Spectral Studies of Azo Dye Degradation Using Selected Biofertilizer: *Pseudomonas Fluorescens*

Sumayya Rehaman*, Aravindan G1, Karthick G1

*Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore, Tamilnadu, India.

**Abstract:** Azo dyes are the azo colorants with about 70% dyestuff. Azo dyes persist in the environment for years and are toxic to human life. In the present study, it was attempted to decolorize the selected azodye by three selected biofertilizers: *Rhizobium* sp., *Azospirillum* sp. and *Pseudomonas fluorescens* whereas also to prove biofertilizer’s degradation property. Initially decolorization of 10% azo dye of silk dyeing effluent was biotreated with above mentioned biofertilizers at 37 °C separately as preliminary studies. It was found that preliminarily decolorization of azo dye with *Pseudomonas fluorescens* with 85% followed by *Azospirillum* sp. with 74%. Based on this, the percentage decolorization was evaluated for various concentrations of 25, 50, 75 and 100% of azo dye of silk dyeing effluent under static conditions with glucose as carbon source. The percentage decolorization was found to be 91% in 5 days with 25% effluent by *Pseudomonas fluorescens* reduced to 68% with crude azo dye effluent which had positive influence on the growth of bacterium in the 0.002g glucose as carbon source as growth rate was increased along with decolorization. In contrast the least percentage decolorization was analyzed as 23% in 5 days with 25% effluent by *Rhizobium* sp. whereas reduced drastically to 11% with 100% effluent. This indicated the dilution is more needed for the better decolorization. The cleavage of azo bond was confirmed through spectral studies such as UV and in HPLC chromatogram of silk dyeing raw industrial bio-treated azo dye. Silk dyeing effluent. Microbial growth has utilized and decolorized the dye wastewater shows its biodegradation potential. The high decolorization ability was observed in *Pseudomonas fluorescens* compared to *Azospirillum* sp., and *Rhizobium* sp., as biofertilizer to convert toxic azo dyes into nontoxic compounds reducing the contaminants will prove dual purpose of usage of biofertilizers in the environment.

**Keywords:** *Azospirillum* sp., biofertilizer, Decolorization, HPLC, *Pseudomonas fluorescens*, *Rhizobium* sp., Spectral studies, UV.

*Corresponding Author

Sumayya Rehaman, Department of Biotechnology, Karpagam Academy of Higher Education, Coimbatore, India.

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1. INTRODUCTION

The man and nature from a few million years are increasingly exposed to the various dyes with hazardous potency. Annually, worldwide the estimated amount of synthetic dyes manufactured were about 700,000 tons with the variation of its classes of about 100,000 types. The chemical xenobiotic compounds are in contact with the body tissues via ingestion, taken up by the inhalation process and absorbed by the dermal tissue. Most of the xenobiotic compounds as dyes are recalcitrant when given out into the public drains and internally contaminates the river resources. Azo dyes are the largest class and found to be resourceful in its nature. The presence of the azo moiety molecule with mono or polycyclic aromatic systems in the dyes named as azo dyes. The first dye was an azo coupling reaction called aniline yellow. Later, with this concept several classes’ such as Direct, Disperse, Direct and Reactive dyes were developed. With intense colors like red, yellow, orange etc. These various types of dyes were used to dye different fibers such as cotton, wool, silk, polyester or synthetic fibers. They have a best application of color reflectance in the food, paper, cosmetic, textile industry and analytical chemistry. They are also antimicrobial agents when used at permissible levels. After the usage of the azo dyes they are let out and the loss of dyes from the fabrics ranges from 2-50% which doesn’t bind to the fabric. While above the limited levels and with an adequate period of time prevalent in the environment. It will be cytotoxic and genotoxic to the flora by devoiding the sunlight to penetrate into the aquatic life to perform the photosynthesis with less oxygen availability and as reducing the water transparency into the aquatic life to perform the photosynthesis with less synthetic fibers. Reactive dyes were developed. With intense colors like red, concept several classes’ such as Direct, Disperse, Direct and azo coupling reaction called aniline yellow. Later, with this systems in the dyes named as azo dyes. The first dye was an azo dye (Azo dye- Dark Pink) of silk dyeing effluent: The untreated and biotreated Azo dyes were subjected to UV VIS Spectrophotometer (Fisher scientific Jenway™ 72) from 200-800nm to check the presence of the various functional groups and Fourier transform infrared spectrum analysis from 400-4000 1/cm wavelength. The untreated and biotreated Azo dyes were subjected to UV VIS Spectrophotometer (Fisher scientific Jenway™ 72) from 200-800nm to check the presence of the various functional groups and Fourier transform infrared spectrum analysis from 400-4000 1/cm wavelength.

2. MATERIALS AND METHODS

2.1 Inoculum preparation from the Biofertilizers:

The nutrient broth was prepared and sterilized in the autoclave for 121 °C. The biofertilizers such as P. fluorescens, Azospirillum sp. and Rhizobium sp., of about 0.02mg/100ml were inoculated in the separate flask and kept in the shaker.

2.2 Preliminary studies on decolorization of the silk dyeing effluent in different concentrations by P. fluorescens, Azospirillum sp. and Rhizobium sp.

Initially the preliminary decolorization test were carried out with 10% of Azo dyes effluent treated with P. fluorescens, Azospirillum sp. and Rhizobium sp. which was inoculated from the pure culture separately in each conical flask. The inoculated flasks were incubated in the shaker for 0-5 days.

2.3 Percentage decolorization of effluent by Rhizobium sp., Azospirillum sp. and P. fluorescens

About 25, 50, 75 and 100%azo dye silk dyeing effluent were prepared in the three batches and inoculated with the pure cultures of Rhizobium sp., Azospirillum sp. and Pseudomonas fluorescens and analyze for the decolorization. The aliquots of the decolorized samples after centrifugation were subjected to UV Vis Spectrophotometer (Fisher scientific Jenway™ 72) at 600nm. The percentage decolorization were calculated by:

\[ \text{Percentage decolorization} = \left( \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \right) \times 100 \]

2.4 UV analysis and FT-IR spectrum of the selected dye (Azo dye- Dark Pink) of silk dyeing effluent

The untreated and biotreated Azo dyes were subjected to UV VIS Spectrophotometer (Fisher scientific Jenway™ 72) from 200-800nm to check the presence of the variety of the functional groups and Fourier transform infrared spectrum analysis from 400-4000 1/cm wavelength.

2.5 HPLC analysis of untreated and bio-treated azo dye of silk dyeing industrial effluent:

The biodegradation of the Azo dyes of the silk dyeing effluent was analyzed through high pressure liquid chromatography which was compared with the untreated azo dyes to identify the dye degradation.

2.6 Chromatographic conditions

The chromatographic system was equipped with column C18 with 3μl particle size (50×4.6 mm I.D) and detector UV-VIS model SPD 20A at specific nanometers at a flow rate of 1ml/min. The solvent HPLC methanol was used with the stream of liquid N2 until it reached nearly 0.5 ml and then some mobile phase was added to reach 1ml. Then 20μl of the untreated and bio-treated azodyes as samples were
injected into the HPLC column. The presence and degradation of the azo compound was determined by comparison of peak area of the samples with that of the standard. The mobile phase with a binary mixture of acetonitrile: water (60:40) was used for crude effluent and biotreated silk dyeing industrial effluent.  

3. RESULTS AND DISCUSSION

Preliminary studies on decolorization of the silk dyeing effluent in different concentrations by P. fluorescens, Azospirillum sp., and Rhizobium sp.

Figure 1 and Plate 2 represents the preliminary studies on percentage decolorization of silk dyeing effluent (with minimum concentrations) by Pseudomonas fluorescens, Azospirillum sp. and Rhizobium sp., respectively for a period of 20 days with an interval of 5 days. The percentage decolorization was found to be least in Rhizobium sp., with 52% decolorization on the 20th day. Within 5 days the
maximum decolorization seen in *P. fluorescens*. On 20th day maximum percentage was observed in *P. fluorescens* of about 85% followed by *Azospirillum* sp., with 74%. A study had also shown similar decolorization % in reactive blue dye (84.4%) by *Trametes hirsuta*16. Among the three biofertilizers used, the azo dye of silk dyeing effluent was more likely degraded by *P. fluorescens* compared to other Biofertilizers. Figure 2 depicts the comparison of preliminary decolorization of silk dyeing effluent with different microorganisms.

### 3.1.2 Percentage decolorization of effluent by *Rhizobium* sp., *Azospirillum* sp. and *Pseudomonas fluorescens*

The decolorization of silk dyeing effluent of varying concentrations (25%, 50%, 75% and 100%) were biotreated with *Rhizobium* sp., *Azospirillum* sp. and *P. fluorescens* with the co-substrate glucose as a carbon source was depicted in Figure 3 and Graph 2. The percentage decolorization was improved by the addition of glucose (0.002g) as co-substrates but with the increasing concentrations of the effluent, the percentage decolorization decreases. In the experimental analysis, about 75% decolorization was read in 25% effluent which in turn reduced by 25% and reached to 50% of decolorization in 100% effluent. The percentage of decolorization was reduced with increasing concentrations of the effluent. Thus the highest percentage of decolorization was evaluated in 25% effluent and the lowest in 100% effluent by *P. fluorescens* compared to other two bacteria with about 91% of decolorization. So it can be recommended than discharging the concentrated effluent it can be diluted to maximum level then decolorized and let out into the environment. The Graph 2 depicts that the percentage decolorization was found to be 91% in 5 days with 25% effluent by *P. fluorescens* whereas reduced to 68% with 100% azo dye effluent which had positive influence on the growth of bacterium in the 0.002g glucose as carbon source as growth rate was increased along with decolorization. In contrast the least percentage decolorization was analysed as 23% in 5 days with 25% effluent by *Rhizobium* sp. whereas reduced drastically to 11% with 100% effluent. This indicated the dilution is more needed for the better decolorization. This results were similar reported that Blue H/C and Red 3B dye were decolorized with two *A. faecalis* species namely *A. faecalis* E5.Cd and *A. faecalis* Fal.3 with the co-substrate glucose17. Similar percentage decolorization was found in acid blue by *Trametes hirsuta*18. A similar study had shown 90% decolorization of acid orange 10 by *Pseudomonas putida*19. Also it is reported that 96.2% decolorization of reactive red 180 anaerobically by *Citrobater* sp., when added with glucose at 4 g l⁻¹18.

The microbial decolourisation could be a viable means in ridding dye wastewater. Dye molecule absorption into the cell surface appears to be quick and is often completed in some hours. The direct reactive dyes could all be cleared out of solution using the same approach 18. In the present study, all the three microorganisms effectively decolorized the effluent at the lower concentration and has been reduced with the increasing concentration of the effluent. Similar to the study the *P. fluorescens* efficiently reduced the physicochemical characteristics of Silk dyeing effluent compared to *Azospirillum* sp.20.
Among the microbes, *Rhizobium* sp., was found to be less effective in decolorizing the effluent and hence the other two microorganisms (*P. fluorescens* and *Azospirillum* sp.) were selected for further study.

**Fig 4. Biotreated Azodye effluent by *Pseudomonas fluorescens* and Silk dyeing effluent subjected to UV and HPLC**

3.1.3 UV analysis of the Azo dye in silk dyeing effluent:

The untreated azo dye and the treated azo dye in the Figure 4 were subjected to the UV Vis analysis from 200-800 nm. In the untreated sample absorbance peak was seen between 400-450 nm of Azodye compound with the impurities or associated trace compounds in the 650-700 nm with a small peak depicted in Graph 3.

Graph 3: Untreated AzoDye compound

Whereas the bio-treated sample was found to have no remarkable peak of absorbance observed indicating the absence of azo dye compound which strongly influences that the azo moiety has been degraded by the microbes on biotreatment as depicted in the Graph 4.

Graph 4: Biotreated AzoDye compound

3.1.4 HPLC of silk dyeing industrial effluent

Graph 5 indicates the chromatogram of Azodye in silk dyeing effluent.

Graph 5. HPLC of Azo dye in Silk dyeing industrial effluent

3.1.5 HPLC of bio-treated effluent

Graph 6 depicts the chromatogram of bio-treated effluent *Pseudomonas fluorescens.*
Thus from the present study, the HPLC chromatogram of silk dyeing industrial effluent at the wavelength of 510nm has shown two peaks of retention time (tR) 4.3, 7.0 minutes with 99.88 % and 0.115 % area (Graph 5). The chromatogram of bio-treated effluent has shown five peaks with retention time (tR) 3.0, 3.1, 3.7, 3.8 and 4.3 minutes depicted in the Graph 6 with reduced percentage area of 39%, 18.5%, 10.1%, 8% and 24.18% which clearly indicates that the Azo dye in the effluent has been degraded by *Pseudomonas fluorescens*. Analogous study of *Sesbania grandiflora* grown in the bio-treated effluent had about 4 peaks when compared with the GLV grown in the freshwater with 6 peaks which proves *P. fluorescens* effect on the biotreated water\(^2\). However comparable analysis of untreated Direct orange 16 and treated sample by *Micrococcus luteus* strain SSN2 were found to be proved with less Rf values at 254nm\(^2\). Analogous results were seen with the retention peak between 3-4 when the Reactive blue dye was decolorized by *Providencia rettgeri* analysed\(^2\). Similar study of *Brassica juncea* grown in the bio-treated effluent water found peaks similar to the freshwater grown green leafy vegetables whereas its peak disappeared in the GLV’s grown in the crude effluent water indicating the effect of *P. fluorescens* in degrading the effluent water\(^2\).  

4. **CONCLUSION**

With all the results and observation proves to conclude strongly that the mentioned Biofertilzers are recommended to be used for the biodegradation of the selected azodye. On experimental evaluation *P. fluorescens* has more degrading capacity compared to the other biofertilizers. Also the percentage decolorization is higher on the diluted azodye effluent than the crude effluent. Further our spectral studies confirm the complete biodegradation of the azodye by reduction of peak area compared with the untreated azodye. Finally our overall findings recommends that the eco-friendly biofertilizer *P.fluorescens* can be used as a potent bacteria for biodegradation of selected Azo dye-(Dark pink) instead of xenobiotics to degrade the azodyes and also it paves the way for the research area of other synthetic dye degradation.

5. **AUTHORS CONTRIBUTION STATEMENT**

Aravindan G and Karthick G, PG research scholars have contributed their efforts by giving the pictorial representation of the observations which gave dynamism to the manuscript.

6. **CONFLICT OF INTEREST**

Conflict of interest declared none.

7. **REFERENCES**


