İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

EFFECT OF FENTON TREATMENT OF ACID AND REACTIVE DYES ON ANAEROBIC, ANOXIC AND AEROBIC BIOLOGICAL PROCESSES

M.Sc. Thesis by
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Programme: Environmental Sciences and Engineering

JUNE 2007

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Date of submission : 7 May 2007

Date of defence examination: 13 June 2007

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JUNE 2007

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ASİT VE REAKTİF BOYALARIN FENTON ARITIMININ ANAEROBİK, ANOKSİK VE AEROBİK BİYOLOJİK PROSESLER ÜZERİNDEKİ ETKİSİ

YÜKSEK LİSANS TEZİ

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Tezin Enstitüye Verildiği Tarih : 7 Mayıs 2007

Tezin Savunulduğu Tarih : 13 Haziran 2007

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HAZİRAN 2007

ACKNOWLEDGEMENTS

This master thesis has been carried out at İstanbul Technical University, Environmental Sciences and Engineering Programme and Denmark Technical University, Institute of Environment and Resources. I would like to thank everyone, involved in my work.

I would like to express my deepest gratitude and thanks to my supervisor Assoc. Prof. Dr. İdil Arslan Alaton, who has supported and encouraged me from beginning of this study and shared her knowledge and experience. Under her supervision, I always felt confident about how I should find the right answers to the problems that I faced to.

I would like to express my deepest appreciation to my co-supervisor Assoc. Prof. Dr. Jens Ejbye Schmidt from DTU for his guidance, recommendations and support throughout this research, who taught me how to search for unknown and enjoy the discovered.

I would like to express my thanks to my technical supervisor Hector Garcia from DTU, for his invaluable support and experience during my time in DTU and also the desperate moments in cases of emergency.

I would finally like to express my ultimate appreciation to my parents, for their encouragement, patient and support about every cases of my life.

June, 2007 **Betül Hande GÜRSOY**

ABBREVIATIONS

AOT	: Advanced Oxidation Technologies
AOP	: Advanced Oxidation Process
AR 183	: Acid Red 183
AO 51	: Acid Orange 51
RB 4	: Reactive Blue 4
COD	: Chemical Oxygen Demand
DOC	: Dissolved Organic Carbon
TOC	: Total Organic Carbon
BOD	: Biochemical Oxygen Demand
NO_3^--N	: Nitrate-Nitrogen
CI	: Color Index
UNEP	: United Nations Environment Programme
EPA	: Environmental Protection Agency
ETAD	: Environmental Technology Advancement Directorate
EC	: Effective Concentration
IC	: Inhibitory Concentration
LC	: Lethal Concentration
ED	: Effective Dose
ID	: Inhibitory Dose
LD	: Lethal Dose
VSS	: Volatile Suspended Solids
TSS	: Total Suspended Solids

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EFFECT OF FENTON TREATMENT OF ACID AND REACTIVE DYES ON ANAEROBIC, ANOXIC AND AEROBIC BIOLOGICAL PROCESSES

SUMMARY

The textile preparation, dyeing and finishing industry generates huge volumes of colored effluents with medium to-high chemical oxygen demand (COD), total dissolved solids (TDS), heavy metals and nonionic surfactances. Especially the textile dyeing process constitutes a major pollution problem due to the variety and complexity of chemicals employed. Among the textile auxiliaries, dyes have attracted most attention since aesthetic deterioration and hindrances of light penetration in to the ecosystem caused by color, some dyes and their degradation products are potentially toxic or even carcinogenic in character. As a consequence, it is essential to treat dyes from textile wastewater prior to discharge in to publicly owned treatment works and/or receiving water bodies.

It is known that conventional treatment of textile wastewater is not effective and not successful decolorization. Advanced Oxidation Processes (AOPs) based on the oxidative degradation of organic compounds using hydroxyl radical ($^{\bullet}OH$) are alternative methods for chemical pre-treatment of recalcitrant wastewater. Among AOP, the Fenton' reagent (Fe^{2+}/ H_2O_2) is one of the most effective and successful on complete color and partial COD removal from textile wastewater.

However, it should be kept in mind that advanced oxidation intermediates might be more toxic and/or inhibitory on anaerobic, anoxic and aerobic biological treatment systems than the original textile dyes.

In the present study, the ability of Fenton's reagent to oxidize two commercial acid (Acid Red 183-AR 183; Acid Orange 51-AO 51) and one fiber reactive dye (Reactive Blue 4-RB 4) as well as the effect of Fenton oxidation on the toxicity of their degradation products was investigated. In the first part of the experimental work, the case-specific catalyst : oxidant (Fe^{2+} : H_2O_2) molar ratio and treatment time were optimized. In the second part of the study, the toxic/inhibitory effect of the

untreated and Fenton-treated dyes was assessed in terms of 1) methane production rate during anaerobic experiments, 2) denitrification (NO_3^- - N abatement) rate during anoxic experiments and 3) glucose (equivalent to 500 mg O_2/L COD; average F/M = 0.13 mg COD_o / mg VSS) degradation rate during aerobic experiments. The optimum Fe^{2+} : H_2O_2 molar ratio and treatment time were determined as 1: 5 (4 mM : 20 mM; pH_o = 3.0) and 10 min, respectively. Under these conditions, 100 %, 100 %, and 93 % color, 58 %, 78 %, and 69 % COD as well as 54 %, 76 %, and 49 % DOC (dissolved organic carbon) removal efficiencies were obtained for AR 183, AO 51 and RB 4 dyes, respectively. Methane production rate was seriously (by 100 %) inhibited in the case of untreated RB 4, whereas 29 % was found for untreated AR 183 and there was no inhibition for untreated AO 51. On the other hand, Fenton treated dyes except RB 4, had no inhibitory effect on methane production. There was 21 % inhibitory effect of treated RB 4 on methane production. For nitrate consumption, neither the untreated nor treated textile dyes showed any inhibitory effect on denitrification. Glucose degradation was slightly inhibited via untreated dyes (23-29 % relative inhibition to control). Fenton-treated dyes had no inhibitory effect on glucose degradation.

ASİT VE REAKTİF BOYALARIN FENTON ARITIMININ ANAEROBİK, ANOKSİK VE AEROBİK BİYOLOJİK PROSESLER ÜZERİNDEKİ ETKİSİ

ÖZET

Tekstil endüstrisi oldukça büyük hacimlerde, yüksek kimyasal oksijen ihtiyacı (KOİ), toplam çözünmüş madde, ağır metal ve aniyonik yüzey aktif maddeler içeren çıkış suları üretmektedir. Estetik açıdan oluşturduğu kirlenme ve ışığın ekosisteme ulaşmasının engellenmesinin yanı sıra, bazı boyar maddeler ve bunların bozunmalarıyla oluşan ürünler potansiyel toksik hatta kanserojen olabilmektedir. Bunun sonucunda tekstil atıksularındaki boyar maddelerin evsel atıksu arıtma tesislerine ve/veya alıcı ortama deşarj edilmeden önce ön arıtımları şarttır.

Konvansiyonel arıtım yöntemlerinin, tekstil atıksuyu arıtımında ve renk gideriminde etkili ve başarılı olmadığı bilinmektedir. Organik maddelerin hidroksil radikali ($^{\bullet}OH$) ile oksidatif arıtımına dayanan İleri Oksidasyon Prosesleri (İOP), arıtmaya dirençli atıksuların kimyasal ön arıtımı için etkili bir yöntemdir. Birçok İOP arasında Fenton reaktanı (Fe^{2+}/ H_2O_2), tekstil atıksulardan renk ve kısmi KOİ gideriminde etkili ve başarılı bir yöntemdir.

Bunların dışında, ileri oksidasyonla oluşan ara ürünlerin, orijinal tekstil boyasına göre anaerobik, anoksik ve aerobik arıtım sistemleri üzerinde daha toksik/inhibisyon etkisine sahip olabilirliği dikkate alınmalıdır.

Bu çalışmada asit ve reaktif boyaların (Acid Red 183-AR 183; Acid Orange 51-AO 51) Fenton reaktanı ile arıtılabilirlikleri ve oksidasyon sonucu oluşan ara ürünlerin toksik etkileri araştırılmıştır. İlk bölümde Fe^{2+}: H_2O_2 konsantrasyonları ve arıtım süresi KOİ, çözünmüş organik madde ve renk giderimi göz önüne alınarak optimize edilmiştir. Çalışmanın ikinci kısmında, arıtılmamış ve Fentonla arıtılmış boyaların toksik/inhibisyon etkisi; 1) anaerobik deneylerde metan üretim hızına, 2) anoksik deneylerde denitrifikasyon hızına (NO_3^- - N azalışına), 3) aerobik deneylerde glikozun (500 mg O2/L eşdeğerinde; F/M = 0,13 mg CODo/mg VSS) degradasyon hızına göre değerlendirilmiştir. Optimum Fe^{2+}: H_2O_2 molar oranları ve artım süresi 1:5 (4 mM : 20 mM, pH_o=3.0) ve 10 dakika olarak belirlenmiştir. Bu şartlar altında

100 %, 100 %, ve 93 % renk, 58 %, 78 %, ve 69 % KOİ ayrıca 54 %, 76 %, ve 49 % çözünmüş organik karbon verimleri sırasıyla AR 183, AO 51 ve RB 4 boyaları için elde edilmiştir. Arıtılmamış boyalarda metan üretim hızı RB 4 varlığında ciddi bir oranda (%100) inhibe olmuşken, 29 % inhibisyon AR 183 varlığında gözlenmiştir, AO 51'in ise inhibisyon etkisi olmamıştır. Diğer taraftan metan üretiminde Fenton ile arıtılmış RB 4 dışında diğer boyaların inhibisyon etkisi gözlenmemiştir. Arıtılmış RB 4 ise 21 % oranında metan üretimi üzerinde inhibisyon göstermiştir. Nitrat gideriminde arıtılmış ve arıtılmamış boyaların hiç birinin denitrifikasyon üzerinde inhibisyon etkisi olmamıştır. Glikoz degradasyonunda ise arıtılmış boyalarda kontrole göre 23–29% inhibisyon etkisi görülse de arıtılmamış boyalarda inhibisyon etkisi yoktur.

1. INTRODUCTION

The textile preparation, dyeing and finishing industry is one of the highest water consumers among different industrial sectors and produces 50 to 100 L wastewater per kg of finished product. From the environmental point of view, particularly the textile dyeing process constitutes a major pollution problem due to the variety and complexity of chemicals (dyes, sequestering agents, tannins, dye carriers, leveling agents, dispersing agents, etc.) employed. Among the textile auxiliaries, dyes have attracted most attention since color in dyehouse effluent not only causes environmental concerns, but also creates a significant aesthetic problem in sewage treatment works and receiving water bodies. Conventional chemical (coagulation-flocculation) and biological (activated sludge, sequential bed reactors, anaerobic/anoxic) processes are widely used for textile wastewater treatment, however, with limited success. Although some of the more biodegradable dye auxiliaries can in some cases be completely eliminated from dyehouse effluent, these treatment systems cannot achieve "destructive" decolorization due to the fact that textile dyes are intentionally/specifically designed to resist aerobic, photolytic and chemical degradation. These effluents often require pre-treatment of segregated process streams (e.g. dyebath effluent) using alternative, advanced oxidation technologies (AOTs) that have more recently been used to treat refractory and/or toxic pollutants. Among the AOTs, the Fenton's process is one of the most well known and established ones being successfully applied for the treatment of industrial wastewater including dyehouse effluent. Moreover, the Fenton's reagent is relatively cheap and easy to handle compared with other AOTs. Fenton's reagent being a mixture of hydrogen peroxide and ferrous iron is effective for complete color and partial COD removal from textile effluent at acidic pH and thus an attractive option to prepare recalcitrant process streams to conventional activated sludge treatment of the combined wastewater. However, it should be kept in mind that advanced oxidation intermediates might be more toxic and/or inhibitory on anaerobic, anoxic and aerobic biological treatment systems than the original textile dyes.

Considering the above mentioned facts, the aim of the present experimental study was to evaluate the effect of Fenton's treatment of frequently employed textile industry dyes on their toxic/inhibitory effect in conventional biological (anaerobic/anoxic/aerobic) treatment systems. For this purpose, two acid dyes, namely Acid Red 183 and Acid Orange 51, and one fiber reactive dye, i.e. Reactive Blue 4, were selected as model textile dyes and subjected to Fenton's treatment under different oxidant (hydrogen peroxide) and catalyst (ferrous/ferric iron) concentrations.

The experimental study comprises two stages; e.g. advanced oxidation of the selected textile dyes and elucidation of their effects on anaerobic, anoxic and aerobic biological treatment systems. In the first part of the study, baseline experiments were conducted at a pH of 3 to optimize case-specific Fenton treatment conditions (i.e. the Fe^{2+}:H_2O_2 molar ratio) in terms of color, COD and DOC removal rates. In the second part of the study, the toxic and/or inhibitory effect of the untreated and Fenton-treated textile dyes was examined in terms of relative (a) methane production, (b) denitrification and (c) activated sludge organic carbon (glucose) abatement rates.

2. THEORETICAL BACKGROUND

The textile industry, apart from being an important contributor to do economy of numerous countries, is also major source of various liquid, solid and gaseous wastes. This kind of industrial activity can have a negative impact on the environment, both in terms of pollutant discharge as well as energy and wastewater is largely depend upon the specific type of operations followed, in general dyeing, washing and finishing operations.

Textile dyeing is the most water consuming and chemically intensive process among all textile preparation, dyeing, washing and finishing stages. In a textile industry about 40-65 L of wastewater is generated per kg of cloth produced (Manu et al., 2001).

Different dyestuffs selected for dyeing material, have different characteristics. Therefore the composition of dyeing effluent varies with the textile produced. Effluent from textile dyeing contains residuals of dyebath auxiliaries and unfixed dyestuff at significant concentrations. The nature of textile effluent depends on fashion, technical and economical factors.

Textile dyes are required to have a high degree of a chemical and photolytic stability in order that they maintain their structure and color. They are designed to resist breakdown attributable to time and exposure to sunlight, water, soap and other parameters such as bleach and perspiration. Anti-microbial agents are frequently used to make textiles, particularly natural fibers such as cotton, resistant to biological degradation.

The color fastness, stability and resistance of dyes to degradation have caused difficulties about color removal as they are not readily degraded under the aerobic conditions prevailing in biological treatment plants and therefore the effluents are in the most cases colored when leaving the plant. Recalcitrant nature of most chemicals

biodegradation and also most physicochemical remediation methods such as adsorption, coagulation or precipitation can inhibit the performance of the treatment plant and cause aesthetic problems and threat aquatic life receiving waters.

2.1. Textile Dyes and Their Classification

Textiles are dyed using a wide range of dyestuffs, techniques and equipment. A dye is used to impart color to materials of which it becomes an integral part. Over 700,000 tons approximately 10,000 types of dyes and pigments are produced annually worldwide. From this amount about 20 % are discharged as industrial effluents during the textile dyeing and finishing processes without previous treatment (Patricia et al., 2006). Dyes used by the textile industry are largely synthetic, typically derived from coal tar and petroleum-based intermediates. Dyes are sold as powders, granules pastes and liquid dispersions, with concentrations of active ingredients ranging typically from 20 to 80 percent.

An aromatic ring structure coupled with a side chain usually required for resonance and thus to impart color. Resonance structures that cause displacement or appearance of adsorption bands in the visible spectrum of light are responsible for color. Correlation of chemical structure with color has been accomplished in the synthesis of dye using a chromogen-chromophore with auxochrome. Chromogen is the aromatic structure containing benzene, naphthalene, or anthracene rings. A chromophore group is a color giver and is represented by the following radicals, which from a basis for the chemical classification of dyes when coupled with the chromogen: azo (-N=N-); carbonyl (=C=O); carbon (=C=C=); carbon-nitrogen (>C=NH or –CH=N-); nitroso (-NO or N-OH); nitro (-NO_2 or =NO-OH); and sulfur (>C=S, and other carbon-sulfur groups). The chromogen-chromophore structure is often not sufficient to impart solubility and cause adherence of dye to fiber. The auxochrome or bonding affinity groups are amine, hydroxyl, carboxyl and sulfonic radicals, or their derivatives. These auxochromes are important in the use classification of dyes (Hunger, 2003).

The primary classification of dyes is based on fibers to which they can be applied and the other classification depends on chemical nature of each dye.Usage classification is advantageous to consider the classification of dyes by use or method of application before considering chemical structures in detail. Most commonly in

use today are reactive and direct types for cotton dyeing; disperse types for polyester dyeing and acid dyes for nylon dyeing. Vat dyes such as indigo are also commonly used for cotton and other cellulosic fibers.

Reactive dyes form a covalent bond with the fiber. Direct dyes are water-soluble anionic dyes, when they dyed from aqueous solution in the presence of electrolytes, are substantive to, i.e., have high affinity for, cellulosic fibers. Disperse dyes are substantially water-insoluble nonionic dyes for application to hydrophobic fibers from aqueous dispersion. Vat dyes are water-insoluble dyes, mainly applied to cellulosic fibers. Sulfur dyes are applied to cotton, although having low cost and good washfastness, they are under pressure from environmental viewpoint.

The most appropriate system for the classification of dyes is by chemical structure, which has many advantageous. First it readily identifies dyes as belonging to group that has characteristic properties, for example azo dyes (strong, good, all-round properties, cost effective) and anthraquinone dyes (weak, expensive). Second, there is manageable number of chemical groups (about a dozen). Most importantly, it is the classification used mostly widely by both the synthetic dye chemist and the dye technologist (Hunger, 2003).

Chemical classification of the dyes is based on their chromophore groups. They are classified as azo, anthraquinone, nitro, nitroso, triphenylmethane, xanthene, acridine, thiazole, sulfur, indigoid, phthalocyanine dyes. Among these, azo type dyes and anthraquinone dyes are mostly used among the others (Haresh, 2003)

Table 2.1: Application classes of dyes and their chemical types (O'Neill et al., 1999)

Dye Class	Substrate	Method of Application	Dye Chromophore Type
Acid	Nylon, wool, silk, paper, inks and leather	Usually from neutral to acidic dyebaths	Azo including premetallised anthraquinone, triphrnylmethane,azine, xanthene,nitro and nitroso
Basic	Paper, polacrylonitrile modified nylon, polyester and inks	Applied from acidic dyebaths	Azo
Direct	Cotton, rayon, paper, leather and nylon	Applied from neutral or slightly alkaline baths containing additional electrolyte	Oxazine
Disperse	Polyester, polyamide, acetate, acrylic and plastics	Often applied by high temperature-pressure or lower temperature carrier methods	Azo, anthraquinone, stryl, nitro and benzodifuranne
Reactive	Cotton, wool, silk and nylon	Reactive site on dye reacts with functional group on fibre to bind dye covalently under influence of heat and pH (alkaline)	Oxazine and basic
Sulfur	Cotton and rayon	Aromatic substrate vatted with sodium sulfide and re-oxidised to insoluble sulfur-containig products on fibre	Intermediate structures
Vat	Cotton, rayon and wool	Water insoluble dyes solubilised by reducing with sodium hydrosulfite, then exhausted fibre and re-oxidised	Anthraquinone (including polycyclic quinones) and indigoids

Table 2.2: Percentage distribution of each chemical class between major application ranges (O'Neill et al., 1999)

% distribution between application ranges									
Chemical Class	*Acid*	*Basic*	*Direct*	*Disperse*	*Mordant*	*Pigment*	*Reactive*	*Solvent*	*Vat*
Acridine		92		4				4	
Aminoketone	11			40	8		3	8	30
Anthraquinone	15	2		25	3	4	6	9	36
Azine	39	39				3		19	
Formazan	70						30		
Indigoid	2					17			81
Metal-complex azo	65		10				12	13	
Methine		71		23		1		5	
Nitro, nitroso	31	2		48	2	5		12	
Oxazine		22	17	2	40	9	10		
Phthalocyanine	14	4	8		4	9	43	15	3
Quinopthalone	30	20		40				10	
Stilbene			98					2	
Thiazine		55			10			10	25

Azo dyes are the first largest class for dyeing of cotton and constitute about 60-70 % of total dyes produced. They are characterized by the presence of one or more azo bonds (-N=N-) in association with one or more aromatic systems, which may also carry sulfonic acid groups. Azo dyes are electronically unstable and have the capacity to receive electrons from the reduced form of mediators. They are mostly used for yellow, orange and red colors.

Anthraquinone dyes are the second largest class of textile dyes. Anthraquinone dyes are electronically stable and as a result, the reduced form of the mediator will likely be less effective in transferring electrons to the dye. They give a wide range of colors in almost the whole visible spectrum, but they are most commonly used for blue and green colors (Fontonet et al., 2002).

Dye fixation and loss of dyes to the discharged effluent is different for each dye and fiber combination. Reactive dyes have rather low rates of fixation while the highest fixation rates are achieved with basic dyes.

Table 2.3: Estimated fixation degree of different dye-fibre combinations and loss to effluent (O'Neill et al., 1999)

Dye application class	*Fibre*	*Degree of fixation (%)*	*Loss to effluent (%)*
Acid	Polyamide	89-95	5-20
Basic	Acrylic	95-100	0-5
Direct	Cellulose	70-95	5-30
Disperse	Polyester	90-100	0-10
Metal-complex	Wool	90-98	2-10
Reactive	Cellulose	50-90	10-50
Sulfur	Cellulose	60-90	10-40
Vat	Cellulose	80-95	5-20

From the table it can be seen that after reactive dyeing process is complete, up to 10-50 % hydrolyzed dye may remain in the spent bath. Therefore up to 50 % of the original color is lost to the effluent.

2.1.1. Acid dyes

Most acid dyes are sodium salts of organic sulfonic acids and the anion is the active colored component. There are a few of acid dyes containing carboxyl groups. The acid dyes have a direct affinity towards polyamide and protein fibers in an acidic dyebath.

Acid added to the dye bath is the most significant assistant in the application of these acid dyes onto desired fabric. Many acid dyes will not exhaust at all if the dye bath has not been acidified. Both the amount of due adsorbed on the fiber and the rate of exhaustion is dependent to the acidity of the dyebath. Dyeing exhaustion is promoted by acid usage. Depending on acid dye molecular size, acid strength and concentration used should be carefully selected. The smaller the dye structure, the more acid or stronger acid is required to have a lower pH. However, exhaustion of high affinity dyes at higher rate or at low pH would cause uneven dyeing because of the difficulty of dye migration which is necessary to have an even dyeing result.

The acid dyes are relatively easy to dissolve in water because of the presence of water-soluble group, SO^{3+}. However, possibility of undissolved particles becoming deposited on the goods should be taken care to avoid. The required amount of dyestuff is dissolved in water better through a pasting step, preferably with a small amount of anionic wetting agent.

Acid dyes are used for dyeing of mainly nylon fibers, as well as wool, silk and modified acrylics. Acid dyeing of nylon results in ionic bonds or salt links between the dye molecules and polymer. The terminal amino group, $-NH_2$ of nylon provides the dye site where the ionic link is formed. The dyeing of nylon with acid dyes can be represented as follows:

$$\text{Nylon-}NH_2 + H^+ \rightarrow \text{nylon-}NH_{3n}^+ \quad \textbf{(2.1)}$$

Nylon polymer with terminal amino group Hydronium ion nylon polymer with + charged terminal amino group

$$\text{Nylon-}NH_3^+ + DSO_3^- \rightarrow \text{nylon-}NH_3^+.^-SO_3D \quad \textbf{(2.2)}$$

Nylon polymer with + charged dye anion terminal amino group → ionic link formed

In addition to dominant ionic bonds, hydrogen bonds and Vander Waals forces play a role in the bonding between the other part of the colored anion and the fiber. Usually, large dye molecular structure will have a higher affinity to the fiber and better wetfastness.

Salt is usually control the evenness of dyeing with large molecule dyes. Glaubert's salt ($Na_2SO_4.10H_2O$) is usually used as a leveling agent. It acts as a retarding agent. When dissolved in water, sodium sulfate will dissociate and generate SO_4^{2-} anion. These smaller anions in the dye liquor are attracted more than the larger dye anions, DSO_3^-, to the positively charged nylon fibers, nylon NH_3^+. The dye anions moving at a much slower rate will gradually replace the sulfate anion on the dye sites due to the high affinity of their molecular structure, resulting in a much more even dyeing (Fan et al., 2004).

2.1.2. Reactive dyes

Over the decades since the commercial introduction of reactive dyes, their use has grown steadily rather than spectacularly. When they first appeared it was predicted that reactive dyes would largely replace azoic combinations, direct dyes and sulphur dyes, displace vat dyes from outlets where fastness to bleaching was not essential and eventually dominate the dyeing of cellulosic materials. This did not occur even in the relatively sophisticated markets they do not account for more than 30 % of all dyes consumed on cellulose. In the USA, however, where vat dyes have been used preferentially for fast dyes cottons, there has been a more gradual trend in favor of reactive dyes. Greater demand for brilliant hues, a shift towards cotton and cotton-rich blends for apparel and a greater prevalence of short-run lots in the US textile industry have all contributed to this trend.

Reactive Systems;

The four characteristic features of a typical reactive dye molecule are:

- the chromophoric grouping, contributing the color and much of the substantivity for cellulose;
- the reactive system, enabling the dye to react with the hydroxyl groups in cellulose;
- a bridging group that links the reactive system to the chromophore;
- one or more solubilising groups, usually sulphonic acid substituents attached to the chromophoric group.

As much as 50 % of the total cost of a reactive dyeing process must be attributed to the washing-off stages and treatment of the resulting effluent. This aspect of the process should be recognized as a major limitation that prevents reactive dyes from achieving the degree of success that was predicted for them at the time of their discovery.

During the early years of development of reactive dyes it was soon recognized that the important reactive systems could be classified into two distinct categories, depending on the mechanism of formation of the dye-fiber bond and the stability of this bond to subsequent treatments. Those based on nitrogen-containing heterocylic rings bearing halogeno substituents undergo nucleophilic substitution (Figure 2.1). The heteroatoms in the arly ring activate the system for nucleophilic attack because of their electronegativity. The attacking nucleophilic can be either a cellulosate anion or a hydroxide ion, the former leading to fixation on the fibre and the latter resulting in hydrolysis of the reactive dye.

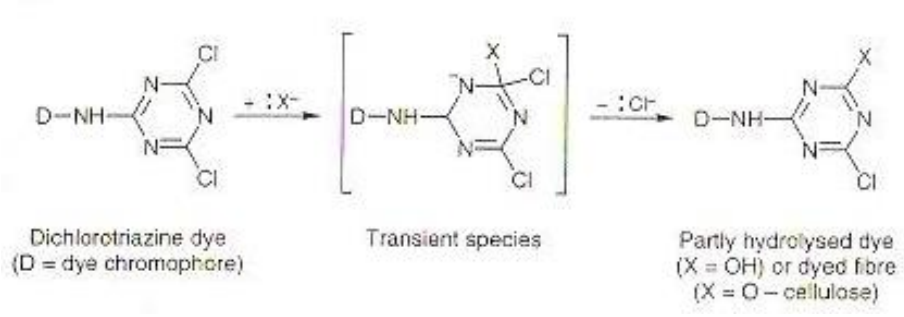


Figure 2.1: Reaction of dichlorotriazinyl group containing reactive dye

Remazol (HOE) dyes, based on the 2-sulphatoethylsulphone precursor of the vinysulphone reactive system (Figure 2.2) or related species, function by a nucleophilic addition mechanism rather than substitution. Before this can occur, however, alkaline 1,2-elimination of the precursor grouping is necessary to release the reactive vinylsulphone system. In this system the carbon-carbon double bond is polarized by the powerfully electron-attracting sulphone group. This polarization confers a positive character on the terminal carbon atom, favoring nucleophilic addition of either a cellulosate anion or a hydroxide ion, again leading to either fixation or hydrolysis respectively.

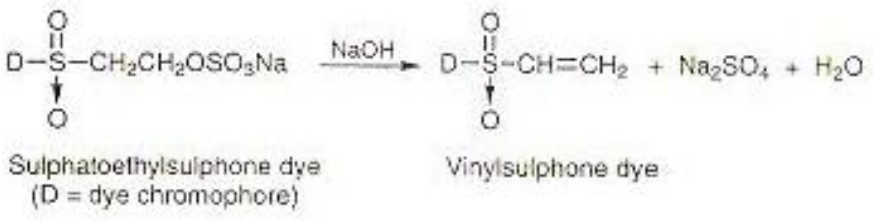


Figure 2.2 :Reaction of vinylsulphone group containing reactive dye

The presence of two types of dye-fibre bond has certain consequences for fastness properties. Mixed bifunctional dyes show superior fastness to acid storage then dichlorotriazine or dichloroquinoxaline systems and better fastness to peroxide washing than difluoropyrimidine or dichloroquinoxaline dyes. Conversely, mixed bifunctional dyes do show some of the weakness to alkaline treatments characteristic of vinylsulphone dyes, although less so than monofunctional dyes relying solely on these groups for fixation.

The design of reactive dye structures almost always involves one or more compromises between conflicting requirements. There is a seldom an ‘ideal’ structure of a desired hue that embodies all possible attractive features with regard to application and fastness properties. The gain in aqueous solubility provided by an extra sulpho group often has to be paid for by a decrease in affinity for cellulose. Enhancement of substantivity is beneficial for high exhaustion but may impair migration of washing-off characteristics. High reactivity offers the possibility of rapid fixation but storage stability may be adversely affected.

Reaction between the dye and cellulose can occur only when the dye has been absorbed into the cellulose phase. Thus the kinetics of the dye-cellulose reaction are

strongly influenced by the rate of absorption of the dye. The ratio of the rate constants for the reaction of the dye with the fibre and with water is a constant for a given dye over a wide range of alkaline pH values.

The efficiency of fixation is a function of:

- the reactivity ratio of rate constants for the fixation reaction and hydrolysis;
- the substantivity ratio, the relative concentrations of the dye absorbed into the substrate and remaining in the dyebath;
- the diffusion coefficient of the dye in the substrate
- the liquor ratio; and
- the surface of the substrate available for absorption of dye.

An increase of dyeing temperature lowers the substantivity ratio and accelerates the rate of hydrolysis of the dye; both of these effects reduce the fixation efficiency. The rates of diffusion into and reaction with the fibre are also accelerated, however, and these factors both favor fixation of the dye. An increase in electrolyte concentration always enhances substantivity without impairing reactivity providing the dye remains completely dissolved.

A valuable classification of reactive dye types has been formulated recently. Three groups relating to the most important control parameter in each case may be distinguished.

Group 1 Alkali-controllable reactive dyes; These dyes have optimal temperatures of fixation between 40 and 60^{o}C. They are characterized by relatively low exhaustion in neutral salt solution before alkali is added. They have high reactivity and care in addition of alkali is necessary to achieve level dyeing, preferably at a controlled dosage rate.

Typical examples of dyes belonging to this group have dichlorotriazine, chlorodifluoropyrimidine, dichloroquinoxaline or vinylsulphone reactive systems.

Group 2 Salt-controllable reactive dyes; Dyes in this group show optimal fixation at a temperature between 80^{o}C and the boil. Such dyes exhibit comparatively high exhaustion at neutral pH, so it is important o add salt carefully to ensure that dyeings

are level. Electrolyte addition is often made portionwise or preferably at a control rate of dosage.

Dyes with these properties typically have low-reactivity systems such as trichloropyrimidine, aminochlorotriazine or bis(aminochlorotriazine). Aminofluorotriazine dyes in the ibacron F (CGY) range have high substantivity and should thus be regarded as salt-controllable but they are sufficiently reactive for fixation at 60^{o}C or even lower temperatures by batchwise application.

Group 3 Temperature-controllable reactive dyes; This group is represented by those dyes that react with cellulose at temperatures above the boil in the absence of alkali, although if desired they can be applied under the same conditions as the salt-controllable group with alkaline fixation at a temperature between 80^{o}C and the boil. Dyes in this group have self-leveling characteristics so there is no need to use auxiliary products to facilitate level dyeing. Good results can be achieved by controlling the rate of temperature rise.

At present only the Kayacelon React (KYK) range of bis (amino-nicotinoatriazine) dyes belong to this group.

Batchwise Application; Reactive dyes can be applied by any conventional batchwise dyeing method for cellulosic materials, including circulating-liquor machines for loose stock, yarn or woven fabrics, as well as jets, winches or jigs for piece dyeing. The conventional dyeing process entails three stages:

1. Exhaustion from an aqueous bath containing electrolyte, normally under neutral conditions;
2. Addition of alkali to promote further uptake and chemical reaction of absorbed dye with the fibre at the optimal pH and temperature;
3. Washing of the dyed material to remove electrolyte, alkali and unfixed dye.

Reactive Dyeing Residues in Waste Liquors; As already discussed in the introduction, approximately half of the cost of a typical reactive dyeing may be attributed to the washing stage and treatment of the resulting effluent. A central objective of research into novel reactive dyes and dyeing process has been the improvement of fixation efficiency and minimization of the proportion of applied

dye that is lost by hydrolysis in the dyebath or by absorption, hydrolysis and removal again at the washing stage.

Reactive dye hydrolysates are not easily adsorbed by sewage sludge in a biological clarification plant. It is desirable to decolorize the liquor at source if practicable. A post-scouring step designed specifically to remove hydrolyzed dye as efficiently as possible, making water treatment of low-load liquors from the rising steps more feasible.

Apart from contamination by unfixed reactive dye residues, probably the most serious ecological problem arising from reactive dyeing is the high salt load in the effluent. Dispensing salt in solid form without significant change in liquor ratio offers wider scope for optimizing quality and cost effectiveness in the package dyeing of yarns and the jet dyeing of fabrics. Improved dye yield and levelness, shorter process times, lower salt usage and environmental benefits are claimed for this technique.

A more fundamental approach to the objective of minimizing contamination of reactive dyeing effluents by excessive salt content is to adjust the dyeing parameters so that only the lowest possible concentrations needs to be added at the dyeing stage. There are four main ways in which that salt consumption can be decreased:

- Lowering the liquor ratio,
- Avoiding high temperatures of dyeing,
- Optimizing the dye recipe,
- Developing novel dyes,

Even under the most favorable circumstances, a concentration of at least 20 g/L was needed in ale depths at relatively low liquor ratio.

2.2. The Problem of Color in Dyehouse Effluent

The presence of even low concentrations of dyes in effluent is highly visible and undesirable. Color and presence of organics in the wastewater are needed to be reduced before disposal. Color is due to the usage of certain dyes during the dyeing process. About 1000 mg/L of dye is present in a typical dyebath (İnce et al., 1999). However due to the poor exhaustion properties of a significant percentage of the

initial dye remains unfixed and ultimately ends up in the dyebath effluent. (Shah, 1998).

Neglecting the aesthetic problem, it prevents penetration of dissolved oxygen in to the natural water bodies is caused by the presence of color and depletion of dissolved oxygen content in water bodies can have a serious effect on aquatic life. Moreover their absorption and reflection of sunlight entering the water interferes with the growth of bacteria to levels sufficient to biologically degrade impurities in the water and start the food chain.

2.3. Toxicity of Textile Dyes

Dye effluent usually contains chemicals, including dye itself, that are toxic, carcinogenic, mutagenic or teratogenic to various microbiological and fish species. Concern arises as many dyes are made from known carcinogens such as benzidine and other aromatic compounds. Also azo and nitro compounds have been reported to be reduced in sediments of aquatic bodies, consequently yielding potentially carcinogenic amines that spreads in the ecosystem. The presence of dyes or their degradation products in water can also cause human health disorders such as nausea, hemorrhage and ulceration of skin and mucous membranes and can cause severe damage to kidney, reproductive system, liver, brain and central nervous system.

Depending on exposure time and dye concentration, dyes can have acute and/or chronic effect on exposed organisms. Some of the dyes, dye precursors and dye degradation products can be also carcinogenic and mutagenic in nature.

Acute toxicity of azo dyes measured by the EU criteria for classification of dangerous substances is rather low, amounting to the LD_{50} values of 250-2000 mg per kg body weight. Some acid, basic and direct azo dyes have been classified as acute toxic to toxic to fish, crustaceans, algae and bacteria, whereas reactive azo dyes have very high effective concentration (EC levels > 100 mg/L) and thus are not considered to be toxic to aquatic organisms. Non-ionic azo dyes are generally classified as toxic and potentially toxic. For anthraquinone dyes there is much less available information on toxicity (Novotny et al., 2006).

Carcinogenic and mutagenic effects are observed with animal experiments. Correlation between the results of mutagenicity test and carcinogenicity of azo dyes

shown in animal experiments is rather poor. Majority of azo dyes require metabolic activation, namely reduction and cleavage of the azo bond to aromatic amines to exhibit mutagenicity in vitro test systems. On the other hand correlation between exposure to aromatic amines and human cancer has been established and documented to be typical occupational risk from Novotny et al. (2006). Mutagenic effect of some anthraquinone dyes has been measured using Ames test (Novotny et al., 2006). Similarly a carcinogenic effect of the anthraquinone derivative Disperse Blue 1, an agent causing induction of red bladder tumors was reported from Atkins, 2000. This compound also found to be mutagenic in bacteria and thus the possibility of a genotoxic mechanism of cancer induction can not be excluded.

Table 2.4: Toxicity of acid dyes, a total of 11 (ETAD 1992b)

Test organism	Time/ unit of	Number of results	Concentration mg/L			
			< 1	1 -10	10 -100	> 100
Zebra fish	96 hr LC_{50}	11	0	2	3	6
Daphnia Magna	48 hr EC_{50}	9	0	0	6	3
Algae	72 hr EC_{50}	9	2	3	3	1
Bacteria	IC_{50}	11	0	0	0	11

Table 2.5: Toxicity of hydrolyzed reactive dyes (ETAD 1992b)

Test organism	Time/ unit of	Number of results	Concentration mg/L		
			1 -10	10 -100	> 100
Zebra fish	96 hr LC_{50}	8	0	0	8
Daphnia Magna	48 hr EC_{50}	8	0	0	8
Algae	72 hr EC_{50}	8	0	7	1
Bacteria	IC_{50}	8	0	0	8

Neither simple chemical nor biological treatment has been proved to be adequate in removing toxicity from textile dyes. Their low biodegradability indicates their resistance to conventional biological treatment. The destruction of toxic pollutants such as biologically recalcitrant compounds in textile wastewater has to be handled in combination non-biological technologies such as special techniques called advanced oxidation processes (AOPs) (Wang et al., 2003)

2.4. Treatment Methods of Dyehouse Effluent

A number of decolorization techniques are being proposed and tested at different stages of commercialization. However due to their synthetic origin and complex structure deriving from the use of different chromophoric groups, dyes are extremely recalcitrant. Along with the recalcitrant nature of dye wastewater, the frequently daily variability of characteristics of such wastewater adds to the difficulty of treatment. Although all the known physicochemical and biological techniques have been explored for decolorization, none has solved the problem completely. Cost-competitive biological options are ineffective, while physicochemical processes are restricted in scale of operation and pollution profile of the effluent. Despite still mostly in laboratory stage of development, a wealth of studies has been reported on implementation of advanced oxidation processes (AOPs). Combinations of conventional physicochemical techniques with the AOPs have also appeared as attractive options. The biological systems, in addition to varieties of combinations among themselves, have also been explored in combination with virtually all sorts of physicochemical and advanced oxidation processes.

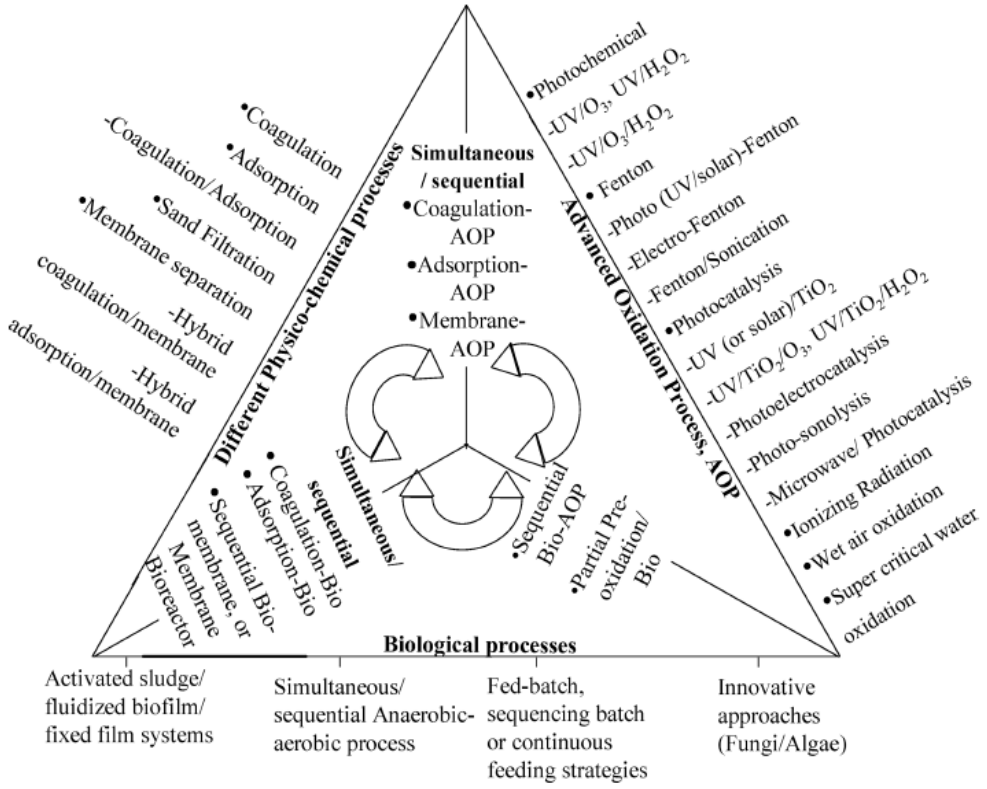


Figure 2.3: Simplified representation of the proposed treatment process combinations in the literature (Hai et al., 2007)

Combinations have been outlined under three broad categories, combination among AOPs, combination of physicochemical treatments among themselves and those with the AOPs, the combination of biological systems with conventional physicochemical processes and AOPs.

2.4.1. Physico-chemical methods for color removal

2.4.1.1. Adsorption

Adsorption techniques, specially the excellent adsorption properties of carbon-based supports, have been utilized for the decolorization of the dyes in the industrial effluents. Activated carbon, either in powder or granular form, is the most widely used adsorbent for this purpose because of its extended surface area, microporous structure, high adsorption capacity and high degree of surface reactivity. It is very effective for adsorbing cationic, mordant and acid dyes and to a slightly less extent dispersed, direct, vat, pigment and reactive dyes. However, the use of carbon adsorption for decolorization of raw wastewater is impractical because of competition between colored molecules and other organic/inorganic compounds.

Therefore it is recommended to be used as polishing step or as an emergency unit at the end of the treatment stage to provide the discharge color standards. The disadvantage of activated carbon adsorption is being expensive and its weight loss is unavoidable during its costly on-site regeneration (10 % loss in the thermal regeneration process) prevents its widespread use.

Adsorption with activated carbon provides to remove a wide variety of dyes as azo, reactive and acid dyes and removal is mostly achieved for basic dyes (Hao et al., 2000).

In using low-cost adsorbents for decolorization of wastewater has been a considerable interest. These molecules include chitosan, zeolite and clay; certain waste products from industrial operations such as fly ash, coal and oxides; agricultural wastes and lignocellusic wastes and so on. Each of the non-regenerable economical has also disadvantages as occurring a disposal problem (Hai et al., 2007).

2.4.1.2. Coagulation-flocculation-precipitation

Coagulation/flocculation/precipitation processes have been used intensively for decolorizing wastewater. These processes may be satisfactory with respect to COD reduction and partial decolorization before discharging wastewater to publicity owned treatment plants. But their stand-alone application in treating textile wastewater is not as efficiently as their combination. In the coagulation process, it is difficult to remove highly water-soluble dyes and even more important, the process produces a large quantity of sludge.

Coagulation is a satisfactory removal for disperse, sulfur and vat dyes and as a result of their high solubility, removal is unsatisfactory for azo, reactive, acid and basic dyes (Hao et al., 2000).

Nevertheless new types of coagulants are investigated such as polyaluminum ferric chloride (PAFC) which has high stability and good coagulating effect for hydrophobic as well as hydrophilic dyes. On the other hand, to avoid massive sludge disposal problem, different novel approaches such as coagulation of low volume segregated dye bath, alum sludge recycling, coagulant recovery from textile chemical sludge, reuse of textile sludge in building materials and coagulation followed by activated carbon adsorption have been proposed (Hai et al., 2007).

2.4.1.3. Membrane filtration

By membrane separation, concentrating dyestuffs and auxiliaries and producing purified water or removing the dyestuff and allowing reuse of water along with auxiliary chemicals or even realizing recovery of considerable portion of dye, auxiliaries and water all together is achieved. These recovery and reuse practice may reduce cost for the treatment. However, the dyeing behavior of the residual dye should ideally be identical to that of the fresh dye may restrict dye recovery and reuse of specific dye classes. On the other hand concentrated sludge production and occurrence of frequent membrane fouling requiring costly membrane replacement prevents widespread use of this technology (Hai et al., 2007).

2.4.2. Advanced oxidation processes for the treatment of textile dyes

Effluent from the textile dyeing can be successfully decolorized with advanced oxidation processes. For advanced oxidation processes, the basic reaction mechanism is the generation of free radicals and subsequent attack by these on the pollutant organic species. Mostly hydrogen peroxide is used to produce free radicals because of its non-selective reaction way on organic compounds leading finally to the mineral end products. Decolorization methods differ in the way in which in hydrogen peroxide is activated. These processes are mostly suitable for all dye classes except for vat dyes where the decolorization is only 50 % (Slokar et al., 1997).

2.4.2.1. Ozonation at high pH

The high chemical energy of ozone is the driving force of its decomposition. Ozone decomposition is a complex function of pH, temperature and concentration of organic solutes as well as inorganic constituents. The reaction of ozone with another substrate may involve both direct, selective reaction of ozone with the substrate (typically electrophilic addition reactions) and that of the substrate with ●OH, the relative proportions of which will depend on various organic and inorganic species being presented or added (Wang et al., 2003). (Eqn 2.1-2.9)

$$O_3 + OH^- \rightarrow HO_2^- + O_2 \qquad k = 70\ M^{-1}\ s^{-1} \qquad \textbf{(2.3)}$$

$$O_3 + R \rightarrow R_{ox} \qquad k = 1\text{-}10^3\ M^{-1}\ s^{-1} \qquad \textbf{(2.4)}$$

$$HO_2^- + O_3 \rightarrow O_3^{\bullet -} + HO_2^{\bullet} \qquad k = 2.8 \times 10^6\ M^{-1}\ s^{-1} \qquad \textbf{(2.5)}$$

$HO_2^{\bullet} \rightarrow O_2^{\bullet-} + H^+$ $pk_a = 4.8$ **(2.6)**

$O_2^{\bullet-} + O_3 \rightarrow O_3^{\bullet-} + \rightarrow O_2$ $k = 1.6 \times 10^9\ M^{-1}\ s^{-1}$ **(2.7)**

$O_3^{\bullet-} + H^+ \rightarrow HO_3^{\bullet}$ $k = 5 \times 10^{10}\ M^{-1}\ s^{-1}$ **(2.8)**

$HO_3^{\bullet} \rightarrow OH^- + O_2$ $k = 1.4 \times 10^5\ M^{-1}\ s^{-1}$ **(2.9)**

$HO_2^{\bullet} + O_2^{\bullet-} \rightarrow HO_2^- + O_2$ $k = 8.7 \times 10^7\ M^{-1}\ s^{-1}$ **(2.10)**

$^{\bullet}OH + R \rightarrow RO^{\bullet}H \rightarrow RO_2^{\bullet} \rightarrow$ Products $k = 10^8 - 10^{11}\ M^{-1}\ s^{-1}$ **(2.11)**

The net reaction for ^{-}OH initiated ozone decomposition is as shown in Eqn 2.10.

$3O_3 + {}^{-}OH \rightarrow 2^{\bullet}OH + 4\ O_2$ **(2.12)**

2.4.2.2. Ozonation in the presence of hydrogen peroxide

The conjugate base of hydrogen peroxide (HO_2^-; $pK_a = 11.8$) can also initiate ozone decomposition resulting in hydroxyl radical formation. The net reaction for the HO_2^- initiated cycle is shown in Eqn 11.

$2O_3 + H_2O_2 \rightarrow 2^{\bullet}OH + 3\ O_2$ **(2.13)**

For textile dyes that are usually not UV-C photolysed with much efficiency, it is preferable to add hydrogen peroxide from an external source instead or also to increase the reaction pH (Arslan-Alaton, 2003).

2.4.2.3. Photolysis of ozone

This process features direct molecular ozonation, ozone decomposition (free radical chemistry) and a photocatalytic reaction (direct photolysis of aqueous ozone producing hydrogen peroxide and indirect photolysis of hydrogen peroxide formed in situ). The principal reaction is given in Eqn 2.12.

$2O_3 + ½\ H_2O(aq) \rightarrow h\nu \rightarrow H_2O_2 \rightarrow h\nu \rightarrow 2^{\bullet}OH$ **(2.14)**

The fact that ozone decomposition is initiated even in dark by OH^-, peroxide anions (HO_2^-) and organic radicals make the O_3/UV-C chain reactions exceedingly complex, and some 84 different reactions have been identified in the kinetically model. The

rate of substrate disappearance with the O_3/UV oxidation system was modeled as a composite of our kinetic terms involving purging, photolysis, molecular ozonation and photolytic ozonation (indirect, free radical oxidation).

Since the bleaching via ozonation is kinetically very fast for almost all types of dye chromophore, a diffusion-limited process where dye oxidation proceeds directly at the gas-liquid interphase is theoretically expected. The high reactivity towards aromatics and its electrophilic nature makes differentiation of direct and indirect oxidation pathways a rather difficult task.

Ozone has a considerably higher molar absorption coefficient (3600 M^{-1} cm^{-1}) at λ 254 nm than hydrogen peroxide (19.6 M^{-1} cm^{-1}). Hence some substrates undergo simultaneously direct UV-C photolysis which contributes significantly to the substrate decay. Of the three AOPs involving ozonation, the O_3/H_2O_2 process should be preferred for the larger treatment plants when substrates of concern are not strong UV absorbers, since the O_3/UV-C process will be difficult to adopt at the scale. The rather is further referred to extensive literature reviews of the O_3/H_2O_2/UV-C AOP combinations and commercialized efforts to treat recalcitrant, problematic waste streams of industrial and agricultural origin (Arslan-Alaton, 2003).

2.4.2.4. Photolysis of hydrogen peroxide

In this process, hydrogen peroxide is activated by UV-C irradiation (at λ = 254 nm) to form $^{\bullet}OH$ (Eqn 2.13):

$$H_2O_2 + h\nu \rightarrow 2\ ^{\bullet}OH \qquad \textbf{(2.15)}$$

with a quantum yield (φ) of 1.0. Principally, the cleavage of hydrogen peroxide by UV-C radiation is the most direct method for $^{\bullet}OH$ generation. However as has been mentioned previously O_3/UV-C yields more radicals and for a sufficient level of $^{\bullet}OH$, high concentration of hydrogen peroxide (≥ 20mM) in the reaction medium are required that in turn will compete with organic substrates for ●OH. Only few applications of H_2O_2/UV-C have gained widespread acceptance so far. The optical transmissivity of most wastewater streams is too low, particularly if solids are present. Another practical limitation is associated with lamp technology. High efficiency lamps that provide photons of high energy and at fast rate are not currently available at reasonable costs (Safarzadeh-Amiri et al.,1997).

2.4.2.5. Power ultrasound (sonolysis)

Sonochemical reactions are induced upon high-intensity acoustic irradiation of liquids at frequencies that produce cavitation (typically in the range 20-1000 kHz). Cavitation is a phenomenon of micro-bubbles formation and their growth and implosion in the irradiated liquid. The extreme temperature and pressure released during adiabatic bubble collapse causes the fragmentation of those gas molecules trapped in the micro bubbles in to radical species. These radical species can either recombine or react with other gaseous molecules within the cavity or in the surrounding liquid after their migration (Vajnhandl et al., 2006; Jiju et al., 1999).

However stand alone application of sonolysis hardly results in complete mineralization of pollutant which has complex mixtures of organic and inorganic compounds. Combined use of sonolysis with other AOPs can prevent severe mass transfer limitation and reduced efficiency of photocatalyst owing to adsorption of contaminants at the surface (Hai et al., 2007).

2.4.2.6. TiO_2-mediated heterogeneous photocatalysis

Titanium dioxide (TiO_2) which has suitable band-gap energy (3.2 eV), is stable and relatively inexpensive. The commercial product Degussa P25 consists of a 75 % anatase and 25 % rutile crystal structure and has an exceptionally high photocatalytic activity for a semiconductor. The composition of organic pollutants in titanium dioxide suspension is due to photogeneration of electrons and holes in this material under near-UV irradiation (at λ = 300-400 nm). Upon irradiation with photons of less than λ 385 nm, electrons migrate from the valance band (VB) to the conduction band (CB) and forms holes in the valence band (h_{VB}) (Eqn 16), which then react with the electron donors (^{-}OH) in the electrolyte to produce $^{\bullet}OH$ (Eqn 2.16):

$$TiO_2 \rightarrow h_{VB}^{+} + e_{CB}^{-}$$

$$h_{VB}^{+} + {}^{-}OH_{surface} \rightarrow \lambda<350\ nm \rightarrow {}^{\bullet}OH \qquad \textbf{(2.16)}$$

TiO_2-mediated photocatalysis so far has been kinetically modeled within the Langmuir-Hinshelwood framework (Eqn 2.17):

$$r_C = dC / dt = k_1 \times (K \times C) / (1 + K \times C) \qquad \textbf{(2.17)}$$

where k_1 is the reactivity constant and C is the time-dependent concentration of the target pollutant/substrate. By the adsorption of the organic substrate at high solute concentrations (when the product $K \times C >> 1$) (i.e. when all available active surface sites are occupied), zero-order abatement kinetics prevail, whereas the equation collapses to a first-order kinetic expression when the product $K \times C << 1$, thus forming a simpler kinetic evaluation format.

The composition of the pollutants can be either accomplished by holes or free radicals. Photogenerated holes and electrons must be trapped by electron donors and acceptors to avoid charge recombination. Surface-adsorbed oxygen or added hydrogen peroxide delay the electron-hole recombination by trapping the conduction band electron to form a superoxide anion or more hydroxyl radicals, respectively. Reduction of oxygen and hydrogen peroxide may proceed according to Eqns 2.18 and 2.19, respectively (Pekakis et al., 2006)

$$O_2 + e_{CB}^- \rightarrow {}^{\cdot}O_2 \quad \textbf{(2.18)}$$

$$H_2O_2 + e_{CB}^- \rightarrow {}^{\bullet}OH + {}^{\cdot}OH \quad \textbf{(2.19)}$$

Transition metal ions, such as Fe^{3+}, also act as an electron scavengers at suitable concentrations and lower charge recombination (Eqn 2.20)

$$Fe^{3+} + e_{CB}^- \rightarrow Fe^2 \quad \textbf{(2.20)}$$

2. 4.2.7. Fenton and photo-Fenton reaction

By Fenton and photo-Fenton oxidation, mineralization of several recalcitrant pollutants like PCBs, chlorinated herbicides, phenolic wastes, chlorophenols, perhalogenated alkenes, dye effluents and many other wastewaters of different origin is effective (Pera-Titus et al., 2004; Arslan et al., 1999).

Fenton's oxidation which hydrogen peroxide is activated with Fe^{2+} salts is very appropriate for the oxidation of wastewaters which inhibit biological treatment and are poisonous. Although COD, color and toxicity are reduced by Fenton oxidation, it involves flocculation and impurities are transferred from the wastewater to the sludge which also needs to be removed. Moreover degradation of organics can cause the formation of intermediates, which can be more toxic than the origin compound

Fenton-type reactions using H_2O_2 as oxidant in the presence of iron ions at acidic pH have been among the most common homogenous systems. The ferrous iron acts as a homogenous catalyst and hydrogen peroxide takes role as an oxidant.

Fenton reaction between dissolved Fe^{2+} and hydrogen peroxide in acidic aqueous solution leads to Fe^{2+} to Fe^{3+} and form the highly reactive hydroxyl radical ($^{\bullet}OH$). The reaction is spontaneous and without the influence of the light. The principal inorganic reactions which are considered common to the Fenton reaction system is shown in Eqn (2.21-2.38).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH \qquad k_1 = 76\ M^{-1}s^{-1} \qquad \textbf{(2.21)}$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow H_2O + HOO^{\bullet} \qquad k_2 = (1.2 - 4.5) \times 10^7\ M^{-1}s^{-1} \qquad \textbf{(2.22)}$$

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + {}^{-}OH \qquad k_3 = (4.3) \times 10^8\ M^{-1}s^{-1} \qquad \textbf{(2.23)}$$

$$Fe^{2+} + HOO^{\bullet} \rightarrow Fe^{3+} + H_2O_2 \qquad k_4 = 1.2 \times 10^6\ M^{-1}s^{-1} \qquad \textbf{(2.24)}$$

$$HOO^{\bullet} \rightarrow {}^{\bullet}O_2^{-} + H^{+} \qquad k_5 = 7.9 \times 10^5\ M^{-1}s^{-1} \qquad \textbf{(2.25)}$$

$$Fe^{2+} + {}^{\bullet}O_2^{-} \rightarrow Fe^{3+} + O_2^{-} \qquad k_6 = 1 \times 10^7\ M^{-1}s \qquad \textbf{(2.26)}$$

$$Fe^{3+} + {}^{\bullet}O_2^{-} \rightarrow Fe^{2+} + O_2^{-} \qquad k_7 = 1.5 \times 10^8\ M^{-1}s^{-1} \qquad \textbf{(2.27)}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\bullet} + H^{+} \qquad k_8 = 0.01\text{-}0.02\ M^{-1}s^{-1} \qquad \textbf{(2.28)}$$

$$Fe^{3+} + HOO^{\bullet} \rightarrow Fe^{2+} + O_2^{-} + H^{+} \qquad k_9 < 2 \times 10^3\ M^{-1}s^{-1} \qquad \textbf{(2.29)}$$

$$2{}^{\bullet}OH \rightarrow H_2O_2 \qquad k_{10} = 5.3 \times 10^9\ M^{-1}s^{-1} \qquad \textbf{(2.30)}$$

$$2HOO^{\bullet} \rightarrow H_2O_2 + O_2^{-} \qquad k_{11} = 8.5 \times 10^5\ M^{-1}s^{-1} \qquad \textbf{(2.31)}$$

$$R\text{-}H^{+} + {}^{\bullet}OH \rightarrow R^{\bullet} + H_2O \qquad k_{12} = 10^9 \times 10^{12}\ M^{-1}s^{-1} \qquad \textbf{(2.32)}$$

$$R^{\bullet} + {}^{\bullet}OH \rightarrow ROH \qquad \textbf{(2.33)}$$

$$R^{\bullet} + H_2O_2 \rightarrow ROH + {}^{\bullet}OH \qquad \textbf{(2.34)}$$

$$R^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + \text{products} \qquad \textbf{(2.35)}$$

$$R^{\bullet} + Fe^{3+} \rightarrow Fe^{2+} + \text{products} \qquad \textbf{(2.36)}$$

$R^{\bullet} + O_2^{-} \rightarrow$ products **(2.37)**

$2\ R^{\bullet} \rightarrow$ products **(2.38)**

The reactions listed above presents some of the multiple parallel processes that occur immediately after $^{\bullet}OH$ generation by Reaction (2.21). The relevance of each reaction in the mechanism is equally dependant of their reaction rate constant (k) at 20^{o}C and the concentration of each participating species. The $^{\bullet}OH$ scavenging by Fe^{2+} and H_2O_2 is shown in Reactions (2.22) and (2.23). The hydroperoxyl radicals ($HOO^{\bullet}$) generated from reaction (2.22) may be transformed to superoxide radical ($^{\bullet}O_2^{-}$) according to Reaction (2.25). Since the k_{pa} of this reaction is 4.8, this process is favored at basic pH, while the equilibrium is shifted towards $HOO^{\bullet}$ at acidic pH. As shown in reactions (2.26) and (2.27), and depending on the reaction pH, either $^{\bullet}O_2^{-}$ or $HOO^{\bullet}$ are linked to the pathways of oxygen generation via reaction with Fe^{2+} and Fe^{3+}, respectively. Oxygen is also generated during the termination reaction of $HOO^{\bullet}$, which also results in H_2O_2 regeneration, Reaction (2.31). $HOO^{\bullet}$ is also generated via reaction of Fe^{3+} and H_2O_2 as shown in Reaction (2.28). Reaction (2.30) is a termination reaction for $^{\bullet}OH$, which regenerates H_2O_2. On the other hand, Reactions (2.32) to (2.33) are the reactions between the organic compounds (R-H), organic radicals ($R^{\bullet}$) and $^{\bullet}OH$, which involve $^{\bullet}OH$ attack on a R-H substrate present in the system and therefore the corresponding reaction rates are a function of substrate properties. The outcome of Reaction (2.32) results in the generation of $R^{\bullet}$, which are responsible for a series of further reactions. Some of them include scavenging of $^{\bullet}OH$ (Reaction 2.33) and Fenton components (Reactions 2.34 and 2.35), Fe^{2+} regeneration (Reaction 2.36), and formation of other products by reaction with oxygen (Reaction 2.37) or other $R^{\bullet}$ radicals via termination reactions. Another case is the reaction between $R^{\bullet}$ and $^{\bullet}OH$ (Reaction 2.33), which constitutes a termination reaction for both radicals.

According to the presented values above, Fenton reaction (Reaction 2.21) has low k value among the reactions of mechanism. This indicates that Reaction (2.21) has a major role only at the beginning of the process at a time when only Fe^{2+} and H_2O_2 and substrate are present. Shortly after the hydroxyl radicals formation, the parallel mechanism is shown by Reaction (2.22) and (2.23) become effective in this process relating on their reaction rate constant values.

Consequently these equations indicate that iron acts as a catalyst, however since the reduction of Fe^{3+} is generally much slower than the oxidation of Fe^{2+}, iron exists mainly in the Fe^{3+} form in these systems. H_2O_2 presence determines oxidation efficiency (Chamorro et al., 2000).

The initial degradation rate depends on form of iron as Fe^{2+} or Fe^{3+}. Initial degradation is slower for Fe^{3+}/ H_2O_2 than for Fe^{2+}/ H_2O_2 since, in the former case, Fe^{3+} must be reduced to Fe^{2+} before hydroxyl radicals are produced.

In the photo-Fenton process, H_2O_2 is utilized more rapidly by three simultaneous reactions, namely the direct Fenton action, photo-reduction of Fe^{3+} ions to Fe^{2+} and H_2O_2 photolysis.

- $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH$ **(2.39)**

- $Fe^{3+} + H_2O + uv \rightarrow Fe^{2+} + {}^{\bullet}OH + H^{+}$ **(2.40)**

- $H_2O_2 + uv \rightarrow 2\ {}^{\bullet}OH$ **(2.41)**

Thus this process produces more hydroxyl radicals when it is compared with conventional Fenton method or to photolysis. If solar radiation is employed instead of artificial UV light, its mostly operating costs would be reduced. However Fenton's process may be preferred because of its lower energy consumption, lower H_2O_2 consumption, lower sludge disposal cost, higher flexibility and lower maintenance requirement.

2.4.3. Biological methods for color removal

2. 4.3.1. Aerobic treatment

Aerobic biodegradation is a process that often takes place in the environment by itself it is often associates with technical systems such as wastewater treatment plants. In case of aerobic bacteria co-metabolic reductive cleavage of azo dyes as well as utilization of azo compounds as sole source of carbon and energy (leading on mineralization) have been reported, dyes are generally very resistant to degradation under aerobic conditions (Banat et al., 1996). Toxicity of dye wastewater and factors inhibiting permeation of dye through the microbial cell membrane reduce the effectiveness of biological degradation.

Hence dyestuff removal currently occurs in the primary settling tank of a wastewater treatment for the water-insoluble dye classes (disperse, vat, sulfur, azoic dyes), while the main removal mechanism for the water-soluble basic and direct dyes in conventional aerobic systems is adsorption to the biological sludge. Reactive and acid dyes however adsorb very poorly to the sludge and are thus major troublemakers in relation to residual color in discharged effluents (Pagga et al., 1994).

Moreover, for dye decolorization under aerobic conditions, pure culture is needed to be used (algal, fungal and bacterial). Using pure culture for dye decolorization is impractical as most of the isolated cultures are dye specific and hence their application on to large scale is not possible because to maintain the pure form in wastewater treatment plant is difficult (Manu et al., 2002).

2. 4.3.2. Anaerobic treatment

Anaerobic biological processes are known as more effective and hold promise of providing a low cost and efficient means to treat the effluent which can be reused and as process water and/or the treatment of textile effluents before their final disposal. Anaerobic biodegradation of dyes and their decolorization is well achieved. Complex nature of the textile wastewater containing dye and various auxiliaries, salts and sulfates might have an inhibitory effect on the anaerobic decolorization.

Microbial anaerobic processes are capable of producing and maintaining low oxidation-reduction potential conditions. Under such conditions, the azo dye bond undergoes cleavage, therefore permanently destroying color (Beydilli et al., 1998; Işık et al., 2004). Cleavage of the dye's azo linkages is resulted in the formation of generally colorless but potentially hazardous aromatic amines (Zee et al., 2005). Bacterial biodegradation of aromatic amines generally can not be achieved under anaerobic conditions. Nevertheless, some aromatic amines such as three isomores of aminobenzoate, 2-aminophenol, 4-aminophenol and 5-aminosalicylic acid have been shown to be mineralized in methanogenic medium (Razo-Flores et al., 1997; Donlon et al., 1996).

Consequently anaerobic biodegradation can be suggested as efficient for decolorization of textile dyes but formation of potentially hazardous aromatic amines and being generally unable to achieve complete mineralization forces to consider. A

treatment process which in anaerobic and aerobic conditions is combined can be most logical concept for removing dyes from wastewater.

2. 4.3.3 Anoxic degradation of dyes

Anoxic degradation is biological reduction of nitrate to nitric oxide, nitrous oxide, and nitrogen gas. Anoxic degradation or biological denitrification is coupled to the respiratory electron transport chain, and nitrate or nitrite is used as an electron acceptor for the oxidation of a variety of organic or inorganic electron donors.

From the previous investigations, it is observed that anoxic degradation for textile dyes is not efficient. Reduction of dye is an oxidation-reduction reaction in which the dye acts as an electron acceptor. The presence of an alternative electron acceptor as nitrate, is shown to slow down the decolorization and is not provide significant changes in the COD removal profile (Laurenço et al., 2000; Panswad et al., 2000). It can be possible to perform decolorization and denitrification in the same treatment step unless hydraulic retention time (HRT) is corresponded to the sum of the retention times required for denitrification and decolorization (Laurenço et al., 2000).

Table 2.6: Advantages and Shortcomings of Individual Dye Wastewater Treatment Techniques (Hai et al., 2007)

Treatment Processes	**Advantages**	**Disadvantages**
Biological	Cost-competitive option. Direct, disperse and basic dyes have high level of adsorption on to active sludge.	Dyes are generally toxic and very resistant to biodegradation. Acid and reactive dyes are highly water soluble and have poor adsorption on to sludge.
Coagulation	Economically feasible, satisfactory removal of disperse, sulfur and vat dyes	Removal is pH dependent; produces large quantity of sludge. May not remove highly soluble dyes; unsatisfactory result with azo, reactive and basic dyes.
Activated carbon adsorption	Good removal of wide variety of dyes, namely azo, reactive and acid dyes, especially suitable for basic dye.	Removal is pH dependent; unsatisfactory for disperse, sulfur and vat dyes. Regeneration is expensive and involves adsorbent loss; necessities costly disposal.
Ion exchange	Adsorbent can be regenerated without loss, dye recovery conceptually possible.	Ion exchange resins are dye-specific, regeneration is expensive, large scale dye recovery cost-prohibitive.
Membrane filtration	Appropriate membrane may remove all types of dyes and thus realize reusable water from dyebath effluent.	Concentration sludge production and costly membrane replacement impede widespread use.
Chemical oxidation	Initiates and accelerates azo-bond cleavage.	Thermodynamic and kinetic limitations along with secondary pollution are associated with different oxidations. Not applicable for disperse dyes. Negligible mineralization is possible.

Table 2.6: Advantages and Shortcomings of Individual Dye Wastewater Treatment Techniques (continued)

Advanced Oxidation Processes (AOPs)	Generate a large number of highly reactive free radicals and by far surpass the conventional oxidants in decolorization.	AOPs in general may produce further undesirable toxic by products and complete mineralization may not be possible. Presences of radical scavengers reduce efficiency of the process some of which are pH dependent. Cost-prohibitive at their present stage of development.
O_3 /UV-C	Applied in gaseous state, no alternation of volume. Good removal of almost all types of dyes; especially suitable for reactive dyes. Involve no sludge formation, necessitates short reaction times.	Removal is pH dependent (neutral to slightly alkaline); poor removal of disperse dyes. Problematic handling, impose additional loading of water with ozone. Negligible or no COD removal. High cost of generation coupled with short half-life and gas-liquid mass transfer limitation; suffers from UV light penetration limitation. Increased level of turbidity in effluents.
H_2O_2 /UV-C	Doesn't involve sludge formation; necessities short reaction times and reduction of COD to some extent may be possible.	Not applicable for all dye types, requires separation of suspended solid and suffers from UV light penetration limitation. Lower pH required to nullify effect of radical scavengers.
Fenton's Reagent	Effective decolorization of both soluble and insoluble dyes; applicable even with high suspended solid concentration. Simple equipment and easy implementation. Reduction COD (except reactive dyes) possible.	Effective within narrow pH range of <3.5; and involves sludge generation. Comparatively longer reaction time required.

Table 2.6: Advantages and Shortcomings of Individual Dye Wastewater Treatment Techniques (continued)

Photocatalysis (mainly based on TiO_2/UV-A)	No sludge production, considerable reduction of COD, potential of solar light utilization.	Light penetration limitation, fouling of catalysts and problem of fine catalyst separation from the treated liquid.
Electrochemical/photoelectrocatalytic processes	Effective decolorization of soluble/insoluble dyes; reduction of COD possible. Not effected by presence of salt in wastewater.	Sludge production and secondary pollution (from chlorinated organics, heavy metals) are associated with electrocoagulation and indirect oxidation, respectively. Direct anodic oxidation requires further development for industrial acceptance. High cost of electricity is an impediment. Efficiency depends on dye nature.
Ionizing radiation	No sludge production; effective oxidation at lab. scale.	Requires a lot of dissolved O_2; complete decolorization and mineralization by stand alone application not possible. Energy efficiency scale-up yet to be achieved.
Photocatalysis (mainly based on TiO_2/UV-A)	No sludge production, considerable reduction of COD, potential of solar light utilization.	Light penetration limitation, fouling of catalysts and problem of fine catalyst separation from the treated liquid.
Wet air oxidation (WAO)	Wet-established technology especially suitable for effluent too dilute for incineration and too toxic and/or concentrated for biological treatment.	Complete mineralization not achieved, as lower molecular weight compounds are not amenable to WAO. High capital and operating costs are associated with elevated pressure and temperature employed.

2.5. Literature Review on Textile Dye Treatment and Toxicity Assessment

2.5.1. Treatment of textile dyes by Fenton and Fenton-like processes

Alaton et al. (2005) assessed a synthetic acid dyebath effluent (SADB) bearing two azo and one anthraquinone dye together with two dye auxiliaries was subjected to pretreatment with Fenton's reagent. Firstly, initial Fe^{2+} and H_2O_2 concentrations as well as pH were optimized to achieve highest COD and color removals during Fenton's treatment of SADB. In the second stage of the experimental work, kinetic studies were conducted to elucidate the effect of operating temperature ($20^oC < T < 60^oC$) on COD, color abatement and H_2O_2 consumption kinetics. Obtained results indicated that 30 % COD and practically complete color removal (99 %) could be achieved at $T = 50^oC$. The kinetic studies revealed that a strong correlation existed between COD removal and H_2O_2 utilization rates. In the final part of the study, the acute toxicity of raw (untreated) and pretreated SADB on heterotrophic biomass was investigated employing a modified (COD-balanced), activated sludge inhibition test. The toxicity experiments demonstrated that the inhibitory effect of SADB towards sewage sludge could be completely eliminated when the effluent was pretreated with Fenton's reagent.

A study by Hsueh et al. (2004) observed Fenton and Fenton-like reactions at low iron concentration (≤10mg/L) to oxidize three commercial azo dyes, namely Red MX-5B, Reactive Black 5 and Orange G. The process was not only significantly decolorizing azo dyes but also minimize sludge creation. It has been found that the solution pH, H_2O_2 concentration and iron concentration in solution were the main factors that influence the degradation of Red MX-5B. The optimum pH of the reaction solution for both Fenton and Fenton-like systems were 2.5–3.0. Additionally, the results showed that dye decolorization could occur at very low iron concentrations (1 mg/L) in both Fenton-like and Fenton reactions. For a Fenton-like system, increasing ferric sulfate dose enhanced the degradation of Red MX-5B. The optimum H_2O_2 concentration was about 200 mg/L for 0.1 Mm Red MX-5B. Although H_2O_2 was essential to generate $^{\bullet}OH$, very high levels of H_2O_2 could reduce decolorization because of scavenging effect. It was also found that the decolorization of azo dyes undergoes a fast reaction than the mineralization of azo dyes, and 37 %, 28 % and 31 % TOC of 0.1mM Red MX-5B, Reactive Black 5 and Orange G can be eliminated

after 480 min of reaction in the presence of 1 mg/L $Fe^{3+,}$ and 100 mg/L H_2O_2 at pH 2.5.

Fongsatitkul et al. (2004) investigated the potential of biological treatment as a single process as well as in association with chemical oxidation to treat a textile wastewater. The experimental part incorporated the following three schemes: biological treatment only (stage 1), chemical oxidation prior to biological treatment (stage 2), and biological treatment followed by chemical oxidation (stage 3). Biodegradation was accomplished employing three identical sequencing batch reactors (SBRs), each having a liquid volume of 10 L, while chemical treatment involved the addition of Fenton's reagent in the range 25–300 mg/L, in a batch-type operation. Following an acclimation period of approximately 60 days, biological treatment resulted in a high percent reduction in chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), and total phosphorus (TP), and in a moderate decrease in color. The process was found to be independent of the variations in the anoxic time period studied; however, an increase in solids retention time (SRT) improved COD and color removal, although it reduced the nutrient (TKN and TP) removal efficiency. Furthermore, both combined treatment alternatives resulted in enhanced color reduction, in comparison to stage 1. Overall, chemical oxidation prior to biological treatment (stage 2) appears to be the best option regarding the treatment of the textile wastewater used.

A study by Meriç et al. (2003) was investigated for decolorization and to reduction of COD content in a mixture of four reactive dyes, i.e., Remazol Black 5 (RB5), Remazol Red RB (RR), Remazol Yellow 84 (RY), Remazol Brilliant Blue (RB) using Fenton Oxidation Process (FOP). Optimum pH, temperature, and the doses of $FeSO_4$ and H_2O_2 were determined. Experiments were conducted on the samples containing a total concentration of 100 mg/L (RB + RY), 200 mg/L (RB5 + RR), 300 mg/L (RB5 + RR + RB + RY), and 400 mg/L (RR + RB + RY) dyes considering their actual application doses in dyehouses. Optimum pH was observed as 2.5 at 30^oC using 400 mg/L $FeSO_4$ and 800 mg/L of H_2O_2 resulting in more than 96 % COD and 99 % Pt-Co unit of color removal for the mixture of RB5 and RR. The optimum conditions determined were 4.0 pH, 50^oC, and 500 mg/L $FeSO_4$ applying 1000 mg/L H_2O_2 for the mixture of (RB5 + RR + RB + RY). A 100 mg/L solution of

a mixture of RB and RY at equal amounts was oxidized using 200 mg/L $FeSO_4$ and 300 mg/L H_2O_2 at 3.0 pH and 50^oC.

Neamtu et al. (2003) studied the degradation of two reactive dyes, C.I. Reactive yellow 84 (RY 84) and C.I. Reactive Red 120 (RR 120) by photo-Fenton and Fenton like oxidation. The effective system conditions were found to be pH of 3, H_2O_2 to Fe^{2+} molar ratio of 20:1 and UV or solar irradiation. The color removal efficiency at the optimum conditions during different Fenton like processes was also evaluated. The results show that the color removal of RY 84 after 15 min reaction time fallows the decreasing order: solar/Fe^{2+}/H_2O_2 > UV/Fe^{2+}/H_2O_2 > UV/Cu^{2+}/Fe^{3+}/H_2O_2 > UV/ Fe^{3+}oxalate/H_2O_2 > UV/ Fe^{2+}/H_2O_2 > dark/ Fe^{2+}/ H_2O_2 > solar/ Fe^{3+}/oxalate/ H_2O_2> UV/ H_2O_2 > UV/ Fe^{2+} = UV. During the same reaction period the relative order for RR 120 removal rate was slightly different: solar/ Fe^{2+}/ H_2O_2 > UV/ Fe^{2+}/ H_2O_2 > UV/ Fe^{3+}/ H_2O_2 > UV/ Fe^{3+}/ H_2O_2 = UV/Cu^{2+}/ Fe^{3+}/ H_2O_2 > UV/ Fe^{3+}/oxalate/ H_2O_2 = UV/ H_2O_2 > UV. The toxic potential of dye's degradation was investigated by the bioluminescence test using the LUMIStox 300 instrument and results were expressed as the percent inhibition of the luminescence of bacteria *Vibrio fischeri.*

2.5.2. Biological treatment of textile dyes

2.5.2.1. Aerobic treatment of dyes

Pourbabaee at al. (2006) evaluated a number of aerobic species capable of decolorizing some of the dyes in a textile mill effluent were isolated. One of the isolates was able to decolorize Terasil Black dye under aerobic conditions in the presence of an exogenous carbon source after five days. Glucose or starch were essential for decolorization but the process proceeds faster in the presence of 0.5 % yeast extract. Results of the BOD_5 showed that the untreated effluent samples had a low BOD_5 value, whereas treated samples showed an initial increase in BOD_5 up to 15 days followed by a decrease after 20 days. FT-IR and GC-MS analyses also revealed that the initial components in the untreated effluent disappear after 20 days of treatment, confirming biodegradation of the dye. Phytotoxicity tests on the untreated effluent samples using the seeds of *Lens orientalis, Triticum aestivum,* and *Triticum boeoticum* indicated that the first one was the most sensitive while the last one was the most resistant. On the other hand the treated effluent allowed 90 % germination in *Triticum boeoticum* seeds and 100 % germination in the other two.

Buitron et al. (2003) investigated aerobic degradation of azo dye acid red 151 in a sequencing batch biofilter (SBR). The azo dye acid red 151 (AR151) was aerobically biodegraded in a sequencing batch biofilter packed with a porous volcanic rock. AR151 was used as the sole source of carbon and energy for acclimated microorganisms. Acclimation was followed using the degradation time and the oxygen uptake rate. A maximal oxygen uptake rate of 0.5 mg O_2 $(L \cdot min)^{-1}$ was obtained. Mineralization studies showed that 73 % (as carbon) of the initial azo dye was transformed to CO_2 by the consortia. A maximal substrate degradation rate of 247 mg AR 151was obtained. Color removal was up to 99 % using an initial concentration of 50 mg/L AR151l. Anaerobic tests suggested that in the interior of the porous material, anaerobic biotransformation can occur, contributing from 14 % to 16 % of the decolorization of the azo dye.

Biological, aerobic degradation of an azo dye and the resulting recalcitrant, aromatic amine products in a constructed wetland was demonstrated by Davies et al. (2006). A vertical flow CW, planted with *Phragmites* sp. was fed with 127 mg/L of acid orange 7 (AO7) at hydraulic loads of 28, 40, 53 and 108 $L\ m^{-2}day^{-1}$. Color removal efficiencies of up to 99 % clearly demonstrate cleavage of the azo bond, also confirmed by the similar AO7 removal and SO_4^{2-} release rates revealing that adsorption onto the matrix was constant. The positive redox potential at the outlet demonstrates that aerobic conditions were present. Chemical oxygen demand and total organic carbon removal efficiencies of up to 93 % were also indicative of AO7 mineralization. The degradation of sulfanilic acid was confirmed by the presence of NO_3^-, SO_4^{2-} and secondary metabolites, which suggest at least two degradation pathways leading to a common compound, 3-oxoadipate.

Senan et al. (2003) studied a microbial consortium capable of aerobic degradation of a mixture of azo dyes consisting of two isolated strains (RRL,TVM) and one known strain of *Pseudomonas putida* (MTCC 1194) was immobilized on laterite stones. The amount of bacterial biomass attached to the laterite stones was 8.64 g per 100 g of the stone on a dry weight basis. The packed bed reactor was filled with these stones and had a total capacity of 850 mL and a void volume of 210 mL. The feed consisted of an equal mixture of seven azo dyes both in water as well as in a simulated textile effluent, at a pH of 9.0 and a salinity of 900 mg/L. The dye concentrations of influent were 25, 50, and 100 μg/mL. The residence time was varied between 0.78 and 6.23

h. It was found that at the lowest residence time 23.55, 45.73, and 79.95 µg of dye was degraded per hour at an initial dye concentration of 25, 50, and 100 µg, respectively. The pH was reduced from 9.0 to 7.0. Simulated textile effluent containing 50 µg /mL dye was degraded by 61.7 %. Analysis of degradation products by TLC and HPLC showed that the dye mixture was degraded to nontoxic smaller molecules. The bacteria-coated pebbles were stable, there was no washout even after 2 months, and the reactor was found to be suitable for the aerobic degradation of azo dyes.

2.5.2.2. Anaerobic treatment of dyes

Santos et al. (2004) evaluated the transformation and toxicity of anthraquinone dyes during incubations with anaerobic granular sludge under mesophilic (30 ◦C) and thermophilic (55 ◦C) conditions. Additionally, the electron shuttling capacity of the redox mediator anthraquinone-2-sulfonic acid (AQS) and subsequent increase on decolorization rates was investigated on anthraquinone dyes. Compared with incubations at 30 ◦C, serum bottles at 55 ◦C presented distinctly higher decolorization rates not only with an industrial wastewater containing anthraquinone dyes, but also with model compounds. Compared with batch assays at 30 ◦C, the first-order rate constant k of the Reactive Blue 5 (RB5) was enhanced 11-fold and 6-fold for bottles at 55 ◦C supplemented and free of AQS, respectively. However, the anthraquinone dye Reactive Blue 19 (RB19) demonstrated a very strong toxic effect on volatile fatty acids (VFA) degradation and methanogenesis at both 30 ◦C and 55 ◦C. The apparent inhibitory concentrations of RB19 exerting 50 % reduction in methanogenic activity (IC_{50}-value) were 55 mg/L at 30 ◦C and 45 mg/L at 55 ◦C. Further experiments at both temperatures revealed that RB19 was mainly toxic to methanogens, because the glucose oxidizers including acetogens, propionate-forming, butyrate-forming and ethanol-forming microorganisms were not affected by the dye toxicity.

Toxicity and intermediates of C.I. Direct Black 38 azo dye in an anaerobic/aerobic sequential reactor system was studied by Işık et al. (2004). An Upflow Anaerobic Sludge Blanket Reactor-Continuous Stirred Tank Reactor was used sequentially to decolorize and mineralize C.I. Direct Black 38 azo dye (C_o = 3200 mg/L) in a synthetic wastewater containing glucose as co-substrate. At the steady state

conditions color was effectively removed under anaerobic condition, while the total aromatic amines and organic fraction could be mainly reduced under aerobic conditions. NO_3− N, COD, BOD_5, aromatic amine, HPLC and GC analyses showed that Direct Black 38 could be chiefly mineralized by the sequential system. The toxicity levels were determined using three different test organism (ATA-anaerobic toxicity, respiration/inhibition and *Daphnia magna* tests) through the continuous operation of anaerobic/aerobic sequential system treating Direct Black 38 dye containing synthetic wastewater. Feed and anaerobic effluent had greater toxicity than the aerobic effluent after mineralization of dye.

An identical investigation with Işık has been done by Sponza et al. (2004) with C.I Direct Red 28. Toxicity and intermediates of C.I Direct Red 28 (DR 28) azo dye was investigated through decolorization and mineralization of DR 28 azo dye in a sequential upflow anaerobic sludge blanket reactor (UASB)/continuous stirred tank reactor (CSTR) system. System performance was determined by monitoring the conventional parameters such as COD, inert COD, TOC, color, ammonia, nitrate, volatile fatty acid (VFA) and total aromatic amine (TAA) analyses. Under steady state conditions color was effectively removed under anaerobic conditions. Total aromatic amines could be mainly removed under aerobic conditions while COD was removed under both anaerobic and aerobic conditions. Samples from effluents of the system were analyzed by high performance liquid chromatography–diode array detection (HPLC–DAD) and gas chromatography–mass spectra (GC–MS) methods to detect the intermediates produced in the effluents of anaerobic and aerobic steps. Benzidine produced under anaerobic conditions was mineralized significantly under aerobic conditions. Simple qualitative analyses, NO_3^-–N, HPLC and GC analyses showed that Direct Red 28 could be chiefly mineralized by the anaerobic/aerobic sequential system. Reduction of toxicity was also observed in anaerobic toxicity assays (ATA) respiration/inhibition and *Daphnia magna* tests through the continuous operation of anaerobic/aerobic sequential treatment system.

Fontenot et al. (2002) assessed the biological decolorization of two reactive anthraquinone dyes (Reactive Blue 4; RB 4 and Reactive Blue 19; RB 19) under methanogenic conditions. Using a mixed, methanogenic culture, batch assays were performed to evaluate the rate and extent of color removal as well as any potential inhibition. The effect of initial dye, biomass, and organic feed concentration, as well

as the effect of repetitive dye addition on color removal kinetics and culture inhibition were assessed. Overall, a lower rate and extent of color removal was observed in RB 4-amended cultures as opposed to the RB 19-amended cultures. For an incubation time of ca. 15 days and an initial dye concentration of 2000 mg/L, the extent of color removal was 50 and 95 % for RB 4 and RB 19, respectively. Inhibition of acidogenesis and to a larger degree of methanogenesis, resulting in accumulation of volatile fatty acids, was observed in both RB 4- and RB 19-amended cultures. Although the degree of inhibition varied among the two dyes tested (RB 19 was more inhibitory than RB 4), an increase of inhibition was observed with increasing initial dye concentration. At an initial dye concentration of 500 mg/L or higher, methane production was lower than 6 % of that of the control culture for both RB 4 and RB 19. However, color removal occurred despite culture inhibition.

Manu et al. (2001) have studied decolorization of the commercial important azo dyes, Orange II (C.I. Acid Orange 7) and Reactive Black 3HN (C.I. Reactive Black 8) under anaerobic conditions in wastewater and ambient temperatures (24-28^{o}C) by maintaining a HRT of 10 days. The dye concentration in wastewater was maintained at 100 mg/L. The reactors were operated for 58 days and Orange II and Black 3HN were easily decolorized under the experimental conditions employed. The performance of the bioreactors was evaluated by monitoring oxidation- reduction potential (ORP) in the reactor, color and COD removal. Color removal of >99 % was achieved in both the dye-containing reactors. COD removals of up to 95 %, 92 %, 94 % were achieved in control, orange and black dye-containing reactors, respectively. Effect of dyes and salts present in textile wastewater on methanogenesis was evaluated based on maximum methane production and methanogenic activity. Based on the maximum methane production data, no inhibition of methanogenesis was observed for dye concentrations of up to 400 mg/L for both the dyes. However from the methanogenic activity data, it was observed that the black dye concentration of 400 mg/L seemed to cause inhibition of methanogenesis.

A study by Beydilli et al. (1998) was investigated six commercial reactive dyes (Reactive Black-5, Red-2 and 120, Yellow-3, 15 and 17) to renovate reactive textile dyebaths and reuse the high-salt containing mixture in the dyeing process. Using an anaerobic, methanogenic culture enriched from municipal sewage sludge batch assays were performed to evaluate potential toxicity of the selected dyes to the

anaerobic microorganisms as well as to determine the anaerobic degradability of these dyes. Total gas and methane production were monitored. No significant toxic effects were observed at a concentration of 300 mg/L for all dyes tested. This was contributed to increased total gas and methane production over that a control. However, higher concentrations of Reactive 2 (500, 1000 and 2000 mg/L) depressed gas production despite continued decolorization. These results suggest that color removal under the low redox conditions maintained by the methanogenic culture occurs irrespective of the culture activity level.

2.5.2.3. Anoxic treatment of dyes

Biological color removal from a simulated cotton textile effluent containing an azo reactive dye, Remazol Brilliant Violet 5R with sequenced anaerobic/aerobic batch reactors was studied by Lourenço et al. (2000). When operating with a sludge retention time (SRT) of 15 days the total COD removal was around 80 %, with 30 % being removed anaerobically. After 40–50 days of acclimatization the color removal efficiency reached a maximum, stable value of 90 % from a feed dye concentration of 90 mg/L, almost all being removed during the anaerobic phase. This color removal was attributed to microbial degradation rather than adsorption and color removal capacity was not lost even after a seven day absence of dye in the fed substrate. The dye-fed reactor experienced a reduction in the ORP values attained during the non-aerated phase, after acclimatization, an effect not observed in the dye-free control. Under denitrifying conditions it was observed that the decolorization levels achieved in the anaerobic phase decreased from 90 % to 70 % after only two cycles with a feed containing 45–60 mg NO_3^--N/L. Reduction of the SRT value from 15 to 10 days reduced the biomass concentration from 2.0 to 1.2 g VSS/L and lowered color removal levels from 90 % to 30–50 %. When the SRT value was increased back to 15 days the color removal capacity of the system was completely recovered, suggesting that with a SRT of 10 days the adequate microbial population could not be installed in the reactors.

Panswad et al. (2000) have studied an anaerobic/aerobic SBR system to treat a synthetic dye wastewater with glucose and acetic acid (1000 mg/L COD) as carbon sources, together with 20 mg/ L of four different reactive dyes; namely bisazo vinylsulphonyl, anthraquinone vinylsulphonyl, anthraquinone monochlorotriazinyl

and oxazine. The color reduction of the first three dyes was 63, 64 and 66 %, respectively. The decolorization efficiency of the last or oxazine dye was not determined because of a strange phenomenon or re-appearance of the color when samples were disturbed. More color removal was achieved in the anaerobic phase than in the aerobic step. The initial decolorization rate was 11.9, 0.37 and 0.48 SU/h for the first three dyes, respectively. The effect of nitrate was observed on color and COD removal. The addition of 5 mM nitrate nitrogen could enhance the COD reduction rate and efficiency. Denitrification by denitrifiers in addition to anaerobic decomposition was accepted as a result of COD reduction enhancement. However more nitrate, 10 mM addition decrease the azo dye decolorization capability, this was explained as competition in reaction reduction between the nitrate, the liquid and the azo bonds in the azo dyes. The addition of nitrate did not have any adverse effect on the color reduction for anthraquinone dyes because these dyes were removed by the adsorption mechanisms not by the microbial degradation.

Carliell et al. (1998) examined effect of nitrate and sulfate which were coming from reactive dye effluent as salts, on the decolorization of reactive azo dye, Reactive Red 141 under anaerobic conditions. Nitrate was found to delay the onset of decolorization for a period of time related to the concentration of nitrates initially present in the system. Sulfate was found to have no discernible effect on the onset or rate of decolorization and it is proposed that the order of reduction of the compounds is nitrate > Reactive Red 141 > sulfate. Measurement of the redox potential in the anaerobic system during decolorization with or without the addition of nitrate or sulfate showed that strictly anaerobic conditions were conducive to rapid decolorization of the azo dye.

Zissi et al. (1996) investigated anoxic degradation of disperse azo-dye, p-aminoazobenzene (p-AAB), a simple azo dye, by a pure culture of *Bacillus subtilis*, growing on a synthetic medium. *Subtilis* is a bacterium capable of using nitrate and/or nitrite as terminal electron acceptor under anoxic conditions. This bacterium lacks the capability for fermentation. The degradation of p-aminobenzene by *Bacillus subtilis* was examined through batch experiments in order to elucidate the mechanism of dye degradation. The results proved that *Bacillus subtilis* co-metabolizes p-aminobenzene under denitrifying conditions in the presence of glucose

as the carbon source, producing aniline and p-phenylenediamine as the nitrogen-nitrogen double bond was broken.

A study by Yongjie et al. (1994) investigated the effect of Acid Orange 7 (AO 7) on the nitrification process was studied using completely stirred tank reactors (CSTR) and batch treatment systems. AO 7 biodegradation was found to be essentially complete when solids retention times (SRT) were maintained above 7.5 days, but systems with lower SRTs were unstable. It was shown that AO 7 inhibited all stages of the nitrification process. Nitrite oxidizers were found to be more sensitive to AO 7 than ammonium oxidizers. The results of kinetic studies indicated that the inhibition of ammonium oxidation was typified by noncompetitive inhibition; the presence of AO 7 decreased the maximum substrate utilization rate and very slightly increased K_s, the half-saturation constant. AO 7 was found to be less toxic to nitrification than some metal and phenolic compounds, but more toxic than some common organic compounds such as formalin, methanol, or acetone.

2.5.3. Toxicity assessment

From the previous studies, 48h – LC_{50} of Acid Red 114 determined by *Pimephales Promelas* (fish organism) as equal to 4 mg/L (Brown et al., 1983). Velegraki et al. (2006) investigated the acute toxicity to marine bacteria using 50 mg/L Acid Orange 7. The inhibitory effect was found 55-58 % corresponding to NaCI containing control solution. The acute toxicity EC_{50} for 15 min value of reacted of Reactive Blue 4 determined by *Vibrio fischeri* was equal to 1108 mg/L, the estimated LC_{50} for fish over 14 d was estimated as 1500 mg/L (Epolitio et al., 2005). Biological toxicity of dyes again measured *vibrio fischeri* test with 30 min exposure time revealed EC_{50} values for Reactive Orange 16 and Congo red (azo dyes) were 1375 and 1623 mg/L, respectively, EC_{50} values were 94 mg/L for Remazol Brilliant Blue R and 488 mg/L for Disperse Blue 3 (anthraquinone dyes) (Novotny et al., 2006).

Toxicity measurement is mostly done with vitro toxicity techniques which are carried on by microorganisms. For instance; Bioluminescence assay with *Vibrio fischeri* has become widely used as fast and reliable preliminary test for risk assessment. *Selenastrum capricornutum,* is most often used algal species, has been found to be sensitive to metals, herbicides and a number of organic chemicals. For standard toxicity assays ciliates is used and most commonly *Tetrahymena pyriformis.* For

acute toxicity assays, *Daphnia magna* is mostly used which are standardized and reliable comparing to biodegradability depending heavily on biodegradability. A number of representative toxicological tests including morphological and physiological assays have been used to provide complementary information for toxicity on the ciliate *Tetrahymena pyriformis*. Ames test with *Salmonella typhimurium* has been widely used for studying genotoxicity of liquid waste, contaminated soil, sewage sludge and sediments (Novotny et al., 2006).

A study by Kumar et al. (2006) investigated decolorization, biodegradation and detoxification of benzidine based azo dye, Direct Black-38 by a mixed microbial culture isolated from an aerobic bioreactor treating textile wastewater. The studies revealed a biotransformation of Direct Black-38 into benzidine and 4-aminobiphenyl followed by complete decolorization and biodegradation of these toxic intermediates. From cytotoxicity studies, it was concluded that detoxification of the dye took place after degradation of the toxic intermediates by the culture.

A bench-scale study combining photo-Fenton reaction with an aerobic sequencing batch reactor (SBR) to degrade a commercial homo-bioreactive dye (Procion Red H-E7B, 250 mg/L) was investigated by Garcia-Montano et al. (2006). The photo-Fenton process was applied as a pre-treatment, avoiding complete mineralization, just to obtain a bio-compatible water able to be treated by means of the SBR in a second step. In this sense, different Fenton reagent concentrations were assessed by following dye solution biodegradability enhancement (BOD_5/COD), as well as the toxicity (EC_{50}), DOC, color and H_2O_2 evolution with photo-Fenton irradiation time. The acute toxicity assessment of the untreated dyestuff by means of Biotox assay show that EC_{50} was greater than 100 % or >44.96 mg/L (this means that the concentration present in solution did not cause the 50 % inhibition of the bacteria). After treatment, there was no evidence of toxic by-products development during the photo-Fenton process.

Chacon et al. (2006) studied a solar photocatalytic degradation of the azo-dye acid orange 24 was carried out by means of a photo-Fenton reaction promoted by solar energy. The dye degradation was monitored during the experimental runs through UV/Vis absorption as well as COD and TOC concentration determination and toxicity reduction. In most cases, a discoloration higher than 85 % was reached using 50kJ/L of accumulated energy. In the case of the best reaction conditions, a

discoloration of up to 95 % and a toxicity reduction from 37 to 5 TU were accomplished with 50kJ/L. In the same experiment, the removal of COD up to 88 % and TOC up to 85 % was reached after 105kJ/L. Results consigned in this work are comparable to others reported in literature for different dyes. The reduction on toxicity values obtained by this methodology was most relevant and present dye degradation by the solar photo-Fenton process as an interesting alternative for coupling with biological processes.

Meriç et al. (2005) have studied the degradation, decolorization and acute toxicity of the solutions containing of Remazol Red 120 (RR) dye and its mixture with Remazol Brilliant Blue (RB) and Remazol Yellow 84 (RY) reactive dyes. The acute toxicity of each dye composition was measured using *Daphnia magna*. Optimum pH for RR and dye mixture was found 3.5 while the optimum temperature was determined 50°C, 40°C and 30°C 100 mg/L of RR, 200 mg/L of RR and the dye mixture, respectively which were chosen on the basis of their toxicity levels on *Daphnia magna*. More than 98 % color and 92 % COD removal were obtained for the dye solutions. For obtaining high color and COD removal H_2O_2 concentrations had to be increased 3 times when RR concentrations was doubled whereas $FeSO_4$ was to be increased 2.5 times for the dye mixture. The results obtained indicate that FR can be assurely used for complete toxicity removal and obtaining high color and COD efficiency with no toxic effluent on *Daphnia magna* for the dye solutions studied. Acute toxicity test with H_2O_2 was useful to evaluate the complete oxidation resulting in practically no residual H_2O_2 (<3 mg/L) in the solution.

Aquatic toxicity evaluation of copper-complexed direct dyes to the *Daphnia magna* was studied by Bae et al. (2005). The aquatic toxicity of a series of copper-complexed direct dyes based on benzidine congeners, 2,2’-dimethyl-5,5’-dipropoxybenzidine and 5,5’-dipropoxybenzidine, were evaluated in acute toxicity studies using *Daphnia magna*. The purpose of the research described was to use bioassays with daphnids to determine the aquatic toxicity of metallized direct dyes synthesized. The results clearly show that all of the copper-complexed dyes examined were highly toxic to daphnids and more toxic than unmetallized new direct dyes as expected. The study also suggested that the assay with *Daphnia magna* was an excellent method for evaluation of dyes for aquatic toxicity.

A study by Neamtu et al. (2004) investigated the degradation of Disperse Red 354 azo dye in aqueous solution with the Fenton. Decolorization, COD removal and acute toxicity with a LUMIStox 300 instrument were observed. After 30 min. reaction time at a 20:1 H_2O_2/Fe^{2+} ratio and pH=3, 76 % decolorization and 90 % COD removal was achieved. Trace concentrations of oxalate, nitrate and sulfate were also identified as reaction products.

In another study, Reactive Black 5 (RB5) was removed from synthetic wastewater using Fenton's oxidation (FO) process (Meriç et al., 2004). Experiments were conducted on the samples containing 100 and 200 mg/L of RB5 to remove the dye toxicity. 75 mg/L of RB5 caused 25 % toxicity on 24-h born daphnids whereas 100 mg/L of RB5 displayed 100 % toxicity on *Daphnia magna*. The study was performed in a systematic approach searching optimum values of $FeSO_4$ and H_2O_2 concentrations, pH and temperature. Optimum pH and temperature for 100 mg/L of RB5 were observed as 3.0 and 40^oC, respectively, using 100 mg/L of $FeSO_4$ and 400 mg/L of H_2O_2 resulted in 71 % chemical oxygen demand (COD) and 99 % color removal. For 200 mg/L of RB5, 84 % COD removal was obtained using 225 mg/L of $FeSO_4$ and 1000 mg/L of H_2O_2 yielding 0.05 molar ratio at pH 3.0 and 40^oC. Color removal was also more than 99 %. Toxicity was completely removed for each concentration of RB5 at optimum removal conditions

A study by Moraes et al. (1999) investigated the minimization of the environmental impact of textile effluents, mainly related to their high coloration and the presence of toxic or carcinogenic reactive dyes, the efficiency of photochemical and ozonation processes, applied in the form of isolated and combined procedures, were evaluated. The investigation was focused on the reduction of total organic carbon content (TOC), color and acute toxicity (monitoring by inhibition of *Escherichia coli* respiration). For a reaction time of 60 min, the anatase TiO_2-assisted photocatalytic process produced color and TOC reduction of about 90 % and 50 %, respectively. Meanwhile, the ozonation process gives a decolorization of about 60 % but negligible TOC reduction. When the processes were applied in a simultaneous form, the decolorization was almost complete and the TOC reduction was higher than 60 %. The three treatments studied yielded an acute toxicity reduction of around 50 %

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Textile dyes

In the present study, two acid (Acid Red 183, AR 183; Acid Orange 51, AO 51) and one reactive (Reactive Blue 4; RB 4) textile dyes were chosen as refractory model pollutants and purchased from Aldrich. The reasons why particularly these three textile dyes were selected for this study are the facts that their molecular structure is exactly known and all three dyes are frequently being applied for the dyeing of cotton and nylon (polyamide) fabrics worldwide as well as in Turkey.

Physicochemical properties of the selected textile dyes are presented in Table 3.1. For the Fenton treatment as well as anaerobic, anoxic and aerobic experiments, aqueous dye solutions were prepared in distilled water. The reason why particularly this concentration was used in the study are formerly published scientific (Tantak et al., 2006; Marco et al., 2006) and literature review papers (O'Neill et al., 1999) as well as private communications with staff from local dyehouses (Turbo Tekstil, 2006) indicating that the concentration typically accounted in effluents from the textile dyeing and finishing factories are in the range of 10-200 mg/L.

Table 3.1: Physicochemical properties of the selected textile dyes AR 183, AO 51 and RB 4

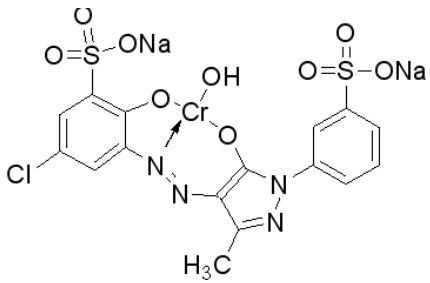
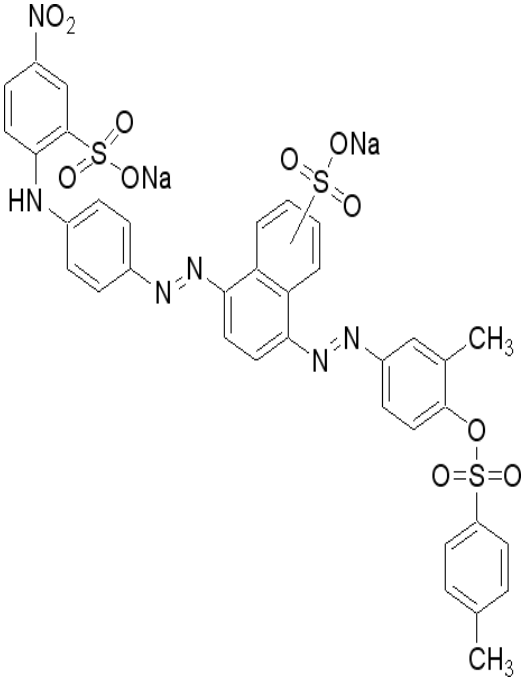
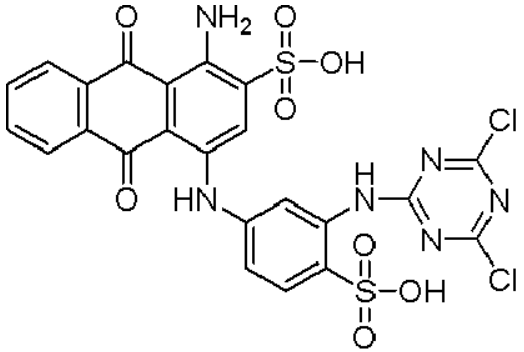
Textile Dye	**AR 183**	**AO 51**	**RB 4**
Molecular Formula	$C_{16}H_{11}ClCrN_4Na_2O_8S_2 \cdot xCr$	$C_{36}H_{26}N_6Na_2O_{11}S_3$	$C_{23}H_{14}Cl_2N_6O_8S_2$
Molecular Weight (g/mol)	584.84	860.80	637.43
Color Index Number	18800	26550	2363639
λ_{max} (nm)	497	463	595
Molecular Structure			

Table 3.2: Environmental characterization of AR 183, AO 51 and RB 4 for 100 mg/L

Textile Dye	**AR 183**	**AO 51**	**RB 4**
COD (mg O_2/g dye)	50	103	63
DOC (mg C/g dye)	14	31	21
Absorbance of 100 mg/L aqueous dye solution at λ_{max} (cm^{-1})	1.018	1.75	0.68

3.1.2. Reagents and other consumables

All chemicals used were reagent grade and obtained from Fluka, Aldrich, Merck and Riedel depending upon the price and availability. The chemicals used for Fenton process were hydrogen peroxide (H_2O_2; 35 % w/w, Fluka), ferrous iron sulfate hepta hydrate ($Fe(SO_4).7H_2O$, Fluka), sodium hydroxide (NaOH pure pellets, Merck), enzyme Catalase (from *Micrococcus Iysodiekticus*, 1 AU destroys 1 μmol H_2O_2 at pH = 7 RTP, 100181 U/mL, Fluka) to destroy residual, unreacted H_2O_2, sulfuric acid (H_2SO_4, d = 1.84 g/cm^3, 96 % w/v, Merck). Peroxide quant sticks (Aldrich) were used to determine the approximate amount of residual (unreacted) H_2O_2 in the reaction solutions. Millipore filters with 0.45 μm cutoffs were used to remove supernatant after ferric hydroxide precipitation to cease the Fenton reaction and remove iron from the reaction solution.

3.2. Experimental Procedures

3.2.1. Fenton experiments

All Fenton reactions were run with 1000 mL, 100 mg/L aqueous dye solutions at T = 20^oC and an initial pH of 3 in 1500 mL capacity beakers. Magnetic stirrers and stir bars were used to provide continuous mixing during the Fenton experiments.

In order to initiate the Fenton reaction, appropriate amounts of H_2O_2 stock solution (10.29 M) to achieve the desired H_2O_2 concentration in the final reaction solution, and likewise, $Fe(SO_4).7H_2O$ from a 10 % w/v (0.36 M) stock solution to achieve the desired Fe^{2+} concentration, were added to the dye solution simultaneously and right after pH adjustment with 1N, 0.5N, 0.1N and 0.02N sulfuric acid.

25 mL sample aliquots were taken from the beakers at pre-determined time intervals (t = 0, 2, 5, 10, 20 and 30 min) during the Fenton reaction. For each sample, the reaction was quenched immediately with the addition of concentrated (6 N) NaOH to increase the pH to around 9-10, i.e. another adjustment was done with 0.5N and 0.1N sulfuric acid for pH = 7-8 to achieve the maximum amount of $Fe(OH)_3$ precipitation. After Fe^{2+} was almost completely precipitated out of the solution in the form of solid $Fe(OH)_3$, the sample was filtered through 0.45 μm-cutoff Millipore filters and spiked with sufficient amounts of enzyme Catalase to destroy any residual/unreacted H_2O_2 in order to prevent its positive interference with COD measurements. Due to the fact that Catalase also contributes to the COD (1.46 mg COD/mL Catalase) and TOC (1.02 mg TOC/mL Catalase) of the samples, separate samples were taken at time t = 0 (prior to Fe^{2+} addition to prevent the initiation of the process) were mixed with exactly the same amount of Catalase that was used to destroy H_2O_2. These samples were identified as "catalase control samples". Samples were taken at the above indicated time intervals for color (absorbance), COD and DOC (dissolved organic carbon) analyses.

3.2.2. Anaerobic experiments

Anaerobic experiments were conducted for 60 days at T = 37^{o}C (under mesophilic conditions) and pH = 6.5 with untreated dyes as well as dyes being subjected to treatment with Fenton's reagent at optimized reaction conditions (pH_o = 3; Fe^{2+}:H_2O_2 ratio = 4 mM: 20mM; t = 10 min). The pH of the dye samples was adjusted to 7.5, that of the sludge to 6.8. N_2 (80 % N_2 and 20 % CO_2) was used to remove all O_2 out that caused pH decrease in the reaction solutions from 7.5 to 6.5.

For the anaerobic experiments, sludge for anaerobic reactors was collected from the anaerobic digestion of Lundtofte Urban Wastewater Plant of Denmark, Lyngby. VSS concentration of the sludge was 16200 mg/L. Sludge was not adapted to the dyes and no substrate was used as external carbon source for the experiments. Only formalin (37 % w/v, Merck) was used for to prevent presence of alive microorganism in the abiotic control reactor to compare with other reactor's culture formation. 1.2μm-cutoff glass microfiber filter papers (Whatman 47 mm).were used for VSS and TSS analyses.

The anaerobic batch reactors consisted of 56, 500-2000 mL capacity glass bottles that 10 of them were used for color, COD, DOC, VSS and TSS analyses (for these reactors, the reactor volume was chosen as Vr_1=1600 mL), the remaining batch reactors were kept for CH_4 analyses (the reactor volume was chosen as Vr_2=300 mL). Samples were taken as the predetermined intervals (t = 0, 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 d). The end of the experiment was decided as the 60th day, i.e. the time when the methane formation rate started to level off. All anaerobic batch reactors were run in triplicate.

Formalin (37 % w/v) was added to abiotic untreated and treated dye samples at 2% (v/v) to kill all the (methanogenic) microorganisms in the inoculum. These samples were called "abiotic untreated and treated" control samples. Samples were defined as untreated, treated, abiotic untreated and treated controls individually for all three dyestuffs. In addition, a Catalase control reactor was prepared. The reactors are listed below;

- Treated samples with optimum molar ratio and optimum time.
- Untreated samples without any chemicals inside.
- Abiotic untreated samples with formalin inside to compare toxic effect with untreated dye solution.
- Abiotic treated samples with formalin inside to compare toxic effect with treated dye solution.
- Control reactor with distilled water
- Catalase control reactor with distilled water, catalase and H_2O_2

The anaerobic batch reactors prepared for methane analysis are summarized below;

- **Reactor 1:** Control — Distilled water and inoculum (290 mL + 10 mL)
- **Reactor 2:** Catalase control — Distilled water, inoculum, Catalase and H_2O_2 (285.6 mL + 10 mL + 3 mL + 1.94 mL)
- **Reactor 3:** Untreated dye — Untreated dye and inoculum (290 mL + 10 mL)

- **Reactor 4:** Treated dye Treated dye, inoculum and Catalase (287 mL + 10 mL + 3 mL)
- **Reactor 5:** Abiotic untreated dye Untreated dye, inoculum and formalin (284 mL + 10 mL + 6 mL)
- **Reactor 6:** Abiotic treated dye Treated dye, inoculum and Catalase and formalin (284 mL + 10 mL + 3 mL +6 mL)

The above given experimental set-up was operated individually for each dyestuff.

The VSS concentration in all reactors was selected as 500 mg/L. During the anaerobic experiments, no external carbon source (substrate) was added to the samples to observe the inhibitory/toxic effect of the untreated and treated dyes more clearly. However, no CH_4 production was observed within the first 48 hours of the anaerobic experiments in all batch reactors including controls. For this reason, 500 mg/L glucose solution was added to the control reactor (containing inoculum and distilled water only) to observe if the delay in expected CH_4 formation was related to the absence of substrate.

3.2.3. Anoxic experiments

Anoxic experiments were conducted for 48 hours at T = 20^{o}C and pH = 8.3 with untreated dyes as well as dyes being subjected to treatment with Fenton's reagent under optimized reaction conditions (pH_o = 3; Fe^{2+}:H_2O_2 = 4 mM: 20mM; t = 10 min). The pH of the untreated and treated dye samples was adjusted to 7.5, that of the sludge to 6.8. Argon (100 %) was used to remove N_2 (or any other gas) out that caused pH increase in the reaction solutions from 7.5 to 8.3.

The anoxic batch reactors consisted of 14, 2000 mL capacity glass bottles that the sample volumes were selected as 1600 mL and the inoculum was added to all reactors at a concentration of 2400 mg/L VSS. Sample aliquots were taken every half an hour during the first 4 hours and after 24 and 48 h to determined changes in color and nitrate after centrifuging (for 10 min at 13,000 rpm in 2 ml Eppendorf tube at Eppendorf Mini Spin table centrifuge).

For the anoxic experiments, sludge was collected from the aeration tanks after the primary settling tank of Lundtofte Urban Wastewater Plant of Denmark, Lyngby. VSS concentration of the sludge was 6400 mg/L. Sludge was not adapted to the dyes prior to the experiments. Acetate (Fluka) and $NaNO_3$ (Riedel) were used as the external carbon and nitrate source.

Formalin was added to abiotic untreated and treated samples at a concentration of 2 % v/v to kill all the microorganisms in the inoculum. These samples were again called "abiotic untreated and abiotic treated" control samples. Samples were identified as untreated, treated, and abiotic untreated and treated sample reactors and prepared individually for each dyestuff. In addition, separate control and Catalase control reactors were prepared for the anoxic experiments.

The reactor set-ups of the anoxic experiments are listed below;

- **Reactor 1:** Control Distilled water and inoculum (1000 mL + 600 mL)
- **Reactor 2:** Catalase control Distilled water and inoculum and catalase and H_2O_2 (990 mL + 600 mL + 8 mL + 3 mL)
- **Reactor 3:** Untreated dye Untreated dye and inoculum (1000 mL + 600 mL)
- **Reactor 4:** Treated dye Treated dye inoculum and catalase (992 mL + 600 mL + 8 mL)
- **Reactor 5:** Abiotic untreated dye Untreated dye, inoculum and formalin (925 mL + 600 mL + 75 mL
- **Reactor 6:** Abiotic treated dye Treated dye, inoculum and catalase and formalin (917 mL + 600 mL + 75 mL + 8 mL)

At the beginning of anoxic experiments, $NaNO_3$ and $NaCH_3COO$ were added to the reactors at a ratio of 1:3 (w/w), corresponding to 111.8 mg/L (1.31 mM) and 335.6 mg/L (5.69 mM), respectively.

The VSS concentration in all reactors was selected as 2400 mg/L.

3.2.4. Aerobic experiments

Experiments were conducted in two stages and individually for all of the 3 investigated textile dyes and the Fenton's treated products to examine their inhibitory effect on glucose degradation via heterotrophic biomass. For that purpose, activated sludge experiments were run in 8, 1000 mL fed-batch reactors for 6 hours at ambient temperature (T = 20^{o}C) and pH = 7.0 with heterotrophic biomass that was obtained different wastewater plants of İstanbul, Turkey and previously acclimated to glucose. During the acclimation period, two 1500 mL fed-batch reactors were daily fed with 516 mg/L glucose solution providing 500 mg O_2/L COD as the carbon source for a period of 30 days. Activated sludge experiments were conducted with samples containing 516 mg/L glucose + 100 mg/L untreated dyes or dye solutions being subjected to treatment with Fenton's reagent under optimized conditions (pH_o = 3; Fe^{2+}:H_2O_2 = 4 mM:20mM; t = 10 min). During the aerobic experiments, sample aliquots were taken at predetermined time intervals (e.g. 0, 10, 20, 30 min and 1, 2, 3, 4, 5 and 6 h) for color and COD after filtering through 0.45 μm-cutoff Millipore membrane filters.

For the aerobic experiments, sludge was collected from aeration tanks of Tuzla Urban Wastewater Treatment Plant, Tuzla; Baltalimanı Urban Wastewater Treatment Plant, Baltalimanı, İstanbul, Turkey. VSS concentration of the sludge was 5425 mg/L for the first aerobic experiment for untreated dyes and VSS concentration was 3365 for the second aerobic experiment for treated dyes. Again sludge was not adapted to the dyes prior to the experiments. Glucose (corresponding to 500 mg/L COD) was used as the external carbon source. 0.45 μm-cutoff sterile filter papers (Millipore) were used to remove microorganism from the sample. For VSS and TSS analyses 1.2μm-cutoff glass microfiber filter papers (Whatman 47 mm).were used.

The reactor set-ups designed for the aerobic experiments are summarized below;

- **Reactor 1:** $Control_1$ Distilled water + inoculum + glucose + A/B*
  (667 mL + 323 mL + 516 mg/L + 5 mL + 5 mL)
- **Reactor 2:** Untreated dye Untreated dye + inoculum + glucose + A/B*
  (667 mL + 323 mL + 516 mg/L + 5 mL + 5 mL)

- **Reactor 3:** $Control_2$ Distilled water + inoculum + glucose and A/B*
  (730 mL + 260 mL + 516 mg/L + 5 mL + 5 mL)
- **Reactor 4:** Treated dye Treated dye + inoculum + glucose + A/B*****
  (730 mL + 260 mL + 516 mg/L + 5 mL + 5 mL)

*A/B is the Solution A contains buffers and Solution B contains micronutrients for feeding of inoculum as a source of N, P, Mg, Fe, Ca, Mn, and Zn.

Table 3.3: Reagents and their amounts for "Sol A"

Reagent		Concentration (g/L)
N	NH_4CI	120
P	KH_2PO_4	160
P	K_2HPO_4	320

Table 3.4: Reagents and their amounts for "Sol B"

Reagent		Concentration (g/L)
Mg	$MgSO_4.7H_2O$	15
Fe	$FeSO_4.7H_2O$	0.5
Ca	$CaCI_2$	2
Mn	$MnSO_4.3H_2O$	0.5
Zn	$ZnSO_4.7H_2O$	0.5

The VSS concentration in all reactors was selected as 3500 mg/L, corresponding to a fixed F/M (food-to-microorganisms) ratio of 0.13 (mg COD / mg MLVSS) in all aerobic reactors.

3.3. Analytical Procedures

3.3.1. Color

In this study, color was referred as the absorbance measured at the maximum absorption bands of the investigated textile dyes (i.e. 497 nm for AR 183; 463 nm for AO 51, and 595 nm for RB 4). Two different spectrophotometers were calibrated and used as a result of carrying out the experiments at DTU, Denmark, and at ITU, Turkey. The absorbance of the untreated and treated samples was measured on a Jenway 6400 model UV-VIS Spectrophotometer and Novespec II, Pharmacia LKB, operating in the wavelength range of 350-900nm, respectively. Absorbance measurements were performed with 1 cm optical path length, reusable glass cells.

3.3.2. COD

For COD measurements that were conducted at DTU, Denmark, the method was based on the Danish Standard DS 217:1991 (1991). Low range COD's were determined in the range of 15-100 mg O_2/L.COD measurements at ITU, Turkey were conducted in accordance with the Standard Methods ISO 6060 (1986) by the closed reflux, titrimetric method.

3.3.3. DOC

DOC was measured on a Shimadzu TOC-TC 5000 A, carbon analyzer. In order to calibrate the analyzer, stock solutions were prepared from a certified reference; hydrogen phthalate ($C_8H_5KO_4$) for TC and potassium hydrogen carbonate ($NaHCO_3$) for IC solutions (QC WW4A Eurofins). For this purpose, an appropriate amount of the originally 500 mg/L stock solutions was thawed before use. Milli-Q-water was used for blank. TC standards were prepared as 5, 10, 25, 50, 100 mg/L and IC standards as 5, 10, 25, 50 mg/L.

3.3.4. Sulfate and nitrate

A ICS-1500 model Dionex Ion Chromatograph, equipped with an Ion Pac AS 14 mm (10-31) Column (P/N 46124) in combination with an anion suppresser (AMMS III 4-mm P/N 56750, chemical regenerating) was used to measure nitrate and sulfate in the

untreated and treated samples. Column and cell heater temperatures were 35^{o}C and the pressure was 1400 psi.

3.3.5. CH_4 formation

A Shimadzu model – 14A GC equipped with a Porapak 60/80 molsiere column (6 ft long and 3mm in inner diameter) and a flame ionization detector (FID) was used for CH_4 measurements during the anaerobic experiments. Nitrogen (100 % v/v) was used as the carrier gas at a pressure of 2.0 kg/cm^2. The injection temperature was set to 110^{o}C, while the detector and oven temperature were 160^{o}C. The retention time for CH_4 was around 50 s. The standard gas mixture used for CH_4 measurements was 70 % N_2 and 30 % CH_4.

3.3.6. Acetate

A GC Shimadzu GC – 2010 equipped with a capillary column (ZB – FFAP, 30 m, 0,53 mm I.D x 1,0 μm) and a flame ionization detector (FID) was used to measure acetate that was expected as an oxidation intermediate or end product of the Fenton experiments.

3.3.7. TSS-VSS

TSS and VSS were measured according to the procedure outlined in Standard Methods (APHA, 1995). TSS concentration was measured at a temperature of 103-105^{o}C and VSS at a temperature of 550^{o}C. For sample filtration, 1.2 μm cut-off glass micro fiber filter papers (Whatman 47 mm papers) were used.

3.3.8. pH

pH was measured using a digital pH meter (692-pH/ionmeter Metroholm at DTU, Denmark and (Thermo Orion, model 520) at ITU, Turkey.

3.3.9. Chromium

The chromium content of AR 183 before and during Fenton treatment (under optimized reaction conditions) was analyzed on a Unicam 929 model atomic absorption spectrometer with and without 50 % v/v nitric acid digestion to determine the amount of chromium released from the originally complexed, organically bound chromium during Fenton oxidation. Unfortunately, chromium directly leached out of

the chromium complex acid dye directly after acidification with 14.5 N nitric acid even before being subjected to Fenton's treatment. The chromium content was determined as 4.5 mg/L in the 100 mg/L digested original dye sample and 3.8 mg/L in the undigested dye sample. Measured chromium levels decreased to 1.2 mg/L for Fenton treated dyes that might be attributable to adsorption of released chromium and/or chromium complex dye on ferric hydroxide sludge that was formed during precipitation of Fe(II)/(III) ion to cease the Fenton reaction and to remove residual soluble iron.

3.4. Determination of inhibitory effect by the presence of treated/untreated textile dyes

Inhibitory effect caused by the presence of treated/untreated textile dyes during anaerobic, anoxic and aerobic experiments is determined as percent inhibition rates by making below calculations.

Calculation of inhibition "I (%)"

●For anaerobic experiments

$$I_R = \left(\frac{(K_c - K_d)}{K_d} \right) \times 100 \qquad \textbf{(1)}$$

I_R = percent inhibition rate (%)

Kc = CH_4 formation rate constant in control (mL CH_4/(mg VSS.d)$^{-1}$)

Kd = CH_4 formation rate constant in sample (mL CH_4/(mg VSS.d)$^{-1}$)

(Anaerobic experiments fits to zero order kinetics)

●For anoxic experiments

$$I_R = \left(\frac{(K_c - K_d)}{K_d} \right) \times 100 \qquad \textbf{(2)}$$

I_R = percent inhibition rate (%)

Kc = Denitrification rate constant in control (mg/(L.h)$^{-1}$)

Kd = Denitrification rate constant in sample (mg/(L.h)$^{-1}$)

(Anoxic experiments fits to zero order kinetics)

●For Aerobic experiments

$$I_R = \left(\frac{(K_c - K_d)}{K_d} \right) \times 100 \quad \textbf{(3)}$$

I_R = percent inhibition rate (%)

Kc = Glucose (COD) degradation rate constant in control $(mg/(L.h)^{-1})$

Kd = Glucose (COD) degradation rate constant in sample $(mg/(L.h)^{-1})$

(Aerobic experiments fit to first order kinetic)

4. RESULTS AND DISCUSSION

4.1. Treatment with Fenton's Reagent

In Fenton process the main factors affecting the rate of Fenton's reaction rate are H_2O_2 and Fe^{2+} concentrations. H_2O_2 concentration is important to obtain high oxidation efficiencies, while the Fe^{2+} concentration is critical for the improvement of reaction kinetics. (Alaton et al., 2007; Vajnhandl et al. 2006; Kim et al., 2004; Kong et al., 2002; Chamarro et al., 2000). During the Fenton reaction (eqn. 4.1.); Fenton's reagent is used to produce hydroxyl radicals ($^{\bullet}OH$) to oxidize organic substances (shown as R);

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + H_2O + {}^{\bullet}OH \qquad \textbf{(4.1)}$$

$$^{\bullet}OH + R \rightarrow \text{Oxidation Products} \quad (k_{20}{}^{o}{}_{C} = 10^{7}\text{-}10^{10}\ M^{-1}s^{-1}) \qquad \textbf{(4.2)}$$

When the reagents Fe^{2+} and H_2O_2 are provided in excess, reduced oxidation efficiency is expected because of the following undesired $^{\bullet}OH$ scavenging reactions (Duran et al., 2006);

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-} \qquad (k_{20}{}^{o}{}_{C} = 4.3 * 10^{8}\ M^{-1}s^{-1}) \qquad \textbf{(4.3)}$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow H_2O + HO_2{}^{\bullet} \qquad (k_{20}{}^{o}{}_{C} = 2.7 * 10^{7}\ M^{-1}s^{-1}) \qquad \textbf{(4.4)}$$

Previous studies have indicated that the highest oxidation efficiency is achieved by reaction (4.2) when neither Fe^{2+} nor H_2O_2 are overdosed (Chamarro et al., 2001; Kang et al., 2002; Kim et al., 2004; Alaton et al., 2007). For this reason, to maximize the treatment efficiency, the case-specific optimum Fe^{2+}: H_2O_2 molar ratio has to be determined.

Generally speaking, the oxidation rate of organic compounds is fast when ferrous ions are present at concentrations > 0.5 mM because more $^{\bullet}OH$ radicals are then produced. However, the Fenton reaction slows down due to the ferric iron production (Fe^{3+}) ion production and consumption of H_2O_2 (Laat et al., 1999);

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH$ $k_{20^oC} = 76\ M^{-1}s^{-1}$ **(4.5)**

$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + {}^{-}OH$ $k_{20^oC} = (4.3) \times 10^8\ M^{-1}s^{-1}$ **(4.6)**

$Fe^{2+} + HO_2{}^{\bullet} + H^+ \rightarrow Fe^{3+} + H_2O_2$ $k_{20^oC} < 2 \times 10^3\ M^{-1}s$ **(4.7)**

Initial Fe^{2+}: H_2O_2 molar ratio

On order to determine the optimum of Fe^{2+}: H_2O_2 molar ratio, a set of experiments was carried out for every each dye (e.g. 100 mg/L aqueous AR 183, AO 51 and RB 4; corresponding to 171 μM, 116 μM, 157 μM dye concentrations) dye. Different molar ratios selected as "2:20, 2:40, 4:20, and 4:40" (in mM) were tested. The optimum molar ratios were determined upon inspection of decolorization, DOC and COD degradation rates obtained for each dye. In order to examine the extend of degradation, NO_3^-, SO_4^{2-} and CH_3COO^- formation were also followed.

4.1.1 Results for Acid Red 183

4.1.1.1 Color abatement

Color abatement efficiencies at the predefined wavelength λ = 497nm were observed for the time intervals set as 0, 2, 5, 10, 20, and 30 minutes for different Fe^{2+} : H_2O_2 molar ratios. Color abatement obtained for different $Fe^{2+:}$ H_2O_2 molar ratios are presented in Figure 4.1.

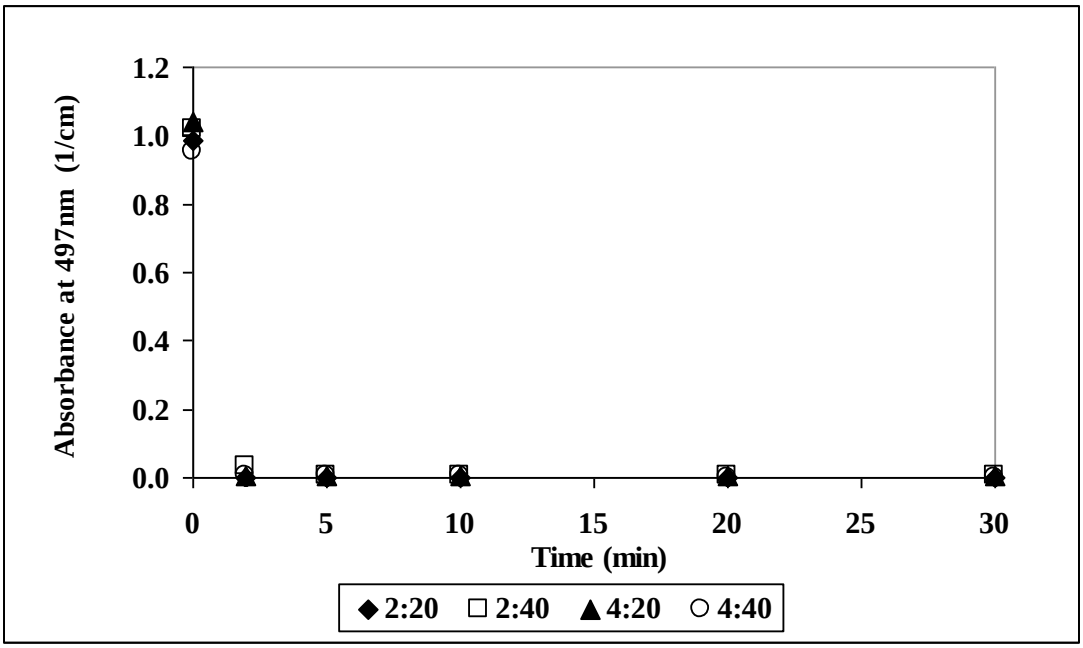


Figure 4.1: Effect of different initial Fe^{2+}: H_2O_2 molar ratios on color abatement at λ= 497 nm for AR 183 (pH_o = 3, $A_{497,\,o}$ = 1.018 cm^{-1})

It may be observed from the figure that color abatement was completed within the first minutes of Fenton's reaction. For each case color abatement rate was more than 99 % after 5 min independent of the applied molar ratio. The rapid decolorization rates can be explained by the fast reaction between ferrous ions and hydrogen peroxide to produce a sufficient amount of $^{\bullet}OH$ that cleaved the dye chromophores easily. In other words, the in-situ formed $^{\bullet}OH$ has enough oxidation capability to completely decolorize AR 183.

From previous studies reported in the literature, fast and complete color removal (> 99.9 %) was achieved by employing Fenton's reagent for 50 mg/L Acid Orange 7

(Tantak et al., 2006). Similarly, color removal was obtained as 96 % (Marco et al., 2006) for 50 mg/L Reactive Black 5 during the first min of Fenton's reaction.

As is evident in the figure, color removal proceeded so fast that is was not possible to fit the color abatement curve to reaction kinetic.

4.1.1.2. COD abatement

COD abatement was followed for the same dye during a time period of 30 min fot the same Fe^{2+}: H_2O_2 . COD abatement rates obtained for AR 183 with different Fe^{2+}: H_2O_2 molar ratios are presented in Figure 4.2.

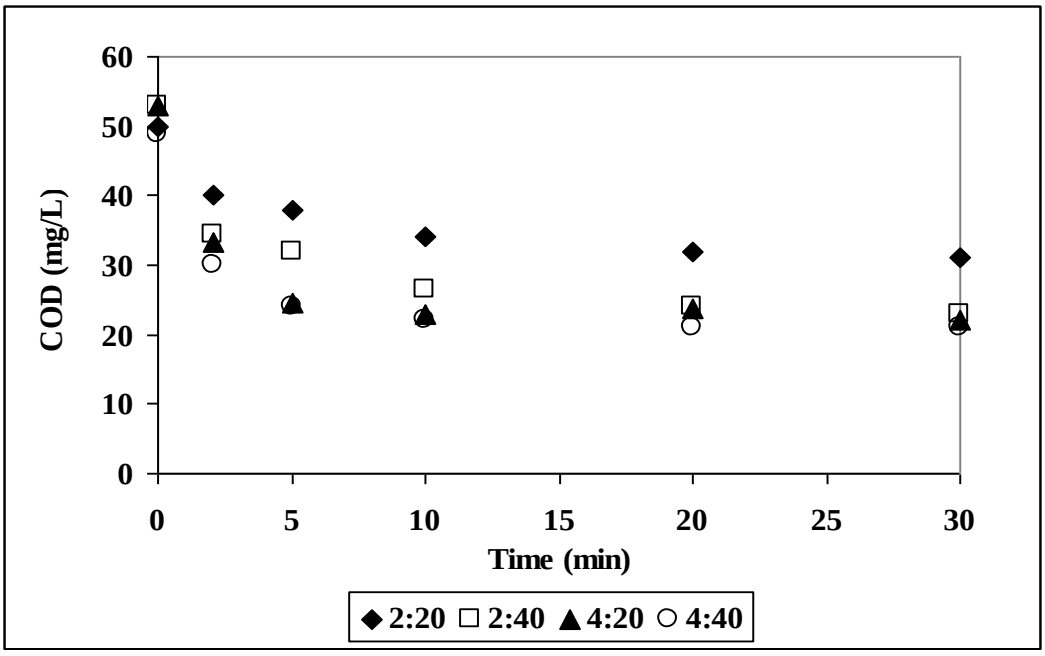


Figure 4.2: Effect of different Fe^{2+}: H_2O_2 molar ratios on COD abatement rates for AR 183 (pH_o = 3, COD_o = 50 mg/L, DOC_o = 14 mg/L)

From the figure it is evident that COD degradation started immediately within the first minutes of the reaction and slowed down after 10 min most probably due to hydrogen peroxide exhaustion and consequent ferric iron accumulation. However, this time different molar ratios resulted in different COD abatement rates. For instance, the COD removal rate was relatively slow for the 2:20 molar ratio resulting in an overall COD removal of only 38 %. Highest and similar COD removals were achieved for the Fe^{2+}:H_2O_2 molar ratios 4:20 and 4:40, resulting in 58 % and 57 % COD reduction after 30 min, respectively. Generally speaking, increasing either the initial H_2O_2 or the Fe^{2+} concentration increased the COD removal rates and efficiencies.

This relatively low COD reduction is most likely due to the fact that highly complex structured dye molecules are only partially oxidized to small organic molecular fragments, such as acetic acids, aldehydes, ketones etc., not being completely mineralized (Wang et al., 2003; Neamtu et al., 2004). For this particular reason, complete degradation of the dyes cannot be achieved.

This observation can be supported by related, previous studies published in the scientific literature, where Fenton oxidation of synthetic acid dyebath effluent, bearing two azo and one anthraquinone dye together with two due auxiliaries resulting in almost 23 % COD removal after 30 min (Alaton et al., 2007). In another investigation which was carried out with textile wastewater using Fenton's reaction, only less than 30 % COD abatement could be achieved. This behavior was attributed to the relatively short retention time selected for the oxidation process leading to incomplete degradation (Fongsatitkul et al., 2004).

4.1.1.3. DOC abatement

DOC abatement rates were also followed for the same Fe^{2+}:H_2O_2 molar ratios and the same time intervals. DOC abatement results obtained for 100 mg/L AR 183 during Fenton's reaction at different molar ratios are presented in Figure 4.3.

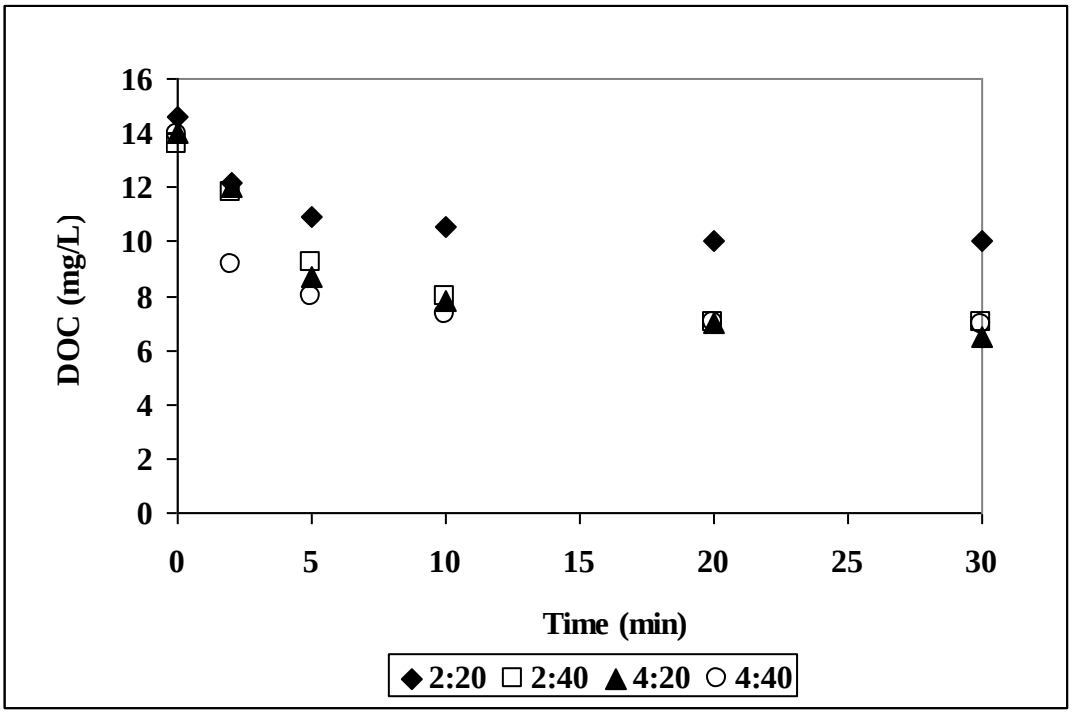


Figure 4.3: Effect of different Fe^{2+}: H_2O_2 molar ratios on DOC abatement for 100 mg/L AR 183 (pH_o = 3, COD_o = 50 mg/L, DOC_o = 14 mg/L)

From the figure is clear that DOC abatement rates were almost parallel with COD abatement. Degradation proceeded fast within in the first 5 minutes of the reaction and almost leveled of after 10 min speculatively due to accumulation of oxidation intermediates and consumption of Fenton's reagent. When different molar ratios were compared, again when the molar ratio was 2:20, relatively less DOC was removal occurred (32 %) after 30 min treatment. This observation may be explained with insufficient oxidation at this particular Fe^{2+}:H_2O_2 molar ratio. Hence, increasing the Fe^{2+} and/or H_2O_2 concentrations increased DOC removal efficiency from 32 % (2:20) to 53 % (4:40).

4.1.1.4. Acetate formation

During oxidation of the AR 183 dye molecules by Fenton oxidation it is expected that some carboxylic acids such as acetate may form and accumulate in the reaction solution at detectable levels. Acetate was measured for t = 0, 10 and 30 min of Fenton's reaction. The different Fe^{2+}: H_2O_2 molar ratios investigated in this part of the study were again 2:20, 2:40, 4:20, and 4:40 mM: mM. The acetate concentrations detected for different molar ratios are presented in Figure 4.4 for 100 mg/L AR 183.

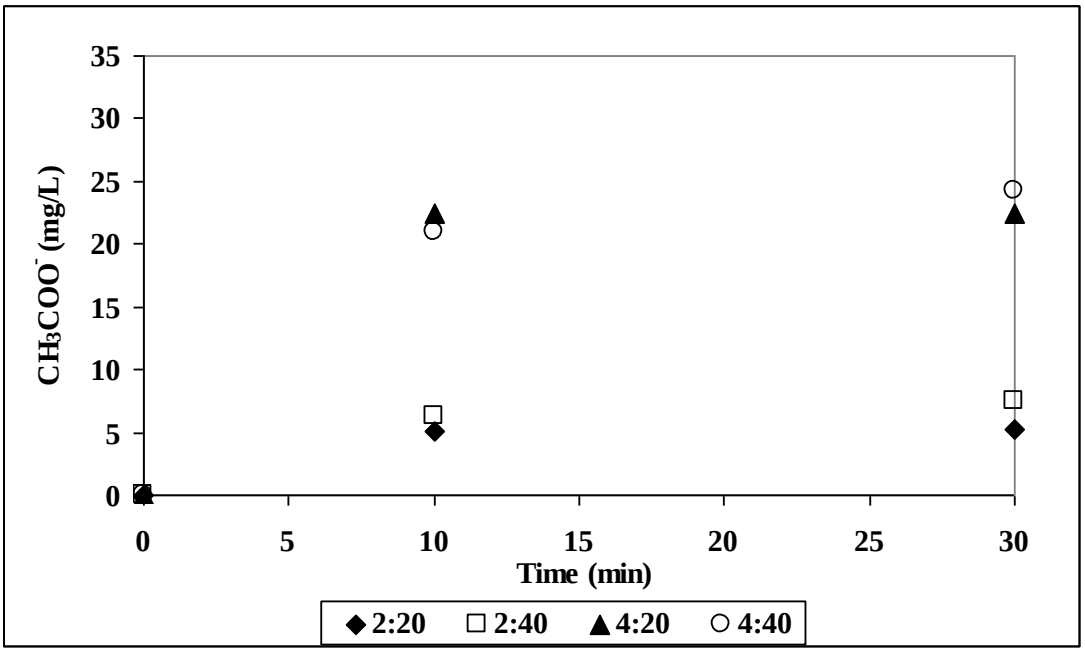


Figure 4.4: Effect of different Fe^{2+}: H_2O_2 molar ratios on acetate formation for 100 mg/L AR 183 (pH_o = 3, COD_o = 50 mg/L, DOC_o = 14 mg/L)

From the figure is it evident that acetate formation was found different for each molar ratio, but increasing the ferrous iron concentration from 2 mM to 4 mM (4:20 mM) increased the acetate concentration to 23 mg/L after 10 min. The acetate concentration remained stagnant thereafter. Increasing the hydrogen peroxide concentration from 20 mM to 40 mM in the presence of 4 mM ferrous iron resulted in having similar formation of 25 and 23 mg/L acetate, respectively and almost leveled after. The reason might be slightly different reaction mechanisms observable under different catalyst: oxidant molar ratios. However, provided that sufficient ferrous iron and hydrogen peroxide were supplied initially, acetate accumulation started, indicating dye breakdown to low molecular weight carboxylic acids, and reached similar concentrations at the end of the studied reaction period. The formation and accumulation of acetate as a dye oxidation intermediate supports the incomplete degradation of the dye molecule as also is evident from partial COD and DOC reduction (Wang et al., 2003; Joseph et al., 1999). Theoretically predicted acetate concentration for 100 mg/L AR 183 was 80.70 mg/L and hence the analyzed acetate levels were less than predicted.

Except acetate, nitrate and sulfate formation was also followed; there was no considerable nitrate formation during during Fenton oxidation. Nitrate formation is mainly due the oxidation and cleavage of the azo bonds and amino groups of dye molecules. However, it is not very surprising that nitrate was not detectable at significant concentrations (measured around detection levels) and hence appreciably lower than predicted or expected, indicating that either complete degradation has not been reached yet or that nitrogen in the dye underwent oxidation to other species such as N_2 or NH_4^+ (Duran et al., 2006). Sulfate was observed in significant amount that was possibly the result of using $Fe_2SO_4.7H_2O$ as iron catalyst source. For this reason nitrate and sulfate formations for dyes was shown in the section of appendix.

4.1.2. Results for Acid Orange 51

4.1.2.1. Color abatement

Color abatement at the maximum absorption band of AO 51 (e.g. λ = 463 nm) was observed for AO 51. Color abatement rates for different Fe^{2+}:H_2O_2 molar ratios is presented in Figure 4.5.

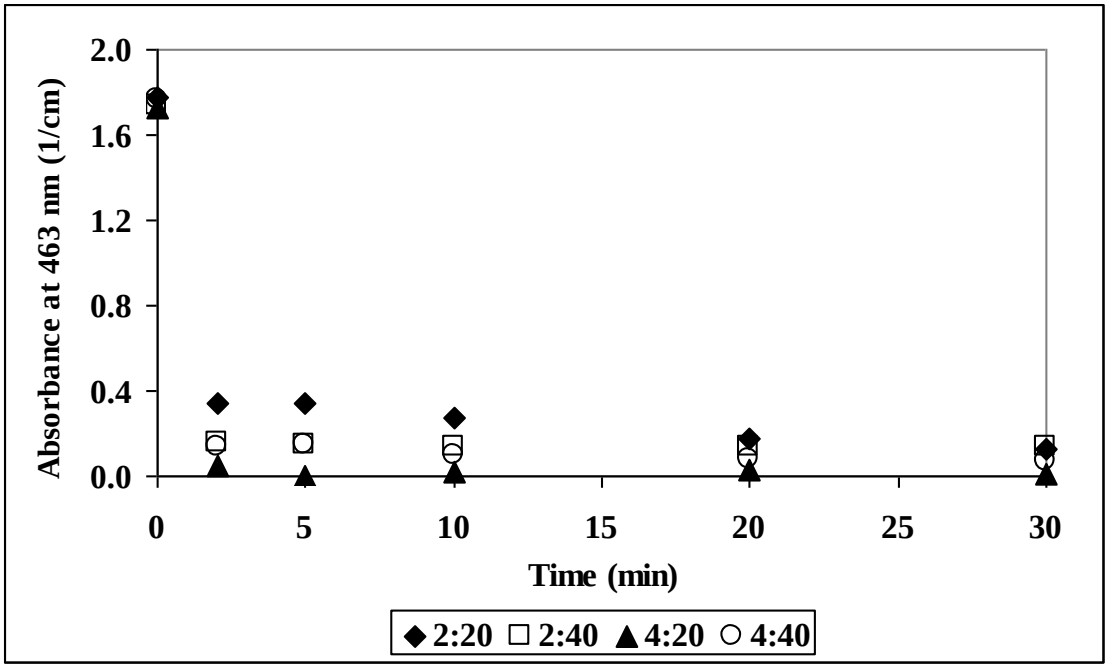


Figure 4.5: Effect of different Fe^{2+}:H_2O_2 molar ratios on color abatement for AO 51 (pH_o = 3, $A_{463,\,o}$ = 1.75 cm^{-1})

From the above figure it is evident that decolorization proceeded fast and was practically complete after 5 min reaction time for all molar ratios, with the exception of the molar ratio 2:20 (mM:mM), e.g. for the lowest Fe^{2+} : H_2O_2 concentrations. For this particular molar ratio, color removal was only 80 % after 5 min oxidation and reached 92 % after 30 min advanced oxidation. Fastest decolorization was obtained for the ratio 4:20 (99.6 %), followed by the molar ratios 4:40 (96 %) and 2:40 (93 %), for which almost identical color abatement rates were obtained. This may be explained as follows; the fast decolorization stage initialized by $^{\bullet}OH$ production due to the reaction of H_2O_2 with Fe^{2+} followed by the instantaneous reaction of $^{\bullet}OH$ with the dye molecules.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + H_2O + {}^{\bullet}OH$$

Thereafter, a slow degradation period (between t = 5-30 min) continues, where the Fenton-like reaction producing $HO_2^{\bullet}$ radicals becomes dominant;

$Fe^{3+} + H_2O_2 \rightarrow FeOOH^{2+} + H^+$

$FeOOH^{2+} \rightarrow HO_2^{\bullet} + Fe^{2+}$

The capability of $HO_2^{\bullet}$ to oxidize organic compounds is by far less than that of $^{\bullet}OH$. As such, it is expected that the reaction slows down. In another study where aqueous solutions of 50 mg/L Reactive Black 5, Reactive Blue 13 and Acid Orange 7 were treated with Fenton's reagent, 90 % color removal occurred within the first 4 min of the reaction. It took another 360 min to achieve complete (e.g. 99 %) decolorization (Tantak et al., 2006). Other reasons of the second, slow reaction stage, wherein the abatement rates almost level of were explained in the previous sections for AR 183.

4.1.2.2. COD abatement

COD abatement rates obtained for oxidation of AO 51 at different Fe^{2+}:H_2O_2 molar ratios is given in Figure 4.6.

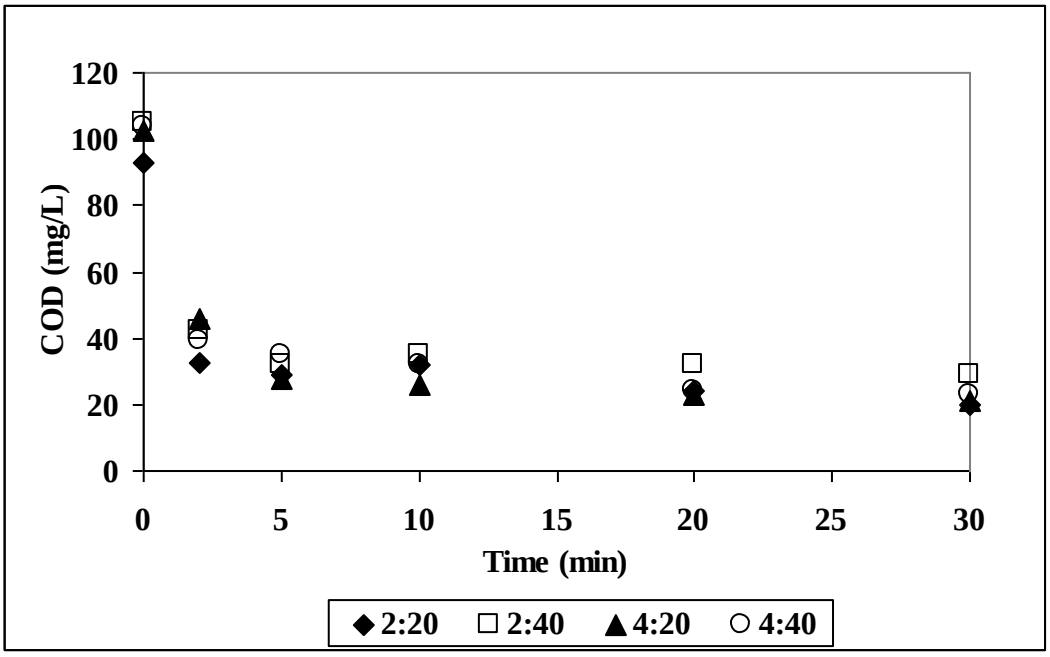


Figure 4.6: Effect of different Fe^{2+}:H_2O_2 molar ratios on COD abatement for 100 mg/L AO 51 (pH_o = 3, COD_o = 103 mg/L, DOC_o = 31 mg/L)

COD abatement rates followed decolorization kinetics and were obtained as 78, 72, 80 and 77 % (t = 30 min), respectively for the molar ratios 2:20, 2:40, 4:20 and 4:40.

Fastest COD removal occurred at the molar ratio of 4:20 Fe^{2+}:H_2O_2, indicating the possibility of radical scavenging side reactions between excess ("overdosed") H_2O_2 and $^{\bullet}OH$ as shown in equation (4.4).

The figure also reveals that compared with the overall COD removal efficiencies after 30 min obtained for AR 183 (e.g. 38, 56, 58 and 57 %, for 2:20, 2:40, 4:20 and 4:40, respectively), degradation of AO 51 is faster and more thorough. This can be explained by the differences in molecular structures and molar concentrations. Although AO 51 has two azo bonds compared to the single azo bond of the chromium complex azo dye AR 183, and an appreciably higher molecular weight than AR 183 (861 g/mol instead of 585 g/mol for AR 183), the molar concentration of AO 51 is conclusively less than that of AR 183.

4.1.2.3. DOC abatement

DOC abatement rates obtained for AO 51 degradation at different Fe^{2+}:H_2O_2 molar ratios are presented in Figure 4.7.

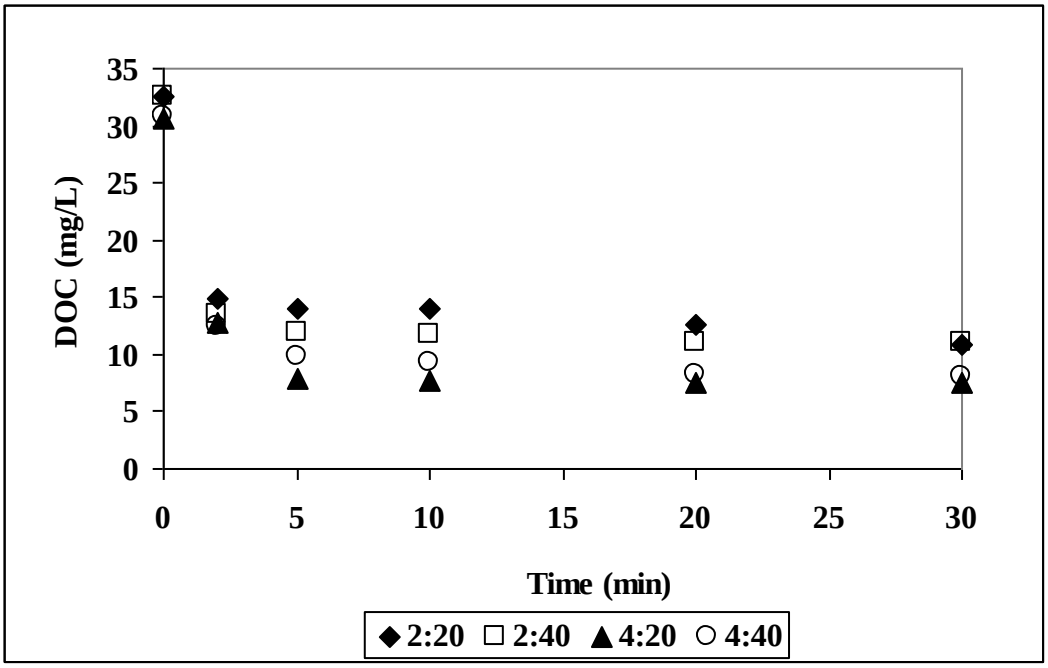


Figure 4.7: Effect of different Fe^{2+}:H_2O_2 molar ratios on DOC abatement rates obtained for 100 mg/L AO 51 (pH_o = 3, COD_o = 103 mg/L, DOC_o = 31 mg/L)

Again, from Figure 4.7 it can be inferred that DOC abatement occurred parallel to color and COD removals; after a brief initial stage of fast degradation, oxidation rates slowed down considerably and almost leveled of after 10 min oxidation. Upon

comparison of DOC removals achieved for different molar ratios, it is evident that the highest and fastest DOC removal was achieved for the ratio 4:20 mM:mM (overall DOC removal was 76%), very closely followed by the DOC removal obtained for the ratio 4:40 mM:mM (74%). Conclusively it can be postulated that the increase in oxidant and catalyst concentration and particularly the concentration of ferrous iron catalyst, an increase in oxidation rate and efficiencies were observed.

4.1.2.4 Acetate formation

Acetate release observed for AO 51 at varying Fe^{2+}: H_2O_2 molar ratios is presented in Figure 4.8.

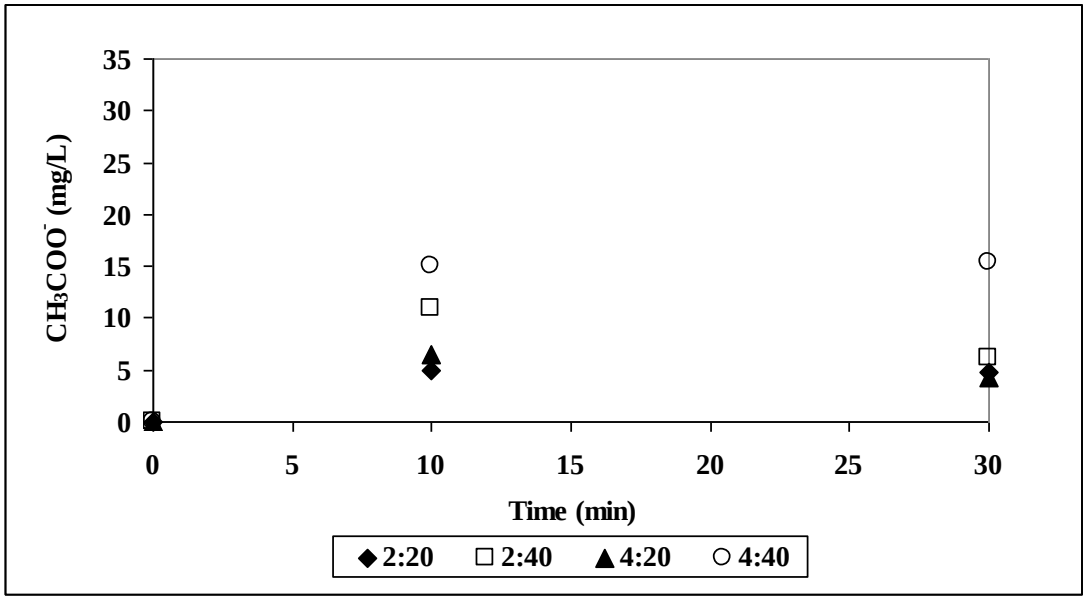


Figure 4.8: Effect of different Fe^{2+}: H_2O_2 molar ratios on acetate formation for 100 mg:/L AO 51 (pH = 3, COD_o = 103 mg/L, DOC_o = 31 mg/L)

From the above figure it is evident that acetate formation was different for all Fe^{2+}: H_2O_2 molar ratios. For instance, the fastest acetate release was obtained at a molar ratio of 4:40, where acetate formation remained almost constant after 10 min at 16 mg/L whereas at a molar ratio of 4:40 acetate formation value was 11 mg/L and decreased thereafter to the concentration of 6.2 mg/L. No significant acetate formation was observed for the Fe^{2+}: H_2O_2 molar ratios 2:20 and surprisingly also at 4:20, at which molar ratio in most cases the highest treatment efficiencies were obtained for Fenton's oxidation of both azo dyes. The theoretically predicted acetate concentration was 123.4 mg/L and the acetate levels obtained during the experiments were less than predicted.

4.1.3. Results for Reactive Blue 4

4.1.3.1. Color abatement

Color abatement rates measured at the maximum absorption band for the anthraquinone dye RB 4 (at 595 nm) during Fenton's oxidation at varying Fe^{2+}: H_2O_2 molar ratios are presented in Figure 4.9.

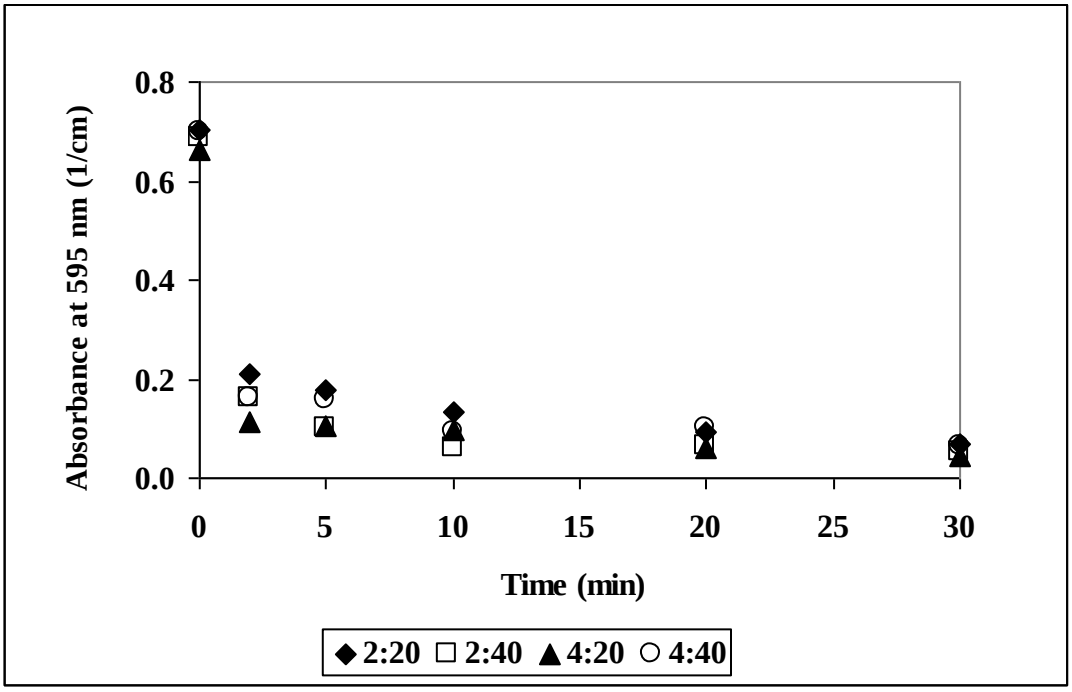


Figure 4.9: Effect of different Fe^{2+}: H_2O_2 molar ratios on color abatement for 100 mg/L RB 4 (pH_o = 3, $A_{595,\,o}$ = 0.68 cm^{-1})

Again, from the above findings it is evident that fastest color removal was obtained for the Fe^{2+}: H_2O_2 molar of 4:20 (mM:mM), followed by the molar ratios 2:40 and 4:40 (mM:mM). As for the other two dye formulations, color removal was comparably slow for the Fenton reaction run at a Fe^{2+}: H_2O_2 molar ratio of 2:20, where both oxidant and catalyst were at their lowest concentration. During the first 5 min of Fenton reaction, color removals were obtained as 75, 85, 84 and 78 % for the molar ratios 2:20, 2:40, 4:20 and 4:40, respectively. After the initial fast reaction period, color removal proceeded slowly and ultimately reached 90, 93, 93 and 91 %, for the molar ratios 2:20, 2:40, 4:20 and 4:40, respectively, at the end of the 30 min Fenton's treatment period.

From the figure it is also evident that compared with the two acid dyes (e.g. the azo dyes AR 183 and AO 51), color removal rates were appreciably slower. A study

which investigated the structural characterization, hydrolysis and Photo-Fenton treatment of RB 4 postulated that pre-hydrolysis of RB 4 had a positive effect of decolorization rates (Epolito et al., 2005).; however, in the present study, pretreatment such a hydrolysis at high T (= 70-90 ^{o}C) and high pH (= pH>11) to simulate real dyebath conditions was not intended. In another study, where the same anthraquinone reactive dye RB 4 was exposed to the photo-Fenton process under solar radiation, complete color removal for an initial dye concentration of 0.1 mM (= 63.74 mg/L) was obtained only after 35 min irradiation (Carneiro et al., 2005).

4.1.3.2. COD abatement

COD abatement rates obtained for Fenton's treatment of 100 mg/L RB 4 at different Fe^{2+}: H_2O_2 molar ratios are presented in Figure 4.10.

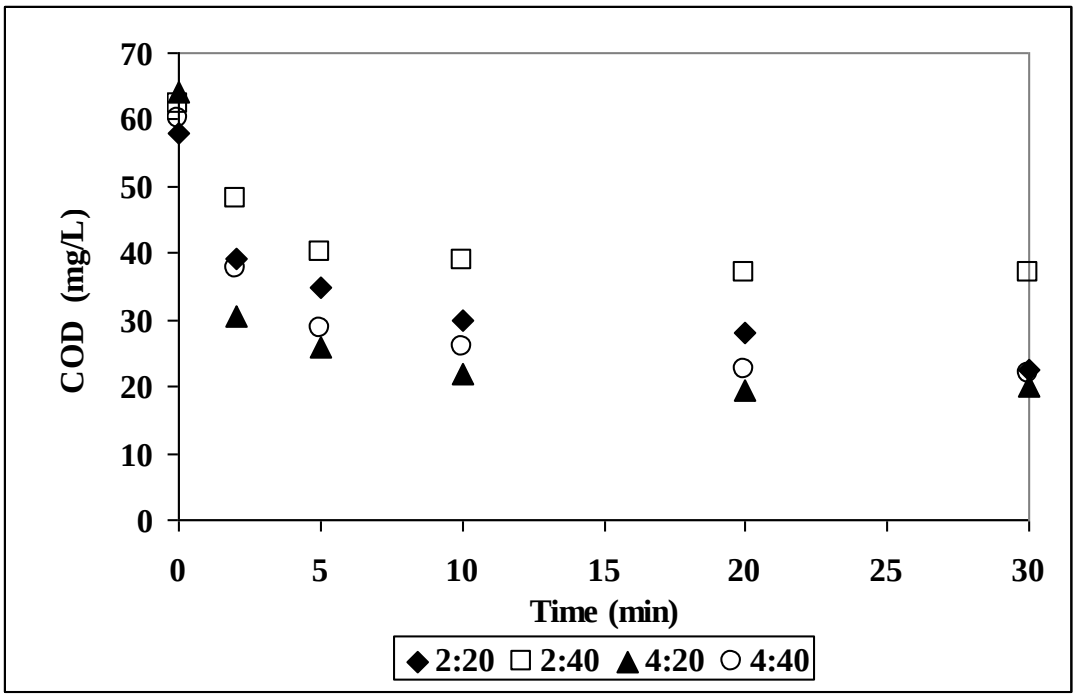


Figure 4.10: Effect of different Fe^{2+}: H_2O_2 molar ratios on the COD abatement for 100 mg/L RB 4 (pH_o = 3, COD_o = 63 mg/L, DOC_o = 21 mg/L)

From the figure it is evident that COD was initially fast and slowed down significantly after 5 min oxidation. Fastest and highest COD removal was obtained for the molar ratio 4:20 (overall COD removal was 69 % after 30 min), whereas COD abatement remained rather slow and incomplete (44 % after 30 min) for the molar ratio 2:40. The COD removals obtained at the other two other two molar ratios were closer to the optimum Fe^{2+}: H_2O_2 molar ratio. When COD abatements of all

three dyes are compared, COD abatement rates were obtained in the following decreasing order AO 51 > RB 4 > AR 183, that is attributable to the differences in their molecular structures and formation/accumulation of different oxidation products/intermediates, e.g. degradation pathways.

4.1.3.3 DOC abatement

DOC abatement rates for RB 4 degradation at different Fe^{2+}: H_2O_2 molar ratios are given in Figure 4.11.

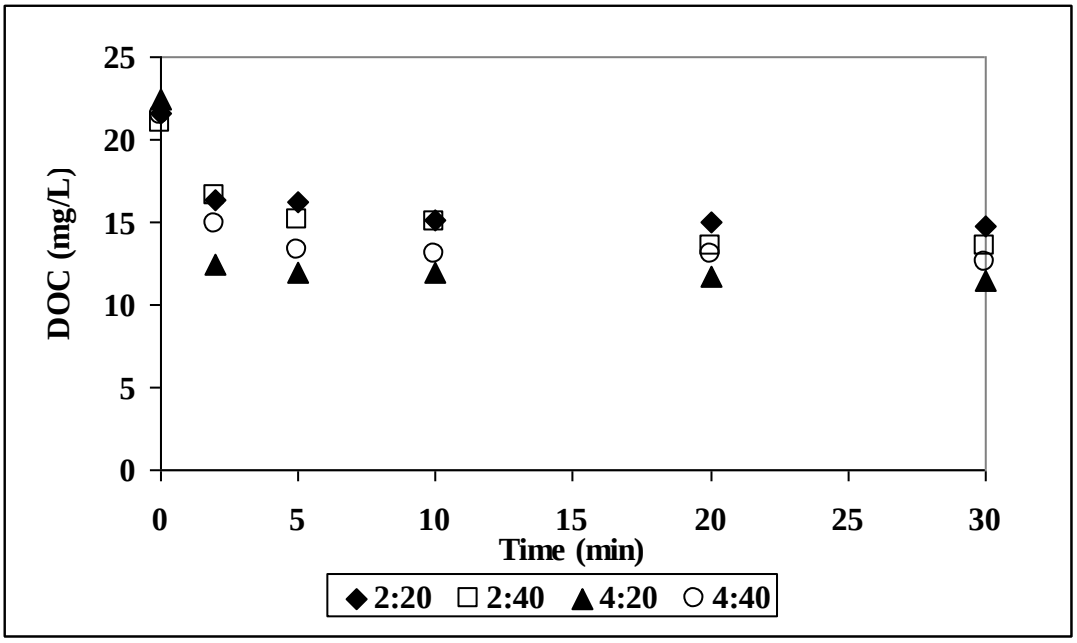


Figure 4.11: Effect of different Fe2+:H2O2 molar ratios on DOC abatement for 100 mg/L RB 4 (pH_o = 3, COD_o = 63 mg/L, DOC_o = 21 mg/L)

Dye degradation started immediately and remained constant after an initial fast oxidation period of 5 min. Lowest DOC removal was obtained for the molar ratio 2:20 (= 35 %) and highest for the molar ratio 4:20 (= 49 %) after 30 min treatment time. The obtained results indicated that ferrous iron catalyst concentration was more effective on oxidation rates than hydrogen peroxide.

4.1.3.4 Acetate formation

Acetate formation at different $Fe^{2+:}$ H_2O_2 molar ratios are presented in Figure 4.12.

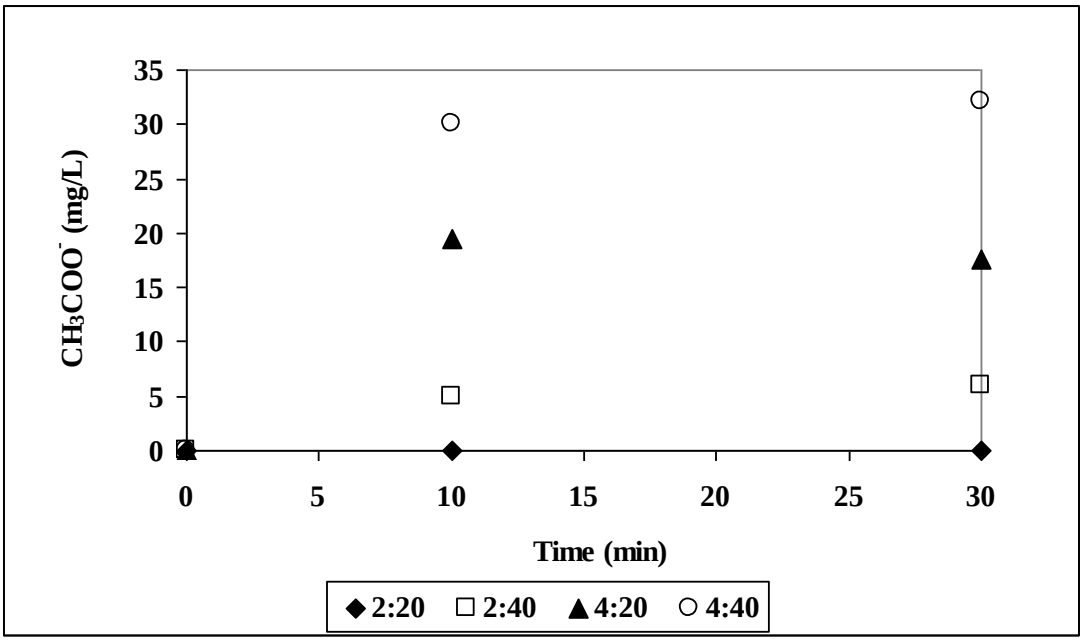


Figure 4.12: Effect of different Fe^{2+}: H_2O_2 molar ratios on acetate formation for 100 mg/L RB 4 (pH_o = 3, COD_o = 63 mg/L, DOC_o = 21 mg/L)

From the figure it can be inferred that for each molar ratio the acetate formation rates were different and no acetate was formed when the reaction was initiated at a molar ratio of 2:20 (mM:mM), e.g. the molar ratio where also color, COD and DOC abatement rates where found relatively slow compared to the other ratios. The highest acetate formation occurred at the molar ratio 4:40 (mM:mM) as 30 mg/L, after 10 min treatment and almost remained stagnant (32 mg/L). The theoretically predicted acetate value was 101.82 mg/L.

Tables 4.1, 4.2, and 4.3 are given to summarize the results of COD, DOC values after 10min and 30 min and their percent removal efficiencies of Fenton treatment on each dyes. Corresponding color removal rate was obtained as; 100% for Acid Red 183 after 10 min; color removal ratio for Acid Orange 51 was observed 92, 93, 100 and 97 % after 30 min by the molar ratios 2:20, 2:40, 4:20 and 4:40 respectively; color removal ratio for Reactive Blue 4 was observed 90, 93, 93 and 91 % after 30 min by the molar ratios 2:20, 2:40, 4:20 and 4:40 respectively.

Table 4.1: COD, DOC values after 10min and 30 min and corresponding percent COD and DOC removal efficiencies obtained after 10 and 30 min Fenton's treatment of 100 mg/L AR 183 at varying Fe^{2+}:H_2O_2 molar ratios.

	COD				**DOC**			
Fe^{2+}:H_2O_2 molar ratios (mM:mM)	COD after 10 min (mg/L)	Removal Efficiency after 10 min (%)	COD after 30 min (mg/L)	Overall Removal Efficiency after 30 min (%)	DOC after 10 min (mg/L)	Removal Efficiency after 10 min (%)	DOC after 30 min (mg/L)	Overall Removal Efficiency after 30 min (%)
2:20	34	32	31	38	11	28	10	32
2:40	26	50	23	56	9	42	7	49
4:20	23	56	22	58	7	50	6	54
4:40	22	55	21	57	8	47	7	50

Table 4.2: COD, DOC values after 10min and 30 min and corresponding percent COD and DOC removal efficiencies obtained after 10 and 30 min Fenton's treatment of 100 mg/L AO 51 at varying Fe^{2+}:H_2O_2 molar ratios.

	COD				**DOC**			
Fe^{2+}:H_2O_2 molar ratios (mM:mM)	COD after 10 min (mg/L)	Removal Efficiency after 10 min (%)	COD after 30 min (mg/L)	Overall Removal Efficiency after 30 min (%)	DOC after 10 min (mg/L)	Removal Efficiency after 10 min (%)	DOC after 30 min (mg/L)	Overall Removal Efficiency after 30 min (%)
2:20	32	66	20	78	14	57	11	67
2:40	35	67	29	72	12	64	11	66
4:20	26	75	21	80	8	75	7	76
4:40	32	70	23	77	9	70	24	74

	COD	**DOC**

Fe^{2+}:H_2O_2 molar ratios (mM:mM)	COD after 10 min (mg/L)	Removal Efficiency after 10 min (%)	COD after 30 min (mg/L)	Overall Removal Efficiency after 30 min (%)	DOC after 10 min (mg/L)	Removal Efficiency after 10 min (%)	DOC after 30 min (mg/L)	Overall Removal Efficiency after 30 min (%)
2:20	24	48	31	38	14	30	10	32
2:40	41	38	23	56	15	28	7	49
4:20	22	66	22	58	12	47	6	53
4:40	26	57	21	57	13	40	8	43

Table 4.3: COD, DOC values after 10min and 30 min and corresponding percent COD and DOC removal efficiencies obtained after 10 and 30 min Fenton's treatment of 100 mg/L RB 4 at varying Fe^{2+}:H_2O_2 molar ratios.

From these findings it can be inferred that the optimum Fe^{2+}: H_2O_2 molar ratios was found 4:20 mM (1:5) for 10 min, coinciding with the highest color, COD, DOC removal efficiencies. Similar studies found a ratio of 1:3 the treatment of acid dyebath effluent which includes azo and anthraquinone dyes (Alaton et al., 2007). The molar ratios for the degradation of different types of textile dyes were determined as 1:5 and 1:7 for the oxidation of Disperse Blue 106 and Disperse Yellow 54, respectively (Kim et al., 2004).

4.2. Anaerobic Experiments

Anaerobic experiments were carried out with 100 mg/L untreated and Fenton's treated samples at pH = 3 for 10 min at a molar ratio of 4:20 mM:mM, e.g. the optimum Fe^{2+}: H_2O_2 molar ratio found for Fenton's oxidation of all three dyes.

During the anaerobic experiments; color, DOC, CH_4, NO_3^-, SO_4^{2-}, VSS and TSS were observed during time period of 0, 1, 3, 5, 7, 10, 15, 20, 25, 30 and every each 5 day until 60th day.

4.2.1. Results for Acid Red 183

4.2.1.1. Color

Changes in color for untreated and treated AR 183 during the anaerobic experiment are presented in Figure 4.13.

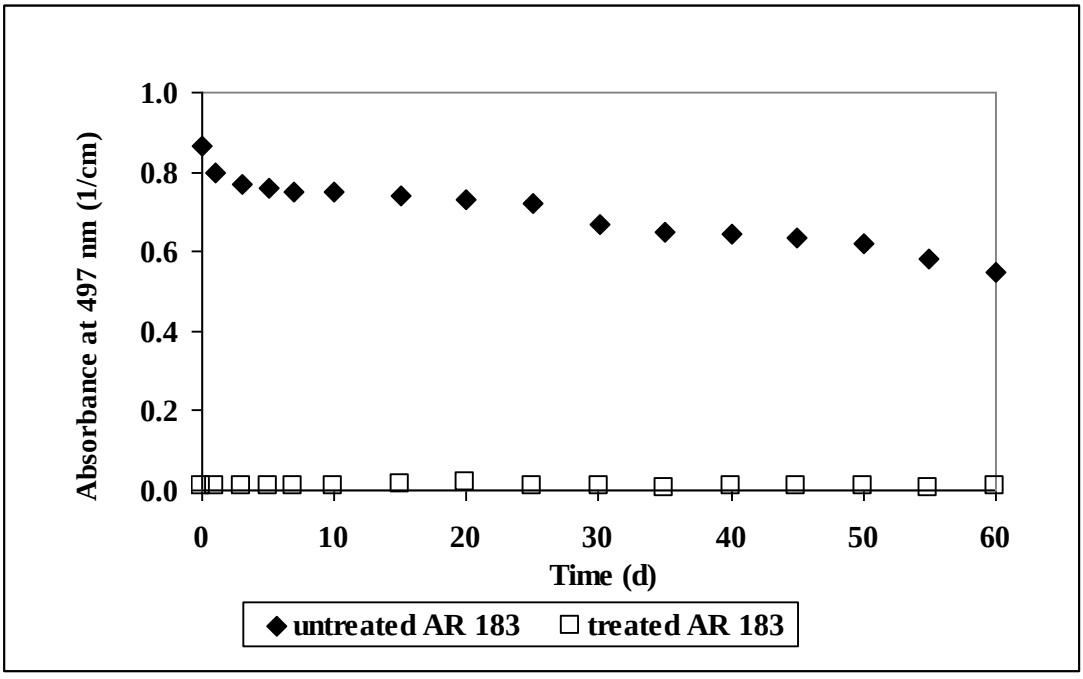


Figure 4.13: Color abatement of untreated and treated AR 183 at 497 nm ($A_{497,\ o\ ,unt\ red}$ = 0.87 cm^{-1}, VSS_o = 500 mg/L)

It may be observed that there was a preliminary adsorption of 15 % on to biomass for untreated AR 183 at the beginning of experiment however color abatement for untreated AR 183 was not efficient under anaerobic conditions such as color removal

on 10th day was 22 %, which increased to 37 % after 60 days of reaction indicating a rather slow decolorization process. Treated AR 183 had no color to be removed after Fenton treatment.

It is known that the decolorization of azo dyes under anaerobic conditions may involve biosorption (adsorption to cell biomass) and reductive of cleavage of the azo bonds.

Some researchers assumed that anaerobic color removal is mainly due to azo dye reduction. (Pinheiro et al., 2004; Cruz et al., 2001). In a study which was conducted with 100-3200 mg/L Direct Black 38 and glucose corresponding to 2000 mg/L COD as co-substrate, color removal was found 80-100% after 15h (Sponza al., 2005); whereas Albuquerque et al. (2005) observed 90 % decolorization of 25 mg/L Acid Orange 7 in the presence of 1150 mg/L starch (1000 mg/L COD) as carbon source after 10h.

On the other hand, in a study of Maas et al. (2005) who studied microbial decolorization of Reactive Red 2 at concentrations of 100 and 200 mg/L under anaerobic conditions, the decolorization rates were found as 78 and 76%, respectively after 27 days. It was suggested biosorption played most important role and accounted for around 80 % of color elimination. Previous published data describing batch experiments conducted with Reactive Red 141 at a concentration range of 100 mg/L (in the presence of sucrose corresponding to 4.8 g/L COD), color abatement was found as 86 %, respectively (Bell et al., 2003). In another study carried out with Reactive Blue 5 at a concentration of 100 mg/L, color abatement obtained as 37 % after 16 days (Luangdilok et al. 2000). Color removal in these studies was thought to be mainly due to biosorption.

Our decolorization results revealed that both of the decolorization processes could take a place during the experiments; regular and more rapidly decolorization until 10th day (22 %) could be related with azo bond reduction however overall decolorization for 60 days could be mainly due to adsorption to cell biomass. If it was just result of azo bond reduction it would not be in this low ranges and it would be occurred more rapidly (Bell et al., 2003). However the yield could be higher even the time period was longer and also insufficient amount of biomass, in other words granule to be attached could be result of low decolorization rate.

Furthermore absence of external co-substrate could result in an inefficient decolorization process. This can be supported with a study which was done with Disperse Blue 79; for low concentrations of the dye (>50 mg/L), it has been observed that to use co-substrate was not necessary, since decolorization efficiencies were 98 % after 72 hours reaction time. But when the concentration was higher than 50 mg/L, the use of co-substrate (114 mg/L of sodium acetate) was required to achieve 98 % of dye decolorization (Cruz et al., 2001). In the absence of an appropriate electron donor, anaerobic color abatement efficiency should be ruled out. But the behaviors of acid and disperse dyes are different hence the effect of co-substrate present for acid dyes could be different.

4.2.1.2. DOC

Since methane formation (assessment of toxicity) could be related with degradation of the dyes, it might be interesting to observe changes in DOC. DOC changes during the anaerobic experiment in the presence of treated and untreated AR 183 is given in Figure 4.14.

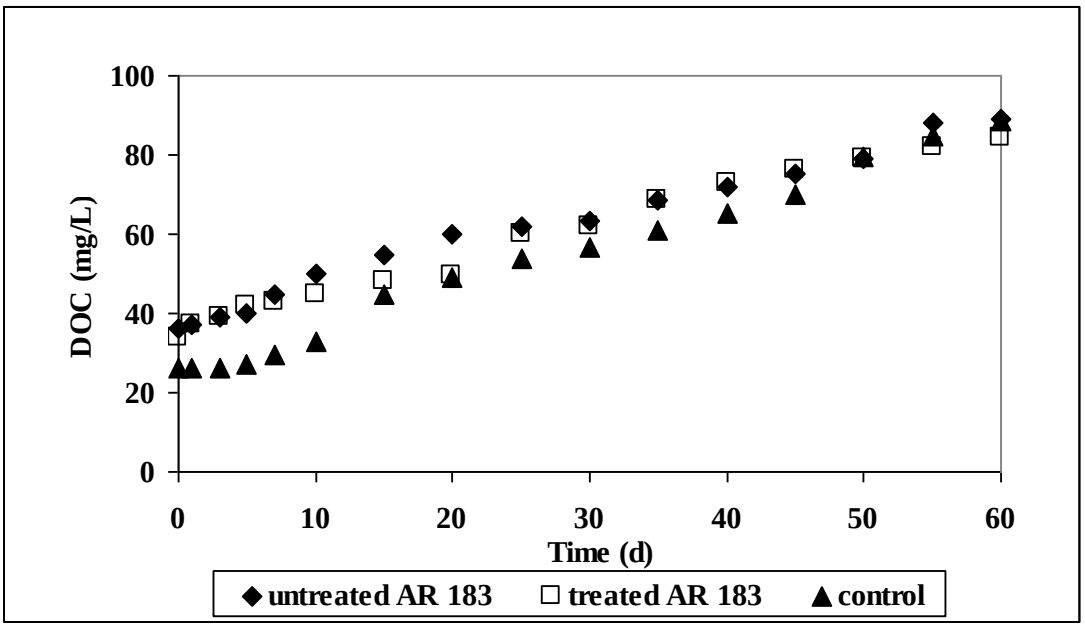


Figure 4.14: Changes in DOC during the anaerobic experiment in the presence of untreated and treated AR 183 (DOCo, control = 26 mg/L, DOCo, unt red = 36 mg/L, DOCo, treated red = 34 mg/L, VSSo = 500 mg/L)

From the figure it is evident that DOC release during the anaerobic experiments proceeded in a similar manner in the presence of untreated and treated AR 183, increasing from 34 and 36 mg/L (almost no change during 10 days) to 84 and 82

mg/L (after 60 days) for treated and untreated AR 183, respectively. For control containing reactor DOC value increased from 26 to 89 mg/L after 60 days and followed a similar path with untreated and treated AR 183. The results indicated that there was no difference in DOC release when the sludge was digested in the presence of untreated and treated AR 183.

Increase in DOC can be explained by endogenous decay and digestion of microorganisms. As a result of not having external carbon source, endogenous decay and digestion started causing continuous carbon release.

4.2.1.3. Nitrate and sulfate

Under anaerobic continuous conditions in other words under absence of oxygen, it is clear that nitrate will decrease and disappear however since reduction of NO_3^-N that was formed at minor concentrations due to oxidation of nitrogen in the form of azo, nitro, amino and triazine present in the original dye solution might be interesting to follow (even it is around insignificant ranges). Therefore, nitrate was measured during anaerobic experiment at t = 0, 20 and 40 days.

NO_3^- concentration changes during anaerobic experiment in the presence of untreated and treated AR 183 is presented in Figure 4.15.

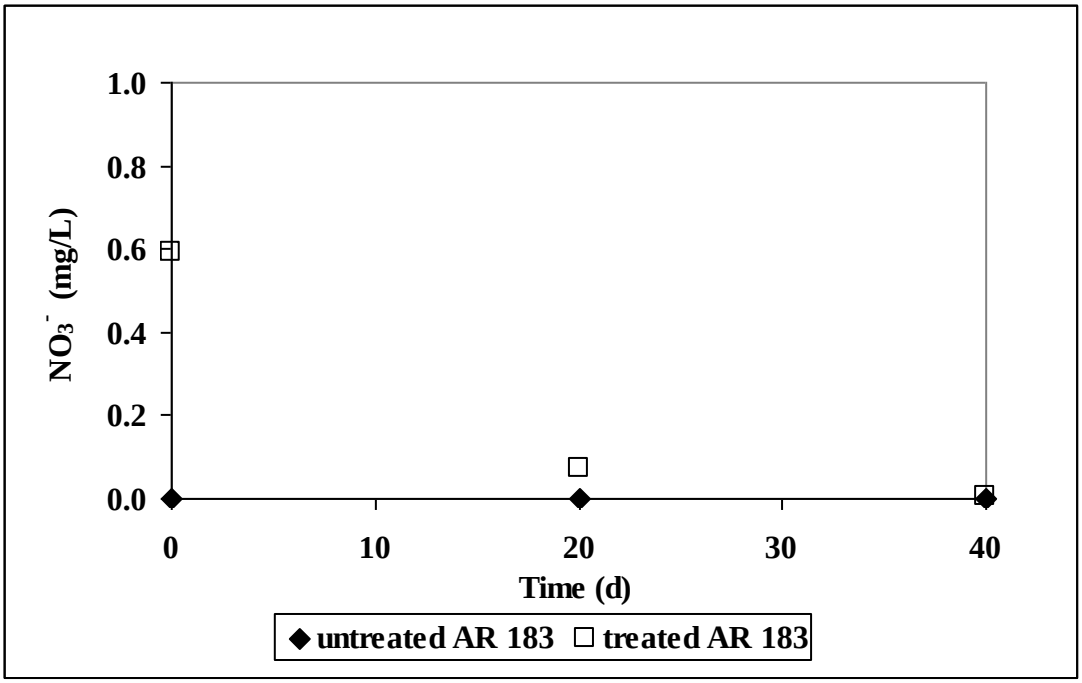


Figure 4.15: Changes in NO_3^- concentration during anaerobic experiment in the presence of untreated and treated AR 183 (VSS_o = 500 mg/L)

As it is obvious from the figure, untreated AR 183 did not contain NO_3^-N and hence there was no nitrate during the anaerobic experiment. In the case of treated AR 183 NO_3^-N decreased to 0.07 mg/L from 0.65 mg/L on the 20th day, and completely disappeared on the 40th day.

Sulfate is again expected to completely disappear under anaerobic continuous conditions like nitrate but especially from Fenton reaction each dyes have significant sulfate accumulation (mostly as result of using $Fe_2SO_4.7H_2O$ as iron catalyst source). Since sulfate presence could affect methanogenic activity, it might be interesting to observe it. Sulfate release was again followed at 0, 20 and 40 day.

Figure 4.16 presents concentration changes of sulfate.

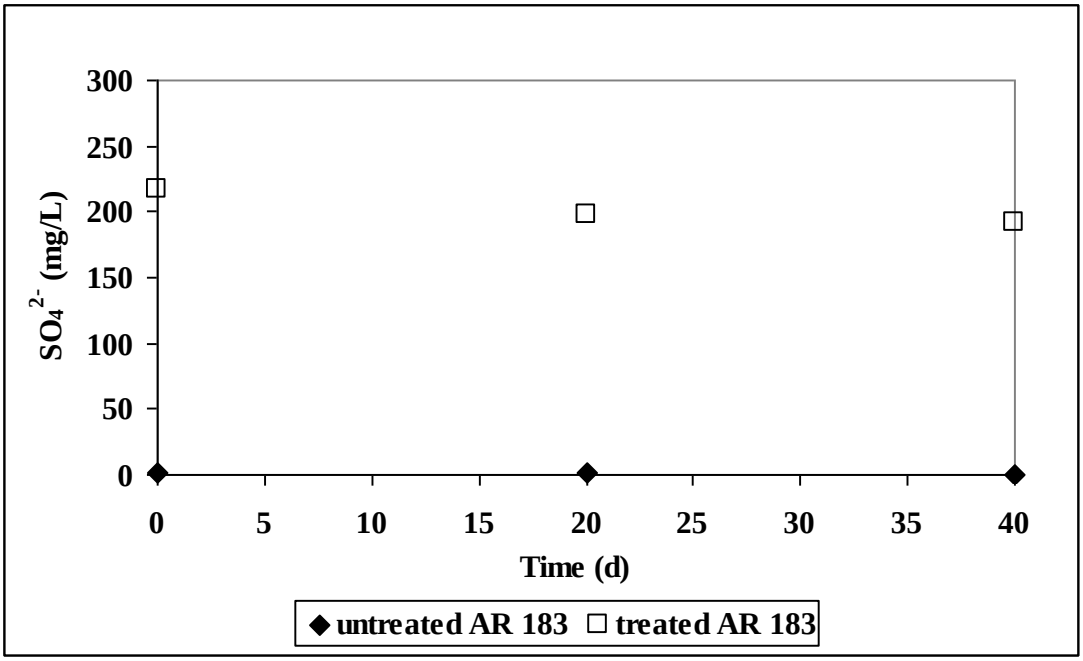


Figure 4.16: Changes in SO_4^{2-} concentrations during anaerobic experiment in the presence of untreated and treated AR 183 (VSS_o = 500 mg/L)

It can be observed from the figure that for treated AR 183, sulfate had a negligible decrease from 216 mg/L to 192 mg/L during the experiment. For untreated one there was no sulfate at the beginning and during the experiment.

The sulfate presence for treated one was coming from Fenton oxidation as $FeSO_4.7H_2O$ and H_2SO_4. When sulfate value is compared between the effluent of Fenton oxidation and influent of anaerobic reactor; anaerobic reactor influent included more sulfate than Fenton oxidation effluent. However previous sulfate

results as 140 mg/L from Fenton oxidation experiment had included HCI instead of H_2SO_4. For this reason the values are different from each other.

Almost no sulfate reduction was another result of inefficient dye degradation.

Because of having similar results for each of dyes, NO_3^- and SO_4^{2-} results for AO 51 and RB 4 have been shown in appendix.

4.2.1.4. CH_4 Formation

Methane production was monitored to assess the toxic effect of chemically treated and untreated dyes on the anaerobic process. Anaerobic digestion is a multi-step process, methane production occurs in the final stage and the methane formation rate is a common tool to assess of industrial pollutants. (Dos Santos et al., 2005; Bell et al., 2000; Donlon et al., 1997)

CH_4 formation was investigated at an initial VSS_o concentration of 500 mg/L for 60 days. A control composed of distilled water and sludge was also run to compare the results with treated and untreated dyes. Figure 4.17 presents methane production in the bioreactors.

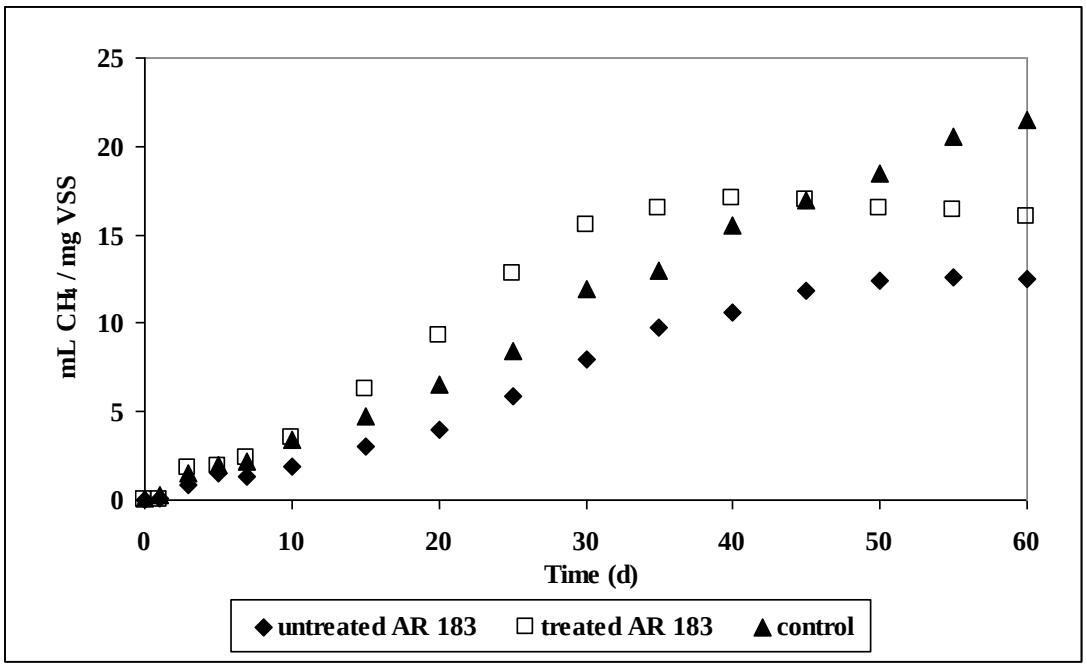


Figure 4.17: Formation of CH_4 during anaerobic experiment in the presence of untreated and treated AR 183 (DOC_o, control = 26 mg/L, DOC_o, unt red = 36 mg/L, DOC_o, treated red = 34 mg/L, VSS_o = 500 mg/L)

From the figure it is evident that methane formation up to the 10th day as 2.8, 1.9, 3.5 mL CH_4/mg VSS for control, untreated and treated AR 183, respectively and then increased by following different paths. Within the first 30 days of anaerobic digestion, methane production rate is significantly faster for treated AR 183 than for the control sample, whereas the methane production rate is comparably slow for sludge digestion in the presence of untreated AR 183. After 30 days specific methane production rates were found as 6.6, 16.2, 11.9 mL CH_4/mg VSS for samples containing untreated, treated AR 183 and control.

The inhibitory effect of untreated AR 183 on methane production can be explained by the occupation of the methanogenic bacteria's active sites by biosorbed dye molecules (Santos et al., 2005). On the other hand, treated AR 183 had no inhibitory effect on methanogenesis. In the presence of untreated/treated AR 183, methane production rates leveled off after 30-40 days of anaerobic digestion, whereas methane production continued in the control sample. After 60 days, 12.5, 16.4 and 20.7 mL CH_4/mg VSS was produced for samples containing untreated, treated AR 183 and anaerobic biomass only, respectively.

Presence of chromium in the chemical structure of AR 183 can be a question for inhibitory effect on methanogenic activity. Although some preliminary results obtained in the literature point release of metal could affect acute toxicity. A study by Osugi et al. (2006) investigated acute toxicity of copper–phtalocyanine dye, Remazol Turquoise Blue 15 for *Vibrio fischeri.* The photoelectrocatalytic oxidized solution showed an increase in the acute toxicity for *Vibrio fischeri* bacteria, explained by copper release up to 55% (0.85 mg/L) in the solution during treatment.

In our case, the chromium content of AR 183 before and during Fenton treatment (under optimized reaction conditions) was analyzed to determine the amount of chromium released from the originally complexed, organically bound chromium during Fenton oxidation. Measured chromium levels decreased to 1.2 from 4.5 mg/L for Fenton treated dyes that might be attributable to adsorption of released chromium and/or chromium complex dye on ferric hydroxide sludge that was formed during precipitation of Fe(II)/(III) ion to cease the Fenton reaction and to remove residual soluble iron. Hence chrome presence in the chemical structure of AR 183 cannot be a possibility for toxic/inhibitory effect on methanogenesis.

4.2.2. Results for Acid Orange 51

4.2.2.1. Color

Color abatement for AO 51 is presented in Figure 4.18.

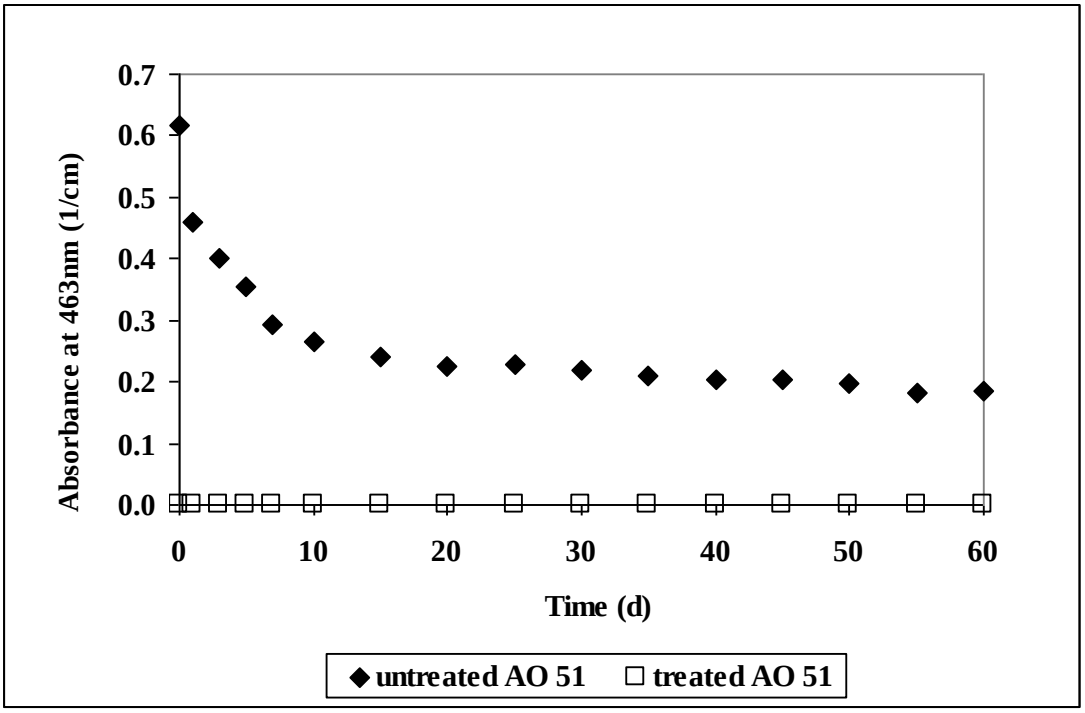


Figure 4.18: Color abatement of untreated and treated AO 51 at 463 nm ($A_{463,\ o,\ unt\ orange}$ = 0.62 cm^{-1}, VSS_o = 500 mg/L)

As is evident in the figure, there was a preliminary adsorption on to biomass as 64% for untreated AO 51 at the beginning of experiment. The color removal for untreated AO 51 was 57 % during the first 10 days of the experiment, and ultimate color removal efficiency was obtained as 70 % after 60 days. Color change was rapidly until 10th day and became slower almost leveling off after 20 days of anaerobic digestion. For treated AO 51, there was no color abatement because its color has already been removed by Fenton oxidation.

The color abatement can be clarified likewise AR 183; the first 10 days azo bond reduction, after 10 days biosorption could take part for decolorization. But differently AR 183 had less (37 %) and slower abatement rate than AO 51. If color abatement is explained with azo bond degradation by biologic activity, AO 51 has two azo bonds compared to the single azo bond of the chromium complex azo dye AR 183. For this reason its abatement rate is higher than AR 183. The rapid decolorization until 10th

day can be related with effectiveness of bacteria under absence of substrate; they have broken down azo bonds and consumed the dye in the first 10 days.

4.2.2.2. DOC

Changes in DOC during anaerobic digestion in the presence of untreated and treated AO 51 are presented in Figure 4.19.

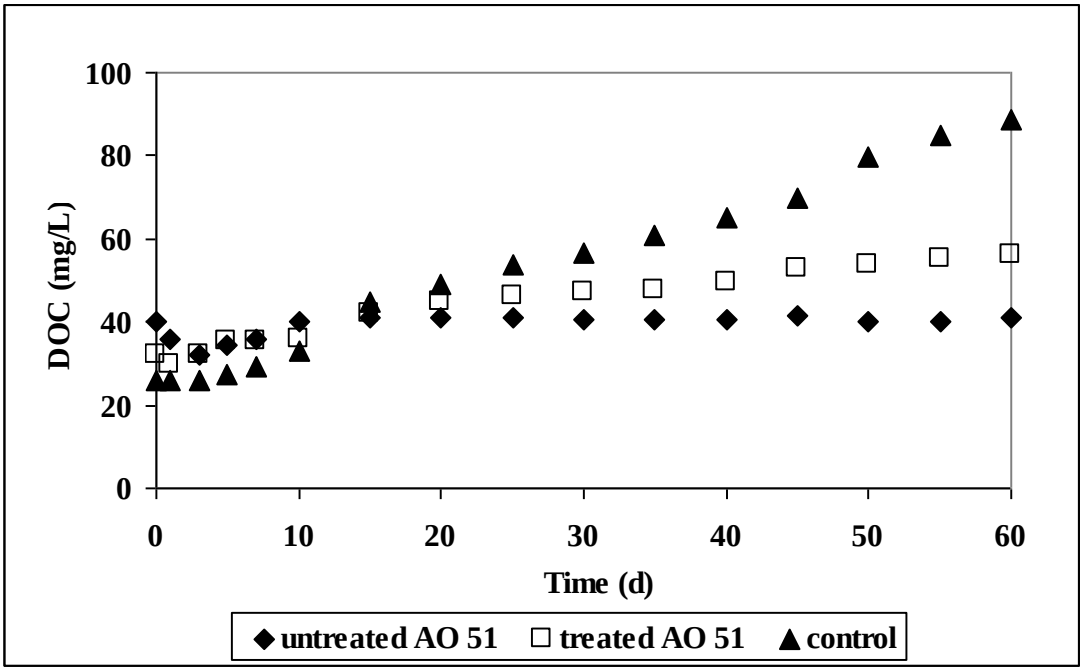


Figure 4.19: Changes in DOC during anaerobic digestion in the presence of untreated and treated AO 51 ($DOC_{o,\ control}$ = 26 mg/L, $DOC_{o,\ unt\ orange}$ = 40 mg/L, $DOC_{o,\ treated\ orange}$ = 32 mg/L, VSS_o = 500 mg/L)

As it is obvious from the figure that DOC for control containing reactor increased from 26 to 89 mg/L whereas, DOC value of untreated AO 51 almost did not alter during the experiment, it was 40 mg/L in the beginning of experiment and 41 mg/L after 60 days. For treated one there was an increase from 32 mg/L (at the beginning of experiment) to 56 mg/L after 60 days.

Increase of DOC can be the result of VSS digestion (in other words organic carbon in solid form converts to the soluble form). Control containing reactor had no substrate to consume, could be reason of VSS digestion and DOC increase. For untreated AO 51, having almost no DOC value change could be reason of dye degradation instead of VSS digestion. Treated dye degradation is not as possible as untreated one

because organic carbon has already been removed by Fenton oxidation (% 80) hence DOC change for treated AO 51 was different from untreated AO 51.

When DOC value changes of AO 51 and AR 183 (from 36 to 82 mg/L for untreated; from 32 to 84 mg/L for treated AR 183 after 60 days) is compared, the results of untreated dyes are different from each other due to their organic molecules content and their degradability. Furthermore, high decolorization efficiency of AO 51 can also support degradation of treated AO 51.

4.2.2.3. CH_4 Formation

CH_4 formation during anaerobic experiments in the presence of untreated and treated AO 51 is presented in Figure 4.20.

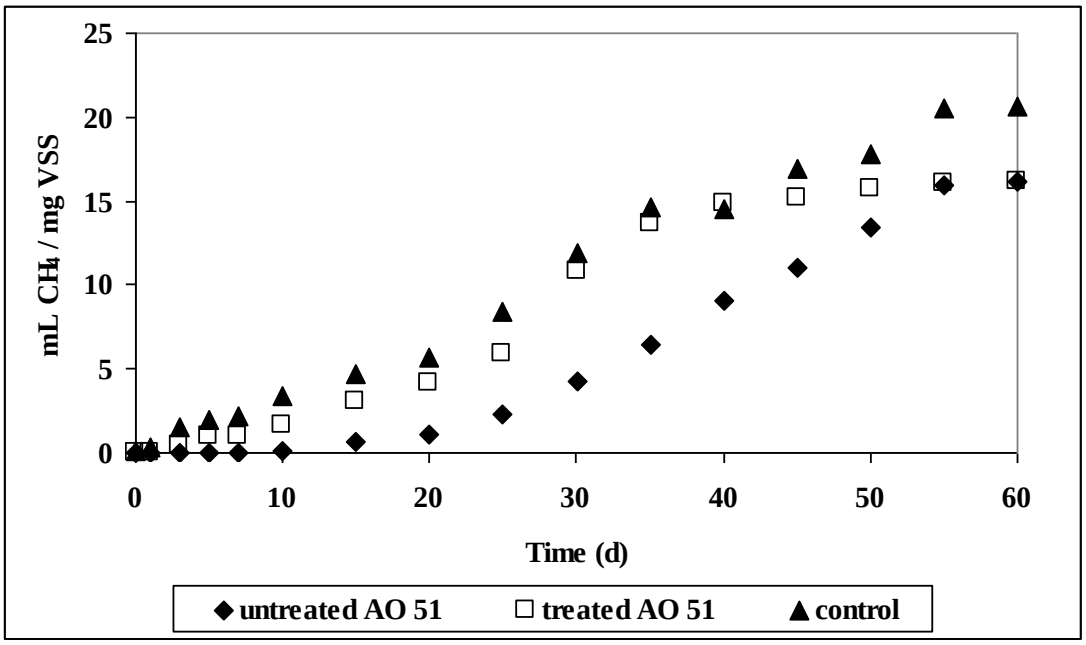


Figure 4.20: CH_4 formation during anaerobic digestion in the presence of untreated and treated AO 51 (DOC_o, control = 26 mg/L, DOC_o, unt orange = 40 mg/L, DOC_o, treated orange = 32 mg/L, VSS_o = 500 mg/L)

From the figure it is evident that methane formation up to 10th day was 0.2, 1.3, 3.5 mL CH_4/mg VSS for untreated, treated AO 51 and control culture. During anaerobic digestion, methane production is significantly lower for untreated AO 51 than for the control sample and treated AO 51. The methane production rate is comparably more for sludge digestion in the presence of treated AO 51 than in the presence of untreated AO 51 until 40th day. Specific methane production rates on 30th day were found as 4.2, 10.9 and 11.9 mL CH_4/mg VSS for samples containing untreated,

treated dyes and control, which on the 60th day changed to 16.2 mL CH_4/mg VSS in untreated AO 51, 16.2 mL/mg in treated AO 51 and 20.7 mL CH_4/mg VSS in control.

Having less methane formation of untreated AO 51 can be attributed to biosorption of color molecules likewise untreated AR 183. However, it should be mentioned that this inhibition could be tolerated during the experiment.

On the other hand, when DOC release rate is compared to CH_4 formation rate, it is not possible to determine an exact relation.

4.2.3. Results for Reactive Blue 4

4.2.3.1. Color

Color abatement for RB 4 during anaerobic experiment is presented in Figure 4.21.

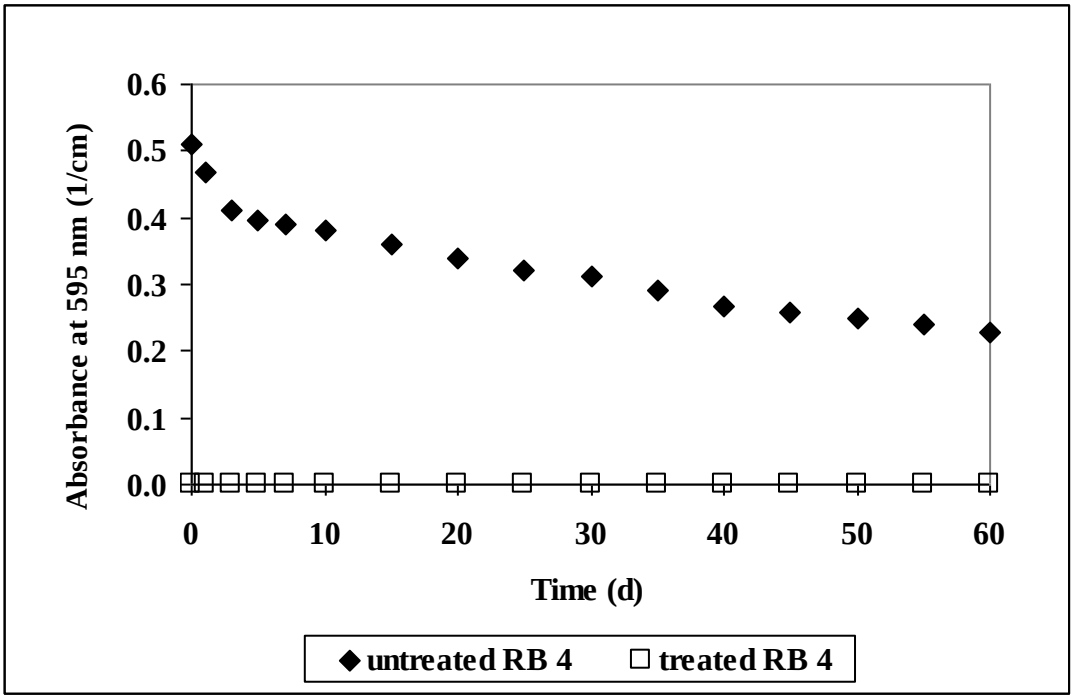


Figure 4.21: Color abatement of untreated and treated RB 4 at 595 nm ($A_{595,\ o,\ unt\ blue}$ = 0.51 cm^{-1}, VSS_o = 500 mg/L)

As it is seen from the figure, there was a preliminary adsorption on to biomass as 25 % for untreated RB 4 at the beginning of experiment. Color removal for untreated RB 4 was 27 % during the first 10 days of the experiment and ultimate color abatement was 55 %. The decolorization proceeded regularly during the experiment.

When color abatement of RB 4 is compared with that of the Acid dyes (37 % for AR 183 and 70 % for AO 51, after 60 days) RB 4 had decolorization between them.

From the previous studies, RB 4 was expected to partition strongly to the aqueous phase due to its high water solubility and also could partition to sediment, in other words, it has affinity to adsorb on to biomass and generally pass through activated sludge systems (Bell, 1998). Compared with azo dyes, anthraquinone dyes were less susceptible to reduction and present very low color abatement capacity (Santos et al., 2005; Epolito et al., 2005; Kim et al., 2004).

An identical experiment with initial dye concentration of 300 mg/L RB 4 to observe anaerobic color abatement was found 78 % after 28 days in the presence of 8400 mg/L VSS and an initial organic feed concentration of 750 mg/L dextrin and 375 mg/L peptone. In the same study decolorization of 300 mg/L RB 19 was found 83 % after 28 days. These decolorization results were attributed to the reductive transformation of dyes (Fontenot et al., 2002). Contrarily, in another study, color removal of Reactive Blue 5 and Reactive Blue 19 was found as 68 % and 57 % for 20 mg/L after 16 days (in the presence of 1000 mg/L COD as carbon source) and was referred to adsorption on to bacterial flock materials (Luangdilok et al., 2000).

In our case the reason of color abatement can be accepted as mainly adsorption on biomass and less efficiency of abatement rate can be explained with insufficient amount of biomass and finite capacity for the biomass to adsorb dye and this will be limited by the generation of new cells. Having no methane production of untreated RB 4 (discussed in the following figure), can also support decolorization reason as adsorption mechanism (Bell et al., 2003). The abiotic mechanism in the current experiment consisted of a considerable adsorption of RB 4 on the biomass (granules) and the formation of precipitates. This is in accordance with Luangdilok et al. (2000) who reported that the decolorization of RB 19 was only due to biomass adsorption and no biotransformation of the dye could be observed. The formation of precipitates is a result of the pure chemical release of the reactive anthraquinone dyes, the release of the free amine from the protonated group of anthraquinone dye incubated under neutral to alkaline solutions (Mccallum et al., 2000).

4.2.3.2. DOC

Changes in DOC during anaerobic experiment in the presence of treated and untreated RB 4 is presented in Figure 4.22.

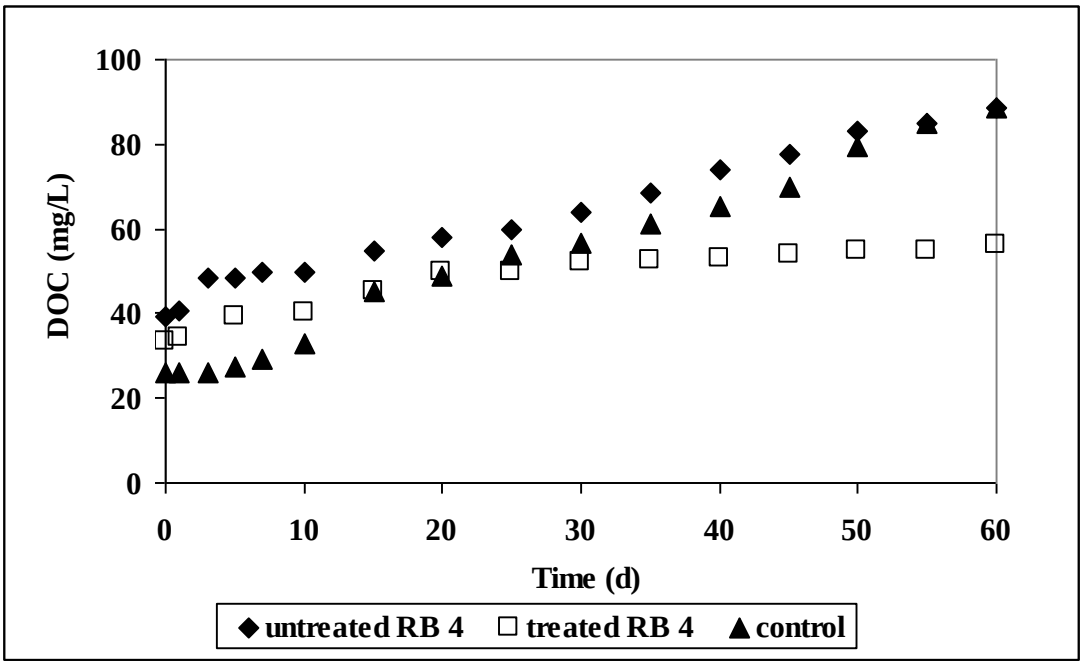


Figure 4.22: Changes in DOC during anaerobic experiment in the presence of treated and untreated RB 4 ($DOC_{o,\ control}$ = 26 mg/L, $DOC_{o,\ unt\ blue}$ = 39 mg/L, $DOC_{o,\ treated\ blue}$ = 33 mg/L, VSS_o = 500 mg/L)

As it is obvious from the figure that both DOC values of untreated and treated RB 4 and control containing reactors increased during the experiment. Change in DOC for treated RB 4 was less then untreated RB 4 and control. DOC for control containing reactor increased from 26 to 89 mg/L while DOC value of untreated RB 4 changed from 39 to 88 mg/L after 60 days. For treated one the change was from 33 mg/L to 56 mg/L.

When DOC value changes of RB 4 and AR 183 (from 36 to 82 mg/L for untreated; from 32 to 84 mg/L for treated AR 183 after 60 days) and AO 51 (from 40 to 41 mg/L for untreated; from 32 to 56 mg/L for treated AO 51 after 60 days) is compared, the results are different from each other mostly due to their organic molecules content and their degradability.

4.2.3.3. CH_4 Formation

CH_4 formation during anaerobic experiment in the presence of treated and untreated RB 4 is presented in Figure 4.23.

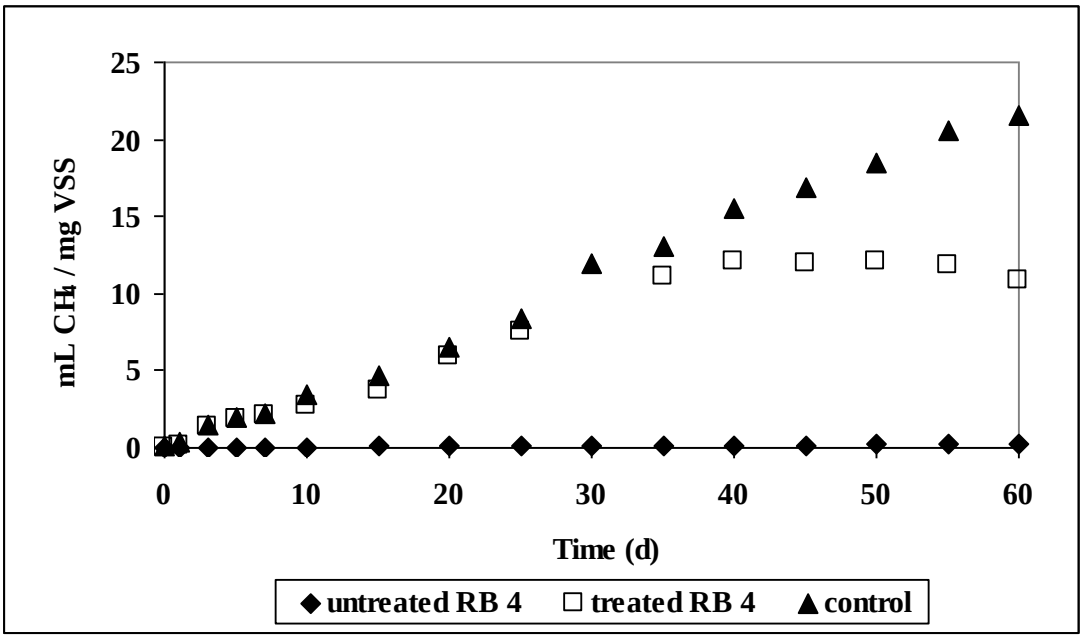


Figure 4.23: CH_4 formation during anaerobic experiment in the presence of treated and untreated RB 4 ($DOC_{o,\ control}$ = 26 mg/L, $DOC_{o,\ unt\ blue}$ = 39 mg/L, $DOC_{o,\ treated\ blue}$ = 33 mg/L, VSS_o = 500 mg/L)

It may be seen from the figure that within the first 30 days of anaerobic digestion, methane production rate of treated RB 4 and control containing reactors were similar with each other whereas methane production rate of treated RB 4 slowed down after 30 days. For untreated RB 4, there was no methane production during the experiment.

Specific methane production rates were found as 2.8 mL CH_4/mg VSS on the 10th day for both samples containing treated RB 4 and control, which on the 30th day changed to 10.8 mL CH_4/mg VSS in treated RB 4 and to 11.9 mL CH_4/mg VSS in control culture, ultimate methane formation was 10.8 and 20.7 mL CH_4/mg VSS after 60 days.

Following a reduced rate of treated RB 4 after 30 days can be explained by endogenous decay and digestion of microorganisms as a result of having no extra carbon source. (If we had observed methane production longer time, we would see not only constant formation but also decrease and already started at 55th day).

Untreated RB 4 had no methane production during the experiment. A previous study focused on characterization of RB 4 found that RB 4 solutions contained 82 % unhydrolyzed, 8 % monohydrolyzed and 10 % dihydrolyzed. Reactive groups of reactive dyes could be hydrolyzed in other words got in to the reaction at high pH values. To be in unhydrolyzed form could cause toxicity for CH_4 formation (Duran et al., 2006).

For our case the toxicity of untreated RB 4 can be result of RB 4's unhydrolyzed form. This is in accordance with Fontenot et al. (2002) who studied inhibition of RB 4 under methanogenic conditions. For incubation time of 15 days and initial dye concentration of 500 mg/L, CH_4 production is 6 % of that the control culture (culture without amendment) for RB 4.

Again it can be inferred that DOC release had no relation with CH_4 formation whereas for untreated RB 4 there was a release from 39 to 88 mg/L (after 60 days), while there was no CH_4 production. This situation can be clarified as inhibitor/toxic effect is not related with VSS digestion.

4.3. Anoxic Experiments

During the anoxic experiments. changes in color and nitrate were followed. Anoxic experiments were carried out with 62.5 mg/L untreated and Fenton's treated samples at pH = 3 for 10 min at a molar ratio of 4mM : 20mM e.g. the optimum Fe^{2+}:H_2O_2 molar ratio found for Fenton's oxidation for all three dyes. A control bearing distilled water and inoculum was also run in parallel to the dye samples.

4.3.1. Results for Acid Red 183

4.3.1.1. Color

Changes in color are presented in Figure 4.24.

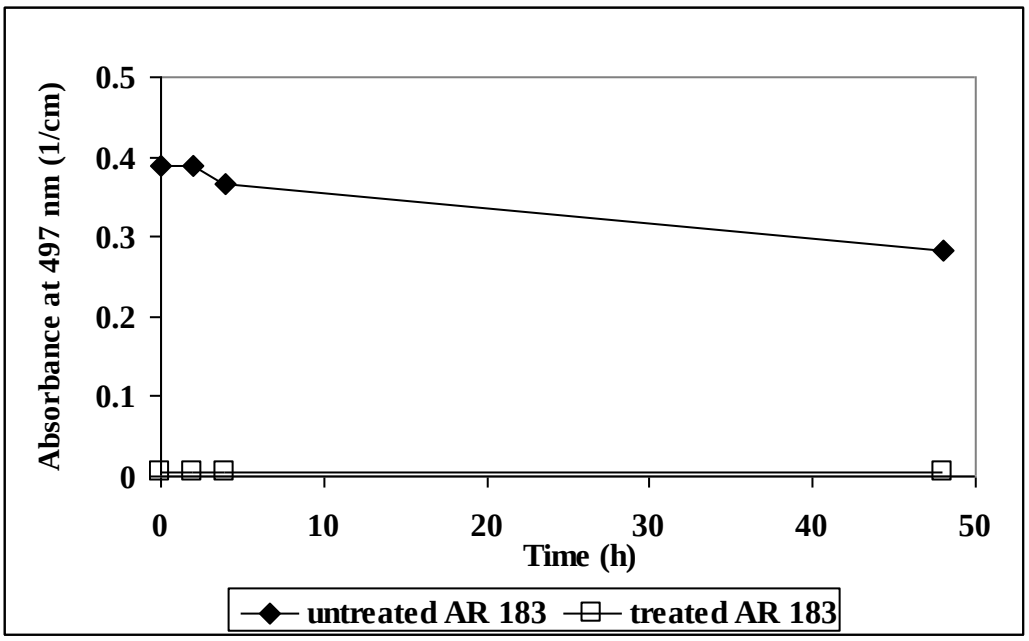


Figure 4.24: Color abatement of untreated and treated AR 183 ($A_{497.\,o}$ = 0.39 cm^{-1}, VSS = 2400 mg/L)

It may be observed from the above figure that there was a preliminary adsorption on to biomass as 40 % for untreated AR 183 at the beginning of experiment**.**

For untreated AR 183 only 6 % color removal was observed during the first 4 hours of anoxic treatment but ultimate color removal was obtained as 28 % after 48 h. Treated AR 183 was already completely decolorized during Fenton treatment.

Provided that the decolorization mechanism was reduction of the azo bond, the low color removal efficiency can be explained as competition between the nitrate nitrogen and azo dyes for electron donors. This may be supported with the previous studies where color abatement under anoxic conditions was investigated. It was observed that NO_3^- acts as an electron acceptor and the presence of an alternative electron acceptor (namely the azo dye) may retard reductive decolorization (Laurenço et al., 2000; Panswad et al., 2000; Carliell et al., 1998).

4.3.1.2. Denitrification

NO_3^- abatement under anoxic conditions is presented in Figure 4.25.

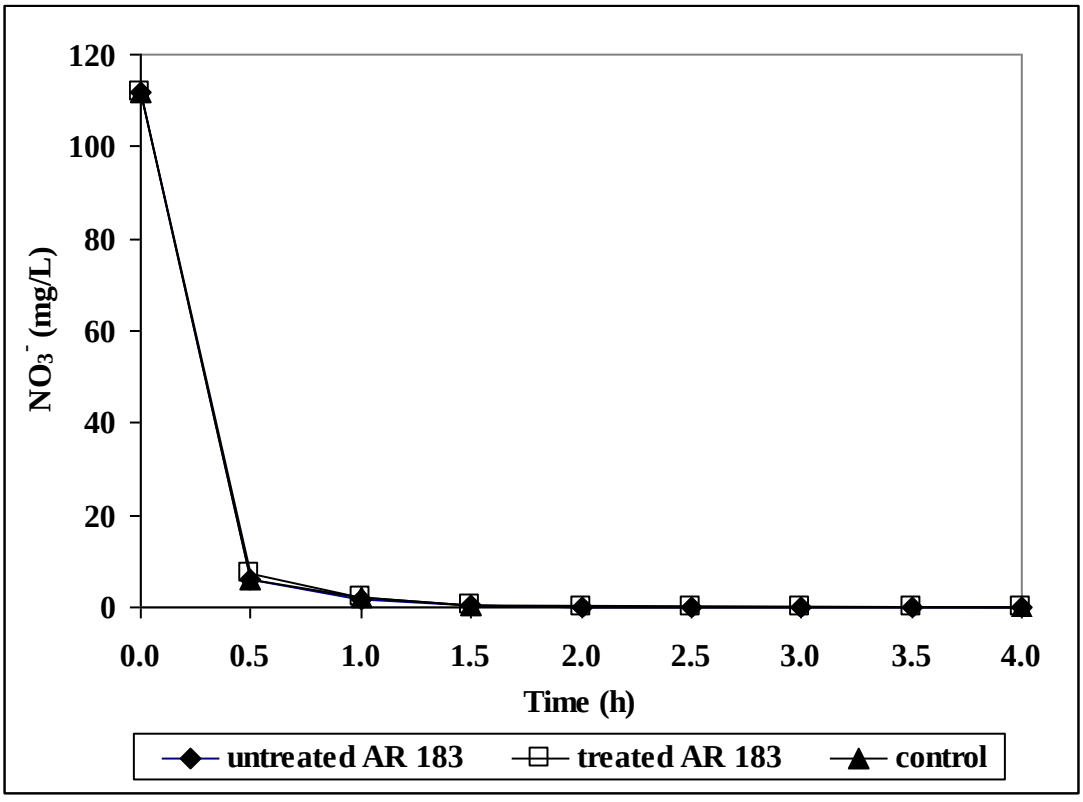


Figure 4.25: NO_3^- abatement in the presence of untreated and treated AR 183 during anoxic experiments. Experimental conditions: AR 183_o = 62.5 mg/L, $NO_3^-._o$ = 112 mg/L, VSS_o = 2400 mg/L

As is evident in the figure, nitrate concentration fell from 112 mg/L at the beginning of the anoxic experiments to 6.0, 7.2 and 6.0 mg/L during the first 30 min in the

presence of untreated, treated dyes and in the control sample, respectively. NO_3^- concentration was 2 mg/L after 1 h and completely consumed after 2 h in all reactors and hence faster than expected. Upon comparison of the denitrification rates within the first 30 min of the anoxic experiments it is clear that denitrification rates were almost identical and untreated/treated AR 183 had no inhibitory effect on denitrification.

Upon comparison of color and nitrate abatement rates it is obvious that denitrification was significantly faster than decolorization; nitrate concentration fell to non-detectable levels after 2 h anoxic treatment, whereas color removal proceeded slow and was not complete even at the end of the experiment. Another explanation of inefficient color removal might be that the decolorization mechanism was biosorption rather than reductive degradation.

4.3.2. Results for Acid Orange 51

4.3.2.1. Color

Changes in the color under anoxic conditions are presented in Figure 4.26.

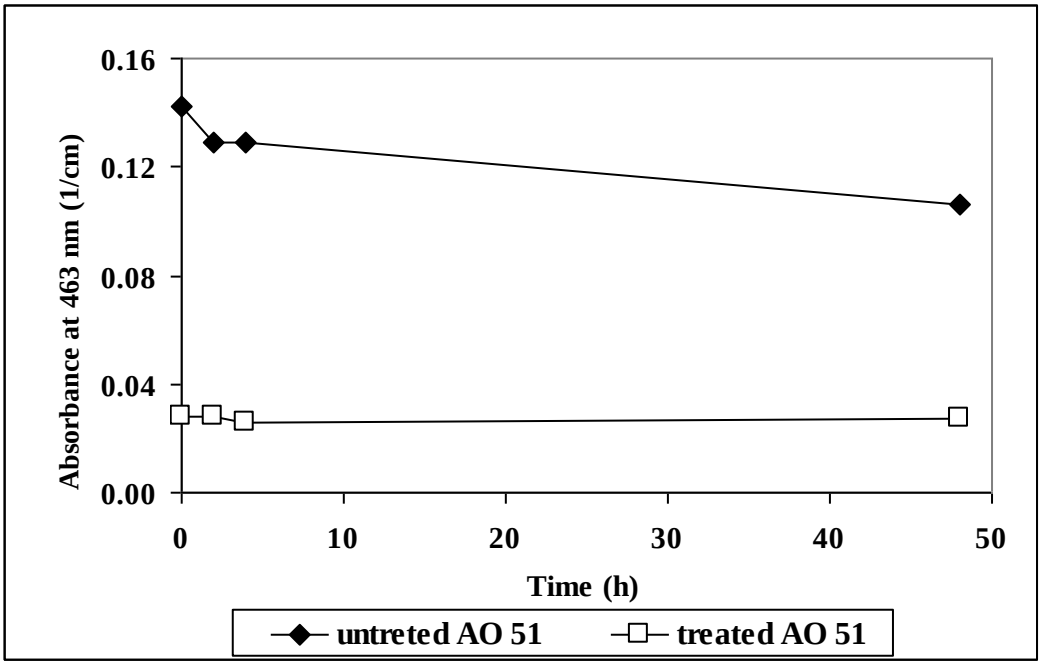


Figure 4.26: Color abatement of untreated and treated AO 51 ($A_{463.\,o}$ = 0.142 cm^{-1}, VSS = 2400 mg/L)

It should be mentioned that there was preliminary color abatement as 80 % for untreated AO 51, as the result of adsorbing on to biomass in the beginning of

experiment. It may be observed from the figure that in the case of untreated AO 51, there was only 9 % color abatement during the first 4 h of anoxic treatment and the ultimate color removal rate was 25 % after 48 h. Again, due to the fact that the treated dye was already completely decolorized during Fenton treatment, no additional color removal was obtained for treated AO 51. The slow and low color abatement rates might be attributable to competition between the azo dye and nitrate for electron acceptors.

4.3.2.2. Denitrification

NO_3^- abatement rates obtained during the anoxic experiments in the presence of untreated and treated AO 51 are presented in Figure 4.27.

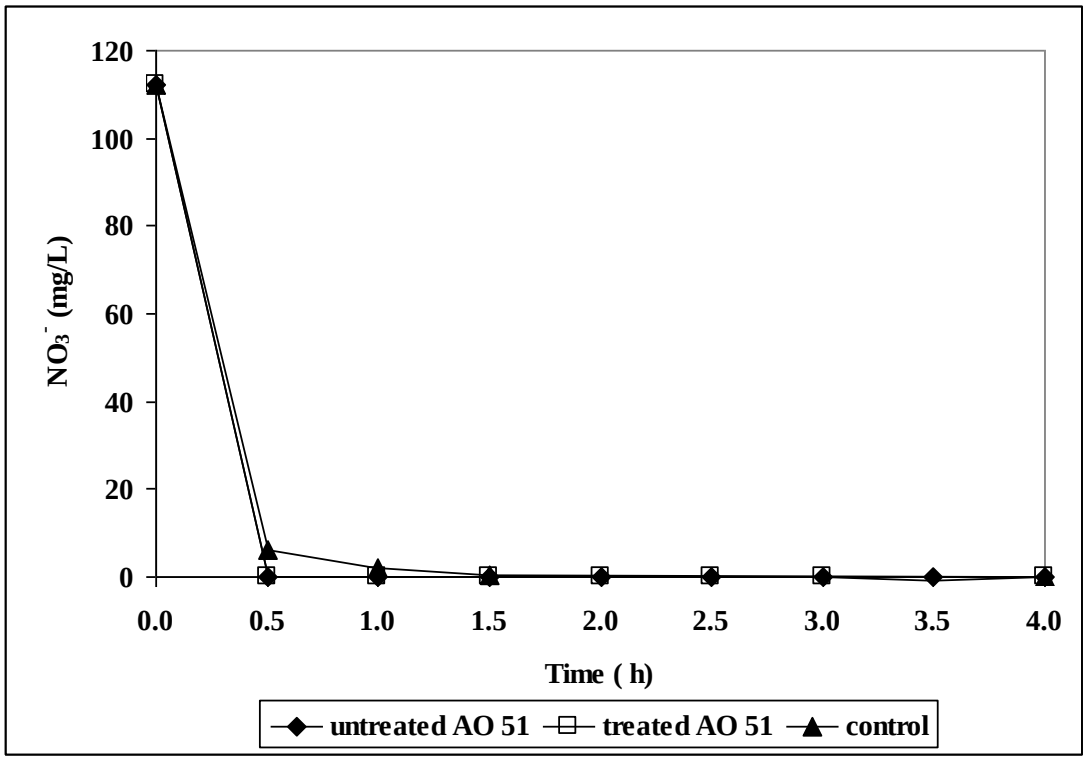


Figure 4.27: NO_3^- abatement during anoxic experiments in the presence of untreated and treated AO 51. Experimental conditions: AO 51_o = 62.5 mg/L, $NO_{3\,.o}^-$ = 112 mg/L, VSS = 2400 mg/L

From the above figure it can be seen that after 30 min there was only 6 mg/L in the control culture, whereas nitrate was already completely consumed in samples containing untreated and treated AO 51.

When compared with the denitrification rates obtained for the chromium complex monoazo dye AR 183, significantly higher NO_3^- abatement rates were obtained for both untreated as well as pretreated disazo dye AO 51. The difference in denitrification rates may be due to the differences in molecular structures and the chromium content (= 4.5 mg/L) of AR 183.

Due to the fact that denitrification rates were found high, it can be concluded that untreated as well as treated AO 51 had no inhibitory effects on denitrification. According to the previous studies, it has been postulated that NO_3^- can act as an alternative electron acceptor and potentially hinder color abatement (Li et al., 2004). According to Yongjie et al. (1991) the presence of nitrate may cause a decrease in the maximum substrate utilization rate.

4.3.3. Results for Reactive Blue 4

4.3.3.1. Color

Decolorization rates obtained for untreated and treated RB 4 during the anoxic experiments are presented in Figure 4.28.

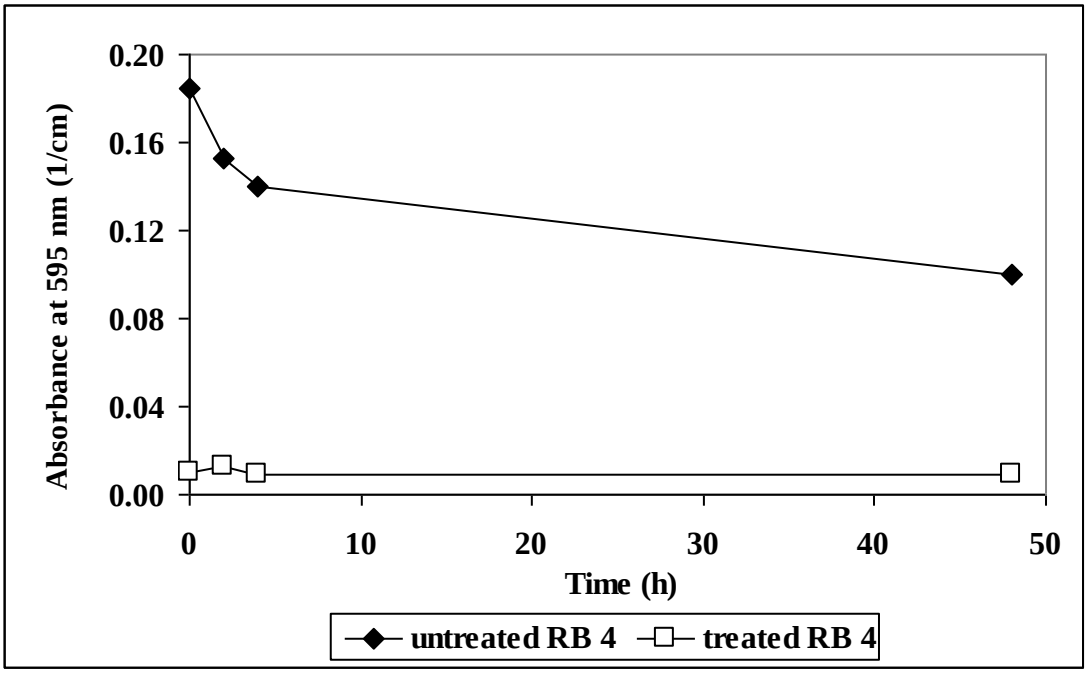


Figure 4.28: Color abatement of untreated and treated RB 4 ($A_{595.\ o}$ = 0.185 cm^{-1}, VSS = 2400mg/L)

In the beginning of experiment, there was a preliminary adsorption on to biomass as 55 % for untreated RB 4. It may be observed from the figure displayed above that for

untreated RB 4, color abatement rates were obtained as 17 %, 26 % and 45 % after 0.5. 1. and 2h respectively. Treated RB 4 was already completely decolorized during Fenton treatment. From the above findings it is obvious that higher and faster decolorization rates were found for RB 4. Considering that the presence of nitrate may hinder reductive azo bond cleavage of azo dyes but not decolorization of anthraquinone dyes it is not surprising that color abatement for acid-azo dyes is related with breaking down of azo bonds and presence of nitrate hinders or slows down this reaction. But for reactive-anthraquinone dye, color abatement probably is not related with azo bond breaking down.

This is in accordance with previous study which has done with 20 mg/L of four different reactive dyes and addition of 5 and 10 mM NO_3^- in an anaerobic/aerobic SBR system with time intervals. The addition of nitrate has not had any adverse effect on the color reduction for anthraquinone dyes because these dyes have removed by the adsorption mechanisms not by the microbial degradation (Panswad et al., 2000). However, it should be kept in mind that the decolorization mechanism for the azo dyes AR 183 and AO 51 might also be due to biosorption, and hence the above explanation is only valid if decolorization is a consequence of azo bond reduction.

4.3.3.2. Denitrification

Denitrification rates observed during the anoxic experiments in the presence of RB 4 are presented in Figure 4.29.

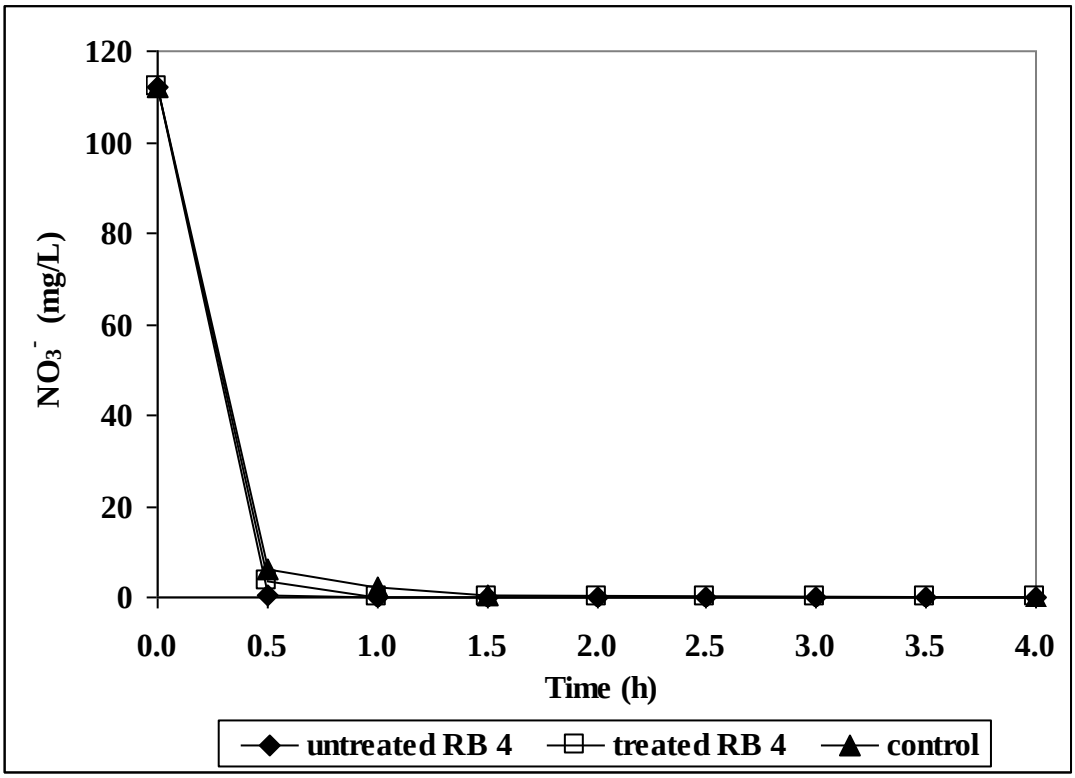


Figure 4.29: NO_3^- abatement during anoxic experiments in the presence of untreated and treated RB 4. Experimental conditions: RB 4_o = 62.5 mg/L, $NO_{3\ .o}^-$ = 112 mg/L, VSS_o = 2400 mg/L

Figure 4.29, indicates that during the first 30 min of the anoxic experiment, the nitrate concentration decreased to 0.3 mg/L, 3.6 mg/L and 6 mg/L in the presence of untreated, treated RB 4 and the control reactor, respectively. After 1 h, NO_3^- was only 2 mg/L in the control and already completely consumed by the anoxic reactors containing untreated/treated RB 4. The above findings revealed that denitrification was faster in the samples containing the dyes than in the control sample. The reason of the slower nitrate consumption in the sample bearing treated RB 4 as compared with the sample bearing untreated RB 4 might be mostly supported by the inhibitory effect of Fenton oxidation products. In summary, the decreasing order of the relative denitrification rates were found as AO 51 > RB 4 > AR 183.

4.4. Aerobic Experiments

Aerobic experiments were carried out with glucose solution equivalent to 500 mg O_2/L COD in the presence of 100 mg/L untreated and Fenton's treated (Fe^{2+}:H_2O_2 = 4mM:20mM; t = 10 min; pH = 3) dye samples at an average VSS concentration of 3500 (in treated bioreactors) and 4000 mg/L (in untreated bioreactors) for 6 h. Control reactors (glucose + heterotrophic biomass) were also run for untreated and treated dye samples. During the aerobic experiments color and COD parameters were followed.

4.4.1. Results for Acid Red 183

4.4.1.1. Color

Changes in color during aerobic treatment of glucose are presented in Figure 4.30.

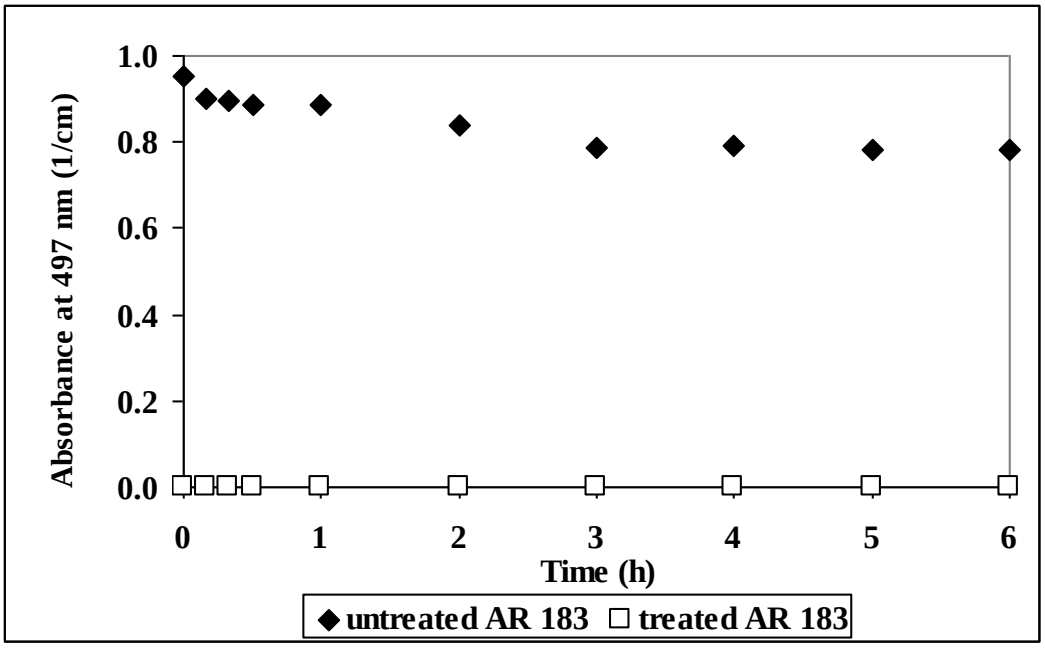


Figure 4.30: Decolorization of untreated and treated AR 183 during aerobic experiments. ($A_{497.\,o}$ = 0.952 cm^{-1}, VSS = 4000 mg/L)

It may be seen from the figure that, there was a preliminary adsorption on to biomass as 5 % for untreated AR 183 at the beginning of experiment. Untreated AR 183 had no significant color removal during the aerobic experiment. Only 7 % of the original absorbance was removed after 1 h biotreatment and the overall color removal efficiency that was obtained after 6 h reached 18 % via degradation or biosorption. Treated AR 183 had no color after Fenton oxidation to be removed. This low

efficiency of decolorization is a result of recalcitrant behavior of dyes towards aerobically microbial degradation. Substitutions such as azo-. nitro- or sulfo- groups are not readily biodegradable under aerobic conditions (Zimmerman et al., 1996).

However, in a study Buitron et al. (2003) investigated aerobic degradation of 25 and 50 mg/L Acid Red 151 in a sequencing batch biofilter packed with porous volcanic rock. Acid Red 151 was used as the sole source of carbon and energy for microorganisms. After 24 hours of reaction time. 50% removal of initial colorant was observed. Pourbabaee et al. (2005) on the other hand studied decolorization of 300 mg/L Terasil Black (a fiber disperse dye) under aerobic conditions in the presence of an exogenous carbon source (e.g. glucose and yeast extract). Decolorization of the effluent containing Terasil Black dye was found to be dependent upon the presence of additional carbon and energy sources, 60 % of the original color disappeared within 30 hours of incubation at a temperature of 30^{o}C in the presence of 10 g/L glucose and 5 g/L yeast extract, whereas only 10 % decolorization occurred in their absence.

In our case, there was a sufficient amount of easily biodegradable external carbon source (i.e. glucose) available. As such, in the presence of glucose, microorganisms preferred to consume this carbon source instead of the carbon being incorporated in the complex dye molecules. Under aerobic conditions, color removal is thought to be a consequence of biosorption.

4.4.1.2. COD

COD abatement was observed to assess the toxic/inhibitory effect of dyes on glucose degradation by aerobic biomass. Glucose degradation was followed for 6 h in terms of COD abatement (COD contribution of dyes was $\leq$ 10 %) at an initial biomass concentration of 3500 and 4000 mg/L. A control composed of the same amount of glucose and biomass and the same sludge was also run to compare the results with untreated and treated dyes. Figure 4.31 presents COD abatement for untreated AR 183 and its control culture.

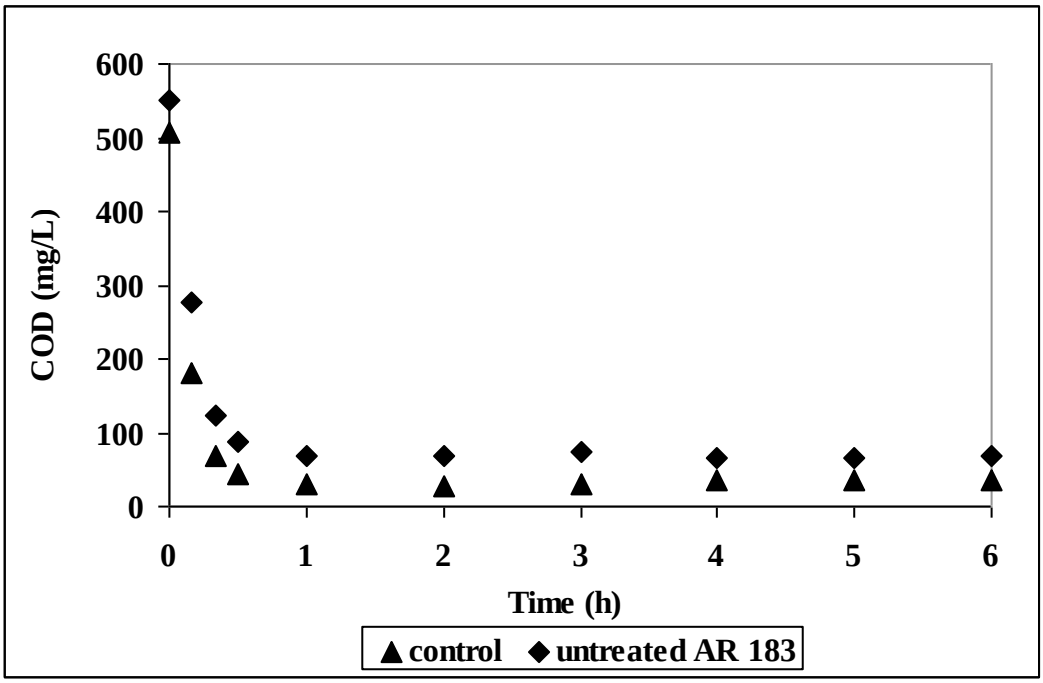


Figure 4.31: Aerobic COD abatement during glucose degradation in the presence of untreated AR 183 as well as its control ($control_1$) culture. Experimental conditions: AR 183_o = 100 mg/L, $glucose_o$ = 516 mg/L (COD_o = 500 mg/L), VSS_1 = 4000 mg/L)

In Figure 4.31., glucose abatement rate in the presence of untreated AR 183 was compared with its respective control culture. Glucose degradation started immediately within the first minutes of the experiment and slowed down after 1 hour mostly due to glucose exhaustion in the reactors. From the figure it is also evident that in the glucose solution bearing untreated AR 183, COD removal reached a steady state after 1 h, but the COD content of the sample was not completely removed during the course of the aerobic experiment. Most probably the remaining COD (= 68 mg/L, 88 %) was the COD of untreated AR 183 + metabolic end products of glucose. In the corresponding control sample ($control_1$), COD fell down to around 37 mg/L (93 %) after 6 h biotreatment. In both cases COD abatement rates were fast and close to each other, indicating that untreated AR 183 did not inhibit aerobic glucose degradation.

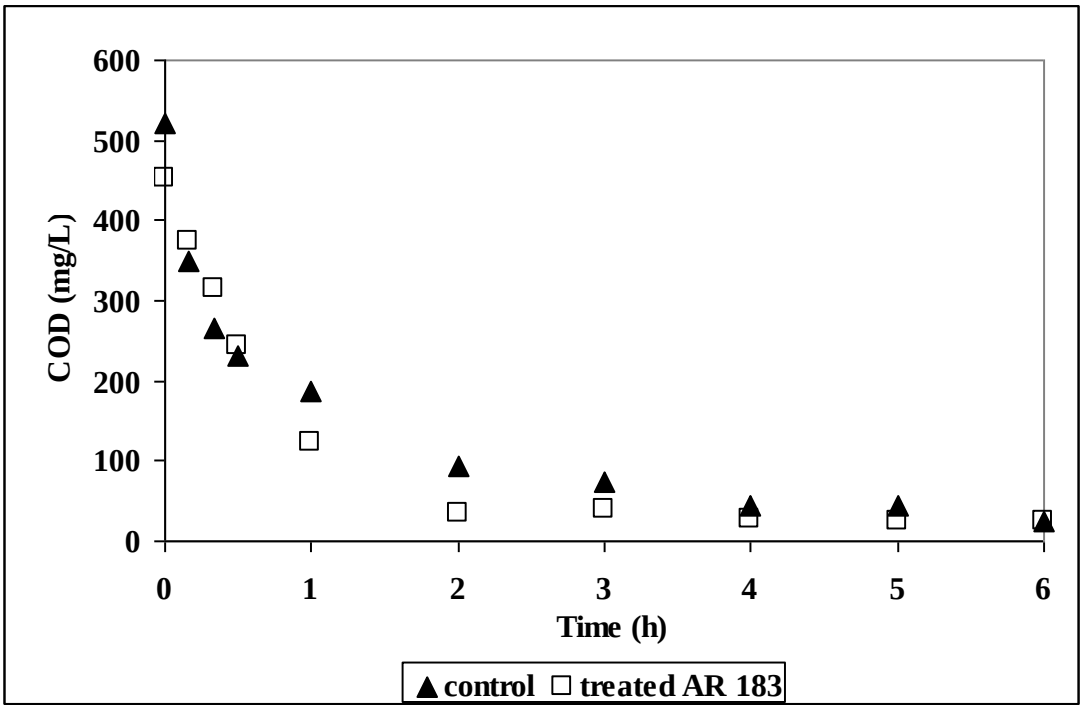


Figure 4.32: Aerobic COD abatement during glucose degradation in the presence of treated AR 183 as well as its control ($control_2$) culture. Experimental conditions: AR 183_o = 100 mg/L, $glucose_o$ = 516 mg/L (COD_o = 500 mg/L), VSS_2 = 3500 mg/L)

In Figure 4.32., glucose abatement rate in the presence of treated AR 183 was compared with its respective control culture. The COD abatement rate of the sample containing glucose + treated AR 183 was faster than that of control2 especially after the first hour of aerobic treatment. The COD content decreased to 375 mg/L (17 %) in the sample with treated AR 183, to 350 mg/L (33 %) in control2 in the first 10 min that changed to 123 (73 %) mg/L in the sample with treated AR 183, and to 187 mg/L (64 %) in control2 in the first hour of aerobic treatment. COD removal leveled off after 2 h in the sample containing treated AR 183 (92 %) and after 6 hours in control2 (final COD = 25 mg/L). Overall COD removal efficiency was 95 % for both reactors.

From the obtained results it is evident that if COD abatement rates were compared with each other, the untreated AR 183 had a slight inhibitory effect on glucose degradation, while this inhibitory effect could be completely disappeared when AR 183 was subjected to Fenton treatment.

4.4.2. Results for Acid Orange 51

4.4.2.1. Color

Color abatement obtained for AO 51 during the aerobic experiment is presented in Figure 4.33.

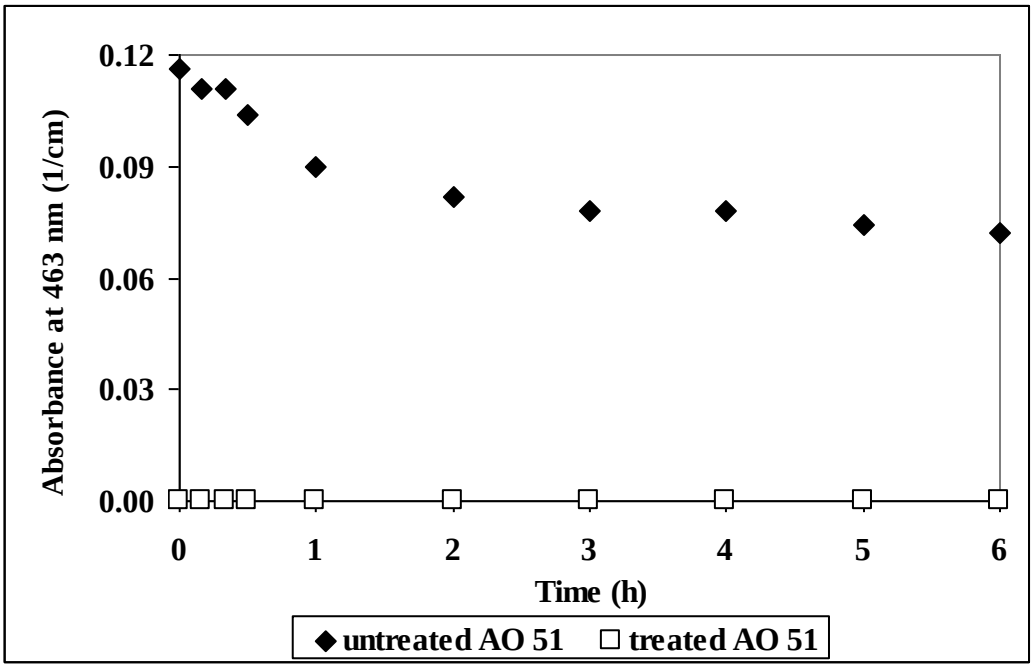


Figure 4.33: Changes in color for untreated and treated AO 51 during aerobic experiments ($A_{463.\,o}$ = 0.116 cm^{-1}; VSS = 4000 mg/L)

In the case of untreated AO 51, there was 22 % color removal during the first hour of aerobic experiment, slowed down after 2 h and overall color removal was 38 % after 6 h. It should be mentioned that, there was a preliminary adsorption on to biomass as 90 % for untreated AO 51 at the beginning of experiment. Upon closer inspection of the results obtained for AO 51 (38 % overall color removal during aerobic glucose degradation) and AR 183 (18 % overall color removal during aerobic glucose degradation), it is clear that more biosorption was observed in the case of AO 51. Davies et al. (2006) studied aerobic decolorization of 127 mg/L Acid Orange 7 (AO 7) in a vertical flow constructed wetland. Color removal efficiencies up to 99 % was found not merely due to biosorption as was also confirmed by a continuous and parallel release of NO_3^- and SO_4^{2-}.

4.4.2.2 COD

COD abatement in the presence of treated and untreated AO 51 under aerobic conditions is presented in Figure 4.34 and 4.35.

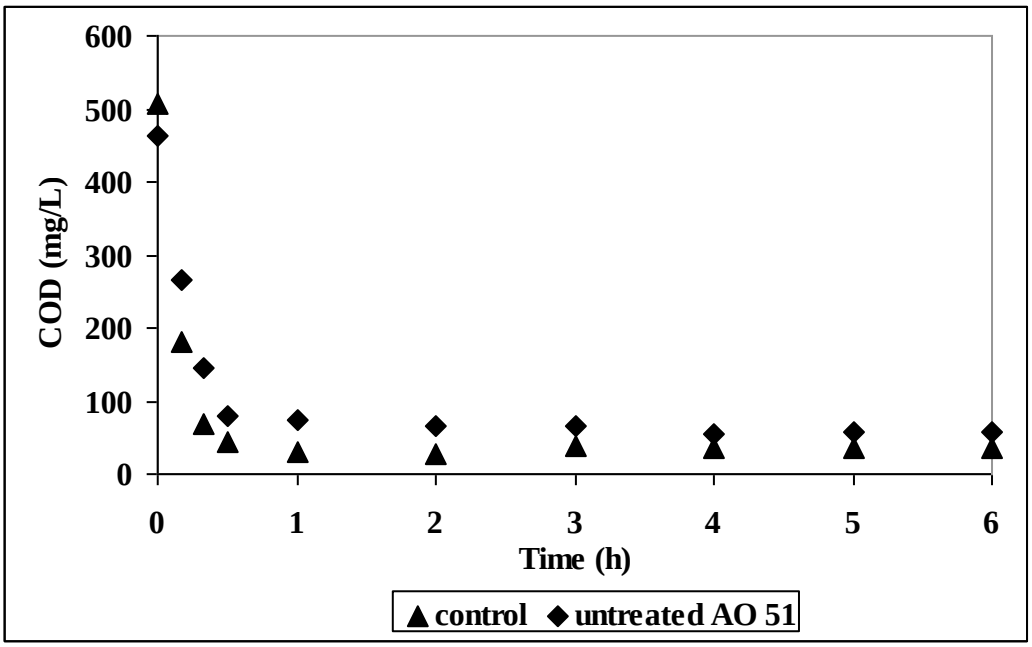


Figure 4.34: COD abatement during aerobic glucose degradation in the presence of untreated AO 51 as well as its corresponding control ($control_1$). Experimental conditions: AO 51_o = 100 mg/L, $glucose_o$ = 516 mg/L (COD_o = 500 mg/L), VSS_1 = 4000 mg/L

As is evident in the figure, glucose degradation rate in the presence of untreated AO 51 was slightly less than in $control_1$ (the same control culture shown for the experiments conducted in the presence of untreated AR 183). COD abatement due to glucose degradation was almost complete in the first hour of the experiment and slowed down thereafter. COD concentration decreased to 265 mg/L (64 % COD removal after 10 min) in the reactor with untreated AO 51 and changed to 75 mg/L (84 %, after 1 h), and ultimately was 56 mg/L (88 %) after 6 h biotreatment.

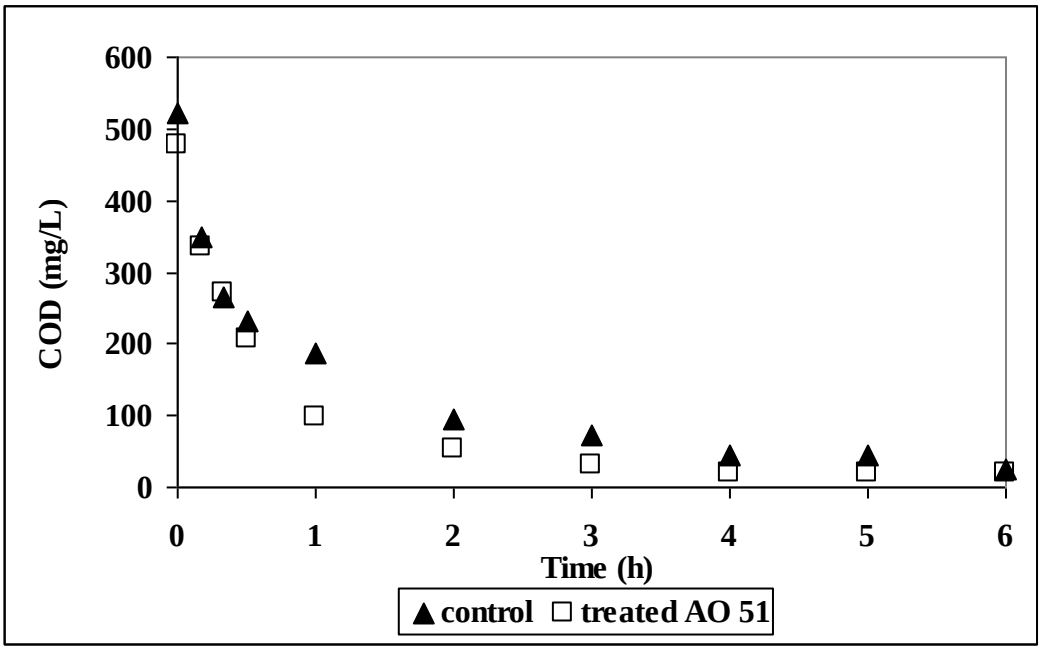


Figure 4.35: COD abatement during aerobic glucose degradation in the presence of treated AO 51 as well as its corresponding control ($control_2$). Experimental conditions: AO 51_o = 100 mg/L, $glucose_o$ = 516 mg/L (COD_o = 500 mg/L), VSS_2 = 3500 mg/L

From the figure, in the sample containing Fenton's treated AO 51; the glucose consumption rate was similar to that in $control_2$ culture, but appreciably higher during the treatment period of 0.5-2 h. The COD value decreased to 335 mg/L (30 % COD removal) in the sample with treated AO 51 and $control_2$ during the first 10 min. to 99 mg/L (79 %) and 187 mg/L (64 %) in the sample containing AO 51 and $control_2$ in the first hour, and to 52 mg/L (89 %) and 94 mg/L (82 %) after 2 hours, respectively. The ultimate COD obtained after 6 hours aerobic degradation was 20 and 25 mg/L (96 % and 95 %) for treated and $control_2$ containing reactors, respectively.

Considering the above experimental findings, it can be concluded that the untreated dyes were not biodegradable, but did not significantly inhibit aerobic glucose abatement. In the case of Fenton's treated acid dyes, glucose degradation was slightly faster than in the control samples and glucose as well as the Fenton's oxidation intermediates of the dyes could be successfully degraded within the investigated aerobic treatment period.

4.4.3. Results for Reactive Blue 4

4.4.3.1. Color

Changes in color of RB 4 during aerobic glucose treatment are presented in Figure 4.36.

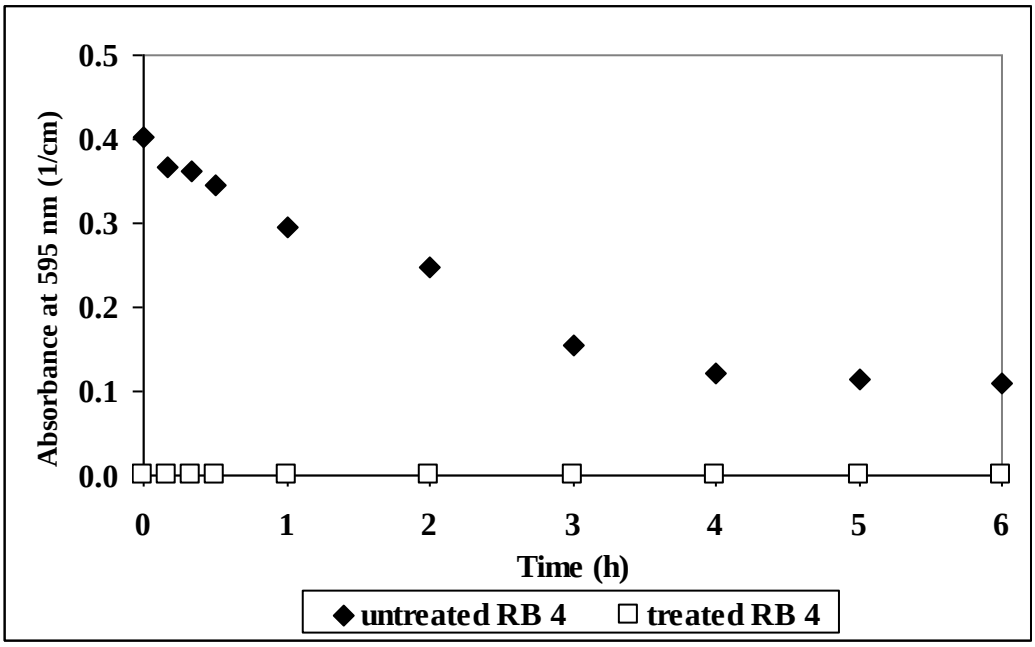


Figure 4.36: Color changes for untreated and treated RB 4 during aerobic experiments ($A_{463.\,o}$ = 0.403 cm^{-1}, VSS = 4000 mg/L)

Due to the fact that color was already removed during Fenton treatment, no changes were observed in the absorbance values of the dye subjected to chemical pretreatment. In the case of the untreated dye, there was a preliminary adsorption on to biomass as 41 % for untreated RB 4 at the beginning of experiment. Color abatement as 27 % was obtained in the first hour and 73 % after 6 hours of aerobic treatment. Again, color removal is thought to be a consequence of sorption onto activated sludge. Upon comparison of color removal efficiencies observed for AR 183. AO 51 and RB 4 during aerobic glucose degradation, the decreasing order of decolorization efficiencies were obtained as RB 4 (73 %) > AR 183 (38 %) > AO 51 (18 %) after 6 h aerobic treatment.

O'Neill et al. (2000) reported an average of 10 % and a maximum of 30 % color (dye) removal for reactive dyes due to sorption onto aerobic biomass, the remainder passing through activated sludge plants. A study conducted by Panswad et al. (2001)

investigated decolorization of 10 mg/L Reactive Black 5 under sequential anaerobic-aerobic conditions. Aerobic treatment accounted for only 2-8 % color removal.

In summary, from the above indicated experimental findings it may be concluded that the dominant color removal mechanism for textile dyes under aerobic conditions is biosorption.

4.4.2.2. COD

COD abatement obtained of untreated RB 4 for aerobic conditions is presented in Figure 4.37.

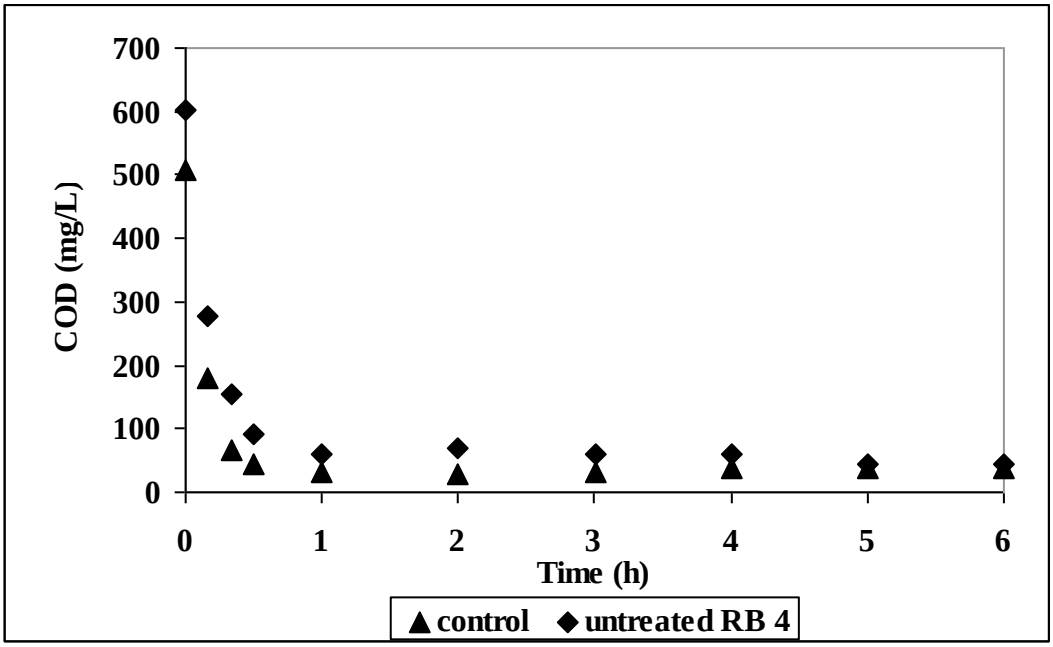


Figure 4.37: COD abatement during aerobic glucose degradation in the presence of untreated RB 4 as well as its corresponding control sample ($control_1$). Experimental conditions: RB 4_o = 100 mg/L, glucose = 516 mg/L (COD_o = 500 mg/L), VSS_1 = 4000 mg/L

From the above figure it is evident that aerobic glucose degradation in the presence of untreated RB 4 showed similar profiles to those obtained in the presence of untreated AR 183 and AO 51. Glucose removal in the reactors containing untreated RB 4 was not significantly inhibited as compared to the control. Again COD removal due to glucose degradation was almost complete in the first hour of the aerobic experiment slowed down thereafter. In the case of untreated RB 4, a COD value of 276 mg/L (54 % COD removal) was reached after 10 min that decreased to 59 mg/L (90 %) after 1 h treatment and ultimately to 45 mg/L after 6 h biotreatment.

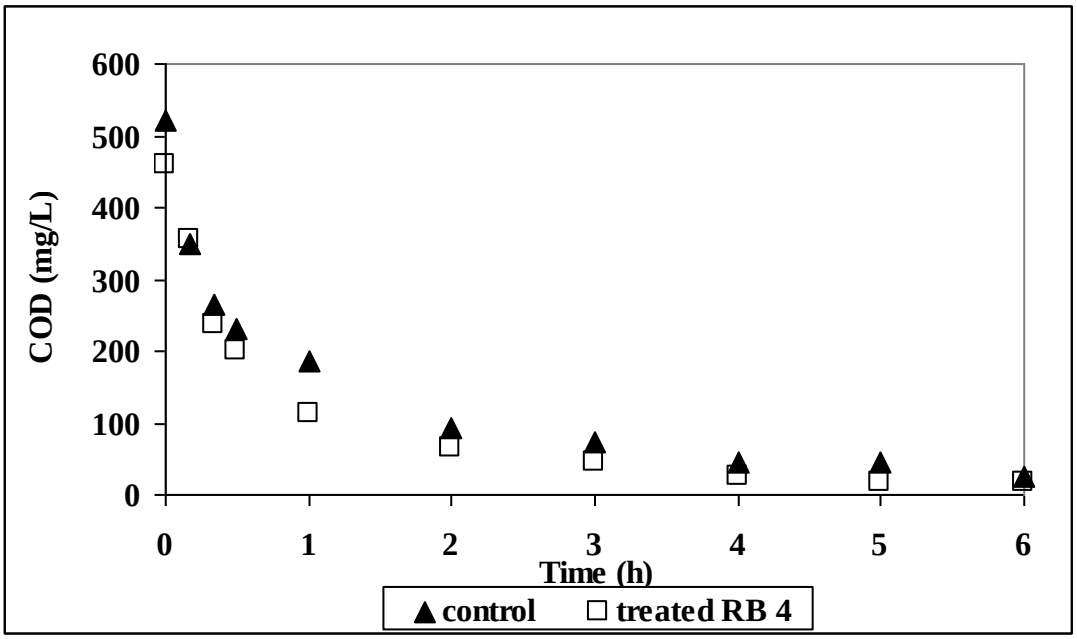


Figure 4.38: COD abatement during aerobic glucose degradation in the presence of treated RB 4 as well as its corresponding control sample ($control_2$). Experimental conditions: RB 4_o = 100mg/L, glucose = 516 mg/L (COD_o = 500 mg/L), VSS_2 = 3500 mg/L

From the figure it is observed that, in the bioreactor containing treated RB 4 glucose consumption being similar to that in $control_2$ reactor was observed; the COD decreased to 355 mg/L (33 %) in the reactor containing treated RB 4 and to 350 mg/L (23 %) in the $control_2$ reactor during the first 10 min. and down to 113 mg/L (75 %) and 187 mg/L (64 %) after 1 hour in the reactor with treated RB 4 and $control_2$, respectively. The ultimate COD was found as 17 mg/L (96 %) and 25 mg/L (95 %) in the presence of treated RB 4 and $control_2$, respectively, after 6 h aerobic glucose treatment.

4.5. Determination of the Inhibitory Effect Caused by the Presence of Treated and Untreated Textile Dyes

4.5.1. Anaerobic experiments

CH_4 formation rates during anaerobic experiment in the presence of treated and untreated AR 183, AO 51 and RB 4 respectively is presented in Figures 4.39., 4.40., .41.

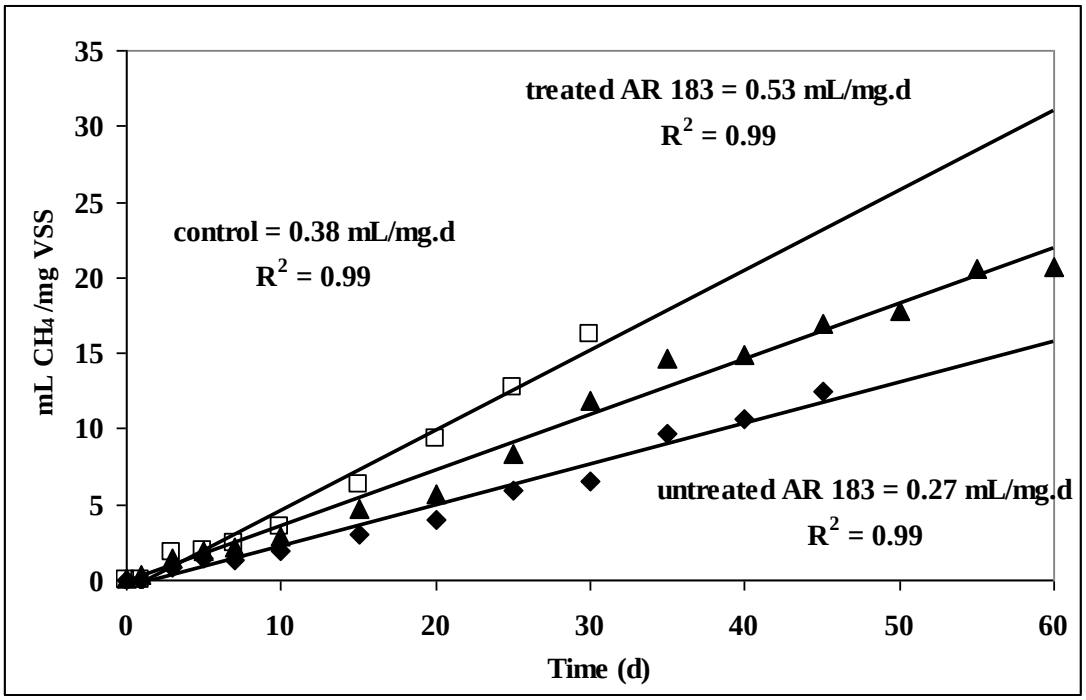


Figure 4.39: CH_4 formation rates during anaerobic experiment in the presence of treated and untreated AR 183

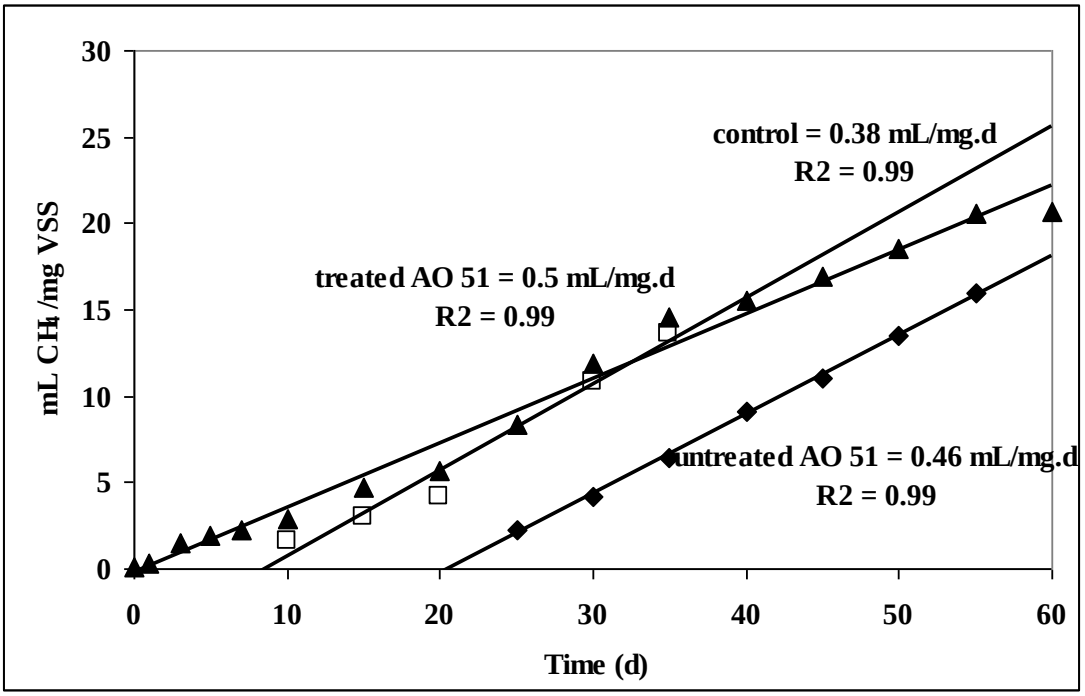


Figure 4.40: CH_4 formation rates during anaerobic experiment in the presence of treated and untreated AO 51

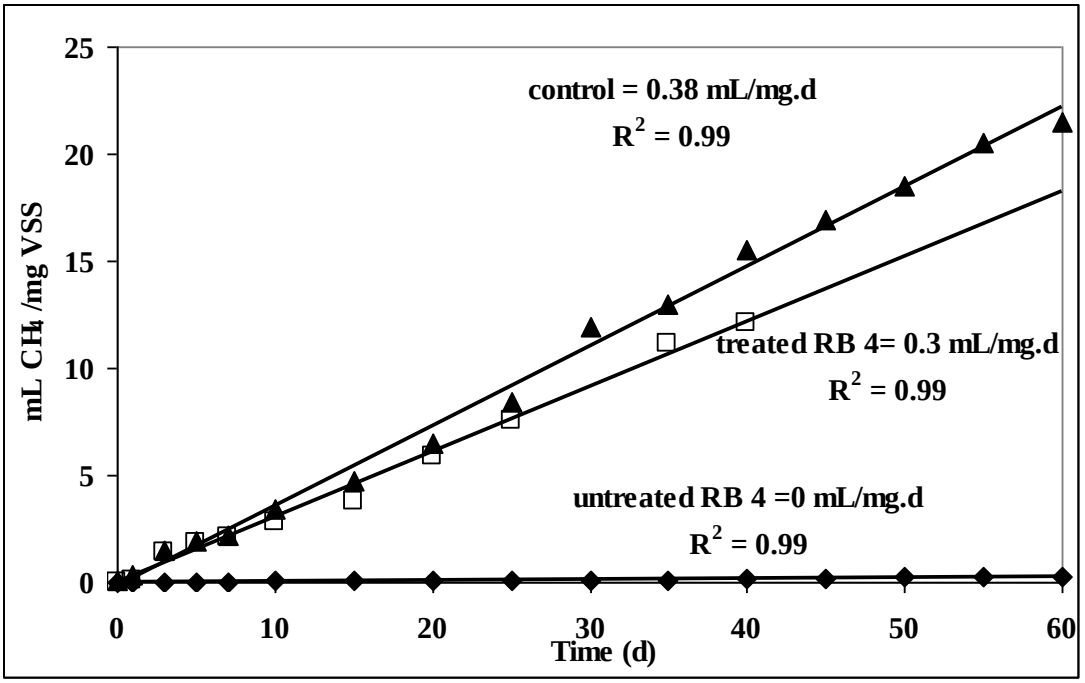


Figure 4.41: CH_4 formation rates during anaerobic experiment in the presence of treated and untreated RB 4

Table 4.5: Inhibition (%) on CH_4 formation rates during anaerobic experiment in the presence of untreated and treated AR 183, AO 51 and RB 4

	Inhibition (%)	
	Untreated	Treated
AR 183	29	0
AO 51	0	0
RB 4	100	21

From the table it is evident that Methane production rate was seriously (by 100 %) inhibited in the case of untreated RB 4, whereas 29 % was found for untreated AR 183 and there was no inhibition for untreated AO 51. Fenton treatment disappeared or decreased inhibition effect of untreated dyes. 29 % inhibition of untreated dye removed and 100 % inhibition of RB 4 increased to 21 % by Fenton treatment.

4.5.2. Anoxic experiments

During anoxic experiments in the presence of treated and untreated AR 183, AO 51 and RB 4 respectively, the results have shown that no inhibition occurred**.** And the reason of having almost complete consumption within the first hour, it is unable to have a kinetic evaluation.

Kd = 210 – 223 mg/L.h

Kc = 212 mg/L.h

Table 4.6: Inhibition (%) on denitrification rates during anoxic experiment in the presence of untreated and treated AR 183, AO 51 and RB 4

	Inhibition (%)	
	Untreated	Treated
AR 183	0	0
AO 51	0	0
RB 4	0	0

4.5.3. Aerobic experiments

Glucose (COD) degradation rates during aerobic experiment in the presence of treated and untreated AR 183, AO 51 and RB 4 respectively is presented in Figures 4.42., 4.43., 4.44. 4.45, 4.46., 4.47.

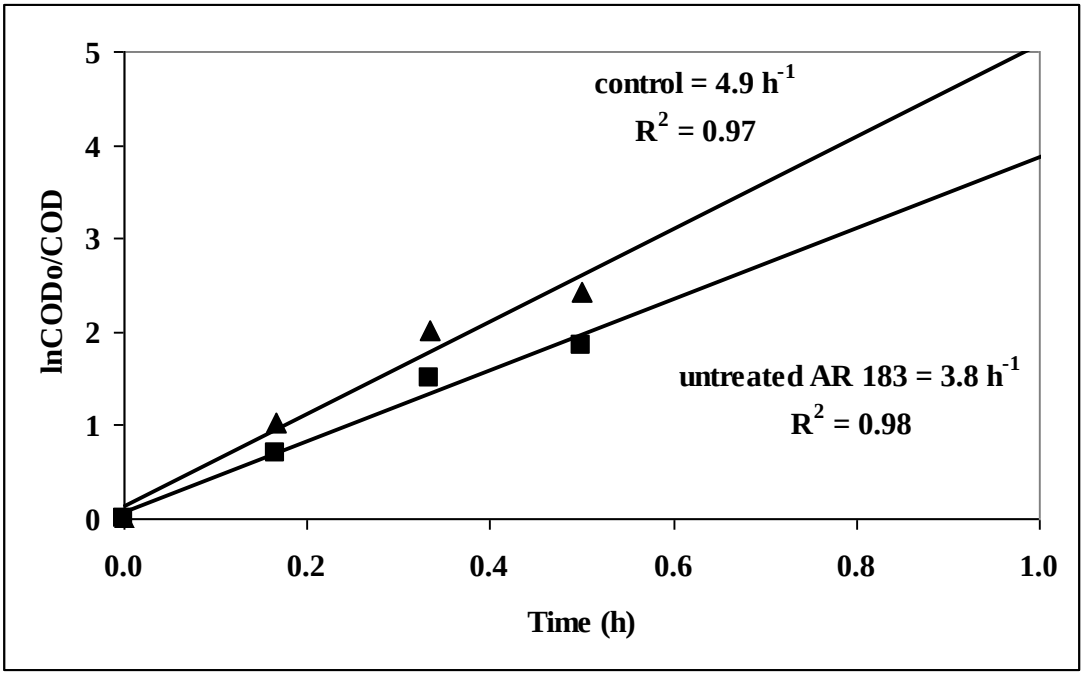


Figure 4.42: Glucose (COD) degradation rates during aerobic experiment in the presence of untreated AR 183

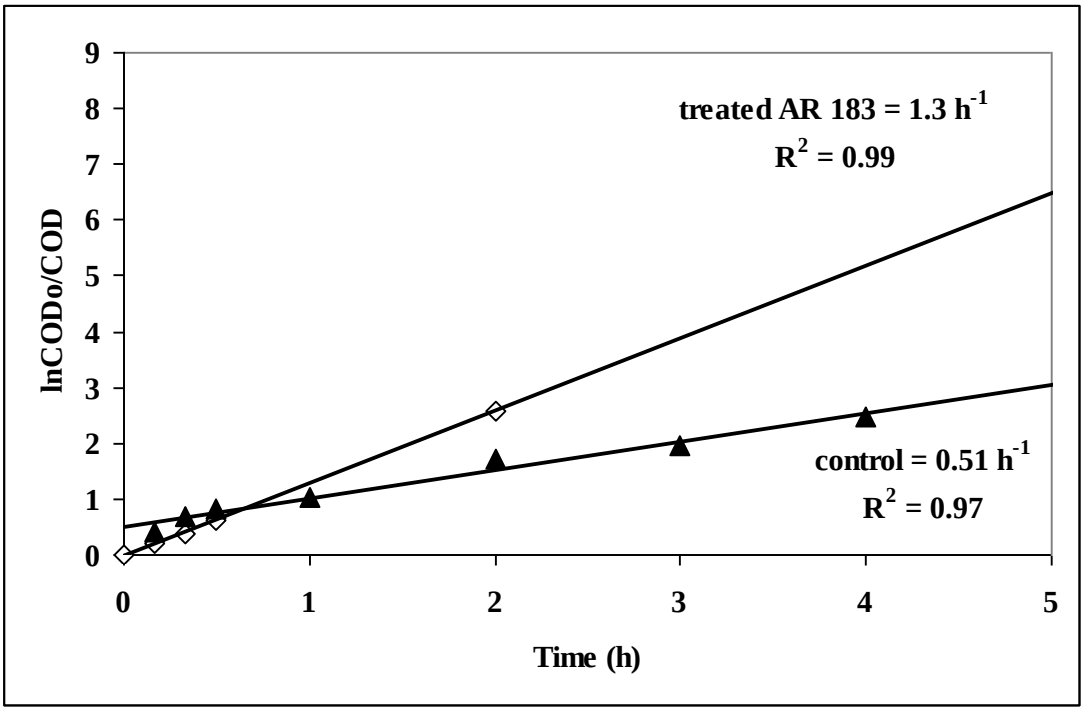


Figure 4.43: Glucose (COD) degradation rates during aerobic experiment in the presence of treated AR 183

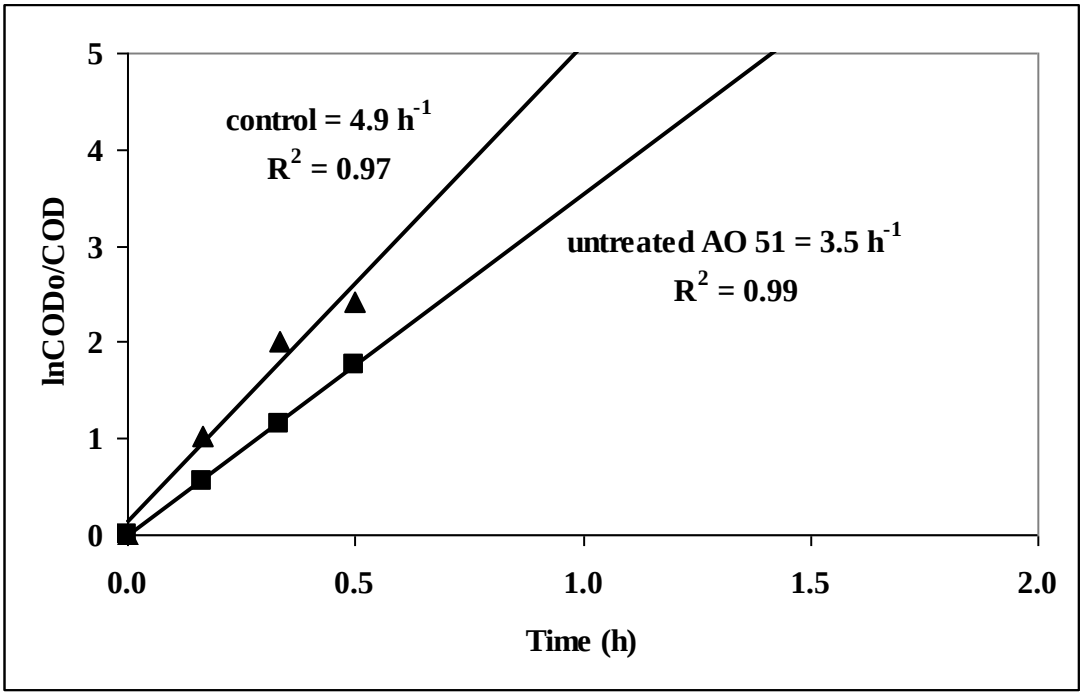


Figure 4.44: Glucose (COD) degradation rates during aerobic experiment in the presence of untreated AO 51

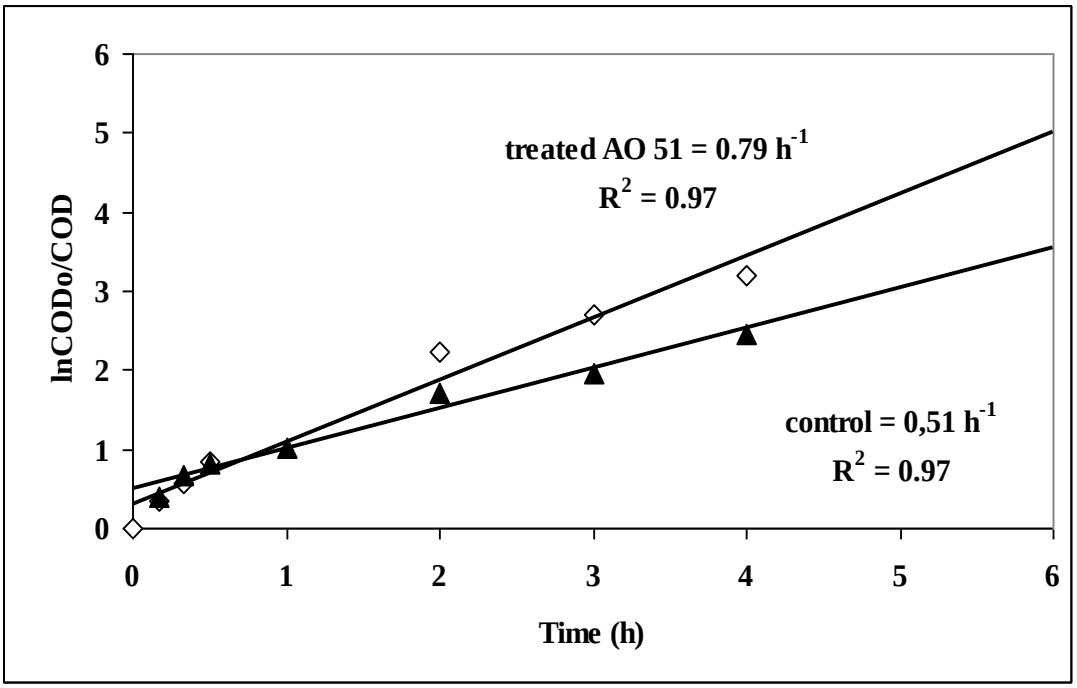


Figure 4.45: Glucose (COD) degradation rates during aerobic experiment in the presence of treated AO 51

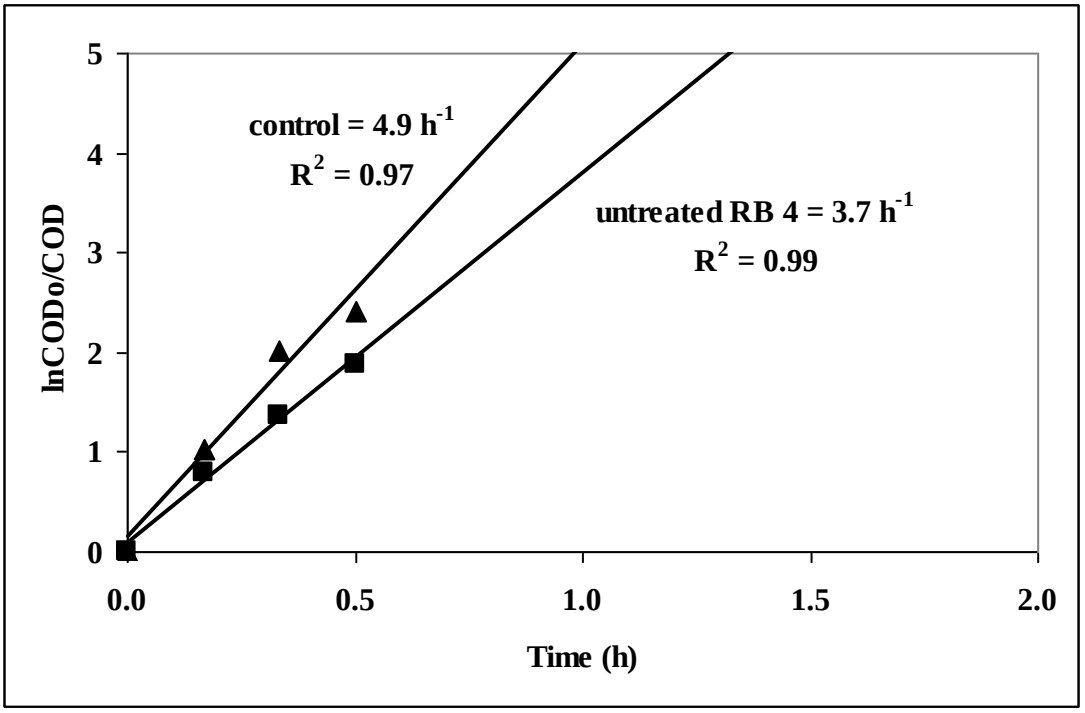


Figure 4.46: Glucose (COD) degradation rates during aerobic experiment in the presence of untreated RB 4

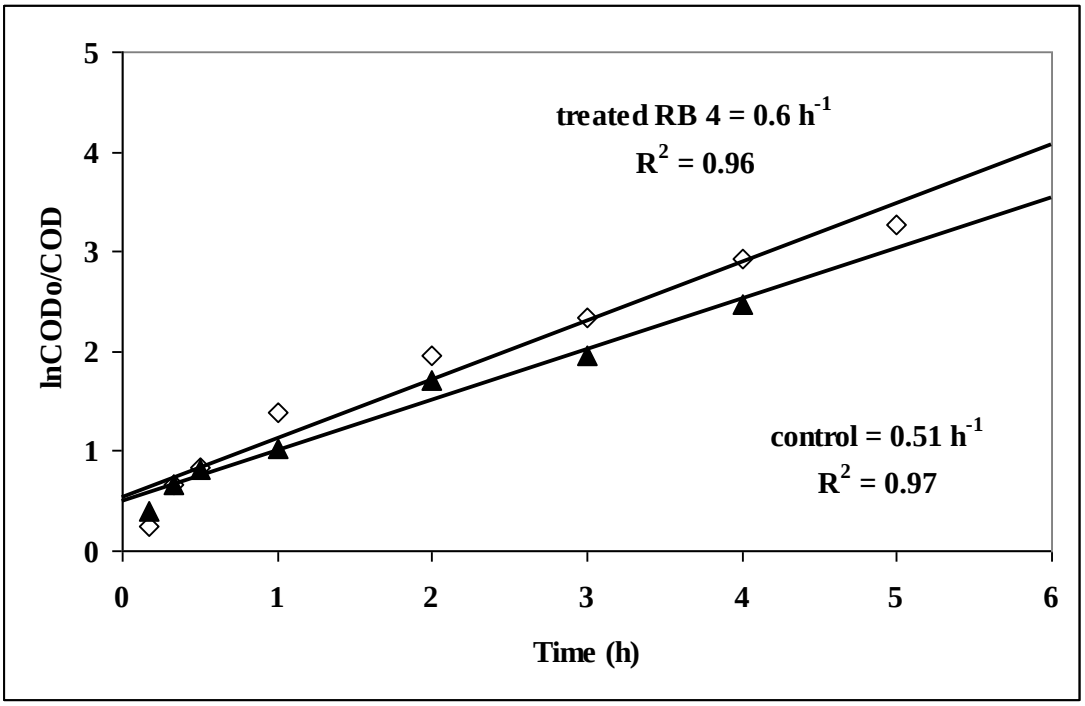


Figure 4.47: Glucose (COD) degradation rates during aerobic experiment in the presence of treated RB 4

Table 4.7: Inhibition (%) on glucose degradation rates during aerobic experiment in the presence of untreated and treated AR 183, AO 51 and RB 4

	Inhibition (%)	
	Untreated	Treated
AR 183	23	0
AO 51	29	0
RB 4	25	0

As it is obvious from the table, glucose degradation was slightly inhibited via both untreated (23-29 % relative inhibition to control) as well as Fenton-treated (21-33 % relative inhibition to control) dyes.

4. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Textile dyestuffs are known for their resistance to biodegradation and conventional physicochemical (adsorption, coagulation, etc.) treatment due to their high degree polarity/hydrophilic nature and complex molecular structure. As a consequence textile dyes tend to sorb on biosludge in activated sludge treatment systems and on sediment soil in receiving water bodies, passing significant environmental, ecotoxicological and ecological problems. With these important issues interview, the present experimental work aims at treating three commercially important textile dyes (Acid Red 183, AR 183; Acid Orange, AO 51; Reactive Blue, RB 4) with Fenton's reagent and studying their behavior in anaerobic, anoxic and aerobic processes. The following conclusions could be drawn from the investigation;

1. Fenton Experiments

- All three investigated textile dyes could be completely decolorized and partially oxidized. COD removal efficiency of 58, 78, 69 % and DOC of 53, 76, and 49 % for AR 183, AO 51, RB 4, respectively. The corresponding color removal was 100 %, 100 %, and 93 %. After 30 min treatment when they subjected to Fenton's reaction at different Fe^{2+}: H_2O_2 molar ratios at pH = 3.
- NO_3^- formation was negligible (around detection limits) for Fenton's oxidation of all studied textile dyes.
- CH_3COO^- formation rate depended upon dye type (up to 23 mg/L for AR 183; 16 mg/L for AO 51 and 32 mg/L for RB 4 at a molar ratio of 4mM:40mM Fe^{2+}: H_2O_2).
- Fastest and highest color, COD and TOC removal was obtained for the diaszo dye, AO 51 because of its higher molecular weight (860.80 mg/mol) and hence lower molecular concentration in the reaction medium.
- Optimum Fenton' oxidation conditions were postulated as pH=3, Fe^{2+}:4mM and H_2O_2: 20mM for all studied textile dyes.

2. Anaerobic Experiments

- Color removal mechanism mainly due to the biosorption. Color removal efficiencies were obtained as 37, 70 and 55 % for AR 183, AO 51 and RB 4 dyes, respectively.
- Methane production was monitored to assess the toxic effect of dyes on the anaerobic process. The biosorbed dyes and intermediate oxidation products may occupy the active sites of the microorganism's enzymes causing toxicity/inhibition of methanogenic bacteria (inhibitory effect on methane formation potential). For untreated dyes, 29 % inhibition, no inhibiton, 100 % inhibition were found for AR 183 AO 51 and RB 4, respectively. For treated dyes, there was no inhibitory effect except for RB4 (21% inhibition).

3. Anoxic Experiments

- Decolorization is not efficient under anoxic conditions. This low efficiency of color abatement can be explained as the competition between nitrate and azo dyes for reduction reaction and dye adsorption on biomass. Nevertheless 28, 9 and 45 % color removal efficiencies were obtained for AR 183, AO 51 and RB 4 dyes, respectively mainly due to adsorption on biomass.
- Nitrate consumption rate was evaluated for determination of the inhibitory effect of dyes/treated dyes. Nitrate was almost consumed within the first hour of anoxic experiment. From this aspect neither the untreated nor treated textile dyes showed any inhibitory effect on denitrification.

4. Aerobic Experiments

- Decolorization is not efficient under aerobic conditions. This low efficiency of decolorization is a result of recalcitrant behavior of dyes to aerobically microbial degradation. However present decolorization as 38, 18 and 73 % for AR 183, AO 51 and RB 4 dyes, respectively can be attributed to adsorption of dyes on biomass.
- COD abatement rate in other words glucose consumption rate was evaluated for determination of the inhibitory effect of dyes/treated dyes. Untreated dyes slightly inhibited as 23-29 % relative to control. Fenton treated dyes had no inhibitory effect on glucose degradation.

Our experimental findings demonstrated that Fenton-treated dyes were not more toxic/inhibitory than untreated (original) dyes to anaerobic/anoxic/aerobic biological treatment systems that are commonly practiced for the treatment of combined dyehouse effluent.

Fenton's reagent can be recommended for complete color and partial COD-DOC removal (reduction in organic load) for pretreatment of dyebaths prior to combined dyehouse effluent

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APPENDIX

A-) Fenton Treatment

A.1. Nitrate

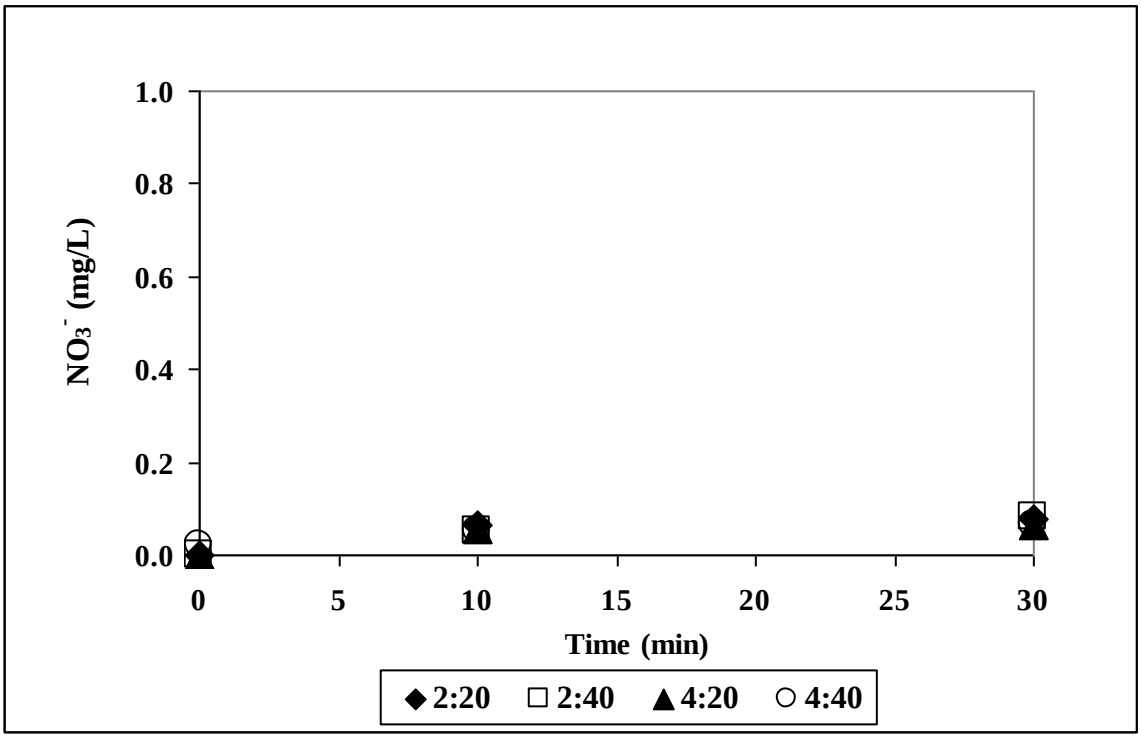

Figure A.1: Effect of different Fe^{2+}: H_2O_2 molar ratios on NO_3^- formation for 100 mg/L AR 183 (pH_o = 3, COD_o = 50 mg/L, DOC_o = 14 mg/L)

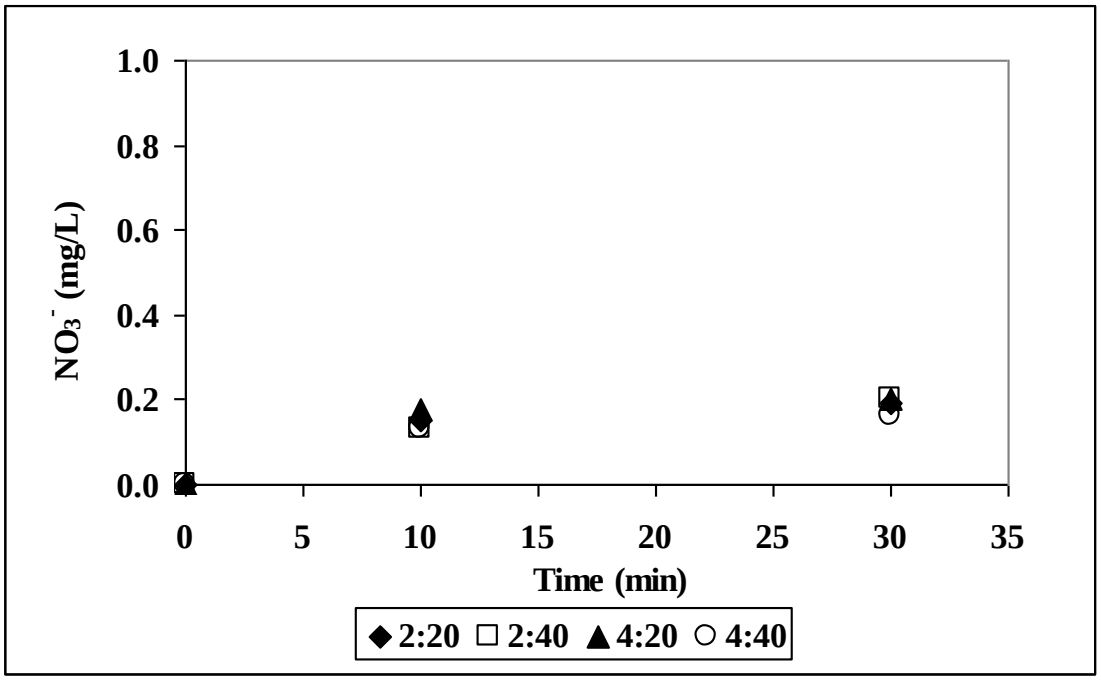

Figure A.2: Effect of different Fe^{2+}: H_2O_2 molar ratios on NO_3^- formation during degradation of 100 mg/L AO 51 (COD_o=103 mg/L, DOC_o=31 mg/L)

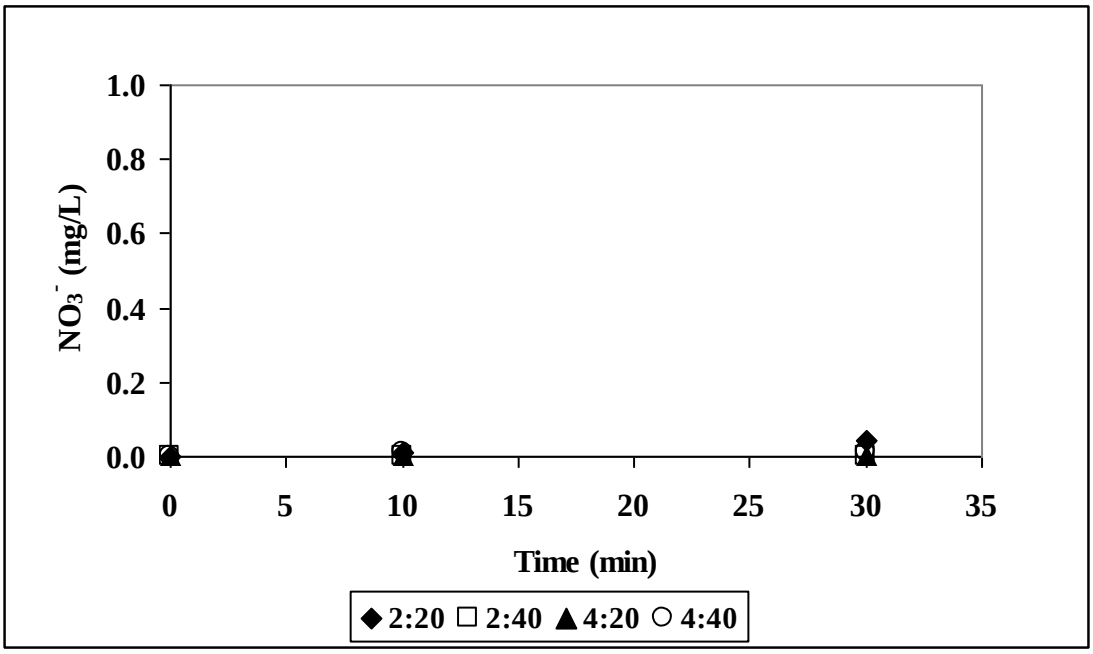

Figure A.3: Effect of different Fe^{2+}: H_2O_2 molar ratios on NO_3^- formation for 100 mg/L RB 4 (COD_o=63 mg/L, DOC_o=21 mg/L)

A.2. Sulfate

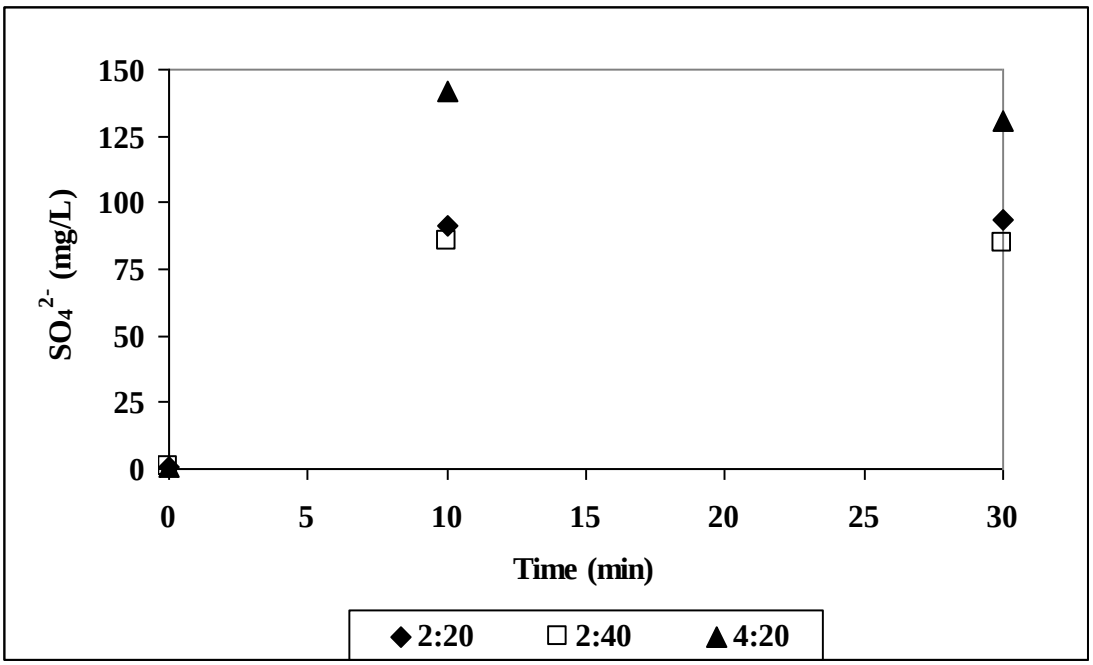

Figure A.4: Effect of different Fe^{2+}: H_2O_2 molar ratios on SO_4^{2-} formation for 100 mg/L AR 183 (pH = 3, COD_o = 50 mg/L, DOC_o = 14 mg/L)

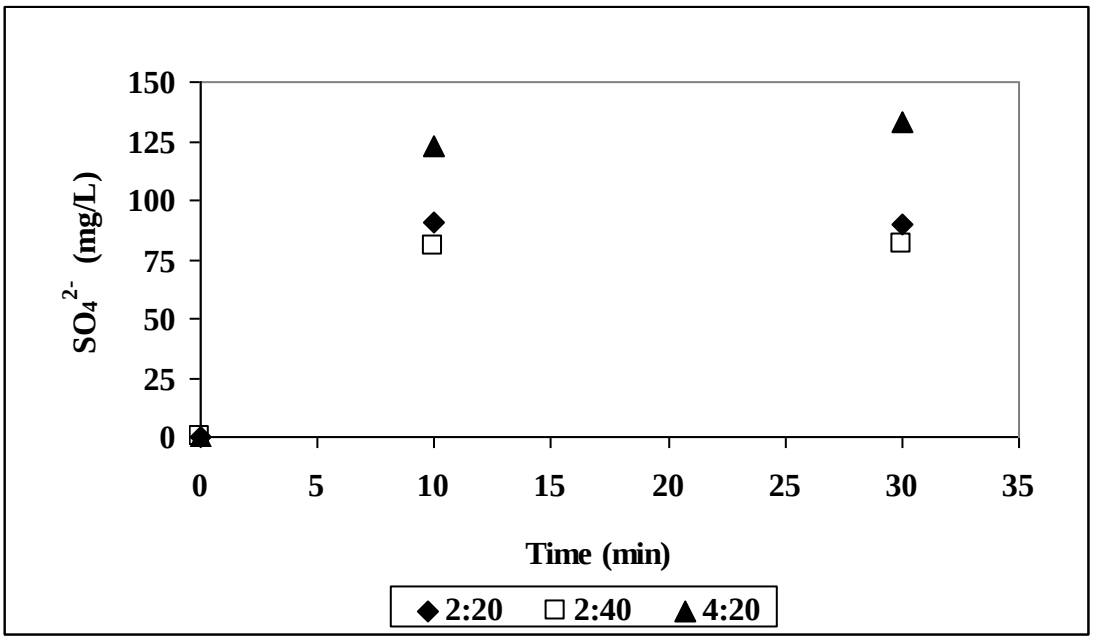

Figure A.5: Effect of different Fe^{2+}: H_2O_2 molar ratios on SO_4^{2-} release for 100 mg/L AO 51 $^{-1}$, (COD_o=103 mg/L, DOC_o=31 mg/L)

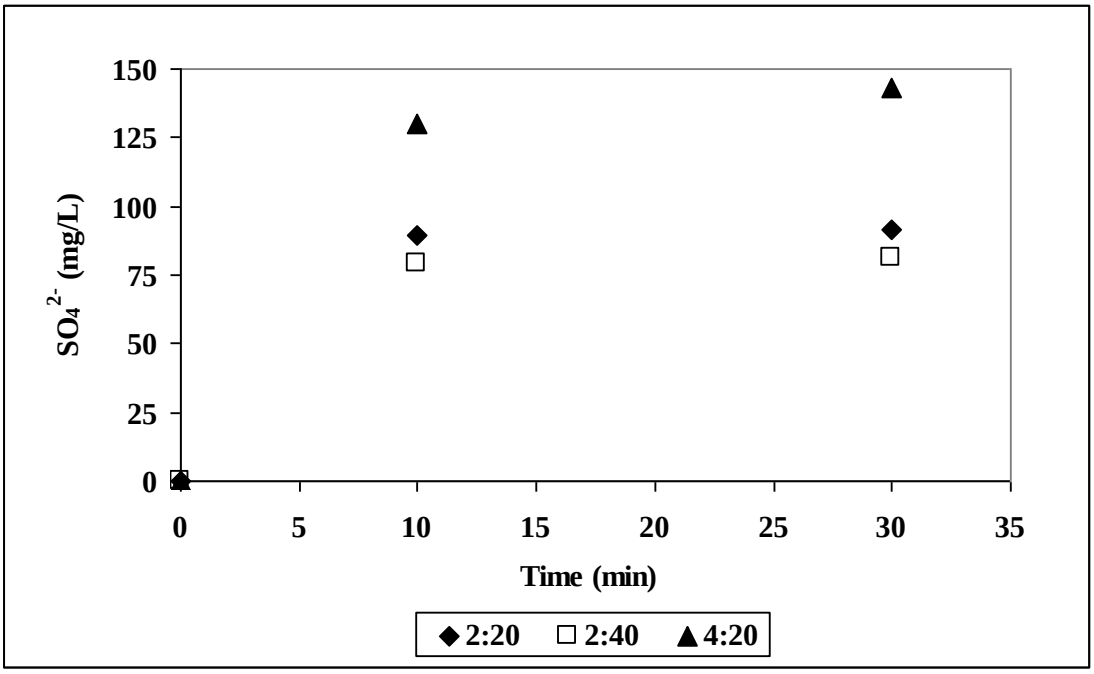

Figure A.6: Effect of different Fe^{2+}: H_2O_2 molar ratios on SO_4^{2-} formation for 100 mg/L RB 4 (pH_o=3, COD_o=63 mg/L, DOC_o=21 mg/L)

B-) Anaerobic Experiments

B.1. Nitrate

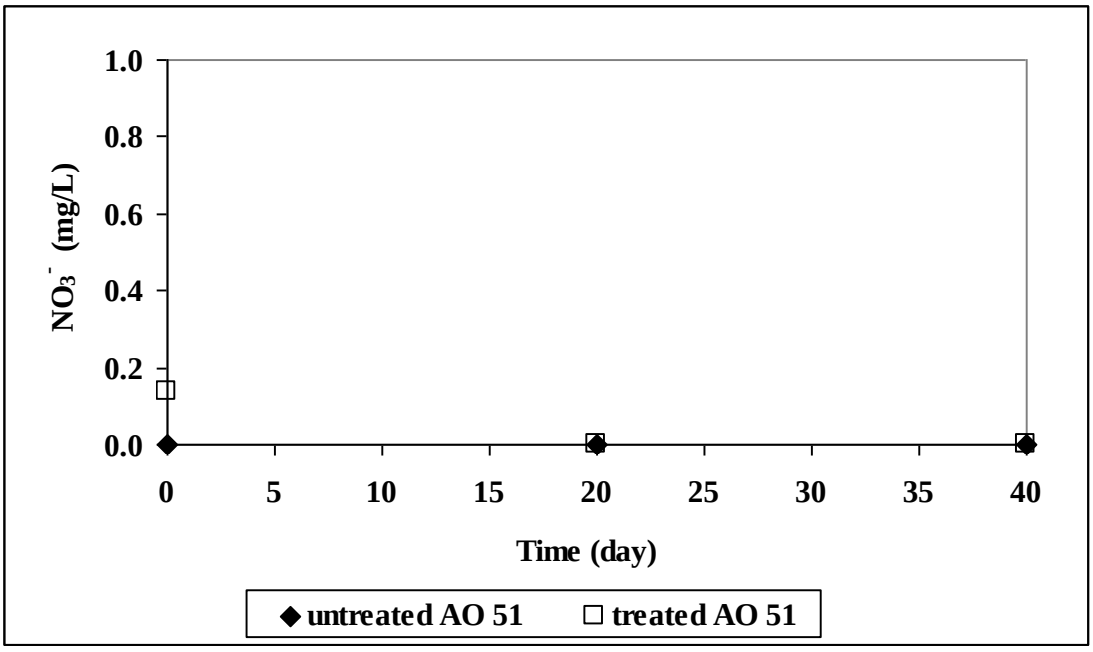

Figure B.1: Changes in NO_3^- concentration during anaerobic experiment in the presence of untreated and treated AO 51 (VSSo = 500 mg/L)

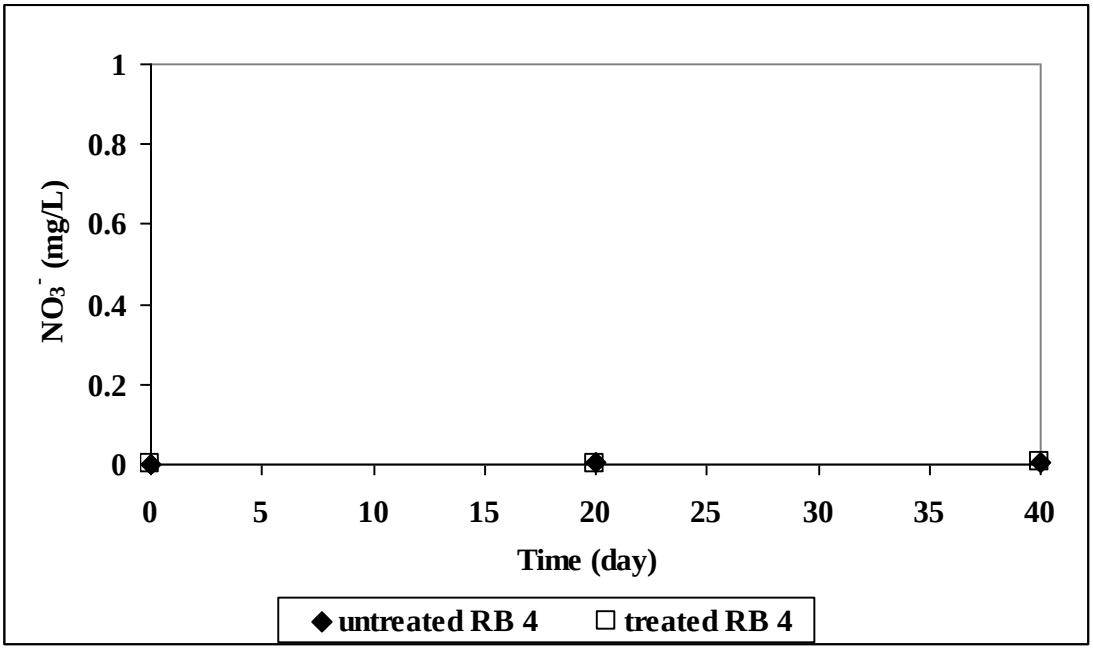

Figure B.2: Changes in NO_3^- concentration during anaerobic experiment in the presence of untreated and treated RB 4 (VSSo = 500 mg/L)

B.2. Sulfate

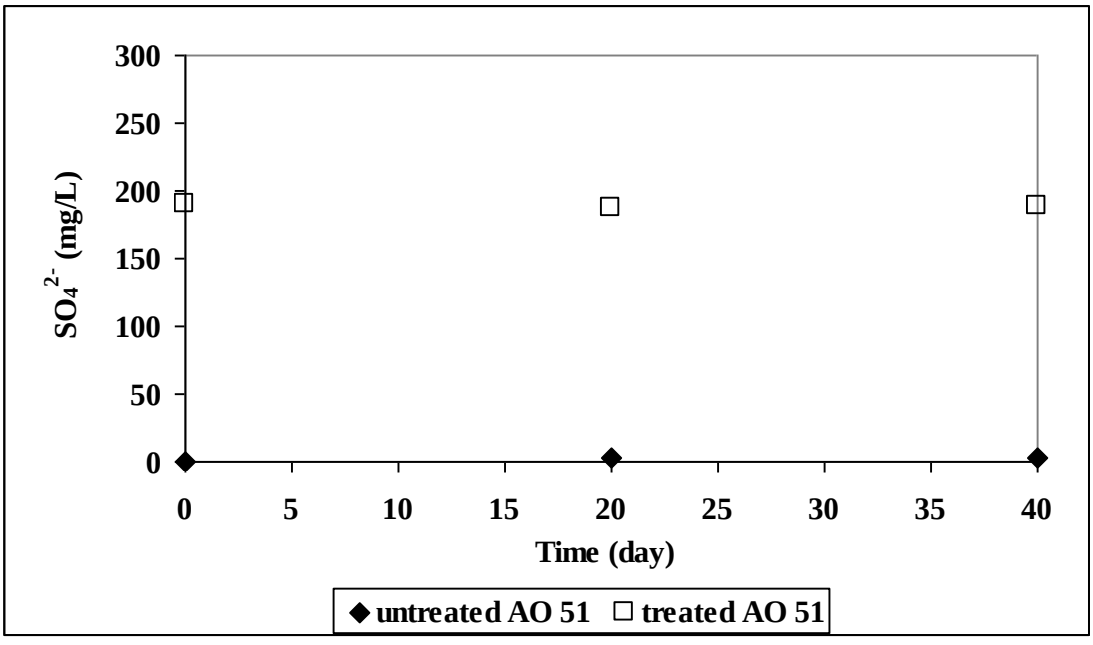

Figure B.3: Changes in SO_4^{2-} concentration during anaerobic experiment in the presence of untreated and treated AO 51 (VSS_o = 500 mg/L)

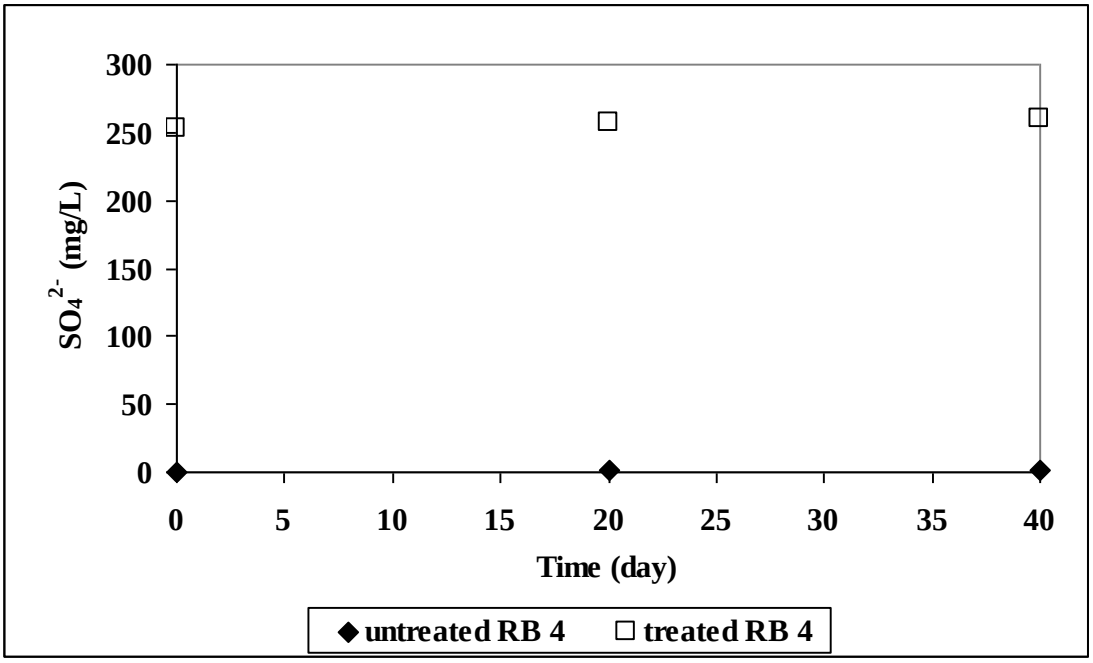

Figure B.4: Changes in SO_4^{2-} concentration during anaerobic experiment in the presence of untreated and treated RB 4 (VSS_o = 500 mg/L)

CURRICULUM VITAE

Betül Hande Gürsoy was born in Erzurum in 1983. She graduated from Ankara Gölbaşı Anatolian High School in 2001. She received her undergraduate degree from İstanbul University (İU), Environmental Engineering Department in 2005. She started her graduate studies at İstanbul Technical University (İTU), Environmental Engineering Department in 2005 and she is currently studying in the Environmental Sciences and Engineering Programme.