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Review

Bacterial biodegradation and decolourization of toxic textile azo dyes

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Textile industry is considered as one of the largest generators of toxic chemical waste water in India. Dyes released by the textile industries pose a threat to environmental safety. Recently, dye decolourization through biological means has gained momentum as these are cheap and can be applied to wide range of dyes. This review paper focuses on the bacterial biodegradation of toxic textile dyes. Along with the early history of dyes, the toxic effects of textile azo dyes also focused in this review. Various biological techniques for the biodegradation of textile dyes by microorganisms are clearly given in this review. The advantages of aerobic biodegradation over anaerobic degradation are also discussed in this review. The studies discussed in this paper indicate biological decolourization by bacteria has a great potential to be developed further as a decentralized wastewater treatment technology for small textile or dyeing units. However, further research work is required to study the toxicity of the metabolites of dye degradation and the possible fate of the utilized biomass in order to ensure the development of an eco-friendly technology.

Key words: Textile dye effluent, azo dyes, biodegradation and bacteria.

INTRODUCTION

Dyes make the world more beautiful through coloured substances, but on the other hand they represent a serious pollution problem for the environment. Almost one million tons of dyes are annually produced in the world, of which azo dyes, characterized by an azo-bond (R_1–N=N–R_2), represent about 70% by weight (Hao et al., 2000). Azo dyes are the most common synthetic colourants released to the environment via textile, pharmaceutical and chemical industries. The discharge of azo dyes in water bodies is problematic not only for aesthetic reasons, but also because azo dyes and their cleavage products (aromatic amines) are carcinogenic (Weisburger, 2002).

India's dye industry produces every type of dyes and pigments. Production of dye stuff and pigments in India is close to 80,000 tones. India is the second largest exporter of dye stuffs and intermediates after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80%. Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment.

Dyes are recalcitrant by design and not readily amendable to common treatment methods, imposing a challenge for closed water systems. Extensive research in the field of biological azo dye decolourization has shown promising results, but much of this work has been done with single model compounds (Cervantes et al., 2001). However, industrial textile wastewater presents the additional complexity of dealing with unknown quantities and varieties of many kinds of dyes (Bisschops and Spanjers, 2003), as well as low biological oxygen demand/chemical oxygen demand (BOD/COD) ratios, which may affect the

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efficiency of the biological decolourization.

HISTORY OF DYES

The history of dyes begins in 2600 BC, according to the earliest written record, with the use of dyestuffs in China. Dyes were originally obtained from animal and vegetable sources. For example, in the 15th century BC, Phoenicians already used Tyrian purple, which was produced from certain varieties of crushed sea snails, and the well-known plant dye indigo has been used since 3,000 BC. Also, Egyptian mummies were discovered to wrapped with dyed clothes made of madder plants. In South America, the Incas elaborated fine textures with different colours before being conquered by Spain. The chemical industry started in 1856 when the Englishman William Henry Perkin accidentally synthesized the first dye, 'Mauve' (aniline), a brilliant fuchsia colour, while searching for a cure for malaria. In the following decades a considerable number of new dyes were synthesized (Welham, 2000).

Azo dyes are the largest and the most important group of dyes, mainly due to their simple synthesis. The production of azo dyes began in 1858 when the German scientist P. Gries discovered the reaction mechanism diazotization for the production of azo compounds. Dyes are classified according to their application and chemical structure. They are composed of a group of atoms responsible for the dye colour, called chromophores, as well as an electron withdrawing or donating substituent that cause or intensify the colour of the chromophores, called auxochromes (Christie, 2001). The most important chromophores are azo (–N=N–), carbonyl (–C=O), methine (–CH=), nitro (–NO_2) and quinoid groups. The most important auxochromes are amine (–NH_3), carboxyl (–COOH), sulfonate (–SO_3H) and hydroxyl (–OH). The auxochromes can belong to the classes of reactive, acid, direct, basic, mordant, disperse, pigment, vat, anionic and ingrain, sulphur, solvent and disperse dye (Welham, 2000).

It is estimated that almost 10^9 kg of dyes are produced annually in the world, of which azo dyes represent about 70% by weight. This group of dyes is characterized by reactive groups that form covalent bonds with OH–, NH–, or SH– groups in fibres (cotton, wool, silk, nylon). Azo dyes are mostly used for yellow, orange and red colours (Christie, 2001). To obtain the target colour, normally a mixture of red, yellow and blue dyes is applied in the dye baths. These three dyes do not necessarily have the same chemical structure. They might contain many different chromophores, in which azo, anthraquinone and phtalocyanine dyes are the most important groups (Hao et al., 2000). Anthraquinone dyes constitute the second most important class of textile dyes, after azo dyes. Anthraquinone dyes have a wide range of colours in almost the whole visible spectrum, but they are most commonly used for violet, blue and green colours (Fontenot et al., 2003).

In alkaline conditions, that is, pH 9 - 12 and salt concentration from 40-100 g/l, and at high temperatures (30-70°C), reactive dyes form a reactive vinyl sulfone (–SO_4–CH=CH_2) group, which forms a bond with the fibres. However, the vinyl sulfone group undergoes hydrolysis, that is, a spontaneous reaction that occurs in the presence of water, and because the products do not have any affinity with the fibres, they do not form a covalent bond. Therefore, a high amount of dye constituents are discharged in the wastewater (Hao et al., 2000). The fixation efficiency varies with the class of azo dye used, which is around 98% for basic dyes and 50% for reactive dyes. Large amount of salts such as sodium nitrate, sodium sulphate and sodium chloride are used in the dye bath, as well as sodium hydroxide is widely applied to increase the pH to the alkaline range. It is estimated that during the mercerising process, the weight of these salts can make up 20% of the fibre weight (EPA, 1997).

DYES AND ENVIRONMENT

The loss of dyes to effluent can be estimated to be 10% for deep shades, 2% for medium shades and minimal for light shades. Dyes are present in the effluent at concentrations of 10 to 50 mg/l with 1 mg/l being visible to the naked eye. They are complex organic compounds which are refractory in aerobic treatment systems. Some contain metals such as Cr, Cu and Zn. Only 50% (m/m) is dye, the remainder is non-hazardous filler and surfactant. There are 2 main factors involved in determining the risk assessment of chemicals, namely, hazard and exposure. Hazard describes the potential biological effects (for example, toxicity and carcinogenicity) that have a dose-response curve. Exposure is a measure of the expected environmental concentration of a chemical over time and distance. The data obtained from hazard and exposure studies will indicate what effects are possible, whereas risk assessment involves determining what is probable.

In the aquatic environment, dyes can undergo bioconcentration, ionization, abiotic oxidation, abiotic and microbial reduction, precipitation and ligand exchange. The ionic dyes such as acid, direct, basic and metal complex dyes will not volatilize whereas, in principle, solvent, disperse, vat and sulphur dyes have the potential to be volatile. Sorption should also play a major role as dyeing is a sorption process. Hydrolytic reactions are not important because if the dyes survive the rigours of biological treatment processes, it is unlikely to degrade rapidly in the environment.

Photochemical reactions may be important as dyes are good adsorbers of solar energy. However, little information is available on this. It is expected that anionic dyes would react with ions such as calcium and magnesium to form insoluble salts and thereby reduce the concentration available for other biological reactions. Similarly for basic

dyes, due to their interaction with humic material and hydrous oxides, redox reactions should also be considered; as in early vat dyeing processes, the dyes were reduced microbially before chemical replacements were introduced. Reduction in the environment would most likely occur under anaerobic conditions, however, the difficulties of working with anaerobic systems has limited research in this area. In general, there is very little literature available on the environmental behavior of dyes. This is probably due to the lack of suitable analytical techniques.

AZO DYES AS ENVIRONMENTAL POLLUTANTS

Dyes contain chromophores, decolourized electron system with conjugated double bonds and auxochromes, electron-withdrawing or electron-donating substituents that cause or intensify the colour of the chromophore by altering the overall energy of the electron system. Usual chromophores are – C = C, - C = N, - C = O, - N = N, - NO_2 and quinoid ring and auxochromes are –NH_3, - COOH, – SO_3H and –OH (Van der Zee, 2002).

Azo dyes are the largest group of dyes. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries (Maximo et al., 2003). Azo dyes are characterized by the presence of one or more azo groups – N = N -, which are responsible for their colouration and when such a bond is broken the compound loses its colour. They are the largest and most versatile class of dye, but have structural properties that are not easily degradable under natural conditions and are not typically removed from water by conventional waste water system. Azo dyes are designed to resist chemical and microbial attacks and to be stable in light and during washing. Many are carcinogenic and may trigger allergic reactions in man. It is estimated that over 10% of the dye used in textile processing does not bind to the fibres and is therefore released to the environment. Some of these compounds cause serious threat because of their carcinogenic potential or cytotoxicity (Adedayo et al., 2004).

Dyeing of textile requires water and generates a substantial quality of effluents containing mineral salts and dyes at high concentration. An estimated 700000 tons of dyes are produced annually worldwide of which 60-70% are azo dyes (Soares et al., 2004). Chronic effects of dyestuffs, especially of azo dyes, have been studied for several decades. Azo dyes in purified form are mutagenic or carcinogenic, except for some azo dyes, leads to formation of aromatic amines and several aromatic amines are known mutagens and carcinogens to human beings (Praveen et al., 2009).

In mammals, metabolic reduction of azo dye is mainly due to bacterial activity in the anaerobic parts of the lower gastrointestinal tract. Various organs, especially the liver and the kidney also reduce azo dyes. After azo dye reduction in the intestinal tract, the released aromatic amines are absorbed by the intestine and are excreted in the urine. The acute toxic hazard of aromatic amines is carcinogenesis, especially bladder cancer. International Agency for Research on Cancer (IARC) summarized the literature on suspected azo dyes, mainly amino-substituted azo dyes, fat soluble dyes and benzidine azo dyes, as well as a few sulphonated azo dyes (Praveen et al., 2009).

BIOLOGICAL DEGRADATION OF TEXTILE DYES

Dyes are stable against breakdown by many microorganisms and most dyes do not biodegrade under the aerobic biological treatments in a municipal sewage plant. Many dyes, including the azo dyes, degrade under anaerobic conditions and the aromatic amines thus formed have been found to degrade further aerobically. Out of several methods that are used in the treatment of textile effluents to achieve decolourization, including physiochemical methods like filteration, specific coagulation, use of activated carbon and chemical flocculation, some of the methods are effective but quite expensive. Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluent (Dubrow et al., 1996). Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. This natural process of bioremediation, which includes bioengineering the capabilities of intrinsic microorganisms, to clean up the environment is an effective alternative to conventional remediation methods (Vidali, 2009).

Advantages of aerobic bacterial degradation over anaerobic degradation

A number of reports discourage the azo dye decolourization by microorganisms under aerobic conditions as it leads to the formation of corresponding aromatic amines. Even though their reductive cleavage is responsible for colour removal, the formation of aromatic amines is highly undesired as they are reported to be carcinogenic. In the presence of oxygen, aromatic amines can be degraded.

Dyes house effluents usually contain azo dyes which are highly resistant to biological treatment and these dyes are considered to be recalcitrant xenobiotic compounds because of the presence of N=N bond and other sulphonic group which are together to be degraded. It was reported that some anaerobic bacteria can biodegrade dye stuffs by azoreductase activity. However, the effluent from biodegradation of dyestuffs could be toxic. Reverse colourization may take place when the degradation process

are exposed to oxygen. Due to the following mentioned problems, full – scale application of bacterial biodegradation is limited and research works have been reported on anaerobic degradation of azo dyes.

The decolourization of azo dyes has been found to be effective under anaerobic conditions. However, the anaerobic degradation yields aromatic amines which are mutagenic and toxic to humans and cannot be metabolized further under the conditions which generated them. Due to these above mentioned problems, the full scale application of bacterial degradation is limited and research works have been reported on aerobic degradation of azo dyes.

BACTERIAL BIODEGRADATION OF TEXTILE DYES

Investigations to bacterial dye biotransformation have so far mainly been focused to the azo dyes. The electron withdrawing nature of the azo linkages obstructs the susceptibity of azo dye molecules to oxidative reaction. Therefore, azo dyes generally resist aerobic bacterial biodegradation. Only bacteria with specialized azo dye reducing enzymes (azoreductase) were found to degrade azo dyes under fully aerobic conditions. This anaerobic reduction implies decolourizations of the dyes to potentially harmful aromatic amines. Aromatic amines are generally not further degraded under anaerobic conditions. Anaerobic treatment must therefore be considered merely as the first stage of the complete degradation of azo dyes. The second stage involves conversion of the produced aromatic amines. For several aromatic amines, this can be achieved by biodegradation under aerobic conditions.

Walker (1970) used facultative anaerobic bacteria where it was suggested that reduced flavins generated by cytosolic flavine–dependent reductase were responsible for the unspecific reduction of azo dyes. Dubin and Wright (1975) investigated the decolourization of azo food dyes by *Proteus vulgaris*. It was shown that independently of the intracellular location of the azo reductase, theoretically, a redox mediator could facilitate the transfer of reducing equivalents from intracellular NADPH to the substrate dye. Meyer (1981) said that the azo dyes may be microbially degraded under anaerobic or aerobic conditions or in aerobic and anaerobic two-stage systems.

Zimmermann et al. (1982) showed the reduction of azo compounds to occur under aerobic conditions. The aerobic azoreductases from the carboxy – orange degrading *Pseudomonas* strains had monomeric flavine – free enzymes that use NADPH and NADH as cofactors and reductively cleave several sulfonated azo dyes. Rafii et al. (1991) developed a plate assay for the detection of anaerobic bacteria that produce azoreductases. With this plate assay, 10 strains of anaerobic bacteria capable of reducing azo dyes were isolated from human faeces and identified as *Eubacterium hadrum, Clostridium clostridiiforme, Butyrivibrio* sp., *Bacteroides* sp., *Clostridium paraputrificum* and *Clostridium nexile*.

Kudlich et al. (1997) suggested a different model for the non-specific reduction of azo dyes by bacteria which does not require transport of the azo dyes or reduced flavins through the cell membranes for *Sphingomonas xenophaga*. It was proposed that in this system, quinone act as redox mediators which are reduced by quinone reducatse located in the cell membrane of *S. xenophaga* and that the formed reduce the azo dyes in the culture supernatant in a purely chemical redox reaction. Hu (1998) reported decolourization of dyes by facultative bacteria. COD/BOD reduction showed that decolourization occurs during the logarithmic growth phase and COD/BOD reduction during the maximum stationary growth phase.

Kapdan et al. (2000) reported that decolourization of azo dye would not take place at a dissolved oxygen concentration higher than 0.45 mg/l and a slight increase in bacterial cell mass at the initial stage. The low colour removal at a temperature beyond 35°C may be attributed to the thermal deactivation enzymes and the low biomass. Yu et al. (2001) isolated different species of *Pseudomonas* sp. and showed a significant improvement on decolourization of recalcitrant non-azo dyes. The optimum decolourization activity was observed in a narrow pH range (7-8), a narrow temperature range (35-40°C), and at the presence of organic and ammonium nitrogen. Chen et al. (2002) isolated and selected *Aeromonas hydrophila* for colour removal from various dyes. More than 90% of red RBN was reduced in colour within 8 days at a dye concentration of 3000 mg/l. This strain could also decolourize the media containing the mixture of dyes within 2 days of incubation. Nitrogen sources such as yeast extract or peptone could enhance strongly the decolourization efficiency.

Nachiyar and Rajkumar (2003) investigated degradation of Navitan Fast Blue using *Pseudomonas aeruginosa*. The organisms required ammonium salts and glucose to co-metabolize the dye. Organic nitrogen sources did not support appreciable decolourization, whereas, combined with inorganic nitrogen, an increasing effect both on the growth and decolourization was observed. An oxygen intensive azoreductase was involved in the decolourization mechanism. Senan and Abraham (2004) reported that an aerobic bacterial consortium consisting of two isolated strains and a strain of *Pseudomonas putida* was developed for the aerobic degradation of a mixture of textile azo dyes and individual azo dyes at alkaline pH (9-10) and salinity (0.9 – 3.8 g/l) at ambient temperature (28±2°C). The degradation efficiency of the strains in different media and at different dye concentrations was studied. The enzyme present in the crude supernatant was found to be reusable for the dye degradation.

Oranusi and Njoku (2005) investigated the biotransformation of food dyes (Tartrazine and Quinoline Yellow) by *Streptococcus faecalis* and *Escherichia coli* isolated from human intestinal microflora. Decolourization of the media containing the dyes was used as an index of biotransformation. Biotransformation was higher under aerobic than under anaerobic condition. The results obtained were attributed to the organism's cytosolic flavin-dependent reductases and redox equivalents generated by metabolism of soluble starch which transfer electrons to the chromophoric group of the dyes. Safia Moosvi et al. (2006) revealed that the organisms like *Paenibacillus polymyxa, Micrococcus luteus* and *Micrococcus* sp. have the ability to decolourize Reactive Violet 5R within 36 h. The culture exhibited good decolourization ability in the pH range from 6.5 to 8.5 and temperature range from 25 to 37°C. The consortium showed complete decolourization utilizing low amount of co-substrates like glucose (0.1% w/v) and yeast extract (0.05% w/v) and could also utilize a cheaper carbon source like starch in place of glucose as an alternative co-substrate.

Kalyanee et al. (2007) investigated the ability of facultative bacterial isolates like *Enterobacter* sp., *Serratia* sp., *Yersinia* sp. and *Erwinia* sp. to degrade C.I. reactive red 195 in solid and liquid dye medium. The best decolourizer, removing dye upto 90% within 2 days of incubation, was *Enterobacter* sp. The dye degradability of *Enterobacter* sp. was evaluated by the decrease of dye concentration. Sandhya et al. (2005) reported that amplified product containing gene responsible for dye decolourization was cloned and expressed in *E. coli*. The resulting recombinant strain *E. coli* decolourized 200 mg/l azo dye (Ramazol Red) at 30°C at 255 mg cell/l/hour, while host *E. coli* had no colour removal ability. The decolourization rate of *E. coli* was optimal at 37-45°C.

Bor-Yann Chen et al. (2009) studied the colour removal efficiency of *Pseudomonas luteola* with Reactive Red 141. The decolourization performance of unacclimated and acclimated pure culture was at most 20 and 70-80%, respectively. It might suggest that combined interactions among decolourizers were crucial for the optimal colour removal. Elisangela et al. (2009) tested the decolourization of four different azo dyes in a sequential microaerophilic-aerobic treatment by a *Klebsiella* sp. isolated from activated sludge process of the textile industry. Dye decolourization was performed under microaerophilic condition until no colour was observed (decolourization percentage >94%).

Saranraj et al. (2010) investigated the decolourization and degradation of direct azo dyes using bacteria isolated from textile dye effluent. In their research, *P. aeruginosa* (97.33%) was identified as the best decolourizer of Congo Red. *Klebsiella pneumoniae* (98.44%) was the best decolourizer of Viscose Orange – A. The best decolourizer of Direct Green-PLS was *Bacillus subtilis* (99.05%). *K. pneumoniae* (87.27%) highly decolourized the Direct Violet-BL. *E. coli* (61.56%) was the best decolourizer of Direct Sky Blue-FF. The best decolourizer of Direct Black-E was *K. pneumoniae* (92.03%).

Recently, Silveira et al. (2011) checked the ability of *Pseudomonas* sp. to remove colour from textile industrial dyes. *Pseudomonas cepacia* exhibited no growth at all on the plates containing dyes (1 g/l), whereas *P. aeruginosa, Pseudomonas oleovorans* and *P. putida* exhibited considerable growth. Decolourization in a liquid culture revealed that *P. oleovorans* was more viable for decolourizing textile dyes.

CONCLUSION

Recent literature reviewed in this paper indicates that although a large number of lab-scale studies have been conducted on decolourization of textile azo dye solutions through bacteria, there is a need to generate relative performance data on industrial effluents. Through this review paper, it is evident that bacteria can be employed as a vital biological tool for developing decentralized wastewater treatment systems for decolourization of dye effluents through biosorption or biodegradation. Such systems could be particularly useful for small-scale textile or dyeing units because most of the microbial strains can be supported on locally available and low-cost growth substrates. Nevertheless, there is a need to formulate the efficient bacterial strains in a product that can be delivered to remote places.

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