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Azo Dyes and Their Metabolites: Does the Discharge of the Azo Dye into Water Bodies Represent Human and Ecological Risks?

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1. Introduction

1.1 History of sintetic dyes

Colorants (dyes and pigments) are important industrial chemicals. According to the technological nomenclature, pigments are colorants which are insoluble in the medium to which they are added, whereas dyes are soluble in the medium. The world's first commercially successful synthetic dye, named mauveine, was discovered by accident in 1856 by William Henry Perkin. These synthetic compounds can be defined as colored matters that color fibers permanently, such that they will not lose this color when exposed to sweat, light, water and many chemical substances including oxidizing agents and also to microbial attack (Rai et al., 2005; Saratele et al., 2011). By the end of the 19th century, over ten thousand synthetic dyes had been developed and used for manufacturing purposes (Robinson et al., 2001a; Saratele et al., 2011), and an estimate was made in 1977 that approximately 800,000 tons of all recognized dyestuffs had been produced throughout the world (Anliker, 1977; Combes & Haveland-Smith, 1982). The expansion of worldwide textile industry has led to an equivalent expansion in the use of such synthetic dyestuffs, resulting in a rise in environmental pollution due to the contamination of wastewater with these dyestuffs (Pandey et al., 2007; Saratele et al., 2011).

The Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry (ETAD) was inaugurated in 1974 with the goals of minimizing environmental damage, protecting users and consumers and cooperating with government and public concerns in relation to the toxicological impact of their products (Anliker, 1979; Robinson et al., 2001a). A survey carried out by ETAD showed that of a total of approximately 4,000 dyes that had

been tested, more than 90% showed LD_{50} values above 2 x 10^3 mg/kg, the most toxic being in the group of basic and direct diazo dyes (Shore, 1996; Robinson et al., 2001a). Thus it appears that exposure to azo dyes does not cause acute toxicity, but with respect to systemic bioavailability, inhalation and contact with the skin by azo dyes is of concern, due to the possible generation of carcinogenic aromatic amines (Myslak & Bolt, 1988 and Bolt & Golka, 1993 as cited in Golka et al., 2004).

Of the approximately 10^9 kg of dyestuffs estimated to be manufactured annually throughout the World, the two most widely used in the textile industry are the azo and anthraquinone groups (Križanec & Marechal, 2006; Forss, 2011). Thus, this chapter is a comprehensive review on the azo dyes and their effects on human and environmental health.

2. Azo dyes

Azo dyes are diazotized amines coupled to an amine or phenol, with one or more azo bonds (–N=N–). They are synthetic compounds and account for more than 50% of all the dyes produced annually, showing the largest spectrum of colors (Carliell et al., 1995; Bae & Freeman, 2007; Kusic et al., 2011). Nearly all the dyestuffs used by the textile industry are azo dyes, and they are also widely used in the printing, food, papermaking and cosmetic industries (Chung & Stevens, 1993; Chang et al., 2001a). An estimate was made in the 80's, that 280,000 t of textile dyes were annually discharged into industrial effluents worldwide (Jin et al., 2007; Saratale et al., 2011). Since the azo dyes represent about 70% by weight of the dyestuffs used (Zollinger, 1987), it follows that they are the most common group of synthetic colorants released into the environment (Chang et al., 2001b; Zhao & Hardin, 2007; Saratale et al., 2011).

One only needs very small amounts of dyes in the water (less than 1 ppm for some dyes) to cause a highly visible change in color (Banat et al., 1996), and colored wastewater not only affects the aesthetic and transparency aspects of the water being received, but also involves possible environmental concerns about the toxic, carcinogenic and mutagenic effects of some azo dyes (Spadaro et al., 1992; Modi et al., 2010; Lu et al., 2010). It can also affect the aquatic ecosystem, decreasing the passage of light penetration and gas dissolution in lakes, rivers and other bodies of water (Saranaik & Kanekar, 1995; Banat et al., 1996; Modi et al., 2010).

The more industrialized the society, the greater the use of azo dyes, and hence the greater the risk of their toxic effects affecting the society. It has already been noted that, as from the 70's, intestinal cancer has been more common in highly industrialized societies, and therefore there may be a connection between the increase in the number of cases of this disease and the use of azo dyes (Wolff & Oehme, 1974; Chung et al., 1978).

Bae and Freeman (2007) already demonstrated the biological toxicity of the direct azo dyes used in the textile industry. The results indicated that C.I. Direct Blue 218 was very toxic to daphnids, with a 48-h LC_{50} between 1.0 and 10.0 mg/L. It must be remembered that toxicity to daphnids is sufficient to suggest potential damage to every receptor ecosystem, and emphasizes the need for the synthetic dye manufacturing industry to carry out toxicological studies (Bae & Freeman, 2007).

2.1 Azo dyes and their mutagenic effects

The azo dyes show good fiber-fixation properties as compared other synthetic dyes, showing up to 85% fixation, but nevertheless this explains why so much dye is released into

the environment, representing the other 10 to 15% of the amount used. Most of the synthetic dyestuffs found in this class are not degraded by the conventional treatments given to industrial effluents or to the raw water (Nam & Reganathan, 2000; Oliveira et al., 2007). Shaul et al. (1991) studied 18 azo dyes, and found that 11 passed practically unchanged through the activated sludge system, 4 were adsorbed by the activated sludge and only 3 were biodegraded, resulting in the release of these substances into bodies of water. Oliveira et al. (2007) showed that even after treatment, effluent from dyeing industries was mutagenic and contained various types of dye. Such data are of concern, especially when one considers that the effluent from the same industry was studied by Lima et al. (2007), who found an increase in the incidence of aberrant crypt in the colon of rats exposed to this sample, this being an early biomarker of carcinogenesis (Lima et al., 2007).

Azo dyes can also be absorbed after skin exposure, and such dermal exposure to azo dyes can occur as an occupational hazard or from the use of cosmetic products. It was postulated in the 80s that the percutaneous absorption of azo dyes from facial makeup could even be a risk factor in reproductive failures and chromosomal aberrations in a population of television announcers (Kučerová et al., 1987; Collier et al., 1993).

Various azo dyes have been shown to produce positive toxic results for different parameters. Tsuboy et al. (2007) analyzed the mutagenic, cytotoxic and genotoxic effects of the azo dye CI Disperse Blue 291, and the results clearly showed that this azo dye caused dose-dependent effects, inducing the formation of micronuclei (MNs), DNA fragmentation and increasing the apoptotic index in human hepatoma cells ($HepG_2$). A variety of azo dyes have shown mutagenic responses in Salmonella and mammalian assay systems, and it is apparent that their potencies depend on the nature and position of the aromatic rings and the amino nitrogen atom. For instance, 2-methoxy-4-aminoazobenzene is an extremely weak mutagen, whereas under similar conditions, 3-methoxy-4-aminoazobenzene is a potent hepatocarcinogen in rats and a strong mutagen in *Escherichia coli* and *Salmonella typhimurium* (Hashimoto et al., 1977; Esancy et al., 1990; Garg et al., 2002, Umbuzeiro et al., 2005a).

According to Chequer et al. (2009), the azo dyes Disperse Red 1 and Disperse Orange 1 increase the frequency of MNs in human lymphocytes and in HepG2 cells in a dose-dependent manner. According to Ferraz et al. (2010), the azo dyes Disperse Red 1 and Disperse Red 13 showed mutagenic activity in the Salmonella/microsome assay with all the strains tested and in the absence of metabolic activation, except for Disperse Red 13, which was negative with respect to strain TA100. After adding the S9 mix, the mutagenicity of the two azo dyes decreased (or was eliminated), indicating that the P450-dependent metabolism probably generated more stable products, less likely to interact with DNA. It was also shown that the presence of a chlorine substituent in Disperse Red 13 decreased its mutagenicity by a factor of about 14 when compared with Disperse Red 1, which shows the same structure as Disperse Red 13, but without the chlorine substituent. The presence of this substituent did not cause cytotoxicity in HepG2 cells, but toxicity to the water flea *Daphnia similis* increased in the presence of the chlorine substituent (Ferraz et al., 2010).

Chung and Cerneglia (1992) published a review of several azo dyes that had already been evaluated by the Salmonella / microsome assay. According to these authors, all the azo dyes evaluated that contained the nitro group showed mutagenic activity. The dyes Acid Alizarin Yellow R and Acid Alizarin GG showed this effect in the absence of metabolic activation (Brown et al., 1978). The dyes C.I. Basic Red 18 and Orasol Navy 2RB, which also contained

nitro groups, were shown to be mutagenic both in the presence and absence of metabolic activation (Venturini & Tamaro, 1979; Nestmann et al., 1981). This review also showed the results obtained in the Salmonella/microsomal test of azo dyes containing benzeneamines, and found that Chrysodin was mutagenic in the presence of a rat-liver preparation (Sole & Chipman, 1986; Chung & Cerneglia, 1992).

Another study applied the micronucleus assay in mouse bone marrow to the azo dye Direct Red 2 (DR2) and the results identified DR2 as a potent clastogen and concluded that excessive exposure to this chemical or to its metabolites could be a risk to human health (Rajaguru et al., 1999).

Al-Sabti (2000) studied the genotoxic effects of exposing the Prussian carp (*Carassius auratus gibelio*) to the textile dye Chlorotriazine Reactive Azo Red 120, and showed its mutagenic activity in inducing MNs in the erythrocytes. They also showed that the dye had clastogenic activity, a potent risk factor for the development of genetic, teratogenic or carcinogenic diseases in fish populations, which could have disastrous effects on the aquatic ecosystem since the fate of compounds found in effluents is to be discharged into water resources (Al-Sabti, 2000).

In addition to the effects caused by exposure to contaminated water and food, workers who deal with these dyes can be exposed to them in their place of work, and suffer dermal absorption. Similarly, if dye-containing effluents enter the water supply, possibly by contamination of the ground water, the general population may be exposed to the dyes via the oral route. This latter point could be of great importance in places where the existent waste treatment systems are inefficient or where there is poor statutory regulation concerning industrial waste disposal (Rajaguru et al., 1999).

2.2 Effects of the azo dyes metabolites

Sisley and Porscher carried out the earliest studies on the metabolism of azo compounds in mammals in 1911, and found sulphanilic acid in the urine of dogs fed with Orange I, demonstrating for the first time that azo compounds could be metabolized by reductive cleavage of the azo group (Sisley & Porscher, 1911 as cited in Walker, 1970).

The mutagenic, carcinogenic and toxic effects of the azo dyes can be a result of direct action by the compound itself, or the formation of free radicals and aryl amine derivatives generated during the reductive biotransformation of the azo bond (Chung et al., 1992; Collier et al., 1993; Rajaguru et al., 1999) or even caused by products obtained after oxidation via cytochrome P450 (Fujita & Peisach, 1978; Arlt et al., 2002; Umbuzeiro et al., 2005a).

One of the criteria used to classify a dye as harmful to humans is its ability to cleave reductively, and consequently generate aromatic amines when in contact with sweat, saliva or gastric juices (Pielesz et al., 1999, 2002). Some such aromatic amines are carcinogenic and can accumulate in food chains, for example the biphenylamines such as benzidine and 4-biphenylamine, which are present in the environment and constitute a threat to human health and to the ecosystems in general (Choudhary, 1996; Chung et al., 2000).

After an azo dye is orally ingested, it can be reduced to free aromatic amines by anaerobic intestinal microflora and possibly by mammalian azo reductase in the intestinal wall or the liver (Walker, 1970; Prival & Mitchel, 1982; Umbuzeiro et al., 2005a). Such biotransformations can occur in a wide variety of mammalian species, including both *Rhesus* monkeys and humans (Rinde & Troll, 1975; Watabe et al., 1980; Prival & Mitchel, 1982;). As

previously mentioned, the main biotransformation products of azo dyes are aromatic amines, and thus a brief description of this class of compounds is shown below.

2.2.1 Aromatic amines

As early as the late nineteenth century, a doctor related the occurrence of urinary bladder cancer to the occupation of his patients, thus demonstrating concern about the exposure of humans to carcinogenic aromatic amines produced in the dye manufacturing industry, since his patients were employed in such an industry and were chronically exposed to large amounts of intermediate arylamines. Laboratory investigations subsequently showed that rats and mice exposed to specific azo dye arylamines or their derivatives developed cancer, mainly in the liver (Weisburger, 1997, 2002). Briefly, as mentioned above, in 1895, Rehn showed concern about the urinary bladder cancers observed in three workers from an 'aniline dye' factory in Germany. This led to the subsequent testing in animals of various chemicals to which these workers were exposed, and, as a result, the carcinogenic activity of the azo dye, 2,3-dimethyl-4-aminoazobenzene for the livers of rats and mice was discovered (Yoshida, 1933 as cited in Dipple et al., 1985). An isomeric compound, N,N-dimethyl-4-aminoazobenzene was also found to be a liver carcinogen (Kinosita, 1936 as cited in Dipple et al., 1985). Only in 1954 was the cause of the bladder tumors observed in the workers in the dye industry established to be 2-naphthylamine. This aromatic amine induced bladder cancer in dogs, but not in rats (Hueper et al., 1938 as cited in Dipple et al., 1985).

In addition, workers in textile dyeing, paper printing and leather finishing industries, exposed to benzidine based dyes such as Direct Black 38, showed a higher incidence of urinary bladder cancer (Meal et al., 1981; Cerniglia et al., 1986). Cerniglia et al. (1986) demonstrated that the initial reduction of benzidine-based azo dyes was the result of azoreductase activity by the intestinal flora, and the metabolites of Direct Black 38 were identified as benzidine, 4-aminobiphenyl, monoacetylbenzidine, and acetylaminobiphenyl (Manning et al., 1985; Cerniglia et al., 1986) . Furthermore, these metabolites tested positive in the Salmonella/microsome mutagenicity assay in the presence of S9 (Cerniglia et al., 1986).

In the opinion of Ekici et al. (2001), although general considerations concerning the kinetics of azo dye metabolism indicate that an accumulation of intermediate amines is not very likely, this possibility cannot be excluded under all conditions. According to legislation passed in the European Community on 17th July 1994, the application of azo dyes in textiles is restricted to those colorants which cannot, under any circumstances, be converted to any of the following products: 4-Aminodiphenyl; 4-Amino-2′,3-dimethylazobenzene (*o*-aminoazo-toluene); 4-Aminophenylether (4,4′-oxydianiline); 4-Aminophenylthioether (4,4′-thiodianiline); Benzidine; Bis-(4-aminophenyl)-methane (4,4′-diaminodiphenylme- thane); 4-chloroaniline (*p*-chloroaniline); 4-Chloro-2-methylaniline (4-chloro-*o*-toluidine); 2,4-Diaminotoluene (2,4-toluylenediamine); 3,3′-Dichlorobenzidine dihydrochloride; 3,3′-Dimethoxybenzidine (*o*-dianisidine); 3,3′-Dimethylbenzidine (*o*-toluidine); 3,3′-Dimethyl-4,4′-diamino-diphenyl methane; 2-Methoxy-5-methylaniline (*p*-kresidine); 4-Methoxy-1,3-phenylenediamine sulfate hydrate (2,4-diaminoanisole); 4,4′-Methylene-bis (2-chloroaniline); 2-Methyl-5-nitroaniline (2-amino-4-nitrotoluene); 2-Naphthylamine; *o*-Toluidine; 2,4,5-Trimethylaniline (Bundesgesetzblatt, 1994 and Directory of Environmental Standards, 1998 as cited in Ekici et al., 2001).

More recently, the scientific community has come to consider the possibility of manufactured azo dyes breaking down generating amines to be a health hazard. The International Agency for Research on Cancer only includes benzidine-based dyes in Group 2A and eight other dyes in Group 2B. Nevertheless, the possibility of azo bond reduction leading to the production of aromatic amines has been demonstrated under a variety of conditions, including those encountered in the digestive tract of mammals (Chung & Cerneglia, 1992; Pinheiro et al., 2004). Therefore, the majority of the attention concerning possible hazards arising from the use of azo dyes is now being directed at their reduction products (Pinheiro et al., 2004).

Nitroanilines are aromatic amines that are commonly generated during the biodegradation of azo dyes under anaerobic conditions, formed by reductive cleavage of the azo bonds (–N=N–) by the action of microorganisms present in the wastewaters (Pinheiro et al., 2004; Van der Zee & Villaverde, 2005; Khalid et al., 2009). Depending on the individual compounds, many aromatic amine metabolites are considered to be non-biodegradable or only very slowly degradable (Saupe, 1999), showing a wide range of toxic effects on aquatic life and higher organisms (Weisburger, 2002; Pinheiro et al., 2004; Khalid et al., 2009).

2.3 Metabolic pathways involved in the reduction and oxidation of azo dyes

Following oral or skin exposure to azo dyes, humans can subsequently be exposed to biotransformation products obtained by the action of intestinal microorganisms or that of others present on the skin, or due to reactions in the liver (Esancy et al., 1990; Chadwick et al., 1992; Chung et al., 1992; Stahlmann et al., 2006). Therefore it is extremely important to study the metabolic pathways of azo dyes that can contaminate the environment, in order to understand the overall spectrum of the toxic effects.

The metabolic pathways the azo dyes actually follow depend on several factors, such as, (a) the mode of administration; (b) the degree of absorption from the gastro-intestinal tract after oral ingestion; (c) the extent of biliary excretion, particularly after exposure to different routes other than the oral one; (d) genetic differences in the occurrence and activity of hepatic reducing-enzyme systems; (e) differences in the intestinal flora; and (f) the relative activity and specificity of the hepatic and intestinal systems, particularly those responsible for reducing the azo link, and all these factors are interrelated (Walker, 1970).

Azo dyes behave as xenobiotics, and hence after absorption, they are distributed throughout the body, where they either exert some kind of action themselves or are subjected to metabolism. Biotransformation may produce less harmful compounds, but it may also form bioactive xenobiotics, ie, compounds showing greater toxicity (Kleinow et al., 1987; Livingstone, 1998). The main routes involved in the biotransformation of dyes are oxidation, reduction, hydrolysis and conjugation, which are catalyzed by enzymes (Zollinger, 1991; Hunger, 1994), but in humans, biological reductions and oxidations of azo dyes are responsible for the possible presence of toxic amines in the organism (Pielesz et al., 2002).

Orange II can be reductively metabolized producing 1-amino-2-naphthol, a bladder carcinogen for rats (Bonser et al., 1963; Chung et al., 1992). This suggests that any toxicity induced by unchanged azo dye molecules should not be accepted as the only effect of these compounds, since the reductive cleavage products from these dyes can be mutagenic/ carcinogenic (Field et al., 1977; Chung et al., 1992).

2.3.1 Oxidative metabolism

Highly lipid-soluble dyes such as azo dyes, with chemical structures containing amino groups, either alkylamino or acetylamino, but without sulfonated groups, are preferentially biotransformed by oxidative reactions (Hunger, 1994).

Oxidation processes are mainly catalyzed by a microsomal monooxygenase system represented by cytochrome P450 (Hunger, 1994), which belongs to a superfamily of heme proteins, present in all living organisms and involved in the metabolism of a wide variety of chemical compounds (Denisov et al. 2005; Mansuy, 2007).

The general mechanism of metabolic oxidation involves an electron transport chain, which first transfers an electron to the P-450-Fe^{3+} complex, which, on reduction, receives an oxygen atom and in the final steps, leads to the formation of an oxidation product in the organism (Furhmann, 1994 as cited in Hunger, 1994).

There are three different oxidation pathways of importance for azo dyes: I) C-Hydroxylation, ring hydroxylation in the case of azo dyes, probably via an epoxidation mechanism and subsequent rearrangement to a phenol. II) N-Hydroxylation at primary or secondary amino groups, or with acetyl amino groups in the liver. This reaction is followed by esterification with glucuronate or sulfate. The activated esters, which are water-soluble, can be excreted, or the ester group can split off with the formation of a nitrenium compound $-NH^{+}$, which can covalently bind to a nucleophilic group of the DNA. III) Demethylation, which is the stepwise oxidation of the methyl groups of dialkylamino compounds, and the N-hydroxy derivative so formed can be further demethylated or react to form a nitrenium compound (Hunger, 1994).

Studies on the metabolism and carcinogenicity of N,N-dimethylaminoazobenzene (Butter Yellow), a classical hepatocarcinogen in rats, have shown that N-methylaminoazobenzenes are mainly metabolized by N-demethylation. In this way, Butter Yellow was first reversibly demethylated to the mono-N-methyl compound, which, in turn, was irreversibly demethylated to form p-aminoazobenzene. These changes were shown to precede reduction of the azo link, by isolating N-methyl-p-amino azobenzene and p-aminoazobenzene from the animal tissues (Miller et al., 1945; Walker, 1970). Radiotracer studies have shown (Miller et al., 1952) that demethylation occurs via the formation of a hydroxymethyl compound, followed by elimination of the methyl group in the form of formaldehyde (Mueller & Miller, 1953; Walker, 1970).

Hydroxylation of the aromatic ring can occur before reductive fission of the azo group, and also on the amines produced by such a reduction, and this pathway appears to be very important in compounds which contain an unsulphonated phenyl moiety (Walker, 1970).

2.3.2 Reductive metabolism

Trypan Blue has been shown to have carcinogenic and teratogenic properties (Field et al., 1977). Although original Trypan Blue is not mutagenic, it was reduced by the cell-free extract of an intestinal anaerobe, *Fusobacterium* sp.2, to a mutagenic product, O-toluidine (3,3'-dimethylbenzidine) (Hartman et al., 1978; Chung et al., 1992). In addition to Trypan Blue, Benzopurpurine 4B and Chlorazol Violet N were also shown to be Ames-positive frame-shift mutagens, but only in the presence of metabolizing systems capable of effecting azo reduction. The activity of these dyes may therefore be attributed to the benzidine metabolite, O-toluidine, which is generated because these amines are themselves indirect frame-shift agents (Hartman, et al., 1978; Matsushima et al., 1978). As mentioned above, the

benzidine produced after the reduction of some dyes can induce bladder cancer in humans and tumors in some experimental animals (Combes & Haveland-Smith, 1982; Chung, 1983). Some azo dyes, such as Brown FK, have been shown to be directly mutagenic in bacterial tests (Haveland-Smith & Combes, 1980a, b; Rafii et al., 1997). However many other azo dyes, such as Congo Red and Direct Black 38, only give a positive result for mutagenicity after chemical reduction or incubation with the contents of the human intestinal tract (Haveland-Smith & Combes, 1980 a,b; Reid et al., 1983; Cerniglia et al., 1986;;Chung and Cerniglia, 1992; Rafii et al., 1997).

Reductive cleavage of the azo linkages is probably the most toxicologically important metabolic reaction of azo compounds. This reaction can be catalyzed by mammalian enzymes, especially in the liver (Walker, 1970; Kennelly et al., 1982) or by intestinal (Chung et al., 1978; Hartman et al., 1978) or skin bacteria such as *Staphylococcus aureus* (De France, 1986; Platzek et al., 1999; Golka et al., 2004). Azo compounds can reach the intestine directly after oral ingestion or via the bile after parenteral administration. They are reduced by azo reductases produced by intestinal bacteria, and to a lesser extent by enzymes from the cytosolic and microsomal fractions of the liver. The first catabolic step in the reduction of azo dyes is the cleavage of the azo bond, producing aromatic amines (Cerniglia et al., 1986), accompanied by a loss of color of the dye, and bacterial azoreductases show much greater activity than hepatic azoreductases (Watabe et al., 1980; Collier et al., 1993; Raffi et al., 1997). This reduction process may produce compounds that are more or less toxic than the original molecule (Collier et al., 1993; Rafii et al., 1997), depending on the chemical structure of the metabolite generated. Although its occurrence in the liver has been regarded as the result of a detoxification reaction, azo reduction may be the first step in azo dye carcinogenesis (Chung et al., 1992).

In addition, Nam & Reganathan (2000) demonstrated that both nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) are capable of reducing azo dyes in the absence of any enzyme, under mildly acidic conditions. The reduced forms of NADH and NADPH are ubiquitous sources of electrons in biological systems, and these function as cofactors in many reductive enzyme reactions. This suggests that the introduction of methyl and methoxy substituents at the 2-,2,3-,2,6-, or 2,3,6-positions of the aromatic ring, accelerates the reduction of phenolic azo dyes by NADH, as compared to that of unsubstituted dyes (Nam & Reganathan, 2000).

It is possible that both the mutagenicity and carcinogenicity of azo dyes are in fact frequently due to the generation of aromatic amines, with subsequent N- and ring hydroxylation and N-acetylation of the aromatic amine (Chung & Cerniglia, 1992). If the azo dyes contain nitro groups, they can also be metabolized by the nitroreductases produced by microorganisms (Chadwick et al., 1992; Umbuzeiro et al., 2005a). Mammalian enzymes in the liver and in other organs can also catalyze the reductive cleavage of the azo bond and the nitroreduction of the nitro group. However, it has been shown that the intestinal microbial azoreductase and nitroreductase play a more important role in this type of metabolism. In both cases, the formation of N-hydroxylamines can cause DNA damage, and if the dyes are completely reduced to aromatic amines, they can then be oxidized to N-hydroxyderivates by P450 enzymes. In addition, N-hydroxy radicals can be acetylated by enzymes such as O-acetyltransferase, generating nitrenium electrophilic ions which are able to react with DNA forming adducts (Chung et al., 1992; Arlt et al. 2002; Umbuzeiro et al., 2005a). Research carried out by Zbaida (1989) showed that the hydroxylation of non-reactive

azo dyes such as azobenzene, increased their binding to microsomal cytochrome P-450 and consequently their rate of reduction (Zbaida, 1989).

Studies with mono-azo dyes have indicated that low electron densities close to the azo bond favor reduction (Walker & Ryan, 1971; Combes & Haveland-Smith, 1982), and this may occur due to hydrogen bonding of an azo N atom together with a proximal naphthol group, producing a keto-hydrazone configuration. It is possible that such a structure, which is present in many food colors, may generate dyes which are more resistant to hepatic and microbial reduction (Parke, 1968 cited as in Combes & Haveland-Smith, 1982).

Kennelly et al. (1984) showed that Direct Black 38**,** Direct Brown 95, Direct Blue 6, Congo Red, Trypan Blue and Chicago Sky Blue were easily reduced by the intestinal microflora when orally administered to rats by gavage, however when administered via the hepatic portal vein, only Direct Black 38**,** Direct Brown 95 and Direct Blue 6 were reduced, all of which are potent liver carcinogens (Robens et al., 1980; Chung et al., 1992).

Sweeney et al. (1994) tested azo dyes for genotoxicity following bacterial reduction of the dye. They found that both reduced amaranth and reduced sunset yellow induced cytotoxicity when incubated with a repair deficient E. coli strain in the absence of hepatic enzymes, indicating DNA damage. On the other hand they failed to mutate the *S. typhimurium* strains TA98 and TA1OO, but in contrast, strain TA102, which detects oxidative mutagens (De Flora et al., 1984), was mutated by reduced amaranth and reduced sunset yellow (Sweeney et al., 1994).

Reduction can also modify the type of activity observed. The direct mutagenicities of Alizarin Yellow GG and Acid Alizarin Yellow R were eliminated by reduction, but in the presence of the exogenous metabolic system (S9), the resulting products were mutagenic and exhibited frame-shift activity (Brown et al., 1978; Combes & Haveland-Smith, 1982).

The Sudan dyes I, II, III and IV are oil-soluble azo dyes (1-amino-2-naphthol-based azo dyes), widely used in coloring plastics, leather, fabrics, printing inks, waxes and floor polishes (An et al., 2007; Xu et al., 2010). Sudan I is a liver and urinary bladder carcinogen in mammals and is also considered as a possible human mutagen, since it can produce the benzenediazonium ion during metabolism catalyzed by cytochrome P450, which could be the mechanism by which Sudan I is activated leading to a carcinogenic final product (Stiborová et al., 2002, 2005; Xu et al., 2010). An et al. (2007) found a dose-dependent increase in DNA migration in the comet assay, and in the frequency of micronuclei with all the concentrations of Sudan I tested (25–100 μM). These data suggest that Sudan I caused breaks in DNA strands and chromosomes. Sudan II causes mutations in Salmonella Typhimurium TA 1538 in the presence of a rat liver preparation (Garner & Nutman, 1977; Xu et al., 2010). Concern about the safety of Sudan III, which is used in cosmetics, has arisen from its potential metabolic cleavage by skin bacteria producing 4-aminoazobenzene and aniline (Pielesz et al., 2002), and Sudan IV has been shown to require reduction and microsomal activation in order to be mutagenic (Brown et al., 1978). These are important mechanisms, since, with the exception of Sudan II, Xu et al. (2010) showed that the bacteria found in the human colon are frequently able to reduce Sudan dyes (Xu et al., 2010).

Almost all the azo dyes are reduced *in vivo*, but the reduction of the ingested dose is frequently incomplete, and thus a certain amount of the dye can be excreted in the unchanged or conjugated form. For instance, in the case of orally dosing rats with Sudan III, none of the expected reduction products was excreted, although p-aminophenol could be detected in the urine after i.p. injection (IARC, 1975; Combes & Haveland-Smith, 1982). This

may have been due to the formation of hydrazones at one of the azo bridges of this diazo dye, which might make it resistant to intestinal reduction (Combes & Haveland-Smith, 1982).

Stahlmann et al. (2006) reported investigations made to evaluate the sensitizing and allergenic potentials of two metabolites expected to be formed by the metabolic activity of skin bacteria and/or by metabolism in the skin. Two metabolites (4-aminoacetanilide and 2-amino-*p*-cresol) of Disperse Yellow 3, an azo dye widely used in the textile industry, were tested using modified local lymph node assay protocols in NMRI mice. The metabolite 2-amino-*p*-cresol gave a clearly positive response in the sensitisation protocol, showing marked increases in lymph node weight and cell proliferation, accompanied by a relative decrease in T-cells and relative increases in B-cells and $1A^+$ cells. Hence, 2-amino-*p*-cresol can be considered to be a stronger allergen in this model. In contrast, 4-aminoacetanilide only led to an increase in lymph node weight and cellularity at the higher concentration of 30%, with no consistent changes in the phenotypic analysis, indicating that this metabolite alone was a weak sensitizer (Stahlmann et al., 2006).

2.4 Dying processing plants effluents and their treatments

The textile industry accounts for two-thirds of the total dyestuff market (Fang et al., 2004; Elisangela et al., 2009). As mentioned before, part of dye used in the textile dyeing process does not attach to the fibers, remaining in the dye baths and eventually being discharged in the wastewater (Fang et al., 2004). The resulting wastewater is usually treated with activated sludge, and the liquid effluent is released to adjacent surface waters (Umbuzeiro et al., 2005 b).

Many dyes do not degrade easily due to their complex structure and textile dye effluent does not decolorize even if the effluent is treated by the municipal wastewater treatment systems (Shaul et al., 1991; Robinson et al., 2002; Forgacs et al., 2004). A study carried out in 1989 showed that the commercial aminoazobenzene dye, C.I. Disperse Blue 79, was not degraded by a conventionally operated activated sludge process and that 85% of the dye remained in the system. Of this 85%, 3% was retained by the primary sludge, 62% by the activated sludge and 20% was found in the final liquid effluent released into the environment (US EPA, 1989; Umbuzeiro et al., 2005 b). The use of an anaerobic system before the activated sludge treatment can result in cleavage of the azo bonds and the release of the corresponding aromatic amines. However, the colourless aromatic amines produced by these anaerobic microorganisms can be highly toxic and carcinogenic (Hu, 1994; Banat et al., 1996; Robinson et al., 2002).

Ekici et al. (2001) tested the stability of selected azo dye metabolites in both activated sludge and water and concluded that they were relatively stable in the aquatic environment and could not be efficiently degraded in wastewater plant systems. With respect to their mutagenicity, Fracasso et al. (1992) showed that dye factory effluents from primary and secondary biological treatments increased their levels of mutagenic activity as compared to the raw (untreated) effluent. The use of activated carbon filtration was beneficial but did not completely remove the mutagenic activity of the final effluent (Fracasso et al., 1992; Umbuzeiro et al., 2005 b).

Azo dyes are usually designed to resist biodegradation under aerobic conditions, the recalcitrance of these compounds being attributed to the presence of sulfonate groups and azo bonds. On the other hand, the vulnerability of reducing the azo bonds by different

mechanisms (e.g. biotreatment in anaerobic conditions) could result in the generation of aromatic amines, which are somewhat toxic and carcinogenic (Öztürk & Abdullah, 2006; Bae & Freeman, 2007; Kusic et al., 2011). It should also be mentioned that azo dyes are associated with various health risks to humans, and therefore colored wastewaters should be efficiently treated prior to discharge into the natural water bodies (Kusic et al., 2011).

Several methods are used to decolorize textile effluents including physicochemical methods such as filtration and coagulation, activated carbon and chemical flocculation (Gogate & Pandit, 2004). These methods involve the formation of a concentrated sludge, which, in turn creates a secondary disposal problem (Maier et al., 2004; Elisangela et al., 2009), since these methods merely transfer the pollution from one phase to another, which still requires secondary treatment (Gogate & Pandit, 2004; Kusic et al., 2011). Recently, new biological processes have been developed for dye degradation and wastewater reuse, including the use of aerobic and anaerobic bacteria and fungi (Elisangela et al., 2009).

The decolorizing of azo dyes using a fungal peroxidase system is another promising method (Hu, 1994). The ligninolytics are the most widely researched fungi for dye degradation (Elisangela et al., 2009) and of these, the white-rot fungi have been shown to be the most efficient organisms for the degrading of various types of dye such as azo, heterocyclic, reactive and polymeric dyes (Novotný et al., 2004). These fungi produce lignin peroxidase, manganese-peroxidase and laccase, which degrade many aromatic compounds due to their nonspecific systems (Forgacs et al., 2004; Revankar & Lele, 2007; Madhavi et al., 2007). However, all these processes for the mineralization of azo dyes need to be carried out in a separate process, since the dye compounds cannot be incorporated into the medium, and this would be impractical due to the great volume of wastewater requiring treatment (Hu, 1994). In addition, the long growth cycle and complexity of the textile effluents, which are extremely variable in their compositions, limit the performance of these fungi. Although the stable operation of continuous fungal bioreactors for the treatment of synthetic dye solutions has been achieved, the application of white-rot fungi for the removal of dyes from textile wastewaters still confronts many problems due to the large volumes produced, the nature of the synthetic dyes and the biomass control (Nigam et al., 2000; Mielgo et al., 2001; Robinson et al., 2001b; Elisangela et al., 2009).

Of the chemical methods under development, advanced oxidation processes (AOPs) seem to be a promising option for the treatment of toxic and non-biodegradable organic compounds in various types of wastewater, including the colored ones (Forgacs et al., 2004; Gogate & Pandit, 2004; Kusic et al., 2011). AOPs have received considerable attention due to their potential to completely oxidize the majority of the organic compounds present in the water. AOPs could serve as oxidative pretreatment method to convert non or low-biodegradable organic pollutants into readily biodegradable contaminants (Mantzavinos & Psillakis, 2004; Kusic et al., 2011). The electron beam (EB) treatment is also included in the class of AOPs, and laboratory investigations, pilot-plant experiments and industrially established technology have shown the efficiency of the EB treatment in destroying textile dyes in aqueous solutions (Han et al. ,2002; Pálfi et al., 2011).

Chlorine has been extensively used as a complementary treatment to remove or reduce the color of industrial effluents containing dyes, and also to disinfect the water in drinking water treatment plants (Sarasa et al., 1998; Oliveira et al., 2010). The discoloration process using sodium hypochlorite (NaOCl) or chlorine gas, is based on the electrophilic attack of the amino group, and subsequent cleavage of the chromophore group (responsible for the

dye color) (Slokar & Marechal, 1998). However, the treatment of textile effluents using the conventional activated sludge method followed by a chlorination step, is not usually an effective method to remove azo dyes, and can generate products which are more mutagenic than the original untreated dyes, such as PBTAs (chlorinated 2-phenylbenzotriazoles). It has been reported that conventional chlorination should be used with caution in the treatment of aqueous samples contaminated with azo dyes (Umbuzeiro et al., 2005b; Oliveira et al., 2010).

Another alternative could be the use of photoelectrocatalysis on titanium supported nanocrystalline titanium dioxide thin film electrodes, where active chlorine is produced promoting the rapid degradation of reactive dyes (Carneiro et al., 2004; Osugi et al., 2009). Osugi et al. (2009) investigated the decolorizing of the mutagenic azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1 by chemical chlorination and photoelectrochemical oxidation on Ti/TiO_2 thin-film electrodes using NaCl and Na_2SO_4 media. After 1 h of treatment, 100% decolorizing was achieved with all the methods tested. After 1 h of photoelectrocatalytic oxidation, all the dye solutions showed complete reduction of the mutagenic activity using the strains TA98 of *Salmonella* in the absence or presence of the S9 mix, suggesting that this process could be a good option for the removal of disperse azo dyes from aqueous media. The results involving conventional chlorination showed that this method did not remove the mutagenic response from the dyes, and in fact promoted an increase in mutagenic activity in the presence of metabolic activity for Disperse Red 13 (Osugi et al., 2009).

3. Conclusions

The discharge of azo dyes into water bodies presents human and ecological risks, since both the original dyes and their biotransformation products can show toxic effects, mainly causing DNA damage. Azo dyes are widely used by different industries, and part of the dyes used for coloring purposes is discharged into the environment. The azo dyes constitute an important class of environmental mutagens, and hence the development of non-genotoxic dyes and investment in research to find effective treatments for effluents and drinking water is required, in order to avoid environmental and human exposure to these compounds and prevent the deleterious effects they can have on humans and aquatic organisms.

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5. References

Al-Sabti, K. (2000). Chlorotriazine Reactive Azo Red 120 Textile Dye Induces Micronuclei in Fish. *Ecotoxicology and Environmental Safety*, Vol. 47, No. 2, pp.149-155, 2000

An, Y.; Jiang, L.; Cao, J.; Geng, C.; Zhong, L. (2007). Sudan I induces genotoxic effects and oxidative DNA damage in HepG2 cells. *Mutation Research*, Vol. 627, No. 2, pp. 164-70, ISSN 1383-5718

Anliker, R. (1977). Color chemistry and the environment, *Ecotoxicology and Environmental Safety*, Vol. 1, No. 2, pp. 211-237

Anliker, R. (1979). Ecotoxicology of dyestuffs - a joint effort by industry. *Ecotoxicology and Environmental Safety*, Vol. 3, No. 1, pp. 59-74

Arlt, V.M.; Glatt, H.; Muckel, E.; Pabel, U.; Sorg, B.L.; Schmeiser, H.H.; Phillips, D.H. (2002). Metabolic activation of the environmental contaminant 3 nitrobenzanthrone by human acetyltransferases and sulfotransferase. *Carcinogenesis*, Vol. 23, No. 11, pp.1937–1945

Bae, J.S.; Freeman, H.S. (2007). Aquatic toxicity evaluation of new direct dyes to the Daphnia magna. Dyes and Pigments, Vol. 73, No. 1, pp. 81-85, ISSN 0143-7208

Banat, I.M.; Nigam, P.; Singh , D. & Marchant, R. (1996). Microbial decolorization of textile-dyecontaining effluents: a review. *Bioresource Technology,* Vol. 58, No. 3, pp. 217-227, ISSN 0960-8524

Bonser, G.M.; Clayson, D.B.; Jull, J.W. (1963). The potency of 20-methylcholanthrene relative to other carcinogens on bladder implantation. *British Journal of Cancer*, Vol.17, No. 2, pp.235-241

Brown, J.P.; Roehm, G.W.; Brown, R.J. (1978). Mutagenicity testing of certified food colours and related azo, xanthene and triphenylmethane dyes with the Salmonella/microsome system. *Mutation Research*, Vol. 56, No. 1, pp. 249-271 (Abstract)

Carliell, C.M.; Barclay S.J.; Naidoo N.; Buckley C.A.; Mulholland, D.A.; Senior, E. (1995). Microbial decolourisation of a reactive azo dye under anaerobic conditions. Water SA, Vol. 21, pp 61-69

Carneiro, P.A.; Osugi, M.E.; Sene, J.J.; Anderson, M.A.; Zanoni, M.V.B. (2004). Evaluation of color removal and degradation of a reactive textile azo dye on nanoporous TiO2 thin-film electrodes *Electrochimica Acta*, Vol. 49, No. 22-23, pp. 3807-3820, ISSN 0013-4686

Cerniglia, C.E; Zhuo, Z.; Manning, B.W.; Federle, T.W.; Heflich, R.H. (1986). Mutagenic activation of the benzidine-based dye Direct Black 38 by human intestinal microflora. *Mutation Research,* Vol. 175, No. 1, pp. 11-16, ISSN 0165-7992

Chadwick, R.W.; George, S.E.; Claxton, L.D. (1992). Role of the gastrointestinal mucosa and microflora in the bioactivation of dietary and environmental mutagens or carcinogens. *Drug Metabolism Reviews*, Vol. 24, No. 4, pp. 425-492

Chang, J.S.; Chien Chou, C.; Yu-Chih Lin, Y.C.; Ping-Jei Lin, P.J.; Jin-Yen Ho, J.Y. & Hu, T.L.(2001a) Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola. Water Research,* Vol. 35, No. 12, pp. 2841–2850.

Chang, J.S.; Chou, C.& Chen, S.Y. (2001b). Decolorization of azo dyes with immobilized *Pseudomonas luteola. Process Biochemistry,* Vol. 36, No. 8-9, pp. 757–763, ISSN 0032-9592

Chequer, F.M.D.; Angeli, J.P.F.; Ferraz, E.R.A.; Tsuboy, M.S.; Marcarini, J.C.; Mantovani, M.S.; Oliveira, D.P. (2009). The azo dyes Disperse Red 1 and Disperse Orange 1 increase the micronuclei frequencies in human lymphocytes and in HepG2 cells. *Mutation Research*, Vol. 676, pp. 83-86, ISSN 1383-5718

Choudhary, G. (1996). Human health perspectives on environmental exposure to benzidine: a review. Chemosphere, Vol. 32, No. 2, pp. 267-291, ISSN 0045-6535(95)00338-X

Chung, K.T.; Fulk, G.E.; Andrews, A.W. (1978). The mutagenicity of methyl orange and metabolites produced by intestinal anaerobes. *Mutation Research,* Vol. 58, No. 2-3, pp. 375-379

Chung, K.T. (1983). The significance of azo-reduction in the mutagenesis and carcinogenesis of azo dyes. *Mutation Research,* Vol. 114, No. 3, pp.269-281, ISSN 0165-111

Chung, K.T. & Cerniglia, C.E. (1992). Mutagenicity of azo dyes: Structure-activity relationships. *Mutation Research/ Reviews in Genetic Toxicology*, Vol. 277, No. 3, pp. 201–220, ISSN 0165-111

Chung, K.T.; Stevens, S.E; Cerniglia, C.E. (1992) The reduction of azo dyes by the intestinal microflora. *Critical Reviews in Microbiology*, Vol. 18, No. 3, pp. 175-190, ISSN 1040-841X

Chung K. T. & Stevens, S. E. (1993). Degradation of azo dyes by environmental microorganisms and helminths. *Environmental Toxicology and Chemistry*, Vol. 12, No. 11, pp. 2121–2132.

Chung, K.T.; Hughes, T.J.; Claxton, L.D. (2000). Comparison of the mutagenic specifity induced by four nitro-group-containing aromatic amines in *Salmonella typhimurium his* genes. Mutation Research, Vol. 465,No. 1-2, pp. 165-171, ISSN 1383-5718

Collier, S.W.; Storm, J.E.; Bronaugh, R.L. (1993). Reduction of azo dyes during in vitro percutaneous absorption. *Toxicology and Applied Pharmacology*, Vol. 118, No. 1, pp. 73-79, ISSN 0041-008X

Combes, R.D.; Haveland-Smith, R.B. (1982). A review of the genotoxicity of food, drug and cosmetic colours and other azo, triphenylmethane and xanthene dyes. *Mutation Research*, Vol. 98, No. 2, pp.101-248, ISSN 0165-1110

De Flora S.; Camoirano A.; Zanacchi C. (1984). Mutagenicity testing with TA97 and TA102 with 30 DNA damaging compounds, negative with other Salmonella strains. Mutation Research, Vol. 134, No. 2-3, pp. 159-165 ISSN 0165-111

De France, B.F.; Carter, M.H.; Josephy, P.D. (1986). Comparative metabolism and mutagenicity of azo and hydrazone dyes in the Ames test. *Food and Chemical Toxicology*, Vol. 24, No. 2, pp.165-169, ISSN 0278-6915

Denisov, I.G.; Makris, T.M.; Sligar, S.G.; Schlichting, I. (2005). Structure and Chemistry of Cytochrome P450. *Chemical Reviews*, Vol. 105, No. 6, pp. 2253-2277, ISSN 10.1021/cr0307143

Dipple, A.; Michejda, C.J.; Weisburger, E.K. (1985) Metabolism of chemical carcinogens. *Pharmacology & Therapeutics*, Vol.27, pp.265-296, ISSN 0163-7258

Ekici, P.; Leupold, G.; Parlar, H. (2001). Degradability of selected azo dye metabolites in activated sludge systems. *Chemosphere,* Vol. 44, No. 4, pp. 721 – 728, ISSN 0045-6535

Elisangela , F.; Andrea, Z.; Fabio, D.G.; Cristiano, R.M.; Regina, D.L.; Artur, C.P. (2009). Biodegradation of textile azo dyes by a facultative *Staphylococcus arlettae* strain VN-

11 using a sequential microaerophilic/aerobic process. *International Biodeterioration & Biodegradation*, Vol. 63, No. 3, pp. 280-288, ISSN 0964-8305

Esancy, J.F.; Freeman, H.S.; Claxton, L.D. (1990). The effect of alkoxy substituents on the mutagenicity of some aminoazobenzene dyes and their reductive-cleavage products. *Mutation Research*, Vol. 238, No. 1, pp. 1–22, ISSN 0165-111

Fang, H., Wenrong, H., Yuezhong, L. (2004). Biodegradation mechanisms and kinetics of azo dye 4BS by a microbial consortium. *Chemosphere*, Vol. 57, No. 4, pp. 293-301, ISSN 0045-6535.

Ferraz, E.R.A.; Umbuzeiro, G.A.; de-Almeida, G.; Caloto-Oliveira, A.; Chequer, F.M.D.; Zanoni, M.V.B.; Dorta, D.J.; Oliveira, D.P. (2010). Differential Toxicity of Disperse Red 1 and Disperse Red 13 in the Ames Test, HepG2 Cytotoxicity Assay, and Daphnia Acute Toxicity Test. *Environmental Toxicology*, pp. 1-9, DOI 10.1002/tox.20576

Field, F.E.; Roberts, G.; Hallowes, R.C.; Palmer, A.K.; Kenneth E. Williams, K.E.; Lloyd, J.B. (1977). Trypan blue: identification and teratogenic and oncogenic activities of its coloured constituents. *Chemico-Biological Interactions*, Vol. 16,No. 1, pp. 69-88

Forgacs, E.; Cserháti, T.; Oros, G. (2004). Removal of synthetic dyes from wastewaters: a review. *Environment International*, Vol. 30, No. 7, pp. 953-971, ISSN 0160-4120

Forss, J.; Welander, U. (2011). Biodegradation of azo and anthraquinone dyes in continuous systems. *International Biodeterioration & Biodegradation*, Vol. 65, No. 1, pp. 227-237, ISSN 0964-8305

Fracasso, M.E.; Leone, R.; Brunello, F.; Monastra, C.; Tezza, F.; Storti, P.V. (1992). Mutagenic activity in wastewater concentrates from dye plants. *Mutation Research*, Vol. 298, No. 2, pp. 91-95, ISSN 0165-1218

Fujita, S. & Peisach, J. (1978). Liver microsomal cytochromes *P*-450 and azoreductase activity *The Journal of Biological Chemistry*, Vol. 253, No. 13, pp. 4512-4513

Garg, A.; Bhat, K.L.; Bock, C.W. (2002). Mutagenicity of aminoazobenzene dyes and related structures: a QSAR/QPAR investigation. *Dyes and Pigments*, Vol. 55, No. 1, pp. 35-52, ISSN 0143-7208

Garner, R.C. & Nutman, C.A. (1977). Testing of some azo dyes and their reduction products for mutagenicity using Salmonella typhimurium TA 1538. *Mutation Research*, Vol. 44, No. 1, pp. 9–19

Gogate, R.; Pandit, B. (2004). A review of imperative technologies for wastewater treatment I: Oxidation technologies at ambient conditions. *Advances in Environmental Research*, Vol. 8, pp. 501-551, ISSN 1093-0191

Golka, K.; Kopps, S.; Myslak, Z.W. (2004). Carcinogenicity of azo colorants: influence of solubility and bioavailability – a Review. *Toxicology Letters*, Vol. 151, No. 1, pp. 203-210, ISSN 0378-4274

Han, B.; Ko, J.; Kim, J.; Kim, Y.; Chung, W.; Makarov, I.E.; Ponomarev, A.V.; Pikaev, A.K. (2002). Combined electron-beam and biological treatment of dyeing complex wastewater. Pilot plant experiments. *Radiation Physics and Chemistry*, Vol. 64, No. 1, pp. 53–59, ISSN 0969-806X

Hartman, C.P.; Fulk, G.E.; Andrews, A.W. (1978). Azo reduction of trypan blue to a known carcinogen by a cell-free extract of a human intestinal anaerobe. *Mutation Research*, Vol. 58, No. 2-3, pp. 125-132

Hashimoto, Y.; Watanabe, H.; Degawa, M. (1977). Mutagenicity of methoxyl derivatives of N-hydroxy-4-amino-azobenzenes and 4-nitroazobenzene. *Gann*, Vol. 68, No. 3, pp. 373-374

Haveland-Smith, R. B.; Combes. R. D. (1980a). Screening of food dyes for genotoxic activity. Food and Cosmetics Toxicology, Vol. 18, No. 3, pp. 215-221

Haveland-Smith, R.B.; Combes, R.D. (1980b). Genotoxicity of the food colours Red 2G and Brown FK in bacterial systems: use of structurally-related dyes and azo-reduction. *Food and Cosmetics Toxicology*, Vol.18, No. 3, pp.223-228

Hu, T.L. (1994). Decolourization of reactive azo dyes by transformation with *Pseudomonas Luteola. Bioresource Technology*, Vol. 49, No. 1, pp. 47-51, ISSN 0960-8524

Hunger, K. (1994). On the toxicology and metabolism of azo dyes. *Chimia*, Vol. 48, pp. 520-522, ISSN 0009-4293

IARC (1975). Working Group Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Vol. 8; Some Aromatic Azo Compounds, Int. Agency for Research on Cancer, Lyon

Jin, X.C.; Liu, G.Q.; Xu, Z.H.; Tao, W.Y. (2007). Decolourisation of a Dye Industry Effluent by Aspergillus fumigatus XC6. *Applied Microbiology and Biotechnology*, Vol. 74, pp. 239-243

Kennelly, J.C.; Hertzog, P.J.; Martin, C.N. (1982). The release of 4,4'-diaminobiphenyls from azodyes in the rat. Carcinogenesis, Vol. 3, No. 8, pp. 947–951

Kennelly, J.C.; Aidan Shaw, A.; Martin, C.N. (1984). Reduction to benzidine is not necessary for the covalent binding of a benzidine azodye to rat liver DNA. *Toxicology,* Vol. 32, No. 4, pp. 315-324, ISSN 0300-483X

Khalid, A.; Arshad, M.; Crowley, D.E. (2009). Biodegradation potential of pure and mixed bacterial cultures for removal of 4-nitroaniline from textile dye wastewater.Water Research , Vol. 43, No. 4, pp.1110-1116, ISSN 0043-1354

Kleinow, K.M.; Melancon, M.J.; Lech, J.J. (1987). Biotransformation and Induction: Implications for Toxicity, Bioaccumulation and Monitoring of Environmental Xenobiotics in Fish. *Environmental Health Perspectives*, Vol. 71, pp. 105-119

Križanec, B., Marechal, A.M.L. (2006).Dioxins and dioxin-like persistent organic pollutants in textiles and chemicals in the textile sector. *Croatica Chemica Acta*, Vol. 79, pp.177-186, ISSN 0011-1643

Kučerová, M.; Polivkowi, Z.; Gregor, V.; Dolanská, M.; Málek, B.; Kliment, V.; Ždárský, E.; Maroušková, A.; Nováková, J. (1987). The possible mutagenic effect of the occupation of TV announcer. *Mutation Research,* Vol. 192, No. 1, pp. 59-63, ISSN 0165-7992

Kusic, H.; Juretic, D.; Koprivanac, N.; Marin, V.; Božić, A.L. (2011). Photooxidation processes for an azo dye in aqueous media: Modeling of degradation kinetic and ecological parameters evaluation. *Journal of Hazardous Materials*, Vol. 185, No. 2-3, pp. 1558–1568, ISSN 0304-3894

Lima, R.O.A.; Bazo, A.P.; Salvadori, D.M.F.; Rech, C.M.; Oliveira, D.P.; Umbuzeiro, G.A. (2007). Mutagenic and carcinogenic potential of a textile azo dye processing plant effluent that impacts a drinking water source. *Mutation Research*, Vol. 626, No. 1-2, pp. 53-60, ISSN 1383-5718

Livingstone, D.R. (1998) The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, Vol. 120, No. 1, pp. 43-49, ISSN 1095-6433

Lu, K.; Zhang, X.L.; Zhao, Y.L.; Wu, Z.L. (2010). Removal of color from textile dyeing wastewater by foam separation. *Journal of Hazardous Materials*, Vol. 182, No. 1-3, pp. 928-932, ISSN 0304-3894

Madhavi, S.; Revankar, S.; Lele, S. (2007). Synthetic dye decolorization by white rot fungus, Ganoderma sp. WR-1. *Bioresource Technology*, Vol. 98, No. 4, pp. 775-780, ISSN 0960-8524

Maier, J.; Kandelbauer, A.; Erlacher, A.; Cavaco-Paulo, A.; Gübitz, M.G., (2004). A new alkali-thermostable azoreductase from Bacillus sp. strain SF. *Applied and Environmental Microbiology*, Vol. 70, No. 2, pp. 837–844, ISSN 0099-2240

Manning, B.W.; Cerniglia, C.E.; Federle, T.W. (1985). Metabolism of the benzidine-based azo dye Direct Black 38 by human intestinal microbiot. *Applied and Environmental Microbiology*, Vol. 50, No. 1, pp. 10-15, ISSN 0099-2240

Mansuy, D. (2007). A brief history of the contribution of metalloporphyrin models to cytochrome P450 chemistry and oxidation catalysis. *Comptes Rendus Chimie*, Vol.10, No. 4-5, pp. 392-413, ISSN 1631-0748

Mantzavinos, D. & Psillakis, E. (2004). Enhancement of biodegradability of industrial wastewaters by chemical oxidation pre-treatment. *Journal of Chemical Technology and Biotechnology*, Vol. 79, pp. 431 – 454

Matsushima, T.; Teichman, B.; Samamura, M.; Sugimura, T. (1978). Mutagenicity of azo-compounds, Improved method for detecting their mutagenicities by the Salmonella mutation test. Mutation Research, Vol. 54, No. 2, pp. 220-221 (abstract)

Meal, P.F.; Cocker, J.; Wilson, H.K.; Gilmour, J.M. (1981). Search for benzidine and its metabolites in urine of workers weighing benzidine-derived dyes. *British Journal of Industrial Medicine*, Vol. 38, No. 2, pp. 191-193

Mielgo, I., Moreira, M.T., Feijoo, G., Lema, J.M., 2001. A packed-bed fungal bioreactor for continuous decolourisation of azo-dyes (Orange II). *Journal of Biotechnology*, Vol. 89, No. 2-3, pp. 99-106, ISSN 0168-1656

Miller, J. A., Miller, E. C.; Baumann, C. A. (1945). On the methylation and demethylation of certain carcinogenic azo dyes in the rat. *Cancer Research,* Vol. 5, pp. 162-168

Miller, E. C., Plescia, A. M., Miller, J. A. & Heidelberger, C. (1952). The metabolism of methylated aminoazo dyes. I. The demethylation of 3'-methyl-4-dimethyl-C^{14}-aminoazobenzene in vivo. *The Journal of Biological Chemistry,* Vol. 196, pp. 863-874

Modi, H.A.; Garima Rajput, G.; Ambasana, C. (2010). Decolorization of water soluble azo dyes by bacterial cultures, isolated from dye house effluent. *Bioresource Technology,* Vol. 101, No. 16, pp. 6580–6583, ISSN 0960-8524

Mueller, G. C. & Miller, J. A. (1953). The metabolism of methylated aminoazo dyes. II. Oxidative demethylation by rat liver homogenates. *The Journal of Biological Chemistry,* Vol. 202, pp. 579-587

Nam, S. & Renganathan, V. (2000). Non-enzymatic reduction of azo dyes by NADH. *Chemosphere*, Vol. 40, No. 4, pp. 351-357, ISSN 0045-6535

Nestmann, E.R.; Kowbel, D.J.; Wheat , J.A. (1981) Mutagenicity in Salmonella of dyes used by defence personnel for the detection of liquid chemical warfare agents, Carcinogenesis, Vol. 2, No. 9, pp. 879-883.

Nigam, P.; Armour, G.; Banat, I.M.; Singh, D.; Marchant, R. (2000). Physical removal of textile dyes from effluents and solid-state fermentation of dye-adsorbed agricultural residues. *Bioresource Technology*, Vol. 72, No. 3, pp. 219-226, ISSN 0960-8524

Novotný, C.; Svobodová, K.; Kasinath, A.; Erbanová, P., 2004. Biodegradation of synthetic dyes by *Irpex lacteus* under various growth conditions. *International Biodeterioration & Biodegradation*, Vol. 54, No. 2-3, pp. 215-223, ISSN0964-8305

Oliveira, D.P.; Carneiro, P.A.; Sakagami, M.K.; Zanoni, M.V.B.; Umbuzeiro, G.A. (2007) Chemical characterization of a dye processing plant effluent—Identification of the mutagenic components. *Mutation Research,* Vol. 626, No. 1-2, pp. 135-142, ISSN 1383-5718

Oliveira, G.A.R.; Ferraz, E.R.A.; Chequer, F.M.D.; Grando, M.D.; Angeli, J.P.F.; Tsuboy, M.S.; J.C. Marcarini, J.C.; Mantovani, M.S.; Osugi, M.E.; Lizier, T.M.; Zanoni, M.V.B.; Oliveira, D.P. (2010). Chlorination treatment of aqueous samples reduces, but does not eliminate, the mutagenic effect of the azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. *Mutation Research*, Vol. 703, No.2, pp. 200-208, ISSN 1383-5718

Osugi, M.E.; Rajeshwar, K.; Ferraz, E.R.A.; Oliveira, D.P.; Araújo, A.R.; Zanoni, M.V.B. (2009). Comparison of oxidation efficiency of disperse dyes by chemical and photoelectrocatalytic chlorination and removal of mutagenic activity. *Electrochimica Acta*, Vol. 54, No. 7, pp. 2086-2093, ISSN 0013-4686

Öztürk, A.; Abdullah, M.I. (2006). Toxicological effect of indole and its azo dye derivatives on some microorganisms under aerobic conditions, Science of the Total Environment, Vol. 358, No. 1-3, pp. 137-142, ISSN 0048-9697

Pálfi, T.; Wojnárovits, L.; Takács, E. (2011). Mechanism of azo dye degradation in Advanced Oxidation Processes: Degradation of Sulfanilic Acid Azochromotrop and its parent compounds in aqueous solution by ionizing radiation. *Radiation Physics and Chemistry*, Vol. 80, No. 3, pp. 462-470, ISSN 0969-806X

Pandey, A.; Singh, P.; Iyengar, L. (2007). Review: Bacterial decolorization and degradation of azo dyes. *International Biodeterioration & Biodegradation* Vol. 59, No. 2, pp. 73-84, ISSN 0964-8305

Pielesz, A. (1999). The process of the reduction of azo dyes used in dyeing textiles on the basis of infrared spectroscopy analysis. *Journal of Molecular Structure* Vol. 511-512, No. 23, pp. 337-344, ISSN 0022-2860

Pielesz, *A.;* Baranowska, I.; Rybak, A.; Włochowicz, A. (2002). Detection and Determination of Aromatic Amines as Products of Reductive Splitting from Selected Azo Dyes. *Ecotoxicology and Environmental Safety*, Vol. 53, No. 1, pp. 42-47, ISSN 0147-6513

Pinheiro, H.M.; Touraud, E.; Thomas, O. (2004). Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. *Dyes and Pigments,* Vol. 61, No.2, pp. 121–139, ISSN 0143-7208

Platzek, T.; Lang, C.; Grohmann, G.; Gi, U.S.; Baltes, W. (1999). Formation of a carcinogenic aromatic amine from an azo dye by human skin bacteria in vitro. *Human & Experimental Toxicology*, Vol. 18, No. 9, pp. 552-559

Prival, M.J. & Mitchell, V.D. (1982). Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavin mononucleotide and hamster liver S9. *Mutation Research*, Vol. 97, No. 2, pp.103-116, ISSN 0165-116

Rafii, F.; Hall, J. D.; Cerniglia, C.E. (1997). Mutagenicity of azo dyes used in foods, drugs and cosmetics before and after reduction by *Clostridium* species from the human intestinal tract. *Food and Chemical Toxicology*, Vol. 35, No. 9, pp. 897-901, ISSN 0278-6915

Rai, H.; Bhattacharya, M.; Singh, J.; Bansal, T.K.; Vats, P.; Banerjee, U.C. (2005). Removal of Dyes from the Effluent of Textile and Dyestuff Manufacturing Industry: A Review of Emerging Techniques with Reference to Biological Treatment. *Critical Review in Environmental Science and Technology*, Vol. 35, pp. 219-238, ISSN 1064-3389

Rajaguru, P.; Fairbairn, L.J.; Ashby, J.; Willington M.A.; Turner,S.; Woolford, L.A.; Chinnasamy, N.; Rafferty, J.A. (1999). Genotoxicity studies on the azo dye Direct Red 2 using the in vivo mouse bone marrow micronucleus test. *Mutation Research*, Vol. 444, pp.175-180, ISSN 1383-5718

Reid, T. M.; Morton, K. C.; Wang, C. Y.; King C. M. (1983) Conversion of Congo red and 2-azoxyfluorene to mutagens following *in vitro* reduction by whole-cell rat cecal bacteria. *Mutation Research,* Vol. 117, pp. 105-112, ISSN 0165-121

Revankar, M.S.; Lele, S.S. (2007). Synthetic dye decolorization by white rot fungus, *Ganoderma* sp. WR-1. *Bioresource Technology*, Vol. 98, No. 4, pp. 775-780, ISSN 0960-8524

Rinde, E.; Troll, W. (1975). Metabolic reduction of benzidine azo dyes to benzidine in the rhesus monkey. *Journal of the National Cancer Institute*, Vol. 55, No. 1, pp. 181-182

Robens, J. F.; Dill, G. S., Ward, J. M.; Joiner, J. R.; Griesemer, R. A.; Douglas, J. F. (1980). Thirteen-week subchronic toxicity studies of direct blue 6, direct black 38 and direct brown 95 dyes. *Toxicology and Applied Pharmacology*, Vol. 54, pp. 431-442, ISSN 0041-008X

Robinson, T.; McMullan, G.; Marchant, R.; Nigam, P. (2001a). Remediation of Dyes in Textile Effluent: A Critical Review on Current Treatment Technologies with a Proposed Alternative. *Bioresource Technology* Vol. 77, No. 3, pp. 247-255, ISSN 0960-8524

Robinson, T.; Chandran, B.; Nigam, P. (2001b). Studies on the production of enzymes by white-rot fungi for the decolorisation of textile dyes. *Enzyme and Microbial Technology*, Vol. 29, pp. 575–579, ISSN 0141-0229

Robinson, T.; Chandran, B.; Nigam, P. (2002). Removal of dyes from a synthetic textile dye effluent by biosorption on apple pomace and wheat straw. Water Research Vol. 36, No. 11, pp. 2824-2830, ISSN 0043-1354

Saranaik, S., Kanekar, P. (1995). Bioremediation of color of methyl violet and phenol from a dye industry waste effluent using Pseudomonas sp. isolated from factory soil. *The Journal of Applied Bacteriology,* Vol. 79, pp. 459-469

Sarasa, J.; Roche, M.P.; Ormad, M.P.; Gimeno, E.; Puig, A.; Ovelleiro, J.L. (1998). Treatment of a wastewater resulting from dyes manufacturing with ozone and chemical coagulation, Water Research, Vol. 32, No. 9, pp. 2721 – 2727, ISSN 0043-1354

Saratale, R.G.; Saratale, G.D.; Chang, J.S. & Govindwar, S.P. (2011). Bacterial decolorization and degradation of azo dyes: A review. *Journal of the Taiwan Institute of Chemical Engineers*, Vol. 42, No. 1, pp. 138–157, ISSN 1876-1070

Saupe, A. (1999). High-rate biodegradation of 3- And 4-Nitroaniline. *Chemosphere,* Vol. 39, No. 13, pp. 2325-2346, ISSN 0045-653

Shaul, G.M.; Holdsworth, T.J.; Dempsey, C.R.; Dostal, K.A. (1991). Fate of water soluble azo dyes in the activated sludge process. *Chemosphere*, Vol. 22, No.1-2, pp. 107-119, ISSN 1045-535

Shore, J. (1996). Advances in direct dyes. *Indian Journal of Fibers and Textile Research*, Vol. 21, pp. 1-29

Slokar, Y.M.; Marechal, A.M.L. (1998). Methods of decoloration of textile wastewaters. *Dyes and Pigments,* Vol. 37, No. 4, pp. 335-356, ISSN 0143-72081

Sole, G.M. & Chipman, J.K. (1986) The mutagenic potency of chrysoidines and Bismark brown dyes. *Carcinogenesis*, Vol. 7, No. 11, pp. 1921-1923.

Spadaro, J.T.; Gold, M.H.; Renganathan, V., 1992. Degradation of azo dyes by the lignin degrading fungus Phanerochaete chrysosporium. *Applied and Environmental Microbiology,* Vol. 58, No. 8, pp. 2397–2401, ISSN 0099-2240

Stahlmann, R.; Wegner, M.; Riecke, K.; Kruse, M.; Platzek, T. (2006). Sensitising potential of four textile dyes and some of their metabolites in a modified local lymph node assay. *Toxicology*, Vol. 219, No. 1-3, pp. 113–123, ISSN 0300-483X

Stiborová, M.; Martínek, V.; Rýdlová, H.; Hodek, P., Frei, E. (2002). Sudan I is a potential carcinogen for humans: evidence for its metabolic activation and detoxication by human recombinant cytochrome P450 1A1 and liver microsomes. *Cancer Research*, Vol. 62, pp. 5678-5684

Stiborová, M.; Martínek, V.; Rýdlová H.; Koblas, T.; Hodek, P. (2005). Expression of cytochrome P450 1A1 and its contribution to oxidation of a potential human carcinogen 1-phenylazo-2-naphthol (Sudan I) in human livers. *Cancer Letters*, Vol. 220, No. 2, pp. 145-154, ISSN 0304-3835

Sweeney, E.A.; Chipman, J.K.; Forsythe, S.J. (1994).Evidence for Direct-acting Oxidative Genotoxicity by Reduction Products of Azo Dyes. *Environmental Health Perspectives*, Vol. 102, No. 6, pp. 119-122, 1994.

Tsuboy, M.S.; Angeli, J.P.F.; Mantovani, M.S.; Knasmüller, S.; Umbuzeiro, G.A.; Ribeiro, L.R. (2007). Genotoxic, mutagenic and cytotoxic effects of the commercial dye CI Disperse Blue 291 in the human hepatic cell line HepG2. Toxicology *in Vitro*, Vol. 21, No. 8, pp. 1650-1655, ISSN 0887-2333

Umbuzeiro, G.A.; Freeman, H.; Warren, S.H.; Kummrow, F.; Claxton, L.D. (2005a). Mutagenicity evaluation of the commercial product C.I. Disperse Blue 291 using different protocols of the Salmonella assay. *Food and Chemical Toxicology,* Vol. 43, No. 1, pp. 49-56, ISSN 0278-6915

Umbuzeiro, G.A.; Freeman, H.S.; Warren, S.H.; Oliveira, D.P.; Terao, Y.; Watanabe, T.; Claxton, L.D. (2005b). The contribution of azo dyes to the mutagenic activity of the Cristais River. *Chemosphere*, Vol. 60, No. 1, pp. 55 – 64, ISSN 0045-6535

US EPA, 1989. Aerobic and anaerobic treatment of C.I. Disperse blue 79. US Department of Commerce, National Technical Information Service (NTIS) (1989) vols. I and II, EPA/600/2-89/051 (PB 90-111642)

Van der Zee, F.P. & Villaverde, S. (2005) Combined anaerobic–aerobic treatment of azo dyes—A short review of bioreactor studies. Water Research, Vol. 39, No. 8, pp. 1425–1440, ISSN 0043-1354

Venturini, S. & Tamaro, M. (1979). Mutagenicity of anthraquinone and azo dyes in Ames' *Salmonella typhimurium* test, Mutation Research, Vol. 68, No. 4, pp. 307-312.

Walker, R. (1970). The Metabolism of Azo Compounds: A Review of the Literature. *Food and Cosmetics Toxicology,* Vol. 8, No. 6, pp. 659-676

Walker, R. & Ryan, A.J. (1971) Some molecular parameters influencing rate of reduction of azo compounds by intestinal microflora. *Xenobiotica*, Vol. 1, No. 4-5, pp. 483-486

Watabe, T.; Ozawa, N.; Kobayashi, F.; Kuruta, H. (1980). Reduction of sulphonated water-soluble azo dyes by micro-organisms from human faeces. *Food and Cosmetics Toxicology*, Vol.18, No. 4, pp. 349-352 ISSN 0015.6264

Weisburger, J.H. (1997). A perspective on the history and significance of carcinogenic and mutagenic N-substituted aryl compounds in human health. *Mutation Research,* Vol. 376, No. 1-2, pp. 261–266, ISSN 0027-5107

Weisburger, J.H. (2002). Comments on the history and importance of aromatic and heterocyclic amines in public health. *Mutation Research*, Vols. 506–507, pp. 9–20, ISSN 0027-5107

Wolff, A. W.; Oehme, F.W. (1974). Carcinogenic chemicals in food as an environmental issue. Journal of the American Veterinary Medical Association, Vol. 164, pp. 623-629

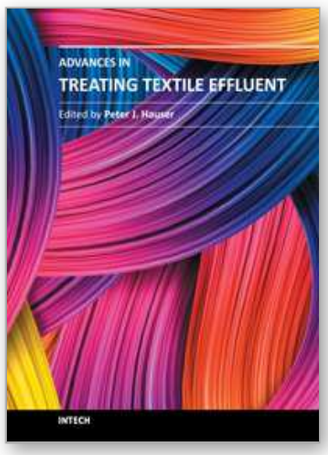
Xu, H.; Heinze, T.M.; Donald D. Paine, D.D.; Cerniglia, C.E.; Chen, H. (2010). Sudan azo dyes and Para Red degradation by prevalent bacteria of the human gastrointestinal tract. *Anaerobe*, Vol. 16, No. 2, pp. 114-119, ISSN 1075-9964

Zbaida, S.; Stoddart, A.M.; Levine, W.G. (1989). Studies on the mechanism of reduction of azo dye carcinogens by rat liver microsomal cytochrome P-450. *Chemico-Biological Interactions*, Vol. 69, No. 1, pp.61-71, ISSN 0009-279

Zhao,X & Hardin, I.R. (2007). HPLC and spectrophotometric analysis of biodegradation of azo dyes by Pleurotus ostreatus. *Dyes and Pigments,* Vol. 73, No. 3, pp. 322-325, ISSN 0143-7208

Zollinger, H (1987). *Colour Chemistry – Synthesis, Properties of Organic Dyes and Pigments*, p. 92-102, VCH Publishers, New York, USA

Zollinger, H. (1991). *Color chemistry: syntheses, properties and applications of organic dyes and pigments*. 3nd ed., Wiley – VCH, ISBN 978 3906 39023 9, New York, USA



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