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Bacterial decolourization of azo dyes

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Abstract

Release of textile effluent into the environment is a matter of health concern. Dyes and pigments that are part of textile effluent generate hazardous wastes which are generally inorganic or organic contaminants. Among the present pollution control strategies, biodegradation of synthetic dyes by microbes is evolving as a promising approach, even more than physico-chemical methods. While both mixed cultures and pure cultures have been used to achieve efficient biodegradation, no conclusive result has been determined. This paper aims at checking the efficiency of mixed culture of sewage and pure isolates in degradation of azo dyes, both simple dyes like methyl red and methyl orange and a more complex dye like Janus green.

Keywords: Bioremediation, Biodegradation, Azo dyes, Mixed culture, Pure isolate

1. Introduction

Environmental pollution is a major issue in the developing world today, as there is a constant raise in pollution rate corresponding to

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increase in development. Almost any process employed by man in any industry results in release of environmental pollutants. One of the main types of pollution is water pollution. It's primarily caused by the discharge of improperly treated industrial effluent. This is left unchecked which harms the environment at alarming rates. The industrial effluent consists of different chemicals like heavy metals, dyes which when released directly into the water body can affect the ecosystem and therein hampers the balance of the ecosystem[1].

Azo dyes are characterized by the presence of one or more azo bonds (–N=N–) with aromatic rings[1].Due to π- delocalization, all azo compounds exhibit colour and are hence used as dyes in textile industries. They are also known as reactive azo dyes as they attach themselves to their substrates by a chemical reaction that forms a covalent bond between the molecule of dye and that of the fibre. Azo dyes are commonly used for dyeing fabrics in textile industry. Effluent that is released out consists of around 50% of dye content[2]. Improper textile dye effluent disposal in aqueous ecosystem leads to the reduction in sunlight penetration which in turn decreases photosynthetic activity dissolved oxygen concentration, water quality and depicts acute toxic effects on aquatic flora and fauna, causing severe environmental problems worldwide[1]. Higher the complexity of the auxochrome in the dye, byproducts formed will be highly complex and therein it will be difficult to remove it from the effluent[3].

Many synthetic azo dyes and their by-products are toxic, carcinogenic and mutagenic, can result in potential health hazard to humankind[3]. Hence, it is necessary to treat this effluent before releasing it into the water body. As of present time, most industries generally use physical or chemical techniques in order to treat the effluent. Some of such techniques that are employed are redox processes, application of ozone, hydrogen peroxide, UV etc.[3] including specialized techniques like photocatalytic decolourization and mediated Fenton reaction [4][5]. It is not always applicable as it involves high cost and it's not very efficient. Hence, there must be alternative to this process to help in obtaining a cheaper and more efficient process of removing the azo dyes.

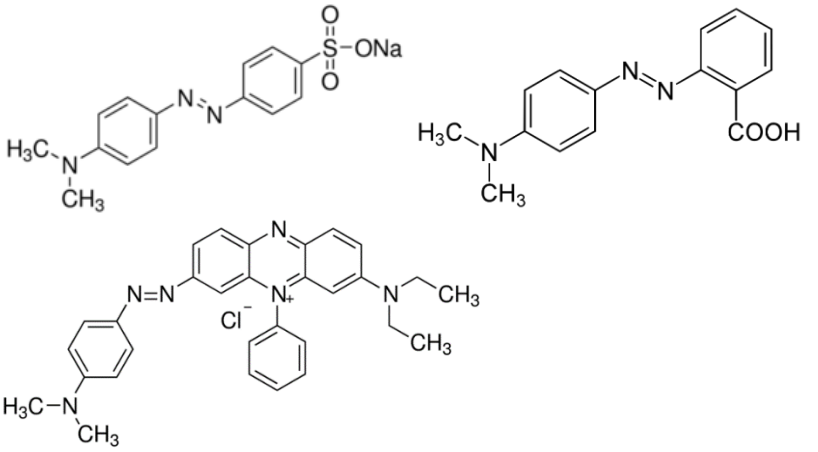

Figure1 Chemical structures of Methyl orange, Methyl red and Janus Green

Current research done in this area focus on treating the effluent using living organisms such as plant and bacteria, process being referred as biological remediation. Use of plants to remove the dyes or phytoremediation, as well as use of fungi has been quite successful[6][7][8], but there are several constraints while using them. They would need a much longer period of time to remove the dyes and would have to be disposed of separately. The organism either breaks the dye into simpler compounds or it adsorbs the dye, therein removing it from the effluent. Bioremediation is currently seen as a specific, less energy intensive, effective and environmentally safe technique since it results in partial/ complete bioconversion of organic pollutants to stable and non-toxic end products[1].

Among the different sources to obtain bacteria, it has been found that effluent release itself has bacteria that could decolorize the dye. It has been seen that when the effluents are released, after a certain while downstream the water body, the colour seems to disappear. This has been attributed to the presence of dye degrading bacteria[9]. Hence, water samples were collected from such a source and bacterial consortium was cultured in order to determine its dye degradation capability. Bacteria have been isolated from this consortium in order to check its efficiency in dye degradation.It has been seen in some studies that pure isolates are quite efficient in degrading dye, whereas some other studies have shown that a

mixed consortium is better at degrading dyes than isolated bacteria [10][11].

Biodegradation of dyes occur in both aerobic and anaerobic condition, but studies have shown that aerobic condition is less effective in terms of degradation of dye. A better alternative that has been devised in place of this is microaerophilic treatment system. Initial decolorisation of azo dyes is through a reductive process therefore it is facilitated by anaerobic static culture condition.[12]Several studies have tried to determine the mechanism of action of azoreductase. The most accepted mechanism currently used states that azoreductase acts as a redox mediator, which produces a primary product, which may be less toxic than the substrate, which is further broken down into harmless compounds by the oxidation by other aerobic microbes, as seen in Figure 2. Enzymatically, the decolorisation of azo dyes is highly dependent on the specificity of azo-reductase for different types of azo dyes.[12]

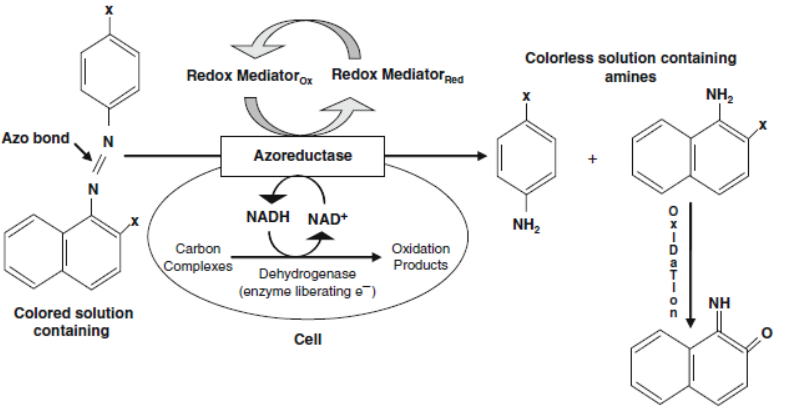

Figure 2 Possible mechanism of degradation of azo dye by bacteria[12]

Research in this area is really important; so as to devise easy and efficient techniques and industries could take up such techniques in order to render the effluent harmless before releasing it into the water body. Thus, it is necessary to devise better efficient techniques such that the industries could take up such methods and therein that would help in protecting the environment from further damage.

2. Materials and methods

2.1 Preparation of mixed culture of sewage bacteria

The mixed culture of bacteria was grown using a water sample that was collected from Agara Lake, Bengaluru. This water sample was acquired from one of the sewage water inlets into the lake which contained colored domestic effluents. The culture was prepared using 100mL of the water sample, which were mixed with 100mg/mL of peptone and yeast extract and 50mg/mL of NaCl, which was incubated in a shaker incubator at 37°C. After 24 hours, 10% of the culture was inoculated in fresh sewage water and incubated at 37°C overnight in a shaker incubator. The culture was stained using Gram staining technique to check for the presence of different types of bacteria. This culture was used for dye decolourization experiments. The collected water sample itself was used for isolation of the bacteria.

2.2 Preparation of dye solutions

The azo dyes Methyl orange, Methyl red and Janus green were used for this study. Solutions of 25ppm, 50ppm, 100ppm and 200ppm concentrations were prepared for each dye using the required amounts of dye in Luria Bertani (LB) broth.

2.2.1 Decolourization experiments

Decolourization experiments were carried out to check the decolourization activity of mixed culture. For this, 5% of the mixed culture was inoculated in each dye solution and kept in the shaker incubator at 37°C until maximum decolourization was seen. The solutions were centrifuged to pellet the bacteria and then the level of decolourization was studied by reading the absorbance of the solutions at the absorption maxima of the dye solutions spectrophotometrically (Methyl orange λ_{max}= 480nm, Methyl red λ_{max}= 580nm, Janus green λ_{max}= 700nm).

The decolourization percentage (efficiency of decolourization) for the respective dyes was calculated using the formula:

$$\text{Percentage of decolourization} = \frac{(\text{Initial absorbance} - \text{Observed absorbance}) * 100}{\text{Initial absorbance}}$$

$$= \frac{(Co - Ct) * 100}{Co}$$

2.2.2 Isolation of pure dye degrading bacteria

The water sample was serially diluted up to 10^8 dilutions and the dilutions were plated on Luria Bertani (LB) Agar and Nutrient Agar (NA) plates, and were incubated at 37°C overnight. Distinct colonies were used for isolation. The colony morphology of the colonies were studied and the bacteria were stained using Gram staining technique. These were then inoculated into dye solutions of 100ppm of Methyl orange and Janus green and incubated overnight in a shaker incubator. The colonies were then checked for efficiency of decolourization.

3. Results

3.1 Preparation of mixed culture and decolourization experiments:

The mixed culture of bacteria was grown using the sewage water sample in peptone, yeast extract and NaCl, without any dyes. The culture obtained was fast growing and gave a high variety of both gram positive and gram negative cocci and bacilli. The culture reached it stationary phase between 8 and 16 hours. Hence, an overnight culture was used as inoculum for the dye decolourization experiments.

In the case of methyl orange, the mixed culture was capable of 100% decolourization within 8 hours for all the concentrations used. Within 4 hours, the bacteria were able to decolourize the 200ppm solution to a level lower than 25ppm (Figure 3). This shows a great reduction capacity, where the bacteria is able to decolourize the dye to such large levels in a very little amount of time.

In the case of methyl red, the mixed culture of bacteria showed gradual decolourization of the dye over a period of 16 hours. In the initial 4 hours, more than 50% of the dye is decolourized even in

the 200ppm solution. This shows that the culture is highly efficient to degrade the dye to level of a 50ppm solution. At this stage, both the 25ppm and 50ppm solutions are completely decolourized. By 16 hours, all the solutions were completely decolourized, hence showing that the culture was 100% efficient in decolourizing the dye (Figure 4).

As methyl red is a pH indicator and may appear yellow if the pH changed to above 5.1 over time, the pH of the solution was checked and seen to be around 7. Acid was added to it and the pH was decreased to around 3, yet the orange-red colour failed to appear. This showed that the dye was actually getting decolourized and not just changing colour due to change in pH.

In the case of Janus green, the absorbance readings showed a gradual decrease as the absorbance was read at 700nm, which is the absorption maxima for Janus Green when it is in its blue form. But visually, the colour only changed from blue to pink and was never completely decolourized to yellow, as was seen in the case of methyl red and methyl orange. Hence, the pink colour is not read accurately at this wavelength. But a gradual decrease in absorption was seen, as in other cases, till it reached a zero value (Figure 5).

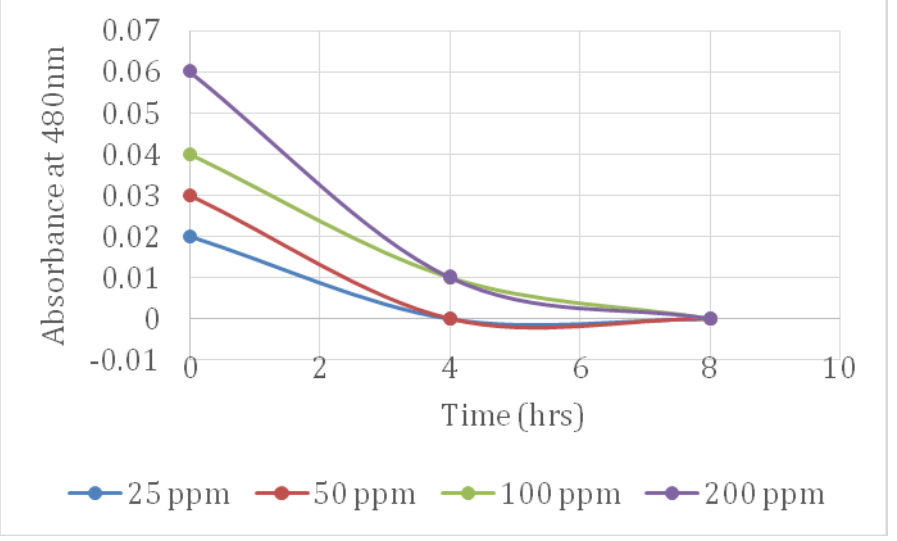

Figure 3 Graph showing net decolourization of different concentrations of Methyl orange with respect to time

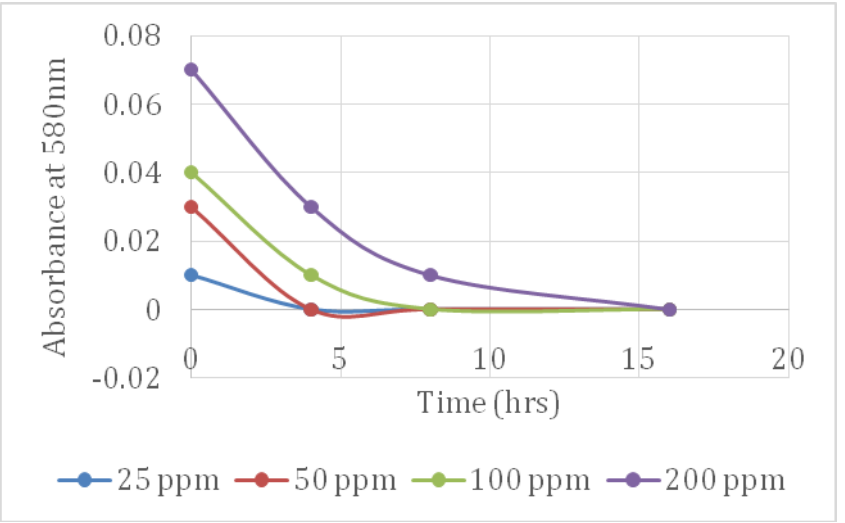

Figure 4 Graph showing net decolourization of different concentrations of Methyl red with respect to time

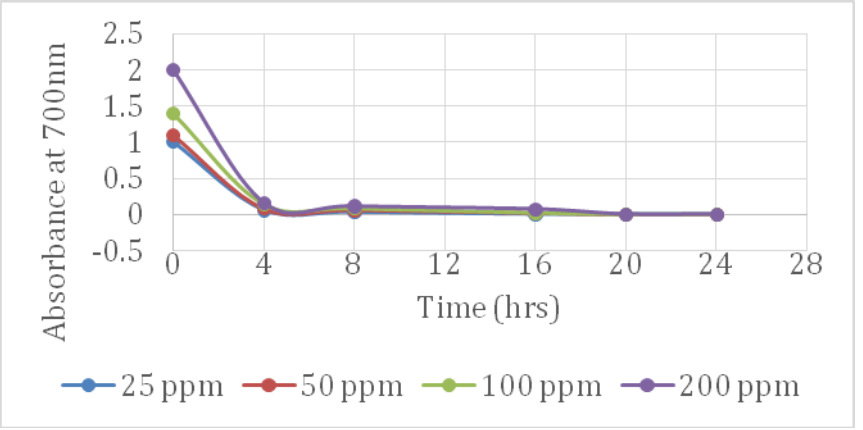

Figure 5 Graph showing net decolourization of different concentrations of Janus green with respect to time

3.2 Isolation of dye degrading bacteria and resultant dye decolourization:

Several types of colonies were observed in the plates of serial dilution, of which 14 distinct pinpoint colonies and 6 filamentous colonies were plated on LB agar and NA plates and incubated overnight. When checked for dye decolourization, the colonies that showed complete and partial decolourization were isolated and patched on a grid plate. While these colonies showed efficient

decolourization in liquid media, they failed to decolourize the dye in solid media. In the end, 2 filamentous colonies and 7 circular colonies were isolated, which had varying Gram staining and colony morphology characteristics. It was seen that the colonies labelled 2F, 6F, 3C, 7C and 11C showed complete decolourization, while 2C, 5C, 9C, 10C, 13C and 14C showed partial decolourization. In the case of Janus green, none of the colonies were able to decolourize the dye.

4. Discussion

This study shows how the mixed culture of bacteria is efficient in decolourizing simple azo dyes like methyl orange and methyl red. But Janus green is a more complex and more toxic dye. Most research done has theorized that the azo dyes may be toxic to some bacteria, hence retard their growth or make them more inefficient [13][1][2][3].This would cause the lag phase to be over a larger period of time, hence delaying the lag phase as the bacteria need to get acclimatized to the new and toxic environment. Previous experiments suggest that the removal of the dyes occurs either by adsorption or by metabolism, which in this study has occurred within 24 hours for simple dyes like methyl orange but took a longer period for Janus Green. [12][14]

The quick removal of the methyl orange and methyl red dyes suggest the presence of enzyme activity, such as azoreductase, which has been found in previous research in species such as *Aeromonas hydrophila*[9].The pelleted bacteria do not appear coloured under the microscope, which suggests that the dye is not being adsorbed or absorbed into the cells.

In the case of Janus green, the difficulty in decolourization could be attributed to the complexity in structure and possible conjugation occurring within the compound, which makes it difficult to be broken down. This dye is also possibly not adsorbed as the cells appear transparent when viewed under the microscope. Also, if adsorption were to take place, then the colour should have been completely removed and not only decolourized to pink. Other studies done on decolourization of Janus green indicate partial decolourization is possible, but they do not state if the blue colour

is lost or if it is changed to pink [15][16]. Other enzyme activity by two or more bacterial species could account for the change in colour, either by breaking the azo bond or by changing the structure of the dye.

In the case of the isolates, it was seen that they took up to 24 hours to achieve complete decolourization for methyl orange and were less efficient than the mixed culture. For decolourization of Janus green, none of the isolates could decolourize the blue dye to pink or to yellow. This shows that in this case, decolourization is not done only by a single species of bacteria, but must happen due to two or more species, acting simultaneously.

5. Conclusion

This study showed that the mixed culture could completely decolorize the dye within a short span of time. Thus, it depicts the potential of the mixed culture in decolorizing the azo dyes. The efficiency of the pure isolates in decolorizing the dye was also analyzed and it was found that pure isolates also had the ability to decolorize dyes, but not as efficient as a mixed consortium.

Several other analyses could be done in order to check the actual efficiency of this concept. There are several physical and chemical parameters which could be analyzed and the actual biodegradation potential could be understood, such as whether the culture is able to break down the toxic form to non-toxic form. Molecular mechanism and enzymes associated with it in this biodegradation could be analyzed by isolating the protein from solution. Various studies done in this field of research have been based on specific bacteria, but the actual mechanism involved and the methodologies may hold the key to using bioremediation as an actual and efficient method of waste disposal. Thus, more detailed studies are required to bring bioremediation into focus as a potential contender for degradation of hazardous chemicals.

References

[1] M. H. Fulekar, R. Khan and P. Bhawana, "Microbial decolorization and degradation of synthetic dyes," *Reviews in Environmental Science and Biotechnology,* vol. 97, pp. 1-24, 2013.

[2] H. Ali, "Biodegradation of Synthetic Dyes—A Review," *Water. Air, Soil Pollution- Springer,* vol. 273, pp. 213-251, 2010.

[3] H. S. Rai, J. Singh, T. K. Bansal, P. Vats and U. C. Banerjee, "Removal of dyes from the effluent of textile and dyestuff manufacturing industry: A review of emerging techniques with reference to biological treatment," *Critical reviews in environmental science and technology,* vol. 35, pp. 219-238, 2005.

[4] B. Goodell, Y. Qian, J. Jellison and M. Richard, "Decolorization and Degradation of Dyes with Mediated Fenton Reaction," *Water Environment Research,* vol. 76, no. 7, pp. 2703-2707, 2004.

[5] R. J. Davis, J. L. Gaine, G. O. and I.-W. Wu, "Photocatalytic Decolorization of Wastewater Dyes," *Water Environment Research,* vol. 66, no. 1, pp. 50-53, 1994.

[6] A. V. Patil, V. H. Lokhande, P. Suprasanna, V. A. Bapat and J. P. Jadhav, "*Sesuvium portulacastrum* (L.) L.: a potential halophyte for the degradation of toxic textiledye, Green HE4B," *Planta- Springer,* vol. 235, no. 5, pp. 1051-1063, 2012.

[7] A. N. Kagalkar, U. B. Jagtap, J. P. Jadhav, S. P. Govindwarand and V. A. Bapat, "Studies on phytoremediation potentiality of *Typhonium flagelliforme* for the degradationof Brilliant Blue R," *Planta,* vol. 232, no. 1, pp. 271-285, 2010.

[8] C. R. Corso and A. C. M. d. Almeida, "Bioremediation of Dyes in Textile Effluents by *Aspergillus oryzae*," *Springer- Microbe Ecology ,* vol. 57, pp. 384-390, 2009.

[9] E. Idaka, H. Horitsu and T. Ogawa, "Some properties of azoreductase produced by *Pseudomonas cepacia*," *Bulletin of Environmental Contamination and Toxicology,* vol. 39, p. 982–989, 1987.

[10] P. P. Vijaya, P. Padmavathy and S. Sandhya, "Decolourization and biodegradation of reactive azo dyes by mixed culture," *Indian journal of Biotechnology,* vol. 2, pp. 259-263, 2003.

[11] E. Zabłocka-Godlewska, W. Przystaś and E. Grabińska-Sota, "Decolourisation of Different Dyes by two *Pseudomonas* Strains," *Water, air, soil pollution,* vol. 225, no. 1846, pp. 1-13, 2014.

[12] D. Barcelo and A. G. Kostianoy, The Handbook of Environmental Chemistry, vol. 9, Berlin: Springer, 2009, pp. 1-38.

[13] A. Stolz, "Basic and applied aspects in the microbial degradation of azo dyes," *Applied microbiology and biotechnology- Springer,* vol. 56, p. 69–80, 2001.

[14] E.F.Kankeu, F.Waanders, P.Njobeh, L.P.Simelane, "Physico-Chemical treatment influenced by bacterial membrane and impact on dye adsorption capacity," in *International Conference on Advances in Science, Engineering, Technology & Natural Resources* , parys, 2016.

[15] S. B. Younes, T. Mechichi and S. Sayadi, "Purification and characterization of the laccase secreted by the white rot fungus *Perenniporia tephropora* and its role in the decolourization of synthetic dyes," *Journal of Applied Microbiology,* vol. 102, pp. 1033-1042, 2007.

[16] L. Levin, E. Melignani and A. M. Ramos, "Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white-rot fungi. Dye decolorization by selected culture filtrates," *Bioresource Technology,* vol. 101, p. 4554–4563, 2010.

[17] C. J. Ogugbue and T. Sawidis, "Bioremediation and Detoxification of Synthetic Wastewater Containing Triarylmethane Dyes by *Aeromonas hydrophila* Isolated from Industrial Effluent," *Biotechnology Research International,* vol. 2011, pp. 1-11, 2011.