

UC Berkeley

UC Berkeley Electronic Theses and Dissertations

Title

Treatment of Trace Organic Contaminants and Nutrients in Open-Water Unit Process Wetlands

Permalink

<https://escholarship.org/uc/item/560472zt>

Author

Jasper, Justin Thomas

Publication Date

2014

Peer reviewed|Thesis/dissertation

Treatment of Trace Organic Contaminants and Nutrients in Open-Water Unit Process Wetlands

By

Justin Thomas Jasper

A dissertation submitted in partial satisfaction of
the requirements for the degree of
Doctor of Philosophy
in
Engineering - Civil and Environmental Engineering
in the
Graduate Division
of the
University of California, Berkeley

Committee in charge:

Professor David L. Sedlak, Chair

Professor Kara L. Nelson

Professor John Coates

Fall 2014

Treatment of Trace Organic Contaminants and Nutrients in Open-Water Unit Process Wetlands

© 2014

Justin Thomas Jasper

Abstract

Treatment of Trace Organic Contaminants and Nutrients in Open-Water Unit Process Wetlands

by

Justin Thomas Jasper

Doctor of Philosophy in Engineering - Civil and Environmental Engineering

University of California, Berkeley

Professor David L. Sedlak, Chair

Treatment wetlands are becoming an increasing popular approach for nutrient removal from municipal wastewater effluent due to their low cost and energy requirements, as well as the ancillary benefits they provide. Recently, they have also been considered as a means of removing wastewater-derived trace organic contaminants. Initial studies of the removal of trace organic contaminants in treatment wetlands indicate that removal is often insignificant and is highly variable among systems. Efforts to improve wetland treatment efficiency have been hampered by a limited understanding of trace organic contaminant removal mechanisms. In this research, a novel type of wetland, consisting of a shallow cell lined with geotextile fabric to prevent the growth of emergent macrophytes, is considered for the removal of wastewater-derived trace organic contaminants and nutrients. When combined with other types of wetland cells in a unit process fashion (i.e., wetland cells designed to remove specific contaminants are arranged in series), open-water unit process wetlands may provide a basis for using treatment wetlands to remove a wide range of trace organic contaminants from municipal wastewater effluent.

To understand how open-water treatment wetlands could be integrated with other types of wetland cells in unit process wetlands, the treatment wetland literature was critically reviewed (Chapter 2). Removal mechanisms of trace organic contaminants and pathogens in treatment wetlands were considered, including sorption, biotransformation, photolysis, sedimentation, predation, and photoinactivation. Methods of enhancing these mechanisms in unit process wetland cells were also identified, both in commonly employed vegetated cells and through the development of novel wetland configurations, such as open-water cells. To further optimize unit process wetlands, the arrangement of wetland cells was evaluated. The application of the unit process concept to a wide range of wastewater contaminants has the potential to make treatment wetlands a more attractive component of urban water infrastructure.

To assess the ability of open-water cells to exploit sunlight photolysis to remove trace organic contaminants from municipal wastewater effluent, a photochemical model was calibrated using measured photolysis rates for atenolol, carbamazepine, propranolol, and sulfamethoxazole in wetland water under representative conditions (Chapter 3). Contaminant transformation by hydroxyl radical and carbonate radical were predicted from steady-state radical concentrations

measured at pH values between 8 and 10. Direct photolysis rates and the effects of light screening by dissolved organic matter on photolysis rates were estimated using solar irradiance data, contaminant quantum yields, and light screening factors. The model was applied to predict the land area required to achieve 90% removal of a suite of wastewater-derived organic contaminants by sunlight-induced reactions under a variety of conditions. Results suggest that during summer, open-water cells that receive a million gallons per day of nitrified wastewater effluent can remove 90% of most compounds in an area comparable to existing full-scale wetland systems.

The bottoms of open-water wetland cells are rapidly colonized by a biomat consisting of an assemblage of photosynthetic and heterotrophic microorganisms. To assess the contribution of biotransformation in this system to the overall attenuation of trace organic contaminants, transformation rates of test compounds measured in microcosms were compared with attenuation rates measured in a pilot-scale system (Chapter 4). Biotransformation was the dominant removal mechanism in the pilot-scale system for atenolol, metoprolol, and trimethoprim, while sulfamethoxazole and propranolol were attenuated mainly via photolysis. In microcosm experiments, biotransformation rates increased for metoprolol and propranolol when algal photosynthesis was supported by irradiation with visible light. Biotransformation rates increased for trimethoprim and sulfamethoxazole in the dark, when microbial respiration depleted dissolved oxygen concentrations within the biomat. These observations are consistent with previous studies in wastewater treatment plants and wetlands at different dissolved oxygen concentrations. During summer, atenolol, metoprolol, and propranolol were rapidly attenuated in the pilot-scale system ($t_{1/2} < 0.5$ d), trimethoprim and sulfamethoxazole were transformed more slowly ($t_{1/2} \approx 1.5\text{--}2$ d), and carbamazepine was recalcitrant ($t_{1/2} > 30$ d). The combination of biotransformation and photolysis resulted in overall transformation rates that were 10 to 100 times faster than those previously measured in vegetated wetlands.

In addition to removing trace organic contaminants, the diffuse biomat formed on the bottom of open-water wetland cells provides conditions conducive to NO_3^- removal via microbial denitrification, as well as anaerobic ammonium oxidation (anammox). To assess this process, nitrogen cycling was evaluated over a 3-year period in an open-water wetland cell (Chapter 5). Approximately two-thirds of the NO_3^- entering the cell was removed on an annual basis. Microcosm studies indicated that NO_3^- removal was mainly attributable to denitrification within the diffuse biomat (i.e., $80 \pm 20\%$), with accretion of assimilated nitrogen accounting for less than 3% of the NO_3^- removed. The importance of denitrification to NO_3^- removal was supported by the presence of denitrifying genes (*nirS* and *nirK*) within the biomat. While modest when compared to the presence of denitrifying genes, the anammox-specific gene hydrazine synthase (*hzs*) was detected at higher concentrations near the biomat bottom. This observation, along with the simultaneous presence of ammonium and nitrate in the biomat, suggested that anammox may have been responsible for some of the NO_3^- removal. The annual temperature-corrected areal first-order NO_3^- removal rate ($k_{20} = 59.4 \pm 6.2 \text{ m yr}^{-1}$) was higher than values reported for more than 75% of vegetated wetlands treating effluent where NO_3^- served as the main nitrogen species (e.g., nitrified secondary wastewater effluent and agricultural runoff). Inclusion of shallow, open-water cells in unit-process wetland systems has the potential to provide simultaneous removal of trace organic contaminants (Chapters 3 and 4) and pathogens, in addition to NO_3^- , in land areas similar to those occupied by existing full-scale treatment wetlands.

TABLE OF CONTENTS

TABLE OF CONTENTS	II
LIST OF FIGURES	IV
LIST OF TABLES	VI
ACKNOWLEDGMENTS	VII
CHAPTER 1. INTRODUCTION	1
1.1 WASTEWATER-DERIVED TRACE ORGANIC CONTAMINANTS	2
1.2 TREATMENT WETLANDS	5
1.3 MOTIVATION AND RESEARCH OBJECTIVES.....	6
1.3.1 <i>Motivation</i>	6
1.3.2 <i>Objective 1: Review of Trace Organic Contaminant Removal in Treatment Wetlands</i>	7
1.3.3 <i>Objective 2: Photolysis of Trace Organic Contaminants in Open-Water Cells</i>	8
1.3.4 <i>Objective 3: Biotransformation of Trace Organic Contaminants in Open-Water Cells</i>	8
1.3.5 <i>Objective 4: Evaluate Nitrate Removal in Open-Water Wetlands</i>	8
CHAPTER 2. UNIT PROCESS WETLANDS FOR REMOVAL OF TRACE ORGANIC CONTAMINANTS AND PATHOGENS FROM MUNICIPAL WASTEWATER EFFLUENTS.	9
2.1 BACKGROUND.....	10
2.2 HYDRAULICS OF SURFACE FLOW WETLANDS	12
2.3 CONTAMINANTS OF CONCERN	13
2.3.1 <i>Trace Organic Contaminants</i>	13
2.3.2 <i>Waterborne Pathogens</i>	14
2.4 REMOVAL MECHANISMS OF TRACE ORGANIC CONTAMINANTS	17
2.4.1 <i>Sorption</i>	17
2.4.2 <i>Biotransformation</i>	18
2.4.3 <i>Photolysis</i>	20
2.5 REMOVAL OF WATERBORNE PATHOGENS	21
2.5.1 <i>Attachment and Sedimentation</i>	21
2.5.2 <i>Predation</i>	21
2.5.3 <i>Photoinactivation</i>	22
2.6 NOVEL UNIT PROCESS WETLANDS FOR REMOVAL OF TRACE ORGANIC CONTAMINANTS AND PATHOGENS	23
2.6.1 <i>Shallow, Open-Water Cells</i>	24
2.6.2 <i>Macrophyte-Dominated Wetland Cells</i>	26
2.6.3 <i>Bivalve Filtration Wetland Cells</i>	27
2.7 CONCLUSIONS	29
CHAPTER 3. PHOTOTRANSFORMATION OF WASTEWATER-DERIVED TRACE ORGANIC CONTAMINANTS IN OPEN-WATER UNIT PROCESS TREATMENT WETLANDS.....	30
3.1 INTRODUCTION	31
3.2 PHOTOLYSIS MODEL	31
3.3 MATERIALS AND METHODS	40
3.3.1 <i>Materials</i>	40
3.3.2 <i>Wetland Water and Conditions</i>	40
3.3.3 <i>Reaction Rate Constants for Carbonate Radical</i>	43
3.3.4 <i>Reaction Rate Constants for Hydroxyl Radical and Carbonate Radical with DOM</i>	43
3.3.5 <i>Photolysis of Representative Compounds in Wetland Water</i>	44
3.3.6 <i>Predicting Photolysis Rates in Unit Process Wetlands</i>	44
3.3.7 <i>Analytical methods</i>	45
3.4 RESULTS AND DISCUSSION	47
3.4.1 <i>Carbonate Radical Reactions with Contaminants</i>	47

3.4.2 Reaction of Hydroxyl Radical and Carbonate Radical with Wetland DOM	49
3.4.3 Photolysis of Test Compounds in Wetland Water.....	52
3.4.4 Photolysis Model Validation and Predictions	54
3.4.5 Estimation of Wetland Area Necessary for Contaminant Photolysis.....	55
3.4.6 Comparison of Photolysis Cells and Existing Wetlands.....	60
3.4.7 Application to Wetland Design.....	61
CHAPTER 4. BIOTRANSFORMATION OF TRACE ORGANIC CONTAMINANTS IN OPEN-WATER UNIT PROCESS TREATMENT WETLANDS.....	62
4.1 INTRODUCTION	63
4.2 MATERIALS AND METHODS	64
4.2.1 Materials.....	64
4.2.2 Monitoring of the Pilot-Scale Wetland.	64
4.2.3 Microcosms.....	69
4.2.4 Determination of Biotransformation Products	70
4.2.5 Photolysis Rate Prediction.....	71
4.2.6 Correcting for Evaporation in the Pilot-Scale Cell.....	71
4.2.7 Microbial Community Characterization.....	73
4.2.8 Analytical Methods	74
4.3 RESULTS AND DISCUSSION	74
4.3.1 Characterization of Wetland Biomat.....	74
4.3.2 Biotransformation in Microcosms	78
4.3.3 Biotransformation Products	82
4.3.4 Compound Attenuation in the Pilot-Scale Open-Water Wetland.....	84
4.3.5 Evaluation of Attenuation Mechanisms in the Pilot-Scale Open-Water Wetland.....	87
4.3.6 Application to Full-Scale Wetland Design.....	92
CHAPTER 5. NITROGEN REMOVAL IN SHALLOW, OPEN-WATER TREATMENT WETLANDS	95
5.1 INTRODUCTION	96
5.2 MATERIALS AND METHODS	97
5.2.1 Materials.....	97
5.2.2 Pilot-Scale Wetland Monitoring	97
5.2.3 Primary Productivity Calculations.....	98
5.2.4 Acetylene Block Microcosms	98
5.2.5 Nutrient Mass Balance.....	98
5.2.6 Measurement of Gene Abundances.....	99
5.2.7 Analytical Methods	99
5.3 RESULTS AND DISCUSSION	100
5.3.1 NO ₃ ⁻ Removal Rates.....	100
5.3.2 NO ₃ ⁻ Removal Mechanisms.....	110
5.3.3 Implications for Design of Open-Water Cells.....	119
CHAPTER 6. CONCLUSIONS.....	121
6.1 SUMMARY	122
6.2 REMOVAL OF TRACE ORGANIC CONTAMINANTS IN OPEN-WATER WETLANDS	122
6.3 REMOVAL OF NITRATE IN OPEN-WATER WETLANDS	123
6.4 FUTURE RESEARCH	124
REFERENCES.....	127

LIST OF FIGURES

Figure 1.1. Box plot comparing trace organic contaminant removal efficiencies	4
Figure 1.2: Comparison of trace organic contaminant removal efficiencies in wetlands and WWTPs.....	6
Figure 2.1: Averages pharmaceutical and personal care product removal efficiencies in treatment wetlands...	15
Figure 2.2: Averages pathogen removal efficiencies in surface flow wetlands.....	16
Figure 2.3: Example of unit process wetland treatment train	24
Figure 2.4: Discovery Bay pilot-scale open-water wetland cell.	25
Figure 2.5: Schematic of bivalve uptake and removal mechanisms of organic contaminants and pathogens....	28
Figure 3.1: Predicted irradiance under clear skies at sea level ($Z(24\text{ h}, \lambda)$).....	33
Figure 3.2: Molar absorption coefficient (ϵ) of test compounds as a function of wavelength.....	34
Figure 3.3: Absorbance at 310 nm ($\alpha(310)$) of wetland water.....	35
Figure 3.4: Typical absorbance of wetland water with 8 mg L ⁻¹ -C DOM	41
Figure 3.5: Inlet and outlet pH values in the shallow, open-water wetland cell	42
Figure 3.6: Typical results from competition kinetics experiment used to measure $k \cdot \text{CO}_3^-$, cont.....	46
Figure 3.7. Measured [$\cdot\text{OH}$] _{ss} and [$\cdot\text{CO}_3^-$] _{ss} in irradiated Discovery Bay wetland water	49
Figure 3.8: Pseudo first-order photolysis rates for test compounds in amended wetland waters	50
Figure 3.9: Pseudo first-order photolysis rates for test compounds in buffered wetland waters	51
Figure 3.10: Predicted and measured pseudo first-order photolysis rates for test compounds	52
Figure 3.11: Predicted contributions of photolysis mechanisms to k_{phot}	53
Figure 3.12: Area predicted to provide 90% removal of contaminants	57
Figure 3.13: Area needed to provide 1-log removal of contaminants from 1 MGD with different [NO_3^-]	58
Figure 4.1: Photograph of the pilot-scale open-water unit process cell.....	64
Figure 4.2: Breakthrough curve of rhodamine-WT in a tracer test in the pilot-scale open-water cell.....	66
Figure 4.3: Spectrum of the red lamp used in microcosm experiments.....	67
Figure 4.4: Percent mass recovery of test compounds amended to wetland microcosms.....	68
Figure 4.5: Average Cl^- concentrations throughout pilot-scale open-water cell.....	72
Figure 4.6: Dissolved oxygen profiles in microcosms and at the pilot-scale open-water cell.....	75
Figure 4.7: Fluorescent imagery, electron microscopy, and illumina sequencing of microcosm biomass	76
Figure 4.8: Scanning electron microscopy of wetland biomat.....	77

Figure 4.9: Fraction of test compound masses remaining in microcosms	79
Figure 4.10: Formation of the biotransformation product metoprolol acid in amended microcosms.....	83
Figure 4.11: Removal of test compounds in pilot-scale open-water wetland	85
Figure 4.12: Seasonal pseudo first-order attenuation rates of test compounds in the open-water cell	86
Figure 4.13: Comparison of measured or predicted removal rate constants of test compounds.....	89
Figure 4.14: Comparison of measured or predicted removal of test compounds corrected for evaporation. ...	91
Figure 4.15: Area predicted to provide 90% removal of contaminants in open-water treatment cells	94
Figure 5.1: Percent evaporation between inlet and outlet of pilot-scale wetland cell.....	101
Figure 5.2: NO_3^- removal along flow-path in Discovery Bay pilot-scale open-water wetland cell.....	102
Figure 5.3: Average NO_3^- removal rates in the Discovery Bay pilot-scale open-water wetland by season.....	103
Figure 5.4: Average inlet and outlet concentrations of nitrogen species	104
Figure 5.5: Natural log of first-order NO_3^- removal rates versus average wetland water temperature	106
Figure 5.6: Typical profile of aqueous species in middle of Discovery Bay open-water pilot scale wetland .	107
Figure 5.7: Organic carbon, nitrogen, and phosphorus mass balance.....	108
Figure 5.8: Conversion of NO_3^- to N_2O in anoxic microcosms amended with acetylene.....	109
Figure 5.9: Abundances of genes involved in denitrification (<i>nirK</i> and <i>nirS</i>) and anammox (<i>hzs</i>).....	112
Figure 5.10: Example of dissolved oxygen profiles used to calculate primary productivity and respiration ...	113
Figure 5.11: Average biomat thickness measured throughout the pilot-scale open-water wetland	114
Figure 5.12: Percent phosphorus, nitrogen, and organic content by mass in wetland biomat	116
Figure 5.13: Concentrations of nitrogen species in amended anoxic microcosms	117
Figure 5.14: Mean area predicted to provide 90% removal of NO_3^- in open-water and vegetated wetlands ...	120

LIST OF TABLES

Table 1.1: Typical Concentrations of Trace Organic Contaminants in WWTP Effluents.....	2
Table 1.2: Potential Ecological Risk of Pharmaceuticals in Environmental Compartments	3
Table 2.1: Examples of Large, Full-Scale Treatment Wetlands in the United States.....	11
Table 3.1: Compound Properties Used in Photolysis Model	36
Table 3.2: Parameters Used to Calculate Wetland Water Absorbance.....	37
Table 3.3: Conditions Employed in Photolysis Model	39
Table 3.4: Second-Order Rate Constants for the Reaction of $\cdot\text{CO}_3^-$ with Test Compounds	47
Table 4.1: Compound-Specific Mass Spectrometry Parameters.....	70
Table 4.2: Test Compound First-Order Transformation Rates	81
Table 4.3: Average Test Compound Concentrations of Trace Organic Compounds in Open-Water Cell	82
Table 4.4: Wetland Temperatures.....	87
Table 4.5: Sorbed Test Compound Concentrations in Open-Water Wetland	90
Table 5.1: QPCR Primers and Thermal Profiles.....	100
Table 5.2: Typical Open-Water Cell Influent Water Quality Parameters.....	105

ACKNOWLEDGMENTS

David Sedlak was an excellent thesis advisor, allowing me to explore my own ideas while ensuring that my work was relevant and rigorous. Kara Nelson, Jim Hunt, Josh Sharpe, John Coates, Gary Sposito, and Lisa Alvarez-Cohen all provided valuable comments and suggestions on my work throughout my time at Berkeley.

This project would not have been possible without Alex Horne's creative genius, who designed the Discovery Bay wetlands and contributed to my work throughout the project. Virgil Koehne, the town manager of Discovery Bay, advocated for the use of treatment wetlands to provide sustainable wastewater treatment, in addition to keeping the wetlands running and in good shape.

I learned a great deal from fellow graduate students and post-doctoral researchers, including but not limited to, Anh Pham, Scott Mansell, Eva Agus, Patrick Ulrich, Erika Houtz, Amanda Greenleaf, Ekrem Karpuzcu, Haizhou Liu, Christy Keenan Remucal, Joonseon Jeong, Jannis Wenk, Tom Bruton, Janel Grebel, Tom Hennebel, Sun Bohit, James Barazesh, Jean Van Buren, Joe Charbonnet, Carsten Prasse, Katie Harding, Andy Torkelson, Shan Yi, Wei Yi, Will Tarpeh, and Aidan Cecchetti. Mi Nguyen and Samantha Bear deserve a special shout out for their long hours spent collecting field samples and maintaining the Discovery Bay wetlands. Zack Jones contributed his insight and ideas into microbiological aspects of all of my work.

My parents, sister, and friends have encouraged me constantly, and I am forever grateful to them.

Marisa, my wife, has been a source of inspiration since the day I met her. I am thankful to have had her support during my time at Berkeley and to have it for the rest of our life together.

CHAPTER 1. Introduction

1.1 Wastewater-Derived Trace Organic Contaminants

Municipal wastewater effluent contains thousands of trace organic contaminants at concentrations ranging from 10^{-9} to 10^{-6} grams-per-liter (Table 1.1). Many of these compounds are classified as pharmaceuticals and personal care products, a family of compounds that can elicit biological responses at relatively low concentrations. During conventional activated sludge treatment, these compounds are typically only partially removed via sorption and biotransformation, due to their relatively low affinity for organic-rich particles and resistance to biotransformation (Daughton and Ternes, 1999; Ternes *et al.*, 2004a). Due to the limited removal of trace organic contaminants during wastewater treatment, wastewater-derived trace organic contaminants are frequently detected in surface waters that receive wastewater effluent (Kolpin *et al.*, 2002; Kim *et al.*, 2007; Kasprzyk-Hordern *et al.*, 2008).

Table 1.1: Typical Concentrations of Selected Trace Organic Contaminants in Wastewater Treatment Plant Effluents^a

Compound name	Concentration ($\mu\text{g L}^{-1}$)	Reference
Benzafibrate	0.02-0.07 <0.25-4.6	(Metcalf <i>et al.</i> , 2003) (Thomas, 1998)
Diclofenac	0.289 0.03-0.07 0.01-0.56	(Roberts and Thomas, 2006) (Metcalf <i>et al.</i> , 2003) (Heberer <i>et al.</i> , 2001)
Ibuprofen	2.97 0.03 <0.005-0.01 <0.05-1.43 0.002-0.081 0.22 \pm 0.15	(Roberts and Thomas, 2006) (Lin <i>et al.</i> , 2005) (Metcalf <i>et al.</i> , 2003) (Gans <i>et al.</i> , 2002) (Buser <i>et al.</i> , 1999) (Khan and Ongerth, 2002)
Naproxen	0.017 0.02-0.30 0.35 \pm 0.12 <LOD-0.12 >0.05-0.52	(Lin <i>et al.</i> , 2005) (Metcalf <i>et al.</i> , 2003) (Khan and Ongerth, 2002) (Heberer <i>et al.</i> , 2001) (Thomas, 1998)
Metoprolol	>0.025-2.2	(Hirsch <i>et al.</i> , 1996; Thomas, 1998)
Carbamazepine	0.042 0.28-1.11 >0.05-6.3	(Lin <i>et al.</i> , 2005) (Metcalf <i>et al.</i> , 2003) (Thomas, 1998)
Triclosan	0.01-0.02 0.2-2.7	(Boyd <i>et al.</i> , 2003) (McAvoy <i>et al.</i> , 2002)

^aAdapted from Nikolaou *et al.* (2007)

Acute toxicity tests often fail to detect the subtle effects of trace organic contaminants on aquatic organisms (Daughton and Ternes, 1999; Webb, 2004). More sensitive approaches, while applied less frequently for toxicity assessments, suggest that certain wastewater-derived trace organic contaminants can detrimentally affect aquatic organisms at environmentally-relevant concentrations. For example, steroid hormones such as ethinyl estradiol, one of the active ingredients in birth control, cause feminization of certain fish species at nanogram-per-

liter concentrations (Purdom *et al.*, 1994). Exposure to microgram-per-liter concentrations of pharmaceuticals, such as metoprolol and carbamazepine, can result in ultrastructural damage to fish organs (Triebkorn *et al.*, 2007). Pharmaceuticals may also cause more subtle effects, such as altering fish feeding and social behavior (Brodin *et al.*, 2013). In the environment, trace organic contaminants are present as a mixture of compounds, which may have an additive effect on aquatic organisms (Schnell *et al.*, 2009). Environmental risk assessments in rivers generally predict significant risk to aquatic organisms downstream of wastewater treatment plants (e.g., Table 1.2), supporting the assertion that trace organic contaminants in wastewater effluent are detrimental to aquatic ecosystems (Jones *et al.*, 2002; Hernando *et al.*, 2006; Ginebreda *et al.*, 2010).

Table 1.2: Potential Ecological Risk of Pharmaceuticals in Environmental Compartments^a

Therapeutic Group	Pharmaceutical	<u>Ecological Risk</u> ^b		
		Wastewater	Surface Water	Sediment
Antibiotics	Erythromycin	High		Medium Medium
	Oxytetracycline			
	Flumequine			
Analgesics	Ibuprofen	High	High	
	Diclofenac	High	High	
	Naproxen	High	High	
	Ketoprofen	High	High	
Lipid	Gemfibrozil	High		
	Clofibrilic acid	High		
β-Blockers	Propranolol	High	Medium	
	Metoprolol	High		
Antiepileptics	Carbamazepine	High	High	

^aAdapted from Hernando *et al.* (2006). ^bEcological risk is calculated by the ratio of the measured environmental concentration of a pharmaceutical to its predicted no-effect concentration. Compounds with ratios of <0.1 are considered low risk; ratios of 0.1-1 are considered medium risk; ratios >1 are considered high risk.

Humans may be exposed to wastewater-derived trace organic contaminants via consumption of drinking water from a source that is subjected to upstream effluent discharge or fish from effluent-impacted surface waters. Thus far, risk assessments have suggested that human pharmaceuticals in drinking waters pose no appreciable health risks to humans (Schwab *et al.*, 2005; Cunningham *et al.*, 2009). However, other wastewater-derived trace organic compounds could be present at concentrations that pose health risks. For example, the carcinogen nitrosodimethylamine (NDMA) is often present in wastewater effluent at concentrations that exceed health guidelines for drinking water (Mitch *et al.*, 2003). The presence of wastewater-derived trace organic contaminants is a particular concern for potable water reuse projects, because the presence of these compounds can cast doubt on their safety (Snyder *et al.*, 2003; Toze, 2006; National Research Council, 2012).

Conventional secondary wastewater treatment (i.e., activated sludge) typically results in less than 50% removal of trace organic contaminants (Figure 1.1). A variety of methods designed to be applied after conventional municipal wastewater treatment can remove wastewater-derived trace organic contaminants, but these treatments are often energy intensive and produce significant amounts of greenhouse gases (Jones *et al.*, 2007). In addition, they may generate disinfection by-products or solid waste that requires disposal. For example, ozone doses similar to those used for disinfection can remove more than 80% of most trace organic contaminants from wastewater effluent (Ternes *et al.*, 2003; Snyder *et al.*, 2006; Hollender *et al.*, 2009). However, the use of ozone requires expensive upgrades to wastewater treatment plant infrastructure, and may result in the formation of by-products such as bromate (von Gunten and Hoigne, 1994). Activated carbon can also remove many trace organic contaminants from wastewater effluent (Snyder *et al.*, 2007), but the sorption capacity of activated carbon is quickly saturated, requiring disposal or energy for regeneration (Suárez *et al.*, 2008). Treatment wetlands have been suggested as an alternative approach for removing trace organic contaminants from wastewater effluent, due to their low operating costs and energy requirements. However, trace organic contaminant removal mechanisms in wetlands are currently not well understood, and most wetlands are not designed to treat trace organic contaminants (see Chapter 2.2).

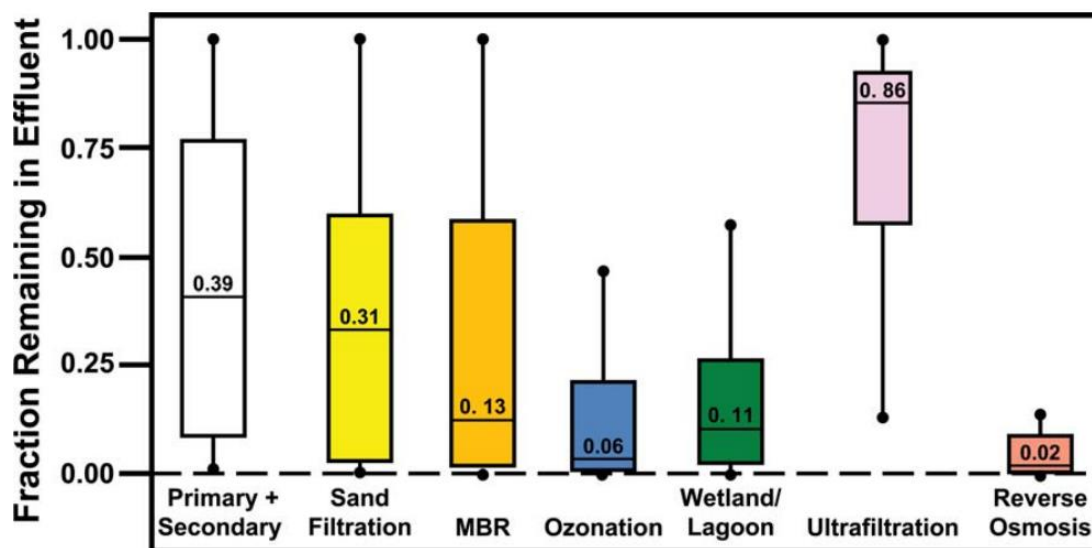


Figure 1.1. Box plot comparing trace organic contaminant removal efficiencies of wastewater treatment technologies (whiskers, 10th-90th percentile; boxes 25th-75th percentile; solid line, median). MBR=Membrane bioreactor. Adapted from Oulton *et al.* (2010).

1.2 Treatment Wetlands

Treatment wetlands have been used to treat wastewater or wastewater effluent for over 50 years (Vymazal, 2010). Due to their long hydraulic residence times (~3-7 days), high productivity, and diverse microbial communities, treatment wetlands can remove a wide range of contaminants from wastewater effluent. Historically, treatment wetlands were used to remove organic matter (i.e., biological oxygen demand), metals, and nutrients from wastewater effluent. Treatment wetlands also offer a number of ancillary benefits including green space for recreational opportunities, wildlife viewing, and habitat creation (Fleming-Singer and Horne, 2006).

Commonly employed wetland designs include free water surface wetlands and subsurface flow wetlands. Subsurface flow wetlands are advantageous in situations in which there is a need to limit access of humans or animals to the water, as when the wastewater has only been partially treated. However, the cost of the additional material necessary to fill the wetland (e.g., gravel) limits the practical size of subsurface flow wetlands. Thus, free-surface flow wetlands are often preferred for large projects (Kadlec, 2009).

Free water surface wetlands are typically planted with emergent macrophytes which provide a carbon source for microbial communities. Macrophyte choice may affect removal rates of certain contaminants. For example, plants with high acid soluble carbon contents may enhance denitrification (e.g., *Typha* sp.) (Bachand and Horne, 2000), while plants rich in lignin may be better suited for sorption of contaminants (e.g., *Scripus* sp.) (Horne and Fleming-Singer, 2005). In addition to macrophyte choice, wetland hydraulics are critical to free water surface wetland treatment efficiency. Preferential flow paths through vegetation can lead to hydraulic short-circuiting, which can drastically reduce wetland performance (See Chapter 2.2).

In the United States, treatment wetlands are commonly employed for the removal of nutrients from wastewater effluent. Nitrate is removed mainly via microbial denitrification in anaerobic zones within wetlands. Phosphate may be removed via sorption, precipitation, or uptake, although in most cases the phosphate removal capacity of a wetland is quickly saturated, and thus wetlands are not typically a sustainable approach for phosphate removal (Vymazal, 2007). Treatment wetlands are also capable of removing many other contaminants from wastewater effluent, including metals via precipitation of metal sulfides (Kadlec and Wallace, 2009), and pathogens (see Chapter 2). In addition to treating wastewater effluent, wetlands are sometimes used to treat primary or secondary wastewater, reducing biological oxygen demand, in addition to removing other contaminants (Solano *et al.*, 2004).

Treatment wetlands have also been considered for the removal of trace organic contaminants from wastewater. Wetlands receiving wastewater effluent have been reported to remove more than 70% of certain trace organic contaminants (Li *et al.*, 2014; Verlicchi and Zambello, 2014). In some situations wetlands can achieve higher trace organic contaminant removal efficiencies than conventional wastewater treatment plants (Figure 1.2). However, trace organic contaminant removal efficiencies differ drastically among wetland systems (see Chapter 2), because wetlands often are not optimized for trace organic contaminant removal. Trace organic contaminant removal in treatment wetlands is typically ascribed to sorption, uptake by plants, biotransformation, and in wetlands with open-water sections, photolysis. However, the

relative importance of these mechanisms is often unknown. Of these mechanisms, only biotransformation and photolysis are sustainable, since sorption and uptake capacities will quickly be saturated if not coupled to biotransformation. Each of these mechanisms is considered in detail in Chapter 2.

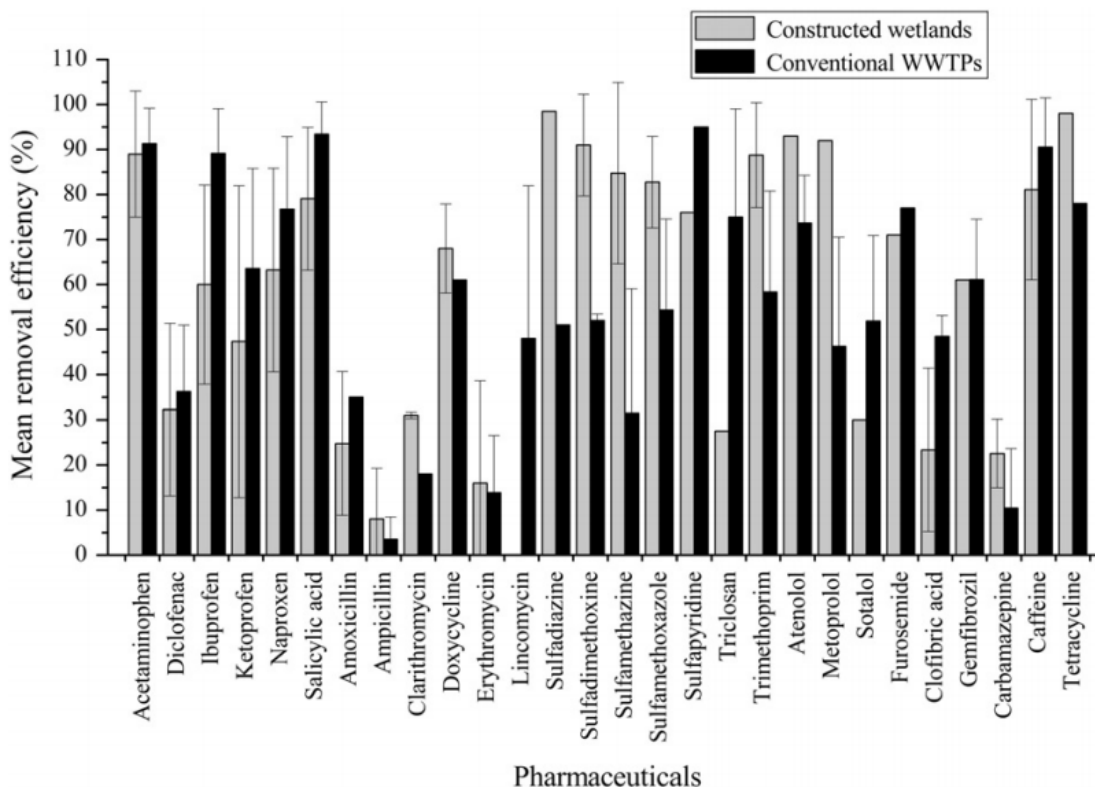


Figure 1.2: Comparison of trace organic contaminant removal efficiencies in treatment wetlands and conventional wastewater treatment plants (WWTPs). Adapted from Li *et al.* (2014).

1.3 Motivation and Research Objectives

1.3.1 Motivation

Treatment wetlands are an attractive option for removing nutrients from wastewater effluent, due to their low cost and energy requirements. In addition, they offer ancillary benefits, such as the creation of wildlife habitat and recreational opportunities. When properly designed, treatment wetlands also may remove wastewater-derived trace organic contaminants. However, wetlands are currently not designed to exploit many potential trace organic contaminant removal mechanisms, such as photolysis and biotransformation.

Photolysis is an important removal mechanism for many trace organic contaminants in lakes and shallow streams. However, in vegetated wetlands, photolysis is ineffective due to shading of the water column by emergent vegetation and deep water. In addition, many trace organic contaminants undergo biotransformation at enhanced rates under aerobic conditions, for example during certain wastewater treatment processes, as compared to under anaerobic

conditions. Vegetated wetlands, however, are typically designed to be dominated by anaerobic zones, which may limit the biotransformation rates of trace organic contaminants.

In order to enhance trace organic contaminant removal, treatment wetlands can be designed as a series of unit process wetland cells, with each cell optimized to remove contaminants via specific mechanisms. In this manner, vegetated wetlands that allow for contaminant removal under anaerobic conditions (e.g., via denitrification) can be put in series with novel wetland cells that enhance other removal mechanisms, such as photolysis and aerobic biotransformation. The resulting unit process wetland can provide more efficient and reliable removal of a wider range of contaminants than a single type of wetland, or than a wetland constructed following “natural design”.

Open-water wetland cells are a novel type of treatment wetland that could provide an effective means of removing trace organic compounds by exploiting photolysis and aerobic biotransformation. These wetland cells consist of shallow basins lined with a geotextile fabric to prevent the growth of emergent macrophytes, allowing sunlight to penetrate the shallow water column. Sunlight transforms trace organic contaminants via photolysis, and it enables the growth of a diffuse photosynthetic biomat on the wetland bottom. Trace organic contaminants can be transformed via biotransformation within the biomat, and transformation rates may be enhanced by the oxic conditions produced by photosynthesis. In addition, nutrients may be removed in the wetland biomat. Incorporation of open-water wetlands into unit process wetlands has the potential to enhance the removal efficiency of trace organic contaminants as well as nutrients in treatment wetlands.

1.3.2 Objective 1: Critically Review Literature on Trace Organic Contaminant Removal in Treatment Wetlands

To provide a foundation for identifying the best approach for removal of trace organic contaminants, a critical review of the literature on treatment wetlands was undertaken. Trace organic contaminants could be broadly categorized as easily removed regardless of treatment wetland design (>60% removal), moderately removed in most studies (40-60% removal), or recalcitrant to removal regardless of wetland design (<40% removal). Possible trace organic contaminant removal mechanisms were evaluated, including sorption, biotransformation, and photolysis. Sorption was found to provide only limited removal of trace organic contaminants, since the sorptive capacity of wetlands is quickly saturated. Biotransformation was determined to be the most important removal mechanism for trace organic contaminants in vegetated wetlands. Nonetheless, removal rates of many contaminants were enhanced under aerobic conditions, as opposed to the anaerobic conditions typical of vegetated wetlands. Due to shading by emergent vegetation, removal of trace organic contaminants and pathogens via reaction with sunlight in vegetated wetlands was found to be insignificant. However, photolysis is known to remove certain photo-labile trace organic contaminants and pathogens in shallow streams or open-water bodies. Based on these conclusions, methods to enhance trace organic contaminant removal efficiencies in wetlands were suggested, including through the development of novel wetland cells, such as open-water cells.

1.3.3 Objective 2: Evaluate Photolysis of Trace Organic Contaminants in Open-Water Wetland Cells

To determine design parameters for open-water cells, photolysis of a suite of trace organic contaminants in open-water wetland cells was evaluated. A model for trace organic contaminant photolysis in wetlands was developed using values from the literature and from laboratory experiments, and this model was validated using a solar simulator. Model parameters, such as wetland depth, pH, dissolved organic carbon content, and nitrate concentration, were then varied to determine the optimal conditions for the removal of trace organic contaminants through photolysis in open-water wetlands. Using this information, the wetland area necessary to achieve efficient removal of trace organic contaminants (i.e., 90% removal from 1 million gallons per day (MGD) of wastewater effluent: A_{90}^1) was estimated and compared to the size of existing full-scale wetlands.

1.3.4 Objective 3: Evaluate Biotransformation of Trace Organic Contaminants in Open-Water Wetland Cells

Trace organic contaminants may be removed by microbial transformation in the diffuse biomat that forms on the bottoms of open-water wetland cells. To evaluate the extent of biotransformation, removal rates of a suite of trace organic contaminants were measured in laboratory microcosms, as well as in a pilot-scale open-water wetland cell. By illuminating microcosms with visible light at wavelengths that did not cause photolysis, it was possible to evaluate the effect of photosynthesis on the biotransformation and sorption of trace organic contaminants. Comparison of removal rates of ambient trace organic contaminants in the pilot-scale wetland to removal rates predicted by microcosm experiments and a photolysis model verified predictions of the removal mechanisms of each test compound. Removal rates in open-water wetlands were compared to rates in vegetated wetland cells, and the area necessary for efficient removal of test compounds (A_{90}^1) was estimated.

1.3.5 Objective 4: Evaluate Nitrate Removal in Open-Water Wetlands

Nitrate removal is one of the primary objectives of many treatment wetlands. Although open-water wetlands were designed to maximize trace organic contaminant and pathogen removal, the removal of nitrate would enable them to be included in unit process wetland systems without sacrificing nitrate removal. To evaluate the potential for nitrate removal in open-water cells, nutrient fluxes were monitored in a pilot-scale system over a 3-year period. Seasonal nitrate removal rates were calculated and their temperature dependence was determined. Microcosm experiments, in addition to mass balances on carbon, nitrogen, and phosphorus, provided a means of calculating the importance of sedimentation, microbial denitrification, and anammox to nitrate removal. The wetland size necessary for efficient wetland removal (A_{90}^1) of nitrate was estimated and compared to A_{90}^1 values for vegetated wetland systems.

CHAPTER 2. Unit process wetlands for removal of trace organic contaminants and pathogens from municipal wastewater effluents.

Reproduced with permission from Jasper, J.T.; Nguyen, M.T.; Jones, Z.L.; Ismail, N. S.; Sedlak, D.L.; Sharp, J.O.; Luthy, R.G.; Horne, A.J.; Nelson, K.L. Unit process wetlands for removal of trace organic contaminants and pathogens from municipal wastewater effluents. *Environ. Eng. Sci.* **2013**, 30, 421-436.

© 2013 Mary Ann Liebert, Inc. publishers.

2.1 Background

Attempts by scientists to estimate the value of ecosystem services provided by natural wetlands rank them among the most valuable land on earth (Costanza *et al.*, 1997). They have been called “nature’s filters”, and the role that natural wetlands play in water purification is part of the justification for their protection and restoration. In an attempt to harness these properties, treatment wetlands have been built for a wide range of applications to improve water quality, including treatment of industrial and municipal wastewater, as well as stormwater, agricultural runoff, and acid mine drainage (EPA, 1993; Vymazal, 2009; Malaviya and Singh, 2012). Wetlands are becoming an increasingly popular option with water agencies because of their low operation cost, energy consumption, and environmental impact (Gearheart, 1999; Fuchs *et al.*, 2011). In addition, wetlands provide ancillary benefits, such as the creation of aesthetically appealing green spaces and wildlife habitat (Fleming-Singer and Horne, 2006). Wetlands specifically designed for treatment of municipal wastewater effluent have been used for at least five decades for the removal of suspended solids, biochemical oxygen demand (BOD), nutrients, metals, and pathogens (Mitsch and Gosselink, 2007; Kadlec and Wallace, 2009; Vymazal, 2010).

Many of the treatment wetlands built in the second half of the 20th century consisted of relatively small plots of land, typically less than 5 hectares (EPA, 2000b; Kadlec, 2012). More recently, the size of treatment wetlands has expanded with systems covering as much as 475 hectares and treating up to $2.5 \times 10^5 \text{ m}^3 \text{d}^{-1}$ (60 MGD) of wastewater effluent, or effluent-dominated river water (Table 2.1). The main purpose of these large wetland systems is typically a combination of nutrient removal and habitat creation. Increasingly, the removal of trace organic contaminants and pathogens is also invoked as a benefit. This new trend, coupled with the continued construction of smaller treatment wetlands, indicates that treatment wetlands are becoming an important part of urban water infrastructure. Despite their increasing popularity, many barriers still prevent them from realizing their full potential for improving water quality and enhancing aquatic habitat.

One of the most significant barriers to the use of treatment wetlands is the difficulty of designing wetlands with predictable performance. Compared to mechanical unit treatment processes, the ecological, transport, and transformation processes occurring in treatment wetlands are even more complex and are not fully understood. For some constituents researchers have made progress understanding the detailed transformation mechanisms, including models that account for the complexity (e.g., Wang and Mitsch, 2000; Howell *et al.*, 2005). However, such complex models cannot be used for design purposes because they are too difficult to parameterize. Nonetheless, the insights provided by mechanistic research can provide the foundation for designing unit process treatment wetlands, with each unit process tailored to the treatment of a specific set of contaminants, by identifying the most important parameters controlling performance. This unit process approach is not meant to undervalue the complexity of wetland ecosystems. Rather, by optimizing specific transformation mechanisms in unit process cells, they can be more easily integrated with other mechanical or natural treatment systems to provide treatment trains with predictable performance.

For example, mechanistic research coupled with studies of full-scale systems has led to robust design approaches for unit process wetlands for denitrification (Kadlec, 2012). Such denitrification wetlands can be used to treat nitrified effluent from mechanical wastewater

treatment plants (e.g., Table 2.1), or they can be staged after shallow aerobic nitrification wetlands (Hammer and Knight, 1994; Vymazal, 2007). Similarly, hybrid wetlands comprised of a vertical flow cell and a cell with calcite media have been shown to be effective at removing both BOD and phosphorus from wastewater (Arias *et al.*, 2003). In addition, deep detention ponds for particle removal and anaerobic digestion of solids prior to vegetated wetlands and slow sand filters have been suggested to provide efficient treatment of municipal wastewater (Horne and Fleming-Singer, 2005).

Table 2.1: Examples of Large, Full-Scale Treatment Wetlands in the United States.

Wetland Name	Location Year Started	Size (ha)	Flow (m³/d)	Stated Purpose
Easterly ¹	Orlando, FL 1987	475	8x10 ⁴	Nutrient removal from wastewater before release to the sensitive St. Johns River; wildlife habitat
Prado ²	Riverside, CA 1992	200	2.5x10 ⁵	Nitrate removal from effluent-dominated Santa Ana River prior to Prado dam, which is used for aquifer recharge; wildlife habitat
George W. Shannon ³	Tarrant County, TX 2002	180	4x10 ⁵	Suspended solid and nutrient removal from effluent-dominated Trinity River prior to reservoirs; wildlife habitat; education

¹ <http://www.trwd.com/Wetlandsoverview.aspx>

² <http://www.ocwd.com/Prado-Wetlands/ca-28.aspx>

³ <http://www.dep.state.fl.us/water/wastewater/dom/oreastwet.htm>

Despite the increasing use of unit process wetlands for nutrient and BOD removal, current understanding of removal mechanisms in wetlands for certain classes of contaminants has not yet been translated into the design of unit process wetlands. This critical review focuses on the application of surface flow unit process wetlands to the removal of two such classes of contaminants, trace organic compounds and pathogens, from wastewater effluent and effluent-dominated river water. Trace organic contaminants are an emerging concern, due to their negative effects on aquatic ecosystems and the inability of conventional wastewater treatment plants to provide adequate removal. Pathogens and indicator organisms, on the other hand, are an historical concern, but removal by wetlands is often poor.

This paper starts with a review of hydraulics in surface flow constructed wetlands, given their central role in treatment performance. Next, the reported removals of trace organic contaminants and pathogens in wetlands is summarized, followed by a review of the main removal mechanisms such as sorption and sedimentation, biotransformation and predation, and photolysis and photoinactivation. Gaps in knowledge are identified for future research that can lead to identifying the controlling factors so that effective unit process wetlands and treatment

trains can be developed. The final section provides suggestions for how these treatment mechanisms can be enhanced in commonly employed unit process wetland cells or how they might be harnessed in novel unit process cells. It is hoped that the application of the unit process concept to a wider range of contaminants will lead to more widespread application of wetland treatment trains as components of urban water infrastructure in the United States and around the globe.

2.2 Hydraulics of Surface Flow Wetlands

Inefficiencies in hydraulics are a major barrier to optimizing the removal of contaminants in treatment wetlands, including trace organics and pathogens. Theoretically, the most effective wetland design would employ plug flow conditions to ensure that all water receives an equal amount of time for treatment. However in practice, plant growth rapidly results in conditions that deviate from ideal. In particular, hydraulic short-circuiting can dramatically decrease the overall performance of a wetland cell. Because this limitation to wetland treatment has been recognized for decades, models have been developed to account for the effects of dispersion due to vegetation, wind, and wetland boundaries (Kadlec, 1994). While these models are an improvement over ideal reactor models and offer insight into flow patterns in wetlands, the complex effects of heterogeneous and dynamic flow patterns are more difficult to model accurately.

Short-circuiting is the result of preferential flow paths through a wetland, which are caused primarily by uneven plant distribution and channelized flow (Kjellin *et al.*, 2007; Lightbody *et al.*, 2008). Short-circuiting results in water having a range of residence times in a wetland, reducing the wetland's treatment efficiency (Keefe *et al.*, 2004; Wörmann and Kronnäs, 2005). This is especially detrimental for wetlands designed to remove waterborne pathogens, which require reductions in concentration of several orders of magnitude to provide effective treatment and thus are severely compromised by even a modest amount of short-circuiting. To demonstrate this point, consider a wetland that is designed to provide 4-log removal (99.99%) of a pathogen under ideal, plug flow conditions. If just 20% of the flow has one-eighth of the nominal residence time, as observed by Lightbody *et al.* (2008) in a recently constructed wetland, the actual removal will only be about 1-log (90%).

The degree of short-circuiting in full-scale wetlands is usually evaluated with tracer studies (Martinez and Wise, 2003; Lin *et al.*, 2003). While tracer studies provide an understanding of how far the system deviates from ideal, more complicated models are necessary to predict contaminant treatment efficiency, as water flowing via different paths may be subjected to diverse biogeochemical conditions, resulting in variable treatment (Kadlec, 2000; Harvey *et al.*, 2005). For example, Keefe *et al.* (2004) modeled the reactive transport of rhodamine WT in three wetlands using a solute transport model with transient storage. Results showed that rhodamine WT loss rates via photolysis and sorption differed in storage and main channel zones, with sorption mass transfer rates being a factor of two higher in storage zones than in the main channel, and photolysis rates in the storage zones being almost an order of magnitude lower than those occurring in the main channel. Thus, an understanding of both the flow distributions and the removal processes at work in these different wetland zones was necessary to accurately interpret tracer test results.

Although short-circuiting cannot be eliminated, it can be reduced by proper wetland design. Consideration of soil conditions prior to wetland construction, such as filling ditches that would channelize flow (Martinez and Wise, 2003), can reduce short-circuiting. Baffles can also be used to increase the wetland's length-to-width ratio (aspect ratio), thereby reducing short-circuiting and encouraging plug flow conditions at a lower cost than building long, narrow wetlands (Reed *et al.*, 1995; Persson, 2000; Shilton and Harrison, 2003). Knight (1987) calculated that an aspect ratio of 2:1 would maximize wetland performance while minimizing construction costs. However, other aspect ratios may be appropriate when there is a need to significantly reduce contaminant concentrations (e.g., in the case of pathogens) and further research is needed to determine the optimal aspect ratio in these cases. The use of a subsurface berm or island placed in front of the wetland inlet also may reduce short-circuiting and improve hydraulic performance (Persson, 2000).

Despite careful wetland design, flow irregularities will still develop over time as plants grow. Flow irregularities may be minimized by using deep transverse mixing zones and other structures to break wetlands into multiple smaller cells. These zones improve wetland performance by mixing water that has traveled through different flow paths, as well as by reducing the likelihood that fast flow paths will be aligned (Lightbody *et al.*, 2007, 2009). Breaking a wetland into multiple cells has a similar effect, disrupting high-speed flow paths and ensuring that water is well mixed between cells (Kadlec, 2000; Horne and Fleming-Singer, 2005).

Periodic maintenance can also be used to control short-circuiting. For example, at the Prado Treatment Wetlands in Southern California, emergent plants are removed during maintenance activities (Scott Nygren, Orange County Water District, personal communication, March 13, 2012). This process involves draining the cell, allowing it to dry for several weeks and using a mower designed for brush removal to cut the plants near the ground surface. At the Easterly Wetlands in Central Florida, wetland plants are occasionally burned to thin the density of accumulated plants.

Incorporating multiple wetland cells is a fundamental component of the unit process wetland design. By linking unit process wetland cells in series, designing cells to have deep zones and baffles, considering the effects of inlet and outlet structures, and providing adequate maintenance, inefficiencies introduced by hydraulic short-circuiting can be minimized. Further research is needed to identify cost-effective maintenance practices that will minimize hydraulic short-circuiting.

2.3 Contaminants of Concern

2.3.1 Trace Organic Contaminants

Municipal wastewater effluent typically contains relatively low levels of organic matter (i.e., most wastewater treatment plants achieve BOD <10 mg L⁻¹). In addition to the biopolymers and residual organic waste that make up the bulk of the biodegradable organic matter, wastewater effluent also contains an assortment of trace organic contaminants, such as pharmaceuticals and personal care products (Kolpin *et al.*, 2002; Ternes *et al.*, 2004b). Trace organic contaminants in wastewater effluent are an issue of concern due to their potential to

cause adverse impacts to aquatic organisms at low concentrations (Daughton and Ternes, 1999; Suárez *et al.*, 2008) as well as their potential to contaminate downstream drinking water supplies (Snyder *et al.*, 2003).

The ability of constructed wetlands to remove trace organic contaminants from wastewater effluent has received growing attention recently (Matamoros and Bayona, 2008). Removal efficiencies for some pharmaceuticals and personal care products in treatment wetlands (Figure 2.1) suggest that trace organic contaminants generally fall into one of three groups of removal efficiency. The first group of compounds is removed efficiently (i.e., > 60% removal) regardless of wetland design and includes substances such as caffeine and naproxen. The second group, which includes the majority of the compounds in Figure 2.1, exhibits partial removal with varying efficiencies depending on wetland design and hydraulic residence times. The final group of compounds, which includes carbamazepine and clofibric acid, are more recalcitrant and exhibit limited removal (i.e., typically < 40% removal) irrespective of wetland design. Note that some values were determined from studies in subsurface wetlands, but they are included here because data from surface flow wetlands has not been reported. Optimization of treatment wetlands has the highest potential for enhancing the removal of the compounds in the second group.

2.3.2 Waterborne Pathogens

Wastewater effluent contains potentially infectious microorganisms, including viruses, bacteria, protozoan (oo)cysts, and helminth eggs. Removal or inactivation of pathogens is therefore necessary before treated effluent is discharged or reused. Treatment wetlands that receive wastewater that has already been disinfected may provide additional treatment of pathogens that are resistant to disinfection (e.g., *Cryptosporidium* oocysts for chlorine or adenovirus for UV). In this case, a wetland may be used to reduce the chemical disinfection requirements and provide an additional treatment barrier. Alternatively, treatment wetlands that receive wastewater effluent that has not been disinfected can play a primary role in pathogen attenuation. In this context, Gersberg *et al.* (1989) suggested that treatment wetlands with hydraulic residence times of 3 to 6 days may be as effective as conventional water treatment systems employing disinfection for the removal of pathogenic bacteria and viruses. Reliance on polishing wetlands for disinfection has the advantage over chlorination of avoiding the production of disinfection byproducts (Buth *et al.*, 2009, 2010).

Most studies on the removal of pathogens in treatment wetlands have measured fecal indicator bacteria rather than actual pathogens. The reported removal efficiency of fecal coliforms by surface wetlands is around 1-log removal (Vymazal, 2005; Kadlec and Wallace, 2009). The few studies that have been conducted with actual pathogens (Figure 2.2) show removal efficiencies up to 2-log, with average values around 1-log. The dominant removal mechanisms vary dramatically among pathogen groups. A better understanding of pathogen removal mechanisms, including attachment and sedimentation, predation, and photoinactivation, and their effectiveness for different pathogen groups is needed to improve the ability to design unit process wetlands for disinfection.

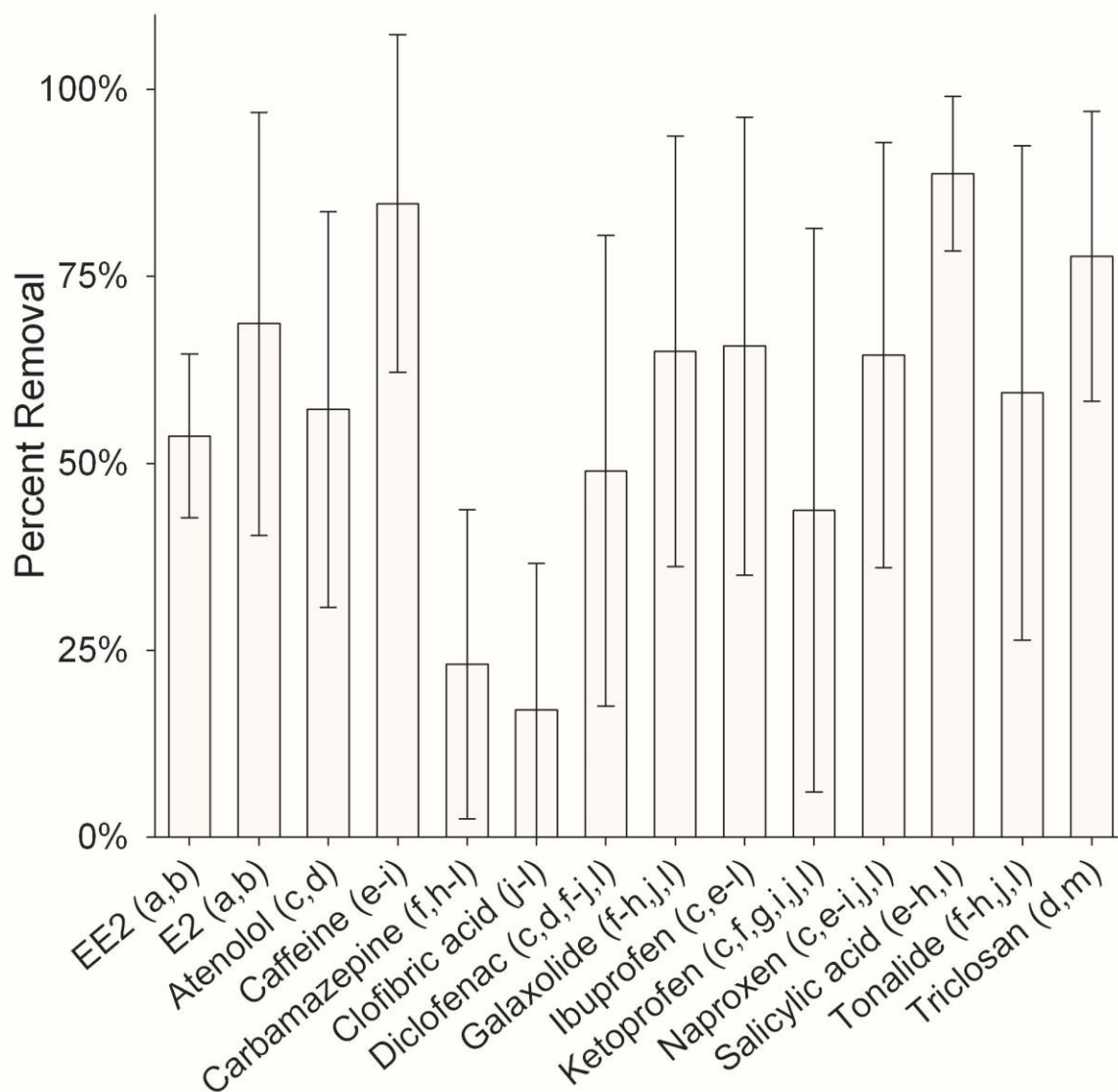


Figure 2.1: Averages pharmaceutical and personal care product removal efficiencies in treatment wetlands. Error bars represent \pm one standard deviation. $n=3-16$. References: a (Gray and Sedlak, 2005); b (Song et al., 2011); c (Breitholtz et al., 2012); d (Park et al., 2009); e (Camacho-Muñoz *et al.*, 2012); f (Hijosa-Valsero et al., 2011); g (Matamoros and Bayona, 2006); h (Matamoros et al., 2007); i (Matamoros et al., 2009); j (Llorens et al., 2009); k (Matamoros et al., 2005); l (Matamoros *et al.*, 2008b); m (Waltman et al., 2006).

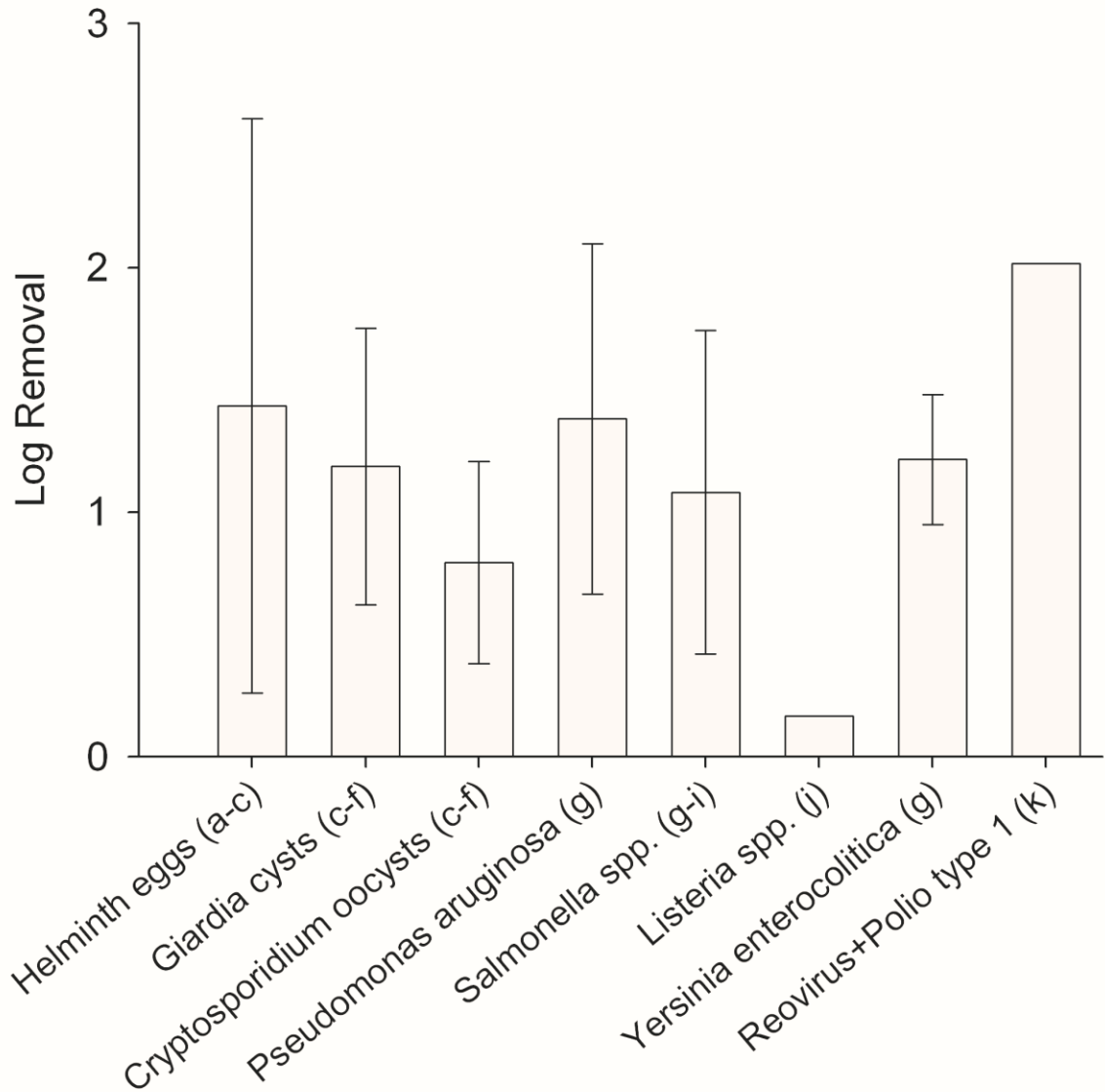


Figure 2.2: Averages with standard deviations of pathogen removal efficiencies in surface flow wetlands receiving non-disinfected influent. References: a (Mandi *et al.*, 1996); b (Mandi *et al.*, 1998); c (Reinoso *et al.*, 2008); d (Falabi *et al.*, 2002); e (Gerba *et al.*, 1999); f (Quiñónez-Díaz *et al.*, 2001); g (Herskowitz, 1986); h (Hill and Sobsey, 2001); i (Song *et al.*, 2010); j (Karpiscak *et al.*, 2001); k (Kadlec and Wallace, 2009).

2.4 Removal Mechanisms of Trace Organic Contaminants

2.4.1 Sorption

Sorption of trace organic contaminants encompasses two distinct processes. Adsorption involves the interaction of a compound with a surface, typically via ion exchange or surface complexation, while absorption entails partitioning into a particle-associated organic phase. Sediments and biofilms in treatment wetlands provide numerous surfaces that may be capable of sorbing trace organic contaminants. If the contaminants exhibit a high affinity for a surface, they will eventually be buried as decaying plant litter accumulates in the wetland. If the sorbent does not degrade, the contaminants will remain in the litter layer until it is removed as part of wetland maintenance activities. If the contaminants are weakly associated with the sorbent, or if the sorbent (e.g., plant litter) degrades, the process may simply slow the movement of contaminants through a wetland, providing more time for other transformation processes to occur.

Although sorption is assumed to be important to trace organic contaminant fate in treatment wetlands (Imfeld *et al.*, 2009), few studies have investigated it specifically. In these studies $\log K_{ow}$, a measure of a contaminant's hydrophobicity, generally predicts which contaminants are most susceptible to absorption. For example, in a bulrush-dominated surface flow wetland with a 30 cm deep gravel bed, the hydrophobic phthalate esters and fragrance molecules ($\log K_{ow} > 4$) were absorbed to organic matter in the gravel bed as well as suspended particles (Reyes-Contreras *et al.*, 2011). Absorption was also found to slow the movement of two steroid hormones ($\log K_{ow} \approx 4$) relative to a conservative tracer in a densely vegetated surface flow wetland (Gray and Sedlak, 2005).

Removal of chemical contaminants by absorption may be more important in subsurface flow wetlands, where flowing water encounters higher densities of particulate organic matter. For example, partial removal of the recalcitrant contaminant carbamazepine ($\log K_{ow} \approx 2.5$) by absorption and negligible sorptive removal of less hydrophobic contaminants was observed in a study of a subsurface flow treatment wetland (Matamoros *et al.*, 2005).

Studies of pesticide sorption in agricultural wetlands show a similar dependence on contaminant hydrophobicity (Kruger *et al.*, 1996; Moore *et al.*, 2002; Reichenberger *et al.*, 2007). For example, sorption of the herbicide atrazine ($\log K_{ow} = 2.75$) to soil, litter, peat, and sediments from three Midwest wetlands was well described for all sorbents by an organic carbon-normalized distribution coefficient ($K_{oc} = 760 \text{ L kg}^{-1} \text{ OC}$) (Alvord and Kadlec, 1995). Given this distribution coefficient, a wetland with about 2 kg m^{-2} litter, containing about 40% organic carbon (Alvord and Kadlec, 1995), and a depth of 40 cm, would be capable of absorbing more than 60% of atrazine from the aqueous phase. This suggests that absorption in wetlands could be significant for compounds with a $\log K_{ow}$ greater than about 2.5, provided that the system is designed properly. For comparison, absorption of trace organic contaminants in activated sludge treatment plants is usually unimportant for compounds with $\log K_{ow}$ values less than about 4 (Ternes *et al.*, 2004a; Wick *et al.*, 2009).

Less hydrophobic compounds have been found to adsorb via specific ionic interactions with activated sludge in treatment plants (Stuer-Lauridsen *et al.*, 2000; Golet *et al.*, 2003) and soils (Tolls, 2001). In wetlands, the relatively hydrophilic fluorescent dye, rhodamine WT, sorbs

significantly to plants and sediments (Lin *et al.*, 2003; Keefe *et al.*, 2004). At neutral pH values, rhodamine WT contains both positively and negatively charged functional groups and is thus likely to be adsorbed via specific interactions with charged functional groups on the sorbents (Kasanavia *et al.*, 1999). Therefore, adsorption of ionic trace organic contaminants in treatment wetlands may be an important loss mechanism for certain compounds. However, additional research is needed to assess the overall importance of this phenomenon and ways in which it could be enhanced through wetland design.

Certain types of wetland vegetation may increase the removal of trace organic contaminants by sorption. For example, it has been suggested that wetlands dominated by bulrush (e.g., *Scripus* spp.) are conducive to sorption of trace organic contaminants due to the large amounts of spongy peat formed by decomposing plants (Horne and Fleming-Singer, 2005). Duckweed (*Lemna* spp.), a floating macrophyte often present in open waters in treatment wetlands, sorbs trace organic contaminants such as halogenated phenols (Tront *et al.*, 2007) and pharmaceuticals and personal care products, including fluoxetine, ibuprofen, and triclosan (Reinhold *et al.*, 2010). However, duckweed grows in a thin layer near the water surface and the relatively small mass of the plant in wetlands likely precludes it from removing a significant fraction of the trace organic contaminants as water passes through a wetland.

Water chemistry also affects sorption of chemical contaminants in constructed wetlands (Hussain and Prasher, 2011). In particular, the pH of wetland water will affect the sorption of contaminants by changing their speciation. This phenomenon has been observed in wastewater treatment plant sludge for the acidic pharmaceuticals diclofenac ($pK_a=4.6$) and ibuprofen ($pK_a=3.5$), which absorbed to primary sludge to a greater extent than to secondary sludge, because a greater fraction of the pharmaceuticals were in their uncharged form at the lower pH value (pH=6.6 in primary versus pH=7.5 in secondary) (Ternes *et al.*, 2004a). Basic contaminants, such as those containing amine functional groups (e.g., the β -blockers), have pK_a values near 9 and are positively charged at neutral pH values. Consequently, their sorption is likely controlled by specific interactions, as was observed in a study by Yamamoto *et al.* (2009). Increasing the pH of wetland water could increase the fraction of the uncharged forms of the compounds, resulting in enhanced sorption by hydrophobic interactions. Further research is needed to determine the potential for enhancing sorption in treatment wetlands through the use of natural processes to alter pH values (i.e., photosynthesis and microbial respiration).

2.4.2 Biotransformation

Microorganisms play a prominent role in the attenuation of trace organic contaminants in constructed wetlands (Matamoros *et al.*, 2008b; Hijosa-Valsero *et al.*, 2010b) due to the diversity of microorganisms and enzymatic activities present (D'Angelo, 2003). In surface flow wetlands, biofilms found on roots, stalks, and detritus are more important to biotransformation than planktonic microorganisms (Gagnon *et al.*, 2007; Truu *et al.*, 2009). Thus it is not surprising that properties affecting biofilm growth, such as the attachment matrix, hydraulic conditions, and composition of the wastewater effluent, can strongly influence microbial ecology and contaminant transformation rates in these systems (Truu *et al.*, 2009).

In vegetated treatment wetlands, the density and type of plants affect microbial community dynamics (Ibekwe *et al.*, 2006; Calheiros *et al.*, 2009) by providing labile forms of

organic carbon, surfaces for biofilm growth, and oxygen gradients (Reddy and D'Angelo, 1997). The ability of decaying plants to create anoxic zones in surface and subsurface flow constructed wetlands is important to the transformation of trace organic contaminants because some compounds are more readily transformed under aerobic conditions (e.g., ibuprofen) while others (e.g., tonalide and galaxolide) are more readily transformed under anaerobic conditions (Hijosa-Valsero *et al.*, 2010b). Further, anoxic, nitrogen-reducing surface flow wetlands have been shown to be capable of transforming certain trace organic contaminants, including atenolol, naproxen, and triclosan, possibly through amide hydrolysis and reductive dehalogenation (Park *et al.*, 2009).

Plant biomass and DOC from plant litter, and to a lesser degree, residual organic carbon from wastewater effluent, provide an important energy source and create selective pressure for microbial community structure and function in wetland systems (Shackle *et al.*, 2000; Gutknecht *et al.*, 2006). Biotransformation of pharmaceuticals in wastewater effluent can be affected by both the abundance and source of organic carbon derived from decaying aquatic plants. For example, gemfibrozil and sulfamethoxazole, two compounds that are poorly removed in wastewater treatment plants, showed better removal in the presence of labile organic carbon derived from wetland plants than in the presence of the labile dissolved organic carbon in wastewater effluent (Lim *et al.*, 2008). Other macrophyte characteristics, such as surface area and litter properties, can also affect microbial density (Bastviken *et al.*, 2005), which may correlate with trace organic contaminant removal rates.

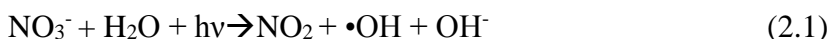
The rhizosphere associated with wetland plants hosts a unique community of microorganisms within aerobic microzones, due to the release of oxygen and nutrient-rich exudates (Brix, 1997; Kyambadde *et al.*, 2004; Münch *et al.*, 2007; Gagnon *et al.*, 2007). Microbially-mediated iron and manganese oxides formed in this region also have the potential for indirect oxidation or enhanced sorption of trace organic contaminants (Mendelssohn *et al.*, 1995; Emerson *et al.*, 1999). Rhizosphere-associated transformation appears to be an important process for trace organic contaminants in subsurface flow wetlands (Zhang *et al.*, 2012). However, the rhizosphere may be less important in surface flow wetlands due to limited contact between the rhizosphere and flowing waters.

Bacteria and fungi transform macromolecules, such as cellulose and lignin, into lower molecular weight compounds through the excretion of extracellular enzymes. Enzyme expression studies in constructed wetlands have provided insight into the importance of extracellular enzyme activity to the processing of these recalcitrant forms of organic carbon (Shackle *et al.*, 2000; Wright and Reddy, 2001; Hill *et al.*, 2006; Francoeur *et al.*, 2006; Rier *et al.*, 2007). Many of the extracellular enzymes used to transform cellulose and lignin (e.g., laccases, phenol oxidases, and peroxidases) also can transform recalcitrant trace organic contaminants (Gianfreda and Rao, 2004; Lu *et al.*, 2009). By modifying the quantity and type of carbon sources in constructed wetlands, it may be possible to increase the activity of extracellular enzymes. Because most extracellular enzymes utilize oxygen or hydrogen peroxide as terminal electron acceptors, their activity is expected to be higher in aerobic environments (Sinsabaugh, 2010; Porter, 2011).

2.4.3 Photolysis

In natural waters, photolysis of trace organic contaminants can occur via direct and indirect mechanisms (Schwarzenbach *et al.*, 2003). Direct photolysis occurs when a contaminant absorbs sunlight and undergoes a transformation reaction. Indirect photolysis involves other chemicals, most often NO_3^- and colored dissolved organic matter (CDOM), which absorb light and produce reactive intermediates that subsequently react with contaminants. With respect to the transformation of organic contaminants, important reactive intermediates include hydroxyl radical ($\bullet\text{OH}$) (Zepp *et al.*, 1987; Brezonik and Fulkerson-Brekken, 1998), singlet oxygen ($^1\text{O}_2$) (Zepp *et al.*, 1977), excited triplet state DOM ($^3\text{DOM}^*$) (Canonica *et al.*, 1995; Boreen *et al.*, 2004), organoperoxy radicals, and carbonate radical ($\bullet\text{CO}_3^-$) (Lam *et al.*, 2003; Canonica *et al.*, 2005).

Treatment wetlands may be conducive to indirect photolysis due to the presence of NO_3^- in nitrified wastewater effluent, which produces $\bullet\text{OH}$ via the reaction:



CDOM derived from wastewater effluent and decaying plants may also promote indirect photolysis through a variety of mechanisms. For example, CDOM was significant to the removal of the pesticides alachlor and carbaryl in wetland waters via indirect photolysis (Miller and Chin, 2002, 2005). For those reactions where CDOM served as a source of $\bullet\text{OH}$, rates of photolysis were not strongly affected by DOM concentrations because it acted as both a source of CDOM and a $\bullet\text{OH}$ scavenger. In addition to generating $\bullet\text{OH}$, CDOM can also serve as a source of the selective oxidants $^1\text{O}_2$ and $^3\text{DOM}^*$, which have been found to be important to the indirect photolysis of certain trace organic chemicals in the environment (Gerecke *et al.*, 2001; Latch *et al.*, 2003b).

Most constructed wetlands are not designed to include shallow open water zones. As a result, few investigators have studied the role of photolysis in trace organic contaminant removal in treatment wetlands. Matamoros *et al.* (2008b) attributed the nearly complete removal of ketoprofen in an engineered treatment wetland with deep (1.5 m) open water zones and a long hydraulic residence time (30 days) to photolysis. While this study demonstrated that photolysis in deep, open ponds can significantly attenuate organic compounds that are particularly susceptible to direct photolysis (ketoprofen has a half-life of 2.5 minutes under near-surface summer-noon conditions (Lin and Reinhard, 2005)), removal efficiencies would be significantly lower for compounds with longer direct photolysis half-lives. For example, consider sulfamethoxazole, a compound that is relatively susceptible to direct photolysis (half-life of about 2 hours under near-surface summer-noon conditions (Lam and Mabury, 2005)), in a 1 m deep wetland with the EPA (2000a) recommended hydraulic residence time of 3 days for open water zones. Given a typical beam attenuation coefficient (α) of 6 m^{-1} at 330 nm, and ideal plug flow conditions, sulfamethoxazole would exhibit a decrease in concentration of only about 10% due to direct photolysis under daily-averaged mid-summer conditions at 40° latitude (Schwarzenbach *et al.*, 2003).

2.5 Removal of Waterborne Pathogens

2.5.1 Attachment and Sedimentation

Some pathogens, like helminth eggs, have settling velocities high enough to be removed by sedimentation in treatment wetlands (e.g., about 0.1 mm s^{-1} ; Sengupta *et al.*, 2011). The presence of rhizomes and macrophyte stalks can further enhance sedimentation (Mandi *et al.*, 1996). However, protozoan (oo)cysts and bacteria have much lower settling velocities (e.g., less than 0.001 mm s^{-1} for *Giardia* cysts (Dai and Boll, 2006)), and viruses are stable in suspension. These organisms will only be removed by sedimentation if they are attached to larger particles. As a result, the removal of cysts such as *Giardia* in some cases has been correlated with particle removal (Quiñónez-Díaz *et al.*, 2001). However, particle association is not always conducive to pathogen removal. For example, in a study conducted by Boutilier *et al.* (2009), the association of *E. coli* with particles appeared to decrease their removal compared to free-floating bacteria. This was because particles with diameters less than $80 \mu\text{m}$ had settling velocities too low to be removed by sedimentation.

In addition to attaching to settling particles, pathogens can be removed by attachment to other surfaces in treatment wetlands. However, the contribution of this mechanism to overall removal is not known. For example, in vegetated surface flow wetlands, viruses were removed by attachment to the biofilm layer of rhizomes and submerged stalks of emergent plants (Gersberg *et al.*, 1987). Biofilms have also been shown to increase the removal of pathogen surrogates (0.1 -, 1.0 -, and 4.5 - μm latex microspheres) in microcosm experiments using surface flow wetland water (Stott and Tanner, 2005). A better understanding of the processes that affect the association of pathogens with particles and surfaces, as well as the size distribution of particles in treatment wetlands, may provide insight into means for enhancing pathogen removal via sedimentation and attachment.

Pathogenic helminth eggs, protozoan (oo)cysts, and viruses that accumulate on wetland surfaces and in sediments will become inactivated over time. In contrast, indicator and pathogenic bacteria have the potential to grow under certain environmental conditions (Ksoll *et al.*, 2007; Ishii *et al.*, 2010), although there is no evidence of growth occurring in treatment wetlands. It should be noted that sloughing or resuspension of sediments has the potential to remobilize viable organisms. In addition, wetland vegetation or sediments that are removed during maintenance activities may contain viable pathogens, which could complicate efforts to reuse the material (e.g., as a soil amendment).

2.5.2 Predation

Predation has the potential to be an important removal mechanism for bacteria and protozoan (oo)cysts in treatment wetlands (Stott *et al.*, 2001; Song *et al.*, 2008), but much less is known about viruses. Grazing of particles in the size range of individual viruses is typically inefficient (Hahn and Höfle, 2001), although viruses attached to larger particles have the potential to be removed by predation. Potential predators of pathogens in treatment wetlands include nematodes, copepods, rotifers, and protozoa (Decamp and Warren, 1998). For example, grazing rates for the ciliated protozoa *Paramecium* were measured to be $111 \text{ E. coli (ciliate-hour)}^{-1}$ and $170 \text{ Cryptosporidium parvum oocysts (ciliate-hour)}^{-1}$ in lab experiments (Decamp and Warren, 1998; Stott *et al.*, 2001)). Hence a population of

2x10⁴ Paramecium per liter (Decamp and Warren, 1998) would have the potential to achieve greater than 6-log removal of *E. coli* or oocysts in one hour, if these were the only particles in the water. Grazing rates in an actual wetland are expected to be significantly lower because pathogens would represent a small fraction of the total particles.

In addition to ciliated protozoa, rotifers may play an important role in the removal of pathogens via grazing due to their dominance in the total zooplankton population in shallow wetlands (Beaver et al., 1998; Fayer et al., 2000; Trout et al., 2002; Proakis, 2003). For example, the rotifer *Brachionus plicatilis* rapidly removed *E. coli* from water under laboratory conditions, with an average feeding rate of almost 700 *E. coli* (rotifer-hr)⁻¹ (Proakis, 2003). Rotifers from six different genera were also found to ingest *Giardia* cysts and *Cryptosporidium* oocysts in simple laboratory experiments (Fayer et al., 2000; Trout et al., 2002). However, ingestion rates in actual wetlands have not been measured, and conditions that promote predation in treatment wetlands are not sufficiently understood.

While predation may provide an effective means for removing indicator organisms and pathogens from water, the viability of the organisms following ingestion is uncertain. In general, protozoan grazing is a major mechanism of bacterial population control (Hahn and Höfle, 2001). However, there is evidence that some pathogenic bacteria are not inactivated as a result of ingestion by protozoan grazers, such as ciliates and amoebas (Barker and Brown, 1994; Meltz Steinberg and Levin, 2007). Indeed, pathogenic bacteria like *Legionella* actually colonize free-living amoeba to protect themselves from unfavorable environmental conditions (Thomas et al., 2010). More research is needed on inactivation of the wide range of pathogens of concern in wastewater by the different types of grazers in treatment wetlands.

2.5.3 Photoinactivation

Sunlight-mediated inactivation is one of the most important disinfection mechanisms in waste stabilization ponds (Davies-Colley *et al.*, 2000; Davies-Colley, 2005), suggesting that open water zones are promising for the removal of pathogens in treatment wetlands. There are three main mechanisms of sunlight-mediated inactivation of microorganisms (Davies-Colley *et al.*, 1999). Analogous to direct photolysis of chemical contaminants, the absorption of UVB radiation (280-320 nm) by DNA causes direct damage to cellular DNA, primarily by pyrimidine dimer formation (Jagger, 1985). Similar to indirect photolysis of chemical contaminants, there are also indirect disinfection mechanisms, in which sensitizers absorb light and produce reactive species that damage organisms. Indirect damage may occur due to absorption of sunlight by cell constituents (endogenous sensitizers) (Davies-Colley *et al.*, 1999; Bosshard *et al.*, 2010) or by sensitizers in water (exogenous sensitizers), such as CDOM. ¹O₂ has been shown to be the most important reactive species produced by exogenous sensitizers during the sunlight-mediated inactivation of MS2 coliphage, which is a model for human enteric viruses (Kohn and Nelson, 2007).

Exogenous inactivation initiated by sensitizers that absorb longer wavelengths, such as CDOM, may be more important than endogenous mechanisms because the UV wavelengths that contribute to endogenous inactivation (direct and indirect) are readily absorbed in wetland water (i.e., λ =280 to 400 nm). However, not all organisms appear to be susceptible to this mechanism (Davies-Colley *et al.*, 1999). Photoinactivation can also be enhanced by high dissolved oxygen and elevated pH (e.g., greater than pH 9) that result from algal photosynthesis (Curtis *et al.*,

1992; Davies-Colley *et al.*, 1999; Ansa *et al.*, 2011).

The importance of sunlight-mediated disinfection in surface flow treatment wetlands was illustrated when *E. coli* concentrations declined significantly after a thick bed of floating duckweed (*Lemna* spp.) was removed from a newly constructed surface flow wetland (MacIntyre *et al.*, 2006). A better understanding of the sunlight-mediated inactivation mechanisms and their roles in pathogen removal is needed to optimize the design and enable prediction of the fate of pathogens in surface flow treatment wetlands.

2.6 Novel unit process wetlands for removal of trace organic contaminants and pathogens

A growing understanding of wetland hydraulics and contaminant attenuation mechanisms in treatment wetlands provides an opportunity to optimize the design and operation of wetlands to remove trace organic contaminants and pathogens in a sequence of unit process cells. The individual unit processes incorporated into a wetland treatment train will depend on a variety of considerations, including influent water quality, contaminants of concern, point of discharge, regulations on effluent water quality, and space constraints. Different unit processes will be needed to address the wide range of trace organic contaminants and pathogens, which are removed by different removal mechanisms. Based on the review of removal mechanisms in Sections 2.4 and 2.5, in this section we further explore several novel designs for unit process cells that target removal of trace organics and wetlands: a shallow, open-water cell, vegetated wetlands that optimize specific enzymes and biodegradation pathways, and a cell incorporating filter-feeding bivalves.

An example of how unit processes could be combined in a treatment train for nitrified wastewater effluent is provided in Figure 2.3. In this example, the first cell provides treatment of trace organics through direct photolysis, indirect photolysis (including $\bullet\text{OH}$ produced from NO_3^-), and sorption and biotransformation in the thick biofilm layer that forms on the cell's bottom. Inactivation of pathogens also occurs through direct and indirect photoinactivation. Next, in the cattail cell, labile organic matter produced by cattails fuels denitrification as well as biodegradation of trace organics, the anaerobic conditions promote precipitation of metal sulfides, and quiescent conditions promote further settling of particle-associated pathogens. Next, in the bulrush cell, non-labile organic carbon accumulates and serves as a sorbent for trace organic contaminants. Finally, in the bivalve cell, particle-associated trace organic contaminants and pathogens are ingested, and transformed or inactivated.

A deeper understanding of the specific mechanisms at play will allow the individual cells to be optimized, as well as their sequential order. For example, if DOM produced by vegetated cells is found to be effective at sensitizing the degradation of specific chemicals or pathogens, then the photolysis cell could be placed after the vegetated cell. The potential for each of these unit processes is explored in greater detail in subsequent sections.

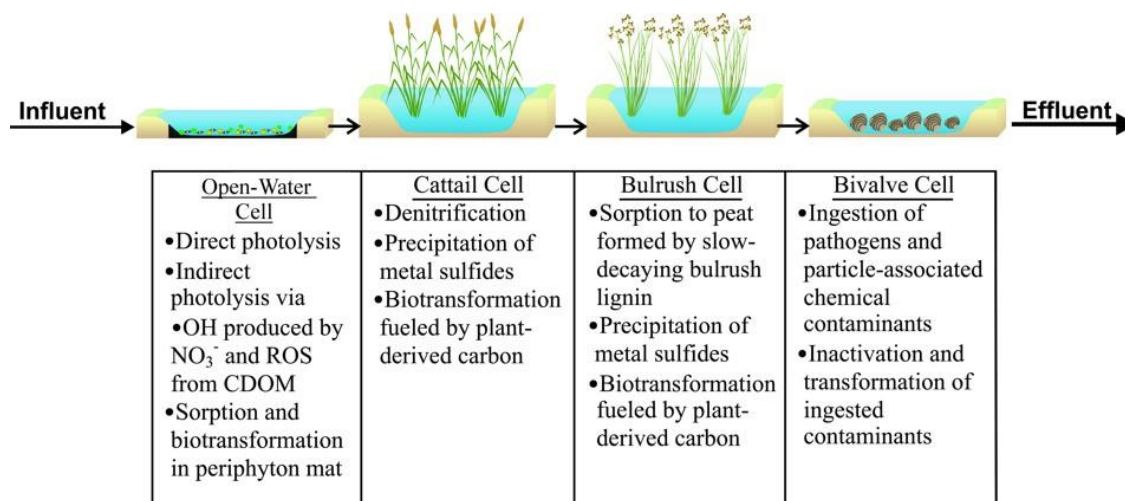


Figure 2.3: Example of unit process wetland treatment train, along with key processes occurring in each unit process cell.

2.6.1 Shallow, Open-Water Cells

Shallow, open-water wetland cells (Figure 2.4) represent a new approach for integrating photochemical processes and aerobic microbes into a unit process wetland. While photolysis can provide a means of removing contaminants, it is difficult to design treatment wetlands for photolysis because emergent macrophytes and floating plants (e.g., duckweed) shade the water. Furthermore, chromophores in wastewater effluent and wetland water strongly absorb sunlight, especially in the important UV region of the solar spectrum, greatly slowing photochemical reactions at depths of more than about 0.5 meters. To circumvent these problems, a shallow, open-water wetland cell can be used. In these cells, concrete or geotextile liners are used on the bottom of the cell to prevent emergent macrophyte growth. Under these conditions, the bottom of the basin is rapidly colonized by a biomat, which is composed of a consortium of organisms dominated by algae, aerobic bacteria, and other eukaryotes (Wetzel, 1983).

A pilot-scale wetland of this design (Figure 2.4) allows sunlight to penetrate throughout the water column, which is typically around 20 cm. The growth of periphyton on the bottom of the cell, rather than suspended in the water column as is the case with high rate algal ponds, prevents shading of the water by algae and the use of relatively high water velocities prevents the accumulation of duckweed on the surface. Inexpensive materials (i.e., wooden boards) are used as baffles to minimize hydraulic short-circuiting.

Photolysis in a shallow, open-water cell with a hydraulic residence time of at least 24 hours would likely result in removal of chemical contaminants that are susceptible to direct photolysis, such as NDMA, ketoprofen, and diclofenac. The shallow cell might also be conducive to removal of certain chemical contaminants by indirect photolysis. Given NO_3^- concentrations of 20 mg N L^{-1} and DOC concentrations of 10 mg L^{-1} , which are typical of secondary wastewater effluent, a daily-averaged $\bullet\text{OH}$ steady-state concentration of about $4 \times 10^{-16} \text{ M}$ would be expected in 20 cm of water during mid-summer at 40° latitude (Zepp *et al.*, 1987; Schwarzenbach *et al.*, 2003). For a typical organic contaminant, which reacts with $\bullet\text{OH}$ at near

diffusion-controlled rates (i.e., about $7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$), approximately 50% removal would be expected after 3 days in the wetland.

The water in periphyton-containing wetland cells exhibits diurnal cycles in which dissolved oxygen concentrations and pH values increase during the day due to photosynthesis (Fletcher and Marshall, 1982; Pollard, 2010). In the pilot-scale wetland shown in Figure 1.4, the pH typically increases to values of between 9 and 10 within 50 meters of the inlet. The alkaline pH conditions in the shallow, open-water cell may affect the rate of direct photolysis of chemical contaminants by changing contaminant speciation (Boreen *et al.*, 2004). Indirect photolysis rates will also be affected by changes in water pH because $\bullet\text{OH}$ is scavenged by inorganic carbon under alkaline conditions forming $\bullet\text{CO}_3^-$, which may then react with contaminants (Lam *et al.*, 2003). This shift in radical formation could lead to selective oxidation of sulfur-containing compounds, which often exhibit elevated reaction rates with $\bullet\text{CO}_3^-$ (Huang and Mabury, 2000c). High concentrations of dissolved oxygen could alter other indirect photolysis pathways, either by quenching intermediate triplet states (Ryan *et al.*, 2011) or by enhancing production of $^1\text{O}_2$ (Latch *et al.*, 2003b).



Figure 2.4: Discovery Bay pilot-scale open-water wetland cell. The cell is about 20 cm deep, 400 m², and has a hydraulic residence time of about 1-3 days.

Pathogens would also be inactivated in a shallow wetland cell, both by direct and indirect mechanisms. Indirect mechanisms would be especially important due to the high $^1\text{O}_2$ concentrations produced by DOM present in wastewater. For example, a daily-averaged steady-state $^1\text{O}_2$ concentration of about $2 \times 10^{-14} \text{ M}$ would be expected during mid-summer at 40° latitude in 20 cm of water (Haag and Hoigné, 1986; Schwarzenbach *et al.*, 2003). Over a 3 day residence time this would result in almost 3-log inactivation of MS2 coliphage, which was reported to be inactivated by $^1\text{O}_2$ with a second-order rate of $1.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Kohn and Nelson, 2007).

In addition to modifying overlying water chemistry, a biomat could remove chemical contaminants and pathogens through sorption, biotransformation, and predation. For example, researchers have found that biomats present in streams are capable of sorbing, and in some cases transforming, trace organic contaminants such as steroid hormones, alkylphenols, non-steroidal anti-inflammatory drugs, and the cyanotoxin microcystin-RR (Wu *et al.*, 2010; Writer *et al.*, 2011b; Dobor *et al.*, 2012). Biotransformation of chemicals may be encouraged by the aerobic conditions encountered at the top of the biomat. In addition, the labile carbon provided by periphyton, which has been shown to enhance denitrification rates in anoxic wetlands (Sirivedhin and Gray, 2006), and the increased activity of extracellular enzymes, such as the phenol oxidases which are associated with photosynthesis (Romani *et al.*, 2004; Francoeur *et al.*, 2006; Rier *et al.*, 2007), might also be important. Pathogens have been found to attach to periphyton as well, although detachment at a later time is possible (Ksoll *et al.*, 2007).

Maintenance activities in a shallow, open-water wetland cell may include removing floating vegetation as well as detritus which accumulates in the periphyton mat. Floating vegetation, such as duckweed, is capable of quickly covering a wetland, limiting the effectiveness of photolysis and potentially altering the microbial community in the periphyton mat. The growth of floating vegetation can be limited by ensuring that hydraulic residence times are less than about three days (EPA 2000a) and that the outlet structure allows floating vegetation to leave with the outflow. If floating vegetation does grow, the wetland can be periodically flushed by increasing the flow rate into the wetland to wash the floating vegetation out. The slow buildup of particulate matter and detritus from decomposing biomat will also need to be removed regularly, as over a few years it may slowly fill in the wetland. This can be accomplished by draining the wetland and removing the dried periphyton mat with a bulldozer. After the old mat is removed, a new biomat will re-grow within weeks on the wetland bottom.

2.6.2 Macrophyte-Dominated Wetland Cells

To enhance contaminant attenuation, vegetated wetland zones can be designed and managed to select for microorganism communities with specific and complimentary composition and functionality. Enhanced attenuation can be achieved through a unit process approach in which different cells are optimized for specific purposes. For example, cells containing cattails provide biomass that is more readily decomposed while bulrush cells would be expected to exhibit higher activities of extracellular enzymes needed to break down the lignin-rich plants (Horne and Fleming-Singer, 2005).

The linking of microbial community dynamics with factors such as plant substrates, temperature, nutrient loading, and dissolved oxygen will allow for wetlands to be actively managed based on environmental indicators. Nutrient availability can also affect microbial community structure and enzyme expression. For example, an abundance of phosphorus can reduce microbial diversity (Ahn *et al.*, 2007) and potentially metabolic diversity. To increase microbial activity or select for reducing conditions, plant biomass harvested during routine maintenance activities can be added back to specific wetland cells. Vegetated wetlands can also be designed to include deep zones to limit plant growth. Other strategies have been suggested, such as raising the water level after plants have senesced to provide additional carbon from the previously un-submerged plant detritus in the winter (Thullen *et al.*, 2005).

Through an increased understanding of wetland microbial communities and the ability to monitor their composition and activity, it may be possible to optimize the performance of unit process cells. Past studies have relied on culture-dependent methods to discern metabolic potential with inherent and often artificial selective pressures (Fortin *et al.*, 2000; Truu *et al.*, 2009) as well as fingerprinting techniques to track spatial and temporal variations in dominant microbes in these systems (Boon *et al.*, 1996; Faulwetter *et al.*, 2009). However, little has been done to thoroughly understand microbial ecology and enzymatic regulation in engineered wetlands. This understanding is necessary to more effectively manage microbial transformation of contaminants and develop enhanced design and monitoring tools for future engineered wetlands. A suite of culture-independent molecular-based methods such as fluorescent in situ hybridization, quantitative PCR (DeJournett *et al.*, 2007; Bacchetti De Gregoris *et al.*, 2011) and high throughput pyrosequencing for phylogenetic analysis and the generation of metagenomes (Jiang *et al.*, 2011), hold immense promise for future studies. Collectively, these molecular tools can further elucidate the microbial structure and function in constructed wetland systems. Studies that characterize enzyme expression in concert with phylogenetic characterization are important to more effectively track these complimentary but not always synonymous variables (Vilchez-Vargas *et al.*, 2010).

2.6.3 Bivalve Filtration Wetland Cells

As a compliment to contaminant attenuation in shallow, open-water wetlands and macrophyte-dominated wetlands, wetland cells can be built to provide a habitat for organisms that remove contaminants through filter feeding, as shown in Figure 2.5. Studies have shown that bivalves such as mussels and clams filter large volumes of water and remove organic particulate matter from the water column (Winter, 1978; Møhlenberg and Riisgård, 1979; Kryger and Riisgård, 1988; Riisgård, 2001). While bivalves occur at low densities in habitats such as coastal estuaries, rivers, and littoral zones of lakes, large populations may be supported in systems with short hydraulic residence times and high primary productivity. Thus, it may be possible to support high densities of filter feeding organisms in a constructed wetland cell where significant concentrations of wastewater- or macrophyte-derived organic matter are available.

While bivalves have not yet been applied for water quality improvement in unit process wetlands, they have been considered for a variety of applications including drinking water treatment (McIvor, 2004), algae and suspended particulate matter removal from river water (Li *et al.*, 2010), and clarification of secondary municipal wastewater effluent (Haines, 1979). Bivalves have also been considered as a means of removing nutrients from aquaculture wastewater (Buttner, 1986; Shpigel *et al.*, 1997) and more recently to remediate surface waters polluted by excessive nutrients in New York City (Cotroneo *et al.*, 2011; NOAA, 2011).

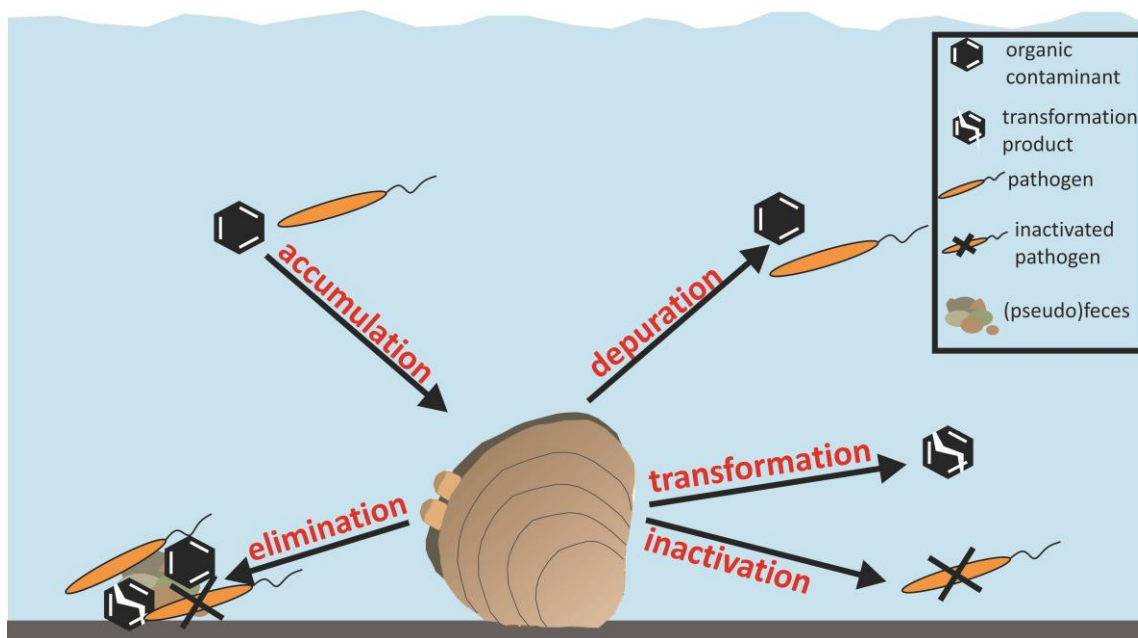


Figure 2.5: Schematic of bivalve uptake and removal mechanisms of organic contaminants and pathogens.

In the process of removing particulate matter, bivalves can also accumulate and in some cases transform particle-associated trace organic contaminants. For example, biotransformation of polybrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs) by a freshwater mussel (*Elliptio complanata*) was observed following exposure through contaminated algae (O'Rourke *et al.*, 2004; Drouillard *et al.*, 2007). In addition, the transformation of crude oil was accelerated more than ten times in the presence of the mussel *Mytilus edulis* (Gudimov, 2002). However, recalcitrant contaminants may not be transformed after ingestion and may instead accumulate in the bivalve tissue (Verrengia Guerrero *et al.*, 2002; Drouillard *et al.*, 2007). These contaminants may then be released through the excretion of pseudofeces and feces (Haven and Morales-Alamo, 1966; Hull *et al.*, 2011). Depending on the affinity of the compound for the (pseudo)feces, the contaminants may then desorb and reenter the water column, be consumed by benthic organisms, or be transformed by microbes. Further research is needed to determine whether particle-associated trace organic contaminants commonly present in wastewater effluent, such as the musk fragrances, are effectively removed and transformed by bivalves in a wetland cell.

Bivalves are also capable of ingesting a wide variety of pathogens (Silverman *et al.*, 1995; Graczyk *et al.*, 2003, 2006; Proakis, 2003; Nappier *et al.*, 2008). Given their ability to efficiently remove particles greater than 0.4 μm in diameter from water, bivalves may filter individual bacteria (0.5-2 μm) and protozoan (oo)cysts (2-15 μm), although not individual viruses (20-100 nm). For example, the zebra mussel (*Dreissena polymorpha*) was found to remove *E. coli* and other bacteria from pond water at a clearance rate of about 6 L (g dry tissue)⁻¹ hr⁻¹ (Silverman *et al.*, 1995). Given this rate, a density of 130 mussels L⁻¹ (approximately 16 mg dry weight (mussel)⁻¹) would result in 90% clearance of bacteria (1-log removal) over a 3 day residence time. Further research is needed, however, to determine whether bivalve grazing results in pathogen inactivation or just accumulation. Limited studies

have shown recovery of viable *Giardia* spp. and infectious *C. parvum* oocysts in *Macoma* spp. and oysters, respectively (Fayer *et al.*, 1998; Graczyk *et al.*, 1999).

Within a unit process wetland, bivalves could be implemented in shallow, open-water cells. Periphyton-derived organic matter or organic matter from wastewater or previous wetland cells would provide sufficient particulate matter for ingestion, and bivalves would enhance contaminant and pathogen removal within the cell. Bivalves could be kept in cages to protect them from predators. Cages would also allow bivalves saturated with recalcitrant contaminants to be easily removed and depurated by exposure to clean water at regular intervals if necessary (Burns and Smith, 1981; Pruell *et al.*, 1986; Peven *et al.*, 1996). Native species should be employed in these wetland cells to avoid invasive species entering receiving surface waters. While further research is necessary to determine appropriate bivalve species, densities, and configurations to maximize treatment efficiency in a unit process wetland, implementation of such a wetland cell could significantly increase attenuation of certain trace organic contaminants and pathogens not effectively removed in other wetland cells, such as particle-associated trace organic contaminants and pathogens that are too small to be removed by sedimentation and that are not susceptible to photoinactivation.

2.7 Conclusions

There is growing interest in integrating large-scale treatment wetlands into urban water infrastructure to improve water quality. Treatment wetlands for polishing effluents may offer advantages over mechanical treatment systems due to their low operating cost and potential to remove a variety of difficult-to-treat contaminants. In addition, treatment wetlands offer aesthetic and habitat benefits in urban spaces. However, a better understanding of how to design these natural barriers to provide predictable treatment of target contaminants is needed.

Applying the unit process approach to wetland design has the potential to contribute to more flexible and predictable treatment. It also provides a framework for applying a mechanistic understanding to system optimization. Nonetheless, there are major challenges associated with harmonizing regulations and avoiding unintended consequences associated with large wetland systems. Active management of treatment wetlands and a better understanding of attenuation processes will be required. After we understand what this encompasses, we will have a better idea of how the technology compares with other options.

CHAPTER 3. Phototransformation of wastewater-derived trace organic contaminants in open-water unit process treatment wetlands

Reproduced with permission from Jasper, J.T.; Sedlak, D.L. Phototransformation of wastewater-derived trace organic contaminants in open-water unit process treatment wetlands. *Environ. Sci. Technol.* **2013**, *47*, 10781-10790.

© 2013 American Chemical Society.

3.1 Introduction

Due to their incomplete removal during biological wastewater treatment, a variety of trace organic contaminants are frequently detected in municipal wastewater effluent (Heberer, 2002; Ternes *et al.*, 2004b). In the absence of significant dilution, some of these contaminants pose risks to aquatic biota (Purdom *et al.*, 1994; Triebskorn *et al.*, 2007) and are an issue of concern for downstream drinking water supplies (Snyder *et al.*, 2003; National Research Council, 2012). While reverse osmosis, ozonation, and granular activated carbon can remove many wastewater-derived trace organic contaminants (Huber *et al.*, 2003; Snyder *et al.*, 2007), this additional treatment is expensive and often produces wastes that require disposal. Chemical oxidation can also produce disinfection byproducts of concern.

Constructed wetlands have been used since the 1970s to remove nitrate and phosphate from municipal wastewater effluent (Kadlec and Wallace, 2009). More recently, researchers have considered using them to remove trace organic contaminants (Chapter 2). In vegetated wetlands, microbes associated with plant surfaces remove many of the trace organic contaminants that are susceptible to biotransformation during activated sludge treatment (Matamoros *et al.*, 2008b). However, rates of biotransformation are often slower in wetlands than in treatment plants.

Sunlight photolysis can transform many wastewater-derived trace organic contaminants detected in wastewater effluent (Andreozzi *et al.*, 2003; Lin and Reinhard, 2005; Fono *et al.*, 2006). This process is usually unimportant in wetlands because shading of the water surface by emergent macrophytes and floating vegetation reduces light penetration. While integration of open waters is often desirable in treatment wetlands as waterfowl habitat (Fleming-Singer and Horne, 2006) and as a means of enhancing mixing (Lightbody *et al.*, 2007), sunlight photolysis in deep, open waters is generally slow (Matamoros *et al.*, 2008b). Alternatively, wetlands constructed in a unit process fashion, with individual cells exhibiting distinctly different characteristics, can integrate shallow, open-water cells that exploit photolysis as a means of removing trace organic contaminants (Chapter 2).

To evaluate the potential of open-water unit process cells to transform trace organic contaminants, a photochemical model was developed and validated under representative conditions using compounds that undergo photolysis by different mechanisms. Using previously published data, the model was used to predict the removal of cimetidine, diuron, NDMA, and 17 β -estradiol. The model was also used to estimate the area needed to reduce the concentration of trace organic contaminants produced by a 1 million gallon per day (MGD) nitrifying wastewater treatment plant by 90%.

3.2 Photolysis Model

The first-order photolysis rate constant for trace organic contaminants (k_{phot}) includes transformation via direct photolysis in addition to indirect photolysis via reaction with hydroxyl radical ($\cdot\text{OH}$) (Zepp *et al.*, 1987; Brezonik and Fulkerson-Brekken, 1998), singlet oxygen ($^1\text{O}_2$) (Zepp *et al.*, 1977; Latch *et al.*, 2003b), excited triplet state dissolved organic matter ($^3\text{DOM}^*$) (Canonica *et al.*, 1995; Gerecke *et al.*, 2001; Boreen *et al.*, 2004), and carbonate radical ($\cdot\text{CO}_3^-$) (Huang and Mabury, 2000c; Canonica *et al.*, 2005), giving:

$$k_{\text{photo}} = k_{\text{direct}} + k_{\cdot\text{OH},\text{cont}}[\cdot\text{OH}]_{\text{ss}} + k_{\cdot\text{CO}_3^-, \text{cont}}[\cdot\text{CO}_3^-]_{\text{ss}} + k_{^1\text{O}_2, \text{cont}}[^1\text{O}_2]_{\text{ss}} + k_{^3\text{DOM}^*, \text{cont}} \quad (3.1)$$

where k_{direct} is the first-order direct photolysis rate constant $k_{\cdot\text{OH},\text{cont}}$, $k_{\cdot\text{CO}_3^-, \text{cont}}$, and $k_{^1\text{O}_2, \text{cont}}$ are the second-order rates constants between contaminants and $\cdot\text{OH}$, $\cdot\text{CO}_3^-$, and $^1\text{O}_2$ respectively; $k_{^3\text{DOM}^*, \text{cont}}$ is the pseudo first-order rate constant for reaction of $^3\text{DOM}^*$ with a contaminant; and $[\cdot\text{OH}]_{\text{ss}}$, $[\cdot\text{CO}_3^-]_{\text{ss}}$, and $[^1\text{O}_2]_{\text{ss}}$ are the steady-state concentrations of each species. For contaminants with relevant pK_a values, k_{photo} is a function of pH, given the quantum yield and reaction rates with reactive species for the protonated and unprotonated contaminant, and the fraction of the contaminant protonated and deprotonated (f) (e.g., $k_{\text{direct}} = f_{\text{deprotonated}}k_{\text{direct, deprotonated}} + f_{\text{protonated}}k_{\text{direct, protonated}}$).

k_{direct} can be calculated from daily-averaged solar irradiance ($Z(24 \text{ hr}, \lambda)$) for conditions at 40° N latitude (Figure 3.1) (Gueymard, 2003); the molar absorption coefficient ($\epsilon(\lambda)$) (Figure 3.2); and the quantum yield (Φ ; see Table 3.1) (Schwarzenbach *et al.*, 2003):

$$k_{\text{direct}} = 2.3 \Phi \sum_{\lambda} S(\lambda) Z(24 \text{ hr}, \lambda) \epsilon(\lambda) \quad (3.2)$$

$S(\lambda)$ is a light-screening factor, which accounts for light absorption in a well-mixed body of water and is calculated by (Schwarzenbach *et al.*, 2003):

$$S(\lambda) = \frac{1 - 10^{-1.2 \alpha(\lambda) z}}{(2.3)(1.2) \alpha(\lambda) z} \quad (3.3)$$

where $\alpha(\lambda)$ is the beam attenuation coefficient and z is the well-mixed depth of the water column. $\alpha(\lambda)$ is estimated by assuming it is related linearly to the concentration of dissolved organic carbon:

$$\alpha(\lambda) = m(\lambda)[\text{DOC}] + b(\lambda) \quad (3.4)$$

Values for $m(\lambda)$ and $b(\lambda)$ (Table 3.2) were obtained using UV/Vis spectra of pilot-scale wetland water taken at dissolved organic carbon concentrations ranging from 5 to 11 $\text{mg L}^{-1}\text{-C}$ (see Figure 3.3 for sample plot). The obtained relationship has a non-zero y-intercept (i.e., $b(\lambda) > 0$), most likely due to differences in types of dissolved organic carbon in different samples (e.g., variations in the fractions of protein-derived organic carbon and humic substances present in the wetland water).

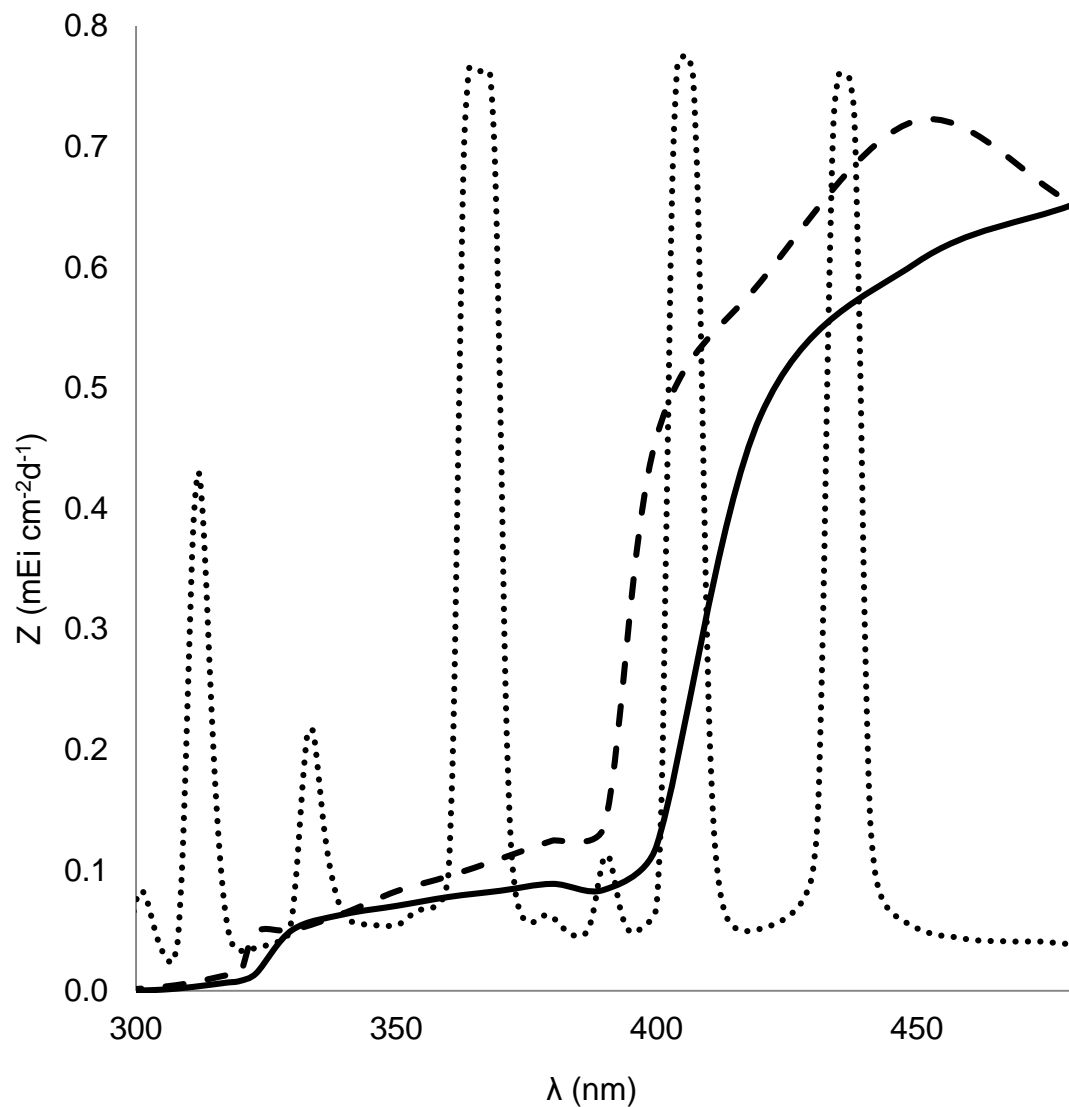


Figure 3.1: Predicted irradiance under clear skies at sea level ($Z(24 \text{ h}, \lambda)$) of June 21st sunlight at 40°N latitude (—) (Gueymard, 2003); irradiance of solar simulator ($Z(\lambda)$) with atmospheric filter (---); and irradiance of 500 W medium-pressure mercury lamp ($\bullet\bullet\bullet$), measured with a Stellarnet spectroradiometer (EPP2000C-SR-100 with CR2 cosine receptor).

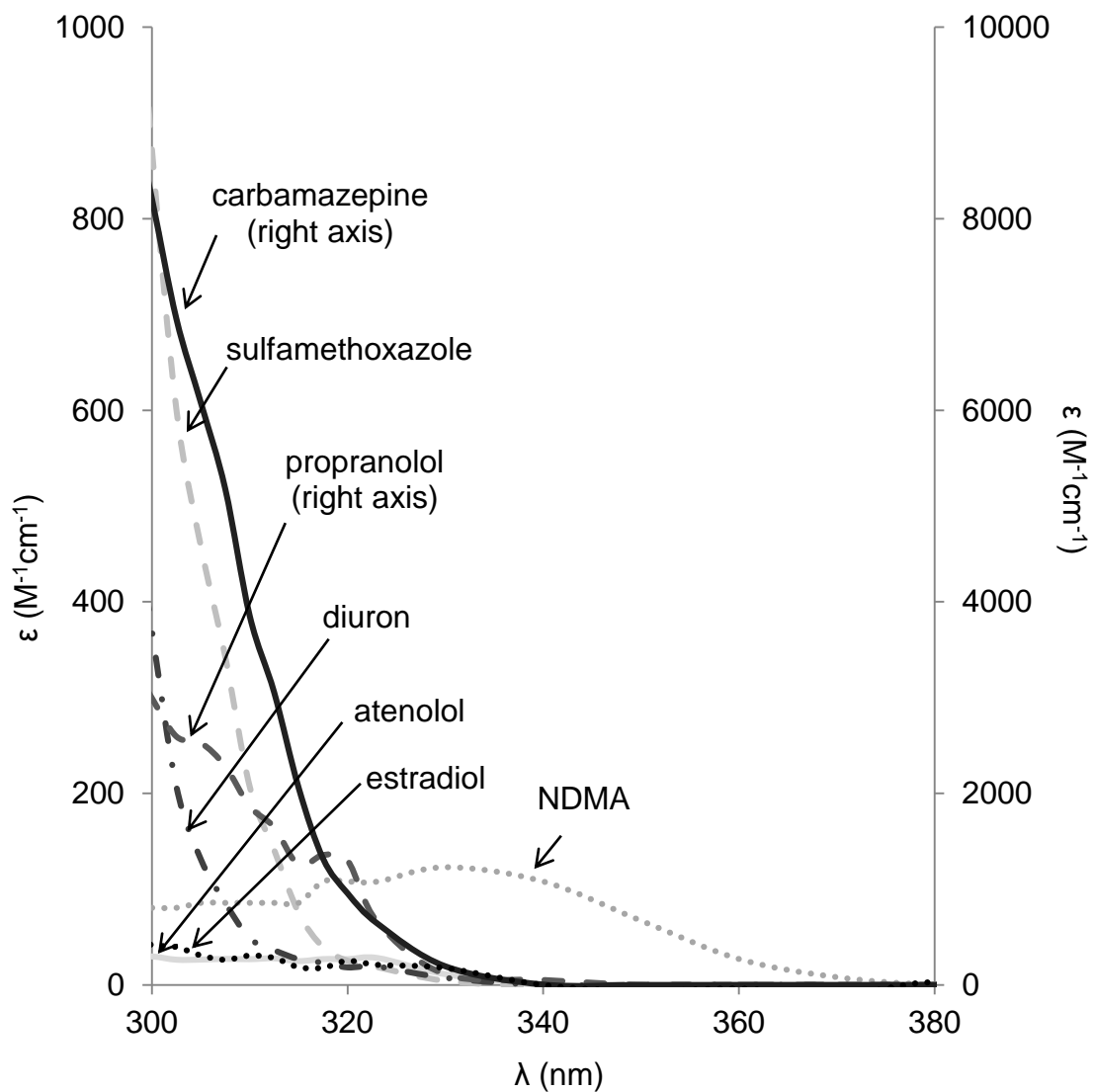


Figure 3.2: Molar absorption coefficient (ϵ) of test compounds as a function of wavelength. Left axis: atenolol, NDMA, diuron, and sulfamethoxazole; right axis: carbamazepine and propranolol.

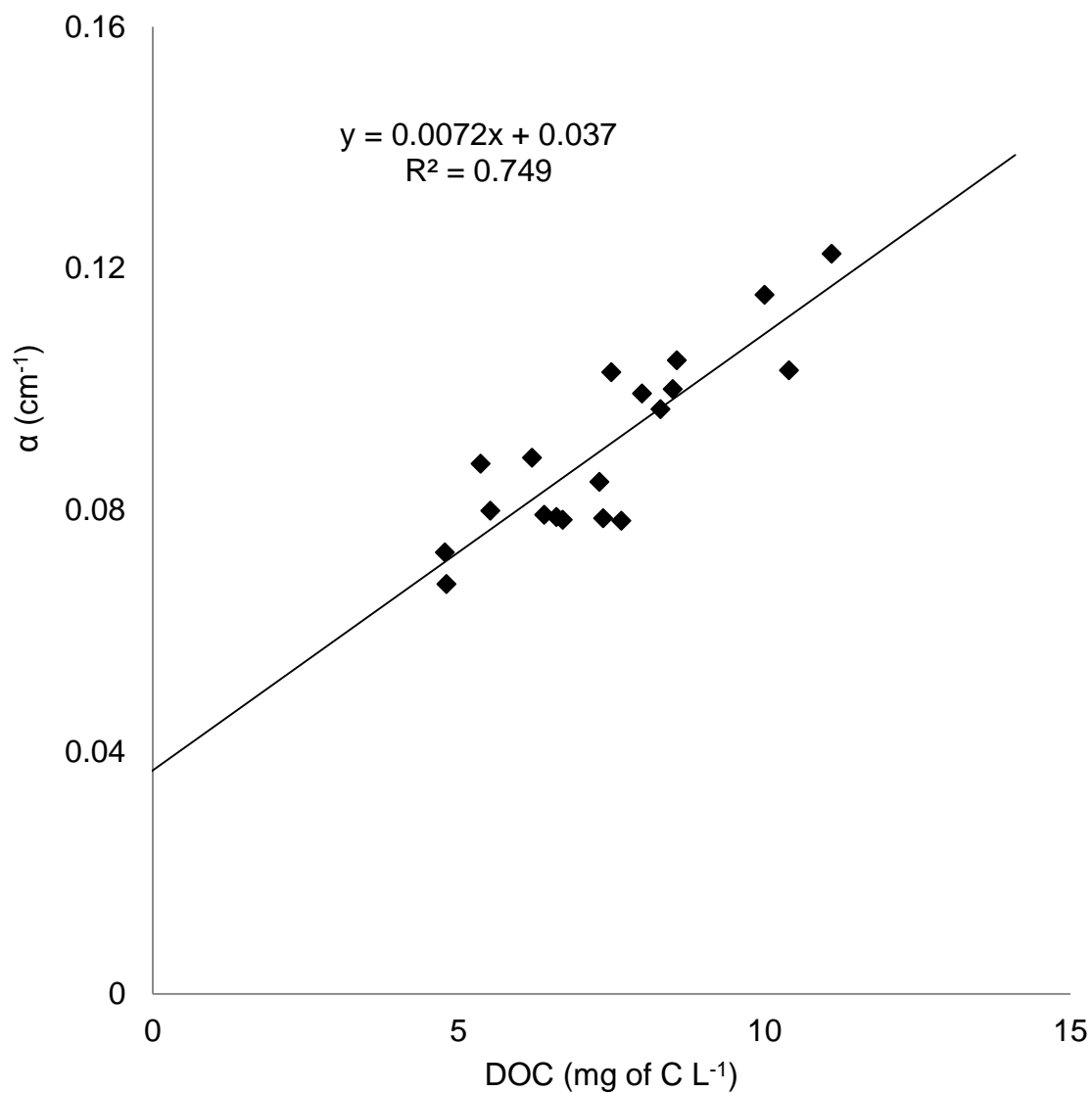


Figure 3.3: Absorbance at 310 nm ($\alpha(310)$) of wetland water collected at different days throughout the wetland with varying concentrations of DOC. Slope and intercept are $m(310 \text{ nm})$ and $b(310 \text{ nm})$ respectively, which are tabulated in Table 3.2.

Table 3.2: Parameters Used to Calculate Wetland Water Absorbance ($\alpha(\lambda)$)^a

λ center	$m(\lambda)$ ($\text{cm}^{-1}(\text{mg L}^{-1}\text{-C})^{-1}$)	$b(\lambda)$ (cm^{-1})
297.5	9.2×10^{-3}	4.2×10^{-2}
300	8.6×10^{-3}	4.2×10^{-2}
302.5	8.1×10^{-3}	4.1×10^{-2}
305	7.8×10^{-3}	4.0×10^{-2}
307.5	7.5×10^{-3}	3.8×10^{-2}
310	7.2×10^{-3}	3.7×10^{-2}
312.5	7.0×10^{-3}	3.5×10^{-2}
315	6.9×10^{-3}	3.3×10^{-2}
317.5	6.6×10^{-3}	3.0×10^{-2}
320	6.5×10^{-3}	2.8×10^{-2}
323.1	6.3×10^{-3}	2.6×10^{-2}
330	6.1×10^{-3}	2.0×10^{-2}
340	5.3×10^{-3}	1.6×10^{-2}
350	4.6×10^{-3}	1.4×10^{-2}
360	4.1×10^{-3}	1.1×10^{-2}
370	3.5×10^{-3}	9.7×10^{-3}
380	3.0×10^{-3}	8.1×10^{-3}
390	2.6×10^{-3}	7.2×10^{-3}
400	2.3×10^{-3}	6.1×10^{-3}
420	1.7×10^{-3}	4.4×10^{-3}
450	1.2×10^{-3}	3.5×10^{-3}
480	8.2×10^{-4}	2.3×10^{-3}

^a $a(\lambda) = m(\lambda)[\text{DOM}] + b(\lambda)$.

$\cdot\text{OH}$ may be formed through the photolysis of colored dissolved organic matter (CDOM) or NO_3^- . $\cdot\text{OH}$ is scavenged in natural waters by DOM, HCO_3^- , and CO_3^{2-} . Thus $[\cdot\text{OH}]_{ss}$ can be calculated by:

$$[\cdot\text{OH}]_{ss} = \frac{R_{\text{form},\cdot\text{OH},\text{NO}_3^-} + R_{\text{form},\cdot\text{OH},\text{DOM}}}{k_{\text{OH},\text{HCO}_3^-}[\text{HCO}_3^-] + k_{\text{OH},\text{CO}_3^{2-}}[\text{CO}_3^{2-}] + k_{\text{OH},\text{DOM}}[\text{DOM}]} \quad (3.5)$$

where $k_{\text{OH},\text{HCO}_3^-}$, $k_{\text{OH},\text{CO}_3^{2-}}$, and $k_{\text{OH},\text{DOM}}$ are the second-order reaction rates between $\cdot\text{OH}$ and water constituents (see Table 3.3 for values). $R_{\text{form},\cdot\text{OH},\text{NO}_3^-}$ and $R_{\text{form},\cdot\text{OH},\text{DOM}}$ are the formation rates (M s^{-1}) of $\cdot\text{OH}$ from NO_3^- and DOM, which may be calculated using:

$$R_{\text{form},\cdot\text{OH},\text{NO}_3^-} = \Phi_{\text{NO}_3^-, \cdot\text{OH}} [\text{NO}_3^-] \sum_{\lambda} 2.3 S(\lambda) \epsilon_{\text{NO}_3^-}(\lambda) Z(\lambda) \quad (3.6)$$

$$R_{\text{form},\cdot\text{OH},\text{DOM}} = \Phi_{\text{DOM}, \cdot\text{OH}} \sum_{\lambda} Z(\lambda) \frac{1 - 10^{-1.2 \alpha(\lambda)z}}{1.2 \alpha(\lambda)} \quad (3.7)$$

$\Phi_{\text{NO}_3^-, \cdot\text{OH}}$ and $\Phi_{\text{DOM}, \cdot\text{OH}}$ are the quantum efficiencies for the production of $\cdot\text{OH}$ from NO_3^- and DOM and were selected from the range of previously reported values to give the best agreement between predicted photolysis rates for test compounds and those measured with a solar simulator (see Table 3.3 for values). It should be noted that in calculating $R_{\text{form},\cdot\text{OH},\text{DOM}}$ it is assumed that DOM is primarily responsible for $\alpha(\lambda)$ and the fraction in the equation represents the proportion of light absorbed by DOM (Schwarzenbach *et al.*, 2003).

$\cdot\text{CO}_3^-$ is produced through the reaction of $\cdot\text{OH}$ with HCO_3^- and CO_3^{2-} and reacts primarily with DOM, so that:

$$[\cdot\text{CO}_3^-]_{ss} = \frac{k_{\text{OH},\text{HCO}_3^-}[\text{HCO}_3^-][\cdot\text{OH}]_{ss} + k_{\text{OH},\text{CO}_3^{2-}}[\text{CO}_3^{2-}][\cdot\text{OH}]_{ss}}{k_{\text{CO}_3^-, \text{DOM}}[\text{DOM}]} \quad (3.8)$$

$k_{\text{CO}_3^-, \text{DOM}}$ is the second-order reaction rate between $\cdot\text{CO}_3^-$ and DOM (see Table 3.3 for values).

$[^1\text{O}_2]_{ss}$ is approximated by correcting near-surface summer-noon values of $[^1\text{O}_2]_{ss}$ per mg of effluent organic matter ($\sim 1 \times 10^{-14} \text{ M (mg of C)}^{-1}$), for screening and light intensity (Haag and Hoigné, 1986):

$$[^1\text{O}_2]_{ss} = 1 \times 10^{-14} S(410 \text{ nm}) \frac{Z(410 \text{ nm})}{Z_{\text{surface, summer-noon}}(410 \text{ nm})} \quad (3.9)$$

Corrections are made at 410 nm, since this wavelength was previously determined to give the best approximation of $[^1\text{O}_2]_{ss}$ (Haag and Hoigné, 1986). $Z_{\text{surface, summer-noon}}(410 \text{ nm})$ is approximately $0.82 \text{ mEi cm}^{-2} \text{ d}^{-1}$ (Gueymard, 2003).

Reaction rates of DOM with trace organic contaminants ($k_{\text{DOM}^*, \text{cont}}$) can be estimated using a wavelength-dependent quantum yield coefficient, $f(\lambda)$ ($\text{Ei}^{-1} \text{ L}$), which accounts for the efficiency of DOM light absorption leading to transformation of a contaminant (Canonica *et al.*, 1995; Gerecke *et al.*, 2001):

$$k_{3\text{DOM}^*,\text{cont}} = \sum_{\lambda} f(\lambda)Z(\lambda) \frac{1-10^{-1.2 \alpha(\lambda)z}}{1.2 \alpha(\lambda)} \quad (3.10)$$

Although $f(\lambda)$ values are not available for most contaminants, and variation between different DOM sources may be significant (Gerecke *et al.*, 2001), this formulation provides a method to account for changes in water depth and DOM light absorption given a known $k_{3\text{DOM}^*,\text{cont}}$ value.

Table 3.3: Conditions Employed in Photolysis Model

<u>parameter</u>	<u>value(s)</u>
depth (cm)	0-50
pH	7-10
[NO ₃ ⁻] (mg L ⁻¹ -N)	0-20
[DOC] (mg L ⁻¹ -C)	1-15
[DIC] (mg L ⁻¹ -C) ^a	60
Z(24 hr, λ) (mEi cm ⁻² d ⁻¹) ^b	throughout year
$\Phi_{\text{NO}_3^-, \cdot\text{OH}}$ ^c	1.6x10 ⁻²
$\Phi_{\text{DOM}, \cdot\text{OH}}$ ^c	3.7x10 ⁻⁵
[¹ O ₂] _{ss, near-surface, noon} (M (mg L ⁻¹ -C) ⁻¹) ^d	1x10 ⁻¹⁴
$k_{\cdot\text{OH}, \text{DOM}}$ ((mg L ⁻¹ -C) ⁻¹ s ⁻¹) ^e	1.7x10 ⁴
$k_{\cdot\text{CO}_3^-, \text{DOM}}$ ((mg L ⁻¹ -C) ⁻¹ s ⁻¹) ^e	3.7x10 ²
$k_{\cdot\text{OH}, \text{HCO}_3^-}$ (M ⁻¹ s ⁻¹) ^{ref a}	8.5x10 ⁶
$k_{\cdot\text{OH}, \text{CO}_3^{2-}}$ (M ⁻¹ s ⁻¹) ^{ref a}	3.9x10 ⁸
$\alpha(\lambda)$ ^f	$m(\lambda)[\text{DOC}] + b(\lambda)$

^aDissolved inorganic carbon. ^b24 hour-averaged solar intensity at 40° N latitude under clear skies on the 21st day of each month (Gueymard, 2003). ^cQuantum yields for formation of $\cdot\text{OH}$ from DOM and NO₃⁻. Values were selected from the range available in the literature (i.e., $\Phi_{\text{NO}_3^-, \cdot\text{OH}}=0.010-0.017$; $\Phi_{\text{DOM}, \cdot\text{OH}}=3.0-9.8 \times 10^{-5}$) (Zepp *et al.*, 1987; Dong and Rosario-Ortiz, 2012) to give the best agreement between model predictions and solar simulator measurements. ^dAverage value for previously studied wastewaters (Haag and Hoigné, 1986). ^eDetermined in this study. ^fLight absorption by wetland water estimated by assuming a linear relationship between dissolved organic carbon ([DOC]) and $\alpha(\lambda)$. Values for $m(\lambda)$ and $b(\lambda)$ are given in Table 3.2. References: a: (Buxton *et al.*, 1988).

3.3 Materials and Methods

3.3.1 Materials

All reagents were purchased from Fisher Scientific (Fairlawn, NJ) at the highest available purity. Isotopically-labeled internal standards were purchased from CDN Isotopes (Quebec, Canada), except for sulfamethoxazole-d4, which was purchased from Toronto Research Chemicals, Inc. (Ontario, Canada). All solutions were prepared using Milli-Q water from a Millipore system.

3.3.2 Wetland Water and Conditions

Photolysis experiments were conducted in water collected from a pilot-scale open-water unit process wetland located in Discovery Bay, CA. The open-water cell received about 10,000 gallons per day ($4.4 \times 10^{-4} \text{ m}^3 \text{ s}^{-1}$) of nitrified wastewater effluent from the adjacent municipal wastewater treatment plant. The 400 m^2 cell was approximately 20 cm deep and divided with 3 baffles to give nearly plug-flow hydraulics, with a hydraulic residence time ranging from 1 to 3 days. The bottom of the cell was lined with concrete or geotextile fabric to prevent the growth of emergent macrophytes. 2- to 5-cm of organic-matter-rich sediments with large numbers of photosynthetic organisms accumulated on the cell bottom.

Wetland water collected from the Discovery Bay open-water pilot-scale wetland contained on average $10\text{-}20 \text{ mg L}^{-1}\text{-N NO}_3^-$, $5\text{-}10 \text{ mg L}^{-1}\text{-C DOC}$, and $60\text{-}80 \text{ mg L}^{-1}\text{-C}$ dissolved inorganic carbon (HCO_3^- and CO_3^{2-}). This variation in dissolved inorganic carbon concentrations was not predicted to affect photolysis rates significantly. The conductivity of wetland water was typically around $1800 \text{ }\mu\text{S cm}^{-1}$. A typical UV/Vis spectrum of the wetland water ($\alpha(\lambda)$) is given in Figure 3.4. The pH in the wetland exhibited diurnal changes (Figure 3.5), varying between values of about 8.5 and 10, due to production and consumption of CO_2 .

Water samples used in this study were collected in clean, amber glass bottles from the mid-point of the wetland, filtered through a $1\text{-}\mu\text{m}$ glass fiber filter, and stored at 5° C prior to experiments.

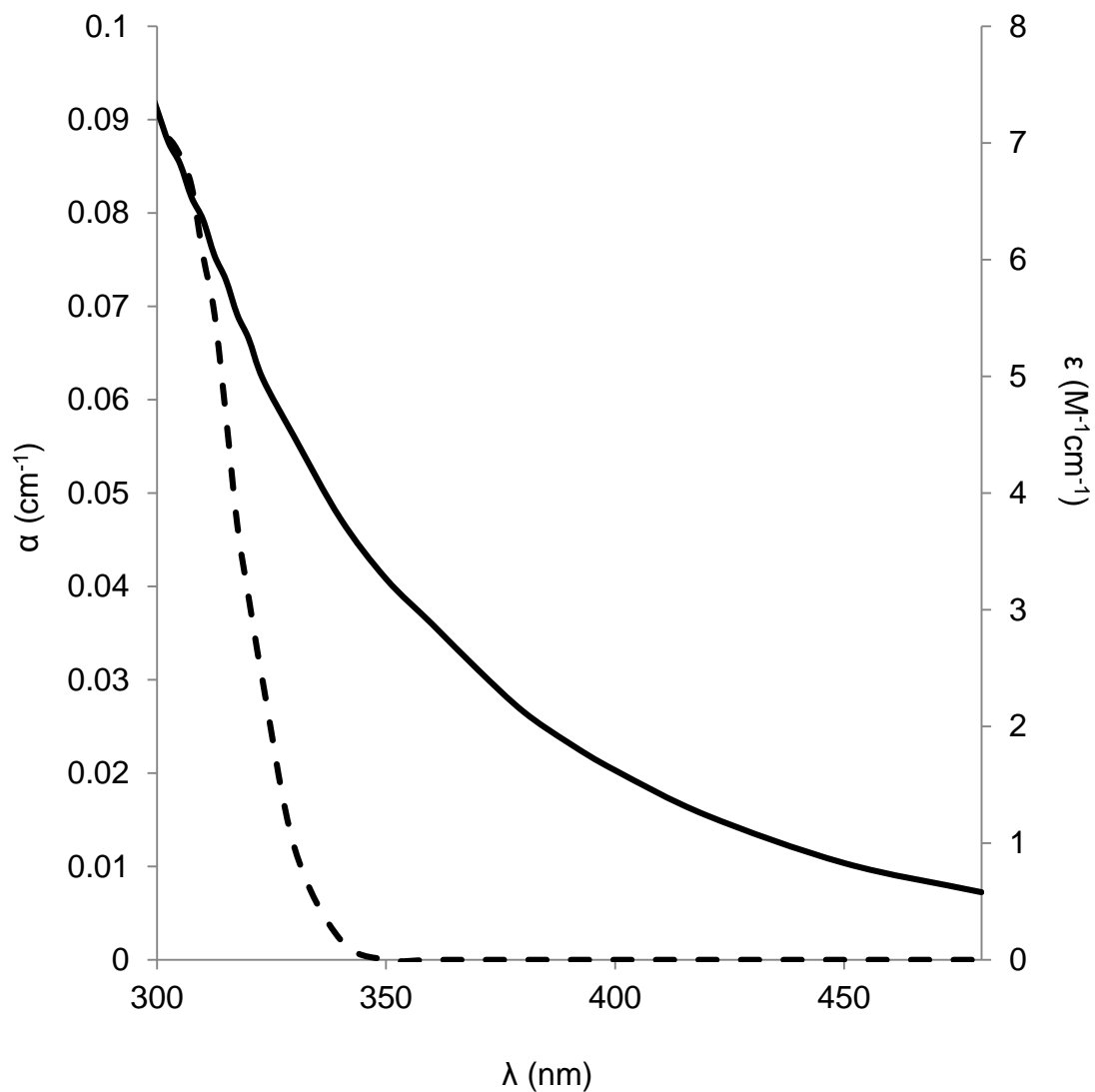


Figure 3.4: Typical absorbance of wetland water with 8 mg L⁻¹-C DOM, calculated with $\alpha(\lambda)=m(\lambda)[\text{DOM}]+b(\lambda)$, using values found in Table 3.2 (left axis, ==) and molar absorption coefficient (ϵ) of NO_3^- (right axis, - - -).

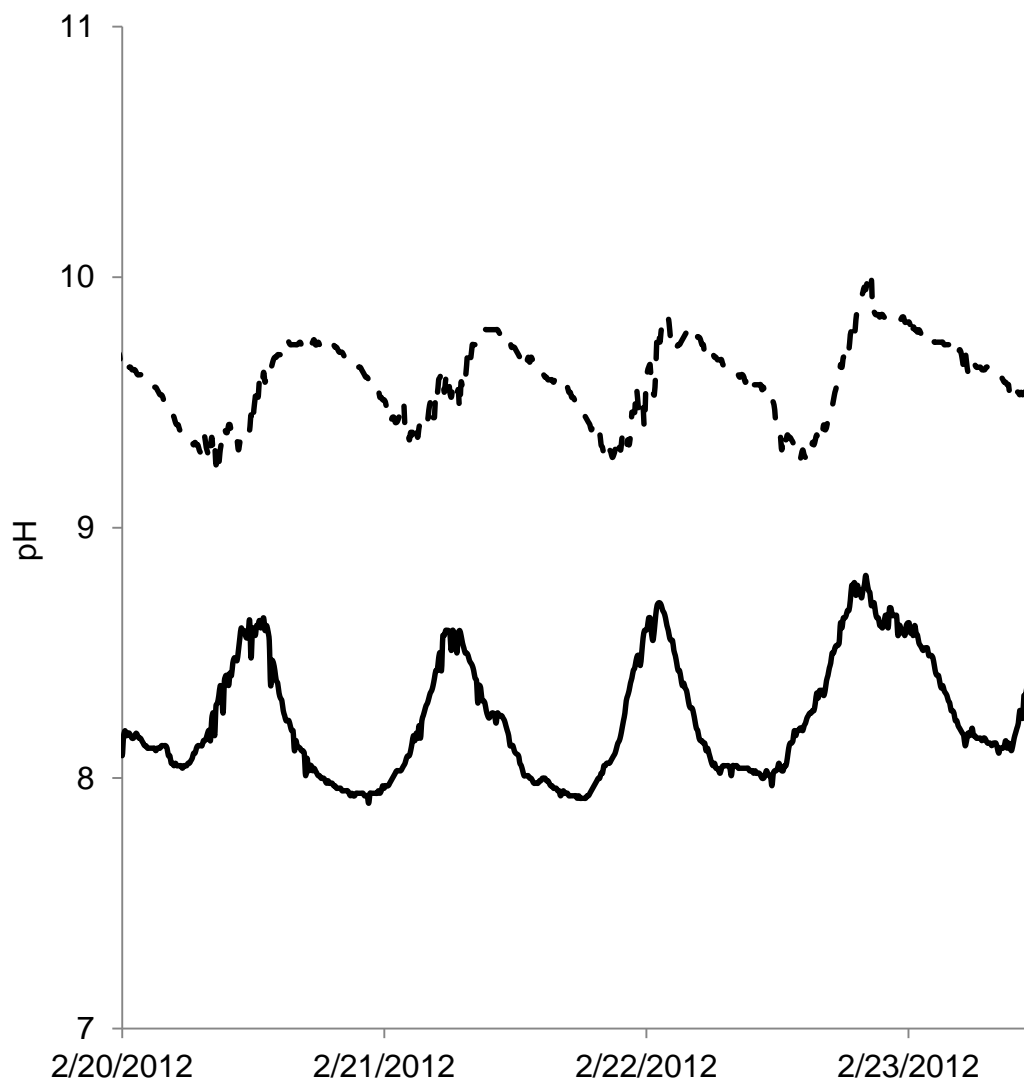


Figure 3.5: Inlet (—) and outlet (---) pH values in the shallow, open-water wetland cell at the Discovery Bay wetlands.

3.3.3 Reaction Rate Constants for Carbonate Radical

Second-order rate constants for the reaction of test compounds with carbonate radical ($\cdot\text{CO}_3^-$) were measured using photosensitizer based competition kinetics methods (Canonica *et al.*, 2005). Briefly, test compounds (~ 2.5 nM) were irradiated in capped, 10-mL borosilicate test tubes with a path length of 1-cm, using a merry-go-round reactor equipped with a 500-W medium-pressure mercury lamp at 25° C. Solutions contained isoproturon as a reference compound ($k_{\cdot\text{CO}_3^-, \text{isoproturon}} = 3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$) (Canonica *et al.*, 2005), along with 4-carboxybenzophenone (CBBP) or duroquinone (DQ) as a photosensitizer (5 μM). Solution pH was adjusted by varying the concentrations of sodium carbonate and sodium bicarbonate buffer solutions (total dissolved inorganic carbon = 0.5-1 M). pH values higher than those typically encountered in sunlit surface waters were used in laboratory experiments to minimize side reactions with sensitizers and to ensure that amine functional groups were predominantly deprotonated. Samples were collected sacrificially at regular intervals. Assuming that losses of test compounds and isoproturon were due only to reaction with $\cdot\text{CO}_3^-$, the following equation could be used to estimate the rate of reaction of a compound with $\cdot\text{CO}_3^-$ ($k_{\cdot\text{CO}_3^-, \text{compound}}$):

$$\ln \left[\frac{[\text{compound}]_t}{[\text{compound}]_o} \right] = \frac{k_{\cdot\text{CO}_3^-, \text{compound}}}{k_{\cdot\text{CO}_3^-, \text{isoproturon}}} \ln \left[\frac{[\text{isoproturon}]_t}{[\text{isoproturon}]_o} \right] \quad (3.11)$$

The rate constant was obtained from a linear regression of $\ln \left[\frac{[\text{compound}]_t}{[\text{compound}]_o} \right]$ versus $\ln \left[\frac{[\text{isoproturon}]_t}{[\text{isoproturon}]_o} \right]$.

3.3.4 Reaction Rate Constants for Hydroxyl Radical and Carbonate Radical with Wetland Dissolved Organic Matter

Steady-state concentrations of $\cdot\text{OH}$ and $\cdot\text{CO}_3^-$ were calculated from the disappearance rates of probe compounds irradiated as described above in wetland water buffered with sodium borate buffer (10 mM) at pH values ranging from 8 to 10. Under these conditions, disappearance of probe compounds exhibited pseudo-first order kinetics ($r^2 > 0.97$). Hydroxyl radical steady-state concentration ($[\cdot\text{OH}]_{ss}$) was measured using *para*-chlorobenzoic acid (*p*CBA) as a probe ($[p\text{CBA}]_o \sim 5 \mu\text{M}$; $k_{\cdot\text{OH}, p\text{CBA}} = 5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) (Buxton *et al.*, 1988). $[\cdot\text{CO}_3^-]_{ss}$ was measured using *N,N*-dimethylaniline (DMA) as a probe ($[\text{DMA}]_o \sim 5 \mu\text{M}$; $k_{\cdot\text{CO}_3^-, \text{DMA}} = 1.8 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) (Neta *et al.*, 1988; Huang and Mabury, 2000a). Data were corrected for direct photolysis of DMA and loss of DMA by reaction with $\cdot\text{OH}$ ($k_{\cdot\text{OH}, \text{DMA}} = 1.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$) (Buxton *et al.*, 1988) using measured $[\cdot\text{OH}]_{ss}$.

Estimates of the rate constants for the reaction of radicals with wetland dissolved organic matter (i.e., $k_{\cdot\text{OH}, \text{DOM}}$ and $k_{\cdot\text{CO}_3^-, \text{DOM}}$) were made by fitting measured steady-state radical concentrations at four or more pH values to the following equations:

$$[\cdot\text{OH}]_{ss} = \frac{R_{\text{form}, \cdot\text{OH}, \text{NO}_3^-} + R_{\text{form}, \cdot\text{OH}, \text{DOM}}}{k_{\cdot\text{OH}, \text{HCO}_3^-} [\text{HCO}_3^-] + k_{\cdot\text{OH}, \text{CO}_3^{2-}} [\text{CO}_3^{2-}] + k_{\cdot\text{OH}, \text{DOM}} [\text{DOM}]} \quad (3.12)$$

and

$$[\cdot\text{CO}_3^-]_{ss} = \frac{k_{\cdot\text{OH},\text{HCO}_3^-}[\text{HCO}_3^-][\cdot\text{OH}]_{ss} + k_{\cdot\text{OH},\text{CO}_3^{2-}}[\text{CO}_3^{2-}][\cdot\text{OH}]_{ss}}{k_{\cdot\text{CO}_3^-, \text{DOM}}[\text{DOM}]} \quad (3.13)$$

where $R_{\text{form}, \cdot\text{OH}, \text{NO}_3^-}$ and $R_{\text{form}, \cdot\text{OH}, \text{DOM}}$ are the formation rates of $\cdot\text{OH}$ from photolysis of NO_3^- and dissolved organic matter, respectively, and $k_{\cdot\text{OH}, \text{HCO}_3^-}$, $k_{\cdot\text{OH}, \text{CO}_3^{2-}}$, $k_{\cdot\text{OH}, \text{DOM}}$, and $k_{\cdot\text{CO}_3^-, \text{DOM}}$ are second-order reaction rates between radical species and dissolved carbon species. Previously measured values of $k_{\cdot\text{OH}, \text{HCO}_3^-}$ and $k_{\cdot\text{OH}, \text{CO}_3^{2-}}$ were used (Table 3.3). The formation rate of $\cdot\text{OH}$ from NO_3^- and DOM when irradiated with a medium-pressure mercury lamp ($R_{\text{form}, \cdot\text{OH}, \text{NO}_3^-} + R_{\text{form}, \cdot\text{OH}, \text{DOM}}$) was calculated by measuring the formation rate of phenol from a solution of benzene in wetland water, given a reaction yield of 0.85 moles of phenol per mole of benzene that reacts with $\cdot\text{OH}$ (Dong and Rosario-Ortiz, 2012). The concentration of benzene employed (i.e., ~3 mM) was calculated to scavenge greater than 99% of the $\cdot\text{OH}$ formed. The method of least squares was used to determine the remaining unknown parameters, $k_{\cdot\text{OH}, \text{DOM}}$ and $k_{\cdot\text{CO}_3^-, \text{DOM}}$.

3.3.5 Photolysis of Representative Compounds in Wetland Water

The contribution of different photolysis mechanisms to contaminant removal in wetland water was investigated by irradiating wetland water as described above. Air-saturated solutions were amended with approximately 50 nM of each of four compounds (i.e., atenolol, propranolol, sulfamethoxazole, and carbamazepine) from a concentrated aqueous stock solution prior to irradiation. Solutions were modified to isolate the contributions of different reactive species: 1% isopropanol (IPA) was used to quench $\cdot\text{OH}$ reactions (Packer *et al.*, 2003); 0.1% isoprene was used to quench $^3\text{DOM}^*$ (Boreen *et al.*, 2008); sparging with nitrogen gas (N_2) was used to evaluate the importance of $^1\text{O}_2$ and $^3\text{DOM}^*$ (Ryan *et al.*, 2011); and adjustment of pH to values between 8 and 10.5 was used to evaluate the effects of pH on photolysis.

3.3.6 Predicting Photolysis Rates in Unit Process Wetlands

Predictions of photolysis rates of trace organic contaminants in open-water unit process cells (k_{photo}) included contributions from direct photolysis, in addition to indirect photolysis via reactions with $\cdot\text{OH}$, $\cdot\text{CO}_3^-$, $^1\text{O}_2$, and $^3\text{DOM}^*$. The conditions employed in the model calculations are summarized in Table 3.3. Properties of contaminants necessary for the estimation of photolysis rates at a given pH value were calculated from experimental data or obtained from previous publications (see Table 3.1).

As described above, direct photolysis rates were calculated using measured molar absorption coefficients and 24-hour averaged solar irradiances for clear skies at 40° N latitude ($Z(24 \text{ hr}, \lambda)$) (Gueymard, 2003) and were corrected for light screening and depth using a screening factor (Schwarzenbach *et al.*, 2003). Steady-state $\cdot\text{OH}$ and $\cdot\text{CO}_3^-$ concentrations were calculated using Equations 3.12 and 3.13. The formation rates of $\cdot\text{OH}$ from NO_3^- ($R_{\text{form}, \cdot\text{OH}, \text{NO}_3^-}$) and dissolved organic matter ($R_{\text{form}, \cdot\text{OH}, \text{DOM}}$) were calculated using $\cdot\text{OH}$ formation quantum

yields from NO_3^- and DOM and were corrected for depth (Zepp *et al.*, 1987; Dong and Rosario-Ortiz, 2012). $^1\text{O}_2$ steady-state concentrations were estimated based on previously measured values from irradiated wastewater effluent (Haag and Hoigné, 1986). Pseudo-first order reaction rates of $^3\text{DOM}^*$ with trace organic contaminants were estimated using a wavelength-dependent quantum yield coefficient ($f(\lambda)$ (L Ei^{-1})), that accounted for the efficiency of light absorption by DOM leading to transformation of a contaminant (Gerecke *et al.*, 2001). A quantum yield coefficient for the reaction of $^3\text{DOM}^*$ with propranolol was used to account for differences between model predictions and rates measured with a solar simulator.

To evaluate the applicability of the photochemical model, predicted rates were compared to photolysis rates of test compounds measured in a solar simulator (Oriel). Test compound photolysis rates were measured in uncovered 1-L borosilicate beakers containing 20 cm of wetland water amended with approximately 50 nM of test compounds. Solutions were irradiated from above with a collimated beam (20 x 20 cm) generated by a 1000-W Xe lamp screened with an atmospheric attenuation filter (typical spectrum shown in Figure 3.1). The solution temperature was maintained at 15°C to minimize evaporation using a recirculating water bath. 1-mL samples were collected at regular intervals for analysis.

3.3.7 Analytical methods

Dissolved organic carbon and dissolved inorganic carbon were measured using a Shimadzu TOC-V analyzer (Standard Method 5310 B) (American Public Health Association, 1995). NO_3^- was analyzed using a Dionex DX-120 ion chromatograph (Standard Method 4500- NO_3^- C) (American Public Health Association, 1995). UV/Vis absorption spectra ($\epsilon(\lambda)$) and wetland water beam attenuation spectra ($\alpha(\lambda)$) were measured using a Perkin Elmer Lambda 35 spectrometer. *p*CBA, DMA, and phenol were separated using an HPLC equipped with a 4.6 mm x 250 mm supelcosil LC-18 5 μm column. *p*CBA was detected at 240 nm, with a mobile phase of 60% acetonitrile and 40% 5mM sulfuric acid. DMA was detected at 252 nm, with a mobile phase of 70% acetonitrile and 30% 5 mM ammonium acetate adjusted to pH 8. Phenol was detected at 270 nm, with a mobile phase of 60% acetonitrile and 40% 10 mM formic acid. A flow rate of 1 mL min $^{-1}$ was used for all analytes.

Pharmaceuticals and isoproturon were separated using a 2.1 mm x 30 mm Zorbax SB-C18 3.5 μm column, eluted with at 0.5 mL min $^{-1}$ acetonitrile and 0.1% acetic acid in water with the following gradient: 0 minutes, 5% acetonitrile; 5.5 minutes 55% acetonitrile; 6 minutes, 100% acetonitrile; 9 minutes, 100% acetonitrile; 10 minutes, 5% acetonitrile. All compounds were detected using electrospray ionization (ESI) with a 60-100 ms dwell time and a gas temperature of 350° C, a gas flow rate of 11 L/min at 50 psi, and a capillary voltage of 3600 V. Compound-specific parameters are given in Table 4.1, although only atenolol, propranolol, carbamazepine, and sulfamethoxazole were analyzed for photolysis studies described in this Chapter.

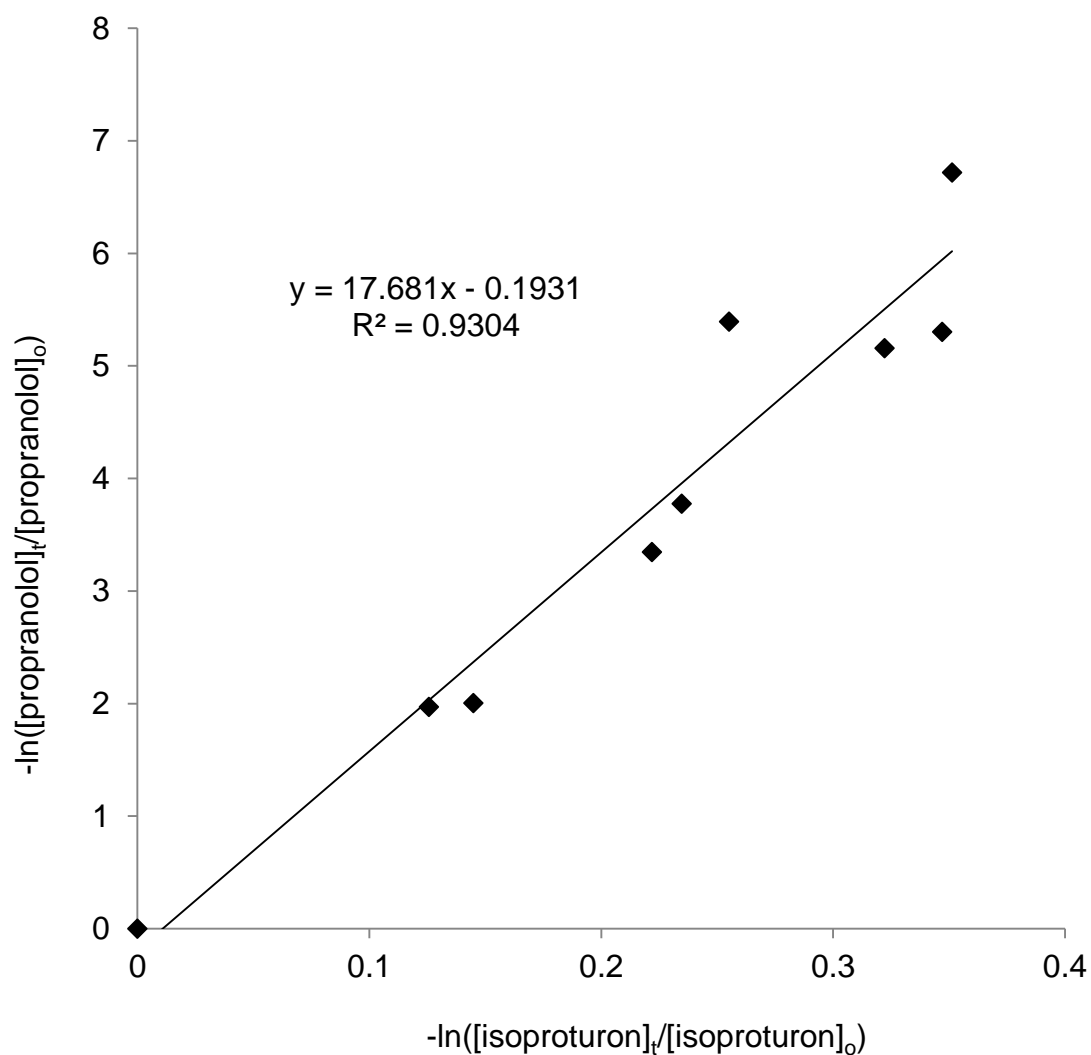


Figure 3.6: Typical results from competition kinetics experiment used to measure $k_{CO_3^{\cdot-}, cont}$. Samples were irradiated in a merry-go-round reactor equipped with a 500 W medium-pressure mercury lamp. pH=11.4; $[isoproturon]_o \approx 2.5$ nM; $[propranolol]_o \approx 2.5$ nM; $[CBBP] \approx 5$ μ M; $[CO_3^{2-}] \approx 0.5$ M.

3.4 Results and Discussion

Wetland water contains chromophores that serve as sources of reactive oxygen species and triplet state species. As a result, photolysis rates of some compounds increased in wetland waters relative to those measured in deionized water. For other compounds, photolysis rates decreased in the presence of chromophores due to light screening. Experiments with probe compounds provided a basis for predicting the magnitude of these effects over a range of conditions.

3.4.1 Carbonate Radical Reactions with Contaminants

The competition kinetics method used to determine second-order $\cdot\text{CO}_3^-$ reaction rates with test compounds exhibited pseudo first-order kinetics as indicated by the linearity of data plotted according to Equation 3.11 ($r^2 > 0.9$; see Figure 3.6). Dark controls indicated that none of the compounds underwent hydrolysis at appreciable rates relative to photolysis. Both CBBP and DQ were used as sensitizers to evaluate the possibility of a side reaction between the sensitizer and the test compound. The observed agreement between rate constants measured with the two sensitizers indicated that side reactions were not important at pH 11.4 (see Table 3.4).

Table 3.4: Second-Order Rate Constants for the Reaction of $\cdot\text{CO}_3^-$ with Test Compounds^a

compound	<u>sensitizer^b ($10^7 \text{ M}^{-1}\text{s}^{-1}$)</u>			
	<u>CBBP</u>		<u>DQ</u>	
	pH 8.2	pH 11.4	pH 8.2	pH 11.4
atenolol	0.9 (± 0.4)	5.9 (± 1.6)	0.3 (± 0.2)	5.4 (± 0.9)
propranolol ^c	7.8 (± 5.6)	46.3 (± 6.8)	7.6 (± 4.5)	42.9 (± 5.1)
sulfamethoxazole ^c	12.9	44.4 (± 12.4)	17.9	32.4
carbamazepine	0.2 (± 0.1)	0.23 (± 0.05)	0.5 (± 0.2)	0.32 (± 0.03)

^a $k_{\cdot\text{CO}_3^-, \text{cont}}$. ^b Errors when available are \pm one standard deviation. ^c Rates corrected for direct photolysis.

The rate constants for reactions of test compounds with $\cdot\text{CO}_3^-$ at pH 11.4 varied over more than two orders of magnitude (Table 3.4), reflecting the selectivity of $\cdot\text{CO}_3^-$. Changes in ionic strength did not significantly affect measured rate constants. As expected, $\cdot\text{CO}_3^-$ reacted rapidly with sulfur-containing functional groups on sulfamethoxazole (Chen and Hoffman, 1975; Huang and Mabury, 2000b). $\cdot\text{CO}_3^-$ also reacted rapidly with the deprotonated amines atenolol and propranolol.

To assess the role of compound speciation on reaction rates with $\cdot\text{CO}_3^-$, experiments were performed at pH 8.2 and 11.4 for atenolol ($\text{pK}_a=9.6$) and propranolol ($\text{pK}_a=9.5$). Attempts to measure $k_{\cdot\text{CO}_3^-}$ at pH 8.2 were complicated by side reactions between sensitizers and test compounds, as evidenced by differences in second-order rate constants measured using the two sensitizers (Table 3.4). In both cases, measured values for $k_{\cdot\text{CO}_3^-}$ were approximately an order of

magnitude lower for the deprotonated β -blockers relative to the protonated species (Table 3.4). As expected, $\cdot\text{CO}_3^-$ reaction rates with carbamazepine did not vary significantly with pH (Table 3.4). Although the protonation state of sulfamethoxazole does not change significantly between pH 8.2 and pH 11.4, measured $k_{\cdot\text{CO}_3^-}$ values varied by approximately a factor of 2.5. This change may have been due to side reactions with sensitizers at pH 8.2. Therefore, $k_{\cdot\text{CO}_3^-}$ values measured at pH 11.4 were used for sulfamethoxazole in subsequent model calculations.

3.4.2 Reaction of Hydroxyl Radical and Carbonate Radical with Wetland Dissolved Organic Matter

Measured $[\cdot\text{OH}]_{\text{ss}}$ and $[\cdot\text{CO}_3^-]_{\text{ss}}$ were strongly affected by pH, with an 80% decrease in $[\cdot\text{OH}]_{\text{ss}}$ and a greater than 100% increase in $[\cdot\text{CO}_3^-]_{\text{ss}}$ as pH increased from 8 to 10 (Figure 3.7). The observed shift in concentrations of radicals was attributed to the faster reaction rate of $\cdot\text{OH}$ with CO_3^{2-} relative to HCO_3^- (Buxton *et al.*, 1988). This was especially relevant in open-water wetlands, where photosynthesis resulted in pH values as high as 10 (see Figure 3.5).

To assess the relative importance of dissolved organic matter and dissolved inorganic carbon as sinks for radicals, $[\cdot\text{OH}]_{\text{ss}}$ and $[\cdot\text{CO}_3^-]_{\text{ss}}$ were modeled using equations 3.12 and 3.13. The measured formation rate of $\cdot\text{OH}$ in wetland water when irradiated with a medium-pressure mercury lamp ($(R_{\text{form},\cdot\text{OH},\text{NO}_3^-} + R_{\text{form},\cdot\text{OH},\text{DOM}}) = 4.3 \times 10^{-10} \pm 0.2 \text{ M s}^{-1}$; data not shown) was similar to previously measured rates (Dong and Rosario-Ortiz, 2012). Steady-state radical concentrations were calculated by fitting the adjustable parameters $k_{\cdot\text{OH},\text{DOM}}$ and $k_{\cdot\text{CO}_3^-,\text{DOM}}$ to equations 3.12 and 3.13 using a least-square model (Figure 3.7). The best-fit value of $k_{\cdot\text{OH},\text{DOM}}$ (i.e., $1.7 \times 10^4 \text{ (mg L}^{-1}\text{-C)}^{-1}\text{s}^{-1}$) was approximately 30% lower than values reported for reactions of $\cdot\text{OH}$ with DOM in natural waters (i.e., $2.3 \times 10^4 \text{ (mg L}^{-1}\text{-C)}^{-1}\text{s}^{-1}$) (Brezonik and Fulkerson-Brekken, 1998) and 80% lower than values for municipal wastewater effluent (i.e., $7.2 \times 10^4 \text{ (mg L}^{-1}\text{-C)}^{-1}\text{s}^{-1}$) (Rosario-Ortiz *et al.*, 2008). The best-fit value for $k_{\cdot\text{CO}_3^-,\text{DOM}}$ (i.e., $370 \text{ (mg L}^{-1}\text{-C)}^{-1}\text{s}^{-1}$) was approximately 30% higher than previously reported values for a hydrophobic Suwannee River fulvic acid extract (i.e., $280 \text{ (mg L}^{-1}\text{-C)}^{-1}\text{s}^{-1}$) (Canonica *et al.*, 2005) and more than 9 times higher than a Suwannee River dissolved organic matter extract (i.e., $40 \text{ (mg L}^{-1}\text{-C)}^{-1}\text{s}^{-1}$) (Larson and Zepp, 1988). The relatively high reactivity of the natural organic matter in this study may be attributed to its source—namely wastewater effluent and the algae, diatoms, and bacteria on the bottom of the cell. Previous research has shown that effluent organic matter may contain abundant reactive moieties associated with soluble microbial products (Dong *et al.*, 2010). Presumably the organisms present in the open-water-cell biofilm released similar reactive compounds.

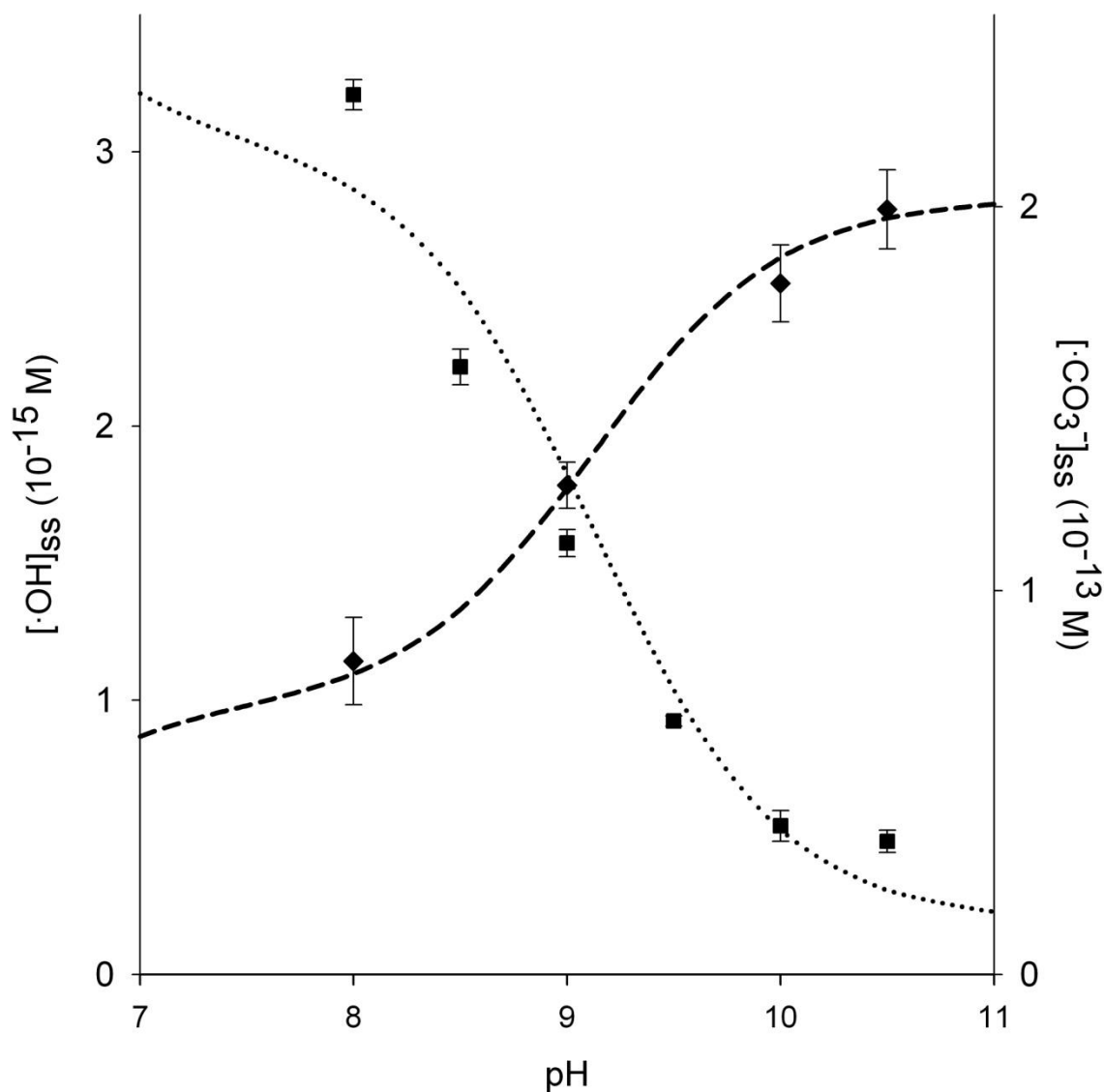


Figure 3.7. Measured $[\cdot\text{OH}]_{ss}$ (■) and $[\cdot\text{CO}_3^-]_{ss}$ (◆) in Discovery Bay wetland water when irradiated with a 500-W medium-pressure mercury lamp. $[\cdot\text{OH}]_{ss}$ (···) and $[\cdot\text{CO}_3^-]_{ss}$ (---) plotted according to Equations 2 and 3. $[\text{NO}_3^-]=16 \text{ mg/L-N}$; $[\text{DOM}]=5.5 \text{ mg of C/L}$; $[\text{HCO}_3^-] + [\text{CO}_3^{2-}]=60 \text{ mg L}^{-1}\text{-C}$; $k_{\text{OH},\text{HCO}_3^-}=8.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$; $k_{\text{OH},\text{CO}_3^{2-}}=3.9 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$; $(R_{\text{form},\cdot\text{OH},\text{NO}_3^-} + R_{\text{form},\cdot\text{OH},\text{DOM}})=4.3 \times 10^{-10} \text{ M s}^{-1}$ (measured); $k_{\text{OH},\text{DOM}}=1.7 \times 10^4 (\text{mg of C L}^{-1})^{-1}\text{s}^{-1}$ (best fit); $k_{\text{CO}_3^-,\text{DOM}}=370 (\text{mg of C L}^{-1})^{-1}\text{s}^{-1}$ (best fit). Error bars represent \pm one standard deviation.

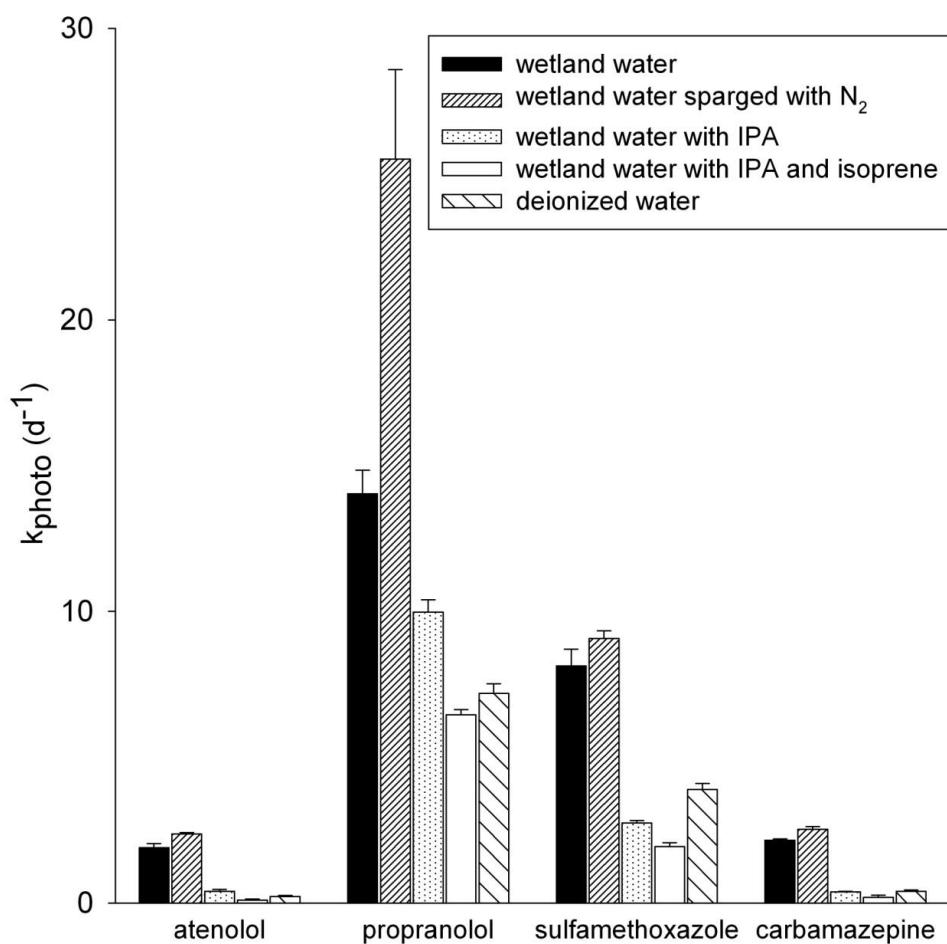


Figure 3.8: Pseudo first-order photolysis rates for test compounds in wetland water, wetland water sparged with N₂, wetland water spiked with 1% IPA, wetland water spiked with 1% IPA and 0.1% isoprene, and deionized water. All solutions were buffered at pH 8.5. Rates were measured in a merry-go-round reactor with a 500-W medium-pressure mercury lamp. Error bars represent \pm one standard deviation.

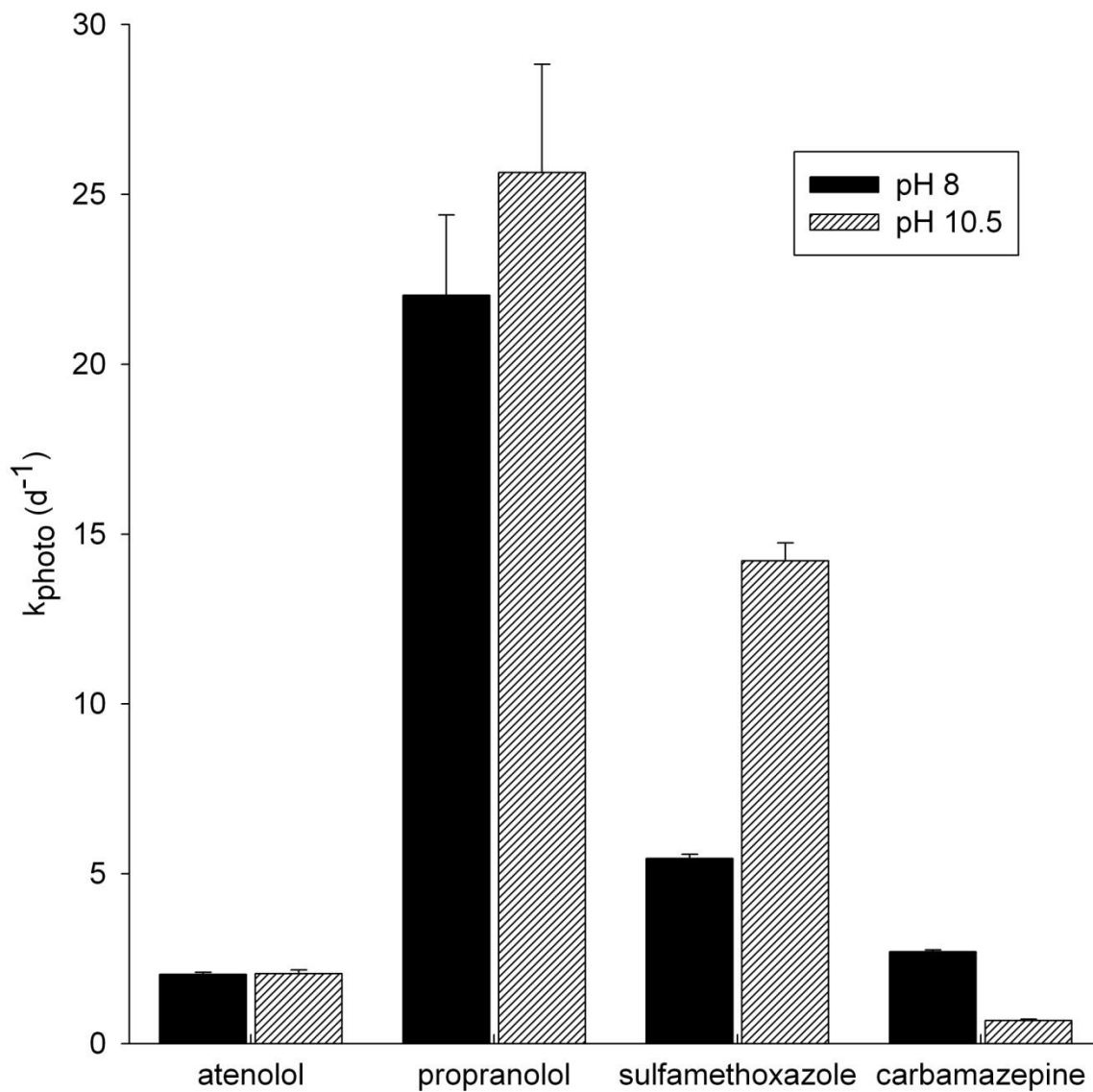


Figure 3.9: Pseudo first-order photolysis rates for test compounds in wetland water buffered at pH 8 and 10.5. Rates were measured in a merry-go-round reactor with a 500-W medium-pressure mercury lamp. Error bars represent \pm one standard deviation.

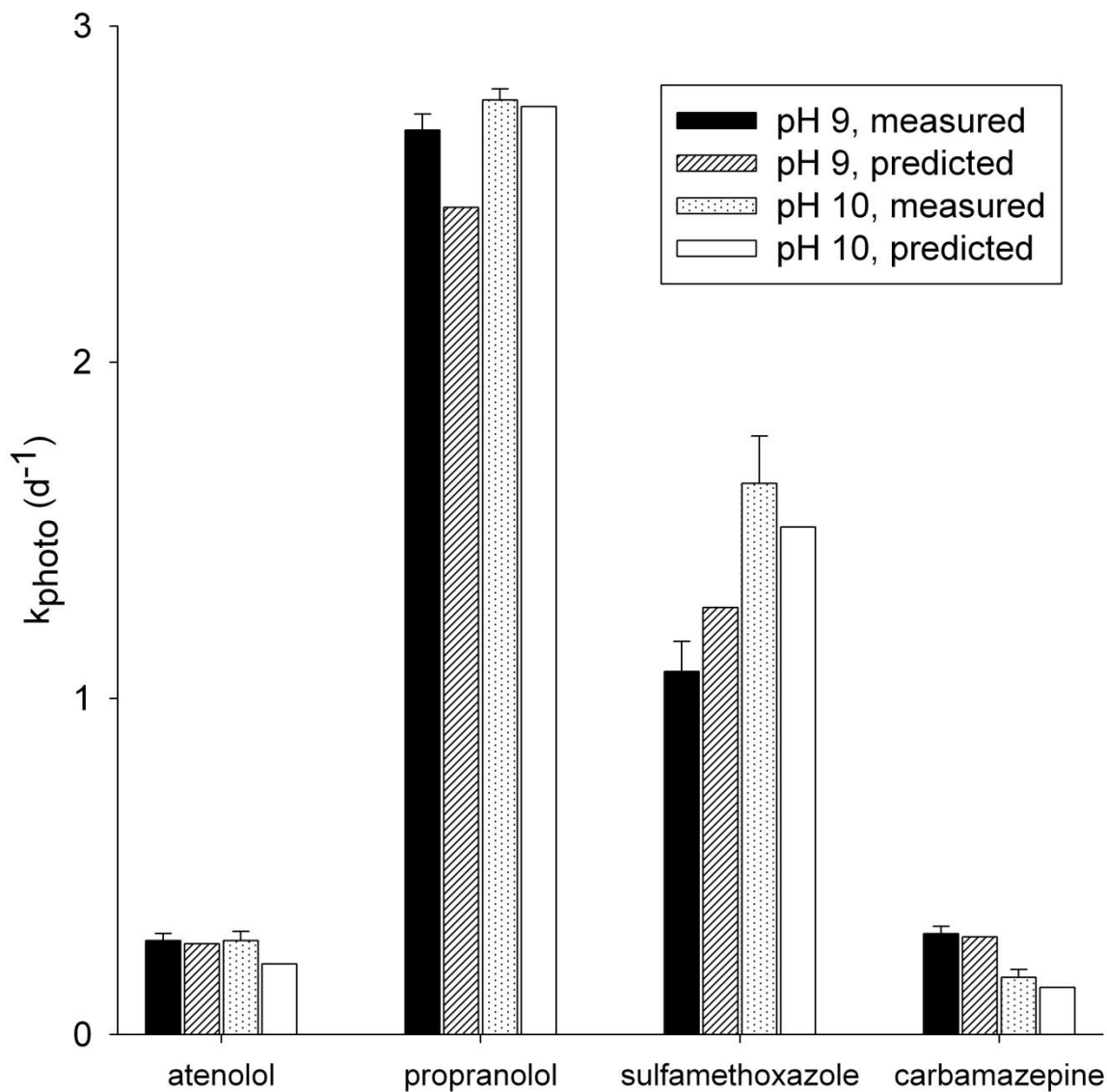


Figure 3.10: Predicted and measured pseudo first-order photolysis rates for test compounds in 20 cm deep wetland water buffered at pH 9 and 10 under a solar simulator. [DOC]=8 mg L⁻¹-C; [NO₃⁻]=16 mg L⁻¹-N; depth=20 cm; [HCO₃⁻]+[CO₃²⁻]=60 mg L⁻¹-C. Error bars represent ± one standard deviation.

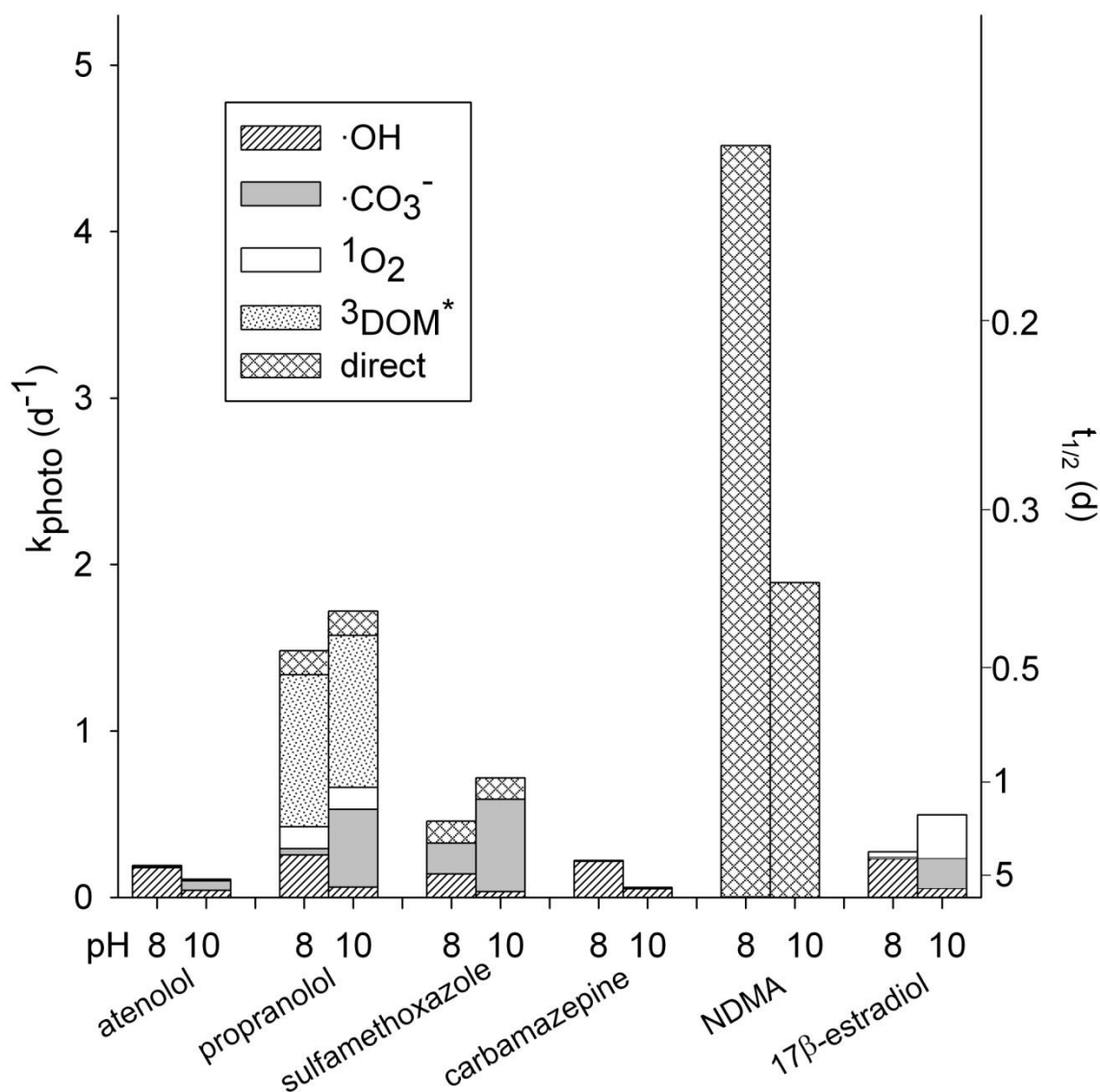


Figure 3.11: Predicted contributions of photolysis mechanisms to k_{phot} at pH values of 8 and 10. [DOC]=8 mg L⁻¹-C; [NO₃⁻]=20 mg L⁻¹-N; depth=30 cm; [HCO₃⁻]+[CO₃²⁻]=60 mg L⁻¹-C; solar intensity ($Z(24 \text{ hr}, \lambda)$) of June 21st under clear skies at 40°N latitude.

3.4.3 Photolysis of Test Compounds in Wetland Water

Each probe compound exhibited different behavior with respect to the relative contributions of different photolysis mechanisms when irradiated with a medium-pressure mercury lamp. Among the compounds studied (atenolol, propranolol, sulfamethoxazole, and carbamazepine), only sulfamethoxazole and propranolol underwent direct photolysis at appreciable rates in deionized water (Figure 3.8), which was consistent with previous findings (Andreozzi *et al.*, 2003; Lin and Reinhard, 2005; Lam and Mabury, 2005). In wetland water at pH 8.5, $\cdot\text{OH}$ accounted for much of the loss of carbamazepine and atenolol, as evidenced by the 80% decreases in transformation rates observed upon addition of IPA (Figure 3.8).

Photosensitized reactions involving $^3\text{DOM}^*$ were important to the loss of propranolol. When wetland water was sparged with N_2 to remove O_2 , a triplet quencher, the rate of loss almost doubled, while addition of the triplet quencher isoprene reduced the rate of propranolol loss by approximately 50% (Figure 3.8). These observations were consistent with previous studies of propranolol photolysis in the presence of humic substances (Chen *et al.*, 2009). The rate of transformation of atenolol, sulfamethoxazole, and carbamazepine increased slightly under N_2 -sparged conditions and decreased slightly but not significantly upon addition of isoprene, implying a small contribution of $^3\text{DOM}^*$ to the photolysis of these compounds. Results from other studies suggest that $^3\text{DOM}^*$ contributes to the photolysis of sulfamethoxazole in municipal wastewater effluent (i.e., 16% of total photolysis rate) (Ryan *et al.*, 2011) and the photolysis of atenolol increases in the presence of fulvic acid isolates (i.e., greater than 50% of the total photolysis rate with $20 \text{ mg L}^{-1}\text{-C DOM}$) (Wang *et al.*, 2012). The smaller contribution of $^3\text{DOM}^*$ observed in this study may have been due to differences in experimental conditions or variations in the properties of $^3\text{DOM}^*$ from different sources, such as antioxidative properties (Canonica and Laubscher, 2008; Wenk *et al.*, 2011). In addition, formation of $\cdot\text{OH}$ and $\cdot\text{CO}_3^-$ from the high concentration of NO_3^- in the nitrified wastewater effluent reduced the relative importance of reactions with $^3\text{DOM}^*$.

To assess the impacts of pH on photolysis rates, experiments were repeated in wetland water buffered at pH 10.5 (Figure 3.9). Atenolol and carbamazepine, which were transformed mainly by $\cdot\text{OH}$ at pH 8.5, exhibited different behavior at pH 10.5 due to the conversion of $\cdot\text{OH}$ to $\cdot\text{CO}_3^-$. The rate of loss of carbamazepine decreased by approximately 70% due to its low reactivity with $\cdot\text{CO}_3^-$, while the rate of loss was almost unchanged for atenolol due to its relatively high rate of reaction with $\cdot\text{CO}_3^-$. As expected, the rate of loss of both sulfamethoxazole and propranolol increased at high pH values, due to relatively fast rates of reaction of these compounds with $\cdot\text{CO}_3^-$.

3.4.4 Photolysis Model Validation and Predictions

Photolysis experiments conducted with a medium-pressure mercury lamp provided a means of obtaining precise data within a period of several hours. However, the conditions used in those experiments were different from conditions encountered in sunlit waters. To assess the potential for artifacts associated with differences in the spectral qualities and light intensity, photolysis rates were measured using a solar simulator and water depth comparable to that of the pilot-scale open-water unit process cell. Comparison of measured photolysis rates of test compounds in the solar simulator agreed within approximately 10% with rates predicted by the

photolysis model at pH values bounding the range typically observed in the pilot-scale system (Figure 3.10).

Due to the large contribution of $^3\text{DOM}^*$ to the indirect photolysis rate of propranolol (e.g., see Figure 3.8), it was necessary to estimate a quantum yield coefficient ($f_{\text{propranolol}}(\lambda)$) for the reaction of $^3\text{DOM}^*$ with propranolol. $f_{\text{propranolol}}(\lambda)$ was assumed to have a similar exponential dependence on wavelength as $f(\lambda)$ values obtained for other organic compounds (i.e., $f(\lambda) = A * e^{-0.02 * \lambda} \text{ L Ei}^{-1}$, where A is a compound-dependent parameter) (Gerecke *et al.*, 2001). The parameter A was estimated for propranolol by minimizing the differences between model predictions and photolysis rates measured using a solar simulator. The obtained value for $A_{\text{propranolol}}$ was about 5 times larger than the reported value for diuron (i.e., $A_{\text{diuron}} = 3.5$; $A_{\text{propranolol}} = 16$) (Gerecke *et al.*, 2001).

The photochemical model was used to predict the contributions of different photolysis pathways to the overall photolysis rates of the test compounds and two additional compounds for which appropriate data were available (i.e., NDMA and 17 β -estradiol). The predicted contributions of each photolysis mechanism to overall photolysis rates of test compounds were similar to those observed using quenchers and the mercury lamp (Figure 3.8). For compounds that reacted mainly with $^{\bullet}\text{OH}$ and $^{\bullet}\text{CO}_3^-$ (i.e., atenolol and carbamazepine), predicted half-lives exceeded 3 days at pH 8 and 5 days at pH 10 (Figure 3.11). Propranolol and sulfamethoxazole were predicted to have half-lives of 0.5 to 1 days, with higher rates expected at pH 10 due to reactions involving $^{\bullet}\text{CO}_3^-$.

Literature data was used to predict the removal of NDMA and 17 β -estradiol in the open-water cell. NDMA was predicted to exhibit half-lives of less than 8 hours at pH 8 due to direct photolysis, with a half-life almost twice as long at pH 10 due to the lower quantum yield of the compound above pH 8.5 (Lee *et al.*, 2005). Predicted half-lives for 17 β -estradiol were about 2.5 days at pH 8 and 1 day at pH 10, due to increased reaction rates of $^1\text{O}_2$ and $^{\bullet}\text{CO}_3^-$ with the deprotonated species at alkaline pH values.

3.4.5 Estimation of Wetland Area Necessary for Contaminant Photolysis

To assess the merits of open-water unit process wetlands as a means of removing chemical contaminants via photolysis it is useful to consider the area necessary to achieve a desired level of treatment. For many wastewater-derived organic contaminants, a 90% decrease in concentration will result in concentrations below a threshold for ecological or human health concerns. For example, a 90% reduction of typical propranolol concentrations in municipal wastewater effluent (i.e., 0.1-0.5 $\mu\text{g L}^{-1}$) (Sedlak and Pinkston, 2001; Huggett *et al.*, 2003) would result in concentrations that are about an order of magnitude lower than the concentration at which reproductive effects in aquatic organisms have been observed (Huggett *et al.*, 2002). Similarly, a 90% reduction would reduce NDMA concentrations in most wastewater effluent samples below the 10 ng L^{-1} notification level set by the California Department of Health Services (Mitch *et al.*, 2003). In addition, a tenfold decrease in concentration of wastewater-derived trace organic contaminant concentrations would result in concentrations in effluent-dominated waters that are comparable to levels typically detected in surface waters (i.e., many surface waters in the United States consist of around 10% wastewater under low-flow conditions) (National Research Council, 2012).

For the purpose of assessing the land required for treatment, it is useful to consider the area needed to achieve 90% reduction in concentration for 1 MGD of wastewater effluent. This provides a simple basis for estimating land areas in different locations (i.e., to estimate the land needed to treat 10 MGD the value is multiplied by 10). This value, which we refer to as A_{90}^1 , is calculated as follows:

We first calculate the nominal hydraulic residence time (HRT) of the system and convert it to units of hectares per MGD (ha MGD^{-1}):

$$\text{HRT} = \frac{z(\text{m}) A(\text{m}^2)}{Q(\text{m}^3 \text{d}^{-1})} = \frac{z(\text{m}) A(\text{ha}) \frac{10,000 \text{ m}^2}{1 \text{ ha}}}{Q(\text{MGD}) \frac{3785 \text{ m}^3/\text{d}}{1 \text{ MGD}}} = 2.64 (\text{MGD d m}^{-1} \text{ha}^{-1}) \frac{z(\text{m}) A(\text{ha})}{Q(\text{MGD})} \quad (\text{A3.14})$$

Where A is the wetland area, Q is the volumetric flow rate, and z is the depth. Since photolysis obeys pseudo-first order kinetics, the time necessary for 90% removal of a contaminant can be replaced by HRT in the equation for first-order degradation:

$$\frac{C}{C_0} = 0.1 = e^{-k_{\text{photo}} t} = e^{-k_{\text{photo}} \text{HRT}} \quad (3.15)$$

By taking the natural logarithm of both sides of Equation 3.15, substituting in Equation 3.14 for HRT, and rearranging yields:

$$A(\text{ha}) = \frac{-\ln(0.1)}{2.64 (\text{MGD d ha}^{-1} \text{m}^{-1})} \frac{Q (\text{MGD})}{z(\text{m}) k_{\text{photo}} (\text{d}^{-1})} = 0.87 (\text{MGD}^{-1} \text{d}^{-1} \text{m ha}) \frac{Q (\text{MGD})}{z(\text{m}) k_{\text{photo}} (\text{d}^{-1})} \quad (3.16)$$

Using a volumetric flow rate of 1 MGD yields:

$$A_{90}^1 (\text{ha}) = \frac{0.87 (\text{MGD}^{-1} \text{d}^{-1} \text{ha m})}{z(\text{m}) k_{\text{photo}} (\text{d}^{-1})} \quad (3.17)$$

Wetland area is an important design criterion for wetland projects because they are often limited by available space, and capital costs for wetland construction increase approximately linearly with wetland area (Kadlec, 2009). To assess the impact of design parameters on wetland performance, the model was used to predict A_{90}^1 for a suite of representative contaminants.

Effect of Depth on Treatment Efficiency. A_{90}^1 decreased with increasing depth for all compounds, with the most significant decreases occurring between 0 and 20 cm (Figure 3.12a). Deeper open-water cells were predicted to be more effective because decreases in photolysis rates due to light screening were compensated for by increases in wetland hydraulic residence times.

Wetland depths beyond 50 cm may be impractical for open-water cells because deeper wetlands result in longer hydraulic residence times that are conducive to the growth of floating aquatic plants, such as duckweed (EPA, 2000a). Furthermore, deep photolysis wetlands will exhibit less growth of photosynthetic organisms at the sediment-water interface—a location where additional contaminant removal occurs via sorption and biotransformation (Chapter 4).

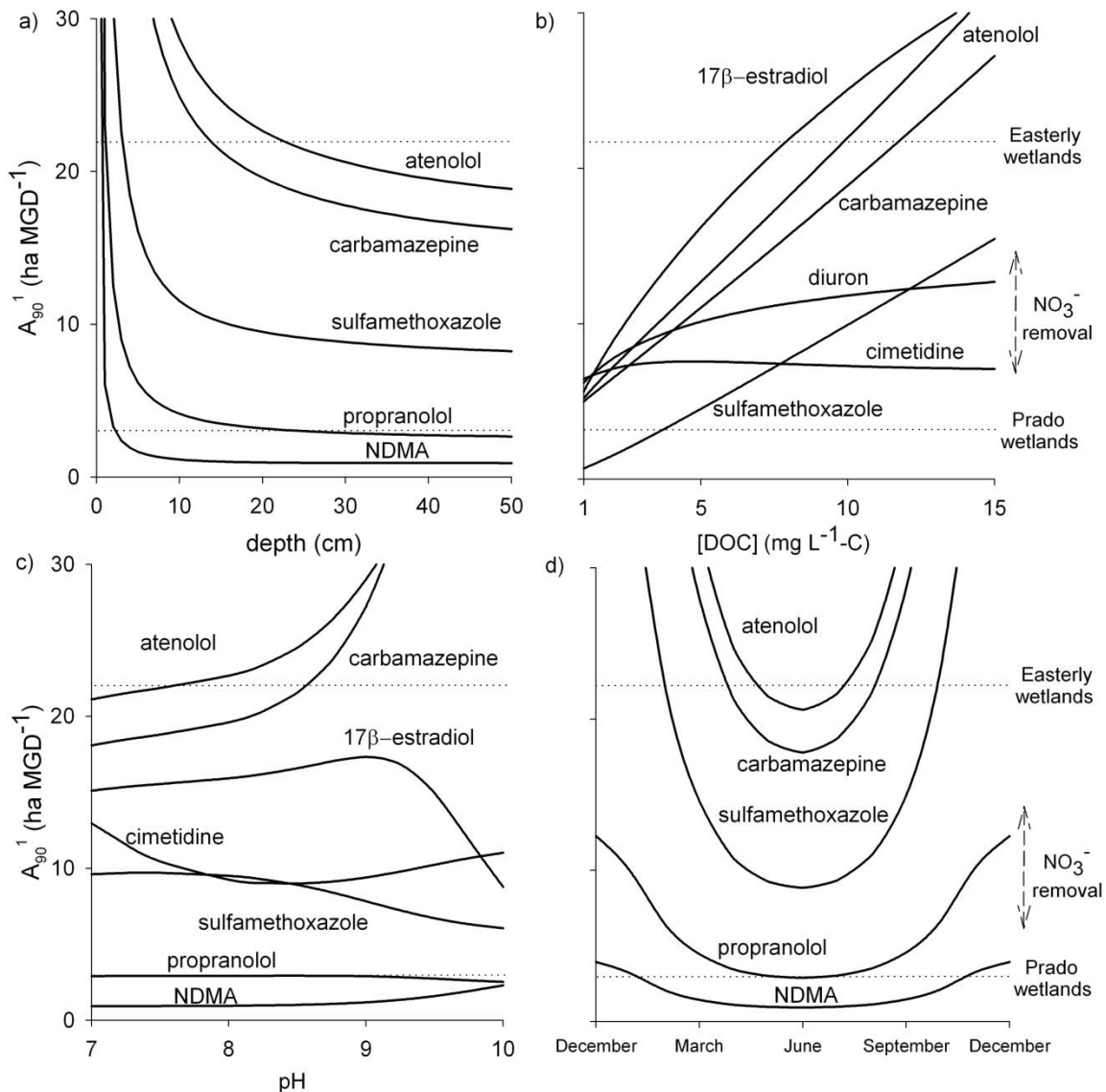


Figure 3.12: Area predicted to provide 90% removal of contaminants from 1 MGD of wastewater effluent in open-water treatment wetlands (A_{90}^1) under varying: (a) depth; (b) [DOC]; (c) pH; and (d) season. If not varied, pH=8; [DOC]=8 mg L $^{-1}$ -C; [NO_3^-]=20 mg L $^{-1}$ -N; depth=30 cm; [HCO_3^-]+[CO_3^{2-}]=60 mg L $^{-1}$ -C; solar intensity ($Z(24 \text{ hr}, \lambda)$) of June 21st under clear skies at 40°N latitude. Dashed lines show the area per MGD of existing full-scale wetland systems and the typical size of wetlands designed for nitrate removal.

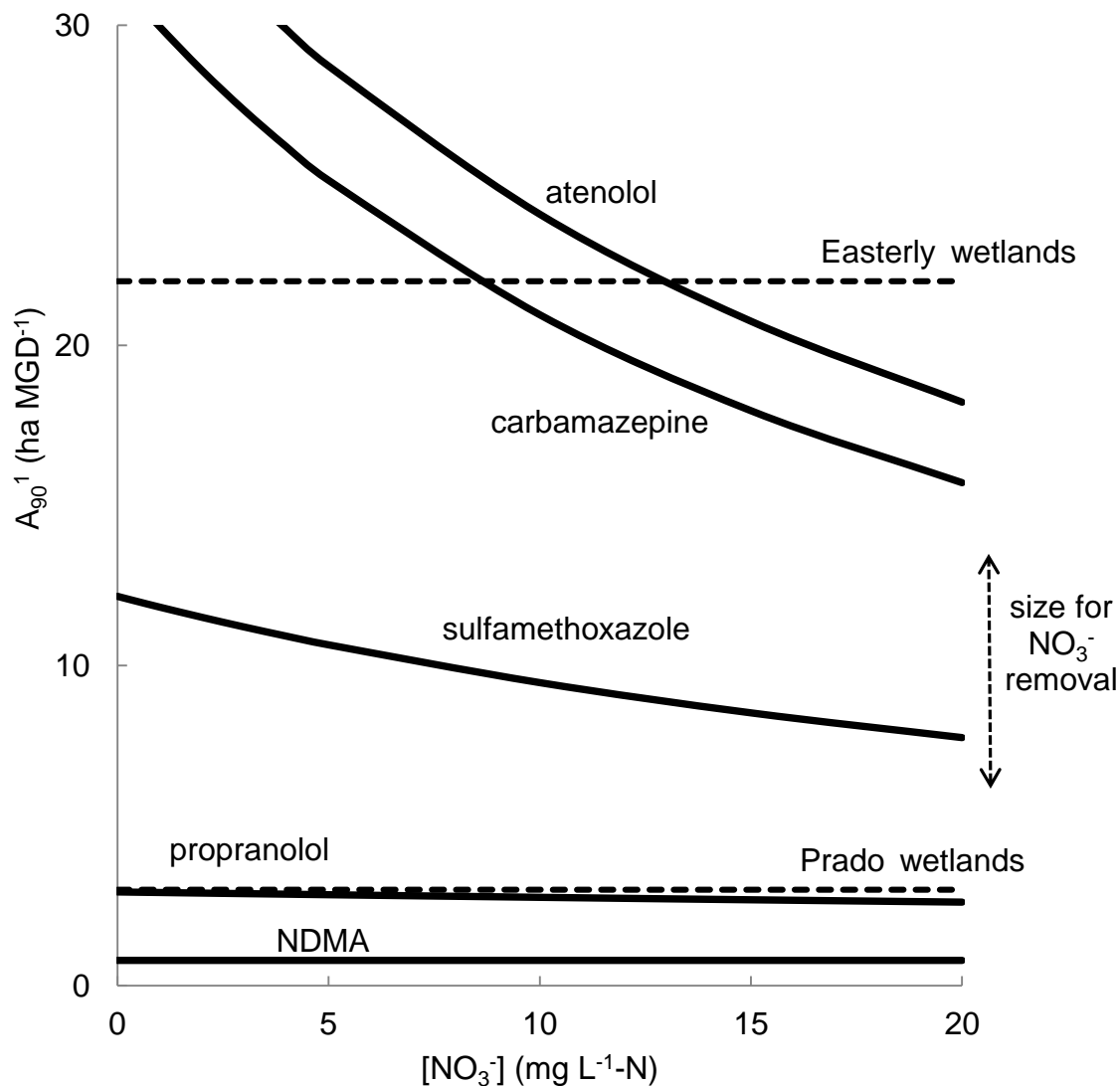


Figure 3.13: Area needed to provide 1-log removal of contaminants from 1 MGD with different $[\text{NO}_3^-]$ under clear skies at 40° N latitude summer sunlight ($Z(24 \text{ hr}, \lambda)$). $[\text{DOC}] = 8 \text{ mg L}^{-1}\text{-C}$; depth = 30 cm; $[\text{HCO}_3^-] + [\text{CO}_3^{2-}] = 60 \text{ mg L}^{-1}\text{-C}$; pH = 8. Dashed lines show the area per MGD of existing full-scale wetland systems.

Effect of Nitrate on Treatment Efficiency. Nitrate is expected to be important to the removal of compounds that react with $\cdot\text{OH}$ and $\cdot\text{CO}_3^-$, such as atenolol and carbamazepine. As a result, removal of these compounds from nitrified wastewater effluent, which typically contains 5 to 20 mg L⁻¹-N NO₃⁻ (Pocernich and Litke, 1997), is predicted to require an area that is about half as large as what would be required if the wastewater had been denitrified prior to treatment (Figure 3.13).

Effect of Dissolved Organic Carbon on Treatment Efficiency. Dissolved organic carbon had a strong effect on A₉₀¹ for most compounds, mainly due to its ability to screen sunlight and scavenge $\cdot\text{OH}$ and $\cdot\text{CO}_3^-$ (Figure 3.12b). For compounds that are removed primarily by reaction with $\cdot\text{OH}$ and $\cdot\text{CO}_3^-$, such as carbamazepine, atenolol, and sulfamethoxazole, A₉₀¹ increased by approximately 50% when dissolved organic carbon concentration increased from 5 to 10 mg L⁻¹. Compounds that react rapidly with ³DOM*, such as diuron, showed the most significant differences in A₉₀¹ at very low concentrations of dissolved organic carbon. This may also be true for compounds such as 17β-estradiol if reaction with ³DOM* were included in model predictions (Leech *et al.*, 2009). Unfortunately, rate constants for reactions of ³DOM* with many compounds are not available, which is a topic that requires further research.

¹O₂ steady-state concentrations were predicted to increase with increasing concentrations of dissolved organic matter. Thus, compounds that react rapidly with ¹O₂, such as cimetidine, exhibited little change in A₉₀¹ as dissolved organic carbon concentrations increased, despite increased light screening at higher dissolved organic carbon concentrations. It should be noted that the supersaturated dissolved oxygen concentrations created by photosynthetic organisms on the bottom of open water cells could have increased the importance of ¹O₂ relative to ³DOM*, due to the scavenging of ³DOM* by O₂. Additional research would be needed to quantify the magnitude of this effect.

Effect of pH on Treatment Efficiency. Open-water unit process cells exhibit wide variations in pH that are determined by the alkalinity, depth, and mixing of the wastewater effluent, in addition to the activity of photosynthetic organisms at the sediment-water interface. For example, in the pilot-scale cell employed in this study, pH increased from values of 8 to 10 as water passed through the system over a two-day residence time (Figure 3.5). In the early morning, or before the photosynthetic organisms were established, pH values remained below 9 throughout the cell.

Changes in wetland pH affected the area needed to remove most contaminants via photolysis (Figure 3.12c). Insight into these trends can be gained by considering dominant photolysis mechanisms for test compounds at pH values of 8 and 10 (Figure 3.11). One important pH-dependent change was the conversion of $\cdot\text{OH}$ to $\cdot\text{CO}_3^-$ (Figure 3.7). As a result of this phenomenon, compounds that react slowly with $\cdot\text{CO}_3^-$ under alkaline conditions (i.e., $k_{\cdot\text{CO}_3^-} < 10^8 \text{ M}^{-1} \text{ s}^{-1}$), such as atenolol and carbamazepine, are predicted to require larger areas for treatment at higher pH values. Conversely, compounds that react with $\cdot\text{CO}_3^-$ at rates above $10^8 \text{ M}^{-1} \text{ s}^{-1}$ (e.g., sulfamethoxazole and propranolol) should require the same or slightly smaller areas at elevated pH values.

Phenolic compounds (e.g., 17 β -estradiol) should need less area for treatment at elevated pH values because their deprotonated forms react with $^1\text{O}_2$ and $^1\text{CO}_3^-$ at rates that are over an order of magnitude faster than their protonated forms (Neta *et al.*, 1988; Tratnyek and Hoigné, 1991).

Direct photolysis rates were also affected by pH due to changes in molar absorption coefficients or quantum yields of different forms of the compounds (Boreen *et al.*, 2004). For example, the rate of direct photolysis of NDMA was predicted to decrease by approximately 75% between pH 7 and 10 (Figure 3.11) due to the effect of protonation of an excited photo-intermediate on the reaction quantum yield (Lee *et al.*, 2005). For NDMA, this effect translated to more than doubling A_{90}^1 as pH increased from 8 to 10.

Effect of Season on Treatment Efficiency. Seasonal variations in solar intensity should also affect A_{90}^1 . For all modeled compounds, A_{90}^1 values were predicted to increase by a factor of 3 to 4 between summer and winter conditions (Figure 3.12d).

Seasonal variation in treatment efficiency is a common challenge for treatment wetlands. For example, microbial denitrification rates slow significantly under cold winter conditions (Bachand and Horne, 2000). In some locations, the summertime period when open-water cells exhibit their best performance will coincide with times of low flow in receiving waters (Loraine and Pettigrove, 2005). The design of open-water unit process cells will need to account for seasonal variations in dilution and conditions in these receiving waters.

3.4.6 Comparison of Photolysis Cells and Existing Wetlands

To gain insight into the feasibility of building open-water unit process cells, it is useful to compare predicted A_{90}^1 values for photolysis with the areas occupied by existing wetlands designed to remove nutrients or to provide wildlife habitat (dashed lines in Figure 3.12). The Prado wetlands in Orange County, California occupy approximately 200 ha. They currently receive up to 65 MGD of water from the effluent-dominated Santa Ana River, yielding a footprint of about 3 ha MGD $^{-1}$ (Orange County Water District, 2008). The Easterly Wetlands, located near Orlando, Florida, receive approximately 20 MGD of wastewater effluent in 475 ha, yielding a footprint of about 22 ha MGD $^{-1}$ (Florida Department of Environmental Protection, 2012). Wetland footprints of between 6 and 14 ha MGD $^{-1}$ have been recommended for the removal of nitrate in surface flow constructed wetlands (Horne, 1995).

Comparison of predicted values of A_{90}^1 with areas of existing full-scale wetlands suggests that open-water cells can provide efficient year-round treatment of photo-labile compounds, such as NDMA and propranolol. During spring and summer, the cells would also provide substantial removal of less reactive compounds, such as 17 β -estradiol and sulfamethoxazole. The least photo-reactive compounds, such as carbamazepine and atenolol, would only be removed to a significant extent through photolysis in relatively large photolysis wetlands during summer months. In practice, A_{90}^1 values may be considerably lower because microbes at the sediment-water interface will remove contaminants by biotransformation (Chapter 4).

3.4.7 Application to Wetland Design

The model described above may also be used to assess the role of photolysis in wetlands that were built without open-water zones designed to enhance photolysis. Many wetlands include open water sections for mixing or waterfowl habitat. In these wetlands, A_{90}^1 values can be estimated by accounting for the fraction of open water (F_{open}) in partially vegetated or shaded wetlands:

$$A_{90,\text{vegetated}}^1 = F_{\text{open}} A_{90}^1 \quad (3.18)$$

For example, the Tarrant Regional Water District's treatment wetlands, located south of Dallas-Ft. Worth, Texas, consist of about 50% open water intermixed with emergent vegetation (Kadlec *et al.*, 2011). The wetland has a footprint of about 8 ha MGD⁻¹, making approximately 4 ha MGD⁻¹ available for photolysis. Thus, according to model predictions depicted in Figure 3.12, photolysis may be remove many photo-labile contaminants in this system.

Ideally, however, open-water unit process cells will be designed as part of a unit process wetland with different types of wetland cells combined in series to maximize treatment efficiency and reliability (Chapter 2). Due to the effect of NO_3^- on the production of $\cdot\text{OH}$ and $\cdot\text{CO}_3^-$, an open-water cell would likely be most effective for removal of trace organic contaminants as the first process in the treatment wetland. However, additional research is needed to determine if wetland-derived dissolved organic carbon is more photoreactive than wastewater-derived dissolved organic carbon, which could result in increased photolysis rates for certain compounds in open-water unit process cells situated downstream of vegetated wetland cells (Pinney *et al.*, 2000).

It is also important to note that most trace organic contaminants will be transformed, and not mineralized, in photolysis wetlands. Although many photo-products will lack the specificity of their parent compounds with respect to biological receptors, further research is needed to determine if additional risks are posed by photo-products. It is also possible that photo-products produced are more amenable to biotransformation in subsequent unit process cells than their parent compounds.

CHAPTER 4. Biotransformation of Trace Organic Contaminants in Open-Water Unit Process Treatment Wetlands

Reprinted with permission from Jasper, J.T.; Jones, Z.L.; Sharp, J.O.; Sedlak, D.L.
Biotransformation of trace organic contaminants in open-water unit process treatment wetlands.
Environ. Sci. Technol. **2014**, 48, 5136-5144.

© 2014 American Chemical Society.

4.1 Introduction

Engineered treatment wetlands are commonly employed as polishing steps for municipal wastewater effluent to remove nutrients and metals that would otherwise require costly upgrades to wastewater treatment plants (Kadlec and Wallace, 2009; Vymazal, 2010). Increasingly, treatment wetlands are also being considered as a cost-effective and sustainable means of removing trace organic contaminants (e.g., pharmaceuticals and personal care products) from municipal wastewater effluent (Oulton *et al.*, 2010; Hijosa-Valsero *et al.*, 2010a; Chapter 2).

Effective trace organic contaminant removal in treatment wetlands is commonly hindered by hydraulic inefficiencies, such as short-circuiting, in addition to a limited understanding of how wetlands can be designed to optimize trace organic contaminant removal. The result is drastic variations in trace organic contaminant removal efficiencies among vegetated wetland systems (Chapter 2; Li *et al.*, 2014). The application of unit process design (e.g., connecting wetland cells optimized for the treatment of specific contaminants in series) could provide more effective removal of contaminants, while reducing hydraulic inefficiencies. However, successful implementation of this design strategy requires both a mechanistic understanding of removal processes in wetland cells as well as the development of novel wetland cells designed specifically for the removal of target contaminants.

As part of an effort to develop new unit process wetland cells, we recently described an open-water cell capable of removing photo-labile trace organic compounds (Chapter 2). Preliminary monitoring studies at the pilot-scale open-water cell indicated that the removal of several trace organic contaminants could not be explained by photolysis alone. As a result, we hypothesized that algae and associated microbes that formed a 2-7 cm thick biomat on the wetland bottom were responsible for some of the contaminant attenuation. Previous studies of algae and bacteria in streams receiving wastewater effluent indicate that microbial communities at the water-sediment interface are capable of removing trace organic contaminants such as 17 β -estradiol, 4-nonylphenol, ibuprofen, and microcystin-RR. In many cases, these biofilms are more important to contaminant attenuation than suspended microorganisms (Winkler *et al.*, 2001; Wu *et al.*, 2010; Writer *et al.*, 2011a, 2011b).

The microbial community that grows in shallow, open-water cells receiving municipal wastewater effluent may be more effective at transforming trace organic contaminants than communities in shallow streams, because high nutrient inputs and low water velocities lead to accumulation of more biomass. Furthermore, continuous exposure to wastewater effluent may lead to selection for organisms capable of metabolizing wastewater-derived contaminants.

The biomat may also be able to transform certain compounds more efficiently than the microbes in wastewater treatment plants. Carbon availability and cycling in this mixed autotrophic/heterotrophic assemblage results in a gradient with respect to electron acceptors and labile carbon compounds that is different from the conditions encountered in a conventional wastewater treatment plant. For example, during the day algal photosynthesis leads to supersaturated dissolved oxygen concentrations, which can enhance the rate of biotransformation of certain compounds (Matamoros *et al.*, 2005; Hijosa-Valsero *et al.*, 2010a, 2010b), while anoxic zones formed by microbial respiration at night or within the biomat may enhance biotransformation of other compounds (Xue *et al.*, 2010; Khunjar *et al.*, 2011).

The purpose of this study was to evaluate the ability of a surface-flow open-water wetland cell receiving fully nitrified wastewater effluent to remove a suite of wastewater-derived trace organic contaminants through sorption and biotransformation. To provide insight into the microbiology of the biomat, the community present on the wetland bottom was characterized by both phylogenetic 16S rRNA gene sequencing and microscopic approaches. Biotransformation rates and sorption capacities were assessed in microcosms utilizing biomass from the pilot-scale wetland and were compared to removal rates of test compounds in a pilot-scale open-water wetland cell.

4.2 Materials and Methods

4.2.1 Materials

All reagents were purchased from Fisher Scientific (Fairlawn, NJ) at the highest available purity. Isotopically-labeled internal standards were purchased from CDN Isotopes (Quebec, Canada), except for sulfamethoxazole-d₄ which was purchased from Toronto Research Chemicals, Inc. (Ontario, Canada). All solutions were prepared using 18 M Ω Milli-Q water from a Millipore system.

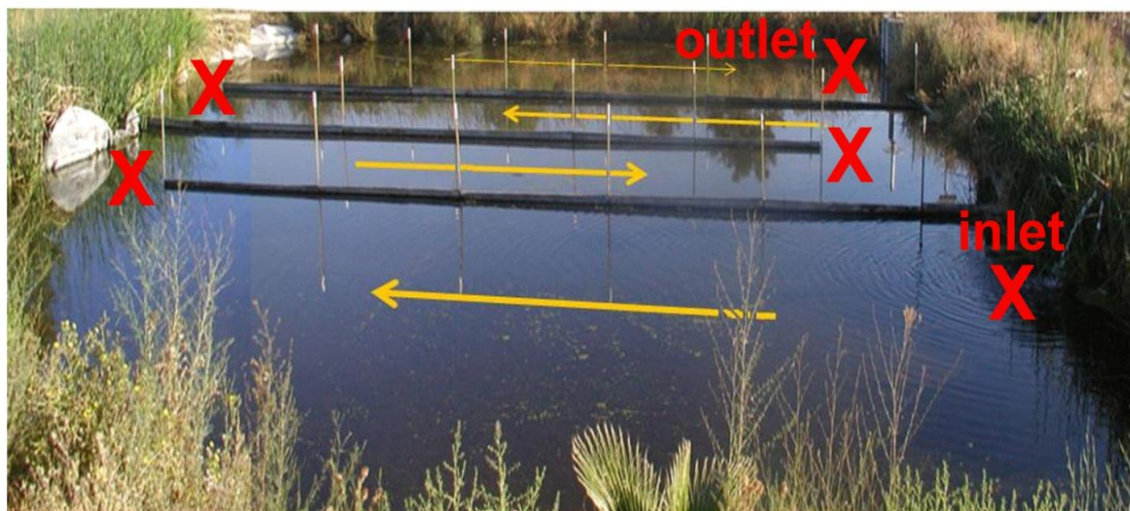


Figure 4.1: Photograph of the pilot-scale open-water unit process cell, located in Discovery Bay, CA (37.9°N, 121.6°W). Arrows indicate flow direct and **X** indicates sampling location.

4.2.2 Monitoring of the Pilot-Scale Wetland.

Ambient test compound concentrations and water quality parameters were monitored in a shallow (20 cm deep), pilot-scale open-water wetland (area=0.04 ha) in Discovery Bay, California that had been operating for 3 years (Figure 4.1). The cell was lined with a non-permeable geotextile fabric to prevent the growth of emergent macrophytes. The bottom of the cell was colonized by a diffuse (90 \pm 5% water by volume) biomat consisting of algae, associated

microorganisms, and detritus. The biomat was approximately 7 cm thick near the cell inlet and tapered in thickness along the flow path of the wetland to 2 cm near the cell outlet, occupying about 20% of the cell volume and corresponding to a total dry mass of approximately 1000 kg. Although the biomat was not firmly attached to the wetland bottom, it was not disturbed at the low flow rates employed. Photosynthesis resulted in daytime dissolved oxygen concentrations as high as 25 mg L⁻¹ during the summer.

The wetland cell received approximately 7x10⁻³ to 2x10⁻² million gallons per day (MGD) (i.e., 2.6x10⁻² to 7.6x10⁻² ML d⁻¹ (MLD)) of un-disinfected, nitrified wastewater effluent from the adjacent oxidation ditch treatment plant. The hydraulic residence time in the cell ranged from 1 to 3 days. Relatively short hydraulic residence times were employed to prevent the growth of floating vegetation (EPA, 2000a). Baffles installed across the cell ensured approximately plug-flow conditions, as indicated by Rhodamine-WT tracer tests (Figure 4.2).

Aqueous samples were collected at the influent, effluent, and at the end of each of the three baffles over a 3-year period (Figure 4.1). Aqueous samples were collected in 1 L, baked amber glass bottles, filtered through 1 µm glass fiber filters and refrigerated prior to analysis. 400 mL samples were amended with approximately 10 ng of isotopically-labeled internal standards and extracted via SPE, as described above, prior to analysis. In some cases, 1 mL samples were amended with 2.5 ng of isotopically-labeled internal standards, and analyzed by HPLC-MS/MS with direct injection of either 100 µL or 800 µL sample aliquots. Samples were analyzed within 3 days of collection.

Test compounds were extracted from biomat samples collected throughout the pilot-scale wetland using methanol, as described below.

Compound attenuation rates were calculated by fitting concentrations measured throughout the wetland to pseudo first-order kinetics ($r^2 > 0.8$) assuming plug-flow hydraulics:

$$C = C_o e^{-k_{\text{pilot}} V/Q} \quad (4.1)$$

where C is the compound concentration; C_o is the compound concentration at the influent; k_{pilot} is the pseudo first-order attenuation constant (d⁻¹); V is the cell volume (i.e., 8x10⁴ L); and Q is the flow rate measured at the wetland inlet (i.e., 2.6x10⁻² to 7.6 x10⁻² MLD).

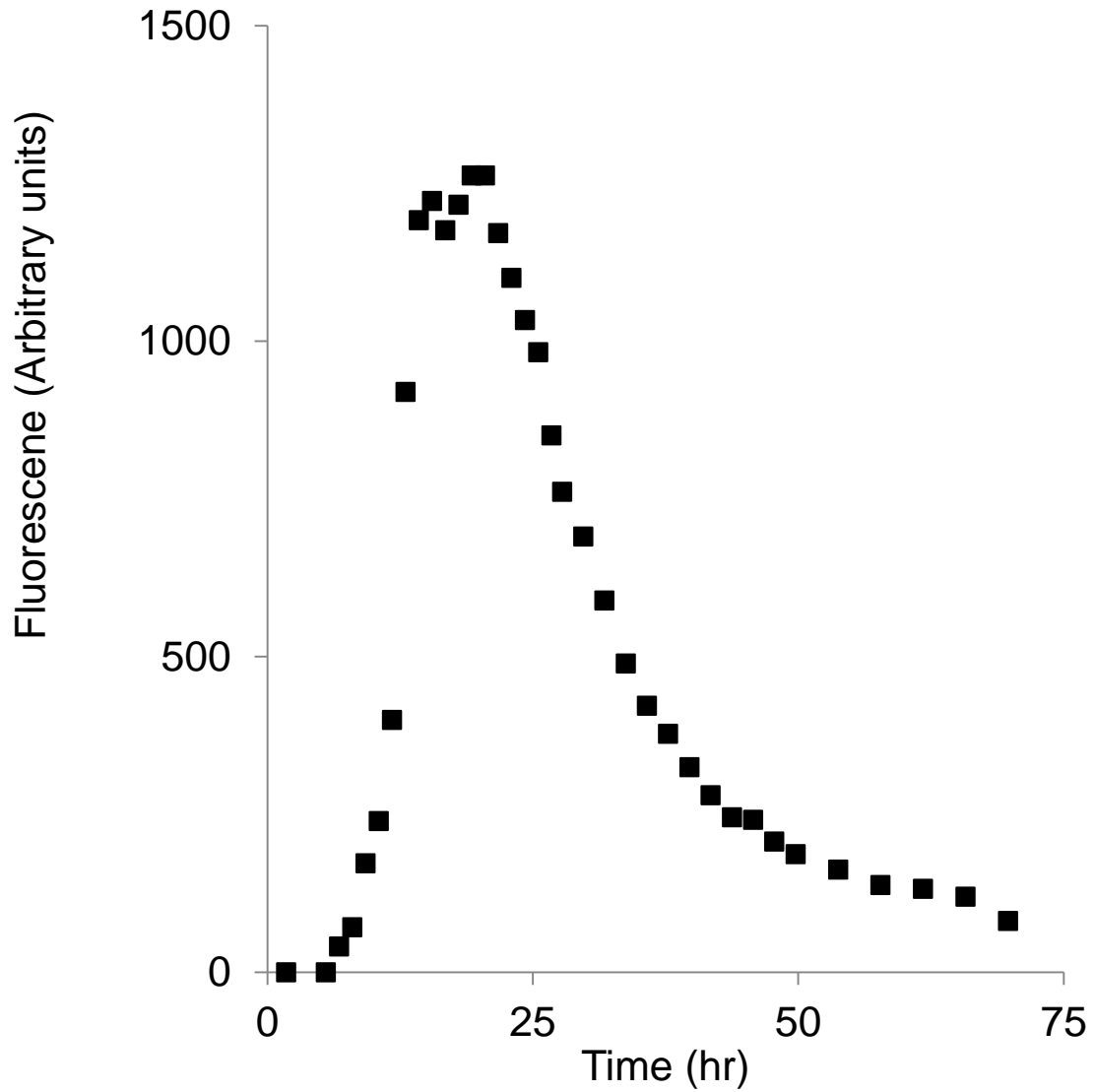


Figure 4.2: Breakthrough curve of rhodamine-WT in a tracer test in the pilot-scale open-water cell. Rhodamine-WT was injected at cell inlet at 0 hours and samples were collected at the cell outlet and analyzed by fluorometry (Turner TD 700). The center of mass was calculated to be 27 hours. The flow into the cell was 1.9×10^{-2} MGD ($8.1 \times 10^{-4} \text{ m}^3 \text{ s}^{-1}$).

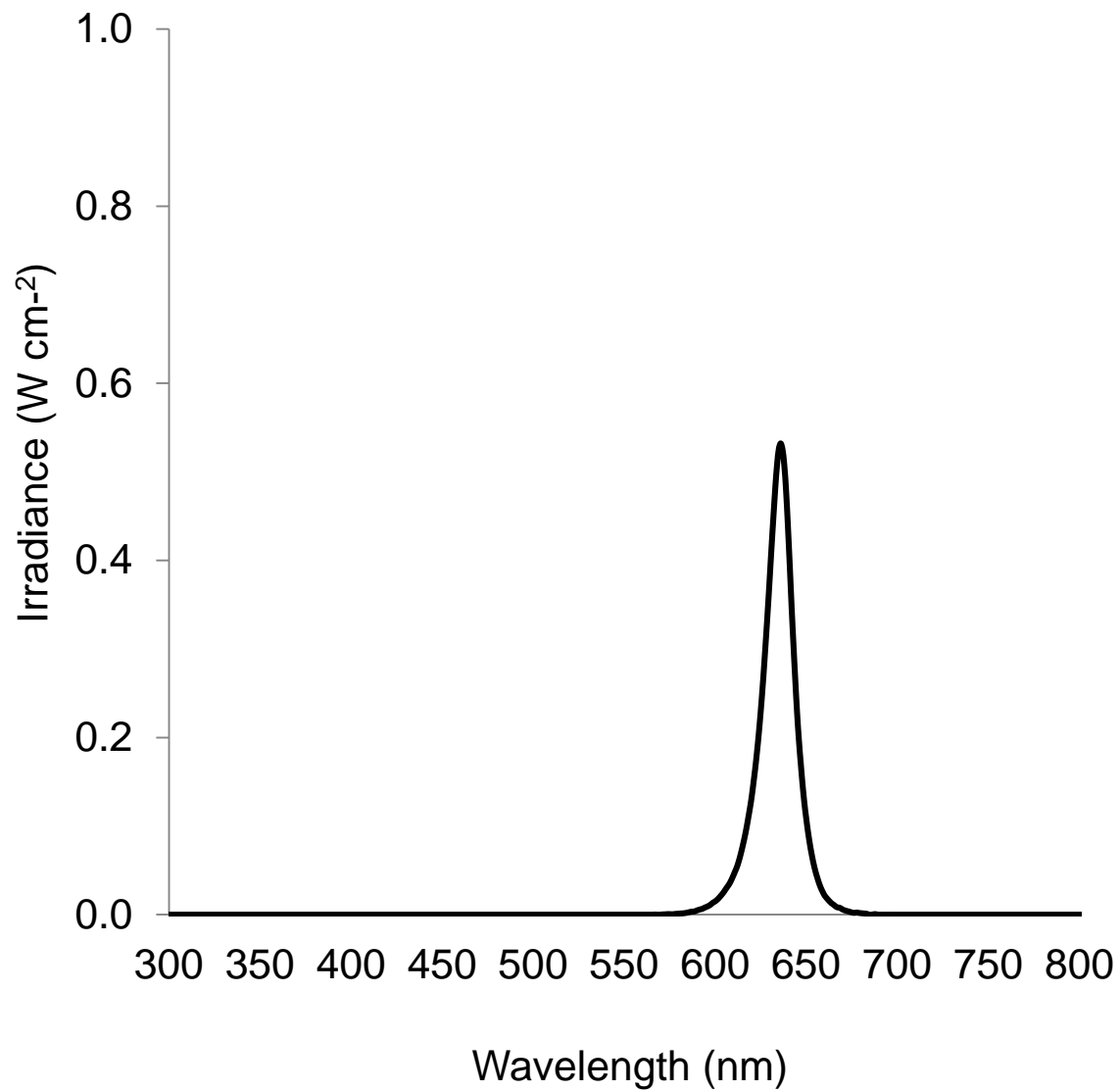


Figure 4.3: Spectrum of the red lamp used in microcosm experiments measured with a spectroradiometer (Stellarnet).

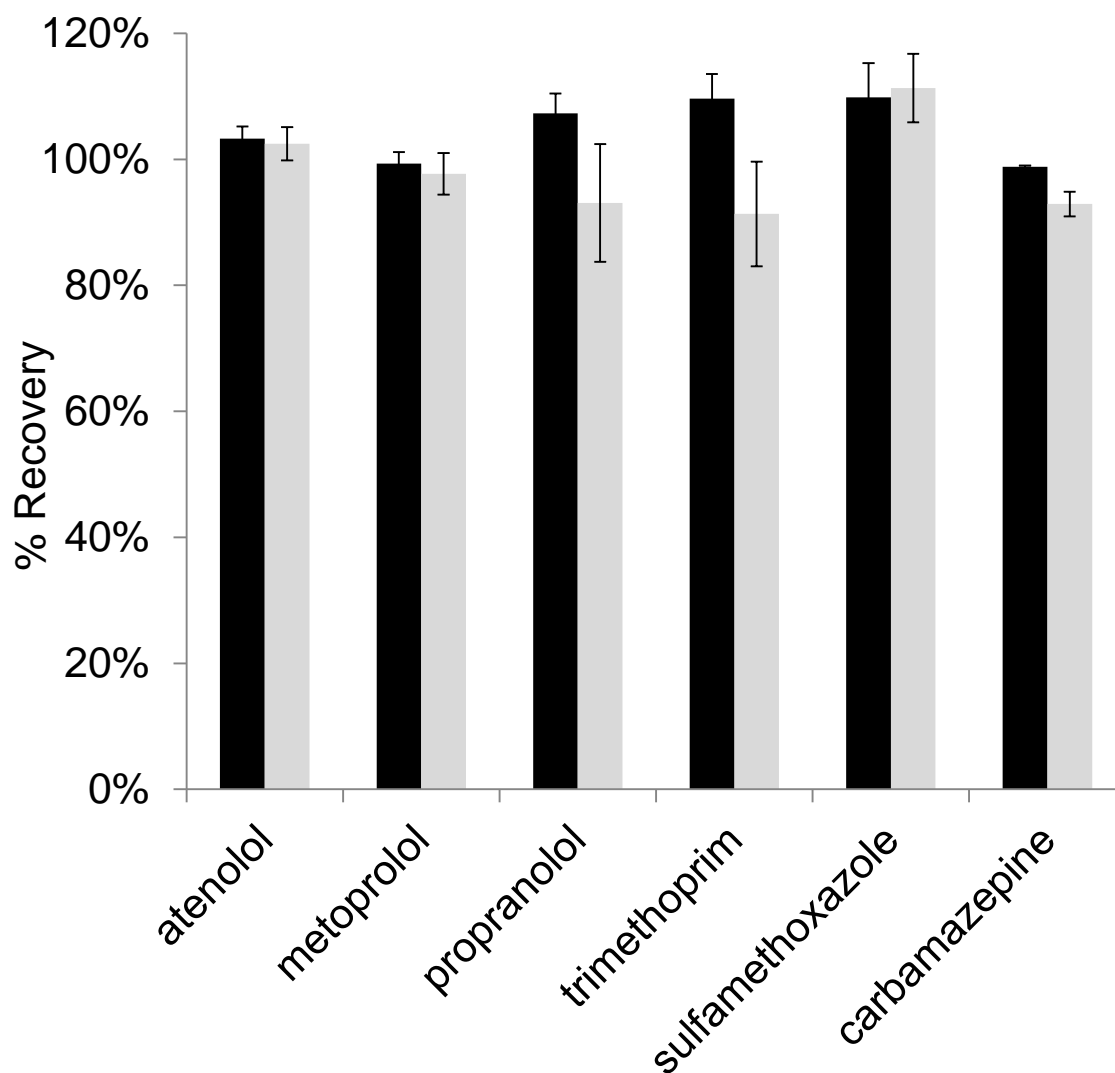


Figure 4.4: Percent mass recovery of test compounds amended to reactors containing autoclaved wetland biomass (13 g L^{-1}) maintained at pH 8.7 (black) and pH 10.0 (gray) by buffering with 20 mM borate. Reactors were gently mixed in triplicate on a rotisserie for 2 hours at 4°C in the dark prior to extraction. Error bars represent \pm one standard deviation.

4.2.3 Microcosms

Experiments to assess test compound sorption and biotransformation were conducted in triplicate in uncovered microcosms containing 1 L of 1- μ m filtered water collected from the midpoint of the Discovery Bay open-water cell ($[\text{DOC}] \approx 8 \text{ mg C L}^{-1}$; $[\text{NO}_3^-] \approx 10 \text{ mg N L}^{-1}$), and freshly collected biomass from the cell bottom (approximately 8 g dry mass). Microcosms were amended with a mixture of six test compounds at a concentration of $5 \mu\text{g L}^{-1}$ each. Microcosms were incubated at room temperature (25-30°C) in the dark or under monochromatic visible light (635 nm; 13.5 W red light, GenCom). The light supported photosynthesis without transforming the test compounds via photolysis (see Figure 4.3 for lamp spectrum). Dark microcosms were gently agitated from above using stir bars suspended on nylon strings to mimic the effects of mixing in wetlands while minimizing perturbation of the biomat. Illuminated microcosms were mixed via bubbles produced by photosynthesis in the biomat. Dark microcosms exhibited a stable pH value of approximately 8.5. In illuminated microcosms, irradiation with visible light increased the pH value to approximately 10 within 24 hours.

1 mL aqueous samples were collected throughout microcosm experiments, and were filtered through 1 μ m glass-fiber Acrodisc syringe filters (Pall Corporation), amended with about 2.5 ng of each isotopically-labeled internal standard, and refrigerated until analysis (within 1 week). Test compounds were extracted from dewatered (centrifuged at 5,000 RPM, 10 minutes), wet biomat samples (about 0.1 g dry weight). Samples were agitated for approximately 8 hours on a rotisserie in 15 mL of methanol amended with about 2.5 ng of each isotopically-labeled internal standard. Methanol extracts were filtered (1 μ m glass-fiber; Millipore, Bellerica, MA) and diluted to 1 L with deionized water prior to clean-up and concentration via solid phase extraction (SPE). The SPE media consisted of 50 mg Waters Oasis hydrophilic-lipophilic balance (HLB) in cartridges pretreated with 10 mL of methanol, followed by 10 mL of Milli-Q water. Cartridges were eluted with 12 mL of methanol, dried under a gentle nitrogen stream, and re-suspended in 1 mL of Milli-Q water prior to analysis via HPLC-MS-MS. 90 to 120% recoveries of analytes from autoclaved biomat samples were achieved Figure 4.4.

Table 4.1: Compound-Specific Mass Spectrometry Parameters^a

compound	precursor ion (amu)	fragmentor voltage (V)	product ions (amu)	collision energy (V)	cell accelerator (V)	ionization mode
1-naphthoxy acetic acid	201	80	143 115	15 45	0 5	negative ^b
4-OH-propranolol	276	115	173 116	15 15	5 5	positive ^c
Atenolol	267	130	145 190	24 16	7	positive
Atenolol-d ₇	274	130	145	24	7	positive
Carbamazepine	237	120	179 194	35 15	7	positive
Carbamazepine-d ₁₀	247	120	204	20	7	positive
Metoprolol	268	130	159 116	17 14	7	positive
Metoprolol-d ₇	275	130	123 159	14 17	7	positive
Metoprolol- α -OH	284	130	116 74	15 15	7	positive
Metoprolol- α -OH-d ₅	289	135	121	15	5	positive
Metoprolol acid	268	130	191 145	17 25	7	positive
Metoprolol acid-d ₅	273	130	196	17	7	positive
Nor propranolol	218	100	155 127	15 45	5	positive
Nor propranolol-d ₇	255	100	189	12	5	positive
Propranolol	260	98	116 183	13 12	7	positive
Propranolol-d ₇	267	98	116	13	7	positive
Sulfamethoxazole	254	110	92 156	25 10	7	positive
Sulfamethoxazole-d ₄	258	110	96	25	7	positive
Trimethoprim	291	140	123 261	20 17	7	positive
Trimethoprim-d ₃	294	140	123	20	7	positive

^aAll compounds were analyzed using a drying gas temperature of 350 °C, a gas flow of 12 L min⁻¹, a nebulizer pressure of 60 psi, a sheath gas temperature of 400 °C, a sheath gas flow of 12 L min⁻¹, and a nozzle voltage of 300 V. ^bCompounds analyzed by positive ionization used a capillary voltage of 3600 V. ^cCompounds analyzed by negative ionization used a capillary voltage of 4500 V.

4.2.4 Determination of Biotransformation Products

Test compound biotransformation products were identified in microcosms amended with a relatively high concentration of a single test compound (i.e., 100 mg L⁻¹). Biotransformation products were identified by high performance liquid chromatography-mass spectrometry (HPLC-MS) in full scan mode from 100-400 amu, in both positive and negative ionization modes. MS conditions were the same as those used for analytes (Table 4.1). Biotransformation products were verified with commercial analytical standards. Methods were subsequently developed for quantification of biotransformation products in microcosms (Table 4.1).

4.2.5 Photolysis Rate Prediction

The contribution of photolysis to the attenuation of test compounds in the pilot-scale system was assessed by extending a previously developed model for photolysis in shallow, open-water wetlands to include trimethoprim and metoprolol (Chapter 3). Briefly, pseudo first-order photolysis rates were calculated for direct and indirect photolysis using solar irradiance values predicted by the Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS) (Gueymard, 2003). Indirect photolysis rates were calculated using predicted steady-state concentrations of hydroxyl radical, carbonate radical, singlet oxygen, and triplet dissolved organic matter, in conjunction with second-order reaction rates of these reactive intermediates with test compounds. Average measured water quality parameters at the middle of the Discovery Bay open-water wetland used for the estimates were as follows: $[\text{NO}_3^-]=10 \text{ mg L}^{-1}\text{-N}$ in the summer and $15 \text{ mg L}^{-1}\text{-N}$ during the remainder of the year; $\text{pH}=9$; $[\text{DOC}]=8 \text{ mg L}^{-1}\text{-C}$; and $[\text{HCO}_3^-]+[\text{CO}_3^{2-}]=60 \text{ mg L}^{-1}\text{-C}$.

4.2.6 Correcting for Evaporation in the Pilot-Scale Cell

The effect of evaporation in the open-water cell was estimated by including an extra term in the calculation of the first-order rate constant, k , according to:

$$\ln \left(F \frac{C}{C_0} \right) = -kt \quad (4.2)$$

where C and C_0 are the concentration at time t and the initial concentration, respectively, and F is the fraction of water remaining after evaporation ($F < 1$). This equation may be rearranged to give:

$$k = -\frac{\ln(F)}{t} - \frac{\ln\left(\frac{C}{C_0}\right)}{t} = -\frac{\ln(F)}{t} + k_{\text{obs}} \quad (4.3)$$

where k_{obs} is the pseudo first-order removal rate of a compound observed in the wetland. Thus,

$$k_{\text{obs}} = k + \frac{\ln(F)}{t} \quad (4.4)$$

where $\frac{\ln(F)}{t} < 0$ and corrects for evaporation. Approximately 10% evaporation (i.e., $F=0.9$), quantified using increases in chloride concentrations, was typical during summer between the cell inlet and outlet (Figure 4.5). Based on a residence time of 1.5 days, $\frac{\ln(F)}{t} = -0.07 \text{ d}^{-1}$.

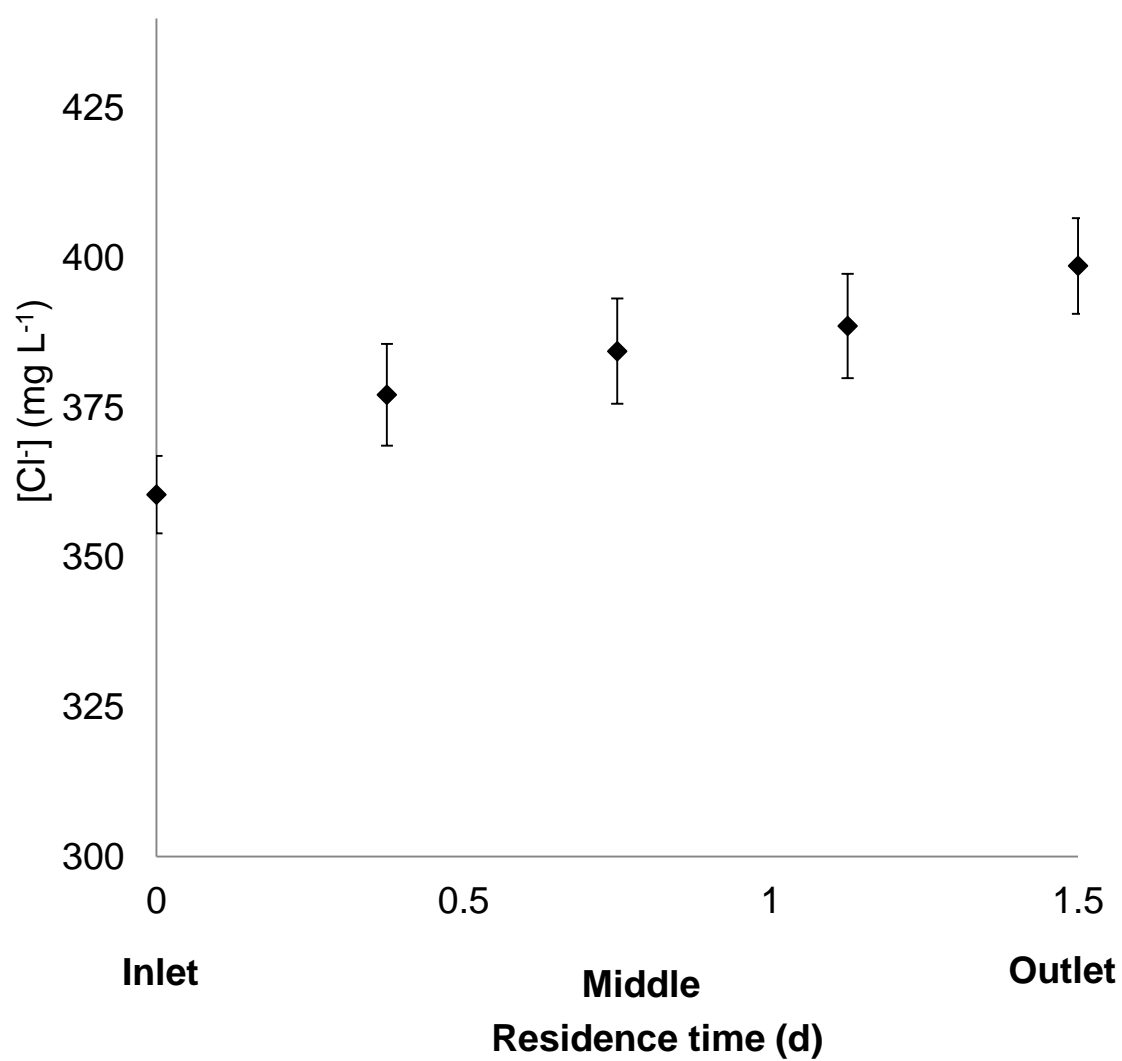


Figure 4.5: Average Cl⁻ concentrations throughout pilot-scale open-water cell during summer 2013. Error bars represent \pm standard error of the mean.

4.2.7 Microbial Community Characterization

Pilot-scale wetland biomat samples (250 μ L) for fluorescent microscopy were washed twice by centrifuging and re-suspending in phosphate buffer solution, before incubating in SYBR Green (stock solution diluted by a factor of 25,000) for 1.5 hours. Fluorescent images were generated using exciting/emission wavelengths of 473/490-540 nm for SYBR Green and 645/664 nm for autofluorescence of chloroplasts (Olympus Fluoview FV10i). Scanning electron microscopy (Hitachi TM-1000 Tabletop Microscope) was conducted on fresh biomass samples dried overnight on foil and placed on carbon tape.

Approximately 1 g of biomass was sampled from dark microcosms and microcosms illuminated with visible light (635 nm) at the beginning, middle, and end of biotransformation microcosm experiments. Samples were shipped overnight on dry ice and stored at -80° C prior to extraction. DNA was extracted from 0.25g of sample using the Mo Bio PowerBiofilm DNA Isolation Kit per manufacturer's protocol. Extracted DNA was amplified in triplicate 25 μ L reactions without Illumina adaptors or primers pads on a Roche LightCycler 480II. A portion of the 16S rRNA gene was amplified using Phusion Master Mix (New England BioLabs, Inc), 3% final volume DMSO, 0.4x final concentration SYBR Green, 200nM 515F (5'GTGYCAGCMGCCGCGGTAA 3') (Hamady *et al.*, 2008), and 12bp Golay barcoded 806R (5'XXXXXXXXXXXXCCGGACTACHVGGGTWTCTAAT 3') (Caporaso *et al.*, 2012). The amplification program was: 94°C 3 min; 94°C 45 sec, 50°C 10 sec, 72°C 90 sec. The program was stopped after all samples had amplified. Triplicates were pooled and purified using Agencourt AMPure XP and quantified using a Life Sciences Qubit 2.0 Fluorometer. Normalized amplicons were sequenced on the Illumina MiSeq platform using NEBNext Ultra DNA Library Prep Kit and a MiSeq Reagent Kits v2 2x250 500 cycle kit.

The sets of 250 bp sequences were stitched together using ea-utils (Aronesty, 2011) fastq-join with a minimum base pair overlap of 100. Stitched sequences were reverse complimented with the fastx toolkit (Pearson *et al.*, 1997) in order to account for sequences that were sequenced in the reverse direction. The resulting sequences were processed in QIIME 1.7 dev (Caporaso *et al.*, 2010) starting with sl_prep_fastq.py to create a barcode .fastq file. The resulting sequence and barcode file were demultiplexed using split_libraries_fastq.py with default parameters, except for "--barcode 12", to negate error correcting of barcodes, as any sequences with errors would have been filtered out by sl_prep_fastq.py. Otus were piked *de novo* using Usearch 6.1 (Edgar, 2010) and chimeras were filtered out using the Greengenes gold database (DeSantis *et al.*, 2006). Representative sequences were aligned using PyNAST (Caporaso *et al.*, 2009) and greengenes 13_5 aligned reference database. Taxonomy was assigned using the RDP classifier and greengenes 13_5 97 otu taxonomy database and the otu table was then rarified to 5050 sequences before further analysis. All phyla with less than 1% relative abundance were filtered out.

DNA for the 23S rRNA gene algal clone library was extracted in the same way as the microcosm samples and amplified with p23SrV_f1 and p23SrV_r1, GGA CAG AAA GAC CCT ATG AA, and TCA GCC TGT TAT CCC TAG AG, respectively, with the published amplification protocol (Sherwood and Presting, 2007). Amplicon was purified via gel electrophoresis using the E. Z. N. A. gel extraction kit (Omega). Purified amplicon was then transformed into electro-competent *E. coli* cells using the TOPO TA Cloning kit (Invitrogen) per

manufacture's instruction. Individual clones were Sanger sequenced by Wyzer Biosciences, Inc (Cambridge, MA). Sequences were analyzed in Geneious v6.0 and trimmed using default quality settings. After trimming any sequences with <90% high quality base scores were excluded from alignment. High quality sequences (16 out of 23) were aligned using the multiple alignment tool with default settings.

4.2.8 Analytical Methods

DOC was measured using a Shimadzu TOC-V analyzer. NO_3^- , Cl^- , and SO_4^{2-} were analyzed using a Dionex DX-120 ion chromatograph (American Public Health Association, 1995). The UV/Vis spectra of wetland water samples were measured using a PerkinElmer Lambda 35 spectrometer.

Trace organic compounds were separated by an Agilent 1200 HPLC using a 2.1 mm x 30 mm Zorbax SB-C18 3.5 μm column, eluted with 0.5 mL min^{-1} acetonitrile and 0.1% acetic acid in water with the following gradient: 0 minutes, 5% acetonitrile; 5.5 minutes 55% acetonitrile; 6 minutes, 100% acetonitrile; 9 minutes, 100% acetonitrile; 10 minutes, 5% acetonitrile. Compounds were quantified in multiple reaction monitoring (MRM) mode using isotope dilution with an Agilent 6460 MS-MS using electrospray ionization (ESI) with a gas temperature of 350°C, a sheath gas temperature of 400°C, a gas flow rate of 11 L/min at 50 psi, and a capillary voltage of 3600 V. Compound-specific parameters are given in Table 4.1.

4.3 Results and Discussion

4.3.1 Characterization of Wetland Biomat

Redox conditions in the wetland varied with depth, ranging from super-saturated with oxygen in the water column during the daytime and approximately 1 cm into the wetland biomat (Figure 4.6), to nitrate-reducing within the biomat, and sulfate-reducing at the bottom of the biomat, based on measurements of nitrate and sulfate (Chapter 5). At night, microbial respiration lowered dissolved oxygen concentrations in the water column to saturation or to as low as 5 mg L^{-1} , depending on the season.

The microbial community within the biomat consisted of an interspersed assemblage of photosynthetic and heterotrophic microorganisms dominated by a single species of diatom, as evidenced by scanning electron microscopy (Figure 4.7 A&B and Figure 4.8) and similarity of high-quality 23S clone library sequences (i.e., 98.8% similar when aligned after trimming). The diatom species was tentatively identified as *Staurosira construens* var. *venter* based on valve morphology (Hamilton *et al.*, 1992) and 16S rRNA gene sequence analysis (as Stramenopiles). Illumina 16S rRNA gene sequencing indicated that in addition to diatoms (30 \pm 3%), the associated sequences were dominated by Proteobacteria (37 \pm 4%; primarily of the β and γ superclasses), Bacteroidetes (7 \pm 1%), and Verrucomicrobia (6 \pm 1%) (Figure 4.7.1 C). Remaining phyla accounted for less than 15% of the total community.

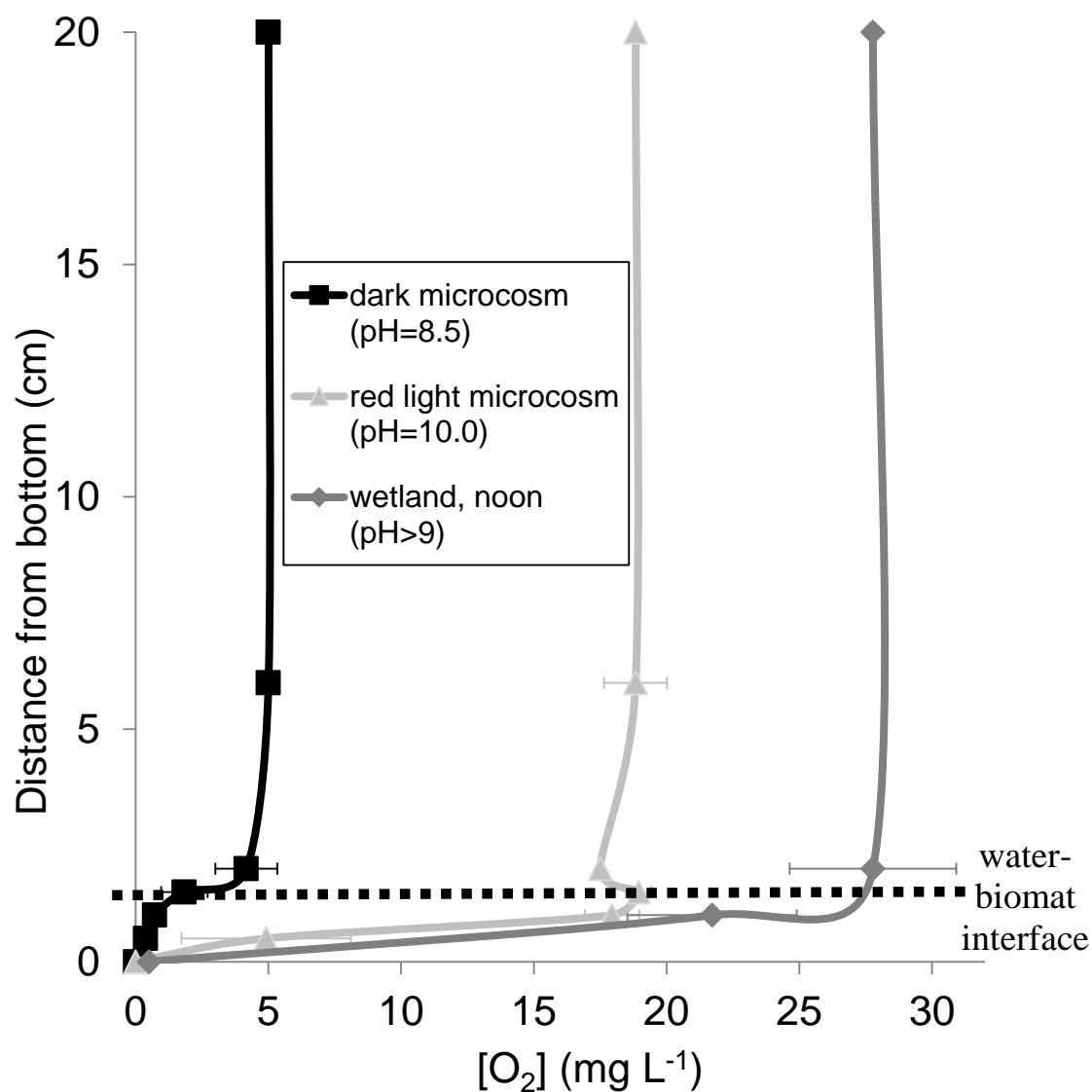


Figure 4.6: Dissolved oxygen profiles in microcosms and at the pilot-scale open-water cell (4-17-13) measured with a micro dissolved oxygen probe (Lazar Research Laboratories). Error bars represent \pm one standard deviation.

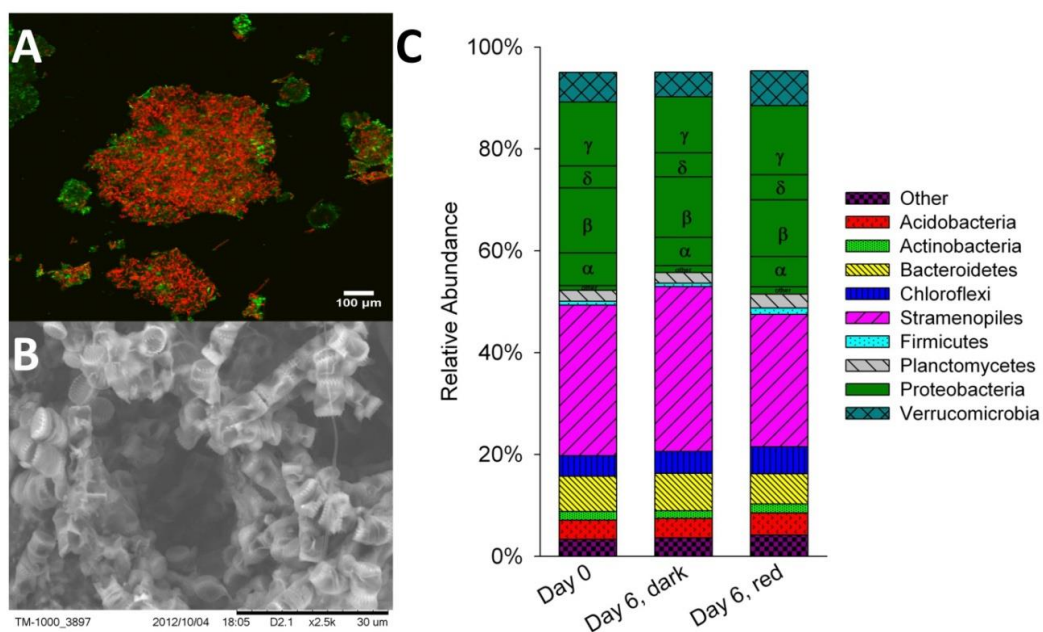
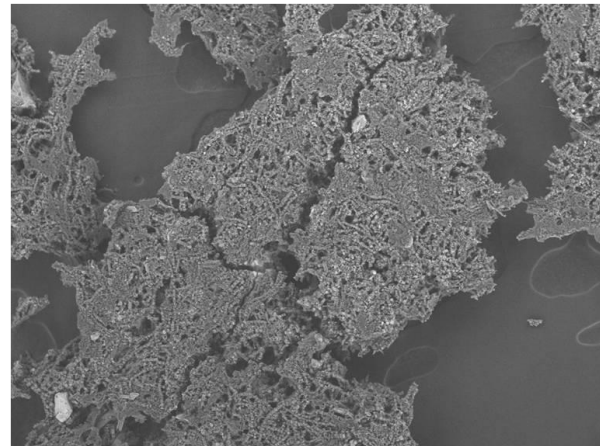
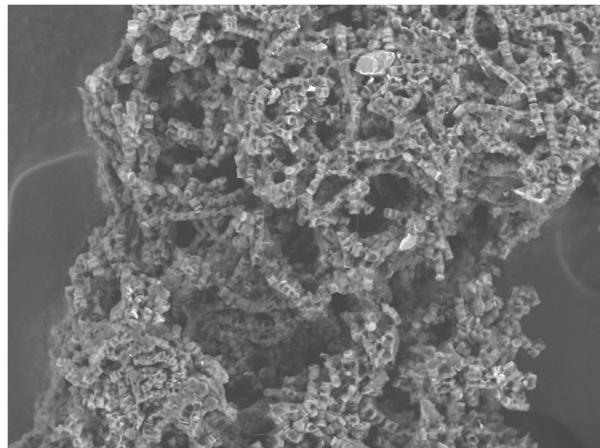


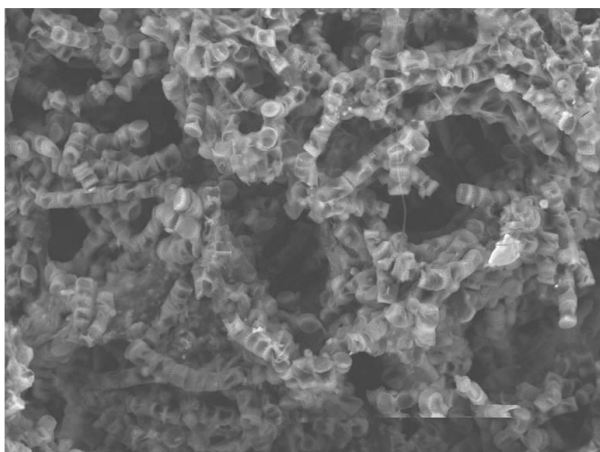
Figure 4.7: A) Fluorescent imagery of fresh biomass showed co-localization of diatoms (red) and bacteria (green). B) Scanning electron microscopy illustrated the presence of filamentous diatoms *Staurosira construens*. C) Illumina sequencing of microcosm biomass fresh from the wetland (Day 0), and after incubation in the dark (Day 6, dark) or incubation under 635 nm visible light (Day 6, red). Note that Stramenopiles is the phylum of the *Staurosira* diatom.



TM-1000_3900 2012/10/04 18:18 D2.1 x180 500 um



TM-1000_3895 2012/10/04 18:00 D2.0 x600 100 um



TM-1000_3896 2012/10/04 18:03 D2.0 x1.2k 50 um

Figure 4.8: Scanning electron microscopy of wetland biomat at 180X (top), 600X (middle), and 1200X (bottom) magnification.

4.3.2 Biotransformation in Microcosms

Microcosms were incubated under visible light to simulate daytime conditions (i.e., pH>9, super-saturated with dissolved oxygen) or in the dark to simulate nighttime conditions (i.e., pH≈8.5, dissolved oxygen below saturation; see Figure 4.6 for dissolved oxygen profiles). As evidenced by 16S-based phylogenetic analysis, microbial community profiles did not change significantly during the course of the experiments, suggesting that the microbes present remained representative of the assemblage in the pilot-scale system (Figure 4.7C). Test compound transformation rates in illuminated microcosms without added biomass were negligible (triangles in Figure 4.9), demonstrating that illumination with red light did not cause transformation of test compounds via photolysis, and that biotransformation in the aqueous phase was negligible.

Sorption to microcosm biomass was important to the removal of propranolol from the aqueous phase, consistent with its relatively high hydrophobicity ($\log K_{ow}=3.4$) (Betageri and Rogers, 1987; Balon *et al.*, 1999). Overall, sorption accounted for up to 60% of the removal of propranolol from the dissolved phase (Figure 4.9). The moderately hydrophobic test compounds carbamazepine ($\log K_{ow}=2.5$) (SRC, 2012) and metoprolol ($\log K_{ow}=1.9-2.3$) (Betageri and Rogers, 1987; SRC, 2012) were sorbed to microcosm biomass to a lesser extent. Sorption accounted for up to 100% of the observed removal of carbamazepine and 20% of the total removal of metoprolol. Sorption of carbamazepine has previously been observed in subsurface flow wetlands, but did not represent a permanent sink after the solids equilibrated with the wetland water (Matamoros *et al.*, 2008a). Sorption accounted for less than 5% of the observed removal of the relatively hydrophilic test compounds atenolol ($\log K_{ow}=0.2-0.5$) (Balon *et al.*, 1999; SRC, 2012), trimethoprim ($\log K_{ow}=0.9$) (SRC, 2012), and sulfamethoxazole ($\log K_{ow}=0.9$) (SRC, 2012).

Based on the results of the microcosm experiments, test compounds could be categorized as undergoing rapid biotransformation (i.e., the β -blockers atenolol, metoprolol, and propranolol; $t_{1/2}<1$ day), moderately amenable to biotransformation (i.e., trimethoprim and sulfamethoxazole; $t_{1/2}=2-20$ days), or recalcitrant (i.e., carbamazepine; $t_{1/2}>40$ days). This classification agreed well with biotransformation rates previously measured in microcosms inoculated with activated sludge from wastewater treatment plants, but the overall rates differed (Table 4.2). For the β -blockers, biotransformation rates in the dark microcosms were approximately an order of magnitude slower than those observed in microcosms inoculated with activated sludge. The lower rates may have been partially attributable to enhanced mass transfer rates of oxygen and trace organic compounds between the aqueous and sludge phases in well-mixed activated sludge microcosms. β -blocker biotransformation rates in microcosms inoculated with biomass from open-water wetlands incubated in the dark increased by approximately a factor of 5 when the biomass was completely mixed into the water column, compared to microcosms that were gently agitated from above (data not shown).

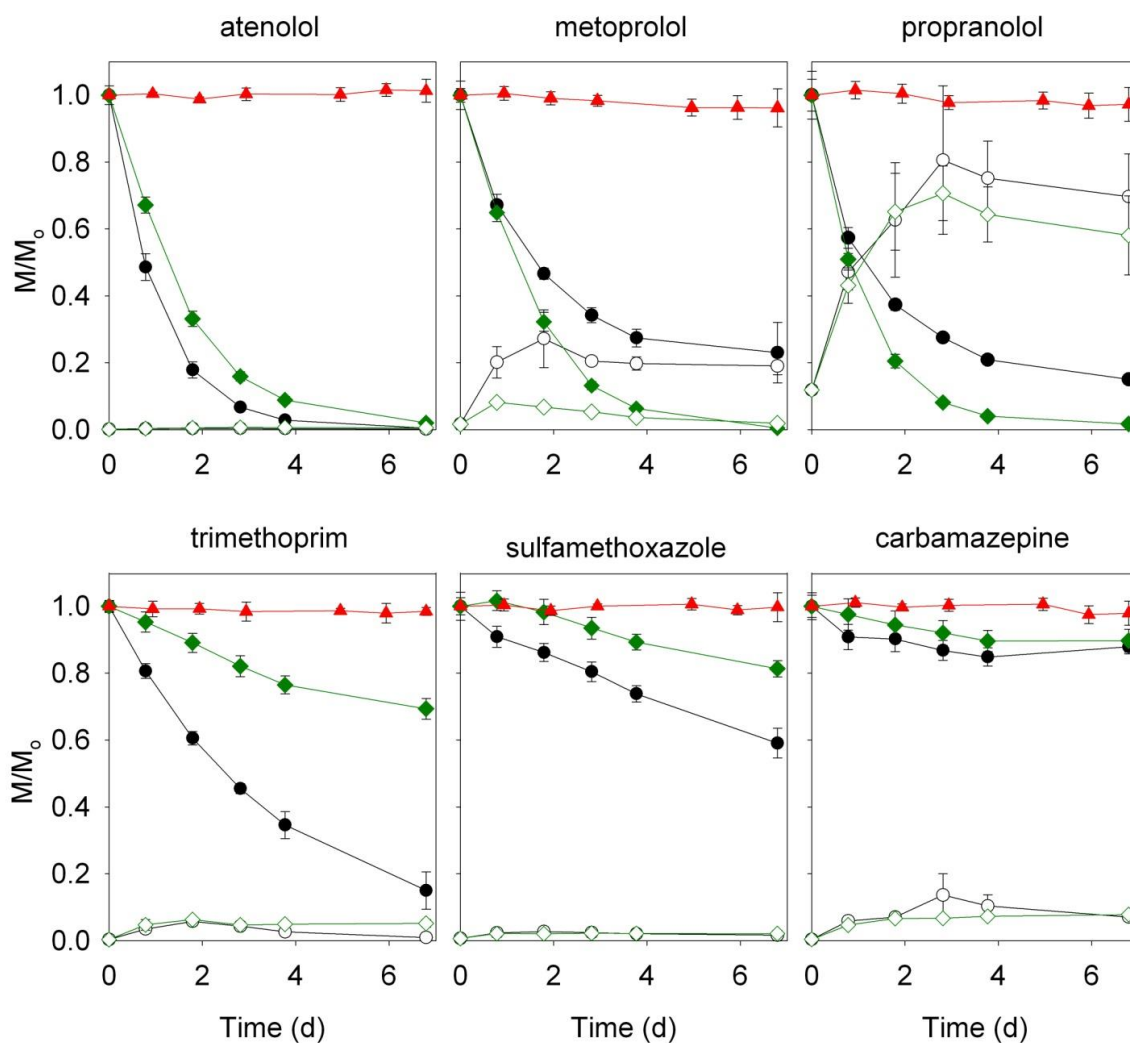


Figure 4.9: Fraction of test compound masses (M/M_0) remaining in microcosms that were: illuminated by visible light (635 nm) without added biomass (▲); in the dark with added biomass (aqueous phase: ●; biomat phase: ○); and illuminated by visible light (635 nm) with added biomass (aqueous phase: ◆; biomat phase: ◇). $M_0 \approx 6 \mu\text{g}$. Error bars represent \pm one standard deviation.

Metoprolol and propranolol biotransformation rates increased by approximately 4 to 8 times in illuminated microcosms compared to dark microcosms. This enhancement may have been attributable to higher concentrations of dissolved oxygen within the biomass: dissolved oxygen concentrations in the irradiated microcosms remained above 15 mg L⁻¹ in the top 1 cm of the biomat, compared to dissolved oxygen concentrations ranging from about 5 mg L⁻¹ at the water-biomat interface to less than 1 mg L⁻¹ deeper than 1 cm in the biomat in the dark microcosms (Figure 4.9 and Table 4.2; see Figure 4.6 for dissolved oxygen profiles). Previous studies have shown that the rates of oxidation of some trace organic compounds were enhanced under aerobic conditions in wetlands (Hijosa-Valsero *et al.*, 2010b) and in activated sludge wastewater treatment systems (Plósz *et al.*, 2010; Xue *et al.*, 2010). For example, biotransformation of metoprolol was up to 5 times faster under aerobic conditions relative to anoxic conditions in full-scale wastewater treatment plants (i.e., $k_{\text{aerobic}} \approx 1.2 \text{ d}^{-1}$ versus $k_{\text{anoxic}} \approx 0.24 \text{ d}^{-1}$) (Xue *et al.*, 2010). Biotransformation rates may also have increased upon illumination with visible light due to enhanced microbial activity caused by the release of organic compounds by autotrophic diatoms during photosynthesis (Cole, 1982), although these effects could not be separated from the effects of dissolved oxygen production by the diatoms.

In contrast, rates of biotransformation of trimethoprim and sulfamethoxazole were more than 5 and 2 times faster, respectively, in the dark microcosms, relative to the illuminated microcosms. This difference in biotransformation rates may have been due to a community metabolic shift caused by anoxic conditions or induced by the absence of primary productivity (Sharp *et al.*, 2007; Patrauchan *et al.*, 2012). Enhanced rates of biotransformation of trimethoprim have been reported at dissolved oxygen conditions below 0.5 mg L⁻¹ in microcosms inoculated with activated sludge (Xue *et al.*, 2010). Furthermore, the biotransformation of trimethoprim has been suggested to involve certain heterotrophic microorganisms with minimal oxygenase activity, which may be inhibited by high dissolved oxygen concentrations (Khunjar *et al.*, 2011).

These results highlight the potential importance of the terminal electron acceptor to rates of transformation of trace organic contaminants. Open-water wetlands exhibit a diurnal fluctuation in redox conditions as well as variations through the vertical profile of the biomat (i.e., oxygen-reducing at the water-biomat interface to sulfate-reducing at the bottom of the biomat), enabling the biotransformation of compounds across terminal electron acceptor gradients. For the removal of compounds that are mainly transformed under anoxic conditions, the use of wetlands with larger anoxic zones (e.g., wetlands with dense macrophytes or subsurface flow wetlands) may be a more effective treatment strategy.

Table 4.2: Test Compound First-Order Transformation Rates (d⁻¹)

Compound	Dark microcosms ^a	Illuminated microcosms ^a	Open-water wetland, summer ^a	Activated sludge microcosms	Surface flow treatment wetlands	Surface waters
Atenolol	0.80±0.03	0.59±0.01	1.34±0.60	6 ^b ref a,b,c	0.05-0.1 ^{ref d}	0.2 ^{ref e} , 0.23 ^{ref f} , 0.30 ^{ref f}
Metoprolol	0.22±0.05	0.82±0.07	1.10±0.3	1.5 ^{ref a} , 2.6 ^b ref c, 1.2 ^{ref g}	0.03 ^{ref d}	0.024 ^{ref f} , 0.039 ^{ref f} , 0.13 ^{ref h} , 0 ^{ref i}
Propranolol	0.11±0.02 ^c	0.86±0.02	1.15±0.53	1.7 ^b ref a	0.09 ^{ref j}	0.021 ^{ref f} , 0.07 ^{ref f}
Trimethoprim	0.29±0.05	0.056±0.004	0.36±0.23	0.2 ^d ref k, 0.4 ^d ref l	0.04-0.2 ^{ref d}	0, 0.2 ^{ref e}
Sulfamethoxazole	0.08±0.01	0.034±0.001	0.45±0.49	0.09 ^{ref d} , 0.26 ^{ref m}	0-0.02 ^{ref d}	0.05 ^{ref e} , 0.04 ^{ref n}
Carbamazepine	0.016±0.004	0.016±0.003	0.02±0.05	<0.4 ^b ref a	0-0.04 ^{ref d} , 0.02 ^{ref o} , 0.01-0.02 ^{ref p}	0.007 ^{ref q} , 0.01 ^{ref r} , 0.8 ^{ref s}

^a Measured in this study. ^b Calculated assuming a total suspended solids concentration of 4 g L⁻¹. ^c Estimated from the linear portion of removal curve, after sorption had reached equilibrium. ^d Measured with nitrifying activated sludge. Negligible loss of trimethoprim has been observed in non-nitrifying activated sludge (Ramil et al., 2009). References: a: (Wick et al. 2009); b: (Helbling et al., 2010); c: (Kern et al., 2010); d: (Breitholtz et al., 2012); e: (Lim et al., 2008); f: (Ramil et al., 2009); g: (Xue et al., 2010); h: (Fono et al., 2006); i: (Kunkel and Radke, 2011); j: (Fono and Sedlak, 2005); k: (Batt et al., 2006); l: (Pérez et al., 2005); m: (Li and Zhang, 2010); n: (Radke et al., 2009); o: (Llorens et al., 2009); p: (Matamoros et al., 2008b); q: (Calza et al., 2013); r: (Tixier et al., 2003); s: (Writer et al., 2013).

4.3.3 Biotransformation Products

Biotransformation products of the β -blockers atenolol, metoprolol, and propranolol were detected in the microcosms. Atenolol was hydrolyzed at the amide group nearly stoichiometrically to form metoprolol acid, a transformation product that has previously been observed in microcosms inoculated with activated sludge (Figure 4.10) (Radjenović *et al.*, 2008; Kern *et al.*, 2010). Metoprolol acid did not undergo further biotransformation. Metoprolol acid was also formed from metoprolol in microcosms, where it accounted for approximately 25% of the metoprolol transformed, which was comparable to the yield observed in microcosms inoculated with activated sludge (Kern *et al.*, 2010). In addition, oxidation of metoprolol produced low concentrations (i.e., $<0.1 \mu\text{g L}^{-1}$) of α -hydroxy-metoprolol, a known human metabolite (Fang *et al.*, 2004). Metoprolol acid is less toxic to mammals than metoprolol (Borg *et al.*, 1975). However, its high stability may warrant further investigation.

The propranolol oxidation product and human metabolite, 4-hydroxy-propranolol (Nałęcz-Jawecki *et al.*, 2008), was detected in microcosms amended with high concentrations of propranolol. 4-hydroxy-propranolol accounted for up to 1% of the propranolol transformed. No biotransformation products were detected in microcosms with high concentrations of trimethoprim, sulfamethoxazole, or carbamazepine (data not shown).

Table 4.3: Average Aqueous Test Compound Concentrations of Trace Organic Compounds in the Pilot-Scale Open-Water Cell

Compound	Concentration (ng L^{-1}) ^a				
	<i>Inlet</i>	<i>Point 1</i>	<i>Middle</i>	<i>Point 3</i>	<i>Outlet</i>
Atenolol	100 \pm 10	31 \pm 4	19 \pm 3	7.7 \pm 1.6	3.3 \pm 0.9
Metoprolol	58 \pm 3	38 \pm 3	30 \pm 3	20 \pm 3	8.6 \pm 0.9
Propranolol	41 \pm 2	23 \pm 3	16 \pm 2	10 \pm 1	4.5 \pm 0.6
Trimethoprim	8.4 \pm 0.9	7.8 \pm 0.8	6.3 \pm 0.3	5.1 \pm 0.7	4.7 \pm 0.7
Sulfamethoxazole	810 \pm 20	750 \pm 20	730 \pm 10	750 \pm 10	710 \pm 20
Carbamazepine	110 \pm 10	110 \pm 10	120 \pm 10	110 \pm 10	110 \pm 10

^aAverage \pm standard error of the mean of 10 samples measured at each sample location in the wetland during the week of August, 20th 2012. See Figure 4.11 for plot of data.

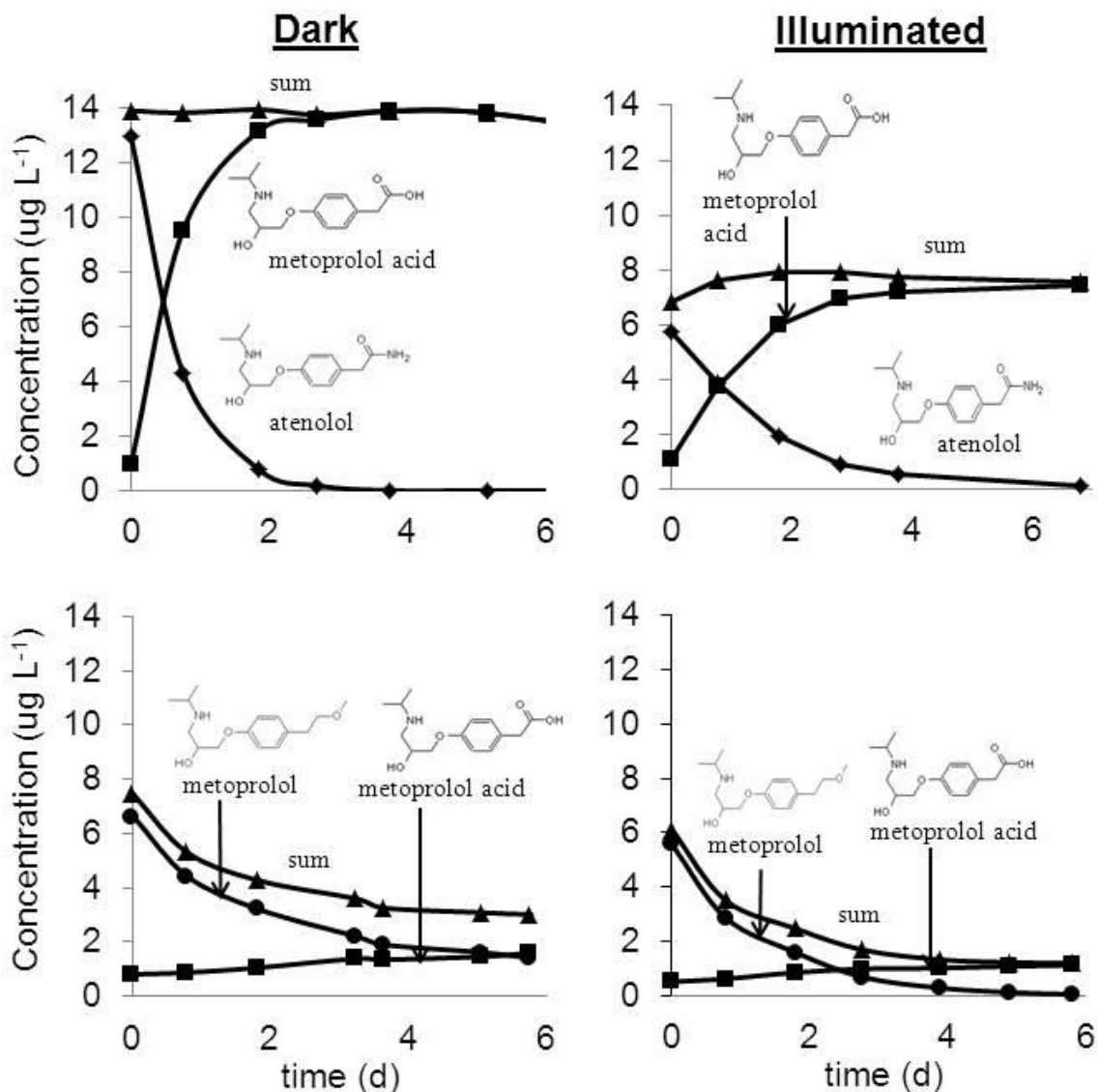


Figure 4.10: Formation of the biotransformation product metoprolol acid (■) in dark (left) and illuminated (635 nm visible light; right) microcosms amended with either atenolol (top; ♦) or metoprolol (bottom; ●). The sum of the concentrations of the parent products and the transformation products are also shown (▲). Sorption accounted for loss of less than 1% of the initial mass of atenolol and from 5% (illuminated microcosm) to 20% (dark microcosm) of the initial concentration of metoprolol.

4.3.4 Compound Attenuation in the Pilot-Scale Open-Water Wetland

Wetland influent concentrations of test compounds typically ranged from 40 to 100 ng L⁻¹ for the β -blockers, 8 to 15 ng L⁻¹ for trimethoprim, 800 to 1000 ng L⁻¹ for sulfamethoxazole, and 90 to 120 ng L⁻¹ for carbamazepine (Table 4.3). Metoprolol acid, the biotransformation product of atenolol and metoprolol, was typically present at influent concentrations that were about an order of magnitude higher than atenolol (i.e., 300-1000 ng L⁻¹). This precluded the observation of the transformation of atenolol to metoprolol acid within the wetland cell. Influent test compound concentrations typically varied by less than 30% based on hourly samples, and removal through the wetland cell typically followed first-order kinetics ($r^2 > 0.8$), except for carbamazepine, which did not exhibit significant removal (see Figure 4.11).

Summer and fall test compound attenuation rate constants in the pilot-scale open-water wetland ranged from greater than 1.4 d⁻¹ for the β -blockers to less than 0.5 d⁻¹ for trimethoprim and sulfamethoxazole, which corresponded to half-lives of 0.5 to 1.5 days (Figure 4.12). These attenuation rates were comparable to rates measured in activated sludge systems, with the exception of atenolol, which was removed at faster rates in treatment plants (Table 4.2). Attenuation rates of all test compounds except carbamazepine were 10 to 100 times faster in the open-water cell than rates previously measured in vegetated surface flow wetlands and surface waters (Table 4.2). Carbamazepine behaved conservatively in the open-water cell, in agreement with previous research showing the compound's stability with respect to photolysis (Chapter 3) and its resistance to biotransformation in activated sludge and surface waters (Tixier *et al.*, 2003; Wick *et al.*, 2009).

Test compound attenuation rate constants decreased by more than 50% for all test compounds during the winter months. Lower attenuation rates of compounds amenable to biotransformation were likely due to reduced microbial activity at colder temperatures. Corrections for the temperature dependence of biotransformation rates of trace organic contaminants were estimated using a modified form of the Arrhenius equation:

$$k_T = k_{T,ref} e^{-\kappa(T_{ref}-T)} \quad (4.5)$$

where k_T and $k_{T,ref}$ are the biotransformation rates at a given temperature (T) and at a reference temperature (T_{ref}), respectively, and κ is the temperature coefficient, which is typically between 0.03 and 0.09 K⁻¹ for trace organic contaminants (Clara *et al.*, 2005; Li *et al.*, 2005; Joss *et al.*, 2006; Wick *et al.*, 2009). Using these values for κ , trace organic biotransformation rates should have decreased by approximately 40-80% during winter (i.e., winter temperatures of 9 \pm 2.5° C versus summer temperatures of 22 \pm 5° C; see Table 4.4), which agreed well with reductions in rate constants observed in the field. Similarly, attenuation rates of compounds amenable to photolysis should have been reduced by up to approximately 75% during the winter due to lower sunlight intensity and shorter days (Chapter 3).

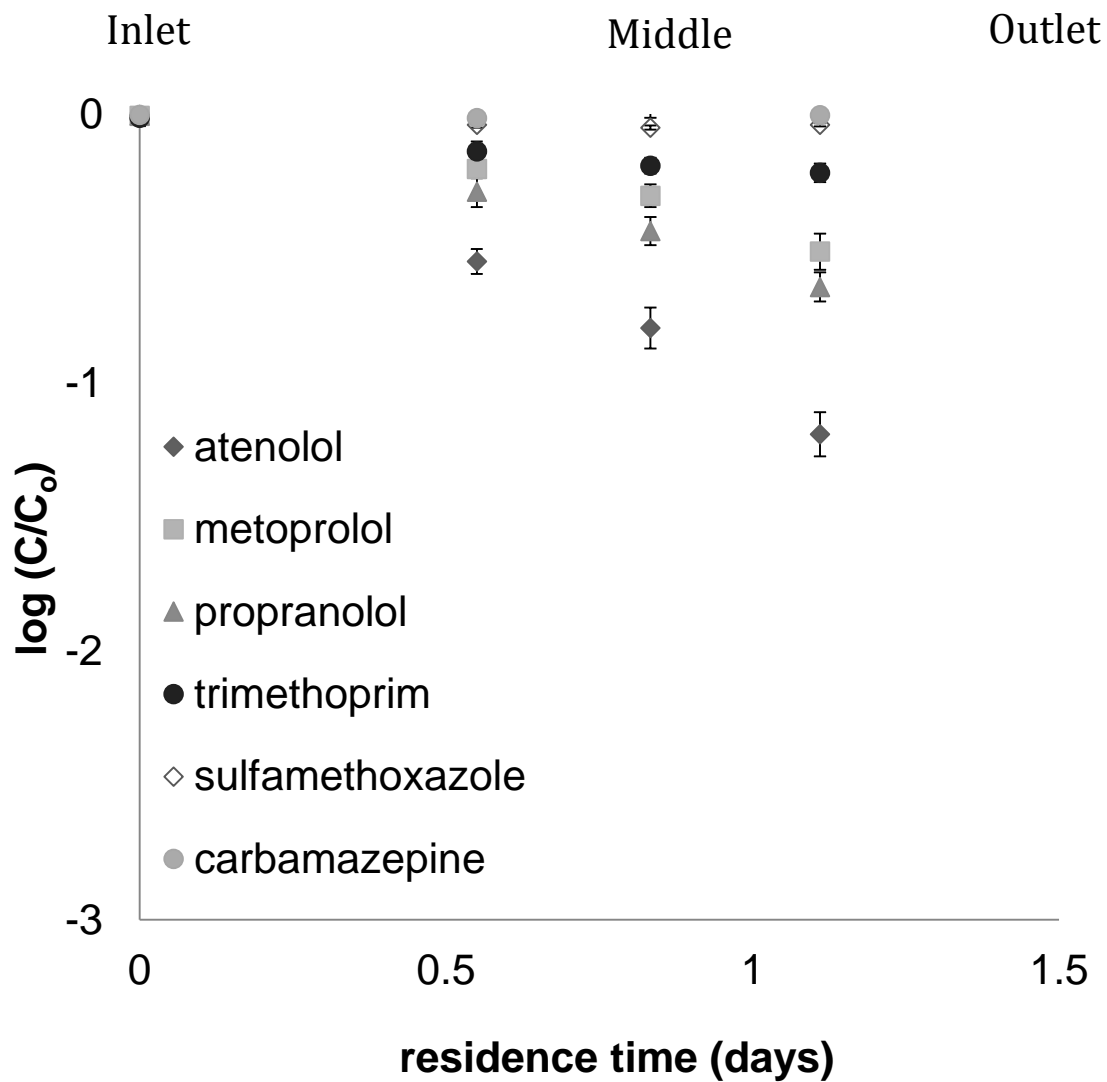


Figure 4.11: Removal of test compounds in pilot-scale open-water wetland during August 2012. Error bars represent \pm standard error of the mean (n=10).

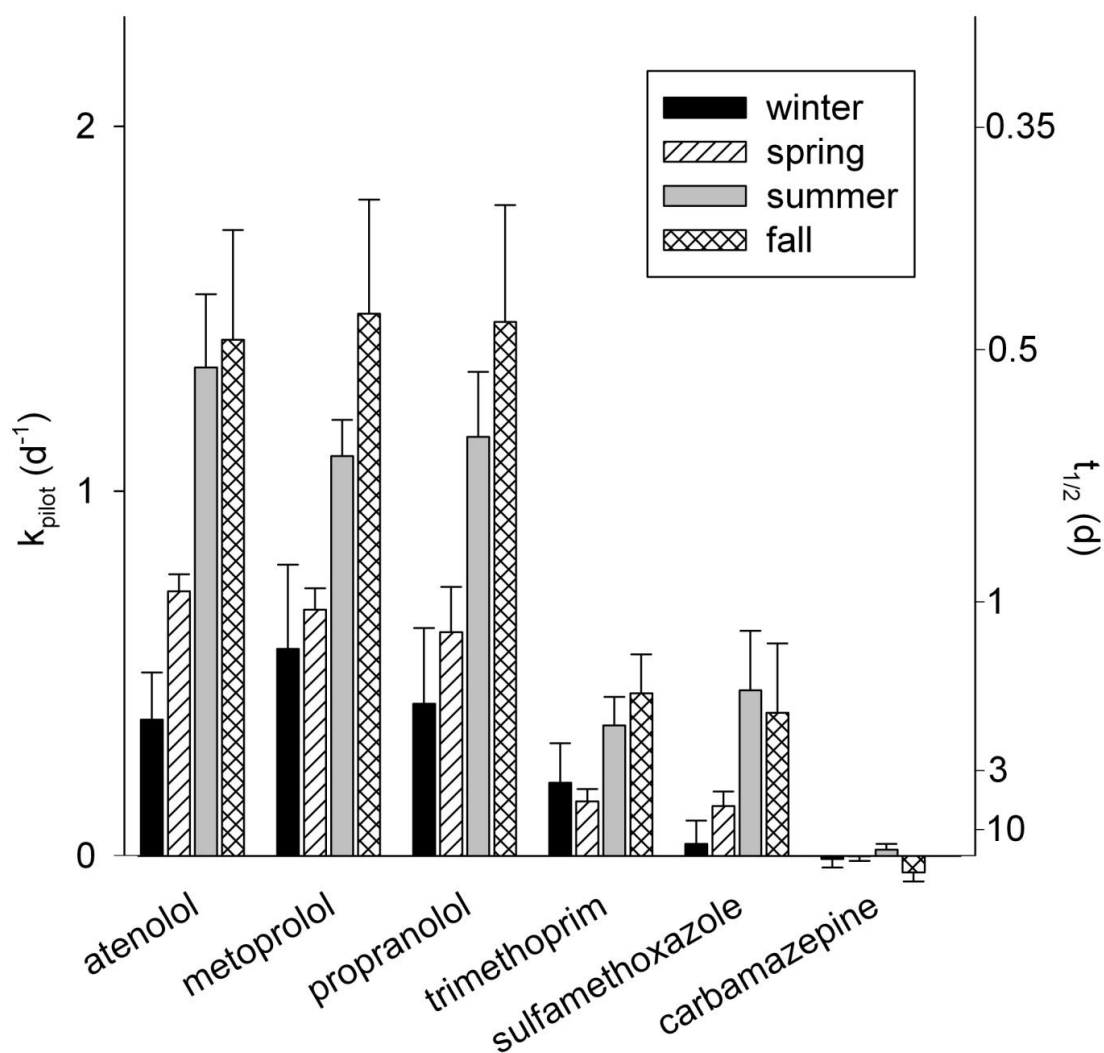


Figure 4.12: Seasonal pseudo first-order attenuation rates of test compounds in the pilot-scale open-water cell. Error bars represent \pm standard error of the mean for measurements made approximately monthly over a 3 year period ($n=5-12$). Negative removal rates are due to water loss via evaporation.

Table 4.4: Wetland Temperatures^a

Month	Average Temperature (°C)
January	8.6±0.4
February	9.8±0.3
March	11.9±0.3
April	14.4±1.0
May	16.8±0.7
June	20.0±0.3
July	22.0±0.2
August	22.3±0.4
September	21.7±0.5
October	17.5±0.3
November	12.4±0.5
December	9.3±0.3

^a Average monthly temperatures reported in Livermore, CA from 2007-2012 ± standard error of the mean (Metar, 2013).

4.3.5 Evaluation of Attenuation Mechanisms in the Pilot-Scale Open-Water Wetland

For non-volatile trace organic compounds, potential attenuation mechanisms in surface-flow open-water wetlands included sorption to the wetland biomat, biotransformation, and photolysis. Each of these mechanisms was evaluated to inform open-water wetland design and to predict the area necessary to removal of trace organic contaminants.

Sorption. The concentrations of test compounds sorbed to the biomat in the open-water cell were less than about 10 µg kg⁻¹, except for propranolol, which was detected at concentrations as high as approximately 70 µg kg⁻¹ (Table 4.5). These concentrations were 10 to 100 times lower than those measured in activated sludge, except for propranolol, which was observed at similar concentrations in activated sludge (Radjenović *et al.*, 2009). Sorbed concentrations corresponded to a mass of approximately 10 mg ha⁻¹ for atenolol and up to approximately 1000 mg ha⁻¹ for propranolol. Over the 3-year period in which the pilot-scale system operated, this corresponded to less than 2% of the mass of propranolol removed and less than 0.1% of the mass of the other test compounds, indicating that sorption is not a significant removal mechanism for the test compounds if it is not coupled to biotransformation.

Sorption was not an important removal mechanism because aqueous test compound concentrations were limited by equilibrium partitioning with the biomat phase. In other words, measured aqueous test compound concentrations were similar to aqueous concentrations predicted by organic carbon-normalized biomat concentrations and log K_{OC} values measured with autoclaved wetland biomass (i.e., measured values were within a factor of 2 of predicted values, except for propranolol which was present at concentrations up to 7 times higher in the biomat phase than predicted; Tables 4.3 and 4.5). Thus, sorption in open-water wetlands may serve as a short-term sink for a pulse of elevated concentrations of trace organic contaminants, which may occur diurnally (Nelson *et al.*, 2011). However, accumulation of fresh biomass was not rapid enough for appreciable attenuation of the test compounds studied.

Photolysis. Photolysis accounted for most of the removal of the photo-labile compounds propranolol and sulfamethoxazole in the pilot-scale wetland during the summer (Figure 4.13). Photolysis has also been suggested to be the dominant removal mechanism for these compounds in lake and river systems (Alder *et al.*, 2010; Bonvin *et al.*, 2011). In contrast, photolysis accounted for only about 10% of the removal of atenolol and metoprolol and less than 40% of the removal of trimethoprim.

The photolysis model over-predicted the removal rate of carbamazepine by about 0.1 d^{-1} , which may be explained by a number of factors. Evaporation in the pilot-scale system resulted in loss of up to 10% of water entering the wetland in summer, as evidenced by increases in chloride concentrations through the wetland (Figure 4.5). Correcting for a water loss of this magnitude yielded a carbamazepine transformation rate of $0.07 \pm 0.03\text{ d}^{-1}$ during summer (i.e., evaporation accounted for approximately 70% of the difference between the photolysis model and field measurements for carbamazepine). Photolysis in the field-scale system may also have been reduced slightly by shading of water passing through the diffuse biomat layer at the wetland bottom, which would have reduced photolysis rates by about 10%. Light screening by floating vegetation and detritus may also have reduced photolysis rates in the pilot-scale wetland as compared to model predictions.

After adjusting photolysis rates for evaporation and the flow of water through the biomat, photolysis accounted for only a small portion of the removal of atenolol (<5%), metoprolol (<5%), and trimethoprim (<20%) in the pilot-scale system (Figure 4.14). Thus, photolysis will only be an important removal mechanism for relatively photo-labile compounds in open-water wetlands (i.e., those compounds with photolysis rates $>0.25\text{ d}^{-1}$), unless the influent wastewater has very high concentrations of NO_3^- (e.g., $>30\text{ mg L}^{-1}$), or low concentrations of DOC (e.g., $<2\text{ mg C L}^{-1}$) for compounds that are transformed by direct photolysis (Chapter 3).

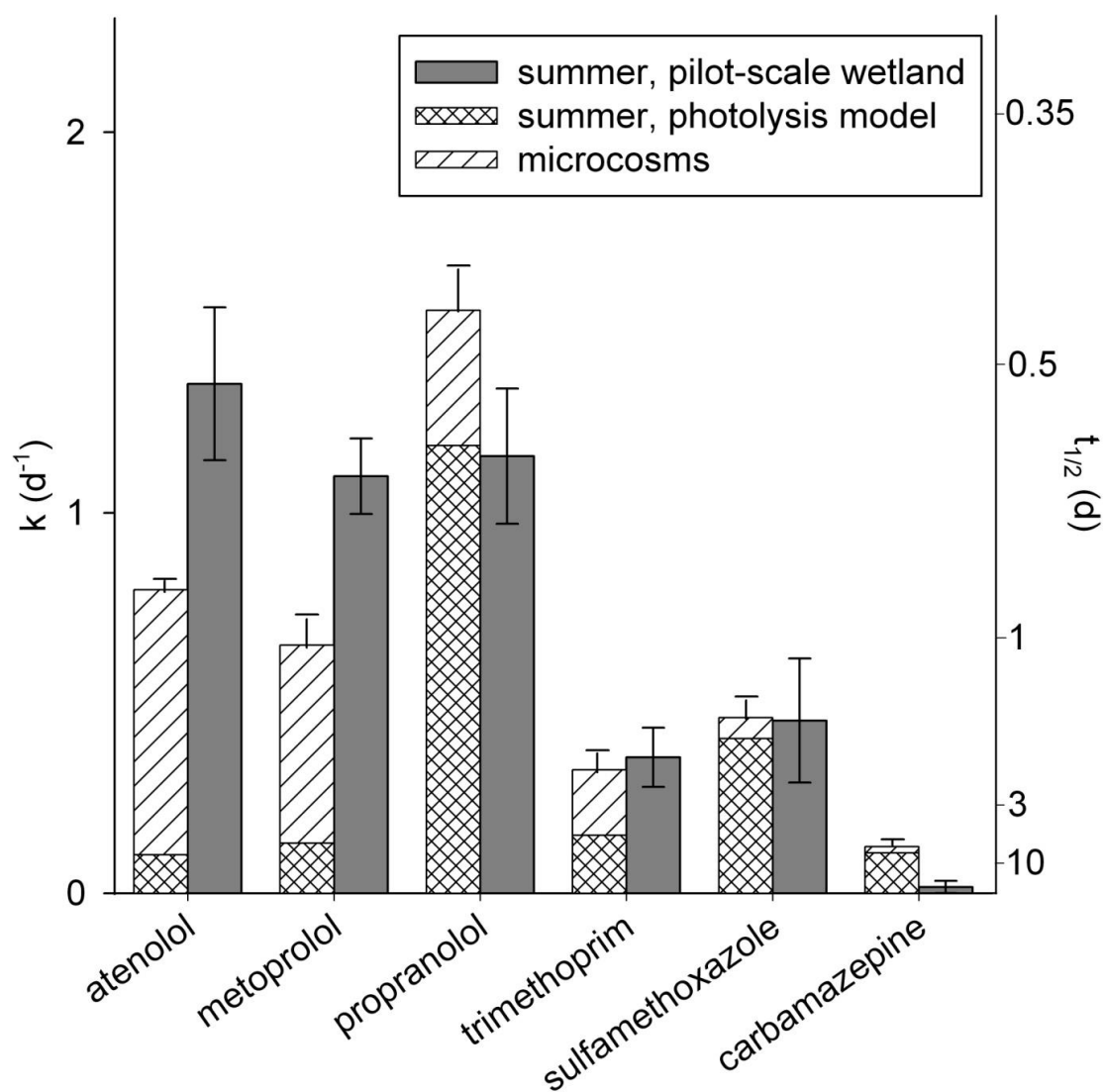


Figure 4.13: Comparison of measured or predicted removal rate constants for test compounds to removal rate constants observed in the Discovery Bay pilot-scale open-water cell during summer. Microcosm removal rates are the average of illuminated and dark microcosms. Error bars represent \pm one standard deviation for photolysis model and microcosms and \pm standard error of the mean for pilot-scale wetland.

Table 4.5: Sorbed Test Compound Concentrations in Open-Water Wetland^a

<u>Compound</u>	<u>Concentration sorbed</u> <u>($\mu\text{g kg}^{-1}$)</u>			<u>Concentration sorbed</u> <u>($\mu\text{g (kg organic carbon)}^{-1}$)^b</u>			<u>Predicted aqueous</u> <u>concentration (ng L^{-1})^c</u>		
	<i>Inlet</i>	<i>Middle</i>	<i>Outlet</i>	<i>Inlet</i>	<i>Middle</i>	<i>Outlet</i>	<i>Inlet</i>	<i>Middle</i>	<i>Outlet</i>
Atenolol	0.2±0.1	0.6±0.1	0.5±0.0	2.9±1.0	1.9±0.3	1.6±0.1	22±8	12±3	12±1
Metoprolol	1.9±0.4	11.3±0.9	6.6±0.7	34±16	37±3	24±3	85±40	83±11	59±10
Propranolol	14±6	69±13	38±12	210±40	230±50	140±50	57±12	52±13	37±14
Trimethoprim	0.2±0.2	1.4±1.3	0.5±0.1	5.1±3.5	4.7±4.4	1.8±0.3	24±17	20±21	8.5±1.5
Sulfamethoxazole	1.8±0.9	2.2±0.8	5.7±1.2	25±3	7.4±2.5	20±5	1220±190	620±140	980±250
Carbamazepine	0.7±0.3	3.0±1.0	2.3±0.1	10±1	10±3	8.1±0.7	130±20	130±40	100±10

^a Average ± one standard deviation. ^b Normalized based on percent organic carbon contents: inlet 7.2±3.7% organic carbon; inlet 30.2±0.2% organic carbon; inlet 28.3±1.3% organic carbon. ^c Aqueous concentration predicted based on organic carbon-normalized concentrations and log K_{oc} values measured in autoclaved test tubes containing wetland biomass (see Figure 4.4) according to: $C_{aq} = C_{sorb} / K_{oc}$.

Biotransformation. To evaluate the contribution of biotransformation to test compound attenuation in the pilot-scale open-water cell, biotransformation rates measured in dark and illuminated microcosms were averaged to approximate the effects of diurnal fluctuations on biotransformation rates. Biotransformation rates measured in bench-scale microcosms accounted for approximately 50% of the average removal rates measured in the pilot-scale wetland for atenolol, metoprolol, and trimethoprim (Figure 4.13; Table 4.2). Biotransformation accounted for approximately 40% of the removal of propranolol and only 10% of the removal of sulfamethoxazole.

Although conditions representative of the pilot-scale system were used to design the microcosms, it is difficult to extrapolate biotransformation rates measured under laboratory conditions to field conditions. Variations in water temperature, biomat thickness, and redox conditions not fully captured in the microcosms affect biotransformation rates. For example, daytime water temperatures in excess of 30°C were common during summer months at the field site and would have likely increased biotransformation rates relative to rates measured in laboratory microcosms. Nonetheless, results from the microcosms suggested that biotransformation was the dominant attenuation mechanism for atenolol, metoprolol, and trimethoprim in the pilot-scale system. Thus, the combination of biotransformation and photolysis accounted for the majority of the observed removal of the test compounds during the summer (Figure 4.13).

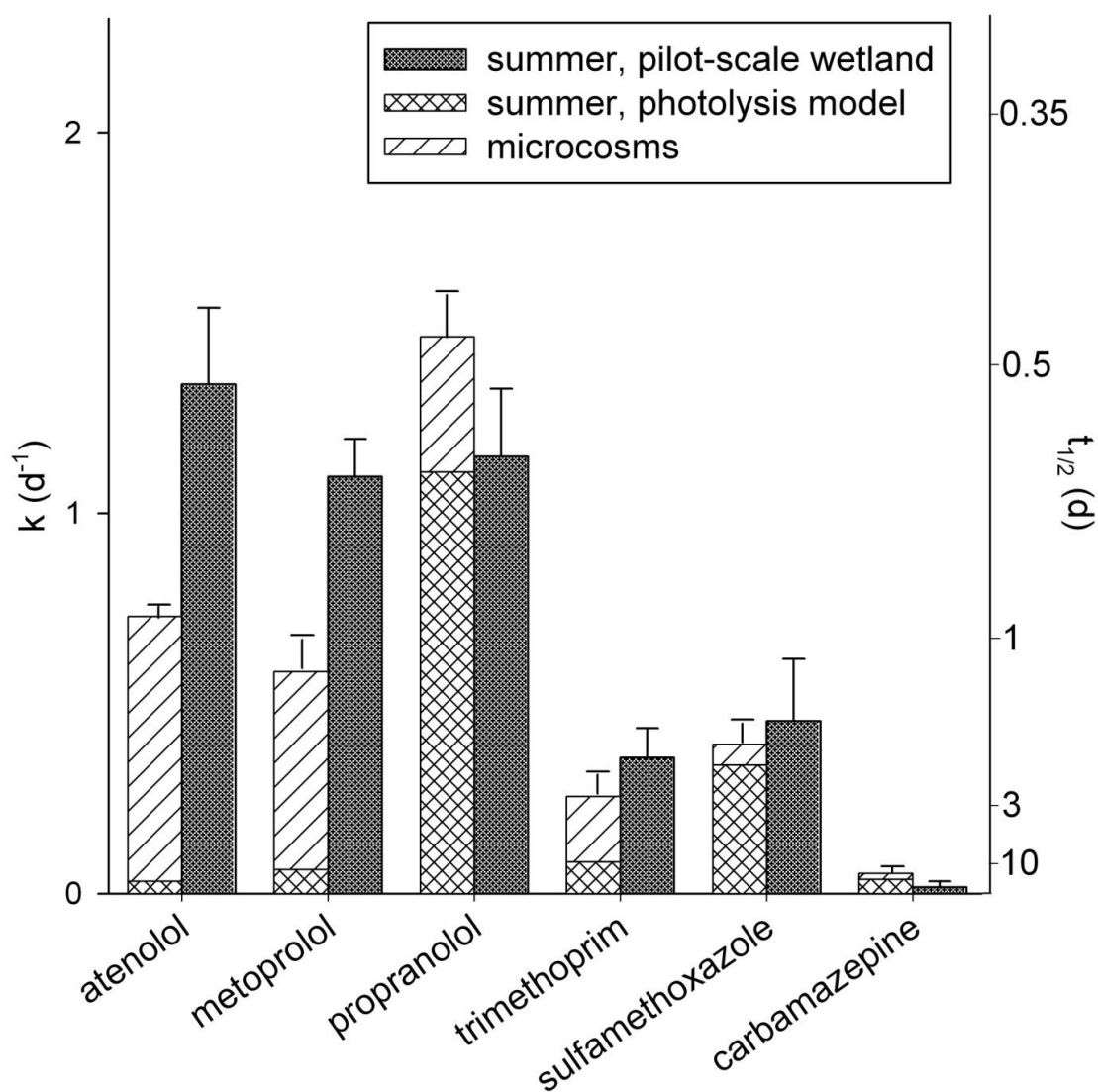


Figure 4.14: Comparison of measured or predicted removal rates of test compounds to removal rates observed in the Discovery Bay pilot-scale open-water wetland during summer, corrected for evaporation. Photolysis rates were corrected assuming 25% of the hydraulic residence time was spent in the biomat and that 7% of the water evaporated per day (see Figure 4.5). Error bars represent \pm one standard deviation for photolysis model and microcosms and \pm standard error of the mean for pilot-scale wetland.

4.3.6 Application to Full-Scale Wetland Design

The wetland area necessary to achieve 90% removal of a contaminant from 1 MGD or 1 MLD of wastewater effluent (A_{90}^1) is a useful metric to evaluate the feasibility of employing open-water cells to remove trace organic compounds (Chapter 3). A one order of magnitude decrease in concentration is a useful metric because it will result in concentrations below the observable effects level for most aquatic organisms (Huggett *et al.*, 2002). Furthermore, many surface waters around the world already consist of 10% wastewater effluent (National Research Council, 2012). Test compound transformation rates were estimated throughout the year using predicted solar irradiances to estimate k_{photo} values (Chapter 3) and the Arrhenius equation to estimate k_{bio} values (Equation 4.5; a mean value of $\kappa=0.06 \text{ K}^{-1}$ was used; see Table 4.4 for average wetland temperatures).

Comparison of A_{90}^1 values of open-water wetlands in temperate climates (i.e., water temperatures of 9-23°C) at 40 N° latitude to existing full-scale wetland systems suggested that test compounds most amenable to biotransformation (i.e., atenolol, metoprolol, propranolol, and trimethoprim) could be removed efficiently in wetlands of a size typically employed for denitrification of wastewater effluent throughout the entire year (Figure 4.15). Open-water wetlands of this size would also result in 90% reductions in the concentration of sulfamethoxazole through most of the year. Of the compounds studied, only carbamazepine would require open-water wetland cells larger than 20 ha MGD⁻¹ (~5 ha MLD⁻¹). Thus, the combination of photolysis and biotransformation mechanisms in open-water wetlands allows for the effective removal of a broader range of trace organic contaminants than can be achieved solely by conventional wastewater treatment plants or vegetated wetlands (i.e., removal of both photo-labile compounds, such as sulfamethoxazole, and compounds amenable to biotransformation, such as atenolol and metoprolol). However, in regions with colder climates, open-water wetland cells would freeze during the winter, limiting their applicability.

The primary factors to consider for the operation and maintenance of full-scale open-water wetlands cells include water depth and the thickness of the biomat on the wetland bottom. Open-water wetland depths of 20-30 cm will maximize photolysis rates (Chapter 3), while ensuring that adequate sunlight reaches the water-biomat interface to support photosynthesis. Pathogen inactivation will also be maximized in open-water cells shallower than 30 cm (Nguyen *et al.*, 2014). At the pilot-scale facility, the biomat accumulated at a rate of approximately 1 cm yr⁻¹ (Chapter 5), which will take up half of the volume of the cell after 10 years of operation, reducing the efficacy of photolysis. To maintain efficient photolysis rates and prevent clogging, sedimentary detritus will need to be removed periodically (e.g., every 5-10 years). This could potentially be accomplished by draining the wetland and removing the dried detritus mechanically, using a pump to suck out the diffuse biomat, or by temporarily increasing flow rates to wash the diffuse biomat out of the system. Removed waste material could be land applied or digested anaerobically. Wetland photolysis rates will also be reduced by the growth of floating vegetation, such as duckweed (*Lemna* sp.) (Chapter 3). Floating vegetation growth may be prevented by keeping hydraulic residence times low enough to ensure that it is washed out (i.e., less than 3 days) (EPA, 2000a), as well as by periodically flushing the wetland with high flows.

Influent water quality may also affect trace organic contaminant attenuation in open-water wetlands. In this study, fully nitrified influent was used to ensure survival of mosquitofish (*Gambusia*), which are needed to control mosquito larvae (Horne and Fleming-Singer, 2005). Influent containing ammonium may affect both the growth of the biomat and the microbial community within the biomat, resulting in changes in trace organic contaminant transformation rates. For example, ammonium removal in wastewater treatment plants is often associated with faster removal of certain trace organic contaminants (Helbling *et al.*, 2012). Photolysis rates are also affected by influent water quality, due to differences in nitrate concentration, pH, and DOC concentration (Chapter 3).

Removing the open-water cell biomat periodically would increase photolysis rates of trace organic contaminants because more of the water column would be illuminated (see *Photolysis* section above). Conversely, biotransformation rates of trace organic contaminants would be reduced due to the reduced contact between the water and the biomat. Biotransformation of compounds that are preferentially transformed in anaerobic zones, such as trimethoprim and sulfamethoxazole, may be especially affected by biomat removal due to a reduced anaerobic zone size. Attenuation of these compounds may be more effective in anaerobic wetland cells (e.g., vegetated cells or subsurface wetland cells) used in series with oxic open-water cells, although further research is necessary to assess the efficiency of different wetland configurations.

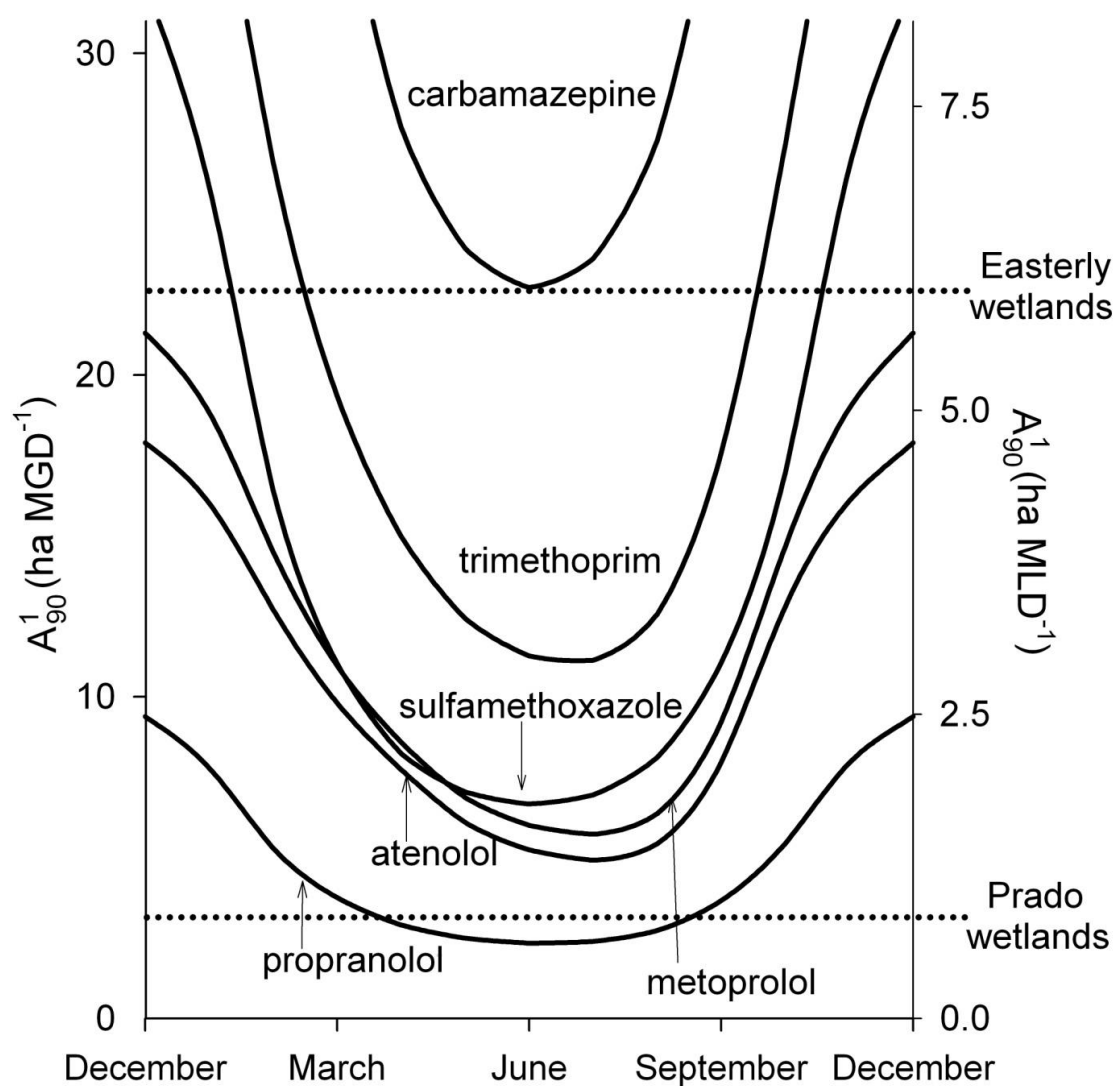


Figure 4.15: Area predicted to provide 90% removal of contaminants from 1 MGD (left axis) or 1 MLD (right axis) of wastewater effluent in open-water treatment cells (A_{90}^1) via biotransformation and photolysis throughout the year. Biotransformation rates were calculated using Equation 4.5 assuming water temperatures ranging from about 9° C in the winter to 23° C in the summer (Table 4.4). Dashed lines show the area per flow rate of existing full-scale wetland systems (Orange County Water District, 2008; Florida Department of Environmental Protection, 2012). Wetland depth=30 cm, pH=9, $[\text{NO}_3^-]=20 \text{ mg-N L}^{-1}$, $[\text{DOC}]=8 \text{ mg-C L}^{-1}$, $[\text{HCO}_3^-]+[\text{CO}_3^{2-}]=60 \text{ mg L}^{-1}\text{-C}$.

CHAPTER 5. Nitrogen Removal in Shallow, Open-Water Treatment Wetlands

Reprinted with permission from Jasper, J.T.; Jones, Z.L.; Sharp, J.O.; Sedlak, D.L. Nitrogen removal in shallow, open-water unit process treatment wetlands. *Environ. Sci. Technol.* **2014** (*in review*).

© 2014 American Chemical Society.

5.1 Introduction

The discharge of effluent from nitrifying wastewater treatment plants (Carey and Migliaccio, 2009) and agricultural runoff (Spalding and Exner, 1993) result in nitrate (NO_3^-) pollution of surface and ground waters. To protect public health the US Environmental Protection Agency and the World Health Organization have established maximum contaminant levels of $10 \text{ mg L}^{-1}\text{-N}$ (0.7 mM) for drinking water (Pontius, 1993; Chilton, 1996). NO_3^- also contributes to the eutrophication of surface waters, in some cases leading to large-scale hypoxia (Diaz, 1995).

Mechanical systems are capable of removing NO_3^- from municipal wastewater, but these systems have high capital costs, and may require energy and substrate inputs (Metcalf & Eddy, 2003). Treatment wetlands offer a low-cost alternative for NO_3^- removal, in addition to removing a range of other contaminants (e.g., metals, pathogens, and trace organic contaminants) (Kadlec and Wallace, 2009). Wetlands are also valued by communities because they provide wildlife habitat and recreational opportunities (Fleming-Singer and Horne, 2006). However, wetland treatment efficiency is often hindered by inconsistent or incomplete removal of contaminants, as well as hydraulic short-circuiting via preferential flow paths. Designing wetlands as a series of unit-process cells optimized to treat specific contaminants (i.e., unit process wetlands) provides a means of enhancing treatment efficiency while reducing the potential for short-circuiting by ensuring water is mixed between cells (Chapter 2).

Nitrate removal in vegetated wetlands is predominantly ascribed to bacterial denitrification, although in wetlands where ammonium (NH_4^+) accumulates from anaerobic decay of organic matter, anaerobic ammonium oxidation (i.e., anammox) can contribute to NO_3^- removal (Zhu *et al.*, 2011). Uptake of NO_3^- by plants and subsequent accretion as sediments typically accounts for only a small fraction of total NO_3^- removal in these systems (Vymazal, 2007).

Wetlands designed for NO_3^- removal are typically planted with emergent vegetation to provide a carbon source and to create conditions where NO_3^- is the most energetically-favorable terminal electron acceptor. Mature vegetated wetlands with a layer of leaf litter and fine, porous sediments typically have higher NO_3^- removal rates than wetlands with only a peat or clay bottom due to close contact between NO_3^- -containing water and active microbes (Horne, 2000; Fleming-Singer and Horne, 2002). The microbial community associated with attached algal communities grown on benthic nets in freshwater aquatic systems also are capable of denitrification (Ishida *et al.*, 2008).

Shallow ($<30 \text{ cm}$), open-water cells designed for photolysis of trace organic contaminants (Chapter 3) and sunlight inactivation of pathogens (Nguyen *et al.*, 2014; Silverman *et al.*, 2014) produce a thick (up to 10 cm), porous, diffuse biomat composed of algae (primarily diatoms), associated heterotrophic microbes, and detritus (Chapter 4). During the daytime, the water column above the biomat is supersaturated with oxygen due to photosynthesis occurring at the biomat surface. Diffusion of oxygen into the biomat may therefore limit denitrification near the water-biomat interface (Risgaard-Petersen *et al.*, 1994). Deeper within the biomat, dissolved oxygen concentrations quickly drop, providing conditions suitable for denitrification (Chapter 4).

Shallow, open-water wetlands differ substantially from open-water treatment ponds, where denitrification rates are typically slower than those observed in vegetated systems (Kadlec, 2012). In treatment ponds, deep water (e.g., >1 m) often limits the interaction between nitrate-containing water and anoxic zones where denitrification occurs, due to thermal stratification. Shallow, open-water wetlands also differ from streams, which usually do not have thick biomats, due to relatively high water velocities (i.e., much greater than $\sim 0.06 \text{ cm s}^{-1}$, the velocity typically employed in shallow, open-water wetlands).

The purpose of this study was to evaluate the potential for using open-water treatment wetlands to remove NO_3^- from municipal wastewater effluent or effluent-dominated streams. NO_3^- removal rates were quantified over a three-year period in a pilot-scale wetland and compared with vegetated treatment wetlands. NO_3^- removal mechanisms, including denitrification, accretion, and anammox, were assessed via a combination of microcosm experiments, functional gene abundance characterization, and field monitoring. Results were used to construct a nutrient mass balance for the pilot-scale cell and to predict wetland areas necessary to ensure removal of NO_3^- from wastewater effluent.

5.2 Materials and Methods

5.2.1 Materials

All reagents were purchased from Fisher Scientific (Fairlawn, NJ) at the highest available purity. Solutions were prepared using 18 M Ω Milli-Q water from a Millipore system.

5.2.2 Pilot-Scale Wetland Monitoring

Ambient concentrations of nitrogen species (NO_3^- , NO_2^- , NH_4^+ , and dissolved organic nitrogen), phosphate, total phosphorus, SO_4^{2-} , and dissolved organic carbon (DOC) were monitored throughout a pilot-scale (400 m²), shallow (20 cm), open-water wetland cell located in Discovery Bay, CA—a system that has been described previously (Chapter 4). The cell was lined with a geotextile fabric to prevent the growth of emergent macrophytes. During the study, a 2-10 cm layer of algae and diffuse biomat comprised of algae, associated heterotrophic microbes, and detritus ($\sim 1500 \text{ kg dry weight}$) was present on top of the liner. The cell received nitrified, non-disinfected wastewater effluent from the adjacent municipal wastewater treatment plant, with a flow rate controlled at 7×10^{-3} to 2×10^{-2} million gallons per day (MGD) (i.e., 2.6×10^{-2} to $7.6 \times 10^{-2} \text{ ML d}^{-1}$ (MLD)). The resulting hydraulic residence time varied from 1 ± 0.1 to 3 ± 0.3 days.

Aqueous samples were collected at the inlet, outlet, and three intermediate points along the flow path of the cell throughout the three-year study period. Samples were stored on ice during transport and were analyzed within 24 hrs. The rate constant for NO_3^- removal was calculated based on the tanks-in-series model (Kadlec and Wallace, 2009):

$$\frac{C_{\text{out}}}{C_{\text{in}}} = \left(1 + \frac{k\tau}{N_h}\right)^{-N} \quad (5.1)$$

where C_{out} is the outlet NO_3^- concentration, C_{in} is the inlet NO_3^- concentration, k is the areal

removal rate (m yr^{-1}), τ is the nominal detention time (yr), h is the water depth (m), and N is the number of tanks-in-series used to describe the cell hydraulics (6.4 based on Rhodamine-WT tracer tests) (Chapter 4). Dissolved oxygen and pH values were monitored near the wetland outlet every 10 minutes.

Biomat samples from the water-biomat interface to the geotextile fabric liner were collected using an acrylic coring device. Samples were analyzed for organic carbon, nitrogen, and phosphorus species. Aqueous samples from within the biomat were collected using a 30 mL pipette. Water was separated from biomat solids by centrifuging at 5000 RPM for 10 minutes.

Reported values represent the mean \pm the standard error of the mean.

5.2.3 Primary Productivity Calculations

Primary productivity was calculated using dissolved oxygen concentrations measured near the wetland outlet during 3 days of each season during 2013. Single curve analysis was used, with the gas transfer coefficient estimated based on dissolved oxygen concentrations measured before sunrise and after sunset. Respiration was estimated from the observed consumption of oxygen at night (Odum, 1956). Respiration and primary productivity rates were calculated as the averages of 3 measurements per season, which were then averaged to provide annual estimates.

5.2.4 Acetylene Block Microcosms

Denitrification rates were measured using the acetylene-block method in stoppered serum bottles (160 mL) amended with acetylene (5 mL) to prevent the conversion of N_2O to N_2 during microbial denitrification (Groffman *et al.*, 1999). Microcosms containing wetland biomass (~ 1.4 g dry weight) and water (~ 85 mL) under a nitrogen headspace at a ratio representative of the pilot-scale wetland (~ 17 g L^{-1}) and were maintained in the dark at $25 \pm 3^\circ \text{C}$. After addition of NO_3^- (~ 45 μmol), NO_3^- , NH_4^+ , NO_2^- , and N_2O were measured over a five-day period. Denitrification rate constants were calculated from observed first-order NO_3^- removal kinetics. Experiments were conducted in triplicate.

5.2.5 Nutrient Mass Balance

To evaluate the contributions of individual NO_3^- removal mechanisms to overall NO_3^- removal, a mass balance was constructed for nitrogen, phosphorus, and carbon. Annually averaged element concentrations were used for the wetland cell influent and effluent. Outlet mass loads were corrected for an average annual evaporation rate of $7.3 \pm 1.1\%$ (Figure 5.1) observed between the inlet and outlet of the wetland cell as determined by Cl^- concentrations. Element losses due to accretion were based on measurements of the wetland biomat thickness, made approximately twice annually, in conjunction with the measured elemental composition of the biomat. Carbon fluxes from the atmosphere were calculated based on primary productivity and oxic respiration rates, as described above. Losses of N_2 and CO_2 gas due to denitrification were calculated based on denitrification rates measured in microcosms, as described above. Carbon requirements for denitrification were calculated assuming that carbon contained in wetland organic matter exhibited a similar composition to that of algae (i.e., $\text{C}_{106}\text{H}_{263}\text{O}_{110}\text{N}_{16}$).

This assumption was consistent with measurements of the chemical oxygen demand of the wetland water.

5.2.6 Measurement of Gene Abundances

DNA was extracted from 0.025 g of freeze-dried biomat using Mo Bio PowerBiofilm DNA Isolation Kit according to the manufacturer's instructions. Extracted DNA was quantified on a NanoDrop Lite and diluted by a factor of 10 prior to amplification, resulting in concentrations ranging from 10-30 ng μL^{-1} .

Amplifications for quantitative PCR were performed on a Roche Light Cycler 480 II using Quanta Biosciences PerfeCTa SYBR Green Super Mix in 25 μL reactions using 2 μL of template DNA. Primer sequences and PCR conditions for *nirS*, *nirK*, *hzs*, and 16S rRNA genes can be found in Table 5.1. Each sample was amplified in triplicate with non-template and negative extraction controls. Standards were generated from amplicons that were purified using Agencourt AMPure XP magnetic beads and quantified using an Invitrogen Qbit 2.0. No nonspecific amplicon bands were observed when run on an Agilent Bioanalyzer 2100. Standard curves were generated from triplicate 1:10 serial dilutions of their purified standards. Each functional gene was normalized by the total number of 16S gene copies.

5.2.7 Analytical Methods

DOC and total dissolved nitrogen were measured using a TOC/TN-V analyzer (Shimadzu) with 1- μm filtered samples. Particulate organic carbon and organic nitrogen content were measured in unfiltered samples. Chemical oxygen demand was determined using standard methods (5220 D). (American Public Health Association, 1995) NO_3^- , Cl^- , phosphate, and SO_4^{2-} were analyzed by ion chromatography (Dionex DX-120). NH_4^+ and NO_2^- were determined using 1- μm filtered samples by standard methods (4500-NH₃ C and 4500-NO₂ B) (American Public Health Association, 1995). Dissolved organic nitrogen was calculated as the difference between total nitrogen and the sum of NO_3^- , NH_4^+ , and NO_2^- . Aqueous phosphate concentrations were measured using unfiltered water samples by alkaline persulfate digestion (Patton and Kryskalla, 2003), followed by colorimetry (method 4500-P C) (American Public Health Association, 1995). Dissolved oxygen and pH measurements were collected with a Manta multi-probe (Eureka Environmental).

Biomat organic content was measured by ashing dried wetland biomass at 550° C (method 2540 E) (American Public Health Association, 1995). Biomat samples were dried, ground, sieved (300 μm), and sent to the UC Davis Analytical Laboratory for elemental analyses. Total nitrogen and carbon content were measured by flash combustion coupled to gas chromatography with thermal conductivity (AOAC, 1997). Organic carbon content was distinguished from total carbon by acid fumigation (Harris *et al.*, 2001). Total phosphorus and iron content of the biomat were measured by nitric acid/hydrogen peroxide closed vessel microwave digestion, followed by coupled plasma atomic emission spectrometry (Sah and Miller, 1992).

N₂O was separated by gas chromatography (GC; Varian 3800) with a packed column (Alltech HayeSep Q 80/100 mesh, 6') held at 50° C, using nitrogen as a carrier gas (20 mL min⁻¹). An electron capture detector (ECD) was used for N₂O detection.

Table 5.1: QPCR Primers and Thermal Profiles

Primer Name	Primer Sequence 5'-3'	[Primer] (nM)	Amplicon Size	Target Gene	Thermal Profile	Ref.
cd3aF	GTSAACGTSAA GGARACSGG	500	425	nirS	95°C 10 min, 6 touchdown cycles [95°C 15 s, 63–58°C (–1°C per cycle) 30 s, 72°C 30 s,] then 35 cycles (95°C 15 s, 58°C 30 s, 72°C 30 s)	(Throbäck <i>et al.</i> , 2004)
R3cd	GASTTCGGRTGS GTCTTGA	500				
nirK876	ATYGGCGGVAY GGCGA	500	164	nirK	95°C 10 min, 6 touchdown cycles [95°C 15 s, 63–58°C (–1°C per cycle) 30 s, 72°C 30 s,] then 35 cycles (95°C 15 s, 58°C 30 s, 72°C 30 s)	(Henry <i>et al.</i> , 2004)
nirK1040	GCCTCGATCAG RTTTRTGGTT	500				
hzsA1597F	WTYGGKTATCA RTATGTAG	400	260	hzs	95°C 3min, 40 cycles (95°C 30 s, 55°C 30 s , 72°C 30 s)	(Harhangi <i>et al.</i> , 2012)
hzsA1857R	AAABGGYGAAT CATARTGGC	400				
EUB338	ACTCCTACGGG AGGCAGCAG	1000	180	16S	95°C 3min, 40 cycles (95°C 60 s, 53°C 30 s , 72°C 60 s)	(Fierer <i>et al.</i> , 2005)
EUB518	ATTACCGCGGC TGCTGG	1000				

5.3 Results and Discussion

5.3.1 NO₃⁻ Removal Rates

Nitrogen species were monitored throughout the pilot-scale open-water wetland to determine removal efficiencies. The average inlet NO₃⁻ concentration was 1.48±0.05 mM throughout the year, which accounted for more than 95% of the nitrogen entering the wetland, as expected for this nitrifying wastewater treatment plant (Table 5.2). On an annual basis, more than 60% of the NO₃⁻ entering the wetland was removed (i.e., 18±4 out of 28±3 kmol yr⁻¹). As typically observed in treatment wetlands, NO₃⁻ removal exhibited first-order kinetics ($r^2=0.8-0.99$; Figure 5.2) (Kadlec, 2012) and showed a significant seasonal dependence (Stein and Hook, 2005; Hernandez and Mitsch, 2007). Removal rates ranged from 18±3 m yr⁻¹ during winter (~30% removal) to 117±14 m yr⁻¹ during summer (>95% removal) (Figure 5.3). NO₂⁻, NH₄⁺, and organic nitrogen concentrations did not change appreciably in the cell ($p>0.1$; Figure 5.4). Particulate organic nitrogen concentrations in unfiltered samples did not differ from organic nitrogen concentrations in filtered samples.

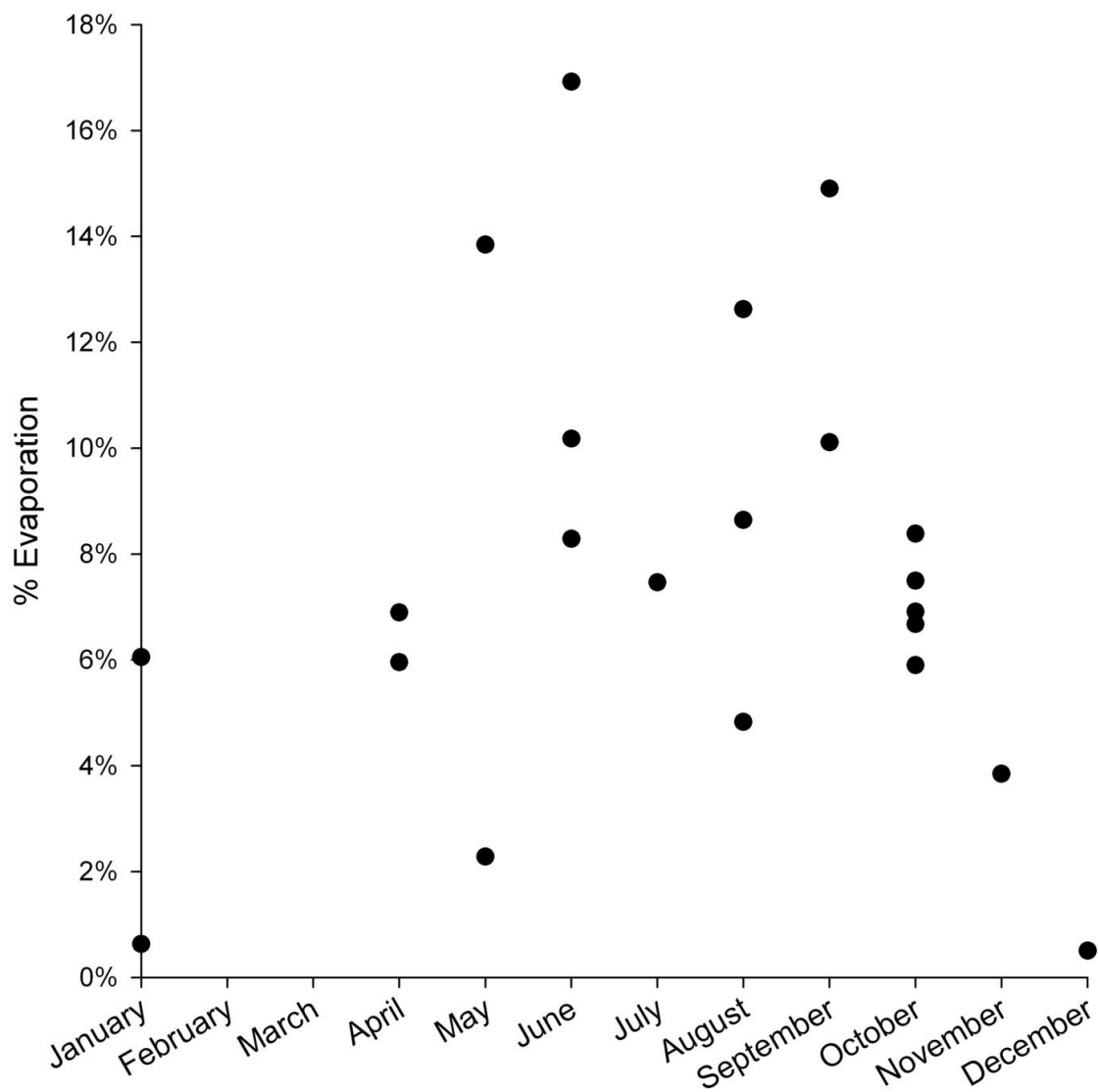


Figure 5.1: Percent evaporation between inlet and outlet of pilot-scale wetland cell throughout the year, calculated based on Cl^- concentrations.

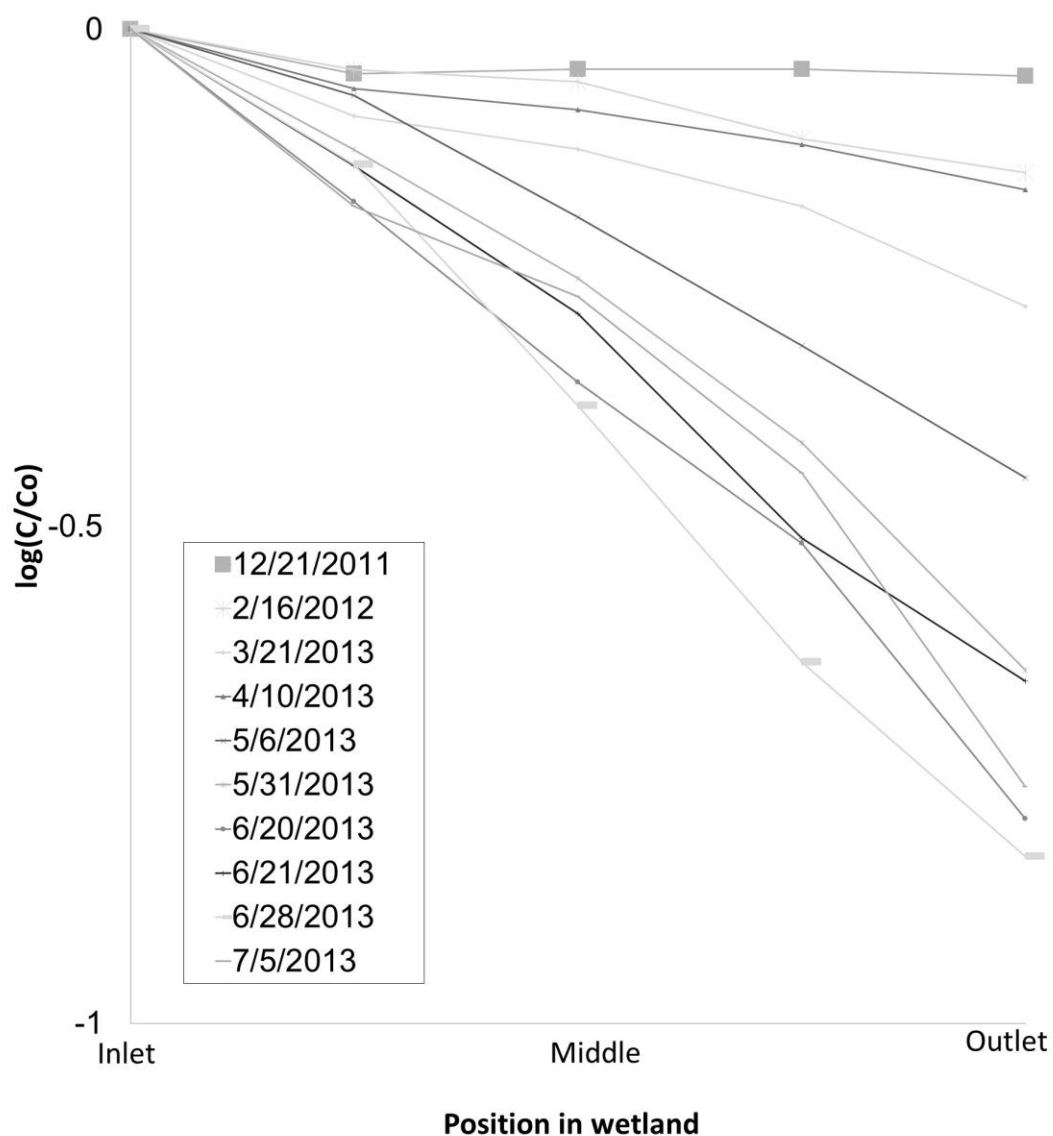


Figure 5.2: Semi-log plot of NO_3^- removal along flow-path in Discovery Bay pilot-scale open-water wetland cell. For clarity, a limited data set is plotted. Other data exhibited similar kinetics.

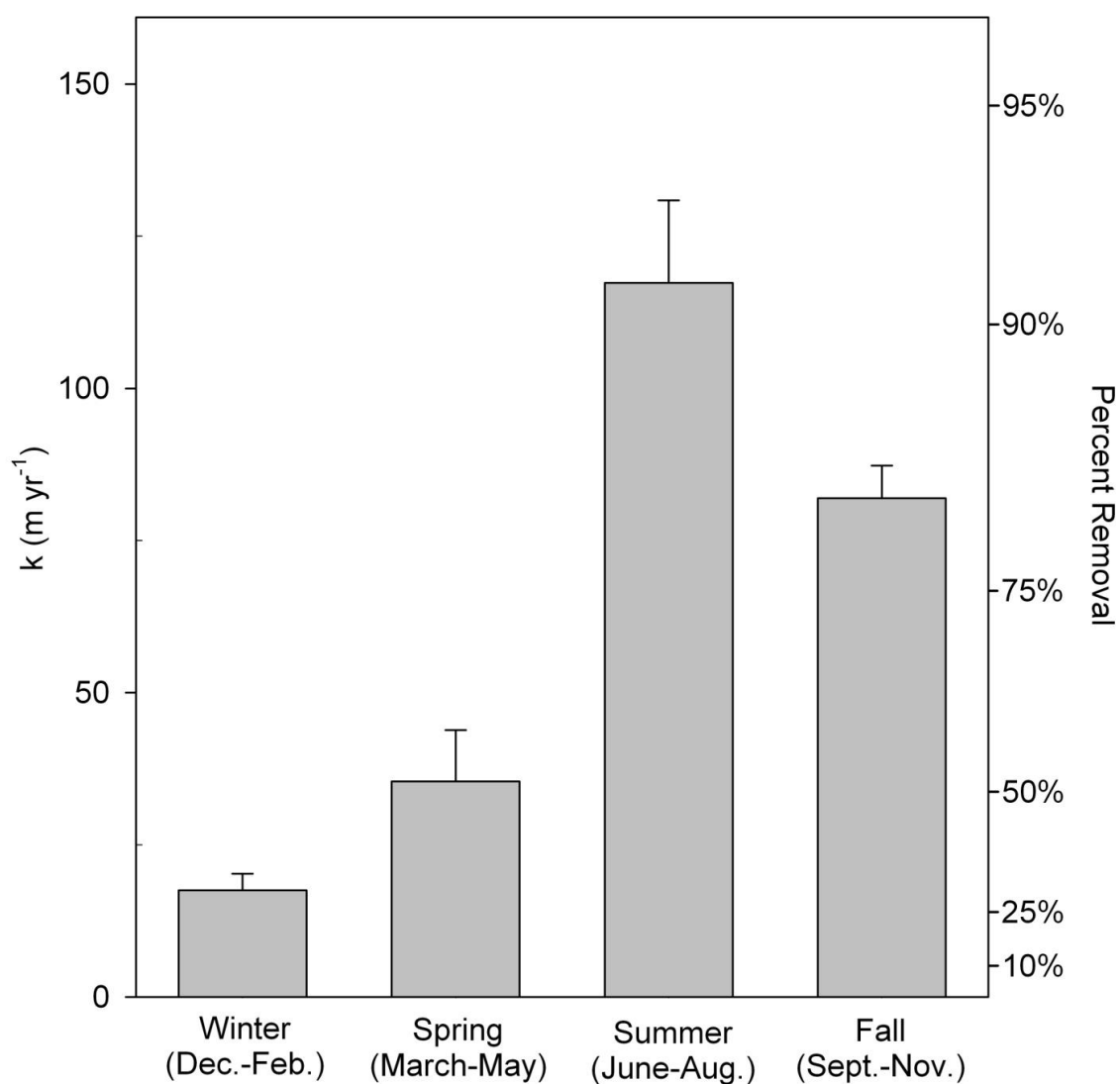


Figure 5.3: Average NO_3^- removal rates in the Discovery Bay pilot-scale open-water wetland by season. Percent removal calculated based on a 1.5 day hydraulic residence time. Error bars represent standard error of the mean (n=9-14).

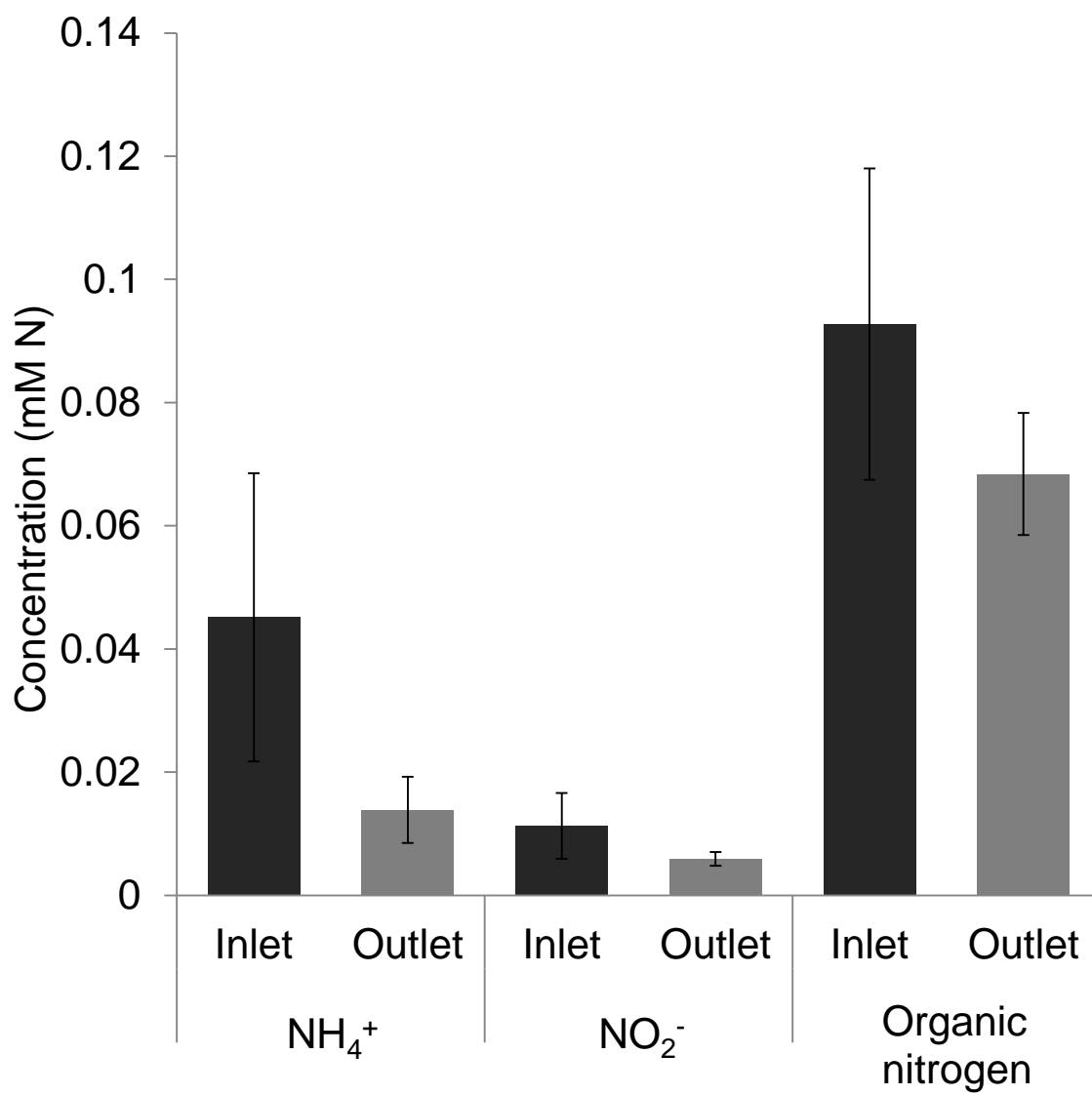


Figure 5.4: Average inlet and outlet concentrations of nitrogen species \pm standard error of the mean (n=3-5).

Table 5.2: Typical Open-Water Cell Influent Water Quality Parameters

Parameter	Value ^a
[NO ₃ ⁻] (mM)	1.48±0.05
[NO ₂ ⁻] (mM)	0.011±0.005
[NH ₄ ⁺] (mM)	0.045±0.023
[Organic N] (mM-N)	0.093±0.025
TOC (mg L ⁻¹ -C)	5.9±0.3
[SO ₄ ²⁻] (mM)	1.5±0.1
[Cl ⁻] (mM)	10.1±0.2
[Total P] (mM)	0.122±0.008
pH	8.6±0.2

^aAverage ± standard error of the mean.

The observed seasonal dependence of NO₃⁻ removal rates was consistent with the effect of water temperature on denitrification rates, as predicted by the modified Arrhenius equation (Kadlec, 2012):

$$k = k_{20}\theta^{(T-20)} \quad (5.2)$$

where θ is the temperature coefficient, k_{20} is the first-order removal rate at 20° C (m yr⁻¹), and T is the water temperature (°C) (Figure 5.5). The obtained θ value agreed with data from surface flow wetlands (i.e., 1.12±0.02 in this study vs. 1.11±0.01 in previous studies) (Kadlec, 2012).

The system-specific parameter k_{20} provided a means of comparing the Discovery Bay open-water wetland performance with other free-water surface flow constructed wetlands. The calculated value (k_{20} =59.4±6.2 m yr⁻¹) was higher than over 75% of the rates compiled in a review of 44 surface flow wetlands (range of 5-168 m yr⁻¹; median of 25±8 m yr⁻¹) (Kadlec, 2012), demonstrating that the open-water wetland cell provided relatively efficient NO₃⁻ removal.

NO₃⁻ concentrations dropped significantly from the top to the bottom of the biomat (i.e., from 0.5 mM at water-biomat interface to <0.05 mM at the wetland bottom; Figure 5.6), demonstrating that NO₃⁻ was attenuated in the diffuse biomat layer. Dissolved oxygen concentrations exhibited a similar profile, ranging from super-saturated in the water column, to anoxic within the biomat. SO₄²⁻ was also depleted within the biomat (Figure 5.6).

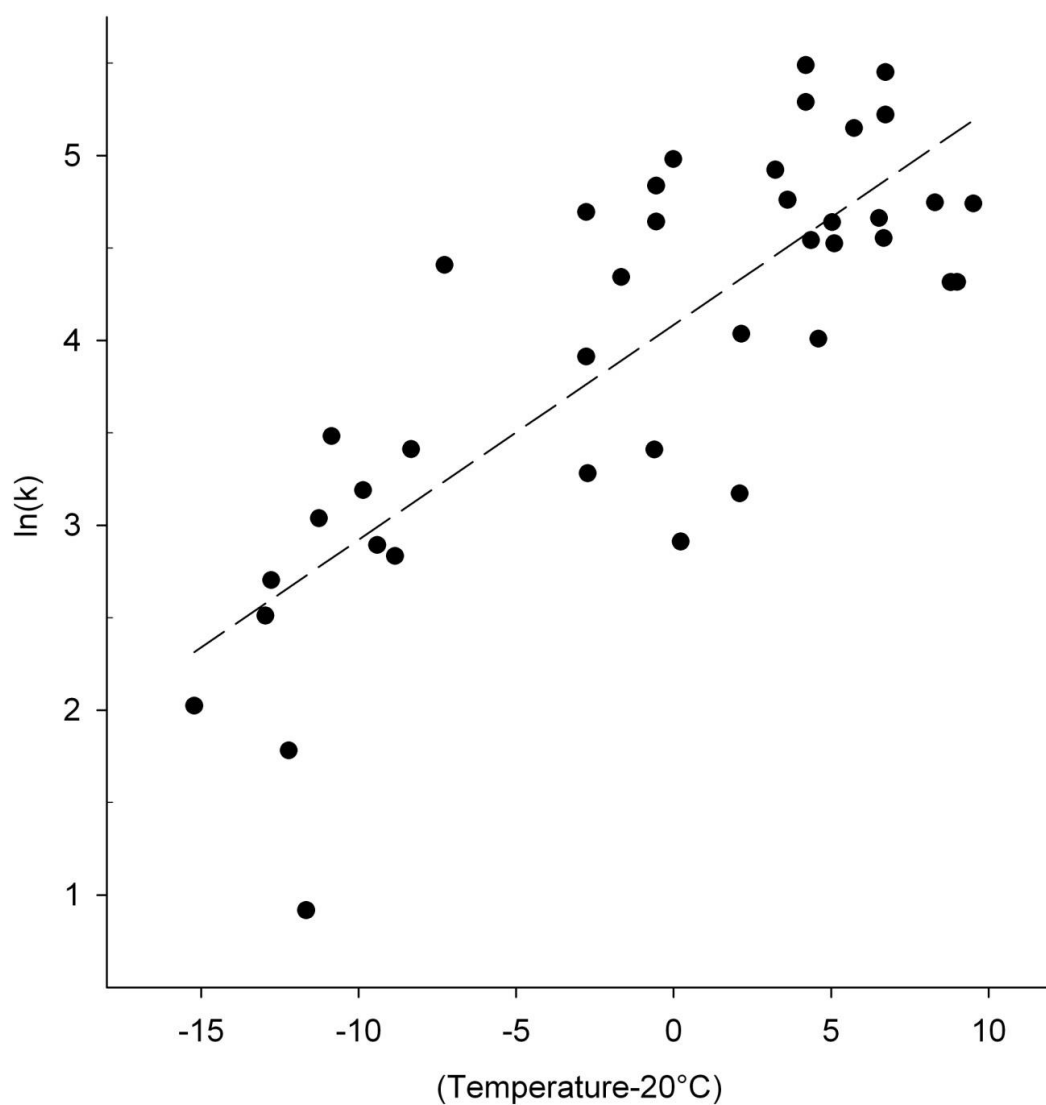


Figure 5.5: Natural log of first-order NO_3^- areal removal rates (k ; m yr^{-1}) in the Discovery Bay pilot-scale open-water wetland versus average wetland water temperature above 20°C . Dotted line represents the best-fit line ($r^2=0.63$; slope= $0.116\text{ }^{\circ}\text{C}^{-1}$; y-intercept= 4.08).

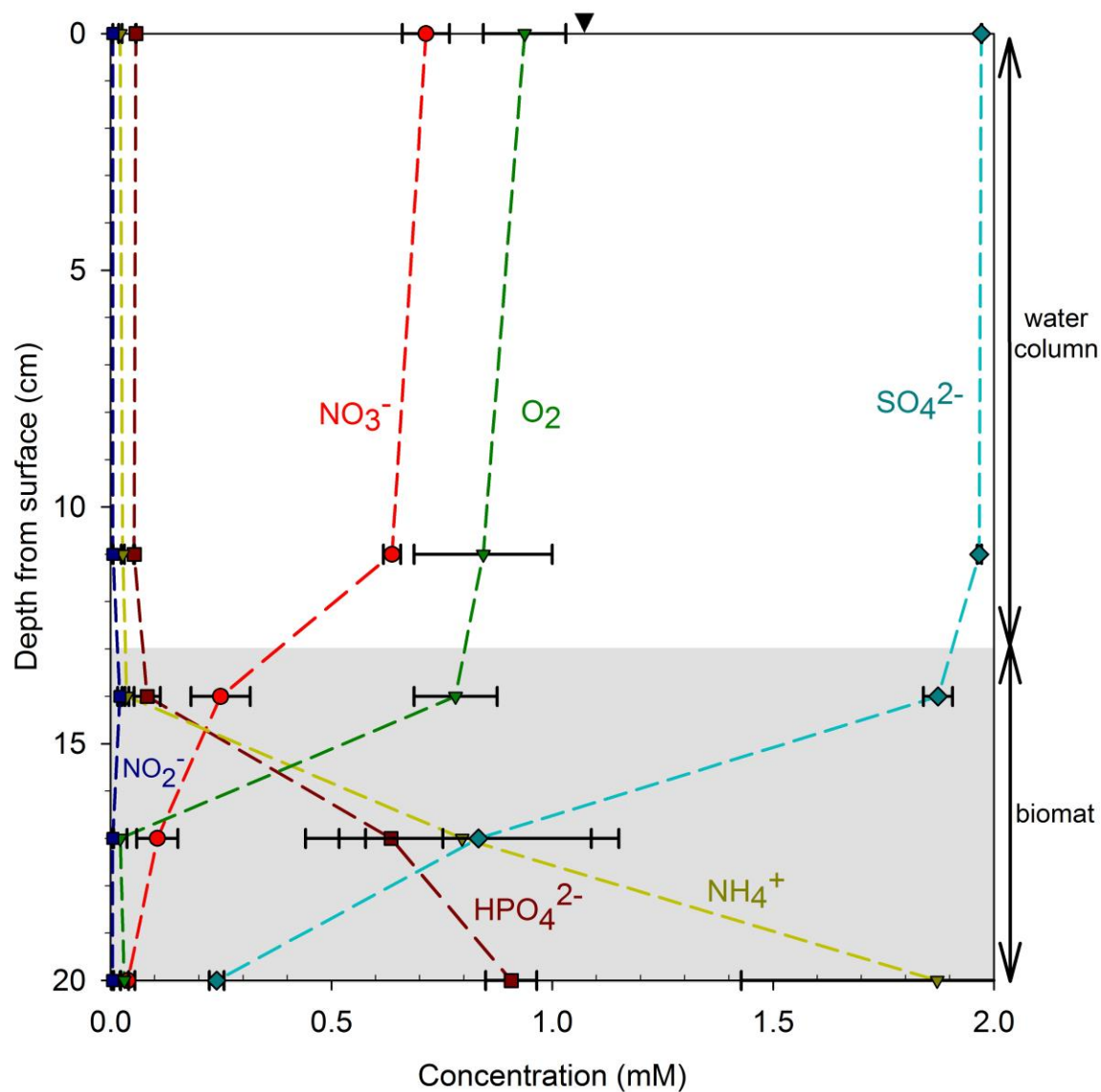


Figure 5.6: Typical profile of aqueous species in middle of Discovery Bay open-water pilot scale wetland, collected on April 2014 at 3:00 PM. Error bars represent \pm standard error of the mean (n=3).

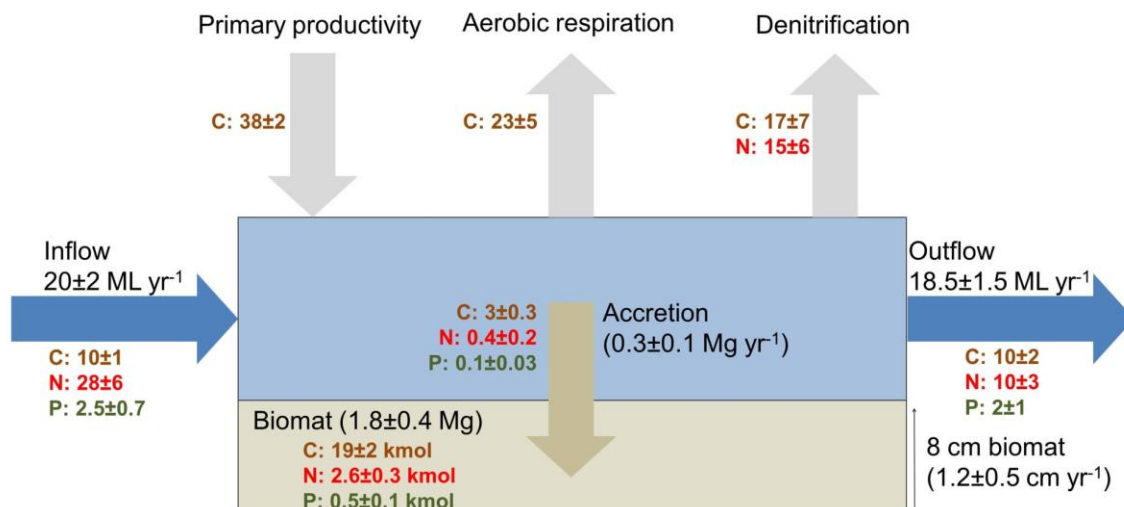


Figure 5.7: Organic carbon (C), nitrogen (N), and phosphorus (P) mass balance (average \pm standard error of the mean; kmol yr^{-1} for fluxes, kmol for biomat) for Discovery Bay open-water wetland during 2012-2013. Accretion fluxes are based on measured biomat elemental composition ($32 \pm 1\%$ organics) and measured accumulation rate. Photosynthesis and aerobic respiration fluxes are based on diurnal oxygen profiles. Denitrification fluxes calculated using rates measured in acetylene-block microcosms, corrected and averaged by monthly average temperatures and Equation 5.2.

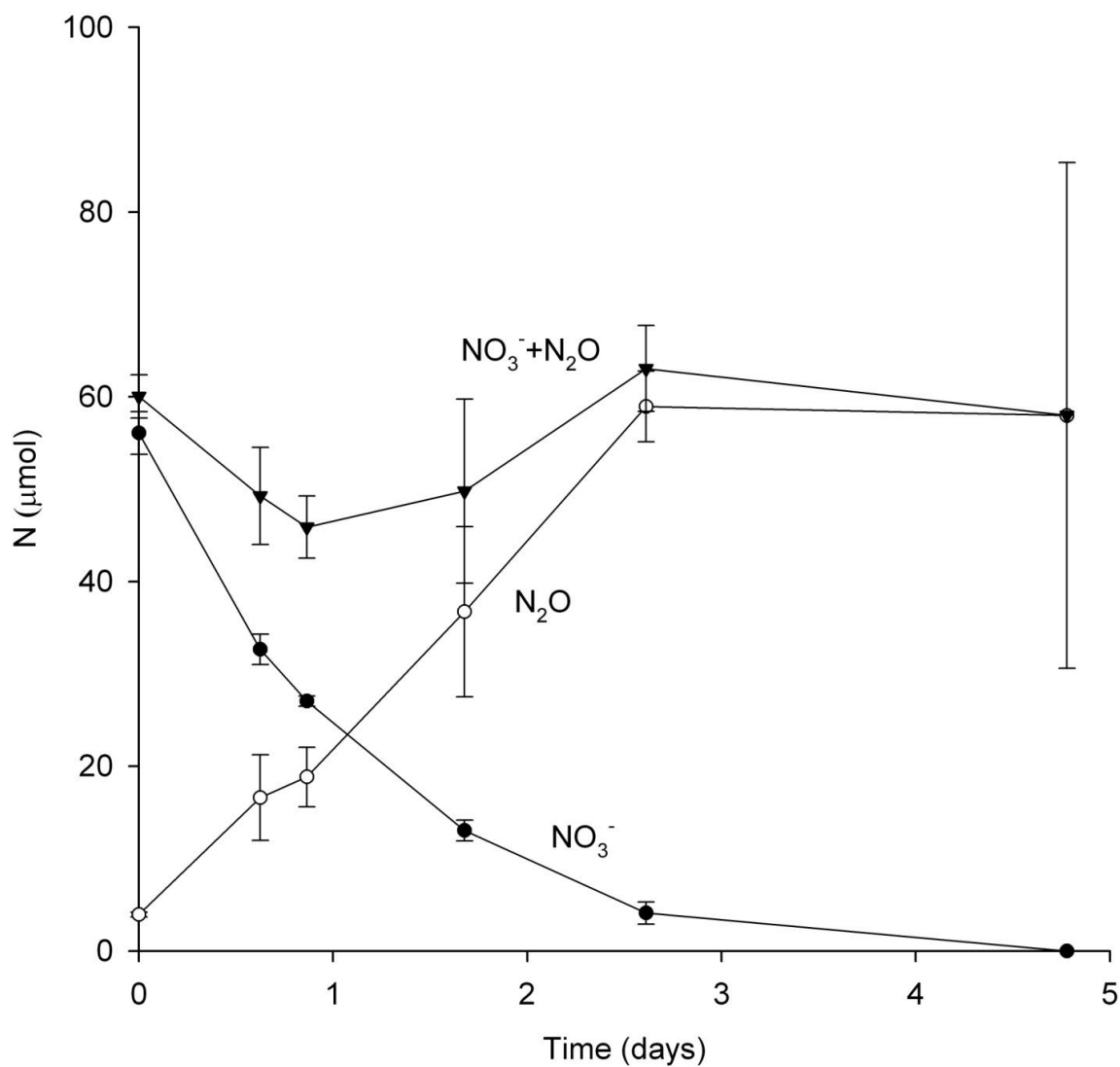


Figure 5.8: Conversion of NO_3^- to N_2O via partial denitrification in anoxic microcosms amended with acetylene gas (5 mL; ~10% v/v). Experiment conducted at 22°C. Error bars represent \pm one standard deviation (n=3).

5.3.2 NO_3^- Removal Mechanisms

Potential NO_3^- removal mechanisms in the open-water wetland included denitrification, assimilatory uptake, and anammox (Burgin and Hamilton, 2007). Although NH_4^+ was formed within the wetland biomat (Figure 5.6), NH_4^+ was not detected in the water column. Therefore, NH_4^+ most likely contributed only to localized cycling of nitrogen. In other words, NH_4^+ produced at the wetland bottom was assimilated, converted to nitrogen gas via anammox, or oxidized to NO_3^- at the biomat-water interface.

To elucidate the contribution of each removal mechanism to the overall loss of NO_3^- , a mass balance was calculated for nitrogen, phosphorus, and carbon in the wetland cell (Figure 5.7). Changes in element concentrations were accounted for by measured denitrification rates, calculations of primary productivity and oxic respiration rates, as well as the accumulation rate and composition of the biomat on the wetland bottom.

Denitrification. In contrast to relatively thin periphyton layers in streams and rivers (i.e., <1 cm thick) where photosynthetic oxygen production slows denitrification rates during the daytime (Risgaard-Petersen *et al.*, 1994), the anoxic conditions necessary for denitrification existed within much of the thick, diffuse biomat layer of the open-water cell throughout the day (Figure 5.6).

To verify that denitrification occurred in the wetland biomat, the final step of denitrification (conversion of N_2O to N_2) was inhibited in microcosms by introducing acetylene. Inorganic nitrogen species were measured during 5-day incubations. Denitrification was the primary loss mechanism of NO_3^- , with greater than 95% of the added NO_3^- recovered as N_2O (Figure 5.8). Denitrification rates in anoxic microcosms, adjusted to 20°C using Equation 2, agreed well with rates measured in the pilot-scale system ($k_{20,\text{microcosm}}=58.7\pm5.4 \text{ m yr}^{-1}$; $k_{20,\text{pilot}}=59.4\pm6.2 \text{ m yr}^{-1}$). Annual denitrification rates based on this measured k_{20} value and average monthly temperatures (Table 4.4) (Metar, 2013) accounted for the NO_3^- removed from the pilot-scale open-water wetland, within uncertainty ($83\pm22\%$; Figure 5.7). N_2O formation in anoxic microcosms not amended with acetylene accounted for less than 1% of the NO_3^- lost and occurred at rates similar to those previously observed in vegetated constructed wetlands ($\sim 7 \text{ mmol m}^{-2} \text{ d}^{-1}$ in this study, versus -0.1 to $140 \text{ mmol m}^{-2} \text{ d}^{-1}$ in previous study) (Søvik *et al.*, 2006).

Further evidence for the importance of denitrification to NO_3^- removal was provided by the presence of the nitrite reduction genes *nirS* and *nirK* near the inlet of the open-water cell (Figure 5.9). Normalized gene abundances were similar to those previously observed in vegetated free surface-flow treatment wetlands (Ligi *et al.*, n.d.; Chon *et al.*, 2011). As expected, the normalized abundance of *nirS* was higher than that of *nirK* (0.01-0.02 copies *nirS* per copy 16S gene copy, versus ~ 0.005 copies *nirK* per copy 16S gene copy), because *nirS* is more widely distributed among denitrifiers (Braker *et al.*, 1998), and is typically in higher abundance than *nirK* in surface waters (Jones and Hallin, 2010).

Denitrification requires an organic carbon substrate at a stoichiometric ratio of approximately one carbon per nitrate (given an organic carbon source such as wastewater-derived organic matter or algae). At lower carbon-to-nitrate ratios, denitrification may be incomplete

(U.S. Environmental Protection Agency, 1993). In wetland systems, organic carbon is typically derived either from influent wastewater or from decaying biomass (i.e., emergent plants and/or algae).

In the pilot-scale open-water cell, $17 \pm 4 \text{ kmol yr}^{-1}$ of organic carbon (with a composition similar to algae) would have been necessary to denitrify the $15 \pm 3 \text{ kmol yr}^{-1} \text{ NO}_3^-$ removed. Average annual primary productivity estimates, calculated based on dissolved oxygen measurements (see Figure 5.10 for example dissolved oxygen profiles), were $38 \pm 2 \text{ kmol yr}^{-1}$. Furthermore, based on measured oxygen consumption rates, $23 \pm 5 \text{ kmol yr}^{-1}$ of organic carbon was calculated to be consumed via aerobic respiration. These values were within the range reported for autotrophic communities in flowing water (Odum, 1956), and the primary productivity estimate was similar to the highest rates reported in wetlands (i.e., $1.1 \text{ kg m}^{-2} \text{ yr}^{-1} \text{ C}$, which corresponds to $37 \text{ kmol yr}^{-1} \text{ C}$ in the 400 m^2 pilot-scale open-water cell) (Azim *et al.*, 2005). A total of $2 \pm 1 \text{ kmol yr}^{-1}$ of organic carbon was predicted to accumulate in the bottom of the cell, based on biomat elemental composition and mass accumulation rates (see below), leaving $12 \pm 5 \text{ kmol yr}^{-1}$ of organic carbon remaining, which is within the range necessary to drive denitrification (Figure 5.7). Thus, microbial denitrification in anoxic regions of the biomat, fueled by algal-derived organic carbon, was the predominant NO_3^- loss mechanism in the Discovery Bay open-water cell.

Modest amounts of NO_3^- may also have been lost by autotrophic denitrification driven by the oxidation of sulfide, which was formed by SO_4^{2-} reduction at the bottom of the biomat (Figure 5.6) (Shao *et al.*, 2010). However, this process would not affect the overall carbon mass balance, because an equivalent amount of carbon would have been required for SO_4^{2-} reduction prior to sulfide oxidation by NO_3^- as was necessary for heterotrophic denitrification. Furthermore, sulfur was not appreciably removed from the system (e.g., via volatilization of H_2S), since SO_4^{2-} concentrations at the wetland outlet were similar to those measured at the wetland inlet.

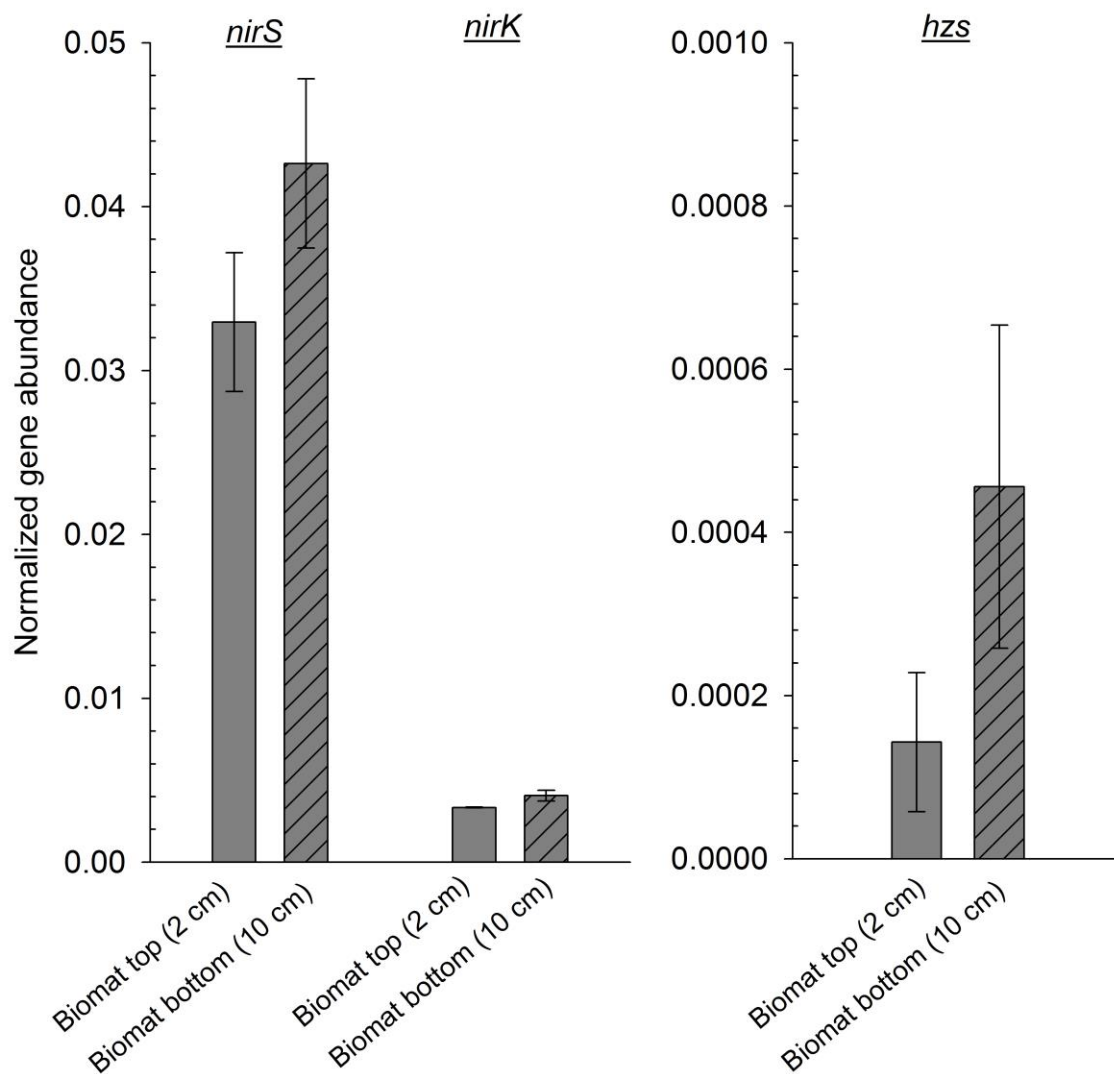


Figure 5.9: Abundances of genes involved in denitrification (*nirK* and *nirS*) and anammox (*hzs*) near the inlet of the open-water wetland cell, normalized to 16S gene copies. Measurements were taken near the biomat-water interface (Biomat top) and at the bottom of the biomat (Biomat bottom). Error bars represent \pm average of the absolute deviation of duplicate measurements.

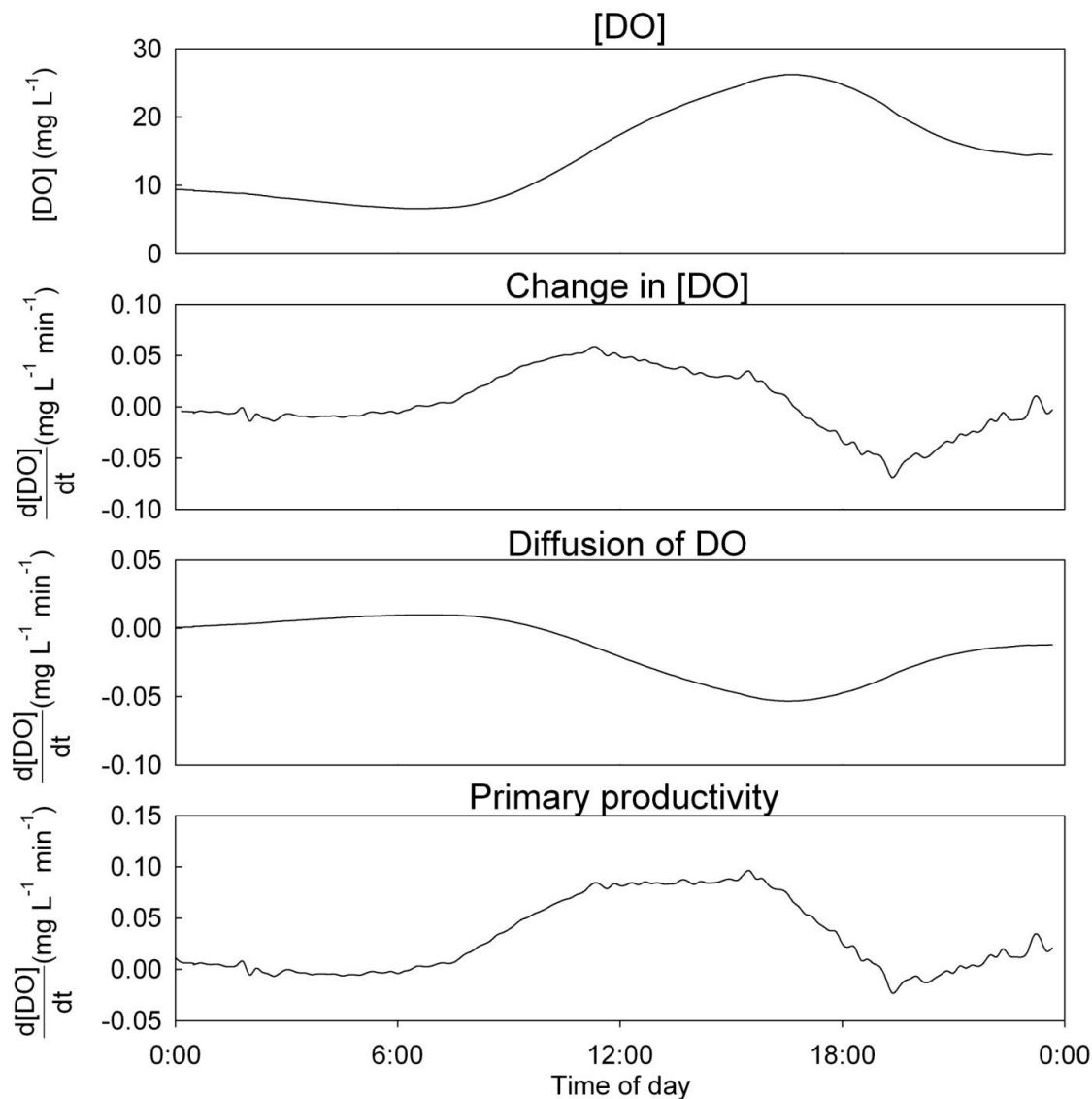


Figure 5.10: Example of dissolved oxygen (DO) profiles used to calculate annual primary productivity and respiration rates. Data shown from April 8th, 2013 at outlet of pilot-scale wetland (Manta MultiProbe, Eureka Environmental, Austin, TX). Diffusion was based on [DO] before sunrise and after sunset; respiration was based on consumption of DO during night; Primary productivity=Change in [DO]+Respiration-Diffusion of DO (Odum, 1956).

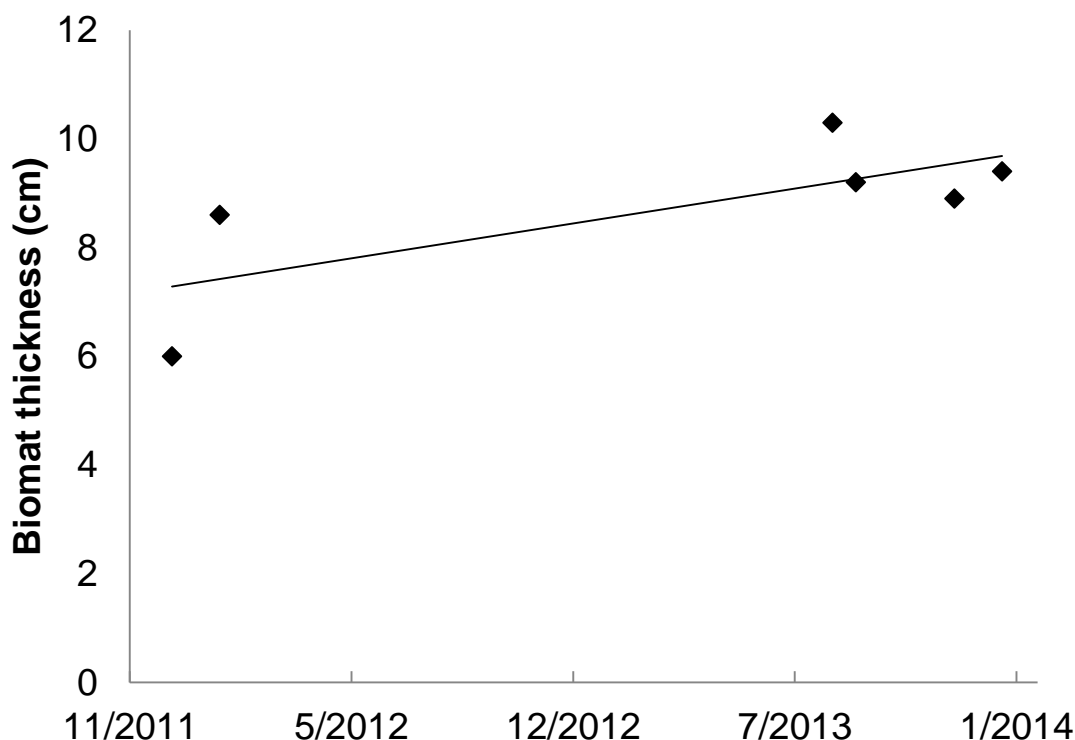


Figure 5.11: Average biomat thickness measured throughout the pilot-scale open-water wetland throughout the study period. Slope= 1.2 ± 0.5 cm yr⁻¹. $r^2=0.55$.

Accretion. In vegetated wetlands, NO₃⁻ uptake by plants and subsequent burial of plant material can account for up to 10% of the observed NO₃⁻ removal (Vymazal, 2007). Accretion is typically more important in systems with low nitrogen loadings (Kadlec and Wallace, 2009). Attached algae can be grown and harvested as a means of removing NO₃⁻ from wastewater effluent (Craggs *et al.*, 1996) and from NO₃⁻-contaminated drinking water (Sládečková *et al.*, 1983). In open-water cells, the majority of NO₃⁻ assimilated by algal and microbial growth will likely be released upon decomposition as organic nitrogen and NH₄⁺ within the biomat. Much of the organic nitrogen and NH₄⁺ will subsequently be recycled as fresh biomass, re-oxidized to NO₃⁻, or converted to N₂ via anammox (see below). Small amounts of biomat could be sloughed off, but this loss was negligible due to the low flow rates employed. Detritus that accumulates as wetland sediments will eventually fill the wetland cell. Permanent nitrogen removal would therefore require periodic sediment removal and disposal (Chapter 2).

The biomat accumulated in the pilot-scale open-water cell at a rate of 1.2 ± 0.5 cm yr⁻¹ during the 3-year study period (Figure 5.11), which is slightly higher than rates reported for vegetated surface flow wetlands (i.e., 0.1-0.9 cm yr⁻¹) (Kadlec and Wallace, 2009). The organic matter content ($32 \pm 1\%$), organic carbon content ($12.7 \pm 0.3\%$), and nitrogen content ($2.0 \pm 0.1\%$) of the wetland biomat were within the range typically observed for vegetated wetland sediments (i.e., 20-35% organic matter; 12-20% organic carbon; 1-3% nitrogen) (Kadlec and Wallace, 2009). The biomat composition did not vary significantly throughout the wetland (Figure 5.12).

The phosphorus content of the biomat was almost 3 times higher than expected based on the Redfield N:P ratio of 16:1 (i.e., $0.8 \pm 0.1\%$; N:P=5.4) (Redfield, 1934; Redfield *et al.*, 1963). While N:P ratios in freshwaters often deviate from values obtained in marine systems, they are commonly enriched with respect to N (i.e., N:P>16:1) (Hecky *et al.*, 1993). Therefore, processes other than assimilation may have contributed to phosphorus accumulation in the biomat, such as adsorption or precipitation of phosphate-containing minerals (Vymazal, 2007). For example, phosphate may adsorb to carbonate- or iron-containing minerals (Ann *et al.*, 1999), both of which were present in the biomat (3% CO_3^{2-} -C and 1% Fe by mass). Phosphate may also precipitate in a variety of minerals (Prochaska and Zouboulis, 2006). For example, the water at the bottom of the biomat was supersaturated with respect to struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$) (Rahaman *et al.*, 2006) at the pH values typical of the wetland (i.e., 8.5-9.5). To assure permanent removal of phosphate from the system, the biomat would need to be harvested periodically, as discussed below.

On the basis of the biomat accumulation rate and its nitrogen content, accretion accounted for only about 2% of the yearly NO_3^- removal observed in the open-water wetland ($0.4 \pm 0.2 \text{ kmol yr}^{-1} \text{ N}$; Figure 5.7). NO_3^- uptake may have been inhibited by the presence of NH_4^+ , which was present at concentrations higher than those known to suppress NO_3^- uptake by phytoplankton throughout the wetland cell (i.e., $\sim 30 \text{ }\mu\text{M}$ versus $0.5\text{-}1.0 \text{ }\mu\text{M}$ to suppress NO_3^- uptake) (Vymazal, 1995).

Effluent total suspended solids concentrations were $4.7 \pm 0.7 \text{ mg L}^{-1}$. Assuming the suspended solids consisted of material from the biomat, this corresponded to less than 1% of the total nitrogen lost in the wetland (i.e., $0.12 \pm 0.02 \text{ kmol yr}^{-1} \text{ N}$). Further, total nitrogen concentrations in unfiltered samples did not differ from those in filtered samples, indicating that particulate nitrogen was not present at significant concentrations. Therefore, export of particulate nitrogen was not a significant nitrogen loss route in the pilot-scale cell.

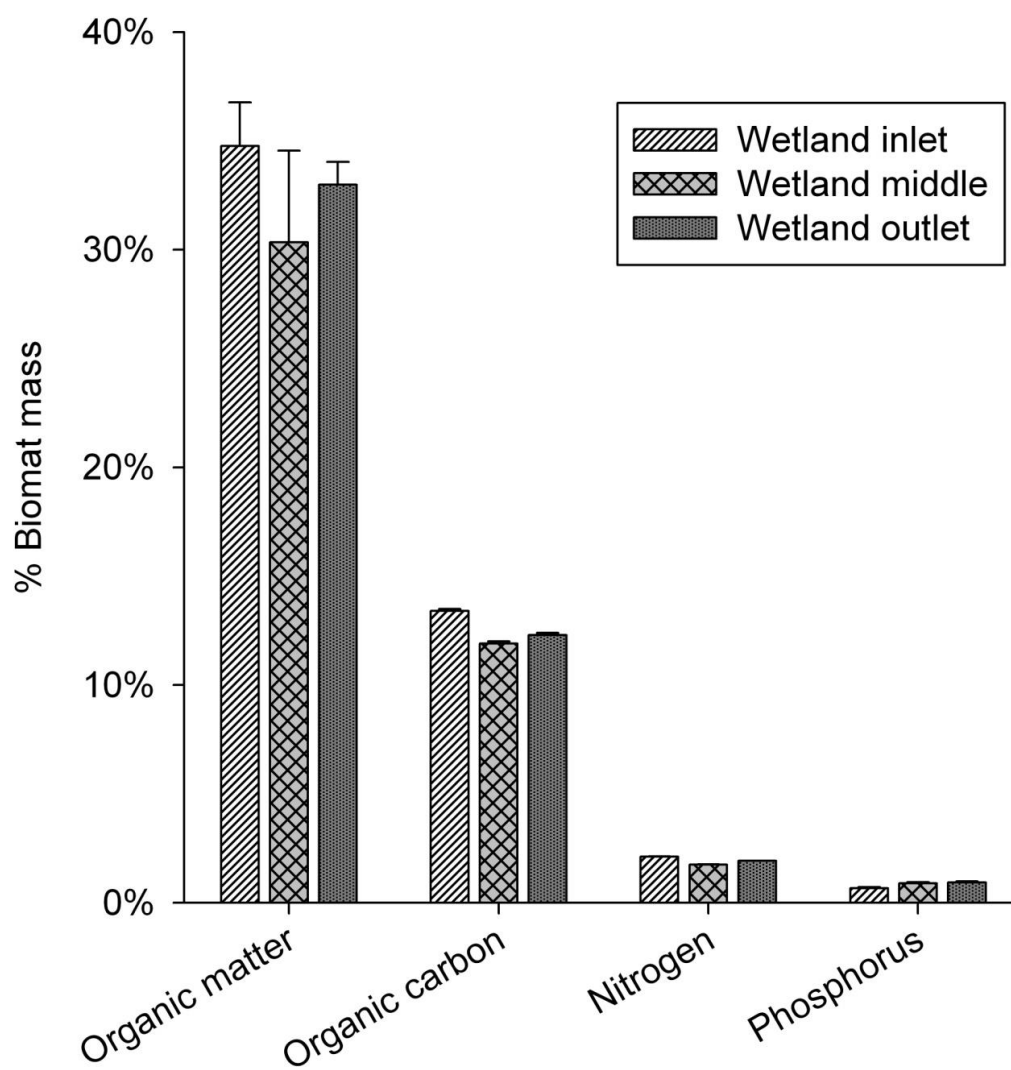


Figure 5.12: Percent phosphorus, nitrogen, and organic content by mass in wetland biomat at the inlet, middle, and outlet of open-water wetland on a dry weight basis. Remainder consisted of non-volatile organic matter (i.e., minerals).

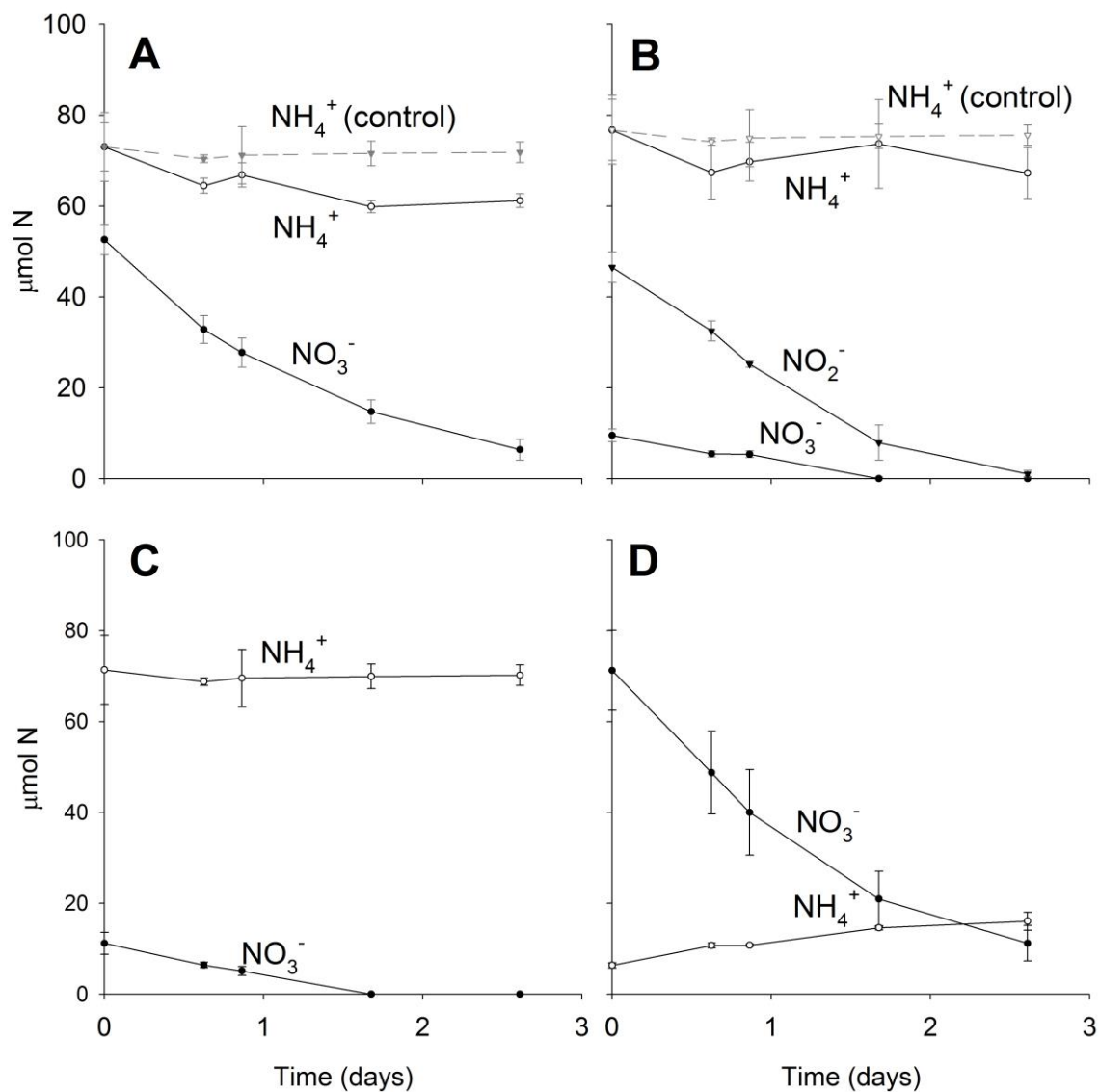


Figure 5.13: Concentrations of nitrogen species in anoxic microcosms amended with A: NH_4^+ and NO_3^- ; B: NH_4^+ and NO_2^- ; C: NH_4^+ ; D: NO_3^- . Experiment conducted at $22 \pm 2^\circ\text{C}$. Error bars represent \pm one standard deviation ($n=3$). NH_4^+ concentrations from the control experiment (NH_4^+ alone; C) are plotted as dashed lines in graphs A and B to emphasize differences.

Anammox. Anammox couples the oxidation of NH_4^+ to the reduction of nitrite under anaerobic conditions (Jetten *et al.*, 2005):



Anammox bacteria have been identified in wetland sediments (Shipin *et al.*, 2005; Dong and Sun, 2007) and have been shown to account for up to 24% of the N_2 production in surface-flow wetlands receiving partially nitrified wastewater effluent (Davison *et al.*, 2006; Erler *et al.*, 2008) and 78% of the N_2 production in vegetated wetland microcosms receiving high concentrations of NH_4^+ and NO_3^- (i.e., 2-20 mM) (Tao *et al.*, 2007). Anammox accounted for up to 33% of the N_2 production in vertical-flow wetland microcosms inoculated with activated sludge and conditioned at elevated NH_4^+ concentrations (i.e., 0.7 mM) (Zhu *et al.*, 2011). In wetlands receiving high concentrations of NH_4^+ (e.g., >0.7 mM), nitrification coupled to anammox may enhance NH_4^+ removal by reducing the amount of oxygen required to oxidize NH_4^+ to N_2 by more than half compared to nitrification followed by denitrification (Tao *et al.*, 2012). In wetlands designed to treat municipal wastewater effluent, such as the Discovery Bay wetlands, low concentrations of NH_3 are required to avoid toxicity to mosquitofish (*Gambusia*) used for vector control (Horne and Fleming-Singer, 2005). Under these conditions, anammox could occur following dissimilatory nitrate reduction to ammonia, or if NH_4^+ is released from decaying organic matter via ammonification.

In the Discovery Bay open-water cell biomat, conditions suitable for anammox were observed in locations where NH_4^+ released at the cell bottom met NO_3^- from the water column, assuming that NO_3^- was partially reduced to transient NO_2^- (Figure 5.6). The presence of *hzs* confirmed that anammox organisms were present within the wetland biomat (Figure 5.9) (Harhangi *et al.*, 2012). Normalized *hzs* abundance was slightly elevated near the bottom of the wetland where the most anoxic conditions existed and NH_4^+ concentrations were highest, although the difference was not statistically significant ($p > 0.25$; Figure 5.6). The normalized abundance of *hzs* was similar to the normalized abundances measured in lake riparian zones (up to about 0.0007 copies *hzs* per 16S gene copy), where anammox accounted for up to 20% of N_2 production (Zhu *et al.*, 2013).

Further evidence of the possibility of anammox activity in the open-water cell biomat was provided by NH_4^+ loss in anoxic microcosms containing wetland biomass. In microcosms amended with NH_4^+ and either NO_3^- or NO_2^- , NH_4^+ concentrations decreased (up to $12 \pm 6 \mu\text{mol}$ NH_4^+ lost; Figure 5.13, A&B), corresponding to 30-40% of total nitrogen lost, possibly due to anammox. In contrast, NH_4^+ concentrations did not change significantly in microcosms that were not amended with NO_3^- or NO_2^- (Figure 5.13, C), demonstrating that volatilization, adsorption, or assimilation were not responsible for NH_4^+ loss. In microcosms amended with only NO_3^- , NH_4^+ concentrations increased by up to $13 \pm 1 \mu\text{mol}$, possibly due to ammonification or dissimilatory nitrate reduction to ammonia (Figure 5.13, D). Further experiments using isotopically-labeled nitrogen species would be necessary to verify and better estimate the contribution of anammox to nitrogen removal in the open-water cell (Thamdrup and Dalsgaard, 2002).

5.3.3 Implications for Design of Open-Water Cells

Due to space constraints and construction costs, wetland area is one of the primary considerations for wetland design (Kadlec, 2009). The wetland area needed to remove 90% of NO_3^- from 1 MGD or 1 MLD (A_{90}^1) provides a useful metric for wetland design (Chapter 3), as this ensures a similar level of NO_3^- treatment as that achieved by the highest technically feasible treatment of municipal wastewater (i.e., effluent concentrations of $<3 \text{ mg L}^{-1} \text{ N}$, given influent $[\text{NO}_3^-] < 30 \text{ mg L}^{-1}$) (U.S. Environmental Protection Agency, 2010; The Department of the Environment, Maryland, 2014).

A_{90}^1 values calculated based on monthly average temperatures using Equation 2 were smaller for open-water wetlands than those calculated based on k_{20} values for the majority of free-water surface flow vegetated treatment wetlands (Figure 5.14) (Kadlec, 2012). Therefore, wetland operators could convert portions of existing vegetated wetlands to shallow, open-water wetlands to enhance removal of trace organic contaminants (Chapters 3,4) and pathogens (Nguyen *et al.*, 2014; Silverman *et al.*, 2014) without sacrificing NO_3^- removal. Shallow, open-water wetlands are also less prone to hydraulic short-circuiting caused by preferential flow-paths in vegetated wetlands (Lightbody *et al.*, 2008), because there is no emergent vegetation to constrict water flow or create channels.

The design and operation of open water cells for NO_3^- removal is relatively simple and requires a shallow cell that is lined to prevent the growth of emergent macrophytes. Shallow water ($<30 \text{ cm}$ deep) and low linear flow rates ($\sim 0.1 \text{ cm s}^{-1}$) are necessary to ensure that the diffuse biomat on the wetland bottom receives sufficient sunlight to support photosynthesis and is not washed out of the system. The biomat thickness will increase over time and will likely provide optimal NO_3^- removal when it is between 4 and 8 cm thick. With deeper biomat thicknesses it is possible that NO_3^- removal via anammox will be enhanced. To prevent clogging, the biomat eventually needs to be harvested (Chapter 4). After removal, the nutrient-rich biomat may be useful as a fertilizer.

In addition to treating nitrified municipal wastewater effluent, shallow, open-water wetlands may be used to treat other NO_3^- -rich waters. The use of shallow, open-water wetland cells to remove NO_3^- , pathogens, and trace organic contaminants, from an effluent-dominated river is currently being investigated in the Prado wetlands in Southern California (Orange County Water District, 2008). Open-water cells could also be applied to treat agricultural run-off containing NO_3^- .

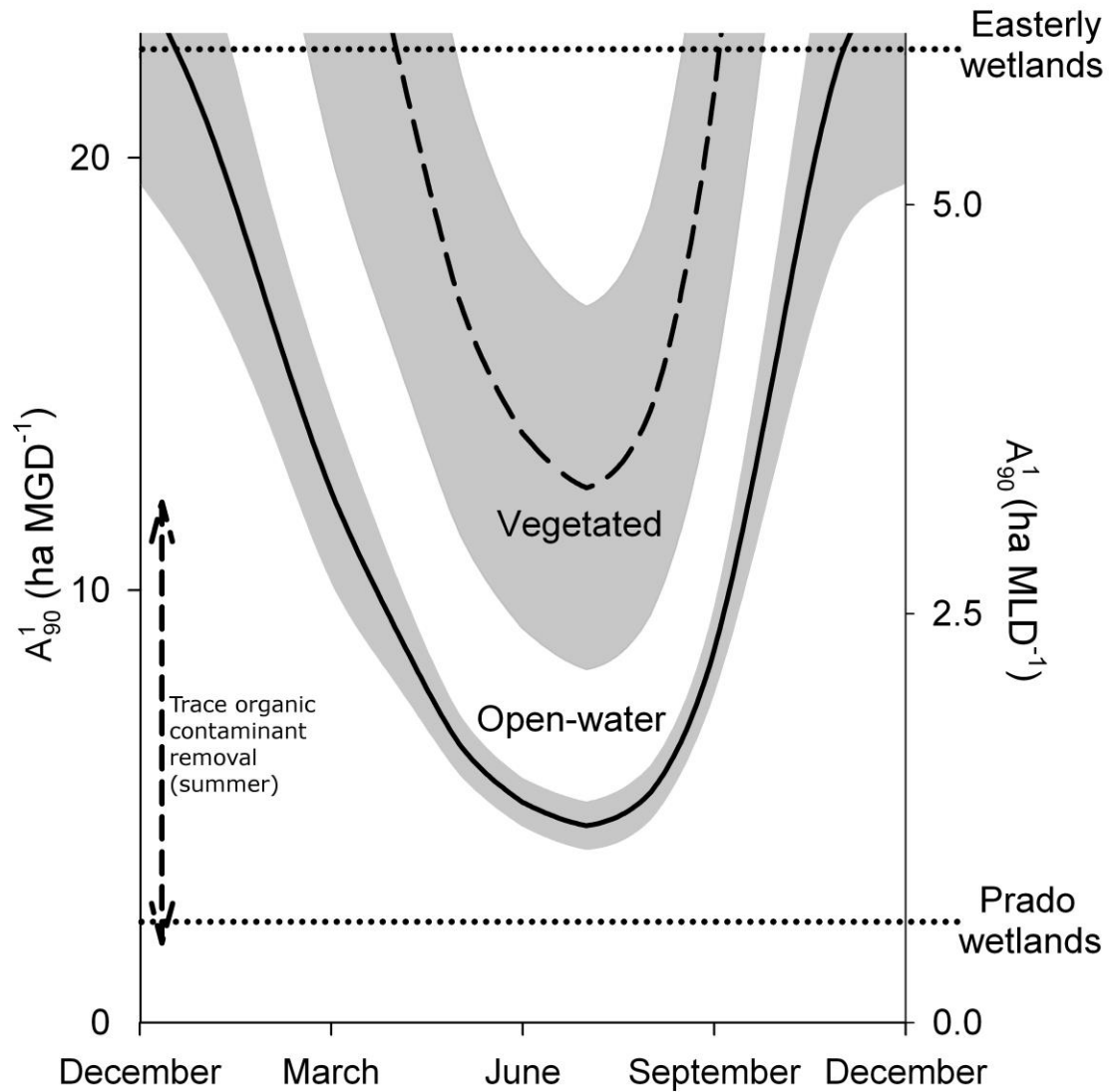


Figure 5.14: Mean area predicted to provide 90% removal of NO_3^- from 1 MGD or 1 MLD of wastewater effluent in open-water (solid line) and vegetated (dashed line) (Kadlec, 2012) treatment wetlands throughout the year (A_{90}^1). Nitrate removal rates were calculated using Equation 2 using average water temperatures in Discovery Bay, CA (i.e., from 10° C in the winter to 23° C in the summer; Table 4.4). Gray area indicates \pm standard error of the mean. Dashed lines show the area per MGD of existing full-scale treatment wetlands (Orange County Water District, 2008; Florida Department of Environmental Protection, 2012) and A_{90}^1 required for trace organic contaminants (atenolol, metoprolol, propranolol, trimethoprim, and sulfamethoxazole) during the summer (Chapter 4).

CHAPTER 6. Conclusions

6.1 Summary

The ability of open-water wetland cells to remove trace organic contaminants and nitrate from wastewater effluent was evaluated in a series of experiments conducted in the laboratory and at a pilot-scale facility. Open-water wetlands are shallow (20-30 cm) cells that are lined with a geotextile fabric to prevent the growth of emergent macrophytes. The unshaded water is well-suited to contaminant removal via photolysis. In addition, a diffuse algal biomat on the wetland bottom supports microorganisms capable of transforming wastewater-derived contaminants and nitrate. To evaluate the ability of open-water wetland cells to treat trace organic contaminants and nutrients, a pilot-scale system receiving nitrified, non-disinfected municipal wastewater effluent was monitored over a 3-year period. To gain further insight into the system, microcosm experiments were conducted using water and biomat samples from the pilot-scale system. The removal of trace organic contaminants via photolysis in open-water wetlands was assessed with a photolysis model based on experiments using simulated sunlight. Results suggested that photolysis could be optimized in shallow water (i.e., ~30 cm) and that photolysis could be enhanced by controlling nitrate concentrations, dissolved organic carbon concentrations, and pH. Monitoring of the pilot-scale system demonstrated that biotransformation in the wetland biomat was also an important removal mechanism for trace organic contaminants. In conjunction with photolysis, this allowed for efficient (>90%) removal of most of the trace organic contaminants studied, in wetland areas similar to existing full-scale wetland systems. In addition to trace organic contaminant removal, the pilot-scale open-water wetland achieved more than 60% removal of nitrate annually, primarily via microbial denitrification. The observed nitrate removal rates were higher than rates typically observed for vegetated wetland systems.

6.2 Removal of Trace Organic Contaminants in Open-Water Wetlands

In Chapter 2, previous studies evaluating the removal of trace organic contaminants in vegetated and subsurface flow treatment wetlands were critically reviewed. The results of this analysis indicated efficient removal of numerous compounds in existing wetlands, but it also revealed a great deal of variability in removal rates among studies. Trace organic contaminant removal in previous studies was typically ascribed to sorption, biotransformation, and in wetlands with open-water zones, photolysis. In most cases, however, trace organic contaminant removal mechanisms were not quantified under carefully controlled conditions, making generalization of results challenging, and limiting their usefulness in optimizing wetland design. Nonetheless, analysis of data in Chapter 2 led to the hypothesis that by designing wetlands in a unit process fashion, with individual wetland cells optimized to remove specific contaminants connected in series, wetland efficiency and reliability could be increased, as compared to a “natural design” approach. In addition, the review indicated that shallow, open-water systems and aerobic conditions would be more likely to result in rapid removal of trace organic contaminants. To assess the unit process concept, novel wetland cells were described, including an open-water wetland cell that exploits photolysis and photosynthesis.

To evaluate the ability of open-water wetlands to remove trace organic contaminants via photolysis, a photolysis model was developed in Chapter 3 based on experiments using simulated sunlight. The model was used to estimate trace organic contaminants photolysis rates under different wetland conditions (e.g., depth, pH, dissolved organic carbon concentration, and nitrate

concentration). The model was also used to determine the wetland area necessary for efficient (i.e., 90%) removal of contaminants. The results indicated that open-water wetland depths of about 30 cm maximize photolysis rates while minimizing the potential for growth of floating vegetation. Further, the model results suggested that photolysis rates were enhanced at high nitrate concentrations, due to the photochemical formation of hydroxyl radical. Dissolved organic carbon reduced photolysis rates for most compounds, but the effect was not as drastic for compounds susceptible to reaction with triplet dissolved organic matter (e.g., diuron and propranolol). Wetland pH affected photolysis rates of test compounds because hydroxyl radical was converted to carbonate radical via reaction with carbonate at alkaline pH values (i.e., >9). Therefore, the photolysis rates of compounds that react rapidly with carbonate radical (e.g., sulfamethoxazole and propranolol) were enhanced at elevated pH values, while the photolysis rates of compounds that react slowly with carbonate radical (e.g., atenolol and carbamazepine) were reduced. Overall, the results of Chapter 3 suggested that most compounds studied could be removed efficiently via photolysis in wetland areas similar to existing systems during summer, and for some compounds, during the spring and fall.

Monitoring of ambient concentrations of trace organic contaminants in a pilot-scale open-water wetland cell indicated that photolysis could not entirely account for the observed removal of trace organic contaminants. To explain this discrepancy, the removal of trace organic contaminants via sorption and biotransformation in the open-water wetland biomat was assessed in Chapter 4. While sorption served as a temporary sink for hydrophobic compounds (e.g., propranolol), the sorption capacity of the biomat was quickly saturated. Therefore, sorption was not a sustainable removal mechanism. Biotransformation rates of trace organic contaminants measured in microcosm experiments displayed differences between illuminated and dark conditions. Certain compounds (e.g., trimethoprim and sulfamethoxazole) were transformed more rapidly under dark conditions (i.e., $\text{pH} \approx 8.5$; $[\text{O}_2] \approx 5 \text{ mg L}^{-1}$) and other compounds (e.g., β -blockers) were transformed more rapidly under illuminated conditions, when algal photosynthesis elevated pH values and dissolved oxygen concentrations (i.e., $\text{pH} \approx 9.5$; $[\text{O}_2] > 20 \text{ mg L}^{-1}$). Measured biotransformation rates were typically about an order of magnitude higher than rates previously measured in vegetated wetlands and surface waters. Predictions for removal of trace organic contaminants in open-water cells via photolysis and biotransformation suggested that efficient removal (i.e., >90%) could be achieved in wetland areas similar to those occupied by existing full-scale systems throughout most of the year.

6.3 Removal of Nitrate in Open-Water Wetlands

Because wetlands are commonly employed for nutrient removal, the ability of open-water wetlands to remove nitrate was evaluated (Chapter 5). On an annual basis, greater than 60% of the nitrate entering the pilot-scale open-water cell was removed. As often observed in vegetated wetlands, nitrate removal was more efficient during warm months and slowed during the cool months, due to changes in microbial activity. Temperature-corrected nitrate removal rates in the pilot-scale open-water cell were higher than values reported for more than 75% of vegetated wetlands ($k_{20} = 59.4 \pm 6.2 \text{ m yr}^{-1}$).

To ensure that nitrate removal in the open-water wetland was sustainable, potential nitrate removal mechanisms were evaluated. Microbial denitrification rates were measured in anoxic microcosms containing water and biomass from the pilot-scale cell. On the basis of

measured denitrification rates, microbial denitrification in the wetland biomat was identified as the primary loss mechanism of nitrate, accounting for more than 80% of the nitrate lost in the open-water cell. Denitrification was driven primarily by algal carbon released as algae decayed in the wetland biomat. The importance of denitrification was supported by the presence of genes necessary for denitrification (*nirK* and *nirS*) within the wetland biomat.

Accretion of assimilated nitrogen was a relatively insignificant nitrogen loss mechanism, accounting for less than 3% of the nitrate removed annually. The phosphorus content of the biomat was greater than expected based on the Redfield ratio, possibly due to the precipitation of phosphate-containing minerals. However, phosphate removal throughout the study was modest (<20%).

Conditions suitable for nitrate removal via anammox (anaerobic ammonium oxidation) existed within the biomat where nitrate mixed with ammonium released from decaying biomass. The removal of ammonium in microcosms amended with nitrate or nitrite suggested that microorganisms capable of anammox were active in the biomat. The higher abundance of the anammox-specific gene hydrazine synthase (*hzs*) near the wetland bottom than adjacent to the biomat surface provided further evidence for the presence of anammox organisms in the pilot-scale wetland cell. Overall, anammox may have been responsible for up to 10% of annual nitrate removal in the system.

Results from Chapter 5 suggested that efficient nitrate removal can be achieved in wetland in areas similar to or smaller than those used for vegetated wetlands. Therefore, portions of vegetated wetlands may be converted to open-water wetlands to enhance removal of trace organic contaminants (Chapters 3 and 4) and pathogens (Nguyen *et al.*, 2014; Silverman *et al.*, 2014) without sacrificing nitrate removal.

6.4 Future Research

As discussed in Chapter 2, it may be possible to enhance contaminant removal efficiency in unit process wetlands by judiciously arranging cells within a wetland treatment train. For example, macrophyte-derived dissolved organic matter may produce compounds that sensitize photolysis of trace organic contaminants better than wastewater-derived dissolved organic matter (Catalán *et al.*, 2013). Therefore, the removal rates of certain trace organic contaminants may be increased in open-water cells positioned after macrophyte-dominated cells. Conversely, nitrate removal in vegetated cells may reduce indirect photolysis of trace organic contaminants in open-water wetlands (Chapter 3).

Photolysis and biotransformation modify trace organic contaminants, but typically do not result in complete mineralization. Transformation products typically do not retain their original biological function and tend to be more hydrophilic which often makes them less toxic and easier for organisms to excrete. However, in some cases transformation products may be more toxic than the parent compounds (Latch *et al.*, 2003a). In Chapter 3, the biotransformation products of several test compounds were identified, but except in the case of atenolol, the identified transformation products only accounted for a small fraction of the transformed parent compounds. To ensure that trace organic contaminant transformation products produced in open-water wetlands are not more toxic than the parent compounds, transformation pathways for

more test compounds should be elucidated. In some cases, this may require the use of ^{14}C -labeled compounds and collection of labeled CO_2 to evaluate the extent of mineralization. While it is not possible to identify the transformation products of all wastewater-derived trace organic contaminants in open-water cells, generalizations may be possible for chemical classes and specific functional groups. Bioassays may also provide guidance as to which test compounds' transformation products are likely to retain biological activity.

Biotransformation rates of certain test compounds were found to differ in illuminated and dark microcosms. As discussed in Chapter 4, it was expected that trace organic contaminant oxidation would be enhanced under the high dissolved oxygen concentrations produced by photosynthesis at the top of the biomat, which was observed for metoprolol and propranolol. Further research is necessary to explain the enhanced rates of transformation of trimethoprim and sulfamethoxazole observed in dark microcosms. Insight into this process may be gained by inhibiting specific types of bacteria, or by adding easily-utilized carbon sources to stimulate bacteria. For example, addition of allylthiourea to microcosms can inhibit nitrifying microorganisms that may be more active when ammonium is released via ammonification in dark microcosms (Batt *et al.*, 2006). The addition of acetate to microcosms could be used to mimic the effect of the release of labile compounds during photosynthesis.

In Chapter 5, nitrogen cycling and removal in open-water wetlands was assessed. While nitrate was primarily removed by denitrification, microcosm incubations and microbial analysis suggested that some nitrate loss may be attributed to anammox. To quantify the importance of anammox in open-water wetlands, studies employing isotopically labeled nitrogen species could be employed (Thamdrup and Dalsgaard, 2002). Understanding the contribution of anammox to nitrogen removal would help inform the management open-water wetland systems. For example, removal of nitrogen via anammox may be enhanced as the wetland biomat thickens, implying that nitrogen removal would be optimized in thicker biomats than predicted based only on nitrate removal via denitrification.

In addition to wastewater-derived contaminants, open-water wetlands may also be capable of removing contaminants present in other wastewaters, such as agricultural runoff and industrial wastewater. Agricultural runoff contains pesticides, which in some cases are removed in vegetated wetlands (Kruger *et al.*, 1996; Moore *et al.*, 2000, 2002). Certain pesticides are photo-labile (Zeng and Arnold, 2012; Remucal, 2014), and their removal may be enhanced via photolysis in open-water wetland cells. Removal of other pesticides via biotransformation may be enhanced under the oxic conditions of open-water wetlands, as compared to in predominantly anaerobic vegetated wetlands. Trace organic contaminants present in industrial wastewater may be removed in vegetated wetlands (Knight *et al.*, 1999; Lin *et al.*, 2006), but their removal in open-water wetlands has not been assessed. Open-water wetlands may also be employed to remove nitrate from agricultural runoff.

Research described in Chapters 2-5 utilized microcosm-scale experiments to gain insight into trace organic contaminant and nitrate removal in the Discovery Bay pilot-scale wetland. Scaling up open-water unit process cells from the pilot- to the demonstration-scale would provide wetland operators and agencies interested in building treatment with further evidence of the utility and feasibility of these systems. It also could help to identify maintenance

requirements and factors affecting wetland performance. To this end, demonstration-scale open-water cells have been built in the Prado Wetlands in Southern California (Orange County Water District, 2008). Three parallel open-water cells, each about 20 times larger than the Discovery Bay pilot-scale cell (i.e., ~0.75 ha) receive approximately 0.3 MGD (~1 MLD) of water from the adjacent, effluent-dominated Santa Ana River. By monitoring the removal of trace organic contaminants, nutrients, and pathogens in these cells, the performance of open-water cells can be assessed under a range of operating conditions. The feasibility of maintenance activities, such as removing floating vegetation and periodically removing the biomat, can also be evaluated.

References

- Ahn, C., Gillevet, P. M., and Sikaroodi, M. (2007). Molecular characterization of microbial communities in treatment microcosm wetlands as influenced by macrophytes and phosphorus loading. *Ecol. Indic.* 7, 852.
- Alder, A. C., Schaffner, C., Majewsky, M., Klasmeier, J., and Fenner, K. (2010). Fate of beta-blocker human pharmaceuticals in surface water. *Water Res.* 44, 936.
- Alvord, H. H., and Kadlec, R. H. (1995). The interaction of atrazine with wetland sorbents. *Ecol. Eng.* 5, 469.
- American Public Health Association. (1995). *Standard Methods for the Examination of Water and Wastewater*, 19th edition. Washington, DC: American Public Health Association, A. W. W. A., Water Environment Federation.
- Andreozzi, R., Raffaele, M., and Nicklas, P. (2003). Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. *Chemosphere* 50, 1319.
- Ann, Y., Reddy, K. R., and Delfino, J. J. (1999). Influence of chemical amendments on phosphorus immobilization in soils from a constructed wetland. *Ecol. Eng.* 14, 157.
- Ansa, E. D. O., Lubberding, H. J., Ampofo, J. A., and Gijzen, H. J. (2011). The role of algae in the removal of *Escherichia coli* in a tropical eutrophic lake. *Ecol. Eng.* 37, 317.
- AOAC. (1997). Microchemical determination of carbon, hydrogen, and nitrogen, automated method. *Official Methods of Analysis of AOAC International*, 16th edition. Arlington, VA: AOAC International, p. 5.
- Arias, C., Brix, H., and Johansen, N. H. (2003). Phosphorus removal from municipal wastewater in an experimental two-stage vertical flow constructed wetland system equipped with a calcite filter. *Water Sci. Technol.* 48, 51.
- Aronesty, E. (2011). *ea-utils: Command-line tools for processing biological sequencing data*.
- Azim, M. E., Verdegem, M. C. J., Van Dam, A. A., and Beveridge, M. C. M. (Eds.). (2005). *Periphyton: Ecology, exploitation, and management*, 1st edition. Oxfordshire, UK: CABI.
- Bacchetti De Gregoris, T., Aldred, N., Clare, A. S., and Burgess, J. G. (2011). Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa. *J. Microbiol. Methods* 86, 351.
- Bachand, P. A. M., and Horne, A. J. (2000). Denitrification in constructed free-water surface wetlands. *Ecol. Eng.* 14, 17.
- Balon, K., Riebesehl, B. U., and Müller, B. W. (1999). Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption. *Pharm. Res.* 16, 882.
- Barker, J., and Brown, M. (1994). Trojan horses of the microbial world. *Microbiology* 140, 1253.
- Bastviken, S. K., Eriksson, P. G., Premrov, A., and Tonderski, K. (2005). Potential denitrification in wetland sediments with different plant species detritus. *Ecol. Eng.* 25, 183.
- Batt, A. L., Kim, S., and Aga, D. S. (2006). Enhanced biodegradation of iopromide and trimethoprim in nitrifying activated sludge. *Env. Sci Technol* 40, 7367.
- Beaver, J., Miller-Lemke, A., and Acton, J. (1998). Midsummer zooplankton assemblages in four types of wetlands in the Upper Midwest, USA. *Hydrobiologia* 380, 209.

- Benner, J., Salhi, E., Ternes, T., and Von Gunten, U. (2008). Ozonation of reverse osmosis concentrate. *Water Res.* 42, 3003.
- Betageri, G. V., and Rogers, J. A. (1987). Thermodynamics of partitioning of β -blockers in the n-octanol- buffer and liposome systems. *Int. J. Pharm.* 36, 165.
- Bonvin, F., Rutler, R., Chèvre, N., Halder, J., and Kohn, T. (2011). Spatial and temporal presence of a wastewater-derived micropollutant plume in Lake Geneva. *Environ. Sci. Technol.* 45, 4702.
- Boon, P., Virtue, P., and Nichols, P. (1996). Microbial consortia in wetland sediments. *Mar. Freshw. Res.* 47, 27.
- Boreen, A. L., Arnold, W. A., and McNeill, K. (2004). Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups. *Environ. Sci. Technol.* 38, 3933.
- Boreen, A. L., Edhlund, B. L., Cotner, J. B., and McNeill, K. (2008). Indirect photodegradation of dissolved free amino acids: The contribution of singlet oxygen and the differential reactivity of DOM from various sources. *Env. Sci Technol* 42, 5492.
- Borg, K. O., Carlsson, E., Hoffmann, K.-J., Jönsson, T.-E., Thorin, H., and Wallin, B. (1975). Act Pharmacol. Toxicol. *Acta Pharmacol. Toxicol. (Copenh.)* 36, 125.
- Bosshard, F., Bucheli, M., Meur, Y., and Egli, T. (2010). The respiratory chain is the cell's Achilles' heel during UVA inactivation in Escherichia coli. *Microbiology* 156, 2006.
- Boyd, G. R., Reemtsma, H., Grimm, D. A., and Mitra, S. (2003). Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada. *Sci. Total Environ.* 311, 135.
- Braker, G., Fesefeldt, A., and Witzel, K.-P. (1998). Development of PCR primer systems for amplification of nitrite reductase genes (nirK and nirS) to detect denitrifying bacteria in environmental samples. *Appl. Environ. Microbiol.* 64, 3769.
- Breitholtz, M., Näslund, M., Stråe, D., Borg, H., Grabic, R., and Fick, J. (2012). An evaluation of free water surface wetlands as tertiary sewage water treatment of micro-pollutants. *Ecotoxicol. Environ. Saf.* 78, 63.
- Brezonik, P. L., and Fulkerson-Brekken, J. (1998). Nitrate-induced photolysis in natural waters: Controls on concentrations of hydroxyl radical photo-intermediates by natural scavenging agents. *Env. Sci Technol* 32, 3004.
- Brix, H. (1997). Do macrophytes play a role in constructed treatment wetlands? *Water Sci. Technol.* 35, 11.
- Brodin, T., Fick, J., Jonsson, M., and Klaminder, J. (2013). Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. *Science* 339, 814.
- Burgin, A. J., and Hamilton, S. K. (2007). Have we overemphasized the role of denitrification in aquatic ecosystems? *Front. Ecol. Environ.* 5, 89.
- Burns, K. A., and Smith, J. L. (1981). Biological monitoring of ambient water quality: The case for using bivalves as sentinel organisms for monitoring petroleum pollution in coastal waters. *Estuar. Coast. Shelf Sci.* 13, 433.
- Buser, H.-R., Poiger, T., and Müller, M. D. (1999). Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater. *Environ. Sci. Technol.* 33, 2529.
- Buth, J. M., Grandbois, M., Vikesland, P. J., McNeill, K., and Arnold, W. A. (2009). Aquatic photochemistry of chlorinated triclosan derivatives. *Environ. Toxicol. Chem.* 28, 2555.

- Buth, J. M., Steen, P. O., Sueper, C., Blumentritt, D., Vikesland, P. J., Arnold, W. A., and McNeill, K. (2010). Dioxin photoproducts of triclosan and its chlorinated derivatives in sediment cores. *Env. Sci Technol* 44, 4545.
- Buttner, J. K. (1986). Corbicula as a biological filter and polyculture organism in catfish rearing ponds. *Progress. Fish-Cult.* 48, 136.
- Buxton, G. V., Greenstock, C. L., Helman, W. P., and Ross, A. B. (1988). Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($\cdot\text{OH}/\cdot\text{O}-$) in aqueous solutions. *J Phys Chem Ref Data* 17, 513.
- Calheiros, C. S. C., Duque, A. F., Moura, A., Henriques, I. S., Correia, A., Rangel, A. O. S. S., and Castro, P. M. L. (2009). Changes in the bacterial community structure in two-stage constructed wetlands with different plants for industrial wastewater treatment. *Bioresour. Technol.* 100, 3228.
- Calza, P., Medana, C., Padovano, E., Giancotti, V., and Minero, C. (2013). Fate of selected pharmaceuticals in river waters. *Environ. Sci. Pollut. Res.* 20, 2262.
- Camacho-Muñoz, D., Martín, J., Santos, J., Aparicio, I., and Alonso, E. (2012). Effectiveness of conventional and low-cost wastewater treatments in the removal of pharmaceutically active compounds. *Water. Air. Soil Pollut.* 223, 2611.
- Canonica, S., Jans, U., Stemmler, K., and Hoigné, J. (1995). Transformation kinetics of phenols in water: Photosensitization by dissolved natural organic material and aromatic ketones. *Env. Sci Technol* 29, 1822.
- Canonica, S., Kohn, T., Mac, M., Real, F. J., Wirz, J., and Von Gunten, U. (2005). Photosensitizer method to determine rate constants for the reaction of carbonate radical with organic compounds. *Env. Sci Technol* 39, 9182.
- Canonica, S., and Laubscher, H.-U. (2008). Inhibitory effect of dissolved organic matter on triplet-induced oxidation of aquatic contaminants. *Photochem. Photobiol. Sci.* 7, 547.
- Caporaso, J. G., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L., and Knight, R. (2009). PyNAST. *Bioinformatics* 26, 266.
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7, 335.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., and Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621.
- Carey, R. O., and Migliaccio, K. W. (2009). Contribution of Wastewater Treatment Plant Effluents to Nutrient Dynamics in Aquatic Systems. *Environ. Manage.* 44, 205.
- Catalán, N., Obrador, B., Felip, M., and Pretus, J. L. (2013). Higher reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake. *Aquat. Sci.* 75, 581.
- Chen, S., and Hoffman, M. Z. (1975). Effect of pH on the reactivity of the carbonate radical in aqueous solution. *Radiat. Res.* 62, 18.
- Chen, Y., Hu, C., Hu, X., and Qu, J. (2009). Indirect photodegradation of amine drugs in aqueous solution under simulated sunlight. *Env. Sci Technol* 43, 2760.

Chilton, J. (1996). Groundwater. In D. V. Chapman, Ed., *Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring*, London. E & Fm Spon, p. 413.

Chon, K., Chang, J.-S., Lee, E., Lee, J., Ryu, J., and Cho, J. (2011). Abundance of denitrifying genes coding for nitrate (narG), nitrite (nirS), and nitrous oxide (nosZ) reductases in estuarine versus wastewater effluent-fed constructed wetlands. *Ecol. Eng.* 37, 64.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O., and Kroiss, H. (2005). The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Res.* 39, 97.

Cole, J. J. (1982). Interactions between bacteria and algae in aquatic ecosystems. *Annu. Rev. Ecol. Syst.* 13, 291.

Costanza, R., D'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R. G., Sutton, P., and Van den Belt, M. (1997). The value of the world's ecosystem services and natural capital. *Nature* 387, 253.

Cotroneo, C., Csoboth, L., Yozzo, D. J., Doss, T., Will, B., and McLaughlin, J. (2011). Working toward nutrient reduction in a eutrophic urban estuary: Ribbed mussel biofiltration in Jamaica Bay, presented at the *4th National Conference on Ecosystem Restoration*, Baltimore, MD.

Craggs, R. J., Adey, W. H., Jessup, B. K., and Oswald, W. J. (1996). A controlled stream mesocosm for tertiary treatment of sewage. *Ecol. Eng.* 6, 149.

Cunningham, V. L., Binks, S. P., and Olson, M. J. (2009). Human health risk assessment from the presence of human pharmaceuticals in the aquatic environment. *Regul. Toxicol. Pharmacol.* 53, 39.

Curtis, T. P., Mara, D. D., and Silva, S. A. (1992). Influence of pH, oxygen, and humic substances on ability of sunlight to damage fecal coliforms in waste stabilization pond water. *Appl. Environ. Microbiol.* 58, 1335.

D'Angelo, E. (2003). Wetlands: Biodegradation of Organic Pollutants. In G. Bitton, Ed., *Encyclopedia of Environmental Microbiology*. New York, USA: John Wiley & Sons, Inc, p. 3401.

Dai, X., and Boll, J. (2006). Settling velocity of *Cryptosporidium parvum* and *Giardia lamblia*. *Water Res.* 40, 1321.

Daughton, C. G., and Ternes, T. A. (1999). Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environ. Health Perspect.* 107, 907.

Davies-Colley, R. J. (2005). Pond disinfection. In A. Shilton, Ed., *Pond treatment technology*. London, UK: IWA Publishing, p. 101.

Davies-Colley, R. J., Donnison, A. M., and Speed, D. J. (2000). Towards a mechanistic understanding of pond disinfection. *Water Sci. Technol.* 42, 149.

Davies-Colley, R. J., Donnison, A. M., Speed, D. J., Ross, C. M., and Nagels, J. W. (1999). Inactivation of faecal indicator micro-organisms in waste stabilisation ponds. *Water Res.* 33, 1220.

Davison, L., Pont, D., Bolton, K., and Headley, T. (2006). Dealing with nitrogen in subtropical Australia. *Ecol. Eng.* 28, 213.

Decamp, O., and Warren, A. (1998). Bacterivory in ciliates isolated from constructed wetlands (reed beds) used for wastewater treatment. *Water Res.* 32, 1989.

DeJournett, T. D., Arnold, W. A., and LaPara, T. M. (2007). The characterization and quantification of methanotrophic bacterial populations in constructed wetland sediments using PCR targeting 16S rRNA gene fragments. *Appl. Soil Ecol.* 35, 648.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., and Andersen, G. L. (2006). Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Appl. Environ. Microbiol.* 72, 5069.

Diaz, R. R. (1995). Marine benthic hypoxia. *Oceanogr. Mar. Biol. Annu. Rev.* 33, 245.

Dobor, J., Varga, M., and Záray, G. (2012). Biofilm controlled sorption of selected acidic drugs on river sediments characterized by different organic carbon content. *Chemosphere* 87, 105.

Dong, M. M., Mezyk, S. P., and Rosario-Ortiz, F. L. (2010). Reactivity of effluent organic matter (EfOM) with hydroxyl radical as a function of molecular weight. *Environ. Sci. Technol.* 44, 5714.

Dong, M. M., and Rosario-Ortiz, F. L. (2012). Photochemical formation of hydroxyl radical from effluent organic matter. *Env. Sci Technol* 46, 3788.

Dong, Z., and Sun, T. (2007). A potential new process for improving nitrogen removal in constructed wetlands—Promoting coexistence of partial-nitrification and ANAMMOX. *Ecol. Eng.* 31, 69.

Drouillard, K. G., Chan, S., O'Rourke, S., G. Douglas, H., and Letcher, R. J. (2007). Elimination of 10 polybrominated diphenyl ether (PBDE) congeners and selected polychlorinated biphenyls (PCBs) from the freshwater mussel, *Elliptio complanata*. *Chemosphere* 69, 362.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460.

Emerson, D., Weiss, J. V., and Megonigal, J. P. (1999). Iron-oxidizing bacteria are associated with ferric hydroxide precipitates (Fe-plaque) on the roots of wetland plants. *Appl. Environ. Microbiol.* 65, 2758.

EPA. (1993). Subsurface Flow Constructed Wetlands For Waste Water Treatment. EPA, Cincinnati, Ohio.

EPA. (2000a, September). Constructed Wetlands Treatment of Municipal Wastewaters. EPA, Cincinnati, Ohio.

EPA. (2000b, October). Guiding Principles for Constructed Treatment Wetlands: Providing for Water Quality and Wildlife Habitat. EPA, Cincinnati, Ohio.

Erler, D. V., Eyre, B. D., and Davison, L. (2008). The contribution of anammox and denitrification to sediment N₂ production in a surface flow constructed wetland. *Environ. Sci. Technol.* 42, 9144.

Falabi, J. a., Gerba, C. p., and Karpiscak, M. m. (2002). Giardia and Cryptosporidium removal from waste-water by a duckweed (*Lemna gibba* L.) covered pond. *Lett. Appl. Microbiol.* 34, 384.

Fang, J., Semple, H. A., and Song, J. (2004). Determination of metoprolol, and its four metabolites in dog plasma. *J. Chromatogr. B* 809, 9.

Faulwetter, J. L., Gagnon, V., Sundberg, C., Chazarenc, F., Burr, M. D., Brisson, J., Camper, A. K., and Stein, O. R. (2009). Microbial processes influencing performance of treatment wetlands: A review. *Ecol. Eng.* 35, 987.

Fayer, R., Graczyk, T. K., Lewis, E. J., Trout, J. M., and Farley, C. A. (1998). Survival of infectious *Cryptosporidium parvum* oocysts in seawater and Eastern Oysters (*Crassostrea virginica*) in the Chesapeake Bay. *Appl. Environ. Microbiol.* 64, 1070.

Fayer, R., Trout, J. M., Walsh, E., and Cole, R. (2000). Rotifers ingest oocysts of *Cryptosporidium parvum*. *J. Eukaryot. Microbiol.* 47, 161.

Fierer, N., Jackson, J. A., Vilgalys, R., and Jackson, R. B. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR Assays. *Appl. Environ. Microbiol.* 71, 4117.

- Fleming-Singer, M. S., and Horne, A. J. (2002). Enhanced nitrate removal efficiency in wetland microcosms using an episodiment layer for denitrification. *Environ. Sci. Technol.* 36, 1231.
- Fleming-Singer, M. S., and Horne, A. J. (2006). Balancing wildlife needs and nitrate removal in constructed wetlands. *Ecol. Eng.* 26, 147.
- Fletcher, M., and Marshall, K. (1982). Are solid surfaces of ecological significance to aquatic bacteria? *Adv. Microb. Ecol.* 6, 199.
- Florida Department of Environmental Protection. (2012). Orlando Easterly Wetlands.
- Fono, L. J., Kolodziej, E. P., and Sedlak, D. L. (2006). Attenuation of wastewater-derived contaminants in an effluent-dominated river. *Env. Sci Technol* 40, 7257.
- Fono, L. J., and Sedlak, D. L. (2005). Use of the chiral pharmaceutical propranolol to identify sewage discharges into surface waters. *Env. Sci Technol* 39, 9244.
- Fortin, D., Goulet, R., and Roy, M. (2000). Seasonal cycling of Fe and S in a constructed wetland: The role of sulfate-reducing bacteria. *Geomicrobiol. J.* 17, 221.
- Francoeur, S. N., Schaecher, M., Neely, R. K., and Kuehn, K. A. (2006). Periphytic photosynthetic stimulation of extracellular enzyme activity in aquatic microbial communities associated with decaying Typha litter. *Microb. Ecol.* 52, 662.
- Fuchs, V. J., Mihelcic, J. R., and Gierke, J. S. (2011). Life cycle assessment of vertical and horizontal flow constructed wetlands for wastewater treatment considering nitrogen and carbon greenhouse gas emissions. *Water Res.* 45, 2073.
- Gagnon, V., Chazarenc, F., Comeau, Y., and Brisson, J. (2007). Influence of macrophyte species on microbial density and activity in constructed wetlands. *Water Sci. Technol.* 56, 249.
- Gans, O., Sattelberger, R., and Scharf, S. (2002). Ausgewählte Arzneimittelwirkstoffe in Zu- und Abläufen kommunaler österreichischer Kläranlagen. *Vom Wasser* 98, 165.
- Gearheart, R. A. (1999). The use of free surface constructed wetland as an alternative process treatment train to meet unrestricted water reclamation standards. *Water Sci. Technol.* 40, 375.
- Gerba, C. P., Thurston, J. A., Falabi, J. A., Watt, P. M., and Karpiscak, M. M. (1999). Optimization of artificial wetland design for removal of indicator microorganisms and pathogenic protozoa. *Water Sci. Technol.* 40, 363.
- Gerecke, A. C., Canonica, S., Müller, S. R., Schärer, M., and Schwarzenbach, R. P. (2001). Quantification of dissolved natural organic matter (DOM) mediated phototransformation of phenylurea herbicides in lakes. *Env. Sci Technol* 35, 3915.
- Gersberg, R. M., Gearheart, R. A., and Ives, M. (1989). Pathogen removal in constructed wetlands. In D. A. Hammer, Ed., *Constructed Wetlands for Wastewater Treatment; Municipal, Industrial and Agricultural*. Chelsea, MI: Lewis, p. 431.
- Gersberg, R. M., Lyon, S. R., Brenner, R., and Elkins, B. V. (1987). Fate of viruses in artificial wetlands. *Appl. Environ. Microbiol.* 53, 731.
- Gianfreda, L., and Rao, M. A. (2004). Potential of extra cellular enzymes in remediation of polluted soils. *Enzyme Microb. Technol.* 35, 339.
- Ginebreda, A., Muñoz, I., De Alda, M. L., Brix, R., López-Doval, J., and Barceló, D. (2010). Environmental risk assessment of pharmaceuticals in rivers. *Environ. Int.* 36, 153.

- Golet, E. M., Xifra, I., Siegrist, H., Alder, A. C., and Giger, W. (2003). Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Env. Sci Technol* 37, 3243.
- Graczyk, T. K., Conn, D. C., Marcogliese, D. M., Graczyk, H. G., and De Lafontaine, Y. (2003). Accumulation of human waterborne parasites by zebra mussels (*Dreissena polymorpha*) and Asian freshwater clams (*Corbicula fluminea*). *Parasitol. Res.* 89, 107.
- Graczyk, T. K., Girouard, A. S., Tamang, L., Nappier, S. P., and Schwab, K. J. (2006). Recovery, bioaccumulation, and inactivation of human waterborne pathogens by the Chesapeake Bay Nonnative oyster, *Crassostrea ariakensis*. *Appl. Environ. Microbiol.* 72, 3390.
- Graczyk, T. K., Thompson, R. C., Fayer, R., Adams, P., Morgan, U. M., and Lewis, E. J. (1999). *Giardia duodenalis* cysts of genotype A recovered from clams in the Chesapeake Bay subestuary, Rhode River. *Am. J. Trop. Med. Hyg.* 61, 526.
- Gray, J. L., and Sedlak, D. L. (2005). The fate of estrogenic hormones in an engineered treatment wetland with dense macrophytes. *Water Environ. Res.* 77, 24.
- Groffman, P. ., Holland, E. A., Myrold, D. ., Robertson, G. P., and Zou. (1999). Denitrification. In G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins, Eds., *Standard soil methods for long-term ecological research*. New York: Oxford University Press, p. 272.
- Gudimov, A. V. (2002). Zooremediation, a new biotechnology solution for shoreline protection and cleanup, presented at the *Environment Canada Arctic and Marine Oil Spill Program Technical Seminar (AMOP) Proceedings*, Ottawa, Canada.
- Gueymard, C. A. (2003). SMARTS: Simple Model of the Atmospheric Radiative Transfer of Sunshine. Solar Consulting Services.
- Von Gunten, U., and Hoigne, J. (1994). Bromate Formation during Ozonization of Bromide-Containing Waters. *Environ. Sci. Technol.* 28, 1234.
- Gutknecht, J. L. M., Goodman, R. M., and Balser, T. C. (2006). Linking soil process and microbial ecology in freshwater wetland ecosystems. *Plant Soil* 289, 17.
- Haag, W. R., and Hoigné, J. (1986). Singlet oxygen in surface waters. 3. Photochemical formation and steady-state concentrations in various types of waters. *Env. Sci Technol* 20, 341.
- Hahn, M. W., and Höfle, M. G. (2001). Grazing of protozoa and its effect on populations of aquatic bacteria. *FEMS Microbiol. Ecol.* 35, 113.
- Haines, K. C. (1979). The use of *Corbicula* as a clarifying agent in experimental tertiary sewage treatment process on St. Croix, US Virgin Islands, presented at the *Proc. 1st International Corbicula symposium*, Texas Christian Univ. Res. Found.
- Hamady, M., Walker, J. J., Harris, J. K., Gold, N. J., and Knight, R. (2008). Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nat. Methods* 5, 235.
- Hamilton, P. B., Poulin, M., Chariest, D. F., and Angell, M. (1992). *Americanarum Diatomarum Exsiccata*. *Diatom Res.* 7, 25.
- Hammer, D. A., and Knight, R. L. (1994). Designing constructed wetlands for nitrogen removal. *Water Sci. Technol.* 29, 15.

Harhangi, H. R., Roy, M. L., Alen, T. Van, Hu, B., Groen, J., Kartal, B., Tringe, S. G., Quan, Z.-X., Jetten, M. S. M., and Camp, H. J. M. O. Den. (2012). Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. *Appl. Environ. Microbiol.* 78, 752.

Harris, D., Horwáth, W. R., and Van Kessel, C. (2001). Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Sci. Soc. Am. J.* 65, 1853.

Harvey, J. W., Saiers, J. W., and Newlin, J. T. (2005). Solute transport and storage mechanisms in wetlands of the Everglades, south Florida. *Water Resour. Res.* 41.

Haven, D. S., and Morales-Alamo, R. (1966). Aspects of biodeposition by oysters and other invertebrate filter feeders. *Limnol Ocean.* 11, 487.

Heberer, T. (2002). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment. *Toxicol. Lett.* 131, 5.

Heberer, T., B., B., Schmidt-Baumler, K., Tsipi, D., V., and Hiskia, A. (2001). Occurrence of Pharmaceutical Residues in Sewage, River, Ground, and Drinking Water in Greece and Berlin (Germany). *Pharmaceuticals and Care Products in the Environment*. American Chemical Society, p. 70.

Hecky, R., Campbell, P., and Hendzel, L. (1993). The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. *Limnol. Oceanogr.* 38, 709.

Helbling, D. E., Hollender, J., Kohler, H.-P. E., Singer, H., and Fenner, K. (2010). High-throughput identification of microbial transformation products of organic micropollutants. *Environ. Sci. Technol.* 44, 6621.

Helbling, D. E., Johnson, D. R., Honti, M., and Fenner, K. (2012). Micropollutant biotransformation kinetics associate with WWTP process parameters and microbial community characteristics. *Environ. Sci. Technol.* 46, 10579.

Henry, S., Baudoin, E., López-Gutiérrez, J. C., Martin-Laurent, F., Brauman, A., and Philippot, L. (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *J. Microbiol. Methods* 59, 327.

Hernandez, M. E., and Mitsch, W. J. (2007). Denitrification in created riverine wetlands. *Ecol. Eng.* 30, 78.

Hernando, M. D., Mezcuca, M., Fernández-Alba, A. R., and Barceló, D. (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta* 69, 334.

Herskowitz, J. (1986). *Town of Listowel Artificial Marsh Project*. Toronto: Ontario Ministry of the Environment.

Hijosa-Valsero, M., Matamoros, V., Martín-Villacorta, J., Bécares, E., and Bayona, J. M. (2010a). Assessment of full-scale natural systems for the removal of PPCPs from wastewater in small communities. *Water Res.* 44, 1429.

Hijosa-Valsero, M., Matamoros, V., Pedescoll, A., Martín-Villacorta, J., Bécares, E., García, J., and Bayona, J. M. (2011). Evaluation of primary treatment and loading regimes in the removal of pharmaceuticals and personal care products from urban wastewaters by subsurface-flow constructed wetlands. *Int. J. Environ. Anal. Chem.* 91, 632.

Hijosa-Valsero, M., Matamoros, V., Sidrach-Cardona, R., Martín-Villacorta, J., Bécares, E., and Bayona, J. M. (2010b). Comprehensive assessment of the design configuration of constructed wetlands for the removal of pharmaceuticals and personal care products from urban wastewaters. *Water Res.* 44, 3669.

Hill, B. H., Elonen, C. M., Jicha, T. M., Cotter, A. M., Trebitz, A. S., and Danz, N. P. (2006). Sediment microbial enzyme activity as an indicator of nutrient limitation in Great Lakes coastal wetlands. *Freshw. Biol.* 51, 1670.

Hill, V. R., and Sobsey, M. D. (2001). Removal of Salmonella and microbial indicators in constructed wetlands treating swine wastewater. *Water Sci. Technol.* 44, 215.

Hirsch, R., Ternes, T. A., Haberer, K., and Kratz, K. (1996). Determination of betablockers and B-sympathomimetics in the aquatic environment. *Vom Wasser* 87, 263.

Hollender, J., Zimmermann, S. G., Koepke, S., Krauss, M., McArdell, C. S., Ort, C., Singer, H., Von Gunten, U., and Siegrist, H. (2009). Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration. *Environ. Sci. Technol.* 43, 7862.

Horne, A. (2000). Phytoremediation by constructed wetlands. In N. Terry and G. Bañuelos, Eds., *Phytoremediation of Contaminated Soil and Water*. Boca Raton, FL: CRC Press,.

Horne, A. J. (1995). Nitrogen removal from waste treatment pond or activated sludge plant effluents with free-surface wetlands. *Water Sci. Technol.* 31, 341.

Horne, A. J., and Fleming-Singer, M. S. (2005). Phytoremediation using constructed treatment wetlands: An overview. In M. Fingerman and R. Nagabhushanam, Eds., *Bioremediation of Aquatic and Terrestrial Ecosystems*. Enfield, NH: Science Publishers, p. 329.

Howell, C. J., Crohn, D. M., and Omary, M. (2005). Simulating nutrient cycling and removal through treatment wetlands in arid/semiarid environments. *Ecol. Eng.* 25, 25.

Huang, J., and Mabury, S. A. (2000a). Steady-state concentrations of carbonate radicals in field waters. *Environ. Toxicol. Chem.* 19, 2181.

Huang, J., and Mabury, S. A. (2000b). A new method for measuring carbonate radical reactivity toward pesticides. *Environ. Toxicol. Chem.* 19, 1501.

Huang, J., and Mabury, S. A. (2000c). The role of carbonate radical in limiting the persistence of sulfur-containing chemicals in sunlit natural waters. *Chemosphere* 41, 1775.

Huber, M. M., Canonica, S., Park, G.-Y., and Von Gunten, U. (2003). Oxidation of pharmaceuticals during ozonation and advanced oxidation processes. *Env. Sci Technol* 37, 1016.

Huggett, D. ., Khan, I. ., Foran, C. ., and Schlenk, D. (2003). Determination of beta-adrenergic receptor blocking pharmaceuticals in United States wastewater effluent. *Environ. Pollut.* 121, 199.

Huggett, D. B., Brooks, B. W., Peterson, B., Foran, C. M., and Schlenk, D. (2002). Toxicity of select beta adrenergic receptor-blocking pharmaceuticals (β -blockers) on aquatic organisms. *Arch. Environ. Contam. Toxicol.* 43, 229.

Hull, M. S., Chaurand, P., Rose, J., Auffan, M., Bottero, J.-Y., Jones, J. C., Schultz, I. R., and Vikesland, P. J. (2011). Filter-feeding bivalves store and biodeposit colloiddally stable gold nanoparticles. *Environ. Sci. Technol.* 45, 6592.

Hussain, S., and Prasher, S. (2011). Understanding the sorption of ionophoric pharmaceuticals in a treatment wetland. *Wetlands* 31, 563.

Ibekwe, A. M., Lyon, S. R., Leddy, M., and Jacobson-Meyers, M. (2006). Impact of plant density and microbial composition on water quality from a free water surface constructed wetland. *J. Appl. Microbiol.* 102, 921.

Imfeld, G., Braeckevelt, M., Kusch, P., and Richnow, H. H. (2009). Monitoring and assessing processes of organic chemicals removal in constructed wetlands. *Chemosphere* 74, 349.

Ishida, C. K., Arnon, S., Peterson, C. G., Kelly, J. J., and Gray, K. A. (2008). Influence of algal community structure on denitrification rates in periphyton cultivated on artificial substrata. *Microb. Ecol.* 56, 140.

Ishii, S., Yan, T., Vu, H., Hansen, D. L., Hicks, R. E., and Sadowsky, M. J. (2010). Factors controlling long-term survival and growth of naturalized *Escherichia coli* populations in temperate field soils. *Microbes Environ.* 25, 8.

- Jagger, J. (1985). *Solar-UV actions on living cells*. New York, NY: Praeger.
- Jasper, J. T., Jones, Z. L., Sharp, J. O., and Sedlak, D. L. (2014a). Biotransformation of wastewater-derived trace organic contaminants in open-water unit process treatment wetlands. *Environ. Sci. Technol.* 48, 5136.
- Jasper, J. T., Jones, Z. L., Sharp, J. O., and Sedlak, D. L. (2014b). Nitrate removal in shallow, open-water treatment wetlands. *Environ. Sci. Technol.* Submitted.
- Jasper, J. T., Nguyen, M. T., Jones, Z. L., Ismail, N. S., Sedlak, D. L., Sharp, J. O., Luthy, R. G., Horne, A. J., and Nelson, K. L. (2013). Unit process wetlands for removal of trace organic contaminants and pathogens from municipal wastewater effluents. *Environ. Eng. Sci.* 30, 421.
- Jasper, J. T., and Sedlak, D. L. (2013). Phototransformation of wastewater-derived trace organic contaminants in open-water unit process treatment wetlands. *Environ. Sci. Technol.* 47, 10781.
- Jetten, M. S. M., Cirpus, I., Kartal, B., Van Niftrik, L., Van de Pas-Schoonen, K. T., Sliekers, O., Haaijer, S., Van der Star, W., Schmid, M., Van de Vossenberg, J., Schmidt, I., Harhangi, H., Van Loosdrecht, M., Gijs Kuenen, J., Op den Camp, H., and Strous, M. (2005). 1994-2004. *Biochem. Soc. Trans.* 33, 119.
- Jiang, C., Li, S.-X., Luo, F.-F., Jin, K., Wang, Q., Hao, Z.-Y., Wu, L.-L., Zhao, G.-C., Ma, G.-F., Shen, P.-H., Tang, X.-L., and Wu, B. (2011). Biochemical characterization of two novel β -glucosidase genes by metagenome expression cloning. *Bioresour. Technol.* 102, 3272.
- Jones, C. M., and Hallin, S. (2010). Ecological and evolutionary factors underlying global and local assembly of denitrifier communities. *ISME J.* 4, 633.
- Jones, O. A. H., Green, P. G., Voulvoulis, N., and Lester, J. N. (2007). Questioning the excessive use of advanced treatment to remove organic micropollutants from wastewater. *Env. Sci Technol* 41, 5085.
- Jones, O. A. H., Voulvoulis, N., and Lester, J. N. (2002). Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. *Water Res.* 36, 5013.
- Joss, A., Carballa, M., Kreuzinger, N., Siegrist, H., and Zabczynski, S. (2006). Chapter 6-Wastewater treatment. In T. A. Ternes and A. Joss, Eds., *Human Pharmaceuticals, Hormones And Fragrances: The Challenge of Micropollutants in Urban Water Management*. London: IWA Publishing, p. 243.
- Kadlec, R. H. (1994). Detention and mixing in free water wetlands. *Ecol. Eng.* 3, 345.
- Kadlec, R. H. (2000). The inadequacy of first-order treatment wetland models. *Ecol. Eng.* 15, 105.
- Kadlec, R. H. (2009). Comparison of free water and horizontal subsurface treatment wetlands. *Ecol. Eng.* 35, 159.
- Kadlec, R. H. (2012). Constructed marshes for nitrate removal. *Crit. Rev. Environ. Sci. Technol.* 42, 934.
- Kadlec, R. H., Bays, J. S., Mokry, L. E., Andrews, D., and Ernst, M. R. (2011). Performance analysis of the Richland-Chambers treatment wetlands. *Ecol. Eng.* 37, 176.
- Kadlec, R. H., and Wallace, S. (2009). *Treatment Wetlands*, 2nd edition. Boca Raton: CRC Press.
- Karpiscak, M. M., Sanchez, L. R., Freitas, R. J., and Gerba, C. P. (2001). Removal of bacterial indicators and pathogens from dairy wastewater by a multi-component treatment system. *Water Sci. Technol.* 44, 813.
- Kasanavia, T., Vu, D., and Sabatini, D. A. (1999). Fluorescent dye and media properties affecting sorption and tracer selection. *Ground Water* 37, 376.

- Kasprzyk-Hordern, B., Dinsdale, R. M., and Guwy, A. J. (2008). The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Res.* 42, 3498.
- Keefe, S. H., Barber, L. B., Runkel, R. L., Ryan, J. N., McKnight, D. M., and Wass, R. D. (2004). Conservative and reactive solute transport in constructed wetlands. *Water Resour. Res.* 40, W01201.
- Kern, S., Baumgartner, R., Helbling, D. E., Hollender, J., Singer, H., Loos, M. J., Schwarzenbach, R. P., and Fenner, K. (2010). A tiered procedure for assessing the formation of biotransformation products of pharmaceuticals and biocides during activated sludge treatment. *J. Environ. Monit.* 12, 2100.
- Khan, S., and Ongerth, J. (2002). Estimation of pharmaceutical residues in primary and secondary sewage sludge based on quantities of use and fugacity modelling. *Water Sci. Technol.* 46, 105.
- Khunjar, W. O., Mackintosh, S. A., Skotnicka-Pitak, J., Baik, S., Aga, D. S., and Love, N. G. (2011). Elucidating the relative roles of ammonia oxidizing and heterotrophic bacteria during the biotransformation of 17 α -ethinylestradiol and trimethoprim. *Environ. Sci. Technol.* 45, 3605.
- Kim, S. D., Cho, J., Kim, I. S., Vanderford, B. J., and Snyder, S. A. (2007). Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. *Water Res.* 41, 1013.
- Kjellin, J., Worman, A., Johansson, H., and Lindahl, A. (2007). Controlling factors for water residence time and flow patterns in Ekeby treatment wetland, Sweden. *Adv. Water Resour.* 30, 838.
- Knight, R. L. (1987). Effluent distribution and basin design for enhanced pollutant assimilation by freshwater wetlands. In K. R. Reddy and W. H. Smith, Eds., *Aquatic plants for water treatment and resource recovery*. Orland, FL, USA: Magnolia Publishing Company, p. 913.
- Knight, R. L., Kadlec, R. H., and Ohlendorf, H. M. (1999). The use of treatment wetlands for petroleum industry effluents. *Environ. Sci. Technol.* 33, 973.
- Kohn, T., and Nelson, K. L. (2007). Sunlight-mediated inactivation of MS2 coliphage via exogenous singlet oxygen produced by sensitizers in natural waters. *Environ. Sci. Technol.* 41, 192.
- Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B., and Buxton, H. T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Env. Sci Technol* 36, 1202.
- Kruger, E. L., Coats, J. R., and Zhu, B. E. (1996). Relative mobilities of atrazine, five atrazine degradates, metolachlor, and simazine in soils of Iowa. *Environ. Toxicol. Chem.* 15, 691.
- Kryger, J., and Riisgård, H. U. (1988). Filtration rate capacities in 6 species of European freshwater bivalves. *Oecologia* 77, 34.
- Ksoll, W. B., Ishii, S., Sadowsky, M. J., and Hicks, R. E. (2007). Presence and sources of fecal coliform bacteria in epilithic periphyton communities of Lake Superior. *Appl. Environ. Microbiol.* 73, 3771.
- Kunkel, U., and Radke, M. (2011). Reactive tracer test to evaluate the fate of pharmaceuticals in rivers. *Env. Sci Technol* 45, 6296.
- Kyambadde, J., Kansime, F., Gumaelius, L., and Dalhammar, G. (2004). A comparative study of *Cyperus papyrus* and *Miscanthidium violaceum*-based constructed wetlands for wastewater treatment in a tropical climate. *Water Res.* 38, 475.
- De Laat, J., Maouala-Makata, P., and Dore, M. (1996). Rate constants for reactions of ozone and hydroxyl radicals with several phenyl-ureas and acetamides. *Environ. Technol.* 17, 707.

- Lam, M. W., and Mabury, S. A. (2005). Photodegradation of the pharmaceuticals atorvastatin, carbamazepine, levofloxacin, and sulfamethoxazole in natural waters. *Aquat. Sci.* 67, 177.
- Lam, M. W., Tantuco, K., and Mabury, S. A. (2003). PhotoFate: A new approach in accounting for the contribution of indirect photolysis of pesticides and pharmaceuticals in surface waters. *Env. Sci Technol* 37, 899.
- Larson, R. A., and Zepp, R. G. (1988). Reactivity of the carbonate radical with aniline derivatives. *Environ. Toxicol. Chem.* 7, 265.
- Latch, D. E., Packer, J. L., Arnold, W. A., and McNeill, K. (2003a). Photochemical conversion of triclosan to 2,8-dichlorodibenzo-p-dioxin in aqueous solution. *J. Photochem. Photobiol. Chem.* 158, 63.
- Latch, D. E., Stender, B. L., Packer, J. L., Arnold, W. A., and McNeill, K. (2003b). Photochemical fate of pharmaceuticals in the environment: Cimetidine and ranitidine. *Env. Sci Technol* 37, 3342.
- Lee, C., Choi, W., and Yoon, J. (2005). UV Photolytic Mechanism of N-Nitrosodimethylamine in Water. *Env. Sci Technol* 39, 9702.
- Leech, D. M., Snyder, M. T., and Wetzel, R. G. (2009). Natural organic matter and sunlight accelerate the degradation of 17 β -estradiol in water. *Sci. Total Environ.* 407, 2087.
- Li, F., Yuasa, A., Obara, A., and Mathews, A. P. (2005). Aerobic batch degradation of 17- β estradiol (E2) by activated sludge. *Water Res.* 39, 2065.
- Li, X.-N., Song, H.-L., Li, W., Lu, X.-W., and Nishimura, O. (2010). An integrated ecological floating-bed employing plant, freshwater clam and biofilm carrier for purification of eutrophic water. *Ecol. Eng.* 36, 382.
- Li, Y., Zhu, G., Ng, W. J., and Tan, S. K. (2014). A review on removing pharmaceutical contaminants from wastewater by constructed wetlands. *Sci. Total Environ.* 468–469, 908.
- Lightbody, A. F., Avenier, M. E., and Nepf, H. M. (2008). Observations of short-circuiting flow paths within a free-surface wetland in Augusta, Georgia, USA. *Limnol. Oceanogr.* 53, 1040.
- Lightbody, A. F., Nepf, H. M., and Bays, J. S. (2007). Mixing in deep zones within constructed treatment wetlands. *Ecol. Eng.* 29, 209.
- Lightbody, A. F., Nepf, H. M., and Bays, J. S. (2009). Modeling the hydraulic effect of transverse deep zones on the performance of short-circuiting constructed treatment wetlands. *Ecol. Eng.* 35, 754.
- Ligi, T., Truu, M., Truu, J., Nõlvak, H., Kaasik, A., Mitsch, W. J., and Mander, Ü. (n.d.). Effects of soil chemical characteristics and water regime on denitrification genes (nirS, nirK, and nosZ) abundances in a created riverine wetland complex. *Ecol. Eng.*
- Lim, M.-H., Snyder, S. A., and Sedlak, D. L. (2008). Use of biodegradable dissolved organic carbon (BDOC) to assess the potential for transformation of wastewater-derived contaminants in surface waters. *Water Res.* 42, 2943.
- Lin, A. Y.-C., Debroux, J.-F., Cunningham, J. A., and Reinhard, M. (2003). Comparison of rhodamine WT and bromide in the determination of hydraulic characteristics of constructed wetlands. *Ecol. Eng.* 20, 75.
- Lin, A. Y.-C., Plumlee, M. H., and Reinhard, M. (2006). Natural attenuation of pharmaceuticals and alkylphenol polyethoxylate metabolites during river transport: Photochemical and biological transformation. *Environ. Toxicol. Chem.* 25, 1458.
- Lin, A. Y.-C., and Reinhard, M. (2005). Photodegradation of common environmental pharmaceuticals and estrogens in river water. *Environ. Toxicol. Chem.* 24, 1303.

- Lin, W.-C., Chen, H.-C., and Ding, W.-H. (2005). Determination of pharmaceutical residues in waters by solid-phase extraction and large-volume on-line derivatization with gas chromatography–mass spectrometry. *J. Chromatogr. A* 1065, 279.
- Llorens, E., Matamoros, V., Domingo, V., Bayona, J. M., and García, J. (2009). Water quality improvement in a full-scale tertiary constructed wetland: Effects on conventional and specific organic contaminants. *Sci. Total Environ.* 407, 2517.
- Loraine, G. A., and Pettigrove, M. E. (2005). Seasonal variations in concentrations of pharmaceuticals and personal care products in drinking water and reclaimed wastewater in Southern California. *Environ. Sci. Technol.* 40, 687.
- Lu, J., Huang, Q., and Mao, L. (2009). Removal of acetaminophen using enzyme-mediated oxidative coupling processes: I. Reaction rates and pathways. *Environ. Sci. Technol.* 43, 7062.
- MacIntyre, M. E., Warner, B. G., and Slawson, R. M. (2006). *Escherichia coli* control in a surface flow treatment wetland. *J. Water Health* 4, 211.
- Malaviya, P., and Singh, A. (2012). Constructed wetlands for management of urban stormwater runoff. *Crit. Rev. Environ. Sci. Technol.* 42, 2153.
- Mandi, L., Bouhoum, K., and Ouazzani, N. (1998). Application of constructed wetlands for domestic wastewater treatment in an arid climate. *Water Sci. Technol.* 38, 379.
- Mandi, L., Houhoum, B., Asmama, S., and Schwartzbrod, J. (1996). Wastewater treatment by reed beds. *Water Res.* 30, 2009.
- Martinez, C. J., and Wise, W. R. (2003). Hydraulic analysis of Orlando Easterly wetland. *J. Environ. Eng.* 129, 553.
- Matamoros, V., Arias, C., Brix, H., and Bayona, J. M. (2007). Removal of Pharmaceuticals and Personal Care Products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter. *Env. Sci Technol* 41, 8171.
- Matamoros, V., Arias, C., Brix, H., and Bayona, J. M. (2009). Preliminary screening of small-scale domestic wastewater treatment systems for removal of pharmaceutical and personal care products. *Water Res.* 43, 55.
- Matamoros, V., and Bayona, J. M. (2006). Elimination of Pharmaceuticals and Personal Care Products in Subsurface Flow Constructed Wetlands. *Env. Sci Technol* 40, 5811.
- Matamoros, V., and Bayona, J. M. (2008). Behavior of emerging pollutants in constructed wetlands. In D. Barceló and M. Petrovic, Eds., *Emerging Contaminants from Industrial and Municipal Waste*. Springer Berlin/Heidelberg, p. 199.
- Matamoros, V., Caselles-Osorio, A., García, J., and Bayona, J. M. (2008a). Behaviour of pharmaceutical products and biodegradation intermediates in horizontal subsurface flow constructed wetland. A microcosm experiment. *Sci. Total Environ.* 394, 171.
- Matamoros, V., García, J., and Bayona, J. M. (2005). Behavior of selected pharmaceuticals in subsurface flow constructed wetlands: A pilot-scale study. *Env. Sci Technol* 39, 5449.
- Matamoros, V., García, J., and Bayona, J. M. (2008b). Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. *Water Res.* 42, 653.
- McAvoy, D. C., Schatowitz, B., Jacob, M., Hauk, A., and Eckhoff, W. S. (2002). Measurement of triclosan in wastewater treatment systems. *Environ. Toxicol. Chem.* 21, 1323.

- McIvor, A. L. (2004). Freshwater mussels as biofilters. Doctoral. United Kingdom: Pembroke College.
- Meltz Steinberg, K., and Levin, B. R. (2007). Grazing protozoa and the evolution of the Escherichia coli O157. *Proc. R. Soc. B Biol. Sci.* 274, 1921.
- Mendelssohn, I. A., Kleiss, B. A., and Wakeley, J. S. (1995). Factors controlling the formation of oxidized root channels. *Wetlands* 15, 37.
- Metar. (2013). Weather history for Livermore, CA.
- Metcalf & Eddy. (2003). *Wastewater Engineering: Treatment and Reuse*, 4th edition. Crawfordsville, IN: McGraw-Hill.
- Metcalf, C. D., Miao, X.-S., Koenig, B. G., and Struger, J. (2003). Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lakes, Canada. *Environ. Toxicol. Chem.* 22, 2881.
- Miller, P. L., and Chin, Y.-P. (2002). Photoinduced degradation of carbaryl in a wetland surface water. *J Agric Food Chem* 50, 6758.
- Miller, P. L., and Chin, Y.-P. (2005). Indirect photolysis promoted by natural and engineered wetland water constituents: Processes leading to alachlor degradation. *Env. Sci Technol* 39, 4454.
- Mitch, W. A., Sharp, J. O., Trussell, R. R., Valentine, R. L., Alvarez-Cohen, L., and Sedlak, D. L. (2003). N-nitrosodimethylamine (NDMA) as a drinking water contaminant: A review. *Environ. Eng. Sci.* 20, 389.
- Mitsch, W. J., and Gosselink, J. G. (2007). *Wetlands*, 4th edition. New York: John Wiley & Sons, Inc.
- Møhlenberg, F., and Riisgård, H. U. (1979). Filtration rate, using a new indirect technique, in thirteen species of suspension-feeding bivalves. *Mar. Biol.* 54, 143.
- Moore, M. T., Rodgers Jr., J. H., Cooper, C. M., and Smith Jr., S. (2000). Constructed wetlands for mitigation of atrazine-associated agricultural runoff. *Environ. Pollut.* 110, 393.
- Moore, M. T., Schulz, R., Cooper, C. M., Smith Jr., S., and Rodgers Jr., J. H. (2002). Mitigation of chlorpyrifos runoff using constructed wetlands. *Chemosphere* 46, 827.
- Münch, C., Neu, T., Kusch, P., and Röske, I. (2007). The root surface as the definitive detail for microbial transformation processes in constructed wetlands--a biofilm characteristic. *Water Sci. Technol.* 56, 271.
- Nałecz-Jawecki, G., Wójcik, T., and Sawicki, J. (2008). Evaluation of in vitro biotransformation of propranolol with HPLC, MS/MS, and two bioassays. *Environ. Toxicol.* 23, 52.
- Nappier, S. P., Graczyk, T. K., and Schwab, K. J. (2008). Bioaccumulation, retention, and depuration of enteric viruses by Crassostrea virginica and Crassostrea ariakensis oysters. *Appl. Environ. Microbiol.* 74, 6825.
- National Research Council. (2012). *Water Reuse*. Washington, D.C.: The National Academies Press.
- Nelson, E. D., Do, H., Lewis, R. S., and Carr, S. A. (2011). Diurnal variability of pharmaceutical, personal care product, estrogen and alkylphenol concentrations in effluent from a tertiary wastewater treatment facility. *Environ. Sci. Technol.* 45, 1228.
- Neta, P., Huie, R. E., and Ross, A. B. (1988). Rate constants for reactions of inorganic radicals in aqueous solution. *J. Phys. Chem. Ref. Data* 17, 1027.

- Nguyen, M. T., Jasper, J. T., and Nelson, K. L. (2014). Photoinactivation of bacterial indicator organisms in open-water treatment wetlands In preparation.
- Nikolaou, A., Meric, S., and Fatta, D. (2007). Occurrence patterns of pharmaceuticals in water and wastewater environments. *Anal. Bioanal. Chem.* 387, 1225.
- NOAA. (2011). Ribbed mussels offer possible natural way to improve urban water quality.
- O'Rourke, S., Drouillard, K. G., and Haffner, G. D. (2004). Determination of laboratory and field elimination rates of polychlorinated biphenyls (PCBs) in the freshwater mussel, *Elliptio complanata*. *Arch. Environ. Contam. Toxicol.* 47, 74.
- Odum, H. T. (1956). Primary production in flowing waters. *Limnol. Oceanogr.* 1, 102.
- Orange County Water District. (2008). Prado Wetlands.
- Oulton, R. L., Kohn, T., and Cwiertny, D. M. (2010). Pharmaceuticals and personal care products in effluent matrices. *J. Environ. Monit.* 12, 1956.
- Packer, J. L., Werner, J. J., Latch, D. E., McNeill, K., and Arnold, W. A. (2003). Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen. *Aquat. Sci.* 65, 342.
- Park, N., Vanderford, B. J., Snyder, S. A., Sarp, S., Kim, S. D., and Cho, J. (2009). Effective controls of micropollutants included in wastewater effluent using constructed wetlands under anoxic condition. *Ecol. Eng.* 35, 418.
- Patrauchan, M. A., Miyazawa, D., LeBlanc, J. C., Aiga, C., Florizone, C., Dosanjh, M., Davies, J., Eltis, L. D., and Mohn, W. W. (2012). Proteomic analysis of survival of *Rhodococcus jostii* RHA1 during carbon starvation. *Appl. Environ. Microbiol.* 78, 6714.
- Patton, C. J., and Kryskalla, J. R. (2003). Evaluation of alkaline persulfate digestion as an alternative to Kjeldahl digestion for determination of total and dissolved nitrogen and phosphorus in water. Water-Resources Investigations Report. U.S. Geological Survey.
- Pearson, W. R., Wood, T., Zhang, Z., and Miller, W. (1997). Comparison of DNA sequences with protein sequences. *Genomics* 46, 24.
- Pérez, S., Eichhorn, P., and Aga, D. S. (2005). Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. *Environ. Toxicol. Chem.* 24, 1361.
- Persson, J. (2000). The hydraulic performance of ponds of various layouts. *Urban Water* 2, 243.
- Peven, C. S., Uhler, A. D., and Querzoli, F. J. (1996). Caged mussels and semipermeable membrane devices as indicators of organic contaminant uptake in dorchester and duxbury bays. *Environ. Toxicol. Chem.* 15, 144.
- Pinney, M. L., Westerhoff, P. K., and Baker, L. (2000). Transformations in dissolved organic carbon through constructed wetlands. *Water Res.* 34, 1897.
- Plósz, B. G., Leknes, H., and Thomas, K. V. (2010). Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in municipal wastewater treatment. *Environ. Sci. Technol.* 44, 734.
- Pocernich, M., and Litke, D. W. (1997). Nutrient concentrations in wastewater treatment plant effluents, South Platte River basin. *J. Am. Water Resour. Assoc.* 33, 205.

- Pollard, P. C. (2010). Bacterial activity in plant (*Schoenoplectus validus*) biofilms of constructed wetlands. *Water Res.* 44, 5939.
- Pontius, F. W. (1993). Legislation/Regulation -- Nitrate and Cancer. *J. Am. Water Works Assoc.* 85.
- Porter, A. J. (2011). Microbial community function in freshwater wetland soils: Using extracellular enzyme analysis to study the effect of moisture and vegetation. Masters. Richmond, Virginia: Virginia Commonwealth University.
- Proakis, E. (2003). Pathogen removal in constructed wetlands focusing on biological predation and marine recreational water quality. *Proc. Water Environ. Fed.* 31-40, 310.
- Prochaska, C. A., and Zouboulis, A. I. (2006). Removal of phosphates by pilot vertical-flow constructed wetlands using a mixture of sand and dolomite as substrate. *Ecol. Eng.* 26, 293.
- Pruell, R. J., Lake, J. L., Davis, W. R., and Quinn, J. G. (1986). Uptake and depuration of organic contaminants by blue mussels (*Mytilus edulis*) exposed to environmentally contaminated sediment. *Mar. Biol.* 91, 497.
- Purdum, C. E., Hardiman, P. A., Bye, V. V. J., Eno, N. C., Tyler, C. R., and Sumpter, J. P. (1994). Estrogenic effects of effluents from sewage treatment works. *Chem. Ecol.* 8, 275.
- Quiñónez-Díaz, M. J., Karpiscak, M. M., Ellman, E. D., and Gerba, C. P. (2001). Removal of pathogenic and indicator microorganisms by a constructed wetland receiving untreated domestic wastewater. *J. Environ. Sci. Health Part A Tox. Hazard. Subst. Environ. Eng.* 36, 1311.
- Radjenović, J., Pérez, S., Petrović, M., and Barceló, D. (2008). Identification and structural characterization of biodegradation products of atenolol and glibenclamide by liquid chromatography coupled to hybrid quadrupole time-of-flight and quadrupole ion trap mass spectrometry. *J. Chromatogr. A* 1210, 142.
- Radjenović, J., Petrović, M., and Barceló, D. (2009). Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water Res.* 43, 831.
- Radke, M., Lauwigi, C., Heinkele, G., Mürdter, T. E., and Letzel, M. (2009). Fate of the antibiotic sulfamethoxazole and its two major human metabolites in a water sediment test. *Environ. Sci. Technol.* 43, 3135.
- Rahaman, M. S., Mavinic, D. S., Bhuiyan, M. I. H., and Koch, F. A. (2006). Exploring the determination of struvite solubility product from analytical results. *Environ. Technol.* 27, 951.
- Ramil, M., El Aref, T., Fink, G., Scheurer, M., and Ternes, T. A. (2009). Fate of beta blockers in aquatic-sediment systems: Sorption and biotransformation. *Env. Sci Technol* 44, 962.
- Reddy, K. R., and D'Angelo, E. M. (1997). Biogeochemical indicators to evaluate pollutant removal efficiency in constructed wetlands. *Water Sci. Technol.* 35, 1.
- Redfield, A. C. (1934). *On the proportions of organic derivatives in sea water and their relation to the composition of plankton*. University Press of Liverpool.
- Redfield, A. C., Ketchum, B. H., and Richards, F. A. (1963). The influence of organisms on the composition of seawater. In M. N. Hill, Ed., *The Sea Volume 2*. New York: Inter Science Publishers, p. 26.
- Reed, S. H., Crites, R. W., and Middlebrooks, E. J. (1995). *Natural systems for waste management and treatment*, 2nd edition. New York: McGraw-Hill.
- Reichenberger, S., Bach, M., Skitschak, A., and Frede, H.-G. (2007). Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review. *Sci. Total Environ.* 384, 1.

- Reinhold, D. M., Vishwanathan, S., Park, J. J., Oh, D., and Michael Saunders, F. (2010). Assessment of plant-driven removal of emerging organic pollutants by duckweed. *Chemosphere* 80, 687.
- Reinoso, R., Torres, L. A., and Bécares, E. (2008). Efficiency of natural systems for removal of bacteria and pathogenic parasites from wastewater. *Sci. Total Environ.* 395, 80.
- Remucal, C. K. (2014). The role of indirect photochemical degradation in the environmental fate of pesticides: A review. *Environ. Sci. Process. Impacts.*
- Reyes-Contreras, C., Matamoros, V., Ruiz, I., Soto, M., and Bayona, J. M. (2011). Evaluation of PPCPs removal in a combined anaerobic digester-constructed wetland pilot plant treating urban wastewater. *Chemosphere* 84, 1200.
- Rier, S. T., Kuehn, K. A., and Francoeur, S. N. (2007). Algal regulation of extracellular enzyme activity in stream microbial communities associated with inert substrata and detritus. *J. North Am. Benthol. Soc.* 26, 439.
- Riisgård, H. U. (2001). On measurement of filtration rates in bivalves--the stony road to reliable data: Review and interpretation. *Mar. Ecol. Prog. Ser.* 211, 275.
- Risgaard-Petersen, N., Rysgaard, S., Nielsen, L. P., and Revsbech, N. P. (1994). Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnol. Oceanogr.* 39, 573.
- Roberts, P. H., and Thomas, K. V. (2006). The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. *Sci. Total Environ.* 356, 143.
- Romani, A. M., Guasch, H., Muñoz, I., Ruana, J., Vilalta, E., Schwartz, T., Emtiazi, F., and Sabater, S. (2004). Biofilm structure and function and possible implications for riverine DOC dynamics. *Microb. Ecol.* 47, 316.
- Rosario-Ortiz, F. L., Mezyk, S. P., Doud, D. F. R., and Snyder, S. A. (2008). Quantitative correlation of absolute hydroxyl radical rate constants with non-isolated effluent organic matter bulk properties in water. *Env. Sci Technol* 42, 5924.
- Ryan, C. C., Tan, D. T., and Arnold, W. A. (2011). Direct and indirect photolysis of sulfamethoxazole and trimethoprim in wastewater treatment plant effluent. *Water Res.* 45, 1280.
- Sah, R. N., and Miller, R. O. (1992). Spontaneous reaction for acid dissolution of biological tissues in closed vessels. *Anal. Chem.* 64, 230.
- Schnell, S., Bols, N. C., Barata, C., and Porte, C. (2009). Single and combined toxicity of pharmaceuticals and personal care products (PPCPs) on the rainbow trout liver cell line RTL-W1. *Aquat. Toxicol.* 93, 244.
- Schwab, B. W., Hayes, E. P., Fiori, J. M., Mastrocco, F. J., Roden, N. M., Cragin, D., Meyerhoff, R. D., D'Aco, V. J., and Anderson, P. D. (2005). Human pharmaceuticals in US surface waters: A human health risk assessment. *Regul. Toxicol. Pharmacol.* 42, 296.
- Schwarzenbach, R. P., Gschwend, P. M., and Imboden, D. M. (2003). *Environmental Organic Chemistry*, 2nd edition. Hoboken, NJ: Wiley.
- Sedlak, D. L., and Pinkston, K. E. (2001). Factors affecting the concentrations of pharmaceuticals released to the aquatic environment. *Water Resour. Update*, 55.
- Sengupta, M. E., Thamsborg, S. M., Andersen, T. J., Olsen, A., and Dalsgaard, A. (2011). Sedimentation of helminth eggs in water. *Water Res.* 45, 4651.
- Shackle, V. J., Freeman, C., and Reynolds, B. (2000). Carbon supply and the regulation of enzyme activity in constructed wetlands. *Soil Biol. Biochem.* 32, 1935.

- Shao, M.-F., Zhang, T., and Fang, H. H.-P. (2010). Sulfur-driven autotrophic denitrification. *Appl. Microbiol. Biotechnol.* 88, 1027.
- Sharp, J. O., Sales, C. M., LeBlanc, J. C., Liu, J., Wood, T. K., Eltis, L. D., Mohn, W. W., and Alvarez-Cohen, L. (2007). An inducible propane monooxygenase is responsible for N-nitrosodimethylamine degradation by *Rhodococcus* sp. strain RHA1. *Appl. Environ. Microbiol.* 73, 6930.
- Sherwood, A. R., and Presting, G. G. (2007). Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. *J. Phycol.* 43, 605.
- Shiga, T., Fujimura, A., Tateishi, T., Ohashi, K., and Ebihara, A. (1993). Differences of chronopharmacokinetic profiles between propranolol and atenolol in hypertensive subjects. *J. Clin. Pharmacol.* 33, 756.
- Shilton, A., and Harrison, J. (2003). Guidelines for the hydraulic design of waste stabilisation ponds website. Massey University, New Zealand.
- Shipin, O., Koottatep, T., Khanh, N. T. T., and Polprasert, C. (2005). Integrated natural treatment systems for developing communities. *Water Sci. Technol.* 51, 299.
- Shpigel, M., Gasith, A., and Kimmel, E. (1997). A biomechanical filter for treating fish-pond effluents. *Aquaculture* 152, 103.
- Silverman, A. I., Nguyen, M. T., Schilling, I., Wenk, J., and Nelson, K. L. (2014). Sunlight inactivation of viruses in an open-water natural wastewater treatment system: Modeling endogenous and exogenous inactivation rates in the presence of light-attenuating compounds and photosensitizers In preparation.
- Silverman, H., Achberger, E. C., Lynn, J. W., and Dietz, T. H. (1995). Filtration and utilization of laboratory-cultured bacteria by *Dreissena polymorpha*, *Corbicula fluminea*, and *Carunculina texasensis*. *Biol. Bull.* 189, 308.
- Sinsabaugh, R. L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol. Biochem.* 42, 391.
- Sirivedhin, T., and Gray, K. A. (2006). Factors affecting denitrification rates in experimental wetlands. *Ecol. Eng.* 26, 167.
- Sládečková, A., Marvan, P., and Vymazal, J. (1983). The utilization of periphyton in waterworks pre-treatment for nutrient removal from enriched influents. In R. G. Wetzel, Ed., *Periphyton of Freshwater Ecosystems*. Springer Netherlands, p. 299.
- Snyder, S. A., Adham, S., Redding, A. M., Cannon, F. S., DeCarolis, J., Oppenheimer, J., Wert, E. C., and Yoon, Y. (2007). Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. *Desalination* 202, 156.
- Snyder, S. A., Wert, E. C., Rexing, D. J., Zegers, R. E., and Drury, D. D. (2006). Ozone-Sci. Eng. *Ozone Sci. Amp Eng.* 28, 445.
- Snyder, S. A., Westerhoff, P., Yoon, Y., and Sedlak, D. L. (2003). Pharmaceuticals, personal care products, and endocrine disruptors in water: Implications for the water industry. *Environ. Eng. Sci.* 20, 449.
- Solano, M. L., Soriano, P., and Ciria, M. P. (2004). Constructed wetlands as a sustainable solution for wastewater treatment in small villages. *Biosyst. Eng.* 87, 109.
- Song, H.-L., Yang, X.-L., Nakano, K., Nomura, M., Nishimura, O., and Li, X.-N. (2011). Elimination of estrogens and estrogenic activity from sewage treatment works effluents in subsurface and surface flow constructed wetlands. *Int. J. Environ. Anal. Chem.* 91, 600.

Song, Z., Sun, Q., Yu, M., Zhou, Y., Kong, X., and Zhao, Y. (2010). Seasonal variation and correlation of *Escherichia coli* and *Salmonellae* in a full-scale constructed wetland for wastewater treatment in China, presented at the 2010 4th International Conference on Bioinformatics and Biomedical Engineering (iCBBE).

Song, Z. W., Wu, L., Yang, G., Xu, M., and Wen, S. P. (2008). Indicator microorganisms and pathogens removal function performed by copepods in constructed wetlands. *Bull. Environ. Contam. Toxicol.* 81, 459.

Søvik, A. K., Augustin, J., Heikkinen, K., Huttunen, J. T., Necki, J. M., Karjalainen, S. M., Kløve, B., Liikanen, A., Mander, ü., Puustinen, M., Teiter, S., and Wachniew, P. (2006). Emission of the greenhouse gases nitrous oxide and methane from constructed wetlands in Europe. *J. Environ. Qual.* 35, 2360.

Spalding, R. F., and Exner, M. E. (1993). Occurrence of nitrate in groundwater—A review. *J. Environ. Qual.* 22, 392.

SRC. (2012). Interactive PhysProp Database.

Stein, O. R., and Hook, P. B. (2005). Temperature, Plants, and Oxygen. *J. Environ. Sci. Health Part A* 40, 1331.

Stott, R., May, E., Matsushita, E., and Warren, A. (2001). Protozoan predation as a mechanism for the removal of cryptosporidium oocysts from wastewaters in constructed wetlands. *Water Sci. Technol.* 44, 191.

Stott, R., and Tanner, C. C. (2005). Influence of biofilm on removal of surrogate faecal microbes in a constructed wetland and maturation pond. *Water Sci. Technol.* 51, 315.

Stuer-Lauridsen, F., Birkved, M., Hansen, L. P., Holten Lützhøft, H.-C., and Halling-Sørensen, B. (2000). Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere* 40, 783.

Suárez, S., Carballa, M., Omil, F., and Lema, J. (2008). How are pharmaceutical and personal care products (PPCPs) removed from urban wastewaters? *Rev. Environ. Sci. Biotechnol.* 7, 125.

Tao, W., Hall, K. J., and Ramey, W. (2007). Effects of influent strength on microorganisms in surface flow mesocosm wetlands. *Water Res.* 41, 4557.

Tao, W., Wen, J., Han, Y., and Huchzermeier, M. (2012). Nitrogen removal in constructed wetlands using nitrification/anammox and nitrification/ denitrification. *Water Environ. Res.* 84, 2099.

Ternes, T. A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., and Joss, A. (2004a). A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. *Water Res.* 38, 4075.

Ternes, T. A., Joss, A., and Siegrist, H. (2004b). Peer reviewed: Scrutinizing pharmaceuticals and personal care products in wastewater treatment. *Env. Sci Technol* 38, 392A.

Ternes, T. A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M., and Teiser, B. (2003). Ozonation. *Water Res.* 37, 1976.

Thamdrup, B., and Dalsgaard, T. (2002). Production of N₂ through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Appl. Environ. Microbiol.* 68, 1312.

The Department of the Environment, Maryland. (2014). The Evolution to Enhanced Nutrient Removal Technology.

Thomas, T. (1998). Occurrence of drugs in German sewage treatment plants and rivers. *Water Res.* 32, 3245.

- Thomas, V., McDonnell, G., Denyer, S. P., and Maillard, J. (2010). Free-living amoebae and their intracellular pathogenic microorganisms. *FEMS Microbiol. Rev.* 34, 231.
- Throbäck, I. N., Enwall, K., Jarvis, Å., and Hallin, S. (2004). Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol. Ecol.* 49, 401.
- Thullen, J. S., Sartoris, J. J., and Nelson, S. M. (2005). Managing vegetation in surface-flow wastewater-treatment wetlands for optimal treatment performance. *Ecol. Eng.* 25, 583.
- Tixier, C., Singer, H. P., Oellers, S., and Müller, S. R. (2003). Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environ. Sci. Technol.* 37, 1061.
- Tolls, J. (2001). Sorption of veterinary pharmaceuticals in soils: A review. *Env. Sci. Technol.* 35, 3397.
- Toze, S. (2006). Water reuse and health risks — real vs. perceived. *Desalination* 187, 41.
- Tratnyek, P. G., and Hoigné, J. (1991). Oxidation of substituted phenols in the environment. *Env. Sci. Technol.* 25, 1596.
- Triebkorn, R., Casper, H., Scheil, V., and Schwaiger, J. (2007). Ultrastructural effects of pharmaceuticals (carbamazepine, clofibric acid, metoprolol, diclofenac) in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). *Anal. Bioanal. Chem.* 387, 1405.
- Tront, J. M., Reinhold, D. M., Bragg, A. W., and Saunders, F. M. (2007). Uptake of halogenated phenols by aquatic plants. *J. Environ. Eng.* 133, 955.
- Trout, J. M., Walsh, E. J., and Fayer, R. (2002). Rotifers ingest giardia cysts. *J. Parasitol.* 88, 1038.
- Truu, M., Juhanson, J., and Truu, J. (2009). Microbial biomass, activity and community composition in constructed wetlands. *Sci. Total Environ.* 407, 3958.
- U.S. Environmental Protection Agency. (1993). Nitrogen Control Manual. *Office of Research and Development* EPA/625/R-93/010.
- U.S. Environmental Protection Agency. (2010). Chesapeake Bay Total Maximum Daily Load for Nitrogen, Phosphorus and Sediment. Section 6. Establishing the Allocations for the Basin-Jurisdictions.
- Verlicchi, P., and Zambello, E. (2014). How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? *Sci. Total Environ.* 470–471, 1281.
- Verrengia Guerrero, N. R., Taylor, M. G., Davies, N. A., Lawrence, M. A. M., Edwards, P. A., Simkiss, K., and Wider, E. A. (2002). Evidence of differences in the biotransformation of organic contaminants in three species of freshwater invertebrates. *Environ. Pollut.* 117, 523.
- Vilchez-Vargas, R., Junca, H., and Pieper, D. H. (2010). Metabolic networks, microbial ecology and ‘omics’ technologies. *Environ. Microbiol.* 12, 3089.
- Vymazal, J. (1995). *Algae and Element Cycling in Wetlands*. Boca Raton, FL: CRC Press.
- Vymazal, J. (2005). Removal of enteric bacteria in constructed treatment wetlands with emergent macrophytes: A review. *J. Environ. Sci. Health Part A Tox. Hazard. Subst. Environ. Eng.* 40, 1355.
- Vymazal, J. (2007). Removal of nutrients in various types of constructed wetlands. *Sci. Total Environ.* 380, 48.

Vymazal, J. (2009). The use constructed wetlands with horizontal sub-surface flow for various types of wastewater. *Ecol. Eng.* 35, 1.

Vymazal, J. (2010). Constructed wetlands for wastewater treatment: Five decades of experience. *Env. Sci Technol* 45, 61.

Waltman, E. L., Venables, B. J., and Waller, W. T. (2006). Triclosan in a North Texas wastewater treatment plant and the influent and effluent of an experimental constructed wetland. *Environ. Toxicol. Chem.* 25, 367.

Wang, L., Xu, H., Cooper, W. J., and Song, W. (2012). Photochemical fate of beta-blockers in NOM enriched waters. *Sci. Total Environ.* 426, 289.

Wang, N., and Mitsch, W. J. (2000). A detailed ecosystem model of phosphorus dynamics in created riparian wetlands. *Ecol. Model.* 126, 101.

Webb, S. F. (2004). A Data-based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals I — Collation of Available Ecotoxicity Data. In A. P. D. K. Kümmerer, Ed., *Pharmaceuticals in the Environment*. Springer Berlin Heidelberg, p. 317.

Wenk, J., Von Gunten, U., and Canonica, S. (2011). Effect of dissolved organic matter on the transformation of contaminants induced by excited triplet states and the hydroxyl radical. *Environ. Sci. Technol.* 45, 1334.

Wetzel, R. G. (1983). *Periphyton of freshwater ecosystems*. The Hague: Dr W. Junk Publishers.

Wick, A., Fink, G., Joss, A., Siegrist, H., and Ternes, T. A. (2009). Fate of beta blockers and psycho-active drugs in conventional wastewater treatment. *Water Res.* 43, 1060.

Winkler, M., Lawrence, J. R., and Neu, T. R. (2001). Selective degradation of ibuprofen and clofibric acid in two model river biofilm systems. *Water Res.* 35, 3197.

Winter, J. E. (1978). A review on the knowledge of suspension-feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. *Aquaculture* 13, 1.

Wörman, A., and Kronnäs, V. (2005). Effect of pond shape and vegetation heterogeneity on flow and treatment performance of constructed wetlands. *J. Hydrol.* 301, 123.

Wright, A. L., and Reddy, K. R. (2001). Phosphorous loading effects on extracellular enzyme activity in everglades wetland soils. *Soil Sci. Soc. Am. Journal* 65, 588.

Writer, J. H., Antweiler, R. C., Ferrer, I., Ryan, J. N., and Thurman, E. M. (2013). In-stream attenuation of neuro-active pharmaceuticals and their metabolites. *Environ. Sci. Technol.*

Writer, J. H., Barber, L. B., Ryan, J. N., and Bradley, P. M. (2011a). Biodegradation and attenuation of steroidal hormones and alkylphenols by stream biofilms and sediments. *Env. Sci Technol* 45, 4370.

Writer, J. H., Ryan, J. N., and Barber, L. B. (2011b). Role of biofilms in sorptive removal of steroidal hormones and 4-nonylphenol compounds from streams. *Env. Sci Technol* 45, 7275.

Wu, Y., He, J., and Yang, L. (2010). Evaluating adsorption and biodegradation mechanisms during the removal of microcystin-RR by periphyton. *Environ. Sci. Technol.* 44, 6319.

Xue, W., Wu, C., Xiao, K., Huang, X., Zhou, H., Tsuno, H., and Tanaka, H. (2010). Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation. *Water Res.* 44, 5999.

- Yamamoto, H., Nakamura, Y., Moriguchi, S., Nakamura, Y., Honda, Y., Tamura, I., Hirata, Y., Hayashi, A., and Sekizawa, J. (2009). Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments. *Water Res.* 43, 351.
- Zeng, T., and Arnold, W. A. (2012). Pesticide Photolysis in Prairie Potholes. *Environ. Sci. Technol.*
- Zepp, R. G., Hoigné, J., and Bader, H. (1987). Nitrate-induced photooxidation of trace organic chemicals in water. *Env. Sci Technol* 21, 443.
- Zepp, R. G., Wolfe, N. L., Baughman, G. L., and Hollis, R. C. (1977). Singlet oxygen in natural waters. *Nature* 267, 421.
- Zhang, D. Q., Gersberg, R. M., Hua, T., Zhu, J., Tuan, N. A., and Tan, S. K. (2012). Pharmaceutical removal in tropical subsurface flow constructed wetlands at varying hydraulic loading rates. *Chemosphere* 87, 273.
- Zhu, G., Wang, S., Feng, X., Fan, G., Jetten, M. S. M., and Yin, C. (2011). Anammox bacterial abundance, biodiversity and activity in a constructed wetland. *Environ. Sci. Technol.* 45, 9951.
- Zhu, G., Wang, S., Wang, W., Wang, Y., Zhou, L., Jiang, B., Op den Camp, H. J. M., Risgaard-Petersen, N., Schwark, L., Peng, Y., Hefting, M. M., Jetten, M. S. M., and Yin, C. (2013). Hotspots of anaerobic ammonium oxidation at land-freshwater interfaces. *Nat. Geosci.* 6, 103.
- Ziegmann, M., and Frimmel, F. H. (2010). Photocatalytic degradation of clofibric acid, carbamazepine and iomeprol using conglomerated TiO₂ and activated carbon in aqueous suspension. *Water Sci. Technol.* 61, 273.