Int. J. Chem. Sci.: 11(2), 2013, 891-910
ISSN 0972-768X
www.sadgurupublications.com

DEVELOPMENT OF FUNGAL CONSORTIUM FOR BIODECOLORIZATION OF TEXTILE WASTE EFFLUENTS: A REVIEW

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ABSTRACT

Water-pollution control is presently one of the major areas of scientific activity. While colored organic compounds generally impart only a minor fraction of the organic load to wastewater, their color renders them aesthetically unacceptable. Effluent discharge from textile and dyestuff industries to neighbouring water bodies and wastewater treatment systems is currently causing significant health concerns to environmental regulatory agencies. Color removal, in particular, has recently become one of major scientific interest, as indicated by the multitude of related research reports. During the past two decades, several physico-chemical decolorization techniques have been reported, however, few have been accepted by the textile industries. But these techniques have high cost of implementation, low efficiency and inapplicability to a wide variety of dyes. The ability of microorganisms to carry out dye decolorization has received much attention. Microbial decolorization of dyes is seen as a cost-effective method for removing these pollutants from the environment. In recent years, there has been an intensive research on fungal decolorization of dye wastewater. It is becoming a promising alternative to replace or supplement to present treatment processes.

Key words: Biodecolorization, waste water, Effluent, Fungal.

INTRODUCTION

Pollution has been defined in various ways. It is considered as the release of unwanted substances to the environment by man in quantities that damage either the health or the resources itself. Industrial effluents from various industries like textile, dye stuffs, paper and pulp, distillery, olive oil mill and metal industries are the major contributors to water pollution.

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Waste waters from textile industries are a complex mixture of many polluting substances such as organo-chlorine based heavy metals, pigments and dyes. The dyestuff usage has been increased day by day because of tremendous increase of industrialization and man's urge for color[1]. Many chemical dyes have been used increasingly in textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness and variety in color compared to that of natural dyes. Color is one of the most obvious indicators of water pollution and discharge of highly colored synthetic dye effluents can be damaging to the receiving water bodies[2]. The effluents of pharmaceutical, textile, printing, photographs, and cosmetics contain dyes[3].

About 100,000 commercial dyes are manufactured including several varieties of dyes such as acidic dye, basic dye, reactive dye, azo-dye, and diazo dye. The world's annual production of dye stuffs amounts to more than 7×10^5 tones[4]. As per the recent data published by the Textile Commissioner's Office, there are 1569 cotton textile industries in India except the small scale industries. Indian dye industries produce every type of dyes and pigments, and the production of dyes is close to 80,000 tones. India is the second largest exporter of dye stuff and intermediates in developing countries after China.

Presence of very low concentration of dyes in effluents is highly visible and undesirable. Some of these dyes are potentially mutagenic and toxic. Contact of dye waste effluents may significantly affect photosynthetic activity in aquatic environment due to the reduced light penetration and may be toxic also to some aquatic lives due to the effluents containing several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes[5]. Some effects of dyes in the environment are as follows: Anthraquinone-based dyes are the most resistant to degradation due to their fused aromatic structures, which remain colored for long periods of time. Basic dyes have high brilliance and therefore, have higher color intensity, making them more difficult to decolorize. While metal-based complex dyes, such as chromium-based dyes, can lead to the release of chromium into water supplies, which is carcinogenic in nature. Some dyes have also been shown to have a tendency to bio-accumulate, when exposed to the environment[6,7]. Heavy-metal ions from textile effluents have also been reported to bioaccumulate at high concentrations in both algae and higher plants exposed to such effluents[8].

The waste water discharges from textile and dyestuff industries have to be treated due to their impact on water bodies and growing public concern over their toxicity and carcinogenicity.

Currently, the major methods of textile waste water treatment involve physical and chemical processes. There is also a possibility that a secondary pollution problem arises

because of excessive chemicals used to decolorize the dyes. Other emerging techniques, such as ozonation, treatment using Fenton's reagent, may have potential for decolorization. However, such technologies usually involve complicated procedures and they are economically unfeasible. There are drawbacks of using physical and chemical methods for treatment of the textile waste effluent. One physical treatment is oxidation, which is used in waste water and it has high energy and many forms of hazardous by-products are released. Another physical method is adsorption and its by-product requires regeneration or disposal. Chemical methods as membrane technologies and coagulation produce high concentration of sludge during treatment.

The limitations of the physical and chemical procedures to remove dyes are: excess amount of chemical usage or sludge generation with obvious disposal problems; costly plant requirements or operating expenses; lack of effective color reduction, particularly for sulfonated dyes; and low sensitivity to a variable wastewater input. Certain treatment schemes may be applicable for some textile mills using one or two types of dyes, but not for other mills or dye mixtures. Other techniques involve chemical oxidation using sodium hypochlorite to remove the color. They, however, release a lot of aromatic amines, which are carcinogenic, or otherwise toxic compounds; these subsequently aggravate the problem[9]. Utilizing the recalcitrant dyes and this affinity to adhere to surfaces as a means of removing them through adsorption, would not involve biodegradation and release of intermediate products, has also been suggested by several authors[10,11].

Alternative approaches to color removal utilize microbial catalysts to reduce the dyes that are present in the effluent. Microbial decolorization and degradation is an environment-friendly and cost-competitive alternative to physical and chemical degradation processes[12]. Many of the textile plants have rural locations and municipal treatment costs are increasing, both industries and scientists are being compelled to search for innovative novel treatments and technologies directed particularly towards the decolorization of dyes in effluents. Dyes usually have a very low rate of removal ratio for BOD to COD (BOD/COD less than 0.1)[10].

Biological methods are cheap and simple to use and have been the main focus of recent studies on dye degradation and decolorization.

Microbial decolorization

The dyes constitute only a small portion of the total volume of waste discharge in

textile processing. These compounds are not readily removed by typical microbial-based waste-treatment processes[13]. Recent fundamental work has revealed the existence of a wide variety of microorganisms capable of decolorizing a wide variety of dyes. Many microorganisms belonging to different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolorize dyes. Bacterial degradation of these dyes requires their intracellular uptake while the fungi degrade these by extracellular enzymes[14]. Recently, a number of studies have shown that some bacteria and fungi are able to biodegrade and bioadsorb the dyes in textile industry effluent[15].

But the main problem with dyes is that they can be detrimental to the microbial population present in such treatment works and may lead to decreased efficiency or treatment failure in such plants[16]. Similar adverse effects have also been detected for aquatic microbial populations and the aquatic environment in general[17,18] or for laboratory cultures[17] exposed to such dyes.

The treatment of textile wastewater by purely biological processes may be possible even without the inclusion of other carbon sources, e.g. municipal wastewater. This has been the subject of intensive research in recent years. Such a situation was predicted earlier by McKay[20], who concluded that "decolorization through biological systems would receive increased attention in the future". Among the many biological systems available are: Aerobic activated-sludge or rotating bioreactors[21,22], Aerobic-anaerobic packed-bed reactors[23,24], Aerobic-anaerobic fluidized-bed reactors[25], Aerobic-anaerobic sequential batch or continuous-flow reactors[26-28], Anaerobic batch reactors[29]. These are the biological systems used in biological treatment plant of textile dyes.

Bacteria decolorization

Numerous bacteria capable of dye decolorization had been reported. *Bacillus subtilis* was isolated from bacterial culture was capable of degrading azo dyes[30], then *Aeromonas hydrophila*[31], followed by a *Bacillus cereus*[32]. Isolating such microorganisms proved to be a difficult task. Extended periods of adaptation in chemostat conditions were needed to isolate the first two *Pseudomonas* strains capable of dye decoiorization[33]. An azoreductase enzyme was responsible for the initiation of the degradation of the Orange II dye by these (*Pseudomonas, Bacillus cereus* and *Aeromonas hydrophila)* strains and substituting any of the groups near the azo group's chemical structure hindered the degradation[34]. Several other decolorizing *Pseudomonas* and *Aeromonas* species were then reported by a Japanese group[35-38]. Many other bacterial strains have been reported that degrade the dyes. The

bacterial consortium degrades and decolorizes the dyes more quickly and efficiently in anaerobic conditions[39].

Fungal decolorization

Fungal strains capable of decolorizing different varieties of dyes have been studied and white rot fungi have also been employed for the decolorization of a number of textile dyes. Bio-decolorization of lignin-containing pulp and paper wastewater, as measured by the decrease in colour absorption, using two white-rot Basidiomycete fungi: *Phanerochaete chrysosporium* and *Tinctoporia sp*., was reported as early as 1980[40,41]. Both were clear examples of colour removal through microbial degradation of polymeric lignin molecules. Since then, the wood-rotting *P. chrysosporium* in particular has been the subject of intensive research related to the degradation of a wide range of recalcitrant xenobiotic compounds, including dyes. The mechanism of color removal involves a lignin peroxidase and Mn-dependent peroxidase or laccase enzymes[42]. Many of the fungal species have been reported, which degrade the dyes. Some of them are *Coriolus versicolor*[43], *Trametes versicolor*[44], *Funalia trogii*[45], *Umbelopsis isabellina* and *Penicillium geastrivous*[46], *Aspergillus sp*., *Trichoderma sp*., *Pycnoporous cinnabarinus*, and *Phanerochaete chrysosporium* fungus. These fungi can be classified into two kinds according to their life state: living cells to biodegrade and biosorb dyes and dead cells (fungal biomass) to adsorb dyes. The term biosorption indicated several metabolism-independent processes (chemical and physical adsorption, complexation, ion exchange, electrostatic interaction, chelation and microprecipitation) taking place in the cell wall rather than oxidation through aerobic or anaerobic metabolism. Both living and dead (dried, heat killed, acid and/or otherwise chemically treated) biomass can be used to eliminate harmful organic compounds.

Living cells

The decolorization capability was tested with species like *Phanerocheate chrysosporium*, *Trametes hirsuta* and an isolated native Marine fungi M1 using synthetic lignin. The results showed that isolated strain decreased the lignin color by up to 20% after cultivation for 7 days. The decolourisation ability of the fungi *Phanerocheate chrysosporium* and *Trametes hirsuta* was observed to be 80 and 70%, respectively[47-50]. The lignin degrading system of white-rot fungi consists of various extracellular enzymes such as laccases, peroxidases and oxidases[51]. They are able to degrade a wide range of organic pollutants e.g. phenolic compounds and synthetic dyes[47,52,53]. The ability of *P. chrysosporium* to degrade a lot of synthetic dyes has been reported[51]. The Funalia trogii ATCC decolorization

mechanisms involved a complicated interaction of biosorption and enzyme activity. The mycelium of *P. oxalicum* rapidly adsorbed RB-19 with high adsorption capacities. Almost up to 60% of dye removal was achieved in 10 min. and 91% in 80 min. The maximum adsorption capacities were found up to 160 mg g^{-1} at 20ºC, *Botrytis cinerea* decolorization mechanism involves biosorption and enzyme degradation[54]. In *Rhizopus arrhizus*, decolorization mechanism involves biosorption[55].

Dead cells

There are only limited studies on dye removal by dead fungal biomass[56-58]. These fungi, which can biosorb diverse dyes, include *Aspergillus niger*[59,60], *Rhizopus arrhizus* and *Rhizopus oryzae*[61], *Fomitopsis carnea* dead cells showed maximum biosorption[62].

Comparison between living cells and dead cells

Compared with live fungal cells, dead fungal biomass possesses various advantages such as absence of nutrient needs and ease of regeneration[63]. Dried, non-living and physically or chemically pretreated fungal biomass would be an attractive biosorbent for removing dyes from dye wastewaters.

Tatrako and Bumps[64] used both living and autoclaved cultures of *P. chrysosporium* to decolorize congo red and observed that that the autoclaved cells had a higher color removal (90%) than the living cells (70%). Living cells have a wide variety of decolorization mechanisms and the dead cells can be effective biosorbents.

Influencing factors

In the decolorization process by fungi (living or dead cells), there are various influencing factors. They can be grouped into two kinds: one is related to fungal growth conditions; the other is related to the characteristics of the dye solution or wastewater. Fungal treatment of textile dyes and effluents has been found to be influenced by temperature, pH, salts, inhibitory molecules (sulphur compounds, surfactants, heavy metals and bleaching chemicals), carbon and nitrogen sources and other nutrients[44]. So the optimization of these parameters is important to study the decolorization of different dyes by fungal isolates.

Fungal growth conditions

As different components possess different abilities to decolorize dyes, it is necessary to create an optimal environment favourable to fungal growth and thus, make the fungi

possess the maximum ability to decolorize dyes in wastewater. Important fungal growth conditions are as follows:

Medium

The fungal strains were used in decolorization experiments with different broth media. The results indicate that *A. nidulan* minimal medium was favorable for fungal growth and it proved to be the best for dye removal studies[65]. This medium has better decolorizing abilities, when compared to those that were taken from non-adapted cultures. Fungi are usually grown in pure nutrient media, without dyes or dye wastewater, to develop a biosorbent using dead fungal biomass. Zhou and Banks[66] studied *R. arrhizus* cultured on four different media: (i) yeast extract, malt extract, sucrose, and agar, (ii) yeast phosphate soluble starch, (iii) malt extract agar and (iv) potato dextrose. The fungal biomass grown in potato dextrose had the highest biosorption capacity for humic acid.

Nutrient concentrations

Baldrian[67] and Miranda *et al*.[68] reported that the highest color removal took place at 1 g K_2HPO_4/L; and 0.5 g $MgSO_4.7H_2O$/L for *A. niger*. Niladevi and Prema[69] obtained maximum decolorizing activity when copper sulphate was used at a concentration of 1mM. Copper sulphate also proved to be a promising inducer for enzyme production *as* similar to that in most of the fungi. It induces the enzymatic mineralization activity.

Ryu and Weon[70] investigated various organic or inorganic nitrogen sources to determine the most suitable nitrogen source for decolorization by *Aspergillus sojae* B-10. Sodium nitrate was the optimal nitrogen source and the highest color removal occurred with NH_4NO_3 at 1.8 g/L for *A. sojae* B-10. But Vasdev *et al.*[71] reported that nitrogen had no effect on decolorization of dyes by the birds-nest fungus *Cyathus bulleri.* Zhang *et al.*[72] investigated the effect of ammonium ion (NH^+_4) concentration on decolorization and observed that the decolorization rate with NH^+_4 was much lower than without the addition of NH^+_4. Tatarko and Bumpus[64] also reported that addition of suplemental nitrogen only inhibited decolorization of congo red in plates containing high amounts of nutrient nitrogen.

As decolorization of dyes by *P. chrysosporium* occurs in secondary metabolic conditions, the important enzyme lignin peroxidase (LiP) is released by fungal cells under either carbon or nitrogen limitation[57,73-75]. Five different nitrogen sources like yeast extract, peptone, beef extract, ammonium chloride and sodium nitrate were tested for extracellular protein and laccase production in *Ganoderma sp*. Yeast extract supported the maximum decolorization activity and maximum extra cellular protein content of 140 μg/mL. The most widely used nitrogen sources for fungal lignolytic enzyme production are ammonium salts

such as tartrate or chloride. Ammonium nitrate favored high laccase production in many white rot fungi, namely, *Basidiomycete* PM1, *Lentinula edodes*[76].

Six different carbon sources such as mannitol, maltose, sucrose, glucose, lactose and starch were tested at 2% for laccase production in *Ganoderma sp.* Among the carbon sources, starch supported a maximum decolorizing activity of laccase with a highest extracellular protein 140 μg/mL. The production of enzyme enhanced using various carbon sources such as mannitol, lactose, maltose, glucose, starch and sucrose. Among the six different carbon sources starch supported good growth and production. Kapdan and Kargi[43] reported that cultivation of *Coriolus versicolor* at 10 g/L glucose resulted in a better fungal growth.

Enhanced decolorization of dyes (50 mg/L) with pre grown fungal biomass of different fungi without additional carbon source might serve as a main carbon source for fungal metabolism[77]. Zhang *et. al.*[72], studied the carbon sources effective co-substrate on decolorization of cotton bleaching effluent by an unidentified white-rot fungus and reported that glucose, starch, maltose and cellobiose were good carbon sources while sucrose, lactose, xylan, xylose, methanol and glyoxal were poor carbon sources. It was also reported that glucose concentration was about 5 g/L. in decolorization of melanoidin by *Coriolus sp.* No. 20, the effective sugars were glucose and sorbose[77].

Miranda et al.[68] reported that using the crystallized sucrose at an initial concentration of 10 g/L produced a maximum color removal of 69%, but using molasses of 5 g/L equivalent sucrose only produced a color removal of 45% Potato Dexxtrose agar for *A. niger*. Belsare and Prasad[78] observed that the decolorization efficiencies of *Schizophyllum* commune with different carbon sources were: sucrose (60%), glucose (48%), cellulose (35%) and pulp (20%). *Myrothecium verrucaria* showed maximum decolorization when influenced by the glucose concentration[58].

pH

pH is an important factor for fungal growth. Fungi can grow at low pH, normally ranging from 4 to 5[79]. The dye decolorizing fungi grow and function best in the pH range of 4.5 to 6.5[65,80]. Higher uptake of dyes obtained at lower pH value may be due to the electrostatic attraction between negatively charged dye anions and positively charged cell surface. Whereas, in another reports, it have been reported that some fungi showed their activity in alkaline pH of 11.5, but, the major dye decolorization is done in the pH range of 4.5 to 6.5.[81,82]

Temperature

Dye removal was influenced by fluctuating temperatures. These results were similar with findings of various researchers[83-85], who explained that fungal growth was supported in a limited temperature range with dye removal. The optimum temperature range lies between 30 to 40°C. Chen et al.[86] reported that optimum temperature for color removal of red azo dye was 30 and 35°C.

Oxygen

Mou et al.[58] studied the effect of shaking and static incubation on decolorization and observed no adverse effect of shaking during the biodecolorization process. Yesilada et al.[45] also observed that there was not much difference in decolorization between static and agitated cultures. Belsare and Prasad[78] studied the effect of intermittent aeration three times a day on decolorization of the effluent. During aeration, dissolved oxygen (DO) ranged between 0.5 and 1.0 mg/L; prior to aeration, DO was zero. They observed that intermittent aeration for a period of three days stimulated lignin breakdown and that color removal was between 82% on the first day and 90% on third day. Soares and Duran[87] reported that agitation was essential for keeping a high rate of decolorization by *Trametes villosa*.

Consortium formation

Four predominant non-adapted fungal strains such as *Aspergillus sp*., *Penicillium sp*., *Fusarium sp*. and *Rhizopus sp*., having the ability of dye decolorization were isolated from different niche[88]. These fungal strains were used to develop consortium for bioremediation efficiency analysis on textile effluent. The *fusarium sp*. is not compatible in the consortium. On analyzing the individual and consortium of non-adapted fungal strains of the treatment trials, the consortium of fungal strains is found to be the very effective in its bioremediation ability. The consortium reduced the color up to 74% and reduces other solids upto 50%[89]. Three triphenyl methane dyes (Crystal violet, Bromophenol blue and Malachite green) were decolorized by six white rot fungi isolated from nature, were evaluated in liquid enrichment media. All the six fungi not only have decolorization capacity and were able to decolorize all three dyes within 72 hours; their growth was even not affected much by the presence of dyes in the medium. Three of these fungi were found to have capacity to decolorize as high as 6 g/L concentration of these dyes[90].

The first attempt on dye decolorization potentials of local microbial consortia isolated from dye contaminated soils in Ngaoundere-Cameroon was determined against two

azo dyes. Decolorization rate and kinetics were monitored by spectrophotometry under different conditions. Effect of process parameters: such as pH, dye concentration and inoculum size on dye decolourisation rate were studied. The process increased with increase in inoculum size, decreased with increase in dye concentration while it was high within a pH range of 7.2 to 8. The full factorial design gave an optimal decolorization of 98.85%, when the pH and the dye concentrations were kept low while the inoculums size was high. It can be concluded that adapted local microbial isolates from textile wastewater contaminated sites can effectively be used in the aerobic treatment of these effluents before discharging into the environment.

Two microbial cultures (Bacterial and fungal cultures) with high decolorization efficiencies of reactive dyes were obtained and were proved to be dominant with fungi consortium in which 21 fungal strains were isolated and 8 of them showed significant decolorization effect to reactive red M-3BE[28]. Higher glucose concentrations in the influents could significantly improve color removal, but was not helpful for dye mineralization. Besides reactive black 5, the bioreactor could effectively decolorize reactive red M-3BE, acid red 249 and real textile wastewater with efficiency of 65%, 94% and 89%, respectively.

Immobilization of microorganisms producing laccase

Immobilization of cells and enzyme also affects the dye degradation capacity. The enzyme immobilization is more efficient than the cell. The enzyme can be used for many times to decolorize the dye[91].

The immobilization of microorganisms can be defined as any technique that limits the free migration of cells. Basically, there are two types of cell immobilization: entrapment and attachment. In the first, the organisms are entrapped in the interstices of fibrous or porous materials or are physically restrained within or by a solid or porous matrix such as a stabilized gel or a membrane. In the latter, the microorganisms adhere to surfaces of other organisms by self-adhesion or chemical bonding. Natural polymers such as alginate, chitosan, chitin and cellulose derivatives have been mostly used as the matrix for the immobilization of cells via the entrapment technique[62], whereas the materials commonly employed for the attachment procedure have been synthetic foams like polyurethane foam[72] and nylon sponge[92]. Recently, both types of carrier materials have shown to be very appropriate for the immobilization of the white-rot fungus *Phanerochaete chrysosporium* in sugar refinery effluent treatment[93]. The selection of the immobilization technique as well as the immobilization material is essential to design an effective system to each particular purpose. Immobilization of the fungal mycelium is done to increase its efficiency of

degradation. The immobilization can be performed in different ways. The main problem with the fungal mycelium immobilization is that the dyes accumulate in the mycelium and block the functioning of the fungus and the extracellular enzyme production is inhibited[94].

Biocatalytic process economics can be enhanced by enzyme reuse and the improvement in enzyme stability afforded by immobilization. The capacity to retain or recover enzymes also allows biocatalyst separation from product; thereby, permitting continuous processes, and prevents carry-through of protein or activity to subsequent process steps[95]. Immobilization can also improve enzyme performance under optimal process reaction conditions (e.g. acidity, alkalinity, organic solvents, and elevated temperatures), a requirement that has often retarded enzyme application in industrial chemical synthesis[96]. In spite of the long history and obvious advantages of enzyme immobilization estimated that only 20% of biocatalytic processes involve immobilized enzymes[97,98]. However, over the last few years, a number of interesting new developments have been reported in the literature and patent applications, indicating that enzyme immobilization has entered an exciting new phase[99].

CONCLUSION

Based on this review, the following conclusions may be drawn -

(i) There are many fungal strains capable of decolorizing dye wastewater. There is a need to develop these fungal strains, which can grow in simple, inexpensive medium and have high production rate and possess high biosorption and degrading capacity.

(ii) Decolorization by living cells involves more complex mechanisms such as intracellular, extracellular oxidases and biosorption, than by dead cells. The process involving living cells is closely related to the operational conditions, such as nutritional requirements, the influent concentration and toxicity. In contrast, decolorization involving dead biomass is easier to operate and dead cells may possess higher biosorption capacity in certain conditions. They can be effective biosorbents.

(iii) The fungal biomass biosorbent can be eluted and regenerated by some organic solvents, surfactants and sodium hydroxide solution.

(iv) There are various factors influencing fungal decolorization related to fungal growth and the characteristics of dye wastewater.

(v) The fungal consortium formation improves the efficiency of dye decolorization of the dye wastewater.

(vi) Fungal decolorization is a promising alternative to replace or supplement present treatment processes. However, using fungal biomass to remove color in a dye wastewater is still in the research stage. More studies are needed to develop a practical application.

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Revised : 18.03.2013 *Accepted : 20.03.2013*