Novel chemical and toxicological studies on textile industry waste streams clean up

by

Svetlana V. Popova

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ABSTRACT

The textile industry is a major water consumer and producer of effluent waste water. A comprehensive review of the literature shows an urgent need to improve the treatment of dyeing and finishing plant effluent. To destroy dyes and purify dye house effluents modern photochemical oxidation methods, such as the UV/hydrogen peroxide process, provide an effective treatment technology. Organic contaminants can be completely mineralised by this method but it is relatively expensive. Photochemical oxidation with subsequent microbiological treatment appears an attractive and cost effective approach in textile waste water treatment.

This research studied the feasibility of combining UV/hydrogen peroxide photochemical oxidation with microbiological treatment to destroy organic dyes and to decolourize waste water. Experiments on the decolourization of a number of mono- and disazo dyes by UV/oxygen and hydrogen peroxide as a function of initial concentration of dyes, dissolved oxygen and hydrogen peroxide, alkalinity and temperature of solutions were carried out. UV/hydrogen peroxide photochemical treatment was shown to rapidly decolourize and decompose organic azo dyes. Increase in oxidant concentration to give a molar ratio between hydrogen peroxide and dye concentrations of 200/1 was found to increase the decolourization rate. Changes in pH to acidic and alkaline also increase dye decolourization rate, the highest decolourization was observed at pH 10.5. Increase in the temperature from 25°C to 60 °C slightly increases decolourization rate.

Chromatographic, spectrophotometric and mass-spectrometric techniques were used to elucidate breakdown pathways and identify intermediates. Suggested type of photo-degradation is destructive oxidation involving rapid breakdown of the azo group with the formation of colourless intermediate substances such as naphthalene (naphthoquinone) and benzene (phenol) derivatives. These intermediates do not accumulate and are further photo-degraded to lower molecular weight substances, organic acids.

Work on establishing ecotoxicological effects and biodegradability of dyes and their photochemical breakdown products, with particularly reference to micro-organisms, was carried out with the biological tests using the freshwater green alga *Chlorella vulgaris*, the bacterium *Pseudomonas putida* and activated sludge. Photochemical treatment of dye solutions with UV/oxygen or UV irradiation alone does not eliminate their toxicity. When the UV/hydrogen peroxide system was used decolourized solutions were non-toxic to micro-organisms. Activated sludge is slow and inefficient in removing residual colour from the dye solutions partially decolourized by photo-oxidation, generally removal of residual colour was in the range of 82 – 85.5 % after 72 hours of biotreatment. An increase in biochemical treatment time from 24 to 144 hours also did not have an influence on colour removal. With an increase in preliminary photochemical treatment time susceptibility of the dye solutions to the biochemical action of activated sludge increases.

The combination of the UV/hydrogen peroxide process with subsequent microbiological treatment is a satisfactory treatment for azo dye solutions suitable for commercial application. Further research to extend the application to other dye classes, particularly reactive dyes in justified.

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"The earth is a garden.
It's a beautiful place.
For all living creatures,
For all the human race.

Helping Mother Earth
We can peacefully roam.
We all deserve a place
We can call our home.

Food is a treasure from the soil and the sea.
Clean, fresh air from the plants and the trees.
The warmth of the sun giving life each day
Turns water into rain, it's nature way.

And I would like to thank you Mother Earth
I like to see you dressed in green and blue
I want to be by you..."

Lorraine Bayes

CHAPTER 1. INTRODUCTION.

Currently, due to long term neglect of the problem, environment protection is one of the global challenges facing mankind. Moreover, there was the perception that natural resources were inexhaustible. Unsustainable exploitation of the environment and the increasing scale of development have upset the ecological balance. Negative consequences of anthropogenic effects have made it necessary to systematically deal with undesirable impacts on the environment and to institute measures to manage the widespread effects.

In recent years the annual increase of anthropogenic loading in the biosphere has been marked, and environmental protection is characterised as failing. Because all pollutants tend to enter water, the aquatic environment is subject to the greatest ecotoxicity pressure [Annual state report 1998, Matoshko 1989]. Important tools in dealing with ecological problems are the minimisation of the pollution of an environment and the generation of biologically dangerous levels of substances by means of constant environmental monitoring, developing modern waste treatment technologies and stringent water environment quality control.

Pollutants of the environment include the systematic discharges and emissions from industry, including the textile industry. The textile industry, a major water consumer for several of its wet processing operations, also produces substantial volumes of waste effluent. These are often highly coloured and contain different chemicals ranging from inorganic compounds and elements, to polymers and organic products which are potentially detrimental to the aqueous environment [Efimov et al 1985]. Consequently,

textile waste water requires extensive treatment before it can be discharged into the aqueous environment.

Effluent discharges from the textile industry to neighbouring water bodies and waste water treatment systems are therefore currently causing significant concern to the public and environmental regulatory agencies in most countries in the world. Government legislation and community pressure are currently forcing the industry to minimised the use and generation of hazardous chemicals in textile operations whilst also increasing waste water treatment efficiency. Very stringent consent limits have been set and the textile industry is finding difficulty in meeting them.

1.1. Characterisation of textile waste water.

The textile industry uses a wide variety of processing operations and equipment, raw materials and chemicals. This leads to waste water which is highly complex and variable in composition including surfactants, organic and inorganic salts, acids, alkalis, oxidising and reducing agents, different solvents and dyes as some of its constituents. The contribution of each type of substances in textile waste water to the total pollution is important but is evaluated differently by various researchers [Melnikov & Vinogradova 1986, Kritchevsky et al 1985, Popova & Nefedova 1980, Laskov et al 1997, Klimenko & Kozshakov 1991].

Two main sources of pollution in textile waste water originally arise from fibres and fabrics being treated, and from textile technological spent bath discharge after desizing, scouring, bleaching, dyeing and finishing operations [Peters 1989]. Washing waters after

these operations, producing about 80 % of the total volume in the textile effluent, might be discharged into environment water bodies or biological treatment plants.

Initially textile materials are subject to a number of purification operations which removes natural colouring and extraneous substances, thus improving whiteness and wetting and giving increases of dye strength and affinity to fabrics. Washing waters from these operations are typically not highly coloured but of high inorganic substance content (chlorides, sulphates, hydroxides, sodium silicate and meta-silicate, acetates, sodium carbonate, inorganic acids and other chemicals). These effluents also contain natural and mineral impurities removed from fibres during the operations and technological impurities, such as lubricants and antistatics, coated on fibres before the spinning process and sizing agents, applied to yarn before weaving, etc.

Bleaching, using oxidants such as sodium chlorite and hypochlorite, introduces chlorine-containing compounds into the effluents which, on interacting with organic substances in natural water bodies and in waste water itself, can lead to the formation toxic chlororganic compounds such as dioxins. Bleaching by chlorine-containing reagents has recently been superseded by ecologically safe peroxide bleaching technology [Kritchevsky 1996].

Dyeing and finishing of textiles is a highly important process in textile manufacturing operations, in terms of both the quality of textile products, especially those with a high fashion content, and environmental concerns. During dyeing process large amounts of waste water are generated from the discharge of spent dye bath solutions themselves and washing waters from the dyeing process. In these, as well as dyes, there can be surfactant and textile auxiliaries added to the dye bath for optimum dispersion of dyes and pigments

in aqueous solutions and for best careering of dyes to fibres and fastening of colour. Effluents from dyeing process are generally characterized as containing considerable colour, a little suspended solid, and mainly a range of soluble compounds such as inorganic and organic salts, surfactants (particularly non-ionic ethoxylated surfactants), other chemicals used in the process including heavy metals and chlorinated compounds, and have high chemical oxygen demand, varied pHs and temperatures.

The finishing process contributes the least pollution loading to total effluent volume, since the majority of modern finishing processes for increase of strength, fire- and waterproofing, etc. of fabrics treated do not require washing operations. However, at the same time, there is a problem of emissions into the air from drying apparatus because chemical products for the finishing process contain free formaldehyde, which escapes during the drying process.

Dyes are not usually major contributors to the chemical oxygen demand (COD) of textile waste water. Data present in table 1. 1 [Reife & Freeman (eds) 1996] indicates that well over 90% of COD load from a typical plant that dyes and finishes woven cotton fabrics come from desizing, scouring agents and bleaches.

Table 1. 1. Typical annual pollution loads from a cotton fabrics textile plant.

Waste source	COD (tons per year)	%
Desizing agents, scouring agents and bleaches	164	86.6
Reducing agents	8	4.2
Detergents and wetting agents	7	3.7
Finishing agents	5	2.6
Organic acids	3	1.6
Dyes and fluorescent brighteners	2.5	1.3

However dyes often receive the most attention from researchers interested in textile waste water treatment processes because the dyes cause both organic pollution and a high coloration of the effluents.

1.1.1. The contribution of dyeing to aqueous environmental pollution.

Coloured effluents result from dyeing and washing operations and the extent of coloration depends on the colour/shade dyed and the type of dye used. There are several thousand chemical products connected with the textile dyeing process listed in the Colour Index [Colour Index 1976] while over 100,000 commercially available dyes and pigments exist, and are produced and used annually in the world textile industry.

Synthetic dyes include several structural varieties based on chemical structure of chromophoric groups and are classified as azo, anthraquinone, indigoid, sulphur, diaryl- and triarylmethane, stilbene and other dyes. Azo dyes account for approximately two-thirds of the dyes available on the market today and constitute the largest group among all

dye classes used in the textile industry. Dyes are classified according to their industrial application as acidic, reactive, basic, disperse and metal-complex dyes, etc. Of the various industrial dye classes, acid and metal-complex azo dyes account for about 12%.

Depending on the industrial application of the dyes, their physical and chemical properties, affinity to the fibre being dyed, and process performance efficiency the extent of dye fixation on different substrates is often on average no more than 70-80% [Peters 1989, Jakovlev 1996, Cooper 1995, Saphonov 1997]. The rest is discharged to municipal treatment plants or environment water bodies. The following Table 1.2 [Cooper 1995] shows the extent to which dyes can be lost to the water environment.

Table 1.2. Extent to which dyes are lost in exhaust and washing water from dyeing.

Dye class	Amount of dye charged lost in waste effluent, %
Reactive	20 – 50
Vat	5 – 20
Direct	5 – 20
Acid	7 – 20
Acid metal-complex	2 – 5
Disperse	20 – 40
Basic	20 – 40
Sulphur	30 – 40

It is clear from the above table that residual amounts of dye in the effluents from the dyebath and washing water differ with dye classes and their chemical structure. Among the

various dye classes, the reactive dyes are of great concern because they have lower rates of fixation of the dye on fibre and up to 50 % of dye charged can be lost into total effluents. However, during the last decade significant improvement in exhaustion and fixation of reactive dyes has been achieved by using bifunctional dyes which contain two reactive centres such as monochlorotriazine and vinyl-sulphone reactive groups. These groups present in dye structure provides higher fixation of these dyes on substrates dyed (to over 80 %) in comparison with other reactive dyes used. This reduces the amount of dye lost into the effluents. In practice, during dyeing of fibres especially cellulosic, up to 20 % of the dye is not fixed in the fibres and dyehouse effluent normally contains about 10 – 50 mg/l of dyes. This concentration is high enough to produce noticeable coloration of water.

Dye lost in effluents after dyeing depends on not only the structure of the dye and the fibres being dyed but also on dyeing method effectiveness and parameters, including the liquor to goods ratio factor. The exhaustion and fixation of the dyes can be increased in some cases by dyeing at lower liquor to goods ratios, for example [Saphonov 1997]:

CI reactive yellow 22: dye take up of 20% - at 30:1 liquor ratio
dye take up of 75% - at 0.6:1 liquor ratio.

Discharge dynamics of spent dye-bath and washing water are well known to also depend on the dyeing technology. If a discontinuous dyeing process is used concentrated effluent will periodically form, and in the case of continuous dyeing processes an effluent, constant in concentration and composition, will be discharged. The main physical and chemical parameters of a coloured waste water discharge are strongly dependent on the dyeing technology and the materials (fibres) to be dyed and finished. The average parameters of

waste water can change in following ranges [Krasnoborodko 1988]: dilution titre of coloured effluents with water to almost complete colour disappearance from 1:25 to 1:4200 (and to 1: 6500 in the case of natural silk dyeing); suspended solid substances from 40 to 2200 mg/l; COD from 50 to 2300 mg/l and pH from 3,3 to 11,4.

The common textile discharge forms from a highly concentrated stream of spent dyebath liquor and the washing water after the dyeing operation. Most washing water (especially in the case of discontinuous dyeing process) contains less pollution than the spent dyebath solutions. In this case, it would be therefore useful to apply separate waste streams systems for these flows and to have preliminary treatment of the spent dyebath solutions with high concentration of dyes in a local waste treatment system.

Investigations [Nazarov et al 1978, Kritchevsky 1996] show that dyeing washing water can have dye concentration levels which on reuse do not affect normal work in some textile technological operations and the quality of goods produced. For example, dyeing of fabrics in dark shades and first-stage washing operation after the dyeing with such reused water were satisfactory.

Separate sewage systems and reuse of wastewater in the textile operations could have the following advantages:

- saving in dyes and chemicals loaded;
- saving water and energy;
- less effluent produced and therefore:
- saving in the cost of effluent treatment technology.

Moreover, dilution of dyehouse effluents with other industrial and domestic discharges in the sewer makes the colour more difficult to treat and creates problems with acceptability of the effluent to the water company.

The major problem with textile waste water is colour produced by the residual dyes during dyeing and washing operations. According to the above data dyes are present in waste water at low concentrations and constitute only a small portion of the pollution in the total volume of textile wastes. While coloured organic compounds contribute only a minor fraction of the organic load to textile effluents, their colour renders them aesthetically unacceptable and these compounds have to be removed before discharging into water bodies. Therefore the removal of dyes from waste water is often more important than the removal of the soluble colourless organic substances.

1.1.2. Textile effluent – environmental concerns and the toxicity of dyes.

In the industry there is considerable debate on what level of environmental hazard is produced by coloured effluent. Beckman and Sewekow [Beckman & Sewekow 1991] have stated that there are "no tenable arguments for the classification of dyestuffs as dangerous substances" in the effluent and "dyestuffs should not be regarded as water pollutants" since "the harmful effect is negligible". The colour problem they have regarded as only aesthetic and the dyes are safe to use. It is only the fact that coloured waters cause public concern that leads them to be treated as pollutants because their presence is so obvious and they do cause aesthetic offence.

Interest in the pollution potential of textile dyes is basically promoted by their possible toxicity, carcinogenicity and mutagenicity. Synthetic dyes, like many organic compounds, are known to be capable of significantly affecting aquatic organisms and the aquatic environment in general [Pohobradski 1982]. This is mainly due to the fact that some raw materials used to synthesize dyes are known carcinogens such as benzidine, naphthylamines (products particularly used to produce azo dyes) and other aromatic compounds. Whilst known human carcinogens, benzidine and naphthylamines, are no longer used as starting materials in dye manufacturing environmental microbial and chemical breakdown of the dye molecules may still give rise to toxic, even mutagenic and carcinogenic products [Clarke & Anliker 1980]. Thus, natural waters and their ecosystems could be seriously affected because of dye presence.

Dyes, independent of their classes, have strong absorbance of light in a wide range of the electro-magnetic spectrum, from the visible to the ultraviolet or infrared regions. Therefore the presence of even small amounts of dyes (at concentrations much less than 1 mg/l for some dyes) in water bodies is inherently highly visible. The visibility of dyes in the waters depends on both their colour and extinction coefficient, and also the clarity of the water. Release of coloured effluents often causes abnormal coloration of surface water and significantly affects their mineralisation level [Wells et al 1994].

Neglecting the aesthetic problems, an environmental concern with dyes is their ability to accumulate in water reducing transparency and light penetration into water and, consequently, reducing photosynthetic activity damaging aquatic plant life. This interferes with the growth of bacteria to levels sufficient to biologically degrade different impurities in the water and to maintain the food chain [Richardson 1983, NIR report 1991].

Depending on exposure time and dye concentration, dyes can have acute and/or chronic effects on exposed organisms.

Not all dyes give rise to the same level of environmental problems. Reports in the literature indicate that most textile dyes do not exhibit significant toxicity to microbial populations [Brown et al 1981, Shaul et al 1991]. However certain dyes which contain azo groups and some substituants such as amino-, nitro- and sulphonic groups are generally found to be recalcitrant to bacterial degradation [Fewson 1988, Kulla et al 1983, Michaels & Lewis 1985, Pagga & Brown 1986, Shaul et al 1991].

Toxicity of dyes to aquatic organisms has been thoroughly tested by different institutions over the world. It has been shown according to data of the Ecological and Toxicological Association of the Dyestuff Manufacturing Industry (USA) that many dyes can inhibit biochemical activity of different aquatic organisms [Anliker 1979, Clarke & Anliker 1980]. Among 3000 commercial dyes in common use 98 % of those tested appeared to be toxic to the fish *Pimephales promelas*. Concentrations causing 50 % fish mortality (LC_{50}) were less than 1 mg/dm^3. Algae are an important part of the aquatic ecosystem because algal photosynthesis is a source of oxygen in surface water and they are a key element in the food chain. Of 56 dyes tested, 15 inhibited algal growth and 13 of these were basic dyes. Anthraquinone-based dyes were shown to inhibit growth of the green algae *Selenastrum capricornutum*, may be toxic for other aquatic organisms and were also shown to be the most resistant to biological degradation due to their complex aromatic structure [Grushko & Timofeeva 1983].

Metal-based complexes of dyes containing chromium, cobalt and occasionally copper in their structure, particularly those containing chromium (often used in the recently introduced low chrome dyeing methods for wool instead of the older after-chrome dyeing) on entering the aquatic environment can lead to the release of the metal, which is very toxic to aquatic micro-organisms [Clarke & Anliker 1980]. In the dyeing process usually both Cr (VI) and Cr (III) are present in the spent dyebath solutions and could be discharged to water, but Cr (VI) is the more toxic of the two species.

Some disperse dyes have been shown to have a tendency to bio-accumulate in aquatic ecosystems, and the octanol/water partition coefficient has been used as an indicator of bio-accumulation of the dyestuffs [Anliker & et al 1981, Baughman & Perenich 1988]. Moreover disperse dyes as well as surfactants and other organic substances in the receiving water bodies can be sorbed onto sediment in higher concentration than in the water [Svenson et al 1996, Takada 1993, Baughman & Perenich 1988]. This also occurs with ionic dyes (acid, basic dyes, etc.) [Baughman & Perenich 1988]. Filter feeding invertebrates in particular will be vulnerable due to this process. It should be also noted that more detailed study of dye toxicity in sediments either using environment conditions or in laboratory conditions has not been carried out.

Investigations of the effects of direct dyes on natural water biota showed that the dye at concentrations above 0.1 mg/dm^3 significantly affect oxygen penetration from air into water and dissolved oxygen concentration, COD and BOD levels, and especially the nitrogen concentration needed for biological activity [Dergatcheva 1980]. It has been also shown, that direct dyes are not easy to biodegrade. After 20 days under environment conditions their concentration in surface water has only fallen by 5-10 % [Udod 1980].

Maximum no observed effect concentrations (NOEC) of direct dyes for aquatic self purification processes in water are less than 0.001 mg/dm^3. According to the EPA data a number of the direct dyes have been included in the "red list" and some of the them (direct azo dyes) are classified as carcinogens.

Reactive dyes are in general less toxic in comparison with other dyes classes because of their not being capable of readily penetrating into living cells [Ivanov 1982]. Nevertheless, active dyes can be toxic to mammals. The role of the dye chromophoric system itself, and reactive groups providing colour intensity and affinity of the dyes to fibres, in toxicity to organisms has not yet been clarified.

Azo dyes of various categories (i. e., acid, basic, direct, others), being products of coupling a diazotized amine with other aromatic or heterocyclic amino-compounds, receive particular attention in their toxicity studies because these dyes are known to release the initial components of dye synthesis under reductive cleavage conditions [Oh et al 1997]. Some investigations reported reductive bio-transformation of the azo linkage in the aquatic environment and abiotic reduction of azo- and nitro- groups in sediments resulting in the regeneration of the toxic parent amines [Brown & DeVito 1993, Weber & Wolfe 1987]. In mammals azo dyes are reduced to aryl amine derivatives mainly in the liver and by intestinal microflora through cytochrome P-450 and by a flavin-dependent cytosolic reductase [Chung et all 1978, Chung & Stevens 1992]. In human intestinal microflora azo dyes are also reduced by a novel flavin-dependent azoreductase.

Investigations on the toxicity of azo dyes of various chemical structure with different numbers of azo groups (mono-, dis-, etc., polyazo), and also mixtures of the dyes used for

leather (synthetic and natural), fur, and textile materials dyeing were thoroughly carried out by a research group from the Ukraine Academy of Sciences [Zvezday et all 1994, 1995 and 1998, Niculina 1996, Niculina et all 1996, 1998, Pchelintschev & Jaskova 1996, Pchelintschev 1996, Deveykis 1998, Vasilenko et al 1998, Kaluzshy 1998]. It was found that the majority of azo dyes tested do not show mutagenic activity using the Ames Test but may be carcinogenic in rats and mice when administered orally or by intraperitoneal injection. Examination of the possible reproductive toxicity of azo dyes in rats revealed the potential of these dyes to cause embryotoxic problems at doses of 400 mg/kg. Aromatic amine intermediates and the metabolites appeared to be more embryotoxic than the colourant substance itself. Thus, although there is ample evidence that many azo dyes are inherently toxic in their pure form, the possible presence of carcinogenic and mutagenic impurities must always be considered in evaluating of azo dye toxicity.

Although it is not proven that all azo dyes are mutagenic or carcinogenic in mammals and man, the azo dyes damage functional activity of kidneys, liver, blood and reproductive systems, are allergens for skin, and affect eyes and respiratory function [Palagina et al 1995, Zvezday & Palagina 1997]. However the relationship between toxic effect and the dye chemical structure, physico-chemical properties, or amount of colorant in commercial dye products is not clear and is currently under investigation.

The previously mentioned classification of dyes (as acid, direct, basic, etc.) based on their dyeing mode is inadequate for environmental evaluation purposes although common properties of dye classes facilitate understanding of their reactions in the aquatic environment. Firstly, the ability of dyes to absorb sunlight results in various photochemical dye transformations. During exposure to sunlight in the environment dyes were found to

become more toxic to hydrobionts [De Lemos & Sugden 1988], and this phenomenon was discussed at a symposium held by the Agrochemical Section of the American Chemical Society [Baughman & Perenich 1988]. Secondly, ionic dyes (in fact, all water soluble dyes) have a tendency to react with substances of opposite charge in water. The main disadvantages of this are that interaction of anionic dyes with magnesium and calcium ions reduces concentrations of these essential elements necessary for biological reactions by forming insoluble precipitates, and the interaction of ionic dyes with humic acids or hydroxides.

A reason for the lack of data on the properties of many dyes in environment risk assessment is that researchers usually investigate very limited ranges of dyes although their variety is constantly being expanded. Those attempting to develop toxicologically relevant data for the dyes are confronted with the difficulty that commercially formulated dye products contain several additives (detergents, salts, oils, etc., including residuals of synthesis intermediates) as well as the actual colorant molecules and they are difficult to purify. Each manufacturer synthesizes the same dye using different parent compounds and adding different types and quantities of additives. Moreover information on dye structure and colorant content is largely confidential and is not freely released.

In addition identification of the dye structures is not always easy. Substance-specific analysis to identify dyes traditionally used thin layer chromatography [Venkataraman 1975]. In recent years liquid chromatography and gas chromatography coupled with mass spectrometry (LC/MS and GC/MS), applied for the analysis of impurities in waste water, have provided only a little more information on this type of effluent. By means of GC/MS only a fraction of the pollutants really existing in the effluents can be detected. The dyes

(like any polar organic compounds) contained in these wastewater are not volatile and accessible to GC separation [Baughman & Perenich 1988]. For more specific information high performance liquid chromatography and mass spectrometry with different desorption ionization techniques including thermospray, laser desorption ionization and fast atom bombardment are considered to be more useful [Straub et al 1992, Jandera et al 1996, Rafols & Barselo 1997, Connely et al 1999].

1.2. Textile effluent treatment technologies: their present role and future potential.

The aforementioned problems have encouraged efforts to minimise environmental releases resulting in lower dye release which could cause detrimental effects at concentrations below visible detection. The treatment of coloured waste water therefore is not restricted to the reduction of ecological parameters, such as chemical oxygen demand (COD), biological oxygen demand (BOD), total organic carbon (TOC), temperature and pH, but also includes the reduction of dye concentrations in waste waters.

Methods of waste water treatment for most effluents are well established, but no one method is successful in every case. Dyes are difficult to treat due to their synthetic origin and complex aromatic molecular structures. Moreover dyes are also designed specifically not to degrade and to resist fading on exposure to water, microbes, detergents, light and oxidising agents. Water soluble dyes have been the main problem, while water insoluble dyes (e. g. disperse and vat dyes) generally exhibit good exhaustion properties and can be removed from the effluent by known and relatively simple physical means such as

coagulation and flocculation, combined with flotation if necessary [Timofeeva 1991, Lin 1993].

To develop effective treatment methods for colour removal from dyehouse effluent, it is important to understand the chemistry of dye molecules. Various bonds and active groups within a dye molecule are responsible for their colour and colour intensity. The colour of water is reduced when the cleavage of the –C=C– bonds, the –N=N– bonds and heterocyclic or aromatic rings occurs.

Dye containing waste water can be treated by different methods of dye removal which refer to the purification process called decolourization. The effectiveness of dye destruction is judged by decolorisation rate. Regarding techniques for textile waste water treatment physical, biological and chemical methods are known.

1.2.1. Microbiological treatment methods.

The most extensively used and cost effective treatment methods for the removal of any pollutants today are **biological systems**. Conventional aerobic biological processes used for the textile effluents containing dyes and surfactants are generally not efficient for colour removal [Pagga & Brown 1986, Doyanycs et al, 1978, Jiang & Bishop 1994, Shvetsova & Morozova 1989]. Biodegradability studies have shown that dyes are generally resistant to biological activity under aerobic growth condition. The little colour removal usually observed can be accounted for by dyes and their metabolities being adsorbed in micro-organisms in the aerobic biological treatment [Ganesh et al 1994, Pagga & Brown 1986] rather than being completely mineralised.

Activated sludge removes no more than 50% of soluble dyes (often basic and direct), but only about 25 % in the case of reactive and certain acid dyes. It also fails to deal with some dyes when in combination with others [Cooper 1995]. The main reason for this is that many synthetic dyes are resistant to microbial degradation under conditions normally found in biological treatment plants. This problem is reflected in the long retention times of textile extended-aeration systems which operate at 2 – 3 days retention whereas municipal treatment systems normally operate at 8 – 12 hours retention.

Moreover variable discharges and effluent composition influence biological process capability. It does not allow the adaption of the activated sludge to each pollutant in effluent for effective bio-chemical degradation and causes the intoxication of sludge micro-organisms [Slokar & Le Marechal 1998]. Most dyes independent of chemical class were found to have increasingly inhibitory effects on sludge micro-organisms at concentrations above 25 mg/dm^3 [Krasnoborodko 1988].

The interest in the study of microbiological destruction of dyes does not, however, weaken. Fundamental investigations have revealed the existence of a wide variety of bacteria capable of decolourising a wide range of dyes. Recent work has focused on the investigation of anaerobic and aerobic conditions in biological treatment, the study of their combination with each other and with physical-chemical waste water treatment methods [Loud et all 1992, Carliell et al 1995, Yatome et al 1991, Seshadri et al 1994, Carliell 1998].

During the past two decades many non biological treatment methods have been investigated and developed. They are divided into three main groups: reagent methods

(coagulation and flocculation, sometimes combined with flotation), separation (adsorption, ultra– and nanofiltration, reverse osmosis) and destructive methods (such as electrochemical destruction, chemical oxidation, reductive chemical processes, etc.) [Mishra & Tripathy 1993]. Each technique has a specific application and distinct advantages and disadvantages.

1.2.2. Physical/chemical treatment methods.

Coagulation / flocculation are amongst the oldest methods of waste water treatment but these methods have varying degrees of success as treatments to remove colour from textile effluents. The efficiency of colour removal is variable, ranging from 20 – 90 % depending on pH, dye class, etc. [Laskov & Vasiljev 1980]. The coagulation process effectively decolourises insoluble dyes (such as disperse dyes), but is not suitable for soluble dyes. For example, reactive dyes are not decolourised by such processes without addition of specially developed organic polymer coagulating agents. Different polymers were evaluated as coagulants and flocculents for treating reactive dye waste water [Runge 1996]. In general, very little colour removal was achieved. The best performer was a strongly cationic, high molecular weight flocculent that achieved approximately 50 % colour removal. However over 7600 mg/dm^3 of flocculent was required to achieve this reduction.

Since effluent varies in terms of dye molecule type, composition and concentration, depending on production requirements and flow, polyelectrolyte has to be overdosed both to improve the efficiency of reaction and to enable the least reactive molecules to be completely removed. This leads to high residual amounts of the polyelectrolyte in the

effluents. Another disadvantage of using coagulation and flocculation is the generation of sludge in large amounts which may become a pollutant itself and require disposal. Sludge formed is difficult to dewater, leading to problems in disposal and in the increase of treatment cost.

Polymer flocculents are favoured in use because they produce less sludge than inorganic ones, but excessive flocculent doses are needed to achieve desirable decolourization. This was pointed out may cause toxicity to bioassay test aquatic organisms as Daphnia pulex and Fathead Minnow [Hall & Mirenda,1991]. Thus, the use of polymer flocculents does not seem to be a practical treatment method of coloured effluents.

Activated carbon adsorption is a widely used technique among the various types of adsorption processes successfully employed for pollutant removal. A problem associated with adsorption processes is the regeneration of the adsorbent applied in both granular and powdered forms. Activated carbon (AC) adsorption, therefore, is most suitable for volatile organic compounds (VOC) which are readily and effectively adsorbed onto the AC as well as being capable of rapid and efficient desorption so that the AC regenerated can be reused a number of times.

Activated carbon is quite effective in removing colour. Average percent colour reduction is of more than 95 % at large doses relative to waste water treated. Although molecules of dyes are reasonably well adsorbed onto the AC, the desorption of these large, inert and high molecular weight molecules is extremely difficult. For regeneration of activated carbon the dyes require to be incinerated (thermally destroyed) resulting in additional technical problems increasing the cost of the process [McKay 1980]. It is a fact that

insoluble dyes such as disperse, sulphur and vat dyes and pigments, are not removed from effluents by activated carbon. The method is also most effective with relatively small volumes of water on a short time-scale, that is not the case with the high volumes involved with dyeing.

Other different adsorbent materials: flaky silicates, mineral, polymeric, natural (including rice grain, chitin, linen straw, dust and even dewatered sludge from a local treatment work) have been studied for dye removal [Tarasevich 1981, Podlesnijuk & Levchenko 1985, Cooper 1995, Najm et al 1991, Timoshenko & Klimenko 1990, Gupta et al 1988, Kievsky 1992, Yakubu & Alhassan 2001]. Colour removal using these adsorbents was generally very effective, giving up to 99 % of reduction, however they also require high doses of adsorbents, or further regeneration or have disposal problems.

Membrane technology, subdivided into the categories: ultrafiltration, nanofiltration and reverse osmosis, is effective in separating dye molecules from the effluent. It actually separates all large molecules, not only dye molecules, and the extent of separation depends on the membrane size used [Runge 1996, Holodckevich et al 1996]. Thus ultrafiltration is of no use in colour removal as the membrane pore size is too large to prevent dye molecules passing through. On the other hand, both nanofiltration and reverse osmosis membranes are effective in separating large dye molecules from effluents.

The technique is capable of treating large volumes fairly quickly but cost is high because membranes rapidly lose selective properties and need to be often cleared [Baran 1990]. To protect the membranes from rapid clogging and fouling a pre-treatment system likely to be involved followed by one or more membrane units or [Buckley, 1992]. The pre-treatment

system, including coagulation and/or adequate filtration, removes the suspended solid making effluent input to membrane cleaner and extending the life-time of the membrane longer. The combined treatment removes 100 % dye colour producing reusable water.

The major drawback to this system is that a coloured residue still remains for disposal [Desai 1982]. Incineration of membrane units is the most effective disposal route, but as some dyes contain chloroaromatic species, there is a risk of dioxin formation. Evaluating membrane technology in general, it can be said the technology is costly, the operation and maintenance intensive, and it generates a concentrated dye stream requiring additional treatment, particularly if the dye recovered cannot be reused.

Electrochemical treatment has been found to remove colour efficiently. Electrochemical destruction method can destroy chromophoric and auxochromic structures of dyes and alter organic dye molecules to more simple organic or new inorganic molecules resulting from reductive / oxidative chemical processes. There is therefore not a problem with sludge formation and disposal.

Colour reduction of 75 to 100% can be achieved with a wide variety of dyes [Ulker & Savas 1994, Beygeldrud 1996, McClung & Lemley 1994, Lin & Peng 1996]. The study of the electrochemical treatment of waste water containing direct and basic dyes has shown that the dyes can be rapidly decolourised [Beygeldrud 1996]. It was however pointed out that chlorine present in the structure of basic dyes, and in metal chlorides applied during dyeing, was oxidised to chlorine dioxide. This participates in the further oxidation of organic substances, increasing the content of chlorites and chlorates in the effluent. Chlorine produced may also generate undesirable chloramines.

The electrochemical process was evaluated for acid anthraquinone and reactive azo dyes [Lin & Peng 1996]. Reactive dyes are most difficult to be decomposed, complete decolourization requires prolonged performance of the process. Also, one of by-products was shown to be aniline, known for its toxic properties, which was not further degraded by prolonged treatment and accumulated in effluent treated. Thus, degradation of dye molecules using electrochemical treatment resulted in the formation of colourless dye fragments which although meeting the requirements of decolourization, sometimes result in the formation of ecotoxic reaction by-products.

Techniques involved **chemical oxidation** using different oxidising agents such as chlorine, ozone, Fenton's reagent or others and their combination, have long provided ultimate solutions for the treatment of both industrial discharges and drinking water [Slipchenko et al 1990].

Oxidation methods with chlorine, in the form of liquid or gas, or chlorine-compounds such as chlorine dioxide, sodium hypochlorite, etc. as a reactive chlorine source, are often used to decolourized wastewater as very simple, low capital and operating cost techniques. Chlorine can also be generated electrochemically. Satisfactory decolourisation of acid and direct dyes has been observed. Reactive dyes required longer treatment, while the solutions of metal-complex dyes have been visibly coloured after treatment [Namboodri et al 1994]. Colour reduction to 97% can be achieved at a high chlorine concentration of 150 mg/l, more than two thirds of which remains in the water. The dyes containing amino- or substituted amino-groups on the naphthalene ring (i. e. derived from aminonaphthol- and naphthylamine-sulphonic acids) are more susceptible to chlorine [Omura 1994], but the

potential of chlorine forming undesirable compounds with nitrogen containing components should be taken into consideration.

Chlorine based decolourization processes are viewed with increasing disfavour because they tend to generate toxic chlorinated organic compounds that are harmful to both humans and the environment [Hodin et al 1994, Chen et al 2001]. There is currently interest in the use of chemical oxidation with "ecologically safe" oxidants such as ozone, oxygen and hydrogen peroxide, which are not detrimental to the environment.

The use of ozone, a powerful oxidising agent for purposes of wastewater treatment dates from the early 1970-s. Although the original purpose of oxidation with ozone was disinfection of water and odour and taste removal ozonation can be used for removing many chemicals from wastewater. It facilitates the decomposition of detergents, chlorinated hydrocarbons, phenols, pesticides and aromatic hydrocarbons [Koboziev 1997, Gontcharuk, et al 1995].

The ability of ozone to destroy dyestuffs has been demonstrated [Snider & Porter 1974, Beszedits 1980, Gahr et al 1994, Strickland & Perkins 1995, Kerzshner et al 1986, Matsui et al 1984, Balcioglu & Arslan 2001]. Ozonation can cause alteration in dye molecular structure and removes colour from effluent by cleaving bonds in the dye molecule to produce colourless fragments. The effectiveness of this process is affected by the presence of salts, pH and temperature of effluents. The method was reported to be effective for the decolourization of different soluble dyes and practically ineffective for insoluble dyes using ozone concentrations of 1 g/l. From the available information [Perkowski 1988, Lin & Liu 1994, Tzitzi et al 1994, Schroder 1996, Tanaka & Ichikawa 1993, Liakou et al 1997]

it is evident that ozonation (sometimes in combination with ultraviolet (UV) irradiation) may improve biodegradability of non-biodegradable dyes.

Nevertheless modern dyes are resistant to ozone oxidation and for their complete conversion to carbon dioxide and water high doses of ozone are required. But the cost of ozone generation is high. Despite the advantages of ozonation, cost is often a barrier in the wide application of the technology. Ozonation could be made more effective and economical if combined with: coagulation / flocculation and subsequent sedimentation / filtration [Ginnocchio et al 1984, Lin & Lin 1993, Lin & Liu 1994] or dissolved air flotation [Kobozev 1997], coagulation and membrane systems [Buckley 1992], ultraviolet irradiation [Hung & Huang 1995] and biological treatment [Liakou et al 1997].

Another important disadvantage of this technology was found to be the possible toxicity of the breakdown products [Dolara et al 1981, Schroder 1996, Lebiedowski 1994, Balcioglu & Arslan 2001]. Dyes often contain nitrogen, chlorine and / or sulphur, and oxidation of such complex molecules could result in metabolites that could be more toxic to the environment than parent molecules.

Unlike ozone treatment, Fenton's reagent (hydrogen peroxide, activated with Fe (II) salts) is also effective for the oxidation of waste waters which inhibit biological treatment. The method is suitable for different dye classes, giving good decolourization rates. Besides offering advantages in COD, colour and toxicity reduction, this process also has disadvantages. Since the mechanism involves flocculation, impurities are transferred from effluent to the sludge, which still needs ecologically questionable land deposition. The Fenton process is preferred for waste water treatment in cases when the release of Fenton

sludge to the sewer is allowed, and can be removed by simple mechanical separation [Slokar & Le Marechal 1998].

Although no waste water treatment technology used today has universal application, chemical oxidation technologies using a combination of ultraviolet light and oxidising agents (activated in some cases if necessary) seem to hold potential for future use in the textile industry. All the above mentioned problems: of sludge formation, regeneration and disposal; increased pollution of waste water caused by ozone or chlorine and offensive odours typically associated with waste water treatment can be avoided using these wastewater treatment technologies.

1.2.3. Advanced oxidative technologies.

Recent developments in chemical waste water treatment have led to an improvement in oxidative methods for organic compound degradation applying photochemical and photocatalytic processes which have presently been referred to as **advanced oxidative processes** (AOPs) according to by Glaze *et al.* in 1987. These processes use a combination of strong oxidants (hydrogen peroxide, ozone or both hydrogen peroxide and ozone), catalysts or photocatalysts (transition metal ions and metal oxides), and ultraviolet (UV) irradiation. Other advanced oxidation process variants also exist using electron beam, ultrasound alone or with UV irradiation, Fenton's reagent with UV irradiation, etc. The expanded list of the AOPs developed have been currently reported in the literature [Huang et al 1993].

The photochemical processes normally use ultraviolet irradiation and can take place either in a homogeneous aqueous medium or in a heterogeneous medium (colloidal suspension of semiconducting particles such as titanium dioxide). The processes in most cases imply generation and subsequent reactions of highly reactive oxidising species. The forms of active oxygen including hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot -}$), peroxyl radicals ($HO_2^{\cdot}$) and also peroxyl anion (HO_2^-) are produced via different pathways and with different efficiencies depending on the AOP system involved.

Ultraviolet light alone may directly degrade organic substances through a weakening of one or more bonds within the pollutant molecule, and breaking the bonds photolytically. This is due to the electronic excitation of the molecule resulting from light absorbtion often completely altering its ability to lose or gain electrons and hence its ability to undergo different photo-reactions such as physical transformations (isomerisation, fluorescence etc.) and/or chemical reactions (including oxidation and reduction). This is especially feasible when the pollutants contain chromophoric groups (for example, carbonyl compounds and other organic substances including dyes).

AOPs have emerged as an important class of technologies for the destruction of a wide range of organic pollutants in different media. Previous researches of water treatment for different organic substances have predominantly been carried out using either ultraviolet (UV) irradiation or hydrogen peroxide alone, or the combination of the ultraviolet light with oxidising agents including hydrogen peroxide (UV/H_2O_2) and/or ozone ($UV/H_2O_2/O_3$ or UV/O_3). Some other types of effluent treatment processes used include titanium dioxide assisted photo-catalytic degradation (UV/TiO_2) and the combination of ultraviolet irradiation with Fenton's reagent or with chlorination [Zhao et al 1993, Gontcharuk et al

1993, Ruppert et al 1993, Tanaka & Ichikawa 1993, Arkchipova et al 1994, David et al 1994, Rao & Dube 1996, Tang & An 1995, Pulgarin & Kiwi 1996, Hachem at al 2001, Nizova et al 1995, Shankar et al 2001].

The results demonstrate that ultraviolet irradiation alone cannot be used as an effective procedure for the removal of complex organic substances from waste water. Complete mineralization requires prolonged irradiation time and a variety of intermediates more toxic than the initial substances could be formed. However, UV treatment seems to be quite efficient when used only for water disinfection purposes. When ultraviolet light and oxidising agents (ozone, oxygen and hydrogen peroxide) are combined, the overall oxidative reaction potential is greatly enhanced. It has been also noted that photochemical oxidation treatment reduces toxicity and improves biodegradability of bio-recalcitrants, hence it can be used as a pre-treatment of waste water prior to discharging to a biological treatment system.

The use of hydrogen peroxide as an oxidant in AOP is preferable among other oxidants and the method can be expected to be more economically efficient [Selukov & Trinko 1988, Jones 1999]. Hydrogen peroxide is commercially available, can be obtained in high purity and has infinite solubility in water. There is also no significant problem with the transport and storage of this reagent and operation procedure. Secondly, hydrogen peroxide is a very effective source of hydroxyl radicals and on UV irradiation, two hydroxyl radicals are formed for each molecule of H_2O_2 photolysed. Also, hydrogen peroxide is a commonly used pre-treating reagent in textile operations, so this abolishes the additional problems associated with its transportation.

To date, only a limited amount of work with specific emphasis on colour removal from textile effluents has been carried out using a combination of ultraviolet light and hydrogen peroxide [Shu et al 1994, Arkchipova 1995, Ince & Gonekc 1997, Namboodri 1996]. UV irradiation/hydrogen peroxide (UV/H_2O_2) treatment was shown to be a suitable degradation and decolourization method for many of the dyes concerned. Colour removal rate of the dyes under UV/H_2O_2 treatment differs between dye classes. Reactive dyes (particularly those of yellow and green colours) need a longer treatment time for their decolourization while direct, metal-complex and acid dyes (excepting anthraquinone-based) are decolourised quite quickly. For disperse dyes and pigments UV/H_2O_2 treatment is not suitable although these colorants can be successfully removed by physical methods as mentioned above. Factors affecting the decomposition and decolourization rate of the dye are the intensity of UV irradiation, hydrogen peroxide concentrations, dye structure and dyebath composition, hardness and alkalinity of solutions.

Nevertheless, a little information is known about the mechanisms of decolourization by photochemical oxidation. It is only known for a certainty [Haag & Mill 1987] that the dyes containing naphthalene rings in their structure are more susceptible to photochemical destruction and have higher decolourization rates compared with those of containing benzene rings. Therefore studies should be carried out on the identification of degradation products after photochemical treatment to provide more specific information about the mechanisms of dye photo-degradation.

In general the destruction of dyes to achieve complete or near-complete mineralization by means of photochemical oxidation treatment is a quite expensive process. This has prompted researchers to investigate innovative methods for coloured waste water.

Thus, from the practical and scientific points of view the combination of photochemical treatment for colour removal/reduction with subsequent biological treatment of formed intermediates, is seen to be a rational approach to waste water treatment technology. Such an approach will reduce treatment costs because of the decrease of photochemical process time, and the increase in biological treatment efficiency when using photochemical treatment followed by a biological one.

1.2.3.1. The principles of the UV/H_2O_2 process.

The fundamental aspects of the UV/H_2O_2 process appear to be quite well understood at present. The basic principle of the process is that UV irradiation combined with hydrogen peroxide significantly increases the destruction rate of organic pollutants as compared with those affecting by UV or hydrogen peroxide alone.

Hydrogen peroxide, being a relatively weak oxidising agent in pure form, needs to be activated. There are several well established ways of intensive activation of H_2O_2 (applying homogeneous and heterogeneous catalysts, or by interaction with ozone or by ultraviolet irradiation). UV irradiation activates H_2O_2 causing the photo-dissociation of one hydrogen peroxide molecule into two hydroxyl radicals [Baxendale & Wilson 1957]:

$$H_2O_2 + h\nu \longrightarrow 2\ OH^{\cdot}\ .$$

Oxidation of the organic substances by the combination of UV irradiation and H_2O_2 implies generation of hydroxyl radicals which are extremely reactive oxidising agents

[Jones 1999]. The oxidation potentials for common oxidants are listed in Table 1. 3. and hydroxyl radicals are in fact the most powerful oxidising species after fluorine.

Table 1.3. Oxidation potentials for a range of oxidants [Jones 1999].

Oxidant	E°, V (vs NHE)
F_2	3.00
$OH^{\cdot}$	2.80
$^1O_2$	2.42
O_3	2.01
H_2SO_5	1.81
H_2O_2	1.76
$KMnO_4$	1,70
$HO_2^{\cdot}$	1,70
HOCl	1,49
Cl_2	1,27
ClO_2	1,27
O_2	1,20

Although the absorption band of hydrogen peroxide extends from the vacuum UV to 365 nm, steady state generation of OH radicals is frequently carried out with UV lamps which emit light at 253.7 nm affording a quantum yield close to unity [Czapski & Bielski 1993]. Photochemical decomposition of hydrogen peroxide dependence on initial concentrations, pH and light intensity has been widely studied [David 1959, Hochanadel 1962, Buxton et al 1988, Bielskiet al 1985]. The photo-decomposition of hydrogen peroxide in water solution at low concentrations and/or relatively high light intensity could be presented by following scheme of consecutive reactions:

$$H_2O_2 + hv \longrightarrow 2\,OH^{\cdot} \quad (1)$$

$$OH^{\cdot} + OH^{\cdot} \longrightarrow H_2O_2 \quad (2)$$

$$OH^{\cdot} + H_2O_2 \longrightarrow HO_2^{\cdot} + H_2O \quad (3)$$

$$OH^{\cdot} + HO_2^{\cdot} \longrightarrow H_2O + O_2 \quad (4)$$

$$HO_2^{\cdot} + H_2O_2 \longrightarrow H_2O + O_2 + OH^{\cdot} \quad (5)$$

$$HO_2^{\cdot} + HO_2^{\cdot} \longrightarrow H_2O_2 + O_2 \quad (6)$$

There is a need to consider $OH^{\cdot}$ radical-radical recombination to hydrogen peroxide (eq. 2) but the reaction is only likely at H_2O_2 concentrations above $3 - 4 \times 10^{-2}$ mol/dm^3 [David 1959]. If an excess of hydrogen peroxide is used hydroxyl radicals $OH^{\cdot}$ can react with hydrogen peroxide (eq. 3, rate constant $k_3 = 2.7 \times 10^7$ dm^{3}mol^{-1}s^{-1}) producing less reactive oxidising agent, hydroperoxyl radicals, and with the newly generated radical itself (eq. 4, $k_4 = 7.1 \times 10^9$ dm^{3}mol^{-1}s^{-1}) [Buxton et al 1988]. The concentration of hydroperoxyl radicals being produced can be controlled by the pH of the reaction system. Their subsequent reactions (eq. 5 and 6) have also been reported to be pH dependent reactions [Hochanadel 1962, Bielskiet al 1985], and the reaction (eq. 6) probably does not occur at all in alkaline solutions (at pH 13 $k_6 = 0.3$ dm^3 mol^{-1}s^{-1}). Thus, as reactions of hydroxyl radicals with hydrogen peroxide and peroxyl radicals can take place, the choice of optimum hydrogen peroxide concentrations and pH is very important for the design and effective performance of the photochemical treatment process.

Hydroxyl radicals are strong oxidising agents, can react rapidly, and cause the chemical oxidation of many organic and inorganic compounds (including alcohols, organic acids, chlorinated hydrocarbons, phenols, naphthols and synthetic organic dyes) with rate

constants which are usually on the order of $10^{8} - 10^{10}$ $dm^3mol^{-1}s^{-1}$ [Legrini et al 1993, Claze 1987]. According to the research data hydroxyl radicals are much less selective oxidising agents than ozone or molecular oxygen, capable of oxidizing organic substances by three different classes of mechanisms:

- hydrogen abstraction when the hydroxyl radical reacts with an organic molecule to produce an organic radical and release of a water molecule:

$$RH + OH^{\cdot} \longrightarrow R^{\cdot} + H_2O$$

The organic radical $R^{\cdot}$ produced reacts quickly with oxygen dissolved in water to yield an organic peroxyl radical which can initiate subsequent oxidation reactions

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot} \longrightarrow \text{products;}$$

- electrophilic addition of hydroxyl radical to a carbon-carbon double bond in organic substances which leads to other radicals the subsequent reactions of which are quite similar to those mentioned above:

$$RH + OH^{\cdot} \longrightarrow RHOH^{\cdot}$$

Electrophilic addition is a commonly accepted mechanistic interpretation of the rapid photodecomposition of chlorinated compounds (such as polychlorobiphenyls, phenols);

- electron-transfer reaction from organic molecule to hydroxyl radical forms an ion-radical pair each component of which can further react under UV irradiation resulting in by-products (this effect will be discussed next chapters):

$$R + OH^{\cdot} \longrightarrow R^{\cdot +} + OH^{-}$$

Reduction of hydroxyl radicals to hydroxyl anions is of particular interest in a case where hydrogen abstraction or electrophilic addition reactions may be disfavoured by multiple halogen substitution or steric hindrance. In this case electron transfer from a organic molecule to hydroxyl radical forms a cation-radical which can be further dramatically converted under UV irradiation resulting in degradation by-products.

1.3. General aspects of dye photochemistry.

Dyes independently of their structure are known to absorb light energy in a wide region of the electro magnetic spectrum, from ultraviolet to infrared. There are three basic areas of photochemical sensitivity in the absorption spectra of each dye which can be isolated as [Terenin 1967]:

1) Visible region (and near UV region) with intensive absorption characteristic of the dye molecule chromogenic system itself;

2) UV region of a spectrum (< 300 nm), with less intensive absorption which is generally characteristic for aromatic rings of dyes with substituents in different positions;

3) UV region of intensive absorption (< 200 nm) due to the presence of saturated hydrocarbon fragments in dye structure.

Absorption of UV-Visible light energy by an organic dye results in electronic excitation of the dye molecule. Promotion of an electron from one molecular orbital to another of higher energy forms excited singlet and triplet states [Bekker 1976]. Dye molecules in excited states are more reactive and differ in chemical and physical properties from the corresponding molecules in the ground state. This is an important principle that excited state species are both better electron acceptors i. e. oxidizing agents and better electron

donors i. e. reducing agents than the corresponding ground state species. The lifetime of an excited state (or of a reactive species) is one of the main factor governing the course of a photoreaction. The excited triplet state is characterised by a relatively long lifetime in comparison with the singlet state and has a greater chance to react in a photochemical process instead of being deactivated to the ground state. The triplet state, therefore, is taken generally as the chemically reactive state in many photoreactions.

Excited states can lose their energy in a variety of photophysical and photochemical transformations, fluorescence, phosphoresence, internal conversation or vibrational cascade [Mejer 1971]. General transformation in a dye molecule can be illustrate with a simple potential energy diagram of a diatomic molecule (Figure 1.1). Excitation energy is dissipated as heat in radiationless transitions between states of like multiplicity (internal conversion) and between singlet and triplet states (intersystem crossing); and as light in radiative transitions to the ground state, fluorescence from singlet state and phosphorescence from triplet state.

In addition, excitation energy may be transferred from excited dye molecule to another molecule (acceptor) which becomes excited and ready for subsequent photoreactions. In such energy-transfer process the excited donor molecules acts as photosensitizer and reverts to the ground state. Excited dye molecules which cannot loose their energy by the above mentioned photophysical processes can liberate excitation energy by undergoing any one of several photochemical reactions such as dissociation, intramolecular rearrangement, redox processes etc.

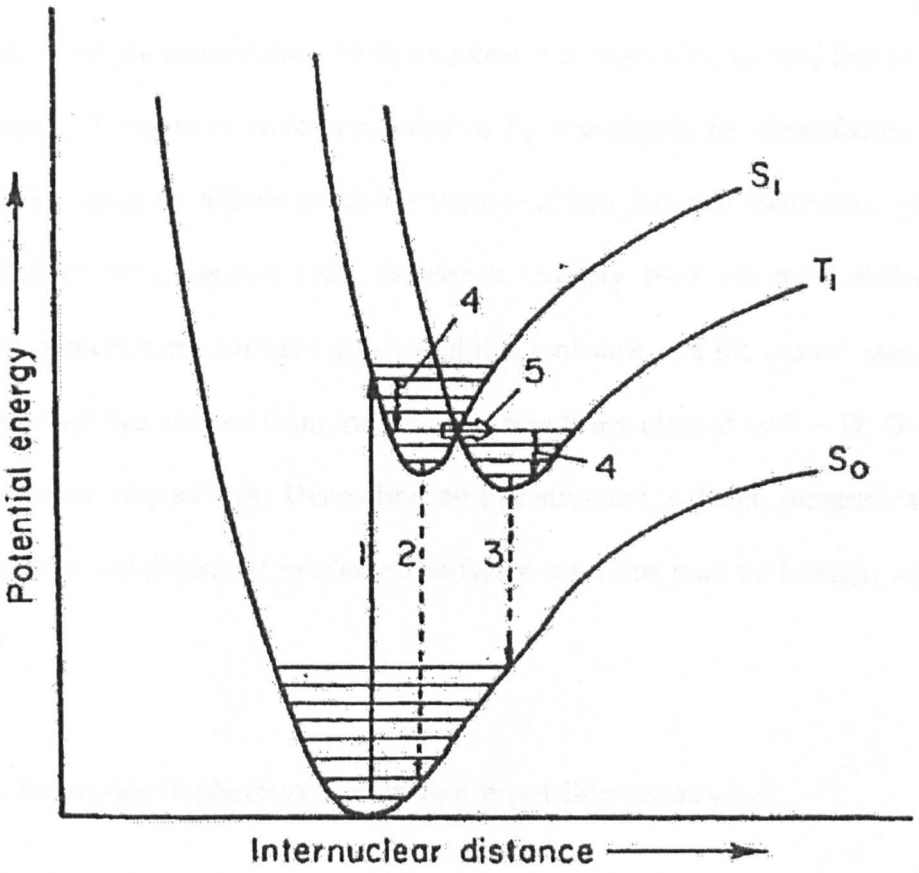


Figure 1.1. Potential energy diagram of a diatomic molecule.

S_o ground state; S_1 singlet state; T_1 triplet state; (1) excitation; (2) fluorescence; (3) phosphorescence; (4) internal conversion of the vibrational states of S_1, T_1; (5) isoenergetic intersystem crossing.

The basic photochemical reactions leading to dramatic changes of the spectral properties of dyes, destruction of their chromogenic part and decolourization are free radical formation, photo-reduction and photo-oxidation. In this context it is interesting to note that as early as 1814 reduction and oxidation were concluded to be responsible for photofading of dyes [Bancroft 1813]. Using the abbreviations for these reactions given in many monographs on the subject [Mejer 1971, Terenin 1967, Gordon & Gregory 1983, Ranby & Rabek 1978, Stepanov 1984] interactions between an excited dye molecule and the ground state dye, or between an excited dye and oxidising or reducing agents are classed as D – D, D – O and D – R mechanisms, respectively. Depending on dye structure, solvent, reagents, additives and wavelength or conditions of irradiation different reactions may be brought about and predominate.

Free radical formation in photoreaction system is possible by the ways:

- Direct photolysis (photo-dissociation) of an exited dye molecule forming two radicals

$$D(R_{(1)} - R_{(2)}) + h\nu \longrightarrow D(R_{(1)} - R_{(2)})^*$$

$$D(R_{(1)} - R_{(2)})^* \longrightarrow {}^{\cdot}R_{(1)} + {}^{\cdot}R_{(2)},$$

$D(R_{(1)} - R_{(2)})^*$ – excited dye molecule

${}^{\cdot}R_{(1)}, {}^{\cdot}R_{(2)}$ – dye fragment radicals.

It should be noted that the spectral region of photochemical interest is between wavelengths of 200 – 700 nm, corresponding to an energy range from E_{200} = 595 kJ/mol to E_{700} = 171 kJ/mol. As energy necessary for breaking chemical bonds (for example, between carbon and carbon, or carbon and oxygen etc.) accounts for ~ 300 – 500 kJ/mol, the formation of radicals in this photochemical reaction is very possible under UV

irradiation (λ = 254 nm). This corresponds to an energy E_{254} = 472 kJ/mol which is comparable with the dissociation energy of many covalent bonds.

- Interaction of an excited dye molecule with neighbouring dye or solvent molecules in the ground state (photosensitization effect):

$$D_{(1)} + hv \longrightarrow D_{(1)}^{*}, \quad D - \text{dye molecule}$$

$$D_{(1)}^{*} + D_{(2)} \longrightarrow {}^{\cdot}D_{(1)} + {}^{\cdot}D_{(2)}, \quad D^{*} - \text{excited dye molecule}$$

$$D^{*} + SH \longrightarrow DH^{\cdot} + S^{\cdot}, \quad SH - \text{solvent molecule}$$

In these processes radical formation does not require high energy and can occur even in visible light as a result of photo-decomposition of a relatively photo-unstable organic molecule interacting with an excited dye molecule. When the solvent is water hydrogen and dye hydroxyl radicals can be formed due to excited dye interacting with water molecules under UV irradiation:

$$D^{*} + HOH \longrightarrow DOH^{\cdot} + H^{\cdot}, \quad HOH - \text{water molecule}$$

Radicals formed by dye photo-decomposition processes or other reactive species transformed in these photoreactions may further react with dye molecules giving many photo-degradation products:

$$D + ({}^{\cdot}D, DH^{\cdot}, DOH^{\cdot}, H^{\cdot} \text{ etc}) \longrightarrow \text{photo-degradation products}$$

In these cases dye itself is presumably decolourized by a photo-reduction mechanism transforming to half-reductive (DH) or complete reductive (DH_2) forms [Kritchevsky 1986, Kuramoto & Kitao 1982, Albini et al 1989]. This fact is not absolutely confirmed and has presently been under investigation.

Photo-reduction reaction of the excited dye molecule may be initiated by certain substances (hydrogen donors) which are present in the reaction solution system as solvent or reducing agent additives. During irradiation the substances are oxidised with a simultaneous reduction (and consequent decolourization) of dye. This reaction involves a hydrogen atom or electron transfer from reducing agents to the excited dye molecule. These process can be simply illustrated as follows [Kritchevsky 1986, Bekker 1976, Ranby & Rabek 1978]:

$$D + hv \longrightarrow D^*$$

$$D^* + RH \longrightarrow D^{-\cdot} + RH^{+\cdot}, \qquad \text{electron transfer}$$

$$D^* + RH \longrightarrow DH^{\cdot} + R^{\cdot}, \qquad \text{hydrogen abstraction}$$

Often for photo-decolourization of a dye two hydrogen atoms must be added to the dye:

$$D^* + RH_2 \longrightarrow DH_2 + R^{\cdot}$$

In the presence of molecular oxygen in an irradiated system half-reduced (or reduced) dye can be returned to the initial dye structure with the same colour properties and other physical and chemical characteristics:

$$DH^{\cdot} + O_2 \longrightarrow D + HO_2^{\cdot}$$

Photo-reduction of dyestuff is often evidently a reversible chemical reaction (for many dyes). Oxidation of half-reduced and reduced forms to the initial form of the dye can occur both on further irradiation and/or in further dark reactions [Kritchevsky 1986]. Thus, both oxidation and reduction of the same dye in aqueous solution are possible under irradiation conditions.

Photochemical oxidation is a main reaction which may cause irreversible decomposition of different dyes. In the literature the study of the influence of oxygen as the oxidising agent on dye decomposition in photochemical processes has received great attention. Primary interaction of the excited dye molecule, usually in a triplet state, with molecular oxygen is believed to generate activated oxygen species such as oxygen radical-anion or singlet oxygen which can further react with dye molecules resulting in photochemical decomposition of the dyes [Terenin 1967, Mejer 1967, Martin & Logsdon 1987, Zollinger 1987]. It is to be expected that dye chemical structure, physico-chemical nature of medium in which a dye is distributed and dissolved oxygen concentration in a solvent should be the principal factors influencing the photodegradation rate of dyes.

The contribution of singlet oxygen to the photofading of some organic dyes, including azo dyes, has been studied by many investigators [Griffiths & Hawkins 1977, Kritchevsky & Gomebkete 1975, Anisimova et al 1983, Gruen et al 1981, Kovaltchuck et al 1986, Kuramoto & Kitao 1982, Albini et al 1989, Tratnyek et al 1994]. Azo dyes were found to undergo self-sensitized photo-oxidation (auto-oxidation) and quenching experiments suggesting a singlet oxygen mechanism. The efficiency of dye photo-oxidation in the presence of dissolved oxygen was reported to depend on the ability of azo dyes both to generate singlet oxygen and to be degraded by the reactive oxygen species. The rates of the photo-oxidation of some azo dyes with oxygen can be increased by adding photosensitizers of singlet oxygen formation (polycyclic aromatic hydrocarbons, fluorescein and phenothiazine derivatives: rose bengal, methylene blue, etc.) [Haag & Mill 1987, Foote 1968, Kuramoto & Kitao 1982, Kovaltchuck et al 1986]. On the other hand, the rates can be retarded by addition of efficient singlet oxygen quenchers (DABCO (1,4-diazabicyclo-[2,2,2]-octane), TPCP (tetraphenylcyclopentadienone), etc.). Additional evidence was

obtained by studying the effects of chemically generated singlet oxygen (formed from hydrogen peroxide and sodium hypochlorite) on dye photochemical oxidation [Foote et al 1968].

In contrast to this evidence as to the possible role of singlet oxygen in photo-oxidation of dyes it was shown that azo dyes produce no singlet oxygen and can undergo minimal photodegradation in oxygenated solutions [Kritchevsky 1986, Albini et al 1989]. Quenching cannot be considered as conclusive evidence for the involvement of singlet oxygen, as quenching of the excited dye state itself, which also initiates chain radical photo-reactions with dye to result in colourless degradation products, could occur [Nonhibell et al 1982, Okada et al 1998, Kovaltchuck & Anisimov 1988].

From these data dye photodegradation process is complex and can be overall described by following scheme:

$$D + h\nu \longrightarrow D^* \quad (1)$$

$$D^* + O_2 \longrightarrow D^{+\cdot} + O_2^{-\cdot} \quad (2)$$

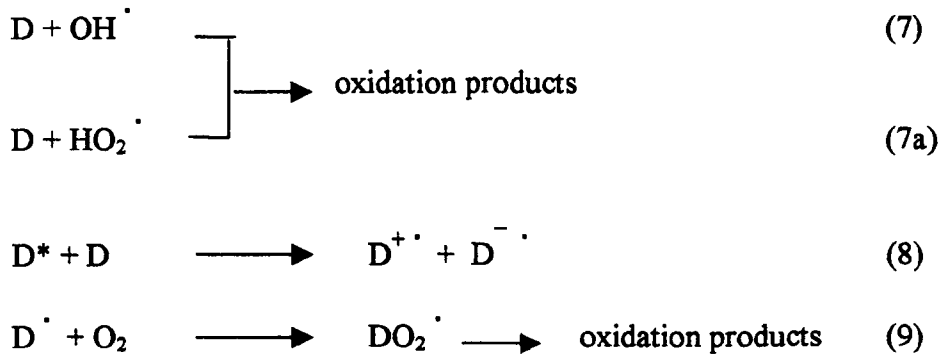
$$D^* + O_2 \longrightarrow D + O_2^* \quad (2a)$$

$$D + O_2^* \longrightarrow \text{oxidation products} \quad (3)$$

$$H_2O + O_2^* \longrightarrow HO_2^{\cdot} + OH^{\cdot} \quad (4)$$

$$2\,HO_2^{\cdot} \longrightarrow H_2O_2 + O_2 \quad (5)$$

$$H_2O_2 + h\nu \longrightarrow 2\,OH^{\cdot} \quad (6)$$



This is only a brief scheme of the photochemical reactions which can bring about photo-degradation of dyes. In the scheme, concentrations of dye, dissolved oxygen and radical formed or/and concentration of substances present being electron donors or acceptors in dye photochemical processes have not been taken into considerations. In real conditions, dye molecules absorbing light energy can undergo more complicated reactions, and often in combination.

According to the scheme, in the photo-decomposition reaction of dye molecules several reactive intermediates can be produced such as semioxidised dye radicals, photo-peroxides unstable to UV irradiation, oxygen reactive species and others. Moreover, the oxygen reactive species are also believed to react with water to produce hydroxyl and peroxy radicals, which may then induce further oxidative breakdown of the dyes. The formation of the dye radicals which are then attacked by molecular oxygen in the usual way can also occur under UV irradiation resulting in dye photo-decomposition. It should be taken into consideration that the dye which is oxidised in the presence of an oxidising agent may act itself as a powerful oxidising agent depending on dye structure and UV irradiation conditions. The oxidised dye can oxidise neighbouring dye molecules or other substances in the solution by a transfer of oxygen from peroxide intermediates or by removing electrons.

Thus, excited dyes in the presence of oxygen can undergo photo-oxidation as well as photo-reduction reactions. At present inconsistent data in the literature on the role of oxygen in the photofading of dyes do not allow the formulation of an accepted overall mechanism of its action. Moreover, when studying dye photo-oxidation processes, dye solutions in organic solvents, in which oxygen solubility and singlet oxygen life-time are higher than in water [Razumovsky 1979, Kaplan 1988, Zinukov et al 1991] and visible light have mainly been used. In the context of examining the feasibility of photochemical treatment the role of oxygen in the decolourization of aqueous dye solutions on UV irradiation is to be studied.

1.4. Effluent quality control.

Traditionally, the estimation of quality of the aquatic environment and effluents in many countries (including Russian Federation) is based on the determination of the concentration of known polluting substances separately and comparison of these values with a legal fixed parameter of pollutant limitation - maximum allowable concentration (MAC) [Sidorenko & Motchshaev 1994, Handbook 1994].

This approach has some advantages such as analytical control of some well-known pollutants (pesticides and related organic chemicals) and perfect economical stimulation for reducing liquid wastes discharged to aquatic environment. Besides the advantages, the approach has however a number of deficiencies.

First, maximum allowable concentration data exists for a only certain number of individual chemical substances. But a serious problem is that there are no simple, sensitive and exact analytical methods to determine many chemical substances, for which an MAC value has been already set up [Kravtchenko & Sobina 1979, Collection of standards1991]. The determination of water pollutants is especially difficult task when the pollution sources are multiple and the polluting effluents are of complex nature. According to data given by the EPA [EPA 1992], some 80% of the synthetic organic compounds found in the water can not be investigated using traditional chemical techniques.

Actually, new complex compounds resulting in reactions between polluting substances and natural organic substances under various environmental condition can be often formed for which MACs do not exist and there are also no techniques to detect and assay the substance. The toxicity of compounds formed in some cases is known might greatly exceed the toxicity of initial substances [Gontcharuk et al 1995, Dolara et al 1981, Okamura et al 1996, Tan & Ami 1991]. For example, ecotoxicity assessment of the water environment around Lake Kojima (Japan) has shown that the toxicity was increased mainly by interaction between some naturally occurring organic compounds in water and effluents from industries including textile effluents [Okamura et al 1996]. Dyes in the environment become photo-reactive during exposure to sunlight and, interacting with humic compounds, can give rise to various toxic substances [Baughman & Perenich 1988]. Determination of dyes present in significant concentration in many environment water bodies have been nevertheless neglected, to a large extent due to the dyes often being mixture of the compounds some of unknown or unpublished structure.

Secondly, the MAC of an harmful substance is the quantitative standard level considered to be safe for humans rather than the biosphere [Rumjantcev & Novikov 1997]. Maximum allowable level of the substances is established according to the principle of causing minimal detrimental effects at this concentration (so called no observed effect concentration - NOEC) not taking into account ecosystem features for all natural and climatic zones. It should be noted that the requirements for water quality for reservoirs used for fish-farming purposes are more strict than used for drinking and domestic purposes.

Thirdly, although analytical methods for the determination of organic chemicals at low concentrations are evolving, they are based on measurements of physico-chemical parameters of effluents and do not solve the problem of risk assessments for the environment in general [Maystrenko et al 1996]. Measurement of pollutant concentration such as suspended solid, temperature, pH, TOC etc. of effluents does not give information on actual effluent toxicity to the environment. It does give a measure of potential hazards and indicate if discharge consent conditions are being complied with for process management and regulatory purposes. The measurements themselves, and even compliance with current standards, does not put an end to the risk of environmental harm.

In this connection, biological methods using different organisms, combined with chemical measurements, are therefore becoming increasingly important to complement the analytical techniques which are conventionally used in evaluating toxic effects of individual chemicals and effluents on the environment [Michaelidou et al 1995] and in monitoring effluent treatment performance.

1.4.1. Biological testing as a method of water treatment quality monitoring.

Living organisms ranging from bacteria to mammalians are known to be susceptible to environment change and, therefore, to be indicators of the harmful effect of any chemicals or effluents [Tumanov 1988]. Classical methods of toxicological investigations using mammalians provide reliable results, however their use is limited by the long time and high cost required for most tests.

Today toxicological screening tests are oriented towards the development of *in vitro* strategies and bioassay [Rozshnov et al 1995]. In toxicity testing a threshold level of substance is defined as the concentration at which the toxicant began to have a detrimental effect on the organism. Since living organisms differ in their response and sensitivity to any pollutant substances, there is therefore no one organism which can serve as an index of what is harmful to aquatic biota.

In many countries (Japan, USA, Canada, Sweden, Germany, France, others) the biological toxicity test methods are included as compulsory ones in the monitoring system for environmental risk assessment of waste water, new chemical and biological substances, chemical commodity products, etc. [Furlong 1995, Russo 1986]. Currently there are more than 50 international recommendations on toxicity testing techniques [Fomin & Cheskis 1992].

Standard toxicity tests are generally conducted with freshwater fish, *Daphnia* and alga *Chlorella* have long played a major role in aquatic hazard and risk assessment, especially at a screening level of evaluation [Korter 1996, Kisljakov 1991, Sazonova et al 1997]. Assessment of potential environmental impact often requires data from other organisms. A number of alternative tests have been proposed for screening due to their experimental simplicity, sensitivity, reproducibility, ease of handling and short exposure time. Some of these tests include the Microtox and Polytox assays, the use of rotifers, brine shrimp cysts, *Paramecium caudatum, Hydra attenuata* and *Daphnia magna* and *pulex* [Janssen & Persoone 1993, Elnabarawy et al 1988, Snell & Persoone 1989, Vanhaeke et al 1981, Hao et al 1995, Hall & Mirenda 1991].

For specific chemicals a variety of techniques for determining toxicity are available, which give information on effects for various species. Thus data on the ecotoxicity and environmental fate of compounds are accumulating. Although, for many wide spread chemicals, such as dyes little, if anything, is known of their possible ecological effects and fundamental ecotoxicological information is still lacking. For complex waste water, containing a more or less unknown mixture of compounds, toxicity testing gives information on the combined effects of the discharge of effluents on the biota, being one further step to secure and preserve the aquatic environment, although it does not identify each chemical in waste water.

Moreover, the studies [Staples et al 1994, Selivanovskaja et al 1993, Lankford & Smith 1994, Pozsharov & Popetchitelev 1996] were performed to illustrate the use of toxicological measurements in assessing industrial effluent treatment processes. The results provided strong evidence of treatment effectiveness because toxicity decreases with

progressive stages of treatment. Biological treatment is commonly used as a post-treatment after physico-chemical treatment of effluents. Since biological treatment simulates degradation processes that occur in the natural environment toxicity assessment of wastewater towards organisms of activated sludge must also be carried out.

Thus the use of toxicity measurements can be useful in evaluating treatment alternatives, modifying existing wastewater treatment and conducting treatment evaluations, and in examining the effects of chemicals and their breakdown products on the micro-organisms which are used in biological treatment plants.

1.5. The aims of this research.

According to the literature textile effluents containing dyes are generally not meeting the requirements for discharges into aqueous environments. In general photochemical oxidation of dye effluents seems to be a satisfactory method for dye destruction. On the other hand, complete mineralization of dyes by means of different biological and physical/chemical methods (including photochemical treatment) is not currently a technologically practicable and cost efficiency process. This has prompted researchers to investigate innovative methods for coloured effluents.

To destroy dyes and decolourize dye house waste water the use of photochemical oxidation combined with subsequent biological treatment of photochemically formed by-products appears to be an attractive approach. Such an approach will potentially reduce treatment cost because of the decrease of photochemical process time, and the increase of biological treatment efficiency as a post-treatment of photochemically breakdown products.

The subject of this research is therefore aimed at the investigation of the feasibility of the combination of the UV/hydrogen peroxide photochemical oxidation treatment as a pre-treatment operation to initially destroy dye chromophore and to decolourise wastewater with subsequent biological treatment. In this connection, experimental work was done:

- to investigate the factors influencing photochemical oxidation such as initial concentration of dyes and oxidant (dissolved oxygen and hydrogen peroxide) and their ratio, temperature of effluents, pH;

- to elucidate the mechanism of dye photo-degradation in the UV/hydrogen peroxide system and to identify intermediate products formed by photochemical oxidation processes using different physical and chemical methods;

- to assess eco-toxicity potential and biodegradability of dyes and their photochemical breakdown products, using biological testing, as analytical methods for detecting by-products do not give information about their toxicological safety level;

- to find optimum conditions for the combination of photochemical and biological decolourisation treatments of dyes.

CHAPTER 2. MATERIALS AND METHODS.

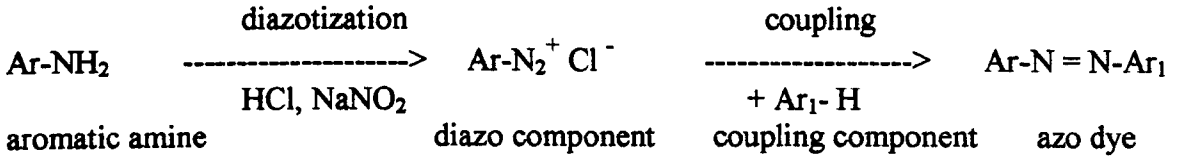
2.1. Introduction.

To study the feasibility of the UV/hydrogen peroxide photochemical oxidation treatment for decolourization of coloured effluents and biodegradability of photooxidation breakdown products water soluble dyes have been chosen. There are important reasons for that. Azo dyes form the largest group, accounting about two-thirds of current total world synthetic colorant production and availability on the market. Water soluble dyes, generally exhibiting lower exhaustion properties, are present in waste water in greater quantity than insoluble dyes (such as disperse and vat dyes), and are difficult to be removed from the effluent by known and relatively simple physico - chemical means.

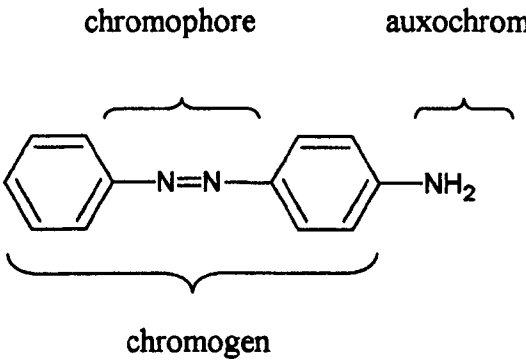
Azo dyes are widely used in the textile industry for dyeing of all natural and synthetic fibres, leather and furs. They are of most interest in technological application and could be found among dye classes (such as acid, reactive, basic, disperse and metal-complex dyes) except vat dyes. Azo dyes are also applicable to the dyeing of rubber, synthetic polymers, paper, food, drugs and cosmetics, and even some stones [Poray-Koshits 1962].

Azo dyes have achieved this prominence due to the variety of their physico-chemical properties and the fact that these dyes are relatively easy to prepare in chemical plants from cheap and readily available raw materials and intermediates. They cover the whole range of shade colour, from yellow to black including different shades of the main colours. Azo dyes have a high colour strength to materials being dyed and quite good light fastness, although their stability may vary depending on dye structure and environment.

As shown in the scheme below azo dyestuffs are obtained by coupling the diazonium ion of a aromatic amine with a coupling component which are aromatic, heterocyclic and enolic compounds and others:



The colour of dyes is attributed to the presence in organic compound structures of chromophoric groups (such as nitro-, nitrozo-, azo, carbonyl groups) and auxochromes which are typically hydroxyl and amino- / alkylamino groups and some others. The coloured organic structures containing chromophores are referred as to chromogens (according to the colour theory formulated by Witt in 1876) and dye structure scheme could be presented as follows:



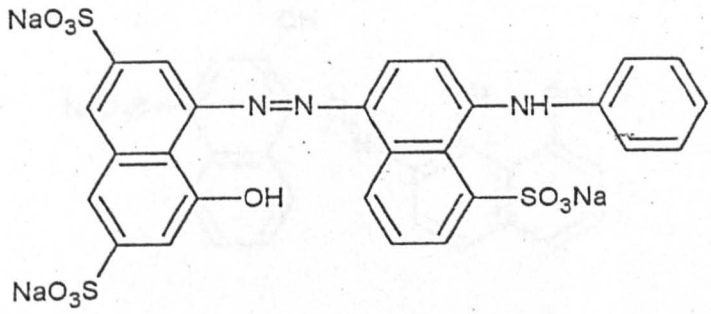
The colour of azo dyes is attributed to the presence in dye chemical structure of conjugated double bonds (aromatic hydrocarbons) including the nitrogen to nitrogen double bond (N=N azo group as a chromophore) associated with auxochromes such as hydroxyl, primary, secondary and tertiary amines. Depending on the number of azo groups present in the molecule azo dyes are divided into mono-, dis- , tris- and polyazo dyes.

The presence of substituents in the molecule of azo dyes such as sulphonic, carboxylic or ammonium groups is characteristic of water soluble azo dyes. Water soluble azo dyes are also subdivided into two groups: anionic and cationic dyes according to whether anionic or cationic charge is delocalised on a specific group in dye molecules when they are dissociated in aqueous solutions.

2.2. Materials.

The choice of dyes used in this study was made based on their solubility, availability and chemical similarity to dyes of commercial importance and potential susceptibility to photolysis. These includes mono- and disazo dyes chemical structures and absorption spectra of which are given below in Figures 2.1 – 2.7.

Structural variations among given azo dyes are significant but most of them contain (phenylazo) naphthalene residues with hydroxyl (-OH) and /or sulphonic ($-SO_3^-$) groups. The dyes, C. I. Acid Blue 158 and C. I. Acid Yellow 99, are characterised by the presence of hydroxyl groups in ortho- positions relative to the azo linkage that allows metal chelate formation.



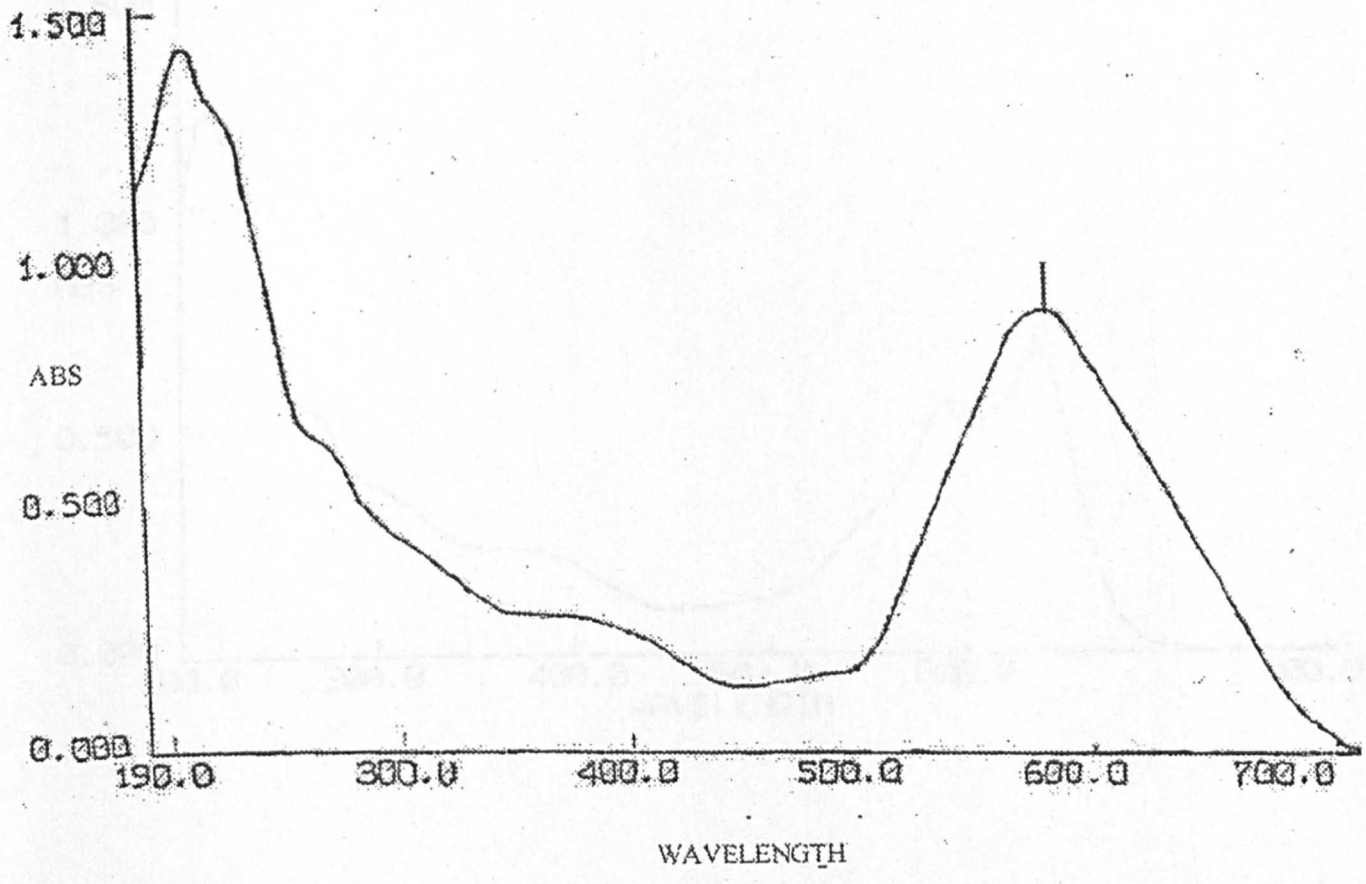
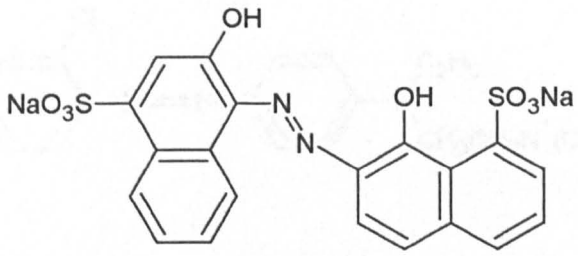


Figure 2.1. Chemical structure and UV-Visible absorption spectrum of dye Acid Blue 92 (C. I. 13390, λ_{max} = 580 nm).



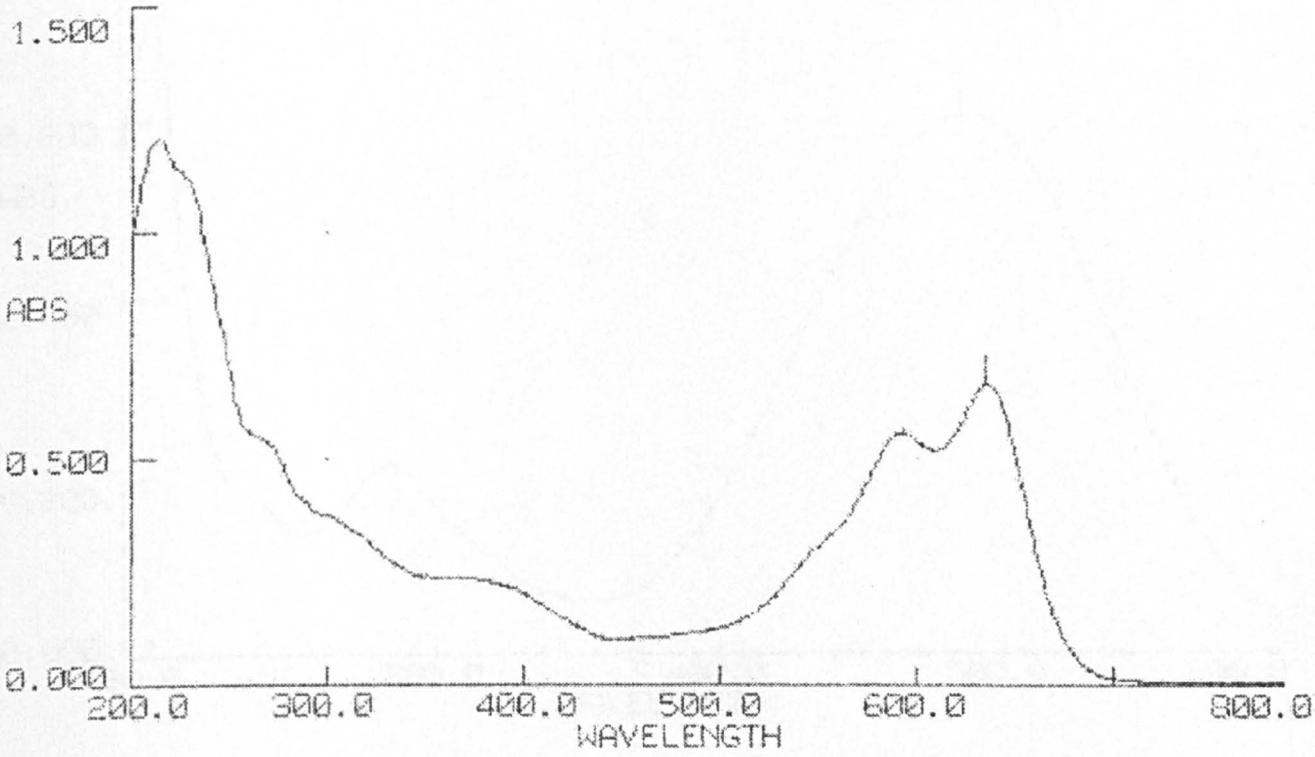
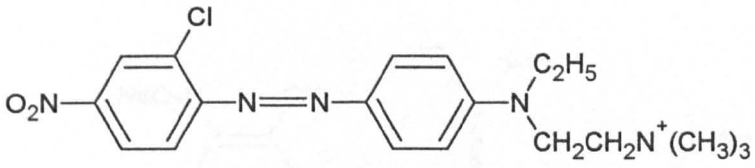


Figure 2.2. Chemical structure and UV-Visible absorption spectrum of dye Acid Blue 158 (C. I. 14880, λ_{max} = 635 nm).



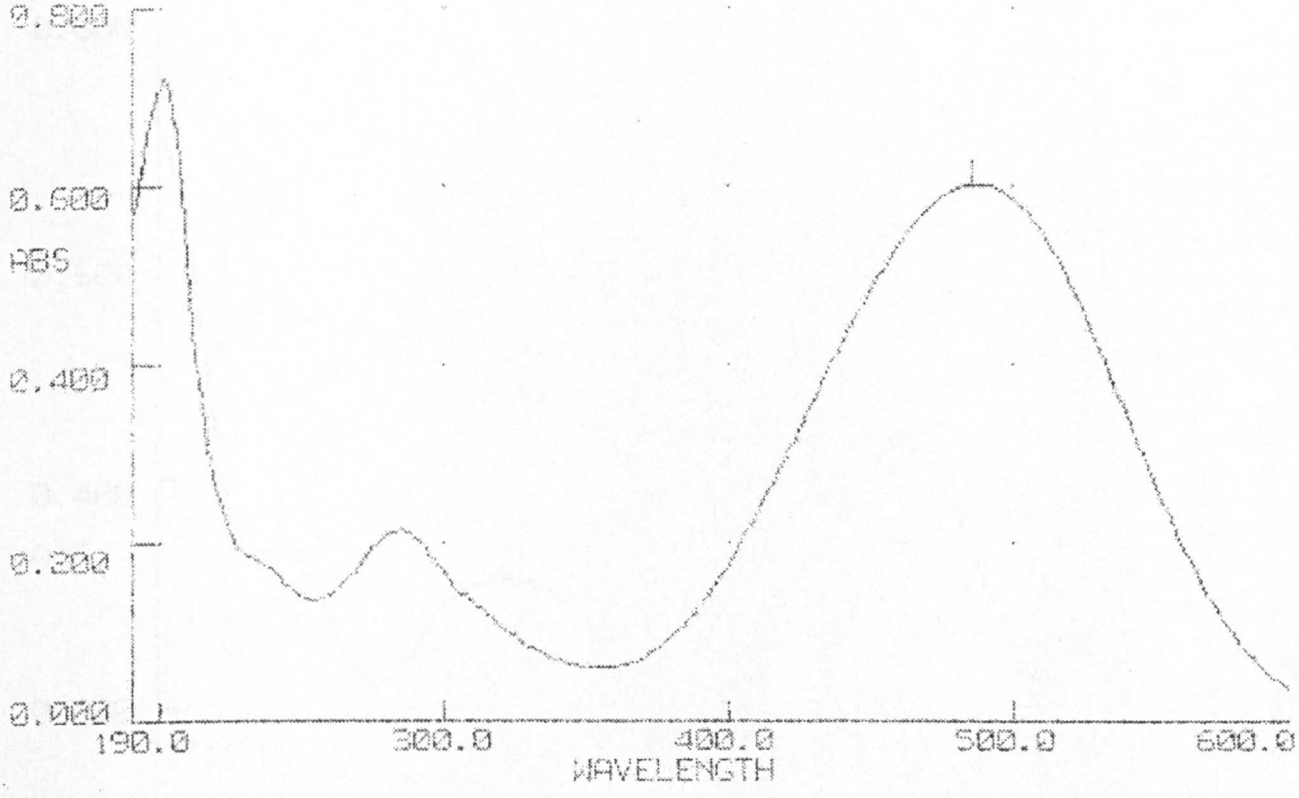


Figure 2.3. Chemical structure and UV-Visible absorption spectra of dye Basic Red 18 (C. I. 11085, λ_{max} = 486 nm).

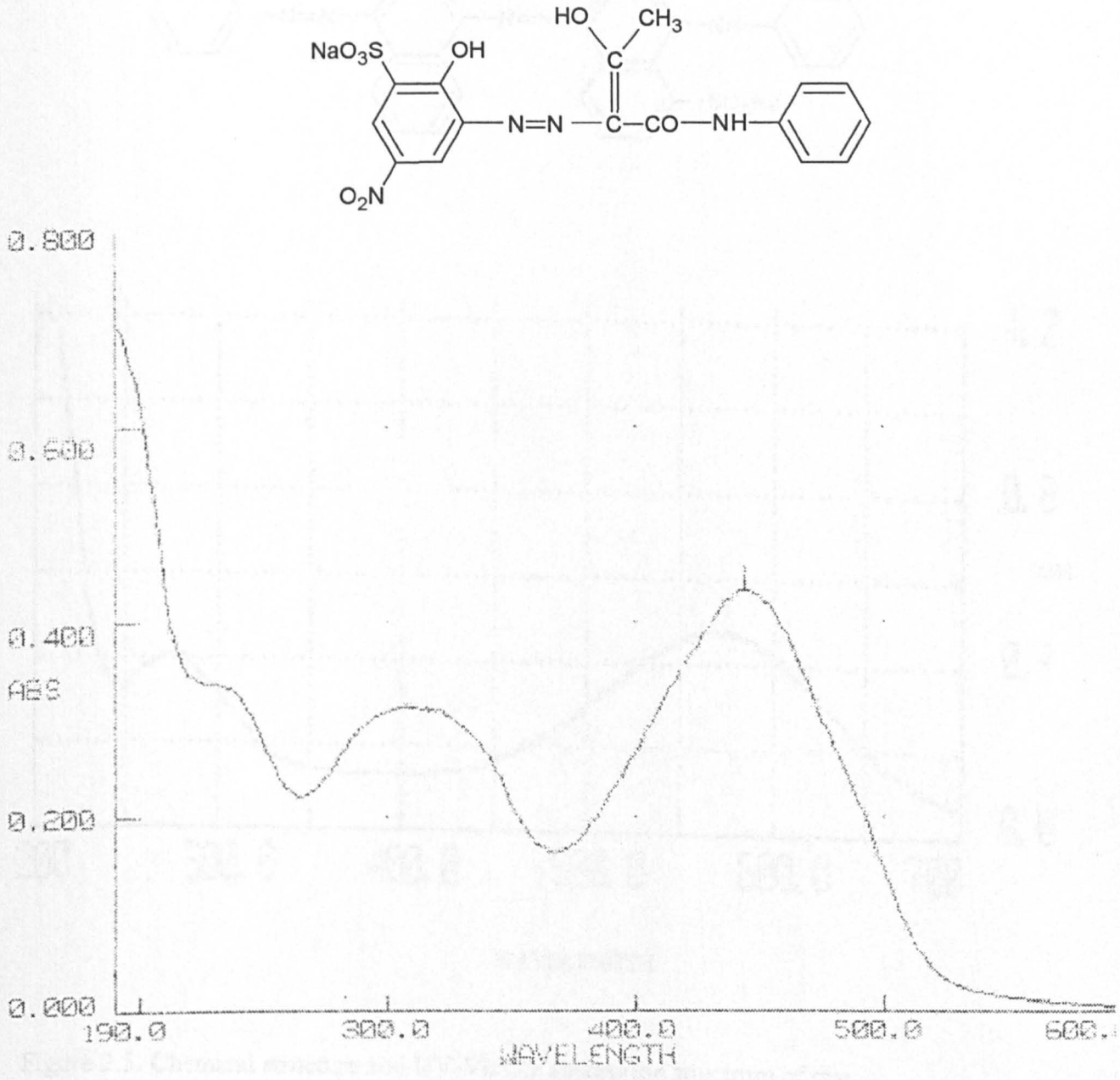
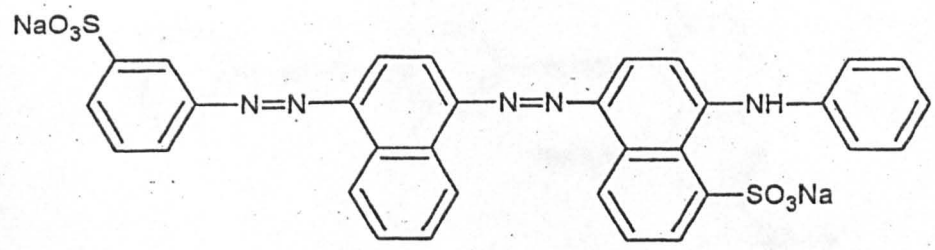


Figure 2.4. Chemical structure and UV-Visible absorption spectrum of dye Acid Yellow 99 (C. I. 13900, λ_{max} = 450 nm).



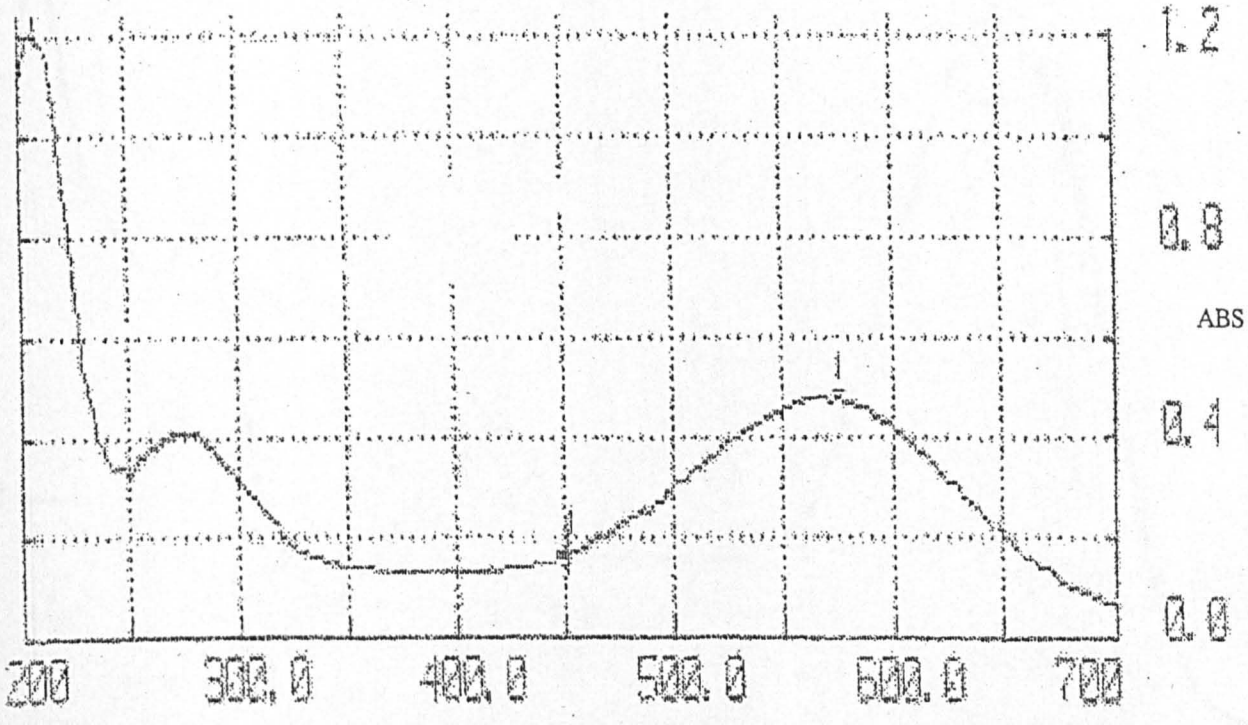
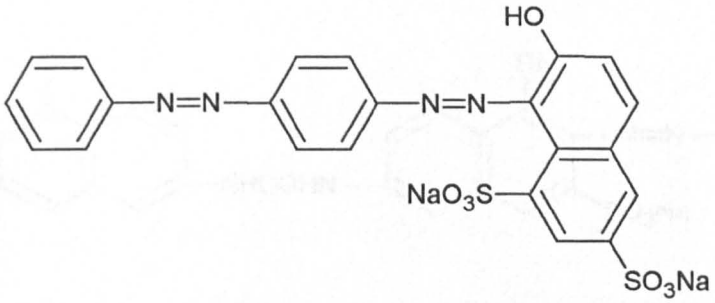


Figure 2.5. Chemical structure and UV-Visible absorption spectrum of dye Acid Blue 113 (C. I. 26360, λ_{max} = 562 nm).



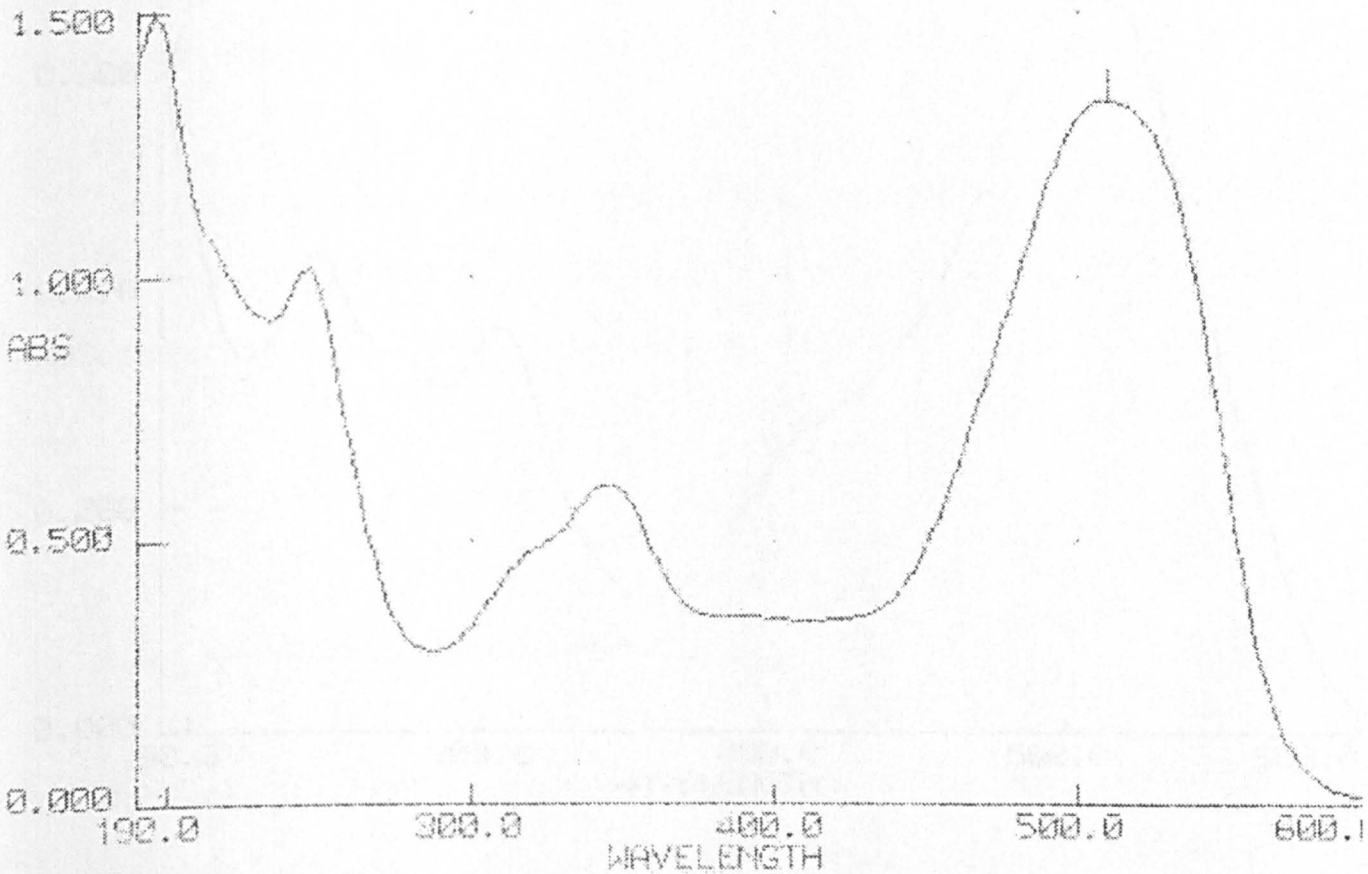
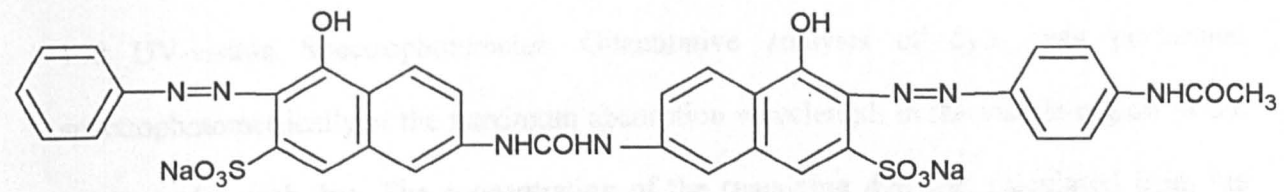


Figure 2.6. Chemical structure and UV-Visible absorption spectra of dye Acid Red 73 (C. I. 27290, λ_{max} = 510 nm).



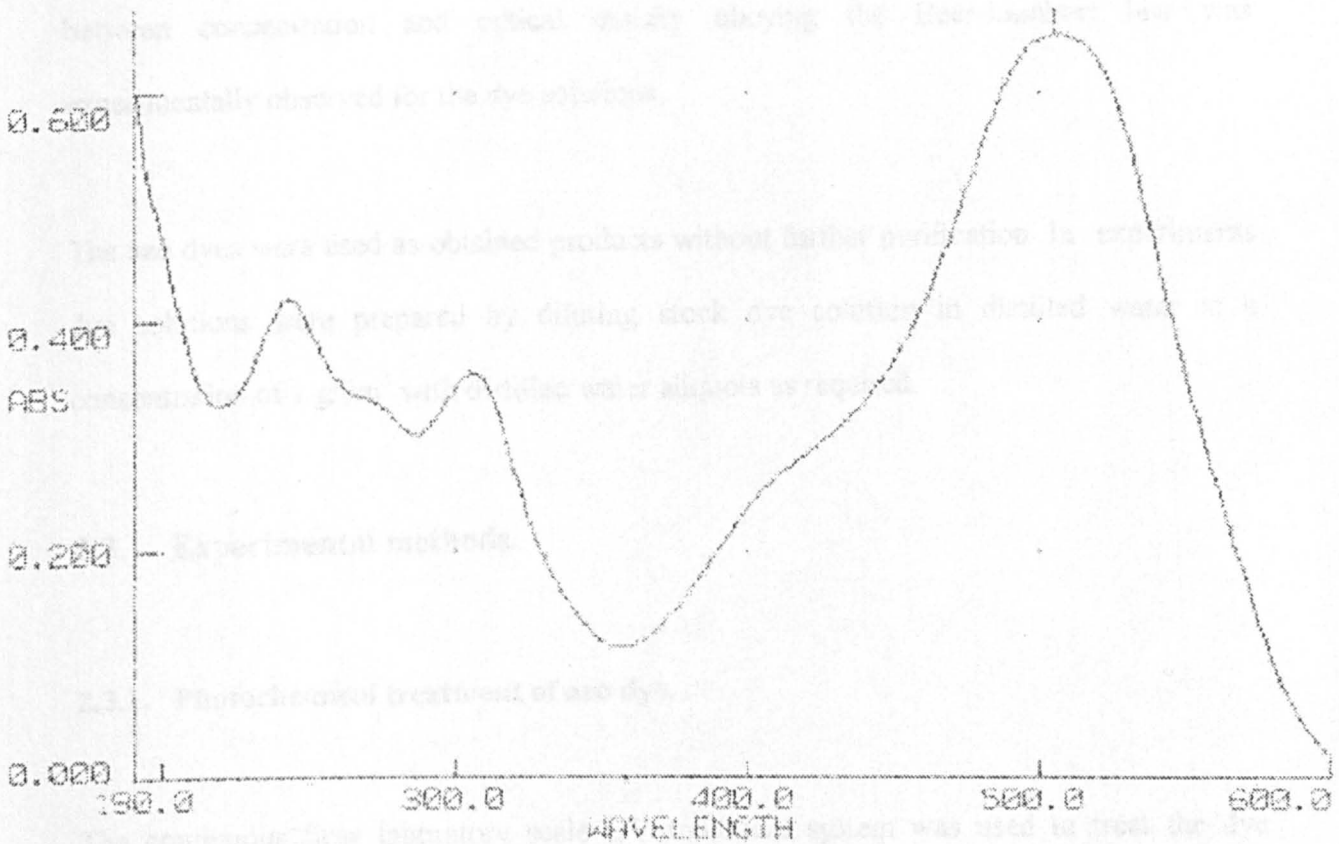


Figure 2.7. Chemical structure and UV-Visible absorption spectrum of dye Direct Red 23 (C. I. 29160, λ_{max} = 506 nm).

UV-Visible absorption spectra for each azo dye were determined using the Unicam UV2-100 UV-visible Spectrophotometer. Quantitative analysis of dyes was performed spectrophotometrically at the maximum absorption wavelength in the visible region of the spectrum for each dye. The concentration of the remaining dye was calculated from the calibration curve. Over the concentration range examined in the study a linear relationships between concentration and optical density obeying the Beer-Lambert law was experimentally observed for the dye solutions.

The azo dyes were used as obtained products without further purification. In experiments dye solutions were prepared by diluting stock dye solution in distilled water at a concentration of 1 g/dm^3 with distilled water aliquots as required.

2.3. Experimental methods.

2.3.1. Photochemical treatment of azo dye.

The continuous flow laboratory scale UV treatment system was used to treat the dye solutions. The laboratory unit (shown in Figure 2.8) consists of photo-reaction chamber connected with a peristaltic pump and a holding reservoir for a test dye solution providing cycled flow during photochemical treatment. The photo-reaction chamber is a cylindrical stainless steel vessel of 52 mm diameter, 250 mm length, and 0.25 dm^3 hold up volume.

UV light source in the system, a low pressure mercury lamp (of 15 mm diameter) with 8 watts output, was placed in the centre of photo-reaction chamber within a quartz sleeve protecting the lamp and preventing interference from contact with reaction solutions.

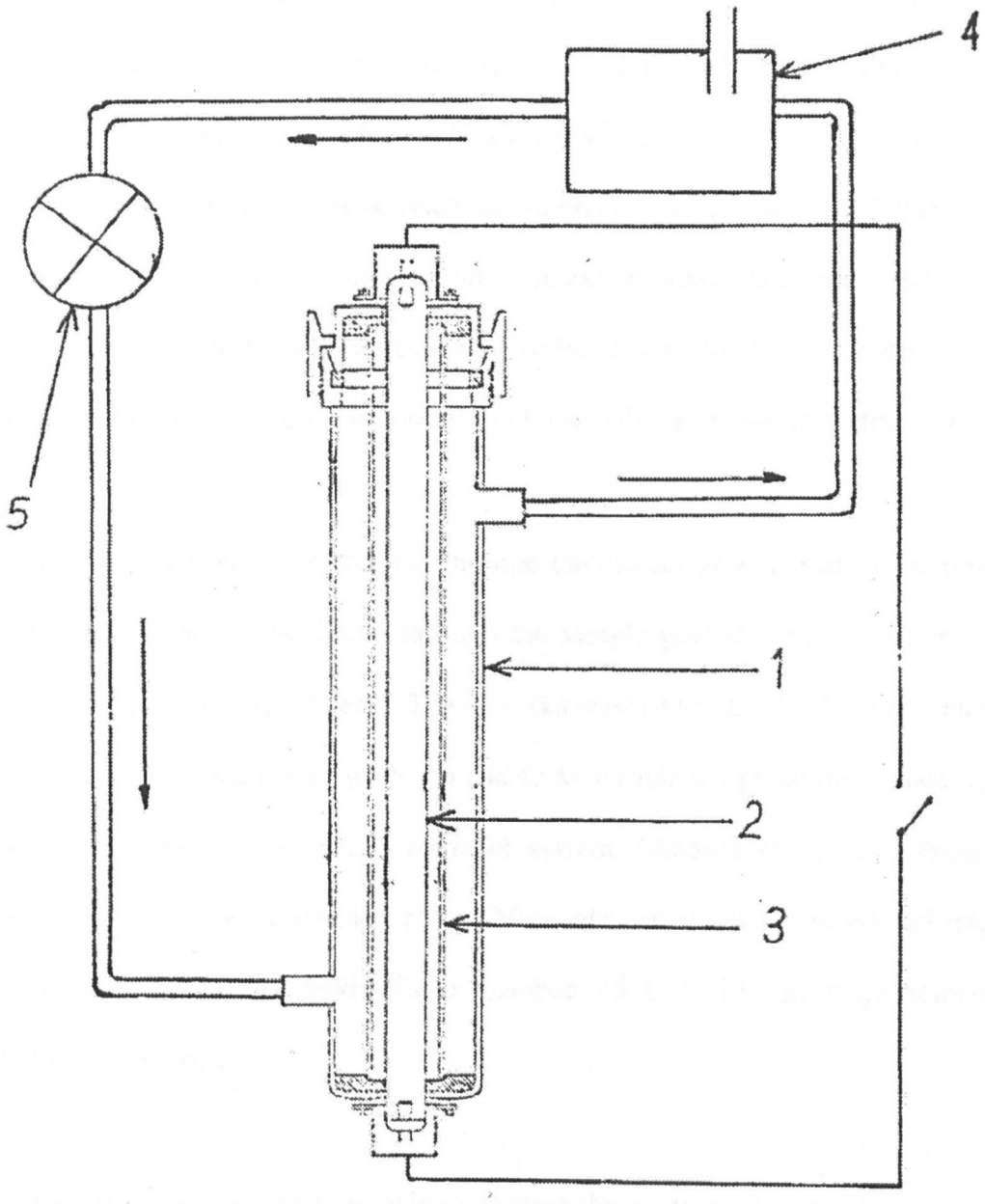


Figure 2.8. Schematic diagram of laboratory scale UV treatment system.

1) photo-reaction chamber, 2) 8 watts low pressure mercury lamp, 3) quartz sleeve, 4) holding reservoir for test dye solution, 5) peristaltic pump.

The lamp emits approximately 90 % of ultraviolet energy at 254 nm (according to safety data sheet, Saratov, Russia). It could be suspected that the actual UV energy reaching the cylindrical photo-reaction chamber was less the than energy output at the lamp's surface. But the energy reaching the photo-reaction chamber was not calculated due to various assumptions that would add to the uncertainty in experimental data interpretation e. g. the effect of reflection from the stainless steel walls, quartz sleeve etc. Before filling the reaction chamber with the test solution the lamp was allowed to warm up for 15 min.

The photolysis solution was circulated through the reactor at a constant flow rate of 350 cm^3/min. Samples were withdrawn through the sample port at regular time intervals for analysis throughout the experiments. The unit was washed thoroughly between each trial to minimize contamination from run to run and to avoid misinterpretation of data caused by the using of different dyes in the treatment system. Changes in dye concentration and optical absorption were determined from UV-visible spectra of untreated and treated dye solutions recorded on the Perkin-Elmer Lambda 15 UV/VIS Spectrophotometer using distilled water as a blank.

In experiments when hydrogen peroxide was used the holding test reservoir was first filled with dye solution. The required amount of 30 % laboratory grade hydrogen peroxide stock solution was added to the dye solution into reservoir just before starting photochemical treatment. Concentrations of hydrogen peroxide were determined ionometrically using a selective electrode (details of measurements are discussed in corresponding chapters). To estimate the role of dark reactions between dye tested and hydrogen peroxide a mixed solution of the same concentrations of dye and hydrogen peroxide was kept in glass vessel wrapped in foil away from UV irradiation sources.

2.3.2. Analytical techniques.

During this research analytical techniques, including UV-Visible spectrometry, high performance liquid chromatography (HPLC) and mass-spectrometry (MS) with the matrix assisted laser desorption ionisation technique (MALDI), were used for attempts to separate and identify photochemical reaction breakdown products to give a preliminary idea about the possible type and mechanism of reactions that azo dye might undergo during photochemical treatment.

It has been pointed out that acid azo dyes containing one or more sulphonic groups in their structure, which provide them with high polarity and water solubility, are difficult to analyse by many analytical methods including liquid chromatography and mass-spectrometry [McLean & Freas 1989, Weatherall 1991, Straub et al 1992, Jandera et al 1996, Rafols & Barselo 1997, Conneely et al 1999]. In HPLC systems with aqueous – organic mobile phases the sulphonated azo dyes are usually strongly adsorbed on reverse phase column media resulting in poor resolution. To achieve good separation of these dyes careful selection of a mobile phase (eluent mixture) and chromatography conditions for each dye are definitely needed. In mass-spectrometry the sulphonic acid groups render the dyestuffs intractable to gas-phase ionisation techniques.

2.3.2.1. High Performance Liquid Chromatography analysis.

The initial azo dye solution and the dye samples withdrawn at regular time intervals during photochemical treatment were analysed by gradient high performance liquid chromatography (AFC-model 352). A reverse phase column C-18 (250 x 4.6 mm) packed with spherical 5 μm particles was used as the stationary phase. The column eluate was passed through a UV detector set at 254 nm. Peak areas were determined using a Merck/Hitachi D-2000 chromato-integrator. Sample volumes of 100 μl were injected into the column.

Samples of dyes from photochemical treatment were eluted from the column with a water and methanol solvent mixture at a flow rate of 0.3 cm^3/min. Initially, the mobile phase consisting of 20 % (v/v) methanol and 80 % (v/v) water was maintained for 5 min (isocratic conditions). A linear gradient with a final composition of 20 % water and 80 % methanol was applied over 15 minutes. The final eluent composition at 80 % methanol was maintained for an additional 15 minutes.

The methanol used was HPLC grade. Water for HPLC analyses was obtained by filtration of distilled water through a Milli-Q water purification system (Waters-Millipore). Before use the mixture of HPLC grade methanol and water was degassed under vacuum to remove dissolved gases interfering with maintenance of a constant pressure in the column. The mobile phase in the solvent reservoirs was continuously stripped with a stream of helium gas during the runs.

2.3.2.2. Mass spectrometry analysis

To identify intermediates and final breakdown products of azo dyes formed during photochemical treatment, mass-spectrometry (MS) with the ionisation technique of matrix assisted laser desorption (MALDI) was used.

This ionization method (MALDI) using photon transfer generated by laser energy is related to methods of «soft» ionization in MS which also includes other ionization techniques such as chemical ionisation (CI), electron impact (EI), fast atom bombardment (FAB), field desorption (FD) etc. [Broun et al 1988]. MALDI– MS relies on rapid energy transfer from photons to the sample via a matrix material which absorbs the laser radiation (typically 532 or 337 nm) resulting in the vaporisation of the matrix and sample embedded in the matrix, leading to sample ionisation. The matrix facilitating the ionisation process also insulates the sample analysed from thermal degradation and fragmentation [Cornett et al 1993, Karas et al 1992]. The method has advantages. It allows to analysis of relatively small quantities of sample at room temperature without their preliminary concentration and/or extraction in organic solvents. Obtaining a clean mass spectrum does not depend strongly on the purity of sample and MALDI technique is known for giving good signals even in the presence of salts, detergents and other impurities.

The MALDI technique in mass spectrometry, first described by Karas and Hillenkamp in 1988 can be used effectively for the MS analysis of involatile, polar and thermally unstable compounds. Using MALDI many classes of biological active substances of high molecular weigh (usually above 200,000 Da) such as proteins and carbohydrates have been widely and successfully analysed [Castoro & Wilkins 1993, Spengler & Cotter 1990, McCloskey

1990, Siugchau 1996, Bekir Salif & Renato Zenobi 1998]. However, the application of this method for the analysis of substances having low molecular weights such as sulphonated azo dyes is very limited [Sullivan & Gaskell 1997].

The matrix material plays a key role in this technique absorbing most of the laser energy, which is transferred to the sample, leading the analyte to ionization. The properties required for a matrix for good MALDI – MS analyses are commonly that it absorbs strongly at the wavelength of the irradiating laser; it can dissolve the sample and effectivly promote the chemistry that leads to sample ionization; it has a background spectrum that does not interfere with the obtaining and interpretation of mass data. The choice of the matrix solution is therefore critical and determines the quality of the mass data.

In the current work, mass spectra of the initial azo dye solution and dye solutions withdrawn at regular time intervals during photochemical treatment for analysis by this method were obtained using a MALDI (Finnigan LaserMAT, Great Britain) time-of-flight mass spectrometer. Desorption / ionisation of the samples was performed with a pulsed nitrogen laser operating at 337 nm. The nitrogen laser was focused onto the sample surface forming spot of about 0.1 – 0.2 mm. in diameter. Laser pulse energies were in the range of 25 – 40 μJ, ion desorption was accelerated to 31 mV and each spectrum corresponds to the sum of 20 – 50 laser shots.

Different types of matrix materials including solutions, commonly used in the MALDI analysis of bio-molecules, and the matrixes, investigated and applied for other substances [Sullivan & Gaskell 1997, Bekir Salif & Renato Zenobi 1998] were used in this study for MALDI – MS analysis of dye solutions. The matrix solutions used are listed below:

- 0.05 M solution of 2,5-dihydroxybenzoic acid in water;
- 0.02 M solution of p-nitroaniline in an ethanol and in a 50 : 50 (v/v) ethanol - water mixture;
- 0.1 M solution of ferulic acid (3-methoxy-4-hydroxycinnamic acid) in a 50 : 50 (v/v) acetonitrile – water mixture;
- 0.1 M solution of nicotinic acid in a 50 : 50 (v/v) ethanol-water mixture;
- 0.1 M solution of 2- [4-hydroxyphenylazo] benzoic acid (HABA) in ethanol with a 0.1 M solution of diammonium citrate in water in the ratio 10:1. In this case 5 μL of diammonium citrate solution was mixed with 50 μL of HABA solution before use.

Sample preparation for MALDI analysis is a simple procedure for any dye solution. It is accomplished by adding (and mixing) 1μL dye solution to the matrix solution previously deposited on a stainless steel probe tip, which is a round surface approximately 2 – 5 mm in diameter. After that the mixture was allowed to dry under atmospheric conditions (at room temperature) the samples are inserted into the mass spectrometer for analysis.

2.3.3. Biological analysis of dyes and their photo-oxidised by-products.

Textile waste waters as mentioned above are strongly coloured and contain dyes that, being poorly biodegradable, can be toxic in natural water systems where they can cause severe damage to zoo- and phyto-plankton. There is no information available in the reference literature about toxicity to aquatic organisms and biodegradability of dyes studied after photochemical treatment using UV/ H_2O_2 system. The analytical techniques used do not also give any information on their ecological impact on the environment.

The ecotoxic potential of the preliminary photo-oxidation products of azo dyes in terms of toxicity to aquatic species, micro-organisms and bacteria (involved in biodegradation), and treatability by particularly sewage micro-organisms was carried out. Eco-toxicological assessment of dyes solutions untreated and after photochemical treatment was performed by (1) an acute toxicity testing using the alga *Chlorella vulgaris*, and (2) bacterial respiration inhibition / stimulation testing using *Pseudomonas putida* or activated sludge from a municipal sewage treatment plant.

2.3.3.1. Alga fluorescence inhibition toxicity test.

The aquatic toxicity tests were carried out using a aquatic organism with a short life cycle and rapid response to environmental changes: the freshwater green alga *Chlorella vulgaris Beijer* following guidelines for testing of chemicals using *Chlorella* [Rus. standard 1990]. This test measures the reduction of alga fluorescence as a result of sample toxicity.

The fluorescence inhibition tests were performed using a Biotester. Details of the instrument and measuring technique have been published [Biotester, Rus. patent 1996]. Briefly, the changes in algal fluorescence following test sample addition are measured dynamically after an excitation flash to the sample.

The cultivation of alga for toxicity testing was performed in accordance to Russian standard RD-118-02-90 [Rus. standard 1990]. Algal cell suspension (20 cm^3) was inoculated into 500 cm^3 mineral salt medium (MS) and cultured at a temperature of 18-20^{O}C under artificial day light (16 hours a day) for about 10 days before using in the toxicity test.

The mineral salt medium (pH 7.0) comprised 3 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 ml micro-element solution (content is given in Table 2.1), 0.5 g $NaH_2PO_4 \cdot 12H_2O$ and 0.03 g $Ca\ Cl_2$.

Table 2.1. Content of micro-element solution for growth of *Chlorella vulgaris*.

Substance	Concentration, mg/100 ml
H_3BO_3	290
$MnCl_2$	180
$ZnSO_4 \cdot 7H_2O$	20
$CuSO_4 \cdot 5H_2O$	8
MoO_3	1.5
$FeSO_4 \cdot 7H_2O$	2

For testing, 8 ml of 10-day cell culture was distributed into glass tubes. Then 2 ml of test sample was further added to each tube and changes in fluorescence were registered. Controls comprised (1) 8 ml of algal cells plus 2 ml mineral salt medium and (2) 8 ml of algal cells with 2 ml of distilled water. The change in fluorescence were also recorded after the same exposure time. Each sample was tested in triplicate as minimum, the average values were used for the evaluation of toxicity.

The effect of tested sample was a coefficient of fluorescence (F coef.) calculated as a change (in %) in alga fluorescence after test sample addition compared with the control according to:

$F = (F\ test / F\ init.) \cdot 100$, where

F init = initial level of algal fluorescence;

F test = algal fluorescence with test sample after exposure time.

Results for acute toxicity of dyes are expressed as EC_{16} which is the lowest observable effective concentration reducing by 16 % the fluorescence of *Chlorella* in a 24 h test. EC_{16} for each sample tested in five dilutions is the highest dilution of the sample that gives statistically significant fluorescence reduction occurring in the exposure time. An end point value of EC_{16} was calculated using a Probit Analysis Program computer software to apply the Litchfield and Wilcoxon method.

2.3.3.2. Bacterial respiration inhibition / stimulation test.

The interaction of parent dyes solutions, and their breakdown products after photochemical treatment was studied using a short-term respiration inhibition / stimulation test with aerobic bacteria. In the experiments the bacterium *Pseudomonas putida* and activated sludge were used. The test measures the change in oxygen uptake by test micro-organisms after sample addition. Oxygen uptake is one of the important parameters reflecting life activity of different micro-organisms. The oxygen uptake parameter is most frequently used in microbiological tests [Slabbert & Morgan 1982, Spanjers et al 1994, Xu & Hasselblad 1996, Ubay et al 1998, Orupold et al 1999] as a rapid method for screening for the toxicity of pollutants since it gives a rapid response which is a direct reflection of cellular metabolism and activity.

Oxygen uptake bioassays were carried out using a biological oxygen monitoring system (Rank Brothers Oxygen Electrode System, Rank Brothers Ltd., UK) consisting of an electronic unit, an oxygen probe (platinum - silver electrode with Teflon membrane) with a saturated solution of potassium chloride as an electrolyte and a standard bath unit equipped with test chamber and a magnetic stirrer. A potentiometric recorder was used to obtain a permanent graphic record of oxygen uptake at a chart speed of 0.5 mm/s. The temperature of the system was maintained at 30°C using a constant temperature circulator. Calibration of the apparatus was carried out with sodium dithionite.

Pseudomonas putida from nutrient agar slope culture was inoculated into 100 ml of mineral salt medium and incubated overnight (16 to 18 hours) at 30°C on a rotary-orbital shaker at 150 rpm. The mineral salt medium (MS) comprised 2 g KH_2PO_4, 2 ml trace element solution (content is given in Table 2.2), 3 g NH_4Cl and 0.4 g $MgSO_4 \cdot 7H_2O$ per litre of distilled water. 100 µL of 20 % arginine to 1 dm^3 solution was provided as carbon source. Sterilisation of medium was at 121°C for 15 minutes. The pH of the medium was adjusted to 7.0 with 0.1 M NaOH solution.

Aliquots (2 ml) of the overnight culture were centrifuged at 13,000 rpm for 5 minutes, washed twice with MS (not containing arginine) to remove exogenous carbon substrate and re-suspended in 3 ml of MS. Washed cell suspensions were held at 30°C with shaking in water bath during biotesting procedure.

Table 2.2. Content of Vishniac & Santer's trace element solution for incubation of *Pseudomonas putida*.

Substance	Concentration, g/L
EDTA	50.0
$ZnSO_4 \cdot 7H_2O$	2.20
$CaCl_2$	5.54
$MnCl_2 \cdot 4H_2O$	5.06
$FeSO_4 \cdot 7H_2O$	5.00
$(NH)_4MoO_{24} \cdot 4H_2O$	1.10
$CuSO_4 \cdot 5H_2O$	1.57
$CoCl_2 \cdot 6H_2O$	1.61

Activated sludge was obtained from a municipal biological treatment plant (Severn Trent Water Ltd, Leicester, UK) on the day of each batch of experiments. The mixed liquid suspended solids concentration was 1.5 g/dm^3 and the sludge was used without further dilution. The activated sludge was also held at 30^oC in a incubator with shaking at 150 rev/min prior to use in the oxygen electrode testing system and during all time of testing. The activated sludge was used within 3 hours of collection from the treatment plant.

Toxicity testing using the oxygen electrode system was carried out according to the procedure described in the literature [Slabbert & Morgan 1982, Slabbert & Grabow 1986]. Oxygen uptake was recorded continuously before (references), during and after sample addition. Test sample (0.2 ml) was added to 3 ml of *Pseudomonas putida* cell suspension

or activated sludge. Four (as minimum) replicate tests were carried out on each test sample and a control. The control was 0.2 ml of distilled water in place of the test sample.

From the chart recorder response, a ratio of average oxygen uptake rate per minute over 3 minutes exposure to 3 minutes pre-exposure (reference oxygen uptake) were determined. All rates were determined over 3 minutes for a total testing period of 7 minutes. The effect of test samples was expressed as percentage inhibition or stimulation calculated as the change in test results in relation to the control.

RESULTS AND DISCUSSIONS.

CHAPTER 3. PHOTOCHEMICAL STUDIES ON AZO DYE SOLUTIONS.

3.1. Introduction.

From a technological point of view colour removal from textile effluent is the main problem in waste water treatment technology development. Azo dyes as the biggest class of colorants used today have received the most attention in this context. These dyes are characterized by high stability to washing agents (detergents), light and different aggressive media including oxidising agents. In addition, water soluble azo dyes are more difficult to destroy.

Aromatic azo dyes under UV irradiation in the presence or absence of oxidising agents can undergo irreversible photo-destruction reactions according to literature data reviewed in Chapter 1. In this research the feasibility of the photochemical treatment of coloured effluents containing azo dyes was studied. The research was intended to elucidate the role of oxygen and hydrogen peroxide, on the decolourization rate of azo dyes in aqueous solutions under UV irradiation, as a function of initial dye and oxidising agent concentrations, temperature and pH. The experiments were aimed also to shed light on the mechanism of the dye photo-degradation and to investigate microbial toxicity and biodegradability of dye photo-degraded products.

3.2. Photo-degradation of azo dyes by UV irradiation.

In this experimental work the dye stock solutions (of 1 g/dm^3 concentration) were diluted with distilled water to produce 1dm^3 of dye solution at a concentration of 25 mg/dm^3. The dye solutions at a concentration of 25 mg/dm^3 were immediately introduced into the laboratory irradiation apparatus and irradiated by UV light without any oxidising agent addition over 120

minutes at a temperature of 25°C and pH 7.0. Dissolved oxygen was not removed from dye solutions in this experimental set. At the start of the experiment and at suitable time intervals samples were withdrawn from the photochemical treatment system and cooled to room temperature for the UV-Visible analysis and determination of residual dye concentration. The data on colour removal against time of photo chemical treatment for dyes studied are summarised in Table 3. 1.

Table 3. 1. Colour removal kinetics of azo dyes by UV irradiation (at 25°C and pH 7)

Dye (C_o = 25 mg/dm^3)	Colour removal (decrease in concentration of dyes after time of UV irradiation*), after time, %:			
	30 min	60 min	90 min	120 min
Acid Blue 158	8.2	12.8	17.5	20.3
Acid Yellow 99	6.9	9.9	13.7	16.7
Acid Blue 92	13.3	31.2	39.0	49.7
Basic Red 18	1.7	2.7	4.0	5.5
Acid Red 73	0.9	1.7	2.1	3.8
Acid Blue 113	3.8	8.7	12.0	14.7
Direct Red 23	1.1	2.1	2.5	4.1

*) UV treatment of the dye solutions in distilled water was carried out without addition of oxidising agent or removal of dissolved oxygen.

In the case of direct photolysis azo dyes are decolourized very slowly, after 2 hours of UV irradiation no more than 50 % of colour removal was observed. From the data (Table 3.1) the

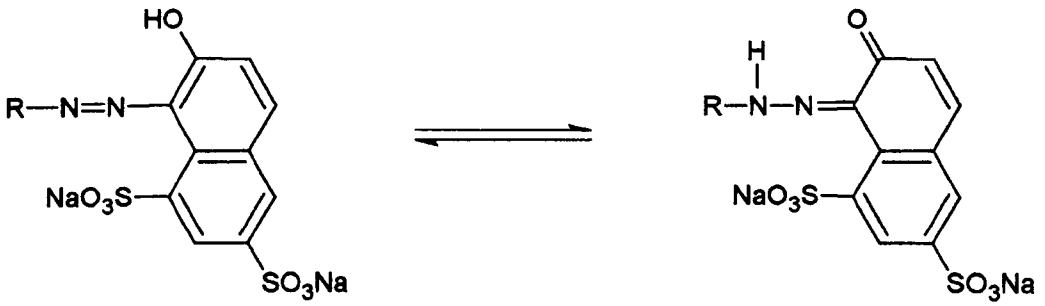
photo-degradation rate for the dyes decreased in the following order: C. I. Acid Blue 92 > C. I. Acid Blue 158 > C. I. Acid Yellow 99 > C. I. Acid Blue 113 > C. I. Basic Red 18 > C. I. Direct Red 23 > C. I. Acid Red 73. In general, the decolourization rate of monoazo dyes is faster than for diazo dyes (except for the dye C. I. Basic Red 18).

The number of azo linkages as well as the presence of other substituents (–OH and $-SO_3$ groups) in the dye molecules seem to be important factors in the photo-degradation of dyes. Under UV irradiation cleavage of all azo groups in the dye structure can occur, but the possibility of simultaneous breakdown of all azo bonds is small. This could be the explanation of dyes with smaller numbers of azo groups becoming colourless faster. This is in accordance with other research [Tang & An 1995] where a similar dependence on azo linkage numbers for decolourization of azo dyes was observed during their photocatalytic degradation. The monoazo dye C. I. Acid Blue 92, which contains the largest number of hydroxy and sulphonic groups was decolourised most rapidly, and the monoazo dye C. I. Basic Red 18 not containing hydroxyl and sulphonic groups was least decolourized.

On the other hand, the presence of aromatic groups, such as phenyl and naphthyl, in the structure of dyes probably strongly affects the decolourization rate of the dyes. The dyes containing a naphthalene ring attached to the azo bond are degraded faster (Table 3.1). It is not surprising because the naphthalene ring has a greater extinction coefficient and absorbs light in UV-Vis region of spectrum more strongly than the benzene ring [Brown et al 1988].

The dyes C. I. Acid Red 73 and C. I. Direct Red 23 were least degraded under UV irradiation. It is probably due to these dyes containing -OH substituents which have tended to stabilise the dyes because of strong internal hydrogen bond formation with the nitrogen atom of the azo

linkage and azo-hydrazone tautomerism [Poray-Koshits 1967, Venkataraman 1967, Zollinger 1987] as follows:



azo tautomer hydrazone tautomer

Strong internal hydrogen bonds stabilising dyes can inhibit different physical and chemical reactions (such as ionization, hydrogen abstraction, photo-isomerisation etc.).

The absorption spectra of dyes studied were recorded over a period of 120 minutes of UV irradiation and given in Figure 3.1. for C. I. Acid Red 73 and C. I. Acid Blue 158 as an examples. UV-Visible analysis shows no significant decrease in optical densities (at maximum absorption of dyes) in the far UV and visible regions of the spectrum with no formation of new absorption peaks during UV treatment. At the same time no changes in optical density in a wide wavelength range from 250 nm to visible were observed. Some photo-transformation of auxochromes in the dye structure may take place, but destruction of the chromogenic part of the dyes does not occur for this duration of UV irradiation.

The results show that decolourization of dyes when irradiated with UV light alone, under the experimental conditions used, is a very slow process. This may be also due to the low concentration of dissolved oxygen in solutions.

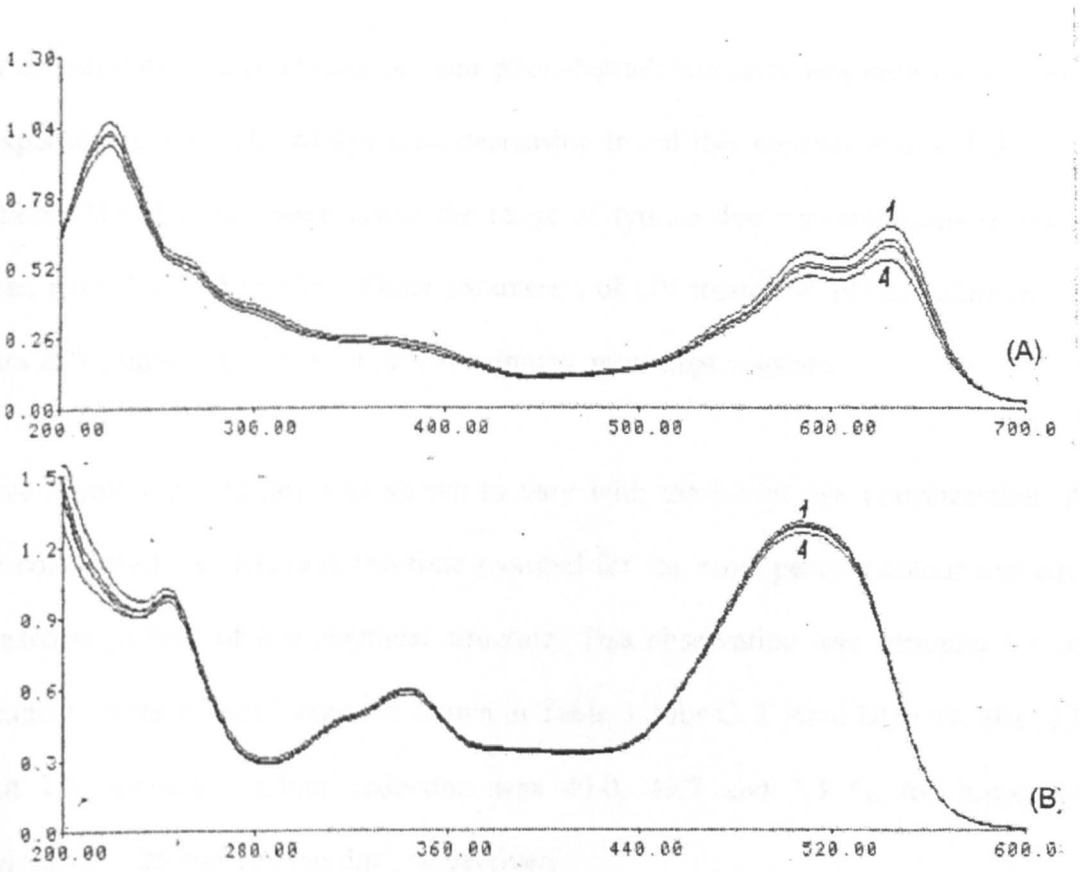


Figure 3.1. UV-Visible absorption spectra of the dyes C. I. Acid Blue 158 (A) and C. I. Acid Red 73 (B), treated by UV irradiation.
(1) – initial dye solution, after time treatment of:
(2) – 30 min., (3) – 60 min., (4) – 120 min.
UV treatment conditions: $[Dye]_o$ = 25 mg/dm^3, 25°C and pH 7.0.

3.2.1. Effect of initial dye and dissolved oxygen concentrations on dye photo-fading.

The effect of initial dye concentration on their photodegradation rates was investigated using a set of experiments for each azo dye with decreasing initial dye concentrations. Initial dye concentrations ($[Dye]_o$) used were inside the range of typical dye concentrations in textile waste water, from 250 to 1 mg/dm^3. Other parameters of UV treatment, pH of solutions 7.0, temperature 25°C, time of UV irradiation 120 minutes, were kept constant.

The relative decolourization rate was shown to vary with the initial dye concentration. As initial dye concentrations increased, the time required for the same percent colour reduction also increased regardless of dye chemical structure. This observation was common for any azo dye studied. In the selected example shown in Table 3.2 for C. I. Acid Blue 92, after 120 minutes of UV treatment colour reduction was 84.0, 49.7 and 7.5 % for initial dye concentrations of 1, 25 and 100 mg/dm^3, respectively.

Relationship between the initial decolourization rate and initial dye concentrations (as illustrated in Figure 3.2) using experimentally obtained data can be described by equation:

$$y = a\, e^{-b\,x},$$

where y – decolourization rate constant (k x 1000, min^{-1}), x – initial dye concentration ($[Dye]_o$, mg/dm^3), a and b are coefficients (according to experimental results equal to 0.0118 and 0.026, respectively). On a logarithmic scale the dependence of the decolourization rate constant against the initial dye concentrations was a straight line.

Table 3.2. The effect of initial dye concentration on colour removal of C. I. Acid Blue 92.

$[Dye]_o$, mg/dm^3	Time of UV treatment, min.	Ct/Co	Colour removal, %
1.0	0	1.0	0.0
	30	0.64	36.0
	60	0.407	59.3
	90	0.252	74.8
	120	0.16	84.0
10.0	0	1.0	0.0
	30	0.76	24.0
	60	0.561	43.9
	90	0.442	55.8
	120	0.325	67.5
25.0	0	1.0	0.0
	30	0.867	13.3
	60	0.688	31.2
	90	0.61	39.0
	120	0.503	49.7
50.0	0	1.0	0.0
	30	0.922	7.8
	60	0.812	18.8
	90	0.731	26.9
	120	0.632	36.8
100.0	0	1.0	0.0
	30	0.979	2.1
	60	0.963	3.7
	90	0.940	6.0
	120	0.925	7.5
150.0	0	1.0	0.0
	30	0.99	0.1
	60	0.995	0.5
	90	0.994	0.6
	120	0.991	0.9

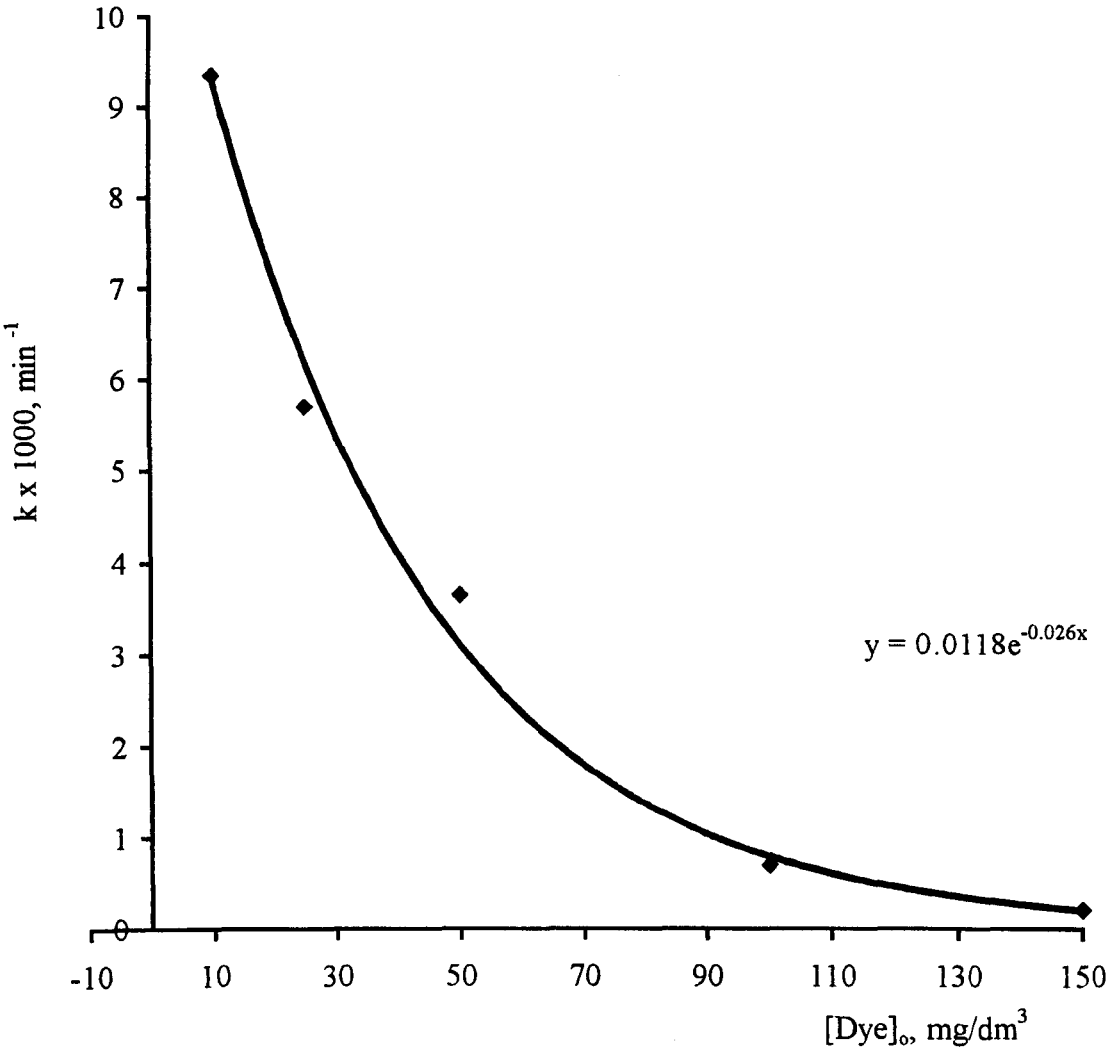


Figure 3.2. Effect of initial concentration of the dye C. I. Acid Blue 92 on decolourization kinetics by UV irradiation.

UV treatment conditions: 25°C, pH 7.0, exposure time to UV irradiation 120 minutes.

The increase of dye photo-fading in aqueous solution with increase of dye concentration can be due to several reasons depending on the complexity of chemical structure and physico-chemical properties of azo dyes.

First, with the increase of dye concentration the average distance between molecules of dye, up to the effective distance for excitation energy transfer to neighbour dye molecules, is decreased [Mejer 1977]. The minimum average distance between dye molecules not quenching the dye excited state and not giving a decrease in dye photo-decolourization rate was established to be 5-10 μm exceeding the average radius of dye molecules themselves by approximately 5 times [Teremin 1967].

Second, if dye concentration is high (more than 10^{-4} mol/dm^3 or about 60 mg/dm^3) an association and aggregation of the dye molecules resulting in dimers, trimers and other dye species formation up to colloidal dimensions, can occur [Terenin 1967]. The dye solution becomes more and more impermeable to UV radiation with the dye acting as a filter and deviating from the Beer Lambert law [Evans & Strapleton 1978, Allen 1994]. The dyes in the form of a monomer are more photo-reactive than those in the aggregated form. The aggregated dye molecules scatter excitation energy before they can photolytically react and the life time of dye excited state is therefore less.

Finally, the decrease in dye photofading rate with the increase of initial concentration may be also due to change in the ratio of dye and dissolved oxygen concentrations. When dye concentration is high the ratio between dissolved oxygen and dye decreases. This can result in the decrease in dye photo-fading. For example, the molar ratios between initial dissolved oxygen concentration of 8.2 mg/dm^3 (2.4×10^{-4} mol/ dm^3) and initial dye concentrations of

100 and 1 mg/dm^3 (8.9 x 10^{-4} and 1.43 x 10^{-6} mol/dm^3) were 0.27 and 168 respectively for C. I. Acid Blue 92. The photo-degradation of the dye with [Oxygen]/[Dye]$_o$ molar ratio of 168 is faster than with the lowest ratio. This suggests that dissolved oxygen may play a part in the decolourization process. The dependence of dye photofading on concentration of dissolved oxygen in solutions was therefore investigated.

The study of azo dye decolourization by UV irradiation as a function of dissolved oxygen content was carried out using saturation of dye solutions with oxygen gas, air and helium gas. Dye solutions at initial concentration 25 mg/dm^3 were photolysed over 60 minutes keeping temperature 25°C and pH 7.0 constant for each run. The saturation of dye solutions with gases or air was continued before and throughout the photolysis. Dissolved oxygen concentrations were measured before and at various time intervals of UV photochemical treatment using an platinum-silver electrode for water oxygen measurements (Aqua-Oxy Electrode Thermooxy-meter, Estonia). Changes in dye concentration during the photolysis were determined spectrophotometrically at maximum absorption wavelength for each dye.

Preliminary experiments to determine optimal gas (or air) levels and the effect of saturation time with a gas (or air) prior to UV treatment showed that an increase in saturation time to 6 hours before photolysis and the increase in gas flow from 5 to 10 dm^3/h do not effect the decolourization rate of dyes. Under atmospheric pressure and the temperature of 25°C the concentration of dissolved oxygen in solutions achieved a constant value over 30 minutes of saturation with gases. When the gassing time before UV treatment was further increased to 6 hours the concentration of the dissolved oxygen did not change. Also the increase of gas (air) flow from 5 to 10 dm^3/h did not influence the dissolved oxygen content in dye solutions. Therefore, dye solutions were further photolysed with saturation with gas (or air) for 30

minutes before and during the photolysis. A flow rate of 5 dm^3/h was maintained constant during each experiment. Results for colour removal of azo dyes as a function of dissolved oxygen content in irradiated solutions are shown in Table 3. 3 for C. I. Acid Blue 92 as an example.

Table 3.3. Results for colour removal of C. I. Acid Blue 92 as a function of dissolved oxygen content in irradiated solutions ($[Dye]_o$ = 25 mg/dm^3, pH 7.0).

Saturation of dye solution before and throughout photolysis with:	T, °C	UV treatment time, min.	O_2, mg/dm^3	Colour removal, %
No bubbling	25	0	8.2	0.0
		10	-	5.4
		20	-	11.2
		30	8.0	13.3
		60	7.3	31.2
	60	0	6.7	0.0
		10	-	1.2
		20	-	6.8
		30	6.5	11.9
		60	6.4	21.5
Air	25	0	8.8	0.0
		10	-	5.5
		20	-	11.8
		30	8.7	14.2
		60	8.5	32.1
Oxygen gas	25	0	46.7	0.0
		10	-	23.1
		20	-	41.8
		30	41.2	54.6
		60	28.8	81.7
Helium gas	25	0 – 60	≈ 0.0	0.0

As shown in Table 3. 3 colour removal rate of dye under UV irradiation becomes higher when initial oxygen concentration in dye solutions increases. On the other hand, negligible changes in decolourization rate of this dye on saturation with air in comparison with colour removal rate without further bubbling of air was observed .

The highest colour removal rate of dye was observed under photolysis with saturation of dye solutions with oxygen gas (Figure 3.3). This may be due to the dissolved oxygen concentration in the oxygen-saturated solutions being much higher than in both air-saturated and unaerated solutions. In the last two cases (air-saturated and unaerated dye solutions) a small change in decolourization rate of the dye in air-saturated solutions in comparison with those photolysed without aeration, was observed. It is probable the initial dissolved oxygen concentrations in these solutions practically coincide (8.8 mg/dm^3 in air-saturated solutions and 8.2 mg/dm^3 in unaerated solutions as the data indicate in the Table 3.3).

In deoxygenated dye solution, while on saturation with helium gas, no decolourization of this dye during the experimental period of UV treatment was observed.

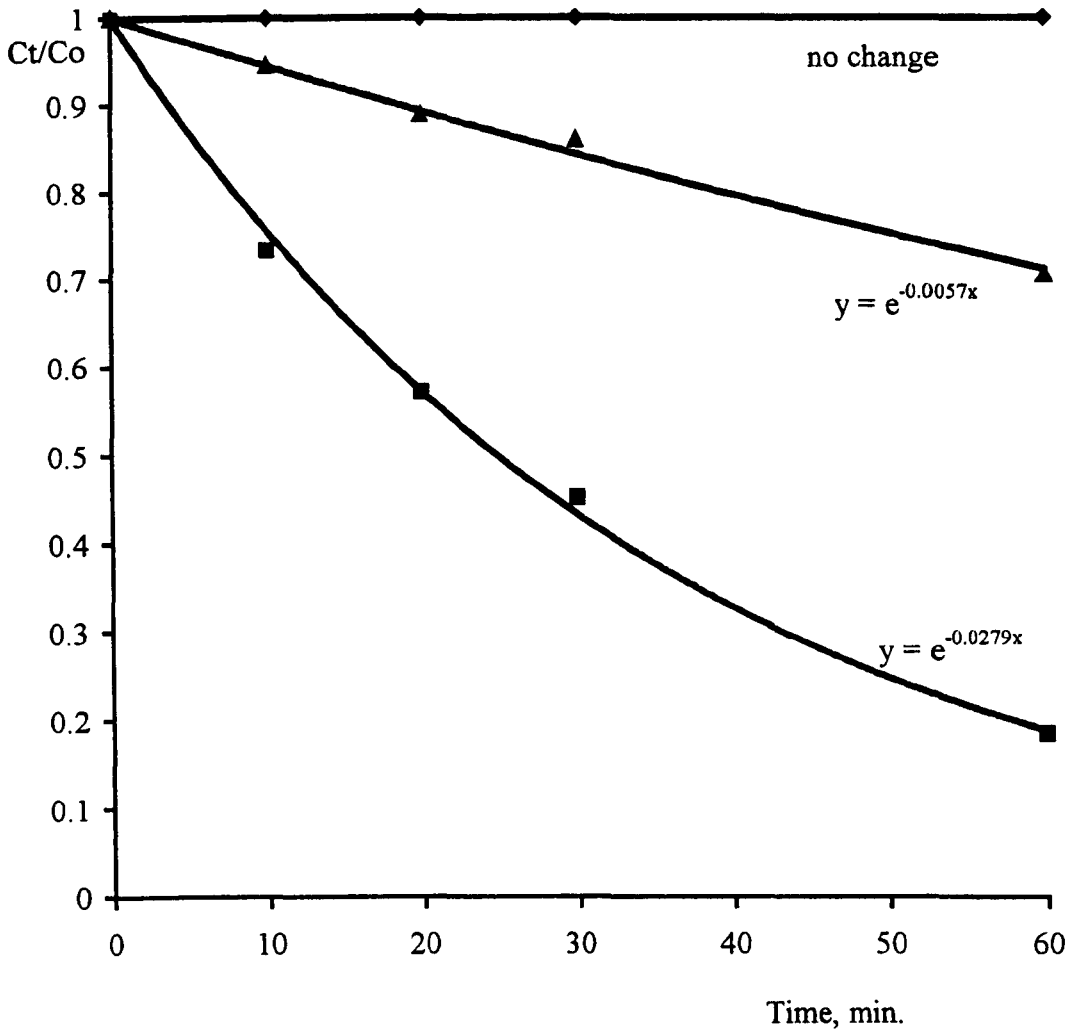


Figure 3.3. Decolourization kinetics of C. I. Acid Blue 92 by UV irradiation in aqueous solutions:

◆ deoxygenated solution (saturation with helium gas)
■ oxygen-saturated solution
▲ air-saturated solution

UV treatment conditions: $[Dye]_o$ 25 mg/dm^3, pH 7.0, 25°C .

Experimental data on the influence of initial dissolved oxygen concentration on decolourization rate of azo dyes in aqueous solutions under UV irradiation, summarized in Table 3.4, show similar results for the majority of the dyes studied (except for C. I. Basic Red 18 and C. I. Acid Blue 113).

Table 3.4. Azo dyes colour removal data as a function of dissolved oxygen content in UV irradiated aqueous solutions (at 25°C and pH 7.0).

Dye ($[Dye]_o$ = 25 mg/dm^3)	Colour removal, % (decrease in dye concentration) after 60 min. of UV irradiation at condition of:			
	saturation of irradiated solutions with:			without further aeration
	helium gas	air	oxygen gas	
C. I. Acid Blue 92	0.0	32.1	81.7	31.2
C. I. Basic Red 18	11.5	4.2	34.5	2.7
C. I. Acid Blue 158	0.0	13.7	41.4	12.8
C. I. Acid Yellow 99	0.0	10.9	37.8	9.9
C. I. Acid Red 73	0.0	3.4	4.4	1.7
C. I. Direct Red 23	0.0	2.3	10.2	2.1
C. I. Acid Blue 113	42.2	15.8	51.6	8.7

From the Table 3.4 the colour removal rate of azo dyes tested increases in oxygen-saturated solutions in comparison with their decolourization rate in air-saturated and unsaturated solutions during photolysis under the same experimental conditions. There are only exceptions for C. I. Basic Red 18 and C. I. Acid Blue 113. In contrast, the colour removal of

these dyes was found to increase on saturation of their solutions not only with oxygen gas and air, but with helium gas as well.

Colour removal rate of azo dyes increases in oxygen-saturated solutions differently for each dye. For example (Table 3.4), the decolourization rate of C. I. Basic Red 18 increases 12.7 times, but C. I. Acid Yellow 99 only 3.8 times. The different sensitivity of the dyes to decolourization by UV irradiation indicates that the chemical structure of the dyes is of primary importance for their photo-reactivity and susceptibility to the destructive action of UV irradiation rather than external physical factors including oxygen concentrations in dye solutions.

Azo dye stability to photochemical destruction is known to be dependent on the number of nitrogen to nitrogen double bonds in the chromophoric part of the dye structure. The higher the azo group content the greater the light stability of the dye. Also the photochemical activity of the dyes can be dramatically changed by the addition of electron-donor and/or electron-acceptor substituents in both azo- and disazo components of dyes and by the presence of dye metal-complexes [Kritchevsky 1986, Gordon & Gregory 1983, Mejer 1971, Stepanov 1984, Zollinger 1987].

The literature data suggest dye photo-oxidation or/and reduction mechanisms at different dissolved oxygen concentrations in aqueous solutions. The activation of molecular oxygen to active oxygen species by photoactive dye molecule, was reported [Bekker (ed) 1976, Kritchevsky 1986, Albini et al 1989] to be dominant in the oxygen-saturated aqueous solutions of azo dyes (at dissolved oxygen concentration of $10^{-3} - 10^{-2}$ mol/dm^3):

$$D + h\nu \longrightarrow D^* \quad (1)$$

$$D^* + O_2 \longrightarrow D^{+\cdot} + O_2^{-\cdot} \quad (2)$$

$$D^* + O_2 \longrightarrow D + O_2^* \quad (3)$$

Rate constants of reactions (eq. 2 and eq. 3) were reported to be in the range of (2.5 – 3.1) x 10^8 dm^3 mol^{-1} s^{-1}[Mejer 1971]. Due to subsequent reaction of photoactive dye molecules with generated reactive oxygen species photo-oxidation of the dyes can take place:

$$D + O_2^* \xrightarrow{h\nu} \text{colourless oxidation products} \quad (4)$$

In air-saturated solutions of azo dyes photo-degradation is a slower process. Under these conditions dye radical formation probably plays the main role. Dye radicals formed can then be attacked by molecular oxygen in the usual way resulting in dye peroxyl radical generation or react with each other giving photo-reduction products:

$$D^{\cdot} + O_2 \xrightarrow{h\nu} DO_2^{\cdot} \longrightarrow \text{colourless oxidation products} \quad (5)$$

$$D^{\cdot} + D^* \longrightarrow \text{reduction products} \quad (6)$$

Dye peroxyl radicals can be further rapidly destroyed photochemically with the formation of colourless by-products (eq. 5). Dye radical formation and their subsequent interactions probably play a dominant role in the decolourization of C. I. Basic Red 18 and C. I. Acid Blue 113 in deoxygenated dye solutions, allowing photo-degradation of these dyes by a photo-reduction mechanism (eq. 6).

The possibility of the dye photo-reduction pathway (eq.6) in aqueous solutions may be confirmed by decolourization of these dyes in the presence of electron donor compounds such as alcohols. The dye photo-decomposition in the air-saturated water solutions with addition of glycerol (initial concentration 15 mg/dm^3) as electron donor substance is shown in Figure 3.4 for dye C. I. Acid Blue 92. In this case the photo-decomposition of the dye by active oxygen forms generated by the excited state of the dye itself under UV irradiation is probably negligible, and dye photo-decomposition occurs due to energy transfer from reducing substance to the dye.

The involvement of oxygen in the photo-fading reactions of dyes is thus confirmed and the absence of decolourization in deoxygenated aqueous solutions of the dyes can be additional evidence to that fact. The photo-fading rate of the azo dyes increases greatly with increasing dissolved oxygen content depending on dye chemical structure. Therefore, in view of feasibility of a UV/H_2O_2 photochemical oxidation system as a treatment step in textile treatment technology to efficiently destroy azo dyes, saturation of dye solutions with air or/and oxygen gas can be seen to accelerate the degradation rate of the dyes.

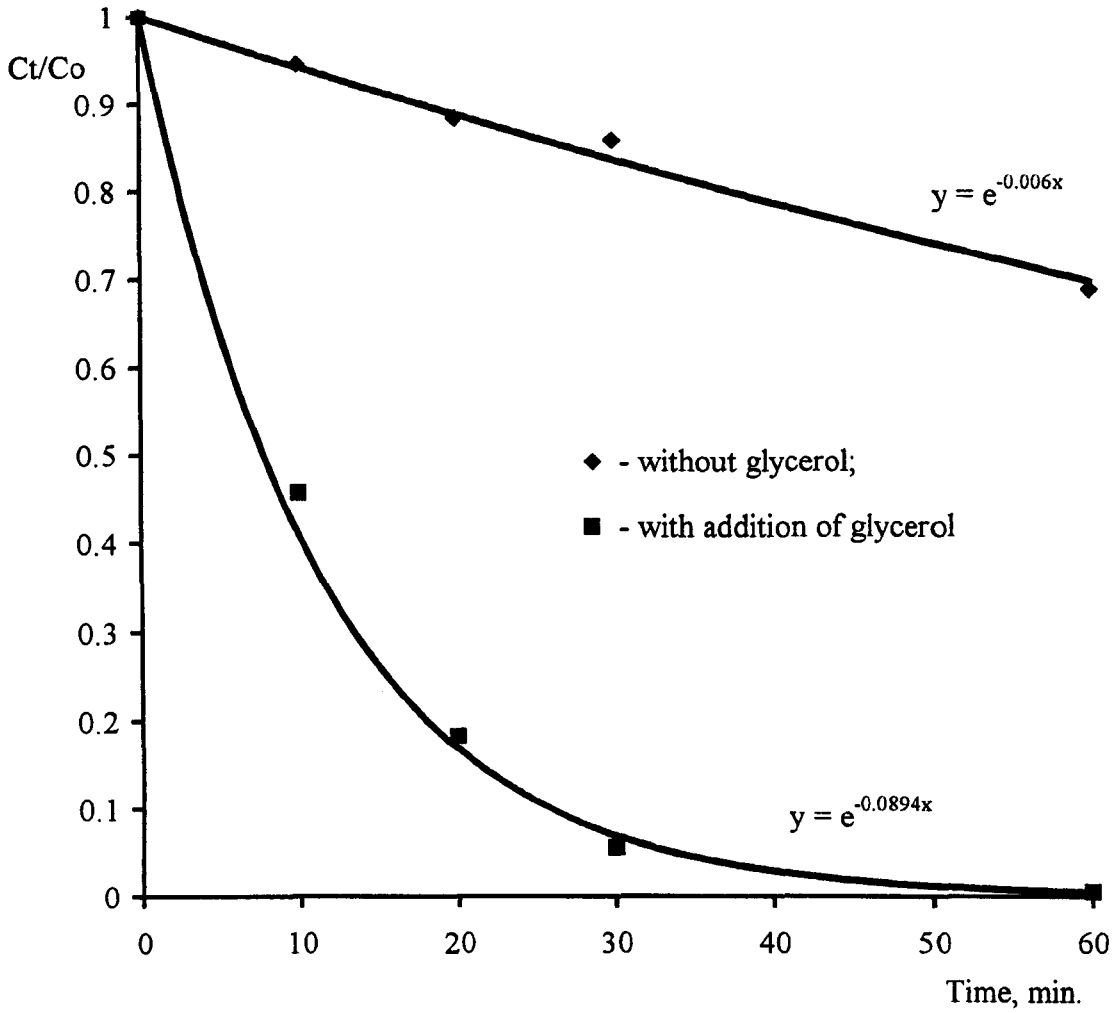


Figure 3.4.Colour removal rate of C. I. Acid Blue by UV irradiation in air saturated solutions.

UV treatment: $[Dye]_o$ 25 mg/dm^3, $[Glycerol]_o$ 15 mg/dm^3, pH 7.0, 25°C.

3.2.2. Effect of temperature on azo dye photo-degradation rate.

During the previous experiments while the influence of initial dye concentrations and dissolved oxygen concentrations on decolourization rates of dyes under UV irradiation conditions were studied, the temperature of the dye solutions was not regulated. Passing repeatedly through the UV irradiation laboratory system (through the peristaltic pump and near the UV lamp) dye solutions being treated warmed up by 2 – 3°C. Therefore, to make sure that this small change in temperature has not a strong effluence on dye decolourization kinetics and to test the effect of temperature on dye photo-degradation rate, experiments on the decolourization of azo dyes were carried out at temperatures of 25°C and 60°C other parameters, $[Dye]_o$ 25 mg/dm^3, pH 7.0, time of UV irradiation 120 minutes, remaining constant.

Experimental results on dye decolourization (Figure 3.5 for C. I. Acid Blue 92) depending on temperature show that colour removal rate of dye decreases slightly with the increasing of temperature from 25°C to 60°C. Similar results were obtained for all dyes studied regardless their chemical structure and physico-chemical properties. This small decrease in dye photo-decolourization rate with increasing of temperature was probably due to decrease in the initial dissolved oxygen concentration in solutions at the higher temperature (Table 3.3). Indeed, according to classical chemistry diminishing the temperature should retard dye fading, but some cases of dye photochemical reactions are reported in which high temperature can cause either rapid fading of dyes or retarding of their photo-fading [Kritchevsky 1986]. It was also noted that there are practically no data on temperature dependence of the decolourization rate of azo dyes in aqueous solutions.

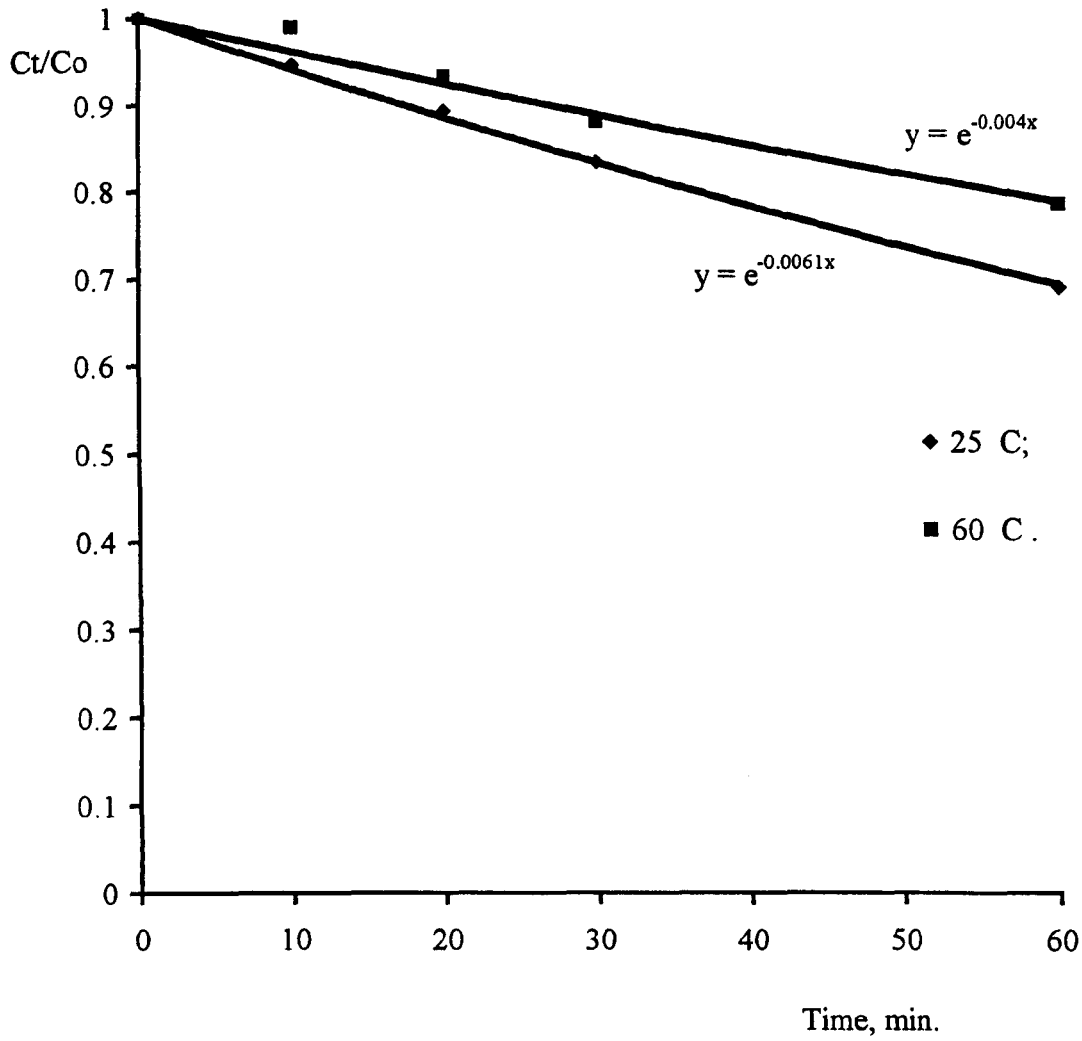


Figure 3.5. Decolourization kinetics of C. I. Acid Blue 92 by UV irradiation as a function of temperature.

UV treatment conditions: $[Dye]_o$ 25 mg/dm^3, pH 7.0

3.2.3. Effect of pH on azo dye photo-degradation rate.

Studies on the decolourization rate dependence on pH were performed over the pH range of 4.0 to 11.0. The photo-degradation of dyes studied were carried out over 120 minutes of UV irradiation at an initial dye concentration $[Dye]_o$ 25 mg/dm^3 and temperature 25°C. The pH of the dye solutions was adjusted using 0.05 M H_2SO_4 solution and 0,1 M NaOH solution. The value of the decolourization rate of the dye at pH 7.0 was used as a reference for each dye. The effect of the pH on the decolourization rate of dyes is summarised in Table 3.5.

Table 3.5. Azo dye colour removal data as a function of pH in UV-irradiated aqueous solutions.

Dye $[Dye]_o$ = 25 mg/dm^3	% colour removal (decrease in dye concentration after 120 min. of UV irradiation), at various pH of irradiated solution:			
	4.0	7.0	9.0	10.5
C. I. Acid Blue 92	60.0	49.7	58.9	72.5
C. I. Basic Red 18	17.1	5.5	3.1	2.5
C. I. Acid Blue 158	7.3	20.3	56.4	91.0
C. I. Acid Yellow 99	3.1	16.7	36.8	56.5
C. I. Acid Red 73	6.5	3.8	9.9	24.6
C. I. Direct Red 23	7.5	4.1	12.2	29.9
C. I. Acid Blue 113	18.0	14.7	21.8	34.3

The decolourization of the dyes on UV irradiation was observed to be more effective in acidic solutions at pH 4.0 and in strong alkaline solutions from pH 9.5 to 11.0 for all dyes used in the research.

UV-Vis absorption spectra of the dyes studied recorded over a period of 120 minutes of UV irradiation show that the change in optical density of the dyes differs depending on pH. As seen in Figure 3.6. (UV-Vis spectra of C. I. Acid Red 73) the decrease in optical densities in the visible range of the dye spectrum at the maximum absorption wavelength was higher in acidic medium at pH 4.0 and particularly in alkaline medium at pH 10.5. Only 3.8 % colour removal for C. I. Acid Red 73 was produced at pH 7.0 in 120 minutes UV irradiation, as compared to 7.0 % and 29.6 % at pH 4.0 and 10.5, respectively. At the same time no formation of new absorption peaks over a wide wavelength range from the far UV to visible were observed.

Similar results were obtained for most dyes except for C. I. Acid Blue 158 and C. I. Acid Yellow 99. UV-Visible spectra of these dyes (as presented in Figure 3.7 for C. I. Acid Blue 158) also show a decrease in optical density in the visible region (at maximum absorption wavelength of dye) at all pH values tested, but in alkaline solutions (at pH 10.5) optical density of these dyes dramatically drops over a wide wavelength range (from 190 nm to 650 nm of the spectrum).

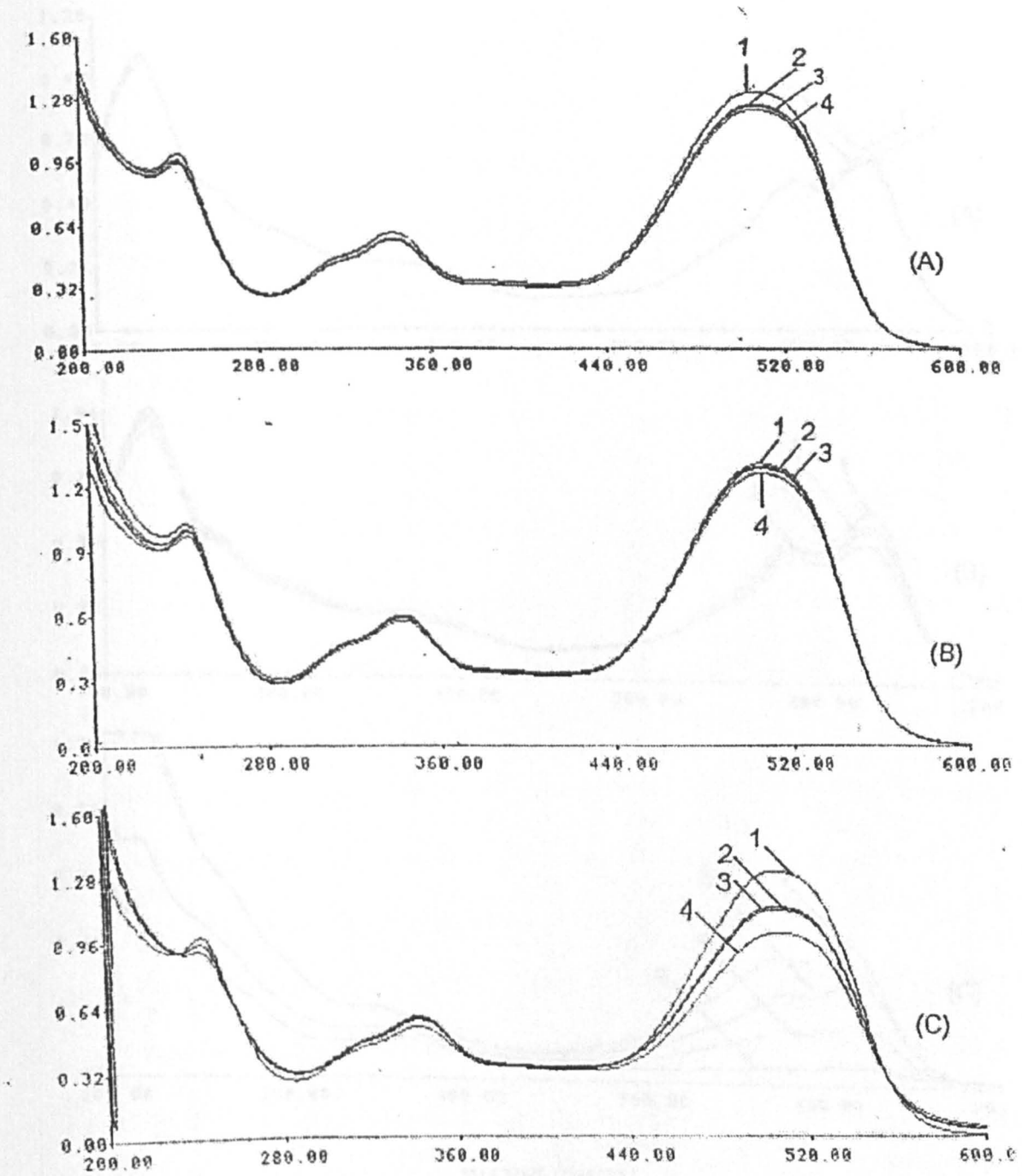


Figure 3.6. UV-Visible absorption spectra of C. I. Acid Red 73 treated by UV irradiation as a function of pH: 4.0 (A), 7.0 (B), 10.5 (C).

(1) – initial dye solution, after treatment time of:

(2) – 30 min., (3) – 60 min., (4) – 120 min.

UV treatment conditions: $[Dye]_o$ 25 mg/dm^3, 25°C.

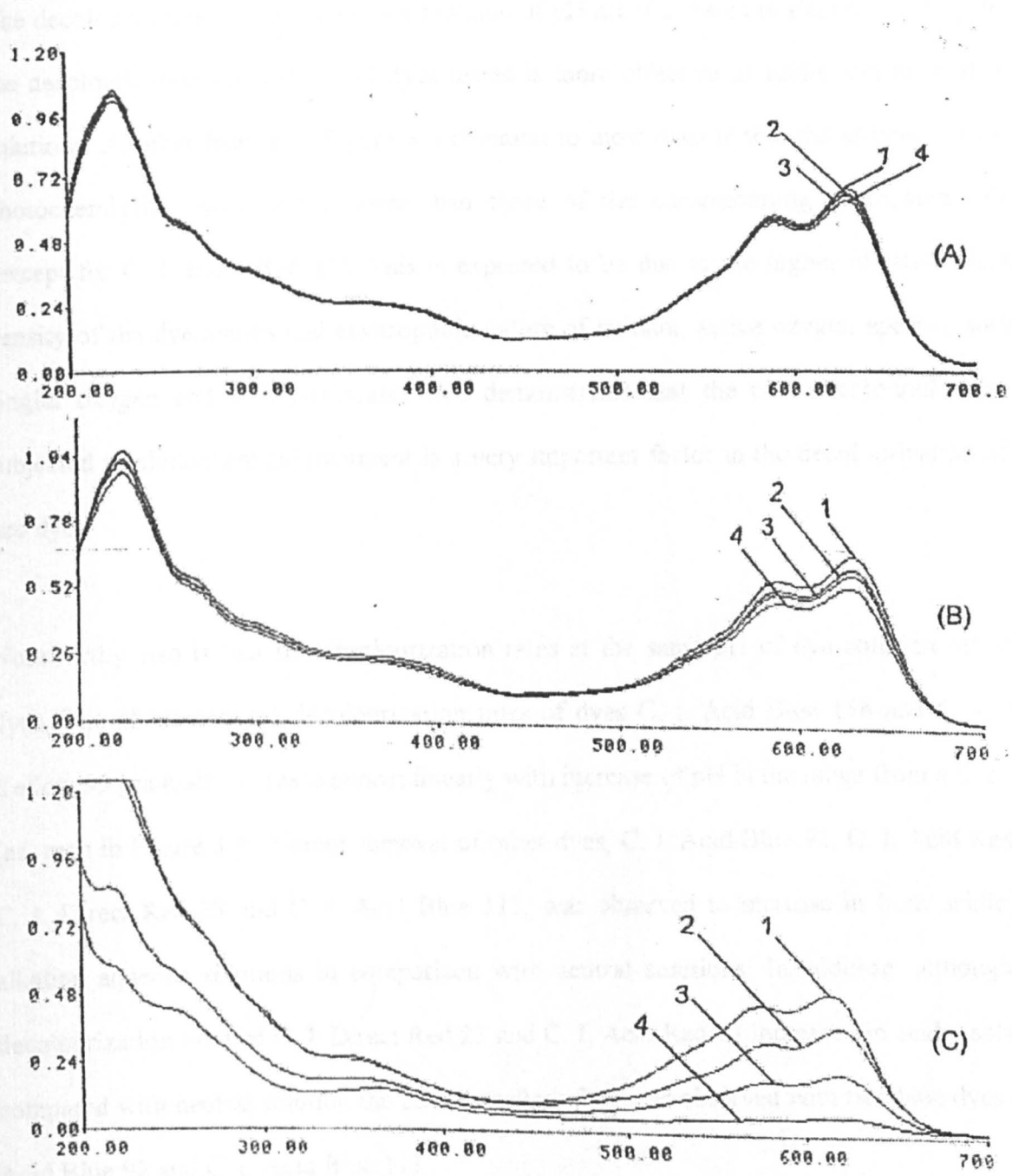


Figure 3.7. UV-Visible absorption spectra of C. I. Acid Blue 158 treated by UV irradiation as a function of pH: 4.0 (A), 7.0 (B), 10.5 (C).

(1) – initial dye solution, after treatment time of:

(2) – 30 min., (3) – 60 min., (4) – 120 min.

UV treatment conditions: $[Dye]_o$ 25 mg/dm^3, 25°C.

The decolourization rates of dyes as a function of pH are illustrated in Figure 3.8. In general, the decolourization process for all dyes tested is more effective in acidic and/or in alkaline solutions. Another feature in Figure 3.8 common to most dyes is that the anionic forms are photochemically decolourized faster than those of the corresponding protonated forms (except for C. I. Basic Red 18). This is expected to be due to the higher negative electron density of the dye anions and electrophilic nature of oxidant, active oxygen species, such as singlet oxygen and/or oxy-radicals. This demonstrates that the pH of coloured effluents subjected to photochemical treatment is a very important factor in the decolourization of the azo dyes.

Noteworthy also is that the decolourization rates at the same pH of dye solution differ for dyes. The photochemical decolourization rates of dyes C. I. Acid Blue 158 and C. I. Acid Yellow 99 gradually increase almost linearly with increase of pH in the range from 4.0 to 10.5 (as seen in Figure 3.8). Colour removal of other dyes, C. I. Acid Blue 92, C. I. Acid Red 73, C. I. Direct Red 23 and C. I. Acid Blue 113, was observed to increase in both acidic and alkaline aqueous solutions in comparison with neutral solutions. In addition, although the decolourization rates of C. I. Direct Red 23 and C. I. Acid Red 73 increased in acidic solution compared with neutral solution the effect was less than was observed with two blue dyes C. I. Acid Blue 92 and C. I. Acid Blue 113.

The behaviour of C. I. Basic Red 18 is unique in that the decolourization rate of this dye gradually decreases (nearly linearly) with the increase of pH and colour removal of this dye is the greatest in pH 4.0 (see data in Figure 3.8).

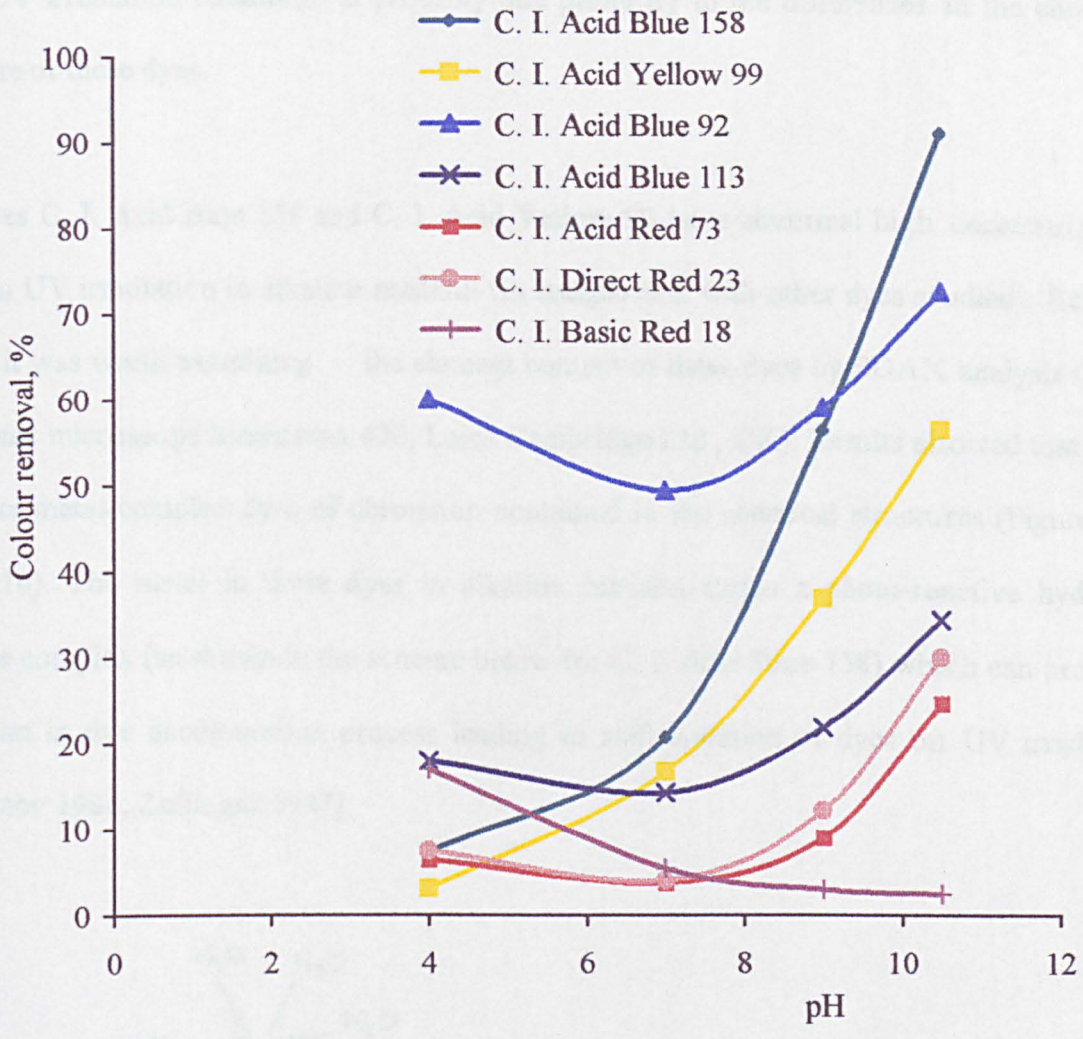
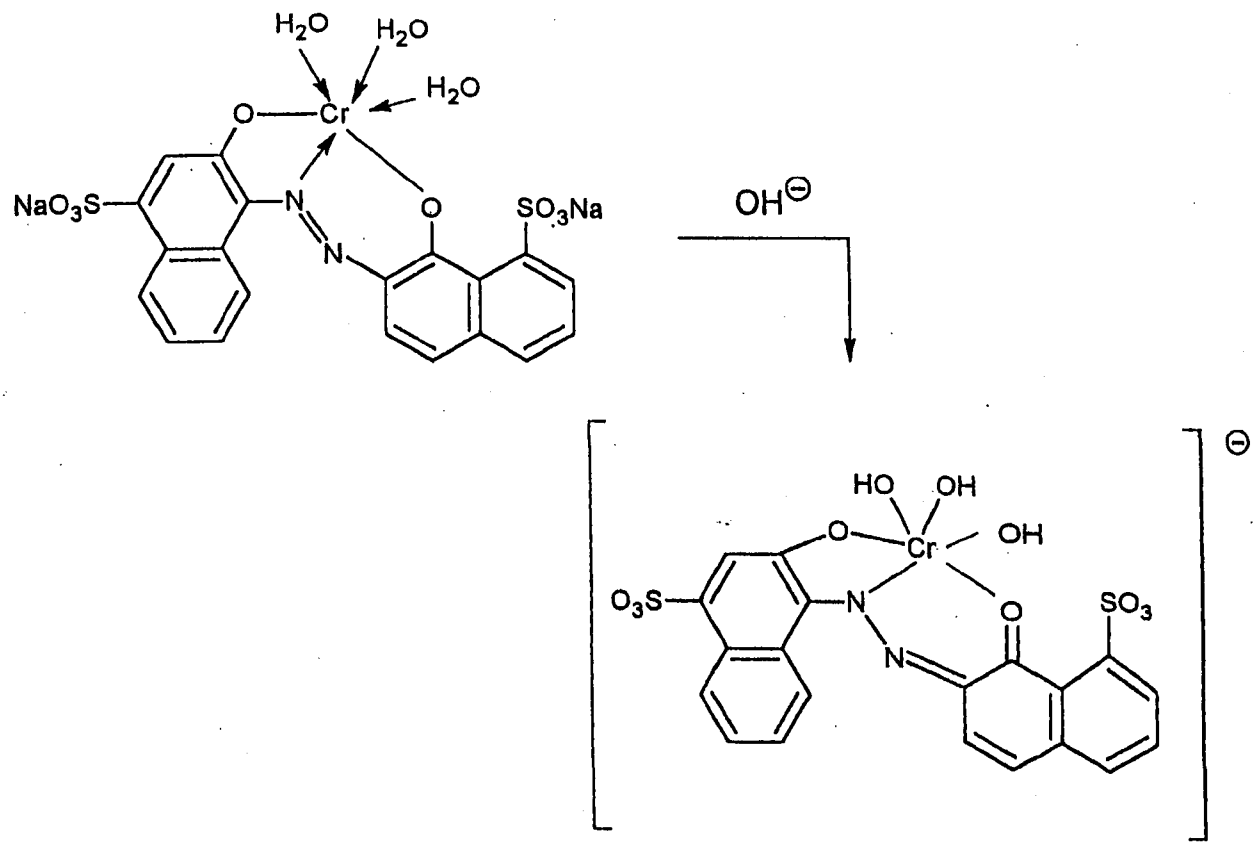


Figure 3.8. Colour removal of azo dyes by UV irradiation as a function of pH.

UV treatment conditions: $[Dye]_o$ 25 mg/dm^3, 25°C, time of UV irradiation 120 min.

The pH dependent differences in photochemical behaviour of the azo dyes observed under the same UV irradiation conditions is probably due primarily to the differences in the chemical structure of these dyes.

The dyes C. I. Acid Blue 158 and C. I. Acid Yellow 99 have abnormal high decolourization rates on UV irradiation in alkaline medium (in comparison with other dyes studied). Because of that it was worth examining the element content of these dyes by EDAX analysis (using electronic microscope Stereoscan 430, Leica Cambridge Ltd., UK). Results showed that these dyes are metal-complex dyes of chromium contained in the chemical structures (Figures 3.9 and 3.10). The metal in these dyes in alkaline solution forms a photo-reactive hydroxy-chrome complex (as shown in the scheme below for C. I. Acid Blue 158) which can probably take part in dye decolouration process leading to self-oxidation of dyes on UV irradiation [Stepanov 1986, Zollinger 1987]:



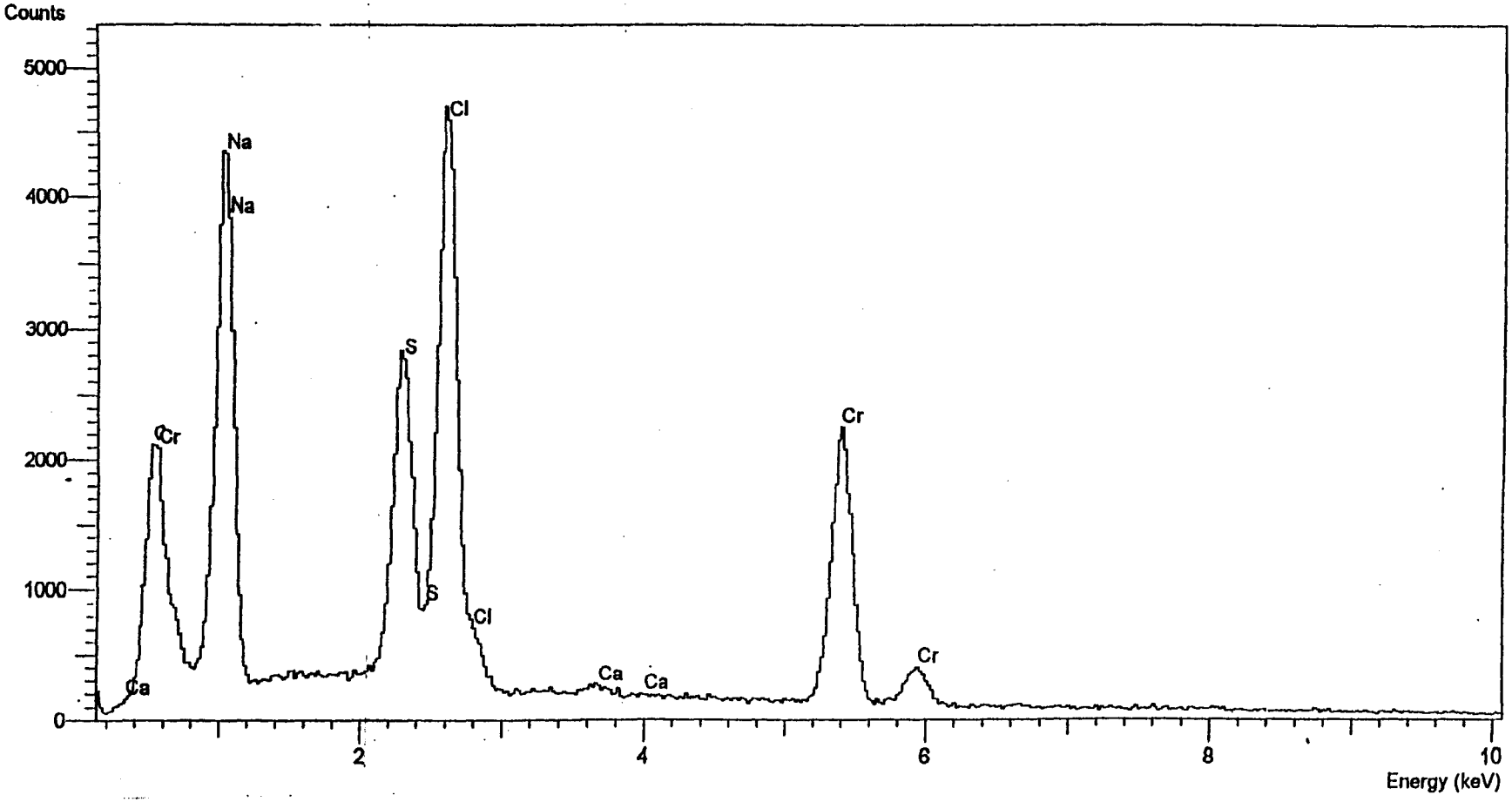


Figure 3.9. EDAX picture of dye C. I. Acid Blue 158.

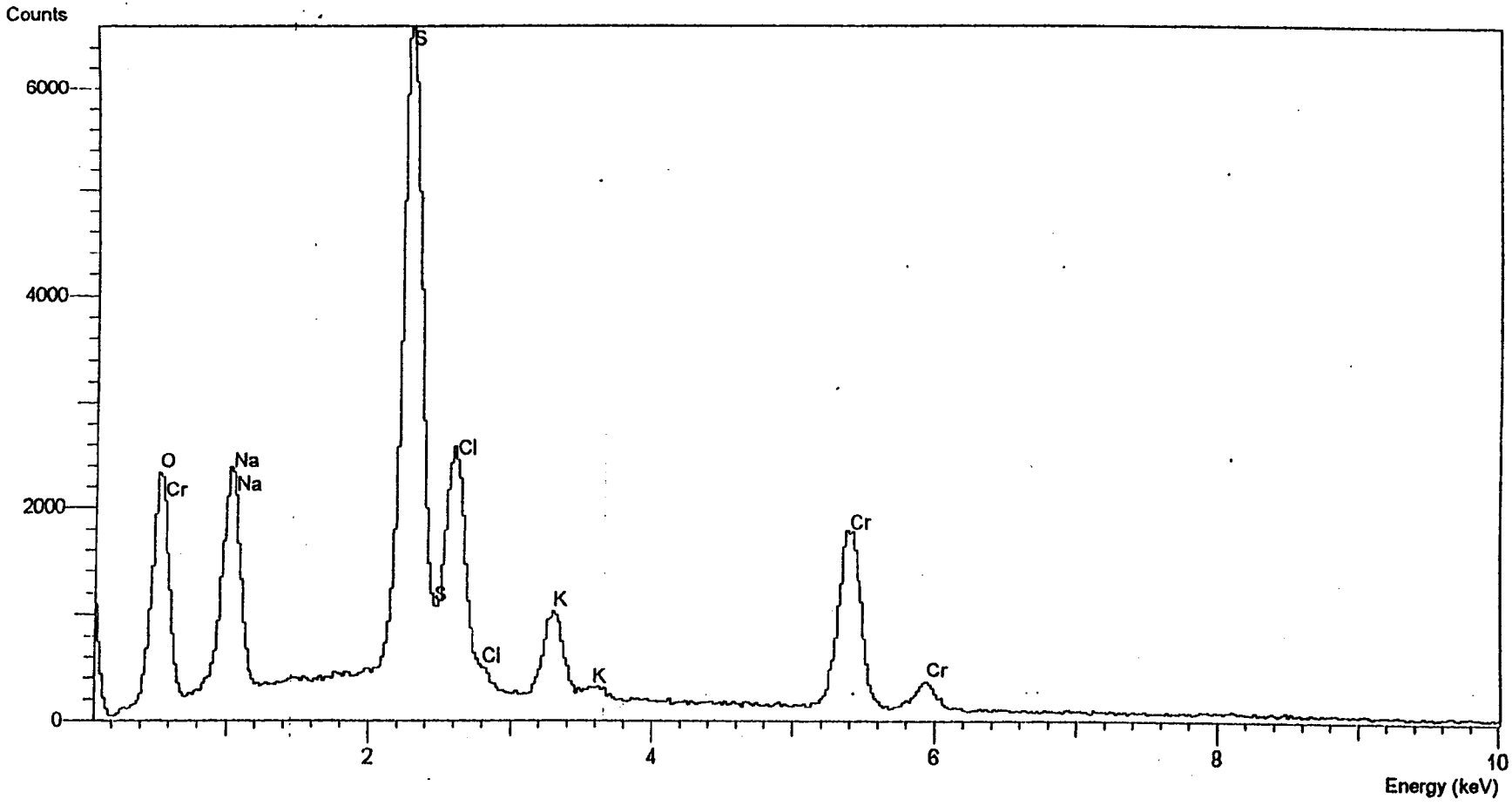


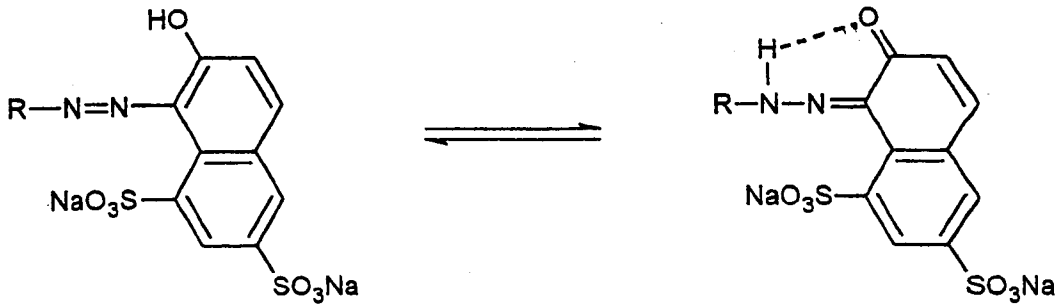
Figure 3.10. EDAX picture of dye C. I. Acid Yellow 99.

Moreover the chromium-complex azo dyes are generally known to have low stability to pH changes over a wide range, and they are relatively stable only in strong acidic aqueous solutions [Stepanov 1986, Zollinger 1987]. It has been also reported that metal in the structure of dyes can play a specific role in the dye decolourization process and can accelerate the photo-decomposition of the dyes in alkaline solutions. This conclusion has been reached in several studies [Oakes & Welch 1997, Oakes & Gratton 1997]. These involved the study of azo dye degradation depending on ability to form dye-metal complexes with copper, and other transition metals such as manganese and cobalt, in alkaline aqueous solutions in the presence and absence of different oxidants such as chlorine, sodium hypochlorite, hydrogen peroxide and others.

In the photochemical process in the current research there might be a similar dependence of azo dye lightfastness on the pH of the dye solutions. Indeed, from the results obtained (data in Figure 3.8) at given experimental conditions of UV treatment catalytic activity of chromium varies with pH resulting in an increase in the decolourization rate of metal-containing dyes with increasing pH.

The dyes C. I. Acid Red 73 and C. I. Direct Red 23 can exist in either one of two tautomeric forms, an azo or hydrazone form, or their equilibrium mixture. The tautomerism is attributed to the presence of hydroxyl groups conjugated with azo group [Poray-Koshits 1967].

The hydroxyl proton of *ortho*-substituted azo dyes is labile and is capable of bonding with a β-nitrogen atom of a nitrogen – nitrogen double bond in the azo compound to form a hydrazone tautomer as follows:

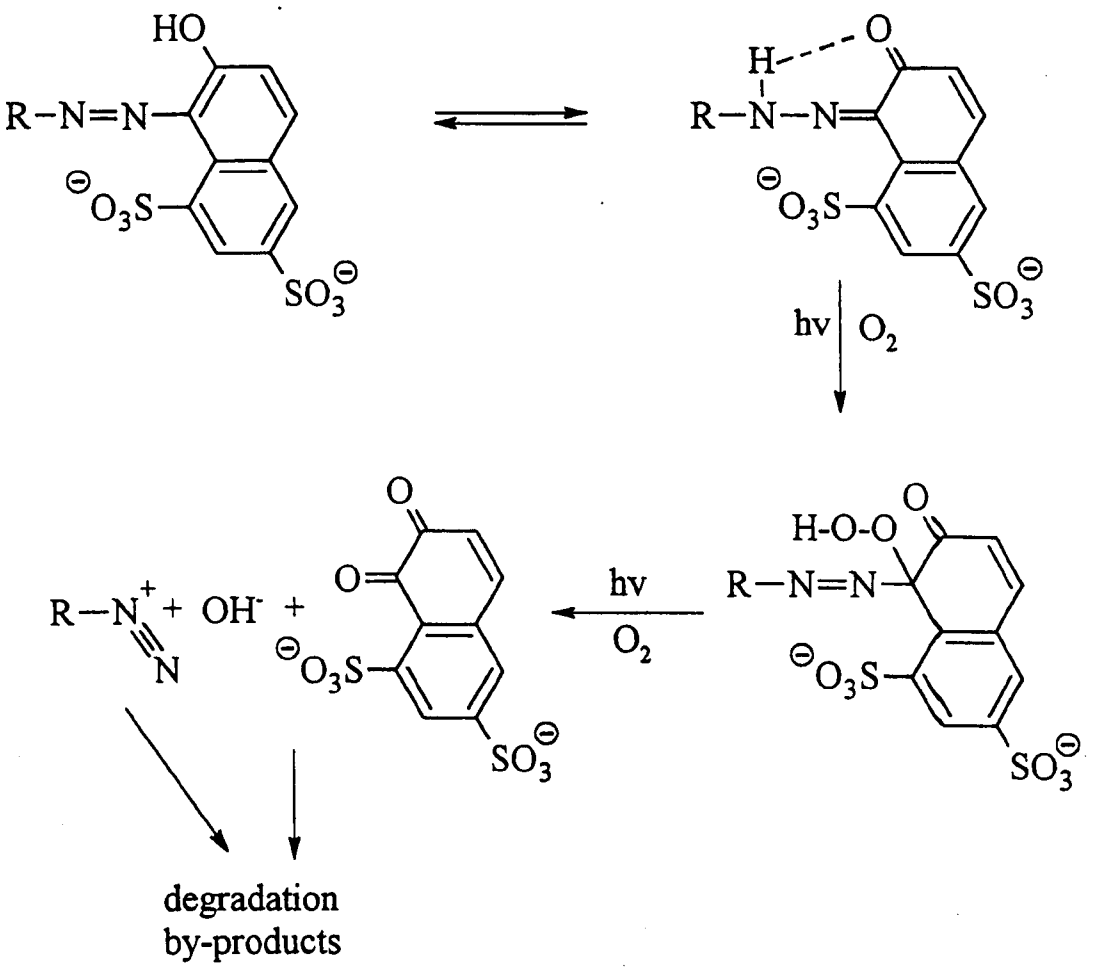


azo tautomer hydrazone tautomer

Although *ortho*-hydroxy substitutions usually tend to stabilize azo dyes because of strong intramolecular hydrogen bond formation (as shown in above scheme for C. I. Acid Red 73) it is generally agreed that the photochemical reactions of *o*-arylazonaphthol dyes in solutions are possible only with the hydrazone forms of the dyes since the hydrazone forms are much less photostable to UV irradiation. Tautomeric equilibrium of the azo dyes differs significantly shifting either to the hydrazone or the azo form depending on many factors (solvent, temperature and others) including pH value.

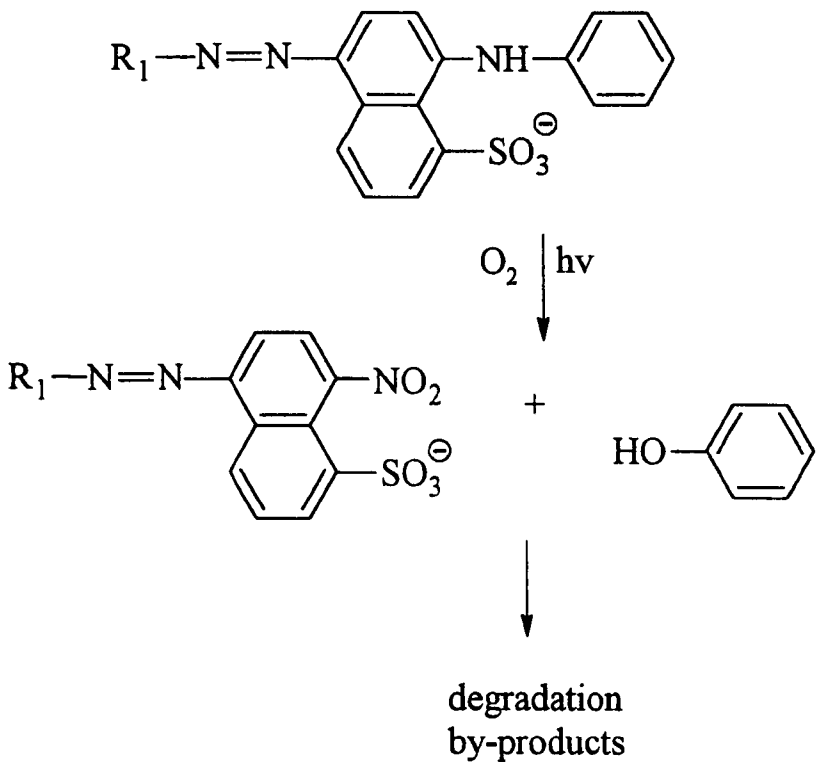
In acidic solution the azo chromogen is protonated at one of the nitrogen atoms forming the dye structure with a positive charge on a nitrogen atom of the azo linkage. Under alkaline conditions, the *o*- substituted arylazonaphthol dyes ionise to form the common anion in which there is electron delocalisation over the whole dye molecule. Earlier conclusions

[Zollinger 1987, Oakes et al 1998] indicate that it has predominantly azo character (i. e. electron density is concentrated upon the hydroxyl group O atom). The destruction of these dyes at an initial stage involves a nucleophilic addition reaction of the oxidant molecule (dissolved oxygen in solution) to a carbon – nitrogen N-C bond coupled to an azo linkage with the hydrazone tautomer of *o*-arylazonaphthol dyes, that can be illustrated in scheme below:



where: R – phenol or azobenzene substitutions in the dye structure.

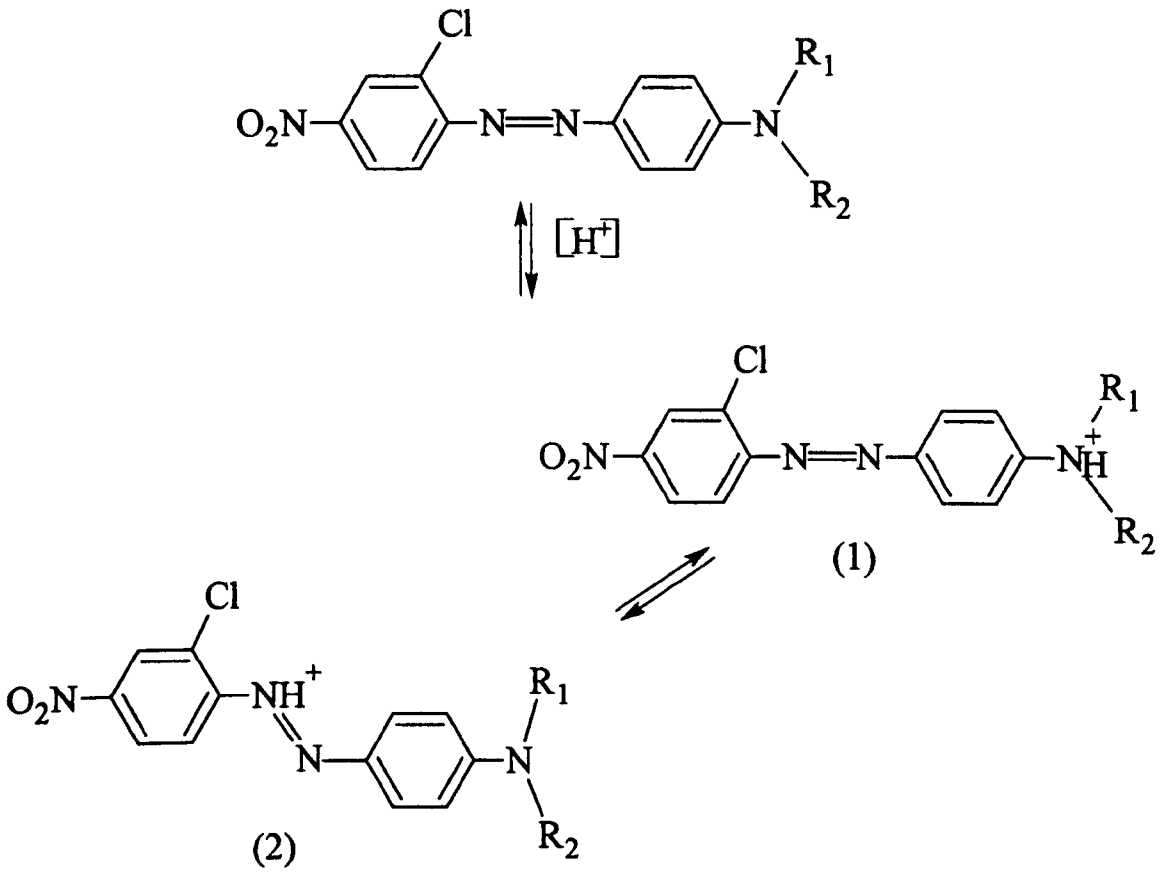
The dyes C. I. Acid Blue 92, and C. I. Acid Blue 113 are not capable of tautomeric transformations, but the behaviour of these dyes in photochemical reactions in alkaline and acidic solutions are similar to those described above. At the given pH values the dyes are not dissociated, the pKa of the dyes found in the literature is about 12 [Haag & Mill 1987, Kritchevsky 1986], and the high reactivity of these dyes is probably attributed to oxidation of the diarylamino nitrogen:



where: R_1 – phenylazonaphthalene (C. I. Acid Blue 113)

or naphthol (C. I. Acid Blue 92).

C. I. Basic Red 18 was already indicated to be significantly less sensitive to photo-induced reactions with dissolved oxygen in both alkaline and acidic solutions than other dyes. The photo-decolourization rate of this dye slows down in alkaline medium but increases at lower pH values. This can be explained by a change in the molecule the structure being subjected to ammonium-azonium tautomerism. In acidic solutions this dye, as any aminoazobenzene dye, is known to be protonated at the amino group as well as at the β-nitrogen of the azo linkage forming ammonium (1) and azonium tautomers (2) according to scheme:



where : R_1 – ethyl group,

R_2 – trimethylamine

Azonium tautomers of aminoazobenzene dyes similar to the hydrazone tautomers of *o*-arylazonaphthol dyes are unstable to UV irradiation. In both cases, the β-nitrogen of the azo linkage has the labile hydrogen atom and the decolourization rate increases. On the whole, the presence of the labile hydrogen atom probably makes dye molecules highly susceptible to oxidation action by active oxygen species.

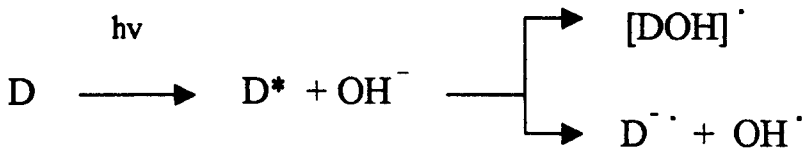
It should be also taken into consideration that the reactive species responsible for initiating degradation of the dyes (under UV irradiation) qualitatively and quantitatively change with pH of dye solutions [Foote et al 1995]. It was already known prior to the 70s of this century [Griffiths 1971, Foote 1968, Terenin 1967]. Later investigations are consistent with earlier conclusions [Kritchevsky 1986, Martin & Logsdon 1987, Kuramoto & Kitao 1982, Okada et al 1998] that the dyes in the presence of oxygen in solutions on UV irradiation can form active species of an ion - radical nature:

$$D \xrightarrow{hv} D^* + O_2 \longrightarrow [D^{+\bullet} \ldots\ldots O_2^{-\bullet}]$$

The rate constant of the excited dye molecule interaction with molecular oxygen in neutral aqueous solutions to form oxy-radicals was reported to be $10^8 - 10^9$ $dm^3\ mol^{-1}\ s^{-1}$ [Terernin 1967]. However, in acidic and alkaline media disproportion of the reactive species results in formation of other active oxidants. In acidic solutions oxy-radicals are capable of directly converting to perhydroxyl radical, a powerful oxidizing agent towards organic substances including dyes.

$$O_2^{-\bullet} + H^+ \longrightarrow HO_2^{\bullet}, \quad k = 2.1 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$$ [Buxton et al 1988].

In alkaline solutions the photo-active dye is believed to abstract an electron from the hydroxyl anion to form reactive hydroxyl radicals and dye radicals [Bamford & Dewar 1949, Terenin 1967, Allen 1994]:



According to this scheme various reactive species (dye, oxy-, hydroxyl and peroxyl radicals, hydroperoxides also) may simultaneously be present in irradiated dye solution, all of which can then induce further oxidative breakdown of the dye.

Thus, pH is an important factor in photo-degradation process for azo dyes which depends not only on chemical structure and physical nature of dyes themselves, but also on the formation and presence of reactive oxidizers in irradiated solution.

3.3. Photo-degradation of azo dyes by UV irradiation in the presence of hydrogen peroxide.

In previous section it was shown that if only dissolved oxygen is present in the dye solutions irradiated azo dyes do not significantly fade or a pronounced effect on colour removal requires long UV irradiation time. To make the photo-fading process of the dyes more effective the use of combined action of UV light and hydrogen peroxide seems to be preferably. It is already indisputable that upon photolysis hydrogen peroxide generates hydroxyl radicals, a very powerful oxidant, the oxidative potential of which is close to that of fluorine. Hydroxyl radicals are able to react with many inorganic and organic compounds with high rate constants $10^{10} - 10^{11}$ dm^3 mol^{-1} s^{-1} [Buxton et al 1988].

In this study using UV/ hydrogen peroxide treatment the influence of different parameters, initial dye and hydrogen peroxide concentrations, temperature and pH, on decolourization rates of azo dyes in aqueous solutions were investigated to achieve an optimal rate of colour removal which can further find practical application for coloured waste water treatment.

During experiments the dye stock solutions of 1 g/dm^3 concentration were diluted with aliquots of distilled water to produce 1 dm^3 of dye solution at the tested concentration. The dye solutions of tested concentration were introduced into the laboratory irradiation apparatus. Then an amount of 30 % hydrogen peroxide as required was immediately added and reaction mixture containing dye and hydrogen peroxide was irradiated by UV light. In each case other parameters, concentration, temperature and pH, were kept constant if it is not mentioned otherwise. At the start of the experiment and at the time intervals samples

were withdrawn from the irradiation apparatus, cooled to room temperature and analysed to determine the residual dye concentration and change in dye absorption spectra.

To determine the role of dark reactions between hydrogen peroxide and dye control experiments were carried out keeping test solutions, containing dye and hydrogen peroxide concentrations equal to those of UV- irradiated samples, in the dark for 180 minutes. It was found for all azo dyes that hydrogen peroxide alone does not cause decolourization of the dyes over this period at pH 7.0.

3.3.1. Effect of initial hydrogen peroxide concentrations on dye photo-fading rate.

In these experiments the dye solutions at a concentration of 25 mg/dm^3 were irradiated by UV light at a temperature of 25°C and pH 7.0 and different initial hydrogen peroxide concentrations. Initial hydrogen peroxide concentrations $[H_2O_2]_o$ were set to give molar proportion between $[H_2O_2]_o$/ $[Dye]_o$ in the range 25/1 – 500/1.

The results on colour removal rate as a function of initial hydrogen peroxide concentration are given in Table 3. 6. (for C. I. Acid Blue 92 as an example). The colour removal rate of dye increases in the presence of hydrogen peroxide in comparison with its direct photolysis and also increases with the increase in H_2O_2 concentrations. However, as can be seen from data in Table 3. 6. decolourization rate of the dye increases at first if the initial hydrogen peroxide concentration increases to give molar ratio between $[H_2O_2]_o$ / $[Dye]_o$ of 200 / 1. After attaining this "optimum" value the dye decolourization rate does not further increase.

Table 3.6. Effect of initial hydrogen peroxide concentration on decolourization rate of azo dye C. I. Acid Blue 92 by UV irradiation (at conditions: 25°C and pH 7.0) .

Initial dye concentration $[Dye]_o$, mg/dm^3	Initial H_2O_2 concentration $[H_2O_2]_o$, mg/dm^3	Molar ratio $[H_2O_2]/[Dye]$ at $t = 0$	Time of UV irradiation, (t) min.	Ct/Co, λ =580 nm	k_{obs}, min^{-1}
25.0	650.0	500	0	1.0	0.187
			5	0.387	
			10	0.159	
			15	0.06	
			20	0.025	
			25	0.009	
25.0	325.0	250	0	1.0	0.216
			5	0.341	
			10	0.114	
			15	0.038	
			20	0.014	
25.0	265.0	200	0	1.0	0.228
			5	0.324	
			10	0.105	
			15	0.032	
			20	0.008	
25.0	200.0	150	0	1.0	0.211
			5	0.346	
			10	0.117	
			15	0.040	
			20	0.014	
25.0	155.0	120	0	1.0	0.186
			5	0.392	
			10	0.166	
			15	0.062	
			20	0.022	
			25	0.01	

Table 3.6 (continued)

25.0	130.0	100	0	1.0	0.176
			5	0.428	
			10	0.186	
			15	0.071	
			20	0.026	
			25	0.019	
25.0	90.0	70	0	1.0	0.151
			5	0.46	
			10	0.224	
			15	0.097	
			20	0.051	
			25	0.028	
			30	0.009	
25.0	65.0	50	0	1.0	0.094
			5	0.562	
			10	0.417	
			15	0.263	
			20	0.164	
			25	0.08	
			30	0.052	
			35	0.027	
			40	0.018	
25.0	32.5	25	0	1.0	0.056
			5	0.785	
			10	0.646	
			15	0.513	
			20	0.407	
			30	0.288	
			40	0.149	
			50	0.080	
			60	0.058	
			70	0.032	

From the results given in Table 3.6 semilogarithmic plots of C*t*/C*o* values (where C*o* and C*t* are the dye concentrations in solution before irradiation and at time *t* of irradiation) against irradiation time produce a straight line for each molar ratio $[H_2O_2]_o$/ $[Dye]_o$ used as shown in Figure 3.11. Dye decolourization reactions under given UV irradiation experimental conditions and at $[H_2O_2]_o \geq [D]_o$ were estimated to be first order with respect to dye concentration and can be described by the equation:

$$\ln (Ct/Co) = - k_{obs}\, t,$$

where: k_{obs} based on first order kinetics is given in units of min^{-1} .

The kinetic rate constants of dye decolourization were calculated for each case of dye-hydrogen peroxide combination (data on k_{obs} are present in Table 3.6). In every experiment, the hydrogen peroxide concentration was high enough not to be represent a limiting factor. A large excess of H_2O_2 in comparison with dye concentrations was added and $[H_2O_2]$ could be considered to have a constant value during the dye decolourization time.

It might be expected that the rate of dye decolourization increases with increasing H_2O_2 concentration. However, the results on the relationship between the initial decolourization rate of dye and $[H_2O_2]o$ indicate there is a threshold value for H_2O_2 amount added to the irradiated solution above which decolourization rate of dyes is inhibited by further increase of H_2O_2 concentration as illustrated in Figure 3.12. Indeed, while the hydrogen peroxide concentration increases to 265 mg/dm^3 (to molar ratio $[H_2O_2]$/[Dye]: 200/1) the colour removal rate increases for the same photoreaction time. But for H_2O_2 initial concentrations exceeding 265 mg/dm^3 the colour removal rate decreases below that for 265 mg/dm^3.

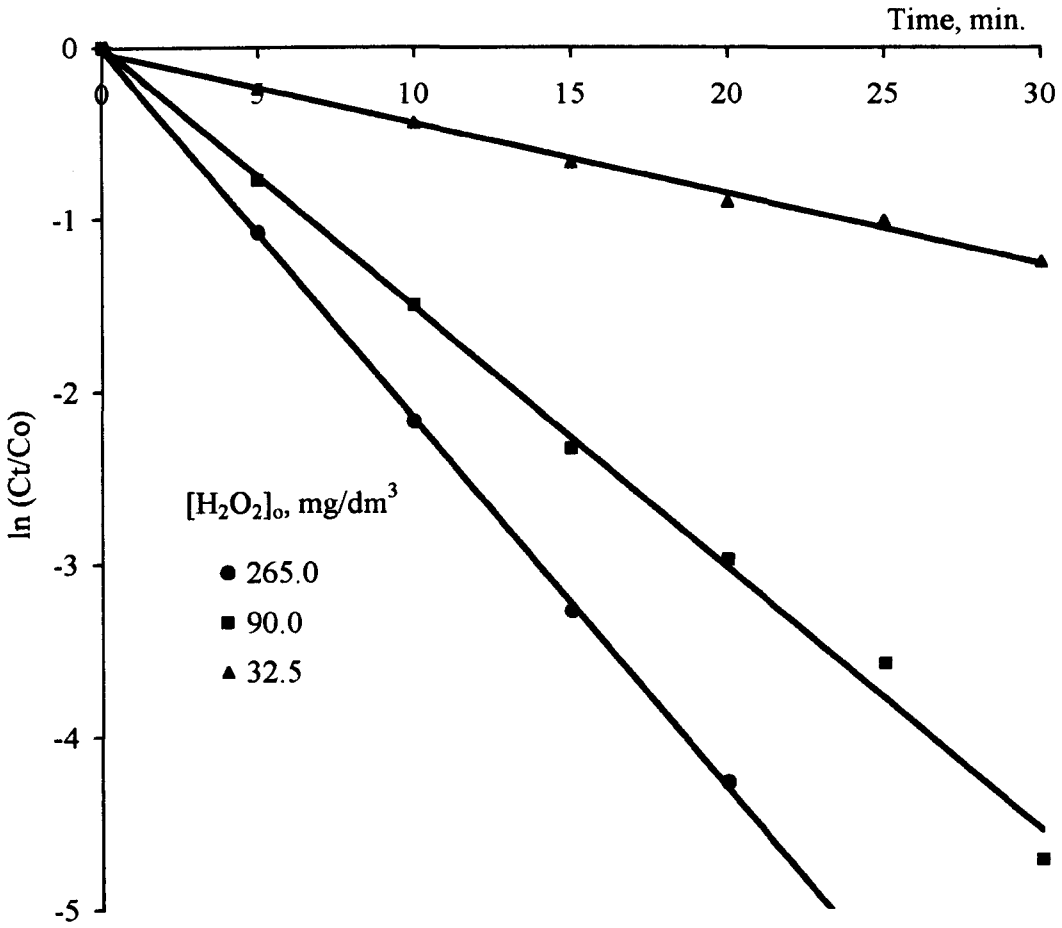


Figure 3.11. Plots of ln Ct/Co for C. I. Acid Blue 92 against irradiation time as a function of initial hydrogen peroxide concentrations.

UV treatment conditions: $[Dye]_o$ 25 mg/dm^3, 25°C and pH 7.0.

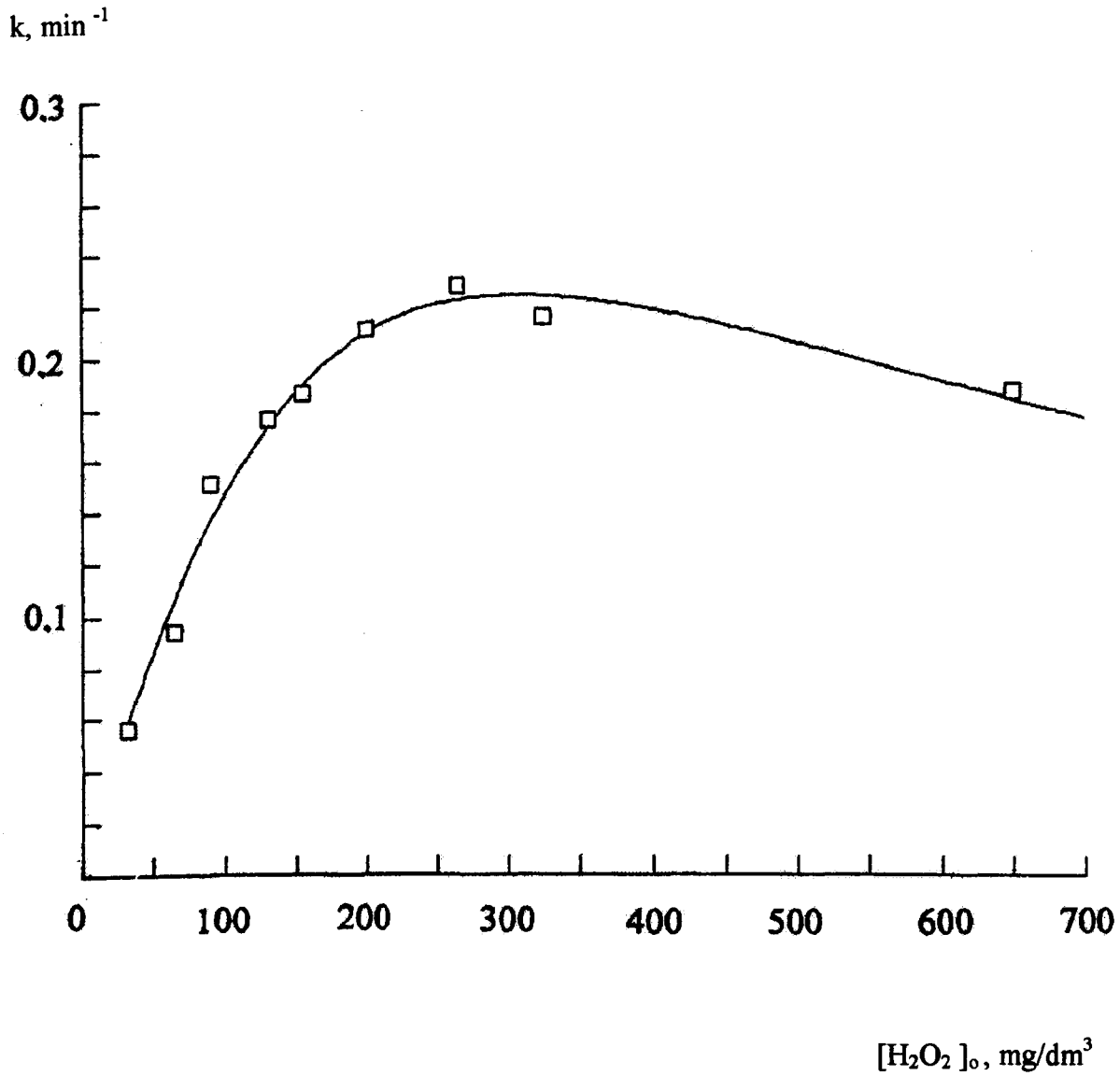


Figure 3.12. Effect of the initial hydrogen peroxide concentrations on the photo-decolourization rate constant of C. I. Acid Blue 92.

UV treatment conditions: $[Dye]_o$ 25 mg/dm^3, 25°C and pH 7.0.

It is due to hydrogen peroxide itself at relative high concentrations acting as a scavenger of hydroxyl radicals according to the equations [Buxton et al 1988]:

$$OH^{\cdot} + H_2O_2 \longrightarrow HO_2^{\cdot} + H_2O, \qquad k = 2.7 \times 10^7\ dm^3\ mol^{-1}\ s^{-1}$$

$$OH^{\cdot} + HO_2^{\cdot} \longrightarrow O_2^{\cdot} + H_2O, \qquad k = 7.1 \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$$

Moreover, $OH^{\cdot}$ radical-radical recombination to hydrogen peroxide could occur if high concentrations of H_2O_2 is used:

$$OH^{\cdot} + OH^{\cdot} \longrightarrow H_2O_2, \qquad k = 5.0 \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$$

Thus, the competition for hydroxyl radicals by hydrogen peroxide and dye causes lower colour removal rates when the hydrogen peroxide concentration exceeds the optimal value. As seen in Figure 3.12 this effect becomes more pronounced at hydrogen peroxide concentrations above $3 - 4 \times 10^{-2}$ mol/dm^3. However, it should be noted that the "optimal value" of hydrogen peroxide for photochemical oxidation of dyes is a very relative value depending on dye class and chemical structure and dye concentration in the dyebath. This value must be determined in each specific case of the dyeing process depending on types of fibres being coloured and the dye used.

For all further studies approximately the stoichiometric mole ratio of hydrogen peroxide, determined from balanced chemical equations representing in theory the photochemical decolourization of the azo dyes studied into their theoretical breakdown products, equal to 70/1 was chosen. This mole ratio corresponds to concentrations in solutions of 90 mg/dm^3 $[H_2O_2]_o$ and of 25 mg/dm^3 $[Dye]_o$.

3.3.2. Photo-degradation of azo dyes studied in UV/H_2O_2 system.

To study the photo-decomposition of seven azo dyes by UV irradiation in the presence of hydrogen peroxide as a function of temperature or pH experiments were carried out using initial dye and hydrogen peroxide concentrations of 25 and 90 mg/dm^3, respectively. One of the parameters either temperature 25°C or pH 7.0 was kept constant. An initial hydrogen peroxide concentration of 90 mg/dm^3 was chosen as "optimum value" for dye decolourization from previous experiments.

Data on the photochemical decolourization of all dyes at pH 7.0 and temperature 25°C are present in Tables 3.7 – 3.13. In general, from these data for all dyes tested the combined action of UV irradiation and hydrogen peroxide induced a fast decrease in the colour of test solutions, greatly increasing dye decolourization rate in comparison with their direct photolysis.

Complete colour removal from solutions was practically achieved after 30 minutes of UV/hydrogen peroxide treatment for all dyes tested. In contrast, after 30 minutes treatment time with the action of UV light alone (at the same treatment conditions) no more than 10 % of the dyes were removed from solutions (data in Table 3.1), despite the fact that the disazo dyes were generally decolourized much more slowly then monoazo dyes giving no more than 4 % colour removal.

Table 3.7. Colour removal kinetics of C. I. Acid Blue 92 in UV/ H_2O_2 system.
($[Dye]_o$ =25 mg/dm^3, $[H_2O_2]_o$=90 mg/dm^3).

$[Dye]_o$, mg/dm^3	T, °C	pH	Time of UV irradiation (t), min.	Ct/Co	Colour removal, %
25.0	25	7.0	0	1.0	0.0
			5	0.457	54.3
			10	0.224	77.6
			15	0.097	90.3
			20	0.051	94.9
			25	0.028	97.2
			30	0.009	99.8
25.0	60	7.0	0	1.0	0.0
			5	0.384	61.6
			10	0.131	86.9
			15	0.040	96.0
			20	0.014	98.6
			25	0.008	99.2
			30	0.002	99.8
25.0	25	4.0	0	1.0	0.0
			5	0.355	64.5
			10	0.119	88.1
			15	0.042	95.8
			20	0.011	98.9
			25	0.006	99.4
25.0	25	10.5	0	1.0	0.0
			5	0.182	81.8
			10	0.028	97.2
			15	0.005	99.5

Table 3.8. Colour removal kinetics of C. I. Acid Red 73 in UV/ H_2O_2 system.
($[Dye]_o$ =25 mg/dm^3, $[H_2O_2]_o$=90 mg/dm^3).

$[Dye]_o$, mg/dm^3	T,°C	pH	Time of UV irradiation, (t) min.	Ct/Co	Colour removal, %
25.0	25	7.0	0	1.0	0.0
			5	0.449	55.1
			10	0.288	71.2
			15	0.166	83.4
			20	0.092	90.8
			25	0.044	95.6
			30	0.018	98.2
25.0	60	7.0	0	1.0	0.0
			5	0.379	62.1
			10	0.168	83.2
			15	0.072	92.8
			20	0.025	97.5
			25	0.016	98.4
			30	0.004	99.6
25.0	25	4.0	0	1.0	0.0
			5	0.442	55.8
			10	0.226	77.4
			15	0.006	89.4
			20	0.048	95.2
			25	0.022	97.8
			30	0.009	99.1
25.0	25	10.5	0	1.0	0.0
			5	0.272	72.8
			10	0.062	93.8
			15	0.018	98.3

Table 3.9. Colour removal kinetics of C. I. Acid Blue 158 in UV/ H_2O_2 system. ($[Dye]_o$ =25 mg/dm^3, $[H_2O_2]_o$=90 mg/dm^3).

$[Dye]_o$, mg/dm^3	T,°C	pH	Time of UV irradiation, (t) min.	Ct/Co	Colour removal, %
25.0	25	7.0	0	1,0	0.0
			5	0.709	21.0
			10	0.428	57.2
			15	0.281	71.9
			20	0.201	79.9
			25	0.077	92.3
			30	0.039	96.1
25.0	60	7.0	0	1.0	0.0
			5	0.493	50.7
			10	0.246	75.4
			15	0.119	88.1
			20	0.053	94.7
			25	0.028	97.2
			30	0.016	98.4
25.0	25	4.0	0	1.0	0.0
			5	0.312	68.8
			10	0.091	90.9
			15	0.022	97.8
			20	0.008	99.2
25.0	25	10.5	0	1.0	0.0
			5	0.107	89.3
			10	0.018	98.2
			15	0.0	100.0

Table 3.10. Colour removal kinetics of C. I. Acid Blue 113 in UV/ H_2O_2 system. ($[Dye]_o$ =25 mg/dm^3, $[H_2O_2]_o$=90 mg/dm^3).

$[Dye]_o$, mg/dm^3	T,°C	pH	Time of UV irradiation, (t) min.	Ct/Co	Colour removal, %
25.0	25	7.0	0	1.0	0.0
			5	0.683	31.7
			10	0.457	54.3
			15	0.313	68.7
			20	0.214	78.6
			25	0.148	85.2
			30	0.092	90.8
25.0	60	7.0	0	1.0	0.0
			5	0.621	37.9
			10	0.411	58.9
			15	0.264	73.6
			20	0.167	83.3
			25	0.106	89.4
			30	0.059	94.1
25.0	25	4.0	0	1.0	0.0
			5	0.651	34.9
			10	0.421	57.9
			15	0.280	72.0
			20	0.183	81.7
			25	0.121	87.9
			30	0.073	92.7
25.0	25	10.5	0	1.0	0.0
			5	0.386	61.4
			10	0.158	84.2
			15	0.074	92.6
			20	0.036	96.4
			25	0.005	99.5

Table 3.11. Colour removal kinetics of C. I. Acid Yellow 99 in UV/ H_2O_2 system. ($[Dye]_o$ =25 mg/dm^3, $[H_2O_2]_o$=90 mg/dm^3).

$[Dye]_o$, mg/dm^3	T,°C	pH	Time of UV irradiation, (*t*) min.	C*t*/C*o*	Colour removal, %
25.0	25	7.0	0	1.0	0.0
			5	0.636	36.4
			10	0.387	61.3
			15	0.224	77.6
			20	0.118	88.2
			25	0.149	85.1
			30	0.051	94.5
25.0	60	7.0	0	1.0	0.0
			5	0.451	54.9
			10	0.208	79.2
			15	0.097	90.3
			20	0.049	95.1
			25	0.019	98.1
			30	0.012	98.8
25.0	25	4.0	0	1.0	0.0
			5	0.217	78.3
			10	0.048	95.2
			15	0.011	98.9
			20	0.001	99.9
25.0	25	10.5	0	1.0	0.0
			5	0.146	85.4
			10	0.024	97.6
			15	0.001	99.9

Table 3.12. Colour removal kinetics of C. I. Basic Red 18 in UV/ H_2O_2 system. ($[Dye]_o$ =25 mg/dm^3, $[H_2O_2]_o$=90 mg/dm^3).

$([Dye]_o$, mg/dm^3	T,°C	pH	Time of UV irradiation, (t) min.	Ct/Co	Colour removal, %
25.0	25	7.0	0	1.0	0.0
			5	0.505	49.5
			10	0.263	73.7
			15	0.138	86.2
			20	0.063	93.7
			25	0.035	96.5
			30	0.016	98.4
25.0	60	7.0	0	1.0	0.0
			5	0.449	55.1
			10	0.193	80.7
			15	0.091	90.9
			20	0.035	96.5
			25	0.017	98.3
			30	0.009	99.1
25.0	25	4.0	0	1.0	0.0
			5	0.284	71.6
			10	0.080	92.0
			15	0.026	97.4
			20	0.005	99.5
25.0	25	10.5	0	1.0	0.0
			5	0.176	82.4
			10	0.038	96.2
			15	0.011	98.9
			20	0.001	99.9

Table 3.13. Colour removal kinetics of C. I. Direct Red 23 in UV/ H_2O_2 system. ($[Dye]_o$ =25 mg/dm^3, $[H_2O_2]_o$=90 mg/dm^3).

$[Dye]_o$, mg/dm^3	T,°C	pH	Time of UV irradiation, (*t*) min.	C*t*/C*o*	Colour removal, %
25.0	25	7.0	0	1.0	0.0
			5	0.536	46.4
			10	0.275	72.5
			15	0.148	85.2
			20	0.081	91.9
			25	0.040	96.0
			30	0.019	98.1
25.0	60	7.0	0	1.0	0.0
			5	0.482	51.8
			10	0.229	77.1
			15	0.111	88.9
			20	0.053	94.7
			25	0.026	97.4
			30	0.009	99.1
25.0	25	4.0	0	1.0	0.0
			5	0.491	50.9
			10	0.243	75.7
			15	0.117	88.3
			20	0.057	94.3
			25	0.041	95.9
			30	0.010	99.0
25.0	25	10.5	0	1.0	0.0
			5	0.219	78.1
			10	0.057	94.3
			15	0.018	98.2
			20	0.001	99.9

However, the relative order in the decrease of the photo-degradation rate of the dyes by UV/ hydrogen peroxide treatment was not the same as that observed by direct photolysis. (given in page 75). For example, C. I. Acid Red 73, which was decolourized more slowly than other dyes by direct photolysis and colour removal was only 3.8 % over 120 min of UV irradiation alone, was decolourized almost completely (98.2 % of colour removal) after 30 minutes treatment by UV irradiation in the presence of hydrogen peroxide. In contrast, C. I. Acid blue 113 was rather more photochemically stable than other dyes and had lost only 90.8 % of colour after the same photo-chemical reaction time.

All azo dyes used in the study strongly absorb light in both the visible and UV ranges (see spectral characteristic for dyes in chapter 2). The absorption spectra of these dyes are generally characterised by the two or three main bands and show that, in addition to their high absorption in the visible region, they also have a strong absorption in the near and far UV light. Hydrogen peroxide solution absorbs only in the UV region not absorbing visible light at all. Figure 3.13 gives a comparison of the absorption spectra of 25 mg/dm^3 dye C. I. Acid Red 73, of 90 mg/dm^3 hydrogen peroxide and their mixture at the same concentrations.

Although the dye absorbs UV light more strongly then hydrogen peroxide, the UV irradiation of the dye and hydrogen peroxide mixture results in a faster decolourization of the dye solutions than UV irradiation alone at the same treatment conditions, reagent concentrations, temperature and pH. For instance Figure 3.14 shows the results obtained with C. I. Acid Red 73. Similar results were obtained for all dyes studied when compared with their direct photolysis.

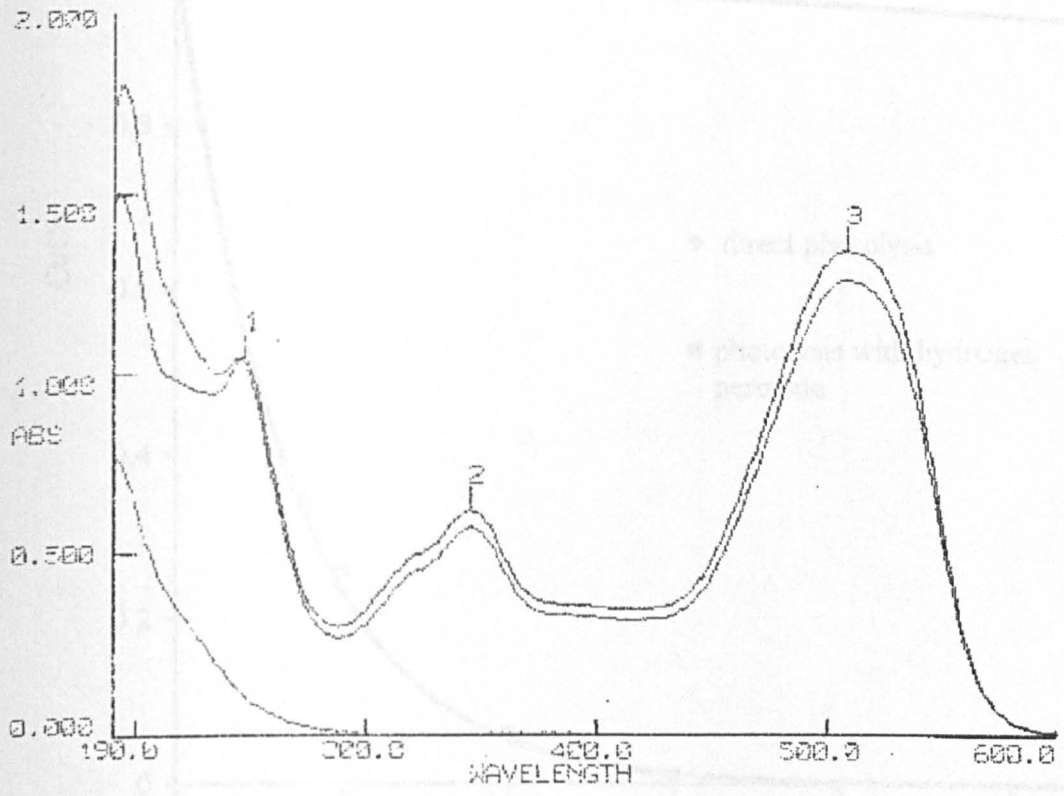


Figure 3.13. UV-Visible absorption spectra of solutions: (a) 25 mg/dm^3 C. I. Acid Red 73, (b) 90 mg/dm^3 hydrogen peroxide, (c) the mixture of 25 mg/dm^3 dye and 90 mg/dm^3 hydrogen peroxide in distilled water.

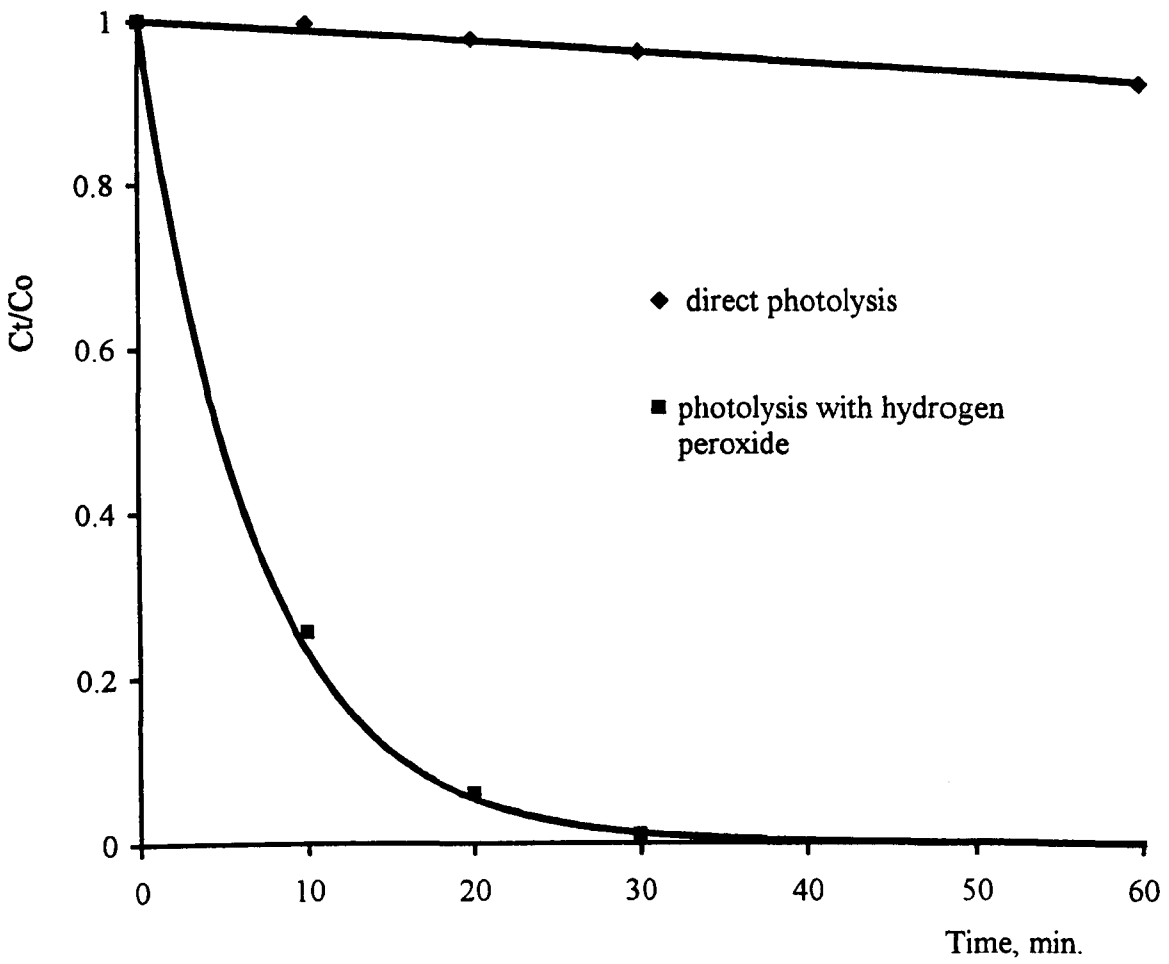


Figure 3.14. Decolourization kinetics of C. I. Acid Red 73 by UV irradiation without and with hydrogen peroxide.

UV treatment: $[Dye]_o$ 25 mg/dm^3, $[H_2O_2]_o$ 90 mg/dm^3, 25°C, pH 7.0.

This effect, observed by irradiating in the presence of hydrogen peroxide, is due to the generation of very reactive hydroxyl radicals by hydrogen peroxide UV photolysis. The quantum yield of hydroxyl radicals is much more than dye radicals at 254 nm wavelength [Venkataraman 1971, Kritchevsky 1987] as described in Chapter 2. Hydroxyl radicals are very reactive oxidising agents and rapidly attack the dye molecules probably initiating fast oxidation of dye to their colourless intermediates:

$$H_2O_2 + hv \longrightarrow OH^{\cdot} + OH^{\cdot},$$

$$\text{Dye} + OH^{\cdot} \longrightarrow \text{colourless intermediate products.}$$

In these reactions the direct photolysis of the dyes also take place but dye decolourization rates by UV irradiation alone are too low to be significant as shown in Chapter 3.1.

The absorption spectra of dyes and hydrogen peroxide mixtures were recorded after various times of UV irradiation and presented in Figure 3.15 for dyes C. I. Acid Blue 158, C. I. Acid Red 73 and C. I. Acid Yellow 99. The spectra show that the characteristic changes in optical density of dye solutions treated by UV/ hydrogen peroxide are similar to the spectra of the dyes treated by UV radiation alone in both visible and UV regions of spectrum. There was a gradual decrease in the optical density in wide range of the spectrum for all dyes and no formation of new peaks during photo-chemical treatment was observed. Similar results were obtained for all dyes (colour removal data in Tables 3.7 – 3.13).

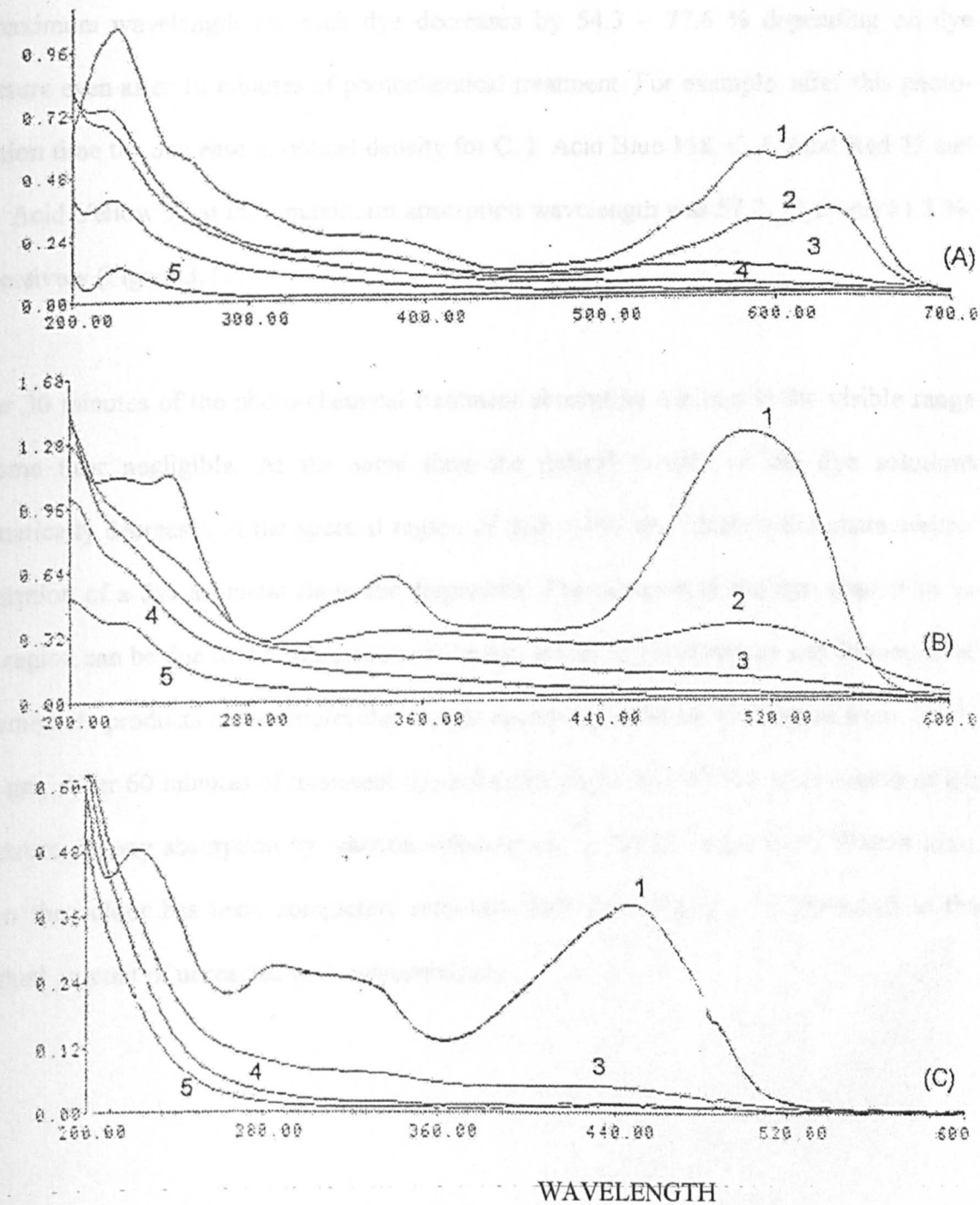


Figure 3.15. UV- Visible absorption spectra of dyes C. I. Acid Blue 158 (A), C. I. Acid Red 73 (B) and C. I. Acid Yellow 99 (C) treated by UV/ H_2O_2.

(1) – initial dye solution with hydrogen peroxide at $t = 0$,

after treatment time: (2) – 10 min., (3) – 20 min., (4) – 30 min., (5) – 60 min.

Treatment conditions: $[Dye]_o$ 25 mg/dm^3, $[H_2O_2]_o$ 90 mg/dm^3, 25°C, pH 7.0.

The spectra analysis also showed that in the visible region of spectrum absorption of dyes at maximum wavelength for each dye decreases by 54.3 – 77.6 % depending on dye structure even after 10 minutes of photochemical treatment. For example, after this photo-reaction time the decrease in optical density for C. I. Acid Blue 158, C. I. Acid Red 73 and C. I. Acid Yellow 99 at their maximum absorption wavelength was 57.2, 71.2 and 61.3 %, respectively (Figure 3.15).

After 30 minutes of the photo-chemical treatment absorption maxima in the visible range become near negligible. At the same time the optical density of the dye solutions dramatically decreases in the spectral region of 280 – 360 nm which is the characteristic absorption of a dye aromatic rings and fragments. The decrease in the dye absorption in this region can be due to decomposition of the azo group in dye structure and formation of intermediate products of low molecular weight absorbing in the far UV region from 190 to 280 nm. After 60 minutes of treatment dye solutions do not absorb in a wide region of the spectrum. Strong absorption by reaction solutions in the far UV after the treatment time, when the colour has been completely removed from dye solutions, is attributed to the residual amount of unreacted hydrogen peroxide.

3.3.3. Effect of temperature on azo dye photo-degradation rate.

To test the effect of temperature on dye photo-degradation rate under UV irradiation in the presence of hydrogen peroxide experiments on the decolourization of azo dyes were carried out at temperatures of 25 and 60°C. Photo-chemical treatment conditions were $[Dye]_o$ 25 mg/dm^3, $[H_2O_2]_o$ 90 mg/dm^3, pH 7.0 and irradiation time 60 minutes.

Experimental results on dye photochemical decolourization at various temperatures (illustrated in Figure 3.16 for C. I. Acid Blue 92) show that colour removal rates for the dye slightly increase with increase of temperature from 25 °C to 60°C. Similar results were obtained for all dyes tested independent of their chemical structure and physico-chemical properties (data are present in Tables 3.7 – 3.13).

This small increase in colour removal rate with increasing temperature is probably due to the well-known fact that photochemically induced reactions have a low activation energy [Cavert & Pitts 1966, Ashton et al 1995]. On the other hand, this increase in dye photo-fading rate could be connected with the instability of hydrogen peroxide solutions strongly depending on change in temperature. It is known that the higher temperature the higher decomposition rate of hydrogen peroxide solutions, both in solutions of UV irradiated and dark conditions [Sharmb 1959].

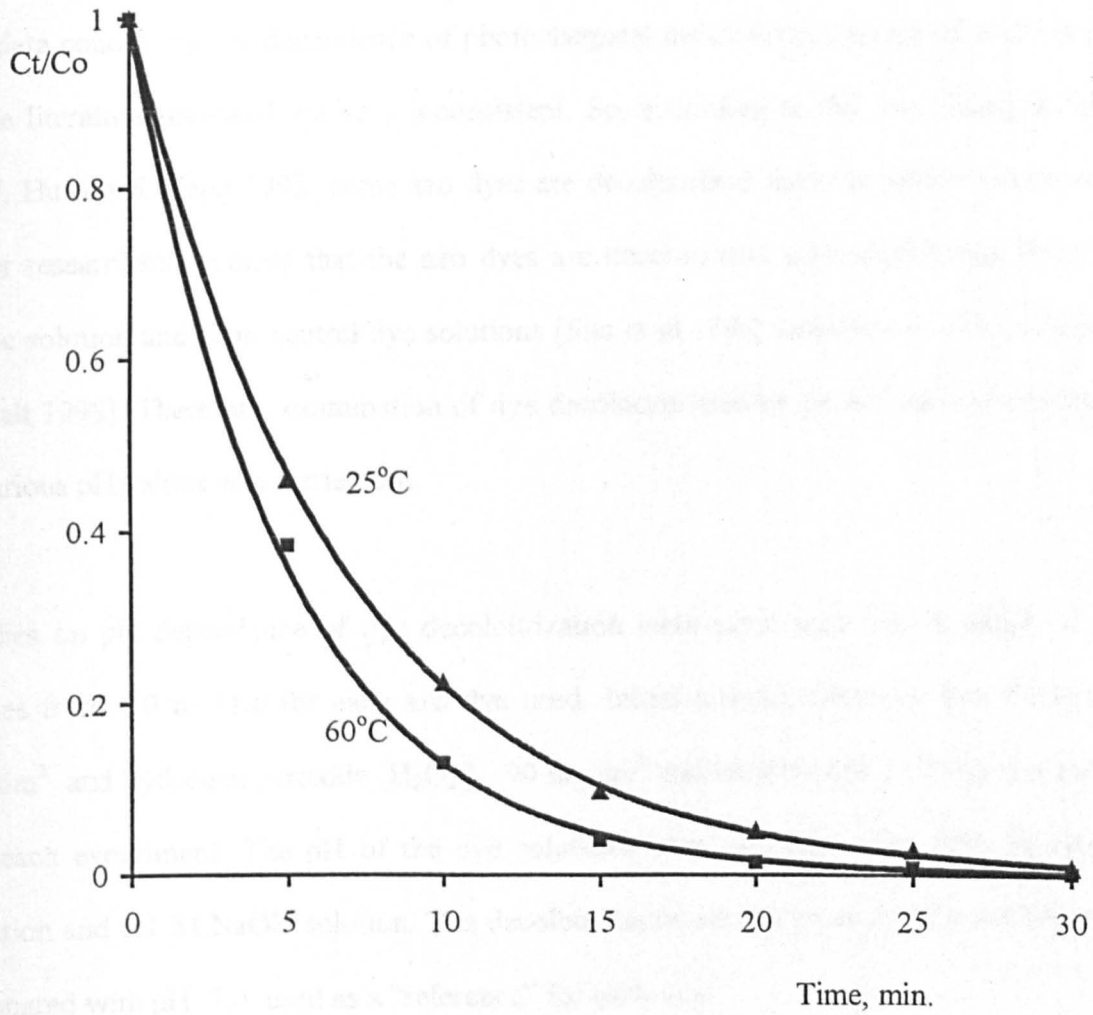


Figure 3.16. Decolourization kinetics of C. I. Acid Blue 92 by UV/H_2O_2 treatment as a function of temperature.

UV treatment $[H_2O_2]_o$ 90 mg/dm^3, $[Dye]_o$ 25 mg/dm^3, pH 7.0.

3.3.4. Effect of pH solution on azo dye photo-degradation rate.

The data concerning the dependence of photochemical decolourization rate of dyes on pH in the literature reviewed are very inconsistent. So, according to the data [Haag & Mill 1987, Hustert & Zepp 1992] some azo dyes are decolourized faster in alkaline solutions. Other researchers reported that the azo dyes are decolourized photochemically faster in acidic solution and/or in neutral dye solutions [Shu et al 1994, Arkchipova 1995, Galindo & Kalt 1998]. Therefore, examination of dye decolourization by photochemical treatment at various pH values was carried out.

Studies on pH dependence of dye decolourization were performed over a range of pH values from 4.0 to 11.0 for each azo dye used. Initial concentrations of dye $[Dye]_o$ 25 mg/dm^3 and hydrogen peroxide $[H_2O_2]_o$ 90 mg/dm^3 and temperature 25°C were constant for each experiment. The pH of the dye solutions were adjusted using 0.05 M H_2SO_4 solution and 0.1 M NaOH solution. The decolourization rate of dyes at different pHs was compared with pH 7.0 used as a "reference" for each dye.

Firstly experiments were done to study pH dependence of possible interactions between azo dyes and hydrogen peroxide without UV irradiation over the same experimental time period as for UV irradiation. It was found that hydrogen peroxide alone does not cause decolourization of the dyes in dark conditions over a wide range of pH except for alkaline solutions. In alkaline dye solutions at pHs from 9.5 to 11.0 all dyes were decolourized by hydrogen peroxide alone (Figure 3.17 for C. I. Acid Blue 158 and C. I. Acid Red 73 as examples). Colour removal level differs for each dye depending on their chemical structure.

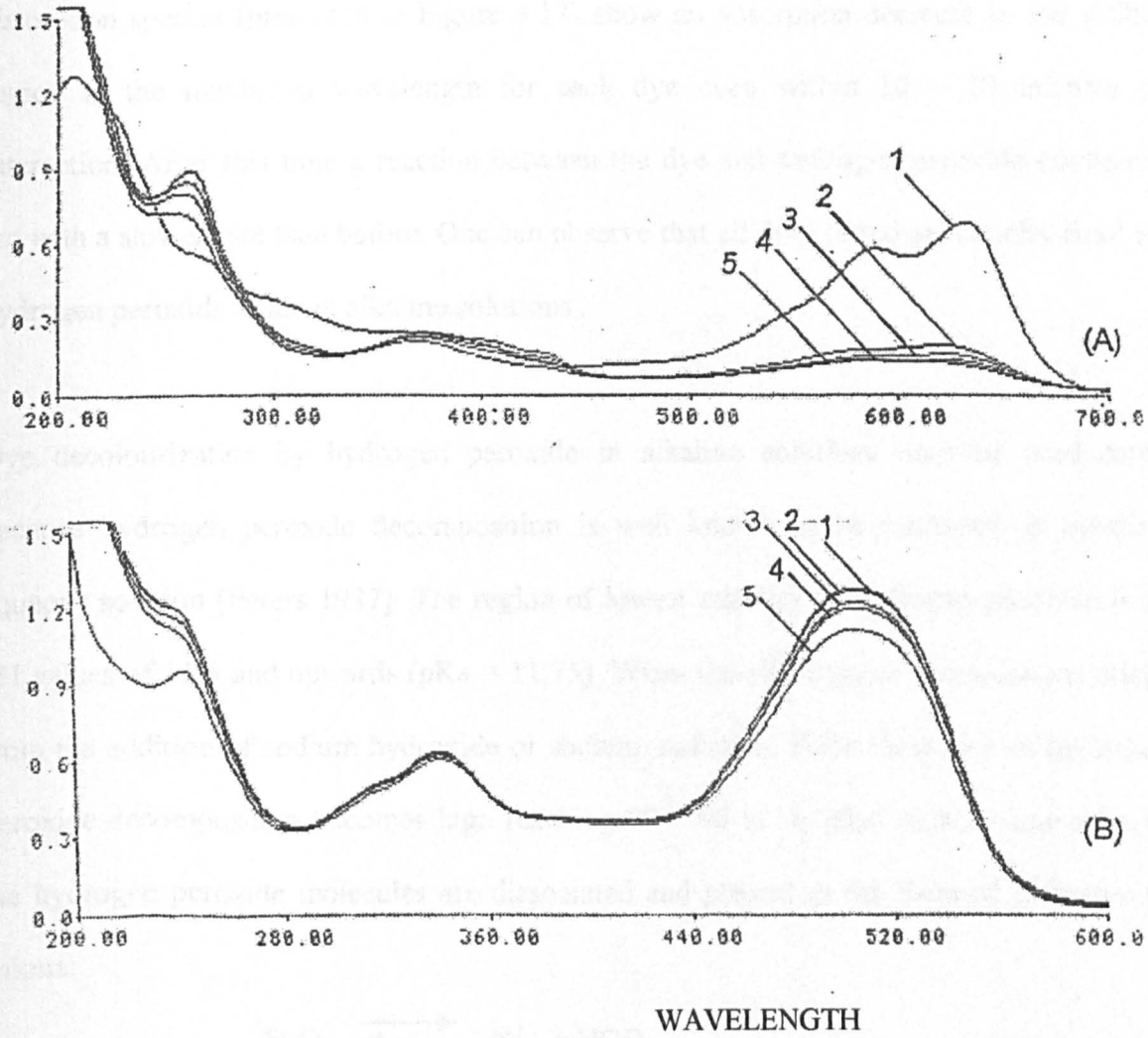


Figure 3.17. UV-Visible spectra of dyes C. I. Acid Blue 158 (A) and C. I. Acid Red 73 (B) over time with hydrogen peroxide in alkaline solutions (pH = 10.5).
(1) – initial dye solution with hydrogen peroxide at $t = 0$,
after reaction time: (2) – 10 min., (3) – 20 min., (4) – 30 min., (5) – 60 min.

Treatment conditions: $[Dye]_o$ 25 mg/dm^3, $[H_2O_2]_o$ 90 mg/dm^3, 25°C.

The UV-Visible spectra of the reaction mixtures of azo dyes and hydrogen peroxide in alkaline solution were recorded over 60 minutes of interaction in dark conditions. The absorption spectra (presented in Figure 3.17) show an absorption decrease in the visible region at the maximum wavelength for each dye even within 10 – 20 minutes of interaction. After this time a reaction between the dye and hydrogen peroxide continued but with a slower rate than before. One can observe that all dyes tested are decolourized by hydrogen peroxide alone in alkaline solutions .

Dye decolourization by hydrogen peroxide in alkaline solutions may be predictable because hydrogen peroxide decomposition is well known to be increased in alkaline aqueous solution [Peters 1987]. The region of lowest stability of hydrogen peroxide is at pH values of 11.5 and upwards (pKa = 11.75). When the alkalinity of the solutions arises from the addition of sodium hydroxide or sodium carbonate alone the extent of hydrogen peroxide decomposition becomes high reaching 80 – 90 %. In alkaline solutions most of the hydrogen peroxide molecules are dissociated and present in the form of perhydroxyl anions:

$$H_2O_2 \rightleftharpoons H^+ + HOO^-$$

Perhydroxyl anion, a powerful nucleophile, reacts with the organic colorants decolourizing dye molecules. It is worth noting that a classical bleaching technology in the textile industry using various organic peroxides and hydrogen peroxide is based on this effect [Stepanov 1986, Peters 1987, Oakes et al 1998, Tokuda & Onura 1999].

The dyes C. I. Acid Blue 158 and C. I. Acid Yellow 99 were decolourized by hydrogen peroxide to greater extent than other dyes in alkaline solutions (Figure 3.17 A for C. I. Acid Blue 158). The mixtures of these dyes with hydrogen peroxide were completely

colourless after 12 hours keeping in dark conditions without UV irradiation. This is due to the presence of chromium in their chemical structure (as illustrated in Figures 3.9 and 3.10). Hydrogen peroxide decomposition is known to be accelerated by the catalytic action of some heavy metal ions such as cobalt, copper, iron, manganese, chromium etc., even in extremely small (trace) amounts in solution [Sharmb 1959, Berdnikov 1973, Peters 1987, Oakes et al 1998]. Chromium has the greatest catalytic activity for hydrogen peroxide decomposition [Jones 1999] which is also pH dependent. In this case, catalytical decomposition of hydrogen peroxide solutions is pronounced in alkaline solutions.

Dye decolourization by UV/hydrogen peroxide treatment as a function of pH was increased in acidic and alkaline solutions for azo dyes containing one azo linkage in their chemical structure. As seen in Figure 3.18 (given for the C. I. Acid Blue 92 as an example of a mono azo dye) practical complete colour removal at pH 10.5 and 4.0 was achieved after 10 and 20 minutes of treatment, respectively, as compared with complete colour removal at pH 7.0 after about 30 minutes treatment. Among monoazo dyes the behaviour of C. I. Acid Blue 158 and C. I. Acid Yellow 99 is very different from others. These dyes are decolourized much faster in alkaline solutions than other monoazo dyes (data in Tables 3.7 – 3.13). UV-Visible spectra of these dyes recorded throughout photochemical treatment (given in Figure 3.19 for C. I. Acid Blue 158 as an example) indicate a rapid decrease in optical density over a wide region of the spectrum. After 10 minutes of the photochemical treatment these dyes in alkaline solution become colourless.

The colour removal rate of disazo dyes is significantly increased only in alkaline solutions and remained almost constant in the pH range from 4.0 to 7.0. The changes in colour removal of disazo dye depending on pH solutions can be seen in UV-Visible spectra shown in Figure 3.20 for the disazo dye C. I. Acid Red 73.

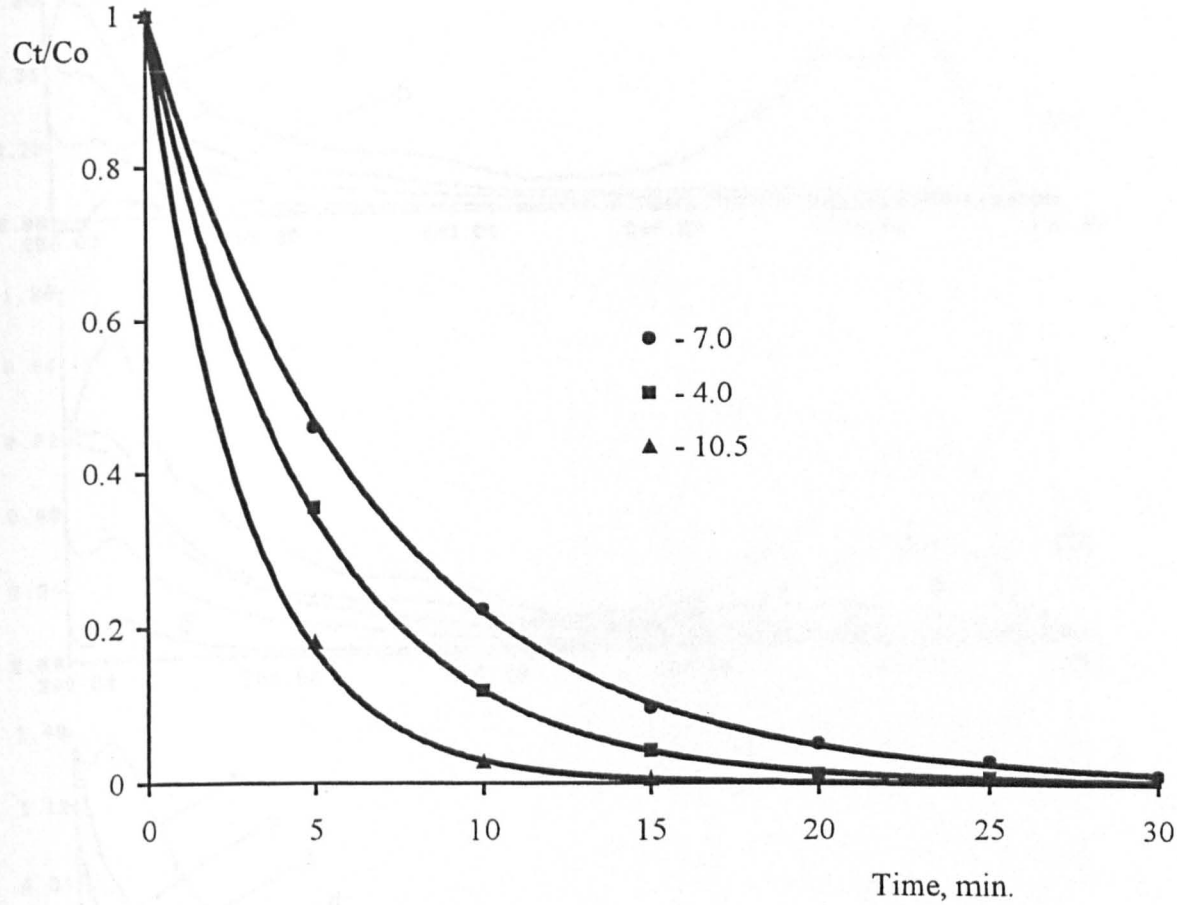


Figure 3.18. Decolourization kinetics of C. I. Acid Blue 92 by UV/H_2O_2 treatment as a function of pH.

UV treatment: $[Dye]_o$ 25 mg/dm^3, $[H_2O_2]_o$ 90 mg/dm^3, 25°C.

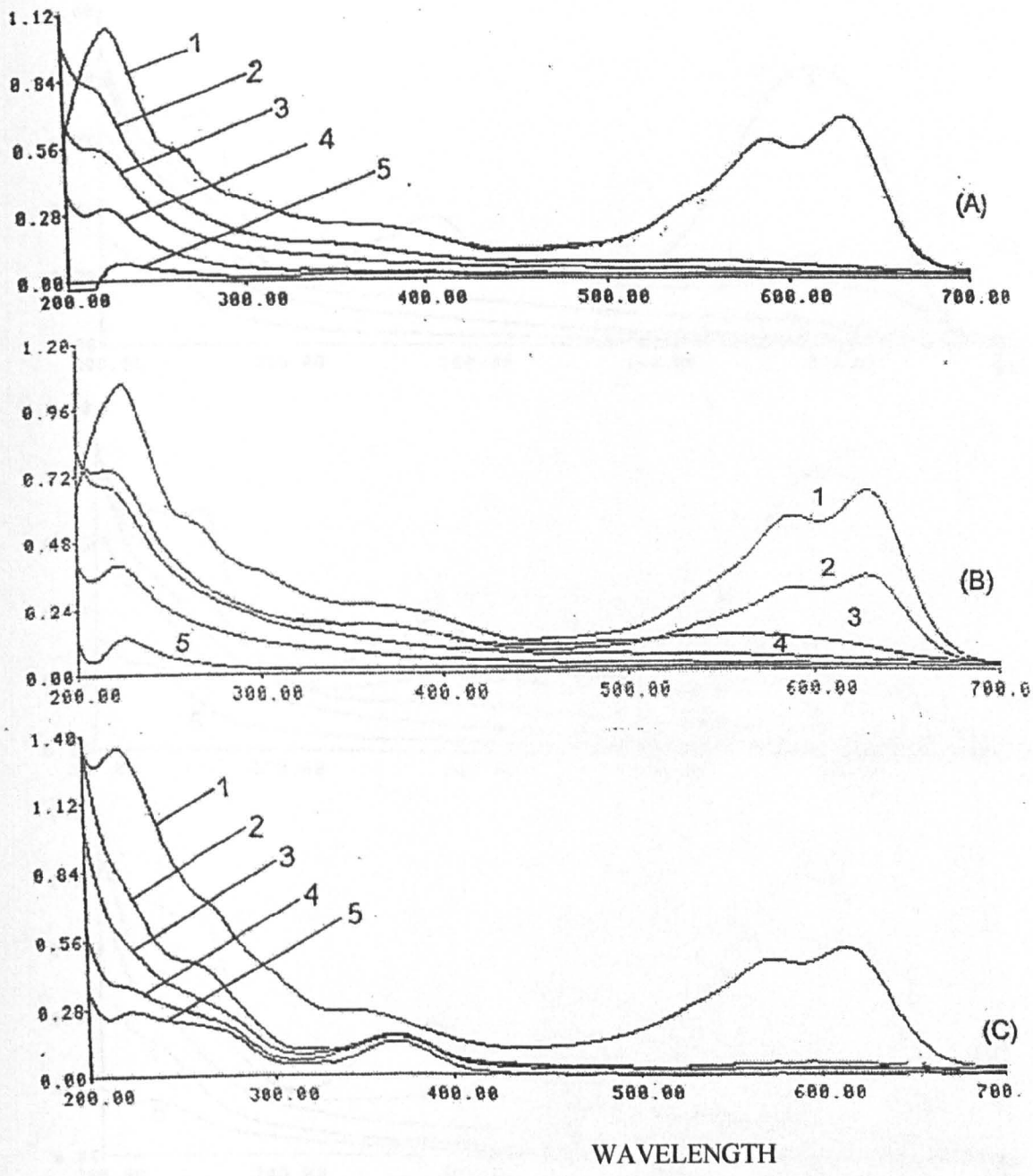


Figure 3.19. UV- Visible absorption spectra of dye C. I. Acid Blue 158 solutions treated by UV/H_2O_2 at pH: 4.0. (A), 7.0 (B) and 10.5 (C).

(1) – initial mixture of dye with hydrogen peroxide at $t = 0$,

after reaction time: (2) – 10 min., (3) – 20 min., (4) – 30 min., (5) – 60 min.

Treatment conditions: $[Dye]_o$ 25 mg/dm^3, $[H_2O_2]_o$ 90 mg/dm^3, 25°C, pH 7.0.

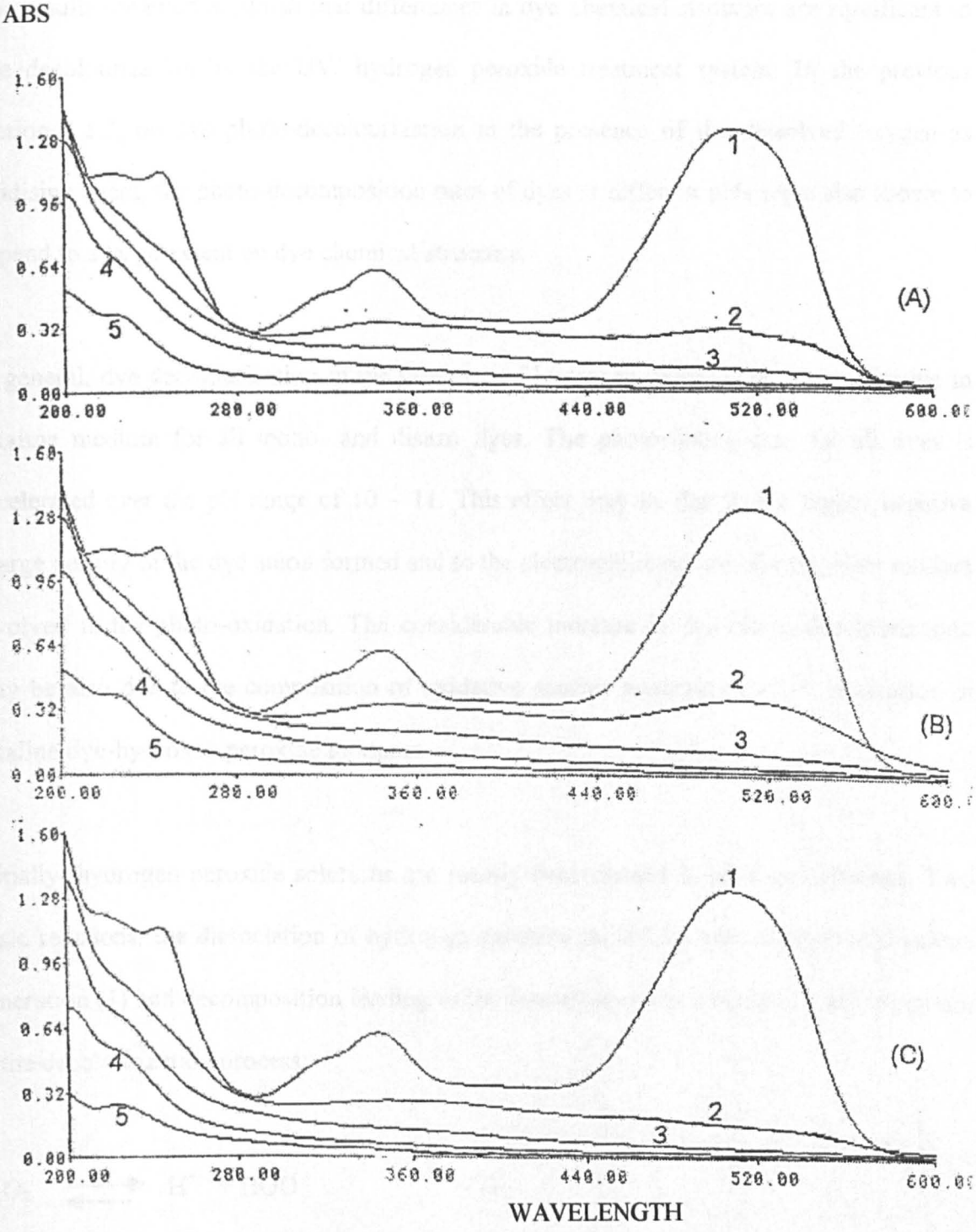


Figure 3.20. UV- Visible absorption spectra of dye C. I. Acid Red 73 solutions treated by UV/H_2O_2 at pH: 4.0. (A), 7.0 (B) and 10.5 (C).

(1)– initial mixture of dye with hydrogen peroxide at $t = 0$,

after reaction time: (2) – 10 min., (3) – 20 min., (4) – 30 min., (5) – 60 min.

Treatment conditions: $[Dye]_o$ 25 mg/dm^3, $[H_2O_2]_o$ 90 mg/dm^3, 25°C, pH 7.0.

The results obtained establish that differences in dye chemical structure are significant in dye decolourization by the UV/ hydrogen peroxide treatment system. In the previous section 3.1.3, on dye photo-decolourization in the presence of the dissolved oxygen as oxidising agent, the photo-decomposition rates of dyes at different pHs were also shown to depend to a large extent on dye chemical structure.

In general, dye decolourization in the presence of hydrogen peroxide is more effective in alkaline medium for all mono- and disazo dyes. The photo-fading rate for all dyes is accelerated over the pH range of 10 – 11. This effect may be due to the higher negative charge density of the dye anion formed and to the electrophilic nature of a transient oxidant involved in the photo-oxidation. The considerable increase in dye photo-decolourization may be also due to the composition of oxidative species generated on UV irradiation in alkaline dye-hydrogen peroxide mixtures.

Initially, hydrogen peroxide solutions are readily decomposed in alkaline solutions. Two basic reactions, the dissociation of hydrogen peroxide molecules with perhydroxyl anions generation (1) and decomposition leading to the formation of the oxygen (2), are important in the decolourization process:

$$H_2O_2 \rightleftharpoons H^+ + HOO^- \qquad (1)$$

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2 \qquad (2)$$

Although the formation of oxygen is classically regarded as an unwanted side reaction and is unlikely to contribute to the bleaching process, in photochemical reactions the presence of oxygen in solutions significantly affects the dye decolourization rate (as shown in Chapter 3.1). In photochemical reactions oxygen formation can be seen an additional source of oxidant in the system effectively destroying dye molecules.

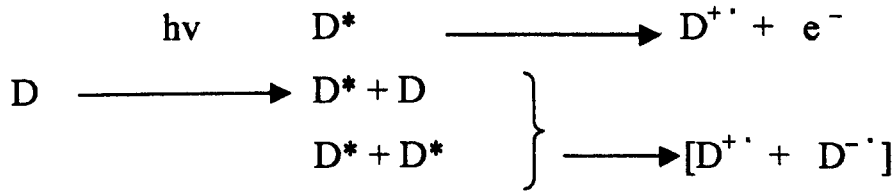
Also, hydrogen peroxide photolysis rate has been found to increase when more alkaline conditions are used [Wardman 1989, Buxton et al 1988]. This might be primarily due to the higher molar absorption coefficient of the perhydroxyl anion at 253.7 nm, which is 240 $dm^3\ mol^{-1}\ cm^{-1}$ in contrast to 18.6 $dm^3\ mol^{-1}\ cm^{-1}$ of hydrogen peroxide itself.

Finally, the presence of alkali (particularly of sodium hydroxide) accelerates the dye photo-decolourization process because of the generation of hydroxyl radicals from the hydroxyl anion by photo-active dye [Allen 1994] as shown:

$$D^* + OH^- \longrightarrow D^{-\cdot} + OH^{\cdot}$$

Thus, photo-fading of azo dyes in alkaline solutions depends on the nature and composition of the oxidative species generated on UV irradiation in alkaline dye-hydrogen peroxide mixtures. Dye decolourization may be due to a oxidative process with the participation of various oxidising species including hydroxyl, superoxide radicals and perhydroxyl anions and/or radicals.

The mechanism for dye photo-decomposition with alkaline hydrogen peroxide solutions is very complex and is not fully understood at present. It is generally believed that the photo-excited dye molecules, through intermediate photo-reactions as shown below:



react then with hydroxyl and/or perhydroxyl anions and oxygen producing intermediate products such as organic peroxide and hydroperoxide radicals [Terenin 1967, Bekker (ed) 1976]. The organic hydroperoxide radicals are very reactive species having the ability to rapidly undergo transformations under UV irradiation [Terenin 1967, Ranby & Rabek 1978) to give substances like ketones, aldehydes, phenols, etc.

On the whole, the results demonstrate that the chemical structure of dyes and pH are important factors influencing dye colour removal from dye solutions under UV irradiation in the presence of dissolved oxygen or hydrogen peroxide.

An important conclusion from the research is that there is in practice no general necessity to adjust pH in order to optimise the UV treatment operating conditions. Dyeing processes are generally carried out either acidic or alkaline solutions and the decolourization rate of azo dyes on UV irradiation also increases in acidic and particularly in alkaline solutions.

3.4. Photochemical degradation mechanism of the azo dyes.

3.4.1. Introduction.

The results on dye decomposition, obtained and described in previous sections, show that it is possible to achieve fast decolourization of dye solutions in the UV/hydrogen peroxide treatment system. However, the decolourization rate of the dyes cannot give information about the degradation mechanisms and the chemical structure of intermediate products formed in irradiated solutions. The fate and toxicity, including ecotoxicity, of these compounds is also important.

Two potential mechanisms of photochemical degradation of azo dyes could be considered, involving symmetric or asymmetric cleavage of the azo groups [Venkataraman 1977]. Cleavage of the azo linkage is known to be able to occur at the N-C bond on the coupler side of the azo linkage, at the C-N of the remaining aromatic fragment or at the double bond between the two N atoms of the azo linkage.

The isolation and characterisation of the dye photo-reaction by-products is extremely complex and represents a major challenge. In particular the dye reaction intermediates in dye-hydrogen peroxide mixtures tend to react further after stopping of UV irradiation. Attempts to isolate azo dye reaction products have met with varying degrees of success [Yang et al 1998, Kritchevsky 1986, Haag & Mill 1987, Griffiths & Hawkins 1977]. Lack of success is primarily because of the relatively low concentration of dye used in the reaction mixture. On the other hand, if a high dye concentration is used this leads to dye aggregation in solutions and hence to the complete change in photo-decolourization

mechanism with the formation of complex reaction products. Moreover, the intermediates of the photochemical decomposition of organic compounds including azo compounds are often too reactive a species to be detectable by any analytic techniques. Quantitative analysis has not been therefore achieved.

Among azo dyes compounds containing sulphonic groups in their structure, which provide them with high polarity and water solubility, are most difficult to analyse by many analytical methods including liquid chromatography and mass-spectrometry [McLean & Freas 1989, Weatherall 1991, Straub et al 1992, Jandera et al 1996, Rafols & Barselo 1997, Conneely et al 1999].

3.4.2. The identification of the photochemical breakdown products of azo dyes.

Experiments were performed to identify of photo-decomposition products of azo dyes after photochemical reaction. To give a preliminary idea about the possible types of intermediates and mechanisms of the reactions that azo dye might undergo during photolysis analytical methods such as thin layer chromatography (TLC), UV-Visible spectrophotometry, high performance liquid chromatography (HPLC) and mass spectrometry with matrix assisted laser desorption ionization technique (MALDI) were used.

Diazo dye C. I. Acid Red 73 was evaluated for identification experiments for intermediate products. The solutions of C. I. Acid Red 73 at a concentration of 25 mg/dm^3 were irradiated by UV light in the presence of hydrogen peroxide of 90 mg/dm^3 concentration at constant temperature (25°C) and pH (7.0). Samples for analyses were withdrawn at regular

time intervals of photochemical treatment throughout the experiments. These analyses to characterise by-products were performed using comparison with standard compounds if these were available commercially.

UV- visible spectrophotometry was carried out throughout the experiments recording spectra of treated dye solutions (shown in sections 3.1. and 3.2.) to assess colour changes occurring as a result of the dye photochemical degradation. This gives information about dye photo-degradation showing rapid decrease in the absorption of dye solutions in the visible and near UV region of the spectrum during the treatment. Nevertheless this does not yield information about the number and nature of intermediate products formed.

Photo-degradation products were analysed initially by thin layer chromatography. TLC analysis was performed using on pre-coated silica gel 60 F254 plates and the mobile phase methanol - methylene chloride increasing from 10:90 to 50:50, respectively. A 254 nm UV detector was used. No separation of dye itself and dye decomposition products after photochemical treatment was observed. This is due to the low concentration of dye in the reaction mixture and the photo-reactivity of by-products formed during UV treatment. Also the use of a less polar eluent to study the breakdown products of this dye probably would be more useful to separate the components and give positive information.

Taking into account the lack of the separation of the photo-degradation products of dye C. I. Acid Red 73 using TLC, experimental work on the separation of photo-degradation products of the dye using HPLC analysis was carried out. The details of HPLC analysis of dye samples after photochemical treatment are described in Chapter 2.

To identify the dye intermediates and the final breakdown products MALDI mass spectra of the solutions of irradiated dye were also obtained giving molecular mass information about by-products.

The matrix material, facilitating the sample ionisation process and insulating the sample analysed from thermal degradation and fragmentation, should not give a background overlapping the peaks of analysed substances which would interfere with obtaining and interpreting their mass data. Among the different types of matrix materials listed in the Chapter 2 and used for MS – MALDI identification analysis of dye solutions the most consistent results were obtained using the matrix solution of 0.1 M solution of 2-[4-hydroxyphenylazo] benzoic acid (HABA) in ethanol with a 0.1 M solution of diammonium citrate in distilled water in the ratio 10:1. The MALDI, in the negative ion operation mode, using the HABA matrix solution was able to detect characteristic anions formed in the photochemically treated dye solutions.

The results on HPLC separation and MS – MALDI identification of photo-degradation products of the dye C. I. Acid Red 73 formed during photochemical treatment are presented in Table 3.14.

Table 3.14. HPLC and mass spectral characteristic of azo dye (C. I. Acid Red 73) photochemical degradation products.

Compound	Molecular ion, $[M - X]^-$	m/z	Rt, min
C. I. Acid Red 73 (unoxidised dye)	$[M - Na]^-$ $[M - 2\,Na]^-$	533 510	17
1-(4`-hydroxyphenylazo)-2-naphthol-6,8-disulphonic acid (sodium salt).	$[M - Na]^-$	443	-
1-(4`-peroxyphenylazo)-2-naphthol-6,8-disulphonic acid (sodium salt)	$[M - Na]^-$	460	-
1-phenylazo-2-naphthol-6,8-disulphonic acid (sodium salt) - C. I. Acid Orange 10.	$[M - Na]^-$ $[M - 2\,Na]^-$	428 405	12
2-hydroxy-6,8-disulphonaphthyl hydroperoxide	$[M - H]^-$ $[MH - Na + 2H]^-$	380 359	-
2-naphthol-6,8-disulphonic acid (sodium salt)	$[M - Na]^-$ $[M - Na + H]^-$	324 325	-
1,2-naphthoquinone-6,8-disulphonic acid (sodium salt)	$[M - Na]^-$	339 318, 320	27 (*)
6,8- disulphonaphthalene	$[M - 2\,Na + H]^-$ $[M - H]^-$	310 285	-
Phthalic acid	-	-	34
Phenol	-	-	19

(*) not confirmed by comparison with standard compound.

HPLC analysis of UV/ hydrogen peroxide oxidation products of C. I. Acid Red 73 (**I**) (here and below product numbers correspond to compounds in Figure 3.28) after 5 – 10 minutes of treatment indicated the formation of an intermediate product with Rt = 12 min. well separated from the initial dye analysed before photochemical treatment (Rt = 17 min). The MALDI mass spectrum of the initial dye solution and dye solutions after photochemical treatment are illustrated in Figures 3.21 – 3. 27. The MALDI mass spectrum of a dye solution analysed after the same treatment time showed mass spectral peaks at m/z 428 and 405. On the basis of these results, the unknown intermediate product was identified as 1-phenylazo-2-naphthol-6,8-disulphonic acid sodium salt (**II**) (Table 3.14).

To confirm the formation of 1-phenylazo-2-naphthol-6,8-disulphonic acid sodium salt (**II**), a standard manufactured compound known as C. I. Acid Orange 10, was analysed by the same HPLC method. Retention times of the products formed after C. I. Acid Red 73 photo-decomposition and the standard compound 1-phenylazo-2-naphthol-6,8-disulphonic acid sodium salt (C. I. Acid Orange 10) were found to coincide.

There were also mass peaks at m/z 443 and 460 in the MALDI spectra attributed to the molecular ions of products formed with the same duration (first 5 – 10 minutes) of photochemical treatment. These products were identified as 1-(4`-hydroxyphenylazo)-2-naphthol-6,8-disulphonic acid and 1-(4`-peroxyphenylazo)-2-naphthol-6,8-disulphonic acid (sodium salts), respectively. These compounds were presumably unstable in the reaction system or present in too low concentration and therefore were not detectable under the HPLC separation conditions used.

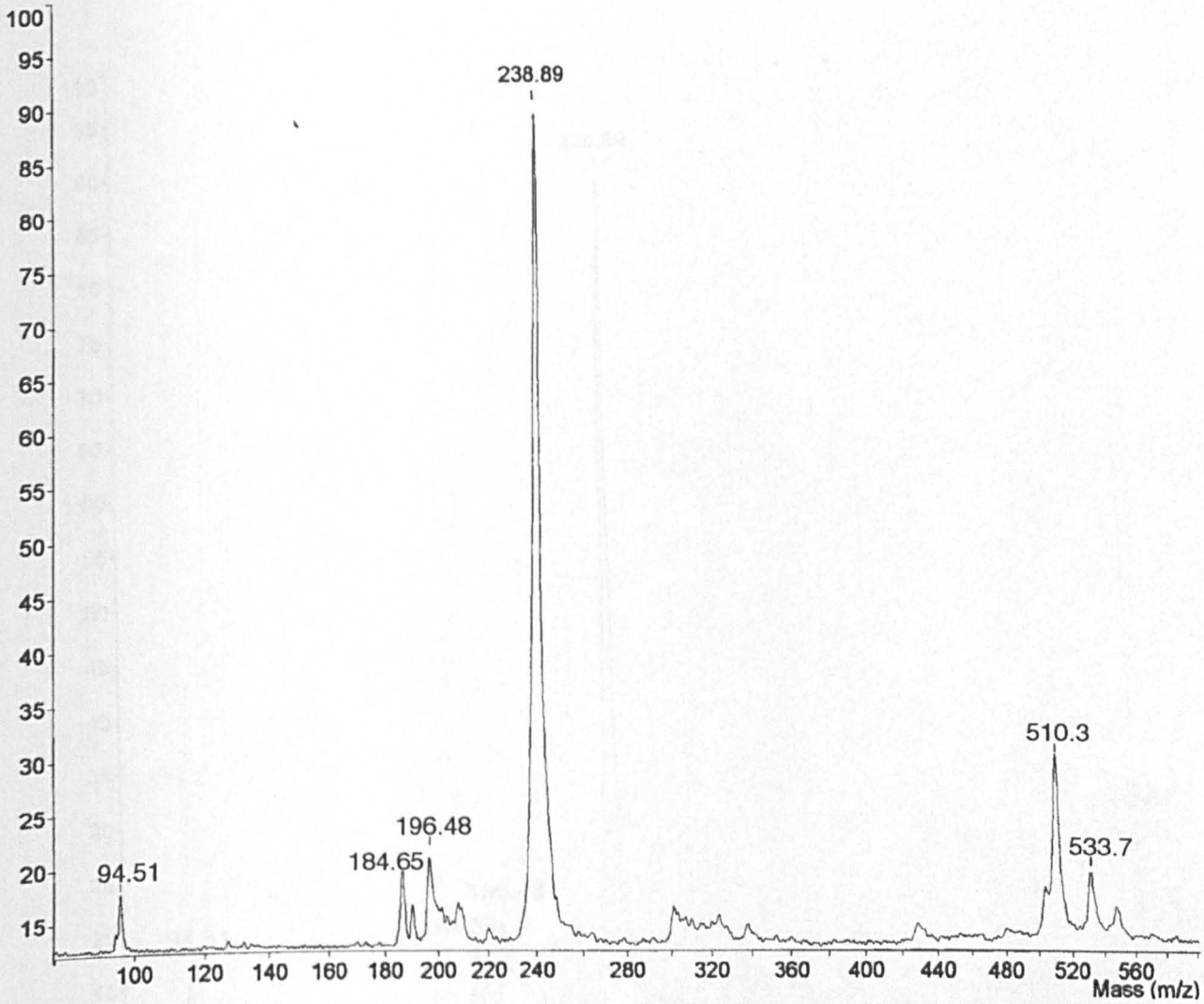


Figure 3.21. The MALDI mass spectrum of initial dye solution of C. I. Acid Red 73.

Note: Mass (m/z) scale for subsequent spectra was chosen to show the peaks which appeared. No relevant peaks were observed in other areas of the spectra.

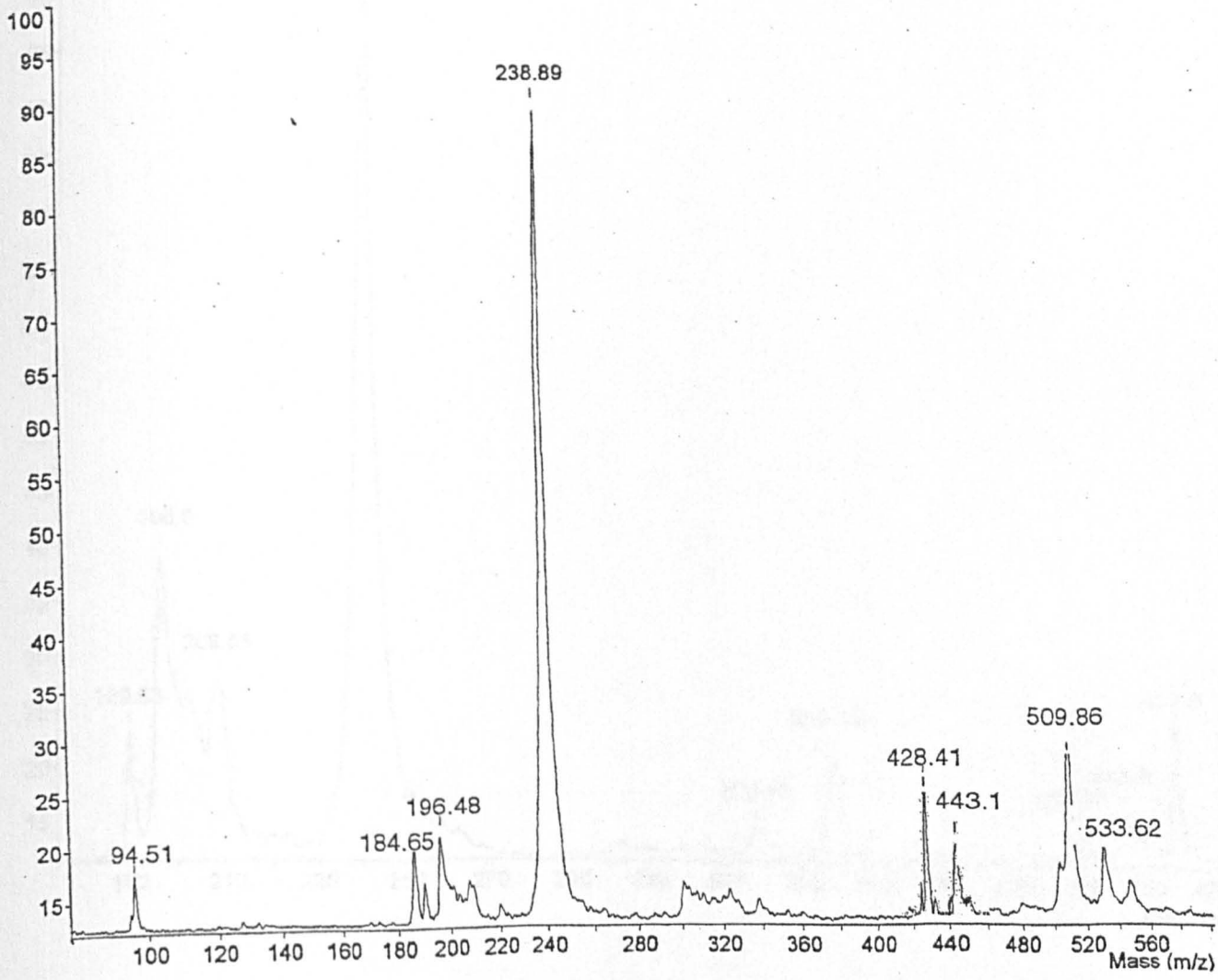


Figure 3.22. The MALDI mass spectrum of C. I. Acid Red 73 solution after 5 minutes of the treatment.

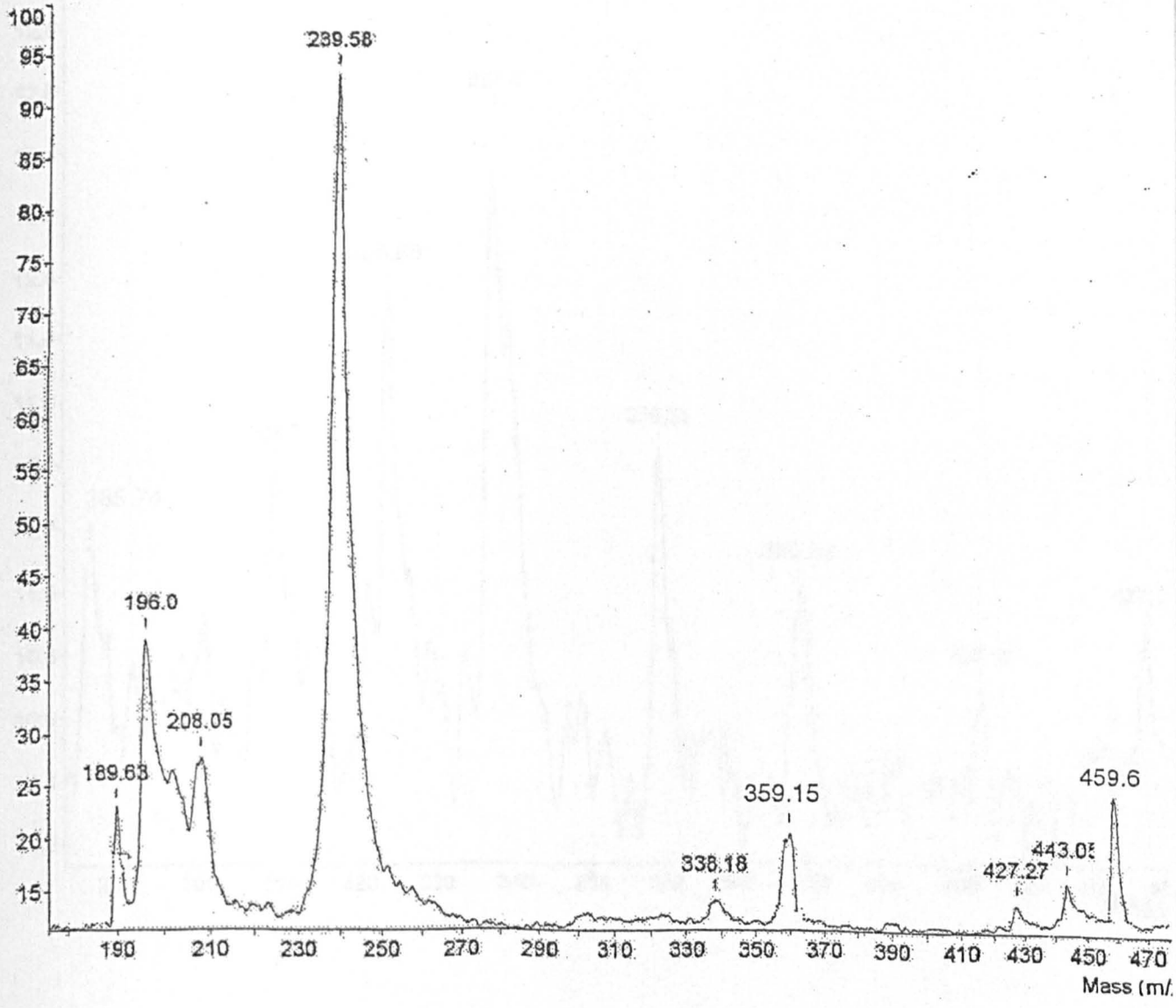


Figure 3.23. The MALDI mass spectrum of C. I. Acid Red 73 solution after 7 minutes of the treatment.

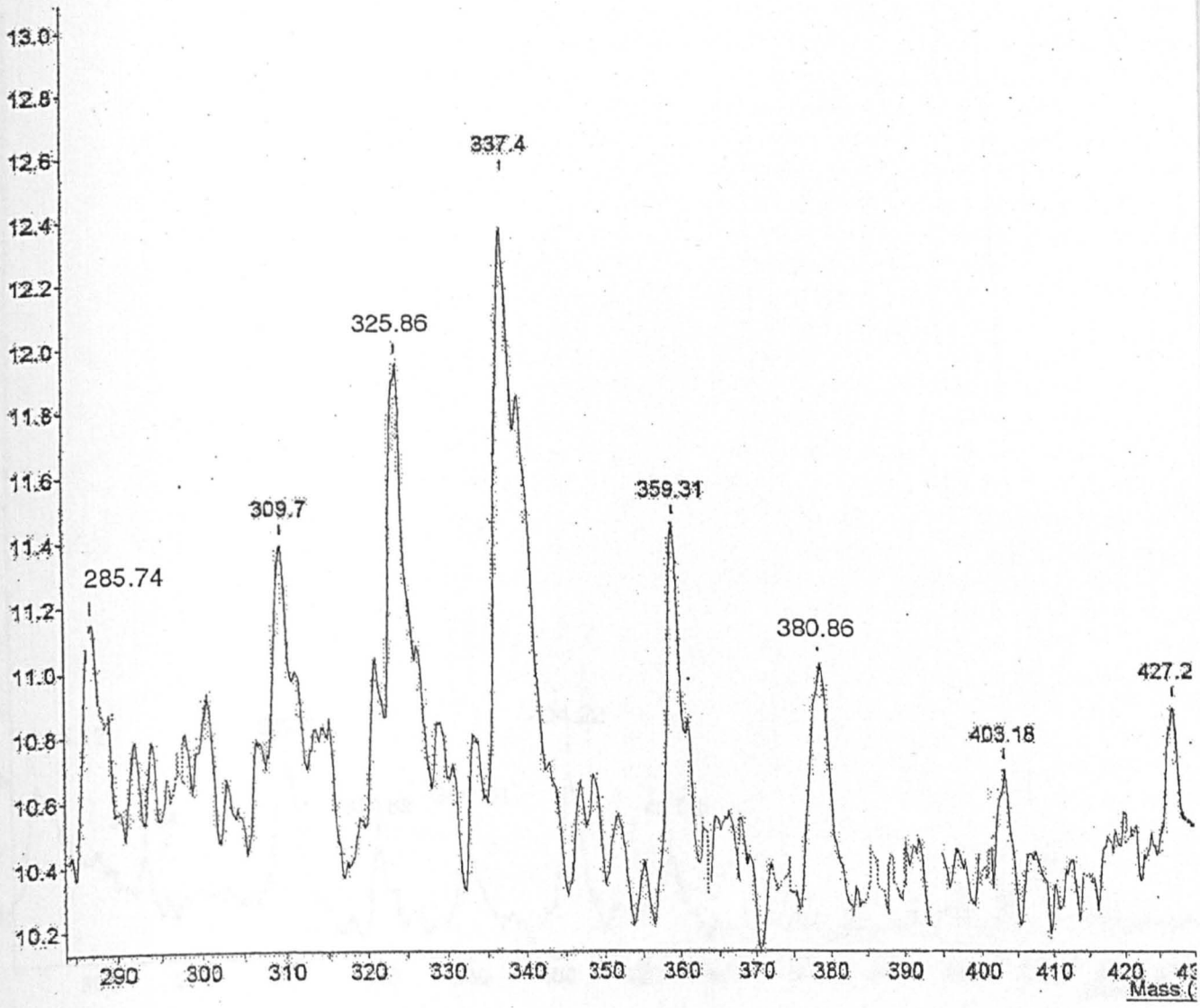


Figure 3.24. The MALDI mass spectrum of C. I. Acid Red 73 solution after 10 minutes of the treatment.

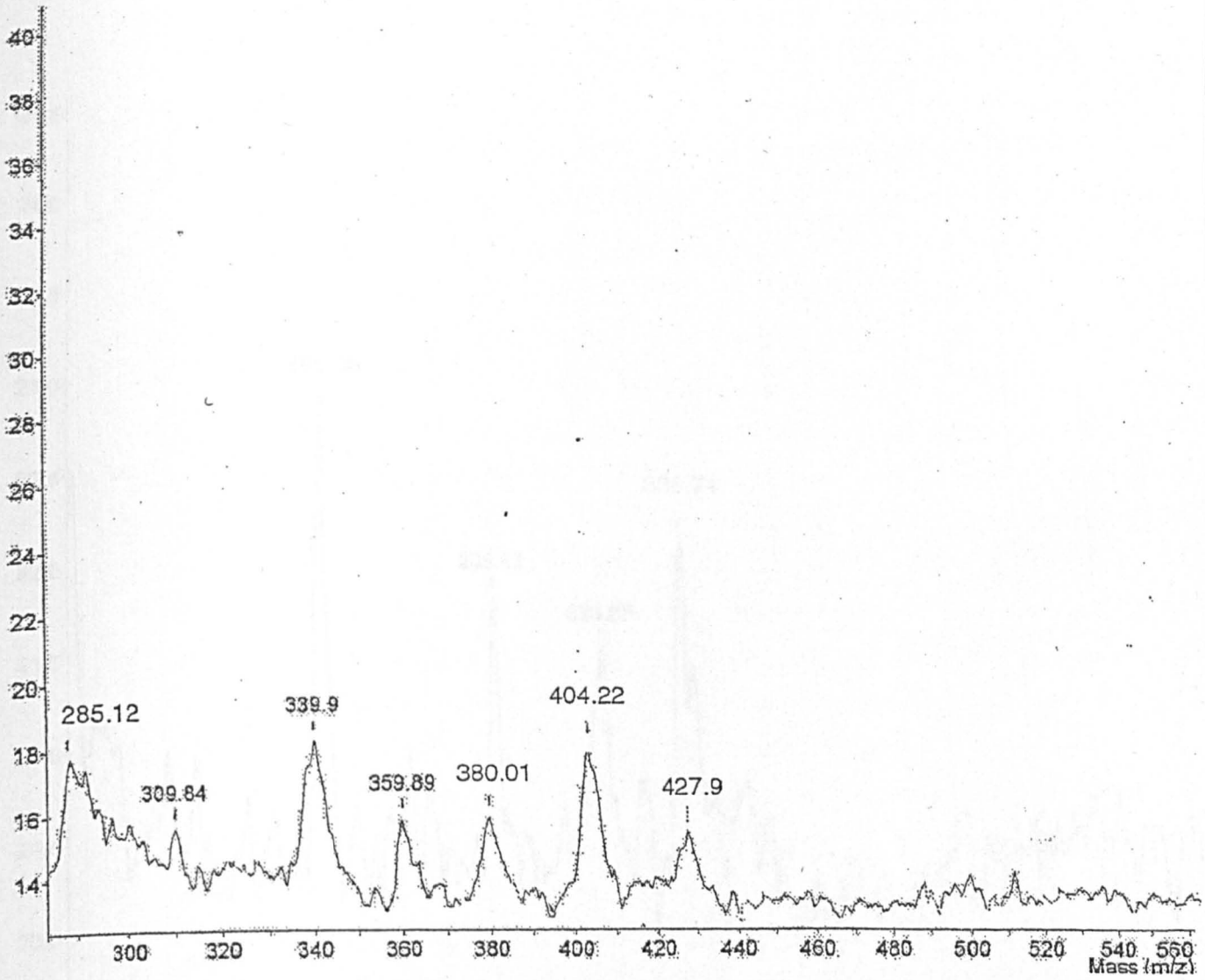


Figure 3. 25. The MALDI mass spectrum of C. I. Acid Red 73 solutions after 15 minutes of the treatment.

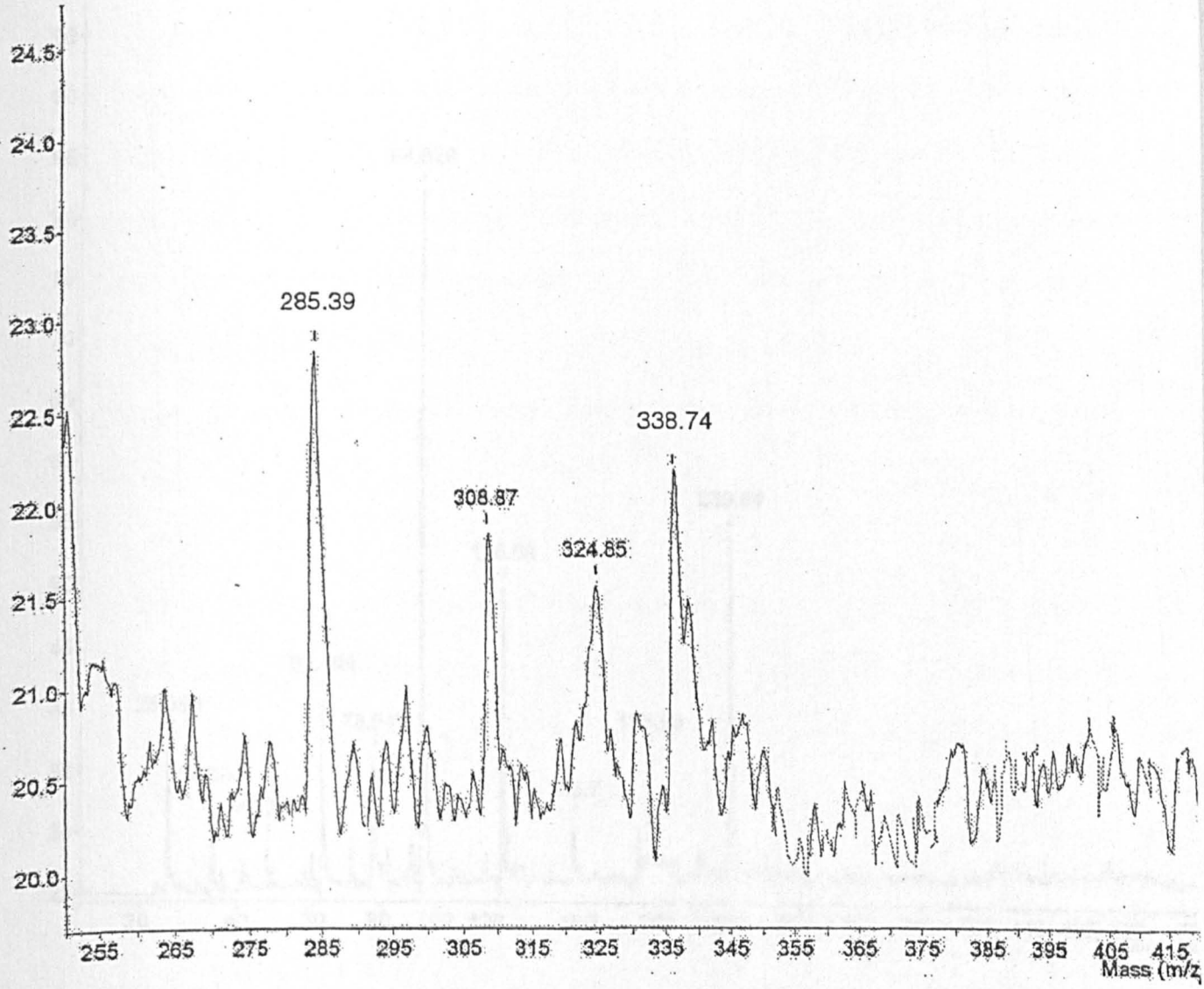


Figure 3.26. The MALDI mass spectrum of C. I. Acid Red 73 solution after 20 minutes of the treatment.

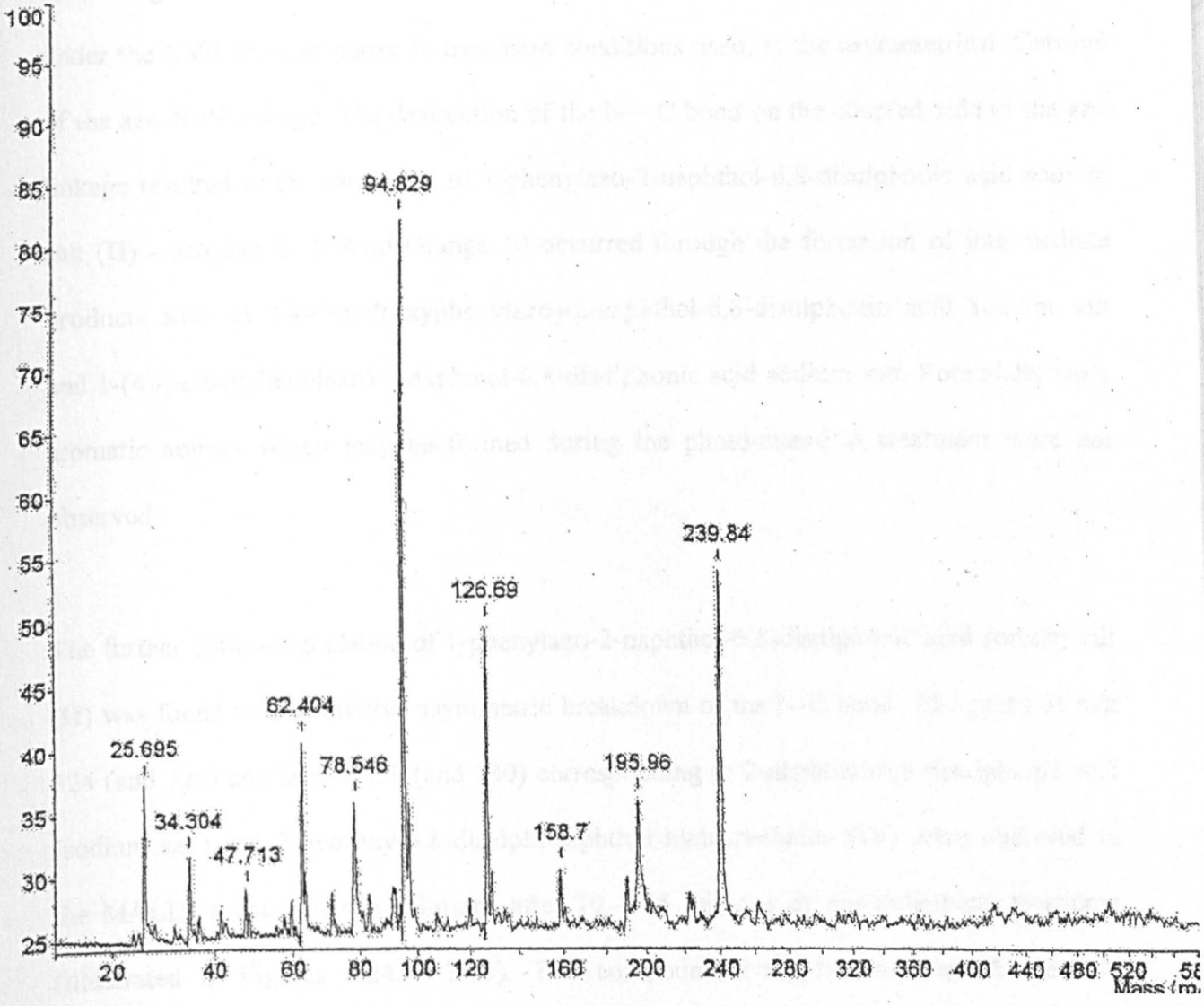


Figure 3. 27. The MALDI mass spectrum of C. I. Acid Red 73 solution after 30 minutes of the treatment.

According to these results the initial stage of photo-oxidation of the dye C. I. Acid Red 73, under the UV/hydrogen peroxide treatment conditions used, is the asymmetrical cleavage of the azo N=N linkage. The destruction of the N – C bond on the coupled side of the azo linkage resulted in the formation of 1-phenylazo-2-naphthol-6,8-disulphonic acid sodium salt (**II**) - azo dye C. I. Acid Orange 10 occurred through the formation of intermediate products such as 1-(4`-hydroxyphenylazo)-2-naphthol-6,8-disulphonic acid sodium salt and 1-(4`-peroxyphenylazo)-2-naphthol-6,8-disulphonic acid sodium salt. Potentially toxic aromatic amines which may be formed during the photo-chemical treatment were not observed.

The further photo-degradation of 1-phenylazo-2-naphthol-6,8-disulphonic acid sodium salt (**II**) was found to also involve asymmetric breakdown of the N-C bond. The peaks at m/z 324 (and 325) and at m/z 359 (and 380) corresponding to 2-naphthol-6,8-disulphonic acid (sodium salt) and 2-hydroxy-6,8-disulphonaphthyl hydroperoxide (**IV**) were observed in the MALDI spectra of dye solutions after 10 – 15 minutes of photochemical treatment (illustrated in Figures 3.24 – 3.25). The compound 2-naphthol-6,8-disulphonic acid probably arises due to the loss of diazonium ion (**III**) from the C. I. Acid Orange 10 molecular ion or azobenzene from C. I. Acid Red 73 molecular ion. To further confirm this proposal photochemical oxidation of the dye C. I. Acid Orange 10 alone (as commercial product) was carried out using the same UV/hydrogen peroxide treatment conditions, and MALDI mass spectra were recorded. Even after 5 – 7 minutes of the treatment the same mass peaks at m/z 325 and 359 were observed.

Compound 2-hydroxy-6,8-disulphonaphthyl hydroperoxide (**IV**) (mass peak at m/z 359) resulting from the photochemical degradation of 1-phenylazo-2-naphthol-6,8-disulphonic acid (C. I. Acid Orange 10) was found to be further photo-oxidised to 1,2-naphthoquinone-6,8-disulphonic acid (**V**) (mass peaks at m/z 339). HPLC analysis of the dye solution after 20 minutes of the treatment indicated the presence of one new intermediate product with a retention time of 27 min., which presumably corresponded to 1,2-naphthoquinone-6,8-disulphonic acid (sodium salt). Unfortunately, this product was not exactly identified by comparison with the standard compound because 1,2-naphthoquinone-6,8-disulphonic acid (sodium salt) was not readily available commercially.

On the other hand, organic hydroperoxides are known to be very unstable on UV irradiation, rapidly degrading with the cleavage of the ArO – OH bond (Ranby & Rabek 1975), and therefore could not accumulate. Simultaneous presence of both products, 2-hydroxy-6,8-disulphonaphthyl hydroperoxide (**IV**) and 1,2-naphthoquinone-6,8-disulphonic acid (**V**), in MALDI spectra of the reaction solutions after identical treatment times could be a possible explanation of this product (**IV**) not accumulating due to quickly decomposing. HPLC analyses also suggested that this product either is formed in trace quantities or has so short a life-time that it was not detectable by the analytical methods used.

Further photochemical oxidation of 1,2-naphthoquinone-6,8-disulphonic acid (sodium salt) resulted in phthalic acid (**VI**) and probably other products of an acidic character. Diazonium salt formed as a result of the previous photo-destruction of the N-C bond on the coupled side of the azo linkage in C. I. Acid Red 73 and/or C. I. Acid Orange 10 was also photo-oxidised rapidly with the formation of phenol (**VII**) and a number of by-products.

The formation of phthalic acid (**VI**) and phenol (**VII**) with the Rt = 34 min. and Rt = 19 min., respectively, was observed in HPLC chromatograms after 20 minutes of photochemical treatment (Table 3.14). The chromatograms of standard (reference) compounds, phthalic acid and phenol, were also obtained. Retention times of analysed products were found to coincide with references ones. The recorded pH data during the photochemical experiments proved the presence of organic acids formation, since the pH of dye solutions dropped from 7.0 (initial dye solution) to 5.87.

The suggested pathway for the photochemical degradation of dye C. I. Acid Red 73 could be illustrated by the following scheme present in Figure 3. 28.

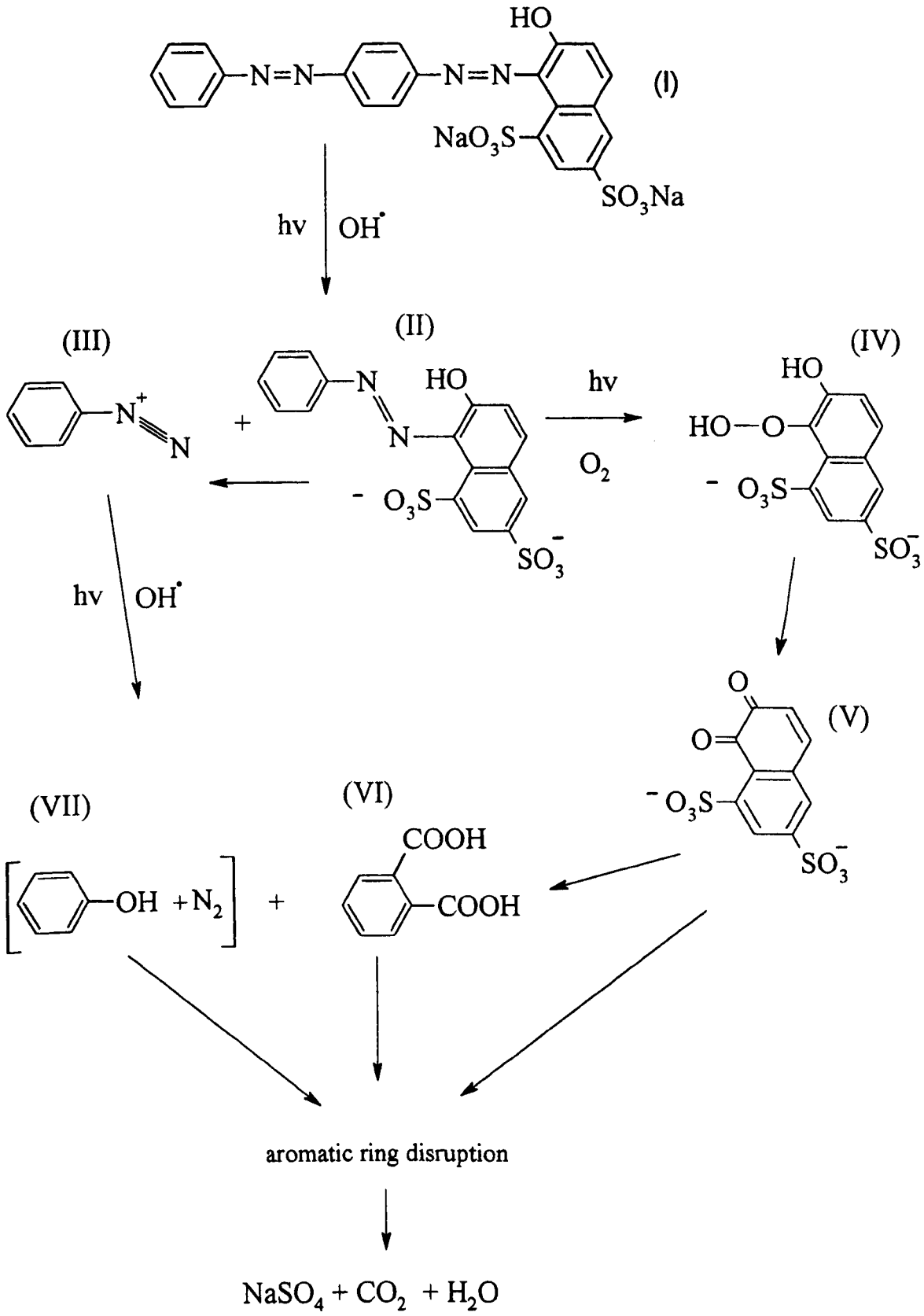


Figure 3.28. Scheme of proposed photo-oxidation mechanism of C. I. Acid Red 73.

Thus, from the experimental data obtained in this study, the formation of potentially carcinogenic aromatic amines during the photochemical treatment of C. I. Acid Red 73 was not observed. The data presented imply that using a UV irradiation period of 20 – 30 min the dye molecules, before complete mineralization, were degraded to colourless intermediate substances such as naphthalene (naphthoquinone) and benzene (phenol) derivatives, and organic acids including phthalic acid.

CHAPTER 4. ECOTOXICOLOGICAL STUDIES ON AZO DYE SOLUTION.

4.1. Introduction.

The dye photochemical decomposition studies described in previous sections show that there is a real possibility of achieving rapid decolourization of azo dye aqueous solutions. Azo dye photochemical degradation using the UV/hydrogen peroxide treatment system occurs through the formation of colourless, low molecular weight breakdown products.

The analytical methods used for the identification of intermediate products formed after the azo dye photochemical treatment were more or less satisfactory for breakdown product identification. However this is not sufficient for an assessment of ecological safety and potential harmful effects on organisms in the aqueous environment and biological treatment plants. Therefore, the use of biological methods in addition to conventional chemical measurements has become established as an increasingly important approach in evaluating the toxic effects of individual chemicals and whole effluents.

Bioassay methods can play an important role in regulating the quality of industrial effluents and in monitoring effluent treatment performance efficiency. A recent UK study on direct toxicity assessment has been carried out on a pilot basis on three UK sites (river Esk near Langholm, river Aire near Bradford and the Lower Tees Estuary) [DTA 2001] which include sites with textile effluent. Results from the UK pilot trials, including the use of *Chlorella* as a test organism are regarded by the UK Environmental Agency as very promising and justifying

further work [McEldowney & McEldowney 2001]. A two year whole effluent toxicity testing evaluation project is also taking place in the USA [US EPA News 2001].

This part of the research was to study the toxicity and biodegradability of azo dyes and their photo-oxidised breakdown products in aqueous solutions using different test-organisms. The biological evaluation of the ecotoxicological potential of the preliminary photo-oxidised products of azo dyes in terms of toxicity to aquatic species and micro-organisms involved in biodegradation and treatability by particularly sewage micro-organisms was carried out.

4.2. Ecotoxicity of parent azo dyes and their photo-oxidised products.

Azo dye solutions before and after photochemical treatment were tested for acute toxicity using the aquatic test-organism, *Chlorella vulgaris*. Growth of algal cells for biotesting was carried out as described previously in Chapter 2. The harmful effects of test samples were estimated by comparing the fluorescence of *Chlorella* after sample addition with the fluorescence of intact *Chlorella* (a control). The EC_{16} end point value, the lowest observable effect concentration causing a reduction in the fluorescence of *Chlorella* by 16 %, was taken for the assessment of acute toxicity. This value was calculated from the data on fluorescence inhibition by setting up a series of dilutions. No less than four dilutions were run on each sample. Results on sample acute toxicity were expressed as a dilution titre.

The solutions of azo dyes were tested before and after UV irradiation with and without hydrogen peroxide at UV treatment conditions constant for all dyes: $[Dye]_o$ 25 mg/dm^3,

$[H_2O_2]_o$ 90 mg/dm^3, pH 7.0 and 25°C Each sample withdrawn for toxicity testing, either initial dye solution or sample after some treatment time, was diluted to produce solutions of different concentration to determine the corresponding EC_{16} value. For testing 2 ml sample was added to 8 ml cell culture distributed into mineral-salt medium in glass tubes. The fluorescence of *Chlorella* after sample addition was observed over 24 hours, recording a change in the fluorescence after immediate sample addition (about 5 sec.) and then after 15, 30, 60, 180 minutes and 24 hours of exposure. To take into account the dilution factor the change in fluorescence of *Chlorella* cells was measured after the addition of 2 ml of distilled water in a control tube over the same period of exposure. In the control the fall in *Chlorella* fluorescence after distilled water addition was not more than 26 % (over 24 hours) in comparison with the fluorescence of intact *Chlorella* cells.

Preliminary experiments demonstrated that *Chlorella* was potentially capable of adapting to the harmful action of the azo dyes and of recovering the fluorescence to some extent over 24 hours. Figure 4.1 shows the change in fluorescence of *Chlorella* with azo dyes (calculated as a % change in algal fluorescence after test sample addition compared with the control) over 24 hours of exposure time. Results on acute toxicity of dye samples were obtained after 60 minutes of the biotesting exposure time showing the maximum fall in *Chlorella* fluorescence (as seen in Figure 4.1). The change in the fluorescence for this exposure period of 60 minutes was further used to calculate EC_{16} values for all dye samples.

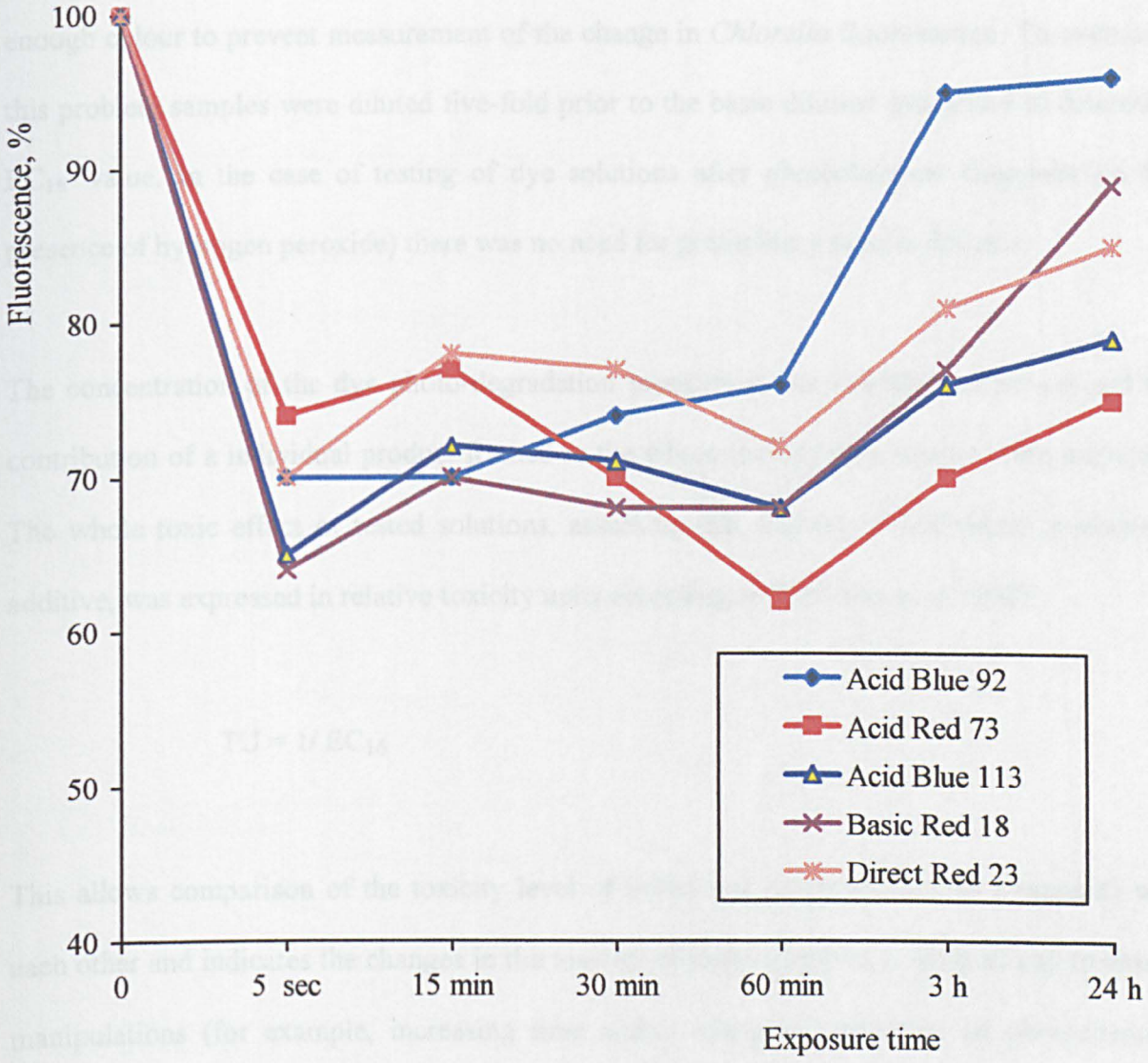


Figure 4.1. The change in fluorescence of *Chlorella vulgaris* with azo dye solutions as a function of exposure time.

The dye samples withdrawn for the toxicity testing before and after UV irradiation alone gave enough colour to prevent measurement of the change in *Chlorella* fluorescence. To overcome this problem samples were diluted five-fold prior to the basic dilution procedure to determine EC_{16} value. In the case of testing of dye solutions after photochemical treatment (in the presence of hydrogen peroxide) there was no need for preliminary sample dilution.

The concentration of the dye photo-degradation products in the irradiated solutions and the contribution of a individual product formed to the whole toxicity of a sample were unknown. The whole toxic effect of tested solutions, assuming that toxicity of individual products is additive, was expressed in relative toxicity units according to [DiGiano et al 1992]:

$$TU = 1/ EC_{16}$$

This allows comparison of the toxicity level of initial dye solutions (before treatment) with each other and indicates the changes in the toxicity of each sample as a result of any treatment manipulations (for example, increasing time and/or changing conditions of photochemical treatment). The data on the toxic effects of azo dye solutions (initial and after photochemical treatment time) to *Chlorella* are present in Table 4.1.

Table 4.1. The toxic effect of azo dye solutions (initial and after photochemical treatment) on the fluorescence of *Chlorella vulgaris*.

Dye	Treatment	Time of treatment, min	EC_{16}, dilution titres	Toxicity Unit (TU)
C. I. Acid Red 73	-	-	0.73	1.36
	UV	30	0.83	1.20
		60	0.71	1.41
	UV / H_2O_2	10	13.6	0.06
		20 – 30	Non-toxic	
C. I. Acid Blue 92	-	-	4.37	0.23
	UV	30	4.89	0.21
		60	4.34	0.24
	UV / H_2O_2	10	48.9	0.02
		20 – 30	Non-toxic	
C. I. Basic Red 18	-	-	1.59	0.63
	UV	30	2.17	0.46
		60	1.99	0.50
	UV / H_2O_2	10 – 30	Non-toxic	
C. I. Direct Red 23	-	-	1.59	0.63
	UV	30	1.19	0.84
		60	1.78	0.56
	UV / H_2O_2	10 – 30	Non-toxic	
C. I. Acid Blue 113	-	-	1.30	0.77
	UV	30	1.55	0.65
		60	3.13	0.32
	UV / H_2O_2	10	48.9	0.02
		20 – 30	Non-toxic	

The data obtained (in Table 4.1) show that the initial solutions of Acid Red 73 and those after photochemical treatment are more toxic than solutions of other dyes to the test organism. It was also observed that the toxicity of solutions of all dyes did not significantly change after the treatment with UV irradiation alone (dissolved oxygen was not removed from dye solutions before the treatment) in comparison with the toxicity of their initial dye solutions. Moreover, the toxicity of some dyes, C. I. Acid Red 73, C. I. Acid Blue 92, C. I. Direct Red 23 and C. I. Acid Blue 113, increased during the treatment time with UV irradiation alone (Figure 4.2). It might be concluded that photo-degradation of azo dyes under UV irradiation in the presence of dissolved oxygen occurred by a mechanism with the formation of compounds more toxic than initial dyes.

On the other hand, these dye solutions irradiated by UV light in the presence of hydrogen peroxide did not cause any inhibition of the fluorescence of *Chlorella* becoming non-toxic after only 10 minutes of photochemical treatment (Figure 4.2). After 20 minutes of photochemical treatment all dye samples were completely non-toxic.

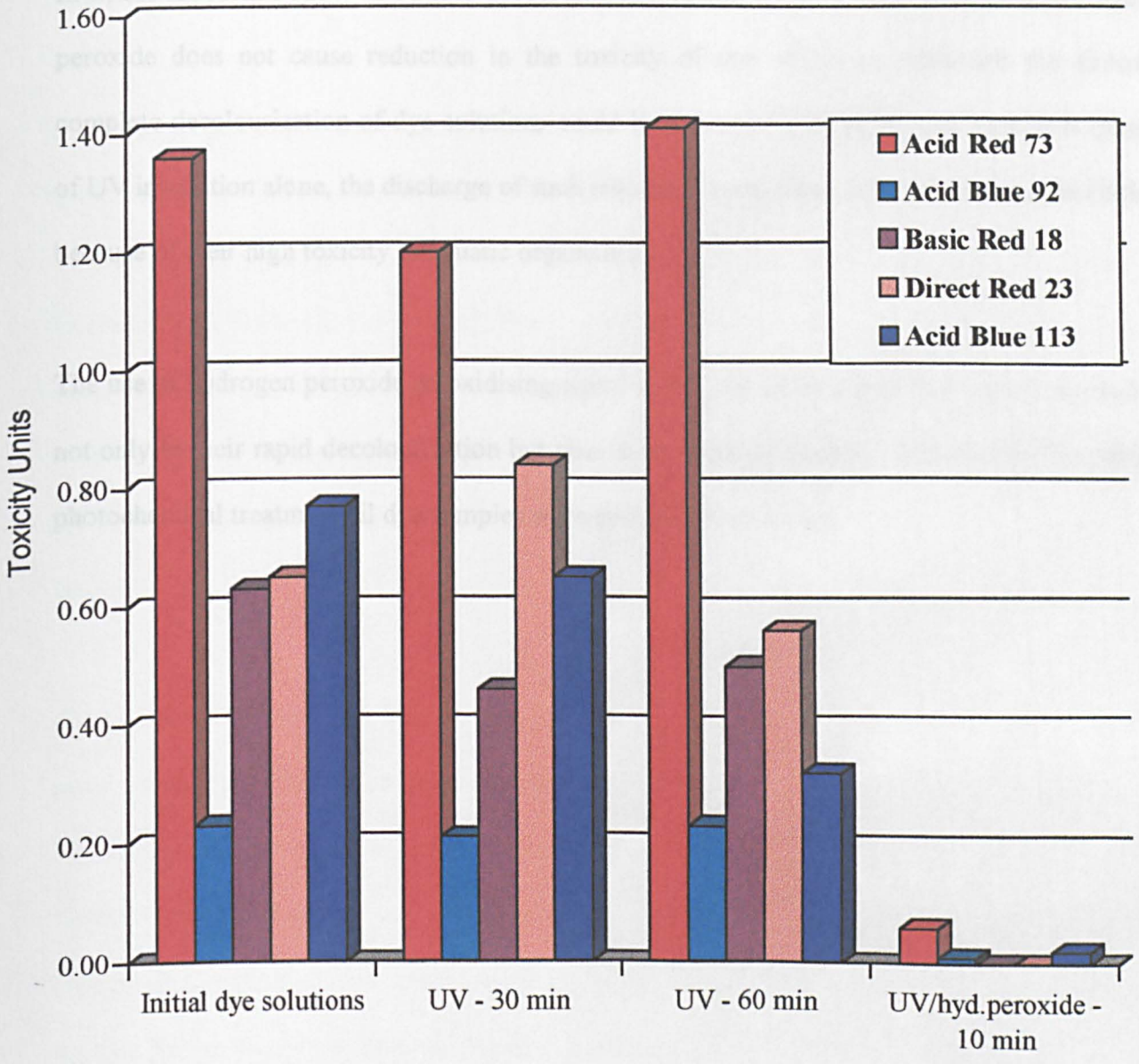


Figure 4.2. The change in toxicity of azo dye solutions after photochemical treatment to *Chlorella vulgaris*.

In whole the results show that photo-decolourization of azo dyes without addition of hydrogen peroxide does not cause reduction in the toxicity of dye solutions. Although the almost complete decolourization of dye solutions could be achieved with prolonged treatment times of UV irradiation alone, the discharge of such solutions to environmental waters is undesirable because of their high toxicity to aquatic organisms.

The use of hydrogen peroxide as oxidising agent in the UV treatment of dye solutions results not only in their rapid decolourization but also in significant reduction in their toxicity. After photochemical treatment all dye samples were completely non-toxic.

4.3. Biodegradability of azo dyes and their photo-oxidative breakdown products.

A bioassay procedure used for the study of the biodegradability of azo dyes and their photochemically breakdown products has been developed previously for the rapid assessment of toxicants in water and described in many reports [Slabbert & Morgan 1982, Slabbert & Grabow 1986, Kirloy & Gray 1995]. The procedure is based on change in the oxygen uptake rates of bacteria. Respiratory response is a sensitive determinant of toxicity and biodegradability, which provides for the possibility of obtaining a faster estimate of toxicity levels for a substance than can be attained from time-consuming lethal or chronic toxicity tests.

Oxygen uptake rates of *Pseudomonas putida* and of activated sludge were employed for the rapid screening of the toxicity and biodegradability levels of azo dye solutions before (initial dye solutions) and after the photochemical treatment in the presence of hydrogen peroxide. Toxicity tests were carried out according to the procedure described by Slabbert and Grabow (1986). Preparation of cell suspensions for analysis and testing of the samples using the oxygen monitoring apparatus were carried out as described in Chapter 2. The effect of test samples on oxygen uptake was examined by injecting 0.2 ml of the samples directly into the chamber containing 3 ml cell suspensions. Four replicate tests were carried out for each test sample and control. The samples were inserted into the test chamber with cell suspension so that all air bubbles were expelled. Oxygen uptake was recorded continuously before, during and after addition of a sample for a total time period of approximately 6–7 minutes. Typical graphic recordings of the effect of samples on oxygen uptake are shown in Figure 4.3.

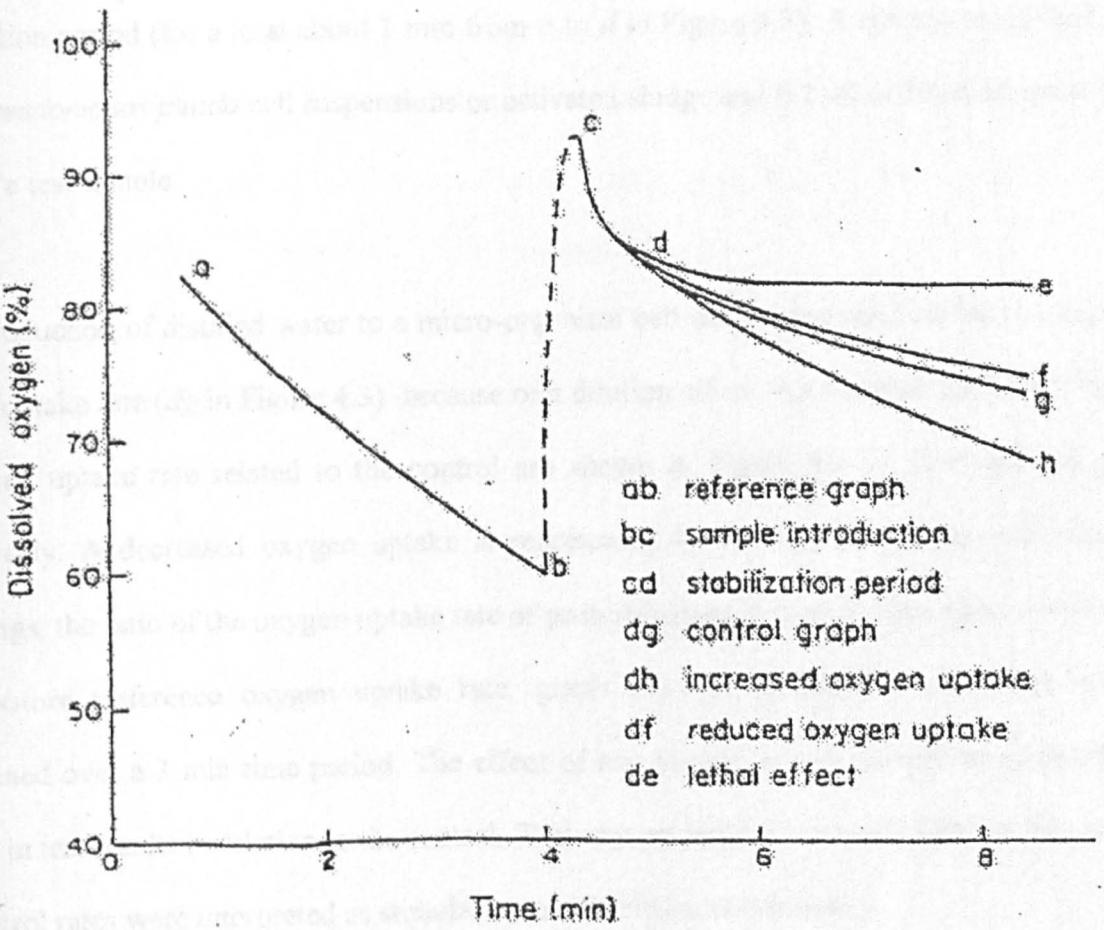


Figure 4.3. Typical recordings of the effect of test solutions on the oxygen uptake of *Pseudomonas putida.*

The time between the sample injection and adjustment of the plunger was allowed for by a stabilisation period (for a total about 1 min from *b* to *d* in Figure 4.3). A control comprised 3 ml of *Pseudomonas putida* cell suspensions or activated sludge and 0.2 ml of distilled water in place of a test sample.

The introduction of distilled water to a micro-organism cell suspension resulted in a changed oxygen uptake rate (*dg* in Figure 4.3) because of a dilution effect. An increase and a decrease in oxygen uptake rate related to the control are shown in Figure 4.3 by lines *dh* and *df*, respectively. A decreased oxygen uptake is represented by the line *de*. Using continuous recordings, the ratio of the oxygen uptake rate of post-stabilisation (test oxygen uptake rate) to pre-exposure (reference oxygen uptake rate, graph *ab*) was determined. All rates were determined over a 3 min time period. The effect of test sample was expressed as percentage change in test results in relation to the control. Test oxygen uptake rates exceeding or less than the control rates were interpreted as stimulation or inhibition, respectively.

The study on biodegradability of parent azo dyes and their breakdown products formed in aqueous solutions after different times of photochemical treatment was firstly carried out, based on change in oxygen uptake of *Pseudomonas putida*. Acid Red 73 and Acid Blue 158 were used in experiments for the rapid screening of the toxicity of azo dyes and their by-products. Aqueous solutions of these dyes at a concentration of 25 mg/dm^3 were irradiated by UV light in the presence of hydrogen peroxide of 90 mg/dm^3 concentration. Dye samples to be tested were withdrawn from the UV apparatus every 10 minutes of photochemical treatment.

Severe inhibition in the oxygen uptake rate of *Pseudomonas Putida* by all dye samples taken at the time intervals of photochemical treatment was observed. After an initial increase in dissolved oxygen concentration following a sample addition, there was no apparent utilisation of oxygen by the bacterial cell suspension. Samples were highly toxic to *Pseudomonas putida* possibly because of residual amounts of hydrogen peroxide.

Therefore, toxicity tests on oxygen uptake of *Pseudomonas putida* were further carried out with the same samples treated by the enzyme catalase. Catalase was used to removed the hydrogen peroxide from photochemically treated dye solutions before these samples were analysed again for their toxicity. The quantity of catalase solution, prepared dissolving 0.04 mg per 5 ml of distilled water, to each sample was 0.1 ml per 5 ml of a dye solution. After addition of catalase solution the samples were kept over 10 minutes at $30^{o}C$ before biotesting. The results on the effect of samples treated with the catalase on oxygen uptake of *Pseudomonas Putida* are summarised in Table 4.2.

Table 4.2. Effect of dye solutions of Acid Red 73 and Acid Blue 158 on oxygen uptake rates of *Pseudomonas putida*.

Dye	Time of UV/H_2O_2 treatment, min	Change in oxygen uptake (test to reference), %	SD	Effect of sample (in relation to control), %
Control (distilled water)		64.3	4.05	-
Acid Red 73	Initial dye solution	44.3	1.73	-20.0
	0*	42.5	3.34	-21.8
	10	44.6	1.04	-19.7
	20	58.7	2.25	-5.6
	30	67.3	5.54	+3.0
	40	71.6	4.09	+7.3
	50	73.2	5.08	+8.9
	60	77.0	0.0	+12.7
Acid Blue 158	Initial dye solution	46.7	2.67	-17.6
	0*	47.4	0.67	-16.9
	10	59.3	2.64	-5.0
	20	75.0	1.38	+10.7
	30	51.7	2.29	-12.6
	40	55.7	3.48	-8.6
	50	54.3	0.36	-10.0
	60	60.0	4.05	-4.3

* Mixture of dye and hydrogen peroxide before photochemical treatment.

SD = standard deviation of four repetitions. Samples were treated with catalase before biotesting. The effect of test sample was presented as "-" inhibition or "+" stimulation of the oxygen uptake of a test sample in relation to the control.

From the Table 4.2 initial dye solutions of both Acid Red 73 and Acid Blue 158, prior hydrogen peroxide addition and UV treatment, caused significant reduction in respiratory rates of *Pseudomonas putida*. An example of a treatment time ("dose") – response curve is shown in Figure 4.4.

After photochemical oxidation, an increase in oxygen uptake rate for C. I. Acid Red 73 solutions was observed with an increase in UV treatment time (Figure 4.4). After about 25 minutes of UV treatment time the inhibition of oxygen uptake caused by the initial dye solutions has been overcome and there was stimulation of oxygen uptake rates with further increase in the treatment time of the dye. Photo-degradation products of this dye (shown in scheme of proposed degradation mechanism in Chapter 3.3) appeared to be non-toxic for the initial concentration of dye used in the experiment.

For solutions of C. I. Acid Blue 158, a near linear increase in oxygen uptake was observed during the first 20 minutes of photochemical treatment as illustrated in Figure 4.4. After this period, considerable inhibition of oxygen uptake rate by dye samples occurred; although these dye samples were completely colourless. This inhibitory effect may be attributed to release of chromium (III or IV) salts into reactive solution during the photochemical degradation of the aromatic structure of the dye.

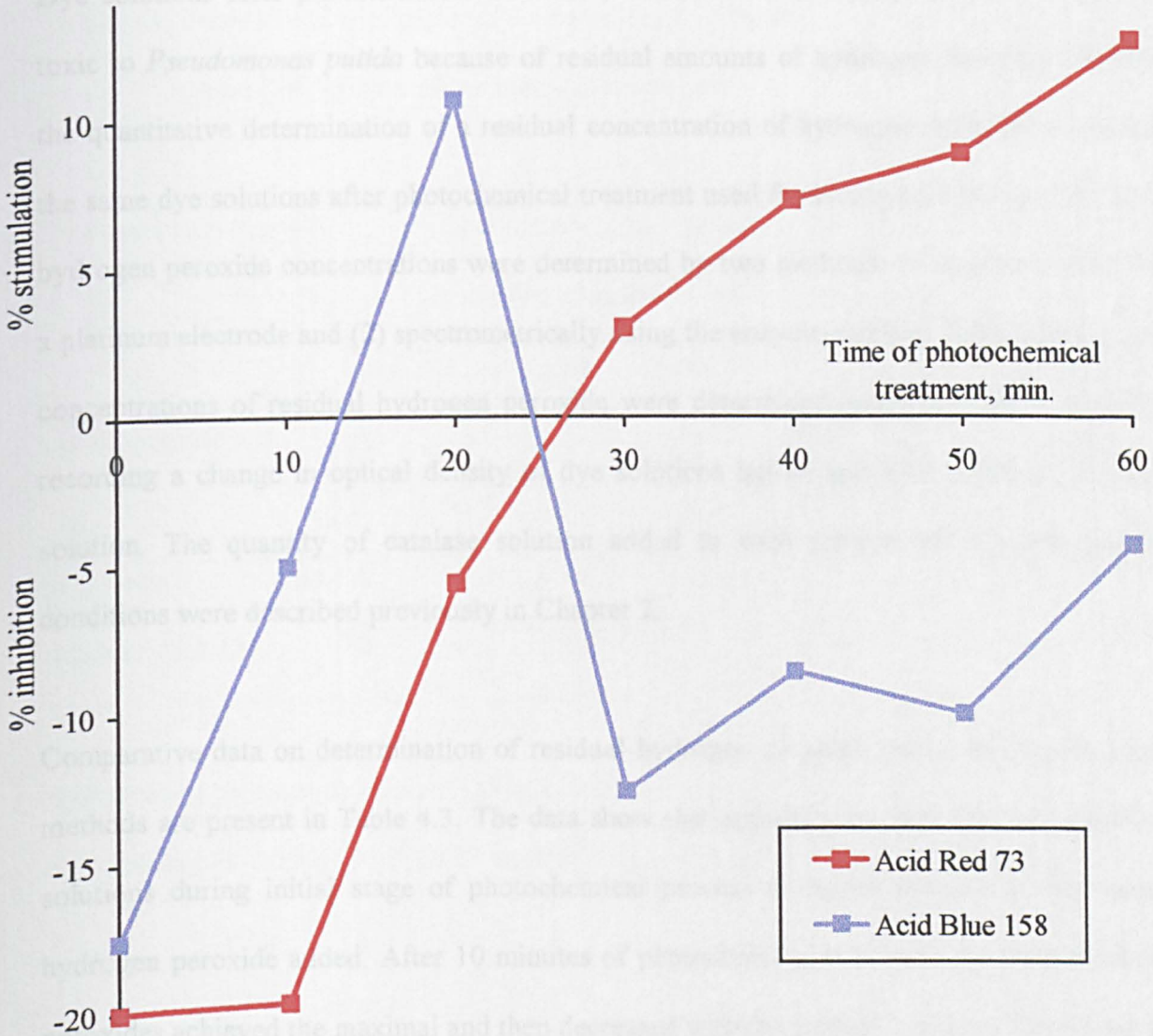


Figure 4.4. Change in oxygen uptake of *Pseudomonas putida* against time of photochemical treatment of dye solutions for Acid Red 73 and Acid Blue 158.

Dye solutions after photochemical treatment, untreated with catalase enzyme, were highly toxic to *Pseudomonas putida* because of residual amounts of hydrogen peroxide. Therefore, the quantitative determination of a residual concentration of hydrogen peroxide was done in the same dye solutions after photochemical treatment used for biological testing. The residual hydrogen peroxide concentrations were determined by two methods: (1) ionometrically using a platinum electrode and (2) spectrometrically using the enzyme catalase. When using catalase concentrations of residual hydrogen peroxide were determined spectrometrically at 240 nm recording a change in optical density of dye solutions before and after addition of catalase solution. The quantity of catalase solution added to each sample and enzyme treatment conditions were described previously in Chapter 2.

Comparative data on determination of residual hydrogen peroxide concentrations by various methods are present in Table 4.3. The data show that apparent concentration of peroxides in solutions during initial stage of photochemical process is higher than an initial value of hydrogen peroxide added. After 10 minutes of photochemical treatment the concentration of peroxides achieved the maximal and then decreased with the further increase of treatment time as shown in Figure 4.5. In contrast, the concentration of hydrogen peroxide solution alone decreased constantly with an increase of UV photochemical treatment time.

Table 4.3. Determination of residual concentrations of hydrogen peroxide in dye solutions of Acid Red 73 after photochemical treatment.

Determination method		Concentration of residual hydrogen peroxide (mg/dm^3) in solutions after the following photo-treatment time, (min):						
		0	10	20	30	40	50	60
Platinum electrode	A	90.2	72.2	61.9	51.0	47.1	38.8	35.5
	B	90.8	107.8	87.0	70.2	49.0	47.7	38.5
Treatment with catalase solution	A	90.0	72.5	60.4	51.1	45.2	38.7	35.1
	B	90.5	107.5	86.2	70.5	50.2	48.7	36.8

A) hydrogen peroxide solution at initial concentration of 90 mg/dm^3.

B) solution of Acid Red 73 dye (25 mg/dm^3) with hydrogen peroxide (90 mg/dm^3)

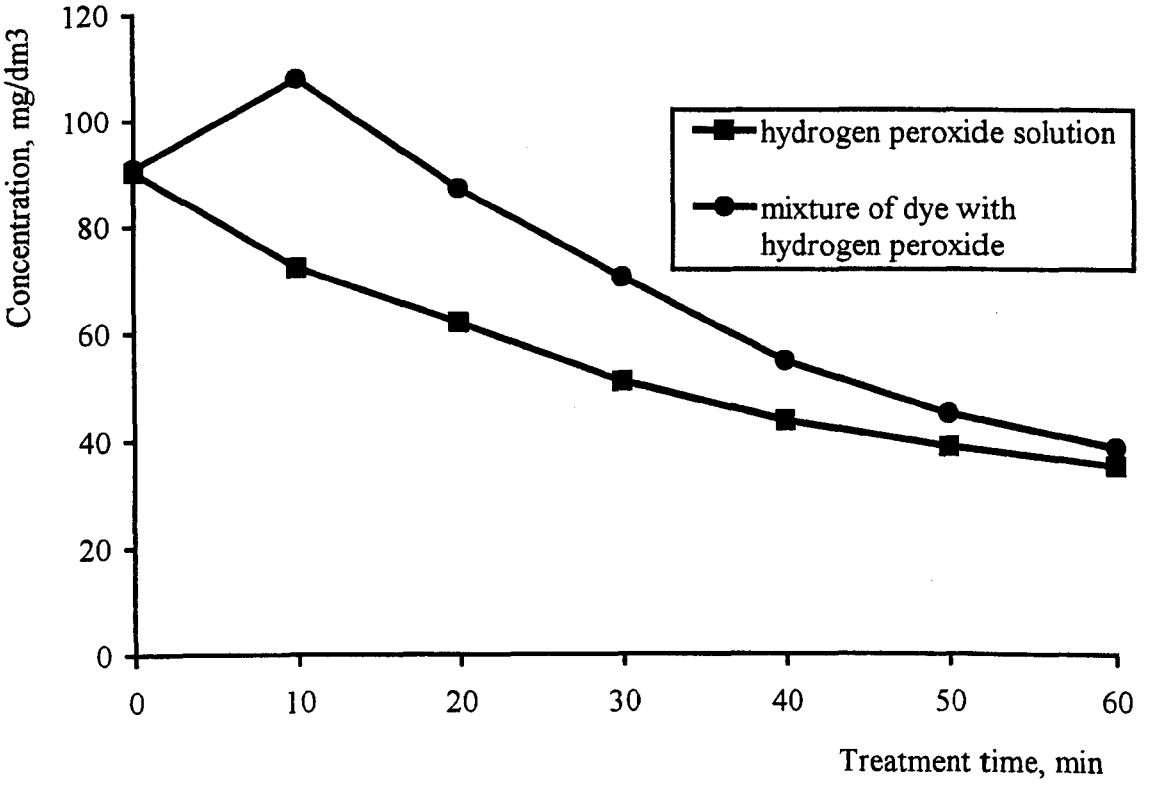


Figure 4. 5. The change in concentration of peroxides during time of UV irradiation of solutions: hydrogen peroxide itself and mixture of dye Acid Red 73 and hydrogen peroxide.

UV treatment conditions: $[Dye]_o$ = 25 mg/dm^3, $[H_2O_2]_o$ = 90 mg/dm^3, T 25°C, pH 7.0.

Thus, the photo-degradation of azo dyes result in the formation of organic peroxides as intermediate products. These compounds did not accumulate in reaction solutions and were destroyed rapidly. The data of this experiment confirmed the mechanism of destructive oxidation of azo dyes (shown in the proposed scheme in Figure 3.28 for C. I. Acid Red 73).

Organisms differ in their response and sensitivity to toxicants so that different organisms in analogous biotest procedures should be used for good correlation in toxicity results. Activated sludge seems to be particularly suitable for the purpose of assessing the damaging action of water polluting substances. In this connection tests on oxygen uptake of activated sludge for the rapid screening of the toxicity and biodegradability levels of azo dye solutions before (initial dye solutions) and after the photochemical treatment in the presence of hydrogen peroxide were done.

The solutions of dyes at a initial concentration of 25 mg/dm^3 were irradiated by UV light in the presence of hydrogen peroxide of 90 mg/dm^3 concentration at constant temperature (25°C) and pH (7.0). Tests were carried out according to the procedure described for testing with *Pseudomonas putida*. Table 4.4 presents the results on the effect of dye samples on the oxygen uptake rate of activated sludge. The effect of tested samples was determined from the change in oxygen uptake rate of test results in relation to the control.

Table 4.4. Effect of azo dyes and their photo-degradation products on oxygen uptake by activated sludge.

Dye	Time of UV/H_2O_2 treatment, min	Change in oxygen uptake rate, %	SD	Effect of sample
Control (distilled water)		89.0	4.90	-
C. I. Acid Red 73	0*	89.3	1.09	+ 0.3
	5	85.4	3.79	- 3.6
	10	79.1	5.84	- 9.9
	20	81.5	2.39	- 7.5
	30	91.1	3.60	+ 2.1
C. I. Acid Blue 158	0	83.4	3.23	- 5.6
	5	83.7	1.00	- 5.3
	10	86.4	2.58	- 2.6
	20	84.6	2.37	- 4.4
	30	93.7	1.56	+ 4.7
C. I. Acid Yellow 99	0	86.2	0.0	- 2.8
	5	85.5	2.04	- 3.5
	10	79.8	1.15	- 9.2
	20	83.8	4.05	- 5.2
	30	89.5	1.32	+ 0.5
C. I. Acid Blue 113	0	94.0	0.0	+ 5.0
	5	84.3	1.81	- 4.7
	10	81.7	0.62	- 7.3
	20	89.4	3.10	+ 0.4
	30	92.1	2.31	+ 3.1

Table 4.4 (continued)

C. I. Acid Blue 92	0	92.7	1.15	+ 3.7
	5	84.1	2.52	– 4.9
	10	83.0	2.38	– 6.0
	20	87.8	0.53	– 1.2
	30	90.3	3.44	+ 1.3
C. I. Basic Red 18	0	89.7	3.29	+ 0.7
	5	90.1	1.72	+ 1.1
	10	90.4	0.81	+ 1.4
	20	93.8	0.0	+ 4.8
	30	96.0	1.67	+ 7.0
C. I. Direct Red 23	0	89.1	1.86	+ 0.1
	5	84.5	4.09	– 4.5
	10	80.6	7.21	– 8.4
	20	82.7	3.17	– 6.3
	30	95.6	1.05	+ 6.6

*) Initial dye solutions at concentration of 25 mg/dm^3 without addition of hydrogen peroxide (before photochemical treatment). SD is standard deviation.

The effect of test samples estimated as a difference in oxygen uptake for test sample in relation to control (units = %): "-" inhibition, "+" stimulation of the oxygen uptake rate.

The results presented in Table 4.4 show that the initial solutions of the majority of azo dyes before photochemical treatment, at a concentration of 25 mg/dm^3, do not cause inhibition of aerobic respiratory function of activated sludge. However, exceptions were the metal (chromium) - containing dyes, Acid Blue 158 and Acid Yellow 99. These dyes, at the same initial concentration tested, inhibited the oxygen uptake rate by 5.6 % and 2.8 % respectively.

Inhibition of oxygen uptake of no more than 10 % was observed for dye samples tested during first 10 minutes of the photochemical treatment, as shown in Figure 4.6. Taking into consideration the results on the determination of residual concentrations of peroxides in irradiated solutions (Table 4.3), the inhibition in the oxygen uptake by these samples was probably due to the increased peroxide concentration for the same treatment period. With further increase of the time of photochemical treatment, a gradual increase in oxygen uptake rate was observed for all azo dyes tested (Figure 4.6).

In contrast, for Acid Blue 158 a decrease in oxygen uptake by 20 min of treatment following an initial increase after 10 min was obtained (Figure 4.6). Nevertheless, the behaviour of this dye was found to be similar comparing the effect on oxygen uptake of *Pseudomonas putida* with activated sludge. Another exception was Basic Red 18. The solutions of this dye, which showed a stimulation of oxygen uptake by activated sludge for the initial dye solution and for all post-treatment samples.

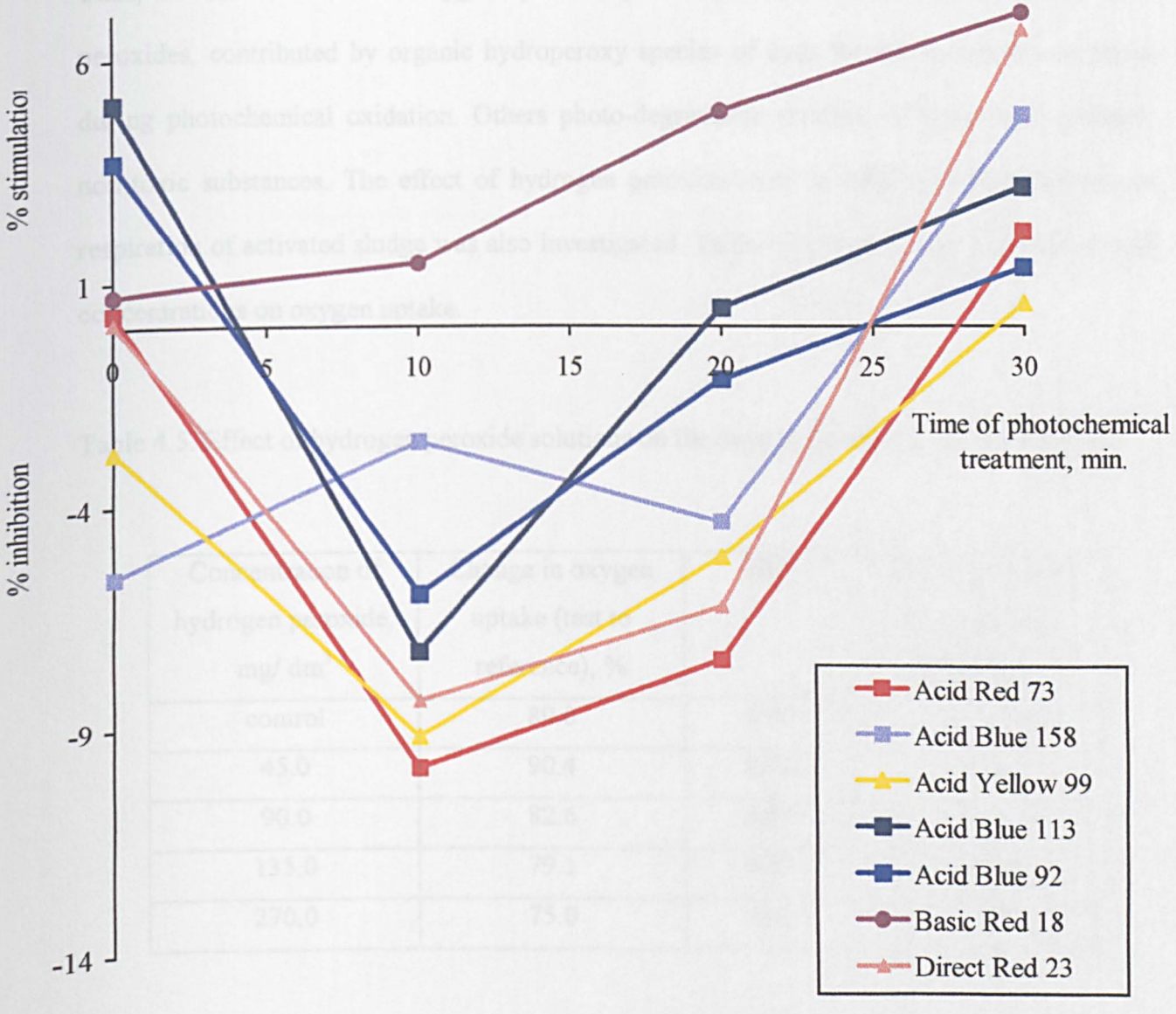


Figure 4.6. Effect of azo dye solutions after photochemical treatment on oxygen uptake rate of activated sludge.

Thus, the initial reduction in oxygen uptake may be caused by an increase in concentration of peroxides, contributed by organic hydroperoxy species of dyes formed in reaction mixtures during photochemical oxidation. Others photo-degradation products of dyes were probably non-toxic substances. The effect of hydrogen peroxide itself at different concentrations on respiration of activated sludge was also investigated. Table 4.5 presents the results of selected concentrations on oxygen uptake.

Table 4.5. Effect of hydrogen peroxide solutions on the oxygen uptake of activated sludge.

Concentration of hydrogen peroxide, mg/ dm^3	Change in oxygen uptake (test to reference), %	SD	Effect* of sample (in relation to control), %
control	89.0	4.90	-
45.0	90.4	2.32	+ 1.4
90.0	82.6	3.27	- 6.4
135.0	79.1	6.87	- 9.9
270.0	75.0	0.0	- 14.0

*) The effect of test: "-" inhibition, "+" stimulation of the oxygen uptake rate. SD – standard deviation. Each result is an average of five repetitions.

Data present in Table 4.5 and the dose – response curve shown in Figure 4.7 demonstrated that hydrogen peroxide at low concentrations (about 45 mg/dm^3) did not inhibit but stimulated the oxygen uptake.

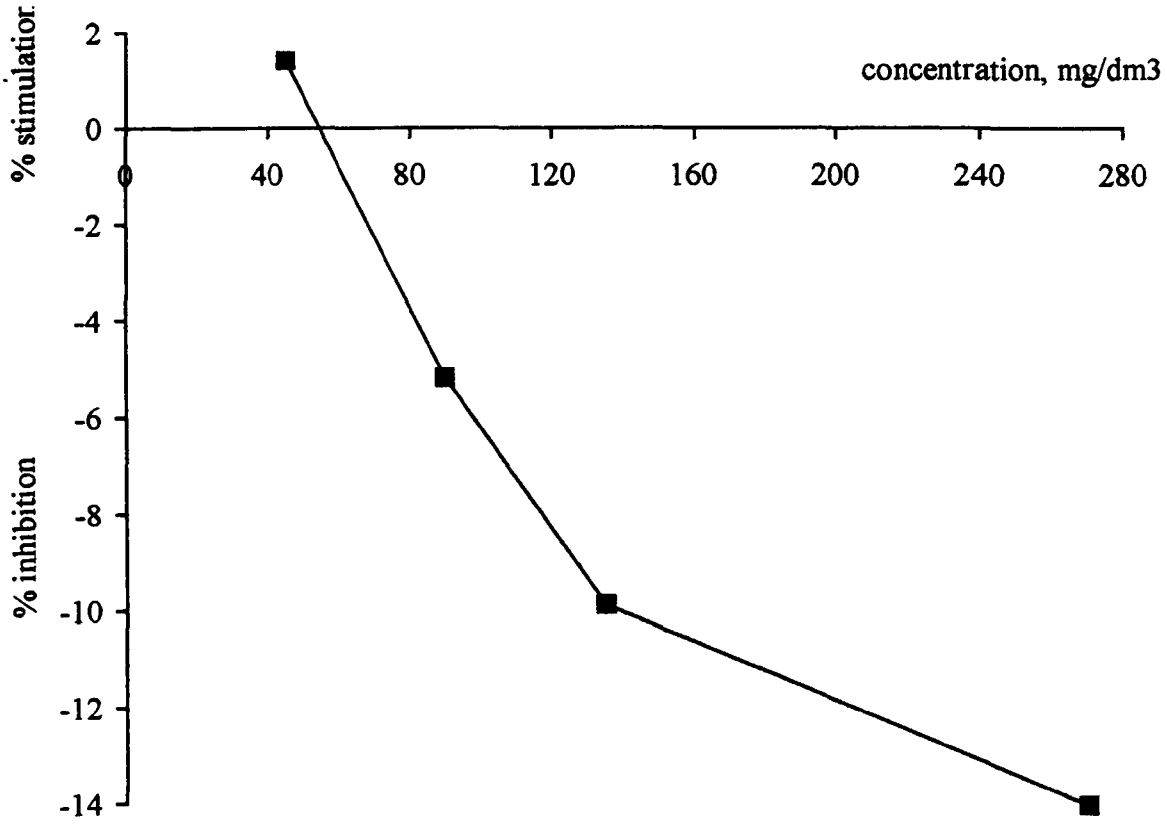


Figure 4.7. Data plotted for effect (% inhibition or stimulation) on the oxygen uptake of activated sludge against initial concentration of hydrogen peroxide.

Hydrogen peroxide at higher concentrations inhibited oxygen uptake and this effect increased with the further increase of hydrogen peroxide concentration. The results showed that the increase in the oxygen uptake rate by dye samples after 20 minutes of treatment (Figure 4. 6) occurs while the concentration of hydrogen peroxide in these solutions reduced to a non toxic level of 60.4 mg/dm^3 (Figure 4.7) which does not inhibit activated sludge respiration.

During the study of toxicity and biodegradability of azo dye solutions (initial and after treatment) using the test based on oxygen uptake of activated sludge the capability of activated sludge to reduce the colour in these dye solutions was also monitored. For these experiments, decolourization of dye solutions was carried out under the same photolysis parameters (initial dye and hydrogen peroxide concentrations were 25 mg/dm^3 and 90 mg/dm^3 respectively, temperature 25°C and pH 7.0). Samples were withdrawn every 5 minutes of the treatment.

The samples collected for testing were mixed with activated sludge to a final volume of 25 ml. Ratios of activated sludge (used without prior dilution) to dye solution of 25, 50 and 75 % were used. Appropriate quantities of each dye sample and activated sludge were distributed into 100 ml flasks and kept at constant temperature of 30°C in a rotary-orbital incubator over a period of 6 days. After certain times of the incubation period 2 ml from the flasks was taken, centrifuged at 13000 rev/min for 5 minutes to separate the aqueous phase (supernatant) for analysing the change in optical density of test solutions. Biodegradation of azo dyes was estimated by the change in dye concentration in solutions in relation to the samples just after photochemical treatment. The change in dye concentration was determined spectrophotometrically at the adsorption maximum for each dye (according to spectrometric

characteristics of dyes reported in Chapter 2). The experimental results are summarized in Table 4. 6.

Table 4.6. Biochemical degradation of initial azo dyes and dye solutions after photochemical oxidation by activated sludge.

Dye C_o = 25 mg/dm^3	Time of photolysis, min	Colour removal [a] at indicated photolysis time, %	Ratio Dye/AS [b] % (v/v)	% Colour removal (of a dye at indicated photolysis time) over treatment time with AS, hours			
				0	24	72	144
Acid Red 73	0	25.0	75 / 25	2.8	6.8	9.1	-
	5	66.3		21.1	21.4	21.5	25.6
	10	78.4		14.1	14.7	15.4	16.4
	15	87.0		9.0	9.7	10.0	10.6
	20	93.1		4.3	5.1	5.6	5.9
	0	50.0	50 / 50	6.2	13.1	19.2	-
	5	77.5		13.2	13.3	13.5	16.7
	10	85.6		9.2	9.7	10.6	11.7
	15	91.3		7.1	7.6	8.1	8.4
	20	95.4		4.6	- [c]	-	-
	0	75.0	25 / 75	2.4	5.8	8.1	-
	5	88.8		4.7	5.0	5.1	9.5
	10	92.8		4.4	5.0	5.9	7.0
	15	95.7		4.3	- [c]	-	-
	20	97.7		2.3	-	-	-

Table 4.6. (continued)

Dye Co = 25 g/dm^3	Time of photolysis, min	Colour removal [a] at indicated photolysis time, %	Ratio Dye/AS [b] % (v/v)	% Colour removal (of a dye at indicated photolysis time) over treatment time with AS, hours		
				0	24	72
Acid Red 73	10	85.6	50/50	9.2	9.7	10.6
Acid Blue 113	10	77.2		12.3	17.1	18.3
Acid Yellow 99	10	85.7		3.8	4.8	6.4
Basic Red 18	10	86.7		6.3	7.8	8.5
Direct Red 23	10	86.3		7.5	9.2	10.6
Acid Blue 158	10	83.7		11.6	12.0	13.1
Acid Blue 92	10	88.8		6.8	7.6	8.7

[a] colour removal (%) observed after mixing the dye solutions taken for tests after indicated time of photochemical treatment with appropriate quantities of activated sludge.

[b] AS – activated sludge.

[c] these solutions did not absorb in the visible region at the absorption maximal wavelength of each dye tested.

From Table 4.6 the initial solutions of C. I. Acid Red 73 were not decolourised significantly for treatment periods of up to 72 hours with different concentration of activated sludge. After photochemical treatment solutions of dye are more susceptible to the biochemical action of activated sludge. However, colour removal from these samples appeared smaller with increase

in photo-treatment time. For instance, the colour removal from solutions of C. I. Acid Red 73 after 5, 10, 15 minutes of photochemical treatment was 55.1, 71.2 and 82.6 % respectively, while colour removal of these solutions after 144 hours of subsequent treatment with activated sludge (at ratio dye/AS of 50/50 % v/v) was 16.7, 11.7 and 8.4 % respectively. The decolourization of dye solutions after photochemical oxidation and subsequent activated sludge treatment for this period did not exceed 91.0 %. An increase of activated sludge biochemical treatment time from 24 to 144 hours also did not have an influence on colour removal as seen in Table 4.6.

Comparing results on colour removal by activated sludge from dye solutions preliminary photochemically treated for 10 minutes none of the azo dyes tested was completely colourless for the period of 72 hours. The dyes C. I. Direct Red 23, C. I. Basic Red 18 and C. I. Acid Blue 92 were decolourized at the same ratio dye/AS to a greater degree than others. Removal of residual colour from these dye solutions as a whole was in the range of 82 – 85.5 % after 72 hours of biotreatment.

The results show that the residual colour present in dye solutions initially decolourized by photochemical oxidation (over 5 – 10 minutes) by 50 – 70 % of initial dye concentration was not rapidly removed by activated sludge. Thus, preliminary photochemical treatment of dyes should be carried out to complete destruction of dye chromophore structure and decolourization of solutions. This will decrease the time of biochemical treatment to achieve the mineralization of photo-degradation products.

The time of photochemical pre-treatment which probably is necessary for effective performance of subsequent biological process is no more than 15 – 20 minutes (depending on dye class and chemical structure). For this photochemical treatment time dye solutions are becoming almost completely colourless. Moreover the concentration of residual hydrogen peroxide and organic peroxides in solutions, which can have negative affect on biochemical activity of activated sludge, dramatically decreases within this treatment time.

Conclusions and recommendations.

1. The photochemical treatment method was demonstrated to be effective for the rapid decolourization and decomposition of some azo dyes.
2. The kinetics of the photo-oxidation reaction has been investigated and was found to be pseudo first order with respect to the dye. In the presence of dissolved oxygen in dye solutions a pronounced effect on colour removal requires long UV irradiation time. Combined action of UV light and hydrogen peroxide significantly enhances photo-fading process of the dyes and reaction order was found to be almost pseudo first.
3. The factors influencing UV/ hydrogen peroxide photochemical oxidation such as initial concentration of dyes and hydrogen peroxide and their ratio, temperature and pH were studied. The optimum value of H_2O_2 added to the irradiated solution below and above which the dye decolourization rate is inhibited is a molar ratio $[H_2O_2]/[Dye]$ of 200/1. Temperature changes in the tested range of 25 - 60°C slightly effect the decolourization rate which increases with increasing temperature. Photochemical decolourization as a function of pH was accelerated in acidic and particularly in alkaline solutions. There is no need to optimise pH before UV treatment as dyeing processes are generally carried out either in acidic or alkaline solutions.
4. A pathway for the oxidation of azo dyes using hydrogen peroxide was suggested. This type of photo-degradation is destructive oxidation involving rapid breakdown of the azo group to produce, before complete mineralization, colourless intermediate substances such as naphthalene (naphthoquinone) and benzene (phenol) derivatives, and organic acids including phthalic acid. Formation of potentially carcinogenic aromatic amines does not occur.

5. The combination of analytical methods to identify photochemical breakdown products with biological methods to assess their biodegradability and potential for causing environment damage was used. Photo-decolourization of azo dyes without addition of hydrogen peroxide does not cause reduction in the toxicity of dye solutions. The use of hydrogen peroxide as an oxidising agent in the UV treatment results not only in rapid decolourization of dye solutions but also in significant reduction in their toxicity to micro-organisms.
6. Screening for the biodegradability levels of dye solutions during the photo-oxidation were done and optimum conditions for the combination of photochemical and biological treatments of dyes were found. Residual colour in dye solutions partially photo-decolourized was not rapidly removed by activated sludge and adequate preliminary photochemical treatment should be carried out to complete destruction of dye chromophore structure and decolourization of solutions. This will increase the effectiveness of subsequent biological process in achieving complete mineralization of photo-degradation products.
7. The common textile effluents form streams with different amounts of pollutants in each case. It would be therefore useful to separate waste streams and to perform photochemical treatment of the spent dye bath solutions in a local treatment system with further water reuse. This approach will save water, chemicals loaded and energy. Investigations should be carried out to establish effects of reused water on quality of fabrics and performance of technological operations in the dyeing process.
8. Textile waste effluents generated from the dyeing process contain, as well as dyes, large amounts of surfactants (particularly non-ionic (phenol)-ethoxylated detergents). The photochemical oxidation method could be used for photo-degradation of these substances included in lists of dangerous compounds, already subject to control and

reported as potential endocrine disruptors. Further studies are needed to evaluate feasibility and efficiency of their photo-degradation.

9. The relative costs of electrical power, waste disposal to landfill, discharge into water and incineration, with national legislation prohibiting or restricting certain disposal routes, will influence the economics of the process. Water costs will also be significant as the photo-oxidation technology allows water recycling. These costs will vary from country to country but increasingly strict environmental legislation can be expected in Russia and European Union. The demands of sustainable development and clean, low environmental impact technology can be met using this process.

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APPENDIX A.

List of publications.

1. Попова С. В., Архипова М. Б., Музыченко Е. А. Разработка и токсикологическая оценка фотоокислительной технологии очистки сточных вод текстильных предприятий. Материалы НТК студентов и аспирантов «СПГУТД Дни науки – 98», С.60-61.

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