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Effect of by-pass and effluent recirculation on nitrogen removal in hybrid constructed wetlands for domestic and industrial wastewater treatment

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ABSTRACT

Hybrid constructed wetlands (CWs) including subsurface horizontal flow (HF) and vertical flow (VF) steps look for effective nitrification and denitrification through the combination of anaerobic/anoxic and aerobic conditions. Several CW configurations including several configurations of single pass systems (HF + HF, VF + VF, VF + HF), the Bp(VF + HF) arrangement (with feeding by-pass) and the R(HF + VF) system (with effluent recirculation) were tested treating synthetic domestic wastewater. Two HF/VF area ratios (AR) were tested for the VF + HF and Bp(VF + HF) systems. In addition, a R(VF + VF) system was tested for the treatment of a high strength industrial wastewater. The percentage removal of TSS, COD and BOD_5 was usually higher than 95% in all systems. The single pass systems showed TN removal below the threshold of 50% and low removal rates (0.6–1.2 g TN/m^2 d), except the VF + VF system which reached 63% and 3.5 g TN/m^2 d removal but only at high loading rates. Bp(VF + HF) systems required by-pass ratios of 40–50% and increased TN removal rates to approximately 50–60% in a sustainable manner. Removal rates depended on the AR value, increasing from 1.6 (AR 2.0) to 5.2 g TN/m^2 d (AR 0.5), both working with synthetic domestic wastewater. On real domestic wastewater the Bp (VF + HF) (AR 0.5 and 30% by-pass) reached 2.5 g TN/m^2 d removal rate. Effluent recirculation significantly improved the TN removal efficiency and rate. The R(HF + VF) system showed stable TN removals of approximately 80% at loading rates ranging from 2 to 8 g TN/m^2 d. High TN removal rates (up to 73% TN and 8.4 g TN/m^2 d) were also obtained for the R(VF + VF) system treating industrial wastewater.

1. Introduction

Constructed wetlands (CWs) have been established as an alternative to technical wastewater treatment systems for the sanitation of small communities, but single stage CWs are not able to get the more stringent discharge limits for nitrogen. Vertical flow (VF) CWs have predominant aerobic conditions, while the subsurface horizontal flow (HF) CWs mainly presented anaerobic conditions. Combining both types of CWs in hybrid systems could achieve complete nitrogen removal so, in the later years the interest in the study of multi-step and hybrid systems has increased (Ávila et al., 2013; Vymazal, 2013; Vymazal and Kröpfelová, 2015).

Vymazal (2013) surveyed 60 hybrid constructed wetlands from 24 countries reported since 2003 to 2012. The most commonly used hybrid system is a VF + HF constructed wetland which has been used for treatment of both sewage and industrial wastewaters. On the other hand, the use of a HF + VF system has been reported only for treatment of municipal sewage. The free water system (FWS) is also used in several hybrid configurations. Vymazal (2013) found that the VF + HF configuration was slightly more efficient in ammonia removal than other hybrid configurations, including HF + VF systems. All types of hybrid CWs are comparable with single VF CWs in terms of NH_3-N removal rates whilst they are more efficient in total nitrogen removal than single HF or VF CWs (Vymazal, 2013). Furthermore, several kinds of industrial wastewaters have been treated in hybrid systems consisting of three (i.e. VF + VF + HF and VF + HF + VF) or more (VF + VF + HF + VF and VF + VF + VF + HF + VF) steps. Some of these multi-step systems directly treated high strength wastewaters such as slaughterhouse (chemical oxygen demand (COD) of 3188 mg/L), milk parlour (up to 5000 mg COD/L), potato starch (24,017 mg COD/L) or tannery (11,500 mg COD/L) wastewaters.

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Focussing on surface removal rate (SRR) of total nitrogen (TN), Vymazal (2013) did not find significant difference neither in NH_3-N removal among the various types of hybrid systems which showed mean values ranging from 2.1 to 2.5 g NH_3-N/m^2 d, nor in TN removal (from 2.3 to 4.3 g TN/m^2 d). The highly variable design and operational conditions of the reviewed systems could be an explanation for the lack of significant differences in nitrogen removal among the hybrid systems. Besides the type of wastewater, environmental conditions, influent concentration and loading rates, the surface area ratio of saturated (i.e. HF units) to unsaturated units (mainly VF units) varied largely. For example, the two step VF + HF and HF + VF systems presented mean HF/VF area ratios (AR) of 2.7 (0.5–7.6) and 1.6 (0.9–3.1), respectively. Early VF + HF hybrid systems presented an even higher AR (Gaboutloeloe et al., 2009). Only some multi-step systems treating high strength wastewater were designed with low ARs ranging from 0.1 to 1.2 (Vymazal, 2013).

Recently, more sophisticated process designs were proposed in order to improve nitrogen removal and to increase the applied loads reducing the land area requirement. Tanner et al. (2012) pointed out that the endogenous organic carbon supply from plant biomass decay and root-zone exudation has often been found to be insufficient to achieve full denitrification in VF + HF hybrid systems. These authors (Tanner et al., 2012) proved the use of carbonaceous bioreactors which incorporate a slow-release source of organic C (e.g. wood chips) aiming to increase denitrification. Multi-feeding or by-pass of untreated influent to the second HF CW unit has also been reported (Stefanakis et al., 2011; Hu et al., 2012; Wang et al., 2014). Recirculation has been employed in various configurations (Brix et al., 2003; Brix and Arias, 2005; Ayaz et al., 2012; Foladori et al., 2013; Vázquez et al., 2013) in order to increase simultaneous nitrification and denitrification processes in either a single CW unit or in the two-step HF + VF systems. Artificial aeration in hydraulic saturated units, attaining to only part of the system or timed has been also considered (Pascual et al., 2016).

The objectives of this research are to compare the performance of various multi-step CW system configurations operating in the same conditions. Several configurations of single pass two-step systems (HF + HF, VF + VF, VF + HF), the VF + HF arrangement with feeding by-pass (Bp(VF + HF)), and the HF + VF system with effluent recirculation (R(HF + VF)) were tested on synthetic domestic wastewater for organic matter, ammonia and TN removal. Two alternatives of the VF + HF (with and without step-feeding) configuration, varying the relative surface area of each unit, were studied. Finally, the VF + VF system with effluent recirculation (R(VF + VF)) was tested for the treatment of a high strength influent simulating food industry wastewater.

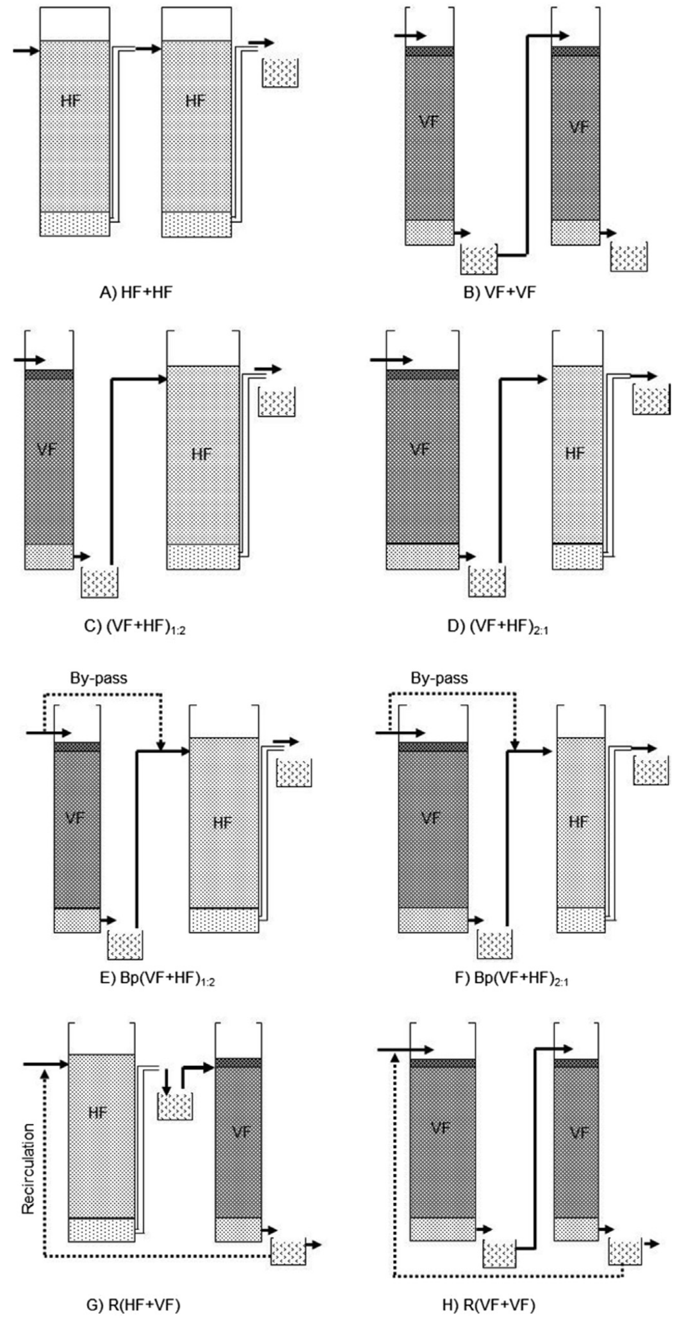

Fig. 1. Schemes of the lab-scale systems: A) Single pass HF + HF system, B) Single pass VF + VF system, C) and D) Single pass hybrid VF + HF systems with different relative surface areas, E) and F) Hybrid VF + HF HF + VF system with effluent recirculation, H) Two-step VF + VF system with effluent recirculation. The first step:second step surface area ratios are 1:1 (A,B), 1:2 (C,E), and 2:1 (D,F,G,H).

2. Materials and methods

2.1. Lab-scale hybrid systems

The schemes of the eight two-step lab systems are shown in Fig. 1. Unsaturated VF CWs were simulated by lab-scale columns, as proposed by Andreottola et al. (2007). Similar cylindrical columns were adapted to simulate subsurface horizontal flow (HF) CWs by applying conditions of continuous hydraulic saturation. Thus, HF columns were similar to VF columns and were also operated in down-flow mode, but changing the conditions required to simulate actual HF CWs, such as hydraulic saturation conditions, continuous flow, bed particle size and influent point below the gravel surface. Both VF and HF columns were made of methacrylate with two optional internal diameters of 10 and 14 cm and a total height of 60 cm. The column cross-sectional areas were used to calculate the hydraulic loading rate (HLR) and the surface loading rate (SLR) for both the VF and HF CWs. Overall HLR was calculated as the influent flow divided by the surface area of both units whilst the SLR was calculated as the product of HLR by the concentration. In this way, effluent recirculation (if the case) did not affected the values of HLR and SLR.

The HF bed consisted of a 10 cm drainage layer of 10–20 mm gravel at the bottom, and a 40 cm filtering layer of 6–12 mm fine gravel (porosity 40%), the influent entering the column 3 cm below the gravel surface. The wastewater flowed downwards until reach the drainage layer and went out upwards through a pipe until reach the water level in the column. This disposition created continuous saturated conditions in the HF columns. The operation of the saturated HF columns simulated a continuous feeding regime by applying small and frequent pulses (at least 16 a day) by using peristaltic pumps.

Except the VF columns used as first unit of the R(VF + VF) system, the bed of all the other VF columns in Fig. 1 consisted of a 10 cm drainage layer of 6–12 mm gravel at the bottom, 32 cm of a main filtering layer of 1–3 mm sand (41% porosity), and a thin 5 cm upper layer of 0–3 mm fine sand. On the other hand, a 37 cm layer of 3–6 mm sand (42% porosity) was used as the main filtering media in the first step of the R(VF + VF) system. In the second part of the operation of this system, the upper 10 cm of the first step filtering media was substituted by a layer of 1–3 mm sand. The influent entered the VF columns over the sand surface.

The VF columns were intermittently operated (several pulses or loads per day), either without resting or with a week regime of 3–4 days feeding and 4–3 days resting, as being detailed in Section 3. In the case of resting, both HLR and SLR were calculated on the basis of the overall cycle including both feeding and resting days. After each influent pulse, the wastewater flows free in the down direction and the effluent was recovered in an open tank located at the bottom of the column, thus providing unsaturated hydraulic conditions.

All bed media were crushed granitic gravel and sand. The units were not planted because of the short operational time of the studies carried out. Timed adjustable flow peristaltic pumps (Dinko Instruments D-21V) were used to pumping the feeding and the recirculation flow. Tanks with a capacity twice the pulse volume were used to store the recirculated effluent. Small basins retaining a volume of about 40 mL were placed at the exit of each column in order to measure some in-situ parameters.

2.2. Synthetic and real wastewater

The synthetic wastewater simulating domestic wastewater (SDWW) was designed based on the proposal of Aiyuk and Verstraete (2004). Suspended solids concentration (obtained from food components) was reduced taking into account that constructed wetlands usually receive pre-treated wastewater or wastewater proceeding from a primary treatment (Álvarez et al., 2008). On the same basis, a synthetic industrial wastewater (SIWW) was prepared by adding commercial vinegar as soluble organic substrate.

A concentrate of SDWW was prepared with the following composition (mg/L): urea (1600), Na-acetate·$3H_2O$ (2250), NH_4Cl (200), peptone (300), $MgHPO_4\cdot 3H_2O$ (500), $K_2HPO_4\cdot 3H_2O$ (400), $FeSO_4\cdot 7H_2O$ (100), $CaCl_2$ (100), Starch (1000), milk powder (2000), dried yeast (900), soy oil (500), $Cr(NO_3)_3\cdot 9H_2O$ (15), $CuCl_2\cdot 2H_2O$ (10), $MnSO_4\cdot H_2O$ (2), $NiSO_4\cdot 6H_2O$ (5), $PbCl_2$ (2) and $ZnCl_2$ (5). This concentrate was maintained at 4 °C until the moment of use, when it was diluted with tap water by a factor of 13 in order to prepare the synthetic DWW.

To prepare the SIWW concentrate, all the amount indicated in the preceding paragraph were multiplied by a factor of 3.5, except Starch (1837 mg/L), milk powder (1750 mg/L), dried yeast (787 mg/L) and soy oil (437 mg/L). Additionally, the SIWW concentrate was supplemented with 371 mL/L of commercial vinegar. Maintained at 4 °C until the moment of use, it was diluted with tap water by a factor of 7 in order to prepare the synthetic IWW. Alkalinity was increased by adding 500 mg $NaHCO_3$/L to the influent.

Real domestic wastewater (RDWW) was obtained from the entrance of the municipal wastewater treatment plant of Bens (A Coruña) prior the primary decanter. It was stored at 5 °C and let to decant for about 2 h, the supernatant being used as the influent to the systems. Dilute influent DWW and IWW as well as real DWW was maintained at 10 °C for a maximum time of 3 days during feeding to the respective system and then a new batch was prepared. The initial composition of the influents to the different lab-scale systems is given in Table 1. Although the ammonification of urea to ammonia has not been investigated in this study, it has been found that about 30% of initial urea was transformed to ammonia after three days of storage in the influent tank. This agree with the fact that urea is readily hydrolysed, chemically or microbially, resulting in the release of ammonia (Kadlec and Wallace, 2009).

2.3. Sampling and analysis

Effluent composite samples were obtained by integrating daily samples and storing them at 4 °C. This procedure was repeated once or twice a week. Obtained samples were analysed in the laboratory for total and volatile suspended solids (TSS, VSS), chemical oxygen demand (COD), biological oxygen demand (BOD_5), ammonium, nitrate and TN. pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO) were determined in situ on the effluent stream. Analytical methods were carried out as described in Standard Methods (APHA, 2005). An integrated pH & redox 26 Crison electrode was used for pH and ORP determination, a selective electrode (Crison 9663) for ammonium and an YSI ProODO electrode for DO. The determination of nitrate nitrogen was made in a Biochrom Libra S6 spectrophotometer. The procedure is based on the second derivative of the UV absorption spectrum for NO_3^-, which has a peak at 228 nm that is proportional to the NO_3^- concentration. To analyze total nitrogen (TN), samples were first digested with potassium persulfate to oxidize all the nitrogenous compounds to NO_3^- and after this the nitrates were determined by spectrophotometry.

2.4. General remarks on system selection and operation

Saturated down-flow columns were used to simulated HF beds. The suitability of saturated down-flow columns to simulate conventional HF beds is derived from the fact that diffusion of atmospheric oxygen is highly reduced due to the presence of sand or gravel substrate when compared to free water surface treatment wetlands and by the limited water-air interface when compared to unsaturated VF treatment wetlands (Nivala et al., 2013). The performance of the simulated VF and HF columns was previously tested by using them as individual systems. Results from these previous test indicated that similar 90–95% COD removal was achieved at higher SLR (80 g COD/m^2 d) for VF columns than for HF columns (20 g COD/m^2 d), whilst the effluent from VF columns was more aerobic (DO 3–7 mgO_2/L, ORP 60–100 mV) than the HF effluent (DO 1–3 mgO_2/L, ORP −110–/−230 mV). In addition, in these conditions, a higher ammonia concentration remained in the HF effluent (66 mg NH_3-N/L) than in the VF effluent (30 mg NH_3-N/L). These results indicate that saturated and unsaturated downflow columns appropriately simulated the performance of conventional HF and VF CWs.

Hybrid CWs (HF + VF, VF + HF) look for effective nitrification and denitrification through the combination of anaerobic/anoxic and aerobic conditions which can be provided by the different types of CW units, depending on its relative surface area and operating conditions. On the other hand, multi-step systems consisting of the same type of units (HF + HF, VF + VF) rely on simultaneous nitrification and denitrification in some parts of the units, depending mainly on the SLR applied. Beside this considerations, the studied two-step systems were classified into tree main types: a) single pass two-step systems (cases A to D in Fig. 1) which are used in current applications of CWs because of its operational simplicity; b) two-step hybrid VF-HF system with influent by-pass to the HF second unit (E and F in Fig. 1), and c) two-step systems

Table 1
Average characteristics of influent wastewaters (mg/L).

Influent	N[a]	pH	TSS	VSS	COD	BOD_5	TN	NH_3-N	NO_3^--N	PO_4^{3-}-P
SDWW[b]	7–14	7.0 ± 0.2	120 ± 32	106 ± 10	539 ± 48	260 ± 49	78 ± 8	8 ± 1	3 ± 1	11 ± 2
RDWW[c]	8	7.2	81 ± 26	73 ± 27	405 ± 49	225 ± 44	57 ± 3	45 ± 7	2 ± 1	5.4 ± 1
SIWW[d]	5	6.4 ± 0.2	272 ± 62	255 ± 61	4007 ± 174	2450 ± 342	439 ± 25	10 ± 1	12 ± 1	83 ± 3

[a] Number of samples (for P-PO_4^{3-}, N = 2).
[b] SDWW: synthetic domestic wastewater.
[c] RDWW: real domestic wastewater.
[d] SIWW: synthetic industrial wastewater.

with recirculation (G and H in Fig. 1). While a) and b) type systems can operate without any energy consumption if wastewater flow by gravity is provided, recirculation in type c) systems always requires energy supply in order to return part of the final effluent to the inlet of the first unit. Although the complexity of implement influent by-pass to the second step in VF + HF hybrid systems seems to be reduced, at the moment this option have not been described in the reviewed literature. Instead of the hybrid HF + VF system, the two-step VF + VF system was selected for the treatment of the high-strength industrial wastewater taking into account that both aerobic and anaerobic conditions would be present in the first VF unit in which nitrification and denitrification can occurs simultaneously due to the high strength of the influent and the application of high SLR (Vázquez et al., 2013).

The average values of the operational parameters and removal results of the three types of systems are shown in Tables 2–4. In these Tables we indicate the HLR and SLR applied which are dependent of the considered system, the removal efficiency and the ammonia and nitrate concentration in the final effluent. The BOD_5 SLR at the first step was used as the criteria in order to set the initial operating conditions (start-up periods). In those systems in which the first step was a HF unit, the SLR applied at this stage was approximately 8–10 g BOD_5/m^2 d. On the other hand, a SLR in the range of 20–35 g BOD_5/m^2 d was applied at the VF units when used as first step. An influent COD/BOD_5 ratio of 2.1 is obtained from Table 1, indicating that the applied SLR in terms of COD was approximately twice of BOD_5 SLR. After the start-up in these conditions, the operating conditions were adapted in order to optimize the TN removal efficiency. Usually, the percentage removal of TSS, COD and BOD_5 were higher than 95% for SDWW (or higher than 85% for RDWW) in all systems, which reached very low effluent concentrations for these parameters. Thus, in order to assess the performance of the different systems, we focused mainly on nitrogen conversion and removal.

Although detailed data about the capacity and efficiency of each individual unit, effluent characteristics including DO and ORP, clogging related parameters (hydraulic infiltration rates and solids accumulation), nitrification and denitrification intensities and gas emissions are available, this data is not presented in this work being only occasionally referred to it.

As indicated in Section 2.1, all the systems were operated without plants. We consider that this fact do not impair the interest of the results and conclusions of the study because of the short period of operation (less than one year) and the high loading rates applied. When necessary, the possible effect of plants presence is pointed out. In general, better results could be expected from plants presence and longer operation times because of the maturation effects (Białowiec et al., 2012; Button et al., 2015; Carballeira et al., 2016). Relating the potential effect of plants on denitrification, several studies stated that endogenous organic carbon supply from plant biomass decay and root-zone exudation are insufficient to achieve full denitrification (Narváez et al., 2011; Tanner et al., 2012).

3. Results and discussion

3.1. Performance of single pass two-step systems

The simplest CW system configuration combining two units are those corresponding to a single pass lineal configuration consisting of step 1 followed by step 2 in series (Fig. 1A, B, C and D), i.e. without by-pass or effluent recirculation. All these systems were feed with SDWW (Table 1) but operated with different HLR and pollutant SLR. Table 2 shows the overall operation characteristics and the efficiency obtained from each one of these systems.

3.1.1. Two-step HF + HF system

The two-step system including only hydraulic saturated conditions (HF + HF) reached limited nitrification rates as indicated by the accumulation of ammonia nitrogen in the final effluent (Table 2). TN removal was about 35% in spite of the reduced overall

Table 2
Performance characteristics of single pass two-step systems treating synthetic domestic wastewater.

System	Period (d[a])	VF op. cond.[b]	HLR[c] (L/m^2 d)	SLR (g/m^2 d)[c]			Removal (%)		Effluent (mg/L)	
				COD	BOD_5	TN	COD	TN	NH_3-N	NO_3^--N
A[d]: (HF + HF)	I (0–76)	n.a.	14.8	8.1	3.9	1.2	95.3	n.d.	36.2	n.d.
	II (77–157)	n.a.	19.1	10.4	5.0	1.5	93.5	n.d.	57.3	n.d.
	III (158–212)	n.a.	20.0	10.8	5.2	1.6	97.5	34.7	46.4	4.7
B[d]: (VF + VF)	I (47–116)	6 (4/3)	68.2	36.7	17.7	5.3	96.5	17.0	19.7	58.0
	II (145–179)	6 (4/3)	72.0	38.8	18.7	5.6	95.1	63.4	6.8	27.0
	III (180–200)	8 (3/4)	46.7	25.2	12.1	3.6	97.9	47.4	2.7	38.8
C[d]: $(VF + HF)_{1:2}$[e]	I (0–40)	8 (3/4)	27.3	16.1	6.9	2.1	97.1	56.3	16.2	23.4
	II (41–69)	9 (3/4)	27.2	16.0	6.9	2.1	98.5	41.2	14.7	35.5
D[d]: $(VF + HF)_{2:1}$[e]	I (0–48)	12 (3/4)	76.5	45.1	19.4	5.8	98.2	19.9	25.3	36.3

[a] Days of operation.
[b] Operational conditions in the VF units: number of pulses per day (days ON/OFF on a week basis).
[c] HLR and SLR were referred to the overall system.
[d] Refers to system description in Fig. 1.
[e] Subscripts indicate the VF:HF area ratio.

Table 3
Performance characteristics of two-step systems with by-pass treating synthetic and real domestic wastewater.

System (wastewater)	Period (d[a])	VF op. cond. [b]	HLR[c] (L/m² d)	By-pass (%)[d]	SLR (g/m² d)[c]			Removal (%)		Effluent (mg/L)	
					COD	BOD_5	TN	COD	TN	NH_3-N	NO_3^--N
E[e]: Bp(VF + HF)$_{1:2}$[f] (SDWW)[g]	III (70–119)	9 (3/4)	33.4	10	19.6	8.4	2.5	98.1	30.9	22.0	37.0
	IV (120–189)	9 (3/4)	35.9	25	21.2	9.1	2.7	97.3	30.9	13.2	42.9
	V (190–230)	9 (3/4)	44.4	50	26.2	11.2	3.4	98.4	47.2	25.1	17.8
	VI (231–258)	9 (3/4)	40.4	50	23.8	10.2	3.1	96.7	50.0	17.2	19.6
F[e]: Bp(VF + HF)$_{2:1}$[f] (SDWW)[g]	II (49–76)	12 (3/4)	95.7	23.4	56.3	24.2	7.2	98.0	20.9	18.2	50.1
	III (77–104)	12 (3/4)	109.0	39.7	64.2	27.6	8.2	97.6	47.3	15.1	34.7
	IV (105–125)	12 (3/4)	126.6	37.3	74.6	32.0	9.6	96.8	53.3	16.2	25.2
F[e]: Bp(VF + HF)$_{2:1}$[f] (RDWW)[h]	V (126–153)	12 (3/4)	125.8	35.6	53.6	29.2	7.1	85.2	44.6	32.9	6.5
	VI (154–165)	12 (3/4)	72.6	18.1	27.9	16.0	4.3	87.5	32.8	20	26
	VII (180)	12 (3/4)	71.0	33.3	27.3	15.6	4.2	88.1	58.7	15	14

[a] Days of operation.
[b] Operational conditions in the VF units: number of pulses per day (days ON/OFF on a week basis).
[c] HLR and SLR were referred to the overall system.
[d] By-pass flow to the second unit as percentage of influent flow to the first unit.
[e] Refers to system description in Fig. 1.
[f] Subscripts indicate the VF:HF area ratio.
[g] SDWW: synthetic domestic wastewater.
[h] RDWW: real domestic wastewater.

Table 4
Performance characteristics of two-step systems with recirculation treating synthetic domestic and industrial wastewater.

Period (d[a])	VF op. cond.[b]	HLR[c] (L/m² d)	Recirculation ratio (%)[d]	SLR (g/m² d)[c]			Removal (%)		Effluent (mg/L)	
				COD	BOD_5	TN	COD	TN	NH_3-N	NO_3^--N
G[e]: R(HF + VF) treating SDWW[f]										
I (0–37)	8 (7/7)	26.6	200	15.7	6.7	2.0	97.4	71.4	3.3	23.3
II (38–68)	8 (7/7)	26.6	300	15.7	6.7	2.0	97.2	75.7	2.3	19.0
III (69–117)	8 (7/7)	33.9	300	20	8.6	2.6	97.6	80.2	2.8	14.9
IV (118–187)	8 (7/7)	35.4	400	20.9	9.0	2.7	97.8	83.7	1.6	11.8
V (188–230)	8 (7/7)	52.2	400	30.7	13.2	3.9	98.8	82.6	3.6	12.2
VI (231–285)	12 (7/7)	68.7	400	40.5	17.4	5.2	97.9	82.3	2.1	14.7
VII (286–338)	16 (7/7)	100.8	400	62.1	26.7	8.0	95.1	78.1	3.8	14.4
H[e]: R(VF + VF) treating SIWW[g]										
I (0–72)	8 (3/4)	13.3	400	53.4	32.7	5.9	96.6	44.8	36.4	103.2
II (73–128)	8 (3/4)	19.1	400	76.5	46.8	8.4	95.0	55.7	50.6	87.6
III (129–184)	8 (3/4)	20.0	400	80.1	49.0	8.8	95.7	58.2	18.6	147.4
IV (185–233)[h]	8 (3/4)	20.4	400	81.8	50.0	9.0	97.0	64.7	25.7	132.1
V (234–261)	12 (3/4)	26.2	400	105.0	64.2	11.5	97.6	73.2	19.6	94.0
VI (262–303)	24 (3/4)	26.3	400	105.3	64.4	11.5	98.9	89.9	12.9	41.4

[a] Days of operation.
[b] Operational conditions in the VF units: number of pulses per day (days ON/OFF on a week basis).
[c] HLR and SLR were referred to the overall system.
[d] Recirculation flow as percentage of final effluent flow.
[e] Refers to system description in Fig. 1.
[f] SDWW: synthetic domestic wastewater.
[g] SIWW: synthetic industrial wastewater.
[h] At the beginning of period IV the filtering media was modified as indicated in Section 2.1.

SLR, which was in the range of 4–5 g BOD_5/m^2 d and 1.2–1.6 g TN/m^2 d. TN removal rate was 0.6 g/m^2 d. All these values are typical of classical horizontal subsurface flow systems (Vymazal, 2007; Tanner et al., 2012). This system also showed low oxygen content and low oxidation-reduction potential (data not shown), indicating that its performance was representative of HF CWs and that HF systems are adequately simulated by saturated down-flow columns.

3.1.2. Two-step VF + VF system

A simple two-step system constituted of two unsaturated units, i.e., simulating classical VF units (VF + VF) was operated at a 3–4 times higher SLR than that of HF + HF system while showing similar or better pollutant removal efficiencies. Nitrification was clearly enhanced in comparison to the operation of the HF + HF system, but nitrate accumulates in the final effluent limiting TN removal. TN removal ranged from 17% to 63% and appeared to be very variable, depending on pH and SLR conditions. Additional alkalis (0.5 g/L of $NaHCO_3$) had to be added to the feeding in order to maintain the effluent pH of both units above 6, otherwise went down to approximately 4.5 (during period I, Table 2) which impaired both nitrification and denitrification. After pH correction, the system reached a high TN removal of 63% at high SLR (19 g BOD_5/m^2 d and 5.6 g TN/m^2 d during period II), but decreased to 47% when the HLR decreased (12 g BOD_5/m^2 d and 3.6 g TN/m^2 d during period III). TN removal rate varied from 1.7 to 3.5 g TN/m^2 d, which was in the range indicated by Vymazal (2013) for single VF units. The lower TN removal achieved at lower SLR was related to the increase in DO and ORP in both columns which impair denitrification, as indicated by the increase of effluent nitrate while the effluent ammonia remained low (Table 2). These results suggest that the system VF + VF can reach efficient TN removal but only when operated at high SLR which create sufficient anoxic zones in the filtering bed.

Most of two-step VF + VF systems combined saturated downflow and upflow units which create anoxic/anaerobic conditions which are similar to HF CWs and showed low to moderate removal efficiencies (Vymazal, 2013). However, Langergraber et al. (2011) combined two vertical downflow constructed wetlands with saturation of the drainage layer of the first-step VF bed whilst the second VF step remained completely unsaturated. In the first two years of operation, the system reached a nitrogen removal efficiency of 53% at SLRs of 40 g COD/m^2 d and 5.2 g TN/m^2 d. This high performance was similar to that found for our VF + VF system at similar high loading rates (period II, Table 2). In the study of Langergraber et al. (2011) the SLR was maintained high and quite stable (95% confidence interval of 1.0 g COD/m^2 d) and thus there are no data about the effect of low loading rates on TN removal.

Besides, the main find of Langergraber et al. (2011) for the two-step VF + VF system was that nitrogen removal efficiency increased over the time. The median removal rate increased from 2.7 g TN/m^2 d (53% TN removal) for the two first years of operation to 3.8 g TN/m^2 d (above 60% TN removal) for the following three years of operation. Langergraber et al. (2011) pointed out that the vegetation as well as the biofilm development in the two-stage VF CW system played the major role for the enhancement of the nitrogen elimination rate. It is not possible to check this effect in our short-term parallel experiments, but one might expect a general improvement over time of the overall nitrogen removal in all the studied systems if they were planted.

3.1.3. Two-step hybrid VF + HF systems with different area ratios

Effective combination of anoxic and aerobic conditions can be offered by a two-step hybrid VF + HF system. Two different surface area ratios were studied for such a system. The first one, $(VF + HF)_{1:2}$, combined a smaller unsaturated first unit and a larger saturated second unit, giving an AR of 2.0. These characteristic determined a relatively low SLR (7 g BOD_5/m^2 d for the overall system) while TN removal ranged from 41% to 56% (Table 2). On the other hand, the second configuration combining a larger unsaturated first unit and a smaller saturated second unit, $(VF + HF)_{2:1}$, giving an AR of 0.5. This system reached a higher SLR but a lower TN removal efficiency. Surface removal rate of nitrogen was similar in both systems, ranging from 0.9 to 1.2 g TN/m^2 d. This rate is in the range but lower than the mean value (2.3 ± 2.1) reported by Vymazal (2013) for VF + HF hybrid systems.

None of the configurations $(VF + HF)_{2:1}$ and $(VF + HF)_{1:2}$ was able to reach an effluent with an enough low nitrate concentration. Probably, the effluent ammonia concentration (15–25 mg/L, Table 2) could be lowered by applying a lower SLR, but this measure would not contribute to the reduction of effluent nitrate, according to the results of Ávila et al. (2013). These authors reported the efficiency of a hybrid VF + HF + FWS system with an equivalent AR of 0.7 (1.3 (FWS + HF)/VF area ratio) treating domestic wastewater at an average SLR 3.8 g BOD_5/m^2 d and 0.8 g TN/m^2 d for the overall system (Ávila et al., 2013). Mean overall removal rates were as 91% BOD_5, 94% NH_3–N and 46% TN, indicating that the VF bed performed very well in ammonia nitrification (74% NH_3–N removal), but the concentration of nitric nitrogen remained almost invariable along the HF and FWS beds. The later was attributed to the lack of organic matter necessary for denitrification and the authors (Ávila et al., 2013) suggested partial by-pass from the Imhoff tank to the HF unit or applying higher loading rates in order to promote denitrification.

Increasing the relative surface area of the saturated second step, as in the configuration $(VF + HF)_{1:2}$, required a lower SLR whilst its effect on TN removal is unclear. As reviewed by Vymazal (2013), the hybrid VF + HF systems reported in the literature presented mean AR in the range of 0.5–7.6 (mean of 2.7). Thus, it could be expected that increasing the AR leads to a high percentage of TN removal because of the high proportion of saturated media and low oxygenation rates. In addition, the presence of plants could also contribute to the denitrification potential due to the exudation of organic matter (Narváez et al., 2011). However, using a large AR will require large surface areas for the overall hybrid system which would be an undesired approach.

These results show that among all single pass two-step configuration systems, the best results were obtained by two unsaturated VF units in series, which reached a nearly complete nitrification and a moderate TN removal but higher than that of the other configurations. An unsaturated unit was always required in order to promote nitrification, unless a large surface area was available (i.e. applying very low SLRs). Furthermore, when the saturated unit is used as the second step following an unsaturated unit, it showed a reduced denitrification capability, probably due to the lack of ready biodegradable organic matter which always was removed in the first step in a large extension.

3.2. Performance of two-step hybrid systems with by-pass

The two-step configuration combining VF and HF units, $Bp(VF + HF)_{1:2}$ and $Bp(VF + HF)_{2:1}$, were operated at different by-pass ratios ranging from 10% to 50% of fresh influent to the second step, defined as the percentage of the influent flow to the first unit. The results obtained are indicated in Table 3. In both cases, the influent flow to the first unit was maintained constant from period to period, and the by-pass was progressively increased by pumping additional volumes of fresh wastewater directly to the second unit.

The system with the larger unit as saturated second step, $Bp(VF + HF)_{1:2}$, required a by-pass ratio of at least 50% in order to significantly increase the TN removal rate. In these conditions, the overall systems received a SLR of 10 g BOD_5/m^2 d and 3 g TN/m^2 d and reached a 50% of TN removal. On the other hand, the system with the smaller unit as saturated second step, $Bp(VF + HF)_{2:1}$, received a three times higher SLR (30 g BOD_5/m^2 d and 9 g TN/m^2 d) and reached approximately 50% of TN removal at a by-pass ratio of 40%. In both cases, the nitrogen remaining in the effluent appeared in similar amounts in both ammonia and nitrate forms (Table 3). The later suggest that these were the optimized conditions relating SLR for TN removal because an increase of SLR would increase the effluent ammonia concentration whilst a reduction of SLR would lead to more oxygenated conditions thus increasing the effluent nitrate concentration.

The hybrid $Bp(VF + HF)_{2:1}$ system has been subsequently operated with real domestic wastewater (Table 3). The results indicate that the RDWW required a slightly lower by-pass ratio and a significantly lower SLR than the SDWW in order to optimize the TN removal. Working at overall SLR of 16 g BOD_5/m^2·d and 4 g TN/m^2·d, the system reached a TN removal slightly higher than 50%.

Overall, these results indicated that a by-pass ratio in the range of 30%–50% increased the TN removal rate to approximately 50% in the two step VF + HF system. However, too much high ammonia and nitrate concentrations remained in the effluent, indicating the difficulties of reaching an advanced treatment in this kind of system configuration.

3.3. Performance of two-step hybrid systems with recirculation

Two configurations of a two-step system provided of effluent recirculation have been studied (Fig. 1), the R(HF + VF) being selected to treat domestic wastewater and the R(VF + VF) to treat a high strength synthetic substrate simulating food industry wastewater. The influent to each system was SDWW and SIWW, respectively (Table 1). The performance characteristics and removal efficiency of these systems are giving in Table 4. The recirculation ratio was defined as the recirculation flow expressed as the percentage of the final effluent flow.

3.3.1. Two-step hybrid HF + VF system with effluent recirculation

The system R(HF + VF) treating SDWW was operated successively at recirculation ratios of 200%, 300% and 400%, while the SLR was maintained in the range of 6.7–9 g $BOD_5/m^2 \cdot d$ (2.0–2.7 g TN/m^2 d) for the overall system and the feeding regime of the VF unit was set at 8 pulses at day without resting (Periods I–IV in Table 4). In these conditions, TN removal ranged from 71% to 83%, slightly increasing with the increase of the recirculation ratio (R^2 0.812, $p < 0.1$).

These results are very similar to that reported by Tanner et al. (2012) for a three step system (HF + HF + VF) with the same AR of 2.0 and recirculation rate of 400%. Receiving SLR of 7.5 g BOD_5/m^2 d and 2.7 g TN/m^2 d on average, this system reached complete nitrification and 73% of TN removal (Tanner et al., 2012). Good performance was also reported by Vymazal and Kröpfelová (2015) when treating municipal sewage in a hybrid $VF_{saturated}$ + VF + HF with recirculation (100%) from the second to the first step. The system presented a large saturated/unsaturated area ratio 5.5 and operated at overall SLRs of 2.5–5.2 g BOD_5/m^2 d and 0.8–1.6 g TN/m^2 d. Overall removal efficiencies amounted to 88.8% and 79.9% for NH_3-N and TN, respectively indicating suitable conditions for nitrification and denitrification.

However, as indicated in Table 4, the system studied here was capable of treat a higher SLR of up to 27 g BOD_5/m^2 d and 8 g TN/m^2 d when the frequency of pulses was increased up to 16 at day, whilst maintaining the overall TN removal above 78% (Periods V–VII, Table 4).

The R(HF-VF) treating SDWW showed a consistently low ammonia concentration in the final effluent (1–4 mg N/L, Table 4), while the nitrate concentration decreased from 23 mg NO_3^--N/L at a recirculation ratio of 200% to 12–15 mg NO_3^--N/L at a recirculation ratio of 400%. The minimum amount of nitric nitrogen in the final effluent equalled 15% of the influent TN which was close to the non-recirculated effluent of 20% at the a recirculation ratio of 400%. This is because nitrification mainly occurred in the unsaturated second VF unit, whilst denitrification was practically limited the saturated first unit. Thus, the recirculation rate determined the effluent nitrate concentration (R^2 0.63, $p < 0.03$).

It is noticeable the stability of the effluent concentrations and of the high removal efficiencies, instead of the variable operational conditions. Although the pH at the final effluent was low during the start-up (mean values of 5.3 and 5.7 during periods I and II), it was recovered to neutral values and self-controlled during the remaining operational periods. Particulate and organic matter removals were always higher than 94% (TSS), 95% (COD) and 98% (BOD_5). All these parameters as well as ammonia and TN were below the regulation limits for the effluent discharge to natural water bodies. Furthermore, even at 16 pulses a day (period VII), the system showed good hydraulic behaviour, maintaining a high infiltration rate in both the HF and VF columns, thus indicating an operation without clogging risks.

3.3.2. Two-step VF + VF system with effluent recirculation treating high strength wastewater

The system R(VF + VF) treating SIWW was operated at a unique recirculation ratio of 400% whilst the SLR increased from 33 to 64 g BOD_5/m^2 d and 6–11.5 g TN/m^2 d for the overall system (Table 4). The feeding regime of both units was of 3 days ON and 4 days OFF (resting) on a week basis whilst the pulsing frequency varied from 8 to 24 cycles at day.

Operating at a pulsing frequency of 8 cycles at day, the removal efficiency of particulate and organic matter was high but not enough to reduce the final effluent concentration below the discharge standards. In fact, effluent concentrations ranged from 13 to 39 mg TSS/L, 120–202 mg COD/L and from 31 to 75 mg BOD_5/L. TN removal efficiency was low, ranging from 45% to 65% and increased with the increase in SLR (Table 4). Nitric nitrogen accumulated in the effluent at a higher concentration than ammonia nitrogen, indicating that the denitrification intensity was the limiting factor. Results not shown suggest that the control of pH favoured the increase of the nitrification intensity in the second unit, whilst applying a higher SLR led to the increase of the denitrification intensity and finally to a higher TN removal.

During the last two periods (Table 4), the pulsing frequency was increased from 8 to 12 and 24 cycles at day. In these conditions, in spite of a slightly higher SLR, the removal efficiency of particulate matter, organic matter and TN clearly increased, reaching effluent concentrations of TSS, COD and BOD_5 below discharge limits, and reaching up to 90% TN removal. However, during the period at 24 pulses at day, the first unit appeared to be close to it hydraulic limit, as indicated by a drastic reduction in the infiltration rate.

3.4. Comparative assessment of the different two-step system configurations

From his review, Vymazal (2013) concluded that hybrid CWs including a VF stage removed substantially more TN as compared to single HF or VF CWs. Our results are in accordance with this statement as we further discusses in this section. Besides, Vymazal (2013) did not find significant difference in NH_3-N and TN removal among the various types of hybrid systems. On the contrary, our results indicated that design and operational features such as the saturated/unsaturated area ratio, the by-pass rate and effluent recirculation introduce significant differences in both nitrification and denitrification rates and TN removal efficiency. In order to further analyze these differences, Fig. 2 summarizes the removal efficiency and the surface removal rate for selected periods of the two-step system configurations studied. TN removal efficiency ranged from 20% to 82% while the SRR ranged from 0.6 to 8.4 g TN/m^2 d.

3.4.1. Single pass two-step systems

Single pass two-step configurations showed the lower SRR, usually bellow 2 g TN/m^2 d, except for the case of the VF + VF system working at high loading rate, which reached 3.6 g TN/m^2 d. Note that this configuration reached a fairly high removal efficiency (63% TN removal) at high loading rate, but decreasing in both TN percentage removal and SRR at low loading rate. Except in this case and conditions, all the other single pass systems including the hybrid VF + HF systems showed percentage TN removal below the threshold of 50%.

3.4.2. VF + HF systems with by-pass

By-pass VF + HF systems showed to be capable of increasing the removal efficiency to the range of 50%–60% TN removal in a sustainable manner. The system $(Bp(VF + HF)_{1:2})$ with the higher AR reached a low SRR of 1.6 g TN/m^2 d whilst the system $(Bp(VF + HF)_{2:1})$ with the lower AR reached a SRR of 5.2 g TN/m^2 d, both working with synthetic domestic wastewater. When the last system was applied for real domestic wastewater, it slightly improved the removal efficiency but at a lower SRR of 2.5 g TN/m^2 d.

Step-feeding setup has been adopted sometimes in single unit HF and VF CWs with the purpose of effective utilization of wetland area or volume through uniform loading distribution, and avoid rapid clogging by distributing suspended solids and organic loading

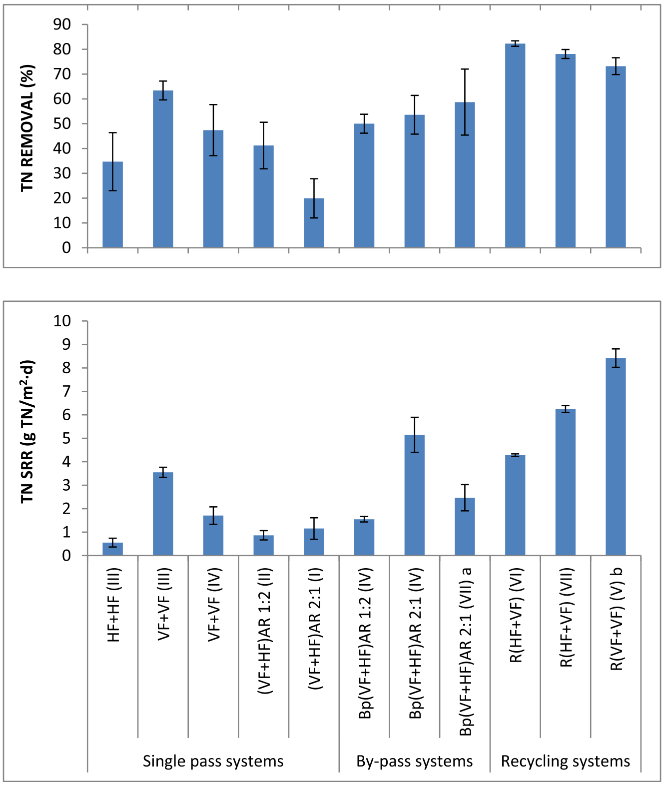

Fig. 2. Removal efficiency (%) and surface removal rate (SRR) for the two-step configurations studied (Selected periods from Tables 2–4; the corresponding period is indicated between brackets. Notes: [a] Treating real domestic wastewater, [b] Treating synthetic industrial wastewater).

in the influent along a greater portion of the wetland (Stefanakis et al., 2011; Wang et al., 2014). However, as pointed out by Wu et al. (2014), few studies valued the role of step-feeding in enhancing denitrification. Li et al. (2014) studied the effect of artificial aeration and step-feeding strategies in a multi-step HF CW which reached high ammonia (99%) and TN (88.1%) removal at low SLR (9.2 g COD/m^2 d and 0.78 g TN/m^2 d). On the other hand, Hu et al. (2012) adopted step-feeding setup in a four stage tidal CW and reported 83% TN removal at 20 g N/m^2 day of loading rate. However, influent by-pass in VF-HF CW system has not yet been studied.

The objective of the by-pass was to increase the COD/TN ratio in the influent to the HF unit. In both systems Bp(VF + HF)$_{1:2}$ and Bp(VF + HF)$_{2:1}$ treating synthetic wastewater, the COD/TN ratio increased from less than 1 (0.3–0.9) when operated without by-pass to about 3.5 at by-pass rates of 40–50%. On the other hand, the COD/TN ratio was 2.7 for the system Bp(VF + HF)$_{2:1}$ treating real domestic wastewater at a bypass of 33%. Thus, TN removal increased with an increase in the COD/TN ratio, but a by-pass of 30–50% provided a COD/TN ratio of about 3–3.5 which appeared to be insufficient for a complete removal of nitrate. These results agree with those of Zhao et al. (2010) who reported that high N removal efficiency only occurred when C/N ratio ranged 2.5–5 in saturated CW. Zhu et al. (2014) reported the greatest removal efficiency of TN at a C/N ratio of 5–7. However, the by-pass is not an adequate measure to further increase the COD/TN ratio in the HF unit of the VF + HF system because higher by-pass rates would also increase the ammonia concentration in the final effluent.

3.4.3. Two-step systems with effluent recirculation

Two-step systems with effluent recirculation significantly improved the TN removal efficiency together with higher SRR. Particularly, the R(HF + VF) system treating synthetic domestic wastewater showed sustainable TN removals of approximately 80% at SLR ranging from 2 to 8 g TN/m^2 d (Table 4). Thus, TN SRR clearly increased with the applied SLR and reached up to 6 g TN/m^2 d (Fig. 2) in sustainable (no clogging) operational conditions.

The higher SRR corresponded to the R(VF + VF) system treating the high strength synthetic industrial wastewater (Fig. 2). This system showed a SRR of 8.4 g TN/m^2 d (73% TN removal) in sustainable (no clogging) operational conditions. However, as indicated in Table 4, the TN removal efficiency of this system decreased when the SLR decreased due to the accumulation of high amounts of nitric nitrogen in the effluent. This behaviour was the same of that observed for the two-step VF + VF without recirculation treating domestic wastewater. Although the R(HF + VF) system for high strength industrial wastewater has not been studied, these results suggest that it could be an interesting alternative because of its capability of offering both anaerobic/anoxic and aerobic conditions at both high and low loading rates.

Recirculation has been proposed in hybrid systems HF + VF to improve nitrogen removal performance, by pumping the nitrified VF effluent to the previous HF step (Foladori et al., 2013; Vymazal, 2013). In other studies, recirculation has been applied in single VF CWs, by returning a fraction of the nitrified VF effluent to the septic tank or the VF inlet where denitrification occurred due to the availability of biodegradable organic matter (Brix and Arias, 2005; Prost-Boucle and Molle, 2012; Vázquez et al., 2013).

Ayaz et al. (2012) reported that a hybrid HF + VF CW with recirculation is an effective method to obtain low nitrogen concentrations in anaerobic pre-treated domestic wastewaters in the warm climate regions. The system (AR of 1.3) received anaerobically pre-treated domestic wastewater which had an average COD/TN ratio of 5.2. Recirculation ratios of 33% (0.25/1), 100% (1/2) and 200% of final effluent to HF influent were subsequently applied. Overall SLR ranged between 7 and 10 g N/m^2 d. TN removal reached 66% at 100% and 200% recirculation, whilst ranges from 19 to 55 without recirculation. According to these results, Vymazal (2013) reported that 100% recirculation was superior to 50% and 200% recirculation in removal of total nitrogen in several CW systems. However, in our system R(HF + VF) treating synthetic domestic wastewater the TN removal efficiency increased as the recirculation rate increased which was explained because more nitrate was returned to the HF unit at higher recirculation rates.

4. Conclusions

The performance of several two-step CW configurations operating under similar design and ambient conditions was studied focussing on nitrogen conversion and removal from synthetic domestic wastewater. The study includes several configurations of single pass systems (HF + HF, VF + VF, VF + HF), the hybrid Bp(VF + HF) arrangement (with feeding by-pass), and the hybrid R(HF + VF) system (with effluent recirculation). Two different AR were tested for the VF + HF and Bp(VF + HF) systems. In addition, the R(VF + VF) system was tested for the treatment of a high strength influent simulating food industry wastewater. The percentage removal of TSS, COD and BOD$_5$ was usually higher than 95% for the synthetic wastewater (or higher than 85% for the real wastewater) in all systems.

Among all single pass two-step systems, the VF + VF system obtained the better results by reaching nearly complete nitrification and a moderate TN removal (1.7–3.5 g TN/m^2 d) but higher than that of the HF + HF (0.6 g TN/m^2 d) and HF + VF (0.9–1.2 g TN/m^2 d) systems. While the single pass systems generally showed TN removal below the threshold of 50%, the VF + VF system can reach up to 63% TN but only when operated at high SLR.

The Bp(VF + HF) system with a by-pass ratio in the range of 40%–50% increased the TN removal rate to approximately 50–60% in a sustainable manner although ammonia and nitrate effluent concentrations remained still high. The TN removal rate depends on the AR value, increasing from 1.6 g TN/m^2 d at AR of 2.0–5.2 g TN/m^2 d at AR of 0.5, both working with synthetic domestic wastewater. On real domestic wastewater the Bp(VF + HF) system with AR of 0.5 and 30% by-pass reached 2.5 g TN/m^2 d removal rate.

Effluent recirculation significantly improved the TN removal efficiency and rate. The R(HF + VF) system treating synthetic domestic wastewater showed sustainable TN removals of approximately 80% at loading rates ranging from 2 to 8 g TN/m^2 d. It is noticeable the stability of this system instead of the variable operational conditions. High TN removal rates (up to 73% TN and 8.4 g TN/m^2 d) were also obtained for the R(VF + VF) system treating the high strength industrial wastewater, although they varied with the applied loading rates.

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