Use of Ozonation and Constructed Wetlands to Remove Contaminants of Emerging Concern from Wastewater Effluent

by

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ABSTRACT

Contaminants of emerging concern (CECs) present in wastewater effluent can threat its safe discharge or reuse. Additional barriers of protection can be provided using advanced or natural treatment processes. This dissertation evaluated ozonation and constructed wetlands to remove CECs from wastewater effluent. Organic CECs can be removed by hydroxyl radical formed during ozonation, however estimating the ozone demand of wastewater effluent is complicated due to the presence of reduced inorganic species. A method was developed to estimate ozone consumption only by dissolved organic compounds and predict trace organic oxidation across multiple wastewater sources. Organic and engineered nanomaterial (ENM) CEC removal in constructed wetlands was investigated using batch experiments and continuous-flow microcosms containing decaying wetland plants. CEC removal varied depending on their physico-chemical properties, hydraulic residence time (HRT) and relative quantities of plant materials in the microcosms. At comparable HRTs, ENM removal improved with higher quantity of plant materials due to enhanced sorption which was verified in batch-scale studies with plant materials. A fate-predictive model was developed to evaluate the role of design loading rates on organic CEC removal. Areal removal rates increased with hydraulic loading rates (HLRs) and carbon loading rates (CLRs) unless photolysis was the dominant removal mechanism (e.g. atrazine). To optimize CEC removal, wetlands with different CLRs can be used in combination without lowering the net HLR. Organic CEC removal in denitrifying conditions of constructed wetlands was investigated and selected CECs (e.g. estradiol) were found to biotransform while denitrification occurred. Although level of denitrification was affected by HRT, similar impact on estradiol was not observed due to a dominant effect from plant biomass quantity. Overall, both modeling and experimental findings suggest considering CLR as an equally important factor with HRT or HLR to design constructed wetlands for CEC removal.

This dissertation provided directions to select design parameters for ozonation (ozone dose) and constructed wetlands (design loading rates) to meet organic CEC removal goals. Future research is needed to understand fate of ENMs during ozonation and quantify the contributions from different transformation mechanisms occurring in the wetlands to incorporate in a model and evaluate the effect of wetland design.

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CHAPTER 1

INTRODUCTION

To improve human life, technological advances bring about new products, tools and applications that become integral parts of everyday lives. While such progress undoubtedly improves the quality of human life, it is logical to raise questions about the impact of these technologies on the environment. Many products and their residues can contaminate the aquatic environment. These contaminants originating mainly from anthropogenic processes are termed contaminants of emerging concerns (CECs) (EPA, 2010) and include pharmaceutical and personal care products (PPCPs), endocrine disrupting compounds (EDCs), disinfection by-products (DBPs), engineered nanomaterials (ENMs) etc. Presence of these CECs in various water sources has confirmed their passage in the environment.

Organic CECs including prescription and non-prescription drugs, antibiotics, pesticides and stimulants were detected in all types of water including drinking water sources, wastewater treatment plant (WWTP) effluents and natural rivers and streams (Hebereer et al., 1997, Kolpin et al., 2002, Kim et al., 2007, Daughton and Ternes et al., 1999, Snyder et al., 2002, 2003, 2008). WWTP effluent is considered as the major source to contaminate water sources with EDCs and PPCPs, although runoffs from agricultural areas and animal feedlots are also considered as potential sources of contamination (Daughton et al, 2001, Snyder et al., 2007). Disinfection by-products (DBPs) are another class of organic CECs that are formed by the reaction between organic matter and chemical disinfectants (e.g. chlorine, chloramines) (Templeton et al., 2009). Among a wide range of DBPs, nitrogenous DBPs such as nitrosamines have been detected in surface water sources (Guo and Krasner, 2009, Krauss et al., 2009, Schreiber and Mitch, 2006). The most commonly known nitrosamine is N-Nitrosodimethylamine (NDMA) which is potentially linked with carcinogenicity. NDMA precursors in wastewater are presumably the secondary amines in PPCPs and polymers which are highly stable and pose significant risk of forming NDMA in streams and rivers where WWTP effluents are discharged (Padhye et al., 2009, Schreiber and Mitch, 200). Halogenated DBPs including trihalomethanes (THMs), haloacetic acids (HAAs) were also detected in effluent dominated streams, (Chen et al., 2009). There is a plethora of organic CECs with potential risks that are mostly derived from wastewater. Researchers have proposed using indicator compounds or surrogates for monitoring the occurrence of CECs and evaluate efficiency of various treatment processes (Dickenson et al., 2009).

Growing concern is also focusing on engineered nanomaterials (ENMs) as pollutants. ENMs currently used in nanotechnology industries include oxide nanomaterials such as silica (SiO_2), titania (TiO_2), ceria (CeO_2), silver (Ag/Ag_2O) and carbonaceous NMs such as fullerenes (C_{60} or C_{70}) or carbon nanotubes (CNTs). ENMs are increasingly being detected in urban runoffs and WWTP effluents (Nowack and Bucheli, 2007, Ju-nam et al., 2008, Kaegi et al., 2008, Kiser et al., 2009). Although, direct evidence on human health is not available yet, toxicological studies suggest possible negative impact on human and ecosystems. Major obstacle of investigating the fate of ENMs in the environment is the limitations in detection and quantification in environmental matrices. In addition, ENMs are often modified by surface functionality which influences their colloidal behavior and may complicate their fate in complex matrices of wastewater effluent or surface water (Jarvie et al., 2009). Because of the analytical challenges and complex behavior, fate of ENMs in the environment remains mostly unknown.

WWTP effluents are considered a valuable resource, and increasingly used for indirect potable reuse, augmentation of potable supplies (e.g., dual distribution systems), agricultural & public irrigation or expansion of surface or ground water. Currently, WWTP effluents are discharged upstream of many rivers which are potential intake sources for DWTPs. Traditional WWTP processes including primary and secondary treatment and activated sludge processes are known to achieve incomplete or low removal of many organic CECs, although for some CECs removal efficiency exceeded 80% (Sui et al., 2011, Nakada et al., 2006, Ternes et al., 1998, Nakada et al., 2006, Kim et al., 2007, Sui et al., 2010). A growing number of studies have reported that even after going through the WWTP processes, organic CECs with varying physico-chemical properties exists in the effluent and their concentration depends on the types, operational conditions and parameters of the treatment processes involved. Thus additional barriers of protections are needed beyond WWTPs to protect water sources from perceived risks from CECs.

Treatment technologies for CEC removal from wastewater effluent

Advanced treatment processes including membrane bioreactor, oxidation or microfiltration/reverse osmosis are excellent options and many of these process were found to increase removal efficiency of CECs from wastewater effluent (Sui et al., 2010, Laera et al., 2011, Hai et al., 2011). Oxidation technologies (e.g. ozone, UV, AOPs) are effective in removing a number of EDCs and PPCPs (Huber et al., 2003, Westerhoff et all. 2005, Snyder et al., 2006). These technologies are favorable over their contemporary ones including the membrane processes, because unlike membranes producing concentrated brine streams that must be disposed, oxidation technologies have no waste streams. But, oxidation process like ozonation is an energy intensive process and requires careful operation and maintenance. Ozonation can also form harmful disinfection by products (DBPs) like bromate (BrO_3^-) when Br^- is present in wastewater (von Gunten and Hoigne, 1994). Besides, the toxicity or the risks associated with the byproducts formed during ozonation of organic compounds is also unknown.

The overall costs, energy and the risks associated with the advanced treatment technologies can be mitigated by adopting sustainable and eco-friendly treatment processes. An example, where contaminants can be removed by natural processes, consuming less energy is constructed wetlands. These wetlands are manmade engineered systems, built specifically to treat contaminants in waste streams. Wetland supports physical, chemical and biological transformation process to remove pollutants from water. Wetlands have been traditionally considered a low-cost alternative to remove bulk pollutants and nutrients from wastewater before discharging into the receiving water sources without consuming any significant amount of energy. There is a growing interest for using these passive technologies for their low energy requirement, operation and maintenance. With the increasing evidence of CECs in wastewater, the use of constructed wetlands as a valid treatment for removing CECs is emerging. Wetlands constructed at or near outfalls of WWTPs or any non-point sources are being investigated for their potential to deal with the CECs. Over the last decade or so, a growing body of research data indicates that constructed wetlands can be considered an effective treatment system for CECs. Wetlands were found to perform better than conventional treatment process for secondary WWTP effluents or small-scale onsite wastewater treatment systems (Matamoros et al., 2005, 2006, 2007, 2008, Hijosa-Valsero et al., 2010 Ávila et al, 2013).

Key Research Needs

Ozonation of wastewater effluent is effective at removing many organic CECs. But, wastewater effluents contain bulk organic matter and inorganic species e.g. ammonia (NH_3), nitrite (NO_2^-) which exerts significant ozone demand. Ozonation also produces hydroxyl radicals (HO•) which non-selectively reacts with organic compounds (von Gunten, 2003). Bulk organic matter present in the WWTP effluent can compete for HO• and reduce removal efficiency of the trace organics. In many cases, ozone dosages are applied based on the organic matter of the wastewater, however when significant levels reduced inorganic constituents are present, the effectively of the applied ozone dose will be reduced consequently lowering the removal efficiency of trace level contaminants. There is a need to identify the demand from the inorganic and organic species of wastewater effluent in order to determine the ozone required to achieve a target goal of trace organic contaminant removal.

Constructed wetlands are mostly used to remove contaminants like nitrate (NO_3^-), chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids (SS), nitrate (NO_3^-), total phosphorus (TP) and the mechanisms for removing these bulk contaminants are well-known. But there is a need to understand the role of different mechanisms responsible for CEC removal in wetlands. There are evidence of CEC removal by a multitude of abiotic and biotic processes (e.g., sorption, photolysis, hydrolysis, and biodegradation), yet the relative importance of these processes for CECs with different physico-chemical properties is unknown (Reed and Crites, 1995; Matamoros et al., 2005, 2006, 2008, 2009; Huang et al., 2004, 2005; Gray and Sedlak, 2005, Conkle et al., 2008, Park et al., 2009, Hijosa-Valsero et al., 2010, Zhang et al., 2012).

Depending on the pollutant loading, size of a wetland can be very large to provide sufficient residence time in order to achieve a desired level of removal. Hydraulic loading rate is one of the main design criteria to estimate the size of a wetland required to achieve a target removal of contaminants. It is critical to investigate the relationship between contaminant removal and hydraulic loading rates based on which appropriate flow rate or area needed to achieve a certain removal rate can be estimated. Carbon loading rate is another important parameter which refers to plant productivity rate of constructed wetlands. It is also important to understand how these loading rates of constructed wetlands influence CEC removal.

Wetlands often receive treated or partially treated municipal wastewater or agricultural runoffs to mainly remove high concentration of nitrate (NO_3^-). In organic rich environment of wetlands with oxygen limitations, high loading of NO_3^- can be successfully removed by denitrification (Vymazal, 2007). Typically, aerobic conditions are considered more favorable for CEC removal. As a result, CEC removal is expected to be low in anaerobic conditions. In denitrifying wetlands, where NO_3^- becomes a substitute for O_2, CECs removal can be benefitted. The key need is to find out whether constructed wetlands currently used to remove NO_3^- from wastewater effluent can also become an effective treatment system for CECs.

Research Objectives

With the increasing reports of CEC occurrences in surface water, drinking water and wastewater effluent, finding effective and sustainable solutions to protect human health and ecosystem from their unknown risks is becoming more important. Research in this dissertation evaluated two common strategies to treat wastewater effluent (Figure 1.1). First method of treatment was advanced but energy consuming ozonation process. The second strategy was a low energy but land-intensive treatment system- constructed wetlands. The goal of this dissertation was to evaluate ozonation and constructed wetlands to remove CECs from wastewater effluent. This was achieved by conducting investigation to answer the following research questions.

1. How does inorganic species of WWTP effluent affect ozone demand and trace organic contaminant oxidation?
2. How does HRT of constructed wetlands impact organic and ENM CEC removal?
3. How does changing design loading rates impact organic CEC removal in constructed wetlands?
4. Does the level of denitrification in constructed wetlands affect organic CEC removal in wetlands?

Based on these research questions the following hypotheses were proposed:

- Accounting for ozone demand by the inorganic species, trace organic removal can be assessed across multiple WWTPs.
- Removal efficiency of caffeine, atrazine and carbamazepine increases with hydraulic retention time (HRT) of wetland microcosms.
- Increasing denitrification in wetland microcosms leads to higher removal of estradiol (E2) and *N*-nitrosodimethylamine (NDMA).
- A-fate-predictive model can show that hydraulic loading rate has greater influence on CEC removal than plant addition or carbon loading rate.

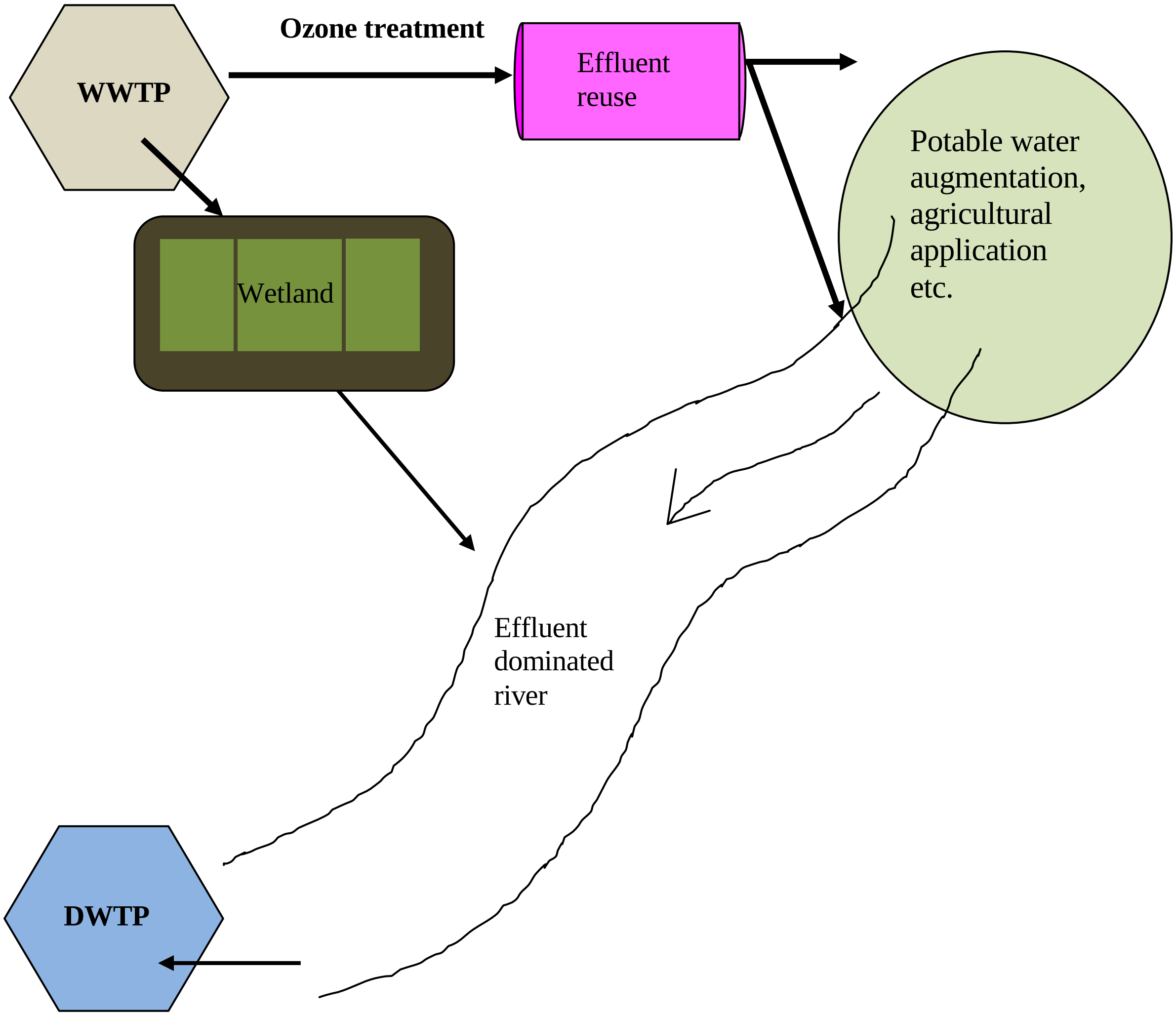

Figure 1.1 Potential applications of ozonation and constructed wetlands to treat wastewater effluent from different sources

The research questions and hypotheses were addresses and tested using two approaches. For the first approach, trace organic removal by ozonation was investigated in the complex matrix of wastewater with varying types and levels of inorganic species. Transformation of bulk organics was also investigated to evaluate other potential benefits of using ozone for WWTP effluent treatment purposes.

In the second approach, a number of PPCPs and ENMs were selected and removal mechanisms for each of these CECs were investigated. Continuous-flow microcosms were established to mimic wetlands where growing plants decay with time and supports multiple removal mechanisms. A fate-transport model was developed to understand the effect of various design loading rates on CEC removal. Finally CEC removal in denitrifying conditions of constructed wetlands was investigated. The effect of loading rates on NO_3^- and CEC removal in denitrifying conditions were parallely investigated using the continuous-flow microcosms. CEC Removal kinetics in anoxic conditions was also evaluated to find out the link between denitrification and CEC removal.

Dissertation Organization

The goal of this dissertation is as to evaluate ozonation and constructed wetlands to remove contaminants of emerging concerns from wastewater effluent. It is organized into 8 chapters. Chapter 2 discusses the research work conducted on ozonation of wastewater effluent. Chapter 3 provides a comprehensive review on CECs and there removal in constructed wetlands. ENM CEC removal in wetlands are discussed in Chapter 4. In Chapter 5, removal mechanisms for organic CECs and the effect of design loading rates are comprehensively summarized. Chapter 6 focuses the role of denitrifying wetlands on organic CEC removal. Field-scale monitoring of selected organic CECs in constructed wetlands are discussed in Chapter 7. Chapter 8 synthesizes the outcome of this dissertation and tests the hypotheses. Finally, Chapter 9 summarizes the conclusions and recommends the future research needs.

CHAPTER 2

EFFECT OF OZONATION ON WASTEWATER EFFLUENT QUALITY*

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Abstract

Ozonation of wastewater has many benefits. The aim of this study is to determine the ozone dosages needed to oxidize organics. Treated effluent from eight full-scale wastewater treatment plants was collected and subjected to lab-scale ozonation. Because both organics and inorganics exert ozone demand, an approach was developed to calculate only the ozone demand associated with organics. This method allowed normalization of parameters including color removal, UV absorbance, and oxidation of trace organics and nitrosamine precursors. We also showed that ozonation effectively reduces the fraction of organic matter characterized as “colloidal organic matter,” thereby reducing the potential for membrane fouling.

Introduction

Municipal wastewater is a complex mixture of organic matter, commonly referred to effluent organic matter (EfOM), which includes residual dissolved organic carbon (DOC) from drinking water, extracellular material, non-degraded organics from industry and other residential wastes (Drewes and Fox, 2001). Conventional wastewater treatment typically involves processes including activated sludge, aerated lagoons, trickling filters or membrane bioreactors, followed by disinfection prior to discharge in surface waters. Ozone is beginning to be employed in wastewater treatment for oxidation, improved membrane fluxes, reduced color and odor, removal of pharmaceutical and personal care products (PPCPs) and other refractory organics (Ternes et al., 2003, Ikehata et al., 2004, Rosal et al., 2008, Wert et al., 2007). Ozonation produces two oxidants- molecular ozone and hydroxyl radicals (HO•). Both these oxidants react with macromolecules and trace organics while also providing disinfection (Westerhoff et al., 1999, Huber et al., 2003, 2005).

One of the issues with disinfection processes are the formation of harmful disinfection byproducts (DBPs). Trihalomethanes (THMs), Haloacetic acids (HAA), or nitrosamines (e.g., N-nitrosodimethylamine (NDMA)) can form during chlorination or chloramination processes (Najm et al., 2001, Lee et al., 2007, Krasner et al., 2009). NDMA formation is controlled by removing its organic precursors and selection of proper disinfectant doses. Ozone or ozone peroxide processes is an effective method to oxidize NDMA because its precursors include aliphatic and aromatic amines are reactive towards ozone and HO• radicals (Hoigné and Bader, 1983; Hollender et al., 2009).

Selection of ozone dose can influence the transformation of organic compounds and formation of DBPs. Ozone dosages are often based on ozone to DOC ratio because of the large ozone demand organic can exert (Westerhoff et al., 1999). However, ozone also reacts with reduced inorganics. For example, nitrite reacts rapidly with ozone at a stoichiometric ratio of 1 mole of O_3 per mole of NO_2^- ($k_{O3} = 3.7x10^5$ $M^{-1}s^{-1}$). Similarly, ammonia can consume significant amount of O_3 at a stoichiometric ratio of 2 mole O_3 per mole of NH_3 ($k_{O3} = 20$ $M^{-1}s^{-1}$). Both nitrite and ammonia also react with HO• radical (with a reaction rate constant of $6x10^9$ and $9.7x10^7$ $M^{-1}s^{-1}$ respectively) (von Gunten, 2003). Because wastewater contains higher levels of reduced nitrogen species than drinking water, these and other inorganics can exert a significant ozone demand in wastewaters and should be considered in selecting ozone dosages.

An approach that includes organic matter content and major reduced inorganic species was used in this study to compare transformations in bulk and trace organics during ozonation of a range of WWTP effluents. Chemical oxygen demand (COD), UV response at 254 nm (UV_{254}), and color were measured to quantify the transformation of organics. Decay of a HO• probe compound, p-chlorobenzoic acid (pCBA), was measured as an indicator of trace organics oxidation in wastewater. Molecular size distribution of DOC was quantified with size exclusion chromatography. Oxidation behavior of NDMA precursors was also investigated by measuring the NDMA formation potential (NDMA FP) of wastewater samples at selected ozone doses.

Material and Methods

Wastewater samples were collected from eight full scale wastewater treatment plants (WWTPs) in Arizona, USA (Table 2.1). These plants covered a broad range of treatment processes including conventional activated sludge treatment, membrane bio reactor, aerated lagoon and wetlands. All samples were filtered with 0.7 µm glass fiber filters (Glass Microfiber filters, Whatman Inc., Piscataway, NJ) to minimize further microbial activities. After filtration, samples were stored at 4^0C prior to measure water quality parameters.

Bench scale ozonation experiment was conducted in batch reactors by adding liquid O_3 stock solution to the wastewater samples (Westerhoff et al., 2006). O_3 gas produced from oxygen fed generator (Osmonics ozone generator, GE Osmonics, Minetonka, MN) was bubbled continuously in a 2L glass reactor filled with nanopure water (18.3 Ω conductivity. Milipore Inc., Billerica, MA) to prepare ozone stock solution. A saturated ozone stock solution with concentration of 40±1 mg/l was prepared. Aliquot of stock solution was injected into a completely stirred 1L glass reactor containing wastewater sample to achieve the selected dose. The surface of the reactor was sealed with teflon lid and samples were drawn from a port near the bottom.

Table 2.1

Wastewater quality parameters of the effluent collected from multiple WWTPs

*[*AS- Activated sludge, MBR- Membrane bioreactor, WT- Wetlands, AL- Aerated Lagoon]*

WWTP		1	2	3	4	5	6	7	8
Treatment process*		AS	MBR	WT	AS	AS	AL	AS	MBR
Parameters	Unit								
Maximum	MGD	18	9	23	204	57	17.2	8	12
pH	-	7.4	7.86	8.26	8. 6	7.81	7.01	7.33	7.48
DOC	(mg/L)	4.31	6.7	5.28	8.6	8	6.8	8.4	6.0
Nitrite NO_2^--N	(mg/L)	0.03	0.09	0.02	0.38	0.13	0.04	0.05	0.05
Nitrate NO_3^--N	(mg/L)	5.26	3.85	0.56	1.73	4.14	11.6	3.11	2.9
Ammonia NH_3-N	(mg/L)	0.02	0.012	0.18	0.99	0.23	0.48	0.16	0.05
UV_{254}	(cm^{-1})	0.08	0.14	0.06	0.13	0.15	0.11	0.13	0.99
True Color	(Pt- Co)	24	40	4	40	44	46	44	28
COD	(mg/L)	12	15	18	24	15	19	16.2	13.8
NDMA FP	(ng/L)	1044	476.4	37.0	1289.5	683.3	672.2	-	-

Ozone concentrations in the stock solution were determined by direct UV absorbance at 258nm (ε_{258nm}=3000 $M^{-1}cm^{-1}$). Residual ozone in wastewater samples were measured at 600 nm using indigo reagent and quenched by purging Nitrogen gas (Bader and Hoigné, 1981). Dissolved organic carbon (DOC) was determined by a Shimadzu^{TM} TOC Analyzer.UV_{254} was measured using a 1 cm cell on a Shimadzu^{TM} UV/VIS spectrophotometer. Nitrite, nitrate, ammonia, COD were measured using Hach DR 5000 spectrophotometer according to the standard methods for examination of wastewater. True color was measured with the same spectrometer at 455nm after filtration with 0.45 µm nylon membrane filters.

HO• exposure in wastewater was quantified by following the oxidation of a probe compound p-chlorobenzoic acid (pCBA), which reacts with HO• with a rate constant of $5x10^9$ (Elovitz and von Gunten, 1999) and slowly with molecular ozone. pCBA (Sigma-Aldrich, St. Louis, MO, USA) stock solution was prepared in nanopure water. Wastewater samples were spiked with pCBA at 1.25 µM prior to ozonation. High performance liquid chromatography (HPLC) was used with reverse-phase (RP18) analytical column to measure pCBA concentration in ozonated wastewater samples. A mobile phase consisting of a mixture of 55% methanol and 45% 10 mM phosphoric acid was used. Detection was made using an UV detector set to a wavelength of 234nm. Method detection limit (MDL) for pCBA for the analytical method was determined to be 4µg/l.

Molecular size distributions of DOC were quantified with size exclusion chromatography system (Waters 2695 Separation Module) connected to a multi-wavelength UVA detector (Waters 2996 photodiode Array Detector) and an online TOC detector (Modified Sievers Total Organic Carbon Analyzer 800 Turbo).This system employed TSK 50S column (Tosho Toyopearl HW-50S resin, Japan) with a phosphate buffer eluent containing 0.025 M Na_2SO_4 to produce an ionic strength of 0.1 M or conductivity of 4.57 mS (Her and Amy, 2002). Before analysis, the conductivity of each sample was adjusted by using 40 times concentrated eluent to approach the conductivity of the mobile phase. UV and DOC data were collected every 0.5 second by using Empower chromatography data software. The system was calibrated by using polyethylene glycol (PEG) standards with molecular weight of 600, 1000, 1450, 3350, 4600, 8000, 10000Da.

NDMA formation potentials were determined on samples after adding excessive amount of chloramines for a specific time period. Samples were first added with borate buffer solution to adjust the pH to 8.2. Chlorine was added based on each sample's DOC level (Cl_2=3×DOC) and sufficient amount of ammonia was added to achieve the chlorine-to-nitrogen ratio of 3:1 (weight basis) (Krasner et al., 2009). Then the samples were stored in a dark room at room temperature (20~25ºC) for 3 days. NDMA formed was then extracted and concentrated using solid phase extraction (SPE) Workstation (Caliper Life Sciences), EPA method 521 SPE Cartridge (Restek U.S.). 200ppt NDMA-d6 was spiked into each sample as an internal standard prior to extraction for calculating recovery rates. Agilent G1530N gas chromatography with mass spectrometry in positive chemical ionization mode was used to measure NDMA after extraction. The detection limit was 2ng/L with analysis variation of <5%. Blank (nano-pure water) and control samples (effluent without free chlorine and ammonia addition) were also prepared and measured to ensure quality control.

Result and Discussion

Ozone decomposition in wastewater

Dissolved ozone was rapidly consumed in wastewater. Residual ozone after 30 seconds ranged 1-2 mg/l for an ozone dose of 0.5 mg-O_3/mg-DOC. Across all the WWTPs studied, instantaneous ozone demand (measured as the difference between the ozone dose and residual ozone concentration at 30 seconds of reaction time) correlated well against DOC of the samples at different ozone to DOC ratio. Slope of the lines clearly indicated the tendency of increasing ozone demand with higher ozone dose (Figure 2.1).

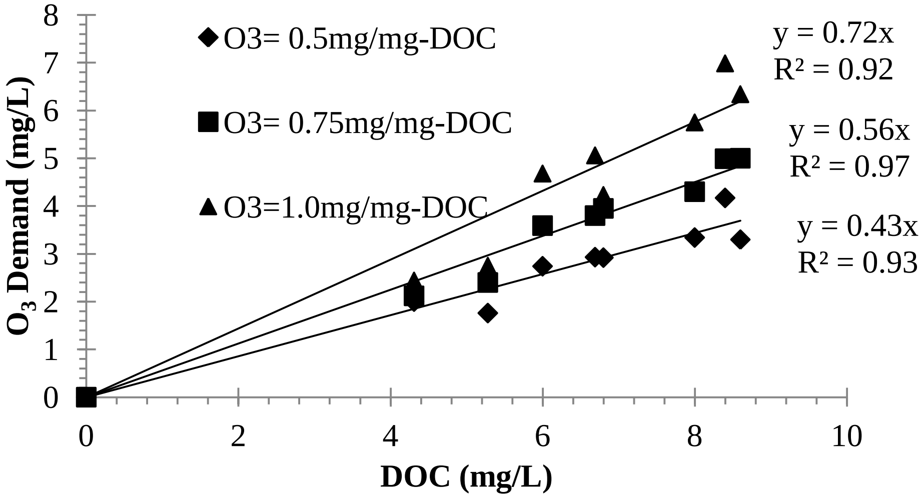


Figure 2.1. Correlation between instantaneous ozone demand and DOC of different wastewater.

This finding is different from studies carried out in surface waters where ozone consumption decreases with increasing ozone dose (van der Helm et al., 2007). Typically wastewaters with higher DOC levels as the result of less effective nitrification or denitrification treatment, which results in more nitrite or ammonia (Krasner et al., 2009b). These reduced nitrogen species are partially responsible for the increased ozone demand in the higher DOC waters.

PCBA removal in wastewater

pCBA was selected as a representative trace organic that reacts slowly with molecular ozone but readily with hydroxyl radicals. pCBA was removed during the first 30 seconds and showed small change over the subsequent 3 minutes. Higher ozone dose caused greater pCBA reduction. For example, pCBA reduction in WWTP 2 increased from 60% to 78% and 84% when ozone dose was increased from 0.5 mg/mg to 0.75mg/mg and 1.0mg/mg ozone dose, respectively.

pCBA removal was plotted against three different parameters- 1) ozone dose, ii) total ozone consumption and iii) ozone consumption by organics only (Figure 2.2 a, b and c). O_3 consumption only by the organics was determined by subtracting the ozone demand of NO_2^- and NH_3 (based on the stoichiometry as described above) from total ozone consumption as shown in equation 1.

Across all wastewater samples, the best correlation was observed between pCBA loss and ozone consumed by the organics (Figure 2.2c). The obtained correlation suggests a tool for predicting trace organic oxidation by HO•. This outcome should not be surprising because organics are good promoters of ozone consumption and initiate chain reactions that yield HO• (Westerhoff et al., 1999), especially in wastewater (Pocostales et al., 2010).

O_3 consumption by organics only = Total O_3 Consumption- $3.43C_{NO2\text{-}N}$-$6.86C_{NH3\text{-}N}$ (2.1)

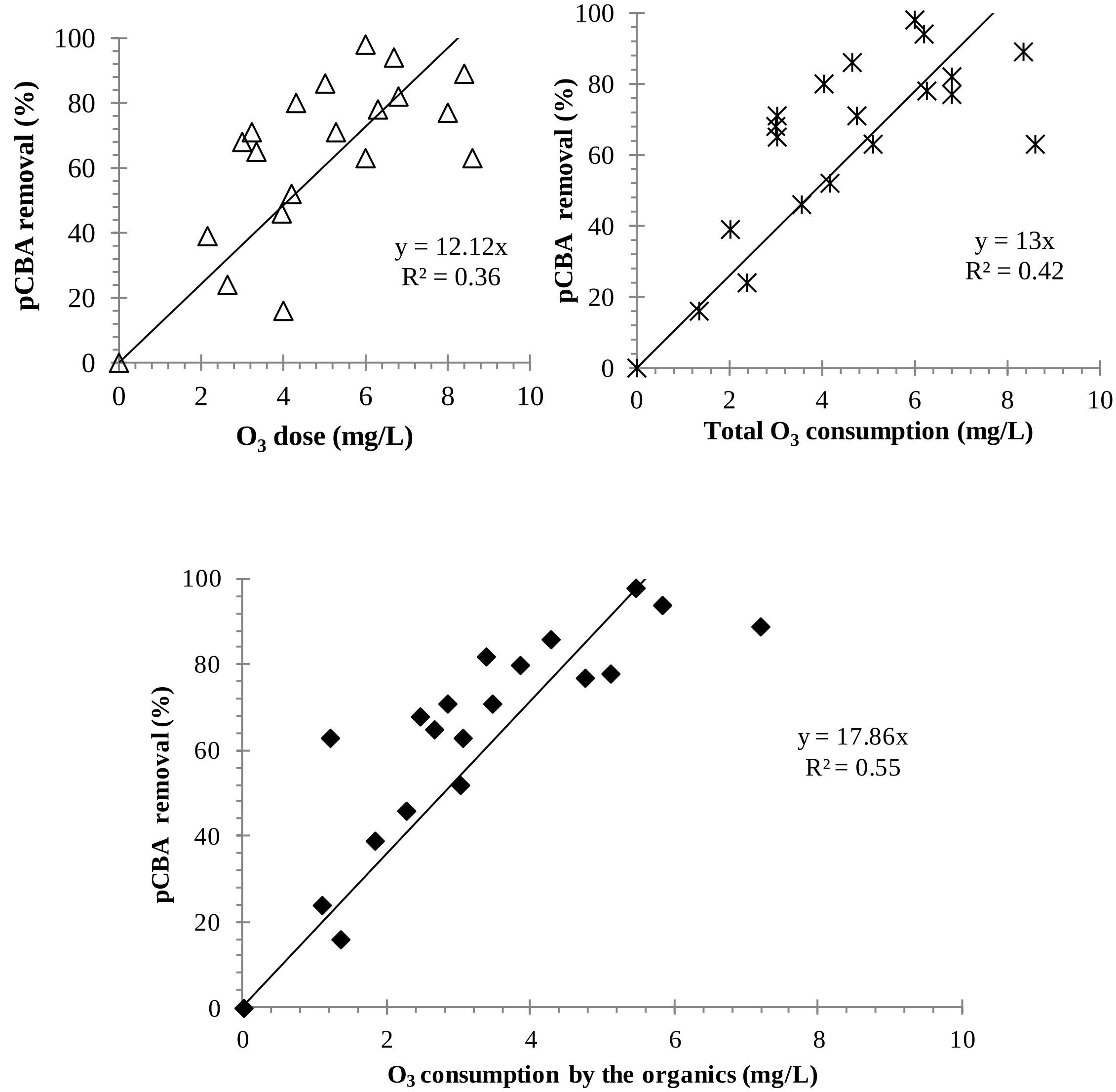


Figure 2.2. Correlation between pCBA removal and O_3 consumption.

Effect of ozonation on color and UV_{254} absorbance

Municipal wastewater has a distinct color that predominantly originates from naturally or artificially occurring carotenoid pigments. Carotenoids are chromophores with double bonds and O_3 can attack double bonds associated with these compounds. Complete color removal was achieved for samples from WWTP 1, 2 and 3 at ozone doses greater than 0.5 mg O_3/mg DOC. Removal of color, and odor, is important as it affects the social acceptance of wastewater for reuse applications.

While we did not directly measure changes in odor, sulfurous and other odors common in wastewater are readily oxidized by ozone (Hwang et al., 1994; Loeb, 2009). Similar to the finding of PCBA removal, ozone consumption by organics only also yielded a good correlation for color removal from wastewater (Figure 2.3).

UV_{254} absorbance was removed only up to 60% compared to the 100 % removal of color at comparable ozone dosages. Good correlation was obtained between UV_{254} removal and ozone consumption by organics only (Figure 2.4), which was better than ozone dose alone. The data suggests that the selected ozone doses were capable of removing aromatic compounds from wastewater regardless of the variation in the treatment processes involved.

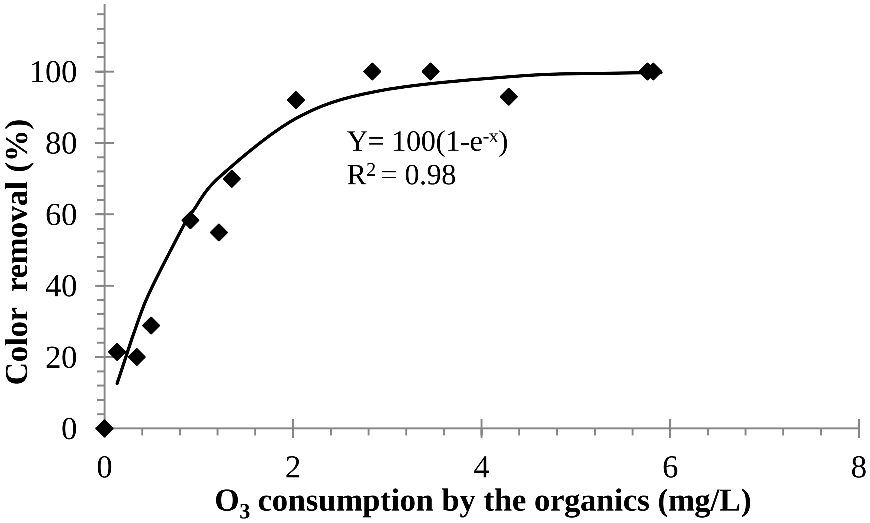


Figure 2.3 Correlation between color removal and ozone consumption.

Ozonation effect on COD of wastewater

COD removal at the selected O_3 doses ranged only from 15% to 45 %. But a unifying relationship between COD removal and ozone consumed by organics alone was not obtained. Within any single wastewater, increasing ozone demand led to greater COD reduction. This is because COD accounts for not only reduced forms of organic carbon, but also reduced nitrogen (e.g., nitrite, ammonia) or other inorganic species which typically occur at lesser concentrations.

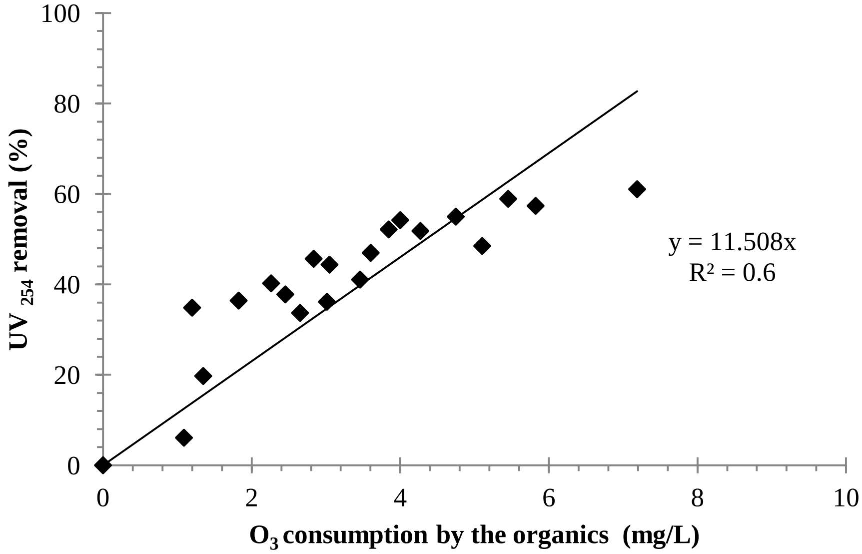


Figure 2.4. Correlation between UV_{254} removal and ozone consumption.

Molecular weight distribution change of ozonated wastewater samples

Ozone oxidized large molecules and produced small size organic compounds which are probably biodegradable. Figure 2.5 shows SEC-TOC chromatograms for different ozone dosages applied to one wastewater sample, but similar trends were observed for the other wastewaters. The distribution can be broadly divided into three groups: colloidal organics above 10,000 Da, medium- 1000 to 10,000 Da and low- 100 to 1000 Da.

Compounds in the high molecular weight range are dominantly colloidal matter (Song et al., 2010). These compounds are responsible for membrane fouling and can sorb micropollutants like PPCPs. The medium range belongs to humic and fulvic substances of effluent organic matter. Low range corresponds to carbohydrates, ketones, aldehydes and other low molecular weight acids. Increasing doses of ozone dramatically reduces the colloidal response and produces low molecular weight organics (rise in the peak around 600 Da). These findings suggest how ozonation could lead to reductions in subsequent membrane fouling, namely by transformation of organic colloids which are well documented membrane foulants (Cohen et al., 1986, Lesjean and Probstein, 2005).

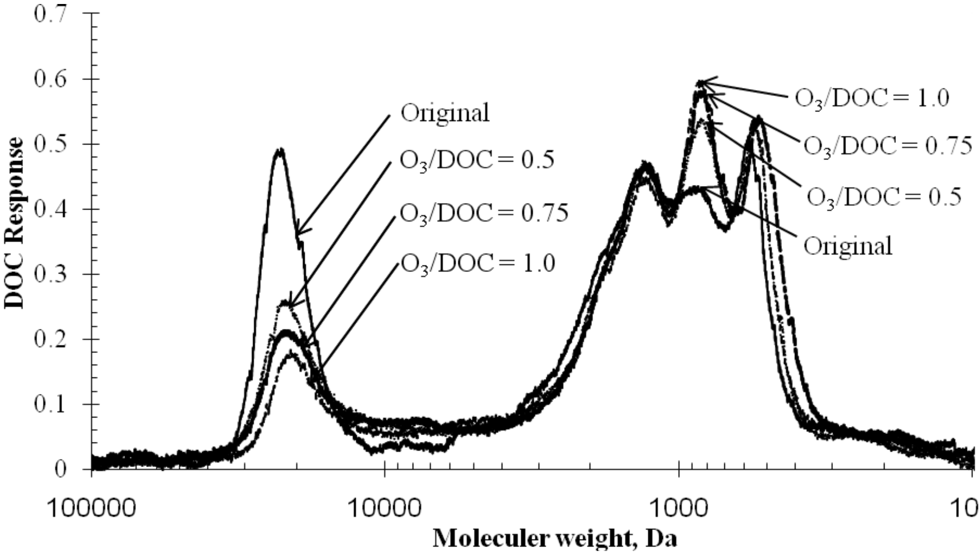


Figure 2.5. SEC-DOC results for ozonated wastewater sample (WWTP 8) at different O_3 doses (mg-O_3/mg-DOC).

Effect of ozonation on NDMA-FP

NDMAFP of wastewater samples (WWTP 1 through WWTP 6) was measured before and after ozonation at four different ozone doses (mg-O_3/mg-DOC = 0.25, 0.5, 0.75

and 1.0). Before ozonation, most of the wastewater samples had NDMAFP values of 400 to 1300 ng/L. For several of the samples NDMAFP was below detection limit at an ozone dose of 1 mg-O_3/mg-DOC and thus are not shown here. Figure 2.6 illustrates that ozonation reduced NDMAFP by 70 to 80% reduction at the lowest applied ozone dose of 0.25 mg-O_3/mg-DOC. Increasing ozone dosages reduced NDMAFP further. The only exception was WWTP 3 which is actually recharged wastewater diluted with native groundwater, and had a low NDMAFP of only 37 ng/L. The results indicated that pre-oxidation by ozone can control the risk of NDMA formation in wastewater effluents.

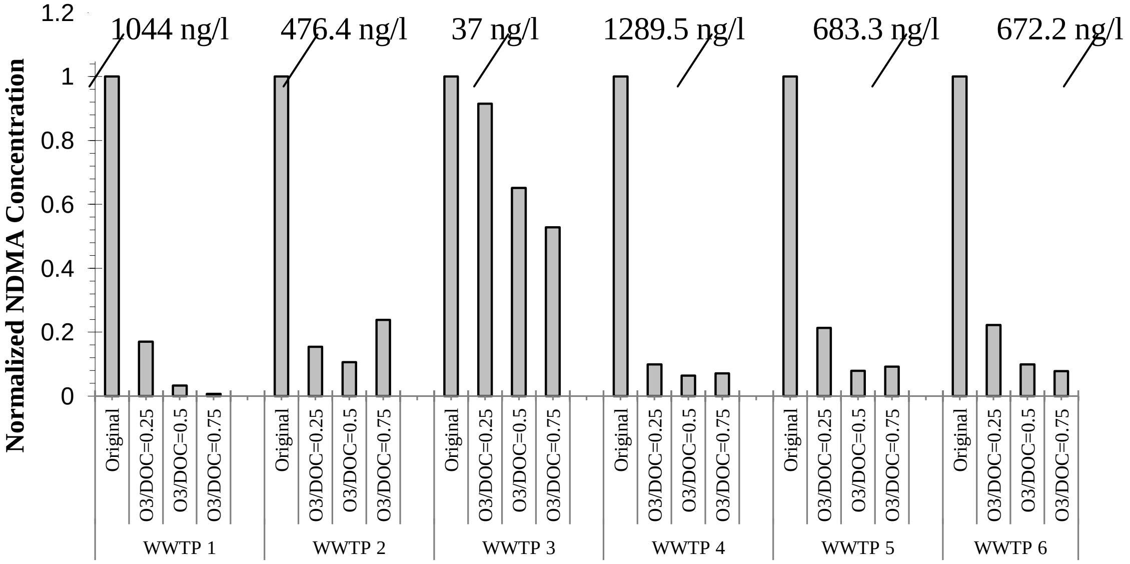


Figure 2.6. NDMAFP reduction of wastewater samples during ozonation (initial NDMAFP prior to ozonation is expressed in ng/L).

Conclusion

This study suggests potential applications and benefits of ozonation on wastewater. Ozonation of wastewater can lead to significant transformation of organic compounds.

However, inorganics present in wastewater exerts significant ozone demand and interferes with ozonation of organic compounds. In this study ozone consumed by organics only was quantified by separating the demand exerted by NO_2^- and NH_3. This provided a unifying series of correlations capable of explaining observed transformation in organics. Transformation of organic compounds during ozonation can lead to several benefits including improved aesthetics (reduced color), reduced potential for membrane fouling (reduced colloidal content), reduced subsequent oxidant demand by chlorine based disinfectants (inferring to reduced UV_{254} response), reduced disinfection byproduct formation (lowering of NDMA FP) and capability to rapidly oxidize trace organics (as indicated by PCBA reduction).

CHAPTER 3

REVIEW ON CONTAMINANTS REMOVAL IN CONSTRUCTED WETLANDS

Introduction

Constructed wetlands are usually designed to remove macro-pollutants and nutrients but these wetlands also hold promises to remove (i.e. polish) different types of CECs. While the removal for macro-pollutants and nutrients were extensively studied, fewer studies exist on CEC removal in constructed wetlands. Wetlands involve natural attenuation processes to treat pollutants and the removal mechanisms for pollutants nitrogen, phosphorus, organic carbon etc. are well-known. Wetlands can remove CECs through multiple removal mechanisms, leading to the removal of a wide range of contaminants. In this chapter, a review is provided on transformation processes involved fin natural attenuation of contaminants, occurrences and removal of CECs in wetlands. The review also highlights the current design criteria used for constructed wetlands including hydraulic loading rates, wetland depth, carbon loading rates, etc. and their effect on contaminant removal.

Natural attenuation process for CECs in constructed wetlands

CEC removal in wetlands is similar to any other natural system which involves in-situ physico-chemical and biological processes that can cause natural attenuation. Several organizations have defined natural attenuation, a comprehensive definition is given by US Environmental Protection Agency (US EPA, 1999):“*The 'natural attenuation processes' that are at work in such a remediation approach include a variety of physical, chemical, or biological processes that, under favorable conditions, act without human intervention to* *reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater. These in-situ processes include biodegradation; dispersion; dilution; sorption; volatilization; radioactive decay; and chemical or biological stabilization, transformation, or destruction of contaminants."*

Even though the definition only includes soil or groundwater, it is also applicable for surface waters or any other places where contaminants are subjected to attenuation processes occurring naturally. Attenuation of chemicals under natural conditions without applying any additional treatment step is cost-effective and environmental benign. Increasing presence of newer classes of contaminants in wastewater effluents enhances the risk of reaching these pollutants to surface waters where they may be attenuated or polished in constructed wetlands in an ecologically friendly way.

There are two major mechanisms that are responsible for attenuation of compounds occurring naturally in rivers/streams or treatment wetlands: (i) abiotic and (ii) biotic. Abiotic processes include volatilization, hydrolysis, sorption and photodegradation. Biotic processes are microbiologically driven mechanisms and commonly referred as biotransformation. The loss of contaminant is represented often by a net reaction term r_T which is the sum of the rates of the various abiotic and biotic processes-, photolysis (r_P), sorption (r_S), volatilization (r_V), hydrolysis (r_H) and biodegradation (r_B) (Equation 3.1). Most CECs especially PPCPs have low Henry's law constants and the resistant against strong bases and acid, so both volatilization and hydrolysis can be neglected for the discussion here. The expression to defines these process are summarized in Table 3.1.

$$r_T = r_v + r_p + r_s + r_H + r_B \qquad (3.1)$$

Table 3. 1

Expressions for contaminant removal mechanisms

Mechanism	Generalized equation	Parameters
Volatilization	$r_v = \frac{k_v}{H_1} . \left(\frac{[C_g]}{H_c} - [C]\right)$	K_v , K_P, K_S, K_H and K_B = Rate constants for volatilization, photolysis, sorption, hydrolysis and biodegradation (1/Time)
Photolysis	$r_p = -K_{P.}[C]$ $= -[C].\varphi_{solar}.\frac{2.303}{j}.\sum_{\lambda} I_\lambda . \varepsilon_\lambda$	
Sorption	$r_s = -K_s.[C] = -K_{ad}.M_S.[C]$	C = Concentration of the contaminant
Hydrolysis	$r_H = -K_H.[C]$ $= -[C].(K_a.[H^+] + K_b.[OH^+])$	φ_{solar} = Quantum yield I_λ, = Flux of solar energy (photons/cm^3-s)
Biodegradation	$r_B = -K_B.[C]$	ε = Molar adsorptivity (L/mol-cm) H_c = Henry's law constant (unit less) K_a and K_b = Hydrolysis rate at acid and base conditions (L/mol-time)

Where, K_v , K_P, K_S, K_H and K_B are rate constant for volatilization, photolysis, sorption, hydrolysis and biodegradation (1/Time) respectively. C is the concentration of the contaminant, φ_{solar} is the quantum yield, I_λ, is the flux of solar energy (photons/cm^3-s), ε is molar adsorptivity (L/mol-cm), H_c is the Henry's law constant (unit less). K_a and K_b are the hydrolysis rate at acid and base conditions (L/mol-time) respectively.

Sorption of Organic and ENM CECs

Sorption of PPCPs to solid sorbents such as sediments, plants, suspended solids is a function of both sorbent media properties including organic content, surface reactivity and specific area and sorbate properties such as log K_{ow} and pK_a (Lorphensri et al, 2003, 2006). The pH of the water matrix has a great influence on sorption. It affects surface charge of the sorbents as well as dissociation of the organic molecules. The pK_a, which establishes the fraction of protonated and deprotonated form of ionizable functional groups of a contaminants, is the pH at which equal concentrations of protonated and deprotonated species of a given ionizable compound are present. Between the protonated and deprotonated species, only the protonated or un-ionized species will be the predominant fraction to partition on to surfaces. The term log D_{OW} considers the effect of both pK_a and pH of the water matrix and it can predict sorption affinity of organic contaminants more accurately. When pH is lower than the pKa values, organic CECs remain mostly in undissociated forms and sorption by hydrophobic partitioning becomes favorable. With increasing pH, dissociated fraction of the ionizable organic CECs increase and sorption can decrease. Sorption of organic CECs with higher pKa values (pKa > 10, e.g. E2, carbamazepine etc.) will not be affected significantly in wetlands with typical pH range of 6 ~ 8.Similarly, diurnal fluctuation will not affect CECs with lower pKa values (< 3, e.g. atrazine) which will remain in dissociated forms within that pH range.

Hydrophobic partitioning was found to be the major reason for sorption of PPCPs including estrogens, actominophen, propranolol, atenolol and others (Scheytt et al., 2005, Lee et al., 2003, Lorphensri et al, 2003, 2006). Among these neutral PPCPs, the ones with high log K_{ow}, for example estrogens (log K_{ow} ~ 3-4) showed greater sorption ability. Alternatively, ionic compounds like nalidixic acid ($pK_a = 5.95$) exhibited strong sorption towards low organics sands, much higher than expected based on its hydrophobicity (log $K_{ow} = 1.54$) supports the fact that electrostatic forces dominates sorption of these compounds.

Partitioning can also occur on organic matter of suspended solids. Wastewater effluents may contain solids of various fractions depending on the treatment processes the raw wastewater was subjected to. Solids are also produced due to death of invertebrates, fragmentation of detritus from plants, production of plankton and microbes. Partitioning on the suspend organics is often quantified using log K_{OW} and f_{OC} (fraction of organic matter in suspended solids).

Over all sorption and mainly partitioning on to surfaces has been considered as one of the major removal mechanisms involved in natural attenuation of organic CECs. The major surface area of constructed wetlands includes plants submerged in the wetland water where sorption or partitioning can take place. It is important to quantify the sorption affinity for organic CECs towards wetland plants and compare with their respective log D_{OW} to find out if the hydrophobic partitioning is the main mode of sorption in constructed wetlands.

Sorption and plant uptake can be a major removal mechanism for ENMs. During long-term exposure, Ag ENM uptake by wetlands plants was reported by Lowry et al., 2012. Plant uptake of ENMs can depend on multiple factors. CeO_2 nanoparticle uptake by rice seedling increased with increasing its concentration (Rico et al. 2013). Size and surface charge were found to influence uptake and subsequent translocation of gold ENMs onto different species of plants (Zhu et al., 2012). Although the mechanisms are unknown, these findings support the role of plants in removing ENMs from constructed wetlands.

Aggregation of ENMs

Aggregation is a combination of both physical (collision) and chemical (attachment) process. For ENMs, two types of aggregation can occur- (i) homoaggregation where NMs can aggregate themselves or (ii) heteroaggregation where NMs deposit on to suspended particles and form aggregates. The first type of aggregation becomes dominant at high ionic strength without the presence of any suspended or dissolved organic matter. The second type of aggregation becomes common when colloidal particles are available. Solid fractions of natural colloids are considered to cause heteroaggregation of NMs followed by sedimentation (Quick et al., 2012). NM aggregation also depends on multiple factors including ionic strength, pH of the solution surface coating or functionalization (Fortner et al., 2005, French et al., 2009). At low concentration of $CaCl_2$, Chen and Elimelech, 2007 found aqueous fullerenes to form stable aggregates by adsorption onto humic acids, however at high concentration of $CaCl_2$, enhanced aggregation occurred by bridging between the nanoparticles and humic acids. Conversely, natural organic matter was found to inhibit aggregation of CNTs and several other metal oxide nanoparticles (Ganesh et al., 2010, Hyung et al., 2007, Gao et al., 2009).

Surface-coating of ENMs can influence aggregation. ENMs are often coated with organic compounds, known as surface functionalization. Once released into the environment, chemical or biological degradation can modify these surface coatings. Coated NMs can also lose their organic coating and undergo rapid aggregation (Jarvie et al., 2009). In a study by Li and Leinhart, 2012, tween coated silver NMs were found to resist aggregation than bare or citrate coated silver NMs in natural waters. However, enhanced aggregation of citrate and polyvinylpyrrolidone (PVP) coated silver NMs were observed when humic acids were present with higher concentration of $CaCl_2$ (Huynah and Chen, 2011). PVP-coated silver NMs, known to be stable in water also failed to resist aggregation when sulfides were present (Levard, et al., 2011). The extent of aggregation also depends on the isoelectric point of the NMs and pH of the solution (Ganesh et al., 2010). The term pH_{ZPC} or the zero pint of charge represents the pH where net surface charge of is zero. For NMs, removal efficiency increases when pH values are close to pH_{ZPC} (Dunphy et al., 2006).

Aggregation is one of the most important mechanisms for ENM removal in wetlands. This process will be dependent on physico-chemical properties of the ENMs as well as the properties of the wetland water. It is important to investigate how ENMs with different characteristics (size, surface charge, coating, etc) will be influenced by the wetland water matrix properties.

Photodegradation of Organic CECs and ENMs

Photodegradation of contaminants in water may occur via two pathways, direct and indirect photolysis. Direct photolysis proceeds as a compound absorbs light, leading to change in its structure through bond cleavage. Indirect photolysis occurs via light absorption by photosensitizers, such as dissolved organic matter (DOM), nitrate and nitrite. These photosensitizers are excited by photons and generate singlet oxygen ($^1O_2$), OH radicals (OH•), DOM-derived peroxy radicals (ROO•) and other photoreactants (Lin and Reinhard, 2005*)*. PPCPs that are resistant towards direct photolysis can be degraded by indirect photolysis.

Direct photolysis rates of organic chemicals vary in natural surface waters, corresponding to the light intensity at different latitudes. Irradiation of the diluted samples is carried out typically with natural sunlight versus solar simulators, such as xenon lamp. The measured rate constants are then converted to the rate constants under near surface sunlight conditions by applying appropriate factors to consider differences of light intensity, light penetration, and the time a chemical spends in the phototransformation layer due to mixing (Liu and Williams, 2007, Robison et al., 2007*)*. Direct photolysis of organic CECs depends on the absorbance spectra which are influenced by pH. Protonation or deprotonation is affected by solution pH and for many CECs, deprotonated forms can react faster than the protonated forms (Tratnyek et al., 1991). Photochemical pathways involving photosensitizers such as dissolved organic matter (DOM) or nitrate can be favored under certain pH conditions (Miller & Chin, 2005). Indirect photolysis by radical species like singlet oxygen ($^1O_2$), OH radicals (OH•) and carbonate radicals ($\bullet CO_3^-$) can also depend on pH (Vione et al., 2011).

The rates of all photochemical processes in a water body are affected by solar spectral irradiance at the water surface, radiative transfer from air into water, and the transmission of sunlight in the water body (Zepp and Cline, 1977). The intensity of sunlight is attenuated in natural waters through absorption and scattering. As sunlight passes down through a water body, the light intensity decreases and its spectral distribution changes. The decrease in photolysis rate with increasing depth depends upon the magnitude and spectral distribution of the attenuation coefficients of the water body, the molar extinction coefficients of the pollutant, and the intensity values of sunlight. PPCPs that are most viable to photodegradation are analgesic drugs including diclofenac, naproxen, ketoprofen, antimicrobials like triclosan, β-blockers such as propranolol, atenolol, and metroprolol (Buser et al., 1998, Tixier et al., 2002, Morrall et al., 2004, Fono et al., 2006, Lin et al., 2007, Liu and Williams, 2007, Robison et al., 2007, Yamamoto et al., 2009). Among these compounds, diclofenac and propranolol are the two most photosensitive compounds, eliminated primarily by photolysis. Estrogens are predominantly removed by biodegradation and sorption; however, direct photolysis of these compounds is also possible.

Photolysis of ENMs can be possible which can change their structure and properties. Sunlight photolysis of Ag ENMs was found to cause reduction in size followed by aggregation (Gorham et al., 2012).Toxicity of Ag ENMs was also reduced when subjected to UV light photolysis (Poda et al., 2103). Photochemical activity was also found in stable aggregates of fullerenes ($n\mathrm{C}_{60}$) leading to generation reactive oxygen species and cytotoxicity (Lee at al., 2009).

In natural waters including constructed wetlands, sunlight photolysis can be a major removal mechanism for CECs. It is most important for the CECs which are not easily susceptible to other removal mechanisms e.g. sorption, biodegradation etc. Existing literature have showed evidences of photolysis for a number of CECs, but the actual contribution of photolysis will be dependent on the penetration of light through the depth and the level of surrounding species in the water that causes light attenuation.

Biotransformation of Organic CECs

Biological processes can also remove CECs from wetlands. Enzymes belonging to different bacterial communities are capable of using these contaminants for metabolism (i.e. as substrates or energy). Organic compounds can also be transformed by co-metabolism, where they are degraded as secondary compounds without being utilized as primary energy or food source.

Biodegradation/biotransformation in wetlands mostly occur in rhizospheres where plant surfaces support biolfilms growth and decaying plants provide organic carbon necessary for biodegradation. Organic rich sediments also support microbial degradation of organic contaminants. Biodegradation can also occur after the organic molecule has sorbed on to sediments. Estrogens were found to be immobilized on sediments due to hydrophobic partitioning, following which they were mineralized via aerobic biodegradation (Bradley et al., 2007). Most CECs like PPCPs are not present at high enough concentrations to support primary metabolism of the microbes. Thus, biotransformation due to co-metabolic activity is supposed to be main mechanism of degrading these compounds biologically. Steroids like EE2 were found to biotransform co-metabollically under conditions that allowed enrichment of nitrifiers (Vader et al., 2000, Shi et al., 2004, Yi et al., 2007, Bradley et al., 2009). EE2 and ammonia were simultaneously degraded in enriched autotrophic or mixed nitrifying cultures mediated probably by coenzyme-referred as ammonium monooxygenase (AMO). Rivers and wetlands receive wastewater effluent containing microbial populations that are capable of degrading various contaminants. The indigenous microbes are also stimulated by the supply of nutrients, cometabolites that are coming with the waste stream. Alternatively, toxins such as biocides, antibiotics, toxic metals can inhibit and compete for carbon substrates or deplete efficient electron acceptors like oxygen. For example, wastewater indicator compounds like caffeine, cotinine, nicotine were consistently found to be inhibited downstream of WWTPs.

Biodegradation can occur in either aerobic or anaerobic conditions. Aerobic processes are faster because of the presence of oxygen whereas, anaerobic processes are slow as the microbes involved in these processes need time to adapt to the existing redox conditions, nutrient availability etc. For example, both triclocarban and triclosan can be degraded by microbial processes in soil under aerobic conditions. But both compounds are highly resistant to biodegradation in soil under anaerobic conditions and tend to persist (Ying et al., 2007, Radke et al., 2010). However, triclosan is more biodegradable than triclocarban in aerobic soil as the OH group in aromatic compounds usually enhances their biodegradability and NH_2 or NH group has a negative effect on biodegradation. In anoxic conditions, reductive dechlorination is a major way of removing organochloride compounds. Triclocarban transformation products were detected in deep sediment zones suggesting that anaerobic degradation of triclocarban is possible however; the process will be slow compared to faster aerobic degradation that may occur in surface sediment layers (Miller et al., 2008).

Due to its versatile nature, biotransformation can support removal of a number of CECs in wetlands or other aquatic environment. Often certain biochemical conditions (nitrifying, denitrifying etc.) of wetlands can be helpful to increase CEC removal. Because the rate and extent of biotransformation depends on multiple factors including types of microbial population, availability of nutrients and labile organic matter, presence of oxygen or other electron acceptors that also controls the different biochemical processes of wetlands.

Occurrence and Removal of CECs in Constructed Wetlands

Pharmaceuticals and personal care products (PPCPs)/Endocrine disrupting contaminants (EDCs)

EDCs and PPCPs represent a wide variety of organic compounds with varying physico-chemical properties. Both naturally occurring and manmade compounds belong to this class including natural or synthetic steroidal hormones, pesticides, herbicides, polyaromatic hydrocarbons, alkyl-phenols (APs), phthalates, alkyl-phenol polyethoxylates (APEs) and many other industrial chemicals. A number of studies evaluated PPCP removal in constructed wetlands (Reed and Crites, 1995, Brix, 1987, Matamoros et al., 2005, 2006, 2008, 2009, Huang et al., 2004, 2005, Conkle et al., 2008). Liner alkylbenzene sulfonates were removed up to ~70% in shallow bed more oxidized zones of wetlands (Huang et al., 2005). Gemifibrozil, sulfamethoxazole, galaxolide, tonalide were partially removed (50~80%) and the least removed compounds were carbamazepine, diclofenac, ketoprofen and clofibric acid; almost showing no removal in the wetlands (Part et al., 2009, Matamoros et al., 2009). Mean removal efficiency of analgesic drugs including ibuprofen, diclofenac, ketoprofen, naproxen, salicylic acid and other PPCPs like caffeine, clofibric acid and methyl dihydrojasmonate exceeded 75% in several constructed wetland studies (Matamoros et al., 2005, 2006, 2009, Hijosa-Valsero et al., 2010). Estrogenic hormones were also detected in wetland water samples with median concentration of 5 to 10 ng/L (Gray and Sedlak, 2005). Shallow wetlands with dense plant roots were ideal for removing estrogens with removal efficiency reaching ~90% (Song et al., 2009).

The combined effect of vegetation and various microscopic environments are considered to cause PPCP/EDC removal in constructed wetlands. However the contributions from specific removal mechanisms supported by the various elements of wetlands are not known. Several of the studies mentioned in previous paragraphs tried to justify which mechanisms were possibly responsible for the removal observed during investigation. Ibuprofen (log K_{OW} 3.97) was found to remove in shallow zones of s submerged wetlands indicating sorption and aerobic biodegradation as the main removal mechanisms (Matamoros et al, 2005, 2006). Clofibric acid (log K_{OW} 2.84), carbamazepine (log K_{OW} 2.45) and ketoprofen (log K_{OW} 3.12) experienced least removal and these CECs were considered refractory (Matamoros et al, 2005, 2006, 2009). Caffeine (log K_{OW}= -0.07) concentration in the influent of wetlands were found very high compared to other PPCPs. Matamoros et al, 2006, 2007 observed efficient removal of caffeine in wetlands although the removal mechanisms were not specified. Salicylic acid (log K_{OW} 2.24) also experienced significant removal in wetlands (Matamoros et al, 2006, 2007, 2009). Both caffeine and salicylic acid are not likely to experience greater removal by sorption because of their low hydrophobicity; their high removal must have been associated with biodegradation or plant uptake. Matamoros et al, 2006, 2007and 2009 presumed that oxic conditions of the vertical flow submerged wetlands was mainly responsible for PPCP removal supported by biodegradation. Salicylic acid is readily biodegradable but fast biodegradation of caffeine is only possible in anaerobic conditions (EPI Suite). Later in another study by the same group, caffeine removal was found higher at low reodx potential indicating anaerobic conditions was likely to cause higher removal of caffeine (Hijosa-Valsero et al., 2010a).

Based on this review, constructed wetlands hold potential to remove a wide range of PPCPs/EDCs to levels that are much comparable with conventional WWTP treatment processes. However, most of these studies reported range of removal efficiencies for a number of contaminants while the actual removal mechanisms and their contribution remained unidentified.

Pesticides

Although pesticide occurrence and removal is separately discussed in this review, a number of these contaminants are considered as EDCs. Growing bodies of literature have shown evidence of endocrine disruption by pesticides detected in WWTP effluent (Hemming et al., 2001, Vajda et al., 2008, Jenkins et al., 2009). Atrazine, a commonly used pesticide was found to be linked with breast cancer occurrences (Hunter et al., 2008).

Constructed wetlands are often used as a buffer between agricultural fields and receiving water bodies to mitigate frequently detected pesticides and herbicides (Brix, 1997, Reed et al., 1995, Moore et al., 2000, 2002). Organophosphate pesticide azinphos-methyl (AZP), chlorpyrifos, endosulfan were found to experience partial removal in wetlands (Schulz et al., 2003,2004, Schulz et al., 2003). Atrazine, a highly persistent pesticide was partially removed in wetlands with effluent concentration varying from 1~2 µg/L (Alvord and Kadlec, 1996). Herbicides, including simazine and metolachlor, were detected in wetland effluents with peak concentrations varying between 750 and 300 µg/L, respectively (George et al., 2003).

Constructed wetlands are well known to remove pesticides. Like PPCPs and EDCs, removal mechanisms for pesticides in wetlands are also not clear. It is also important to find out whether certain biochemical conditions of wetlands favor their removal.

Engineered Nanomaterials (ENMs)

Unlike the organic contaminants, fewer studies exist relating to occurrence or fate of ENMs in wetlands. However, Ag NMs have been extensively studied to understand their fate in the environment (Kaegi et al., 2010, Lowry et al. 2012, Levard et al., 2012a, 2012b). Ag NMs was found unstable in oxic environments and vulnerable to dissolution (Levard et al., 2012a). Once released, sulfidation of Ag^+ is considered to be one of the main transformation mechanisms (Levard et al., 2012a, 2012b). Ag_2S was found as the main products in WWTP effluent and biosolids (Kaegi et al., 2010, Lowry et al. 2012). At high concentrations, no significant effect of Ag NMs was observed on anaerobic digestion processes or methanogenic bacteria (Yang et al., 2012). This was probably due to rapid transformation of Ag NMs into Ag_2S because of high levels of sulfide being present in anaerobic digestors. Sulfidation of Ag NMs is also probably one of the most important transformation mechanisms in natural environment including wetlands. In oxygen limiting conditions of wetlands where decaying plants produces organic sulfides, sulfidation can become an important transformation mechanism for Ag NMs. Fate of Ag NMs in wetlands was very recently studied by Lowry et al., 2012. Ag NMs in the water column was mostly found as Ag_2S and Ag-sulfhydryl compounds. However, sulfidation of Ag NMs is considered to be slow process and depends on the quantity of sulfide species present. Despite of their transformation, Lowry et al., 2012 found a significant amount of Ag NMs to accumulate in plants and aquatic organisms of wetlands. Interestingly, at low concentration of sulfide, dissolution to Ag^+ and subsequent formation of Ag_2S is considered to be the main mechanism for Ag NM transformation, however, at high concentrations, sulfidation occurs by direct conversion of Ag NMs to Ag_2S NMs (Liu et al., 2007).

The existing literature supports the role of constructed wetlands to remove ENMs. Multiple mechanisms are associated with ENM removal. For Ag NMs, accumulation on plants and organisms and sufidation has been identified as the major processes to affect their fate in wetlands. However, there are many other types of metallic or carbonaceous NMs and their transformation or removal in wetlands need investigation. It is also important to find out which parameters of wetlands influence these transformation processes. Research efforts are also needed to understand the contribution from the mechanisms which are known to influence organic CEC removal but currently unknown for ENMs. In constructed wetlands, where plants provide dominant surface area, it is important to investigate the interaction of wetland plants with ENMs parallely with the organic CECs.

Selection of CECs (Organics and ENMs)

Wastewater effluent contains plethora of organic CECs but our goal was to select as few compounds as possible having different physico-chemical properties because variation in the properties can determine the relative strength of the CECs for undergoing certain attenuation mechanisms. Selection of compounds from different classes (e.g. pesticides, pharmaceuticals, steroids) also provides an opportunity to represent other similar compounds belonging to the same classes that are also found in environment. Organic CECs used in this dissertation included atrazine, 17β-estradiol (E2), testosterone, caffeine, carbamazepine and N-Nitrosodimethylamine (NDMA). Atrazine is one the most widely used pesticides/herbicides in United States and often detected in concentrations greater than the maximum contaminant level (MCL) for human consumption regulated by EPA. It is also one of the most common compounds detected in influents of wetlands treating exclusively agricultural runoff (Vanderford et al., 2006, Loos et al., 2007). 17β-estradiol (E2) is widely considered in studies for its toxicological effects on aquatic organisms. Even at low concentration level (~ng/L) range, estrogenic activity from this organic CECs can be present. Unlike the natural or synthetic estrogens, much less effort has been directed for studying behavior of testosterone in natural waters. Testosterone can represent androgenic hormones, which will most likely be considered in toxicological studies for its endocrine disrupting effects.

Caffeine has been identified as an anthropogenic marker for wastewater contamination of surface waters (Guo and Krasner, 2009, Young et al., 2008). Constructed wetlands were also found to remove caffeine to a good extent (Matamoros and Bayona, 2006). Carbamazepine is ubiquitous in surface waters because of its low removal rate in WWTPs and persistent behavior (Herber et al., 2001, Ternes, 2008, Guo and Krasner, 2009). It is not easily biodegradable and can be removed predominantly by sorption to hydrophobic surfaces. It is considered to be a good indicator compound for WWTP effluent due its stability and persistence in the environment, exhibited long half-lives for photolysis and biodegradation processes. Finally, carcinogenic disinfection by-products (DBPs) like N-nitrosodimethylamine (NDMA) are likely to form during disinfection of WWTP effluent (Drewes at el., 2006). Even NDMA is known to have a short half-life when subjected to photolysis, it has been detected in effluent dominated streams and other aquatic environments (Schreiber and Mitch, 2006, Guo and Krasner, 2009). The selected CECs and ENMs with their principal physico-chemical properties are summarized in Table 3.2.

Table 3. 2

*Physico-chemical properties of selected organic and ENM CECs**

Trace organic contaminants/ ENMs (Class)	Molecular weight (MW) (gm/mole)/Size	pK_a/Charge	Log K_{OW} [Log D_{OW} @ pH 7.5]
17β-Estradiol (E2) (Steroid)	272.4	10.4	3.94 [2.46]
Atrazine (Pesticides)	215.7	1.7	2.61 [0.84]
Testosterone (Steroidal hormones)	288.4	-	3.32
Carbamazepine (Pharmaceuticals)	13.9	2.25	2.25 [0.44]
Caffeine (Stimulant)	194.1	10.4	0.16 [-1.31]
Primidone (Pharmaceuticals)	218.25	11.6	0.91 [-0.66]
Functionalized nano silver (Carboxy coated)	10 nm	Zeta potential = -47.1 mV (@ pH = 7.0)	NA
Aqueous fullerene (aq-nc_{60})	80 nm	pH= 6.5(in water)	NA

* Source: EPI Suite^{TM} (U.S. EPA)

To study ENM removal in constructed wetlands, aqueous fullerenes (aq-nC_{60}) and functionalized nanosilver was selected. Fullerene and nanosilver are the most frequently used ENMs that can be found in a variety of consumer and commercial products (Woodrow Wilson International Center for Scholars. 2013). Researchers are now focusing to use this NM for many novel applications. With the increasing use, possibility is there that fullerenes will reach WWTPs and may contaminate surface waters. Functionalized silver nanoparticles have wide applications for their antimicrobial, antibiotic antifungal and antiviral properties. They can accumulate and spread toxicity in aquatic environments. Removal of silver ENMs in WWTPs, their occurrence in environmental matrices and toxicological effect recently received significant interests (Lowry et al., 2012).

Design Criteria Used for Constructed Wetlands

Constructed wetland design parameters can influence pollutant removal including CECs. Among the many parameters used in wetland design, few are critically important which links the size of the wetlands with contaminant removal. Hydraulic residence time (HRT) or hydraulic loading rate (HLR) is probably the most influential design parameter of wetlands. HRT, also expressed as τ is an estimate of the average time water requires to flow completely through the wetland. The actual residence time may be less than the calculated value, due to preferential flow. HLR, also expressed as ‘q’ refers to flow rate per unit area or depth divided by HRT.

$$\text{HRT: } \tau = V/Q \tag{3.2}$$

$$\text{HLR: } q = Q / A = h / \tau \tag{3.3}$$

Where, V = wetland volume, Q = average flow rate, h= depth of water and A = wetland area

HRT for constructed wetlands are suggested from 4 to 15 days depending on types of contaminants and treatment goals (Tchobanoglous and Burton, 1991). HLRs typically vary from 0.7–5 cm/d for constructed surface flow wetlands and 2–20 cm/d for constructed subsurface flow wetlands (USEPA, 2000). Treatment efficiency can be improved by decreasing HLR by operating wetlands at longer HRTs. This can lower loading rate, provide more contact time and consequently result into higher efficiency in removal of pollutants. However, HRT, as shown in the above expressions is directly related to the size of the wetlands and need to be optimized to obtain adequate removal at reasonable cost. Also, increasing HRT beyond a certain level may not always improve removal rates considerably.

Longer HRT has been found to cause greater nutrient removal by several studies (Metcalf and Eddy, 1991, Tanner et al., 1995, Ghosh and Gopal, 2010). Typical HRT is ranged from 4 to 15 days, however, wetlands with relatively shorter HRTs (3-4 days) were also found to achieve good removal of pollutants (Metcalf and Eddy, 1991, Ghosh and Gopal, 2010). HRT affects the presence of oxic and anoxic zones in wetlands, which influence biodegradation of organic contaminants including PPCPs and EDCs. For example, removal of liner alkylbenzene sulfonates declined when HRT was increased as it changed the geochemical conditions of wetland from aerobic to anaerobic (Park et al., 2009). But increasing HRT also increases the organic carbon in wetlands which drives biodegradation. Ibuprofen removal in wetlands were significantly affected by HRT (Matamoros et al., 2009).

Water depth is an influential factor especially in the performance of sub-surface flow wetlands. Beds with a smaller depth generally remove more COD, BOD, ammonia and dissolved reactive phosphorus (Matamoros et al, 2008, Huang et al., 2004, 2005). Shallow depth creates aerobic region near the surface wetlands and enhance removal of organic pollutants (Huang et al., 2004, 2004). Vegetated wetlands with shallow depth were found to outperform better than other conventional secondary WWTP effluent treatment processes including biological filtration (Matamors et al.,2009, and Hijosa-Valsero et al., 2010b). Depth can affect redox status of a wetland consequently relative importance of different biochemical reactions. Redox potential is an important physico-chemical parameter of the medium influencing especially biodegradation of organic compounds. For example, in shallow depth wetlands, denitrification (which is energetically favorable for biodegradation) was found to dominate, whereas in deeper wetlands, sulfate reduction and methanogenesis were prevailing (Huang et al., 2004). Deeper wetlands also experience clogging, and face a decline in HRT leading to a decrease in the removal efficiency of pollutants (Matamoros et al, 2008).

Carbon loading rate represents the productivity of productivity of emergent macrophytes in wetlands. Carbon loading rates are usually based on the productivity of emergent macrophytes in wetlands. For example, this rate can vary from 3,000-4,500 g dry weight m^{-2} $yr^{-1}$for temperate climate and 6,500-8,500 g dry weight m^{-2} yr^{-1} for tropical climates (Ingersoll and Lawrence, 1998). The role of wetland plants other than providing surface area for microbial growth has not been investigated yet. Highly vegetated wetlands showed better performance in removing nutrients and pollutants (BOD, COD, TSS, TKN, TP) when compared to unplanted systems (Naylor et al., 2003, Kaseva, 2004). However the specific role of organic carbon released by the plants was not considered for CEC removal. The effect of carbon addition was only considered in studies dealing with nutrient (NO_3^-) removal which found that denitrification rates proportionately increase with carbon supply (Gersber et al., 1985, Ingersoll and Baker, 1998). Areal nitrate removal rate exceeded 4 g N m^{-2} d^{-1} for a carbon loading rate of 12,000 g dry weight m^{-2} yr^{-1} which is almost 10 times more than the average nitrogen removal rate in treatment wetlands (Gersberg et al., 1983, Ingersoll and Lawrence, 1998). It should be mentioned that areal loading rate (ALR) is another common design parameter used in designing wetlands. The ALR is the maximum removal rate of a pollutant mass per unit surface area of a wetland per daily input. The equation to determine ALR is shown below

$$ALR = Q \times C / A_{surface} \quad (3.4)$$

Where C is the concentration of the pollutant, $A_{surface}$ is the surface area of the wetland.

Other design parameters such as aspect ratio, macrophyte species, and filter bed materials were not found to influence removal efficiency in constructed wetlands significantly (Huang et al., 2004, 2005, Brisson and Chazarnec, 2009). Difference in experimental conditions and wetland morphology makes it harder to generalize or come to an agreement whether these parameters need to have more importance in designing wetlands to remove CECs.

Summary

Based on the literature review on CEC occurrences and removal in wetlands, few major areas where research is needed to resolve key issues include the followings:

- Sorption on to surfaces has been considered as one of the potential removal mechanisms for organic CECs. The major surface area of constructed wetlands includes plants submerged in the wetland water where sorption or partitioning can take place. There is a need to quantify the sorption affinity for organic CECs towards wetland plants.
- Sunlight photolysis can be a major removal mechanism for organic CECs in constructed wetlands. Existing literature have showed evidences of photolysis for a number of CECs in aquatic environment. However, it is important to investigate

photolysis in the context of wetlands where actual light intensity is reduced by multiple factors e.g. depth, absorbance by inorganic and organic matter of wetland water etc.

- Biotransformation can support removal of a number of organic CECs in wetlands. The rate and extent of biotransformation depends on multiple factors that also controls different biochemical processes of wetlands. It is needed to investigate how key biochemical conditions dominant in wetlands (nitrifying, denitrifying etc.) of wetlands can improve CEC removal.
- Aggregation of ENMs is dependent on physico-chemical properties of ENMs as well as the properties of wetland water. It is important to investigate how fate of ENMs with different characteristics (e.g. size, surface charge, coating, etc) are influenced by the wetland water matrix properties (e.g. pH, ionic strength, dissolve organic matter etc.).
- Constructed wetlands hold potential to remove a wide range of PPCPs/EDCs to levels that are much comparable with conventional WWTP treatment processes. However, the existing studies reported range of removal efficiencies for a number of these contaminants while the actual removal mechanisms and their contribution needs to be identified.
- Existing wetlands are designed primarily to remove bulk pollutants. Currently, it is unknown how design criteria of constructed wetlands can affect CEC removal. There is a need to find out relationship between design parameters of wetlands (e.g. loading rates) and CEC removal.
- ENMs have been detected in sewage and wastewater effluents. Literature sources studying fate of ENMs have shown potential transformation occurring in constructed

wetlands. Thus it is equally important to find out the effect of design parameters on ENM removal.

CHAPTER 4

ENGINEERED NANOMATERIAL REMOVAL IN CONSTRUCTED WETLANDS*

*Majority of this chapter was published: Sharif, F., Westerhoff, P. Sorption of Trace Organics and Engineered Nanomaterials onto Wetland Plant Materials, Environmental Science: Process and Impacts, 15, 267-274, 2013.

Abstract

Wastewater treatment plant (WWTP) effluents are sources for emerging pollutants, including organic compounds and engineered nanomaterials (ENMs), which then flow into aquatic systems. In this study, ENM removal in constructed wetlands was investigated using continuous-flow laboratory microcosms and batch scale experiments. The microcosms were operated at varying hydraulic residence times (HRTs) and contained wetland plant materials. Representative organic compounds and ENMs are simultaneously spiked into the microcosm influent, along with a conservative tracer (bromide), and then monitored in the effluent over time. It was observed that a more hydrophobic compound-natural estradiol (E2) achieved better removal than a polar organic compound-parachlorobenzoic acid (pCBA), which mimics the behaviour of the tracer. The two ENMs spiked into the microcosm: carboxy-coated nano-silver (CAR-Ag) and aqueous fullerenes (aq$n$C$_{60}$) experienced comparable removal. Relative to the tracer, the highest removal of ENMs and trace organics was 60% and 70%, respectively. A two-fold increase in HRT, 10~30 % increase in mass removal was observed for the contaminants. With accumulation of plant materials in the microcosms, average removal increased from 49 % to 62 % for CAR-Ag and 47 % to 70% for aq-nC$_{60}$at comparable HRTs.

Batch sorption studies with wetland plant materials were conducted to understand the behaviour of the contaminants in the microcosms. Batch sorption studies found higher

sorption affinity of aq$n$C$_{60}$ than CAR-Ag which explained the relatively better removal of aq$n$C$_{60}$ than Car-Ag when the plant biomass quantity increased in the microcosms. Ag NMs were found to destabilize in microcosm influent water due to high conductivity, but in the presence of wetland plants they were stabilized. The effect of surface coating on NM stability and sorption was also investigated. Gum-arabic coated Ag NMs (Ga-Ag) was found to be more stable than CAR-Ag and Polyvinylpyrrolidne coated Ag NMs (PVP-Ag). However, surface coating of the NMs had no effect on sorption onto the wetland plants.

Introduction

Engineered nanomaterials (ENMs) represent a potential new class of emerging contaminants (Westerhoff et al., 2009, Oberdörster et al., 2005).Nano-scale titanium dioxide, silica/silica oxide, cerium oxide, zinc oxide, silver, fullerenes, and carbon nanotubes are being used, or will be in the near future, in a wide range of personal care products, pharmaceuticals, clothing, paints, industrial processing, and other consumer products. These ENMs are likely to enter sewage and storm waters (Nowack and Bucehli, 2007, Ben and Westerhoff, 2008).Currently, the fate of ENMs in wetlands is ill-defined, although the removal of many metals is well understood. For example, wetland environments, especially the rhizospheres of macrophytes, are well-known for phytoremediation of heavy metal particles. Similarly, common wetland plants, including reeds have been found to biomineralize copper into metallic nanoparticles in and near roots, thus preventing copper from entering the food chain (Manceau et al., 2008). More recently, silver NMs with different surface coatings were found to transform and react with surrounding chemical species of the aquatic environment (Levard et al., 2012). The process of binding with plant exudates is also considered an important abiotic reaction in the removal of silver NMs from water columns (Unrine et al., 2012).

Constructed wetlands are often designed to remove nutrients (nitrogen, phosphorous, organic carbon) and/or suspended solids from storm water runoff or wastewater effluent. However, emerging pollutants, including a variety of trace-level organic compounds - PPCPs, plasticizers, stimulants and manufacturing agents) can also be found in constructed wetlands and other effluent-dominated waters (Grey and Sedlak, 2005, Fono et al., 2006, Vazquez-Roig et al., 2011).Constructed wetlands are often designed for a particular hydraulic retention time (HRT) as a means to remove biochemical oxygen demand (BOD), nitrogen, and phosphorus. The effect of HRT on the removal of micropollutants, such as PPCPs, is not well investigated. Typically, longer HRTs are found to achieve greater nutrient removal (Metcalf and Eddy, 1991, Tanner et al., 1995, Manceau et al., 2008, Ghosh and Gopal, 2010). Treatment efficiency improves in these cases due to the lowering of the loading rate, which is inversely proportional to HRT. However, HRT is directly related to the size of the wetlands and needs to be optimized to obtain adequate removal at reasonable costs. Most often, adequate removal is achieved for an optimal HRT, beyond which further removal has been found to be negligible (Ghosh and Gopal, 2010, Chen et al., 2006, Akratos et al., 2007, Kotti et al., 2010).

The aim of this chapter is to evaluate ENM removal in constructed wetlands using continuous-flow laboratory microcosms and batch scale experiments. The continuous-flow microcosms studies were designed to (i) investigate removal of ENMs of different sizes and composition (CAR-Ag and aq-nC_{60})) against removal of tracer organic contaminants of different polarities (17β -estradiol (E2) and *p*-chlorobenzoic acid (pCBA)) (ii) evaluate the effect of HRT and plant biomass quantity on ENM removal. These microcosms were operated in the dark and pre-equilibrated for more than six months using water with nutrient constituents as the water supply, and a weekly addition of dried, chopped bulrushes (*Scirpus validus*) as dry plant biomass. This addition of plants mimic the growing plants that senesce in wetland water. A conservative tracer (Br^-) was simultaneously added to obtain estimate HRT and other hydraulic parameters of the microcosms. The similar types of microcosms were previously used to represent trends that have been observed in full-scale wetlands for nitrogen and dissolved organic carbon (DOC) (Ingersoll and Baker, 1998, Pinney et al., 2000).

Batch experiments were conducted using similar wetland plant materials to (i) study sorption affinity of the contaminants towards the plant surfaces existing in the water phase of wetlands (ii) investigate stability of Ag NMs in wetland (iii) identify the effect of surface coating on Ag NM stability and sorption onto the wetland plants.

With the help of these two experimental approaches, the primary objectives were to answer the following questions: (i) To what extent can ENMs be removed in the microcosms, along with the trace organic contaminants? (ii) Does increasing HRT improve the removal efficiency of ENMs? (iii) Are batch sorption experiments reasonable predictors for the fate of ENMs in simple microcosms? (iv) Is surface-coating an important factor for ENM sorption in constructed wetlands?

Materials and Methods

Stock preparation

E2 and pCBA were obtained from Sigma-Aldrich, located in St. Louis, MO, USA. Potassium bromide was obtained from ICN Biomedicals Inc., Ohio. The stock solution of E2 was prepared in an HPLC grade methanol since it has very limited solubility in water. Potassium bromide stock solutions and pCBA were prepared in nanopure water (18.3Ω conductivity; Milipore Inc., Billerica, MA). The aq-*n*C_{60} suspension was prepared by directly adding 99.9% C_{60} powder (MER Corporation; Tucson, AZ) to ultrapure water and mixing for a prolonged period of three months. The suspension was filtered through a 0.7µm GF/F filter and preserved in an amber bottle covered with aluminum foil to prevent any chances of photolysis.

The CAR-Ag suspension was a concentrated carboxy-coated Ag (CAR-Ag) suspension (Vive; Ontario, Canada). A detailed characterization of the aqueous fullerenes and functionalized nano-silver can be found in a previously published article (Kier et al., 2010). The Ag NMs used to investigate the coating effect were polyvinylpyrrolidne (PVP) and Gum-arabic (GA) coated Ag NMs. These two NMs were obtained from Center for the Environmental Implications of Nanotechnology (CEINT). Ionic and non non-ionic (or nano size Ag) fractions were determined by ultrafiltration with 1000 Da molecular weight cut-off (MWCO) membrane (Millipore,MA) by a stirred ultrafiltration cell unit (Amicon 8050, Millipore, MA). This method is described in Yang et al., 2012. The non-ionic or the NM fractions were 99.1±0.02%, 98±0.13% and 98.2±0.04% for PVP-Ag, GA-Ag and CAR-Ag respectively. Table 4.1 summarizes properties of the organic and ENM contaminants used in this study.

Table 4.1

Properties of target organics and ENMs

Trace organic contaminants or ENMs (Class)	Molecular weight (MW) (g/mole) or Size	pKa or charge	Log K_{OW} [Log D_{OW} @ pH = 7.5]
17 β- estradiol (E2) (Steroid)	272.4	10.4	3.94 [2.46]
P-chlorobenzoic acid, (pCBA) (Process Intermediate)	236.3	3.98	2.52 [0.96]
Aqueous fullerene (aq-nC_{60})	80 nm ‡	-	NA*
Carboxy coated nano silver (CAR-Ag)	10 nm	Zeta potential = -47.1 mV (@ pH = 7.0)	NA*
polyvinylpyrrolidne coated nano silver (PVP-Ag)	8 nm	-	NA*
Gum-arabic coated nano silver (GA-Ag)	6 nm	-	NA*

‡ Measured with DLS in solution, NA* - Not applicable

Microcosm studies

The continuous-flow microcosms are multiple acrylic tanks (20cm x 25cm x 13cm), each with a 6.4 L volume capacity. Figure 4.1 is the schematic of the microcosm which shows the positions of the inlet and outlet from where influent water was continuously added and effluent samples were taken for measuring the concentration of the contaminants respectively. A model feed water was pumped (Monistat cassette peristaltic pump) through the microcosms at three different flow rates. The bulk residence time (HRT) was calculated as volume divided by flow rate. The feed water was used was dechlorinated granular activated carbon (GAC) filtered tap water (Tempe, AZ), with added nutrient constituents (0.2 mM K_2HPO_4, 1 mM NH_4Cl). The reason for using tap water is that it contains a blend of common anions and cations.

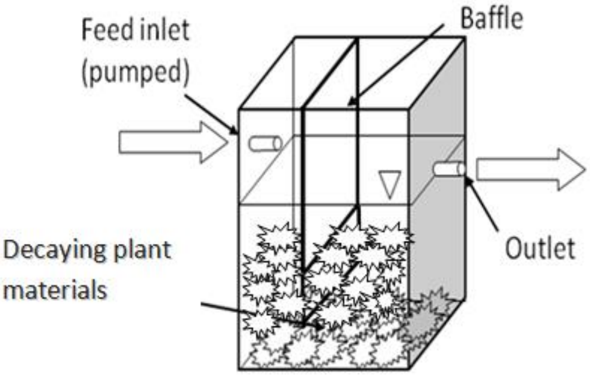


Figure 4.1. Schematics of the continuous-flow microcosms.

Plant addition rates were determined based on a review of the productivity of emerging macrophytes. Temperate climates were found to yield 3,000-4,500g DW m^2/yr and tropical wetlands yielded 6,500-8,500g DW m^2/yr (Ingersoll and Baker, 1998). For this study, 6,000g DW m^2/yr was considered a reasonable estimate for the wetlands in Sonoran deserts of Arizona, USA which translated into an addition of 3 g of dried plant material per week (Ingersoll and Baker, 1998). Due to decomposition, the added plants leach DOC and accumulate as biomass in the microcosms. Decaying plant materials is a major source of labile organic carbon in full-scale wetlands. With organic carbon, nutrients and trace metal ions being available, biological activity is likely to occur in the microcosms. The microcosm studies were conducted in two phases. During the first phase, the microcosms were operated at three HRTs: 2.1, 2.6, and 4.8 d. Four different pollutants were spiked into the microcosms to achieve an initial concentration of 500 µg/L. Br^- was spiked in the form of potassium bromide stock solution to obtain a concentration of 30 mg/L. A 100 ml volume of a solution containing all the target pollutants and tracer was spiked into the microcosms with a syringe pump, while simultaneously withdrawing the same volume out. This was done to minimize any turbulence and sudden changes in microcosm volume. Effluent samples were taken and filtered with 0.7µm GF/F filters and stored at 4^0C until analyzed.

The second phases of microcosm studies were conducted five months later. During this period, plant addition of into the microcosms was continued at the same rate. The HRT of the microcosms were 2.3, 3.1 and 7.4 d respectively. ENMs were spiked into these microcosms following the similar method described above to find out if the accumulation of plant materials in the microcosms improves removal.

Average influent and effluent water qualities of the microcosms in both phases are presented in Table 4.2. Higher levels of DOC in the effluent water indicate plant decomposition activity and consequent leaching of DOC. Nitrogen transformation was also observed in the microcosms. The effluent water ammonia (NH_3) is probably the fraction of the influent NH_3 that was not transformed within the microcosms and also any new NH_3 formed by the plants. NH_3 can form when organic nitrogen is released during plant decomposition. The presence of nitrate (NO_3^-) in the effluent water also supports the evidence of nitrogen transformation process occurring in the microcosms.

Sorption studies with wetland plants

Sorption is one of the major abiotic processes influencing the fate of contaminants in wetlands. Contaminants can sorb onto sediments, plants, or suspended solids in wetlands. Much of the current effort related to sorption studies has focused mainly on soils and sediments, but not plants. The microcosms of this study contained plants, but no sediments, and thus batch experiments were conducted using the wetland plants.

Table 4.2

Effluent water quality parameters of the microcosms

			1st phase HRT (d)			2nd phase HRT (d)		
Parameters	Unit	Influent water	2.1	2.6	4.8	2.3	3.1	7.4
DOC	mg/L	2.0	4±1.0	4±0.7	5.5±0.6	4± 0.5	5± 0.7	6.8± 1
Ammonia (NH_3- N)	mg/L	14.0	10. 53	10.20	11.90	10.5	9.2	8.6
Nitrate (NO_3^- - N)	mg/L	0	0.12	0.12	0.1	0	0	0.26
pH	-	7.2	7.33	7.10	7.40	7.5	7.4	7.8
Conductivity	mS/cm	1.2	0.90	0.98	0.94	1.0	0.94	0.95

To quantify the interaction of organic pollutants or ENMs with plant materials, experimental data were fit by Freundlich's model. While the partitioning mechanism of organic pollutants to plant or soil surfaces differs from the aggregation-like mechanism of ENMs with the same surfaces, Freundlich models were still found to fit both pollutant classes (Kiser et al., 2010). An empirical model was used to describe the solid-liquid distribution coefficient K_d (L/g)

$$q = K_f C_e^{1/n} \tag{4.1}$$

$$K_d = K_f C_e^{1/n-1} \tag{4.2}$$

Where q (μg/g) and Ce (μg/L) are the sorbed and aqueous phase concentrations of the contaminant, respectively. K_f (($\mu g^{1-1/n} L^{1/n} g^{-1}$) and n are the Freundlich constant and Freundlich exponent, respectively.

Sorption experiments were conducted using the bottle-point method. The background solution contained 1 mM $NaHCO_3$. Two types of controls – (i) no plant material (only contaminants) and (ii) plant material only (without contaminants) were used. Experiments were carried out in 40 mL glass vials with Teflon-lined screw caps. The air-dried wetland plants, similar to those used in the microcosms were first hydrated in the background solution for 24 hours. One exception between the plants used in the microcosms and the sorption experiments is that the plants were autoclaved for 15 minutes at 10.33 MPa (1500 PSI) after being air-dried prior to conduct the batch sorption experiments. Autolcaving was done to minimize any chance of plant decomposition and consequent microbial activity during the equilibration period. pH of the background water was adjusted to 7 prior to the addition of contaminants. The mass of plants added into the vials was selected to obtain a concentration of 10 g/L. Another parameter that varied between the microcosm and sorption studies is the presence of ions in the water, commonly measured as ionic strength. Wetland influent water has higher ionic strength compared to the pH buffered nanopure water. Ionic strength can significantly impact ENM stability which is explained in the discussion section of this article.

Target compounds were added into the vials containing the background solution and plant materials. Vials were then covered with aluminum foil to avoid photolysis and then equilibrated for 72 hours at room temperature. The equilibration time was selected based on the findings from previous experiment with E2, which showed that no further improvement could be attained if equilibration time was increased beyond 48 hours. Subsequently, the vials were left undisturbed for 6 hours to allow any large particles to settle. Samples were then filtered with 0.7µm GF/F filters and kept at 4^0C before analysis.

The amount sorbed by the plant material was calculated from the difference between the initial and final concentrations of the solute.

Tracer studies

Tracer studies are conducted to characterize hydraulic conditions inside a reactor. In many practical cases, mean residence time ($\bar{t}$) is less than the theoretical residence time (τ) or HRT, which is determined by dividing the reactor volume with the bulk flow rate. This occurs due to short circuiting when fluid elements travel through a short path leaving behind unutilized spaces in the reactor. In the microcosms, this phenomenon is obvious because of the dead zones created by the plants. In our study, the pulse input of Br- was introduced as a tracer in the reactors. Effluent samples were collected over time. $\bar{t}$ was calculated using equation (4.3):

$$\bar{t} = \frac{\sum tC\Delta t}{\sum C\,\Delta t} \quad . \quad (4.3)$$

Where C is the concentration of the tracer exiting from the reactor at time t (mg/L) and Δt is the time interval for sampling (hr). Dimensionless variance (σ^2) is a parameter needed to calculate the dispersion coefficient (D) and the equivalent number of CSTRs (n), which closely model the mixing and flow characteristics within the microcosm. With a greater value for n, the system will approach a plug-flow hydraulic condition. For wetlands, D typically varies from 0.13 to 0.46 (Kadlec and Wallace, 2008).

$$\sigma^2 = \frac{\sum t^2 C\Delta t}{\sum C\,\Delta t} - \left(\frac{\sum tC\Delta t}{\sum C\,\Delta t}\right)^2 \quad (4.4)$$

$$n = \frac{\sigma^2}{\bar{t}^2} \quad (4.5)$$

$$D = \frac{\sigma^2}{2 * \bar{t}^2} \quad (4.6)$$

Analytical methods

Determination by HPLC-fluorescence/UV

Details of E2 and pCBA analysis with HPLC can be found elsewhere.26 In brief, our liquid chromatography analysis was performed on a Waters600 series (Milford, MA, USA). Detection was accomplished with a Waters 2475 fluorescence detector at an excitation wavelength of 280 nm and an emission wavelength of 310 for E2. A Waters 5-mm LiChrosorbs RP18 analytical column (4.6 mm x100 mm) connected to a Waters 5-mm LiChrosorbs RP18 guard column (4.6mm x10 mm) was used for reverse-phase separations with a 200-mL sample loop. The mobile-phase solvent profile was 45% deionized (DI) water acidified with 10mM H_3PO_4 and 55% methanol for 30 minutes at a constant flow rate of 1 mL/min. The pCBA was analyzed simultaneously adopting the same method, but was detected with a Waters2990 UV detector set to a wavelength of 234 nm. Method detection limit (MDL) for the pCBA and E2 were 4 and 1µg/L, respectively.

Determination of CAR-Ag with inductively coupled plasma-optical emission spectroscopy (ICP-OES)

Samples were digested with ultrapure nitric acid prior to analysis of CAR-Ag ions with ICP-OES (Icap 6000 Series, Thermo Scientific; Cambridge, UK) at a wavelength of 328.0nm and 338.2nm. To understand the matrix suppression effect on recovery, appropriate amounts of CAR-Ag suspension were spiked in different microcosm water matrices to obtain a concentration of 500 µg/L. These matrix-spiked samples were digested and then analyzed at the time of measuring all the other samples. Recovery of CAR-Ag ions exceeded 95 %.

Hydrodynamic chromatography (HDC) interfaced with ICP-mass spectrometry (MS)

Unlike the “dissolved” organic pollutants, a thorough understanding of the environmental fate of ENMs cannot be obtained by measuring the concentration alone. Rather, it is also important to characterize the ENMs in terms of their particle size distribution. Hydrodynamic chromatography (HDC) is a separation technique used to separate small particles based on their flow through a packed bed of nonporous beads. Particle separation is accomplished as a result of velocity gradients that develop in the capillaries between beads. This technique can be used in tandem with metal detection methods, such as ICP-MS which is then known as HDC-ICP-MS, to investigate the fate of metal-containing ENMs in environmental samples.

Thermo X-Series 2 Quadruple ICP-MS (Thermo Fisher Scientific Inc., Waltham, MA) was used for Ag NM detection. The separation system consisted of a PL-PSDA Type-1 hydrodynamic chromatography column (Varian, Inc., Shropshire, UK) attached to a Thermo Spectra Series P100 HPLC pump (Thermo Fisher Scientific Inc., Waltham, MA) fitted with a manual injection valve. The reported size-separation range of the PL-PSDA column used here was 5-300nm. The mobile phase used was a water based mixture of salts and surfactants: 0.5 mM Na_2HPO_4, 0.05% non-ionic surfactant (Triton-X100), 0.0125% $C_{12}H_{25}SO_4Na$ (SDS), 0.05% formaldehyde, adjusted to pH ~ 7.5 using HCl and NaOH. Injection volumes of 20 μL were used with a mobile phase flow of 1.7ml/min at a constant pressure of 9 MPa. A tee joint was installed between the column and the ICP-MS nebulizer to reduce the amount of sample reaching the plasma.

aq-nC$_{60}$ liquid-liquid extraction and HPLC analysis

The supernatant of the effluent samples, toluene, and acetic acid were combined in a ratio of 1:1:2.5, respectively. The solutions were then mixed for at least 2 hours prior to removing the toluene phase for HPLC analysis. The toluene extracts were diluted with an equal volume of acetonitrile. The molecular C_{60} in toluene-acetonitrile mixtures was then separated by a Supelco RP C-18 HPLC column, 15cm × 4.6mm I.D., 5µm particle size (St. Louis, MO, USA), with detection occurring at 336nm. The HPLC mobile phase was to be a 1/ 1 (v/v) mixture of toluene and acetonitrile, running isocratically at 1mL/min. The extraction procedure was able to recover 107 ± 3% of known concentrations of aq-*n*C_{60} based on the mass balance calculation.

Results and Discussion

Continuous-flow microcosm studies

Hydraulic behavior of the continuous-flow microcosms

Preliminary trials were conducted on the microcosms to improve the hydraulic behavior, and finally, an injection pump was used for pulse input of target compounds to minimize any turbulence and sudden changes in volume. Tracer responses in the microcosms used in both phases are plotted in Figure 4.2.

In the 1st phase, $\bar{t}$ of 2.1 d, 2.6 d, and 4.8 d microcosms were1.92 d, 1.96 d, and 3.86 d, respectively. The n values for the same order of microcosms were 1.82, 1.71, and 1.82, respectively. The D values of the microcosms were 0.3, 0.27, and 0.27, respectively, which are within the typical range mentioned in the previous section. $\bar{t}$ for the microcosms studied in 2nd phase were 1.62 d, 2.7 d and 4.9 d respectively for the HRTs of 2.3 d , 3.1 d and 7.4 d respectively. The corresponding n values 3.3, 3.54 and 2.33 respectively. D values were 0.15, 0.14 and 0.21 respectively.

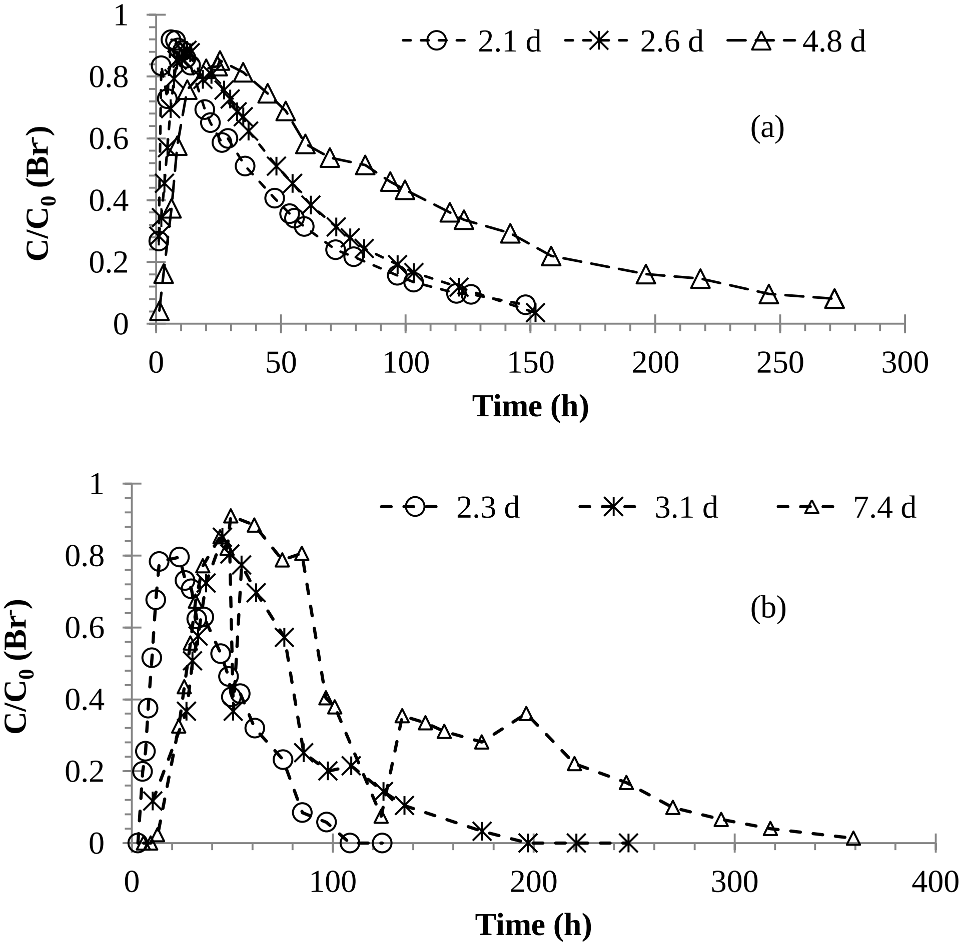


Figure 4.2. Tracer responses with time from the microcosms operated at different HRTs. a: 1st phase; b: 2nd phase.

The mass recovery of tracer in the microcosms varied between 80~85% during the 1st phase. During the 2nd phase , mass recovery of trace was lower and varied between 68~85 %. Typically, treatment wetland tracer recoveries in the range of 80~120% are acceptable (Kadlec and Wallace, 2008). However, recovery is often subdued by retaining the tracer within the wetlands. In the second phase, the lowest recovery for tracer was found in the microcosm operated at the longest HRT of 7.4 d which also had a significant lower value of actual residence time (4.9 d).

Contaminant removal in the microcosms

Concentration of the contaminants in the effluent samples collected with time was measured for each of the microcosms. Mass fraction of the contaminants at time t was estimated by multiplying the concentration with flow rate and the time interval. Mass recovery was then calculated using the simple equation below and compared for each HRT. Figure 4. 3 shows the recovery of ENMs and trace organics parallely studied during the 1st phase of the microcosms studies.

In all of the microcosms, regardless of a variation in HRT, the highest cumulative mass recovery in the effluent was observed for Br^- and pCBA. The least mass recovery was seen for E2; thus, E2 was found as the most efficiently removed pollutants in the microcosms. The recovery of the two ENMs occurred between the two organics. Removal of E2 increased from 56 % to 66 % to 70 % when HRT was increased from 2.1d to 2.6d and then to 4.8 d. Removal of aq-nC_{60} increased from 37% to 43% to 60% when HRT was increased to the higher values. For CAR-Ag, the removal increased from 50% to 60% when HRT was increased from 2.1d to 4.8d; however, removal dropped to 38% when HRT was 2.6d. Mass removal percentage of the target contaminants is compared in Figure 4.4. Removal was adjusted to account for the loss of Br- in the microcosms. For example, if the removal of a contaminant was found to be 70% and the loss of Br^- in the microcosm was 10%, then the actual removal of that contaminant would become 60%.

$$\text{Mass recovery (\%)} = \frac{[\text{Mass in the effluent}]_t}{[\text{Mass in the microcosm}]_{t=0}} * 100\% \qquad (4.7)$$

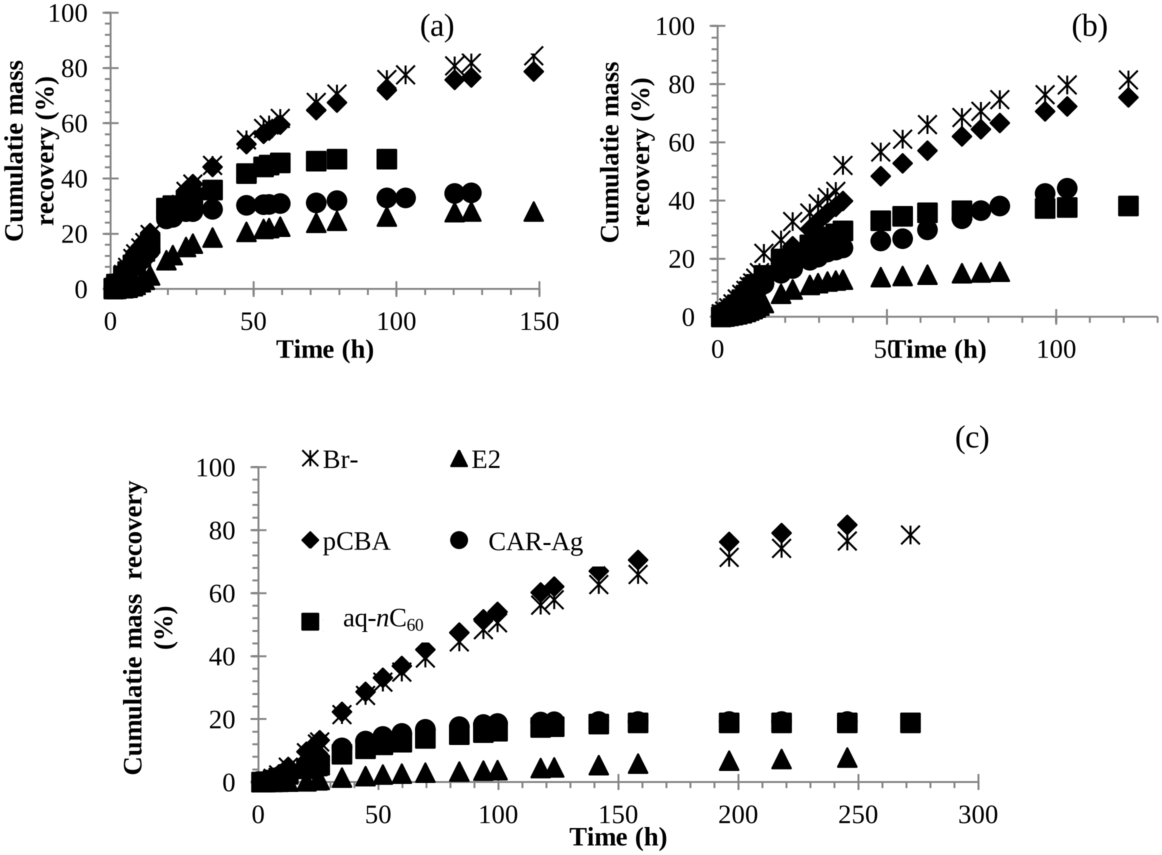


Figure 4.3. Cumulative mass recovery (%) of the contaminants in the microcosms. a: HRT= 2.1 d, b: 2.6 d and c: 4.8 d.

In the 2nd phase of the microcosm studies, pulse input studies were conducted with CAR-Ag and aq-nC_{60} in the microcosms operated at 2.3 d, 3.1 d and 7.4 d HRTs. The mass removal was calculated using the similar approach and showed in Figure 4.5. Due to higher tracer loss (32%) in the longest HRT microcosm, mass removal of contaminants dropped compared to other microcosms experiencing lower loss in tracer (16 % and 19 %).

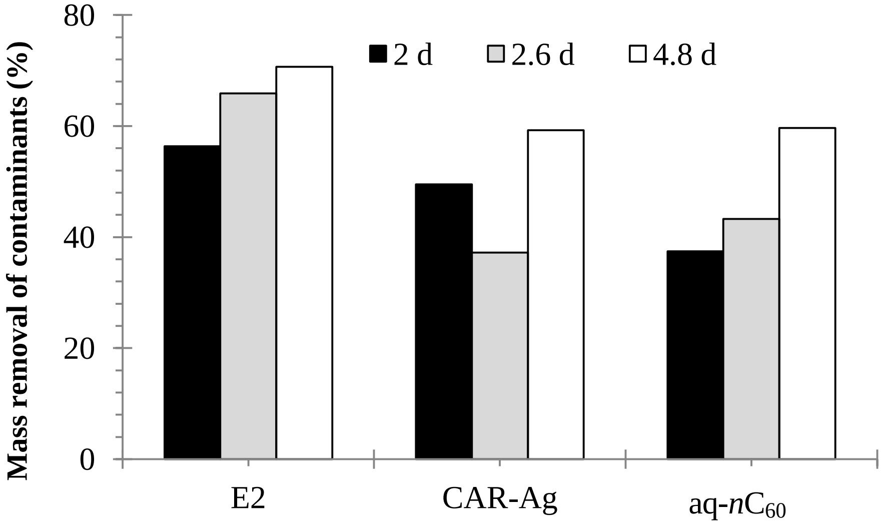


Figure 4.4. Mass removal of ENMs in microcosms (1st phase studies).

Figure 4.6 compares ENM removal observed during the two phases of microcosms studies. The HRT values used in this plot are actual HRT obtained from tracer studies. Higher removal was observed during the 2nd phase studies and except for the HRT 4.9d of the 2nd phase studs, increasing HRT increased removal. As mentioned in the previous paragraph, due to higher tracer loss the net removal was reduced at the 4.9 d HRT. Without adjusting the tracer loss, the actual removal data is plotted in Figure 4.6 B. The effect of HRT had less impact on aq-nC_{60} than CAR-Ag when the plant material quantity was relatively higher during the 2nd phase studies. Increasing HRT beyond 2.7 d, aq-nC_{60} removal did not change. When HRT was lengthened by two fold, additional removal for aq-nC_{60} and CAR-Ag in the 1st phase studies was 29 % and 25 % respectively. During the 2nd phase, lengthening HRT by three fold, 11 % and 29 % more mass of aq-nC_{60} and CAR-Ag was removed.

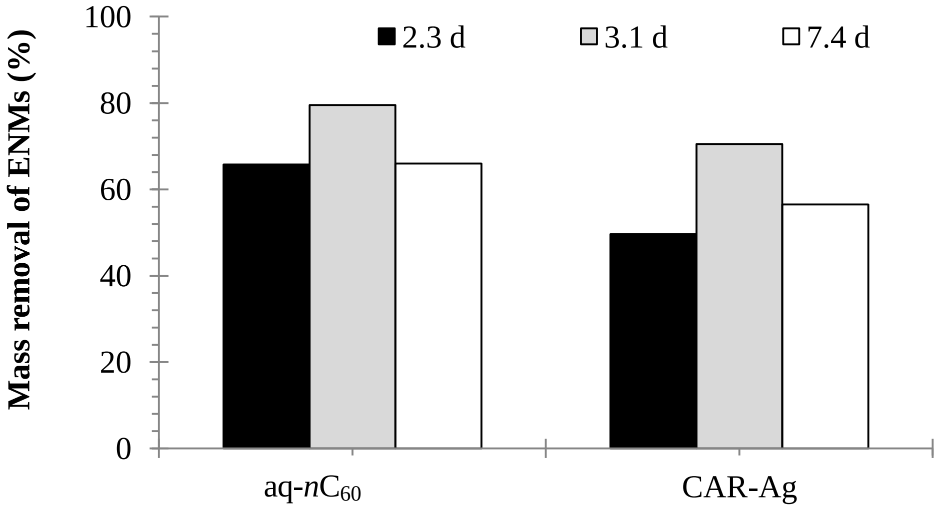


Figure 4.5. Mass removal of ENMs in microcosms (2nd phase studies).

Size distribution of Ag NMs in microcosm effluent

In addition to determining total Ag concentration, an analysis of the size distribution of the nanoparticles was conducted using HDC-ICP-MS method. The current method only allows characterization of metal-based NMs. Figure 4.7 A shows the size distribution of Ag in CAR-Ag stock and an effluent sample from the microcosm operated at HRT of 4.8 d. Size calibration was done with Au colloids of 5, 20, 50, and 100nm (Figure 4.7 B), which shows that the Ag NMs in the stock were < 20nm in size. Elution of the Ag peak and its shape from the microcosm effluent sample clearly indicate an increase in the size of the particles. Mean diameter of these particles ranged between 50 and 100 nm.

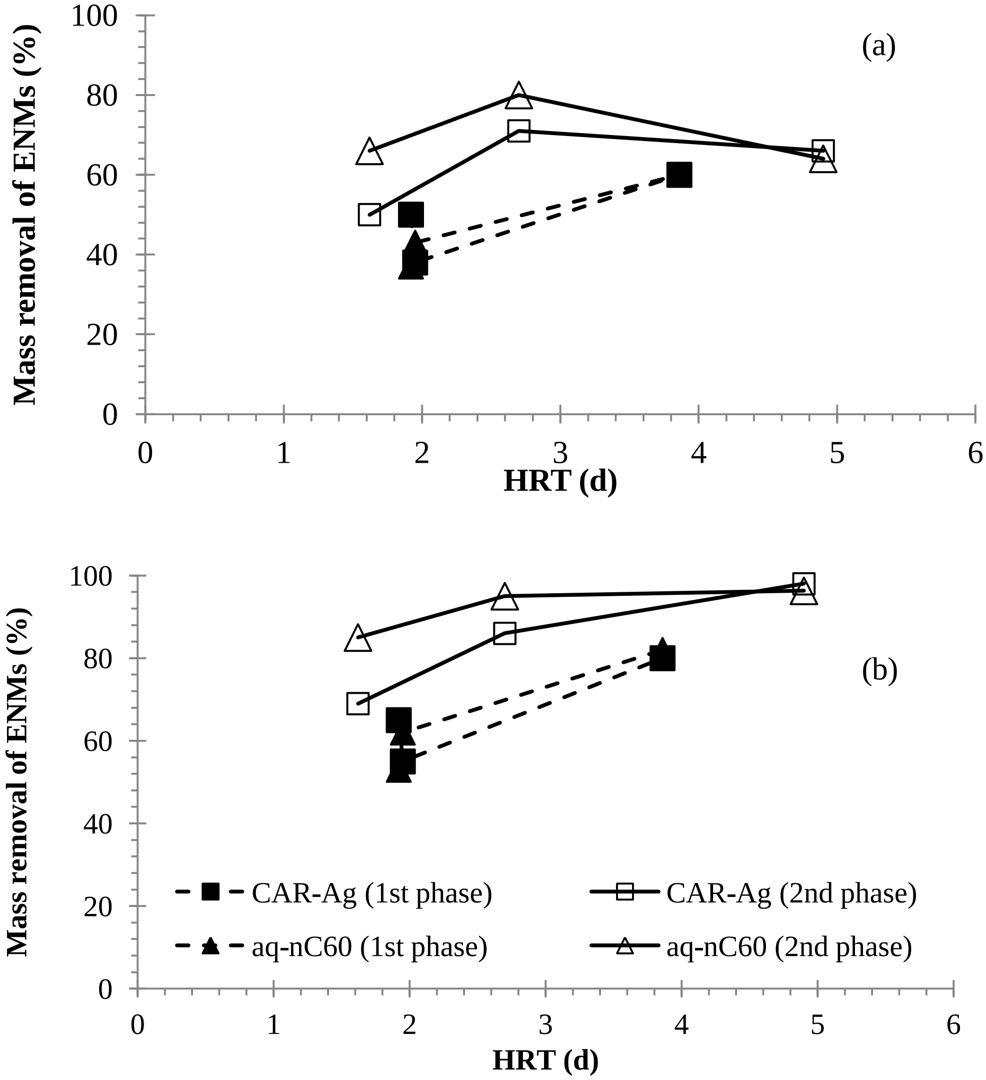


Figure 4.6. Mass removal of ENMs during 1^{st} and 2^{nd} phase of microcosms studies . a. With tracer loss. b. Without considering tarcer loss.

The probable mode of interaction for Ag NMs in the microcosms include (i) sorption to the bulrushes, (ii) association with organic colloids, (iii) aggregation and subsequent settling, or (v) physical straining and entrapment with suspended solids. Among these potential interaction mechanisms, sorption to the plants that influence the removal of Ag NMs has already been discussed. Partitioning to the colloids is the next possible option and to support this assumption, molecular weight distributions of the effluent samples were analyzed to investigate the presence of organic colloids which were released from decaying plant materials in the microcosms.

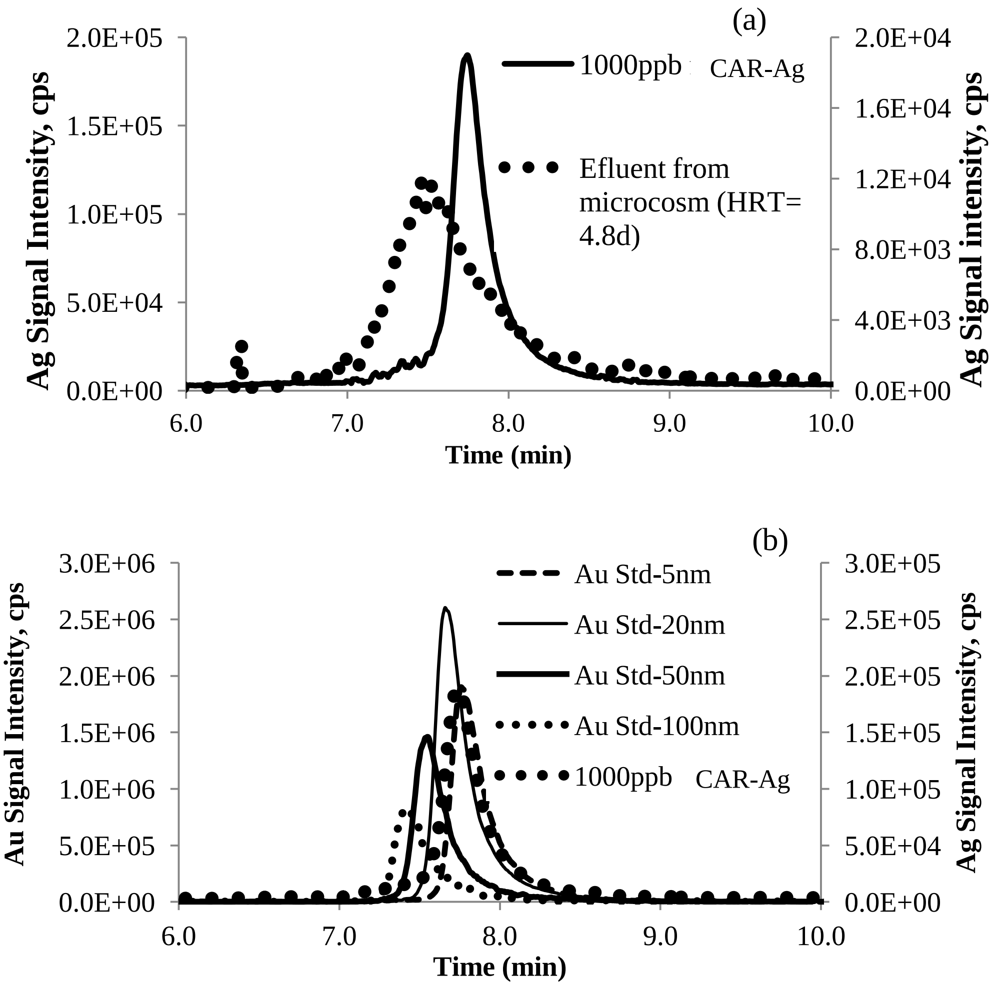


Figure 4.7. HDC-ICP-MS diagram. a: Ag nanoparticle in CAR-Ag stock and microcosm effluent sample. b: Au standards calibration.

Figure 4.8 shows size exclusion chromatography (SEC) chromatograms for DOC n the microcosm effluent samples from 1st phase microcosm studies. DOC values are the same as shown in Table 4.2. The analytical method is explained elsewhere (Song et al., 2010). Compounds in the high-molecular-weight range (above 10,000 Da) are predominantly colloidal matter (Song et al., 2010). The peaks in this region for the microcosm effluents indicate the presence of organic colloids. The effluent from microcosm 3, which had the longest HRT and highest DOC level in the 1st phase studies, had the tallest peak. The size distribution data for Ag NMs (Figure 4.7 A) also belonged to this effluent. It is possible that CAR-Ag NMs partitioned onto the organic colloids generated in this microcosm which increased their size.

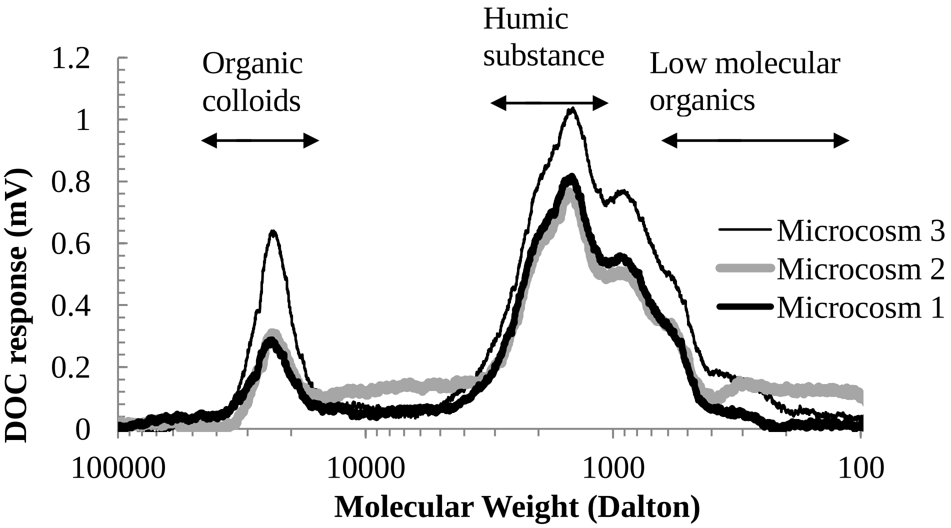


Figure 4.8. SEC-DOC data of the microcosm effluent samples.

Batch-scale studies

Sorption studies with wetland plants

Batch experimental data for interaction of the pollutants with bulrushes are presented in Figure 4. 9. Between the two organic compounds, the higher K_d value of E2 (0.26 L/g) suggests its higher sorption tendency than pCBA (0.11 L/g). Sorption on the plant materials must have been caused by hydrophobic partitioning as E2, with higher hydrophobicity (represented by higher value of log K_{OW}), sorbed more compared with *pCBA.*

Natural and synthetic estrogen removal in wetlands are thought to be dominated by sorption (William set al.,2003, Grey and Sedlak, 2005) and these batch experiments also suggest plants as a major sorbent in the wetlands. Between the two ENMs, aq-*n*C_{60} (0.10 L/g) has a higher affinity for sorption than CAR-Ag (0.10 L/g) expressed by its higher K_d value. Unlike the organic contaminants, hydrophobicity of the ENMs cannot be estimated by means of parameters such as log K_{OW}.

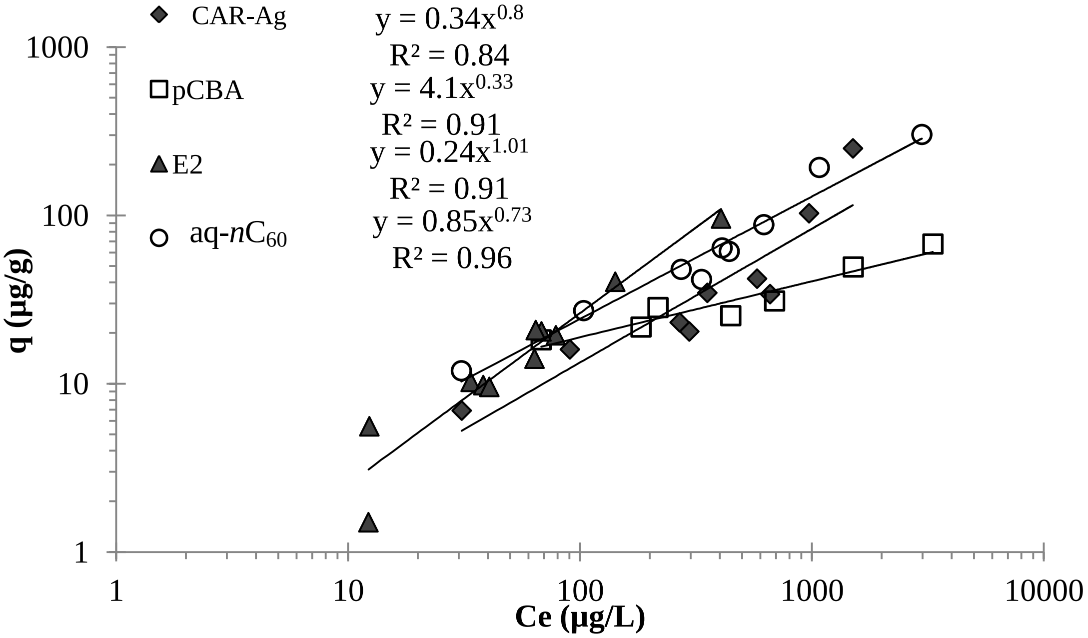


Figure 4.9. Sorption isotherms for E2, PCBA, CAR-Ag, and aq-*n*C_{60} with bulrush (*Scirpus validus*).

The sorption tendency of the two ENMs can be attributed to the differences in the material and surface properties. The aq-*n*C_{60}, prepared by the prolonged stirring method, is relatively hydrophobic and its sorption is considered to be driven by hydrophobic interactions (Brant et al., 2005). However, CAR-Ag used in this study had carboxylates as functionalizing agents. Carboxylates are low molecular weight polar molecules used for stabilizing particles in water, which, in turn, makes the NMs more hydrophilic. Based on their surface properties, therefore, aq-*n*C_{60}, being more hydrophobic, is likely to experience greater sorption onto the plant materials than CAR-Ag.

Effect of salt on ENM aggregation

Control experiments were set up to investigate contaminants loss n the microcosms. E2 was mixed with CAR-Ag in de-ionized water and wetland microcosm influent water. Their concentrations were measured after 4 d to find out how much loss can actually occur. Figure 4.10 shows E2 concentration at the beginning and end of these control experiments. While E2 was found stable, CAR-Ag was only stable in DI water and significant loss occurred in the microcosm influent water.

Microcosm influent water includes trace level metal ions and nutrients that might have affected stability of the Ag NMs. The relatively high increased ionic strength of the water (1.2 mS/cm) could have caused aggregation and subsequent sedimentation of the NMs. To further investigate the effect of ionic strength or conductivity, CAR-Ag NMs were introduced at a concentration of 1000 µg/L in nanopure water (2 µS/cm), 1 mM $NaHCO_3$ (100 µS /cm), microcosm influent water (1300 µS/cm) and 100 X dilution-wetland microcosm influent (13 µS/cm). The sample vials were shaken for 48 hours and the concentrations were measured. Figure 4.11 A shows CAR-A*g* stability in different types of water with varying conductivity.

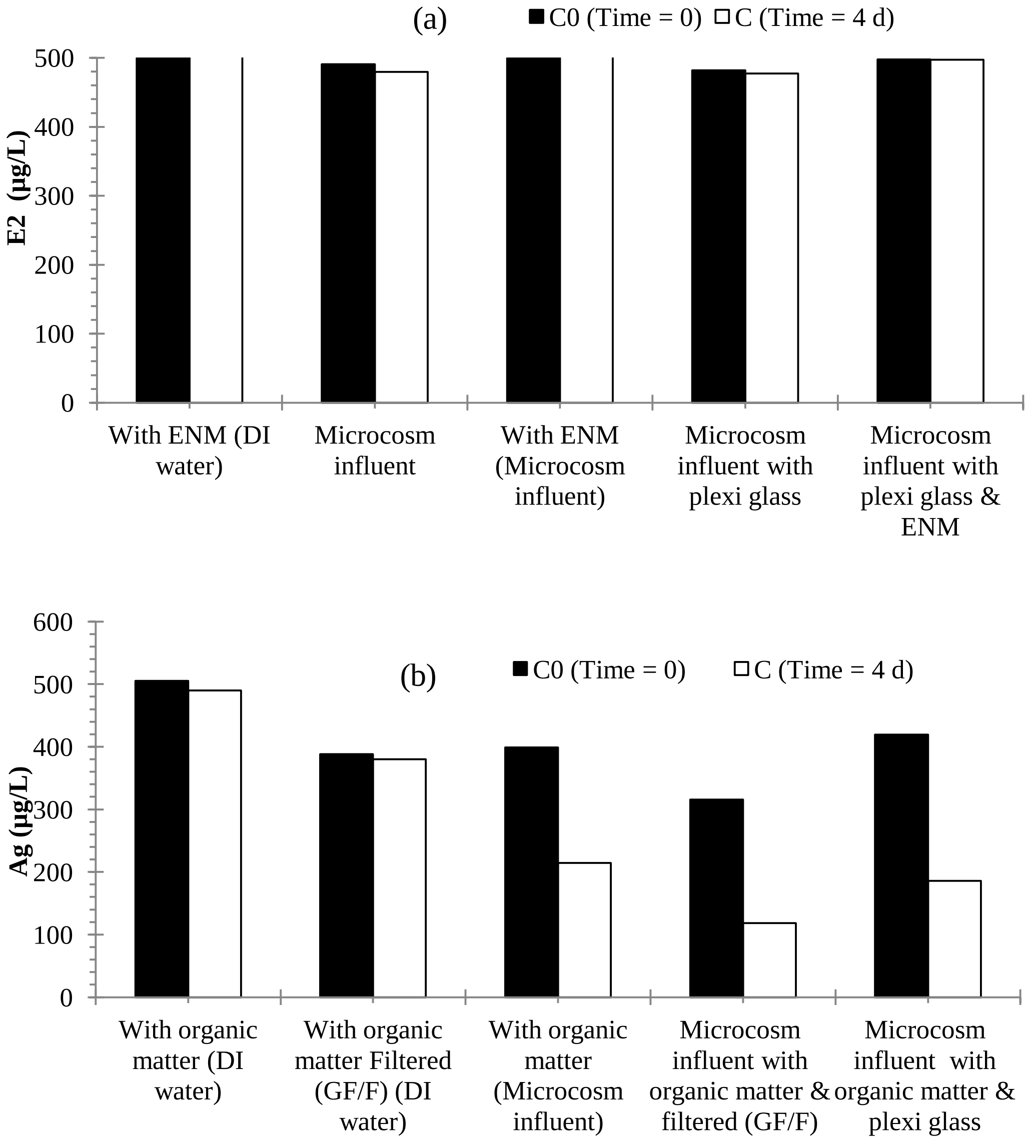


Figure *4.10.* Concentrations of (a) E2 and (b) CAR-Ag in control experiments.

Significant loss in Ag NM occurred in the water with highest conductivity and this loss decreased by diluting the water with DI water which lowered the conductivity. This supports the pronounced effect of salt or ions in water or wastewater that influences fate of metallic ENMs including Ag. Ag NMs have been also reported to undergo significant dissolution under environmental conditions. Once dissolved, these ions can precipitate by coming into contact with other anions in the water matrix. Oxidation of Ag^0 to Ag^+ is thermodynamically favored at room temperature and once oxidized; precipitation in the form of AgCl is very likely. Even though NH_4Cl was used as one of the nutrients in the microcosm influent water, average concentration of Cl^- in tap water is 100 mg/L. Such a level of Cl^- is common in the freshwater sources of Southwest US.

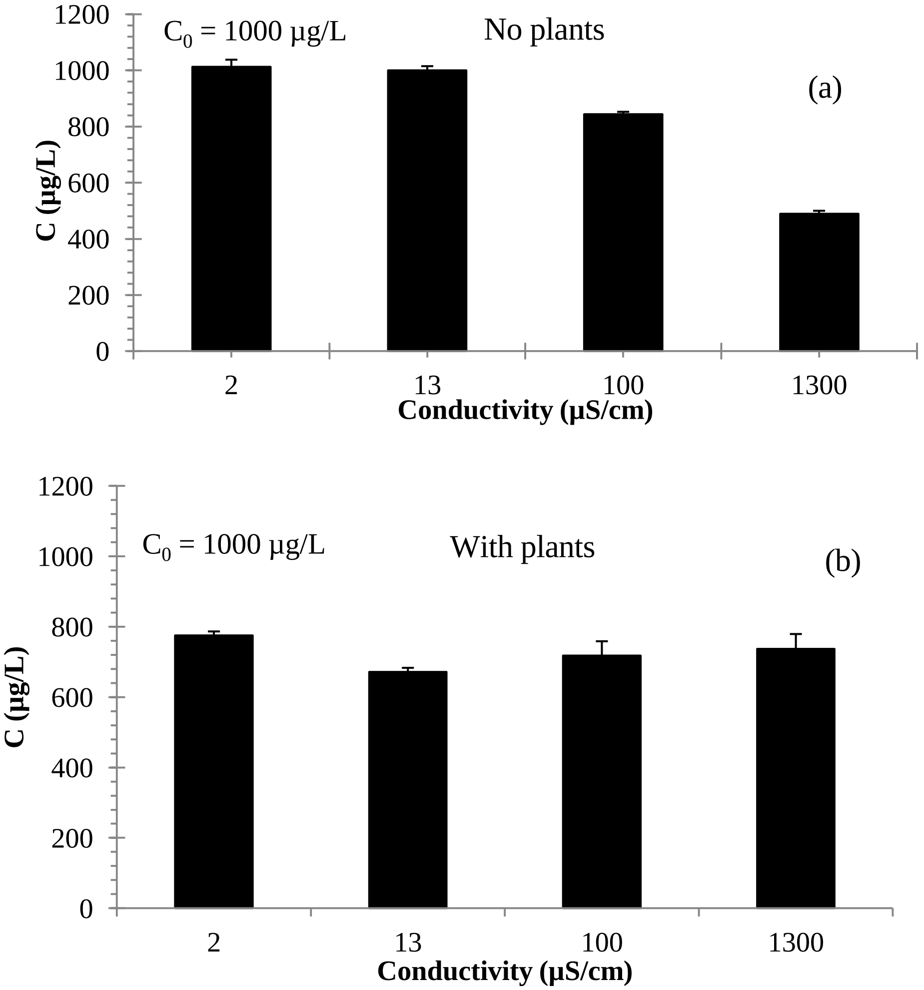


Figure 4.11. CAR-Ag stability in water with varying conductivity. a: Without addition of plants, b: with plants.

Figure 4.11 B represents similar conditions except that CAR-Ag was spiked into the waters with varying conductivity containing hydrated plant materials. Natural organic matter (NOM) is known to stabilize NMs and resist the effect of high ionic strength (Fabrega at al, 2009, Delay et al., 2011). Hydrated plants leach organic matter including humic substances. Figure 4.8 showed the presence of humic material in microcosm effluent which is a major component of natural organic matter (NOM). Thus DOC leached from plants in wetlands can also act like NOM and reduce the effect of ionic strength and consequent aggregation of NMs.

Effect of coating on Ag NM stability and sorption onto wetland plants

Among the three different coated Ag NMs (CAR-AG, PVP-Ag and GA-Ag) selected in this study, macromolecule like PVP and polysaccharides like GA are considered to perform better than relatively smaller molecules like carobyxylic acid as stabilizing agents. However, once exposed to the aquatic environment, these surrounding organic molecules can be substituted by organic matter present in the natural systems. As mentioned in the previous paragraphs, stabilization due to NOM has been observed in several studies; however the actual associations of NOM with ENM surfaces coated with different organic molecules are unknown.

Figure 4.11 shows that plant-leached DOC, like NOM can stabilize Ag NMs and counteract the effect of conductivity. The three Ag NMs: PVP-Ag, GA-Ag, CAR-Ag were spiked to obtain a concentration of 1000 µg/L in 1 mM $NaHCO_3$ solution (100 µS/cm) and microcosm influent water (1300 µS/cm). The experiment was conducted with addition of plants in two different quantities (10 g/L and 100 g/L). Controls were prepared with similar types of water with no addition of plants. After equilibration period, concentrations in the supernatants were measured.

Figure 4.12 shows Ag concentrations in the controls prepared with the two types of water of varying conductivity. As previously shown, higher conductivity causing destabilization and dissolution of Ag ENMs is primarily the main reason of loss of Ag NMs in the controls. Among the three types of Ag NMs, GA-Ag followed by CAR-Ag was found more resistant to the effect of conductivity and subsequent destabilization. Although PVP is known to be effective in stabilizing Ag ENMs (Levard et al., 2012), more Ag was found to release from PVP or other satirically stabilized Ag NMs when exposed to natural environment (Li and Lenhart, 2012). Thus, the significant loss observed for PVP-Ag in this study was probably due to the rapid release and subsequent interaction with inorganic ions of microcosm influent water.

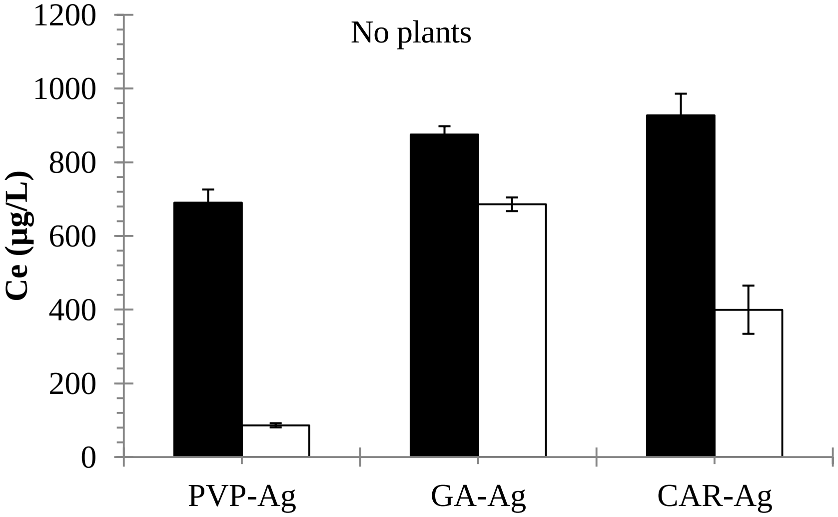


Figure 4.12. Coated Ag NMs in water with varying conductivity with no plant materials,

■ 100 µS/cm, □1300 µS/cm.

When wetland plants were present, all three Ag ENMs with different coatings experienced similar removal. Figure 4.13 shows the concentration of the Ag ENMs after equilibrating with wetland plants in microcosm influent water using two different plant concentrations. More ENMs were removed when higher concentrations of plant materials were used (Figure 4.12). This is in agreement with the better removal observed in the microcosms with higher quantity of accumulated plants. However, there was no significant difference in sorption affinity among the coated Ag ENMs. It should be noted here that after equilibration, the vials were shaken and samples were taken from the vials and digested without any filtration. Any Ag ENMs associated with dissolved or suspended organic matters was released during digestion and the measured concentrations represent the total amount of Ag that were not associated with any plant materials. The amount sorbed onto the plants was determined from mass-balance. From Figure 4.13, these amounts were comparable for all three types of coated Ag ENMs. We believe that, once exposed into the sample vials containing hydrated plant materials, for a fraction of the NMs, the coatings were no longer stable and probably substituted by the organic matter released from plants. The remaining fractions either directly associated with the plant surfaces or released as Ag^+ which then interacted with the plant surfaces. Regardless of the actual transformation mechanism, the effect of coating can be considered less important to evaluate the fate of Ag NMs in wetlands.

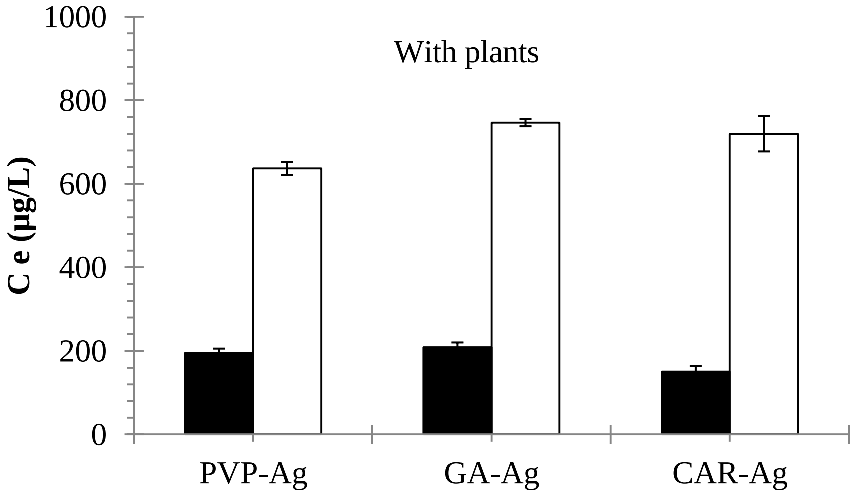


Figure 4.13. Coated Ag NMs in water with varying conductivity with plant materials, conductivity = 100 µS/cm. Plant material concentration ■ 100 g/L, □10 g/L.

Discussion

The continuous flow microcosms and batch-scale studies lead to the following answers to the key questions addressed at the beginning of this chapter.

(i) To what extent can engineered nanomaterials be removed in the microcosms, along with the trace organic contaminants? According to the removal data from the microcosms, hydrophobic organic compounds, e.g., E2 will be removed better than polar organic compounds. ENMs entering into microcosms can experience comparative removal alongside organic contaminants. However, ENM removal cannot be predicted with log K_{OW} as it is the case for E2 and pCBA. Despite the differences in properties between CAR-Ag and aq-nC_{60}, both experienced very similar removal.

(iii) Does increasing HRT improve the removal efficiency of contaminants? By increasing the HRT, the overall removal of the target contaminants improved by 10~30 %

by lengthening the HRT by two or three folds. This improvement was less significant for the organic contaminants than ENMs. Based on its highest sorption affinity, E2 was predominantly removed by sorption onto a stationary phase (plants). However with time more mass of E2 can sorb onto plant materials resulting into a slightly better removal in the microcosms with longer HRTs. ENMs can be subjected to various removal processes including sorption. aq-$n\mathrm{C}_{60}$ could be associated with organic colloids or be physically entrapped in various locations of the microcosms. Aggregation followed by sedimentation can be a potential removal mechanism for ENMs in the microcosms that might have been impacted by HRT. Sulfidation of Ag NMs can be an important transformation mechanism in the microcosms with decaying plant materials (Levard et al., 2012a, 2012b). A recent study by Lowry et al., 2012 showed Ag NMs in the water columns of wetland mesocosms mostly remain associated with sulfide species (Ag_2S and Ag-sulfhydryl compounds). Regardless of the change in removal with the change in HRT, our observations agree with other studies that also found that wetlands with short HRTs of 3~4 days are also able to achieve good removal of pollutants (Tanner et al., 1995; Ghosh and Gopal, 2010).

(iii) Are batch sorption experiments reasonable predictors for the fate of emerging pollutants in simple microcosms? Experiments with wetland plants and fitting the data with sorption models were useful in predicting the trend for pollutant removal in the microcosms. E2 had the highest value for K_d (0.26 L/g) and was also the most efficiently removed compound in the microcosms. Similarly, pCBA with the lower K_d (0.11 L/g) value showed negligible removal in the microcosm. Based on the higher sorption affinity and better removal of E2, partitioning to plant surfaces can be considered an important removal mechanism for more hydrophobic compounds. However, the microcosms are biologically active because of the plant decomposition and simultaneous removal of contaminants by any biological process is possible.

Batch experiments also showed that ENMs can be removed by sorption onto plant the materials similar to the organic contaminants. With plant materials accumulating in the microcosms, greater removal experienced by the ENMs can be explained by the increase in plant surface area available for sorption. Higher removal of aq-nC_{60} than CAR-Ag with increased quantity plant materials in the microcosms can be explained by its greater sorption affinity towards the plant materials. But for CAR-Ag, sulfidation or other processes were also active because when HRT was lengthened, removal of CAR-Ag became comparable with that of aq-nC_{60}.

(iv) Is salt present in wetland water and surface-coating of the Ag ENMs affect their fate in constructed wetlands?

Salts in the microcosm water increased the ionic strength which caused aggregation of Ag ENMs. However, when plant leached DOC was present Ag NMs behaved similarly in water with varying conductivity. Batch experiments with coated Ag NMs revealed that some coated NMs can resist destabilization caused by high ionic strength but it did not affect sorption onto the wetland plant materials. All three coated Ag NMs experienced similar sorption tendencies. The possible reason could be substitution of the original coatings by dissolve organic matter in the water. In a previous study investigating gold NM interaction with NOM did not find any significant difference in the stability of different types of coated NMs when NOM was present (Stankus et al., 2011).

Conclusion

This chapter investigated ENM removal in constructed wetlands. Microcosms studies were used to identify the effect of HRT and plant biomass accumulation, whereas batch-scale studies were used to investigate sorption, stability and the effect fo surface coating on ENM behavior in wetlands. Although the microcosms do not contain all the major components of a wetland including living aquatic plants or sediments, they portray the importance of wetland plants and its ability to remove both trace organic compounds and ENMs. Existing wetlands are designed and operated at particular HRTs primarily to remove bulk pollutants and nutrients. With the help of the microcosms, this study shows that wetlands when operated at relatively short HRT can remove both categories of pollutants. We acknowledge that both plants and sediments provide surface area available for sorption in wetlands and estimating sorption affinity towards the plants can be considered as a screening tool to identify which organic pollutants are more likely to be removed. Future research is necessary to uncover details of ENM interaction within wetland matrices. Apart from their role as sorbents, wetland plants decaying in the microcosms initiate biological processes that can be responsible for contaminant removal. Thus the microcosms used in study could represent the complex environment of wetlands where multiple removal processes occur simultaneously. The major conclusions of this chapter are summarized below.

- ENMs can experience comparable removal with trace organic contaminants in wetlands with plants although the mechanisms for removal of these two categories of contaminants might be different (e.g. organics were mainly removed by sorption and biodegradation, but ENMs were mainly removed by sorption and other physico-chemical transformation mechanisms like sulfidation or precipitation etc.

- Ag NMs in the microcosm effluent were found to associate with colloidal materials produced by the decaying plants.
- Increasing the plant material quantity in the microcosms, the effect of HRT on ENM removal was reduced. At comparable HRTs, removal increased for both ENMs when plant material quantity became higher. HRT had relatively higher impact on CAR-Ag than aq-nC_{60}.
- ENMs sorption occurs onto the wetland plant materials. aq-nC_{60} had better sorption affinity than CAR-Ag NMs towards wetland plants.
- Higher conductivity due to dissolved salts can aggregate ENMs, where higher DOC leached from plant stabilizes ENMs. Conductivity had no influence on organic contaminants.
- Among the three coated Ag NMs investigated, GA-Ag was most stable (prevented aggregation and subsequent dissolution) followed by CAR-Ag and PVP-Ag NMs. In the presence of hydrating wetland plants, effect of coating on sorption was not evident.

CHAPTER 5

IMPACT OF HYDRAULIC AND CARBON LOADING RATES ON CONTAMINANTS OF EMERGING CONCERN (CECs) REMOVAL IN CONSTRUCTED WETLANDS*

*The work in this chapter was submitted: Sharif, F., Westerhoff, P., Herckes, P. Impact of Hydraulic and Carbon Loading Rates on Contaminants of Emerging Concerns (CECs) Removal in Constructed Wetlands (Environmental Pollution).

Abstract

Constructed wetlands remove trace organic contaminants via synergistic processes involving plant biomass that include hydrolysis, volatilization, sorption, biodegradation, and photolysis. Wetland design conditions, such as hydraulic loading rates (HLRs) and carbon loading rates (CLRs), influence these processes. Contaminant of emerging concern (CEC) removal by wetland plants was investigated at varying HLRs and CLRs. Rate constants and parameters obtained from batch-scale studies were used in a mechanistic model to evaluate the effect of these two loading rates on CEC removal. CLR significantly influenced CEC removal when wetlands were operated at HLR > 5 cm/d. High values of CLR increased removal of estradiol and carbamazepine but lowered that of testosterone and atrazine. Without increasing the cumulative HLR, operating two wetlands in series with varying CLRs could be a way to improve CEC removal.

Introduction

Contaminants of emerging concern (CECs) are discharged into the environment from sources including wastewater treatment plant (WWTP) effluent, agricultural runoff, over land flow from animal feedlots, storm water outfall, and recreational activity. Constructed wetlands can be operated at or near many of these pollution sources to protect the receiving water bodies from pathogens and traditional bulk pollutants (e.g., biological oxygen demand (BOD), nitrate (NO_3^-)). However, constructed wetlands may also facilitate CEC removal, which is becoming more important as concerns about CECs reaching aquatic environments grow.

CECs are a broad category of contaminants that includes pharmaceuticals and personal care products (PPCPs), endocrine-disrupting contaminants (EDCs), perfluorinated compounds, and engineered nanomaterials. CEC removal was not investigated in constructed wetlands until recently. Many studies have shown that wetlands can remove similar levels of PPCPs and EDCs as do conventional wastewater treatment systems (Reed and Crites, 1995; Matamoros et al., 2005, 2006, 2008a, b, 2009; Huang et al., 2004, 2005; Gray and Sedlak, 2005; Conkle et al., 2008; Park et al., 2009; Hijosa-Valsero et al., 2010; Zhang et al., 2012). Although these studies identified wetlands as a potential treatment system for CECs, few investigated how the design of constructed wetlands especially loading rates (e.g. carbon loading rates) affect CEC removal.

Contaminant removal in wetlands occurs by natural attenuation, which involves multiple removal mechanisms e.g. sorption, biodegradation, and photolysis supported by plants and soils/sediments (Kadlec and Wallace, 2009). The design parameters of wetlands can influence contaminant removal. Hydraulic loading rate (HLR), which refers to flow rate per unit area, is a parameter commonly used to determine the size of a constructed wetland. Constructed wetlands are often operated at low HLR, which results in long hydraulic retention times (HRTs) and thus requires that the wetland be larger. HLR affects bulk pollutants including ammonia (NH_4-N), nitrate (NO_3^-), and chemical oxygen demand (COD) (Trang et al., 2010). Carbon loading rate (CLR) or plant productivity is another parameter that is not directly linked with size but influence contaminant removal processes in wetlands (Ingersoll and Baker, 1998). Wetland plants maintain aquatic habitats and perform critical functions in contaminant removal. For example, plants provide surface area on which contaminant sorption can occur. Bacteria can attach to and grow on plant surfaces (Kadlec and Wallace, 2009). Plant uptake has been also implicated as a potential removal mechanism for PPCPs (Reinhold et al., 2012; Matamoros et al., 2012). Exudates from plant decomposition and biofilms on plant surfaces can support contaminant removal (Matamoros et al., 2012). Microbial activities supported by the exudates of vegetated wetlands cause removal of polar contaminants that are not amenable to uptake or sorption processes (Matamoros et al., 2008a, 2012). In contrast, dissolved organic carbon (DOC) leached from decaying plants, which increases with a higher CLR, can reduce the actual intensity of light and lower the contribution of contaminant photolysis (Jasper et al., 2013). Although DOC is a photosensitizer that generates radical oxidant species including singlet oxygen ($^1O_2$) and OH radicals (OH•) (Lin and Reinhard, 2005), it also inhibits photo-induced reactions by self-reacting with the radical species (Wenk et al., 2011).

In this study, sorption, biodegradation, and photolysis of selected CECs were investigated in the presence of wetland plants (Table 5.1). Wastewater effluent contains plethora of organic CECs but our goal was to select as few compounds as possible with different physico-chemical properties because variation in the properties determined the relative strength for undergoing certain attenuation mechanisms. Dried wetland plants (*Scripus* spp.) were added into continuous-flow type lab-scale microcosms to mimic the areas where growing plants senesce over time and facilitate pollutant removal. Batch-scale studies were conducted using the plant materials to study the different removal mechanisms. To understand the effect of HLR and CLR on CEC removal, a fate-transport model was developed in AQUASIM, a software program used to simulate aquatic systems. The removal mechanisms were incorporated into the model with the help of rate expressions that were parameterized with partitioning coefficients and rate constants obtained from batch–scale studies. Model predictions were used to investigate how varying HLR and plant addition rates affect removal of different types of CEC.

Table 5.1

*Physico-chemical properties of selected organic CECs**

CECs (Class)	Molecular weight MW (g/mole)	pK_a	Log K_{OW}
17 β - estradiol (E2) (Steroid hormones)	272.4	10.4	4.02
Testosterone (Steroid hormones)	288.4	-	3.32
Atrazine (Pesticides)	215.7	1.7	2.61
Carbamazepine (Pharmaceuticals)	236.3	13.9	2.45

* Source: EPI Suite^{TM} (U.S. EPA)

Material and methods

Materials

17β- estradiol (E2), testosterone, atrazine, carbamazepine, and sodium azide (NaN_3) were obtained from Sigma-Aldrich, St. Louis, MO, USA. Potassium bromide was obtained from ICN Biomedicals Inc., Ohio, USA. HPLC-grade acetonitrile and formic acid were obtained from Fisher Scientific, New Hampshire, USA. The water used for analytical purposes was nanopure water with 18.3 Ω conductivity (Milipore Inc., Billerica, MA). Isotope-labeled compounds (99 % purity) -Estradiol-[$^{13}C_6$], Testosterone-[d_5] , Atrazine-

[d_5] and Carbamazepine-[d_{10}] were purchased from Cambridge Isotope Laboratories, Inc (Tewksbury, MA, USA).

Lab-sale microcosms

The microcosms used in this study were acrylic tanks (20 cm x 25 cm x 13 cm) with internal baffles to reduce short circuiting (Schematics shown in Chapter 4). They represent areas of free water surface wetlands with floating and submerged vegetation. Similar microcosms were previously used to study transformation of DOC, nitrate and investigate sorption of trace organics and engineered nanomaterials onto wetland plants (Ingersoll and Baer, 1998, Pinney and Westerhoff, 2000, Sharif et al., 2013). The influent water was activated carbon filtered dechlorinated filtered tap water (Tempe, AZ) to provide trace level metal ions essential for bacterial growth. The water (pH 7.3 ± 0.2) was pumped through a Monistat cassette peristaltic pump at different flow rates. Air-dried bulrushes (*Scirpus validus*) collected from the Tres Rios wetlands, AZ, were added into the microcosms at a rate of 3 g/week. Plant addition rates were based on the productivity of emerging macrophytes in the Sonoran deserts of Arizona; 6000 g DW (dry weight) m^2/yr were considered a reasonable estimate, which translated into an addition of 3 g of plants per week (Ingersoll and Baker, 1998).Two different microcosms were operated at an HLR of 3.4 cm/d and 5.6 cm/d respectively to obtain effluent water with different DOC levels.

Sorption experiments

Sorption experiments were conducted using the method described elsewhere (Sharif et al., 2013). In brief, several initial concentrations of CECs from 0.1 μM to 1 μM. Air-dried wetland plant materials were autoclaved for 15 minutes at 1500 PSI prior to conduct the experiments to minimize microbial activity. The ratio of plant materials to volume of water was 100 mg: 10 mL. Experiment was conducted with a ratio of 300 mg: 10 mL. The measured amount of plants was placed in 40 mL amber vials with Teflon-lined screw caps and hydrated in microcosm influent water. The pH was adjusted to 7 using sodium hydroxide (NaOH) prior to the addition of the target CECs. The vials were then covered with aluminum foils to avoid photolysis and shaken at 150 rpm for 72 hours at room temperature. The equilibration time was selected on a previous study (Sharif et al., 2013). After the equilibration period, samples were taken, filtered with GF/F filters (0.7 micron), and kept at 4°C until analysis. The amount of a CEC sorbed by the plant material was calculated from the difference between the initial and final concentrations of the CEC in the samples.

A desorption study was conducted immediately after sorption equilibration was complete. The water in the sample vials used in the sorption studies was replaced with fresh microcosm influent water. Vials were then shaken, and supernatants were sampled to

Biodegradation experiments

Batch experiments were conducted in 100 mL volume serum bottles (prepared in triplicates) containing decaying plant biomass and effluent water from microcosms. The ratio of the mass of decaying plant biomass to volume of water was 1 g: 100 mL and 3 g: 100 mL. 1mM ammonia (NH_3-N) was added as a nutrient in these experiments. The amount of plant biomass represents bulk weight, not actual dry weight. Target CECs were spiked to obtain an initial concentration of 1 µM, samples were taken periodically and filtered. Controls were prepared using the same plant biomass except autoclaved prior to addition into the serum bottles. The serum bottles were covered with aluminum foil to prevent photolysis and kept at room temperature (25^oC). To further stop microbial activity, samples were spiked with NaN_3 to a concentration of 100 mg/L. Biodegradation experiments were conducted in aerobic conditions and at room temperature, but no stirring or external addition of O_2 was provided.

Photolysis experiments

Batch photolysis experiments were conducted with simulated natural sunlight irradiation system equipped with a temperature controlled water bath. The schematic of the apparatus is shown in Appendix A (Figure A1). The simulated light was provided by a 300-W xenon arc lamp (Spectraphysics Oriel, 91160A), and the output of the arc lamp is filtered through a standardized air mass 1.5 filter (AM 1.5 Global), which yields a spectrum representative of the solar spectrum at ground level when the sun is at a zenith angle of 48.2°, The lamp output power is variable but was set at a typical ambient value of 1325 W/m^2 (Chen at al., 2010). The CECs were spiked in two types of water (i) microcosm influent water (without any plant-leached DOC) and (ii) filtered microcosm effluent water with different levels of plant-leached DOC. These waters were placed in a thermostated and jacketed reactor vessel (100 ml volume) and irradiated using the solar light stimulator. Samples were taken from the reactor vessel with time to measure the photolysis kinetics. Water temperature was maintained to be 25^0C. Dark controls were also prepared using the same types of water.

Liquid Chromatography/Tandem Mass spectrometry (LC-MS/MS)

A Varian ProStar 210 HPLC (Palo Alto, CA) was used for chromatography. Analytes were separated with a Synergi (250×4mm) column with a 4 μm particle size (Phenomenex, Torrance, CA). A binary isocratic phase was used with 0.1% formic acid, 25% deionized water and 75% methanol as eluent. An injection volume of 20 μL was used for all analytes. The ionization sources to analyze the CECs used were electrospray ionization (ESI) for atrazine and carbamazepine and atmospheric chemical ionization (APCI) for E2 and testosterone. To identify the precursor and product ions, the HPLC employed a Varian 1200L triple quadrapole mass spectrometer. Method detection limits (MDLs) and Limit of quantifications (LOQs) for E2, testosterone, atrazine and carbamazepine were- 0.02 and 0.08 nM, 0.02 and 0.05 nM, 0.03 and 0.11 nM and 0.05 and 0.15 nM respectively. The precursors and product ions, retention time and other parameters are summarized in Appendix A (Table A1). To compensate matrix effect, isotopically labeled compounds were spiked into the samples prior to analysis. Matrix spike recovery was determined for each of the analytes. Recovery (n = 6) of E2, testosterone, atrazine and carbamazepine in microcosm effluent water was 109±8%, 95±9%, 99±7%, 104±3% respectively.

Fate-transport model using AQUASIM

A fate predictive model was developed in AQUASIM, programmed by EAWAG (Swiss Federal Institute of Aquatic Science and Technology). The basic fate-transport equation (eqn. 5.1) included the different removal mechanisms. Rate expressions were used to define the mechanisms (eqn. 5.2 to eqn. 5.6) and parameterized with constant values obtained from batch-scale studies. Table 5.2 includes the definitions of the different parameters used in these equations.

$$\frac{\mathrm{dC_d}}{\mathrm{dt}} = \frac{\mathrm{Q}}{\mathrm{V}}\mathrm{C_0} - \frac{\mathrm{Q}}{\mathrm{V}}\mathrm{C_d} - u\frac{dC_d}{dx} + D\frac{d}{dx}\left(\frac{dC_d}{dx}\right) \pm \mathrm{r_T} \tag{5.1}$$

Where, $\mathrm{r_T} = \mathrm{r_{hydro}} + \mathrm{r_{sorp}} + \mathrm{r_{bio}} + \mathrm{r_{photo}}$

$$\mathrm{r_{vol}} = -\mathrm{k_{vol}C_d} = \frac{\mathrm{k_L}}{\mathrm{H}}\mathrm{C_{d}} = \frac{\mathrm{K_l}}{\mathrm{H}}\frac{\mathrm{H_e}}{\mathrm{H_e} + \mathrm{RT}\,(\frac{\mathrm{K_l}}{\mathrm{K_g}})}\mathrm{C_d} * \alpha_0 \tag{5.2}$$

$$r_{hydro} = -k_{hydro} C_d \tag{5.3}$$

$$r_{sorp} = -k_{ad} C_p C_d + K_{des} C_p C_s = -K_{ad} C_p C_{sd} + \frac{K_{ad}}{K_d} C_p C_s \tag{5.4}$$

Table 5.2

Definition and values of parameters used in the model

Definition	Values (unit)
Length, L	20.5 (cm)
Width, w	12.7 (cm)
Depth of water, H	25.2 (cm)
Cross-sectional area, A_X	320 (cm^2)
Surface area, A_S	260 (cm^2)
Volume, V	6561 (cm^3)
Hydraulic loading rate, HLR	1, 3, 6, 10, 15, 20 (cm/d)
Hydraulic residence time, HRT	25, 10, 5, 2.5, 1.7, 1.3 (d)
Flow rate	260, 651, 1302, 2604, 3905, 5207 (cm^3/d)
Longitudinal velocity, u	Q/A_x (calculated by the model) (cm/d)
Dispersion, D	Dispersion number × u × L (cm/d) (Dispersion number = 0.2)
C_d	Dissolved-phase concentration (ng/L)
C_S	Sorbed-phase concentration (ng/g)
K_d	Partitioning coefficient (Table 5.3)
K_{ad}	Adsorption rate constant (L/g-d) (Table 5.3)
K_{des}	Desorption rate constant (1/d) (Table 5.3)
K_{hydro}	Hydrolysis rate constant (negligible, Figure A2, Appendix A)
Carbon loading rate, CLR	2×10^3 , 6×10^3,1.2×10^4 kg-DW /ha-yr equivalent to 0.14, 0.43, 0.86 g-DW/d in microcosms (Ingersoll and Baker, 1998)
DOC loading rate, Y	Using equation (5.8) (g DOC/cm^2-d)
Plant carbon addition rate, B	50% of the dry weight of plants added g C/d
DOC leaching constant, K_{PL}	0.08 g DOC/g C added (Pinney et al., 2000)
DOC biodegradation rate, K_p	0.06 1/d (Pinney et al., 2000)
Plant decomposition rate, K_{decomp}	0.0028 1/d (Debusk and Reddy, 2005)
Concentration of plant biomass in the microcosms, C_p	$\sum_{i=0}^{t} \frac{\left(CLR * e^{-K_{decomp} * t_i}\right)}{V}$ g/L

Table 5.2

Definition and values of parameters used in the model (Continued)

Definition	Values (unit)
Molar adsorptivity of a chemical at wavelength λ, ε_λ	Absorbance/l.C (L/mol-cm) Path length, l = 2 cm, Concentration in phosphate buffer, C = 10 mg/L
Quantum yield, φ	0.0002–0.03
Flux of solar energy of wavelength λ, I_λ	6.5×10^{11} to 3.54×10^{15} (mol photons/cm^2-s) (Zepp and Cline (1977))
Conversion constant, j	6.023×10^{23} (Lyman and Rosenblatt, 1982)
Light attenuation coefficient, α_D	$\alpha_D = D[\alpha_W + \alpha_a \times a + \alpha_c \times DOC + \alpha_s \times m]$ (1/cm) α_w = attenuation coefficient for water 0.141 1/cm α_a , α_c , α_s = concentration-specific attenuation coefficients for chlorophyll a, DOC, and inorganic suspended solids, 69, 6.25, and 0.35 L/mg-m, respectively a, m = concentrations of chlorophyll a and inorganic suspended solids, 0.001 and 5 mg/L, respectively (Chapra, 1997)
K_L, K_l, K_g	Over-all, liquid and gas film mass-transfer coefficients (m/d)
Fraction of the chemical in the nonionized form α_0	$\alpha_0 = \frac{[H^+]}{[H^+] + 10^{-pK_a}}$ Where, pKa= Acid dissociation coefficient (Table 5.1) $[H+] = 10^{-pH}$
He	Henry's law constant (atm m^3 $mole^{-1}$)
M	Molecular weight of the organic CEC
$K_{l,O2}$	Oxygen transfer coefficients (m/d)
U_w	Wind velocity (mps)
R, T	Universal gas constant [8.206x10-5 atm m^3 $(K\ mole)^{-1}$], absolute temperature (K)

$$r_{photo} = -k_{photo}C_d = -C_d\frac{2.303}{j}\varphi\sum\varepsilon_\lambda I_\lambda = -C_d\frac{2.303}{j}\frac{\varphi}{\alpha_D H}\sum I_{0,\lambda}(1-e^{-\alpha_D * H})\varepsilon_\lambda \qquad (5.5)$$

$$r_{bio} = -k_{bio,p}C_pC_d \tag{5.6}$$

$$DOC = \frac{Y_{DOC}}{\frac{H}{\tau}+K_p*H} \tag{5.7}$$

$$Y_{DOC} = \frac{K_{PL\,(g\,DOC)}}{g\,C_{added}} * B\left(\frac{(g\,C_{added})}{d}\right) * \frac{1}{A_{surface}} \tag{5.8}$$

The dimensions of the microcosms were used to define the geometry of a wetland system (Table 5.2). HLR values are within the typical range (0.1~ 20 cm/d) used in constructed wetlands (The Interstate Technology & Regulatory Council wetland tem, 2003). The selected plant addition rates were previously used by Ingersoll and Baker, 1998 to represent low, medium and high plant productivity in wetlands of Arizona. DOC leached from the plants was predicted by the model using eqn. 5.7 and 5.8 adopted from Pinney et al., 2000. A sample calculation to determine plant leached DOC is provided in Appendix A.

The selected CEC influent concentration, 10 nM (equivalent to few µg/L), is comparable with the concentration range of PPCPs detected in constructed wetlands receiving secondary effluent (Matamoros et al., 2008b). Parameters defining the rate expressions, other than the partitioning coefficients and rate constants were calculated or obtained from literature sources and listed in Table 5.2. Among the five different mechanisms, hydrolysis and volatilization were not included during simulation. Hydrolysis of the CECs in microcosm influent water was found negligible (Figure A2, Appendix A). Volatilization of the CEC is expected to be insignificant because of the low values of Henry's law constants, H (< 10^{-7} atm-m^3/mol). It should be noted that volatilization is considered less unimportant when H is less than $3x10^{-7}$ atm-m^3/mol (Lyman and Rosenblatt, 1982). The amount of plant materials accumulated in the microcosm in a 2 year period was estimated using a plant decomposition rate of 0.0028 d^{-1} (Debusk and Reddy, 2005). Dispersion number, D was selected to be 0.2. Typical values of D in wetlands varies from 0.13 to 0.46 (Kadlec and Wallace, 2008). Quantum yield (φ) was determined by solving eqn. 5.6 for the photolysis rate constant obtained in microcosm effluent water. The irradiation intensity of the xenon lamp was used to calculate φ. Molar adsorptivity of CECs were calculated by measuring their UV-VIS spectra in 0.05M phosphate buffer at pH 7.0 (Figure 5.1a). Solar irradiation intensity for a typical summer season (40^0N latitude, midday and midseason) was adopted from Zepp and Cline, 1977 (Figure 5.1b).

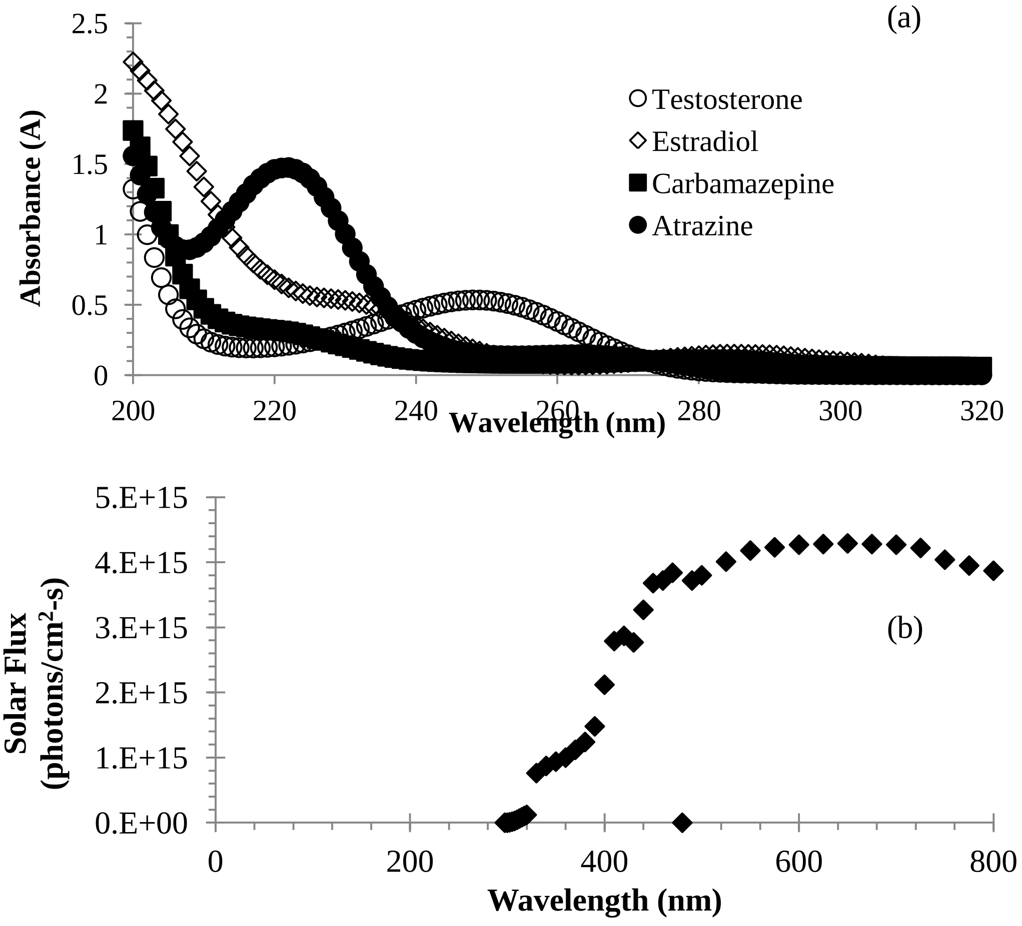


Figure 5.1. UV-vis spectra of the CECs in 0.05M phosphate buffer (pH=7.0, C_0= 10 mg/L) (a). Solar spectral distribution (Zepp and Cline, 1977) (b).

Results and Discussion

Batch sorption experiments

Figure 5.2 (upper panel) shows the sorption isotherms for E2, testosterone, atrazine and carbamazepine in microcosm influent water. Isotherms obtained with 30 g/L of plant materials are shown in Figure A3 of supplementary data. Partitioning coefficients, K_d were determined as the slope of the isotherms lines when it was linear (using the initial data points) and summarized in Table 5.3 with their corresponding log D calculated at pH 7. The parameter Log D represents the un-ionized species of an organic compound that can sorb onto organic matter (Leo et al., 1995). It is calculated based on the log K_{OW} and pK_a of the organics ($\log D = \log K_{OW} - \log[1+10\{abs(pH-pK_a)\}]$). Comparing the K_d values against Log D, CECs with higher log D showed greater sorption affinity which indicates that sorption of CECs on wetland plants are dominated by hydrophobic partitioning.

Sorption of organic CECs onto soils have been widely studied (Alvord and Kadlec, 1995, Casey et al., 2003, Lee at al., 2003, Dori et al., 2005Fan et al., 2007, Stein et al., 2008, Bradley et al., 2009, Yamamoto et al., 2009), however, interaction and removal by wetland plant biomass have not been investigated. Casey et al., 2003 reported K_d for E2 to range between 0.01 ~ 0.332 L/g onto soils with varying texture and organic matter. K_d values for testosterone onto the similar types of soils ranged between 0.01 ~ 0.8 L/g (Casey et al., 2004). Sorption of the steroidal hormones was found largely dependent on the organic matter content of the soil. For both steroids, K_d increased in soils with higher organic matter. With low organic matter content (0.2 ~ 2.4 %), Lee et al., 2003 obtained highly variable K_d values for E2 (0.003 ~ 0.08 L/g) and testosterone (0.004 ~ 0.04 L/g). In plant materials, organic content is usually much higher than sediments and consequently, our experimentally determined K_d values are in the higher end of the range observed from the literature.

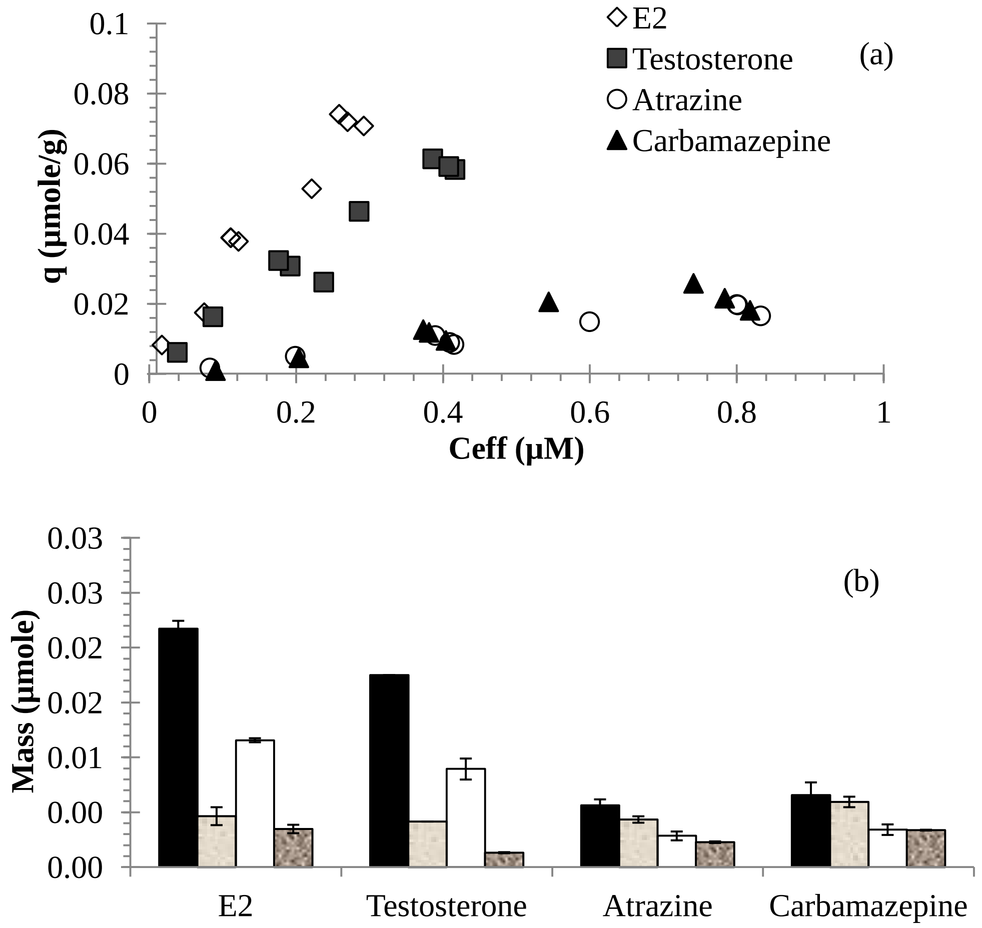


Figure 5.2. Sorption isotherm for CECs with hydrated wetland plant materials (10g/L) (a). Mass of CECs adsorbed and desorbed from plants at different initial concentrations (b). ■ Mass adsorbed (C_0= 1μM),□ mass adsorbed (C_0= 0.5 μM), ░ mass desrobed (C_0= 1μM), ▩ mass desorbed (C_0= 0.5 μM).

K_d for atrazine sorption onto wastewater irrigated soil was found to very low (0.0003 ~ 0.0005 L/g) (Dori et al., 2005). Chung et al., 1996 found a K_d value of 0.01 L/g for atrazine onto wetland sediments and observed a very slow rate of biotransformation

(0.003 d^{-1}). Like atrazine, sorption of carbamazepine onto soil is also possible and K_d values reported by Stein et al., 2008 was 0.0017 and 0.012 L/g in two different sediments with varying organic content. Yamamoto et al., 2009 found K_d values of 0.014 and 0.018 L/g for carbamazepine in two types of river sediments.

Rate constants for desorption was determined based on the equilibrium relationship between sorption and desorption (equation 5.4). Using sorption kinetics sand partitioning coefficient values, desorption rate constants were determined (Table 5.3). Sorption kinetics of the CECs is shown in Figure A4 of Appendix A. To investigate the extent of sorption and desorption, adsorbed and desorbed mass of the CECs at two different solute concentrations (0.5 µM and 1 µM) were compared in Figure 5.2 b. Desorption increased with increasing solute concentration. At C_0 of 1 µM, the desorbed mass for E2 was 0.063µmole, but at C_0 of 0.5 µM, the desorbed mass was 0.027 µmole. Similar phenomenon was observed in previous studies considering soils as sorbents. With increasing solute concentration, more sorption sites were occupied and amount sorbed increased. But with the increasing demand for sites, less favorable or low energy sites were occupied from where desorption occurred easily (Drori et al., 2005; Zhang et al., 2010).

CECs with higher sorption tendency showed less desorption. E2 experienced least desorption followed by testosterone, carbamazepine and atrazine. For example, only 20 % of the adsorbed mass of E2 desorbed from the plants but for atrazine the desorbed mass was 82 %. For carbamazepine, with the lowest sorption affinity, 99 % of the mass desorbed. Thus hydrophobic CECs may strongly bound to the sorption sites of the organic rich plant surfaces form where desorption is less likely to occur.

Biodegradation experiments

Figure 5.3 shows kinetic data from the batch CEC biodegradation experiments with the decaying biomass to water ratio of 1 g: 100 mL. The difference in removal between the abiotic control and the biologically active batches can be attributed to biologically mediated removal. Concentrations in the control were higher than in the biologically active batches for E2 until 3 d and for testosterone until 5 d. Such behavior of CECs in the abiotic controls leads to the possibility that the bacteria in the decaying plant biomass were not completely inactive even after autoclaving or addition of sodium azide (NaN_3). However, ammonia was added as a nutrient in these experiments and no loss in its concentration was observed in the control whereas 4 mg/L of NH_3-N was removed the in the bioactive batch (Figure A5). Physical controls were also prepared with the organic CECs spiked in water (without any addition of plant biomass) and $NaN_3$was added to prevent microbial activities. CECs were found to remain stable in these controls which remove any possibilities of significant reaction occurring between NaN_3 and the CECs. Based on these observations, sterilization was probably maintained in the abiotic controls and reactions between NaN_3 and the CECs were not significant. Consequently, the loss in the concentrations can be explained by sorption or any abiotic degradation. Sorption must have occurred but the actual dry weight of the biomass was only 10 ~30 % of the dry weight of plant materials used in sorption isotherm studies and it does not explain the high removal observed in the abiotic controls. However, NaN_3 can also alter the water chemistry and influence sorption (Stevens-Garmon et al., 2011, Maeng et al, 2013). But the complete removal observed in the abiotic controls was not only due to any enhanced sorption; abiotic degradation was also possible. Colucci et al. (2001) found 75 % of E2 added into autoclaved soil microcosms to transform to E1.

Transformation of E2 in autoclaved controls with the presence of Hg Cl_2 was also reported by Fan et al. (2007). In summary, the controls used in these studies should not be referred as ‘abiotic controls’ instead they can be termed as ‘azide spiked /autoclaved control’ where biological reactions were minimized but abiotic removal was still occurring.

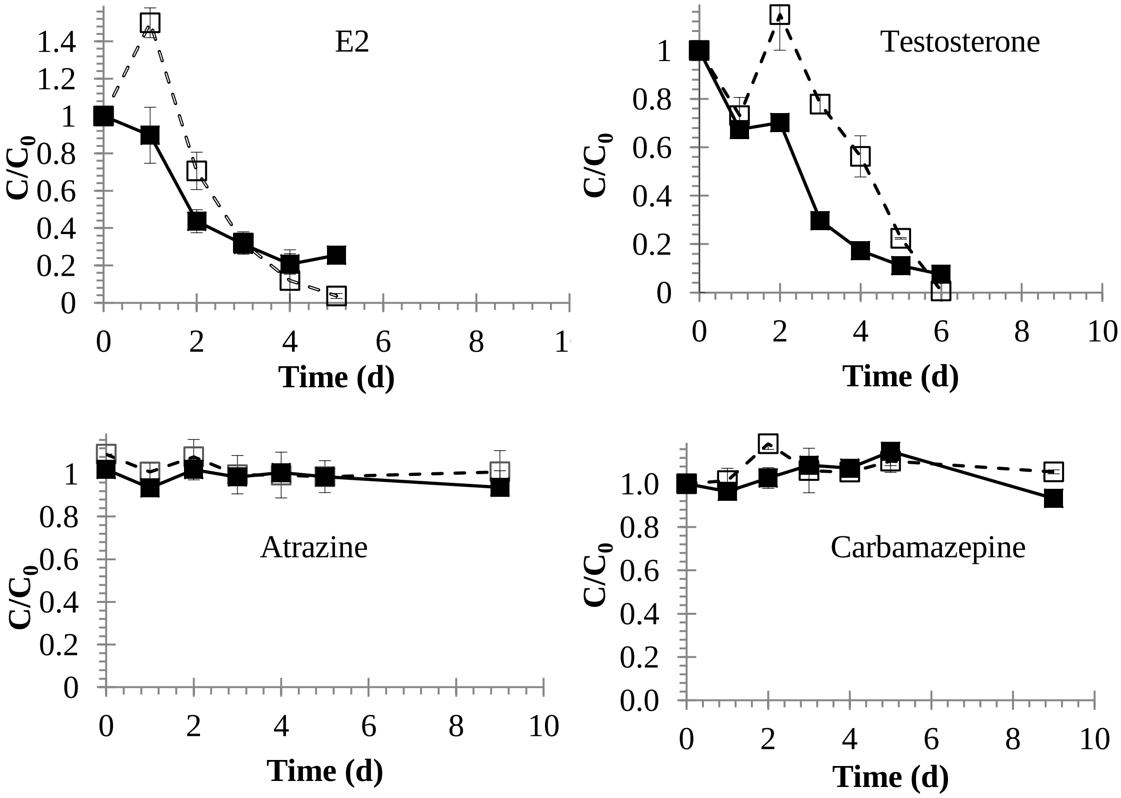


Figure 5.3. Removal kinetics of the CECs in biodegradation experiments with 10 g/L of decaying plant biomass. Symbols: Close circle: biologically active; open circle: Autoclaved – azide spiked control.

First-order reaction rate constants (K_{bio}) were obtained using the data shown in Figure 5.3. For E2 and testosterone, data up to 3 and 4 days respectively were used and values obtained in the controls were deducted. Comparing K_{bio} of the steroids, testosterone was more biodegradable than E2. Both atrazine and carbamazepine showed negligible removal by any biological process supported by the decaying plant biomass. The experiments were also conducted using a different quantity of plant biomass (3 g: 100 mL) and the degradation kinetics for E2 and testosterone are shown in Figure 5.4.

Increasing the amount of plant biomass can increase the number of bacteria available for biodegradation of CECs which causes faster removal. To reflect the effect of biomass on the biodegradation rate, second-order reaction rate constants, $K_{bio,p}$ (L g plant biomass^{-1} d^{-1}), were obtained by dividing K_{bio} by the dry weight of the plant biomass used. These were used in the model developed later in the study. This method of deriving a pseudo-first order reaction rate constants has been applied by Painter et al. (1968) and Paris et al. (1981). The assumption behind using these constant values was that the plant biomass concentration is substantially greater (g/L) than the concentration of CECs (nM or ng/L). Table 5.3 summarizes the values of rate constants used later in the model.

Biodegradation of steroidal hormones especially E2 onto soils and sediments was investigated by Fan et al., 2007, Bradley et al., 2009, Mashtare et al., 2013 and Li et al., 2013. E2 was found to experience rapid biodegradation in agricultural soils (1.4~2.8 d^{-1}) (Colucci et al., 2001). Biodegradation rates of E2 and testosterone in packed columns with different types of soils were found to vary between 0.07 ~ 0.18 d^{-1} and 0.07~0.36 d^{-1} respectively (Das et al. 2004). Mass of soil used in these studies was high compared to the amount of plant biomass used in our experiments. Using aerobic soil microcosms, Mashtare et al., 2013 studied biodegradation of E2 and the rate constants varied between 1.6 and 2 d^{-1} for different types of soils. The actual dry weight of the soil was 10 ± 0.1 g. The normalized biodegradation rates from this study are comparable with our observed rates. Li et al. (2013) studied E2 removal in soil-water systems and found biodegradation rates to vary between 0.24 ~ 0.4 d^{-1} which are also comparable with our observations.

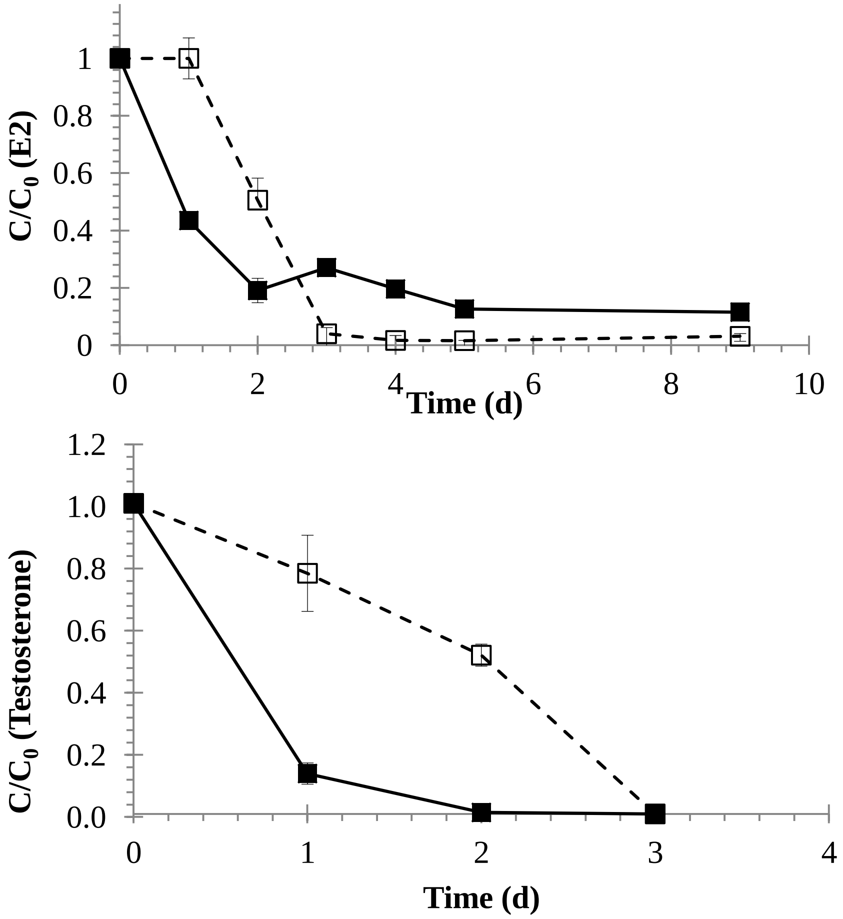


Figure 5.4. Removal kinetics of the CECs in biodegradation experiments with 30 g/L of decaying plant biomass. Symbols: Close circle: biologically active; open circle: Autoclaved – azide spiked control.

Photolysis experiments

Figure 5.5 shows the photolysis kinetics of CECs in microcosm influent and effluent water. Both atrazine and testosterone underwent greater photolysis in microcosm influent water compared to the others. But photolysis was significantly reduced in the microcosm effluent water which contained higher DOC (6.5~8.5 mg/L) than the influent water (DOC 1.5mg/L). Table 5.4 summarizes the first order reaction rate constants of photolysis for the CECs. Between the two types of microcosm effluent water used, rate constants were slightly lower for the effluent water with higher DOC. Torrents et al. 1997 found significant reduction in the rate of atrazine photolysis when surface water DOC was present. Slower photolysis of nitrosamines were also observed in water with effluent organic matter (Chen at al., 2007). Thus instead of causing any indirect photolysis by producing radical species, plant-leached DOC, similar to the DOC derived from surface or wastewater sources can interfere and reduce the light intensity which will ultimately reduce direct photolysis of CECs in wetlands.

Table 5.3

Isotherm parameters and rate constants obtained from batch sorption and biodegradation experiments

CECs	Sorption isotherm experiments				Biodegradation experiments
Parameter	Log D (pH=7)	K_d (r^2)	K_{ad} (r^2)	K_{des} (r^2)	$K_{bio,p}$ (r^2)
Unit	-	(L/g)	(L/g-d)	(1/d)	(L/g plant biomass-d)
E2	2.4	0.32 (0.91)	7.8E-02 (0.91)	2.4E-01	1.2E-01 (0.92)
Testosterone	1.2	0.16 (0.72)	4.5E-02 (0.94)	2.8E-01	2.8 E-01 (0.9)
Atrazine	0.88	0.024 (0.87)	1.5E-02 (0.99)	6.3E-01	-
Carbamazepine	0.40	0.025 (0.9)	1.6E-02 (0.94)	6.4E-01	-

φ was determined using the photolysis rate constants obtained in microcosm effluent water. φ for E2 measured in river water by Lin & Reinhard, 2004 was 0.0048 which is comparable with our experimental value of 0.0085. Borch et al., 2008 studied photodegradation of steroidal hormones in Colorado river water including E2 and testosterone and observed higher loss rate of testosterone than E2 under simulated natural conditions which is similar to the findings of our study. Photolysis of carbamazepine in natural water was found to be very slow with φ values of 0.00013 (Lam & Mabury, 2005) and 0.000027 (Yamamoto et al., 2009). Palm & Zetzsch, 2012 estimated φ of atrazine (0.05) in buffer solution with pH 9 which is slightly higher than what we have observed in microcosm effluent water under the presence of plant-leached DOC (0.024).

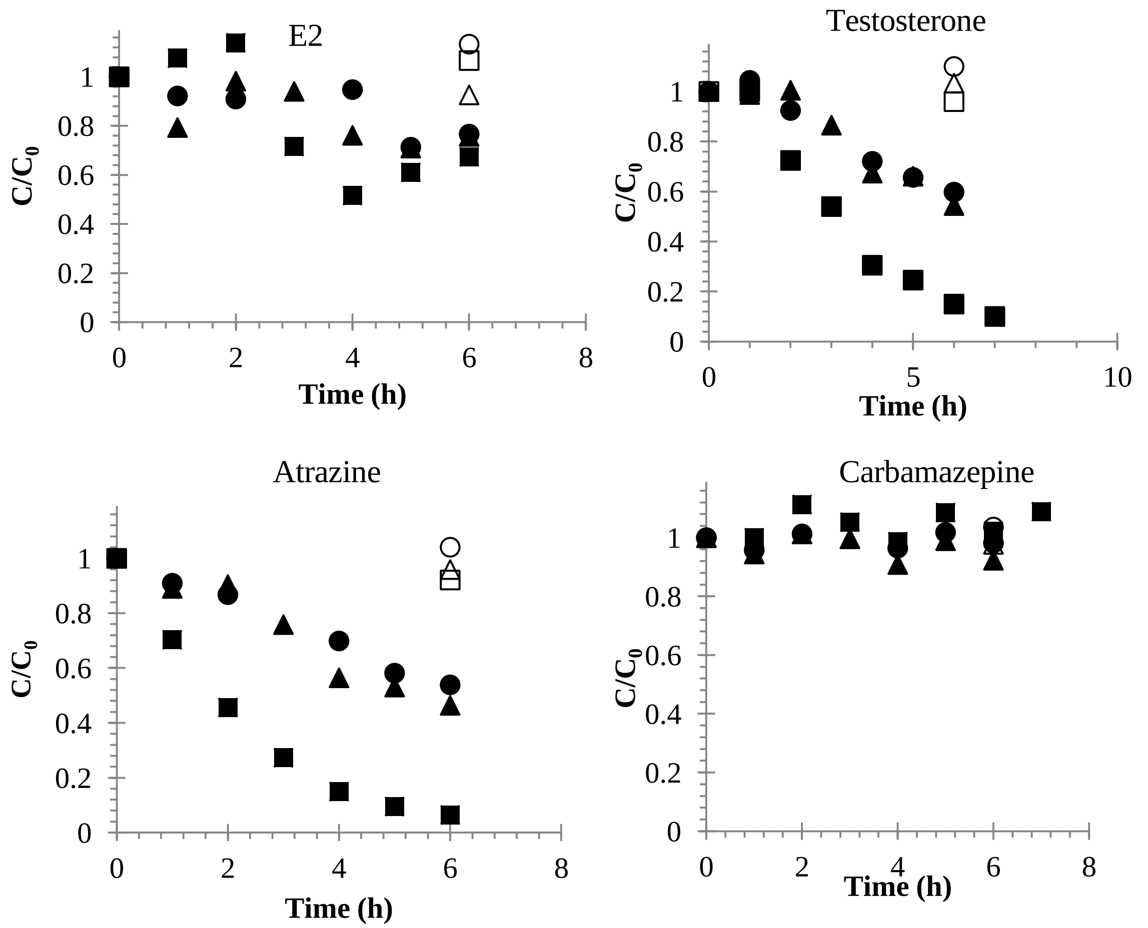


Figure 5.5. Photolysis kinetics of CECs in microcosm influent water (organic free) and effluent water (with plant-leached organic matter).Symbols: ■ -microcosm influent water, ▲ - microcosm effluent 1, ● - microcosm effluent 2. Open symbols (□, △,○) represent dark controls.

Model predictions for CEC removal

The CEC removal at varying HLR and plant addition rates predicted by the model were converted into areal removal rates and summarized in Figure 5.6. Areal removal rate represents contaminant removal per unit surface area of a wetland per daily input. It is often used to estimate size of wetlands from projected pollutant loading. The equation used to calculate areal removal rate is shown below.

$$\text{Areal removal rate} = \frac{Q*(C_0 - C_{eff})}{A_{surface}} \tag{5.9}$$

Table 5.4

Photolysis rate constants (d^{-1}) for organic CECs

	K_{Photo} (d^{-1})			
Water quality	Estradiol (r^2)	Testosterone (r^2)	Atrazine (r^2)	Carbamazepine (r^2)
Microcosm influent DOC= 1.5 mg/L NO_3^-= 0 mg/L-N	2.4 (0.7)	9.0 (0.77)	15.6 (0.99)	-
Microcosm effluent 1 DOC= 6.5 mg/L NO_3^-= 0.25 mg/L-N	1.3 (0.6)	2.1 (0.84)	3 (0.93)	-
Microcosm effluent 2 DOC= 8.5 mg/L NO_3^-= 1.3 mg/L-N	1.1 (0.66)	2.0 (0.91)	2.2 (0.97)	-

Where, Q is the flow rate, C_0 and C are the influent and effluent concentration of a contaminant and $A_{surface}$ is the surface area of the wetland. Areal removal rates of the CECs increased with HLR. However, increasing the HLR beyond 10 cm/d, removal of carbamazepine did not improve. The corresponding HRT of these loading rates can be found in Table 5.2. Since HRT is inversely related to HLR, areal removal rates decreased with HRT (Figure A6, Appendix A).

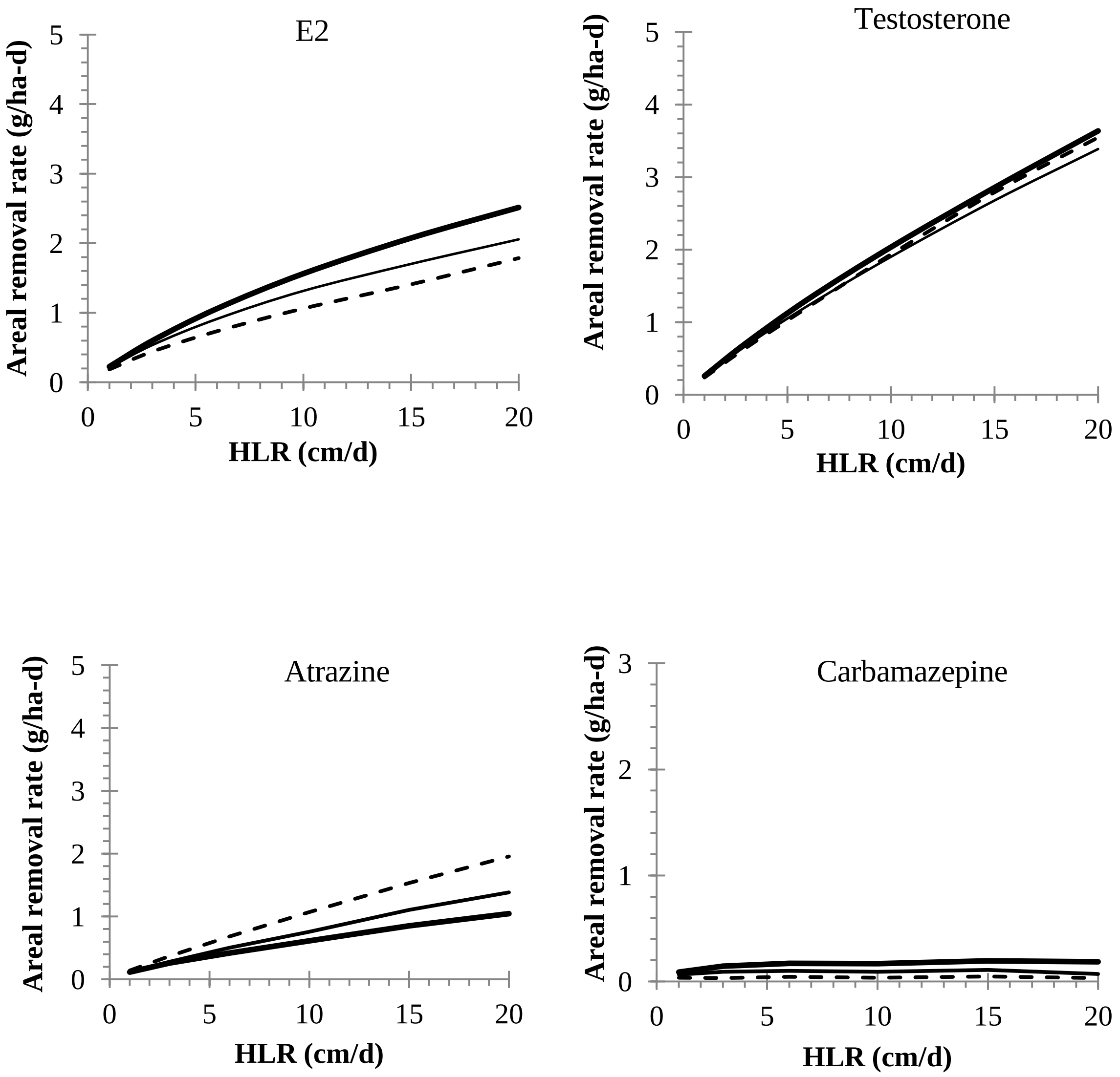


Figure 5.6. Model predicted areal removal rates for CECs at varying hydraulic loading and plant addition rates. – – –2x10^3 Kg-DW/ha-yr, —— 6x10^3 Kg-DW/ha-yr, ━━ 1.2x10^4 Kg-DW/ha-yr.

For all CECs except for carbamazepine, the effect of CLR on areal removal rates was evident beyond HLR > 5 cm/d. Increasing CLR from 6 × 10^3 kg-DW/ha-yr to 1.2 × 10^4 kg-DW/ha-yr increased the areal removal rates for E2 from 1.8 g/ha-d to 2.5 g/ha-d at the highest HLR of 20 cm/d. CLR is likely to influence removal because E2 has a high sorption affinity toward wetland plants.

The areal removal rate of testosterone increased from 3.55 g/ha-d to 3.64 g/ha-d between the lowest and highest CLRs. Higher CLR not only provided more surface area for sorption or increased the quantity of bacteria available for biodegradation but also increased the DOC level, which significantly reduced photolysis of testosterone. Because of this counter effect, net impact of CLR on testosterone was not significant. Figure A7, Appendix A explains how different removal mechanisms are linked with the physico-chemical properties of the CECs). However, areal removal rates of testosterone were still greater than those of E2 at the highest CLR; thus, the high productivity of plants in a wetland is an advantage for overall steroid removal. Finally, CLR had a major impact on atrazine removal because atrazine was removed mainly by photolysis. The areal removal rate of atrazine decreased from 2 g/ha-d to 1 g/ha-d when CLR increased from 2×10^3 kg-DW/ha-yr to 1.2×10^4 kg-DW/ha-yr.

Finally for carbamazepine, increasing CLR improved removal. Thus, regardless of the hydraulic conditions, a densely planted wetland can remove more carbamazepine than a wetland with a small amount of planted area. Areal removal rates of carbamazepine increased from 0.03 g/ha-d to 0.15 g/ha-d when CLR increased from 2×10^3 kg-DW/ha-yr to 1.2×10^4 kg-DW/ha-yr at the HLR of 20 cm/d.

Experimental data from the continuous-flow through microcosms were used to validate the model. The selected CECs were introduced in the microcosms with HRT of 4.5 d (HLR = 5.6 cm/d) and 7.4 d (HLR = 3.4 cm/d). These HRT represent actual residence time obtained from tracer studies. Data from these studies are shown in Figure A8 and A9 of

Appendix A. Plants were added at 3 g DW/week to represent the productivity rate of $6x10^4$kg-DW/ha-yr. Steady state conditions were confirmed by monitoring DOC in the effluent of these two microcosms. At this stage the wetlands microcosms had been operated for more than eighteen months. The average water quality parameters for the two effluents are shown in Table 5.5.

Table 5.5

Average concentration of water quality parameters in the effluent of the microcosms in CEC 1 study (mg/L or mg-N/L)

	DOC	NH_3-N	NO_2^--N	NO_3^--N
	mg/L	mg-N/L	mg-N/L	mg-N/L
HRT (4.5 d)	4.8±0.7	7.86	0.32	0.84
HRT (7.4 d)	6.8±1.0	6.2	0.63	2.6

CEC removal data from this experiment is shown in Figure 5.7. The microcosms were operated in dark; thus the contribution from photolysis was excluded during model simulation. The model predicted CEC removal at the two HLR and $6x10^4$ kg-DW/ha-yr of plant addition rate was compared against the experimental removal data (Figure 5.8). Percent accuracy values for E2, testosterone, atrazine and carbamazepine for the two HRTs were- 108 % and 103 % ; 99.6 % and 104 %; 128.5 % and 127 %.; 175 % and 80 % respectively. Higher accuracy for carbamazepine was due to its negligible removal observed in the microcosms (4 % and 13 %) as well as predicted by the model (7 % and 10 %).

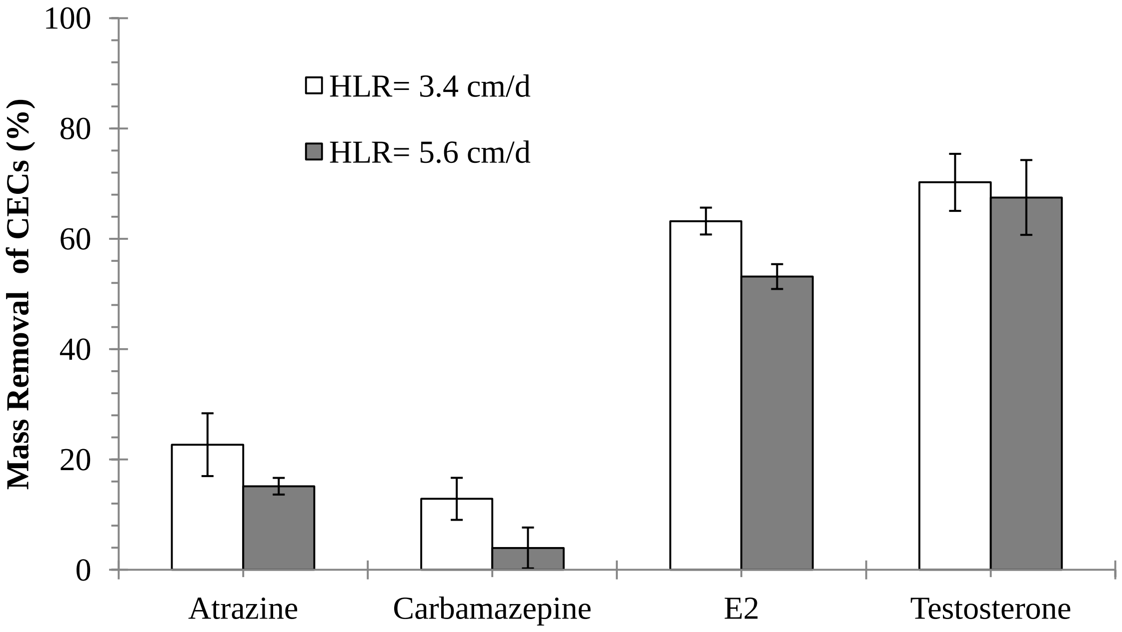


Figure 5.7. Mass removal of CECs in microcosms operated at HLR of 3.4 and 5.6 cm/d.

Based on the model predictions, higher CLR will increase removal of one group of CECs but may lower removals of other CECs. To overcome such a situation, a hybrid design for constructed wetlands is recommended. In this design, the wetland consists of two cells, one that is maintained at lower CLR to facilitate removal of CECs susceptible to photolysis, followed by a densely planted second cell to facilitate removal of other CECs. To investigate the benefits of such design, we simulated a scenario in which the first cell had the lowest CLR in this study of 2×10^3kg-DW/ha-yr and the CLR of the second cell was 1.2×10^4 kg-DW/ha-yr. An HRT of 5 d was divided equally, resulting in an HLR of 10 cm/d for each of the cells. The effluent concentration of the first cell was used as the influent concentration of the second cell.

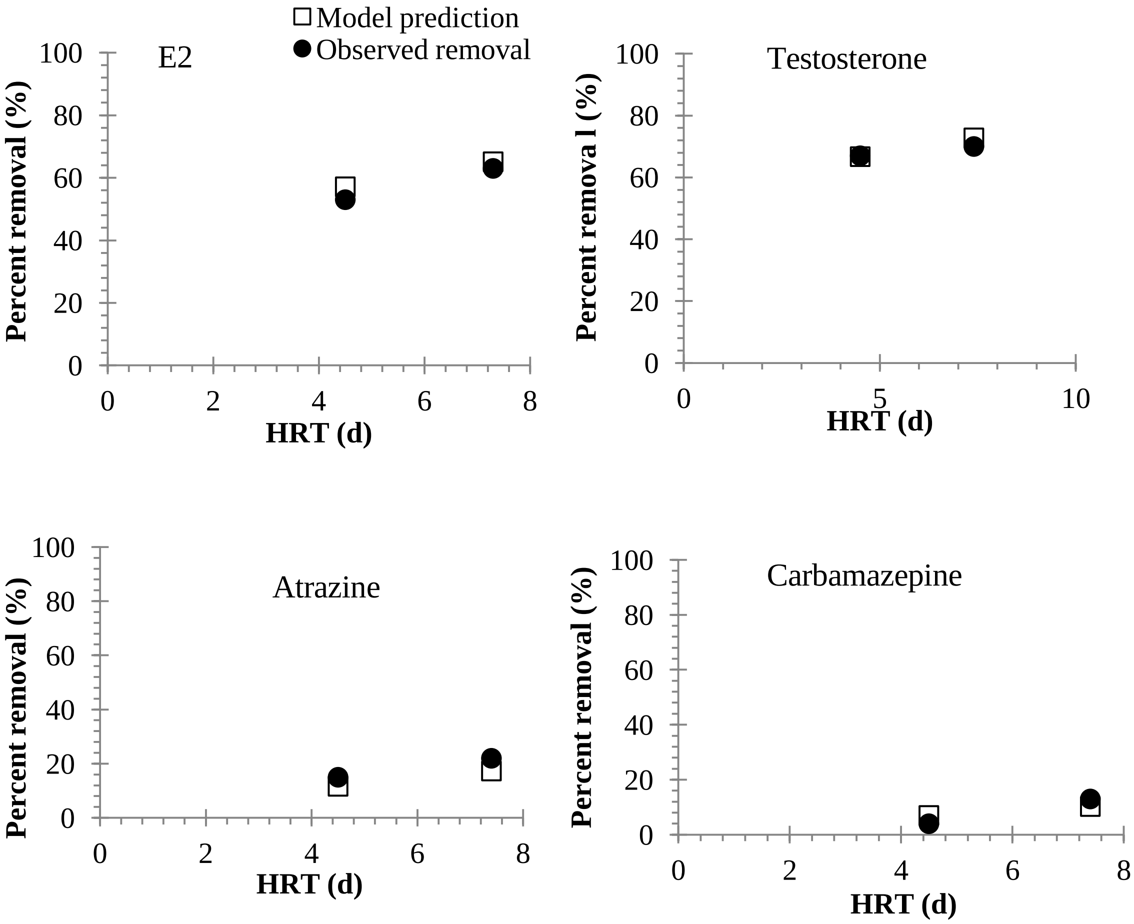


Figure 5.8. Model validations with experimental results from the microcosms.

The removal was compared (Figure 5.9) against the maximum removal observed for each of the CECs in the previous scenarios at the HLR of 20 cm/d. By connecting two wetlands with different plant quantity, removal was optimized for all the CECs without exceeding the net HLR. E2 and testosterone removal was reduced by 18 % and 27 % from their maximum removal observed at the highest CLR. Removal decreased by 20 % for atrazine, which experienced maximum removal at the lowest CLR. It should be noted that at the highest CLR where E2 and testosterone removal is maximum, atrazine removal becomes lower by 40 % from its maximum removal at the lowest CLR .Finally for carbamazepine, removal was increased by 28 %. Thus, maintaining the same HLR, two wetland cells with varying CLRs can be combined to optimize organic CEC removal.

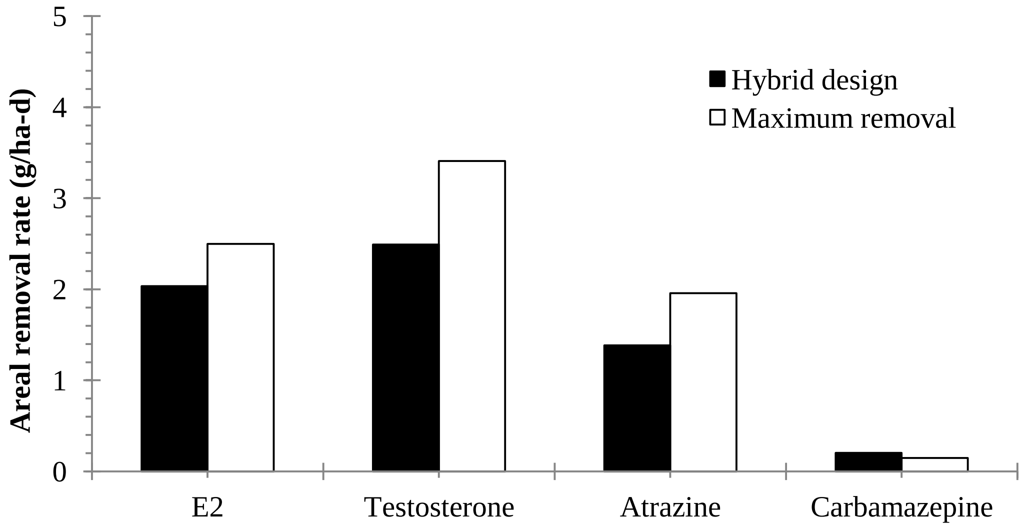


Figure 5.9. Comparison of areal removal rates predicted by the model.

Conclusion

This study evaluated the impact of hydraulic and carbon loading rates (HLRs and CLRs) on the removal of contaminants of emerging concern (CECs) in constructed wetlands. A fate-predictive model incorporated sorption, biodegradation, and photolysis as functions of the loading rates, which were parameterized with rate constants obtained from batch-scale removal mechanism studies**.** The findings from this study are summarized below.

- A fate –predictive model was developed to evaluate the effect of wetland design loading rates on CEC removal.

- Carbon loading rates (CLRs) can influence CEC removal when wetlands are operated at higher hydraulic loading rates (HLR). Except for carbamazepine, areal removal rates of the CECs increased significantly with hydraulic loading rate. It is particularly important where only smaller size wetlands can be constructed due to land area limitations and/or the wetlands need to be operated at HLR > 5 cm/d.
- Depending on the dominant removal mechanisms, the effect of CLRs varied among CECs with different physico-chemical properties. CLR had the least impact on testosterone. But higher CLR reduced areal removal rates of atrazine but improved E2 and carbamazepine removal.
- Adoption of a single design condition (e.g., high or low carbon addition rate) can favor removal of one group of CECs while decreasing that of others, Combination of wetlands with different CLRs can optimize CEC removal without lowering the net HLR.
- Although the number and variety of removal mechanisms can be greater in full-scale wetlands, the model developed in this study can be used as a preliminary tool to determine the wetland design conditions needed to achieve a particular CEC removal target.

CHAPTER 6

CONTAMINANTS OF EMRGING CONCERN (CECS) REMOVAL IN DENITRIFYING CONDITIONS OF CONSTRUCTED WETLANDS

*The work in this chapter is planned for submission to Water Research: Sharif, F., Westerhoff, P., Zhang, J., Herckes, P. Fate of Organic Micro-pollutants in Denitrifying Conditions of Constructed Wetlands.

Abstract

Constructed wetlands receiving wastewater effluents containing nitrate (NO_3^-) can support denitrification and synergistic removal of organic contaminants. In oxygen (O_2) limiting conditions of wetlands, contaminant removal can be improved if NO_3^- is available as an alternative election acceptor. In this study, contaminant of emerging concerns (CECs){17*β* - estradiol (E2), *N*-Nirtrosodimethyl amine (NDMA), atrazine, caffeine and carbamazepine} removal in continuous-flow microcosms established with wetland plants and operated at varying hydraulic residence times (HRTs) were assessed. NO_3^- removal increased with HRT and complete denitrification was observed at an HRT of 5 d. E2 removal efficiency (> 80 %) was higher than NDMA, atrazine, caffeine or carbamazepine removal which were 56 ± 6 %, 43 ± 10 %, 43 ± 20 % and 4 ± 6 % respectively. CEC removal except for atrazine was not influenced by HRTs between 1 to 5 days. To investigate the relationship between NO_3^- removal and biotransformation of CECs, batch experiments with decaying plant biomass from the microcosms were conducted. Only E2 was found to biotransform significantly while denitrification was occurring. Results from these experiments also showed that increasing the amount of plant biomass, rates of denitrification and E2 removal increased and nitrite (NO_2^-) accumulation was prevented. Using a relatively lower quantity of plant biomass, E2 removal was found to increase with

increasing denitrification. When a higher plant biomass quantity was used, E2 was quickly removed (due to greater sorption and biotransformation) while denitrification was still occurring. This suggests a dominant effect of plant biomass on E2 removal as a consequence there was no trend between removal and the level of denitrification in the microcosms. For the remaining CECs, removal behavior in the continuous-flow microcosms was different from that of the batch experiments. Mass ratio of CECs added to plant biomass was compared and a higher value existing in the microcosms is considered to have caused the difference in the removal behavior observed in these two approaches.

Introduction

Constructed wetlands effectively remove bulk pollutants from wastewater effluent, agricultural or animal feedlot runoffs including chemical oxygen demand (COD), bioclogical oxygen demand (BOD), suspended solids (SS), nitrate (NO_3^-), total phosphorus (TP) (Brix and Arias, 2005, Hench et al., 2003, Vymazal, 2007, 2009, Trang et al., 2010 In organic rich environment of constructed wetlands, diffusion from atmospheric creates a thin layer of oxygen (O_2) at the water surface, but a gradient is formed which is rapidly consumed by the microbes in the wetland water (Rackoczy et al., 2011). At low O_2 conditions, NO_3^- becomes the alternative electron acceptor for microbial activities and denitrification proceeds.

Constructed wetland receiving wastewater effluent and runoffs also contains trace organic contaminants commonly known as contaminants of emerging concern (CECs). A number of CECs including pharmaceuticals and personal care products (PPCPs) endocrine disrupting contaminants (EDCs) have been detected in wetlands (Matamoros and Bayona, 2006, 2008, Matamoros et al., 2009). Biodegradation is considered as one of the major removal processes of these CECs (Matamoros et al., 2005, Hijosa-Valsero et al., 2010, Ávila et al., 2013). Typically, aerobic biodegradation is considered more favorable for CECs as O_2 is energetically favorable compared to other electron acceptors. Accordingly, in O_2 limiting conditions of wetlands, CEC removal is expected to be low. When NO_3^- is present as a substitute electron acceptor, CEC removal may be improved by the denitrifying microbial communities of constructed wetlands.

Denitrification can facilitate CEC removal. For example, denitrifying conditions in activated sludge treatment process significantly (> 65 %) transformed estrogen, musk fragrances and antidepressants (Suarez et al., 2010). A number of studies observed steroidal hormone removal in anoxic conditions (Fan et al., 2011, Ke et al., 2012, Czajika and Londry et al., 2006). 17β -estradiol (E2) and estrone (E1) degraded faster under NO_3^- reducing conditions of lake sediments compared to anaerobic conditions with sulfate as the electron acceptor (Czajika and Londry et al., 2006). Matsui et al. (2000) found major reduction in estrogenecity (in terms of E2 equivalent) during the anoxic stage of a conventional sewage treatment plant. Andersen et al. (2003) found highest removal of E2 and E1 occurring in the denitrification tank of an activated sludge type treatment process. Apart from the steroidal compounds, carcinogenic disinfection by-products (DBPs) formed during disinfection of WWTP effluent including *N*-nitrosodimethylamine (NDMA) can be removed under anoxic conditions (Gunnison et al., 2000, Drewes at el., 2006, Chung et al. 2008, Padhye et al., 2009). NDMA biodegradation rate under anoxic conditions of a simulated aquifer was found comparable with the rate observed in aerobic conditions (NalinaKumari, et al., 2010). The importance of complete denitrification to prevent NDMA formation was highlighted by Padhye et al. (2009). Nitrite (NO_2^-), a byproduct of denitrification process is considered to react with precursors like dimethylaime (DMA) or commonly occurring organic compounds in wastewater and form NDMA.

In constructed wetlands, denitrification depends on multiples factors and the most important ones are NO_3^- loading, redox conditions and carbon availability. High NO_3^- loading of wetlands was found to increase areal removal rates for NO_3^- (Gersberg et al., 1983, Ingersoll and Baker, 1998). Carbon availability is necessary to support the metabolic activity of heterotrophic bacteria causing denitrification. Organic carbon becomes available in wetlands when emerging and submerged plants decay in water. Plant exudates or litter supplies the biodegradable carbon needed to drive denitrification (Ingersoll and Baker, 1998, Gebremariam and Beutel, 2008). However, the quantity of plant derived organic carbon depends on the hydraulic residence time (HRT). Plant-leached DOC decreases at short HRTs which can lower the NO_3^- removal efficiency (Ingersoll and Baker, 1998, Pinney et al., 2000).

In this study, CEC removal in denitrifying conditions of constructed wetlands was investigated. Continuous-flow microcosms receiving NO_3^- (2 mM of NO_3^-- N) were established with wetland plants. A total of five organic CECs with varying physico-chemical properties (Table 6.1) were premixed with the influent water and supplied into the microcosms. To investigate the effect of HRT on denitrification and CEC removal, these microcosms were operated at varying HRTs (1.5, 2.5, and 5 d). Concentrations of NO_3^- and CECs in microcosm effluents were monitored. Batch experiments with partially decayed plant biomass were conducted to study CEC removal kinetics and discover relationships between denitrification and CEC removal.

Table 6.1

*Physico-chemical properties of selected CECs**

Organic CECs	Molecular weight	pK_a	Log K_{OW}
17*β* -estradiol (E2)	MW (g/mole)	10.4	
	272.4		4.02
N-nitrosodimethylamine (NDMA)	74.1	8.5	-0.57
Atrazine	215.7	1.7	2.61
Carbamazepine	236.3	13.9	2.45
Caffeine	194.2	14.0	0.16

*Source: EPI Suite^{TM} (U.S. EPA)

Material and Methods

Materials

17*β*- estradiol (E2) (≥ 98 % purity), atrazine (≥ 98.8 % purity), caffeine (≥ 98.5 % purity), carbamazepine (≥ 98 % purity), *N*-nitrosodimethylamine (NDMA) (≥ 98 % purity) and sodium azide (NaN_3) (≥ 99.5 % purity) were obtained from Sigma-Aldrich, St. Louis, MO, USA. HPLC-grade acetonitrile, methanol, dichloromethane and formic acid were obtained from Fisher Scientific, New Hampshire, USA. Nanopure water with 18.3 Ω conductivity (Milipore Inc., Billerica, MA) was used for analytical purposes. Isotope-labeled compounds (99 % purity) -Estradiol-[$^{13}C_6$], Atrazine-[d_5], Caffeine-[$^{13}C_3$] and Carbamazepine-[d_{10}], NDMA-[d_6] were purchased from Cambridge Isotope Laboratories, Inc (Tewksbury, MA, USA).

Continuous loading of CECs in microcosms

The details of the microcosms used for this study were previously described in Chapter 5. The only exception is that instead of NH_4Cl, KNO_3^- was fed into the microcosms to have an influent NO_3^- concentration of 2 mM. The feed solution contained a mix of the CECs at a concentration of 10 nM. A control microcosm with no addition of plant materials was operated at 4.5 d to estimate any loss of the contaminants that are not associated with the decaying plant biomass. Effluent samples were collected weekly to measure NO_2^-, NO_3^- and DOC. pH of the effluent samples were 7.6 ± 0.2.

Batch experiments in anoxic conditions

Batch experiments were conducted in 100 mL volume serum bottles containing decaying plant biomass and water from the denitrifying microcosms. The ratio of bulk weight of decaying plant biomass to water was 1 g: 100 mL and 3 g: 100 ml. Appendix B includes photographs of dry plant materials supplied into the microcosms and the plant biomass decaying with time. Abiotic controls were prepared by adding 100 mg/L sodium azide (NaN_3) to stop microbial activity. NaN_3 was selected instead of any non-chemical inactivation method e.g. autoclave which may alter the structure of the plant biomass content. However, azide can also alter the water chemistry and increase conductivity (Stevens-Garmon et al., 2011). After placing the plant biomass and water into the serum bottles, a mixture of CECs prepared in deionized water was spiked to obtain a concentration of 1 to 1.5 µM. Serum bottles were prepared in triplicates and then divided into three groups: (1) bioactive with 2mM NO_3^-, (ii) bioactive without NO_3^- (iii) abiotic control with 2 mM NO_3^-. KNO_3^- solution was added to obtain the concentration of 2 mM NO_3^--N. Serum bottles were then purged with N_2 to flush any nitrogen and then sealed. The bottles were covered with aluminum foil to avoid photolysis and the kept in an anaerobic glove box. Samples were collected over time to measure the concentration of NO_3^-, NO_3^- and the CECs remaining in solution.

Analytical methods for CECs

CESs in the samples collected from the microcosms were extracted through solid-phase extraction (SPE) based on EPA method 1694 for PPCPs and 521 for NDMA (EPA, 2004, 2007). After collection, samples were preserved with $NaN_{3.}$ For PPCPs, A 1000 mL of sample volume were filtered with 0.7 GF/F and pH was reduced to 2 by adding aliquots of concentrated sulfuric acid. For NDMA, a 500 ml volume of samples were extracted. Both extractions were performed on Caliper Life Sciences Auto Trace extraction manifold (Zymark Corp., Hopkington, MA). Matrix suppression in the samples was corrected by performing isotope dilution. 200 to 400 ng/L of isotopes were added into the samples prior to the analysis. Waters Oasis hydrophilic-lipophilic balance (HLB) cartridges (Waters Corp. Milford, MA) were used for PPCPs. Cartridges were conditioned with MTBE, methanol, dichloromethane and reagent water. Samples were loaded onto the cartridges at 10 mL/min and soaked with a solution of methanol and MTBE mixture. The resulting extract were concentrated with a gentle stream of nitrogen and brought to a final volume of 1 ml using methanol. Analyte recoveries were measured by spiking the PPCPs at a concentration of 10 nM in effluent water from the microcosm before extraction. The recoveries (n=6) were 93±22 % for E2, 106±11 % for atrazine, 81±12 % for caffeine and116±7 % for carbamazepine. The LC-MS/MS conditions of the CECs can be found in Chapter 5. Additional information on the analytical methods e.g. standard calibration curves, chromatogram peaks, can be found in Appendix B. Extracted NDMA was analyzed by Agilent G6890N gas chromatography with mass spectrometry in positive chemical ionization mode was used to measure NDMA after extraction. The detection limit was 2 ng/L with variation of <5 %.

Extraction of E2 from decaying plant biomass

Decayed plant biomass used in batch experiments were decanted and then dried at 105^0C. Mass used in the batch experiment described above with the ratio 1 g: 100 mL was taken out from the serum bottles to extract E2. The dry weight of the plant biomass was 0.1±0.02 g. Internal standards for E2 (estradiol-$^{13}C_6$) was added to obtain a concentration of 100 µg/L in the final volume of 1 mL. The method for extraction used was adopted from Miller et al. (2008) and Venkatesan et al. (2012). In brief, 10 ml volume of methanol and acetone (50:50) was added into vials containing the dried biomass and then shaken for three hours at 150 rpm. The samples were decanted and filtered with 0.7 µm GF/F and concentrated to 1mL by a gentle nitrogen stream at 25^oC.

Results

NO_3^- and CEC removal in the microcosms

Effluent samples from the microcosms were collected (in triplicate) over an eight month period and analyzed for DOC, NO_3^- and the selected CECs. Figure 6.1 and 6.2 shows the effluent concentrations for the water quality parameters and CECs during the operation of these microcosms. The average DOC concentration in the effluents were 3 ±1, 4±1 and 7± 2 mg/L for the 1.5, 2.5 and 5 d HRT microcosms respectively (Figure 6.3 a) . NO_3^- removal was > 99 % (< 0.5 mg/L NO_3^--N remaining in the effluent) in the microcosm with 5 d HRT (Figure 6.3 b). 22 ± 6 % (6 ±2 mg/L NO_3^--N) and 45± 7 % (13 ±2 mg/L

NO_3^--N) influent NO_3^- was removed in the microcosms operated at 1.5 and 2.5 d HRT respectively. NO_3^- removal increased by lengthening HRT of the microcosms. Longer HRT increased the level of leached DOC and enabled more NO_3^- removal in the microcosms. This observation is consistent with prior work which observed > 90 % NO_3^- removal in similar microcosms operated at 5 d HRT (Ingersoll and Baker, 1998, Pinney and Westerhoff, 2000).

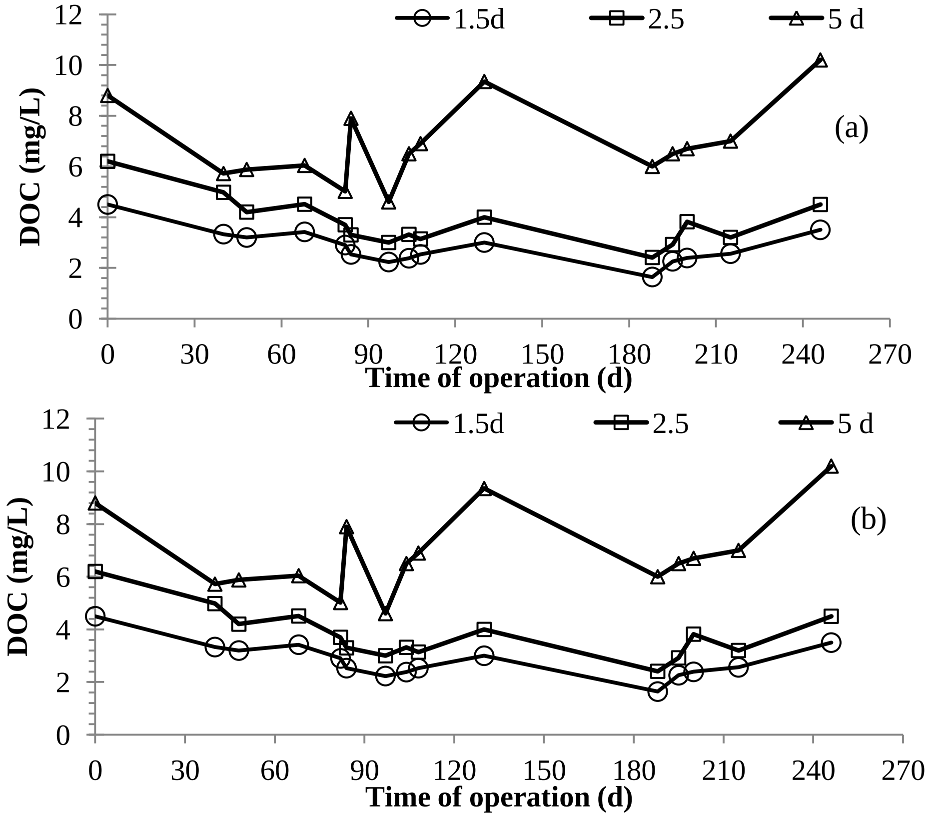


Figure 6.1. Concentrations of NO_3^- (a) and DOC (b) in effluent of the microcosms receiving a nitrate loading of 2 mM (28 mg/L) NO_3^--N.

Figure 6.2 shows normalized effluent concentrations of the organic CECs in the microcosms. A control microcosm with no plant materials was operated at an HRT of 5 d and CEC concentrations were measured in the effluent. No significant loss (< 10 %) was observed for any of the CECs (Figure 6.2).

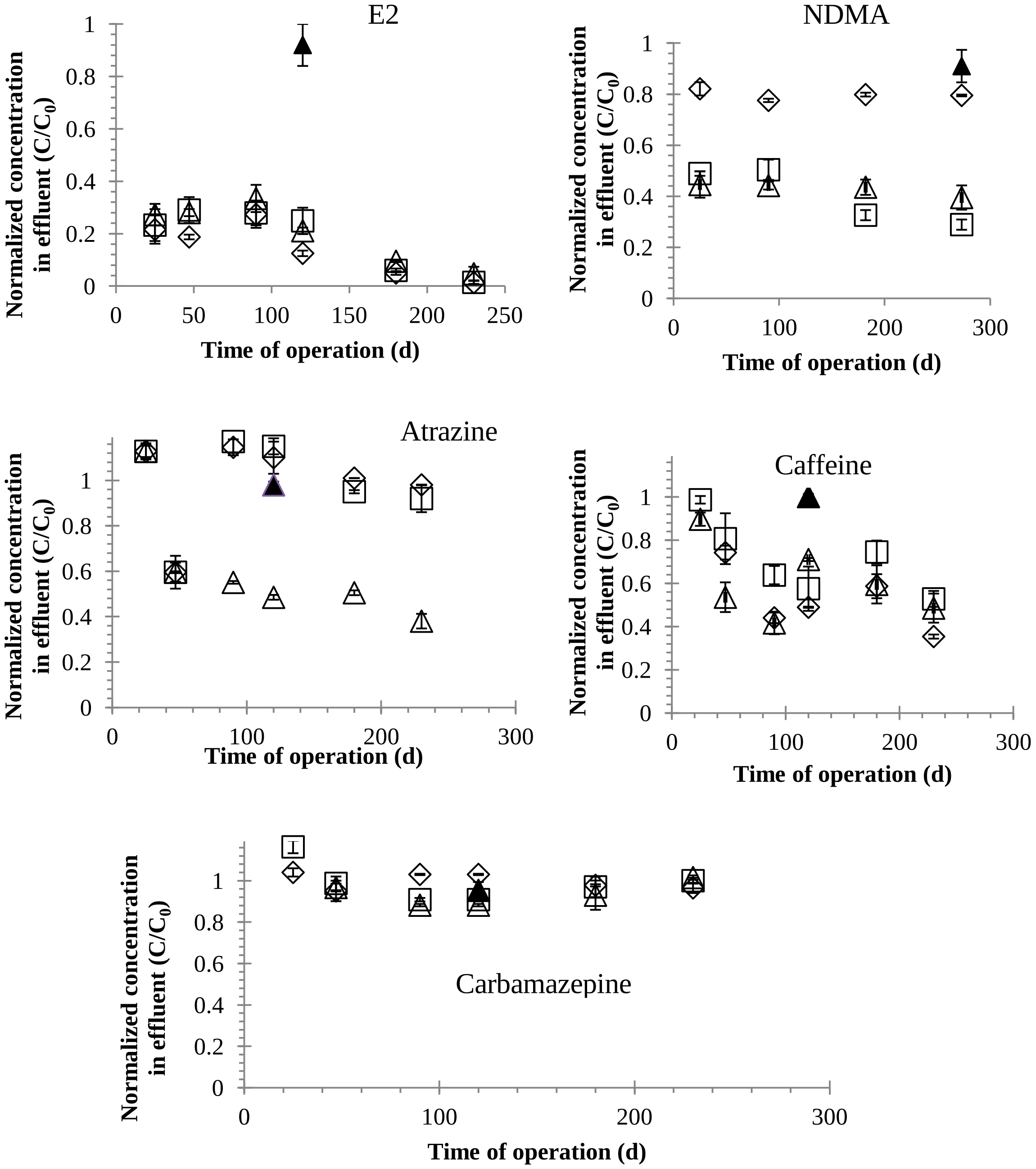


Figure 6.2. Effluent concentration of CECs in the microcosms with time.

HRT : 1.5 d (◇), 2.5 d (□), 5 d (△, control microcosm 5 d (▲.

Organic CEC removal in the denitrifying microcosms is summarized in Figure 6.3. E2 removal did not vary at different HRTs and average removal was 83 ± 11 %, 82 ± 13%, and 80 ± 11% in the 1.5 d, 2.5 d and 5 d HRT microcosms respectively. High elimination of estrogenic compounds including E2 (> 98 %) was observed under denitrifying conditions of conventional sewage treatment (> 98 %) and activated sludge processes (> 70 %) (Andersen et al., 2003, Suarez et al., 2010). Comparable removal observed in our study supports the use of constructed wetlands as a similarly effective treatment option to remove E2 from wastewater effluent.

NDMA removal was 20 ± 3 %, 57 ± 10 %, and 56 ± 6 % in the 1.5 d, 2.5 d and 5 d HRT microcosms. Lengthening HRT from 1.5 d to 2.5 d, increase in removal was observed ($p < 0.05$), but there was no significant change in removal when HRT was lengthened from 2.5 d to 5 d ($p > 0.05$). NDMA has been reported to form due to the reaction of secondary and tertiary amines with nitrite which is an intermediate of denitrification via process called nitrosation. Although optimal conditions for nitrosation are acidic, NDMA was also found to form in alkaline conditions (Keefer et al., 1980, Kaplan et al., 1983). Microbial formation of complex organic matter or enzymatic reaction can catalyze nitrosation and support NDMA formation in neutral or alkaline pH levels (Ayanaba and Alexander, 1974, Yang et al., 1977, Leach et al., 1987). Wolfe et al. (1976) found atrazine (which contains two secondary amines) to form nitrosamines when nitrite was present at pH 2~ 4.5. It may be possible that byproducts of atrazine biodegraded under denitrifying conditions reacted with nitrite and form NDMA though microbial process. Consequently, instead of any increase in removal, no significant change in NDMA concentration was observed in the in the longest HRT microcosm (pH 7.3 ± 0.2). Although, removal in constructed wetlands has not been investigated yet, NDMA was found to remove in simulated sub-surface environment. Nalinakumari et al., 2004 found > 70 % NDMA removal in soil columns with HRT of 20 days where NO_3^- concentration was reduced from 5.1 mg/L to 1.7 mg/L. Patterson et al., 2012 found 50 % removal of NDMA in an anaerobic aquifer with a residence time of 10 days. Our study shows comparable NDMA removal at relatively short residence times of denitrifying wetlands.

Atrazine was only removed (43 ± 10 %) in the 5 d HRT microcosm. Mean removal of atrazine in the other two microcosms were 2.5± 6 % and 1.5±3 %. Atrazine removal in denitrifying conditions of bioreactors was studied by Crawford et al., 1998, where it was used at high concentration (mM level) to serve as a carbon source for the denitrifying bacteria. Stuck et al., 1995 also used atrazine as a carbon source for denitrifying bacteria and observed high removal. Simultaneous NO_3^- and atrazine removal also occurred in biological activated carbon (BGAC) reactors with removal exceeding 80 % with a very short hydraulic residence time of 4.5 h (Herzberg et al., 2004). This efficient removal was due to the large surface area available for biofilm growth and inoculation of the carbon media with atrazine-degrading bacteria. In our study, concentrations of CECs were low (nM) and not enough to serve as a carbon source. However, removal in the longest HRT microcosm which had the highest effluent DOC and achieved maximum NO_3^- removal indicates that atrazine was probably co-metabolized by the denitrifying bacteria but a threshold HRT was needed for removal to occur.

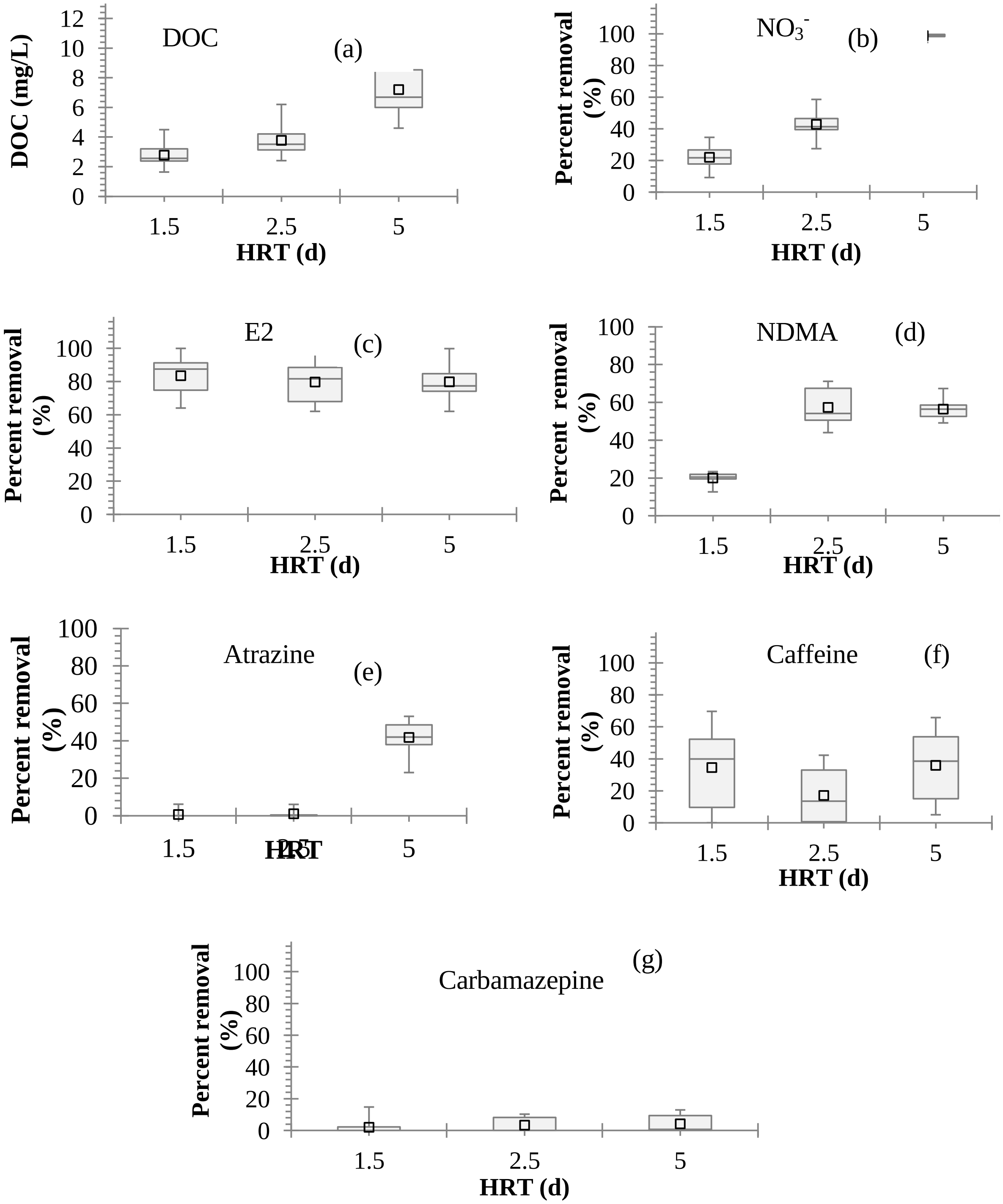


Figure 6.3. Effluent DOC (mg/L), percent removal of NO_3^- and CECs in the microcosms at different HRTs. The square inside the box represents mean value; maximum and minimum values are represented by the far end of the upper and lower whiskers respectively.

Average removal for caffeine in the microcosms was 52 ±28 %, 31 ± 18 % and 47±12 %. Compared to the low or partial removal observed in this study, high (> 90 %) removal of caffeine was reported in constructed wetlands by Conkle et al., 2008, Hijosa-Valsero et al., 2010, Matamoros et al., 2012, and Zhang et al., 2012. Unlike these wetlands, plant uptake was not present in the microcosms which could have been a potential removal mechanism for caffeine. Other biological processes enhanced by aerobic conditions could be also responsible for the high caffeine removal in constructed wetlands. Consequently, the relatively anaerobic environment of the microcosms supporting denitrification was not favorable for caffeine.

Carbamazepine experienced negligible removal in the microcosms; average removal was 2 ±4 %, 3 ±5 % and 4 ±6 %. Carbamazepine is considered a refractory compound and not removed by conventional wastewater treatment processes (Herber et al., 2001, Ternes, 2008, Guo and Krasner, 2009, Suarez et al., 2010). Low removal (0 ~ 14 %) in constructed wetlands was reported by Clara et al., 2005 and Zhang et al., 2011 which is comparable with the findings from this study. Slightly greater removal (> 20 %) was reported by Hijosa-Valsero et al., 2010 in pilot-scale wetlands.

NO_3^- and CEC removal kinetics in batch experiments

NO_3^- removal kinetics with various quantities of decaying plant biomass is shown in Figure 6.4. The bulk concentrations of the plant biomass were 10 and 30 g/L. Figure 6.4 (a and c) shows that with increasing plant biomass from 10 g/L to 30 g/L, NO_3^- removal was complete within 5 days. Figure 6.4 (b and d) shows NO_2^- formation during the batch experiments. NO_2^- is the first by-product of the denitrification reaction which is converted to nitric oxide (NO) then nitrous oxide (N_2O) and to finally gaseous nitrogen (N_2). With the lower plant biomass concentration (Figure 6.2 b), NO_2^- accumulated with time. However at the higher concentration of plant biomass (Figure 6.4 d), concentration of NO_2^- reached a peak value of 0.2 mg/L NO_2^--N and gradually decreased to zero. Although the concentration of other by products (NO or N_2O) were not measured, the difference in the behavior of NO_2^- indicates that higher plant biomass quantity is important to achieve faster NO_3^- removal as well as complete denitrification. The effluent water from the microcosms did not have any NO_2^- demonstrating that complete denitrification might have occurred in the microcosms.

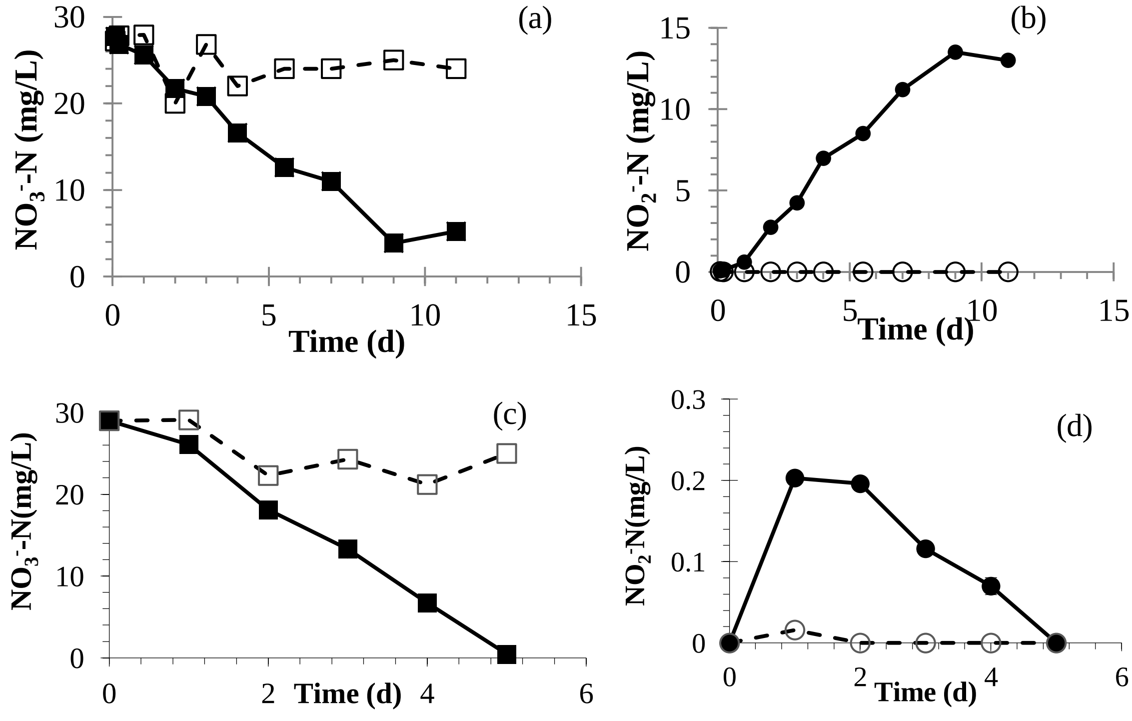


Figure 6.4. NO_3^- removal and NO_2^- formation in batch experiments with decaying plant biomass 10 g/L (panes a and b) and 30 g/L(panes c and d). Closed symbols represent biologically active samples and open symbols represent abiotic controls. Initial NO_3^- concentration is 2 mM (28 mg/L) NO_3^- -N.

CEC removal kinetics in the parallel batch experiments with 10 and 30 g/L of plant biomass are shown in Figure 6.5. The abiotic controls were prepared by spiking NaN_3 into serum bottles containing the biomass, water, NO_3^- and the contaminants at the same initial concentration used in the biologically active samples. Removal observed in these controls was due to sorption and greater removal was observed when higher amount of decaying plants were used. The absence of any biotic activity in abiotic controls was confirmed from the unchanged concentration of NO_3^- in the controls (Figure 6.4). A physical control was also prepared by spiking CECs and NO_3^- in de-ionized water with no plant biomass to verify if hydrolysis was occurring.

E2 removal was consistently higher in batch experiments with 10 g/L (b) nt biomass and containing 2 mM NO_3^--N compared to the bottles where NO_3^- was not present (Figure 6.5 a). At the beginning of the experiment, concentration of E2 in the serum bottles without any NO_3^- was comparable with the bottle containing 2 mM NO_3^-. E2 concentration gradually increased, forming a peak and then finally decreased to the same concentration level observed in the abiotic control. This could be reversible transformation occurring between E2 and its by-product estrone (E1). Although the concentration of E1 was not measured, previous studies showed that in NO_3^- reducing condition of sediments, reversible transformation from E2 to E1 is possible (Czajka and Londry, 2006).

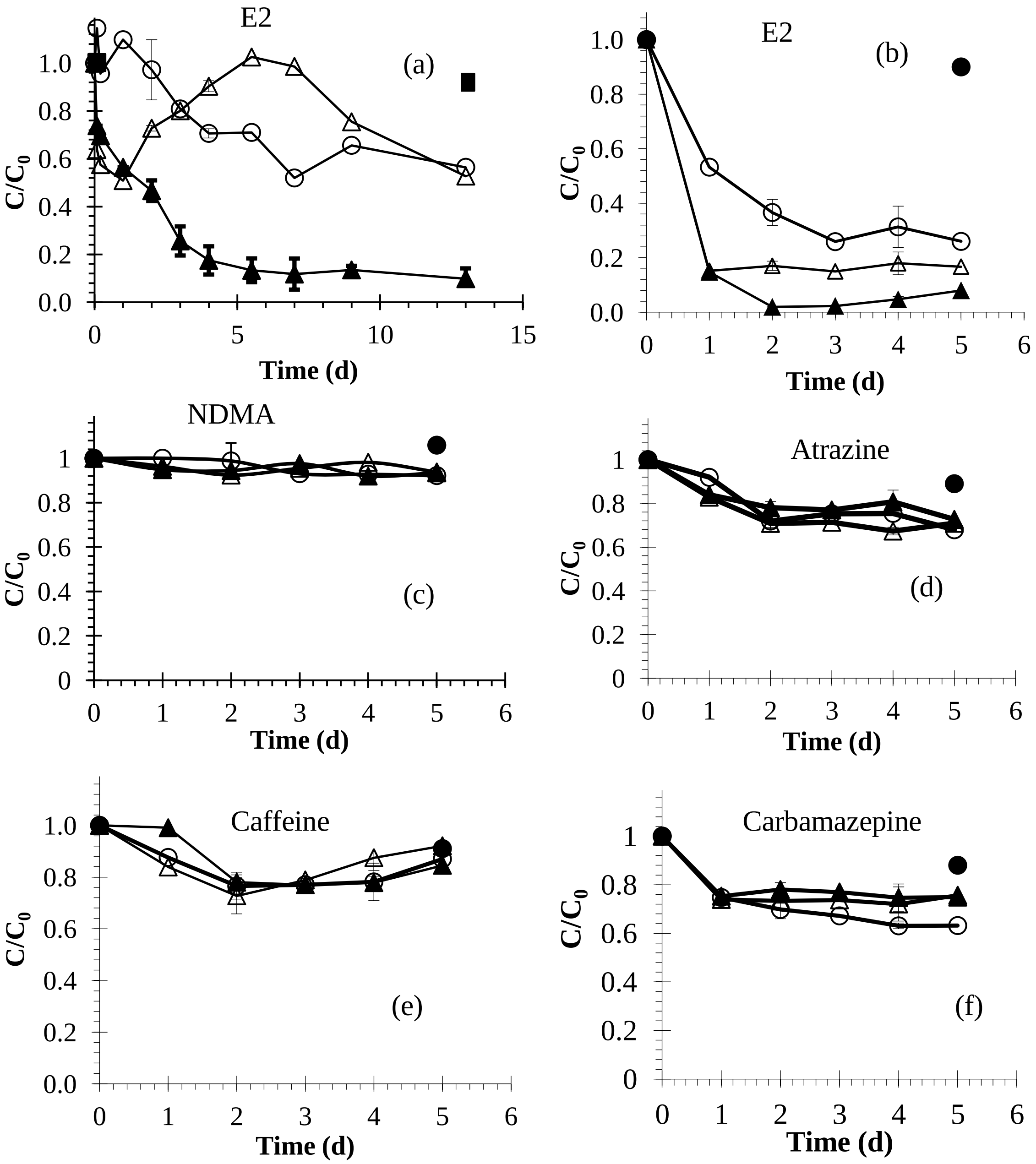


Figure 6.5. CEC removal in anoxic biodegradation experiments. a. E2 with 10 g/L plant biomass. b. E2 with 30 g/L plant biomass. c, d, e and f. NDMA, atrazine, caffeine and carbamazepine with 30 g/L plant biomass. Symbols-▲ 2 mM NO_3^- △ 0 mM NO_3^- ○ abiotic control ● physical control. Error bars represent ± one standard deviation. Except for Figure 6.5 a (with 2 mM NO_3^-), serum bottles were prepared in triplicates and data were collected at 2 and 4d, in places where not visible, error bars are smaller than the symbol.

Due to increased sorption with a higher amount of plant biomass quantity (30 g/L), E2 removal increased in both biologically active samples and abiotic controls. The difference in removal in abiotic controls and bioactive conditions (0 and 2 mM NO_3^-) were less than what was observed with 10 g/L of plant biomass. At the end of this experiment, only 8 % of the initial E2 added was remaining in the serum bottles containing 2 mM NO_3^- while, 17 % was remaining in the bottles without containing any NO_3^- and in the abiotic control 26 % E2 was remaining (Figure 6.4 b). Removal was also faster with higher plant biomass quantity. With 10 g/L of plant biomass, concentration of E2 became less than 10 % of the initial value at 14 days. With 30 g/L of plant biomass, the concentration reached the same level within 5 days only.

Removal kinetics for NDMA, atrazine, caffeine and carbamazepine with 30 g/L of plant biomass in the batch experiments are also shown in Figure 6.5. NO_3^- supplement did not improve removal for any of these CECs during these batch experiments. NDMA concentrations in all three types of experiments (abiotic control, 0 mM NO_3^- and 2 mM NO_3^-) were similar and did not change with time. Among the remaining CECs, atrazine removal at the end of the 5 d was comparable for all three types of conditions. 70% of atrazine added was remaining in the abiotic control as well as in the bioactive batches. Carbamazepine experienced greater removal in the abiotic control than in the bioactive samples. Sorption of atrazine and carbamazepine onto plant biomass should be low based on their partitioning coefficients determined in Chapter 5. The comparable or greater removal in the abiotic controls could be associated with NaN_3. NaN_3 was found to react with atrazine and other triazine herbicides by replacing the chlorine atom with an azide group (Chefetz et al., 2006). Maeng et al, 2013 found greater sorption of acetaminophen, caffeine and phenacetine in samples prepared with NaN_3.

Mass balance of E2 in the batch experiments

Based on the removal kinetics observed in the batch experiments, only E2 was influenced by denitrification. However, at the higher plant biomass quantity, a low difference in removal was observed in the experiments with or without the addition of NO_3^-. To investigate whether E2 experienced enhanced sorption (biosorption) onto the active biomass under the presence of NO_3^- or biotransformed, mass of E2 associated with the plant biomass in the batch experiments was determined by a mass-balance. Figure 6.6 shows the mass fractions of E2 associated with the biomass and remaining in the aqueous phase at the end of the experiments with 10 g/L of decaying plant biomass. The total mass (in solution and associated biomass) should be 100 % if E2 was not biotransformed. From the batch experiments with 0 mM NO_3^- 90 % mass was recovered. Only 25% mass was recovered from the 2 mM NO_3^-. In the abiotic control, 73% mass was recovered instead of a theoretical 100% recovery. The lowest mass recovery was from the serum bottle where NO_3^- was added and denitrification was occurring (shown in Figure 6.4 A) which supports that E2 removal was due to biotransformation not bio-sorption.

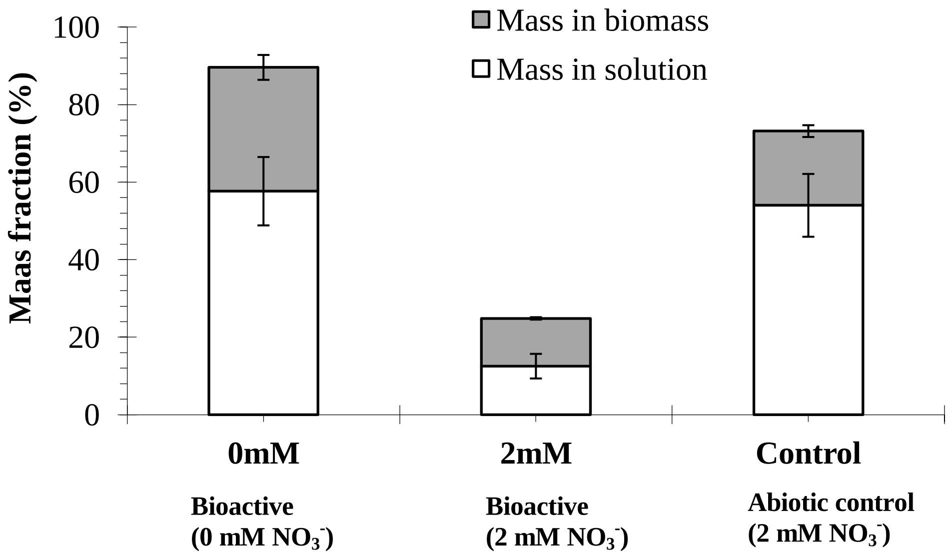


Figure 6.6. Mass balance of E2 in anoxic biodegradation experiment with plant biomass concentration of 10 g/L.

Discussion

This study used two experimental approaches to investigate organic CEC removal in denitrifying conditions of wetlands. The continuous flow microcosm studies showed that denitrification was dependent on the operating HRT, however, CEC removal was not affected significantly ($p>0.05$). Batch experiments conducted with decaying plant biomass confirmed biotransformation of E2 under denitrifying conditions. Plant biomass had a major impact on E2 removal. The difference between removal by sorption and biotransformation was reduced when a higher amount of plant biomass was used in the batch experiments. Figure 6.7 shows cumulative removal of E2 against NO_3^- during batch experiments. At the lower plant biomass quantity, E2 removal increased with NO_3^- removal until the concentration remaining became low (< 10 % of the initial amount of E2 added). At the higher biomass quantity, E2 was quickly removed leaving a negligible amount to react. Greater removal with a high plant biomass quantity supports that E2 removal in the microcosms was dominated by the amount of plant biomass available; therefore the effect of HRT and denitrification on E2 removal was not noticeable.

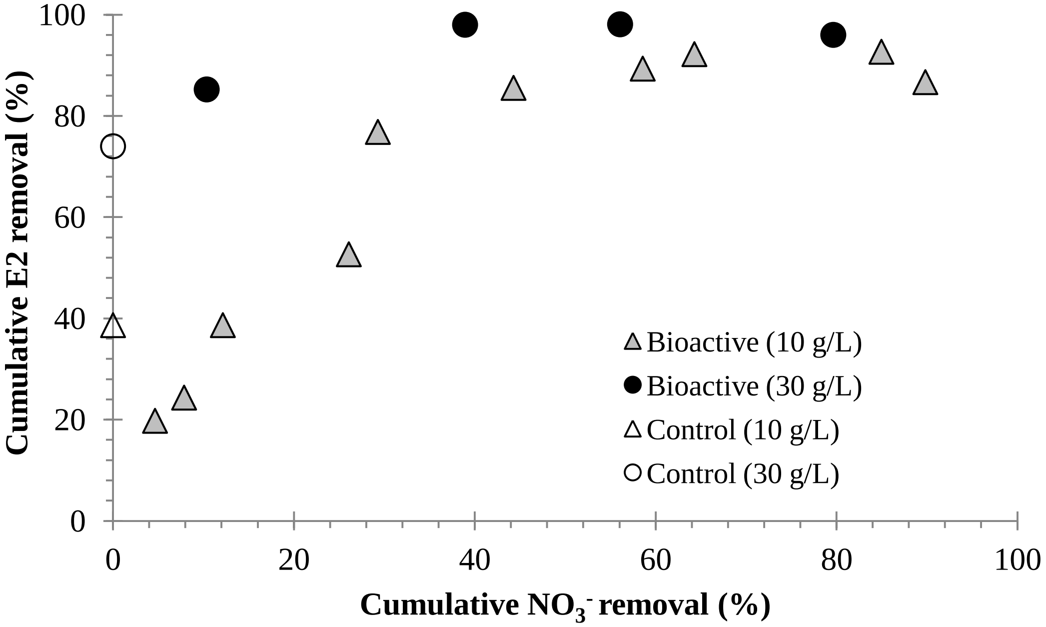


Figure 6.7. Cumulative E2 and NO_3^- removal during batch experiments at 10 g/L and 30 g/L of plant biomass.

For NDMA, atrazine and caffeine, removal was observed in the continuous-flow microcosms but not in the batch experiments. In both of these experimental approaches, the type of biomass, nutrients and contaminants were similar. The only difference is the amount of CECs and plant biomass used. The mass ratio of CECs added to plant biomass was estimated for both continuous-flow microcosms and batch experiments. Mass added into the microcosms was calculated based on the initial concentration, flow rate and HRT of the microcosms. Plant biomass added into the batch experiments were calculated from the initial concentration and the volume of the water used in the serum bottles. Plant biomass quantity in the microcosms was estimated considering a continuous addition of 3 g/ week of dry plant materials for a year. Table 6.2 summarized these values and the estimated ratios for the organic CECs.

Table 6.2

Calculation of mass ratio of CECs added to plant biomass in continuous–flow microcosms and batch experiments

	E2	NDMA	Atrazine	Caffeine	Carbamazepine
Continuous-flow microcosms					
Mass of CECs added (µg)	17.4	4.7	13.8	12.4	15.2
Plant biomass (g)*	90.0	90.0	90.0	90.0	90.0
Mass of CECs added/plant biomass (µg/g)	0.2	0.1	0.2	0.1	0.2
Batch experiments					
Mass of CECs added (µg)	27.2	7.4	21.6	12.4	23.7
Plant biomass (g)$^+$	0.3	0.3	0.3	0.3	0.3
Mass of CEC added/mass of plant biomass (µg/g)	90.7	24.7	72.0	41.4	79.0

* Mass calculated for an accumulation period of 1year with a decomposition rate of 0.0028d^{-1} and 10 % residual moisture content.

$^+$ Dry weight (at 105 ^{0}C) of plant biomass used in the batch experiment with bulk weight of 30 g/L.

The mass ratio of CECs added to plant biomass was significantly higher in the batch experiments. The mass of CECs added into both of these experiments were comparable. The difference is in the amount of plant biomass in these two approaches which affected these ratios. The higher ratio value did not cause any effect on E2 removal because of its higher biodegradability and sorption affinity for plant materials than the other CECs. This finding indicates that a balance is needed between CEC loading and plant biomass available in constructed wetlands. This ratio can be considered an equivalent term for constructed wetlands which is similar to the food to microorganism ratio (F/M) used in activated sludge systems. It also supports that denitrifying wetlands can polish WWTP effluent by removing trace levels of organic CECs that do not have high sorption affinity or highly biodegradable.

Conclusions

This study investigated the feasibility of using constructed wetlands as a strategy for CEC removal while removing bulk pollutants like NO_3^-. The key findings are summarized below.

- Organic CECs were removed in denitrifying conditions of continuous-flow microcosms with decaying plant biomass. Maximum removal of E2, NDMA, atrazine, caffeine and carbamazepine in these microcosms were 83 ± 12 %, 56 ± 6 %, 43 ± 10 %, 47 ± 12 %, 4 ±6 % respectively.
- Lengthening HRT increased NO_3^- removal efficiency but most CECs except for atrazine ($p < 0.05$) were unaffected.
- Mass balance on aqueous and plant-sorbed phases showed that a greater removal of E2 in NO_3^- amended conditions of batch experiments was due to biotransformation not bio-sorption.
- Plant biomass quantity had a dominant effect on E2 over HRT . Consequently, level of denitrification in the microcosms was not found to influence E2 removal.

- Compared with the batch experiments, NDMA, caffeine and atrazine removal was only observed in the microcosms and it was due to a higher mass ratio of CECs added to plant biomass.

CHAPTER 7

MONITORING ORGANIC CECS IN FULL-SCALE WETLANDS

Introduction

Field-scale sampling studies are useful to investigate CEC occurrences and removal in full-scale constructed wetlands. In this chapter, sampling studies conducted at two different constructed wetlands of Arizona were discussed. These wetlands were City of Kingman wetlands and Sweetwater wetlands in Tucson, AZ. The primary goal of these studies was to evaluate the wetlands' removal potential for a selected number of CECs and find out the role of hydraulic retention time (HRT). However, because of the flow fluctuations, diurnal variations and limited quantitative data on the flow path of the water, drawing any major conclusions was not possible.

Sampling in City of Kingman wetlands

Constructed treatment wetland system in Kingman, AZ consists of nine equal size cells (700 x 50 m) densely planted with mixture of bulrushes (*Scirpus*) and cattails (*Typha*). The schematic diagram of the wetland cells are shown in Figure 7.1. The cells are connected in series and the depth of water level at the time of sample collection was 0.5 m. The wetlands received effluent from activated sludge treatment plant that treats municipal wastewater for the city of Kingman. Discharge from the wetlands is conveyed to infiltration basins that recharge local groundwater.

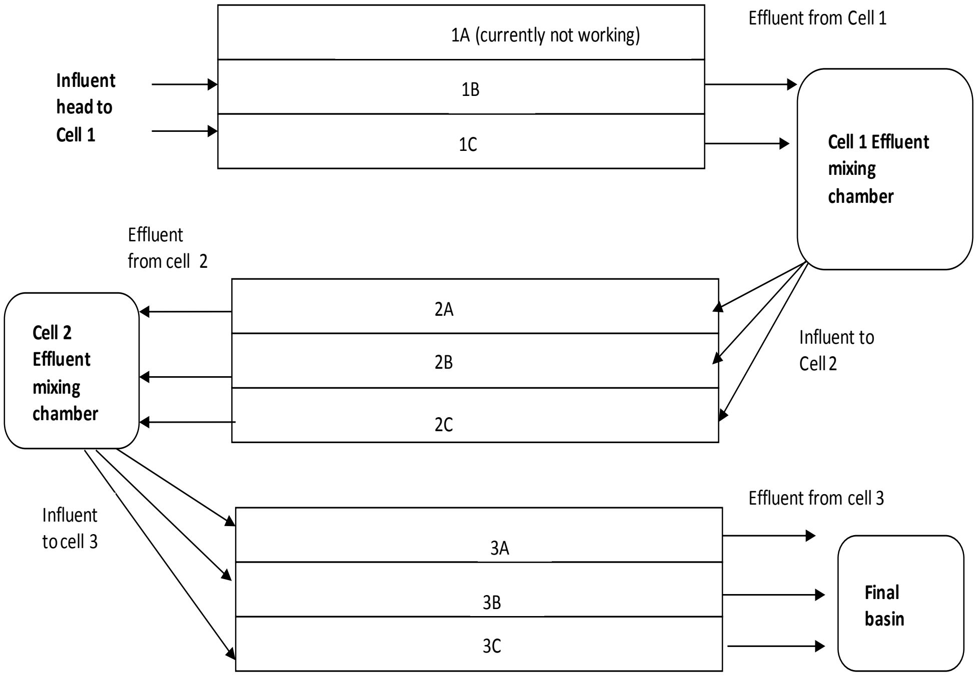


Figure 7.1. Schematic digramn of wetland cells in Kingman, AZ.

1st Sampling Campaign in Kingman Wetlands

The 1st sampling study was conducted in May 2011. During the sampling event, the average HRT of the wetland was approximately 15 days. At the day of sample collection, the wetlands received water at a rate of 1.5 MGD, which was equally dived into the first set of two cells. Discharge from the wetlands was conveyed to infiltration basins to recharge local groundwater. Grab samples were collected at several locations including influent and effluent locations with weirs. Except for these designated influent and effluent points, samples at all other locations were collected near the edge of the wetlands and close to water surface. Water quality parameters and selected organic CECs were monitored at all sampling locations but data from the major locations especially the influent and effluent to the cells are summarized in Table 7.1 and Figure 7.2. The increase in DOC and UV_{254}

absorbance is due to the leaching of organic matter from the plants. The influent NH_3 and NO_3^- concentrations were low. The increase in NH_3 concentration in the intermediate locations was due to the conversion of organic nitrogen released by the decaying plants or living organisms to ammonia.

Table 7.1

Water quality parameters in major locations of Kingman wetlands (1st campaign)

	DOC	TDN	NO_2^- -N	NO_3^--N	NH_3-N	UV_{254} absorbance
Sample names			mg/L			(AU)
Influent-Head	8.8	4.8	0.3	2.0	2.5	0.16
Effleunt-Cell1mixing basin	12.0	19.4	0.1	0.3	19.0	0.30
Effleunt-Cell2 mixing basin	40.5	14.7	0.1	2.2	12.5	0.66
Final basin	20.5	3.5	0.1	2.0	1.4	0.62

Among all the monitored CECs, primidone had the highest concentration followed by carbamazepine. Both primidone and carbamazepine are stable in the environment and considered conservative tracers of wastewater. However, concentrations of primidone observed in Kingman wetlands were higher than the reporting ranges available from literature (Guo and Krasner, 2009). Typical concentrations of primidone were reported to be ~ 100 ng/L. Carbamazepine concentrations in the samples were in a more typical range of 100 - 2000 µg/L (Guo and Krasner, 2009, Clara et al., 2005, Kim et al., 2007). Caffeine was found to vary from 50 - 1800 µg/L in wastewater effluents. Both atrazine and estradiol concentrations were low and it is not unusual to see such low concentrations. Steroids including 17β - estradio (E2) and testosterone were reported as ‘not detected’ in various literature sources (Ternes et al., 1999, Kim et al., 2007). However, testosterone in Kingman wetlands was much higher than the reported range. Sucralose was found in very high concentrations compared to the other CECs and that is why it was plotted separately (Figure 7.2, lower panel).

Although concentration of the CECs varied at intermediate locations, removal was calculated using the concentrations observed at the influent and final effluent locations of the wetlands. Based on that approach, testosterone experienced highest removal of 89 % ($p < 0.05$). No significant removal of E2, carbamazepine, primidone and sucralose was observed ($p > 0.05$).

Atrazine concentration was almost similar throughout the different sampling locations ($p > 0.05$). Unexpectedly, caffeine in the final sampling location was found greater than the influent concentration ($p < 0.05$). However, if diurnal variations were the main reason then concentrations of the other PPCPs should have also shown the similar behavior. Partial removal was observed for carbamazepine (45 %) which is comparable with what was observed by Matamoros et al., 2009, 2011, 2012 (30 ~ 50 %).

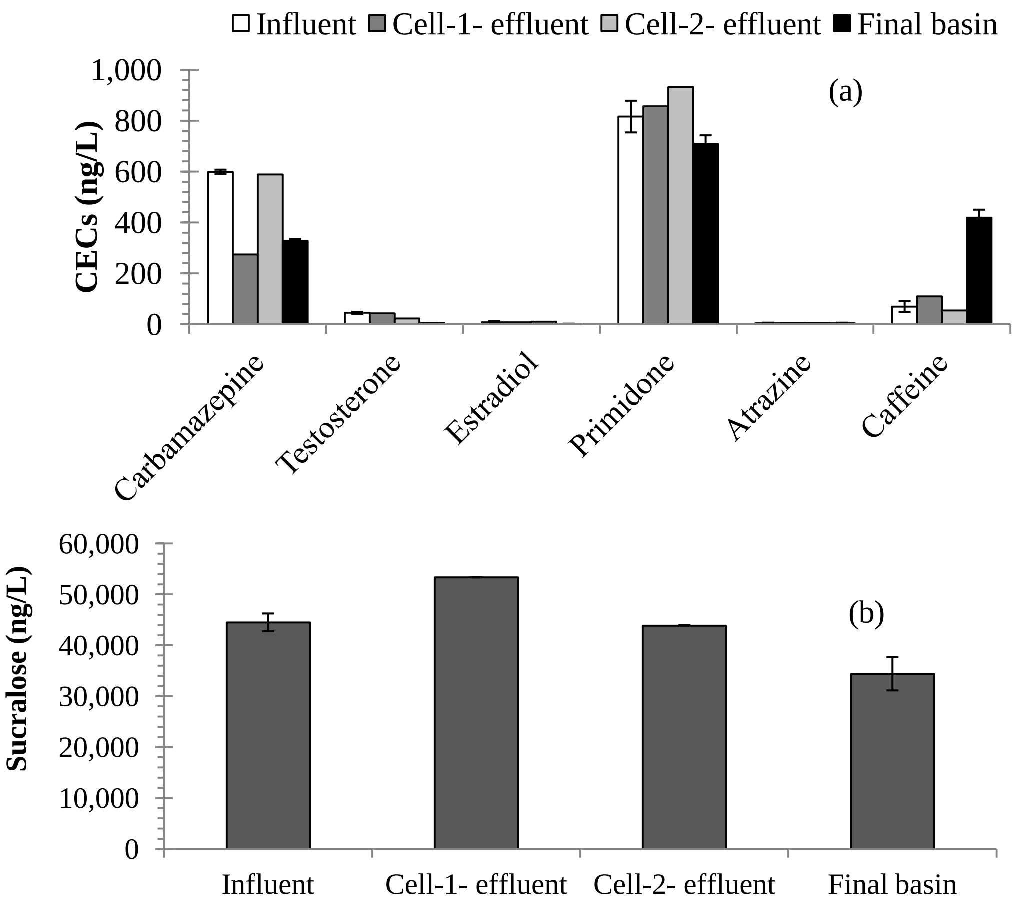


Figure 7.2. Target CEC concentrations observed at the influent and effluent locations of Kingman wetlands during 1st campaign. a: Carbamazepine, testosterone, estradiol, primidone, atrazine, caffeine). b: Sucralose.

2nd Sampling Campaign in Kingman Wetlands

During the 2nd sampling study of Kingman wetlands, a composite sampling method was adopted instead of the previously used grab sampling technique. One particular cell was studied comprehensively and samples from the influent and effluent locations of that cell were collected. In order to make that cell representative of the wetland, a cell receiving water directly from the WWTP was chosen for sampling.

Samples were collected for four consecutive days. Sample bottles were shipped to the City of Kingman wetland operation team and with their help samples were collected for the first three days. On the 4th day, samples were collected from the same location in duplicates and brought back to the lab. The bottles shipped to the Kingman wetlands were clean amber bottles containing sodium azide (NaN_3) to minimize bacterial activity and they were refrigerated during storage at the wetland operation plant. Composite samples were prepared by taking a fixed volume of water from the samples from Day 1 to Day 4 and mixing, prior to measuring the water quality parameters and subsequent extraction of target analytes. The DOC, ammonia, nitrate concentration of the influent and effluent samples collected at Day 4 and the composite sample prepared are summarized in Table 7.2.

Table 7.2

Water quality parameters in major locations of Kingman wetlands (2nd campaign)

	DOC	NH_3-N	NO_3^--N
Sample names		(mg /L)	
Cell influent (Collected on 4th day of sampling)	6±0.13	0.07±0.01	1.2±0.04
Cell influent (4 day composite)	6.2	0.07	3.2
Cell effluent (Collected on 4th day of sampling)	10±0.14	0.8±0.01	0.07±0.1
Cell effluent (4 day composite)	10.1	0.96	0.01

Before scheduling the sampling event, the wetland operation team of Kingman was requested to discharge the WWTP effluent to the selected cell at a consistent rate for duration of at least two weeks to ensure that the cell would be in steady-state by the time of sampling (HRT of the cell is 3.5 d). Concentrations measured in the composite samples and the samples collected during the 4-day sampling period are shown in Figure 7.3. The influent concentrations of DOC, NH_3 and NO_3^- were low compared to the previous sampling campaign. The WWTP discharging the water to the wetland was upgraded to include MBR process as a result the effluent water quality improved significantly. The increase in the effluent DOC and NH_3 concentration is due to the plant activity in the wetland cells. Although the influent NO_3^- concentration was low, a slight denitrification was noticed which almost completely removed the influent NO_3^-.

Caffeine concentrations in the influent and effluent samples from the cell indicated insignificant removal ($p > 0.05$). Atrazine was found in very low concentration (< 5 ng/L) and both the average and composite sample data showed a slightly higher concentration in the effluent compared to the influent ($p > 0.05$). Primidone and carbamazepine were the two highest concentrations of CECs detected in the wetland samples, which is commensurate with the data from previous monitoring studies. Primidone appeared to experience insignificant removal in the wetland cell ($p > 0.05$). Carbamazepine had the highest concentration among the monitored CECs and experienced removal to some extent ($p < 0.05$). Between the steroids- E2 and testosterone, E2 was detected in slightly higher concentration than testosterone. The maximum concentration of E2 was less than < 20 ng/L, whereas testosterone was detected <10 ng/L. Concentration detected at the influent and effluent locations were comparable for both of these CECs.

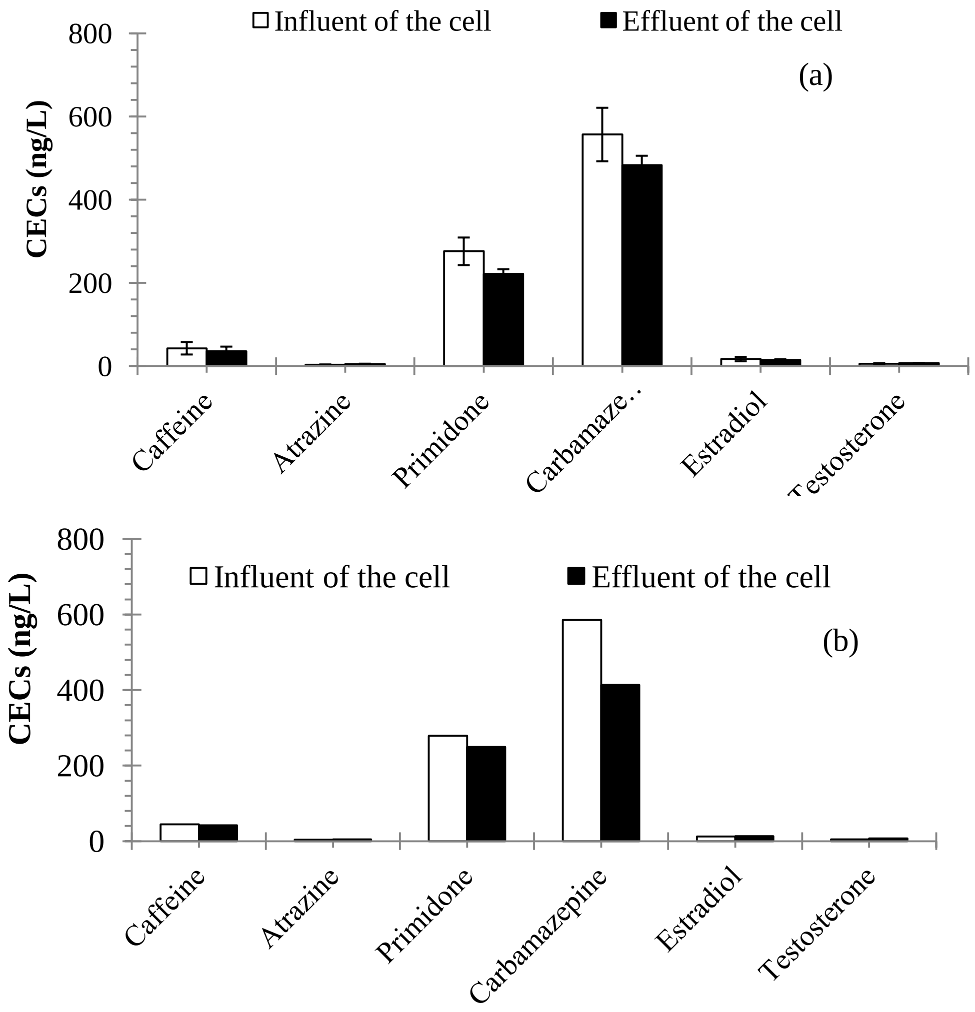
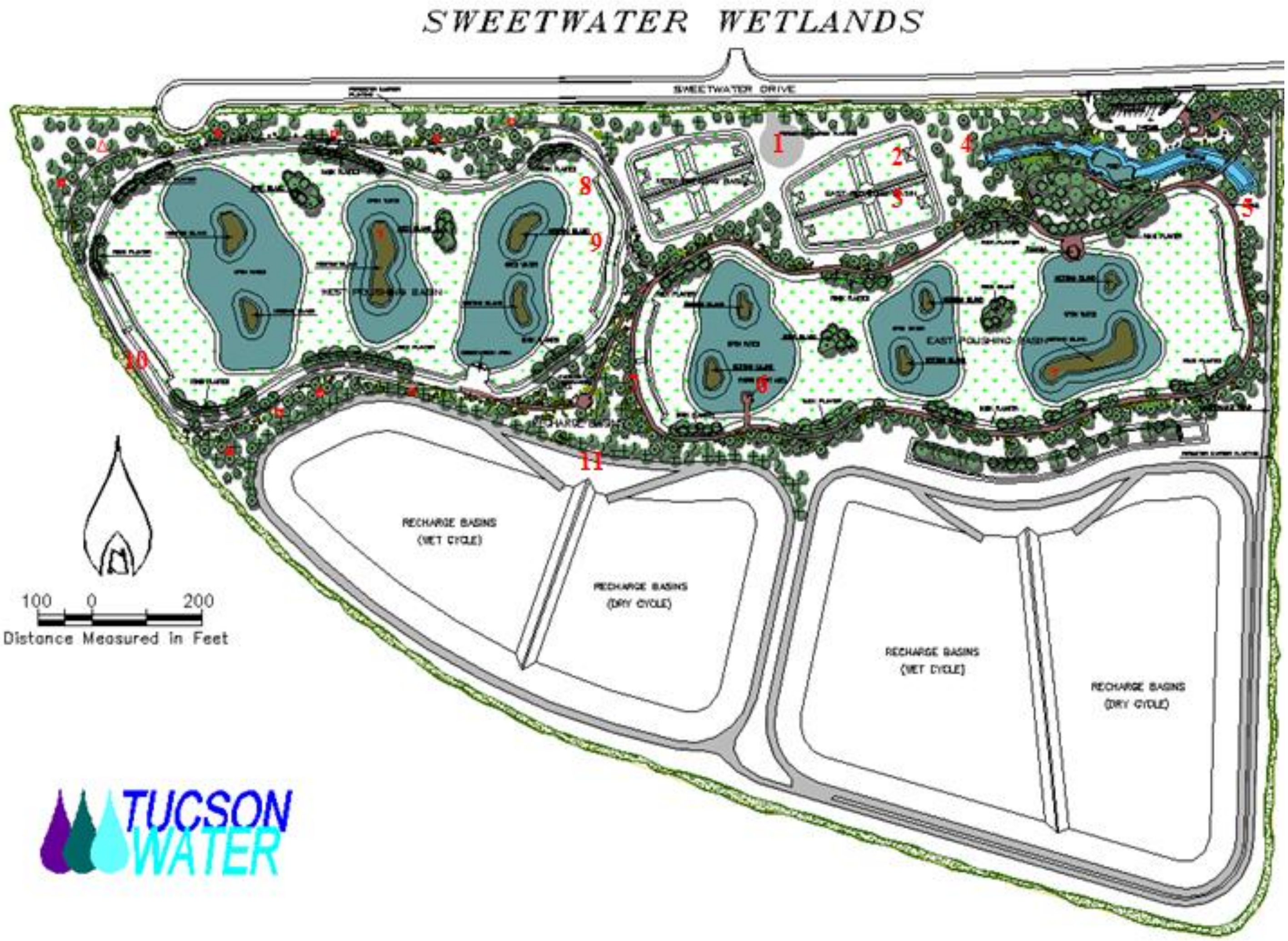


Figure 7.3. Target CEC concentrations observed at the influent and effluent locations of Kingman wetlands during 2nd campaign. a: Average concentrations. b: concentrations in composite samples.

Sampling in Sweetwater wetlands

Sampling in Sweetwater wetlands of Tucson, AZ was conducted in September of 2011. Samples were collected throughout the wetlands, mainly influent and effluent locations of the different polishing and settling basins (Figure 7.4).



F*igure 7.4.* Sampling locations (numbered in red) in Sweetwater wetlands (City if Tucson).

The sampling locations are numbered from 1 to 10 represents as follows: 1-Splitter, 2-upstream of stream feature, 3- downstream of stream feature, 4-sedimentation basin –east (north), 5-sedimentation basin-east (south), 6- structure at east polishing basin, 7-east polishing basin effluent, 8- west polishing basin influent (north), 9- west polishing basin influent (south), 10- east polishing basin effluent, 11-effluent mixing basin. However, detections of CECs were sporadic among the different sampling locations (Figure 7.5). The erratic concentrations obtained from the wetlands needed further investigation. To find out, operational conditions especially flow or loading pattern data were needed. Unfortunately, no data on the flow or volume of water entering the wetlands were available from the wetland operators. It was found that the wetlands were not operated at a regular or consistent pattern and the cells at the two sides (east and west) did not receive the same blend of water. The influent flow rate to the wetlands also varied from one day to another.

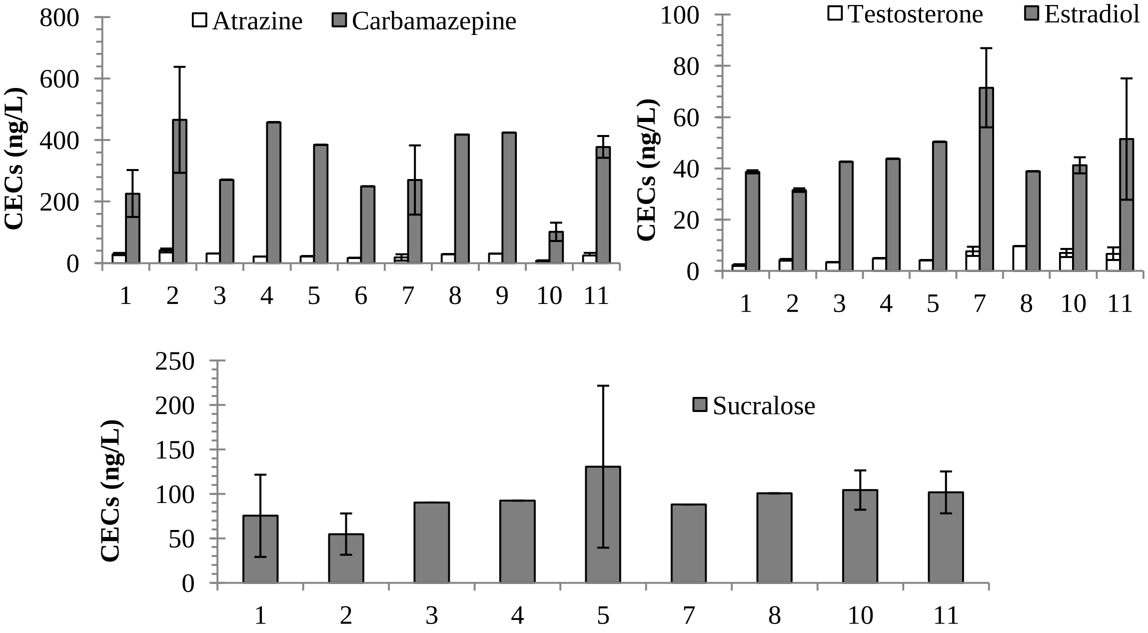


Figure 7.5. Organic CEC concentrations detected in Sweetwater wetlands.

Summary

The major findings from the field sampling studies are summarized as below:

- Monitoring studies showed that organic CECs enter wetlands when WWTP effluents are discharged.
- Atrazine concentrations detected in the wetlands were very low. This is attributable to the ability of atrazine to undergo efficient phototransformation (observed in batch-scale photolysis studies). Once exposed to sunlight, atrazine is probably photodegraded quickly enough to result into low concentrations at the influent locations of the cells.
- Carbamazepine experienced partial removal ($p < 0.05$) during both of the sampling studies conducted in the wetlands.
- Primidone was found refractory and experienced insignificant removal ($p > 0.05$).
- In the City of Kingman wetlands, most of the target organic CECs were present at very low levels including the steroids. Although steroids were the most efficiently removed CECs in lab-scale studies, their very low concentrations detected in the Kingman wetlands did not allow investigating their removal in full-scale wetlands.
- Wetland sampling at the full-scale is challenging and removal information cannot be readily extracted. When sampling is conducted using grab sampling techniques, the effect of variation in contaminant loading and absence of the accurate knowledge of the hydraulic path of water can affect the results. Even with

composite sampling strategies, where samples are taken for several days to understand the loading variation, contaminants that are present at very low concentration, estimating their removal in wetlands becomes challenging.

CHAPTER 8

DISSERTATION SYNTHESIS

Introduction

With the advancement in analytical chemistry and epidemiological studies, contaminants of emerging concern (CECs) are increasingly found in the environment. This creates potential threats to the watersheds used for potable water sources or sustaining downstream ecosystems. It also creates barrier for safe discharge of wastewater and its reuse. To deal with the unavoidable presence of CECs, existing WWTP treatment process and systems needs to be evaluated for their potential role and should be taken into consideration.

Recently, the WWTP industry have grown significantly and adopted newer treatment processes to become more efficient and support mass population, while the natural treatment systems are left behind. Natural systems provide passive treatment and occasionally used to remove heavy loadings of organic and inorganic contaminants from wastewater. Recently, in many parts of the world, the concept of using natural systems as sustainable treatment processes is emerging. The challenges of using such systems include land area limitations, complexity and less control in the operation. However, there are multiple advantages that may outweigh the disadvantages including less maintenance (consequently low operation cost), low energy requirement (no aeration or pressurized filtration needed), providing habitats for wild lives and recreational areas.

In this dissertation, constructed wetlands, a natural system was investigated for its potential as a CEC treatment system while an advanced treatment process – ozonation was also evaluated to remove trace organic contaminants (representing CECs) from WWTP effluent. This chapter will present and synthesize the results obtained described in the previous chapters and answer the research questions addressed in Chapter 1. At the end, a guideline will be provided integrating both natural and advance treatment processes with a goal to remove CECs from WWTP effluent.

Research question 1

How does inorganic species of wastewater effluent affect ozone demand and trace organic contaminant oxidation?

In Chapter 2, trace organic removal was investigated during ozonation of wastewater effluent collected from multiple WWTPs. These WWTP effluents were ozonated based on their corresponding DOC level and trace organic oxidation was estimated by measuring the loss of a HO• probe compound (pCBA). The two reduced nitrogen species of WWTP effluent-NH_3 and NO_2^- showed significant O_3 demand. Based on their stoichiometry with O_3, demand from NH_3 and NO_2^- were estimated and excluded from total ozone consumption in order to find out ozone consumed by organics only. When pCBA removal was plotted against (i) O_3 dose, (ii) total O_3 consumption and (iii) O_3 consumption by organics only, a better correlation (higher R^2 value) was observed when ozone consumption by organics only was used (Figure 2.2). This implied that if the O_3 demand from NH_3 and NO_2^- are excluded trace organic removal in municipal WWTP effluent can be predicted.

Trace organic compounds are predominantly removed by HO• and organic matter in WWTP effluent (known as EfOM) promotes HO• production (Wert et al., 2009). Wert et al., 2009 found comparable O_3 exposure in municipal WWTP effluent when O_3 dosages were selected based on O_3 to DOC ratio. This indicates a similarity in EfOM characteristics of municipal WWTP effluent. Because of this similarity, when O_3 is applied based on DOC level and demand from varying levels of inorganic constituents are removed, the remaining ozone consumed by the organics can be estimated to develop relationships to predict trace organic removal. This finding supports hypothesis 1- ‘Accounting ozone demand from inorganic species, trace organic removal can be predicted across multiple WWTPs’.

Research question 2

How does HRT of constructed wetlands impact organic and ENM CEC removal?

Constructed wetlands are operated at long HRTs to ensure adequate time for contaminants to experience removal. But HRT is related to the size and increasing HRT can also increase the area. HRT also controls the effluent water quality and higher levels of plant-leached DOC found at longer HRT can impact the safety of reusing wastewater treated by constructed wetlands (Pinney and Westerhoff, 2000). Sampling studies conducted in full-scale wetlands also showed increase in DOC and UV_{254} absorbance in wetland effluents (Chapter 7).

Continuous flow microcosms with wetland plants were built and operated at varying HRTs to investigate the effect of HRT on CEC removal. Chapter 4 discussed ENM removal in these microcosms. Pulse-input studies showed that removal increased for ENMs (aq-nC_{60} and CAR-Ag) by lengthening HRT (Figure 4.6). However with accumulation of plant materials removal was improved in the microcosms at similar HRTs. Enhanced removal with increasing plant biomass quantity was verified by batch sorption studies conducted with dry wetland plant materials (Figure 4.12).

Organic CEC removals in the microcosms were conducted using pulse-input and continuous loading approaches. During pulse-input studies (Chapter 5), organic CECs were injected into the microcosms and doubling the HRT from 4.5 d to 9 d, an additional 3~13 % more mass of CECs was removed (Figure 5.7). During the continuous-loading studies, for most organic CECs, HRT did not change removal significantly ($p < 0.05$) except for atrazine (Figure 6.3) which was not removed in the microcosms with an HRT of less than 5 d. Therefore, hypothesis 2-“Removal efficiency of caffeine, atrazine and carbamazepine increases with hydraulic retention time (HRT) of wetland microcosms” was partially validated (for atrazine only).

Ideally prolonged exposure by lengthening HRT should increase removal of the organic CECs. One of the potential reasons is that the time dependent removal mechanisms can be benefitted with longer time period. However, when multiple removal mechanisms are active then the net effect of lengthening the time of exposure may not be significant. For example, CECs which have high sorption tendency that are also biodegradable, lengthening HRT may not create any major impact on their removal. It is also possible that HRT will not have any effect on CECs which are removed by spontaneous processes. For example, caffeine removal observed in the microcosms was comparable. Although, caffeine is a polar compound ($\log K_{OW} = 0.16$), sorption onto the decaying plant biomass can occur to some extent. Another potential effect of HRT is the change in the biochemical processes that also can impact specific CEC removal. For example, atrazine removal occurred only in the longest HRT microcosm achieving complete NO_3^- removal. It is also possible that a threshold HRT was needed for atrazine to experience removal in the microcosms. Another explanation for not seeing any effect of HRT on selected CECs (e.g. carbamazepine) could be a prolonged exposure needed than the tested range of HRT. It is also possible that bacteria living under denitrifying conditions were not able to acclimate towards these CECs (e.g. carbamazepine) thus lengthening HRT was not found helpful.

Research question 3

How does changing the design loading rates impact organic CEC removal in constructed wetlands?

Organic CECs in wetlands are removed by multiple mechanisms. These mechanisms can be influenced by wetland design parameters including loading rates. A fate predictive model was developed in Chapter 5, where different removal mechanism (hydrolysis, sorption, biodegradation, and photolysis) was included which were parameterized with rate constants obtained from batch experiments. The model was simulated to find out the effect of hydraulic and carbon loading rates (HLRs and CLRs) on organic CEC removal. The model also showed that depending on the dominant removal mechanisms, CLR can have both positive and negative impact on CECs. CLR controls surface area and biomass available for sorption and biodegradation, but it increases DOC which reduces contribution from photolysis. For example, atrazine can be predominantly removed by photolysis and removal decreased with increasing values of CLR. In contrast, E2 and carbamazepine removal increased with increasing CLR. Finally for testosterone, which responded to all removal mechanisms (e.g. sorption, biodegradation and photolysis) was least affected by CLR.

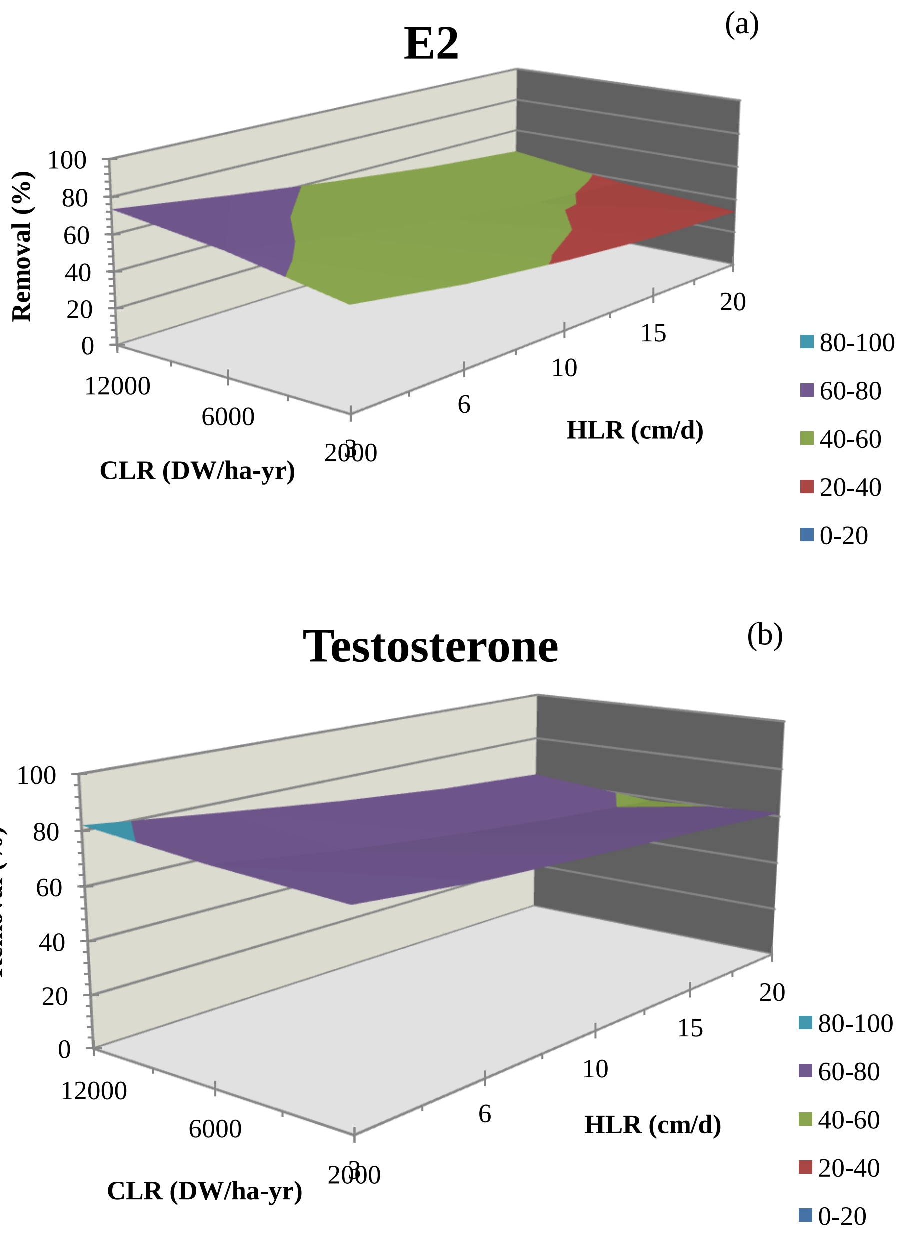


Figure *8.1*. CEC removal (%) with varying hydraulic and carbon loading rates. a : E2, b: testosterone

Figure 8.1 and 8.2 are three dimensional surface plots drawn to show how removal of the selected CECs varies with HLR and CLR. Based on the shape of the plots for E2 and testosterone, it appears that E2 is slightly more influenced by the loading rates than testosterone (Figure 8.1 a and b). The surface plot for atrazine (Figure 8.2 a) shows that negative effect of CLR and less impact of HLR on atrazine removal. Figure 8.2 b shows carbamazepine removal is influenced when HLRs are low and CLRs are high.

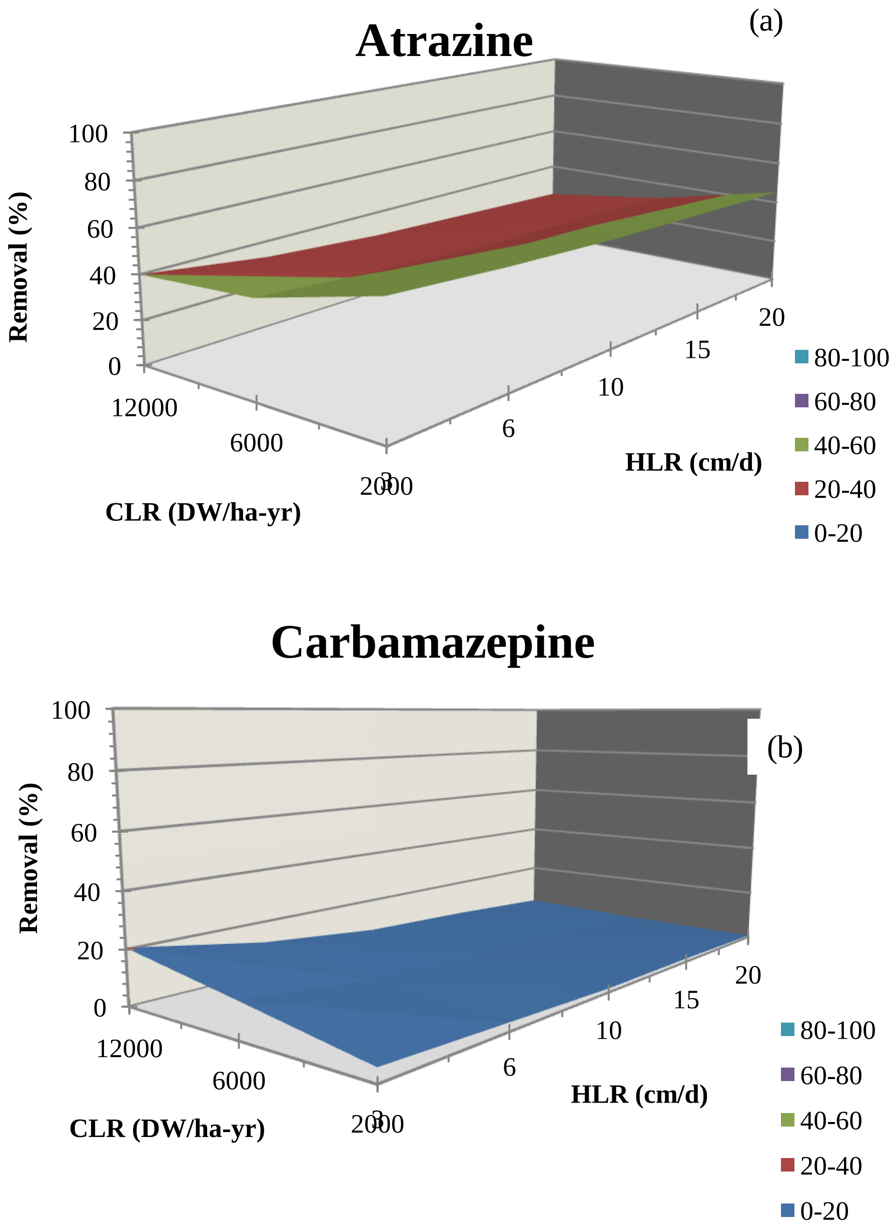


Figure *8.2*. CEC removal (%) with varying hydraulic and carbon loading rates. a: atrazine, b: carbamazepine.

In general, both HLR and CLR can affect organic CEC removal in constructed wetlands. HLR which represents water loading into constructed wetlands per unit area over time is a function of residence time or the time that the volume of water containing the contaminants is allowed to occupy in the wetland. Thus HLR controls the time frame of the different removal mechanisms occurring in the wetlands. CLR controls surface area and biomass quantity available for sorption and biodegradation. It also controls the leached DOC from plants affecting photolysis. Plant-leached DOC also depends on HLR. Thus, these two loading rates create an integrated effect and one loading rate cannot be proclaimed as more influential for overall CEC removal. This finding does not support hypothesis 3 “A-fate-predictive model can show that hydraulic loading rate has greater influence on CEC removal than plant addition or carbon loading rate”.

Research question 4

‘Does the level of denitrification in constructed wetlands affect organic CEC removal in wetlands?

Constructed wetlands can remove NO_3^- from wastewater effluent. NO_3^- removal was observed in continuous-flow microcosms with plant biomass (Chapter 6). The level of denitrification in the microcosms were dependent on HRT of the microcosms as plant leached DOC increased with lengthening HRT which was in agreement with a previous study by Ingersoll an Baker, 1998.

Organic CECs were also removed in the denitrifying microcosms which support the use of denitrifying wetlands as a promising option to remove CECs. It may appear that denitrification had no role on CEC removal since HRT controlling the level of denitrification in the microcosms was not found to significantly affect CEC removal (Figure 6.1). However, batch experiments conducted with the plant biomass from the microcosms showed that E2 was biotransformed while denitrification was occurring

(Figure 6.3 and 6.4). With higher plant biomass quantity available, both NO_3^- and E2 removal became faster. Because of the overarching effect of plant biomass quantity on E2 removal, the effect of HRT or denitrification was not observed in the microcosms. Plant biomass quantity also affected the other CEC removals. Mass of CECs relative to the plant biomass quantity available favored NDMA, atrazine and caffeine removal in the microcosms over the batch experiments. Overall, these findings support the hypothesis that increasing denitrification increased E2 removal but the similar effect on NDMA removal was not observed.

Guidelines for choosing a feasible treatment system for CEC removal

The goal of this dissertation was to investigate the two different approaches of wastewater effluent treatment for CEC removal. In this section, an integrated approach is provided here as a guideline to select appropriate treatment process for CEC removal.

The first step of selecting the right treatment process or system for CEC removal is to measure water quality parameters (e.g. DOC, reduced nitrogen species) and a suite of CECs in the wastewater effluent that needs to be further treated for safe discharge or reuse. The selected CECs can be indicator or surrogate compounds commonly found in wastewater effluent. This step is critical to make decisions on which of the two treatment systems should be selected. Four hypothetical scenarios are discussed here involving different levels of DOC and reduced nitrogen species (Figure 8.3). The first scenario is for a higher quality effluent with low levels of DOC, NH_3 and NO_3^- and ozonation can be selected for CEC removal for this scenario. O_3 dosages required to remove the target CECs can be estimated by measuring the demand from the reduced organic species and find out O_3 consumed by organics. The guideline includes a figure where CEC removals are plotted against O_3 consumed by organics. The line drawn for pCBA was obtained from the findings of Chapter 2. Similar lines were obtained for a number of commonly detected CECs of wastewater effluent based on their HO• rate constants in comparison with pCBA. This figure shows how the effect of O_3 varies among the different CECs and for some CECs (e.g. atrazine, iopromide) significant O_3 dosages may be needed to achieve higher removal. It should be noted here that pCBA is non-reactive with ozone but the CECs selected can react with ozone to a varying extent. However it was assumed here that in wastewater ozone will be quickly consumed and these CECs will be only oxidized by HO• that forms during ozonation.

In the second scenario, only NO_3^- level is high and constructed wetlands can be considered as the suitable treatment process for CEC removal. High NO_3^- loading can support denitrification in constructed wetlands where CECs can be naturally removed parallely with NO_3^-. Third scenario involves WWTP effluent with high levels of DOC. Constructed wetlands can be selected because higher value of DOC will make ozonation a costly and energy consuming treatment option. The fourth scenario involves effluent with high levels of NH_3 and again constructed wetlands should be selected to avoid large O_3 dosages. In this scenario, if the effluent form the wetland contains significant amount of NO_3^- then it should be discharged into another wetland connected in series where denitrification can remove the influent NO_3^-. For the scenarios where constructed wetlands have been selected to be the right treatment system for CECs, design parameters e.g. HLRs and CLRs should to be selected in the next step. The model developed in Chapter 5 can act as a useful tool to select these parameters based on the types of CECs measured in the effluent in the first step.

The final step in these guidelines is to monitor the water quality of the effluents. For selected CECs, removal may not be significant by the preceding treatment process and additional treatment may need to be used. For example, if in the first step CECs like triazine pesticides e.g. atrazine were detected at elevated levels and ozonation was selected as the effluent treatment option, additional treatment by wetlands may need to follow to remove these CECs that are not prone to ozone. Similarly, if refractory CECs (e.g. carbamazepine) were not removed by wetlands then ozonation process may be required to remove them. These two treatment processes/systems can be integrated in this way, to maximize CEC removal from wastewater effluent.

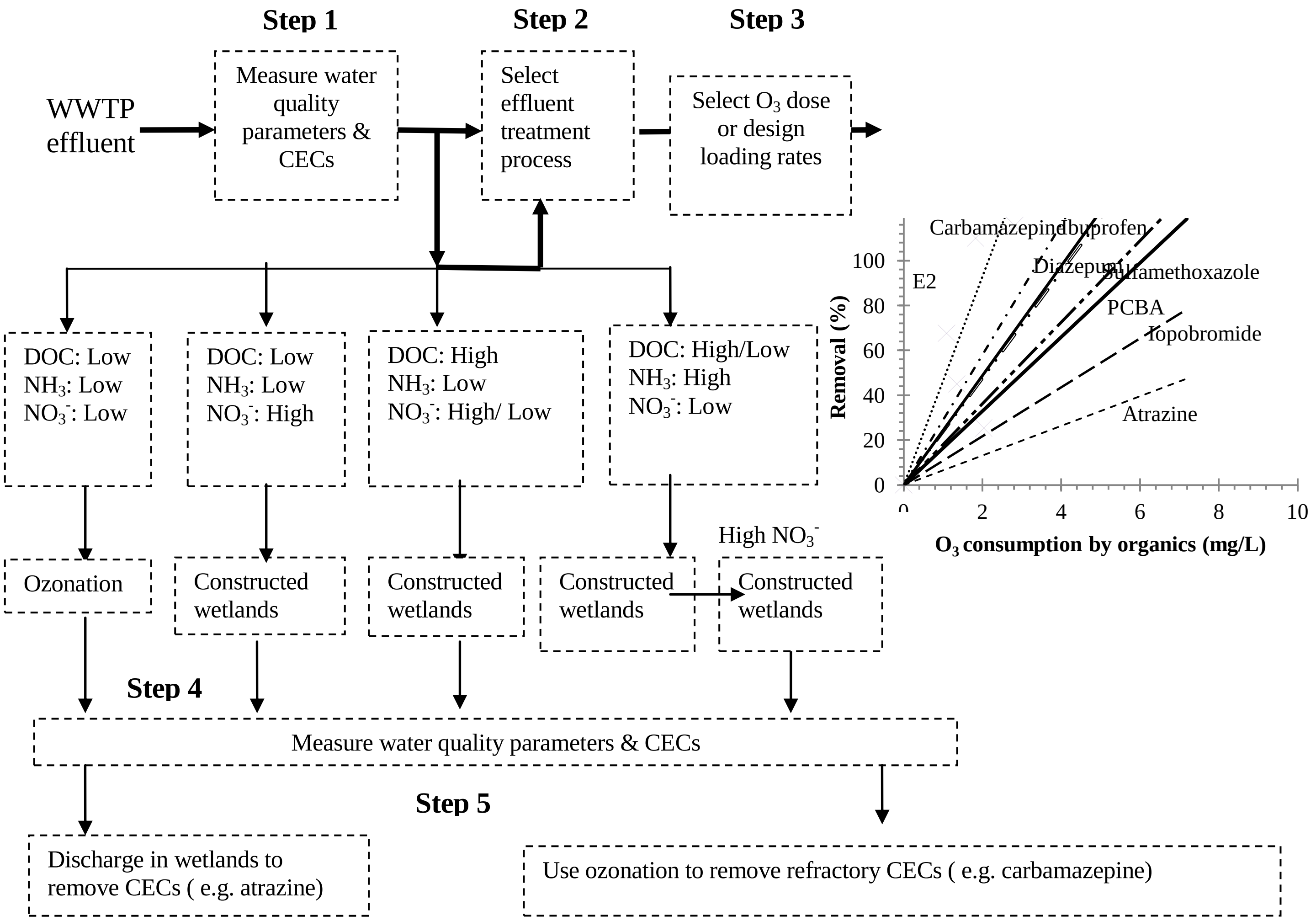


Figure 8.3. An integrated approach using ozonation and constructed wetlands as feasible treatment options for CECs.

CHAPTER 9

SUMMARY, CONCLUSIONS, LIMITATIONS, AND FUTURE RECOMMENDATIONS

Summary

The goal of this dissertation was to evaluate ozonation and constructed wetlands to remove CECs from wastewater effluent. Research work conducted to meet this goal was discussed in the previous chapters. The major observations from these chapters are summarized below.

Chapter 2: Effect of ozonation on wastewater effluent quality

- Ozone consumed by dissolved organic compounds was quantified by separating the demand from NO_2^- and NH_3 which helped to obtain a series of correlations to explain transformation in bulk and trace organics.
- Better correlations were obtained for pCBA, UV_{254} and color removal by accounting on ozone consumption by organics rather than using ozone consumption by both organics and inorganics.
- Ozonation reduced NDMA formation potential (FP) by 70 to 80 % at the lowest applied dose of 0.25 mg-O_3/mg-DOC.
- Molecular size distribution analysis of ozonated wastewater showed reduced response from colloidal organic matter which indicates that ozonated water will cause less membrane fouling.

Chapter 4: Engineered nanomaterial removal in constructed wetlands

- Continuous-flow microcosms were used to evaluate simultaneous removal of ENMs and trace organic contaminants.

- ENM (aqueous fullerenes (aq-nC_{60}) and carboxylated nanosilver (CAR-Ag) removal was comparable with that of 17β - estradiol (E2). The range of removal efficiencies in the microcosms (considering tracer loss) for aq-nC_{60}, CAR-Ag and E2 were 38 ~ 60 %, 37 ~ 60 % and 56 ~ 70 % respectively.
- By increasing the plant material quantity, the effect of HRT on ENM removal was reduced. Pulse-input studies of ENMs were conducted in two phases (five months apart) while plant materials were consciously added into the microcosms at 3 g DW/week. Between the two phases of studies, lengthening HRT by twofold in the 1st phase where a relatively lower quantity of plant materials were present, aq-nC_{60} and CAR-Ag removal increase was 30 % and 25 % respectively. During the 2nd phase studies with greater quantity of plant materials, lengthening the HRT by three fold, additional mass removal for aq-nC_{60} and CAR-Ag was found to be 11 % and 30 % respectively.
- Size of CAR-Ag ENMs increased in microcosm effluent compared to their original size. This could be due to aggregation or association with organic colloids produced by the decaying plant biomass.
- Batch sorption studies showed higher sorption affinity of aq-nC_{60} (K_d = 0.2 L/g) than CAR-Ag NMs (K_d = 0.1 L/g) towards wetland plants.
- Conductivity influenced stability of Ag NMs. But sorption of Ag ENMs in water with varying conductivity level was found similar. This indicates the possibilities of a stabilizing effect from DOC leached from hydrating plant materials.

- Gum arabic coated Ag NMs (GA-Ag) was found more stable in microcosm influent water (1300 µs/cm conductivity) than CAR-AG and polyvinylpyrrolidne coated Ag NMs (PVP-Ag).
- Increasing the plant material quantity, Ag ENM sorption increased. However, surface coating did not affect sorption. All three coated Ag NMs experienced similar sorption onto wetland plants.

Chapter 5: Impact of hydraulic and carbon loading rates on Contaminants of emerging concern (CECs) removal in constructed wetlands

- Batch experiments were conducted to understand the role of different removal mechanisms for organic CECs in the continuous-flow microcosms.
- Sorption isotherm studies showed sorption affinity for the organic CECs was related to their corresponding Log D. Partitioning coefficients for E2, testosterone, atrazine and carbamazepine were 320, 160, 24 and 25 L/Kg respectively.
- Organic CECs with higher sorption tendency (higher values of K_d) showed less desorption. Desorption was least for E2 followed by testosterone, carbamazepine and atrazine.
- Plant-leached DOC reduced photolysis of organic CECs. Rate of photolysis was lower in microcosm effluent water (DOC = 6.5~8.5 mg/L) compared to the influent water (DOC 1.5 mg/L). The trend of photolysis among the CECs was atrazine > testosterone > E2 > carbamazepine.
- Decaying plant biomass supported biodegradation of organic CECs. The rate of biodegradation was higher for testosterone followed by E2.Atrazine and carbamazepine experienced negligible biodegradation.

- Hydrolysis of organic CECs in microcosm influent water was found negligible.
- A fate-predictive model was developed using AQUASIM to incorporate the different removal mechanisms and parameterized with rate constants and parameters obtained from batch experiments.
- Except for carbamazepine, areal removal rates of the organic CECs increased with hydraulic loading rate (HLR).
- Carbon loading rates (CLRs) influenced organic CEC removal with increasing HLRs.
- The effect of CLR varied among CECs with different physico-chemical properties. Higher CLR reduced areal removal rates of atrazine but improved E2 and carbamazepine removal. On the other hand, testosterone was least impacted by CLR. Combination of wetlands with different CLRs can optimize removal of different types of CECs without reducing the design HLR.

Chapter 6: Contaminants of emerging concern (CECs) removal in denitrifying conditions of constructed wetlands

- Denitrifying conditions of continuous-flow microcosm supported organic CEC removal. Removal efficiencies was low for carbamazepine (< 10%) and varied between 40 ~ 85 % for E2, NDMA, atrazine and caffeine.
- Denitrification in the microcosms increased by lengthening HRT, however, no trend was observed between HRT and organic CEC removal.
- E2 removal was improved while denitrification was occurring. Biotransformation of E2 was confirmed from batch removal kinetics and conducting a mass-balance between aqueous and sorbed phases.

- Decaying plant biomass quantity controlled the rate of denitrification and E2 removal. With higher plant biomass quantity available, both NO_3^- and E2 removal became faster. The difference between sorption and biotransformation of E2 was reduced by increasing the plant biomass quantity.
- Plant biomass quantity also affected other CEC removal. Because of a lower mass ratio of organic CECs added to plant biomass, removal was not observed in the batch-scale studies for NDMA, atrazine and caffeine.

Chapter 7: Monitoring organic CECs in full-scale wetlands

- Field-scale monitoring studies showed that organic CECs can enter constructed wetlands when WWTP effluents will be discharged.
- The increase in DOC, UV_{254} absorbance and NH_3 level in the effluent samples collected from the Kingman wetlands indicate plant activity including leaching of organic matter and ammonification during decay of the plant materials. During the second sampling campaign, significant improvement in influent water quality was due to the upgrade in the WWTP discharging the effluent into the wetlands.
- Most of the CECs (E2, testosterone and atrazine) monitored in the City of Kingman wetlands were present at low levels. Because of such low background concentration, removal was not investigated.
- Higher concentration levels were observed for sucralose (35 ~ 55 µg/L), carbamazepine (275 ~ 600 ng/L) and primidone (250 ~ 800 ng/L) during the various sampling studies conducted in the City of Kingman wetlands.
- Obtaining removal information from full-scale wetlands is complicated due to the lack of knowledge on contaminant loading and actual hydraulic residence time. Composite

sampling strategies can be helpful to understand the loading variation but even with such strategies estimating removal of CECs that are present at very low level is challenging.

Conclusions

Presence of CECs and the perceived risks associated with them can be a major obstacle to promote use of wastewater effluent. Current treatment technologies need to adapt with the increasing presence of CECs in wastewater effluent. This dissertation evaluated two types of wastewater effluent treatment processes (ozonation and constructed wetlands) to gain a better understanding on their role in CEC removal. A method was developed to estimate organic CEC oxidation across different wastewater sources based upon ozone demand attributed to dissolved organics alone by separating the ozone demand from inorganic species.

Constructed wetlands can be used as a vehicle to remove both organic and ENM CECs from wastewater effluent. ENM removal increases with increasing the mass of plant materials or lengthening hydraulic residence time of wetlands. Thus wetlands operated at short hydraulic residence times can still achieve good removal of ENMs if plant material quantity is high. Due to higher sorption affinity, higher quantity of plant materials can have greater impact on ENMs like aqueous-nC_{60} than Ag. However, lengthening hydraulic residence time; Ag ENMs experienced comparable removal with aqueous-nC_{60} which indicates possibilities of other removal mechanisms (e.g. sedimentation, sulfidation, etc.). A fate-predictive model was developed to evaluate the role of design loading rates on organic CEC removal which showed that areal removal rates increases with hydraulic loading rates except for the CECs experiencing very low removal (e.g. carbamazepine).It also concludes that removal rate increases with increasing the carbon loading rates except for the CECs dominantly removed by photolysis (e.g. atrazine). In order to optimize overall CEC removal, wetlands with different carbon loading rates can be connected in series and operated at same hydraulic loading rates.

Organic CECs can be also removed in wetlands designated to remove high levels of nitrate. Higher denitrification can lead to greater removal of CECs (e.g. estradiol) due to biotransformation. Both denitrification and CEC removal is influenced by the decaying plant biomass quantity of wetlands. On the other hand, impact of hydraulic residence time can be more pronounced on level of denitrification than CEC removal and complete denitrification can be achieved within a relative short residence time (5 days). Mass of CECs entering the wetlands compared to the plant biomass quantity available to support denitrification and simultaneous CEC removal is critical to understand the removal behaviors. Based on the findings from both modeling and experimental studies, carbon loading rate or plant biomass availability can be considered as an equally important design parameter as hydraulic loading rate.

Limitations and Future Recommendations

The role of growing aquatic plants or plants rooted into the sediments was not considered in this dissertation. Consequently removal predicted by the model is expected to improve if additional removal mechanisms supported by the plants are included.

Microcosms were kept at a constant temperature of $30^{o}C$ and they mainly simulate performances during the summer season. Similar studies need to be conducted at lower temperature to reflect performance in cold temperature. Since biological processes slow down during cold season, contaminant removal in constructed wetlands can be expected to decrease during winter.

pH of the microcosm water was 7.3 ± 0.2 which falls within the typical pH range observed in constructed wetlands. However, diurnal fluctuation in pH due to the activities from growing plants can influence the different removal mechanisms and need to be considered in batch-scale studies. pH in natural waters are controlled by photosynthesis and respiration activities. These processes influence net addition (respiration) or removal (photosynthesis) of CO_2 and as a result pH can either fall or rise. Typically, photosynthesis exceeds respiration and pH rises during day time. When sunlight is stronger or plant productivity is high, pH in aquatic environments like constructed wetlands can rise significantly. Specifically, in open water zones of wetlands, high pH can occur due to algal activity. Oppositely, organic substances generated during plant decomposition can raise acidity. But in most cases, influent alkalinity buffers the pH and consequently diurnal variation is not observed especially in the vegetated zones of constructed wetlands (Kadlec & Wallace, 2009). Annual average pH of free water surface flow type wetlands treating municipal wastewater effluent was reported to be 7.2 ± 0.35 which included wetlands of Arizona (Kadlec & Wallace, 2009). Subsurface flow wetlands are also known to buffer pH variations.

Batch-sale studies showed that photolysis can be an important removal mechanism for organic CECs in constructed wetlands. However it was not included in the microcosm studies as these microcosms were kept inside the laboratory environment. We need to use microcosms established in outdoors to study photolysis and other removal mechenaisms such as volatilization. Organic CECs can transport from wetland water to the surrounding air by volatilization and this process is influenced primarily by the contaminants' Henry's law constant values (H) and wind velocity (Kent, 2012). Organic CECs with Henry's Law constant values greater than approximately 10^{-4} atm-m^3/mol can experience significant loss due to volatilization (Kent, 2012). Increasing wind velocity can increase mass transfer from gas film and improve volatilization rate. Thus future research work is needed to study relatively volatile organic CEC removal in constructed wetlands (e.g. micro/mesocosms, pilot/full-scale units) that are open to the atmosphere.

Rate constants for biodegradation (K_{bio}) used in the model were determined from the batch experiments where loss observed in the controls were deducted. Due to continuous dropping of the concentration level in these controls, K_{bio} for E2 and testosterone probably underestimated the actual contribution from biodegradation. The unexpected removal behaviors of the CECs in the controls are presumed to be any abiotic loss associated with the autoclave procedure or the addition of $NaN_{3.}$ However loss in the autoclaved controls or controls where inactivation was achieved with chemical agents (e.g. NaN_3, Hg Cl_2) was reported in literature (Colucci et al., 2001, Fan et al., 2007).

Advanced treatment processes like ozonation has benefits of achieving higher removal efficiency but these processes can form unknown by-products with potential risks towards human health and aquatic organisms. Ozonation can transform the properties of ENMs that may increase their toxicity or facilitate movement in the environment. Research work is needed to understand fate of ENMs during ozonation of wastewater effluent.

In order to use constructed wetlands as a feasible treatment system for wastewater effluent, it is critical to understand the effect of design loading rates on CEC removal. To quantify the impact of wetland design on CEC removal, a mechanistic model incorporating removal mechanisms as functions of the loading rates is the best approach. It is also important to validate the model with actual data. To do this, appropriate rate constants are needed to represent the actual removal observed so that the developed model can simulate accurately. However, using rate constants obtained from batch experiments may not represent the actual conditions. To avoid this, *in-situ* rate constants need to be obtained. Areas of pilot or full-scale wetlands can be isolated to build *in-situ* wetland microcosms where removal mechanisms can be closely studied under field conditions.

Plant biomass has major impact on CEC removal and accurate estimation is important. In the model developed, estimation was made based on annual productivity and cattail leaf decomposition rates obtained from literature. But the decay process controlling the amount of plant biomass available in a wetland can be subjected to the design parameters including hydraulic loading rates or HRT. It is important to investigate plant decay process and how it is tied with other parameters of wetlands in order to accurately estimate the actual quantity of plant biomass.

Wetland plant produces suspended solids during the decay process which may influence fate and transport of CECs. The concentration levels of these suspended solids depend on the decay process and HRT of the wetlands. Relationships between HRT, plant decomposition and production of these suspended solids need to be established to incorporate in the fate-predictive model. The empirical relationships exiting in literature considers spherical shapes of suspended solids which are also not applicable for the plant produced suspended solids.

A fate-predictive model is also needed for ENMs to understand the effect of design loading rates on removal. The first step would be to explore the different transformation mechanisms that are possible for ENMs in wetlands. Uptake by living plants and translocation, photolysis in wetland water under the influence of plant-leached DOC, biological transformation by microbes residing on the biolfilms could be some of the potential transformation mechanisms for ENMs in wetlands. These mechanisms can be studied exclusively to understand kinetics and obtain rate constants to accurately represent in a model.

The decision to construct wetlands should be driven by the wastewater effluent quality. Discharging high quality effluent will not allow to gain benefits from using constructed wetlands as a treatment system for CECs. In Kingman wetlands, the incoming wastewater was treated by tertiary treatment processes including membrane bioreactors consequently the influent concentrations for most CECs were very low. Wetlands should be constructed to receive partially treated wastewater effluent to naturally remove both bulk and micro-pollutants. An additional advantage of these wetlands could come from by monitoring the relative changes between water quality parameters and CEC levels that may provide surrogate parameters to use as an alternative and quick way to asses CEC removal.

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APPENDIX A

SUPPLEMENTARY DATA FOR CHAPTER 5

Table A1

Precursor, product ions, LC-MS parameters, and MDLs for selected organic micropollutants

Analytes	Isotopically labeled compounds	Precursor ion	Product ion	Retention Time (min)	Collision energy (eV)	Capillary voltage (V)	MDL (µg/L)
Atrazine	Atrazine-d_5	216.2	175.1	11.6	-16	65	7.5
Carbamazepine	Carbamazepine-d_{10}	237.2	194.0	14.3	-21	80	11.3
17β -estradiol	[$^{13}C_6$]-estradiol	255.0	159.1	14.2	-14	60	6.8
Testosterone	Testosterone-d_5	261.0	165.0	11.6	-16	55	4.8

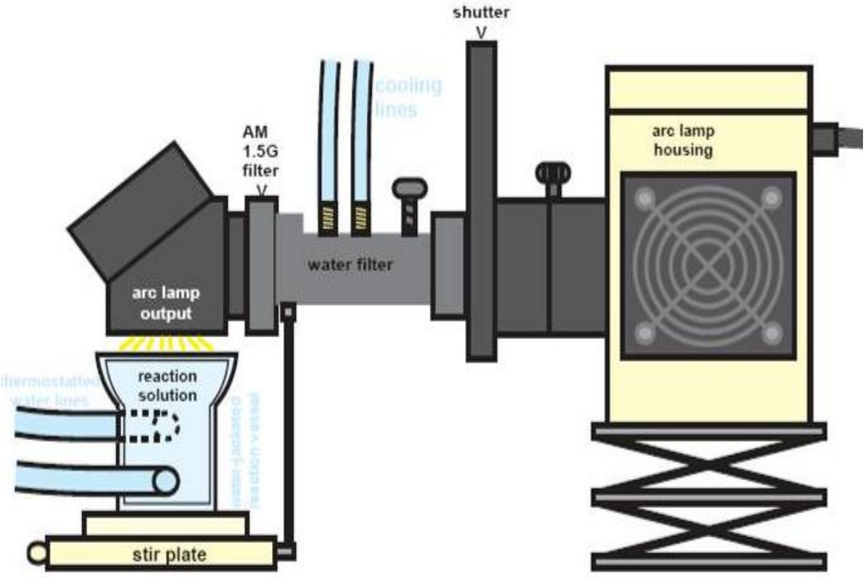


Figure A1. Schematics of the solar stimulator

Sample calculation to predict DOC leaching from decaying plants

Parameters	Unit	Values
Plant addition in wetland	g DW/ha-yr	6.00E+03
Plant addition in wetland	g/week	3
Plant addition in wetland	g/d	0.43
Plant C added	g C/d	0.21
Mass leacing rate, K_{PL}	g DOC/g C added	0.08
DOC biodegradation rate, K_p	1/d	0.06
Depth, H	cm	25.00
volume, V	cm^3	6.40E+03
Flow rate, Q	cm^3/d	1562
HRT	d	4.10
A surface	cm^2	2.60E+02
K_{PL}	(g DOC)/g C added	3.08E-04
Y	$g/cm^3.d$	6.59E-05
C (DOC)	g/cm^3	8.67E-06
C (DOC)	mg/L	8.67

Hydrolysis of CECs

Target CEC were spiked in the microcosm influent water (pH = 7.3, conductivity = 1.3 mS/cm) and samples were taken periodically to measure the concentration. Experiments were conducted at room temperature and in the dark to limit any potential photolysis. Figure A2 shows the concentration of selected organic CECs with time. None of the CECs were hydrolyzed during the 8 days of the experiment, thus ruling out hydrolysis as representing a major mechanism of removal in wetland microcosm influent water for these analytes under the conditions investigated.

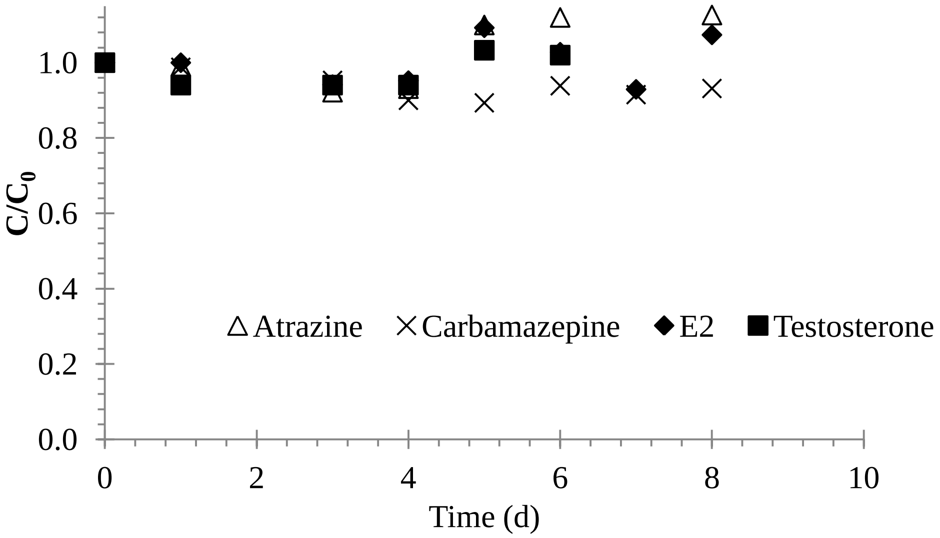


Figure A2. Hydrolysis kinetics of CECs in microcosm influent water at pH= 7.3

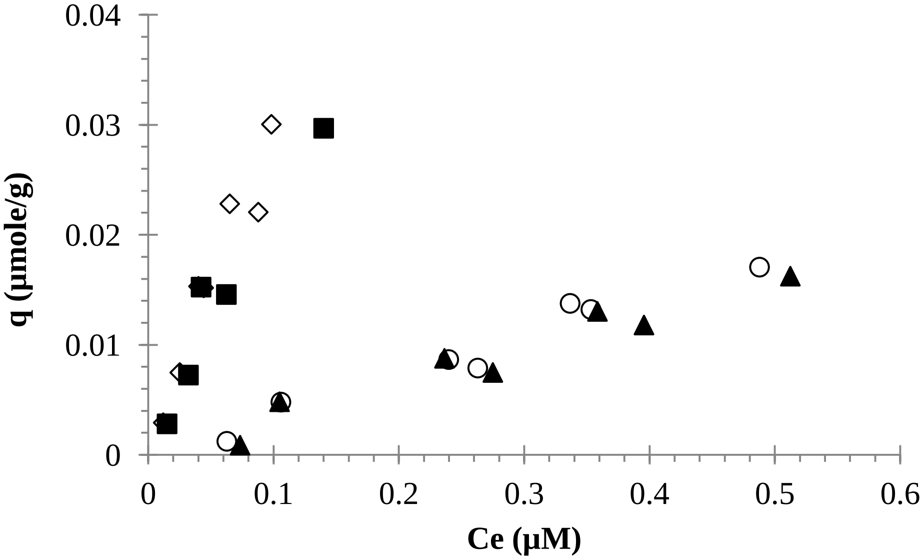


Figure A3. Sorption isotherms of CECs using 30 g/L of hydrated wetland plant materials.

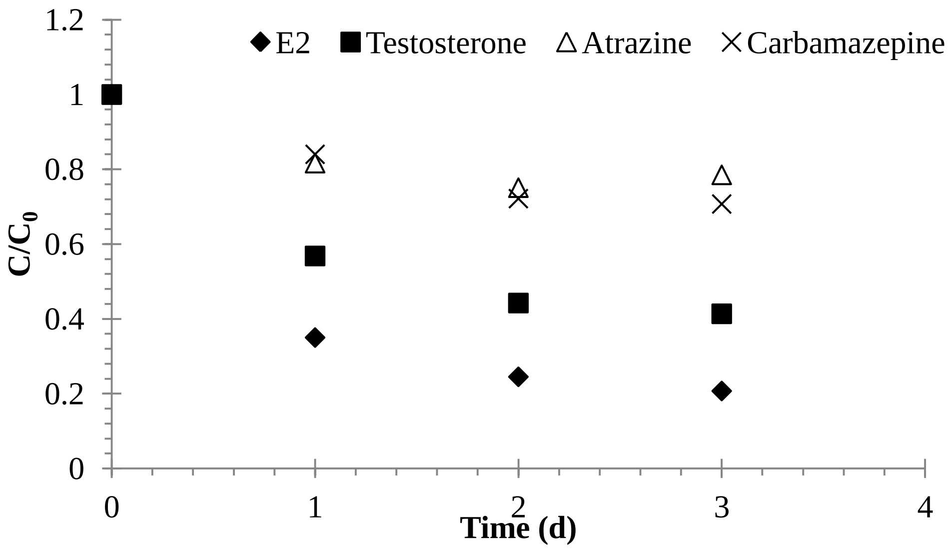


Figure A4. Sorption kinetics of the organic CECs at C_0 = 1µM.

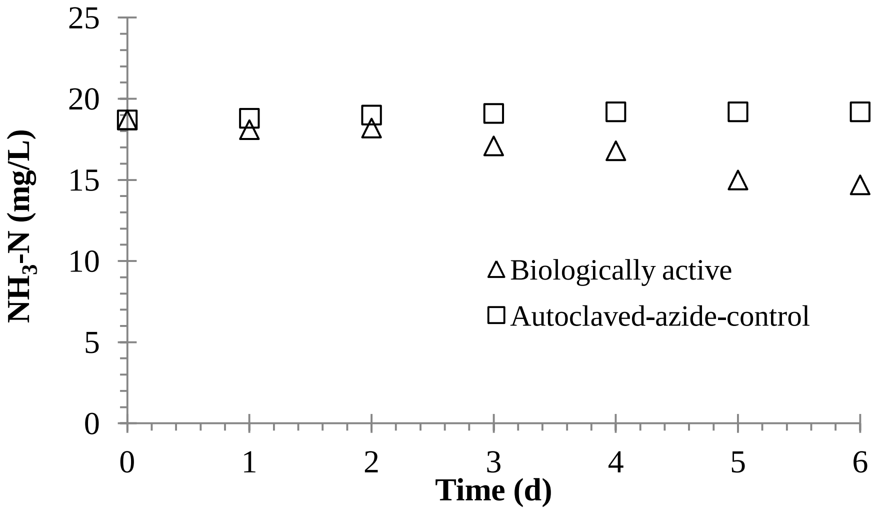


Figure A5. NH_3-N concentration in biodegradation experiments with 30 g/L of decaying plant biomass.

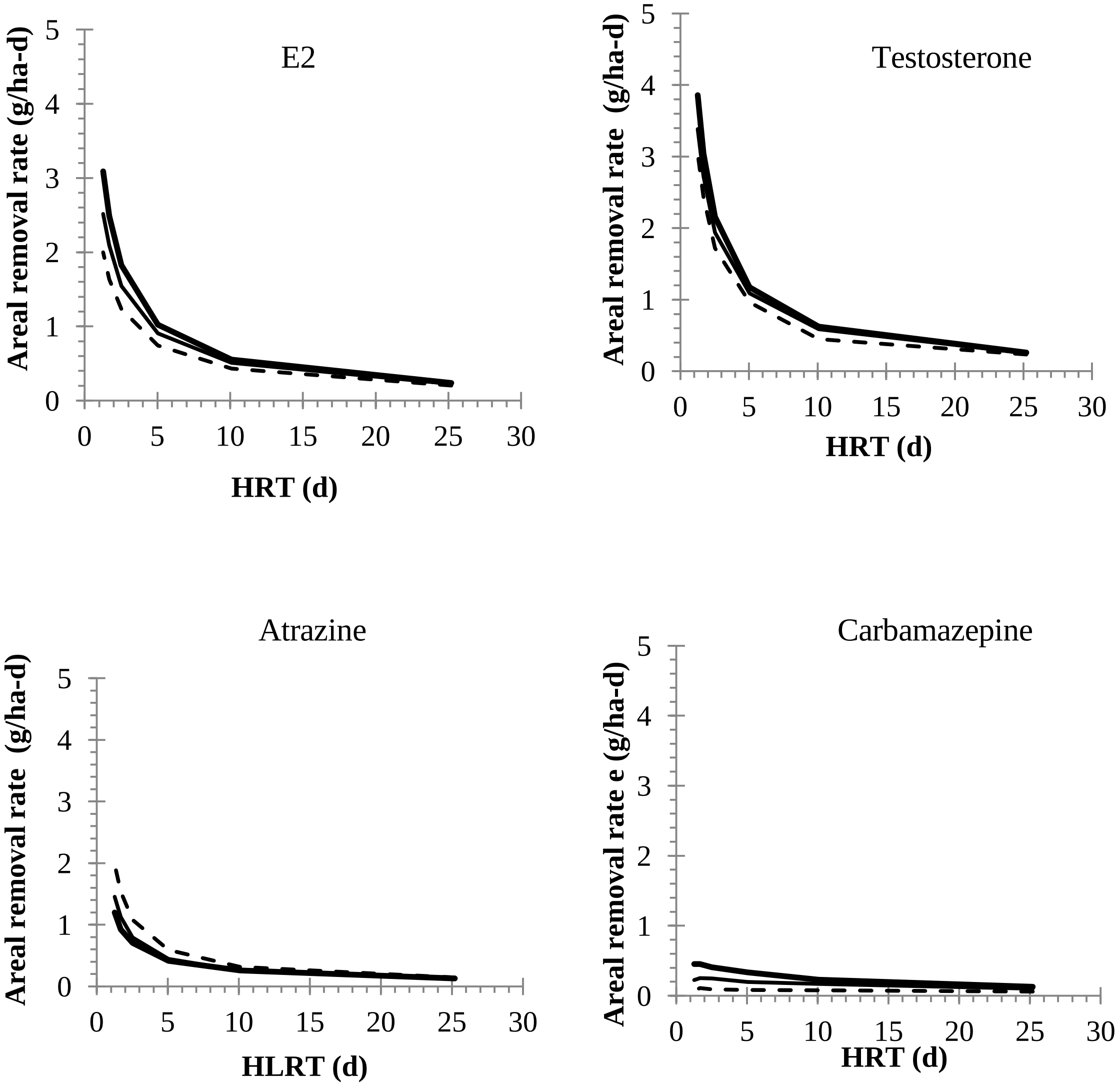


Figure A6. Model predicted areal removal rates for CECs at varying HRTS and plant addition rates. – – – – $2x10^3$ Kg-DW/ha-yr ——— $6x10^3$ Kg-DW/ha-yr ▬▬▬ $1.2x10^4$ Kg-DW/ha-yr.

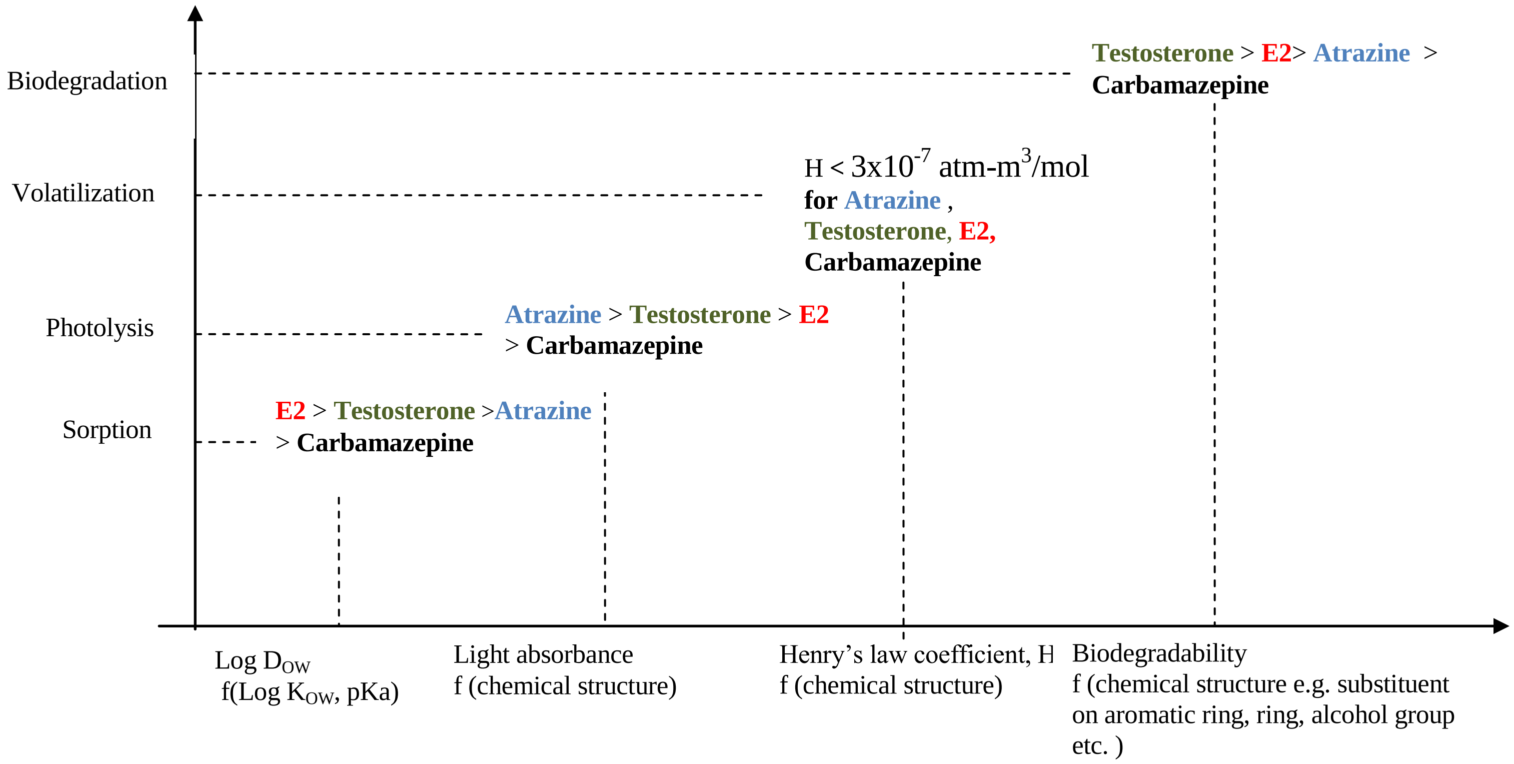


Figure A7. Schematic diagram showing the link between physico-chemical properties with CEC removal mechanisms

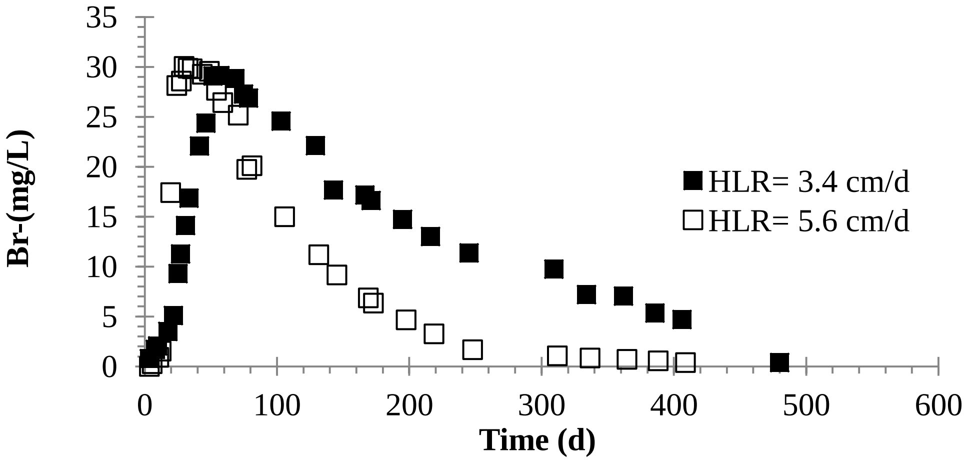


Figure A8. Tracer date from microcosms operated at HLR of 3.4 and 5.6 cm/d.

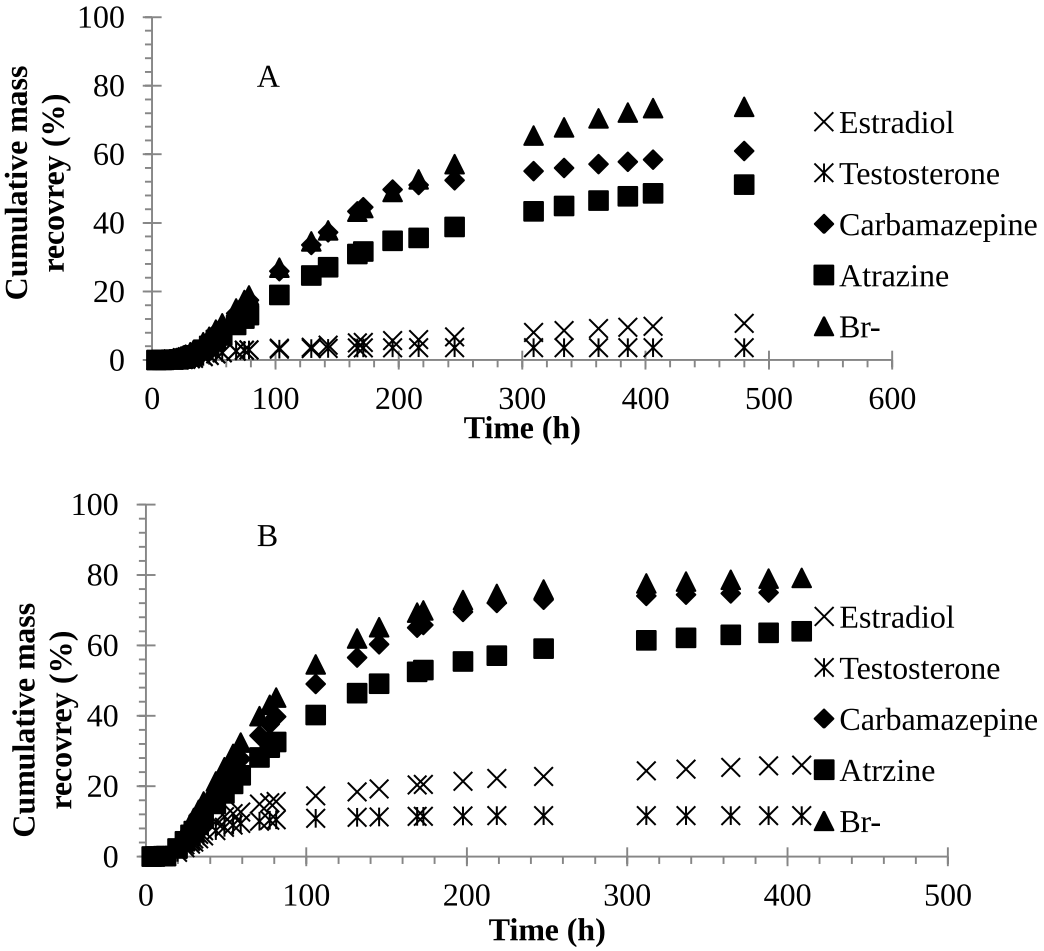


Figure A9. Mass recovery of organic CECs from the microcosms. A. HLR = 3.4 cm/d and B. HLR= 5.6 cm/d.

APPENDIX B

SUPPLEMENTARY DATA FOR CHAPTER 6 AND 7

Figure B1. Wetland plants used in the microcosms. a. Dry plant materials. b. decayed plant biomass from the microcosms.

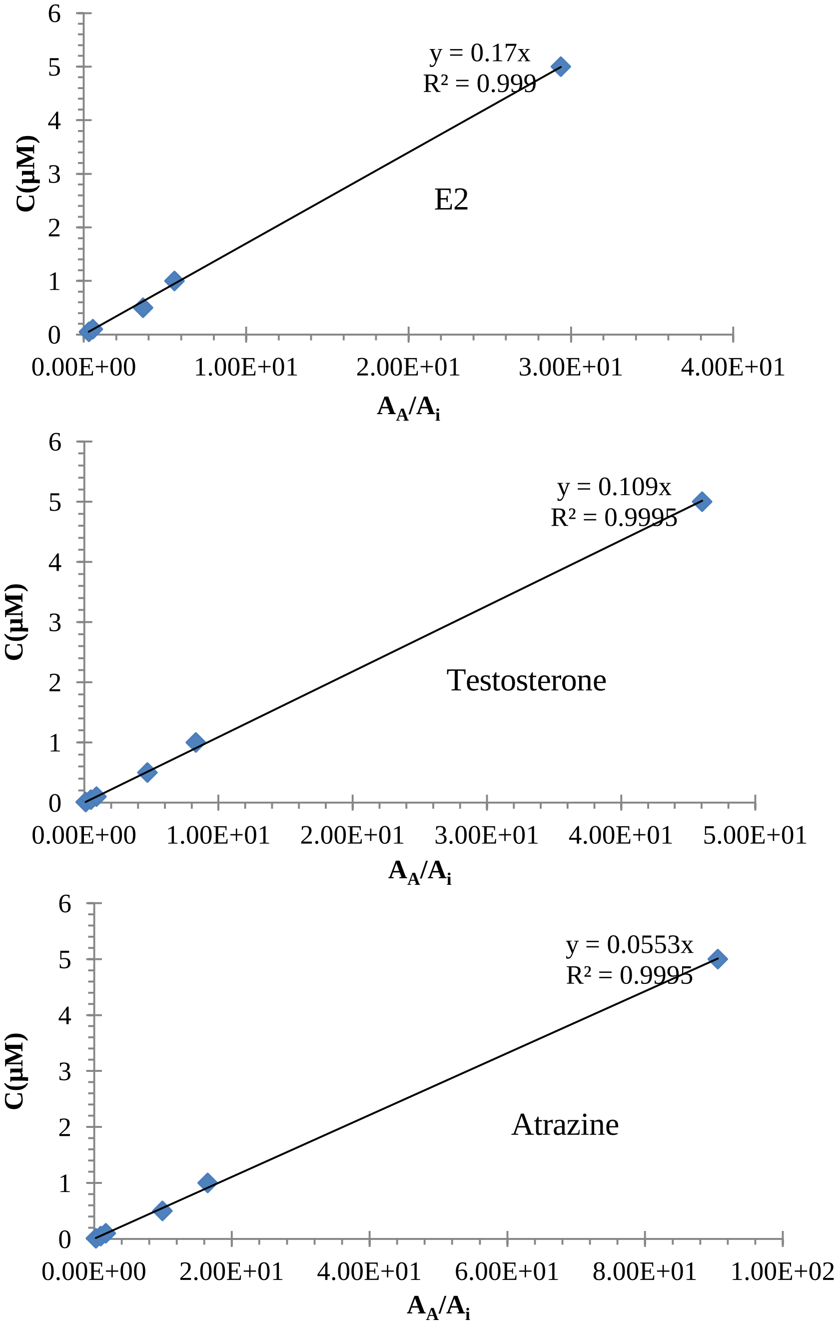


Figure B2. Standard calibration curves for organic CECs

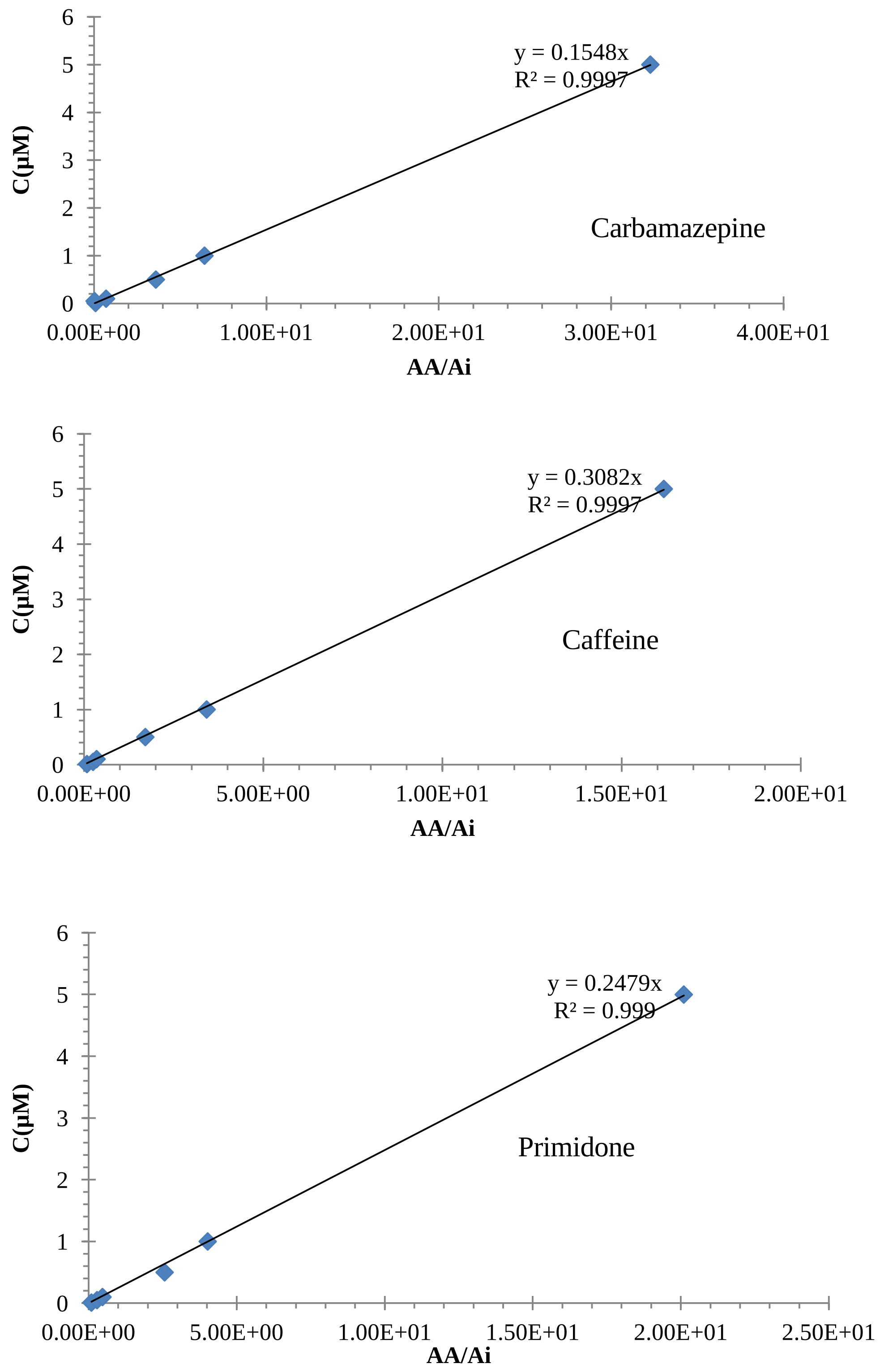


Figure B3. Standard calibration curves for organic CECs

Table B1

LC-MS/MS source parameters

	ESI positive	ESI negative	APCI positive
Detector voltage (V)	1660	1660	1660
Needle voltage (V)	4050	-3500	N/A
Shield voltage (V)	475	-400	325
Nebulizing gas Pressure (psig)	55	55	55
Nebulizing current (µA)	3	-7	5
Turbo gas (psig)	81	81	81
Collison gas pressure (mTorr)	1.5	1.5	1.5
Drying gas pressure (psig)	20	20	20
Drying gas temperature (^{0}C)	300	300	300
Probe *X*-axis position (mm)	-7	-7	-7
Probe *Y*-axis position (mm)	+4	+4	+4
Vaporizing gas temperature (^{0}C)	N/A	N/A	300
Vaporizing gas Pressure (psig)	N/A	N/A	20

Compound-specific parameters

Analytes	Isotopically labeled compounds	Precursor ion	Product ion	Retention time (min)	Collision energy (eV)	Capillary voltage (V)	MDL (µg/L)
Atrazine	Atrazine-d_5	216.2	175.1	6.8	-16	65	7.5
Carbamazepine	Carbamazepine-d_{10}	237.2	194.0	5.3	-21	80	11.3
17β -estradiol	[$^{13}C_6$]-Estradiol	255.0	159.1	5	-14	60	6.8
Testosterone	Testosterone-d_5	261.0	165.0	6.2	-16	55	4.8
Caffeine	Caffeine-[$^{13}C_3$]	195.1	109.7	4.5	-17	50	19
Primidone	Primidone-d_5	224.0	167.1	4.5	-10.5	70	19

Table B2

Standard calibration for organic CECs

CECs	Calibration standard (μM)	Area of analytes, A_A	Area of isotopes, A_i	Ratio A_A/A_i
E2	0.05	1.16E+05	3.65E+05	3.17E-01
	0.1	1.97E+05	3.54E+05	5.56E-01
	0.5	1.10E+06	3.02E+05	3.65E+00
	1	2.06E+06	3.70E+05	5.58E+00
	5	1.16E+07	3.94E+05	2.94E+01
Testosterone	0.01	1.07E+05	9.83E+05	1.09E-01
	0.05	5.15E+05	1.05E+06	4.91E-01
	0.1	9.99E+05	1.10E+06	9.10E-01
	0.5	5.13E+06	1.09E+06	4.70E+00
	1	9.61E+06	1.16E+06	8.31E+00
	5	5.66E+07	1.23E+06	4.60E+01
Atrazine	0.01	2.57E+06	9.69E+06	2.66E-01
	0.05	9.99E+06	1.04E+07	9.61E-01
	0.1	1.79E+07	1.06E+07	1.70E+00
	0.5	9.88E+07	9.96E+06	9.93E+00
	1	1.67E+08	1.01E+07	1.65E+01
	5	7.63E+08	8.42E+06	9.06E+01
Carbamazepine	0.01	1.07E+06	1.18E+07	9.03E-02
	0.05	4.31E+05	1.20E+07	3.60E-02
	0.1	8.18E+06	1.17E+07	7.01E-01
	0.5	3.89E+07	1.08E+07	3.59E+00
	1	6.80E+07	1.06E+07	6.41E+00
	5	2.75E+08	8.51E+06	3.23E+01
Caffeine	0.01	2.60E+05	3.24E+06	8.01E-02
	0.05	8.90E+05	3.56E+06	2.50E-01
	0.1	1.19E+06	3.43E+06	3.49E-01
	0.5	5.67E+06	3.32E+06	1.71E+00
	1	1.13E+07	3.30E+06	3.42E+00
	5	5.37E+07	3.32E+06	1.62E+01

Matrix spike recovery of CECs during denitrification studies:

To understand the influence of matrix on recovery of the analytes, at first the background concentrations in the effluent water were determined. Spike recovery study was conducted using effluent from each of the microcosms operated at 1.5, 2.5 and 5 d respectively. 10 nM mix of CECs were added into a 1L of the filtered effluent samples from each of these microcosms and prepared in duplicates. The background concentration was deducted prior to calculate the recovery.

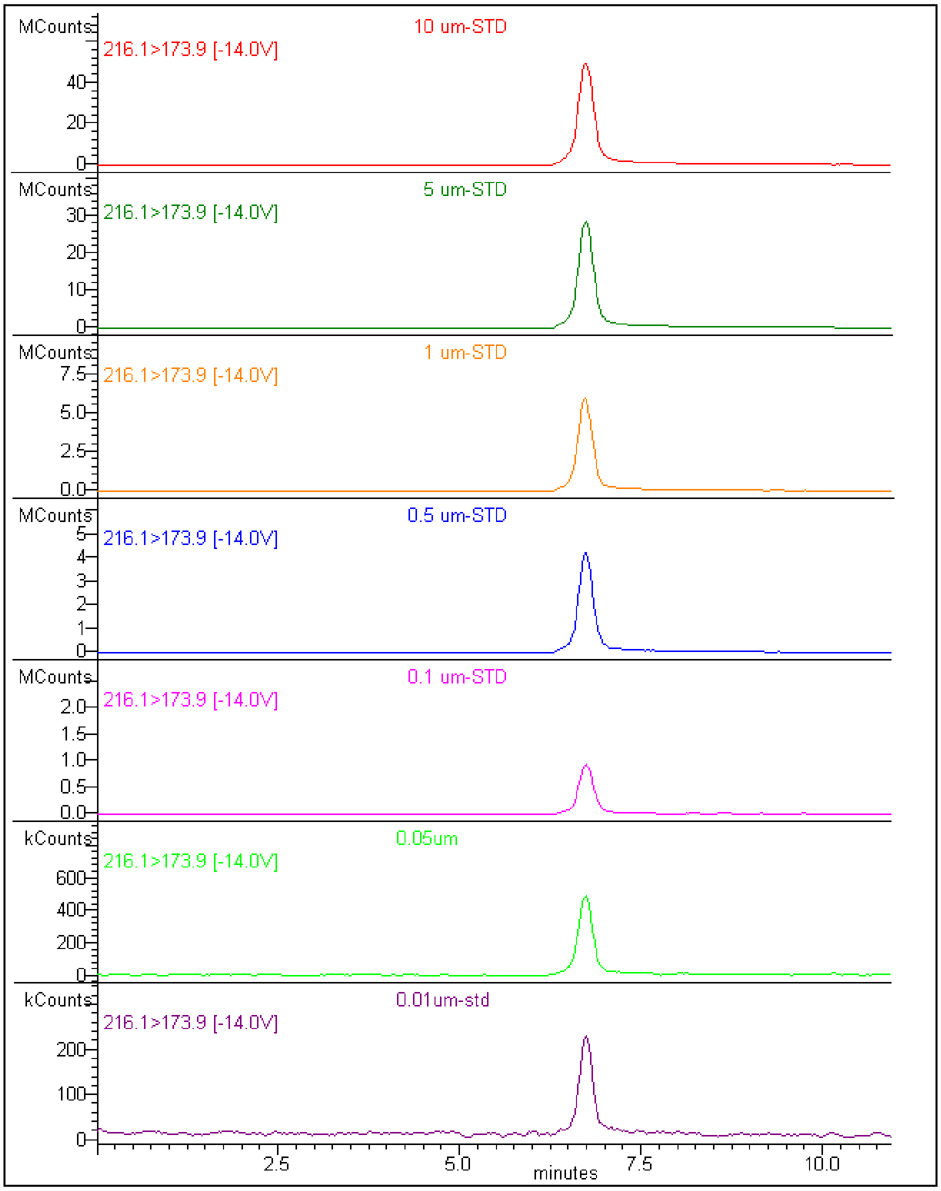
Table B3

Recovery (%) of selected CEC from microcosm effluent water during denitrification studies

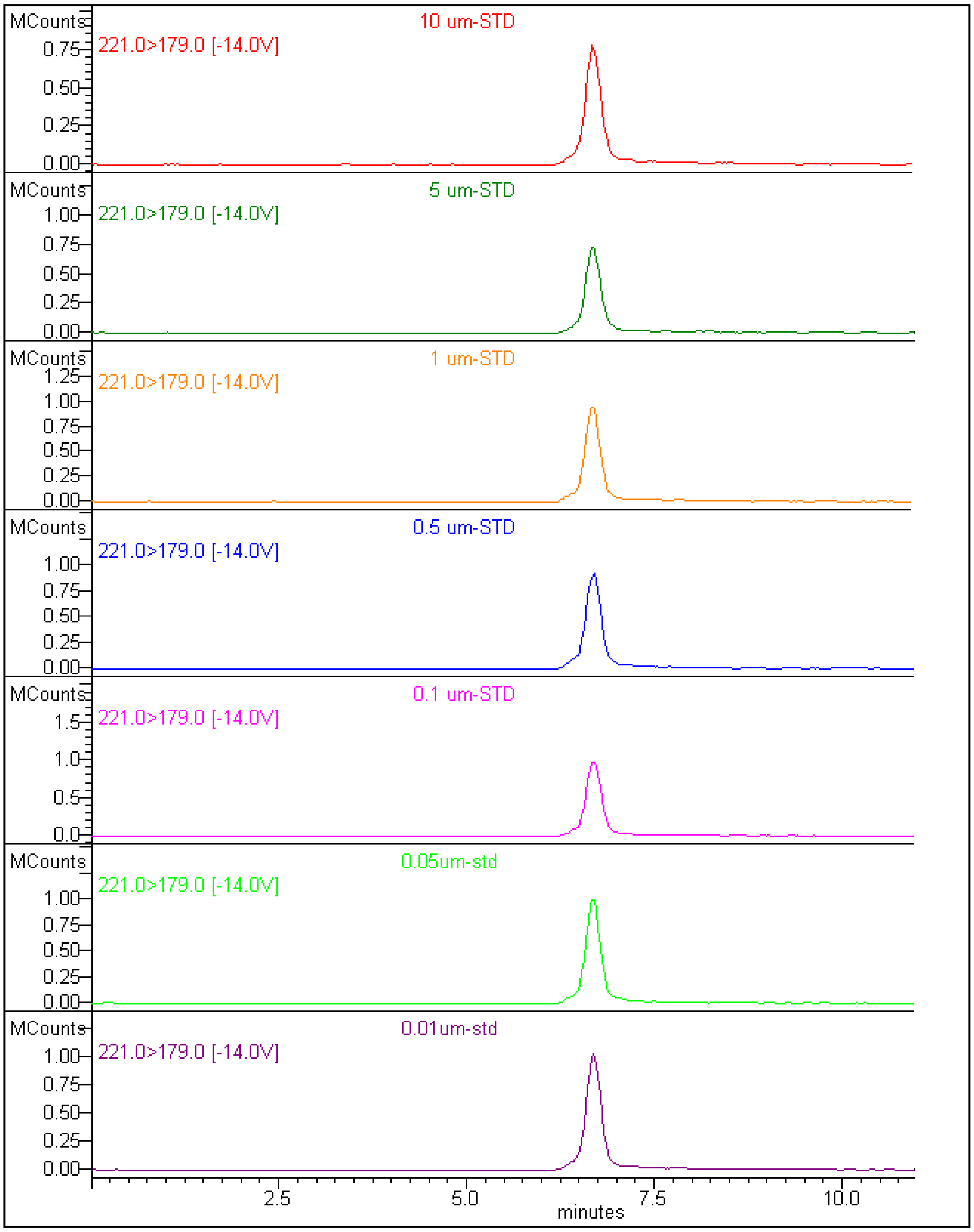
	Caffeine	Atrazine	Carbamazepine	E2
Microcosm influent water	99	110	123	120
Microcosm influent water-dup	103	105	125	125
Microcosm 1 Effluent	78	90	106	86
Microcosm 2 Effluent-dup	75	104	109	75
Microcosm 2	77	103	115	96
Microcosm 2Effluent-dup	72	116	111	87
Microcosm 3	73	124	117	75
Microcosm 3 Effluent-dup	71	94	122	78
Average	81	106	116	93
STDEV	12	11	7	22

Chromatogram Peaks:

Atrazine

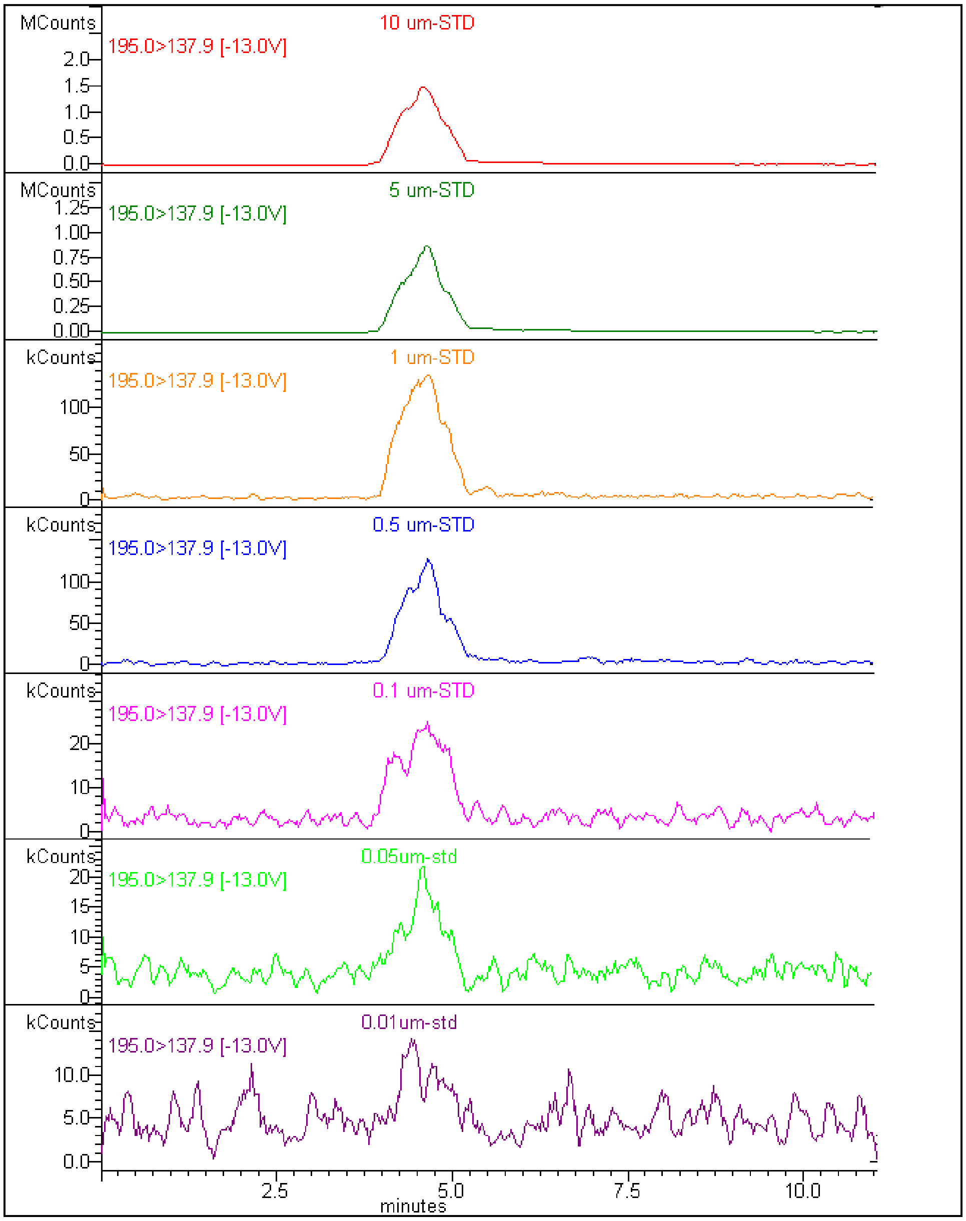


Atrazine - d_6

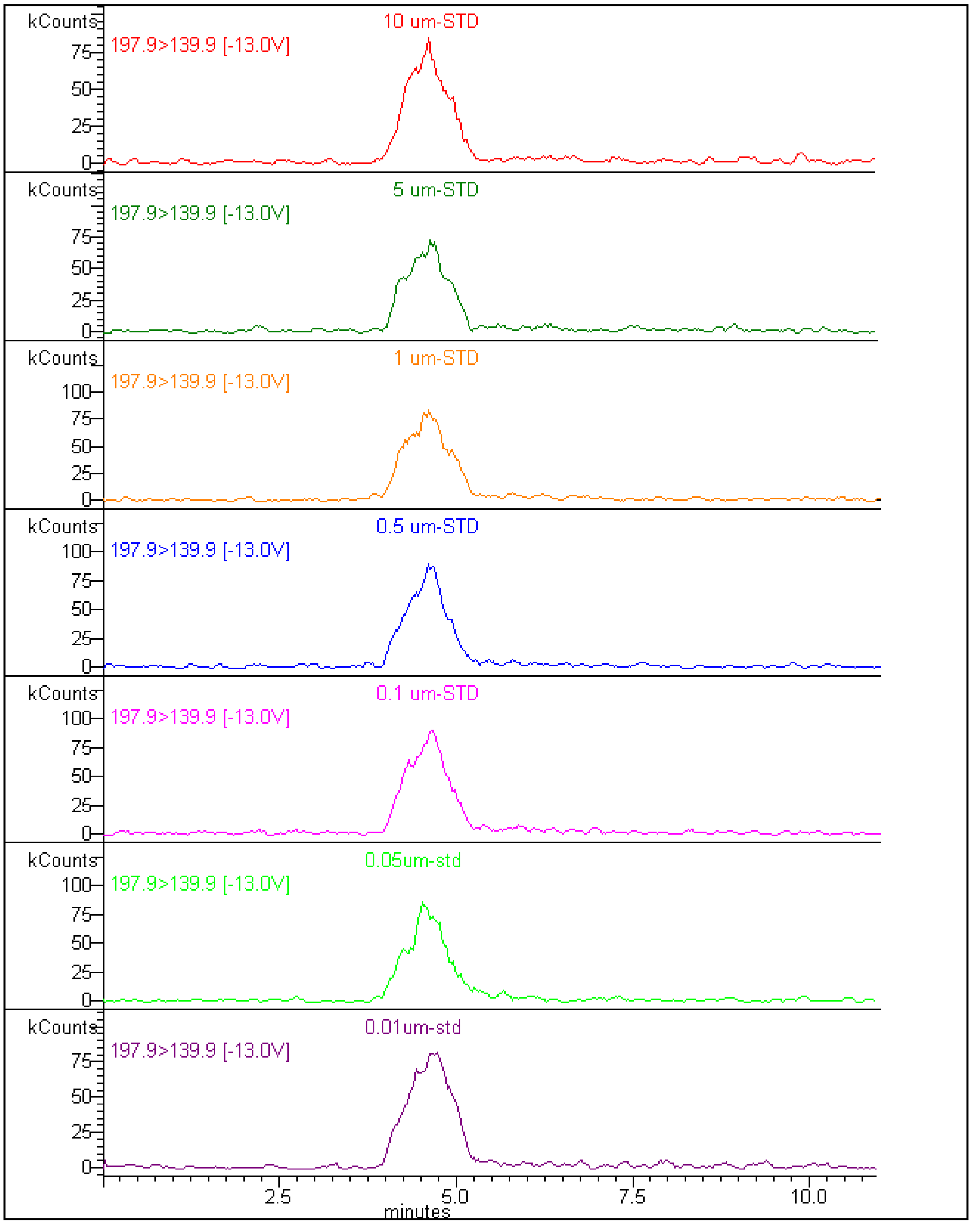


MCounts
10 um-STD
221.0>179.0 [-14.0V]
0.75
0.50
0.25
0.00
5 um-STD
1.00
1 um-STD
1.25
0.5 um-STD
0.1 um-STD
1.5
1.0
0.5
0.0
0.05um-std
0.01um-std
2.5
5.0
7.5
10.0
minutes

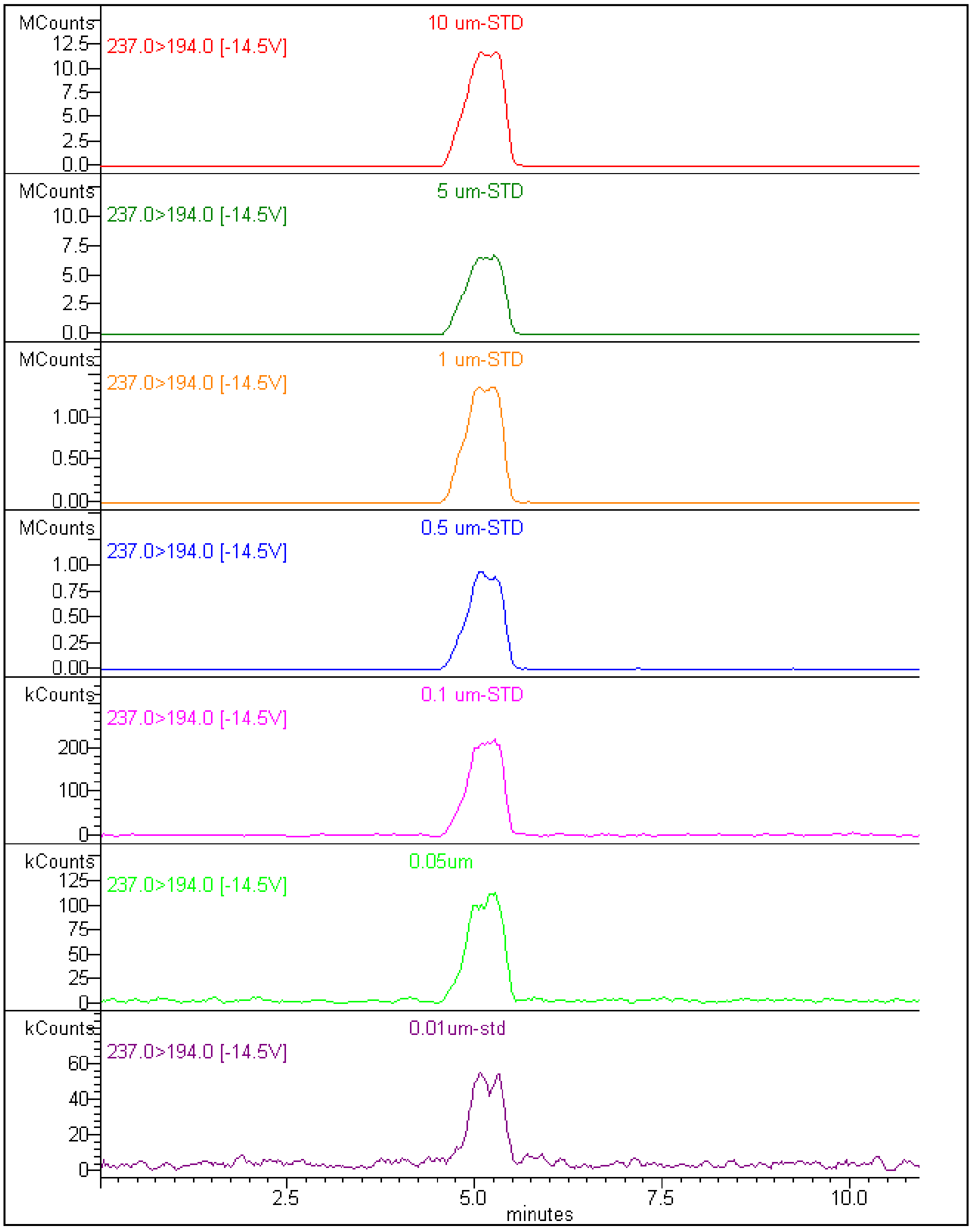
affeine


MCounts
10 um-STD
195.0>137.9 [-13.0V]
2.0
1.5
1.0
0.5
0.0
MCounts
5 um-STD
195.0>137.9 [-13.0V]
1.25
1.00
0.75
0.50
0.25
0.00
kCounts
1 um-STD
195.0>137.9 [-13.0V]
100
50
0
kCounts
0.5 um-STD
195.0>137.9 [-13.0V]
100
50
0
kCounts
0.1 um-STD
195.0>137.9 [-13.0V]
20
10
0
kCounts
0.05um-std
195.0>137.9 [-13.0V]
20
15
10
5
0
kCounts
0.01um-std
195.0>137.9 [-13.0V]
10.0
5.0
0.0
2.5
5.0
7.5
10.0
minutes

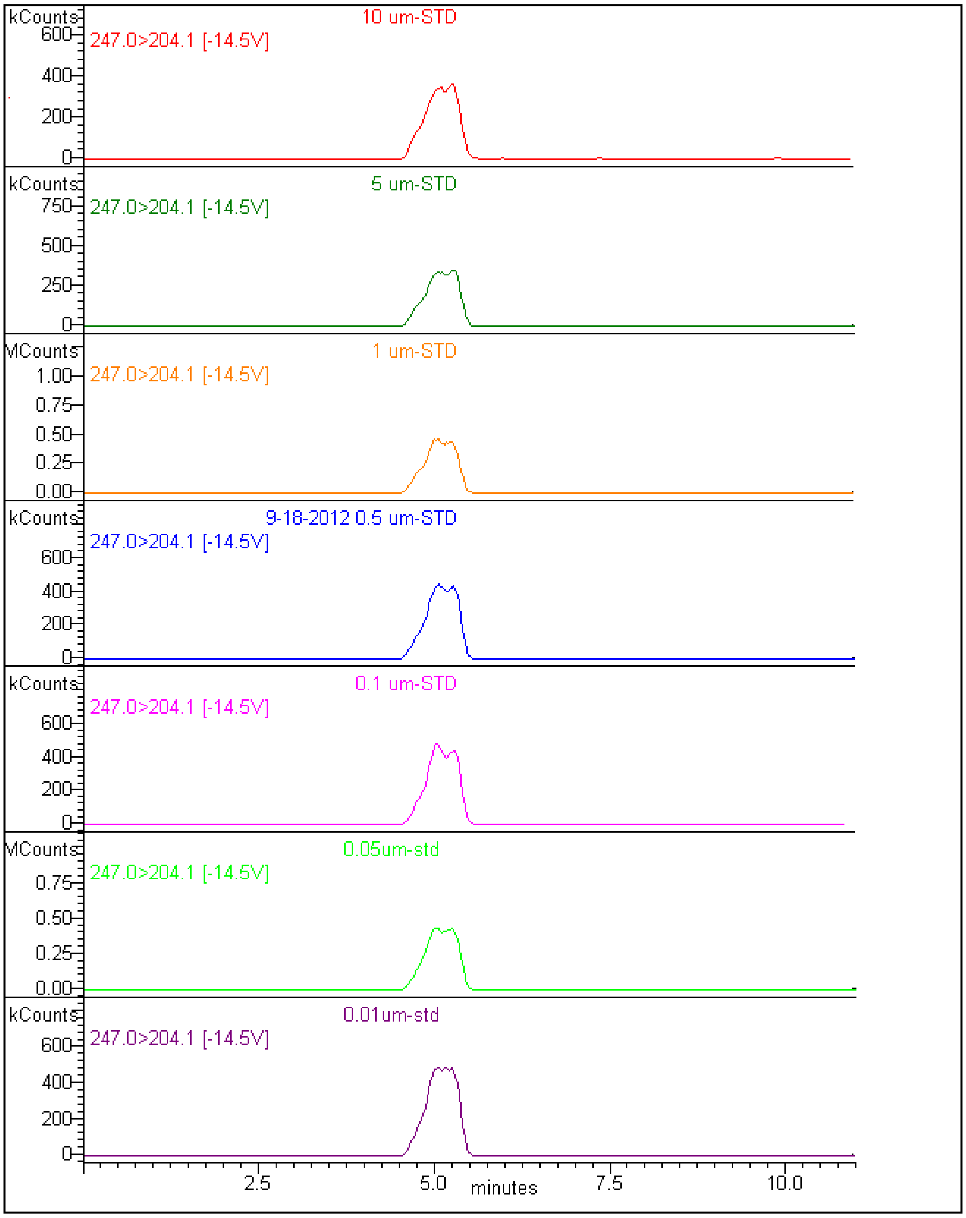
Caffeine - $^{13}C_3$



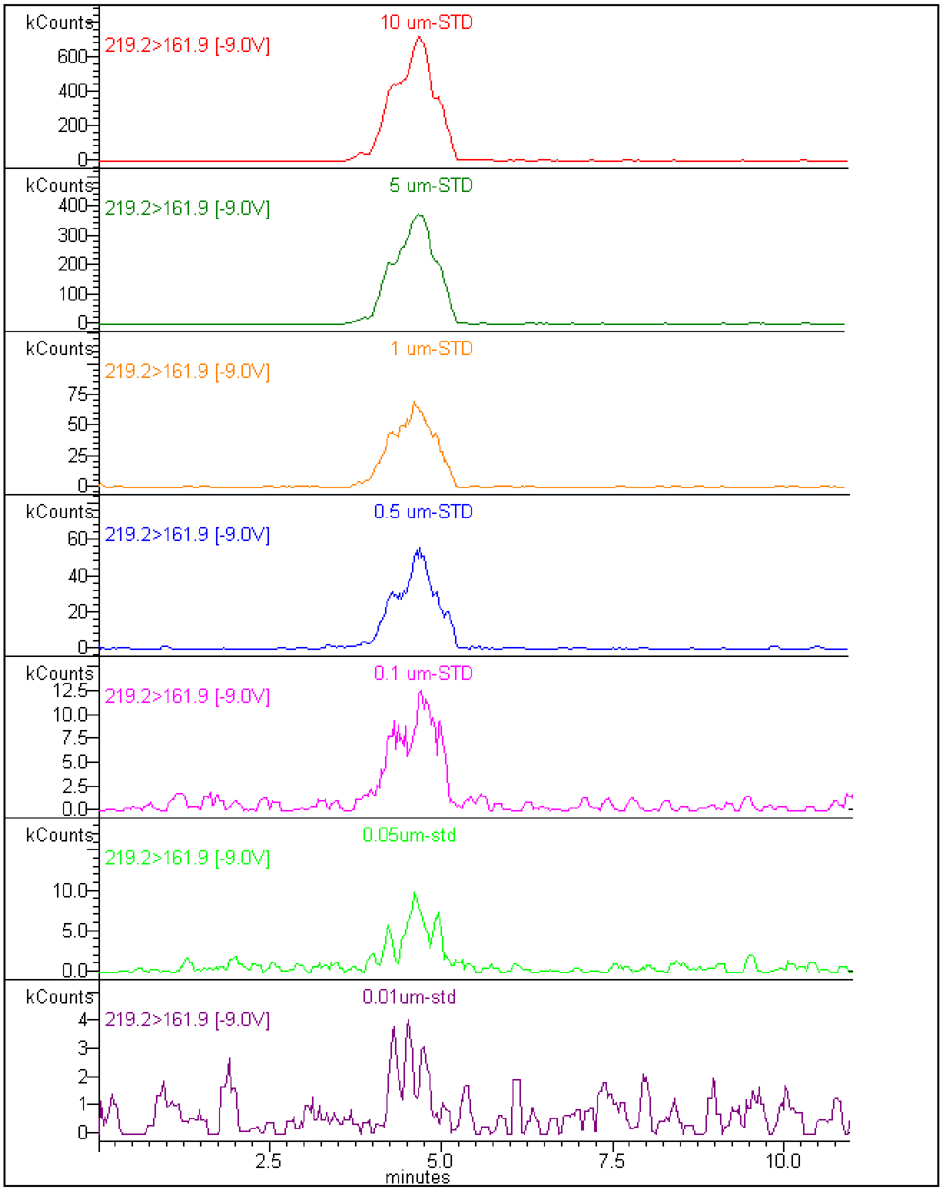
Carbamazepine



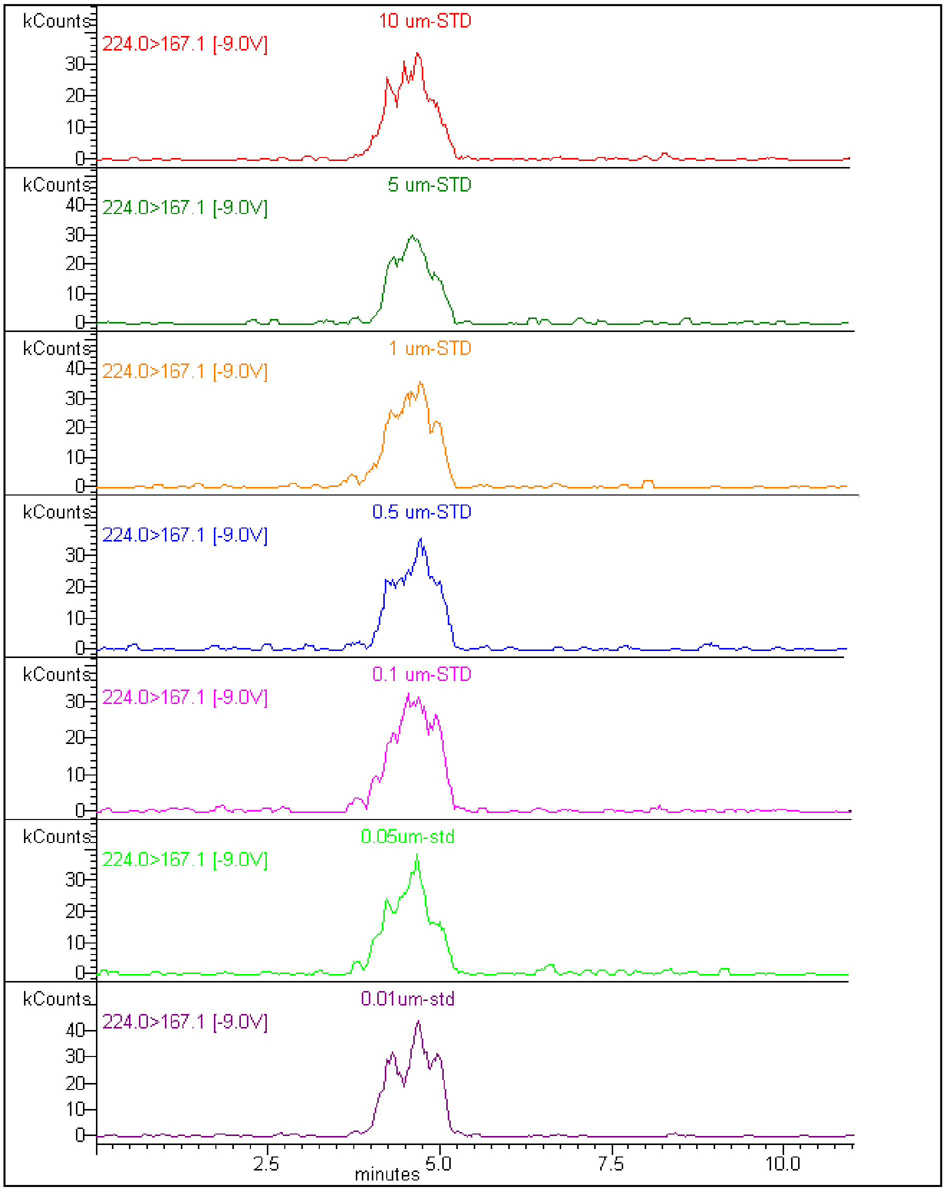
Carbamazepine - d_{10}



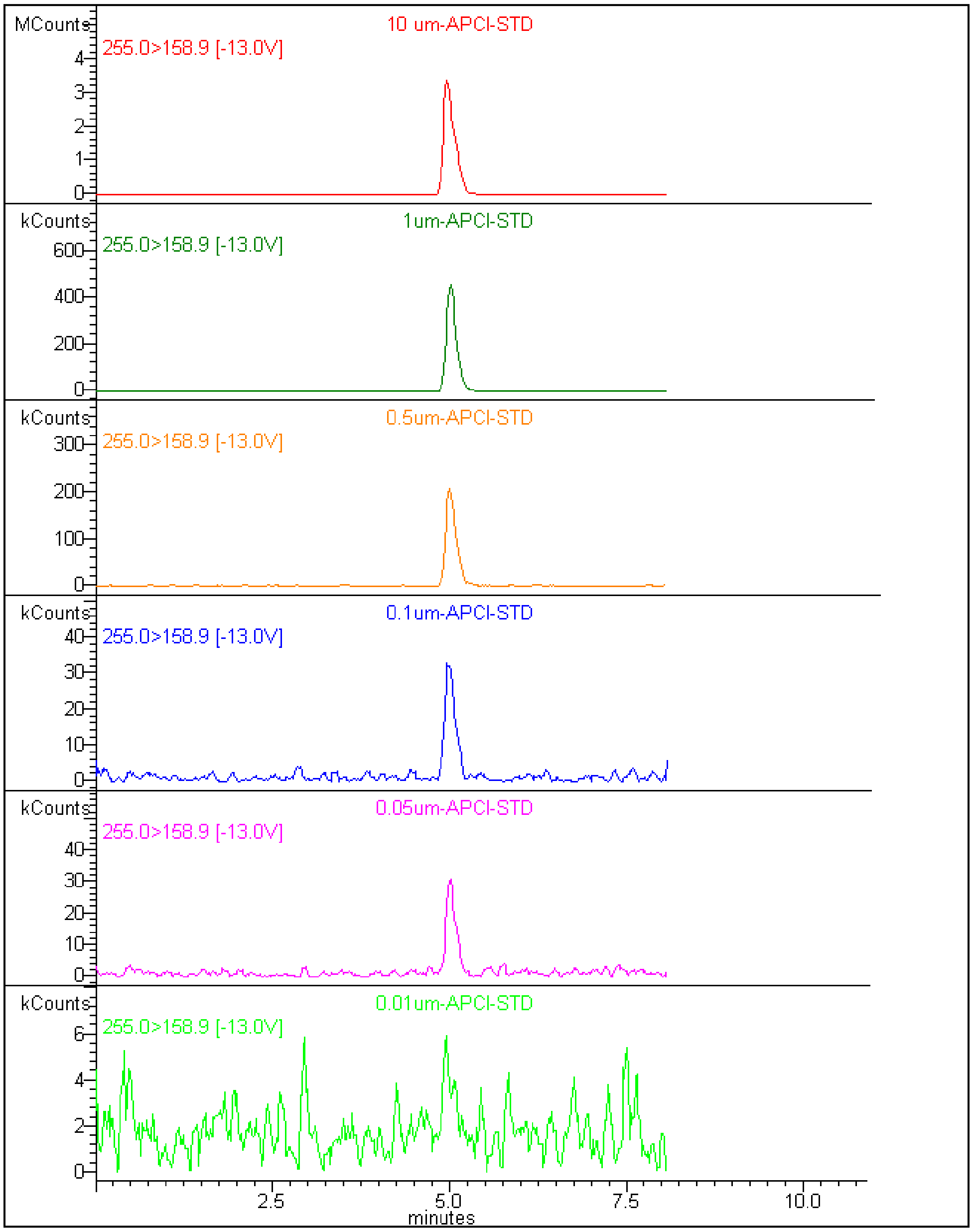
Primidone


kCounts
10 um-STD
219.2>161.9 [-9.0V]
600
400
200
0
kCounts
5 um-STD
219.2>161.9 [-9.0V]
400
300
200
100
0
kCounts
1 um-STD
219.2>161.9 [-9.0V]
75
50
25
0
kCounts
0.5 um-STD
219.2>161.9 [-9.0V]
60
40
20
0
kCounts
0.1 um-STD
219.2>161.9 [-9.0V]
12.5
10.0
7.5
5.0
2.5
0.0
kCounts
0.05um-std
219.2>161.9 [-9.0V]
10.0
5.0
0.0
kCounts
0.01um-std
219.2>161.9 [-9.0V]
4
3
2
1
0
2.5
5.0
7.5
10.0
minutes

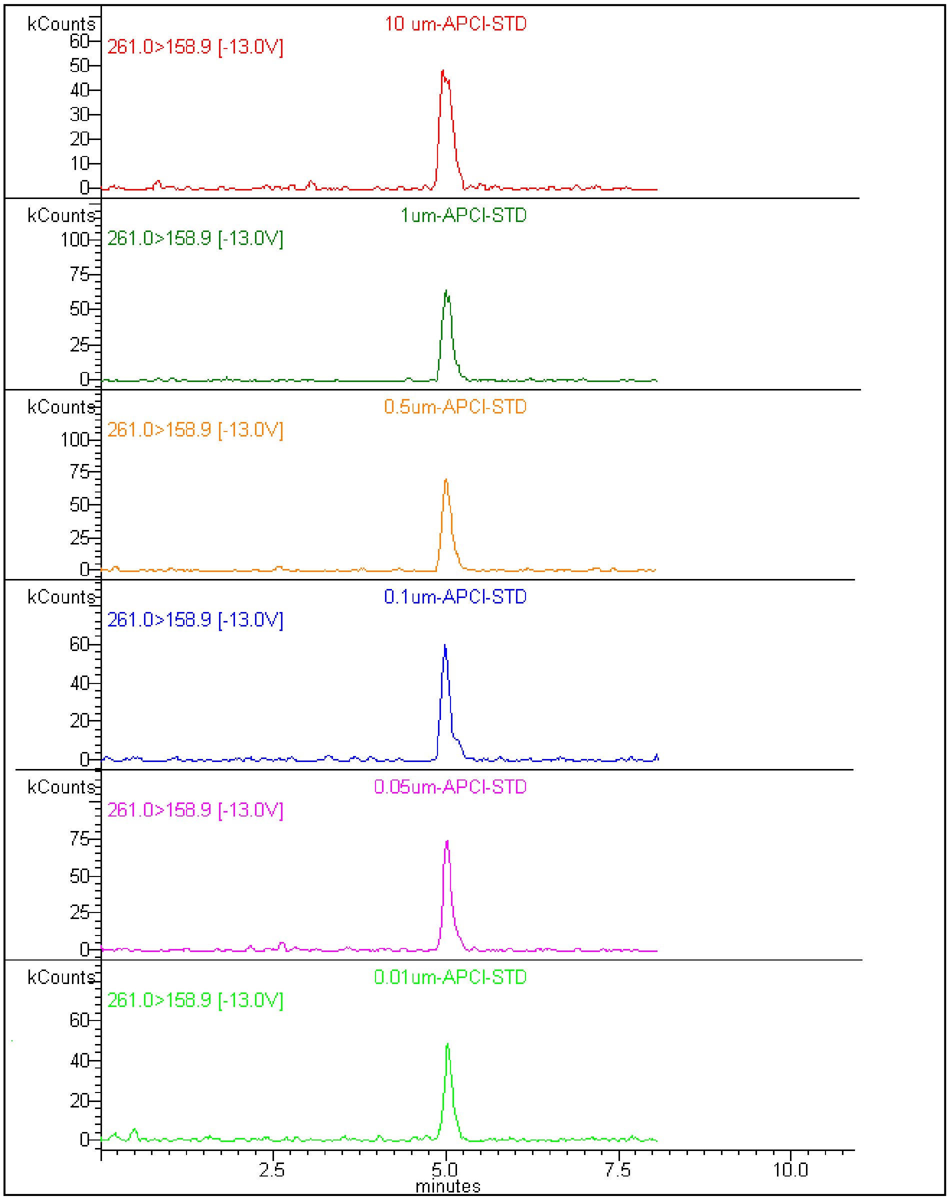
Primidone - d_5



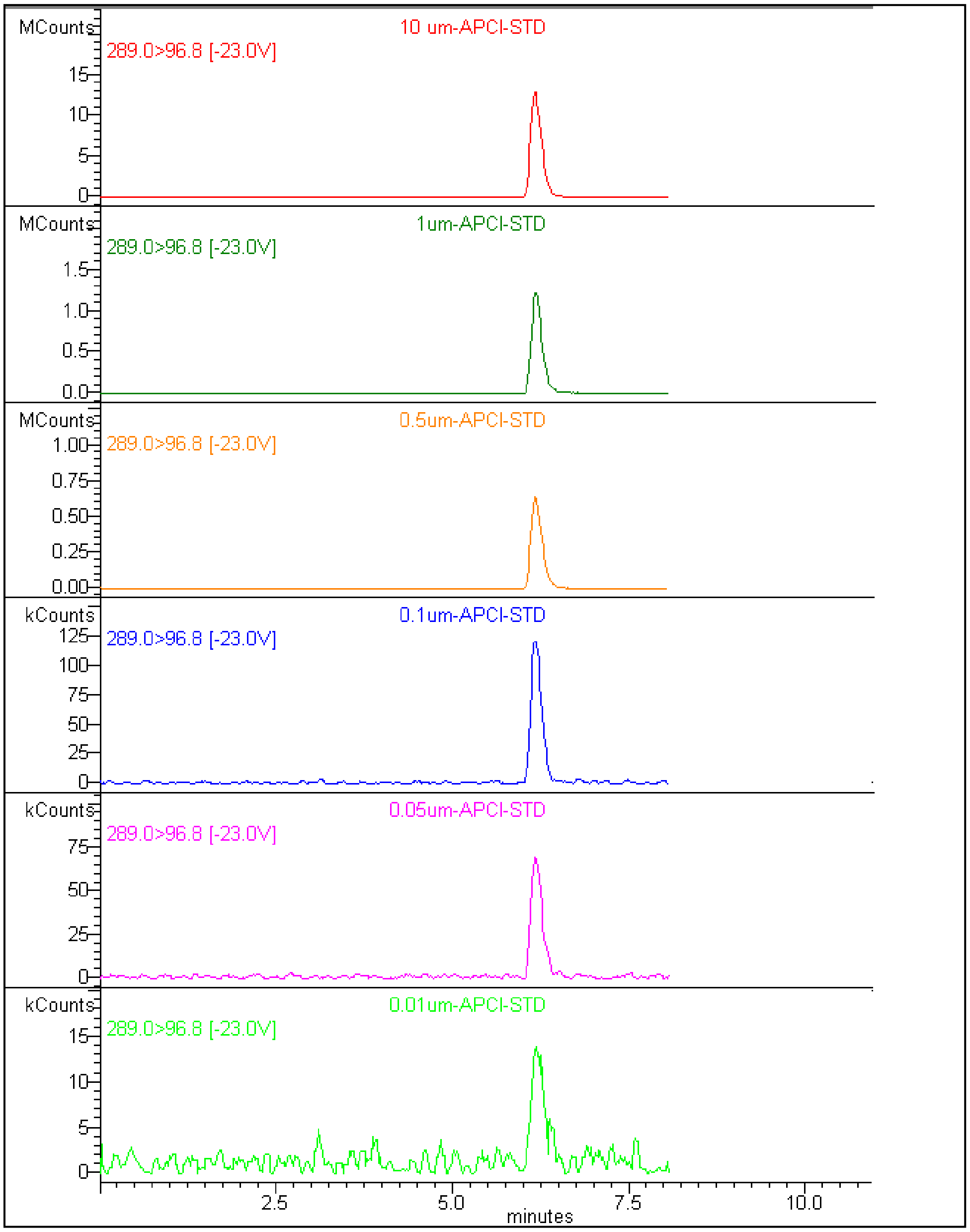
Estradiol (E2)



Estradiol - d_6



Testosterone



Testosterone - d_6