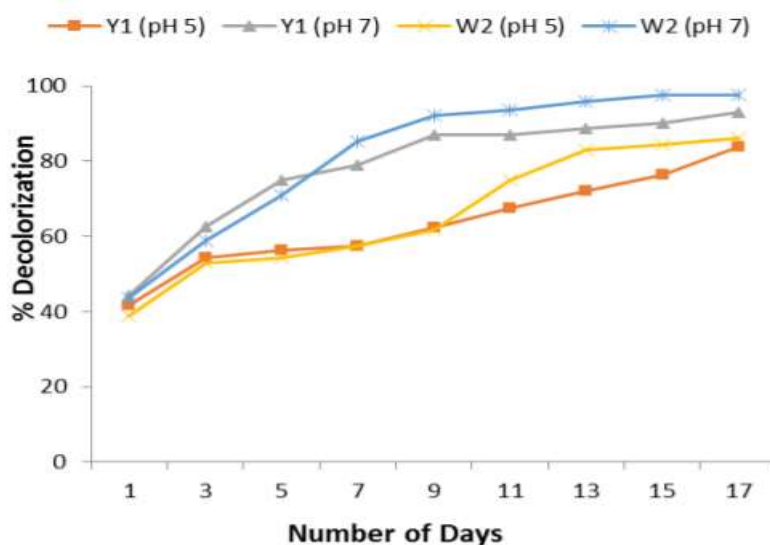


Figure 3: Decolorization of Scarlett RR by bacterial isolates (Y1 and W2) at pH (5 and 7) at 37 °C temperature

8. CONCLUSION

The initial tests showed that 2 out of 4 isolates of the three tested RLB, RN, and SGL dyes could be fully decolorated throughout this study. The degradation of azo-dyes seems to be common among the bacteria examined, showing decoloration range 57 to 100 per cent in 2 hours. Based on the findings of the screening, two most efficient isolates were chosen for future research, showing the greatest decolorative capability for all tested anaerobic azo dyes. In many other ways, bacteria are beneficial and heavy metal soil-isolated bacteria have the ability to promote plant development. Different zinc solubilizers for development and nourishment of rice plants have been discovered. Several research on scarlet RR azo colors and certain plants have been performed, along with helpful information on these features. RR azo dye and other textile colourings. Whether the enzyme system in bacteria is constitutive, inductible or repressible in bacteria must be determined. A better azoreductase

understanding leads scientists to look for novel azoreductases with a wide substrate selectivity and high activity. Although a number of azo-colour-decreasing microorganisms have been discovered, only a tiny fraction of azo-color-free bacteria have so far been screened and thoroughly researched. Thus, novel bacteria with a high ability to breakdown azo dyes may be isolated. Azo dye mineralization's metabolic processes remain unclear in bacteria. The completeness of azo degradation shows that further study is needed.

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