

PhD Thesis

EFFECT OF DESIGN AND OPERATIONAL FACTORS ON THE REMOVAL EFFICIENCY OF EMERGING ORGANIC CONTAMINANTS IN CONSTRUCTED WETLANDS FOR WASTEWATER TREATMENT

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—¡Buenos días! —dijo el principito.

—¡Buenos días! —respondió el comerciante.

Era un comerciante de píldoras perfeccionadas que quitan la sed. Se toma una por semana y ya no se sienten ganas de beber.

—¿Por qué vendes eso? —preguntó el principito.

—Porque con esto se economiza mucho tiempo. Según el cálculo hecho por los expertos, se ahorran cincuenta y tres minutos por semana.

—¿Y qué se hace con esos cincuenta y tres minutos?

—Lo que cada uno quiere... "

"Si yo dispusiera de cincuenta y tres minutos —pensó el principito— caminaría suavemente hacia una fuente..."

El principito

Antoine de Saint Exupéry

Preface

The current thesis is framed within the context of two Spanish National Projects: the NEWWET project “Design criteria of constructed wetlands: new configurations for the removal of conventional and emerging pollutants in wastewater” (CTM-2008-06676) by the Spanish Ministry of Innovation and Science; and the project entitled “Integrated solution for the treatment of wastewater, stormwater and sludge in small communities through constructed wetland systems” by the Spanish Ministry of Environment (085/RN08/03.2). This research has been also partially supported by NaWaTech FP7 project (Grant Agreement N°: 308336). Cristina Àvila kindly acknowledges a predoctoral fellowship from the Universitat Politècnica de Catalunya-BarcelonaTech.

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List of acronyms

ACE	Acetaminophen
AHTN	Tonalide
AOPs	Advanced oxidation processes
BOD	Biochemical oxygen demand
BPA	Bisphenol A
COD	Chemical oxygen demand
C _u	Uniformity coefficient
CW	Constructed wetland
D ₁₀	Size of the sieve in which 10% of a gravel sample pass through
D ₆₀	Size of the sieve in which 60% of a gravel sample pass through
DC	Doxycycline
DCF	Diclofenac
DO	Dissolved oxygen
EC	Electrical conductivity
EDCs	Endocrine disrupting chemicals
EE2	17 α -ethinylestradiol
E _H	Redox potential
ENR	Enrofloxacin
EOCs	Emerging organic contaminants
EPA	Environmental Protection Agency
ETM	Erythromycin
FAO	Food and agriculture organization of the United Nations
FWS	Free water surface constructed wetland
GC-MS	Gas chromatography coupled to mass spectrometry
HF	Horizontal subsurface flow constructed wetland
HLR	Hydraulic loading rate
HRT	Hydraulic retention time
HUSB	Hydrolytic upflow sludge blanket reactor
IB	Ibuprofen
IT	Imhoff tank
K _{ow}	Octanol-water partition coefficient
LIN	Lincomycin
LOD	Limit of detection
LOQ	Limit of quantification
MXR	Multixenobiotic resistance
NH ₄ -N	Ammonium nitrogen
NO _x -N	Nitrate and nitrite nitrogen
NPX	Naproxen
NSAID	Non-steroidal anti-inflammatory drug
O&M	Operation and maintenance
OLR	Organic loading rate

OTC	Oxygen transfer capacity
OXY	Oxybenzone
PCPs	Personal care products
PE	Population equivalent
PPCPs	Pharmaceutical and personal care products
RSD	Relative standard deviation
SMZ	Sulfamethoxazole
SPE	Solid phase extraction
TCS	Triclosan
TKN	Total Kjeldahl nitrogen
TMSH	Trimethylsulfonium hydroxide
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket reactor
UNDP	United Nations development programme
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
VAp	Actively aerated saturated vertical subsurface flow wetland
VF	Vertical subsurface flow constructed wetland
VGp	Gravel-based vertical subsurface flow constructed wetland
VS1p	Sand-based vertical subsurface flow constructed wetland fed every hour
VS2p	Sand-based vertical subsurface flow constructed wetland fed every two hours
WHO	World Health Organization
WWTP	Wastewater treatment plant

Abstract

Water of quality constitutes a scarce and essential resource for life and public health, and its sustainable management is of crucial importance. For this reason wastewater treatment, reuse and reclamation represent a key practice in that approach. Nevertheless, there is a generalized concern about the occurrence and possible adverse effects of emerging organic contaminants such as pharmaceutical and personal care products in the environment, which in many occasions are not well removed in conventional wastewater treatment plants. To this regard, constructed wetlands have shown promise in their ability to remove a variety of these contaminants, and represent a good alternative for wastewater treatment in small communities, having low operational and maintenance expenses. However, there is a need in increasing the knowledge about the conditions that promote the removal of these compounds within this ecotechnology.

This thesis aims at evaluating the capacity of different wetland configurations (vertical subsurface flow, horizontal subsurface flow and free water surface wetlands), as well as the effect of various design and operational factors (primary treatment, operation strategy, loading frequency, grain size, use of active aeration, hydraulic loading rate) on the removal of a variety of emerging organic contaminants, including three non-steroidal anti-inflammatory drugs (ibuprofen, diclofenac and acetaminophen), three personal care products (tonalide, triclosan and oxybenzone) and two endocrine disrupting compounds (ethinylestradiol and bisphenol A). Several assays were carried out at experimental and pilot scale in constructed wetland systems in Barcelona and Seville (Spain), and in Leipzig (Germany). In order to achieve approximate steady state conditions of the influent concentrations of the target emerging organic contaminants and to obtain a more reliable estimate of the removal efficiency of the systems, continuous injection experiments were performed in the experimental-scale systems.

The elimination of target contaminants in horizontal subsurface flow wetlands exhibited a seasonal pattern, presumably due to enhanced biodegradation, volatilization and plant uptake at higher water temperatures. In this wetland type the use of a HUSB reactor -as opposed to a conventional settler- for the primary treatment of wastewater conferred more reduced conditions to the constructed wetland system, which resulted in reduced performance. Conversely, operating the horizontal wetlands in batch, alternating cycles of saturation and unsaturation, promoted the existence of higher redox conditions, which greatly enhanced the removal of target compounds. The identification of an intermediate product of bisphenol A in this treatment system (promoted by the operation in batch) suggests that aerobic biodegradation could constitute a principal removal mechanism of this substance when a higher redox status prevail.

In vertical flow wetlands the loading frequency (hourly vs. bi-hourly) showed significant differences on the removal of some compounds. This could be attributed to the lower contact time and reduced oxygen renewal at lower loading frequencies. Moreover, the occurrence of gravel (4-8 mm) as opposed to sand (1-3 mm) for the main bed media of vertical flow wetlands exhibited a significantly lower treatment performance. The smaller grain size of sand would result in smaller pores, providing a better filtering capacity, a higher surface area for biofilm growth and a longer contact time, and hence increased efficiency. Conversely, the use of active aeration in a saturated vertical flow wetland did not show to enhance contaminant removal in respect to the typical unsaturated vertical wetland type.

A hybrid treatment system based on a vertical wetland stage and a horizontal subsurface flow and free water surface wetlands in series has proved to be a very robust technology for wastewater treatment in small communities, producing a final effluent suitable for its reuse in various applications. Overall removal efficiency of emerging contaminants was very high ($90 \pm 11\%$), even under high hydraulic loads, presumably due to the combination and synergy of various abiotic/biotic removal mechanisms (e.g. biodegradation, sorption, volatilization, hydrolysis, photodegradation). Toxicological assays performed together with the injection of antibiotics during the highest hydraulic loading rate campaign showed that general toxicity, estrogenicity and dioxin-like activities were well removed along the different units of the treatment system.

Resumen

El agua constituye un recurso escaso y esencial para la vida y la salud pública, y por ende su gestión sostenible es de vital importancia. Por esta razón, el tratamiento del agua residual y su reutilización constituyen una práctica clave en este planteamiento. Sin embargo, existe una preocupación generalizada por la presencia y posibles efectos adversos de contaminantes emergentes orgánicos en el medio natural, tales como los fármacos y productos del cuidado personal, los cuales, en muchos casos, no son eliminados de manera eficiente en las plantas de tratamiento de agua residual convencionales. Los humedales construidos han demostrado ser eficientes en su capacidad para eliminar muchos de estos contaminantes, y representan una buena alternativa para el tratamiento de agua residual en pequeñas comunidades ya que suponen bajos costes de operación y mantenimiento. Sin embargo, existe poca información acerca de las condiciones (ya sean las características de diseño o las estrategias de operación) que promueven la eliminación de estos compuestos en esta ecotecnología.

Este trabajo de investigación tiene como objetivo evaluar la capacidad de diferentes configuraciones de humedales construidos (vertical de flujo subsuperficial, horizontal de flujo subsuperficial y de flujo superficial), así como el efecto de varios factores de diseño y funcionamiento (tratamiento primario, estrategia de operación, frecuencia de alimentación, granulometría, uso de aireación activa, carga hidráulica) en la eliminación de un conjunto de contaminantes emergentes orgánicos, incluyendo tres fármacos anti-inflamatorios no-esteroides (ibuprofeno, diclofenaco y acetaminofen), tres productos del cuidado personal (tonalide, triclosan y oxibenzona) y dos disruptores endocrinos (etinilestradiol y bisfenol A). Se realizaron varios ensayos a escala experimental y piloto en sistemas de humedales construidos en Barcelona y Sevilla (España), y en Leipzig (Alemania). Para lograr aproximar condiciones de estabilidad en las concentraciones de contaminantes emergentes orgánicos en el afluente, y obtener así una estimación más fiable de la eficiencia de eliminación de los sistemas, se realizaron experimentos de inyección continua en los sistemas a escala experimental.

La eliminación de los contaminantes estudiados en humedales horizontales de flujo subsuperficial exhibió un patrón estacional, posiblemente debido a una mayor biodegradación, volatilización e incorporación por las plantas a mayores temperaturas. En este tipo de humedal el uso de un reactor anaeróbico de flujo ascendente para el tratamiento primario -en vez de un decantador convencional- confirió condiciones más reducidas al sistema, lo cual promovió un menor rendimiento. En cambio, al operar el humedal horizontal en batch, alternando ciclos de saturación e insaturación, promovieron la existencia de mayores condiciones redox, lo cual mejoró notablemente la eliminación de los compuestos estudiados. La identificación de un intermedio de degradación del bisfenol A en este sistema de tratamiento (en la línea operando en batch) sugiere que la biodegradación aeróbica podría constituir un mecanismo predominante de eliminación de esta sustancia cuando prevalecen condiciones más oxidantes.

En humedales de flujo vertical, la frecuencia de alimentación (horaria/bihoraria) mostró diferencias significativas en la eliminación de algunos compuestos. Esto podría atribuirse a un menor tiempo de contacto y reducida renovación del oxígeno que se dan a frecuencias de alimentación menores. Además, la presencia de grava (4-8 mm) en lugar de arena (1-3 mm) en el lecho principal de los humedales verticales exhibió una eficiencia de eliminación significativamente menor. El tamaño más pequeño de la arena se traduce en poros más pequeños que proporcionan una mejor capacidad de filtración, un área superficial mayor para el crecimiento de biofilm y un tiempo de contacto mayor. Por el contrario, el uso de aireación activa en un humedal de flujo vertical saturado no demostró mejorar la eliminación de los contaminantes con respecto al tipo convencional insaturado.

Un sistema de tratamiento híbrido basado en una primera fase de humedal vertical y un humedal horizontal de flujo subsuperficial y uno de flujo superficial en serie, ha demostrado ser una tecnología muy robusta para el tratamiento de agua residual en pequeñas comunidades, produciendo un efluente final adecuado para su reutilización para varios fines. La eficiencia general de eliminación de contaminantes emergentes fue muy alta ($90 \pm 11\%$), incluso bajo cargas hidráulicas altas, posiblemente debido a la combinación y sinergia de varios mecanismos de eliminación abióticos/bióticos (e.g., biodegradación, sorción, volatilización, hidrólisis, fotodegradación). Ensayos toxicológicos realizados junto con las inyecciones de antibióticos durante la campaña de cargas hidráulicas más altas demostraron que la toxicidad general, estrogenicidad y actividades de tipo dioxina fueron eliminadas satisfactoriamente a lo largo de las diferentes unidades del sistema de tratamiento.

Resum

L'aigua constitueix un recurs escàs i essencial per a la vida i la salut pública, i per tant la seva gestió sostenible és de vital importància. Per aquesta raó, el tractament de l'aigua residual i la seva reutilització constitueixen una pràctica clau en aquest plantejament. No obstant això, hi ha una preocupació generalitzada per la presència i possibles efectes adversos de contaminants emergents orgànics en el medi natural, com ara els fàrmacs i productes de la cura personal, els quals, en molts casos, no són eliminats de manera eficient a les plantes de tractament d'aigua residual convencionals. Els aiguamolls construïts han demostrat ser eficients en la seva capacitat per eliminar molts d'aquests contaminants, i representen una bona alternativa per al tractament d'aigua residual en petites comunitats ja que suposen baixos costos d'operació i manteniment. No obstant això, hi ha poca informació sobre les condicions (ja siguin les característiques de disseny o de les estratègies d'operació) que promouen l'eliminació d'aquests compostos en aquesta ecotecnologia.

Aquest treball de recerca té com a objectiu avaluar la capacitat de diferents configuracions d'aiguamolls construïts (vertical de flux subsuperficial, horitzontal de flux subsuperficial i de flux superficial), així com l'efecte de diversos factors de disseny i funcionament (tractament primari, estratègia d'operació, freqüència d'alimentació, granulometria, ús de ventilació activa, càrrega hidràulica) en l'eliminació d'un conjunt de contaminants emergents orgànics, incloent tres fàrmacs antiinflamatoris no-esteroïdals (ibuprofè, diclofenac i acetaminofè), tres productes de la cura personal (tonalide, triclosan i oxibenzona) i dos disruptors endocrins (etinilestradiol i bisfenol A). Es van realitzar diversos assajos a escala experimental i pilot en sistemes d'aiguamolls construïts a Barcelona i Sevilla (Espanya), i a Leipzig (Alemanya). Per aconseguir aproximar condicions d'estabilitat en les concentracions de contaminants emergents orgànics en l'afluent, i obtenir així una estimació més fiable de l'eficiència d'eliminació dels sistemes, es van realitzar experiments d'injecció contínua en els sistemes a escala experimental.

L'eliminació dels contaminants estudiats en aiguamolls horitzontals de flux subsuperficial exhibeixen un patró estacional, possiblement a causa d'una major biodegradació, volatilització i incorporació per les plantes a majors temperatures. En aquest tipus d'aiguamoll l'ús d'un reactor anaerobi de flux ascendent per al tractament primari - en comptes d'un decantador convencional - conferir condicions més reduïdes al sistema, el qual va promoure un menor rendiment. En canvi, en operar l'aiguamoll horitzontal en batch, alternant cicles de saturació i insaturació, van promoure l'existència de majors condicions redox, la qual cosa va millorar notablement l'eliminació dels compostos estudiats. La identificació d'un intermedi de degradació del bisfenol A en aquest sistema de tractament (en la línia operant en batch) suggereix que la biodegradació aeròbica podria constituir un mecanisme predominant d'eliminació d'aquesta substància quan prevalen condicions més oxidants.

En aiguamolls de flux vertical, la freqüència d'alimentació (horària/bihorària) va mostrar diferències significatives en l'eliminació d'alguns compostos. Això es podria atribuir a un menor temps de contacte i reduïda renovació de l'oxigen que es donen a freqüències d'alimentació menors. A més, la presència de grava (4-8 mm) en lloc de sorra (1-3 mm) al llit principal dels aiguamolls verticals van exhibir una eficiència d'eliminació significativament menor. La mida més petita de la sorra es tradueix en porus més petits que proporcionen una millor capacitat de filtració, una àrea superficial més gran per al creixement de biofilm i un temps de contacte més gran. Per contra, l'ús de ventilació activa en un aiguamoll de flux vertical saturat no va demostrar millorar l'eliminació dels contaminants respecte al tipus convencional insaturat.

Un sistema de tractament híbrid basat en una primera fase de l'aiguamoll vertical i un aiguamoll horitzontal de flux subsuperficial i un flux superficial en sèrie, ha demostrat ser una tecnologia molt robusta per al tractament d'aigua residual en petites comunitats, produint un efluent final adequat per a la seva reutilització per a diversos fins. L'eficiència general d'eliminació de contaminants emergents va ser molt alta ($90 \pm 11\%$), fins i tot sota càrregues hidràuliques altes, possiblement a causa de la combinació i sinergia de diversos mecanismes d'eliminació abiòtics/biòtics (e.g. biodegradació, sorció, volatilització, hidròlisi, fotodegradació). Assaigs toxicològics realitzats juntament amb les injeccions d'antibiòtics durant la campanya de càrregues hidràuliques més altes van demostrar que la toxicitat general, estrogenicitat i activitats de tipus dioxina van ser eliminades satisfactòriament al llarg de les diferents unitats del sistema de tractament.

1. Introduction

Water constitutes the major part of planet Earth's surface (over 70%), giving it its blue appearance from space. However, only about 2.5 % of all water is freshwater, while the remaining is salt water. About two-thirds of this freshwater is contained in glaciers and permanent snow cover. The remaining available freshwater is unevenly distributed throughout the world (UNESCO, 2003). Although there is no global water scarcity as such, this is unevenly distributed and a lot of it is wasted, polluted, or unsustainably managed.

Almost one-fifth of the world's population (around 1.2 billion people) lives in areas where the water is physically scarce, while other 500 million people are approaching this scenario. Another 1.6 billion people live in developing countries, which suffer "economic" water shortage due to the lack of the necessary infrastructure to take water from rivers and aquifers. Additionally, 2.6 billion people nowadays lack basic sanitation. About 70% of those without sanitation live in rural areas, where 90% of open defecation takes place (UNDP, 2006; WHO-UNICEF, 2013).

Nowadays an increasing number of regions of the world have a chronic shortage of water (Fig. 1.1).

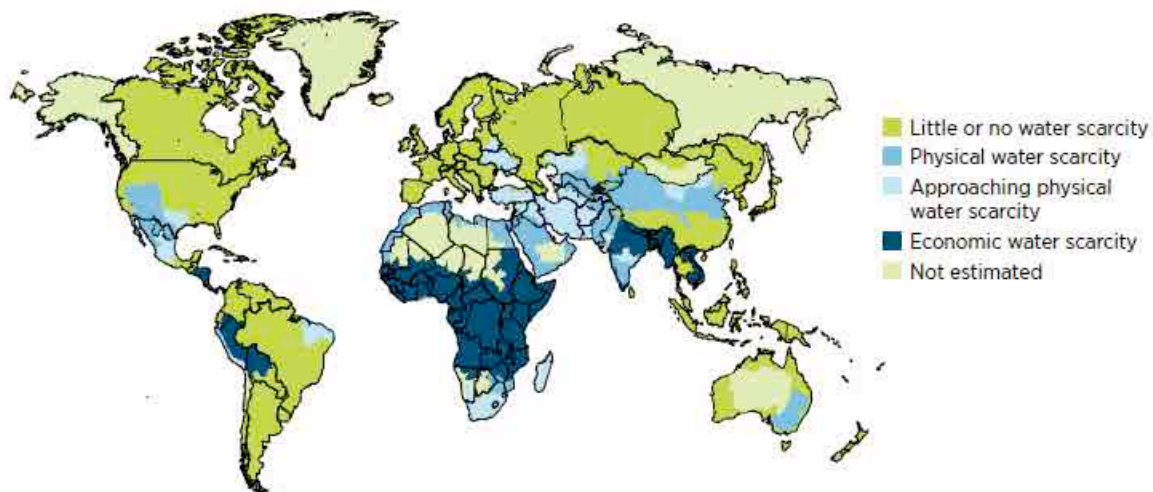


Figure 1.1. Global physical and economic water scarcity (Source: adaptation of UNESCO (2012) from: Comprehensive Assessment of Water Management in Agriculture (2007), map 2.1, p. 63. IWMI, <http://www.iwmi.cgiar.org/>).

In this scenario, the water crisis is among the main problems to be faced by humanity in the XXIst century. This is especially pressing in arid and semi-arid places (UNDP, 2006; Scott, 2013). Moreover, it is probably in rural areas that water scarcity affects people most. In a lot of developing countries, irrigation in agriculture remains the backbone of the economy or rural areas, where most of the population lives. One in five people in the developing world have not access to sufficient clean water (minimum of 20 L d⁻¹), while average water consumption in Europe and the USA ranges 200 to 600 L d⁻¹ (FAO, 2007).

Water scarcity forces people to rely on unsafe sources of drinking water. The use of poor water quality can increase the risk of waterborne diseases (e.g. cholera, typhoid fever, etc.). Every year almost 2 million child deaths are counted related to unclean water and poor sanitation (UNDP, 2006).

Although Millenium Development Goal (MDG) number 7 aims to halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation, the situation is getting worse. Water scarcity is an increasing problem, as needs for water rise along with population growth, urbanization and higher industrial and domestic water demand. On national and local scales, there is a need for appropriately funded infrastructure and robust governance mechanisms in order to protect water resources and ensure a sustainable development and distribution of water-derived benefits. Initiatives are emerging worldwide to attain this matter. For that reason, wastewater treatment, reuse and reclamation represent key practices in that approach (UNESCO, 2012).

More than one-third of the planet's accessible renewable freshwater is used for agricultural, industrial and municipal purposes. Most of the activities eventually lead to the contamination of freshwater systems worldwide with thousands of industrial and natural chemical compounds. Some 2 million tons of waste, derived from all industrial and human activities (e.g. synthetic compounds used in industrial and consumer products; diffuse agricultural pollution from the application of fertilizers and pesticides; oil and gasoline components through accidental spills, etc.) are released into receiving waters everyday with incomplete or no treatment at all (Fig. 1.2) (UNESCO, 2003; Schwarzenbach et al., 2006).



Figure 1.2. Ratio of treated to untreated wastewater discharged into water bodies. (Source: adaptation of UNESCO, 2012).

During the last decade, the occurrence of organic micropollutants in the environment has attracted great interest since a generalized concern arise about the possible undesirable effects of many of these pollutants in the environment and to living organisms (Cunningham et al., 2006; Kümmerer et al., 2009). The trace pollutants referred to as 'emerging organic contaminants' (EOCs) mainly comprise a group of man-made compounds such as pharmaceutical and personal care products (PPCPs), pesticides, plasticizers and antiseptics that are continuously discharged into the environment as a result of consumer activities, waste disposal, accidental releases and purposeful introduction (Daughton, 2004a).

One of the main sources of EOCs into the environment is the discharge of effluents from wastewater treatment plants (WWTPs), where their removal is often incomplete (Heberer, 2002a). Although a whole array of advanced treatment technologies have lately appeared aiming at the elimination of EOCs, such as ozonation, chemical advanced oxidation or UV radiation (Kim et al., 2009; Liu et al., 2009; Rosal et al., 2010), and have shown to enhance their elimination, the cost of these treatments is oftentimes too expensive and not justified under the current concept of sanitation and wastewater treatment. Moreover, they could lead to the generation of transformation products being in some cases more persistent or toxic than the parental compound (Fatta-Kassinos et al., 2011).

To this regard, decentralized, extensive low-cost treatment technologies, such as constructed wetlands, emerge as a great alternative for wastewater treatment and reuse, which require almost no maintenance and energy consumption. Constructed wetlands (CWs) are natural wastewater treatment systems that emphasize the processes happening in natural wetlands in order to improve their treatment capacity (Kadlec and Wallace, 2009). They constitute an alternative cost-effective technology to conventional WWTPs in the context of small communities with less than 2000 people equivalent (PE) (Puigagut et al., 2007). Apart from the removal of conventional wastewater pollutants (e.g. biochemical oxygen demand, total suspended solids, nitrogen, phosphorus, etc.), CWs have proven to have a great potential for the removal of EOCs. The existing literature includes mostly studies on the occurrence and behavior of pharmaceuticals at horizontal flow (HF) wetlands at micro and meso-scale (Matamoros et al., 2008a; Hijosa et al., 2010b; Dordio et al., 2010; Hijosa et al., 2011b; Zhang et al., 2011; Reyes-Contreras et al., 2012; Zhang et al., 2012b), as well as pilot-scale (Matamoros and Bayona, 2006; Matamoros et al., 2005). Moreover, several studies have been carried out at vertical flow (VF) systems (Matamoros et al., 2007; Matamoros et al., 2009a; Song et al., 2009) and others at full-scale tertiary treatment systems, which include the use of a free water surface (FWS) wetland (Matamoros et al., 2008b; Llorens et al., 2009; Hijosa et al., 2010a; Matamoros and Salvadó, 2012). Couple of other examples comprises the use of a CW after a previous treatment system like a conventional WWTP or an anaerobic digester (Reyes-Contreras et al., 2011; Verlicchi et al., 2013).



Figure 1.3. Aerial view of the activated sludge WWTP and tertiary treatment constructed wetland system at the Natural Park of Aiguamolls de l'Empordà, Girona, Spain (photo: Consorci Costa Brava).

The removal of contaminants in CWs occurs as a result of complex physico-chemical and microbial interactions. The rates of these processes depend on a variety of design and operational factors such as depth of the bed, type and size of media, hydraulic and organic loading rates, feeding strategy and artificial/external aeration, among others. The design of CWs is often carried out using the black box concept, and reduced treatment efficiency may occur when wetlands are constructed without considering the influence of these parameters. Most of the available research concerning design and operational parameters' influence on treatment performance focuses on conventional water quality parameters. Only in the last decade, the effect of these parameters on the removal of EOCs has been investigated. Those include very few studies evaluating the influence of water depth (Matamoros et al., 2005; Matamoros and Bayona, 2006), type of organic matter (Matamoros et al., 2008a); type of granular media (Dordio et al., 2009; Dordio and Carvalho, 2013) and other different design parameters and modes of operation (Hijosa-Valsero et al., 2010b, 2011b; Zhang et al., 2012a,b). Therefore the optimization of the performance of CWs in terms of EOC removal stands as a necessity that can be achieved through the identification of the optimal design and operational factors of these treatment systems.

In this chapter, the author will present the state of the art of relevance to the topic, followed by the description of the objectives of this research.

1.1. Emerging organic contaminants

EOCs are, in most cases, unregulated contaminants whose emissions have emerged as an environmental problem. They comprise a diverse collection of thousands of chemical substances, such as pharmaceuticals, personal care products (PCPs), pesticides, hormones, surfactants, flame-retardants, plasticizers, industrial additives and agents, as well as their transformation products (Farré et al., 2008). The steady increase in the manufacture and consumption of this type of compounds (Campbell, 2007), together with the development of new analytical techniques that have allowed their detection (González et al., 2007, Farré et al., 2012), attracted the attention and concern of the scientific community around a decade ago (Halling-Sorensen et al., 1998). Since then, a number of authors have done extensive research about their occurrence, fate and ecotoxicological effects of these pollutants in the environment (Kolpin et al., 2002; Cunningham et al., 2006; Barceló and Petrović, 2007; Santos et al., 2010; Brooks et al., 2012).

Europe stands as a pioneer in this field and many programs/projects are carried out, which investigate the occurrence, fate and ecotoxicology of these pollutants in all the different compartments of the aquatic environment, as well as potential solutions concerning the elimination of these in wastewater. Some examples of finalized projects are:

- POSEIDON (2001-2004): Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care Products in Sewage and Drinking Water Facilities to Improve Indirect Potable Water Reuse (at: <http://undine.bafg.de/servlet/is/2884/>)
- P-THREE (2002-2005): Removal of Persistent Polar Pollutants through Improved Treatment of Wastewater Effluents (at: http://cordis.europa.eu/projects/rcn/64765_en.html).
- EMCO (2004-2007): Reduction of Environmental Risks, Posed by Emerging Contaminants, through Advanced Treatment of Municipal and Industrial Wastes. (at: <http://wbc-inco.net/object/news/3582>)
- ERAPharm (2004-2007): Environmental Risk Assessment of Pharmaceuticals (at: <http://www.erapharm.org/>)
- KNAPPE (2007-2008): Knowledge and Need Assessment on Pharmaceutical Products in Environmental Waters (at: http://cordis.europa.eu/search/index.cfm?fuseaction=result.document&RS_LANG=ES&RS_RCN=12458418&q=).
- PILLS (2009-2012): Pharmaceutical Input and Elimination from Local Sources (at: <http://www.pills-project.eu/>).

Though major strides have been made in increasing knowledge about the matter, one of the main conclusions obtained from the review of the state of the art in the matter is that this is, however, fragmentary, dealing with only a part of the problematic, thus preventing from a holistic understanding of the issue of EOCs in the environment as a whole (KNAPPE, 2008).

In September 2005 the NORMAN network (Network of reference laboratories for monitoring of emerging environmental pollutants) was created with the financial support of the European Commission, with the purpose of being a self-sustaining network of reference laboratories, research centers and related organizations around Europe for the monitoring and biomonitoring of EOCs. The aim is to enhance the exchange of information, to harmonize methodologies and to stimulate coordinated, interdisciplinary projects on problem-oriented research to address identified needs.

The Environmental Protection Agency (EPA, USA) has also made great efforts to raise attention about the potential problems derived from the ubiquity of pharmaceuticals in the environment, not only asking scientist to increase the research on the field, but also calling the attention of authorities, health care providers, pharmaceutical companies and patients (Daughton and Ternes, 1999; Daughton, 2004b). The ultimate goal is to develop mitigation practices to tackle this environmental problem and to avoid the risk associated with these pollutants (Barceló, 2003).

Out of the rich array of EOCs, the ones receiving the greatest attention up to date are the following: antibiotics, due their reported increased antibiotic resistance genes in aquatic microbiota and potentially human pathogens (Díaz-Cruz et al., 2003; Knapp et al., 2010; Patra et al., 2012; Rodríguez-Rojas et al., 2013); then, X-ray contrast media, which have shown high persistence in the environment; cytostatic drugs, which, being majorly used in the chemotherapy of oncological patients, are designed to prevent the growth and proliferation of cells, and thus are considered highly hazardous compounds due to their genotoxic properties (Zhang et al., 2013); and finally endocrine disrupting compounds (EDCs) such as estrogens, PCBs and organochlorine pesticides (e.g. DDT), among others, which have been found to induce disruption of the reproductive system, female and male reproductive health in humans and wildlife, sex ratio (in wild fish and mollusks), neurodevelopment in children and wildlife, as well as to cause hormone-related cancers, bone disorders, metabolic disorders, and even immune function, diseases and disorders in humans and wildlife (Bergman et al., 2013).

Daughton (2004b) conducted a great discussion about the universe of chemicals that occur in the environment, both known (regulated or not) and unknown, so as to put efforts in widening the understanding of the exposure and risk of chemicals in ecosystems and human health. A large portion of the chemicals occurring in analyzed water samples are not (or cannot be) identified, due to limitations in the repertoire of available analysis tools. Everyday, and for decades now, a whole array of new drugs are released into the environment with mechanisms of action never before encountered by biological systems, and only a very small percentage of commercially used contaminants are being investigated. Definitively, industrial and technological advances in respect to the production of chemicals poses substantial challenges to the evolution and design of regulatory practices (Bolong et al., 2009).

The limited information on the occurrence, fate and effects of EOCs makes it difficult for authorities to regulate their disposal and limit their concentrations. Some groups of compounds have received

regulation, such as pesticides and other priority pollutants (EC, 1976). Moreover, the European Commission is working on the preparation of a candidate list of 553 endocrine disrupting compounds (EDCs) so as to prioritize further detailed review of these, being the industrial chemical bisphenol A and the estrogenic steroid hormone 17α -ethinylestradiol included, together with many new pesticides and other industrial chemicals (EC, 2000b). This list is subjected to changes in response to developments in scientific knowledge or changes in patterns of chemical usage. On the other hand, in the USA the EPA holds a list of compounds called the Contaminant Candidate List (CCL), which comprises a list of contaminants that although are not subject to any drinking water regulation, are being monitored with the aim of possibly being included in future regulations. The last one (CCL3, 2008) includes several EDCs, and some flame-retardants.

However, there are no laws, acts or regulations of any kind, which include guidelines for PPCPs concentrations in WWTPs, drinking water facilities or the environment. The lack of regulations allows unlimited and widespread discharge of EOCs in the environment, which have unknown consequences for the environment and public health. Efforts should be harmonized and integrated between the different actors involved in PPCPs' lifecycle, as well as researchers and public authorities, so as to obtain more productive outcomes and understanding of the problematic and to take action to limit their presence in the environment (Schwarzenbach et al., 2006; KNAPPE, 2008; Daughton, 2009).

1.1.1. Description of studied emerging organic contaminants in this work

There are a large number of EOCs that are continuously released to the environment due to their wide use in society. A subclass underneath this class is PPCPs, which in turn comprise an extraordinarily diverse group of chemicals used in veterinary medicine, agricultural practice, human health and cosmetic care.

PPCPs include prescription and non-prescription medications, nutritional supplements, diagnostic agents, as well as other consumer products such as disinfectants, fragrances, sunscreen and cosmetics. Even though most of them are polar, have a short half-lifetime in water, and are found in trace concentrations, they are considered pseudo-persistent pollutants (Barceló and Petrović, 2007). This is because their universal, frequent usage by multitude of individual and animals causes a continuous discharge into the environment often times sourcing from either non-treated or insufficient wastewater treatment.

The selected EOCs which were studied in this work consisted in the most part of PPCPs and these were chosen following two criteria: 1) to have a wide spectrum of molecular physico-chemical characteristics of compounds, which would presumably make them behave in different ways under the same treatment (i.e. acidic, basic and neutral compounds); and 2) have a high production volume and widespread use.

Target PPCPs included three anti-inflammatory drugs (ibuprofen, diclofenac, acetaminophen), three personal care products (tonalide, oxybenzone, triclosan) and a synthetic estrogen (17α -ethinylestradiol). Moreover, some of the chemicals extensively used in the industry could have an impact on public health by disrupting the endocrine system. For that reason Bisphenol A, a high-production chemical widely used in epoxy resins lining food and beverage containers, was also added to the list of target compounds.

The molecular structures of EOCs are typically large and complex, and differ a lot among substances, containing functional groups such as hydroxyl, carboxyl, ketone and amine. It is important to note that the classification of pharmaceuticals by their active substances within subgroups of pharmaceuticals does not imply that they follow a certain chemical behavior. Small changes in the chemical structure may have significant effects on solubility and polarity, and other properties, which will in turn determine their environmental distribution in air, water, sediments, soils and animals (Kümmerer, 2009).

One of the physico-chemical properties of organic compounds most applied in the field of environmental chemistry is the n-octanol water partition coefficient (K_{ow}), which is defined as the ratio of the compound's concentration in a known volume of n-octanol to its concentration in a known volume of water at equilibrium and at constant temperature (Eq. 1).

$$K_{ow} = \frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}} \quad (\text{Equation 1})$$

K_{ow} values are constant for a given compound and reflect the lipophilicity of a compound. Since octanol is in many ways fat-like in many of its physico-chemical characteristics, it imitates the biota lipid-water partition process. In relation to this, it has been found to be related to water solubility, soil/sediment adsorption coefficients, and bioconcentration factors for aquatic life. Hence, the higher the K_{ow} of a chemical, the higher its lipophilicity. K_{ow} values are generally directly proportional to molecular weight.

K_{ow} values can be measured in the laboratory with shake-flask systems, or can be estimated by models. K_{ow} values have been measured for a wide variety of chemicals and range from about 0.001 to over 10,000,000. Since the range of K_{ow} values encompass ten orders of magnitude, $\log K_{ow}$ values are used instead, and range -3 to 7. A substance is usually considered lipophilic at $\log K_{ow}$ values ranging 3-4 to 6.5 ($K_{ow} > 100$) (Connell, 2005).

However, K_{ow} assays are done adjusting the pH of the aqueous phase so that the predominant form of the compound is un-ionized. In this way, although some substances could be expected to be fairly lipophilic when having $\log K_{ow}$ values ranging 3-4, the occurrence of carboxylic groups or other polar groups in their structure are prone to be ionized and thus, increasing their hydrophilicity. Since many pharmaceuticals are ionizable at environmental pH conditions, $\log K_{ow}$ might not always be an appropriate predictor of the hydrophobicity of a compound. In particular, most non-steroidal anti-inflammatory drugs (NSAIDs) are ionized at environmental pH, and behave as highly polar compounds. Hence, for weak acids the hydrophobicity also depends on its pK_a and the occurring pH of the media where they are contained (Eq. 2).

The ionization constant (K_a) is a measure of (weak) acid strength as expressed by the concentration of ionized molecules divided by the concentration of unionized molecules. In this way, the lower the pK_a , the higher the K_a and the stronger the acid.

where,

$$\log \frac{[A^-]}{[AH]} = \text{pH} - \text{p}K_a \quad (\text{Equation 2})$$

Hence, for a given pK_a of a compound, the higher the pH, the higher the ionization of the compound.

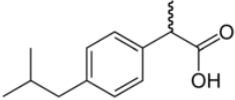
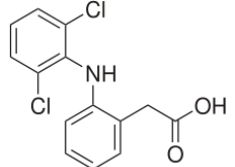
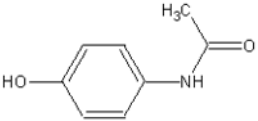
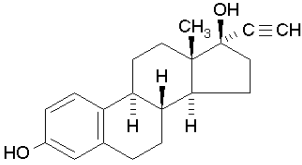
To this regard, the octanol-water distribution coefficient (D_{ow}), which is defined as the ratio of the sum of all forms of the compound (ionized + unionized) in each of the two phases, constitutes a better descriptor for ionizable compounds.

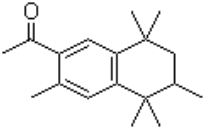
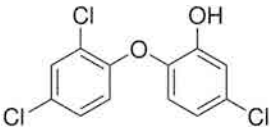
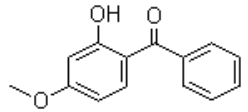
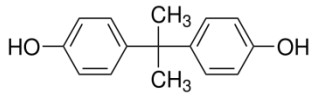
$$\log D_{ow} = \log \left(\frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{ionized water}} + [\text{solute}]_{\text{neutral water}}} \right) \quad (\text{Equation 3})$$

In this case the pH of the aqueous phase is buffered to a specific value such that the pH is not significantly perturbed by the introduction of the compound. The $\log D_{ow}$ is pH dependent, and hence one must specify the pH at which the parameter was measured. For un-ionizable compounds, $\log K_{ow} = \log D_{ow}$ at any pH.

In Table 1.1 a list of the selected organic compounds and a layout of the main characteristics of these substances is given.

Table 1.1. Use and physicochemical properties of the studied compounds.

Trade name	Acronym	CAS RN MW Formula	pK _a	Log K _{ow} ^a	Log D _{ow}	Molecular structure	Application
Ibuprofen	IB	15687-27-1 206.28 C ₁₃ H ₁₈ O ₂	4.31	3.97	0.28	 <p>(<i>RS</i>)-2-(4-(2-methylpropyl)phenyl)propanoic acid</p>	Analgesic/anti-inflammatory
Diclofenac (Voltaren)	DCF	15307-86-5 296.15 C ₁₄ H ₁₁ Cl ₂ NO ₂	4.2	4.02	0.70	 <p>2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid</p>	Analgesic/anti-inflammatory
Acetaminophen (Paracetamol)	ACE	103-90-2 151.17 C ₈ H ₉ NO ₂	-	0.46	-	 <p><i>N</i>-(4-Hydroxyphenyl)acetamide;</p>	Analgesic
17 α -ethinylestradiol	EE2	57-63-6 296.41 C ₂₀ H ₂₄ O ₂	-	4.12	-	 <p>(17α)-19-Norpregna-1,3,4(10)-trien-20-yne-3,17-diol</p>	Oral contraceptive

Tonalide	AHTN	1506-02-1 258.40 C ₁₈ H ₂₆ O	-	5.9	-	 <p>7-Acetyl-1,1,3,4,4,6-hexamethyltetraline</p>	Musk fragrance
Triclosan	TCS	3380-34-5 289.54 C ₁₂ H ₇ Cl ₃ O ₂	-	4.66	-	 <p>5-Chloro-2-(2,4-dichloro-phenoxy)phenol</p>	Antibacterial and antifungal agent
Oxybenzone (Benzonphenone-3)	OXY	131-57-7 228.24 C ₁₄ H ₁₂ O ₃	-	3.52	-	 <p>(2-Hydroxy-4-methoxyphenyl)-phenylmethanone</p>	Sunscreen agent
Bisphenol A	BPA	80-05-7 228.29 C ₁₅ H ₁₆ O ₂	-	3.64	-	 <p>4-[2-(4-hydroxyphenyl)propan-2-yl]phenol</p>	Monomer used in hard plastic and epoxy resins

Within the group of pharmaceuticals, three non-steroidal anti-inflammatory drugs (NSAIDs) were chosen. Ibuprofen (IB) is a NSAID that is prescribed for the treatment of pain or inflammation associated to osteoarthritis and rheumatoid arthritis. It is also used to relieve mild to moderate pain (e.g. headaches, muscle ache, menstrual pain, etc.) and to reduce fever. Diclofenac (DCF) (or Voltaren) is a NSAID also used to relieve pain caused by osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and pain from other causes. Acetaminophen (ACE) (or paracetamol) is an analgesic used to relieve mild to moderate pain (e.g. headaches, muscle aches, menstrual periods, colds and sore throats, toothaches, backaches) and to reduce fever. Finally, 17- α -ethynylestradiol (EE2) is an orally bioactive estrogen used in almost all modern formulations of oral contraceptive pills. This type of estrogenic hormone are potent endocrine disruptors that have been proven to cause feminization in male fishes at concentrations as low as 1 ng L⁻¹ (Thorpe et al., 2001).

When it comes to PCPs, fragrances are generally semi-volatile organic compounds, which have been for many centuries used for multiple purposes (e.g. perfumes, deodorants, washing and cleaning agents, cosmetics). Typical concentrations of fragrances range from 0.3% in face creams to as much as 20% in perfume extracts (Cadby et al., 2002). Most of the fragrances used nowadays are synthesized by industry, and nitro musks and polycyclic musks are the two main categories. The latter are the major musks used today, accounting for almost two-thirds of worldwide production, with an estimated worldwide production volume of 6000 t year⁻¹ (Rimkus, 1999). Tonalide (AHTN) is one of the most widely used polycyclic musks around the world and is used in cosmetics and detergents. It is designed to be very lipophilic by design, in order to sorb to organic materials such as the skin, and in consequence is poorly soluble in water, as indicated by its high octanol-water coefficient (Table 1.1). Moreover, oxybenzone (OXY) is an organic ultraviolet filter used in sunscreen and other cosmetics. Finally, triclosan (TCS) is an antibacterial and antifungal agent present in soaps, deodorants, toothpastes, shaving creams, and in a number of consumer products (MedlinePlus, 2010). Personal care products differ from pharmaceuticals in that large amounts can be directly introduced in the environment, by direct release into recreational waters or volatilized into the air (e.g. musks).

To end with, bisphenol A (BPA) is an industrial chemical monomer with estrogenic activity that is used in the production of food packaging, dental sealants, polycarbonate plastic and many other products.

As it can be observed in Table 1.1, the EOCs selected for this work cover a wide range of molecular structures, containing different functional groups, which may cause them have different chemical behaviors. These small changes in the chemical structure may have significant effects on polarity and other properties and eventually determine the fate of these substances within constructed wetlands.

1.1.2. Emerging organic contaminants in the environment

1.1.2.1. Sources and fate of emerging organic contaminants

EOCs enter the environment through multitude of sources, whose components are depicted in Fig. 1.4.

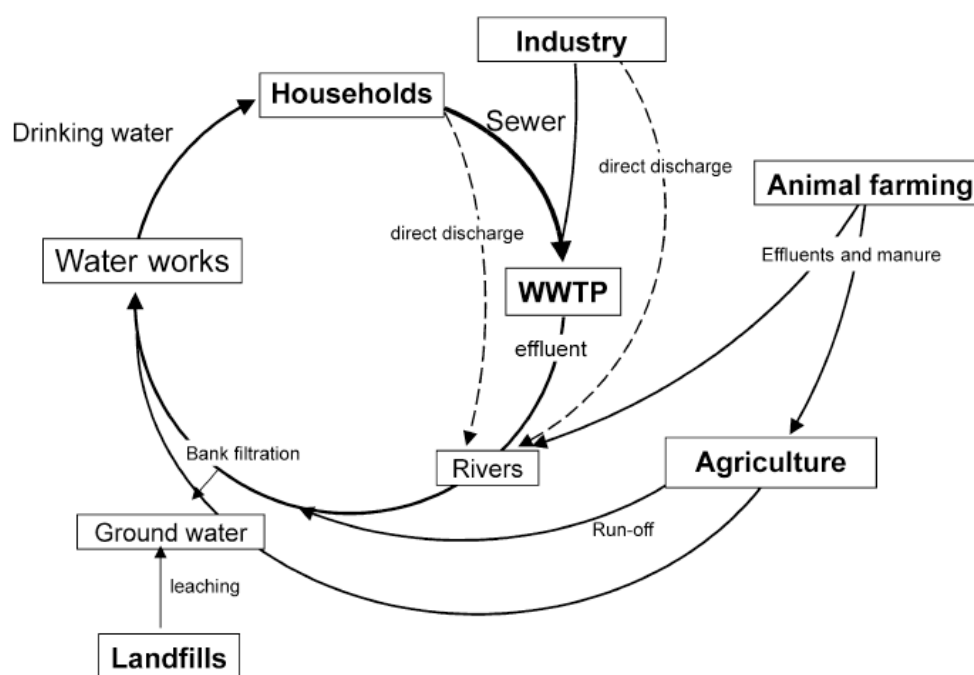


Figure 1.4. Sources and fate of contaminants in the (partially) closed water cycle with indirect potable re-use (Petrović et al., 2003).

Consumption of PPCPs is highly worldwide. As an example of human consumption, it is worth noting how the consumption of non-steroidal anti-inflammatory drugs (NSAIDs) in Spain raised from 26.30 DHD (defined daily doses/1000 inhabitants.day) to 45.81 DHD from 1992 to 2006 (AEMPS, 2006). Out of the NSAIDs, one of the compounds attaining the greatest increase was ibuprofen, whose consumption increased from 0.29 DHD in 1992 up to 21.30 in 2006. The NSAID DCF (commonly known as voltaren) however showed rather stable values of consumption overtime, averaging 7 DHD. According with IMS Health data 942 tons of the anticonvulsant carbamazepine and 877 tons of DCF were sold in 2007 in 76 major countries, which are believed to account for 96% of the global pharmaceutical market. Antibiotic consumption did not fluctuate a lot during the last decade in Spain as it is shown in the report of the AEMPS (2009). Values ranged 0.1 DHD for most studied quinolones up to a maximum value of 6.6 DHD found for the peniciline amoxicilin. As it can be seen in the report by the OECD, most countries in the world have experimented a significant increase in consumption of pharmaceuticals in the last decade (OECD, 2013). To this regard, Daughton and Ruhoy (2013) have called to note the importance of prescribing the “lowest effective dose” of pharmaceuticals on patients as a strategy to diminish EOCs concentrations in the environment and eventually avoid potential adverse environmental impacts.

Once pharmaceuticals are ingested and a fraction metabolized, they are excreted and reach the urban sewer network up to the wastewater treatment plants. The current treatment processes applied for municipal wastewater treatment often fail to completely remove EOCs, which leads to their subsequent release in the terrestrial and aquatic ecosystems through disposal and reuse applications.

On the other hand, the second major source of PPCPs in the environment after sewage effluent discharge is terrestrial run-off derived from the agrifood (and especially meat) industry. To have an idea of the levels of consumption of antibiotics on livestock farms, while human antibiotic use in the USA is maintained below 8 billion pounds per year, livestock farms have been increasing their use, constituting to date nearly four-fifths of the total amount of antibiotics used in the USA (FDA, 2011).

In addition, since the chemical BPA started to be used in the 1940s as a monomer of the polycarbonate plastic and in the manufacture of epoxy resin, everyday people are exposed to it through a vast array of products, which include plastic drinking bottles, canned beverage drinks and metal food containers, eyeglass wear, toys and medical devices. Leaching of BPA occurs continuously in the pasteurization and canning process, sterilizing, microwave heating and washing of the containers into the products that are consumed (Krishnan et al., 1993; Le et al., 2008). Thus, it enters the human body, being excreted almost exclusively in the urine (Wolfgang and Wolfgang, 2008).

A significant source of concern for EOCs in groundwater is the leaching from municipal landfills, and BPA constitutes a major contaminant due to plastic and metal waste disposal (Eggen et al., 2010; Erler and Novak, 2010). Improper disposal of unused or expired drugs (either through the toilet or landfill) constitute another source of these contaminants in the environment.

Depending on the physico-chemical characteristics of the organic contaminant (and their transformation products) and the characteristics of the soil, EOCs can either reach groundwater and pollute aquifers or remain retained within the soil and eventually accumulate, thus affecting the ecosystem and human beings through the food-web.

As a matter of fact, EOCs have been detected at trace concentrations in the freshwater environment for decades (Halling-Sorensen et al., 1998; Kolpin et al., 2002; Loos et al., 2009, 2010; González et al., 2012; Jurado et al., 2012). As a particular example, Kim et al. (2007) documented the frequent detection of many pharmaceuticals, hormones, antibiotics and flame-retardants in surface waters in South Korea. BPA A and other EDCs, as well as toxicological effects over caged male fathead minnows, were found in 90% of sediment and water samples of 11 studied lakes across Minnesota, USA (Writer et al., 2010). Stackelberg et al. (2004) found pharmaceuticals compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant before and after treatment. Pharmaceuticals were also found at the ng L^{-1} level in the tap water of Berlin (Heberer, 2002b). The authors attribute this phenomenon to be characteristic of conurbations like Berlin, with high municipal wastewater discharges and low surface water flows, when groundwater recharge is used in drinking water production.

Some other scattered examples, which show the ubiquity of the occurrence of EOCs in various compartments of the aquatic environment around the world, are displayed in Table 1.2.

Table 1.2. Minimum, maximum and average concentrations (in ng L⁻¹) of target emerging organic contaminants in natural water bodies around the globe.

	Min	Max	Avg	Sample source	Reference
Diclofenac	10	50	29	River Elbe and at the mouth of its tributaries (Germany)	Wiegel et al. (2004)
Ibuprofen	<20	70	35		
Acetaminophen	<5	65	16		
Bisphenol A	16	100	40		
Ibuprofen	11	38	28	Han, Nakdong and Youngsan rivers (South Korea)	Kim et al. (2007)
Acetaminophen	4	73	33		
Diclofenac	1	6.8	3.0		
Oxybenzone	1	3	2		
Acetaminophen	<LOD	712	-	Ebro River basin (Spain)	López-Serna et al. (2012)
Triclosan	<LOD	8	n.a.	Surface waters of Greenwich Bay (USA)	Katz et al. (2013)
Acetaminophen	2	17	5	Near-shore habitats of Lake Michigan - river discharges into the lake from predominantly urban watersheds (USA)	Ferguson et al. (2013)
Ibuprofen	2	84	8		
Carbamazepine	2	118	40	Aquifers at the delta area of the Llobregat River (NE Spain)	Teijon et al. (2010)
Ibuprofen	<LOD	185	185		
Diclofenac	35	477	256		
Acetaminophen	-	1890	180	Groundwater used for drinking-water supply in California (USA)	Fram and Belitz (2011)
Diclofenac	<LOD	1.2	0.2	Urban groundwater in the district of Poble Sec, Barcelona (Spain)	López-Serna et al. (2013)
Ibuprofen	<LOD	2	0.2		
Acetaminophen	<LOD	<LOD	<LOD		
Erythromycin	<LOD	<LOD	<LOD		
Enrofloxacin	12	264	75		
Ibuprofen	<LOD	200	-	Berlin groundwater wells (Germany)	Heberer (2002a)
Diclofenac	<LOD	380	-		
Clofibric acid	<LOD	7300	-		

Concentrations of organic contaminants in surface waters (primarily due to their incomplete elimination in WWTPs) as well as groundwater (due to filtration through the soil) are in the range of ng L⁻¹ to µg L⁻¹.

The pathways of PPCPs in the environment, from resource to drinking water, are set out by Halling-Sørensen et al. (1998) and Mompelat et al. (2009). The polar nature and low volatility of PPCPs prevents their escape from the aquatic realm, being primarily distributed through aqueous transport and food chain dispersal (Daughton and Ternes, 1999). Although the half-life in aquatic ecosystem for most EOCs is relatively low (Yamamoto et al., 2009), some substances are fairly recalcitrant and may

accumulate in soil, reaching concentrations of g kg^{-1} . As an example, clofibric acid has been found to have a half-life of 21 years in soil (Díaz-Cruz and Barceló, 2005; Hernando et al., 2006).

In relation to this, the application of sewage sludge or agricultural pesticides, could eventually contribute to soil, surface and groundwater contamination, either by accumulation of these contaminants in soil in the long term, or by possible leaching and migration of contaminants through the soil zone (Wilson et al., 1996; Chen et al., 2011; Stuart et al., 2012).

Another major pathway of groundwater contamination is due to groundwater-surface water interaction through infiltration from losing reaches of rivers, especially near industrial premises and sewage works (Stuart et al., 2012). The contamination of the groundwater could pose serious challenges to safety regarding drinking water supply. A recent study by the GAO (2011) showed that most drinking water samples grabbed in USA were contaminated by pharmaceuticals, mostly estrogen-based hormones and antibiotics.

Moreover, there is a clear need to include irrigation as an additional exposure route for chemicals in terrestrial ecosystems, so as to assess the potential risks derived. Driven by the imposing water scarcity, irrigation through water reclamation is being widely implemented in Europe for multiple purposes, mainly agriculture, due to the advantages related to nutrient recovery, socio-economic implications. The practice of wastewater reuse without proper management poses potential risks to human health and the environment due to the potential occurrence of pathogens and chemical pollutants, such as nanoparticles (Muñoz et al., 2009; Fatta-Kasinos et al., 2011).

1.1.2.2. Toxicity and evaluation of risks associated to emerging organic contaminants

The ubiquity of EOCs in the environment has created a great concern among scientists due to the possible toxicological effects that these substances may pose on ecosystems and public health. In the last years numerous studies are evaluating the possible toxicological effects related to the occurrence of EOCs, mainly on aquatic organisms (Fent et al., 2006), as well as the management of risk (Enick and Moore, 2007) with the purpose of giving support to decision taking by stakeholders and eventually policy-making.

Although occurring concentrations of EOCs in the environment are oftentimes very low (ranging ng L^{-1} to $\mu\text{g L}^{-1}$), and may not seem to pose an appreciable risk to human health, these are continuously released to the environment, constituting so-called “pseudo-persistent” contaminants. In many cases the possible consequences of their occurrence in the environment are not well understood, but in others the effects seems evident and alarming.

BPA, which might be the most controversial substance among selected compounds, was banned in children’s products in several states of the USA in 2008 due to its well-documented endocrine-disrupting effects. It has been found to be already ubiquitous in the human body and it represents a potent developmental toxin at very low doses, causing harmful effects on animals and humans. There is a strong body of literature about BPA effects, which include structural and neurochemical changes, behavioral changes, disruption of hormone production and fertility, immune disorders, increased growth rate, among others (Vom Saal and Hughes, 2005; Vom Saal and Welshons, 2005; Keri et al., 2007; Lang et al., 2008; Padmanabhan et al., 2008; Eler and Novak, 2010). BPA is one of the so-called EDCs, a class of EOCs, which has attracted particular attention, since they, made to mimic biological hormones, disrupt an organism’s natural processes.

What is more, musk fragrances are found to be ubiquitous, persistent, bioaccumulative pollutants in aquatic organisms that are sometimes highly toxic (Daughton and Ternes, 1999; Luckenbach et al., 2004; Zhang et al., 2013). Amino musk transformation products are toxicologically significant.

Polycyclic musks fragrances have been found to accumulate in several aquatic organisms, such as fish and mussels, but also in high trophic level aquatic organisms and human breast milk (Kannan et al., 2005; Yin et al., 2012).

It is important to note that PPCPs are designed to be biologically active, by modulating endocrine and immune systems and cellular signal transduction, in order to serve their therapeutic purposes. This in consequence will potentially affect the physiological and biochemical functions of biological systems and ecosystems. Moreover, some of them are estrogen hormones (such as EE2), which belong to the category of EDCs, showing toxicity at ng L^{-1} levels (Larsson et al., 1999; Ingerslev et al., 2003). However, PPCPs are quite diverse among them and therefore they may have very different effects over ecosystems (Halling-Sorensen et al., 1998). Although PPCPs have received less scrutiny than pesticides (many are EDCs of concern), these differ from the latter in that the disposal of PPCPs is widespread, long-term and in lower doses. Thus aquatic organisms receive a more chronic exposure. In fact, total loads of PPCPs disposal could exceed those of antibiotics (Daughton and Ternes, 1999).

Although little is known about the occurrence and effects of pharmaceuticals in the environment, more data exist for antibiotics than for any other therapeutic class. This is a result of their extensive use in human therapy and animal husbandry, as well as plant agriculture and aquaculture. It has become clear that the intense use and misuse of antibiotics has increased and spread the occurrence of highly resistant pathogenic bacteria. There is real concern about the effects on aquatic microbiota and consequent alteration of the structure of the microbial community, as well as on the development of resistance in potential human pathogens. Although occurring bacteria in freshwater sources are commonly non pathogenic, there could be exchange of genetic material between pathogenic and non-pathogenic bacteria, conferring them increased antibiotic resistance (Witte, 1998; Barbosa and Levy, 2000; Cabello, 2006; Schlüter et al., 2007; Kelly et al., 2009; Zhang et al., 2009; Middleton and Salierno, 2013).

Research efforts aiming at elucidating the potential toxicological significance of EOCs in ecosystems and eventually in public health requires documentation of contaminant uptake, modes of action and biological endpoints, as well as exposure. Most of the published literature is based on acute effects on aquatic organisms, and in hazard and risk assessments. Assessment factors are applied to the acute effects data for extrapolation to chronic (long-term) effects. This approach is oftentimes non realistic, given that the ratio between acute and chronic toxicity shows to differ depending on the pharmaceutical (Cunningham et al., 2006; Fent et al., 2006). In general, pharmaceuticals do not show high acute toxicity, but chronic toxicity and potential subtle effects are marginally known.

Cleuvers (2003) found how IB, DCF and carbamazepine were toxic for algae, and discuss that although acute effects of single substances in the aquatic environment are very unlikely due to the small concentrations, a combination effect of several compounds and/or their transformation products can occur. Brausch and Rand (2011) evaluated acute and chronic toxicity data available for personal care products, including TCS and AHTN. Only TCS and Triclocarban presented any hazard. Moreover, Breitholtz et al. (2012) assessed the ecotoxicity of various micropollutants on macroalgae and crustaceans in a free water surface wetland in Sweden and reported a good quality of the effluent water, comparable to that treated under advanced tertiary treatment processes.

Conversely, Fent et al. (2006) found how a few substances such as DCF, was the compound with the highest acute toxicity within the class of NSAIDs, and average concentrations found at the effluent of conventional WWTPs were in the range of those being toxic for fish. What is more, Lai et al. (2002), using a food-web model found that natural and synthetic estrogens, such as EE2, could be bioaccumulated in fish in river systems, although to a lesser extent than other EDCs such as DDT (Lai et al., 2002). Likewise, AHTN and other fragrances, which are made hydrophobic so as to adsorb to tissue might accumulate in aquatic biota (Rimkus, 1999).

Although acute toxicity in the aquatic systems due to the occurrence of EOCs is unlikely to occur, there is a lack of chronic toxicity data and effects from long-term, low-level environmental exposures should be further addressed, so as to have a better understanding of the environmental risk of these compounds.

What is more, since pharmaceuticals are not present alone in the environment, but as multi-component mixtures, an accurate prediction of the chronic mixture toxicity is indispensable for an environmental risk assessment. Significant synergistic toxic effects may occur through additive exposures; therefore they should not be handled in isolation. The effects and interaction of a cocktail of commonly used pharmaceuticals, including carbamazepine, ibuprofen and the antibiotic sulfamethoxazole were studied using *in vitro* tests on human and zebrafish cells by Pomati et al. (2008), and they found that the mixture of drugs at ng L^{-1} can inhibit cell proliferation on aquatic life. However, the research in this new field of ecotoxicology is just starting and much remains to be learned (Daughton and Ternes, 1999; Kümmerer, 2009; Stuart et al., 2012).

Another issue of very recent concern is the inhibition of multixenobiotic resistance (MXR) in aquatic organisms, which has especially been found in environmental samples from polluted locations. This mechanism is used as a “first line of defense” against endogenous and exogenous potentially toxic xenobiotics, which are expelled out of the cell by various transmembrane transport proteins. It is still unclear whether the so-called MXR inhibitors or chemosensitizers (Smital and Kurelec, 1998) are natural or man-made substances. It is also unknown whether these are constituted by a few powerful MXR inhibitors, or if the inhibition is caused by the mere presence of a large number of chemicals in polluted water. It is also discussed whether these substances could play key roles in potentiating the effects of other xenobiotics (Daughton and Ternes, 1999). Research on this topic has also just been initiated, such as the study of the inhibition of MXR by musk fragrances (Luckenbach et al., 2004) and still multiple aspects need to be addressed on this topic (Smital et al., 2004).

Finally, it has been discussed that even though ecological effects caused by PPCPs may not be manifest, it does not mean that they do not exist. Potential subtle effects of these substances over time may occur (Daughton and Ternes, 1999). In order to have a better understanding of mechanisms of action of these contaminants in the environment, the target- or biomolecule-oriented, or mode-of-action-based investigation activities are preferred over traditional standard ecotoxicological assays.

In general, assessing the impact of EOCs in aquatic systems remains a major challenge, requiring improved analytical and modeling tools, the development of methods to classify existing and new chemicals on the basis of their potential to harm humans and ecosystems, as well as the development of attenuation technologies and other strategies which minimize their introduction into the environment (Schwarzenbach et al., 2006).

1.1.2.3. Degradation products

The compounds resulting from the structural change within the human body are called metabolites. As with metabolism, when a substance is introduced to the environment or a treatment plant, it can undergo different structural changes by a variety of biotic (e.g. biodegradation, biotransformation) and non-biotic processes (e.g. phototransformation, hydrolysis), resulting in partial or complete transformation of the original compound. This results in changes in their physico-chemical properties, which in turn affects their behavior and toxicity. Although the transformation of parent compounds could be expected to reduce toxicity, in some cases transformation products could have higher persistence and/or more harmful effects than the parent substance. This has particularly been found during natural and technical photolytic processes and advanced oxidation processes (AOPs) (Vogna et al., 2004; Kümmerer, 2009; Fatta-Kassinos et al., 2011; Benitez et al., 2013).

Little is known about the degradation pathways and products of most EOCs in the environment, and less is known about their intermediates of degradation, which just started to be explored (Miao and Metcalfe, 2003; Farré et al., 2008). Gómez et al. (2012) just recently found transformation products of various EOCs at the Henares River basin (central Spain) at concentrations much higher than those of their parent compounds. Various transformation products of IB and other pharmaceuticals were identified by Quintana et al. (2005) in municipal wastewater treated by a membrane bioreactor.

These findings highlight the importance of including degradation products in monitoring and research programs so as to have a better understanding of the behavior and potential toxicology of EOCs in the environment. However, it is important to have in mind that given the universe of chemicals that are everyday released into the environment and, the difficulty and expense involved in their analysis, it would be impossible (if not infinite) to take them all into consideration for research programmes or rutinary monitoring. Instead, well-balanced efforts must be put based on predicted risks associated to each specific contaminant in order to find a compromise solution.

1.1.3. Ocurrance and fate of emerging organic contaminants in conventional wastewater treatment plants

The occurrence and fate of EOCs have been extensively assessed in many conventional treatment technologies, such as activated sludge systems, which are often times unable to degrade EOCs, and thus constitute the major source of EOCs into the environment. The activated sludge process is the most common type of secondary treatment used in municipal wastewater treatment plants worldwide. It consists of a two-stage suspended growth biological treatment process designed to majorly remove organic matter. The first stage is comprised by an aerated reactor in which organic matter is eliminated by a mixed microbial population, while the second stage consists of a settling tank or clarifier that separates solids (activated sludge) from water. A portion of the activated sludge is wasted while the rest is returned back to the aerated reactor. While hydraulic retention time (HRT) of the water could be of about 8 hours, for conventional activated sludge, the average solids retention time is 5 to 10 days. Conventional WWTPs are an intensive wastewater treatment technology. They are able to treat great amounts of water, occupying a relatively small surface area (Tchobanoglous and Burton, 1991).

Concentrations of EOCs in raw wastewater are in the range of ng L^{-1} to $\mu\text{g L}^{-1}$ (ppt-ppb) and a compilation of results of various studies including influent and effluent concentration of EOCs (those selected in the present work) at conventional WWTPs is displayed in Table 1.3.

Table 1.3. Minimum, maximum and (average) concentrations of the studied compounds (in ng L⁻¹) and percentage of detection (% detect.) at influent and effluent of various conventional municipal wastewater treatment plants around the world.

EOC	WWTPs influent	% detect.	WWTP effluent	% detect.	Country	Ref.
IB	1200-2679 (2021)	n.a.	<LOD-2400 (489)	n.a.	Austria	1
	968-2986 (1681)	100	131-424 (263)	100	UK	2
	2800-25400 (12500)	n.a.	500-2600 (1500)	n.a.	Greece	3
	Max: 16500 (8450)	n.a.	Max: 773 (384)	n.a.	Canada	4
	170-83500 (14600)	97	2-24600 (1960)	93	n.a.	5
	3730-353000 (69700)	n.a.	<LOD-26500 (4130)	n.a.	Spain	6
	(1900)	n.a.	(250)	n.a.	USA	7
DCF	905-4114 (2572)	n.a.	780-1680 (1366)	n.a.	Austria	1
	57-1161 (260)	100	6-496 (179)	100	UK	2
	<LOD-3900 (2000)	n.a.	<LOD-2600 (1300)	n.a.	Greece	3
	Max:1010 (204)	n.a.	Max: 748 (194)	n.a.	Canada	4
	105-4110 (1340)	81	35-1950 (680)	85	n.a.	5
	<LOQ-561 (232)	n.a.	6-431 (220)	n.a.	Spain	8
	<LOD	0	<LOD	0	Spain	6
ACE	68107-482687 (211380)	100	1826-24525 (11733)	100	UK	2
	108383-246641 (178116)	100	>80-1575 (353)	86	UK	2
	4700-52500 (20600)	n.a.	500-1700 (900)	n.a.	Greece	3
	1571-37458 (23202)	n.a.	<LOQ	n.a.	Spain	8
	(960)	n.a.	<LOD	n.a.	USA	7
AHTN	210-1106 (760)	n.a.	144-170 (158)	n.a.	Austria	1
	Max: 2000 (804)	n.a.	Max: 600 (274)	n.a.	Canada	4
	210-1690 (990)	100	144-200 (162)	100	n.a.	5
OXY	n.a.	n.a.	1-30 (11)	71	South Korea	9
TCS	33-463 (228)	100	13-82 (57)	100	UK	2
	<LOD-1000 (800)	n.a.	<LOD	n.a.	Greece	3
	4010 (1930)	n.a.	Max: 324 (108)	n.a.	Canada	4
	n.a.	n.a.	1.3-32 (12)	57	South Korea	9
	1520-4430	n.a.	(810)	n.a.	USA	10
	<LOQ-2417 (860)	n.a.	<LOQ-512 (219)	n.a.	Spain	8
EE2	0.4-70 (4.2)	91	0.2-5 (0.9)	59	n.a.	5
	<LOD	0	<LOD	0	Sweden	11
	n.a.	n.a.	1.3	14	South Korea	9
BPA	222-727 (416)	100	>105-237 (86)	75	UK	2

1. Clara et al. (2005), 2. Kasprzyk-Hordern et al. (2009), 3. Kosma et al. (2010), 4. Lishman et al. (2006), 5. Miège et al. (2009), 6. Santos et al. (2009), 7. Yu et al. (2006), 8. Rosal et al. (2010), 9. Kim et al. (2007), 10. Katz et al. (2013), 11. Zorita et al. (2009). N.a.: not available.

As we already commented, the NSAIDS take part of a therapeutical group widely used nowadays, and consequently ACE and IB are the ones being found at higher concentration levels in raw wastewater out of the studied compounds. In the lower concentration level of EOCs in the environment have been reported the detection of steroid hormones, generally at low ng L⁻¹ and oftentimes below detection limits (Daughton and Ternes, 1999; Carballa et al., 2004). However, it should be noted that in spite of the low concentrations, estrogens could induce serious chronic effects, such as endocrine disruption (Ingerslev et al., 2003). The occurrence of antibiotics in WWTPs has also been reported in various studies (Yang and Carlson, 2004).

Activated sludge WWTPs are not specifically designed to eliminate EOCs and thus removal rates are very variable and compound-dependent. A recent report by the EPA includes a review of recent literature on wastewater treatment technologies and their ability to remove 16 EOCs. Average removal efficiencies were calculated from a collection of as many as 41 full-scale WWTPs based on activated sludge, and removal rates for selected EOCs are displayed in Table 1.4. Note that the treatment systems used to obtain the presented data on the table did not include activated sludge systems that reported design modifications from the conventional activated sludge WWTPs (e.g. those that aim to remove nutrients). Although there are many variations of this process, further division of activated sludge categories was impractical (EPA, 2010).

Table 1.4. Minimum, maximum and average removal efficiencies of target emerging organic contaminants at full-scale activated sludge WWTPs treating municipal wastewater (adapted from: EPA, 2010).

	Removal efficiency (%)			# of WWTPs used to calculate removal
	Min	Max	Avg	
Ibuprofen	43	100	90	32
Acetaminophen	>90	100	97	4
Diclofenac	7.1	>99	44	23
Tonalide	13	97	67	20
Oxybenzone	>8.0	>96	76	6
Triclosan	>67	100	89	22
Ethinyl estradiol	>1	>99	66	13
Bisphenol A	11	100	78	41
Sulfamethoxazole	9.0	99	58	15

Treatment performance was found to vary among the conventional WWTPs under study, majorly as a function of the characteristics of inflowing wastewater. Average removal efficiencies were very variable among target compounds and ranged from 66% for EE2 to 97% for ACE.

EOCs may be removed from wastewater during activated sludge treatment by mechanisms of biodegradation and/or adsorption to the particulate matter. Some compounds undergo rapid biodegradation (e.g. ACE) in conventional WWTPs (Rosal et al., 2010; Kosma et al., 2010; Miège et al., 2009; Gros et al., 2009; Yu et al., 2006; Sim et al., 2010). Likewise, IB and TCS are consistently removed in most treatment plants (Clara et al., 2005; Lishman et al., 2006; Terzić et al., 2008). However, some compounds exhibit variable removal, (e.g. DCF, BPA), while others, such as carbamazepine, have shown to be very recalcitrant (Kosma et al., 2010). In fact, in some occasions

higher concentrations have been found in the effluent than in the influent, which is attributed to desorption and/or deconjugation processes (Kasprzyk-Hordern et al., 2009; Zorita et al., 2009). The more hydrophobic substances may attach to suspended solids or activated sludge. To this respect, Carballa et al. (2005) found how compounds with high sorption properties such as AHTN and DCF were significantly removed during coagulation-flocculation assays encompassing different doses and types of coagulants at different temperatures, whereas hydrophilic compounds (e.g. IB and carbamazepine) were not affected. Conversely, Jelić et al. (2011) found no accumulation of DCF in sludge samples from three conventional WWTPs.

Treated effluent of WWTPs can either be disposed in surface waters or be reused for multiple applications. Additionally, the waste sludge, after its drying and treatment, could be applied to the land as fertilizers. In this way, remaining concentrations of EOCs may transfer to soil and surface waters.

1.1.4. Advanced treatment technologies

Since conventional WWTPs have shown to be unable to completely eliminate EOCs, as well as other specific and priority substances included in Water Framework Directive (EC, 2000a), a number of new advanced treatment technologies have lately emerged in this sense. A whole array of fairly standardized unit processes is available so as to remove EOCs by transformation or removal by physical methods, including adsorption or filtration. These include activated carbon (Ternes et al., 2002; Snyder et al., 2007), ozonation (Ternes et al., 2003; Rosal et al., 2010), photo-Fenton (Rodríguez-Gil et al., 2010), UV radiation (Kim et al., 2009), ultrasonic irradiation (Naddeo et al., 2009), chemical AOPs (involving hydroxyl radicals), which generally use a combination of oxidization agents (e.g. H₂O₂, O₃, TiO₂, etc.), irradiation (UV or ultrasound) and catalysts (Klavarioti et al., 2009; Liu et al., 2009), chlorine dioxide oxidation (Huber et al., 2005), membrane technologies (Yoon et al., 2006; Snyder et al., 2007; Al-Rifai et al., 2011), and membrane bioreactors (Kimura et al., 2005; Quintana et al., 2005; Abegglen et al., 2009; Shariati et al., 2010). The anti-inflammatory DCF has consistently shown to be well photodegraded under UV treatment (Andreozzi et al., 2003; Zhang et al., 2008; Kim et al., 2009). DCF also gets well mineralized under ozonation (Rosal et al., 2010) and chlorine disinfection (Kosma et al., 2010).

Average removal efficiencies of a wide range of EOCs achieved under different advanced treatment technologies were calculated by the EPA (2010) and a summary for the selected organic compounds is displayed in Table 1.5.

Table 1.5. Min, Max and average removal efficiencies of selected emerging organic contaminants under different advanced treatment technologies for municipal wastewater treatment at full-scale (EPA, 2010).

Compound	Technology	Min	Max	Avg (%)	# systems used to calculate removal
Ibuprofen	Chlorine disinfection	>43	100	78	5
	Ozonation	>90	100	95	2
	Reverse Osmosis	-	-	72	1
	UV disinfection	>81	100	90	6
Acetaminophen	Chlorine disinfection	>90	>99	95	2
	Reverse Osmosis	-	-	90	1
	UV disinfection	-	-	90	1
Diclofenac	Chlorine disinfection	>18	>90	66	3
	Reverse Osmosis	-	-	90	1
	UV disinfection	>86	>91	89	3
Tonalide	Chlorine disinfection	64	93	79	2
	UV disinfection	-	-	52	1
Oxybenzone	Chlorine disinfection	>8	>95	51	2
	Reverse osmosis	-	-	95	1
	UV disinfection	>89	>96	92	3
Triclosan	Chlorine disinfection	>67	>99	83	4
	Ozonation	99	100	99	2
	Reverse Osmosis	-	-	67	1
	UV disinfection	>71	>99	90	5
Ethinylestradiol	Chlorine disinfection	1	72	42	4
Bisphenol A	Chlorine disinfection	>20	>96	72	8
	Ozonation	90	100	96	3
	UV disinfection	>72	>92	85	4
	Granular activated carbon	-	-	100	1

Although advanced treatment technologies have shown to enhance the elimination of these contaminants, they are very unlikely to be implemented in the context of wastewater treatment of small communities, even if the technology is improved. To this regard, decentralized, extensive low-cost treatment technologies, such as CWs, emerge as a great alternative for wastewater treatment and reuse, which require almost no maintenance and energy consumption. Their successful implementation at full-scale has shown their capacity to degrade all kind of contaminants, from conventional parameters to heavy metals or pathogens. Just in recent years their potential on the removal of EOCs is being explored. The characteristics, advantages and disadvantages and performance of CWs in the treatment of municipal wastewater are shown in the following section.

1.2. Constructed wetlands as a decentralized wastewater treatment technology

CWs are natural wastewater treatment systems that consist of a properly designed shallow basin, which contains a substrate that is planted with aquatic vegetation. Other components such as microorganisms and aquatic invertebrates develop naturally. These systems are constructed to mimic the microbiological, biological, physical and chemical processes that occur in a natural wetland but in a more controlled environment to treat wastewater (Kadlec and Wallace, 2009).

These natural treatment systems constitute a cost-effective and technically feasible approach for the treatment of wastewater for several reasons:

- Can be less expensive to build than other treatment options.
- Low operational and maintenance (O&M) expenses (energy and supplies). Easy to maintain (do not require highly qualified staff).
- Able to tolerate fluctuations in flow and load.
- Promote biodiversity. As an example, restored wetlands are becoming common in agricultural landscapes of northern Europe so as to increase nutrient retention as well as increasing species diversity (Thiere et al., 2009; Matamoros et al., 2012a).
- Can be easily integrated into the natural and rural landscape, and provide aesthetic, commercial and habitat value (Llorens et al., 2009).
- Possible commercial revenue from flower harvesting and/or aquaculture (Jana, 1998).

However, they have some limitations:

- Generally high land area requirement: they may be more economical than other technologies, but only if land is available and affordable. While activated sludge technology requires approximately 0.06 m²/PE (Cooper, 2005), vertical subsurface, horizontal subsurface and free water surface CWs require 2, 5 and 8 m²/PE, respectively to attain the same contaminant removal efficiencies (Cooper, 2005; Vymazal, 2005; Kadlec, 2009).
- Poor maintenance or design can lead to excessive clogging, which can affect hydraulics and treatment performance (Knowles et al., 2011).

Since the implementation of the Water Framework Directive 2000/60/EC (EC, 2000a), as well as the Directive 91/271/CEE concerning urban wastewater treatment (EC, 1991), there is a need to comply with stringent regulations in many countries around Europe, especially for the treatment of small communities. This has attracted the focus to decentralized wastewater treatment ecotechnologies, existing an increasing demand for economical, aesthetic and ecologically sustainable treatment systems.

To this regard CWs, and other biological treatment technologies such as waste stabilization ponds, require very low to no energy consumption, and are easy to operate. Thus, they constitute a good alternative for wastewater treatment in small communities. Indeed, constructed wetlands have been extensively used at full-scale across the world since the 80s, for the treatment of not only municipal wastewater but all kinds of wastewater including stormwater, landfill leachate, tannery wastewater, and other industrial wastewaters (Schulz et al., 2001; Bulc, 2006; Behrends et al., 2007; Nivala et al., 2007; Vymazal, 2009). They have been widely implemented in countries like France (Molle et al., 2005), UK (Weedon, 2003; Cooper, 2005), Denmark (Brix and Arias, 2005) or Czech Republic (Vymazal, 2002), and their implementation is increasing in countries like Spain (Vera et al., 2011) or

Italy (Masi et al., 2007). Numerous studies have shown their capability to maintain hydraulic, technical, economic, environmental and ecological benefits (Vymazal, 2002; Dixon et al., 2003; Zhou et al., 2009).

1.2.1. Types of constructed wetlands

There are several types of constructed wetlands. They can mainly be divided into subsurface flow (horizontal or vertical), surface flow constructed wetlands, and hybrid systems, which are a combination of different types.

1.2.1.1. Horizontal subsurface flow constructed wetlands

This type of wetlands consist of gravel beds planted with wetland vegetation, where the wastewater is intended to remain below the top of the substrate and to flow horizontally in and around the roots and rhizomes of the plants. These wetlands are always flooded and water depth usually remains between 0.3 and 0.9 m (between 0.05 and 0.1 m beneath the surface of the bed). The recommended organic loading rate is around 6 g of biochemical oxygen demand (BOD₅) m⁻² d⁻¹ (García and Corzo, 2008). The biofilm that grows on the granular media and the roots and rhizomes of the vegetation plays a major role in the removal of contaminants from wastewater.

HF wetlands are typically composed by: inlet piping, an impervious liner, filter media, emergent vegetation and outlet piping. A diagram of a conventional HF wetland is depicted in Fig. 1.5.

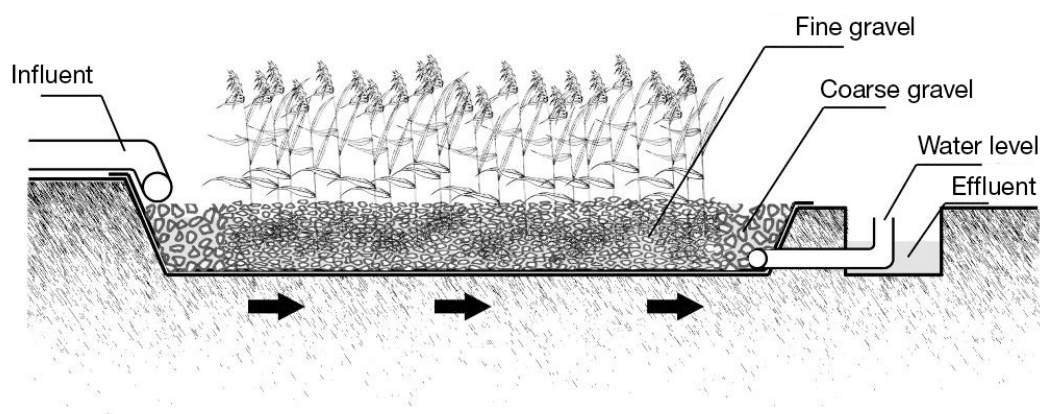


Figure 1.5. Horizontal subsurface flow constructed wetland schematic (adapted from Pedescoll, 2010).

They are good for total suspended solids (TSS), BOD₅ and bacteria removal and denitrification. However, they are poor at nitrification because of limited oxygen transfer capacity (OTC) (Cooper, 1999; Tyroller et al., 2010). Since the water is not exposed, the risk associated with exposure to pathogenic organisms is minimized and they do not provide suitable habitat for mosquitos (Kadlec and Wallace, 2009).

1.2.1.2. Vertical subsurface flow constructed wetlands

In this type of wetlands water is distributed across the surface of a sand or gravel bed planted with emergent vegetation, and the effluent is collected from the bottom of the media, where the water is freely draining. The treatment of the water occurs as it percolates through the filter media and the plant root zone. There exist several variations of VF wetlands. In the most common design the bed is

usually comprised of several layers of different grain size and contains a network of draining perforated pipes. They are usually fed intermittently and frequently loading and resting periods are applied. This is the case of many systems in France, where 2 or 3 beds work alternatively. In the VF French systems, the wastewater is fed without a previous primary treatment (Molle et al., 2008), causing the accumulation of a layer of solids on the top of the bed, which in turn acts as a filter. The alternation of cycles of feed and rest promote mineralization of the solid deposits during resting phases (Molle et al., 2005). In VF wetlands in general, it is the special operating conditions (i.e. intermittent dosing and unsaturated bed) that allow a higher organic loading rate to be applied in comparison to HF beds. Applied OLR values vary significantly from place to place and range from 20 g BOD₅ m⁻² d⁻¹ in Denmark (Brix and Arias, 2005) up to 180 g BOD₅ m⁻² d⁻¹ in French systems (raw wastewater). The components of a typical VF wetland are shown in Fig. 1.6.

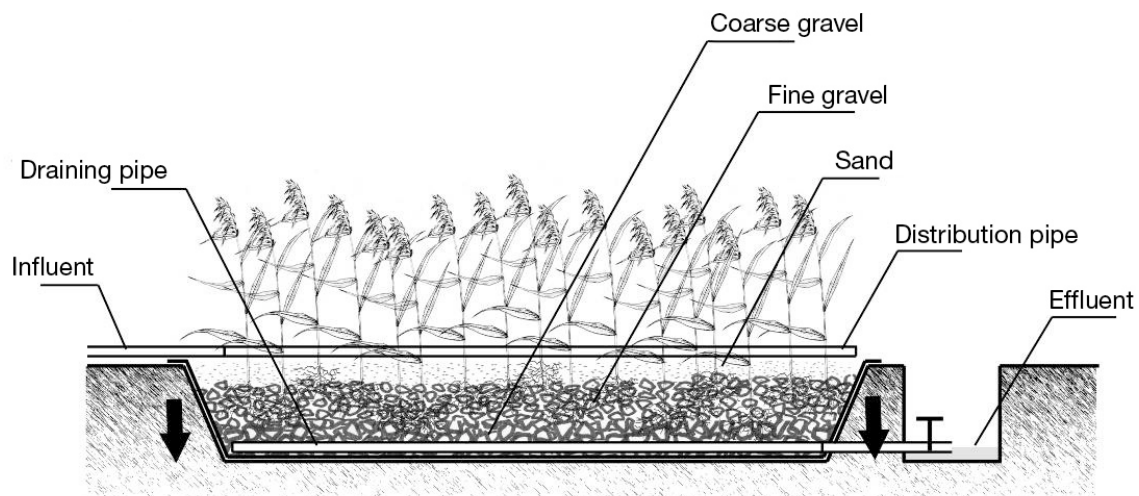


Figure 1.6. Basic elements of a vertical subsurface flow constructed wetland (adapted from Pedescoll, 2010).

The configuration of these systems confers very different properties to those of HF wetlands. They have a much greater oxygen transfer capacity (OTC) than HF beds, which makes them good for nitrification. Oxygen transfer is achieved by means of diluted oxygen present in wastewater, convection while intermittent loading, and diffusion processes occurring between doses (Torrens et al., 2009). The high OTC also leads to good removal of BOD₅ and chemical oxygen demand (COD); they are also able to remove some bacteria (Headley et al., 2013) and are also considerably smaller (about 2-3 m²/PE) than HF systems (about 5 m²/PE) (Cooper, 2005; Vymazal, 2005). Nevertheless, they are less good for suspended solids removal and, more specially for single VF systems where no resting periods are allowed, clogging of the media may occur rapidly if the granular material selection and the hydraulic loading rates are not correct (Cooper, 1999; Platzer and Mauch, 1997; Kayser and Kunst, 2005).

1.2.1.3. Free water surface wetlands

In this type of wetlands, the wastewater flows above the top of the media (with some kind of impervious layer underneath), which can be of many types (e.g. clay, soil, gravel, etc.), since the wastewater is not intended to be filtered through it. These wetlands contain areas of open water, floating vegetation, and emerging plants, either by design or as an unavoidable consequence of the design configuration. A schematic of a conventional FWS wetland is depicted in Fig. 1.7.

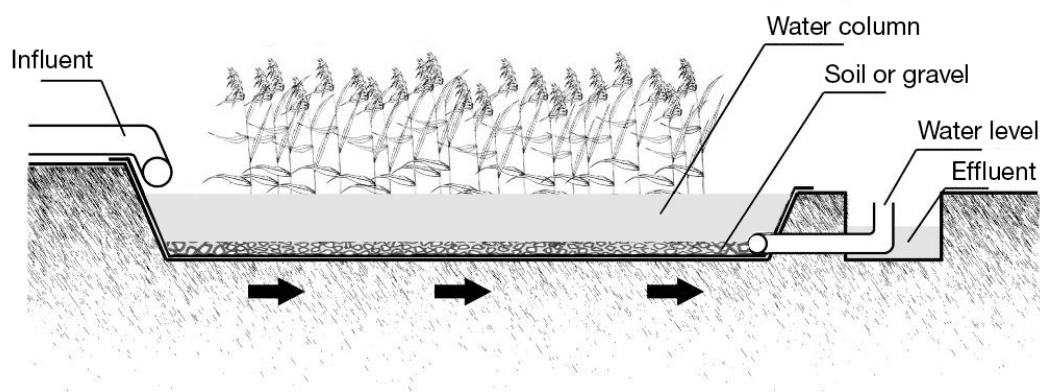


Figure 1.7. Free water surface constructed wetland schematic (adapted from Pedescoll, 2010).

Because the water is exposed during the treatment process, it is common that these types of wetlands attract a variety of wildlife (i.e. insects, mollusks, fish, amphibians, reptiles, birds and mammals) (Kadlec and Knight, 1996). Moreover, since they represent a risk for public health due to human exposure to pathogens, they are commonly applied for tertiary treatment of secondary or tertiary treatment effluents (e.g. activated sludge systems, waste stabilization ponds, SSF wetlands, etc.). They are especially appropriate for restoration of deteriorated areas or attenuation of agricultural runoff at tertiary treatment level (Seguí et al., 2009; Thiere et al., 2009; Matamoros et al., 2012a; Matamoros and Salvadó, 2012).

1.2.1.4. Hybrid systems

Various types of constructed wetlands may be combined in order to achieve higher treatment efficiency, especially for nitrogen and pathogens. These hybrid systems are normally comprised of VF and HF systems arranged in different possible manners. While in HF wetlands nitrification is not achieved due to a lack of oxygen, VF wetlands can provide good conditions for nitrification but no denitrification occurs in these systems. Thenceforward, the strengths and weaknesses of each type of system balance each other out and in consequence it is possible to obtain an effluent low in BOD and in total nitrogen (TN) concentrations (Vymazal, 2005). Different combinations are possible, including HF followed by VF wetlands, VF followed by HF wetlands and other stages of filters including water recirculation from one stage to another (Brix and Arias, 2005).

Given the special interest in hybrid CW systems for water treatment and reclamation in decentralized areas, in the context of the present thesis a collaborative project between the Universitat Politècnica de Catalunya-BarcelonaTech (UPC) and the Foundation Centre for New Water Technologies (CENTA, Seville, Spain) emerged in 2009, being financed by the Spanish Ministry of Environment (085/RN08/03.2). The project aimed at integrating the treatment of combined sewer effluent as well as the produced sludge, while providing a final effluent suitable for its reuse through the sole use of constructed wetlands of different configurations. The specific aim was to evaluate the capacity of a combination of VF, HF and FWS wetlands operating in series, for the treatment of wastewater in terms of conventional water quality parameters, as well as EOCs.

An experimental meso-scale (flow = $0.2 \text{ m}^3 \text{ d}^{-1}$) constructed wetland system following the cited configuration (VF, HF and FWS wetlands operating in series) was constructed at the facilities of the UPC, in Barcelona. Parallely, a pilot-scale (flow = $14 \text{ m}^3 \text{ d}^{-1}$) system based on the exact same configuration was put in operation at the facilities of the CENTA. Both plants were monitored over a period of about 1.5 years. Chapters 5, 6, 7 and 8 are a result of this collaboration.

1.2.2. Removal and behavior of emerging organic contaminants in constructed wetlands

The elimination of EOCs in CWs takes place as a result of the simultaneous occurrence of various removal mechanisms, including biodegradation, adsorption, photodegradation, plant uptake, or hydrolysis. Some removal mechanisms will predominate in some wetland configurations in respect to others (e.g. photodegradation will only take place in FWS wetlands).

Average removal efficiencies of target EOCs at several pilot and full-scale CW systems of different configurations are shown in Table 1.6.

Table 1.6. Average removal efficiencies (R.E.) of target emerging organic contaminants at different pilot and full-scale constructed wetland systems of various configurations.

Compound	Range	Avg R.E. (%)	Type of treatment	Ref
Ibuprofen	95-96	96	Restoration FWS wetland (receives secondary effluent from a WWTP)	1
	48 (shallow)- 81 (deep)	n.a.	Pilot-scale HF wetlands (different depths) with injection of contaminants	2
	n.a.	99	Experimental pilot -scale VF wetland (5m ²)	3
	n.a.	<15	Restored wetland system fed with natural waters impacted by urban and agricultural run-off	4
Diclofenac	n.a.	73	Experimental pilot -scale VF wetland (5m ²)	3
	n.a.	<15	Restored wetland system fed with natural waters impacted by urban and agricultural run-off	4
	65-87	77	Three hybrid treatment Systems (ponds and CWs)	5
	73-96	n.a.	FWS wetland (receives secondary effluent from WWTP)	6
	n.a.	21	Household HF wetland	7
Acetaminophen	>90	>90	HF wetland	8
Tonalide	88-90	89	Restoration FWS wetland (receives secondary effluent from a WWTP)	1
	n.a.	82	Experimental pilot-scale VF wetland (5m ²)	3
	n.a.	15-40	Restored wetland system fed with natural waters impacted by urban and agricultural run-off	4
Oxybenzone	n.a.	98	Household HF wetland	7
	n.a.	90	Household VF wetland	7
		77	Tertiary treatment by Pond + FWS wetland	9
Triclosan	n.a.	>40	Restored wetland system fed with natural waters impacted by urban and agricultural run-off	4
	n.a.	86	Tertiary treatment by Pond + FWS wetland	9
Bisphenol A	n.a.	15-40	Restored wetland system fed with natural waters impacted by urban and agricultural run-off	4

1. Llorens et al. (2009); 2. Matamoros et al. (2005); 3. Matamoros et al. (2007); 4. Matamoros et al. (2012a); 5. Hijosa-Valsero et al. (2010a); 6. Matamoros et al. (2008b); 7. Matamoros et al. (2009a); 8. Ranieri et al. (2011); 9. Matamoros and Salvadó (2012).

The anti-inflammatory drug IB has shown to be readily removed in the three types of CWs (HF, VF and FWS). Results by Matamoros et al. (2005) and Matamoros and Bayona (2006) show high removal efficiencies in HF wetlands (60 - 80%), observing that the elimination rates are higher in shallower (0.27 m) than deeper beds (0.5 m). Matamoros et al. (2008a) found a prevalence of OH-IB over Ca-IB metabolite in a HF wetland, which would be explained by its predominant aerobic biodegradation. A comparison between different seasons by Hijosa-Valsero et al. (2011b) in HF beds shows a seasonal variability, with lower removal efficiencies in winter (<40%) than in summer (>90%). The removal of IB is also high in FWS wetlands, as documented by Llorens et al. (2009) and Matamoros et al. (2008b), where efficiencies of over 90% are detected. High elimination rates were also reported for VF beds, with efficiencies of around 99% for unsaturated vertical wetlands (Matamoros et al., 2007). Likewise, a high elimination (90%) of the analgesic ACE was reported by Ranieri et al. (2011) in a HF wetland.

Conversely, although DCF has been reported to be recalcitrant in activated sludge WWTPs (Heberer, 2002a), this substance shows a variable removal in CWs, ranging from 21% in HF beds (Matamoros et al., 2009a; Matamoros and Bayona, 2006) up to 73% in a VF wetland in Denmark (Matamoros et al., 2007). Average removal efficiencies in FWS wetlands have been reported to be as high as 96% and 73-96% in studies carried out by Llorens et al. (2009) and Matamoros et al. (2008b), respectively. This could be attributed to its large removal through photodegradation, as reported by Andreozzi et al. (2003). This substance is fairly hydrophilic and was not detected in the particulate matter associated to the gravel matrix of CWs, as reported by Matamoros and Bayona (2006). A high variability on the elimination of DCF was also observed by Hijosa-Valsero et al. (2011b) in various experimental meso-scale HF wetlands, who showed that its degradation was favored by high temperatures (summer VS winter) and found variable removal efficiencies in Barcelona (around 70%) and in León (<12%) wetlands.

In regards to the removal of the estrogen EE2 in constructed wetlands, Gray and Sedlak (2005) found a 41% removal after spiking it in concentrations slightly above background concentrations in a full-scale FWS wetland (750 m²) with a depth of 0.5 m and a hydraulic retention time of 84 h, and reported sorption as a major mechanism for its removal in the aqueous phase. Song et al. (2009) evaluated the influence of saturated vs. unsaturated conditions in VF units in regards to the removal of estrogens, and found out that unsaturated conditions (and thus more oxidized conditions) were better for the removal of this type of pollutants.

What is more, the fate of AHTN in constructed wetlands has been previously investigated by several authors, such as Matamoros et al. (2008b) who reported elimination rates of 88 - 90% in a full-scale FWS wetland, which did not show a dependence on the season, presumably due to their low biodegradability properties (i.e. major removal mechanism through interaction with organic matter and sediment). In fact, in another study, this time in two HF wetlands at Les Franqueses del Vallès (Barcelona), Matamoros and Bayona (2006) found this fragrance together with galaxolide to be the most abundant on the gravel bed and the suspended particulate matter, attributing the removal of these musks to hydrophobic interactions with the organic matter and the biofilm. Moreover, no water depth effect dependence was found for this compound. On the contrary, Hijosa-Valsero et al. (2011b) reported a possible dependence of this compound on temperature, since the removal efficiencies were significantly higher in summer than in wintertime, presumably due to the release of hydrophobic compounds in winter (i.e. galaxolide and AHTN), under a lower biofilm and plant activity. Removal efficiencies in VF wetlands were also reported by Matamoros et al. (2007) to be moderately removed with elimination rates ranging from 75 - 82%.

The behavior of the sunscreen agent OXY in CWs has been reported by Matamoros et al. (2007) in a VF wetland, where it was very efficiently removed (>95%), and by Matamoros et al. (2009a), where the removal was also >95% in HF wetlands and 90% in VF wetlands. The authors suggest that it could be

partially removed by sorption onto the particulate matter, although further experiments to confirm this hypothesis are needed.

In regards to the elimination of TCS, Park et al. (2008) reported removal efficiencies in FWS wetlands in South Korea of above 60%. Matamoros et al. (2012a) found TCS to be the substance achieving the highest removal efficiency (>40%) among all studied compounds in a restored wetland in Denmark.

1.2.3. Design and operational factors affecting efficiency

This section provides an overview of the major factors affecting the performance (out of the available published literature in terms of both conventional water quality parameters and EOCs) of CWs, in particular those having to do with design characteristics but also with operation strategies. These include the type of primary treatment, granular media characteristics, use of aeration, hydraulic loading rate, intermittent loading frequency and loading-resting cycles, water depth, vegetation and seasonality.

1.2.3.1. Primary treatment

After a preliminary treatment (pretreatment), which consists of removing large solids and heavy mineral solids from the raw wastewater, a primary treatment usually takes place in facilities based on CWs. This basically consists of creating quiescent flow conditions for sedimentation within a fairly deep tank with the aim of reducing the high concentration of total solids of the wastewater. Settled matter is removed as sludge from the bottom of the tank and the clarified water will be removed from the top of the tank to be further treated in secondary treatment, i.e. constructed wetlands.

Although septic and Imhoff tanks have been the most typically applied technology as a previous step to constructed wetlands, there exist other primary treatment systems, such as the upflow anaerobic sludge blanket (UASB) reactor or the hydrolytic upflow sludge blanket (HUSB) reactor. Its effect in treatment performance of constructed wetlands have been little investigated until recently and may constitute good alternative to conventional primary treatments, producing effluents with a lower organic matter content and a greater removal of suspended solids (Álvarez et al., 2008; Barros et al., 2008). Recently, Reyes-Contreras et al. (2011) evaluated the removal of PPCPs at a full-scale system consisting in a UASB reactor followed by a HF and a FWS wetlands operating in series. Findings show how the UASB was in general found to be more efficient in winter than in summer. Authors discuss that this seasonality is especially important for those compounds whose major removal mechanism is adsorption, which would adsorb to suspended solids and organic matter at a higher rate at lower temperatures.

An experimental treatment plant was designed for the development of research on this specific topic (primary treatment) (NEWWET) (Chapters 3 and 4), and constructed at the facilities of the GEMMA group (Department of Hydraulic, Maritime and Environmental Engineering of the Universitat Politècnica de Catalunya-BarcelonaTech, Spain). The system consisted of three different treatment lines, which had different primary treatment and operation strategies. In particular, two primary treatment options were compared: a HUSB reactor and a conventional settler. After data collection for 2.5-year period, Pedescoll et al. (2011a) found that the existence of the HUSB reactor did not enhance the treatment performance in HF wetlands, being the elimination rates of COD, BOD₅ and ammonium slightly lower than the ones found at the lines with the conventional settler. Moreover, lower redox potentials in the effluent of the treatment line containing this reactor were detected in comparison to conventional settling. However, the removal of TSS in the HUSB reactor was 20% higher than in the settlers, presumably helping prevent or delay clogging processes in the wetlands. The study by Hijosa et al. (2011b) in this experimental treatment system showed how the settler produced lower effluent concentrations of IB, DCF, and AHTN than the HUSB reactor.

In general, primary treatment represents a key step on the efficiency of contaminant removal in wetlands and its influence should be an object of further investigation. This is to be contrasted for the removal of EOCs, especially those being more hydrophobic (e.g. fragrances, etc.), since their removal depends majorly on their sorption to particulate matter.

1.2.3.2. Media characteristics

The hydraulic conductivity of a porous media is very sensitive to media size, as well as particle size distribution and particle shape (Knowles et al., 2011).

In regards to HF wetlands, it is a usual practice to have coarse stones in the inlet and outlet of the wetland in order to increase the hydraulic conductivity and to allow a better flow of the water in those areas. However, a wide range of materials and size of the main bed media materials of CWs have been applied around the world, and its selection is in many cases dictated by the availability, price and local practices of a certain region. Recommendations by García and Corzo (2008) suggest hard, durable and homogeneous materials that do not contain fine grains, which might clog the media (Pedescoll et al., 2009).

When it comes to size, the Specialists Group of the IWA has stated the size range of 8-16 mm as the most commonly applied in HF systems (IWA, 2000). However, other authors such as García and Corzo (2008) have suggested finer grain size, 5-8 mm, as more appropriate for main bed material. Other design guidelines relevant to these systems can be found in US EPA (2000) and Wallace and Knight (2006).

In general, finer materials have been recommended in HF beds when low organic loads are applied since they provide a greater surface area for microbial biofilms. Moreover, results by García et al. (2005), who conducted an experiment at a pilot-scale system based on 8 HF wetlands (54-56 m² each) at the municipality of Les Franqueses del Vallés (Barcelona, Spain), to compare coarse ($D_{60}= 10$ mm, $C_u= 1.6$) to small granitic gravel ($D_{60}= 3.5$ mm, $C_u= 1.7$), as well as different hydraulic loading rates and water depths, show that finer gravel promotes a higher growth of the vegetation and in conjunction a higher removal of pollutants such as ammonia. However, it is important to have in mind that the finer the material, the greater the risk of clogging and hydraulic problems. As an example, soil based horizontal flow CWs started to be implemented in Austria in the 1980s for wastewater treatment in rural areas, and problems concerning the hydraulic conductivity took place, which resulted in clogging of the filter and poor performance (Haberl and Perfler, 1990).

During the 1990s the research focus changed to the development of VF sand based CWs with intermittent loading to promote nitrification whose standards are compiled in the Austrian Standard norms (ÖNORM B 2505, 2009). These systems typically have a sand layer of 0.06-4mm (Langergraber et al., 2003).

On the other hand, the structure of the bed media of VF wetlands typically consists of different layers of materials that gradually increase in size from the top to the bottom of the bed. As an example, typical Danish systems (Brix and Arias, 2005) consist of an insulation layer of 0.20 m followed by a thick layer of sand ($D_{60} = 1-4$ mm, $C_u < 3.5$; <0.5% clay and silt, washed materials), which constitutes the major part of the bed (around 1.0 m deep), and a drainage layer of 0.2 m of gravel. These are very similar to those reported by Masi et al. (2007) in Italy and also to the ones applied by Weedon (2003) in the UK (Table 1.7).

On the contrary, commonly employed VF systems at France are lacking the insulation layer, and they have a main layer of fine gravel (2-8 mm), followed by a layer of gravel (5 mm), just about 0.10-0.20 m deep. However, French systems differ greatly from the others since receive raw wastewater (without

any primary treatment) and usually three beds in parallel alternating operation are implemented (one of them is fed for 4 days while the others rest for about a week (Molle et al., 2008). Indeed, these multiple-cell wetland network is designed to accumulate a solids layer on the surface of the bed, through which is beneficial for treatment performance, without being detrimental to hydraulic performance (Chazarenc and Merlin, 2005).

A study carried out at a full-scale experimental plant at Aurignac (France) by Torrens et al. (2009), which comprises the two-year evaluation of six French-style VF systems in parallel (50 m² each) receiving the effluent from a facultative pond, found that the crushed sand beds performed significantly worse than those using river sand. They attribute it to the shape of the crushed sand (more angular), which would make the attachment of the biomass more difficult. This data is supported by data exhibiting lower biomass content in the systems having crushed sand. Moreover, in Estonia Öövel et al. (2007) have reported light-weight-aggregate (LWA) as the bed material of the VF wetlands. All systems coincide in that they have a drainage layer of coarser material at the bottom so as to avoid clogging. Further details on the structure and grain size of the main types of VF beds applied at different parts of Europe can be found in Table 1.7.

Table 1.7. Media specifications of several variants of vertical flow beds implemented at full-scale for the treatment of domestic wastewater worldwide.

Design	Weedon (2003) -UK-	Brix and Arias (2005) -Denmark-	Öövel et al. (2007) -Estonia-	Masi et al. (2007) -Italy-	Molle et al. (2008) -France-
Structure and grain size of the layers of the beds (from top to bottom)	0.15 m insulation layer; 0.80 m layer of sand-gravel (0.5 - 9 mm); 0.05 m layer of gravel (10 mm); 0.15 m drainage layer of stone (40 mm)	0.20 m insulation layer of wood pieces or sea shells; 1.0 m layer of sand (D ₆₀ = 1-4 mm); 0.20 m drainage layer of coarse gravel (8 - 16 mm) with passive aeration pipes	0.20 m layer of soil and grass; geomembrane permeable to water; 0.30 m layer of LWA (light-weight aggregates of 2 - 4 mm); 0.50 m layer of LWA (10-20 mm)	0.10 m gravel layer (4 - 8 mm); 0.60 m sand layer (0.06 - 4 mm); 0.20 m drainage layer (4 - 8 mm)	> 0.30 m main layer of fine gravel (2 - 8 mm); 0.10 - 0.20 m gravel layer (5 mm); 0.10 - 0.20 m drainage layer (20-40 mm) with passive aeration pipes

As it can be observed, the size of the layers varies for different VF variants, but in general it is established to use a top layer of fine material such as sand or fine gravel that will act as a filter for suspended solids and will decelerate the downflow, allowing for a better distribution of the wastewater (Molle et al., 2006; Torrens et al., 2009; Stefanakis and Tsihrintzis, 2012). However, especially at the non-French style VF systems, where just a single cell is applied, the selection of this layer is of crucial importance because, if too fine, it might cause a fast clogging of the surface of the wetland (Cooper, 2003).

Studies like the one carried out by Prochaska et al. (2007) evaluate the effect of the nature of the gravel on the treatment efficiency of pilot-scale VF wetlands, which compared the effect of the addition of dolomite to sand to the treatment performance. Nevertheless, the addition of dolomite did not significantly improve the overall phosphorus removal. Neither did the use of special materials such as zeolite and bauxite improve the performance of some pilot-scale VF wetlands (Stefanakis and Tsihrintzis, 2012). The use of materials with special properties would be more appropriate for their use in HF or FWS wetlands, where a longer contact time is applied. The nature of the granular material

is especially important for non-biodegradable organic pollutants, as it can play a major role in their removal through sorption phenomena (Dordio et al., 2009; Dordio and Carvalho, 2013).

1.2.3.3. Use of active aeration

One of the limitations of HF wetlands is their low oxygen transfer capacity (OTC). With the realization of this fact, active aeration aroused in North America as a practice that improves the OTC and therefore leads to an increase of the treatment performance of these types of systems. Wallace et al. (2006) reported a significant improvement of the system in terms of BOD and TSS removal when the HF beds were aerated. Aerated wetlands consist of a network of drip-irrigation tubing on the bottom of the wetland cell that is connected to an air blower. The blower is sized to provide to meet the nitrogenous and biochemical oxygen demand of the incoming wastewater. However, active aeration represents an added-up cost of operation and maintenance, which is just justified if the improvement in the performance of the wetland, and therefore reduced wetland size needed, offsets the cost of aeration equipment and energy needs (Kadlec and Wallace, 2006; Nivala et al., 2012b).

A variation of the VF wetland recently implemented at the treatment facility of Langenreichenbach (UFZ, Germany) consists of an actively aerated saturated VF wetland. The difference with the aerated HF wetlands is that the feeding is done from the top and draining is done from the bottom. Since this type of system will be objective of study of the current thesis (Chapter 9), further details on it can be found in Nivala et al. (2013).

To the best of our knowledge still there are no comparative analysis on the use and benefits of active aeration in CWs. However, Nivala et al. (2007) showed the performance of an aerated CW for the treatment of landfill leachate generated at small landfills at Iowa, a fairly cold climate. Though it was necessary to upgrade the system with a pretreatment chamber for iron removal since the aeration pipes and holes became clogged with ferric hydroxide precipitates, the system performed very well after the renovation with removal rates for BOD₅ and NH₄-N, which were above 90% most of the time. At the period where the system was not properly aerated (6 months), removal efficiencies were sporadic for BOD₅, ranging from 0 to 100%.

1.2.3.4. Hydraulic loading rate

Hydraulic loading rate (HLR) is inversely proportional to the hydraulic retention time for a given wetland depth, and varies from site to site and also depending on the wetland configuration. Due to the loading-resting operational regime implemented in most VF wetlands, this type of systems can accept significantly higher HLRs (and hence organic loading rate (OLR)) compared to the other types of configurations (HF and FWS). In Table 1.8, the organic loading rates applied at the constructed wetlands mentioned in Table 1.7 are shown.

Table 1.8. Organic and hydraulic loading rates applied at different typical vertical subsurface flow constructed wetlands at full-scale.

Weedon (2003) -UK-	Brix and Arias (2005) -Denmark-	Öövel et al. (2007) -Estonia-	Masi et al. (2007) -Italy-	Molle et al. (2008) -France-
OLR: 42 g BOD ₅ m ⁻² d ⁻¹ ; 80 g COD m ⁻² d ⁻¹	OLR: 20 g BOD ₅ m ⁻² d ⁻¹	OLR: 3-8 g BOD ₇ m ⁻² d ⁻¹	OLR: 3 g COD m ⁻² d ⁻¹	OLR: 180 g COD m ⁻² d ⁻¹ ;
HLR: 0.039 m d ⁻¹	HLR: 0.05 m d ⁻¹	HLR: 0.03 to 0.08 m d ⁻¹	HLR: 0.018 m d ⁻¹	HLR: 0.37 m d ⁻¹

The 216-m² VF wetland operated at a schoolhouse in Estonia received variable HLRs depending on its use, and values ranged 0.03 to 0.08 m d⁻¹ (Öövel et al., 2007). Additionally, Weedon (2003) reported a HLR of 0.039 m d⁻¹ in VF wetlands in UK and found how bed blockage was triggered by an increase of 6 times the typical dry weather flow, occurring after periods of prolonged rainfall.

In a study carried out by Prochaska et al. (2007) different hydraulic loads were compared (0.08 and 0.17 m³ m⁻² per batch, every 2-3 days) at VF units at laboratory scale (0.24 m²), and results showed that under the low HLR higher removal efficiencies were achieved for various water quality parameters (e.g. average COD effluent concentrations were 9 mg L⁻¹ and 37 mg L⁻¹, respectively). Moreover, Stefanakis and Tsihrintzis (2012) carried out monitoring of pilot-scale VF wetlands (0.57 m² each) for three consecutive years, applying HLRs of 0.19, 0.26 and 0.44 m d⁻¹, in the three consecutive years, respectively. They found the system to be more stable in terms of BOD₅ and COD removal during the second and third year despite the higher loads, and highlighted the capacity of VF beds of receiving high loads when operating as the first treatment step (up to 200 g COD m⁻² d⁻¹).

In regards to HF wetlands, findings from García et al. (2005) at the treatment plant of Les Franqueses del Vallès abovementioned, involving several wetland cells, which received different HLRs (0.02, 0.036, and 0.045 m d⁻¹), found that HLR was controlling the effluent concentrations of ammonium, COD and BOD₅, showing a higher removal efficiency at lower loads. More recently, Zhang et al. (2012a) investigated six mesoscale HF wetlands (0.72 m²) fed with synthetic wastewater and spiked pharmaceuticals (including IB and DCF), operating at HLRs of 0.06 m d⁻¹ (HRT: 2 d) and 0.03 m d⁻¹ (HRT: 4 d), and removal efficiencies did not seem to differ much.

However, it is important to note that studies which evaluate the effect of operating the system under a certain HLR (as well as other operational parameters) on the treatment performance, as well as those which aim at assessing clogging in CWs, are usually carried out at an early stage of the life of the wetland or for a period of time which is too short to properly evaluate the development of clogging within the bed. Clogging may result in hydraulic malfunction and/or reduced treatment performance, and this has been shown to happen in systems as young as eight years old. This phenomenon is estimated through field-based methods based on hydraulic conductivity, tracer testing and physico-chemical characterization of clog matter. Models which can predict clogging are still in a stage of development (Samsó and García, 2013) and should eventually be improved so as to allow assessment of clogging dynamics under varying scenarios. Still the matter remains little understood, and so to date the best approach involves to minimize its development through improved design and operational and maintenance practices (Nivala et al., 2012a).

1.2.3.5. Intermittent loading frequency and loading-resting cycles

Most VF wetlands are pulse-loaded at a rapid rate, where the water is discharged as a flood on the bed surface and is freely drained at the bottom of the media. The number of doses per day is as variable as are VF wetland configurations across Europe. As an example, in France most of these vertical systems are fed with 8 pulses of water per day (Molle et al., 2008), while other authors like Brix and Arias (2005) have documented as many as 16-24 pulses per day in Denmark, but also in this case half of the effluent is recirculated to the start point.

As the feeding frequency is decreased, in order to allow for a longer time for mineralization, a greater volume is discharged at every pulse. Then the HRT is also decreased and there is reduced contact time between media and pollutants. Nevertheless, at higher feeding frequencies (and lower volume per pulse), the OTC into the bed can get reduced and result in lower nitrification rates in VF beds (Torrens et al., 2009).

On the other hand, HF wetlands can also be operated at loading-resting cycles. This case differs from the one in VF wetlands in that the HF beds would be saturated, while the VF would not. This strategy also aims at maintaining, as much as possible, aerobic conditions within the wetlands, and in fact a higher performance of this strategy over the continuous-feeding strategy have been previously shown (Stein et al., 2003; Caselles-Osorio and García, 2007). This is also the case of the operation strategy of a treatment line in one of the experimental treatment plants that was used for experiments for this thesis. The NEWWET treatment plant (Section 4.1.1.) has a treatment line consisting of two HF wetlands operated with cycles of 4 days of filling-resting-draining phases. Pedescoll et al. (2011b), have reported a higher performance in terms of COD and ammonium removal under this strategy, as opposed to the continuous one, particularly in winter (up to 50% higher than a continuously fed system). Additionally, Zhang et al. (2012b) evaluated the same strategy in HF beds (0.72 m²), exhibiting significantly better performance on pharmaceutical removal (including IB and DCF, among others) under batch operation as compared to continuous operation.

1.2.3.6. Water depth

Several investigations have clearly demonstrated that water depth constitutes a key design parameter affecting the redox condition, oxygen supply and hence the removal efficiency of constructed wetlands.

Results of the study carried out by García et al. (2005) at the pilot-scale HF wetland system at Les Franqueses del Vallès (Barcelona), showed how wetlands with a mean water depth of 0.27 m, performed consistently better in terms of COD, BOD₅ and ammonia removal than those with a water depth of 0.5 m. Findings indicate that water depth influences biochemical reactions responsible for the degradation of organic matter.

During a continuous injection experiment carried out at this same experimental facility, which counted on HF cells of about 54-56 m² each, Matamoros et al. (2005) showed how the removal efficiency for IB was significantly higher (81%) in the shallow than the deeper bed (48%). Redox potentials were higher in the shallow (-144 to -131 mV) than in the deep ones (from -183 to -151 mV), which would promote more energetically favorable biochemical reactions, leading to a higher removal of organic matter as well as other pollutants.

In fact, results by Huang et al. (2005) at the same pilot plant at Les Franqueses, which examines the behavior of volatile fatty acids and volatile alkylsulfides, arrive to the same conclusions. Water depth appears to be a determining factor in HF wetlands, which affects surface reaeration, which in turn drives the redox conditions and biochemical reactions occurring within the beds. Shallower wetlands appear to have higher redox potential and thus promote more variate and energetically favorable reactions. Other authors have shown evidence, which support this hypothesis (Headley and Davison, 2005; Matamoros and Bayona, 2006; Tietz et al., 2007).

However, this may be different and less relevant for VF wetlands. Results provided by Torrens et al. (2009) in parallel VF beds in the full-scale treatment system at Aurignac (French style VF wetlands), which compared 25-cm filters to 65-cm ones, found how the deeper ones performed significantly better than the shallower ones in respect to all physicochemical parameters, especially organic matter. The shorter retention time in shallower beds (1 to 4 h) as compared to the longer (4 to 11 h) found in the tracer tests, would be responsible for reduced treatment efficiency.

At laboratory scale, Song et al., (2009), which compared three VF microcosms (0.15 m²) with various depths of 7.5, 30 and 60 cm, respectively, on the removal of various estrogens, including EE2, found how shallower VF wetlands increased removal efficiency of all studied compounds. Conditions of

unsaturation in respect to an operation under permanently water-saturated conditions also enhanced treatment performance.

1.2.3.7. Vegetation

The role of vegetation in CWs has proven to be of great importance as plants participate in the assimilation of nutrients, provide surface for biofilm growth, pump and provide oxygen to the belowground zone of the systems, retain suspended particles and insulate against low temperatures. Although plant uptake represents a relatively small proportion of total nutrient removal, plants play a major role in enhancing nitrification and denitrification activities due to root-zone aeration and supply of organic matter (Tanner, 2001). These phenomena seem to prevail more in HF wetlands as compared to the VF wetland type (Torrens et al., 2009).

Commonly found plant species in CWs include the common reed (*Phragmites australis*), cattail (*Typha angustifolia*), but the list is very large (Vymazal, 2013).

Additionally, the type of plant species does not seem to affect much the performance of VF systems (Stefanakis and Tsihrintzis, 2012), contrarily to what has been found in HF and FWS wetlands (Akratos and Tsihrintzis, 2007; Kotti et al., 2010). Hijosa-Valsero et al. (2010b) studied the presence of vegetation, as well as the type of vegetation (*P. australis* vs. *T. angustifolia*) in several mesoscale HF wetlands (located at a WWTP facilities in León, Spain) (of about 1 m²), and found that the presence of plants enhanced the removal efficiency of various PPCPs, such as IBU, DCF, and AHTN. In this study, *P. australis* showed better performance, at least during summer season.

There is a good bunch of literature evaluating the influence of plant species in treatment performance (Brisson and Chazarenc, 2009). In general, differences have been found to take place (occasionally large) between different macrophyte species at studies at all scales, suggesting that vegetation type does matter. However, results from different studies are sometimes contradictory and oftentimes little effort is done to elucidate the mechanisms that could explain that one species is better than other, and thus the topic deserves better attention.

1.2.3.8. Seasonality

Several authors have repeatedly observed the variability of treatment performance of constructed wetlands depending on the season. Recently Matamoros and Salvadó (2012) evaluated the effect of the season at a tertiary treatment restoration wetland consisting of two parallel polishing ponds (2 ha surface area, about 1 m water depth) and a three parallel FWS cells (0.8 ha each, 0.5 m water depth) on the removal of a number of EOCs, and found that removal efficiencies were generally greater during the warm season. This effect was especially prevailing in the ponds.

The study by Hijosa-Valsero et al. (2010b), which evaluated the removal of PPCPs (including IB, DCF and AHTN) at several HF mesocosms (León, Spain) containing different design and modes of operation, assessed the seasonal variability in treatment performance and found that consistently better performances in summer than in winter for almost all of the studied compounds. Reyes-Contreras et al. (2012) reported similar findings in the same treatment system. Moreover, Hijosa-Valsero et al. (2011b) studied the seasonality of the treatment systems in León as well as the system in Barcelona (in the context of the NEWWET project) and observed that water temperature was crucial for a good removal of PPCPs. In simple words, colder season means colder water temperature, which will in turn affect the rates at which microbial populations degrade pollutants, which is especially evident for organic matter and nitrogen compounds. The dependence of CWs treatment performance on water temperature was also examined by Stefanakis and Tsihrintzis (2012).

2. Objectives and structure

A major challenge for the development of the constructed wetland technology is to improve our understanding of process mechanisms occurring within the wetlands, and which design and operational factors are affecting these, so as to optimize their treatment performance.

Most of the scientific evidence on the behavior of emerging organic contaminants in constructed wetlands is based on studies aiming at monitoring their occurrence and removal, while just a few attempt at finding which are the key design and operational factors influencing treatment performance. Moreover, while most of the comparisons are based on non-replicated units, well-replicated controlled experiments are needed so as to obtain more reliable outcomes on the topic.

The major objective of the present thesis dissertation is to evaluate the potential of constructed wetlands of different configurations for the removal of emerging organic contaminants in urban wastewater.

In order to optimize this ecotechnology, the influence of design and operational parameters of constructed wetlands should be further addressed.

For that purpose, the specific objectives of this thesis are:

- To assess the behavior in different wetland configurations (i.e. horizontal subsurface flow vs. vertical subsurface flow vs. free water surface constructed wetlands) on the removal of emerging organic contaminants.

- To evaluate the effect of the type of primary treatment implemented before the wetlands (i.e. conventional settler vs. hydrolytic upflow sludge bed (HUSB) reactor) on the removal of emerging organic contaminants.

- To study the influence of different design and operational factors (i.e. operation strategy—permanently saturated vs. cycles of saturation and unsaturation—, loading frequency, hydraulic loading rate, use of active aeration, grain size) on the removal of emerging organic contaminants.

Target emerging organic contaminants consisted of widespread used compounds often found in urban wastewater, including mostly pharmaceuticals and personal care products as well as endocrine disrupting compounds. These were three non-steroidal anti-inflammatory drugs (ibuprofen, acetaminophen and diclofenac), three personal care products (tonalide, triclosan and oxybenzone), and two endocrine disrupting compounds (ethinylestradiol and bisphenol A).

To attain these objectives, several experiments at both experimental and pilot-scale were carried out at treatment systems based exclusively on constructed wetlands. These were located in Barcelona, Seville (Spain) and Leipzig (Germany).

Concentrations of organic micropollutants found at the influent wastewater are often very variable, since their disposal and consumption varies a lot depending on the time of the day and period of the year. Therefore, and given the hydraulic retention time of the treatment systems (which is usually in the range of several days), it is challenging –if not impossible– to assess the removal efficiency of the treatment system at a given moment. Thus, in order to achieve approximate steady state conditions of the influent concentrations of the target compounds and to obtain a more reliable estimate of the removal efficiency of the systems, continuous injection experiments of emerging organic contaminants were performed, when possible.

The study shown in Chapter 3, served as the development of the methodology of injection of emerging organic contaminants in constructed wetlands, which was carried out at a HF wetland system in Barcelona having a HUSB reactor as a primary treatment. Once the experimental setup was calibrated, we proceeded with an injection of emerging organic contaminants in three treatment lines of HF constructed wetlands of the same experimental treatment plant (Chapter 4), differing in their primary treatment and mode of operation (alternation of saturated-unsaturated conditions vs. permanently saturated).

What is more, a meso-scale hybrid constructed wetland system consisting of a combination of VF, HF and FWS wetlands operating in series was designed, constructed at the UPC facilities in Barcelona with research purposes. In Chapter 5, an introduction to this treatment system and treatment performance in terms of conventional water quality parameters during a one-year monitoring period is covered. Moreover, Chapter 6 shows the injection of emerging organic contaminants and antibiotics, as well as a toxicity assessment, at the same treatment system when being submitted to different hydraulic loading rates. The performance under dry and wet weather conditions (and the evaluation of a first-flush event) in terms of water quality parameters of a full-scale hybrid constructed wetland system of the same cited configuration located in Seville when treating combined sewer effluent is evaluated in Chapter 7. Chapter 8 shows the results of a monitoring campaign evaluating the behavior emerging organic contaminants in that same treatment system. The effect of feeding regime, grain size, and aeration on the removal of water quality parameters and emerging organic contaminants was evaluated in VF constructed wetlands at mesoscale at the treatment facility located in Leipzig (Germany) and results are shown in Chapter 9. Chapters 3-9 correspond to peer-reviewed articles, which have been published during the research period, or have been sent and are under review. Chapter 10 contains a general discussion of all results, and finally, in Chapter 11 the conclusions of the doctoral thesis are presented.

3. Capacity of an experimental horizontal subsurface flow constructed wetland system for the removal of emerging organic contaminants: an injection experiment



This chapter is based on the article:

Ávila, C., Pedescoll, A., Matamoros, V., Bayona, J.M., García, J., 2010. Capacity of a horizontal subsurface flow constructed wetland system for the removal of emerging pollutants: An injection experiment. *Chemosphere* 81, 1137-1142.

A continuous injection experiment was implemented in a pilot-scale horizontal subsurface flow constructed wetland system to evaluate the behavior of four pharmaceuticals and personal care products (i.e. ibuprofen, naproxen, diclofenac and tonalide) and for the first time a phenolic estrogenic compound (i.e. bisphenol A). The treatment system consisted of an anaerobic reactor as a primary treatment, followed by two 0.65 m² wetlands (B1 and B2) working in parallel and connected to a 1.65 m² wetland (B3) operating in series. Overall removal efficiencies for the selected compounds ranged from 97 to 99%. The response curves of the injected pollutants show that the behavior of these compounds strongly depends on their sorption and biodegradation characteristics. While about 50% of ibuprofen was removed in B1 and B2, 99% was achieved at B3, where the dissolved oxygen concentration was significantly higher (O₂ B1-B2 = 0.5 mg L⁻¹ and O₂ B3= 5.4 mg L⁻¹). Naproxen and diclofenac were efficiently removed (93%) in B1 and B2, revealing anaerobic degradation as a probable removal mechanism. Moreover, tonalide and bisphenol A were readily removed in the small wetlands (94% and 83%, respectively), where the removal of total suspended solids was 93%. Therefore, given their high hydrophobicity, sorption onto the particulate matter stands for the major removal mechanism. However, the tentative identification of carboxy-bisphenol A as an intermediate degradation product in B3 suggested biodegradation as a relevant bisphenol A removal pathway under aerobic prevailing conditions.

3.1. Introduction

During the last decade, the occurrence of organic micropollutants in the environment has attracted great interest since a generalized concern arise about the possible undesirable effects of many of these pollutants in the environment and to living organisms (Cunningham et al., 2006). These trace pollutants, usually known as EOCs, mainly consist of compounds of anthropogenic origin such as pharmaceutical and personal care products (PPCPs), pesticides, surfactants and plasticizers that are continuously discharged into the environment as a result of consumer activities, waste disposal, accidental releases and purposeful introduction (Daughton 2004). There is a high degree of toxicological evidence of these compounds in the aquatic environment as well as in humans. As example, BPA, present in polycarbonate polymers and PVC stabilizer, represents an EOC of public health concern because of its reported association with the increase of cancer risk in humans (Keri et al., 2007).

One of the main sources of these pollutants into the environment is the discharge of effluents from WWTPs, where their removal is often incomplete (Heberer, 2002). Constructed wetlands (CWs) constitute an alternative cost-effective technology for the treatment of urban wastewater that has attracted increasing interest in the last decades in the context of small communities with less than 2000 PE (Puigagut et al., 2007). While the role of WWTPs and other advanced treatment technologies for the removal of these organic compounds, such as ozonation and membrane bioreactors has been examined (Kimura et al., 2005), available information about the performance of CWs is currently limited, and most of the literature is basically related to herbicides, pesticides and surfactants (Schulz and Peall, 2001). Just recently the occurrence and behavior of EOCs such as PPCPs in these systems have attracted increased attention (Matamoros and Bayona, 2006; Matamoros et al., 2008a; Dordio et al., 2009).

The goal of this study was to evaluate the response curves at steady state and the removal behavior of three non steroidal antiinflammatory drugs (NSAIDs) named IB, naproxen (NPX) and DCF, a musk fragrance (AHTN) and for the first time a phenolic estrogen (BPA) in a pilot-scale HF CW system fed with urban wastewater. In order to achieve steady state conditions and to obtain a more reliable estimate of the removal efficiency of the system, a continuous injection experiment was implemented, where bromide was used as a conservative tracer. Furthermore, intermediate degradation products of the selected injected compounds were tentatively identified in order to get a further insight in the processes involved in the pollutant removal.

3.2. Materials and methods

3.2.1. Description of the treatment system

The pilot-plant consisted of a mesoscale HF wetland system located at the Technical University of Catalonia Campus (Barcelona, Spain). It started operation in March 2007 and received wastewater from a nearby municipal sewer. The water was first coarsely screened, before it flowed into a hydrolytic upflow sludge bed reactor (HUSB) as primary treatment. The effluent of the reactor was split into two small HF beds (B1 and B2) with a surface area of 0.65 m² each, working in parallel. Finally, the effluent of these two units flowed into a 1.65 m² HF wetland operated in series (B3). The final effluent was discharged into a tank (flow meter) with a water sensor level that was activated every 5 L effluent (Fig. 3.1). Flow measurements were recorded on a daily basis.

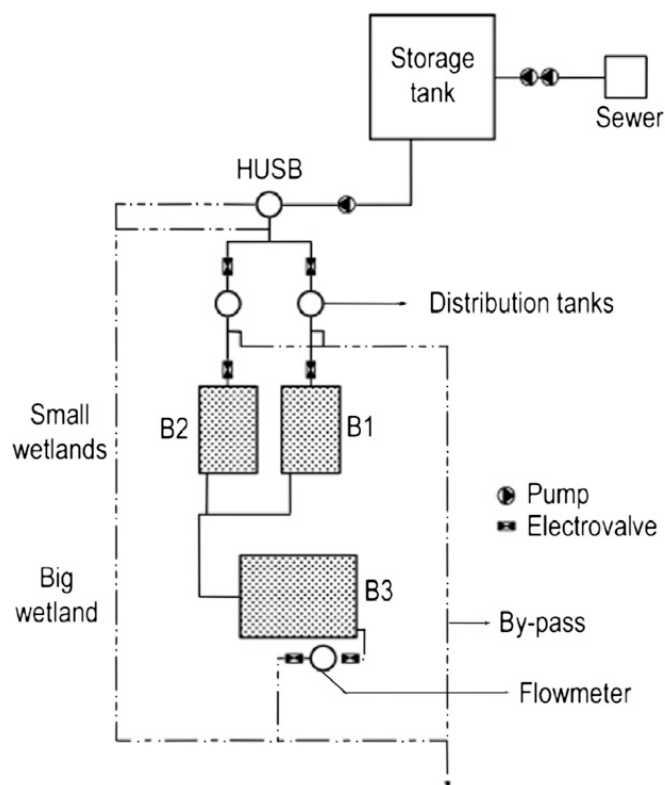


Figure 3.1. Layout of the experimental horizontal subsurface flow constructed wetland system in Barcelona.

The granular medium of the wetlands consisted of a 30 cm high gravel layer with a D_{60} of 5 mm and an initial porosity of 40%. Water depth was maintained at 25 cm and all of the wetlands were planted with *Phragmites australis*. The system received a total flow of 84 L d^{-1} (hydraulic loading rate = 0.028 m d^{-1}) and was designed to treat an organic loading rate of $4.7 \pm 1.5 \text{ g BOD m}^{-2} \text{ d}^{-1}$. The theoretical hydraulic retention time was 3.5 days (Corzo et al., 2008).

3.2.2. Experimental design

For the injection of the target compounds, a glass bottle of 20 L of distilled water was spiked with IB, NPX, DCF, AHTN and BPA, which were previously dissolved in methanol. Potassium bromide (KBr) was also added as a conservative tracer. This mixture was homogenized and injected into the distribution pipes of the influent of the two parallel wetlands using two peristaltic pumps that supplied a flow rate of 3.2 L h^{-1} each, obtained from Damova (Barcelona, Spain) which were synchronized to the primary effluent discharge of the HUSB reactor). Note that pollutants were injected at the effluent of the HUSB reactor and not at the influent of the entire system. Flow rates of the injection pumps were adjusted so as to obtain the desired influent concentration of the EOCs and the tracer (i.e. IB = $75 \mu\text{g L}^{-1}$; NPX = $30 \mu\text{g L}^{-1}$; DCF = $2.5 \mu\text{g L}^{-1}$; AHTN = $1.25 \mu\text{g L}^{-1}$; BPA = $1 \mu\text{g L}^{-1}$; KBr = 15 mg L^{-1}). Those concentrations corresponded to 250% of the maximum concentration of the EOCs detected in previous campaigns (Hijosa-Valsero et al., 2011b). The injection experiment was run for 24 days throughout May 2009.



Figure 3.2. View of the doped solution, which was not exposed to light (left), the peristaltic pumps (center) and the injection wires which delivered the solution in the inlet pipe of the horizontal subsurface flow constructed wetlands (right).

3.2.3. Sampling strategy

In order to reach steady state conditions and to obtain a more reliable estimate of the removal efficiency of the system, sampling started once the concentration of the tracer (bromide) had stabilized at the effluent of the system. Then, 12h-composite samples of the effluent of each constructed wetland were collected by taking 80 mL of sample every four hours, three times a day. Samples of the effluent of the HUSB reactor were also taken into account target compounds concentration in the influent wastewater. Sampling was carried out four times a week for two weeks (n=8) during May 2009. All samples were collected in 250 mL amber clean glass bottles, which were transported refrigerated to the laboratory where they were stored at 4 °C until analysis. The sample holding time was less than two days. Samples were analyzed for the organic micropollutants and conventional water parameters as described below. Note that during the whole experiment, no rainfall events were recorded. What is more, the registered flow rates at the effluent of the system were much lower than the expected ones. For that reason, evapotranspiration rates were calculated and correction factors were applied as explained in Section 3.3.1.

3.2.4. Chemicals

Gas chromatography (GC) grade (Suprasolv) hexane, ethyl acetate, methanol and acetone were obtained from Merck (Darmstadt, Germany) and analytical-grade hydrochloric acid was obtained from Panreac (Barcelona, Spain). Analytical grade ($\geq 98\%$) ibuprofen, naproxen, diclofenac, tonalide, bisphenol A, 2,2'-dinitrophenyl, dihydrocarbamazepine, and triphenylamine were purchased from Sigma-Aldrich (Steinheim, Germany). The 2,4,5-trichlorophenoxypropionic acid (2,4,5-TPA) was obtained from Riedel-de Haen (Seelze, Germany); potassium bromide and trimethylsulfonium hydroxide (TMSH) were supplied from Fluka (Buchs, Switzerland) and 0.7 μm glass fiber filters of $\phi = 47$ mm (GF/F) were purchased from Whatman.

3.2.5. Analytical methodology

Conventional wastewater quality parameters, including ammonium, TSS and COD were determined by using Standard Methods (APHA-AWWA-WPCF, 2001). Sulfate (SO_4^{2-}) and bromide (Br^-) were analyzed using a DIONEX ICS-1000 ion chromatographer (IC). Onsite measurements of water temperature, dissolved oxygen (DO), pH and electrical conductivity (EC) were taken using a Checktemp-1 Hanna thermometer, a Eutech Ecoscan DO6 oxymeter, a Crison pH-meter and an EH CLM 381 conductivity meter, respectively. Redox potential (E_H) was also measured in situ by using a Thermo Orion 3 Star redox meter. E_H values were corrected for the potential of the hydrogen electrode.

The concentrations of the selected EOCs in wastewater samples were analyzed after the samples had been filtered and processed as previously described by Matamoros et al. (2005). More details on analytical procedure and analytical quality parameters are described in the Supplementary Material section.



Figure 3.3. View of the solid phase extraction and the GC-MS equipment used for the determination of target emerging organic contaminants.

3.2.6. Statistical analyses

The experimental results were statistically evaluated using the SPSS 13 package (Chicago, IL). Normal distributions were obtained by the Kolmogorov–Smirnov test after outliers were excluded from data sets. The concentration comparison between treatment systems corrected by evapotranspiration (Section 3.1) was analyzed by using the ANOVA test for two independent samples (parametric statistics) and the Tukey post-hoc. Statistical significance was defined as a $p < 0.05$.

3.3. Results and discussion

3.3.1. General parameters

During the experimental period, the existence of high temperatures and a dense plant biomass, lead to a high evapotranspiration rate and therefore fairly concentrated effluents were found. For this reason data have been corrected using bromide concentrations so as to take into account the effect of evapotranspiration. In order to ensure that no significant plant uptake of bromide occurred, a mass balance was calculated, and recoveries were always above 90%. The corrected and non-corrected values obtained along the different CW units for the main characteristics of the wastewater are shown in Table 3.1. The average removal efficiencies achieved for COD, TSS and ammonium in the two small CWs (B1 and B2) were 63, 93 and 67%, respectively and the overall removal efficiencies for the entire system were 81, 93 and 99%, respectively.

Table 3.1. Average concentrations and standard deviations of water quality parameters and injected emerging organic contaminants along the horizontal subsurface flow constructed wetland system in Barcelona (n=8). Values are corrected in respect to evapotranspiration rates. Non-corrected parameters are shown in parentheses. Any significant differences ($p < 0.05$) among treatment units are displayed in Table 3.2. (*) Concentrations marked with an asterisk correspond to the background plus the injected concentration of target compounds.

		HUSB	B1	B2	B3
Water quality parameters	Temperature (°C)	22.7±2.7	22.1±1.7	22.3±1.8	21.4±1.5
	DO (mg L ⁻¹)	<LOD	0.9±0.5	0.4±0.4	5.4±1.8
	pH	7.3-7.6	7.0-7.2	7.0-7.2	6.9-7.1
	E _h (mV)	-123.2±17.1	-97.3±13.2	-102.5±17.4	126.1±42.1
	EC (mS cm ⁻¹)	2.6±0.5 (2.6±0.5)	2.8±0.5 (4.2±0.4)	2.6±0.4 (4.1±0.4)	2.8±0.3 (7.9±1.5)
	COD (mg L ⁻¹)	255±42 (255±42)	90±23 (141±29)	98±14 (155±15)	47±7 (131±29)
	TSS (mg L ⁻¹)	48±5 (48±5)	3±1 (5±1)	3±1 (5±2)	3±1 (9±3)
	NH ₄ ⁺ (mg N L ⁻¹)	27.5±3.6 (27.5±3.6)	8.9±2.9 (13.3±3.4)	9.3±3.8 (14.4±4.7)	0.02±0.01 (0.06±0.03)
	SO ₄ ²⁻ (mg L ⁻¹)	102.2±25.9 (102.2±25.9)	70.2±27.6 (108.7±45.9)	20.0±10.6 (31.7±16.7)	115.7±33.4 (312.9±76.4)
Injected contaminants	Ibuprofen (µg L ⁻¹)	132.4±7.35*	53.1±13.2	56.5±14.0	1.5±0.5
	Naproxen (µg L ⁻¹)	35.7±0.8*	2.2±0.3	3.0±0.8	0.3±0.1
	Diclofenac (µg L ⁻¹)	3.2±0.1*	0.3±0.1	0.2±0.1	0.003±0.001
	Tonalide (µg L ⁻¹)	1.8±0.14*	0.1±0.1	0.1±0.1	0.04±0.01

3.3.2. Occurrence of emerging organic contaminants in raw wastewater

The target compounds were chosen according to their widespread use and high frequency of detection reported in previous studies (Matamoros and Bayona, 2006). Background concentrations of

the selected contaminants during the sampling campaign at the effluent of the HUSB reactor, that is, before injection, were detected in the range of 0.05-46.8 $\mu\text{g L}^{-1}$. The NSAIDs IB, DCF and NPX, were found in the ranges 23.6-46.8, 0.02-0.26 $\mu\text{g L}^{-1}$ and 1.53-3.94 $\mu\text{g L}^{-1}$. The obtained values are in accordance with those reported by different authors in domestic raw wastewaters of several countries (Kosma et al., 2010). The polycyclic musk, AHTN, was quantified with concentrations ranging from 0.14-0.50 $\mu\text{g L}^{-1}$. If we compare these with other studies reported in raw wastewater from Spain, these values are in conformity with the ones reported by Matamoros and Bayona (2006). The influent concentrations of BPA ranged from 0.005 $\mu\text{g L}^{-1}$ to 0.445 $\mu\text{g L}^{-1}$. Again these values fall within the range previously reported by Kasprzyk-Hordern et al. (2009) in the UK.

3.3.3. Removal of selected emerging organic contaminants

Table 3.1 summarizes EOCs concentrations at each treatment unit of the experimental system. These values were corrected by evapotranspiration factors as mentioned in Section 3.3.1.

In general, all of the selected compounds were efficiently removed, with overall efficiencies ranging from 97 to 99%. Removal efficiencies for the selected compounds range from 98 to 99 for IB, 99 for NPX, 97 to 98 for AHTN and 85 to 99% for BPA. DCF stands out for its high elimination rates, around 99% in most cases. Overall, these high rates are attributable to the high influent concentrations of these compounds due to the injection as well as high spring temperatures at the time of the experiment, enhancing biodegradation processes and/or plant uptake. Furthermore, these efficiencies are comparable to those documented for IB by Gómez et al. (2007) by membrane technologies. Conversely, the high elimination rates of DCF completely contrast with its recalcitrance reported by Matamoros and Bayona (2006) in HSSFCWs. Regarding the synthetic fragrance AHTN, the removal efficiencies are slightly higher than the 85% reported by Rosal et al. (2010) in a Spanish WWTP. The elimination achieved for BPA is in accordance with those reported for a WWTP in the UK (Kasprzyk-Hordern et al., 2009). However, the removal efficiencies for naproxen are not directly comparable to those detected in WWTPs since the inlet concentration of these contaminants is usually lower than in our experiment.

Table 3.2. P-values obtained among the treatment units of the horizontal subsurface flow constructed wetland system by the statistical analysis of corrected concentrations of water quality parameters and injected contaminants. *Statistically significant differences at a significance level of 0.050.

		HUSB vs B1	HUSB vs B2	HUSB vs B3	B1 vs B2	B1 vs B3	B2 vs B3
Water quality parameters	DO	0.224	0.788	0.000*	0.731	0.000*	0.000*
	pH	0.000*	0.000*	0.000*	0.999	0.514	0.584
	E _h	0.182	0.359	0.000*	0.975	0.000*	0.000*
	EC	0.000*	1.000	0.839	0.000*	0.000*	0.836
	COD	0.000*	0.000*	0.000*	0.946	0.012*	0.003*
	TSS	0.000*	0.000*	0.000*	1.000	1.000	1.000
	NH ₄ ⁺	0.000*	0.000*	0.000*	0.989	0.000*	0.000*
	SO ₄ ²⁻	0.083	0.000*	0.763	0.004*	0.014*	0.000*
Injected pollutants	IB	0.000*	0.000*	0.000*	0.915	0.000*	0.000*
	NPX	0.000*	0.000*	0.000*	0.070	0.000*	0.000*
	DCF	0.000*	0.000*	0.000*	0.342	0.000*	0.000*
	AHTN	0.000*	0.000*	0.000*	0.999	0.331	0.402
	BPA	0.000*	0.000*	0.000*	0.957	0.007*	0.025*

3.3.4. Behavior of selected emerging organic contaminants along the experimental constructed wetland system

Response curves at the steady state of the injected contaminants along the different units of the CW system are shown in Fig. 3.4. Effluent concentrations of the target compounds were normalized by their influent concentration (background plus injected contaminant). Furthermore, these values were corrected in respect to the calculated evapotranspiration rate (Section 3.3.1).

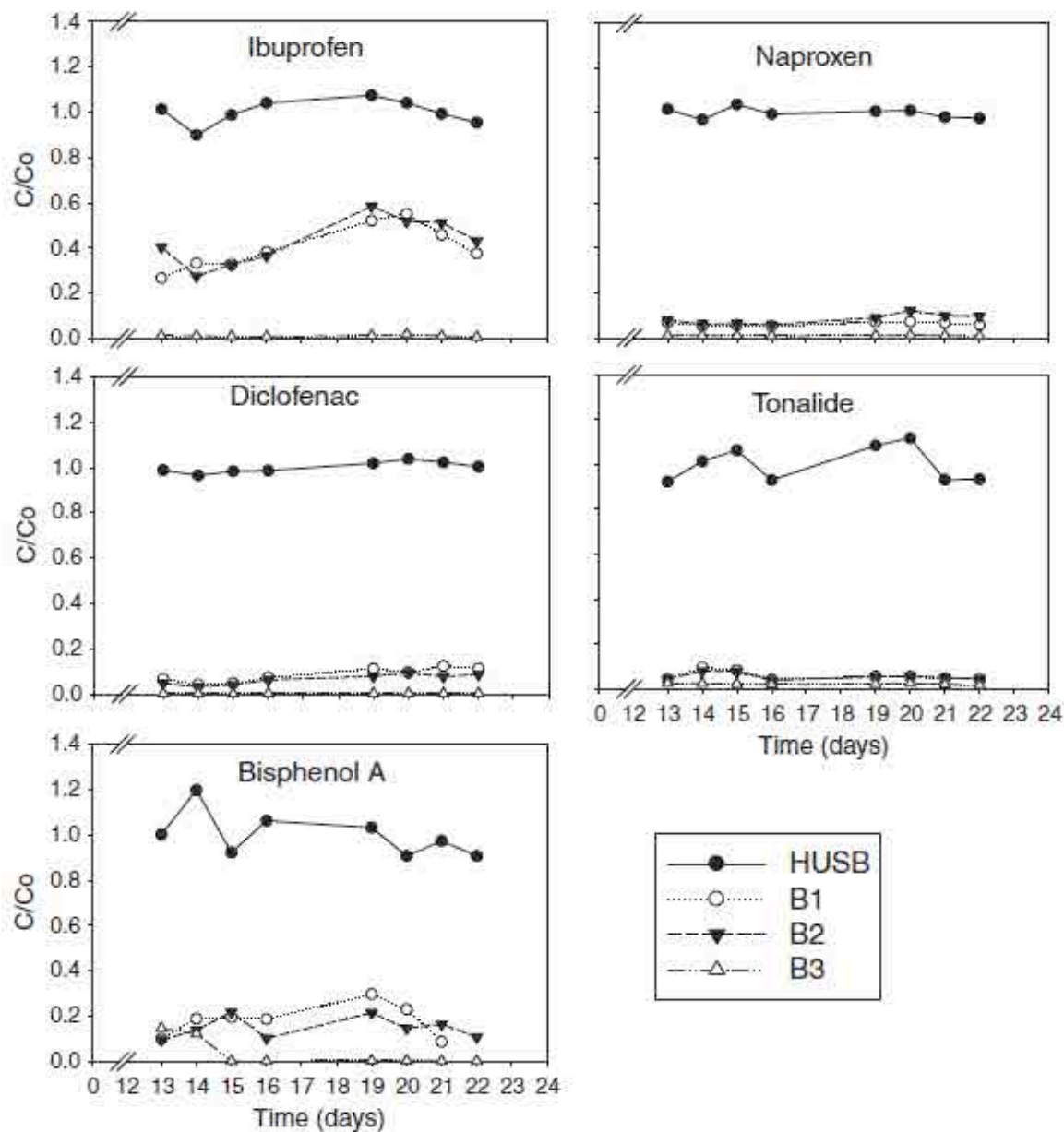


Figure 3.4. Concentration of the selected emerging organic contaminants normalized by their total influent concentration (background concentration + injected concentration) in the influent of the wetlands (effluent of the HUSB), effluent of the intermediate wetlands (B1 and B2) and of the final effluent of the horizontal subsurface flow constructed wetland system (B3). Initial concentrations (C_0) correspond to the HUSB concentrations of Table 3.1.

An analysis of the response curve of IB reveals that this contaminant's behavior clearly differs from those of the other compounds. It can be observed that while above 80% of the incoming concentration of all investigated compounds is readily removed in the first two wetlands (B1 and B2), only 50 to 60% of the incoming IB is eliminated. This behavior is presumably linked to its selective aerobic biodegradation pathway. From the observation of Table 3.1, it is noticeable that B1 and B2 are still working under anaerobic conditions, indicated by low values of DO and E_H , as well as a high degree of ammonium. Conversely, in the last constructed wetland (B3) the environment is already much more aerobic. Fig. 3.5 shows the correlation between DO and IB concentrations during the sampling campaign. This curve reveals a negative correlation between these two parameters, with a high correlation coefficient ($R^2=0.845$). These results are in accordance with IB's aerobic biodegradation dependence previously reported by Zwiener and Frimmel (2003). Moreover, no sorption of IB onto the organic matter retained in the substrate is expected by its low octanol-water partition coefficient (Table 1.1).

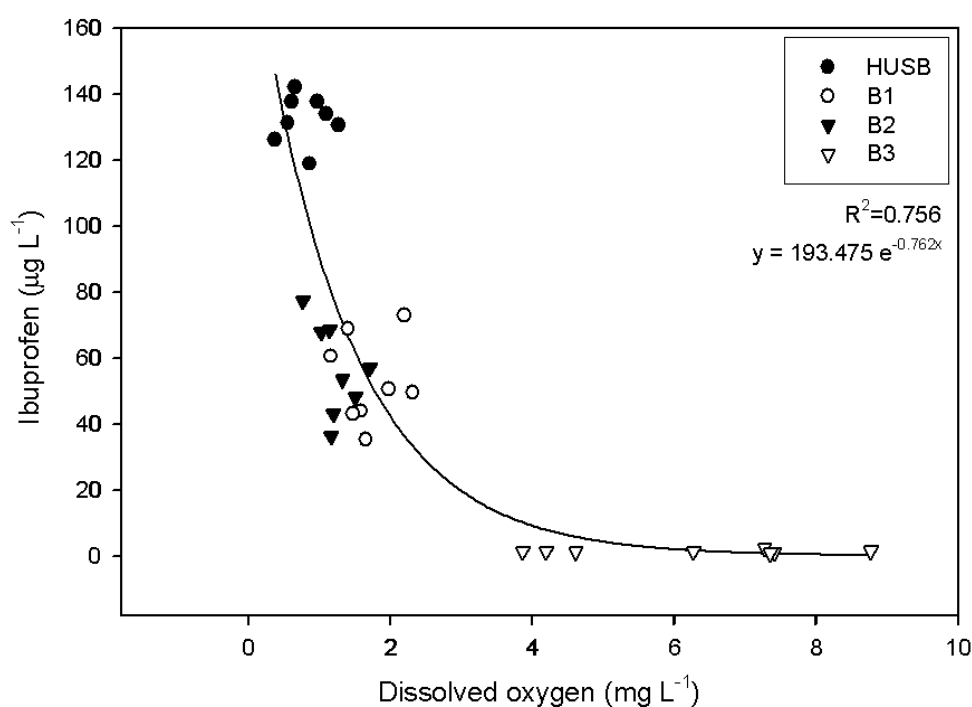


Figure 3.5. Nonlinear regression between ibuprofen and dissolved oxygen concentration at the different units of the experimental horizontal subsurface flow constructed wetland.

Regarding the behavior of naproxen, a very significant reduction of its concentration was already achieved in wetlands B1 and B2. A comparison of the removal of naproxen under two CWs with different redox potential values by Matamoros and Bayona (2006) reported a higher removal under more oxidized conditions. However, in our study naproxen removal was achieved under fairly reducing conditions. As far as the mechanism of removal is concerned, laboratory degradation tests performed by Quintana et al. (2005) showed naproxen to be removed by metabolic transformation. Sorption onto organic matter has not been considered as a removal pathway due to its high hydrophilicity (Table 1.1).

The analgesic DCF, a contaminant that has been reported several times as recalcitrant for a variety of wastewater treatment technologies (Quintana et al., 2005; Matamoros and Bayona, 2006) attained very high elimination rates in B1 and B2. Matamoros and Bayona (2006) have shown sorption onto the organic matter retained in the gravel not to constitute a major removal mechanism, given the hydrophilic character of its structure. Moreover, Kimura et al. (2005) have suggested that the presence of chlorine in its molecular structure makes it difficult to be degraded. However, research shows that polyhalogenated compounds such as DCF can be biodegraded by reductive dehalogenation (Schwarzenbach et al., 2003). Given the anoxic conditions of wetlands B1 and B2 (Table 3.1), our results are consistent with this anaerobic degradation pathway. Nevertheless, although the bulk water does not appear to have DO, it does not imply that the system is entirely anoxic. Several authors (Cooper et al., 1996; Matamoros et al., 2008a) have reported the existence of oxidized microenvironments within the bed of HF wetlands where aerobic degradation of pollutants takes place. This may give rise to the co-existence of several pathways of degradation of DCF.

An analysis of the response curve of the musk AHTN shows that this compound is readily removed during the first stage of the system (B1 and B2). The behavior of this compound is attributable to its high lipophilicity, indicated by its high octanol-water partition coefficient ($\log K_{ow} = 5.9$), and its refractory behavior to biodegradation. From the observation of suspended solid removal along the treatment system (Table 3.1), it is evident that these two parameters are closely related, indicating that adsorption onto solid particles is the key mechanism involved in the polycyclic musk removal. In fact, the accumulation of this compound in the particulate phase has been well reported (Carballa et al., 2004; Matamoros and Bayona, 2006).

BPA is the one showing the highest variability among sampling days, with removal efficiencies ranging from 70 to 90% for CWs B1 and B2. Although several authors have shown the elimination of BPA through physical and biological processes, still residual concentrations of this chemical compound have been addressed at the final effluent of various processes including activated sludge WWTPs (Kasprzyk-Hordern et al., 2009). Furthermore, Wintgens et al. (2004) has observed the presumable association of BPA with particulate material, a process that is likely given its fairly high octanol-water partition coefficient (Table 1.1). Other authors such as Spivack et al. (1994) have studied the metabolic pathway of BPA by some aerobic bacteria that undergo through several intermediate metabolites, which have been found in our experiment (Section 3.3.5). Therefore, an analysis of the results obtained in our study suggests biodegradation and association to the particulate matter as the most likely processes involved in the elimination of BPA.

To sum up, the two main removal mechanisms postulated in this study are biodegradation (i.e. IB, NPX, DCF and BPA) and sorption onto the substrate (i.e. AHTN and BPA). Whereas sorption of AHTN and BPA onto the organic matter retained in the substrate is supported by their high $\log K_{ow}$ value, plant uptake is not considered to be significant, at least for negatively charged compounds like analgesics (Matamoros and Bayona, 2006) or highly hydrophobic ones (i.e. AHTN). More studies are required in order to achieve more information about BPA.

3.3.5. Intermediate degradation products

Intermediate degradation products from injected EOCs were investigated along the treatment plant. Since Carboxy-Bisphenol A was the only tentatively identified compound in all sampling points, it was monitored. Fig. 3.6 shows the mass to charge ratio spectrum of BPA and its identified degradation product. CA-BPA was not identified in the HUSB effluent. Fig. 3.7 shows the behavior of CA-BPA as relative area abundance in comparison with BPA concentration decay along treatment plant. Although the removal efficiency of BPA was much higher in wetlands B1 and B2 than in B3, formation of CA-BPA is higher in B3. This fact together with the higher oxidation structure of CA-BPA and the previously

identification of CA-BPA in aerobic bacteria cultures (Spivack et al., 1994) reveals that the presence of CA-BPA might be clearly associated with aerobic degradation pathways. The presence of intermediate oxidized compounds in aerobic conditions has been previously described for other EOCs such as IB (Zwiener and Frimmel, 2003; Matamoros et al., 2008a) but this is the first time that CA-BPA has been identified in a wastewater treatment system.

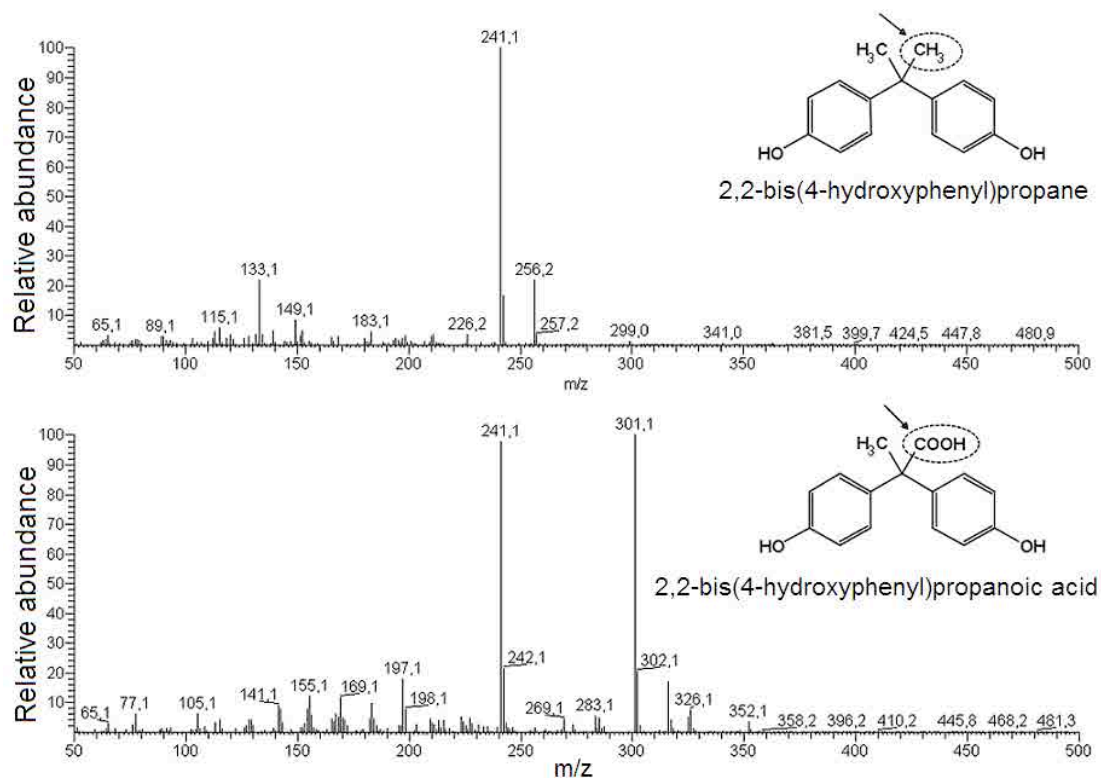


Figure 3.6. Mass to charge ratio spectrum of bisphenol A and Carboxy-bisphenol A.

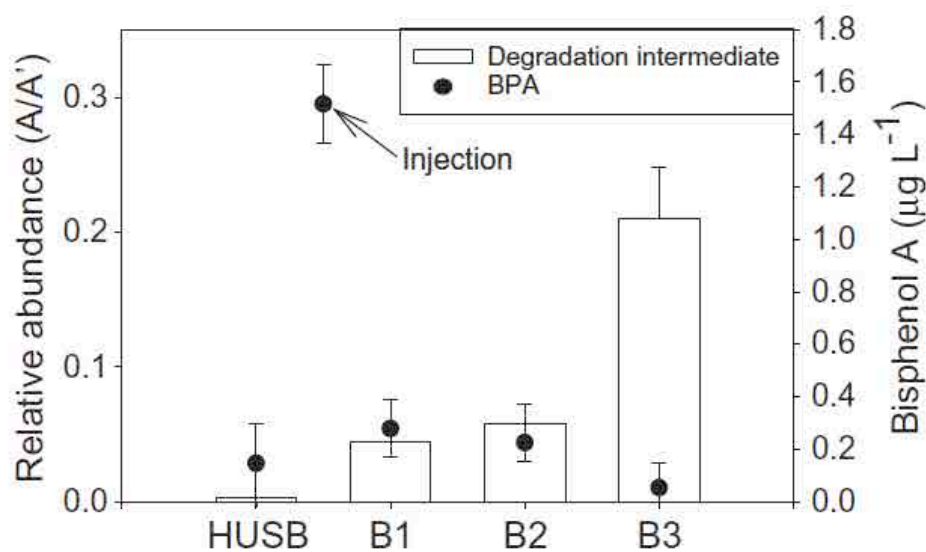
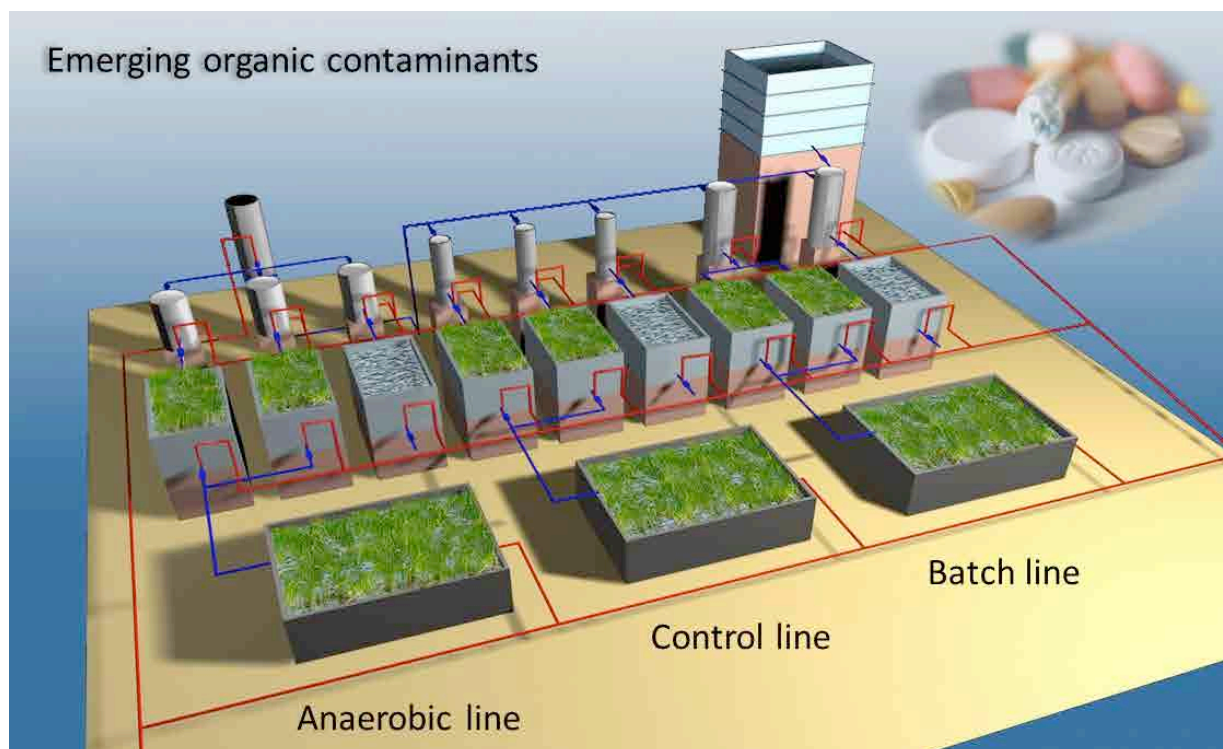


Figure 3.7. Relative abundance of Ca-BPA A/area abundance of internal standard in respect to BPA concentration decay along the horizontal subsurface flow constructed wetland system.

3.4. Conclusions

In summary, results indicate that the behavior of the EOCs in HF beds differs among the selected organic compounds, owing to their sorption and biodegradation characteristics. In particular, from the results of the present study we can conclude that aerobic conditions are crucial for the IB biodegradation. Furthermore, with the exception of IB, most of the EOC removal took place in the first stage of the system, where most of the particulate matter was removed and conditions were still fairly anoxic. However, although the presence of oxygen has been previously addressed as a key factor for the removal of most EOCs, the high efficiency could be owed to the coexistence of various microenvironments in CWs with different physico-chemical conditions. That would allow both aerobic and anaerobic metabolic pathways in the removal of EOCs take place. In general, HSSFCWs have proven to be useful for the degradation of EOCs from urban wastewater. Further attention should be paid to the biodegradation metabolites produced in the degradation pathway of EOCs, in order to obtain a better understanding of the major processes involved in their removal.

4. Removal efficiency of emerging organic contaminants within experimental horizontal subsurface flow constructed wetlands operating under different primary treatment and operation strategies



This chapter is based on the article:

Ávila, C., Reyes, C., Bayona, J.M., García, J., 2013. Emerging organic contaminant removal depending on primary treatment and operational strategy in horizontal subsurface flow constructed wetlands: Influence of redox. *Water Research* 47, 315-325.

This study aimed at assessing the influence of primary treatment (hydrolytic upflow sludge blanket (HUSB) reactor vs. conventional settling) and operational strategy (alternation of saturated/unsaturated phases vs. permanently saturated) on the removal of various EOCs (i.e. ibuprofen, diclofenac, acetaminophen, tonalide, oxybenzone, bisphenol A) in horizontal subsurface flow constructed wetlands. For that purpose, a continuous injection experiment was carried out in an experimental treatment plant for 26 days. The plant had 3 treatment lines: a control line (settler-wetland permanently saturated), a batch line (settler-wetland operated with saturate/unsaturated phases) and an anaerobic line (HUSB reactor-wetland permanently saturated). In each line, wetlands had a surface area of 2.95 m², a water depth of 25 cm and a granular medium $D_{60} = 7.3$ mm, and were planted with common reed. During the study period the wetlands were operated at a hydraulic and organic load of 25 mm d⁻¹ and about 4.7 g BOD m⁻² d⁻¹, respectively. The injection experiment delivered very robust results that show how the occurrence of higher redox potentials within the wetland bed promotes the elimination of conventional quality parameters as well as emerging microcontaminants. Overall, removal efficiencies were always greater for the batch line than for the control and anaerobic lines, and to this respect statistically significant differences were found for ibuprofen, diclofenac, oxybenzone and bisphenol A. As an example, ibuprofen, whose major removal mechanism has been reported to be biodegradation under aerobic conditions, showed a higher removal in the batch line (85%) than in the control (63%) and anaerobic (52%) lines. Bisphenol A showed also a great dependence on the redox status of the wetlands, finding an 89% removal rate for the batch line, as opposed to the control and anaerobic lines (79 and 65%, respectively). Furthermore, diclofenac showed a greater removal under a higher redox status (70, 48 and 32% in the batch, control and anaerobic lines). Average removal efficiencies of acetaminophen, oxybenzone and tonalide were almost >90% for the 3 treatment lines. The results of this study indicate that the efficiency of horizontal flow constructed wetland systems can be improved by using a batch operation strategy. Furthermore, we tentatively identified 4-hydroxy-diclofenac and carboxy-bisphenol A as intermediate degradation products. The higher abundance of the latter under the batch operation strategy reinforced biodegradation as a relevant bisphenol A removal pathway under higher redox conditions.

4.1. Introduction

The presence of EOCs in the aquatic environment has attracted increasing interest in the last decade due to the possible undesirable effects of many of these compounds in the environment and to living organisms (Enick and Moore, 2007). One of the main sources of these contaminants into the environment is the discharge of effluents from WWTPs where their removal is often incomplete. WWTPs are not currently designed to cope with these compounds, and although many advanced treatment technologies, such as ozonation, ultrasound, activated carbon or membrane bioreactors have been evaluated with promising results (Rosal et al., 2010; Shariati et al., 2010), these processes are not widely used nowadays mainly due to their high cost.

Constructed wetlands (CWs) have proven to constitute an alternative cost-effective technology to conventional WWTPs in the context of small communities (Puigagut et al., 2007). Apart from the removal of conventional wastewater quality parameters (e.g. BOD, TSS, nitrogen, phosphorus, etc.), CWs have shown to have a potential for the removal of EOCs (Hijosa-Valsero et al., 2010a; Matamoros et al., 2005; Dordio et al., 2009).

The removal of contaminants in CWs occurs as a result of complex physico-chemical and microbial interactions. The rates of these processes depend on a variety of design and operational factors such as depth of the bed, substrate, hydraulic and organic loading rates, feeding strategy and artificial/external aeration. The design of CWs is often carried out using the black box concept, and reduced treatment efficiency can occur when wetlands are constructed without considering the influence of some of these parameters. Most of the available research concerning design and operational factors' influence on treatment performance focuses on conventional water quality parameters (Aguirre et al., 2005; García et al., 2003b, García et al., 2005) and only in recent years the effect of these parameters on the removal of EOCs has started to be investigated. Matamoros and Bayona (2006) found how shallow bed horizontal subsurface flow CWs performed generally better than deeper ones on the removal of EOCs due to its higher redox potential (note that these CWs received the same wastewater and operated with the same areal organic loading rates). The tendency was opposite for carbamazepine in the study by Matamoros et al. (2005), which was attributed to the higher surface area available at deeper beds which would enhance the sorption of this compound in the organic matter and biofilm present in the gravel bed.

In general, it has been found that the prevalence of high redox potentials (in the oxidizing range) in wastewater treatment systems promotes aerobic respiration that is more efficient in removing most EOCs than anaerobic degradation pathways (Froehner et al., 2011; Onesios et al., 2009). Likewise, in CW systems a high redox status has shown to enhance the removal of most compounds (Hijosa-Valsero et al., 2010a; Hijosa-Valsero et al., 2011c; Matamoros and Bayona, 2006). Moreover, Matamoros et al. (2007) reported a better performance of vertical flow wetlands as opposed to horizontal flow ones owed to more oxidized conditions promoted by the unsaturated operation of the vertical beds.

In order to optimize the treatment performance in CWs it is therefore necessary to further investigate how operational and design parameters affect the removal of EOCs. Therefore, the aim of the present study was to carry out a continuous injection experiment that can allow for a reliable estimate of the removal efficiency of the system, so as to assess the influence of primary treatment (anaerobic reactor VS conventional settler) and operation strategy (continuously saturated conditions VS operation in batch with unsaturated periods) on the removal of various EOCs (i.e. IB, DCF, ACE, AHTN, OXY, BPA). Moreover, intermediate degradation products of the selected injected contaminants were attempted to be identified in order to get a further insight in the processes involved in their removal.

4.2. Materials and Methods

4.2.1. Description of the treatment plant

The pilot-plant used is set outdoors at the experimental facility of the GEMMA group (Department of Hydraulic, Maritime and Environmental Engineering of the Universitat Politècnica de Catalunya-BarcelonaTech, Spain). It started operation in February 2007 and treats urban wastewater pumped directly by means of 2 pumps from a nearby municipal sewer. Firstly, the wastewater is coarsely screened and subsequently stored in a 1.2 m³ polyethylene tank, which is continuously stirred in order to avoid sedimentation of solids. Wastewater retention time in this tank is approximately 12 h. From the storage tank, the wastewater is conveyed to 3 different treatment lines, which for ease of understanding, have been named batch, control and anaerobic (Fig. 4.1). Differences between treatment lines are related to the type of primary treatment and the operation strategy applied. All three lines include HF wetlands.

The layout of the wetlands is the same in all three lines: three small wetlands in parallel (0.65 m² each), two of them connected to a big wetland in series (1.65 m²) (Fig. 4.1). One of the three small wetlands was left unplanted and discharges directly to the sewer and, strictly speaking, does not belong to the treatment lines. These unplanted wetlands were constructed in order to study plant influence on clogging processes (Pedescoll et al., 2011a). However, clogging processes fall outside the scope of this paper and unplanted wetlands will therefore not be considered here. The two small planted parallel wetlands were necessary for the operation of the batch line. This system was also adopted in the other two lines for comparative purposes. These two small wetlands have a joint surface area (1.3 m²), which is approximately 45% of the total surface area of the entire treatment line (2.95 m²). Each of the three lines received a flow of 84 L d⁻¹ (not including the unplanted wetlands), which corresponds to a hydraulic loading rate of 0.028 m d⁻¹. The wetlands of each line were designed to have a maximum organic loading rate of approximately 6 g BOD m⁻² d⁻¹, as recommended by Kadlec and Knight (1996) and García et al. (2005).

4.2.1.1. Primary treatment

Batch and control lines have cylindrical PVC static settlers as primary treatment and are filled with screened wastewater pumped from the storage tank every 4 h. The control line has three settlers, one for each small wetland (planted and unplanted), with an effective volume of 7 L. The batch line has only two settlers because the small planted wetlands operate alternately, having each an effective volume of 14 L. In the control line, after 2 h of settling from each settler, 7 L of wastewater are discharged into the small wetlands (in total 14 L to the planted wetlands at each discharge). In the batch line, after 2 h of settling, 14 L of wastewater are discharged into one small wetland.

On the other hand, the anaerobic line has a cylindrical PVC HUSB reactor as primary treatment, which has an effective volume of 105 L. The reactor is continuously fed with wastewater from the storage tank by means of a peristaltic pump that supplies a known flow. The HUSB reactor was operated at a HRT of 5 h at the time of the study. Every 4 h the content of the upper part of the HUSB reactor was discharged into three distribution tanks (one per each small wetland) with an effective volume of 7 L. Without time for particle settling (tanks were charged and discharged in 10 min), wastewater was discharged from these tanks into the small wetlands by means of electrovalves. This set-up ensured a flow of 7 L to each small wetland every 4 h.

4.2.1.2. Wetlands

Big and small wetlands consist of polyethylene containers 1.5 m long, 1.1 m wide and 0.50 m high, and 0.95 m long, 0.70 m wide and 0.45 m high, respectively. Wastewater from the primary treatment was discharged by means of perforated pipes located along the width of the wetlands. Each container had a drainage pipe on the flat bottom for effluent discharge. The uniform gravel layer ($D_{60} = 7.3$ mm, $C_u = 0.83$, 40% initial porosity) was 0.3 m deep and the water level was kept 0.05 m below the gravel surface to give a water depth of 0.25 m. The theoretical mean HRT was 3.5 d per line. Wetlands were planted with *Phragmites australis*, which covered the entire surface of the wetlands.

In anaerobic and control lines all the wetlands remained permanently saturated. The batch line, however, operated under a scheme of a four-day cycle and the small wetlands were not permanently saturated. Accordingly, for the first two days of the cycle the small wetlands were fed in the same way as the control line, on the third day the wetland rested under saturated conditions but received no influent and on the fourth day the wetland was drained and rested under unsaturated conditions. Note that the two small wetlands of the batch line are at different phases at any time. Flow measurements were recorded on a daily basis at the effluent of each line with a water sensor level. Further technical and operational details of the experimental plant can be seen in Pedescoll et al. (2011b).

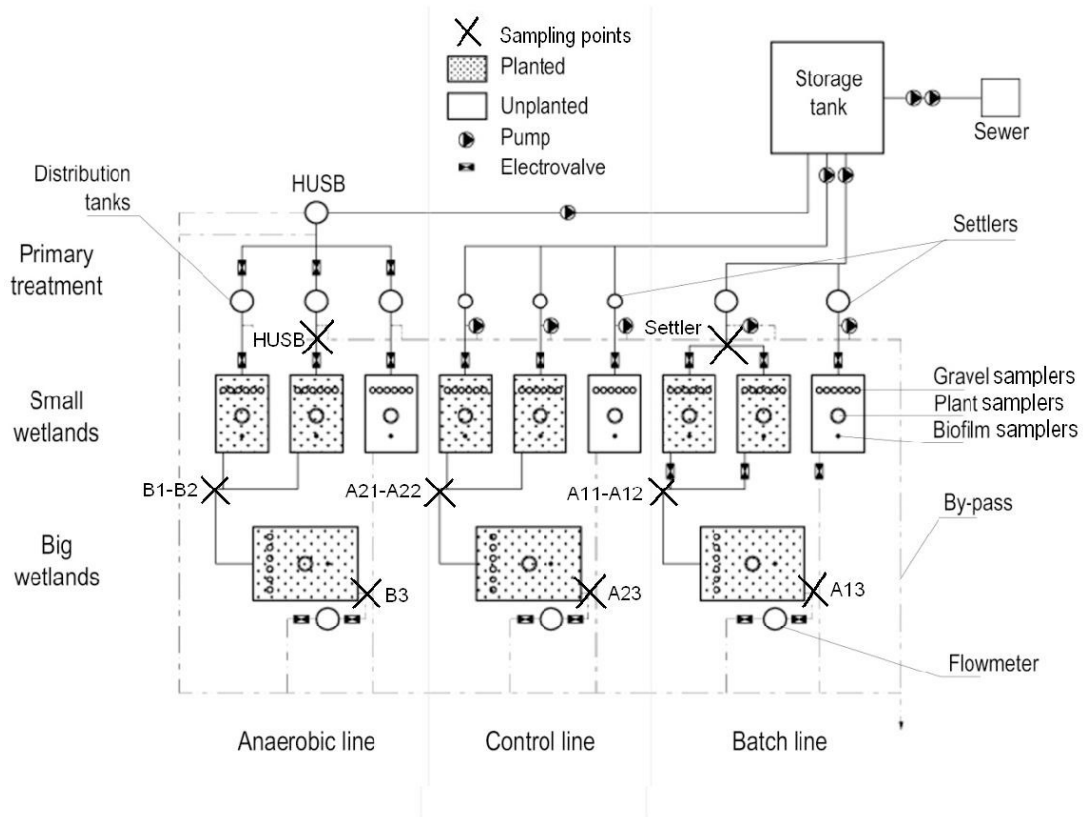


Figure 4.1. Schematic diagram of the experimental horizontal subsurface flow constructed wetland system (three-treatment lines) in Barcelona and sampling points (Pedescoll et al., 2011b). Sampling points for the present study are indicated with a cross.



Figure 4.2. View of the experimental horizontal subsurface flow constructed wetland system in Barcelona. a) summer; b) winter.

4.2.2. Injection experiments

About 20 L of distilled water contained in a glass bottle protected from sunlight was spiked with IB, DCF, ACE, AHTN, OXY and BPA, previously dissolved in methanol, together with KBr as a conservative tracer. This blend was homogenized and injected into the influent pipes of the small planted wetlands of the 3 lines by means of peristaltic pumps, obtained from Damova (Barcelona, Spain). They supplied a flow rate of 3.2 L h⁻¹ (for batch and anaerobic lines) and 8 L h⁻¹ (for control line). These flow rates were selected so as to synchronize the duration of the injection of the doped solution to that of the wastewater, and therefore obtain a homogenous mixture at the wetland influent. Concentrations of the doped solution were adjusted in order to obtain the desired influent concentration of the EOCs and the tracer (i.e. IB = 75 µg L⁻¹; DCF = 2.5 µg L⁻¹; ACE = 35 µg L⁻¹; AHTN = 3 µg L⁻¹; OXY = 8 µg L⁻¹; BPA = 1.8 µg L⁻¹, KBr = 15 mg L⁻¹). Those concentrations corresponded to about 250% of the maximum concentration of the EOCs detected in previous campaigns (Hijosa-Valsero et al., 2011b; Ávila et al., 2010). The injection experiment lasted 26 days from November to December 2009.

4.2.3. Sampling strategy

Sampling was started only when KBr concentrations had stabilized at the effluents of the 3 treatment lines, so as to obtain a more reliable estimation of the removal efficiency of the systems under steady state conditions. Effluent 12-h composite samples were collected manually by taking 80 mL of sample every four hours, three times a day. Final effluents of the three treatment lines were grabbed. For sampling of the small wetlands, blended volumes of the two-planted wetlands' effluents of each line were collected. Effluents of the HUSB reactor and one of the conventional settlers were also sampled so as to measure the background concentration of the selected compounds in the influent wastewater (Fig. 4.1). Likewise, several samples of the spiked solution containing the EOCs injected were also grabbed, so as to ensure that no interaction among the selected compounds occurred and that incoming concentrations were as desired.

Although initially sampling was intended to take place during 6 consecutive days, various punctual technical problems with the pumps caused the incoming KBr and EOC concentrations to vary slightly from the expected influent concentrations. This variation occurred after the second sampling day, so we decided to proceed with the sampling in a different manner (i.e. two sampling days per week during three consecutive weeks), hence ensuring a stable incoming concentration. This fact was key in the experimental procedure, since only a correct injection experiment can give us robust results. Sampling was carried out two times a week for three weeks (n = 6) during December 2009. All samples were collected in 250 mL amber glass bottles and brought to the laboratory where they were stored at -4 °C until analysis, which took place within 2 days timespan. Samples were analyzed for the selected microcontaminants and conventional water parameters as described below. Note that during the whole experiment, no rainfall events were recorded.

4.2.4. Chemicals

GC grade (Suprasolv) hexane, ethyl acetate, methanol and acetone were obtained from Merck (Darmstadt, Germany) and analytical-grade hydrochloric acid was obtained from Panreac (Barcelona, Spain). Analytical grade (≥98%) ibuprofen, naproxen, diclofenac, tonalide, bisphenol A, 2-2'-dinitrophenyl, dihydrocarbamazepine, and triphenylamine were purchased from Sigma-Aldrich (Steinheim, Germany). The 2,4,5-trichlorophenoxypropionic acid (2,4,5-TPA) was obtained from Riedel-de Hën (Seelze, Germany); potassium bromide and trimethylsulfonium hydroxide (TMSH) were

supplied from Fluka (Buchs, Switzerland) and 0.7 μm glass fiber filters of $\phi = 47$ mm (GF/F) were purchased from Whatman.

4.2.5. Analytical methodology

Onsite measurements of water temperature, DO, pH and EC were taken using a Checktemp-1 Hanna thermometer, a Eutech Ecoscan DO6 oxymeter, a Crison pH-meter and an EH CLM 381 conductivity meter, respectively. E_H was also measured in situ by using a Thermo Orion 3 Star redox meter. E_H values were corrected for the potential of the hydrogen electrode. Conventional wastewater quality parameters, including ammonium nitrogen ($\text{NH}_4\text{-N}$), TSS and COD were determined by using Standard Methods (APHA-AWWA-WPCF, 2001). Sulfate (SO_4^{2-}), nitrate nitrite nitrogen ($\text{NO}_x\text{-N}$) and bromide (Br^-) were analyzed using a DIONEX ICS-1000 ion chromatographer (IC).

The concentrations of the selected EOCs in wastewater samples were analyzed after the samples had been filtered and processed as previously described by Matamoros et al. (2005). The linearity range was from 0.01 to 4 mg L^{-1} . The correlation coefficients (R^2) of the calibration curves were always higher than 0.99. The limit of detection (LOD) and limit of quantification (LOQ) were compound dependent in the range from 0.11 to 0.47 $\mu\text{g L}^{-1}$ and 0.12 to 0.80 $\mu\text{g L}^{-1}$, respectively.

4.2.6. Statistical analyses

Experimental results were statistically evaluated using the SPSS 13 package (Chicago, IL). Data normality was checked with a Kolmogorov-Smirnoff test. For the evaluation of the anaerobic line assay carried out in summer time, comparisons of differences in removal efficiencies between the three treatment lines were performed with parametric ANOVA tests and Tukey post-hoc tests. On the other hand, for the assays developed in winter time aiming at comparing the three different treatment lines, the concentration comparison between treatment systems corrected by evapotranspiration (Section 3.1.x?) was analyzed by using the ANOVA test for two independent samples and the Tukey post-hoc. Statistical significance was defined as a $p < 0.05$.

4.3. Results

4.3.1. Background concentrations of emerging organic contaminants and primary treatment efficiencies

Background concentrations of EOCs found at the effluent of the primary treatment units (before injection) are shown in Table 4.1. It is important to mention that EOC concentrations were slightly higher at the effluent of the HUSB reactor when compared to the settler almost in every case (except for ACE). Background concentrations of the selected pharmaceuticals -before injection- for the effluent of both primary treatment units ranged 11.1 to 32.0, 0.4 to 1.3, 1.8 to 13.5, 1.0 to 4.2, 0.2 to 1.0 and 0.5 to 1.6 $\mu\text{g L}^{-1}$ for IB, DCF, ACE, AHTN, OXY and BPA, respectively. Those are in the range to those found by other authors as referenced in Table 4.1, except for AHTN and OXY, whose concentrations were below and above the literature, respectively. In addition, Hijosa-Valsero et al. (2011b) reported for the same pilot plant similar incoming concentrations for IB and DCF, while slightly lower AHTN after a sampling campaign carried out in winter 2008.

Table 4.1. Background concentrations (in $\mu\text{g L}^{-1}$) of selected emerging organic contaminants at the effluent of the two types of primary treatment (before injection).

Compound/Type of primary treatment	HUSB reactor (this study)	Conventional settler (this study)	Other studies	References
Ibuprofen	18.11±7.29	14.57±2.72	18.24 (settler), 24.11 (HUSB)	Hijosa-Valsero et al., 2011b
			1.2-2.6	Clara et al., 2005
			<LOQ-4.11	Rosal et al., 2010
			8.3-17.2 (11.7)	Matamoros et al., 2007
			0.17-83.5	Miège et al., 2009
			0.98-6.33	Kasprzyk-Hordern et al., 2009
Diclofenac	0.76±0.29	0.68±0.18	0.56 (settler), 0.77 (HUSB)	Hijosa-Valsero et al., 2011b
			0.90-4.11	Clara et al., 2005
			0.48-1.28	Matamoros et al., 2007
			<LOQ-0.56	Rosal et al., 2010
			0.10-4.11	Miège et al., 2009
			0.06-1.16	Kasprzyk-Hordern et al., 2009
Acetaminophen	6.39±3.64	7.70±3.54	1.57-37.46	Rosal et al., 2010
			5.53-292	Miège et al., 2009
			108.38- 246.64	Kasprzyk-Hordern et al., 2009
Tonalide	2.87±1.23	2.22±0.45	0.32 (settler), 0.63 (HUSB)	Hijosa-Valsero et al., 2011b
			0.21-1.11	Clara et al., 2005
			0.66-1.83	Matamoros et al., 2007
			<LOQ-1.93	Rosal et al., 2010
			0.21-1.69	Miège et al., 2009
Oxybenzone	0.53±0.25	0.45±0.13	8.58-22.1	Matamoros et al., 2007
Bisphenol A	1.04±0.35	0.85±0.45	0.72-2.38	Clara et al., 2005
			<0.14-2.14	Stasinakis et al., 2008

Water quality parameters of effluents obtained in the HUSB reactor were compared to those of the conventional settler and are shown in Table 4.2. Redox potentials were much lower in the effluent of the HUSB reactor than in the settler (in average -196 ± 56 and -87 ± 84 mV, respectively). Sulfate concentrations were also slightly lower in the HUSB reactor (119 ± 21 vs. 134 ± 32 mg L^{-1}), which indicates a slightly higher sulfate reduction activity in accordance with the lower redox potentials. HUSB reactor achieved a slightly greater retention of solids than the settler as shown by the effluent TSS concentrations (98 ± 24 and 107 ± 32 mg L^{-1} , respectively). COD and $\text{NH}_4\text{-N}$ concentrations were

higher at the effluent of the HUSB reactor. These results are in accordance with those shown previously by Pedescoll et al. (2011b) in this same pilot plant, where after 2 years of monitoring of these two primary treatment units, the HUSB reactor produced effluents with significantly lower redox potentials than the settler (average of -103 ± 100 and $+172 \pm 103$ mV, respectively). Likewise, in the same study the retention of solids was found to be greater at the HUSB reactor, with effluent concentrations of 62 ± 30 mg L⁻¹, as opposed to 99 ± 47 mg L⁻¹ outflowing the settler.

Table 4.2. Water quality parameters and effluent concentrations of emerging organic contaminants along the different treatment units of the horizontal-sbusurface flow constructed wetland system. B1-B2, A21-A22 and A11-A12 represent the small wetlands of the anaerobic, control and batch lines, respectively. B3, A13 and A23 stand as the final effluents of the anaerobic, control and batch lines, respectively (see Fig 4.1). Statistically significant differences for final effluent concentrations of the three lines for the selected EOCs are marked with an asterisk (*). Note: a single value for settler is shown, since it constituted the same primary treatment for control and batch lines.

		Anaerobic line			Control line			Batch line	
		HUSB	B1-B2	B3	Settler	A21-A22	A23	A11-A12	A13
Water quality parameters	Temperature	14.8±3.5	13.2±3.0	13.6±3.9	14.9±4.3	13.4±4.0	12.9±2.8	13.6±4.1	13.6±4.1
	pH	7.3±0.1	7.0±0.1	6.9±0.1	7.6±0.1	7.0±0.1	6.9±0.1	6.9±0.1	6.9±0.1
	E _h (mV)	-196±56	-225±47	-96±28	-87±84	-209±35	-78±42	-117±45	-11±32
	COD (mg L ⁻¹)	317±44	150±20	82±9	278±40	108±15	68±9	89±12	59±9
	TSS (mg L ⁻¹)	98±24	8±3	24±8	107±32	9±6	21±7	11±3	9±3
	NH ₄ -N (mg L ⁻¹)	28.8±4.0	17.7±3.1	2.1±0.1	26.4±5.1	15.9±1.3	0.8±0.6	13.0±2.0	0.1±0.2
	NO _x -N (mg L ⁻¹)	<LOD	<LOD	0.1±0.2	<LOD	<LOD	<LOD	4.6±3.7	0.1±0.3
	SO ₄ ²⁻ (mg L ⁻¹)	119±21	24±11	25±16	134±32	26±10	31±15	114±15	98±25
Injected EOCs (µg L ⁻¹)	Ibuprofen*	83.9±2.8	55.2±8.3	39.9±6.6	83.6±2.7	40.2±5.1	30.5±5.7	30.3±7.9	12.5±2.2
	Diclofenac*	2.8±0.3	2.3±0.3	1.9±0.2	2.7±0.2	2.0±0.3	1.4±0.2	1.2±0.2	0.8±0.1
	Acetaminophen	34.0±3.6	6.7±6.3	1.5±0.6	34.3±4.0	3.9±2.5	1.0±0.4	3.3±2.2	0.6±0.1
	Tonalide	3.4±1.5	1.8±0.9	0.4±0.1	3.4±0.4	1.2±0.4	0.3±0.06	0.7±0.1	0.3±0.02
	Oxybenzone*	8.3±0.2	1.8±0.6	1.1±0.3	8.2±0.1	2.3±0.5	0.4±0.2	0.7±0.3	<LOD
	Bisphenol A*	1.9±0.4	1.2±0.6	0.6±0.08	1.8±0.5	0.8±0.4	0.4±0.07	0.3±0.2	<LOD

4.3.2. Treatment line efficiencies

Average final effluent redox potentials varied significantly between the 3 treatment lines, with a higher value found in the batch line (-11 ± 32 mV), as opposed to the control and anaerobic lines (-78 ± 42 and -96 ± 28 mV, respectively). Although the number of samples undertaken at this study was low ($n = 6$), these values are reinforced by those obtained by Pedescoll et al. (2011b) at this pilot plant, where sampling was much more intensive (2 years) and large ($n = 70$). Those reflect how the redox potentials found at the final effluent of the anaerobic line were significantly lower than those found at the control and batch lines. (-45 ± 78 , -5 ± 71 and $+3 \pm 93$ mV for anaerobic, control and batch lines, respectively). Furthermore, results of our study show that the batch line had slightly better COD removal efficiencies (79%) than the control and anaerobic lines (75 and 74%, respectively), as well as for TSS removal (92, 80 and 75% for batch, control and anaerobic lines, respectively). What is more, in terms of $\text{NH}_4\text{-N}$ removal, statistically significant differences were found between the three lines, where the batch line performed again better (97.6%) than the control (97%) and anaerobic (93%) lines, reaching a final concentration of 0.1 mg L^{-1} .

Table 4.2 shows selected EOC concentrations within the 3 treatment lines. As it can be observed, elimination rates were high for most of the studied compounds (Fig. 4.3). ACE showed great removal efficiencies in the three lines (above 95%). AHTN showed also no great variation between the three treatment lines, achieving 88, 91 and 91% in the anaerobic, control and batch lines, respectively. The rest of the compounds had a greater removal found always in the batch line, where more oxidized conditions took place. In fact, statistically significant differences in terms of removal efficiencies achieved in the effluent of the three lines were found for IB, DCF, OXY and BPA. IB removal was much higher in the batch line (85%) than in the control (63%) and anaerobic lines (52%). Even greater differences were found for DCF, which achieved just 32% of removal in the anaerobic line, a figure far lower than the 70% accomplished in the batch line. OXY presented, in general, high removal efficiencies (87, 94 and 97% in the anaerobic, control and batch lines, respectively). Likewise, BPA showed a great dependence on the type of treatment received, with 65, 79 and 89% removal efficiencies in the anaerobic, control and batch lines, respectively.

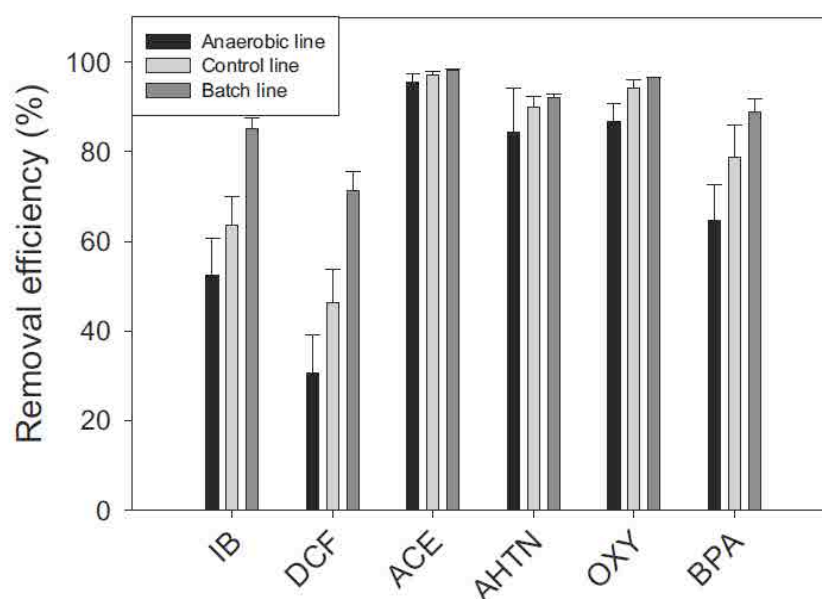


Figure 4.3. Removal efficiencies achieved in the three treatment lines of the experimental horizontal subsurface flow constructed wetland system for the selected emerging organic contaminants. Target contaminants have been displayed by their acronyms.

4.4. Discussion

4.4.1. Removal of the selected emerging organic contaminants

Average removal efficiencies were in general high for all the EOCs studied. In a study carried out prior to this one (May 2009) in the anaerobic line of this pilot plant it was observed however higher removal efficiencies for these substances (Ávila et al., 2010). It is important to note that it also consisted of an injection experiment with similar injected EOCs concentrations. Average removal efficiency of IB obtained in the present study in the anaerobic line (52%) was lower than those reported by Ávila et al. (2010), where a range of 98 - 99% removal occurred. Removal efficiencies for DCF were in the present study much lower (32%) than those obtained in the prior one (99%). Removal of AHTN was also lower in this study (88%) than in the previous one (97 - 98%). Likewise, the elimination of BPA was much higher in Ávila et al (2010), ranging 85 - 99%, than in this work (65%). Lower efficiencies found in the present study are attributable to the fact that the sampling campaign performed by Ávila et al. (2010) took place in spring season, as opposed to the current one which occurred in winter. To this regard, it should be mentioned that higher temperatures were found in spring (23°C) than in winter (15°C), which would promote a higher microbial activity.

Hijosa-Valsero et al. (2011b) performed a sampling campaign in the three lines of this pilot plant (with no injection of contaminants) one year prior to this one (winter 2008) and results differ substantially, reporting lower values of removal for IB, DCF and AHTN. In fact, in the study by Hijosa-Valsero et al. (2011b) the concentration of DCF increased in respect to the influent in the effluent of the control and batch lines, and only 23% of removal was found in the anaerobic line. For AHTN, an increase in the concentration occurred in the three lines. This could be attributed to the much lower incoming concentrations in the CWs, due to the fact that no injection was realized, which would result in lower elimination rates.

However, in general removal efficiencies observed in our study are in accordance with other studies documented in CW systems. Hijosa-Valsero et al. (2010a) reported a range of 42 - 99% removal in three full-scale HF wetlands systems of Spain, and a range of 65 - 78% for DCF. Ranieri et al. (2011) showed removal efficiencies above 90% for ACE after an injection experiment in HF wetlands. The removal of OXY was comparable (97%) as reported by Matamoros et al. (2009a) in HF beds.

Response curves at the steady state for the selected compounds at the effluent of the three treatment lines in respect to the incoming concentration are displayed in Fig. 4.4. In general, it can be observed how the experimental setup based on a continuous injection delivered results stable over time, which gives a good approximation of the behavior of the compounds in the treatment system. The fact that incoming concentrations varied significantly for some of the substances (i.e. AHTN, BPA) in respect to the average incoming concentration is due to the fact that for these substances the injected concentrations were not much higher than background concentrations, resulting therefore in the occurrence of some noise in the curves.

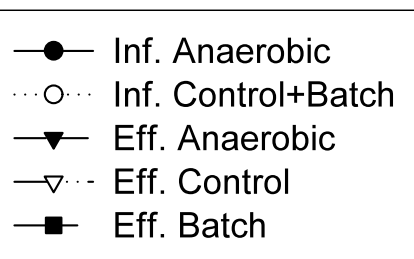
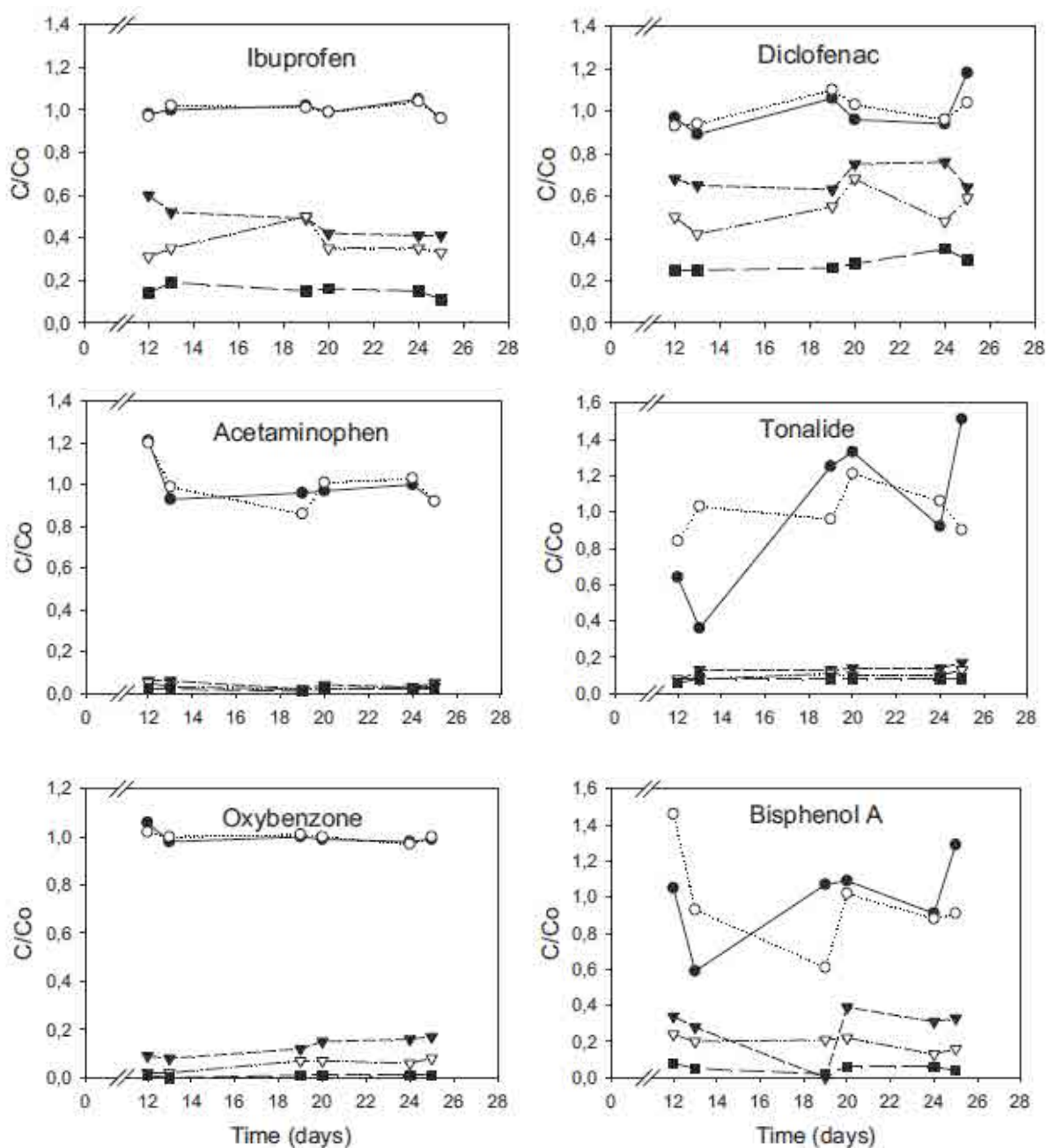


Figure 4.4. Concentration of the selected emerging organic contaminants normalized by their total influent concentration (background concentration + injected concentration) in the influent and final effluent of the three treatment lines of the horizontal subsurface flow constructed wetland system. Initial concentrations (C_o) correspond to the HUSB and settler concentrations of Table 4.2.

4.4.2. Effect of primary treatment and operation strategy

Some of the EOCs showed a high dependence on the primary treatment and mode of operation. In general, it has been observed that using a HUSB reactor as a primary treatment conferred more reducing conditions to the wetland environment along the anaerobic treatment line, if compared to the control line. Likewise, operating the wetlands in a batch mode, that is alternating cycles of saturation and unsaturation, promotes more oxidized conditions of the wetland bed, which has proven to be beneficial for the removal of conventional water quality parameters as well as for EOCs. In this sense, we can obtain into conclusion that the redox status taking place within the wetlands stands as a key parameter that controls the removal of the studied compounds. To this regard, results for EOCs are remarkably in accordance with previous literature referring to their mechanisms of removal, which would be promoted in most cases by a higher redox potential.

To start with, effluent concentrations of IB differed significantly between the 3 treatment lines, showing a high dependence of this substance on the redox status of the system. To this regard, aerobic biodegradation has been documented as the major removal pathway for this compound (Zwiener and Frimmel, 2003; Abegglen et al., 2009). Hijosa-Valsero et al. (2010a) showed how high redox potentials in CWs promoted its removal. In addition, Matamoros et al. (2005) and Matamoros and Bayona (2006) found a higher removal efficiency of this substance when a shallow wetland 0.27 m-deep was used (62 – 80%) as opposed to a deeper one 0.5 m-deep (17 – 52%), due to the occurrence of more oxidized conditions. IB is a hydrophilic substance with a low octanol-water partition coefficient, and for that reason sorption onto the substrate does not constitute a removal mechanism (Clara et al., 2005; Joss et al., 2005).

DCF seemed to be also highly dependent on the treatment received. Although moderate removal efficiencies (32 – 70%) were achieved in this study, the literature has reported how the removal of DCF has been in many cases limited, as regarded by the presence of chlorine in its structure, which would make it difficult to be degraded (Zorita et al., 2009). In fact, Zorita et al. (2009) showed how an increase in the concentration of DCF in the effluent water in respect to the influent occurred, presumably due to its de-conjugation or desorption from particles. The prevailing removal pathway has been reported to be the anaerobic biodegradation through dehalogenation in anoxic conditions (Park et al., 2009). To this regard, Zwiener and Frimmel (2003) found that the elimination of this compound was higher in an anoxic reactor rather than in the oxic one. However, the existence of high redox potentials has been regarded as beneficial for the removal of this substance in CWs, as shown by Hijosa-Valsero et al. (2010a) and Hijosa-Valsero et al. (2011b), being the latter one carried out in this pilot plant. The results obtained in this study pose therefore a new hypothesis that contrasts with the previous hypotheses about its removal pathway consisting on anaerobic biodegradation. In this case, its degradation seems to be promoted by a high oxidation status of the wetland bed.

The removal of ACE has been reported to be very high, not only in CWs but also in conventional WWTPs (Kosma et al., 2010). To this regard, its elimination has been attributed to happen majorly through biodegradation (Ranieri et al., 2011; Shariati et al., 2010). In fact, it has been observed how well bacteria are adapted to its degradation, since the removal occurred rapidly with a minimum lag phase (Yu et al., 2006). Its degradation in our study was very large in the 3 treatment lines. Statistical differences were found not to be significant, thus it can be concluded that its degradation does not seem to depend on the redox status of the system at the ranges tested in our study.

The elimination of AHTN has been repeatedly attributed to occur through sorption onto the organic matter retained in the gravel bed (Joss et al., 2005; Matamoros and Bayona, 2006), given its high lipophilicity as indicated by its high octanol-water partition coefficient ($\log K_{ow} = 5.9$). The fact that elimination rates were lower in this case as compared to the study undertaken by Ávila et al. (2010) in the anaerobic line in spring season, could be explained by a release of hydrophobic compounds in

winter (Hijosa-Valsero et al., 2011b). Removal efficiencies have not differed significantly between the 3 treatment lines, and therefore the primary treatment and mode of operation does not seem to affect the behavior of this substance in CWs.

Likewise, the level of removal of OXY was very high as previously reported for CWs (Matamoros et al., 2009a), where biodegradation has once again been attributed as the possible removal mechanism. In this case, removal efficiencies were found to vary significantly between the 3 treatment lines, finding a dependency of this compound on the redox status of the system. Greater oxidized conditions promoted its removal.

In regards to BPA, it is important to note the high difference on the removal efficiencies found in the 3 treatment lines. BPA has been observed to be removed by sorption by Stasinakis et al. (2008) in a proportion of 15% of the influent mass. However, biodegradation stands as the major pathway of removal in several studies, being promoted under aerobic conditions (Al-Rifai et al., 2011; Spivack et al., 2004). Ávila et al. (2010) reported the occurrence of carboxy-BPA, a metabolite of degradation of BPA, occurring in a high proportion under higher redox potentials. The significant differences found for its removal in the batch line (89%), as compared to the control (79%) and anaerobic (65%) lines, is in accordance with the hypothesis standing that the major removal mechanism of BPA in CWs is the biodegradation that would be promoted by a higher redox status of the wetland bed.

4.4.3. Emerging organic contaminants' removal kinetics

Areal-based first-order removal rate constants (k_A) have been calculated for each EOC and are shown in Table 4.3. The k_A values obtained in this study are in every case higher than those obtained in the same pilot plant by Hijosa et al. (2011b). This would be explained by the much higher concentrations entering the treatment system during this study due to the injection of EOCs. k_A obtained in the present study are however about an order of magnitude smaller than those obtained by Matamoros et al. (2007) in VF wetlands. This is attributed to the higher efficiency of this type of systems, whose bed is much more oxidized, which would result in higher degradation rates.

Table 4.3. Areal-based first-order removal rate constants obtained for the target emerging contaminants in the three treatment lines of the horizontal subsurface flow constructed wetland system and comparison with other literature.

Compound	k_A (m d ⁻¹)									
	This study			Hijosa et al. (2011b)			Matamoros et al. (2007)			
	Loading (mg m ⁻² d ⁻¹)	Anaerobic line	Control line	Batch line	Loading (mg m ⁻² d ⁻¹)	Anaerobic line	Control line	Batch line	Loading (mg m ⁻² d ⁻¹)	VFCW
Ibuprofen	2.38	0.021	0.029	0.054	0.86	0.0007	0.0061	0.0102	0.65	0.40
Diclofenac	0.08	0.011	0.019	0.035	0.02	-0.0076	-0.0042	-0.0020	0.08	0.10
Acetaminophen	0.97	0.089	0.101	0.115	na ^a	na	na	na	na	na
Tonalide	0.09	0.061	0.069	0.069	0.01	-0.0153	-0.0298	-0.0154	0.11	0.11
Oxybenzone	0.23	0.057	0.086	0.096	na	na	na	na	0.72	0.26
Bisphenol A	0.05	0.033	0.043	0.064	na	na	na	na	na	na

^aNot available.

4.4.4. Intermediate degradation products

Intermediate degradation products from the injected EOCs were investigated along the treatment plant. Two metabolites of degradation of DCF and BPA were tentatively identified, namely 4-hydroxy-DCF (4-OH-DCF) and Carboxy-BPA (CA-BPA), respectively. Values of relative area abundance of 4-OH-DCF (A) in respect to its parental compound DCF (A') were very low (0.01-0.04) in all treatment units and no statistical differences between treatment lines were observed. In contrast, the A/A' ratios of CA-BPA in respect to BPA differed greatly between treatment units as it is shown in Fig. 4.5.

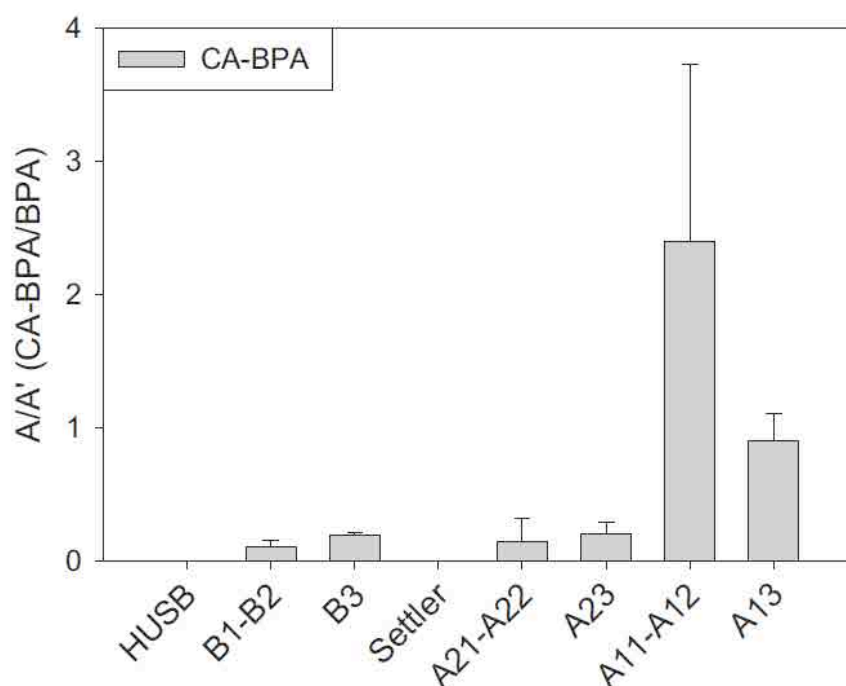


Figure 4.5. Relative area abundance of the intermediate degradation product Carboxy-Bisphenol A in respect to the area abundance of its parental compound (i.e. Bisphenol A) along the horizontal subsurface flow constructed wetland system (three treatment lines).

It can be observed how CA-BPA was not identified in the primary treatments' effluents (Fig. 4.5). However, it has been previously reported that CA-BPA is generated within CWs presumably through aerobic bacterial metabolism (Ávila et al., 2010). In this study is confirmed that CA-BPA is formed under higher redox potentials (and therefore less anaerobic conditions), given the high A/A' ratio found in the small wetlands of the batch line (A11-A12) as well as in the big wetland of the same line (A13), constituting these significantly high values in respect to the other treatment wetlands.

4.5. Conclusions

The use of a HUSB reactor, as opposed to a conventional settler for the primary treatment of wastewater, was not found to promote the removal of the selected EOCs. In fact, the occurrence of more reduced conditions within the HUSB reactor diminished the removal efficiency of the studied compounds.

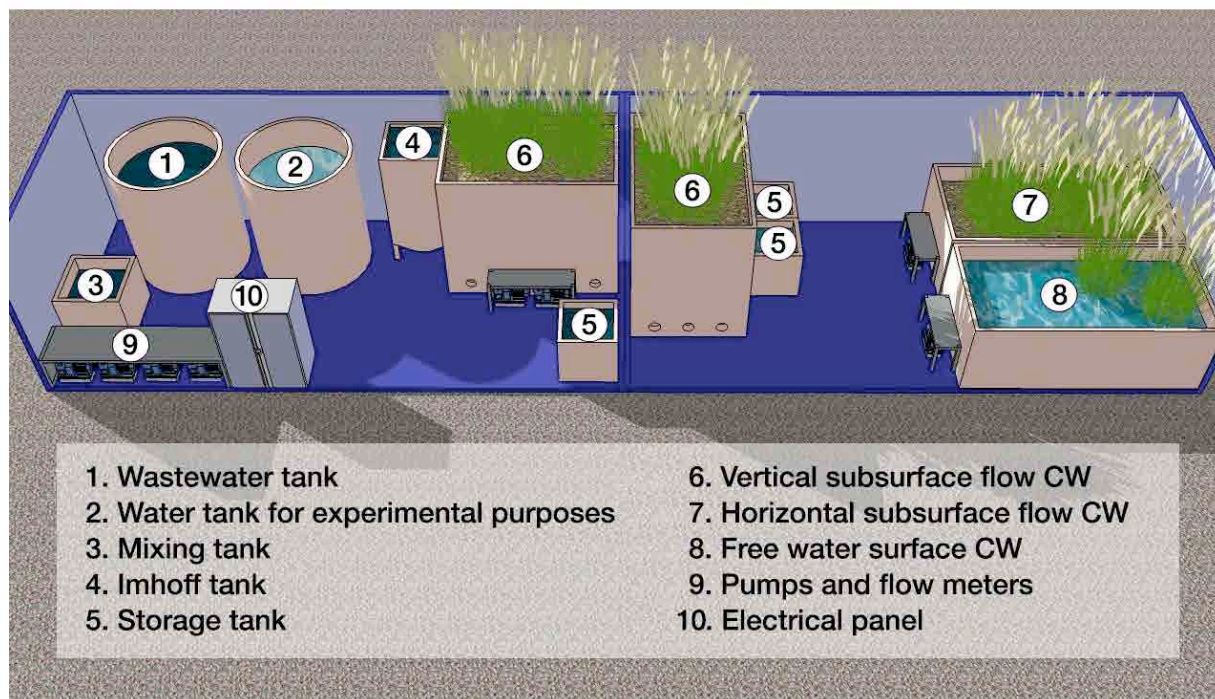
The mode of operation in batch, alternating phases of saturation and unsaturation, promoted the existence of a higher redox status, as compared to functioning under saturated conditions, which in turn enhanced significantly the elimination of the studied compounds.

The continuous injection experiment delivered very robust results that have shown how the occurrence of high redox potentials within the CW bed promotes the elimination of conventional quality parameters, as well as EOCs. In this study, out of the 6 studied EOCs, 4 of them (i.e. IB, DCF, OXY, BPA) were found to be dependent on the redox status of the system. Substances whose major removal mechanism seems to be the biodegradation under more oxidized conditions (i.e. IB, BPA) are those that behave most differently among the 3 treatment lines. DCF, although previously reported to be removed by anaerobic biodegradation was found in this study to depend highly on the existence of oxidized conditions for its removal. To this regard, the operation under cycles of saturation and unsaturation seems to enhance the treatment performance of CWs. Further attention should be paid to the biodegradation metabolites produced in the degradation pathway of EOCs, so as to get a better insight of the major processes involved in their removal.

5. Experimental three-stage hybrid constructed wetland system for wastewater treatment in warm climate regions

Hybrid Constructed Wetland System

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This chapter is based on the article:

Ávila, C., Garfí, M. and García, J., 2013. Three stage hybrid constructed wetland system for wastewater treatment and reuse in warm climate regions. *Ecological Engineering*, in press. At: <http://dx.doi.org/10.1016/j.ecoleng.2013.09.048>

An experimental hybrid constructed wetland system consisting of 3 stages of different wetland configurations (i.e. two vertical flow beds (1.5 m² each) alternating feed-rest cycles followed by a horizontal subsurface flow (2 m²) and a free water surface (2 m²) wetlands in series) and the quality of its final effluent were evaluated for about one year. Mean overall removal rates were as 97% TSS, 78% COD, 91% BOD₅, 94% NH₄-N, 46% TN and 4% PO₄-P. Vertical flow beds achieved high organic matter retention (77% BOD₅) and great nitrification capacity (74% NH₄-N removal). Although horizontal and free water surface wetlands accomplished little denitrification, they enabled water disinfection to produce an effluent suitable for various reuse applications. Authors suggest partial bypass from the Imhoff tank to the horizontal subsurface flow wetland so as to provide a carbon source to promote denitrification. The treatment system performed equally well in terms of organic matter and ammonium removal both in warm and cold seasons. However, reduced nitrate retention took place in horizontal and free water surface wetlands in the cold season, presumably due to low denitrification activity at low water temperatures. In general, the three-stage hybrid constructed wetland system has proven to constitute an appropriate ecotechnology for wastewater treatment and reuse in small communities of warm climate areas.

5.1. Introduction

Since the implementation of the Water Framework Directive 2000/60/EC (EC, 2000), as well as the Directive 91/271/CEE concerning urban wastewater treatment (EC, 1991) and their stringent regulations and guidelines, especially for the treatment of small communities of less than 2000 PE, the interest for decentralized wastewater treatment ecotechnologies has steadily increased. There is therefore an increasing demand for economical, esthetic and ecologically sustainable treatment systems.

Constructed wetlands (CWs) are wastewater treatment ecotechnologies that emphasize the processes happening in natural wetlands in order to improve their treatment capacity (Kadlec and Wallace, 2009). They are used worldwide to treat various types of wastewater, such as domestic wastewater (Vymazal, 2005), combined sewer overflow (Ávila et al., 2013b) or refinery effluent (Wallace and Kadlec, 2005), among others. They have proven to be efficient at removing not only the conventional water quality parameters but also to have a great potential for the elimination of EOCs (Ávila et al., 2013a; Hijosa-Valsero et al., 2010a).

Various constructed wetland configurations may be combined so as to increase their treatment efficiency, especially for nitrogen. These hybrid systems are normally comprised of VF and HF wetlands arranged in many possible manners. While in HF wetlands nitrification is usually not achieved due to its limited oxygen transfer capacity (OTC), VF wetlands can provide good conditions for nitrification of ammonium into nitrate. Then, within an anoxic environment and in the presence of an organic substrate, denitrifying bacteria can reduce nitrate into nitrogen gas (N₂). Thenceforward, the strengths and weaknesses of each type of system balance each other out and in consequence it is possible to obtain an effluent low in BOD and in TN concentrations (Vymazal, 2007). Many combinations are possible, including HF followed by VF wetlands, VF followed by HF wetlands and other stages, including water recirculation from one stage to another (Brix and Arias, 2005). Moreover, the presence of FWS beds may act as water storages as well as it conferees a buffer capacity to the system since it allows for high flow fluctuations.

Although the benefits of hybrid constructed wetlands in small communities have been proven for the treatment of various types of wastewater, such as dairy (Comino et al., 2011; Kato et al., 2013), tannery (Saeed et al., 2012) or winery (Serrano et al., 2011) wastewaters, further studies reporting their performance on the treatment of domestic wastewater are still lacking. What is more, most of domestic wastewater hybrid CW systems have been constructed and evaluated in cold climate regions of central and northern Europe (Norvee et al., 2005; Öövel et al., 2007; Vymazal, 2005; Vymazal and Kröpfungová, 2011) and only a few examples of these type of treatment systems in Mediterranean regions have been reported (Abidi et al., 2009; Ávila et al., 2013b; Ayaz et al., 2011; Herrera-Melián et al., 2010; Masi and Martinuzzi, 2007; Tunçsiper, 2009). In sight of the water scarcity scenario and stringent regulations, the enlargement of knowledge on this matter may only enhance their acceptance and future implementation of this ecotechnology in small communities of warm regions.

With the purpose of evaluating the treatment capacity of a hybrid constructed wetland system and potential synergies in treatment processes, we designed, operated and monitored an experimental hybrid constructed wetland system based on three stages of different wetland configurations treating urban medium strength domestic wastewater for a period of about one year.

5.2. Materials and methods

5.2.1. Description of the treatment system

The experimental treatment plant is set outdoors at the experimental facility of the GEMMA group (Department of Hydraulic, Maritime and Environmental Engineering of the Universitat Politècnica de Catalunya-BarcelonaTech, Spain). The system was constructed in 2010, and after a commissioning period of establishment of the vegetation and gradual start-up of the treatment system under similar operation conditions than those of this study, the experimental plant was monitored periodically from July 2012 to May 2013. All of the elements of the treatment system are integrated on two 11 m² skids, which can be easily transported on a truck. Urban wastewater is pumped directly to the system by means of 2 pumps from a nearby municipal sewer. Firstly, the wastewater is coarsely screened and subsequently conveyed to a 1.2 m³ polyethylene storage tank, which is continuously stirred so as to avoid settling of solids and completely integrated in the experimental plant. Subsequently, the water is conducted by means of peristaltic pumps in continuous operation into an Imhoff tank (0.2 m³) with a nominal design HRT of 24 hours (for a design flow of 200 L d⁻¹). Its effluent then flows into a 0.25 m³ stirred storage tank (or distribution tank) and from this point water flows into 3 stages of different constructed wetland configurations. These are two VF beds alternating its operation followed by a HF and a FWS wetlands operating in series. The treatment line can be seen in Fig. 5.1.

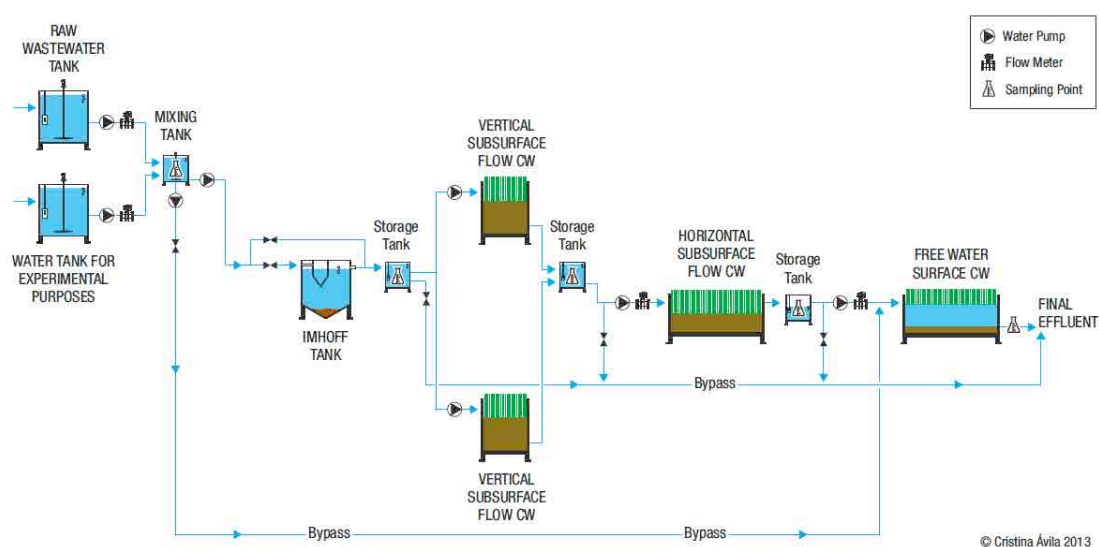


Figure 5.1. Layout and sampling points of the experimental three-stage hybrid constructed wetland system.



Figure 5.2. View of the experimental hybrid constructed wetland system in Barcelona.

The two VF wetlands have a surface area of 1.5 m^2 each and operate alternatively in cycles of 3.5 days. This alternation of phases of feed and rest allow for controlling the growth of the attached biomass, to maintain aerobic conditions within the filter bed and to mineralize the organic deposits accumulated on the bed surface (Molle et al., 2008). VF wetlands were intermittently fed from a 0.25 m^3 stirred storage tank (distribution tank) by means of hydraulic pulses so as to improve oxygen renewal. For this purpose two pressure pumps work alternatively, depending on the day of the cycle, to feed each of the VF beds. Each of these is ruled by a level float switch, which is programmed so as to provide about 13 pulses per day. Every time the level float activated the pump, a volume of approx. 15 L (from a stirred storage tank) is discharged on a VF bed during about 10 s. A polyethylene pipe distributes the pumped water 0.1 m above the top of the bed. This pipe contains 5 perforations with diffusers that provide a true 360° radial horizontal water pattern, thus ensuring an evenly distribution of the wastewater over the whole surface of the filter. Moreover, each VF container has a metal trames 0.1 m above floor level and a number of holes situated underneath it so as to allow for passive aeration of the bed.

Effluent of the VF beds –regardless of the one in operation- is accumulated in a 0.25 m^3 tank and sent by a pump which runs continuously to the second stage of wetlands, which consists of a 2 m^2 HF wetland. Finally, the effluent of this unit is collected in another 0.25 m^3 storage tank from which is continuously pumped into a 2 m^2 FWS wetland to complete the treatment and to produce an effluent of quality for its further reuse. The continuous feeding of the HF and FWS wetlands is done by means of peristaltic pumps. To this regard, the above-mentioned intermediate 0.25 m^3 storage tanks are necessary for sampling of the effluents as well as for overall functioning of the treatment system. Except for the distribution tank feeding VF beds, which holds the water until there is enough volume for a pulse, volume and holding time in the remaining storage tanks is minimum as controlled by level floats, to ensure a fresh sample. These are not exposed to light. Moreover, all CWs were constructed on polypropylene and were planted with *Phragmites australis*. Vegetation was well established in all

VF and HF wetlands. Only about a third of the area of the FWS wetland was covered with vegetation in order to allow for sunlight penetration.



Figure 5.3. View of the vertical subsurface flow constructed wetlands and their distribution pipes (a); horizontal subsurface flow and free water surface wetlands (b) in the experimental hybrid constructed wetland system located in Barcelona.

The treatment plant operated at a constant flow of approximately 200 L d⁻¹ during the whole experimental period, giving an average HLR and OLR for VF units of 0.06 m d⁻¹ and 8.9 g BOD₅ m⁻² d⁻¹. An electromagnetic flow meter (SITRANS F M MAGFLO®) was installed at the inlet of the primary treatment so as to assist on the follow up of the flow values entering the treatment system. Further system features are detailed in Table 5.1.

Table 5.1. Main characteristics of the experimental hybrid constructed wetland system.

Parameter	Unit	Value
Average Inflow	L d ⁻¹	200
Dimensions VF beds	m (W x L x D)	1.0 x 1.5 x 1.3
VF filling media	Depth of layers: m	Upper layer: 0.1 m of sand (1-2 mm)
	Grain size Ø: mm	Bottom layer: 0.7 m of fine gravel (3-8 mm)
Dimensions HF bed	M	1.0 x 2.0 x 0.3
HF water level	M	0.25
HF filtering media	Main media: mm	Main media: 0.3 m of gravel (4-12 mm)
	Inlet and outlet: cm	Inlet and outlet: stone (3-5 cm)
Dimensions FWS unit	M	1.0 x 2.0 x 0.5
FWS free water column	M	0.3
Average OLR	g BOD ₅ m ⁻² d ⁻¹	8.9*
Average HLR	m d ⁻¹	0.06*

*These values were calculated taking into consideration only the area of VF wetlands (i.e. 3 m²).

5.2.2. Sampling strategy

Since the purpose of this study was to assess the overall treatment performance of the system over a long period of operation, as well as its dependence on the seasonality, the treatment plant was monitored from July 2012 to May 2013 on a weekly basis. Grab samples were taken the same day of the week (at about 10 pm) by taking about 1.5 L of sample at the effluent of the different treatment units (Fig. 5.1). In particular, the effluent of VF beds was sampled at the subsequent storage tank, just after a feeding pulse to the VF unit occurred, so as to ensure a fresh sample. Samples of the final effluent were taken at the outlet of the FWS wetland. All samples were taken to the adjacent laboratory for the analysis of the following parameters: COD, BOD₅, TSS, NH₄-N, nitrate and nitrite nitrogen (NO_x-N), orthophosphate phosphorus (PO₄-P). Analysis of sulphate (SO₄²⁻), Total Kjeldahl nitrogen (TKN), *Escherichia coli* and helminth eggs were carried out once a month.

The influence of climatic conditions on the treatment efficiency of the system was evaluated by dividing the dataset into two periods: the warm season, from March to August, and the cold season, from September to February.

5.2.3. Analytical methods

Onsite measurements of water temperature, DO, pH and EC were taken by using a Checktemp-1 Hanna thermometer, a Eutech Ecoscan DO6 oxymeter, a Crison pH-meter and a EH CLM 381 conductivity meter, respectively. E_H was also measured in situ by using a Thermo Orion 3 Star redox meter. E_H values were corrected for the potential of the hydrogen electrode.

Conventional wastewater quality parameters, including COD, TSS and NH₄-N were determined by using Standard Methods (APHA, 2001). BOD₅ was measured by using a WTW® OxiTop® BOD Measuring System. SO₄²⁻, PO₄-P and NO_x-N were analyzed using a DIONEX ICS-1000 ion chromatograph. TKN was determined according to EN 25663 (1993). Isolation and enumeration of *E. coli* was made by using the Most Probable Number method (APHA, 2001). The enumeration of helminth eggs was done by the Baillenger method (Ayres and Mara, 1996).

5.3. Results and discussion

5.3.1. Performance of the treatment system

Average values and standard deviations of water quality parameters after each stage of the wastewater treatment system are shown in Table 5.2. To start with, it is important to note the high influent bulk water DO concentrations in the raw wastewater (3.5 ± 1.6 mg O₂ L⁻¹). The fact that the wastewater arrived so oxygenated was owed to the frequent pumping and prolonged stirring of the water in its transport from the sewer system up until the experimental treatment plant and, more particularly, to its wastewater tank. This oxygen input was found to be unavoidable and was linked to a high redox status of the wastewater ($+249 \pm 133$ mV). However, it can be seen how DO concentrations slightly decreased as the wastewater passed through the Imhoff tank (2.0 ± 1.1 mg O₂ L⁻¹), where oxygen was presumably consumed by bacterial activity in the process of degradation of some of the influent organic matter (as we will see later on), conferring likewise less oxidized conditions to its effluent ($+89 \pm 189$ mV). Subsequently, as the wastewater percolated through the filter media and plant root zone of the VF beds, it recovered its oxidizing potential up to values similar to the original ones (i.e. 4.4 ± 0.9 mg O₂ L⁻¹ and $+205 \pm 84$ mV). DO and Eh values dramatically decreased in its passage through the HF wetland (i.e. 0.4 ± 0.2 mg O₂ L⁻¹ and -85 ± 52 mV), and finally increased within the free water column of the FWS unit, obtaining high values in the final effluent (5.7 ± 1.9 mg O₂ L⁻¹ and $+241 \pm 61$ mV).

Table 5.2. Average concentrations (\pm s.d.) of water quality parameters along the experimental hybrid treatment system (n = 57).

Parameter	n	Influent	Imhoff tank	VF	HF	FWS
T (°C)	57	20.0 \pm 5.1	20.1 \pm 5.2	19.4 \pm 5.1	20.0 \pm 5.0	19.1 \pm 5.4
DO (mg L ⁻¹)	53	3.5 \pm 1.6	2.0 \pm 1.1	4.4 \pm 0.9	0.4 \pm 0.2	5.7 \pm 1.9
E _h (mV)	57	249 \pm 133	89 \pm 189	205 \pm 84	-85 \pm 52	241 \pm 61
pH	56	7.8 \pm 0.2	7.8 \pm 0.2	7.8 \pm 0.2	7.6 \pm 0.2	7.8 \pm 0.4
EC (mS cm ⁻¹)	57	2.4 \pm 0.7	2.4 \pm 0.6	2.3 \pm 0.6	2.3 \pm 0.6	2.3 \pm 0.4
Turbidity (NTU)	57	112 \pm 83	81 \pm 48	8 \pm 5	3 \pm 2	2 \pm 2
PO ₄ -P (mg L ⁻¹)	21	4.6 \pm 1.3	5.6 \pm 1.4	4.9 \pm 1.0	4.9 \pm 1.2	4.1 \pm 1.1
SO ₄ ²⁻ (mg L ⁻¹)	46	157 \pm 40	126 \pm 55	143 \pm 33	141 \pm 34	141 \pm 33
BOD ₅ (mg L ⁻¹)	51	164 \pm 67	133 \pm 61	26 \pm 14	16 \pm 13	13 \pm 11
COD (mg L ⁻¹)	15	205 \pm 84	189 \pm 83	61 \pm 34	47 \pm 27	49 \pm 24
TSS (mg L ⁻¹)	17	161 \pm 68	25 \pm 18	13 \pm 12	2 \pm 2	4 \pm 3
NH ₄ -N (mg L ⁻¹)	53	24.4 \pm 9.4	20.2 \pm 10.8	5.3 \pm 1.2	2.4 \pm 1.7	0.9 \pm 0.8
NO _x -N (mg L ⁻¹)	29	0.3 \pm 0.2	0.2 \pm 0.3	16.3 \pm 3.2	12.6 \pm 5.1	13.6 \pm 6.8
TKN (mg L ⁻¹)	30	34.0 \pm 12.8	27.1 \pm 5.5	11.4 \pm 3.1	6.3 \pm 3.0	3.1 \pm 1.9

Water quality parameters (and their removal efficiencies) were calculated in concentration units and not in mass loads per unit area, as evapotranspiration rates were not measured. Fig. 5.4 shows the decrease in the concentration of TSS and the two forms of organic matter (COD, BOD₅) along the treatment line. The removal of TSS was very efficient and consistent within the Imhoff tank ($83 \pm 8\%$). This emphasizes the importance of the primary treatment on TSS removal, crucial for avoiding clogging of the bed media in both VF and HF wetlands (Langergraber et al., 2003; Pedescoll et al., 2011a). The entrapment of solids in the VF beds was efficient showing a removal rate of $57 \pm 26\%$ and concentrations remained low during its passage through the rest of the treatment plant up to an overall removal rate of $97 \pm 1\%$.

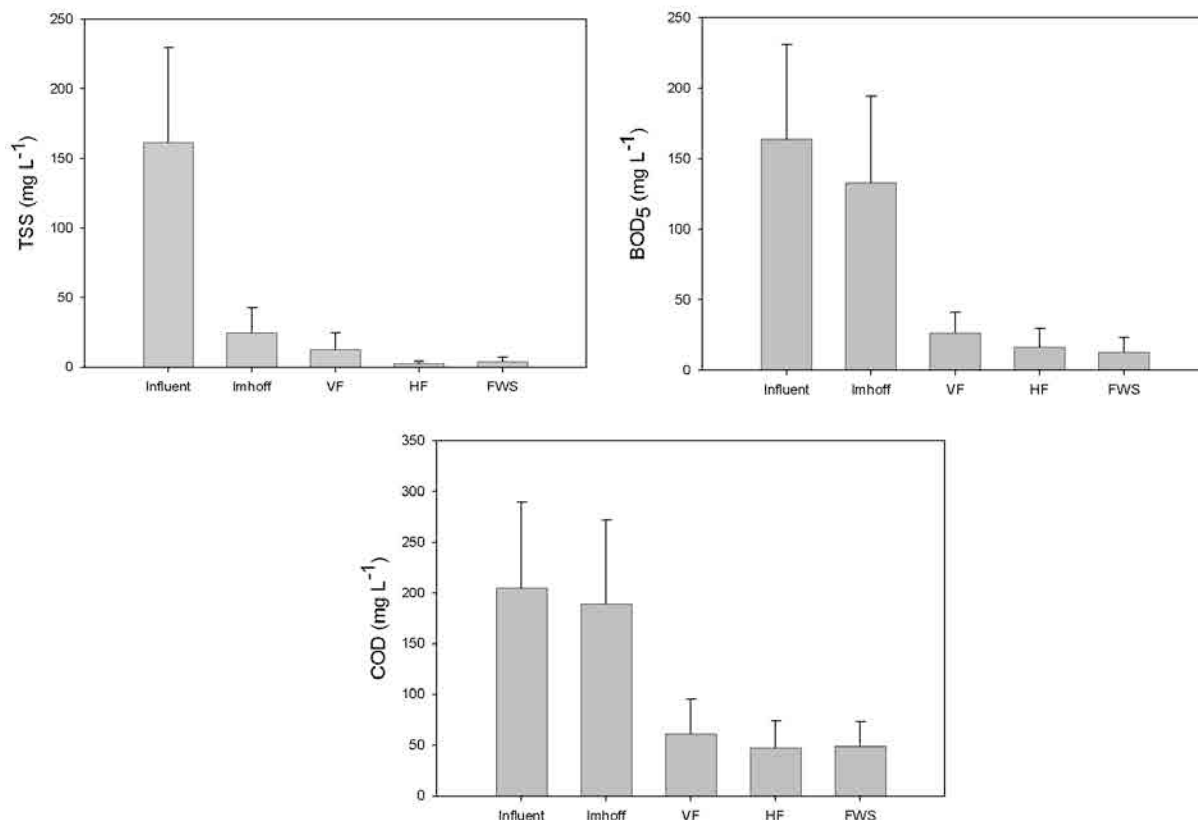


Figure 5.4. Average values (\pm s.d.) of water quality parameters in the effluent of the different treatment units of the experimental hybrid constructed wetland system in Barcelona.

Influent COD and BOD₅ concentrations were fairly low (205 ± 84 mg L⁻¹ and 164 ± 67 mg L⁻¹, respectively) and therefore it was a medium strength wastewater. It is to be mentioned that the neighborhood that produced the wastewater is residential and holds high water consumption derived from its use in gardens and swimming pools. Moreover, there is a high presence of schools in the area.

Removal rates for COD and BOD₅ in the Imhoff tank were of $11 \pm 28\%$ and $15 \pm 33\%$, respectively. Subsequently, a high organic matter removal took place in the VF wetlands, with elimination rates of $65 \pm 19\%$ for COD and $77 \pm 15\%$ for BOD₅. These rates are in accordance with those reported by Norvee et al. (2005) in a treatment plant of similar characteristics consisting of a VF followed by a HF wetland treating a hospital effluent in Estonia (66% of BOD₇ removal in the VF bed). The configuration of this type of systems confers a great oxygen transfer rate, which greatly enhances the removal of organic matter. It is the special operating conditions of VF wetlands (i.e. intermittent loading and

resting periods) that allow high organic loading rates to be applied, while avoiding clogging of the granular media (Molle et al., 2006; Weedon, 2003). Moderate organic matter removals took place within the HF unit ($27 \pm 14\%$ for COD and $41 \pm 33\%$ for BOD_5). However, overall elimination rates were very high ($78 \pm 7\%$ for COD and $91 \pm 7\%$ for BOD_5) and laid within the range reported by Herrera-Melián et al. (2010) in a similar treatment system in the warm climate of the Canary Islands (Spain).

Fig. 5.5 exhibits the balance of different nitrogen species in the effluent of each treatment system unit on a yearly basis. The VF bed performed very well in removing NH_4-N (74%), which was in most part transformed into oxidized nitrogen species (NO_x-N). Again it is manifest the high level of oxygenation taking place within the VF bed, which allowed for the nitrification of the major part of NH_4-N . Concentrations of NH_4-N further decreased during its passage through the HF and FWS wetlands, to achieve an excellent overall removal rate of $94 \pm 6\%$.

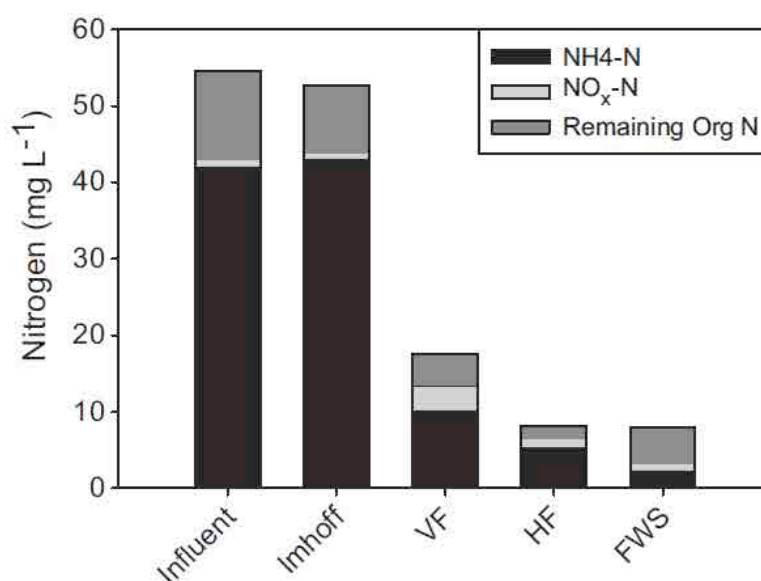


Figure 5.5. Concentration of the different nitrogen species at the effluent of the different treatment units of the experimental three-stage hybrid treatment system.

On the other hand, concentrations of NO_x-N remained almost invariable along the HF and FWS beds. The fact that such a low denitrification occurred in the HF wetland could be attributed to the lack of organic matter necessary for heterotrophic bacteria to accomplish the denitrification reaction (Kadlec and Wallace, 2009). The high performance of VF beds produced an effluent with a very low organic matter content to enter the HF wetland (26 ± 14 mg BOD_5 L⁻¹), which could constitute a restraining factor for the denitrification process in the HF wetland. To this regard, Behrends et al. (2007) showed how the lack of organic matter in terms of labile organic carbon (COD) resulted in reduced denitrification in an integrated constructed wetland system treating medium strength wastewater from a pilot-scale intensive fish farm. The addition of an external carbon source was also helpful for the denitrification activity in a system consisting of coupled VF and HF wetlands in China. Xinshan et al. (2010) documented in this system a TOC/TN ratio ranging 2.5 to 5.0 for a good TN removal efficiency (above 92%). On the other hand, Masi and Martinuzzi (2007) obtained a fully nitrified effluent in a system consisting of a HF followed by a VF bed and recommended its recirculation to the primary treatment unit so as to promote the presence of anoxic conditions that could enhance the denitrification capacity of the treatment system. The removal of TN in their treatment system was of 60%, a slightly higher rate than the $46 \pm 22\%$ obtained in our treatment plant.

The presence of nitrates in the final effluent is not of great concern since there are not fixed limits for this parameter in the guidelines of the European Directive (EC, 1991) regarding wastewater treatment of small communities. However, we believe that TN removal efficiency could be improved by bypassing part of the effluent of the Imhoff tank straight into the HF wetland, which would increase the organic matter source availability and thus promote denitrifying bacteria activity. Previous experiences have proved the benefits of bypass or recirculation for the enhancement of the treatment capacity of VF wetlands (Brix and Arias, 2005) as well as hybrid systems (Ayaz et al., 2011; Ayaz et al., 2012; Kato et al., 2013; Tunçsiper, 2009), especially in terms of TN removal. Another option would be to operate the treatment plant at higher HLRs, hence forcing its treatment capacity and limiting the OTC within the VF bed, and consequently generating conditions more favourable for an efficient denitrification in the HF and FWS units. Feeding the VF beds with raw wastewater without any primary treatment such as the common French systems (Molle et al., 2006) could also constitute a good strategy. At the moment we are planning assays, which consist in the application of higher HLRs to the treatment system so as to evaluate its influence on treatment performance.

Retention of PO₄-P along the treatment system was very low with overall removal efficiencies of approx. 11%. In general, phosphorus removal in CWs ranges 10 – 20%, and no significant differences are found between HF and VF wetlands (Kadlec and Wallace, 2009). A similar tendency was observed for the concentration of sulphates, with total elimination rates of 4,8%.

On the other hand, the seasonal variability of treatment efficiency was evaluated and results for some water quality parameters are shown in Table 5.3. The treatment system was found to perform equally well throughout the year in terms of organic matter and NH₄-N removal, showing no significant differences in both individual and overall average removal efficiencies of BOD₅ or NH₄-N between warm and cold seasons. However, it is to be noted how, while in the warm season water NO_x-N concentrations in water slightly decreased along its passage through the HF and FWS units, no elimination of NO_x-N was found during the cold season. These results demonstrate the high dependence of denitrification rates, as well as period of maximum plant assimilation, on water temperature (15 ± 5 and 23 ± 2 °C in cold and warm seasons, respectively), with a reduced denitrification activity at lower temperatures (Spieles and Mitsch, 2000; Boulêtreau et al., 2012).

Table 5.3. Average concentration (± s.d.) along the experimental hybrid treatment system and overall removal efficiency (RE) of various water quality parameters in warm and cold seasons.

	Influent	Imhoff tank	VF	HF	FWS	Total RE (%)
Warm season (Mar-Aug)						
T (°C)	23.5 ± 2.5	23.7 ± 2.4	22.3 ± 2.4	23.0 ± 2.6	22.9 ± 2.3	n.a.
DO (mg L ⁻¹)	3.0 ± 1.2	2.1 ± 1.0	4.2 ± 0.8	0.4 ± 0.2	4.7 ± 1.4	n.a.
BOD ₅ (mg L ⁻¹)	127 ± 44	118 ± 45	20 ± 10	14 ± 7	12 ± 5	90
NH ₄ -N (mg L ⁻¹)	22.3 ± 10.5	21.9 ± 12.4	5.2 ± 1.4	3.0 ± 2.0	0.8 ± 1.1	96
NO _x -N (mg L ⁻¹)	0.3 ± 0.3	0.1 ± 0.1	15.9 ± 3.6	11.8 ± 3.7	8.2 ± 6.9	n.a.
Cold season (Sept-Feb)						
T (°C)	16.1 ± 5.0	16.2 ± 5.0	15.7 ± 5.3	15.7 ± 4.7	15.5 ± 5.1	n.a.
DO (mg L ⁻¹)	4.0 ± 1.6	1.9 ± 1.1	4.5 ± 0.6	0.5 ± 0.2	6.6 ± 1.6	n.a.
BOD ₅ (mg L ⁻¹)	193 ± 57	183 ± 70	29 ± 12	16 ± 16	12 ± 14	94
NH ₄ -N (mg L ⁻¹)	24.1 ± 7.4	18.3 ± 5.3	5.4 ± 1.2	2.3 ± 1.1	1.1 ± 0.7	95
NO _x -N (mg L ⁻¹)	0.2 ± 0.1	0.3 ± 0.3	15.7 ± 3.8	14.5 ± 5.0	15.2 ± 5.2	n.a.

n.a. - not applicable.

5.3.2. Quality of final effluent and potential reuse applications

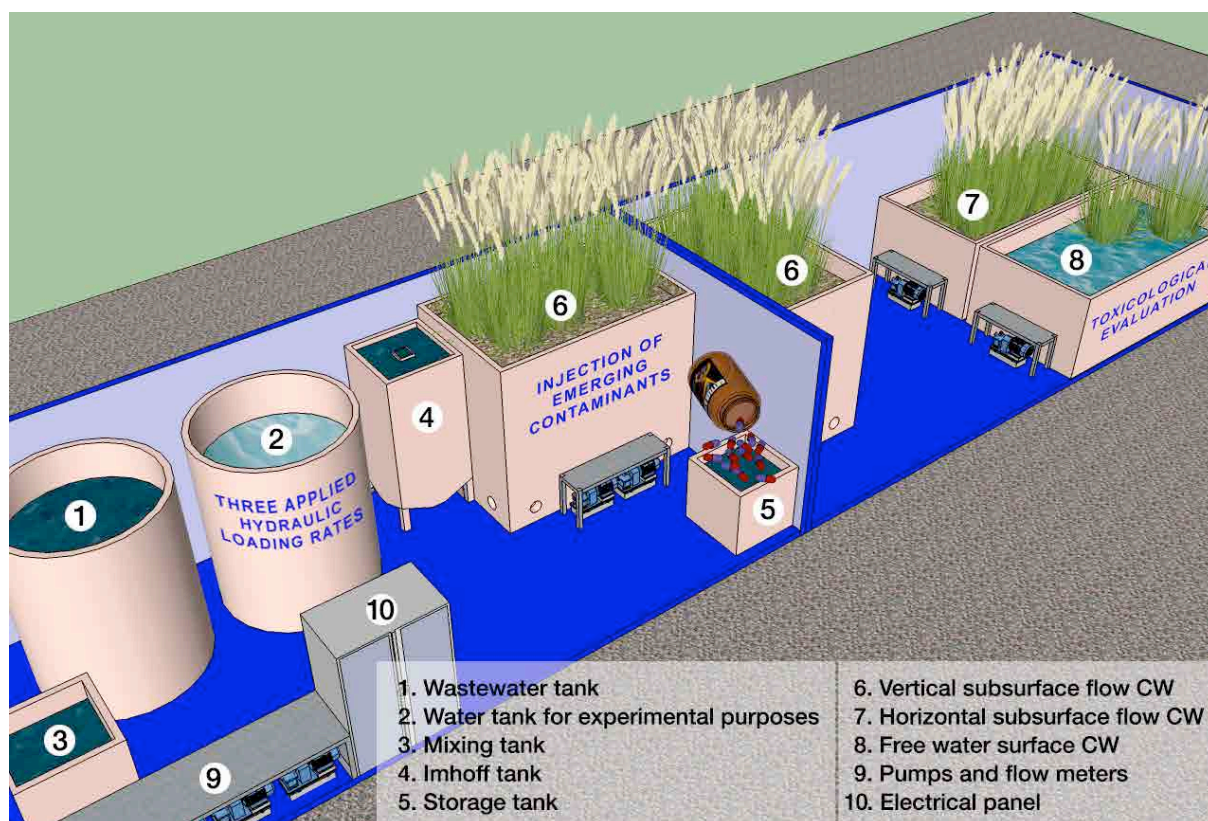
E. coli was used as an indicator of pathogen microorganisms at the final effluent so as to assess its possible reuse applications. Although no samples of the influent wastewater were analyzed for this pathogen and thus no removal efficiencies could be calculated, the general performance of the treatment system was satisfactory and the final effluent had *E. coli* values of approximately 2.3 ± 2.5 log-units 100 mL^{-1} . No helminth eggs were found throughout the monitoring period. This value is very similar to that reported by Ávila et al. (2013b) in a treatment system with the exact same configuration but at full-scale.

The final effluent of the treatment plant fulfilled the Spanish regulation limits (BOE, 2007) for some water reuse applications as regards the pathogens indicator *E. coli*, TSS concentration and helminth eggs. According to maximum admitted values equal to 1000 CFU 100 mL^{-1} for *E. coli*, 35 mg L^{-1} for TSS and no helminth eggs, the potential reuse applications allowed for the final effluent of the treatment system are the following: agricultural (irrigation of crops of ornamental flowers, plant nurseries, greenhouses with no contact of regenerated water with crops; irrigation of non-alimentary industrial crops, cereal and oilseed crops), industrial (process and cleaning water except for food industry), recreational (recharge of water bodies with no access to the public) and environmental uses (recharge of aquifers by percolation through the ground). As in the study by Ávila et al. (2013b) in a pilot-scale hybrid system with the exact same configuration, the HF and FWS wetlands would probably prove crucial to achieve a water quality suitable for its further reuse.

5.4. Conclusions

VF wetlands achieved great BOD_5 removal and nitrification of most part of ammonium nitrogen. However, almost negligible denitrification occurred within HF and FWS wetlands presumably due to the lack of organic matter necessary for denitrifying bacteria metabolism. A bypass of part of the wastewater from the Imhoff tank to the HF bed is recommended to promote denitrification. Disinfection processes occurring within the HF and FWS wetlands made final effluent suitable for reuse in various applications. The treatment system performed equally well in terms of organic matter and $\text{NH}_4\text{-N}$ removal both in warm and cold seasons. However, lower denitrification activity took place in HF and FWS wetlands in the cold season, presumably due to the low water temperature. In general, the hybrid constructed wetland treatment system has proven to be a very robust ecotechnology adequate for the treatment of wastewater of similar characteristics in small communities of warm climate areas such as the Mediterranean region. Further experiments should be made in the future so as to assess the treatment capacity of the system when working under higher organic loads.

6. Attenuation of emerging organic contaminant in an experimental hybrid constructed wetland system under different hydraulic loading rates and their associated toxicological effects in wastewater



This chapter is based on the article:

Ávila, C., Matamoros, V., Reyes-Contreras, C., Piña, B., Casado, M., Mita, L., Rivetti, C., Barata, C., García, J. and Bayona, J.M., 2013. Attenuation of emerging organic contaminants in a hybrid constructed wetland system under different hydraulic loading rates and their associated toxicological effects in wastewater. *Science of the Total Environment*, accepted.

The capacity of a hybrid CW system consisting of two VF wetlands working alternatively (3 m²), one HF wetland (2 m²) and one FWS wetland (2 m²) in series to eliminate 13 EOCs under three different HLRs (0.06, 0.13 and 0.18 m d⁻¹ – considering the area of the two VF beds-) was studied through a continuous injection experiment. General toxicity, dioxin-like activity, antimicrobial activity and estrogenicity were also measured under the highest hydraulic loading rate. The hybrid system was highly efficient on the removal of total injected EOCs (except for antibiotics, 43 ± 32%) at all three HLRs (87 ± 10%). The removal efficiency in the hybrid CW system showed to decrease as the HLR increased for most compounds. The VF wetland removed most of the injected EOCs more efficiently than the other two CWs, which was attributable to the predominant aerobic degradation pathways of the VF beds (70 ± 21%). General toxicity was reduced up to 90% by the VF unit. Estrogenicity and dioxin-like activity were similarly reduced by the VF and the HF wetlands, whereas antimicrobial activity was mainly removed by the FWS unit. Bearing this in mind, this injection study has demonstrated that the use of hybrid CW systems is a suitable wastewater technology for removing EOCs and toxicity even at high HLRs.

6.1. Introduction

EOCs are a large, relatively new group of unregulated compounds such as pharmaceuticals, personal care products, plasticizers, surfactants and herbicides about which there is relatively limited ecotoxicological and human health information (Murray et al., 2010). The presence of these new contaminants in both fresh and reclaimed waters is a matter of major concern with largely unknown consequences (Daughton, 2005; Kümmerer, 2009). Among the most well known effects, it has been reported that triclosan impairs algal growth and develops bacterial resistance (Orvos et al., 2002) whereas pharmaceutical mixtures containing carbamazepine, ibuprofen, and clofibrac acid have also been found to be toxic for algae (Cleuvers, 2003). Estrogenicity has already been demonstrated for many contaminants such as natural and synthetic hormones and alkylphenols commonly detected in wastewaters (Bergman et al., 2013, Dagnino et al., 2010).

Although the removal of EOCs may be partially achieved by the application of conventional wastewater treatment plants (WWTPs) combined to advanced tertiary treatment processes (Rosal et al., 2010), their high cost limits the widespread application of these treatment technologies. In this regard, non conventional technologies with low O&M cost such as CWs increased their interest as decentralized WWTPs and also for wastewater sanitation of small populations (Kadlec and Wallace, 2009), which are highly efficient on the removal of EOCs (Matamoros and Bayona, 2013). CWs are effective in treating polluted waters arising from a wide range of domestic, industrial and agricultural operations. Such eco-technology enables the water to be reused in a cost-effective way, whilst at the same time creating small areas of wetland wildlife habitat (Randerson, 2006).

CWs can be classified as either surface flow (SF) or subsurface flow (SSF) systems. SSF wetlands are subdivided into VF and HF wetlands, depending on the water flow direction. HF units are continuously fed and the wastewater flows horizontally through a vegetated gravel bed. Conversely, in VF wetlands the feeding is done intermittently and water is distributed across the surface of a planted sand/gravel bed, and effluent is collected from the bottom of the media, where the water is freely draining. In VF systems sometimes two or three parallel beds are used, which alternate phases of feed and rest, so as to promote mineralization of the deposit of solids during resting phases (Molle et al., 2005). In this regard, the HF bed works under saturated conditions and the removal of organic matter is mostly through anaerobic pathways, whereas the VF system works under unsaturated water conditions and the aerobic environment prevails (Vymazal, 2007). FWS wetlands are generally used for treating secondary treated wastewater effluents and permit the water reuse with healthy guaranties due in part to photodegradation processes (Llorens et al., 2009). In this way, while some substances such as ibuprofen, naproxen and diclofenac exhibit better removal in VF than HF wetlands, hydrophobic compounds like tonalide and galaxolide exhibit similar elimination rates at the two CW configurations due to sorption processes onto the organic matter (Matamoros et al. 2005, 2007). Moreover, FWS wetlands have shown to increase the removal efficiency of photolabile compounds like triclosan and ketoprofen as a consequence of direct sunlight exposure (Breitholtz et al., 2012; Conkle et al., 2008; Matamoros et al., 2008b; Matamoros and Salvadó, 2012).

Despite the great performance of CWs, one of their main limitations is that they typically require low HLRs, which is translated in a large surface area. In order to exploit different degradation pathways and hence improve overall water effluent quality, CWs of different configuration can be operated in series, and therefore they can work at greater HLRs (Vymazal, 2005). Masi et al. (2004) studied the capacity of a hybrid system consisting of a VF and a HF wetlands and found that it was capable of removing estrogens up to 90%. Reyes-Contreras et al. (2012) found that a hybrid system consisting of an upflow anaerobic sludge blanket reactor (UASB), FWS and a HF wetlands in series led to an

improvement in the removal of various EOCs. Nevertheless, little attention has been paid to the capacity of hybrid systems based on VF-HF-FWS configuration for the attenuation of EOCs and their associated biological effects in wastewater.

The aim of this study was to assess the capacity of an experimental-scale hybrid CW system for urban wastewater treatment consisting of two parallel VF units alternating cycles of feed and rest, one HF and one FWS wetland in series for removing EOCs and to evaluate potential synergies in treatment processes. We performed continuous injection experiments at three different HLRs (i.e. once, twice and three times the design flow) of three non-steroidal anti-inflammatory drugs (IB, DCF and ACE), three personal care products (AHTN, OXY and TCS), two endocrine disruptors (BPA and EE2) and five antibiotics (lincomycin, erythromycin, doxycycline, sulfamethoxazole and enrofloxacin). Given the lack of information on the removal of EOCs in hybrid CW systems, the aim was to test whether a simple operating approach can lead to an enhanced treatment capacity. Focus was put onto the removal efficiency and processes (e.g. biodegradation, sorption, photodegradation) of EOCs at different HLRs. Moreover, and so as to have a further insight into the biological effects of targeted EOCs and antibiotics, we carried out assays of general toxicity, estrogenicity, dioxin-like and antimicrobial activities during the highest HLR campaign. Compounds were selected on the basis of their concentration and high frequency of detection in WWTPs (Hijosa-Valsero et al., 2011a; Miège et al., 2009), whereas toxicological analyses were selected according to the expected biological effects of previous selected compounds. To the best of our knowledge this is the first comprehensive study involving EOCs and their biological effects in CWs.

6.2. Materials and methods

6.2.1. Pilot plant description

Fig. 6.1 shows the experimental treatment plant, which was set outdoors at the experimental facility of the GEMMA's group (Universitat Politècnica de Catalunya-Barcelona Tech, Spain). The system started up operation in May 2010. Urban wastewater collected from a municipal sewer is pumped into an Imhoff tank (0.2 m³) with a nominal design HRT of 24 hours (design flow of 200 L d⁻¹). Its effluent then flows into a 0.25 m³ stirred storage tank (distribution tank) and from this point water flows into 3 stages of different constructed wetland configurations. These consist of two parallel VF beds alternating their operation followed by a HF and a FWS wetlands operating in series. The two VF wetlands have a surface area of 1.5 m² each and operate alternatively in cycles of 3.5 days. They are intermittently fed from the distribution tank by means of hydraulic pulses so as to improve oxygen renewal (about 15 L per pulse), thus administering about 13, 27 and 37 pulses d⁻¹ for the three applied HLRs in ascending order, respectively.

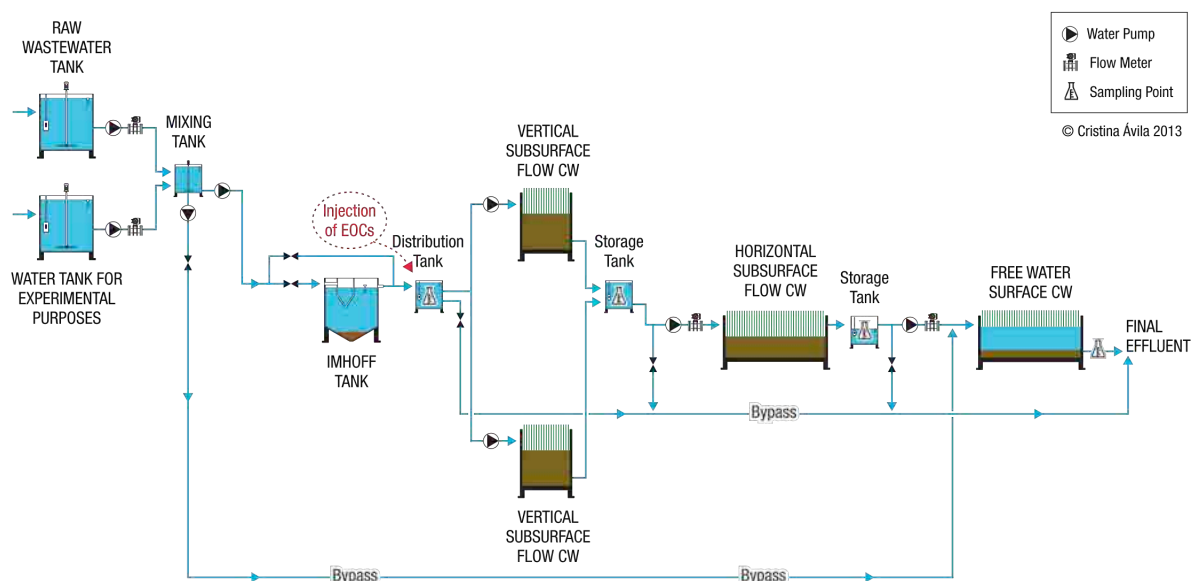


Figure 6.1. Layout, injection of emerging organic contaminants and sampling points of the experimental hybrid treatment system.

Effluent of the VF beds is accumulated in a 0.25 m³ tank and sent to the second wetland stage, which consists of a 2 m² HF wetland. Subsequently, the effluent of this unit is collected in another 0.25 m³ storage tank and finally pumped into a 2 m² FWS wetland to complete the treatment. Feeding of the HF and FWS wetlands is in a continuous mode and is performed by means of peristaltic pumps. To this regard, the above-mentioned intermediate 0.25 m³ storage tanks are necessary for sampling of the effluents as well as for overall functioning of the treatment system. Retention time within these tanks is <1h and they do not interfere in water characteristics. They are not exposed to light. The filling media of the VF beds consists of an upper 0.1 m layer of sand (1-2 mm) and a main layer of fine gravel (3-8 mm). Each VF container has a metal grating 0.1 m above floor level and a number of holes situated underneath it so as to allow for passive aeration of the bed. The HF wetland contains gravel media (4-12 mm), which is 0.3 m deep (water depth = 0.25 m) and inlet and outlet zones of stone (3-5 cm) (Ávila et al., 2013a). The FWS unit contains about 0.1 m of gravel media (4-12 mm) for establishment of the vegetation. All CWs were constructed in polyethylene and were planted with *Phragmites australis* a year before. Vegetation was well established in both VF and HF wetlands at the time of the injection experiment. Only about a third of the area of the FWS wetland was covered with vegetation in order to allow for sunlight penetration.

6.2.2. Experimental design

6.2.2.1. Hydraulic loading rate assays

Since the main scope of this study was to evaluate the treatment capacity of the hybrid CW system under high organic and hydraulic loads, the system was submitted to three campaigns of different HLRs (i.e. 0.06, 0.13 and 0.18 m d⁻¹). Considering VF beds are the treatment stage receiving the major part of the organic load, and aiming at evaluating their treatment potential when submitted to increasing HLRs, only the surface area of the VF beds was considered for the calculation of the mentioned HLRs. Although only one VF wetland is operating at a time, the need to have another one for allowance of resting periods represents additional surface area, and therefore the surface of both VF beds was taken into consideration (3 m²).

These HLRs corresponded to the design flow (200 L d⁻¹), twice (400 L d⁻¹) and almost three times (550 L d⁻¹) the design flow of the treatment plant, respectively. These experiments were carried out from March to April 2011. The applied HLRs were controlled by increasing the frequency of the peristaltic pump, which feeds the Imhoff tank, and these were validated with the corresponding flow meter. This peristaltic pump worked a total of 8, 16 and 24 h d⁻¹ distributed throughout the day for the small, medium and high HLRs, respectively. Since hydraulic batches were applied to the VF wetlands with the same flow (approx. 15 L per pulse) at every HLR campaign, a number of 13, 27 and 37 pulses per day were administered to the VF units for the three HLR campaigns in ascending order, respectively. The treatment plant was submitted to the 3 HLR campaigns in ascending and consecutive order. Acclimation of the CW treatment system to each of the HLRs was allowed, before sampling was carried out, for a time span of a minimum of 3 times the theoretical HRT of the entire treatment system. The HRTs within the CW system were approximately 4, 2 and 1.5 d during the small, medium and large HLRs' campaigns, respectively. These were calculated taking into consideration HF and FWS units, since the HRT in VF beds is expected to be of hours.

6.2.2.2. Injection of emerging organic contaminants

For the injection of the target compounds during the 3 HLR campaigns, a glass bottle of 20 L of refrigerated distilled water protected from sunlight was spiked with IB, DCF, ACE, AHTN, OXY, BPA, TCS, EE2, which were previously dissolved in methanol. KBr was also added as a conservative tracer. This mixture was homogenized and injected into the stirred storage tank distributing the primary effluent into the hybrid CW system (Imhoff tank effluent, also called distribution tank), using a peristaltic pump that supplied a flow rate of 1.3 L h⁻¹, obtained from Damova (Barcelona, Spain). This was synchronized to the peristaltic pump feeding wastewater into the Imhoff tank.

Injected concentrations entering the CW system corresponded to about 250% of the maximum concentration of the EOCs detected in wastewater in previous campaigns (Ávila et al., 2013b; Hijosa-Valsero et al., 2011b). The injection of EOCs was continuously run for 42 days from March 5th to April 15th 2011 at a constant concentration. After the acclimation period for each HLR campaign and only when effluent concentrations of the tracer were equilibrated, effluent samples were grabbed as explained in Section 6.2.3.

6.2.3. Sampling strategy

Only after acclimation period and stabilization of the conservative tracer at the effluent, grab samples were taken once a day for a total of 5 consecutive days (for each of the HLR campaigns) at effluent of the different treatment units (Fig. 6.1). Although a high variability in EOC concentrations depending on the time of the day and on the period of the year is expected to take place in the influent wastewater (Ort et al., 2010), the performance of a continuous injection experiment can solve this issue. Previous injection experiments have shown to achieve approximate steady state conditions and to obtain a fairly reliable estimate of the removal efficiency of a CW system (Ávila et al., 2010; Ávila et al., 2013b).

This sampling strategy was carried out in the same manner for each of the three HLR campaigns in consecutive and ascending order, during March and April 2011. Samples were collected in 250 mL amber clean glass bottles, which were taken to the laboratory where they were stored at 4°C until analysis. The sample holding time was less than 48 h. Samples were analyzed for conventional water quality parameters and EOCs as described in Section 6.2.5.

6.2.4. Chemicals and Reagents

GC grade (Suprasolv) hexane, methanol, ethyl acetate and acetone were supplied by Merck (Darmstadt, Germany). Analytical-grade hydrogen chloride was obtained from Panreac (Barcelona, Spain). IB, ACE, DCF, AHTN, OXY, BPA, TCS, EE2 and 2,2'-dinitrophenyl were purchased from Sigma-Aldrich (Steinheim, Germany). 2,4,5-trichlorophenoxypropionic acid (2,4,5-TPA) was from Riedel-de-Haën (Seelze, Germany). Potassium bromide and trimethylsulfonium hydroxide (TMSH) were purchased from Fluka (Buchs, Switzerland). Strata-X polymeric SPE cartridges (200 mg) were obtained from Phenomenex (Torrance, CA, USA) and the 0.7 μm glass fiber filters of $\varnothing = 47$ mm were supplied by Whatman (Maidstone, UK).

For determination of antibiotics, SMZ, lincomycin hydrochloride monohydrate, erythromycin hydrate and ENR were all purchased from Fluka (BUCHS, Switzerland). Doxycycline hydrochloride was obtained from SYVA S.A. laboratories. Enrofloxacin-d5 hydrochloride (ENR-d5) was obtained from Sigma-Aldrich (Steinheim, Germany). Sulfamethoxazol-d4 (benzene D4) (SMZ-d4) was bought from Toronto Research Chemicals (Toronto, Canada). GC grade (SupraSolv) methanol and HPLC grade water (Lichrosolv) were obtained from Merck (Darmstadt, Germany). The following chemicals were all purer than analytical grade formic acid (purity 99%) from Merck (Darmstadt, Germany), ethylenediaminetetraacetic acid disodium salt 2-hydrate (Na_2EDTA) from Panreac (Barcelona, Spain). Nylon membrane filters 0.45 μm ($d=47$ mm) and 0.22 μm ($d=17$ mm) were obtained from Millipore (Ireland) and Scharlab (Barcelona, Spain) respectively. Oasis HLB solid-phase extraction cartridges (200 mg 6 mL^{-1}) were obtained from Waters (Milford, MA, USA).

6.2.5. Analytical methodology

6.2.5.1. Conventional water quality parameters and emerging organic contaminants' determination

Conventional wastewater quality parameters, including $\text{NH}_4\text{-N}$, TSS and COD were determined by using Standard Methods (APHA, 2001). Bromide was analyzed using a DIONEX ICS-1000 ion. Onsite measurements of water temperature, DO, pH and turbidity were taken using a Checktemp-1 Hanna thermometer, a Eutech Ecoscan DO6 oxymeter, a Crison pH-meter and a Hanna HI 93703 turbidimeter, respectively. E_{H} was also measured in situ by using a Thermo Orion 3 Star redox meter. E_{H} values were corrected for the potential of the hydrogen electrode.

Concentrations of the selected EOCs in wastewater samples were analyzed after the samples had been filtered and processed as previously described by Matamoros et al. (2005) and Hijosa-Valsero et al. (2011a). Briefly 250 mL of wastewater from Imhoff and VF wetland effluents and 500 mL from HF and FWS wetland effluents were filtered and percolated through a SPE cartridge by duplicate. One cartridge was used for analytical determination of EOCs in water samples whereas the other was used for the toxicological analysis. The instrumental linearity range was from 0.005 to 10 mg L^{-1} . The correlation coefficients (R^2) of the calibration curves were always higher than 0.99. The limit of detection (LOD) and limit of quantification (LOQ) were compound dependent in the range from 9 to 151 ng L^{-1} and from 36 to 169 ng L^{-1} , respectively. RSD was lower than 20% and recoveries were above 80%.

6.2.5.2. Determination of antibiotics

For the determination of antibiotics, aqueous samples were filtered through a nylon membrane filter with a pore size of 0.45 μm . A sample volume of 200 mL (influent and effluent) was spiked with 113 ng SMZ-d4 and 101 ng ENR-d5. 2.5 mL Na_2EDTA 5% (m/v) was added to all samples after filtration.

The spiked sample was percolated through a solid-phase extraction cartridge Oasis-HLB (200mg 6mL⁻¹) previously conditioned with 6 mL methanol 6 mL HPLC grade water 6 mL Na₂EDTA 10 mmol. The flow rate was adjusted to approximately 5 mL min⁻¹. After the preconcentration of sample, cartridges were rinsed with 8 mL of HPLC grade water and allowed to dry for 15 min. The extract was eluted with 5 mL methanol. The extract was evaporated until 100 µL under a gentle nitrogen stream and reconstituted to a final volume of 1000 µL with LC mobile phase (water/methanol, 98/2, v/v; 0.1% formic acid).

A TSQ Quantum triple-stage quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Thermo Fisher Scientific, San Jose, CA, USA), a Finnigan Surveyor MS Pump Plus and an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland) were used for the UPLC-MS/MS analysis.

The chromatographic separation was conducted on a C18 Kinetex column (2.6 µm × 5 cm × 2.1 mm ID) Phenomenex (Torrance, CA, USA) preceded by an XBridge C18 guard column (2.5 µm × 5 mm × 2.1 mm ID) Waters (Milford, MA, USA). The mobile phase was Milli-Q water as eluent A and methanol as eluent B both containing 0.1% formic acid at flow rate of 350 µL min⁻¹. The elution started at 2% B for 1 min and was then linearly ramped up to 99% B in 18 min, where it was held for 1 min before being returned to the initial conditions in 1 min. The injection volume was 5 µL, and the column was maintained at 35°C.

The MS/MS determination was carried out in ESI positive ion mode with the spray voltage at 5.0 kV and the optimum tube lens voltage (TL) for each m/z. The ion transfer temperature was set at 250°C. Nitrogen (purity > 99.999%) was used as a sheath gas, ion sweep gas and auxiliary gas at a flow rate of 70 psi. Argon gas was used for collision-induced dissociation at a pressure of 1.3 mTorr, and the optimum collision energy (CE) was selected for each transition. The target compounds were analyzed in the MRM mode, monitoring two transitions for each compound. The quantifier and qualifier ions, CE and LODs, LOQs are shown in table 1SM. Data acquisition was performed with Xcalibur 2.0.7 software (Thermo Fisher Scientific). The linearity range was from 0.002 to 3 µg mL⁻¹. The correlation coefficients (R²) of the calibration curves were always higher than 0.992. The surrogate recoveries were always higher than 78% for ENR-d5 and 87% for SMZ-d4.

To optimize the source and MS/MS conditions, a 10 ng µL⁻¹ stock solution of each compound in methanol was infused at a flow rate of 10 µL min⁻¹ by a syringe pump integrated into the TSQ instrument and mixed with the mobile phase (400 µL min⁻¹, MeOH:Milli-Q water (50:50, v/v), both containing 0.1% HCOOH).

Table 6.1. LOD, LOQ and monitoring ions in LC-MS/MS (ESI) for determination of antibiotics.

Analyte	Monitoring ions (m/z) and collision energy						Quality parameters	
	TL (V)	Precursor	Quantifier	CE (V)	Qualifier	CE (V)	LOD (ng L ⁻¹)	LOQ (ng L ⁻¹)
ENR	201	360	245	26	316	17	0.8	1.2
SMZ	236	254	92	22	107	21	6.3	7.8
DC	236	445	267	28	428	9	37	57
	225	734	158	12	576	5	30	52
ETM	240	716	157	20	558	5	1.0	2.2
	238	407	126	31	359	17	0.8	1.2
ENR-d5	233	365	245	16	321	8	n.a.	n.a.
SMZ-d4	240	258	96	21	111	17	n.a.	n.a.

TL: Tube lens; CE: collision energy; LOD: limit of detection; LOQ: limit of quantification; n.a.: not applicable

6.2.6. Toxicological evaluation

For toxicological evaluation, we decided to carry out an assay during the highest HLR campaign (i.e. 0.18 m d⁻¹). For this campaign, the antibiotics enrofloxacin (ENR), sulfamethoxazole (SMZ), doxycycline (DC), erythromycin (ETM) and lincomycin (LIN) were also spiked to the doped solution with the rest of EOCs so as to test general toxicity, dioxin-like activity, antimicrobial activity and estrogenicity within the treatment system.

The SPE cartridges analyzed for the toxicological effects were eluted with 10 mL of methanol. General toxicity was performed by using Zebra fish toxicity assay (ISO 15088) and *Daphnia magna* feeding assay (Barata et al., 2008). Estrogenicity and dioxin-like activities in samples were carried out using already described protocols (Noguerol et al., 2006). Antimicrobial activity of samples was tested by using disk diffusion method according to EUCAST rules for antimicrobial susceptibility testing (www.eucast.org).

The Estrogen Receptor Assay (ER-RYA) was performed using the yeast strain BY4741 (MAT_{aura3D0} leu2D0 his3D1 met15D0) from EUROSCARF (Frankfurt, Germany) transformed with the plasmids pH5HEO (hER) and pVitBX2 (ERE-LacZ) (Noguerol et al., 2006). For the AhR yeast assay (AhR-RYA), we used the YCM4 yeast strain (Miller, 1997), harbouring a chromosomally integrated construct that co-expresses the hAHR and ARNT genes under the Gal1-10 promoter and the pDRE23-Z (XRE5-CYC1-LacZ) plasmid.

Antimicrobial activity of samples was tested by using disk diffusion method according to EUCAST rules for antimicrobial susceptibility testing (www.eucast.org). Wild-type *Escherichia coli* was purchased from CECT (Valencia, Spain, CECT n° 434 -_ATCC. 25922) and was grown and used following CECT instructions. Antimicrobial susceptibility disks (6 mm diameter, Becton Dickinson SG-Servicios Hospitalarios, Barcelona Spain) were impregnated with 20 µl of sample extracts using glass micropipettes (Microcaps®, Drummond Scientific Co., Sigma-Aldrich) and dried for 2 hours under a laminar flow cabinet. Ampicillin-impregnated disks (10 µg of ampicillin) and blank disks impregnated with 20 µl of sample solvent (methanol) were used as positive and negative control respectively. An inoculum suspension equivalent to 0.5 McFarland turbidity standard was spread evenly over a Mueller-Hilton agar plate using a sterile cotton swab (BD Difco, SG, Servicios Hospitalarios, Barcelona, Spain) to obtain a confluent lawn of growth. Disks were placed onto the plate within 15 min of inoculation, carefully spaced to prevent overlapping of inhibition zones. Experiments were performed at least in triplicate. Test plates were incubated for 20 h at 37°C within 15 min after the application of the disks. The diameter of circular inhibition zones around the disks were measured with a calliper from the back, against a black background illuminated with reflected light. Inhibition zones of positive controls always filled perfectly with acceptable ranges reported (www.eucast.org) while negative controls never showed inhibition zone.

6.2.7. Statistical analysis

Experimental results were evaluated using the SPSS v.15 package (Chicago, IL, USA). Data normality was checked with a Kolmogorov-Smirnov test and comparisons of differences between the three HLRs in removal efficiencies of the injected EOCs were performed with parametric ANOVA tests and Bonferroni post-hoc tests. Differences were considered significant when $p < 0.05$. A Principal Component Analysis (PCA) was conducted on the wastewater composition and toxicity. It is worth mentioning that only EOCs concentrations collected simultaneously to those samples used for the toxicological study were used for running the PCA. Once the data matrix was completed, it was autoscaled to have zero mean and unit variance (correlation matrix) in order to avoid problems arising from different measurement scales and numerical ranges of the original variables. A Varimax rotation was also included in the analysis.

6.3. Results and discussion

6.3.1. General water quality parameters

The present study was conducted in spring season with a water temperature ranging 14-19°C and well-developed vegetation. Concentrations of COD, TSS and NH₄-N at the effluent of the Imhoff tank were in accordance with those found in raw urban wastewaters (Table 6.2). Actual organic loading rates (OLRs) in terms of COD being applied to the VF beds were 37 ± 6 , 110 ± 13 and 159 ± 27 g COD m⁻².d⁻¹ for the three campaigns in ascending HLR order, respectively. For ease of understanding, these values correspond to 22, 65 and 93 g BOD₅ m⁻² d⁻¹, respectively, assuming a COD/BOD₅ ratio of 1.7, according the large dataset obtained for the same wastewater source by Pedescoll et al. (2011b).

Table 6.2. Average concentration and standard deviation of general quality parameters achieved at the three hydraulic loading rate campaigns (n=5 per campaign) at the experimental hybrid constructed wetland system. Overall removal efficiencies (R.E.) achieved at each treatment unit are shown in brackets.

	Imhoff tank	VF	HF	FWS	R.E. (%)
HLR = 0.06 m d⁻¹					
T (°C)	14 ± 1	13 ± 1	14 ± 1	14 ± 1	-
DO (mg L ⁻¹)	0.8 ± 0.2	3.6 ± 0.4	0.6 ± 0.2	5.9 ± 0.7	-
E _h (mV)	-88 ± 23	+128 ± 28	-59 ± 57	+171 ± 28	-
pH	7.8 ± 0.0	7.8 ± 0.1	7.7 ± 0.1	7.8 ± 0.1	-
Turbidity (NTU)	101 ± 38	12 ± 2 (88%)	4 ± 3 (67%)	3 ± 1 (25%)	97
COD (mg L ⁻¹)	549 ± 96	188 ± 23 (66%)	123 ± 19 (35%)	50 ± 6 (59%)	91
TSS (mg L ⁻¹)	27 ± 4	9 ± 1 (67%)	3 ± 1 (67%)	2 ± 2 (33%)	91
NH ₄ -N (mg L ⁻¹)	31 ± 3	8 ± 1 (74%)	3 ± 1 (62%)	1 ± 1 (67%)	96
HLR = 0.13 m d⁻¹					
T (°C)	15.6 ± 0.8	15.4 ± 1.1	15.8 ± 1.3	15.8 ± 0.9	-
DO (mg L ⁻¹)	0.3 ± 0.0	3.2 ± 0.4	0.4 ± 0.2	5.2 ± 0.7	-
E _h (mV)	-139 ± 6	+120 ± 9	-99 ± 40	+158 ± 8	-
pH	7.8 ± 0.1	8.0 ± 0.1	7.8 ± 0.1	8.0 ± 0.1	-
Turbidity (NTU)	113 ± 19	14 ± 2 (88%)	4 ± 1 (71%)	3 ± 1 (25%)	97
COD (mg L ⁻¹)	828 ± 80	295 ± 16 (64%)	235 ± 19 (20%)	89 ± 8 (62%)	89
TSS (mg L ⁻¹)	53 ± 10	9 ± 4 (83%)	4 ± 2 (56%)	2 ± 1 (50%)	96
NH ₄ -N (mg L ⁻¹)	47 ± 8	19 ± 4 (60%)	13 ± 3 (26%)	13 ± 2 (0%)	71
HLR = 0.18 m d⁻¹					
T (°C)	19 ± 1	19 ± 1	19 ± 1	18 ± 1	-
DO (mg L ⁻¹)	0.2 ± 0.0	2.7 ± 0.3	0.3 ± 0.2	3.7 ± 0.4	-
E _h (mV)	-168 ± 17	+110 ± 19	-115 ± 42	+156 ± 23	-
pH	7.5 ± 0.0	7.8 ± 0.1	7.6 ± 0.1	7.6 ± 0.0	-
Turbidity (NTU)	96 ± 15	29 ± 9 (70%)	6 ± 3 (79%)	4 ± 2 (33%)	96
COD (mg L ⁻¹)	868 ± 146	354 ± 100 (59%)	177 ± 54 (50%)	74 ± 17 (58%)	91
TSS (mg L ⁻¹)	60 ± 12	17 ± 10 (72%)	3 ± 2 (82%)	3 ± 2 (0%)	95
NH ₄ -N (mg L ⁻¹)	43 ± 9	15 ± 7 (65%)	12 ± 2 (20%)	6 ± 3 (50%)	86

Regardless of the HLR applied, VF beds increased the redox potential and oxygen concentration of the water due to the unsaturated water conditions of the system. They were highly efficient on the removal of TSS, COD and $\text{NH}_4\text{-N}$ under the three studied HLRs. The configuration of this type of systems confers a great oxygen transfer capacity (OTC), which greatly enhances the removal of organic matter and $\text{NH}_4\text{-N}$. It is the special operating conditions of VF beds (i.e. intermittent loading and/or resting periods) that allow high OLRs to be applied, while avoiding clogging of the granular media (Molle et al., 2006; Weedon, 2003). However, in the long run the higher feeding frequency of the VF bed at the highest HLR could limit the OTC and in turn hinder organic matter degradation as well as the nitrification capacity of the beds (Molle et al., 2006; Torrens et al., 2009). This is in accordance with Prochaska and Zouboulis (2009) who compared different HLRs (0.08 and 0.17 m per batch, every 2-3 days) in VF wetlands at laboratory scale and found how the alteration of HLRs from higher to lower improved the wetland overall performance. Similar results were reported by Tunçsiper (2009) in a hybrid treatment system in Turkey, who found how nitrogen removal efficiencies decreased as the HLR increased and the recycle ratios increased. Although in our study $\text{NH}_4\text{-N}$ removal did not show any trend in respect to applied HLRs (74, 60 and 65% removal of $\text{NH}_4\text{-N}$ for the three HLRs in ascending order, respectively), reduced nitrification could happen at high HLRs in the longer-term if oxygen renewal and mineralization of the organic matter is not allowed. Although during our study no accumulation of solids was observed on the bed surface, the treatment system was quite young and the experimental period at high HLRs was too short. Caution should be put so as to avoid clogging of the filter beds, which could eventually jeopardize the efficiency and long-term performance of these systems (Kayser and Kunst, 2005; Langergraber et al., 2003; Platzer and Mauch, 1997). Nevertheless, in French style VF wetlands, where various beds alternate cycles of feed and rest, it has been observed that very high hydraulic overloads ($0.4\text{-}1.8\text{ m d}^{-1}$) can be applied without observing a decrease in the infiltration capacity or the treatment performance of the system (Molle et al., 2006, 2008).

On the other hand, saturated conditions taking place within the HF bed caused E_H and DO concentrations to steadily decrease in the wastewater during its passage through it, at the three HLRs. Thus, limited organic matter removal occurred within this wetland (35, 20 and 50% removal of COD at the three HLRs in ascending order, respectively), which was especially efficient in particulate matter entrapment (67, 56 and 82% TSS removal). Remaining $\text{NH}_4\text{-N}$ reduction was also low, achieving 62, 26 and 20% removal rates under the three HLRs in ascending order, respectively.

Finally, more oxidized conditions were recuperated within the FWS wetland, and hence organic matter, COD and $\text{NH}_4\text{-N}$ were further removed at variable elimination rates, which showed no dependence on HLR. The FWS wetland was built to polish the wastewater before it could be reused. Overall removal efficiencies for the three HLRs were in general very high and ranged 91-96% for TSS, 89-91% for COD, and 71-96% for $\text{NH}_4\text{-N}$, being in the range of those found in other hybrid CW systems based in VF and HF wetlands in series (Vymazal, 2013).

6.3.2. Behaviour of emerging organic contaminants

The concentration of spiked compounds in the distribution tank was constant during the whole injection period (RSD < 20% except for OXY) and it ranged from 1 to $59\text{ }\mu\text{g L}^{-1}$, depending on the compound (Fig. 6.2). It is worth mentioning that since we only studied the dissolved phase, the removal efficiencies of some hydrophobic compounds such as AHTN ($\log K_{ow} = 5.9$) and EE2 ($\log K_{ow} = 4.2$) might be underestimated because they are more likely sorbed onto the particles. On the other hand, influent concentrations of injected antibiotics (which were injected only during the highest HLR campaign, i.e. 0.18 m d^{-1}) were $1.8 \pm 0.35\text{ }\mu\text{g L}^{-1}$ for ENR, $2.5 \pm 0.58\text{ }\mu\text{g L}^{-1}$ for SMZ, $3.7 \pm 0.64\text{ }\mu\text{g L}^{-1}$ for DC, $1.9 \pm 0.53\text{ }\mu\text{g L}^{-1}$ for ETM and $2.9 \pm 0.50\text{ }\mu\text{g L}^{-1}$ for LIN.

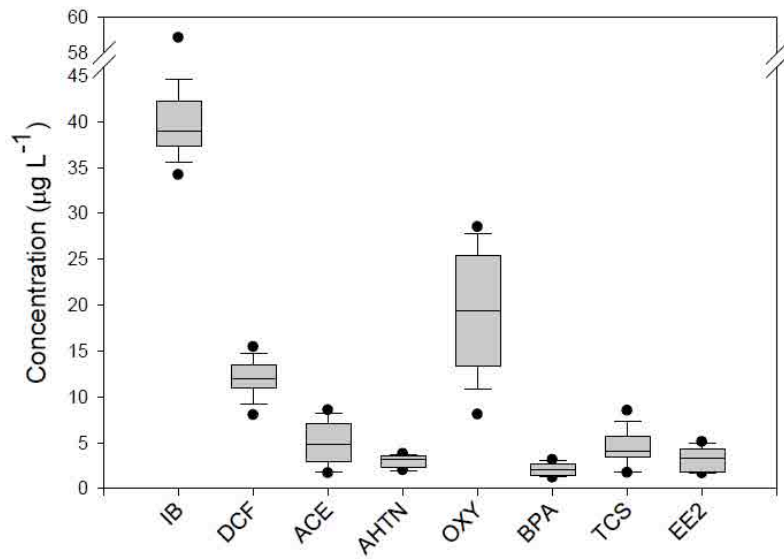


Figure 6.2. Box plot of target emerging organic contaminants in the distribution tank (effluent of the Imhoff tank) after those have been injected in the experimental hybrid constructed wetland system. Plots the median, 10th, 25th, 75th, and 90th percentiles (n=15).

Fig. 6.3 and Fig. 6.4 show the accumulated removal efficiencies of the studied EOCs along the CW system for the three applied HLRs. Target EOCs were grouped in relation to their overall removal efficiency in the hybrid system into (i) very efficiently removed (>90%, ACE, OXY, TCS, BPA and ENR), (ii) moderately removed (from 50 to 90%, IB, DCF, AHTN, EE2 and DC), and (iii) poorly removed (<40%, SMZ, ETM and LIN). The average removal efficiency of all selected EOCs was high ($87 \pm 10\%$) presumably due to the simultaneous occurrence of biodegradation, sorption, hydrolysis and photodegradation processes within the hybrid CW system. Removal efficiencies were similar to those reported previously by CWs and conventional WWTPs (Matamoros and Bayona, 2008; Miège et al., 2009).

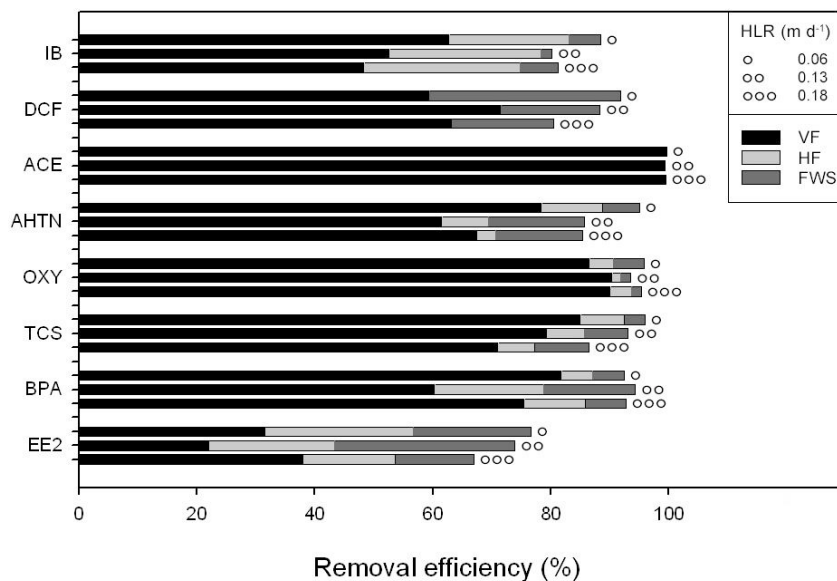


Figure 6.3. Accumulated average removal efficiencies of studied emerging organic contaminants along the different units of the experimental hybrid constructed wetland system at the three experimental hydraulic loading rates.

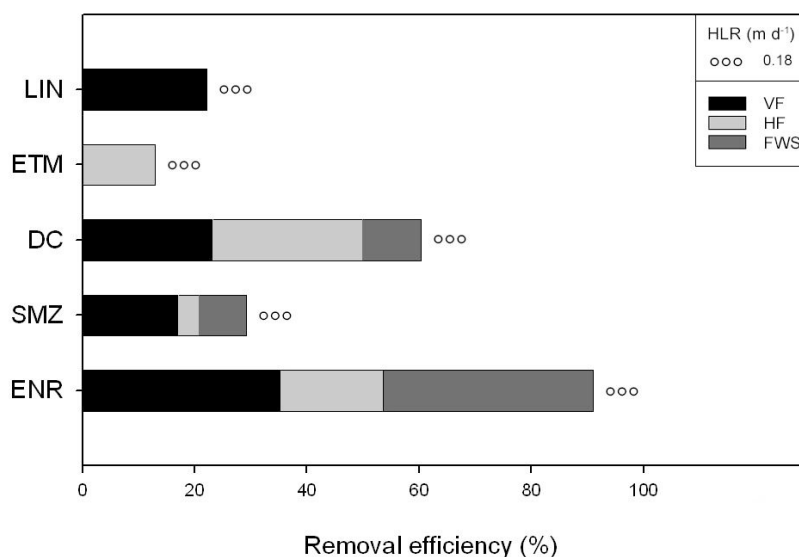


Figure 6.4. Accumulated average removal efficiencies of studied antibiotics along the different units of the experimental hybrid constructed wetland system.

In general, the removal efficiency in the hybrid CW system showed to decrease as the HLR increased for most studied compounds (except for BPA, OXY, ACE). Statistically significant differences ($p < 0.05$) were found between the three different HLRs on EOCs removal efficiencies for DCF, AHTN and TCS. Particularly, with regards to the VF wetlands performance, mass removal in general increased proportionally with mass loading rates, which is in accordance to what was observed by Matamoros et al. (2007), who applied HLRs of 0.013, 0.03, 0.07 and 0.16 m d^{-1} to a pilot-scale (5 m^2) VF wetland. Although higher removal efficiencies were observed at their study at the design HLR of 0.070 m d^{-1} (99% for IB, 82% for AHTN, 97% for OXY, 73% for DCF), influent mass loading rates were significantly lower than the current study since no injection of contaminants was performed (0.82 vs. 2.9 $\text{mg m}^{-2} \text{d}^{-1}$ for IB, 0.07 vs. 0.2 $\text{mg m}^{-2} \text{d}^{-1}$ for AHTN, 1.0 vs. 1.2 $\text{mg m}^{-2} \text{d}^{-1}$ for OXY, and 0.06 vs. 0.8 $\text{mg m}^{-2} \text{d}^{-1}$ for DCF, at Matamoros et al. (2007), and the current study, respectively). This study has demonstrated that the use different CW typologies connected in series, even when they operate at high HLRs, are useful for increasing the removal efficiency of EOCs due to the simultaneous occurrence of aerobic/anaerobic biodegradation, sorption and photodegradation processes.

6.3.2.1. Vertical subsurface flow beds performance

It is easily noticeable (Fig. 6.3) how the VF stage was responsible for the major part of the overall removal of all EOCs, especially for ACE (99%), OXY (89%), TCS (78%) and BPA (72%), if considering all three HLRs. This could be attributable to the unsaturated conditions of VF wetlands, which proportionate a higher oxygenation of the beds, hence favoring aerobic microbial processes (Vymazal, 2007). In fact, this is in accordance with previous laboratory-scale studies carried out by Zwiener and Frimmel (2003) and Conkle et al (2012) who found that aerobic mediated biodegradation was more effective at removing pharmaceutical compounds than anaerobic pathways. High elimination rates have already been reported for IB, DCF and OXY in VF CWs (Matamoros et al., 2007) and activated sludge WWTPs (Miège et al., 2009), whereas sorption to the organic matter is the most plausible removal process for EE2 and AHTN due to their high $\log K_{ow}$, of 4.2 and 5.9 respectively. Sorption could also constitute an important removal mechanism for TCS ($\log K_{ow} = 4.7$), although its biodegradation has already been demonstrated in an aerobic soil (Ying et al., 2007). What is more, Ávila et al. (2013b) found how HF wetlands operated under unsaturated conditions resulted in higher

redox conditions and consequently enhanced removal efficiency of various EOCs (especially BPA, IB and DCF). Additionally, Matamoros et al. (2007) found a high elimination of OXY (>90%) operating in a VF at much lower HLRs (0.03 - 0.07 m d⁻¹). This is the first time that it has been demonstrated that VF wetlands operating at high HLRs (up to 0.18 m d⁻¹) are useful for removing these compounds. Apart from the abovementioned, the large removal efficiencies found at this wetland unit might also have to do with the fact that the VF wetland was the first treatment stage of the system, where most of the total removal would take place, as observed by Hijosa-Valsero et al. (2010) and Matamoros et al. (2008).

It can also be observed that for some compounds the removal rates within the VF bed decreased as the HLR increased (i.e. IB, 63, 53 and 48% and TCS, 85, 79, 71% for the small, medium and large HLRs, respectively). Matamoros et al. (2007) found how HLR in VF wetlands did not affect the removal of IB or OXY, however it had an effect on naproxen, an anti-inflammatory drug, but also on AHTN and DCF, whose removal efficiencies were reduced as the HLR increased (0.013, 0.03, 0.07 and 0.16 m d⁻¹). To this regard, IB has repeatedly been documented to be eliminated at fairly high rates through aerobic biodegradation processes, being highly dependent on redox conditions (Ávila et al., 2013b; Zwiener and Frimmel, 2003). AHTN, a highly hydrophobic compound, exhibited variable removal, in general lower at higher HLRs (78, 62, 67%). Higher HLRs mean higher number of pulses per day, hence lower OTC, as well as a decreased entrapment of hydrophobic compounds onto particulate matter due to lower contact time. Thus, operating the VF beds at high HLRs for a longer period of time could have an effect on the elimination of some substances, especially those having a high dependence on the redox status of the system or on the adsorption onto particles. No clear patterns were found for BPA, DCF and EE2 within this unit between HLRs.

6.3.2.2. Horizontal subsurface flow constructed wetland performance

The HF wetland, under saturated water conditions, performed the best results for ETM (13%), DC (27%), IB (24%), EE2 (21%) and BPA (11%) in average for the three HLRs (note that these values correspond to accumulated rates, i.e. the additional removal within this treatment wetland, and not to individual rates). In general, EOCs removal efficiencies within the HF wetland were low, as was the redox status of the wetland bed. Hijosa-Valsero et al. (2011a) found similar low removal efficiencies for ETM (47 - 79%) and DC (no removal) in HF wetlands and suggested that DC was probably eliminated by means of adsorption/retention processes. The low removal efficiencies obtained in this injection study for LIN, DC and ETM were already observed in HF wetlands with different design characteristics and in conventional WWTPs (Hijosa-Valsero et al., 2011a). Moreover, no DCF removal occurred in the HF unit at any of the studied HLRs. This is in accordance with Matamoros and Bayona (2006) who reported a recalcitrant behavior of DCF under anaerobic conditions, in a study carried out in a full-scale HF wetland system located in Barcelona (Spain). In fact, Ávila et al. (2013b) reported a high dependence of the removal of this substance on a high redox status of the system in an experimental meso-scale HF wetland system with three different treatment lines (71% vs. 31% under a batch operation vs. anaerobic primary treatment strategy, respectively). Removal rates obtained in the anaerobic line of the cited study were slightly higher than those obtained in the current one for IB, AHTN, OXY, BPA and DCF. Although mass loading rates of injected EOCs were similar for most compounds with the exception of DCF (whose rates were 6 times higher in this study) (1.6-2.4 mg m⁻² d⁻¹ of IB, 0.08-0.1 mg m⁻² d⁻¹ of AHTN, 0.24-0.25 mg m⁻² d⁻¹ of OXY, 0.04-0.05 mg m⁻² d⁻¹ of BPA), the applied HLR was lower (0.028 m d⁻¹), and these worked as a first treatment step, which would explain higher treatment performance.

If we look into differences between the three HLRs within the HF unit, we find especially particular the cases of hydrophobic substances, such as again AHTN, which showed a decrease in removal efficiency at higher HLRs (10, 8 and 3% for the three HLRs in ascending order, respectively). The same pattern was shown for EE2 with elimination rates of 25, 23 and 15% in the three HLRs, respectively. This

could be explained by low removal of particulate matter by the HF reactor where these compounds would be attached. No patterns between HLR and EOC removal in the HF unit was observed for BPA, OXY or TCS. BPA removal showed a high variability on the performance at all treatment units and HLRs.

6.3.2.3. Free water surface wetland performance

The FWS unit performed the best removal rates (accumulated) for ENR (37%), DCF (22%), EE2 (21%) and AHTN (12%), presumably to the greatest part due to the direct sunlight exposure of this CW, which permits the photodegradation of these molecules. This is in agreement with the high photodegradation rates already reported for some of these compounds in surface waters (Babić et al. 2013; Matamoros et al., 2009b; Matamoros and Salvadó, 2012). Conversely, the removal of IB and OXY was especially poor within this system ($5 \pm 2\%$ and 3 ± 2 , respectively). The effect of HLR on EOCs removal in the FWS unit was negligible.

6.3.3. Toxicity assessment

Table 6.3 shows that more than 90% of the initial generic toxicity (*D. magna* feeding and Zebra fish embryo toxicity assays) present in the wastewater was eliminated after passing through the VF bed, which is in agreement with the best performance of this CW for removing most of the studied EOCs. The estrogenicity (RYA/ER) and dioxin-like activity (RYA/YCM) were reduced similarly in the VF and the HF wetlands, whereas antimicrobial activity (Gram + and Gram -) was mainly removed in the FWS unit. These results are in agreement with those found by Shappell et al. (2006), who observed similar high removal of estrogenic activity (83-93%) from swine wastewater in a lagoon-CW treatment system.

Table 6.3. Toxicity and effects of wastewater samples according with the treatment stage at the experimental hybrid treatment plant. Note: toxicity tests and injection of antibiotics were carried out at the highest hydraulic loading rate campaign (i.e. 0.18 m d⁻¹).

	Imhoff tank	VF	HF	FWS
Zebra fish embryo toxicity assay (%)	100	7.0	7.0	10
RYA/ER (%)	58	34	22	0.0**
RYA/YCM (%)	65	29	0.0**	30
GRAM-* (cm)	9.3	8.5	7.8	0.0**
GRAM+* (cm)	9.8	6.8	7.6	0.0**
<i>D. magna</i> feeding inhibition (%)	100	3.8	5.1	32

*Semi-quantitative bactericidal assay (Kirby-Bauer method, EUCAST –European Committee on Antimicrobial Susceptibility Testing), ** below the quantification limit.

These findings cannot be directly correlated with the behavior of the EOCs because degradation products from EOCs and other compounds that were already present in the wastewater may be also the responsible for the biological effects found in the samples evaluated. However, the increase in dioxin-like activity and *D. magna* feeding toxicity in the FWS unit may be due to the presence of oxidized compounds produced by the sunlight exposure. This is in agreement with previous studies, which demonstrated that the photodegradation of some pharmaceuticals increases toxicity. Trovó et al. (2009) found that SMZ irradiation increase *D. magna* toxicity from 60% to 100%. Furthermore, it has already been observed that photodegradation of TCS can form dioxin by-products which would increase dioxin-like activity (Mezcua et al., 2004).

6.3.4. Principal Component Analysis (PCA) study

A Principal Component Analysis (PCA) was performed from the whole data set in order to get a further insight on the effect of the different reactors on the elimination of spiked contaminants and toxicity. It is worth mentioning that the performance of the PCA study was indispensable for the adequate assessment of the correlation between compounds and toxicity assays. The PCA reduced the 19 measured variables to three principal components with eigenvalues greater than 1, which explains the 99% of the variability observed (Table 6.4).

Table 6.4. Principal component analysis of 19 individual variables.

	PC1	PC2	PC3
Percentage of variance (%)	72	21	7
Enrofloxacin	.707	.643	.294
Sulfamethoxazole	.436	.607	.665
Doxycycline	.819	.571	.058
Erythromycin	-.143	.062	-.988
Lincomycin	.950	.256	.182
Ibuprofen	.833	.551	.051
Tonalide	.779	.542	.314
Oxybenzone	.844	.394	.364
Triclosan	.816	.492	.303
Diclofenac	.695	.520	.497
Bisphenol A	.834	.480	.272
Ethinylestradiol	.763	.641	.082
Zebra fish embryo toxicity assay	.850	.337	.405
RYA/ER	.630	.744	.223
RYA/YCM	1.000	.026	.005
GRAM-	.142	.987	-.081
GRAM+	.223	.966	.128
<i>D. magna</i> feeding inhibition	.886	.084	.456

The first principal component (PC1), which accounted for the 72% of the variance, had high positive loadings (>0.8) for DC, IB, LIN, OXY, TCS, BPA, general toxicity and dioxin-like activity, indicating a compound-toxicity correlation. PC2, which explained the 21% of the variance, had positive loadings for ENR, SMZ, EE2, Gram + activity, Gram - activity, and estrogenicity, indicating an association of enrofloxacin and sulfamethoxazole with antimicrobial activity (Gram + and Gram -), and EE2 with estrogenicity, which is in agreement with the effect of both antibiotics and the estrogen, respectively. Finally, the PC3, which only explained the 7% of the variance, had high positive loadings for ETM.

The score plots of the PC1 vs. PC2 separates the treatment unit effluents in three clusters (Fig. 6.5).

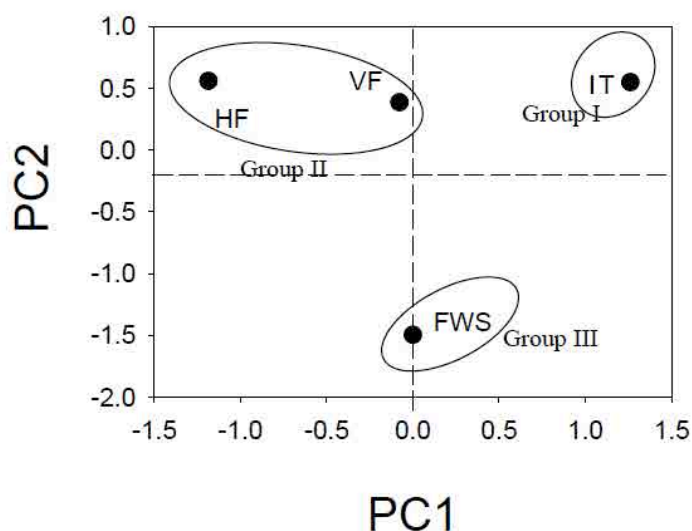


Figure 6.5. Score plot of the combination of the PC1 and PC2.

The cluster I includes the water samples from the Imhoff tank effluent, which have a high content of EOCs and high toxicological activity for all studied biological effects, the group II includes samples from the HF and VF wetlands effluents, which are presenting low content of EOCs but only high biological responses for estrogenicity and antimicrobial activity. Finally, the group III includes the water samples from the FWS wetland effluent, which are those presenting the lowest estrogenicity and antimicrobial activity, but still having some toxicological effects due to the presence of oxidized compounds.

6.4. Conclusions

A hybrid CW system consisting of two VF wetlands operating alternatively, followed by a HF and a FWS wetlands in series has shown to be a reliable and robust technology for the removal of conventional water quality parameters, EOCs, and associated adverse biological effects from domestic wastewater.

The greatest part of solids entrapment, organic matter and $\text{NH}_4\text{-N}$ removal was attained within the VF beds (60-83%). However, caution should be put to the observation of accumulation of solids and clogging of the filter bed as well as reduced OTC when operating at high HLRs.

The average overall removal efficiency of all selected EOCs (except for antibiotics, $43 \pm 32\%$) was high ($87 \pm 10\%$), presumably due to the simultaneous occurrence of biodegradation, sorption, hydrolysis and photodegradation processes in the hybrid CW system. VF beds were responsible for the removal of the major part of EOCs, especially for ACE, OXY, TCS, BPA and AHTN ($>80\%$), whereas HF was especially important for EE2 removal and remaining IB removal. FWS wetland performed particularly well on the elimination of EE2, AHTN and DCF.

General toxicity (Zebra fish and *D. magna*) was removed up to 90% by the VF wetland. Estrogenicity (ER) and dioxin-like (YCM) activities were similarly reduced by the VF and the HF units, whereas antimicrobial activity was mainly removed by the FWS wetland. PCA study grouped samples in relation to their toxicology and EOC content, but also correlated compounds with their toxicological effects. Although further studies are necessary, this work has proved the suitability of hybrid CWs as a wastewater cost-effective treatment solution due to their capacity to improve water quality at high HLRs.

7. Integrated treatment of combined sewer wastewater and stormwater in a pilot three-stage hybrid constructed wetland system in southern Spain



This chapter is based on the article:

Ávila, C., Salas, J.J., Martín, I., Aragón, C. and García, J., 2013. Integrated treatment of combined sewer wastewater and stormwater in a hybrid constructed wetland system in southern Spain and its further reuse. *Ecological Engineering* 50, 13-20.

An integrated pilot-scale treatment system consisting of a vertical subsurface flow (317 m²), a horizontal subsurface flow (229 m²) and a free water surface (240 m²) constructed wetlands operating in series for the treatment of a combined sewer effluent was put into operation and monitored over a period of about 1.5 years. The goal of the treatment system was to provide effluents suitable for various water reuse applications. Moreover, the influence of pulses of high flow resulting from several rain events over the treatment performance of the system was evaluated. An intensive sampling campaign was also carried out following an intense storm (45 mm in one-hour span) to have a further insight into the characteristics of the inflowing water at the early part of it or so-called 'first-flush'. Results under dry weather conditions showed a good performance on the removal of BOD₅, COD and TSS taking place already in the vertical flow wetland (94, 85 and 90%, respectively). A high removal of total nitrogen occurred also in the vertical flow wetland (66%) suggesting both nitrification and denitrification to take place, presumably due to the existence of both aerobic and anoxic microenvironments within the bed. Removal of *Escherichia Coli* along the treatment system was of almost 5 log units. To this respect, the horizontal flow and free water surface wetlands proved to be crucial treatment units to achieve a water quality suitable for further reuse (e.g. recharge of aquifers by percolation through the ground, silviculture and irrigation of green areas non accessible to the public). Although the occurrence of the storm event caused a prompt raise of COD and TSS within the first 30 min of rainfall (868 and 764 mg L⁻¹, respectively), it was soon followed by a dilution effect. In general the storm events did not jeopardize the correct functioning of the system, proving its robustness for the treatment of a combined sewer effluent.

7.1. Introduction

Nowadays, it is estimated that the load of wastewater receiving inappropriate treatment in Spain (mostly small communities with less than 2,000 PE corresponds to 3 to 4 million PE. Although this load represents just a small percentage of the total load to be treated (6 - 8%), more than 6,000 communities have been counted to contribute (Aragón et al., 2013). This fact highlights the complexity on the development of Sanitation Plans to be introduced.

The Spanish National Plan for Water Quality (2007-2015) was created to comply with the quality objectives and requirements of Directive 91/271/CEE concerning urban wastewater treatment (EC, 1991), as well as the EU Water Framework Directive (EC, 2000a). Although no specific criteria or prescribed technologies for communities with less than 2,000 PE have been specified, the plan aims at boosting the establishment and use of low-cost solutions to provide wastewater treatment to small communities.

To this regard, constructed wetlands constitute the most commonly used treatment technology in the last years (Vera et al., 2011). Numerous studies have shown their capability to maintain hydraulic, technical, economic, environmental and ecological benefits (Zhou et al., 2009; Dixon et al., 2003; Vymazal, 2002). Although the number of these systems is still not so large in Spain (Puigagut et al., 2007), it is important to note that this technology has a promising prospective in the coming years.

In Spain most of the sewer catchments are combined, collecting both urban wastewater and urban runoff (Diaz-Fierros et al., 2002). The original aim of this is to prevent flooding in urban areas and to protect public health. Mediterranean weather, which is characteristic in south Spain, is distinguished by periods of low or no rainfall followed by stormy periods. It is now fully recognized that combined sewer overflows are themselves significant sources of pollution of receiving water systems (Lau et al., 2002).

Constructed wetlands (CWs) have been widely used for the treatment of wastewater but also for stormwater, separately (Carleton et al., 2001; Somes et al., 2000). In stormwater wetlands there is in many cases a need to compensate for evapotranspiration in dry periods. To that regard, the use of CWs for the treatment of combined sewer wastewaters as well as stormwaters could solve this problem. However, only a few examples of the influence of high rainfall events in this type of system have been assessed (Van de Moortel et al., 2009; Green et al., 1999).

In this context a collaborative project between the Universitat Politècnica de Catalunya (Barcelona) and the Foundation Centre for New Water Technologies (Seville) emerged in 2009, aiming at integrating the treatment of a combined sewer effluents containing wastewater and urban runoff in small communities through the use of constructed wetlands.

Hybrid systems are a combination of various types of CWs so as to balance out the strengths and weaknesses of each type of system (Vymazal, 2005). In this study, the performance of an integrated approach for the treatment of a combined sewer system based exclusively on CWs was assessed. A pilot-scale treatment system consisting of a series of different types of wetlands was put into operation and monitored over a period of about 1.5 years. An interpretation of the functioning of the system under dry weather, wet weather conditions and for the beginning of an intense storm event was made, so as to evaluate its performance on the treatment of a combined sewer effluent.

The influence of some intense storm events over the treatment performance was examined so as to evaluate the buffer capacity of the wetland system under extraordinary high flow conditions. Albeit

characterizing contaminant loads in storm water is a complex issue due to spatial and temporal variations in weather conditions and rainfall pattern, we have proceeded to feature the composition of the early part of an intense storm event or so-called 'first-flush event', which has been identified as a relatively high proportion of the total storm pollution load, through the implementation of an intensive sampling campaign and assessment of the water quality. Moreover, the final goal of the treatment system was to provide effluents suitable for its reuse. For that purpose, the final effluent was contained in a water tank and its quality was monitored and compared to the Spanish guidelines' requirements so as to estimate its possible reuse in various environmental applications.

7.2. Materials and Methods

7.2.1. Description of the treatment system

The constructed wetland treatment system was part of a larger pilot-scale treatment plant that received the wastewater from 2,500 PE from the municipality of Carrión de los Céspedes (Seville) together with the runoff collected in a combined sewer system. Research activities of the Foundation Centre for New Water Technologies (CENTA) are developed within this 41,000-m² experimental plant, which contains a great variety of both extensive and intensive technologies for its analysis and validation, but also for knowledge dissemination and outreach (www.centa.es). Pretreatment chambers are common to all technologies in the plant and its effluent is diverted towards each of them.

The constructed wetlands started operation in spring 2005, however the treatment line as it is now began its operation in July 2009. In particular, a hybrid system consisting of a combination of various types of constructed wetlands was set so as to balance out the strengths and weaknesses of each type of system. The raw wastewater is firstly screened through two sieves of 3 cm and 3 mm wide, followed by a pretreatment chamber for sand and grease removal. The water is then conveyed towards a pumping chamber, from which the water is conducted through submersible pumps into a distribution system. This last one consists of a chamber, equipped with electromagnetic flow meters (Sigma 950). The pretreated water is next led through gravity towards an Imhoff tank, with a treatment capacity of 40 m³ d⁻¹ (for the feeding of 3 different lines of wetlands, just one belonging to this study treatment system), a settling area volume of 3.5 m³ and a digestion area volume of 25 m³. The primary effluent is then pumped towards 3 lines of constructed wetlands of different configurations operating in series, including the studied hybrid constructed wetland system (Fig. 7.1). Each of the treatment lines is fed 20 times per day. Pumped flow was measured by means of an electromagnetic Sigma 950 flow meter of 50 mm of diameter. The hybrid wetland consisted of a VF wetland VF, which was connected in series to a HF wetland and finally to a FWS wetland. Both the VF and the HF beds were planted with *Phragmites australis* with a density of 5 plants m⁻².

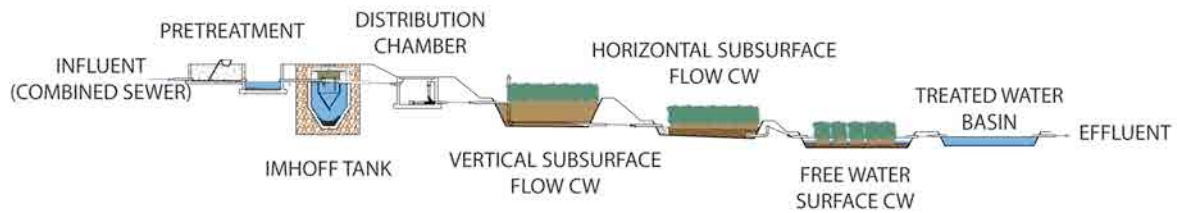


Figure 7.1. Layout of the pilot-scale hybrid constructed wetland system in Seville.

The VF unit had a surface area of 317 m² (23.5 x 13.5 m) and received an average flow of 14 m³ d⁻¹, an average organic loading rate (OLR) of about 9 g BOD₅ m⁻² d⁻¹ and an average hydraulic loading rate (HLR) of 44 mm d⁻¹. The bed consisted of a top layer of 0.05 m of sand (grain size = 1 - 2 mm), followed by a 0.6 m layer of gravel (grain size = 4 - 12 mm) and an underlying 0.15 m stone layer (grain size = 25 - 40 mm). Feeding of the VF was done through five lengthwise pipes of 32 mm of diameter, perforated with 1 cm diameter holes every 1.8 m distance. Five draining pipes (diameter = 125 mm) were installed lengthwise at the bottom of the wetland within the 15 cm-thick gravel layer. Every draining pipe had three 1 m-tall chimneys so as to provide oxygen transfer into the wetland beds.

The HF bed had a surface area of 229 m² (26 x 8.8 m) and consisted of a gravel bed of 0.4 m depth (grain size = 4 - 12 mm), with an inlet and outlet zone of stones of 40 - 80 mm of diameter. Feeding of the bed was done through 63 mm diameter polyethylene pipes perforated with 1 cm holes located every 1 m distance. The outlet of the wetland consisted in two 125 mm-diameter draining pipes buried at the bottom of the outlet stone layer and connected to a flexible pipe that held the water level 5 cm below the top of the gravel.

The FWS wetland had a surface area of 240 m² (24 x 10 m) and a water depth of 30 cm. A mixture of *Typha* spp., *Scirpus* spp., *Iris pseudacorus*, *Carex flacca*, *Cyperus rotundus* and *Juncus* spp. were planted on a 0.2 m gravel bed (grain size = 4 - 12 mm). Different indigenous wetland macrophytes were selected so as to improve biodiversity and enhance the treatment capacity of the wetland. Since the CW system had been working for several years, it was mature and the vegetation was well developed in all treatment units. The final effluent of the treatment line was collected in a 20 m³ open-air water tank working as a raft for irrigation so as to measure periodically the quality of the treated water and assess its possible reuse application.





Figure 7.2. View of the different units of the pilot hybrid treatment system: vertical subsurface flow (a); horizontal subsurface flow (b); free water surface wetland (c); water tank for reuse (d).

7.2.3. Sampling strategy

In order to have a better understanding of the sampling strategy, details are displayed in Table 7.1. Water effluents (24-h composite samples) of the different treatment units of the experimental plant (i.e. influent wastewater, Imhoff tank, VF, HF, FWS units and reuse water tank) were monitored once a week from July 2009 to April 2011. For the adequate separation of those so-called dry and wet periods, we extracted from the 1.5-year sampling period 2 stages of wet weather where an extraordinary high precipitation period took place (19th December 2009 to 29th March 2010 and 9th December 2010 to 5th April 2011).

Table 7.1. Sampling strategy at the pilot hybrid constructed wetland system in Carrión de los Céspedes (Seville).

Scope	Period	Frequency	Sampling days (n)	Average inflow (m ³)	Average HLR (mm d ⁻¹)	Average OLR (g BOD ₅ m ⁻² d ⁻¹)
Dry weather conditions	07/07/2009- 18/12/2009 30/03/2010- 08/12/2010	Weekly	58	14	44	9.0
Wet weather conditions	19/12/2009- 29/03/2010 9/12/2010- 05/04/2011	Weekly	6	27	86	0.75
First-flush event	11/05/2011	Every 15 min for influent and Imhoff tank, 30 min for VF, 120 min for HF and 240 min for FWS	1	-	-	-

For the correct assessment of the treatment performance of the constructed wetland system under high flow pulses caused by storm events, we selected from the wet periods a total of 6 sampling days where high rainfall was followed by an inflow exceeding 20 m³ d⁻¹. The sampling days picked occurred after intense rainfall events in winter 2010 (i.e. 13th, 19th, 26th January, 9th, 16th February and 6th March) which caused an inflow reaching the VF unit ranging 20 - 37 m³ d⁻¹, with an average inflow of 27 m³ d⁻¹, a figure far higher than the usual average of 14 m³ d⁻¹ occurring under dry weather conditions. The storms that took place for about one week before the selected sampling days had rainfall depths ranging from 41 to 120 mm. It is important to indicate that samples were not taken at the early part of a storm event, but at different times during and after the rain event, ranging from some hours up to 6 days.



Figure 7.3. View of the autosamplers (left) and the 24 bottles (right) used for water sampling at the different units of the pilot hybrid treatment system.

Moreover, and so as to attempt elucidating the characteristics of the water entering the treatment system in the early part of an intense high rainfall event, or so-called ‘first-flush’, we proceeded to an intensive single sampling campaign of the different treatment units for its further analysis on water quality parameters. The storm event took place on May 11th 2011 at around 18pm and discharged 45 mm of precipitation in a one-hour span. Sampling was made immediately after the beginning of the storm with an autosampler following a 15-min frequency for the effluents of influent wastewater and Imhoff tank, a 30-min frequency for VF wetland, a 120-min frequency for HF unit and a 240-min frequency for the FWS unit. Sampling frequencies were selected according to their theoretical HRT.

Samples were analyzed for organic matter (BOD₅, COD), TSS, TN, total phosphorus (TP), NH₄-N, NO_x-N, PO₄-P and *Escherichia Coli*. Onsite measurements such as DO, pH, turbidity, water temperature and EC were also taken at the time of sample collection.



Figure 7.4. View of a) the pilot hybrid treatment system and b) the adjacent highway during the monitored intense storm event.

7.2.4. Analytical methods

Conventional wastewater quality parameters, including TSS and COD were determined by using Standard Methods (APHA, 2001). BOD₅ was measured by using a WTW® OxiTop® BOD Measuring System. TN, TP, NH₄-N, NO_x-N and PO₄-P were determined by using a Bran Luebbe AutoAnalyzer 3. Isolation and enumeration of *E. coli* was made using a Chromogenic Membrane Filtration technique (APHA, 2001).

Onsite measurements of DO, pH and turbidity were taken using a Hach HQ 30d oxymeter, a Hach Senslon i30 pH-meter and a Hach 2100Q turbidity meter, respectively. Water temperature and EC were also measured in situ by using a Hach Senslon i30 thermometer and conductivity meter.

7.3. Results and discussion

7.3.1. Treatment performance under dry weather conditions

Mean values and standard deviations of water temperature, DO, pH, turbidity and EC are shown for the different treatment units in Table 7.2.

Table 7.2. Wastewater physico-chemical characteristics under dry weather conditions at the pilot hybrid constructed wetland system in Seville. Mean values and standard deviations are shown.

	Influent	Imhoff tank	VF	HF	FWS	Water tank
T (°C)	22.1 ± 3.4	21.4 ± 2.8	20.9 ± 3.8	19.2 ± 4.1	16.1 ± 3.7	14.8 ± 2.4
DO (mg L ⁻¹)	0.8 ± 0.8	0.3 ± 0.1	1.4 ± 0.8	2.7 ± 2.1	1.9 ± 1.5	12.0 ± 4.0
pH	7.9 ± 0.3	7.5 ± 0.4	7.0 ± 0.3	7.1 ± 0.2	7.1 ± 0.3	8.0 ± 0.7
Turbidity (NTU)	237 ± 92	111 ± 46	8 ± 4	15 ± 16	23 ± 22	14 ± 4
EC (µS cm ⁻¹)	1396 ± 200	1297 ± 209	1222 ± 276	1302 ± 300	1098 ± 197	943 ± 82

Under dry weather conditions, influent TSS, COD and BOD₅ concentrations (Table 7.3) fall within the range of recently documented average concentrations for these parameters in small communities in the region of Andalucía (south Spain) with 2,000 - 2,500 PE, as indicated by Ausencio et al. (2011). Results of the study undertaken in this pilot plant show the great potential of CWs as a suitable technology for the treatment of domestic wastewater from small communities. Very efficient COD and BOD₅ reductions were already achieved in the VF wetland in respect to the Imhoff tank (85 ± 6% and 94 ± 4%, respectively). Concentrations remained low for the rest of the wetland units. TSS were also readily retained within the VF wetland bed to a removal efficiency of 90 ± 8% and no significant levels of solids were detected later on.

Table 7.3. Dry and wet periods' average concentrations and standard deviations of water quality parameters at the effluent of the different units of the pilot hybrid constructed wetland system.

	Influent	Imhoff tank	VF	HF	FWS
Dry period					
TSS (mg L ⁻¹)	287 ± 108	98 ± 25	8 ± 6	8 ± 3	6 ± 2
COD (mg L ⁻¹)	539 ± 200	294 ± 123	46 ± 18	30 ± 16	50 ± 16
BOD ₅ (mg L ⁻¹)	393 ± 127	204 ± 80	10 ± 5	5 ± 5	7 ± 3
TN (mg L ⁻¹)	54.6 ± 12.5	52.7 ± 18.1	17.6 ± 6.0	8.2 ± 4.6	7.9 ± 5.1
NH ₄ -N (mg L ⁻¹)	42.1 ± 11.2	43.1 ± 15.2	10.4 ± 5.5	5.5 ± 4.8	2.3 ± 2.7
NO _x -N (mg L ⁻¹)	0.9 ± 0.4	0.8 ± 0.6	3.1 ± 2.6	0.7 ± 0.2	0.9 ± 0.5
TP (mg L ⁻¹)	8.1 ± 2.5	6.8 ± 2.6	5.7 ± 2.9	4.7 ± 3.8	5.3 ± 2.7
PO ₄ -P (mg L ⁻¹)	5.4 ± 2.0	4.8 ± 2.5	4.8 ± 2.3	3.7 ± 3.2	4.7 ± 2.6
Wet period					
TSS (mg L ⁻¹)	1953 ± 2813	26 ± 28	9 ± 1	1302 ± 1245	12 ± 3
COD (mg L ⁻¹)	138 ± 57	59 ± 21	29 ± 1	35 ± 10	37 ± 6
BOD ₅ (mg L ⁻¹)	49 ± 25	17 ± 7	10 ± 5	12 ± 0	12 ± 0
TN (mg L ⁻¹)	54.4 ± 23.7	45.6 ± 12.4	36.3 ± 5.3	23.2 ± 7.3	15.2 ± 5.3
NH ₄ -N (mg L ⁻¹)	10.5 ± 5.7	5.8 ± 3.2	1.2 ± 1.1	0.7 ± 0.2	1.2 ± 0.4
NO _x -N (mg L ⁻¹)	30.7 ± 5.5	37.1 ± 8.8	35.3 ± 3.7	23.2 ± 8.2	13.5 ± 5.3
TP (mg L ⁻¹)	2.1 ± 0.8	1.2 ± 0.3	1.3 ± 0.2	0.9 ± 0.0	0.9 ± 0.1
PO ₄ -P (mg L ⁻¹)	1.2 ± 0.7	1.0 ± 0.1	1.2 ± 0.3	0.9 ± 0.0	0.9 ± 0.0

Removal of NH₄-N was also high within the VF bed, achieving removal efficiencies of 74 ± 11%. The major removal mechanism for this N species could be explained by its nitrification into NO_x-N, given the sufficient DO concentration for its transformation by microorganisms (Xinshan et al., 2010). However, the concentration of NO_x-N at the effluent of the VF bed represents just a small proportion of the NH₄-N that could be transformed into NO_x-N by nitrification (Fig. 7.5). Added to that, it is remarkable the elevated removal of TN within this CW bed (66 ± 9%). To this respect, we can obtain into conclusion that NO_x-N is being removed within the same wetland bed by further mechanisms (i.e. assimilation by microbial or plant biomass, adsorption onto the media and denitrification) as explained by Collins et al. (2010). Although these authors state that short HRT might be a critical factor influencing removal by denitrification, it has been proved that a strong denitrification activity of microorganisms at redox potentials below 300 mV occur at microscale (Yu et al, 2007). Moreover, Akratos and Tsihrintzis (2007) found a decrease of N concentrations without a significant increase of NO_x-N species. This leads us to think that the removal of TN within the VF wetland could be majorly explained by the occurrence of both aerobic and anoxic microsites within the wetland bed (Cooper et al, 1996; Ávila et al., 2010), which could allow for both aerobic and anaerobic reactions to occur. That would lead to the assumption that nitrification could occur near the roots after which the formed nitrate would diffuse to the surrounding anaerobic sites where denitrification would take place (Flynn et al., 1999). To this respect, it is important to note that the vegetation was very well developed in the wetlands, since these assays were carried 4 years after its planting and start of operation.

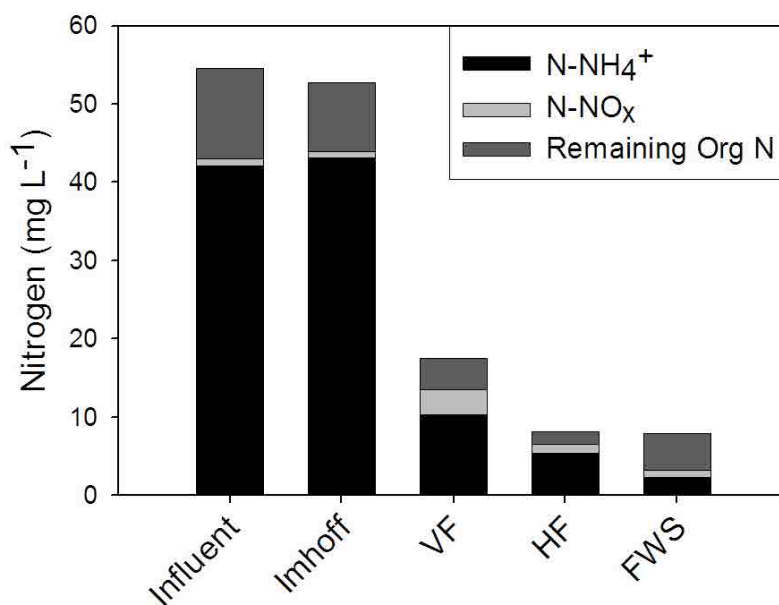


Figure 7.5. Effluent concentration of the different species of nitrogen under dry weather conditions at the pilot hybrid constructed wetland system in Seville.

Adsorption of NH₄-N onto negatively charged particles of the media and its further release and eventual plant uptake could be another possible mechanism of TN removal as explained by Connolly et al. (2004). To this respect, the resting conditions between feeding pulses would allow for desorption of ammonia nitrogen from the media and its assimilation by plants. That would prevent saturation of the sorption sites of the media for the next feeding pulse. However we believe this mechanism to be very unlikely to happen due to the high concentration of NH₄-N being removed in the wetland in this system (Van de Moortel et al., 2009). TN slightly decreased along the HF wetland (56 ± 19%). The fraction of the TN constituted by remaining NH₄-N (about 5 mg L⁻¹) could be further removed presumably by its nitrification, followed by denitrification processes of that NO_x-N formed together with the one formed at the VF bed. The decrease of TP was almost negligible, achieving a 22% of removal along the whole CW system.

7.3.2. Treatment performance under wet weather conditions

The characteristics of water quality at the different treatment units after the selected 6 rainfall events are displayed in Table 7.3 and can be compared to those occurring in dry weather conditions. The amount of TSS in the influent wastewater was extremely high, which is attributed to runoff sediment transport and mobilization of material accumulated in the sewer system during antecedent dry periods (Skipworth et al., 2000). These solids are of mineral origin, as indicated by the low values of COD and BOD₅ entering the system as compared to the system under dry weather conditions. To this regard it is important to remind that sampling was not made at the early part of a storm event, but at different times during and after the rain event, ranging from some hours up to 6 days. This fact does not therefore demonstrate the characteristics of the water in the first-flush event, which is evaluated in the punctual sampling campaign shown further on in this study (Section 7.3.3). The level of entrapment of TSS in the Imhoff tank was remarkable. However, a steady increase of their concentration took place in the HF bed, which could be owed to the washout of particles retained in the gravel bed of the wetland at high HLRs (dry period = 70 ± 20 mm d⁻¹; wet period = 120 ± 50 mm d⁻¹).

As it has been mentioned, the influent concentration of both the organic matter and nutrients is significantly lower than in the normal period due to the dilution of the wastewater occurring after the first-flush occurred. The removal efficiencies for COD and BOD₅ along the treatment system are lower than those found in the dry period (73 and 75%, respectively, in wet period versus 91 and 98%, respectively, in dry period). Removal of TP was slightly higher than in the dry period, due to the low incoming concentration (2.1 mg L⁻¹). However, although the concentrations of TN appear to stay similar, the contribution of different N species differs, observing high concentrations of NO_x-N at the influent wastewater presumably due to the washout of fertilizers applied in surrounding agricultural soils.

7.3.3. Characteristics of a first-flush event

Many authors have defined a 'first-flush' or 'rinsing flush' event as a relatively high load of pollutants in the initial phases of combined sewer flow as compared with the latter stages of a storm event (Suárez and Puertas, 2005; Skipworth et al., 2000). The nature of these events is very diverse and their occurrence is random. This can be observed within the results of the intensive sampling campaign carried out right after the beginning of the storm (Fig. 7.6).

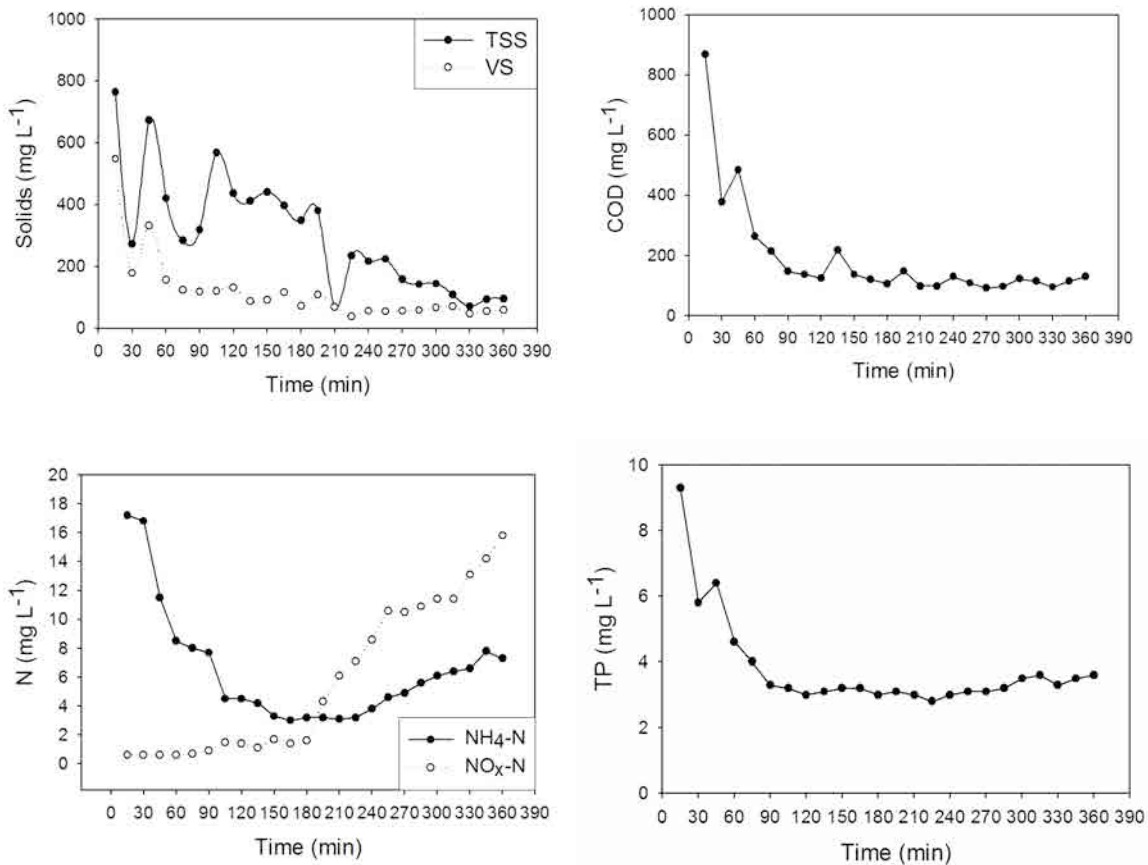


Figure 7.6. Evolution of the concentrations of water quality parameters over time at the influent of the wastewater treatment plant after an intense storm event (t = 0, beginning of the storm) at the pilot hybrid system.

It is remarkable how the peak values of TSS occurring at the first 30 min consisted mainly of organic solids, as shown by the high proportion of volatile solids (VS) fraction (65 - 71%), similar to that reported by Ahyerre et al. (2000). That is reasserted by the high load of COD (starting at 870 as opposed to 540 mg L⁻¹ in the dry season) at the very beginning of the rain, which decreased down to around 250 mg L⁻¹ in just 60 min. The same pattern occurred for TP, observing very high concentrations at the start of the rainfall (above 9 mg L⁻¹). Values for TP are in accordance with those reported by Lee and Bang (2000) for several watersheds receiving urban stormwater. The high loads of organic matter and TP at the early part of the rainfall can be attributed to the erosion of the organic layer of pipe deposits (Ahyerre et al., 2000) due to the increased flow rate under storm weather conditions. Combined sewer catchments are often oversized to accommodate storm flow. Low velocities within the catchment would therefore cause sediments to accumulate (Barco et al., 2008). As discussed by Somes et al. (2000), a wide range of hydrodynamic and pollutant loadings can be expected to occur in a combined sewer due to the stochastic nature of stormwater quantity and quality. Gupta and Saul (1996) found peak rainfall intensity, peak inflow, rainfall duration and the antecedent dry weather period to be the most important parameters influencing the first-flush load of suspended solids. In this case, the initial and maximum organic load in terms of COD entering the system lays within the range or maximum concentration reported by Suárez and Puertas (2005) for 10 storm events recorded at a river catchment in Seville, south Spain (506 - 3,260 mg L⁻¹). The same can be applied, although results were found to a small extent below the range (921 - 3,394 mg L⁻¹), to the maximum concentration of TSS. The slightly smaller concentrations in our study could be explained by the existence of a short antecedent dry period as indicated by previous storms which had occurred soon before, having therefore removed a lot of the accumulated pipeline deposits.

Equally interesting was the steady decrease in the NH₄-N concentration, which followed the same pattern over time as TSS and COD. It can be observed that, although occurring at the beginning of the storm with a lower concentration than in the dry period (17 mg L⁻¹ as opposed to 42 mg L⁻¹, respectively), NH₄-N is gradually replaced by an increase in the NO_x-N concentration. The source of NO_x-N could be once again explained by the washout of the soil that had been fertilized for agricultural purposes.

Fig. 7.7 shows the evolution of different water quality parameters overtime in the different treatment units from the beginning of the storm event up to several days after the rain event. Effluent of Imhoff tank showed the same TSS, VS and COD tendency than in the influent, although slightly delayed in respect to time and in smaller concentrations since much of it gets settled within the Imhoff tank. The concentration of NO_x-N started to increase 330 min (2 mg L⁻¹) after the start of the storm, although only the beginning of the rise could be observed due to the end of the sampling period. Likewise, NH₄-N levels decreased throughout the sampling period from 22 mg L⁻¹ at t = 0 min to 8 mg L⁻¹ at t = 360 min. Both in the effluent of the VF and HF wetlands high TSS concentrations were found at the beginning of the sampling, which is attributed to the washout of solids contained in the bed due to the high HLR. The concentration of NO_x-N raised steadily both in the VF and HF wetlands from the beginning of the sampling up to an average concentration of 9.9 ± 2.6 mg L⁻¹ and 3.3 ± 2.2 mg L⁻¹, respectively. Within the FWS wetland, the remaining NO_x-N was almost completely removed and COD levels remained similar, tentatively due to the external contribution of organic matter (i.e. plants and algae).

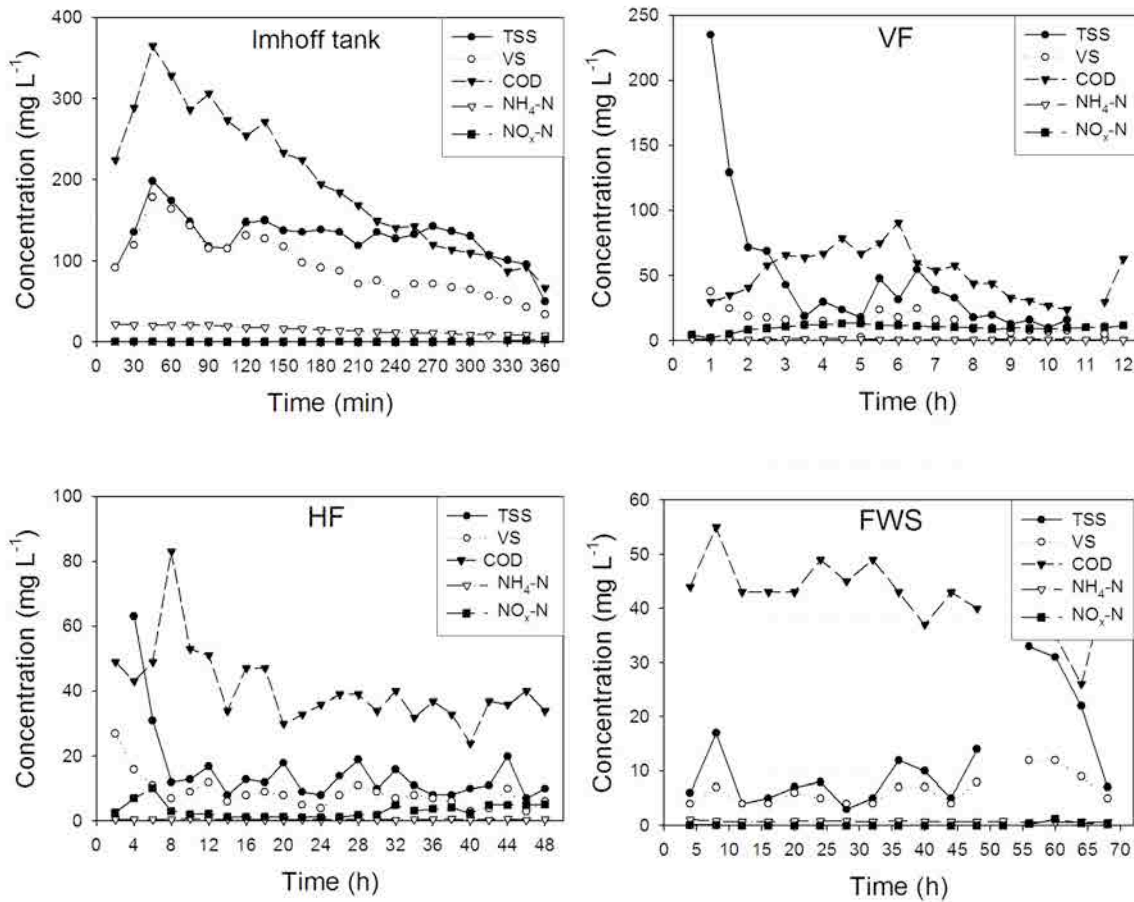


Figure 7.7. Evolution of water quality parameters in the different treatment units of the pilot-scale hybrid constructed wetland system after an intense storm event ($t=0$, beginning of the storm).

However, the intense storm events recorded at this treatment plant did not significantly affect the efficiency of the system, showing its robustness in the recovery from peak to average values of water quality parameters. Moreover, no signs of clogging were observed within the CWs.

7.3.4. Quality of treated water

E. coli was used to determine the disinfection efficiency of the treatment system (Fig. 7.8). The hybrid system achieved an overall average disinfection efficiency of 4.7 logarithmic units (average effluent of 2.3 ± 2.4 log-units 100 mL^{-1} or 200 ± 275 CFU 100 mL^{-1} for *E. Coli*). This value lays within the range found in similar studies, such as that of Masi and Martinuzzi (2007) where an average removal efficiency of 2.2 log-units was achieved after treatment in a HF unit followed by a VF one. The removal efficiency varied considerably not only from unit to unit, but also within the same unit. This variation can be attributed to the complex combination of physical, chemical and biological processes that govern the removal of pathogenic organisms within the wetlands (García et al., 2003b; IWA, 2000). During the passage through the VF wetland, *E. Coli* were reduced 1.4 log-units, whereas the reduction in the HF bed reached 1.6 log-units. The subsequent increase of almost 1 log-unit of *E. Coli* in the FWS wetland can be attributed to its resuspension as well as the presence of wildlife. However, a 2.2 log-units decrease took place in the water basin where sun penetration was complete, disinfecting the water to a final *E. Coli* concentration in every case below $1000 \text{ CFU } 100 \text{ mL}^{-1}$.

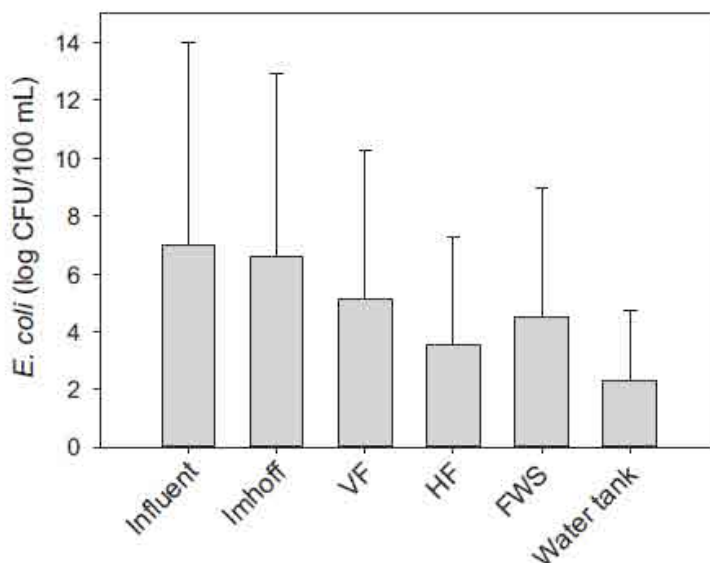


Figure 7.8. Average concentration and standard deviations of *E. Coli* along the different treatment system units of the pilot hybrid treatment system.

The treated wastewater contained in the water basin fulfilled the Spanish regulation limits (BOE, 2007) for some water reuse applications as regards the pathogens indicator *E. Coli* and the TSS concentration (maximum admitted values equal to 1000 CFU 100 mL⁻¹ for *E. Coli* and 35 mg L⁻¹ for TSS). These applications are the recharge of aquifers by percolation through the ground, silviculture and irrigation of forests and other green areas non accessible to the public. To this respect, the functions provided by the HF and FWS wetlands proved crucial to achieve a water quality suitable for its further reuse.

7.4. Conclusions

The experimental system based on constructed wetlands appears as an integrated approach capable of accomplishing a good treatment of a combined sewer effluent. During dry weather conditions, the VF wetland guarantees an efficient mineralization of organic matter as well as a satisfactory TN removal. However, the presence of the HF and FWS wetlands has proven crucial for the further purification and disinfection of the water. In such manner, the quality of the final treated wastewater fulfills the requirements for its reuse in various environmental applications, those are the recharge of aquifers by percolation through the ground, silviculture and irrigation of forests and other green areas non accessible to the public.

The occurrence of an intense storm event causes a steady increase in TSS and COD contents at the beginning of the rainfall, owed to the washout of sewer-system materials that accumulate during antecedent dry periods. This increase is followed by a dilution of the wastewater, resulting in low concentrations of organic matter and NH₄-N soon after the beginning of the rainfall. Moreover, NO_x-N concentrations increase due to the washout of fertilizers applied to the land. It is important to note the short time taken for peak to average concentrations, which emphasizes the robustness of the design approach and justifies the risk taken in choosing the system instead of storm retention tanks.

The large database of samples has shown this system able to operate over a wide range of hydrodynamic and pollutant loading conditions. This reinforces the idea of hybrid CWs as very robust systems for combined sewer effluent treatment in small communities with varying loading rates.

8. Emerging organic contaminant removal in a pilot three-stage hybrid constructed wetland system in south Spain



This chapter is based on the article:

Ávila, C., Bayona, J.M., Aragón, C., Martín, I., Salas, J.J. and García, J., 2014. Emerging organic contaminant removal in a full-scale hybrid constructed wetland system for treatment and reclamation of wastewater from small communities of warm Mediterranean climates. In preparation.

A pilot-scale hybrid constructed wetland (CW) system based on three-stages of different wetland configurations showed to be a very robust ecotechnology for domestic wastewater treatment and reuse in small communities. It consisted of a 317-m² VF bed, a 229-m² HF bed and a 240-m² FWS wetland operating in series. VF and HF wetlands were planted with *Phragmites australis* and the FWS unit contained a mixture of plant species. An excellent overall treatment performance was exhibited on the elimination of conventional water quality parameters (99% average removal efficiency for TSS, BOD₅ and NH₄-N; n=8), and its final effluent proved to comply with existing guidelines for its reclamation in various reuse applications. The removal of studied EOCs, which included various pharmaceuticals, personal care products and endocrine disruptors, was also very high (average 90 ± 11%), being compound dependent (n=8). The high rates were achieved due to high temperatures as well as the differing existing physico-chemical conditions occurring at different CW configurations, which would allow for the combination and synergy of various abiotic/biotic removal mechanisms to occur (e.g. biodegradation, sorption, volatilization, hydrolysis, photodegradation). While aerobic metabolic pathways and solids retention are enhanced in the VF bed, other removal mechanisms such as anaerobic biodegradation and sorption would predominate in the HF bed. At last, photodegradation through direct sunlight exposure, and less importantly, sorption onto organic matter, seem to take an active part in organic contaminant removal in the FWS wetland.

8.1. Introduction

The occurrence of EOCs, such as pharmaceutical and personal care products (PPCPs), pesticides or antiseptics in poorly treated wastewater and eventually in other watercourses constitutes nowadays an increasing concern worldwide due to their possible toxicological effects to the environment and living organisms (Daughton, 2005; Cunningham et al., 2006; Kümmerer et al., 2009).

On the other hand, constructed wetlands (CWs) are natural wastewater treatment systems that emphasize the processes happening in natural wetlands so as to improve their treatment capacity (Kadlec and Wallace, 2009). They constitute an alternative cost-effective technology to conventional WWTPs, especially in the context of small communities with less than 2000 PE (Puigagut et al., 2007). Various types of constructed wetlands have been combined in order to achieve higher treatment efficiency, especially for nitrogen removal. These hybrid systems are normally comprised of VF and HF beds arranged in many possible manners, including recirculation from one stage to another. While in HF wetlands nitrification is not achieved due to a lack of oxygen, in VF units aerobic conditions prevail, which provide good conditions for nitrification, but little to negligible denitrification occurs in these systems. Thenceforward, the strengths and weaknesses of each type of system can balance each other out and it in consequence it is possible to obtain an effluent low in TN concentrations, as well as other pollutants (Cooper, 1999; Vymazal, 2007; Masi and Martinuzzi, 2007).

Since EOCs are often poorly removed in conventional WWTPs (Heberer, 2002), advanced water reclamation technologies have been studied (e.g. AOPs such as photo-Fenton, ozonization) (Klavarioti et al., 2009; Rosal et al., 2010). However, these AOPs often require a high level of energy consumption and O&M cost, and thus are very unlikely to be implemented in the context of wastewater treatment of small communities. To this regard, several studies have shown a great capacity for EOC removal of constructed wetland systems at full-scale for domestic wastewater treatment of small communities in warm climates. These studies were conducted at systems consisting of a single wetland configuration at a time, namely VF, HF (Matamoros et al., 2009a) or FWS (Matamoros et al., 2008b; Llorens et al., 2009). However, studies which evaluate the contribution to EOC removal of different wetland types within a hybrid system through potential synergies in treatment processes are very scarce, and include other types of treatment units (e.g. conventional WWTPs, waste stabilization ponds) as a treatment step prior to CWs (Hijosa-Valsero et al., 2010a; Matamoros and Salvadó, 2012). Evaluating the physicochemical properties and behavior of EOCs in different constructed wetland units belonging to hybrid systems remain, thus, a future challenge to be developed. This will help in refining CW design and operation modes, which in turn may increase CW acceptance and implementation as a cost-effective and operational alternative to conventional wastewater treatment technologies in decentralized areas (Imfeld et al., 2009).

In the context of a collaborative project between the Universitat Politècnica de Catalunya-BarcelonaTech (Barcelona) and the Foundation Centre for New Water Technologies (Seville), which aimed at the treatment of wastewater up to quality standards appropriate for reuse through the sole use of CWs, an experimental meso-scale hybrid constructed wetland system was constructed. The system combined different CW configurations (VF, HF and FWS) and showed an excellent performance, both in terms of water quality parameters but also on EOC removal (Ávila et al., 2013a,b). Parallely, and in the context of the same collaboration, a comprehensive approach implemented at full-scale with identical wetland configuration (VF, HF and FWS) in a Mediterranean climate area of south Spain (Seville) proved to be a highly efficient ecotechnology for an integrated sanitation of small communities in warm climates, holding very low O&M requirements (Ávila et al., 2013d). The treatment technology, which received combined sewer effluent, exhibited a great

performance on solids, organic matter and TN removal, and showed to be very resilient to water flow fluctuations when evaluated during stormy periods and first-flush events. The final effluent of this proved to be of sufficient quality for its further reuse in various applications (i.e. silviculture and irrigation of forests and other green areas non accessible to the public. etc.).

However, the disposal into the aquatic environment of EOCs due to incomplete wastewater treatment has been of great concern for more than a decade (Kolpin et al., 2002; Cunningham et al., 2006). Additionally, in recent times there is a clear need to include irrigation as an additional exposure route for chemicals in terrestrial ecosystems. As an example, recent research is being done to explore whether these contaminants can be incorporated to crops irrigated with reclaimed water (Matamoros et al., 2012b; Calderón-Preciado et al., 2013). Although concentrations are low, questions have been raised about the potential impacts of these substances in the environment and animal and public health after long-term exposure (Matamoros et al., 2012b).

In this scenario, the aim of this study was to evaluate the treatment performance of a pilot-scale hybrid CW system located in a Mediterranean climate from southern Spain on the elimination of various EOCs from a combined sewer effluent. The selected compounds consisted of various commonly used pharmaceuticals and personal care products (PPCPs), as well as a high-production chemical widely used in epoxy resins lining food and beverage containers. These were: three non-steroidal anti-inflammatory drugs (IB, DCF, ACE), three personal care products (AHTN, OXY, TCS) and two endocrine disrupting compounds (BPA and EE2).

8.2. Materials and methods

8.2.1. Pilot-plant description

The hybrid treatment system was part of a larger pilot treatment plant (41,000-m² experimental plant of the Foundation Centre for New Water Technologies, CENTA) that received the wastewater from 2500 PE from the municipality of Carrión de los Céspedes (Seville) together with the urban runoff collected in a combined sewer system. Average annual rainfall in the area is around 650 mm and the average temperature is 17.4°C (AEMET, 2011). The pilot plant contains a great variety of both extensive and intensive technologies for wastewater treatment from small rural communities in the Mediterranean area (Fahd et al., 2007), which are submitted to analysis and validation, and are used for knowledge dissemination and outreach (<http://www.centa.es>). Pretreatment chambers are common to all technologies in the plant and its effluent is diverted towards each of them. Pretreatment consists of screening (3 cm and 3 mm), and sand and grease removal. After pretreatment, the effluent is conveyed towards a pumping chamber, from which the water is distributed through submersible pumps to the different treatment technologies present in the plant. The constructed wetland system started operation in 2005, though the treatment line as it is now began to operate in July 2009. In particular, a hybrid system consisting of a combination of various CW configurations was set in order to balance out the strengths and weaknesses of each type of system. The treatment line consisted of an Imhoff tank followed by a VF wetland, a HF wetland and a FWS wetland connected in series (Fig. 8.1).

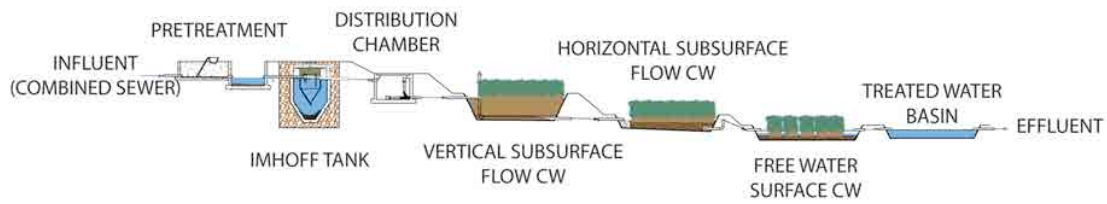


Figure 8.1. Layout of the pilot hybrid constructed wetland system.



Figure 8.2. Aerial view of the 41000-m² pilot-scale experimental treatment facility of the Foundation Centre for New Water Technologies (CENTA) located Carrión de los Céspedes (2300 PE), south Spain.

The VF wetland had a surface area of 317 m² and was designed for an organic loading rate (OLR) of about 9 g BOD₅ m⁻² d⁻¹. It was fed intermittently at about 20 pulses d⁻¹ to an average inflow of 14 m³ d⁻¹. The bed consisted of a top layer of 0.05 m of sand (1-2 mm), followed by a 0.6 m layer of gravel (4-12 mm) and an underlying 0.15 m stone layer (25-40 mm). Feeding of the VF bed was done through five lengthwise pipes (diameter = 125 mm) perforated with 1 cm diameter holes every 1.8 m distance. Five draining pipes were installed lengthwise at the bottom of the wetland within the 15 cm-thick gravel layer. Every draining pipe had three 1m-tall chimneys so as to provide oxygen transfer into the wetland bed.

The HF unit had a surface area of 229 m² and consisted of a gravel bed of 0.4 m depth (4-12 mm), with an inlet and outlet zone of stones (40-80 mm) to facilitate the flow. Feeding of the bed was done through a 63 mm diameter polyethylene pipe perforated with 1 cm holes every 1 m distance. The

outlet of the wetland was done by means of two 125 mm-diameter draining pipes located at the bottom of the stone layer and connected to a flexible pipe, which held the water level 5 cm below the top of the gravel. Both the VF and the HF beds were planted with *Phragmites australis*. Vegetation was very well developed at the time of the study.

Finally, the FWS wetland had a surface area of 240 m² and a water depth of 30 cm. A mixture of *Typha* spp., *Scirpus* spp., *Iris pseudacorus*, *Carex flacca*, *Cyperus rotundus* and *Juncus* spp. were planted on a 0.2 m gravel bed. Since the treatment system had been working for several years, it was mature and the vegetation was well developed in all units. The final effluent of the treatment line was collected in an open-air water tank with a capacity of 20 m³ so as to store the treated water for its further reuse. Further details on the system can be found in Ávila et al. (2013d).

8.2.2. Sampling procedure

Concentrations of EOCs found at urban wastewaters are often very variable, since their disposal and consumption varies a lot depending on the time of the day and period of the year (Ort et al., 2010; Nelson et al., 2011). Therefore, and given the hydraulic retention time of the treatment systems (which is usually in the range of several days), it is challenging –if not impossible– to assess the removal efficiency of the treatment system at a given moment. In order to minimize this problem, non flow-dependent 24-h composite samples were taken at this study. Sampling was performed twice a week instead of daily so as to minimize variability and to distribute sampling and analysis efforts overtime. Although these experiments were carried out at summer season, further experiments should be carried out in colder conditions to evaluate possible reduction in treatment performance.

Sampling was performed twice a week for four consecutive weeks (n = 8) during May and June 2011. Effluent 24-h composite samples of the influent, Imhoff tank, VF, HF and FWS wetlands were collected by autosamplers (about 500 mL every 1 h; no refrigeration was applied). Grab samples from the final water tank were also taken so as to measure the final quality of the stored water, which was exposed to direct sunlight. Samples for the evaluation of EOCs were transported to the laboratory in 250 mL amber glass bottles and kept refrigerated at 4°C until analysis. The sample holding time was less than 24 h. Conventional water quality parameters and studied EOCs were analyzed as described in Section 2.4. Moreover, it should be noted that no rainfall events were recorded two weeks before or during the sampling period.

8.2.3. Chemicals

GC grade (Suprasolv) hexane, methanol, ethyl acetate and acetone were obtained from Merck (Darmstadt, Germany) and analytical-grade hydrogen chloride was supplied by Panreac (Barcelona, Spain). ENR, SMZ, DC, ETM, LIN, IB, ACE, DCF, AHTN, OXB, BPA, TCS, EE2 and 2,2'-dinitrophenyl were obtained from Sigma-Aldrich (Steinheim, Germany). 2,4,5-trichlorophenoxypropionic acid (2,4,5-TPA) was from Reidel-de-Haen (Seelze, Germany). Trimethylsulfonium hydroxide (TMSH) was supplied by Fluka (Buchs, Switzerland). Strata-X polymeric SPE cartridges (200 mg) were purchased from Phenomenex (Torrance, CA, USA) and the 0.7 µm glass fiber filters of ø = 47 mm were obtained from Whatman (Maidstone, UK).

8.2.4. Analytical methods

Onsite measurements of DO, E_H, water temperature, EC, pH and turbidity were taken using a Hach HQ 30d oxymeter, a Hach Senslon i30 multi-meter and a Hach 2100Q turbidity meter, respectively. E_H values were corrected for the potential of the hydrogen electrode. Conventional wastewater quality parameters, including TSS and COD were determined by using Standard Methods (APHA, 2001).

Biochemical oxygen demand at 5 days (BOD₅) was measured by using a WTW® OxiTop® BOD Measuring System. TN, TP, NH₄-N, NO_x-N and PO₄-P were determined by using a Bran Luebbe AutoAnalyzer 3. Isolation and enumeration of *E. coli* was made using a Chromogenic Membrane Filtration technique (APHA, 2001).

Determination of EOCs in water samples was carried out after samples had been filtered and processed as previously described by Matamoros et al. (2005). The linearity range was from 0.01 to 3 mg L⁻¹. The correlation coefficients (R²) of the calibration curves were always higher than 0.99. The limit of detection (LOD) and limit of quantification (LOQ) were compound dependent in the range from 0.009 to 0.08 µg L⁻¹ and 0.02 to 0.27 µg L⁻¹, respectively.

8.3. Results and discussion

8.3.1. General water quality parameters

Table 8.1 shows concentrations of the studied water quality parameters along the hybrid CW system. Temperatures were fairly high at the time of the study (24 ± 2 °C), as expected for the hot summers of the Mediterranean climate from southern Spain. Indeed, EC values showed to increase as water passed through the FWS unit and the water tank, which could be explained by the high evapotranspiration taking place in the systems. Experimental OLR and HLRs entering the VF wetland were of about 6 g BOD₅ m⁻².d and 0.044 m d⁻¹, respectively.

Average overall removal efficiencies achieved in the treatment system up to the water tank were unquestionably high for most water quality parameters (99% TSS, 89% COD, 99% BOD₅, 98% NH₄-N). These results are in conformity with those obtained by Ávila et al. (2013d) in this treatment plant, after a 1.5-year monitoring period under dry and wet weather conditions, including an intensive sampling campaign during a first-flush event. Solids entrapment and organic matter removal was very high within the VF wetland (90 and 91% for TSS and BOD₅, respectively). Their concentrations remained low along the treatment system. The elimination of NH₄-N was also fairly high within the VF (67%), where the high values for TN removal (65%) together with the low concentrations of NO_x-N suggest once again both nitrification and denitrification processes to take place within this wetland type, due to the coexistence of aerobic and anoxic microsites within the wetland bed (Cooper et al, 1996; Ávila et al., 2013c). Further nitrification and denitrification occurred within the HF and FWS wetlands, up to an overall TN removal of 94%. This removal rate is much higher than most values reported by full-scale hybrid CWs of similar configuration at warm climates, such as the one by Masi and Martinuzzi (2007) at a system consisting of a 160-m² HF followed by a 180-m² VF, which treated the wastewater from a medium scale tourist facility in Italy (60% TN removal). To this regard, Ayaz et al. (2012) performed some experiments at a pilot-system in Turkey consisting of a HF (18 m²) and a VF (14 m²) wetland in series, and found how recirculation from the VF to the HF unit enhanced the treatment efficacy, especially in terms of nitrogen removal (up to 79% TN removal). Overall removal efficiencies of TP and PO₄-P were 47 and 16%, respectively.

The hybrid treatment system proved to have a great disinfection capacity, exhibiting overall *E.coli* reductions of about 5 log-units, which is in conformity with previous long-term microbiological pathogen evaluations in this system (Ávila et al., 2013d). Final effluent concentrations complied with Spanish regulation limits for some water reuse applications. The function made by the HF and FWS wetlands proved crucial to achieve a water quality appropriate for its reclamation.

Table 8.1. Mean values and standard deviations of water quality parameters along the pilot hybrid constructed wetland system (n=8).

	Influent	Imhoff tank	VF	HF	FWS	Water reuse tank	Overall removal efficiency (%)
Temperature (°C)	24 ± 2	24 ± 2	23 ± 2	22 ± 2	20 ± 2	21 ± 2	-
DO (mg L ⁻¹)	0.2 ± 0.0	0.2 ± 0.0	2.0 ± 1.7	4.2 ± 0.4	2.7 ± 0.3	3.8 ± 0.6	-
E _h (mV)	+62 ± 8	+2 ± 31	+115 ± 62	+139 ± 44	+129 ± 61	+210 ± 63	-
pH	7.8 ± 0.1	7.5 ± 0.5	7.4 ± 0.4	7.5 ± 0.4	7.8 ± 0.1	7.8 ± 0.2	-
EC (mS cm ⁻¹)	1.5 ± 0.1	1.5 ± 0.08	1.5 ± 0.05	1.5 ± 0.03	1.7 ± 0.09	1.8 ± 0.09	-
Turbidity (NTU)	228 ± 33	108 ± 30	22 ± 18	28 ± 13	8 ± 3	4 ± 2	98
TSS (mg L ⁻¹)	212 ± 59	114 ± 33 (46%)	11 ± 4 (90%)	13 ± 4 (-18%)	6 ± 2 (54%)	3 ± 1 (50%)	98
COD (mg L ⁻¹)	405 ± 106	258 ± 42 (36%)	44 ± 14 (83%)	29 ± 7 (34%)	47 ± 8 (-62%)	43 ± 8 (8%)	89
BOD ₅ (mg L ⁻¹)	320 ± 57	125 ± 7 (61%)	11 ± 8 (91%)	7 ± 2 (36%)	6 ± 2 (14%)	4 ± 3 (33%)	99
NH ₄ -N (mg L ⁻¹)	25.5 ± 5.4	24.2 ± 6.6 (5%)	8.0 ± 2.2 (67%)	2.5 ± 1.8 (69%)	0.7 ± 0.11 (72%)	0.6 ± 0.1 (14%)	98
NO _x -N (mg L ⁻¹)	0.3 ± 0.3	0.2 ± 0.2	0.8 ± 0.9	0.5 ± 0.2	0.2 ± 0.2	0.1 ± 0.1	-
TN (mg L ⁻¹)	40.1 ± 8.8	38.5 ± 6.1 (4%)	13.3 ± 3.6 (65%)	3.6 ± 1.4 (73%)	2.4 ± 0.6 (33%)	2.2 ± 0.5 (8%)	94
TP (mg L ⁻¹)	5.9 ± 1.2	5.9 ± 1.6 (0%)	5.3 ± 1.8 (10%)	4.2 ± 2.0 (21%)	3.1 ± 0.4 (26%)	3.1 ± 0.6 (0%)	47
PO ₄ -P (mg L ⁻¹)	3.2 ± 0.7	3.2 ± 0.6 (0%)	2.9 ± 0.4 (9%)	2.2 ± 0.9 (24%)	2.7 ± 0.3 (-23%)	2.7 ± 0.7 (0%)	16
<i>E. coli</i> (CFU 100 mL ⁻¹)	1:107	5:106	9:105	3:103	<40	<40	99.999

8.3.2. Emerging organic contaminants

8.3.2.1. Occurrence and overall treatment performance

Background concentrations of studied EOCs in influent wastewater ranged 13.5-24.5 $\mu\text{g L}^{-1}$ for IB, 0.4-1.9 $\mu\text{g L}^{-1}$ for DCF, <LOD-8.5 $\mu\text{g L}^{-1}$ for ACE, 0.4-0.9 $\mu\text{g L}^{-1}$ for AHTN, 0.1-0.2 $\mu\text{g L}^{-1}$ for TCS and 1.4-5.7 $\mu\text{g L}^{-1}$ for BPA (Table 8.2). Those values were in the range previously reported in raw wastewater by other authors (Matamoros et al., 2007; Miège et al., 2009; Hijosa-Valsero et al., 2010b; Ávila et al., 2013c). The sunscreen agent OXY and the synthetic estrogen EE2 were not detected at any of the sampling days. The analgesic paracetamol (ACE) was detected in 50% of the influent samples and its concentrations varied significantly. The rest of compounds were detected at every sample of influent wastewater.

The hybrid constructed wetland system (up to the effluent of the FWS unit) performed remarkably well also in the removal of EOCs, achieving very high overall removal efficiencies for the majority of the studied compounds (average of $90 \pm 11\%$). These rates are in agreement with those reported by Ávila et al. (2013b) at an injection experiment conducted at a meso-scale experimental hybrid CW system consisting of the same wetland configurations, and with those found by Hijosa-Valsero et al. (2010a) at three-full scale hybrid CW systems consisting of different combinations of waste stabilization ponds and FWS and HF wetlands in series. Moreover, Matamoros and Salvadó (2012) observed very high removal efficiencies (around 90%) for most studied compounds (e.g. IB, DCF, AHTN, TCS) at a full-scale reclamation pond-FWS wetland system in Girona, Spain, treating secondary effluent from a conventional WWTP. However, influent concentrations were much lower than this study.

Final effluent concentrations of target EOCs were very low, being below the limit of detection for various contaminants (i.e. ACE, BPA). The rest were in the ng L^{-1} order (20-100), which is in the range of those found in the environment, such as those reported by Matamoros et al. (2009b) in small ponds or lagoons. These concentrations were also in the range of those obtained in advanced treatment technologies applied at full-scale, such as ozonation or membrane filtration (Snyder et al., 2007; Rosal et al., 2010).

The high removal efficiencies can be explained by differing existing physico-chemical conditions at different CW configurations, which would allow for the combination and synergy of various physicochemical and biological removal mechanisms to occur (e.g. biodegradation, sorption, volatilization, hydrolysis, and photodegradation) and thus achieve improved treatment efficiency of most pollutants (Imfeld et al., 2009). In this sense, while aerobic metabolic pathways and solids retention are enhanced in VF wetlands, other removal mechanisms such as anaerobic biodegradation and sorption would predominate in HF beds. At last, the FWS wetland would be responsible for potential photodegradation of compounds, and less importantly through adsorption onto organic matter and uptake of plant material (Matamoros and Salvadó, 2012). However, although the experimental design and analysis and eventually data were insufficient for the deepening into the elucidation of EOCs removal mechanisms occurring within a CW, the purpose of this study was rather to attempt to identify possible removal pathways for different EOCs occurring in different CW configurations, based on their observed experimental behavior, together with the support from previous literature.

8.3.2.2. Vertical subsurface flow constructed wetland performance

Fig. 8.3 shows average removal efficiencies of selected EOCs accumulated at each stage of the hybrid constructed wetland system. The Imhoff tank achieved a good removal of the musk fragrance AHTN (40%), presumably due to a high degree of attachment to the particulate matter. The removal of the biodegradable substance IB was also not negligible in this tank (20%). The VF bed showed variable removal of EOCs, being compound dependent. It performed best for ACE (94%), IB (58%) and TCS (50%), while lower removal efficiencies were achieved for BPA (44%), AHTN (17%) and DCF (36%).

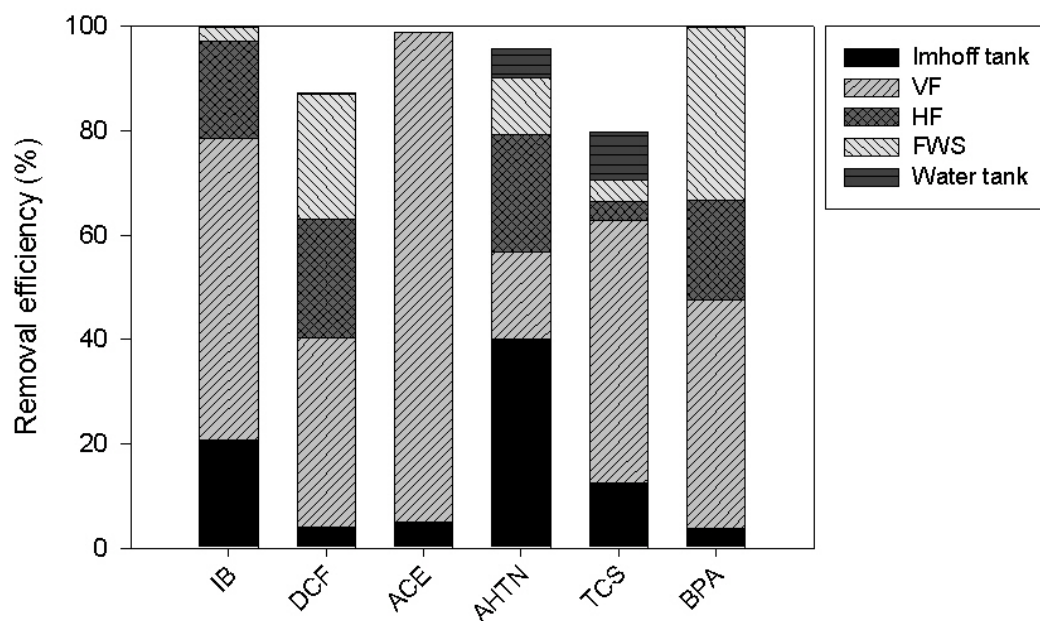


Figure 8.3. Accumulated removal efficiencies for the selected emerging organic contaminants at the different units of the pilot hybrid treatment system.

In particular, ACE was completely removed within the VF bed. This analgesic has shown to be readily biodegraded (Yamamoto et al., 2009) to concentrations below the limit of detection in all types of wetlands, including HF (Ávila et al., 2013c), VF (Ávila et al., 2013b) and hybrid treatment wetlands (Conkle et al., 2008; Ávila et al., 2013b), as well as conventional WWTPs (Miège et al., 2009). Average elimination rates achieved for IB (58%) did not fluctuate much during the sampling campaign and are in agreement with the attributed aerobic biodegradation of this compound, being more oxidized conditions provided by the unsaturated operation, as well as intermittent feeding, of the VF bed of key importance for an enhanced degradation (Zwiener and Frimmel, 2003; Matamoros et al., 2008a). To this regard, Matamoros et al. (2007) showed how removal rates for IB were higher for unsaturated ($99 \pm 1\%$) than for saturated ($55 \pm 1\%$) VF wetlands (5 m^2) at an experimental pilot plant in Denmark. Similar results were obtained by Ávila et al. (2013c) at an injection experiment at HF wetland system comparing permanently saturated operation vs. operation on cycles of saturation/unsaturation (63 and 85%, respectively). What is more, Ávila et al. (2013b) reported an average of 55% of IB elimination at two alternating VF beds (3 m^2) at an experimental hybrid system in Spain, and treatment performance in terms of IB removal seemed to be negatively correlated to hydraulic loading rates. In that particular case, higher HLRs would translate into a higher number of feeding pulses and shorter resting times, resulting in a decrease of aeration into the system and hence a lower treatment performance (Torrens et al., 2009). However, this compound seems to be fairly easily biodegraded, and is well attenuated also in conventional WWTPs (Lishman et al., 2006; Miège et al., 2009).

Moreover, the prevailing aerobic conditions of the VF bed also seemed to be especially important for the elimination of TCS (50%). These results are in agreement with those by Ávila et al. (2013b) in a VF bed (3 m²), who reported an average removal of 78% and observed how the operation under higher HLRs decreased treatment performance (from 85 to 71%). Although sorption onto the substrate could constitute a relevant removal mechanism for TCS, given its hydrophobic characteristics ($\log K_{ow} = 4.7$), a negligible removal took place within the HF bed (Fig. 8.4). While other substances, like AHTN, show a great reduction within the HF bed presumably due to sorption processes, results for TCS would exhibit little sorption capacity. The behavior of TCS within the three different wetland configurations indicate aerobic biodegradation as the major removal mechanism involved in the elimination of this compound (Singer et al., 2002; Ying et al., 2007).

Conversely, the removal of DCF within the VF wetland was lower (36%) and more variable. Although DCF has been reported to be recalcitrant in activated sludge WWTPs (Heberer, 2002a; Miège et al., 2009), variable (from negligible to very high) removal efficiencies of this substance have been reported at CW systems (Matamoros and Bayona, 2006; Matamoros et al., 2009a). Well-replicated experimental mesoscale studies carried out in HF wetlands have demonstrated that higher redox conditions enhance the removal of this compound, among other EOCs (Hijosa-Valsero et al., 2010b; Ávila et al., 2013b,c). In this way, Matamoros et al. (2007) reported a better elimination in an unsaturated VF bed (73%) if compared to a saturated VF (53%). Zhang et al. (2012b) found significantly better performance of experimental mesoscale HF beds operating in pulses than those continuously fed. What is more, Ávila et al. (2013b) recently found how DCF was efficiently removed within the first stage (VF) of the experimental hybrid CW system (around 65%), but conversely no removal of DCF occurred at the following HF bed, where degenerated oxygen conditions prevailed. Nevertheless, very high (99%) removal rates were found by Ávila et al. (2010) during a continuous injection experiment in summer in an experimental HF wetland system operating under anaerobic conditions ($E_H = -123$ mV; DO < LOD). The similarly high removal efficiencies achieved in the current study in the HF bed (23%) if compared to the VF (36%) suggest that various alternative mechanisms may determine the elimination of this compound, and to that respect, anaerobic biodegradation through reductive dehalogenation could constitute a predominant degradation pathway of DCF when anaerobic conditions prevail (Park et al., 2009; Hijosa-Valsero et al., 2010a; Ávila et al., 2010). Average removal rate of the musk fragrance AHTN in the VF wetland was lower (17%), than that found by Matamoros et al. (2007) in unsaturated and saturated VF wetlands (82 and 75%, respectively), and Ávila et al. (2013b) (average of 69%), which found a dependence of this substance on the applied HLR, suggesting that decreased entrapment of hydrophobic compounds onto particulate matter occurred due to reduced contact time at higher HLRs. The removal of this compound occurs mainly through sorption on the particulate matter, given its high hydrophobicity (Matamoros et al., 2007). Moreover, the elimination of the endocrine disruptor BPA was significantly higher in this wetland (44%) if compared to the HF wetland (19%), which is in conformity with the previously observed dependence of this substance on aerobic conditions (Ávila et al., 2013b). However, the removal of this substance has also been achieved under anaerobic conditions of HF wetlands (Ávila et al., 2010) and thus the degradation of this substance could be owed to multiple mechanisms which seem to vary significantly in time, including association to the particulate matter (Wintgens et al, 2004), and biodegradation (Ávila et al., 2010).

The superior treatment performance of the VF bed over the other treatment units could be owed to energetically favorable aerobic microbial reactions, as well as hydrolysis reactions, taking place within this wetland type and provided by its design and operation strategy, conferring high effluent E_H ($+115 \pm 62$ mV) and DO concentrations (2.0 ± 1.7 mg O₂ L⁻¹). This wetland type is characterized by holding a great oxygen transfer capacity due to the unsaturated conditions of the bed, with passive aeration, as well as to the intermittent feeding operation. Oxygen transfer is achieved mainly by means of convection while intermittent loading and diffusion processes occurring between doses (Torrens et al.,

2009). In fact, significantly higher enzymatic activities and microbial biomass have been found in the upper layer of the sand of VF wetlands, where there is more substrate and nutrient availability, indicating favorable redox conditions for aerobic metabolism, including carbon, nitrogen and other pollutants' degradation (Zhou et al., 2005; Tietz et al., 2007; Imfeld et al., 2009). However, the synergic nitrification-denitrification activity observed in the VF bed within this study (Section 3.1) suggest the co-existence of both aerobic and anaerobic microenvironments within this wetland bed, which would allow both processes to take place. Similarly, this finding indicates that although aerobic biodegradation and sorption onto organic matter may be the major removal mechanisms contributing to EOCs reduction in VF wetlands, alternative processes based on anaerobic metabolism could simultaneously be occurring at anoxic microsites or micropores within the wetland bed (i.e. in lower layers) (Cooper et al., 1996; Ávila et al., 2010), which contribute to its elimination. Finally, it is worth noting that the large removal rates achieved at this wetland in comparison to the other wetland units are also influenced by the fact that this bed was the first stage of the system, where the major part of the removal would occur (Hijosa Valsero et al. 2010a; Ávila et al., 2013b).

8.3.2.3. Horizontal subsurface flow constructed wetland performance

Target EOCs within the HF bed as a second stage of the hybrid CW system exhibited removal efficiencies, which were best for DCF (23%), AHTN (23%) and BPA (19%) and IB (19%) (Fig. 8.4). Note that these were not individual efficiencies in respect to the influent of the HF wetland, but the further proportion of elimination in respect to the previous treatment unit (% accum.). The purpose was to ease the comparison of the efficiency of removal within this bed in respect to the other two units. The remaining IB was considerably reduced up to average effluent concentrations of $0.5 \pm 0.3 \mu\text{g L}^{-1}$. The fragrance AHTN, due to its high hydrophobicity, was moderately removed, presumably by sorption onto the particulate material of the gravel matrix (Carballa et al., 2005; Matamoros and Bayona, 2006). Removal of TCS was extremely poor within this wetland bed (3%), as compared to the VF bed (50%). Similarly low removal efficiencies (<10%) were obtained at the HF bed of the mesoscale hybrid CW system studied by Ávila et al. (2013b). Although TCS has been detected in plant and sediments of a HF wetland and its concentration generally decreased from inflow to outflow (Zárate et al., 2012), sorption and plant uptake does not appear to constitute a principal mechanism of TCS removal in constructed wetlands. Otherwise, higher degradation rates would have been expected at the HF beds (Singer et al., 2002). The degradation of BPA was only moderate (19%), especially if compared to the reduction rates achieved at the VF (44%) and particularly FWS (33%) wetlands. This would be explained by the occurrence of less energetically-favorable metabolic processes occurring at lower redox conditions, which would result in lower degradation kinetics and hence lower elimination rates (Ávila et al., 2013c).

In general, HF wetlands have exhibited lower treatment capacity on the removal of EOCs than VF beds, which could be attributed to the dependence of their transformation processes on a high redox status of the system (Matamoros et al., 2009a; Ávila et al., 2013b). However, the treatment performance of HF wetlands has been found to be significantly enhanced in respect to the removal of EOCs (including IB, DCF, or BPA) at experiments at micro, meso and pilot-scale by optimized operation strategies resulting in high redox potentials, such as operating these wetlands in cycles of feed and rest, providing a shallow depth of the water table, or a using a primary treatment based on a conventional settler rather than anaerobic treatments (Matamoros et al., 2005; Song et al., 2009; Ávila et al., 2013c).

8.3.2.4. Free water surface wetland performance

The FWS wetland performed especially well for BPA (33%), followed by DCF (24%). The removal of BPA within this wetland was fairly high if compared to the HF unit, which could be explained by enhanced

biodegradation of this substance under higher E_H and DO conditions within the water column of the FWS wetland (Liu et al., 2009; Ávila et al., 2013c). Sorption onto particulate matter (Stevens-Garmon et al., 2011) and photodegradation (Matamoros et al., 2012a) could further contribute to BPA removal in FWS wetlands. Moreover, the reduction of DCF was in accordance with elimination rates reported by Llorens et al. (2009) at a full-scale FWS wetland receiving secondary effluent. The reduction of DCF was also significantly high (24% accum.) at a mesoscale FWS unit (Ávila et al., 2013b). These results support photodegradation as a principal removal mechanism involved in DCF attenuation in water bodies (Buser et al., 1998; Andreozzi et al., 2003; Matamoros and Salvadó, 2012, 2013; Ávila et al., 2013b), together with less predominant mechanisms (i.e. aerobic/anaerobic biodegradation, plant uptake) (Ávila et al., 2010). Removal of remaining IB took place in this wetland (3%), presumably due to biodegradation since low photodegradation rates are expected for this compound (Yamamoto et al., 2009; Szabó et al., 2011). Although the removal of TCS was negligible (around 4%), some more reduction within the water reuse tank seemed to occur, indicating that photooxidation processes may constitute a small contribution to its removal (Mezcua et al., 2004; Ávila et al., 2013b; Matamoros and Salvadó, 2012, 2013). Additionally, the further reduction of AHTN concentrations within this wetland bed was significant (11%), accumulating an overall removal efficiency of 90%. Although the reduction of AHTN concentrations in the FWS wetland could be attributed to sorption onto particulate matter and sediment, further reduction was achieved at the water reuse tank, suggesting photodegradation through sunlight exposure as one of the principal mechanisms of AHTN's removal within this type of wetland configuration. Similarly high removal efficiencies were obtained at other FWS wetlands operating as a tertiary treatment step (Matamoros et al., 2008b; Llorens et al., 2009; Matamoros and Salvadó, 2012; Ávila et al., 2013b).

8.4. Conclusions

A hybrid CW system at pilot-scale, which consisted of a VF wetland, a HF wetland and a FWS wetland operating in series, showed to be a very robust ecotechnology for wastewater treatment and reuse in small communities. Excellent overall treatment performance was exhibited on the elimination of conventional water quality parameters (99% average removal efficiency for TSS, BOD_5 and NH_4-N), and its final effluent proved to comply with existing guidelines for its reclamation in various reuse applications (e.g. recharge of aquifers by percolation through the ground, silviculture and irrigation of forests and other green areas non accessible to the public). The removal of EOCs, which included various PPCPs and EDCs, was also very high ($90 \pm 11\%$).

Most organic matter as well as EOC removal took place in the first stage of the treatment system (VF), where aerobic conditions are expected to prevail. The intermittent feeding and unsaturation of the bed constitute key practices in that approach. However, significant denitrification was also found to occur within this wetland bed, suggesting that although aerobic degradation and sorption onto organic matter might constitute the major removal mechanisms contributing to EOCs removal in VFs, alternative processes based on anaerobic microbial metabolism could simultaneously be occurring at anoxic microsites or micropores within the bed, possibly at lower layers. Conversely, a lower removal efficiency was found for the HF bed, where mostly anaerobic degradation and sorption onto the gravel matrix are expected to occur. Finally, photodegradation through direct sunlight exposure and sorption to organic matter seem to take an active part in EOC elimination in FWS wetlands.

The combination of different wetland configurations has shown to optimize a number of important treatment processes, achieving an excellent overall EOC reduction, as well as removal of conventional water quality parameters. This has been possible thanks to the occurrence of complementary abiotic/biotic removal pathways taking place under differing physico-chemical conditions existing at wetlands of different configuration.

9. Removal of emerging organic contaminants in pilot-scale vertical subsurface flow constructed wetlands as a function of media size, loading frequency and use of active aeration



This chapter is based on the article:

Ávila, C., Nivala, J., Olsson, L., Kassa, K., Headley, Müller, R., T., Bayona, J.M. and García, J., 2014. Influence of media size, loading frequency and use of active aeration on the removal of EOCs in vertical subsurface flow constructed wetlands. In preparation.

The relative importance of a particular degradation process in constructed wetlands (CWs) can vary significantly, as a function of numerous environmental factors, the organic contaminant being treated, wetland configuration, and specific design and operational parameters. In this study several side-by-side pilot-scale vertical subsurface flow CWs (VF) (surface area: 6.2 m²; hydraulic loading rate: 95 mm d⁻¹) differing in loading frequency (hourly vs. bi-hourly fed), grain size (1-3 mm vs. 4-8 mm), as well as on the use of active aeration (under saturated conditions), were evaluated for their treatment efficiency on the removal of eight widely-used EOCs, including mostly pharmaceuticals and personal care products, in municipal wastewater. All VF beds received experimental organic and hydraulic loading rates (HLR) of 7-16 g TOC m⁻².d and 95 mm d⁻¹, respectively. In general, sand-based VFs performed significantly better than gravel-based wetlands both in the removal of water quality parameters (TSS, TOC, NH₄-N) and target EOCs (85 ± 17% vs. 74 ± 15%, respectively). A higher contact time between water and biomass, a greater filtering capacity, and in general higher oxygen availability and redox potentials in sand-based VFs would promote the elimination of substances whose removal is expected to happen through aerobic biodegradation (bisphenol A, triclosan, oxybenzone, ibuprofen), as well as those typically adsorbed onto particulate matter (tonalide). Although the loading frequency did not show to affect the removal of water quality parameters, significantly lower removal efficiencies were found for tonalide and bisphenol A at the less frequently dosed VF wetland (higher volume per pulse). In general, similar conditions were found at these two wetland units, except for redox and oxygen values, which were lower in the bi-hourly fed system. This suggests that the higher velocity of the water and reduced contact time at higher loading volumes would decrease oxygen renewal during the feeding pulse, hence resulting in lower treatment efficiencies. However, diclofenac was the only contaminant showing an opposite trend to the rest of compounds, achieving higher elimination rates at the less oxidized wetlands (bi-hourly and gravel-based VFs). The coexistence of various microenvironments could allow for the combination of various anaerobic and aerobic degradation pathways to take place within these wetland units, which could be important for an improved removal of some contaminants. Moreover the use of active aeration in a saturated-VF bed did not seem to improve any aspect of treatment performance in comparison with the typical passive unsaturated VF wetland. In practice, grain size as well as loading frequency should be carefully selected, as well as HLRs controlled, in order to ensure that enough oxygen renewal and mineralization of organic matter takes place so that no clogging develops in the filter beds.

9.1. Introduction

EOCs mainly comprise a group of man-made compounds such as pharmaceuticals and personal care products (PPCPs), endocrine disruptors, pesticides, surfactants and antiseptics, which are continuously discharged into the environment as a result of agricultural, industrial and municipal activities. Their occurrence has been reported in all compartments of the aquatic environment (Kolpin et al., 2002; Loos et al., 2009; Jurado et al., 2012), being found even at the effluent of drinking water treatment plants (Heberer et al., 2002). Although their concentrations are usually low (from parts per trillion to low parts per billion level), many of them have raised toxicological concerns on living organisms and human beings, particularly when occurring as complex mixtures of compounds (Schwarzenbach et al., 2006; Pal et al., 2010). One of the main sources of these contaminants in the environment is the discharge from conventional WWTPs, where their elimination is often insufficient (Heberer, 2002). Although a whole array of advanced tertiary treatment technologies have been lately evaluated for the removal of EOCs (e.g. ozonation, advanced chemical oxidation, UV radiation, etc.) (Kim et al., 2009; Liu et al., 2009; Rosal et al., 2010), the high O&M cost of these technologies is oftentimes not justified under the current concept of wastewater treatment. Particularly in decentralized areas, where water flow may vary significantly overtime, an easy-to-operate treatment technology is needed. In this regard, extensive low-cost technologies such as constructed wetlands (CWs) constitute a great alternative for wastewater treatment and reuse. Their use has become increasingly important in recent decades, due to its simplicity of operation and maintenance, low environmental impact, low or no energy cost, low waste production and good integration within the environment. CWs have been widely implemented worldwide, in the treatment of various types of wastewater, originating from almost every conceivable contamination source (Vymazal, 2005; Wallace and Kadlec, 2005; Comino et al., 2011; Serrano et al., 2011; Saeed et al., 2012). These are natural wastewater treatment systems that consist of a properly designed –impervious- shallow basin, which contains a substrate that is planted with aquatic vegetation. Other components such as microorganisms and aquatic invertebrates develop naturally. These systems are constructed to mimic the microbiological, biological, physical and chemical processes that occur in a natural wetland for the primary purpose of water quality improvement. CWs have traditionally been classified into two main types, surface flow and subsurface flow. In turn, subsurface flow wetlands are divided into horizontal flow and vertical flow, depending on the water flow direction (Kadlec and Wallace, 2009). The wetland type will determine occurring physico-chemical conditions of the wetland and in turn existing degradation processes (Imfeld et al., 2009). As a matter of fact, it is well known that in subsurface flow CWs the oxygen demand exerted by incoming wastewater for pollutant removal generally exceeds the amount of oxygen available within the wetland bed (Kadlec and Wallace, 2009; Nivala et al., 2012b). While subsurface oxygen limitation is one of the main rate-limiting factors for traditional HF wetlands (Brix and Schierup, 1990), VF wetlands were developed so as to increase the oxygen transfer capacity, which is enhanced due to its specific design and operational conditions (Torrens et al., 2009). Hence, these require less area (2-3 m²/PE) than other CW configurations (i.e. about 5 and 8 m²/PE in HF and FWS wetlands, respectively) to attain the same contaminant efficiencies (Cooper, 2005; Vymazal, 2005; Kadlec, 2009).

In particular, many variants of VF wetlands have been implemented and evaluated worldwide, but they are usually unsaturated and intermittently pulse-loaded, and the main media layer is typically sand. In particular, wastewater is supplied in hydraulic batches, thus ensuring an even distribution of water and suspended solids across the whole infiltration area, and flows vertically from the top to the bottom. Drainage pipes at the bottom are usually connected to ventilation pipes, to provide a pathway for air to be drawn in to the substrate from the bottom of the bed. Occasionally various beds alternate their functioning in phases of feed and rest (Molle et al., 2005). Oxygen transfer is achieved by means

of diluted oxygen present in wastewater, convection while intermittent loading, and diffusion processes occurring between doses (Torrens et al., 2009). In this way not only does it promote the mineralization of organic matter, but also the nitrification step, which is essential for the subsequent process of denitrification (Cooper, 1999; Kayser and Kunst, 2005). VF wetlands have gained considerable importance in wastewater treatment in small communities and at household-scale in the last decades, such as in the United Kingdom (Weedon, 2003), Denmark (Brix and Arias, 2005), Estonia (Öövel et al., 2007), the Netherlands, the United States, Germany, or Austria, achieving effluents that satisfy the legislations (Langergraber et al., 2003). Sometimes they are combined with HF wetlands, constituting hybrid systems, with the aim of complementing contaminant removal processes, especially those aiming at TN retention (nitrification-denitrification) (Cooper, 1999; Öövel et al., 2007; Molle et al., 2008). Moreover, France has over 20 years of experience with VF systems, which in 2005 recorded a total of 350 treatment plants in operation in small communities. The French systems differ significantly on the mode of operation from the others, in that raw wastewater is applied, causing the accumulation of a layer of solids on the top of the bed, which in turn acts as a filter. Usually three beds work in parallel so as to allow for the alternation of cycles of feed and rest, and thus promote mineralization of the deposit of solids during resting phases (Molle et al., 2005). In general, these various design and operational variants represent a broad spectrum of technologies, which range from moderately engineered systems (VF with intermittent feeding) up to highly intensified systems (with intensified pumping, water level fluctuation, or active aeration) (Fonder and Headley, 2013). Across this gradient of systems, there are trade-offs between the system footprint and energy requirement. In this way, a decrease in required surface area typically comes at a cost of increased O&M costs.

The relative importance of a particular degradation process in CWs can vary significantly, as a function of numerous environmental factors, the organic contaminant being treated, wetland configuration, and specific design and operational parameters. For example, in unsaturated intermittently-loaded VF wetlands, diffusion and convection processes depend on the loading regime, being affected by the number of pulses, the volume of the pulse, and the duration of surface filter dewatering (Molle et al., 2006; Torrens et al., 2009). The choice of the filter media (i.e. grain size, grain material, depth of layers) is also crucial, and should simultaneously satisfy treatment needs, while avoiding clogging of the bed and maintaining oxygen renewal in the media (Cooper, 2003; Kayser and Kunst, 2005; Stefanakis and Tsihrintzis, 2012). Moreover, the use of active aeration (e.g., an air pump connected to subsurface network of air distribution pipes), which has been often successfully applied in HF wetlands (Wallace, 2001), has been recently applied in saturated VF wetlands and its treatment performance should be evaluated (Murphy and Cooper, 2011).

Thus, attempting to evaluate the influence of these and other factors on the elimination of EOCs in CWs may help to optimize the technology by refining constructed wetland design and operation modes. Most of the available research focuses on conventional water quality parameters. Only in the last decade, the effect of these factors on the removal of EOCs has been investigated. These include studies evaluating the influence of water depth (Matamoros et al., 2005; Matamoros and Bayona, 2006), type of organic matter (Matamoros et al., 2008a), type of support matrix (Dordio et al., 2009; Dordio and Carvalho, 2013), and other different design parameters and modes of operation (Hijosa-Valsero et al., 2010; Hijosa-Valsero et al., 2011b; Zhang et al., 2012a,b). However, they have been carried out majorly in HF wetlands. To date, no study has investigated recent advances in VF CW design in respect to EOC removal in an actual side-by-side comparison.

The aim of this study was to investigate the effect of filter media (coarse sand vs. fine gravel), loading regime (hourly with 4 mm/pulse or bi-hourly with 8 mm/pulse) and active aeration on the removal efficiency of various EOCs, including PPCPs (i.e. IB, ACE, DCF, AHTN, TCS, OXY) and endocrine disrupters (i.e. EE2, BPA) in four pilot-scale VF CWs working in parallel treating primary treated domestic wastewater from the municipality of Langenreichenbach, Eastern Germany.

9.2. Materials and Methods

9.2.1. Facility description

The four VF pilot systems evaluated in this study are part of the Langenreichenbach ecotechnology research facility, which is located in the village of Langenreichenbach, Leipzig (Germany). It contains 15 individual pilot-scale treatment systems of eight different designs or operational variants differing in terms of flow direction, degree of media saturation, media type, loading regime, and aeration mechanism. The facility is adjacent to the wastewater treatment plant for the nearby village, enabling all of the pilot-scale systems to receive the same (domestic) wastewater. The surrounding villages can be classified as rural-residential. For a detailed description of the overall research facility and each specific design, the reader is referred to Nivala et al. (2013). Air temperature, rainfall, humidity and evaporation were monitored on an hourly basis by an automatic weather station at the site.

In particular, our study focused on the four-planted VF CWs differing in design parameters and operational modes. Raw wastewater is taken from a pressure sewer line before it enters the adjacent WWTP and passes through a sedimentation tank (16.5 m³; HRT: 2 d) for primary treatment. The effluent then passes through a bank of two commercial-size septic tank filters (screen size: 0.8 mm) to prevent carry-over of large solids into the pump chamber from which the treatment systems are loaded. Subsequently water is pumped through two submersible pumps, which deliver the primary treated wastewater to the VF constructed wetlands, in pulses with a duration of about 30-60 seconds.



Figure 9.1. View of one of the pilot-scale vertical subsurface flow constructed wetlands of the experimental wastewater treatment facility of Langenreichenbach, Germany.

9.2.1.1. Vertical subsurface flow constructed wetlands

Each VF bed measures 2.75 m by 2.4 m, but since it contains an outlet shaft for monitoring purposes, the effective surface area is 6.2 m² per bed. Main design and operational parameters of the studied vertical flow wetlands are shown in Tables 9.1 and 9.2. These variants differ on media type, loading regime and aeration mechanism. In particular, they consisted of two typical passive coarse sand-based (1-3 mm) VF units (VS1p and VS2p, which differed in the loading frequency), a fine gravel-based (4-8 mm) VF wetland (VGp), and an intensified system consisting of a saturated VF wetland receiving active aeration containing medium gravel (8-16 mm) (VAp). Each bed was loaded intermittently every one hour (except for VS2p, which was fed every 2 h), with a predefined volume of wastewater. A 15-cm layer of coarse gravel was used as the drainage layer at the bottom of all VF beds. All systems received the same hydraulic loading rate (HLR) (i.e. 0.095 m d⁻¹), which were accurately measured through a control system.

Table 9.1. Design and operational details for the studied pilot-scale vertical flow constructed wetlands.

System Abbreviation ¹	System Type	Depth of Main Media (m)	Saturation Status	Main Media Type	Loading Interval (hour)	Surface Area (m ²)	Hydraulic Loading Rate ^b (mm d ⁻¹)
Vertical Flow							
VS1p	VF	0.85	Unsaturated	Coarse sand	1.0	6.20	95
VS2p	VF	0.85	Unsaturated	Coarse sand	2.0	6.20	95
VGp	VF	0.85	Unsaturated	Fine gravel	1.0	6.20	95
Intensified							
VAp	VF + Aeration	0.85	Saturated	Medium gravel	1.0	6.20	95

¹The suffix "p" in the system abbreviation denotes the presence of plants (*Phragmites Australis*) in the treatment systems.

^bAverage values for August – September 2010.

Table 9.2. Grain size characteristics of the sand and gravel used at the different pilot vertical flow wetlands.

Media Type	Nominal Size (mm)	d ₁₀ (mm)	d ₆₀ (mm)	Uniformity Coefficient
Coarse sand	1 – 3	0.8	1.8	2.3
Fine gravel	4 – 8	3.5	5.5	1.6
Medium gravel	8 – 16	5.0	9.6	1.9
Coarse gravel ^a	16 – 32	10.5	11.2	1.1

The reasoning behind the choice in loading regime (hourly vs. bi-hourly) was so as to investigate the difference between smaller, more frequent doses and larger, less-frequent doses. Loading frequency is a potentially important operational design variable that is rarely stipulated in design guidelines and that varies a lot in practice, ranging once per day (large doses) to every 20 min (microdoses) (Brix and Arias, 2005; Torrens et al., 2009). Since they received the same HLR, the volume per pulse differed,

being of approx. 4 L m⁻².pulse and of 8 L m⁻².pulse for VS1p and VS2p, respectively. On the other hand, in practice, well-graded sand that is typically used in VF wetlands is not available everywhere in the world, and fine gravel (well-sorted and free of fines) in the order of 4 to 8 mm is often the next most suitable material. For that reason, the gravel-based VF wetland (VGp) was also evaluated as opposed to sand-based VF unit (VS1p). Details on these units are depicted in Fig. 9.2 and Fig. 9.3.

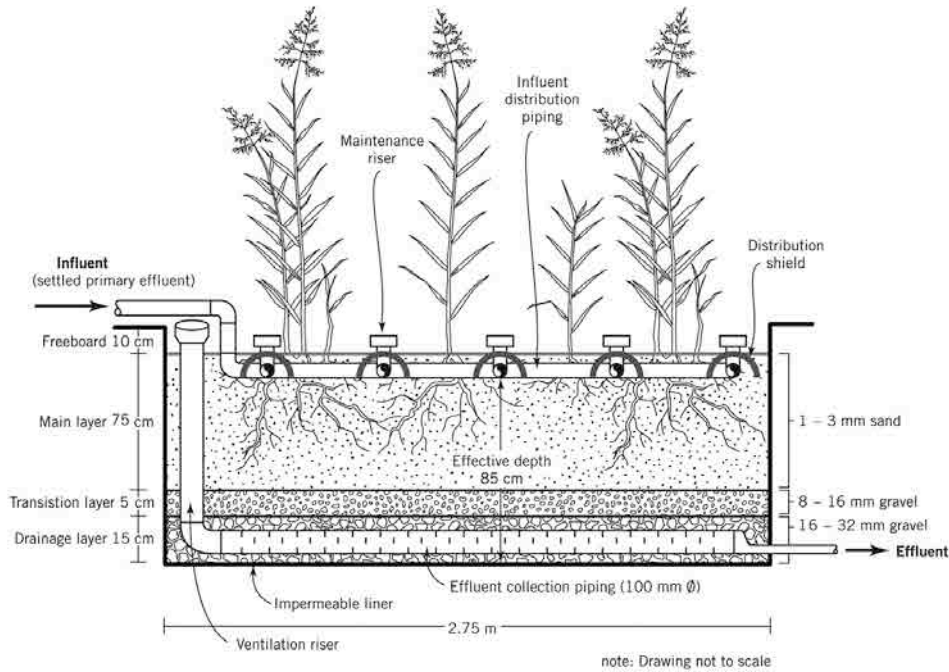


Figure 9.2. Profile view of the sand-based unsaturated vertical subsurface flow systems (VS1p and VS2p) Obtained from: Nivala et al. (2013).

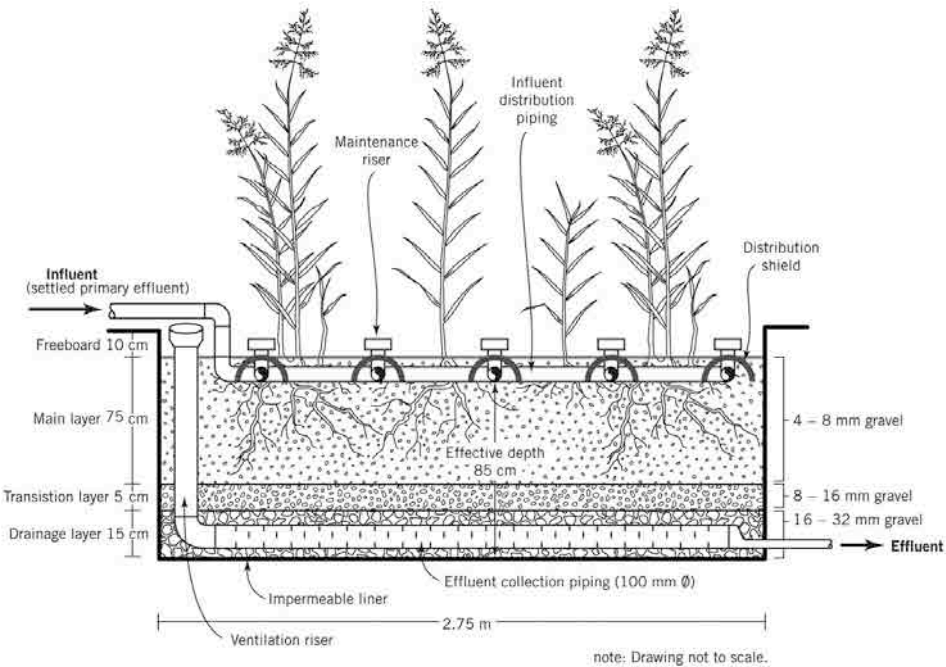


Figure 9.3. Profile view of the gravel-based unsaturated vertical subsurface flow system (VGp). Obtained from: Nivala et al. (2013).

Moreover, the ability to mechanically aerate saturated HF wetlands has shown to greatly exceed the oxygen transfer rate possible in passive wetland designs, with proportional increases in treatment efficiency (Wallace, 2001; Nivala et al., 2012b). However, questions remain so as to elucidate whether a typical sand-based unsaturated VF bed can be enhanced through the operation of the unit in saturated conditions equipped with an integrated aeration system (Nivala et al., 2013). Although this VF design variation could seem not to differ much from aerated HF wetlands, the vertical downflow configuration of the cell offers two process advantages over HF ones. First of all, the cross-sectional influent organic loading rate over the distribution area ($\text{g BOD m}^{-2}\cdot\text{d}$) is much lower in this configuration than in horizontal flow designs, which might minimize the potential for clogging (Wallace and Liner, 2011). Moreover, since the water is applied at the top of the bed and flows downwards to the collection systems, while air bubbles flow in the opposite direction, water in the bed should be extremely well mixed (Headley et al., 2013). For the vertical flow aerated system of this study (VAp), the density of the aeration orifices was $0.078 \text{ m}^2/\text{orifice}$. Drip irrigation tubing (Geoflow, Inc.) with known airflow-pressure drop characteristics was utilized so that the aeration grid could operate in a “balanced” condition, being energy efficient while ensuring uniform coverage of the air distribution network. This wetland was filled with medium gravel (8-16 mm). Further details on the VAp design are shown in Fig. 9.4.

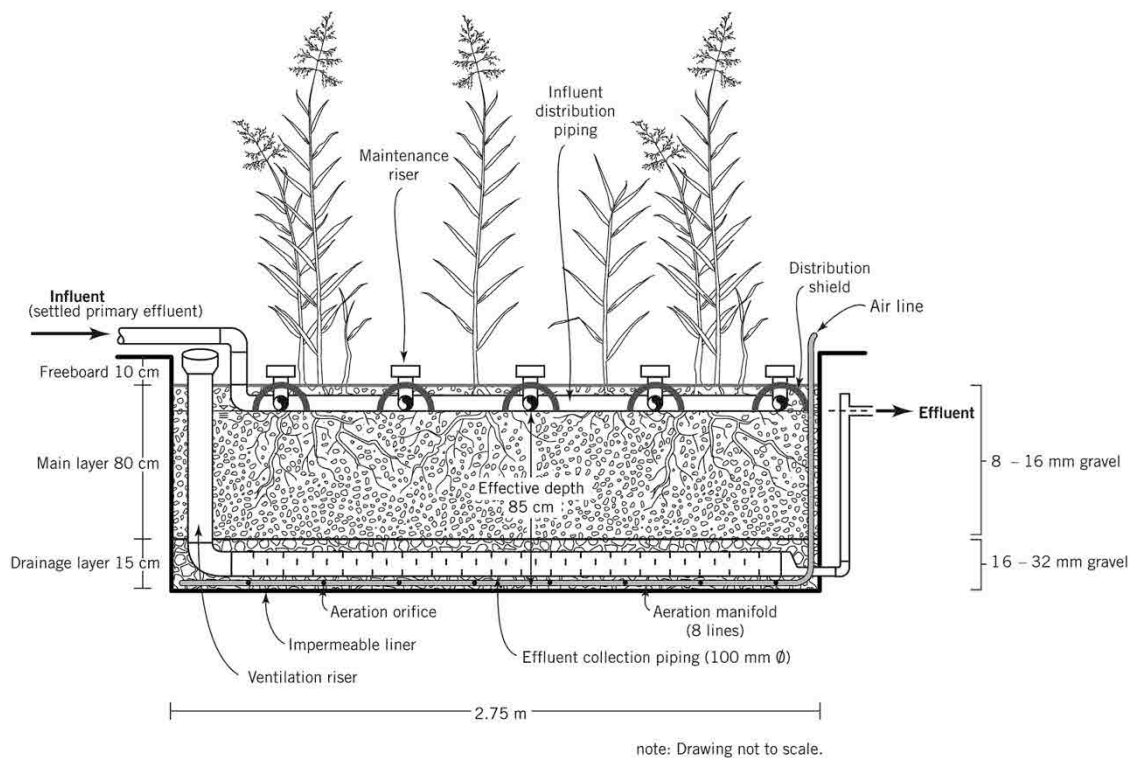


Figure 9.4. Profile view of the gravel-based saturated vertical subsurface flow constructed wetland with an integrated aeration system (VAp). Obtained from: Nivala et al. (2013).

Due to risk of freezing during winter, which could damage the distribution system, the wastewater could not be dosed to the surface of the beds. Influent distribution pipes were covered by a 10-cm layer of the main filter media (sand or gravel) over the top of the bed. The design modification included a setting with shields, which allowed for an enhanced distribution of the wastewater and increased surface area over which the wastewater is received. These pipes contains perforations spaced every 50 cm, as well as a single downwards-facing drainage hole so as to drain out remaining wastewater between loading events, and thus avoiding freezing and damage of the same (Wallace,

2000). Fig. 9.5 also shows the collection system at the bottom of the VF beds, which consists of 100 mm diameter perforated pipes, which are connected to a 100 mm ventilation riser. The outflow from each wetland bed returned to the main control building via gravity, for effluent flow measurement and sampling before it was discharged to the adjacent WWTP for final disposal.

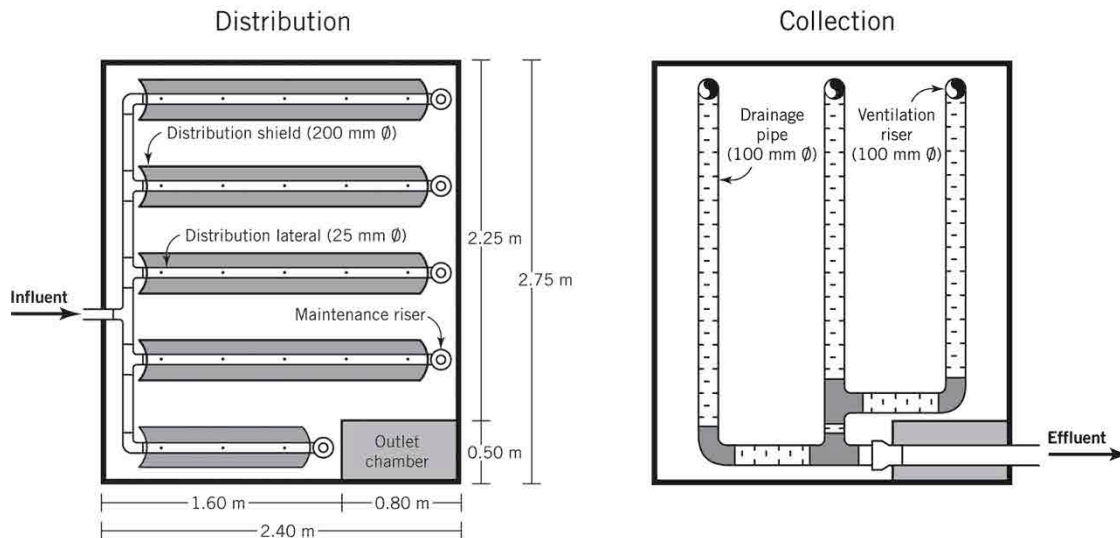


Figure 9.5. Plan view of the distribution and collection systems of all studied vertical flow constructed wetland variants (i.e. VS1p, VS2p, VGp, VAp). Obtained from: Nivala et al. (2013).

All systems were planted with *Phragmites australis* in September 2009 at a density of 5 plants per square meter. During the commissioning period, treatment units were fed with a nutrient solution consisting of tap water and a soluble plant fertilizer. The facility started operation with real raw wastewater in June 2010, and the treatment systems of this study were monitored from August to September 2010 as explained in Section 9.2.2. Total rainfall at the site during August 2010 was 130 mm, and mean air temperature was 18°C, ranging 9 to 30°C. However, due to the mode operation of VF, effect of dilution could be considered negligible since samples were taken right after a considerable volume of water was fed in a pulse. Although mean air temperatures differ a lot in the wintertime are sub-zero (min recorded T: -19°C), the only exposed pipes were thermally insulated to prevent the water from freezing, thus enabling year-round operation.

9.2.2. Sampling strategy

Sampling points included outlet of the septic tank (influent), and effluent of the different VF beds (VS1p, VS2p, VGp, VAp). The sampling campaign took place twice a week from August 3 to September 7 2010 (i.e. August 03, 05, 10, 12, 17, 19, 26, 31 and September 02, 07 2010). Grab samples ($n = 10$) were collected at the effluent of the VF beds at the time of a loading pulse so as to acquire fresh samples.

After onsite measurement of water temperature, E_H , DO, pH and EC within 3 hours after sampling, samples were brought back to Leipzig (UFZ) and analyzed immediately upon arrival. Influent and effluent water quality analyses included TSS, total organic carbon (TOC), TN, NH_4-N , nitrate nitrogen (NO_3-N) and nitrite nitrogen (NO_2-N).

Samples for EOCs were collected in 250 mL amber clean glass bottles, which were transported to the laboratory where they were stored at 4°C until analysis. The sample holding time was less than 24 h.

Target EOCs included various non-steroidal anti-inflammatory drugs, namely IB, ACE and DCF; various personal care products, including the musk fragrance AHTN, the sunscreen agent OXY, and the antiseptic TCS; and two endocrine disrupting compounds, the oral contraceptive EE2 and the widely used industrial chemical BPA. Samples were analyzed for organic micropollutants and conventional water quality parameters as described in Section 9.2.4.

9.2.3. Chemicals

GC grade (Suprasolv) hexane, ethyl acetate, methanol and acetone were obtained from Merck (Darmstadt, Germany) and analytical-grade hydrochloric acid was obtained from Panreac (Barcelona, Spain). Analytical grade ($\geq 98\%$) IB, DCF, ACE, AHTN, OXY, BPA, EE2, TCS and triphenylamine were purchased from Sigma-Aldrich (Steinheim, Germany). The 2,4-dichlorobenzoic acid was obtained from Riedel-de Haen (Seelze, Germany). 4-n-nonylphenol was obtained from Dr. Ehrenstorfer GmbH. (Augsburg, Germany). BPA-d14 was prepared by the research group of Dr. Monika Moder (UFZ, Leipzig). β -estradiol 17-acetate was purchased from ICN Biomedicals Inc. (Aurora, Ohio). Antipyrine-d3 was obtained from CDN Isotopes (Quebec, Canada). Trimethylsulfonium hydroxide (TMSH) was supplied from Fluka (Buchs, Switzerland) and 0.7 μm glass fiber filters of $\phi = 47$ mm (GF/F) were purchased from Whatman.

9.2.4. Analytical methodology

E_H , DO, EC and water temperature were measured by using the multimeter WTW Multi 350i. pH values were measured using a pH-meter of the model WTW pH96. Analyses of remaining water quality parameters were carried out at the UBZ laboratory within 10 h after sampling. The content of TOC was determined according to the European Standard DIN EN 1484, using the Total Organic Carbon Analyzer TOC-V_{CSN} from Shimadzu. TN was determined according to the European Standard DIN EN 12660, using the Total Nitrogen Measuring Unit TNM-1 from Shimadzu. $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ concentrations were analyzed colorimetrically according to the German standards DIN 38 406 E5, DIN 38 405 D9 and DIN 38 405 D10, respectively. Test-kits from Merck were used for $\text{NH}_4\text{-N}$ (Spectroquant test no. 114752) and test-kits from HACH-Lange for $\text{NO}_3\text{-N}$ (LCK 339/340) and $\text{NO}_2\text{-N}$ (LCK 341/342). The content of TSS was determined by following Standard Methods (APHA, 2001).

Concentrations of target EOCs were analyzed after samples had been filtered and processed following a previously described GC-MS methodology (Matamoros et al., 2005). The linearity range was from 0.2 to 10 mg L^{-1} . The correlation coefficients (R^2) of the calibration curves were always higher than 0.99. The limit of detection (LOD) and limit of quantification (LOQ) were compound dependent in the range from 0.6 to 189 ng L^{-1} and 8 to 216 ng L^{-1} , respectively. RSD was lower than 20% and recoveries were above 80%.

9.2.5. Statistical analysis

Experimental results were statistically evaluated using the SPSS 13 package (Chicago, IL). Data normality was checked with a Kolmogorov-Smirnoff test. Comparisons of differences in removal efficiencies between the different VF wetland variants for water quality parameters (i.e. TSS, TOC, TN, $\text{NH}_4\text{-N}$) and for target EOCs (i.e. IB, DCF, AHTN, OXY, TCS, BPA) were performed with parametric ANOVA tests and Tukey post-hoc tests. Differences were considered significant when $p < 0.05$.

9.3. Results and Discussion

9.3.1. Conventional water quality parameters

Table 9.3 shows average concentrations for water quality parameters at the influent and effluent of all treatment units. During the experimental period influent wastewater temperature was 19 ± 2 °C. Applied HLRs were 95 mm d^{-1} for all VF beds and average surface organic load ranged 7 to $16 \text{ g TOC m}^{-2} \text{ d}^{-1}$ (average of $12 \text{ g TOC m}^{-2} \text{ d}^{-1}$).

Table 9.3. Water quality parameters and target emerging organic contaminant concentrations (\pm s.d.) at the influent (septic tank outlet) and effluent of the different pilot-scale vertical flow wetland variants ($n = 10$). Removal efficiencies (%) are shown in parentheses. Sampling points: Influent: outlet of the septic tank; VS2p: bi-hourly fed sand-based VF; VS1p: hourly fed sand-based VF; VGp: gravel-based VF; VAp: aerated saturated VF.

	Influent	V2Sp	V1Sp	VGp	VAp
<i>Conventional water quality parameters</i>					
Water T (°C)	18.8 ± 1.8	18.8 ± 2.0	18.8 ± 2.0	18.7 ± 2.2	19.0 ± 2.0
pH	7.3 ± 0.1	6.6 ± 0.4	6.6 ± 0.6	7.2 ± 0.2	6.1 ± 0.7
Eh (mV)	-263 ± 45	155 ± 38	169 ± 36	98 ± 31	172 ± 40
DO (mg L^{-1})	0.2 ± 0.1	4.1 ± 1.1	5.5 ± 1.3	3.4 ± 1.0	5.2 ± 0.9
EC ($\mu\text{S cm}^{-1}$)	1555 ± 298	1259 ± 157	1240 ± 177	1320 ± 172	1197 ± 125
TSS (mg L^{-1})	96 ± 43	2 ± 2 (98%)	1 ± 1 (99%)	13 ± 6 (86%)	2 ± 2 (98%)
TOC (mg L^{-1})	125 ± 36	16 ± 2 (86%)	13 ± 2 (89%)	26 ± 6 (78%)	14 ± 1 (88%)
TN (mg L^{-1})	63 ± 13	44 ± 9 (29%)	42 ± 10 (33%)	40 ± 4 (36%)	45 ± 9 (29%)
NH ₄ -N (mg L^{-1})	45.7 ± 9.7	1.0 ± 0.5 (98%)	0.6 ± 0.5 (99%)	5.8 ± 2.1 (87%)	0.1 ± 0.1 (>99%)
NO _x -N (mg L^{-1})	0.6 ± 0.6	39.2 ± 5.7	37.5 ± 7.9	27.4 ± 4.8	42.3 ± 9.7
<i>Emerging organic contaminants (in $\mu\text{g L}^{-1}$)</i>					
Ibuprofen	44.5 ± 13.0	0.44 ± 0.22	0.14 ± 0.05	2.43 ± 0.95	0.20 ± 0.05
Diclofenac	5.58 ± 2.95	1.66 ± 0.68	2.55 ± 0.64	1.93 ± 0.49	2.37 ± 0.50
Acetaminophen	<LOD	<LOD	<LOD	<LOD	<LOD
Tonalide	0.18 ± 0.02	0.06 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.03 ± 0.00
Oxybenzone	2.58 ± 1.74	0.07 ± 0.03	0.10 ± 0.07	0.29 ± 0.08	0.24 ± 0.05
Bisphenol A	2.80 ± 1.32	0.77 ± 0.33	0.14 ± 0.11	1.20 ± 0.33	0.06 ± 0.03
Triclosan	0.44 ± 0.15	0.06 ± 0.01	0.05 ± 0.01	0.12 ± 0.01	0.06 ± 0.01
Ethinylestradiol	<LOD	<LOD	<LOD	<LOD	<LOD

Fig. 9.6 shows DO profiles for influent and effluent of each VF unit, measured right after a loading event, during the experimental period.

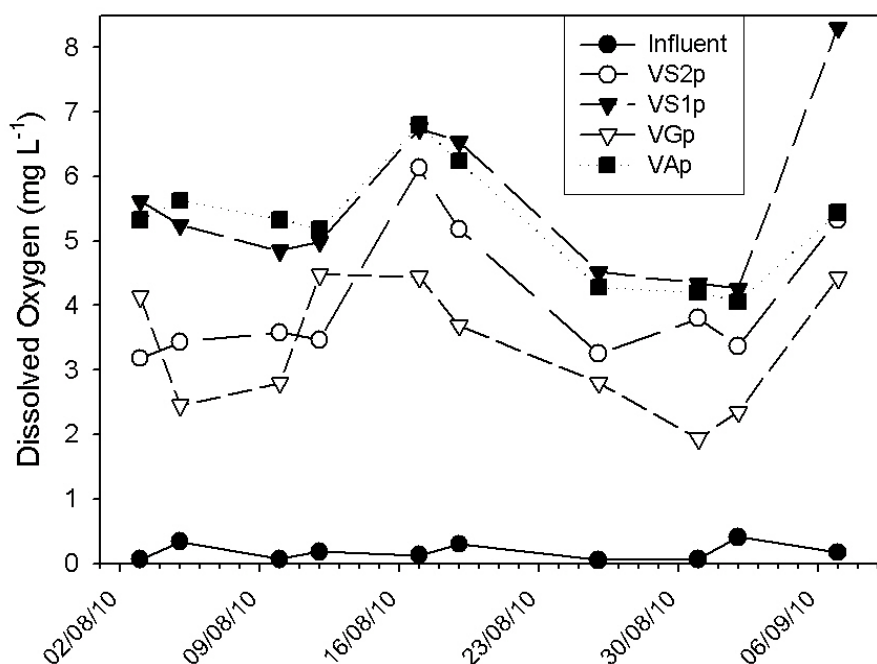


Figure 9.6. Dissolved oxygen concentrations at the influent and the effluent of the different vertical flow constructed wetland variants during the experimental period. Influent: outlet of the septic tank; VS2p: bi-hourly fed sand-based VF; VS1p: hourly fed sand-based VF; VGp: gravel-based VF; VAp: aerated saturated VF wetland.

Similar high values (above 5 mg L⁻¹) were found for the hourly-operated sand-based (VS1) wetland and the saturated-aerated one (VAp). Oxygenation of the water was slightly lower in the bi-hourly sand-based wetland (VS2p), with values around 4 mg L⁻¹, which could be explained by a reduced retention time due to the higher volume of the dose if compared to VS1p (hourly loading). DO values were consistently lower in the gravel-based wetland (VGp), with average values of about 3.4 mg L⁻¹. Redox values reflected the same tendency. These rapidly recuperated from -263 mV in the influent up to fairly oxidized conditions in all VF CWs. However, sand-based (VS2p and VS1p) and the aerated saturated (VAp) VFs seemed to provide a higher redox status (from +155 to +172 mV) than the gravel-based VF wetland (+98 mV).

Removal rates for organic and NH₄-N were in general very high, being consistent with the prevailing oxidized conditions of the filters, suggesting that aerobic pathways are predominating (Brix and Arias, 2005). Significantly lower entrapment of TSS and NH₄-N removal was found at the gravel-based VF (86% TSS and 87% NH₄-N), in comparison with the other three VF units (>98% for both parameters) ($p < 0.05$). Removal of TOC was also lower at the gravel-based wetland (78%) as compared to the sand-based (89%). In general, removal rates for the sand-based systems are in accordance with those observed for NH₄-N and BOD₅ (about 98%) in a 5-m² pilot-scale VF CW of similar characteristics (Matamoros et al., 2007), regardless of the applied HLR (i.e. 13, 30, 70, 160 mm d⁻¹).

The greater performance of the sand-based VFs compared to the gravel-based VF wetland could be owed to the smaller grain size of sand. This results in smaller pores, hence providing a better filtering capacity, a higher surface area for biofilm growth and a longer hydraulic retention time. The higher surface area per unit volume of media in sand-based filters would also enhance oxygen diffusion due to a larger air-water interface (Lahav et al., 2001), which in turn would enhance microbial reactions. In fact, higher oxygen consumption rates were found in this experimental plant in sand-based when

compared to gravel-based units (Nivala et al., 2012b), indicating higher microbial activities (and consequently higher nitrifying bacteria activity).

The reduction of TN was in general low, ranging- 29-33%, typical for this type of CW configuration, where low denitrification activity is expected. Although apparently a lower TN removal seemed to take place in sand-based units (VS2 and VS1) and aerated wetland (VAp) as compared to the gravel-based wetland (VGp), differences were not statistically significant. However, Nivala et al. (2012b) reported the same trend in this treatment plant when monitored over a longer period of time and attributed this to the fact that lower oxygen availability in gravel-based wetlands would promote a higher denitrification activity. On the other hand, in the present study, the loading frequency and the use of active aeration did not show any statistically significant effect on the removal of any of the studied water quality parameters.

9.3.2. Emerging organic contaminants

9.3.2.1. Background concentrations and overall removal efficiencies

Fig. 9.7 shows average concentrations of studied EOCs at the influent of the VF beds (effluent of septic-tank). These ranged 27.7-64.2, 1.74-9.86, 1.2-6.2, 1.0-4.8, 0.23-0.71 and 0.13-0.20 $\mu\text{g L}^{-1}$ for IB, DCF, OXY, BPA, TCS and AHTN, respectively. These were in the range of those reported at other studies for all target contaminants, except for the anti-inflammatory drug DCF and the sunscreen agent OXY, whose concentrations were significantly higher than those typically found at the influent of WWTPs at various countries (Clara et al., 2005; Miège et al., 2006; Matamoros et al., 2007; Kasprzyk-Hordern et al., 2009; Kosma et al., 2010; Ávila et al., 2013b). The analgesic ACE and the synthetic estrogen EE2 were found below the limit of detection in all influent samples.

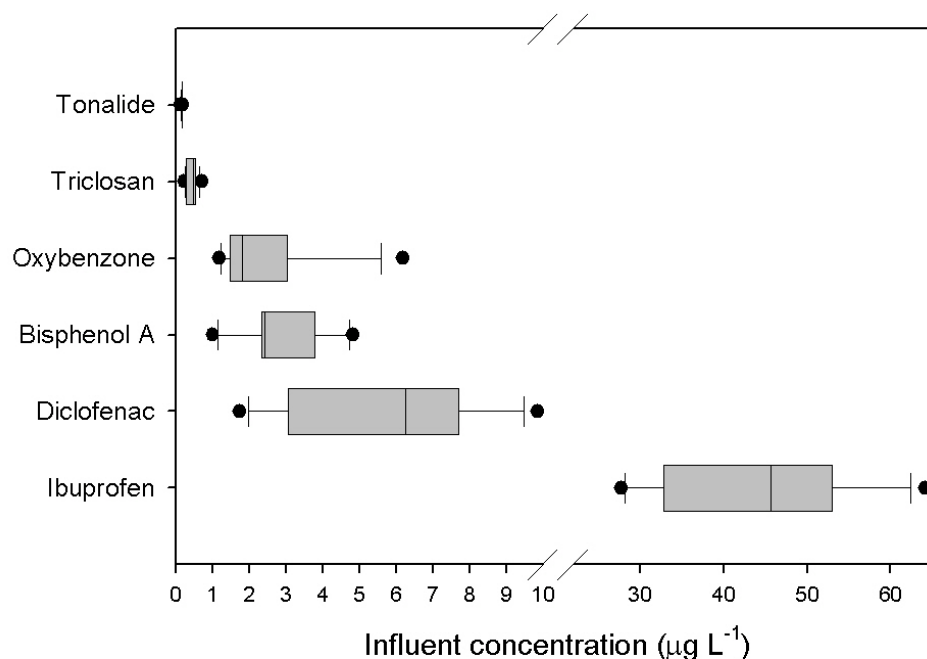


Figure 9.7. Box plot of target emerging organic contaminant concentrations in the influent wastewater (effluent of the septic tank) during the experimental period ($n = 10$) in the experimental facility in Langenreichenbach, Germany.

Fig. 9.8 shows average removal efficiencies achieved at the different VF units. Target EOCs were grouped in relation to their removal efficiency into (i) very efficiently removed, that is >95% removal in at least one of the systems (IB, OXY, BPA); and (ii) those moderately removed, which include removals from 55 to 90% (AHTN, TCS, DCF). In general, removal efficiencies were very high, as previously observed in this type of CWs. However, it is important to note that this experiment was carried out during the summer season and further campaigns should be hold in winter season to evaluate possible decrease in treatment performance at low temperatures. The anti-inflammatory drug IB has been reported to be well biodegraded under aerobic-prevailing conditions in unsaturated VF wetlands. Matamoros et al. (2007) found removal rates of 99% in a pilot-scale VF wetland operating at various HLRs up to 160 mm/d. Slightly smaller removal rates (89%) were observed at various household VF systems in Denmark operating at 30 mm/d (Matamoros et al., 2007). Moreover, Ávila et al. (2013a) found lower removal efficiencies of this substance at an experimental VF wetland, yet HLRs were fairly higher (63, 53 and 48% for 60, 130 and 180 mm d⁻¹). Removal of OXY was also very high and averaged 93%, which is in conformity with values observed by Matamoros et al. (2007, 2009a). Ávila et al. (2013a) found an average of 89% OXY removal in VF beds alternating operation, but its elimination did not show dependence on the HLR. The removal of the industrial chemical BPA was, however, very variable and differed a lot between treatment systems (57-98%). These were in accordance with those reported by Ávila et al. (2013a), ranging 69-80%. Average removal of the musk fragrance AHTN was of 71%, and showed to depend much on the wetland design and operational parameters, ranging from 63 to 80%. These rates are in the range of other studies in VF beds (Matamoros et al., 2007; Ávila et al., 2013a). The elimination of the antiseptic TCS was generally high (average of 84%). Its removal has been found to depend on operating conditions, as it was also reported by Ávila et al. (2013a) in a VF bed, who found how its elimination decreased as the HLR increased (85, 79 and 71% for 60, 130 and 180 mm/d, respectively). Finally, the removal of DCF ranged from 54 to 70%, depending on the VF bed. These were in the range of those reported by Matamoros et al. (2007) and Ávila et al. (2013a).

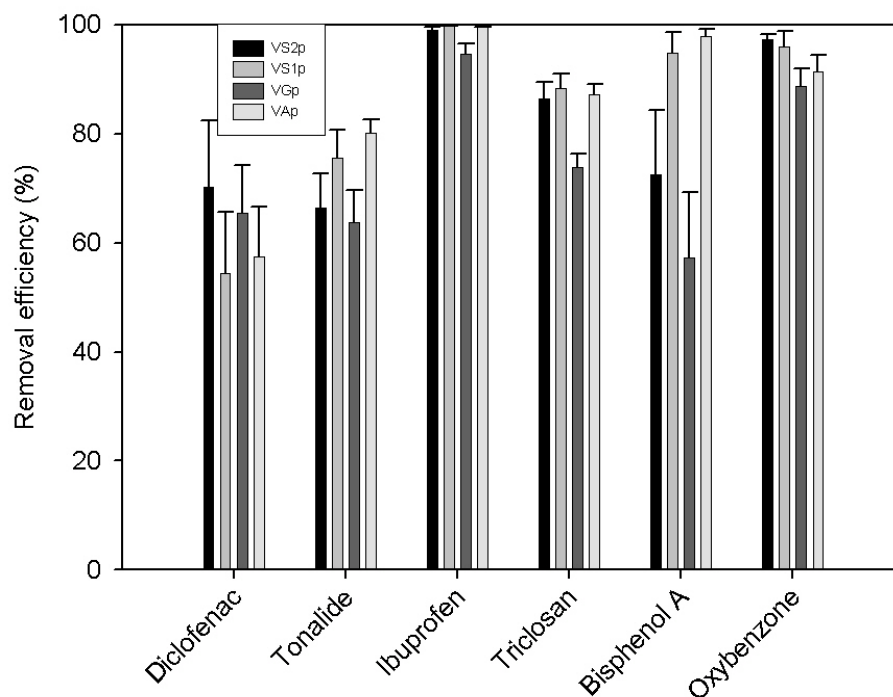


Figure 9.8. Removal efficiencies (\pm sd) achieved in the different pilot-scale vertical flow wetland variants on the removal of target organic micropollutants (n=10). VS2p: bi-hourly fed sand-based; VS1p: hourly fed sand-based; VGp: gravel-based; VAp: aerated saturated.

9.3.2.2. Effects of loading frequency

For identical HLRs, the bi-hourly sand-based VF wetland (VS2p) received double the volume of wastewater per pulse (8 mm/pulse) than the hourly-based one (4 mm/pulse) (VS1p). In this way, a higher volume of batch dosed at lower feeding frequencies (VS2p) should favor hydraulics, oxygen transfer into the media due to diffusion and mass convection, as well as lower residual water content. However, if the volume is too high, it could lead to decreased removal efficiency due to reduced contact time between the water and the biomass. On the other hand, lower volumes at higher frequencies could enhance treatment efficiency, but if loading frequency is too high oxygen renewal occurring between pulses could be limited, thus affecting removal processes such as nitrification. Differences could be more notable in the long-term (Molle et al., 2006; Torrens et al., 2009).

Although the two VF wetlands (VS2p and VS1p) performed equally well in terms of water quality parameters, statistically significant differences ($p < 0.05$) were found for DCF, AHTN and BPA. In general, similar conditions were found at these two wetland units, except for E_H and overall DO values, which were lower in the bi-hourly fed system (Fig. 9.6). This suggests that the higher velocity of the water and reduced contact time at higher loading volumes would decrease oxygen renewal during the feeding pulse, hence resulting in lower treatment efficiencies. The lower contact time of the water in VS2p showed to be detrimental for the removal of AHTN (66 ± 6 and $76 \pm 5\%$ in VS2p and VS1p, respectively), as well as for other hydrophobic substances that get primarily removed by sorption onto organic matter. In fact, very similar AHTN removal values were observed in VS2p if compared to the gravel-based VF (VGp), where particle retention is expected to be lower than in sand-based (VS1p). On the other hand, even greater differences were found for BPA (72 ± 12 and $95 \pm 4\%$ in VS2p and VS1p, respectively), whose elimination has been attributed to occur through biodegradation enhanced at aerobic conditions as well as possible sorption onto particulate matter (Ávila et al., 2010, 2013a,b). However, other presumably redox-sensitive species such as TCS (Chapters 5 and 8) did not show significant differences between these two modes of operation, suggesting that differences in EOC removal rates found at the different two treatment types were also influenced by the kinetics of each pollutant degradation (Matamoros et al., 2007; Ávila et al., 2013b). In that way, while some substances would be rapidly degraded as soon as more oxidized conditions are available, others would need a longer retention time within the media. On the contrary, DCF showed the opposite trend than the other compounds, exhibiting higher removal in the bi-hourly fed VF ($70 \pm 12\%$) as opposed to the hourly-fed one ($54 \pm 11\%$). Note that although without statistical significance, removal efficiencies of DCF were also higher (like those of VS2p) at the less oxygenated gravel-based wetland (VGp), but however similarly low at the well-aerated VAp. Although in general high redox conditions have been found to enhance DCF removal in CWs (Matamoros and Bayona, 2006; Matamoros et al., 2007, 2009a; Ávila et al., 2013a,b), other studies, which show high removal efficiencies in HF wetlands operating at very low redox and DO status (Chapters 3 and 8) have suggested that alternative anaerobic processes, such as reductive dehalogenation under anoxic conditions might also actively participate in the elimination of this compound within CWs. Indeed, it seems like the combination of different removal pathways taking place through aerobic and anaerobic microbial reactions occurring at various wetland microenvironments may enhance the removal of this and other compounds (Quintana et al., 2005; Park et al., 2009; Hijosa-Valsero et al., 2010a).

In general, the loading frequency is an important operational parameter to guarantee the good treatment performance of this type of treatment systems, where sufficient oxygen renewal and mineralization of the organic matter should be allowed so as to avoid clogging of the filter. Applied HLRs and potential accumulation of solid deposits on the bed surface should be carefully supervised and controlled, especially in non-rested VF wetlands, so as to avoid filter clogging and surface ponding, which would eventually decrease the efficiency and lifetime of the treatment system (Platzer and Mauch, 1997; Langergraber et al., 2003).

9.3.2.3. Effects of media size

The effect of the media size was however more remarkable. The gravel-based VF (VGp) wetland performed significantly worse ($p < 0.05$) than the sand-based hourly-fed wetland (VS1p) in the elimination of all studied EOCs, except for DCF. Differences were especially important for BPA (95 vs. 57% for VS1p and VGp, respectively), TCS (88 vs. 74%) and AHTN (76 vs. 63%).

As previously observed, redox and DO conditions were significantly lower in the gravel-based VF compared to the sand-based (5.5 and 3.4 mg L⁻¹ DO in VS1p and VGp, respectively). In gravel-based VFs, lower HRT together with a lower filtering capacity due to higher pores between grains would result in reduced treatment efficiency. In fact, it has been observed that a rapid flow downwards does not favor ammonia sorption onto the gravel bed, which might eventually decrease nitrification activity (Molle et al., 2006; Torrens et al., 2009). The lower surface area per unit volume in gravel-based as compared to sand-based VFs, provided by a larger grain size, reduces oxygen diffusion and entrapment of solids, and may provide insufficient contact time between the biofilm and pollutants. On the other hand, in sand-based VFs, the higher surface area for biofilm attachment and higher oxygen availability may promote the existence or larger biomass and more diverse microbial communities. In fact, higher microbial biomass and activities have been found to take place in the upper part (about first 10 cm of a 50 cm VF) of the filter bed (Tietz et al., 2007).

In general, sand-based VF wetlands have shown to perform substantially better than gravel-based wetlands, due to conditions provided by a small grain size. Increased HRT together with higher oxygen availability and redox conditions would promote the elimination of substances whose removal is majorly achieved through aerobic biodegradation (BPA, TCS, OXY, IB), as well as those -typically hydrophobic ones- which are mainly removed by sorption onto the particulate matter (AHTN). This is in accordance with previous literature which shows similar behavior of these substances in VF beds (Matamoros et al., 2007, 2009a; Chapters 5 and 8).

However, it is to note that these experiments were carried out at the beginning of the systems functioning with real wastewater, when the systems were still not mature. For this reason, further observations should be made at a later stage so as to assess the development of biomass and treatment performance in these two wetland types (gravel vs. sand). Although higher elimination rates were found at sand-based systems, their long-term operation may make them more vulnerable to clogging. For that reason, grain size as well as loading frequency should be carefully selected, as well as HLRs well controlled, in order to ensure that enough oxygen renewal and mineralization of organic matter takes place so that no clogging develops in the filter beds (Platzer and Mauch, 1997; Cooper, 2005; Kayser and Kunst, 2005).

9.3.2.4. Effects of active aeration (under saturated conditions)

In general, the passive unsaturated typical VF bed (VS1p) and the actively aerated saturated VF bed (VAp) performed in a very similar way throughout the whole experimental period. The two VF wetland variants exhibited almost identical effluent water quality for all studied EOCs as well as conventional water quality parameters. DO and E_H conditions were identical. The removal of OXY was the only substance whose removal seemed to depend on the use of aeration. In this way, its degradation was significantly lower in the VAp than in the VS1p. Hence, unlike in HF wetlands, the use of active aeration in the saturated-VF bed did not seem to improve any aspect of treatment performance in comparison with the typical unsaturated VF.

9.4. Conclusions

Three pilot-scale unsaturated VF wetland units (6.2 m²) differing in grain size and loading frequency, as well as an intensified VF unit with active aeration working under saturated conditions, receiving wastewater from the municipality of Langenreichenbach (Germany), proved to be very efficient on the removal of various EOCs, consisting mainly of pharmaceuticals and personal care products. The hydraulic loading rate was 95 mm/d for all vertical flow variants and surface organic load ranged 7 to 16 g TOC m⁻² d⁻¹.

In general, sand-based VF wetlands showed to perform substantially better than gravel-based wetlands on the removal of water quality parameters as well as most studied EOCs, due to conditions provided by a small grain size. At the sand-based type, increased hydraulic retention time together with higher oxygen availability and redox conditions would promote the elimination of substances whose removal is majorly achieved through aerobic biodegradation (BPA, TCS, OXY, IB), as well as those –typically hydrophobic ones– which are mainly removed by sorption onto the particulate matter (AHTN).

Moreover, whereas the loading frequency (hourly vs. bi-hourly) did not show to affect the removal of conventional water quality parameters, lower E_H and DO values were yet occurring at the bi-hourly fed. In fact, decreasing loading frequency (and thus higher volume of the pulse) significantly affected the removal of DCF, AHTN and BPA removal. This suggests that the higher velocity of the infiltrating water and reduced contact time at higher loading volumes would decrease oxygen renewal as well as particle entrapment, hence resulting in lower treatment efficiencies (AHTN, BPA). However, the removal of DCF was the only contaminant showing an opposite trend to the rest of compounds, achieving higher elimination rates at the less oxidized wetlands (bihourly and gravel-based VFs). Presumably, the coexistence of various microenvironments would allow for the combination of various anaerobic and aerobic degradation pathways to take place within these wetland units, which could be important for an improved removal of some contaminants. Moreover, unlike in horizontal flow wetland type, the use of active aeration in a saturated-VF bed did not seem to improve any aspect of treatment performance in comparison with the typical passive unsaturated VF wetland.

Although higher elimination rates were found at sand-based systems, their long-term operation may make them more vulnerable to clogging, and thus gravel-based systems may satisfy treatment needs when good quality sand is not available. In general, studies should be carried out at a more mature stage of the treatment plant to evaluate potential clogging development. In practice, grain size as well as loading frequency should be carefully selected, as well as HLRs controlled, in order to ensure that enough oxygen renewal and mineralization of organic matter takes place so that no clogging develops in the filter beds.

10. General discussion

The current chapter discusses the different aspects held along the previous ones, which correspond to the objectives of the thesis. The general discussion is structured in four points which aim at giving response to the specific objectives of the thesis exposed in Chapter 2. It is important to note that all studied CW systems were operated with real wastewater. However, a continuous injection of EOCs was performed in the experimental treatment systems (Chapters 3, 4, 6).

10.1. Behavior of emerging organic contaminants in horizontal subsurface flow constructed wetlands

Aspects regarding the first, second and third objective of the thesis are discussed in this section, which explain the behavior of EOCs in HF wetlands, as well as the effects of type of primary treatment, and the operation strategy on EOC removal. The two experiments developed within the HF wetland type were carried out in an experimental mesoscale HF CW system consisting of three different treatment lines. This was located at the facilities of the GEMMA group (Universitat Politècnica de Catalunya-BarcelonaTech), where a Mediterranean climate prevails. The three treatment lines were differentiated on one hand by the primary treatment applied (HUSB vs. conventional settler), and on the other hand by the loading strategy (alternation of saturated-unsaturated conditions vs. permanently saturated). For ease of understanding these were called 'anaerobic' (HUSB, continuous operation), 'control' (conventional settler, continuous operation), and 'batch' (conventional settler, operation in cycles of feed and rest).

In both of the experiments carried out in this treatment plant, continuous injections of EOCs were performed. The first assay was carried out during spring (May 2009) in only one of the treatment lines (Chapter 3) -the 'anaerobic line'-, while the second experiment was conducted in winter (November 2009) in the three treatment lines at the same time (Chapter 4). The methodology of the continuous injection of EOCs proved to deliver results, which were stable overtime, which gives a good approximation of the behavior of these substances in the treatment system.

The capacity of HF wetlands for the removal of EOCs showed to be variable and compound-dependent. If we compare the anaerobic line in the two experiments, overall removal rates were much higher during the spring season than in the winter season campaign (Fig. 10.1). It is to note that injected concentrations were very similar in the two campaigns.

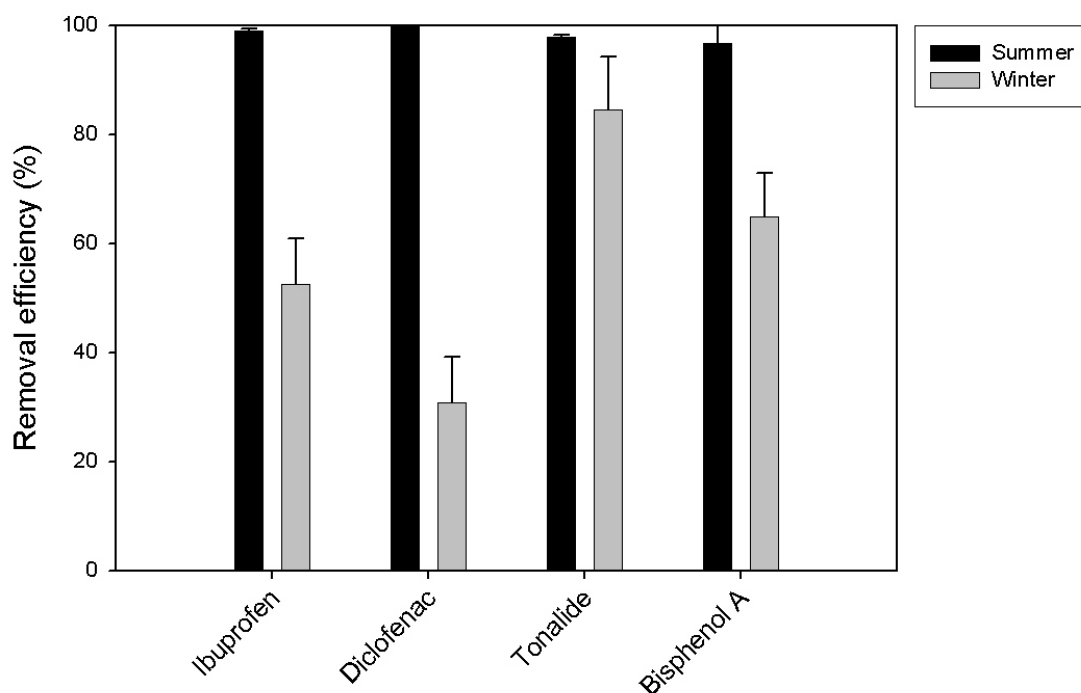


Figure 10.1. Removal efficiency of several studied emerging organic contaminants in the anaerobic line of the experimental horizontal subsurface flow constructed wetland system during summer and winter seasons.

HF wetlands display seasonal effects for EOCs removal, with lower removal efficiencies during the cold season at lower water temperatures. Similar seasonal patterns have been observed at almost identical HF wetland mesocosms in León (NW Spain) (Hijosa et al., 2010b) and by the same author at this same treatment system (Hijosa et al., 2011b) (with no injection of EOCs), being removal efficiencies consistently better in summer than in winter for almost all of the studied EOCs. Reyes-Contreras et al. (2012) observed similar trends at the treatment system in León.

It is generally known that microbial activity is highly influenced by temperature. Higher water temperatures were registered in the warm season (23°C) as opposed to the cold season (15°C). Microorganisms in wetlands usually reach their optimal activity at warm temperatures (15-25°C), especially nitrifying and proteolytic bacteria (Truu et al., 2009). The same trend has been found to occur for denitrifying bacteria (Spieles and Mitsch, 2000; Boulétreau et al., 2012). In this study, the elimination of EOCs has been found to depend on water temperature, being especially important for biodegradable compounds (e.g. IB, DCF, BPA). Moreover, since the vegetation was fully developed during the summer campaign, it could have also contributed to the removal of EOCs, either by plant assimilation, and to a higher extent by the transfer of oxygen to the rhizosphere (Brix, 1993; Tyroller et al., 2010). This should allow the creation of microenvironments with different redox conditions, which in turn would promote the development of microbial biofilms with functionally different respiration processes (Wiessner et al., 2005; Kadlec and Wallace, 2009).

In this way, it is also important to mention that during the time of the experiment carried out in the warm season, very high evapotranspiration rates were measured (about 40% in the first stage and up to 70% in the second stage) due to the high temperatures and to the occurrence of very dense plant biomass. To this respect, we believe that the effect of the scale of the experiment could have to some extent triggered the high evapotranspiration rates, which were especially high in the second stage of the system, where a lower oxygen demand occurred. These conditions would significantly be

responsible for the oxygenation of the HF wetland bed due to the diurnal fluctuation of the water table, and should eventually enhance EOC removal.

What is more, it is widely known that removal of most pollutants in CWs occur primarily due to microbial activity (García et al., 2010). In fact, the removal efficiency of EOCs in CWs is oftentimes higher than that achieved in conventional WWTPs, which could presumably be due to their higher microbial diversity occurring in wetland ecosystems (Kadlec and Wallace, 2009). CWs can be designed to favor a wide range of redox conditions, therefore enhancing a variety of biological processes and removal of multiple contaminants in the same CW bed. In turn, CW technology can be optimized if the factors influencing their performance are identified.

Generally, HF wetlands include a primary treatment step, which typically consists of a settler or a septic tank. The use of a HUSB reactor as opposed to a conventional settler has not shown to improve general treatment performance (Chapter 4). Although HUSB reactor may enhance the retention of TSS overtime, which could help avoiding clogging development in the HF bed (Pedescoll et al., 2011b), this confers low redox potentials to the water, which results in lower removal efficiency of water quality parameters and EOCs removal. Results of the current thesis show that the treatment line containing a HUSB reactor (anaerobic line) performed slightly worse than the one having a conventional settler (control line), as is depicted in Fig. 10.2.

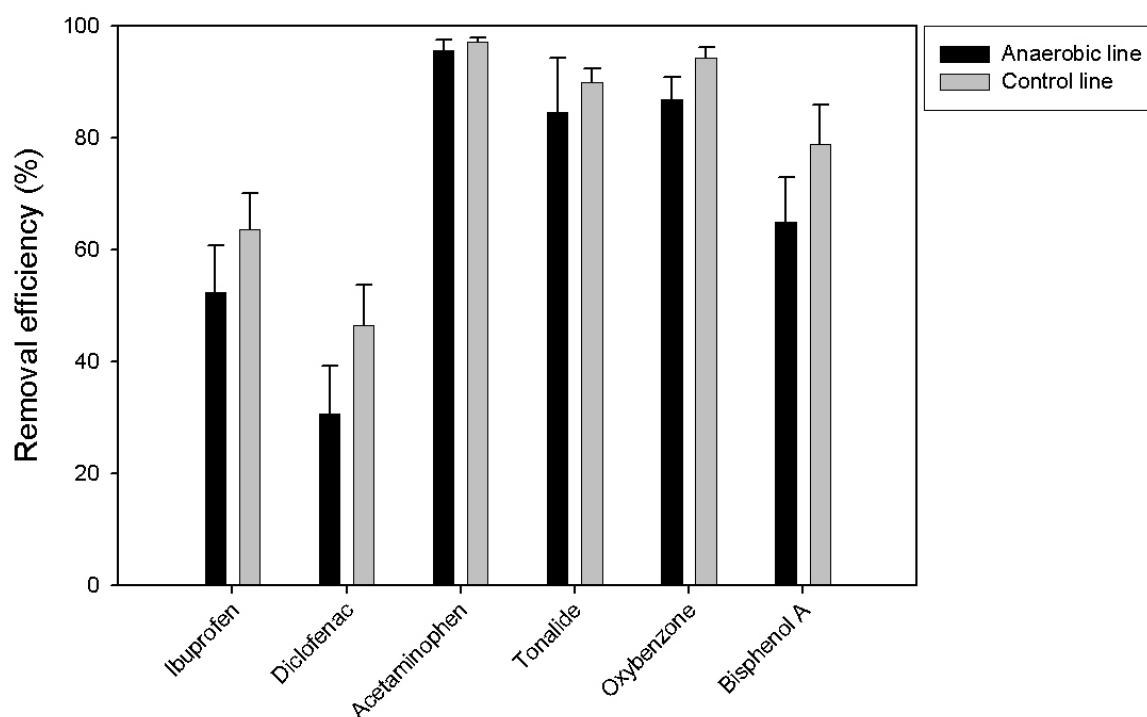


Figure 10.2. Removal efficiencies of target emerging organic contaminants as a function of the primary treatment: anaerobic line (HUSB reactor); control line (conventional settler) in horizontal subsurface flow constructed wetlands.

Nevertheless, it is important to note that in our experiments mostly polar compounds were studied, which have a low interaction with suspended solids (except for the musk fragrance AHTN). Therefore,

it might be possible that hydrophobic substances get more efficiently removed within these primary treatments where TSS retention is higher.

On the other hand, operating the HF wetlands in batch seems to be a very important factor promoting the elimination of EOCs. The alternation of phases of saturation and unsaturation promotes the existence of a higher redox status (average of -11 ± 32 mV) as compared to functioning under saturated conditions (-78 ± 42 mV), which in turn enhance significantly the elimination of the studied compounds. This can be seen in Fig. 10.3.

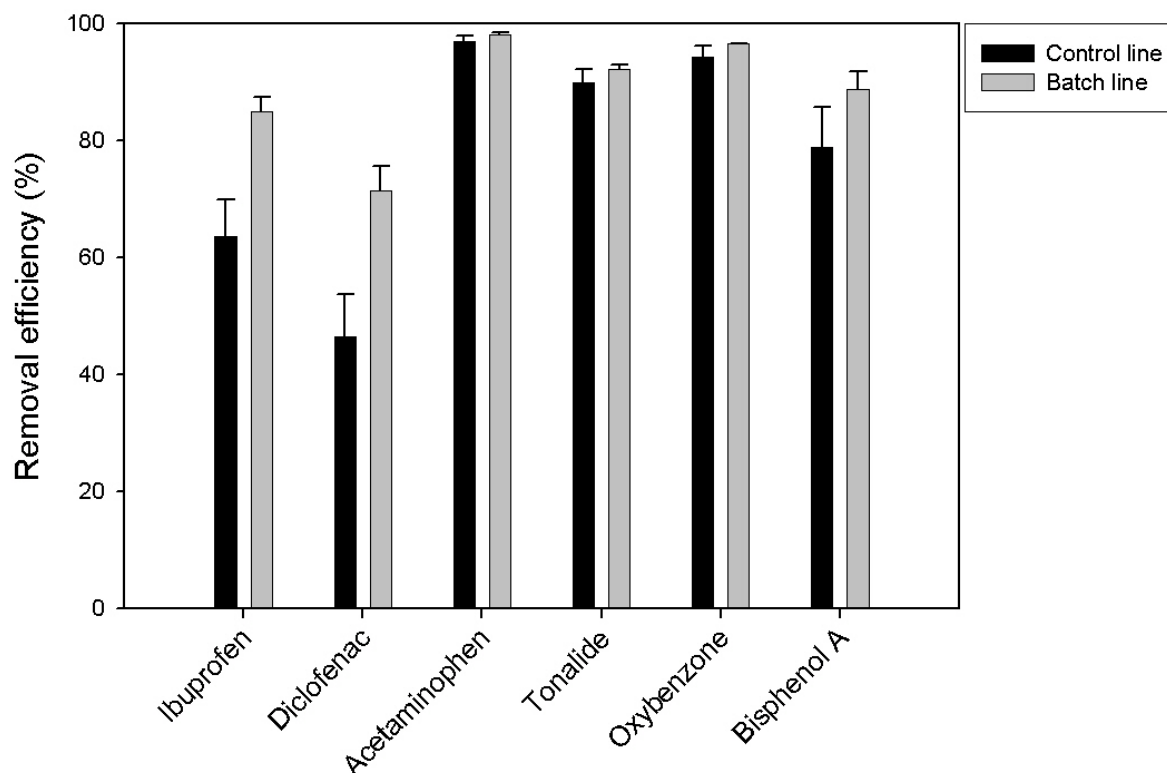


Figure 10.3. Removal efficiencies of target emerging organic contaminants as a function of the loading strategy (permanently saturated (control) vs. alternation of cycles of saturation-unsaturation (batch)) in horizontal subsurface flow constructed wetlands.

Similar results were observed by Zhang et al. (2012b), who found significantly better performance on EOC removal (e.g. DCF, IB, etc.) at experimental mesoscale HF beds operating in pulses than at those continuously fed. In general, the continuous injection experiments delivered very robust results that have shown how the occurrence of high redox potentials within the CW bed promotes the elimination of conventional quality parameters, as well as EOCs. In the present study, out of the 6 studied EOCs, 4 of them (i.e. IB, DCF, OXY, BPA) were found to be dependent ($p < 0.05$) on the redox status of the system. Substances whose major removal mechanism is thought to be biodegradation under aerobic conditions (i.e. IB, BPA) are those that behave most differently between the 3 treatment lines. Moreover, DCF, although previously reported to be recalcitrant in conventional WWTPs, or with variable removal efficiencies in HF wetlands, was found to also depend on more oxidized conditions. To this regard, the operation under cycles of saturation and unsaturation seems to be a good strategy to enhance the treatment performance of HF CWs, by promoting the fluctuation of redox conditions, where robust aerobic facultative biofilms can operate (Stein et al., 2003; Faulwetter et al., 2009).

Moreover, the tentative identification of an intermediate product of BPA in the two experiments, shed light into a possible majoritary removal mechanism for BPA. The higher occurrence of this metabolite in the wetlands containing the highest redox and oxygen values, suggests aerobic biodegradation as an important removal pathway for this compound. In this study is confirmed that CA-BPA is formed under higher redox potentials. Further attention should be paid to the biodegradation metabolites produced in the degradation pathway of EOCs, so as to get a better insight of the major processes involved in their removal.

Other design factors for HF wetlands, which were not an object of study of the current thesis are water depth, HLR, grain size and type of media. In particular, water depth seems to be a determining factor in this type of wetlands, which affects the redox conditions, oxygen supply and thus the treatment performance of the wetlands. Shallower wetlands appear to have higher redox potential and thus promote more variate and energetically favorable reactions, which in turn enhance the degradation of EOCs, especially biodegradable pharmaceutical compounds (Matamoros et al., 2005). Other authors have shown experimental evidence, which support this hypothesis (García et al., 2003a, 2005; Headley and Davison, 2005; Huang et al., 2005; Matamoros and Bayona, 2006).

However, one of the main limitations to the implementation of CWs is that they typically require high land area in comparison to conventional WWTPs, since they operate at relatively low HLRs to achieve efficient treatment performance (Fountoulakis et al., 2009). The influence of the HLR on removal efficiency of HF wetlands was evaluated for water quality parameters by García et al. (2005) in a pilot-scale HF CW system involving several wetland cells in parallel, where different HLRs were applied (0.03, 0.036 and 0.045 m d⁻¹). Higher removal rates of organic matter and NH₄-N were found at lower HLRs. More recently Zhang et al. (2012a) investigated six mesoscale HF wetlands fed with synthetic wastewater containing pharmaceuticals, operating at HLRs of 0.03 m d⁻¹ and 0.06 m d⁻¹, and observed very little differences between them. Moreover, Zhang et al. (2011) found that NSAIDs IB and NPX were removed at a higher rate under lower HLRs. Further studies should be done so as to address the capacity of HF wetlands for EOC removal at high HLRs.

In regards to the granular media of HF beds, in general a wide range of materials and sizes have been applied around the world, and its selection is in many cases dictated by the availability, price and local practices of a certain region. Some studies indicate the importance of using hard, durable and homogeneous materials that do not contain fine grains, which might clog the media (García and Corzo, 2008; Pedescoll et al., 2009). In general, finer materials (D₆₀ = 3.5 vs. 10 mm) have been recommended in HF beds when low organic loads are applied since they provide a greater surface area for microbial biofilms. Finer gravel promotes a higher growth of the vegetation and in conjunction a higher removal of pollutants such as ammonia (García et al., 2005). However, it is important to have in mind that the finer the material, the greater the risk of clogging and hydraulic problems. Moreover, although usually granite gravel is used in HF wetlands, other alternative sorbents have emerged so as to increase the adsorption capacity of these wetlands, such as light expanded clay aggregates (Dordio et al., 2009, 2010, 2011). This is expected to be especially important for hydrophobic compounds.

Finally, it is well known that in HF wetlands the oxygen demand exerted by the degradation of pollutants in wastewater exceeds the amount of oxygen available within the wetland bed. In general, subsurface oxygen limitation is one of the main rate-limiting factors for traditional HF wetlands (Brix and Schierup, 1990; Kadlec and Wallace, 2009; Tyroller et al., 2010). For that reason, other variants such as the use of active aeration are being developed and implemented worldwide and have shown to enhance the treatment performance of this type of CWs (Wallace, 2001; Nivala et al. 2013). However, this practice come at a heavy operational and maintenance cost, which is only justified if the improvement in the performance of the wetland offsets the cost of operation equipment and energy needs (Kadlec and Wallace, 2009; Nivala et al., 2012b).

In conclusion, temperature and sunlight variations are relevant factors to take into consideration for CW design and O&M, especially at places showing high seasonality. In general, it has been observed that the treatment performance of HF Cws on EOC removal is enhanced by the adoption of strategies that promote a higher redox status of the system. The use of a certain primary treatment, such as anaerobic reactors, so as to promote TSS retention, might be relevant for the removal of hydrophobic compounds, but in general lower contaminant removals are achieved due to lower redox conditions within the treatment line. On the other hand, operating HF wetlands in cycles of saturation and unsaturation stands as a very relevant strategy, which greatly enhances EOC removal, by promoting a higher redox status of the wetland bed. Water depth is also a critical factor influencing performance, being shallower wetlands of about 0.25-0.30 cm more efficient in EOC removal than deeper HF Cws.

10.2. Behavior of emerging organic contaminants in vertical subsurface flow constructed wetlands

Aspects regarding the first, and third objective of the thesis are discussed in this section, which explain the behavior of EOCs in VF wetlands, as well as the effects of loading frequency, grain size, and use of aeration on EOC removal (Chapter 9). The experiment developed within this CW type was carried out in four parallel pilot-scale VF beds located at the experimental facility of Langenreichenbach (east Germany). The VF units were intermittently fed, and these were differentiated by the loading frequency (hourly vs. bi-hourly fed), the media size (coarse sand (1-3 mm) vs. fine gravel (4-8 mm)), and the use of active aeration under saturated conditions.

The evaluation of the VF systems was conducted during a monitoring campaign carried out in summer 2010. During the time of the experiment, water temperature was 19° C and applied HLRs were 0.095 m d⁻¹. Average surface OLR ranged was 12 g TOC m⁻² d⁻¹.

The removal efficiency of EOCs was very high, and target contaminants were grouped in relation to their removal efficiency into (i) very efficiently removed, that is >95% removal (IB, OXY, BPA), and (ii) those moderately removed, which include removal from 55 to 90% (AHTN, TCS, DCF). These rates are in the range of other studies in VF wetlands Matamoros et al. (2007, 2009a). In general, it has been observed that VF wetlands are more efficient on the removal of EOCs than HF wetlands, which agrees with other laboratory studies which suggest that aerobic pathways are in general more efficient in the degradation of these substances than anaerobic ones (Zwiener and Frimmel, 2003; Conkle et al., 2012).

However, the relative importance of a particular degradation process in VF CWs can vary as a function of environmental factors, organic contaminant being treated, wetland configuration, and specific design and operational parameters. For example, in unsaturated intermittently-loaded VF wetlands (typical), diffusion and convection processes depend on the loading strategy, being affected by the number of pulses, the volume of the pulse, and the duration of surface filter dewatering (Molle et al., 2006; Torrens et al., 2009). The choice of the filter media (i.e. grain size, grain material, depth of layers) is also crucial, and should simultaneously satisfy treatment needs, while avoiding clogging of the bed and maintaining oxygen renewal in the media (Cooper, 2003; Kayser and Kunst, 2005). Moreover, the use of active aeration, which has been often successfully applied in HF wetlands (Wallace, 2001), has been recently been applied in saturated VF wetlands and this strategy should be evaluated.

In regards to the loading frequency, a higher volume of batch dosed at lower feeding frequencies should favor oxygen transfer into the media due to diffusion and mass convection. However, if the volume is too high, it could lead to decreased removal efficiency due to reduced contact time between

the water and the biomass. On the other hand, if the loading is done too frequent, the capacity of oxygen renewal occurring between pulses could be limited, thus affecting removal processes such as nitrification. To this respect, differences could be more notable in the long-term (Molle et al., 2006; Torrens et al., 2009). In our study, although the two VF wetlands performed equally well in terms of water quality parameters, statistically significant differences ($p < 0.05$) were found for some of the substances (DCF, AHTN and BPA). Removal efficiencies at these two VF beds are displayed in Fig. 10.4.

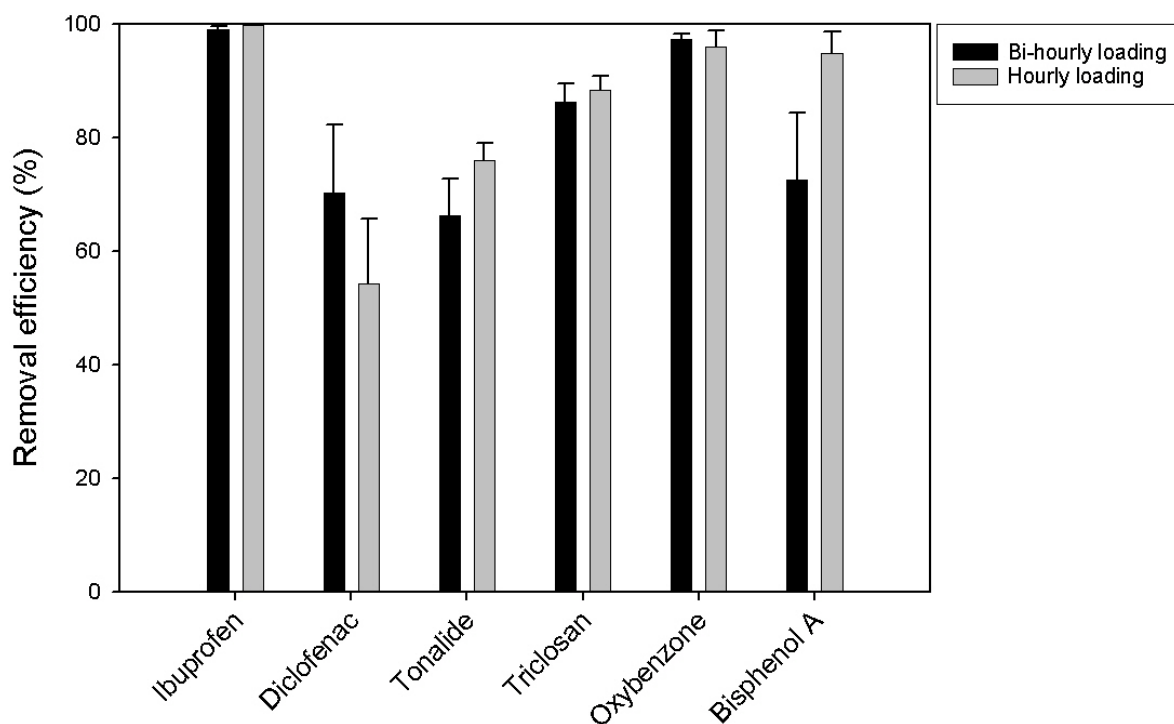


Figure 10.4. Removal efficiency of target emerging organic contaminants as a function of the loading frequency (hourly vs. bi-hourly) in vertical subsurface flow constructed wetlands.

In general, similar conditions were found at these two wetland units, except for redox potential and overall DO values, which were lower in the bi-hourly fed system. This suggests that the higher velocity of the infiltrating water and reduced contact time at higher loading volumes would decrease oxygen renewal during the feeding pulse, as well as particle entrapment capacity, hence resulting in lower treatment efficiencies. The possible lower contact time of the water in the bi-hourly fed VF wetland could be detrimental for the removal of hydrophobic substances (e.g. AHTN) that get primarily removed by sorption onto organic matter. On the other hand, greater differences were found for BPA, whose elimination has been attributed to occur through biodegradation enhanced at aerobic conditions as well as possible sorption onto particulate matter in SSF CWs (Chapters 3, 4, 6, 8). However, other presumably redox-sensitive species such as TCS (Chapter 4, Chapter 8) did not show significant differences between these two modes of operation, suggesting that differences in EOC removal rates found at the different two treatment types were also influenced by the kinetics of each pollutant degradation (Matamoros et al., 2007). In that way, while some substances would be rapidly degraded as soon as oxidizing conditions are available, others would need a longer retention time within the media.

On the contrary, DCF showed the opposite trend than the other compounds. Although in general high redox conditions have been found to enhance DCF removal in CWs (Chapters 4 and 6), other studies, which show high removal efficiencies in HF wetlands operating at very low redox and DO status (Chapter 3) have suggested that alternative anaerobic processes, such as reductive dehalogenation under anoxic conditions might also actively participate in the elimination of this compound within CWs. Indeed, it seems like the combination of different removal pathways taking place through aerobic and anaerobic microbial reactions occurring at various wetland microenvironments may enhance the removal of this and other compounds (Quintana et al., 2005; Amon et al., 2007; Park et al., 2009; Hijosa-Valsero et al., 2010a).

In general, the loading frequency is an important operational parameter to guarantee the good treatment performance of this type of treatment systems, where sufficient oxygen renewal and mineralization of the organic matter should be allowed. Applied HLRs and potential accumulation of solid deposits on the bed surface should be carefully supervised and controlled, especially in non-rested VF wetlands, so as to avoid filter clogging and surface ponding, which could eventually decrease the efficiency and lifetime of the treatment system (Platzer and Mauch, 1997; Langergraber et al., 2003).

On the other hand, the effect of the media size showed to be more remarkable. The gravel-based (4-8 mm) VF wetland performed significantly worse ($p < 0.05$) than the sand-based (1-3 mm) VF wetland in the elimination of all studied EOCs, except for DCF, as it can be seen in Fig. 10.5. Differences were especially important for BPA, TCS and AHTN.

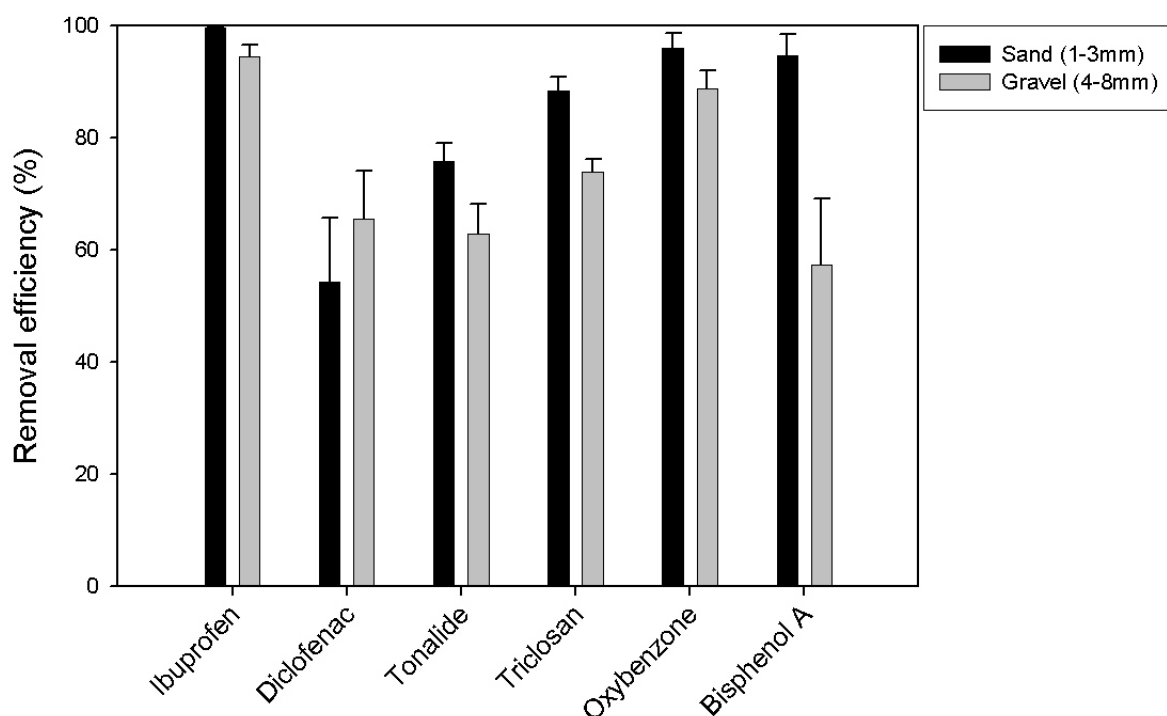


Figure 10.5. Removal efficiency of target emerging organic contaminants as a function of the grain size (coarse sand: 1-3 mm; fine gravel: 4-8 mm) in vertical subsurface flow constructed wetlands.

The greater performance of the sand-based VF beds compared to the gravel-based VF wetland can be attributed to the smaller grain size of sand. This results in smaller pores, hence providing a better filtering capacity, a higher surface area for biofilm growth and a longer contact time. The higher

surface area per unit volume of media in sand-based filters would also enhance oxygen diffusion due to a larger air-water interface (Lahav et al., 2001), which in turn would enhance microbial reactions. In fact, higher oxygen consumption rates were found in this experimental plant in sand-based when compared to gravel-based units (Nivala et al., 2012b), indicating higher microbial activities. E_H and DO values were significantly lower in the gravel-based VF wetland compared to the sand-based (3.4 and 5.5 mg L⁻¹ DO, respectively).

In other words, in gravel-based VF wetlands, lower contact together with a lower solids retention capacity due to higher pores between grains would result in reduced treatment efficiency. In fact, it has been observed that a rapid flow downwards does not favor ammonia sorption onto the gravel bed, which might eventually decrease nitrification activity (Molle et al., 2006; Torrens et al., 2009). The lower surface area per unit volume in gravel-based as compared to sand-based VF beds, provided by a larger grain size, reduces oxygen diffusion and entrapment of solids, and may provide insufficient contact time between the biofilm and pollutants. On the other hand, in sand-based VFs, the higher surface area for biofilm attachment and higher oxygen availability may promote the existence or larger biomass and more diverse microbial communities. In fact, higher microbial biomass and activities have been found to take place in the upper part (about first 10 cm of a 50 cm VF) of a VF wetland (Tietz et al., 2007). Due to this, sand-based VF wetlands have shown to promote the elimination of substances whose removal is majorly achieved through aerobic biodegradation (BPA, TCS, OXY, IB), as well as those, typically hydrophobic ones, which are mainly removed by sorption onto the particulate matter (AHTN).

However, it is to note that these experiments were carried out at the beginning of the systems functioning with real wastewater, when the systems were still not mature. For this reason, further observations should be made at a later stage so as to assess the development of biomass and treatment performance in these VF wetlands. Although higher elimination rates were found at sand-based systems, their long-term operation may make them more vulnerable to clogging. For that reason, grain size as well as loading frequency should be carefully selected, as well as HLRs well controlled, in order to ensure that enough oxygen renewal and mineralization of organic matter takes place so that no clogging develops in the filter beds (Platzer and Mauch, 1997; Cooper, 2005; Kayser and Kunst, 2005).

Moreover, the use of active aeration in a saturated-VF bed did not seem to improve any aspect of treatment performance in respect to the typical passive unsaturated sand-based VF wetland (Fig. 10.6). Similar DO (above 5 mg L⁻¹) and E_H values were observed between the two wetland units.

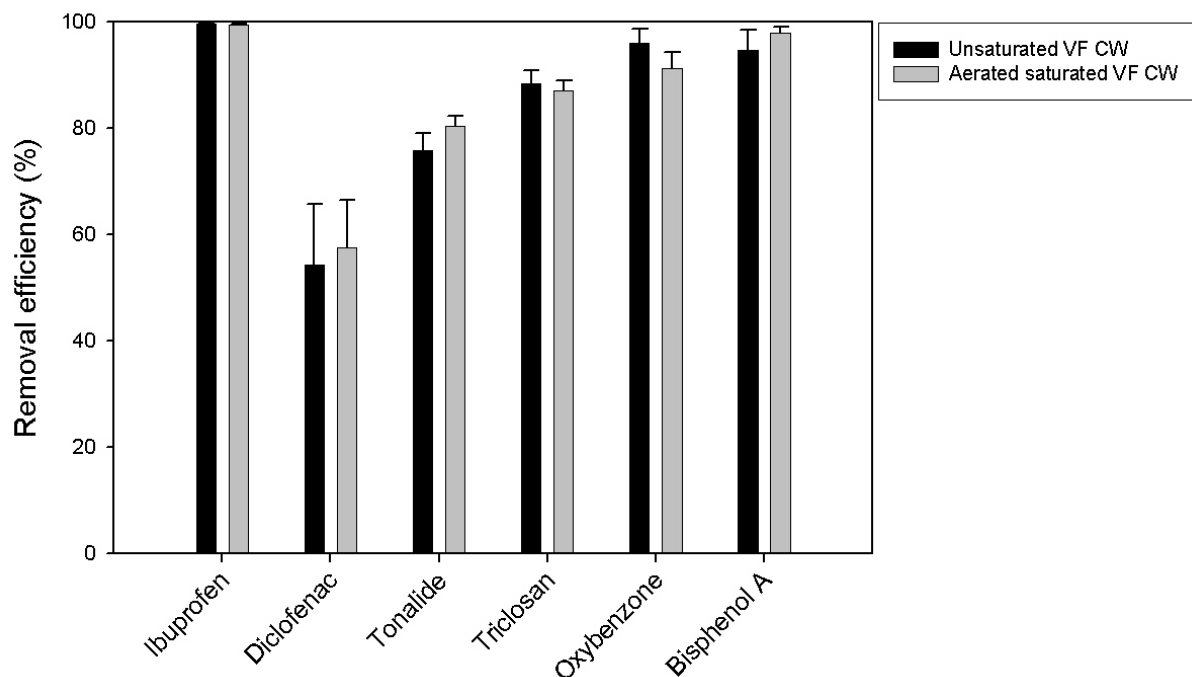


Figure 10.6. Comparison of the removal efficiency of emerging organic contaminants of a typical sand-based vertical subsurface flow constructed wetland and an actively aerated saturated vertical subsurface flow wetland.

The two VF wetland variants exhibited almost identical effluent water quality for all studied EOCs as well as conventional water quality parameters. The removal of OXY was the only substance whose removal seemed to depend on the use of aeration. In this way, its degradation was significantly lower in the aerated VF wetland. In general, as opposed to what has been observed for HF wetlands, the use of active aeration in the saturated-VF bed does not seem to improve any aspect of treatment performance in comparison with the typical unsaturated VF.

In practice, grain size as well as loading frequency should be carefully selected, as well as HLRs controlled (Matamoros et al., 2007), in order to ensure desired removal of EOCs, as well as enough oxygen renewal and mineralization of organic matter so that no clogging develops in the filter beds. Nevertheless, in French-style VF wetlands, where various beds alternate cycles of feed and rest, it has been observed that very high hydraulic overloads can be applied without observing a decreased in the infiltration capacity or treatment performance of the system at very high HLRs (0.4-1.8 m d⁻¹).

In conclusion, in unsaturated intermittently-fed VF wetlands, a loading frequency of one and two hours has not shown great differences in treatment performance, although both strategies seemed to be very efficient on the removal of EOCs. Moreover, sand-based VF wetlands perform significantly better than gravel-based ones in the reduction of target compounds. Nevertheless, in particular case scenarios the grain and type of media used should be adapted to available local materials, and the use of gravel could constitute an acceptable solution. What is more, the use of active aeration in VF wetland operating under saturated conditions has not shown to enhance the removal of the studied EOCs.

10.3. Behavior of emerging organic contaminants in hybrid constructed wetland systems

The aspects regarding the first and third objective of the thesis are discussed in this section, which explain the behavior of EOCs in hybrid CW systems (Chapters 6 and 8). For this purpose, experiments were carried out at two hybrid constructed wetlands at experimental and at pilot-scale. An injection of EOCs at three HLRs was carried out in the experimental hybrid CW system located at the facilities of the GEMMA group (Universitat Politècnica de Catalunya-BarcelonaTech) in spring 2011. For the evaluation of the pilot-scale hybrid CW system at the treatment facility of the Foundation Centre for New Water Technologies (CENTA) located in Carrión de los Céspedes, Seville (Spain), a monitoring campaign was carried out in summer 2011.

The selection of the hybrid CW systems configuration was the result of a collaborative project with the CENTA, aiming at the integrated treatment of wastewater, stormwater and sludge in small communities through the use of constructed wetland systems, so as to produce a final effluent suitable for its reuse in various applications. The two hybrid CW systems (Chapters 6 and 8) had the exact same wetland configuration, and consisted of a VF wetland stage, followed by a HF and a FWS wetlands operating in series. While in the system at Barcelona two parallel VF wetlands operated in cycles of feed and rest, in the pilot-scale system in Seville, a single-cell VF wetland stage was applied.

The study at the experimental hybrid CW system was conducted in spring season with a wastewater average temperature varying 14-19°C and well-developed vegetation. Actual OLRs in terms of COD being applied to the VF beds were 37 ± 6 , 110 ± 13 and 159 ± 27 g COD m⁻².d⁻¹ for the three campaigns in ascending HLR order, respectively. For ease of understanding, these values correspond to 22, 65 and 93 g BOD₅ m⁻² d⁻¹, respectively, assuming a COD/BOD₅ ratio of 1.7, according the large dataset obtained for the same wastewater source by Pedescoll et al. (2011b).

Fig. 10.7. shows the accumulated removal efficiencies of the studied EOCs along the CW system for the three applied HLRs. Target EOCs were grouped in relation to their overall removal efficiency in the hybrid system into (i) very efficiently removed (>90%, ACE, OXY, TCS and BPA), and (ii) moderately removed (from 50 to 90%, IB, DCF, AHTN and EE2).

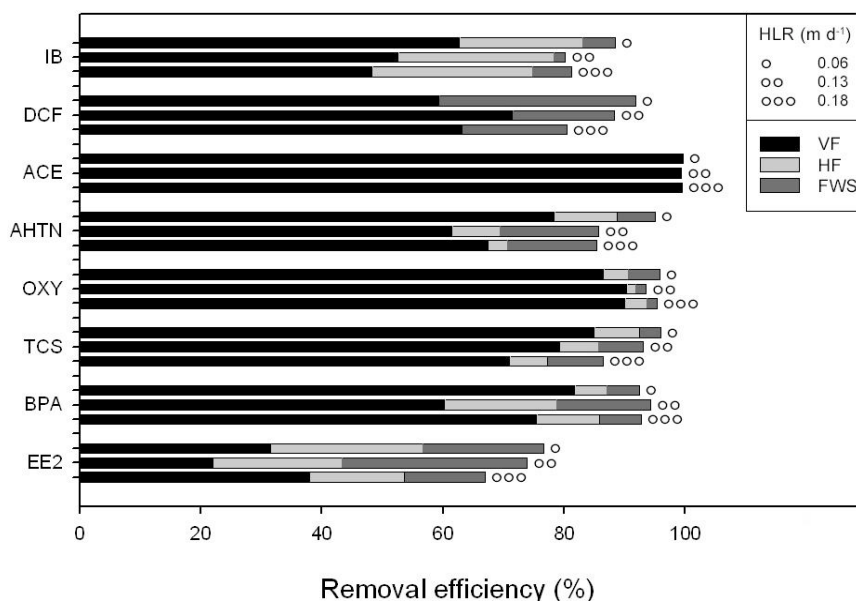


Figure 10.7. Accumulated average removal efficiencies of studied emerging organic contaminants along the different units of the experimental hybrid constructed wetland system at the three experimental hydraulic loading rates.

In general, the removal efficiency in the hybrid CW system showed to decrease as the HLR increased (0.06, 0.13 and 0.18 m d⁻¹) for most studied compounds (except for BPA, OXY, ACE). Statistically significant differences ($p < 0.05$) were found between the three different HLRs on EOCs removal efficiencies for DCF, AHTN and TCS. Particularly, with regards to the VF wetlands performance, mass removal in general increased proportionally with mass loading rates, which is in accordance to what was observed by Matamoros et al. (2007), who applied HLRs of 0.013, 0.030, 0.070 and 0.160 m d⁻¹ to a pilot-scale (5 m²) VF wetland.

The VF stage was responsible for the major part of the overall removal of all EOCs, especially for ACE, OXY, TCS and BPA, if considering all three HLRs. This could be attributable to the unsaturated conditions of VF wetlands, which proportionate oxidizing conditions, hence favoring aerobic microbial processes. Apart from the abovementioned, the large removal efficiencies found at this wetland unit might also have to do with the fact that it the VF wetland was the first stage of the system, where most of the total removal would take place (Hijosa-Valsero et al., 2010a; Matamoros et al., 2008b).

It can also be noticed that for some compounds the removal rates within the VF bed decreased as the HLR increased, which is in accordance with results obtained by Matamoros et al. (2007). Higher HLRs result in lower OTC and a decreased entrapment of hydrophobic compounds onto particulate matter due to lower contact time. Thus, operating the VF beds for a longer period of time could have an effect on the elimination of some substances, especially those having a high dependence on the redox status of the system or on the adsorption onto particles.

In general, EOCs removal efficiencies within the HF wetland were low, as was the redox status of the wetland bed. Moreover, no DCF removal occurred in the HF unit at any of the studied HLRs. This is in accordance with Matamoros and Bayona (2006) who reported a recalcitrant behavior of DCF under anaerobic conditions, in a study carried out in a full-scale HF wetland system located in Barcelona province. In general, observed EOC removal rates in this HF wetland are slightly lower than those obtained in the HF wetland system located in Barcelona (Chapter 4). Although mass loading rates of injected EOCs were similar in the two studies for most compounds with the exception of DCF (whose rates were 6 times higher in this study), the fact that the applied HLR was lower (0.028 m d⁻¹) in the HF CW system in Barcelona, and that those worked as a first treatment step, could explain higher treatment performance. If we look into differences among the three HLRs within the HF unit, we find especially particular the cases of hydrophobic substances, such as again AHTN, which showed a decrease in removal efficiency at higher HLRs. The same pattern was shown for EE2. This could be explained by low removal of particulate matter by the HF reactor where these compounds would be attached.

The FWS unit performed the best removal rates (accumulated) for DCF, EE2 and AHTN, presumably to the greatest part due to the direct sunlight exposure of this CW, which permits the photodegradation of these molecules. Photooxidation of EOCs has previously been reported to be a major removal mechanism of EOC removal in surface waters (Babić et al. 2013; Matamoros et al., 2009b; Matamoros and Salvadó, 2012).

Moreover, in collaboration with the Department of Environmental Analytical Chemistry of the CSIC, a toxicity assessment was carried out, and results show that more than 90% of the initial generic toxicity (*D. magna* feeding and Zebra fish embryo toxicity assays) present in the wastewater was eliminated after passing through the VF bed, which is in agreement with the best performance of this CW for removing most of the studied EOCs. These findings cannot be directly correlated with the behavior of the EOCs because degradation products from EOCs and other compounds that were already present in the wastewater may be also the responsible for the biological effects found in the samples evaluated. However, the increase in dioxin-like activity and *D. magna* feeding toxicity in the FWS unit may be due to the presence of oxidized compounds produced by the sunlight exposure. Previous

studies have shown that the photodegradation of some pharmaceuticals increases toxicity (Mezcua et al., 2004; Trovó et al., 2009). A PCA study grouped samples in relation to their toxicology and EOC content, but also correlated compounds with their toxicological effects.

In general, the experimental hybrid CW system has shown to be a reliable and robust technology for the removal of a large range of EOCs, general toxicity, dioxin-like activity, antimicrobial activity and estrogenicity from domestic wastewater. Caution should be put to the observation of accumulation of solids and clogging of the filter bed as well as reduced oxygen transfer capacity when operating at high HLRs. Although further studies are necessary, this work has proved the suitability of hybrid CWs as a wastewater cost-effective treatment solution due to their capacity to improve water quality as well as to remove EOCs and their associated adverse biological effects at high HLRs.

On the other hand, during the assays carried out at the pilot-scale hybrid CW system in Seville, water temperatures were fairly high (24 ± 2 °C), as expected for the hot summers of the Mediterranean climate from southern Spain. Indeed, EC values showed to increase as water passed through the FWS and the water tank, which could be explained by the high evapotranspiration taking place in the systems. Experimental OLR and HLRs entering the VF wetland were of about 6 g BOD₅/m².d and 0.044 m d⁻¹, respectively.

Average overall removal efficiencies were unquestionably high for most water quality parameters (99% TSS, 89% COD, 99% BOD₅, 98% NH₄-N), as observed in Chapter 7, after a 1.5-year monitoring period under dry and wet weather conditions, including an intensive monitoring campaign during and after a first-flush event. Solids entrapment and organic matter removal was very high within the VF wetland. The elimination of NH₄-N was also fairly high within the VF bed (67%), where the high values for TN removal (65%) together with the low concentrations of NO_x-N suggest both nitrification and denitrification processes to take place within this wetland type, due to the coexistence of aerobic and anoxic microsites within the wetland bed (Cooper et al, 1996). Further nitrification and denitrification occurred within the HF and FWS wetlands, up to an overall TN removal of 94%. This removal rate is much higher than most values reported by full-scale hybrid CWs of similar configuration at warm climates, such as the one by Masi and Martinuzzi (2007) at a system consisting of a 160-m² HF followed by a 180-m² VF, which treated the wastewater from a medium scale tourist facility in Italy (60% TN removal). The hybrid treatment system proved to have a great disinfection capacity, exhibiting overall E.coli reductions of about 5 log-units, and complied with Spanish regulation limits for some water reuse applications. The function made by the HF and FWS wetlands proved crucial to achieve a water quality appropriate for its reclamation.

The hybrid constructed wetland system (up to the effluent of the FWS unit) performed remarkably well also in the removal of EOCs, achieving very high overall removal efficiencies for the majority of the studied compounds (average of $90 \pm 11\%$). The accumulated removal efficiencies in each treatment unit are displayed in Fig. 10.8.

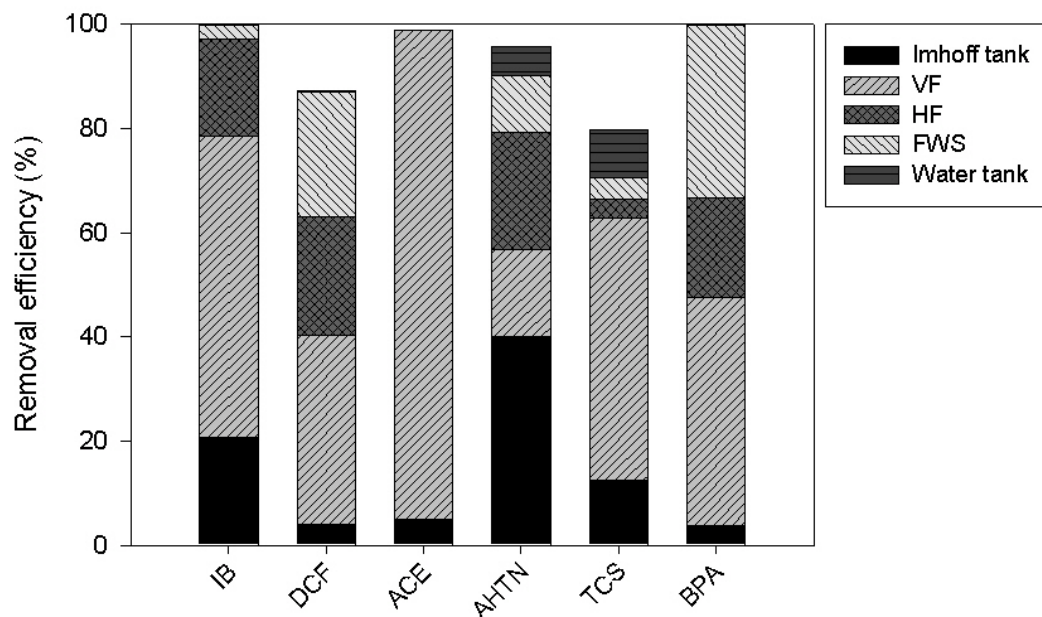


Figure 10.8. Accumulated removal efficiencies for the selected emerging organic contaminants at the different units of the pilot hybrid treatment system.

Final effluent concentrations of target EOCs were very low, being below the limit of detection for various contaminants (i.e. ACE, BPA). The rest were in the ng L^{-1} order (20-100), which is in the range of those found in the environment, such as those reported by Matamoros et al. (2009b) in small ponds or lagoons. These concentrations were also in the range of those obtained in advanced treatment technologies applied at full-scale, such as ozonation or membrane filtration (Snyder et al., 2007; Rosal et al., 2010).

The high removal efficiencies can be explained by differing existing physico-chemical conditions at different CW configurations, which would allow for the combination and synergy of various physicochemical and biological removal mechanisms to occur (e.g. biodegradation, sorption, volatilization, hydrolysis, and photodegradation) and thus achieve improved treatment efficiency of most pollutants (Faulwetter et al., 2009; Imfeld et al., 2009). In this sense, while aerobic metabolic pathways and solids retention are enhanced in VF wetlands, other removal mechanisms such as anaerobic biodegradation and sorption would predominate in HF beds. At last, the FWS wetland would be responsible for potential photodegradation of compounds, and less importantly through adsorption onto organic matter and uptake of plant material (Matamoros and Salvadó, 2012).

The Imhoff tank achieved a good removal of the musk fragrance AHTN, presumably due to a high degree of attachment to the particulate matter. The VF bed showed variable removal of EOCs, being compound dependent. It performed best for ACE, IB and TCS, while lower removal efficiencies were achieved for BPA, AHTN and DCF. While other substances, like AHTN, show a great reduction within the HF presumably due to sorption processes, results for TCS would exhibit little sorption capacity. The behavior of TCS within the three different wetland configurations indicate aerobic biodegradation as the major removal mechanism involved in the elimination of this compound (Singer et al., 2002; Ying et al., 2007). Although TCS has been detected in plant and sediments of a HF CW and its concentration generally decreased from inflow to outflow (Zárata et al., 2012), sorption and plant uptake does not appear to constitute a principal mechanism of TCS removal in constructed wetlands.

The similarly high removal efficiencies achieved for DCF in the current study in the HF bed if compared to the VF wetland suggest that various alternative mechanisms may determine the elimination of this compound, and to that respect, anaerobic biodegradation through reductive dehalogenation could constitute a predominant degradation pathway of DCF when anaerobic conditions prevail (Park et al., 2009; Hijosa-Valsero et al., 2010a; Chapter 3). On the other hand, the elimination of the endocrine disruptor BPA was significantly higher in the VF wetland if compared to the HF wetland, which is in conformity with the previously observed dependence of this substance on aerobic conditions (Chapter 4). However, the removal of this substance has also been achieved under anaerobic conditions of HF wetlands (Chapter 3) and thus the degradation of this substance could be owed to multiple mechanisms which seem to vary significantly in time, including biodegradation and association to the particulate matter (Wintgens et al, 2004).

As previously observed for the experimental hybrid CW system (Chapter 6), the superior treatment performance of the VF over the other treatment units could be owed to energetically favorable aerobic microbial reactions, as well as hydrolysis reactions, taking place within this wetland type and provided by its design and operation strategy, which results in high effluent redox potentials and DO concentrations. However, the synergic nitrification-denitrification activity observed in the VF bed within this study suggest the co-existence of both aerobic and anaerobic microenvironments within this wetland bed, which would allow the occurrence of both processes. This could be partially explained by the occurrence of a saturated layer at the bottom of the wetland bed, below draining pipes. Similarly, this finding indicates that although aerobic biodegradation and sorption onto organic matter may be the major removal mechanisms contributing to EOCs reduction in VF wetlands, alternative processes based on anaerobic metabolism could simultaneously be occurring at anoxic microsites or micropores within the wetland bed (i.e. in lower layers) (Cooper et al., 1996; Ávila et al., 2010), which contribute to its elimination.

The FWS wetland performed especially well for BPA, followed by DCF. The removal of BPA within this wetland was fairly high if compared to the HF unit, which could be explained by enhanced biodegradation of this substance under higher redox and DO conditions within the water column of the FWS (Liu et al., 2009). Sorption onto particulate matter (Stevens-Garmon et al., 2011) and photodegradation (Matamoros et al., 2012a) could further contribute to BPA removal in FWS wetlands. These results support photodegradation as a principal removal mechanism involved in DCF attenuation in water bodies (Buser et al., 1998; Andreozzi et al., 2003; Matamoros and Salvadó, 2012, 2013), together with less predominant mechanisms (i.e. aerobic/anaerobic biodegradation, plant uptake). Although the removal of TCS was negligible, some more reduction within the water reuse tank seemed to occur, indicating that photooxidation processes may constitute a small contribution to its removal (Mezcua et al., 2004; Ávila et al., 2013b; Matamoros and Salvadó, 2012, 2013). Although the reduction of AHTN concentrations in the FWS could be attributed to sorption onto particulate matter and sediment, further reduction was achieved at the water reuse tank, suggesting photodegradation through sunlight exposure as one of the principal mechanisms of AHTN removal within this type of wetland configuration. Similarly high removal efficiencies were obtained at other FWS wetlands operating as a tertiary treatment step (Matamoros et al., 2008b; Llorens et al., 2009; Matamoros and Salvadó, 2012).

In conclusion, hybrid CW systems consisting of a combination of CW configurations, including a VF CW, a HF wetland, and a FWS wetland operating in series, has shown to be a very robust ecotechnology for wastewater treatment and reuse in small communities. The combination of different wetland configurations has shown to optimize a number of important treatment processes, achieving an excellent overall removal efficiency of EOC (average of $90 \pm 11\%$), conventional water quality parameters ($>90\%$), as well as a high disinfection capacity. This has been possible thanks to the occurrence of complementary abiotic/biotic removal pathways taking place under differing physico-chemical conditions existing at wetlands of different configuration.

11. Conclusions

In this PhD study the performance of CWs on the removal of an array of EOCs was evaluated. The effect of different CW configurations, as well as various design and operational factors was also assessed. For that purpose, several assays were carried out in experimental (continuous injections of EOCs) and pilot-scale (monitoring campaigns) CW systems. In accordance to obtained results, we can conclude:

- The elimination of EOC in HF wetlands showed to be compound-dependent (32 and 99% for DCF and ACE as min and max values, respectively) and exhibited a seasonal pattern, with higher removal rates during the warm season, presumably due to enhanced biodegradation, volatilization and plant uptake at higher water temperatures.
- In HF wetlands, the use of a HUSB reactor as opposed to a conventional settler for the primary treatment of wastewater conferred more reduced conditions within the CW system, which in turn resulted in reduced removal efficiency of most target EOCs.
- In HF wetlands, the mode of operation in batch, alternating cycles of saturation and unsaturation, promoted the existence of a higher redox status as compared to operation under continuously saturated conditions, which in turn greatly enhanced the removal of all target EOCs.
- The tentative identification of an intermediate product of BPA under more oxidized conditions in HF wetlands (promoted by the alternation of cycles of saturation/unsaturation) suggests that aerobic biodegradation could constitute a principal removal mechanism of this substance when a higher redox status prevail.
- In VF wetlands, which are intermittently-fed, a higher loading frequency (bi-hourly vs. hourly) showed to perform significantly worse on the removal of some EOCs (DCF, AHTN, BPA). This could be attributed to the lower contact time and reduced oxygen renewal at lower loading frequencies.
- The occurrence of gravel (4-8 mm) as opposed to sand (1-3 mm) for the main bed media of VF wetlands exhibited a significantly lower elimination of studied EOCs. The smaller grain size of sand would result in smaller pores, hence providing a better filtering capacity, a higher surface area for biofilm growth and a longer contact time, and in consequence increased treatment performance.
- The use of active aeration in a saturated VF wetland variant did not show to enhance any aspect of EOC removal in respect to the typical unsaturated VF wetland type. Dissolved oxygen and redox conditions were almost identical in the two VF beds.
- A hybrid treatment system based in a VF wetland stage, followed by a HF and a FWS wetland operating in series has proved to be a very robust technology for the treatment of wastewater in small communities, which is able to produce a final effluent suitable for its reuse in various applications (e.g. irrigation of green areas non-accessible to the public, recharge of aquifers by percolation through the ground, etc.).
- The experimental hybrid CW system, performed very well on the removal of EOCs ($87 \pm 10\%$), even at high HLRs. Three HLRs were applied (0.06, 0.13 and 0.18 m d⁻¹) and the removal efficiency of injected EOCs showed to decrease as the HLR increased. Moreover, general toxicity, estrogenicity and dioxin-like activities were reduced by the VF and the HF units, whereas antimicrobial activity was mainly removed by the FWS wetland.

- The pilot-scale hybrid CW system, which received combined sewer effluent, exhibited a great performance on water quality parameters removal, and showed to be very resilient to water flow and quality fluctuations during stormy periods and first-flush events. Most contaminant removal took place within the first stage of the hybrid CW system (VF stage), which is in accordance with the higher redox status of this type of Cws, which enhances microbial activity. Very high EOC overall removal efficiencies were achieved ($90 \pm 11\%$), presumably due to the combination and synergy of various abiotic/biotic removal mechanisms (e.g. biodegradation, sorption, volatilization, hydrolysis, photodegradation).
- In general, the combination of different constructed wetland configurations has proven to be a very competitive alternative for wastewater treatment in small communities, which could become especially attractive if legislation in regards to water quality and guidelines for new contaminants becomes more stringent.

12. References

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Curriculum Vitae

Cristina Ávila Martín was born in Málaga, Andalusia (Spain), in 1983. She obtained her Bachelor degree in Environmental Science from the University of Málaga and holds a M.Sc. in Applied Ecology from the University of Halmstad, Sweden. Her M.Sc. thesis was carried out in wastewater stabilization ponds for wastewater treatment and reclamation through aquaculture at the University of Kalyani, West Bengal, India. She then started her PhD research at the Universitat Politècnica de Catalunya · BarcelonaTech (Spain) in 2009.

Articles in referred journals

- Ávila, C., Bayona, J.M., Aragón, C., Martín, I., Salas, J.J., García, J., 2014. Emerging organic contaminant removal in a full-scale hybrid constructed wetland system for treatment and reclamation of wastewater from small communities of warm Mediterranean climates. In preparation.
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Conference contributions

Oral communications

- Ávila, C., Aragón, C., Martín, I., Salas, J.J., Bayona, J.M., García, J., 2013. Removal of emerging organic contaminants in hybrid constructed wetlands for the treatment of wastewater of small communities of warm climate regions. 5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL). October 2013, Nantes, France.
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Posters

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Aragón, C., Martín, I., Ávila, C., García, J., Salas, J.J., 2012. Evaluación de la calidad de los lixiviados generados en un humedal para el secado de fangos durante su puesta en marcha. In: López-Geta, Ramos, Fernández and Lorca (Eds.). Instituto Geológico y Minero de España. Madrid: ISBN 978-84-7840-863-4.

Participation in R+D Projects

- Safeguarding Water Resources in India with Green and Sustainable Technologies (SWINGS). Financed by European Commission (FP7), coordinated by Aimen Technology Centre (2012 – 2015; 21 research groups-subprojects). www.swingsproject.eu
- Natural water systems and treatment technologies to cope with water shortages in urbanized areas in India (NaWaTech). Financed by the European Commission (FP7), coordinated by Technologie-Transfer-Zentrum (TTZ) Bremerhaven (2012 – 2015; 13 research groups-subprojects). www.nawatech.net
- Biotechnology for Africa's Water Supply (WATERBIOTECH). Financed by the European Commission (FP7) and coordinated by Technologie-Transfer-Zentrum (TTZ) Bremerhaven (2011 – 2014; 20 research groups-subprojects). www.waterbiotech.eu
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