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Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters

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Abstract

The present status of origins, known hazards, release restrictions and environmental fate of aromatic amines is reviewed. The specific case of aromatic amines arising from the reduction of the azo bond of azo colorants is addressed, with emphasis on the recalcitrance of azo dyes, their demonstrated vulnerability to azo bond reduction through different mechanisms and the lack of data on the biodegradability of the resulting amines. The evolution and present array of analysis methods for aromatic amines in water samples is reviewed, highlighting the increased sophistication and sensitivity attained, and referring a few efforts towards fast analysis methodologies. The case for the application of direct ultraviolet spectral analysis with advanced deconvolution techniques for the monitoring of aromatic amines in textile effluent treatment is presented.

Keywords: Aromatic amines; Azo dyes; Hazards; Textile wastewater; Analysis; UV spectra

1. Origin and types of aromatic amines in the environment

Aromatic amines are generally identified as those chemical compounds having in their molecular structure one or more aromatic rings, bearing one or more amino substituents. They range from the simplest aniline to highly complex molecules with conjugated aromatic or heterocyclic structures and multiple substituents. Table 1 lists some examples of environmentally or health-related important amines together with their major origins and potential impact.

Major sources of amines in the environment include several chemical industry sectors such as oil refining, synthetic polymers, dyes, adhesives and rubbers, pharmaceuticals, pesticides and explosives [1]. Non-industrial sources under intense study due to their role in cancer development in humans include the application of hair dyes, automobile exhaust fumes, the burning/pyrolysis of protein-rich vegetable matter, such as in forest fires and tobacco smoking and the cooking and subsequent consumption of meats [3].

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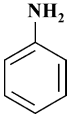
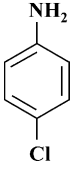
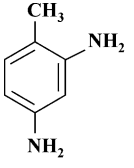
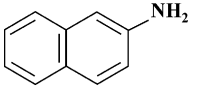
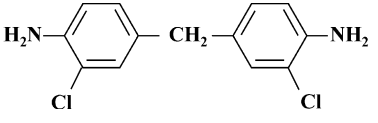
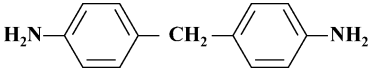
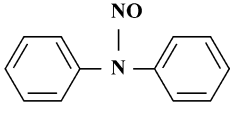
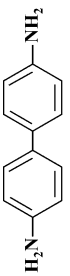
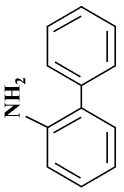
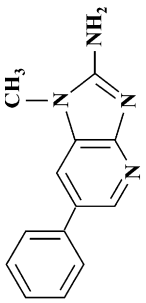
Table 1
Some examples of aromatic amines known to be potential hazards to human health and the environment [1–3]

Name/CAS number	Chemical structure	Major origins	Potential impact
Aniline		Manufacture of isocyanates, rubber, dyes, explosives, pesticides, pharmaceuticals. Oil refining. Tobacco smoke. Forest fires.	VOC with ozone-forming potential; Toxic to aquatic life; Possibly carcinogenic and genotoxic
4-Chloroaniline		Manufacture of dyes, pesticides, various chemicals	Toxic to humans Carcinogenic and genotoxic
Toluene 2,4-diamine		Manufacture of toluene diisocyanate (for elastomers), dyes, resins, fungicides	VOC Toxic to humans and aquatic life Possibly carcinogenic and genotoxic
2-Naphthylamine		Manufacture of dyes	Toxic to humans Carcinogenic
4,4′-Methylenebis(2-chloroaniline)		Manufacture of polyurethanes	VOC Toxic to humans and aquatic life Possibly carcinogenic and genotoxic
4,4′-Methylenedianiline		Manufacture of polyurethanes, dyes, epoxy resins	VOC. Recalcitrant adsorption onto particulate matter Toxic to humans and aquatic life Possibly carcinogenic and genotoxic
N-Nitrosodiphenylamine		Manufacture of dyes, pharmaceuticals, rubber	Harmful to humans Possibly carcinogetic

(*continued on next page*)

Table 1 (*continued*)

Name/CAS number	Chemical structure	Major origins	Potential impact
Benzidine		Manufacture of dyes	Toxic to humans Carcinogenic
2-Aminobiphenyl		Cigarette smoking	Genotoxic and carcinogenic
2-Amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine		Cooking of meats	Genotoxic and carcinogenic

In relation to human and ecosystem exposure to chemicals, the EEA and UNEP [4] point out that most of the several thousand chemicals presently being manufactured and used lack toxicity and eco-toxicity data for adequate risk assessment. However, evidence of long-term and irreversible health effects from many chemicals is building up and the precautionary principle is increasingly encouraged in regulatory measures. This principle aims at reducing the discharge of chemicals in the environment and is based on the assumption that a chemical poses a potential hazard through its persistent and bio-accumulating nature, even though toxicity data may not be available [4]. Initiatives have been undertaken to promote less hazardous use of chemicals and the build up of emission knowledge allowing the exposure assessment of chemicals based on their persistence and spatial range, a process which is faster and less costly than toxicity and eco-toxicity testing. Among these initiatives is the inventory and public information of chemicals' releases to the environment, such as the OECD Pollutant Release and Transfer Register (PRTR) and national actions such as the US Toxics Release Inventory (TRI). Table 2 lists amines currently included in several hazardous chemicals release inventory and restriction regulations, at national and international level, which reflect the current knowledge of their hazardous character, with particular emphasis on their carcinogenicity. The inclusion of aromatic amines among hazardous chemicals under the surveillance of precautionary principle agents worldwide is thus recognised.

The first concerns with human exposure to carcinogenic aromatic amines arose in the dye manufacturing industry as early as the late nineteenth century [9]. Dye production intermediates, and later amines involved in other chemical manufacturing industry sectors (see Table 1), were therefore the major object of attention in aromatic amine toxicity and carcinogenicity studies and occupational health improvement actions. More recently, the possibility of manufactured azo dyes breaking down during use to their constituent amines has been considered a health hazard. Although the International Agency for Research on Cancer includes only benzidine-based dyes in

Table 2
Aromatic amines listed in some hazardous substances emission inventories and restriction of use regulations, complemented with their status in the carcinogenic substances list of the International Agency for Research on Cancer [1,5–8]

CAS number	Name	TRI (USA)[a]	Pollution Inventory (UK)[b]	Directive 76/769/EEC[c]	Directive 2002/61/EC[d]	IARC Classification[e]
92-67-1	4-Aminobiphenyl	carcinogen	–[f]	carcinogens: cat. 1	√[g]	Group 1
62-53-3	Aniline	toxic	√	–	–	Group 3
90-04-0	*o*-Anisidine	carcinogen	–	carcinogens: cat. 2	√	Group 2B
134-29-2	*o*-Anisidine hydrochloride	carcinogen	–	–	–	–
104-94-9	*p*-Anisidine	toxic	–	–	–	Group 3
92-87-5	Benzidine	carcinogen	–	carcinogens: cat. 1	√	Group 1
106-47-8	*p*-Chloroaniline	carcinogen	–	carcinogens: cat. 2	√	Group 2B
95-69-2	*p*-Chloro-o-toluidine	carcinogen	–	–	√	Group 2A
120-71-8	*p*-Cresidine	carcinogen	–	–	√	Group 2B
615-05-4	2,4-Diaminoanisole	carcinogen	–	–	√	Group 2B
39156-41-7	2,4-Diaminoanisole sulfate	carcinogen	–	–	–	–
101-80-4	4,4′-Diaminodiphenyl ether	carcinogen	–	–	√	Group 2B
2687-25-4	2,3-Diaminotoluene	–	√	–	–	–
95-80-7	2,4-Diaminotoluene	carcinogen	√	carcinogens: cat. 2	√	Group 2B
823-40-5	2,6-Diaminotoluene	–	√	–	–	–
496-72-0	3,4-Diaminotoluene	–	√	–	–	–
25376-45-8	Diaminotoluene (mixed isomers)	carcinogen	–	–	–	–
99-30-9	2,6-Dichloro-4-nitroaniline	toxic	–	–	–	–
91-94-1	3,3′-Dichlorobenzidine	carcinogen	–	carcinogens: cat. 2	√	Group 2B
612-83-9	3,3′-Dichlorobenzidine dihydrochloride	carcinogen	–	carcinogens: cat. 2	–	–
64969-34-2	3,3′-Dichlorobenzidine sulfate	carcinogen	–	carcinogens: cat. 2	–	–
119-90-4	3,3′-Dimethoxybenzidine	carcinogen	–	carcinogens: cat. 2	√	Group 2B
20325-40-0	3,3′-Dimethoxybenzidine dihydrochloride	carcinogen	–	carcinogens: cat. 2	–	–
111984-09-9	3,3′-Dimethoxybenzidine hydrochloride	carcinogen	–	carcinogens: cat. 2	–	–
838-88-0	3,3′-Dimethyl-4,4′-diaminodiphenylmethane	–	–	carcinogens: cat. 2	√	Group 2B
121-69-7	*N*,*N*-Dimethylaniline	toxic	–	–	–	Group 3
119-93-7	3,3′-Dimethylbenzidine	carcinogen	–	carcinogens: cat. 2	√	Group 2B
612-82-8	3,3′-Dimethylbenzidine dihydrochloride	carcinogen	–	carcinogens: cat. 2	–	–
41766-75-0	3,3′-Dimethylbenzidine dihydrofluoride	carcinogen	–	carcinogens: cat. 2	–	–
122-39-4	Diphenylamine	toxic	–	–	–	–
101-14-4	4,4′-Methylenebis (2-chloroaniline)	carcinogen	√	carcinogens: cat. 2	√	Group 2A
101-61-1	4,4′-Methylenebis (*N*,*N*-dimethyl)benzenamine	carcinogen	–	–	–	Group 3
101-77-9	4,4′-Methylenedianiline	carcinogen	√	carcinogens: cat. 2	√	Group 2B
134-32-7	1-Naphthylamine	carcinogen	–	–	–	Group 3
91-59-8	2-Naphthylamine	carcinogen	–	carcinogens: cat. 1	√	Group 1
100-01-6	*p*-Nitroaniline	toxic	–	–	–	–
99-59-2	5-Nitro-o-anisidine	toxic	–	–	–	Group 3
99-55-8	5-Nitro-o-toluidine	toxic	–	–	√	Group 3
55-18-5	*N*-Nitrosodiethylamine	carcinogen	–	–	–	–
86-30-6	*N*-Nitrosodiphenylamine	toxic	–	–	–	Group 3
156-10-5	*p*-Nitrosodiphenylamine	toxic	–	–	–	Group 3
95-54-5	1,2-Phenylenediamine	toxic	–	–	–	–
108-45-2	1,3-Phenylenediamine	toxic	–	–	–	Group 3
615-28-1	1,2-Phenylenediamine dihydrochloride	toxic	–	–	–	–
624-18-0	1,4-Phenylenediamine dihydrochloride	toxic	–	–	–	–
106-50-3	*p*-Phenylenediamine	toxic	–	–	–	Group 3
139-65-1	4,4′-Thiodianiline	carcinogen	–	–	√	Group 2B
636-21-5	*o*-Toluidine hydrochloride	carcinogen	–	–	–	–
95-53-4	*o*-Toluidine	carcinogen	–	carcinogens: cat. 2	√	Group 2A
137-17-7	2,4,5-Trimethylaniline	–	–	–	√	Group 3
87-62-7	2,6-Xylidine	carcinogen	–	–	–	Group 2B
95-68-1	2,4-Xylidine	–	–	–	–	Group 3
60-09-3	4-Aminoazobenzene	carcinogen	–	carcinogens: cat. 2	√	Group 2B
97-56-3	*o*-Aminoazotoluene	—		carcinogens: cat. 2	√	Group 2B

Table 2 (*continued*)

CAS number	Name	TRI (USA)[a]	Pollution Inventory (UK)[b]	Directive 76/769/EEC[c]	Directive 2002/61/EC[d]	IARC Classification[e]
122-66-7	1,2-Diphenylhydrazine	carcinogen	–	carcinogens: cat. 2	–	–
60-11-7	4-Dimethylaminoazobenzene	carcinogen	–	–	–	Group 2B
16543-55-8	*N*-Nitrosonornicotine	carcinogen	–	–	–	Group 2B

[a] List of the toxic chemicals for which reporting is required under Section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA) (also referred to as the Toxics Release Inventory (TRI)), US EPA, 2001.

[b] The Pollution Inventory (PI) is an annual record of pollution in England and Wales from activities regulated by the Environment Agency, UK, 2002.

[c] Classification on the annex of EC Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations.

[d] 19th amendment to Directive 76/769/EEC, which specifically restricts the use in textiles of certain azo dyes which, by reductive cleavage of azo bonds, can produce any of the 22 aromatic amines of the given list.

[e] Classification of agents or mixtures according to the International Agency for Research on Cancer. Group 1: carcinogenic to humans; Group 2A: probably carcinogenic to humans; Group 2B: possibly carcinogenic to humans; Group 3: not classifiable as to carcinogenicity in humans.

[f] – not included in list.

[g] √included in list.

its Group 2A and 8 dyes in Group 2B, of which a few are azo dyes, the possibility of azo bond reduction giving aromatic amines has been demonstrated for a variety of ecological conditions, including those encountered in the digestive track of mammals [10]. Therefore, most of the attention concerning the possible hazards arising from the use of azo dyes has been transferred to their reduction products. The 19th amendment to Directive 76/769/EEC (Directive 2002/61/EC, Table 2), to be enforced throughout the EC member states by September 2003, has established, at European level, the attention due to the particular case of azo dyes as they are used in textile manufacturing. Directive 2002/61/EC follows the earlier initiative of the German Consumer Goods Ordinance and the subsequent regulations appearing in several member states, constituting a first, harmonised, international approach to the control of aromatic amine emissions specifically arising from the dyeing of textiles with azo dyes.

2. Carcinogenicity, toxicity and biodegradability of aromatic amines

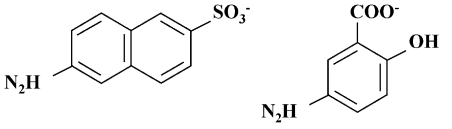
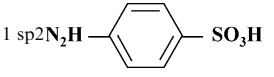
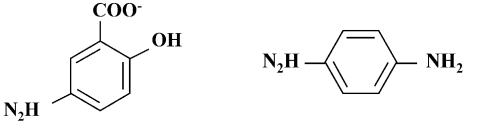
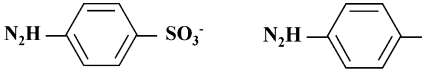
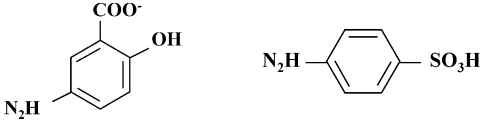
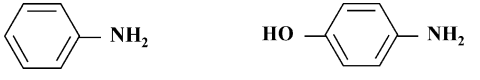
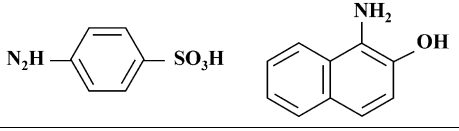
Following the studies establishing the correlation between exposure to N-substituted aryl compounds and occurrence of human cancers, the second part of the twentieth century has seen key developments in the identification of the mechanisms of toxicity and carcinogenicity in these compounds [9]. Biochemical activation through *N*-hydroxylation, followed by sulfation, esterification or acetylation reactions, generating reactive intermediates able to bind to DNA molecules are presently established as the main routes of genotoxicity (mutagenecity) and carcinogenicity of arylamines. Nitrosoaryl or *N*-hydroxyaryl intermediates have also been found to be involved in amine toxicity through interaction with hemoglobin, causing methemoglobinemia. Rapid screening tests for likely carcinogens were designed using DNA-binding assays, using the developed knowledge and the resulting linkage found between the genotoxic and carcinogenic characters. Following the discovery of powerful mutagens (heterocyclic aromatic amines) in the by-products of proteinaceous food processing [11] the subject of carcinogenic/mutagenic N-substituted aryl compounds has seen increasingly rapid developments, covered in already eight editions of a dedicated international conference [3]. Also, the availability of rapid tests for mutagenic character has enabled manufacturers of dyes and other chemicals to screen new products and intermediates and efficiently select those which pose the least risk of human toxicity [9]. Among other structural characters, sulfonation, carboxylation and complexation with copper ions, as well as avoidance of

substitution of the hydrogens in the amine moiety with ethyl alcohol or an acetyl group, were found to decrease mutagenicity in *p*-phenylenediamine and benzidine derivatives [10]. The use of quantitative structure–activity relationship (QSAR) analysis is also widespread in the prediction of toxicity and carcinogenicity of aromatic amines. This is a result of the massive amount of experimental data accumulated on these aspects and also responds to the need for reliable prediction methods due to the wide range of amine structures involved in the manufacture of chemicals, particularly in dye chemistry [12]. Benigni and Passerini [12] recently reviewed QSAR models related to the aromatic amine carcinogenicity in mice and rats and compared it with models for mutagenecity in *Salmonella*, for general toxicity in organisms ranging from procariotes to animals, and for enzymatic and chemical oxidation reactivity. For mutagenic/carcinogenic potency as well as general toxicity, amine hydrophobicity was the major factor in the QSAR models, indicating an aspecific mode of action through interaction with cell membranes. However, for the distinction between non-carcinogenic and carcinogenic amines and for the enzymatic and chemical oxidations QSAR models were mostly based on molecular electronic and steric parameters, with hydrophobicity playing a minor role. Razo-Flores and co-workers [13] also found a good correlation between the hydrophobicity and the toxicity of amino- and nitro-substituted aromatic compounds towards methanogenic granular slugde. These authors proposed that azo dye and nitro-aromatic reduction in anaerobic conditions could function as a detoxifying mechanism.

A major fraction of the aromatic amines which have been considered most environmentally relevant has been found to be biodegradable, though in diverse extents and strongly depending on factors such as the type of microbial population and its adaptation conditions and the availability of oxygen. Ekici et al. [14] investigated the biodegradability of 8 of the aromatic amines listed in Table 1 (identified under the Directive 2002/61/EC column) in activated sludge systems. Under aerobic conditions, measured half-degradation times ranged from 10 to 140 h while in anaerobic conditions the range was from less than 0.5 h to 205 h. Börnick et al. [15] and Worch et al. [16] both measured degradation rates of aromatic amines in river water fed to biofilm water treatment systems. The 20 tested substances included aniline and several of its chloro, bromo, methyl, nitro and mixed derivatives, *N*,*N*-dimethylaniline, *N*-ethylaniline and 1-naphthylamine. Half-degradation times from 0.5 h for 1-naphthylamine to 35 h for 2,4,5-trichloroaniline were reported. For the latter compound, a biofilm adaptation period of 6 weeks resulted in a 20-fold increase in the biodegradation rate. Six amines, including aniline and *o*-toluidine, showed half-degradation times under 1 h. Razo-Flores et al. [13] tested the biodegradability potential of aniline, aminophenols, aminobenzoates and 5-aminosalicylate by methanogenic sludge and found that all compounds, except aniline, were at least partially biodegraded, though with lag-phases between 25 and 110 days and in some cases requiring pre-adaptation of the culture to 2-nitrophenol. Recently, Coughlin et al. [17] successfully demonstrated the biodegradation of sulfanilic acid and 1-amino-2-naphtol by a bacterial co-culture established in an aerobic rotating biological contactor, following the reduction of the azo dye Acid Orange 7, fed as the sole carbon and nitrogen source. Similarly, Tan et al. [18] reported complete degradation of aniline by the facultative fraction of methanogenic granular sludge exposed to oxygen. However, sulfanilic acid and 5-aminosalicylic acid mineralisation required the adition of an aerobic enrichment culture obtained from river sediments exposed to sulfonated aromatic compounds. Stolz [19] reviewed extensive work on the biodegradative pathway of amino-naphthalene sulfonates by *Sphingomonas* and *Pseudomonas* strains, isolated from environments under exposure to sulfonated contaminants. These studies included the investigation of the functional enzymes, their purification and characterisation and cloning of some of the correspondent genes.

Tan and co-authors [18] also pointed out that 4-aminophenol rapidly suffered auto-oxidation to oligomeric or polymeric humic-like substances, a phenomena likely to happen with aromatic amines bearing hydroxyl substituents. The same phenom-

Table 3
Published reports of demonstrated bio-mineralisation of aromatic amines following the reductive cleavage of azo compounds [18,32–35]

Initial azo compound	Examined aromatic amine intermediates	Microbial culture used	Amine biodegradation conditions and results	References
Mordant Yellow 3		Co-culture of 2 bacterial isolates	Two-step anaerobic/aerobic both amines mineralised	[32]
Acid Orange 7		Bacterial strains isolated from a wastewater treatment plant	Aerobic growth; sulphanilic acid degraded only in the presence of an external carbon source	[33]
Acid Orange 8				
Mordant Orange 1		Methanogenic granular sludge from a bioreactor treating petrochemical wastewater	Anaerobic conditions; 5-aminosalicylate degraded after a period of culture adaptation	[34]
Azodisalicylate				
4-Carboxy-4′-sulfoazobenzene		Bacterial isolate, adapted to aromatic amines	Aerobic growth on azo compound as sole carbon and energy source	[35]
Mordant Yellow 10		Methanogenic granular sludge from a bioreactor treating petrochemical wastewater;	Aerobic conditions; sulfanilic acid and 5-aminosalicylate degraded only in the presence of the aerobic culure	[18]
4-Phenylazophenol		Aerobic culture from river sediments, enriched by exposure to amines	Aniline degraded in aerobic conditions by the facultative bacteria in the methanogenic sludge	
Acid Orange 7		Aerobic biofilm; co-culture of 2 bacterial strains isolated from the biofilm	Aerobic conditions; sulfanilic acid and 1-amino-2-naphtol both degraded	[17]

enon was mentioned for 1-amino-2-naphtol [17] and 5-aminosalicylic acid [18], an effect which could effectively compete with biodegradation, increasing aromatic amine recalcitrance. Kudlich and co-workers [20] also specified that sulfonated *o*-aminohydroxybenzenes and *o*-aminohydoxynaphthalenes were easily decomposed upon exposure to oxygen and studied this effect with 1-amino-2-hydroxynaphthalene-6-sulfonate, 1-amino-2-hydroxynaphthalene-3,6-disulfonate and 1,2,7-triamino-8-hydroxynaphthalene-3,6-disulfonate. Dimers and quinone derivatives were found among the oxidation products, some of which could be biodegraded by activated sludge.

Thus, though very significant work has been done to assess and understand the biodegradation of aromatic amines, the most part of the presently employed compounds of this group remains to be assessed and their fate in wastewater treatment systems and in the environment remains largely unknown.

3. Azo dye origin of aromatic amines

Dye manufacturing is one of the sectors in chemical industry dealing with the greatest variety of products and intermediates. Due to this, textile and dye manufacturer's associations such as EURATEX and ETAD [21] have expressed great concern for the proposed EU strategy for environmental management of chemicals laid out in the White Paper "Strategy for Future EU Chemical Policy". In particular, the requirement for registry of marketed substances (and the breakdown products they can release in the environment) used in amounts exceeding 1 ton is estimated by ETAD to involve 2000–2500 existing colorants plus their production intermediates, and cost around 1000 million ∈. Over 3000 individual azo colourants (including dyes and pigments) are presently in use [22]. In case of dyes, they represent 60–70% of all dyes used [8]. Azo colorants can enter the environment from their own manufacture processes but the most significant routes include their use in subsequent industrial sectors, such as textile, paper, plastics, food and drugs colouring or the production of paints and lacquers. Roughly two-thirds of the dyestuff market is directed to the textile sector, and it is estimated that around 12% of the used colorants are loss in wastewaters [23]. Emissions can also arise further downstream in the life cycle of azo colorants, such as in the landfilling of painted or dyed materials and in the washing of dyed textiles. Current knowledge on the mechanisms of dye toxicity and the use of QSAR tools has led to the development of azo dyes with low direct toxicity [9] and azo dyes which could breakdown to carcinogenic aromatic amines are largely phased out in Europe [8]. Due to the general association of dye toxicity with hydrophobic character [12] low toxicity has often been achieved through the introduction of polar moieties in the dye structure, also resulting in higher aqueous solubility, sulfonation being a widespread character [23] particularly in the reactive dye group. However, this feature can hinder their removal in wastewaters treatment works. Increased hydrophilicity has been described as unfavourable for dye bioelimination in activated sludge systems, through absorption onto the biomass. Greaves et al. [22] conducted a chemometric assessment of the structural functions favouring water soluble dye bioelimination, for reactive, direct and acid dyes, which indicated that higher molecular size/ionic charge ratios and fewer sulfonate or sulfonamide substituents increased dye bioelimination. Azo dyes have also been long known to resist effective biodegradation in aerobic conditions with the exception of a few simple-structured dyes [24,25]. The recalcitrance of azo dyes has been attributed to the presence of sulfonate groups and azo bonds, two features generally considered as xenobiotic [26].

The azo bond in azo colorant molecules is however vulnerable to reductive cleavage. Due to its significance in toxicological and eco-toxicological terms, and its potential for eliminating colour impact in azo dye containing effluents, the biologically-mediated decolourisation of azo dyes through azo bond reduction has been extensively investigated in the past 20 years. Several reviews have been published on or including this subject, with emphasis on wastewater purification [25,27–31]. Basic, identified mechanisms [27] include non-specific reduction by electron transporters from

the cellular metabolic pathways (flavins, quinones), eventually accelerated by external redox mediators (naphthoquinones, humic substances), dye-specific reduction by azo reductase enzymes which can occur in the presence of oxygen, and chemical reduction by sulfide generated in the process of microbial sulfate reduction. However, only a few of the studies on microbial azo dye reduction included a clear demonstration of total or partial, subsequent biodegradation of the resulting metabolites, aromatic amines. Table 3 lists some of these reports.

Although the possibility of total elimination of azo dye reduction metabolites through biotransformation has been demonstrated, the process appears clearly less straightforward than the primary reduction step. Basic limitations are the need for specific, adapted microbial strains and, in some cases, co-cultures of several strains [26], the tendency of some metabolites to undergo chemical oxidation to more recalcitrant products, and the lack of knowledge on the bio-reactivity of the majority of the aromatic amines which can be formed in wastewater treatment plants or water courses as a result of the reduction of the discharged azo dyes presently in use [36]. The identified carcinogenic amines (Table 2) have been found to pose a significant risk of bioaccumulation in the environment [36], though, again, little is known of the more hydrophilic amines which could result from azo bond cleavage in water soluble dyes.

Thus, the likely persistence and largely unknown effect of azo dye derived aromatic amines in the environment makes them a desirable target for discharge monitoring and environmental distribution studies, in accordance with the precautionary principle basis.

4. Analysis of aromatic amines in aqueous samples

Efforts towards the development of accurate, reproducible and low detection limit methods for the detection and quantification or aromatic amines started with the first recognition of the health hazards they represent, target matrices including air, natural water, wastewater, soils, foods and body fluids. Methods based on voltammetry [37], potentiometry with specific electrodes [38] and spectrophotometric quantification after a specific colour-generating reaction or derivatisation to a chromophore [39–41] were proposed. However, the need for sample characterisation with respect to their content on different amine molecules and isomers soon directed most efforts to the separative domains, namely chromatography and electromigration [42]. Thin-layer and paper chromatography [43] tests were reported in the 1980s, together with developed methods in gas chromatography (GC) [44–46] and high performance liquid chromatography (HPLC) [47,48]. These were soon followed by capillary electrophoresis techniques [49].

To achieve higher levels of separation and lower detection limits for the target amines, particularly from complex matrices such as wastewater and foods, an array of sample pre-treatment methods have been proposed, aiming both at reducing interferences (sample cleanup) and pre-concentration, of which some have been duly validated and integrated in standardised analysis procedures and automated devices [50,51]. These pre-treatments include basic liquid–liquid extraction [52] and solid-phase extraction [52,53], involving transfer of the analytes to a second, liquid or solid, phase, and their recovery into a third liquid phase, through evaporation of the extraction solvent and re-dissolution or through elution from the solid phase. Both these procedures have been miniaturised into so-called solid-phase microextraction (SPME) [54,55] and liquid-phase microextraction [56] systems, achieving minimal sample volume requirements, avoidance of analyte losses through evaporation, faster analyses and better reproducibility. Such microextraction systems make use of the immense variety of chemical functions now available in the form of solid adsorbent packed cartridges or pre-columns [50,57], as well as supported liquid membranes [58]. Among applications to amine analysis, the promising use of immunosorbents based on antibodies against benzidine and nitroaniline has been described [50]. Also, efforts to avoid the use of organic solvents have resulted in the development of membrane separation and surfactant micelle-

mediated separation techniques for the pre-purification and pre-concentration of samples in aromatic amine analysis [59].

Diverse chemical possibilities have been applied for the main step of aromatic amine separation for analysis and quantification.

Due to the polar nature of aromatic amines, GC methodologies generally require derivatisation into apolar, volatile, thermally stable products prior to injection into the chromatographic column, though recent columns and equipments can allow separation of some aromatic amines without derivatisation [60]. This reaction step can be combined with pre-extraction, such as in solid-phase analytical derivatisation (SPAD) [61] and the obtained aducts are adapted to the post-column detection method used [62,63]. For amines, derivatisation via the amino group is most reported, examples being perfluoroacylation [45,61], the formation of thiophosphoryl [62] or *N*-permethylated [44] derivatives, the replacement of the amino group by iodine [63] or the bromination of the aromatic ring [64]. In very complex mixtures common in samples for aromatic amine analysis, GC offers better peak carrying capacity in chromatograms than liquid chromatography techniques.

Separation of aromatic amines by HPLC is presently a common practice for the analysis of these substances in water, avoiding the need for pre-derivatisation and the danger of thermal degradation in GC. Both reversed-phase (octyl or octadecyl silica derivatives) and ion chromatography (cation exchange resins) methodologies have been developed, with fine tuning of separations being achieved by temperature, mobile phase composition and gradient adjustments [50,56,65,66]. For reversed-phase separations, mixtures of aqueous buffers with acetonitrile or methanol are widely used, though, for the separation of sulfonated amines, the addition of ion-pair reagents such as quaternary ammonium salts or tertiary amines has been reported [67–69].

Taking advantage of the polar, ionisable nature of aromatic amines, capillary electrophoresis (CE) methods, both in the capillary zone electrophoresis and the micellar electrokinetic chromatography modes, have also been proposed for their fractionation in environmental samples [42,53,70]. The technique uses fused silica capillaries under an electrical field, and separation occurs either solely on the basis of molecular weight and charge (electrophoretic mobility) or associated with differential solubilisation in surfactant micelles. Associated with pre-concentration, pre-derivatisation or in-capillary derivatisation techniques [42,69], in order to overcome the problem of inherent low sensitivity, this method has shown potential for excellent separation efficiency [53,70].

A wide range of techniques have been applied for post-separation identification and quantification of chromatographic or CE peaks in aromatic amine analysis.

Devices reported for simple quantification or aromatic amine peaks in GC include flame ionisation [44,55], nitrogen-selective [46], flame photometric [62] and electron capture detectors [45,63,64], usually after pre-conversion to the required derivatives. HPLC and CE of aromatic amines have been used with UV spectrophotometric [47,54,56], electrochemical (amperometric) [48,70] and fluorescence [50] detection. Signal enhancement can be obtained through pre- or post-column derivatisation reactions with a chromophore or fluorophore [70,71]. In CE, indirect detection using a background electrolyte bearing also a chromophore or a fluorophore offers potentially higher sensitivity levels [42].

Peak identification is often a challenge in chromatographic aromatic amine analysis, due to the occurrence of multiple amine structures, interfering substances such as other aromatics and products of amine condensation. Though sample cleanup and specific derivatisation reactions at pre- or post-column level can minimise interferences, the coupling to spectral analysis methods, mass spectrometry (MS) or diode array spectrophotometry, has emerged as a powerful aid. Several reports on applications of GC–MS [60,61], HPLC-MS [51,72] and UV diode array detection [52,69] to aromatic amine analysis have been published.

Standardised analysis methodologies for aromatic amines in different matrices are available, either included in procedures adapted to a range

of organic analytes, such as EPA methods 1625, 625 and 8270D [73] or APHA method 6410B [74], or specific for given amines, such as EPA method 605 for benzidines in water. These methods usually combine liquid–liquid extraction for sample cleanup and pre-concentration with analysis and quantification by GC–MS or HLPC. In the particular case of amines arising from the reductive cleavage of azo dyes, several standard methods have been established in Europe for the detection of the 22 amines restricted by EC regulations (see Table 2), such as the French norm AFNOR XP G08-014 for dyed textiles [75] or the German method DIN 53316 for dyed leather [76]. These norms are presently under revision, with specific drafts or pre-drafts being available for textiles, leather and toys, such as the German draft standards DIN EN 14362-1/2, for dyed textiles [77,78]. The older methods use a reduction procedure with dithionite as an amine-generating step, followed by liquid–liquid extraction and GC with flame ionisation detection, GC–MS or HPLC with diode array detection. Some reports have been published testing the application of the whole or part of these methodologies to marketed materials, such as leather [79] and toys [80], and directly to azo dyes, examining the effects of experimental conditions [81–83].

In parallel to the development of the more sophisticated analysis methods mentioned in the previous paragraphs, some efforts have been published on faster and simpler detection methods for aromatic amines as a chemical group, with less emphasis on individual compound identification. In addition to the potentiometric and spectrophotometric methods mentioned above [37–41], reported techniques include chemiluminescence adapted to flow injection analysis (FIA) systems [84], spectrophotometry with FIA, after specific derivatisation to a chromophore [85], and an enzymatic, amperometric biosensor with amine oxidase [86]. Other amine detection and identification techniques recently reported are Fourier transform infrared spectroscopy applied to reduced azo dye extracts [87] and an electrochemical DNA biosensor for aromatic amines, successfully tested on wastewater samples [88].

5. Direct ultraviolet spectrophotometry of aromatic amines

Although the importance of detailed analytical methods for aromatic amine detection, identification and quantification at levels down to trace is unquestionable in environmental and health risk assessment of this class of substances, the costs involved, particularly in instrumentation and skilled staff requirements, are high. This factor tends to restrict the use of these methods in routine monitoring of industrial effluent discharges to the amines which have been proven as health hazards, namely the carcinogens. In the specific case of the wastewaters from modern textile industries in Europe, the risk of discharge of these amines is avoided through the selection of dye manufacturers which offer guarantees of compliance with Directive 2002/61/EC [8]. However, other aromatic amine structures, namely those included in the broader class of aromatic sulfonated compounds, are widely used as components of textile azo dyes with little direct information being available on their toxicity and ecotoxicity [23,57,69]. Sulfonated aromatics have been found in many natural aqueous environments, exhibiting high solubility and mobility in these media due to their ionic nature [18,57], and being often recalcitrant to biodegradation due to the xenobiotic character of the sulfonic group [26]. Thus, despite their apparent lack of toxicity, aromatic sulfonates and, in particular, sulfonated aromatic amines, are among the contaminants which should be monitored in industrial wastewater treatment systems. In the case of the textile industry, there is a driving force for the inclusion of anaerobic units or stages in wastewater treatment systems, for the purpose of high organic load abatement, nutrient removal or colour removal through reductive decolourisation of residual dyes [31,89], therefore increasing the opportunities for aromatic amine generation inside the treatment plant. Monitoring, and eventual elimination, of these amines can be best carried out at this stage, before they enter the environment and undergo several-fold dilutions making their detection and quantification increasingly difficult [57]. For this, fast and inexpensive detection methodologies are required.

Spectrophotometry in the ultraviolet (UV) range has repeatedly proven to be a fast, inexpensive and reliable method for the monitoring of both aggregate (oxygen demand, suspended solids) and specific (nitrate, surfactants, phenols) analytical parameters in urban and industrial wastewaters [90–92]. Through the application of spectral analysis based on deconvolution techniques [93,94], quantitative and qualitative wastewater parameters can be estimated on direct samples in just a few minutes, using portable or online field instrumentation. In the case of textile effluents, the use of the UV range of the spectra (200–350 nm) could be particularly useful to avoid interference by visible colour which would limit the applicability of other proposed spectrophotometric methods for amine analysis, based on a chemical reaction to produce quantifiable colour [39–41]. Table 4 briefly lists the potential advantages and disadvantages of the direct UV spectrophotometric method for the detection of aromatic amines in textile industry effluents.

Direct UV spectra examination in relation to the detection of aromatic amines resulting from azo compound reduction has not been investigated in depth, though publications on azo-bond cleavage sometimes include spectra in the UV–visible range, as supporting evidence for the accumulation or elimination of azo dye metabolites. Razo-Flores and co-workers [34] used UV–visible spectra together with GC–MS to demonstrate the elimination of only one of the aromatic amine metabolites resulting from the reduction of two azo compounds in a methanogenic bioreactor. On the other hand, Brás et al. [95] present UV–visible spectra as evidence that the bio-decolourisation process of Acid Orange 7 proceeds through molecular structural changes and not simple adsorption. In a different context, that of wastewater monitoring in a chemical industry, Perez [96] has successfully applied UV spectral deconvolution to the estimation of aniline derivative concentrations. By defining characteristic average spectra for global and chlorinated aniline mixtures, amine concentrations could be estimated from direct UV spectra, in the presence of interferences by a background organic matrix and by monochlorobenzene, with good correlation to HPLC determined amine levels.

Table 4
Brief assessment of the potential for aromatic amine monitoring in textile effluent by direct UV spectrophotometry, as compared with other analysis techniques

Assessment factors	GC, HPLC, CE with advanced detection methods (MS, diode array)	Rapid methods (chromophore-based spectrophotometry; titration with specific electrodes)	Direct UV spectrophotometry
Instrumentation	Complex; high cost	Simple; low-cost	Simple; low-cost
Chemicals	Multiple requirements; expensive; hazardous	Some requirements (derivatisation reactants; titrants); moderate cost	None, apart from UV cell cleaning
Sample preparation	Multiple requirements	Required to ensure high yield chemical conversion or avoid titrant side reactions	None, apart from dilution
Speed of analysis	Low	Moderate	High
Adaptability for online monitoring	Difficult	Demonstrated for other parameters, with online sample preparation	Easy; demonstrated for other parameters
Maintenance requirements	High	Moderate	Low (UV cell cleaning)
Skilled labour requirements	High	Low	Low
Analyte detection limit	Low to very low	Moderate	Moderate
Specific compound identification	Yes	No	No
Susceptibility to interferences	Minimised by sample treament	Moderate	Moderate

Within a typical textile dyeing process, aromatic amines of azo dye origin will mostly result from the wastewater treatment process, except for those contained as manufacture impurities in the commercial dyes employed [54]. In the effluent treatment process, conditions allowing azo dye reduction can occur in poorly aerated zones in equalisation tanks, particularly if retentions times are long and a significant concentration of biodegradable organic matter is also present. These conditions are certainly present in anaerobic treatment bioreactors, and can occur also in anoxic zones deliberately created in aerobic reactors for phosphorus and nitrogen removal. In addition, if dyes have been significantly transferred to the biological sludge, by natural adsorption for the less hydrophilic dyes, or with the aid of adsorbents like powdered activated carbon or polyelectrolytes, their reduction to aromatic amines can take place in the sludge processing stages, where high organic load and oxygen deficient conditions largely prevail. Contaminated waters from sludge processing operations are usually recycled to the equalisation tank, thus potentially returning amines to the liquid phase. Therefore, depending on the fate of dyes in the treatment process and the objectives of aromatic amine monitoring, e.g., registry of discharged amounts or the control of elimination processes such as advanced oxidation, sampling points should be chosen so as to maximise the possibilities of detection and allow mass balances to be carried out. For these sampling points, other present solutes or suspended matter should be assessed for their potential to cause UV spectral interferences in amine detection.

UV spectral characteristics of aromatic amines have been extensively studied within the broader domain of aromatic compounds, and have been duly included theoretical and empirical descriptions of the electronic transitions which give rise to characteristic absorption wavelengths [97]. Prediction methods for the effect of substituents, conjugation and heteroatoms on the characteristic spectra of benzene and napththalene have been established, though the accumulation of such features renders predictions increasingly difficult. The effect of pH is also to be considered, particularly with ionisable substituents, such as the amine, sulfonate and carboxyl groups. Table 5 gives some characteristic peak wavelengths in the UV range for aromatic amines.

In textile wastewaters, the array of chemicals used as auxiliaries in textile preparation, dyeing and finishing could interfere in aromatic amine UV spectral detection. Those likely to be rejected in strong concentrations are, however, mostly organic or inorganic acids, bases and salts. The use of alkyl benzene sulfonates or alkyl benzene ethoxylates as surfactants in the formulation of wetting and washing agents could also lead to interferences in the UV spectra, the sulfonates having been found to absorb in the 220–230 nm region [99]. Mineral sulfide which could be formed in anaerobic conditions as a result of the metabolism of sulfate-reducing bacteria, shows a peak at 231 nm, at neutral and alkaline pH [100], while carboxylic acids and aldehydes have been found to absorb also in the 190–240 range [92]. Fig. 1 presents some examples of spectra from chemicals currently used in large amounts in textile dyehouses. It can be seen that all significant interferences from this group of compounds lie in the 200–240 nm range, which effectively rules out the use of absorbance peaks in this region for the detection of aromatic amines (see Table 5). However, the aromatic amine spectra in Table 5 show extensive structuration in the 260–300 nm range, which would be free from interference from contaminants such as those above mentioned and exemplified in Fig. 1. Thus, the main interfering substances which should be considered in this context are the residual dyes themselves. Unfortunately, azo dye spectra show also varied and intense structuration in the near UV region, as can be seen from the three examples in Fig. 2. However, their characteristic absorption in the visible region (400–700 nm), where most aromatic amines show little absorption (nitro-substituted amines excepted, Table 5), provides a way to independently assess their residual concentration levels. By spectral deconvolution in the visible range, residual dye concentrations can be estimated and this information used to process the raw spectra in the exploitable UV region (Fig. 2) so as to eliminate dye interferences. This same principle was

successfully used by Perez [96] to remove nitrophenol interferences in UV-spectra based estimation of the concentrations of other, colourless phenol derivatives. The success of this procedure in dyehouse effluents however relies on a basic knowledge of the dyes discharged at the time of measurement, allowing the use of a database of dye spectra for the estimation of interferences in the aromatic amine detection range. In this context, the possibility of occurrence of absorption in the visible range from the products of the auto-oxidation of aromatic amines [20] should be assessed. On the other hand, provided spectral structuration in the 240–350 nm range for the parent dyes and their derived amines shows sufficient differences (see examples of Fig. 2), direct deconvolution with representative spectra can be carried out. The latter procedure can include spectra describing the suspended matter and dissolved organic matter matrices also present in raw samples, a technique which has already proven its usefulness in the monitoring of urban and industrial wastewaters [90–92]. In addition to potentially allowing the survey of aromatic amine emissions, direct UV spectrophotometry can also be an ideal technique for the monitoring of advanced treatment processes (oxidation, photo-oxidation, photolysis, adsorption) operated for the removal of residual

Table 5
Main spectral regions of absorbance in the UV–visible range for some aromatic amines

Aromatic amines	Main absorbance regions (nm)														Sources
Aniline derivatives															
Aniline	196				230					281					[97]
Anilinium ion	196–206			239–260											[97]
m-Phenylenediamine			211		238					289					[97]
o-Phenylenediamine			210		239						295				[97]
p-Phenylenediamine	197					241						305			[97]
p-nitroaniline				229							297–310			373	[97]
Sulfanilic acid	191		215				258–269								[97]
Sulfanilate ion	199					249				282					[97]
Metanilic acid					235			260–270		290					[98]
2,4-Diaminophenol		207										301			[97]
2,4-Diaminophenol anion			213			244							315		[97]
2,6-Diaminophenol			213							280					[97]
2,6-Diaminophenol anion				220							291				[97]
2-Aminoresorcinol		205			234				271						[97]
2-Aminoresorcinol	198			225-230						294					[97]
o-Methoxyaniline					238					288					[98]
p-Methoxyaniline					234				297						[96], [98]
p-Chloroaniline						242				295					[98]
2,3-Dichloroaniline					236					292					[96]
m-Methylaniline					234				284						[96]
5-Aminosalicylic acid												325			[34]
m-Trifluoromethylaniline					235					290					[96]
Biphenyl amines															
4-Aminobiphenyl								270–280							[98]
Benzidine									280–290						[98]
Naphthylamine derivatives															
1-Naphthylamine		211				242						320–327			[97]
1-Naphthylamine cation		219–220					260–289								[97]
2-Naphthylamine		213			237				272–291				340		[97]
2-Naphthylamine cation			220				267–283								[97]
4-Nitro-1-naphthylamine						247–259								406	[98]
1-Amino-2-naphthol-4-sulfonic acid		217				242					310		345		[a]

[a] Spectrum obtained in the authors' laboratory.

amines and other aromatics from textile industry wastewaters.

6. Conclusions and perspectives

In the present context of increasing concern for the survey of chemicals' releases to the environment, aromatic amines have an unquestionable place. During the past century, the developed knowledge of their hazards, the resulting regulations and the efforts of synthesis chemists have led to remarkable improvements in the safe manufacture, handling, use and disposal of products involving aromatic amines, in particular azo dyes. However, market pressure towards the continual introduction of new products has led to the present, extensive range of commercialised dyes, for many of which adequate environmental and health impact data is still lacking. On the other hand, the quantification of chemicals in the environment is a major issue, leading to the development of increasingly sensitive analysis methods and providing each day new data pointing to the

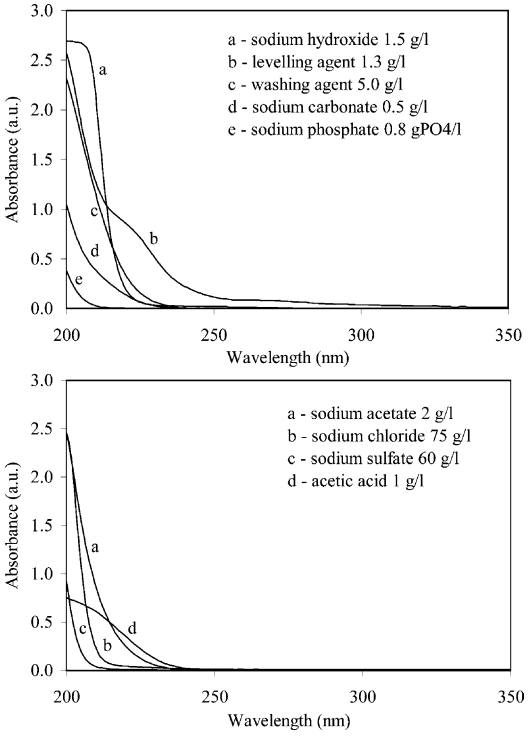


Fig. 1. UV spectra of some examples of salts, acids, bases and auxiliary agents likely to occur in textile wastewaters.

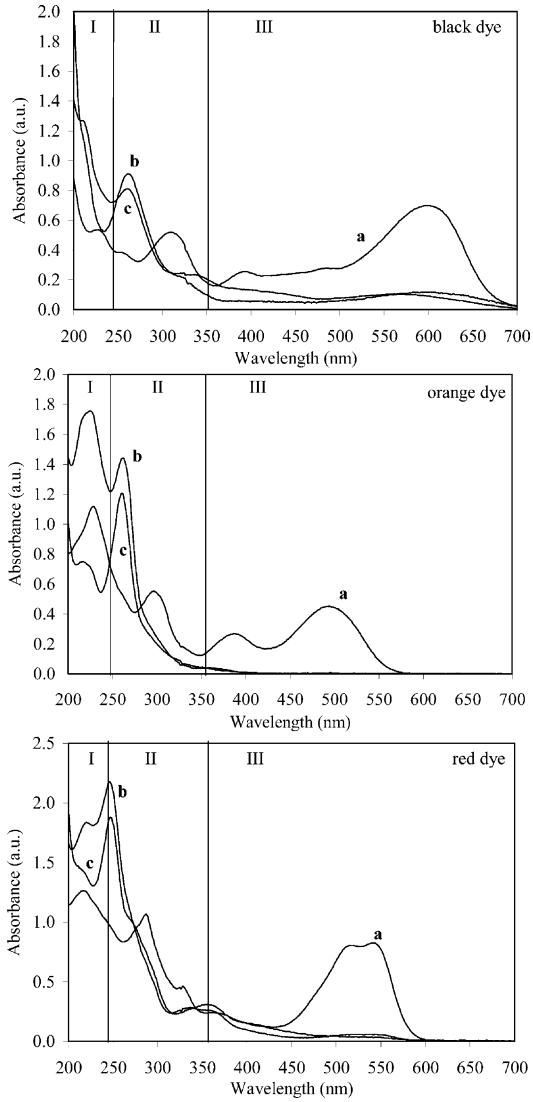


Fig. 2. UV–visible spectra of three commercial azo dyes of the reactive type presently in use for cotton-based textile dyeing (a), together with spectra obtained after their chemical reduction with sodium sulfide (b) and after their biological reduction with activated sludge in anaerobic conditions (c). The vertical lines roughly identify the spectral zones where severe interferences are likely to occur in textile wastewaters (zone I), according to Fig. 1, where spectra exploitation for amine detection is possible (zone II), according to Table 5, and where spectra exploitation for the quantification of residual dye levels is possible (zone III).

persistence of many compounds, among which the sulfonated aromatics. Thus, in anticipation of renewed actions in the enforcement of the precautionary principle, rapid, inexpensive methods for the monitoring of aromatic amine emissions in wastewaters, particularly in the case of azo dye reduction in textile effluents, need to be developed. Direct UV spectral examination, combined with advanced deconvolution methodologies, has a definite potential in this domain.

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