ISTANBUL TECHNICAL UNIVERSITY « GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

OXIDATION OF AQUEOUS BISPHENOL A WITH THE FENTON'S REAGENT: THE EFFECTS OF OPERATING PARAMETERS ON PROCESS PERFORMANCE AND TOXICITY EVALUATIONS

M.Sc. THESIS

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Environmental Engineering Department

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

SULU BİSFENOL A ÇÖZELTİSİNİN FENTON REAKTİFİYLE OKSİDASYONU: İŞLETME PARAMETRELERİNİN PROSES PERFORMANSI ÜZERİNDEKİ ETKİLERİ VE TOKSİSİTE DEĞERLENDİRMELERİ

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HAZİRAN, 2013

Ece Aytaç, a M.Sc. student of ITU Graduate School of Science Engineering and Technology student ID 501111712, successfully defended the thesis/dissertation entitled "**OXIDATION OF AQUEOUS BISPHENOL A WITH THE FENTON'S REAGENT: THE EFFECTS OF OPERATING PARAMETERS ON PROCESS PERFORMANCE AND TOXICITY EVALUATIONS**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury

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Date of Submission : 03 May 2013
Date of Defense : 05 June 2013

To my family and colleagues,

FOREWORD

This thesis was written for my Masterdegree during the period of Autumn 2012 and Spring 2013, under the co-operation of Technical University of Denmark (DTU) and Istanbul Technical University (ITU), in Department of Environmental Engineering.

The intent of the thesis is to assess oxidation of aqueous bisphenol A with the Fenton's reagent, the effects of operating parameteres on process performance and toxicity.Bisphenol A and total organic carbon removals were observed as well as hydrogen peroxide consumptions depending on time. The toxicity was analyzed by acute toxicity test on the freshwater crustacean *Daphnia magna*, biotox test method based on measurement of light emission from the photobacteria *Vibrio fischeri* and growth inhibition test with *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*).

I would liketo express my deepest appreciation to my thesis supervisor, İdil Arslan-Alaton, and my project supervisors at DTU, namely Kresten Ole Kusk and Signe Qualmann, of being great help during the development of this thesis.

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I would like to dedicate my thesis to my beloved parents who always support me materially and morally.

June, 2013

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ABBREVIATIONS

AOP	**:** Advanced Oxidation Process
BPA	**:** Bisphenol A
COD	**:** Chemical Oxygen Demand
DO	**:** Dissolved Oxygen
DOM	**:** Dissolved Organic Matter
EDCs	**:**Endocrine Disrupting Compounds
EC_{50}	**:** Median Effective Concentration
LD_{50}	**:**Lethal Concentration causing 50% inhibition in the test organism
HPLC	**:** High Performance Liquid Chromatography
LOEC	**:**Lowest Observed Effect Concentration
NOEC	**:**No Observed Effect Concentration
PAEs	**:** Phthalic Acid Esters
ROS	**:**Radical Oxygen Species
TDS	**:** Total Dissolved Solids
TOC	**:** Total Organic Carbon
TSS	**:**Total Suspended Solids

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OXIDATION OF AQUEOUS BISPHENOL A WITH THE FENTON'S REAGENT: THE EFFECTS OF OPERATING PARAMETERS ON PROCESS PERFORMANCE AND TOXICITY EVALUATIONS

SUMMARY

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane; BPA), is a chemical being widely used as a monomer for the production of epoxy resins and polycarbonate, unsaturated polyester-styrene resins and flame retardants. The final industrial products are used as coating materials on cans, as powder paints, additives in thermal paper, in dental fillings and as antioxidants in plastics. It has been postulated that BPA has estrogenic activity and is classified as an endocrine disrupting compound (EDC). Due to its high consumption rate and adverse health effects on wildlife, BPA is known as one of the industrial pollutants that have generated significant academic as well as public interest recently. BPA is being released into the natural environment as well as surface water during its manufacturing and by leaching from final products If not treated properly, effluent containing BPA is a potential source of contamination in the aquatic environment. Due to the fact that biotreatment requires long retention times and cannot degrade BPA completely. rapid and efficient treatment processes including advanced oxidation processes (AOPs) have successfully been developed for the efficient treatment of BPA. Among the studied AOPs, the Fenton's reagent is kinetically attractive, less expensive in terms of operating costs, well-known and established. However, as for all AOPs, more toxic/mutagenic oxidation products than the original pollutant could potentially form during the application of Fenton's treatment to oxidize BPA and its organic carbon content. Consequently, it is of major importance to follow changes in toxicity during the application of AOPs, preferrably by conducting battery tests to examine potential toxic effects on tests organisms belonging to different trophic levels.

Considering the above mentioned issues, the present study aimed at investigating the effect of Fenton's treatment of aqueous BPA solution under varying reaction conditions on its toxicity using three different test organisms, namely the photobacterium *Vibrio fischeri* (decomposer level), the freshwater cladoceran *Daphnia magna* (consumer level) and the microalgae *Pseudokirchnerialle subcapitata* (prodcuer level). Fenton experiments were conducted in pure water, real freshwater samples, real lakewater samples as well as the growth medium of the test organisms.

In the first part of the study which was conducted at Istanbul Technical University, the effect of different process variables of the Fenton's reagent including the initial BPA, H_2O_2 and Fe^{2+} concentrations, pH, temperature and water matrix (pure and real

freshwater) on the treatment performance (BPA and TOC removal rates) being observed during the application of the Fenton's reagent to degrade BPA as well as its TOC content, was investigated. Acute toxicity changes in pure and real freshwater samples spiked with BPA indicated that in the presence of 0.4 mM Fe^{2+} and 2.0 mM H_2O_2 at room temperature (20 ^oC) and pH=5, complete BPA removals could be achieved within 1-2 min. The corresponding TOC removal efficiencies ranged between 34-41% at at room temperature after 90 min treatment and could not be enhanced significantly by increasing the reaction temperature. However, in the presence of 0.4 mM of Fe^{2+} and 4.0 (higher initial concentration) mM of H_2O_2 at pH=5 and elevated temperatures (T=50 ^oC), the overall TOC removal could be increased to 60% in pure water and 75% in real freshwater after 120 min treatment, whereas BPA degradation occurred promptly within the first min of the reaction.The enhancement was mainly due to reagent concentrations and not temperature.

Toxicity test results obtained at Istanbul Technical University indicated that the luminescence inhibition rate of *Vibrio fischeri* could be reduced from 70% (original BPA solution) to 12% in pure water and 23% in real freshwater after only 1 min Fenton's treatment. Complete detoxification was achieved after 40-50 min and 90 min treatmentin the pure and real freshwater samples, respectively. Detoxification patterns generally paralleled BPA degradation profiles.A similar toxicity pattern was ovserved for *V. fischeri* responses at the Technical University of Denmark, although the Fenton process was significantly inhibited in the saltwater medium.

Acute toxicity test results obtained with*Daphnia magna at* at the Technical University of Denmarkdemonstrated that percent immobilization dropped from 70% to around 10% (24 h) and from 100% to around 20% (48 h) after 30 min and 60 min Fenton's reaction, respectively andcomplete detoxification could be achieved after 90 treatment. The growth inhibition test conducted with the microalgae*Pseudokirchneriella subcapitata*in its growth mdium and real lakewater revealed that the relative inhibition value was 100% in the original BPA solution and did not drop below 40% during 90 min Fenton's treatment. Removal rates and toxicity were significantly affected by the water matrix and decreased appreciably in real lakewater samples. Considering the toxicity test results obtained during Fenton's treatment of BPA in pure water, different water matrices, and growth media it could be concluded that the relative sensitivity of the test species used in the present work was *Pseudokirchneriella subcapitata*>*Daphnia magna* >*Vibrio fischeri.* The battery test results demonstrated not only BPA, but also the oxidation products of the Fenton's reagent caused inhibitory effects.

SULU BİSFENOL A ÇÖZELTİSİNİN FENTON REAKTİFİYLE OKSİDASYONU: İŞLETME PARAMETRELERİNİN PROSES PERFORMANSI ÜZERİNDEKİ ETKİLERİ VE TOKSİSİTE DEĞERLENDİRMELERİ

ÖZET

İki fenol ve polikarbonat moleküllerinin birleşmesiyle oluşan bisfenol A (BFA), günümüzde genellikle plastik, naylon, polyester, epoksi reçine gibi maddelerin üretilmesinde ara ürün olarak kullanılmaktadır. Fenolik kokulu, krem-beyaz renkte ve kristal yapıda olan bir tür organik bileşiktir. Sudaki çözünürlüğü düşük olmakla birlikte (120 mg/L) etanol ve aseton gibi çözücülerde iyi çözünmektedir.

BFA'nın çevreye salınımının BFA içeren zararlı atık depolama sahaları; BFA üretimi sonucu oluşan partiküller; depolama, proses ve üretim faatliyetleri sonucu oluşan atıksu deşarjları ve bu süreçlerde oluşan kazalar gibi çeşitli yollarla olduğu tespit edilmiştir. Yapılan toksisite çalışmalarıyla, BFA'nın özellikle hamile kadınlarda ve çocuklarda üreme, büyüme ve gelişme üzerinde toksik etki yarattığı; dişi cinsiyet hormonu olan östrojeni taklit ettiği; meme, prostat ve testis kanserini tetiklediği; teratojenik etkileri olabildiği bulunmuş ve endokrin bozucu bileşik olarak nitelendirilmiştir.

Biyolojik olarak ayrışabilirliğinin zor olması, mikrobiyolojik proseslerde toksik etkisi yaratması ve ayrıştığında ara ürünlerin kendisinden daha toksik etkide olabilmesinden dolayı BFA'nın çeşitli arıtım yöntemleri üzerine çalışmalar yapılmıştır. Adsropsiyon, membran bazlı filtrasyon ve ozonlama gibi proseslerle istenilen verim elde edilememiştir. BFA'nın etkin giderimi için alternatif arıtım yöntemleri araştırılmıştır. Çeşitli çalışmalar, ileri oksidasyon proseslerinin endokrin bozucu bileşiklerin arıtılmasında, konvansiyonel proseslerden daha etkili olduğunu göstermiştir.

Dirençli ve toksik organik/inorganik maddelerin arıtımında yaygın olarak kullanılan ileri oksidasyon teknikleri çok güçlü bir oksitleyici olan hidroksil radikalinin (HO•) oluşmasına ve bunun reaksiyonlarına dayanmaktadır. HO• oluşumuna dayanan prosesler arasında ozonlama, Fenton, perokson (O_3/H_2O_2) ya da TiO_2 ve ZnO gibi katalizlerle UV'nin kombinasyonu sayılabilmektedir. Uygulama kolaylığı, maliyet ve başarılı olarak uygulanabilirliği açısından daha çok Fenton ve UV/H_2O_2 prosesleri dikkat çekmektedir. Bu çalışmada da yürütülen Fenton prosesi, Fe^{+2} ve Fe^{+3}'nin H_2O_2 ile birlikte asidik şartlarda katalitik olarak reaksiyonuna dayanmaktadır.

Günümüzde, BFA'nın atıksular, sedimentler ve kontamine olmuş sular için ekotoksikolojik durumu çevre kirliliğinin önemli bir bölümünü oluşturmaktadır. BFA'nın toksik etkilerinin incelenmesi için hızlı, kolay, hassas ve maliyet açısından da uygun olan çeşitli toksisite testleri yapılmıştır. Bu testler için ise

mikroorganizmalar, bitki ve algler, omurgasızlar, kemirgenler ve balık türleri kullanılmıştır.

Bu çalışmada; BFA, H_2O_2 ve Fe^{2+} konsantrasyonları, pH ve sıcaklık gibi işletme parametrelerinin Fenton prosesi üzerindeki etkileri,Fenton prosesi boyunca BFA'nın toksisitesinin saf suda, gerçek sularda ve çeşitli organik ve inorganik maddeler içeren farklı test ortamlarında gösterdiği değişikliklerin incelenmesi amaçlanmıştır. Bu amaçla, çalışmanın birinci kısmı, İstanbul Teknik Üniversitesi'nde (İTÜ) yürütülmüş, Fenton prosesinin, BFA ve TOK giderimleri üzerinde H_2O_2 (0.50-5.00 mM) ve Fe^{2+}(0.05-1.0 mM) konsantrasyonları, pH (3-6), sıcaklık (20˚C-70˚C), saf ve gerçek ham su üzerindeki etkileri araştırılmıştır. Akut toksisite çalışmaları fotobakteri *Vibrio fischeri*(çürütücü) kullanılmıştır. Bu çalışmalar, 20 mg/L BFA için, 120 dakikalık Fenton prosesi boyunca, pH 5'te, H_2O_2:Fe^{2+}= 10:1 (H_2O_2=4.0 mM; Fe^{2+}=0.4 mM) oranında ve 50˚C'de saf ve gerçek ham su ortamlarında yürütülmüştür. Aynı zamanda, H_2O_2 miktarı iki katına çıkarıldığında (4 mM) ve sıcaklık 50˚C iken, BFA ve TOK giderim verimlerinin gösterdiği değişimler de incelenmiştir.

Danimarka Teknik Üniversitesi'nde (DTÜ) yürütülen çalışmanın ikinci kısmında ise, fotobakteri *Vibrio fischeri*, tatlı su piresi *Daphnia magna* (tüketici) ve mikroalg *Pseudokirchneriella subcapitata* (üretici) kullanılarak BFA'nın farklı test ortamlarında ve göl suyundaki toksisitesi araştırılmıştır. Toksisite deneyleri, 20 mg/L BFA için, 90 dakikalık Fenton prosesi boyunca, pH 5'te, H_2O_2:Fe^{2+}= 5:1 (H_2O_2=2.0 mM; Fe^{2+}=0.4 mM) oranında ve oda sıcaklığında yürütülmüştür.

Yapılan deneyler, 20 mg/L BFA'nın, 90 dakikalık Fenton prosesi süresince en uygun arıtım verimininde H_2O_2=2 mM, Fe^{2+}=0.4 mM, pH 5 ve oda sıcaklığı (20˚C) şartlarında elde edildiğini göstermiştir. Bu şartlarda %100 BPA giderimi 1-2 dakike içinde sağlanırken, 90 dakikalık arıtım sonunda ise %50 civarında TOK giderimi elde edilmiştir. Sıcaklık 20˚C'nin üzerine çıkarıldığında ise, birinci dakika TOK giderim hızının arttığı gözlenmiş, bununla birlikte 90 dakika sonunda toplam TOK giderimi %34-41 arasında kalmıştır. Sıcaklık artışının proses verimini arttırmadığı, en uygun sıcaklık değerinin oda sıcaklığı (20˚C) olduğu görülmüştür. 4.0 mM of H_2O_2 ve0.4 mM of Fe^{2+} kullanılarak pH 5'te ve 50˚C'de yürütülen çalışmalarda, 120 dakikalık arıtım sonunda, saf su için %60 ve gerçek ham su için 75% TOK giderimleri elde edilmiştir. Saf su yerine kullanılan test ortamlarına bağlı olarak, sırasıyla, M2 ortamı, göl suyu, M1 ortamı ve %2 NaCl ortamı için %22, %45, %58 and %62 TOK giderimleri elde edilmiştir. Tuzlu suyun (%2 NaCl), TOK giderimini inhibe ettiği sonucuna varılmıştır.

Akut toksisite testlerinden elde edilen sonuçlar, orijinal BFA'nın (20 mg/L)*Vibrio fischeri*'ye karşı %70 inhibisyon etkisi gösterdiğini, 1 dakikalık Fenton prosesi sonunda bu değerin saf su için %12'ye, gerçek su için ise %23'e düştüğünü göstermiştir. 40-50 dakika sonunda ise saf suyun, 90 dakika sonunda ise gerçek ham sudaki toksisitenin tamamen giderildiği gözlenmiştir. DTÜ'de *Vibrio fischeri* ile yürütülen inhibisyon testinde farklı olarak saf su yerine, test organizmasının yaşama ortamı olan %2'lik NaCl çözeltisi kullanılmıştır. Bu şartlarda, orijinal BFA (20 mg/L) %50 inhibisyon etkisi gösterirken, 1 dakikalık Fenton prosesi sonunda bu değer %11'e düşmüştür. 90 dakika sonunda ise toksisitenin tam giderilemediği, hala %8 inhibisyon olduğu gözlemlenmiştir. *Daphnia magna* ile akut toksisite çalışamaları için Fenton prosesi test organizmasının yaşama ortamında (M1 ortamı) gerçekleştirilmiştir. Test organizamasının 24 saatlik inkübasyonu için, 30 dakikalık

Fenton prosesi sonunda, immobilizasyon %70'ten %10'a düşerken; 48 saatlik inkübasyon süresi için, 60 dakikalık Fenton prosesi sonunda, immobilizasyon %100'den %20'ye düşmüştür. Her iki inkübasyon süresi için, 90 dakikalık arıtım sonunda toksisitenin tamamen giderildiği gözlemlenmiştir. *Pseudokirchneriella subcapitata* ile inhibisyon testleri için Fenton prosesi test organizmasının yaşama ortamında (M2 ortamı) ve göl suyunda yürütülmüştür. Orijinal BFA çözeltisi her iki ortam için %100 inhibisyon gösterirken, 90 dakikalık arıtım sonunda bu değerin %40 civarında kaldığı gözlemlenmiştir.

Toksisite deney sonuçları, BFA'ya karşı olan hassasiyet sıralamasının; *Pseudokirchneriella subcapitata*>*Daphnia magna* >*Vibrio fischeri* şeklinde olduğunu göstermiştir. Toksisite sonuçları aynı zamanda BFA'nın tek başına toksik etkiye neden olmadığını, arıtım ve reaksiyonlar boyunca farklı ve daha toksik ara ürünlerin oluşabileceğini göstermiştir.

1. INTRODUCTION

BPA is being predominantly used in various industries as an intermediate in the production of polycarbonate plastics, the majority of epoxy and polysulfonate resins (Kang et al., 2006; Fiege et al., 2002; Staples et al., 1998). It has been demonstrated that BPA is being distributed into the environment through a number of routes, including discharge of wastewater and washwater produced from BPA production facilities, discharge of effluent from wastewater treatment plants, leaching from consumer products containing BPA at hazardous waste landfill sites, residual of particulates or dust from BPA production, processing, or storage facilities, and accidental discharge (Garoma and Matsumoto, 2009). According to the USEPA (2010) "BPA is a reproductive, developmental, and systemic toxicant in animal studies and weakly estrogenic, there are questions about its potential impact particularly on children's and pregnant women's health and the environment." BPA has been found to mimic the primary female sex hormone; oestrogen. Therefore, BPA is categorized in a group of so-called "endocrine disrupting compounds-EDCs". Moreover, it has been reported that BPA may cause a decline in sperm counts and potentially increase the rates of hormone related cancers, such as cancers of the breast, testicular and prostate cancer. BPA may also cause teratogenic effects and defects in the reproductive tract, as well as other hormone related effects, such as earlier puberty in girls (Lyons, 2000).

BPA is a solid, such as crystals and flakes at room temperature. It has a low vapor pressure of 3.91×10^{-7} mm Hg, indicating that it exists in both the vapor and particulate phases in the atmosphere. Because of its low volatility based on its Henry's Law constant of 1.0×10^{-11} atm.m^3/mol, volatilization from the water surfaces is not expected to have an important impact on its fate in natural waters. When BPA released into water, BPA can be adsorbed to suspended solids and sediments because of its low mobility in the soil based upon an estimated K_{oc} (soil organic carbon-water partition coefficient) of 796. Biodegradation is also accepted to have minor contribution to its degradation in the environment. Moreover, according to Stasinakis

(2008), some of EDCs cause severe problems in biological treatment systems because of their resistance to biodegradation or/and toxic effects on microbial processes. The partial oxidation of compounds may result in the generation of intermediates being more toxic than the parent compounds. Hydrolysis of BPA is negligible under ambient conditions since it lacks functional groups that are susceptible to hydrolysis (Hazardous Substances Data Bank, HSDB, 2009).

Several treatment methods including adsorption, membrane based filtration, ozonation, biological and enzymatic processes have been proposed for the removal of BPA (Tessora et al., 2013; Husain and Qayyum, 2012; Jing and Yongqiang, 2010; Garoma et al., 2010; Cui et al., 2009; Liu et al., 2008; Zhang et al., 2006), however with limited success. Consequently, there is an urgent need for alternative treatment processes for efficient removal of BPA and its endocrine disrupting and/or toxic properties. Several studies have demonstrated that so-called advanced oxidation processes (AOPs) were more effective in the degradation of EDCs than conventional treatment processes (Rosenfeldt and Linden, 2004; Snyder et al., 2004).

AOPs are based on the generation of free radicals such as the hydroxyl radical (HO•) which is the strong, highly reactive and hence non-selective oxidant (E^o= 2.8 V). HO• reacts very rapidly with most organic as well as inorganic pollutants (Rizzo, 2011; Carey, 1992). One of the well-known and established AOPs is the Fenton's reagent, which relies on the catalytic decomposition of Fe^{2+} and Fe^{3+} by H_2O_2 under acidic pH's (2-5). The chemicals used for the Fenton's reagent are highly abundant and non-toxic, easy to handle and thus environmentally safe (Munter, 2001). Prior to selection of the most appropriate AOP, it should be considered that the efficiency and performance of these processes may change dramatically when they are applied to real water and wastewater matrices that contain significant amounts of organic as well as inorganic substances, besides the target pollutant. Consequently, it is important to test the selected AOP under real treatment conditions, namely in the natural environment of the pollutant under investigation.

The ecotoxicological situation of BPA for wastewater, sediments and contaminated water bodies in the aquatic and terrestrial environment is crucial part of environmental pollution. Assessment of biological effects using rapid, simple, sensitive and cost effective tests can provide specific information on toxicity and ecotoxicity. Traditionally, microorganisms, plants and algae, invertebrates and fish

are used for this purpose. It should be kept in mind that during the application of AOPs, there is always the risk of producing degradation intermediates that could potentially be more toxic than the original/parent pollutant (Marugán et al., 2012; Arslan-Alaton and Olmez-Hanci, 2011; Munter, 2001;). Consequently, toxicity tests serve as integral tools to decide whether a treatment process is ecotoxicologically safe or not. There is a significant gap in the scientific literature regarding the application of Fenton's reagent and battery toxicity test protocols in real water and wastewater matrices to degrade EDCs including BPA.

1.1 Aim and Motivation of the Study

Considering the above mentioned issues, the aim and motivation of the present experimental study was to examine the changes in toxicity of BPA during application of the Fenton's reagent in pure water and real surface water samples.

In the first part of the study which was conducted at Istanbul Technical University (ITU), Department of Environmental Engineering, the effect of different process variables of the Fenton's reagent including the initial BPA, H_2O_2 and Fe^{2+} concentrations, pH, temperature and water matrix (pure or real freshwater) on the performance of the Fenton's reagent to degrade BPA as well as its TOC content was investigated. During Fenton's treatment of BPA, H_2O_2 consumption rates were also determined. Acute toxicity changes in pure and real freshwater samples spiked with BPA were followed by employing the photobacteria *Vibrio fischeri.*

In the second part of the study, which was conducted in the laboratory facilities of Environmental Engineering Department of Denmark Technical University (DTU), a series of battery tests were conducted on untreated and Fenton-treated pure (growth mediums) and real freshwater (lake) samples spiked with BPA. In the battery tests, (1) the photobacteria *Vibrio fischeri* (decomposer level), (2) the freshwater crustacean*Daphnia magna* (consumer level) and (3) the microalgae *Pseudokirchneriella subcapitata* (formerly as *Selenastrum capricornutum*, producer level) were used as the toxicity test organisms. The originality of the present study mainly comes from performing the acute toxicity tests in the growth medium and habitat of the test species.

1.2 Scope of the Study

Within the scope of the experimental study, the most suitable values of process parameters for Fenton's reagent was firstly determined on the basis of BPA and TOC removal rates and efficiencies which were conducted at ITU. For this purpose, experiments were carried out with aqueous 20 mg/L of BPA solutions under varying treatment conditions at a fixed reaction duration of 90 min. For these experiments, the application ranges were as follows; BPA= 20 and 50 mg/L; H_2O_2= 0.50-5.00 mM; Fe^{2+}= 0.05-1.00 mM; pH= 3-6; and T: 20-70 ^{o}C.The effects ofprocess parametersontreatment efficiency was evaluated on the basis of BPA and TOC removals. At the same time, H_2O_2 consumption rates were compared under different process conditions. After these experimental studies, an experimental set was conducted for 120 min in the presence of 4 mM H_2O_2 and 0.4 mM Fe^{2+} at pH=5 and T=50˚C by dissolving 20 mg/L of BPA in pure water and real (raw) freshwater in order to evaluate the acute toxicity of BPA and its intermediate products using the *Vibrio fischeri* as the test organism (decomposer level). During this experimental set, the effects of increasing H_2O_2 concentration two times were also examined.

The second part of the study which was carried out at DTU, the acute toxicity of BPA and its intermediate products in pure water and real freshwater was examined in more detail employing a battery test. Three test protocols were selected for the acute toxicity assessment, namely (1) determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (decomposer level); (2) acute toxicity test on the freshwater crustacean *Daphnia magna* (consumer level), as well as(3) freshwater algal growth inhibition test with unicellular green microalgae *Pseudokirchneriella subcapitata* (producer level). In the battery test, it was aimed at comparatively evaluating the toxic effects of BPA and its advanced oxidation products on different test organisms from the responses of the tests were also evaluated considering the differences in treatment efficiencies and rates obtained in different test media and real freshwater samples.

2. LITERATURE REVIEW

2.1 Bisphenol A

2.1.1 History of bisphenol A

Bisphenol A (BPA; 4,4'-(propane-2,2-diyl)diphenol, *p,p'*-isopropylidenebisphenol, 2,2-bis(4-hydroxyphenyl)propane) is a colorless and white solid, has a mild phenolic odor and is soluble in organic solvents, but only fairly soluble in water. It was first synthesized in 1891 by the Russian chemist Aleksandr Dianin, and identified as an artificial oestrogen by the British chemist Charles Edward Dodds in the early 1930s (Erler and Novak, 2010). During that time, the purpose of first use of BPA was to increase the growth of cattle and poultry, and the second was in the middle of the 1930s as an oestrogen replacement therapy for women. The research groups at Bayer and General Electric have used BPA since the 1950s to make polycarbonate plastics and obtain epoxy resin, and in the coating of food and beverage containers (Walsh, 2010; Erler and Novak, 2010).

Dodds and Lawson (1936) determined the oestrogenicityof BPA by experiments on rats conducted during growth and pregnancy. The National Toxicology Program, (NTP, 1982) tested the safety of BPA due to increasing popularity of BPA-containing products and according to these test results, carcinogenicity effects were not convincing, however reproductive toxicity was reported. Vom Saal et al. (1998) studied the effects of low-dose (below 50μg/kg) BPA exposure on mice and reported some changes in male reproductive organs, as well as increased prostate weights. Eventually, it was decided that especially when BPA exposed during the main developmental phase, there was a growing risk of development disorders in the behavioural, metabolic and reproductive systems.

According tothe above findings, research keeps going and the discussions have started about prohibition of BPA, and to what extent, all over the world. In 2009, BPA appeared on the potential candidate contaminant list used during its

development of the third Candidate Contaminant List of substances by the United States Environmental Protection Agency (USEPA, 2009).

In other governmental studies, human health risk assessments were also conducted for BPA in the recent past. Japan's National Institute of Advanced Industrial Science and Technology (AIST, 2007), The European Union Risk Assessment Report (2008), The European Food Safety Administration (EFSA, 2008) and The United States Environmental Protection Agency (USEPA, 2010) all concluded within the past four years that the most recent studies stating low-dose, endocrine-related effectswere inadequate for the purposes of hazard evaluation and risk assessment.

In 2010, Canada's Department of the Environment declared BPA to be a toxic compound andit was prohibited under the *Hazardous Products Act* of polycarbonate baby bottles containing BPA (Sheffield and Burgham, 2010). In 2010, a temporary ban was declared by the Health Ministry of Denmark. In 2011, The Ministry of Agriculture and Rural Affairs (MARA) banned the use of BPA in baby bottles and other polycarbonate items produced for babies in Turkey.

In 2012, The United States Food and Drug Administration (USFDA) decided that "the scientific evidence at this time does not suggest that the very low levels of human exposure to BPA through the diet are unsafe." Although, the USFDA is continuing to additional research to resolve the potential uncertainties in the interpretation of the studies including route of exposure used in the studies and the relevance of animal models to human health.

2.1.2 Synthesis and use of bisphenol A

As can be seen from **Fig. 2.1**, BPA is synthesized by the condensation of acetone with two moles of phenol under low pH, high temperature conditions and the presence of catalysts, such as hydrochloric acid (HCl) or a sulfonated polystyrene resin. Unrefined BPA is then purified via distillation technology. Afterwards, molten purified product is filtered and dried (Staples et al., 1998). BPA is a high-volume production chemical compound and its global production capacity was around one million tons in the 80s (Fiege et. al, 2002), and around five million tons in 2008 (Dow Chemical Company, 2012).

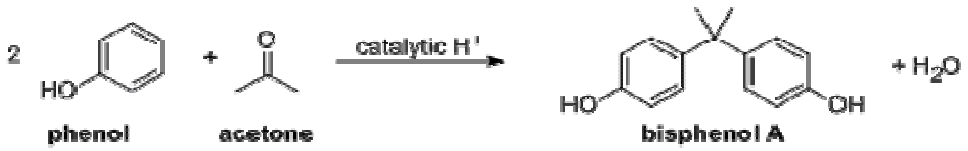


Figure 2.1 : Synthesis of bisphenol A.

Polycarbonate plastics and epoxy resins contain BPA as an indermediate compound. Polycarbonates are used to make various consumer products, such as food and drink packaging, compact discs, automotive lenses, medical devices, sports equipments, thermal paper, paper coatings, encapsulation of electronic parts, powder paints, adhesives, household electronics, dental fillings and sealants, motorcycle helmets, and safety glasses (Kang et al., 2006; Fiege et al., 2002; Staples et al., 1998). Epoxy resins are used as coating for protection of food and beverage cans, PVC pipes, aerospace applications, car coatings and anti-corrosion coatings for floors (Kang et al., 2006).

Various studies reported by the USEPA and around the world showed the widespread occurrence of BPA in the environment, including leachates from hazardous waste landfills (Yamamoto et al., 2001), surface water (Kolpin et al., 2002), effluent and sludge from wastewater treatment plants, sediment samples from rivers, lakes, and channels as well as tissues of aquatic animals (Fromme et al., 2002), treated drinking water (Rodríguez et al., 2004) as well as rainwater (Vethaak et al., 2005).

In numerous studies, the measured concentrations of BPA in streams and rivers in Japan, Europe and the United States have been reported. The reported BPA concentrations in surface waters varied between 0.016 and 0.500 μg/L (Cousins et al., 2002). According to the Japan Environmental Agency (JEA, 2001), detectable BPA concentrations in 67 out of 124 water samples were reported which have been selected from “Water Quality Monitoring” sites of downstream rivers. The average concentration of BPA was 0.01 μg/L and 95% of the samples contained less than 0.24 μg/L of BPA in these studies. The concentration of BPA in the natural aquatic environment is in the ng/L-μg/L range (Jafari et al., 2009).

2.1.3 Properties of bisphenol A

Some of the chemical and physical properties of BPA are summarized in **Table 2.1**. (Kang et al., 2006; Staples et al., 1998).

Table 2.1 : Physicochemical properties of bisphenol A.

Physicochemical Parameters	
IUPAC name	4,4'-(propane-2,2-diyl)diphenol
Molecular structure	
CAS No.	80-05-7
Molecular formula	$C_{15}H_{16}O_2$
Purity	99.85% (min)
Molecular weight	228.29 g/mol
Water solubility	120 mg/L at 25 ˚C
Vapour pressure	3.91 x 10^{-7} mmHg at 25 ˚C
Octanol-Water partitioning coefficient (log K_{ow})	3.32 (2.20-3.82)
Henry's Law constant	1.0 x 10^{-11} atm.m^3/mol
Soil Organic Carbon-Water partitioning coefficient (log K_{oc})	2.9
Melting point	150-155 ˚C
Boiling point (4 mmHg)	220 ˚C
Acid dissociation constant (pK_a)	9.59-11.30
Density	1.2 g/cm^3 at 25 ˚C
Flash point	213 ˚C
Freezing point	-156.5 ˚C

2.1.4 Environmental fate of bisphenol A

BPA is expected to disperse into degrade to some extent in all available environmental phases due to its measurable vapor pressures, aqueous solubility and octanol-water partition coefficient (K_{ow}). BPA has a pK_a value between 9.59 and 11.30 which means it is not expected to ionize at a pH of 7 or less. BPA is a

moderately hydrophobic compound (log K_{ow} of 3.32) and fairly soluble in water which means it partitions to organic phases such as soils and sediments, however, the dissolved phase contains a perceptible fraction of BPA. It is not expected to appreciably volatilize or hydrolyze in natural waters (Staples et al., 1998).

Cousins et al. (2002) reported that BPA has an insignificant level in the atmosphere because of its low volatility. Moreover, they concluded that BPA is relatively rapidly degraded with half-lives in water and soil of about 4.5 days and less than 1 day in air. Therefore, it was evident that aerobic biodegradation of BPA is the dominant removal mechanism process in all media except the atmosphere. BPA vapor is susceptible to atmospheric photooxidation reaction by hydroxyl radicals (Staples et al., 1998). Aerobic biodegradation of BPA is thought likely to occur rapidly in surface benthic sediments, but anaerobic biodegradation is thought to be a slow removal process in deeper sediments, depending on environmental conditions such as temperature and the condition of the microbial community (Cousins et al., 2002).

2.1.5 Toxicity of bisphenol A

Most research on toxic effect of BPA in the environment which can affect the survival, reproduction, growth and development fitness has been examined by using short-term (acute toxicity) or long-term (chronic toxicity) experiments focusing on the aquatic organisms, including fish, algae, bacteria, invertebrates and plants. For evaluating survival fitness, the lethal effects of BPA for aqueous exposure were investigated in studies with exposure periods changing from 48 h to more than 400 days (Tabata et al., 2001; Yokota et al., 2000; Kloas et al., 1999; Alexander et al., 1988). The chronic effects of BPA on growth and development have been evaluated by observing length and weight of organs (relative to whole body weight), structural deformities, population growth (for algae), as well as secondary sexual characteristics with the exposure duration ranged from 28 to 120 days (Schäfers and Wenzel, 2000; Yokota, 2000; Bayer AG, 1999). Ecotoxicity tests on reproductive system of aquatic organisms have been conducted by observing sperm motility and length, production of eggs, time of hatch, hatching ability of eggs, survival of embryos and differentiation of sexes (Oehlmann, 2000; Anderson et al., 1999; Alexander, 1988; Caspers, 1998).

In the literature, Alexander et al. (1988) found that BPA has an acute toxicity for freshwater and marine species with LC_{50} (median lethal concentration causing death in half of the test organisms) values were in the range of 1-10mg/mL.Staples et al. (2002, 1998) reported that BPA exhibited toxicological effects on the survival, growth and reproduction system of the aquatic organisms, including freshwater and saltwater algae, invertebrates and fish, such as *Selenastrum capricornutum*, *Pimephalespromelas*, *Daphnia magna*, *Mysidopsis bahia*. The results showed that LC_{50} values were in the range of 1000-20,000 μg/L. Genetic toxicity and physiological effects of BPA was investigated by Park et al. (2006) in aquatic sentinel species, freshwater crustacean *Daphnia magna* and larva of aquatic midge *Chironomus tentans*. It was found that *Daphnia magna* was more sensitive than *Chironomus tentans.* Stasinakis (2008) and Olmez-Hanciet al. (2013) investigated possible acute toxicity of BPA on the photobacteria *Vibrio fischeri* by calculating percent relative inhibition values and it was concluded that there were toxic effects of BPA as well as its toxic intermediate products.

In this experimental study, it is focused on the photobacteria*Vibriofischeri*, the freshwater crustacean *Daphnia magna* and freshwater microalgae *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*).

2.1.6 Health effects of bisphenol A

BPA in food and beverages accounts for the majority of daily human exposure, especially when they are heated at high temperatures. AlthoughNTP (2008) reported that the sources of exposure to BPA are air, dust and water. There have been concerns declared about the mechanism underlying negative harmful effects, such as reproductive and developmental effects of BPA since the 1930s and these were proved by several studies on freshwater and marine life, humans, and laboratory animals (Office of Environmental Health Hazard Assessment, (OEHHA), 2009).

The oestrogen-like activity of BPA is of major concern. It closely imitates the structure and function of the hormone estradiol with the ability to bind and activate the same “oestrogen receptor” as the natural hormone, which is the main sex hormone in humans and other animals, and is essential for the menstrual cycle and controlling fertility (Beverly, 2011; Okada et al., 2008; Vom Saal and Myers, 2008; O'Connor and Chapin, 2003). Moreover, studies on humans and laboratory animals

demonstrated that BPA causes abnormal sexual development, decreasing in male fertility and other adverse health effects, such as pancreatic β-cell function disruption (Ropero et al., 2008), obesity-promoting effects (Newbold et al., 2008), increases the prevalence of cardiovascular disease, diabetes, and liver-enzyme abnormalities (Lang et al., 2008), liver damage (Bindhumol et al., 2003) and thyroid hormone disruption (Moriyama et al., 2002).

Additionally, the NTP (1982) concluded that "there was no convincing evidence that BPA was carcinogenic to F344 rats or B6C3F1 mice of either sex." based upon analysis of data from a two-year carcinogenicity bioassay of BPA. Grun and Blumberg (2007) had proved that BPA can potentially cause or contribute to human obesity that are now mentioned under the "environmental obesogen" hypothesis. Human obesity is also a risk factor for diabetes, coronary heart disease, hypertension, and gall bladder disease. BPA appears to stimulate or suppress the immune system and may also change immune response ways BPA also influences the hippocampus, which means BPA has adverse effects memory and learning (OEHHA, 2009). While these studies provide some evidence that BPA has the potential to directly or indirectly affect the health, much further investigation is needed.

2.1.7 Treatability and removal of bisphenol A

Due tothe adverse healtheffectsdescribed above, BPA has to be removed from the water before it can be used for domestic applications. Until now, various data in the literature related with removal and treatability of BPA from contaminated water have been reported.

In a laboratory study conducted by Lu et al. (1990), about 99% BPA removal was achieved in 14 days by an activated sludge treatment process, which had a much longer residence time compared to the hydraulic residence time for conventional wastewater treatment plants. West and Goodwin (1997) studied the biodegradability of BPA by using a manometric respirometry test and results indicated that BPA degrades slowly, achieving 81-93% BPA degradation in 28 days. Vethaak et al. (2005) conducted a survey on influents and effluents from conventional wastewater treatment plants with biological treatment processes. It could be demonstrated that BPA was not completely and effectively removed by these treatment processes. On the other hand, Lee et al. (2008) conducted a pilot-scale study with an integrated

membrane bioreactor, nanofiltration, and reverse osmosis unit with which around 93, 95, and 96% BPA removals were achieved, respectively. The capital, operation and maintenance costs associated with these processes rendered them less attractive compared to conventional treatment process.

BPA's adsorption onto activated carbon surfaces is also restricted (Choi et al., 2005), and thus activated carbon must be replaced or regenerated very often to remove BPA effectively from contaminated water. Due to its very low Henry's constant of 1.0×10^{-11} atm.m^3/mol **(Table 2.1)**, air stripping is also not suitable for BPA removal.

Considering all these described above, advanced and alternative treatment processes need to be developed and applied for effective BPA removal.

2.2 Advanced Oxidation Processes

2.2.1 Definition and general principles

Advanced oxidation processes (AOPs) have been a matter of recent scientific and technological interest in water treatment technologies. AOPs aremainly employed to effectively oxidize, transform or remove recalcitrant compounds from industrial and municipal wastewater including resistant organics (pesticides, surfactants, coloring matters, pharmaceuticals and endocrine disrupting chemicals)based on the parameters assessed, removal efficiencies and the degradation mechanisms of pollutants (Arslan-Alaton and Olmez-Hanci, 2012; Wang and Xu, 2011; Stasinakis, 2008; Munter, 2001). Oxidative destruction of compounds resistant to conventional ozonation or H_2O_2 oxidation can be achieved by AOPs. Additionally, AOPs have been successfully applied as pretreatment methods to decrease toxicity and increase biodegradability that inhibit biological wastewater treatment processes (Stasinakis, 2008).

Among various radicals, such as superoxide radical ($O_2^{\bullet -}$), hydroperoxyl radical ($HO_2\bullet$), hydroxyl radical (HO•), and alkoxyl radical (RO•), HO• is known to playthe most important role in AOPs for wastewater treatment (Gomes et al., 2005; Tai et al., 2002).

Several experimental studies have shown that the degradation of organic compounds by AOPs mainly depends on the HO• reaction mechanisms (Boonrattanakij et al., 2009; Liu et al., 2009; Rao and Chu, 2009; Song et al., 2007; Peller et al., 2001). However, these chain reactions can be inhibited by the presence of free radical scavengers, such as CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-} and PO_4^{3-} (Wang and Xu, 2011; Li and Crittenden, 2009).

Table 2.2 summarizes some oxidizing agents and their relative oxidation power as well as oxidation potential. As can be seen from **Table 2.2**, HO• is a powerful, and hence non-selective oxidizing agents.

Table 2.2 : The relative oxidation power and oxidation potential of some oxidizing agents (Trapido, 2008; Carey, 1992).

Oxidizing species	**Relative oxidation power***	**Oxidation potential (eV)**
Cl_2	1.00	1.38
HOCl	1.10	1.49
MnO_4^-	1.24	1.69
H_2O_2	1.31	1.77
O_3	1.52	2.07
O•	1.78	2.42
HO•	2.05	2.80

*Reference compound: Chlorine

The second-order reaction rate constants of O_3 and HO• with different organic compounds are also given in **Table 2.3**.These constants may differ in quite a wide range from 0.01 to 10^4 M^{-1} s^{-1} .

Table 2.3 : Comparison of second-order reaction rate constants (in M^{-1} s^{-1}) of O_3 and HO• with different organics (Munter, 2001).

Compound	**k (M^{-1} s^{-1})**	
	O_3	**HO•**
Chlorinated alkenes	10^3-10^4	10^9-10^{11}
Phenols	10^3	10^9-10^{10}
N-containing organics	10-10^2	10^8-10^{10}
Aromatics	1-10^2	10^8-10^{10}
Ketones	1	10^9-10^{10}
Alcohols	10^{-2}-1	10^8-10^9

Practically, the rate of degradation of a contaminant is almost proportional to the reaction rate constant for the contaminant with HO•. As can be seen from **Table2.3**, chlorinated alkenes are treated most efficiently because the double bond is very sensitive to HO•. Saturated molecules (i.e., alkanes) are more difficult to oxidize which react at a much lower rate constants (Solarchem Environmental Systems, (SES), 1994).

HO• attack all compounds in two ways; it can abstract H^+ atom from water **(2.1)**,as with alkanes or alcohols, or it can add itself to the contaminant **(2.2)**, as in the case ofolefins or aromatic compounds (Munter, 2001). Moreover, electron transfer reactions can occur depending upon structures of compounds **(2.3)** (Hullar and Anastasio, 2011).In the following equations, the reacting organic compound is described by R;

Hydrogen Abstraction: $RH + HO\bullet \rightarrow R\bullet + H_2O$ (aliphatics) **(2.1)**

Addition: $R + HO\bullet \rightarrow ROH$ (aromatics) **(2.2)**

Electron Transfer: $R^n + HO\bullet \rightarrow R^{n-1} + OH^-$ **(2.3)**

2.2.2 Advantages and disadvantages of employing AOPs

A brief summary indicating the major advantages and disadvantages of some AOPs is presented in **Table2.4**.

Table 2.4 : Advantages and disadvantages of some AOPs (Kommineni et al., 2008).

AOP	Advantages	Disadvantages
H_2O_2/O_3	Additional disinfectant	Necessary treatment of excess H_2O_2 due to potential for microbial growth
	More effective than O_3 or H_2O_2 usedalone	Potential for bromate formation (controllable through adjustment of O_3/H_2O_2 ratio and pH)
	Established technology based on remediation applications	Necessity of ozone off-gas treatment
O_3/UV-C	Additional disinfectant	Capital costs and energy requirements are high
		Potential for bromate formation (controllable through adjustment of the O_3/H_2O_2 ratio and pH)
	More effective than O_3 or UValone used	Turbidity which can interfere with UV light influence
		Mass transfer limitations because of gaseous ozone diffusion
	More efficient at formation HO• than H_2O_2/UV process for same oxidant concentrations	Necessary of ozone off-gas treatment
		Interfering compounds (such as nitrate, iron) can absorb UV light
		Contaminate water with mercury because of UV lamp and quartz sleeve failures
		Potential increase in THM formation after pre- and/or post-chlorination
H_2O_2/UV-C	No potential for bromate formation	Turbidity/suspended solids content may interfere with UV light absorption by the oxidant
	Pulsed-UV irradiation may work as disinfectant	Less stoichiometrically efficient in the formation of HO• than O_3/UV process
	Full-scale drinking water treatment facilities are available	Interfering compounds (such as nitrate) ay compete with the oxidant for UV light
	Does not require off-gas treatment	Potential increase in THM formation after pre- and/or post-chlorination
	Not limited by mass transfer compared to O_3 processes	

Table 2.5 (continued)

TiO_2/UV-A	No potential for bromate formation	No full-scale applications present
		Pre-treatment requirement to avoid impurity of the TiO_2 catalyst
	Can be performed at higher (300-380 nm) wavelengths than other UV oxidation processes.	A separation step requirement after addition of TiO_2 as a slurry
		Potential for rapid loss of TiO_2 effectiveness, necessity of catalyst on-site storage or regeneration method
		Strict studies needed to calculate the optimum TiO_2 dose
	No off-gas treatment requirement	Necessity of oxygen sparging
		Reaction efficiency is highly pH-dependent, requiring close observing and control
Fenton's reagent	No potential for bromate formation	Necessity of iron extraction system
	Practically no energy requirements as compared to AOPs that utilize O_3 or UV	Acidic pH (<2.5) is required to provide the iron in solution (not always) since Fe can form soluble complexes in water
	No off-gas treatment requirement	Increasing operation and maintenance costs because of pH adjustment

2.2.3 Major AOP types and applications

The most well-known and established AOPs are summarized in **Table2.5**. They consist of a combination of strong oxidizing agents, such as H_2O_2 and O_3 with catalysts including transition metal ions and ultraviolet (UV), near-UV, visible light irradiation. TiO_2/UV-A, H_2O_2/UV-C and the Fenton's reagent seem to be some of the most known AOPs for water and wastewater (Stasinakis, 2008).

Table 2.5 : List of most known and well established AOPs.

Photochemical AOPs	H_2O_2/UV-C
	H_2O_2/Fe^{2+}/UV-C
	TiO_2/UV-A, H_2O_2/TiO_2/UV-A
	O_3/UV, H_2O_2/O_3/UV-C
	$S_2O_8^{2-}$/UV-C
Non-photochemical AOPs	Fe^{2+}/H_2O_2, Fe^{3+}/H_2O_2
	$Fe^{2+}/S_2O_8^{2-}$
	TiO_2/O_3, H_2O_2/O_3, H_2O_2/O_3/ TiO_2
	Ozonation at high pH
	Wet air oxidation (WAO)
	Sonolysis
	Supercritical water oxidation (SCWO)
	Electrochemical oxidation

Most of the above given AOPs have been employed in laboratory-, pilot-scale, and full-scale studies. Some of these studies are summarized below, mainly focusing on Fenton's reagent.

Samet et al. (2012) examinedthe degradation of chlorpyrifos (insecticide) in wastewater by Fenton (H_2O_2/Fe^{2+}) and Photo-Fenton (H_2O_2/Fe^{2+}/UV-C) processes in a laboratory-scale reactor. %90 COD removal could be achieved with Photo-Fenton's process which was 50% less time than that used in the Fentonprocess.

Jamil et al. (2011) investigated H_2O_2/UV, Fenton and Photo-Fenton processes for the treatment of paper mill wastewater. From the treatability studies using these processes. It was concluded that the Photo-Fenton process was the most efficient process in the biodegradability enhancement of organic matter in the effluent.

The dark Fenton-like process with persulfate used as the oxidant ($Fe^{2+}/S_2O_8^{2-}$) as well as in the presence UV light was applied for degradation of C.I. Reactive Red 45 (RR45, reactive azo dye) by Kusic et al. (2011) and results showed a high accuracy in predicting the degradation, decolorization and mineralization of C.I. Reactive Red 45.

Papadopoulos et al. (2007) examined the effectiveness of the Fenton process for the degradation of the organic content of wastewater generated from a textile industry. Experimental results indicated that 45% COD removal was achieved, as well as 71.5% color removal. During the process, organic substances were not completely mineralized depending on structural changes of intermediate organic products.

Torres et al. (2007) evaluated the involvement of the HO• in the sonochemical degradation of BPA. Ultrasound action was compared to Fenton process for deionised acidic water and natural water. Experimental results showed that both processes exhibited the same BPA elimination rate and same primary intermediates in deionised water. According to the COD and TOC results, the Fenton process which conducted in deionised water was slightly more efficient than ultrasonic treatment for the removal of BPA by-products. Additionally, experiments which conducted in natural water indicated that the inhibition of the Fenton process while the ultrasound action was not hampered.

Arslan-Alaton et al. (2002) compared the treatment efficiency of ozonation, H_2O_2/UV-C and TiO_2/UV-A processes for the oxidation of a simulated reactive dyebath effluent. The ozonation reaction showed almost instantaneous decolorization kinetics and a reasonable TOC removal rate.They concluded that ozonation and H_2O_2/UV-C were superior and in view of the electrical energy efficiencycould be selected for full-scale dyehouse effluent decolorization.

Lucas et al. (2010) investigated the effectiveness of different ozone-based AOPs, including ozonation, O_3/UV-C and H_2O_2/O_3/UV-C on the treatment of winery wastewater in a pilot-scale, bubble column reactor. It was concluded that the effectiveness of each AOP as follows: H_2O_2/O_3/UV-C>O_3/UV-C>O_3 at the natural pH of the wastewater (pH 4).

In pilot-scale applications, Moreira et al. (2012) conducted experiments with different AOPs types, including TiO_2/UV-A, H_2O_2/TiO_2/UV-A, H_2O_2/UV-C, H_2O_2/Fe^{2+}/UV-C and H_2O_2/Fe^{2+} processes focusing on the treatment of a pesticide-containing wastewater using a pilot plant. It was concluded that despite the Fenton process showed a slower mineralization profile, it was quite efficient for significant pesticide abatement compared to the other AOPs.

Vilar et al. (2012) reported an application of H_2O_2/Fe^{2+}/UV and H_2O_2/Fe^{2+} processes to the treatment of a sanitary landfill leachate in a pilot plant. Experimental results showed that H_2O_2/Fe^{2+}/UV reaction was much more efficient than the respective dark reaction under same experimental conditions, resulting 86% mineralization and 94% degradation of aromatic content of the leachate after 4 days of UV exposure.

Additionally, severeal full-scale installations H_2O_2/O_3 systems worldwide, treating different contaminants in process wastewater, groundwater, and drinking water are available. For instance, in the U.S. (Los Angeles, CA), wellwater was treated with the H_2O_2/O_3 (peroxone) oxidation process for trichloroethylene (TCE) and tetrachloroethylene (PCE) removal at a scale of 450 m^3/h. In France (Paris), a peroxone treatment system was employed for atrazine removal from the water of the River Seine at a scale of 5,000 m^3/h (Munter, 2001). Since 2004, a drinking water treatment plant at a scale of 4,500 m^3/h in Andijk (Holland) have been employing for both disinfection and removal of organic pollutants which were mainly pesticides, endocrine disruptors and pharmaceuticals by the application of H_2O_2/UV process (Kruithof et al., 2007)

Full-scale Fenton reagent's applications in South Africa (Gravelet-Blondin et al., 1996) and in Italy (Antonelli and Rozzi, 2001) have been carried out to treat textile effluents. Barbusinski (2009) also reported about a full-scale chemical treatment plant which is based on the Fenton's reagent has been working since 2000 in southern Poland. It is used for decolorisation and degradation of dye wastewater from production of matches and it has been observed that the Fenton's reagent at high efficiencies, can achieve high removal efficiency for COD, color removals and complete detoxification (in relation to photobacteria *Vibrio fischeri*). Barbusinski also recommended that AOPs can be also applied successfully for other water treatment applications, especially for water containing biorefractory compounds.

2.2.4 The Fenton's reagent

The Fenton's reagent is mainly used as a pre-treatment of wastewater containing resistant to biological treatment or/and toxic to biomass (Mantzavinos and Kalogerakis, 2005). The reaction is exothermic, and however, in large scale plants, the reaction is commonly run out at ambient temperature using a large excess Fe^{2+} as well as H_2O_2.

The Fenton's reagent is originally defined as the catalytic decomposition of H_2O_2 under acidic pH (2-5). Fenton-like processes generally refer to the catalytic decomposition of H_2O_2 which utilize metal ions including Co, Cu and Fe ions. The homogeneous Fenton's reagent consists of H_2O_2 and Fe^{2+} catalyst that is used to oxidize contaminants and can be used to destroy organic compounds by the generation of HO• (Wang and Xu, 2011). It was developed in the 1894 by Henry John Horstman Fenton as an analytical reagent.

2.2.4.1 Mechanisms of the Fenton's reagent

In Fenton process, Fe^{2+}/Fe^{3+} and H_2O_2 are quite stable at acidic conditions. HO• are generated via free radical chain reactions (Ileri and Karaer, 2011). Fe^{2+} ions are oxidized by H_2O_2 to Fe^{3+} in a few seconds to minutes **(2.4)**.After that, Fe^{3+} is reduced back to Fe^{2+}, generating a hydroperoxyl radical (HO_2•) by the same H_2O_2 (**2.5 and 2.6)**.

Reactions are shown below (Lewkiewicz et al., 2008; Walling, 1975; Fenton, 1894);

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + OH^- \qquad (k_1 = 76\ M^{-1}\ s^{-1}) \qquad \textbf{(2.4)}$$

$$Fe^{3+} + H_2O_2 \rightarrow HO_2\bullet + Fe^{2+} \qquad (k_2 = 0.01\ M^{-1}\ s^{-1}) \qquad \textbf{(2.5)}$$

$$Fe^{2+} + HO\bullet \rightarrow Fe^{3+} + OH^- \qquad (k_3 = 3 \times 10^8\ M^{-1}\ s^{-1}) \qquad \textbf{(2.6)}$$

The reaction of H_2O_2 with Fe^{3+}**(2.5)** is called the "Fenton-like" reaction and described as similar to the Fenton's reaction. The modified Fenton's reagent is frequently defined as introducing Fe^{3+} ions instead of Fe^{2+} and excessive H_2O_2 in comparison to the amount of iron used. In the Fe^{3+}/H_2O_2 system, Fe^{2+} ions are reproduced and HO• as well as other free radicals are generated.According to the reactions **(2.7 and 2.8)**, produced Fe^{3+} ions act as a catalyst in the decomposition of H_2O_2 into O_2 and H_2O (Lewkiewicz et al., 2008; Fenton, 1894).

$Fe^{3+} + HO_2\bullet \rightarrow Fe^{2+} + O_2 + H^+$ ($k_4 = 3.3x10^5\ M^{-1}\ s^{-1}$) **(2.7)**

$HO\bullet + H_2O_2 \rightarrow H_2O + HO_2\bullet$ ($k_5 = 3.3x10^7\ M^{-1}\ s^{-1}$) **(2.8)**

Alkyl radicals (R•) are created when HO• oxidize organic compounds (RH) by severing protons. These radicals are highly reactive and may undergo further oxidization (Lewkiewicz et. al, 2008).

$RH + HO\bullet \rightarrow H_2O + R\bullet \rightarrow$ further oxidization ($k_6 \approx 10^7 \sim 10^{10}\ M^{-1}\ s^{-1}$) **(2.9)**

The general notation of the Fenton's reaction was simplified and water dissociation was explained by Walling (1975);

$2Fe^{2+} + H_2O_2 + 2H^+ \rightarrow 2Fe^{3+} + 2H_2O$ **(2.10)**

This reaction suggests that H_2O_2 decomposition may occur if H^+ ions are present. It means that an acidic environment will be favorable for the production of the maximum number of HO•. In the presence of organic matter (RH), excessive Fe^{2+} and low pH, the HO• may be connected to aromatic or heterocyclic compounds (**2.11and2.12**). They may also separate the H atom, triggering out a chain reaction of radicals oxidization (Walling, 1975);

$RH + HO\bullet \rightarrow H_2O + R\bullet$ (radical substitution) **(2.11)**

$R\bullet + H_2O_2 \rightarrow ROH + HO\bullet$ **(2.12)**

Free organic radicals produced **(2.11)** can be oxidized by Fe^{3+}, reduced by Fe^{2+} or polymerized (Tang and Tassos, 1997);

$R\bullet + Fe^{3+}$ - oxidation $\rightarrow R^+ + Fe^{2+}$ **(2.13)**

$R\bullet + Fe^{2+}$- reduction $\rightarrow R^- + Fe^{3+}$ **(2.14)**

$2R\bullet$ - polymerization $\rightarrow R - R$ **(2.15)**

Fundamentally, the procedure of Fenton's reagent requires; adjusting the pH (3-5), adding the iron catalyst (as a solution of $FeSO_4$), adding the H_2O_2 and afterthat oxidization reactions, neutralization-coagulation processes and precipitation to remove Fe ions as ferric hydroxide ($Fe(OH)_3$) after the reactions. If the pH is too

high, the iron precipitates as $Fe(OH)_3$ and catalytically decomposes the H_2O_2 to O_2, which potentially creats a hazardous situation (Bishop, 1968).

The advantage of the Fenton's reagent is the requirement of very little energy input compared to other AOPs to activate H_2O_2 (Lücking et al., 1998). The application of this oxidation process results in a cost-effective source of HO•, which is H_2O_2, using easy-to-handle and store reagents. Iron is a highly abundant and non-toxic element for the environment. The process does not produce vapour emissions. Therefore, it requires no off-gas treatment or air permits (Kommineni et al., 2008).

However, disadvantages in using the Fenton's reagent include that the production of a significant amount of iron sludge due to the $Fe(OH)_3$ precapitation, additional water pollution that may be caused by the homogeneous catalyst (Fe ion added in the form of its soluble salt), which can not be kept in the process, requirement of pH adjustment before and after the treatment. Therefore, an iron extraction system is necessary for removing residual iron from the water medium, an acidic pH environment is also necessary to provide the iron in solution. As a result, the requisite acid-base injections and removal of iron sludge increase the operation and maintenance costs (Kommineni et al., 2008; Chou et al., 1999; Lücking et al., 1998). Fenton's reagent has also the relatively high cost and risks related to the storage and transportation of H_2O_2 (Brillas et al., 2009).

2.2.4.2 The parameters affecting the Fenton's reagent

The major parameters affecting the Fenton's reagent consist of H_2O_2, Fe^{2+}, concentrations, their reactions, pH and temperature.

§ H_2O_2 concentration

Degradation rate increases with increasing of H_2O_2 concentration. However,in case of excessively high H_2O_2 concentrations,H_2O_2 reacts with HO• and itis not recommended (Kang and Hwang, 2000) because the oxidation reaction may be inhibited. As a consequence, the introduced H_2O_2 concentration has to be optimized. Further addition of H_2O_2 may result in a rapid decrease in wastewater toxicity. The maximum efficiency of the process related with the stoichiometric rates between Fe^{2+}, Fe^{3+} and RH (Bishop, 1968).

§ Fe^{2+} concentration

Degradation rate increases with increasing of Fe^{2+} ions concentration until a point is reached where further addition of iron becomes inefficient. In other words, degradation rate decreases at above certain concentration and suspended and/or dissolved Fe^{2+} ions concentration increase (Kang and Hwang, 2000). In some studies, it is stated that in the presence of H_2O_2 and organic materials the catalytic cycle is very fast and used iron type does not matter. Redox cycle of the iron following the reaction is possible by raising the pH, separating the iron floc, and re-acidifying the iron sludge. However, some studies indicate that when very low dosage of Fenton's reagent (e.g., < 10-25 mg/L H_2O_2) is used, Fe^{2+} ions are preferred (Bishop, 1968).

§ pH

The optimum pH range for the Fenton's reagent has been reported as 2.5-3.5 (Eyad et al., 2007). Theredox system works better and hence the removal of organic compounds is more efficientunder acidic conditions. When the pH is lower than 2.5, the treatment performance and oxidation efficiencies drop dramatically depending mainly on Fe^{2+} solubility and speciation. However, H^+ becomes the main acceptor of HO• radicals at pH<3 (Barbusinski and Koscielniak, 1999). When pH is > 5, Fe^{2+} ions are not stable and these ions are converted to colloidal ferric species which generate hydroxo complexes, without forming HO• (Bishop, 1968). Additionally, H_2O_2 is unstable at basic pH medium and in the presence of oxygen, it may be degraded and lose its oxidation ability. Under these conditions, redox reactions between H_2O_2 and Fe^{2+} ions are difficult (Kuo, 1992). Moreover, H_2O_2 is a weak acid, with a pK_a value of 11.6 and dissociates at elevated pH's (Sundura, 1998).

$$H_2O_2 + OH^- \leftrightarrow H_2O + HO_2^- \quad (pK_a = 11.6) \qquad \textbf{(2.16)}$$

§ Temperature

Elevating thetemperatureincreasesthe reactionrate of the Fenton process. When the temperature, whichis lower than20˚C,all chemical processes showa significant decrease inremoval efficiencies. When the temperature is above 40-50˚C, the generation rate of HO• is enhanced. However, when the temperature approaches

60˚C, H_2O_2 undergoesself-decomposition to H_2O and O_2 (Khamaruddin et al., 2011). The optimum temperature is 20-40˚C for the Fenton process.

Additionally, optimum pH, H_2O_2, Fe^{2+} and temperature exists depending on the pollutant concentration or type, water matrix, post treatment and reaction time. (Bishop, 1968).

2.2.5 Application of AOPs for BPA removal

Several non-conventional treatment techniques and AOPs have been investigated for the efficient degradation of BPA have been studied recently. The purpose of these studies was principally focused on treatment kinetics and mechanism of degradation of BPA. Process and operational parameters, such as water quality, pH, initial BPA concentration, and oxidant dosage, on the rate of degradation of BPA are important variables in establishing the effectiveness and efficiency of any treatment process.

Wang et al. (2009) examined the photocatalytic degradation of BPA with the TiO_2/UV-A photocatalytic process in a self-designed horizontal circulating bed photocatalytic reactor (HCBPR). The effects of initial BPA concentration, initial pH, TiO_2concentration and temperature on photocatalytic degradation of BPA were investigated to obtain the optimum treatment conditions which showed the high BPA removal efficiencies were achieved BPA in HCBPR. 95% TOC and 97% BPA wereobtained after 6h treatment.

Garoma and Matsumoto (2009) studied the degradation of BPA by ozonation in a a semi-batch reactor. The experimental results under different operating conditions such as inlet ozone concentration (1.4-5.1 mg/L), initial BPA concentration (23-42 µM), pH (2-10), and bicarbonate concentration (1-20 mM) revealed that ozonation could be a potential choice for effective removal of BPA from contaminated water.

Torres et al. (2007) evaluated the sonochemical degradation of BPA comparing Fenton's reagent in deionised acidic (pH 3) and natural water (pH 7.6). BPA degradation rate and primary intermediates were identical in both processes. However, COD and TOC analyses showed that the Fenton's reagent was more efficient and faster than ultrasonic treatment for the removal of BPA intermediates in the case of deionised water.

Chen et al. (2006) investigated H_2O_2/UV-Coxidation for the degradation of BPA and changes in its estrogenic activitiy. BPA removal rates and formation of degradation products, which were determined by high performance liquid chromatography (HPLC) analysis demonstrated that the H_2O_2/UV-C process was effective as compared UV alone for reducing larval lethality in treated BPA solutions.

2.2.6 Applications of the Fenton's reagent for BPA removal

Zheng et al. (2009) studied Fenton processindissolved organic matter (DOM) removal of EDCs including phthalic acid esters (PAEs) and BPA from the young and mature landfill leachate. BPA removal was around 60% and 37% from the mature leachate and young leachate, respectively. Results also showed that removing efficiency of BPA and treatment capacity of the Fenton's reagent had a relationship with its concentration.

Sajiki and Yonekubo (2004) investigated BPA degredation in seawater using Fenton's reagent. These results indicated that BPA degradation occured in the presence of radical oxygen spices and accelerated by the formation of hypochlorite (OCl^-) in salt containing water samples. Degradation threshold of BPA was observed when Fenton process was employed in seawater spiked with high amount of BPA.

Ioan et al. (2007) carried out Fenton's reagent with and without additional sonochemical treatment of 25 mg/L BPA. Complete degradation of BPA was achieved after 60 min under both treatment conditions and the other experimental results showed that ultrasonically treatment method could enhance the degradation rate as compared Fenton's reagent alone.

2.3 Toxicity Tests

2.3.1 The need for toxicity tests

The toxicological situation of wastewater, sediments and contaminated water bodies is a crucial part for environmental pollution. Commonly, some collective environmental parameters such as pH, DO, COD, BOD, TOC, TDS and TSS are used for pollution monitoring and evaluation of effluent quality. However, these parameters only demonstrate the nature of the pollutants and do not

provideinformation about the biological and toxicity effects of them in the environment/receiving body (Parvez et al., 2006; Movahedian et al., 2005).

Assessment of biological effects using rapid, simple, sensitive and cost effective methods can define specific information on toxicity and ecotoxicity effects. Traditionally, different organisms such as fish, algae, bacteria, invertebrates and plants are used in aquatic biotoxicity tests (Movahedian et al., 2005). These tests are based on measuring the reaction of organisms exposed to contaminants relative to a control and have been used to determine the toxicity levels of target compounds and complex aqueous matrices such as surface water, groundwater or wastewater (Rizzo, 2011).

2.3.2 Biotoxluminescent bacteria test with photobacteria *Vibrio fischeri*

The Biotox luminescence test is based on light emission (luminescence) from the marine photobacteria *Vibrio fischeri* which is often preferred as the first test in a test battery after the treatment of phenol, pesticides, cyanides, drugs etc., considering its speed and cost (Marugàn et al., 2012; Parvez et al., 2006). If the light emission which is a result of different life/metabolic processes is reduced, it means that a toxic compound inhibits one or more of these processes. This test is a short-term test, where the results of the test is ready in a few hours from the start. Less time consuming than most other toxicity tests and simplicity of operation are the advantages of those toxicity tests. The bacteria are provided by producers in a lyophilized form, and moreover, they can be stored for several months to be used depends on demand (Rizzo L., 2011). It has been standardized (ISO, 1998) and it is commercially available in different versions as well.

2.3.3 Acute toxicity test on the freshwater crustacean *Daphnia magna*

Daphnia magna is a characteristic and common representative of the freshwater invertebrates which is used to evaluate the acute and chronic toxicity of freshwater ecosystems. The tests based on acute effects of compounds on *Daphnia magna* are standardized by USEPA (2002) and ISO Standard (ISO 6341-2010). The organisms are exposed to target contaminants or aqueous matrices under static conditions and mobile daphnids are counted after 24 and 48 hours exposure of acute toxicity or 21 days exposure of chronic toxicity (Tisler et al., 2004). The use of daphnids has many advantages for toxicity tests, such as high sensitivity to toxic chemicals, short

reproduction time and they reproduce parthenogenetically (virgin birth) when the conditions are optimal (Tothill and Turner, 1996).

2.3.4 Algal growth inhibition test with green microalgae *Pseudokirchneriella subcapitata*

Their ubiquity and short life cycle make algae appropriate for toxicological experiments. Toxicity tests related on algae have been studied (Pehlivanoglu and Sedlak, 2004; Radix et al., 2000; Wong et al., 1995; Joubert, 1980). The biomass of algae ismeasured using an automatic particle counter and growth inhibition is used as the indicator of toxicity. The difficulty in culturing and/or lack of reproducibility are main disadvantage of algal tests (Farrè and Barcelò, 2003).

Used organisms in this experimental study, their methods used in literature and some applications to water, wastewater and liquid waste such as landfill leachate are summarized in **Table 2.6.**

Table 2.6 : Major organisms being used in toxicity tests protocols, their methodology and some selected some applications.

Test Group	Test Organism	Test Method	Application/references
Microrganisms	*Vibrio fischeri*	ISO, 2008	Disinfection of hospital wastewater (Emmanuel et al., 2004) Industrial wastewater treatment (Tisler et al., 2004) Urban wastewater treatment (Hernando et al., 2005)
Invertebrate	*Daphnia magna*	USEPA, 2002 ISO, 2010	Disinfection of hospital wastewater (Emmanuel et al., 2004) Drinking water treatment (Rizzo et al., 2005) Industrial wastewater treatment (Oral et al., 2007) Advanced treatment of urban wastewater (Rizzo et al., 2009) Urban wastewater treatment (Hernando et al., 2005) Landfill leachate treatment (Marttinen et al., 2002)
Plants and algae	*Pseudo. subcapitata*	ISO, 2012	Industrial wastewater treatment (Walsh et al., 1980; Tisler et al., 2004; Oral et al., 2007) Urban wastewater treatment (Hernando et al., 2005)

2.3.5 Toxicity tests conducted with bisphenol A and its degradation products

Frontistis et al. (2011) investigated the estrogenic properties of BPA measuring the bioluminescence inhibition of *Vibrio fischeri*. Experimental results showed 38% inhibition of bioluminescence for 300 mg/L BPA which decreased to 26% after 120 min photoelectrocatalytic oxidation. They also concluded that oxidation products were less toxic and estrogenic than BPA.

Rodríguez et al. (2010) studied the degradation of BPA using different AOPs focusing on the removal of BPA and the formation of phenolic intermediates. Toxicity was determined by measuring the luminescence inhibition tests with of *Vibrio fischeri*. The results indicated that there was a relationship between mineralization, TOC conversion and toxicity, moreover, some of the phenolic

intermediates formed could be more toxic than BPA. When mineralization was 20%, toxicity reduced from 70% to 30%.

Alexander et al. (1988) reported the BPA toxicity results of a series of short term tests using fresh and salt water algae, invertebrates and fish. They concluded that 2.7 and 3.1 mg/L of 96-h EC_{50} based on cell count and cell volume, respectively, for the freshwater microalgae *Selanastrum capricornutum*. It was reported that 48-h EC_{50} was 10 mg/L for *Daphnia magna*.

Stephenson (1983) reported 48-h EC_{50} was 3.9 mg/L for *Daphnia magna* and 96-h EC_{50} was 2.5 mg/L based on cell growth for *Selanastrum capricornutum*.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Bisphenol A

Bisphenol A (BPA; 4,4'-isopropylidenediphenol, 228.287 g/mole) was supplied from Sigma Aldrich, Germany. Some of the chemical and physical properties of BPA are summarized in **Table 2.1**.

All other chemicals used were at least reagent grade and obtained from Fluka, Sigma Aldrich or Merck depending upon their price, purity and availability.

3.1.2 Real freshwater samples

Experiments were also run with real freshwater sample taken from 1) the inlet **(Table 3.1)** of a water treatment plant located in Kagithane, Istanbul; 2) from Sjælsø lake **(Table 3.2)** located in Birkerød, Denmark .

Table 3.1 : Environmental characterization of raw freshwater sample taken from inlet of Kagithane water treatment plant.

Parameter	Unit	Value
TOC	mg/L	6.9
DOC	mg/L	6.2
Alkalinity	mg $CaCO_3$/L	92
Hardness	mg $CaCO_3$/L	120
Colour	Pt-Co	26
Turbidity	NTU	3
SS	mg/L	bdl*
pH	-	8.3
UV_{254}	-	0.234
UV_{280}	-	0.185
UV_{350}	-	0.076
Cl^-	mg/L	21
Br	mg/L	3.6
NO_2^-	mg/L	0.52
NO_3^-	mg/L	0.27
SO_4^{2-}	mg/L	12
PO_4^{3-}	mg/L	2.7
BPA	mg/L	bdl*

*below the detection limit

Table 3.2: Environmental characterization of the raw lake water sample taken from Sjælsø lake.

Parameter	Unit	Value
TOC	mg/L	10.4
TKN	mg/L	1.1
pH	-	8.4
PO_4-P	mg/L	0.076

Real raw freshwater sample was taken from a local water treatment plant of the Istanbul Metropolitan Municipality. The selected water treatment plant serves over 5 million inhabitants of the European Part of Istanbul and practices aeration, ozonation, coagulation-flocculation and sand filtration for elimination of odor and taste (iron and manganese), micropollutants, organic carbon, turbidity, suspended matter and microorganisms. For disinfection purposes the treatment facility also features intermittent- and post-chlorination. However, Sjælsø lake in Denmarkhas recently served as adrinking water reservoir for Gentofte Municipality.

Both collected real freshwater samples were stored in plastic carboys in a cool room at 4^{o}C prior to use. For the sample taken in Istanbul, 450 nm cut-off cellulose nitrate membranes filters (Sartorius Stedim Biotech No. 11406-47-ACN) and for the sample taken in Denmark, glass microfibers filters with 150 nm cutoffs (VWR European Cat. No. 516-0875) as well as cellulose acetate membrane filters with 450 nm cutoffs (Q-Max CA-S Syringe Filters Cat. No. CA250450S) were used prior to analysis in order to obtain the supernatant.

3.1.3 Fenton Experiments

In the Fenton experiments, the pH (3-6) and temperature (20-70^{o}C) of 20 mg/L and 50 mg/L aqueous BPA solution was adjusted using NaOH (1N) or/and H_2SO_4 (1N or 6N) in a 2-liter beaker. Then an appropriate amount of H_2O_2 (0.5-5.0 mM) was added to the pH-adjusted BPA solution from a 35% w/w stock solution to obtain a final H_2O_2 concentration of 0.5-5.0 mM in the reaction solution. The reaction was initiated by adding the Fe^{+2} (0.05-1.00 mM) ions from a $Fe(SO_4).7H_2O$ (10% w/v) stock solution. The stock solution was daily prepared by dissolving $Fe(SO_4).7H_2O$ in

distilled water. The Fenton reaction was continued for 90 min and samples were taken at regular time intervals. The reaction was ceased by spiking the sample with 1N NaOH solution to increase the pH to around 10±1. Thereafter, the pH was adjustedto 7.0-7.5 which is the optimum pH value for maximum $Fe(OH)_3$ precipitation and removing Fe^{2+} from solution. In order to remove the formed $Fe(OH)_3$ flocs, the samples was filtered through 450 nm cut-off membrane filters prior to all measurements. The samples were analyzed for BPA and TOC abatements, residual (unreacted) H_2O_2 consumption and toxicity tests.

3.2 Experimental Procedures

3.2.1 BPA

The amount of BPA in the aqueous solution was measured by high-performance liquid chromotography (HPLC; Agilent 1100 Series, USA) equipped with a UV Detector (G1314A, Agilent Series) and Symmetry C18 (3.9 × 150 mm, 5 mm, Waters) column. The mobile phase was acetonitrile-water solution (50% v/v) used at flow rate 0.5 mL/min. The eluent was monitored at 214 nm. The column temperature was set at 25 ˚C during the measurements. Quantification of BPA was achieved through the use of calibration curve. Using this methodology, a detection limit of 0.14 mg/L of BPA was reached.

3.2.2 TOC

Changes in the TOC content of the samples was monitored on a Shimadzu VPCN and ASI-V model organic carbon analyzer at ITU and DTU, respectively. The instruments were equipped with autosamplers and Infrared (IR) detectors, and were calibrated with standard potassium hydrogen phthalate solutions.

3.2.3 H_2O_2

At ITU, the residual (unreacted) H_2O_2 was measured by the molybdate catalyzed iodometric method accordance with Horwitz (1980), whereas at DTU, the OPDV (oxo-peroxo-pyridine-2,6-dicarboxylato-vanadate) colorimetric method was employed in accordance with Tanner and Wong (1998).

3.2.4 Acute toxicity tests

At ITU, 4 Mm H_2O_2 was used at 50 ^oC. Before all toxicity tests, enzyme Catalase derived from *Micrococcus lysodeikticus* (solution, dark brown, ≈170000 U/mL, Fluka, USA) was used for destroying residual H_2O_2 (around 6 U/mL for each sample), whereas, at DTU, acute toxicity analyses was carried out with samples being subjected to Fenton's reagent under the following optimized reaction conditions; 20 mg/L BPA, 2 mM H_2O_2, 0.4 mM Fe^{2+}, pH 5 and room temperature.

At ITU, acute toxicity was measured in all untreated and Fenton-treated samples using the photobacteria *Vibrio fischeri* as the test organism. The test ISO 11348-3 test protocol(2008) was used to measure the acute toxicity of untreated and Fenton-treated freshwater samples spiked with BPA.

At DTU, acute toxicity was measured in all untreated and Fenton-treated samples using the photobacteria *Vibrio fischeri*, *Daphnia magna* and *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) as the test organisms. The ISO 11348-3 test protocol (2008), the ISO 6341 test protocol (2010) and the ISO 8692 test protocol (2012) were used to measure the acute toxicity of*Vibrio fischeri, Daphnia magna* and *Pseudokirchneriella subcapitata*, respectively, in untreated and Fenton-treated freshwater samples spiked with BPA.

3.2.4.1 Biotox-Luminescence: Test method based on measurement of light emission from the marine photobacteria *Vibrio fischeri*

The biotox test was performed according to the ISO 11348-3:2008. The BioTox^{TM} Kit was derived as freeze dried material from Aboatox, Turko, Finland.

The test organism marine bacteria *Vibrio fischeri* emits light under normal conditions. This emission of light is used as the test parameter in the biotox test. The light emission from the bacteria is measured photometrically by a photomultiplier. The light emission is a result of different life processes (metabolic reactions) within the bacterial cells. If a toxic compound inhibits one or more of these processes the light emission is reduced.

Before starting the test, the freeze-dried bacteria were dissolved in 12.5 mL dilution water and were temperated to room temperature for about 30 min. Two replicates of each sample were tested. After adding 100 µL of the bacteria suspension into each of

the test vials, the vials were placed in the luminometer (Luminoskan TL Plus, Thermo Lab Systems, Finland) starting with controls and measured their luminescence (t_0). Thereafter, 100 µL of the samples were added into the vials and measurements were repeated after 5 (t_5), 15 (t_{15}) and 30 min (t_{30}). The results were noted and inhibitory effect on *Vibrio fischeri* were evaluated by using following equations;

$$f_{kt}=I_{kt} / I_0 \quad \textbf{(3.1)}$$

f_{kt} is the correction factor for the contact time of 5, 15 or 30 min; I_{kt} is the luminescence intensity in the control sample after the contact time of 5 min, 15 min or 30 min, in relative luminescence units; I_0 is the luminescence intensity of the control test suspension, immediately before the addition of the diluent, in relative luminescence units **(3.1)**.

$$I_{ct}=I_0 \times f_{kt*} \quad \textbf{(3.2)}$$

I_{ct} is the corrected value of I_0 for test sample tubes immediately before the addition of the test sample; I_0 is the luminescence intensity of the test sample suspension, immediately before the addition of the sample or the diluted sample, in relative luminescence units; f_{kt*} is the mean of f_{kt}**(3.2)**.

$$H_t=(I_{ct}-I_t)/I_{ct} \times 100 \quad \textbf{(3.3)}$$

H_t is the inhibitory effect of a test sample after the contact time of 5, 15 or 30 min, in percent; I_t is the luminescence intensity of the test sample after the contact time of 5, 15or 30 min, in relative luminescence units. In this experimental study, 15 min of incubation time was evaluated **(3.3)**.

3.2.4.2 Acute toxicity test with the freshwater crustacean *Daphnia magna*

The acute toxicity test was performed according to the ISO 6341 test protocol (2010). The Danish clone of *Daphnia magna* was isolated in Langedammen in Birkerød in 1978 and has since then been kept as a clone in the laboratory. Under normal laboratory conditions (20°C) the Danish clone started to reproduce when animals were 8 days old. UVP/White light transilluminator (light table) was used to count dead animals and to control the number of animals in the beakers. For this test,

Daphnia magna medium (M1 medium) was used as dilution water. Nutrients required and their amounts for M1 media are shown in **Table 3.3.**

Table 3.3 : M1 media for *Daphnia magna* (ISO 6341, 2010).

Ingredients for M1 media	
Chemical	**Concentration (mg/L)**
$CaCl_2.2H_2O$	293.8
$MgSO_4.7H_2O$	123.3
KCl	58
$NaHCO_3$	64.8
Na_2SIO_3	4.3
$NaNO_3$	0.274
$KHPO_4$	0.143
K_2HPO_4	0.184
$C_{12}H_{17}ClN_4OS$	0.075
$C_{63}H_{88}CoN_{14}O_{14}P$	0.001
$C_{10}H_{16}N_2O_3S$	0.00075

The test was performed with animals less than 24 h old which were exposed to various concentrations of the test substance for up to 48 h. All studies were conducted at 20 ± 2 °C, pH 7.8 ± 0.2, with darkness period, without aeration and feeding. It was provided 5 animals per beaker, at least 2 mL medium/animal and 25 % of the air saturation value (≥2 mg O_2/L). During the test, dissolved oxygen and pH was measured in the control and all test samples. After 24 and 48 h, the number of dead and/or immobilized animals were counted and inhibitions were reported.

3.2.4.3 Growth Inhibition Test with the green microalgae*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)

The growth inhibition test was performed according to the ISO 8692 test protocol (2004). *Pseudokirchneriella subcapitata* was derived from the algal culture collection at Norwegian Institute of Water Research, (NIVA), Oslo, Norway. For this test, synthetic freshwater medium (M2 medium) was used as dilution water. Chemicals required and their amounts for M2 media are shown in **Table 3.4**.

Table 3.4 : M2 media for the algal test (ISO 8692, 2004).

Ingredients for M2 media	
Chemical	**Concentration (mg/L)**
NH_4Cl	15
$MgCl_2.6H_2O$	12
$CaCl_2.7H_2O$	18
$MgSO_4.7H_2O$	15
KH_2PO_4	1.60
$FeCl_3.6H_2O$	0.064
$Na_2EDTA.2H_2O$	0.10
H_3BO_3	0.185
$MnCl_2.4H_2O$	0.415
$ZnCl_2$	0.003
$CoCl_2.6H_2O$	0.0015
$CuCl_2.2H_2O$	0.00001
$Na_2MoO_4.2H_2O$	0.007
$NaHCO_3$	50

The pre-culture was set up three days before the start of the test to secure exponential growth in the inoculum culture. The cell density was measured on the Coulter multisizer. The flasks used in the measurements contained approximately 10^4 cells/mL at test initiation in 25 mL of each concentration.Three replicates was prepared containing 4 mL of each concentration. The replicates were placed in an algal growth chamber under continuous fluorescent illumination (60-120 $\mu mol \cdot m^2 \cdot s^{-1}$) and incubated at 22 ± 1 ˚C and pH 8 ± 0.2. At the start, after 24 and 48 h, 0.4 mL of sample with 1.6 mL acetone were mixed for biomass measurement and stored in dark for at least 12 hours. All acetone extracted samples including a blind and all replicates were measured at 420 nm on Cary Eclipse Fluorescence spectrophotometer. The measured fluorescence (relative units) was used directly as the biomass parameter. The growth of biomass was calculated as follows;

Growth of biomass: $N_n = N_0 \cdot \exp(\mu \cdot t_d)$ **(3.4)**

Growth rate of biomass (normally expressed as μ): $\mu = (\ln N_n - \ln N_0)/t_d$ **(3.5)**

Inhibition of growth (in %): $I\mu_i(\%) = (1 - \mu_i/\mu_c) \times 100$ **(3.6)**

According to above equations, N_0 is the initial biomass (inoculum - measurement of fluorescence at the start); N_n is the final biomass (measurement of fluorescence at the end); t_d is the length of the test period (in days); $I\mu_i$is the percentage inhibition of growth rate for concentration *i*; $\mu_i(d^{-1})$is the mean growth rate for concentration *i* and $\mu_c$is the mean growth rate for the control.

4. RESULTS AND DISCUSSION

4.1 The Effects ofOperating Parameters on Fenton's Reagent

As mentionedin the previous sections, during Fenton's process H_2O_2 and Fe^{2+} concentrations must be adjusted appropriately in order to provide high treatment efficiencies and to prevent undesired HO• scavenging (side) reactions occuring in the presence of an excess of one of the two reagents (Tang and Huang, 1996);

$$Fe^{2+} + HO\bullet \rightarrow Fe^{3+} + OH^- \qquad (k_1 = 3.0 \times 10^8\ M^{-1}s^{-1}) \qquad \textbf{(4.1)}$$

$$H_2O_2 + HO\bullet \rightarrow H_2O + HO_2\bullet \qquad (k_2 = 2.7 \times 10^7\ M^{-1}s^{-1}) \qquad \textbf{(4.2)}$$

The most suitable molar ratio between H_2O_2 and Fe^{2+} (the H_2O_2:Fe^{2+} molar ratio) must be predetermined in order to minimize the HO• scavenging effects. As indicated before, pH and temperature have also a significant effect on the performance of the Fenton's reagent. All these parameters need to be investigated before selection of most appropriate Fenton's reagent conditions for the acute and subchronic toxicity tests.

Accordingly, the first part of the experimental study involved the determination of BPA concentration (20 mg/L (87.6 μM) or 50 mg/L (219 μM) and most appropriate values of H_2O_2 (0.50-5.00 mM) and Fe^{2+} (0.05-1.00 mM) concentrationsranges as well as pH (3-6) and temperature (20-70˚C).

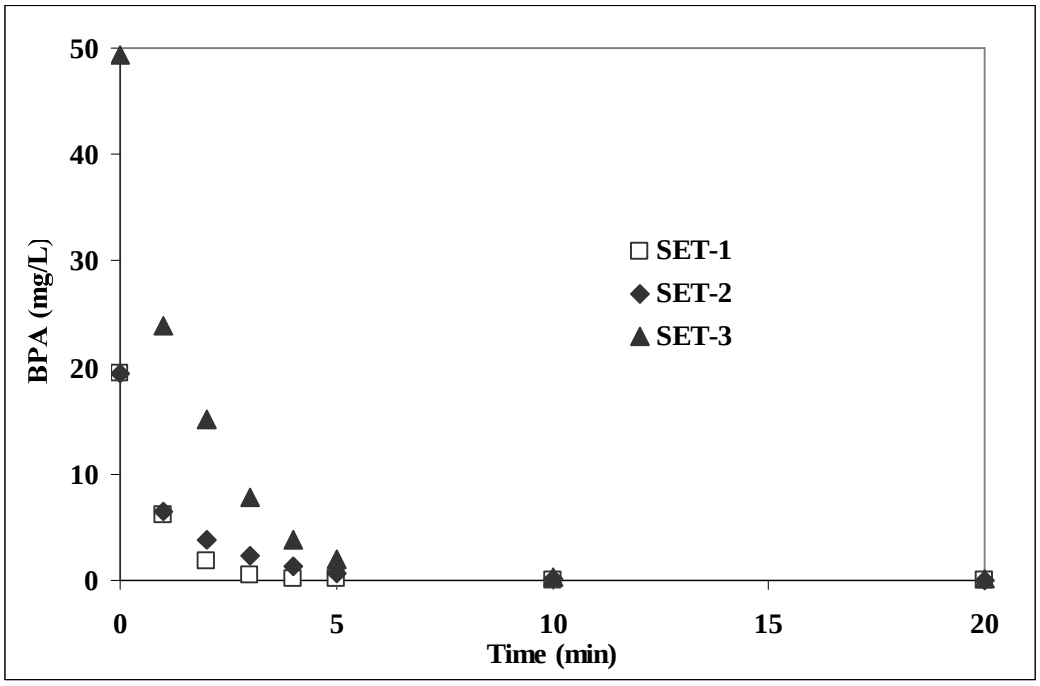
4.1.1 Baseline experiments

For the baseline experiments, the operating conditions were as follows;

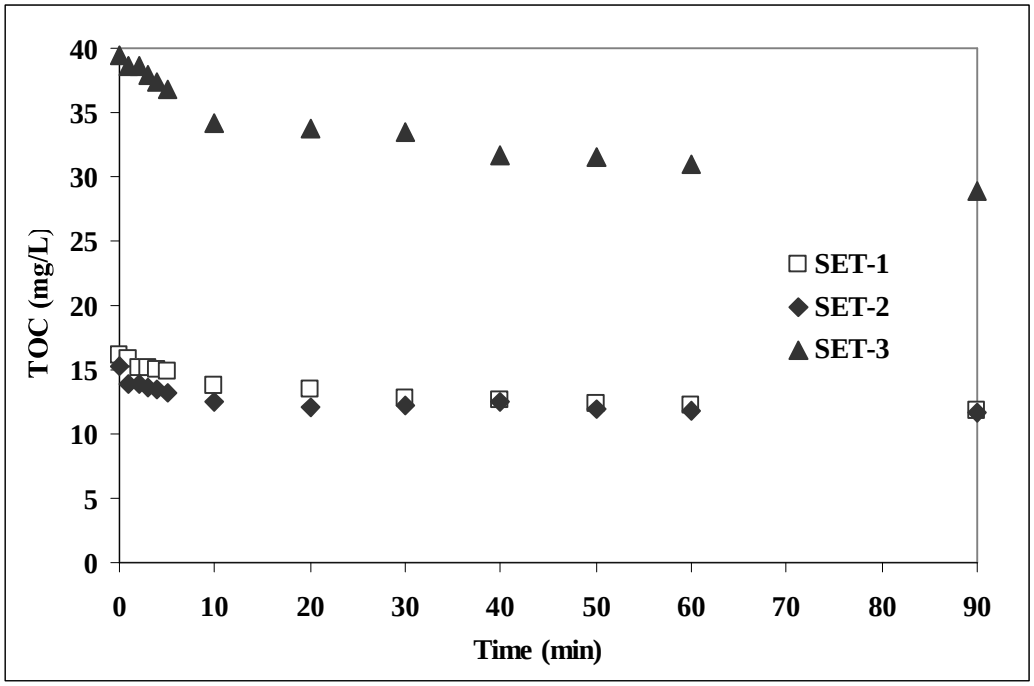
- SET-1) BPA= 20 mg/L, H_2O_2= 5.0 mM;
- SET-2) BPA= 20 mg/L, H_2O_2= 1.0 mM;
- SET-3) BPA= 50 mg/L, H_2O_2= 5.0 mM;

while maintaining the concentration of Fe^{2+} constant at an arbitrary value of 0.1 mM. Other reaction conditions were an initial pH of 3 and room temperature. All experiments were conducted for 90 min.

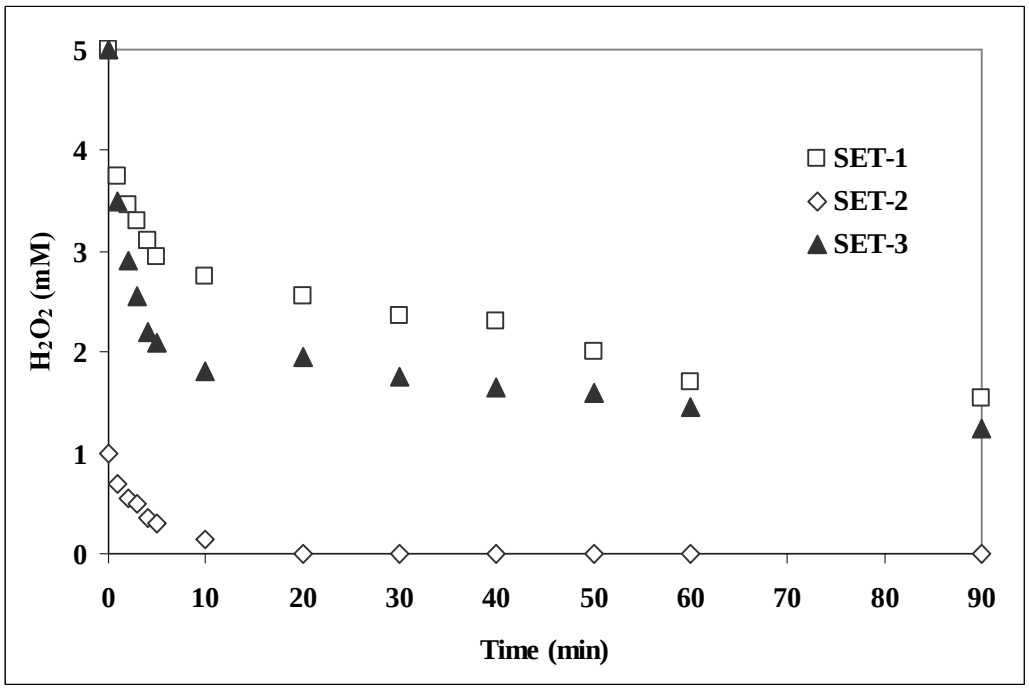
Fig. 4.1 displays changes in BPA (a), TOC (b) and H_2O_2 (c) as a function of treatment time.



(a)



(b)



(c)

Figure 4.1 :BPA (a),TOC (b),H_2O_2(c) abatements for SET-1,2 and 3. Experimental conditions: SET-1: BPA=20 mg/L; H_2O_2=5 mM,SET-2: BPA=20 mg/L;H_2O_2=1 mM, SET-3: BPA=50 mg/L;H_2O_2=5 mM.

As can be seen from **Fig. 4.1a**, even though complete BPA removal was achieved after 40 min, removal of 50 mg/L of BPA was slower than that of 20 mg/L BPA, as expected. It was concluded that this concentration was slightly higher for Fenton's process. Because of highest BPA concentration, SET-3 (BPA=50 mg/L; H_2O_2=5 mM) showed faster TOC removal rate in first 10 min which was shown in **Fig. 4.1b**. However, there was nopronounced difference in TOCremoval rates after first 10 min. It was observed from **Fig. 4.1c** that H_2O_2 consumption of SET-2 (BPA=20 mg/L, H_2O_2=1 mM) was faster than those observed in the other sets because of its low initial H_2O_2 concentration. Additionally, when the H_2O_2 concentration was 5 mM, there was still a residual H_2O_2 even after the treatment of 90 min indicating that 5 mM was an excessive concentration for these experimental conditions. It could be concluded that H_2O_2 consumption rate was related to pollutant concentration and Fe^{2+} concentration which may be increased for complete H_2O_2 consumption and oxidation.

As can be clearly seen in **Fig. 4.2**, TOC removal efficiencies were similar to each other (around 27%) for 90 min treatment time. However, the specific H_2O_2 consumption (per removed TOC) was 27, 9 and 12 mg/mg for SET-1, SET-2 and SET-3, respectively, which have been calculated as shown in**(4.3)**.

Specific H_2O_2 consumption rates, Y_{H2O2} have been calculated for t=90 min as follows;

Y_{H2O2} (in mg/mg) = mg/L H_2O_2 consumed / mg/L TOC removed **(4.3)**

It was evident that the results of SET-2 (BPA=20 mg/L, H_2O_2=1 mM) should be preferred in terms of efficient H_2O_2 usage and TOC removal.

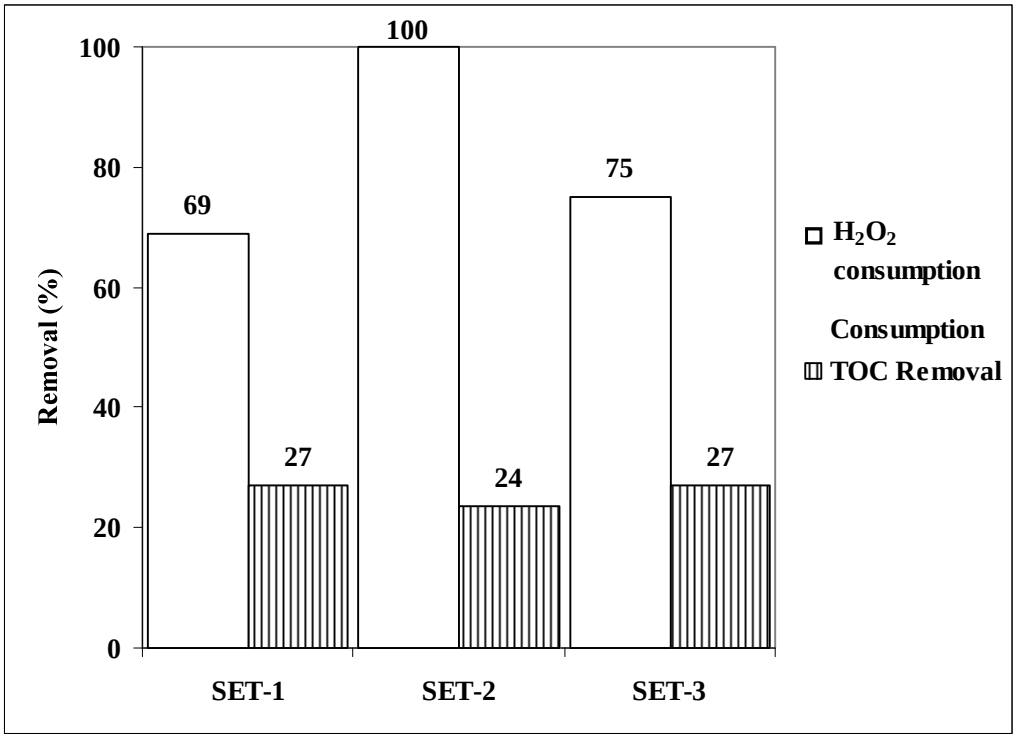


Figure 4.2 : Percent TOC removal and H_2O_2consumptions obtained for SET-1,2 and 3.Experimental conditions:
SET-1: BPA=20 mg/L; H_2O_2=5 mM,
SET-2: BPA=20 mg/L;H_2O_2=1 mM,
SET-3: BPA=50 mg/L;H_2O_2=5 mM.

BPA, TOC and H_2O_2 abatement rates followed first-order kinetics **(4.4 and 4.5)** which were displayed in **Table 4.1**.

$$r = -\frac{d[C]}{dt} = k[C] \quad \textbf{(4.4)}$$

$$\ln[\frac{C_0}{C}] = kt \quad \textbf{(4.5)}$$

k= The first order rate coefficient (min^{-1})

C= Concentration of the compound (mg/L)

A semi-logarithmic plot of C_o/C (normalized concentration) versus treatment time gives the pseudo-first order abatement rate constant (min^{-1}).

As is evident in **Table 4.1**, SET-1 (BPA=20 mg/L, H_2O_2=5 mM) had highest rate coefficient (1.012 min^{-1}) for BPA removal. However, SET-2 (BPA=20 mg/L, H_2O_2=1 mM) had highest rate coefficients in terms of TOC removal and H_2O_2

consumption. Considering all the results obtained, it was decided for other experimental sets that H_2O_2<5 mM and Fe^{2+}>0.1 mM could be the mostappropriate conditions for Fenton's reagent of 20 mg/L of BPA.

Table 4.1 : First-order rate coefficients for the baseline experiments. Experimental conditions: SET-1: BPA=20 mg/L; H_2O_2=5 mM, SET-2: BPA=20 mg/L; H_2O_2=1 mM, SET-3: BPA=50 mg/L; H_2O_2=5 mM.

Baseline Experiments	***k* (min^{-1})**		
	BPA	**TOC**	**H_2O_2**
SET-1	1.012	0.005	0.008
SET-2	0.524	0.012	0.184
SET-3	0.188	0.003	0.007

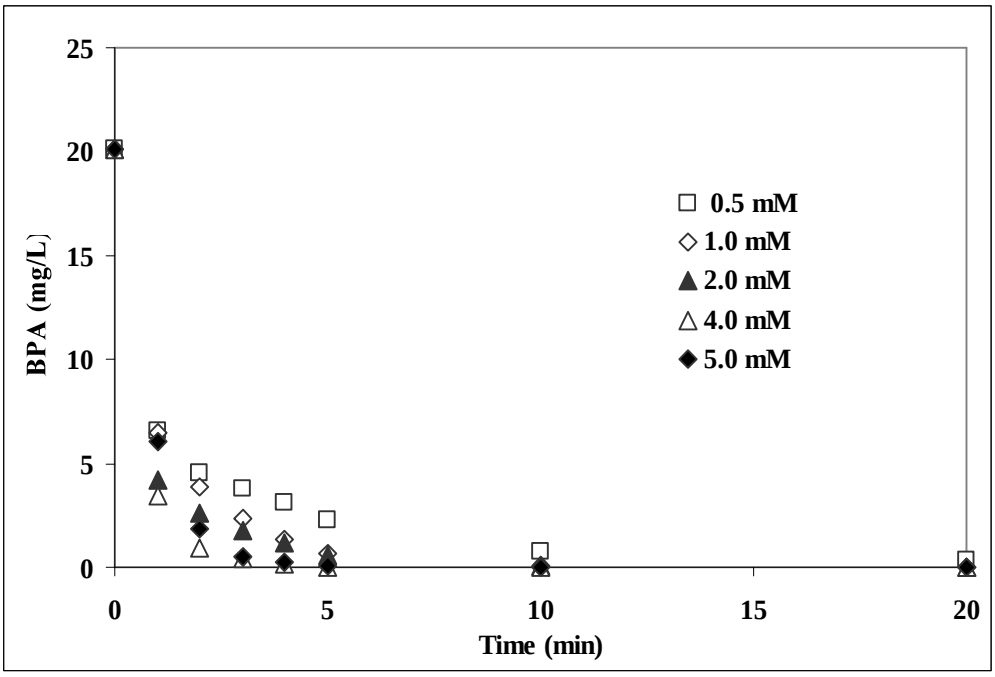
Arslan-Alaton et al. (2012a) investigated treatment of 17 mg/L aqueous BPA solution using H_2O_2:Fe^{2+}=5:1 molar ratio at pH 3. In the case of Fenton'sreagent, 94% BPA abatement was achieved after 50 min with a reaction rate coefficient of 0.11 min^{-1}. However,41% BPA and 30% TOC removal were obtained withthe Fe^{2+}/persulfate treatment and the corresponding first-order rate coefficients for BPA and TOC removals were calculated as 0.005 min^{-1}and 0.0024 min^{-1}, respectively.

4.1.2 Effect of H_2O_2

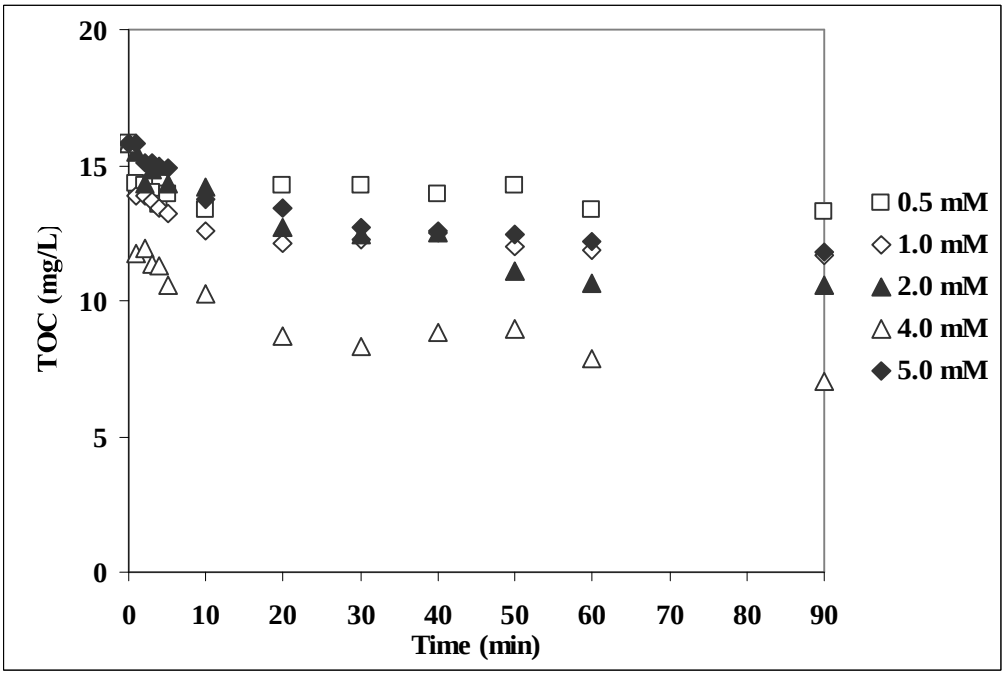
In order to determine the most suitable initial H_2O_2 concentration, a set of experiments was carried out in which initial H_2O_2 concentration (0.5-5.0 mM) was progressively increased while maintaining the concentration of Fe^{2+}constant at an arbitrary value of 0.1 mM. All experiments were conducted with 20 mg/L initial BPA concentration at an initial pH of 3 as well as room temperature during 90 min treatment time.

Fig. 4.3. presents BPA (a), TOC (b) and H_2O_2 (c) abatements at varying initial H_2O_2 concentrations. As is evident in **Fig.4.3a**, except the experimental set which was conducted with 0.5 mM H_2O_2, complete BPA removalwas obtained before 20 min Fenton process at varying initial H_2O_2 concentrations. presented in **Fig.4.3a**. After 90 min, practically complete BPA removal was achieved in the presence of 0.5 mM H_2O_2. It is also evident from **Figs.4.3a** and **4.3b** that BPA and TOC removal rates increased with increasing initial H_2O_2 concentrations, respectively, except for the

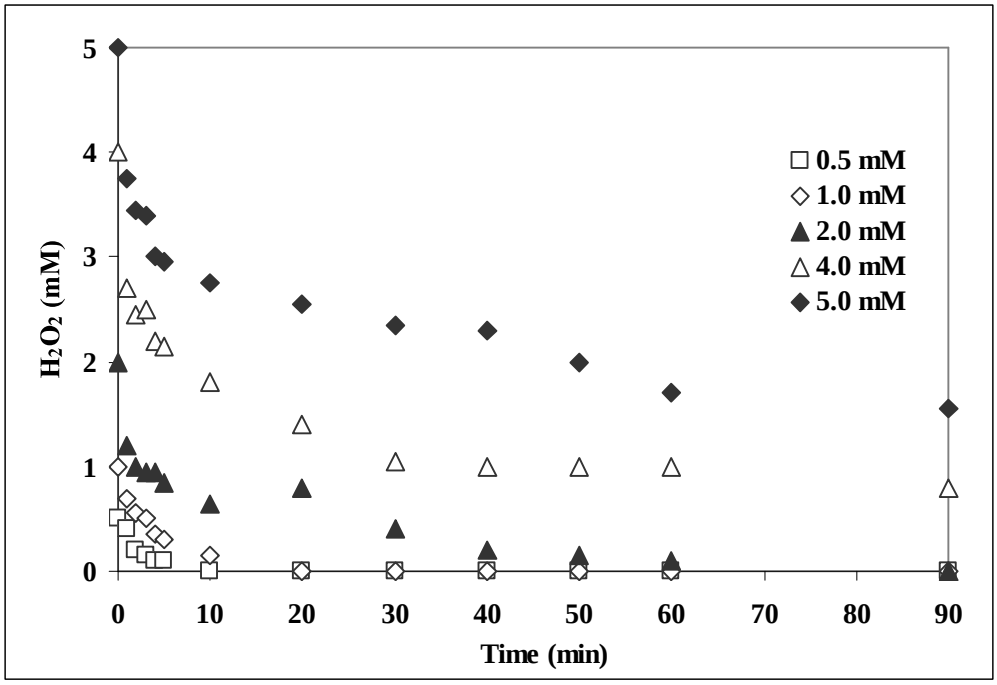
experimental run with the highest initial H_2O_2 concentration. It was concluded that excessive concentrations of H_2O_2 with respect to Fe^{2+} resulted in •OH scavenging reactions (undesired, inefficient side reactions), speaking for inefficient H_2O_2 consumption rates.



(a)



(b)



(c)

Figure 4.3 : The effect of initial H_2O_2 concentration on BPA (a), TOC (b), H_2O_2 (c) abatement rates. Experimental condition: BPA_0=20 mg/L; TOC_0=16 mg/L; Fe^{2+}=0.1 mM.

As can be seen from **Fig.4.3c**, all H_2O_2 was consumed within the first 5, 10 and 60 min for 0.5 mM, 1.0 mM and 2 mM H_2O_2 concentrations, respectively. However, 4 mM and 5 mM of H_2O_2 was not entirely consumed after 90 min under the same conditions.

Table 4.2 and **Fig. 4.4** summarize BPA, TOC and H_2O_2 abatements in percent after 90 min treatment at varying initial H_2O_2 concentrations. As can be clearly seen in **Table 4.2** and **Fig. 4.4**, the most appropriate H_2O_2 concentration was selected as 2 mM corresponding to 20:1 ratio of H_2O_2:Fe^{2+}, 100% BPA removal (BPA_0=20 mg/L) and 33% TOC removal (TOC_0=16 mg/L) Fenton process with a final TOC value of 11 mg/L after 90 min. The experimental set with highest removal efficiency for TOC (56%) was not selected as most suitable condition corresponding to 4.0 mM H_2O_2 concentration, because there were residual H_2O_2 in the medium after 90 min.

Table 4.2 : Summary of the obtained results for BPA, TOC and H_2O_2 abatements in percent at varying initial H_2O_2 concentrations. Experimental conditions: BPA_0=20 mg/L; TOC_0=16 mg/L; Fe^{2+}=0.1 mM; t=90 min.

H_2O_2 Concentration (mM)	BPA		TOC		H_2O_2 Consumptions (%)	Y_{H2O2} (mg/mg)
	Final BPA (mg/L)	Removal Efficiency (%)	Final TOC (mg/L)	Removal Efficiency (%)		
0.5	0.2	99	13	16	100	6
1.0	0	100	12	26	100	9
2.0	**0**	**100**	**11**	**33**	**100**	**14**
4.0	0	100	7	56	80	12
5.0	0	100	12	25	69	29

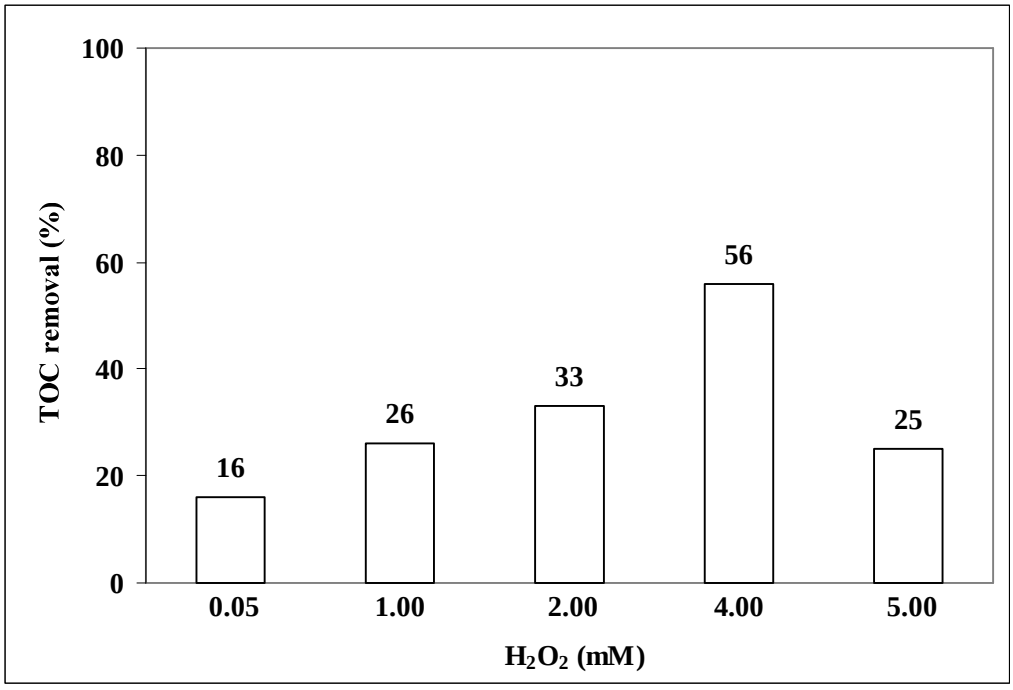


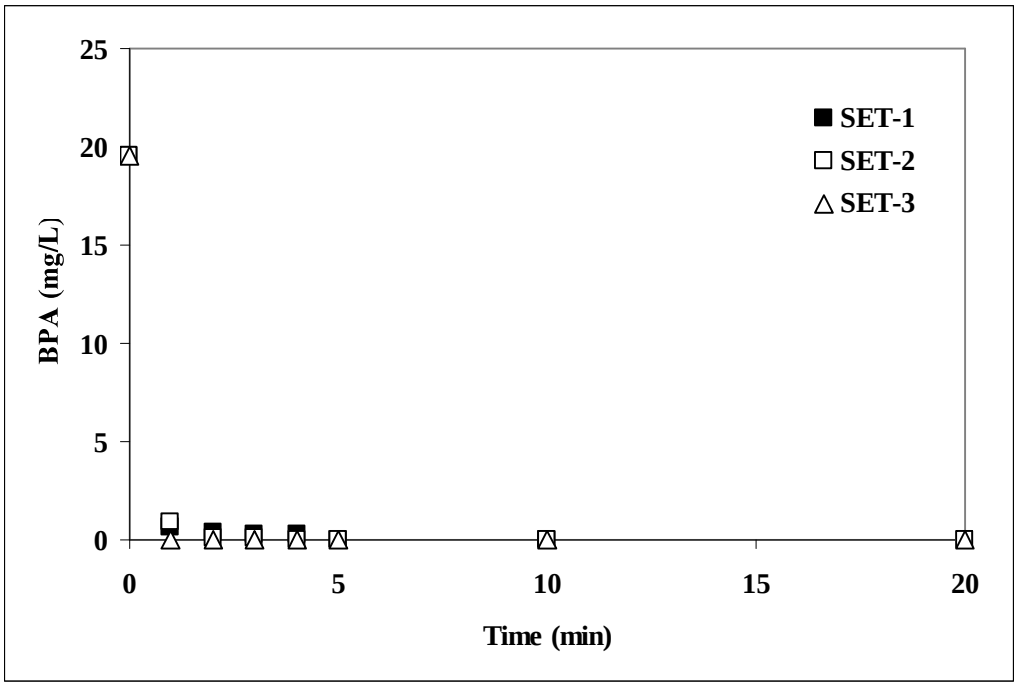
Figure 4.4 : The effect of initial H_2O_2 concentration on percent TOC removal efficiencies. Experimental condition: BPA_0=20 mg/L; TOC_0=16 mg/L; Fe^{2+}=0.1 mM; t=90 min.

Similarly, Tessora et al. (2013) investigated the degradation of BPA with Fenton's reagent. They used H_2O_2:Fe^{2+}=2:1 ratio for 40 mg/L BPA solution. The complete removal of BPA was achieved in less than 1 min. In another study conducted by Ioan et al. (2007) the degradation of BPA was examined by sono-Fenton and Fenton's reagent. Complete degradation of 25 mg/L of BPA was achieved after 60 min using

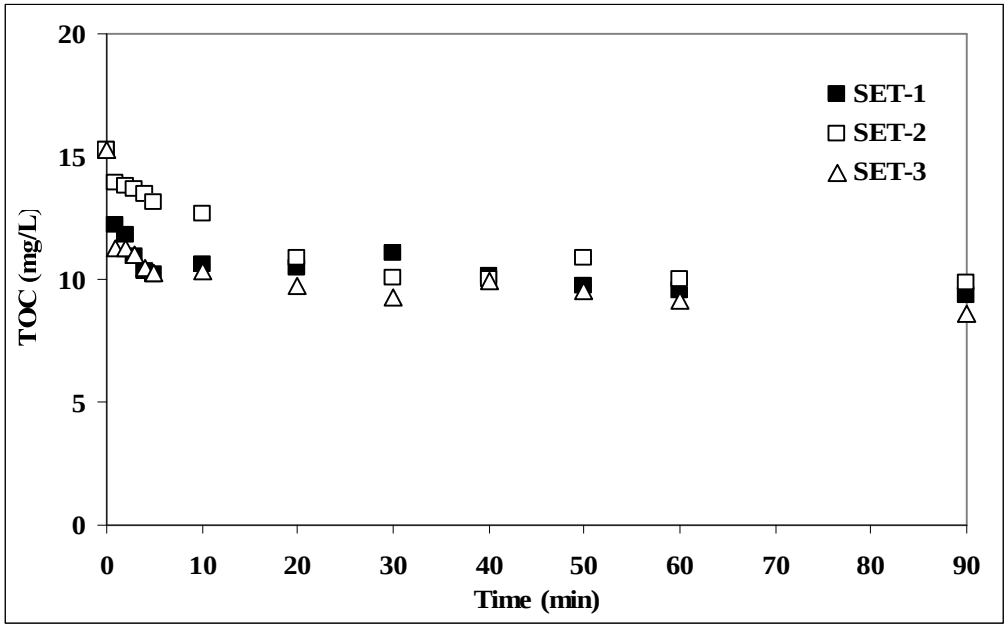
H_2O_2:Fe^{2+}5:1 ratio. Ling and Nai-Yun (2011) examined the degradation of 5 mg/L of aqueous BPA solution using H_2O_2:Fe^{2+}=2:1 and 99.12% BPA removal was achieved in 30 min at pH 4.

Additional experiments were conducted in order to evaluate effect of H_2O_2 addition under the following conditions; SET-1) 2 mM H_2O_2 addition directly, SET-2) 1 mM H_2O_2 addition and second addition of 1 mM H_2O_2 after 10 min, SET-3) 2 mM H_2O_2 addition and second addition of 2 mM H_2O_2 after 10 min while maintaining the concentration of Fe^{2+}constant at an arbitrary value of 0.4 mM at pH=5 and room temperature for 90 min reaction time.

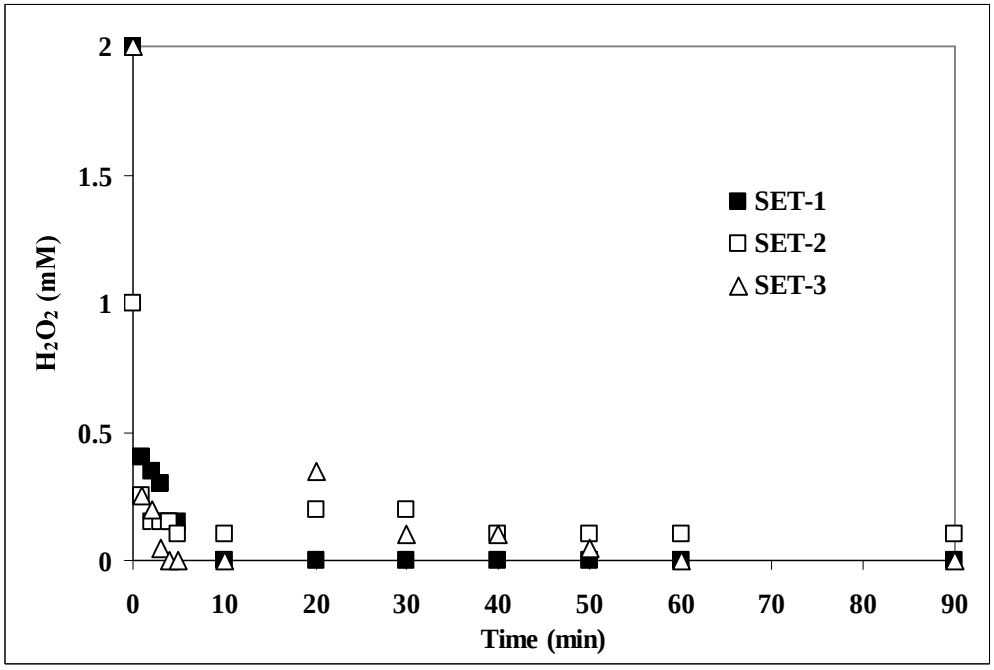
BPA (a), TOC (b) and H_2O_2 (c) abatements rates for these additional experiments are shown in **Fig. 4.5**. There was no significant difference on BPA removal rate and complete BPA removal was already achieved after 5 min which can be seen from **Fig. 4.5a**. It can be obviously seen from **Fig.4.5b**, in SET-3, the majority of TOC removal was achieved in first 10 min, whereas, it was not affected with second addition of 2 mM H_2O_2 after 10 min. In SET-1 and SET-3, TOC removal was almost same and there was no difference significantly after 10 min. It was concluded that addition of H_2O_2 directly or gradually was not very effective.



(a)



(b)



(c)

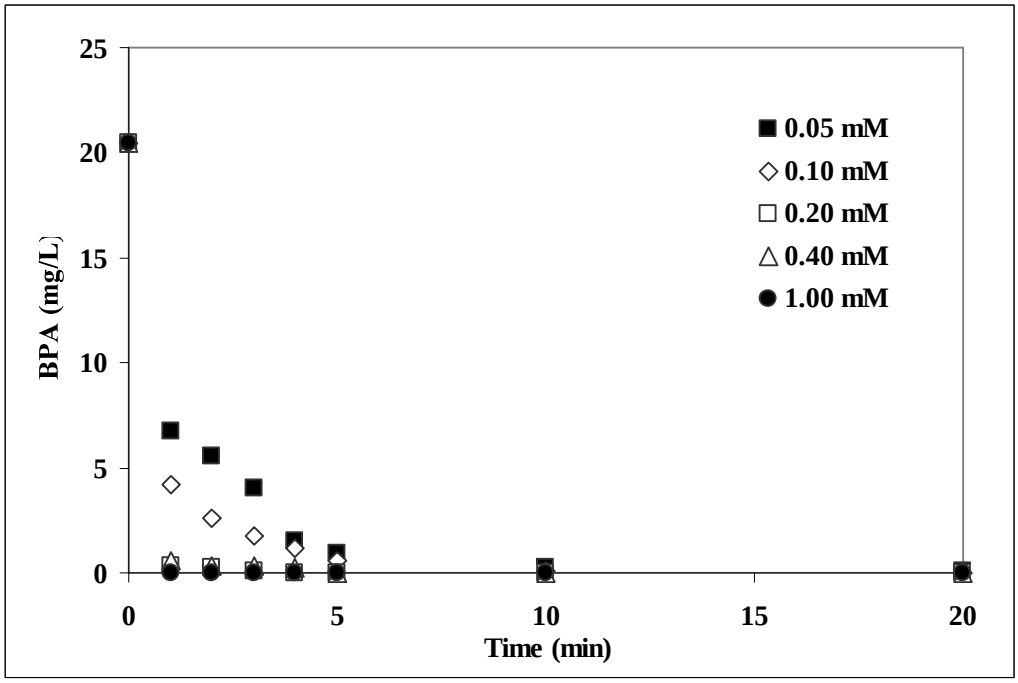
Figure 4.5 : The H_2O_2addition effects; SET-1) 2 mM H_2O_2 addition directly, SET-2) 1 mM H_2O_2 addition and second addition of 1 mM H_2O_2 after 10 min, SET-3) 2 mM H_2O_2 addition and second addition of 2 mM H_2O_2 after 10 min. Experimental conditions: BPA=20 mg/L, TOC= 15 mg/L; Fe^{2+}=0.4 mM

4.1.3 Effect of Fe^{2+}

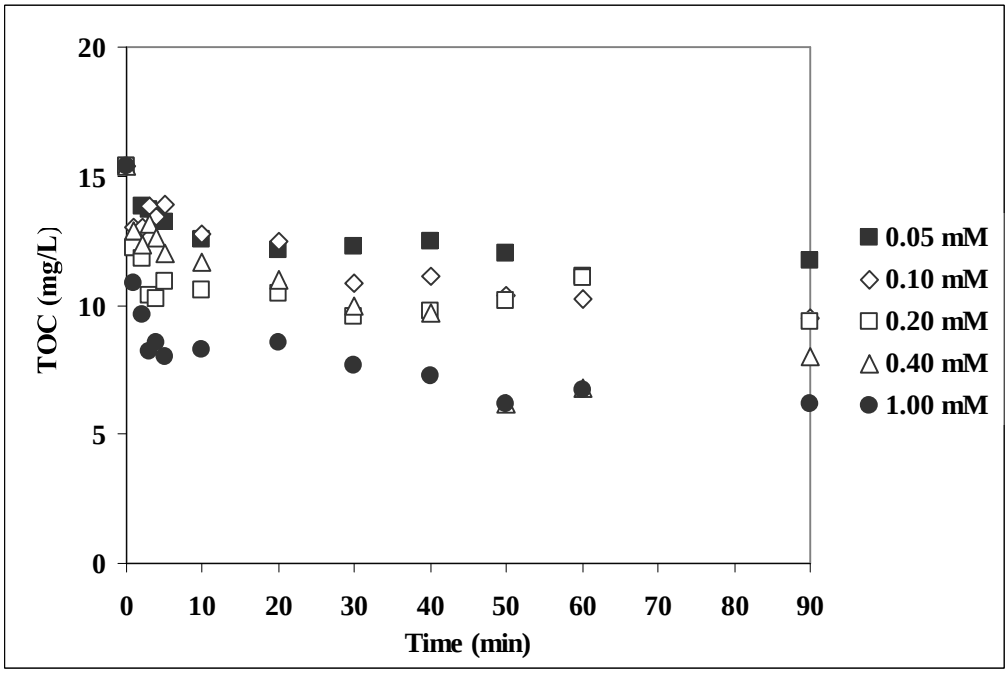
Fe^{2+} effect of Fenton's reagent was reported in various studies. In a study conducted by Ioan et al. (2007), the degradation rate of 25 mg/L of BPA increased with increasing initial Fe^{2+} concentration (H_2O_2=0.2 mM). Chan and Chu (2003) investigated the dose and ratio effects of Fe^{2+} and H_2O_2 for Fenton's reagent and they found that Fenton's reagent ratio had little effect on the oxidation capacity at high Fe^{2+} concentration, but it was more critical at lower Fe^{2+}.

In this part, the effect of Fe^{2+} concentration on Fenton process efficiency was investigated at different initial Fe^{2+} concentrations (0.05-1.00 mM) and a H_2O_2 concentration previously determined to be suitable (2.00 mM) at pH=3 and at room temperature.

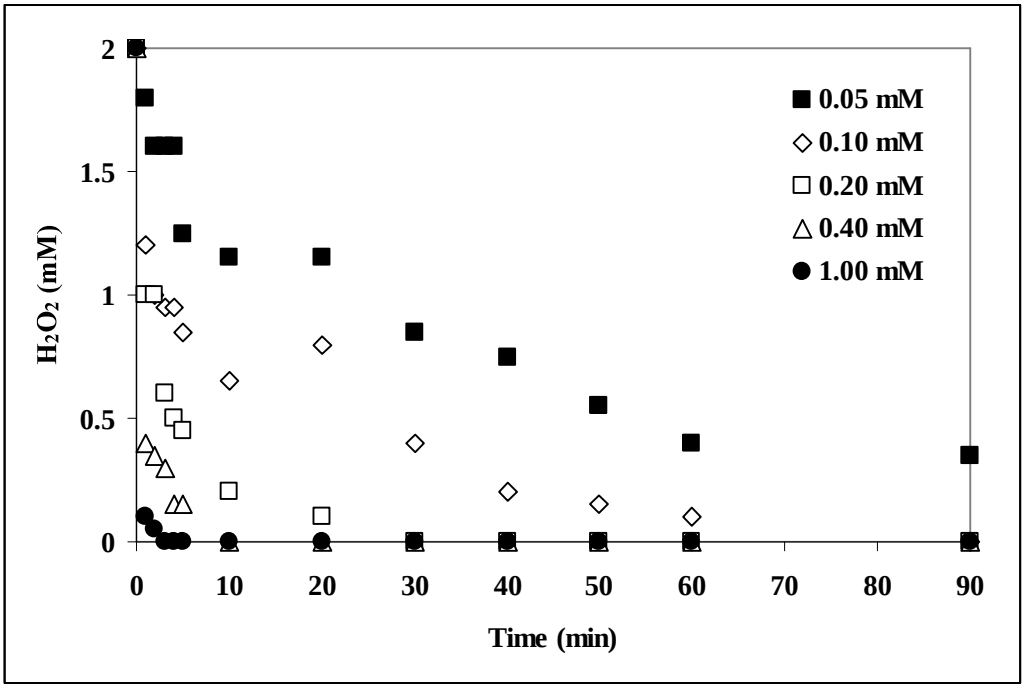
BPA (a), TOC (b) and H_2O_2 (c) abatements are displayed in **Fig. 4.6**. BPA and TOC removal rates increased with increasing initial Fe^{2+} concentrations as can be seen from **Figs. 4.6a** and **4.6b**, respectively. After 30 min, BPA removal was 100% in all experimental sets and increasing Fe^{2+} concentrations increased the TOC removal efficiencies. As is evident in **Fig. 4.6c**, H_2O_2 consumption rate increased with increasing initial Fe^{2+} concentrations.H_2O_2 was completely consumed within 60, 20, 5, 2 min of Fenton process for the initial Fe^{2+} concentrations of 0.10, 0.20, 0.40 and 1.00 mM, respectively. The experimental set which was conducted with 0.05 mM of Fe^{2+} showed that there was H_2O_2 residual at the end of the treatment, which showed that 0.05 mM of Fe^{2+} was not enough to achieve complete consumption of 2 mM H_2O_2.


25
20
15
10
5
0
BPA (mg/L)
0
5
10
15
20
Time (min)
0.05 mM
0.10 mM
0.20 mM
0.40 mM
1.00 mM

(a)


20
15
10
5
0
TOC (mg/L)
0
10
20
30
40
50
60
70
80
90
Time (min)
0.05 mM
0.10 mM
0.20 mM
0.40 mM
1.00 mM

(b)



(c)

Figure 4.6: The effect of initial Fe^{2+} concentration on BPA (a), TOC (b), H_2O_2 (c) abatement rates. Experimental conditions: BPA_0=20 mg/L; TOC_0= 15 mg/L, H_2O_2=2 mM.

The effect of varying initial Fe^{2+} concentrationsin percent BPA, TOC and H_2O_2 abatements are displayed in **Table 4.3** and **Fig. 4.6**. The most efficient Fe^{2+} concentration was selected as 0.40 mM corresponding to 100% BPA removal (BPA_0=20 mg/L) and 48% TOC removal (TOC_0= 15 mg/L) resulting in an effluent TOC of value of 8 mg/L after 90 min. The highestTOC removal efficiency (60%)was obtained the following reaction conditions: 2 mM H_2O_2 and 1 mM Fe^{2+}. However, instead of 1 mM Fe^{2+} resulting in the highest TOC removal efficiency and rate under the studied reaction conditions, a lower Fe^{2+} (0.4 mM) was selected, because of excessive Fe^{2+} concentrations in the environment are not are not in the environment.

Therefore, the most suitable H_2O_2:Fe^{2+} molar ratio in terms of BPA and TOC removal as well as H_2O_2 consumption after Fenton process at pH=3 was determined to be 5:1. Similarly, Zheng et al. (2009) used a ratio of 7:1 for the removal of EDCs in landfill leachate containing 0.8 mg/L of BPA. Poerschmannet al. (2010) used a ratio of 10:1 for degradation of 10 mg/L of BPA which was close to the theoretical optimum ratio of 11:1 which had found by Tang and Huang (1997). Differently,

Sajiki and Yonekubo (2004) used molar ratio of 250:1 when they examined inhibition of seawater (3% w/v NaCl) on BPA degradation. They observed that BPA degradation could be achieved by an addition of radical oxygen species (ROS) and further accelerated by the formation of OCl^- in salt containing water samples.

Table 4.3 : The effect ofvarying initial Fe^{2+} concentrations on BPA, TOC removals and H_2O_2consumption rates. Experimental conditions: BPA_0=20 mg/L; TOC_0= 15 mg/L; H_2O_2=2 mM.

Fe^{2+} Concentration (mM)	BPA	TOC		H_2O_2 Consumption (%)	Y_{H2O2} (mg/mg)
	Removal Efficiency (%)	Final TOC (mg/L)	Removal Efficiency (%)		
0.05	100	12	24	62.5	11
0.10	100	9	39	90	10
0.20	100	9	39	100	11
0.40	**100**	**8**	**48**	**100**	**9**
1.00	100	6	60	100	7

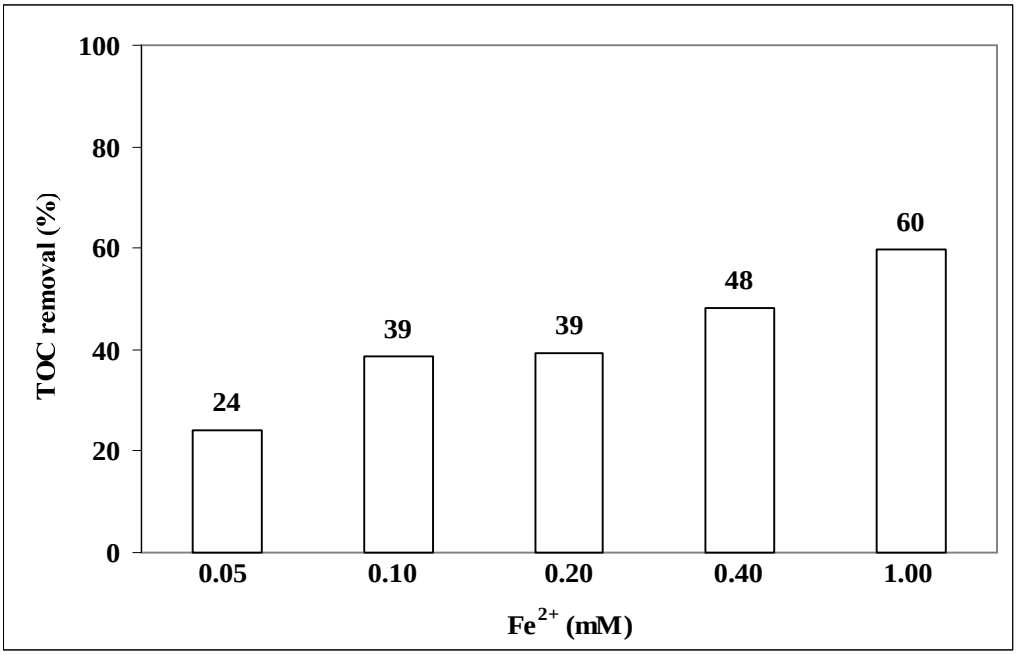
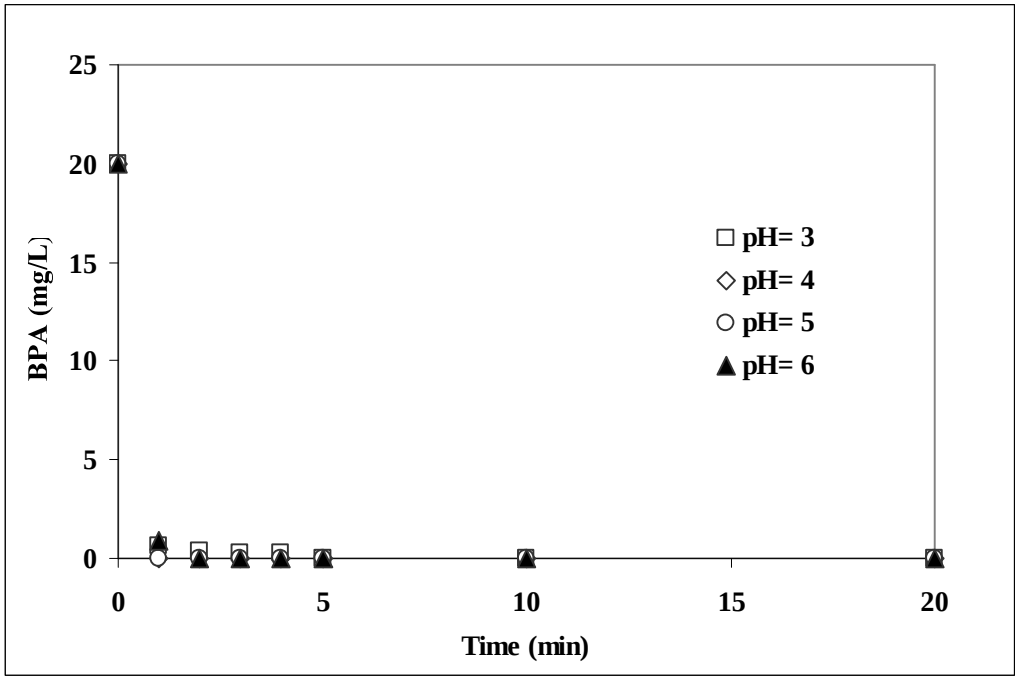


Figure 4.7 : The effect of Fe^{2+}concentration on percent TOC removal efficiencies. Experimental conditions: BPA_0=20 mg/L;TOC_0= 15 mg/L; H_2O_2=2 mM.

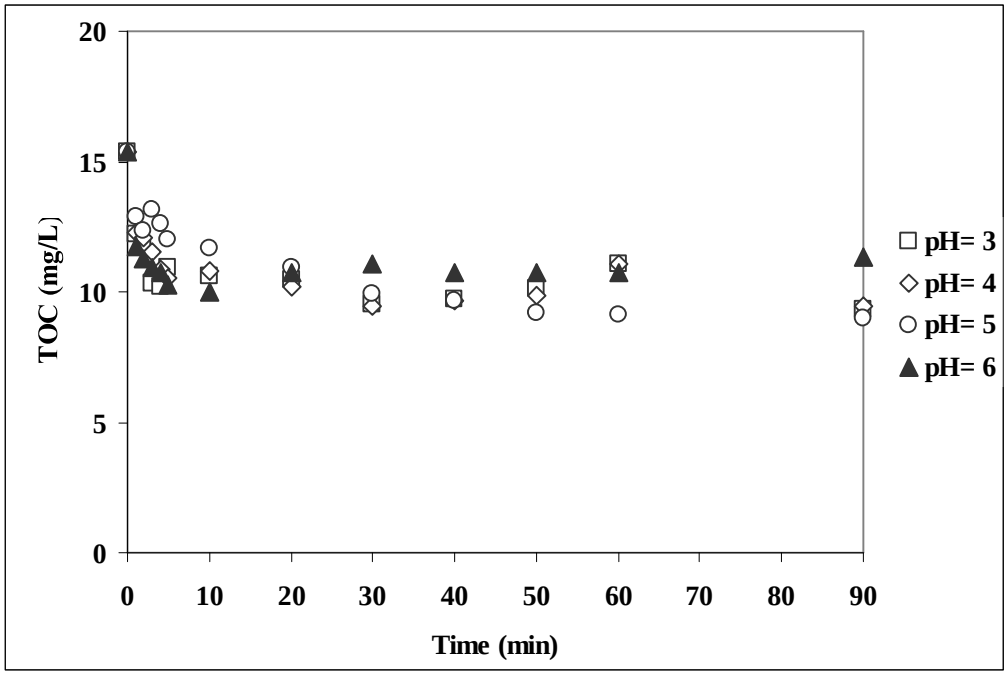
4.1.4 Effect of pH

As mentioned before, the most suitable pH range for the removal of various organics with Fenton's reagent was found between 2 and 5 (Khamaruddin et al. 2011; Duesterberg et al. 2008; Niaounakis and Halvadakis, 2006; Ijpelaar et al., 2001; Bishop, 1968). The effect of pH on BPA and TOC removals during Fenton process were examined for already optimized initial H_2O_2 (2 mM) and Fe^{2+} (0.4 mM). The tested pH values ranged between 3 and 6.

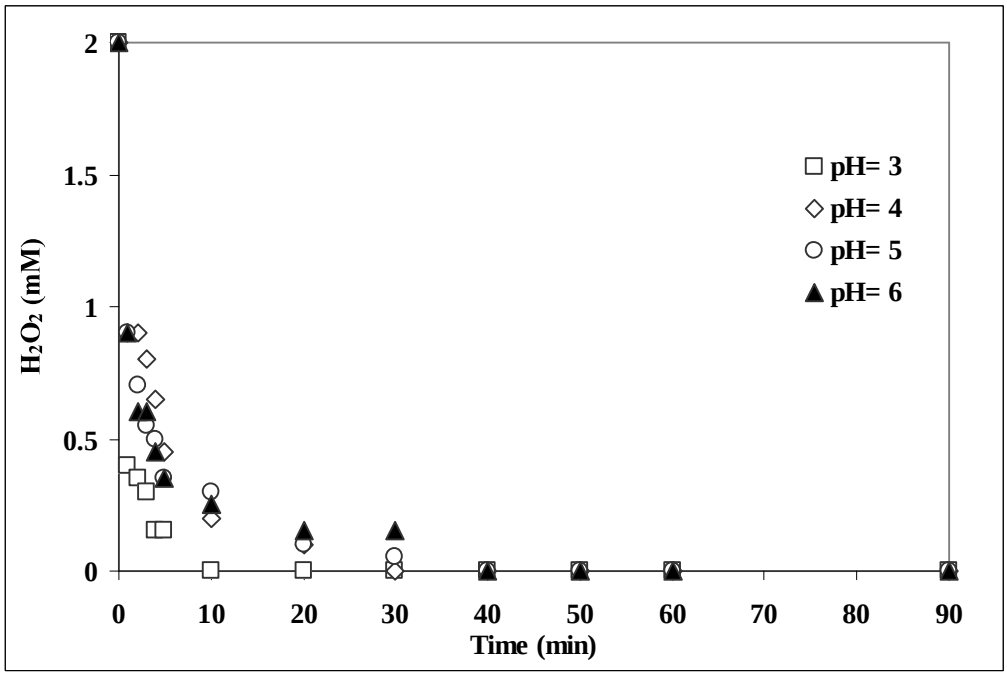
Fig. 4.8 presents changes in BPA (a), TOC (b) and H_2O_2 (c) abatements at varying initial pH values. As can be seen from **Figs. 4.8a**and **4.8b**, BPA and TOC removals were 100% and 30% after the first 5 min, respectively. As is obvious in **Fig. 4.8b**, most suitable pH to obtain the highest TOC removal (42%) was found at around pH=5, whereas, the lowest TOC removal (26%) was achieved at pH=6 after 90 min. It was observed from **Fig. 4.8c**, there was no residual H_2O_2 after 90 min Fenton process. All H_2O_2 was consumed after 30 min at pH= 4, 5 and 6, whereas, it was consumed faster within 5 min at pH=3.



(a)



(b)



(c)

Figure 4.8: The effect of pH on BPA (a), TOC (b), H_2O_2 (c) abatement rates. Experimental conditions: BPA_0=20 mg/L; TOC_0= 15 mg/L; Fe^{2+}=0.4 mM; H_2O_2=2.0 mM.

Table 4.4and **Fig. 4.9** summarize BPA, TOC and H_2O_2 abatements in percent after 90 min. As can be clearly seen in **Table 4.4** and **Fig. 4.9**, the most suitable pH was 5 achieving the highest TOC removal efficiencies (42%) with a final TOC value of 8 mg/L after 90 min. However, increasing the pH above 5 resulted in slightly worsening effects of pH in terms of TOC removal rates, whereas BPA abatement was not appreciably higher at pH values between 3 and 6.

Table 4.4 : The effect ofpH on BPA, TOC removals and H_2O_2consumption rates. Experimental conditions: BPA_0=20 mg/L; TOC_0= 15 mg/L; Fe^{2+}=0.4 mM;H_2O_2=2.0 mM.

pH	BPA	TOC		H_2O_2 Consumptions (%)	Y_{H2O2} (mg/mg)
	Removal Efficiency (%)	Final TOC (mg/L)	Removal Efficiency (%)		
3	100	9	39	100	11
4	100	9	38	100	11
5	**100**	**8**	**42**	**100**	**9**
6	100	11	26	100	17

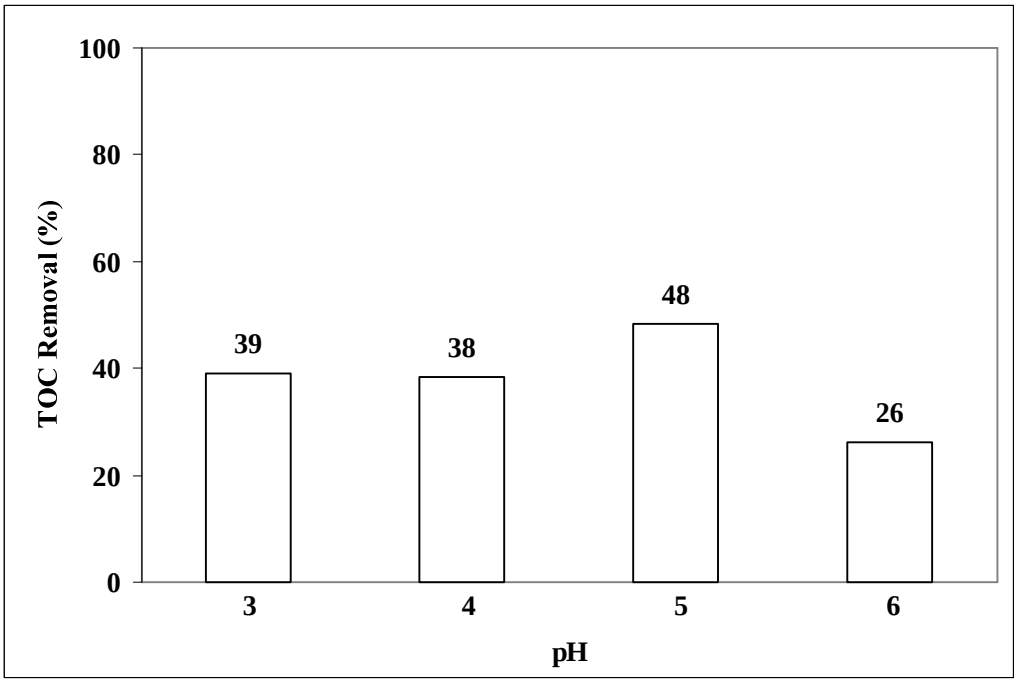


Figure 4.9 : The effect of pH on percent TOC removal. Experimental conditions: BPA_0=20 mg/L; TOC_0= 15 mg/L;Fe^{2+}=0.4 mM; H_2O_2=2.0 mM.

Considering all the results and because of being closer to pH of natural water, the most suitable pH was chosen 5 corresponding to 100% BPA removal (BPA_0=20 mg/L) and 48% TOC removal (TOC_0= 15 mg/L) for 90 min Fenton process of BPA. Similarly, Lunar et al. (2000) searched the degradation of photographic developers and they concluded that when the initial pH adjusted between 3 and 5, the degree of oxidation of the organic matter was maximum. Ioan et al. (2007) found that the degradation efficiency of BPA was the highest at pH 4 and the degradation rate of BPA increased with decreasing initial pH.

4.1.5 Effect of temperature

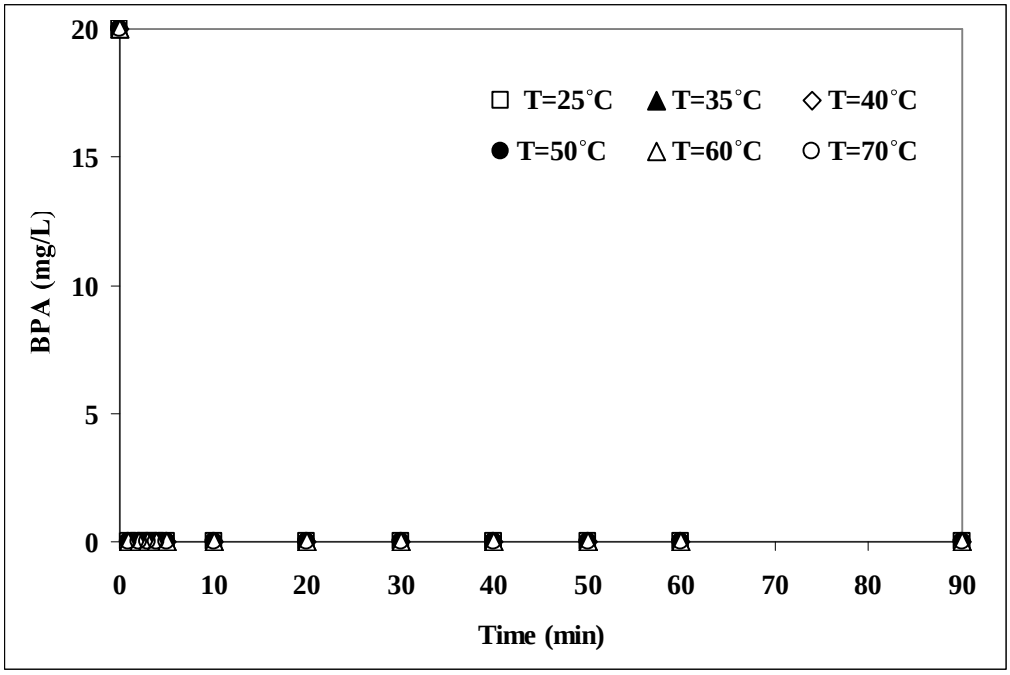
As mentioned before, some Fenton's reagent studies showed that there is an optimum temperature beyond which the treatment efficiency decreases dramatically. Wang et al. (2011a) found the optimum temperature 20-30 ˚C for the treatment of landfill leachate. Similarly, Guedes et al. (2003) reported that the optimal temperature was 30 ˚C for the degradation of cork cooking wastewater. On the other hand, Wu et al. (2010) found the optimum temperature at 45 ˚C and they concluded that when the temperature approaches to 60 ˚C, H_2O_2decomposition is enhanced to $H_2O$and O_2**(4.5)**. When temperature increases around 70 ˚C, O_2 solubility decreases with an expectednegative effect on the availability of O_2 for the H_2O_2replacement (Utset et al., 2000);

$2H_2O_2 \rightarrow 2H_2O + O_2$ (thermal decomposition) **(4.5)**

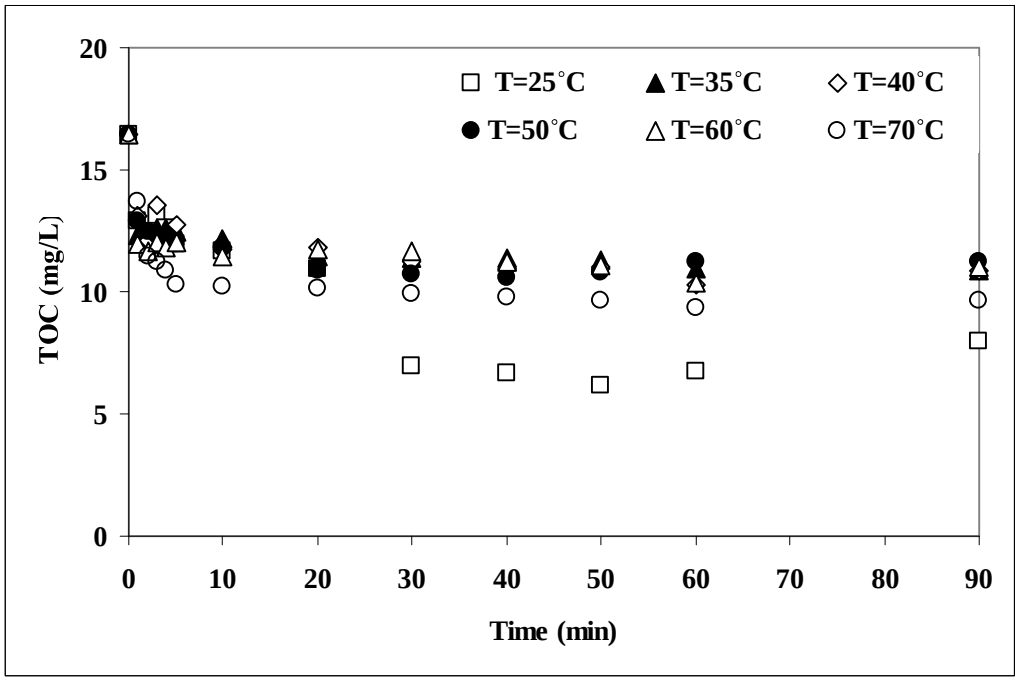
In this experimental study,in order to examine the effect of temperature (20-70˚C) on BPA, TOC abatements and H_2O_2 consumptions, experiments were conducted for 90 min under the following reaction conditions; H_2O_2=2.0 mM; Fe^{2+}=0.4 mM; pH=5. It was aimed at determining TOC and BPA removals efficiencies as well as H_2O_2 consumptions at varying reaction temperatures.

Fig. 4.10 displays changes in BPA (a), TOC (b) and H_2O_2 (c) abatements at varying temperature values. As can be seen from **Fig. 4.10a**, complete BPA removal was achieved before first min. It can be said that BPA removal rates were very fast. It was observed from **Fig. 4.10b** that TOC removal leveled off after 60 min treatment for all operating temperatures. It is also important to note that the positive effect in TOC removal rates with increasing reaction temperature was more pronounced

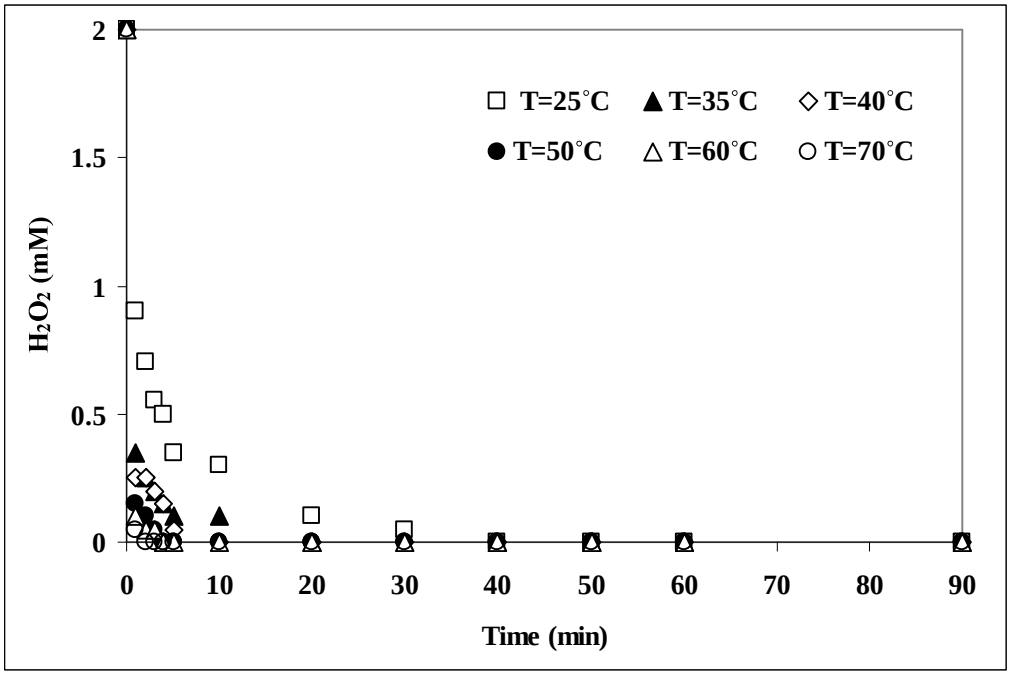
within the first minute of Fenton process. H_2O_2 consumptions were shown in **Fig. 4.10c**. Complete H_2O_2 consumption was obtained after 20, 10, 5, 3, 3, 1 min at T=20, 35, 40, 50, 60 and 70 ˚C, respectively.



(a)



(b)



(c)

Figure 4.10 : The effect of temperature on BPA (a), TOC (b), H_2O_2 (c) abatement rates. Experimental conditions: BPA_0=20 mg/L; TOC_0= 16 mg/L; Fe^{2+}=0.4 mM; H_2O_2=2.0 mM; pH=5.

Table 4.5 and Fig. **4.11** summarize BPA, TOC and H_2O_2 abatements in percent for different reaction temperature values after 90 min Fenton process. As can be seen clearly seen in **Table 4.5** and **Fig. 4.11**, the highest TOC removal efficiency was obtained at T=20˚C as 51% with a final TOC value of 8 mg/L after 90 min. It could be said that TOC percent removal did not change dramatically with increasing temperature. Similarly, Rodríguez et al. (2011) examined nicotine removal at 20 and 50˚C and they concluded that the degradation rate increased with increasing temperature, whereas final pollutant conversion, TOC percent removal, and detoxification were identical. On the other hand, Lopez et al. (2005) investigated 4-chloro-3-methyl phenol (CMP) degradation by Fenton's reagent at 25 and 70˚C and the maximum TOC removal (85%) was achieved at 70˚C.

Table 4.5 : The effect oftemperature on BPA, TOC removals and H_2O_2 consumption rates. Experimental conditions: BPA_0=20 mg/L; TOC_0= 16 mg/L; Fe^{2+}=0.4 mM; H_2O_2=2.0 mM; pH=5.

Temperature (˚C)	BPA	TOC		H_2O_2 Consumption (%)	H_2O_2 consumed/TOC removed (mg/mg)
	Removal Efficiency (%)	Final TOC (mg/L)	Removal Efficiency (%)		
20	**100**	**8**	**51**	**100**	**8**
35	100	11	34	100	12
40	100	11	34	100	12
50	100	11	32	100	13
60	100	11	33	100	13
70	100	10	41	100	10

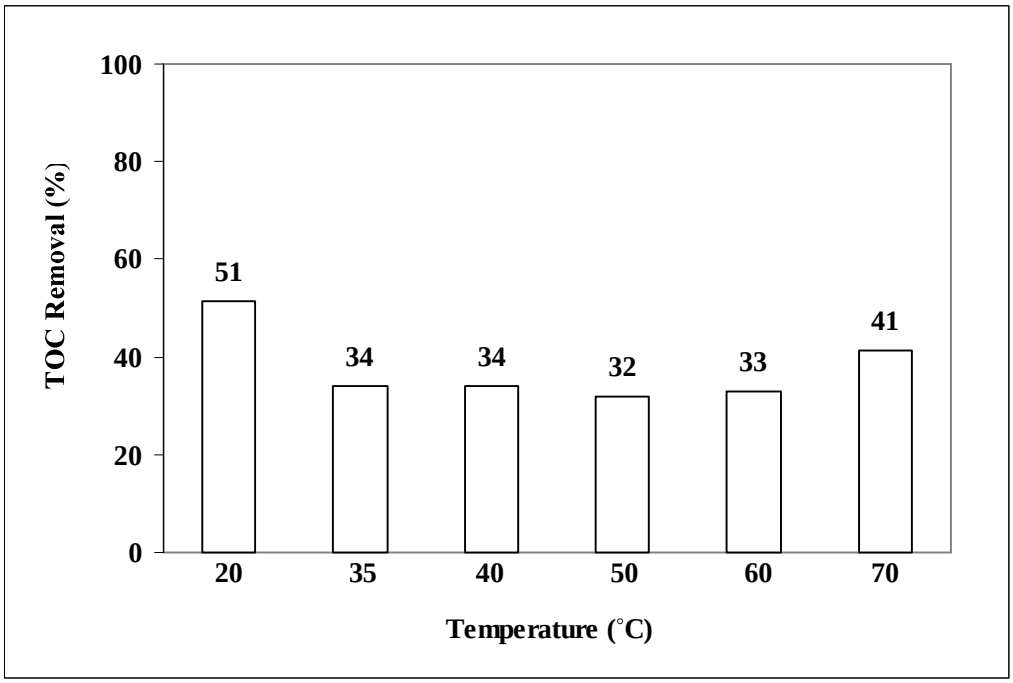
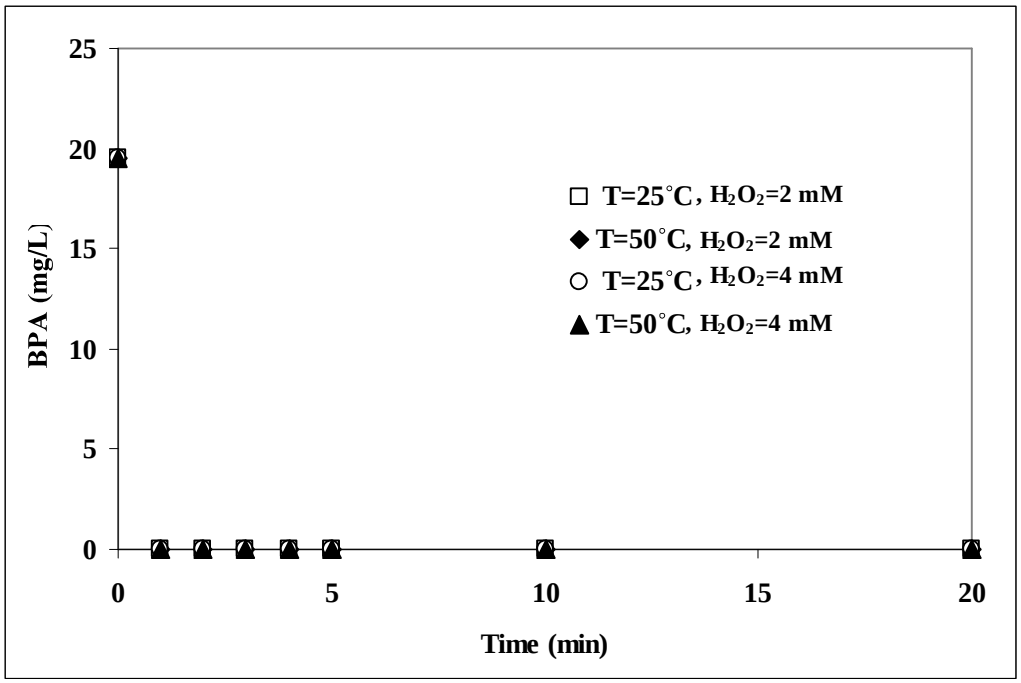


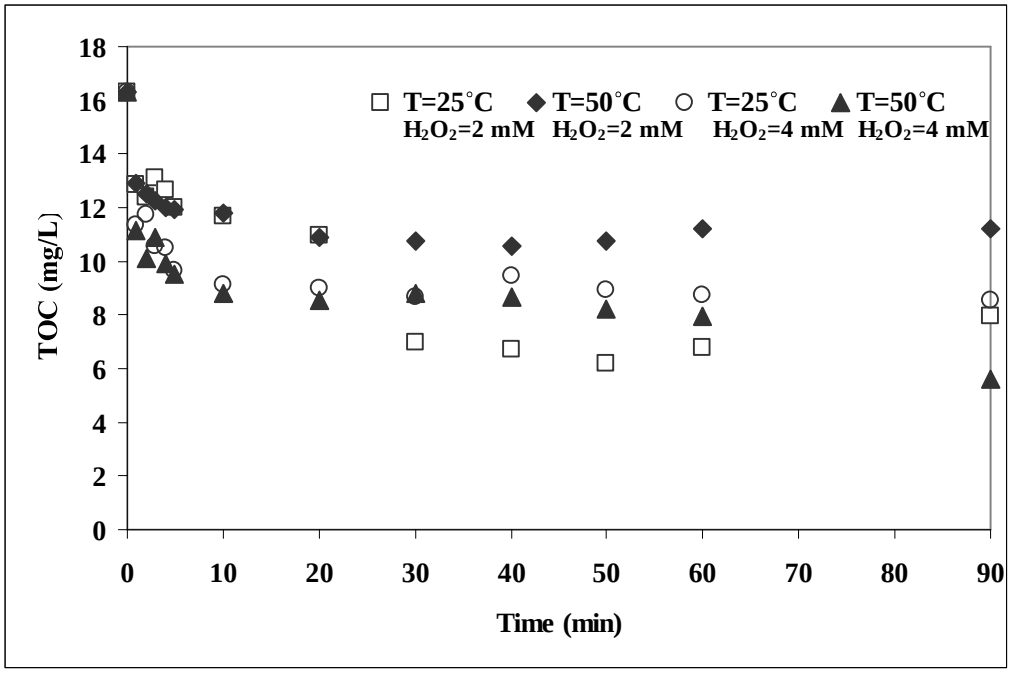
Figure 4.11 : The effect of temperature on percent TOC removal efficiencies. Experimental conditions: BPA_0=20 mg/L; TOC_0= 16 mg/L; Fe^{2+}=0.4 mM; H_2O_2=2.0 mM; pH=5.

4.1.6 Preliminary experiments before the toxicity tests

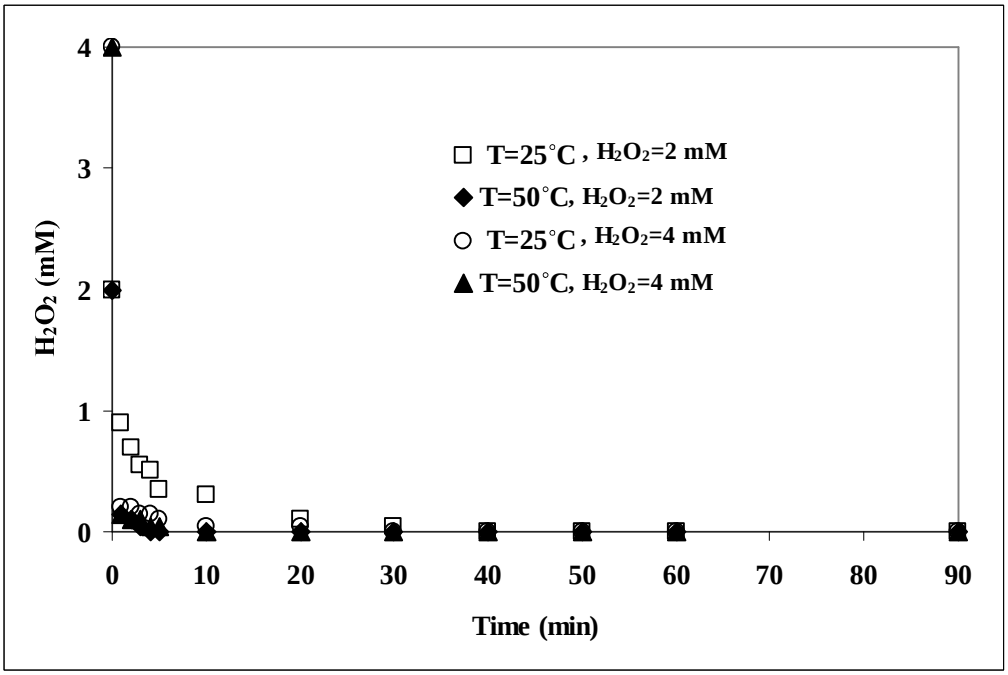
Considering all optimized conditions described in previous sections, it was decided to increase the initial H_2O_2 concentration as well as temperature in order to observe an improvement. For this reason, the separate experimental sets were conducted before starting luminescent bacteria test with *Vibrio fischeri*. Fenton process was carried with 2 and 4 mM H_2O_2 at 25 and 50 ˚C while maintaining the initial concentration of Fe^{2+} constant at a value of 0.4 mM and at an initial pH of 5. As can be seen from **Fig. 4.11**, BPA removal was identical for all experimental sets, whereas, the highest TOC removal was observed at the conditions of 0.4 mM of H_2O_2 at 50 ˚C. H_2O_2 consumption rates were increased with increasing temperature and increasing H_2O_2concentration as previously mentioned.



(a)



(b)



(c)

Figure 4.12 : The effect of temperature and initial H_2O_2concentration on BPA (a), TOC (b), H_2O_2 (c) abatement rates. Experimental conditions: BPA=20 mg/L; TOC= 16 mg/L; Fe^{2+}=0.4 mM; pH=5.

4.2 Acute Toxicity Tests

Since the aquatic environment could receive discharges of BPA from production, processing, and sewage treatment plant effluents,BPA has been the subject of considerable aquatic toxicity tests in recent years. As already known, these studies have included both sub-chronic and chronic tests using conventional test methodologies and organisms, as well as with non-standard test species (Olmez-Hanci et al., 2013; Ileri and Karaer, 2011; Rodríquez et al., 2010; Park et al., 2006; Chiang et al., 2003; Alexander et al., 1989; Stephenson et al., 1983).

In this part of experimental study, the acute toxicity of BPA was evaluated with different battery test using organisms of different trophic levels, such as the photobacteria*Vibrio fischeri* selected as decomposers, the freshwater crustaceans*Daphnia magna* selected as consumers, and freshwater green microalgae *Pseudokirchneriella subcapitata*selected as producers.

Toxicity tests were conducted under following conditions; at ITU, BPA=20 mg/L (88 μM), H_2O_2= 4.0 mM, Fe^{2+}= 0.4 mM, pH=5, T=50˚C and t= 120 min, whereas at DTU, BPA=20 mg/L (88 μM), H_2O_2= 2.0 mM, Fe^{2+}= 0.4 mM, pH=5, T=20˚C and t= 90 min.

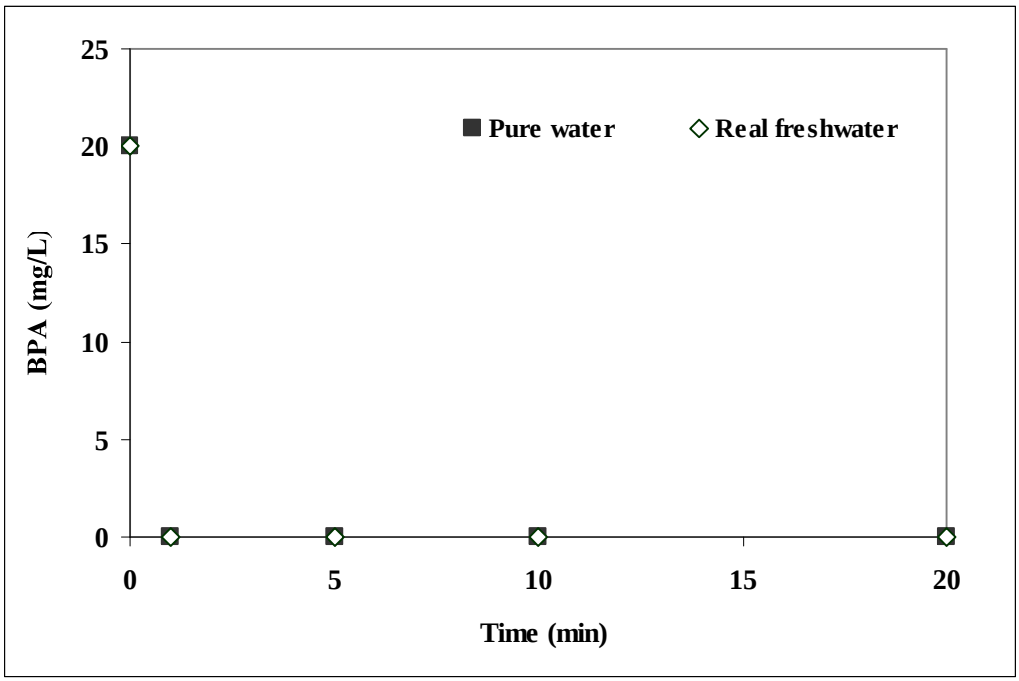
4.2.1 Biotox-Luminescence: Test method based on measurement of light emission from the photobacteria *Vibrio fischeri*

4.2.1.1 Biotox tests conducted in pure and raw freshwater

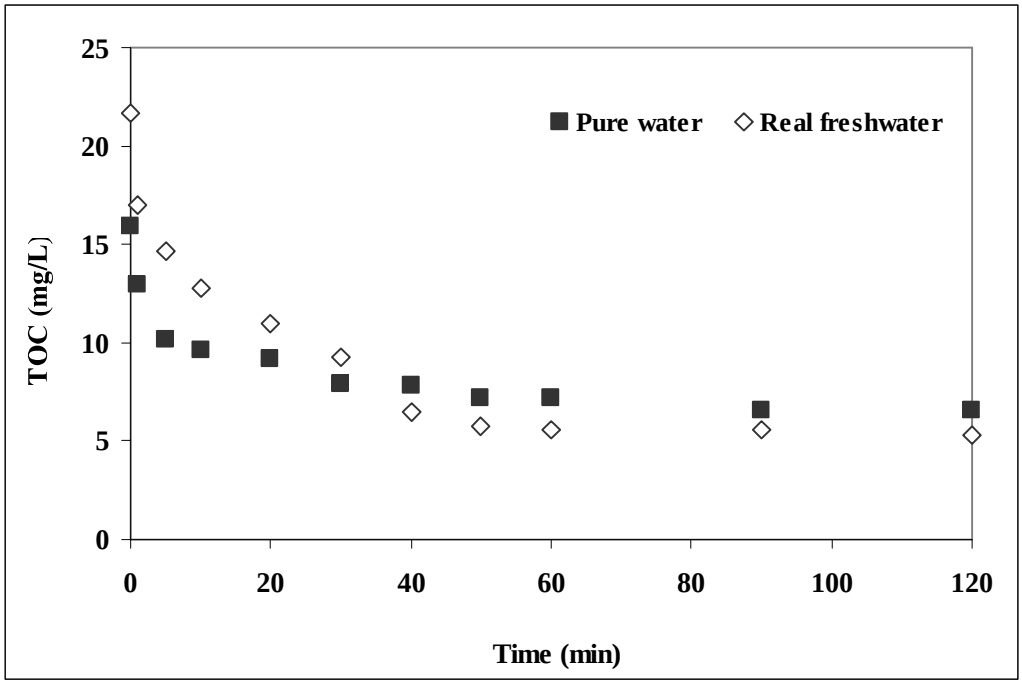
At ITU, biotox tests were conducted with pure water and real freshwater (from Kagithane),according to the principles recommended by the ISO 11348-3, (2008).

The effects of pure water and real freshwater onBPA (a), TOC (b) and H_2O_2 (c) abatements as well as % relative inhibitions (d) are shown in **Fig. 4.13**. As can be seen from **Fig. 4.13a**, complete BPA removal was achieved within 1 min as expected. **Fig. 4.13b** shows that freshwater had 6 mg/L higher TOC and TOC removal rate was slower than pure water. After 120 min, the overall TOC removal was 60% in pure water and 75% in freshwater. As is evident from **Fig. 4.13c**, H_2O_2 abatement rates were parallel with TOC abatement rates. After 120 min, there was no residual H_2O_2 in the media.

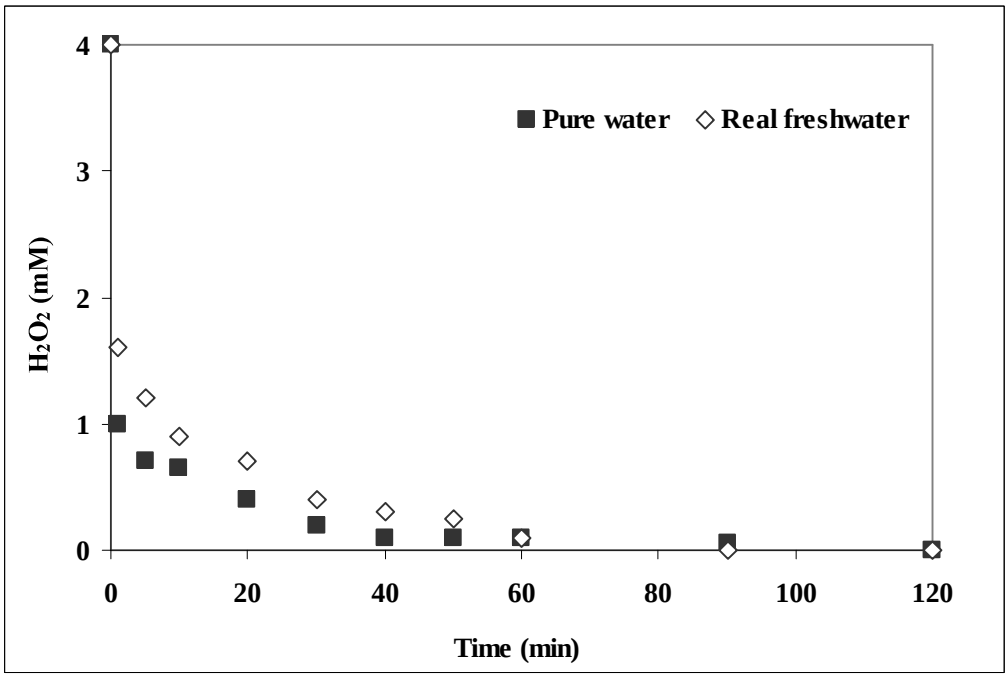
As can be clearly seen from **Fig. 4.13d**, the inhibition decreased steadily during Fenton process reaching negative (stimulative) values after 50 min in pure water and after prolonged Fenton process in the real freshwater sample (at 90 min). The % relative inhibition value was around 70% in the original BPA solution and dropped to 12% and 23% after 1 min Fenton treatment in pure and real freshwater, respectively. This immediate reduction in acute toxicity coincided with complete BPA removal after 1 min. Generally speaking, the decrease in the inhibitory effect of BPA was faster and more pronounced in the pure water sample, whereas in for Fenton treatment in the real freshwater, the reaction time had to be extended to beyond 90 min. In pure water, complete detoxification was achieved after 40-50 min oxidation.



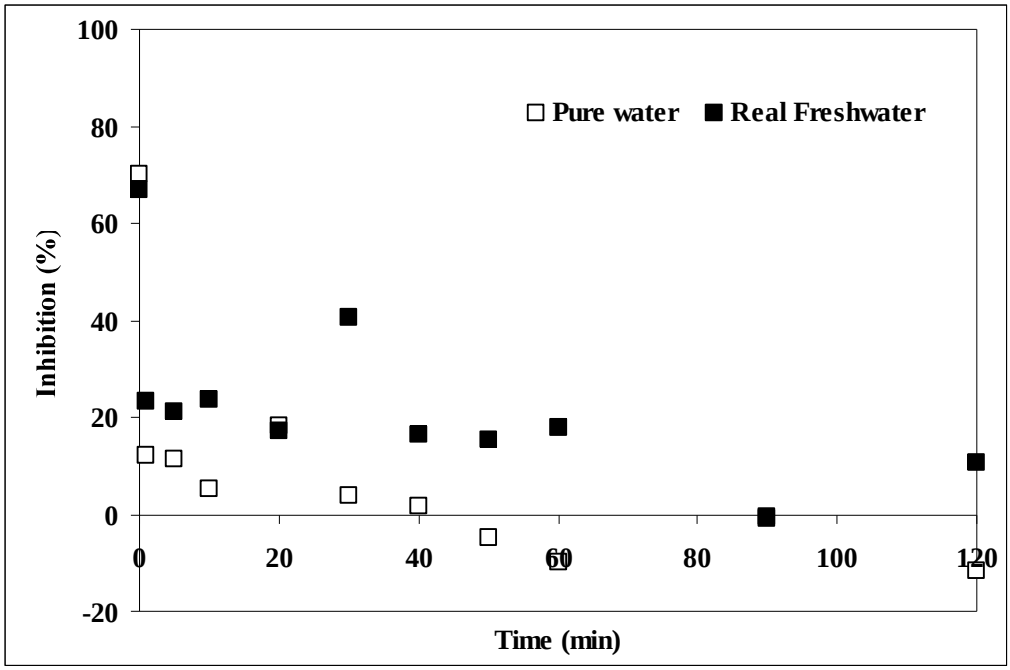
(a)


25
20
15
10
5
0
TOC (mg/L)
Pure water
Real freshwater
0
20
40
60
80
100
120
Time (min)

(b)


4
3
2
1
0
H2O2 (mM)
Pure water
Real freshwater
0
20
40
60
80
100
120
Time (min)

(c)



(d)

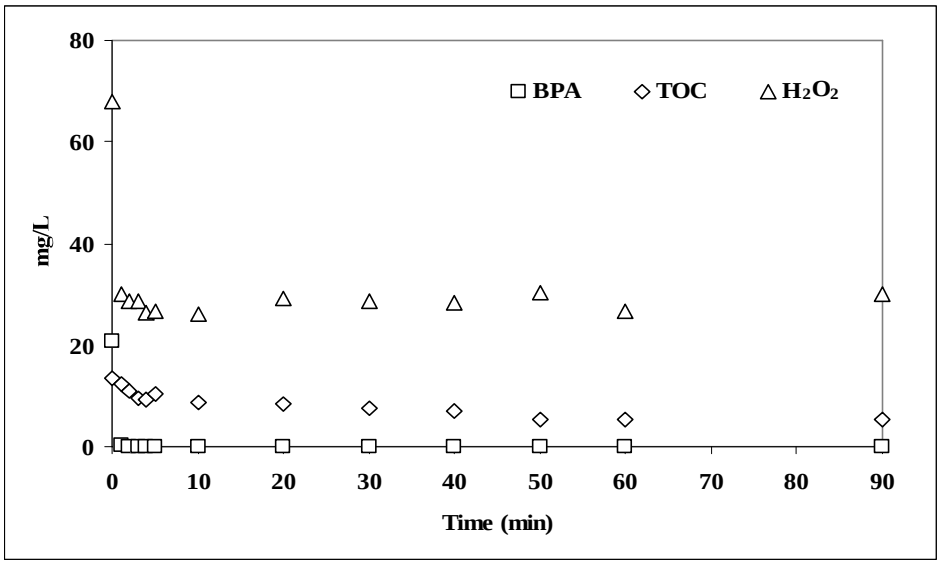
Figure 4.13 : Changes in BPA (a), TOC (b) and H_2O_2 (c) abatement rates and percent relative inhibitions (d) during Fenton treatment inpure water and real freshwater. Experimental conditions: BPA=20 mg/L; $TOC_{purewater}$= 16 mg/L; $TOC_{freashwater}$= 22 mg/L; Fe^{2+}=0.4 mM; H_2O_2= 4.0 mM; pH=5; T=50˚C; t= 120 min.

4.2.1.2 Biotox tests conducted in 2% NaCl medium

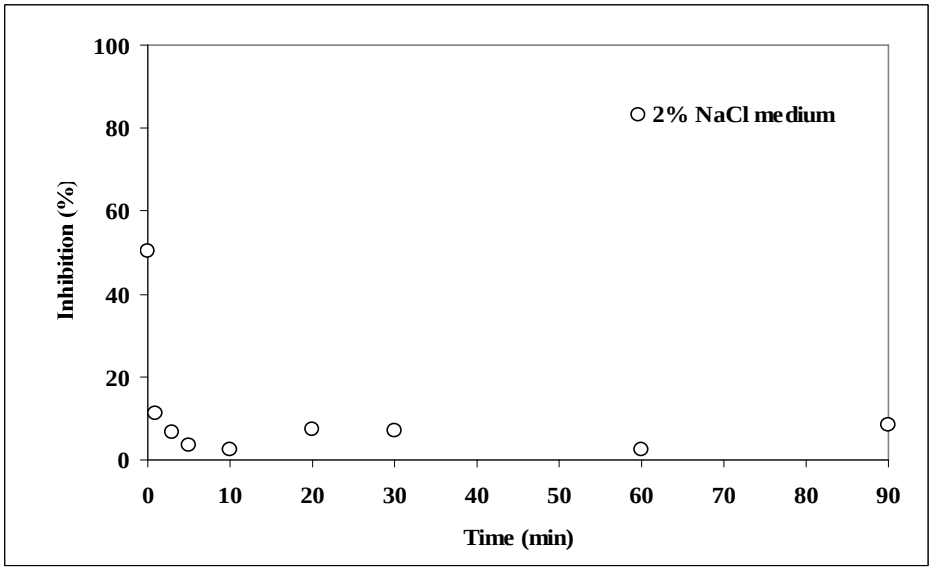
At DTU, biotox tests were conducted in 2% (w/v) NaCl medium. **Fig. 4.14** presents changes in BPA, TOC and H_2O_2 abatement rates (a) and % relative inhibitions(b) obtained during Fenton treatment of BPA in in 2% NaCl medium.

As can be seen from **Fig.4.14a**, complete BPA removal was achieved within 1 min as expected. During the treatment, 1.2 mM H_2O_2 was consumed within 10 min, whereas after 90 min there was 0.85 mM residual H_2O_2 in the medium. However, TOC decreased steadily during 90 min and complete TOC removal was around 9 mg/L. As is evident in **Fig. 4.14b**, the inhibition decreased during Fenton treatment reaching 2.5% after 10 min. After 30 min, it was observed that the acute toxicity increased to around 7% and decreased again to 2.5% after 60 min. The % relative inhibition value was around 50%in the original BPA solution and dropped to 11% after 1 min Fenton treatment. Similar to the toxicity tests conducted at ITU, this immediate reduction in acute toxicity coincided with complete BPA removal after 1

min.The inhibitory effect of BPA was lower in 2% NaCl medium than in real freaswater (at ITU), whereas complete detoxification could not achieved after 90 min in the 2% NaCl medium. Therefore, the reaction time had to be extended to beyond 90 min for Fenton treatment in 2% NaCl medium.



(a)



(b)

Figure 4.14 : Changes in BPA, TOC and H_2O_2 abatement rates (a) and percent Relative inhibitions (b) during Fenton treatment of BPAin 2% NaCl medium. Experimental conditions: BPA= 20 mg/L; $TOC_{2\%NaCl}$=14 mg/L; Fe^{2+}=0.4 mM;H_2O_2=2.0 mM; pH=5; T=20˚C; t= 90 min;incubation time for photobacteria=15 min.

Similarly, Olmez-Hanci et al. (2013) investigated a thermally activated persulfate oxidation and acute toxicity of BPA (88 μM) using *Vibrio fischeri*. The inhibitory effect of original BPA solution was 58% which increased to 84% after 30 min and decreased to 22% after 90 min during the treatment.

Rodríguez et al. (2010) evaluated acute toxicity of BPA (50 μM) after different solar AOPs. They found that toxicity dropped from 70% to 30% when mineralization was 20%, regardless of the process conducted. However, there was an increase in toxicity at the beginning of the Fenton and photo-Fenton treatments and therefore, they concluded that the formation and accumulation of reaction intermediate products could be more toxic than BPA.

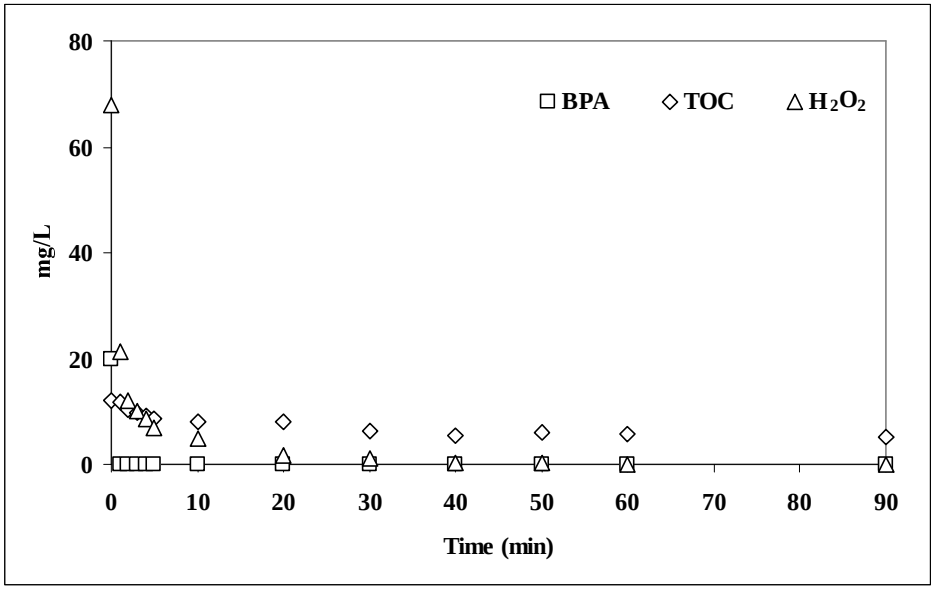
Chiang et al. (2003) searched photocatalytic degradation and mineralization of BPA (88 μM) as well as toxicity of BPA. They concluded that there was a gradual decrease in toxicity for BPA degradation at pH 10. However, they found that more toxic intermediates than BPA were generated during the early stage of the oxidation at pH 3. After a series of toxicity tests, an estimated EC_{50} value of 3.46±0.52 mg/L was obtained which was similar with the EC_{50} value of BPA reported in the literature.

4.2.2 Acute toxicity test on the freshwater crustacean *Daphnia magna*

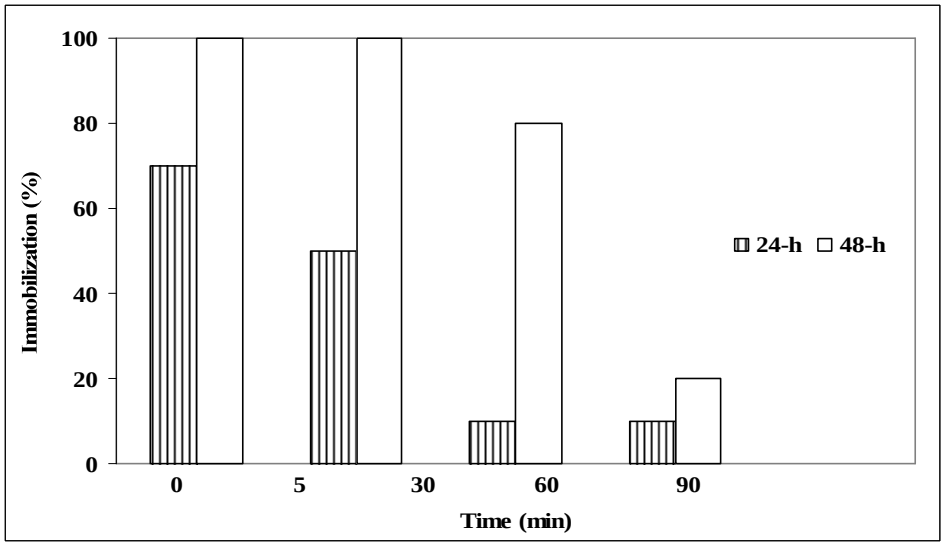
The crustacean toxicity test to evaluate the immobilizationof *Daphnia magna* was performed by hatching neonates from ephippia where animals <24 h oldat20˚C in M1 medium (synthetic freshwater), according to the principles recommended by the ISO 6341, (2010).

Fig. 4.15presents changes in BPA, TOC and H_2O_2 abatement rates (a) and % relative immobilizations (b) obtained during Fenton treatment of BPA in M1 medium. As can be seen from **Fig. 4.15a**, complete BPA removal was achieved within 1 min. TOC removal rate was faster in first 10 min and continued to decrease slowly after 10 min. Final TOC removal was 7 mg/L after 90 min. As is evident in **Fig. 4.15a**, H_2O_2 was consumed in parallel with TOC removal in first 10 min. The overall H_2O_2 was consumed in M1 medium after 20 min.As can be seen clearly from **Fig. 4.15b**, immobilizations of *Daphnia magna* were 70% and 100% for 24 h and 48 h, respectively. However, immobilizations reached around 10% (24 h) and 20% (48 h) after 30 min and 60 min, respectively. It was concluded that the acute toxicity of

BPA was very high and decreased slowly during Fenton treatment compared to other toxicity tests conducted in this experimental study. After 90 min, there was no immobilized *Daphnia magna* in M1 medium. Additionally, it could be said that Fenton treatment time had to be extended to beyond 90 min because of variability of toxicological effects of BPA during the treatment.



(a)



(b)

Figure 4.15 : Changes in BPA, TOC and H_2O_2 (a) abatement rates and immobilizations (%) (b) during Fenton treatment in M1 medium. Experimental conditions: BPA=20 mg/L,$TOC_{M1medium}$= 12 mg/L, Fe^{2+}=0.4 mM; H_2O_2=2.0 mM; pH=5; T=20˚C; t= 90 min.

The acute toxicity of waste water of a cotton textile plant was investigated using *Daphnia magna*and Fenton treatment by Ileri and Karaer (2011). The acute toxicity scale LD_{50}values were determined as %50 in raw water and %80 in water treated. As a result, they concluded that Fenton treatment had a positive contribution to the removal of acute toxicity in textile wastewaters.

Park et al. (2006) evaluated the toxicity of envrionmental pollutants, such as nonylphenol (NP), chloropyriphos (CP) and BPA usind *Daphnia magna* and *Chironomus tentans* (larva of aquatic midge). They observed that *Daphnia magna* was more sensitive than *Chironomus tentans*and the order of acute toxicity was CP > NP > BPA in *Daphnia magna*.

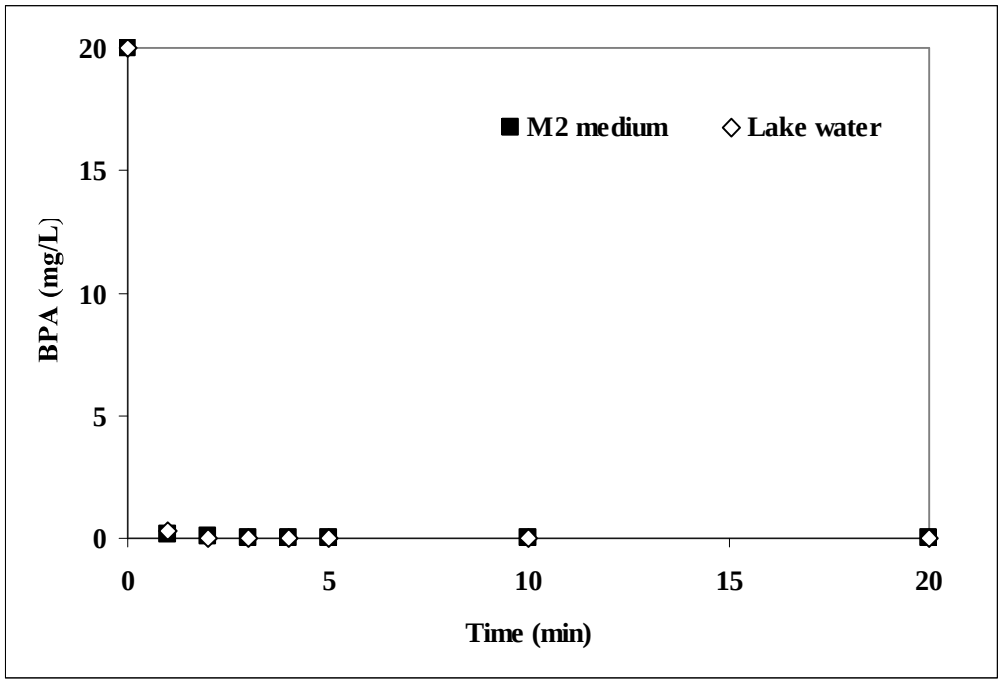
4.2.3 Growth inhibition test with green microalgae*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)

The algalgrowth inhibition test using*Pseudokirchneriella subcapitata* was performed by measuring the fluorescence of biomass of algae at20˚C in M2 medium and lake water (from Sjælsø), according to the principles recommended by the ISO 8692 (2012).

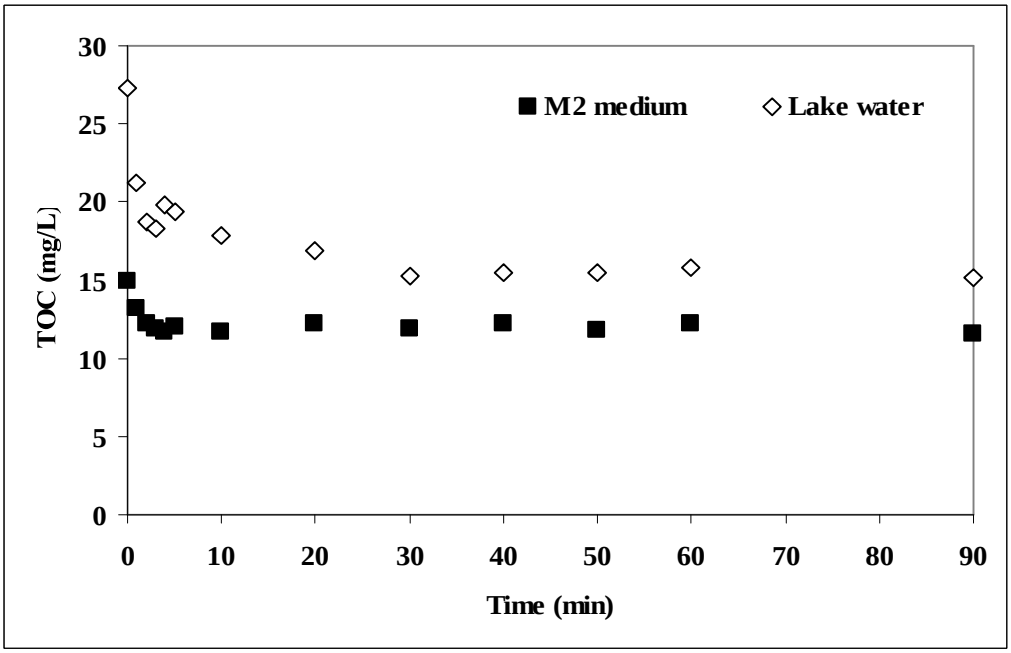
Fig. 4.16 shows changes in BPA (a), TOC (b), H_2O_2 (c) abatement rates, % relative inhibitions for 24 h (d)and 48 h (e) incubation periods during Fenton treatment of BPAin M2 medium and lake water.

As is evident in **Fig. 4.16a**, **4.16d** and **4.16e** the reductions in acute toxicity coincided with complete BPA removal after 1 min. However, it could be said that acute toxicity did not change significantly during 90 min Fenton treatment. It was also observed from **Figs. 4.16b**, **4.16d** and **4.16e**, changes in acute toxicity and TOC removal were parallel. 4 mg/L and 10 mg/L TOC removal was achieved after 10 min in M2 medium and lake water, respectively. Because of organic and inorganic materials content in M2 medium and high alkalinity in lake water, TOC removal rate was slow in both medium. After 10 min and during Fenton treatment, TOC removal did not change dramatically. Additionally, **Fig. 4.16c** shows that H_2O_2 consumption rates were identical and consumed completely after 40 min in both medium. As can be clearly seen from **Figs. 4.16d** and**4.16e** the % relative inhibition value was 100% in the original BPA solution in both medium. The % relative inhibition dropped to 60% and 44% (24 h) after 1 min Fenton treatment, ranging between 55-67% and 28-

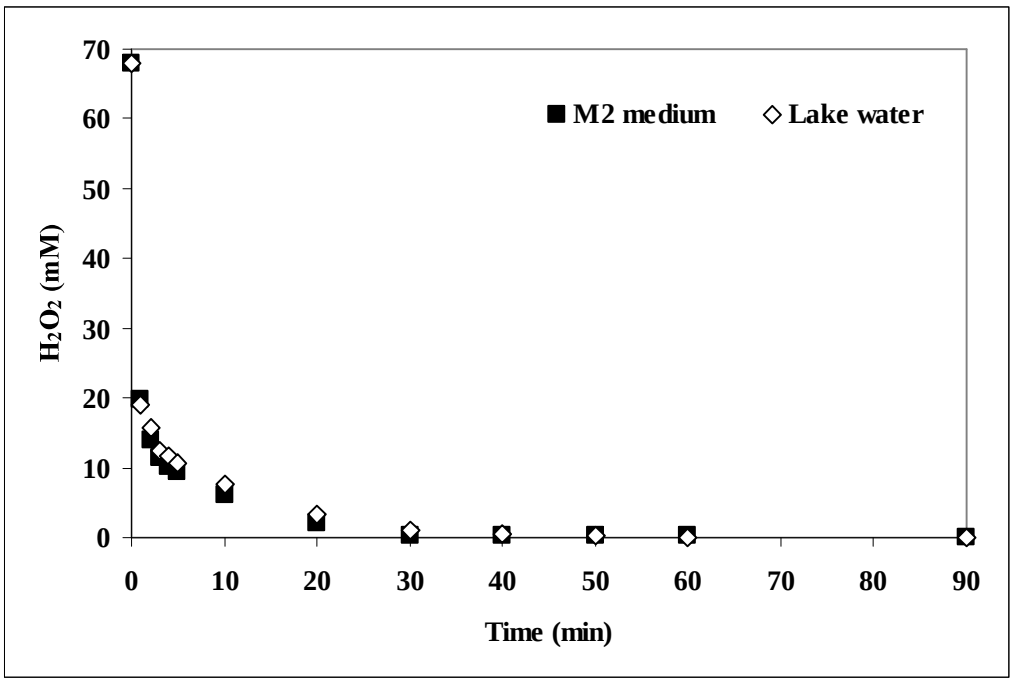
47% (24 h) during 90 min Fenton treatment in M2 medium and lake water, respectively, which were shown in **Fig. 4.16d**. According to **Fig. 4.16e**, the % relative inhibition value dropped from 100% to 44% and 46% (48 h) after 1 min Fenton treatment, changing between 40-55% and 41-68% (48 h) during 90 min Fenton treatment in M2 medium and lake water, respectively.



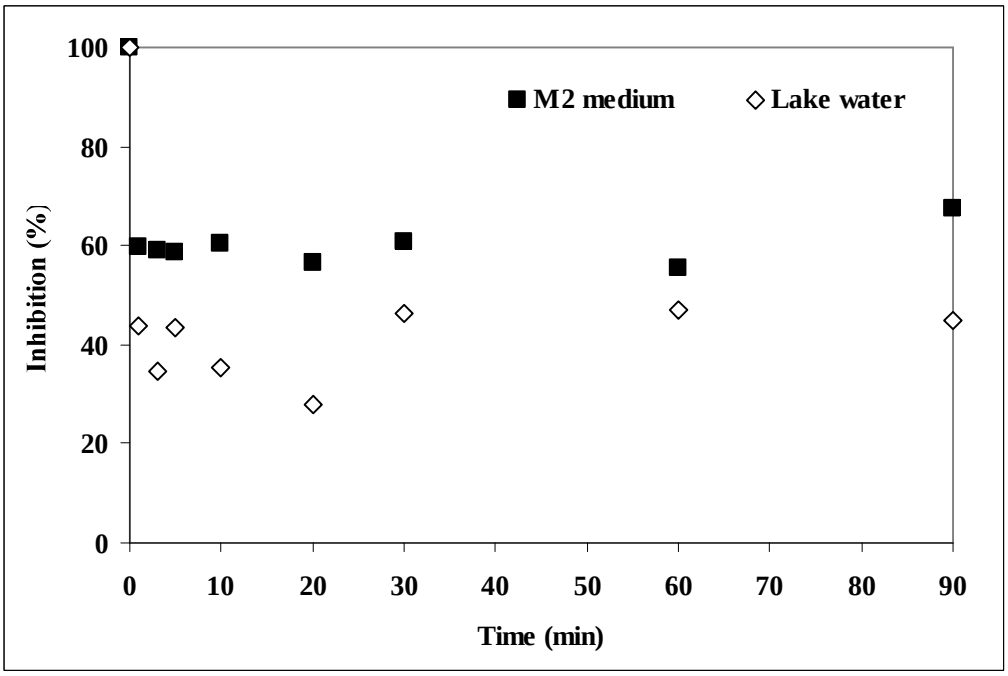
(a)



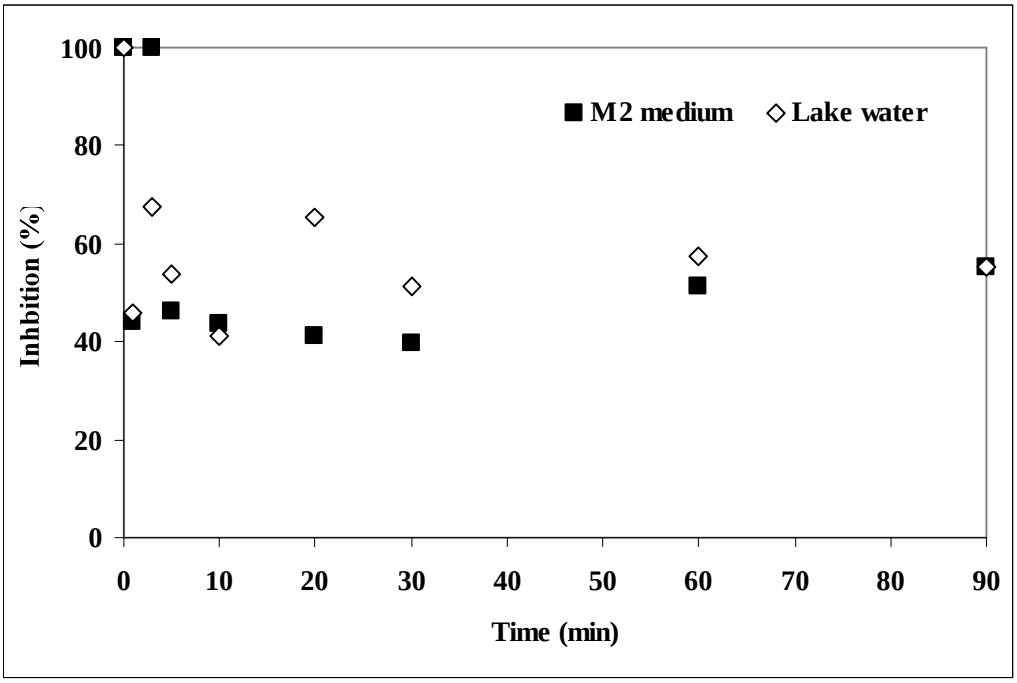
(b)


70
60
50
40
30
20
10
0
H2O2 (mM)
M2 medium
Lake water
0
10
20
30
40
50
60
70
80
90
Time (min)

(c)


100
80
60
40
20
0
Inhibition (%)
M2 medium
Lake water
0
10
20
30
40
50
60
70
80
90
Time (min)

(d)



(e)

Figure 4.16 : Changes in BPA (a), TOC (b) and H_2O_2 (c) abatement rates and % relative inhibitionsfor 24 h (d) and 48 h (e) incubation periods during Fenton treatment of BPAin M2 medium and lake water. Experimental conditions: BPA= 20 mg/L;$TOC_{M2medium}$=15 mg/L; $TOC_{Lakewater}$=27 mg/L; Fe^{2+}=0.4 mM; H_2O_2=2.0 mM; pH=5; T=20˚C; t= 90 min.

There are various bioassays reported of a series of short term toxicity tests of BPA in literature. Stephenson (1983)reported a 96-h EC_{50} of 2.5 mg/L based on cell growth and 48-h EC_{50} of 3.9 mg/L using *Pseudokirchneriella subcapitata* and *Daphnia magna*, respectively. Alexander et al. (1989) reported a 96-h EC_{50} of 2.7 mg/L based on cell count and 48-h EC_{50} of 10 mg/L using *Pseudokirchneriella subcapitata* and *Daphnia magna*, respectively. On the other hand, Hendriks et al. (1994) reported 48-h EC_{50} of 20 mg/L using *Daphnia magna*.

In this experimental study, it was found that the relative sensitivity of the test species usedwas *Pseudokirchneriella subcapitata*>*Daphnia magna*>*Vibrio fischeri*. Similarly, a battery of bioassays was conducted by Antunes et al. (2007) to evaluate the acute toxicity of the different compartments of uranium mine pit (such as Mn, Fe, Al, U, Sr) using algae and crustaceans. They found that *Pseudokirchneriella subcapitata*was more sensitive than *Daphnia magna*. Isidori et al. (2003) evaluated

toxicity of leachates from municipal solid waste landfills using, *Brachionus calyciflorus* (the freshwater rotifer) and *Thamnocephalus platyurus* (the freshwater crustaceans), *Vibrio fischeri* and *Daphnia magna*. They found that the least sensitive organism was *Vibrio fischeri.*

4.2.4 Comparison of the response of the test organisms and the Fenton reaction mode

Fig. 4.17 displays comparison of the all toxicity test results.It could be said that the most sensitive organism to BPA and its degradation products was *Pseudokirchneriella subcapitata* which showed inhibition between 40-60% during 90 min treatment. However, the complete detoxification was achieved for *Daphnia magna* and *Vibrio fischeri*after 60 min treatment. *Vibrio fischeri* was the least sensitive organism which showed immediate decrease in toxicity after 1 min treatment.

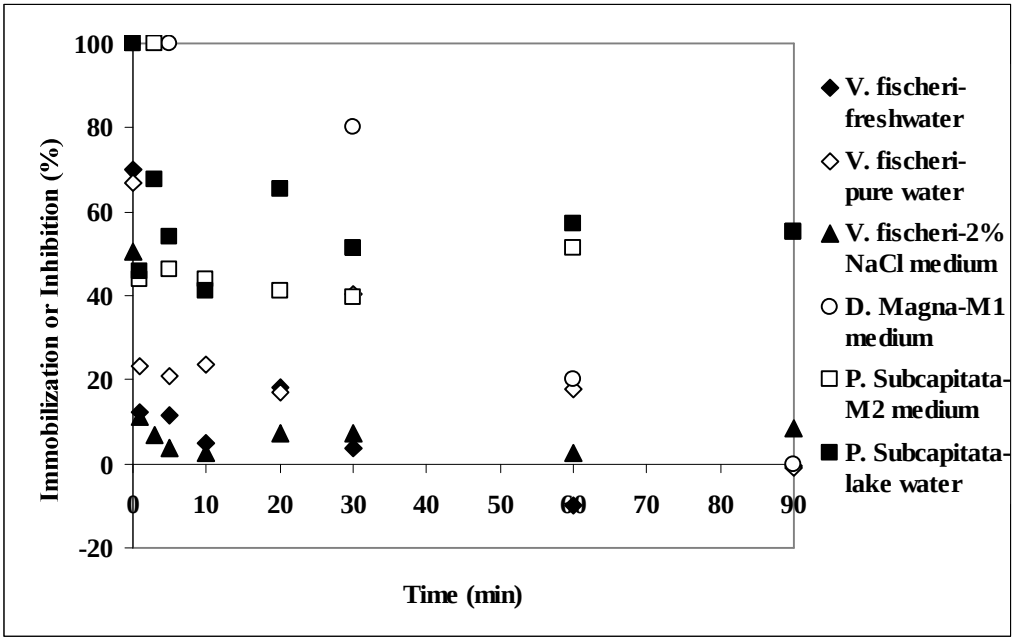


Figure 4.17 : Comparison of the changes in percent relative immobilization or inhibitionsof the test organisms and the Fenton reaction mode.

5. CONCLUSIONS AND RECOMMENDATIONS

In the present study, it was aimed at exploring the effect of Fenton pretreatment on the degradability and toxicity of a simulated BPA solution. The following conclusions may be drawn from this experimental study:

1. Most suitable experimental conditions for the BPA solution were established as follows: H_2O_2=2 mM, Fe^{2+}=0.4 mM and pH=5 at room temperature (T=20˚C) during 90 min Fenton treatment. Under these conditions, which yielded an overall BPA removal efficiency was 100% in 1-2 min. The corresponding TOC removal efficiency was around 50% after 90 min.
2. The overall TOC removal efficiency was between 34-41% above 20˚C after 90 min treatment time. The accelerating thermal effect on TOC removal was pronounced at the beginning of Fenton process (first minute). TOC and BPA abatements did not show any improvement upon further increase in temperature and reaction time confirming a most suitable value for Fenton pretreatment of the BPA solution.
3. Treatment efficiencies obtained for 20 mg/L BPA in real freshwater samples did not differ significantly from those found in pure water because of low alkalinity and presence of small amounts of Cl^- ions. After 120 min, total TOC removal was around 60% in pure water and 75% in freshwater.
4. treatment efficiencies obtained for 20 mg/L BPA in different growth media showed some differences in terms of TOC removal. Depending on medium used, 22%, 45%, 58% and 62% TOC removals were obtained in M2 medium, lake water, M1 medium and 2%NaCl medium, respectively. H_2O_2 consumption rate was significantly slow in 2%NaCl medium and only half amount of H_2O_2 was consumed because of inhibition effects of Cl^- ions. However, there was no inhibition in TOC removal due to low pH.

5. Acute toxicity results, which obtained at ITU, it could be demonstrated that the percent relative luminescence inhibition of photobacteria *Vibrio fischeri* could be reduced from 70% (20 mg/L BPA) to 12% in pure water and 23%in real freshwater after only 1 min Fenton treatment. Complete detoxification was achieved in 40-50 min and 90 min in the pure and real freshwater samples, respectively. Detoxification patterns generally paralleled BPA degradation profiles.
6. Acute toxicity results, which obtained at DTU, demonstrated that the percent relative luminescence inhibition of photobacteria *Vibrio fischeri* could be reduced from 50% (20 mg/L BPA) to 11% after only 1 min Fenton treatment. Even though complete detoxification could not be achieved after 90 min, 8% relative inhibition was obtained after 90 min Fenton treatment.
7. At DTU, acute toxicity results obtained using*Daphnia magna* demonstrated thatimmobilizationsdropped from 70% to around 10% (24 h) and from 100% to around 20% (48 h) after 30 min and 60 min, respectively. Complete detoxification was achieved after 90 min Fenton treatment.
8. The growth inhibition test results with *Pseudokirchneriella subcapitata* indicated thatthe % relative inhibition value was 100% in the original BPA solution in M2 medium and lake water. Generally speaking, during 24 h of incubation time, % relative inhibition dropped to around 55-60% and 30-45%, during 48 h of incubation time% relative inhibition was around 40-55% and 40-65% in M2 medium and lake water, respectively.
9. All acute toxicity results showed that the only factor causing toxicity was not only BPA, but also could be some oxidation products.

Overall speaking, the toxicity test results revealed that the most sensitive organism to BPA and its degradation products was *Pseudokirchneriella subcapitata*, whereas *Vibrio fischeri* was the least sensitive organism.

It was observed that Fenton's reagent can be recommended which was very successful in removing BPA from aqueous solution within minutes and could lead to complete removal of BPA from contaminated water. Fenton's reagent was also capable of reducing the TOC concentration of the water.

The treatment time can be extended to provide complete detoxification in relative luminescence inhibition test with *Vibrio fischeri*. Further analysis can be conducted to observe transformation products during Fenton process and after that the toxicity test results will be evaluated more clearly.In order to observe toxicological effects of BPA, acute toxicity test should be preferred with *Pseudokirchneriella subcapitata* which has sensitivity compared to other organisms.It could be said that Fenton treatment time had to be extended to beyond 90 min because of variability of toxicological effects of BPA during the treatment.

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APPENDICES

APPENDIX A: First-order rate coefficients calculations for SET-1, 2 and 3.

Table A.1 : Operating parameters used for baseline experiments.

Parameters	**SET-1**	**SET-2**	**SET-3**
BPA (mg/L)	20	20	50
H_2O_2 (mM)	5	1	5
Fe^{2+} (mM)	0.1	0.1	0.1
Initial pH	3	3	3
Temperature (˚C)	20	20	20
$TOC_{BPA,theoretical}$=	15.8	15.8	39.4

Table A.2 : Summary of calculations of first-order rate coefficient for SET-1. BPA=20 mg/L; Fe^{2+}=0.1 mM; H_2O_2=5.0 mM.

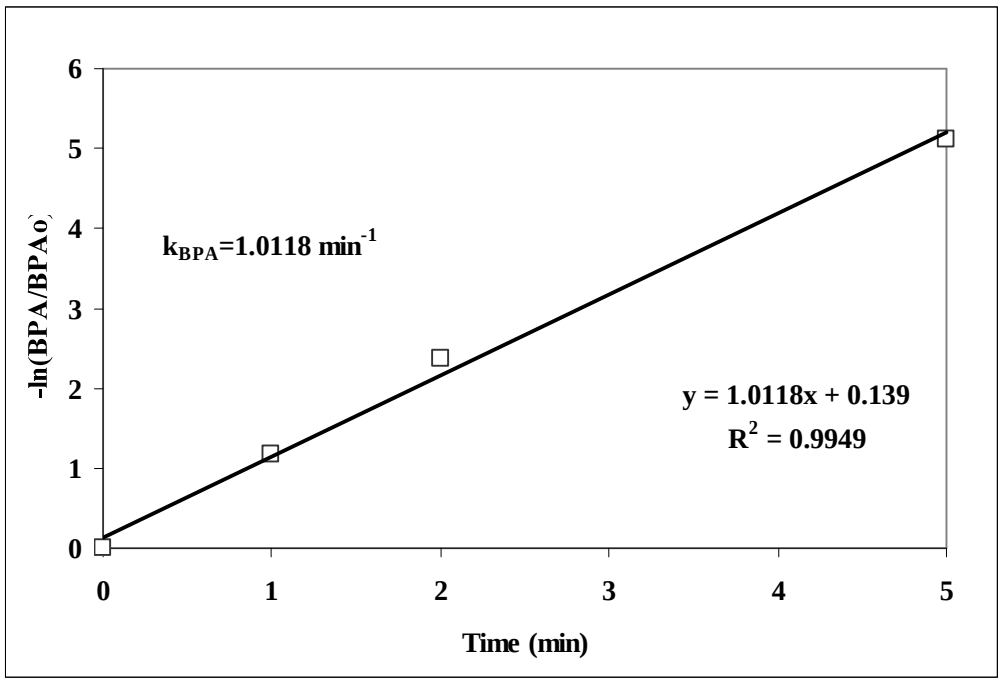
Time (min)	BPA	BPA/BPA$_0$	TOC	TOC/TOC$_0$	H_2O_2	$H_2O_2/(H_2O_2)_0$	ln(BPA/BPA$_0$)	ln(TOC/TOC$_0$)	ln(H_2O_2/H_2O_{20})
0	19.50	1.00	16.13	1.00	5.00	1.00	0.00	0.00	0.00
1	6.08	0.31	16.06	1.00	3.75	0.75	1.16	0.00	0.29
2	1.81	0.09	15.12	0.94	3.45	0.69	2.38	0.06	0.37
5	0.12	0.01	14.88	0.92	2.95	0.59	5.11	0.08	0.53
10	0.00	0.00	13.77	0.85	2.75	0.55	-	0.16	0.60
20	0.00	0.00	13.45	0.83	2.55	0.51	-	0.18	0.67
30	0.00	0.00	12.71	0.79	2.35	0.47	-	0.24	0.76
40	0.00	0.00	12.43	0.77	2.30	0.46	-	0.26	0.78
50	0.00	0.00	12.18	0.76	2.00	0.40	-	0.28	0.92
60	0.00	0.00	12.12	0.75	1.70	0.34	-	0.29	1.08
90	0.00	0.00	11.82	0.73	1.55	0.31	-	0.31	1.17

Table A.3 : Summary of calculations of first-order rate coefficient for SET-2. BPA=20 mg/L; Fe^{2+}=0.1 mM; H_2O_2=1.0 mM.

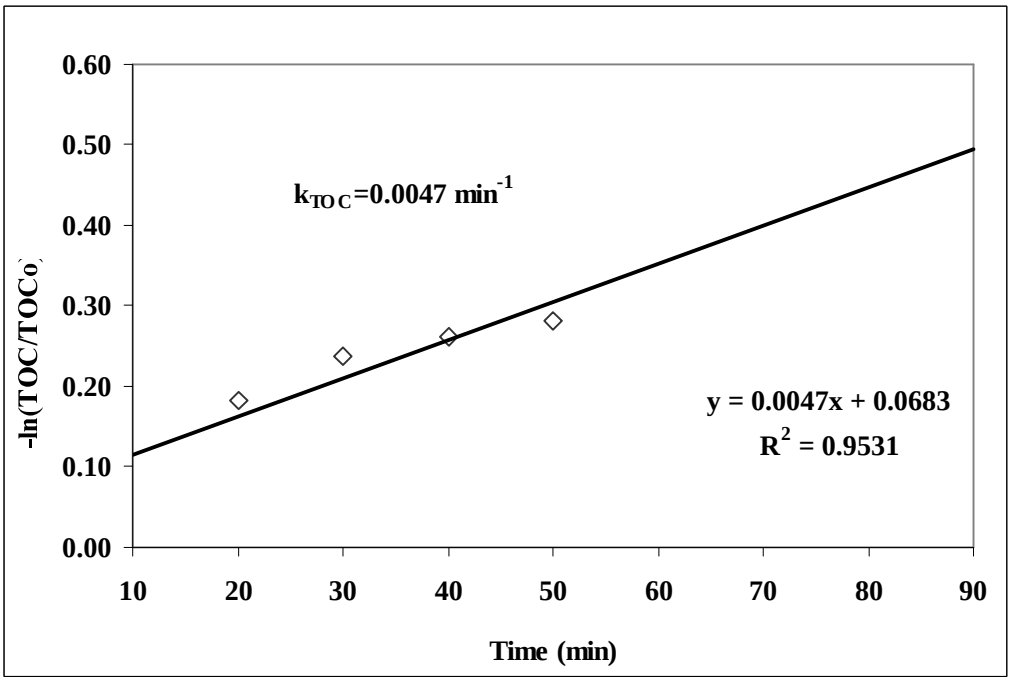
Time (min)	BPA	BPA/BPA_0	TOC	TOC/TOC_0	H_2O_2	$H_2O_2/(H_2O_2)_0$	ln(BPA/BPA_0)	ln(TOC/TOC_0)	ln(H_2O_2/H_2O_{20})
0	19.36	1.00	15.32	1.00	1.00	1.00	0.00	0.00	0.00
1	6.50	0.34	13.89	0.91	0.70	0.70	1.09	0.10	0.60
2	3.90	0.20	13.84	0.90	0.55	0.55	1.60	0.10	0.60
3	2.34	0.12	13.68	0.89	0.50	0.50	2.11	0.11	0.69
4	1.36	0.07	13.44	0.88	0.35	0.35	2.66	0.13	1.05
5	0.67	0.03	13.25	0.86	0.30	0.30	3.36	0.15	1.20
10	0.08	0.00	12.55	0.82	0.15	0.15	5.45	0.20	1.90
20	0.04	0.00	12.15	0.79	0.00	0.00	5.45	0.23	-
30	0.00	0.00	12.26	0.80	0.00	0.00	-	0.22	-
40	0.00	0.00	12.50	0.82	0.00	0.00	-	0.20	-
50	0.00	0.00	11.99	0.78	0.00	0.00	-	0.25	-
60	0.00	0.00	11.85	0.77	0.00	0.00	-	0.26	-
90	0.00	0.00	11.70	0.76	0.00	0.00	-	0.27	-

Table A.4 : Summary of calculations of first-order rate coefficient for SET-3. BPA=50 mg/L; Fe^{2+}=0.1 mM; H_2O_2=5.0 mM.

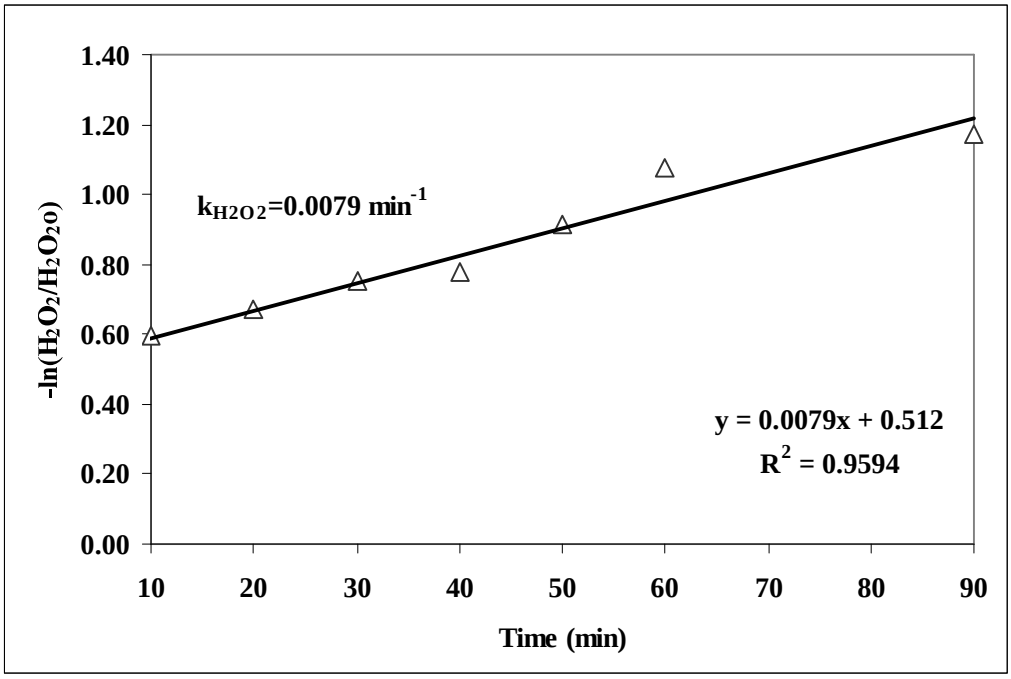
Time (min)	BPA	BPA/BPA_0	TOC	TOC/TOC_0	H_2O_2	$H_2O_2/(H_2O_2)_o$	ln(BPA/BPA_0)	ln(TOC/TOC_0)	ln(H_2O_2/H_2O_{20})
0	49.32	1.00	39.49	1.00	5.00	1.00	0.00	0.00	0.00
1	23.87	0.48	38.61	0.98	3.50	0.70	0.73	0.02	0.36
2	15.05	0.31	38.60	0.98	2.90	0.58	1.19	0.02	0.54
3	7.79	0.16	37.89	0.96	2.55	0.51	1.85	0.04	0.67
4	3.87	0.08	37.33	0.95	2.20	0.44	2.55	0.06	0.82
5	1.99	0.04	36.76	0.93	2.10	0.42	3.21	0.07	0.87
10	0.27	0.01	34.15	0.86	1.80	0.36	5.21	0.15	1.02
20	0.24	0.00	33.77	0.86	1.95	0.39	5.31	0.16	0.94
30	0.09	0.00	33.53	0.85	1.75	0.35	6.36	0.16	1.05
40	0.04	0.00	31.63	0.80	1.65	0.33	7.19	0.22	1.11
50	0.00	0.00	31.56	0.80	1.60	0.32	-	0.22	1.14
60	0.00	0.00	31.03	0.79	1.45	0.29	-	0.24	1.24
90	0.00	0.00	28.82	0.73	1.25	0.25	-	0.31	1.39


-ln(BPA/BPA$_o$)
k_{BPA}=1.0118 min^{-1}
y = 1.0118x + 0.139
R^2 = 0.9949
Time (min)

(a)

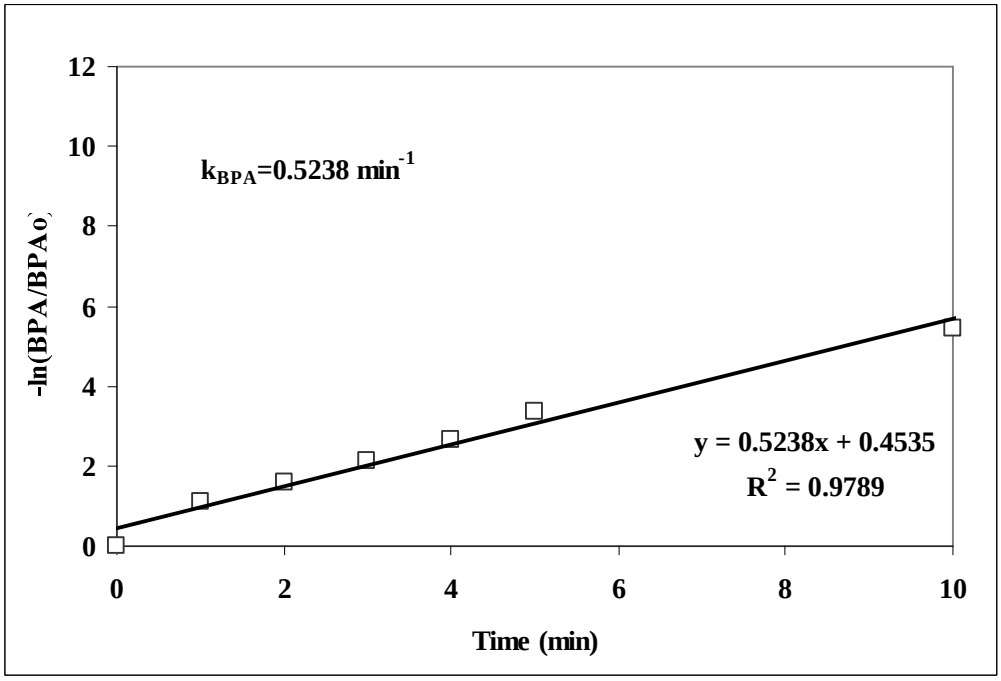

-ln(TOC/TOC$_o$)
k_{TOC}=0.0047 min^{-1}
y = 0.0047x + 0.0683
R^2 = 0.9531
Time (min)

(b)

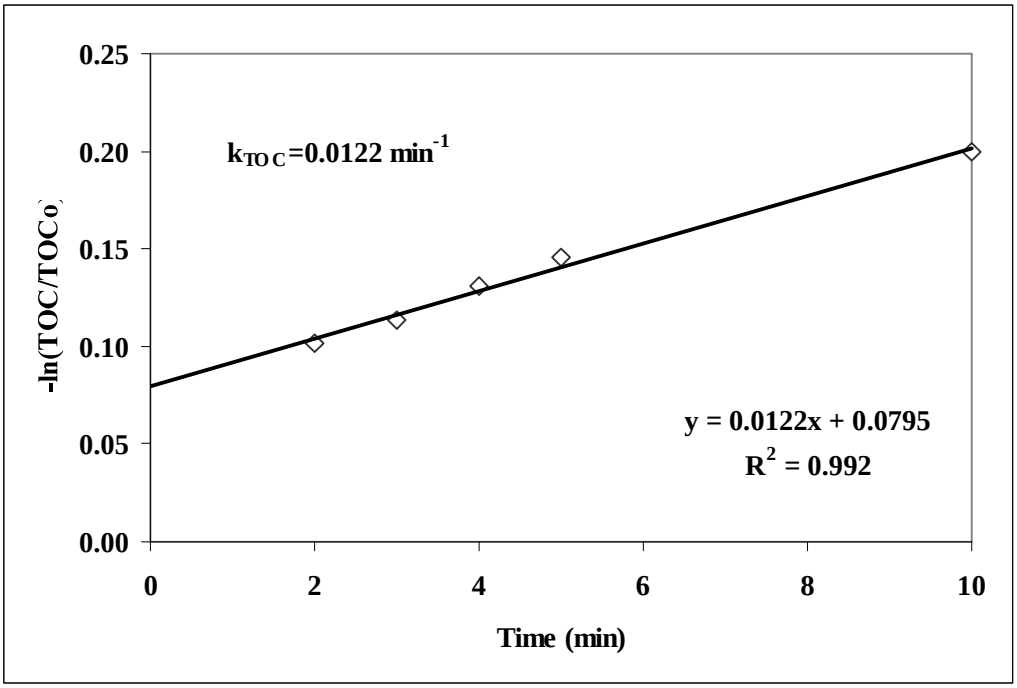


(c)

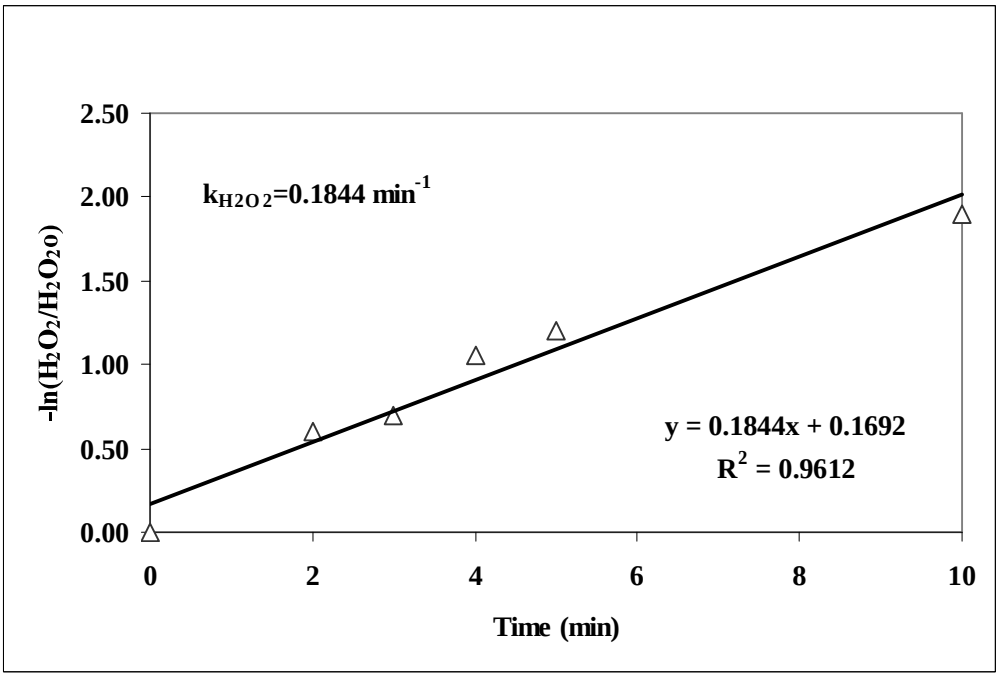
Figure A.1 : First-order rate coefficients of BPA (a), TOC (b) and H_2O_2 (c) abatement rates for SET-1. Experimental conditions: BPA=20 mg/L; Fe^{2+}=0.1 mM; H_2O_2=5.0mM.



(a)

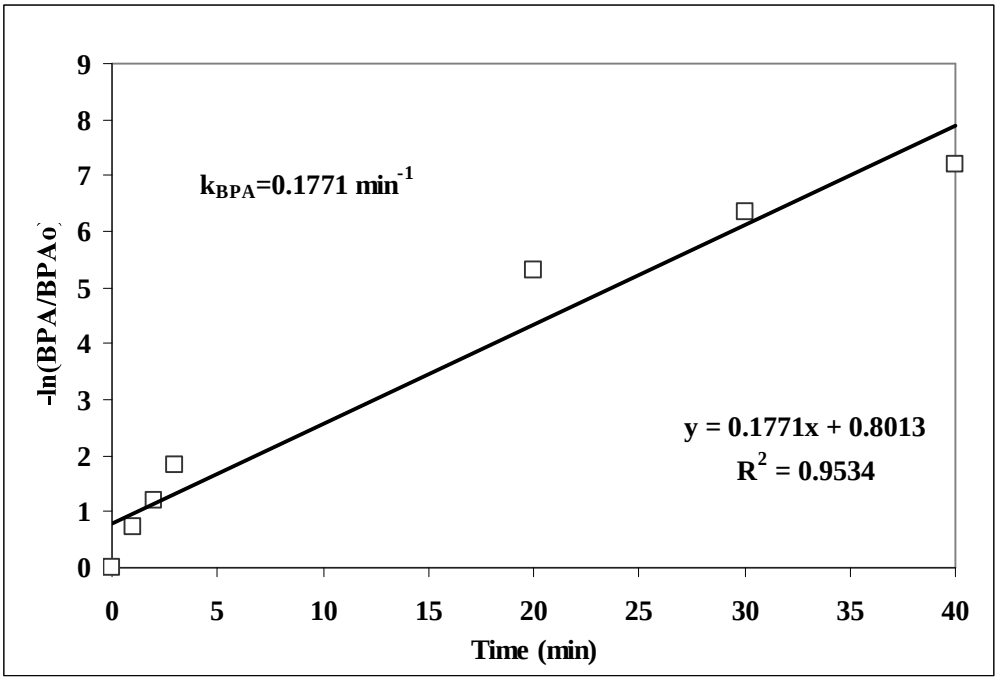


(b)

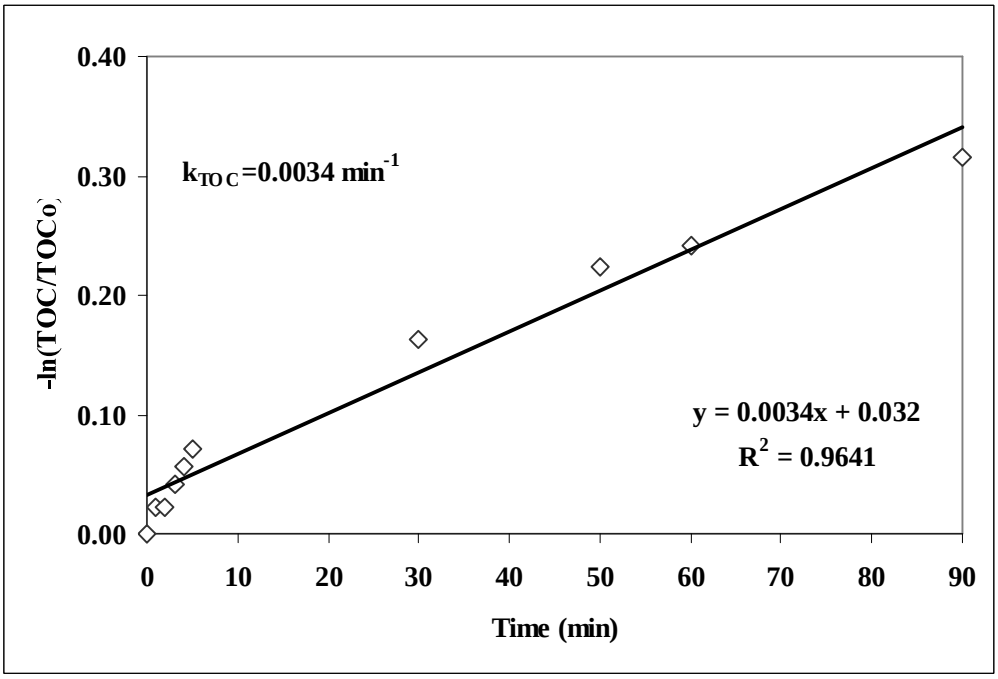


(c)

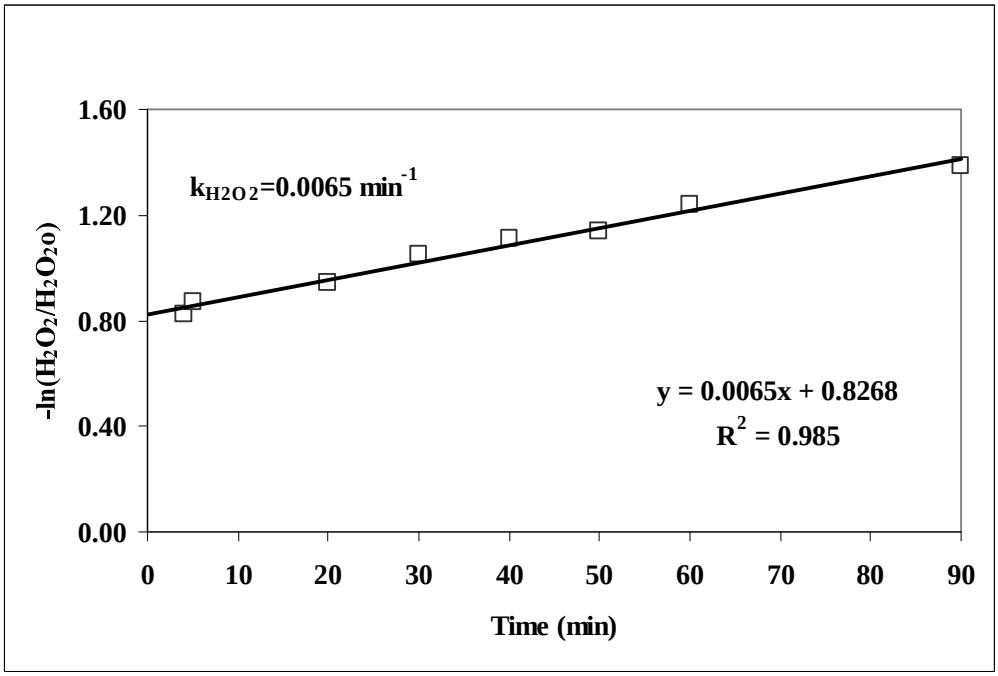
Figure A.2 : First-order rate coefficients of BPA (a), TOC (b) and H_2O_2 (c) abatement rates for SET-2. Experimental conditions: BPA=20 mg/L; Fe^{2+}=0.1 mM; H_2O_2=1.0mM.


-ln(BPA/BPAo)
Time (min)
k_{BPA}=0.1771 min^{-1}
y = 0.1771x + 0.8013
R^2 = 0.9534
0
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
30
35
40

(a)


-ln(TOC/TOCo)
Time (min)
k_{TOC}=0.0034 min^{-1}
y = 0.0034x + 0.032
R^2 = 0.9641
0.00
0.10
0.20
0.30
0.40
0
10
20
30
40
50
60
70
80
90

(b)



(c)

Figure A.3 : First-order rate coefficients of BPA (a), TOC (b) and H_2O_2 (c) abatement rates for SET-3. Experimental conditions: BPA=50 mg/L; Fe^{2+}=0.1 mM; H_2O_2=5.0mM

APPENDIX B: The effects of operating parameters on BPA, TOC and H_2O_2 abatement rates.

§ Effect of H_2O_2

Table B.1 : Effect of initial H_2O_2concentration on BPA removal. Experimental conditions: BPA_{Ort}= 20.1 mg/L; TOC_{Ort} = 15.81 mg/L; Fe^{2+}= 0.1 mM; pH=3; T= 20˚C; t=90 min.

Time (min)	0.5 mM of H_2O_2	1.0 mM of H_2O_2	2.0 mM of H_2O_2	4.0 mM of H_2O_2	5.0 mM of H_2O_2
0	20.44	19.36	20.77	20.23	19.50
1	6.54	6.50	4.20	3.48	6.08
2	4.51	3.90	2.64	0.95	1.81
3	3.76	2.34	1.79	0.43	0.53
4	3.14	1.36	1.14	0.20	0.21
5	2.30	0.67	0.55	0.00	0.12
10	0.79	0.08	0.10	0.00	0.00
20	0.34	0.04	0.00	0.00	0.00
30	0.23	0.00	0.00	0.00	0.00
40	0.22	0.00	0.00	0.00	0.00
50	0.20	0.00	0.00	0.00	0.00
60	0.21	0.00	0.00	0.00	0.00
90	0.20	0.00	0.00	0.00	0.00

Table B.2 : Effect of initial H_2O_2concentration on TOC removal. Experimental conditions: BPA_{Ort}= 20.1 mg/L; TOC_{Ort} = 15.81 mg/L; Fe^{2+}= 0.1 mM; pH=3; T= 20˚C; t=90 min.

Time (min)	0.5 mM of H_2O_2	1.0 mM of H_2O_2	2.0 mM of H_2O_2	4.0 mM of H_2O_2	5.0 mM of H_2O_2
0	15.38	15.32	16.33	15.90	16.13
1	14.34	13.89	15.50	11.72	15.80
2	14.23	13.84	14.33	11.94	15.12
3	13.99	13.68	14.86	11.37	15.10
4	13.56	13.44	14.99	11.28	14.95
5	13.91	13.25	14.31	10.57	14.88
10	13.38	12.55	14.18	10.23	13.77
20	14.24	12.15	12.74	8.72	13.45
30	14.23	12.26	12.43	8.32	12.71
40	13.93	12.50	12.50	8.87	12.60
50	14.25	11.99	11.07	8.98	12.43
60	13.33	11.85	10.63	7.90	12.18
90	13.28	11.70	10.58	7.01	11.82

Table B.3 : Effect of initial H_2O_2concentration on H_2O_2 consumption. Experimental conditions: BPA_{Ort}= 20.1 mg/L; TOC_{Ort} = 15.81 mg/L; Fe^{2+}= 0.1 mM; pH=3; T= 20˚C; t=90 min.

Time (min)	0.5 mM of H_2O_2	1.0 mM of H_2O_2	2.0 mM of H_2O_2	4.0 mM of H_2O_2	5.0 mM of H_2O_2
0	0.50	1.00	2.00	4.00	5.00
1	0.40	0.70	1.20	2.70	3.75
2	0.20	0.55	1.00	2.45	3.45
3	0.15	0.50	0.95	2.50	3.40
4	0.10	0.35	0.95	2.20	3.00
5	0.10	0.30	0.85	2.15	2.95
10	0.00	0.15	0.65	1.80	2.75
20	0.00	0.00	0.80	1.40	2.55
30	0.00	0.00	0.40	1.05	2.35
40	0.00	0.00	0.20	1.00	2.30
50	0.00	0.00	0.15	1.00	2.00
60	0.00	0.00	0.10	1.00	1.70
90	0.00	0.00	0.00	0.80	1.55

§ Effect of Fe^{2+}

Table B.4 : Effect of initial Fe^{2+}concentration on BPA removal. Experimental conditions: BPA_{Ort}= 20.44 mg/L; TOC_{Ort} = 15.41 mg/L; H_2O_2= 2.00 mM; pH=3; T= 20˚C; t=90 min.

Time (min)	0.05 mM of Fe^{2+}	0.10 mM of Fe^{2+}	0.20 mM of Fe^{2+}	0.40 mM of Fe^{2+}	1.00 mM of Fe^{2+}
0	20.96	20.10	19.96	20.67	20.54
1	6.71	4.20	0.33	0.59	0.00
2	5.57	2.64	0.22	0.34	0.00
3	4.02	1.79	0.08	0.30	0.00
4	1.51	1.14	0.00	0.25	0.00
5	0.97	0.55	0.00	0.00	0.00
10	0.23	0.10	0.00	0.00	0.00
20	0.06	0.00	0.00	0.00	0.00
30	0.05	0.00	0.00	0.00	0.00
40	0.00	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	0.00

Table B.5 : Effect of initial Fe^{2+}concentration on TOC removal. Experimental conditions: BPA_{Ort}= 20.44 mg/L; TOC_{Ort} = 15.41 mg/L; H_2O_2= 2.00 mM; pH=3; T= 20˚C; t=90 min.

Time (min)	0.05 mM of Fe^{2+}	0.10 mM of Fe^{2+}	0.20 mM of Fe^{2+}	0.40 mM of Fe^{2+}	1.00 mM of Fe^{2+}
0	15.32	15.71	15.72	15.02	15.28
1	12.19	13.03	12.21	12.88	10.85
2	13.84	13.03	11.78	12.36	9.60
3	13.68	13.85	10.35	13.14	8.20
4	13.44	13.39	10.23	12.64	8.55
5	13.25	13.90	10.93	11.99	8.02
10	12.55	12.74	10.61	11.69	8.24
20	12.15	12.45	10.44	10.97	8.57
30	12.26	10.84	9.56	9.95	7.63
40	12.50	11.09	9.76	9.69	7.23
50	11.99	10.34	10.14	6.17	6.20
60	11.15	10.23	11.08	6.76	6.70
90	11.70	9.46	9.35	7.97	6.20

Table B.6 : Effect of initial Fe^{2+}concentration on H_2O_2 consumption. Experimental conditions: BPA_{Ort}= 20.44 mg/L; TOC_{Ort} = 15.41 mg/L; H_2O_2= 2.00 mM; pH=3; T= 20˚C; t= 90 min.

Time (min)	0.05 mM of Fe^{2+}	0.10 mM of Fe^{2+}	0.20 mM of Fe^{2+}	0.40 mM of Fe^{2+}	1.00 mM of Fe^{2+}
0	2.00	2.00	2.00	2.00	2.00
1	1.80	1.20	1.00	0.40	0.10
2	1.60	1.00	1.00	0.35	0.05
3	1.60	0.95	0.60	0.30	0.00
4	1.60	0.95	0.50	0.15	0.00
5	1.25	0.85	0.45	0.15	0.00
10	1.15	0.65	0.20	0.00	0.00
20	1.15	0.80	0.10	0.00	0.00
30	0.85	0.40	0.00	0.00	0.00
40	0.75	0.20	0.00	0.00	0.00
50	0.55	0.15	0.00	0.00	0.00
60	0.40	0.10	0.00	0.00	0.00
90	0.35	0.00	0.00	0.00	0.00

§ Effect of pH

Table B.7 : Effect of initial pH on BPA removal. Experimental conditions: BPA_{Ort}= 19.08 mg/L; TOC_{Ort} = 15.41 mg/L; H_2O_2= 2.00 mM; Fe^{2+}= 0.40 mM; T= 20˚C; t= 90 min.

Time (min)	pH= 3	pH= 4	pH= 5	pH= 6
0	20.67	18.65	18.41	18.61
1	0.59	0.04	0.03	0.87
2	0.34	0.04	0.00	0.00
3	0.30	0.00	0.00	0.00
4	0.25	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00
40	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00

Table B.8 : Effect of initial pH on TOC removal. Experimental conditions: BPA_{Ort}= 19.08 mg/L; TOC_{Ort} = 15.38 mg/L; H_2O_2= 2.00 mM; Fe^{2+}= 0.40 mM; T= 20˚C; t= 90 min.

Time (min)	pH= 3	pH= 4	pH= 5	pH= 6
0	15.72	15.71	15.02	15.07
1	12.21	12.27	12.88	11.77
2	11.78	12.10	12.36	11.25
3	10.35	11.56	13.14	10.97
4	10.23	10.87	12.64	10.77
5	10.93	10.56	11.99	10.30
10	10.61	10.79	11.69	10.02
20	10.44	10.22	10.97	10.75
30	9.56	9.45	9.95	11.07
40	9.76	9.66	9.69	10.73
50	10.14	9.87	9.17	10.72
60	11.08	11.06	9.15	10.71
90	9.35	9.46	8.97	11.35

Table B.9 : Effect of initial pH on H_2O_2 consumption. Experimental conditions: BPA_{Ort}= 19.08 mg/L; TOC_{Ort} = 15.38 mg/L; H_2O_2= 2.00 mM; Fe^{2+}= 0.40 mM; T= 20˚C; t= 90 min.

Time (min)	pH= 3	pH= 4	pH= 5	pH= 6
0	2.00	2.00	2.00	2.00
1	0.40	0.90	0.90	0.90
2	0.35	0.90	0.70	0.60
3	0.30	0.80	0.55	0.60
4	0.15	0.65	0.50	0.45
5	0.15	0.45	0.35	0.35
10	0.00	0.20	0.30	0.25
20	0.00	0.10	0.10	0.15
30	0.00	0.00	0.05	0.15
40	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00

§ Effect of temperature

Table B.10 : Effect of temperature on BPA removal. Experimental conditions: BPA_{Ort}= 20.00 mg/L; TOC_{Ort} = 16.42 mg/L; H_2O_2= 2.00 mM; Fe^{2+}= 0.40 mM; pH= 5; t= 90 min.

Time (min)	T=25˚C	T=35˚C	T=40˚C	T=50˚C	T=60˚C	T=70˚C
0	18.41	19.60	19.43	20.03	20.34	20.60
1	0.03	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00
40	0.00	0.00	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	0.00	0.00

Table B.11 : Effect of temperature on TOC removal. Experimental conditions: BPA_{Ort}= 20.00 mg/L; TOC_{Ort} = 16.42 mg/L; H_2O_2= 2.00 mM; Fe^{2+}= 0.40 mM; pH= 5; t= 90 min.

Time (min)	T=25˚C	T=35˚C	T=40˚C	T=50˚C	T=60˚C	T=70˚C
0	15.02	16.27	15.93	16.70	17.62	17.03
1	12.88	12.33	13.09	12.91	11.93	13.72
2	12.36	12.19	12.13	12.49	11.70	11.46
3	13.14	12.62	13.56	12.29	12.02	11.26
4	12.64	12.58	11.97	12.01	11.83	10.90
5	11.99	12.48	12.77	11.93	12.03	10.27
10	11.69	12.20	11.71	11.81	11.48	10.20
20	10.97	11.43	11.80	10.89	11.71	10.12
30	6.95	11.38	11.26	10.76	11.70	9.91
40	6.69	11.37	10.94	10.56	11.21	9.79
50	6.17	11.27	10.98	10.78	11.09	9.66
60	6.76	10.95	10.31	11.20	10.34	9.34
90	7.97	10.85	10.86	11.21	10.99	9.62

Table B.12 : Effect of temperature on H_2O_2 consumption. Experimental conditions: BPA_{Ort}= 20.00 mg/L; TOC_{Ort} = 16.42 mg/L; H_2O_2= 2.00 mM; Fe^{2+}= 0.40 mM; pH= 5; t= 90 min.

Time (min)	T=25˚C	T=35˚C	T=40˚C	T=50˚C	T=60˚C	T=70˚C
0	2.00	2.00	2.00	2.00	2.00	2.00
1	0.90	0.35	0.25	0.15	0.10	0.05
2	0.70	0.25	0.25	0.10	0.05	0.00
3	0.55	0.20	0.20	0.05	0.05	0.00
4	0.50	0.15	0.15	0.00	0.00	0.00
5	0.35	0.10	0.05	0.00	0.00	0.00
10	0.30	0.10	0.00	0.00	0.00	0.00
20	0.10	0.00	0.00	0.00	0.00	0.00
30	0.05	0.00	0.00	0.00	0.00	0.00
40	0.00	0.00	0.00	0.00	0.00	0.00
50	0.00	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	0.00	0.00

APPENDIX C: Acute toxicity test results.

§ Acute toxicity test with photobacteria *Vibrio fischeri*at ITU

Table C.1 : Luminescence intensity of *Vibrio fischeri* after 120 min Fenton's treatment in pure water.

Time (min)	T= 0' min	T= 0 min	T= 15 min	T= 30 min
Control	16220	13312	10196	8926
Control	15425	12824	10119	8825
Control	15240	12449	10160	8306
Control	16338	12414	9653	8581
0	16588	3294	3112	3105
0	16232	3234	3149	3076
1	15322	12118	8687	6944
1	15444	12322	8475	7350
5	14531	11927	8103	7357
5	14381	11529	8170	7172
10	14110	10982	8589	6959
10	13541	11557	8100	6866
20	13810	11212	7188	6424
20	13448	11252	6961	6624
30	13605	10789	8311	7155
30	13266	10231	8107	7233
40	12637	10174	7871	6881
40	12453	9118	7803	6709
50	8751	5931	7950	6624
50	11954	9735	5092	4661
60	10821	9636	8024	6869
60	11946	10211	7849	6642
90	10763	8283	7230	6056
90	11714	8683	7078	6125
120	11852	8369	7740	6916
120	10914	8532	8392	6977

Table C.2 : The correction factors for the contact time of 5 min, 15 min or 30 min after 120 min Fenton's treatment in pure water.

$f_{kt}=I_{kt}/I_0$	T= 0 min	T= 15 min	T= 30 min
f_{kt1}	0.82071517	0.62860666	0.5503083
f_{kt2}	0.83137763	0.65601297	0.5721232
f_{kt3}	0.81686352	0.66666667	0.5450131
f_{kt4}	0.75982372	0.59083119	0.5252173
f_{kt*}	0.80719501	0.63552937	0.5481655

Table C.3 : The corrected values of I_0 (I_{ct}) and the inhibitory effect of the test sample (H_t) after the contact time of 5 min, 15 min or 30 min in pure water.

Time (min.)	T= 0 min			T= 5 min			T= 15 min		
	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$
Control	13092.70307	-1.67	0.00	10308.28639	1.09	0.00	8891.24379	-0.39	0.00
Control	12450.98303	-3.00		9803.040541	-3.22		8455.45224	-4.37	
Control	12301.65196	-1.20		9685.467607	-4.90		8354.04163	0.58	
Control	13187.95208	5.87		10383.27886	7.03		8955.92731	4.19	
0	13389.75083	75.40	75.36	10542.1612	70.48	69.98	9092.96868	65.85	65.64
0	13102.38941	75.32		10315.91274	69.47		8897.82177	65.43	
1	12367.84195	2.02	1.59	9737.581015	10.79	12.22	8398.9912	17.32	15.25
1	12466.31974	1.16		9815.115599	13.65		8465.86739	13.18	
5	11729.35069	-1.69	-0.50	9234.877283	12.26	11.43	7965.39232	7.64	8.33
5	11608.27144	0.68		9139.547878	10.61		7883.1675	9.02	
10	11389.52159	3.58	-1.08	8967.319418	4.22	5.05	7734.61466	10.03	8.76
10	10930.22763	-5.73		8605.703207	5.88		7422.70852	7.50	
20	11147.36309	-0.58	-2.12	8776.660607	18.10	18.33	7570.16502	15.14	12.64
20	10855.1585	-3.66		8546.598975	18.55		7371.72913	10.14	
30	10981.88811	1.76	3.11	8646.377086	3.88	3.86	7457.7911	4.06	2.30
30	10708.24901	4.46		8430.93263	3.84		7271.96301	0.54	

Table C.3 : (continued)

Time (min.)	T= 0 min			T= 5 min			T= 15 min		
	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$
40	10200.52334	0.26	4.78	8031.184656	1.99	1.70	6927.16694	0.67	1.19
40	10051.99946	9.29		7914.247251	1.41		6826.30449	1.72	
50	7063.763535	16.04	7.57	5561.517522	-42.95	-4.99	4796.99595	-38.09	-4.61
50	9649.209153	-0.89		7597.118095	32.97		6552.76993	28.87	
60	8734.657206	-10.32	-8.11	6877.063319	-16.68	-10.03	5931.69846	-15.80	-8.62
60	9642.751593	-5.89		7592.03386	-3.38		6548.3846	-1.43	
90	8687.839896	4.66	6.41	6840.202615	-5.70	-0.39	5899.90486	-2.65	0.98
90	9455.48235	8.17		7444.591047	4.92		6421.21022	4.61	
120	9566.875262	12.52	7.84	7532.2941	-2.76	-11.87	6496.85705	-6.45	-11.54
120	8809.726342	3.15		6936.16755	-20.99		5982.67785	-16.62	

Table C.4 : The inhibitory effect and the gama values of the test sample after the contact time of 5 min, 15 min or 30 min in pure water.

Time (min)	T= 5 min		T= 15 min		T= 30 min	
	H_{t5}	r_{t5}	H_{t15}	r_{t15}	H_{t30}	r_{t30}
Control	0.00	0.00	0.00	0.00	0.00	0.00
Control						
Control						
Control						
0	75.36	3.06	69.98	2.33	65.64	1.91
0						
1	1.59	0.02	12.22	0.14	15.25	0.18
1						
5	-0.50	0.00	11.43	0.13	8.33	0.09
5						
10	-1.08	-0.01	5.05	0.05	8.76	0.10
10						
20	-2.12	-0.02	18.33	0.22	12.64	0.14
20						
30	0.00	0.00	0.00	0.00	0.00	0.00
30						
40	4.78	0.05	1.70	0.02	1.19	0.01
40						
50	7.57	0.08	-4.99	-0.05	-4.61	-0.04
50						
60	-8.11	-0.07	-10.03	-0.09	-8.62	-0.08
60						
90	6.41	0.07	-0.39	0.00	0.98	0.01
90						
120	7.84	0.09	-11.87	-0.11	-11.54	-0.10
120						

Table C.5 : Luminescence intensity of *Vibrio fischeri* after 120 min Fenton's treatment in real freshwater.

Time (min)	T= 0' min	T= 0 min	T= 15 min	T= 30 min
Control	184363	162058	127142	119733
Control	180510	156750	128685	118575
0	189700	50210	41973	45239
0	179865	48947	43499	44026
1	173905	126655	97640	92263
1	177143	129275	91503	90406
5	186249	130052	97213	98472
5	171146	128299	100403	92401
10	177051	136599	97614	88378
10	175604	127086	91142	85204
20	170670	131048	102989	93524
20	168366	125524	94105	89864
30	168599	120436	96721	85667
30	238	142	62	46
40	159437	113263	87948	85848
40	156450	121795	97394	88250
50	157646	120132	91752	89645
50	156493	110811	94438	86270
60	163182	108326	91818	88909
60	150420	108781	88702	90686
90	148807	112228	110157	103755
90	154483	108287	104015	107058
120	157002	112902	98475	86330
120	152283	112635	95542	92303

Table C.6 : The correction factors for the contact time of 5 min, 15 min or 30 min after 120 min Fenton's treatment in real freshwater.

$f_{kt}=I_{kt} / I_0$	T= 0 min	T= 15 min	T= 30 min
f_{kt1}	0.87902	0.68963	0.64944
f_{kt2}	0.86837	0.7129	0.65689
f_{kt*}	0.87369	0.70126	0.65317

Table C.7 : The corrected values of I_0 (I_{ct}) and the inhibitory effect of the test sample (H_t) after the contact time of 5 min, 15 min or 30 min in real freshwater.

Time (min)	T= 0 min			T= 5 min			T= 15 min		
	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$
Control	161076.9205	-0.61	0.00	129287	1.66	0.00	120419	0.57	0.00
Control	157710.576	0.61		126585	-1.66		117903	-0.57	
0	165739.8275	69.71	69.28	133030	68.45	66.98	123905	63.49	63.01
0	157147.0431	68.85		126133	65.51		117482	62.53	
1	151939.8245	16.64	16.56	121953	19.94	23.14	113589	18.77	20.32
1	154768.8469	16.47		124224	26.34		115704	21.86	
5	162724.7081	20.08	17.14	130609	25.57	20.96	121651	19.05	18.20
5	149529.3016	14.20		120018	16.34		111787	17.34	
10	154688.467	11.69	14.43	124159	21.38	23.68	115644	23.58	24.65
10	153424.2312	17.17		123145	25.99		114698	25.71	
20	149113.4231	12.12	13.39	119685	13.95	17.12	111476	16.10	17.19
20	147100.4312	14.67		118069	20.30		109971	18.28	
30	147304.002	18.24	24.98	118232	18.19	40.52	110123	22.21	46.31
30	207.939267	31.71		166.901	62.85		155.453	70.41	
40	139299.2139	18.69	14.79	111807	21.34	16.28	104139	17.56	15.60
40	136689.4887	10.90		109713	11.23		102188	13.64	

Table C.7 : (continued).

Time (min)	T= 0 min			T= 5 min			T= 15 min		
	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$
40	139299.2139	18.69	14.79	111807	21.34	16.28	104139	17.56	15.60
40	136689.4887	10.90		109713	11.23		102188	13.64	
50	137734.4272	12.78	15.87	110551	17.01	15.48	102969	12.94	14.27
50	136727.0576	18.95		109743	13.95		102216	15.60	
60	142571.1994	24.02	20.62	114433	19.76	17.84	106585	16.58	12.14
60	131421.1115	17.23		105484	15.91		98249.1	7.70	
90	130011.8424	13.68	16.72	104353	-5.56	-0.79	97195.6	-6.75	-6.42
90	134970.9318	19.77		108333	3.99		100903	-6.10	
120	137171.768	17.69	16.52	110100	10.56	10.55	102548	15.82	11.51
120	133048.8042	15.34		106790	10.53		99466	7.20	

Table C.8 : The inhibitory effect and the gama values of the test sample after the contact time of 5 min, 15 min or 30 min in real freshwater.

Time (min)	T= 5 min		T= 15 min		T= 30 min	
	H_{t5}	r_{t5}	H_{t15}	r_{t15}	H_{t30}	r_{t30}
Control	0.00	0.00	0.00	0.00	0.00	0.00
Control						
0	69.28	2.26	66.98	2.03	63.01	1.70
0						
1	16.56	0.20	23.14	0.30	20.32	0.26
1						
5	17.14	0.21	20.96	0.27	18.20	0.22
5						
10	14.43	0.17	23.68	0.31	24.65	0.33
10						
20	13.39	0.15	17.12	0.21	17.19	0.21
20						
30	0.00	0.00	0.00	0.00	0.00	0.00
30						
40	14.79	0.17	16.28	0.19	15.60	0.18
40						
50	15.87	0.19	15.48	0.18	14.27	0.17
50						
60	20.62	0.26	17.84	0.22	12.14	0.14
60						
90	16.72	0.20	-0.79	-0.01	-6.42	-0.06
90						
120	16.52	0.20	10.55	0.12	11.51	0.13
120						

Table C.9 : The perfent relative inhibitions (%) in pure and real freshwater.

Time (min)	Percent Relative Inhibition (%)	
	Pure water	Real Freshwater
0	70	67
1	12	23
5	11	21
10	5	24
20	18	17
30	4	41
40	2	16
50	-5	15
60	-10	18
90	0	-1
120	-12	11

Table C.10 : Changes in BPA, TOC and H_2O_2 abatements after 120 min Fenton's treatment in pure and real freshwater.

Time (min)	BPA (mg/L)		TOC (mg/L)		H_2O_2 (mM)	
	Pure water	Real freshwater	Pure water	Real freshwater	Pure water	Real freshwater
0	18.77	20.13	15.91	21.69	4.00	4.00
1	0	0	12.95	17.01	1.00	1.60
5	0	0	10.14	14.67	0.70	1.20
10	0	0	9.64	12.8	0.65	0.90
20	0	0	9.21	10.94	0.40	0.70
30	0	0	7.90	9.25	0.20	0.40
40	0	0	7.78	6.46	0.10	0.30
50	0	0	7.18	5.78	0.10	0.25
60	0	0	7.17	5.56	0.10	0.10
90	0	0	6.58	5.61	0.05	0.00
120	0	0	6.56	5.27	0.00	0.00

§ Acute toxicity test with photobacteria *Vibrio fischeri* at DTU

Table C.11 : Luminescence intensity of *Vibrio fischeri* after 90 min Fenton's treatment in 2% NaCl medium.

Time (min)	T= 0' min	T= 0 min	T= 15 min	T= 30 min
Control	660212	686687	611927	526292
Control	669578	711054	645222	523377
0	630097	290564	289316	283802
0	671428	317855	320662	309489
1	668851	642038	564985	472107
1	672229	637685	562762	473569
3	673040	675274	581884	514733
3	697957	698346	626762	546699
5	683004	700533	635722	560138
5	684237	687749	609178	497960
10	682270	698203	629835	276893
10	671896	692104	617024	538158
20	679401	677839	609792	542096
20	658220	644673	561551	476947
30	687230	662880	562018	486952
30	670755	652188	552386	453232
60	644000	662050	583547	485186
60	668917	692504	627360	550858
90	645126	655498	559654	480744
90	634602	648001	548147	459605

Table C.12 : The correction factors for the contact time of 5 min, 15 min or 30 min after 90 min Fenton's treatment in 2% NaCl medium.

$f_{kt}=I_{kt} / I_0$	T= 0 min	T= 15 min	T= 30 min
f_{kt1}	1.040100756	0.926864401	0.797156065
f_{kt2}	1.061943493	0.96362485	0.781652026
f_{kt*}	1.051022124	0.945244626	0.789404045

Table C.13 : The corrected values of I_0 (I_{ct}) and the inhibitory effect of the test sample (H_t) after the contact time of 5 min, 15 min or 30 min in 2% NaCl medium.

Time (min)	T= 0 min			T= 5 min			T= 15 min		
	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$	I_{ct}	H_t	$H_{average}$
Control	693897.4186	1.04	0.00	624061.845	1.94	0.00	521174.0236	-0.98	0.00
Control	703741.2918	-1.04		632915.006	-1.94		528567.5819	0.98	
0	662245.8874	56.12	55.54	595595.803	51.42	50.45	497401.1207	42.94	42.28
0	705685.6828	54.96		634663.709	49.48		530027.9793	41.61	
1	702977.1988	8.67	9.21	632227.813	10.64	11.04	527993.6851	10.58	10.67
1	706527.5515	9.74		635420.849	11.43		530660.292	10.76	
3	707379.9304	4.54	4.67	636187.443	8.54	6.77	531300.4987	3.12	1.95
3	733568.2487	4.80		659740.103	5.00		550970.0793	0.78	
5	717852.3149	2.41	3.39	645605.86	1.53	3.67	539166.1206	-3.89	1.96
5	719148.2252	4.37		646771.347	5.81		540139.4558	7.81	
10	717080.8646	2.63	2.31	644912.051	2.34	2.59	538586.698	48.59	23.56
10	706177.5611	1.99		635106.083	2.85		530397.4204	-1.46	
20	714065.4822	5.07	5.94	642200.144	5.05	7.40	536321.8978	-1.08	3.57
20	691803.7826	6.81		622178.918	9.74		519601.5307	8.21	
30	722293.9344	8.23	7.86	649600.464	13.48	13.18	542502.1421	10.24	12.32
30	704978.3449	7.49		634027.559	12.88		529496.7104	14.40	
60	676858.2479	2.19	1.84	608737.539	4.14	2.46	508376.2052	4.56	0.12
60	703046.5662	1.50		632290.199	0.78		528045.7858	-4.32	
90	678041.6989	3.32	3.09	609801.884	8.22	8.42	509265.0741	5.60	6.93
90	666980.742	2.85		599854.13	8.62		500957.386	8.25	

Table C.14 : The inhibitory effect and the gama values of the test sample after the contact time of 5 min, 15 min or 30 min in 2% NaCl medium.

Time (min.)	T= 5 min.		T= 15 min.		T= 30 min.	
	H_{t5}	r_{t5}	H_{t15}	r_{t15}	H_{t30}	r_{t30}
Control	0.00	0.00	0.00	0.00	0.00	0.00
Control						
0	55.54	1.25	50.45	1.02	42.28	0.73
0						
1	9.21	0.10	11.04	0.12	10.67	0.12
1						
3	4.67	0.05	6.77	0.07	1.95	0.02
3						
5	3.39	0.04	3.67	0.04	1.96	0.02
5						
10	2.31	0.02	2.59	0.03	23.56	0.31
10						
20	5.94	0.06	7.40	0.08	3.57	0.04
20						
30	7.86	0.09	7.10	0.08	12.32	0.14
30						
60	1.84	0.02	2.46	0.03	0.12	0.00
60						
90	3.09	0.03	8.42	0.09	6.93	0.07
90						

Table C.15 : Changes in BPA, TOC and H_2O_2 abatements for 90 min Fenton's treatment in 2% NaCl medium.

Time (min)	BPA (mg/L)	TOC (mg/L)	H_2O_2 (mM)
0	20.64	13.57	2.00
1	0.22	12.35	0.88
2	0.00	10.97	0.84
3	0.00	9.56	0.84
4	0.00	9.23	0.78
5	0.00	10.46	0.78
10	0.00	8.65	0.77
20	0.00	8.45	0.86
30	0.00	7.65	0.84
40	0.00	6.92	0.83
50	0.00	5.32	0.89
60	0.00	5.27	0.78
90	0.00	5.22	0.89

§ **Acute toxicity test with freshwater crustacean *Daphnia magna***

Table C.16 : Changes in BPA, TOC and H_2O_2 abatements for 90 min Fenton's treatment in M1 medium.

Time (min)	BPA (mg/L)	TOC (mg/L)	H_2O_2 (mM)
0	20.00	12.00	2.00
1	0.12	11.74	0.63
2	0	10.40	0.36
3	0	9.64	0.29
4	0	9.16	0.26
5	0	8.71	0.21
10	0	8.12	0.14
20	0	8.17	0.05
30	0	6.43	0.03
40	0	5.47	0.01
50	0	6.18	0.00
60	0	5.67	0.00
90	0	5.10	0.00

Table C.17 : Percentage of immobilized *Daphnia magna* for 24 h and 48 h incubation period after 90 min Fenton's treatment in M1 medium.

Time (min)	Total number of animals per concentration	Number per beaker A B C D	Number of dead (24 h) A B C D	Total number of dead (24 h) A B C D	Percentage of Immobilized animals (%) (24 h)	Number of dead (48 h) A B C D	Total number of dead (48) A B C D	Percentage of Immobilized animals (%) (48 h)
Control	20	5 5 5 5	0 0 0 0	0	0	0 0 0 0	0	0
0	10	5 5	3 4	7	70	5 5	10	100
1	10	5 5	0 0	0	0	0 0	0	0
3	10	5 5	0 0	0	0	0 0	0	0
5	10	5 5	2 3	5	50	5 5	10	100
10	10	5 5	0 0	0	0	0 0	0	0
20	10	5 5	0 0	0	0	0 0	0	0
30	10	5 5	0 1	1	10	4 4	8	80
60	10	5 5	0 1	1	10	1 1	2	20
90	10	5 5	0 0	0	0	0 0	0	0

§ **Acute toxicity test with freshwater green microalgae *Pseudokirchneriella subcapitata***

Table C.18 : Changes in BPA, TOC and H_2O_2 abatements after 90 min Fenton's treatment in lake water and M2 medium.

Time (min)	BPA (mg/L)		TOC (mg/L)		H_2O_2 (mM)	
	Lake water	M2 medium	Lake water	M2 medium	Lake water	M2 medium
0	20.95	20.40	27.34	14.95	2.00	2.00
1	0.30	0.13	21.21	13.22	0.56	0.58
2	0.00	0.10	18.79	12.29	0.46	0.41
3	0.00	0.00	18.34	11.90	0.37	0.34
4	0.00	0.00	19.80	11.68	0.34	0.30
5	0.00	0.00	19.34	12.02	0.31	0.28
10	0.00	0.00	17.85	11.73	0.23	0.18
20	0.00	0.00	16.88	12.27	0.09	0.06
30	0.00	0.00	15.26	11.91	0.03	0.01
40	0.00	0.00	15.44	12.19	0.02	0.01
50	0.00	0.00	15.47	11.77	0.00	0.01
60	0.00	0.00	15.84	12.24	0.00	0.01
90	0.00	0.00	15.16	11.63	0.00	0.00

Table C.19 : Biomass readings of the test samples for 24 h and 48 h incubation period after 90 min Fenton's treatment in M2 medium.

Time (min)	Replicate	Day 0 Biomass reading	Day 1 Biomass reading	Day 2 Biomass reading	Day 1 Growth Rate (μ_1)	Day 2 Growth Rate (μ_2)	Day 1 Average ($\mu_{Ave.1}$)	Day 2 Average ($\mu_{Ave.2}$)	Day 1 Inhibition $I\mu i_1$ (%)	Day 2 Inhibition $I\mu i_2$(%)
Control	A	0.359	3.671	5.673	0.10	0.12	0.10	0.15	0.00	0.00
Control	B	0.359	3.762	17.670	0.10	0.16				
Control	C	0.359	3.632	16.306	0.10	0.16				
Control	D	0.359	3.360	14.280	0.09	0.15				
Control	E	0.359	3.357	15.902	0.09	0.16				
0	A	1.089	0.385	0.310	-0.04	-0.05	-0.03	-0.04	128.37	129.19
0	B	1.089	0.831	0.558	-0.01	-0.03				
0	C	1.089	0.574	0.322	-0.03	-0.05				
1	A	1.149	3.075	8.354	0.04	0.08	0.04	0.08	59.84	43.92
1	B	1.149	3.247	9.058	0.04	0.09				
1	C	1.149	2.404	8.405	0.03	0.08				
3	A	1.114	2.787	2.460	0.04	0.03	0.04	-0.02	59.07	112.18
3	B	1.114	3.033	0.290	0.04	-0.06				
3	C	1.114	2.728	0.522	0.04	-0.03				
5	A	1.031	2.765	7.598	0.04	0.08	0.04	0.08	58.62	46.34
5	B	1.031	2.698	6.395	0.04	0.08				
5	C	1.031	2.528	7.287	0.04	0.08				

Table C.19 : (continued).

Time (min)	Replicate	Day 0 Biomass reading	Day 1 Biomass reading	Day 2 Biomass reading	Day 1 Growth Rate (μ_1)	Day 2 Growth Rate (μ_2)	Day 1 Average ($\mu_{Ave.1}$)	Day 2 Average ($\mu_{Ave.2}$)	Day 1 Inhibition $I\mu i_1$ (%)	Day 2 Inhibition $I\mu i_2$(%)
10	A	1.083	2.586	8.804	0.04	0.09	0.04	0.08	60.28	43.77
10	B	1.083	2.749	7.802	0.04	0.08				
10	C	1.083	2.744	7.883	0.04	0.08				
20	A	1.034	2.882	15.165	0.04	0.11	0.04	0.09	56.57	41.18
20	B	1.034	2.669	6.094	0.04	0.07				
20	C	1.034	2.847	6.736	0.04	0.08				
30	A	1.147	2.745	10.207	0.04	0.09	0.04	0.09	60.76	39.76
30	B	1.147	2.674	8.985	0.04	0.09				
30	C	1.147	3.053	10.797	0.04	0.09				
60	A	1.002	2.753	5.991	0.04	0.07	0.04	0.07	55.39	51.30
60	B	1.002	2.927	6.086	0.04	0.08				
60	C	1.002	2.682	5.228	0.04	0.07				
90	A	1.243	3.048	6.622	0.04	0.07	0.03	0.07	67.34	55.35
90	B	1.243	3.041	5.867	0.04	0.06				
90	C	1.243	1.958	6.056	0.02	0.07				

Table C.20 : Biomass readings of the test samples for 24 h and 48 h incubation period after 90 min Fenton's treatment in lake water.

Time (min)	Replicate	Day 0 Biomass reading	Day 1 Biomass reading	Day 2 Biomass reading	Day 1 Growth Rate (μ_1)	Day 2 Growth Rate (μ_2)	Day 1 Average ($\mu_{Ave.1}$)	Day 2 Average ($\mu_{Ave.2}$)	Day 1 Inhibition $I\mu i_1$ (%)	Day 2 Inhibition $I\mu i_2$(%)
Control	A	0.541	1.564	5.290	0.04	0.10	0.07	0.18	0.00	0.00
Control	B	0.457	1.410	5.755	0.05	0.11				
Control	C	0.923	0.944	10.504	0.00	0.10				
Control	D	0.400	1.726	7.773	0.06	0.12				
Control	E	0.388	1.675	8.546	0.06	0.13				
0	A	1.609	0.500	0.465	-0.05	-0.05	-0.04	-0.04	161.98	122.41
0	B	0.793	0.503	0.480	-0.02	-0.02				
0	C	1.635	0.344	0.474	-0.06	-0.05				
1	A	0.416	1.111	6.562	0.04	0.11	0.04	0.10	43.69	45.72
1	B	0.677	1.483	4.348	0.03	0.08				
1	C	0.428	1.319	5.781	0.05	0.11				
3	A	0.568	1.536	5.272	0.04	0.09	0.05	0.06	34.51	67.69
3	B	0.484	1.430	5.369	0.05	0.10				
3	C	0.435	1.572	0.311	0.05	-0.01				
5	A	0.596	1.124	2.208	0.03	0.05	0.04	0.09	43.59	53.88
5	B	0.575	1.521	5.349	0.04	0.09				
5	C	0.503	1.827	6.747	0.05	0.11				

Table C.20 : (continued).

Time (min)	Replicate	Day 0 Biomass reading	Day 1 Biomass reading	Day 2 Biomass reading	Day 1 Growth Rate (μ_1)	Day 2 Growth Rate (μ_2)	Day 1 Average ($\mu_{Ave.1}$)	Day 2 Average ($\mu_{Ave.2}$)	Day 1 Inhibition $I\mu i_1$ (%)	Day 2 Inhibition $I\mu i_2$(%)
10	A	0.457	1.394	6.217	0.05	0.11	0.05	0.11	35.25	41.28
10	B	0.428	1.423	5.897	0.05	0.11				
10	C	0.516	1.415	6.804	0.04	0.11				
20	A	0.499	1.203	0.197	0.04	-0.04	0.05	0.06	28.04	65.17
20	B	0.255	1.272	5.687	0.07	0.13				
20	C	0.492	1.647	5.750	0.05	0.10				
30	A	0.437	1.337	5.035	0.05	0.10	0.04	0.09	46.23	51.18
30	B	0.510	1.396	3.500	0.04	0.08				
30	C	0.549	1.037	4.597	0.03	0.09				
60	A	0.575	1.318	4.593	0.03	0.09	0.04	0.08	46.96	57.32
60	B	0.454	1.359	2.957	0.05	0.08				
60	C	0.599	1.330	3.367	0.03	0.07				
90	A	0.516	1.125	3.941	0.03	0.08	0.03	0.08	62.34	55.37
90	B	0.556	0.523	3.905	0.00	0.08				
90	C	0.435	1.467	3.075	0.05	0.08				

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PUBLICATIONS/PRESENTATIONS ON THE THESIS

§ Arslan-Alaton I., Aytac, E., 2012: Aqueous Bisphenol A Treatment With The Fenton's Reagent: Effect of Operating Parameters and Water Matrix. *1st International Industrial Water Technologies Symposium and Fair*, December 6-9, 2012, Bursa, Turkey.