

## Study on Degradation of Azodyes through Bioremediation

<sup>1</sup>Akshaya A, <sup>2</sup>Prabhakar B, <sup>3</sup>Jones Felix CK, <sup>4</sup>Ramesh M, <sup>5</sup>Kumaravel S  
<sup>1,2,3,4,5</sup>Sri Shakthi Institute of Engineering and Technology, Coimbatore-641062.

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### ABSTRACT

Azo colours are widely used in textile, leather, and other projects, but because of their toxic, mutagenic, and carcinogenic qualities, they pose a serious environmental risk. Conventional methods of treating effluents containing colour are often wasteful, expensive, or irrational. Utilising microorganisms such as parasites, green growth, and microscopic creatures, bioremediation is an eco-friendly and a stute alternative that transforms these artificially coloured substances into non-toxic outcomes. Pseudomonas species have demonstrated remarkable potential among microorganisms due to their adaptability to various environments and metabolic flexibility. The main focus of this work is the enhanced conditions-based degradation of azo colours using Pseudomonas microorganisms. The azo reductase enzyme's enzymatic breaking of the azo bond (-N=N-) is one of the microbial debasement processes that reduces the colour atoms to less hazardous, less lustrous amines. Depending on external factors, these intermediaries can be biotransformed into simpler mixes via either anaerobic or oxygen-consuming processes. Important factors influencing the efficiency of debasement, such as pH, temperature, oxygen concentrations, and the availability of supplies of carbon and nitrogen, were simplified. The results demonstrate that Pseudomonas may effectively contaminate azo colours at neutral pH levels (about 7), moderate temperature ranges (25°C to 37°C), and in the presence of electron donors. To ensure complete colour disintegration, replacing anaerobic-high-impact conditions was very persuasive. This investigation demonstrates the potential of bioremediation as a cost-effective solution for treating wastewater contaminated with azo colours, providing a workable approach to natural administration and pollution management. Additional research on growing and improving microbial consortiums can improve the effectiveness of contemporary wastewater treatment.

**Keywords:** Hazardous, Carcinogenic, Biodegradation, Remediation, Microbe, Azo reductase, Temperature, Biotransformation, and Wastewater Management.

### I. INTRODUCTION

From antiquity to the present, dyeing technology has advanced due to the synthesis of new pigments and dyes, as well as the discovery of new matrices and raw materials. In 1856, William Henry Perkins made a crucial discovery when he accidentally produced mauvein, the first synthetic dye ever. Because of their wide range of colors, low cost, and resistance to fading from different materials, water, sunlight, and perspiration, synthetic dyes have largely replaced natural dyes over time. An estimated 10,000 distinct colors are currently produced on a contemporary scale, with an annual total production volume of about 700,000 tons; additionally, 10 to 15% of those are released into the environment. Serious consequences result from this situation for the degraded climate, such as hindering the flow of daylight into the water, harming photosynthetic organisms, lowering the water's oxygen content, causing metabolic pressure, neurosensorial damage, greenery rot, death, and reduced faunal development, among other things. Furthermore, when these mixtures are released into the environment untreated, humans may also become victims. They can be extremely toxic if consumed orally or inhaled, or simply come into contact with the skin [1].

Both the color itself and the metabolite s they release when they break or degrade such as sweet-smelling amines are linked to the toxic effects of azo colors, particularly their ability to cause changes. One of the factors considered when classifying the colors as potentially harmful to health is the possibility that the color will separate and release these carcinogenic amines when it comes into contact with saliva or stomach juice. However, the activity of digestive microorganisms and possibly the liver or intestinal wall's azoreductase catalyst can also

reduce the color when consumed, demonstrating how difficult it can be to remove these toxins [2]. Therefore, it's critical to understand the risks associated with releasing these colors into the environment without first treating them and how using microorganisms to bioremediate these pollutants is a workable alternative. This survey aims to evaluate the factors that influence these organic cycles, the microbial components involved, and the explicit debasement of azo colors by microorganisms. A few examples where tainting caused by these poisons was found in effluents are also presented to highlight the importance of bacterial bioremediation in this field and to highlight the importance of preventive estimates in the release of untreated colors.

## II. AZODYE

The distinctive class of artificial colors known as "azo colors" is defined by the presence of at least one azo gathering ( $-N=N-$ ) that connects fragrantrings. This specific azo useful group is crucial to the substance structure that confers various shades and forms the basis of their vibrant varieties. When azo colors are combined, a substance response called azo coupling occurs, in which a diazonium salt reacts by releasing a fragrant compound, which causes the azo linkage to be arranged. Because of the vast array of varieties that can be produced by this reaction, azo colors are definitely adaptable and generally suitable for a variety of business settings. Azo colors are well-known in the material industry for their magnificent and diverse range of tones, which range from intense blues and greens to brilliant reds and yellows. They are preferred due to their ease of use, moderateness, and ability to produce unique varieties at a high variety speed. The colors are suitable for use in upholstery, rugs, and clothing because they adhere well to both regular and engineered strands. Additional uses for azo colors include the food and beverage industry, where they are used to add variety to products, and the cosmetics industry, where they enhance the aesthetic appeal of high-quality products. Their versatility and usefulness are further demonstrated by the fact that they are used in the creation of paints, inks, plastics, and other contemporary applications. Azo colors are not without concerns, despite their widespread use. Certain azo colors have the ability to degrade into aromatic amines, which are known to cause cancer.

Administrative investigation, the cornerstone of wellbeing principles in many countries, has also been prompted by this potential for destructive debasement. For example, the European Association has strict regulations that limit the use of certain azo colors in consumer goods, particularly those that may come into direct contact with the skin.

India was the first nation in Asia to regulate chemicals, including azo dyes, when it outlawed the handling of 112 dyes, including those in the azo group, in 1997. The list of prohibited aromatic amines in this nation is identical to that found in REACH Regulation 1907/2006. Other Asian nations, like China (2005), South Korea (2010), Taiwan (2011), and Egypt (2012), have implemented their own laws that place limitations on azo dyes and/or aromatic amines. Japan joined this list in 2014 when it declared azo dyes to be dangerous and prohibited the use of 24 aromatic amines derived from these dyes in any leather, fur, or textile product at concentrations of 30 mg/kg or higher. In a Vietnamese regulation, the same concentration was utilized to limit 22 aromatic amines [3-6].

Regulations about industrial effluents are also in place in other nations, such as Canada, France, Australia, Brazil, Pakistan, Malaysia, Turkey, and Morocco. These regulations include specifications regarding the color of these effluents, which in turn affect the number of dyes that are permitted in them [7].

## BACTERIAL DEGRADATION

The bioremediation of dye effluents using more environmentally friendly methods, like microorganisms, has drawn attention from researchers and industry. Numerous novel techniques for dye biodegradation have emerged recently, and they are thought to be both economical and ecologically benign. Therefore, in comparison to other treatment approaches, biological treatments that use microorganisms to break down synthetic dyes are being researched as feasible and affordable alternatives. Numerous toxic compounds can be broken down and converted into non-toxic end products by the bacterial strains. Enzymes like laccase, peroxidase, azoreductase, veratryl alcohol oxidase, and oxidoreductive enzymes enable bacteria to degrade dyes found in industrial wastewater and effluents. Azo dye degradation by bacteria in tanning and textile effluents typically consists of two steps. The first step involves the

azoreductase enzyme reducing the dye's azo linkage, which produces aromatic amines. Toxic by nature, these products (aromatic amines) are either fully mineralized or further broken down into less toxic metabolites in the second step. The literature has documented the mechanism by which *P. aeruginosa* causes the degradation of other azo dyes [8].

### III. MATERIALS AND METHODOLOGY

**Sample collection:** In India, soil and effluent samples were taken from various discharge points from dyeing units and locations where idols are permanently painted to isolate the bacterial samples for dye degradation in the current study [9].

#### Isolation and Identification of Dye-Degrading Bacteria:

To isolate individual cultures, sterile water blanks were used to serially dilute soil and effluent samples. The diluted sample, 100 microliters in volume, was then spread-plated on nutrient agar media. As pure cultures were kept on nutrient agar, individual colonies were thus obtained following incubation. The textile dye samples were combined in a 1:100 ml v/v ratio with minimal salts basal medium. The experiment was conducted in triplicate, with the dye-mixed medium being inoculated with the isolated bacterial culture and then incubated at room temperature on an incubator shaker at a speed of 200 rpm. A UV-Vis spectrophotometer was used to measure absorbance at 590 nm every two days to assess the degree of dye degradation.

According to Bergy's manual of Determinative Bacteriology, the bacterial isolates that could completely degrade the dye were identified through morphological and biochemical characterization

[9].

**Optimization:** Using the basal media as constant, the test organism's degradation of various textile dye samples under various physical conditions was optimized. In the current study, dye concentrations ranging from 10 mg/l to 80 mg/l were employed to optimize degradation. The pH range that was optimized was 4–8. For optimization, the temperatures were 28°C, 37°C, and 40°C. The UV-Vis spectrophotometer was used to measure the absorbance at 590 nm to ascertain the degree of degradation of the dye samples under various optimization conditions [9].

### IV. RESULTS AND DISCUSSION

To identify the potential isolates, tests were performed using a variety of morphological and biochemical characterization techniques, selective media, and methods that were found to maximally degrade the dyes. The isolated microbe was determined to be *Pseudomonas aeruginosa* based on all tests and growth on selective media. The following outcomes were obtained by optimizing the conditions for dye degradation. The degree of degradation decreased as the dye concentration rose. The toxic activity of the dye on the test organism may be the reason why dye degradation was significantly reduced as dye concentration increased. Figure 1 shows that maximum dye degradation occurred at a concentration of 40 mg/l. Optimization studies were conducted using the dye concentration that resulted in greatest degradation for the isolate. Figure 2 shows that using ammonium nitrate as a nitrogen source led to faster breakdown than alternative sources [9].

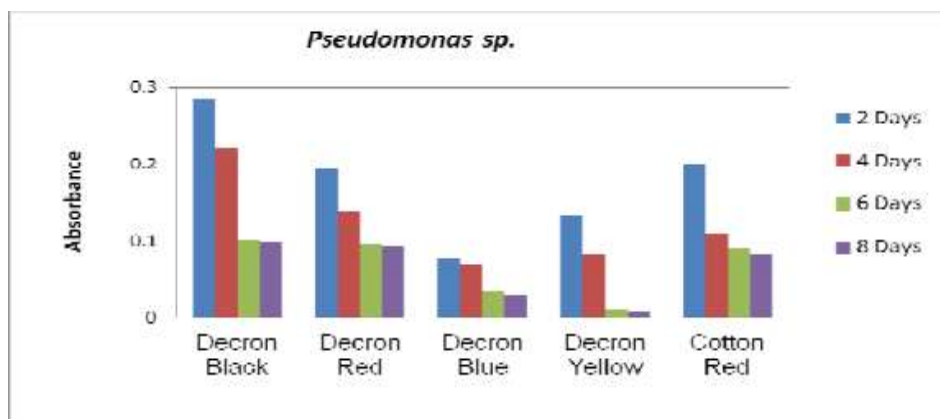


Figure 1: *Pseudomonas aeruginosa* degradation of dye (40 mg/l). Prasad, M. P. (2014)

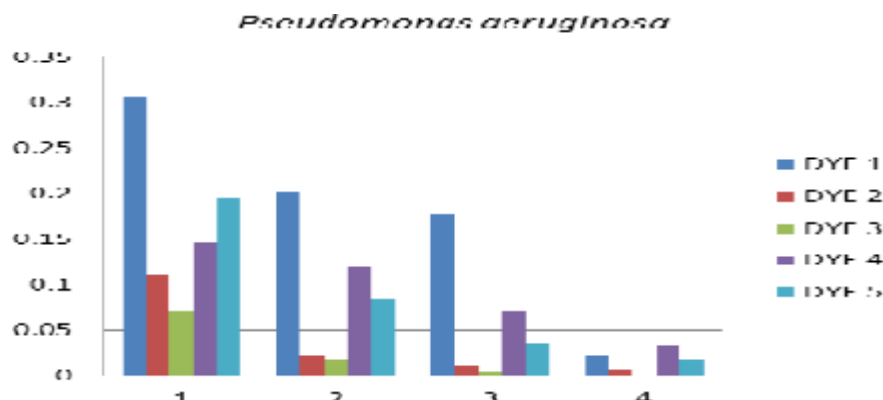


Figure 2: Nitrogen- Ammonium Nitrate. Prasad, M. P. (2014)

## V. CONCLUSION

The study indicates that *Pseudomonas aeruginosa* is an excellent bioremediation agent for azo dye degradation, producing considerable decolorization under ideal conditions. The bacterium's enzymatic activity, particularly azoreductase, effectively degrades azo bonds, resulting in detoxification and mineralization of hazardous chemicals. Environmental parameters such as pH, temperature, and dye concentration influence degradation efficiency, but acclimation increases resistance to higher dye concentrations. Although scaling up the process for industrial applications is challenging, the findings emphasize the potential of *Pseudomonas aeruginosa* for sustainable and eco-friendly wastewater treatment, providing a possible alternative to traditional chemical approaches.

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