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# Azo Dyes: Characterization and Toxicity– A Review

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## Abstract

The emission of effluents from textile industries has been a major concern of the modern world, due to the great pollution that these effluents promote on the water resources. Among the synthetic dyes released in effluents from textile industries, azo dyes is one of the more detrimental classes because it is highly persistent in the aquatic environment, due to its chemical composition, involving aromatic rings, azoic linkages and amino groups. This review aimed to gather information on the importance of the production and industrial use of azo dyes, as well as present some studies that have been developed to evaluate the toxicity of such chemical compounds and their metabolites on different living organisms. This paper presents some considerations on the importance of the biological treatment of textile effluents, the discovery of microorganisms capable of degrading azo dyes efficiently in order to reduce potential risks of these dyes to organisms and environment.

## Keywords

*Dyes; Textile Effluents; Cytotoxicity; Genotoxicity; Mutagenicity; Carcinogenicity; Bioremediation*

## Introduction

The use of dyes is a very mature practice used to modify the colour characteristics of different substrates, such as fabric, paper, leather, among others [1, 2]. Before the mid nineteenth century, substances with colouring properties were extracted from natural sources, mainly from animals or vegetables. However, natural dyes were almost completely replaced by the synthetic in the beginning of the twentieth century. Today, virtually, all dyes and pigments commercially available are synthetic substances, with exception of some inorganic pigments. Every year, hundreds of new coloured compounds flooded the market and developed into a series of different applications [3].

Dyes and several organic compounds used for dyeing which are chemical substances have been already incorporated by the technology of our daily life. The

global consumption of dyes and pigments approximates  $7 \times 10^5$  tons/year and only in the textile industry it consumes about two-thirds of all the world production [4, 5]. According to Guaratini and Zanoni [6], in Brazil, a decade ago, 26,500 tons of dyes were consumed every year, which corresponded to 3.8% of all the dye produced in the world.

During the textile process, inefficiency in the colouring generates large amounts of dyes residues, which are directly released into water bodies, consequently, contaminating the environment. In the dyeing processes, enormous quantities of pollutants are discharged in aquatic bodies, resulting from impurities of the removal of the crude material and residual chemical reagents used in such processes [7]. Residues of dyes either are discharged in waters that pass by treatment systems of the companies or are released directly into the environment, causing a severe contamination of water bodies, which is mainly observed and aggravated next to areas with the presence of many textile industries [8, 9].

Among the residues of dyes that pollute environment, there are azo dyes that are discharged in large quantities, directly in water bodies, characterizing an important way of environmental contamination [10]. According to Nam and Renganathan [11] and Jarosz-Wilkolazka et al. [12], about 10 to 15% of the total dye used by the industries are lost during the dyeing process and, thus, are being released into the environment. According to O'Neill et al. [13], these values can be even higher, reaching until 50%. However, the exact data of the amount of dyes released into the environment are not yet fully known [14]. In Brazil, the textile industry is responsible for the generation of great volumes of residues, with high organic load and strong colouration, which represents a major environmental problem generated by the textile sector.

According to Kirk-Othmer [15], dyes can be classified into:

- acid dyes: anionic dyes, soluble in water, with one or more sulphonic or carboxylic acid groups in their molecules and, chemically, constituted by compounds azo, anthraquinones and triarylmethanes, iminoacetone, nitro, nitrous and quinoline, with application in nylon, silk, modified acrylic, wool, paper, food and cosmetics;

- basic dyes: cationic dyes, soluble in water, producers of colouring cationic compounds in solution and chemically constituted by compounds azo, anthraquinone, triarylmethane, methane, thiazine, oxazine, acridine and quinoline, with application in modified acrylic, modified nylon, modified polyesters and papers, and some of them having biological activity are used in medicine as antiseptics;

- direct dyes: anionic compounds, soluble in water, when in the presence of electrolytes (salts that increase their affinity for the fibre). Chemically they are constituted by azo compounds, with thiazoles, phtalocyanines and oxazines, with application in the dyeing of cotton and regenerated cellulose, paper, leather and nylon;

- fluorescent dyes (group of the xanthenes): colourless compounds that absorb incident ultraviolet light and re-emit in the visible region (blue) of the spectrum. In fact, they are not dyes, but due to the wide application in fabrics and other materials, the Colour Index made their classification within this group of chemicals;

- reactive dyes: compounds of very simple chemical structure, with absorption spectrum presenting narrow range of capitation and dyeing possessing brilliant characteristics. Chemically they are constituted by azo compounds, anthraquinones and phtalocyanines, with high fixing property by simple dyeing methods, making covalent bridges with the fibre (cotton, wool or nylon), by the compatible hydroxyl group of cellulose;

- sulphurous dyes: small group of dyes, however, with low cost and good fixing properties. They are applied to cotton, after alkaline reduction bath, with sodium sulphite as reducing agent;

- vat dyes: insoluble compounds in water and applied, mainly, to cellulosic fibres, such as leuco-soluble salts, after reduction in alkaline bath, normally with sodium hydrosulphite. After exhaustion of the fibre, they are re-oxidized to the keto-insoluble form and after

treatment normally by soda, develop crystalline structure. Chemically they are the anthraquinones and indigo;

- dye precursors: dyes obtained from raw materials. This group has simple chemical characteristic, such as benzene and naphthalene, whose colour is given by a variety of chemical reactions. Normally they are cyclic aromatic compounds and derivatives, mainly of petroleum and coal.

According to Guaratini and Zanoni [6], there are still the dispersive dyes which are water insoluble products, applied to cellulose fibres and other hydrophobic fibres by suspension. During the dyeing process, the dye suffers hydrolysis and the originally insoluble formation is slowly precipitated in the disperse form on the cellulose acetate. Generally, the process occurs in the presence of dispersing agents of long chains which stabilize the dye suspension, and facilitate the contact with the hydrophobic fibre. This class of dyes is mainly constituted by azo dyes, and has been used in the dyeing of synthetic fibres, such as cellulose acetate, nylon, polyester and polyamide.

According to Majcen-le Marechal et al. [3], there are more than 3,000 different dyes available in the market and half of them belong to the azo dyes compounds class. These dyes are used in the textile industry for the colouring of polyester, nylon, cellulose diacetate and triacetate and acrylic fibres [9], and are also used as additive in products derived from the petroleum and in the dyeing of leather, paints, plastics, papers, wood, oils, cosmetics, pharmaceuticals, metals and food [8]. In addition to its versatility, due to the diversity of applications, there are other advantages in using azo dyes in industries. These chemical compounds are easily synthesized, have excellent fixative and permanency properties and present a great variety of colours, when compared to natural dyes [16, 17].

Azo dyes are compounds characterized with the presence of one or more azo groups ( $-N=N-$ ), usually in number of one or four, linked to phenyl and naphthyl radicals, which are usually replaced with some combinations of functional groups including: amino ( $-NH_2$ ), chlorine ( $-Cl$ ), hydroxyl ( $-OH$ ), methyl ( $-CH_3$ ), nitro ( $-NO_2$ ), sulphonic acid and sodium salts ( $-SO_3Na$ ) [18]. Azo dyes, synthesized from aromatic compounds, are not basic in aqueous solution (due to the presence of the linkage  $N=N$ , which reduces the possibility of unpaired electron pairs in nitrogen atoms), are readily reduced to hydrazines and primary

amines, functioning as good oxidizing agents [19].

On the one hand, the azo dyes meet the needs of man, on the other hand, they entail ecological and sanitary changes in the hydric resources, soil and atmosphere. The presence of dyes in the aquatic bodies leads to an aesthetic problem and can have a negative impact on public health [20]. However, several liquid and solid effluents of textile industries are treated before being released into the environment, which reduces the impact of these agents on the aquatic environment.

Despite the difficulty in the treatment of the residues generated and the adverse indications for their use, azo dyes, especially sulphurous, are widely used for dyeing fibres. This is mainly due to its affordable cost and its good fixative characteristics [21].

Several countries have adopted environmental legislation and requirements to restrict the use of hazardous chemicals in the production of textiles and clothing and one of the most known laws in this issue is the Second Amendments to the Consumer Protection Act, elaborated by the German government in 1994, prohibiting the use of azo dyes. According to the German legislation, some azo dyes are considered allergenic (Disperse Yellow 1/3; Disperse Orange 3/37/76; Disperse Red 1; Disperse Blue 1/35/106/124) and some are considered carcinogenic (Acid Red 26, Basic Red 9, Basic Violet 14, Direct Black 38, Direct Red 28, Direct Blue 6, Disperse Yellow 3, Disperse Orange 11, Disperse Blue 1) [22]. In addition, other European countries, such as Sweden, France and Denmark, formulated their own legislation for azo dyes [22]. The Portuguese government, for example, published the Decree-Law nº 208/2003 [23], which states: Azo dyes that, by reductive cleavage of one or more azo groups, can release one or more aromatic amines in detectable concentrations, i.e., higher than 30 ppm, cannot be used in textile and leather articles susceptible to enter in direct and prolonged contact with human skin or with the oral cavity.

It was stated that azo dyes, after cleavage, present the capacity to release aromatic amines considered as carcinogenic, the European Union, by the Directive 2002/61/EC, reformulated by the Directive 2004/21/CE, has banned the use of these dyes used in the production of textile articles that enter in contact with skin or mouth. These Directives also establish that the referred textile articles cannot contain the 22 amines listed in the legislation, in a concentration higher than 30 ppm and, if the articles are made of recycled fibres, they cannot contain more than 70 ppm [22, 24].

According to Umbuzeiro et al. [25], the black commercial dye (BDCP - Black Dye Commercial Product), widely used in the dyeing industry of synthetic fibres, is composed by 3 dyes belonging to the group of nitro-aminobenzenes: C.I. Disperse Blue 373, C.I. Disperse Violet 93 and C.I. Disperse Orange 37. According to Oliveira (2005) [2] and USEPA (1994) [17], BDCP is an organic compound that belongs to the class of disperse dyes with azo function, which has, at least, one azo bond, besides presenting insolubility in water and good fixing to natural and synthetic fibres. Guaratini and Zanoni [6] cited that in the dyeing process of fibres with azo dyes, there is impregnation of the fibre with a compound soluble in water (coupling agent), which presents high affinity for cellulose. The addition of a diazonium salt ( $RN_2^+$ ) provokes a reaction with the coupling agent already fixed to the fibre, producing a dye insoluble in water. Thus, the dye is formed directly on the fibre, allowing that this process provides good results, such as high fixation pattern and high resistance to light and humidity. Due to the fact that these compounds are insoluble in water, dispersing agents are added to the dye to produce finely divided particles. This mixture results in a stable dispersion in the dye bath.

### Toxicity of Azo Dyes

A great variety of substances derived from dyes have been tested, in laboratorial animals, to determine the real toxic effects of these compounds on living organisms [26]. Studies that assess the toxicity of azo dyes and metabolites related to their degradation are important for the establishment of strategies to reduce the harmful effects of these chemicals [27, 2].

The evaluation of the toxicity of textile dyes is very important, mainly due to the different effects that they cause in the environment and the organisms exposed to them. The biological activities also differ greatly between the dyes and, despite the similarities of the structures, the toxicological properties cannot be generalized according to the reference of only one chemical group [3].

The uncontrolled discharge of azo dyes in water bodies causes serious environmental problems, such as: reduction of the light absorption due to the organisms that inhabit the aquatic environments and production of different amines under anaerobic conditions [28-30].

The acute toxicity of azo dyes, according to the criteria of the European Union for the classification of

dangerous substances, is low and the values of LD<sub>50</sub> are 250-2000 mg/Kg body weight [31]. Some azobasic, acid and direct dyes are classified into very toxic or toxic to fishes, crustaceans, algae and bacteria, while reactive azo dyes are toxic only at very high concentrations (Effective Concentration Levels >100 mg/L), therefore, excluded from considering toxic for aquatic organisms. [32].

Acute toxicity of azo dyes, defined by the criteria of the European Union for the classification of dangerous substances, is very low, and only few of them have values of LD<sub>50</sub> below 250 mg/kg body weight [33]. However, the occupational sensitivity to azo dyes has been shown in textile industries since 1930 [34], like, for some disperse dyes (monoazo or anthraquinone) that were involved with allergic reactions [35].

Studies showed the presence of some azo dyes in certain algae and plants [36], and also have shown the adverse effects for aquatic microbial populations exposed to effluents containing dyes [37].

Several studies shows that the release of azo dyes into the environment is alarming due to the toxic, mutagenic and carcinogenic characteristics of these dyes and of their biotransformation products [38], which can cause different damages to the organisms exposed.

Amin et al. [39] evaluated the toxic effects of two azo dyes used as food additives, tartrazine and carmoisine, by oral administration of two concentrations (one low and other high), in albino male rats, for 30 days. It was measured the quantities of ALT, AST, ALP, urea, creatinine, total protein, albumin, lipid profile, blood glucose in serum, and estimated the activities of GSH, catalase, SOD and MDA in the hepatic tissue of the animals. Data showed a significant increase in the rates of ALT, AST, ALP, urea, creatinine, total protein and albumin in the serum of rats treated with tartrazine and carmoisine, especially in the higher concentrations. The activities of GSH, SOD and catalase decreased and MDA increased in the tissues of rats fed with the high dose of tartrazine and different doses of carmoisine. It is concluded, therefore, that both azo dyes affected adversely and altered the biochemical markers of vital organs such as liver and kidney, not only in higher concentrations but also in the lowers. Tartrazine and carmoisine not only cause changes in the hepatic and renal parameters but their effects become a risk to the organisms at higher doses, since it can induce oxidative stress by means of the formation of free radicals.

Studies have also shown the presence of azo dyes in different samples of water and sediments. Studies performed by Umbuzeiro et al. [40], in the Salmonella/Microsome test, showed a low to moderate mutagenic activity in Cristais River (Cajamar/SP), due to the presence of azo dyes, nitroaromatic compounds and aromatic amines. Another study carried out by Umbuzeiro et al. [25] detected the presence of dyes in all the samples collected (effluent of the dyeing industry, raw water and water treatment station), and associated the mutagenicity of these samples mainly of the raw water with the presence of dyes and colourless polycyclic nitroaromatic compounds, possibly generated during the treatment of the effluent. Oliveira [2] also showed the presence of components of the black commercial dye (BDGP) and aromatic amines in the raw and treated effluents discharged by a dyeing industry, indicating that the industrial treatment was not efficient for the removal of these compounds, which corroborated some studies performed by some authors [14, 28, 41] showing that activated sludge systems were not efficient in the removal of azo dyes present in industrial effluents.

Maguire and Tkacz [42] detected 15 different dyes in samples of water, suspended solids and sediments of a river of Canada, and 3 of which were identified as: C.I. Disperse Blue 79, C.I. Disperse Blue 26 and C.I. Disperse Red 60. Oliveira [2] showed that the presence of about 1 µg of C.I. Disperse Blue 373 and 10 µg C.I. Disperse Orange 37, for each 1 g of the sediment of two distinct environmental samples (one located immediately below the discharge of the effluent of a textile industry and the other from a collection site situated at the entrance of the water treatment station for public supply), which characterizes high rates of mutagenic activity for these two samples. These same dyes were detected in water samples in the same area analyzed by Umbuzeiro et al. [25].

Some azo dyes only exhibit mutagenic activity when the azo bond is reduced. The aromatic amines formed can be more or less carcinogenic and/or mutagenic, in relation to the original compound, depending on their chemical structure [25]. According to Plumb et al. [43] and Yoo et al. [44], these aromatic amines are always more dangerous than the original compounds and may have toxic [45, 46]), mutagenic and carcinogenic actions [47]. The reduction of these azo dyes can generate DNA adducts [48, 49], which can lead to toxic effects, even for the microorganisms that act in the discolouration of azo dyes [29, 50-53].

According to Biswas and Khuda-Bukhsh [54], the azo dye *p*-dimethylaminobenzene (*p*-DAB) caused cytotoxic and genotoxic effects in the chromosome aberrations test, micronucleus test and mitotic index in bone marrow cells and spermatozooids of rats. Rats fed chronically with *p*-DAB resulted in an increase in the number of chromosome aberrations and nuclear abnormalities in germ cells, when compared to the control group.

Al-Sabti [55] observed mutagenic effects for a textile azo dye, the "chlorotriazine reactive azo red 120", by the induction of micronuclei in erythrocytes of fishes. Some authors [56] concluded, according to studies performed with mammalian cells, that some textile dyes induce the formation of micronuclei by mechanisms of clastogenicity.

Researchers [57] showed genotoxicity and mutagenicity of the C.I. Disperse Blue 291 dye, based on the induction of fragmentation in the DNA, formation of cell bearing micronuclei and increase in the index of apoptosis in mammalian cells (HepG2).

Caritá and Marin-Morales [58] showed the mutagenic potential of certain concentrations of industrial effluents contaminated by azo dyes, by the means of micronucleus and chromosome aberration tests with the *Allium cepa* test-organism.

Matsuoka et al. [59] showed that the compounds of PBTA1 and PBTA2 and their respective precursor azo dyes are cytotoxic for the hamster cells CHL and V79-MZ, inducing the formation of micronucleated cells, multilobulated nuclei and highly condensed and binucleated cells. The precursor azo dye of PBTA1 also induced the endoreduplication in V79-MZ hamster cells. Probably, these compounds affect not only the DNA, but also structural and regulatory proteins involved in the cell division process.

In a literature review [60], it was described the mutagenic activity of several azo dyes by the Ames test. C.I. Solvent Yellow 14, C.I. Pigment Solvent Yellow 7, C.I. Pigment Orange 5, C.I. Pigment Red 4 and C.I. Pigment Red 23 were considered mutagenic, while C.I. Pigment Red 3 was considered weakly mutagenic. C.I. Pigment Red 53:1 C.I. Pigment Red 57:1 did not present mutagenic action and this must be associated with the formation of sulphated aromatic amines that are not genotoxic.

Chequer et al. [61] showed the mutagenicity of the azo dyes C.I. Disperse Red 1 and C.I. Disperse Orange 1, extensively used in several countries, by the increase

in the dose-response of the micronuclei frequency in human lymphocytes and mammalian cells (HepG2), when compared to the negative control group.

Some studies [62] performed with the assays of *Salmonella*, micronuclei and comet, showed that 10 commercial products containing azo dyes presented genotoxic action for bacteria and human keratinocytes. Another study [63], also using the *Salmonella* assay, besides the mouse lymphoma assay, showed that 15 of 53, i.e., approximately 28% of the samples of textile dyes tested were positive for the Ames test and 60% of the samples that presented positive responses to *Salmonella*, also induced genotoxic effects in the mouse lymphoma assay.

Sudan azo dyes induce genotoxic effects and the monoazo Sudan 1 dye is considered a carcinogen of liver and bladder for mammals and of mutagenic potential for humans, while Sudan 2 is considered genotoxic for hepatic cells of rats. Now, Sudan 4 requires reduction and microsomal activation to be mutagenic [64].

Ventura-Camargo et al. [65] showed, through chromosome aberration assay, chromosome banding and FISH, that a commercial azo dye that confers black colouration (BDCP – Black Dye Commercial Product) to textile products, in concentrations of 1, 10, 100 and 1000 µg/L, is cytotoxic, genotoxic and mutagenic to meristematic cells of *Allium cepa*, by inducing cell death, chromosome aberrations, variations in the quantity of nucleoli and micronuclei. It was also observed that the azo dye presents aneugenic and clastogenic actions for the test organism studied, which prevailed even after the recuperation treatments. Moreover, the techniques of C-banding CMA3/DAPI and FISH showed chromosome sites more susceptible to breaks, which confirmed the aneugenic action of the azo dye. Ventura [66] also observed, by assays with *A. cepa*, that when the black commercial dye pass through biodegradation treatment with a pool of heterotrophic bacteria, yeast of the species *Candida viswanathii* or by the basidiomycete fungus *Phanerochaete chrysosporium*, its toxic potential increases, which proves that the biodegradation of this azo dye produces metabolites potentially more toxic than that the original dye, probably due to the cleavage of the azo bonds. The genotoxic effects observed in the meristematic cells of *A. cepa* before and after the biodegradation were similar, however, higher frequencies of genotoxic damages were observed after the microbial treatment.

Recent toxicological studies with the azo dye Red HE3B (Reactive Red 120), before and after bacterial treatments, showed that the dye was able to induce oxidative stress and a high frequency of chromosome aberrations and micronuclei in root cells of *A. cepa*, when compared to the effects caused by its metabolites. Moreover, by the comet assay, performed with the same test organism, it was possible to detect that the rate of damages induced by the dye in the DNA in a significantly higher form than that induced by its metabolites, indicating that the microbial treatments were favourable to the detoxification of Red HE3B [67].

A recent study [68] assessed the efficiency of the conventional chlorination treatment to remove the genotoxicity and mutagenicity of the azo dyes Disperse Red 1, Disperse Orange 1 and Disperse Red 13 in aqueous solutions, using the comet assay and the micronucleus test with HepG2 cells, and the *Salmonella* assay. The comet assay showed that the three dyes studied induced damages in the DNA of HepG2 cells in a dose-dependent form and that, even after chlorination, these azo dyes remain genotoxic, although they had induced a lower index of damage. The micronucleus test showed that the mutagenic activity of the azo dyes was completely removed after chlorination, under the conditions tested. The *Salmonella* assay showed that chlorination reduced the mutagenicity of the three compounds tested with the strain YG1041, but enhanced the mutagenicity of Disperse Red 1 and Disperse Orange 1 with the strain TA98. In general, it was concluded that the chemical treatment used must be performed with caution for the treatment of aqueous samples contaminated with azo dyes.

Toxicity of the azo dye Direct Red 28 is mainly related to its intermediate metabolites, benzidine and 4-aminobiphenyl, since they are capable of inducing a high frequency of damages in the DNA and apoptosis in human cells from promyelocytic leukaemia cell line (HL-60) [69]. It was also showed that culture of *Bacillus velezensis* is able to degrade and detoxify completely the toxicity presented by the metabolites of the azo dye Direct Red 28.

Oral exposure of humans to azo dyes can lead to the formation of aromatic amines, both by the intestinal microflora and by liver azoreductases and some of these amines have presented carcinogenic properties [70].

Several azo dyes present genotoxic, mutagenic and

carcinogenic activity in tests with microorganisms and mammalian cells [71-75]). The 3-methoxy-4-aminobenzene, for example, is mutagenic for bacteria and carcinogenic for rats, while 2-methoxy-4-aminobenzene is weakly mutagenic for bacteria but not carcinogenic for rats [76]. Thus, the genotoxicity, mutagenicity and carcinogenicity of dyes are closely related with the nature and position of the substituent bond to the azo group [25].

Exposure to some azo dyes has been related to the development of bladder cancer, splenic sarcomas, hepatocellular carcinomas, cell anomalies and chromosome aberrations [61, 77-80]. These effects can be derived from the direct action of dyes on cells or mainly from the formation of products of the metabolism resulting from the reduction of the azo bond [28], which are capable of interaction with the molecule of DNA, damaging it [25, 81-82].

Studies performed by Alves de Lima et al. [83], in the aberrant crypt test, showed that a sample of a certain effluent containing azo compounds (C.I. Disperse Blue 373, C.I. Disperse Violet 93 and C.I. Disperse Orange 37) of the Black Dye Commercial Product (BDGP), induced an increase of pre-neoplastic lesions in the colon of rats exposed to concentrations of 1% and 10% of this effluent.

Some authors [60] described carcinogenic activities for the azo dye C.I. Pigment Red 53:1, through observation of lung tumours in male rats. They also reported that, according to IARC, the azo dye C.I. Solvent Yellow 14 is also considered carcinogenic, since it is able to induce hepatocellular carcinoma, besides tumours in the urinary tract of rats.

It was proven the hepatocarcinogenic action of the azo dye *p*-dimethylaminobenzene (*p*-DAB) for mice and rats, when they were fed by long periods with low doses of this compound. Chronic intake of these animals with *p*-DAB resulted in a significant increase in the mitotic activity of the liver parenchyma cells in relation to the negative control [54].

Besides the carcinogenic and teratogenic effects, azo dyes cause dysfunction in the reproductive organs of rodents. For example, pre-natal exposure to Congo Red permanently reduced the number of germ cells in male and female rats and mice [84]. Another study showed adverse effects of the exposure of the gonads of young males and females of rats, but there was only reduction of the fertility for young females [85]. Suryavathi et al. [86] studied the toxic effects in short

term (15 days) of textile effluents on the male reproductive system of adult rats and mice. The effluents containing azo dyes caused the decrease of the body weight (7-25%) and length of the reproductive organ (testes, epididymis, prostate and seminal vesicles) (2-48%) of the animals treated. Histopathological analyses also showed alterations in the spermatogenesis process with various abnormalities in sperm, such as reduction in the quantity of spermatozooids (10-59%) and altered mobility (14-56%), which affected the fertility of the animals.

## Treatments of Textile Effluents

### *General Considerations*

Aquatic environments are of extreme importance for the world population, since they are used as source to obtain water, agricultural activities and for animal production, and are also associated to recreational activities. The final destination of a large quantity of pollutants, derived from industrial, agricultural and domestic activities, are the rivers, lakes and oceans. This water pollution puts at risk all the population that lives near to hydric resources [87].

Dyestuff industries and textile industries are, respectively, the largest producers and users of azo dyes, producing tons of residues which are released into the environment and cause serious problems, due to the chemical and photolytic stability, which elapses in its high persistence in natural environments [26, 88-89]. Installation of efficient treatment of effluents in textile industries that use azo dyes is a growing concern due to the visible aesthetic impact caused by the discharge of residues that reaches water bodies, as well as the possible toxic effects that these compounds can promote on the biota associated to these hydric resources. As the environmental legislation becomes more demanding, the effectiveness and reduction of the cost of the treatment processes become more important [1].

Environmental contamination resulted from the emission of effluents of dyeing industries is a global problem [9], therefore different methods of effluents treatment have been used in an attempt to minimize the problems resulted from this contamination [2]. The textile dyes can be removed, physically by processes of flocculation, adsorption, activated coal, wood chips, silica gel, filtration by membranes, ion exchange, UV radiation, electrokinetic coagulation and filtration, or

chemically by processes of oxidation, peroxidation of salts of iron II (Fenton reaction), ozonization, photochemical processes of electrochemical destruction, UV-peroxide system, cucurbituril and by sodium hypochlorite [5]. However, most of these methods, which simply accumulate or concentrate the dyes [5], present high cost and trigger secondary pollution, caused by the excessive use of chemical substances [90].

### *Azo dyes biodegradation*

Bioremediation, i.e., biological degradation of these dyes is a treatment process that has been highlighted, since it degrades the pollutants and do not accumulate these chemical compounds into the environment. However, for the bioremediation constitutes an efficient treatment, it is necessary to take into considerations which are the enzymes able to degrade certain azo dyes [29, 90, 91], since they are synthetic compounds relatively resistant to biological degradation processes, due to the complex chemical structure [26, 92]. It should be also performed tests that evaluate the toxic or inhibitory effects of these pollutants in the microbial population [93]. Due to the fact that most of the synthetic dyes are recalcitrant to the microbial degradation, effluents of the textile industries are normally resistant to the biological treatment, both with microorganisms and plants [94-97].

Mcmullan et al. [98] stated that the ability of the microorganisms to discolour and metabolize dyes is known for a long time, and the use of technologies based on the bioremediation, for the treatment of textile effluent, has aroused great interest. Researches performed in the last decades have shown an increase in the number of microorganisms that are able to discolour and degrade artificial dyes [99].

Azo dyes are scarcely biodegradable organic compounds due to their high stability to light and resistance to microbial attack. These dyes are resistant to conventional biodegradation [100, 101], however, under anaerobic conditions, they have been associated with the generation of metabolites. According to Saratale et al. [102], there are some species of microorganisms that, under certain environmental conditions, are able to completely mineralize several types of azo dyes. In the initial stage of anaerobic degradation of azo dyes, a reductive cleavage of the azo bonds begins to occur, originating from aromatic amines, which are recalcitrant for anaerobic bacteria



[103, 104], with exception of few aromatic amines substituted by hydroxyl and carbon-hydroxyl groups, which are degraded under methanogenic conditions [105]. On the other hand, aromatic amines are readily degraded anaerobically [106, 107].

Although azo dyes represent a potentially important class of pollutants, little is known about their fate [108]. For about 30 years, several studies have been carried out aiming to use microorganisms as agents in the bioremediation treatment of aquatic bodies containing textile dyes [109]. According to Saratale et al. [102], a great variety of organisms are able to discolour dyes, such as bacteria, basidiomycete fungi, yeasts, algae and plants. Biological methods used in the treatment of effluents that contain dyes use different organisms [2], and some microorganisms have received great attention, regarding their capacity and ability in the process of discolouring effluents of textile industries [29]. Discolouring of dyes by microorganisms is commonly performed by bacteria and basidiomycete fungi. However, there are other organisms able to degrade azo dyes, such as some algae [110-112] and plants [113, 114].

Contaminations by dyes of the azo type can characterize a great danger to exposed organisms, besides being toxic due to their own chemical properties, they can be transformed into even more toxic compounds by their metabolization of microorganisms present in the environment. There is an urgency to assess the effectiveness of biological treatments of industrial effluents, since, normally, the biodegradation products are even more detrimental to the environment, due to the high toxicity of the metabolites produced during the biodegradation processes [66].

Due to the diversity, concentration and composition of the dyes present in the effluents, there is a great motivation for researchers to study the biodegradation of hazardous compounds as well as to discover microorganisms that are able to degrade efficiently a great number of pollutants at a very low operational cost [9].

Bacteria group used in the degradation of dyes is considered, particularly, useful in the degradation of azo dyes, since they have the capacity to perform the reductive cleavage of the azo bonds, present in this type of compound [9].

Metabolization of azo dyes by bacteria, under anaerobic conditions, may occur in different ways: 1.

cleavage of the azo bond, catalyzed by azoreductases (cytoplasmic enzymes with low specificity to the substrate) [8, 9]; 2. non-specific reduction by electron carriers (redox reaction), from cell metabolic pathways (ex: release of flavins, quinines, hydroquinones) [115-117]; 3. action of reduced inorganic compounds, such as  $\text{Fe}^{2+}$ , which are formed as final product of certain metabolic reaction by strictly anaerobic bacteria [8, 117-119]; 4. chemical reduction by sulphur radicals, generated in the reduction of sulphate salts [116, 117].

According to Brown and Hamburger [120], total mineralization of non coloured aromatic amines, formed from the bacterial degradation of azo dyes, is not possible under anaerobic conditions and, therefore, these amines are accumulated in the environment, which may have toxic, mutagenic actions and, possibly, carcinogenic actions to the exposed animals [1]. It is important to consider that the bacterial treatment, in aerobic conditions, is generally efficient to mineralize totally these aromatic amines [121, 122].

In the State of São Paulo - Brazil, most industries of dye processing, mainly use systems of activated sludge to treat their effluents. However, some studies showed that the activated sludge systems are not efficient to remove all the dyes and aromatic amines present in industrial effluents. According to the studies of Van der Zee et al. [118], it was observed that the application of activated sludge in ascending laminar flow hood, in anaerobic conditions, caused the significant reduction in the colouration in only 8 of the 20 types of azo dyes. Shaul et al. [101], studying 18 types of azo dyes, observed that 11 of them were not altered by the activated sludge treatment, 4 (Acid Blue 113, Acid Red 151, Direct Violet 9 and Direct Violet 28) were adsorbed into the activated sludge (composed by different species of bacteria) and only 3 (Acid Orange 7, Acid Orange 8 and Acid Red 88) were biodegraded. Studies [123] showed that 20% of the dye C.I. Disperse Blue 79 remained in the final effluent after treatment with activated sludge. Detection and quantification of components of black commercial dye (BDGP – Black Dye Commercial Product), present in samples of raw and treated effluents of Cristais River, showed that this commercial product is recalcitrant even after aerobic treatment [124].

Bacterial ability to biodegrade azo dyes has been reported for many species, such as: *Aeromonas* sp., *Bacillus* sp., *Pseudomonas* sp., *Rhodococcus* sp., *Shigella* sp., *Klebsiella* sp., *Proteus mirabilis*; *Pseudomonas luteola* and *Mycobacterium avium* [125-128]. Studies performed

by Wong and Yuen [46] showed that the bacteria *Klebsiella pneumoniae* was efficient in the degradation of an azo dye, the methyl red, inferring that it could be used in the treatment of industrial effluents containing other azo dyes. Zissi and Lyberatos [129] showed that the bacteria *Bacillus subtilis* was able to degrade azo dyes present in effluents of textile industries. Studies performed by many researchers [98, 130-133] showed that certain bacteria of the genus *Streptomyces*, known to produce extracellular peroxidases that acted in the degradation of lignin, were effective in the degradation of dyes. However, in aerobic conditions, azo dyes are more resistant to the bacterial attack [125]. The bacteria *Kocuria rosea* presents high potential of discolouring and degrading the sulphonated azo dye methyl orange, besides originating degradation products (4-amino sulphonic acid and N,N'-dimethyl-*p*-phenylenediamine) that were not toxic for plants (*Sorghum vulgare* and *Phaseolus mungo*) and bacteria (*Kocuria rosea*, *Pseudomonas aeruginosa* and *Azotobacter vinelandii*) [134].

According to Kakuta et al. [135], some yeasts are able to degrade synthetic dyes. Some studies [136-139] showed that some species of yeasts, such as *Candida zeylanoides*, *Saccharomyces cerevisiae* and *Issatchenkia occidentalis*, have the ability to cleave the azo bonds of azo dyes, originating aromatic amines, by reduction mechanism similar to those of several bacteria. Vitor [140] showed that the yeast *Candida albicans* has the ability to degrade an azo dye, the "Direct Violet 51", indicating that the more vulnerable sites for disruption of this compound are the bonds of nitrogen with secondary amines. According to Meehan et al. [141], colour removal of Remazol Black-B by the yeast *Kluyveromyces marxianus* occurred due to the chemical adsorption of these azo dyes by the cell biomass but not due to any chemical enzymatic activities. Studies [90] showed that the yeast *Saccharomyces cerevisiae* degraded efficiently, the azo dye methyl red, considered toxic, may be used in the biodegradation processes of dyes present in the environment.

According to Ramalho et al. [139], yeasts, mainly *Saccharomyces cerevisiae*, are good agents in the bioremediation of azo dyes, since their growth and viability are not affected by the presence of dyes and their metabolites (potentially carcinogenic and mutagenic), besides organisms being able to perform the complete mineralization of azo compounds. The yeast *Saccharomyces cerevisiae* represents, besides a low cost biological material, a promising organism in the removal of a toxic azo dye, the methyl red, was found

in effluents from dyeing industries, since it was able to rapidly and totally degrade the referred dye [142].

Kunz [143] confirmed that studies concerning the biodegradation of toxic effluents have increased in the last years, and there is a great highlight for the basidiomycetes fungi. These fungi are used in the food industry, in the production of enzymes, treatment of effluents and other activities.

The degradation process of dyes by basidiomycete fungi, mainly the white-rot fungi, which are microorganisms with great ability to biodegrade dyes, are linked to the action of exoenzymes that act on lignin (structural polymer found in the cell wall of plants), such as laccases, ligning peroxidases (LiP), peroxidases dependent on manganese (MnP) [144, 145], as well as enzymes that produce H<sub>2</sub>O<sub>2</sub> [5, 6, 146], which has the ability to degrade several recalcitrant pollutants, including the synthetic dyes [147-148].

Among the white-rot fungi that have the capacity to degrade dioxins, polychlorinated biphenyls (PCBs), other chlorinated organic compounds and azo dyes, this capacity is directly related to the nature of the substituent groups of the aromatic rings, we can mention *Phanerochaete chrysosporium* [96, 146, 149-152], *Trametes versicolor* [153-155], *Coriolus versicolor* [156, 157] and *Bjerkandera adusta* [153, 158]. Other groups of fungi that have also shown to be efficient in discolouring dyes are: *Aspergillus niger* [159], *Geotrichum candidum* [92, 160], *Pleurotus ostreatus* [97]; [161, 162] and *Cunninghamella elegans* [163, 164], among others.

Martins et al. [95, 96, 97] have carried out several assays on the biodegradation of azo dyes of textile applications by the filamentous fungus *Phanerochaete chrysosporium* to assess its recalcitrance, mainly, until at which point they would be recalcitrant. It was verified that the concentrations of nutrients and the azo dyes, as well as the structures of the azo dyes, interfere in the processes of their biodegradation. Studies [162] showed that white-rot fungi degrade the azo dye Disperse Orange 3, originating aromatic amines, by an oxidative mechanism, which is different from the anaerobic bacterial way. Kirby et al. [146] showed that the fungi *Phlebia tremellosa* were able to degrade 8 synthetic textile dyes present in artificial effluents, at the concentration of 200 mg/L, reducing about 96% its colour. Studies [165] showed the fragmentation of different azo dyes by the action of the fungi *Neurospora crassa*, originating, at least, two amino compounds, besides producing aniline in great

quantities, which explains the increase of the toxicity of azo dyes after degradation. According to these studies, the dyes "Procion Red MX-5B" and "Acid Red 151" were considered the most toxic and the dyes "Direct Red 23" and "Erythrosine B", the least toxic.

Anastasi et al. [166] performed a study on the degradation of industrial dyes, among which the azo in 17 species of basidiomycete fungi belonging to different ecophysiological groups, meanwhile he demonstrated the efficiency of fungi in performing the discolouration of simulated effluents composed by one or several dyes together. According to the above, the ecotoxicity test with *Lemna minor* showed a significant reduction in the toxicity of dyes after treatment by the fungi *Bjerkandera adusta*, indicating that the discolouration induced by this fungus was followed by detoxification events.

There are some algae species able to degrade azo dyes, with the help of reduction mechanisms [167]. The ones form the genus *Chlorella* [110, 112] and *Oscillatoria* [110] and *Spirogyra* [111] are able to degrade these compounds into aromatic amines, which, in turn, can be further metabolized into organic compounds or CO<sub>2</sub> [112].

Among the organism that are able to degrade azo dyes, some species of plants can be cited, such as *Rheum rabarbarum* [113] *Brassica juncea*, *Sorghum vulgare* and *Phaseolus mungo* [168] and several typical plants of flooded environments [114]. Studies performed by Kagalkar et al. [169] showed the efficiency of *Blumea malcommi* (an herbal plant from the Asteraceae family) in degrading a textile azo dye, the Reactive Red 5B. Deniz and Saygideger [170] showed that princess tree leaves (*Paulownia tomentosa*) can be used in the removal of another textile azo dye, the Basic Red 46.

Although plant species are able to biodegrade dyes, their large-scale application is still not absolutely feasible, mainly due to the limited tolerance of the plants to concentration of pollutants as well as the need of large areas for implanting phytoremediation [168].

### Final Considerations

The major environmental problems in the textile industry are related to the use of azo dyes, a large family of synthetic dyes highly resistant to natural degradation and proved toxic potential due to their ability to induce various toxic, cytotoxic, genotoxic, mutagenic and carcinogenic effects on different

organisms when exposed to such compounds.

In addition to the problems related to the release of toxic substances or to the discharge of compounds which can be converted into metabolites which are most harmful to the environment, the effluents derived from dye activities have strong staining. Besides being a source of visual pollution, that feature offers serious environmental risks; mainly due to the interference in the natural photosynthetic processes that causes incalculable losses in medium and long term for all aquatic biota.

From the data presented here, it can be concluded that the immediate development of dyes free from toxic potential as well as dyes with low toxicity is urgently required; so does the increased investment in the research aiming at developing effective methods for the biological treatment of effluent textiles, in order to avoid or reduce the possibility of harmful effects of these chemical compounds on the environment and the exposed organisms, including human health.

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