



LUND UNIVERSITY

Treatment of Textile Wastewater by Combining Biological Processes and Advanced Oxidation

Punzi, Marisa

2015

[Link to publication](#)

Citation for published version (APA):

Punzi, M. (2015). *Treatment of Textile Wastewater by Combining Biological Processes and Advanced Oxidation*. [Doctoral Thesis (compilation)].

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Treatment of textile wastewater by combining biological processes and advanced oxidation

Marisa Punzi



LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Engineering, Lund University, Sweden.

To be defended at Center for Chemistry and Chemical Engineering.

Date 8th of June 2015 at 10:00.

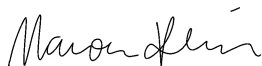
Faculty opponent

Professor Anna Ledin, Environmental Department of the City of Gothenburg,
Gothenburg, Sweden

Organization LUND UNIVERSITY Department of Biotechnology P.O Box 124 SE 221 00 Lund, Sweden Author(s): Marisa Punzi	Document name DOCTORAL DISSERTATION	
	Date of issue 2015-06-08	
	Sponsoring organization Swedish International Development Cooperation Agency (SIDA)	
Title and subtitle: Treatment of textile wastewater by combining biological processes and advanced oxidation		
<p>Abstract</p> <p>Treatment of textile wastewater is challenging because the water contains toxic compounds that have low biodegradability. Dyes, detergents, surfactants, biocides and more are used to improve the textile process and to make the clothes resistant to physical, chemical and biological agents. New technologies have been developed in the last decades and in particular Advanced Oxidation Processes (AOPs) have shown considerable potential for treatment of industrial effluents. These processes however are expensive and full scale applications are still scarce. In addition, the complex oxidation chemistry transforms the pollutants into a very large number of degradation intermediates which may be even more toxic than the original compounds.</p> <p>This thesis presents a novel treatment approach where two AOPs, photo-Fenton oxidation and ozonation, are used after an anaerobic biofilm process for treatment of textile wastewater, azo dyes degradation and removal of toxicity. The biological treatment cleaves the azo bonds of the dyes and consumes the biodegradable compounds whereas the following advanced oxidation degrades the aromatic amines and other by-products that are recalcitrant to biological degradation. The approach that includes photo-Fenton oxidation resulted in higher reduction of chemical oxygen demand (COD) than that with ozonation when treating real textile wastewater. The latter however resulted in higher reduction of toxicity towards the bacteria <i>Vibrio fischeri</i> and the shrimp <i>Artemia salina</i>. Mutagenic effects were detected in the untreated and biologically treated effluent, but not after photo-Fenton oxidation and ozonation.</p> <p>Environmental impact and costs of the two treatment strategies, at the operating conditions used in this study, are high compared to the full-scale biological process used in the Netherlands, where the wastewater is actually treated. Substitution of artificial light with sunlight and upscaling would result in great improvements in terms of electricity requirements and ozone consumption and brings down the impact to values that are comparable to the biological process, suggesting that full scale implementation can be achieved. Further research should look into combining this treatment approach with technologies that allow water and salt recovery and reuse, to make the textile industry more sustainable.</p>		
Key words: Azo dye; textile wastewater; biological treatment; biofilm; microbial community; DGGE; green algae; photo-Fenton; ozone; toxicity; mutagenicity; LCA; cost analysis		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title		ISBN 978-91-7623-351-1
Recipient's notes	Number of pages	
	Security classification	

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature



Date

2015-04-27

Treatment of textile wastewater by combining biological processes and advanced oxidation

Marisa Punzi



LUND
UNIVERSITY

Cover photo: Dyeing with the scarlet resinous secretion of a lac insect. Avani
Uttarakhand, India. Fall 2012. Photo by Lina Sofia Lundin.

Doctoral thesis

© Marisa Punzi

Faculty of Engineering, Biotechnology

ISBN 978-91-7623-351-1 (printed)

ISBN 978-91-7623-352-8 (pdf)

ISRN LUTKDH/TKBT – 15 / 1164 – SE

Printed in Sweden by Media-Tryck, Lund University
Lund 2015



KLIMATKOMPENSERAT
PAPPER



Table of contents

Popular summary	7
List of papers	9
My contribution to the papers	10
Abbreviations	11
1. Introduction	12
Scope of the thesis	12
2. Textile production and environmental implications	13
The textile process	14
Textile wastewater	15
3. Textile dyes	17
4. Analysis of textile wastewater	19
5. Biological treatment of textile wastewater	21
Anaerobic and aerobic processes	21
Biofilm process	23
Analysis of the microbial community	27
Algae and cyanobacteria	32
6. Physical-chemical processes for treatment of textile wastewater	35
Overview of AOPs	35
Photo-Fenton oxidation	37
Ozonation	40
How do we combine physical-chemical and biological processes	41
7. Assessment of toxicity	47
Acute toxicity	48
Mutagenicity	52
8. An ideal treatment - implications	54
Environmental impact and cost analysis of photo-Fenton and ozonation	54
Cost analysis	56
Environmental impact	58
Textile wastewater treatment in the future	59
9. Conclusions and future perspectives	61
Acknowledgments	63
References	65

Abstract

Treatment of textile wastewater is challenging because the water contains toxic compounds that have low biodegradability. Dyes, detergents, surfactants, biocides and more are used to improve the textile process and to make the clothes resistant to physical, chemical and biological agents. New technologies have been developed in the last decades and in particular Advanced Oxidation Processes (AOPs) have shown considerable potential for treatment of industrial effluents. These processes however are expensive and full scale applications are still scarce. In addition, the complex oxidation chemistry transforms the pollutants into a very large number of degradation intermediates which may be even more toxic than the original compounds.

This thesis presents a novel treatment approach where two AOPs, photo-Fenton oxidation and ozonation, are used after an anaerobic biofilm process for treatment of textile wastewater, azo dyes degradation and removal of toxicity. The biological treatment cleaves the azo bonds of the dyes and consumes the biodegradable compounds whereas the following advanced oxidation degrades the aromatic amines and other by-products that are recalcitrant to biological degradation. The approach that includes photo-Fenton oxidation resulted in higher reduction of chemical oxygen demand (COD) than that with ozonation when treating real textile wastewater. The latter however resulted in higher reduction of toxicity towards the bacteria *Vibrio fischeri* and the shrimp *Artemia salina*. Mutagenic effects were detected in the untreated and biologically treated effluent, but not after photo-Fenton oxidation and ozonation.

Environmental impact and costs of the two treatment strategies, at the operating conditions used in this study, are high compared with the full-scale biological process used in the Netherlands, where the wastewater is actually treated. Substitution of artificial light with sunlight and upscaling would result in great improvements in terms of electricity requirements and ozone consumption hence would bring down the environmental impact to values that are comparable to the biological process, suggesting that full scale implementation can be achieved. Further research should look into combining this treatment approach with technologies that allow water and salt recovery and reuse, to make the textile industry more sustainable.

Popular summary

Colors have always been part of human life and culture. Today natural pigments have been substituted by synthetic dyes that usually end up threatening nature itself by polluting the environment. To what extent can we use chemistry and biology to minimize the environmental impact of the textile industry?

During the dyeing process a certain amount of dye, 2 to 50 percent, does not bind to the fabric and is washed away. Companies are working on improving this process. Meanwhile something needs to be done to avoid the dye effluents ending up untreated in the natural environment.

Discharge of untreated textile effluents in the natural environment is a widespread problem where the production of textiles is concentrated, for example in the south of Asia. This is caused by lack of care and awareness, and defective regulations. Much research has been performed to reduce the environmental impact of the dyeing process units and appropriate technologies are now available. However, most units are very small, sometimes just the size of a family company, and they do not have money for treating their effluent.

Why are colors threatening the environment? Aquatic plants and algae are indispensable for aquatic ecosystems. They use carbon dioxide and light to produce oxygen and food, which are consumed by higher organisms like fish. The presence of colors in water tends to stop the penetration of sunlight, hence the gradual decrease in living organisms in rivers. Dyes can be harmful for humans, acting as irritants for eyes and skin. Moreover, the problem does not disappear with the color! In fact, in time the dye molecules will eventually break down and become colorless. The resulting compounds are even more harmful than the original dyes and in some cases carcinogenic.

Researchers all around the world are looking for an optimal treatment for textile effluents. Common solutions for wastewater treatment include aerobic biological processes, adsorption and coagulation. Can these be used for textile effluents as well? Microorganisms are the main actors in aerobic biological processes because they remove nutrients like organic carbon, nitrogen and phosphorous from water without the addition of chemicals. The downside is that a lot of oxygen is needed for this process and excess sludge that may contain pollutants is generated and needs to be handled in an appropriate way. In addition, most dyes used in textile industry are not fully degradable and the treated water could contain dangerous amines. Adsorption and

coagulation remove dyes and other pollutants by transferring them from a liquid phase, the wastewater, to a solid phase, the adsorbent or coagulant. This is however only a temporary solution. In fact, the resulting sludge produced contains dyes and needs to be properly disposed of, with additional costs.

Our research group is dealing with textile wastewater treatment. The project started some 10 years ago after visiting several textile dyeing units in the south of India. The research goal was to devise an ideal treatment which was cheap, easy to operate and efficient in terms of producing a good-quality effluent. The strategy is simple. First, the wastewater is treated with anaerobic microorganisms, which can reduce the total organic content of the effluent by metabolizing the degradable compounds without the need for oxygen. Secondly, an advanced oxidation process based on the activity of a powerful oxidant is applied to facilitate the removal of harmful amines. We have evaluated a few options for this last step. One is ozonation, energy intensive but “clean”. This means that it does not produce any sludge or secondary waste. In fact, ozone, apart from being a well-known pollutant, can oxidize aromatic compounds releasing oxygen. A very successful process is photo-Fenton oxidation. The effluent is treated with iron and hydrogen peroxide in the presence of light and what remains at the end is a mixture of salts rich in iron. To verify the effectiveness of the treatment, we used a series of toxicity tests. The costs and environmental impact assessment of the treatments suggested in this work indicate that the technology is ready to be implemented.

Money cannot buy happiness, but it can certainly help to preserve our beautiful world. The technology for industrial wastewater treatment is mature enough to offer solutions to avoid pollutants from escaping into the environment. Dyestuff and other chemicals found in textile effluents are no exception. However, industries are often not ready to put money into waste handling, something that will not make them richer. Therefore, the future of wastewater should be centered on the concept of water reuse, which is profitable for industries as well as the environment.

List of papers

This thesis is based on the following papers, which are referred to by Roman numerals in the text. The papers are provided in the end of the thesis.

- I. Punzi M., Mattiasson B. and Jonstrup M. Treatment of synthetic textile wastewater by homogeneous and heterogeneous photo-Fenton oxidation. *Journal of Photochemistry and Photobiology A: Chemistry* 248 (2012) 30-35
- II. Jonstrup M., Punzi M. and Mattiasson B. Comparison of anaerobic pre-treatment and aerobic post-treatment coupled to photo-Fenton oxidation for degradation of azo dyes. *Journal of Photochemistry and Photobiology A: Chemistry* 224 (2011) 55– 61
- III. Punzi M., Anbalagan A., Aragão Börner R., Svensson B.-M., Jonstrup M. and Mattiasson B. Degradation of a textile azo dye using a biological treatment followed by photo-Fenton oxidation: evaluation of toxicity and microbial community structure. *Chemical Engineering Journal* 270 (2015) 290-299
- IV. Punzi M., Nilsson F., Anbalagan A., Svensson B.-M., Jönsson K., Jonstrup M. and Mattiasson B. Combined anaerobic-ozonation process for treatment of textile wastewater: evaluation of acute toxicity and mutagenicity removal. *Journal of Hazardous Materials* 292 (2015) 52-60
- V. Punzi M., Jabari A. and Mattiasson B.. Screening of green algae for their ability to degrade azo dyes. (Manuscript)
- VI. Punzi M. and Börjesson P. Analysis of environmental impact and costs of treatment of textile wastewater using a biological process followed by advanced oxidation. (Manuscript)

My contribution to the papers

- I. I planned the experiments together with the co-authors. I performed all the experiments and wrote the manuscript with the help of comments from the co-authors.
- II. The work was mostly planned by Maria Jonstrup and I performed the experiments together with her. I helped in the writing and editing of the manuscript.
- III. I designed the study together with Maria Jonstrup. Many of the experiments were performed by Anbarasan Anbalagan. Rosa Aragão Börner helped in designing the molecular biology study which was performed by Anbarasan Anbalagan and me. I designed the toxicity study with Britt-Marie Svensson and performed the tests. I also wrote the manuscript with the help of comments from the co-authors.
- IV. I designed the study together with the co-authors and performed the biological tests, together with Anbarasan Anbalagan, and the toxicity tests, planned with Britt-Marie Svensson. The ozonation experiments were performed by Filip Nilsson. I also wrote the manuscript with the help of comments from the co-authors.
- V. I designed the study together with the co-authors. Part of the experimental work was performed by Amir Jabari and part by me. I wrote the first draft of the manuscript.
- VI. I designed and performed the study with the assistance of Pål Börjesson. I also wrote the manuscript with the help of comments from the co-author.

Abbreviations

AOP	Advanced Oxidation Process
AP	Acidification Potential
AU	Absorbance Units
BOD	Biological Oxygen Demand
CETP	Centralized Effluent Treatment Plant
CMC	Carboxy Methyl Cellulose
COD	Chemical Oxygen Demand
CPC	Compound Parabolic Collector
DGGE	Denaturing Gradient Gel Electrophoresis
EC	Energy Consumption
EC ₂₀	Concentration that gives an Effect in 20% of the population
EP	Eutrophication Potential
FISH	Fluorescence In Situ Hybridization
FU	Functional Unit
GWP	Global Warming Potential
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
LCA	Life Cycle Assessment
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance
PCR	Polymerase Chain Reaction
PM	Particulate Matter formation
POCP	Photochemical Ozone Creation Potential
PVA	Polyvinyl Alcohol
RFLP	Restriction Fragment Length Polymorphism
RR	Remazol Red (azo dye)
SEM	Scanning Electron Microscopy
TE	Real Textile Effluent
TOC	Total Organic Carbon
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
WD	Water Depletion
ZDTS	Zero Discharge Treatment System

1. Introduction

Synthetic dyes have been produced since 1800s. They have been optimized so that they can withstand physical and chemical agents and last for many years without fading. Thanks to these characteristics the durability of fabrics has improved significantly and the textile industry has flourished (Porter et al. 1972). However, a large part of the synthetic dyes used in the dyeing of fabrics ends up in water. It is very difficult to eliminate these molecules, which nowadays represent a danger for the environment and for the people working in textile production. In fact, in many of the producing countries, dyes from wastewater are adsorbed to available materials or precipitated so that the water becomes clear, but large amounts of toxic sludge are generated and used, for example, to build roads (Blomqvist 1996). In the last years the legislation has become stricter in order to meet the growing concern for the conditions of the workers and the environment. Thus, new and effective strategies to clean the water from dyes need to be developed so that no toxic by-products are generated.

Scope of the thesis

The aim of this research project was to evaluate different biological and physical-chemical processes for their ability to degrade azo dyes to non-toxic compounds and use the results to develop a novel approach for treatment of textile wastewater which can provide high efficiency in terms of water purification at low cost and environmental impact. Textile industry is in fact in great need to reduce its negative impact on the environment and to become more “green”. Improved treatment of textile wastewater is one of the ways to pursue this objective.

To achieve this goal, synthetic and real textile effluents were treated at lab-scale using photo-Fenton oxidation as stand-alone treatment (**Paper I**) and in combination with biological treatment (**Paper II and III**), as well as ozonation in combination with biological treatment (**Paper IV**). A preliminary experimental evaluation of the possibility of using algae as an alternative biological process was also conducted (**Paper V**). These treatments were first compared in terms of dye degradation, removal of organic compounds and toxicity. Then, environmental impact and costs of the most promising strategies were assessed (**Paper VI**). The results are ready to be applied in the planning of a new treatment plant.

2. Textile production and environmental implications

The textile industry is one of the largest and most ancient in the world. The production of textiles, once present all over the world, has been moved to countries where the costs of materials and labor are low. More than 50% of the clothing and textiles of the USA and EU are imported from Asia, in particular China, India and Bangladesh (Adhikari 2008). In some of these countries the textile industry has become one of the most important business sectors, and significant efforts are continuously being made to increase production. The textile industry utilizes substantial amounts of water. Thus, textile production and other industries, agriculture and people compete for access to clean water. In some areas, for example the Tirupur district situated in the south of India, textile production is the main occupation among the local population. The textile industry developed in Tirupur after 1970 and by the mid-1980s it accounted for 85% of India's total export of textiles. In 1995 virtually all dyeing and bleaching effluents were discharged in the Noyyal River and its tributaries without any treatment. The same river was used as a source of drinking and agricultural water. This uncontrolled situation resulted in severe water pollution in the Tirupur area, increased salt content in the groundwater and also presence of many toxic compounds, which meant both industry and people had to buy water from outside the area (Blomqvist A 1996). Later, the law obliged factories to treat dyeing and bleaching effluents and the situation changed. Common Effluent Treatment Plants (CETPs) were built and used by most textile factories so that clear water was discharged into the environment (Pophali et al. 2003, Moosvi and Madamwar 2007). But the problem was not solved. In fact, CETPs consist typically of two treatment steps: coagulation/flocculation followed by the use of an aeration tank. The water is clarified after each step and the sludge generated is collected. Thus, most dyes and pollutants present in textile effluents are precipitated with aluminum, iron, magnesium salts and lime and separated from the water in the form of sludge. This sludge is then stored in outdoor fields or used to build roads, but the risk of it being spread around during heavy rainfall is high (Balasubramanian et al. 2006). Most textile dyes are water soluble and when free in the environment will absorb sunlight preventing it to be used by photosynthetic organisms. In addition, some dyes are toxic and can release compounds that are more toxic and even mutagenic (Pinheiro et al. 2004). An interesting example can be found in the study by de Aragão Umbuzeiro and colleagues (2005) where mutagenicity was detected

in the River Cristais, São Paulo, Brazil and could be clearly correlated with the presence of azo dyes released by a nearby textile processing plant.

The textile process

The industrial production of textiles is a rather long and complex process. In short, the fibers, natural or man-made, are transformed into yarn and then fabric. The fabric undergoes so-called “wet processing” during which chemicals are used to provide the required properties of durability and color. Finally, the final product is assembled (Verma et al. 2012).

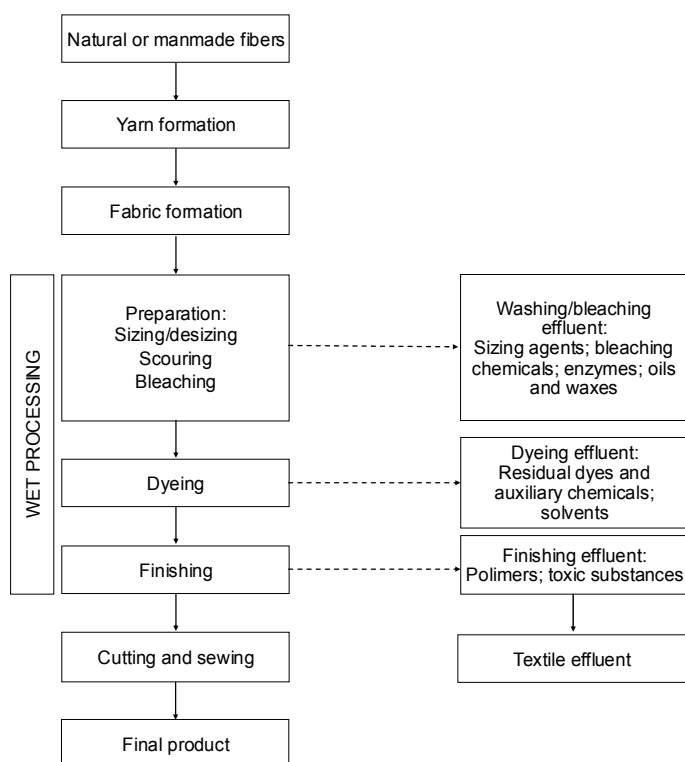


Figure 1

A simplified scheme of the textile process: from the production of the fibers to the final product. The wastewater generated during the wet processing is highlighted. Adapted from Kocabas and colleagues (2009) and Verma and colleagues (2012).

Figure 1 summarizes the main steps of a typical textile production and highlights the generation of wastewater during the wet processing.

Textile wastewater

Large amounts of high-quality water are required for wet processing of fabrics. Depending on the shade, the technique and the chemicals used in a certain textile process, the estimated consumption goes from 50 to 240 l of water per kilogram of finished textile (Blomqvist 1996, Kocabas et al. 2009) or in a city like Tirupur as much as 90 million liters of water per day (Blomqvist 1996).

The content of the wastewater can vary, but the main characteristics are: high organic content; presence of dyes, dyestuff and other toxic chemicals; inorganic compounds like sodium hydroxide, sodium hypochlorite, sodium sulfide, hydrochloric acid and sodium chloride; solvents and detergents. In addition, textile effluents often have high temperature and pH between 4 and 12 (Blomqvist 1996, Verma et al. 2012). Insoluble fibers are often present in the effluent and can cause clogging of pipes. As shown in Figure 1, wastewater streams generated in different steps of the textile process are blended to form the so-called textile wastewater. The effluent generated during the preparation steps usually consists of sizing agents such as starch, polyvinyl alcohol (PVA), carboxymethyl cellulose (CMC) and polyacrylic acid, enzymes used to remove the sizing agents, soaps and detergents used to remove wax and other impurities from the fabrics and bleaching agents. This wastewater stream is biodegradable at least to a certain extent and gives the highest contribution to the organic load of the textile wastewater. The dyeing effluent has limited biodegradability as up to 50% of the applied dye along with sodium chloride and other auxiliary chemicals are lost to the effluent (O'Neill et al. 1999). The dyes used are in fact heterogeneous preparations containing inactivated dyes and additives that enhance the binding of the dye to the fibers. The finishing effluent contains mostly low or non-biodegradable compounds such as oil repellents, flame retardants and all that makes the fabric ready to be used (Porter et al. 1972). This complexity, together with the large volumes generated, makes the treatment of textile effluents very challenging and at the same time crucial for the protection of the environment. Discharge of textile wastewater into natural water bodies would decrease the oxygen content because of the lower penetration of sunlight and, at the same time, because of the increased biological oxygen demand (BOD) that would lead to an increase in oxygen consumption. As a consequence, the life in the water body would drastically decrease. In addition, the many toxic compounds in the textile wastewater would also cause a reduction of organisms in the receiving water and in the nearby soil. It is important to notice that removing the color does not necessarily mean that the negative effects on the environment are avoided. Toxic molecules and compounds that may cause other problems such as eutrophication may not absorb visible light.

In this work both a synthetic and a real textile effluent were used. The synthetic effluent was prepared so as to mimic the real composition of textile wastewater in terms of chemical oxygen demand (COD) and salt content and for that purpose a summary of

the average composition of textile wastewater based on the literature and real data made by Jonstrup (2011) was used. The real textile wastewater was received from Ten Cate, the Netherlands, a company that produces protective textiles and other items. Table 1 compares the composition of the wastewaters used in this work and the average composition found by Jonstrup (2011).

Table 1

Composition of the wastewaters used in this work with an average from literature and real data (Jonstrup 2011).

	AVERAGE - LITERATURE	SYNTHETIC WASTEWATER - THIS STUDY	REAL WASTEWATER - THIS STUDY
Dye conc (mg/l)	700	100-1000	
COD (mg/l)	1781	608-1536	1714
BOD (mg/l)	363		
pH	10	7-8.5	10
Chloride (mg/l)	15867	182-5723	
Sulfate (mg/l)	1400		400
NH ₄ (mg/l)	17	101.2	1.7
NO ₃ (mg/l)	2		3.6
Total N (mg/l)	23		50
PO ₄ (mg/l)	17	36.5-292.1	16.8
Ca (mg/l)	43	30	50
Mg (mg/l)	4	25.5	
Na (mg/l)	2900	118-3934	
Fe	1.2	0.6	

3. Textile dyes

Dyes and pigments are everywhere around us. People learnt early in their history how to extract colors from natural resources and use them to paint everyday objects and to express their skills and spirituality.

In 1856, a young student called William Perkin synthesized by mistake the first synthetic dye, mauveine, using coal tar. He was in fact trying to synthesize quinine, a compound used to cure malaria. He soon realized that the purple solution obtained could color silk and was quick to understand the importance of his finding. That “accident” made him famous and gave a great impulse to the growth and development of textile and printing industry. In fact, the technology used up till then to dye fibers was the same as in Roman times and synthetic dyes constituted a great improvement because they are stable molecules, their binding to the fabrics was much more resistant than that of natural dyes and their colors more vivid (Holme 2006).

Synthetic dyes may be toxic to humans by causing allergies and skin- or lung sensitization. In addition, some dyes and their derivatives are mutagenic and have therefore been indexed (European Parliament 2002). However, some carcinogenic dyes may still be in use and the products dyed with them may be sold also where the compounds are banned. The main concern is for the workers, but as a consequence of inadequate disposal of wastewater and waste material there can also be consequences for living organisms that come into contact with such wastes.

Dyes are classified by their chemical type (azo, anthraquinone, indigo,...) and by their method of application (acid, direct, reactive,...). Azo dyes are the most widespread dyes and also the ones that raise the biggest concern in terms of mutagenicity and carcinogenicity (O'Neill et al. 1999). Their main feature is the presence of one or more azo bonds ($-N=N-$) linking aromatic structures. The azo bond can be cleaved chemically or biologically to release aromatic amines, which are often more toxic than the mother compound and mutagenic. Azo dyes have a more intense color than anthraquinone dyes and this advantage, together with the fact that they are relatively cheap to produce, has resulted in their dominance of the market (Koh 2011). Figure 2 shows the chemical structure of the azo dye 4-aminoazobenzene and the aromatic amines released after cleavage of the azo bond, aniline and phenylenediamine. The figure also highlights the chromophore and auxochrome of the dye. A chromophore is a conjugated chemical structure and is the part of the molecule that is responsible for its color. Other examples of chromophores are the nitro and carboxyl groups.

Auxochromes are all those groups that are attached to the chromophore and change its ability to absorb light, and thus change the color.

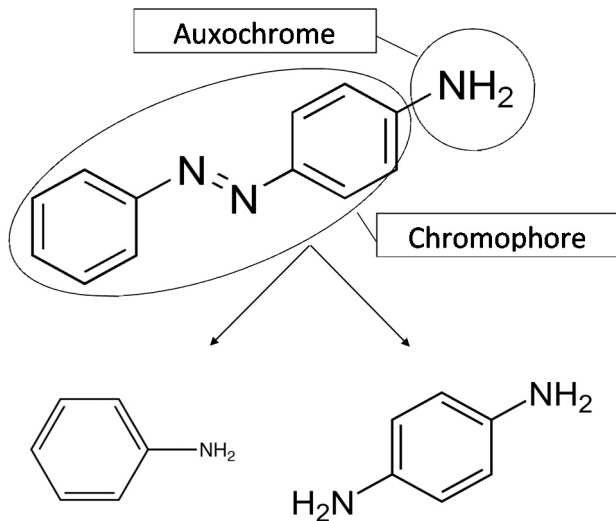


Figure 2

Chemical structure of the azo dye 4-aminoazobenzene and the aromatic amines released after cleavage of its azo bond, aniline and phenylenediamine.

Textile industries often use commercial dyestuff produced by formulations protected by trademark, which is an additional challenge for the treatment of their effluents. These formulations contain mixture of dyes, salts and other additives that facilitate and improve the binding to the fabrics. The dyes used in this work are an example of commercial preparations with an unknown chemical formula. The dyes used, called Remazol Red, Remazol Blue and Remazol Yellow, were received from Greetings Knitwear, an end user from Tirupur, India. According to the manufacturer of the dyes, Remazol dyes contain 50- to 60% dyestuff, 30- to 40% inorganic salts and up to 5% functional additives. Remazol Red contains a mixture of azo dyes; Remazol Blue contains a mixture of diazo-divinylsulfone dyestuff and formazan-vinylsulfone-copper complex dyestuff. Some azo dyes contain coordination metals in their structure, which is an additional problem for the environment and may affect wastewater treatment. It is thus essential to install appropriate wastewater treatment facilities in correspondence of textile factories so as to avoid discharging water containing azo dyes in the environment.

4. Analysis of textile wastewater

The complex nature of textile wastewater makes its analysis challenging. However, knowledge regarding the composition of the effluent is important for choosing an appropriate treatment strategy. Analysis of the effluents is essential to determine the efficiency of the treatment. Such analyses should focus on measuring the removal of organic content and nutrients, but also on the degradation of the dyes and of other toxic or recalcitrant organic compounds if present. Analysis of the dye preparations is important since they contain not only active dye molecules but also by-products. In addition, toxicity should be monitored to ensure that the treated effluent will not harm the receiving water body and surrounding ecosystems.

Conventional analyses are used to measure and evaluate the removal of the organic content, for example BOD, COD and total organic carbon (TOC). BOD is the amount of oxygen required for biological oxidation of the organic matter present in an effluent. The COD is a measure of the amount of oxygen needed to oxidize chemically the organic and inorganic compounds present in the effluent. Thus, BOD is always lower than COD and their ratio is an indication of the biodegradability of the contaminants present in the water. The TOC measures all the organic carbon present in an effluent, including the part that cannot be oxidized. In addition to the organic content, the concentration of other nutrients such as inorganic ions should be measured. Special attention should be paid to the presence of salts, in particular sodium chloride, which are used at high concentrations to improve the binding of dyes to fabric and are therefore abundant in the resulting wastewater.

A simple but reliable method to evaluate the degradation of dyes is based on their ability to absorb light. UV-visible absorbance is useful for evaluating the quality of a textile effluent and the performance of a treatment process. Dyes can absorb light in the visible range, but when they are degraded they lose this ability. Azo dyes in particular undergo cleavage of the azo bond rather easily which results in the formation of aromatic amines and at the same time the disappearance of color. These amines, although colorless, are toxic and mutagenic and it is necessary to degrade them further, possibly into inorganic compounds. Most dyes and also the aromatic amines produced as a consequence of decolorization can also absorb light in the UV range thanks to the aromatic structures. Thus, it is possible to follow the degradation of these structures by measuring the UV absorbance. Each dye has a specific absorbance spectrum, a property that is very useful for model systems in which only one dye molecule is involved. Figure 3 shows the UV-visible absorbance spectrum of the azo dye Remazol Red, one of the dyes used in the

study, with two characteristic peaks at 290 nm and 516 nm. When a specific wavelength cannot be followed, the degradation of aromatic structures can be estimated by measuring the absorbance at 254 nm. In fact, this wavelength is conventionally used to monitor the presence of aromatic compounds in water.

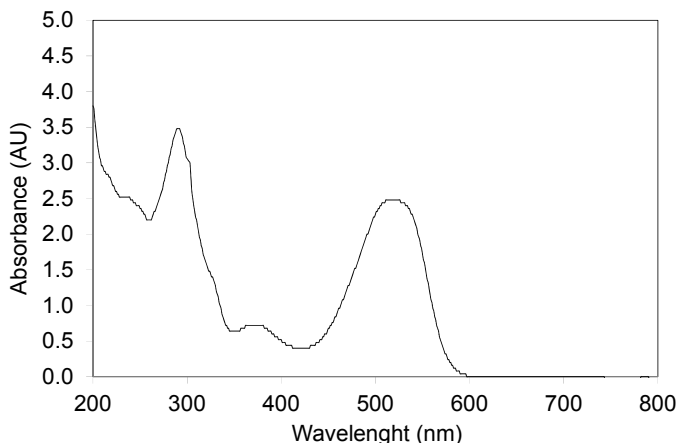


Figure 3.
UV-visible spectrum of the commercial azo dye Remazol Red.

The degradation of azo dyes and other complex organic compounds can also be monitored by using HPLC coupled with a diode array detector (DAD) followed by mass spectrometry (MS). However, textile effluents are usually composed of such a wide variety of compounds, most of them of unknown chemical structure, that a sound and detailed analysis would take considerable time and funds. In addition, exact identification of the intermediates would not be possible, especially when advanced oxidation processes are used for treatment, because of the lack of standards hence coupling of LC-MS with nuclear magnetic resonance (NMR) would be necessary (Hisaindee et al. 2013). Nevertheless, it would be of great interest to find out to what extent dyes are degraded biologically and what remains in the effluent after treatment. It would be even more interesting to match this information with data on toxicity, so that the compounds responsible for toxicity and especially mutagenicity could be identified. On the other hand, the composition of real textile effluents varies a lot from day to day depending on the chemicals used. Thus, simpler and quicker methods for the evaluation of treatment processes are required.

5. Biological treatment of textile wastewater

Biological processes are often the preferred choice for treatment of wastewater. They are considered to have low environmental impact and costs in comparison with other types of treatments, because they require only slight or no addition of chemicals and reasonable amounts of energy. They are based on the ability of microorganisms to transform the contaminants and use them as sources of energy, carbon and other minerals which are essential for their growth. Biological treatment is the most common treatment for textile wastewater nowadays (Carneiro et al. 2010). Microorganisms that can degrade azo dyes are necessary for efficient treatment of textile wastewater. These microorganisms need to have enzymes such as azoreductases and oxidases. The first enzyme is required to cleave the azo bonds and make the aromatic amines more accessible. Oxidases are fundamental to break down the previously released aromatic amines. The challenge is to find microorganisms endowed with oxidases that can break down all azo dyes and also thrive in the presence of salts and other conditions typical of textile effluents. For this reason, it is important to keep on looking for interesting microorganisms in the most diverse natural environments.

Anaerobic and aerobic processes

Aerobic and anaerobic microorganisms can both be useful for treatment of textile wastewater. Enzymes with azoreductase activity have been found in many types of aerobic and anaerobic microorganisms including bacteria, fungi and algae. The most interesting oxidases have been identified in microorganisms that live under aerobic conditions.

Anaerobic processes using consortia of microorganisms (anaerobic sludge or biofilm) as the active agents have often been used for textile wastewater treatment (Firmino et al. 2010, Spagni et al. 2012). Such processes are effective in reducing the organic content of the effluent and also in removing the color by reducing the azo bonds (**Papers II, III, and IV**). Their advantage compared to aerobic processes is that they can handle higher organic loads, they do not need aeration and they generate less sludge. In this work, the possibility of using anaerobic mixed cultures for removal of color and organic

content was tested first in batch mode with the three azo dyes Remazol Red, Remazol Yellow and Remazol Blue (**Paper II**). Figure 4 shows that the color is readily removed within three days of incubation.

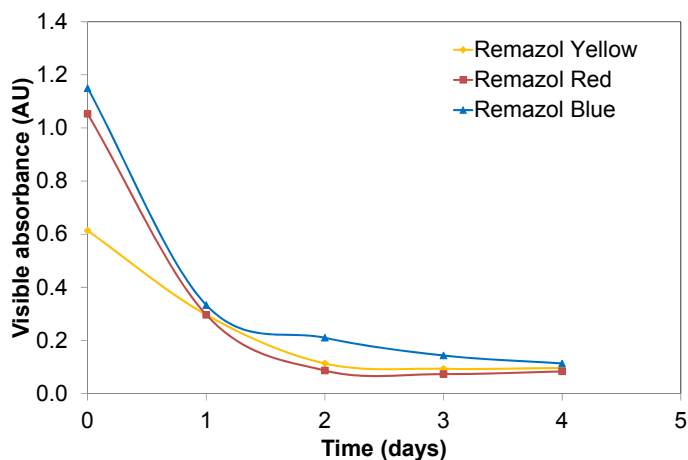


Figure 4.

Reduction of absorbance in the visible range during anaerobic batch treatment of azo dyes. The wavelengths registered were the absorbance maxima at 416 nm (Remazol Yellow), 516 nm (Remazol Red) and 603 nm (Remazol Blue). AU = absorbance units.

Many studies report that aerobic processes based on activated sludge can be used to degrade dyes further after anaerobic treatment (Kapdan and Alparslan 2005; Frijters et al. 2006, Jonstrup et al. 2011). The idea is that the aerobic microorganisms have oxidative enzymes that can break down the aromatic amines released during anaerobic color removal (Figure 2). Depending on the chemical structures of the degradation products and on the enzymes present in the system, the aerobic degradation may or may not work and in some cases toxic organic molecules remain in the effluent (Garcia-Montano et al. 2008a, Rodrigues da Silva et al. 2012). The remaining degradation products can be aromatic amines, but also carboxylic acids and other small organics (Kudlich et al. 1999, Isik 2004, Ozdemir et al. 2013). Under aerobic conditions, the aromatic amines may also undergo autoxidation and turn back into colored molecules (Kudlich et al. 1999, O'Neill et al. 2000, Rehorek and Plum 2006). The dyes used in this study could not be degraded by anaerobic-aerobic treatment. In fact, as shown in Figure 5, the reduction of absorbance in the UV range, used to evaluate the degradation of aromatic structures, was very similar in the samples containing activated sludge compared with the controls in which the anaerobically treated solutions were inoculated with autoclaved sludge or left non-inoculated. These results indicate that biological treatments may have to be complemented with physical-chemical processes to obtain effluents that are free from toxic compounds.

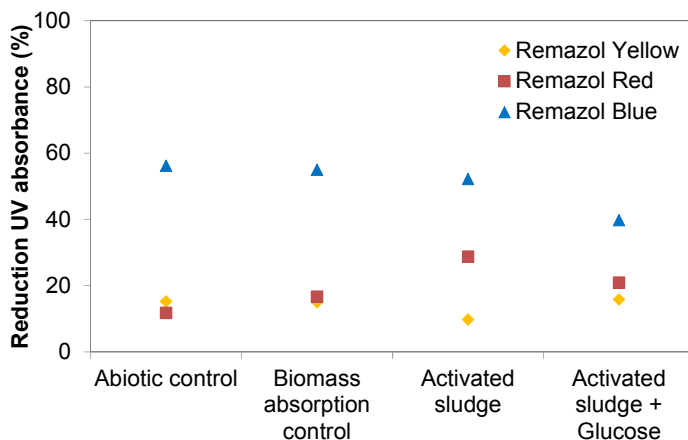


Figure 5.

Reduction of absorbance in the UV range during aerobic batch treatment of anaerobically treated azo dyes. The wavelength registered were the absorbance maxima at 264 nm (Remazol Yellow), 271 nm (Remazol Red) and 261 nm (Remazol Blue).

Pure cultures have also been used for degradation of azo dyes. Promising results have been achieved with white-rot fungi, a type of basidiomycete also known as wood-decaying fungi (Axelsson et al. 2006). These fungi produce extracellular enzymes to decompose lignin and thus find access to cellulose, which they use as a carbon source. Lignin is a complex organic molecule composed of many aromatic rings linked to one another. Azo dyes somewhat resemble the structure of lignin and researchers have suggested that lignin-degrading enzymes might also be able to degrade azo dyes. This idea proved correct, and thus both white-rot fungi and their oxidative enzymes have been used for dye degradation (Mendoza et al. 2011, Jonstrup et al. 2013). However, using pure cultures for treatment of wastewater remains a challenge. In fact, unwanted microorganisms found in water will start to grow and eventually overtake the white-rot fungi thereby destabilizing the system (Libra et al. 2003).

Biofilm process

Textile wastewater contains compounds that can be toxic to microorganisms. In addition, its composition varies a lot from day to day. For these reasons it can be advantageous to use biofilm reactors for biological treatment. Biofilms can be of several types: granules of microorganisms that have aggregated spontaneously, a layer of microorganisms attached to a static surface like a pipe, or a layer of microorganisms growing on suspended carriers (Nicolella et al. 2000). All these forms find application in water- and wastewater treatment and the most common example is trickling filters

(Nicolella et al. 2000). Biofilms provide several advantages, in particular for wastewater treatment. First, they allow biomass retention without need for sedimentation tanks, which means smaller areas and less energy required compared with conventional systems. In addition, the compounds in the water have to cross a barrier made by the biofilm itself and thus create a concentration gradient within the biofilm (Nicolella et al. 2000, Cresson et al. 2006). This gradient stimulates the growth of different microorganisms at different depths of the biofilm, depending on which molecules reach that particular depth. The innermost layer is most likely protected against toxic molecules, whereas the top-most layer is more exposed and thus colonized by less sensitive microorganisms. Overall, and also thanks to the extracellular polysaccharides produced by the microorganisms, the biofilm will be more resistant to stress than a suspended cell system (Cresson et al. 2006).

The biological studies described in **Papers III and IV** were performed in two anaerobic biofilm reactors in which Poraver carriers (porous glass beads received from Dennert Poraver GmbH, Schlüsselfeld, Germany) were used as support material for the development of a biofilm starting with an inoculum of anaerobic sludge. Figure 6 shows a schematic view of a reactor.

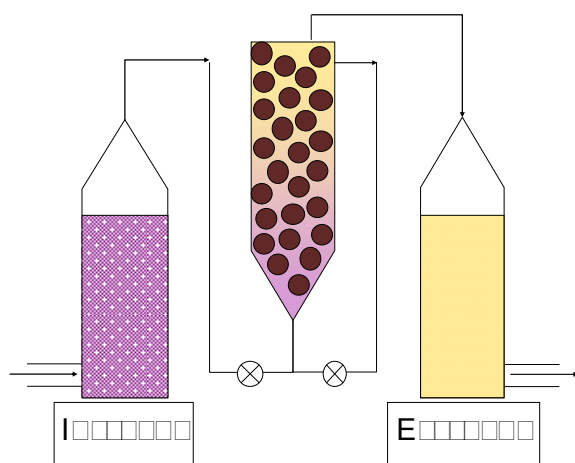


Figure 6.

Schematic view of an anaerobic biofilm reactor used in this work. The reactors were operated in continuous mode at a HRT of 2-6 days for 2 years. The feeding was first synthetic textile wastewater containing different concentrations of the azo dye Remazol Red, sodium chloride and starch. In the last 6 months the reactors were fed with real textile wastewater received from the textile factory Ten Cate, Nijverdal, the Netherlands.

The system was operated in continuous mode and fed first with a synthetic effluent containing starch as the carbon source. The robustness of the biofilm process in relation to dye concentration (Remazol Red, **Paper IV**) and salinity (sodium chloride, **Paper III**) was also studied since those are the main characteristics of real textile effluents, as

can be seen in the summary of the composition of textile wastewater in Table 1. Both sodium and chloride ions can inhibit microbial functions by increasing the osmotic pressure and thus causing cell rupture and consequent death (Vyrides and Stuckey 2009). In the last part of the work, the biofilm reactors were used for treatment of a real textile wastewater received from the company Ten Cate, the Netherlands. Figure 7 chronologically summarizes the results in terms of COD removal obtained during the different stages of the study. Each time the feeding composition was changed, the reactors were given three hydraulic retention times (HRTs) to stabilize. Then, data were collected for three HRTs (0.3 g/l - 10 g/l NaCl) in the first part of the study, but for 23 HRTs (100 mg/l - 1000 mg/l Remazol Red and real wastewater) in the rest of the study.

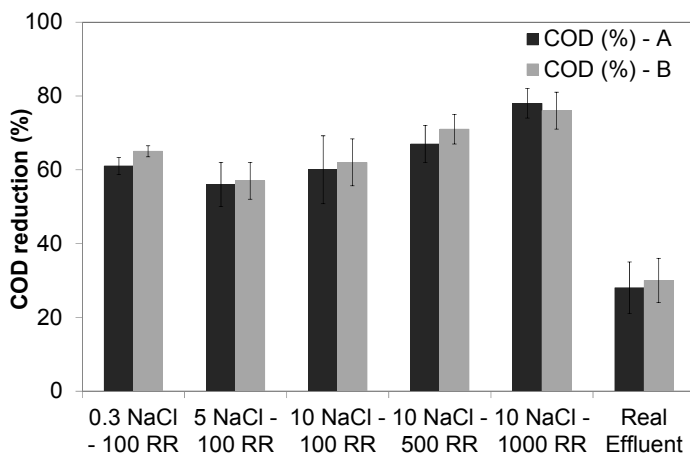


Figure 7.

COD removal during biological treatment of synthetic and real textile wastewater in the two anaerobic biofilm reactors (A and B). The different concentrations of sodium chloride and dye (Remazol Red) are indicated on the x-axis in g/l (NaCl) and mg/l (Remazol Red, RR). Values are average of measurements taken from samples collected over 3 or more HRTs. Error bars represent standard deviation.

The increasing concentration of sodium chloride had only a small influence on the COD removal. The process became more unstable, as the longer error bars show, but the removal decreased only slightly compared with the lowest sodium chloride concentration. The study continued with the evaluation of the influence of higher dye concentrations. As the dye content increased, so did the initial COD and also the COD removal. This result was rather surprising since previous studies with the same dye showed that the COD associated with the dye itself is not really removed during the biological process (**Paper II**). In addition, other works report a decrease in COD removal as the dye content increases (Farabegoli et al. 2010, Jonstrup et al. 2011). Several explanations can be found. The microbial community might have adapted to the high salinity as the reactors were run with the same feeding for several HRTs. A sodium chloride concentration of 10 g/l may inhibit microbial growth, but after 30

HRTs the microbial community might have changed, which may have resulted in improved COD removal. Similarly, adaptation to metabolizing starch as a carbon source may have occurred and caused increased starch utilization. Finally, the dyestuff Remazol Red was not pure but a mixture of azo dyes, inorganic salts and organic additives. The latter may be biodegradable, and since their concentration in the synthetic effluent increased proportionally to the concentration of the dye, the higher COD reduction may also be owed to their degradation. A solution of 100 mg/l Remazol Red has COD of 60 mg/l, which is small compared to the 600 mg/l COD associated with starch (0.465 g/l), but the contribution of Remazol Red and starch becomes comparable once the dye concentration is increased to 1000 mg/l. The COD reduction during treatment of the real textile effluent was significantly lower, but in agreement with the results obtained at the full scale plant, giving on average 30% of COD reduction (Frijters et al. 2006). The impurities in this wastewater have low biodegradability as the water contains a mixture of organic and inorganic compounds and more specifically starch, polyvinyl alcohol, carboxy-methyl cellulose, azo dyes and other types of dyes.

In addition to the COD, it is important to monitor the degradation of the aromatic compounds because the toxicity is most likely due to their presence. No UV reduction was obtained during the biological treatment, but only a minor change in the wavelength of maximum absorbance (**Paper III**). Azo dyes are rather resistant to biological degradation and the role of the biological treatment is mostly to lower the organic content of the effluent as much as possible so that the following treatment steps, required to mineralize the by-products from degraded dyes and detoxify the effluent, can be more effective.

A closer look at the biofilm and also at the suspended microorganisms was taken through scanning electron microscopy (SEM). Figure 8 shows that the surface of the carriers, after seven months of operation, is well colonized and different types of microorganisms could be seen such as bacteria and fungi. In contrast, no biofilm developed on the inner part of the carriers (**Paper III**). Part of the liquid content of the reactors was filtered through a special dark membrane, which is suitable for SEM analyses, and nets of interconnected clumps of microorganisms were observed.

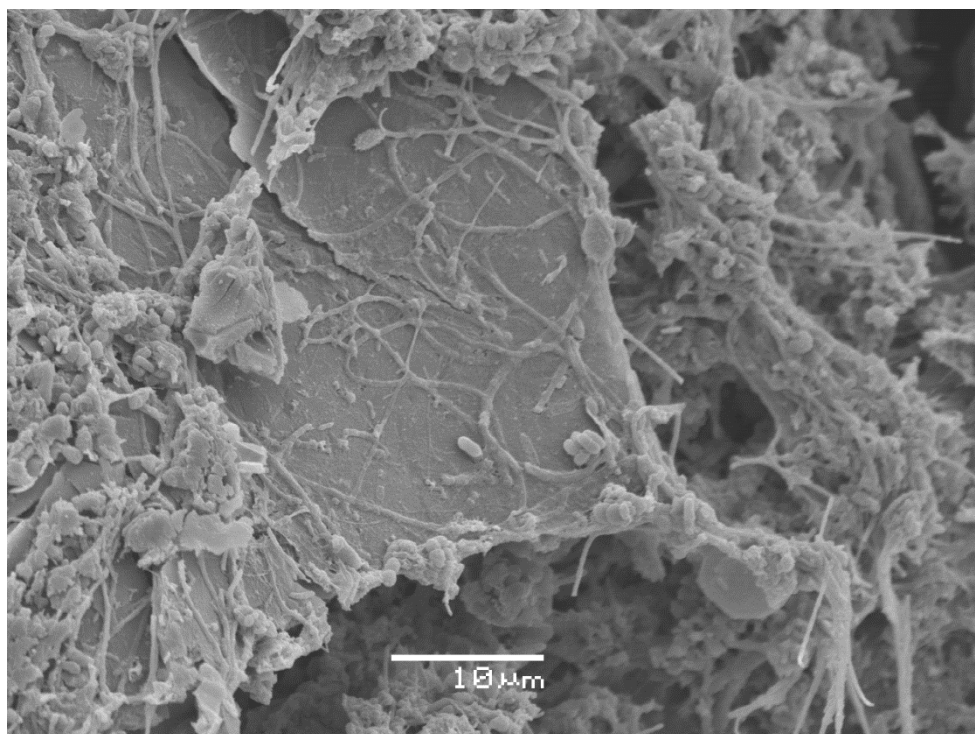


Figure 8.

SEM micrograph of the surface of a colonized carrier taken from one of the reactors used for treatment of textile wastewater. The scale bar is 10 μm.

Analysis of the microbial community

Knowledge about the structure and diversity of the microbial community of a biological process facilitates the understanding of the process itself. In wastewater treatment, this can help explaining why some compounds are degraded whereas others are not and can be used to identify operating parameters that require adjustment. Analysis of the microbial community of biological systems is not routine, but it is often used to find an explanation for process failures. In the future, knowledge about the main actors of a certain biological process should be collected before execution of the process itself, so that its design and operation can be optimized (Koch et al. 2014). Although in recent years more attention has been paid to these issues, most of the collected information concerns system for production of biogas and bioenergy (Agler et al. 2012, Koch et al. 2013) and little is known about biological processes for treatment of wastewater, in particular textile (Yang et al. 2012).

Molecular biology tools, based on DNA amplification, were used to analyze the composition and structure of the microbial community in the anaerobic biofilm reactors. It is well known today that cultivation techniques only allow isolation of a small number of the microorganisms that constitute a community (Pace 1997, Rappe and Giovannoni 2003). Studies based on DNA analyses instead can give a broader insight into the diversity of biological systems such as sludge used for wastewater treatment.

One way to classify DNA-based methods is to distinguish between PCR-based and PCR-independent methods, as in the scheme in Figure 9 (Dorigo et al. 2005, Desai et al. 2010, Nagarajan and Loh 2014). In this research project, PCR coupled with denaturing gradient gel electrophoresis (DGGE) was used to analyze the bacterial and eukaryotic community present in the anaerobic biofilm reactors used for textile wastewater treatment.

PCR-based methods require amplification of a DNA fragment, usually from molecular genetic markers and in particular 16S or 18S RNA genes, for prokaryotes and eukaryotes, respectively. The fragments can then be processed in different ways: separated through electrophoresis (DGGE), cloned and sequenced, digested with restriction enzymes and subsequently separated and sequenced (restriction fragment length polymorphism, RFLP) or directly sequenced by next-generation sequencing technologies such as pyrosequencing. All these techniques give information regarding the diversity and structure of the community and allow for partial quantification of the dominant species. More recently, real-time quantitative PCR (qPCR) and next-generation sequencing have become more popular as they overcome most of the limitations of fingerprinting and clone libraries. However, these methods are still very expensive and available only in a limited number of laboratories. The most common PCR-independent method is fluorescence in-situ hybridization (FISH) which uses fluorescent probes targeting rRNA at species, group or kingdom level. FISH is used for *in situ* monitoring and allows for both functional and phylogenetic identification (Desai et al. 2010). Another hybridization technology is DNA microarray, which is sensitive and allows quantification. DGGE was chosen for this project because it is simple to set-up and less expensive than other PCR-based methods. DGGE and the other fingerprinting techniques have a great advantage, the results are obtained quickly and in the form of images, which directly reflect the diversity of the analyzed community (Kim et al. 2013). In addition, DGGE is widely used in the water treatment field and to detect changes in the microbial diversity caused by the presence of environmental pollutants (Desai et al. 2010).

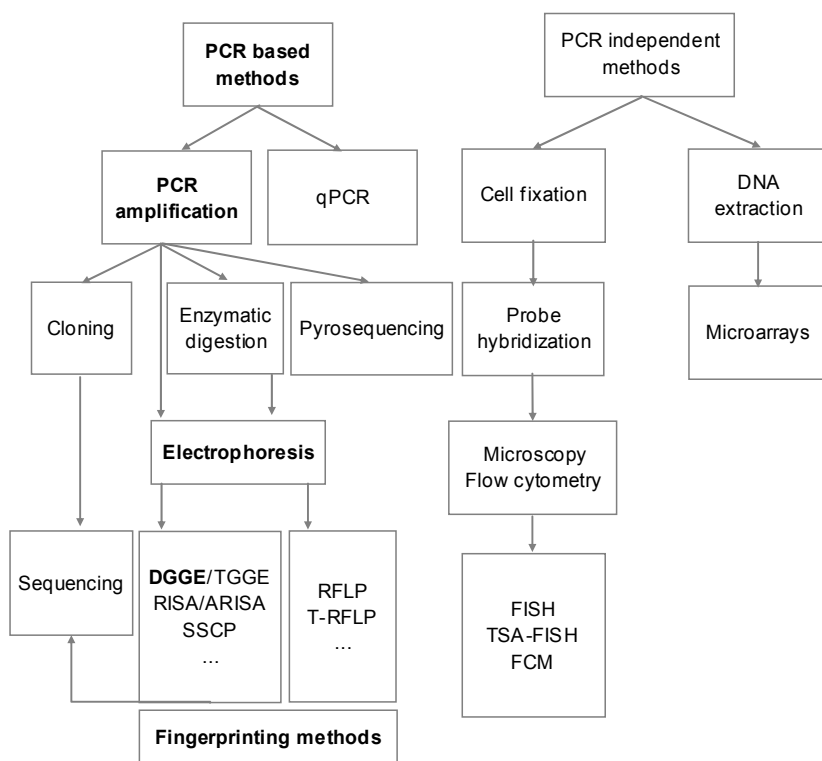


Figure 9.

Schematic view of various DNA-based techniques that can be used today to study microbial communities. Adapted from Dorigo and colleagues (2005) and Desai and colleagues (2010).

In **Paper III**, PCR followed by DGGE was used to analyze the composition of the microbial community in the biofilm and of the suspended microorganisms exposed to synthetic textile wastewater. The goal of using this fingerprinting method was to obtain information about the structural diversity and complexity of the prokaryotic and eukaryotic communities involved in the treatment and to identify the most abundant members. In the last few years, this method has been used in several studies to investigate the diversity existing in biological (textile) wastewater treatment plants (Bellucci and Curtis 2011, Yang et al. 2012) and in lab-scale reactors for azo dyes degradation (Tan et al. 2010, Cui et al. 2012, Forss et al. 2013). One or more hypervariable regions (V) of the genes coding for ribosomal RNA, 16S rDNA for prokaryotes and 18S rDNA for eukaryotes, are amplified through PCR by special primers attached to a guanine-cytosine (GC)-clamp on one extremity (Muyzer and Smalla 1998, Yu and Morrison 2004). The obtained fragments have the same length but different sequence and can be separated through DGGE. The DGGE gel is a polyacrylamide gel containing a linear gradient of increasing concentrations of urea and formamide. The fragments, because of their different sequences, have different melting points and thus migrate to different lengths on the gel (the sooner the double strand

melts, the shorter it migrate). Thanks to the GC-clamp the two DNA filaments do not separate completely and can be extracted from the gel and identified through sequencing. When looking at the gel, several bands can be seen in each line, each representing a different operational taxonomic unit (Muyzer and Smalla 1998). In our study, primers that amplify the V3-V5 regions of the bacterial 16S rDNA were used as well as primers that amplify the ITS2 region of the eukaryotic 18S rDNA. Up to 17 bands were found for the bacterial DNA and up to 13 bands for the eukaryotic DNA.

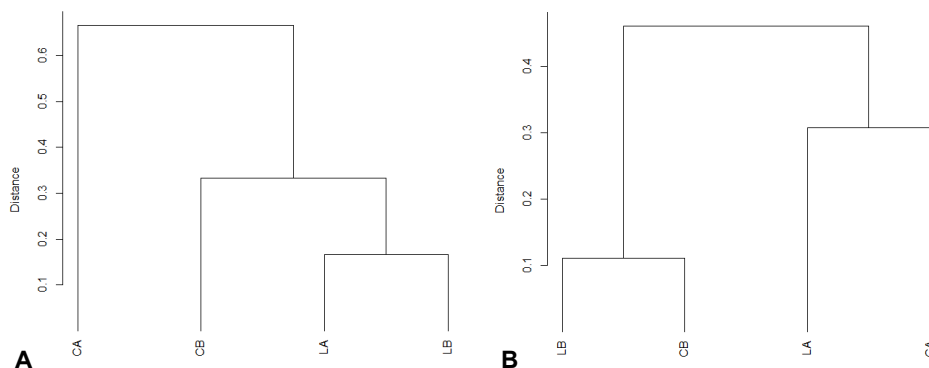


Figure 10.

Dendrograms showing hierarchical clustering based on UPGMA algorithm and Jaccard distance estimation using band patterns from DGGE of 16S rDNA of the bacterial fraction (a) and ITS2 of the eukaryotic fraction (b) of the microbial community of biofilm and suspended biomass of the anaerobic reactors.

DGGE fingerprints were used to calculate the Shannon-Wiener diversity index (H) (Gafan et al. 2005) and also for hierarchical clustering using unweighted pair group comparison method with arithmetic means (UPGMA) algorithm and Jaccard distance estimation (Vasileiadis et al. 2012). The analysis shows that both the biofilm and the suspended biomass were composed of bacteria and eukaryotes (then found to be fungi and algae). A higher bacterial diversity and richness (number of bands) was found in comparison with the eukaryotic diversity. In a study by Yang and colleagues (2012) on the bacterial and fungal community of a full-scale plant for treatment of wastewater generated in a dyeing process, predominance of bacteria over fungi was also found. In our study, the diversity and richness of the biofilm community developed on the carriers was higher than that of the suspended biomass for the eukaryotes, but lower for the bacteria. High similarity was found between the communities that developed on the biofilm and suspended biomass of both reactors, with the only exception being the bacterial community on the biofilm of reactor A, which showed some differences (Figure 10). Feeding the reactors with an azo dye and also a rather high concentration of sodium chloride certainly applied selective pressure and pushed the composition within the communities in the same direction.

Table 2.
Sequence identity of the excised eukaryotic DGGE bands.

DGGE Band	Closest cultured relative in GenBank	Phylum	GenBank accession number
	(<i>Accession number</i>) (% Identity)		
2	<i>Rhodotorula mucilaginosa</i> strain Y8-2 (KJ183057.1) (99%)	Basidiomycota	KM577621
3	<i>Rhodotorula mucilaginosa</i> strain Y8-2 (KJ183057.1) (99%)	Basidiomycota	KM577622
5	<i>Chlorella sorokiniana</i> strain UTEX 2714 (LK021940.1) (99%)	Chlorophyta	KM577623
6	<i>Chlorella vulgaris</i> strain UTEX2714 (KJ676110.1) (98%)	Chlorophyta	KM577624
7	<i>Chlorella sorokiniana</i> strain UTEX 2714 (LK021940.1) (98%)	Chlorophyta	KM577625
10	<i>Penicillium roqueforti</i> strain 94 (KJ608120.1) (96%)	Ascomycota	KM595066

Previous studies have focused on identifying the main bacterial strains involved in dye degradation or textile wastewater treatment, whereas eukaryotes have been somewhat neglected. In our study, representative bands of eukaryotic DNA were cut from the DGGE gel and sequenced. Table 2 summarizes the results of the sequencing: of the six microorganisms identified, three were algae belonging to the genus *Chlorella* and three fungi belonging to either *Penicillium* or *Rhodotorula*. The most interesting outcome of the analysis was the detection of algae for the first time in an anaerobic process for treatment of textile wastewater. Algal species belonging to the genus *Chlorella* are often found in soil and water. They are unicellular photosynthetic microorganisms and some members can grow in absence of light by using organic molecules as carbon source (Yoshida et al. 2006). The reactors used in the study were not strictly anaerobic and were almost completely covered with dark isolating material. Such conditions allow mostly heterotrophic growth, but the possibility of photosynthesis cannot be excluded because some light could enter the reactors through the top-most and bottom-most parts. The presence of *Rhodotorula*, a fungus previously isolated from textile wastewater (Ertugrul et al. 2009) and also employed for decolorization of azo dyes (Onat et al. 2010) is also interesting. It is likely that it played a role in the decolorization of the dyes. The fungus similar to *Penicillium roqueforti* is known for growing on starch (Bergenholtz and Nielsen 2002), which was used in our process as carbon source, but no connection with wastewater or degradation of pollutants was found in the literature.

Algae and cyanobacteria

The search for microorganisms bearing novel enzymes that can degrade pollutants is a fascinating and never-ending activity. Diversity is the key factor, thus the most odd and unusual sources should be considered. With this in mind, in **Paper V** samples of freshwater from an alkaline Ethiopian lake, a Colombian lake, some Swedish ponds and lichens, and from the outlet water of the previously discussed anaerobic biofilm reactors, were screened for the presence of unicellular algae and cyanobacteria.

Microalgae, a class of microorganisms that includes both microscopic eukaryotic algae and cyanobacteria, are interesting because of their unique biochemistry. They are photosynthetic microorganisms and, in addition, they use phosphorus and nitrogen for their growth, a characteristic that is now broadly investigated as it would allow recovery of both minerals from wastewater instead of the conventional transformation into phosphate sludge and nitrogen gas, respectively (Christenson and Sims 2011). Perhaps even more interesting is their ability to produce lipids that can be used for production of biodiesel and other products such as bioplastics (Christenson and Sims 2011).

Less attention has been given to the ability of microalgae to degrade organic compounds, although they certainly play a role in wastewater treatment and bioremediation of polluted sites thanks also to their ability to produce oxygen and thus enhance the activity of aerobic microorganisms (Semple et al. 1999, Muñoz and Guieysse 2006). Some researchers have investigated the potential of microalgae for azo dyes degradation and found that some strains can cleave azo bonds (Saha et al. 2010, Priya et al. 2011, Liu et al. 2013) and some may even be able to biotransform aromatic by-products of dye decolorization (Cerniglia et al. 1981). More research focused on the use of microalgae as adsorbent for azo dyes (Mohan et al. 2008, Ergene et al. 2009, Lim et al. 2010), mostly because no carbon source is required for their mass cultivation which is therefore cheaper than cultivation of other microorganisms (Solis et al. 2012).

Several algae and cyanobacteria were isolated and 14 of them were screened for degradation of the azo dye 4-aminoazobenzene (Figure 2). The dye had a negative influence on the growth of all the isolates and six of them did not grow at all. Nevertheless, eight isolates resulted in a color reduction of 10-82 %, as shown in Table 3. Growth inhibition has been previously reported (Parikh and Madamwar 2005) and can be caused by the lower light transmission due to the color, but also by eventual toxicity of the dye towards the tested strains. HPLC analysis also indicated that the azo bond in the 4-aminoazobenzene molecules had been cleaved as a peak for aniline was detected after incubation.

Table 3.

Microalgae screened for removal of 4-aminoazobenzene. Chlorophyll *a* is the difference between initial and final chlorophyll *a* content; each isolate was exposed to the dye for 14 days. L1-L3: Cyanobacteria isolated from lichens. P1-P6: cyanobacteria isolated from ponds in Lund. A1-A5: algae isolated from (1-2) the effluent of the anaerobic biofilm reactors, (3) a Colombian lake and (4-5) an Ethiopian alkalyne lake.

Isolate name	Preliminary identification	Chlorophyll <i>a</i> content in dye treated samples (mg/l \pm SD)	Chlorophyll <i>a</i> content in biotic controls (mg/l \pm SD)	Dye removal (%)
L1	<i>Scytonema</i> sp.	ND	2.7 \pm 0.4	5 \pm 1
L2	<i>Chroococcidiopsis</i> sp.	ND	1.8 \pm 0.4	7 \pm 2
L3.	<i>Aphanocapsa</i> sp.	ND	0.2	8 \pm 2
P1	<i>Nostock</i> sp.	0.5 \pm 0.2	2.1 \pm 0.2	12 \pm 3
P2	<i>Nostock</i> sp.	1.2 \pm 0.4	2.2 \pm 0.3	41 \pm 6
P3	<i>Anabaena</i> sp.	0.7 \pm 0.2	1.5 \pm 0.3	9 \pm 2
P4	<i>Synechococcus</i> sp.	ND	1.5 \pm 0.5	5 \pm 2
P5	<i>Phormidium</i> sp.	ND	2.4 \pm 0.3	51 \pm 2
P6	LPP-group A	0.5 \pm 0.4	1.9 \pm 0.4	37 \pm 5
A1	<i>Chlorella</i> sp.	2.7 \pm 0.4	3.8 \pm 0.2	70 \pm 4
A2	<i>Scenedesmus</i> sp.	1.6 \pm 0.3	3 \pm 0.2	82 \pm 3
A3	<i>Stichococcus</i> sp.	2 \pm 0.5	3.5 \pm 0.5	40 \pm 3
A4	<i>Ankistrodesmus</i> sp.	1.8 \pm 0.3	4.1 \pm 0.4	55 \pm 7
A5	Unknown	ND	4 \pm 0.5	8 \pm 1

ND = the final chlorophyll *a* content was equal to or lower than the initial value.

SD = standard deviation

The algal isolates A2 and A4 were further tested for degradation of the azo dye methyl red. Both strains could grow in presence of 5 mg/l of the dye although the chlorophyll *a* content after 14 days of incubation was 30% and 50% lower than when the A2 and A4, respectively, were cultivated in absence of the methyl red. Figure 11 shows that both strains achieved high removal of methyl red, even when cultivated in absence of nitrogen and thus under non-growing conditions.

The results of both degradation tests indicate that some isolates possess powerful and stable azoreductase activities, which function even when the cells are not growing. Such characteristics are promising and encourage further studies on the isolates. It would be interesting to explore the substrate specificity of these enzymes and test the isolates for degradation of other pollutants. In fact, stable and powerful enzymes are very useful for biotechnological applications. For example, these isolates could be cultivated under optimal conditions and then put in contact with the contaminant to be degraded. The degradation would thus take place without the necessity to supplement additional nutrients and the eventual toxicity of the contaminant would not affect the growth of the microorganisms.

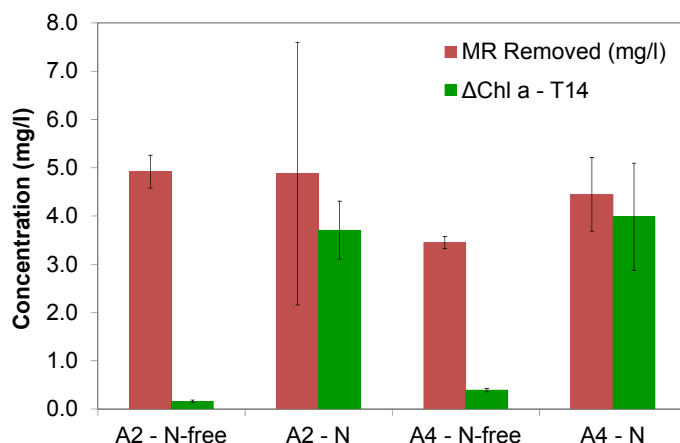


Figure 11.

Removal of methyl red (mg/l) and growth of the algal isolates A2 and A4 (change in chlorophyll *a* content, mg/l) after incubation for 14 days. MR = methyl red; N-free = medium without nitrogen; N = medium with nitrogen. Values are average of 3 independent measurements. Error bars represent standard deviation.

Another interesting application of algae is their co-cultivation with aerobic microorganisms. Algae can in fact provide the oxygen required for the growth of the bacteria and remove the costs for aeration. Similar systems where algae are used as source of oxygen for bacteria with good degradation capacity (Borde et al. 2003, Munoz et al. 2003) or for activated sludge for municipal wastewater treatment (Su et al. 2011, Posadas et al. 2013) have been studied. The results of our study suggest that even the algae can be exploited for their degradation capacity. In particular, the algae can cleave the azo bonds and the bacteria or fungi could degrade the released aromatic amines, in a similar way to the more commonly used anaerobic-aerobic azo dye degradation process (Frijters et al. 2006, Jonstrup et al. 2011).

6. Physical-chemical processes for treatment of textile wastewater

Biological treatment alone can remove color and reduce the organic content of textile effluents, but complete degradation and mineralization of the dyes is unlikely, as shown by the previous results and confirmed by previous studies (Isik and Sponza 2007, Murali et al. 2013). Two strategies can be followed to obtain an effluent that will not harm the receiving bodies. The first possibility is the addition of a pre-treatment step that transforms the recalcitrant organic compounds into biodegradable molecules, so that the biological process can transform them into inorganic compounds (Guieysse and Norvill 2014). The second possibility is the addition of a post-treatment that can mineralize the compounds remaining after biological treatment. In the last few decades, advanced oxidation processes (AOPs) have been extensively studied for their ability to degrade all sorts of organic compounds, especially aromatics. These physical-chemical processes have one main disadvantage: high energy and chemicals requirements compared with biological processes. To overcome this problem, they can be used in combination with biological processes, as both pre- and post-treatment, which was the topic of **Papers II, III and IV**.

Overview of AOPs

Advanced oxidation processes are based on the use of *in situ* generated hydroxyl radicals ($\text{HO}\cdot$), a much more reactive molecule compared with the oxidants conventionally used in water and wastewater treatment, e.g. chlorine and hydrogen peroxide (von Sonntag 2008). Hydroxyl radicals can be generated in several ways and AOPs can be classified according to the mechanism involved in the generation of the radicals which can be chemical, photochemical, electrochemical and sonochemical (Table 4) (Hai et al. 2007). Ozonation is a special case, although there is no added catalyst involved and therefore it is not classified as AOP, hydroxyl radicals are generated as a consequence of the decomposition of ozone in water (Staehelin and Hoigne 1985). Hydroxyl radicals are the strongest oxidants in water (von Gunten 2007) and in the presence of organic molecules, they behave like electrophiles and readily add to unsaturated bonds (Buxton et al. 1988). Although they oxidize preferentially aromatics, hydroxyl radicals have very

low selectivity; therefore they find broad application in water and wastewater treatment (Buxton et al. 1988).

Many AOPs have been tested for their ability to degrade pollutants that are present in textile wastewater both as stand-alone treatment and in combination with biological processes. Table 4 presents a summary of the most commonly used AOPs and those evaluated in this study are indicated in bold and further explained in the next sections “Photo-Fenton oxidation” and “Ozonation”.

Table 4.

AOPs used for treatment of textile wastewater both alone and in combination with biological processes. In bold the processes investigated in this research project.

Ozonation (O₃)			Paper IV (Constapel et al. 2009, Guelli Ulson de Souza et al. 2010, Lorito et al. 2012, Somensi et al. 2010)
AOPs	Chemical	Fe²⁺/H₂O₂	Paper II (Liu et al. 2007, Bae et al. 2015)
		O ₃ /H ₂ O ₂	(Tosik and Wiktorowski 2001, Bauman et al. 2011)
	Photochemical	H ₂ O ₂ /UV	(Nagel-Hassemer et al. 2012)
		Fe²⁺/H₂O₂/UV	Paper I, II, III (Liu et al. 2007, Blanco et al. 2014)
		O ₃ /UV	(Zhao et al. 2011)
		TiO ₂ /UV	(Kositzki et al. 2004, Prieto et al. 2005, Jonstrup et al. 2010)
	Electrochemical	Anodic oxidation	(Cruz-Gonzalez et al. 2012, Yu et al. 2013, Shwe et al. 2014)
		Electro-Fenton	
	Sonochemical		(Ashwin et al. 2011, Pang and Abdullah 2013)

Photo-Fenton oxidation

Fenton reaction refers to a reaction in which hydrogen peroxide oxidizes transition metal ions in their low oxidation state (von Sonntag 2008) and it got its name from H.J.H. Fenton who first extensively described it in 1894. According to von Sonntag (2008), the reaction had already been discovered by Schönbein in 1857. The Fenton reaction is a phenomenon that can be observed in nature when ferrous ions are oxidized by by-products of aerobic respiration such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (Imlay et al. 1988, Lemire et al. 2013). It was Brown and colleagues (1964) who reported the application of Fenton reaction for the first time for degradation of organic pollutants.

The classic homogeneous Fenton reaction starts when ferrous ions and hydrogen peroxide are in solution together, at low pH. The ferrous ions catalyze the decomposition of hydrogen peroxide which results in the production of hydroxyl radicals and hydroxyl ions, while the iron is oxidized to ferric ions (Equation 1).



A chain of free radicals reactions take place whereby various forms of oxygen radicals are generated and the iron is reduced back to ferrous ions (Pignatello et al. 1999). Addition of UV to near-visible light (< 380 nm) increases the production of hydroxyl radicals by direct photolysis of hydrogen peroxide (Equation 2) or by reducing ferric ions to ferrous ions (Equations 3 and 4), which will then be ready to catalyze the decomposition of other hydrogen peroxide molecules (Pignatello et al. 1999). This photo-reaction is usually called photo-Fenton.

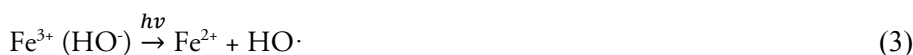
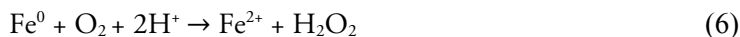


Photo-Fenton oxidation has been tested for wastewater treatment and several studies report that it can extensively mineralize organic pollutants (Jimenez et al. 2011). In this work the photo-Fenton reaction was used for degradation of the azo dyes Remazol Red and Remazol Blue as stand-alone treatment (**Paper I**) and in combination with a biological process (**Papers II and III**).

In **Paper I**, homogeneous photo-Fenton reaction, in which Fe(II) is used as catalyst, was compared with heterogeneous photo-Fenton reaction, in which Fe(0) is used as catalyst. In fact, a usual concern regarding large-scale application of the photo-Fenton oxidation is the fact that iron salt is added to the wastewater. The reaction is performed at low pH, 2-3, which implies that the pH of the water has to be adjusted before addition of iron and again after the reaction is completed. Iron ions are not soluble at neutral pH; therefore a precipitate, often called iron sludge, is formed as soon as the

pH is neutralized. This iron precipitate or sludge can be seen as a secondary pollutant generated during the wastewater treatment. To overcome this problem, the amount of iron used in the photo-Fenton reaction should be minimized. An alternative is to use insoluble iron instead and recover it at the end of the treatment. For this reason the potential of Fe(0) as catalyst of the photo-Fenton reaction was investigated. Fe(0) can release ferrous ions when exposed to hydrogen peroxide (Equation 5) (Bergendahl and Thies 2004) or oxygen (Equation 6) (Lee et al. 2007).



Similar results were obtained by using both reactions for treatment of a solution of Remazol Red in water. However, homogeneous photo-Fenton oxidation had a higher reaction rate than the heterogeneous reaction (Figure 12) and could reduce UV absorbance and COD to a higher extent when applied to solutions of the dye in the presence of sodium chloride. The reason for the lower reaction rate is that first ferrous ions have to leach from the surface of the Fe(0) and only after that can the Fenton reaction start. The leaching of ferrous ions (Equations 5 and 6) is fast, but their concentration remains rather stable in the solution, in contrast to what occurs in the homogeneous photo-Fenton where the concentration of ferrous ions is highest at the beginning. As a consequence, the decomposition of hydrogen peroxide and the generation of hydroxyl radicals is faster in the homogeneous photo-Fenton than in the heterogeneous reaction (Lucking et al. 1998, Papíć 2009).

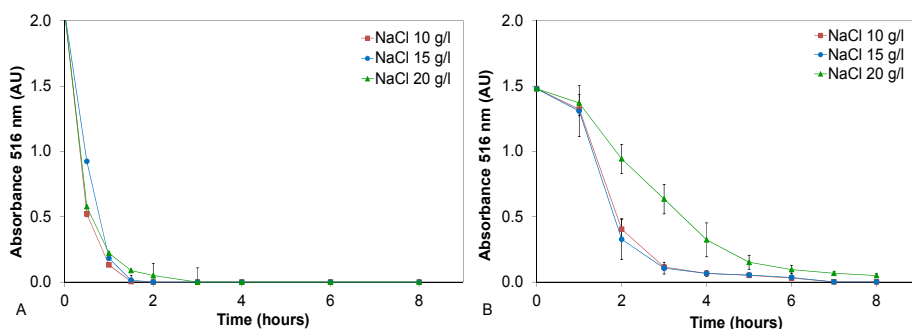


Figure 12.

Reduction of absorbance at 516 nm during (a) homogeneous and (b) heterogeneous photo-Fenton oxidation of solutions of Remazol Red (100 mg/l) and sodium chloride (10-20 mg/l). Initial conditions: 0.25 mM Fe^{2+} , 3 mM H_2O_2 , pH 3. Values are average of 3 independent measurements. Error bars represent standard deviation.

Similar amounts of iron sludge were produced during homogeneous and heterogeneous photo-Fenton oxidation, 0.42 g/l, which are lower than what would be obtained through coagulation with lime and/or iron sulfate, from 0.6-1.4 mg/l (Georgiou et al. 2003). There were two advantages with the heterogeneous reaction. First, the lower

concentration of iron ions left in the treated solution: 2.9 mg/l in contrast to 5.8 mg/l found in the solution treated with the homogeneous process. In addition, the catalyst remaining in the treated water could be reused for other batches of photo-Fenton. The catalyst was reused 20 times and a reduction of UV and visible absorbance higher than 85% was achieved. The reduction of COD though was higher than 80% only in the first four batches and then decreased to 60% or less.

This study was also carried out with the aim of assessing the interference of sodium chloride on the photo-Fenton reaction. Sodium chloride is abundant in textile wastewater because it is used to improve the binding of the dyes to the fibers, but the chloride ion, like other anions, can interfere with photo-Fenton oxidation. Anions like chloride and phosphate can interact with ferric and ferrous ions and decrease their availability (Lu et al. 1997). Chloride ions are also radical scavengers (Kiwi et al. 2000, Orozco et al. 2008). Figure 13 shows that the negative effect of sodium chloride can be clearly seen by monitoring the reduction of UV absorbance. Both reaction rate and extent of degradation were affected by the presence of chloride ions. The heterogeneous photo-Fenton oxidation resulted in a more negative outcome, which may be caused by the fact that lower concentration of ferrous ions and thus hydroxyl radicals are present in the solution (Tang and Chen 1996).

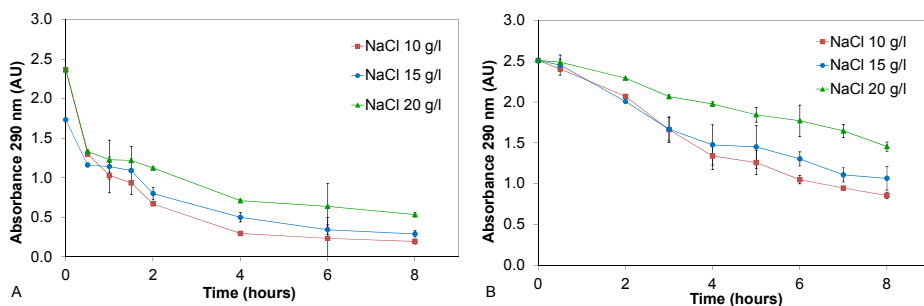


Figure 13. Reduction of absorbance at 290 nm during (a) homogeneous and (b) heterogeneous photo-Fenton oxidation of solutions of Remazol (Red 100 mg/l) and sodium chloride (10-20 mg/l). Initial conditions: 0.25 mM Fe^{2+} , 3 mM H_2O_2 , pH 3. Values are average of 3 independent measurements. Error bars represent standard deviation.

Increasing the initial concentration of Fenton reagents resulted in improved reduction of COD and UV absorbance. Nevertheless, the COD reduction was rather low, 11-13%, when a solution was treated which contained Remazol Red and starch and had initial COD of 3300 mg/l. Starch is a non-aromatic biopolymer rich in hydroxyl groups and is in fact a hydroxyl radical scavenger (Yang et al. 2010). Thus, much higher initial concentrations of hydrogen peroxide and iron are needed to degrade aromatics in presence of starch.

To summarize, photo-Fenton oxidation showed promising results for degradation of azo dyes. However, some issues need to be addressed: the low pH and thus the generation of iron sludge; the scavenging effect of anions and organic matter like starch; the light, which is important to improve the reaction efficiency, but is a cost and also makes this technology less environment-friendly. Solar-Fenton, which uses sunlight instead of artificial light, should be preferred in those areas characterized by high sunshine levels.

Ozonation

Ozone is used throughout the world for disinfection and oxidation of water (von Gunten 2003). Although ozonation is not considered an AOP, it has the unique feature of decomposing into hydroxyl radicals (Equations 7 and 8), which are the main oxidants in AOPs and the strongest oxidants in water (von Gunten 2003). Hydroxyl radicals are an added value as they are non-selective oxidants whereas ozone has high selectivity towards free amines and benzene derivatives with electron-donating substituents (von Gunten 2003, Nöthe et al. 2009).



Ozone decomposes in the presence of hydroxyl ions, and thus the reaction can be accelerated by increasing the pH or adding hydrogen peroxide. Ozone combined with hydrogen peroxide, as well as UV light or granulated activated carbon, is considered an AOP (Sonntag and Gunten 2012). Ozonation can potentially degrade all organic pollutants, but the matrix plays a very important role, as it does in photo-Fenton oxidation. In fact, dissolved organic carbon and ions such as chloride and bicarbonate can scavenge most hydroxyl radicals and compete with the target organic pollutants (Nöthe et al. 2009). The main advantage of ozonation compared with photo-Fenton oxidation is that no secondary waste is generated; the only products of the treatment are water and oxygen. In addition, it can easily be implemented in existing plants and its operation is automated already at lab and pilot scale.

When it comes to textile wastewater, researchers have highlighted that ozonation can efficiently remove color, but little success has been shown with regard to the reduction of COD or organic content in general (Radetski et al. 2002, Khadhraoui et al. 2009, Tabrizi et al. 2011, 2013). Thus, many studies have focused on using ozonation as pre-treatment before a biological process.

An additional challenge with ozonation is that the generation of ozone requires considerable amount of energy and has to be done *in situ* using liquid or compressed

oxygen, which has to be transported to the plant and stored there, or by separating oxygen from air.

How do we combine physical-chemical and biological processes

Both ozonation and photo-Fenton oxidation have great potential when it comes to mineralization of organic compounds. However, they also have a common obstacle: the water matrix. Dissolved organic matter and ions compete with target compounds for the oxidants and scavenge most of the radicals and this leads to higher reagents consumption and higher costs. Thus, to improve the efficiency of these treatment processes, the negative effect of the matrix needs to be reduced, which can be done by using biological processes. In **Paper II** two strategies were tested: first photo-Fenton oxidation was used to improve the biodegradability of an azo dye solution, which was then treated with an aerobic biological process. Then, an anaerobic batch process was used to remove the biodegradable organic content and the obtained effluent was further treated through photo-Fenton. This last approach was further studied in **Papers III and IV**, where an anaerobic biofilm process was used as first treatment step followed by ozonation or photo-Fenton oxidation.

The first option, photo-Fenton oxidation followed by biological degradation, was applied to a solution of Remazol Red with COD of 60 mg/l. The photo-oxidized solution was used as such for biological treatment (batch activated sludge), without addition of minerals or carbon source. The amount of reagent required for the photo-Fenton oxidation (0.25 mM Fe^{2+} and 1 mM H_2O_2) was optimized experimentally so that only partial reduction of COD and UV absorbance would be achieved and no hydrogen peroxide would be left in solution. The results, in Figure 14a, shows that an overall 82% of reduction of UV absorbance and 85% reduction of COD were achieved with this combined treatment. Use of AOPs to enhance the biodegradability of wastewater is a promising approach when compounds that are toxic and can inhibit biological processes are present; strategies and challenges in combining AOPs and biological processes have recently been reviewed by Guieysse and Norvill (2014).

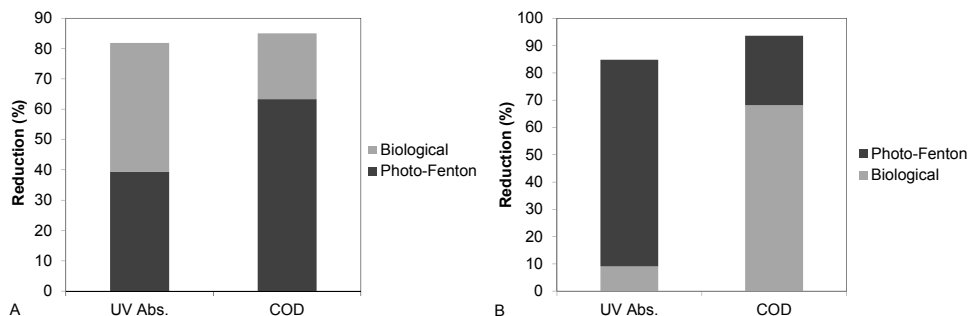


Figure 14.

Reduction of COD and UV absorbance during (a) photo-Fenton oxidation followed by biological treatment and (b) biological treatment followed by photo-Fenton oxidation, of a solution of Remazol Red 100 mg/l. The initial COD was *a.* 60 mg/l and *b.* 220 mg/l. The initial UV absorbance was 3.3 absorbance units.

For the second treatment strategy, a solution of Remazol Red was prepared in an anaerobic mineral medium containing glucose as carbon source. The COD was 220 mg/l. The biologically treated water (batch anaerobic treatment) was photo-oxidized (2 mM Fe^{2+} and 1 mM H_2O_2) and an overall 85% reduction of UV absorbance and 94% reduction of COD obtained (Figure 14b). Both treatments were successful and the set-up in which photo-Fenton was used as first step had a lower consumption of reagents, however, this reaction was performed on a pure solution of Remazol Red. But when photo-Fenton was used as post-treatment, the reaction was performed on anaerobically treated water containing the by-products of the dye but also salts and glucose, which were added to sustain the previous biological treatment. Thus, the increased consumption of reagents was caused by a more complex water composition. In fact, the matrix is what determines the reagent requirements, as discussed in the sections “Photo-Fenton oxidation” and “Ozonation”. If the same solution were used in both set-ups, higher initial Fenton reagents concentration would be necessary to obtain the same removal of COD and UV absorbance in the first set-up, where photo-Fenton is used as first treatment step. Hence, biological treatment followed by photo-Fenton, was performed in the subsequent studies.

In **Paper III** the treatment process was scaled up and two anaerobic biofilm reactors were used for the biological treatment of a synthetic textile effluent composed of Remazol Red 100 mg/l, starch 0.47 g/l, mineral medium and sodium chloride, 10-20 g/l. The effluent was further treated by photo-Fenton oxidation. High reduction of UV absorbance and COD was obtained under all tested conditions; the final COD was in fact below 18 mg/l. However, clear differences were observed in the rate of removal; in particular, the higher the sodium chloride concentration, the slower was the reduction of UV absorbance and COD (Figure 15).

In a work by Bacardit and colleagues. (2007), a similar effect of sodium chloride on the reaction rate of the photo-Fenton oxidation was observed. They were investigating its

performance for treatment of industrial wastewater containing between 3 and 50 g/l of sodium chloride. The authors also found that the removal of organic compounds (measured as TOC in their study) was the same no matter which sodium chloride concentration was in the system. It may be that the chloride ions interfere with the hydroxyl radicals by reducing their production rate, but not the total yield. Chloride ions can quench hydroxyl radicals and so decrease the decomposition rate of the available hydrogen peroxide molecules. In addition, chloride ions can interact with ferric and ferrous ions reducing their reaction rate with hydrogen peroxide (Maciel et al. 2004, Bacardit et al. 2007). Maciel and colleagues (2004) show in their work that sodium chloride concentrations up to 10 g/l do not significantly affect the removal of organic carbon, thanks also to the UV light which can break iron hydroxyl and iron complexes with organic compounds.

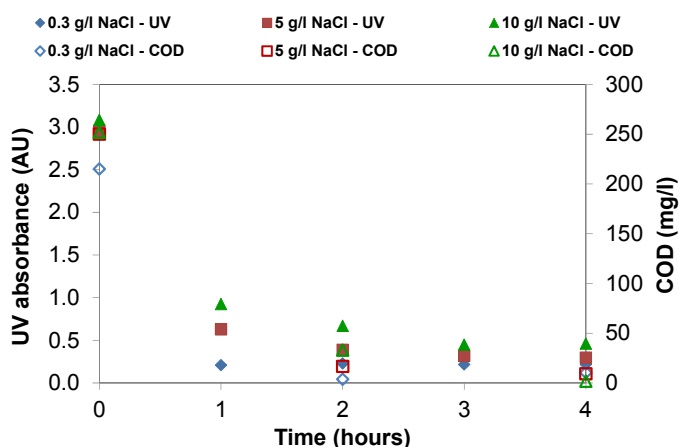


Figure 15. Reduction of UV absorbance and COD during photo-Fenton oxidation of biologically treated synthetic textile wastewater. Initial conditions: 3 mM Fe^{2+} , 15 mM H_2O_2 , pH 3. AU = absorbance units.

The photo-Fenton reaction is very effective in degrading complex organic pollutants; however, it has long reaction time and high chemical requirements. Thus, its use in combination with other treatment processes is recommended, so that costs and environmental impact can be minimized. The use of photo-Fenton as pre-treatment before biological degradation of textile wastewater has been suggested (Rodriguez et al. 2002, Lucas et al. 2007, Rodrigues et al. 2009). However, a better solution is to use photo-Fenton after biological treatment (Blanco et al. 2014) (**Paper III**) or after other pre-treatment processes such as electrocoagulation (Manenti et al. 2015).

A similar approach was used in **Paper IV**, where ozonation was used as post-treatment after the anaerobic biological process instead of photo-Fenton oxidation. The synthetic textile wastewater had the same composition as in the previous study, the sodium chloride content was kept at 10 g/l, but the dye concentration was increased from 100

to 500 and 1000 mg/l, which means that the COD increased as well from 650 to 900 and 1290 mg/l. The biologically treated effluent was exposed to ozone for 1, 2 and 4 minutes and the reduction of both COD and UV absorbance increased together with the exposure time. Nevertheless, the initial dye concentration was the main factor affecting the treatment and large differences, especially in terms of COD removal, were observed (Figure 16). The fact that the UV absorbance was reduced to a large extent indicates that most aromatic rings were opened during the ozone treatment. On the other hand, as the dye concentration increases, a major number of non-degraded organic compounds accumulate in the effluent, which therefore needs to be treated further.

The same ozonation process was also used as post-treatment for real textile wastewater; the exposure time was further increased to six minutes. Figure 16 shows that both UV absorbance and COD were reduced only to a limited extent. In particular, no significant difference in COD was observed between the effluents exposed to ozone for one to six minutes. The UV absorbance decreased slightly as the exposure increased, indicating a progressive destruction of the aromatic structures. The aromatics were most likely transformed to more simple aromatic compounds and not mineralized.

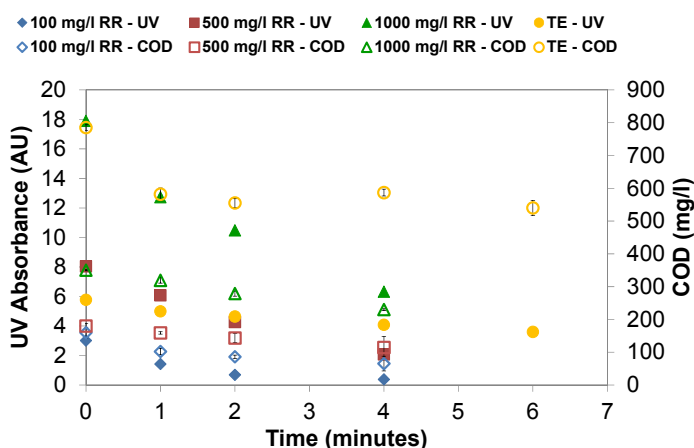


Figure 16.

Reduction of UV absorbance and COD during ozonation of biologically treated real (TE) and synthetic textile wastewater containing 100, 500 and 1000 mg/l Remazol Red (RR). AU = absorbance units.

The real textile wastewater was characterized by a rather high concentration of dissolved organic matter (DOC), 333 mg/l, compared with 5-20 mg/l in municipal wastewaters (Sonntag and Gunten 2012), and DOC has inhibitory effects for ozonation and advanced oxidation (Nöthe et al. 2009, Qian et al. 2013).

Large-scale installations for photo-Fenton treatment are still scarce and mostly use solar light rather than artificial (Zapata et al. 2010, Michael et al. 2014, Velegraki and Mantzavinos 2015). The challenge is to build a reactor which allows exposure of large

volumes of water to sunlight without using excessively large areas. A common solution is the use of compound parabolic solar collectors (CPC) technology (Blanco-Galvez et al. 2007), but other reactor set-ups have been used as reviewed by Bahnemann (2004). Typically, the reactors for photocatalysis are tilted to better face the sun and have small depth so that the light reaches a larger volume of water. Full-scale ozone installations are more common, probably because their operation is simpler than photo-Fenton oxidation. Ozonation is used routinely for treatment of drinking water in many countries (von Gunten 2007), and it is becoming more and more common for treatment of industrial wastewater, also from textile industries (Lotito et al. 2012, Bertanza et al. 2013).

Figure 17 compares the results obtained in this project for synthetic and real wastewater using both photo-Fenton oxidation and ozonation as post-treatment after a biological process.

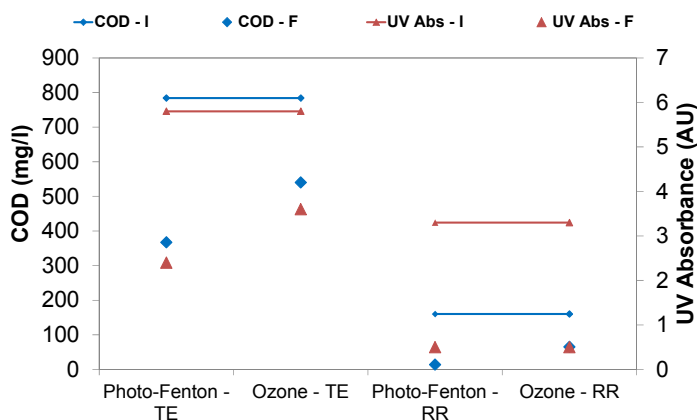


Figure 17.

Reduction of UV absorbance and COD during ozonation and photo-Fenton oxidation of biologically treated real textile wastewater (TE) and synthetic textile wastewater (RR). COD-I = initial COD; COD-F = final COD; UV Abs-I = initial UV absorbance; UV Abs-F = final UV absorbance; AU = absorbance units.

Higher mineralization of the organic compounds in wastewater was obtained with photo-Fenton oxidation, as the reduction of both COD and UV absorbance indicates. Nevertheless, studies in which the degradation mechanism of azo dyes during photo-Fenton oxidation has been investigated report that complete mineralization is difficult to achieve, transformation products like triazine may accumulate, and prolonged exposure is needed (Feng et al. 2000, Garcia-Montano et al. 2008b, Thomas et al. 2014). Transformation products of azo dyes and other organic compounds accumulate also during ozonation of textile wastewater. In fact, the possibility of obtaining

complete mineralization using ozonation is still under discussion (Hong and Zeng 2002, Somensi et al. 2010).

One important factor affecting the degradation of organic compounds during ozonation of wastewater is the actual ozone consumption. Ozone has in fact limited solubility in water (mg/l range) (von Gunten 2007). The ozone consumption in the process used in this project was measured and a rather high amount of ozone went through the process unreacted: 0.15 g/l and 0.40 g/l when 0.52 g/l and 0.78 g/l, respectively, were fed (4 and 6 minutes of exposure to ozone). This inefficiency should be addressed to avoid unnecessary costs and possibly improve the degradation. A solution, already in use in the pilot studies performed by Primozone (Jürgen Bischhaus, personal communication), is to let ozone and wastewater be in contact for some time after exposure to the ozone flow; the residual ozone concentration is reduced by about 10 times. The water first flows in an exposure tank where the ozone is fed for a few minutes. Then it is transferred to a reaction tank, where the ozone can react with the organic compounds in the water for a longer time. The only drawback is the necessity for an additional tank or reactor, but this strategy may allow reduction of the amount of the ozone required for treatment of a certain type of water, which is a big advantage.

7. Assessment of toxicity

The pollution caused by textile wastewater is linked to several factors. As for all wastewaters, the release of nutrients in a natural water body will increase the oxygen consumption and thus decrease the oxygen availability and the life in the water basin. In addition, the colored effluent affects the photosynthetic capacity of the ecosystem by reducing the penetration of sunlight into aquatic environments. That affects the growth of plants and other organisms living in the same ecosystem. The dyes and their degradation products may have genotoxic and mutagenic effect and thus interfere with the genetic material and the reproductive system of vertebrates and invertebrates (Pinheiro et al. 2004, Sharma et al. 2007, Bafana et al. 2011).

Biological processes and advanced oxidation have been studied for treatment of textile wastewater. The results were very encouraging when the treatment was performed on azo dye solutions but their interpretation became more difficult as the wastewater became more and more similar to real textile effluents. Chemical analysis of the composition of these effluents is challenging, especially after treatment, when a large number and variety of transformation products may be produced (Weschenfelder et al. 2007, Constapel et al. 2009, Hisaindee et al. 2013). Thus, it is essential to assess the quality of treated effluents with other tools. Ecotoxicity studies can be used to determine the effects that an effluent would produce if it entered an ecosystem. One of the major advantages of toxicity tests over chemical analysis is their ability to assess a complex mixture and not only known, targeted compounds (Escher and Fenner 2011).

Tests targeting different organisms should be used to assess the effect on different trophic levels. There are a great number of tests to choose from, but not all tests are suitable for all effluents. The toxicity of textile wastewater, for example, should be tested on organisms that are tolerant to, or even thrive on, high salinity. Toxicity tests can be of different types and be used to evaluate the acute or chronic effects as well as mutagenicity of a certain solution. Nevertheless, they all aim at finding a relation between amount of chemicals to which an organism is exposed and the type and degree of response produced, in other words a dose-response relationship. Acute toxicity is the result of a short exposure to a certain amount of a chemical and is detected shortly after the exposure. In contrast, chronic toxicity results from prolonged or repeated exposure to a certain chemical, even at very low doses, and is detected long time after the first exposure. Some dyes and their transformation products are mutagenic; they can increase the rate of DNA or RNA mutations and thus change their composition. Some

mutations can cause cancer, and therefore some mutagenic compounds are also carcinogenic.

Acute toxicity

The most commonly used toxicity tests are those assessing the acute toxicity of a dissolved chemical, because they provide a response in a short time. In **Papers II, III and IV**, three different toxicity tests were used. The target organisms were the brine shrimp *Artemia salina*, the edible herb *Lepidium sativum* and the bioluminescent bacterium *Vibrio fischeri*. In Figure 18 they are arranged according to their sensitivity to pollutants; *Artemia salina* is the least sensitive followed by *Lepidium sativum* and *Vibrio fischeri*, known for its high sensitivity (Rizzo 2011).

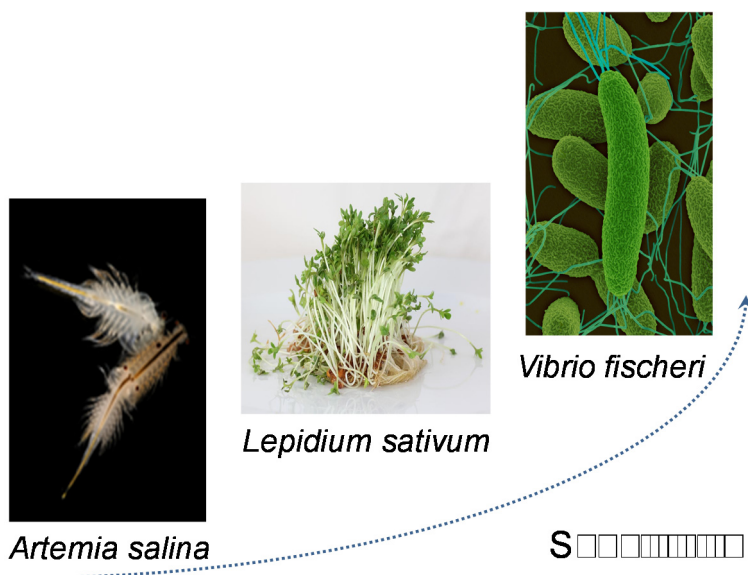


Figure 18.

Sensitivity scale of target organisms used for assessment of acute toxicity. Photos: *V. fischeri* Image copyright Dennis Kunkel Microscopy, Inc.; *L. sativum* gemuese-info.de; *A. salina* © Hans Hillewaert.

The toxicity tests were chosen in an attempt to target those (micro)organisms that would eventually be in contact with the effluent, treated or untreated. Assays that evaluate the performance of a biological wastewater treatment process such as inhibition of nitrification (Arvin et al. 1994) and respirometry assay (Gendig et al. 2003) were initially considered, but could not be used because of their sensitivity to salinity.

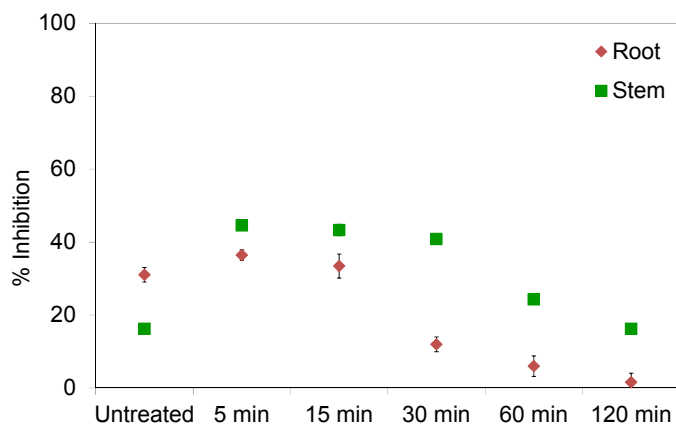


Figure 19.

Growth inhibition of stems and roots of *Lepidium sativum* after exposure to untreated and photo-oxidized solutions of Remazol Red (the initial concentration was 100 mg/l). The time on the x-axis indicate for how long the Remazol Red solution has undergone photo-Fenton oxidation. Each dot is the average of 5 independent measurements. Error bars represent standard deviation.

The plant *Lepidium sativum* was used in **Paper II** to evaluate the effects of the azo dye Remazol Red and of its degradation products formed during photo-Fenton oxidation on plants. Plants are used less often, but reports can be found where they have been used to assess toxicity of organic pollutants and the products of their degradation during biological and advanced oxidation processes (Moradas et al. 2008, Ellouze et al. 2010). The growth inhibition of both stems and roots was calculated and, interestingly, it was found to be higher after 5- to 15 minutes of photo-Fenton oxidation than for the untreated Remazol Red solution. Figure 19 also shows that the inhibition decreased as the treatment continued. Similar results have been reported for other azo dyes (de Luna et al. 2014). An increase in acute toxicity after a short photo-Fenton treatment or when very low Fenton reagents are used has been reported also by Zhang and colleagues (2014) in a study on the effect of photo-Fenton and coagulation for degradation of the azo dye red 3BS, using zebrafish as target organism.

The toxicity of Remazol Red was measured also with *Vibrio fischeri* and *Artemia salina* (**Paper IV**). *Vibrio fischeri*, a bioluminescent bacterium, was chosen because it is the most widely used acute toxicity test (Dalzell et al. 2002, Ferraz et al. 2011a, de Luna et al. 2014). In addition, it grows in 2% sodium chloride, so the salinity of the textile effluents does not affect the response. *Artemia salina* was chosen instead of the more commonly used invertebrate *Daphnia magna*, because it is tolerant to salinity. Solutions of Remazol Red were moderately toxic at a concentration of 100 mg/l, but increased concentrations led to higher toxicity. The toxicity increased even more after biological treatment, except for the solution containing 1000 mg/l of Remazol Red. Figure 20 confirms that the sensitivity of the brine shrimp is much lower than that of *Vibrio*

fischeri and some toxicity was detected only for the dye solution with the highest concentration and for the real textile wastewater. It is also interesting that the untreated real textile effluent has rather high toxicity, which decreases slightly after biological treatment.

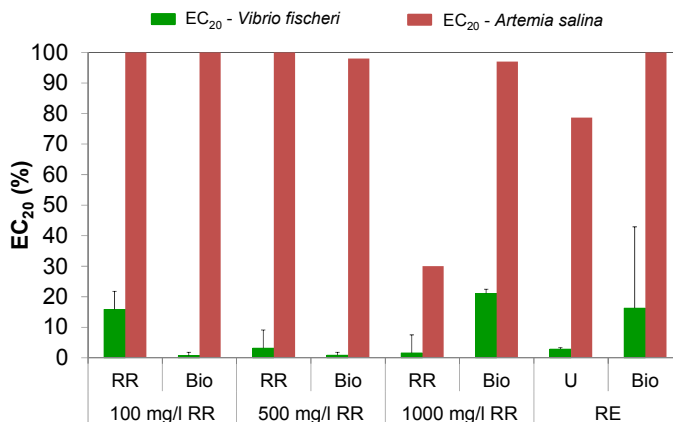


Figure 20.

Toxicity (EC₂₀) of untreated and biologically treated synthetic and real textile wastewater to *Vibrio fischeri* and *Artemia salina*. RR = Remazol Red; Bio = bio-treated effluent; RE = real textile effluent; U = untreated textile effluent. EC₂₀ = concentration at which 20% of the target organisms give the described response. Values are average of at least 3 independent measurements. Error bars represent standard deviation.

Azo dyes are not usually seen as toxic, and it is rather their degradation products which are considered harmful for humans and the environment (Novotny et al. 2006, Bafana et al. 2011). These results suggest that this is not always true and the dye itself, depending on its chemical structure and especially on the type and position of the groups attached to the aromatic rings (Ferraz et al. 2011b), is a potential high risk for the people working with it and the environments contaminated with it.

The untreated real wastewater had rather high toxicity towards *V. fischeri*, which decreased after biological treatment in agreement to what reported by the full scale treatment plant (Frijters et al. 2006). The toxicity towards *A. salina* was low and completely removed after biological treatment.

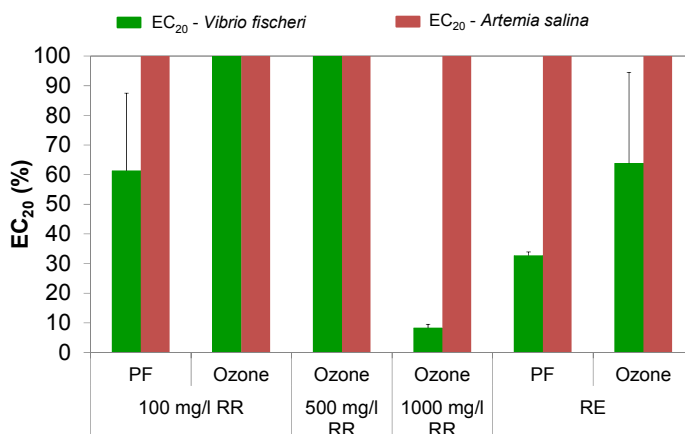


Figure 21.

Toxicity of biologically treated synthetic and real textile wastewater after photo-Fenton oxidation or ozonation to *Vibrio fischeri* and *Artemia salina*. RR = Remazol Red; RE = real textile effluent; PF = photo-Fenton oxidation. EC₂₀ = concentration at which 20% of the target organisms give the described response. Values are average of at least 3 independent measurements. Error bars represent standard deviation.

Photo-Fenton oxidation and ozonation, used as post-treatment after the anaerobic biological process, were successful in removing the toxicity (**Papers III and IV**). Better reduction was obtained with ozonation (Figure 21), although the reduction of COD and absorbance was higher after photo-Fenton oxidation (Figure 17). These results, apparently in contrast, highlight the necessity of combining chemical analysis with toxicity assessment. Toxic compounds may be present at very low concentration, and especially when advanced oxidation techniques are used, a large number of toxic transformation products may be produced (Schröder 1996, Constapel et al. 2009). It is very challenging to detect their presence in effluents when measuring parameters like COD and TOC, but also when more sensitive techniques such as HPLC-MS are used (Weschenfelder et al. 2007). Thus, bioassays play a very important role and their use should be encouraged also to monitor the performance of full scale treatment plants. It would be interesting to be able to associate the remaining toxicity in the treated effluents with some compounds that are difficult to degrade even with AOPs. There is a lack of studies, though; some data can be found regarding chlorophenols where it is reported that the toxicity observed during photo-Fenton treatment is due to the generation degradation by-products (Karci et al. 2012). In some studies on azo dye degradation, accumulation of triazines which may be toxic has been observed (Garcia-Montano et al. 2008b, Thomas et al. 2014).

Mutagenicity

Assessment of mutagenicity of untreated and treated textile effluents is of great importance as some azo dyes and the aromatic amines produced during their degradation can be mutagenic. Potential mutagenicity effects related to textile wastewater have already been detected in receiving water bodies (de Aragão Umbuzeiro et al. 2005) and also in biological and chemical sludge generated in a full scale wastewater treatment plant (Mathur et al. 2007).

Common methods used to evaluate the ability of chemicals and mixtures to cause DNA damages are the Comet assay and Ames test. The Comet assay measures DNA strands breaks in eukaryotic cells which have been exposed to potentially genotoxic agents (Collins 2004, Olive and Banath 2006). The Ames test is an assay designed to detect chemically induced gene mutations using histidine-dependent strains of *Salmonella typhimurium*. It can be performed in agar plates or in microplates, and the latter, used in this work, is shown in Figure 22 (Reifferscheid et al. 2012). A culture stored at -80°C is cultivated overnight in growth medium in the presence of histidine. Thereafter, a 24-well microplate filled with a solution containing exposure medium (lacking histidine) and target compound/wastewater is inoculated with the culture. S9-fraction of rat liver extract can be added to simulate metabolic activation that would occur if the target compounds were ingested by mammals and went through their liver. After 90 minutes' incubation, indicator medium is added and the cultures are transferred into a 384-well microplate. The results are read after a further 48 hours' incubation: the revertant wells are those with a certain absorbance at 425 nm (purple in Figure 22). The more revertant wells, the higher is the mutagenicity potential of a certain sample. To improve the sensitivity of the assay to different types of mutagens, several strains have been developed. The most commonly used strains are TA98 and TA100, which are most sensitive to frame shift mutations and GC pair substitutions, respectively (McCann et al. 1975, Mathur et al. 2007, Shehzadi et al. 2014).

In **Paper IV**, the Ames test without metabolic activation was used to estimate the mutagenicity potential of real textile wastewater before and after treatment. The strain used, YG7108, was developed by Yamada and colleagues (1995) and it is special because it lacks two genes encoding for O6-methylguanine DNA methyltransferases, which respond to DNA damages caused by alkylating agents (Yamada et al. 1997). For this reason, its use to detect mutagenicity after advanced oxidation of water and wastewater has been suggested (Magdeburg et al. 2014, Mestankova et al. 2014). The untreated effluent, as well as the effluent after biological treatment and even after one minute's exposure to ozone, was positive to the test (*p-values* of $5.4e^{-10}$, 0.03 and $2.2e^{-16}$, respectively).

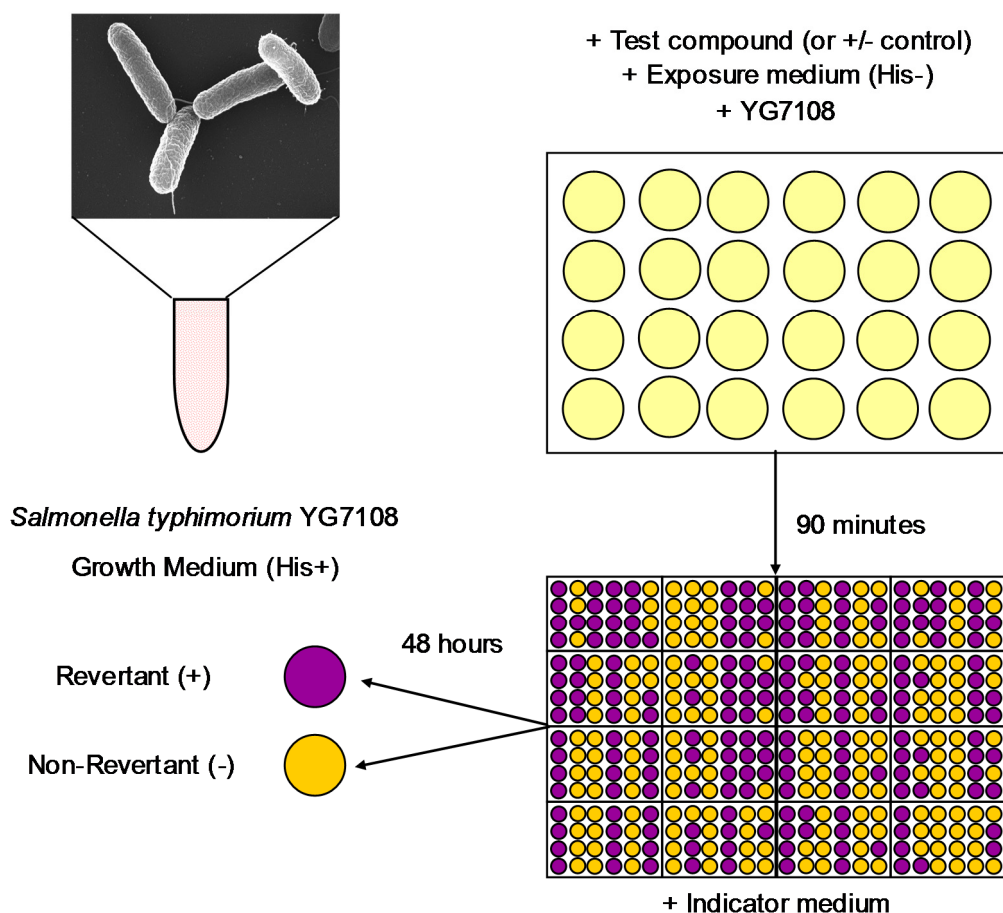


Figure 22. Fluctuation Ames test for estimation of mutagenicity potential of chemicals and/or wastewater. The SEM image of *Salmonella typhimurium* was taken by Volker Brinkmann, Max Planck Institute for Infection Biology, Berlin, Germany (doi:10.1371/journal.pbio.0030166.g001).

There was non-significant difference between the untreated and biologically treated effluents, but the mutagenic effects of the water after one minute of exposure to ozone were significantly higher than those of the biologically treated water (p -values of 0.05). These results suggest that compounds with alkylating activity, for example compounds that can methylate DNA, may be present in textile wastewater and that they may be generated during ozonation. Exposure to ozone for a longer period of time resulted in complete removal of mutagenic effects. Other studies on industrial and municipal wastewater treatment where ozone was used as tertiary treatment reported a reduction of mutagenicity potential after ozonation to a different extent depending on the chemical composition of the effluent and on the ozone dose applied (Misik et al. 2011, Bertanza et al. 2013).

8. An ideal treatment - implications

The experimental results of this project indicate that textile wastewater can be cleaned by using several treatment processes. The choice of the technology to be used should take into account the characteristics of the specific textile effluent to be treated. However, other factors are also very important in deciding upon the configuration of a new treatment plant and these factors are the costs and the environmental impact. In **Paper VI**, the environmental impact and the costs of the two combined treatment approaches studied in this research project, anaerobic treatment followed by photo-Fenton oxidation or ozonation, were assessed and discussed. In addition, the treatment processes studied in this project do not tell the whole story. Physical pre-treatment to separate the textile fibers is usually necessary and additional polishing steps may be used at the end before discharging the treated effluent or possibly re-using the water in the textile production. Ideally, salt and dyes could be separated from the dyeing effluent and the water reused, but this is not the reality yet. The missing steps that need to be taken to reach green textile production will also be discussed later in this chapter.

Environmental impact and cost analysis of photo-Fenton and ozonation

Life cycle assessment (LCA) methodology (ISO 14044:2006) was used to evaluate the environmental impact of the two combined strategies studied in this thesis:

- a. anaerobic biological process followed by photo-Fenton oxidation
- b. anaerobic biological process followed by ozonation

The LCA methodology is based on the “cradle-to-grave” concept, following the life of a product from the origin of its raw materials to the disposal of the product itself and its by-products, as shown in Figure 23.

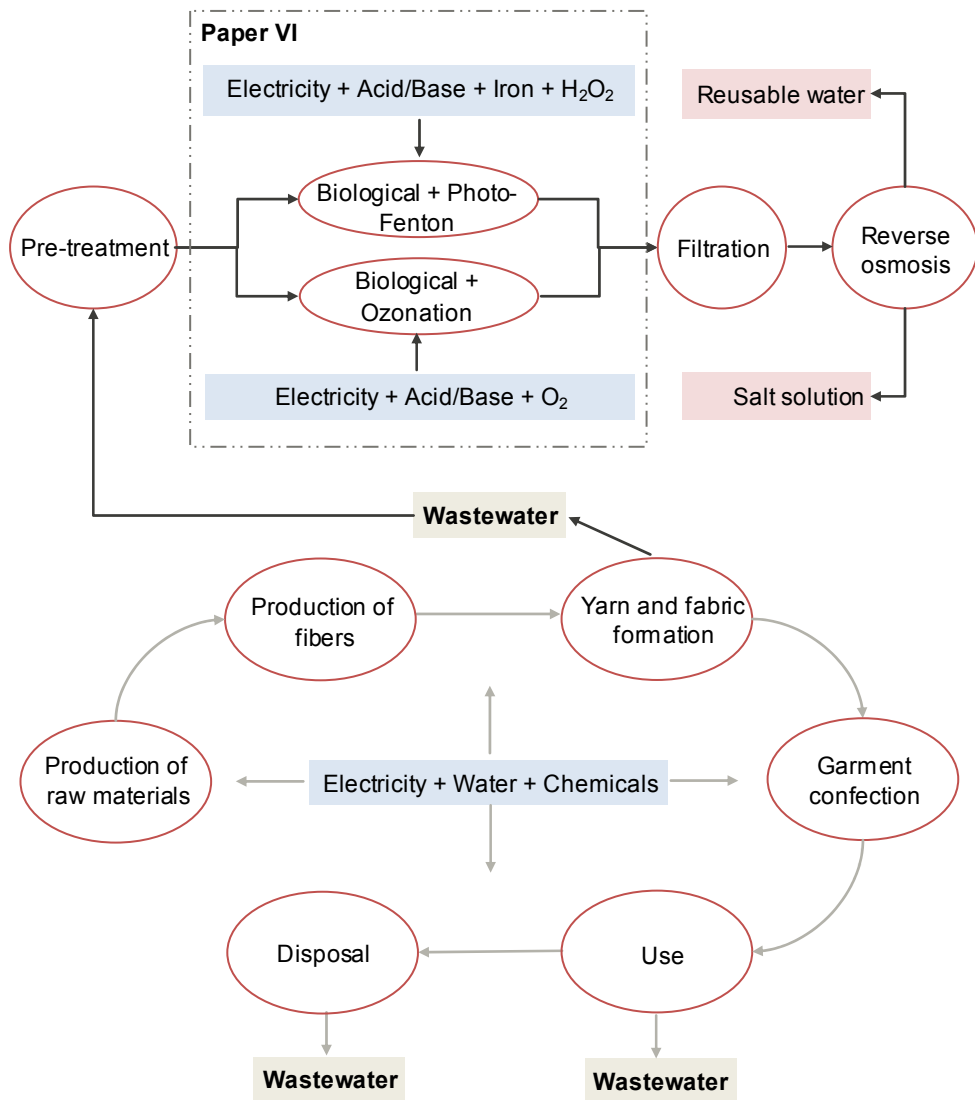


Figure 23.
Simplified life cycle of textile production with details about an ideal treatment for the wastewater produced during manufacture of the textile product.

In this case, the product is a certain amount of textile produced and the treatment of the wastewater is only a small part of the entire life cycle, the squared section in Figure 23. For this reason, it is only possible to assess the environmental impact of the different treatment approaches and not the entire life cycle. Nevertheless, treatment of textile wastewater has quite an impact on the entire life cycle of textiles, as discussed by Yuan and colleagues (2013). As Figure 23 shows, wastewater is produced not only when the textile products are manufactured, but also during their normal use and when they are

disposed as waste. However, these streams are usually handled as municipal waste and wastewater and conventional methods are used for their treatment.

A crucial step in the assessment of the costs and environmental impact of a certain process is the definition of the functional unit (FU), which is a measure of the service provided by the process and which for water treatment is usually a certain volume of water treated or the elimination of a certain pollutant to a predetermined extent. The role of the functional unit is to allow quantification and comparison of the consumptions and emissions of one or more processes. In this study the functional unit was defined as the reduction of acute toxicity of at least 57% according to the EC₂₀ calculated at 15 minutes using the *V. fischeri* bioluminescence assay of 1 m³ of textile wastewater, that is the volume of wastewater produced when manufacturing an average of 7.7 kg of textiles (35-40 cotton t-shirts). All the data and results presented in the following sections have been normalized to this unit.

Cost analysis

The costs of advanced oxidation processes depend on the degree of mineralization that is achieved, the treatment time and the scale of the plant (Carra et al. 2013, Sánchez Pérez et al. 2013). In fact, for a higher level of mineralization one of two strategies can usually be followed: either the total amount of reagent is increased or the length of the treatment is increased. Both strategies will therefore lead to higher costs. These operating costs were the object of the economic analysis performed at the end of this research project. The costs associated with capital investment were not considered as they depend on plant size and volume of water to be treated per day.

Experimental data collected for treatment of the textile wastewater from the Netherlands, as described in the previous chapters, were used to calculate the requirement for raw materials and energy for the oxidation processes as well as the disposal of the precipitate generated during photo-Fenton. The inventory of the data is summarized in Table 5.

The biological process has a negligible cost (0.1 €/FU) compared with photo-Fenton oxidation and ozonation (3.4 €/FU for both processes). Electricity, hydrogen peroxide and iron sulfate are the main factors affecting the cost of the photo-Fenton oxidation. The results in Table 5 show that the disposal of the precipitate rich in iron would have a relatively low cost, 4% of the total, in contrast to that reported in some previous studies (Wang et al. 2012, Bokare and Choi 2014). Substitution of artificial light with sunlight would significantly reduce the electricity requirements of the process and thus the overall costs. In fact, given a solar-Fenton oxidation and the lowest prices for the reagents (according to Table 5), the total cost is as low as 0.9 €/FU. To decrease the costs for the reagents, the entire process would need to be optimized. The reagents concentrations are in fact crucial for the outcome of the treatment and can be reduced only if the organic content of the wastewater is further reduced by other means.

Alternatives are to find cheaper sources for the reagents, for example the hydrogen peroxide remaining in the side stream produced during bleaching of the textiles could be exploited, or to recover a reagent such as iron after the treatment and reuse it.

The costs of ozonation are high because ozone generation is a rather energy-intensive process. As discussed in the end of the section entitled “How to combine physical-chemical and biological processes”, the ozone utilization in the lab-scale process was not efficient. The ozone utilization and its oxidation efficiency can be improved by increasing the reaction time after ozone has been fed into the wastewater. That is currently done at pilot scale and has resulted in a large reduction in the ozone required for the treatment. Therefore, the costs can be potentially reduced from 3.4 to 0.5 €/FU.

Table 5.

Inventory data and cost analysis of biological, photo-Fenton and ozonation processes.

	Input/output	Amount	Cost/FU (€/m ³)
Biological	Electricity	0.002 kWh/FU	0.10 ^a
Photo-Fenton	Hydrogen peroxide	1.7 kg/FU	0.37-1.45 (Garcia-Montano et al. 2006)
	Sodium hydroxide	0.59 kg/FU	0.11-0.32 (Rodrigues et al. 2014)
	Sulfuric acid	0.43 kg/FU	0.03-0.06 ^b
	Iron disposal	8.4 kg/FU	0.14 (Kalliala and Talvenmaa 2000)
	Iron sulfate	3.8 kg/FU	0.04-0.94 ^b
	Electricity	15.5 (1.7) kWh/FU	0.52 (0.06) ^a
	Total		3.43 (0.75)
Ozonation	Oxygen (liquid)	5.8 (0.7) kg/FU	1.22 (0.14) ^c
	Electricity	17.4 (2.2) kWh/FU	2.09 (0.26) ^a
	Total		3.31 (0.4)

EUR/USD = 1.13, average for Jan- 22 to Feb 22,2015 from XE.com

^ahttp://ec.europa.eu/eurostat/statistics-explained/index.php/Energy_price_statistics Accessed on 20/2/2015 - Average for half 2013, industrial

^b<http://www.icis.com/chemicals/channel-info-chemicals-a-z/> Accessed on 20/2/2015 -

^cSpace Shuttle Use of Propellants and Fluids. National Aeronautics and Space Administration. September 2001. Retrieved December 16, 2007. "NASAFacts FS-2001-09-015-KSC"

Advanced oxidation processes are usually considered expensive. The cost estimation of the treatments tested in this research project only partially confirms their high costs. In fact, our results show that upscaling and optimization can bring down reagents and electricity consumption costs, resulting in overall costs that are in the same order of magnitude as those estimated for biological processes, considered to be a cheap treatment alternative. Investments in installation of advanced oxidation pilot plants on sites where textile wastewater is treated are thus encouraged as they allow further

optimization and costs reduction of these new technologies. It is important to implement new treatment technologies to improve the quality of the treated effluent and reduce the risk of toxic and mutagenic compounds being released in the environment.

Environmental impact

The environmental impact assessment of the combined biological-ozonation process as well as biological-photo-Fenton process was performed using the inventory data represented in Table 5. The results are shown in detail in **Paper VI**, Figure 2.

The biological process resulted in a small environmental impact when compared with that of the oxidation processes because of the low electricity consumption and the fact that no chemicals are added during biological treatment. Ozonation was characterized by high electricity consumption and water utilization. Oxygen is needed to generate ozone and this process requires large amounts of water and energy. In fact, ozonation resulted in extremely high water usage, 17 m³ per FU. This is a particularly discouraging value because the volume of wastewater to be treated in the functional unit is only 1 m³. As a result, the environmental impact of ozonation was higher than that of photo-Fenton in all categories. The production of electricity is the factor most affecting the impact of the photo-Fenton oxidation. Production of hydrogen peroxide has also a substantial influence, especially on eutrophication potential, particulate matter formation and global warming potential. The disposal of the iron-rich precipitate has a major effect only on the eutrophication potential.

These results confirm that the high energy requirement is the main drawback of AOPs. The same conclusions were drawn by Muñoz and colleagues (2006) in a study that compared several AOPs for treatment of a Kraft mill effluent. They also indicated electricity consumption as the main factor affecting the environmental impact and therefore ozonation as least friendly process.

A sensitivity analysis was carried out to consider the uncertainties of the processes, for example how the electricity is produced, which type of light is used to perform the photo-Fenton oxidation, the amount of reagents required for the oxidation and so on. A detailed summary of the different factors considered and how the environmental impact of the processes would change can be found in **Paper VI**, Table 4. The most interesting part was the evaluation of potential improvements of the processes. Substitution of artificial light with sunlight for the photo-Fenton oxidation results in a great improvement and it is feasible in certain areas of the world. Data collected in Almeria, Spain (Ignacio Maldonado Rubio, personal communication) and in a study by Durán and colleagues (2012) indicate that the electricity requirement would decrease by 90% and cause a significant reduction of the environmental impact in all the categories. Ozonation can also be optimized to reduce its environmental impact. In particular, upscaling from lab to pilot resulted in a decrease in ozone consumption from

790 g/m³ to 100 g/m³ with consequent reduction in oxygen and electricity requirements. The overall environmental impact was consistently improved. In addition, the consumption of water and energy in both oxidation processes was reduced and, particularly for ozonation it became similar to that of the biological treatment.

The outcome of the economic and environmental analysis confirms that the biological process has substantial advantages in terms of consumption of electricity and chemicals compared with advanced oxidation technologies. Nevertheless, photo-Fenton oxidation and ozonation can be optimized to be cost-efficient and to minimize emissions. The recommendation is to use biological processes where possible so that only what is non-biodegradable is taken care of with advanced oxidation technologies.

Textile wastewater treatment in the future

In the last decade effort has been put to into changing the way we think about waste and wastewater so that we learn to see them as resources. The so-called “paradigm shift” from wastewater treatment to resource recovery should also involve the textile sector. Salt, dyes and water could be separated and reused in textile manufacture with economic and environmental advantages. Water reuse is becoming a reality and, together with desalination, represents the solution to water scarcity around the world (Elimelech and Phillip 2011).

A large quantity of salt and dyes end up in the first rinse after dyeing. Separate treatment of this effluent would allow salt recovery and enhance the treatability of the effluent (Vishnu et al. 2008). Membrane technology is a good option for recovery of salts (Koyuncu et al. 2004, Allègre et al. 2006, Vishnu et al. 2008). Several dyeing units in Tirupur have recently installed zero discharge treatment systems (ZDTSs), which allow recovery of salt and reuse of water (Vishnu et al. 2008). Dyeing and washing effluents are treated separately, the first through nanofiltration, which results in up to 70% salt recovery, and the second through flocculation/coagulation and reverse osmosis, for a total water recovery close to 90%. Although this system provides good recovery and good economic revenue, it is not the optimal solution. There has been a lot of discussion regarding the generation of toxic sludge from treatment of textile wastewater and the ZDTSs do not tackle that problem. In fact, coagulation/flocculation is the step that removes most of the color and organic content from the wastewater, but that means that for each 100 l of wastewater treated, 13-17 l of toxic (wet) sludge are produced. The solution that emerges from this research is that biological treatment followed by advanced oxidation can remove color, organic content and toxicity from textile effluents and prepare them for an eventual further treatment, for example reverse osmosis, so that reusable water is obtained with very low sludge production. A simple scheme representing this idea is depicted in Figure 23 in the beginning of this chapter.

One issue that still requires a lot of research is the recovery of dyes. Only a few studies are found in the literature where methods to separate dyes from aqueous solutions are tested and they are based on liquid-liquid extraction (Muthuraman et al. 2009) or liquid membrane process (Othman et al. 2011). Novel technologies need to be developed and better cooperation between industries and research is necessary. Dyes can have many different chemical structures, which until now has not helped us to find a way to recover them all.

9. Conclusions and future perspectives

In this thesis biological and physical-chemical methods have been evaluated for their ability to degrade azo dyes and treat textile wastewater.

The chosen biological process was an anaerobic biofilm reactor. High reduction of COD was achieved and the process was stable even under harsh conditions such as high concentrations of sodium chloride and azo dyes. The azo dyes were biotransformed into colorless but toxic compounds, probably aromatic amines. The microbial community was dominated by the presence of bacteria, but also eukaryotic microorganisms, in particular fungi and algae. The role of algae in azo dyes degradation was also confirmed in a separate study where isolates from the reactors and from the natural environment showed ability to cleave azo bonds.

The physical-chemical methods evaluated were photo-Fenton oxidation and ozonation. Photo-Fenton was first tested as a stand-alone treatment and demonstrated to have the potential to mineralize azo dyes. Some advantages may result from the use of solid iron instead of soluble iron sulfate, but the latter was preferred for its fast reaction kinetics. Although efficient when applied to aqueous dye solution, the photo-Fenton reaction needed high initial concentrations of reagents to degrade azo dyes in synthetic textile wastewater. Thus, its use in combination with biological treatment was investigated.

A two-step treatment was set up by combining the advantages of the biological process, high reduction of COD, and of photo-Fenton reaction, mineralization of azo dyes. The same approach was used to evaluate the potential of ozonation. An anaerobic biofilm process was used as first step followed by photo-Fenton oxidation or ozonation. Both strategies were successful in treating textile wastewater in terms of degradation of organic compounds including the dyes.

The azo dyes were toxic to the shrimp *Artemia salina* and to the bacterium *Vibrio fischeri*. The toxicity was even higher after biological treatment, but was reduced by the oxidative post-treatment. The real textile wastewater was also toxic and had a high mutagenic effect. The mutagenicity was removed only after appropriate post-treatment, suggesting that this type of analysis should be performed before the discharge of treated textile wastewater into the environment.

Because of the toxic nature of the dyes, use of advanced oxidation or other methods before biological treatment is suggested in the literature. This work shows that biological biofilms are robust and can thrive even under such conditions. Use of biological treatment as a first step offers considerable advantages in terms of process economy and environmental impact as it has low energy and chemical requirements.

In addition, the reagents requirement of photo-Fenton oxidation and ozonation depends on the pollution-level of the water to be treated, hence if used as post-treatment after biological degradation, their costs and environmental impact is reduced.

A treatment that includes several biological processes combined in series may provide additional benefits in terms of removal of organic compounds and pollutants compared with the treatment tested in this study. In fact, different types of microorganisms thrive under different conditions and may express their degradation potential better if cultivated alone than in a mixed culture. White-rot fungi, for example, possess powerful oxidative enzymes that can degrade many aromatic molecules. Their use after the anaerobic process applied in this study may lead to further degradation of the dyes by-products. As a consequence, the effluent to be treated with advanced oxidation would have low organic content hence less electricity and chemicals would be needed for the final treatment step. Biological and advanced oxidation processes can be combined in different ways and the treatment set-up should be optimized so that microbial degradation is exploited at the expenses of advanced oxidation, which use should be minimized.

The assessment of costs and environmental impact highlighted even more the importance of reducing electricity and reagents consumption of AOPs. Use of solar light and upscaling resulted in promising improvements for photo-Fenton and ozonation, respectively. Nevertheless, more efforts should be put into optimizing the use of resources: improving the ozone consumption, reusing the iron remaining after photo-Fenton and finding alternatives to hydrogen peroxide. These are just some of the issues that need to be addressed to accelerate the implementation of new and effective technologies for textile wastewater treatment.

The suggested novel approach for textile wastewater treatment composed of an anaerobic biofilm process followed by photo-Fenton or ozone oxidation would fit well into the zero water discharge concept and improve it greatly by reducing the sludge production. In such a system, the effluent of the oxidation process would be treated with reverse osmosis so that water and salt would be recovered and reused in the manufacturing of textiles. The future of wastewater treatment is in fact water reuse.

Textile wastewater treatment would benefit considerably from technologies that allow separation of dyes. Dyes are in fact the main concern in terms of toxicity and their presence in the environment in water or as solid sludge should be avoided at all costs.

With the improvement of the analytical tools, more and more pollutants are detected in wastewater and degradation of emerging contaminants has become a major issue in the wastewater sector. The novel treatment strategy suggested in this research has two main advantages: a very small amount of sludge is generated and the advanced oxidation applied on the bio-treated wastewater is very effective in degrading complex organic molecules. Therefore, application of such a treatment approach is recommended also for treatment of other types of industrial wastewater as well as for degradation of a variety of organic pollutants present in municipal effluents.

Acknowledgments

The Swedish International Development Cooperation Agency (SIDA) and Vetenskapsrådet (VR) are gratefully acknowledged for providing financial support. The Royal Physiographic Society of Lund is acknowledged for providing funding to buy essential laboratory equipment.

These years in Lund have been very important to me and when I look back I can see that I have really grown up as a person and as a scientist. If I reached the end of the PhD-journey it is (also) because of all the people that have surrounded me and whom I want to sincerely thank:

My supervisor Bo Mattiasson, for believing in me as a researcher, giving me the opportunity to be a PhD student and develop my own ideas. Thank you also for pointing towards the right direction when I needed it and for teaching me the importance of looking at things from a broader perspective.

Maria Jonstrup, for teaching me all what I needed to know to get through this PhD: how to perform an experiment, how to network, how to write my thesis. So many things have changed in your life since we first met, but no matter what you have always been there for me. I cannot tell you enough how grateful I am!

Rajni Hatti-Kaul, for accepting to step in and become my main supervisor. Thank you for being always willing to listen to my progress and questions and for doing your best to support me.

Anbu, you have been a great student, always helpful and in a good mood. Thank you for working hard and putting so much enthusiasm into our projects.

Amir, thank you for doing your best and not giving up on a very challenging project!

The researchers with whom I have been collaborating: Rosa from Biotechnology, Filip Nilsson and Karin Jönsson from Chemical Engineering, Britt-Marie Svensson from Kristianstad Högskola, Pål Börjesson from Environmental and Energy Systems Studies. And those who have been helping me: Daniel Stalter from the University of Queensland, Australia; Marc van Coeverden from Maan Group, the Netherlands and Jürgen Bischhaus from Primozone.

Emma and Siv, thank you for the irreplaceable assistance with all sorts of bureaucratic issues. Thank you also for taking the time to talk in Swedish with me, it helped a lot! Thanks also to Frans-Peder for keeping my computer in a good shape, to Ulla for the

help with ordering and shipping of various materia and to Sanel for helping me with budget and other financial matters.

To all the members of the Environmental group, thank you for building such a friendly atmosphere and...for working for a better environment! I could not wish for nicer colleagues!

To all the Biotechnology people. I keep many good memories of our times together at work, after work, on a spring trip. Thank you for your smiles and kind words and for being willing to share your knowledge.

To all the people I met here in Lund, you taught me so much about the entire world! For all these years I have been on a journey without even the need of traveling!

To all my friends in Italy, thank you for your kindness. A big thank you in particular to Angela & Angela, Claudia, Leo, Luisa, Gigi, Marco, Maria Giovanna, Mino, Piero, Raffaele, Rosy, Vittorio e la piccolo Giorgia, thank you for being close even when you are far away (or better when I am far away). It is very reassuring to know that I can always count on you. A special thank you to Reno for being a great friend since the day we met in Pavia and for giving me a lot of confidence.

The “Icelandic” family for making me feel at home from the first moment and for showing me a new way of living: you are brave and fearless in a way I could not even imagine.

Tutta la mia mia famiglia, spero davvero di passare più tempo con voi nel futuro. Un grazie speciale a Marcella e Clara, siete le migliori sorelle che potessi desiderare, vi ammiro e mi rendete orgogliosa ogni giorno. Giorgia ed Eleonora grazie per i vostri sorrisi, i disegni e tutto l'amore che una zia può desiderare. Basta sentirvi via telefono per riempirmi di gioia. Alessandro, grazie per prenderti cura di loro!

Mamma e papà, grazie per il vostro amore incondizionato. Con il vostro esempio mi avete insegnato a fare sempre del mio meglio, essere generosa ed onesta. Grazie per ricordarmi ogni giorno cosa è che conta davvero nella vita.

Sverrir, my husband and best friend, thank you for making me feel invincible. Thank you for your endless love and support. You make meaningful every day of my life.

“If you want to go fast, go alone, but if you want to go far, go together”

African proverb

References

- Adhikari, R.Y., Yumiko (2008) Textile and clothing industry - Adjusting to the post-quota world. From: Studies in trade and investment. United Nations Economic and Social Commission for Asia and the Pacific (ESCAP).
- Agler, M.T., Werner, J.J., Iten, L.B., Dekker, A., Cotta, M.A., Dien, B.S. and Angenent, L.T. (2012) Shaping reactor microbiomes to produce the fuel precursor n-butyrate from pretreated cellulosic hydrolysates. *Environmental Science & Technology* 46(18), 10229-10238.
- Allègre, C., Moulin, P., Maisseu, M. and Charbit, F. (2006) Treatment and reuse of reactive dyeing effluents. *Journal of Membrane Science* 269(1–2), 15–34.
- Arvin, E., Dyreborg, S., Menck, C. and Olsen, J. (1994) A mini-nitrification test for toxicity screening, minntox. *Water Research* 28(9), 2029–2031.
- Ashwin, R., Athinthra, K.S., Sriramvignesh, M., Kathiravan, M.N. and Gopinath, K.P. (2011) Treatment of textile effluent using SBR with pre and post treatments: Kinetics, simulation and optimization of process time for shock loads. *Desalination* 275(1–3), 203–211.
- Axelsson, J., Nilsson, U., Terrazas, E., Aliaga, T.A. and Welander, U. (2006) Decolorization of the textile dyes Reactive Red 2 and Reactive Blue 4 using *Bjerkandera* sp Strain BOL 13 in a continuous rotating biological contactor reactor. *Enzyme and Microbial Technology* 39(1), 32–37.
- Bacardit, J., Oller, I., I. Maldonado, M., Chamarro, E., Malato, S. and Esplugas, S. (2007) Simple models for the control of Photo-Fenton by monitoring H_2O_2 . *Journal of Advanced Oxidation Technologies* 10(2), 219–228.
- Bae, W., Won, H., Hwang, B., de Toledo, R.A., Chung, J., Kwon, K. and Shim, H. (2015) Characterization of refractory matters in dyeing wastewater during a full-scale Fenton process following pure-oxygen activated sludge treatment. *Journal of Hazardous Materials* 287(0), 421–428.
- Bafana, A., Devi, S.S. and Chakrabarti, T. (2011) Azo dyes: past, present and the future. *Environmental Reviews* 19, 350–370.
- Bahnmann, D. (2004) Photocatalytic water treatment: solar energy applications. *Solar Energy* 77(5), 445–459.
- Balasubramanian, J., Sabumon, P.C., Lazar, J.U. and Ilangoan, R. (2006) Reuse of textile effluent treatment plant sludge in building materials. *Waste Management* 26(1), 22–28.

- Bauman, M., Lobnik, A. and Hribernik, A. (2011) Decolorization and modeling of synthetic wastewater using O_3 and H_2O_2/O_3 processes. *Ozone-Science & Engineering* 33(1), 23-30.
- Bellucci, M. and Curtis, T.P. (2011) *Methods in Enzymology*. Martin, G.K. and Lisa, Y.S. (eds), pp. 269-286, Academic Press.
- Bergendahl, J.A. and Thies, T.P. (2004) Fenton's oxidation of MTBE with zero-valent iron. *Water Research* 38(2), 327-334.
- Bergenholtz, K.P. and Nielsen, P.V. (2002) New improved method for evaluation of growth by food related fungi on biologically derived materials. *Journal of Food Science* 67(7), 2745-2749.
- Bertanza, G., Papa, M., Pedrazzani, R., Repice, C., Mazzoleni, G., Steimberg, N., Feretti, D., Ceretti, E. and Zerbini, I. (2013) EDCs, estrogenicity and genotoxicity reduction in a mixed (domestic and textile) secondary effluent by means of ozonation: A full-scale experience. *Science of the Total Environment* 458-460(0), 160-168.
- Blanco, J., Torrades, F., Moron, M., Brouta-Agnesa, M. and Garcia-Montano, J. (2014) Photo-Fenton and sequencing batch reactor coupled to photo-Fenton processes for textile wastewater reclamation: Feasibility of reuse in dyeing processes. *Chemical Engineering Journal* 240, 469-475.
- Blanco-Galvez, J., Fernandez-Ibanez, P. and Malato-Rodriguez, S. (2007) Solar photocatalytic detoxification and disinfection of water: Recent overview. *Journal of Solar Energy Engineering-Transactions of the Asme* 129(1), 4-15.
- Blomqvist, A. (1996) *Food and Fashion*, TEMA, Linköping, Sweden.
- Bokare, A.D. and Choi, W. (2014) Review of iron-free Fenton-like systems for activating H_2O_2 in advanced oxidation processes. *Journal of Hazardous Materials* 275(0), 121-135.
- Borde, X., Guieysse, B., Delgado, O., Munoz, R., Hatti-Kaul, R., Nugier-Chauvin, C., Patin, H. and Mattiasson, B. (2003) Synergistic relationships in algal-bacterial microcosms for the treatment of aromatic pollutants. *Bioresource Technology* 86(3), 293-300.
- Brown, R.F., Jamison, S.E., Pandit, U.K., Pinkus, J., White, G.R. and Braendlin, H.P. (1964) the reaction of Fenton's reagent with phenoxyacetic acid and some halogen-substituted phenoxyacetic acids. *The Journal of Organic Chemistry* 29(1), 146-153.
- Buxton, G.V., Greenstock, C.L., Helman, W.P. and Ross, A.B. (1988) Critical-review of rate constants for reactions of hydrated electrons, hydrogen-atoms and hydroxyl radicals ($OH/O\cdot$) in aqueous-solution. *Journal of Physical and Chemical Reference Data* 17(2), 513-886.
- Carneiro, P.A., Umbuzeiro, G.A., Oliveira, D.P. and Zanoni, M.V.B. (2010) Assessment of water contamination caused by a mutagenic textile effluent/dyehouse effluent bearing disperse dyes. *Journal of Hazardous Materials* 174(1-3), 694-699.
- Carra, I., Ortega-Gómez, E., Santos-Juanes, L., Casas López, J.L. and Sánchez Pérez, J.A. (2013) Cost analysis of different hydrogen peroxide supply strategies in the solar photo-Fenton process. *Chemical Engineering Journal* 224(0), 75-81.

- Cerniglia, C.E., Freeman, J.P. and Baalen, C. (1981) Biotransformation and toxicity of aniline and aniline derivatives in cyanobacteria. *Archives of Microbiology* 130(4), 272-275.
- Christenson, L. and Sims, R. (2011) Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances* 29(6), 686-702.
- Collins, A. (2004) The comet assay for DNA damage and repair. *Molecular Biotechnology* 26(3), 249-261.
- Constapel, M., Schellentraeger, M., Marzinkowski, J.M. and Gaeb, S. (2009) Degradation of reactive dyes in wastewater from the textile industry by ozone: Analysis of the products by accurate masses. *Water Research* 43(3), 733-743.
- Cresson, R., Carrère, H., Delgenès, J.P. and Bernet, N. (2006) Biofilm formation during the start-up period of an anaerobic biofilm reactor—Impact of nutrient complementation. *Biochemical Engineering Journal* 30(1), 55-62.
- Cruz-Gonzalez, K., Torres-Lopez, O., Garcia-Leon, A.M., Brillas, E., Hernandez-Ramirez, A. and Peralta-Hernandez, J.M. (2012) Optimization of electro-Fenton/BDD process for decolorization of a model azo dye wastewater by means of response surface methodology. *Desalination* 286, 63-68.
- Cui, D., Li, G., Zhao, D., Gu, X., Wang, C. and Zhao, M. (2012) Microbial community structures in mixed bacterial consortia for azo dye treatment under aerobic and anaerobic conditions. *Journal of Hazardous Materials* 221, 185-192.
- Dalzell, D.J.B., Alte, S., Aspichueta, E., de la Sota, A., Etxebarria, J., Gutierrez, M., Hoffmann, C.C., Sales, D., Obst, U. and Christofi, N. (2002) A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge. *Chemosphere* 47(5), 535-545.
- de Aragão Umbuzeiro, G., Freeman, H.S., Warren, S.H., de Oliveira, D.P., Terao, Y., Watanabe, T. and Claxton, L.D. (2005) The contribution of azo dyes to the mutagenic activity of the Cristais River. *Chemosphere* 60(1), 55-64.
- De Luna, L.A.V., da Silva, T.H.G., Pupo Nogueira, R.F., Kummrow, F. and Umbuzeiro, G.A. (2014) Aquatic toxicity of dyes before and after photo-Fenton treatment. *Journal of Hazardous Materials* 276, 332-338.
- Desai, C., Pathak, H. and Madamwar, D. (2010) Advances in molecular and "-omics" technologies to gauge microbial communities and bioremediation at xenobiotic/anthropogen contaminated sites. *Bioresource Technology* 101(6), 1558-1569.
- Dorigo, U., Volatier, L. and Humbert, J.-F. (2005) Molecular approaches to the assessment of biodiversity in aquatic microbial communities. *Water Research* 39(11), 2207-2218.
- Durán, A., Monteagudo, J.M. and San Martín, I. (2012) Photocatalytic treatment of an industrial effluent using artificial and solar UV radiation: An operational cost study on a pilot plant scale. *Journal of Environmental Management* 98(0), 1-4.
- Elimelech, M. and Phillip, W.A. (2011) The future of seawater desalination: Energy, technology, and the environment. *Science* 333(6043), 712-717.

- Ellouze, M., Aloui, F. and Sayadi, S. (2010) Study on the influence of high salts content on fungal treatment of saline wastewaters. *Desalination and Water Treatment* 13(1-3), 411-417.
- Ergene, A., Ada, K., Tan, S. and Katircioglu, H. (2009) Removal of Remazol Brilliant Blue R dye from aqueous solutions by adsorption onto immobilized *Scenedesmus quadricauda*: Equilibrium and kinetic modeling studies. *Desalination* 249(3), 1308-1314.
- Ertuğrul, S., San, N.O. and Dönmez, G. (2009) Treatment of dye (Remazol Blue) and heavy metals using yeast cells with the purpose of managing polluted textile wastewaters. *Ecological Engineering* 35(1), 128-134.
- Escher, B.I. and Fenner, K. (2011) Recent advances in environmental risk assessment of transformation products. *Environmental Science & Technology* 45(9), 3835-3847.
- European Parliament, C.o.t.E.U. (2002) Directive 2002/61/EC of the European Parliament and of the Council of 19 July 2002 amending for the nineteenth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (azocolourants).
- Farabegoli, G., Chiavola, A., Rolle, E. and Naso, M. (2010) Decolorization of Reactive Red 195 by a mixed culture in an alternating anaerobic-aerobic sequencing batch reactor. *Biochemical Engineering Journal* 52(2-3), 220-226.
- Feng, W., Nansheng, D. and Helin, H. (2000) Degradation mechanism of azo dye C. I. reactive red 2 by iron powder reduction and photooxidation in aqueous solutions. *Chemosphere* 41(8), 1233-1238.
- Ferraz, E.R.A., Grando, M.D. and Oliveira, D.P. (2011a) The azo dye Disperse Orange 1 induces DNA damage and cytotoxic effects but does not cause ecotoxic effects in *Daphnia similis* and *Vibrio fischeri*. *Journal of Hazardous Materials* 192(2), 628-633.
- Ferraz, E.R.A., Umbuzeiro, G.A., de-Almeida, G., Caloto-Oliveira, A., Chequer, F.M.D., Zandoni, M.V.B., Dorta, D.J. and Oliveira, D.P. (2011b) Differential toxicity of Disperse Red 1 and Disperse Red 13 in the Ames test, HepG2 cytotoxicity assay, and *Daphnia* acute toxicity test. *Environmental Toxicology* 26(5), 489-497.
- Firmino, P.I.M., da Silva, M.E.R., Cervantes, F.J. and dos Santos, A.B. (2010) Colour removal of dyes from synthetic and real textile wastewaters in one- and two-stage anaerobic systems. *Bioresource Technology* 101(20), 7773-7779.
- Forss, J., Pinhassi, J., Lindh, M. and Welander, U. (2013) Microbial diversity in a continuous system based on rice husks for biodegradation of the azo dyes Reactive Red 2 and Reactive Black 5. *Bioresource Technology* 130(0), 681-688.
- Frijters, C., Vos, R.H., Scheffer, G. and Mulder, R. (2006) Decolorizing and detoxifying textile wastewater, containing both soluble and insoluble dyes, in a full scale combined anaerobic/aerobic system. *Water Research* 40(6), 1249-1257.
- Gafan, G.P., Lucas, V.S., Roberts, G.J., Petrie, A., Wilson, M. and Spratt, D.A. (2005) Statistical analyses of complex denaturing gradient gel electrophoresis profiles. *Journal of Clinical Microbiology* 43(8), 3971-3978.

- Garcia-Montano, J., Domenech, X., Garcia-Hortal, J.A., Torrades, F. and Peral, J. (2008a) The testing of several biological and chemical coupled treatments for Cibacron Red FN-R azo dye removal. *Journal of Hazardous Materials* 154(1-3), 484-490.
- Garcia-Montano, J., Ruiz, N., Munoz, I., Domenech, X., Garcia-Hortal, J.A., Torrades, F. and Peral, J. (2006) Environmental assessment of different photo-Fenton approaches for commercial reactive dye removal. *Journal of Hazardous Materials* 138(2), 218-225.
- Garcia-Montano, J., Torrades, F., Perez-Estrada, L.A., Oller, I., Malato, S., Maldonado, M.I. and Peral, J. (2008b) Degradation pathways of the commercial reactive azo dye Procion Red H-E7B under solar-assisted photo-Fenton reaction. *Environmental Science & Technology* 42(17), 6663-6670.
- Gendig, C., Domogala, G., Agnoli, F., Pagga, U. and Strotmann, U.J. (2003) Evaluation and further development of the activated sludge respiration inhibition test. *Chemosphere* 52(1), 143-149.
- Georgiou, D., Aivazidis, A., Hatiras, J. and Gimouhopoulos, K. (2003) Treatment of cotton textile wastewater using lime and ferrous sulfate. *Water Research* 37(9), 2248-2250.
- Guelli Ulson de Souza, S.M.d.A., Santos Bonilla, K.A. and Ulson de Souza, A.A. (2010) Removal of COD and color from hydrolyzed textile azo dye by combined ozonation and biological treatment. *Journal of Hazardous Materials* 179(1-3), 35-42.
- Guieysse, B. and Norvill, Z.N. (2014) Sequential chemical–biological processes for the treatment of industrial wastewaters: Review of recent progresses and critical assessment. *Journal of Hazardous Materials* 267(0), 142-152.
- Hai, F.I., Yamamoto, K. and Fukushi, K. (2007) Hybrid treatment systems for dye wastewater. *Critical Reviews in Environmental Science and Technology* 37(4), 315-377.
- Hisaindee, S., Meetani, M.A. and Rauf, M.A. (2013) Application of LC-MS to the analysis of advanced oxidation process (AOP) degradation of dye products and reaction mechanisms. *TRAC Trends in Analytical Chemistry* 49(0), 31-44.
- Holme, I. (2006) Sir William Henry Perkin: a review of his life, work and legacy. *Coloration Technology* 122(5), 235-251.
- Hong, P.K.A. and Zeng, Y. (2002) Degradation of pentachlorophenol by ozonation and biodegradability of intermediates. *Water Research* 36(17), 4243-4254.
- Imlay, J.A., Chin, S.M. and Linn, S. (1988) Toxic DNA damage by hydrogen-peroxide through the Fenton reaction *in vivo* and *in vitro*. *Science* 240(4852), 640-642.
- Isik, M. (2004) Efficiency of simulated textile wastewater decolorization process based on the methanogenic activity of upflow anaerobic sludge blanket reactor in salt inhibition condition. *Enzyme and Microbial Technology* 35(5), 399-404.
- Isik, M. and Sponza, D.T. (2007) Fate and toxicity of azo dye metabolites under batch long-term anaerobic incubations. *Enzyme and Microbial Technology* 40(4), 934-939.
- ISO (14044:2006) Environmental management - Life cycle assessment - Requirements and guidelines.

- Jimenez, M., Oller, I., Maldonado, M.I., Malato, S., Hernandez-Ramirez, A., Zapata, A. and Peralta-Hernandez, J.M. (2011) Solar photo-Fenton degradation of herbicides partially dissolved in water. *Catalysis Today* 161(1), 214-220.
- Jonstrup, M. (2011) Treatment of textile wastewaters using combinations of biological and physico-chemical methods, Lund University, Lund.
- Jonstrup, M., Kumar, N., Guieysse, B., Murto, M. and Mattiasson, B. (2013) Decolorization of textile dyes by *Bjerkandera* sp BOL 13 using waste biomass as carbon source. *Journal of Chemical Technology and Biotechnology* 88(3), 388-394.
- Jonstrup, M., Kumar, N., Murto, M. and Mattiasson, B. (2011) Sequential anaerobic-aerobic treatment of azo dyes: Decolourisation and amine degradability. *Desalination* 280(1-3), 339-346.
- Jonstrup, M., Waerjerstam, M., Murto, M. and Mattiasson, B. (2010) Immobilisation of TiO₂ for combined photocatalytic-biological azo dye degradation. *Water Science and Technology* 62(3), 525-531.
- Kalliala, E. and Talvenmaa, P. (2000) Environmental profile of textile wet processing in Finland. *Journal of Cleaner Production* 8(2), 143-154.
- Kapdan, I.K. and Alparslan, S. (2005) Application of anaerobic-aerobic sequential treatment system to real textile wastewater for color and COD removal. *Enzyme and Microbial Technology* 36(2-3), 273-279.
- Karci, A., Arslan-Alaton, I., Olmez-Hanci, T. and Bekbölet, M. (2012) Transformation of 2,4-dichlorophenol by H₂O₂/UV-C, Fenton and photo-Fenton processes: Oxidation products and toxicity evolution. *Journal of Photochemistry and Photobiology A: Chemistry* 230(1), 65-73.
- Khadhraoui, M., Trabelsi, H., Ksibi, M., Bouguerra, S. and Elleuch, B. (2009) Discoloration and detoxification of a Congo red dye solution by means of ozone treatment for a possible water reuse. *Journal of Hazardous Materials* 161(2-3), 974-981.
- Kim, J., Lim, J. and Lee, C. (2013) Quantitative real-time PCR approaches for microbial community studies in wastewater treatment systems: Applications and considerations. *Biotechnology Advances* 31(8), 1358-1373.
- Kiwi, J., Lopez, A. and Nadtochenko, V. (2000) Mechanism and kinetics of the OH-radical intervention during Fenton oxidation in the presence of a significant amount of radical scavenger (Cl⁻). *Environmental Science & Technology* 34(11), 2162-2168.
- Kocabas, A.M., Yukseler, H., Dilek, F.B. and Yetis, U. (2009) Adoption of European Union's IPPC Directive to a textile mill: Analysis of water and energy consumption. *Journal of Environmental Management* 91(1), 102-113.
- Koch, C., Fetzer, I., Schmidt, T., Harms, H. and Mueller, S. (2013) Monitoring functions in managed microbial systems by cytometric bar coding. *Environmental Science & Technology* 47(3), 1753-1760.
- Koch, C., Mueller, S., Harms, H. and Harnisch, F. (2014) Microbiomes in bioenergy production: From analysis to management. *Current Opinion in Biotechnology* 27, 65-72.

- Koh, J. (2011) Textile Dyeing. Chapter 10 - Dyeing with Disperse Dyes, CCBY 3.0.
- Kositzi, M., Antoniadis, A., Poullos, I., Kiridis, I. and Malato, S. (2004) Solar photocatalytic treatment of simulated dyestuff effluents. *Solar Energy* 77(5), 591-600.
- Koyuncu, I., Topacik, D. and Yuksel, E. (2004) Reuse of reactive dyehouse wastewater by nanofiltration: process water quality and economical implications. *Separation and Purification Technology* 36(1), 77-85.
- Kudlich, M., Hetheridge, M.J., Knackmuss, H.J. and Stolz, A. (1999) Autoxidation reactions of different aromatic o-aminohydroxynaphthalenes that are formed during the anaerobic reduction of sulfonated azo dyes. *Environmental Science & Technology* 33(6), 896-901.
- Lee, J., Kim, J. and Choi, W. (2007) Oxidation on zerovalent iron promoted by polyoxometalate as an electron shuttle. *Environmental Science & Technology* 41(9), 3335-3340.
- Lemire, J.A., Harrison, J.J. and Turner, R.J. (2013) Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat Rev Micro* 11(6), 371-384.
- Libra, J.A., Borchert, M. and Banit, S. (2003) Competition strategies for the decolorization of a textile-reactive dye with the white-rot fungi *Trametes versicolor* under non-sterile conditions. *Biotechnology and Bioengineering* 82(6), 736-744.
- Lim, S.-L., Chu, W.-L. and Phang, S.-M. (2010) Use of *Chlorella vulgaris* for bioremediation of textile wastewater. *Bioresource Technology* 101(19), 7314-7322.
- Liu, G., Zhou, J., Meng, X., Fu, S.Q., Wang, J., Jin, R. and Lv, H. (2013) Decolorization of azo dyes by marine *Shewanella strains* under saline conditions. *Applied Microbiology and Biotechnology* 97(9), 4187-4197.
- Liu, R., Chiu, H.M., Shiau, C.S., Yeh, R.Y.L. and Hung, Y.T. (2007) Degradation and sludge production of textile dyes by Fenton and photo-Fenton processes. *Dyes and Pigments* 73(1), 1-6.
- Lotito, A.M., Fratino, U., Bergna, G. and Di Iaconi, C. (2012) Integrated biological and ozone treatment of printing textile wastewater. *Chemical Engineering Journal* 195, 261-269.
- Lu, M.C., Chen, J.N. and Chang, C.P. (1997) Effect of inorganic ions on the oxidation of dichlorvos insecticide with Fenton's reagent. *Chemosphere* 35(10), 2285-2293.
- Lucas, M.S., Dias, A.A., Sampaio, A., Amaral, C. and Peres, J.A. (2007) Degradation of a textile reactive azo dye by a combined chemical-biological process: Fenton's reagent-yeast. *Water Research* 41(5), 1103-1109.
- Lucking, F., Koser, H., Jank, M. and Ritter, A. (1998) Iron powder, graphite and activated carbon as catalysts for the oxidation of 4-chlorophenol with hydrogen peroxide in aqueous solution. *Water Research* 32(9), 2607-2614.
- Maciel, R., Sant'Anna Jr, G.L. and Dezotti, M. (2004) Phenol removal from high salinity effluents using Fenton's reagent and photo-Fenton reactions. *Chemosphere* 57(7), 711-719.

- Magdeburg, A., Stalter, D., Schliuesener, M., Ternes, T. and Oehlmann, J. (2014) Evaluating the efficiency of advanced wastewater treatment: Target analysis of organic contaminants and (geno-)toxicity assessment tell a different story. *Water Research* 50, 35-47.
- Manenti, D.R., Modenes, A.N., Soares, P.A., Boaventura, R.A.R., Palacio, S.M., Borba, F.H., Espinoza-Quinones, F.R., Bergamasco, R. and Vilar, V.J.P. (2015) Biodegradability and toxicity assessment of a real textile wastewater effluent treated by an optimized electrocoagulation process. *Environmental Technology* 36(4), 496-506.
- Mathur, N., Bhatnagar, P., Mohan, K., Bakre, P., Nagar, P. and Bijarnia, M. (2007) Mutagenicity evaluation of industrial sludge from common effluent treatment plant. *Chemosphere* 67(6), 1229-1235.
- McCann, J., Spingarn, N.E., Kobori, J. and Ames, B.N. (1975) Detection of carcinogens as mutagens - bacterial tester strains with r-factor plasmids. *Proceedings of the National Academy of Sciences of the United States of America* 72(3), 979-983.
- Mendoza, L., Jonstrup, M., Hatti-Kaul, R. and Mattiasson, B. (2011) Azo dye decolorization by a laccase/mediator system in a membrane reactor: Enzyme and mediator reusability. *Enzyme and Microbial Technology* 49(5), 478-484.
- Mestankova, H., Schirmer, K., Canonica, S. and von Gunten, U. (2014) Development of mutagenicity during degradation of N-nitrosamines by advanced oxidation processes. *Water Research* 66, 399-410.
- Michael, I., Panagi, A., Ioannou, L.A., Frontistis, Z. and Fatta-Kassinos, D. (2014) Utilizing solar energy for the purification of olive mill wastewater using a pilot-scale photocatalytic reactor after coagulation-flocculation. *Water Research* 60, 28-40.
- Misik, M., Knasmueller, S., Ferk, F., Cichna-Markl, M., Grummt, T., Schaar, H. and Kreuzinger, N. (2011) Impact of ozonation on the genotoxic activity of tertiary treated municipal wastewater. *Water Research* 45(12), 3681-3691.
- Mohan, S.V., Ramanaiah, S.V. and Sarma, P.N. (2008) Biosorption of direct azo dye from aqueous phase onto *Spirogyra* sp 102: Evaluation of kinetics and mechanistic aspects. *Biochemical Engineering Journal* 38(1), 61-69.
- Moosvi, S. and Madamwar, D. (2007) An integrated process for the treatment of CETP wastewater using coagulation, anaerobic and aerobic process. *Bioresource Technology* 98(17), 3384-3392.
- Moradas, G., Auresenia, J., Gallardo, S. and Guieysse, B. (2008) Biodegradability and toxicity assessment of trans-chlordane photochemical treatment. *Chemosphere* 73(9), 1512-1517.
- Muñoz, I., Rieradevall, J., Torrades, F., Peral, J. and Domènech, X. (2006) Environmental assessment of different advanced oxidation processes applied to a bleaching Kraft mill effluent. *Chemosphere* 62(1), 9-16.
- Muñoz, R. and Guieysse, B. (2006) Algal-bacterial processes for the treatment of hazardous contaminants: A review. *Water Research* 40(15), 2799-2815.

- Munoz, R., Kollner, C., Guieysse, B. and Mattiasson, B. (2003) Salicylate biodegradation by various algal-bacterial consortia under photosynthetic oxygenation. *Biotechnology Letters* 25(22), 1905-1911.
- Murali, V., Ong, S.-A., Ho, L.-N. and Wong, Y.-S. (2013) Evaluation of integrated anaerobic-aerobic biofilm reactor for degradation of azo dye Methyl Orange. *Bioresource Technology* 143(0), 104-111.
- Muthuraman, G., Teng, T.T., Leh, C.P. and Norli, I. (2009) Extraction and recovery of methylene blue from industrial wastewater using benzoic acid as an extractant. *Journal of Hazardous Materials* 163(1), 363-369.
- Muyzer, G. and Smalla, K. (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 73(1), 127-141.
- Nagarajan, K. and Loh, K.-C. (2014) Molecular biology-based methods for quantification of bacteria in mixed culture: perspectives and limitations. *Applied Microbiology and Biotechnology* 98(16), 6907-6919.
- Nagel-Hassemer, M.E., Coral, L.A., Lapolli, F.R. and Souza Pessoa de Amorim, M.T. (2012) UV/H₂O₂ process as post-treatment for removal of color and polishing end in textile wastewater. *Quimica Nova* 35(5), 900-904.
- Nicolella, C., van Loosdrecht, M.C.M. and Heijnen, J.J. (2000) Wastewater treatment with particulate biofilm reactors. *Journal of Biotechnology* 80(1), 1-33.
- Nöthe, T., Fahlenkamp, H. and von Sonntag, C. (2009) Ozonation of wastewater: Rate of ozone consumption and hydroxyl radical yield. *Environmental Science & Technology* 43(15), 5990-5995.
- Novotny, C., Dias, N., Kapanen, A., Malachova, K., Vandrovcova, M., Itavaara, M. and Lima, N. (2006) Comparative use of bacterial, algal and protozoan tests to study toxicity of azo- and anthraquinone dyes. *Chemosphere* 63(9), 1436-1442.
- Olive, P.L. and Banath, J.P. (2006) The comet assay: a method to measure DNA damage in individual cells. *Nat. Protocols* 1(1), 23-29.
- Onat, T.A., Gumusdere, H.T., Guvenc, A., Donmez, G. and Mehmetoglu, U. (2010) Decolorization of textile azo dyes by ultrasonication and microbial removal. *Desalination* 255(1-3), 154-158.
- O'Neill, C., Hawkes, F.R., Hawkes, D.L., Lourenco, N.D., Pinheiro, H.M. and Delee, W. (1999) Colour in textile effluents - sources, measurement, discharge consents and simulation: a review. *Journal of Chemical Technology and Biotechnology* 74(11), 1009-1018.
- O'Neill, C., Lopez, A., Esteves, S., Hawkes, F.R., Hawkes, D.L. and Wilcox, S. (2000) Azo-dye degradation in an anaerobic-aerobic treatment system operating on simulated textile effluent. *Applied Microbiology and Biotechnology* 53(2), 249-254.
- Orozco, S.L., Bandala, E.R., Arancibia-Bulnes, C.A., Serrano, B., Suarez-Parra, R. and Hernandez-Perez, I. (2008) Effect of iron salt on the color removal of water containing

- the azo-dye reactive blue 69 using photo-assisted Fe(II)/H₂O₂ and Fe(III)/H₂O₂ systems. *Journal of Photochemistry and Photobiology a-Chemistry* 198(2-3), 144-149.
- Othman, N., Zailani, S.N. and Mili, N. (2011) Recovery of synthetic dye from simulated wastewater using emulsion liquid membrane process containing tri-dodecyl amine as a mobile carrier. *Journal of Hazardous Materials* 198, 103-112.
- Ozdemir, S., Cirik, K., Akman, D., Sahinkaya, E. and Cinar, O. (2013) Treatment of azo dye-containing synthetic textile dye effluent using sulfidogenic anaerobic baffled reactor. *Bioresource Technology* 146(0), 135-143.
- Pace, N.R. (1997) A molecular view of microbial diversity and the biosphere. *Science* 276(5313), 734-740.
- Pang, Y.L. and Abdullah, A.Z. (2013) Fe³⁺ doped TiO₂ nanotubes for combined adsorption-sonocatalytic degradation of real textile wastewater. *Applied Catalysis B-Environmental* 129, 473-481.
- Papic, S. (2009) Decolourization and mineralization of commercial reactive dyes by using homogeneous and heterogeneous Fenton and UV/Fenton processes. *Journal of Hazardous materials* 164 (2-3), 1137-45.
- Parikh, A. and Madamwar, D. (2005) Textile dye decolorization using cyanobacteria. *Biotechnology Letters* 27(5), 323-326.
- Pignatello, J.J., Liu, D. and Huston, P. (1999) Evidence for an additional oxidant in the photoassisted Fenton reaction. *Environmental Science & Technology* 33(11), 1832-1839.
- Pinheiro, H.M., Touraud, E. and Thomas, O. (2004) Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. *Dyes and Pigments* 61(2), 121-139.
- Pophali, G.R., Kaul, S.N. and Mathur, S. (2003) Influence of hydraulic shock loads and TDS on the performance of large-scale CETPs treating textile effluents in India. *Water Research* 37(2), 353-361.
- Porter, J.J., Nolan, W.F. and Lyons, D.W. (1972) Water uses and wastes in textile industry. *Environmental Science & Technology* 6(1), 36-41.
- Posadas, E., Garcia-Encina, P.-A., Soltan, A., Dominguez, A., Diaz, I. and Munoz, R. (2013) Carbon and nutrient removal from concentrates and domestic wastewater using algal-bacterial biofilm bioreactors. *Bioresource Technology* 139, 50-58.
- Prieto, O., Feroso, J., Nunez, Y., del Valle, J.L. and Irusta, R. (2005) Decolouration of textile dyes in wastewaters by photocatalysis with TiO₂. *Solar Energy* 79(4), 376-383.
- Priya, B., Uma, L., Ahamed, A.K., Subramanian, G. and Prabakaran, D. (2011) Ability to use the diazo dye, C.I. Acid Black 1 as a nitrogen source by the marine cyanobacterium *Oscillatoria curvipes* BDU92191. *Bioresource Technology* 102(14), 7218-7223.
- Qian, F.-y., Sun, X.-b. and Liu, Y.-d. (2013) Effect of ozone on removal of dissolved organic matter and its biodegradability and adsorbability in biotreated textile effluents. *Ozone-Science & Engineering* 35(1), 7-15.

- Radetski, C.M., Rosa, S.M.C., Rosa, E.V.C., Sierra, M.M.D. and Simionatto, E.L. (2002) Ozonation of textile wastewater: Physicochemical and phytotoxic aspects. *Environmental Technology* 23(5), 537-545.
- Rappe, M.S. and Giovannoni, S.J. (2003) The uncultured microbial majority. *Annual Review of Microbiology* 57, 369-394.
- Rehorek, A. and Plum, A. (2006) Online LC-MS-MS process monitoring for optimization of biological treatment of wastewater containing azo dye concentrates. *Analytical and Bioanalytical Chemistry* 384(5), 1123-1128.
- Reifferscheid, G., Maes, H.M., Allner, B., Badurova, J., Belkin, S., Bluhm, K., Brauer, F., Bressling, J., Domeneghetti, S., Elad, T., Fluckiger-Isler, S., Grummt, H.J., Guertler, R., Hecht, A., Heringa, M.B., Hollert, H., Huber, S., Kramer, M., Magdeburg, A., Ratte, H.T., Sauerborn-Klobucar, R., Sokolowski, A., Soldan, P., Smital, T., Stalter, D., Venier, P., Ziemann, C., Zipperle, J. and Buchinger, S. (2012) International round-robin study on the Ames fluctuation test. *Environmental and Molecular Mutagenesis* 53(3), 185-197.
- Rizzo, L. (2011) Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. *Water Research* 45(15), 4311-4340.
- Rodrigues da Silva, M.E., Milen Firmino, P.I., de Sousa, M.R. and dos Santos, A.B. (2012) Sequential anaerobic/aerobic treatment of dye-containing wastewaters: Colour and cod removals, and ecotoxicity tests. *Applied Biochemistry and Biotechnology* 166(4), 1057-1069.
- Rodrigues, C.S.D., Madeira, L.M. and Boaventura, R.A.R. (2009) Treatment of textile effluent by chemical (Fenton's Reagent) and biological (sequencing batch reactor) oxidation. *Journal of Hazardous Materials* 172(2-3), 1551-1559.
- Rodrigues, C.S.D., Madeira, L.M. and Boaventura, R.A.R. (2014) Decontamination of an industrial cotton dyeing wastewater by chemical and biological processes. *Industrial & Engineering Chemistry Research* 53(6), 2412-2421.
- Rodriguez, M., Sarria, V., Esplugas, S. and Pulgarin, C. (2002) Photo-Fenton treatment of a biorecalcitrant wastewater generated in textile activities: biodegradability of the photo-treated solution. *Journal of Photochemistry and Photobiology a-Chemistry* 151(1-3), 129-135.
- Saha, S.K., Swaminathan, P., Raghavan, C., Uma, L. and Subramanian, G. (2010) Ligninolytic and antioxidative enzymes of a marine cyanobacterium *Oscillatoria willei* BDU 130511 during Poly R-478 decolourization. *Bioresource Technology* 101(9), 3076-3084.
- Sánchez Pérez, J.A., Román Sánchez, I.M., Carra, I., Cabrera Reina, A., Casas López, J.L. and Malato, S. (2013) Economic evaluation of a combined photo-Fenton/MBR process using pesticides as model pollutant. Factors affecting costs. *Journal of Hazardous materials* 244-245(0), 195-203.
- Schröder, H.F. (1996) Polar organic pollutants from textile industries in the wastewater treatment process — biochemical and physicochemical elimination and degradation

- monitoring by LC-MS, FIA-MS and MS-MS. *TRAC Trends in Analytical Chemistry* 15(8), 349-362.
- Semple, K.T., Cain, R.B. and Schmidt, S. (1999) Biodegradation of aromatic compounds by microalgae. *FEMS Microbiology Letters* 170(2), 291-300.
- Sharma, K.P., Sharma, S., Singh, P.K., Kumar, S., Grover, R. and Sharma, P.K. (2007) A comparative study on characterization of textile wastewaters (untreated and treated) toxicity by chemical and biological tests. *Chemosphere* 69(1), 48-54.
- Shehzadi, M., Afzal, M., Khan, M.U., Islam, E., Mobin, A., Anwar, S. and Khan, Q.M. (2014) Enhanced degradation of textile effluent in constructed wetland system using *Typha domingensis* and textile effluent-degrading endophytic bacteria. *Water Research* 58(0), 152-159.
- Shwe, M.T., Dimaculangan, M.M. and de Luna, M.D.G. (2014) *Frontiers in Materials and Minerals Engineering*. Othman, R., Baharun, N. and Pung, S.Y. (eds), pp. 40-45.
- Solis, M., Solis, A., Perez, H.I., Manjarrez, N. and Flores, M. (2012) Microbial decolouration of azo dyes: A review. *Process Biochemistry* 47(12), 1723-1748.
- Somens, C.A., Simionatto, E.L., Bertoli, S.L., Wisniewski, A., Jr. and Radetski, C.M. (2010) Use of ozone in a pilot-scale plant for textile wastewater pre-treatment: Physico-chemical efficiency, degradation by-products identification and environmental toxicity of treated wastewater. *Journal of Hazardous Materials* 175(1-3), 235-240.
- Von Sonntag, C. and von Gunten, U. (2012) *Chemistry of ozone in water and wastewater treatment: from basic principles to applications*. IWA Publishing.
- Spagni, A., Casu, S. and Grilli, S. (2012) Decolourisation of textile wastewater in a submerged anaerobic membrane bioreactor. *Bioresource Technology* 117, 180-185.
- Staehelin, J. and Hoigne, J. (1985) Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. *Environmental Science & Technology* 19(12), 1206-1213.
- Su, Y., Mennerich, A. and Urban, B. (2011) Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. *Water Research* 45(11), 3351-3358.
- Tabrizi, M.T.F., Glasser, D. and Hildebrandt, D. (2011) Wastewater treatment of reactive dyestuffs by ozonation in a semi-batch reactor. *Chemical Engineering Journal* 166(2), 662-668.
- Tabrizi, M.T.F., Glasser, D. and Hildebrandt, D. (2013) Ozonation of textile Reactive Red 198 dye in a CSTR. *Journal of Advanced Oxidation Technologies* 16(1), 159-167.
- Tan, L., Qu, Y., Zhou, J., Ma, F. and Li, A. (2010) Microbial community shifts in sequencing batch reactors for azo dye treatment. *Pure and Applied Chemistry* 82(1), 299-306.
- Tang, W.Z. and Chen, R.Z. (1996) Decolorization kinetics and mechanisms of commercial dyes by H₂O₂/iron powder system. *Chemosphere* 32(5), 947-958.

- Thomas, S., Sreekanth, R., Sijumon, V.A., Aravind, U.K. and Aravindakumar, C.T. (2014) Oxidative degradation of Acid Red 1 in aqueous medium. *Chemical Engineering Journal* 244(0), 473-482.
- Tosik, R. and Wiktorowski, S. (2001) Color removal and improvement of biodegradability of wastewater from dyes production using ozone and hydrogen peroxide. *Ozone-Science & Engineering* 23(4), 295-302.
- Vasileiadis, S., Coppolecchia, D., Puglisi, E., Balloi, A., Mapelli, F., Hamon, R.E., Daffonchio, D. and Trevisan, M. (2012) Response of ammonia oxidizing bacteria and archaea to acute zinc stress and different moisture regimes in soil. *Microbial Ecology* 64(4), 1028-1037.
- Velegriaki, T. and Mantzavinos, D. (2015) Solar photo-Fenton treatment of winery effluents in a pilot photocatalytic reactor. *Catalysis Today* 240, 153-159.
- Verma, A.K., Dash, R.R. and Bhunia, P. (2012) A review on chemical coagulation/flocculation technologies for removal of colour from textile wastewaters. *Journal of Environmental Management* 93(1), 154-168.
- Vishnu, G., Palanisamy, S. and Joseph, K. (2008) Assessment of field scale zero liquid discharge treatment systems for recovery of water and salt from textile effluents. *Journal of Cleaner Production* 16(10), 1081-1089.
- Von Gunten, U. (2003) Ozonation of drinking water: Part I. Oxidation kinetics and product formation. *Water Research* 37(7), 1443-1467.
- Von Gunten, U. (2007) The basics of oxidants in water treatment. Part B: ozone reactions. *Water Science and Technology* 55(12), 25-29.
- Von Sonntag, C. (2008) Advanced oxidation processes: mechanistic aspects. *Water Science and Technology* 58(5), 1015-1021.
- Vyrides, I. and Stuckey, D.C. (2009) Effect of fluctuations in salinity on anaerobic biomass and production of soluble microbial products (SMPs). *Biodegradation* 20(2), 165-175.
- Wang, C., Liu, H. and Sun, Z. (2012) Heterogeneous photo-Fenton reaction catalyzed by nanosized iron oxides for water treatment. *International Journal of Photoenergy* 2012, 1-10.
- Weschenfelder, S.E., Jose, H.J., Gebhardt, W. and Schroeder, H.F. (2007) Monitoring the physicochemical and chemical treatment of textile wastewater using GC/MS, LC/MS and -MS/MS techniques. *Separation Science and Technology* 42(7), 1535-1551.
- Yamada, M., Sedgwick, B. and Sofuni, T. (1995) Construction and characterization of mutants of *Salmonella typhimurium* deficient in DNA-repair of O6-methylguanine. *Journal of Bacteriology* 177(6), 1511-1519.
- Yamada, M., Matsui, K., Sofuni, T. and Nohmi, T. (1997) New tester strains of *Salmonella typhimurium* lacking O6-methylguanine DNA methyltransferases and highly sensitive to mutagenic alkylating agents. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis* 381(1), 15-24.

- Nohmi, T., Yang, Q., Wang, J., Wang, H., Chen, X., Ren, S., Li, X., Xu, Y., Zhang, H. and Li, X. (2012) Evolution of the microbial community in a full-scale printing and dyeing wastewater treatment system. *Bioresource Technology* 117, 155-163.
- Yang, S., Guo, Z., Miao, F., Xue, Q. and Qin, S. (2010) The hydroxyl radical scavenging activity of chitosan, hyaluronan, starch and their O-carboxymethylated derivatives. *Carbohydrate Polymers* 82(4), 1043-1045.
- Yoshida, N., Ikeda, R. and Okuno, T. (2006) Identification and characterization of heavy metal-resistant unicellular alga isolated from soil and its potential for phytoremediation. *Bioresource Technology* 97(15), 1843-1849.
- Yu, R.-F., Lin, C.-H., Chen, H.-W., Cheng, W.-P. and Kao, M.-C. (2013) Possible control approaches of the electro-Fenton process for textile wastewater treatment using on-line monitoring of DO and ORP. *Chemical Engineering Journal* 218, 341-349.
- Yu, Z.T. and Morrison, M. (2004) Comparisons of different hypervariable regions of *rrs* genes for use in fingerprinting of microbial communities by PCR-denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology* 70(8), 4800-4806.
- Yuan, Z.-W., Zhu, Y.-N., Shi, J.-K., Liu, X. and Huang, L. (2013) Life-cycle assessment of continuous pad-dyeing technology for cotton fabrics. *International Journal of Life Cycle Assessment* 18(3), 659-672.
- Zapata, A., Oller, I., Sirtori, C., Rodriguez, A., Sanchez-Perez, C.A., Lopez, A., Mezcua, M. and Malato, S. (2010) Decontamination of industrial wastewater containing pesticides by combining large-scale homogeneous solar photocatalysis and biological treatment. *Chemical Engineering Journal* 160(2), 447-456.
- Zhang, J., Chen, S., Zhang, Y., Quan, X., Zhao, H. and Zhang, Y. (2014) Reduction of acute toxicity and genotoxicity of dye effluent using Fenton-coagulation process. *Journal of Hazardous Materials* 274(0), 198-204.
- Zhao, W., Liao, Q., Zhang, J., Yang, Y., Dai, J. and Zhao, D. (2011) Oxidation of Cationic Red X-GRL by ozonation combined with UV radiation in aqueous solution: Degradation, kinetics, and modeling. *Chemical Engineering Journal* 171(2), 628-639.