UNIVERSITÀ DEGLI STUDI DI PADOVA

Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente
DAFNAE

SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE DELLE PRODUZIONI VEGETALI
INDIRIZZO: AGRONOMIA AMBIENTALE
CICLO: XXV

Performance of hybrid constructed wetland for piggery wastewater treatment

Direttore della Scuola: Ch.mo Prof. Angelo Ramina
Coordinatore d'indirizzo: Ch.mo Prof. Antonio Berti
Supervisore: Ch.mo Prof. Maurizio Borin

Dottorando: Marco Politeo

Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

Marco Politeo, January 31st 2013

A copy of the thesis will be available at [*http://paduaresearch.cab.unipd.it/*](http://paduaresearch.cab.unipd.it/)

Dichiarazione

Con la presente affermo che questa tesi è frutto del mio lavoro e che, per quanto io ne sia a conoscenza, non contiene materiale precedentemente pubblicato o scritto da un'altra persona né materiale che è stato utilizzato per l'ottenimento di qualunque altro titolo o diploma dell'università o altro istituto di apprendimento, a eccezione del caso in cui ciò venga riconosciuto nel testo.

Marco Politeo, 31 gennaio 2013

Una copia della tesi sarà disponibile presso [*http://paduaresearch.cab.unipd.it/*](http://paduaresearch.cab.unipd.it/)

A mio nonno Gino

Table of contents

Supplementary pictures, technical draw and information in the additional CD attached to this thesis.

Riassunto

L'impatto ambientale del settore zootecnico nel Nord-Est Italia colpisce direttamente l'acqua (accumulo di nitrati e eutrofizzazione). Le limitazioni introdotte dopo l'applicazione della Direttiva CE 91/676 si traducono spesso in un aumento della richiesta di superfici disponibili per la distribuzione dei reflui. In alcune zone, la carenza di terreni e l'elevato carico zootecnico hanno comportato un costo aggiuntivo che gli allevatori devono sostenere per potere disporre di terreni di altri agricoltori dove potere delocalizzare gli effluenti prodotti. Per valutare le possibilità applicative della fitodepurazione per il trattamento dell'azoto nella frazione liquida degli effluenti suini è stato condotto un monitoraggio di un impianto ibrido operante a scala aziendale. L'impianto ibrido di fitodepurazione occupa un'area di 130 m^2, è costituito da tre vasche a flusso sub superficiale verticale (VF) operanti in parallelo seguite da una vasca a flusso sub superficiale orizzontale (HF). Durante la sperimentazione (2010-2012) le prestazioni dell'impianto di fitodepurazione sono state valutate variando: condizioni ambientali, concentrazioni e volumi in ingresso, modalità e tempi di carico e scarico delle vasche verticali. Nello specifico durante il primo periodo (Aprile 2010-Luglio 2010) il sistema è stato caricato con 5 m^3/giorno di liquame pre-trattato. L'unità verticale ha funzionato come "filtro biologico". Nel secondo periodo (Ottobre 2010-Aprile 2011) il sistema è stato caricato con 5 m^3/giorno di liquame non pre-trattato in modalità sequenziale batch, alternando fasi di "tutto pieno" e "tutto vuoto" grazie a un sistema di temporizzatori modulari pausa/lavoro a tempi indipendenti. Per valutare l'influenza delle basse temperature sui processi di rimozione dell'azoto, nel terzo periodo (Maggio 2011-Luglio 2012) il sistema è stato caricato con 1.7 m^3/giorno di refluo ricostruito in modalità sequenziale batch. Nel complesso, il sistema ibrido di fitodepurazione ha ridotto le concentrazioni in ingresso del COD dal 46 al 56%, del azoto totale dal 40 al 54%, del azoto ammoniacale dal 43 al 60%, del azoto nitrico dal 21 al 55%, del fosforo totale dal 32 al 35% e del ortofosfato dal 24 al 34%.

Summary

The environmental impact of the livestock sector in North-East Italy directly affects water (nitrates accumulation and eutrophication). Through the “Nitrate Directive” (91/676/EEC), the EU aims to reduce water pollution caused or induced by nitrates from agricultural sources. Disposal of animal wastewater is a common problem among local farmers. Land spreading is the usual disposal method but requires sufficient land area in close proximity to the farm. Problems associate with animal wastewater treatment and land application has prompted an urgency to find alternative treatment systems that are technically feasible and economically viable. Hybrid constructed wetlands (HCW) are being considered as an alternative method for livestock wastewater disposal which could reduce the amount of land necessary for terminal land application. This work presents the results of monitoring a full-scale hybrid wetland system operating on a swine farm. The HCW system was composed of three vertical-subsurface flow wetlands (VF) in parallel with a total area of 30 m^2, followed by one horizontal-subsurface flow wetland (HF) connected in series (100 m^2). During the experimentation (2010-2012), HCW operated under different conditions: seasonal variations (temperature), pollutants concentrations, hydraulic loading rate (HLR), hydraulic retention time (HRT), feeding mode and operational regimes. During the first period (April 2010-July 2010) the system was loaded with 5m^3/d of pre-treated piggery wastewater and VF system worked like a “biological filter”. During the second period (October 2010-April 2011), the system was loaded with 5m^3/d of raw piggery wastewater, a sequential batch (feed-stay-drain-rest) feed mode in VF system was used. To determine if winter climate conditions influence treatment effectiveness, during the third period (May 2011-July 2012) the system was loaded with 1.7 m^3/d of synthetic wastewater. VF system was fed with sequential batch mode with two different operational regimes. Overall concentration reduction obtained by HCW system for COD ranged from 46 to 56%, for total nitrogen from 40 to 54%, for ammonia nitrogen from 43 to 60%, for nitric nitrogen from 21 to 55%, for total phosphorus from 32 to 35% and for orthophosphate from 24 to 34%.

Chapter I

General background and objectives of the thesis

Introduction

In many situations the harmful environmental effects of livestock rearing are caused by the high concentration of effluents produced in a limited area. Animal manure nutrients in excess of crop uptake accumulate and even saturate soils. At saturation, nutrients are lost to either surface or ground waters (Martinez et al., 2009). Nitrogen and phosphorus are two nutrients with the greatest potential to create water pollution (EEC, 1991). Through the "Nitrate Directive" (91/676/EEC), the EU aims to reduce water pollution caused or induced by nitrates from agricultural sources. The Directive imposes the individuation of vulnerable zones in the different countries in which action plans have to be adopted to reduce nitrate losses. In these zones a maximum of 170 kg per hectare per year from animal wastes is allowed. The recent Derogation No. L 287/36 granted to Italy raised this limit to 250 kg during the 2012-2015 period in Regions on the Padana Plain (Piedmont, Lombardy, Veneto and Emilia Romagna) for crops with high nitrogen demand and long growing season (Official Journal of the European Union, 2011). Veneto Region is characterized by high N input farming systems, the designated vulnerable zones to which the action programmes apply cover about 87% of the utilised agricultural area (UAA) and include over 740,000 pigs that produced an average of 1,805,000 m^3/year of manure (ISTAT, 2011).

Piggeries produce large volumes of manure with high nutrient concentrations and have less land available compared to dairy cattle or sheep farms as the animals are housed in large industrial units (Kumm, 2003; Healy et al., 2007; Meers et al., 2008; Harrington and Scholz, 2010). Low dry matter content (usually 2-5%), increases transportation costs and makes the application of these manures to land and crops difficult and limits the periods of application.

Spreading the excess slurry over arable land may result in contamination of groundwater and eutrophication of surface waters (Smith et al., 2000; Martinez et al., 2009). Once distributed on the fields, ammonium nitrogen, which represents the main form of nitrogen in slurry, is readily oxidized to nitrate that is poorly absorbed by soil colloids, so is easily moved into surface waters (Luo et al., 2002). Land spreading is often a viable option where there are large areas of farmland (Harrington and Scholz, 2010) however, if sufficient land is not available, pig slurries have to be treated in order to reduce the high nutrient concentration.

Piggery manure management consists of three main phases: the first is solid-liquid separation that is the primary treatment process used to improve liquid manure handling properties and to generate solids (Zhang and Westerman, 1997). Several methods are available to separate solids from liquids, including sedimentation (solids settle by gravity), mechanical separation (screen separators, centrifuge, screw press and belt press), evaporation ponds, dehydration, coagulation and flocculation (Zhang and Westerman, 1997). The second phase converts the solid fraction into an exportable product used for composting (Brito et al., 2008; Ross et al., 2006), re-feeding, or generating biogas (methane) (Worley and Das, 2000; Demirer and Chen, 2005). The third phase aims to reduce nutrient content in the liquid fraction to meet discharge criteria or the remaining nutrients are spread on arable land (Meers et al., 2005). Nutrient content reduction in the liquid fraction can be obtained via different techniques: activated sludge treatment (Ten Have et al., 1994), sequencing batch reactors (Zhang et al., 2006), anaerobic codigestion (Verstraete and Vandevivere, 1999), reverse osmosis (Masse et al., 2010), or ultrafiltration (Fugère et al., 2005).

Constructed wetlands (CWs) are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and the associated microbial assemblages to assist in treating wastewaters (Vymazal, 2004). Since the 1990s, CWs have been used in the treatment of several wastewaters such as domestic sewage, urban runoff and storm water, industrial and agricultural wastewater, and leachate (Scholz and Lee, 2005).

As reported by Szogi et al. (1999), CWs have the potential to remove organic compounds and nutrients from piggery wastewater. Indeed, over the last 25 years CWs to treat liquid swine slurry have been heavily researched in the United States, with several designs being implemented and varying results obtained. CWs provide an environment for the physical/physico-chemical retention and biological reduction of organic matter and nutrients by promoting uptake, transformation and inactivation of nutrients and pathogens by microorganisms and plants, filtration, adsorption and chemical precipitation by contact with plants, substrate and litter, settling of suspended solids, chemical transformation (e.g. nitrification, denitrification), predation and natural die-off of pathogens. (Geary and Moore, 1999; Knight et al., 2000). Treatment efficiency differs for different pollutants and varies considerably spatially and temporally, depending predominantly on the type of CW used, its design, age of the system, feeding mode (how wastewater is applied), hydraulic loading rate (HLR) and hydraulic retention time (HRT) (Karpiscak et al., 1999). However, due to the effect of different temperatures, the treatment efficiency of these systems tends to change over the year (Bachand and Horne, 2000; Healy and Cawley, 2002).

In Italy, application of this technology is encouraged by the legislation (‘‘Water Framework Regulation’’, D.Lgs 152/99 and modifications), which introduced the concept of ‘‘adequate treatment’’ (Annex 5, paragraph 3) by advising that: ‘‘for all settlements with population equivalent (p.e.) between 50 and 2000 p.e. the application of techniques for natural depuration such as long-term storage or CWs is thought to be favourable . . .’’ (Mantovi et al., 2003). Depuration of wastewater through a CW system is attracting interest for its potential application to agricultural wastewaters, although the literature reports only one example of the use of an FWS system to treat agricultural drainage waters (Borin and Tocchetto, 2007). Applications of CWs for livestock wastewater management are not widespread in Italy.

Existing treatment wetlands for piggery wastewater have a wide variety of engineering designs, wetted areas, flow rates, inflow water qualities, plant communities, hydrologic regimes. A piggery wastewater treatment wetlands database (PWDB) was developed to summarize existing treatment wetland information about all types of treatment wetlands. Scientific journal articles, monitoring reports to agencies, consultant reports, private databases and proceedings of CW conferences have been used as sources. In particular, only a selection of the papers describing full-scale CW case studies for swine wastewater treatment was considered.

Piggery wastewater treatment wetlands database (PWDB)

The PWDB includes 13 treatment wetland sites with a total of 18 case studies, containing a total of 300 individual treatment cells. For each site, state, herd sizes and form of pre-treatment are reported. In this database case studies are defined as single or parallel wetland treatment areas. Information entered for each case study includes design area in hectares (ha), hydrologic type (surface flow-SFW, subsurface flow-SSFW or Hybrid system-HCW), average daily flow rate (Q) in m^3/d, Hydraulic Retention Time (HRT) and number of cells. Cells are wetland areas that are clearly delineated from other treatment areas by dikes or uplands and that have recognizable inlet and outlet points. For each cell, surface area, type (vertical subsurface flow wetlands-VF, horizontal subsurface flow wetland-HF or marsh), plant species names for resident vegetation and substrate size and material are reported. Over 500 operational data records are summarized in the database. These data indicate for each cell, median inlet and outlet concentration for chemical oxygen demand (COD), total kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4-N), nitric nitrogen (NO_3-N) and total phosphorus (TP). Concentration abatements for each cell (A%) were calculated on the second quartile (Q2; median) and third quartile (Q3) concentration values as: A% = [(Cin – Cout)/Cin] ×100, where Cin is median inflow concentration (mg/L) and Cout is median outflow concentration (mg/L). In some cases, no published information is available for operating treatment wetland systems. Based on design and hydraulic wastewater flow characteristics, CWs treating piggery wastewater in the PWDB are classified into three major groups: surface flow wetland (SFW), subsurface flow wetland (SSFW) and hybrid CWs (HCW). Table 1.1 summarizes the key data of 18 examined case studies in the piggery wastewater treatment wetlands database (PWDB). It can be observed that an SFW system was used in fifty percent of case studies, and the rest are SSFW systems, with HCW being used in only one case study (Kato et al., 2010).

Table 1.1 – Summary of key data for 18 examined case studies in the piggery wastewater treatment wetlands database (PWDB).

Location	Wetland type	COD (mg/L)		TKN (mg/L)		NH_4-N (mg/L)		NO_3-N (mg/L)		TP (mg/L)		Reference
		IN	OUT	IN	OUT	IN	OUT	IN	OUT	IN	OUT	
Australia	SSFW	629	399	261	183.5					19.4	15.7	Finlayson et al. (1987)
USA	SFW			70	6	55	3.5			25.8	6.20	Hammer et al. (1993)
USA	SFW					24.1	7.15	2.18	2.88			Hunt et al. (1993)
USA	SFW			130	43.1	112	37.3			29	17.4	Chathcart et al. (1994)
USA	SFW			54.4	16.2	45.1	13.1			25.8	13	McCaskey et al. (1994)
USA	SFW			416	249	405	244	1.06	2.35	17.4	14	Reaves et al. (1994)
China	SSFW	1847	246									Wang et al. (1994)
USA	SSFW	667	421			201	155					Parkes et al. (1998)
China	SSFW	1865	246									Junsan et al. (2000)
USA	SSFW			261	31.5	198	25.5	59	5.50			Poach et al. (2003)
Lithuania	SSFW	374	68	31	19.1	16.6	11.6			9.6	0.8	Strusevičius and Strusevičiene (2003)
Taiwan	SSFW	1115	198	197	159	161	151	3.42	1.21	43.6	18	Lee et al. (2004)
USA	SFW	808	464	175	109					73	55	Poach et al. (2004)
USA	SFW			86	53					56	48	Stone et al. (2004)
USA	SFW	308	148	63.8	19.6	40.8	12.4			51.8	49.4	Hunt et al. (2007)
USA	SFW	445	246							71	66	Poach et al. (2007)
Korea	HCW	7100	6333	1492	1383	1408	1307			81	60	Kato et al. (2010)
Spain	SSFW	11656	9752	2629	1991	2028	1546	39	28	30.5	28.4	Sánchez-García et al. (2010)

Median inlet and outlet concentration of COD, TKN, NH_4-N, NO_3-N and TP for each case study.

Surface flow wetland treating piggery wastewater

Surface flow wetland (SFW) sometimes called free water surface (FWS) wetland, consists of a shallow basin, soil or other substrate to support the emergent wetland vegetation and a water control structure that maintains a shallow water depth (Lee et al., 2009) **(Figure 1.1).**

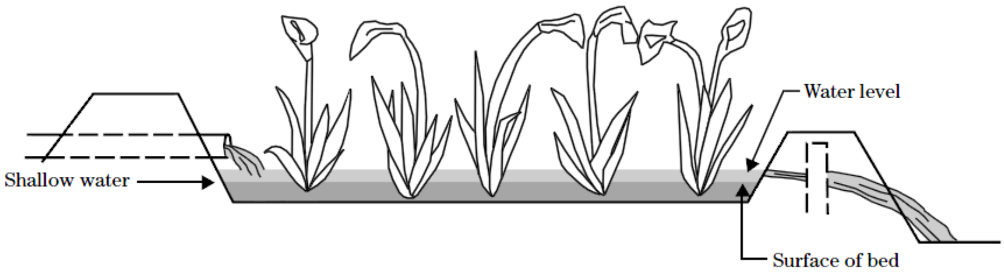


Figure 1.1 - Basic elements of an SFW system from USDA Environmental Engineering Handbook, 2002.

The majority of SFW engineered for piggery wastewater treatment required significant land area with a median system size of 0.14 ha. The large pig waste treatment wetland in the PWDB is a combination of five SFW at the Sand Mountain Experiment Station at Crossville (USA), the combined treatment area of all of the cells is 0.36 ha (McCaskey et al., 1994). This wetland system treated 32.8 m^3/d of diluted piggery wastewater after a passage through a two-stage lagoon.

The whole surface of an SFW is generally divided in multiple cells, with a number comprised between 6 (median surface of 110 m^2 each cell) (Poach et al., 2004) and 16 (median surface 22 m^2 each cell) (Reaves et al. 1994). The multiple cells configuration has the advantage of providing greater flexibility in design and operation, and of enhancing the performance of the system overall by decreasing the potential for short-circuiting. SFW systems are usually used for piggery wastewater treatment to avoid clogging. Chen et al. (2008) assessed a conventional three-stage treatment scheme followed by a modified free water surface wetland (with or without plants) with a two-day hydraulic retention time.

The raw effluent from most swine facilities must be pre-treated, mainly to reduce the total solids, organic matter and nutrients concentration before entering an SFW system

(US.EPA, 1997). Primary settling is necessary and pre-treatment is desirable upstream of the wetland. All of the wetland systems in the PWDB have some form of pre-treatment. The most widespread form of pre-treatment in examined case studies is an aerobic lagoon, and in order of their occurrence: storage ponds and activated sludge systems. Aerobic lagoons are quite deep (a few meters), typically have short residence times and allow settling and a significant reduction of the organic load, and are usually designed on a volume basis. An SFW is generally considered anoxic, with a thin aerobic layer at the surface due to passive aeration of the water (IWA, 2000). Aerobic lagoons allow nitrification and reduce ammonia volatilization to be achieved due to reduced concentrations of ammonia in the wastewater (Poach et al., 2003). As a result, these reduced concentrations help to minimize the potential risk of ammonia toxicity to wetland plants.

Piggery wastewater flows across the surface at depths that typically range between 0.2 and 0.6 m, depending on the type of vegetation and other design factors. Carty et al. (2008) highlighted that shallower systems help to increase nitrification by increasing the aerobic conditions present in the cells. The bottom slope must be flat from side to side, but may be flat or have a slight gradient from inlet to outlet (from 0 to 0.5%). The substrate is usually compacted clay or hydric soils, if available. Wastewater in the SFW wetland flows slowly across the surface of the bed in a horizontal direction, with a hydraulic flow rate that ranges from 5 to 9 m^3/d and a hydraulic retention time from 7 (Reaves et al., 1994) to 36 days (Hunt et al., 2007).

SFW systems treating swine wastewater have generally been configured either as a continuous marsh or a marsh-pond-marsh (m-p-m) (Figure 1.2)

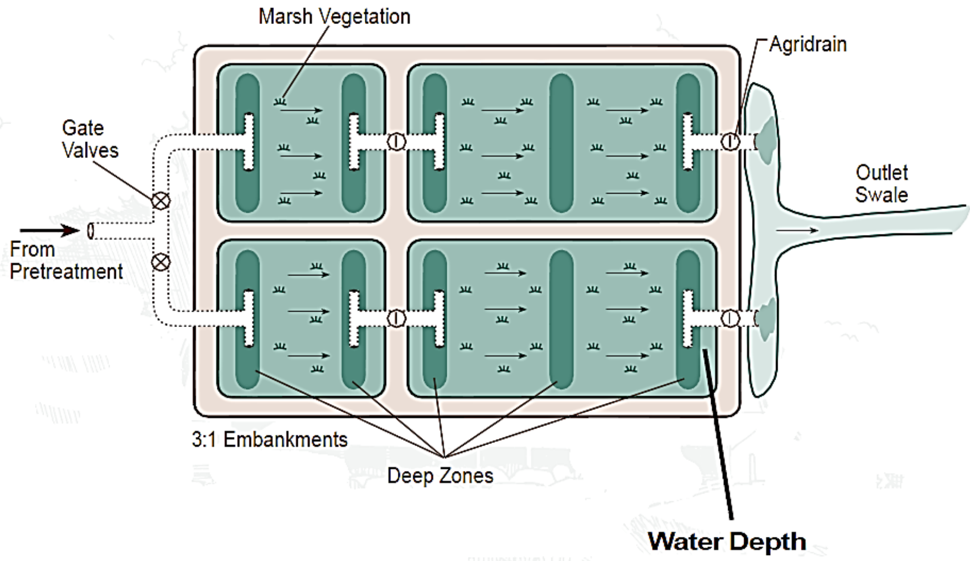


Figure 1.2 – marsh-pond-marsh SFW configuration from Constructed Wetlands and Animal Wastewater Management, 1998.

As the name implies, the marsh-pond-marsh design has a combination of open water and fully vegetated areas (marsh) with variable water depth that are usually recommended to create anaerobic and aerobic zones. Well-oxygenated open water enhances nitrification, while more anaerobic, densely vegetated areas promote denitrification, sediment settling and phosphorus retention (Kadlec and Knight, 1996; Braskerud, 2001; Thullen et al., 2005).

Components of the marsh-pond-marsh surface flow wetland are as follows:

1. Marsh: a shallow basin with dense emergent vegetation such as cattail (*Typha latifolia* L.). The marsh's function is the ammonification and removal of BOD_5, TSS and pathogens.
2. Pond: constructed pond of 0.5-1 m depth that is similar to an aerobic lagoon. Vegetation includes surface species such as duckweed (*Lemna minor* L.) and submerged species such as pondweed (*Potamogeton* spp.). The pond's purpose is to increase the dissolved oxygen concentration and oxidation status of swine wastewater and promote nitrification (Cathcart et al., 1994; Reddy et al., 2001).
3. Meadow: overland flow system with reed canary grass (*Phalaris arundinacea* L.) or other inundation-tolerant grasses. The meadow receives effluent from the pond in a shallow sheet flow having a depth from 1 to 5 cm. The meadow's purpose is to

remove TSS and further promote nitrification and denitrification. Each cell should have a length to width ratio of 3-5:1 (Cronk, 1996).

In CWs, plants provide a substrate and carbon source for microbes. Wetland plants oxygenate the substrate immediately adjacent to their roots and increase the aerobic portion of an otherwise anaerobic zone (Brix, 1993). In addition, plants remove nutrients from the incoming wastewater during the growing season. While plant nutrient uptake is usually not the major pathway of nitrogen and phosphorus removal, it has been credited with 16-75% of total N removal and 12-73% removal of total P in wastewater treatment wetlands (Reddy and DeBusk, 1987). Emergent herbaceous plants (EHPs) are the dominant vegetation type used in SFW for piggery wastewater treatment because they have high productivity, rapid nutrient uptake, rhizome colonization and finally, in order to survive, tolerance to high nutrient inputs (especially for ammonia nitrogen).

SFW beds are vegetated with several mixtures of different macrophyte species. In order of their occurrence these are cattail (*Typha* spp.*),* bulrush (*Scirpus* spp.*)* and common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), which are all water-tolerant macrophytes that are rooted in the soil but emerge above the water surface.

SFW performance summary

Few data are reported on changes in chemical oxygen demand (COD) through SFW treatment wetlands. Median concentrations decreased from 445 to 246 mg/L for an abatement of 44.7%, the highest median COD reductions were reported by Hunt et al. (2007) with values from 308 to 148 mg/L for an efficiency of 52% (Table 1.2).

The majority of TKN in most of the piggery wastewater systems is in the ammonium form. At the North Carolina A&T State University farm near Greensboro, the ammonium fraction was 64% (Hunt et al., 2007), and at Auburn University's Sand Mountain Agricultural Experiment Station in Alabama averaged 82% (McCaskey et al., 1994) (Table 1.1). Median TKN inflow and outflow concentrations were 175 and 104 mg/L respectively, with a median percentage abatement of 40%. A marsh–pond–marsh site in Greensboro at North Carolina A&T State University reported in numerous publications (Hunt et al., 1994; Reddy et al., 2001; Stone et al., 2002; Poach et al., 2003; Stone et al., 2004) had a TN concentration reduction from 36 to 51.3%. Nitrogen removal in SFW is supported by means of sedimentation, adsorption, organic matter accumulation, microbial assimilation, nitrification, denitrification, and ammonia volatilization (Brix, 1994; Poach et al., 2003). Hunt et al. (2002) reported that denitrifying enzyme activity in SFW increases over time with the maturity of constructed wetlands, an increased rate of nitrogen application, and water depth which leads to increased ammonia volatilization. Data collected by PWDB reported that median NH_4-N inflow and outflow concentrations were 86 and 39 mg/L respectively, with a median abatement of 55%. Poach et al. (2004) highlighted the fact that the pond sections in SFW configured as m-p-m had significantly higher proportions (23 to 36%) of volatilization than the marsh areas (<12%). Volatilization was the dominant nitrogen removal mechanism in the pond sections (54 to 79%). NO_3-N inflow concentration was lower than the other parameters, and generally increased through SFW passage. Nitrogen processing in surface swine wetlands is quite temperature dependent. However, Maddux (2002) explained that if constructed properly, SFW can be used successfully in subarctic conditions during the unfrozen season.

Phosphorus is partly removed in CWs by plant uptake, accretions of wetland soils, microbial immobilization, retention by root bed media, and precipitation in the water column (Reddy et al., 2001; Scholz, 2006). Kadlec and Knight (1996) reported that treatment efficiency for phosphorus may be higher during the initial years of operation and decline to a lower level at system maturity. The median concentration was reduced from 29 to 18.1 mg/L, with a median abatement of 38.2% (Table 1.2).

Table 1.2 – Treatment performances of SFW systems for piggery wastewater. Data from PWDB.

SFW	COD (mg/L)			TKN (mg/L)			NH_4-N (mg/L)			NO_3-N (mg/L)			TP (mg/L)		
	IN	OUT	A%	IN	OUT	A%	IN	OUT	A%	IN	OUT	A%	IN	OUT	A%
median	445	246	44.7	175	104	40.5	86	39	54.6	1.06	2.18		29	18.1	38.2
Q3	808	464	42.5	416	240	42.1	405	234	42.2	1.06	3.39		56	49.4	11.7
n.	14	14		43	43		45	45		27	27		49	49	

Subsurface flow wetland treating piggery wastewater

Subsurface flow wetland (SSFW), sometimes called engineered reed beds, typically consists of a ditch or a bed, sealed by an impermeable substance to block leakage, and media that assist the growth of emergent plants (Lee et al., 2009) (Figure 1.3).

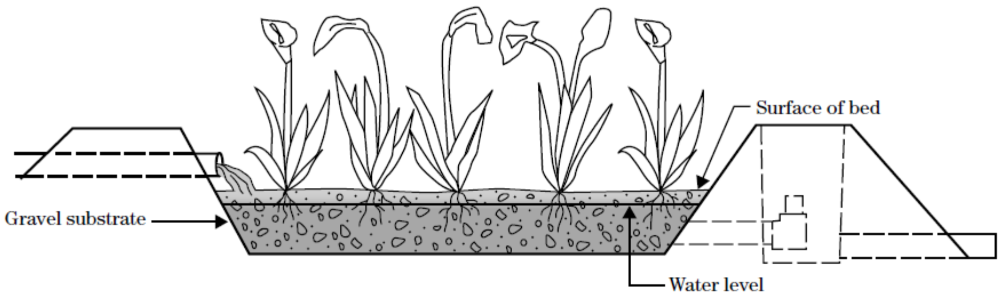


Figure 1.3 - Basic elements of an SSFW from USDA Environmental Engineering Handbook, 2002.

SSFW systems for piggery wastewater are primarily used in China and Europe (Lithuania and Spain). SSFW systems are subdivided into horizontal and vertical flow systems according to the flow direction of the wastewater. In the horizontal-subsurface flow wetland (HF) wastewater flows horizontally through the substrate, whilst in the vertical-subsurface flow wetlands (VF) wastewater is dosed intermittently onto the surface and gradually drains through the filter media before collecting in a drain at the base. Among various types of SSFW systems, the HF type has been most commonly used for piggery wastewater. The oldest recorded HF system is at Cooper County Hog Farm, in Springs (Australia) which started in 1987 (Finlayson et al., 1987). The majority of other systems started operating from 2000.

The size of a subsurface treatment for piggery wastewater is based on daily flow rate. Data analyzed from PWDB reported a median required land of 290 m^2. HF systems have a very small external oxygen transfer and a smaller inlet compared to a vertical flow constructed wetland. Therefore they require a larger area. The large SSFW for swine wastewater treatment is reported by Junsan et al. (2000), who used a 4-stage HF constructed wetland with a total surface area of 449 m^2 in China.

Subsurface flow systems are susceptible to clogging, the accumulation of solids shortens the effective life of a constructed wetland, making solids removal a necessary pre-treatment step. Upstream storage ponds or solids separators can remove solids and ideally release only liquid effluent for treatment within the wetlands. Solids separators collect solids and pass the liquid portion of the wastewater to another treatment such as activated sludge reactors for additional nitrogen reduction by nitrification and denitrification (Meers et al., 2008). Solids separators remove 40 to 60% of solids and a significant fraction of the nutrients in wastewater (Zhang and Westerman, 1997). As reported by Harrington et al. (2005), the dilution of piggery wastewater with clean water is a common practice in SSFW operation. The dilution of wastewaters is important in promoting good nutrient removal within constructed wetlands. Moreover, if the organic loading rates are excessive, this can result in decreased removal performances (Kantawanichkul et al., 2003) and an increase in the risk of ammonia toxicity to some constructed wetland plants (Hunt et al., 2002, 2004).

The average daily flow rate in SSFW vary widely, from 2.3 m^3/d (Finlayson et al., 1987) to 81 m^3/d (Junsan et al., 2000), with a hydraulic retention time from 5 (Finlayson et al., 1987) to 28 days (Sánchez-García et al., 2010).

Faulwetter et al. (2009) classified the VF system feeding mode in three distinct categories depending on the wastewater management strategy:

1. Batch feed mode: sequential process in which the VF system is filled with wastewater for a determined period of time (incubation time) and then completely drains before the next batch of effluent is applied (Caselles-Osorio and Garcia, 2007). This method of distributing inflow favours more aerobic processes. Alterations to this design include reciprocating or tidal flow wetlands (Tanner et al., 1999; Austin et al., 2003).
2. Intermittent flow feed: similar to batch feed mode, but the VF system is not completely drained before a new batch of wastewater is added to the system.
3. Continuous flow feed: this is the simplest and therefore most common technique. VF system is continuously fed, hence the media substrate of the system is never drained. This method of distributing inflow has been considered less effective than batch and intermittent flow mode for aerobic pollutant removal (Stein et al., 2003).

The filter bed used for HF system is wide and shallow with a slope of about 1%. The most widespread media material is gravel (ranging from 10-32 mm in diameter), used to fill the bed in the central area to a depth of 0.5-1 m. The inlet and outlet zone are filled with coarse-rock material (grain size 50-100 mm). VF systems are typically composed of rock or crushed gravel of 10-15mm diameter, or in various combinations with sand.

The VF wetlands are usually planted with *Phragmites* spp. and *Typha* spp. (Scholz, 2006), whereas HF contain a number of other emergent macrophytes, including *Iris* spp., *Shenoplectus* spp., *Carex* spp. and *Scirpus* spp. Among macrophytes, *Phragmites* species are preferred due to four principal reasons: (a) resistance to environmental conditions (long flooding periods of the filter surface, dry periods, high organic matter contents) (Brix, 1987), (b) resistance at high pollutant concentrations (Jingtao et al., 2012), (c) fast growth of aboveground biomass, (d) rigorous root penetration into the media (Barbera et al., 2009; Wang et al., 2012). More recently, *Glyceria* spp. are being used because of their high tolerance to the toxicity of ammonia in piggery wastewater (Tylova-Munzarova et al., 2005).

SSFW performance summary

Data from PWDB reported changes in chemical oxygen demand (COD) through SSFW that decreased from 1847 to 246 mg/L for a median abatement of 86.7%. Strusevičus and Strusevičiene (2003) presented the results from a 50 m^2 HF constructed wetland designed to treat pig-breeding farm wastewaters in Lithuania. COD was abated by 68% (inflow 722 and outflow 374 mg/L) (Table 1.3).

Median TKN inflow and outflow concentrations were 801 and 291 mg/L respectively, with a median abatement of 63.7%. High TKN concentrations in pig slurry cause problems with respect to oxygen supply in HF systems since these are considered as anoxic systems (IWA, 2000) and are generally unable to transfer oxygen at sufficient rate to achieve full nitrification (Cooper, 1999). According to Hunt et al. (2006), HF wetlands showed high efficiency in denitrification that dropped the median nitric nitrogen concentration from 39 to 13 mg/L. VF wetlands are generally considered to be highly aerobic systems, since wastewater drains vertically through the planted substrate, allowing for unsaturated conditions and excellent oxygen transfer (IWA, 2000).

Therefore, vertical flow systems have also been piloted (Kantawanichkul et al., 1999; Sezerino et al., 2003). Nitrification was significantly higher in vertical flow compared to surface and subsurface flow wetlands, but denitrification was low (Cooper et al., 1996; Vymazal, 2007; Li et al., 2008). Parkes et al. (1998) reported a NH_4-N abatement of 34.3% by a series of vertical flow reed beds (Table 1.3).

Unlike nitrogen forms abatements, the phosphorus removal mechanism is mainly provided by HF system substrate accumulation. Median phosphorus inflow and outflow concentrations were 31 and 18 mg/L respectively, with a median abatement of 41% (Table 1.3).

Table 1.3 – Treatment performances of SSFW systems for piggery wastewater. Data from PWDB.

SSFW	**COD** (mg/L)			**TKN*** (mg/L)			**NH_4-N** (mg/L)			**NO_3-N*** (mg/L)			**TP*** (mg/L)		
	IN	OUT	A%	IN	OUT	A%	IN	OUT	A%	IN	OUT	A%	IN	OUT	A%
median	1847	246	86.7	801	291	63.7	230	151	34.3	39	13	67	31	18	41
Q3	1865	445	76.2	2629	1506	42.7	249	238	4.42	39	23.5	40	31	20.4	33.1
n.	19	19		9	9		9	9		6	6		7	7	

* Inflow and outflow concentrations data were available from HF system.

Hybrid constructed wetlands treating piggery wastewater

A hybrid constructed wetland (HCW) system incorporates two or more different types of wetlands, frequently being comprised of VF and HF units that are arranged in a two-stage pattern to achieve complete TN removal (Kadlec and Wallace, 2009) (Figure 1.4).

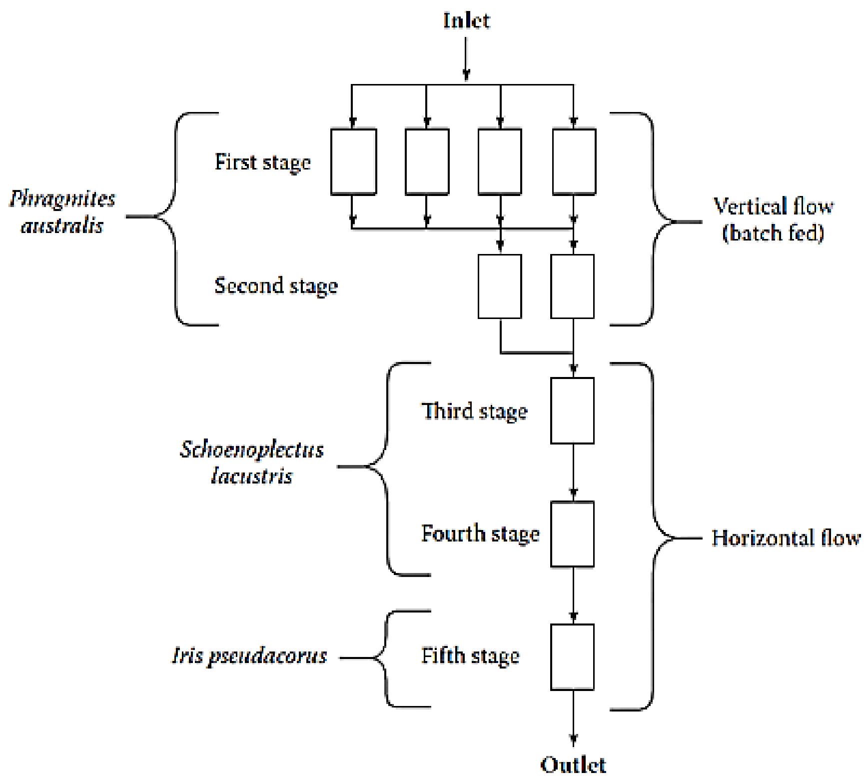


Figure 1.4 - Sample HCW layout from Cooper and Findlater (1990) Constructed Wetlands in Water Pollution Control.

Higher concentration reductions are obtained using the nitrification potential of a VF unit and the denitrification potential of an HF one. Indeed, HF units are known to usually achieve only limited TN removal due to a lack of oxygen flux for the nitrification step (Vymazal, 2005). Many combinations are possible, including vertical followed by horizontal flow wetlands, horizontal followed by vertical and other stages of wetlands including water recirculation from one stage to another (Brix et al., 2003; Kadlec and Wallace, 2009).

- VF - HF combinations. VF wetlands (often two or more stages in combination) are used for TSS filtration, BOD removal, and nitrification; subsequent HF stages are used for effluent polishing (Figure 1.5).

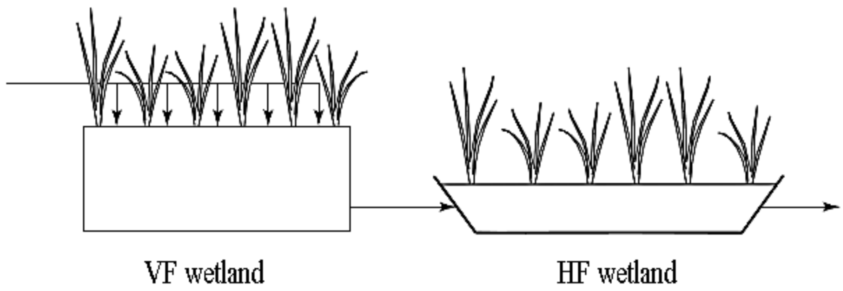


Figure 1.5 – Hybrid wetland system scheme (VF+HF) from Kadlec and Wallace (2009).

- HF - VF combinations. HF initial stage is used to remove BOD and TSS, both of which can be removed in an anaerobic environment. This reduces the overall oxygen demand on the subsequent VF stage, which is designed primarily for nitrification (Brix, 1998).

HCW system has been used to treat domestic or municipal sewage (Brix et al., 2003). However, it has recently been used for many other types of wastewater including agro-industrial (Comino et al., 2011) and landfill leachate (Mæhlum et al., 1999). HCW treating piggery wastewater is an innovative technology. The literature reports mainly pilot-scale and meso-scale experiments (Harrington and Scholz, 2010; Dong and Reddy, 2010; Meers et al., 2008). Only one full-scale case study is reported.

Kato et al. (2010) assessed the performance of a full-scale HCW system to treat raw piggery wastewater. The hybrid system was designed to treat 15 m^3/d, was built in 2010 on a private pig farm in Hokkaido, Northern Japan. The system was composed of four vertical-subsurface flow wetlands (VF), (VF1 of 572 m^2, VF2 of 446 m^2, VF3 of 184 m^2, VF4 of 75 m^2) without circulating pump, working in series, followed by one horizontal-subsurface flow wetland (HF) connected in series (195 m^2). All systems were filled with volcanic pumiceous gravel and planted with *P. australis*. During seven months of operation the HCW system average concentration reduction efficiencies were: COD 70%, TN 36%, NH_4-N 36% and TP 77%.

The high TN concentrations contained in swine effluents caused problems with respect to oxygen supply in VF cells of the HCW system for piggery wastewater. As an alternative to vertical flow, a fill-and-drain mode has been employed with good success (Behrends et al., 2003; Rice et al., 2005). This concept involves continuously pumping wastewater back and forth between adjacent cells in a two-hour cycle. This vertical flow design provides aeration of the gravel substrate and exposes the internal biofilms to atmospheric oxygen. During the "drain phase" of the cycle, atmospheric oxygen causes enhanced oxidation of ammonia and organic matter (Kadlec and Wallace, 2009)

A hybrid arrangement of aerated VF wetland followed by non-aerated HF wetland can provide substantially improved nitrogen removal rates over a single aerated wetland due to: (a) higher oxygenated environment of aerated VF wetland can nitrify the incoming NH_4-N; and (b) the nitrified products can be denitrified in the following anaerobic environment of HF wetland.

Research structure and objectives

The main objective of this PhD dissertation is to assess, at full-scale, the performance of an HCW system with combined VF and HF units for treating pig farm effluents.

The sub-objectives for achieving the main goal are:

I. To compare the performance of the HCW with those observed in its start-up phase (Borin et al., 2013).
II. To compare piggery wastewater treatment performance of VF and HF units.
III. To evaluate if different combinations of bed media matrix and macrophyte plants can affect system performance.
IV. To test and compare different wastewater feeding management (intermittent and batch) in order to determine optimal loading, management schemes and operational regimes for VF system.
V. To determine if winter climate conditions influence treatment effectiveness, and clarify the effect of temperature on nitrification and denitrification process.
VI. To make sustainable recommendations for HCW system design, maintenance operations and macrophyte growth in order to optimize piggery wastewater treatment performance.

Chapter II

The case study

Materials and methods

Site description

The experiment was conducted from 2010 to 2012 at a private pig fattening farm in Carmignano di Brenta, Padova, in Veneto Region, NE Italy (E: 11419.58 m; N: 453745.16; 46 m a.s.l). The piggery housed approximately 1000 pigs (with a fattening cycle of one year, from 15 to 175 kg of live weight). The hybrid constructed wetland (HCW) was built in 2008 and was designed to provide tertiary treatment of pre-treated liquid fraction of pig slurry effluent. The design guidelines provided by APAT were based on municipal wastewater (ARPAT-APAT, 2005). Starting from 2010 the HCW system was adjusted to the needs of the experiment. In September 2010 a mechanical water meter with five digit mechanical counter was installed at the inlet of each vertical-subsurface flow wetland cell to measure the volume of wastewater applied. In October 2010, a submersible water pump controlled by timers was placed inside the water level control structure of each VF cell to manage the VF system in batch feed mode. In May 2011 water meters were also installed at the inlet and outlet of the horizontal-subsurface flow wetland.

Hybrid constructed wetland basic configuration and characteristics

The HCW system was composed of three vertical-subsurface flow wetlands (VF) in parallel with a total area of 30 m^2 followed by one horizontal-subsurface flow wetland (HF), connected in series (100 m^2) (Figure 2.1).

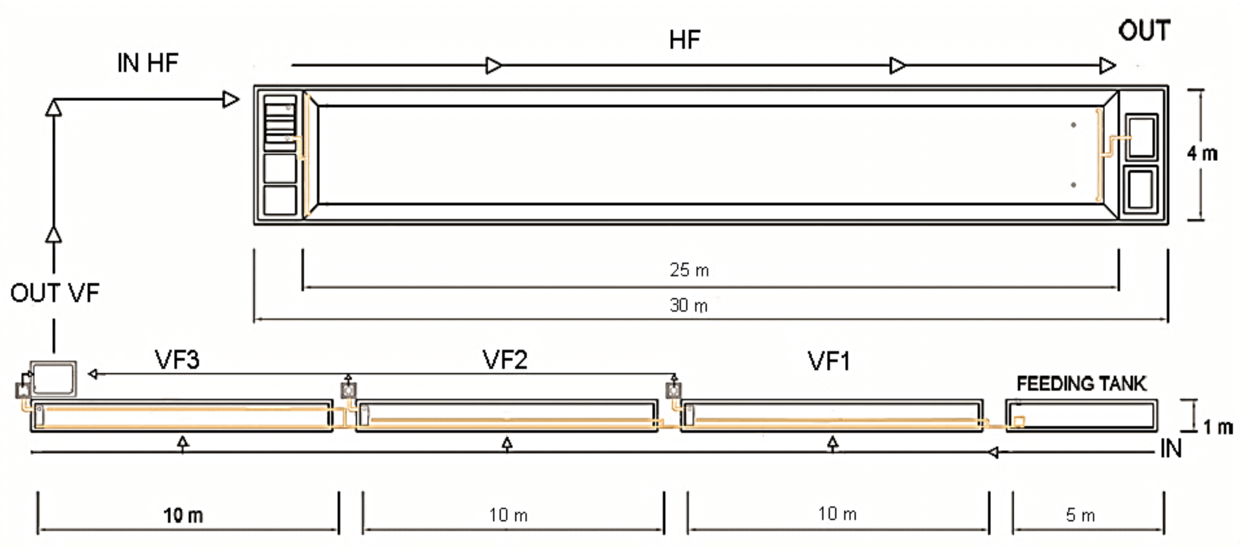


Figure 2.1 - Overhead view of HCW system.

Each VF cell was built in concrete (length: 10 m; width: 0.7 m; depth: 0.7 m) (Figure 2.2a). Three plastic sheet liners were placed inside each cell to prevent leakage and contact of wastewater with groundwater. These were two layers of nonwoven geotextile sheeting with a basic weight of 400-800 g/m^2 and thickness 1 mm with an interlayer EPDM geo-membrane. The first two cells were filled with washed gravel, diameter 10–20 mm (d_{10} = 8.5 mm; d_{60} = 9.7 mm) with porosity of 40%. The first one (VF1) was vegetated with *Canna x generalis*, the second (VF2) with *Phragmites australis* (Cav.) Trin. Ex Steud. (Common reed). The third (VF3), planted as VF2 cell, was filled with a 0.10 m deep gravel layer (diameter 10–20 mm) overlying a 0.10 m deep coarse sand (diameter 3–5 mm) and zeolite (diameter 5–10 mm) transition layer and a 0.30 m deep gravel drainage layer (10–20 mm in size). The main components of the zeolite are: chabasite 60%, K-feldspar 13%, phillipsite 5%, mica 5% and augite 2%. Zeolite was used by the HCW system owner as a test to assess if it would increase the ammonium nitrogen abatement performance according to what is reported in the literature (Nguyen and Tanner, 1998).

The piggery wastewater was distributed evenly over the surface of the VF beds by a pressurized PVC distribution pipe 75 mm in diameter that ran along the VF wetland unit. A drainage pipe (diameter 75 mm, length 10 m) was located on the bottom of each VF cell in order to facilitate effluent collection. The drainage pipe of each VF cell was connected on one side to a 100 mm diameter collection pipe that discharges the effluent from the bed to a manhole that has a water level control structure. The siphon maintained water level at 0.30 m from the surface in each VF cell allowing prevailing unsaturated conditions in the upper part and saturated in the lower part. The wastewater discharged from each VF cell was collected in a common sump (OUT VF) (length 1.2 m, width 0.8 m, depth 0.60 m). The VF effluent was then transferred by a submersible sump pump with an integrated float switch to the horizontal subsurface flow wetland.

The horizontal-subsurface flow wetland (HF) was a basin of 25 m long, 4 m wide and depth 0.7 m with a bottom slope of 1%. The bottom and walls of the basin were waterproofed with the same plastic sheets as those used for VF cells. The inlet and outlet sections were filled using two strips of coarse-rock material (diameter 50–100 mm) along two opposite edges of the basin, with washed gravel: grain size 10-20 mm (d_{10} = 8.5 mm; d_{60} = 9.7 mm) with porosity of 40% placed in the central area. At the HF inlet a distributor pipe was buried immediately below the surface (diameter 100 mm), placed horizontally and perpendicular to the direction of flow. At the outlet, a similar collector pipe was buried at the bottom. The HF effluents were collected in an interred tank and recycled for cleaning the piggery. The wetland was planted in April 2008 with *Phragmites australis* (Cav.) Trin ex. Steudel (Common reed) using 8 stems per m² (Figure 2.2b).

Figure 2.2a - General view of VF unit and cell details **Figure 2.2b** - General view of HF unit and cell details, August 2010.

Experimental set up and management

During the experimentation, there were three distinct periods depending on: presence or not of specific swine slurry pre-treatment, different hydraulic loading rate (HLR), different hydraulic retention time (HRT), different feeding mode and operational regimes.

1. April 2010-July 2010 (first period)
2. October 2010-April 2011 (second period)
3. May 2011-July 2012 (third period)

April 2010-July 2010 (first period)

During the first monitoring period, pig manure and daily yard washings passed through the floor of the barn that is 1/3 grid and 2/3 brick, and were collected in a central storage facility consisting of a holding tank where the slurry underwent some sedimentation. The amount of raw manure from the tank effluent treated daily was set at 8-10 m^3. A mechanical separator with a rotating 120 μm mesh screen separated this into liquid (4-5 m^3/d) and solid fractions (4-5 m^3/d). The liquid phase passed through a biological aerobic treatment with the capacity to treat up to 5 m^3/d and a residence time of 24 h. During the whole investigation period this system did not seem to provide the expected reliability. The effluent from the reactor was collected and stored in an underground storage settling tank (400 m^3 capacity). A submersible sump pump with an integrated float switch transferred pre-treated wastewater from the settling tank into the feeding cell (pump chamber). The feeding cell consisted of a 5 m x 1 m concrete tank 0.7 m deep, with a submersible sump pump inside to feed the HCW. The pump was controlled by two programmable timers in series installed in the service building of the farm. One timer dictated the time of loading cycles and was set to work twice per day, with one cycle in the morning from 7am to 9am and one in the evening from 6pm to 8pm. The other timer dictated the intermittent pulse feeding time within each loading cycle (alternation of one minute load and 4 minutes stop). During the rest (recuperation) period, the wastewater percolation favoured the transport of oxygen from the surface downwards. In addition, intermittent flushing allowed the surface to dry out for certain periods of time (Von Felde and Kunst, 1997). Flow rate (Q) of about 2.5 m³ was evenly distributed to all three VF cells per loading cycle for a total of 5 m^3/d. Hydraulic loading rate (HLR) was 3.8 cm/d. Hydraulic retention time (HRT) was a minimum of 4 days (an optimal value is between 4 and 5 days).

The water level control structure of each VF cell was equipped with a siphon pipe (Figure 2.3). The siphon maintained water level at 0.30 m from the surface, allowing prevailing unsaturated conditions in the upper part and saturated in the lower part.

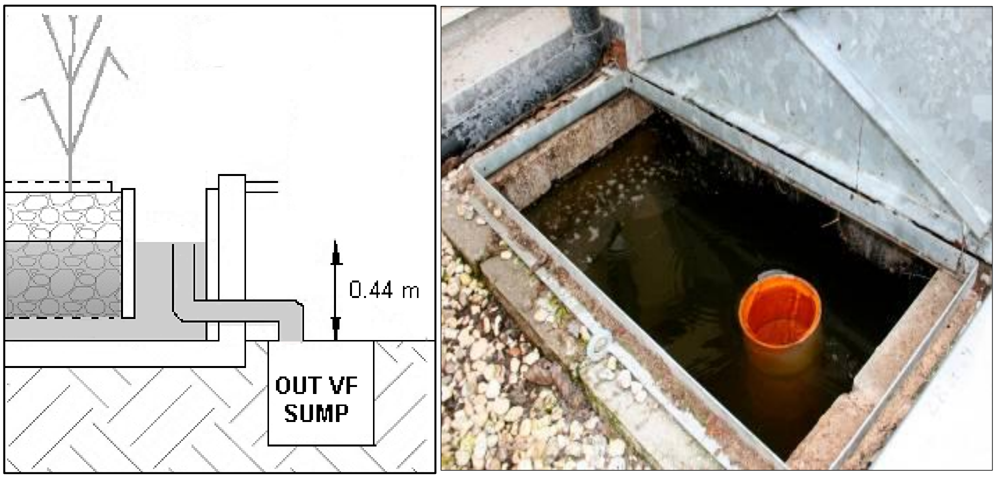


Figure 2.3 - Schematic representation (not to scale) of water level control structure of each VF cell during the first period (April 2010-July 2010).

October 2010-April 2011 (second period)

During the second period, pre-treatment consisted only of a mechanical separator which removed the solid phase with the largest particle size. The HCW system was fed daily with 5 m^3 of liquid swine slurry. To enhance oxygen transfer for the nitrification process and minimize the adverse effect of clogging, a sequential batch (feed-stay-drain-rest) feed mode was used. This is a sequential process in which when the VF unit was filled with wastewater, air was repelled from the substrate and anoxic conditions developed over a determined period of time (incubation time). During the draining process, an additional flux of oxygen was sucked into the VF substrate, which aerobic treatment processes favoured. Sequencing batch operation has the potential to enhance the removal of COD through aerobic processes and the removal of ammonia nitrogen through nitrification, as the maximum pollutant-biofilm contact is established and the rate of oxygen transfer is increased during the operation (Sun et al., 1999).

Draining of the VF substrate was generated by submersible water pumps controlled by timers placed inside the water level control structure of each VF cell (Figure 2.4).

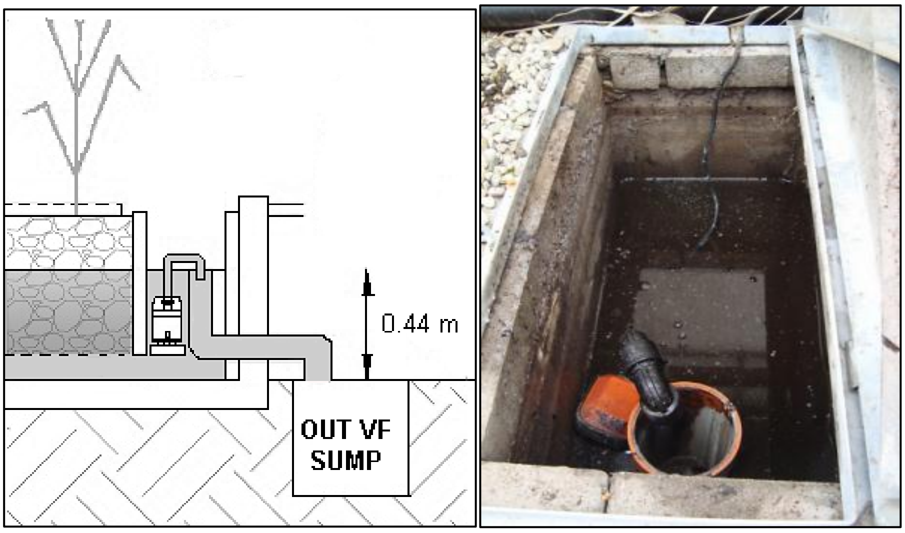


Figure 2.4 - Schematic representation (not to scale) of water level control structure of each VF cell during the second period (October 2010-April 2011).

The sequential batch feed mode was characterized by predetermined feeding and rest periods, summarized in Table 2.1. The operational regime (OR) was set as: intermittent pulse feeding of 4 hours to improve wastewater distribution and maintain sufficient aeration, followed by a fill period of 8 hours (incubation time). From 6 am to 10 am a draining period allowed air to be drawn from the atmosphere into the substrate of each VF cell. A subsequent rest (recuperation) period was initiated, in which the enhanced decomposition of organic particles using O_2 transferred into the substrate when it was unsaturated with the wastewater (Zhao et al., 2004).

Table 2.1 – Operational regime (OR) scheme VF unit period 2 (October 2010-April 2011).

Step	VF unit period	Duration (hour)	Schedule	Eh range (mV)	Conditions
I	feeding period	4	6 pm - 10 pm		
II	fill period	8	10 pm - 6 am	+30 to +40	anoxic
III	draining period	4	6 am - 10 am		
IV	rest period	8	10 am - 6 pm	+200 to +270	oxic

May 2011-July 2012 (third period)

During the third period, the system was loaded daily with synthetic wastewater (a commercial fertilizer was used) to simulate the characteristics of pre-treated piggery wastewater. The wastewater was prepared daily in the feeding tank just before the feeding by dissolving ammonium nitrate fertilizer 26% N (13% Nitrate and 13% Ammoniacal) in 1.7 m³ of freshwater maintained at a temperature above 9 °C to avoid freezing. The average concentration of synthetic wastewater used was 250.3 (±3.7) mg/L of TN, 124.5 (±2.5) mg/L of NO_3-N and 124.9 (±3.2) mg/L of NH_4-N. Water meters with a five digit mechanical counter were attached: at the inlet of each VF cell, at the outlet of VF common sump and at the outlet of HF unit in order to measure wastewater volumes. Daily flow rate (Q) of applied to HCW system was 1.7 m³ that was evenly distributed to all three VF cells (average flow rate of 565 L/day per VF cell). Hydraulic loading rate (HLR) of the entire HCW system was 1.3 cm/d and the hydraulic retention time (HRT) was 7 days as minimum (an optimal value is placed between 7 and 8 days). From May to July 2011 and from January to 11th July 2012 the wastewater operational regime (OR1) was set as presented in Table 2.2. Intermittent pulse feeding of 2 hours was adopted in the wastewater application of VF cells, followed by a fill period (incubation time) of 6 hours. From 6 to 8 pm, VF system was drained to provide a rest period of 14 hours in order to assist the oxidation of accumulated organic particles within the substrate.

Table 2.2 – Operational regime 1 scheme VF unit period 3 (From May to July 2011 and from January to 11th July 2012).

Step	VF unit period	Duration (hour)	Schedule	Eh range (mV)	Conditions
I	feeding period	2	10 am - 12 am		
II	fill period	6	12 am - 6 pm	+30 to +40	anoxic
III	draining period	2	6 pm - 8 pm		
IV	rest period	14	8 pm - 10 am	+200 to +270	oxic

A different operational regime was used (OR2) in the last two monitored cycles of the investigation period (11^{th} and 18^{th} July 2012). As summarized in Table 2.3, fill (incubation) period was set from 12 am to 2 pm for 14 hours and rest period for 6 hours in order to assist the anoxic condition and promote denitrification.

Table 2.3 – Operational regime 2 scheme VF unit period 3 (11^{th}-18^{th} July 2012).

Step	VF unit period	Duration (hour)	Schedule	Eh range (mV)	Conditions
I	feeding period	2	10 am - 12 am		
II	fill period	14	12 am - 2 pm	+0.5 to +10	anoxic
III	draining period	2	2 pm - 4 pm		
IV	rest period	6	4 pm - 10 am	+150 to +170	oxic

Monitoring activities and analysis

During the whole investigation period, wastewater samples were collected from inflow (1; IN), outflow of VF1 (2; OUT VF1), outflow of VF2 (3; OUT VF2), outflow of VF3 (4; OUT VF3), inflow of HF (5; IN HF), outflow of HF, final effluent (6; OUT HF) (Figure 2.5).

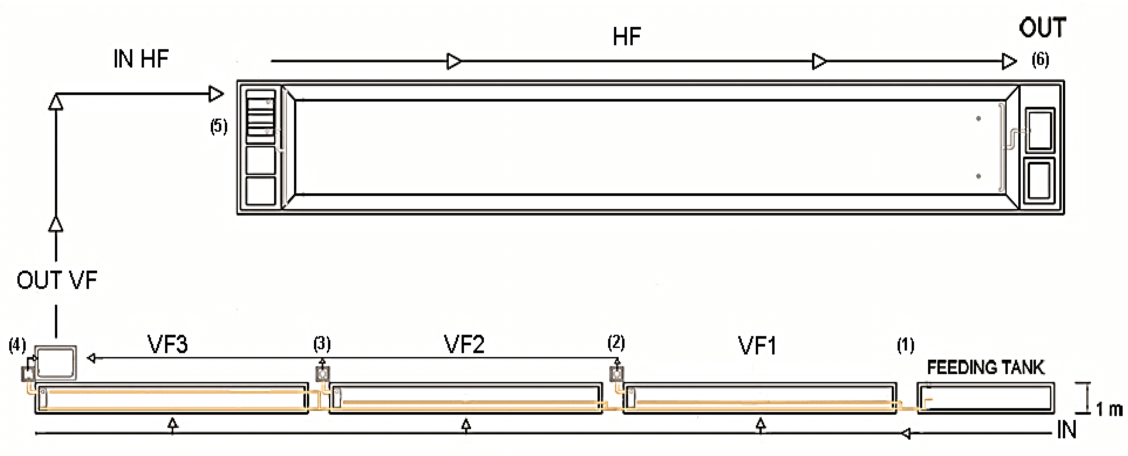


Figure 2.5 - Sampling points: (1) influent, (2) VF1 effluent, (3) VF2 effluent, (4) VF3 effluent, (5) influent of HF, (6) HF effluent (final effluent).

During the first period, wastewater samples were collected weekly, for a total of 15 samplings with a total of 90 samples. For each sample 18 parameters were evaluated (Table 2.4).

During the second period, wastewater samples were collected twice per month, for a total of 12 samplings with a total of 72 samples. For each sample 18 parameters were evaluated (Table 2.4).

During the third period, wastewater samples were collected and volumes passed through HCW system were also measured. These operations were carried out weekly. The entire period was divided in two distinct sub-periods: the first from May to July 2011 with 11 samplings and the second from January to July 2012 with 26 samplings, for a total of 37 samplings and a total of 222 samples. For each sample 9 parameters were evaluated (Table 2.4).

Table 2.4 - Parameters monitored in the three experimental periods

Parameters	**Unit**	**Period 1**	**Period 2**	**Period 3**
Wastewater temperature (T)	°C	x	x	x
Electrical Conductivity (EC)	µS/cm	x	x	x
pH	-	x	x	x
Dissolved Oxygen (DO)	mg/L	x	x	x
Redox potential (E_h)	mV	x	x	x
Turbidity	NTU	x	x	
Chemical Oxygen demand (COD)	mg/L	x	x	x[a]
Total Nitrogen (TN)	mg/L	x	x	x
Ammonia Nitrogen (NH_4-N)	mg/L	x	x	x
Nitric Nitrogen (NO_3-N)	mg/L	x	x	x
Nitrite Nitrogen (NO_2-N)	mg/L	x[a]	x[a]	
Total phosphorus (TP)	mg/L	x	x	
Ortho phosphate (PO_4-P)	mg/L	x	x	
Sodium (Na^+)	mg/L	x	x	
Potassium (K^+)	mg/L	x	x	
Magnesium (Mg^{2+})	mg/L	x	x	
Calcium (Ca^{2+})	mg/L	x	x	
Chloride (Cl^-)	mg/L	x	x	
Sulphate (SO_4^{2-})	mg/L	x	x	

[a] parameter measured occasionally

Water chemical analysis

Wastewater temperature (T), pH, dissolved oxygen (DO), redox-potential (E_h) and electrical conductivity (EC) were tested on site at a depth of 5 cm below the water surface with a Hach Lange HQD 40d multi-parameter meter with interchangeable probes according to standards methods (APHA, 1998). Before testing, each probe was carefully calibrated according to the manufacturer's procedures. Samples were collected, preserved at 4 °C and then analyzed within a short time. Turbidity was measured on site (with adequate sample dilutions) with a portable Hach Lange 2100P turbidimeter.

Chemical Oxygen Demand (COD), total nitrogen (TN), ammonia nitrogen (NH_4-N), nitric nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), total phosphorus (TP) and orthophosphate (PO_4-P) were determined photometrically using a Hach-Lange DR-2800 spectrophotometer and adequate cuvette test kits (cuvette-tests LCK 338, 302, 340, 414, 342). At the exit from the biological aerobic treatment, the nitrite nitrogen (NO_2-N) measurements were abandoned because the concentrations detected during the initial investigation period were negligible. The ISO 7150-1 method was used for ammonium analysis and the ISO 7890-1-2-1986 method for nitric analysis (Hach-Lange, 2008), according to DIN (1985). Adequate sample dilutions were made with a stock supply of deionised water. For the cations determination of Sodium (Na^+), Potassium (K^+), Magnesium (Mg^{2+}), Calcium (Ca^{2+}) and anions of Chloride (Cl^-) and Sulphate (SO_4^{2-}), an Ion chromatography system (ICS-900, Dionex) equipped with an anion exchange column (Ion Pac AS23) and a cation exchange column (Ion Pac CS12A) was used, following Methods 300.0 (Pfaff et al., 1989).

Vegetation sampling and analysis

Vegetation was completely harvested from VF and HF units in December 2010, November 2011 and October 2012 by cutting the stems at a height of 10 cm. The collected aboveground biomass was weighed on site for total fresh weight. Samples were dried at 65 °C in a forced draught oven for 36 hours, then 1 g powered samples were dried at 130 °C to measure the residual moisture content. Samples were then analyzed to determine nitrogen content using the AOAC official method (AOAC, 2002). In December 2010 a dry sample of aboveground biomass from HF unit was mechanically pulverized and a pill of 1 g in weight was prepared for the heating value determination. The Higher Heating Value (HHV [MJ/Kg]) was evaluated directly with the Mahler bomb, the Lower Heating Value (LHV [MJ/Kg]) was estimated from the HHV and from the moisture content of the samples at harvest. Combustion energy (C.E.%) was calculated as: C.E. = [Total fresh weigh × LHV]. Experimental tests were done at the Experimental and Didactic Centre in Cadriano (Bologna) using a commercial boiler (Alpina 35, ALPI, Poggio Rusco, Italy) that can produce a thermal energy power of 29 kW.

Tracer test with rhodamine WT

Tracer experiments (Dierberg and DeBusk, 2005) using the organic fluorescent tracer Rhodamine WT (RWT) were performed during 14 days in March 2011 to describe the hydraulic behaviour of the HF system. The test was carried out in collaboration with Environmental System Analysis Lab (LASA) of Department of Chemical Processes Engineering of Padova University. RWT tracer has already been used in numerous hydrological studies (Turner et al., 1991); applications of RWT as a tracer have also been reported for free water surface wetlands (Lin et al., 2003) and horizontal subsurface flow constructed wetland (Sandoval-Cobo and Pena, 2007). The HCW system was loaded daily with 1.7 m^3 of fresh water. At the beginning of the loading cycle an RWT tracer injection of 15 ml was performed every thirty minutes in VF system. At the end of the loading cycle, sixteen RWT tracer injections suitable for obtaining the desired inlet tracer concentration were performed (Figure 2.6).

Figure 2.6 – Wastewater with rhodamine WT (RWT) tracer at sampling point (5) (influent of HF) – March 2011.

An ISCO® 6712 portable auto-sampler was installed at the outlet of HF unit in order to collect a water sample every three hours from day 0 (start of loading cycle) to day 14 (end of tracer test). By the end of the measurement campaign 143 water samples had been collected at HF outlet. Each water sample was analyzed with a portable fluorometer (SCUFA by Turner Designs, USA) to measure the RWT concentration. The results are discussed in chapter IV.

Meteorological data

Air temperature, humidity, solar radiation, rainfall volumes, wind speed and direction were recorded on site, from 11th June 2010 at 24 hour intervals, using a meteorological station (CR 800 series, Campbell Scientific). ET_O with the FAO 56 approach (Allen et al., 1998) for short reference crop ET_{os} was then calculated (Allen et al., 2005).

Microbiological analysis

Analysis of wetland biodegradation level was evaluated following a novel method and using a purposely defined device (University of Padova, Italy, PCT/IB2012/001157, June 13, 2012, Squartini, Concheri, Tiozzo) measuring the degradation of cotton threads that are placed in the gravel layer of VF and HF units for a week in June 2010. A small vertical hole was made in the gravel with a spade, then threads (15 cm long) were carefully placed vertically and the hole was filled with gravel. In each VF cell and at the inlet and outlet sections of HF unit, three sets of cotton threads (untreated- yellow label, pre-treated with nitrogen- red label and pre-treated with phosphorus- green label) were buried for a total of 15 threads (three treatments x five points) (Figure 2.7).

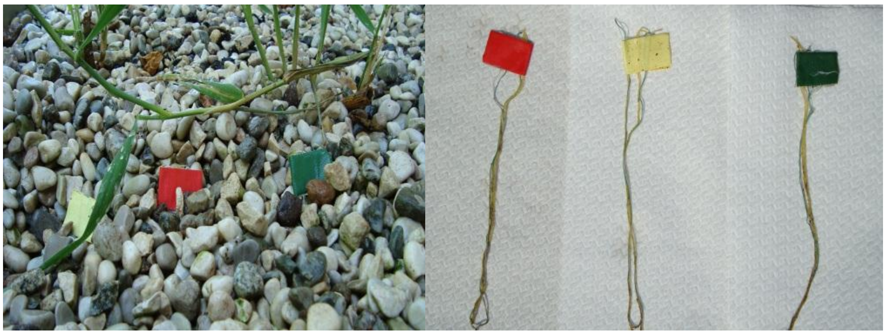


Figure 2.7 - Analysis of soil biodegradation level of hybrid system. Yellow label control, red label N-treated and green label P-treated. (June 2010).

The nitrogen or phosphorus pre-treated versions of the threads, as defined by the above quoted patent, are used to assess to what extent such additions can further stimulate microbial activity in comparison to the plain untreated threads, i.e. whether availability of nitrogen or phosphorus limited thread decomposition. For this purpose they had previously been impregnated with different solutions containing 37.5 mM NH_4NO (N-treated) or 42.3 mM Na_2HPO_4, 22 mM KH_2PO_4 (P-treated), respectively. After a week, threads were gently extracted from the gravel and air-dried. Their residual resistance to breakage was determined using a digital dynamometer (IMAD ZP, ELIS Electronic Instruments and Systems, Rome, Italy) to measure the peak force required to rupture them by applying progressive tractional force. The following formula was adopted to determine the thread resistance: R = (Ri / Rni) x 100 (where: R= resistance percentage; Ri = rupture value of the thread buried in the soil; Rni = rupture value of a control filament when new). Resistance was converted into the degradation percentage (D) by subtracting resistance percentage values from 100.

Data elaboration

All statistical analyses were carried out using the computer software package STATISTICA 7.0 (Statsoft Inc., 2004). The data series of the parameters did not follow normal distribution even after transformations. Thus, statistical analyses were carried out with the Kruskal–Wallis non parametric test to compare the six sampling positions (position 1; IN), (position 2; OUT VF1), (position 3; OUT VF2), (position 4; OUT VF3), (position 5; IN HF) (Gilbert, 1987) and Box and Whiskers were used to present the data. Different letters were used to indicate significant differences at ($p < 0.05$) by Kruskal–Wallis test.

For the whole investigation period (first – second – third monitored period), pollutants reduction performance of the HCW system and performance comparison of each unit was expressed in percentage of concentration abatement (A). Concentration abatement was calculated on second quartile (Q2; median) concentration values as: A = [(Cin – Cout)/Cin] ×100, where Cin is inflow concentration (mg/L) and Cout is outflow concentration (mg/L).

During the third monitored period, the performance comparison of HCW system units was also based on two different approaches:

1. Mass reduction efficiency (MRE) calculated as: MRE = [(Cin*Vin)–(Cout*Vout)/(Cin*Vin)]×100, where Cin is the inflow concentration (mg/L), Vin is the average inflow volume of synthetic wastewater applied (m^3/d) with daily rainfall volume (mm/d) included, Cout is the outflow concentration (mg/L), Vout is the outflow volume detected at the outlet of the unit (m^3/d);
2. Surface load reduction (SLR) is the difference between inlet pollutants loading rate with daily rainfall volume and outlet loading rate expressed per unit of surface. TN concentrations associated with rainfall ranged from 2.3 to 3.5 mg/L (U.S. EPA, 1993), with more than half present as ammonia (Kadlec and Wallace, 2009). SLR is usually expressed as the removed pollutants mass per CW surface unit area and time (g/m^2/d), and represents a useful parameter to assess system efficiency (Kadlec and Wallace, 2009).

In the third period data set, segmented linear regression analysis (or broken-line regression) with a non-parametric approach developed by Pettitt (1979) was used to identify a change-point of wastewater temperature that caused variations in nutrient removal performance. Partial F-test in one-way analysis of variance was used to determine any significant differences at ($p < 0.05$).

Chapter III

Results first period: April 2010-July 2010

Results

On site parameters

Wastewater temperatures measured through the system did not vary noticeably, the median value was above 15 °C. According to Reddy and Patrick (1984), water temperatures lower than 15 °C or higher than 30 °C can drastically reduce the growth rate of nitrifying bacteria, thus limiting the denitrification rate (Figure 3.1).

pH measured at HCW influent showed values ranging from 7.5 to 8.06, (slightly alkaline conditions). Inflow median of 7.89 dropped to 7.6 after passage through VF cells that exhibited values ranging from 7.31 to 8.01, probably due to the nitrification process consuming alkalinity (Kadlec and Knight, 1996). The following passage to HF system caused a slight increase in pH to a median value of 7.81 linked to the reduction in nitrate (Sezerino et al., 2003) (Figure 3.1). As nitrification proceeds optimally at pH between 7.5 and 8.5 (Platzer, 1996), the pH values were optimal in all three VF cells. Literature reports show that the denitrification process can be hampered at pH < 6.0 and pH > 8.0, with the optimal range being between 7 and 7.5 (U.S. EPA, 1975), hence the pH value measured in the HF unit (7.81) results as suitable for the process. Furthermore ammonia nitrogen loss through volatilization would probably be negligible since it generally requires a pH of 9.3 (Jing and Lin, 2004).

The median inflow concentration of DO (1.87 mg/L) slightly decreased after passage through VF cells to 1.57 mg/L. This is surprising because it is well known that vertical flow systems are aerated, but could be due to the particular design and management of the vertical cells. In fact the presence of the syphon maintains the deepest 0.4 m of the bed in predominantly saturated conditions that probably ensure less than optimal substrate aeration (Figure 3.1). As the nitrification process only occurs under aerobic conditions (DO concentration at least ≥ 1.5 mg/L) (Ye and Li, 2009), DO values were sufficient in all three VF cells. Median DO concentration measured at HF inflow (0.04 mg/L) was significantly lower than the previous passage, probably because the residence of the wastewater in the pipes connecting vertical cells and sump might have further decreased oxygen concentration. DO concentration then increased significantly to 0.29 mg/L, probably linked to the *P. australis* root system oxygen released into the surrounding rhizosphere, which facilitates aerobic degradation of pollutants (Moshiri, 1993). Biddlestone et al. (1991) stated that *P. australis* has the ability to pass oxygen, from its leaves through stems and rhizomes and out from its fine hair roots into the root zone or rhizosphere. Literature reports on the oxygen release ability of *P. australis* give different value ranges. Armstrong et al. (1990) recorded oxygen release (per unit wetland area) by *P. australis* to be in the range of 5-12 g $O_2/m^2/d$, whereas Bavor et al. (1988) estimated oxygen release by *P. australis* species to be approximately 0.8 g O_2/m^2on gravel wetland substrates.

Moderately reduced redox conditions (+126 mV) were detected at HCW inflow (Fig. 3.1). Median influent wastewater redox potential slightly decreased to 112 mV after VF cells passage, this suggests that the VF system maintains aerobic conditions suitable for nitrification (+100 mV < E_h < +350 mV) (Vymazal, 2005) but not for the denitrification process(+50 mV <E_h< -50 mV) (Knowles, 1981).The E_h value measured at HF inflow decreased drastically in the range from -53 to -33 mV probably due to oxygen-limited condition suitable for anaerobic microbial populations (Kadlec and Knight, 1996; Faulwetter et al., 2009) (Figure 3.1).

Electrical conductivity (EC) of the influent varied widely from 1875 to 2670 µS/cm. The median value of 2240 µS/cm was significantly reduced (36%) after passage through the VF unit. No statistical differences were found between the three VF cells, though median EC values measured in VF1 and VF2 effluent were similar (1436 and 1426 µS/cm respectively) and higher than inVF3 with 1126 µS/cm. HF unit dropped the EC value further to 1068 µS/cm. (Figure 3.1).

Turbidity measured at the system inlet ranged between 250 and 540 NTU, the median value of 380 NTU did not differ significantly after VF unit passage, being 320 NTU. HF unit further reduced it significantly to 200 NTU, which might be linked to higher contact time between the influent and the gravel bed media in the HF unit (Figure 3.1).

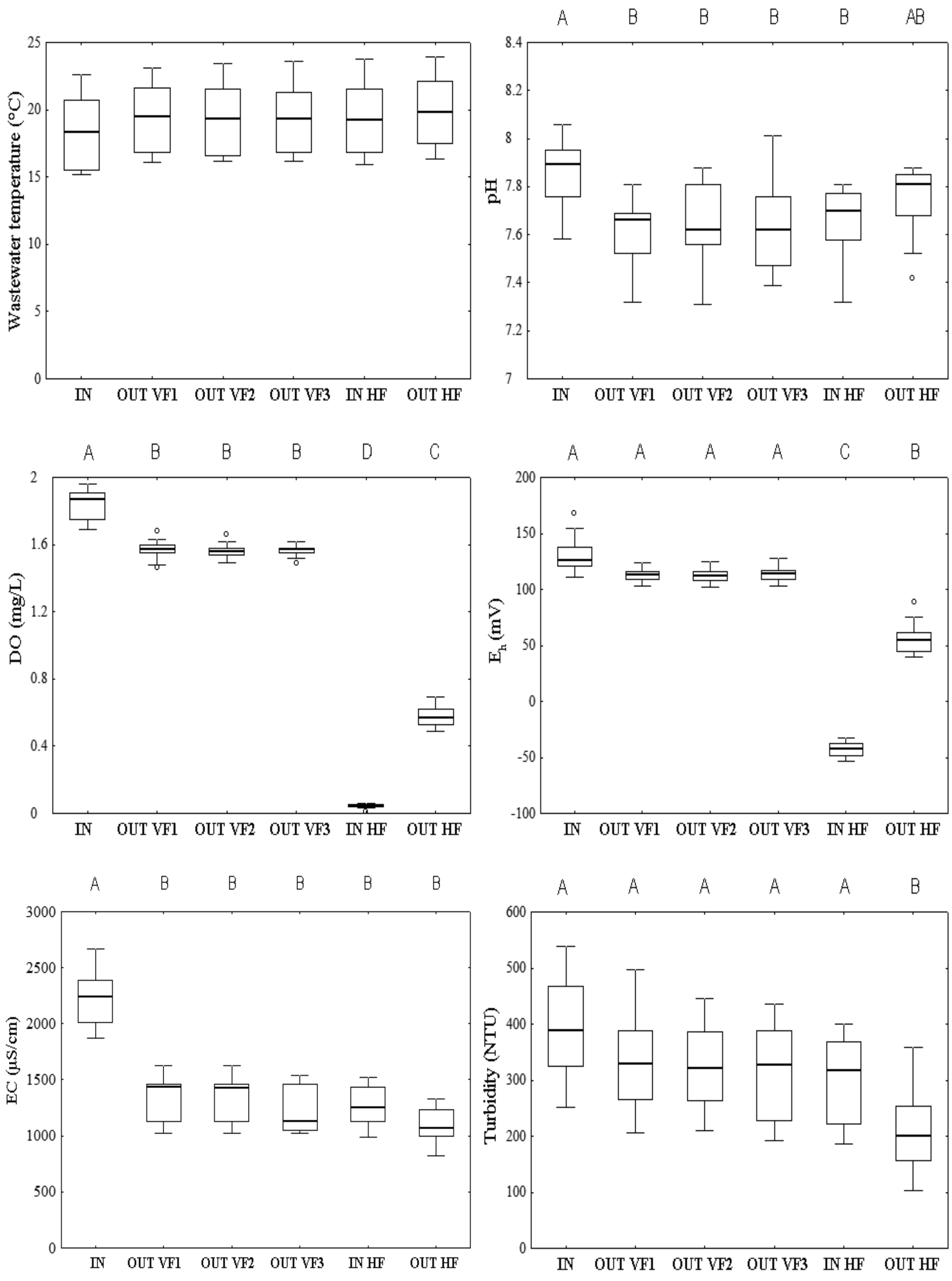


Figure 3.1 - Box-plot diagrams of wastewater temperature (T), pH, dissolved oxygen (DO), redox potential (Eh), electrical conductivity (EC) and turbidity in the sampling points of the hybrid system during the first monitored period (April-July 2010).

COD

COD influent concentration fluctuated over time. This might be related to the low and unsteady pre-treatment efficiency performed by the biological aerobic treatment. At the lower COD input (140 mg/L) detected on 7th and 14th May, similar concentrations were measured in the effluent of both units: 100 and 73 mg/L from VF and HF respectively. HF unit enhanced the COD concentration reduction provided by VF cells for all the whole period, except on 30th April when higher effluent concentration was measured (103.5 mg/L) (Figure 3.2).

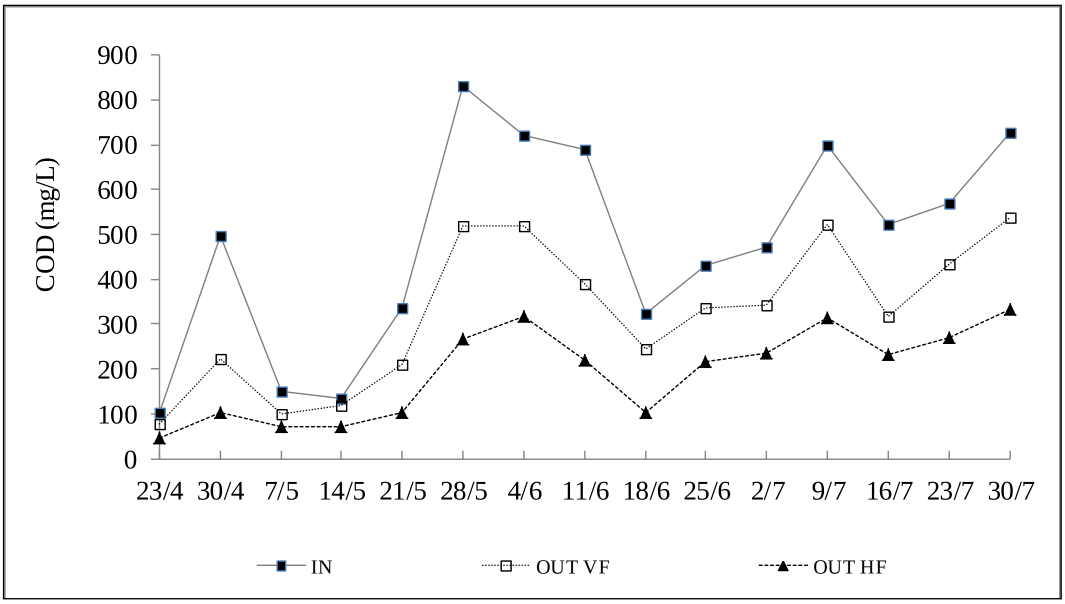


Figure 3.2 - COD concentration at the sampling points of the hybrid system during the first monitored period (April-July 2010).

COD concentration measured at the inlet varied widely between 104 and 831 mg/L. The median influent value was 498 mg/L, the same order of magnitude reported by Poach et al. (2007) in a marsh-pond-marsh (m-p-m) constructed wetland treating pig effluent in Greensboro, NC, USA. VF cells gave a greater contribution to the abatement, dropping the median value to 330 mg/L and sharply reducing the variability. No statistically significant difference was found between VF cell effluent concentrations. HF cell reduced the concentration to a final discharge of 221 mg/L. Variability in HF was lower than in VF cells (Figure 3.3).

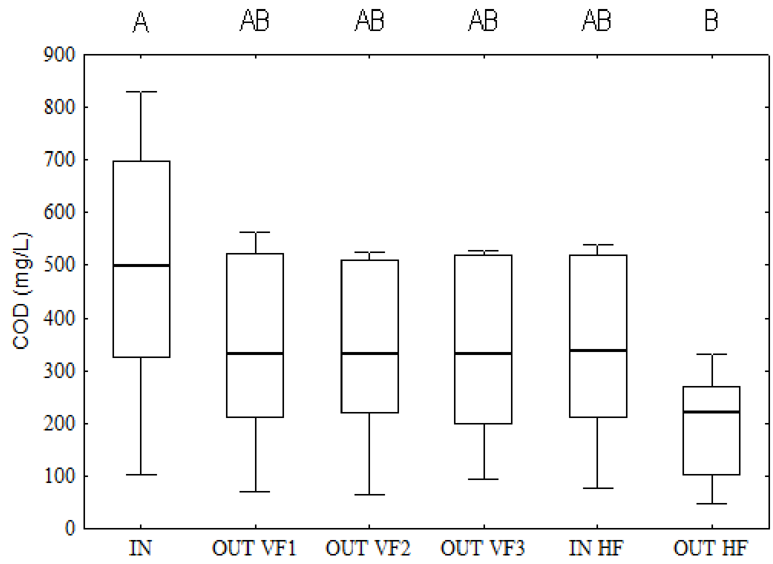


Figure 3.3 – Box-plot diagrams of COD concentration (mg/L) in the sampling points of the hybrid system during the first monitored period (April-July 2010).

Nitrogen forms

TN influent concentration ranged from 203 to 668mg/L, with a median value of 416mg/L;a similar inlet value was reported by Reaves et al. (1994) from the Livestock Wastewater Treatment Wetland Database (420 mg/L) with a system composed of sixteen parallel unlined wetland cells. The measured values were always above 200 mg/L with inconstant fluctuations, the highest concentrations being measured on 4th June and 30th July (>660 mg/L). These results were mainly attributable to differences in pig-waste composition, farm management and storage conditions (Figure 3.4a).The abatement achieved by VF and HF did not show a clear time pattern: on certain dates the higher contribution was given by the VF system, on others the two systems gave similar contributions and the HF only had best performance once. VF system dropped the median value to 290 mg/L but maintained high variability. No statistically significant differences were found for TN effluent concentration between VF cells. HF system accomplished abatement to a final discharge concentration of 198 mg/L and a marked reduction in the interquartile (Figure 3.5).

As expected (Chastain et al., 2003), the ammonia form represented the major fraction (55%) of total nitrogen loaded in the HCW system. From 7th May to 4th June a progressive increase of NH_4-N influent concentration was measured (from 110.6 to

398.8 mg/L). After that, the concentration followed the same time pattern shown by TN form (Figure 3.4b). Ammonia removal is pH and temperature dependent (Wallace et al., 2006). During the monitored period, pH ranged from 7.5 and 8, therefore ammonia loss through volatilization from VF unit can be considered negligible because the volatilization process generally requires a pH of 9.3 (Jing and Lin, 2004). VF unit gave a reduction in ammonia concentration (39%) despite the limited oxygen content (1.55 mg/L median) detected in each cell. Several studies (Yalcuk and Ugurlu, 2009; Saeed and Sun, 2011) reported that zeolite could be an effective substrate in terms of NH_4-N removal from wastewater. However its presence in VF3 cell did not significantly enhance the ammonia nitrogen removal with respect to the other two cells. This is surprising because Ling et al. (2011) stated that NH_4-N sorption capacity of the zeolite media is related to a high presence of Fe oxides and Na and Al ions which often promotes substantial ion exchanges. In our study median inlet Na concentration was high (247 mg/L). So the lack of performance enhancement was probably due to the undersized zeolite layer (0.10 m) that provided less surface for ion exchanges at the daily flow rate (5m^3/d). This probably caused a short contact time between the zeolite media and the fluid. Similar observations were made by Stefanakis and Tsihrintzis, (2012) HF unit lowered the NH_4-N median inlet concentration of 163.2 to the final discharge of 114.3 mg/L. Lower NH_4-N concentration reduction capacity of HF than VF unit is probably due the continuous loading of the HF beds and the water saturated conditions that principally favour denitrification (Figure 3.5).

NO_3-Ninfluentconcentration was lower than 6 mg/L during the entire monitoring period; the highest concentration was reached on 28thMay with 5.23 mg/L. Despite efficient NH_4-N abatement in VF cells, NO_3-N concentration did not increase, except on 19th June (Figure 3.4c). A lower denitrification rate in VF cells may result from the lack of availability of organic carbon or an insufficient supply of NO_3-N (Spieles and Mitsch, 2000). HF unit gave greater abatement than VF cells with a median outflow value of 1.62 mg/L that might be due to the action of common reed, which probably provided wastewater oxygenation by diffusion through aerenchymatous tissues or roots (Furniss, 1992). It also provides a substrate for the growth of microorganisms and enhances their contact with wastewater (Figure 3.5).

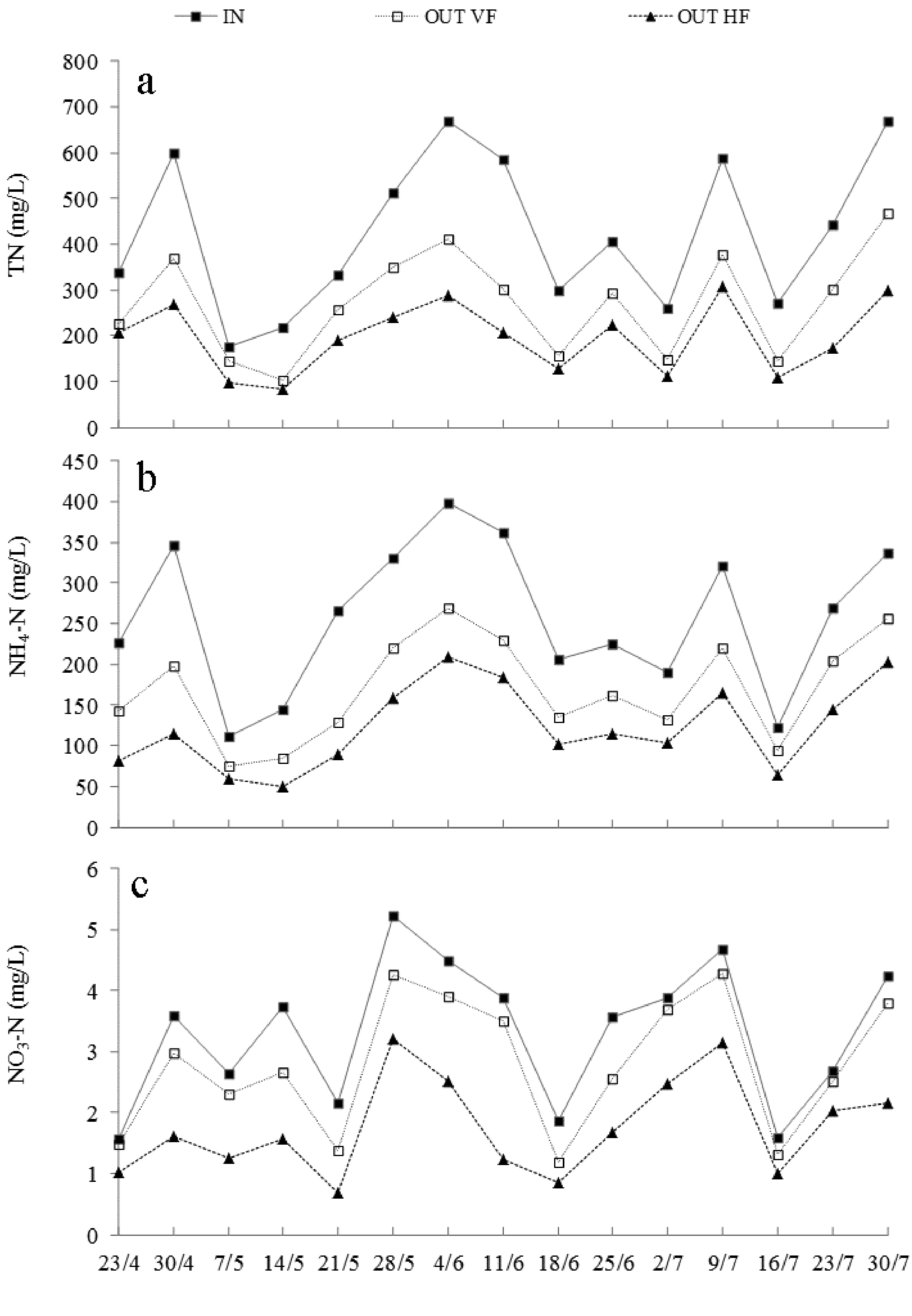


Figure 3.4 – Nitrogen forms concentration at the sampling points of the hybrid system during the first monitored period (April-July 2010).

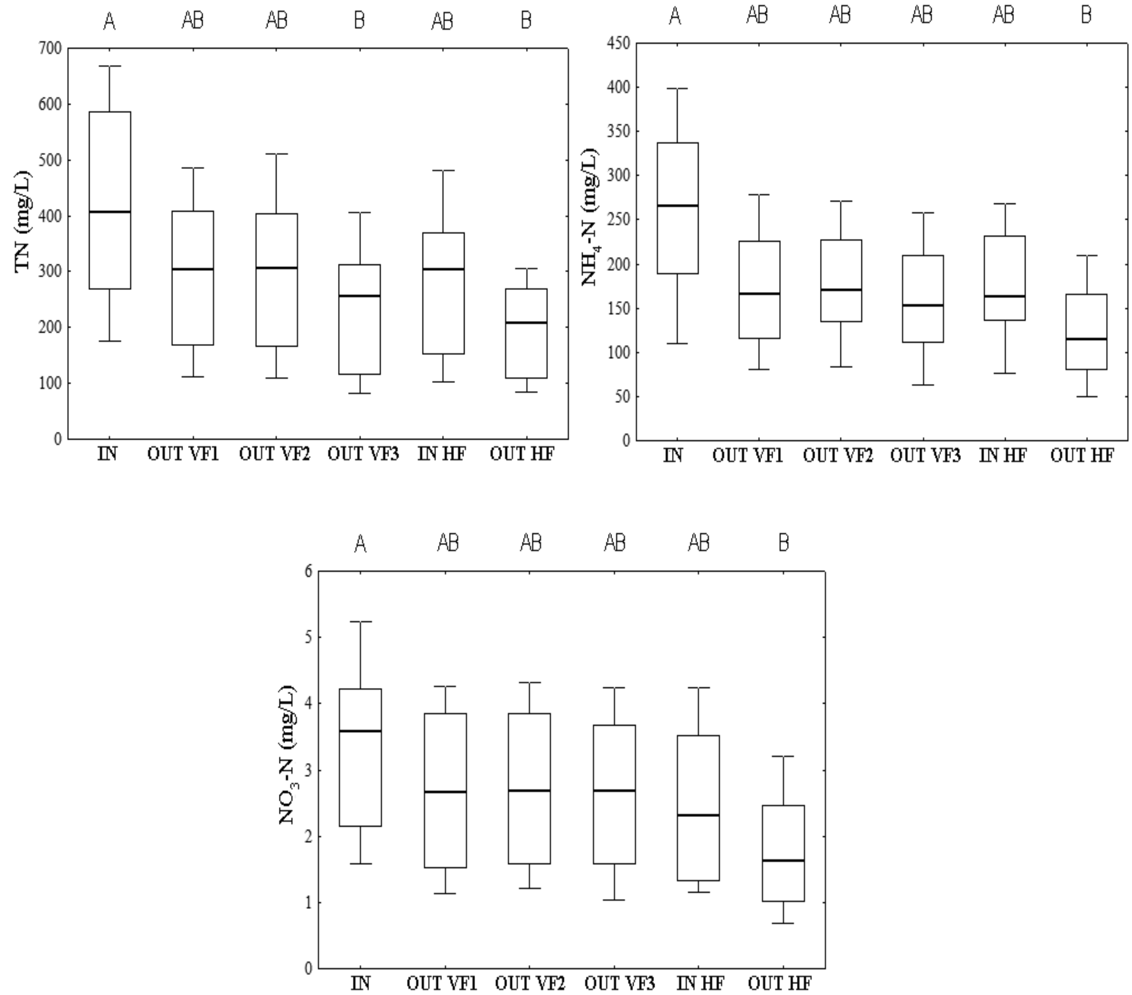


Figure 3.5 – Box-plot diagrams of nitrogen forms concentration (mg/L) in the sampling points of the hybrid system during the first monitored period (April-July 2010).

Phosphorus forms

A fluctuating TP inlet concentration was measured from the beginning of monitoring. Inlet concentration did not exceed 26 mg/L, a similar finding (28.7 mg/L) was reported by Cathcart et al. (1994) in the Pontotoc, Mississippi experiment. TP concentration effluent from VF unit was generally slightly lower than at the inlet, except on 18^{th} and 25^{th} June 2010, when 18.5 and 17.3 mg/L were measured respectively (Figure 3.6a).VF unit provided not statistically significant abatement that dropped the median value from 21 to18.5 mg/L, maintaining a wide variability (from 10 to 26.4 mg/L). HF cell achieved the best abatement to a final discharge concentration of 14.3 mg/L (Figure 3.7).

Orthophosphate is the dissolved inorganic form of phosphorus (Kadlek and Wallace, 2009) and represented more than 90% of TP inlet concentration in this study. VF and HF effluent concentration of PO_4-P time pattern was in accordance with TP, however lower concentrations were measured at different sampling points (Figure 3.6b). A statistically significant difference was found only between median PO_4-Pconcentration from influent (19.6 mg/L) and HF effluent (13.2 mg/L) (Figure 3.7). This probably occurs through three parallel paths, with reaction rates of: sorption to substrate, biofilm assimilation and *P. australis* uptake (Lantske et al., 1999).

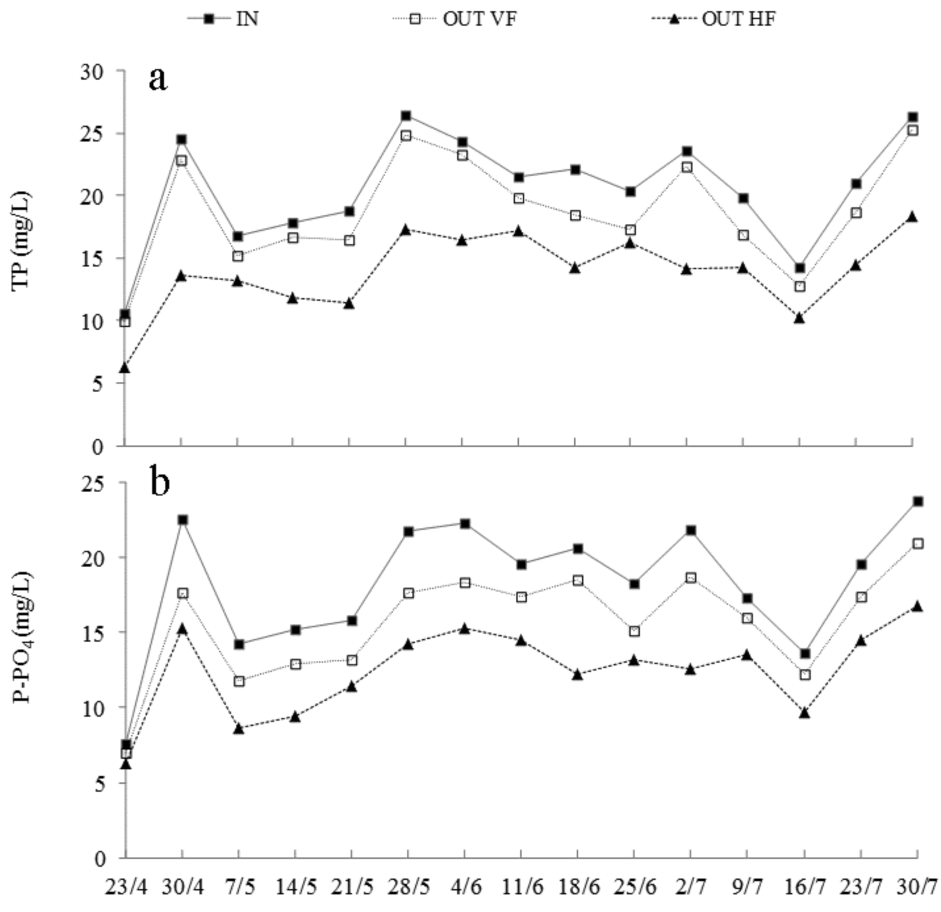


Figure 3.6 – Phosphorus forms concentration at the sampling points of the hybrid system during the first monitored period (April-July 2010).

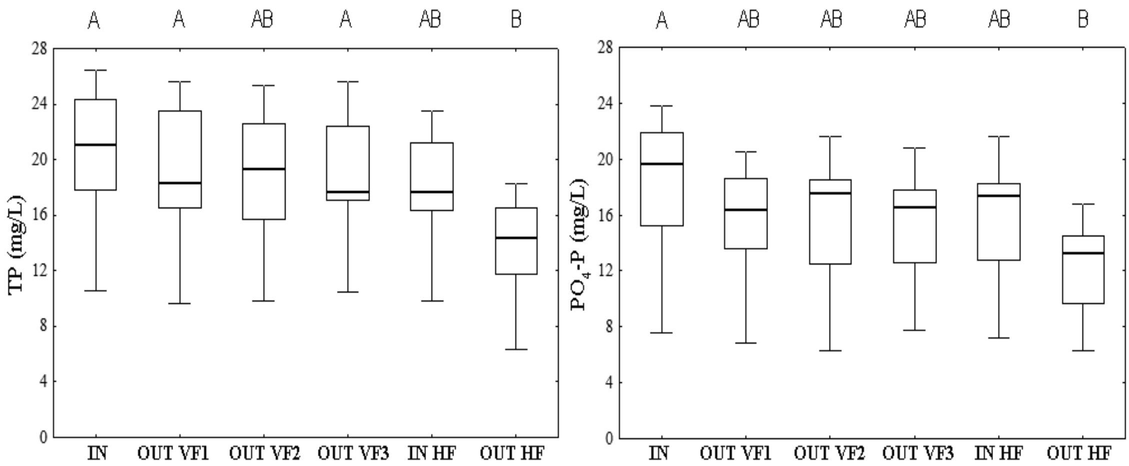


Figure 3.7 – Box-plot diagrams of phosphorus forms concentration (mg/L) in the sampling points of the hybrid system during the first monitored period (April-July 2010).

Ions

Swine manure contains different ions originating from the feed, supplements, medications, and water consumed by the animals.

Because most freshwater wetland species have low sodium requirements, the dissolved sodium content of wastewater passing through wetlands changes little (Kadlek and Wallace, 2009). During the monitored period, the median influent concentration of 247 mg/L passing through the HCW system decreased negligibly (Figure 3.8). The factors that contributed most to the failure of sodium removal were probably the solubility of this chemical element and the low cation association with organic material, which is efficiently removed by physical processes in CWs (Basil et al., 2005). Because wetland species have low dissolved sodium absorption this cation could be used as a conservative tracer for tracking groundwater discharges from wetlands, being easily monitored by an electrical conductivity sensor (Torrens et al., 2009). Chazarenc et al. (2003) used high concentrations (67,000 mg/L) of sodium chloride solution as a tracer in an HF system.

Potassium concentration detected at the VF and HF effluents, 610 and 609 mg/L respectively, was not very different from the influent 614 mg/L. As reported by Lo Monaco et al. (2009), CWs are able to remove only small portions of this nutrient due to the high solubility of Potassium and its non-association with organic material (Figure 3.8).

During the first monitored period the influent Mg^{2+} concentration ranged from 4.8 to 7.3 mg/L, after passage through the HCW system the outlet concentration ranged from 4.1 to 7 mg/L. No statistically significant concentration reduction was observed. Kadlec and Wallace (2009) stated that Magnesium concentration in surface water almost always exceeds the requirements for plant growth and elevated concentrations are not affected when wastewater travels through wetland treatment systems (Figure 3.8).

Calcium concentration does not change appreciably in HCW treatment system, as observed for Sodium, Potassium, Magnesium (Figure 3.8).

Median Chloride inflow concentration of 439 mg/L was slightly reduced to 422 mg/L. Cl- is used as tracer of water movement in the wetland because it is considered conservative in wetland environments, and it is also commonly used in estimating evapotranspiration (Juang and Johnson, 1967), groundwater recharge (Allison and Hughes, 1983) and lake water balance (Lin et al., 1987) (Figure 3.8).

Median inlet Sulphate concentration of 90 mg/L wasn’t reduced after passage through VF cells, however the HF unit slightly contributed to abating the concentration (82 mg/L). As reported by Eger (1994), the process of Sulphate reduction proceeds best at pH between 5 and 9. Hence, the measured pH range in VF and HF units were sufficient for Sulphate reduction. On the other hand, Sulphate-reducing bacteria are obligate anaerobes, and become active only under anaerobic conditions (Hao, 2003). The DO concentration measured at HF outflow exceeded 0.60 mg/L, which implies that the presence of reeds enhances the oxygen transfer to the bed, thus limiting Sulphate reduction (Figure 3.8).

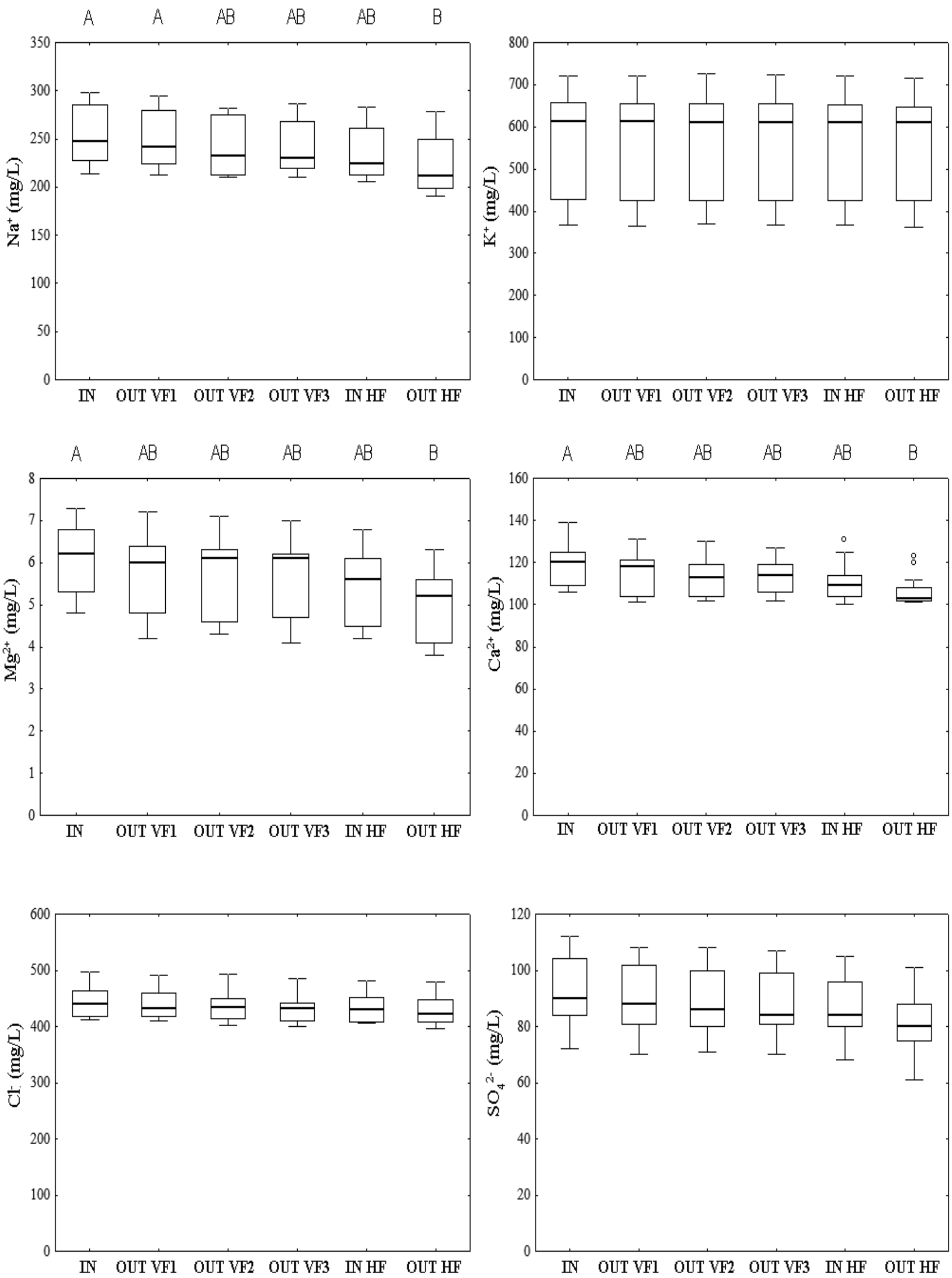


Figure 3.8 – Box-plot diagrams of Sodium (Na^+), Potassium (K^+), Magnesium (Mg^{2+}), Calcium (Ca^{2+}), Chloride (Cl^-) and Sulphate (SO_4^{2-}) forms concentration (mg/L) in the sampling points of the hybrid system during the first monitored period (April-July 2010).

Pollutants abatement

Percentage abatements (A) for the monitored parameters in HCW are presented in Table 3.1. HCW systems showed a good treatment performance, despite the fact that the monitoring regarded its second functioning season (start-up phase from May to December 2009). During the start-up phase (Borin et al., 2013), median COD concentration decreased from 1126 at the inlet to 235 mg/L at the outlet (79% abatement). TN concentration was abated by 64% (671-240 mg/L). NH_4-N and NO_3-N median inflow concentrations (390 and 6.61 mg/L) were reduced by 63% and 53%, respectively, with median outflow concentrations of 145 and 3.14 mg/L. TP inflow concentration of 23 mg/L was abated by 61%. Whilst during this first monitoring period lower abatement performances were found: COD was abated by approx. 56%, TN by 53%, NH_4-N by 50%, NO_3-N by 55% and TP by 32%.

Kantawanichkul et al. (2001) investigated the efficiency of a pilot combined wetland system (VF unit followed by an HF unit) in treating raw swine waste without recirculation of the effluent and a hydraulic loading rate (HLR) of 3.7 cm/d. The combined system reduced the average COD inlet concentration from 2800 to 43mg/L, with an abatement of 98%. Lee et al. (2004) reported a median COD concentration reduction from 1160 to 264 mg/L (77% abatement) obtained with a horizontal subsurface wetland with an HLR of 12 cm/d fed with pig manure after pre-treatment by solid separation followed by anaerobic digestion and aerobic oxidation. During the whole monitored period HCW system reduced the median inlet COD concentration (about 500 mg/L) by 50 to 80%, with a median of 56% and an HLR of 3.8 cm/d. These findings were generally lower than those reported by Lee et al. (2004) and Kantawanichkul et al. (2001). The wide range of organic matter removal found in different studies was probably related to several reasons: (a) the great variation of pig-waste composition, which is, in turn, highly dependent on farm management and storage conditions (Boursier et al., 2005), (b) different HLR and operative conditions.COD concentration reduction reached in the VF unit (33%) could be improved by providing higher oxygen availability as observed by Kouki et al.(2009). Indeed previous studies have demonstrated that intermittent wetland drainage improved removal of COD

(Tanner et al., 1999). Furthermore, increased age is also associated with an increase in microbial population (Kadlec, 1999).

Nitrogen abatement within constructed wetland systems includes different processes, mainly: uptake from plants and other living organisms, sedimentation, nitrification, denitrification, ammonia volatilization, and cation exchange for ammonium (Newman et al., 1999; Yang et al., 2001; Wallace and Knight, 2006; Mitsch and Gosselink, 2007). During the monitoring period, TN abatement ranged from 50% to 71.5% with a median value of 53%. TN concentration reduction achieved by VF and HF units differed by just 5%. Meers et al. (2004) observed higher TN percentage reductions ranging from 73% to 83% for a CW treating pre-treated slurry wastewater with a residence time of 8-18 days. Lower TN abatement performances might result from the inadequate contact time between the influent and the gravel bed media of both wetland units. As supported by the literature on wastewater treatment in CWs, the lack of well-established vegetation could cause slightly lower TN treatment efficiency than that obtained in planted beds.

The system reduced ammonia nitrogen with efficiency ranging from 40% to 68%. Despite the median dissolved oxygen content of 1.57 mg/L measured in the VF cells effluent, the unit gave a median reduction in ammonia nitrogen of 39%. Parker et al. (1998) observed a NH_4-N reduction of 32% with a VF system planted with *P. australis* that treated diluted piggery effluent from a lagoon (inflow concentration of 242 mg/L and outflow of 165 mg/L).

Phosphorus removal efficiency was lower than the other constituents. TP abatement ranged from 20% to 44.7% with a median value of 32%. TP abatement observed in HF unit was higher (19%) than VF (12%), mainly due to two reasons: first through adsorption by the porous media as reported by Bonomo et al. (1997) and second by precipitation where phosphorus reacts with the porous media and with minerals such as ferric oxyhydroxide and carbonate (Kadlec and Knight, 1996; Yang et al., 2001).

Orthophosphate was mainly removed by plant uptake and by adsorption on the porous media (Kadlec and Knight, 1996). HF unit had a high concentration reduction (24%), while VF only gave 11%. This was maybe linked to reducing conditions in the HF unit (i.e., lack of oxygen, DO concentrations below 0.5 mg/L) that can lead to solubilisation of minerals and release of dissolved phosphorus (Reed et al., 1995; Kadlec and Knight, 1996).

In general, concentration abatement reached by the system was very low for investigated ions. The ion with lowest concentration reduction was K^+ with 1%, while the highest was SO_4^{2-} with 9%. Na^+ and K^+ were mainly reduced by VF cells activity with 1% and 4% respectively, while Mg^{2+} and SO_4^{2-} by the HF unit with 3% and 6% respectively. Ca^{2+} and Cl^- concentrations were reduced by the same percentage in both units, 3% and 2% respectively.

Table 3.1 – Concentration reduction efficiency calculated on median value of VF, HF unit and HCW system for all measured parameters – first monitored period (April-July 2010).

	VF			HF			HCW		
Parameter	Inflow (mg/L)	Outflow (mg/L)	A (%)	Inflow (mg/L)	Outflow (mg/L)	A (%)	Inflow (mg/L)	Outflow (mg/L)	A (%)
COD	498	336	33	336	221	34	498	221	56
TN	417	293	30	303	198	35	417	198	53
NH_4-N	266	163	39	163	114	30	226	114	50
NO_3-N	3.58	2.67	25	2.30	1.62	30	3.58	1.62	55
TP	21	18.5	12	17.6	14.3	19	21	14.3	32
PO_4-P	20	17.4	11	17.3	13.2	24	20	13	34
Na^+	247	242	2	239	238	0.4	247	238	4
K^+	614	611	1	611	609	0.3	614	609	1
Mg^{2+}	6.2	6.17	1	6	5.8	3	6.2	5.8	6
Ca^{2+}	120	116	3	114	111	3	120	111	8
Cl^-	439	432	2	430	422	2	439	422	4
SO_4^{2-}	90	89.3	1	88	82.3	6	90	82.3	9

Analysis of wetland biodegradation level

Percentage degradation measured on cotton threads placed in the gravel layer of three VF cells showed high activity of nitrogen microbial degradation (Figure 3.9a). There was a less degradation of N-treated threads (43.6 %) if compared with the untreated control (63.5%). This finding suggests that the substrate of VF cells was saturated with nitrogen pollutants due to the daily piggery wastewater load, thus a depressive effect was observed on N-treated threads. A higher degradation of 59.6% was obtained on P-treated threads, probably due to lower phosphorus retention by root bed media in VF cells.

In HF substrate a similar depressive effect was observed on N-treated threads, whilst on P-treated threads lower degradation than the control suggests that more phosphorus accumulated in the HF substrate (Figure 3.9b).

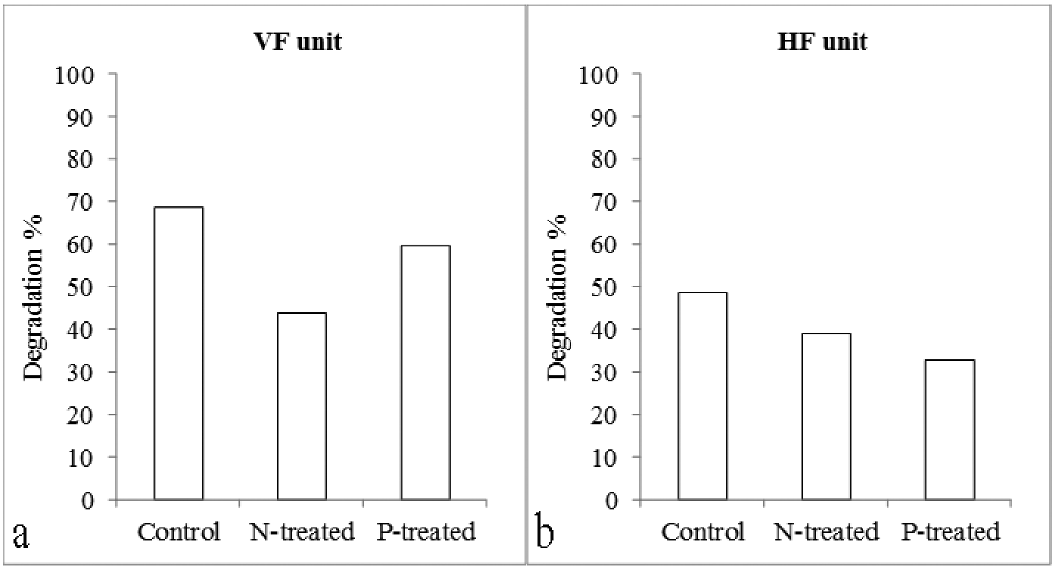


Figure 3.9 – Microbial percentage degradation observed in VF and HF system

Conclusions

During the first monitoring period variable concentrations of pollutants were measured in the HCW influent. This might result from differences in pig-waste composition, farm management and storage conditions but could also be related to inefficiencies of the pre-treatments used on the raw piggery wastewater. This fluctuation might have influenced the performance of the HCW. Another limiting factor for HCW abatement performance was the daily flow rate of 5 m^3 (HLR of 3.8 cm/d) that overloaded the system causing short-circuiting of the wastewater through the VF and HF units, negatively impacting the residence time and therefore treatment efficiency. Despite these operating conditions, the HCW system successfully treated piggery wastewater.

The highest abatement efficiency was attained on COD (56%), NO_3-N (55%) and TN (53%), TP decreased from 21 mg/L to 14.3 mg/L, achieving 32% abatement. VF system gave the main reduction in ammonia concentration (39%), despite a median dissolved oxygen content of 1.57 mg/L measured in VF cells effluent. This suggests that VF treatment performance could be improved with a fill-and-drain operational mode that provided rest periods to achieve a higher oxygen level in substrate. There were no significant differences in any of the measured parameters outlet concentration among different vegetation and substrates of the VF cells. HF unit was more efficient than VF in reducing TN, NO_3-N, TP and PO_4-Pconcentration; VF and HF gave similar abatements for COD of 33 and 34% respectively. Concentration abatement reached by HCW system was very low for the investigated ions. The preliminary tests on biodegradation level in VF and HF system confirmed the presence of microbial consortia, responsible for the removal of specific pollutants in CW.

Chapter IV

Results second period: October 2010-April 2011

Results

Meteorological data

Average temperatures at the experimental site varied between 8.6 and 12.4°C in late autumn, and from 2.8 to 9.2°C in winter. Precipitation was marked by heavy rainfall in autumn with monthly values of 170 and 323 mm for October and November 2010, then decreased in December 2010. The precipitation trend decreased during the colder season to level around 50.6 mm in January 2011 (Figure 4.1).

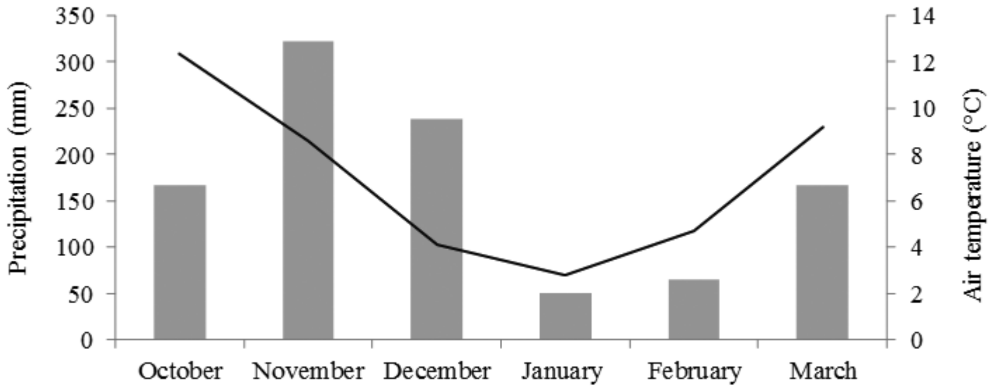


Figure 4.1 – Meteorological data for the test site : bars givemonthlyprecipitation in millimeters (left Y axis), line graph indicates average day air temperature (right Y axis).

On site parameters

Despite the low air temperatures measured in the whole investigation period (0.5-14°C) the wastewater inflow temperature ranged from 15.2 to 17.5 °C. Effluent measured from the VF system maintained a median value of 14.6°C, nevertheless at HF outlet the temperature decreased drastically from 14th January to 25th February with a median of 11.4°C (Figure 4.2).

Since the nitrification process consumes alkalinity (Kadlec and Knight, 1996), significant nitrification could have resulted in the substantial drop of pH in wastewater after VF passage, from 7.91 to 7.65. However, as denitrification is an alkalinity producing process this increased the median value to 7.74 at HF outflow (Figure 4.2).

Median DO inflow concentration showed a slightly higher value (1.91 mg/L) than in the first period (1.87 mg/L). The unsaturated condition of VF system substrate provided by the sequential batch feed mode promoted higher atmospheric oxygen diffusion in the matrix bed media (Sun et al., 1998; Noorvee et al., 2007), which probably boosted the nitrification process and organics removal. Indeed, median DO concentration measured at VF outflow showed an increase of 19% compared with the previous monitored period. In contrast, a lack of oxygen (0.05 mg/L) measured at HF inflow unit in conjunction with the high content of a carbon source, promoted denitrification. As reported in the first monitored period, DO concentration at the outlet of HF unit increased significantly to 0.60 mg/L, probably released by the common reed root system. Bavor et al. (1988) estimated oxygen release by *Phragmites* species to be approximately 0.8 g O_2/m^2 in gravel wetland substrates (Figure 4.2).

Batch feeding created a temporal redox variation in VF system, as observed by Allen et al. (2002). There was first a major drop in the redox potential when the wastewater was added (131 mV) and then a gradual increase with time and pollution removal (median value 160 mV). The E_h median value measured at HF inflow decreased drastically from -28 to -44 mV. The alternation between reduced and oxidized conditions probably influenced microbial activity by favouring robust aerobic facultative biofilms that can operate under varying nutrient concentrations (Stein et al., 2003) (Figure 4.2).

EC values of the influent wastewater varied widely from 2350 to 3260 µS/cm. After passage through the VF unit, the median dropped from 3000 µS/cm to 2640 µS/cm. Statistical significant differences were found between first two VF and the third. HF cell further abated EC to 1965 µS/cm, which was lower than the limit for irrigation purposes recommended by the FAO Guidelines (1987) (Figure 4.2).

Turbidity measured at the system inlet was higher than in the first monitored period, due to the higher inlet pollutants concentration and the presence of only a solid separation as pre-treatment. However HCW system reduced the inflow turbidity from 694 to 247 NTU (Figure 4.2).

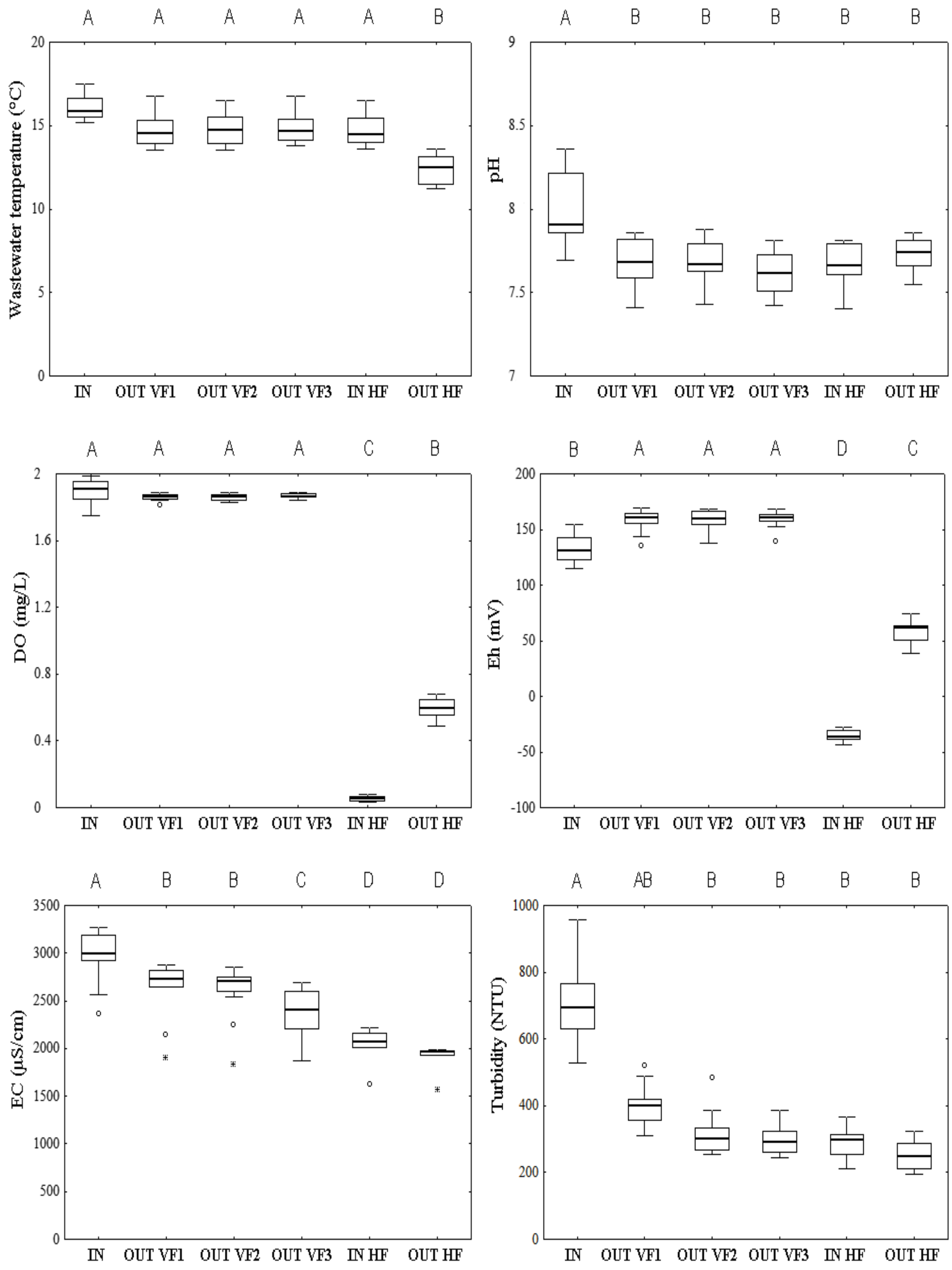


Figure 4.2 - Box-plot diagrams of Wastewater temperature, pH, Dissolved Oxygen (DO), Redox potential (E_h) and Electrical Conductivity (EC) in the sampling points of the hybrid system during the second monitored period (October 2010-April 2011).

COD

Higher COD influent concentration than the previous period was observed. From 12th November 2010 to 14th January 2011, the incoming COD concentration decreased from 1416 to 904 mg/L and subsequently from 28th January 2011 to 25th March decreased from 1148 to 702 mg/L. Figure 4.4 shows that during the beginning of the monitoring period till 28th January 2011 a similar COD concentration reduction trend was observed in VF and HF system.

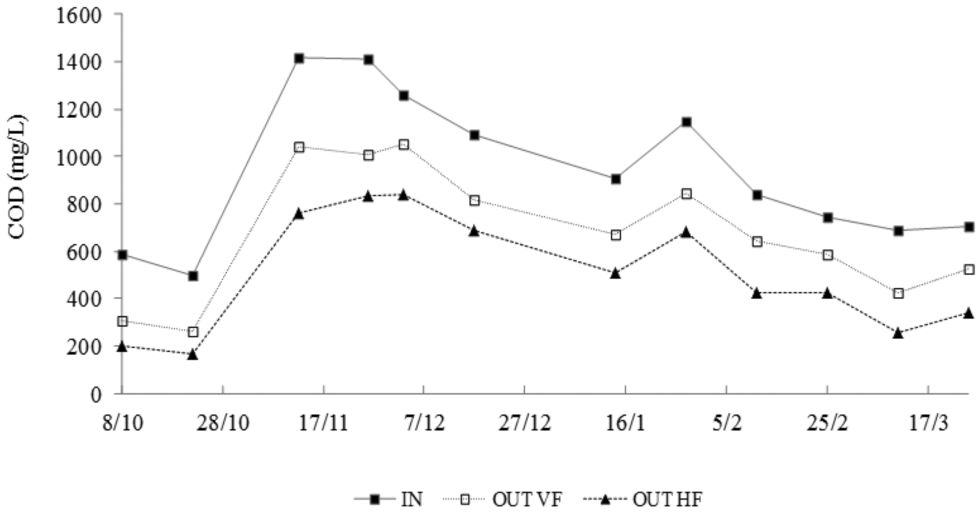


Figure 4.4 - COD concentration at the sampling points of the hybrid system during the second monitored period (October 2010-April 2011).

COD inflow concentrations varied widely between 498 and 1416 mg/L, with a median value of 870 mg/L (74% higher than the first monitored period). VF system reduced the median (656 mg/L) maintaining high variability. No difference was found among the three VF cells. HF cell gave a significant contribution to the abatement, dropping the median inflow from 656 to 465 mg/L (Figure 4.5).

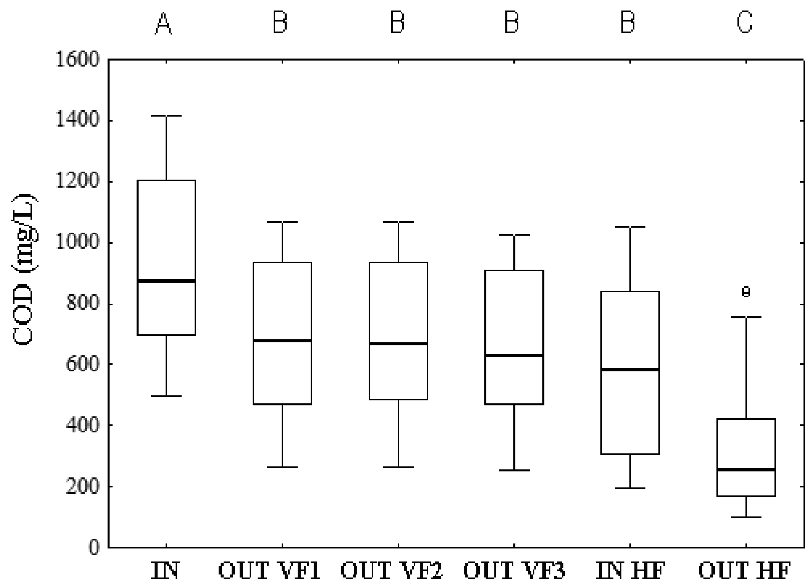


Figure 4.5 – Box-plot diagram of COD concentration (mg/L) in the sampling points of the hybrid system during the second monitored period (October 2010-April 2011).

Nitrogen forms

Median TN inflow concentration of 692 mg/L was generally higher than that reported in several studies on piggery wastewater treatment in CWs (ranging from 108 to 364 mg/L: Meers et al., 2008; Dong and Reddy, 2010; Harrington and Scholz, 2010; Morand et al., 2011). Highest values were measured on 12th November 2010 and 28th January 2011, with 983 and 926 mg/L respectively. TN concentration reduction achieved by VF system (30%) was quite constant for the whole monitored period, while from 14th January 2011, HF cell showed lower abatement performances with a decrease from 8 to 17% (Figure 4.6a). VF system lowered the median value to 494 mg/L but maintained a high variability. As reported in the first monitored period, no significant differences were found between VF cells for TN concentration. The HF system accomplished abatement to a final discharge concentration of 413 mg/L (Figure 4.7).

Median NH_4-N inflow concentration (366 mg/L) represents 53% of median TN concentration. A similar inlet value was reported by Knight et al. (2000) from the Livestock Wastewater Treatment Wetland Database (366 mg/L). Inlet concentration remained higher than 270 mg/L except on 22nd October 2010 (195mg/L). Although the HCW system operated with higher ammonia nitrogen than in the first monitored period, there were no signs of ammonium toxicity to plants. VF and HF effluent concentration trend was in accordance with the TN trend (Figure 4.6b). NH_4-N concentration was mainly reduced by VF system (32%); the best performance was obtained by VF3 cell (40%), but no significant differences were found between VF cells. In VF and HF the pH was well buffered within a range of 7.41 to 8.36, hence, ammonia nitrogen loss through volatilization was negligible since this generally requires a pH of 9.3 (Jing and Lin, 2004). Air convection within the VF substrate is a consequence of the batch feeding, which is directly related to the wastewater flow within the unsaturated substrate. The entering wastewater pushes the gas present in the porous media and at the same time creates an aspiration effect of atmospheric air when the VF substrate is unsaturated (Forquet et al., 2009). Thus during the 8 hours rest period O_2 level in the VF porous media was re-generated and the aerobic conditions improved nitrification process performance (Figure 4.7). Instead, HF system showed a very low nitrogen ammonia reduction probably due to the lack of dissolved oxygen measured at the outflow of the cell (from 0.49 to 0.68 mg/L).

NO_3-N input concentration ranged between 3.68 and 9.15 mg/L, the median value of 5.17 mg/L was 44% higher than in the first period. Many researchers have found that high nitrate concentration enhances denitrification rate in wetlands (Howard-Williams and Downes, 1989; Hanson et al., 1994; Lowrance et al., 1995; Willems et al., 1997; Sartoris et al., 2000). However during the monitored period, lower NO_3-N concentration reduction than the first period was observed in both systems from 14thJanuary to 25th February 2011, probably linked to lower wastewater temperature that ranged from 11.2 to 11.8°C at HF cell outlet. Starting from 11th March 2011, with higher wastewater temperature (12.4°C) NO_3-N concentration abatement ameliorated (Figure 4.6c).

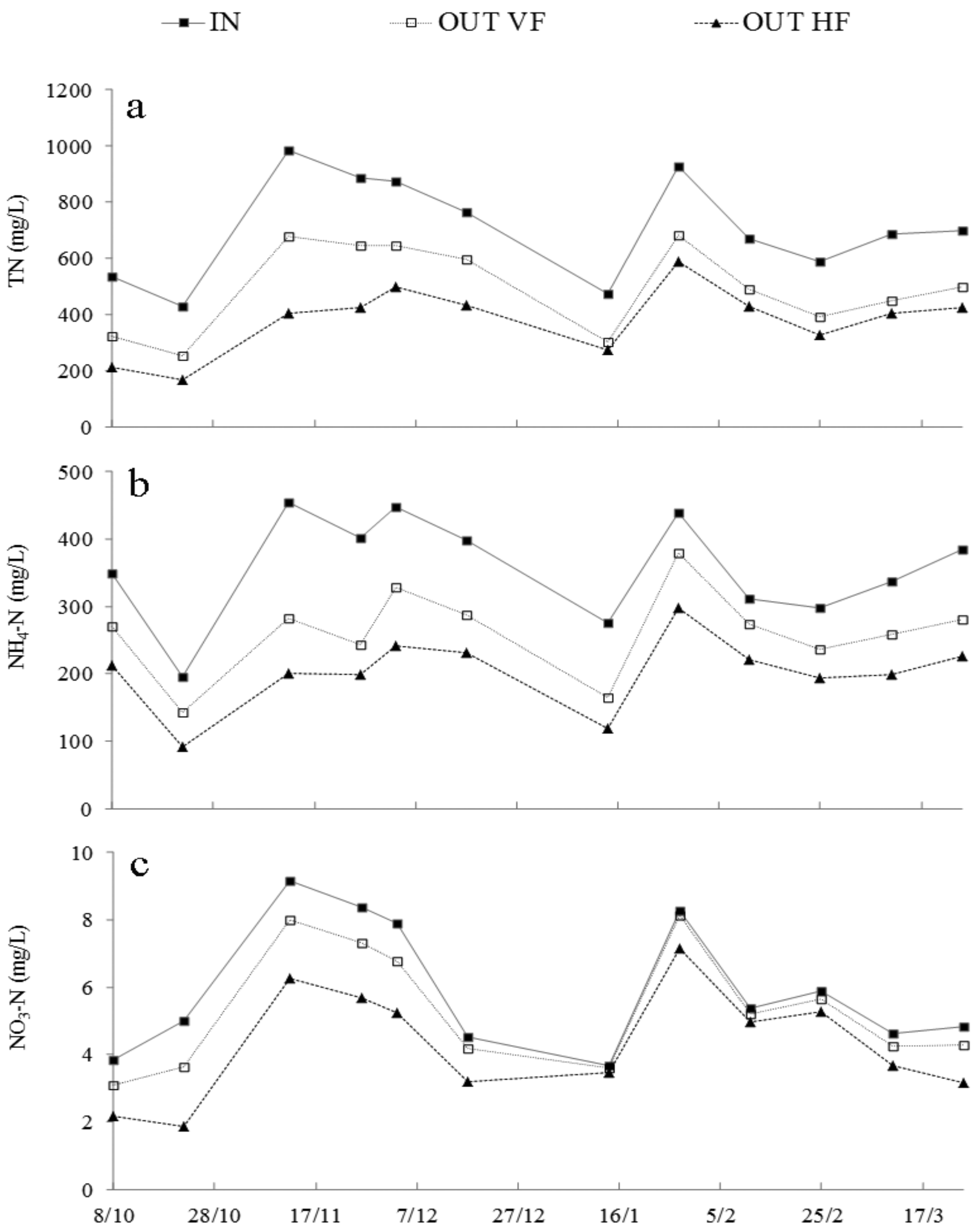


Figure 4.6 – Nitrogen forms concentration at the sampling points of the hybrid system during the second monitored period (October 2010-April 2011).

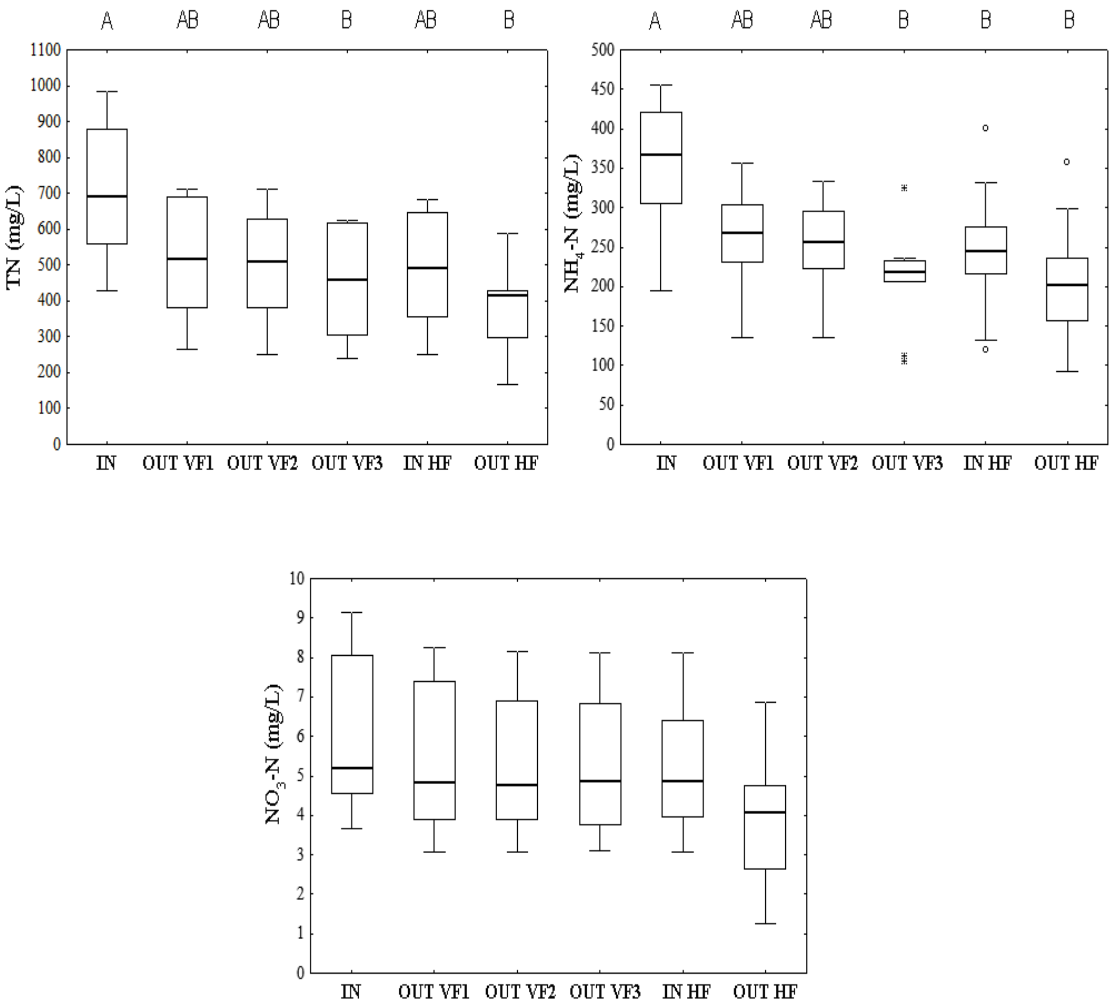


Figure 4.7 – Box-plot diagrams of nitrogen forms concentration (mg/L) in the sampling points of the hybrid system during the second monitored period (October 2010-April 2011).

Phosphorus forms

TP influent concentration did not exceed 34 mg/L during the monitoring. Similar conditions (30 mg/L) were obtained by Sànchez-Garcìa et al. (2010) from CWs composed by three HF units of 67 m^2 treating 8m^3 pig slurry per charge. On some dates (i.e. 12th and 26th November, 17th December 2010) VF system reduced the median TP inflow concentration by 20%. HF cell abatement was higher than VF in the period between 22nd October and 17th December 2010. Starting from 14th January 2011, worse abatement was measured in the HF system, probably due to lowering wastewater temperature during the cold season. Indeed, Nichols (1983) stated that phosphorus adsorption to the substrate is wastewater temperature correlated due to its extreme redox sensitivity (Figure 4.8a). VF system provided not significant abatement that dropped the median value from 21.4 to 18.3 mg/L, (a similar finding was reported in the first monitored period). HF system achieved best abatement to a final discharge of 14 mg/L (Figure 4.9).

Median orthophosphate inflow concentration (15 mg/L) was 25% lower than in the first period and represents 70% of median TP concentration. PO_4-P time pattern was in accordance with TP, however as in the first monitored period, lower influent and effluent concentrations were measured at different sampling points (Figure 4.8b).

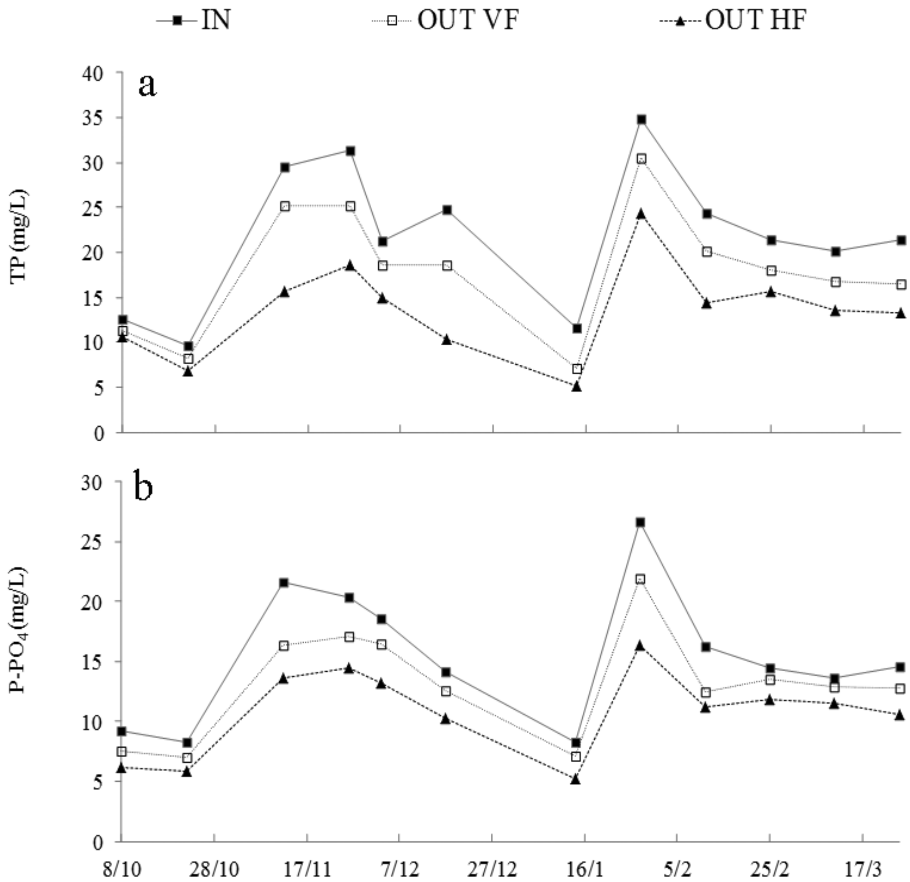


Figure 4.8 – Phosphorus forms concentration at the sampling points of the hybrid system during the second monitored period (October 2010-April 2011).

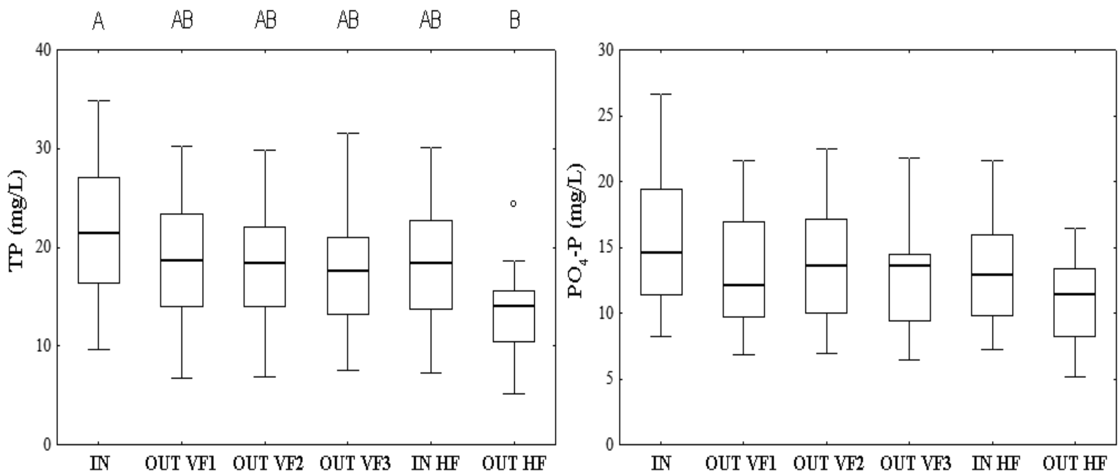
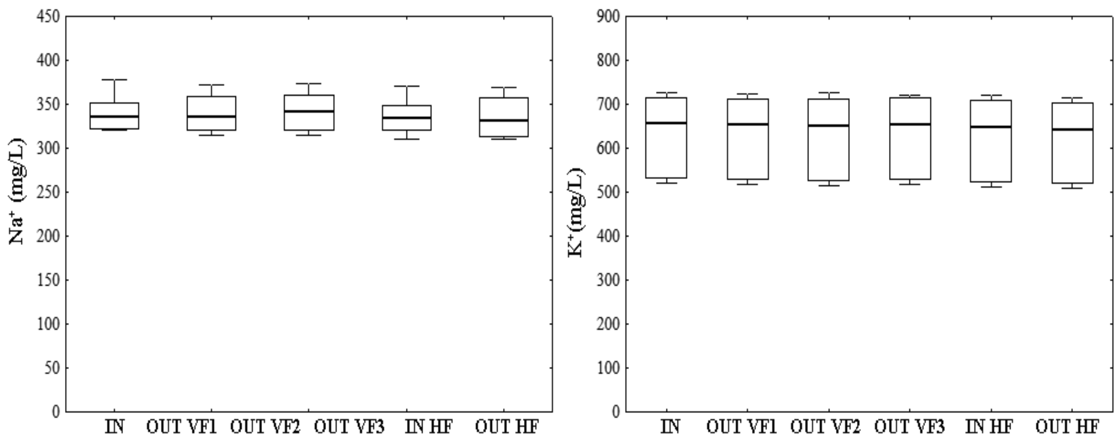


Figure 4.9 – Box-plot diagrams of phosphorus forms concentration (mg/L) in the sampling points of the hybrid system during the second monitored period (October 2010-April 2011).

Ions

Figure 4.10 reports cations and anions concentration variations provided by HCW system during the second monitored period. Both inflow and outflow concentrations detected for ions forms were higher than the previous period. Statistically significant differences were found between inlet and outlet of HCW system for: Mg^{2+} (IN 7.6 mg/L; OUT 6.8 mg/L), Ca^{2+} (IN 142 mg/L; OUT 129 mg/L), and Cl^- (IN 520 mg/L; OUT 495 mg/L).



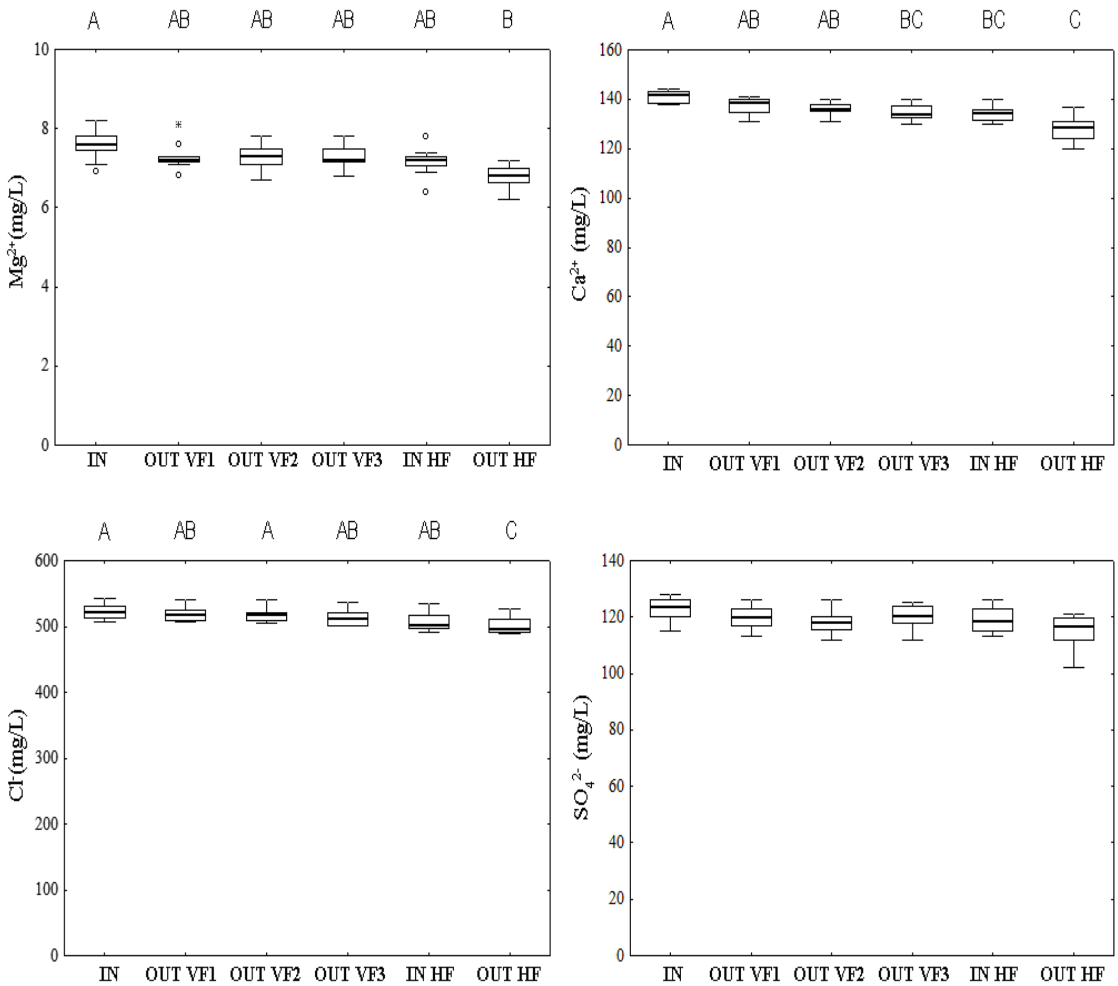


Figure 4.10 – Box-plot diagrams of Sodium (Na^{+}), Potassium (K^{+}), Magnesium (Mg^{2+}), Calcium (Ca^{2+}), Chloride (Cl^{-}) and Sulphate (SO_4^{2-}) forms concentration (mg/L) in the sampling points of the hybrid system during the second monitored period (October 2010-April 2011).

Pollutants abatement

Percentage abatements (A) for the monitored parameters in HCW are presented in Table 4.1. HCW system showed a good treatment performance, despite the absence of a suitable pre-treatment. The HCW system removed approx. 46% of COD, 40% of total nitrogen, 43% of ammonia nitrogen, 21% of nitrate nitrogen and 35% of total phosphorus.

The overall COD percentage abatement achieved during the monitoring ranged between 33.1 and 51.4%. Although in the first period the VF system was able to obtain an abatement of 33%, in the second one the abatement was only 25%. This is probably linked to the higher concentration of recalcitrant organic matter measured at the inlet sampling point. The literature reports (Calheiros et al., 2007; Sun and Saeed, 2009) that an increasing organic loading often coincides with greater organics removal rate, although excessive loading rates can cause organic matter accumulation, reduction of void space, and lower pollutant removal efficiencies in constructed wetlands. Jia et al. (2010) observed that a VF system planted with *P. australis* with intermittent loading exhibited a greater COD removal performances >96% (363-14.3 mg/L) if compared with a continuous loading system that removed 92% (363-30 mg/L).On the contrary, in this study an increase of oxidizing conditions in the VF cells substrate provided by alternating wet and dry periods didn't show an increasing COD reduction. A worsening in COD percentage abatement was also observed in HF system compared to the first monitoring period, probably linked to: (a) not suitable contact time between wastewater and attached biofilms in the root zone; (b) lower temperature (autumn-winter season). Stefanakis and Tsihrintzis, (2012) showed that the efficiency in reducing COD effluent concentrations in VF and HF units is enhanced (from 2 to 13%) as temperature increases.

TN abatement ranged from 36.5% to 60.7% with a median percentage value of 40%. TN concentration reduction achieved by VF system was 29% and by HF 16%. These differences were probably related to the ammonia form representing the major fraction of total nitrogen loaded in the HCW system. Note that the VF system performed better in ammonia reduction. Increased nitrogen load typically coincides with greater removal rates in subsurface flow wetland systems (Lee and Scholz, 2007; Albuquerque et al., 2009; Tuncsiper, 2009; Dan et al., 2011), within the tolerable limits. However, wetlands receiving well-treated wastewater tend to have a higher potential for biochemical removal due primarily to lower concentrations of those chemicals in the influent wastewater. Conversely, wetlands receiving untreated or poorly treated wastewater tend to have lower potential for biochemical removal due to their high concentrations in the influent water (Hammer and Knight, 1994; Knight et al., 2000; Knight, 2003). Indeed lower TN abatement percentage was obtained (40%) than in the first period (53%) despite higher median inlet concentration being measured.

The HCW system reduced ammonia nitrogen with efficiency that ranged from 30% to 57%. VF system was able to abate 32% of NH_4-N influent compared to only 16% by the HF. Batch mode provided higher DO level in VF cells by increasing oxidizing conditions that can improve nitrification, however higher concentration level (median 360 mg/L) limited the removal performance. Several studies reported that excessive ammonia in wastewater could be toxic and could hamper growth and photosynthesis of certain wetland plants. Tanner et al. (1995) stated that ammonium toxicity tends to differ between species, although the mechanism of ammonia toxicity to wetland plants remains unclear. Jingtao et al. (2012) reported that *P. australis* grew well at ammonia concentrations of up to 160 mg/L, but growth was inhibited at levels higher than 640 mg/L.

Reduction of nitric nitrogen obtained by the HCW system was lower than the other nitrogen forms (21%). As reported by several studies (Huang et al., 2000; Ansola et al., 2003; Demin and Dudeney, 2003; Mars et al., 2003), higher hydraulic retention time (HRT) typically facilitates nitrogen removal from wastewater due to a longer contact period of nitrogen pollutants with microorganisms. HRT within the range 4-5 days was probably too brief to achieved high performances. HF system provided major abatement (16%) if compared with VF cells (8%). This finding is probably related to the aerobic-unsaturated substrate conditions of VF wetlands that hamper the denitrification process.

The abatement level of phosphorus was constant (32-35%) during the first and second monitored periods. This suggests that, contrary to what was reported by Rustige et al. (2003), low temperatures decrease the TP sorption capacity of the substrate, and secondly that the system's porous media was not yet saturated. Significantly higher TP abatement occurred in the HF unit (19-24%), probably due to a higher absorption capacity than that of VF (related to the higher HRT).

For orthophosphate, HF unit gave a 24% concentration reduction, while VF cells only 11%. A higher result in PO_4-P abatement was expected in the HF system because during the second period, vegetation growth was homogeneous and one of the best ways to reduce it is by plant uptake (Brooks et al., 2000; Vymazal, 2004).

Similar concentration reductions of cations and anions to first period were found. Lowest percentage abatement was obtained for Na^+ and K^+ with 2% while the highest was obtained for Ca^{2+} with 9%.K^+, Ca^{2+} and SO_4^{2-} were mainly abated by the VF system with 1%, 5% and 4% respectively. Mg^{2+} was mainly abated by the HF cell with 6%. Cl^- concentration was reduced by 1% in both systems.

Table 4.1 – Concentration reduction efficiency calculated on median value of VF, HF unit and HCW system for all measured parameters – second monitored period (October 2010-April 2011).

	VF			HF			HCW		
Parameter	Inflow (mg/L)	Outflow (mg/L)	A (%)	Inflow (mg/L)	Outflow (mg/L)	A (%)	Inflow (mg/L)	Outflow (mg/L)	A (%)
COD	870	656	25	653	466	29	870	466	46
TN	692	494	29	492	413	16	692	413	40
NH_4-N	366	250	32	246	207	16	366	207	43
NO_3-N	5.17	4.75	8	4.86	4.08	16	5.17	4.08	21
TP	21.4	18.3	14	18.4	14	24	21.4	14	35
PO_4-P	15	12.8	12	12.9	11.4	12	15.0	11	24
Na^+	337	335	1	334	330	1.2	337	330	2
K^+	657	652	1	648	643	0.8	657	643	2
Mg^{2+}	7.60	7.25	5	7.20	6.80	6	7.60	7.20	5
Ca^{2+}	142	135	5	134	129	4	142	129	9
Cl^-	520	515	1	502	495	1	520	495	5
SO_4^{2-}	123.5	119	4	119.0	116.5	2	124	116.5	6

Tracer test

Figure 4.11 presents the progressively increased tracer concentration detected at HF outlet with a fluorescence detector from day 0 (start of loading cycle) to day 14 (end of tracer test). Strictly connected to the concentration, the fluctuating volumes of fresh water applied for each loading cycle are shown,which ranged between 1.71 to 1.79 m^3/d. From the beginning of the loading cycle the first tracer concentration was detected on day 4 with a value of 10.3 µg/L. From days 4 to 7 the rhodamine WT concentration increased drastically to a maximum value of 117.9 µg/L. After day 7, the concentration detected at HF ouflow decreased progressively until the end of tracer test on day 14 with 5.3 µg/L.

The tracer test result confirmed that the water remained in the HF cell for a minimum of 7 days (an optimal value is set at between 7 and 8 days).

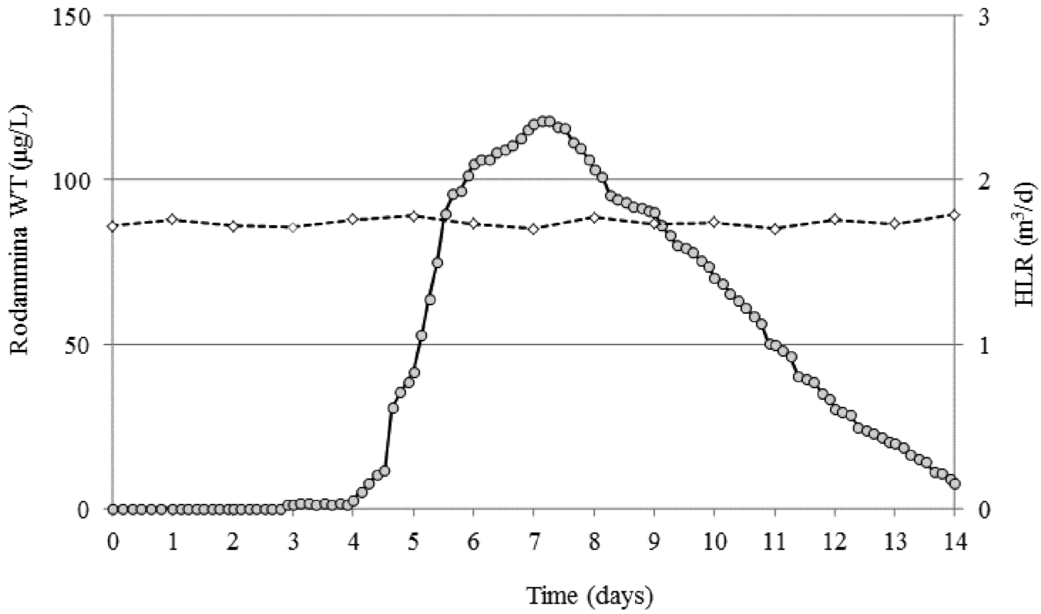


Figure 4.11 – Tracer test in HF system: line graph indicates tracer concentration (µS/cm) detected every three hours at HF outlet by the fluorescence detector (left Y axis), dashed line indicates hydraulic loading rate (right Y axis) (March 2011).

Conclusions

Pig manure must be pre-treated by a primary (odour reduction, volume reduction, energy recovery) and a secondary treatment (nutrient reduction, adding value to the manure) before CWs application. With respect to the previous monitoring two external conditions have changed and may affect the performance: (a) higher pollutant load (the raw pig manure was pre-treated by only a solid-separation); (b) lower temperature (autumn-winter season).

To improve the performance of VF system found in the previous period, a batch feed (wet – dry period) was adopted. During the wet period, piggery wastewater was applied until the VF substrate was fully saturated, thereby allowing enhanced contact between media, biomass, and the bulk liquid. After the saturation phase, the wastewater was left to drain, thereby allowing the diffusion of atmospheric oxygen into the VF system substrate, and enriching the biofilms with oxygen. However as in the previous period a daily flow rate of 5 m^3/d (HLR of 3.8 cm/d) was applied, this overloaded the system and caused the lack of wastewater VF treatment. A teach loading cycle of 2.5 m^3, an average of 0.6-0.8 m^3

was not treated since wastewater was discharged by the syphon. Results reported by the tracer test indicate that HCW system has a design flow of less than 2 m^3/d, hence a decrease of HLR was expected to enhance removal efficiency. In addition, a decreasing of HLR could prolong the contact period of wastewater pollutants with microorganisms in the HF substrate. Hydraulic retention time in HF system with a daily flow rate (Q) of 5 m^3/d ranged between 4-5 days, instead a Q of 1.7-1.8 m^3/d could prolong the HRT to 7-8 days, this operation probably facilitated nitrogen removal from wastewater. Akratos and Tsihrintzis (2007) reported that in an HF wetland system an 8 day HRT is required at above 15°C, with 14-20 days being recommended as optimal.

Another limiting factor for HCW abatement performance was the colder temperature measured from January 2011 at HF outflow. Zhang et al. (2011), Zhao et al. (2011) and several other authors documented the negative impact of lower temperature on nitrogen and organics removal in treatment wetlands.

Despite these operating conditions, the HCW system successfully treated piggery wastewater. The HCW system removed approx. 46% of COD, 40% of total nitrogen, 43% of ammonia nitrogen, 21% of nitrate nitrogen, and 35% of total phosphorus. HF system was more efficient than VF in reducing COD, NO_3-N and TP inlet concentration, whilst VF system gave higher concentration reduction for TN and NH_4-N. Similar abatements (12%) were achieved for PO_4-P in both wetland units. As reported for the first monitored period, cations and anions depuration efficiency attained in HCW was very low. Lowest percentage abatement was obtained for Na^+ and K^+ with 2%, while the highest was obtained for Ca^{2+} with 9%. K^+, Ca^{2+} and SO_4^{2-} were mainly abated by VF system with 1%, 5% and 4% respectively. Mg^{2+} was mainly abated by HF cell with 6%. Cl^- concentration was reduced by 1% in both systems.

Chapter V

Results third period: May 2011-July 2012

Results

Meteorological data

During the third monitored period, average air temperatures varied between 2.3 °C (February 2012) and 26.3 °C (August 2011). From February to July 2012 a progressive increasing of air temperature was measured. Precipitation was marked by heavy rainfall in July 2011 with 133 mm, October 2011 with 138 mm and May 2012 with 154 mm. (Figure 5.1).

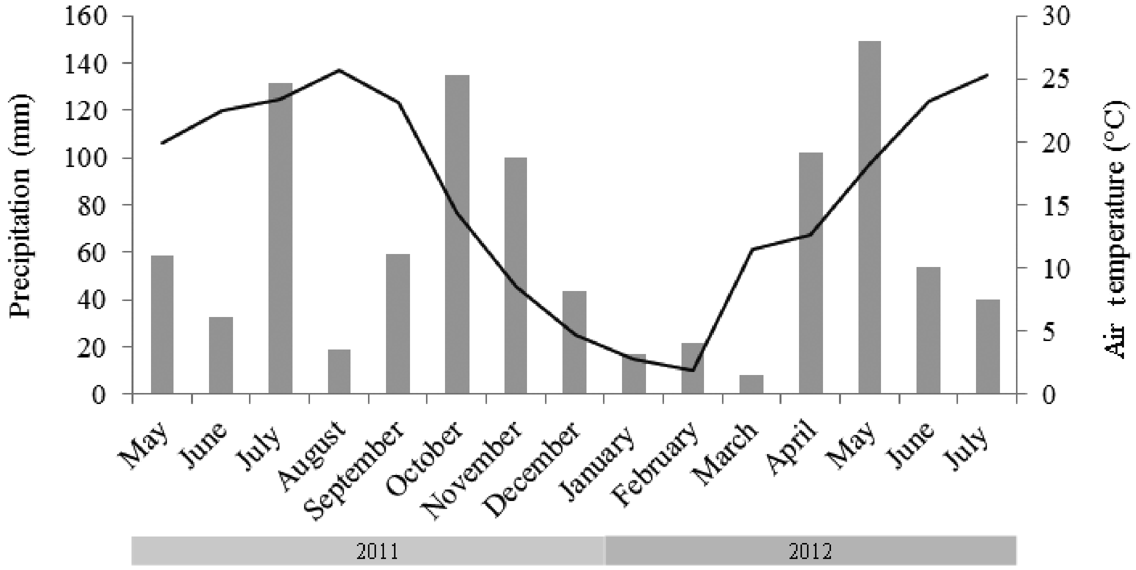


Figure 5.1 – Meteorological data for the experimental site during the third monitored period (May 2011 - July 2012). Bars give monthly precipitation in millimeters (left Y axis), line graph indicates average daily air temperature (right Y axis).

From May to July 2011, slight differences were measured between air and wastewater temperatures. Inlet wastewater temperature ranged between 20.5 and 22.3 °C. These values favour a more efficient biological N removal (range of 20-25 °C) as reported by Sutton et al. (1975) since warmer temperatures positively influence both microbial activity and oxygen diffusion rates in constructed wetlands (Phipps and Crumpton, 1994). From January to July 2012, based on air and wastewater temperature, two different sub-periods could be identified: the first one from January to March 2012, with mean daily air temperatures between -2 and 13.8 °C. The mean inlet synthetic wastewater temperature was loaded at 11.8 °C (the freshwater temperature used to prepare synthetic wastewater was never below 9 °C) and it was discharged at 5.4 °C. The second sub-period from April to July 2012 had mean air temperatures increasing from 13.8 to 26.7 °C and median inlet wastewater temperature of 20 °C (Figure 5.2).

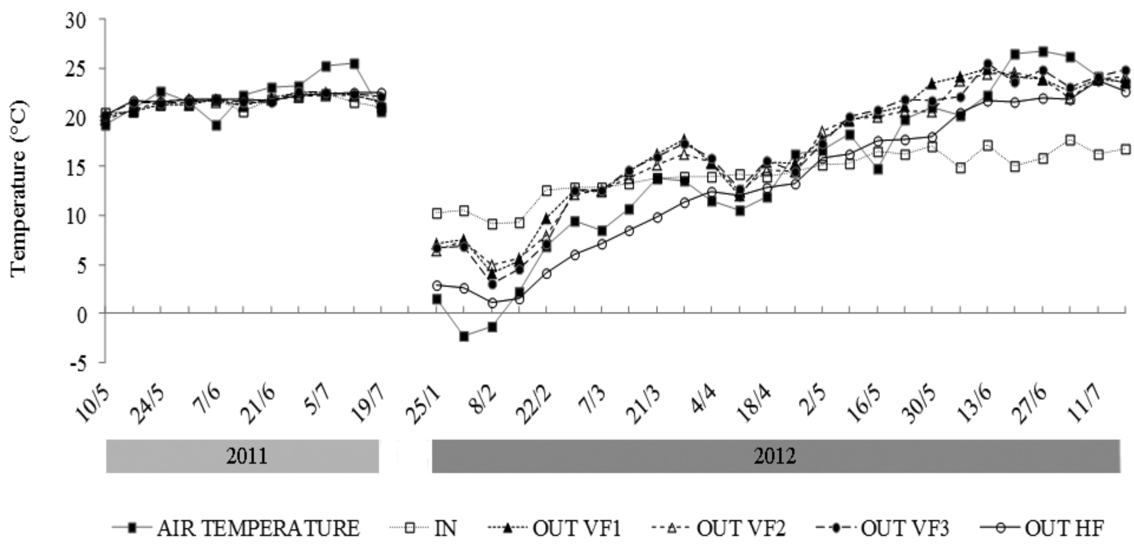


Figure 5.2 – Mean air and wastewater temperature measured at HCW system during the third monitored period (May 2011-July 2012).

Cumulative ETos time pattern distinguishes three sub-phases: (a) initial phase from May to September 2011, characterized by high temperatures and intensive plant growth; (b) central phase, from October 2011 to March 2012, with low air temperatures and quiescent plants; (c) late phase, from April 2012 until the end of the monitoring period, with increasing temperatures and intensive plant growth (Figure 5.3). In the first phase, average daily ETos was 5.90 mm; in the second one, the system consumed 1.97 mm/day on average. The lowest value (0.04 mm/day) was detected on 17th December 2012. In the winter-spring phase, daily ET was 5.04 mm. The daily ETos values were not very different from those found in the literature in similar conditions (latitude and measurement method) (Salvato and Borin, 2010).

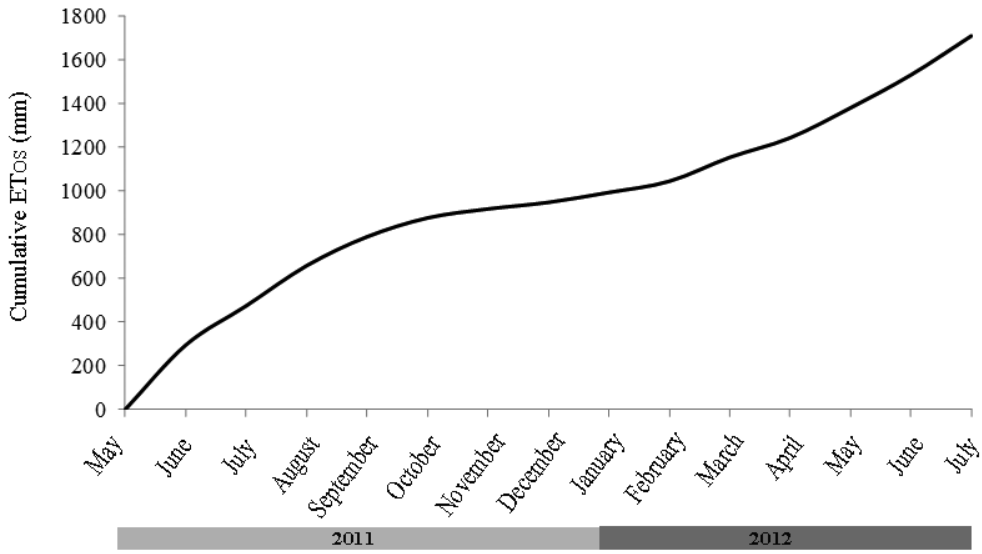


Figure 5.3 – Cumulative ETos during the third monitored period (May 2011-July 2012).

Knowing the ET for each cell and ETos it was possible to calculate the K_c (crop coefficient) with a similar meaning of K_c as that for agricultural crops (Allen et al., 1998). From May to July 2011, the K_c of VF1 cell planted with *Canna x generalis*, was higher (average of 4.20) than VF2 and VF3 (planted with *P. australis)* with 3.93 and 3.97 respectively. The K_c calculated for HF unit (average 4.04) planted with *P. australis* was similar to that obtained for VF2 and VF3 (Table 5.1).

Table 5.1 – Monthly K_c of VF and HF units from May to July 2011.

	VF1	**VF2**	**VF3**	**HF**
May	3.26	3.11	3.16	3.19
June	4.21	4.12	4.15	4.25
July	5.13	4.55	4.61	4.68
average	4.20	3.93	3.97	4.04

Starting from January 2012, during the winter months, low K_c values were calculated in both VF and HF units. The minimum value was for VF3 with 0.61. The K_c values reached during the 2012 summer months were higher than that calculated for 2011, probably due to plant development. The maximum values reached by cells planted with *P. australis* were 6.57 for HF, followed by 6.44 and 6.41 for VF2 and VF3 respectively. A higher K_c value for *P. australis* (7.8) was obtained by Borin et al. (2011) during the last 10 days of July 2009 from vegetated plastic tanks in similar conditions (latitude and measurement method). High K_c values reached in July 2012 were probably correlated to the high ET measured for each cell during the month (Table 5.2).

Table 5.2 – Monthly K_c of VF and HF units from January to July 2012.

	VF1	**VF2**	**VF3**	**HF**
January	0.84	0.67	0.61	0.71
February	1.57	1.35	1.32	1.38
March	2.49	2.31	2.33	2.36
April	3.68	3.56	3.68	3.47
May	4.23	4.18	4.21	4.27
June	5.24	5.11	5.13	5.17
July	6.77	6.44	6.41	6.57
average	3.55	3.37	3.38	3.42

On site parameters

The median influent pH was neutral or slightly alkaline (7.64) during the warm and cold season. VF cells treatment step caused a slight pH decrease (7.6), which is probably related to the nitrification process alkalinity consumption (approx. 3 g of bicarbonate is produced for g NO_3-N reduction) (Seed and Sun, 2012). The same finding was obtained during the first and second monitored periods. pH measured at HF effluent exhibited alkaline values from 21st March 2012 till the end of monitoring period (Figure 5.4). pH values measured at VF and HF effluent were optimal both for nitrification ($7.5 < pH < 8.5$) (Sanchez and Porter,1994; Platzer, 1996) and denitrification processes ($7 < pH < 7.5$) (U.S. EPA, 1975). No significant seasonal variations were found for pH during the whole investigation period, as also observed by Akratos and Tsihrintzis (2012) with a long-term evaluation of five HF wetlands operating for synthetic wastewater treatment.

From May to July 2011, a high median inflow DO concentration of 2.20 mg/L was measured. This inflow value increased to 2.60 mg/L after passage through VF cells. This gain was probably due to a rest period of 14 hours (OR1) that promotes greater oxidized conditions in the gravel pore substrate of VF unit at each loading cycle. DO concentration detected in HF effluent varied from 0.47 to 0.58 mg/L, with a median value of 0.53 mg/L, and were in the range (<0.3-0.5 mg/L) considered optimal by Bertino (2010) to accomplish nitrate reduction. From January to March 2012 DO concentrations measured in VF (2.77 mg/L) and HF effluent (0.74 mg/L) were higher than values measured in the warm period. This result was probably due to oxygen solubility in water increasing as temperature decreases (Stefanakis and Tsihrintzis, 2012). From the beginning of March to July 2012, DO concentration at VF and HF did not decrease possibly due to plant growth that enhanced oxygen transfer to the plant roots. Indeed, Lawson (1985) calculated a possible oxygen flux from roots of *P. australis* of up to 4.3 $g/m^2/d$ during the warm season. Reversing the operational regime (OR2) in VF system during the last two loading cycles (6 hours of rest period) caused a DO concentration decrease of 10% and 38% in VF and HF effluent respectively (Figure 5.4).

Median E_h inflow value was constant for the whole investigation period (+260 mV). From May to July 2011 thanks to VF passage, E_h slightly decreased to +206.6 mV maintaining suitable conditions for the nitrification process (+100 mV < E_h < +350 mV) (Vymazal, 2005); whilst median E_h decreased drastically from +10 to 0 mV at HF outflow. From January to March 2012, with lower wastewater temperature, Eh measured at VF effluent was 7% higher than in the warmer period; the same pattern was measured for HF unit, where a five-fold increase was obtained (>53 mV). This finding was higher than the optimal substrate conditions required for denitrifying bacteria (+50 mV < E_h < -50 mV) (Knowles, 1981). Starting from 4th April 2012 with a rise in wastewater temperature, similar E_h values were found in both VF and HF effluent. During the last two loading cycles of the monitored period, E_h measured in VF effluent decreased drastically (35.1 mV) due to the prolonged full stage (14 hours) promoted by OR2 (Figure 5.4).

During the warm season (May-July 2011), influent synthetic wastewater EC was higher than 1600 µS/cm, and increased after passage through VF cells. Wastewater passage through HF cell also slightly increased the median value to 1840 µS/cm. During the colder sub-period (January-March 2012), incoming EC didn't exceed 1600 µS/cm until 28th March, when the values rose until the end of monitoring. This result is probably related to freshwater temperature increasing from 12 to 18 °C, which promotes dissolving of ammonium nitrate in water. After 18th April 2012, the same trends were observed in both VF and HF units (Figure 5.4). EC seasonal variations were observed during the summer, probably due to increasing evapotranspiration (May-September 2011 and April-July 2012) and plant growth as reported by Hench et al. (2003).

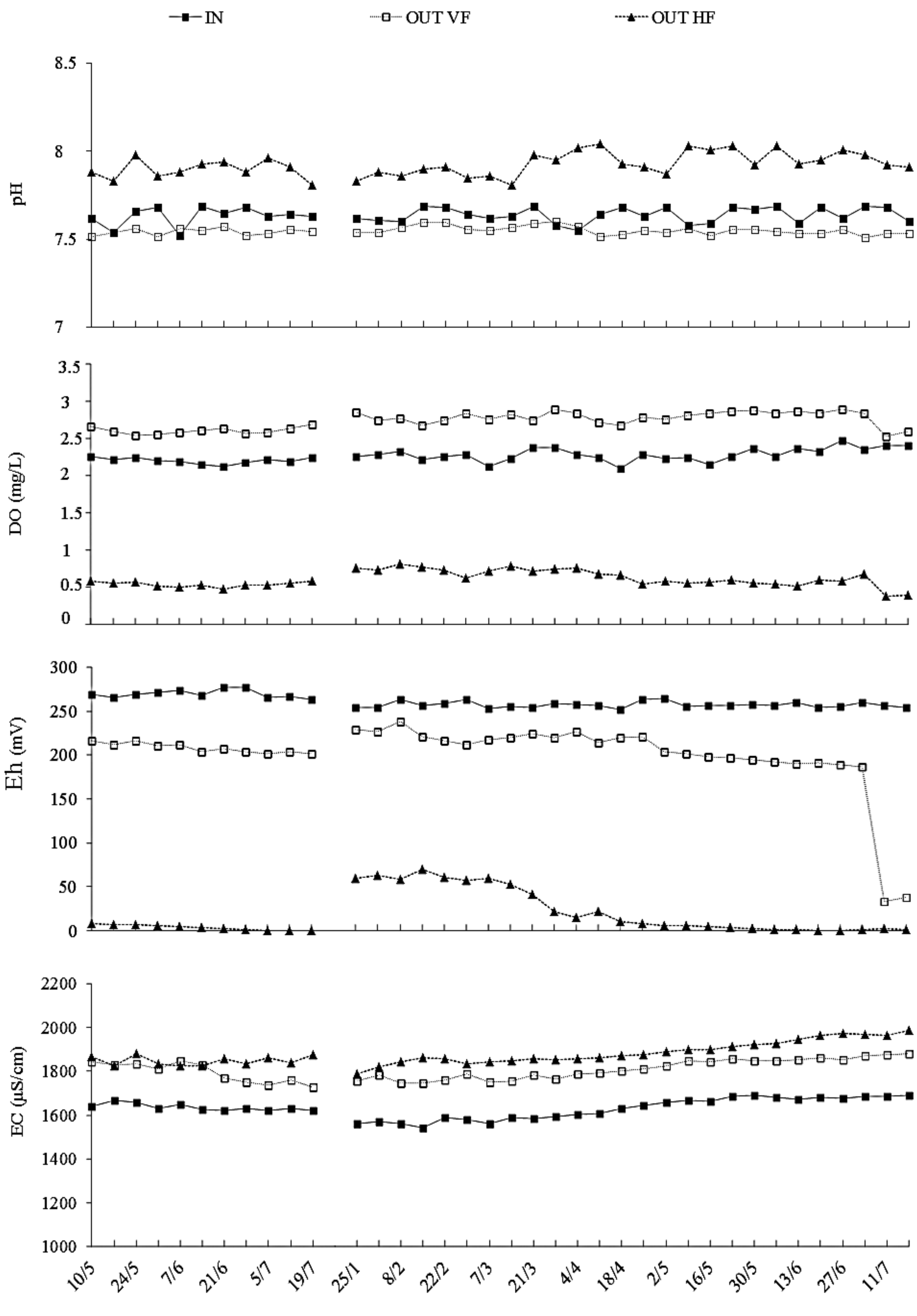


Figure 5.4 – Variation of pH, DO, E_h, EC, during the third monitored period (May 2011-July 2012)

COD

COD inlet concentration was very low during the whole investigation period (median 6.8 mg/L) because an organic carbon source was not added to the synthetic wastewater daily load. Starting from 11th April 2011 an increasing COD concentration was measured at VF effluent. The values rose from 8.3 to 11 mg/L at the end of monitored period. A progressive COD concentration was measured in HF effluent from 28th March 2012. The highest value was reached on 4th July 2012 with 26.8 mg/L (Figure 5.5).

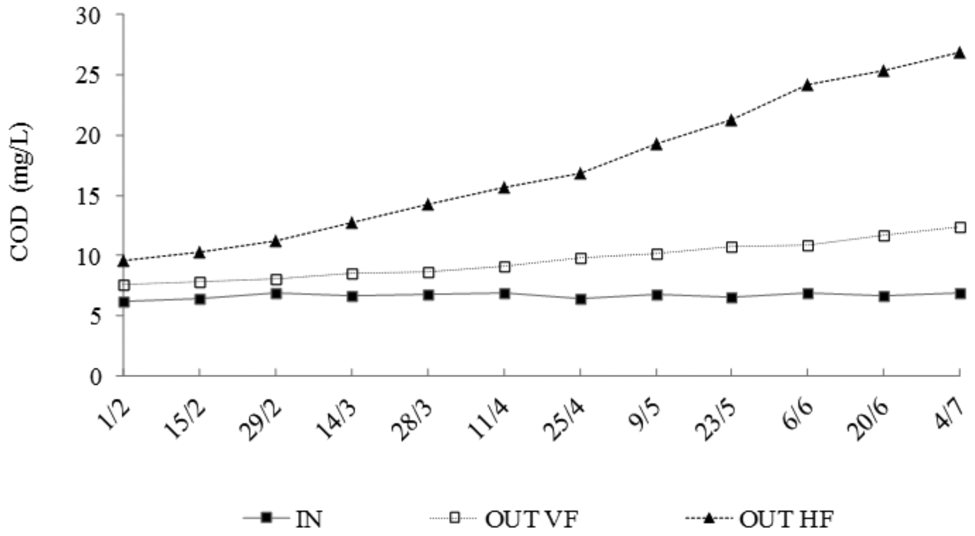


Figure 5.5 – COD concentration at inlet (IN) outlet of VF unit (OUTVF) and outlet of HF unit (OUT HF) of the hybrid system during the monitoring period (May 2011-July 2012).

The relationship of COD concentration and temperature was linearly fitted, resulting in high correlation coefficients of $R^2 > 0.88$ (Figure 5.6). This result was probably related to two main reasons: first, with warmer wastewater and air temperature *P. australis* provides carbon from root exudates (due to photosynthetically fixed carbon, within a range of 5-25% C) (Brix, 1997); second, the progressively decaying plant materials from the colder season increase the bioavailable organic carbon available to denitrifiers.

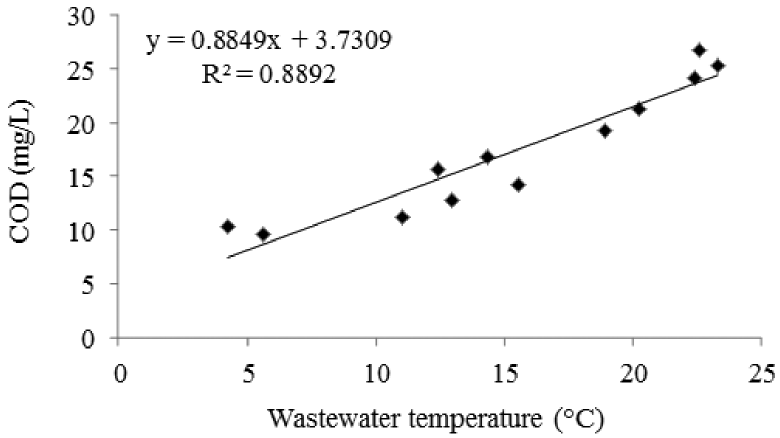


Figure 5.6 – Wastewater temperature and COD concentration correlation (January-July 2012). Correlation is significant at $p < 0.05$.

Nitrogen forms

Daily TN inflow concentration of 250 mg/L was reduced differently during the monitored period. From May to July 2011, TN was abated to median values of 173.4 and 105.6 mg/L by VF and HF units respectively. During the colder season, from January to March 2012, TN concentration reductions accomplished by both VF and HF were negligible. Starting from 21st March 2012 a progressive increasing of TN abatement performances was measured in HCW units. From 25th April 2012 a TN abatement performance similar to the previous year was obtained. A comparison was made between two loading cycles (27th June and 4th July 2012) managing the VF unit with OR1 (fill period 6 hours) and another two (11th and 18th July 2012) managed with OR2 (fill period 14 hours) under similar environmental conditions. During the last two loading cycles (11th and 18th July) higher TN effluent concentrations were measured from VF (>17.2%) and HF (> 2.41%) (Figure 5.7a). Considering the whole investigation period TN effluent concentration measured for the VF unit provided a statistically significant abatement from the inlet. Significant differences were also found between VF1 and VF3 effluent concentrations, probably related to the different types of plants and substrate media. The HF system lowered (not significantly) the inlet median value from VF unit from 146 mg/L to a final discharge concentration of 114 mg/L, but with an increase in variability (Figure 5.8).

From May to July 2011, ammonia nitrogen concentration followed a decreasing trend for both VF and HF units. NH_4-N concentration detected in VF unit effluent decreased slightly from 72 to 61.3 mg/L; the same time pattern was observed for HF unit effluent, with a concentration decrease from 53.8 to 40.6 mg/L. During the colder season, a drop of median inlet concentration trends was measured in both VF and HF cells. The effluent concentration from VF unit decreased from 74.3 mg/L (January 2012) to 68.6 mg/L (March 2012) and from HF unit from 59.6 to 50.3 mg/L. Passing from spring to summer a gradual decrease in NH_4-N concentration was detected for VF (from 68.6 to 56.3 mg/L) and HF units (from 50.4 to 39.5 mg/L). As a result of a change of OR in VF cells (on 11th and 18th July 2012) a drastically increasing ammonia concentration was measured in VF and HF effluent respectively, 17.5% and 2.2% higher than the previous loading cycle (Figure 5.7b). Considering the whole investigation period, NH_4-N effluent concentration measured for the VF system provided a statistically significant abatement from the inlet. Furthermore, unlike in first and second years of monitoring, significant differences were found between the first two VF cells and the third. This was probably due to the contribution of the zeolite substrate medium in VF3 that could absorb NH_4^+ (Nguyen and Tanner, 1998). An enhanced performance by zeolite substrate was probably reached since the average daily flow rate in the VF cell was significantly lower (0.56 m^3/d) during the third monitored period than previously. HF cell passage slightly reduced the concentration to a final discharge of 49.6 mg/L (Figure 5.8).

NO_3-N concentration trend for the first warm season (May-July 2011) was in accordance with the TN and NH_4-N trend. From January to March 2012, NO_3-N concentration measured in VF and HF effluent exceeded influent. Median influent concentration was 124.6 mg/L, while median effluent was 181 and 185 mg/L for VF and HF units respectively, probably due to a conversion of ammonia nitrogen into nitric nitrogen. From 11th April 2012, the median NO_3-N effluent concentration measured from VF cells (123.5 mg/L) decreased with the increasing wastewater temperature until 9th May 2012 (100 mg/L); after that date the concentration was constant and ranged between 95 and 10. Instead the drop in concentration measured for HF unit continued until the end of monitoring, probably due to absorption of nitric nitrogen by growing vegetation.

During the last two loading cycles of 11th and 18th July 2012, the fill period (14 hours) in OR2 promoted an increase of 11% the NO_3-N concentration from VF unit and a decrease of 30% by HF unit (Figure 5.7c). Considering the whole investigation period, VF system lowered the median NO_3-N inlet concentration to 101 mg/L. Furthermore no significant differences were found between VF cells. HF unit accomplished abatement to the discharge value of 76.1 mg/L. An increasing of lower and upper quartiles was observed at HF inflow and outflow (Figure 5.8).

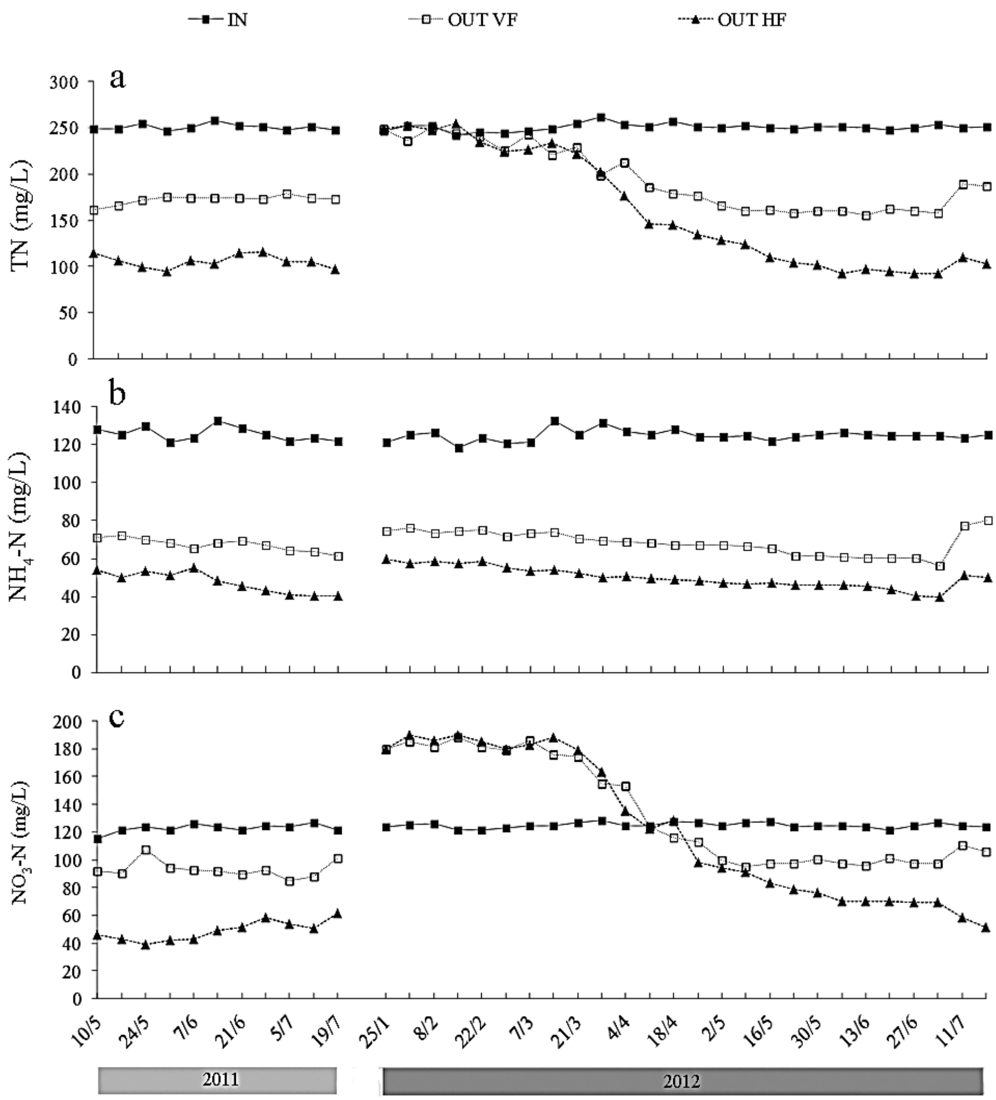


Figure 5.7 – Nitrogen forms concentration at the sampling points of the hybrid system during the third monitored period (May 2011-July 2012).

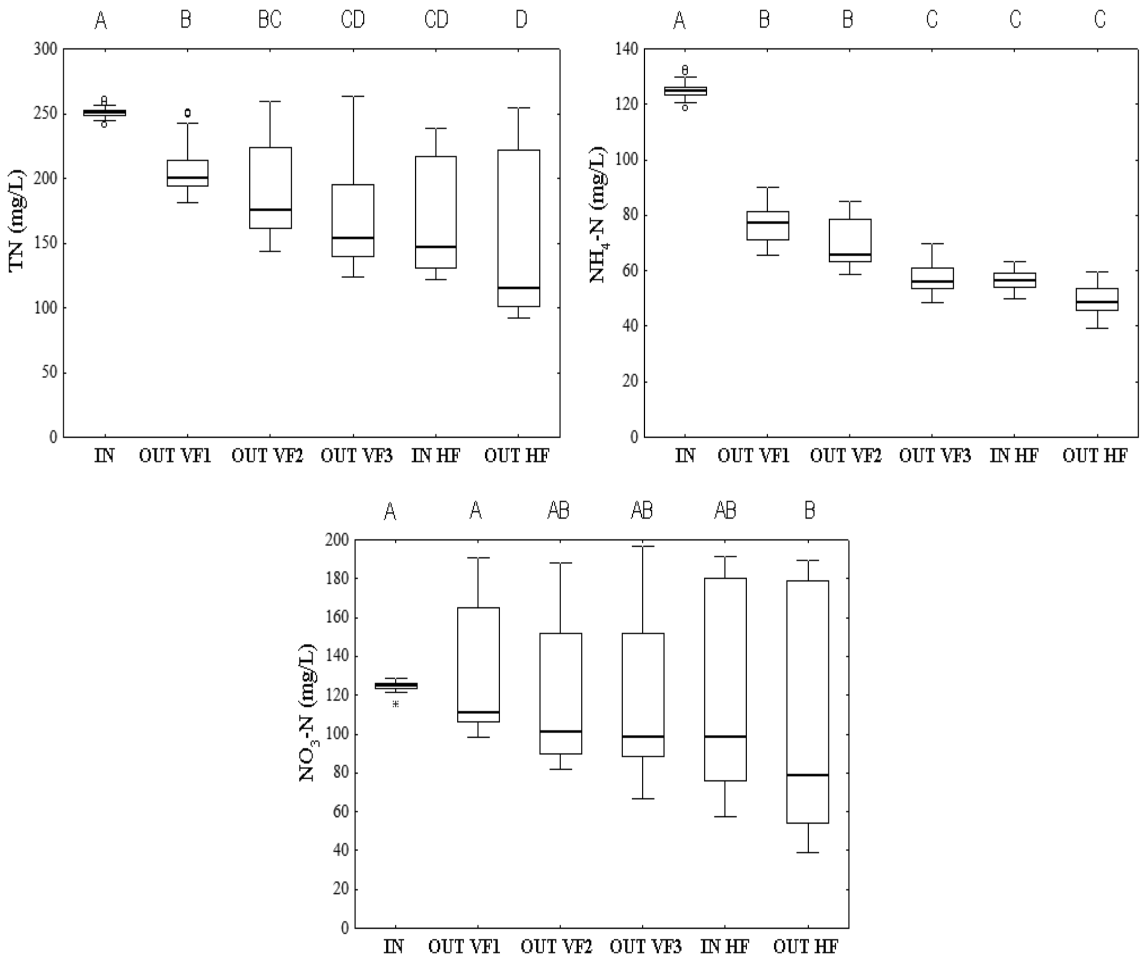


Figure 5.8 – Box-plot diagrams of nitrogen forms concentration (mg/L) in the sampling points of the hybrid system during the third monitored period (May 2011-July 2012).

Overall nitrogen abatement

Percentage abatements (A) for nitrogen forms in the HCW are presented in Table 5.3. The system removed approx. 54% of total nitrogen, 60% of ammonia nitrogen and 39% of nitric nitrogen.

TN abatement ranged from -5.3% to 63.7% with a median value of 54%. If compared with the second monitored period, higher TN concentration reduction was achieved by VF and HF system with 30 and 34% respectively. Note that median TN inlet concentration during the second monitored period was 692 mg/L, instead during the third one it was 250 mg/L.

Overall ammonia nitrogen reduction was 60%. VF system performed better (45%) than HF (27%) probably due to the OR management strategy that promoted oxygen diffusion through the substrate.

NO_3-N concentration reduction was lower than other nitrogen forms. The overall median abatement of 39% was mainly provided by HF unit with 25%. The presence of organic matter in wastewater is essential to drive the denitrification reaction (Beauchamp et al., 1989), nevertheless COD concentration increased slightly from 8.3 to 11 mg/L at the end of the monitored period due to slow decomposition of plants.

Table 5.3 – Concentration reduction efficiency calculated on median value of VF, HF unit and HCW system for nitrogen forms – third monitored period (May 2011-July 2012).

	VF			HF			HCW		
Parameter	Inflow (mg/L)	Outflow (mg/L)	A (%)	Inflow (mg/L)	Outflow (mg/L)	A (%)	Inflow (mg/L)	Outflow (mg/L)	A (%)
TN	250	174	30	174	114	34	250	114	54
NH_4-N	125	68	45	68	50	27	125	50	60
NO_3-N	125	101	19	101	76	25	125	76	39

Effect of the operational regime

During the two last loading cycles managed with OR1 (on 27th June and 4th July 2012) and the two managed with OR2 (on 11th and 18th July 2012), different concentration abatement was measured. As reported by several studies (McBride and Tanner, 2000; Austin et al., 2003), nitrogen removal through sequential nitrification and denitrification in a VF system are mainly based on adsorption processes. Batch operation in a VF system has the potential to enhance the removal of nitrogen thanks to: temporal redox variation (between aerobic and anoxic conditions), maximum pollutant-biofilm contact and increasing oxygen transfer during the operation (Sun et al., 1999). Generally speaking, in this study, the VF system managed with OR1 gave higher percentage concentration reduction for all nitrogen forms (Table 5.4).

TN concentration abatement achieved by VF system managed with OR1 was 20% higher in comparison with OR2. Higher TN abatement of 48.4 and 30.5% was measured in VF3 with OR1 and OR2 management respectively. This finding was probably related to a prolonged unsaturated condition ensuring better oxygenation of substrate, which favours aerobic processes.

NH_4-N abatement in VF system managed with OR1 was 32.3% higher than with OR2. During the fill period of 6 hours in OR1, ammonium ions (NH_4^+) present in wastewater were probably absorbed onto a negatively charged biofilm. While, during the draining process, and subsequently during the rest period of 14 hours, the atmospheric oxygen was drawn down into the VF cell substrate, resulting in rapid aeration of the biofilm and NH_4^+ nitrification. The essential factor for nitrogen removal with VF system batch operation management is the absorption of NH_4^+ ions (McBride and Tanner, 2000; Austin et al., 2003). The absorption efficiency depends on different characteristics: one of the most important is the cation exchange capacity of the substrate used in the treatment system (Austin, 2006). Higher NH_4-N abatement of 60.3 and 45.8% was measured in VF3 with OR1 and OR2 management respectively. This could be linked to the 0.10 m transition layer of zeolite stones used in VF3. A number of research studies (Yalcuk and Ugurlu, 2009; Saeed and Sun, 2011) used a zeolite substrate to optimize treatment performances in VF wetland systems.

Nitrate ions rapidly desorbed from the biofilm into the wastewater during the fill period of the VF and they are used as an electron acceptor during denitrification (Austin et al., 2003). VF system managed with OR2 showed no enhanced denitrification performance. A prolonged fill period of 14 hours did not significantly improve nitrate-nitrogen abatement.

Table 5.4 – Comparison of mean percentage abatement of TN, NH_4-N and NO_3-N obtained by three VF cells with different operational regime (values in bold are significantly different at $p < 0.05$ by Kruskal–Wallis one-way analysis of variance).

	VF1		VF2		VF3	
	OR1[a]	OR2[b]	OR1[a]	OR2[b]	OR1[a]	OR2[b]
A (%)						
TN	**24.6**	18.3	**37.4**	25.8	**48.4**	30.5
NH_4-N	**46.2**	28.5	**50.1**	35.6	**60.3**	45.8
NO_3-N	**12.4**	7.5	**27.2**	15.1	**27.9**	16.1

[a]OR1 - operational regime 1 – with 6 hours of fill period (VF cell substrate saturated conditions) and 14 hours of rest period (VF cell substrate unsaturated conditions). [b]OR2 - operational regime 2 – with 14 hours of fill period (VF cell substrate saturated conditions) and 6 hours of rest period (VF cell substrate unsaturated conditions).

Effect of wastewater temperature

Many individual wetland processes, such as microbially mediated reactions, are affected by temperature. Processes regulating organic matter decomposition and all nitrogen cycling reactions (mineralization, nitrification and denitrification) display cyclic seasonal variations (Kadelc and Reddy, 2001).

A review of the literature illustrates that seasonal differences in CWs nitrification are notable (Kuschk et al., 2003; Song et al., 2006), Wittgren and Maehlum (1997) stated that nitrogen cycling was inhibited in colder months due to the decrease of oxygen availability. The optimal temperature for nitrifying bacteria is 28-36 °C, however, Katayon et al. (2008) stated that a temperature range between 16.5 and 32 °C is favourable for nitrification in CWs. Cookson et al. (2002) suggested that nitrifying communities can adapt to temperature changes and may maintain their activity at lower temperatures by metabolic adaptation. However, other studies have shown that nitrification is inhibited by water temperatures <10 °C (dropping off rapidly below 6 °C) and >40 °C (Hammer and Knight, 1994; Herskowitz et al., 1987; Werker et al., 2002 Xie et al., 2003).

Seasonal and temperature effects have also been examined on the denitrification process (Reddy et al., 2001; Trias et al., 2004). The activity of denitrifying bacteria in CWs sediments is generally more robust in spring and summer than in autumn and winter (Herkowitz, 1986) and the overall removal rate of nitrate is significantly higher in summer than in winter (Christensen and Sorensen, 1986; van Oostrom and Russell, 1994; Stober et al., 1997). Denitrification can occur between 5 and 30 °C and the reaction rate increases exponentially with increasing temperature, reaching a plateau between 20 and 25 °C, as long as other environmental factors do not restrict the rate (U.S. EPA, 1975). Some researchers report that denitrification ceases below 5 °C (Stanford et al., 1975), others have measured some denitrification activity at 4 °C, albeit at much slower rates (Limmer and Steele, 1982; Pfenning and McMahon, 1996; Richardson et al., 2004). Summarizing, the microbial activities related to nitrification and denitrification can decrease considerably at water temperatures below 15 or above 30 °C (Kruschk et al., 2003; Vymazal, 1999).

As observed by Akratos and Tsihrintzis (2012), for all pollutants, lower temperatures generally correspond to lower reduction efficiency and the opposite. Correlation charts drawn up between percentage abatements and wastewater temperatures were linearly fitted, resulting in high correlation coefficients of $R^2 > 0.90$ for TN and $R^2 > 0.83$ for NO_3-N (Table 5.5). Whilst in this study, although a slightly increased NH_4-N abatement was achieved with warmer wastewater temperature there was no significant correlation ($R^2 < 0.60$), which implies that there was also biological activity during the cold season.

Many researches (Akratos and Tsihrintzis, 2007; Kuschk et al., 2003 Vymazal, 1999) reported that at temperatures below 15 °C, neither the bacteria responsible for N removal nor the vegetation functioned properly. To confirm this value or individuate another wastewater temperature that caused variations in TN and NO_3-N abatement performances, a segmented linear regression analysis (or broken-line regression) was applied to percentage abatement measured during the whole period. The analysis fits a least squares regression line in each segment and assumes a linear relationship between the independent variable and the outcome within each segment. Figure 5.9, shows the existence of a wastewater temperature breakpoint at 14.2 °C that caused variations in reduction efficiencies for TN and NO_3-N.

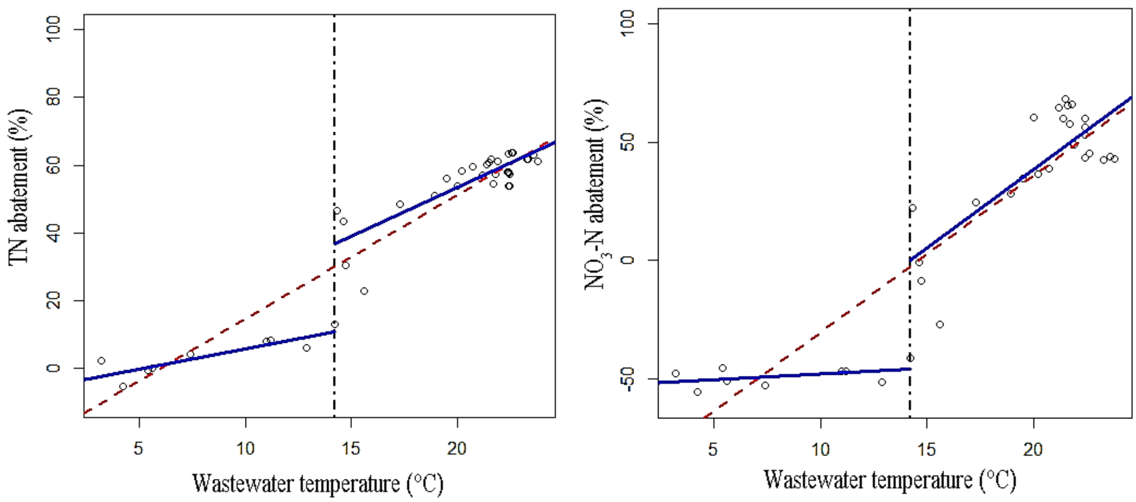


Figure 5.9 – Wastewater temperature and concentration abatement correlation charts with change-point for TN and NO_3-N forms. Correlations are significant at $p < 0.05$ and breakpoints are significant at $p < 0.05$ by partial F-test.

Table 5.5 – Slope and (correlation coefficient) R^2 for the linear relationships and for values below and above the individuated change-point.

Nitrogen Form	linear relationships		Values below breakpoint		Values above breakpoint	
	Slope	R^2	Slope	R^2	Slope	R^2
TN	3.69	0.96	1.19	0.76	2.9	0.7
NO_3-N	6.62	0.83	0.49	0.21	6.64	0.62

For TN and NO_3-N, it seems that wastewater temperature of 14.2 °C is the key value to allow the activation of microbial processes. This value is very closed to the threshold of 15 °C found by Akratos and Tsihrintzis (2007), who reported that with wastewater temperature below 15 °C the mean TN removal was 58.8% and above 15 °C 73.9%. In our study, with wastewater temperature below 14.2 °C, median TN abatement achieved by HCW system was about 4%. VF system lowered the inlet concentration by 3.5%. No significant differences were found among the three VF cells and VF3 reached high TN abatement performance (6.3%). HF system concentration reduction was negligible (negative values was measured) (Figure 5.10). During the colder sub-period (January-March 2012) VF system became a net source of nitric-nitrogen, thus negative concentration reduction was measured (Figure 5.10). As observed in previous studies

(Brodrick et al., 1988; Werker et al., 2002; Burchell et al., 2007), NO_3-N negligible abatement was measured with wastewater temperature range 3-8 °C probably related to a slowdown in denitrification activity (Limmer and Steele, 1982; Pfenning and McMahon, 1996). Another important factor that could have limited denitrification activity was the lack of organic carbon in synthetic wastewater used to feed the HCW system (Gersberg et al., 1983; Lin et al., 2002). However Burchell et al. (2007) highlighted the fact that with winter temperatures (7.5 °C) denitrification activity did not respond to added organic matter.

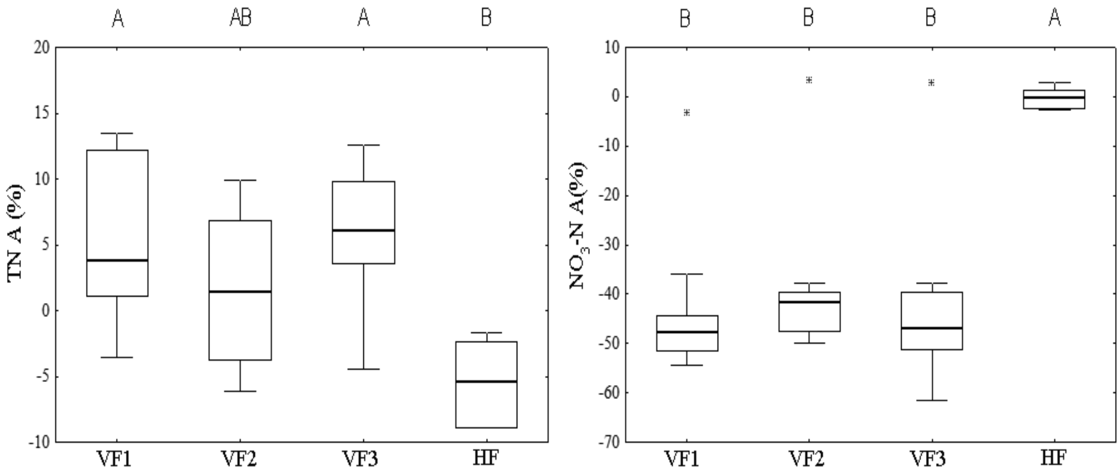


Figure 5.10 – Box-plot diagrams of TN and NO_3-N concentration (mg/L) at the sampling points of the hybrid system during the colder sub-period (wastewater temperature <14.2 °C) of the third monitored period.

Higher wastewater temperature than the individuated change-point ameliorated the abatement of both nitrogen forms, reaching a median value of 57.5 and 45.4% for TN and NO_3-N respectively with the HCW system (Figure 5.11). TN concentration reduction increased noticeable in VF system, significant differences were obtained by the three VF cells. As observed in TN abatement with temperature <14.5 °C VF3 cell reached higher abatement performance (43.2%). Significantly higher nitric nitrogen abatement was obtained if compared with monitoring at wastewater temperature <14.5 °C. VF1 abatement (13.4%) was significantly lower than the other VF cells (26%). NO_3-N abatement obtained by HF cell was in accordance with that obtained by VF2 and VF3.

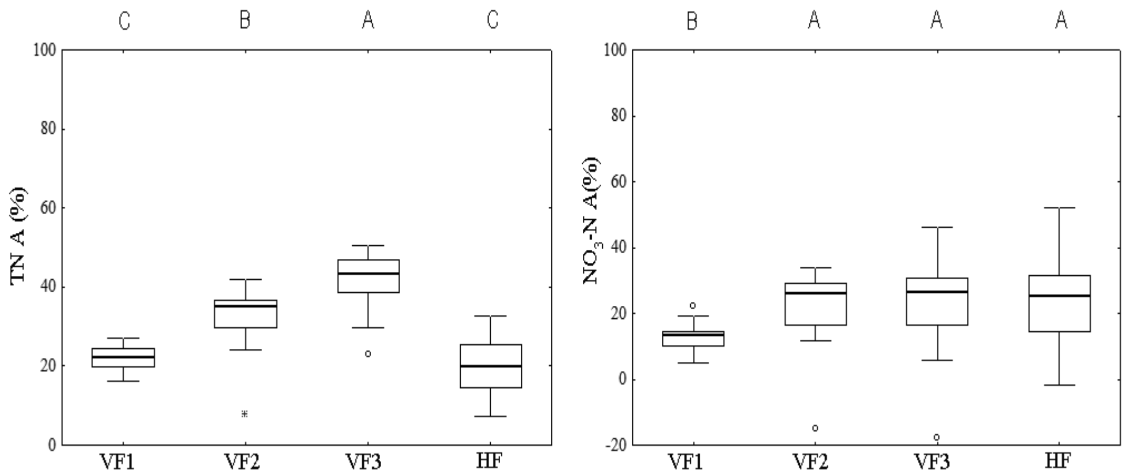


Figure 5.11 – Box-plot diagrams of TN and NO_3-N concentration (mg/L) in the sampling points of the hybrid system during the warmer sub-period (wastewater temperature >14.2 °C) of the third monitored period.

HCW hydraulic conditions

The average flow rate (Q) in the HCW system during the third monitored period was 1.7 m^3/d, which was slightly less than the system design limit of 2 m^3/d. The HLR for the entire system during the whole period was 1.3 cm/d (lower than that used for the first and second monitoring periods of 3.8 cm/d). The average flow rate in VF cells was 0.56 m^3/d. Cumulative wastewater volume discharged by HF unit from January to July 2012 was 25.2 m^3 (Figure 5.12). Outflow exhibited a seasonal pattern, decreasing as wastewater temperature increased due to evapotranspiration. Indeed during the cold season (January-March 2012) HF outflow decreased from 1.62 to 1.35 m^3/d, and during the hot season (May-July 2012) it decreased from 0.70 to 0.51 m^3/d. The hydraulic retention time (HRT) of the entire system was 7 days.

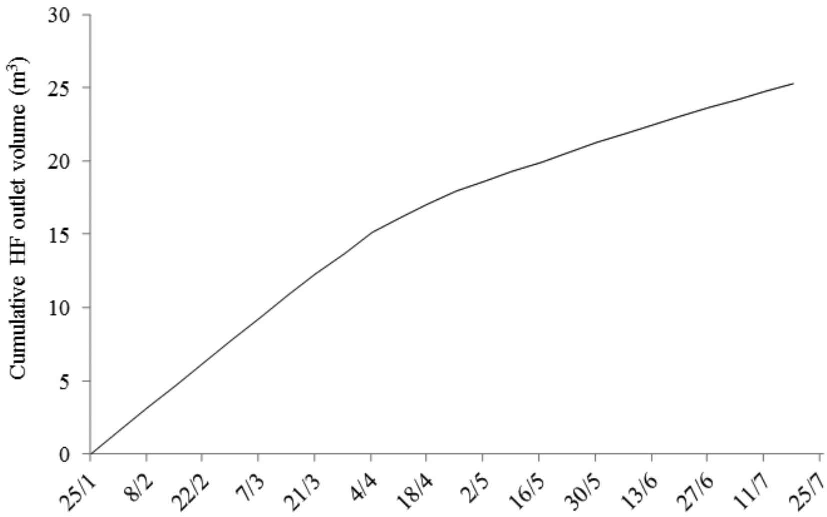


Figure 5.12 – Cumulative discharged wastewater volumes from HF cell during the third monitored period (January-July 2012).

Mass reduction efficiency (MRE)

Figure 5.13 presents mass removal efficiency of nitrogen forms obtained during the third monitored period. The differences between seasons may also be partly attributable to discharge differences. Indeed during the summer, higher evapotranspiration rate reduced the outflow rates as compared to winter when, with plant senescence, temporary sequestering of nutrients would also be released rather than taken up (Nicholson, 1983; Richardson and Craft, 1993).

From May to July 2011, TN mass reduction was quite similar for both VF and HF units (60%), during the colder sub-period (January-March 2012) VF system provided lower MRE that ranged between 2 and 30%. Starting from April 2012, MRE increased significantly from 30 to the highest measured value of 64% (4^{th} July 2012). From January to February 2012, negative MRE values were obtained from the HF cell. However, from April to the end of monitoring the HF cell reached a similar value to VF system. (Figure 5.13a).

A slightly increasing NH_4-N mass reduction was observed in VF unit during the warm and cold sub-periods. From January to July 2012 ammonia-nitrogen MRE increased from 40.3 to 70% but remarkable seasonal differences were not found. Instead, Song et al. (2006) reported that the rate of NH_4-N reduction was about 40% less efficient in spring and winter than in summer. HF unit showed lower mass reduction (8-25%) from January to March 2012 and ameliorated from April to July (30-55%) (Figure 5.13b).

From May to July 2011, NO_3-N mass reduction was mainly provided by HF system with a median value of 59.3% and VF system with 52%. During the colder sub-period, from January to 14th March 2012 a negligible MRE was obtained from HF unit (-1.2 to 5.2%). After that period an increasing trend occurred, reaching the maximum MRE value of 70%. From January to April 2012 a negative trend was measured (from -41.2 to -7.5%). With the increase of temperature MRE of VF unit rose to the value observed in HF system (Figure 5.13c).

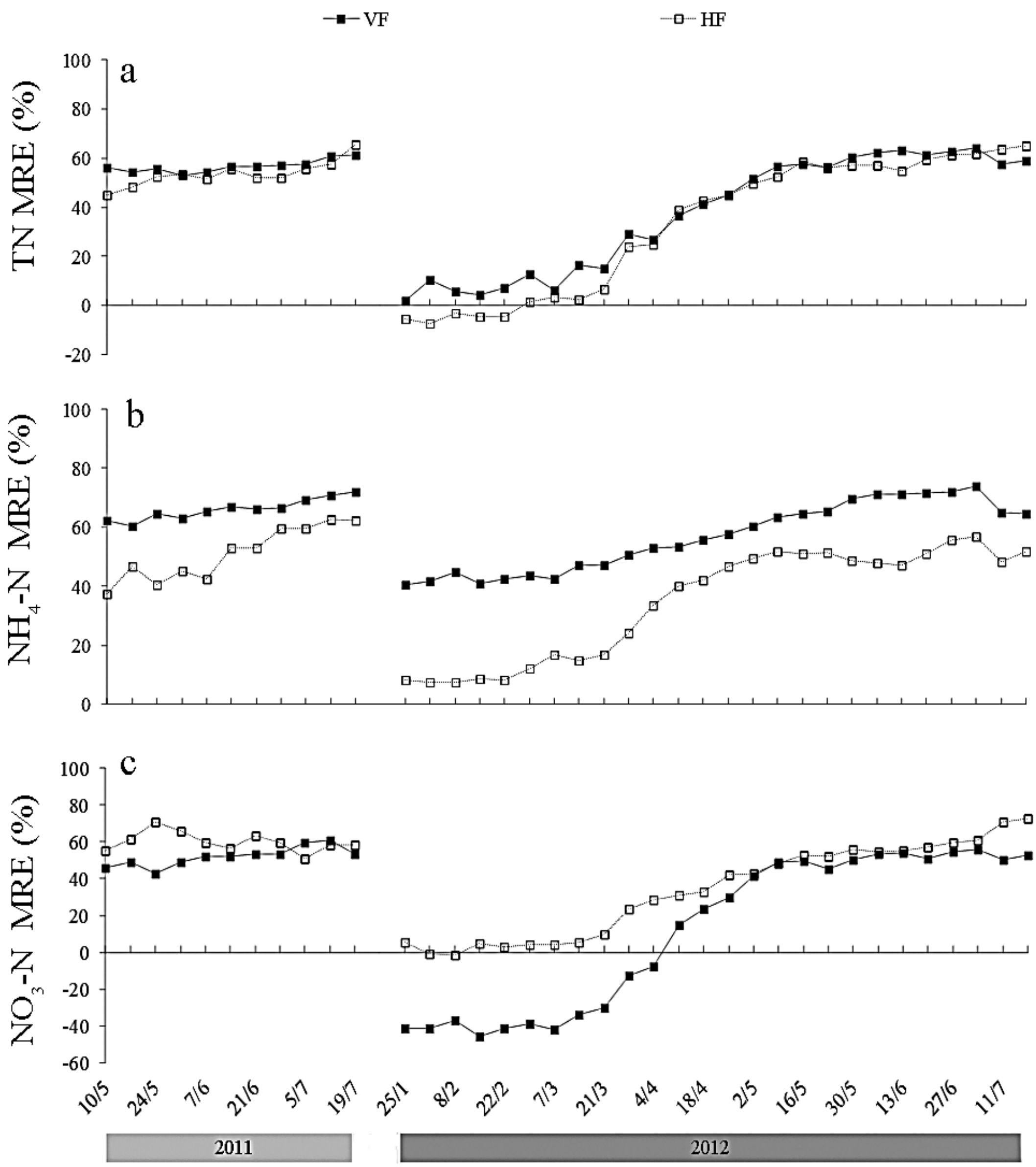


Figure 5.13 – Nitrogen forms mass removal efficiency (MRE) at the sampling points of the hybrid system during the third monitored period (May 2011-July 2012).

Surface load reduction (SLR)

During the first sub-period (May-July 2011), TN load reduction obtained by the HCW system ranged from 2.6 to 2.91 g/m^2/d. From 25th January to 15th February 2012 a negligible SLR (ranging from 0.14 to 0.27 g/m^2/d) was calculated. From 22nd February to 16th May 2012 it increased noticeably from 0.41 to 2.75 g/m^2/d; after this date the trend was constant till the end of monitoring (Figure 5.14)

No significant variation in the NH_4-N load reduction trend occurred between the cold and warm sub-periods of 2012, however a slight increase was calculated from 0.84 to 1.52 g/m^2/d. Higher values were therefore reported during the warm sub-period of 2011 (1.47 to 1.60 g/m^2/d) (Figure 5.14).

During the warm sub-period of 2011, a constant NO_3-N SLR was obtained, the value ranged from 1.26 to 1.45 g/m^2/d. Instead, from 25th January to 28th March 2012, SLR values were negative (-0.61 to -0.11 g/m^2/d). From 4th April to the end of monitoring a progressive SLR increasing occurred from 0.18 to 1.41 g/m^2/d (Figure 5.14).

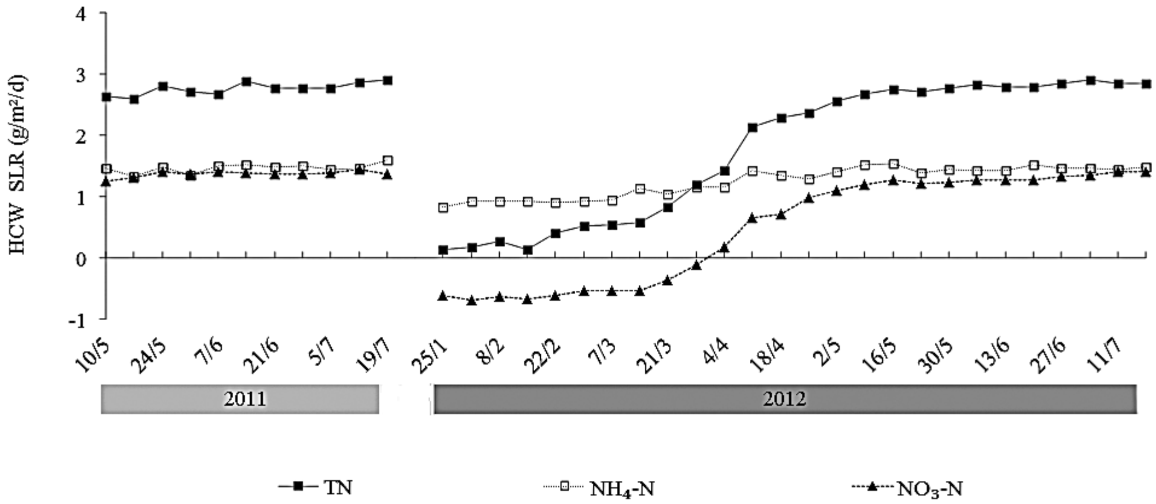


Figure 5.14 – Surface load reduction (SLR) of HCW system for nitrogen forms during the third monitored period (May 2011-July 2012).

Median TN SLR of 2.5 g/m^2/d was obtained by HCW system throughout the investigation period. Median SLR obtained by VF unit was 11 g/m^2/d; lower values (9 g/m^2/d) of SLR were reported by (Stefanakis and Tsihrintzis, 2012). A higher TN load reduction (13 g/m^2/d) was calculated in VF2, followed by VF1 and VF3 with 11 and 10 g/m^2/d respectively. HF unit removed significantly lower TN rates than VF unit, with a median value of 0.72 g/m^2/d and the variability decreased (Figure 5.15).

Ammonia nitrogen SLR obtained by HCW system ranged from 0.84 to 1.60 g/m^2/d, the median value was 1.43 g/m^2/d. This finding is in accordance with what was reported by different studies that demonstrated efficient wetland performance at NH4-N range 0.15 to 30 g/m^2/d (Farahbakhshazad and Morrison, 1997; Zachritz et al., 2008; Saeed and Sun, 2011). As observed for TN, VF system was able to remove a higher rate of NH_4-N than HF. Not significant differences in NH_4-N SLR were found between VF cells, however VF2 median value (7 g/m^2/d) was higher than VF1 and VF3, with 6.2 and 6 g/m^2/d respectively. HF unit provided negligible NH_4-N load reduction (between 0.07 and 0.52 g/m^2/d) (Figure 5.15).

Median NO_3-N SLR value provided by HCW system was 1.26 g/m^2/d. No statistically significant differences were found among VF cells. Higher SLR was obtained by VF2 (5.73 g/m^2/d), followed by VF1 and VF3 with 4.63 and 4.04 respectively. HF system was able to remove a median NO_3-N rate of 0.31 g/m^2/d (Figure 5.15).

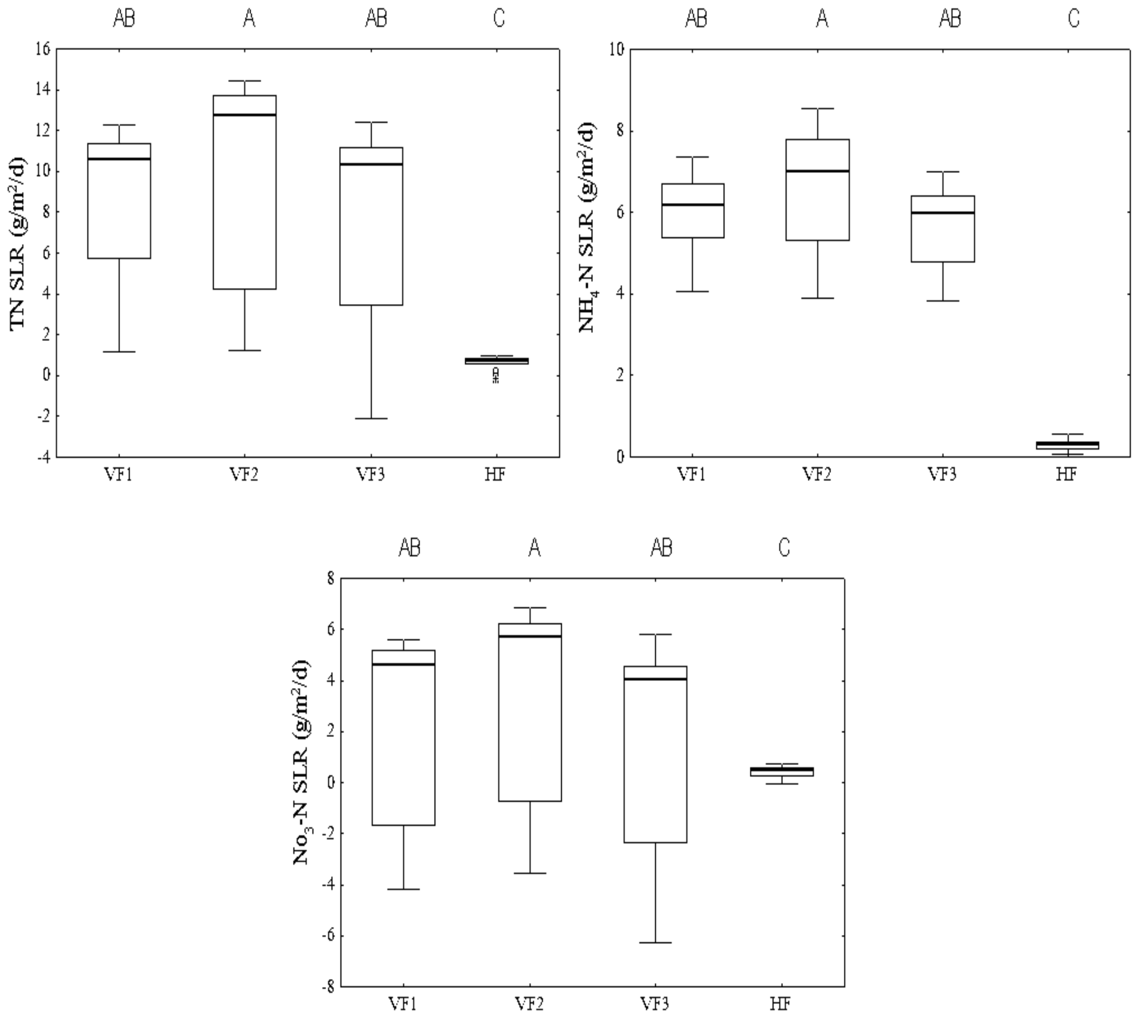


Figure 5.15 – Box-plot diagrams of surface load reduction (SLR) for nitrogen forms during the third monitored period (May 2011-July 2012).

Conclusions

The observed fluctuating flow and concentrations during the two previous monitored periods also varied seasonally and their influence could be confused with that of temperature (IWA, 2000). For this reason, a synthetic wastewater was used to standardize the HCW system inlet in order to better study the effects of temperature under the same nitrogen load. The average flow rate was 1.7 m^3/d, which was slightly less than the HCW system design limit. Batch feed mode was used to test two different operational regimes (OR). VF system managed with operational regime 1 (OR1) (fill period 6 hours – rest period 14 hours) promoted unsaturated condition in VF unit substrate that ensured better oxygenation (> 2.70 mg/L) and high E_h (> 180 mV). VF system managed with OR2 (fill period 14 hours – rest period 6 hours) promoted the lowering of DO concentration in VF and HF effluent to 2.5 and 0.35 mg/L respectively. Eh value measured at VF system effluent drastically decreased from 216 to 35 mV. TN and NH_4-N concentration abatements achieved by VF system managed with OR1 were 20% and 32.2% higher than OR2 respectively.

Overall the HCW system abatement performances were 54% of total nitrogen, 60% of ammonia nitrogen and 39% of nitric nitrogen (calculated on median concentrations). In this third monitoring, the use of zeolite porous media as substrate material significantly enhanced the NH_4-N concentration reduction in VF3 unit in comparison with first and second monitored period.

Seasonal variations affected the HCW system performance. Higher effluent concentrations were observed during the cold period, especially for TN and NO_3-N. NO_3-N median outflow concentrations (181 and 185 mg/L from VF and HF unit respectively) exceeded influent (124.6 mg/L) between January and March 2012. The performance ameliorated with increasing wastewater temperature. NH_4-N concentration reduction was quite similar in both the warm and cold season. Segmented regression analysis individuated 14.2 °C as a breakpoint temperature that discriminated different abatement rates for TN and NO_3-N. With wastewater temperature lower than 14.2 °C, 4.1% and a negative value (-47.3%) were obtained for TN and NO_3-N. With wastewater temperature higher than 14.2 °C, abatement of 57.5 and 45.4% was reached by HCW system for TN and NO_3-N respectively. Unlike in other studies, in this case a synthetic wastewater was used containing only urea as source of nitrogen. Instead, the literature reported (Stefanakis and Tsihrintzis, 2012; Akratos and Tsihrintzis, 2012) that in addition to the nitrogen source, an organic load was utilized to simulate a COD inflow. The lack of organic carbon availability in addition to the temperature effect could affect the denitrification process (Beauchamp et al., 1989; Gale et al., 1993; Weier et al., 1993). The differences between seasons may also be partly attributable to discharge differences. Indeed during the summer, a higher evapotranspiration rate reduced the outflow rates as compared to winter when, with plant senescence, temporary sequestering of nutrients would also be released rather than taken up.

TN SLR ranged from 0.14 to 2.91 $g/m^2/d$. In general, HF cell showed low TN elimination rates ranging from a negligible value due to the colder period (-0.20 $g/m^2/d$) to 0.98 $g/m^2/d$. As reported by other studies (e.g. Canga et al., 2011), higher N elimination rates were attributed predominantly to VF system. Thus median VF unit SRL was 11 $g/m^2/d$.

Chapter VI

HCW vegetation

Plant development and aerial biomass production

As suggested by Brix (1994), different species of plant were used in VF and HF systems taking into account factors such as the rooting depth, plant productivity and tolerance to high wastewater loads.

Canna x generalis used in the first cell of VF system is a robust perennial rhizomatous ornamental plant; the literature (Zhu et al., 2004; Zhang et al., 2007) mentioned the use of this species in different VF systems according to its root growth potential. Planting with *C. x generalis* was performed in April 2010, using 40 branching rhizomes. For the first two weeks the water level in the vertical units was kept at a few centimetres below the gravel surface to allow plant development. The life cycle of *Canna x generalis* began in the first week of May 2010, when buds from underground rhizomes started to produce the first green leaves. The sympodial rhizome curves its direction upwards to form the aerial part of the plant after producing 5-6 nodes. These “active” nodes produced up to three aerial branches, so three new plants grew very close to each other. As documented in Figure 6.1, plants developed very dense, upright stems (160-180 cm tall in August 2012) that were sturdy, glabrous and green in colour. At the end of July 2010, a clustered spike of flowers appeared at the top of the stalks, with petals ranging from blood red to orange or yellow streaked with red. pH measured in the VF substrate, particularly during the first and second monitored period was slightly alkaline (7.58 to 8.36). This range of value was outside the *C. x generalis* optimum development range, which is generally considered to be between 5.5 and 7.5 (Jett, 2005). The high concentrations of pollutants with which HCW system was fed during the second monitored period did not influence plant development and propagation.

Figure 6.1 – *Canna x generalis* development during three years of monitoring in VF1 cell.

As summarized in table 6.1, *C. x generalis* produced a large amount of aerial biomass in a short time. During the first and second year, fresh weight values were low (they rose from 7.14 to 11.8 Kg/m^2). During the third growing season the fresh weight increased significantly to 14 Kg/m^2. Dry weight ranged from 1.14 during the first year, to 1.67 in the second and 1.96 Kg/m^2 in the last: no significant differences were observed among years. However, measured dry weights were higher than the 0.67 Kg/m^2 reported by Zhang et al. (2007). Dry matter percentage was not significantly different among the years, but values were strictly connected to harvest time and so to the water content in the stem and leaves. Nitrogen concentration in the aerial parts of *C. x generalis* was similar during the first and second growing seasons (1.98 and 1.8%) but an increase in concentration was measured (2.6%) during 2012, probably due to the different wastewater composition. The nitrogen accumulation by *C. x generalis* was relatively high if compared with *Phragmites australis* used in the other two vertical cells.

Table 6.1 – Fresh biomass, dry weight, dry matter and nitrogen stored the aerial parts of *Canna x generalis* harvested form VF1 cell at the end of autumn of each year. Different letters indicate significant differences at $p < 0.05$ by Kruskal–Wallis test.

Species	Year	Fresh weight (Kg/m^2)	Dry weight (Kg/m^2)	Dry matter (%)	Nitrogen (%)	N uptake in aerial part (kg/m^2)
Canna x generalis	2010	7.14 b	1.14 a	17 a	1.98 b	0.02 a
	2011	11.8 b	1.67 a	15.4 a	1.8 b	0.03 a
	2012	14 a	1.96 a	14.9 a	2.6 a	0.05 a

VF2 and VF3 cells were planted in April 2010 with *Phragmites australis* (Cav.) Trin ex. Steudel at a density of 5 plants/m^2. The plants grew in a normal way and proved to be extremely robust and to survive shock dosing (Figure 6.2). Regarding the effect of pH on the growth of *P. australis*, a fluctuating pH did not harm the growth of this macrophyte, if it is considered that the optimum pH for plant development is between 3.7 and 8 (U.S. EPA, 2000). *P. australis* increasing biomass (Table 6.2) indicated that its development was not inhibited when exposed to high concentrations of NH_4-N, especially during the second monitored period.

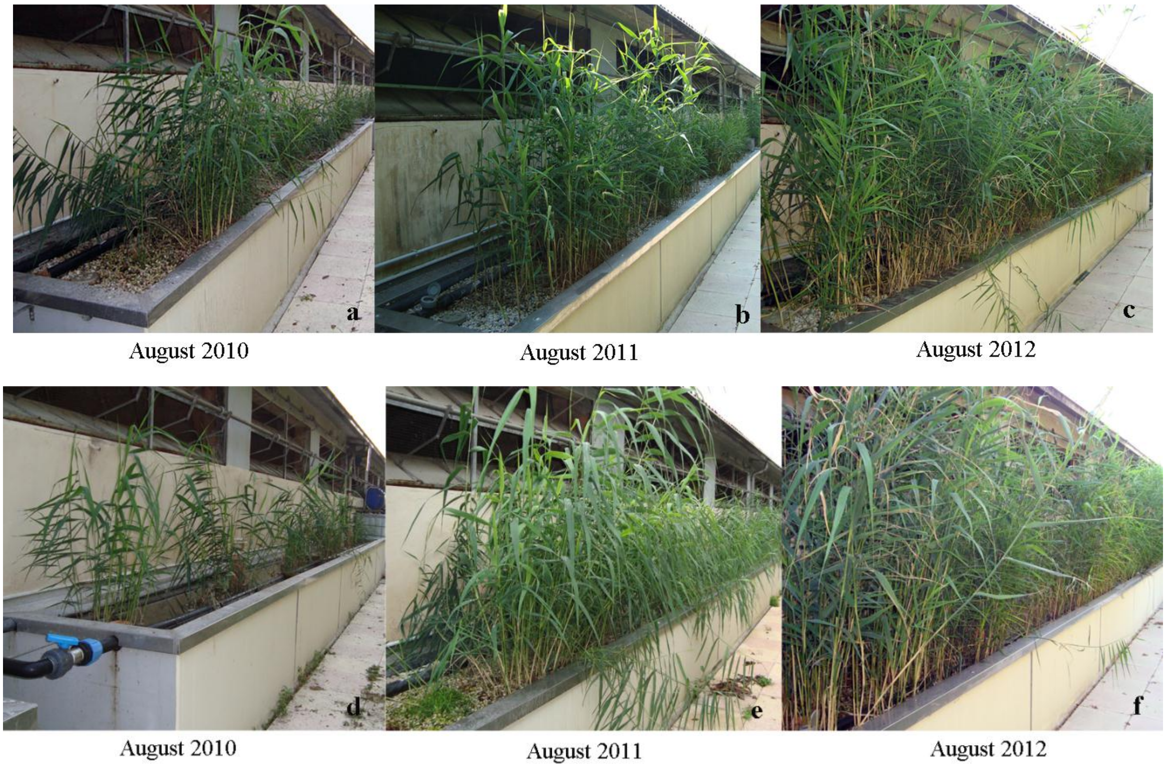


Figure 6.2 – *P. australis* development during three years of monitoring in VF2 (a-b-c) and VF3 cells (d-e-f).

During the first year, fresh weight values from VF2 and VF3 cells were low, at 0.29 and 0.60 Kg/m^2 respectively, probably due to the start-up period (Table 6.2). There was a significant difference in fresh biomass production between the first and last two years in VF2, instead significant differences were found in VF3 in each of the three growing seasons. Generally fresh weight production in VF3 (ranging from 0.6 to 2.2 Kg/m^2) was higher than in VF2 (from 0.29 to 1.2 Kg/m^2).

Healthy and mature reed stands are highly productive (Windham and Lathrop, 1999). Maximum dry weight values of aboveground *P. australis* biomass reported in the literature vary between 1.17 kg/m² (Rothman and Bouchard, 1990) to 7.70 kg/m² (Hartzendorf and Rolletschek, 2001). Soetaert et al. (2004) stated that typical production of mature *P. australis* is around 1 kg/m² of dry weight. In our study, lower biomass yields were obtained in both VF cells (0.39 – 0.79 kg/m² during the third year) probably due to: a) the VF unit was shaded by rows of trees; b) three to four growing seasons are usually needed to reach maximum standing crop, but it may take even longer in some systems as reported by Vymazal and Kröpfelovà (2005). Dry matter content varied from 35.1 to 36.3%. Nitrogen stored in the aboveground biomass of *P. australis* harvested from VF2 cell was lower than VF3 during the first and second year, but the opposite trend occurred in the third, with 2.22% in VF2 and 1.9% in VF3. The nitrogen accumulation in stems and leaves ranged from 0.001 Kg/m^2 (VF2 during the first year) to 0.015 Kg/m^2 (VF3 during the third year).

Table 6.2 – Fresh biomass, dry weight, dry matter and nitrogen stored in the aerial parts of *P. australis* harvested from VF2 and VF3 cells at the end of autumn in each year; different letters indicate significant differences at $p < 0.05$ by Kruskal–Wallis test.

Species	Year	Fresh weight (Kg/m^2)	Dry weight (Kg/m^2)	Dry matter (%)	Nitrogen (%)	N uptake in aerial part (kg/m^2)
P. australis (VF2)	2010	0.29 b	0.1 b	35.1 a	1.82 b	0.001 b
	2011	1.14 a	0.38 a	35.6 a	1.85 b	0.007 a
	2012	1.2 a	0.39 a	33.7 a	2.22 a	0.008 a

Species	Year	Fresh weight (Kg/m^2)	Dry weight (Kg/m^2)	Dry matter (%)	Nitrogen (%)	N uptake in aerial part (kg/m^2)
P. australis (VF3)	2010	0.6 c	0.2 b	35.6 a	1.97 a	0.003 b
	2011	1.24 b	0.41 b	35.4 a	1.69 b	0.006 b
	2012	2.2 a	0.79 a	36.3 a	1.9 a	0.015 a

HF unit was planted with *P. australis* (Cav.) Trin ex. Steudel in April 2009 (one year before the start of this experimentation) at a density of 8 plants/m^2. Regional guidelines suggest that water levels should be maintained between 100 and 200 mm for at least the first 6 weeks after planting, and the wetland should not be allowed to dry out below the surface. However, due to HF system management problems and high evapotranspiration rate during the *P. australis* growing period shallow waters (<100 mm) restricted the growth of the cell vegetation. *P. australis* thus covered a small proportion of the HF bed surface (Figure 6.2a). During May 2010, an additional transplanting (about 150 mature plants) was therefore done to establish a dense cover. The water level for the first 4 weeks after transplanting was raised to a few centimetres below the gravel surface. The reeds developed normally, without apparent disease symptoms (Figure 6.2c).

Figure 6.2 –Vegetative growth of *P. australis* during three years of monitoring in HF cell.

During the first year, low fresh weight was obtained (0.95 Kg/m^2), probably due to the low engraftment levels (Figure 6.3). During the second and third years there was a progressive increase in aboveground biomass production from 1.96 to 2.76 Kg/m^2 respectively. A statistically significant increase in dry weight was obtained from the first (0.33 Kg/m^2) to second year (0.7 Kg/m^2), however the yield obtained in the third year (0.93 Kg/m^2) suggests that one or two more years will be necessary to reach maximum standing crop (Kadlec et al., 2000). Dry matter was in accordance with what was obtained for *P. australis* in VF unit. Nitrogen stored in the aerial parts was slightly lower than that obtained from VF unit, with similar values during each of three growing seasons, ranging between 1.44 and 1.8%. Similar nitrogen accumulation was obtained

(0.016 Kg/m^2) if compared with *P. australis* harvested in the third year from VF unit (0.015 Kg/m^2).

Table 6.3 – Fresh biomass, dry weight, dry matter and nitrogen stored in the aerial parts of *P. australis* harvested from HF unit at the end of autumn in each year; different letters indicate significant differences at $p < 0.05$ by Kruskal–Wallis test.

Species	Year	Fresh weight (Kg/m^2)	Dry weight (Kg/m^2)	Dry matter (%)	Nitrogen (%)	N uptake in aerial part (kg/m^2)
P. australis	2010	0.95 a	0.33 a	36.8 a	1.77 a	0.005 b
(HF)	2011	1.96 b	0.7 b	36.8 a	1.44 a	0.009 b
	2012	2.76 c	0.93 b	34.3 a	1.8 a	0.016 a

P. australis heating value

Plant harvesting can provide a possible economic profit from constructed wetlands. CWs provide plant biomass that could be a valuable feedstock for bioenergy. Biomass can be converted into energy (heat or electricity) using both thermochemical and biochemical conversion technologies. Combustion is the best developed and most frequently applied process used for solid biomass fuel because of its low costs and high reliability. Burning new biomass contributes no new carbon dioxide to the atmosphere, because replanting harvested biomass ensures that CO_2 is absorbed and returned for a cycle of new growth (McKendry, 2002).

Almost all kinds of herbaceous biomass feedstock used for combustion have an energy content that falls within the range 14-19 GJ/t (compared to the 17-30 GJ/t of coal). In our study, the common reed LHV (Lower Heating Value) was 10.4 GJ/t, significantly lower than those reported in other studies, which ranged from 15 to 20 GJ/t (Barz et al., 2008; Gravalos et al., 2010) (Table 6.4). Moisture content essentially reduces the heating value of fuel, increases the volume of flue gases, and deteriorates ignition and combustion (Vares et al., 2005). Unsuitable climate conditions at harvesting time prevented getting a harvested aboveground biomass with a moisture content lower than 64.6%. Due to these conditions, a higher LHV value wasn't obtained. The ash amount obtained in the combustion tests (8.7%) was in accordance with the literature. As reported by Komulanein et al. (2008), when the energy content (heating value) of harvested common reed is ca.15 GJ/t at operating moisture content, the energy content of a one hectare reed bed can be approximately 21MW/h (with a dry matter yield of 5 ton/ha). This is equivalent to the energy consumption of one detached house per year.

Table 6.4 – Average aboveground biomass production and heating value of *P. australis* from HF cell in December 2010.

Species	LHV (GJ/t)	HHV (GJ/t)	Ash %	Combustion energy (GJ/t)
P. australis (HF)	10.4	16.6	8.7	103.1

LHV: Lower Heating Value; HHV: Higher Heating Value;

Harvested nitrogen

As shown in Figure 6.3, from 25th January to 25th July 2012, for 183 days, HCW system was fed with a TN median inlet concentration of 250 mg/L in 1.7 m³ of freshwater. Nitrogen total amount was 77.7 Kg. Also taking into account the precipitation in that period, 78 Kg of TN entered the HCW system. 35 Kg of the total loaded 78 Kg of TN were discharged with the output water, and 2.19 kg were removed with the aboveground biomass harvested in October 2012. The missing amount of TN was about 40.8 Kg/ha. We can hence suppose that 47.6% of TN loaded to the system was removed via denitrification.

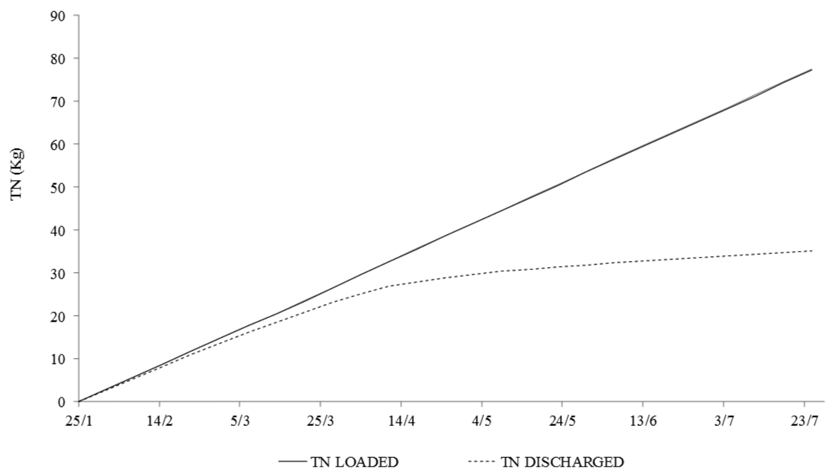


Figure 6.3 – Cumulative loaded and discharged TN (Kg) during the third monitored period from 25th January to 25th July 2012, for 183 days.

Chapter VII

General discussion and conclusions

A full scale HCW system with combined VF and HF units for treating pig farm effluents was tested for three years (2010-2012). During the experimentation, HCW operated under different conditions: seasonal variations (temperature), pollutants concentrations, hydraulic loading rate (HLR), hydraulic retention time (HRT), feeding mode and operational regimes.

In general, the best abatement performances were achieved during the third period. This was probably due to several reasons: (a) steady and lower inlet concentrations (250.3 mg/L of TN, 124.5 mg/L of NO_3-N and 124.9 mg/L of NH_4-N) than the other two periods; (b) a lower flow rate (1.7 m^3/d) and HLR (1.30 cm/d) than the other two periods (5 m^3/d and HLR of 3.8 cm/d); (c) the sequential batch (feed-stay-drain-rest) feed mode for VF unit without overloading. Despite the improvements made to the VF system (submersible water pump placed inside the water level control structure) in the second period (October 2010), worse abatement performances were measured in comparison with the other periods. This was probably correlated to: (a) high pollutant load (caused by only one pre-treatment stage for processing the inflow raw pig slurry); (b) high daily flow rate (5 m^3/d) that overloaded the VF unit and affected the duration of contact between pollutants and the microbial population in the wetland system (HRT of 4-5 days); (c) the winter monitoring period (October 2010-April 2011).

Comparing HCW start-up phase (May to December 2009) abatement performances (Borin et al., 2013) with those observed in the three years of monitoring, greater concentration lowering was observed for all investigated pollutants (organic matter, nitrogen and phosphorus).

Regarding the comparison of VF and HF units piggery wastewater treatment performance, HF unit was more efficient than VF in reducing COD, TN, NO_3-N, TP and PO_4-P concentration; whilst VF gained higher concentration reduction for NH_4-N. These findings confirmed that VF systems promote high atmospheric oxygen diffusion that favours aerobic pollutant removal mechanisms; on the contrary, HF systems favour anoxic pollutant removal mechanisms.

Overall COD concentration reduction obtained by HCW system ranged from 46% (from 870 to 446 mg/L, second period) to 56% (from 498 to 221 mg/L first period). Contrary to what was reported in the literature (Stein and Kakizawa, 2005), HF unit showed 34% and 29% higher COD concentration abatement than VF unit during the first and second period respectively.

Median inlet TN concentration was abated in the range between 40% (from 692 to 413 mg/L second period) and 54% (from 250 to 114 mg/L third period). Similar abatement performances were obtained in the first (53%) and third periods (54%), despite different median inflow concentrations being measured (417 and 250 mg/L in the first and third period respectively). HF unit gave a greater contribution (34-35%) than VF (29-30%) to lowering the TN inlet concentration. This is not surprising because VF system typically provides little denitrification. Total nitrogen removal in these systems could consequently be limited (Kadlec and Wallace, 2009).

HCW system reduced ammonia nitrogen by 43% (from 366 to 207 mg/L second period) to 60% (from 250 to 114 mg/L third period). During the first period the inlet concentration of 266 mg/L was lowered to 114 mg/L with a median reduction of 50%. The majority of NH_4-N concentration reduction occurred within the VF unit (32-45%). This was probably because the unsaturated media substrate conditions promote higher atmospheric oxygen diffusion inside the matrix pores which can boost nitrification (Sun et al., 1998; Noorvee et al., 2007).

The nitric nitrogen reduction obtained with the HCW system ranged between 21% (from 5.17 to 4.08 mg/L second period) and 55% (from 3.58 to 1.62 mg/L first period). During the third period, lower NO_3-N abatement than in the second period was obtained (39%), probably due to: a) high inlet concentration (125 mg/L); b) low wastewater temperature (9.2-13.8 °C) applied to the system during winter that could affect denitrification rates; c) low organic matter concentration (from 9.6 to 26.8 mg/L). HF unit provided the major contribution (16-30%) to lowering median NO_3-N inflow concentration. This is probably related to predominant anoxic conditions in conjunction with the presence of an organic matter source (first and second period), which can promote denitrification (Haberl et al., 1995; Rousseau et al., 2008).

A constant abatement level for phosphorus (32-35%) was maintained during the first and second period. This suggests that, contrary to what was reported by Rustige et al. (2003), low temperatures decrease the TP sorption capacity of the substrate, and secondly that the system's porous media were not yet saturated. Significantly higher TP abatement occurred in HF unit (19-24%) probably due to its higher absorption capacity than VF (related to the higher HRT)

Median inlet orthophosphate concentration was abated by 24% (from 15 to 11 mg/L) to 34% (from 20 to 13 mg/L). During the first period, HF unit had a high concentration reduction (24%) perhaps linked to reducing conditions (i.e., lack of oxygen, DO concentrations below 0.5 mg/L) that can lead to solubilisation of minerals and release of dissolved phosphorus (Reed et al., 1995; Kadlec and Knight, 1996), whilst during the second period, both systems gave the same abatement value (12%).

In general, concentration abatement reached by the system during the first and second period for investigated ions was very low. The ion with the lowest concentration reduction was K^+ with 1-2% while the highest was SO_4^{2-} with 6-9%. Ca^{2+} was mainly reduced by VF cells activity with 3-5%, while Mg^{2+} and SO_4^{2-} by the HF unit with 3-6% and 2-6% respectively. Cl^- concentrations were reduced by 1-2% in both units.

During the first two monitored periods, there were no significant differences in measured parameters outlet concentration among VF cells for different vegetation (*Canna x generalis* – *Phragmites australis*) and substrates (only gravel – gravel with sand and zeolite). During the third period significant differences in NH_4-N effluent concentration were found between first two VF cells and the third one. This result was probably due to the use of a zeolite layer (0.10 m) in the porous media substrate of VF3 cell that enhanced NH_4-N concentration reduction, as reported by several other studies (Yalcuk and Ugurlu, 2009; Saeed and Sun, 2011). The difference in performance between the first two periods and the third was probably correlated to the daily flow rate. With a daily flow rate of 5 m^3/d used during the first and the second periods the zeolite layer was probably undersized, this provided less surface for ion exchanges and caused a short contact time between the zeolite media and the fluid. Similar observations were made by Stefanakis and Tsihrintzis, (2012).

Sequential batch feed mode (feed-stay-drain-rest) was adopted during the second period in VF unit. This improvement: (a) promoted greater oxidized conditions (> 19% than first period) allowing atmospheric oxygen diffusion inside the substrate during the rest phase; (b) created a temporal redox variation (stay phase + 30/40 mV and rest period +200/270 mV) that influenced microbial activity by favouring robust aerobic facultative biofilms; (c) prevented clogging of the VF media substrate. Batch feed mode ensures better oxygenation of VF unit substrate, which favours aerobic processes and could therefore ensure better organic matter and ammonia nitrogen abatement. Nevertheless, COD and NH_4-N abatement measured in VF unit during the second period (25% and 32% respectively) were lower than those measured in first one (33% and 39% respectively). This result was probably related to the higher inlet concentrations of COD and NH_4-N and the lower temperatures during the second period.

An abatement performance comparison of two operational regimes (OR) was tested in VF unit during the third period. TN and NH_4-N concentration abatement achieved by VF system managed with OR1(6 hours fill period-14 hours rest period) was 20% and 32.2% respectively higher than OR2 (14 hours fill period-6 hours rest period). VF unit managed with OR2 achieved low percentage reduction, which ranged from 18.3 to 30.5% for TN and from 28.5 to 45.8% for NH_4-N. A prolonged fill period of 14 hours applied in OR2 showed no significant improvement in nitrate-nitrogen abatement.

A period of cold weather (January-March 2012) resulted in reduced microbial activity and severe die-back of the macrophytes in each unit. Nitrogen removal rates have been shown to be temperature dependent, thus resulting in seasonal variation (Knight et al., 2000; Kuschk et al., 2003; Trias et al., 2004). Wastewater temperature mainly affected denitrification rates as demonstrated by NO_3-N median outflow concentration (181 and 185 mg/L from VF and HF unit respectively) that exceeded influent (124.6 mg/L) during a period in which inlet wastewater temperatures ranged from 9 to 14.2 °C. Segmented regression analysis individuated a breakpoint temperature at 14.2 °C that discriminated different abatement rates for TN and NO_3-N. With wastewater temperature lower than 14.2 °C, 4.1% for TN and a negative value (-47.3%) for NO_3-N were obtained. With wastewater temperature higher than 14.2 °C, abatement of 57.5 and 45.4% was reached by the HCW system for TN and NO_3-N respectively. The higher nitrogen removals at high temperatures were probably due to: (a) plants, which are growing in spring and summer and block nitrogen as nitrates and ammonia; and (b) bacteria responsible for nitrogen removal, which work better in temperatures above 15 °C (Reed et al., 1995; Yang et al., 2001; Vymazal, 2002; Kuschk et al., 2003).

During three years of monitoring, both plant species (*C. x generalis* and *P. australis*) proved to be extremely robust and survived shock dosing, long periods of total immersion and cold. Fresh biomass of the aerial part, measured in the third growing season (October 2012 at the end of experimentation), showed that the most productive was *C. x generalis* in VF1 cell with an average weight of 14 Kg/m^2, followed by *P. australis* in VF3 and VF2 cells with 2.2 and 1.2 Kg/m^2 respectively. Estimated fresh weight of aboveground *P. australis* biomass in HF reached 2.76 Kg/m^2. As regards dry weight, for *C. x generalis* this ranged from 1.14 to 1.96 Kg/m^2 and for *P. australis* from 0.1 (initial period in VF2 cell) to 0.93 Kg/m^2 (third growing season in HF cell). Nitrogen accumulation in the aboveground tissues of harvested plants showed an increase during the last year in comparison with the first two years. Indeed, nitrogen in *C. x generalis* increased significantly from 1.89 (average of first and second year) to 2.6% (measured in the third year) with an increase of 37.5% , followed by *P. australis* in VF2 with an increase of 21.3%. The amount of nitrogen removed by harvesting VF cells showed that the most efficient was *C. x generalis* in VF1 cell with an average of 0.05 Kg/m^2 followed by *P. australis* in VF3 and VF2 cell with 0.015 and 0.008 Kg/m^2 respectively. Average nitrogen amount removed by harvesting aboveground biomass of *P. australis* in HF was 0.016 Kg/m^2. This higher value if compared with those reached by VF2 and VF3 cells was probably related to high aboveground biomass production and mature bed conditions in HF. The amount of N harvested in the aerial part of the plant is very low if compared to the amount of N abated by the wetland system.

The monitoring of the HCW system performance revealed useful suggestions for optimal management:

- Pig manure must be pre-treated by a primary (odour reduction, volume reduction, energy recovery,) and a secondary treatment (nutrient reduction, adding value to the manure) before CWs application.
- The water level can affect HRT and atmospheric oxygen diffusion through the media substrate. When water levels are reduced to their lowest during summer, the water temperature is often high, maximizing plant productivity and oxygen diffusion rates (Kadlec and Knight, 1996).

- The operational regime (time between the fill and drain phase) of batch feed mode in the VF unit can be modified when the HCW system is used for treatment of heavy pollutant loads.
- To allow bio-reaction it is necessary to foster the optimal range of process parameters.

  For nitrification these are: (a) wastewater temperature higher than 5 °C; (b) pH between 7.5 and 8.5, nitrification process can be hampered at pH below 6; (c) DO levels of 1.0 mg/L or more; (d) E_h value between +100 mV and +350 mV.

  For denitrification they are: (a) wastewater temperature higher than 14.2 °C; (b) pH between 7 and 7.5, denitrification process can be hampered at pH above 8; (c) DO levels lower than 0.5 mg/L; (d) E_h value between -50 mV and +50 mV.
- For the climate conditions of the experimental site, the plants need a minimum of three years to reach the maximum density value.

In conclusion, the HCW system proved to be quite efficient in reducing inlet concentration of the pollutants (organic matter, nitrogen and phosphorus) and could be an effective tertiary treatment in combination with efficient pre-treatments for the management of swine wastewater in small piggeries where sufficient land is not available for spreading.

Acknowledgements

The research was carried out with the financial support of:

- Veneto Agricoltura, project “RIDUCAREFLUI”. AZIONE 6: Studi applicativi di fito-bio-depurazione inerenti lo smaltimento controllato, su superfici forestate, del digestato proveniente da impianti per la produzione di biogas e biometano; la fitodepurazione produttiva per il trattamento degli EA e del digestato; l’analisi di innovative proposte tecniche e tecnologiche; SOTTOAZIONE 6.3: Trattamento di liquami zootecnici tramite fitodepurazione per l’abbattimento del carico azotato.
- AGER, project “SEES PIG” – Solutions for Environmental and Economic Sustainability of PIG manure. Grant n° 2010-2220 filiera del suino.

I am grateful to my supervisor, Prof. Maurizio Borin, for the opportunity to undertake my PhD and Luigi Guarnieri for the assistance.

The study benefited greatly from the contribution of many people. I acknowledge technical support of Dr. Giovanni Marco Carrer (Environmental System Analysis Lab -LASA- of Department of Chemical Processes Engineering) University of Padova for tracer test analysis, Prof. Andrea Squartini, and Prof. Giuseppe Concheri University of Padova for wetland biodegradation analysis, Prof. Andrea Berti and PhD Nicola Dal Ferro University of Padova, provided much guidance with statistical analysis, PhD Gianluca Simonetti and PhD Michela Salvato University of Padova assisted me with water chemical analysis.

The data collection would not have been possible without the field assistant Simone Breschigliaro. Alberto Modena assisted me with the design drawing. I am thankful to Elia Scudiero, Elisa Cocco, Gianmarco Tardivo, Giulia Florio, Jessica Tamiazzo, Anna Mietto, Carmelo Maucieri, Matteo Passoni, Giovanna De Stefani, Gino Malimpensa.

I would especially like to thank my family, Doretta, Paolo, Giulio and Alessandra who have supported, helped and encouraged me during this experience.

References

Albuquerque, A., Oliveira, J., Semitela, S., Amaral, L., 2009. Influence of bed media characteristics on ammonia and nitrate removal in shallow horizontal subsurface flow constructed wetlands. Bioresource Technology. 100, 6269–6277.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration: guidelines for computing crop water requirements. In: Proceedings of the Irrigation and Drainage Paper No. 56. Food and Agricultural Organization, United Nations, Rome, Italy.

Allen, W.C., Hook, P.B., Biederman, J.A., Stein, O.R., 2002. Temperature and wetland plant species effects on wastewater treatment and root zone oxidation. J. Environ. Qual. 31, 1010–1016.

Allen, R.G., Walter, I.A., Elliott, R.L., Howell, T.A., Itenfisu, D., Jensen, M.E., Snyder, R.L., 2005. ‘‘The ASCE Standardized Reference Evapotranspiration Equation’’. Am. Soc. Civil Eng., Reston, VA, 59 p. (with supplemental appendices).

Allison, G.B., Hughes, M.W., 1983. The use of natural tracers as indicators of soil-water movement in a temperate semi-arid region, J. Hydrol. 60, 157–173.

Akratos, C.S., Tsihrintzis, V. A., 2007. Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot scale horizontal subsurface flow constructed wetlands, Ecol. Eng. 29, 173–191.

Ansola, G., Gonzalez, J.M., Cortijo, R., Luis, E.D., 2003. Experimental and full scale pilot plant constructed wetlands for municipal wastewater treatment. Ecological Engineering. 21, 43–52.

Armstrong, W., Armstrong, J., Beckett, R.M., 1990. Measurement and Modeling of Oxygen Release from Roots of Phragmites Australis. Constructed Wetlands for Water Pollution Control. Pergamon Press, Oxford, UK, pp. 41–52.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20^{th} ed. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

ARPAT-APAT, 2005. Linee Guida per la progettazione e gestione di zone umide artificiali per la depurazione dei reflui Civili. A cura di Mazzoni M., Litografia I.P., Firenze.

AOAC, 2002. Official Methods of Analysis. Method 990.03. Protein (crude) in Animal Feed Combustion Method (Dumas method). 17th edition 2002. J AOAC, 72–770.

Austin, D., Lohan, E., Verson, E., 2003. Nitrification and denitrification in a tidal vertical flow wetland pilot. In: Proceedings of the Water Environment Federation Technical Conference, Los Angles, California. Water Environment Federation, Alexandria, VA, USA.

Bachand, P.A.M., Horne, A.J., 2000. Denitrification in constructed wetland free-water surface wetlands: II. Effects of vegetation and temperature. Ecol. Eng. 14, 17–32.

Barbera, A.C., Cirelli, G.L., Cavallaro, V., Silvestro, I., Pacifici, P., Castiglione, V., Toscano, A., Milani, M., 2009. Growth and biomass production of different plant species in two different constructed wetland systems in Sicily. Desalination. 246, 129–136.

Barz, M., Ahlhaus, M., Wichtmann, W., Timmermann, T., 2008. Production and energetic utilization of biomass from rewetted peatlands. Heat & Power and Thermal Physics. 1, 47–55.

Bavor, H.J., Roser, D.J., McKersie, S.A., Breen, P., 1988. Treatment of Secondary Effluent. Report to Sydney Water Board, Australia.

Beauchamp, E.G., Trevors, J.T., Paul, J.W., 1989. Carbon sources for bacterial denitrification. Adv. Soil Sci. 10, 113–142.

Behrends, L. Bailey, E. Ellison, W. Houke, L. Jansen, P. Shea, C. Smith S. and T. Yost. 2003. Reciprocating Constructed Wetlands for Treating High Strength Anaerobic Lagoon Wastewater In: Ninth International Symposium on Animal, Agriculture and Food Processing Waste. Durham, N.C. pp. 113–124.

Biddlestone, A.J., Gray, K.R., Job, G.D., 1991. Treatment of dairy farm wastewaters in engineered reed bed systems. Process Bio. 26, 265–268.

Bonomo, L., Pastorelli, G., Zambon, N., 1997. Advantages and limitations of duckweed-based wastewater treatment systems. Wat Sci Technol. 35, 239–246.

Borin, M., Tocchetto, D., 2007. Five year water and nitrogen balance for a constructed surface flow wetland treating agricultural drainage waters. Sci. Total Environ. 380, 38–47.

Borin, M., Milani, M., Salvato, M., Toscano, A., 2011. Evaluation of Phragmites australis (Cav.) Trin. evapotranspiration in Northern and Southern Italy. Ecol. Eng. 37, 721–728.

Borin, M., Politeo, M., De Stefani, G., 2013. Performance of a hybrid constructed wetland treating piggery wastewater. Ecol. Eng. 51, 229–236.

Boursier, H., Bèline, F., Paul, E., 2005. Piggery wastewater characterisation for biological nitrogen removal process design. Bioresour. Technol. 96, 351-358.

Brasil, M.S., Matos A.T., Soares A.A., Ferreira, P.A., 2005. Qualidade do efluente de sistemas alagados construídos, utilizados no tratamento de esgoto doméstico. Revista Brasileira de Engenharia Agrícola e Ambiental, v. 9, supl., p. 133–137.

Braskerud, B.C., 2001. The influence of vegetation on sedimentation and resuspension of soil particles in small constructed wetlands. Journal of Environmental Qual. 30, 1447–1457.

Brito, L.M., Coutinho, J., Smith, S.R., 2008. Methods to improve the composting process of the solid fraction of diary cattle slurry. Bioresour. Technol. 99 (18), 8955–8960.

Brix, H., Arias, C., Johansen, N.H., 2003. Experiments in a two stage constructed wetland system: nitrification capacity and effects of recycling on nitrogen removal. In: Vymazal, J. (Ed.), Wetlands: Nutrients, Metals and Mass Cycling. Backhuys Publishers, Leiden, The Netherlands, pp. 237–258.

Brix, H., 1987. Treatment of wastewater in the rhizosphere of wetland plants - the root-zone method. Water Science and Technology. 19, 107–118.

Brooks A.S., Rozenwald, M.N., Geohring, L.D., Lion L.W., Steenhuis, T.S., 2000. Phosphorus removal by wollastonite: A constructed wetland substrate, Ecol. Eng. 15, 121–132.

Burchell II, M.R., Skaggs, R.W., Lee, C.R., Broome, S., Chescheir, G.M., Osborne, J., 2007. Substrate organic matter to improve nitrate removal in surface-flow constructed wetlands. J. Environ. Qual. 36, 194–207.

Calheiros, C.A., Rangel, A., Castro, P.M.L., 2007. Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater. Water Research. 41, 1790–1798.

Canga, E., Dal Santo, S., Pressl, A., Borin, M., Langergraber, G., 2011. Comparison of nitrogen elimination rates of different constructed wetland designs. Water Sci Technol. 64, 1122–1129.

Carty, A., Scholz, M., Heal, K., Gouriveau, F., Mustafa, A., 2008. The universal design, operation and maintenance guidelines for Farm Constructed Wetlands (FCW) in temperate climates. Bioresource Tech. 99, 6780–6792.

Caselles-Osorio, A., Garcia, J., 2007. Impact of different feeding strategies and plant presence on the performance of shallow horizontal subsurface-flowconstructed wetlands. Sci. Total Environ. 378, 253–262.

Cathcart, T.P., Hammer, D.A., Triyono, S., 1994. In: DuBowy, P.J., Reaves, R.P. (Eds.), Performance of a constructed wetland vegetated strip system used for swine waste treatment. Constructed Wetlands for Animal Waste Management, Purdue Research Foundation, West Lafayette, IN, USA, 9–22.

Chastain, J.P., Camberato, J.J., Albrecht, J.E., Adams, J., 2003. Swine manure production and nutrient content. Chapter 3a in Confined Animal Manure Managers Certification Program Manual B Swine Version 3. Clemson University Cooperative Extension Service.

Chazarenc F., Gérard, M., Gontheir, Y., 2003. Hydrodynamics of horizontal subsurface flow constructed wetlands. Ecol. Eng. 21, 165–173.

Chen, S.W., Kao, C.M., Jou, C.R., Fu, Y.T., Chang, Y.I., 2008. Use of a constructed wetland for post-treatment of swine wastewater. Environmental Engineering Sci. 25, 407–417.

Christensen, P.B., Sorensen, J., 1986. Temporal variation of denitrification activity in plant-covered, littoral sediment from Lake Hampen, Denmark. Appl. Environ. Microbiol. 51, 1174–1179.

Clarke, E., Baldwin, A.H., 2002. Responses of wetland plants to ammonia and water level. Ecol. Eng. 18, 257-264.

Comino, E., Riggio, V., Rosso, M., 2011. Mountain cheese factory wastewater treatment with the use of a hybrid constructed wetland. Ecol. Eng. 37, 1673–1680.

Cookson, W.R., Cornforth, I.S., Rowarth, J.S., 2002. Winter soil temperature (2–15 ◦C) effects on nitrogen transformations in clover green manure amended or unamended soils; a laboratory and field study. Soil Biol. Biochem. 34, 1401–1415.

Cooper, P.F., Findlater, B.C., 1990. Constructed Wetlands in Water Pollution Control. Pergamon Press: New York.

Demirer, G.N., Chen, S.L., 2005. Anaerobic digestion of dairy manure in a hybrid reactor with biogas recirculation. World J. Microbiol. Biotechnol. 21 (8–9), 1509–1514.

Dan, T.H., Quang, L.N., Chiem, N.H., Brix, H., 2011. Treatment of high-strength wastewater in tropical constructed wetlands planted with Sesbania sesban: horizontal subsurface flow versus vertical down flow. Ecol. Eng. 37, 711–720.

Daniels, R., 1998. You're now entering the root zone—investigation: the potential of reed beds for treating waste waters from leather manufacture. World Leather 11, 48–50.

Demin, O.A., Dudeney, A.W.L., 2003. Nitrification in Constructed Wetlands Treating Ochreous Mine Water. A Technical Article Published in Mine Water and the Environment. IMWA Springere Verlag, pp. 15–21.

Dierberg, F.E., DeBusk, T.A., 2005. An evaluation of two tracers in surface flow wetlands: rhodamine-WT and Lithium. Wetlands. 25, 8–25.

DIN (Deutsches Institut für Normung), 1985. German Standard Methods for the Examination of Water, Wastewater and Sludge. Deutches Institut für Normung, Berlin.

Dong, X., Reddy, G.B., 2010. Soil bacterial communities in constructed wetlands treated with swine wastewater using PCR-DGGE technique. Bioresour. Technol. 101, 1175–1182.

Dong, X., Reddy, G.B., 2012. Ammonia-oxidizing bacterial community and nitrification rates in constructed wetlands treating swine wastewater. Ecol. Eng. 40, 189–197.

EEC/91/676, O.J. NL 375, 31.12.1991.p1. Protection of waters against pollution caused by nitrates from agricultural sources.

Eger, P., 1994. Wetland treatment for trace metal removal from mine drainage: the importance of aerobic and anaerobic processes. Water Sci. Technol. 29, 249–257.

FAO 1987. Soil and water conservation in semi-arid areas. N.W. Hudson. Soil Bullettin 57. FAO, Rome.172.

Farahbakhshazad, N., Morrison, G.M., 1997. Ammonium Removal Processes for Urine in an Up flow Macrophyte System. Environmental Science & Technology, Vol. 31, No. 11, pp. 3314–3317.

Faulkner, S.P., Richardson, C.J., 1989. Physical and chemical characteristics of freshwater wetland soils. In Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural, ed. D. A. Hammer, 41–72.

Faulwetter, J.L., Gagnon, V., Sundberg, C., Chazarenc, F., Burr, M.D., Brisson, J., Camper, A.K., Stein, O.R., 2009. Microbial processes influencing performance of treatment wetlands: a review. Ecol. Eng. 35, 987–1004.

Finlayson, M., Chick, A., Von Oertzen, I., Mitchell, D. 1987. Treatment of piggery effluent by an aquatic plant filter. Biol. Wastes. 19, 179–196.

Forquet, N., Wanko, A., Molle, P., Mosé, R., Sadowski, A.G., 2009. Two-Phase flow modelling for oxygen renewal estimation in vertical flow filter: luxury or necessity? Water Science and Tech. 59, 2311–2319.

Fugère, R., Mameri, N., Gallot, J.E., Comeau, Y., 2005. Treatment of pig farm effluents by ultrafiltration. J. Membr. Sci. 255 (1–2), 225–231.

Furniss, P., Lane, A., 1992. Practical Conservation: Water and Wetlands. Hodder and Stoughton, London, UK, p. 128.

Gale, P.M., De´vai, I., Reddy, K.R., Graetz, D.A., 1993. Denitrification potential of soils from constructed and natural wetlands. Ecol. Eng. 2, 119–130.

Gambrell, R.P., Patrick W.H., 1978. Chemical and microbiological properties of anaerobic soils and sediments. In Plant Life in Anaerobic Environments, eds. D. D. Hook and R. M. Crawford, 375–425.

Geary, P.M., Moore, J.A., 1999. Suitability of a treatment wetland for dairy wastewaters. Wat. Sci. Tech. 40, 179–185.

Gersberg, G.M., Elkins, B.V., Goldman, C.R., 1983. Nitrogen removal in artificial wetlands. Water Res. 17, 1009–1014.

Hach-Lange, 1989. Water Analysis Handbook. HACH Company, Loveland, CO, USA.

Hammer, D.A., Pullin, B.P., McCaskey, T.A., Eason, J., and Payne, V.W.E. 1993. Treating livestock wastewaters with constructed wetlands, in: Constructed Wetlands for Water Pollution Improvement, G.A. Moshiri, ed., CRC Press/Lewis Publishers, Boca Raton, Florida, pp. 343–347.

Hammer, DA; RL Knight, 1994. Designing constructed wetlands for nitrogen removal. Water Science and Tech.15–27.

Hanson, G.C., Groffman, P.M., Gold, A.J., 1994. Denitrification in riparian wetlands receiving high and low groundwater nitrate inputs. J. Environ. Qual. 23, 917–922.

Hao, O.J., 2003. Sulphate-reducing bacteria. In: Mara, D., Horan, N.J. (Eds.), The Handbook of Water and Wastewater Microbiology. Academic Press, London, pp. 459–469.

Harrington, R., Dunne, E.J., Carroll, P., Keohane, J., Ryder, C., 2005. The concept, design and performance of integrated constructed wetlands for the treatment of farmyard dirty water. In: Dunne EL, Reddy KR, Carton OT (eds) Nutrient management in agricultural watersheds: a wetlands solution. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 179–188.

Harrington, C., Scholz, M., 2010. Assessment of pre-digested piggery wastewater treatment operations with surface flow integrated constructed wetland systems. Bioresour. Technol. 101, 7713–7723.

Hartzendorf, T., Rolletschek, H., 2001. Effects of NaCl-salinity on amino acid and carbohydrate contents of Phragmites australis. Aquat. Bot. 69, 195–208.

Healy, M.G., Cawley, A.M., 2002. The nutrient processing capacity of a constructed wetland in western Ireland. J. Env. Qual. 31, 1739–1747.

Healy, M.G., Rodgers, M., Mulqueen, J., 2007. Treatment of dairy wastewater using constructed wetlands and intermittent sand filters. Bioresour. Technol. 98 (12), 2268–2281.

Herskowitz, J., Black, S., Sewandowski,W., 1987. Listowel artificial marsh treatment project. In: Reddy, K.R., Smith, W.H. (Eds.), Aquatic Plants for Water Treatment and Resource Recovery. Magnolia Publishing Co., Orlando, FL, USA.

Herskowitz, J., 1986. Listowel Artificial Marsh Project Report, Ontario Ministry of the Environment, Water Resources Branch, Toronto. P. 251.

Howard-Williams, C., Downes, M.T., 1989. Short term nitrogen dynamics in a small Brazilian wetland (Lago Infernao Sao Paulo). J. Trop. Ecol. 5, 323–335.

Huang, J., Reneau, R.B., Hagedorn, C., 2000. Nitrogen removal in constructed wetlands employed to treat domestic wastewater. Water Research 34, 2582–2588.

Humenik, F.J, Szogi A.A., Hunt, P.G., Broome, S., Rice, J.M., 1999. Wastewater utilization: a place for managed wetlands. Asian-Australian Journal of Animal Sci. 12, 629–632.

Hunt, P.G., Humenik, F.J., Szogi, A.A., Rice, J.M., Stone, K.C., Cutts, T.T. and Edwards, J.P., 1993. Constructed Wetlands Treatment of Swine Wastewater. Written presentation at 1993 International Winter Meeting sponsored by American Society of Agricultural Engineers (ASAE). Paper no. 93–2616.

Hunt, P.G., Szogi, A.A., Humenik, F.J., Rice, J.M., Matheny, T.A., Stone, K.C., 2002. Constructed wetlands for treatment of swine wastewater from an anaerobic lagoon. Transactions of the American Society of Agricultural Eng. 45, 639–647.

Hunt, P.G., Matheny, T.A., Stone, K.C., 2004. Denitrification in a coastal plain riparian zone contiguous to a heavily loaded swine wastewater spray field. Journal of Environmental Quality. 33, 2367–2374.

Hunt, P.G., Poach, M.E., Shappel, N.W., Matheny, T.A., Reddy, G.B., Kyoung S.R., Szogi, A.A., 2007. Swine Lagoon Wastewater Treatment in Marsh-Pond/Floating Wetland-Marsh Constructed Wetland. International Symposium on Air Quality and Waste Management for Agriculture, Broomfield, Colorado pp.16–19.

ISTAT (Istituto Nazionale di Statistica), 2010. Verified on January 12th, 2013 and available on: http://agri.istat.it/sag is pdwout/jsp/dawinci.jsp?q= plB020000020000063200&an=2011&ig=1&ct=759&id=8A|9°

IWA, 2000. Specialist Group on Use of Macrophytes in Water Pollution Control, Constructed Wetlands for Pollution Control: Processes, Performance, Design and Operation. Kadlec R.H., Knight R.L., Vymazal J., Brix H., Cooper P.F., Haberl R. (eds.) IWA Publishing: London, United Kingdom. p.156.

Jia, W., Zhang, J., Wu, J., Xie, H., Zhang, B., 2010. Effect of intermittent operation on contaminant removal and plant growth in vertical flow constructed wetlands: a microcosm experiment. Desalination 262, 202–208.

Jingtao, X., Jian, Z., ; Congcong, Z., Cong, L., Huijun, X., Shanshan, W., 2012. Effect of Ammonia Stress on Physiological and Biochemical Character of Phragmites australis in Constructed Wetland. Third International Conference on Digital Manufacturing and Automation (ICDMA). DOI 10.1109 /ICDMA.2012.83, pp.343–346.

Jett, J.W., 2005. Plant pH preferences. West Virginia University Extension Service, USA.

Jing, S.R., Lin, Y.F., 2004. Seasonal effect on ammonia nitrogen removal by constructed wetlands treating polluted river water in southern Taiwan. Environmental Pollution 127 (2), 291–301.

Juang, F.H.T., Johnson, N.M., 1967. Cycling of chlorine through a forested watershed in New England. J. Geophys. Res. 72, 564–5647.

Junsan, W., Yuhua, C., and Qian, S. 2000. The application of constructed wetland to effluent purification in pig plant, in: Proc. 7th Internat. Conf. on Wetland Systems for Water Pollution Control, Lake Buena Vista, Florida. University of Florida, Gainesville and Int. Water Association. pp. 1470–1480.

Kadlec, R.H., 1999. The limits of phosphorus removal in wetlands. Wetland Ecol. Manag. 7, 65–175

Kadlec, R.H., Knight, R.L., 1996. Treatment Wetlands. Lewis Publishers, Boca Raton, FL, USA, p. 893.

Kadlec R.H., Reddy K.R., 2001. Temperature effects in treatment wetlands. Water Environment Research. 73, 543–557.

Kadlec, R.H., Wallace S.D., 2009. Treatment Wetlands, Second edition, CRC Press, Taylor & Francis Group, New York.

Kantawanichkul, S., Pilaila, S., Tanapiyawanich, W., Tikampornpittaya, W., Kamkrua, S., 1999. Wastewater treatment by tropical plants in vertical flow constructed wetlands. Water Science and Tech. 40, 173–178.

Kantawanichkul, S., Neamkam, P., 2001. Nitrogen removal in a combined system: vertical vegetated bed over horizontal flow sand bed. Water Sci. Technol. 44, 137-142.

Kantawanichkul, S., Somprasert, S., 2005. Using a compact combined constructed wetland system to treat agricultural wastewater with high nitrogen. Water Science and Tech. 51, 47–53.

Karpiscak, M.M., Freitas, R.J., Gerba, C.P., Sanchez, L.R., Shamir, E., 1999. Management of dairy waste in the Sonoran Desert using constructed wetland technology. Wat. Sci. Tech. 40, 57–65.

Katayon, S., Fiona, Z., Noor, M.J.M.M., Halim, G.A., Ahmad, J., 2008. Treatment of mild domestic wastewater using subsurface constructed wetlands in Malaysia. International Journal of Environmental Studies. 65 87–102.

Kato, K., Inoue, T., Ietsugu, H., Koba, T., Sasaki, H., Miyaji, N., Yokota, T., Sharma, P.K., Kitagawa, K., Nagasawa, T., 2010. Design and performance of hybrid reed bed systems for treating high content wastewater in the cold climate Proceedings of 12th International Conference on Wetland Systems for Water Pollution Control. October 4 – 8, 2010. San Servolo Island, Venice, Italy. Vol. 1, pp. 511–517.

Knight, R.L., Payne Jr., V.W.E., Borer, R.E., Clarke Jr., R.A., Pries, J.H., 2000. Constructed wetlands for livestock management. Ecol. Eng. 15, 41–55.

Knight, R.L., Gu, B.H., Clarke, R.A., Newman, J.M., 2003. Long-term phosphorus removal in Florida aquatic systems dominated by submerged aquatic vegetation. Ecol. Eng. 20, 45–63.

Knowles, R., 1981. Denitrification. In: Soil Biochemistry, Paul, E.A. and Ladd, J.N. (eds.), Marcel Dekker, Inc., NY. Volume 5, pp. 323–369.

Komulainen, M., Simi, P., Hagelberg, E., Ikonen, I., Lyytinen, S., 2008. Reed energy-Possibilities of using the Common Reed for energy generation in Southern Finland. TurKu University of Applied Sciences, Report 67, 16–21.

Kouki, S., M'hiri, F., Saïdi, N., Belaïd, S., Hassen, A., 2009. Performances of a constructed wetland treating domestic wastewaters during a macrophytes life cycle. Desalination. 246, 452–467.

Kuschk, P., Wießner, A., Kappelmeyer, U., Weißbrodt, E., Kästner, M., Stottmeister, U., 2003. Annual cycle of nitrogen removal by a pilot-scale subsurface horizontal flow in a constructed wetland under moderate climate. Water Res. 37, 4236–4242.

Kumm, K.I., 2003. Ways to reduce nitrogen pollution from Swedish pork production. Nutr. Cycl. Agroecosyst. 66 285–293.

Lantske, I.R., Mitchell, D.S., Heritage, A.D., Sharma, K.P., 1999. A model of factors controlling orthophosphate removal in planted vertical flow wetlands. Ecol. Eng. 12, 93–105.

Lawson G.J., 1985. Cultivating Reeds (Phragmites australis) for Root Zone Treatment of Sewage, Contract report to the Water Research Centre (IRE Project 965), Cumbria, United Kingdom.

Limmer, A.W., Steele, K.W., 1982. Denitrification potentials: measurement of seasonal variation using a short-term anaerobic incubation technique. Soil Biol. Biochem. 14, 179–184.

Lin. J.C., Schnoor. J.L., Glass, G.E., 1987. Ion budgets in a seepage lake. In: Sources and Fates of Aquatic Pollutants. Adv. Chem. Ser. 216. American Chemical Society. Washington, DC, pp. 209–227.

Lin, A.Y.C., Debroux, J.F., Cunningham, J.A., Reinhard, M., 2003. Comparison of rhodamine WT and bromide in the determination of hydraulic characteristics of constructed wetlands. Ecol. Eng. 20, 75–88.

Lin, Y.F., Jing, S.R., Wang, T.W., Lee, D.Y., 2002. Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. Environ. Pollut. 119, 420–423.

Ling, Z., Hua, C.L., Ying, O., Fen, L., Dan, T., 2011. Kinetic adsorption of ammonium nitrogen by substrate materials for constructed wetlands. Pedosphere 21, 454–463.

Lee, C.G., Fletcher, T.D., Sun, G., 2009. Nitrogen removal in constructed wetland systems. Eng. Life Sci. 9, 11–22.

Lee, C-Y., Lee, C-C., Lee, F-Y., Tseng, K-S., Liao, C-J. 2004. Performance of subsurface flow constructed wetland taking pretreated swine effluent under heavy loads. Bioresource Tech. 92, 173–179.

Lee, B.L., Scholz, M., 2007. What is the role of Phragmites australis in experimental constructed wetland filters treating urban runoff? Ecol. Eng. 29, 87–95.

Lo Monaco, P.A.V., Matos A.T., Sarmento, A.P., Lopes Jùnior, A.V., Lima J.T., 2009. Desempenho de filtros constituídos por fibras de coco no tratamento de águas residuárias de suinocultura. Engenharia na Agricultura, 17, 473–480.

Luo, A., Zhu, J., Ndegwa, P.M., 2002. Removal of carbon, nitrogen, and phosphorus in pig manure by continuous and intermittent aeration at low redox potentials. Biosyst. Eng. 82, 209–215.

Lowrance, R., Vellidis, G., Hubbard, R.K., 1995. Wetlands and aquatic processes. J. Environ. Qual. 24, 808–815.

Mantovi, P., Marmiroli, M., Maestri, E., Tagliavini, S., Piccinini, S., Marmiroli, N., 2003. Application of a horizontal subsurface flow constructed wetland on treatment of dairy parlor wastewater. Bioresour. Technol. 88, 85–94.

Maehlum, T., Warner, W.S., Stålnacke, P., Jenssen, P.D., 1999. Leachate treatment in extended aeration lagoons and constructed wetlands in Norway, in: Constructed Wetlands References 499 for the Treatment of Landfill Leachates, G. Mulamoottil, E.A. McBean, and F. Revers, eds., Lewis Publisher/CRC Press, Boca Raton, 151–163.

Maddux, D.C., 2002. Constructed Wetlands for Wastewater Treatment in the Subarctic. Ph.D. Dissertation, University of Alaska (Fairbanks, Alaska).

Mars, R., Taplin, R., Ho, G., Mathew, K., 2003. Greywater treatment with the submergent Triglochin huegelii e a comparison between surface and subsurface systems. Ecol. Eng. 20, 147–156.

Martinez, J., Patrick, D., Barrington, S., Burton, C., 2009. Livestock waste treatment systems for environmental quality, food, safety, and sustainability. Bioresource Tech. 100, 5527–5536.

Masse, L., Massè, D.L., Pellerin, Y., Dubreuil, J., 2010. Osmotic pressure and substrate resistance during the concentration of manure nutrients by reverse osmosis membranes. J. Membr. Sci. 348, 28–33.

McBride, G.B., Tanner, C.C., 2000. Modelling biofilm nitrogen transformations in constructed wetland mesocosms with fluctuating water levels. Ecol. Eng., 14, 93–106.

McCaskey, T.A., Britt, S.N., Hanhah, T.C., Eason, J.T., Payne, V.W.E. and Hammer, D.A. 1994. Treatment of swine lagoon effluent by constructed wetlands operated at three loading rates. In: P.J. DuBowy and R.P. Reaves (Editors), Proc. of a Workshop on Constructed Wetlands for Animal Waste Management, 4-6 April 1994, Lafayette, pp. 23–33.

McKendry, P., 2002. Energy production from biomass (part 1): overview of biomass. Bioresource Tech. 83, 37–46.

Meers, E., Rousseau, P.L., Blomme, N., Lesage, E., Laing, D.G., Tack, G.M., Verloo, M.G., 2005. Tertiary treatment of the liquid fraction of pig manure with Phragmites australis. Water Air Soil Pollut. 160, 15–26.

Meers, E., Tack, F.M.G., Tolpe, I., Michels, E., 2008. Application of a full-scale constructed wetland for tertiary treatment of piggery manure: monitoring results. Water Air Soil Pollut. 193, 15–24.

Mitsch, W.J., Gosselink, J.G., 2007. Wetlands. 4th edn. Wiley, New York.

Morand, P., Robin, P., Escandec, A., Picot, B., Pourcher, A.M., Jiangping, Q., Yinsheng, L., Hamon, G., Amblard, C., Luth Fievet, S., Oudart, D., Pain, C., Cluzeau, D., Landrain, B., 2011. Biomass production and water purification from fresh liquid manure - use of vermiculture, macrophytes ponds and constructed wetlands to recover nutrients and recycle water for flushing in pig housing. Proc. Environ. Sci. 9, 130–139.

Moshiri, G.A., 1993. Constructed Wetlands for Water Quality Improvement. CRC press Inc., USA.

Newman, J.M, Clausen, J.C, Neafsey, J.A, 1999. Seasonal performance of a wetland constructed to process dairy milkhouse wastewater in Connecticut. Ecol. Eng. 14, 181–198.

Nguyen, M. L., Tanner, C.C., 1998. Ammonium removal from wastewaters using natural New Zealand zeolites. New Zealand Journal of Agricultural Research 41: 427-446.

Nichols, D.S., 1983. Capacity of nature wetlands to removal nutrients from wastewater. J. Water Pollut. Contr. Fed. 55, 495–505.

Noorvee, A., Poldvere, E., Mander, U., 2007. The effect of pre-aeration on the purification process in the long term performance of a horizontal subsurface flow constructed wetland. Science of the Total Environment 380, 229–236.

Official Journal of the European Union, 2011. Commission implementing decision of 3 November 2011 on granting a derogation requested by Italy with regard to the Regions of Emilia Romagna, Lombardia, Piemonte and Veneto pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources. Volume 54 L287/36.

Parkers, M.E., Mc Bride, A.D., Waalkens, A. 1998. Treatment of dilute piggery effluent with vertical flow reed beds. Journal of environmental quality. 27, 783–788.

Phipps, R.G., Crumpton, W.G., 1994. Factors affecting nitrogen loss in experimental wetlands with different hydrologic loads. Ecol. Eng. 3, 399-408.

Platzer, C., 1996. Enhanced nitrogen elimination in subsurface flow artificial wetlands - a multi tage concept. in: Proceedings of the 5th IAWQ Conference on Wetland Systems in Wa.t Poll. Control. Wien.

Pfaff, J.D., Brockhoff, C.A., O'Dell, J.W., 1989. Method 300.0: the determination of inorganic anions in water by ion chromatography. Cincinnati, Ohio: U.S. Environmental Protection Agency, Inorganic Chemistry Branch, Chemistry Research Division, Environmental Monitoring Systems Laboratory, A.W. Breidenbach Environmental Research Center.

Pfenning, K.S., McMahon, P.B., 1996. Effect of nitrate, organic carbon, and temperature on potential denitrification rate in nitraterich riverbed sediments. J. Hydrol. 187, 283–295.

Pettitt A. N., 1979. A Non-Parametric approach to the Change-Point problem. Applied statistics, 28, 126–135.

Poach, M.E., Hunt, P.G., Vanotti, M.B., Stone, K.C., Matheny, T.A., Johnson, M.H., Sadler, E.J., 2003. Improved Nitrogen Treatment By Constructed Wetlands Receiving Partially Nitrified Swine Wastewater. Ecol. Eng. 20, 183–197.

Poach, M.E., Hunt, P.G., Reddy, G.B., Stone, K.C., Johnson, M.H., and Grubbs, A.M., 2004. Swine wastewater treatment by marsh-pond-marsh constructed wetlands under varying nitrogen loads, Ecol. Eng. 23, 165–175.

Poach, M.E., Hunt, P.G., Reddy, G.B., Stone, K.C., Johnson, M.H., Grubbs, A., 2007. Effect of intermittent drainage on swine wastewater treatment by marsh-pond-marsh constructed wetlands. Ecol. Eng. 30, 43–50.

Reaves, R.P., DuBowy, P.J. and Miller, B.K., 1994. Performance of a constructed wetland for dairy waste treatment in Lagrange County, Indiana. In: P.J. DuBowy and R.P. Reaves (Editors), Proc. of a Workshop on Constructed Wetlands for Animal Waste Management, 4-6 April 1994, Lafayette, pp. 43–52.

Reddy, K., Patrick, W., 1984. Nitrogen transformations and loss in flooded soils and sediments. Critical Reviews in Environmental Control. 13, 273–309.

Reddy, G.B., P.G. Hunt, R. Phillips, K. Stone, and A. Grubbs. 2001. Treatment of swine wastewater in marsh-pond-marsh constructed wetlands. Water Sci. Tech. 44, 545–550.

Richardson, W., Strauss, E., Bartsch, L., Monroe, E., Cavanaugh, J., Vingum, L., Soballe, D., 2004. Denitrification in the upper Mississippi River: rates, controls, and contribution to nitrate flux. Can. J. Fish. Aquat. Sci. 61, 1102–1112.

Ross, M., Garcia, C., Hernández, T., 2006. A full-scale study of treatment of pig slurry by composting: kinetic changes in chemical and microbial properties. Waste Manage. 26, 1108–1118.

Rothman, E., Bouchard, V., 2007. Regulation of carbon processes by macrophyte species in a great lakes coastal wetland. Wetlands 27, 1134–1143.

Rustige, H., Tomac, I., Höner, G., 2003. Investigations on phosphorus removal in subsurface flow constructed wetlands, Water Sci. Technol. 48, 67–74.

Saeed, T., Sun, G., 2011. Enhanced denitrification and organics removal in hybrid wetland columns: comparative experiments. Bioresource Tech. 102, 967–974.

Salvato, M., Borin, M., 2010. Effect of different macrophytes in abating nitrogen from a synthetic wastewater. Ecol. Eng. 36, 1222–1231.

Sanchez, C.A., Porter, P.S., 1994. Nitrogen in the organic soils of the EAA. In: Bottcher, D.B., Izuno, F.T. (Eds.), Everglades Agricultural Area (EAA). Univ. Press of Florida, Gainesville, FL, pp. 42–84.

Sànchez-Garcìa, P., Caballero, A., Faz, A., Lobera, J.B., 2010. A Pig slurry purification option: Constructed wetland. Proc. of a 14th Ramiran Internation conference on treatment and use of organic residues in agriculture, 12-15 September 2010, Lisboa, Portugal pp. 30.

Sandoval-Cobo, J.J., Pena, M.R., 2007. Performance analysis of SSF wetlands in tropical regions based on first-order kinetic models for the removal of organic matter. In: Mander, Ü., Kóiv, M., Vohla, C. (Eds.), Proceedings of the 2nd International Symposium on “Wetland Pollutant Dynamics and Control WETPOL 2007”–Extended Abstracts. 16–20 September, Tartu, Estonia; I, pp. 268–270.

Sartoris, J.J., Thullen, J.S., Barber, L.B., Salas, D.E., 2000. Investigation of nitrogen transformations in a southern California constructed wastewater treatment wetland. Ecol. Eng. 14, 49–65.

Szogi, A.A., Rice, J.M., Humenik, F.J., Hunt, P.G., Stem, G., 1999. Constructed wetlands for confined swine wastewater treatment. In: Proceedings of 1999 Animal Waste Management System Symposium, North Carolina, pp. 379–383.

Scholz, M., Lee, B.H., 2005. Constructed wetlands: A review, Int. J. Environ. Stud. 62, 421–447.

Scholz, M., 2006. Wetland Systems to Control Urban Runoff. Elsevier, Amsterdam.

Sezerino P.H., Reginatto V., Santos M.A., Kayser K., Kunst S., Philippi S., Soares H.M., 2003. Nutrient removal from piggery effluent using vertical flow constructed wetlands in southern Brazil. Water Science and Technology 48(2): 129–135.

Skarda SM, Moore JA, Niswander SF, Gamroth MJ (1994) Preliminary results of a wetland for treatment of dairy farm wastewater. In: DuBowy PJ, Reaves RP (eds) Constructed wetlands for animal wastewater management. Proceedings of workshop, 4–6 Apr 1994, Purdue University, West Lafayette, IN. pp. 34–42.

Smith, K.A., Charles, D.R., Moorhouse, D., 2000. Nitrogen excretion by farm livestock with respect to land spreading requirements and controlling nitrogen losses to ground and surface waters. Part 2: Pigs and poultry. Bioresour. Technol. 71 (2), 183–194.

Soetaert, K., Hoffmann, M., Meire, P., Starink, M., Oevelen, D., Regenmortel, S., Coxc, T., 2004. Modeling growth and carbon allocation in two reed beds (Phragmites australis) in the Scheldt estuary. Aquat. Bot. 79, 211–234.

Solano, M.L., Soriano, P., Ciria, M.P., 2004. Constructed wetlands as a sustainable solution for wastewater treatment in small villages. Biosystems Eng. 87, 109–118.

Song, Z., Zheng, Z., Li, J., Sun, X., Han, X., Wang, W., Xu, M., 2006. Seasonal and annual performance of a full-scale constructed wetland system for sewage treatment in China. Ecol. Eng. 26, 272–282.

Spieles, D.J., Mitsch, W.J., 2000. The effects of season and hydrologic and chemical loading on nitrate retention in constructed wetlands: a comparison of low and high nutrient riverine systems. Ecol. Eng. 14, 77–91.

Stanford, G., Dzienia, S.,Van der Pol, R., 1975. Effect of temperature on denitrification rate in soils. J. Soil Sci. Soc. Am. 39, 867–870.

StatSoft, Inc., 2004. STATISTICA, Version 7, www.statsoft.com. 29/06/2011.

Stein, O.R., Hook, P.B., Biederman, J.A., Allen, W.C., Borden, D.J., 2003. Does batch operation enhance oxidation in subsurface constructed wetlands? Water Sci. Technol. 48, 149–156.

Stefanakis, A.I., Tsihrintzis, V.A., 2012. Effects of loading, resting period, temperature, porous media, vegetation and aeration on performance of pilot-scale vertical flow constructed wetlands. Chemical Engineering Journal, Volume 181-182, p. 416-430.

Stober, J.T., O'Connor, J.T., Brazos, B.J., 1997. Winter and spring evaluations of a wetland for tertiary wastewater treatment.Water Environ. Res. 69, 961–968.

Stone, K.C., Poach, M.E., Hunt, P.G., Reddy, G.B. 2004. Marsh-pond-marsh constructed wetland design analysis for swine lagoon wastewater treatment. Ecol. Eng. 23, 127–133.

Struseviĉius, Z., and Struseviĉiene, S.M. 2003. Investigations of wastewater produced on cattle-breeding farms and its treatment in constructed wetlands, in: Proc. Internat. Conf. Constructed and Riverine Wetlands for Optimal Control of Wastewater at Catchment Scale, Ü. Mander, C. Vohla and A. Poom, eds., University of Tartu, Institute of Geography, Tartu, Estonia, Publ. Instituti Geographici Universitatis Tartuensis. Vol. 94, pp. 317-324.

Sun, G., Gray, K.R., Biddlestone, A.J., 1998. Treatment of agricultural wastewater in down flow reed beds: experimental trials and mathematical model. Journal of Agricultural Engineering Research 69, 63–71.

Sun, G., Gray, K.R, Biddlestone, A.J., 1999. Treatment of agricultural wastewater in a pilot-scale tidal flow reed bed system. Environ. Technol. 20, 233–237.

Sun, G., Saeed, T., 2009. Kinetic modelling of organic matter removal in 80 horizontal flow reed beds for domestic sewage treatment. Process Biochemistry 42, 717–722.

Surrency, D. 1993. Evaluation of aquatic plants for constructed wetlands. In: GA Moshiri, ed. Constructed wetlands for water quality improvements. Lewis Publishers, Boca Raton, Florida, USA.

Sutton, P.M., Murphy, K.L., Jank. B.E., Monaghan, B.A., 1975. Efficacy of Biological Nitrification. J. Water Pollut. Control Fed. 47, 2665.

Tanner, C.C., D'Eugenio, J., McBride, G.B., Sukias, J.P.S., Thompson, K., 1999. Effect of water level fluctuation on nitrogen removal from constructed wetland mesocosms. Ecol. Eng. 12, 67–92.

Tanner, C.C., Clayton, J.S., Upsdell, M.P., 1995. Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands – II. Removal of nitrogen and phosphorus. Water Resources 29, 27–34.

Ten Have, P.J.W., Willers, H.C., Derikx, P.J.L., 1994. Nitrification and denitrification in an activated-sludge system for supernatant from settled sow manure with molasses as an extra carbon source. Bioresour. Technol. 47, 135–141.

Thullen, J.S., Sartoris, J.J. and Nelson, S.M., 2005. Managing vegetation in surface-flow wastewater treatment wetland for optimal treatment performance. Ecol. Eng. 25, 583–593.

Torrens, A., Molle, P., Boutin, C., Salgot, M., 2009. Removal of bacterial and viral indicators in vertical flow constructed wetlands and intermittent sand filters. Desalination. 247, 170–179.

Trias M, Hu Z, Mortula MM, Gordon RJ, Gagnon GA (2004) Impact of seasonal variation on treatment of swine wastewater. Environ. Tech. 25, 775–781.

Tylova-Munzarova, E., Lorenzen, B., Brix, H., Votrubova, O., 2005. The effects of NH_4 and NO_3 on growth, resource allocation and nitrogen uptake kinetics of Phragmites australis and Glyceria maxima. Aquatic Botany 81, 326–342.

Tuncsiper, B., 2009. Nitrogen removal in a combined vertical and horizontal subsurface-flow constructed wetland system. Desalination 247, 466–475.

Turner, E.G., Netherland, M.D., Getsinger, K.D., 1991. Submersed plants and algae as factors in the loss of rhodamine WT dye. J. Aquat. Plant Manage. 29, 113–115.

USDA, United States Department of Agriculture, 2002. Environmental Engineering National Engineering Handbook, Part 637, Chapter 3, Constructed Wetlands. Pp 3-3.

U.S. Environmental Protection Agency, US EPA, 1975. Process Design Manual for Nitrogen Control. Office of Technology Transfer, Washington, DC.

U.S. Environmental Protection Agency, US EPA, 1993. Design manual: Nitrogen control, EPA 625/R- 93/010, U.S. EPA Office of Research and Development: Washington D.C.

U.S. Environmental Protection Agency, US EPA, 1994. A Handbook of Constructed Wetlands, A Guide to Creating Wetlands for: Agricultural Wastewater, Domestic Wastewater, Coal Mine Drainage, Stormwater in the Mid- Atlantic Region. Volume 1: General Considerations. United Sates Environmental Protection Agency, Washington DC, New York, USA.

U.S. Environmental Protection Agency, US EPA, 1997. Constructed Wetlands and Wastewater Management for Confined Animal Feeding Operations. CH2MHILL, Gulf of Mexico Program (U.S.). Nutrient Enrichment Committee. EPA Number: 855K97001.

U.S. Environmental Protection Agency, US EPA, 1998. Constructed Wetlands and Animal Wastewater Management. CH2MHILL, Gulf of Mexico Program (U.S.). Nutrient Enrichment Committee.

Verstraete, W., Vandevivere, P., 1999. New and broader applications of anaerobic digestion. Crit. Rev. Environ. Sci. Technol. 29, 151–173.

Vares, V., Kask, Ü., Muiste, P., Pihu, T., Soosaar., 2005. Manual for biofuel users. Tallinn University of Technology. pp. 30–35.

Vymazal, J., 1999. Nitrogen removal in constructed wetlands with horizontal sub-surface flow can we determine the key process? In: Vymazal, J. (Ed.), Nutrient Cycling and Retention in Natural and Constructed Wetlands. Backhuys Publishers, Leiden, pp. 1–17.

Vymazal, J., 2004. Removal of phosphorus in constructed wetlands with horizontal sub-surface flow in the Czech Republic, Water, Air and Soil Pollution: Focus, 4, 657-670.

Vymazal, J., 2005. Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. Ecol. Eng. 25, 478–490.

Vymazal, J., KrÖpfelovà , L., 2005. Growth of Phragmites australis and Phalaris arundinacea in constructed wetlands for wastewater treatment in the Czech Republic. Ecol. Eng. 25, 606–621.

Van Oostrom, A.J., Russell, J. M., 1994. Denitrification in constructed wastewater wetlands receiving high concentrations of nitrate, Water Sci. Technol. 29, 7–14.

Von Felde, K. and Kunst, S. 1997. N- and COD-removal in vertical-flow systems. Wat. Sci. Tech., 35, 79–85.

Wang, R., Baldy, V., Périssol, C., Korboulewsky, N., 2012. Influence of plants on microbial activity in a vertical-down flow wetland system treating waste activated sludge with high organic matter concentrations. Journal of Environmental Management 95, S158–S164.

Wang, J., Cai, X., Chen, Y., Yang, Y., Liang, M., Zhang, Y., Wang, Z., Li, Q., Liao, X., 1994. Analysis of the configuration and the treatment effect of constructed wetland wastewater treatment system for different wastewaters in South China, in: Proc. 4th Internat. Conf. Wetland Systems for Water Pollution Control, ICWS'94 Secretariat, Guangzhou, P.R. China, pp. 114–120.

Weier, K.L., Doran, J.W., Power, J.F., Walters, D.T., 1993. Denitrification and the dinitrogen:nitrous oxide ratio as affected by soil water, available carbon, and nitrate. Soil Sci. Soc. Am. J. 57, 66–72.

Werker, A.G., Dougherty, J.M., McHenry, J.L., Van Loon, W.A., 2002. Treatment variability for wetland wastewater treatment design in cold climates. Ecol. Eng. 19, 1–11.

Willems, H.P.L., Rotelli, M.D., Berry, D.F., Smith, E.P., Reneau Jr., R.B., Mostaghimi, S., 1997. Nitrate removal in riparian wetland soils: effects of flow rate, temperature, nitrate concentration and soil depth. Water Res. 31, 841–849.

Windham, L., Lathrop, R.G., 1999. Effects of Phragmites australis (common reed) invasion on aboveground biomass and soil properties in brackish tidal marsh of the Mullica River. New Jersey. Estuaries 22, 927–935.

Wittgren, H.B., Maehlum, T., 1997. Wastewater treatment wetlands in cold climates. Water Sci. Technol. 35, 45–53.

Worley, J.W., Das, K.C., 2000. Swine manure solids separation and composting using alum. Appl. Eng. Agric. 16 (5), 555–561.

Xie, S.-G., Zhang, X.-J., Wang, Z.-S., 2003. Temperature effect on aerobic denitrification and nitrification. Journal of Environmental Sciences 15, 669–673.

Yang, L., Chang, H.T., Huang, M.N.L., 2001. Nutrient removal in gravel and soil based wetland microcosms with and without vegetation. Ecol. Eng. 18, 91–105.

Yalcuk, A., Ugurlu, A., 2009. Comparison of horizontal and vertical constructed wetland systems for landfill leachate treatment. Bioresource Tech. 100, 2521–2526.

Ye, F., Li, Y., 2009. Enhancement of nitrogen removal in towery hybrid constructed wetland to treat domestic wastewater for small rural communities. Ecol. Eng. 35, 1043–1050.

Zachritz II, W.H., Hanson, A.T., Sauceda, J.A., Fitzsimmons, K.M., 2008. Evaluation of submerged surface flow (SSF) constructed wetlands for recirculating tilapia production systems. Aquacultural Engineering 39, 16–23.

Zhang, R.H. and P.W. Westerman. 1997. Solid-liquid separation of annual (sic) manure for odor control and nutrient management. Applied Engineering in Agriculture, 13, 385–393.

Zhao, Y.Q., Babatunde, A.O., Hu, Y.S., Kumar, J.L.G., Zhao, X.H., 2011. Pilot field-scale demonstration of a novel alum sludge-based constructed wetland system for enhanced wastewater treatment. Process Biochemistry 46, 278–283.

Zhao, Y.Q., Sun, G., Allen, S.J., 2004. Anti-sized reed bed system for animal wastewater treatment : a comparative study. Water Research. 38, 2907–2917.

Zhang, R.H., Westerman, P.W., 1997. Solid–liquid separation of animal manure for odor control and nutrient management. Appl. Eng. Agric. 13 (5), 657–664.

Zhang, L., Xia, X., Zhao, Y., Xi, B., Yanan, Y., Guo, X., Xiong, Y., Zhan, J., 2011. The ammonium nitrogen oxidation process in horizontal subsurface flow constructed wetlands. Ecol. Eng. 37, 1614–1619.

Zhang, Z.J., Zhu, J., King, J., Li, W.H., 2006. A two-step fed SBR for treating swine manure. Proc. Biochem. 41 (4), 892–900.

Zhang, Z., Rengel, Z., Meney, K., 2007. Nutrient Removal from Simulated Wastewater Using Canna indica and Schoenoplectus validus in Mono- and Mixed-Culture in Wetland Microcosms. Water Air Soil Pollut. 183, 95–105.

Zhu, X., Cui, L., Liu, W., & Liu, Y. (2004). Removal efficiencies of septic tank effluent by simulating vertical flow constructed Canna indica Linn. wetlands. Journal of Agro-Environment Science, 23, 761–765.