Study on Bioremediation Potential of Various Fungi Species for the Removal of Textile Azo Dye from Dye Contaminated Siteds

*Swati Sharma
**Usha Sharma

Abstract

Synthetic dyes are known as the azo dyes. These are the industrially synthesized organic compounds. the azo dyes are basically identified by the presence of their azo bonds (N=N). These synthetic dyes are chiefly used in the industries like textile, food, paper, cosmetics and pharmaceutical industries. Out of all the available synthetic dyes, azo dyes are widely used in the textile industries. But combinations of these dyes which are free (unbounded) with the fibre, when released into the environment causes several environmental pollutions, most commonly bioaccumulation. These are various physico-chemical methods that are employed for the treatment of these azo dyes before discharging into the nearby waste streams, but these methods are not widely used as these methods have various limitations like high cost, low effectiveness and they cause several secondary pollutions as well. Biological methods are known as the alternative methods for the physico- chemical technologies, in which with the help of various microorganisms like bacteria, fungi, algae, yeast along with some plants and their enzymes lots of toxic azo dyes are decolourized. Biological methods get lots of attention because these methods have high efficiency, cost effective and environmental friendly as it does not cause any secondary pollution. So in relation with this, this paper provides an overview on the dye degradation and decolourization via means of biological methods.

Keywords: Azo dye; bio sorption; decolourization; dye degradation; enzyme; microorganism

Introduction:

Dyes are known to be an important chemical compound widely used in industries as colouring agents. The use of these chemical compounds are the essential step in all industrial or manufacturing processes. From various studies, it was reported that more than 10,000 dyes such as Congo red have been integrated in color index (Jalajaa *et al.,* 2009).

It is estimated that approximately 10-15% dyes are released during the manufacturing process into processing water (Selvam, *et al.,* 2003), which ultimately causing serious environmental issues as during the process large amount of polluted effluents (wastewater) are released which are directly discharged into the nearby waterbodies which affects the photosynthetic activity in aquatic life by decreasing the light penetration into the water and in addition it causes reduction of dissolved oxygen, which leads to "Oxygen Sag" in the receiving water (Delclos *et al.,* 1984; O'Neil *et al.,* 1999). The dyes are not easily biodegradable because of which they may also be toxic, mutagenic and carcinogenic to some animals lives in water and the water plants due to the presence of aromatic materials, chlorides, heavy metals like Cd, Cr, Co, Cu, Hg, Ni, Mg, Fe and Mn (Dhaneshvar *et al.,* 2007). So it is very essential to treat the polluted water coming from the industries before releasing it into the environment or waterbodies in order to reduce the risk of the environmental contamination from such pollutes waste (Arami *et al.,* 2005).

Singh *et al.,* in 2012 categorized the dyes into two major groups by (i) chromophore groups which is responsible for color or dye, includes azo dyes, anthraquinone dyes and phthalocyanine dyes etc. and secondly which is based upon their application or on their usage method. Dyes which are rich in organic or chemical compounds are mainly classified by the presence of chromophores (basically the unsaturated groups) such as –N=N–, –C≡N– and –C=C–. These unsaturated groups are mainly responsible for the colouring whereas for their fixation of fibres, basically the functional groups named as –NH2, –OH, –COOH and –SO3H are responsible (Molinari *et al*., 2004).

Various studies showed the potential of fungus for the treatment of wastewater coming from textile industries. Numerous combined microbial treatments (anaerobic as well as aerobic) had been recommended to improve the textile dyes biodegradation. (Huag, *et al.,* 1991). According to Ramya *et al.,* 2007, they suggested that decolourization of dye present in wastewaters by fungus, can be an promising alternative to replace the treatment which are using recently (Ramya *et al.,* 2007).

Microbial Degradation of Azo dyes:

From various researches it is identified that numerous microorganisms including fungi, bacteria, yeasts and algae can be able to decolorize or even mineralize many azo dyes completely in assured environmental surroundings. It was also reported that bacteria cleave the azo bond by use of enzyme namely azoreductase, which causes the decolourization of dye (Saratale *et al.,* 2011).

Fungi and bacteria, both are known to be the main degraders of organic matters, but out of both fungi are widely used for the decolourization of dyes because of their pre-eminence in the production of enzyme. Usually, fungi are categorized as white rot fungi, brown rot fungi, or soft-rot fungi depending upon the decay and descriptions (Nilsson, 1988) irrespective of their taxonomic locus.

Bacteria:

It was reported that various bacteria can also be used for the decolourization of dyes, as they cleave the azo bond which ultimately causes the dye decolourization (Parshetti *et al.,* 2011). For example, in industries mainly in textile industries various types of dyes are used as main compound in their manufacturing area, which are afterwards disposed off into natural waterbodies through wastewater. With this content a study was done which deals with the dye degradation mainly of Methyl red (MR) and Carbol fuchsin (CF) by the means of bacteria and its consortium which are isolated from effluents of textile industry. In this study, three different species of bacteria were isolated and subjected to the dye for the dye decolourization method. Later on the identified bacterial isolates were found as Proteus spp., Pseudomonas spp. and Acinetobacter spp. and it was also reported that these bacterial isolates are able to decolourize the methyl red and Carbol fuchsin dye by 0.002 gm/L. During this study, the bacterial consortium showed the highest dye decolourization rates which was approximately up to 100% and 96%, respectively within 24 h instead of pure bacterial isolates (Joshi *et al.,* 2015).

Algae:

The azo dyes degradation by the help of various algae was also studied and evaluated and it was reported that different types of algae can degrade various types of azo dyes. The dye degradation rate is totally dependent upon the dyes molecular structure and the type of algae which is going to be used. It was proposed that the enzyme azo reductase of algae is mainly responsible for the azo dye degradation by breaking the azo linkage into aromatic amine. Then these aromatic amines are exposed for further breakdown by algae. Algae plays a major role in azo dyes degradation in stabilization ponds, rather than only providing oxygen for bacterial growth (Jingi and Houtian, 1992; Banat *et al.***,** 1996).

Yan and Pan in 2004 stated that with the help of the various algal species like *Chlorella pyrenoidosa, Chrorella vulgaris* and *Oscillateria tenius*, more than thirty azo compounds can be decolorized and biodegraded into simpler aromatic amines (Yan and Pan, 2004).

Fungus:

According to Hofrichter, in 2002, it was studied and reported that fungi are also capable to degrade the pollutants because of having extracellular, nonspecific and non-stereo-selective enzyme system, which include various enzymes like lignin peroxidase (LiP), laccase and manganese peroxidase (MnP) (Hofrichter, 2002). Because of the secretion of extracellular enzymes, Fungi are known for the dye degradation better than bacteria (Forss and Welander, 2009).

It has been investigated that various fungal strains can be used for the degradation of dyes like for degradation of Congo red mainly *Gliocladium virens* is used (Singh, 2008), different harmful dyes degradation such as, Congo red, Acid red, Basic blue and Bromophenol blue dye directly, fungus species namely *Trichoderma harzianum* can be used (Singh and Singh, 2010).

Extracellular Enzymatic Degradation of Textile Dyes:

1) **Laccases:**

Laccases have been widely studied for their azo dye degradation. Laccase is the extracellular enzyme that showed a significant interest in the decolourization of coloured textile wastewaters (Husain, 2006). Laccase enzyme is essential as it oxidizes the toxic and nontoxic compounds. Laccases are the oxidoreductases enzymes which have a major role in various processes as they capable for bioremediation due to their different features like they have non-specific oxidation capacity, they do not require any co-factors and also they do not use the oxygen as an electron acceptor which is readily available (Telke *et al.,* 2011; Kalyani *et al.,* 2012).

Laccase belongs to the blue multicopper oxidases and contributes in cross-linking of monomers, degradation of polymers, and ring cleavage of aromatic compounds. Laccases are broadly distributed in higher plants and fungi like Ascomycetes, Deuteromycetes and Basidiomycetes and they are plentiful in lignin-degrading white-rot fungi. It is also used for the production of organic substance, which mainly includes amines and phenols. During this the result of the whole reaction are dimers and oligomers resulting from the coupling of reactive radical intermediates. Nowadays, these enzymes are widely used in the field of textile, pulp and paper, and food industry as well as for the formation of biosensors, biofuel cells, in medical tools that are used in diagnostic purpose and as bioremediation agent to clean the herbicides, pesticides and some explosives that are present in soil. It has been identified that two main forms of laccases are found, known as laccase-1 and laccase-2 which are used in different industrial fields for different purposes (Shraddha *et al.,* 2011).

2) Peroxidases:

Apart from laccases another extracellular enzyme known as peroxidases are also widely used for the azo dye treatment (Ezeronye and Okerentugba, 1999). The fungi are capable for the degradation of azo dyes as they synthesises the exo enzymes (peroxidases and phenol oxidases). Peroxidases are known as the hemoproteins, which are able to catalyse the reactions in the presence of hydrogen peroxide (Duran *et al.,* 2002)

Heme peroxidases are the enzymes which are produced by almost all organisms. For example, the present study deals with the decolourization, detoxification and bioremediation of Reactive Blue 160 textile dye by the help of T. patula enzymes. Patel and Jadhav (2013) studied the capability of some enzymes which are produced from the roots of Tagetes patula plant named as lignin peroxidase, laccase, tyrosinase and DCIP reductase Tagetes patula is an annual flowering plant which generally belongs to the family of Asteraceae. During this study it was found that 90% of dye can be decolourize within 4 d of incubation, confirmed by UV–vis, HPLC and FTIR analysis. Enzymes like lignin peroxidase, tyrosinase, laccase and NADH–DCIP reductase, found in tissues of the roots of the plant are responsible for the remediation. From GC–MS examination, it was also revealed that six types of various metabolites are also formed after phytoremediation of Reactive Blue 160 process such as sodium benzenesulfonate, 6-chloro 1,3,5-triazine-2,4-diamine, disodium benzene-1,4-disulfonate, sodium 3-amino-4-hydroxybenzenesulfonate, 1-phenylmethanediamine and sodium 4-amino-3-carboxybenzenesulfonate.

3) Polyphenol oxidase:

Plant Polyphenol oxidase (PPO) are categorized into group of copper proteins that are extensively distributed from bacteria to mammals (Shi *et al.,* 2001). Polyphenol oxidase or (PPO) is known to be a tetramer enzyme which comprises of four copper atoms per molecule, along with binding sites for two aromatic compounds and oxygen. On the other hand, Plant polyphenol oxidases are the simple and inexpensive alternate for the decolourization of aromatic pollutants as it utilizes the free molecular oxygen that act as an oxidant (Wada *et al.,* 1995; Duran and Esposito, 2000). PPO produced from various plants are widely used for decolourization of dye (Prabha and Patwardhan, 1982; Khan and Husain, 2007; Jadhav *et al.,* 2011; Arnnok *et al.,* 2010).

Along with this, the potential of PPO of three fast growing plants; Parthenium hysterophorus, Alternanthera sessilis and Jatropha curcas were tested, out of which the P. hysterophorus (Congress grass) is known to be the world's worst weed that grows fast and this weed is also very well known for pesticide activity (Ramanujam *et al.,* 2011; Apurva *et al.,* 2010).

The potential of the PPO which was extracted from Parthenium hysterophorus, Alternanthera sessilis and Jatropha curcas was estimated by Shinde *et al.,* 2012. In this study the potential of polyphenol oxidase (PPO) was studied which was extracted from leaves of these three particular plants, used for the decolourization two textile dyes named as Yellow 5G and Brown R. This was done under optimum temperature, pH and substrate concentration. In which the optimum temperature for all the three was set at 50^0C and their pH lies in acidic range.

Decolourization and Biodegradation of Azo Dyes Specifically by Various Types of Fungal Strains:

Azo dyes are existing in almost every color, as they are present in a group which are color-fast and can be structurally altered to bind with various natural and synthetic fabrics (Kirk-Othmer Encyclopedia of Chemical Technology 1992). But unfortunately, the azo dyes are unaffected by microbial degradation. The reason behind this is that the linkages of azo dyes are hardly present in the environment. Thus various micro-organisms that produces some enzymes are found to be capable in abolishing the azo dyes. The azo dyes are very toxic in nature as well as they are carcinogenic, so it very necessary to found some ways by which the effluents from the dye industries are treated. In contrast with this, the white rot fungi are able to degrade some artificial chemicals, such as dyes. The very well-known white rot fungus named as *Phanerocheate chrysosporium* has been tested for dye decolourization along with enzymes which are involved in lignin degradation, such as lignin peroxidase (Lip), manganese peroxidase (MnP) and laccase (Swamy *et al.,* 1999; Robinson *et al.,* 2001). Another fungal strain named as *Trichoderma harzianum* has also been studied and reported for the textile dye degradation (Singh and Singh, 2010).

In 2010, Ali *et al.,* investigated the potential of *Aspergillus flavus* for degradation or decolourization of textile dyes, Bromophenol blue and Congo red. In this study the bio removal of each dye was tested in two different culture media and in two different conditions (stationary and shaking conditions). After taking the inocula from various fungi, the fungal strains showed different ability of dye removal, which was found to be high in Acid Red (which was known as di-azo) as compared to orange dye (which was known as mono-azo dye). During this study it was found that apart from *Aspergillus tereus*, all the isolates of fungal strains showed high dye removal efficiency in SDB. In stationary state, *Aspergillus flavus* and *Alternaria* spp. showed 67% and 57% of decolourization efficiency in Orange II, in SDB and STE, respectively, while *Penicillium* spp showed 34 and 33 % decolourization efficiency in same. When the experiment was shifted to shaking state from stationary state, it was found that dye removal efficiency was also increased specifically with the *Penicillium spp., A. flavus and Alternaria spp.*

In present study the seven various fungal strains which were isolated from effluent of textile dye, were tested for the azo dye decolourization. The isolated seven various fungal strains were *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Penicillium chrysogenum, Mucor* sp. and *Trichoderma viride*. During the experiment, the inolum of fungus was added to the flask which already contains the azo dye (500 mg/l) along with the little amount of yeast extract, glucose and sucrose, which was incubated for 12 days after sterilization. As a result, it was found that the fungal strain i.e. *Aspergillus niger* decolourize the Congo dye completely in only 6 days. *Aspergillus fumigatus* decolourize the orange A dye nearly about 88.70%, while *Mucor* sp. Decolourize the Direct Green – PLS dye by 69.73%. apart from this, *Aspergillus niger* was identified to decolourize the Violet-BL, *Aspergillus flavus* found to decolourize the Direct Sky Blue-FF dye completely in 9 days whereas the *Trichoderma viride* decolourize the Direct Violet-BL dye and *Mucor* sp found to decolourize the Direct Sky Blue-FF dye completely in 12 days (Saranraj *et al.,* 2010).

From various researches it was found that the waste effluent coming from the dye industries contains the azo dye in it which without any pre-treatment, directly discharged into the nearby water streams, ultimately causes the water and soil pollution. To prevent these contamination, the process of removal of dye is very important step. To resolve this problem, Rani along with her collegues in 2011, studied the efficiency of white rot fungi for the dye degradation in the presence of an external carbon source, which is commonly found in the wastewater. During the study, fungal strains named as, potential of D. flavida along with white rot fungi has been studied for azo dye decolourization (Amaranth, Metanil yellow, Trypan blue and Chlorazole black) in absence of external carbon source. This experiment was setup at 36°C in 250 ml

Erlenmeyer flasks, put at stationary state along with this the N-limited medium (50 ml) with dye (50 mg/l) in the absence and presence of glucose. As a result, it was found that in the absence and presence of glucose the Amaranth dye was decolourize about 99% after 5 days of treatment, then after 10 days of treatment Metanil yellow, Trypan blue and Chlorazole black E was found to decolourize about 82.5%, 99% and 72% respectively.

Two fungal strains named as *Aspergillus niger,* and *Phanerochaete chrysosporium*, secluded from dye effluent soil. These fungal strains were used for detoxification and biodegradation of Malachite green, Nigrosin and Basic fuchsin dyes. For biodegradation of dye, agar overlay and liquid media methods in static and shaking conditions at 25°C were used. As a result, it was recorded that Basic fuchsin, Nigrosin, Malachite green and dye mixture was decolourize by 81.85%, 77.47%, 72.77% and 33.08% respectively by *Aspergillus niger* fungal strain while *P. chrysosporium* decolourize Nigrosin, Basic fuchsin, Malachite green and dye mixture by 90.15%, 89.8%, 83.25% and 78.4% respectively. These fungal strains were found to perform better in shaking conditions when compared to static conditions (Rani *et al.,* 2014).

Apart from this the fungal strains known as *Penicillium expansm* and *Aspergillus niger* are also used for the degradation of two dyes such as Safranin dye and Carcinogenic dyes. Out of which Safranin dye is also known as industrial dye as it is widely used is various industries for colouring purpose. Particularly in biodegradation of Safranin dye it was found that the fungal strains named as *P. expansum* was found to be more efficient than *A. Niger.* The results from this particular experiments showed that *P. expansum* showed about 41.035% of Bio decolonization whereas *A. Niger* Bio decolonize about 38.952% when incubation was done for 120 hours (Mohammed, 2016).

Recently, the potential of fungal strain *Aspergillus flavus* was studied by Singh and Singh in 2017 for decolourization of azo (Acid red) and anthraquinonic (Basic blue) dyes**.** During the experiment the selected fungal cultures were grown and then filtered with the help of Whatman filter paper No. 1 and after filteration the filterate were centrifuged at 6000 rpm for about 5 minutes and the supernant which was left after centrifugation was used for spectrophotometric analyses. spectrophotometric analyses for Acid red and Basic blue dyes, were done at 494 nm and 630 nm, respectively. From spectrophotometric analyses it was recorded that the selected fungal species are able to degrade and decolourize the dyes by 62% and 77% for Basic blue and for Acid red respectively.

Conclusion:

Mostly the industries produce a lot amount of waste effluent which contains dye in it is directly discharged into the nearby water streams which ultimately causes the serious environmental issues. So it is very necessary to have a proper waste water treatment facility or method for treating the dye effluent so that it cannot hamper the aquatic life forms. There are different methods like physical, chemical and biological treatment are used for the treatment of decolourization of azo dyes. Out of which it was found that majority of the treatment techniques work in mainly two ways either by concentrating the colour intensity into the effluent produced from dye industry or either by complete elimination of dye molecule. From the three major methods the physical and chemical methods are found to be insufficient for the treatment of textile wastewater because these physical and chemical methods are not very eco-friendly method as well as these methods produce some secondary pollution also. Biological method that is bioremediation method was found to be clean and alternative source for the bioremediation of wastewater containing dyes. As in biological method various types of microorganisms are used which have a capability to decolourize and metabolize the dye. The techniques based upon bioremediation are widely used nowadays as it was found to be more successful and viable method for the treatment of textile azo dyes. However, the decolourization mechanism (dye reduction) is not clear till now. So the further research on dye reduction mechanism would be helpful in the improvement of bioremediation method.

*Department of Botany
Govt. PG College, Tonk
**Department of Zoology
BBD Govt. College, Chimanpura (Raj.)

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