Bioremediation concepts for treatment of dye containing wastewater: A review

Haresh Keharia & Datta Madamwar*

Post Graduate Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, India

Synthetic dyes are extensively used in wide range of industries amongst which textile processing industries are the major consumers. Large amounts of dyes are lost in wastewaters of these industries during dyeing and subsequent washing steps of textiles. These dyes are resistant to degradation by conventional wastewater treatment plants and are released into environment untreated thus causing pollution of surface and ground waters in the areas of the world harboring such industries. Presence of color in wastewaters has become major environmental concern and stringent discharge standards are being enforced on release of colored wastewaters in environment. The seriousness of the problem is apparent from the magnitude of the research done in this field in last decade. Increasing number of microorganisms are being described for their ability to decolorize and degrade artificial dyes and novel bioremediation approaches for treatment dye bearing wastewaters are being worked out. In this review we have investigated potential microbial processes for developing feasible remediation technology to combat environmental pollution due to dye bearing wastewaters.

Keywords: Biological treatment, Degradation of textile dyes, Dyes in wastewater, Environmental pollution, Textile waste

Around 10,000 different dyes with an annual production of more than \(7 \times 10^5\) metric tones worldwide are commercially available and are extensively used in textile processing, paper printing, pharmaceutical, food and other industries\(^1\). Amongst various applications of synthetic dyes about 300,000 tons of different dyestuffs are used per year for textile dyeing operations, thus dye houses are the major consumers of synthetic dyes and consequently are the major cause of the water pollution\(^1\).

During textile processing, inefficiencies in dyeing results in large amounts of dyestuff being directly lost to the wastewater, which ultimately finds its way to the environment. These dyes are highly resistant to microbial degradation under aerobic conditions normally found in wastewater treatment plants. This is because dyestuffs are designed to resist chemical fading and light induced oxidative fading\(^2\). Other factors involved in reducing biological degradation of dyes include properties such as high water solubility, high molecular weight and fused aromatic ring structures, which inhibit permeation through biological cell membranes. Textile dyes are classified as azo, diazo, cationic, basic, anthraquinone based, metal complex dyes based on the nature of their chemical structure. Further, depending on their application dyes are classified as reactive, disperse, vat and mordant etc. Amongst all reactive azo dyes are most problematic due to their excess consumption and high water solubility. Furthermore, the sulfonic acid and the azo group are rare amongst natural products and thus both confer xenobiotic character on this class of compounds\(^3\). Anthraquinone based dyes are most resistant to bacterial degradation due to their fused aromatic structures, which remain colored for long periods of time. Basic dyes have high brilliance and therefore 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, which is carcinogenic in nature, into water supplies\(^4\). Algae and higher plants exposed to dye containing effluents have been shown to accumulate high concentrations of certain disperse dyes and heavy metals\(^5\).

The recalcitrance of the dyestuff to biodegradation is apparent from the fact that these compounds escape various stages of wastewater treatment plants and finally enter into environment. As a result rivers and ground waters in those areas of the world with high concentration of dyeing industries suffer severe color contamination\(^6\). The presence of even a small fraction of dye in water (less than 1 ppm for some dyes) is highly visible due to the color and affects the aesthetic merit of the streams and other water resources. It also interferes with the penetration of light in water bodies and may affect the aquatic biota. Some of the dyes
and/or their degradation products have proved to be toxic, mutagenic and/or carcinogenic in nature. Thus removal of dyes from effluents has been given utmost importance.

The environmental issues regarding the presence of color in wastewaters has become a major survival problem for dyestuff manufacturers, dyers, textile finishers and sewers due to stringent color consent standards being enforced by regulatory bodies for release of treated effluents in the ecosystem. Scientists and industrialists have been researching new technologies to remove color from solution for over four decades, and many solutions have been proposed. However, very few of these techniques are used in water treatment works thus reflecting the complexity of the problem.

Numerous physical and chemical techniques based on coagulation-flocculation, precipitation, membrane filtration, ion-exchange, electrochemical destruction, photochemical degradation, ozonation and adsorption are available for removal of color from textile wastewaters. The majority of color removal techniques work either by concentrating the color into a sludge, or by partial to complete breakdown of the colored molecule. Although some of the physicochemical processes have been shown to be effective, their application is limited due to excess usage of chemicals, sludge generation with subsequent disposal problems, high installation as well as operating costs and sensitivity to a variable wastewater input.

Bioremediation constitutes an attractive alternative to physicochemical methods of remediation, mainly due to its reputation as low-cost, eco-friendly and publicly acceptable treatment technology. A large number of microorganisms have been isolated in recent years that are able to degrade dyes previously considered non-degradable. This suggests that, under selective pressure of environmental pollution, a microbial capacity for the degradation of recalcitrant textile dyes is developing that might be harnessed for dye removal by biotechnological processes. Biological treatment for decolorization of textile effluents may be either aerobic, anaerobic or combination of both depending on the type of microorganisms being employed.

Aerobic decolorization and degradation of textile dyes

Aerobic biological treatment of color in dye house effluents is extremely inefficient for two reasons.

Firstly, the dyes are highly stable to biological oxidation; indeed they are designed to be oxidatively stable. The second reason is the frequently poor adsorption of dyes onto the activated sewage sludge. Highly water-soluble anionic dyes have been reported to pass directly through an aerobic biological treatment plants. It was found in a study on bioelimination of various dyes that all the three anionic water soluble reactive dyes (CI Reactive Violet 15; Reactive Blue 19 and Reactive Black 5) were neither removed or biodegraded by activated sewage sludge even after 20 days of incubation. Similar findings have been reported for other sulphonated water soluble anionic dyes. Nonetheless various aerobic fungi and bacteria capable of aerobic oxidation of dyes have been described. Majority of the fungi belonging to white rot group have been shown to degrade wide variety of dyes. The dye decolorizing activity of these fungi in most cases has been correlated to their ability to produce ligninolytic enzymes such as laccase, lignin peroxidase, manganese peroxidase and manganese independent peroxidases, which exhibit broad substrate specificity. One of the most studied white rot fungus is Phanerochaete chrysosporium, which has been shown to degrade large spectrum of azo, anthraquinone and triphenylmethane dyes, with decolorization efficiencies exceeding 90% in most cases. Dyes having hydroxyl, amino, acetamido or nitro functional groups substituted in their aromatic groups are more susceptible to degradation by P. chrysosporium. Other white rot fungi such as Geotrichum candidum, Trametes versicolor, T. modesta, T. pocus, C. citrinula Citocybulla duseni, Pleurotus ostreatus, Bjerkandera adusta and Pycnoporus sinnabarinus, Dattronia concentria have also been shown to degrade industrially relevant complex azo dyes.

Application of white rot fungi for treatment of textile and dyestuff industrial wastewaters has been worked out based on two strategies: (1) use of the dye degrading enzymes extracted from the culture medium and (2) use of whole cell active cultures directly for transformation of dyes. The use of enzymes for dye decolorization offers higher stability towards presence of growth inhibitory chemicals as well as variable wastewater composition that may affect the growth of fungi, e.g. high salinity. Based on this approach Abadulla et al. demonstrated use of immobilized laccase for decolorization and detoxification of textile dyes. The toxicity of dyes was found...
to be reduced by 80%. The reuse of textile effluents, decolorized with immobilized laccase, for dyeing could be successfully demonstrated. Thus a closed-loop technology with reuse of enzymatically treated dyeing effluent to reduce water consumption was proposed. Recently López and coworkers reviewed and demonstrated application of enzymatic membrane reactors for treatment of dye containing wastewaters. However, the main disadvantage is the higher costs resulting from the need for extraction and cleaning steps. The process based on production of ligninolytic enzymes by solid state fermentation of neem hulls (an agrowaste) and its use in decolorization of synthetic dye solution has been reported with a view to cut down the cost involved in enzyme production.

Different reactor configurations have been tested for application of white rot fungi in treatment of dye bearing wastewaters, including stirred tank reactors, packed bed reactors, fluidized bed reactors, and rotating disk reactors. Kirby and coworkers investigated the remediation of actual textile effluent by \textit{P. chrysosporium} using rotating tube bioreactor system. The reactor configuration was found to be robust enough to continuously decolorize three different mixed azo dye effluents by greater than 90% over a 38 day operating period.

Amongst various configurations fed batch fluidized bioreactor was found to be most efficient and showed 97% color removal at dye (Orange II) loading rate of 1000 mg L\(^{-1}\) d\(^{-1}\). Our work also justified suitability of fluidized bed bioreactor, employing mycelial beads of \textit{T. versicolor} for decolorization of sulfonated azo dyes. The reactor performance was found to be highly stable when operated continuously with over 95% dye removal efficiency at dye loading rates of 100 mg L\(^{-1}\) d\(^{-1}\). The application of white rot fungi in wastewater treatment plants still suffers a severe set back due number of reasons: (1) white rot fungi are not the normal inhabitants of wastewater treatment plants and may not be able to survive competition by other fast growing microorganisms, (2) degradation rates are quite low and (3) optimal pH for growth and degradation is found to be between 4.5 and 5.0 for most white rot fungi, which may be unfavourable for other desirable microorganisms. These crucial problems need to be solved in order to develop a economically feasible treatment process using white rot fungi.

Aerobic bacteria belonging to \textit{Streptomyces} species and a \textit{Flavobacterium} ATCC 39723 also produce extracellular peroxidases having ability to degrade various xenobiotic compounds including dyestuffs. Several bacteria including \textit{Citrobacter sp.}, \textit{Kurthia sp.}, \textit{Corynebacterium} and \textit{Mycochromobacterium} sp., and a mixed culture consisting of \textit{Pseudomonas mendocina} and \textit{Pseudomonas alcaligenes} has been shown to degrade triphenyl methane dyes. Recently a strain of yeast \textit{Candida zeylanoides} was shown to reductively decolorize azo dyes under aerobic conditions. Also a \textit{Bacillus sp.} was isolated for treatment of printing ink wastewater and its commercial significance was demonstrated by developing a novel internal airlift loop bioreactor using bacteria immobilized on ceramic honeycomb support. However, except for white rot fungi, \textit{Streptomyces} species and a \textit{Flavobacterium} sp., azo dyes are generally recalcitrant to degradation by aerobic microorganisms. In many reports on the aerobic metabolism of azo dyes, the bacterial strains (e.g. \textit{Aeromonas sp.}, \textit{Bacillus subtilis}, \textit{Proteus mirabilis}, \textit{Pseudomonas pseudomallei} 13NA and \textit{Pseudomonas luteola}) were grown aerobically with complex media or sugars and then incubated under static conditions in the presence of different azo dyes. These static cultures presumably become rapidly oxygen depleted and the reactions observed should therefore be viewed as an anaerobic decolorization of azo dyes.

**Anaerobic decolorization and degradation of textile dyes**

Under anaerobic conditions many bacteria have been reported to readily decolorize azo-dyes. Azo dye reduction is an ubiquitous capacity of most anaerobic microorganisms. The main interest in this field has been focused on bacteria from human intestine that are involved in the metabolism of azo dyes ingested as food additives. Baughmann and Weber (1994) demonstrated that in anoxic sediment environments uncharged azo dyes readily undergo biologically mediated reduction to the corresponding amines. It appears that almost every compound that has been tested is biologically reduced under anaerobic conditions, although there are some reports that metal-ion containing dyes are less susceptible. Application of various anaerobic processes has been claimed for treatment of dye containing wastewaters. Most investigators have applied up-flow anaerobic sludge blanket (UASB) reactor for investigating treatment of dye containing wastewaters. UASB offers various advantages such as allows retention of high concentration of active microbial biomass.
allowing very high organic loading rates and excellent COD removal, however development of granular sludge requires a long time and is difficult which is main limiting factor in its successful field application.

Keharia developed an up-flow anaerobic fixed film bioreactor using bonechar as a support matrix and cattle dung slurry as a source of anaerobic bacteria for decolorization of reactive dyestuff industrial effluent. Average color removal and COD removal efficiency was found to be 70% and 50% respectively at organic loading rate of 7.88 Kg COD m^-3 d^-1. The main advantage of fixed film bioreactor is that it allows retention of active biomass in form of a biofilm attached to the support media without recirculation of biomass or addition of fresh biomass during operation of the reactor thus offering efficient mass transfer and waste stabilization.

Fed-batch processes using Pseudomonas luteola was shown to be effectively decolorize Reactive Red 22 dye. The culture was aerated and agitated during growth phase and then shifted to gentle agitation with no aeration during decolorization phase. The yeast extract to dye ratio of 0.5 was found to be critical for dye decolorization to occur.

A combination of dye adsorption on cellulose anion exchange resin and anaerobic dye reduction was demonstrated for effective removal of anionic dyes from wastewater. Anionic dyes have strong affinity for cellulose or lignocellulosic ion exchangers, which makes subsequent regeneration of adsorbent bed difficult. The adsorbed dye can be reduced by chemical methods which would be uneconomical. Instead effective regeneration of dye-adsorbed resin by anaerobic reduction process using cells of Burkholderia cepacia was demonstrated. The bacterial cells (1x 10^10) were filled in dialysis tubing and suspended in a large column containing glucose mineral medium along with 1 mM of sodium anthraquinone-2-sulfonate (AQS), which was connected to a small column loaded with a MOPS buffer washed Quaternary ammonium cellulose. The resin was then loaded with Remazol Red R3B dye. The medium from the large column after about 12 hr of incubation was then pumped through the dye loaded resin, which resulted in 80% dye reduction within 50 min, demonstrating the continuous use of the resin bed for dye adsorption with periodic brief interruptions for a regeneration cycle involving the passing of microbially reduced AQS. The AQS served as electron shuttle between bacterium and dye thus eliminating the requirement of having microorganisms compatible with variety of dyes typically present in textile wastewaters.

Bacteria under anaerobic conditions mediate decolorization of azo dyes by causing reductive cleavage of azo linkage. The process of azo bond reduction is catalyzed by a variety of soluble nonspecific cytoplasmic enzymes, known as azo reductases. These azoreductases facilitate the transfer of electrons via soluble flavins or other redox mediators to the azo dye, which is then reduced. The role of cytoplasmic azo reductases in dye reduction in vivo is uncertain because transport of polymeric dye molecule or highly charged sulfonated azo dyes across cytoplasmic membrane is unlikely. Probably reduced flavins that are products of cytoplasmic flavin reductases may function as intermediates in azo dye degradation. It is also possible that reduced inorganic compounds such as Fe^2+, H_2S etc. that are formed extracellularly as end products of certain strictly anaerobic bacterial metabolism may cause azo dye reduction.

In addition to azo dyes bacterial metabolism of other dye molecules under anoxic conditions has also been studied. Henderson et al. demonstrated ability of range of aerobic bacterial cultures as well as consortia of microbes from human, mouse, and rat intestines to decolorize triphenylmethane dyes using malachite green as a model compound. The metabolite produced leuco malachite green, is a suspected carcinogen and raises concerns over wide use of malachite green for various purposes worldwide. Gonçalves et al. (2000) investigated anaerobic degradation of anthraquinone dyes and were unsuccessful in decolorizing anthraquinone based disperse dye, CI Disperse Blue 56 even at 35 mg l^-1.

The anaerobic process offer several advantages in comparison to aerobic ones in terms of no aeration requirements, low sludge generation and methane gas production. However the main limitation is that azo dye reduction under anaerobic conditions leads to the formation of colorless aromatic amines, which are generally recalcitrant to further anaerobic degradation. The amino groups concentrate electrons into the ring, which greatly interferes with the nucleophilic strategies of aromatic compound degradation used by anaerobic microorganisms. Nonetheless evidence is accumulating that some aromatic amines are mineralized by anaerobic microorganisms and consortia. Aromatic amines with carboxy, hydroxy, and methoxy substitutions are potentially mineralizable in methanogenic consortia. Razo-Flores et al. for
the first time demonstrated complete mineralization of an azo dye Azodesalicylate under anaerobic conditions. Donlon et al.\textsuperscript{77} reported partial mineralisation of the azo dye Mordant Orange I by a methanogenic granular sludge in a continuous-upflow anaerobic sludge blanket reactor. In this study, however, complete mineralisation of only one of the azo cleavage products, 5-aminosalicyclic acid, was possible, with other product (1,4-phenylenediamine) accumulating in the reactor.

Generally aromatic amines that are not mineralized under anaerobic conditions accumulate in such environments and pose a major threat to human health and environment because of their well-documented toxic, mutagenic and/or carcinogenic activity\textsuperscript{85,77}.

### Anaerobic/aerobic treatment of azo dyes

Whilst anaerobic decolorization of azo dyes occurs easily, complete mineralization of molecule is difficult and generally ends up in accumulation of aromatic amines\textsuperscript{69}. With exception of few aromatic amines, most are generally recalcitrant to anaerobic degradation and pose a threat to human and animal health as their toxic, mutagenic and/or carcinogenic nature is well documented\textsuperscript{69}. Aromatic amines and also sulfonated aminoaromatics, which are recalcitrant to anaerobic degradation, are easily degraded by aerobic bacteria\textsuperscript{62,85}. Thus aerobic treatment after anaerobic dye reduction would result in complete mineralization of dyestuffs in textile wastewaters and the feasibility of this strategy was first demonstrated for the sulfonated azo dye Mordant Yellow 3\textsuperscript{57,86,89}. Two-stage treatment process have been used by several workers wherein during first anaerobic stage of treatment process, azo dye is readily reduced to corresponding amines, which are then metabolized relatively easily under aerobic conditions\textsuperscript{57,86,89}.

The anaerobic/aerobic treatment can be carried out either sequentially or simultaneously. Sequential processes may combine the anaerobic and the aerobic step either alternatively in the same reaction vessel or in a continuous system in separate vessels. A sequencing batch reactor namely the "anaerobic+aerobic" system was used to demonstrate decolorization of Reactive Turquoise Blue (RTB) containing synthetic dye wastewater. The anaerobic and aerobic phases were either performed alternatively in the same reactor (system 1) or separately in two different reactors (system 2). The overall RTB removal efficiency for system 2 (80.8%) was higher than that for system 1 (66%). The differences of the two systems in RTB removal may be due to their dissimilar microorganism compositions and different process conditions. System 1 was an anaerobic-aerobic alternative system, where aerobic and anaerobic processes were in turn operated in the same reactor which as inoculated with 1:1 mixture of anaerobic and aerobic activated sludge. System 2 was an anaerobic-aerobic tandem system, where the anaerobic and aerobic processes were operated in two reactors that were separately inoculated with anaerobic and aerobic microorganisms and appropriate growth conditions were maintained. This led to development of specific stable microbial communities in each reactor, which enabled both anaerobic and aerobic phases of the system 2 to play marked roles in RTB removal\textsuperscript{57}.

Similar observations have been made by Wen and coworkers\textsuperscript{77} while investigating long term performance of two-stage (anaerobic-aerobic) sequencing batch reactors for treatment of RTB containing wastewaters. Overall dye removal rates were found to be 81% and 92.5% after anaerobic and aerobic stages.

Simultaneous treatment systems utilize anaerobic zones within basically aerobic bulk phases such as observed in biofilms, granular sludge or biomass immobilized in other matrices. Tan et al.\textsuperscript{29} demonstrated degradation of Mordant Orange I by methanogenic granular sludge exposed to oxygen utilizing ethanol or acetate as co-substrates. They suggested that co-substrates stimulate oxygen respiration, which lowers oxygen penetration into the biofilm and thereby creates anaerobic microniches where azo dye reduction can occur. In another simultaneous anaerobic/aerobic treatment system two bacterial cultures were immobilized in calcium alginate beads. One bacterial strain caused the reduction of Mordant Yellow 3 in the anaerobic zones leading to the formation of 6-aminonaphthalene-2-sulfonate (6-ANS) and 5-aminosalicyclic acid (5-ASA). Subsequently, the same strain could degrade 6-ANS to 5-ASA and the second strain could mineralize 5-ASA in the aerobic zones\textsuperscript{67}.

In the sequential and simultaneous treatment systems, auxiliary substrates are required, which supply the bacteria in the anaerobic zones with a source of carbon and energy and a source of reduction equivalents for the cleavage of the azo bond. Although at least certain azo dyes can be mineralized by anaerobic/aerobic treatment systems,
However it has a serious limitation. Many of the aromatic amines, which are formed during the anaerobic reduction of the azo dyes, are rather unstable under aerobic conditions and undergo auto-oxidation reactions is a major drawback if a true mineralization of azo dyes is intended.

Thus, a single step treatment process would be ineffective for remediation textile and dyestuff industry wastewater and a combination of more than one process may be useful. This is because the composition of textile dyeing industry is highly variable as more than one dyes may be used in single dyeing operation in order to achieve desired color shade as well as dyeing depends on the fashion profiles and thus keeps changing. In general sequential anaerobic-aerobic process seems to be more practicable since during initial anaerobic stage, most azo dyes get decolorized producing aromatic amines which along with anthraquinone and triphenylmethane dyes would get degraded during subsequent aerobic treatment process.

References


KEHARIA & MADAMWAR: BIOREメディAITION OF DYE CONTAINING WASTEWATER


