SOLAR DEGRADATION OF ESTRONE AND 17β-ESTRADIOL

(Spine title: Solar Degradation of Estrone and 17β-Estradiol)

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by

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Abstract

The presence of steroid estrogens in surface water is an issue of intense concern due to their endocrine disruption properties on aquatic wild life. Amongst the steroid estrogens, the natural estrogens estrone (E1) and 17β-estradiol (E2) are potential sources of endocrine disruption due to high estrogenic activity. Although these steroid hormones are known to be well suited to solar degradation in wetlands due to shallow depth and their extended absorbance in the 300-400 nm range, which overlaps with the solar spectrum of UVB and UVA. Limited information is available concerning their fate caused by direct and indirect photolysis in aquatic environments. Hence, this study investigated the photochemical behavior of E1 and E2 under natural sunlight (290−700 nm) produced by a solar simulator in Milli-Q water in the presence of different water constituents, e.g. pH, NO_3^-, Fe^{3+}, HCO_3^-, humic acid and turbidity in order to mimic the natural aquatic environment.

Both E1 and E2 were found to be degraded in simulated solar light due to direct photolysis and photo-oxidation following pseudo-first-order reaction kinetics. The photodegradation rate of E1 compared to E2 was considerably faster due to high molar absorbance of E1 in the solar-UV region (λ>290 nm to 380 nm). The half-life of E1 at 1 sun intensity was found to be ≈ 50 minutes, whereas it was 10 hours for E2 under the same conditions; accordingly about 67% degradation of E1 occurred due to direct photolysis compared to 48% degradation of E2. The degradation rate of both E1 and E2 decreased slightly with increasing initial steroid concentration and varied linearly or with square root dependence on the light intensity, respectively in the region of 25−100 mW

cm^{-2}. The rate of mineralization based on the total organic carbon (TOC) reduction was always lower than E1 and E2 degradation rates, while the TOC of the solution decreased steadily with increased irradiation time.

In the presence of NO_3^-, Fe^{3+} and humic acid, the photodegradation rate increased significantly attributed to photosensitization by the reactive species such as hydroxyl radical ($OH^\bullet$). HCO_3^- slowed down the degradation rate attributed to $OH^\bullet$ scavenging. Turbidity also reduced photodegradation of E2 by decreasing transmittance due to light attenuation. The solution pH also had a considerable effect on the degradation rate with maximum degradation occurring around neutral pH of 7 for both E1 and E2.

Co-authorship

The content of this thesis is based on the following two manuscripts:

1. Chowdhury, R. R.; Charpentier, P. A.; Ray, M. B. Photodegradation of Estrone in Solar Irradiation. Industrial & Engineering Chemistry Research. 2010, 49 (15), 6923 – 6930.

2. Chowdhury, R. R.; Charpentier, P. A.; Ray, M. B. Photolysis of 17β-Estradiol in Natural Aquatic Environment under Solar Irradiation. Submitted to Journal of Photochemistry and Photobiology A: Chemistry, 2010.

Rajib Roy Chowdhury is the principal author of both manuscripts, which were reviewed by Dr. Paul A. Charpentier and Dr. Madhumita B. Ray who provided additional recommendations for further improvement.

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Introduction

1.1 Background

The ubiquitous presence of emerging persistent organic contaminants in the aquatic environment is of worldwide concern.[1] Many of these contaminants are suspected endocrine disrupting compounds (EDCs) that can interfere with the normal function of hormones by interacting with the endocrine system presenting a potential threat to aquatic life and human health.[1–3] This emerging group of EDCs includes natural and steroid hormones (E1, E2, E3, EE2, MeEE2 etc.), pharmaceuticals (ibuprofen, naproxen, gemfibrozil etc.) and industrial chemicals (bisphenol A, dioxins, triclosan, atrazine, DDT etc.).[3,4]

Among all EDCs, natural and synthetic steroid hormones (estrogens), excreted by livestock in the conjugated form but are present in surface water as the free steroid, are considered to be responsible for the majority of endocrine-disruption in aquatic environments due to their high estrogenic activity.[4,5] Both natural and synthetic steroid hormones have been detected at elevated levels in soil, ground water as well as surface water adjacent to agricultural fields fertilized with animal manure in countries such as Canada, Brazil, Germany and the United States.[6]

A number of studies in the 1990s have shown that the concentration of these steroid hormones in natural aquatic environment is very low in the ng/l range (10-1830 ng/l). However, due to their extremely high biological potency and procreation toxicity, this

trace level still has sufficient potential to alter sexual behavior and development of both vertebrates and invertebrates.[6,7] For example, steroid estrogens at a concentration of 5-6 ng/l could destroy the entire fathead minnow population in a lake study in northwestern Ontario within three years due to feminization of male fish.[8] It was found in a recent study that short-term exposure to natural and environmental estrogens may impair smolt development and their survival while delaying subsequent seaward migration of juvenile Atlantic salmon.[9]

There are many aspects of aqueous environments that contribute to their ability to breakdown these steroid hormones. Sediments provide sorption sites and habitat for anaerobic microbial processes. The direct and indirect photolysis in the presence of photosensitizers is probably the primary abiotic degradation pathway to dictate the fate of these compounds in surface water. They degrade rapidly in the presence of high intensity UV-C (254 nm),[10] and many studies have investigated using advanced oxidation processes (AOP) such as semiconductor photocatalysis, UV/H_2O_2 UV/O_3, O_3/H_2O_2 etc. to study the degradation of these hormones in water.[4,11,12] However, there is little information available on the direct photolysis and indirect photolysis of natural steroid hormones (E1 and E2) in aquatic environments under solar irradiation indicating a considerable gap in the scientific knowledge. Sound knowledge in this area is essential to determine the environmental fate, toxicity and risk assessment of these steroid estrogens in natural echo systems. Thus, an understanding of the factors that contribute to the degradation of contaminants in aquatic environments is critical for the development of accurate models on the environmental fate of these constituents.

1.2 Goals of the research

The primary objective of this research is to improve our knowledge and understanding of the fate of E1 and E2 in an aquatic environment in the presence of sunlight and various water constituents. The specific goals are as follows:

1. Develop an extraction method for E1 and E2 from aqueous solution;

2. Develop an analytical technique for detecting E1 and E2 in low concentrations by means of GC-MS and HPLC-UV detection;

3. To investigate the kinetics of photodegradation of E1 and E2 in aquatic environments due to direct solar irradiation (i.e. UV-B, UV-A, and visible radiation, 290-700 nm) using a solar simulator with a controlled dose of sun light;

4. To investigate the effect of natural photosensitizers (dissolved uncharacterized organic matter or humic acid, Fe^{3+} and NO_3^-) and other water constituents (HCO_3^- and turbidity) providing complete information on the photochemical behavior of steroid hormones in an aquatic environment;

5. To investigate the evolution of intermediates and extent of mineralization of E1 and E2.

1.3 Thesis overview

Chapter 2 describes the available literature pertinent to this research study.

Chapter 3 describes the photodegradation of estrone in solar irradiation including a mineralization study and the detection of possible intermediates.

Chapter 4 describes the photolysis of 17β-estradiol in natural aquatic environment under solar irradiation, including the effect of water constituents and influencing factors on photodegradation such as pH, NO_3^-, Fe^{3+}, HCO_3^-, humic acid and turbidity.

Chapter 5 presents the major findings and conclusions of this study followed by environmental significance and recommended future work.

1.4 References

1. Zuo, Y.; Zhang, K.; Deng, Y. Occurrence and Photochemical Degradation of 17α-Ethinylestradiol in Acushnet River Estuary. *Chemosphere*. **2006**, *63*, 1583.

2. Zhang, K.; Zuo, Y. Pitfalls and Solution for Simultaneous Determination of Estrone and 17α-Ethinylestradiol by Gas Chromatography–Mass Spectrometry after Derivatization with N,*O* -bis (trimethylsilyl)trifluoroacetamide. *Anal. Chim. Acta.* **2005**, *554*, 190.

3. Wicks, C.; Kelley, C.; Peterson, E. Estrogen in a Karstic Aquifer. *Ground Water*. **2004**, *42*, 384.

4. Liu, X. L.; Wu, F.; Deng, N. S. Photodegradation of 17α-Ethynylestradiol in Aqueous Solution Exposed to a High-pressure Mercury Lamp (250 W). *Environ. Pollut*. **2003**, *126*, 393.

5. Desbrow, C.; Routledge, E.J.; Brighty, G.C.; Sumpter, J.P.; Waldock, M.J. Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical Fractionation and in Vitro Biological Screening. *Environ. Sci. Technol*. **1998**, *32*, 1549.

6. Ying, G. G.; Kookana, R. S.; Ru, Y. J. Occurrence and Fate of Hormone Steroids in the Environment. *Environ. Int.* **2002**, *28*, 545.

7. Li, J.; Mailhot, G.; Wu, F.; Deng, N. Photochemical Efficiency of Fe (III)-EDDS Complex: •OH Radical Production and 17β-Estradiol Degradation. *J. Photochem. Photobiol. A*. **2010**, *212*, 1.

8. Pelley, J. Estrogen Knocks out Fish in Whole-Lake Experiment. *Environ. Sci. Technol.* **2003**, *37,* 313A.

9. Madsen, S. S.; Skovbolling, S.; Nielson, C.; Korsgaard, B. 17-β Estradiol and 4-nonylphenol delay smolt development and downstream migration in Atlantic salmon. *Aquat. Toxicol.* **2004**, *68,* 109.

10. Liu, B.; Liu, X. Direct Photolysis of Estrogens in Aqueous Solutions. *Sci. Total Environ.* **2004**, *320*, 269.

11. Lin, Y.; Peng, Z.; Zhang, X. Ozonation of Estrone, Estradiol, Diethylstilbestrol in waters. *Desalination*. **2009**, *249*, 235.

12. Zhang, Y.; Zhou, J. L.; Ning, B. Photodegradation of Estrone and 17β-Estradiol in Water. *Water Res*. **2007**, *41*, 19.

Literature Review

2.1 Endocrine disrupting compounds and steroid estrogen

Over the last decade, the presence of biologically active persistent contaminants in surface water is of major concern. Many of these environmental contaminants are suspected as endocrine disrupting compounds due to their ability to modulate the action of hormones by interacting with hormone receptors. This mimics or antagonizes the production and activities of endogenous hormones by interacting with the endocrine system presenting a potential threat to aquatic life and human health.[1,2] An endocrine disrupting compound (EDC) has been defined as "an exogenous substance or mixture that alters the function(s) of the endocrine systems and consequently causes adverse health effects in an intact organism, or its progeny or (sub) populations".[3] In 1999, the Canadian Environmental Protection Act (CEPA, 1999) defined an EDC as a substance that has the ability to disrupt the synthesis, secretion, transport, binding, action or elimination of hormones in an organism, or its progeny, that is responsible for the maintenance of homeostasis, reproduction, development or behavior of an organism." Although the topic of endocrine disruption is considered as an "emerging issue" in environmental research, scientists have recognized the ability of natural and synthetic compounds to interfere with natural hormone systems of animals for over 80 years.[4] The ability of estrogenic and androgenic compounds to interfere with the natural metamorphosis of amphibians was reported as early as 1948.[5]

From various literatures, these chemicals can be classified broadly in six categories as follows:

- synthetic and natural hormones (e.g. E1, E2, E3, EE2);

- pesticides (e.g. DDT, vinclozolin, TBT, atrazine);

- pharmaceuticals and personal care products (PPCPs) (e.g. diclofenac, ibuprofen, sulfamethoxazole, oxybenzone);

- heavy metals (e.g. lead, mercury, cadmium);

- industrial chemicals (e.g. bisphenol A, phthalate, nonylphenol);

- combustion byproducts (e.g. dioxin).

Over the last several decades, estrogenic compounds which are either produced endogenously by animals or used as pharmaceutical products in human or veterinary medicine, are of emerging concern due to their high endocrine disruption potential and threat to aquatic lives. Both natural and synthetic estrogens, estrone (E1), 17β-estradiol, estriol (E3), 17α-ethinylestradiol (EE2) and mestranol MeEE2 (as shown in Figure 2.1) can create detrimental estrogenic effects. This could be due to the common phenol ring of their molecules that is regarded as one of the essential functional groups to interact with the estrogen receptor.[6] Among all steroid estrogens, both natural estrogens such as E1 and E2 are among the most potent of all EDCs, which have been detected at elevated levels in soil, ground water as well as surface water adjacent to agricultural fields fertilized with animal manure. These hormones make their way into the aquatic environment through sewage discharge and animal waste disposal due to both human and animal excretions.[7] It has been estimated that around 2.7 mg/L in urine per capita on a daily basis is one of the

principal sources of these types of compounds in the aquatic environment, whereas the

animal excretion of these hormones is predominantly confined in livestock waste from

animal feeding operations (AFOs) such as sheep, cattle, pigs and poultry. The

concentration of E1 and E2 was found to be 44 ng/g on average in dry poultry waste.[1]

Estrone (E1)   17β-Estradiol (E2)

Estriol (E3)   17α-Ethinylestradiol (EE2)

Mestranol (MeEE2)
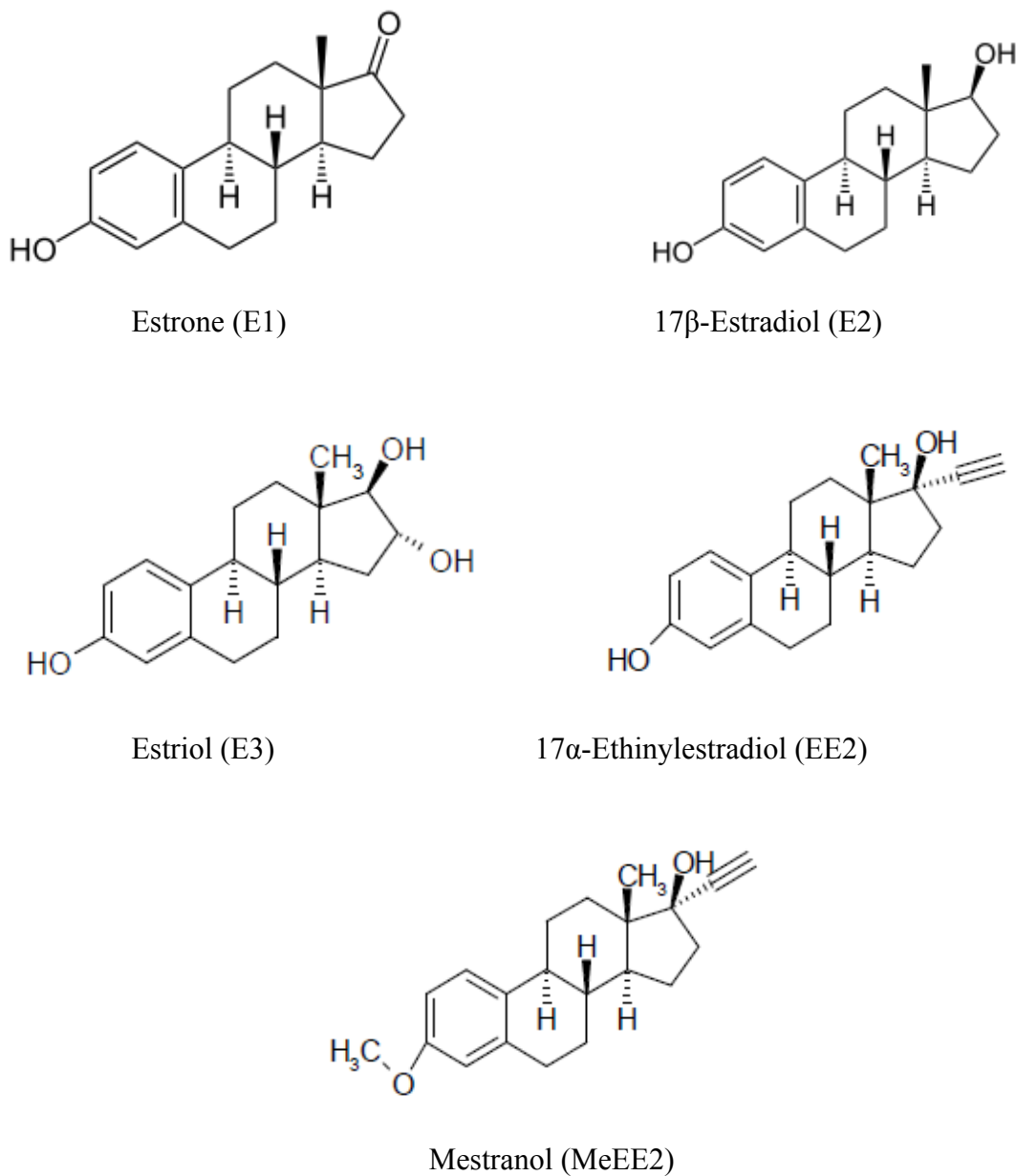
Figure 2.1 Molecular structures of steroid estrogens.

2.1.1   Mechanism of endocrine disruption

Most of the known environmental chemicals with hormonal activity derive that activity through interference and interaction with one or more steroid/thyroid/retinoid gene family of nuclear receptors. It is believed that the endocrine disruption occurs due to the abnormal binding of a hormone-like compound with one of the nuclear receptors of the endocrine system and its subsequent adverse effects.[8]
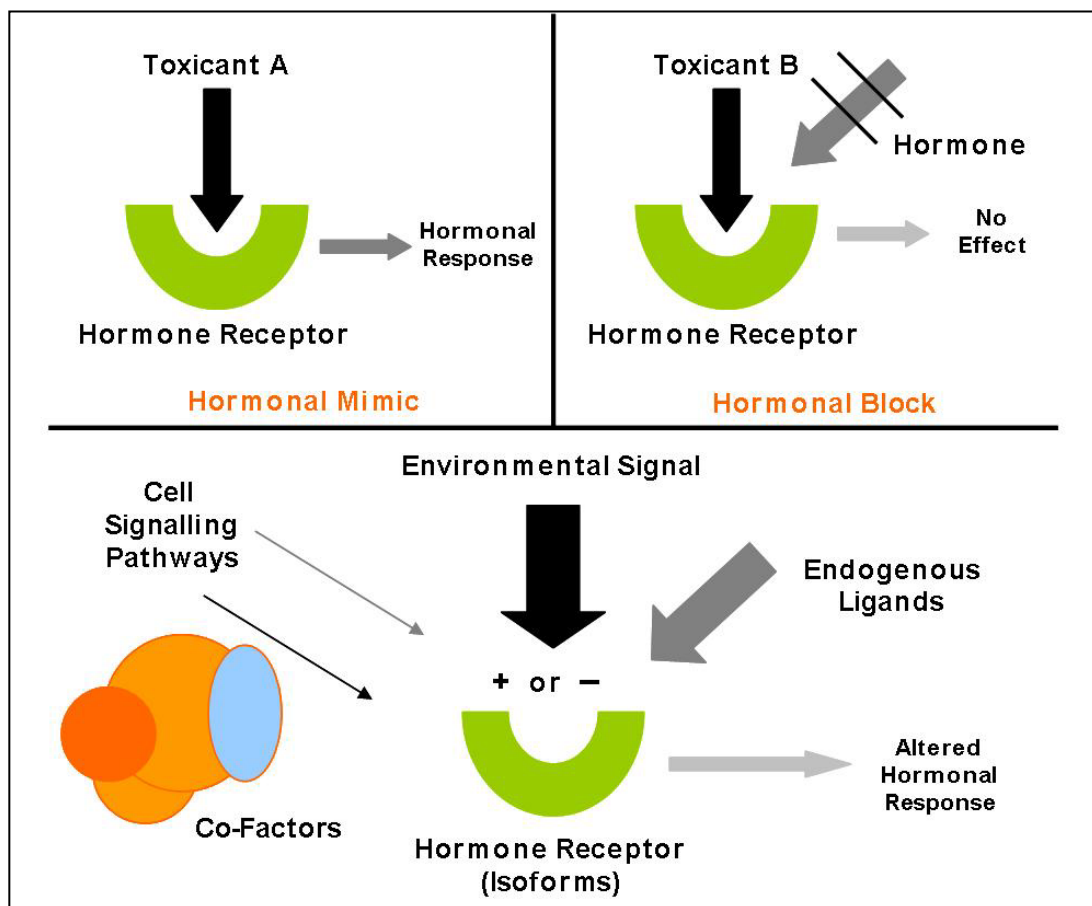
Figure 2.2 Mechanism of endocrine disruption processes.[8]

These EDCs can interact with endocrine systems and cause a disruption to normal functions through three possible routes (Figure 2.2): i) mimicking the effect of natural hormones via binding to the hormone receptors, known as an agonist response; ii) blocking the receptors in target cells for these hormones and therefore preventing the normal response of natural hormones, known as an antagonistic response; iii) altering the synthesis and function of hormone receptors and interfering with the synthesis, metabolism and excretion of hormones.[9]

2.1.2   Physiochemical properties of steroid estrogens

Steroidal compounds, including estrogens, represent a hormonal class generally synthesized from cholesterol. Therefore, E1, E2, E3, EE2 and MeEE2 display molecular structures similar to cholesterol, with a five-carbon ring attached to three six-carbon rings (Figure 2.1). Natural steroid estrogens, namely E1, E2, and E3, have water solubility of approximately 13 mg/L, whereas synthetic estrogenic steroids have much lower solubilities of 4.8 mg/L for EE2 and 0.3 mg/L for MeEE2, respectively. All these steroid hormones have very low vapor pressures ranging from 2.3×10^{-10} to 6.7×10^{-15} mm Hg, indicating the low volatility of these compounds. The log K_{ow} values of natural steroids are 3.94 for E2, 3.43 for E1 and 2.81 E3, respectively. Synthetic steroids have higher log K_{ow} values, 4.15 for EE2 and 4.67 for MeEE2. From the physicochemical properties of these steroids, it can be seen that steroid estrogens are hydrophobic organic compounds of low volatility.[10]

Table 2.1 Physicochemical properties of steroid estrogens[10]

	E1	E2	E3	EE2	MeEE2
Molecular formula	$C_{18}H_{22}O_2$	$C_{18}H_{24}O_2$	$C_{18}H_{22}O_3$	$C_{20}H_{24}O_2$	$C_{21}H_{26}O_2$
Molecular weight (g/mol)	270.4	272.4	288.4	296.4	310.4
Water solubility (mg/L @ 20 ^{0}C)	13	13	13	4.8	0.3
Vapor pressure (mm Hg)	2.3×10^{-10}	2.3×10^{-10}	6.7×10^{-15}	4.5×10^{-11}	7.5×10^{-10}
log K_{ow}	3.43	3.94	2.81	4.15	4.67

2.2 Environmental fate of steroid estrogens

2.2.1 Photodegradation

In natural surface waters, photochemical reactivity is very much limited to the photic zone, i.e. the upper most region of the water column which is affected by depth and attenuation. The depth of this photic zone varies widely with the indivisual water body. Surface water with a high algal content or sediment loading will have a very shallow photic zone due to light absorption and scattering. In addition, humic substances can absorb or attenuate sunlight, which also decreases the depth of light penetration, while colored dissolved organic matter is the main UV-absorbing constituent in surface water and controls the UV light penetration.[11]

Solar phototransformation or degradation of organics in an aquatic environment may occur from either direct or indirect photolysis within the photic zone. Direct photolysis occurs due to photon absorption by a pollutant, which becomes excited to its singlet state, then undergoes chemical transformation to generate one or more different product species. This process is governed by the structure of the molecule and is directly related to the pollutant's structure. Molecular moieties that absorb photons are defined as chromophores, and include functional groups such as alkenes, carbonyls, aromatics, heterocyclic, and nitro groups. Direct photolysis in natural light is only possible if the contaminant of interest absorbs light in the UV-visible range (290-750 nm).[12]

In indirect photolysis, abundant photosensitizers NO_3^-, Fe^{3+} and humic substances, which are ubiquitous in surface water, absorb solar radiation to reach an excited state and generate free radicals comprised of reactive oxygen species (ROS) (e.g., hydroxyl radicals ($OH^\bullet$), peroxyl radicals ($ROO^\bullet$), singlet oxygen ($^1O_2$), etc.) and other non-ROS transients.[13] Among these reactive photochemically generated species in surface waters in the presence of solar irradiation, $OH^\bullet$ plays a very important role in the phototransformation of organic pollutants due to the reaction between most organics and $OH^\bullet$, which occurs with rate constants that are essentially diffusion controlled.[14] The major sources of $OH^\bullet$ in natural water have been identified as NO_3^-, Fe^{3+} and humic substances, while HCO_3^- plays an important role due to its scavenging effect of $OH^\bullet$ in surface water.[15] Another important water parameter is turbidity, which affects the efficiency of the photochemical reactivity as the suspended material limits the light attenuation.

2.2.2   Sorption

Sorption which includes adsorption, i.e., if the compounds attach to a two-dimensional surface, and absorption i.e.; if the compounds penetrate into a three-dimensional matrix; is the process in which chemicals become associated with solid-phases.[16] While partitioning, which results in the distribution of organic contaminants between the aqueous and solid phases, is governed by equilibrium; i.e. it represents a principal mechanism of controlling contaminant mobility in the natural environment.[17]

Different studies have shown that sorption of estrogens in the soil and sediment of aquatic environments is moderate to high with typical sorption coefficients (K_d) ranging from 26 to 108 L/Kg for E1 and 30 to 123 L/Kg for E2, respectively. Sorption of estrogens usually exhibits non-linear behavior in soil and sediment, similar to other hydrophobic organic compounds with the sorption behavior being modeled using the Freundlich isotherm with log K_F=1.71 and sorption constants ranging from 0.57 to 0.83.[1] The hydrophobic nature of E1 and E2 likely promotes an affinity towards humic substance, yielding moderate organic carbon normalized distribution coefficients (K_{OC}) ranging 4882 for E1 and 3300 for E2, respectively.[1]

Hence, a number of soil properties and environmental factors contribute to the sorption behavior of free estrogens into a solid phase. While humic substance plays an important role, the impact of soil minerals cannot be ignored and they may contribute to the commonly observed sorption non-linearity of estrogens in soils.[18] In aquatic environments, higher ionic strength solutions not only lead to greater estrogen sorption,

higher salt concentrations also enhance the particle aggregation and flocculation. The coupling of these two natural phenomena likely promotes sedimentation of hormones out of the water column.[19]

2.2.3   Microbial degradation

The ability of potential breakdown of E1 and E2 from five different soil types by microorganisms was reported by Turfitt as early as 1947.[20] It was reported that a strain of *Proactinomyces spp.* isolated from an acid and arable soil degraded E2 as a carbon source, while E1 was metabolized by a strain of bacterial genus *Proactinomyces* in arable soil.[21] Studies have shown that *Novosphingobium tardaugens*, *Rhodococcus zopfii* and *Rhodococcus equi* isolated from activated sludge from wastewater treatment plants could degrade all the four principal estrogens within 24 hours.[22] However, it was found that both E1 and E2 degraded rapidly, but mineralization occurred slowly in soils receiving swine manure.[23]

Although the literature reveals that estrogens undergo degradation by microbial populations than simple transformation in soil systems,[24] very limited information is available on environmental parameters such as nutrient levels, pH and other recognized variables impacting microbial activity etc. that influence or inhibit the degradation of estrogen in the environment.

2.3 References

1. Ying, G. G.; Kookana, E. S.; Ru, Y. J. Occurrence and Fate of Hormone Steroids in the Environment. *Environ. Int.* **2002**, *28*, 545.

2. Pelley, J. Estrogen Knocks out Fish in Whole-Lake Experiment. *Environ. Sci. Technol.* **2003**, *37*, 313A.

3. IPCS, 2002. The International Programme on Chemical Safety (IPCS): Global assessment of the state-of-the-science of endocrine disruptors. WHO/PCS/EDC/02.2.

4. Cook, J. W.; Dodds, E. C.; Hewett, C. L.; Lawson, W. Estrogenic Activity of Some Condensed Ring Compounds in Relation to Their Other Biological Activates. *Proc. Roy. Soc.* **1934**, *B114*, 272.

5. Sluczewski, A.; Roth, P. Effects of Androgenic and Estrogenic Compounds on the Experimental Metamorphoses of Amphibians. *Gynecol. Obstet.* **1948,** *47*, 164.

6. Kustrin, S. A.; Turner, J. V.; Glass, B. D. Molecular Structural Characteristics as Determinants of Estrogen Receptor Selectivity. *J. Pharm. Biomed. Anal.* **2008**, *48*, 369.

7. Desbrow, C.; Routledge, E.J.; Brighty, G.C.; Sumpter, J.P.; Waldock, M.J. Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical Fractionation and in Vitro Biological Screening. *Environ. Sci. Technol*. **1998**, *32*, 1549.

8. McLachlan, J. A. Environmental Signaling: What Embryos and Evolution Teach Us about Endocrine Disrupting Chemicals. *Endocr. Rev.* **2001**, *22*, 319.

9. Ropero, A. B.; Alonso-Magdalena, P.; Ripoll, C.; Fuentes, E.; Nadal, A. Rapid Endocrine Disruption: Environmental Estrogen Actions Triggered Outside the Nucleus. *J. Steroid Biochem. Mol. Biol*. **2006**, *102*, 163.

10. Lai, K. M.; Johnson, K. L.; Scrimshaw, M. D.; Lester, J. N. Binding of Waterborne Steroid Estrogens to Solid Phases in River and Estuarine Systems. *Environ. Sci. Technol.* **2000**, *34*, 3890.

11. Markager, S.; Vincent, W. F. Spectral Light Attenuation and the Absorption of UV and Blue Light in Natural Waters. *Limnol. Oceanogr.* **2000**, *45*, 642.

12. Zepp, R. G.; Cline, D. M. Rates of Direct Photolysis in Aquatic Environment. *Environ. Sci. Technol.* **1977**, *11*, 359.

13. Chin, Y. P.; Miller, P. L.; Zeng, L.; Cawley, K.; Weavers, L. K. Photosensitized Degradation of Bisphenol A by Dissolved Organic Matter. *Environ. Sci. Technol.* **2004**, *38*, 5888.

14. Lam, M. W.; Tantuco, K.; Mabury, S. A. Photofate: A New Approach in Accounting for the Contribution of Indirect Photolysis of Pesticides and Pharmaceuticals in Surface Waters. *Environ. Sci. Technol.* **2003**, *37*, 899.

15. Vione, D.; Falletti, G.; Maurino, V.; Minero, C.; Pelizzetti, E.; Malandrino, M.; Ajassa, R.; Olariu, R. L.; Arsene, C. Sources and Sinks of Hydroxyl Radicals upon Irradiation of Natural Water Samples. *Environ. Sci. Technol*. **2006**, *40*, 3775.

16. Schwarzenbach, R. P.; Gschwend, P. M; Imboden, D. M. Environmental Organic Chemistry, 2nd ed.; John Wiley & Sons: New Jersey, 2003.

17. Ehlers, G. A. C.; Loibner, A. P. Linking Organic Pollutant (Bio)Availability with Geosorbent Properties and Biomimetic Methodology: A Review of Geosorbent Characterisation and (Bio)Availability Prediction. *Environ. Pollut.* **2006** *141*, 494.

18. Bonin, J. L.; Simpson, M. J. Sorption of Steroid Estrogens to Soil and Soil Constituents in Single- and Multi-Sorbate Systems. *Environ. Toxicol. Chem.* **2007**, *26*, 2604.

19. Lai, K. M.; Johnson, K. L.; Scrimshaw, M. D.; Lester, J. N. Binding of Waterborne Steroid Estrogens to Solid Phases in River and Estuarine Systems. *Environ. Sci. Technol.* **2000**, *34*, 3890.

20. Turfitt, G. E. Microbiological Agencies in the Degradation of Steroids: II. Steroid Utilization by the Microflora of Soils. *J. Bacteriol.* **1947**, *54*, 557.

21. Fujii, K.; Kikuchi, S.; Satomi, M.; Ushio-Sata, N.; Morita, N. Degradation of 17β-Estradiol by a Gram-Negative Bacterium Isolated from Activated Sludge in a Sewage Treatment Plant in Tokyo, Japan. *Appl. Environ. Microbiol.* **2002**, *68*, 2057.

22. Yoshimoto, T.; Nagai, F.; Fujimoto, J.; Watanabe, K.; Mizukoshi, H.; Makino, T.; Kimura, K.; Saino, H.; Sawada, H.; Omura, H. Degradation of Estrogens by *Rhodococcus zopfii* and *Rhodococcus equi* Isolates from Activated Sludge in Wastewater Treatment Plants. *Appl. Environ. Microbiol.* **2004**, *70*, 5283.

23. Jacobsen, A.M.; Lorenzen, A.; Chapman, R.; Topp, E. Persistence of Testosterone and 17β-Estradiol in Soils Receiving Swine Manure or Municipal Biosolids. *J. Environ. Qual.* **2005**, *34*, 861.

24. Colucci, M. S.; Bork, H.; Topp, E. Persistence of Estrogenic Hormones in Agricultural Soils: I. 17Beta -Estradiol and Estrone. *J. Environ. Qual.* **2001**, *30*, 2070.

Photodegradation of Estrone in Solar Irradiation

3.1 Introduction

The ubiquitous presence of emerging persistent organic contaminants in the environment is of world-wide concern.[1,2] Many of these compounds are suspected endocrine disrupting compounds (EDCs) that can interfere with the normal function of hormones by interacting with the endocrine system presenting a potential threat to aquatic life and human health.[1–3] Besides industrial chemicals such as bisphenol-A, DDT, atrazine, methoxychlor, chlordecone, alkylphenols, PCBs and phthalic esters, some natural steroid estrogens such as estrone (E1), estradiol (E2), estriol (E3) and mestranol (MeEE2) and synthetic pharmaceuticals such as diethylstilbestrol (DES), ibuprofen, norfloxacin and 17α-ethynylestradiol (EE2) are found to be the most potent EDCs.[4,5]

Estrogenic steroids are detected in the influent and effluent of sewage treatment plants in different countries in various concentrations.[6–8] These steroid hormones make their way into the aquatic environment through sewage discharge and animal waste disposal due to both human and animal excretions. It has been estimated that around 2.7 mg/L in urine per capita on a daily basis is one of the principal sources of these types of compounds in the aquatic environment, whereas the animal excretion of these hormones is predominantly confined to livestock waste from the animal feeding industry including sheep, cattle, pigs and poultry.[1,9] The concentration of E2 was found to be 44 ng/g on

average in dry poultry waste.[10] These steroids have also been detected at elevated levels in soil, ground water as well as surface water adjacent to agricultural fields fertilized with animal manure.[11,12] The degradation time of these compounds in the environment may vary from a few days to months depending on the environmental parameters.[13] Although the concentration of these steroid hormones in natural aquatic environments are in the very low ng/L range (10-1830 ng/L), it is very important to understand their environmental fate due to their extremely high biological potency and procreation toxicity.[5,14–16]

Steroid hormones are known to degrade rapidly in the presence of high intensity UV-C (254 nm), and many degradation studies of these hormones are available in the literature using advanced oxidation processes (AOP) such as semiconductor photocatalysis, UV/H_2O_2 UV/O_3, O_3/H_2O_2 etc.[17,18] However very little information is available about their environmental fate, transport and degradation in natural water. The objective of this study is to determine the environmental degradation of steroid E1 (See Figure 3.1) in aquatic environment due to direct solar irradiation (i.e. UV-B, UV-A, and visible radiation, 290-700 nm), including the factors affecting the photodegradation and in the presence of natural photosensitizers such as dissolved uncharacterized organic matter, or the humic substances.
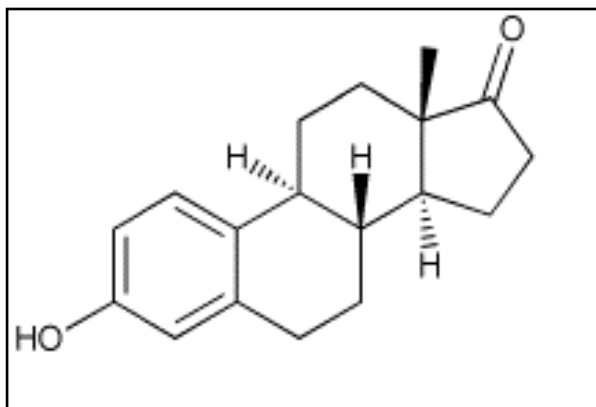
Figure 3.1 Structure of Estrone (E1).

3.2 Experimental

3.2.1 Chemicals

E1 (MW: $C_{18}H_{22}O_2$) was obtained from Sigma-Aldrich (Oakville, Ontario, Canada) and used without further purification. All organic solvents including methanol (HPLC grade), acetone, dichloromethane (DCM) were of distilled-in-glass grade and purchased from Fisher Scientific (Ottawa, Ontario, Canada). The derivatizing reagent *N,O*-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) was purchased from Supelco (Oakville, Ontario, Canada). Humic acid (Technical grade, CAS registry number: 1415-93-6) and other chemicals including sodium hydroxide, hydrochloric acid, sodium sulphate, potassium hydrogen phthalate and sodium hydrogen carbonate were obtained from Sigma-Aldrich (Oakville, Ontario, Canada). Laboratory grade water (LGW, 18 MΩ) was prepared from an in-house Millipore purification system (Billerica, MA).

3.2.2   Standard and sample preparation

Working solutions were prepared by dissolving 5 ± 0.05 mg E1 in 1 L of Milli-Q water by stirring for 2 hours to ensure complete dissolution. The natural pH of Milli-Q water is 6.0 and this is also the pH of E1 solution. All experiments were carried out at pH 6.0 except for those evaluating the effect of pH on degradation of E1, where NaOH or HCl was used to adjust the pH. The working solution was wrapped with aluminum foil and stored at 4 ^{0}C to prevent any degradation.

3.2.3   Photodegradation experiments

Photodegradation was carried out using a solar simulator (Model: SS1KW, Sciencetech, ON, Canada) with 1000 watt xenon arc lamp and air mass filter (AM filter) AM1.5G, which produces identical simulated 1 SUN irradiance of 100 mWcm^{-2} at full power that matches the global solar spectrum (Class A standards as per JIS-C-8912 & ASTM) at sea level and zenith angle 37 degree (Refer to Appendix A). The absorption spectram of E1 was measured and is shown in Figure 3.2.
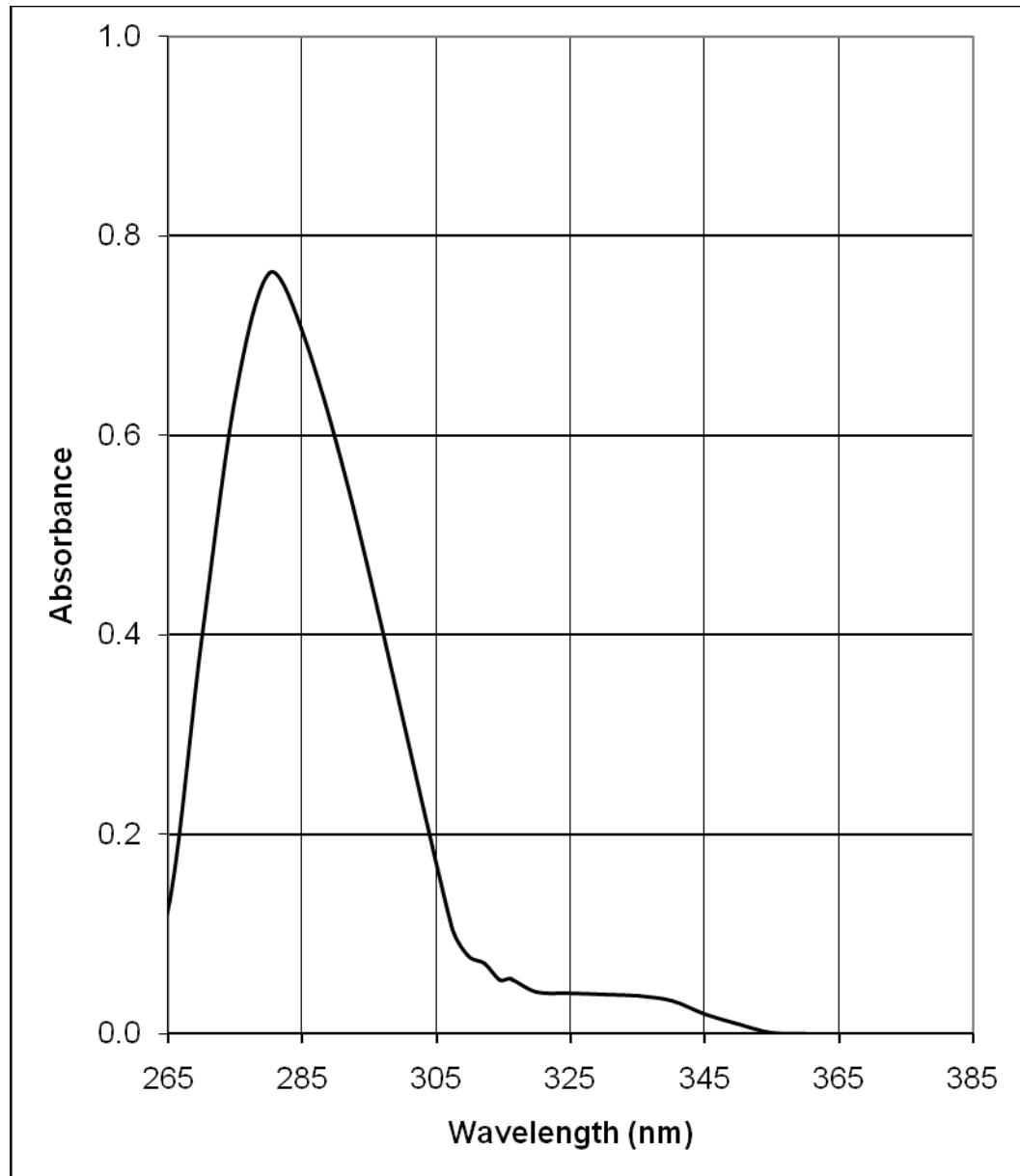
Figure 3.2 Absorption spectrum of E1 over 265-385 nm at pH 6.0.

Since E1 exhibits considerable absorption in the 300-360 nm wavelength region, photon flux from the solar simulator was calculated in the 300-400 nm range, which was 5.3 x 10^{-5} Einstein m^{-2} s^{-1} at 1 SUN.

An open water-jacketed vertical glass vessel (Length: 16 cm x Diameter: 12 cm) was used as the solar photo-reactor, which was placed on a magnetic stirrer during all experiments, under aerated conditions @ 400 rpm and a temperature of 20 ± 2 ^{0}C. The aqueous solution was irradiated directly from the top using a vertical solar beam of 8 inch (20.32 cm) diameter from the solar simulator. In all experiments, the total irradiated solution volume was 500 mL. The irradiation intensity was measured at the top surface of the experimental solution by a Broadband Thermopile Detector (Model: UP19K-15W, Sciencetech, ON, Canada), which allows measurement of the radiation emitted by a light source between 190 nm-11 μm (UV-VIS-IR) and the power density on a surface (in mW/cm^2). The schematic of the experimental setup is shown in Figure 3.3. The experiments were performed using different solar intensities, solution pH, initial concentrations of E1, concentration of humic acid and dissolved oxygen. All the experiments were conducted in triplicate with average error around 5% and the results presented in the Figures and Tables are the average of three experiments with reported standard deviations or error bars.
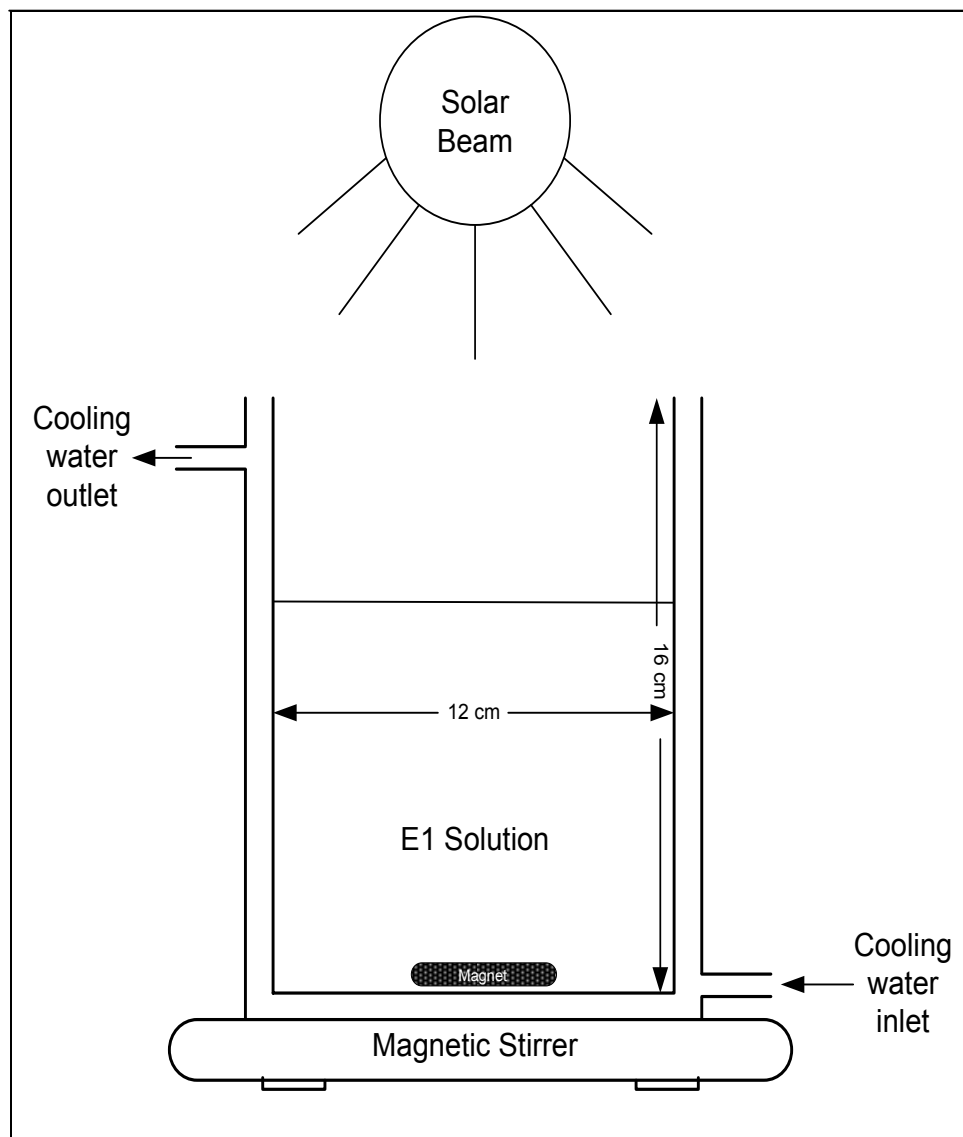
Figure 3.3 Schematic of the experimental setup.

3.2.4   Extraction and sample analysis

Liquid-liquid extraction is one of the most preferred methods to extract organic pollutants from aqueous samples.[19] In this work, 50 mL of the E1 sample was extracted (liquid-liquid extraction) in three stages using 10, 10, and 5 mL of DCM to maximize the recovery. The reproducibility of triplicate extractions was very high with overall recovery

of $92 \pm 2\%$. The calibration curve (5 points calibration: 10, 5, 2.5, 1.125 and 0.625 mg/L) was generated by using extracted E1 dissolved in DCM to eliminate the effect of loss due to extraction. Since the system is well mixed, the effect of concentration due to volume reduction by sampling was minimized. In addition, the increase in distance of the solution from the solar beam due to volume reduction by sampling is about 3 cm, subsequently the incident intensity at the top of water solution drops by only 3%. Therefore, the effect of sampling on experimental results is insignificant.

After the final stage of extraction, sodium sulphate was added to remove any remaining water from the sample, which was subsequently transferred to a round bottom flask through a funnel containing additional sodium sulphate to remove any remaining water. The water free sample in 25 mL DCM was then concentrated to 2 mL using a rotary turbo-evaporator (Heidolph, Germany) operating at 50 rpm and a heating bath set at 45^0C. Then 100 µl of the derivatizing reagent *N,O*-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) was added to the sample extract in a 2 mL GC vial, which was capped and sealed with a PTFE lid. The samples were then allowed to react one hour at 60^0C in a water bath before injecting into the GC-MS. After derivatization the sample was ready for injection into the GC-MS for analysis.

Analysis was performed using a gas chromatograph (GC-2010, Shimadzu) coupled with a quadrupole mass spectrometer (GCMS-QP2010S, Shimadzu) equipped with an auto-injector (AOC-5000, Shimadzu). Chromatographic separation was achieved using a BPX5 (5% phenyl polysilphenylene-siloxane) type capillary column of 30 m x 0.25 mm

i.d. (film thickness of 0.25 μm) obtained from SGE (Austin Texas, USA). Helium was used as the carrier gas at a constant flow rate of 1.54 mL/min. The injector temperature was maintained at 320^0C, and the injection volume was 1 μl in splitless mode. The column head pressure was set at 90 kPa. The GC oven temperature was programmed as: 2 min at 50 ^{0}C, first ramp at 20 ^{0}C/min to 100 ^{0}C (held for 5 min), second ramp 10 ^{0}C/min to 200 ^{0}C and third ramp at 20 ^{0}C/min to 300 ^{0}C (held for 14 min). The mass spectrometer was operated in selected ion monitoring (SIM) mode with positive ionization by electron impact (EI). The interface temperature between the inlet and MS transfer line was maintained at 320^0C, and the ion source temperature was also maintained at 230^0C. The SIM ions (m/z) for E1 were 347, 218 and 257 with GC retention time of 25.156 min.

Total organic carbon (TOC) was measured on selected samples by means of a Shimadzu TOC-V$_{CPN}$ analyzer with an ANSI-V auto sampler. TOC of the initial and oxidized samples was determined with the TOC analyzer using operating conditions of 150 mL/min gas flow rate and 300 kPa of gas pressure based on the oxidation of the sample in a combustion chamber heated at 680°C using platinum as a catalyst with detection of carbon by IR-CO_2 analysis (calibration curve was generated using reagent grade potassium hydrogen phthalate).

3.3 Results and Discussion

3.3.1   Solar photodegradation kinetics of E1 in aqueous solution

To confirm that all degradation occurred by photodegradation, a 10 hour control experiment was carried out in the dark by covering the reactor with aluminum foil at an E1 concentration of 5 mg/L at pH 6.0. There was no evidence of E1 degradation at ambient conditions in the absence of solar light. Thereafter, the kinetic experiments were carried out with an initial E1 concentration of 5 mg/L (18.50 μmol/L) at 1 SUN solar irradiation at solution pH 6.0 under normal dissolved air concentration, aerated and nitrogen purged conditions. The results show that the E1 concentration decayed exponentially with time. As shown in Figure 3.4, a plot of time t against $\ln(\frac{C}{C_0})$ follows a pseudo-first-order kinetics model with respect to E1 concentration in water:

$$\ln(\frac{C}{C_0}) = -kt \tag{3.1}$$

where C_0 and C are the concentrations of E1 at time zero and reaction time t in min., k is the pseudo-first-order degradation rate constant (min^{-1}) and the half life of E1 was determined by:

$$t_{1/2} = \frac{\ln 2}{k} \tag{3.2}$$
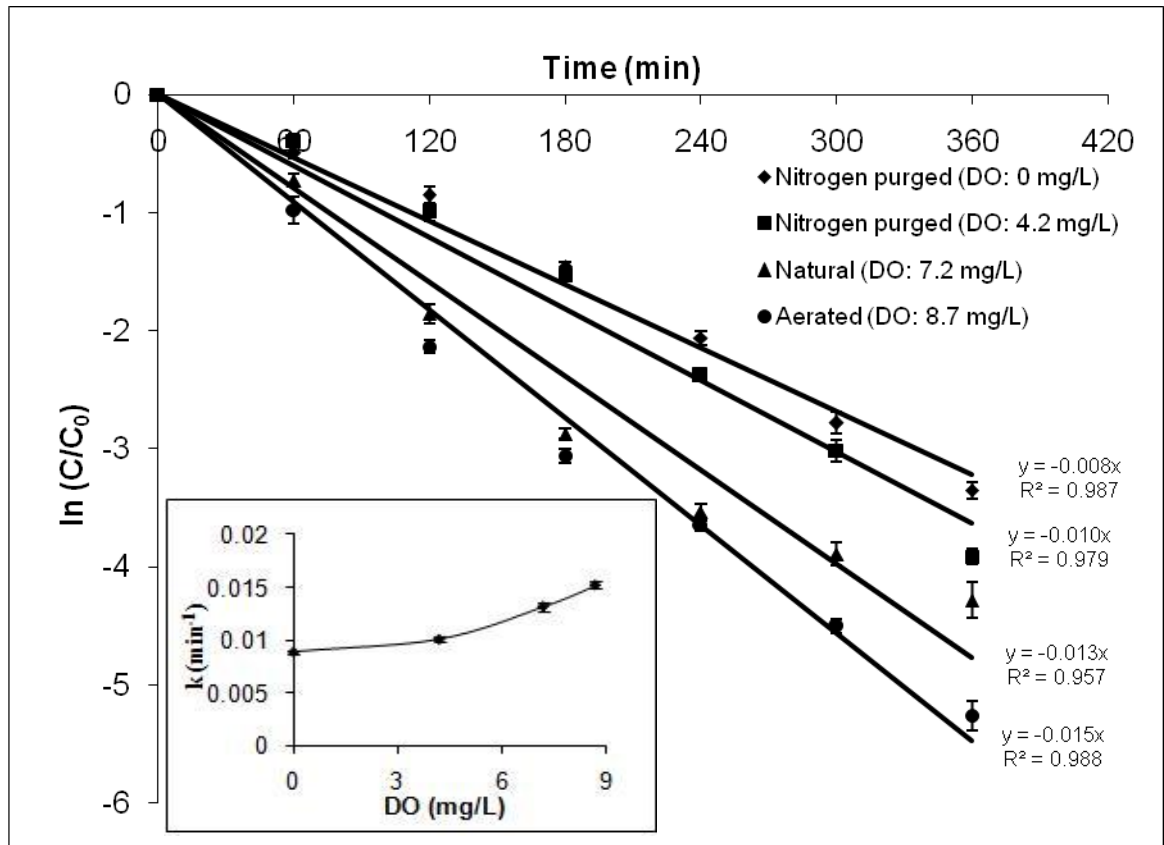
Figure 3.4 Solar photodegradation of E1 and pseudo-first-order rate constant k at

normal, nitrogen purged and aerated conditions.

C_0 (E1) = 5 mg/L, pH = 6.0, solar intensity= 1 SUN and irradiation time = 6 hours

Under solar radiation in the range of 290-700 nm, E1 degraded rather quickly. The value of rate constant k for E1 under 1 SUN irradiation in the natural condition is 0.0132 $\pm$ 0.0004 min^{-1}. Similar pseudo-first-order kinetics for estrogenic compounds in artificial UV-light were also reported by other researchers.[18,20]

Solar degradation of steroid hormones may occur by either direct or indirect photolysis. Direct photolysis occurs due to the absorbance of photons of certain energy

by the substrate, and depends on both the rate of light absorption and the reaction quantum yield of the excited state. For indirect photolysis, the reaction occurs due to free radical formation from the photosensitizers such as natural organic substances.[21] The photodegradation of E1 in natural sunlight occurs mostly due to direct solar photolysis. In order to determine the extent of photolysis, control experiments were conducted by purging air from the reactor using nitrogen. It can be seen in Figure 3.4 that degradation of E1 decreased in the presence of nitrogen (DO: 0 mg/L and 4.2 mg/L). Comparing the degradation in the presence of nitrogen with that of in the presence of natural dissolved oxygen (k =0.0132 ± 0.0004 min^{-1}@ DO: 7.2 mg/L), it can be inferred that about 67% degradation (k =0.0089 ± 0.0002 min^{-1}@ DO: 0 mg/L) occurred due to direct photolysis. Although, the absorption maxima of E1 is at 280 nm, E1 has an extended light absorption band within 290-350 nm (see Figure 2); i.e. UV-B and UV-A region, which is present in natural solar irradiation (UV-A: 6.3% and UV-B:1.5%).[22] The direct photolysis of E1 occurs in the region where the solar spectrum overlaps with the E1 light absorption band. The degradation rate constant increased by ≈15% in the presence of additional air (DO: 8.7 mg/L) due to photooxidation as shown below:

$$E1 + h\upsilon \xrightarrow{\;O_2\;} Photoproducts \qquad\qquad (3.3)$$

Although reactive oxygenated species (ROS) such as $\cdot OH$, $^1O_2$, $HO_2\cdot/O_2^{\cdot-}$ are only formed in the presence of nitrate ions, humic substances and Fe (III)-oxalate complexes, etc., the presence of molecular oxygen promotes further oxidation of the products by the primary photochemical process. This is further shown by the extent of mineralization

determined by measuring TOC at various conditions, which indicates that only about 17

± 0.9% TOC degraded in 6 hours in the presence of nitrogen (DO: 0 mg/L) as opposed to

27 ± 1.1%, and 30 ± 1.2% TOC removal in the presence of normal dissolved oxygen (DO:

7.2 mg/L) and additional aeration (DO: 8.7 mg/L), respectively.   As anticipated, the

presence of oxygen thus helps the degradation of both E1 and its intermediates.

Experiments were also conducted at several initial E1 concentrations of 3.70, 7.40,

11.10 and 14.80 µmol/L to determine the effect of concentration on the degradation rate.

As shown in Table 3.1, the solar photodegradation of E1 in aqueous solution followed 1st

order kinetics for all initial concentrations tested, although the rate constant decreased

slightly (about 10% reduction in rate-constant for five-fold increase in concentration)

with increasing E1 initial concentration. This is a common trend for photochemical

degradation of organic compounds, where the photolysis rate can be decreased due to

photon limitations occurring at higher initial concentrations of the organics.[20] The

transmittance of E1 solution was measured at 290 nm and it varied from 98% for 3.7

µmol/L (1 mg/L) to 87% for 18.5 µmol/L (5 mg/L) to E1 solution; a drop of 11% in

transmittance corresponds well with the 8% drop in reduction in rate constant of E1.

From the above, the molar absorption coefficient of E1 at pH 6.0 and 290 nm was

measured as 6216 ± 1146 cm^{-1} M^{-1}. This value corresponds well with the literature value

of 9260 cm^{-1} M^{-1} at alkaline pH of 11.5.[23]

Table 3.1 Pseudo-first-order rate constant, k and half-life for solar photodegradation at different E1 initial concentration (intensity 1 SUN, 100 mWcm^{-2})

E1 Concentration (μmol L^{-1})	k (min^{-1})	Half-life (min)
3.70	0.0144 ± 0.0003	48.13
7.40	0.0140 ± 0.0002	49.50
11.10	0.0137 ± 0.0004	50.58
14.80	0.0134 ± 0.0003	51.72
18.50	0.0132 ± 0.0004	52.50

3.3.2   Effect of solar intensity on photodegradation of E1

Solar intensity is an important parameter to determine the photodegradation of organic compounds, because the photon generation rate changes with different solar intensities. To determine the effect of solar intensity on photodegradation of E1, experiments were conducted at four different solar intensities of 1 SUN (100 mWcm^{-2}), ¾ SUN (75 mWcm^{-2}), ½ SUN (50 mWcm^{-2}) and ¼ SUN (25 mWcm^{-2}) simulated by adjusting the power output of the xenon arc lamp, and using the same experimental conditions discussed above. As shown in Table 3.2, the solar photodegradation of E1 follows pseudo-first-order kinetics for all solar intensities, and the rate constant decreases with decreasing light intensity. The results are also shown in Figure 3.5 where the rate constant k is plotted versus solar intensity. It is apparent that the degradation rate constant is linear with solar intensity in the range tested, and follows the equation:

$$k = 0.0001I \qquad\qquad\qquad\qquad (3.4)$$

where I is the solar intensity in $mWcm^{-2}$.

Table 3.2 Pseudo-first-order rate constant, k and half-life for solar photodegradation at different solar intensities of E1

Solar Intensity	k (min^{-1})	Half-life (min)
1 SUN (100 mWcm^{-2})	0.0132 ± 0.0004	52.50
¾ SUN (75 mWcm^{-2})	0.0108 ± 0.0002	64.09
½ SUN (50 mWcm^{-2})	0.0076 ± 0.0003	91.45
¼ SUN (25 mWcm^{-2})	0.0056 ± 0.0002	122.75
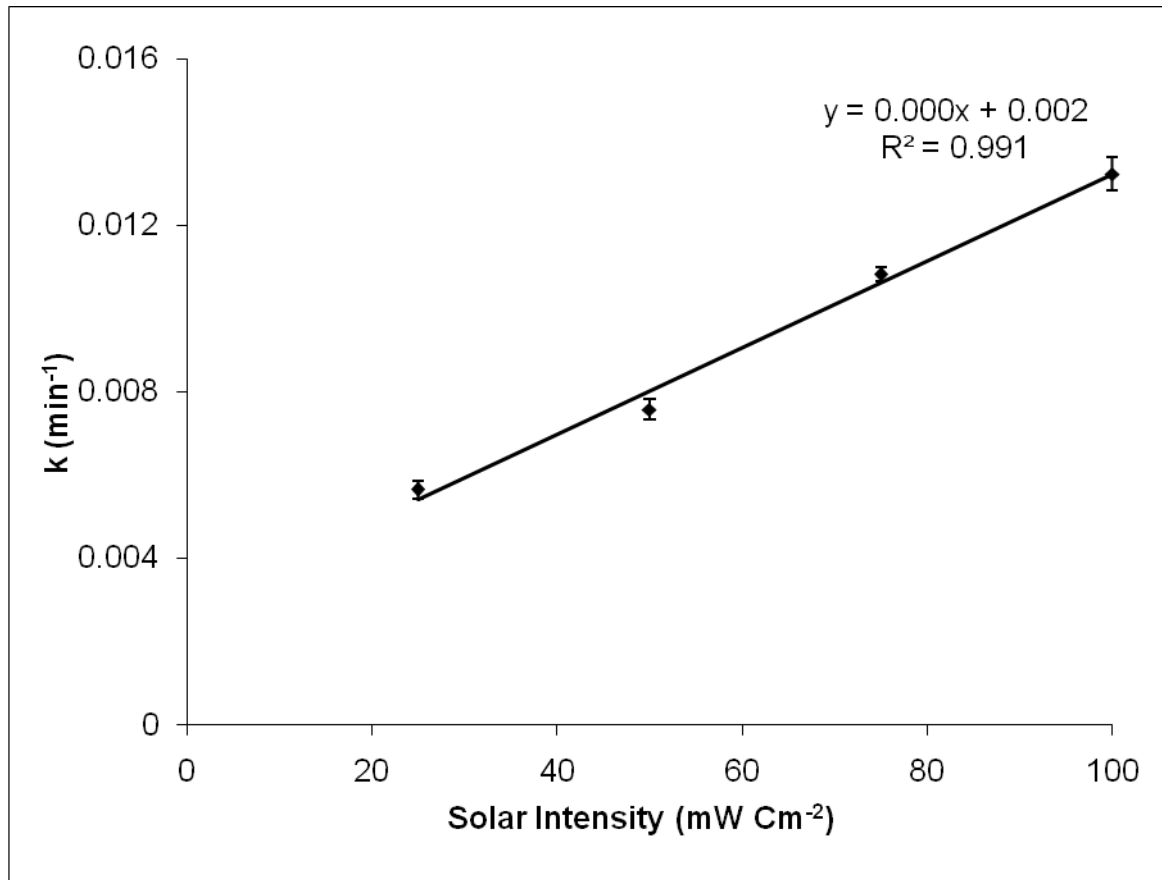
Figure 3.5 Effect of solar intensity on photodegradation of E1.

C_0 (E1) = 5 mg/L, pH = 6.0 and irradiation time = 6 hours

The enhanced solar photodegradation rate at higher intensity is obviously due to the availability of a greater amount of photons at the higher solar intensity. However, the change in rate of degradation is not directly proportional to the intensity, e.g., for a 4 times increase in intensity causes about 2.35 times increase in the rate of degradation of E1. Since the quantum yield of the complex molecules in water is fairly independent of wavelength and seldom exceeds one, the dependence of the rate of photolysis on intensity is solely due to absorption of photons.[24] However, the absorption of photons in these

experiments should remain constant at all intensities as no other chromophore besides E1 was used. The relatively lower dependence of photodegradation on intensity is due to photooxidation in the presence of oxygen, as it was seen earlier that the photolysis corresponds to only 67% of the total degradation in the presence of naturally dissolved air/oxygen.

3.3.3   Mineralization of E1 in solar photodegradation

In order to determine the extent of mineralization of E1, TOC values of the solution were monitored during solar photodegradation. Due to the variation of solar light intensity during the day and over the year, the TOC removal of E1 solution was evaluated as a function of four different solar intensities (1 SUN, ¾ SUN, ½ SUN and ¼ SUN) with initial concentration of E1 = 5 mg/L (initial TOC = 4 mg/L) and solution pH 6.0 for 6 hours irradiation time. The results indicate that TOC removal increases with time as well as solar intensity as shown in Figure 3.6.

Although both mineralization and the degradation efficiency of E1 decreased with decreasing irradiation intensity as shown in Figure 3.7, the mineralization efficiency was always significantly lower than the degradation of E1 itself. Even at the maximum solar intensity of 1 SUN, mineralization efficiency was only $27 \pm 1.1\%$, whereas the degradation of E1 was $98.6 \pm 1\%$ after 6 hours. The significant difference between degradation and mineralization efficiency implied that the intermediates of E1 degrade slower than E1.
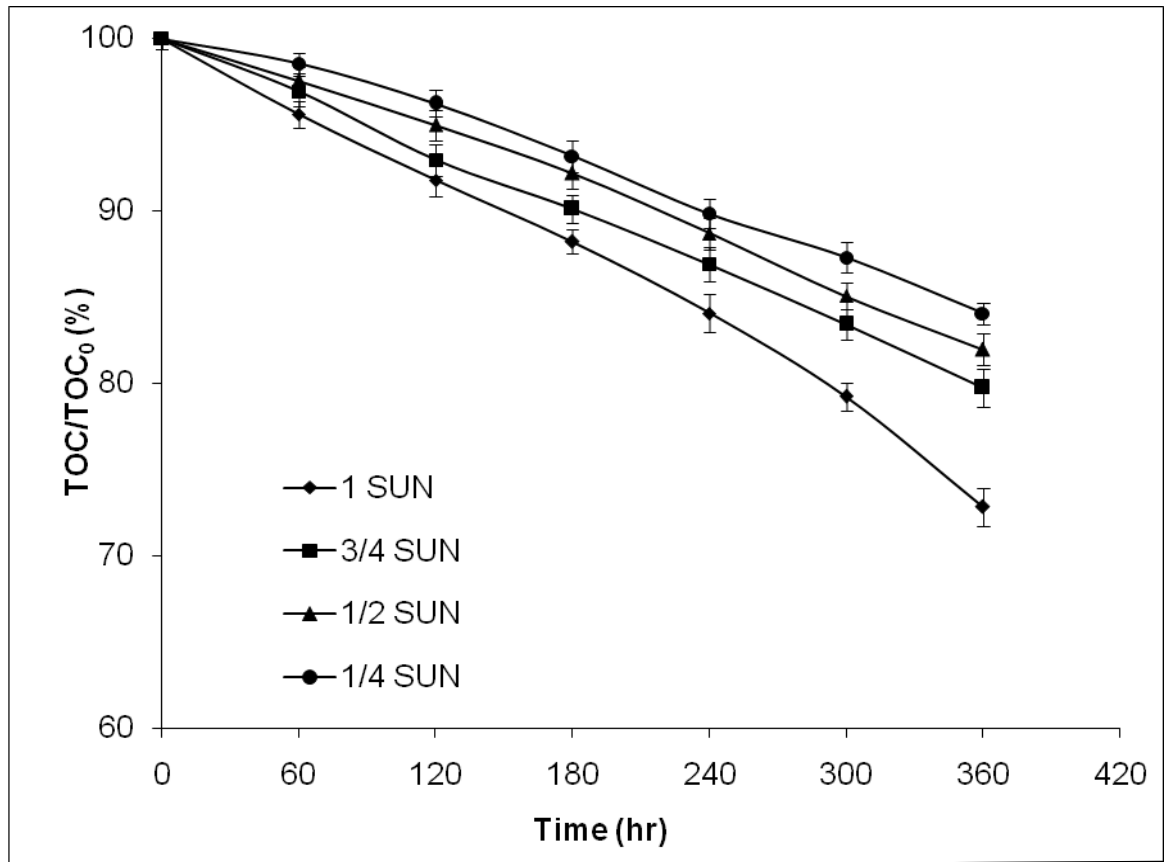
Figure 3.6 Removal of TOC at different solar intensities.

C_0 (E1) = 5 mg/L, initial TOC = 4 mg/L and pH = 6.0
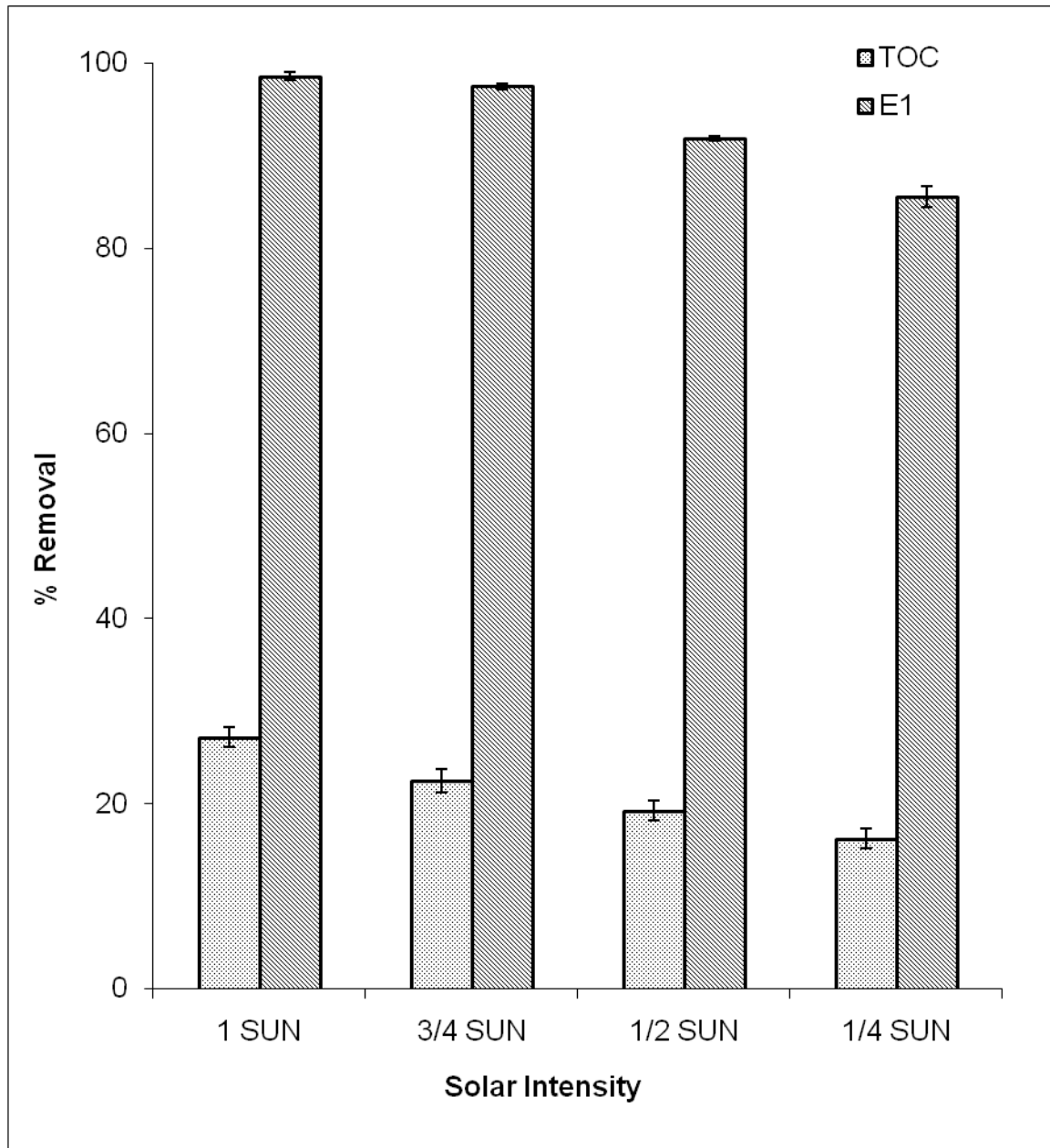
Figure 3.7 Comparison between mineralization and degradation efficiencies of E1 at

different solar intensities.

C_0 (E1) = 5 mg/L, pH = 6.0, initial TOC = 4 mg/L and irradiation time = 6 hours

Several unidentified peaks in the GC-MS chromatogram indicated the increasing concentration of intermediates with time, reaching a plateau and then declining with time, corresponding to consecutive reactions as shown in Figures 3.8a and 3.8b. The most abundant intermediate shown in Figure 3.8a is identified as benzeneacetic acid/phenylacetic acid (m/Z: 472, 355 and 73). The maximum concentration of the intermediate and the time to reach the maximum concentration are both dependent on the solar intensity (Figure 3.8b). Figure 3.8b also indicates that phenylacetic acid breaks down following 1st order kinetics.
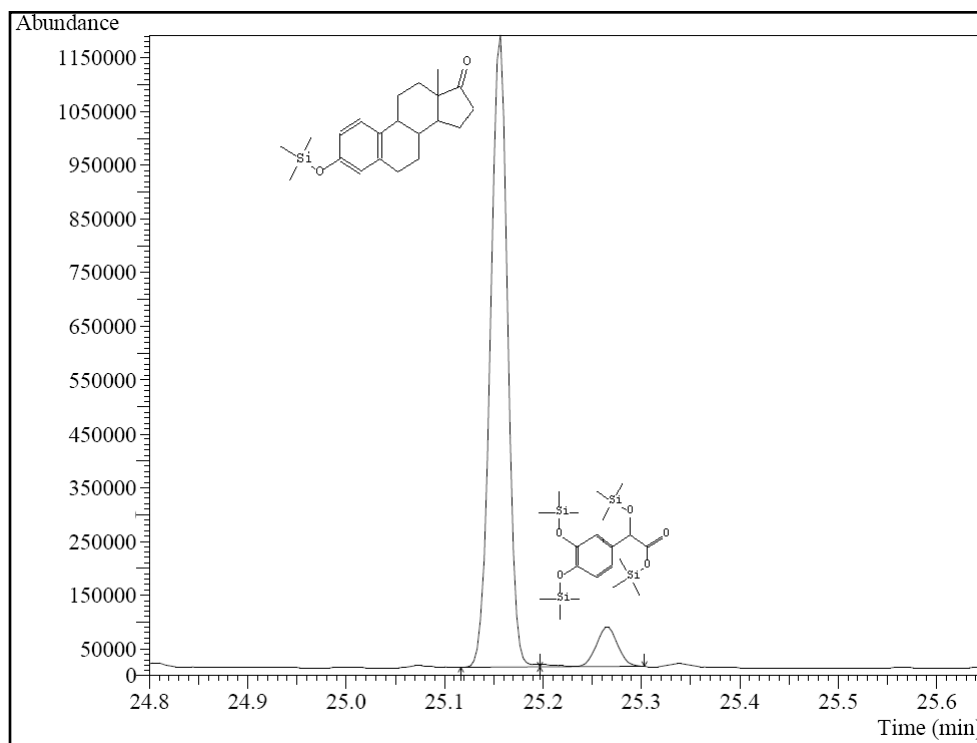
Figure 3.8a Chromatogram of E1 and its photoproduct after

3 hrs of solar irradiation at 1 SUN.

C_0 (E1) = 5 mg/L, pH = 6.0, solar intensity= 1 SUN, irradiation time = 3 hours
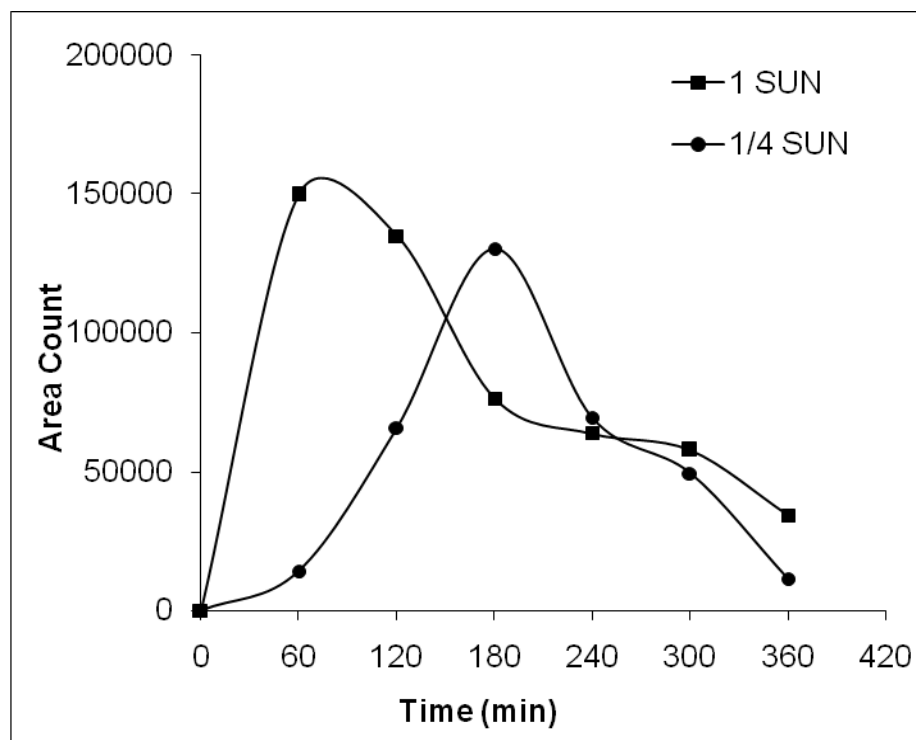
Figure 3.8b Evolution of intermediate with time for different solar intensities.

C_0 (E1) = 5 mg/L, pH = 6.0, solar intensity= 1 SUN and ¼ SUN, irradiation time = 6

hours

3.3.4   Effect of humic acid concentration on solar photodegradation of E1

Sunlight transmittance through water is primarily regulated by the color, concentration and chemical structure of dissolved organic matter (DOM). Humic substances are the largest fraction of DOM in natural water and are categorized as humic acids, fulvic acids, and humin depending on their solubility. Humic substances contain benzene, carboxyl groups, and carbonyl-type chromophores.[25,26] The chromophoric humic substances absorb solar radiation to reach an excited state, hence generate free radicals that indirectly cause photodegradation of organic compounds. These free radicals

include reactive oxygen species, hydroxyl radicals, super oxide anions etc.[27] Although the humic acid fraction in humic substances varies depending on the origin, they are good chromophores in the visible range and can be effective photosensitizers, and are widely studied in natural environments.[28,29]

Hence, experiments were carried out for several humic acid concentrations in the range of 0-10 mg/L at 30 minutes solar irradiation and an intensity of 1 Sun, while the E1 initial concentration was maintained at 5 mg/L. The percentage removal of E1 with humic acid concentration after 30 minutes of solar irradiation is shown in Figure 3.9. The results show that enhanced degradation of E1 occurs with increasing humic acid concentration up to 8 mg/L, due to both direct and indirect solar photolysis as per reaction (3.3) for direct photolysis and reactions (3.5) – (3.9) for indirect photolysis.

$$HS \xrightarrow{\ h\upsilon\ } HS^{\bullet-} \tag{3.5}$$

$$HS^{\bullet-} + O_2 \longrightarrow Oxidized - HS + O_2^{\bullet-} \tag{3.6}$$

$$2O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2 \tag{3.7}$$

$$H_2O_2 \xrightarrow{\ h\upsilon\ } 2OH^{\bullet} \tag{3.8}$$

$$E1 + OH^{\bullet} \longrightarrow Photooducts \tag{3.9}$$
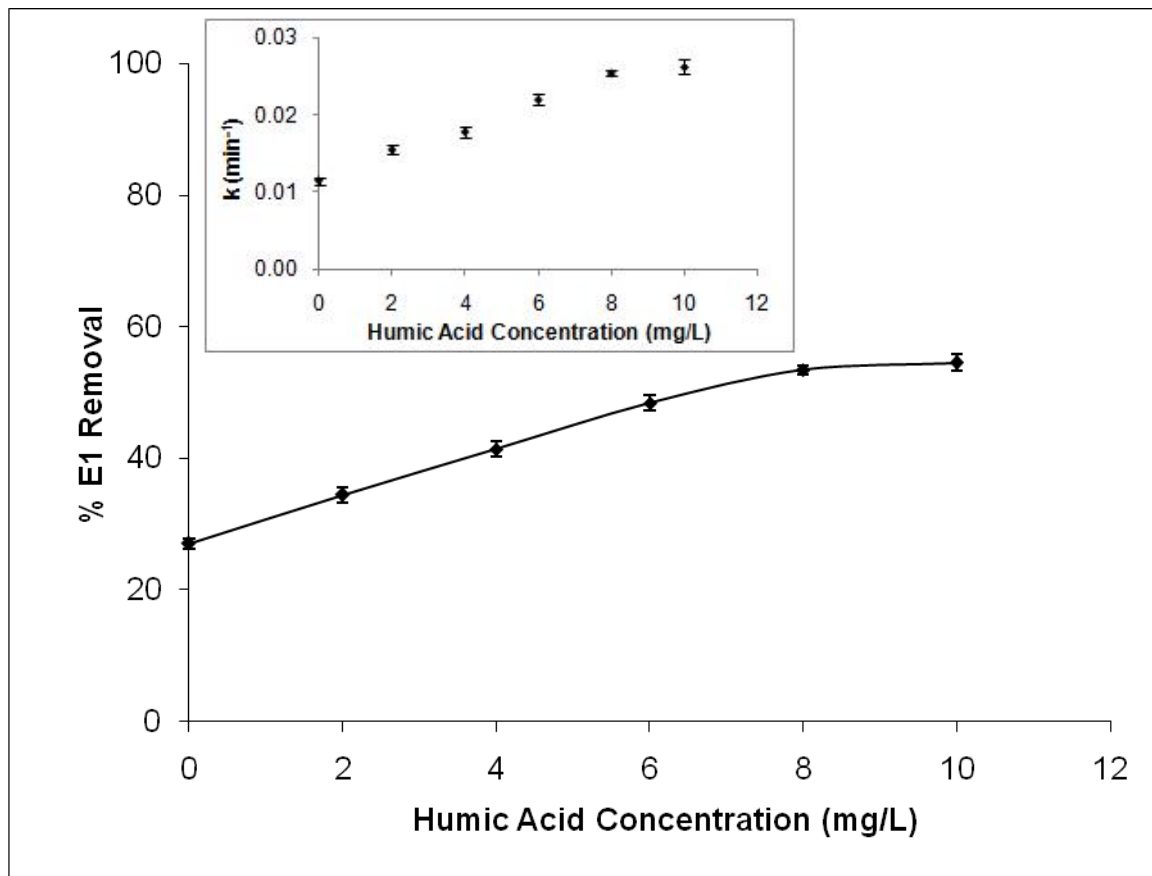
Figure 3.9 Effect of humic acid concentration on solar photodegradation of E1.

$C_0(E1) = 5$ mg/L, solar intensity= 1 SUN and irradiation time = 30 minutes

At higher concentrations of humic acid, the degradation efficiency was reduced due to the scavenging of reactive oxygen species.[29]   Dark adsorption studies with humic acid at various concentrations showed insignificant adsorption of E1 on humic acid.   In addition, both E1 and humic acid concentration in the solution was low to detect any significant changes due to adsorption. The transmittance of 10 mg/L humic acid solution is 69% at 290 nm indicating good absorption of light by humic acid. The degradation of E1 followed zero-order kinetics with respect to E1 concentration in the presence of humic

acid, in comparison to the pseudo-first order kinetics described earlier without humic acid. This indicates the presence of photosensitized reactions induced by humic acid, and the rate was doubled in the presence of humic acid. However, it should be noted that in the presence of carbonate alkalinity, hydroxyl radicals are scavenged causing a lower rate of photodegradation; thus the rate of E1 degradation in the presence of humic acid presented in this work is higher than what it would be encountered in the natural environment. Further studies are required to determine the intermediates and mechanism of E1 degradation due to photosensitization and photolysis in natural water in the presence of other ions and dissolved organic matter.

3.3.5   Effect of initial pH on solar photodegradation of E1

The solution pH is an important parameter that affects the solar photodegradation of organic compounds in natural aquatic environments. In this work, the solution pH was varied in a range relevant to the natural environment, with any extreme pH changes avoided. As humic acid is a very weak acid, the solution pH does not change significantly due to its addition; pH only varies from 6.7 for 2 mg/L to 6.2 for 10 mg/L of humic acid in water, whereas the natural pH of E1 in Milli-Q water is around 6. The experiments were carried out at several pH values in the range of 3.0-9.0 using a solar intensity of 1 SUN and E1 initial concentration of 5 mg/L. The results provided in Figure 3.10 show an optimum pH range of 6.0-7.0 giving a maximum degradation rate. At lower pH values, the degradation efficiency decreased sharply. Since, these experiments were conducted in the absence of anions such as NO_3^- or dissolved organic matter, which are known to produce hydroxyl radicals in water in the presence of sunlight, the effect of pH seen in

this work cannot be related directly to the hydroxyl radical. The acid dissociation constant for E1 is $\approx$10.4,[23] therefore E1 remains protonated in the test pH range, although some dissociation would occur at higher pH. At pH's above the pKa, the phenol group on E1 structure would form phenoxide ions, facilitating faster degradation than the un-dissociated E1. Similar trends were reported for the degradation of 17α-ethynylestradiol (EE2) in an engineered system[30] as well as solar degradation of phenol and chlorophenol where the photolysis rate of phenols was much lower at a pH below the pKa due to lower rate of photolysis of the nonionized form relative to the phenoxide ion.[31] The drop in photolysis rate at acidic pH is mainly due to the lower molar absorbance as the molar absorbance of E1 is decreased from 9.26 x10^3 cm^{-1} M^{-1} at pH 12.3 to 1.85 x10^3 cm^{-1} M^{-1} at pH 3.6.[23] Since the experiments with different pH were conducted in deionized water, there was no other oxidants present other than molecular oxygen in the reaction media. The rate constant reaches a plateau around pH 8, which indicates the complexity of subsequent photooxidation processes of primary photoproducts in different pH, and cannot be established without a detailed mechanism study. Although the effect of alkalinity was not tested in this work, based on the experimental results at higher pH, it is expected that higher photolysis would possibly occur at higher alkalinity. It should also be noted that in this work the effect of pH was evaluated on direct photolysis only, and the effect of pH on phootoxidation due to the hydroxyl radical was not tested.

Figure 3.10 Effect of pH on solar photodegradation of E1.

C_0 (E1) = 5 mg/L, solar intensity= 1 SUN and irradiation time = 1 hour

3.4 Conclusions

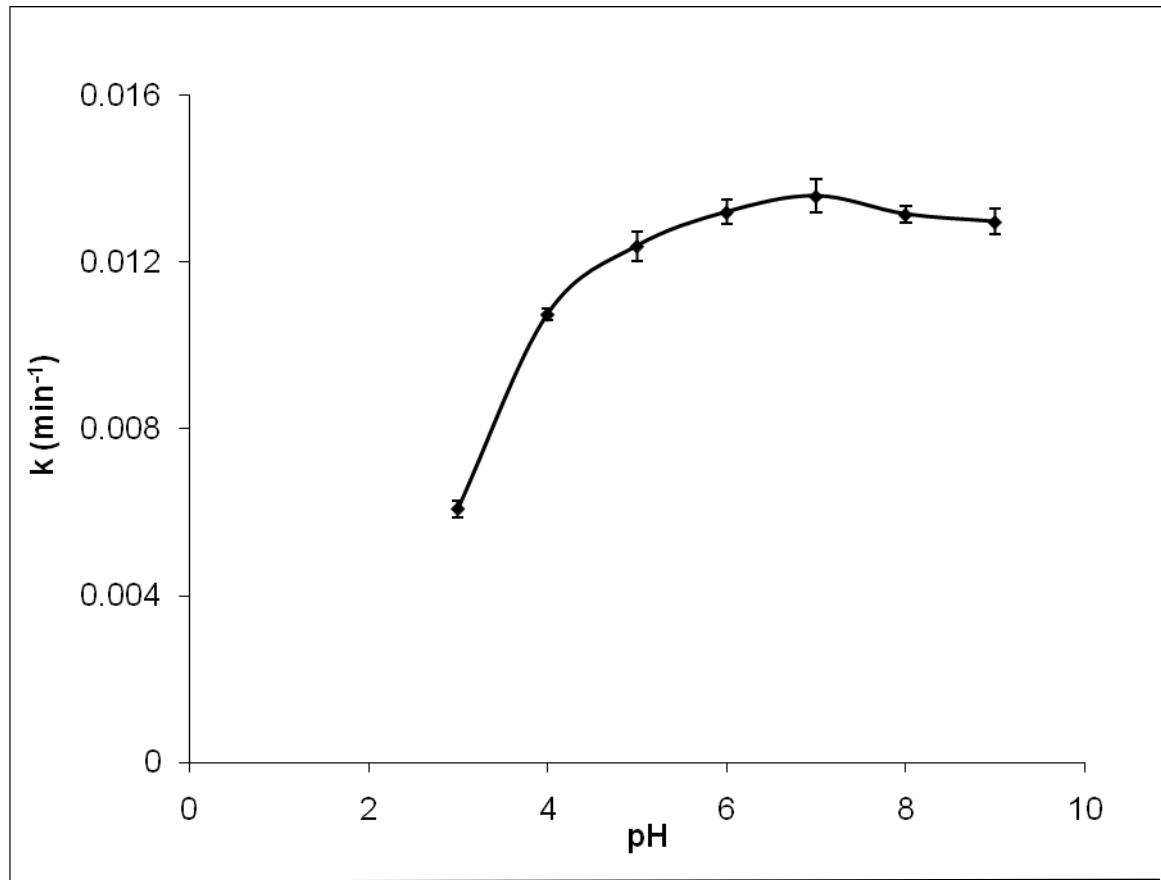Photodegradation of E1 was studied in aqueous solution under natural solar irradiation using a solar simulator. E1 degraded rapidly in simulated solar light due to both direct photolysis and oxidation. The half life of E1 varied from 48-123 minutes depending on the solar intensity and concentration. The effects of several parameters such as initial concentration, solar intensity, pH and the effect of humic substances on

phtodegradation of E1 were studied. The degradation rate increased for increasing humic acid concentration up to 8 mg/L due to photosensitization, and maximum degradation occurred at neutral pH. In water with high carbonate alkalinity, the rate of degradation would be lower than what was found in this work due to the scavenging of hydroxyl radical, however, about 50% of E1 degrades due to direct photolysis and subsequent photooxidation in the presence of molecular oxygen, somewhat reducing the effect of alkalinity. The major intermediates detected in E1 photodegradation were benzeneacetic acid/phenylacetic acid, which also photodegraded with time; and TOC analysis indicates a steady degradation of TOC indicating a gradual mineralization of E1.

3.5 References

1. Zuo, Y.; Zhang, K.; Deng, Y. Occurrence and Photochemical Degradation of 17α-Ethinylestradiol in Acushnet River Estuary. *Chemosphere*. **2006**, *63*, 1583.

2. Zhang, K.; Zuo, Y. Pitfalls and Solution for Simultaneous Determination of Estrone and 17α-Ethinylestradiol by Gas Chromatography–Mass Spectrometry after Derivatization with N,*O* -bis (trimethylsilyl)trifluoroacetamide. *Anal. Chim. Acta.* **2005**, *554*, 190.

3. Wicks, C.; Kelley, C.; Peterson, E. Estrogen in a Karstic Aquifer. *Ground Water*. **2004**, *42*, 384.

4. Liu, X. L.; Wu, F.; Deng, N. S. Photodegradation of 17α-Ethynylestradiol in Aqueous Solution Exposed to a High-pressure Mercury Lamp (250 W). *Environ. Pollut*. **2003**, *126*, 393.

5. Desbrow, C.; Routledge, E.J.; Brighty, G.C.; Sumpter, J.P.; Waldock, M.J. Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical Fractionation and in Vitro Biological Screening. *Environ. Sci. Technol*. **1998**, *32*, 1549.

6. Baronti, C.; Curini, R.; D'Ascenzo, G.; Di Corcia, A.; Gentili, A.; Samperi, R. Monitoring Natural and Synthetic Estrogens at Activated Treatment Plants and in Receiving River Water. *Environ. Sci. Technol*. **2000**, *34*, 5059.

7. Nasu, M.; Goto, M.; Kato, H.; Oshima, Y.; Tanaka, H. Study on Endocrine Disrupting Chemicals in Wastewater Treatment Plants. *Water Sci. Technol*. **2001**, *43*, 101.

8. Snyder, S. A.; Keith T. L.; Verbrugge, D. A.; Snyder, E. M.; Gross, T. S.;, Kannan, K.; Giesy, J. P. Analytical Methods for Detection of Selected Estrogenic Compounds in Aqueous Mixtures. *Environ. Sci. Technol*. **1999**, *33*, 2814.

9. Kolpin, D.W.; Furlong, E.T.; Meyer, M.T.; Thurman, E.M.; Zaugg, S.D.; Barber, L.B.; Buxton, H.T. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in US Streams, 1999–2000: A National Reconnaissance. *Environ. Sci. Technol*. **2002**, *36*, 1202.

10. Ying, G. G.; Kookana, R. S.; Ru, Y. Occurrence and Fate of Hormone Steroids in the Environment. *Environ. Int*. **2002**, *28*, 545.

11. Casey, F. X. M; Larsen, G. L.; Hakk, H.; Simunek, J. Fate and Transport of 17β-Estradiol in Soil–Water Systems. *Environ. Sci. Technol*. **2003**, *37*, 2400.

12. Hanselman, T. A.; Graetz, D. A.; Wilkie, A. C. Manure-Borne Estrogens as Potential Environmental Contaminants: A review. *Environ. Sci. Technol.* **2003**, *37*, 5471.

13. Ying, G. G.; Kookana, R. S. Degradation of Five Selected Endocrine-Disrupting Chemicals in Seawater and Marine Sediment. *Environ. Sci. Technol*. **2003**, *37*, 1256.

14. Purdom, C. E.; Hardiman, P. A.; Bye, V. J.; Eno, N. C.; Tyler, C. R.; Sumpter, J. P. Estrogenic Effects of Effluents from Sewage Treatment Works. *Chem. Ecol*. **1994**, *8*, 275.

15. Brion, F.; Tyler, C. R.; Palazzi, X.; Laillet, B.; Porcher, J. M; Garric, J.; Flammarion, P. Impacts of 17β-Estradiol, Including Environmentally Relevant Concentrations, on Reproduction after Exposure during Embryo-Larval-, Juvenile- and Adult-Life Stages in Zebrafish (Danio Rerio). *Aquat. Toxicol*. **2004**, *68*, 193.

16. Pelley, J. Estrogen Knocks Out Fish in Whole-Lake Experiment. *Environ. Sci. Technol*. **2003**, *37*, 313A.

17. Lin, Y.; Peng, Z.; Zhang; X. Ozonation of Estrone, Estradiol, Diethylstilbestrol in Waters. *Desalination*. **2009**, *249*, 235.

18. Coleman, H. M.; Routledge, E. J.; Sumpter, J. P.; Eggins, B. R.; Byrne, J. A. Rapid Loss of Estrogenicity of Steroid Estrogens by UVA Photolysis and Photocatalysis over an Immobilised Titanium Dioxide Catalyst. *Water Res*. **2004**, *38*, 3233.

19. Moeder, M.; Schrader, S.; Winkler, M.; Popp, P. Solid-Phase Microextraction–Gas Chromatography–Mass Spectrometry of Biologically Active Substances in Water Samples. *J. Chromatogr. A*. **2000**, *873*, 95.

20. Liu, B.; Liu, X. Direct Photolysis of Estrogens in Aqueous Solutions. *Sci. Total Environ*. **2004**, *320*, 269.

21. Schwarzenbach, R. P.; Gschwend, P. M; Imboden, D. M. *Environmental Organic Chemistry*, 2nd ed.; John Wiley & Sons: New Jersey, 2003.

22. Gibson, J. H. UVB Radiation: Definition and Characteristics. http://uvb.nrel.colostate.edu/UVB/publications/uvb_primer.pdf (accessed Feb 21, 2010).

23. Hurwitz, A. R.; Liu, S.T. Determination of Aqueous Solubility and pKa Values of Estrogens. *J. Pharm. Sci*. **2006**, *66*, 524.

24. Zepp, R. G; Cline, D. M. Rates of Direct Photolysis in Aquatic Environment. *Env. Sci. and Technol.* **1977**, *11*, 359.

25. Zhan, M.; Yang, X; Yang, H.; Kong, L. Effect of Natural Aquatic Humic Substances on the Photodegradation of Bisphenol A. *Front. Environ. Sci. Eng. Chin*. **2007**, *1*, 311.

26. Chen, J.; Gu, B.; LeBoeuf E. J.; Pan, H.; Dai, S. Spectroscopic Characterization of Structural and Functional Properties of Natural Organic Matter Fractions. *Chemosphere*. **2002**, *48*, 59.

27. Hoigné, J.; Faust, B. C.; Haag, W. R.; Zepp, R. G. *Aquatic Humic Substances*; American Chemical Society: Washington DC, 1989.

28. Wetzel, R. G. Solar Radiation as an Ecosystem Modulator. In *UV Effects in Aquatic Organisms and Ecosystems*; Helbling, E. W., Zagarese, H., Eds.; Royal Society of Chemistry: Cambridge, 2003.

29. Ma, J.; Graham, N. J. D. Degradation of Atrazine by Manganese-Catalysed Ozonation: Influence of Humic Substances. *Water Res*. **1999**, *33*, 785.

30. Liu, B.; Wu, F. S.; Deng, N. S. UV-Light Induced Photodegradation of 17α-Ethynylestradiol in Aqueous Solutions. *J. Hazard. Mater*. **2003**, *98*, 311.

31. Hwang, H. M.; Hodson, R. E.; Lee, R. F. Photolysis of Phenol and Chlorophenols in Estuarine Water. In *Photochemistry of Environmental Aquatic Systems*; Zika, R. G., Cooper, R. G., Eds.; ACS Symposium Series 327; American Chemical Society: Washington, DC, 1987; pp 27-43.

Photodegradation of 17β-Estradiol in Natural Aquatic Environment under Solar Irradiation

4.1 Introduction

The ubiquitous presence of emerging contaminants (ECs), which include a diverse collection of chemical substances, in aquatic environments is a major worldwide concern. Among the ECs, special importance is given to endocrine disrupting compounds (EDCs), as they can interfere with the normal function of hormones by interacting with the endocrine system presenting a potential threat to aquatic life and human health.[1,2] Besides industrial chemicals such as bisphenol-A, DDT, atrazine, methoxychlor, chlordecone, alkylphenols, PCBs and phthalic esters, some natural steroid estrogens such as estrone (E1), 17β-estradiol (E2), estriol (E3) and mestranol (MeEE2) and synthetic pharmaceuticals such as diethylstilbestrol (DES), ibuprofen, norfloxacin and 17α-ethynylestradiol (EE2) are found to be the most potent EDCs.[1,2-4]

Among the EDCs, natural estrogens are thought to be most likely to cause estrogenic effects on aquatic life due to their very potent estrogenic activity, even at very low concentrations; 17β-estradiol is the most potent natural estrogens among estrone and estriol.[4] Estrogenic steroids are detected in the influent and effluent of sewage treatment plants in different countries at various concentrations.[2] These steroid hormones make

their way into the aquatic environment through sewage discharge and animal waste disposal due to both human and animal excretions. The concentration of E2 was found to be 44 ng/g on average in dry poultry waste.[1,2] These steroids have also been detected at elevated levels in soil, ground water as well as surface water adjacent to agricultural fields fertilized with animal manure.[5]

Although the concentrations of the steroid hormones in natural aquatic environments are in the low ng/L range (10-1830 ng/L), it is very important to understand the fate of these EDCs in aquatic environments. This helps to determine the environmental impact and potential threat to aquatic life due to their extremely high biological potency and procreation toxicity.[6,7] The degradation time of these organic pollutants in the environment may vary from a few hours to months depending on various environmental parameters.[8] Among the different environmental degradation processes (abiotic/biotic), photodegradation from solar irradiation is one of the most important factors for determining the ultimate fate of the persistent pollutants in aquatic environments.[9]

Solar phototransformation or degradation of organics in aquatic environment may occur from direct or indirect photolysis. Direct photolysis is the result of light absorbance by the pollutants. As all steroid estrogens have considerable sunlight absorbance in the ultraviolet and blue spectral region (290-360 nm),[10] it is considered that direct solar photolysis might play an important role for the photodegradation of steroid estrogens in aquatic environments.[11] In indirect photolysis, NO_3^-, Fe^{3+} and humic substances play very

important roles, which are ubiquitous in surface water and absorb solar radiation to reach an excited state and subsequently generate free radicals comprised of reactive oxygen species (ROS) (e.g., hydroxyl radicals ($OH^•$), peroxyl radicals ($ROO^•$), singlet oxygen ($^1O_2$), etc.) and other non-ROS transients.[12] Among these reactive photochemically generated species in surface waters in the presence of solar irradiation, $OH^•$ plays a very important role in the phototransformation of organic pollutants because of its very high oxidizing potential. In addition, reaction between most organics and $OH^•$ occurs with rate constants that are essentially diffusion controlled.[13] The major sources of $OH^•$ in natural water have been identified as NO_3^-, Fe^{3+}, and humic substance, while HCO_3^- plays a negative role due to its scavenging effect on $OH^•$ in surface water.[14] Another important water parameter is turbidity, because it controls light attenuation through the water.

Steroid hormones are known to degrade rapidly in the presence of high intensity UV-C (254 nm), and many degradation studies of these hormones are available in the literature using advanced oxidation processes (AOP) such as semiconductor photocatalysis, UV/H_2O_2, UV/O_3, O_3/H_2O_2 etc.[15,16] Although photodegradation of steroid hormones has been studied in engineered systems, comprehensive studies documenting their fate in environmental conditions in the presence of sunlight are still limited. Earlier, Chowdhury et al.[11] reported the solar degradation of Estrone (E1) in water, however, environmental photodegardation of 17β-estradiol (E2), the most potent of natural estrogens has never been studied. The objective of this study is to determine the kinetics of photodegradation of steroid E2 (See Figure 4.1) in aquatic environment due to direct solar irradiation (i.e. UV-B, UV-A, and visible radiation, 290-700 nm) using a solar

simulator with controlled doses of sun light under different environmental conditions including solar intensity, initial concentration of E2, pH, natural photosensitizers (dissolved uncharacterized organic matter or the humic substances, Fe^{3+} and NO_3^-), and other water constituents (HCO_3^- and turbidity). The extent of mineralization of E2 under various various conditions was also evaluated. In addition, wherever possible, a comparative analysis of the photodegradation of E1 and E2 is provided.
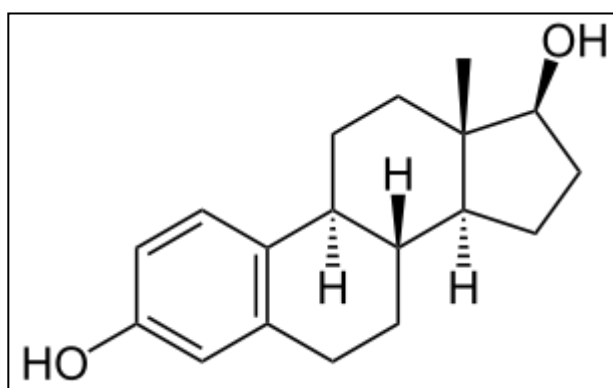
Figure 4.1 Structure of 17β-estradiol (E2).

4.2 Experimental

4.2.1 Chemicals

E2 (MW: $C_{18}H_{24}O_2$, CAS registry number: 50-28-2) was obtained from Sigma-Aldrich (Oakville, Ontario, Canada) and used without further purification. Organic solvent acetonitrile (AcN) for HPLC analysis was of HPLC grade and purchased from Fisher Scientific (Ottawa, Ontario, Canada). Humic acid (Technical grade, CAS registry number: 1415-93-6) was also obtained from Sigma-Aldrich (Oakville, Ontario, Canada).

AMCO clear turbidity standard, 1000 NTU was purchased from Fisher Scientific (Ottawa, Ontario, Canada). All other reagents used for solutions were reagent grade and used without further purification. Laboratory grade water (LGW, 18 MΩ) was prepared from an in-house Millipore purification system (Billerica, MA).

4.2.2   Standard and sample preparation

Stock solutions (5 ± 0.05 mg/L) of E2 (solubility: 13 mg/L @ 20 ^{0}C) were prepared by dissolving an appropriate amount of E2 in Milli-Q water in a volumetric flask by stirring for 2 hours to ensure complete dissolution. The working water samples were prepared by spiking the stock solution in Milli-Q water to obtain the desired initial concentration. The stock and working solutions were wrapped with aluminum foil and stored at 4 ^{0}C to prevent any degradation. The natural pH of Milli-Q water is 6.5, which is also the pH of the E2 solution. All experiments were conducted at pH 6.5 except for evaluating the effect of pH and HCO_3^- on the degradation of E2, where NaOH or HCl were used to adjust the pH.

4.2.3   Photodegradation experiment

Photodegradation experiments were carried out using a solar simulator (Model: SS1KW, Sciencetech, ON, Canada) with a 1000 watt xenon arc lamp. An air mass filter (AM filter) AM1.5G was installed in the radiation beam to produce identical simulated 1 SUN irradiance of 100 mWcm^{-2} at full power that matches the global solar spectrum (Class A standards as per JIS-C-8912 & ASTM 927-05) at sea level and zenith angle 37 degree (Refer to Appendix A). The light absorption spectra of E2 was measured and

shown in Figure 4.2. In spite of λ_{max}=278 nm, E2 exhibits slight absorption in the 300-350 nm wavelength region, which can induce photolysis of E2. Hence, photon flux from the solar simulator was calculated in the 300-400 nm range, which was 5.3 x 10^{-5} Einstein $m^{-2} s^{-1}$ at 1 SUN irradiation.
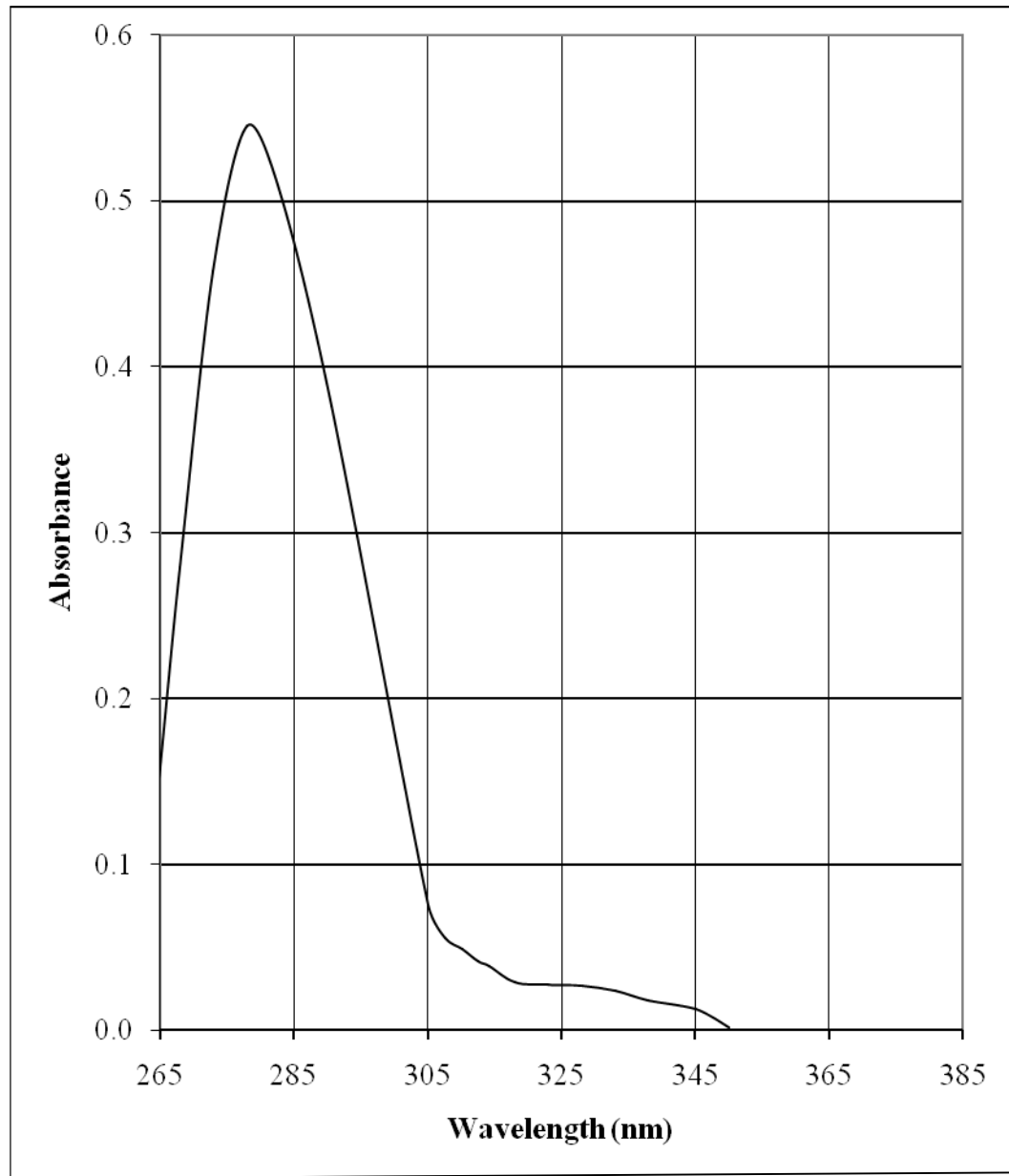
Figure 4.2 Absorption spectrum of E2 over 265-385 nm at pH 6.5

An open water-jacketed vertical glass vessel (Length: 11cm x Diameter: 9 cm) was used as the solar photo-reactor, which was placed on a magnetic stirrer during all experiments, under aerated conditions @ 350 rpm and a temperature of 22 ± 2 ^{0}C. The aqueous solution was irradiated directly from the top using a vertical solar beam of 8 inch (20.32 cm) diameter from the solar simulator. In all experiments, the total irradiated solution volume was 300 mL. The irradiation intensity was measured at the top surface of the experimental solution by a Broadband Thermopile Detector (Model: UP19K-15W, Sciencetech, ON, Canada), which allows measurement of the radiation emitted by a light source between 190 nm-11 μm (UV-VIS-IR) and the power density on a surface (in mWcm^{-2}). The schematic of the experimental setup is shown in Figure 4.3. The experiments were performed using different solar intensities, initial concentrations of E2, dissolved oxygen and in presence of different water constituents, such as pH, NO_3^-, Fe^{3+}, HCO_3^-, humic acid and turbidity.
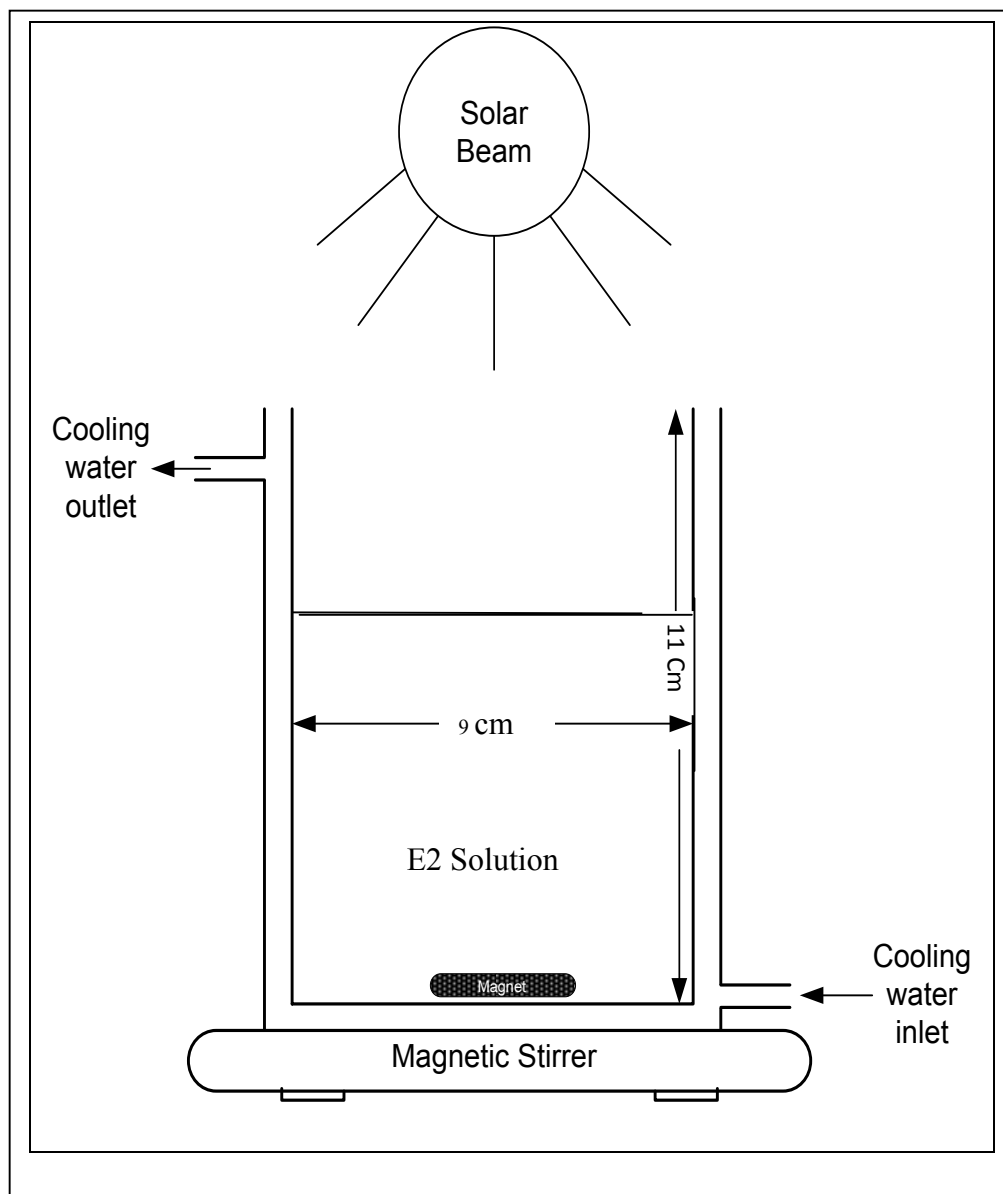
Figure 4.3 Schematic of the experimental setup.

In each experiment, a maximum of 5 irradiated samples (2 mL each) were withdrawn from the photo-reactor at different irradiation times for the kinetic study. Hence, the volume variation due to sampling was negligible. All experiments were conducted in triplicate with average error around 5% and the results presented in the

following Figures and Tables which are the average of three experiments with reported

standard deviations or error bars.

4.2.4   Analytical methods

4.2.4.1 HPLC analysis

The E2 concentration was measured by HPLC (ICS 300, Dionex), which included a

DP pump, an AS auto sampler, a DC column oven and PDA UV detector, connected to

Chromeleon software. Separations were carried out with an Acclaim 120 C_{18} reversed-

phase column (150 mm × 4.6 mm i.d., 5 μm particle size, Dionex, USA). The injection

volume was 40 μL from 2 mL HPLC vial, capped and sealed with PTFE lid. The mobile

phase was a mixture of AcN and Milli-Q water (50:50 v/v) at a flow rate of 1 mL/min by

the HPLC pump.   The column temperature was maintained at $30^{0}C$ and detection

wavelength was set at 280 nm, the maximum absorbance of E2. The retention time of E2

was 5.04 min shown in the HPLC output of Figure 4.4.
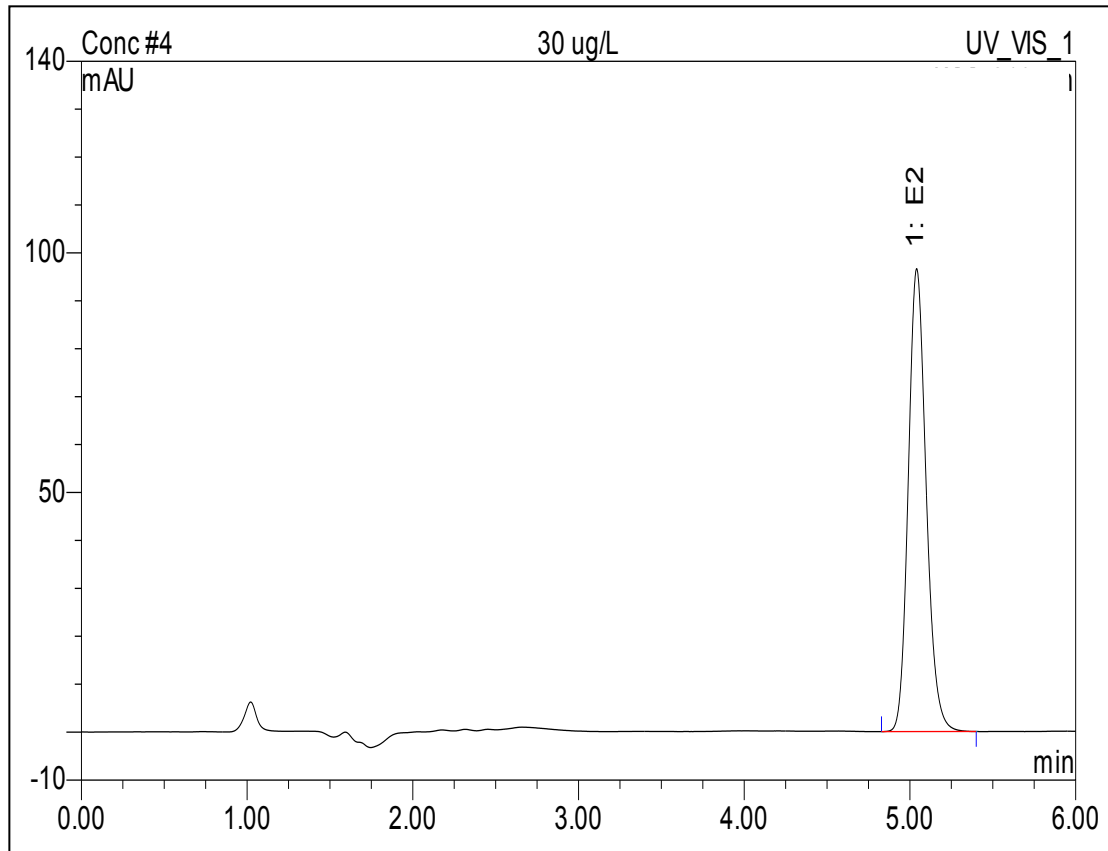
Figure 4.4 HPLC Chromatogram of E2.

4.2.4.2 Other analyses

The UV-Vis spectra and absorbance at wavelength 290 nm of the experimental samples were recorded using a UV-Vis spectrophotometer (UV-3600, Shimadzu) in a 1 cm path length quartz cuvette. Total organic carbon (TOC) was measured on selected samples by means of a Shimadzu TOC-V$_{CPN}$ analyzer with an ANSI-V auto sampler, and the pH and turbidity were measured using a Beckman Coulter pH meter (model number: pHi 460) and a Micro 100 Laboratory Turbidimeter, respectively.

4.3 Results and Discussion

4.3.1 Kinetics of solar photolysis of E2 in aqueous solution

Before the kinetics study, a 10 hours control experiment was carried out in the dark by covering the reactor with aluminum foil at an E2 concentration of 5 µg/L at pH 6.5 to determine the extent of dark reaction. There was no evidence of E2 degradation at ambient conditions in the absence of solar light. Thereafter, the kinetic experiments were carried out with an initial E2 concentration of 5 µg/L at 1 SUN solar irradiation, pH 6.5, and using normal dissolved air, nitrogen purged and aerated conditions. The results show that the E2 concentration decayed exponentially with time. All experiments produced linear plots of t against $\ln(\frac{C_{E2}}{C_{0_{E2}}})$ as shown in Figure 4.5, indicating the photodegradation of E2 in aqueous solution under solar irradiation followed pseudo-first-order kinetics. The pseudo-first-order degradation rate constant and half-life of E2 can be calculated as per equations 4.1 and 4.2:

$$\ln(\frac{C_{E2}}{C_{0_{E2}}}) = -kt \tag{4.1}$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{4.2}$$

where, $C_{0_{E2}}$ and C_{E2} are the concentrations of E2 at time zero and reaction time t in hr., k is the pseudo-first-order degradation rate constant (hr^{-1}) and $t_{1/2}$ is the half-life.
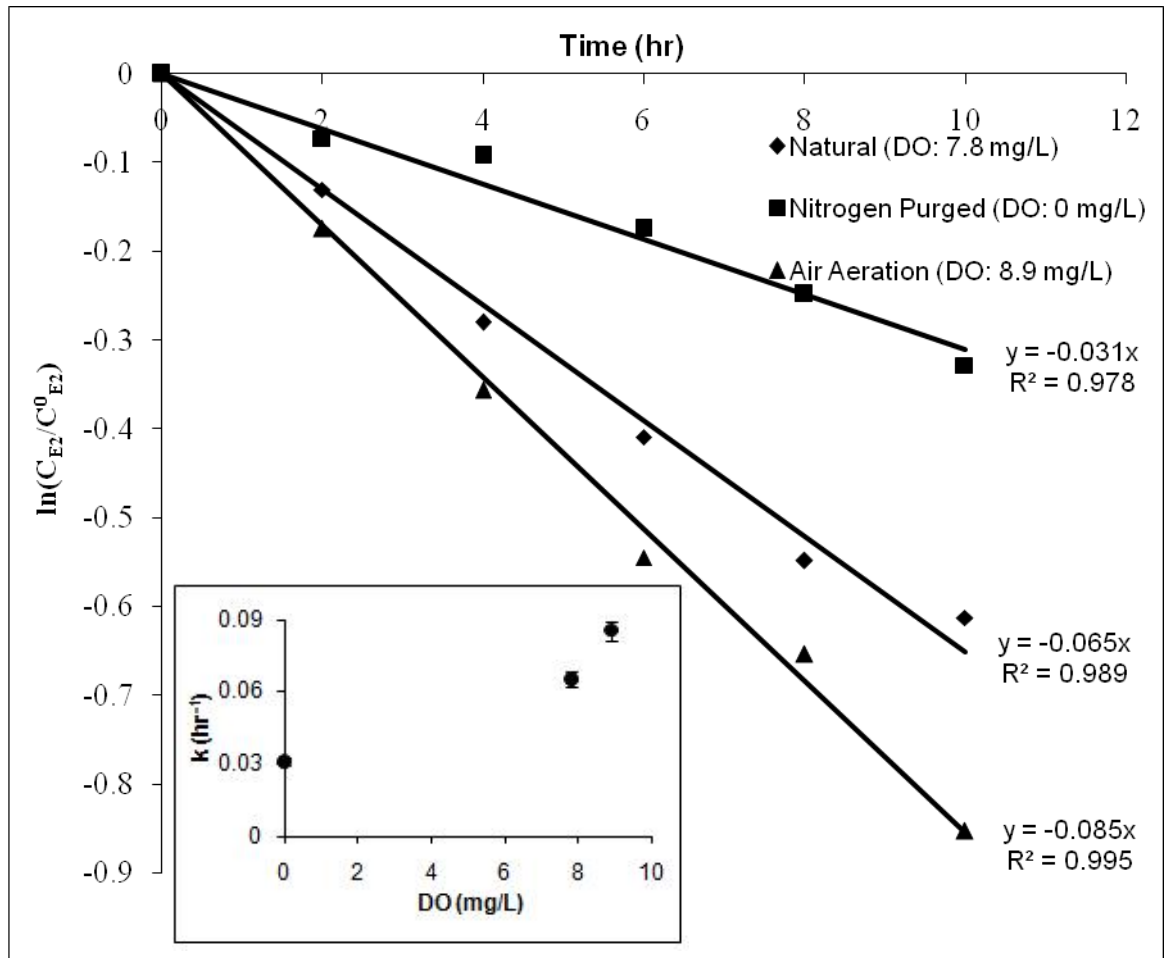
Figure 4.5 Solar photodegradation of E2 and pseudo-first order rate constant k at

normal, nitrogen purged and aerated conditions.

$C_{0_{E2}}$ = 5 μg/L, pH = 6.5, solar intensity= 1 SUN and irradiation time = 10 hours

E2 degraded due to direct photolysis under solar irradiation in the range of 290-700 nm in the absence of free radicals, which are generally produced in the presence of photosensitizers. The value of the pseudo-first-order rate constant k of E2 under 1 SUN irradiation in natural conditions was 0.0652 ± 0.0033 hr^{-1}. Although, the absorption maxima of E2 is at 278 nm, E2 has extended light absorption band within 290-340 nm

(see Figure 4.2); i.e. UV-B and UV-A region, which is present in natural solar irradiation (UV-A: 6.3% and UV-B:1.5%).[17]

The direct photolysis of E2 occurs in the region where the solar spectrum overlaps with the E2 light absorption band. In order to determine the extent of direct photolysis of E2, control experiments were conducted by purging air from the reactor using nitrogen. It can be seen in Figure 4.5 that the degradation of E2 is substantially decreased in the absence of air (DO: 0 mg/L for nitrogen purged condition). Comparing the degradation in the presence of nitrogen ($k = 0.0311 \pm 0.0016$ hr^{-1}@ DO: 0 mg/L) with that of in the presence of naturally dissolved oxygen ($k = 0.0652 \pm 0.0033$ min^{-1}@ DO: 7.8 mg/L), it can be inferred that about 48% degradation of E2 occurred due to direct photolysis.

The degradation rate constant increased by ≈32% in the presence of additional air ($k = 0.0855 \pm 0.0040$ hr^{-1}@ DO: 8.9 mg/L) than that in naturally dissolved air due to photooxidation as shown below:

$$E2 + h\upsilon \xrightarrow{\quad O_2 \quad} Photoproducts \qquad\qquad (4.3)$$

The extent of mineralization was also determined by measuring the total organic carbon (TOC) at various experimental conditions, which indicates that only about $8 \pm 0.3\%$ TOC degraded in 10 hours in the presence of nitrogen (DO: 0 mg/L) as opposed to $13 \pm 0.6\%$, and $15 \pm 0.7\%$ TOC removal in the presence of naturally dissolved oxygen (DO: 7.8 mg/L) and additional aeration (DO: 8.9 mg/L), respectively. As anticipated, the

presence of oxygen helps the degradation of both E2 and its intermediates. Mineralization of estrone (E1) under similar operating conditions was found to be much higher than E2 indicating that the primary photochemical process of photon absorption is important for total degradation of estrogenic compounds.[11] This is also reflected by the much higher photolysis rate of E1 (0.534 ± 0.012 hr^{-1}), which is about 6.5 times higher than E2. The half-life of E2 at 1 sun intensity is $\approx$ 10 hours, whereas it is only about 50 minutes for E1; accordingly about 67% degradation of E1 occurred due to photolysis as opposed to 48% degradation by photolysis of E2.[11]

Quantum yield (Φ) is an important parameter to measure the efficiency of photodegradation and is defined as the number of moles of reactant transformed divided by the number of moles of photons absorbed by the reactant, in this case by E2:

$$\Phi \quad = \quad \frac{\text{Number of molecules reacted (or produced)}}{\text{Number of photons of light absorbed}} \qquad (4.4)$$

Under polychromatic irradiation in dilute aqueous solution, the rate of disappearance of an absorbing compound E2 is given by following equation 4.5:[10]

$$-\frac{dC_{E2}}{dt} = \sum \Phi_\lambda I_{0,\lambda}(1 - 10^{\varepsilon\lambda C_{E2}z}) \qquad (4.5)$$

where Φ_λ is the quantum yield (in mol Einstein[-1]), ε_λ (M[-1] cm^{-1}) the molar absorption coefficient, $I_{0,\lambda}$ (Einstein L[-1] s^{-1}) the photon flux at the wavelength λ, z (cm) and C_{E2} (M) are the reactor optical path length and the concentration of the compound E2,

respectively. When the concentration of the absorbing compound is low enough (this is usually the case in natural waters), equation 4.5 can be simplified by integrating to equation 4.6:

$$\sum \Phi_\lambda = \frac{k}{2.303 \times I_{0,\lambda} \times \varepsilon_\lambda \times z} \qquad (4.6)$$

where k (s^{-1}) is the pseudo-first-order rate constant. The average quantum yield can be calculated by integrating the 1 SUN solar intensity over the wavelength range of 300-400 nm, which gave the value of 0.0069 mol Einstein^{-1} for E2 in Milli-Q water, which is comparable to the quantum yield of E2 (Φ= 0.0048 mol Einstein^{-1}) earlier determined by Lin et al.;[18] the difference in quantum yield can be attributed to the wavelength of light used and other experimental conditions used in the work of Lin et al.[18] The quantum yield of E1 is 0.0356 mol Einstein^{-1}, which is 5 times higher than E2, attributed to its higher absorbance in the UV-A and UV- B regions of solar light (ε_{290}(E2)= 1010 M^{-1} cm^{-1} and

ε_{290}(E1)= 2186 M^{-1} cm^{-1}).

4.3.2   Effect of initial concentration on photodegradation of E2

As E2 has been detected at various concentrations in natural surface water in recent year, it is important to investigate the effect of E2 concentration on its photolysis in solar irradiation. Experiments were carried out at different initial E2 concentrations of 5, 10, 20, 30, 40 and 50 µg/L to investigate the effect of concentration on the degradation rate. As shown in Table 4.1, solar photodegradation of E2 in aqueous solution decreased only

slightly (about 10% reduction in rate-constant for ten-fold increase in concentration) with increasing E2 initial concentration. This is a common trend for photochemical degradation of organic compounds, where the photolysis rate can be decreased due to photon limitations occurring at higher initial concentrations of the organics.[19] However, due to the small absorbance of E2, the small range of concentration tested, and the presence of sufficient solar photon flux, the effect of concentration of E2 is minimal.

Table 4.1 Pseudo-first-order rate constant, k and half-life for solar photodegradation at different E2 initial concentrations (intensity 1 SUN, 100 mWcm^{-2})

E2 Concentration (µg/L)	k (hr^{-1})	$t_{1/2}$ (hr)	R^2
5	0.0652 ± 0.0033	10.63	0.9896
10	0.0633 ± 0.0024	10.94	0.9787
20	0.0616 ± 0.0025	11.24	0.9864
30	0.0603 ± 0.0017	11.49	0.9693
40	0.0598 ± 0.0026	11.59	0.9723
50	0.0589 ± 0.0022	11.76	0.9815

4.3.3   Effect of solar intensity on photodegradation of E2

The variation of solar light intensity during the day and over the year is an important parameter to consider when evaluating solar driven processes, because the

photon generation rate changes with different solar intensities. Therefore, experiments were carried out at four different solar intensities of ¼ SUN (25 mWcm^{-2}), ½ SUN (50 mWcm^{-2}), ¾ SUN (75 mWcm^{-2}) and 1 SUN (100 mWcm^{-2}) simulated by adjusting the power output of the xenon arc lamp, and using the same experimental conditions discussed earlier to determine the effect of solar intensity on the photodegradation of E2. As shown in Table 4.2, the solar photodegradation of E2 follows pseudo-first-order kinetics for all solar intensities, and the rate constant increases with increasing light intensity. Figure 4.6 plots the degradation rate constant k versus solar intensity, which shows that k is directly proportional to the square root of solar intensity over the range tested as per the following equation:

$$k = 0.006 \times I^{0.5} \tag{4.7}$$

where I is the solar intensity in mWcm^{-2}. The enhanced solar photodegradation rate at higher intensity is obviously due to higher photon flux. The relatively lower dependence of photodegradation on intensity is due to photooxidation in presence of the oxygen as was seen earlier. The photolysis corresponds to only 48% of the total degradation in the presence of naturally dissolved air/oxygen, and the lower molar absorbance of E2. E1 with higher light absorption and higher photolysis extent (67% as compared to 48% of E2) shows a stronger dependence of the degradation rate on solar intensity.

Table 4.2 Pseudo-first-order rate constant, k and half-life for solar photodegradation at different solar intensities of E2

Solar Intensity	k (hr^{-1})	$t_{1/2}$ (hr)	R^2
¼ SUN (25 mWcm^{-2})	0.0329 ± 0.0016	21.07	0.9829
½ SUN (50 mWcm^{-2})	0.0433 ± 0.0022	16.01	0.9746
¾ SUN (75 mWcm^{-2})	0.0575 ± 0.0026	12.05	0.9909
1 SUN (100 mWcm^{-2})	0.0652 ± 0.0033	10.63	0.9896
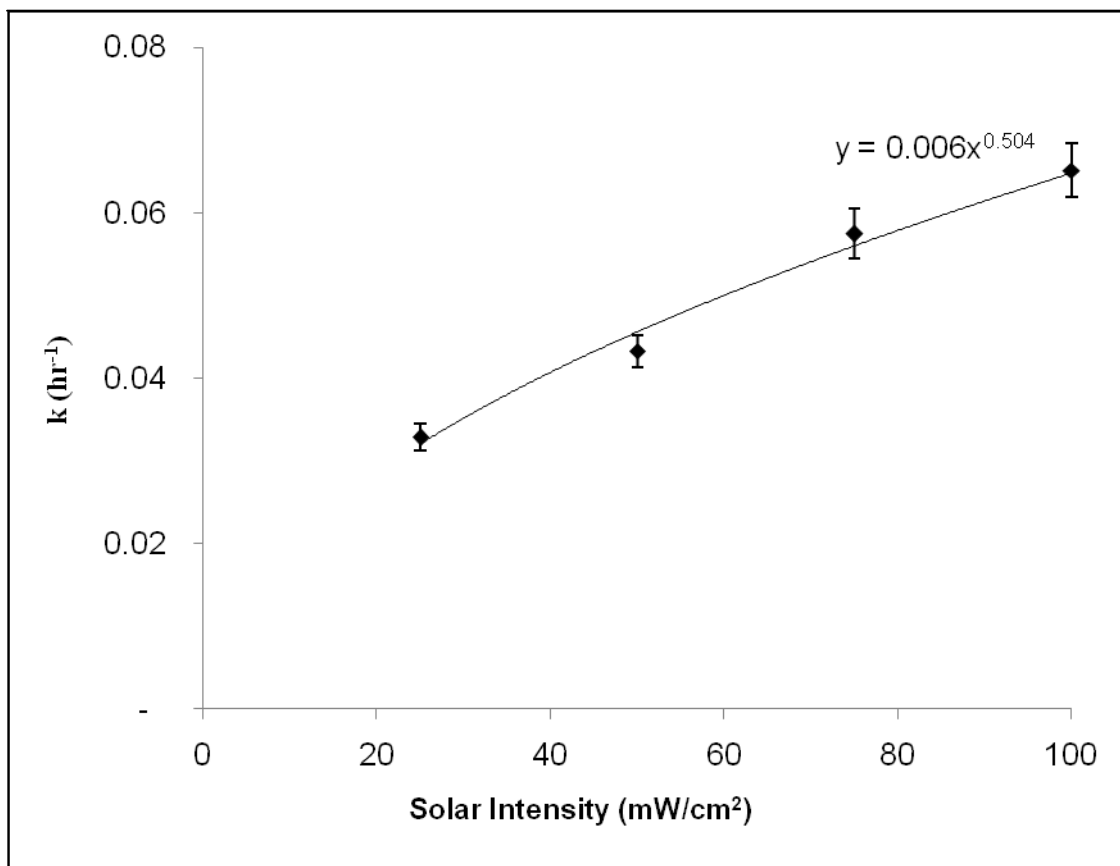
Figure 4.6 Effect of Solar intensity on solar photodegradation rate constant of E2.

$C_{0_{E2}}$ = 5 μg/L, pH = 6.5 and irradiation time = 10 hours

4.3.4 Mineralization

Complete mineralization is an important parameter in the fate of environmental pollutants as the degradation photoproducts or intermediates may exhibit more toxicity than the parent organic pollutants. In order to determine the extent of mineralization of E2, TOC values of the solution were monitored during solar photodegradation. Due to the variation of solar light intensity during the day and over the year, the TOC removal of E2 solution was evaluated as a function of four different solar intensities (¼ SUN, ½ SUN, ¾ SUN and 1 SUN) with initial concentration of E2 = 5 μg/L (initial TOC = 0.004 mg/L)

and solution pH 6.5 for 10 hours of irradiation. The results indicate that TOC removal increases with solar intensity as shown in Figure 4.7. It was found that mineralization of E2 increased with solar intensity as well as the irradiation time. But the mineralization of E2 was always significantly lower than the degradation of E2 itself. Even at the maximum solar intensity of 1 SUN, the extent of mineralization was only $13 \pm 0.6\%$, whereas degradation was $46 \pm 0.23\%$ after 10 hours of irradiation. The significant difference between the rates of degradation and mineralization implies that the photoproducts of E2 oxidation are much slower than E2 photodegradation under the present experimental conditions.[11] It is possible that the aromatic ring of E2 was easily broken due to photolysis, while the alicyclic rings were not destroyed due to the higher stability.
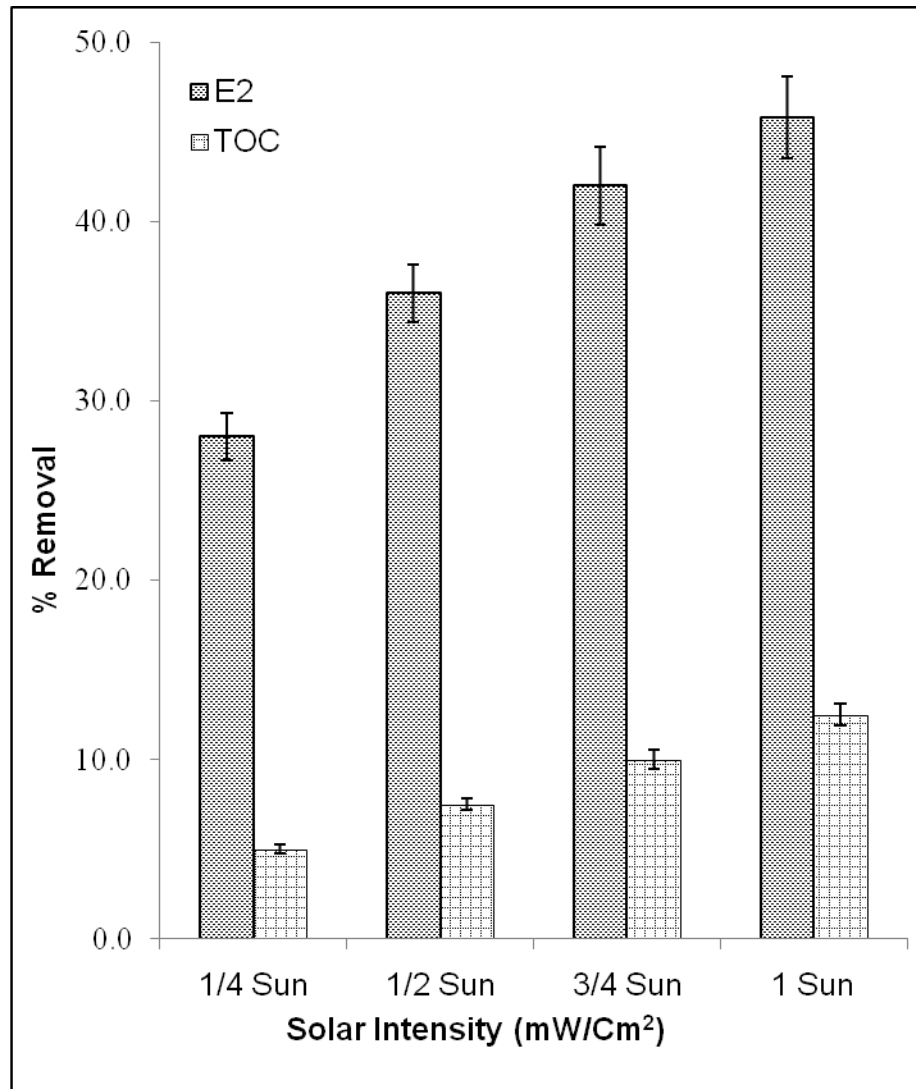
Figure 4.7 Comparison between mineralization and degradation efficiencies of E2 at

different solar intensities.

$C_{0_{E2}}$ = 5 μg/L, pH = 6.5, initial TOC = 0.004 mg/L and irradiation time = 10 hours

4.3.5   Effect of water parameters on solar photodegradation of E2

4.3.5.1 Influence of pH

Water pH is one of the most important parameters influencing the solar photodegradation of organic compounds in natural aquatic environments. To evaluate the effect of pH on photodegradation, the experiments were carried out at several pH values in the range of 3.0-9.0 using a solar intensity of 1 SUN and E2 initial concentration of 5 µg/L. The results are presented in Figure 4.8. In this work, any extreme pH changes were avoided to maintain environmentally relevant conditions. The results show that the photodegradation rate of E2 was significantly dependent on the solution pH; the photodegradation rate constant in the alkaline regime was higher than that in the acidic regime, with maximum degradation occurring around pH 7. Since these experiments were conducted in the absence of anions such as NO_3^-, Fe^{3+} or humic acid, which are known to produce $OH^\bullet$ in surface water in the presence of sunlight, the effect of pH seen in this work cannot be related to $OH^\bullet$. The acid dissociation constant for E2 is ≈ 10.4,[21] therefore E2 remains protonated in the test pH range, although some dissociation would occur at higher pH values. At pH's above the pKa, the phenol group on the E2 structure would form phenoxide ions, facilitating faster degradation than the un-dissociated E2. Similar trends were reported for the degradation of estrone and 17α-ethynylestradiol in engineered systems[11,22] as well as for the solar degradation of phenol and chlorophenol. Here, the photolysis rate of phenols was much lower at a pH's below the pKa due to a lower rate of photolysis of the nonionized form relative to the phenoxide ion.[23] The drop in photolysis rate is due to a lower molar absorbance of light ($\geq$290 nm) with the nonionized E2 than that of ionized E2. The molar absorbance of E2 decreased from 9.07

$x10^3$ cm^{-1} M^{-1} at pH 12.3 to 1.81 $x10^3$ cm^{-1} M^{-1} at pH 3.6, therefore ionized E2 is easier to be excited and degraded than nonionized E2.[21]
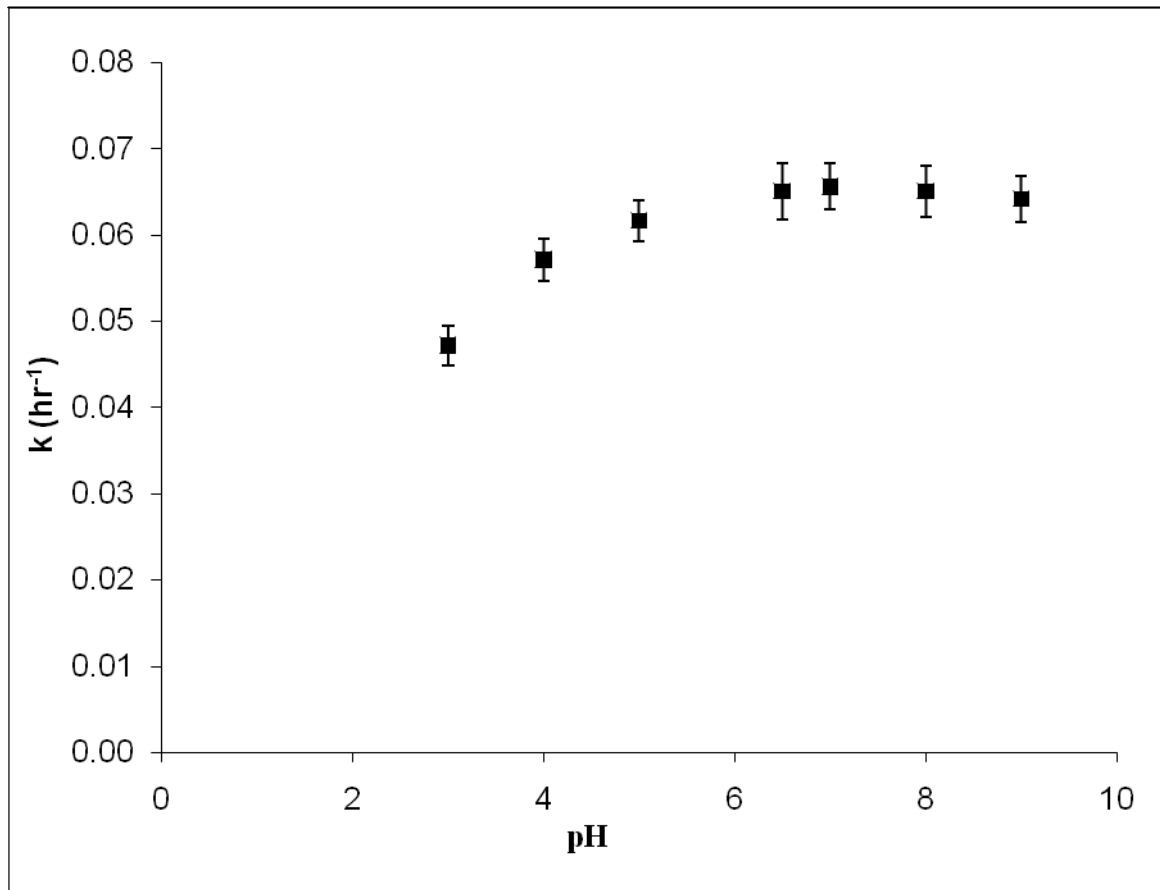
Figure 4.8 Influence of pH on solar photodegradation of E2.

$C_{0_{E2}}$ = 5 µg/L, solar intensity= 1 SUN and irradiation time = 2 hours

4.3.5.2 Influence of NO$_3^-$

NO$_3^-$ is generally present in natural surface water at various concentrations depending on the agricultural and geographic location.[24] Hence experiments were carried out for several NO$_3^-$ concentrations using NaNO$_3$ stock solution in the range of 0-40

mg/L, which are similar to natural surface water conditions, using a solar intensity of 1 SUN and E2 initial concentration of 5 µg/L with the results shown in Figure 4.9. Solar photodegradation of E2 in the presence of NO_3^- also follows pseudo-first-order kinetics and the degradation efficiency was enhanced markedly with increasing concentration of NO_3^- and the degradation rate increased proportionately with increasing concentrations of NO_3^-. It is well known that NO_3^- produces $OH^\bullet$ when excited by solar ultraviolet light at wavelengths between 290 and 330 nm with quantum yield (Φ) ranging from 0.009 to 0.017.[25] The mechanism of $OH^\bullet$ generation from NO_3^- photolysis at λ_{max} 302 nm ($\varepsilon = 7.2$ M^{-1} Cm^{-1}) results in two primary photochemical processes as per reactions 4.8 - 4.9, and according to reaction 4.10, $OH^\bullet$ should be proportional to NO_3^- and if its reaction with E2 is equimolar, the degradation rate of E2 should also be proportional to NO_3^-.[24-26] Similar results were obtained for the photodegradation of diuron and monolinuron in the presence of NO_3^- by other researchers.[24,25]

$$NO_3^- \xrightarrow{h\upsilon} NO_3^{\bullet-} \longrightarrow NO_2^- + O(^3P) \qquad \qquad (4.8)$$
$$\longrightarrow NO_2 + O^{\bullet-} \qquad \qquad (4.9)$$

Followed by:

$$O^{\bullet-} + H_2O \rightarrow OH^\bullet + OH^- \qquad \qquad (4.10)$$

$$E2 + OH^\bullet \longrightarrow Photooducts \qquad \qquad (4.11)$$
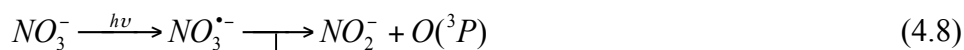
Increasing the nitrate concentration by 4 times, the photodegradation rate constant increased by 1.8 times, with the rate constant following the linear relationship with NO_3^- concentration.

$$k = 0.0014C + 0.0682 \hspace{3cm} (4.12)$$

where, k is the pseudo-first order rate constant of E2 degradation in hr^{-1}, and C is the nitrate concentration in mg/l.
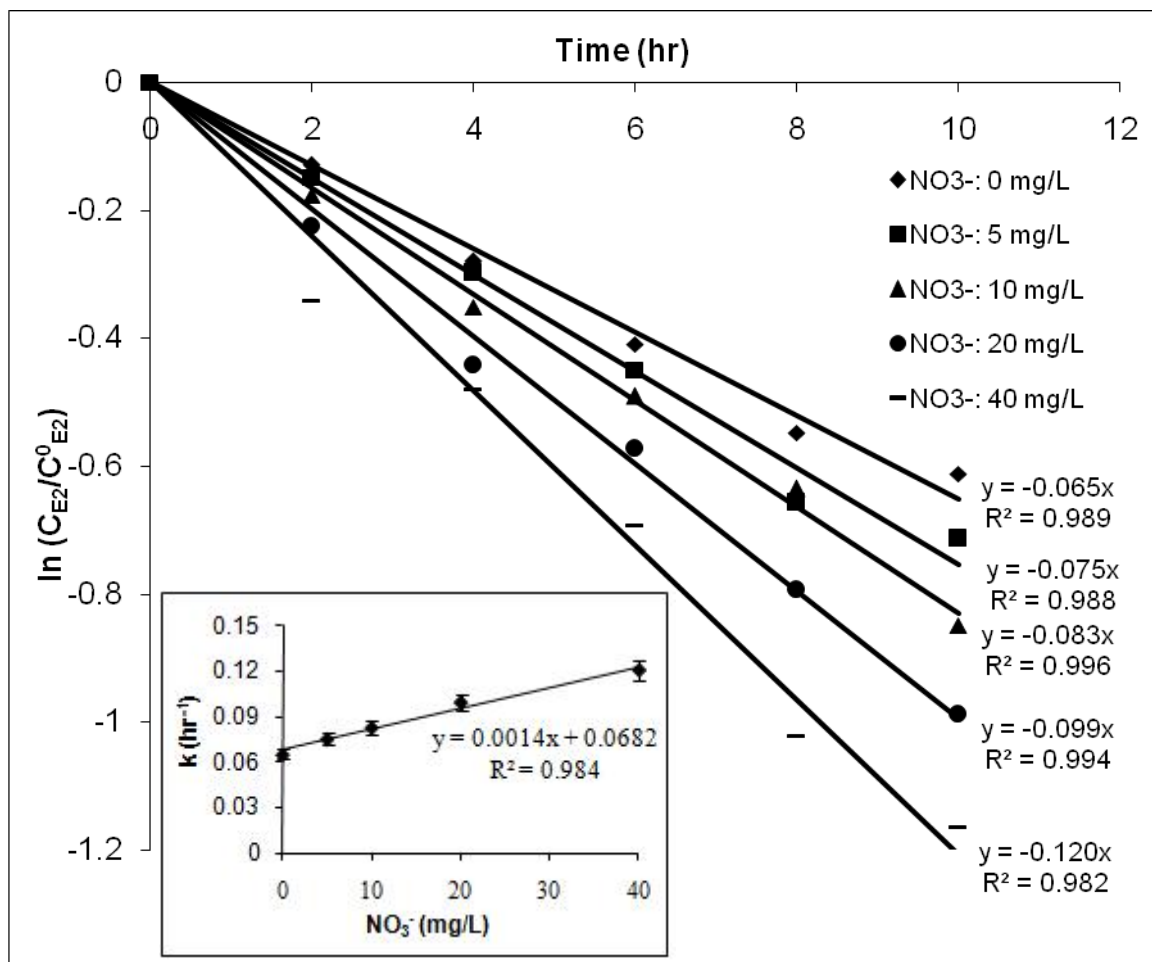
Figure 4.9 Influence of NO_3^- concentration on solar photodegradation of E2.

$C_{0_{E2}}$ = 5 µg/L, pH = 6.5, solar intensity= 1 SUN and irradiation time = 10 hours

4.3.5.3 Influence of Fe^{3+}

Dissolved iron is often present in natural surface water at various concentrations depending on the geographical location. It has been shown that Fe^{3+}-aquo complexes strongly absorb solar irradiation (λ>290 nm) at acidic pH's between 2.5-5 and yield OH$^{\bullet}$ and Fe^{2+} according to the following reaction:[27]

$$\text{Fe}^{3+}\text{-OH} \underset{[O]}{\overset{h\upsilon}{\rightleftarrows}} \text{Fe}^{2+} + \text{OH}^{\bullet} \qquad\qquad (4.13)$$

Among the Fe^{3+}-aquo complexes (Fe^{3+}, $Fe(OH)^{2+}$, $Fe(OH)_2^+$, dimer $Fe(OH)_2^{4+}$), Fe(OH)$^{2+}$ is the predominant photoreactive species in terms of OH$^{\bullet}$ generation with the highest quantum yield as per the following reaction:[27]

$$Fe(OH)^{2+} \xrightarrow{h\upsilon} Fe^{2+} + OH^{\bullet} \qquad\qquad (4.14)$$

Hence experiments were conducted using 30 μmol/L and 50 μmol/L Fe^{3+} concentrations (by spiking FeCl$_3$ stock solution) at three different pH values of 3, 4 and 5 using a solar intensity of 1 SUN and E2 initial concentration of 5 μg/L with the results shown in Table 4.3. The results show that the photodegradation rate of E2 was enhanced considerably in the presence of Fe^{3+} for all pH's as predicted due to the presence of OH$^{\bullet}$. The effect is more pronounced at pH 3 where a 30% increase in rate occurred by increasing the Fe^{3+} concentration from 30 μmol/L to 50 μmol/L. In an earlier study, the photodegradation rate increased about 8% by increasing Fe^{3+} concentration by 67% at pH 3-5 due to increasing OH$^{\bullet}$ production due to increasing the Fe^{3+} concentration.[28]

Table 4.3 Influence of Fe^{3+} on solar photodegradation of E2 (intensity 1 SUN)

pH	k (hr^{-1}) Without Fe^{3+}	k (hr^{-1}) $Fe^{3+}= 30\mu M$	k (hr^{-1}) $Fe^{3+}= 50\mu M$
3	0.0472 ± 0.0023	0.0564 ± 0.0027	0.0616 ± 0.0027
4	0.0571 ± 0.0025	0.0629 ± 0.0033	0.0678 ± 0.0035
5	0.0616 ± 0.0023	0.0660 ± 0.0031	0.0731 ± 0.0032

4.3.5.4 Influence of HCO_3^-

Carbonate and bicarbonates, which are responsible for alkalinity, are the most common inorganic salts present in natural surface water. Studies have shown that CO_3^- and HCO_3^- do not absorb solar UV radiation, but act as $OH^\bullet$ scavengers.[29] Since inorganic carbon exists mainly in the form of HCO_3^- at natural pH, experiments were carried out for several HCO_3^- concentrations (using $NaHCO_3$ stock solution) in the range of 50-200 mg/L as $CaCO_3$ in the presence of 40 mg/L NO_3^-, which produces $OH^\bullet$ when excited by ultraviolet light at wavelength 290 - 330 nm.[25,30] The experiments were conducted at an intensity of 1 SUN and E2 initial concentration of 5 µg/L. The results shown in Table 4.4 indicate that the degradation rate decreased markedly with increasing HCO_3^- concentration. This is due to the fact that HCO_3^- acts as $OH^\bullet$ scavengers according to reaction 4.15 with a second order rate constant 8.5 x 10^6 M^{-1} S^{-1}; the reaction produces $CO_3^\bullet$, which is a weak oxidizing agent that hardly reacts with E2. [29,31]

$$HCO_3^- + OH^{\bullet} \rightarrow CO_3^{\bullet-} + H_2O \tag{4.15}$$

Although the solution pH increased from 6.47 to 8.76 due to the increase in alkalinity, the effect of increasing pH on degradation rate is minimal in this pH range as can be seen in Figure 4.8.

Table 4.4 Influence of HCO_3^- on solar photodegradation of E2 in presence of 40 mg/L NO_3^- (intensity 1 SUN)

HCO_3^- (mg/L as $CaCO_3$)	0	50	100	200
pH	6.47	7.96	8.40	8.76
k (hr^{-1})	0.1210 ± 0.0054	0.1167 ± 0.0057	0.1021 ± 0.0042	0.0834 ± 0.0043
% Reduction	-	3.55%	12.51%	18.32%

4.3.5.5 Influence of humic acid

Humic substances (HS), the largest fraction of dissolved organic matter (DOM) are ubiquitous in the aquatic environment. They are formed during the abiotic and microbiological transformations of plant and animal materials, and can be categorized as humic acids, fulvic acids, and humin depending on their solubility.[11,32] Humic acid is the predominant constituent in HS with an average molecular weight of 2,000 to 5,000 g/mol containing a high portion of oxygen-containing functional groups (e.g. phenolic hydroxyl, carboxyl groups, and carbonyl-type chromophores). The chromophoric HS

absorbs solar radiation mostly between 300 - 500 nm to reach an excited state, hence generating free radicals (e.g., hydroxyl radicals ($OH^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), singlet oxygen ($^1O_2$), etc.) that cause photooxidation of organic contaminants.[32,33] Since the presence of humic acid has a significant effect in natural aquatic environments, it is very important to study the influences of humic acid on the photodegradation of E2 to predict the transport and fate of organic contaminants in natural water.

Prior to photodegradation studies, the dark adsorption of E2 by humic acid was conducted in batch mode. In these experiments, 10–400 µg/L of E2 was put in 0.1 mg of humic acid in 100 ml solutions in 150 ml bottles. The temperature of all solutions was controlled at 24 ^{0}C. The tests were conducted in an orbital-shaker at 100 rpm. Then the sample was taken out at various times for analyzing in HPLC. The sorption equilibrium data of E2 on humic acid were plotted in Figure 4.10 and modeled using the Freundlich equation 4.16 and 4.17 as follows (Refer Appendix B for C_{E2} vs t plot):

$$q_e = K_F C_e^n \tag{4.16}$$

$$ln q_e = ln K_F + n ln C_e \tag{4.17}$$

where, q_e is the equilibrium solid-phase solute concentration (µg/mg), C_e is the aqueous-phase solute concentration (µg/L), K_F is the Freundlich capacity parameter, and n is the isotherm nonlinearity index. The parameter K_F has units of $(µg/mg)/(µg/L)^n$ and n is

unitless. Figure 4.10 is the adsorption isotherms of E2 on humic acid modelelled by the Freundlich equation with K_F is 1.02 $(\mu g/mg)/(\mu g/L)^n$ and n=0.04.
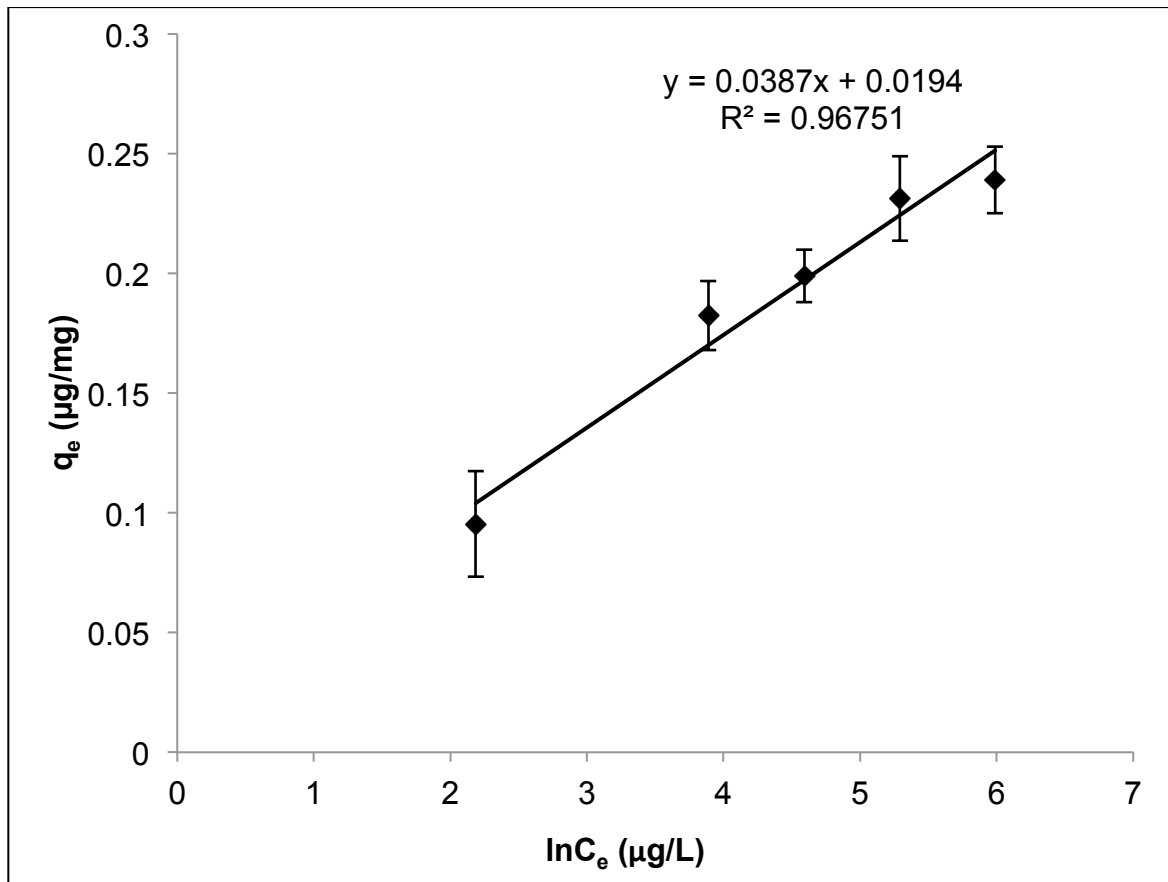
Figure 4.10 Freundlich adsorption isotherm of E2 on humic acid.
$C_{0_{E2}}$ = 10, 50, 100, 200 and 400 µg/L, time = 30 min. and temperature = 24 ^{0}C

Experiments were carried out at several humic acid concentrations in the range of 0-10 mg/L at 2 hours solar irradiation and an intensity of 1 SUN, while the E2 initial concentration was maintained at 5 µg/L. The degradation rate of E2 with humic acid concentration after 2 hours of solar irradiation is shown in Figure 4.11. The

photodegradation rate increased considerably (≈22%) by the addition of 2 mg/L humic acid compared to pure E2 solution due to photoxidation resulting from OH$^{\bullet}$ as per reactions 4.17 - 4.21. Further degradation was also observed with increasing humic acid concentration up to 8 mg/L, but the rate of increase was much lower at higher concentration.

$$HS \xrightarrow{h\upsilon} HS^{\bullet -} \qquad\qquad (4.17)$$

$$HS^{\bullet -} + O_2 \longrightarrow Oxidized - HS + O_2^{\bullet -} \qquad\qquad (4.18)$$

$$2O_2^{\bullet -} + 2H^+ \longrightarrow H_2O_2 + O_2 \qquad\qquad (4.19)$$

$$H_2O_2 \xrightarrow{h\upsilon} 2OH^{\bullet} \qquad\qquad (4.20)$$

$$E2 + OH^{\bullet} \longrightarrow Photooducts \qquad\qquad (4.21)$$
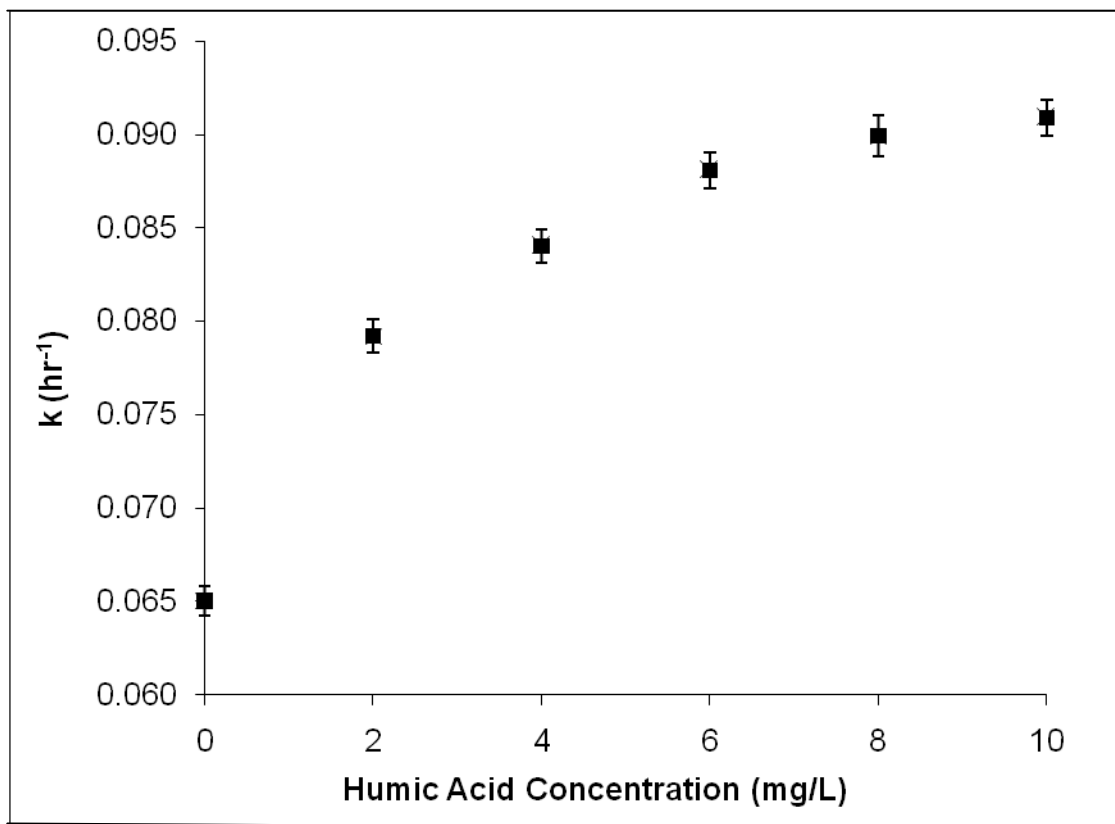
Figure 4.11 Influence of humic acid concentration on solar photodegradation of E2.

$C_{0_{E2}}$ = 5 µg/L, solar intensity= 1 SUN and irradiation time = 2 hours

Beyond 8 mg/L humic acid concentration, the degradation rate reached a plateau due to the scavenging of reactive oxygen species[34] as well as increased light attenuation with increasing humic acid concentration. The transmittance of humic acid solution was decreased from 89% to 71% at 290 nm for 2 mg/L to 10 mg/L humic acid concentration, respectively, indicating absorption of light by humic acid. Although indirect photolysis probably increases with humic acid concentration, the direct photolysis rate is decreased due to the absorption of photons by humic acid. As humic acid is a very week acid, solution pH does not change significantly due to its addition; solution pH only varied

from 6.46 for 2 mg/L to 6.17 for 10 mg/L of humic acid in water, whereas the natural pH of E2 in Milli-Q water is around 6.5. Hence, the effect of pH change due to different humic acid concentrations is minimal. The effect of humic acid concentration on the degradation rate was higher for E1 than E2 (a maximum difference of 56% was observed for similar condition), indicating that the sensitized photooxidation rate of E1 is also higher than E2, and the primary photochemical process of proton absorption by the parent compound enhances the overall degradation.

4.3.5.6 Influence of turbidity

Turbidity measured in Nephelometric Turbidity Units (NTU) is an optical property of a liquid that causes light to be scattered and absorbed rather than transmitted and is the ultimate measure of water clarity and cloudiness. In natural aquatic system sunlight penetration depends on the reflection from the water surface and attenuation in water by absorption and scattering. The movement of the water body and angle of incidence for sunlight are responsible for reflection, while attenuation is greatly influenced by the water depth and turbidity, which is caused by organic matter (OM), phytoplankton, color, mineral content and suspended sediment.[35] Here, simple experiments were carried out for several solution turbidity values in the range of 0-60 NTU (desired experimental turbidity was produced by adding clear turbidity standard, 1000 NTU to E2 solution) at an intensity of 1 SUN with E2 initial concentration of 5 µg/L and the results are shown in Table 4.5. The degradation of E2 in different turbid solutions decreased with increasing turbidity attributed to the reduction in light penetration. The transmittance of E2 solution was measured at 290 nm and it varied from 99.1% for 0.1 NTU ($k = 0.0652 \pm 0.0033$ hr^{-1})

to 51.7% for 60 NTU (k=0.0472 ± 0.0019 hr^{-1}); a drop of 47.8% in transmittance corresponds well with the 38% drop in reduction in the rate constant of E2, which is consistent with the intensity of ½ Sun (k=0.0433 ± 0.0022). Therefore the effect of turbidity is directly related to photon attenuation and subsequent decrease in photolysis. Since these experiments were not conducted in the presence of a photosensitizer, the effect of turbidity on photooxidation cannot be characterized.

Table 4.5

Influence of turbidity on solar photodegradation of E2 (intensity 1 SUN)

Turbidity (NTU)	0.1	5	10	20	40	60
k (hr^{-1})	0.0652 ± 0.0033	0.0616 ± 0.0027	0.0594 ± 0.0029	0.0549 ± 0.0026	0.0494 ± 0.0023	0.0472 ± 0.0024
Transmittance @ 290 nm	99.1%	95.4%	91.4%	83.5%	67.6%	51.7%

4.4 Conclusions

Photodegradation of E2 in aqueous solution occurs under simulated solar irradiation as per pseudo-first-order reaction kinetics. The quantum yield was evaluated to be 0.0069 in Milli-Q water in direct photolysis. About 48% of E2 degraded due to the direct photolysis and subsequent photo-oxidation in the presence of molecular oxygen. The

half-life of E2 varied from 10-21 hours depending on the solar intensity and concentration. The effects of several water constituents such as pH, NO_3^-, Fe^{3+}, HCO_3^-, humic acid and turbidity on phtodegradation of E2 were evaluated. The degradation rate increased in the presence of NO_3^-, Fe^{3+} and humic acid concentration due to photosensitization, whereas HCO_3^- slowed down the degradation rate because of the $OH^{\bullet}$ scavenging effect with the maximum degradation occurring at neutral pH. Turbidity also reduced the photodegradation of E2 due to a reduction in light penetration. Although the TOC analysis showed a steady degradation of TOC indicating a gradual mineralization of E2, TOC removal was always much lower than the degradation of E2, indicating stability of the photoproducts of E2. As all natural estrogens have very similar structures and properties of E2, the other steroid hormones also are expected to be removed from the natural aquatic system by direct and indirect photoreactions, and the half-life may be rather short in full sun and in clear water.

4.5 References

1. Zuo, Y.; Zhang, K.; Deng, Y. Occurrence and Photochemical Degradation of 17α-Ethinylestradiol in Acushnet River Estuary. *Chemosphere.* **2006**, *63*, 1583.

2. Ying, G.; Kookana, R. S.; Ru, Y. J.; Occurrence and Fate of Hormone Steroids in the Environment. *Environ. Int.* **2002**, *28*, 545.

3. Liu, X. L.; Wu, F.; Deng, N. S. Photodegradation of 17α-Ethynylestradiol in Aqueous Solution Exposed to a High-Pressure Mercury Lamp (250 W). *Environ. Pollut.* **2003**, *126*, 393.

4. Li, J.; Mailhot, G.; Wu, F.; Deng, N. S. Photochemical Efficiency of Fe(III)-EDDS Complex: •OH Radical Production and 17β-Estradiol Degradation. *J. Photochem. Photobiol., A* **2010**, *212*, 1.

5. Casey, F. X. M.; Larsen, G. L.; Hakk, H.; Simunek, J. Fate and Transport of 17β-Estradiol in Soil–Water Systems. *Environ. Sci. Technol.* **2003**, *37*, 2400.

6. Purdom, C. E.; Hardiman, P. A.; Bye, V. V. J.; Eno, N. C.; Tyler, C. R.; Sumpter, J. P. Estrogenic Effects of Effluents from Sewage Treatment Works. *Chem. Ecol.* **1994**, *8*, 275.

7. Brion, F. ; Tyler, C. R. ; Palazzi, X.; Laillet, B. ; Porcher, J. M. ; Garric, J. ; Flammarion, P. Impacts of 17β-Estradiol, Including Environmentally Relevant Concentrations, on Reproduction after Exposure During Embryo-larval-, Juvenile- and Adult-Life Stages in Zebrafish (Danio rerio). *Aquat. Toxicol.* **2004**, *68*, 193.

8. Ying, G. G.; Kookana, R. S. Degradation of Five Selected Endocrine-Disrupting Chemicals in Seawater and Marine Sediment. *Environ. Sci. Technol.* **2003**, *37*, 1256.

9. Zafiriou, O. C.; Joussot-Dubien, J.; Zeep, R. G.; Zika, R. G. Photochemistry of Natural Waters. *Environ. Sci. Technol.* **1984**, *18*, 358A.

10. Leifer, A. *The Kinetics of Environmental Aquatic Photochemistry: Theory and Practice*; American Chemical Society: Washington, DC, 1988.

11. Chowdhury, R. R.; Charpentier, P.; Ray, M. B. Photodegradation of Estrone in Solar Irradiation. *Ind. Eng. Chem. Res*. **2010**, *49*, 6923.

12. Chin, Y. P.; Miller, L.; Zeng, L.; Cawley, K.; Weavers, L. K. Photosensitized Degradation of Bisphenol A by Dissolved Organic Matter. *Environ. Sci. Technol.* **2004**, *38*, 5888.

13. Lam, M. W.; Tantuco, K.; Mabury, S. A. Photofate: A New Approach in Accounting for the Contribution of Indirect Photolysis of Pesticides and Pharmaceuticals in Surface Waters. *Environ. Sci. Technol.* **2003**, *37*, 899.

14. Vione, D.; Falletti, G.; Maurino, V.; Minero, C.; Pelizzetti, E.; Malandrino, M.; Ajassa, R.; Olariu, R. L.; Arsene, C. Sources and Sinks of Hydroxyl Radicals upon Irradiation of Natural Water Samples. *Environ. Sci. Technol.* **2006**, 40, 3775.

15. Coleman, H. M.; Routledge, E. J.; Sumpter, J. P.; Eggins, B. R.; Byrne, J. A. Rapid Loss of Estrogenicity of Steroid Estrogens by UVA Photolysis and Photocatalysis over an Immobilised Titanium Dioxide Catalyst. *Water Res.* **2004**, *38*, 3233.

16. Lin, Y.; Peng, Z.; Zhang, X. Ozonation of Estrone, Estradiol, Diethylstilbestrol in Waters. Desalination. **2009**, *249*, 235.

17. Gibson, J. H. UVB Radiation: Definition and Characteristics. http://uvb.nrel.colostate.edu/UVB/publications/uvb_primer.pdf (accessed June 24, 2010).

18. Lin, A. Y.; Reinhard, M. Photodegradation of Common Environmental Pharmaceuticals and Estrogens in River Water. *Environ. Toxicol. Chem.* **2005**, *24*, 1303.

19. Liu, B.; Liu, X. Direct Photolysis of Estrogens in Aqueous Solutions. *Sci. Total Environ.* **2004**, *320*, 269.

20. Zepp, R. G.; Cline, D. M. Rates of Direct Photolysis in Aquatic Environment. *Env. Sci. and Technol.* **1997**, 11, 359.

21. Hurwitz, A. R.; Liu, S. T. Determination of Aqueous Solubility and pKa Values of Estrogens. *J. Pharm. Sci.* **2006**, *66*, 624.

22. Liu, B.; Wu, F.; Deng, N. S. UV-light Induced Photodegradation of 17α-Ethynylestradiol in Aqueous Solutions. *J. Hazard. Mater.* **2003**, *98*, 311.

23. Hwang, H.M; Hodson, R. E.; Lee, R. F. Photolysis of Phenol and Chlorophenols in Estuarine Water, in: R.G. Zika, R.G. Cooper (Eds.), Photochemistry of Environmental Aquatic Systems, ACS Symposium Series 327, American Chemical Society: Washington, DC, 1987.

24. Shankar, M. V.; Nélieu, S.; Kerhoas, L.; Einhorn, J. Photo-induced Degradation of Diuron in Aqueous Solution by Nitrites and Nitrates: Kinetics and Pathways. *Chemosphere.* **2007**, *66*, 767.

25. Nélieu, S.; Kerhoas, L.; Sarakha, M.; Einhorn, J. Nitrite and Nitrate Induced Photodegradation of Monolinuron in Aqueous Solution. *Environ. Chem. Lett*. **2004**, *2*, 83.

26. Zepp, R. G.; Hoigne, J.; Bader, H. Nitrate-induced Photooxidation of Trace Organic Chemicals in Water. *Environ. Sci. Technol.* **1987**, 21, 443.

27. Feng, W.; Nansheng, D. Photochemistry of Hydrolytic Iron (III) Species and Photoinduced Degradation of Organic Compounds. A Minireview. *Chemosphere*. **2000**, *41*, 1137.

28. Zhang, C.; Wang, L.; Wu, F.; Deng, N. Quantitation of Hydroxyl Radicals from Photolysis of Fe(III)-Citrate complexes in Aerobic Water. *Environ Sci. Pollut. R.* **2006**, *13*, 156.

29. Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. Critical Review of Rate Constants for Reactions of Hydrated Electrons, Hydrogen Atoms and Hydroxyl Radicals ($\cdot$OH/$\cdot$O$^-$) in Aqueous Solution. *J. Phys. Chem. Ref. Data.* **1988**, *17*, 513.

30. Ma, J.; Graham, N. J. D. Degradation of Atrazine by Manganese-Catalysed Ozonation-Influence of Radical Scavengers. *Water Res.* **2000**, *34*, 3822.

31. Haarstrick, A.; Kut, O. M.; Heinzle, E. TiO_2-Assisted Degradation of Environmentally Relevant Organic Compounds in Wastewater Using a Novel Fluidized bed Photoreactor. *Environ. Sci. Technol.* **1996**, *30*, 817.

32. Hoigné, J.; Faust, B. C.; Haag, W. R.; Zepp, R. G., Aquatic Humic Substances, American Chemical Society: Washington DC, 1989.

33. Wetzel, R. G. Solar Radiation as an Ecosystem Modulator, in: E.W. Helbling, H. Zagarese (Eds.), UV Effects in Aquatic Organisms and Ecosystems, Royal Society of Chemistry: Cambridge, 2003.

34. Ma, J.; Graham, N. J. D. Degradation of Atrazine by Manganese-Catalysed Ozonation: Influence of Humic Substances. *Water Res.* **1999**, *33*, 785.

35. Dunlop, J.; McGregor, G.; Horrigan. N. Potential Impacts of Salinity and Turbidity in Riverine Ecosystems, http://www.wqonline.info/Documents/Report_WQ06_Review_reduced.pdf (accessed May 25, 2010).

Conclusions

5.1 Summary of results

The present research showed that both natural steroids E1 and E2 degraded under simulated solar irradiation following pseudo-first-order reaction kinetics due to direct photolysis and indirect photolysis in the presence of photosensitizers. The quantum yield was evaluated to be 0.0356 and 0.0069 in Milli-Q water for E1 and E2, respectively in direct photolysis.

The half-life of E2 at 1 sun intensity was found to be $\approx$ 10 hours, while it was only 50 minutes for E1 under the same conditions; accordingly about 48% degradation of E2 occurred due to direct photolysis compared to 67% degradation for E1. The photodegradation rate decreased with increasing initial steroid concentration due to photon limitations, and the maximum degradation occurred at natural pH.

The photodegradation rate of both steroids increased in the presence of humic acid concentration, whereas the rate of E2 also increased in presence of NO_3^- and Fe^{3+} due to photosensitization, whereas HCO_3^- slowed down the degradation rate as it scavenges $OH^{\bullet}$ radicals. Turbidity also reduced the photodegradation of E2 due to a reduction in light penetration.

Although the TOC analysis showed a steady degradation of TOC indicating a gradual mineralization of both steroids, TOC removal was always much lower compared

to the degradation of parent compounds, as the photoproducts of steroid oxidation are stable and their degradation rates are much slower. Whether, the photoproducts are more geno-toxic or mutagenic than the parent compounds remains to be determined.

As all natural and synthetic estrogens have very similar structures and properties of E1 and E2, the other steroid hormones also are expected to be removed from the natural aquatic system by direct and indirect photoreactions, and their half-lifes may be rather short in full sun and in clear water.

## 5.2 Recommendations for future work

During the present study, some areas were revealed to be of significant interest for future research. They are listed as follows:

1. Based on preliminary GC-MS data, additional photodegradation byproducts and intermediates of both E1 and E2 photolysis exist. These need to be identified to understand the environmental fate of E1 and E2 in aquatic environments. Ideally, toxicity of each byproduct should be evaluated.

2. The identity of the reactive oxygen species (ROS) responsible for E1 and E2 degradation in the presence of photosensitizers remains unclear. Future research using of molecular probes, such as 2,4,6-trimethylphenol, can determine the specific chemical species responsible (i.e., phenoxyl and peroxyl radical scavengers). It would

also be valuable to estimate the second order rate constant between •OH and steroids (E1 and E2).

3. This study was conducted under controlled environment in the laboratory. Further research is required for establishing actual environmental fate of these steroid hormones by conducting experiments in surface water of creeks, lakes, rivers, etc. from various locations. This will help to find out the effect of different water matrices.

Appendix A: The spectral irradiance from solar simulator (Air mass filter: AM1.5G)
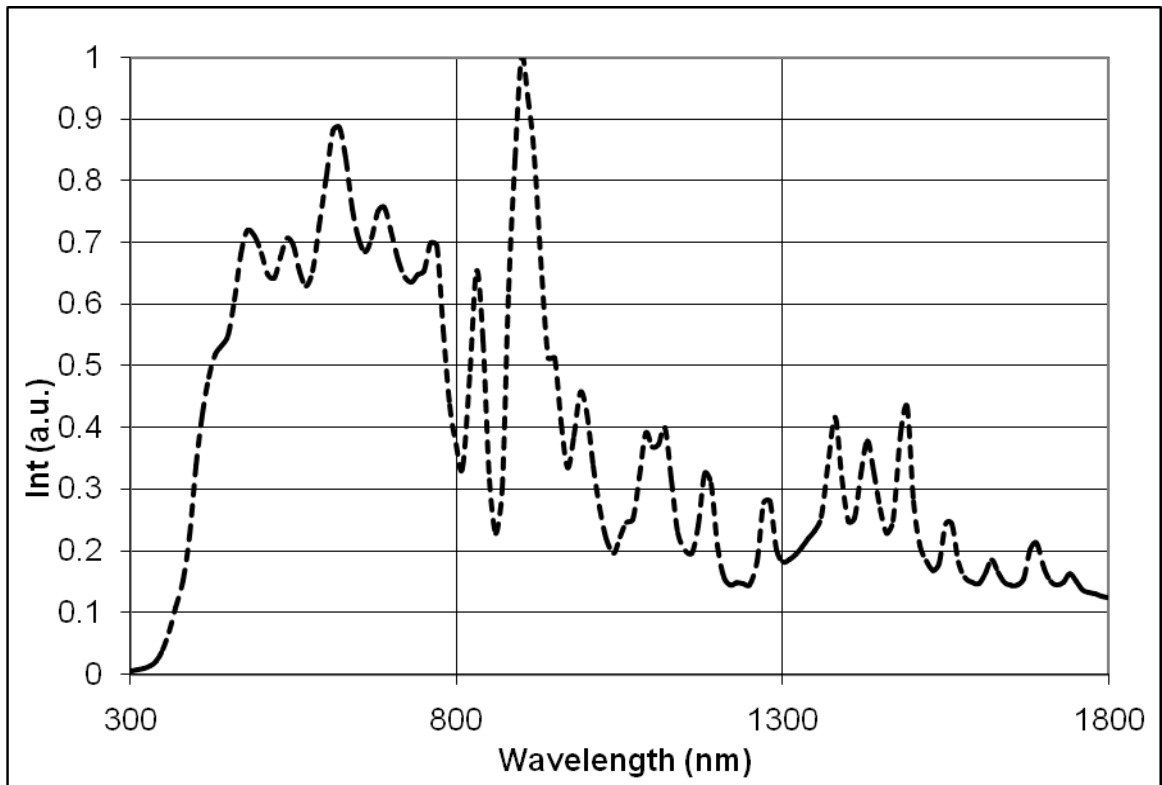
Appendix B: E2 adsorption in humic acid
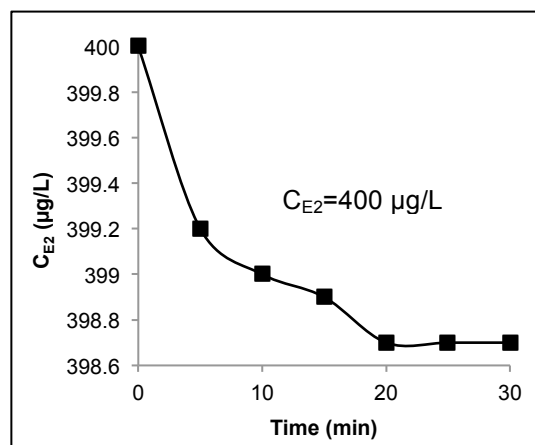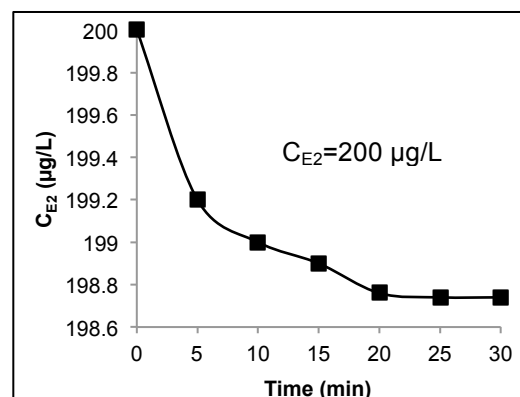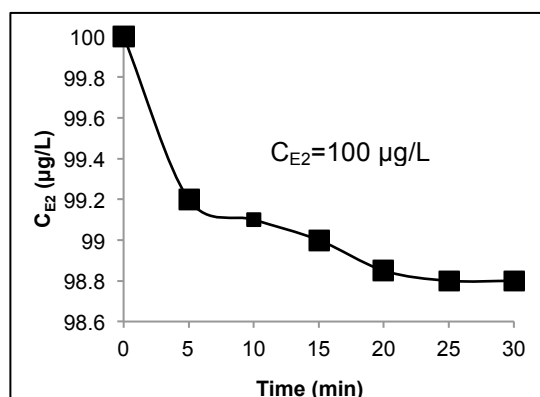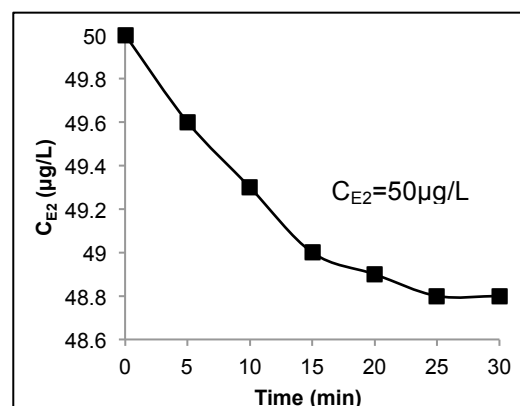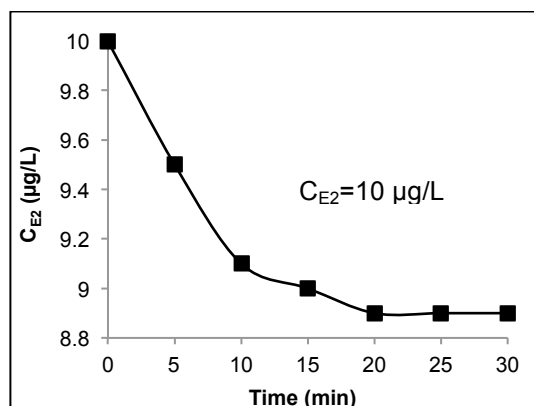
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Journal Articles:
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Chowdhury, R. R.; Charpentier, P. A.; Ray, M. B. Photodegradation of Estrone in Solar Irradiation. Industrial and Engineering Chemistry Research. 2010, 49 (15), 6923 – 6930.

Referred Conference Proceedings and Poster Presentations:
Chowdhury, R. R.; Charpentier, P. A.; Ray, M. B. Fate of Estrone in Solar Photodegradation. 7th Annual Earth Day Colloquium, London, ON, April, 2010.

Chowdhury, R. R.; Charpentier, P. A.; Ray, M. B. Fate of Steroid Estrone (E1) in Natural Aquatic Environment. 25th EASTERN Canadian Symposium on Water Quality Research, Ottawa, ON, October, 2009.

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