ijlpr 2020; doi 10.22376/ijpbs/lpr.2021.11.1.L73-79

International Journal of Life science and Pharma Research

Research Article

Biotechnology for Prospective Medical Science

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

Sumayya Rehaman*, Aravindan G[1], Karthick G[1]

*[1]*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 biofertlizer'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.

Recieved On 17 April 2020
Revised On 28 August 2020
Accepted On 01 September 2020
Published On 04 January 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Sumayya Rehaman*, Aravindan G1, Karthick G1 , Spectral studies of Azo Dye degradation using selected Biofertilizer of Pseudomonas fluorescens..(2021).Int. J. Life Sci. Pharma Res.11(1), L73-79 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.1.L73-79

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[1]. 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 inturn contaminates the river resources[2]. 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[3]. 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[4]. While above the limited levels and with an adequate period of time prevalent in the environment[5]. It will be cytotoxic and genotoxic[6] 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[7] and in fauna penetrating the cell by absorption through skin or ingestion and destroying its nature creating cell mutagenicity and leads to the cancer due to the presence of nitrates, chlorides, heavy metals, aromatic and aliphatic xenobiotic compounds[8]. Finding an alternative solution of biomolecules was proved to decolorize the dyes either by the whole cells or by releasing the enzymes by bacteria and Fungi by absorbing in the cell matrix by algae/ yeast[9]. As earlier said enzymes such as azoreductase, laccases and peroxidases also degrade the azo dyes in their respective mechanism[10]. Also the nontoxic products are the outcomes of the biotreatment makes this technology a promising one [11] which gives the oxygen and water in turn converting the organic compounds with a low cost effective one. Biofertilizers of the genus *Pseudomonas, Azospirillum* and *Rhizobium* are the microorganisms which decompose organic pollutants through cometabolism in natural water and soil environments. These microbes are having the capacity of biodegradation of complex chemical compounds and also act as PGPR (Plant growth promoting rhizobacteria) which produce antibiotics as well as secondary metabolites such as siderophore, phytohormones, volatile compounds and hydrogen cyanide (HCN). The *Azospirillum* sp., and *Pseudomonas* sp., belong to the plant growth promoting rhizobacteria that are the bacteria capable of promoting plant growth by colonizing the plant's root[12]. So far the biofertilizers were not used for degradation of azo dye effluents. With these literature review, this research paper is designed for the degradation of the azodyes by selected biofertilizers like *Azospirillum sp*., *Pseudomonas fluorescens*and *Rhizobium* sp., and to evaluate its effect on varying concentration of azo dyes.

2. MATERIALS AND METHODS

2.1 Inoculum preparation from the Biofertilizers:

The nutrient broth was prepared and sterilized in the autoclave for 121 ^{0}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[13].

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 analyse 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:

Percentage decolorization = [(Initial absorbance- Final absorbance)/ Initial absorbance] *100[14].**

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 various functional groups and Fourier transform infrared spectrum analysis from 400-4000 1/cm wavelength [15].

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[15].

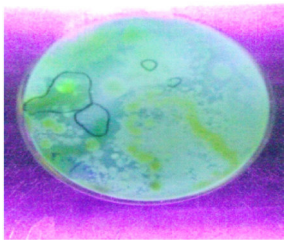
2.6 Chromatographic conditions

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. [15]

3. RESULTS AND DISCUSSION

PF 1- Pseudomonas fluorescens isolates from Biofertilizers, maintained in nutrient slants
PF 2 - Pseudomonas fluorescens cultures isolated from Preliminary decolorization Silk dyeing effluent
A 1- Azospirillum sp., maintained in nutrient slants

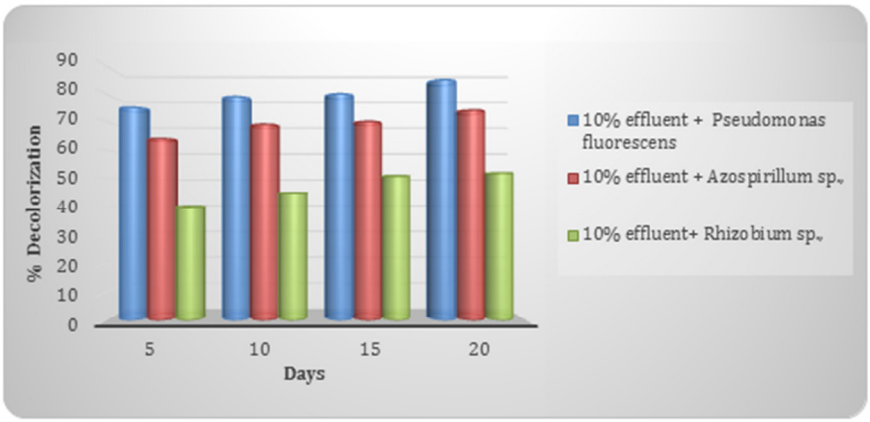


PF 2 - Pseudomonas fluorescens strain 1 cultures in UV transilluminator

Fig 1. Biofertilizers Culture Inoculum to perform degradation

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

Fig 2. Pseudomonas fluorescens, Azospirillum sp. and Rhizobium sp., inoculated in 10% effluent



Graph 1. Values are the mean of three replicates

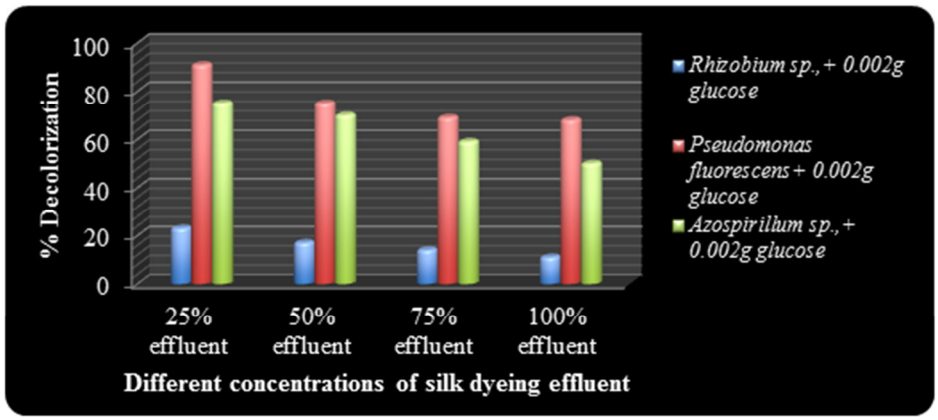
3.1.1 *Preliminary decolorization of the silk dyeing effluent*

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 sho wn similar decolorization % in reactive blue dye (84.4%) by *Trametes hirsute*[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 glucose[17]. 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^{-1} [18].



Values are the mean of three replicates

Graph 2. Percentage decolorization of the silk dyeing effluent by *P. fluorescens, Azospirillum sp.* and *Rhizobium sp.*, with the co-substrate (glucose)

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 [19]. 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].

Rhizobium sp., with different concentrations of the effluent (25% -100%)

P. fluorescens with different concentrations of the effluent (25%-100%)

Azospirillum sp., with different concentrations of the effluent (25%-100%)

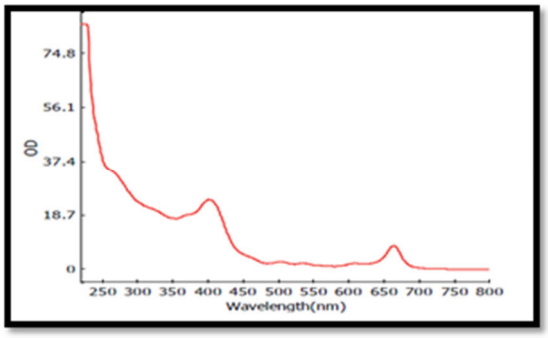
Fig 3. Decolorization of the silk dyeing effluent with different concentrations by P. fluorescens, Azospirillum sp. and Rhizobium sp., with the co-substrate (glucose).

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.

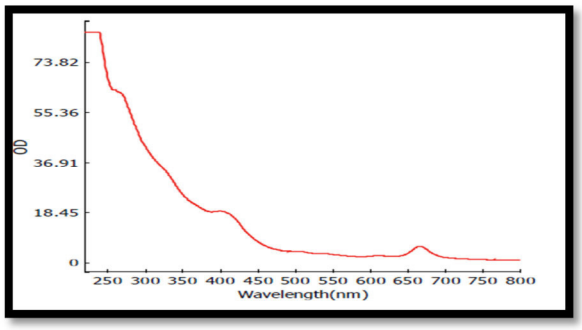
Fig 4. Biotreated Azodye effluent by Pseudomonas fluorescens and Silk dyeing effluent subjected to UV and HPLC

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

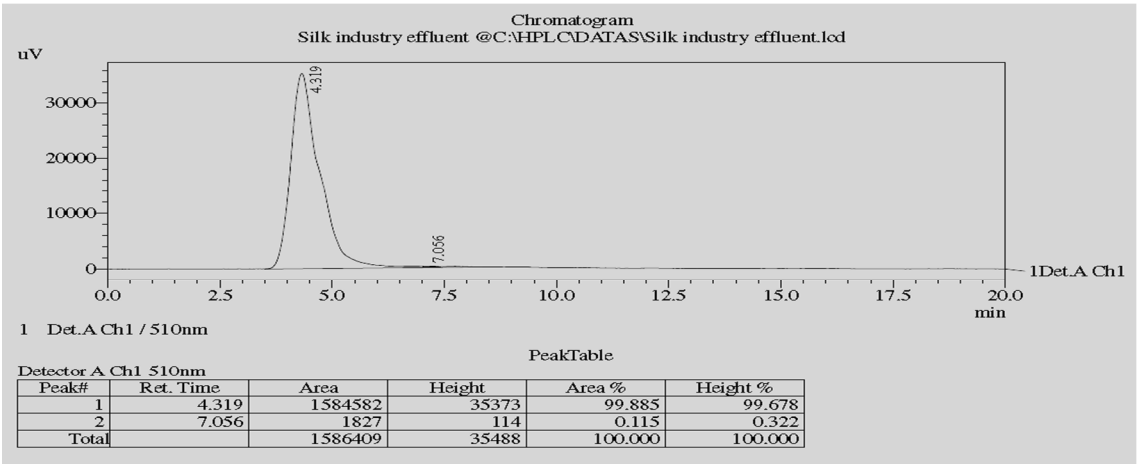


Graph 4: Biotreated AzoDye compound

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.

3.1.4 HPLC of silk dyeing industrial effluent

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



PeakTable

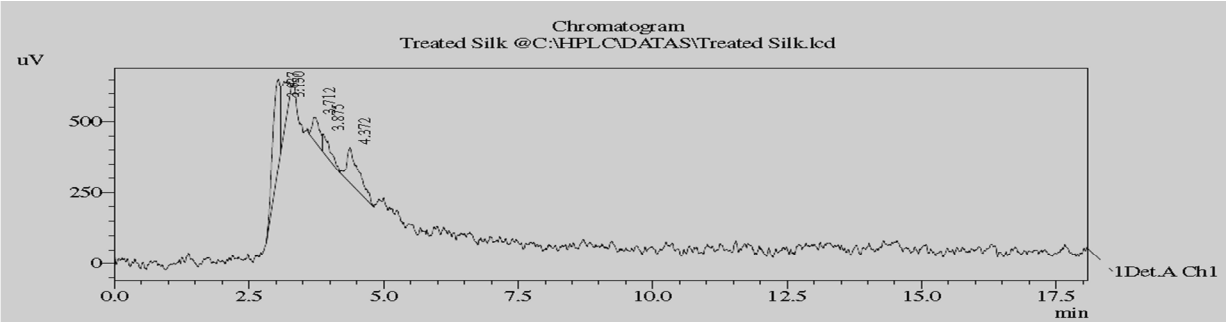
Detector A Ch1 510nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.319	1584582	35373	99.885	99.678
2	7.056	1827	114	0.115	0.322
Total		1586409	35488	100.000	100.000

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.*



PeakTable

Detector A Ch1 510nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.037	3271	319	39.010	41.232
2	3.150	1559	178	18.596	23.013
3	3.712	850	84	10.141	10.838
4	3.875	677	65	8.070	8.410
5	4.372	2028	128	24.182	16.507
Total		8386	773	100.000	100.000

Graph 6. HPLC of azo dye of bio-treated effluent

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[21]. However comparable analysis of untreated Direct orange 16 and treated sample by *Micrococcus luteus* strain SSN2 were found to be proved with degradation with less Rf values at 254nm [22].Analogous results were seen with the retention peak between 3-4 when the Reactive blue dye was decolorized by *Provedencia rettgeri* analysed.[23] 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 [24,25].

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

1. Adedayo O, Javadpour S, Taylor C, Anderson WA, Moo-Young M. Decolourization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant. World J Microbiol Biotechnol. 2004 Aug 1;20(6):545-50. doi: 10.1023/B:WIBI.0000043150.37318.5f.
2. Mathur N, Bhatnagar P. Mutagenicity assessment of textile dyes from Sanganer (Rajasthan). J Environ Biol. 2007 Jan 1;28(1):123-6. PMID 17717998.
3. Weglarz-Tomczak E, Gorecki L. Azo dyes—biological activity and synthetic strategy. Chemik. 2012;66(12):1298-307.
4. Pandey A, Singh P, Iyengar L. Bacterial decolorization and degradation of azo dyes. Int Biodeterior Biodegrad. 2007 Mar 1;59(2):73-84. doi: 10.1016/j.ibiod.2006.08.006.
5. Chung KT, Cerniglia CE. Mutagenicity of azo dyes: structure–activity relationships. Mutat Res. 1992 Sep 1;277(3):201-20. doi: 10.1016/0165-1110(92)90044-A, PMID 1381050.
6. Olukanni OD, Osuntoki AA, Gbenle GO. Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria. Afr J Biotechnol. 2006;5(20).
7. Corso CR, Maganha de Almeida AC. Bioremediation of dyes in textile effluents by Aspergillus oryzae. Microb Ecol. 2009 Feb 1;57(2):384-90. doi: 10.1007/s00248-008-9459-7, PMID 18989608.
8. Daneshvar N, Ayazloo M, Khataee AR, Pourhassan M. Biological decolorization of dye solution containing malachite green by microalgae *Cosmarium sp*. Bioresour Technol. 2007 Apr 1;98(6):1176-82. doi: 10.1016/j.biortech.2006.05.025, PMID 16844368.

9. Waghmode TR, Kurade MB, Kabra AN, Govindwar SP. Biodegradation of rubine GFL by *Galactomyces geotrichum MTCC 1360* and subsequent toxicological analysis by using cytotoxicity, genotoxicity and oxidative stress studies. Microbiology. 2012 Sep 1;158(9):2344-52. doi: 10.1099/mic.0.060467-0, PMID 22723285.
10. Joshni TC, Subramaniam K. Enzymatic degradation of azo dyes-a review. Int J Environ Sci. 2011;1(6):1250-60.
11. Wang H, Su JQ, Zheng XW, Tian Y, Xiong XJ, Zheng TL. Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by Citrobacter sp. CK3. Int Biodeterior Biodegrad. 2009 Jun 1;63(4):395-9. doi: 10.1016/j.ibiod.2008.11.006.
12. Prakash P, Karthikeyan B. Isolation and purification of Plant Growth Promoting Rhizobacteria (PGPR) from the rhizosphere of *Acorus calamus* grown soil. Indian Streams Res J. 2013 Apr 3;3:1-13.
13. Bashan Y. Migration of the Rhizosphere Bacteria Azospirillum brusilense and Pseudomonas fluorescens Towards Wheat Roots in the Soil. Microbiology. 1986 Dec 1;132(12):3407-14. doi: 10.1099/00221287-132-12-3407.
14. Neifar M, Jaouani A, Kamoun A, Ellouze-Ghorbel R, Ellouze-Chaabouni S. Decolorization of solophenyl red 3BL polyazo dye by laccase-mediator system: optimization through response surface methodology. Enzyme Res. 2011;2011:179050. doi: 10.4061/2011/179050, PMID 21869923.
15. Sumayya AR, Saranya R S, Sivagami srinivasan, Mohammed Rafiqkhan, Brindha PS, Mohammed sabeek M, Sangeetha S. Fourier Transform Infrared (FTIR) Spectroscopy and High-Performance Liquid Chromatography Analysis of Brassica juncea (Mustard) and Silk dye-ing effluent's impact on the spectral studies. ijrps;10(2):1266-72. doi: 10.26452/ijrps.v10i2.419.
16. Abadulla E, Tzanov T, Costa S, Robra KH, Cavaco-Paulo A, Gübitz GM. Decolorization and detoxification of textile dyes with a laccase from Trametes hirsuta. Appl Environ Microbiol. 2000;66(8):3357-62. doi: 10.1128/aem.66.8.3357-3362.2000, PMID 10919791.
17. Saha AK, Sultana N, Mohanta MK, Mandal A, Haque MF. Identification and characterization of azo dye decolourizing bacterial strains, *Alcaligenes faecalis* E5. Cd and *A. faecalis Fal. 3* isolated from textile effluents. Am Sci Res J Eng Technol Sci (ASRJETS). 2017 May 13;31(1):163-75.
18. Abadulla E, Tzanov T, Costa S, Robra KH, Cavaco-Paulo A, Gübitz GM. Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta*. Appl Environ Microbiol. 2000 Aug 1;66(8):3357-62. doi: 10.1128/AEM.66.8.3357-3362.2000, PMID 10919791.
19. Tripathi A, Srivastava SK. Eco friendly treatment of azo dyes: bio decolorization using bacterial strains. Int J Biosci Biochem Bioinformatics. 2011 May 1;1(1):37-40. doi: 10.7763/IJBBB.2011.V1.7.
20. Wang H, Su JQ, Zheng XW, Tian Y, Xiong XJ, Zheng TL. Bacterial decolorization and degradation of the reactive dye Reactive Red 180 by Citrobacter sp. CK3. Int Biodeterior Biodegrad. 2009 Jun 1;63(4):395-9. doi: 10.1016/j.ibiod.2008.11.006.
21. Sumayya AR, Srinivasan S. Functional groups and compounds analysis using chromatographic and spectroscopic techniques of untreated and bio remediated silk dyeing effluent. Int J Curr Res. 2018 Oct 30;10(10):74083-91. doi: 10.24941/ijcr.32609.10.2018.
22. Singh S, Chatterji S, Nandini PT, Prasad ASA, Rao KVB. Biodegradation of azo dye Direct Orange 16 by *Micrococcus luteus* strain SSN2. Int J Environ Sci Technol. 2015 Jul 1;12(7):2161-8. doi: 10.1007/s13762-014-0588-x.
23. Lade H, Govindwar S, Paul D. Low-Cost Biodegradation and Detoxification of Textile Azo Dye C.I. Reactive Blue 172 by Providencia rettgeri Strain HSL1. J Chem. 2015 Jan 1;2015:1-10. doi: 10.1155/2015/894109.
24. Sumayya AR, Saranya R S, Sivagami srinivasan, Mohammed Rafiqkhan, Brindha PS, Mohammed sabeek M, Sangeetha S. Fourier Transform Infrared (FTIR) Spectroscopy and High-Performance Liquid Chromatography Analysis of Brassica juncea (Mustard) and Silk dye-ing effluent's impact on the spectral studies. ijrps;10(2):1266-72. doi: 10.26452/ijrps.v10i2.419.
25. Sumayya AR, Srinivasan S. Analysis of Chemical characteristics in untreated and biotreated Silk dyeing effluent by *Azospirillum sp.* and *Pseudomonas fluorescens*. Int J Adv Pharm Biol Chem. 2014 Mar 1;3(1):176-9. ISSN: 2277 – 4688.