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Mycoremediation for the Treatment of Dye Containing Effluents

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Abstract - Mycoremediation is an innovative biotechnology that uses living fungus for in situ and ex situ cleanup and management of contaminated sites. It is a cost effective process and the end products are non-hazardous. The process typically begins with field collection of fungi from a local area and continues with steps of culturing, screening, and preconditioning of native species to remediate specific contaminants. Industrial effluents entering into the surface waters are perhaps the most important sources of toxic contaminants in the environment. Textile is one of the largest industries which results in pollution contributed by untreated effluent discharge, which contains high concentrations of consumed metal based dyes, phenol, aromatic amines etc. The presence of metal based coloured dyes and foaming chemicals in textile waste water not only retards biological activity by reducing the light penetration but also causes metal toxicity to both aquatic and terrestrial life. There are various methods for the treatment of textile wastewater for the removal of dye. These broadly fall into three categories namely physical, chemical and biological. The biological treatment of effluent has become an economically and environmentally attractive alternative to the present physico-chemical methods of treatment. The major disadvantage of physico-chemical methods has been largely due to the high cost, low efficiency, limited versatility, interference by other wastewater constituents and the handling of the waste generated. Microbial decolourisation can be achieved by using various naturally occurring microorganisms such as bacteria, fungi and their enzymes. Use of fungi is economical and eco-friendly technique for the fine tuning of waste water treatment. Fungi have proved to be suitable organisms for the treatment of textile effluent and dye removal. The fungal mycelia have an additive advantage over single cell organisms by solubilising the insoluble substrates by producing extracellular enzymes. Due to an increased cell-to-surface ratio, fungi have a greater physical and enzymatic contact with the environment. Among the different fungi white- rot fungi play an important role for dye degradation. White rot fungi have been studied for nearly three decades and new species are being developed to decolourize various textile dyes with their lignin-degrading enzymes.

Keywords:Textile industry, dye effluent, mycoremediation, white rot fungi

I. INTRODUCTION

Our biosphere is under constant threat from continuing man made activities on water by domestic, industrial, agriculture, shipping, radio-active, aquaculture wastes; on air by industrial pollutants, mobile combustion, burning of fuels, agricultural activities, ionization radiation, cosmic radiation, suspended particulate matter; and on land by domestic wastes, industrial waste, agricultural chemicals and fertilizers, acid rain, animal waste. These pollutants have negative influence over biotic and abiotic components on different natural eco-systems. Some of the recent environmental issues include green house effect, loss in bio-diversity, rising of sea level, abnormal climatic change and ozone layer depletion etc. In recent years, different approaches have been discussed to tackle man made environmental hazards. Thus a clean technology, eco-mark and green chemistry are the need of the hour in preventing and reducing the adverse effect on our surroundings.

II. ENVIRONMENTAL POLLUTION DUE TO DYES

Dyes are extensively used for several industrial applications and approximately 5% of them end up in effluents.Textile sector are a complicated industrial chain with a high diversity in terms of raw materials, processes, productions and equipment. Textile is one of the important sectors which produces excessive waste. The annual world production of textile industry is about 30 million tonnesrequiring 700,000 tonnes of different type dyes which causes considerable environmentalpollution problems (Talarposhti*et al.,* 2001).

The important negative impact of textile dye is the releasing of colour effluents into the environment (Stolz, 2001). Out of various activities in textile industry, chemical processing contributes about 70% of pollution. The nature of pollution that accompanies this industry is primarily due to the non-biodegradable nature of the dyes along with the strong presence of toxic trace metals/acid/alkali/ carcinogenic aromatic amines traceable in the effluents. The following table illustrates the pollutants associated with some of the very popular dyes.

a. Sources and Causes of Generation of Textile Effluent

Textile industry involves wide range of raw materials, machineries and processes to engineer the required shape and properties of the final product. Waste stream generated in this industry is essentially based on water-based effluent generated in the various activities of wet processing of textiles. The main cause of generation of this effluent is the use of huge volume of water either in the actual chemical processing or during re-processing in preparatory, dyeing, printing and finishing that is 45% material in preparatory processing, 33% in dyeing and 22% are re-processed in finishing.

The fact is that the effluent generated in different steps is well beyond the standard and thus it is highly polluted and dangerous. Various chemical and physical processes such as adsorption, air stripping, flocculation, precipitation, reverse osmosis and ultra-filtration can be used for colour removal textile effluents (Robinson*et al*, 2001, Zamora *et al*., 1999, Ladakowicz*et al*., 2001, Georgiou *et al*., 2002). Treatment of dye-based effluents is considered to be one of the challenging tasks in environmental fraternity.

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Table 1: Types of Pollution that is associated with various dyes

Class	Fiber	Nature of Pollution
Direct	Cotton	Salt, unfixed Dyes, copper salts, cationic fixing agents
Reactive	Cotton	Salt, unfixed Dyes, Alkali
Vat	Cotton	Alkali, oxidizing agent, reducing agent
Sulphur	Cotton	Alkali, oxidizing agent, reducing agent, unfixed dyes
Acid	Wool	Unfixed dyes, organic dyes
Disperse	Polyester	Carriers, reducing agent, organic acids
1:2 Metal complex dyes	Wool	Metals, organic acid

b. Impact of Dyes

The textile industry accounts for two-thirds of the total dyestuff market (Riu*et al.*,1998) and consumes large volumes of water and chemicals for wet processing of textiles. The discharges of wastewater are the main cause of the negative environmental impact of the textile industry. Azo dyes and nitrated aromatic hydrocarbon are the two groups of chemical that are abundant in the environment. Large amounts of chemically different dyes are used for various industrial applications including textile dyeing. A significant proportion appears in the form of wastewater and is spilled into the environment (Meyer, 1981).

Conventional wastewater treatment is not efficient to remove recalcitrant dyestuffs from effluents (Shaul*et al*., 1991). Major pollution textile waste water comes from dying and pollutant textile wastewater are high suspended solids, chemical oxygen demand (COD), heat, colour, acidity, and other soluble substances. The most toxic dyes for algae and fishes are basic and acid dyes. Algae growth and fish mortality are not affected by dye concentrations below 1 mg/l. The chemicals present in the textile industry effluents thus affect the normal life of animals. Toxic compounds from dye effluent get into aquatic organisms, pass through food chain and ultimately reach man and cause various physiological disorders like hypertension, sporadic fever, renal damage, cramps, etc.

III. COLOURISTIC CLASSIFICATION

Classification by usage or application is the principle system adopted by the colour index (Trotman, 1990). The most important and widely used textiles fibres are cotton and polyester, and because of this the dyes predominantly used are those for dyeing these two fibres, and to some extent polyester-cotton blends.

a. Acid dyes
Acid dyes are applied to fibres such as nylon, wool and silk, also to some extent to paper, leather and cosmetics. The main chemical classes of these dyes are azo, anthraquinone, triphenylmethane, azine, xanthenes, nitro and nitroso and are soluble in water (O'Neill *et al*., 1999).

b. Cationic (Basic) dyes
Basic dyes are mainly applied to paper, polyacrylonitrile, modified nylons, polyesters, cat ion dyeable polyethylene terephthalate and medicine. The main chemical classes are diazahemicyanine, triarylmethane, cyanine, hemi cyanine, thiazine, oxazine and acridine (O'Neill *et al*., 1999).

c. Direct dyes
Direct dyes are applied to cotton, rayon, paper, leather, wool, silk, nylon and are also used as pH indicators and biological stains. The dyes belonging to this class are polyazo compounds, along with some phthalocyanines and oxazines (O'Neill *et al*., 1999).

d. Disperse dyes
Disperse dyes were originally developed for the dyeing of cellulose acetate but they are mainly applied to polyester, nylon, cellulose and acrylic fibres. They are water insoluble non-ionic dyes used for hydrophobic fibres from aqueous dispersion. They generally contain azo, anthraquinone, styryl, nitro and benzodifuranone groups (O'Neill *et al*., 1999).

e. Mordant dyes
Mordant dyes are used for wool, leather and natural fibres. Most natural dyes are mordant dyes. Some mordant dyes such as heavy metal category are hazardous to health. They consist of azo and anthraquinone groups (O'Neill *et al*., 1999).

f. Vat dyes
Vat dyes are water insoluble dye used mainly in cotton and are incapable of direct dyeing of fibres. These dyes groups contain antraquinone and indigoids (O'Neill *et al*., 1999).

g. Reactive dyes
Reactive dyes are generally used for cotton, wool and nylon. Their chemical structures are simpler than other dyes; their absorption spectra show narrow absorption bands and final dyeing colour is brighter than when using direct dyes (O'Neill *et al*., 1999).

h. Sulfur dyes
Sulphur dyes are commonly used for cotton and rayon and have limited use with polyamide fibres, silk, leather, paper and wood. Sulfur Black 1 is the largest selling dye by volume because of its low cost and properties (O'Neill *et al*., 1999).

i. Solvent dyes
Solvent dyes are non-polar water insoluble dyes mainly used for plastics, gasoline, lubricants, oils and waxes (O'Neill *et al*., 1999). Dyes classified according to method of application maybe anionic, direct or disperse depending on their terminal application i.e. either uses on protein, cellulose or polyamide fibres (Hunger, 2003).

IV. DYE REMOVAL METHODS

Commonly applied treatment methods for colour removal from coloured effluents involves various combinations of biological, physical and chemical decolourization methods (Galindo and Kalt, 1999; Robinson *et al*., 2001; Azbar*et al*., 2004). Generally in physical method dyes are removed by adsorption, in chemical method chromophore has been modified through chemical reaction, biological decolourization occur through biosorption and degradation. The major disadvantage of physico-chemical methods has been largely due to the high cost, low efficiency, limited versatility, interference by other wastewater constituents and the handling of the waste generated. Microbial decolourization being cost-effective is receiving much attention for treatment of textile dye waste water (Banat *et al.*, 1996; Stolz, 2001; Zee and Villaverde, 2005). Biological dye degradation technique are either based on partial or complete biodegradation of dyes by pure and mixed culture of bacteria, fungi, algae (Bhatt *et al.*, 2000). A number of biotechnological approaches have been suggested by recent research in combating this pollution source in an eco-efficient manner, which includes the use of bacteria or fungi, often in combination with physicochemical processes (Willmott*et al*., 1994; McMullan *et al*., 2001; Robinson *et al*., 2001). The biodegradation of dyes was first reported in white rot fungi (Glen and Gold, 1983) and since then many scientists have explored the biomineralization properties of different fungi.

V. MYCOREMEDIATION

Mycoremediation is an innovative biotechnology that uses living fungus for *in situ* and *ex situ* cleanup and management of contaminated sites.Mycoremediation is an economically and environmentally sound alternative for extracting, transporting and storing toxic waste.Mycelia can destroy these toxins in the soil before they enter our food supply. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular ndacids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant.

a. Mycoremediation Method

The overall method of mycoremediation is quite simple: Overlay straw or woodchips infused with the right mycelium to create a 'living membrane of enzymes that will rain down' on the toxins in the top soil. Replenish annually with additional mycelium-treated substrate. Depending on the situation, several sequential applications may be the necessary norm to reduce toxins to acceptable levels.

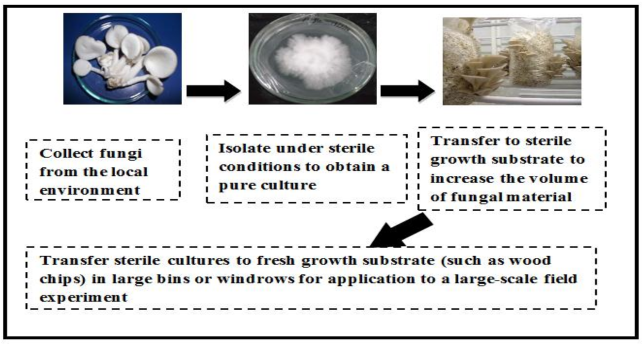

Figure 1: Mycoremediation Preparation Process for Application to Field Settings

VI.DYE DECOLOURIZATION BY WHITE ROT FUNGI

White rot fungi are the most intensively studied dye-decolorizing microorganisms to decolourize various textile dyes with their lignin-degrading enzymes (Cripps et al., 1990; Champagne and Ramsay, 2005; Bhatti et al., 2008). The decolourization of dyes by white rot fungi was first reported by Glenn and Gold (1983).Uptake effects or dye sorption by WRF mycelia without real degradation are generally minimal (Glenn and Gold, 1983). These effects are, rather, seen in applications of non-WRF, such as Aspergillusniger, whose (dead) biomass could be used as an adsorbent (Fu and Viraraghavan, 2000)and serve as part of a technical solution in water pollution control.Tien and Kirk (1983) reported the first dye decolourization by Phanerochaetechrysosporium. WRF are superior dye-decolorizers in comparison with prokaryotes. The rate of dyes decolourization depends on the composition of medium and on the dye microorganism combination. Some other physical factors that influence dye decolourization are pH, temperature, dye concentration and agitators. Lignin-modifying enzymes play a significant role in the dye decolourizationmetabolism by white rot fungus (McMullan et al., 2001). The ligninolytic enzymes are excreatedextracellularlyby white rot fungi through the oxidation of lignin in the extracellular environment of the fungal cell.The ligninolytic enzymes produced by white-rot fungi have been grouped into two: peroxidases (which includesMnP and LiP) and laccases.Ligninolytic enzymes are also involved in the degradation of various xenobiotic compounds including dyes (Glenn and Gold, 1983).

a. Mechanism of dye decolourization by white rot fungus

For the complete degradation of lignin and a wide variety of environmental pollutants several mechanisms are used by white rot fungi. Both oxidative and reductive reactions are required for the metabolism of both lignin and environmental pollutants. The ferric form of the enzyme, usually referred to as resting enzyme, is oxidizedby two electrons by hydrogen peroxideto a form of peroxidases referred to ascompound I. Compound I can be reducedby one electron by chemicals having a suitablereduction potential. The enzyme isreduced to a form called compound IIwhereas the chemical is oxidized by oneelectron. This one-electron oxidized chemical,or the original chemical, can thenreduce compound II back to restingenzyme. In order to be oxidized by twoelectrons (compound I), one electron isremoved from ferric iron to form ferrylwhile the second electron is withdrawnfrom the porphyrin ring. The latter isreduced first to form compound II. Thereduction potential for compound I is thusslightly higher than for compound II. The major mechanisms involved in the dye decolourization by white rot fungi are Biodegradation, Biosorption, various reactor designs and Immobilised fungal cells. In this biodegradation and biosorption plays a major role in dye decolourization.

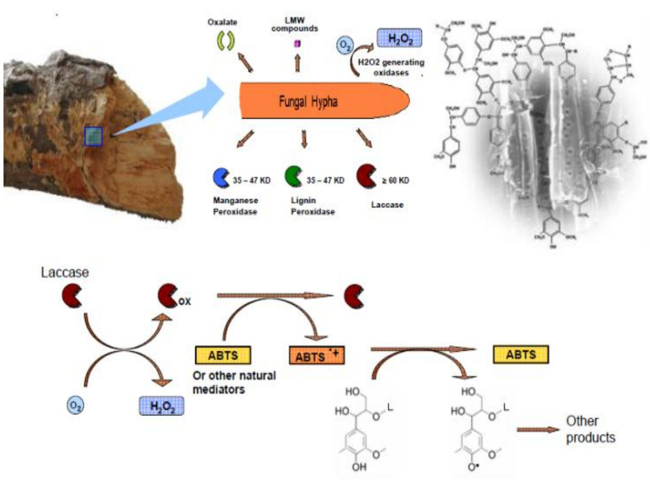

Figure 2:Dye decolourization mechanism of white rot fungus

Due to the ability to produce ligninolytic enzymes to mineralize the dyes.Biosorption is a method used for removal of heavy metal contaminants from waste water. It includes both adsorption and absorption. The extent of dye bio sorption depends on the chemical structure and functional group of the dye molecules (Fu and Viraraghavan, 2000).

The decolourization activity was significantly affected by dye concentration, amount of pellet, temperature and agitation of the media. With live cells, the decolourization involved adsorption of the dye compound by the fungal pellet at the initial stage, followed by fungal catabolism (Yesilada*et al*., 2003). Microbial cell immobilization refers to the systems or techniques in which "there is a physical confinement or localization of microorganisms that permits their economic reuse" (Anderson, 1975). Immobilized fungal cells have several advantages over dispersed cells such as simple reuse of the biomass, easier liquid-solid separation and minimal clogging in continuous-flow systems (Arica *et al*., 1993; Tieng and Sun, 2000).

In addition, immobilized cultures tend to have a higher level of activity and are more resilient to the environment perturbations such as pH or exposure to toxic chemical concentrations than suspension cultures (Shin *et al*., 2002) and immobilization protects the cell from shear damage (Abraham *et al*., 1991; Fiedurek and Ilczuk, 1991; Vassilev and Vassileva, 1992). Moreover, cell immobilization lowers the apparent broth viscosity and makes the rheological features more favourable for oxygen supply and mass transfer.

VII. CONCLUSION

The literature reviewed revealed the fact that there are varying methods to assess decolourization. Further studies are essential for optimizing conditions for large scale production of enzymes and dye decolourization. Last two decades vast number of research work has under gone on efficiency of white rot fungus on dye decolourization. Even though various methods are used to assess decolourization of dyes researchers have paid more attention on using lignin modifying enzymes of white rot fungi. Although lignin modifying enzymes give good decolourization results, this approach is not optimal. Combination of physical, chemical and biological processes are more efficient for textile dye removal but are expensive. Thus further studies should be conductedto elucidate the catabolic processes involved in the degradation of distinct dye groups by the LME of WRF. Progress in the field of nanotechnology could also provide tools for studying cell surface and topology to better understand the importance of membrane-bound oxidoreductases and their role in growth-associated degradation of organic dyes by WRF in the near future.

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