

Biotechnological Approaches To Combat Textile Effluents

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Abstract

The treatment of textile effluents is of interest due to their toxic and esthetic impacts on receiving waters. While much research has been preformed to develop effective treatment technologies for wastewaters containing azo dyes, no single solution has been satisfactory for remediating the broad diversity of textile wastes. To ensure the safety of effluents, proper technologies need to be used by treatment facilities when degrading fiber-reactive azo dyes. Previous research efforts have focused on various biological, chemical, and physical techniques for treating azo dye wastes. There is evidence that all three areas have potential for remediating dyehouse wastes. However, chemical treatment is often cost and application limited, while physical removal can lead to extra solid wastes and increased overhead. A very promising area for removing unwanted color from textile wastewater is biotreatment that is targeted at breaking down the dye molecules to basic elements (mineralizing them), and doing so in a way that has much less potential for environmental impact than conventional methods.

INTRODUCTION

Textile and dyeing industry is one of the most important industries and it consumes substantial volume of water and chemicals for wet processing. During textile processing, inefficiencies in dyeing results in large amount of dye being lost to the waste water which ultimately finds its way into the environment. The amount of dye lost is dependent upon the type of dye application varying from only 2% loss when using basic dyes to a 50% loss when certain reactive dyes are used (O'Neill et al., 1999).

The treatment of textile effluents is of interest due to their toxic and esthetic impacts on receiving waters. While much research has been preformed to develop effective treatment technologies for wastewaters containing azo dyes, no single solution has been satisfactory for remediating the broad diversity of textile wastes. Human and ecological health concerns have prompted the government to require textile effluent discharges to have increasingly lower color and nitrogen levels. Despite being aware of the problem, many textile manufactures have failed to adequately remove azo dye compounds from their wastewaters. Until dye and textile manufactures are able to develop efficient technologies, allowing for increased dye- fiber bonding and lower dyehouse losses (Lewis, 1999), the problem of treating these types of wastes will fall to the wastewater treatment facilities.

To ensure the safety of effluents, proper technologies need to be used by treatment facilities when degrading fiber-reactive azo dyes. Previous research efforts have focused on various biological, chemical, and physical techniques for treating azo dye wastes. There is evidence that all three areas have potential for remediating dyehouse wastes. However, chemical treatment is often cost and application limited, while physical removal can lead to extra solid wastes and increased overhead. Biological treatment has been effective in reducing dyehouse effluents, and when used properly has a lower operating cost than other remediation processes. Combinations of chemical and biological or physical and biological treatment have also proven to be effective. A very promising area for removing unwanted color from textile wastewater is biotreatment that is targeted at breaking down the dye molecules to basic elements, and doing so in a way that has much less potential for environmental impact than conventional methods. The biological process is reported to be the possible solution due to its low-cost, environmentally friendly and publicly accepted technology.

AZO DYES AND INTERMEDIATES

Azo dyes, which account for approximately one-half of all known dyes, are commonly used as coloring agents in the food, pharmaceutical, and textile industries. As a result, they are the most common synthetic colorants released into the environment. Because they are highly colored, azo dyes are readily visible in effluent water and can be the focus of

significant environmental complaints centered on that visibility. These compounds are also of concern because some of the dyes, dye precursors or their biotransformation products, such as aromatic amines, have been shown to be carcinogenic (Razo-Flores et al., 1997).

Azo dyes contain at least one nitrogen-nitrogen (N=N) double bond, however many different structures are possible (Zollinger, 1991). Monoazo dyes have only one N=N double bond, while diazo and triazo dyes contain two and three N=N double bonds, respectively. The azo groups are generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocycles or enolizable aliphatic groups (Zollinger, 1991). These side groups are necessary for imparting the color of the dye, with many different shades and intensities being possible (McCurdy, 1991). When describing a dye molecule, nucleophiles is referred to as auxochromes, while the aromatic groups are called chromophores. Together, the dye molecule is often described as a chromogen. The absorption and reflection of visible and UV irradiation is ultimately responsible for the observed color of the dye (Zollinger, 1991).

Synthesis of most azo dyes involves diazotization of a primary aromatic amine, followed by coupling with one or more nucleophiles. Amino- and hydroxy- groups are commonly used coupling components (Zollinger, 1991). Because of the diversity of dye components available for synthesis, a large number of structurally different azo dyes exist and are used in industry (McCurdy, 1991). World wide production of organic dyes is currently estimated at nearly 450,000 tons, with 50,000 tons being lost in effluents during application and manufacture (Lewis, 1999).

Fiber-reactive azo dyes exhibit a high wet- fastness, due to their ability to covalently bond to substrates. However, dyes that hydrolyze in solution prior to bonding to a substrate are often lost in the washing processes (Loyd, 1992). The bridging group serves to combine the chromogen with the reactive group of the dye molecule. The bridging group must be stable, soluble in water, and exhibit a certain degree of flexibility. Amino and alkylamino groups are generally used for this purpose. The reactive group serves to bond the dye molecule to a substrate via nucleophilic substitution or addition. Mono-, di-, and trichlorotriazinyl are all examples of reactive functional groups.

TOXICITY CONSIDERATIONS

The potential for toxic effects to the environment and humans, resulting from the exposure to dyes and dye

metabolites, is not a new concern. As early as 1895 increased rates in bladder cancer were observed in workers involved in dye manufacturing (Rehn, 1895). Since that time, many studies have been conducted showing the toxic potential of azo dyes. As mentioned previously, azo dyes are primarily composed of aromatic amines. Substituted benzene and naphthalene rings are common constituents of azo dyes, and have been identified as potentially carcinogenic agents (IARC, 1982). Further examples of toxic aromatic amines, which could be created from the degradation of azo dye compounds is shown in the table-1 (Cartwright R.A., 1983).

Figure 1

Table-1: Toxic aromatic amines created from azo dyes

AROMATIC AMINE GROUP	HUMAN CARCINOGEN EVIDENCE (Cartwright R.A., 1983)
1-Naphthylamine	Slight/ Mixed
2-Naphthylamine	Good
2,5-Diaminotoluene	Slight
3,3'-Dichlorobenzidine	Slight/ Mixed
3,3'-Dimethoxybenzidine	Slight/ Mixed
3,3'-Dimethylbenzidine	Slight
4-Biphenylamine	Good
4-Nitrobiphenyl	Slight/ Mixed
4,4'-Methylenebis(2-chloroaniline)	Slight
Auramine	Slight
Benidine	Good
Magenta	Slight
N-Phenyl-2-naphthylamine	Slight
N,N-Bis(2-chloroethyl)-naphthylamine	Good

BIODEGRADATION - ANAEROBIC TREATMENT

Anaerobic reduction of azo dyes using microbial sludges can be an effective and economic treatment process for removing color from dyehouse effluents. Although this effectively alters the chromogen and destroys the observed color of the dye, many aromatic groups are not susceptible to anaerobic reduction. However, there is evidence that some azo dye metabolites may be fully stabilized in anaerobic environments (Razo-Flores, 1997; Weber et al., 1987).

Chung et al., (1978) conducted a study measuring the degradability of seven azo dyes using intestinal and other major anaerobes. The studies were carried out using isolated strains of bacterium in suspended cell mediums containing the different azo dyes. Although the dyes studied were not fiber-reactive dyes, their findings showed that the reduction of azo compounds could be accomplished by intestinal and

other major anaerobes. Furthermore, the presence of aromatic intermediates was also detected in measurable amount for each dye and some of the intermediates had been previously determined to be mutagenic.

In a three-part research series, Brown et al. (1983, 1987) studied the degradability of various azo dyes in both anaerobic and aerobic systems. In the first study, Brown and Laboureur, (1983a) investigated the anaerobic degradability of 22 commercial dyes. Of the dyes studied, four monoazo and six diazo dyes showed substantial biodegradation, while two polyazo dyes showed moderate to variable reductions.

Later in 1987, Brown and Hamburger conducted a study on 14 azo dyes subjected to anaerobic sludge digestion followed by aerobic treatment. Brown and Hamburger's results confirmed the findings from earlier research, showing decolorization of the azo dyes.

Razo-Flores et al., (1997) investigated the fate of Mordant Orange 1 (MO1) and Azodisalicylate (ADS) under methanogenic conditions using continuous upflow-anaerobic-sludge-blanket (UASB) reactors. Their research focused on the reduction by-products, 5-aminosalicylic acid (5-ASA) and 1,4-phenylenediamine. Co-substrates, glucose were also fed to the reactors in order to supply the reducing equivalents needed for the reduction of the azo bonds. The results of this study demonstrated the ability of an anaerobic consortium to completely mineralize some azo dye compounds.

Chinwetkitvanich et al., (2000) performed a study on various reactive dyebath effluents. The research examined the effect of co-substrate and initial color concentrations on fiber-reactive dye reduction efficiencies in UASB reactors. Five different experiments were conducted using a variation of red, blue, and black dye synthetic wastewaters and also real dyehouse effluents composed of red, blue, and black dyes. Their results showed that by adding a co-substrate, such as tapioca, increased reduction efficiencies could be achieved. However, at high levels of tapioca addition no enhancement was observed. Furthermore, Chinwetkitvanich et al., (2000) concluded that higher initial color concentrations might be deleterious to acid forming bacteria, resulting in a lower dye removal. Additionally, the authors suggest that sulfate-reducing bacteria might out-compete other anaerobic microorganisms for available organic carbon, but contribute minimally to decolorization. This could serve to limit the reduction equivalents necessary for dye degradation.

AEROBIC TREATMENT

Conventional activated sludge treatment of wastes is often an effective and highly economic system for reducing organic pollutants in wastewater. A fair amount of research has been conducted assessing the viability of using activated sludge to treat textile effluents (Zissi et al., 1997; Loyd and Chapman, 1992; Shaul et al., 1991). However, aerobic treatment of azo dye wastes has proven ineffective in most cases, but is often the typical method of treatment used today (Yang et al., 1998). Because aerobic microbes cannot reduce azo linkages, their ability to destroy dye chromogens is less than anaerobic bacterium. However, aerobic sludges have been successfully used to stabilize dye metabolites (Brown and Laboureur, 1983b).

The aerobic biodegradability of aniline, o-toluidine, p-anisidine, p-phenetidine, o-dianisidine, and 3,3'-dichlorobenzidine, was investigated by Brown and Laboureur (1983b). These compounds are all lipophilic aromatic amines and possible by-products of azo dyes. Because many aromatic structures are nonbiodegradable in anaerobic environments and are not hydrophilic, they can accumulate in the adipose tissues of organisms. Many aromatics have been identified as possible carcinogens, which make their release into the environment a concern. Previous work by Brown and Laboureur (1983a) indicated that azo dyes may be broken down to their intermediate structures in a reductive environment, but were not amenable to further degradation by anaerobes.

Brown and Laboureur (1983b) concluded that aniline, p-anisidine, p-phenetidine and o-toluidine were readily biodegradable by aerobes, while o-dianisidine and 3,3'-dichlorobenzidine were inherently biodegradable. They suggested that these compounds could be stabilized if released into the environment or directly from a dyehouse into a conventional wastewater treatment plant.

Zissi et al., (1997) investigated the biological oxidation of p-aminoazobenzene (pAAB) by *Bacillus subtilis*. This was carried out in batch experiments using a suspension medium supplemented with glucose, ammonium chloride, and pAAB under sterile conditions. Cellular growth rates and inhibition, glucose utilization, pAAB degradation, and by-product formation were observed. The results proved that *Bacillus subtilis* could cometabolize pAAB in the presence of glucose, breaking the N=N double bond and producing aniline and p-phenylenediamine. Furthermore, evidence was found that suggested pAAB was inhibitory to microbial

growth, and that glucose was the growth-limiting substrate. The degradation of the dye was the direct result of an oxygen-insensitive azo reductase enzyme found to be present in the soluble fraction of the biomass. This enzyme was also synthesized independently of the presence of pAAB.

The majority of previous research suggests that aerobic biodegradation of most azo dyes is not effective. While there are certainly exceptions to the case, it would appear that conventional activated sludge systems are not adequate for treating azo dye wastewaters. Evidence does show that the aerobic biodegradation of azo dye intermediates is possible and is perhaps an effective treatment process for stabilizing these compounds after anaerobic reduction.

WHITE ROT FUNGI

Various investigations have shown that white rot fungi can efficiently degrade various organic pollutants other than lignin. Examples include polycyclic aromatic hydrocarbons, persistent environmental pollutants such as DDT (Bumpus et al., 1985, Bumpus, 1989), alkyl halide insecticides (Kennedy et al., 1990), xenobiotic compounds (Paszczynski et al., 1995), and dinitrotoluene (Valli et al., 1992).

The similarities in the molecular structures of the above compounds to those of dyes were obvious to the early investigators. This initiated interest in using the fungi for decolorization of dye wastewater. This application to the decolorization of azo dyes using the white rot fungus *Phanerochaete chrysosporium* was first described by Cripps et al., 1990. They showed that three azo dyes, Orange II, Tropaeolin O, and Congo red, could be decolorized by *Phanerochaete chrysosporium*. Other white rot fungi, such as *Bjerkandera adusta* and *Trametes versicolor* also showed the ability to degrade azo dyes efficiently (Heinfling et al., 1997). This and other work showed that *Pleurotus ostreatus*, had high potential for decolorizing a variety of azo dyes (Cao, 2000, Shin et al., 1998). Despite the known ability of the fungi to break down dyes and render them colorless, there have been few attempts to comprehensively examine the dye effluent systems after biotreatment by white rot fungi and determine the molecular nature of the process. Decolorization, by itself, demonstrates only the transformation of the chromophoric group of a dye, but does not reveal much about the mode of degradation of the dye molecules.

FACTORS AFFECTING DYE BIODEGRADATION

Due to the highly variable nature of biological treatment systems and especially textile effluents, there are a number of factors that may affect the biodegradation rate of azo dyes. Non-dye related parameters such as temperature, pH, dissolved oxygen or nitrate concentrations, type and source of reduction equivalents, bacteria consortium, and cell permeability can all affect the biodegradation of azo dyes and textile effluents. Dye related parameters such as class and type of azo dye (i.e. reactive-monoazo), reduction metabolites, dye concentration, dye side-groups, and organic dye additives could also affect the biodegradability of azo dye wastewaters.

Wuhrmann et al., (1980) investigated the effects of pH, temperature, type and concentration of respiration substrates, and oxygen tension on the rate of biological reduction of a variety of azo dyes. A consortium of microbes was used, including *Bacillus cereus*, *Sphaerotilus natans* and two others isolated from sewage-activated sludge. Also, activated sludge was used in experiments with mixed biocenoses. Temperatures, which are too high or too low, can result in the exclusion of a particular group of microorganisms. Using activated sludge, Wuhrmann et al., (1980) determined that temperature has an increasing linear relationship with the reduction rate of Orange II and Lanasyll violet up to 28 °C. In general however, most studies have been conducted at set temperatures, offering minimal data on temperature effects.

The wastewater pH can affect the proper functioning of both anaerobic and aerobic organisms (Grady et al., 1999). Wuhrmann et al., (1980) also investigated the effect of pH on dye reduction rates, but were unable to conclusively establish a relationship. However, they did state that an exponential increase in the decolorization rate was observed by decreasing the pH, but this relationship depended on the dye being tested. Loyd, (1992) observed an indirect increase in the rate of decolorization of Navy- 106, with decreased pH values in anaerobic batch tests.

A final non-dye related factor is the cell permeability and the cell wall adsorption of azo dyes. Wuhrmann et al., (1980) investigated the effects of dye adsorption by the cell wall and concluded the following: (1) dye adsorption follows Freundlich adsorption isotherms at low dye loads per weight of biomass, but exhibits a high variability; (2) depending on the dye, subsequent reduction may take place or the dye may remain in the cell wall; (3) adsorption does not inhibit the reduction rate of microbes that exhibit the ability to reduce azo dyes. While these conclusions are limited based on the testing performed, they do give an indication of the

variability that is possible when dealing with azo dyes and biological treatment systems. Ganesh, (1992) concluded that very little of the dye added to a biological reactor will be leached from the biomass when placed in a landfill. This might suggest that the dye is effectively reduced after adsorption to the cell wall or that very little dye is actually adsorbed.

Cell permeability might play an important role in dye biodegradation. In the study conducted by Wuhrmann et al., (1980), all dyes that were not reduced by whole cells were effectively degraded by cell extracts from both facultative anaerobes and obligate aerobes. This suggests that many cells might be capable of dye biodegradation, but are limited by the permeability of their cell walls.

The azo dye structure can play a significant role in the dye biodegradation rate. Depending on the number and placement of the azo linkages, some dyes will biodegrade more rapidly than others. In general, the more azo linkages that must be broken will cause the reduction rate to be slower.

Fiber-reactive azo dyes often contain solubilizing side groups, as well as a nucleophilic reactive group. Depending on the nature of these groups, biodegradation might be inhibited. The production of toxic by-products or the presence of toxic dye additives may also inhibit biodegradation. High salt concentrations are not uncommon in textile effluents and may result in adverse conditions for biodegradation. Dispersing and solubilizing agents may also create inhibitory conditions for dye reduction (Cariell et al., 1994).

A final and important factor to evaluate is the initial dye concentration of the wastewater. Seshadri et al., 1994 performed a study investigating the effect of different influent dye concentrations on the color removal efficiency. They concluded that elevated dye concentrations may cause a drop in percent dye removal. Furthermore, the inhibition may be directly related to the effects of increased dye metabolite formation due to higher dye concentrations. Less pronounced reductions were seen at lower concentration levels. It should be noted that tolerable influent concentrations are likely specific to individual or related groups of dyes. Cariell et al., (1995) also states that toxicity assays showed that C.I. Reactive Red 141 was inhibitory to anaerobic organisms at concentrations greater than 100 mg/L, however prior biomass adaptation increased their resistance to elevated dye concentrations.

In this, various biological treatment systems were evaluated for their ability to degrade textile waste waters containing reactive azo dyes. In biological methods, the dyes were broken down into simpler compounds. As on date the most reliable strategy is biodegradation by eco – friendly microbes, which is generally accepted as an environmentally sound and economically feasible protocol for the treatment of hazardous waste and effluents.

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