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ORIGINAL PAPER

Clogging reduction and removal of hormone residues with laboratory-scale vertical flow organic-based filter and hybrid wetland

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Abstract A laboratory-scale, intermittently fed, organic-based vertical flow filter was tested as a pre-treatment of high-strength urban wastewater to reduce the risk of clogging in treatment wetlands. At an average hydraulic loading rate of 815 L/m^2 day and average surface loading rates of biological oxygen demand of 458 g/m^2 day, chemical oxygen demand of 594 g/m^2 day and suspended solids of 310 g/m^2 day, the organic-based vertical flow filter achieved removal efficiencies of 48 % of biological oxygen demand, 45 % of chemical oxygen demand, 69 % of suspended solids and 51 % of turbidity. For this unit, removals were significantly correlated with organic surface loading rates but not with hydraulic loading rate. Additionally, the organic-based vertical flow filter removed almost completely the hormone residues studied: estrone, 17β-estradiol, 17β-ethynyl estradiol, diethylstilbestrol, estriol, norethisterone and testosterone, most probably by the combination of adsorption onto the organic substrate and biodegradation. The efficiency of the combined system was remarkable for biological oxygen demand (97 %), chemical oxygen demand (89 %), suspended solids and turbidity (99 %), fecal coliforms and *E. coli* (99.9 %) and fecal enterococci (99 %).

Keywords Hybrid treatment wetland · Clogging · Organic substrate · Hormones

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Introduction

Treatment wetlands (TWs) are one of the most recommended on-site technologies for wastewater depuration for small communities, i.e., those with <2,000 population equivalent (Turon et al. 2009). In TWs, water flow is a key factor which determines the substrate to be used, operation mode and performance of the system. Thus, TWs can be classified as horizontal flow (HF) or vertical flow (VF) and surface or subsurface flow. The horizontal subsurface flow TW, the most commonly used configuration, can achieve high removal efficiency of organic matter and suspended solids (SS) even under strong flow fluctuations. In the VF, the influent is usually intermittently dosed to attain a high degree of oxygenation of the substrate and higher aerobic degradation rates (Cooper 2005; Vymazal 2009; Serrano et al. 2011). Coupling VF with HF has been proposed as a powerful combination to improve performance and minimize water loss by evapo-transpiration in hot-climate countries (Masi and Martinuzzi 2007).

In addition to this, one of the main problems associated with the use of TWs is the clogging of the substrate. In fact, clogging can drastically reduce the life span of these systems, with predictions of their longevity gradually reduced from almost a century to about 10 years (Knowles et al. 2011). Regarding the features of the wastewater to be treated, two basic parameters must be considered: the concentrations of SS and chemical oxygen demand (COD). The effect of the concentration of solids seems obvious as high SS loading rates would lead to faster clogging by means of the physical filling of the substrate pores. It is also known that most solids settle near the inlet zone of TWs (Caselles-Osorio et al. 2007). For high-strength wastewaters, a pre-treatment aimed at reducing the concentration of SS should be employed before their treatment with subsurface flow TWs. However, low SS alone does not prevent clogging as biodegradable organic matter can contribute to develop biofilm clogging (Caselles-Osorio and García 2006). Thus, in addition to SS loading rate, that of COD should also be low enough to control the development of biofilm. Clogging problems in VF seemed to be minimized with COD and SS loading rates below 20 and 5 g/m^2 day, respectively (Winter and Goetz 2003).

Gravel and sand have been used as substrate for TWs. These or any other mineral medium have a fixed "hosting capacity" for solids which is given by their porosity. In fact, only the surface of the gravel is useful from the water treatment viewpoint as it provides room for the biofilm development, while the inner volume of gravel does not provide any treatment improvement. Alternative substrates such as charcoal, slag, peat and compost, for instance, have been tested to achieve higher phosphorus removals (Korkusuz et al. 2005; Koiv et al. 2009) or to increase the removal of phenol (Tee et al. 2009). Compared to mineral substrates (gravel and sand), organic substrates can enhance plant settlement and growth, adsorb more organic pollutants and host a larger amount of SS. Thus, using an organic-based TW previously to a mineral-based TW could prolong the life span of the latter. However, considering the potential advantages of organic-based TWs, the existing literature on their use for wastewater is still scarce.

Endocrine disrupting compounds (EDCs) pose a threat to wildlife and human population because of their ability to disrupt the normal function of their endocrine systems. Steroid hormones belong to the most potent active EDCs present in the environment and can be classified as estrogens, gestagens and androgens, according to their chemical structure and pharmacological effects (Aufartová et al. 2011). Wastewater treatment plants, dairy wastewaters and manure are among the main sources of EDCs to natural streams (Cai et al. 2012; Song et al. 2009). TWs and ponds have shown to be very efficient in the removal of these emerging pollutants from wastewater with efficiencies ranging from 68 % (Song et al. 2009) up to 93 % (Shappell et al. 2007). In these systems, sorption and biodegradation might be the main removal mechanisms of hormone residues (Khanal et al. 2006; Song et al. 2009), the role of sorption being particularly important due to their low volatility and hydrophobic character (Lai et al. 2000).

The main goal of this work was to test the efficiency of an organic-based pre-filter under high hydraulic and organic surface loadings on the removal of SS and organic matter with the goal of reducing the risk of clogging in TWs. Additionally, to determine whether the combination of the organic-based pre-filter with a hybrid TW could satisfactorily treat high-strength urban wastewaters containing hormone residues. The experiments included in this work were performed in the Campus of Tafira (University of Las Palmas de Gran Canaria, Canary Islands, Spain) between November 2009 and May 2011.

Materials and methods

Analysis of the elemental composition of the organic substrate

The organic substrate used in this study was mulch obtained by triturating dry branches of the autochthonous palm tree (*Phoenix canariensis*). The elemental composition of the substrate was determined after sterilization in autoclave, drying (110 °C for a week), trituration and sieving of the mulch. The fraction with particle size between 106 and 250 µm was washed with milli-Q water in a vacuum filtration system to remove color and other impurities. Then, the so-treated material was dried again at 100 °C for 24 h. The elemental analysis was performed with a 1,112, Flash EA Elemental Analyzer.

Analysis of chemical and biological parameters

Chemical water quality parameters (BOD, COD, SS and turbidity) were measured in unfiltered, homogenized samples as described by standard methods (APHA 1998). Biological oxygen demand (BOD_5) can include nitrification as no inhibitor was added. Fecal indicators (fecal coliforms, *E. coli* and fecal enterococci) were analyzed by the membrane filtration method. The agars for fecal coliforms (Chapman TTC-tergitol 7) and fecal enterocci (Slanertz-Barley) were purchased from Panreac (Spain). For the identification and quantification of *E. coli* (purple colonies), a *Chromogenic E. coli/coliform Selective Agar* from Oxoid (UK) was employed.

Determination of hormones

Instrumentation

The HPLC system consisted of a Varian pump fitted with a Varian Autosampler 410 with a volume selector, a column valve module with an internal oven and a Varian photodiode array (PDA) and fluorescence (FL) detectors. The system and the data management were controlled by Star software from Varian (Varian Inc., Madrid, Spain).

LC/MS/MS was performed by a Varian 320-MS TQ reversed-phase liquid chromatography coupled to a triple quadrupole (TQ) mass spectrometer equipped with an electrospray interface (LC/ESI/MS/MS). Chromatographic conditions and characteristics of ESI/MS/MS parameters for each compound studied are described in Vega-Morales et al. (2010).

Reagents and solutions

Hormones selected in the present study include the steroids estrone (E1), 17β-estradiol (E2), 17β-ethynyl estradiol (EE2), estriol (E3), norethisterone (NOR) and testosterone (TET), and the nonsteroid diethylstilbestrol (DES). All of these were purchased from Sigma–Aldrich (Steinheim, Germany). All standards were 98–99 % pure. Individual standard solution of these compounds was prepared in methanol at a concentration of 1,000 mg/L. The solutions were stored at 4 °C and diluted to the required concentrations with pure water prior to use. HPLC-grade methanol was obtained from Panreac (Spain) and glacial acetic acid was obtained from Scharlau Chemie S.A. (Spain). Ultra-high-quality water obtained from a Milli-Q water purification system (Millipore, USA) was used for all experiments and solutions.

Columns, mobile phase and chromatographic conditions

A universal C_{18} (4.6 × 250 mm and 5 μm particle diameter, from Fortis Technologies Ltd.) column with a column guard of the same sorbent was used for separations of the analytes.

Chromatographic separations of the selected analytes were conducted by using high-performance liquid chromatography with UV and fluorescence detectors. Extract samples volumes of 100 μL were injected into the chromatographic system. The mobile-phase composition was optimized to achieve the best separation. Initial mobile phase was water–methanol (55:45 (v/v)) and reached 50:50 (v/v) in a time of 20 min. After that, separation was in isocratic mode until 45 min. Flow rate was 1.0 mL/min. The temperature in the thermostated column compartment was set at 30 ± 0.2 °C.

E1, E2, E3 and EE2 were detected by fluorescence, with excitation wave number of 228 nm and emission wavelength of 315 nm. Different wavelengths were used to monitor NOR (246 nm), TET (260 nm) and DES (263 nm) in DAD detector.

In-tube solid-phase microextraction

The configuration used for in-tube SPME has been the one published by Aufartová et al. (2011). The capillary column was placed directly behind the injection needle and in front of the injection valve of the autosampler. Capillary connections were facilitated by the use of a 4 cm × 1/16 in sleeve. Polyetheretherketone (PEEK) tubing was placed at each end of the capillary. Stainless steel nuts, ferrules and connectors were used to complete the connections.

To carry out the preliminary experiences of in-tube SPME, two different capillary columns were used: Supel-Q^{TM} PLOT fused-silica capillary column (30 m × 0.32 mm i.d., Supelco, Boston, USA) and Carboxen^{TM} 1006 PLOT fused-silica capillary column (30 m × 0.32 mm i.d., Supelco, Boston, MA, USA).

The autosampler software was programmed to control the in-tube SPME extraction, desorption and injection. Vials (2 mL) were filled with 1 mL of standard solution or sample for extraction. Additional vials (2 mL) containing 1.5 mL of methanol were used for desorption of the target compounds.

The extraction of the analytes onto the capillary coating (by aspirating the sample into the injection loop) was performed by 40 repeated draw/eject cycles of 100 μL of the samples using 60 cm of Supel-Q capillary column at a flow rate of 0.31 mL/min. The extracted compounds were desorbed from the capillary coating by 50 μL of methanol, transported directly to the LC column and detected by PDA and fluorescence detectors. The capillary column and injection needle were washed in one step and conditioned by 10 repeated draw/eject cycles of 90 μL of methanol between each sample extraction. In all cases, a section of 60 cm of capillary column was used.

Sampling and sample preparation

Samples were collected in pre-cleaned amber glass bottles, filtered through a 0.45-μm filter, acidified to a pH <3 and stored at 4 °C until extraction. They were introduced in the chromatograph and subjected to the optimized in-tube SPME method.

Description of the wastewater treatment system

The system employed in this study (Fig. 1) was a mesocosm hybrid (vertical–horizontal–vertical flow) TW designed for the treatment of raw wastewater from the Campus. Following the water flow, it consisted of:

– An organic-based vertical filter (OVF). This unit was a 70-L cylindrical plastic recipient with a surface area of 0.1 m^2 (diameter: 36 cm, height: 70 cm) containing palm tree mulch as substrate. Inside the OVF, two void spaces consisting of two vertical perforated tubes were prepared. The first tube was used as an accumulation zone where the larger particles would be retained and the second one was used to favor the effluent evacuation and inspection (Fig. 1). The elemental analysis of the organic substrate revealed a high content of carbon (41.57 %), low contents of sulfur (0.37 %) and nitrogen (2.55 %). The concentration of hydrogen was 4.52 % and that of oxygen (50.99 %) was obtained by subtraction from the total and the summation of the other elements. The porosity of the wet substrate was determined to be 56 %.

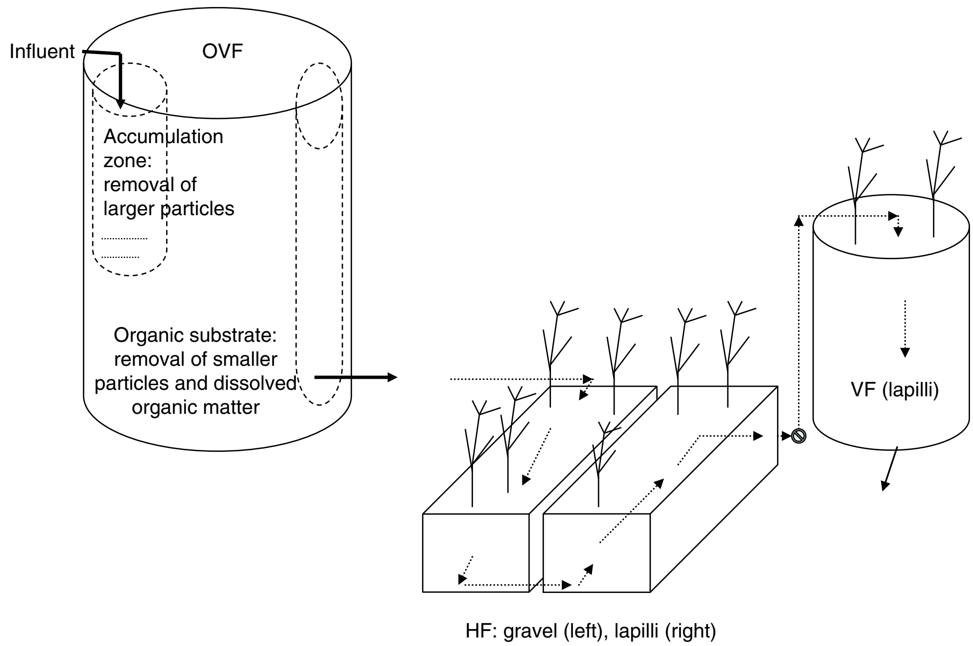

Fig. 1 Scheme of the system employed (not to scale) and water flow. Note that the OVF dimensions have been enlarged to show details

- The subsurface horizontal flow TW (HF) was built with two rectangular recipients (length: 125 cm, width: 57 cm, height: 56 cm) made of polypropylene (Pandora, Italy). The total volume of each recipient was 265 L, the net volume was 110 L and the surface area was 0.67 m^2. The first recipient contained gravel (99.8 % gravel, 0.1 % sand) consisting of locally available triturated basaltic rock with a porosity of 49 %. The average particle diameter was 6.5 mm and the values of d_{10}–d_{60} were 4.11 and 5.51 mm, respectively. The second recipient contained volcanic lapilli (gravel: 68.9 %, sand: 30.9 % and mud: 0.2 %) with a porosity of 54 %. The average particle size was 2.7 mm, and the d_{10}–d_{60} were 1.09 and 2.53 mm, respectively. The substrates had a depth of 37 cm, and both reactors were planted with common reed (*Phragmintes australis*).
- The VF was a 250-L cylindrical recipient with a surface area of 0.26 m^2. The substrate was lapilli with a depth of 80 cm and was also planted with common reed. The HF effluent was continuously pumped with a peristaltic pump into the VF.

The HF and VF had been used in previous experiments since November 2007 but with a different tank arrangement (Herrera-Melián et al. 2010). The hybrid TW of this work was set in October 2009 to treat raw wastewater from the Campus. However, the concentrations of organic matter and SS of the influent were very high, and clogging symptoms, i.e., ponding, were soon observed in the HF. Thus, the OVF as described above was introduced in November 2009 with the main goal of examining its efficiency at reducing SS as the most important cause of clogging in TWs.

Hydraulic regime

The wastewater used in these experiments was collected from a 17-m^3 tank which receives raw wastewater from a part of the Campus. A dilacerating pump controlled with a timer is located at the bottom of the tank. The timer was programmed for the pump to work for 3 min every 3 h with the goal to alternate fill-and-dry periods, particularly for the OVF. Under these experimental conditions and because of the high porosity of the organic substrate, dissolved oxygen supply to the substrate would be granted, minimizing anaerobiosis and the consequent greenhouse gas emissions (Maltais-Landry et al. 2009). Additionally, aerobic conditions speed up mineralization of the organic matter that clogs the substrate. Hence, intermittent dosage of the influent has been recommended to reduce the risk of clogging in TW (Knowles et al. 2011).

The HF effluent was continuously pumped with a peristaltic pump into the VF. The average daily inflow was measured by collecting the treated water in graduated recipients.

Statistics

The statistic treatment of the data was performed with the free program R-Commander which is developed under R environment and allows the most usual statistic tests to be performed. The initial treatment consisted of detecting outliers by means of a box-plot. Once the outliers were conveniently removed, the average concentrations of each parameter (BOD, COD, SS, turbidity and fecal indicators) at the successive sampling points (influent-OVF effluent, OVF effluent-HF effluent and HF effluent-VF effluent) were compared to determine whether they were statistically different. A one-way ANOVA was usually employed after determining that the data were normally distributed by means of the Shapiro–Wilk test and homoscedastic with the Bartlett test. If the data were not normally distributed, the Shapiro–Wilk test was applied to their logarithmic values. If this did not work, the nonparametric Kruskal–Wallis test was used to determine significant differences. The significance level used was 0.05, i.e., if p value >0.05, the null hypothesis of no difference between means was accepted. Regarding correlation, the Pearson test was used if the data were homoscedastic and normally distributed. Otherwise, the Spearman's rank correlation test was employed. Correlation was accepted as significant when p value <0.01.

Results and discussion

The comparison of the average concentrations of BOD, COD, SS, turbidity and fecal indicators among successive sampling points showed that they were significantly different with p values ranging between 0.009 and 1.7×10^{-13}.

Characteristics of the influent

The influent was raw wastewater generated in the buildings (laboratories, cafeterias, toilets …) of the Campus of Tafira

Table 1 Concentrations of the influent (average ± standard deviation, n of data), concentrations (± standard deviation) of the effluents, SLR and removals

Parameter	Influent Average concentration ± standard deviation, number of data	OVF Effluent conc. SLR Removal	HF Effluent conc. SLR Removal	VF Effluent conc. SLR Removal	Global Removal
BOD, mg O_2/L	579 ± 378, n: 28	281 ± 119	99 ± 59	16 ± 15	–
		534 ± 490	18 ± 10	35 ± 22	–
		48	62	84	97
COD, mg O_2/L	923 ± 568, n: 33	455 ± 213	134 ± 56	87 ± 32	–
		757 ± 555	28 ± 16	47 ± 31	–
		45	66	31	89
SS, mg/L	627 ± 913, n: 34	112 ± 75	14 ± 10	4 ± 3	–
		618 ± 1,086	46 ± 82	39 ± 31	–
		69	84	68	99
Turbidity, NTU	303 ± 210, n: 38	120 ± 76	11 ± 7	3 ± 2	–
		$2.5 (\pm 2.2) \times 10^5$	$7.2 (\pm 5.2) \times 10^3$	$3.5 (\pm 2.7) \times 10^3$	–
		51	89	69	99
pH	7.13 ± 0.46, n: 11	7.07 ± 0.25	7.07 ± 0.13	6.7 ± 0.22	–
		–	–	–	–
		–	–	–	–
EC, µS/cm	1,504 ± 418, n: 11	1,556 ± 380	1,623 ± 289	1,588 ± 299	–
		–	–	–	–
		–	–	–	–
E. coli,		–	$3(\pm 3.9) \times 10^5$	$4.1(\pm 5.9) \times 10^3$	
CFU/100 mL	$9.9 (\pm 14) \times 10^6$, n: 11	$7.2 (\pm 9.9) \times 10^{10}$	$5.4 (\pm 9.3) \times 10^9$	$3.3 (\pm 3.6) \times 10^8$	
		–	95	95	99.9
Fecal coliforms, CFU/100 mL	$2.1 (\pm 2.3) \times 10^7$, n: 23	–	$4.9(\pm 5.1) \times 10^5$	$2.6(\pm 3.3) \times 10^4$	
		$8.1 (\pm 7.8) \times 10^{10}$	$6.0 (\pm 5.9) \times 10^9$	$1.5 (\pm 1.7) \times 10^9$	
		–	92	87	99.9
Fecal enterococci, CFU/100 mL	$1.7 (\pm 2) \times 10^6$, n: 32	–	$1(\pm 1.2) \times 10^5$	$1.7(\pm 2.7) \times 10^4$	
		$6.8 (\pm 4.) \times 10^9$	$5.1 (\pm 3.3) \times 10^8$	$3.3 (\pm 3.6) \times 10^8$	
		–	88.6	85	99

(Gran Canaria, Canary Islands, Spain). The main features of the wastewater used in this study are provided in Table 1.

The high concentrations of the influent were caused by the accumulation of sludge in the 17-m^3 tank where the pump is located. This pump pit is emptied and cleaned every 5–7 years, but the experiments reported in this work were performed before cleaning to achieve highly concentrated wastewaters as those from small communities in Spain (Salas et al. 2006). According to the characteristics of the three standardized types of wastewater (high loaded, moderate loaded and low loaded) (Metcalf and Eddy 2004; Molinos-Senante et al. 2012), the influent can be considered to be high loaded for BOD (>450 mg/L), between high (>1,250 mg/L) and moderate loaded (>750 mg/L) for COD and high loaded (>350 mg/L) for SS.

Surface loading rates and hydraulic retention time

The average daily inflow was 79 ± 29 L/day (*n*: 77). The resulting surface hydraulic loading rates (HLR) for each unit were 861 ± 158 L/m^2 day for the OVF, 64 ± 12 L/m^2 day for the HF and 344 ± 85 L/m^2 day for the VF. As can be observed in Table 1, the surface loading rates (SLR) applied to the OVF were remarkably high in comparison to those applied to the other units in this study and those found in the literature. Additionally, the high values of the standard deviation show the high variability of the influent concentrations. In the particular case of SS for the influent of the OVF, the standard deviation is greater than the average of the concentrations which varied between 59 and 4,850 mg/L. As indicated above, one of the goals of this study was to determine the OVF performance at high and varying hydraulic and organic loadings in a similar way to those found in small communities.

The hydraulic retention time (HRT) of the OVF can be considered to be equal or lower than 3 h as it was fed every 3 h, and we could observe that just before each dose, the unit had been completely drained. The nominal HRT of the HF was 2.8 days while that of the VF was 1.42 h. For the VF, a NaCl pulse trace was performed to determine the experimental or average HRT. The obtained value was 87 min. and the tracer recovery was 115 %. Regarding the validity of the experiments, Kadlec and Wallace (2009) refer to mass recoveries of 80–120 % as indicators of successful wetland hydraulic tracer studies.

Performance of the OVF

The main goal for the OVF was to achieve a substantial reduction in the influent concentration of SS, which is the first cause of clogging in TWs. Nowadays, this is usually done with Imhoff and septic tanks and more recently with UASB reactors (de la Varga et al. 2013), but these anaerobic systems require large volumes and generate methane, a greenhouse gas that reduces their environmental sustainability. In consequence, the selected system should be aerobic or at least minimize anaerobic conditions, perform well under high organic loadings without clogging and offer similar or better efficiencies to the already existing Imhoff and septic tanks. After several preliminary experiments with gravel, organic substrate and their mixtures, the selected system was an organic-based vertical flow filter.

The organic substrate was prepared from a forest waste and consisted of triturated, dry palm tree (*Phoenix canariensis*) branches. This material is renewable, locally available and very abundant in the Canary Islands and has been introduced as an ornamental plant in many places around the world (Nehdi et al. 2010). The dried branches fall from the palm, accumulate around the tree and increase the risk of fire, particularly in locations with long, dry summers.

The OVF was in operation from December 2009 to May 2011. The average HLR applied to this unit was 861 L/m^2 day and those of BOD, COD, SS and turbidity were 534 g/m^2 day, 757 g/m^2 day, 618 g/m^2 day and 2.5 × 10^5 NTU × L/m^2 day, respectively (Table 1). Such loading rates were remarkably high if compared to other studies using VF. For instance, Ye et al. (2012) employed HLRs of 300 and 500 L/m^2 day in studies on the oxygenation of VFs. Molle et al. (2008) applied HLRs in the range 380–510 L/m^2 day, while those of COD and SS were 167–180 and 53–64 g/m^2 day, respectively, when studying nitrogen removal in a full-scale hybrid TW.

Though the high loads applied and short HRT estimated (<3 h), the obtained average removals for the OVF were remarkable, being 48 % for BOD, 45 % for COD, 51 % for turbidity and 69 % for SS (Table 1). These results are comparable to those obtained with anaerobic reactors such as the UASB, which is an improved version of the conventional septic tank, but with remarkably lower HRT in the present case. For example, Al-Jamal and Mahmoud (2009) studied the efficiency of two UASB-septic tanks for the treatment of high-strength wastewaters in Palestine during wintertime (17.3 °C). The average total COD concentration was 905 mg/L, similar to that of this work. Average removals for total COD, BOD and SS for both systems were 51–54, 45–49 and 74–78 % with HRT of 2 and 4 days, respectively. After almost 1 year of continuous operation and monitoring, Sabry (2010) achieved removals of 84 % for COD, 81 % for BOD and 89 % for SS with an up-flow septic tank/baffled reactor (USBR) with an average retention time of 20 h in rural Egypt. The author compared these results with a conventional septic tank/anaerobic filter unit that provided removals of 52.1, 56, and 53.6 % for COD, BOD, and SS removals, respectively, at an average retention time of 22.5 h (Panswad and Komolmethee 1997). The low TRH (<3 h) of the OVF in comparison with those of the anaerobic pre-treatment reactors (8 h to 4 days) is an advantage of the former, as lower TRH implies lower reactor volume to achieve similar efficiencies. Another potential advantage of the OVF over the anaerobic reactors is the fact that the influent became remarkably deodorized after passing through the reactor. This could be attributable to the fact that the organic material of the mulch could act as an odor adsorbent and suggests that the OVF was well aerated and aerobic conditions prevailed.

Nevertheless, the OVF presented two drawbacks. One of them was that mainly during the first week of operation, the organic substrate released a brownish color to the effluent. This was most probably caused by the partial leaching of lignin from the organic substrate (Namasivayam et al. 1998). After this time, coloring was progressively reduced and after 2–3 weeks had completely ceased. This suggests the adequacy of cleaning the organic substrate before use, as recommended for gravel and other mineral substrates. The second drawback was that because of the extremely high surface loadings applied to the OVF, the sludge accumulation zone had to be emptied every 2–3 months, leading to increased maintenance. However, accumulated sludge must also be periodically removed from anaerobic reactors. The retained SS tended to accumulate as a surface layer of sludge and did not penetrate deeply into the organic substrate. This helped the removal of the accumulated sludge by simply replacing the perforated tube located in the accumulation zone and avoided having to change all the mulch. Figure 2 illustrates the surface loading rates removed versus surface loading rates of SS for the OVF. The dotted line ($x = y$) indicates maximum removal. Similar results were obtained for BOD, COD, SS and turbidity and are not shown for clarity.

The results shown in Fig. 2 reveal a correlation between surface loading rates and surface loading rate removed, indicating an accurate predictability of the amount that could be removed by this reactor. In the particular case of SS, the proximity of most of the values to the $x = y$ line, i.e., maximum achievable removal indicate a remarkably high and stable performance even under the high hydraulic and organic loadings applied.

Removal of BOD, COD, SS and turbidity was positively correlated with organic surface loading rate and influent concentration but not with HLR. Figures 3 and 4 show the obtained correlation of BOD removal with surface loading rate and HLR, respectively. Removals of BOD, COD and turbidity ranged from 30 to 40 % at the lowest loadings and 70–80 % at the highest ones. Better values were obtained for SS, for which the lowest removals were around 40–50 % for the lowest loads and 80–90 % for those with

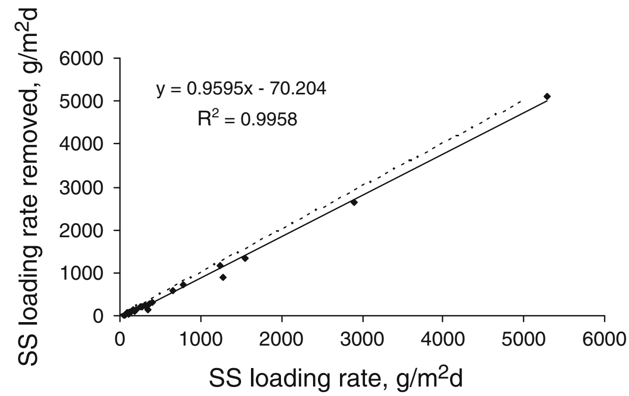

Fig. 2 Surface loading rate removed versus loading rate of SS for the OVF. The *dotted line* ($x = y$) indicates maximum removal

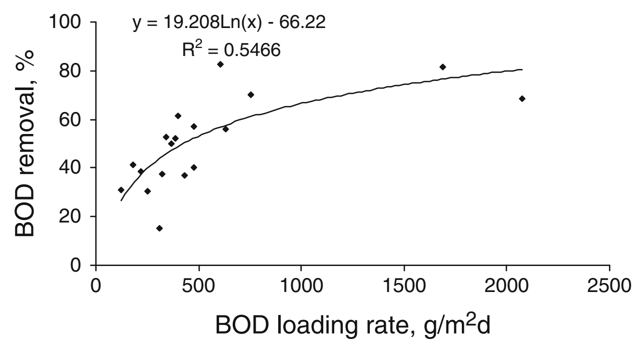

Fig. 3 Removal (%) of BOD versus surface loading rate for the OVF

the highest loads. The logarithmic fit of the data provided the best correlation with R^2 values ranging between 0.358 and 0.55. The fact that the best fit was logarithmic seems to indicate that although removal was stronger for the higher loads there is an upper limit (about 80 %) that cannot be surpassed, indicating the boundaries of the efficiency of the reactor. However, these performance results can be improved by incrementing the OVF depth used in this study (70 cm) to achieve longer HRTs for this reactor. Correlation between influent concentration and removal was significant for all the parameters studied (p values <0.003).

No correlation was found between HLR and removal (Fig. 4) as the values of the R^2 obtained were very low in all cases: BOD (R^2: 0.138), COD (R^2: 0.0022), SS (R^2: 0.0419) and turbidity (R^2: 0.0092). This and the fact that good correlations were found for removal versus influent concentrations suggest that the highest efficiencies were obtained by the OVF with highly concentrated influent independently from the HLR but within the limits studied in this work. Thus, the OVF seems to be suitable for the pre-treatment of high-strength wastewaters such as those from small communities which are characterized by high

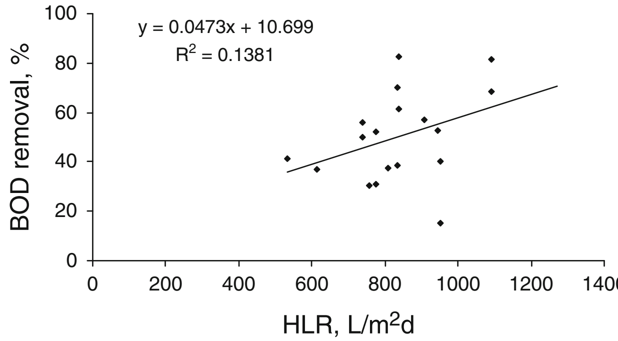

Fig. 4 Removal (%) of BOD versus hydraulic loading rate for the OVF

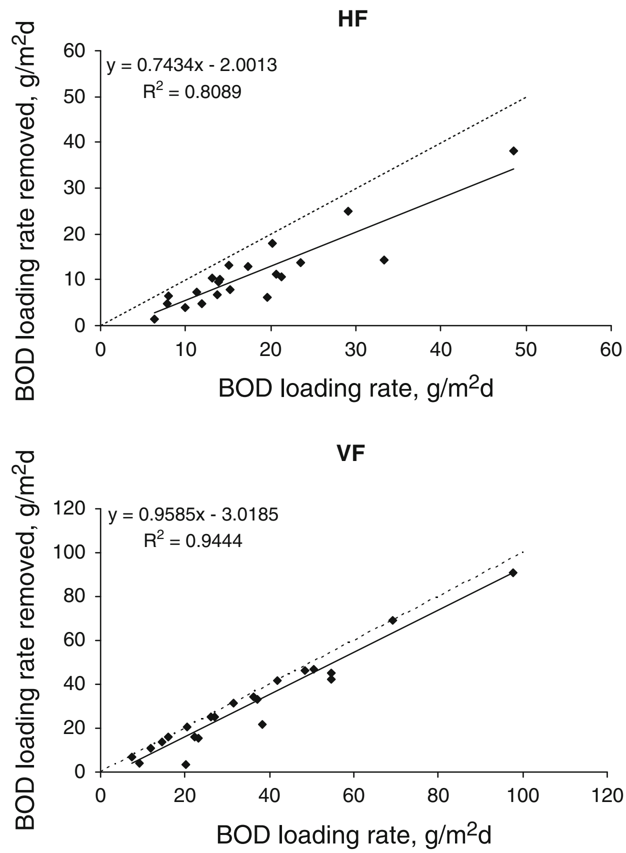

Fig. 5 BOD surface loadings removed versus organic surface loadings for the HF and VF

concentrations and high variability in production to reduce the risk of clogging of TWs.

Considering the average BOD loading rate applied to the OVF (534 g/m^2 day, Table 1), the surface used for this unit (0.11 m^2/he) was remarkably reduced. Additionally, the corresponding surface required for the subsequent TW would be also diminished. Barros et al. (2008) considered that the required area for a TW processing the effluent of a UASB can be reduced by the same percentage of the BOD percentage reduction achieved by the pre-treatment method. Thus, according to the results of this study (Table 1), the subsequent TW area could be reduced by 48 % in which the BOD removal achieved. Additionally, these authors considered this approach as conservative because if the average SS removal is taken into account (69 % in the present study) the possible surface area reduction would be greater.

Removal efficiency of the HF and VF

The overall efficiencies achieved by the complete system were particularly high as the least effective removal was that of COD (89 %), lower than those of BOD (97 %), SS (99 %) and turbidity (99 %). As indicated above, Table 1 provides the concentrations of the effluent and the removals achieved for the HF, VF and the complete system. Note that the removals provided in this work are the average of removals calculated for each reactor and might not coincide with removals calculated from the concentration averages presented on Table 1.

Efficiency of the HF In this work, the goal of placing the HF before the VF was to protect the latter from clogging, by minimizing the SS load received by the VF as suggested by Masi and Martinuzzi (2007). The average surface hydraulic load on the HF was 64 L/m^2 day, while those of BOD, COD and SS were 18, 28 and 46 g/m^2 day, respectively (Table 1). The recommended BOD loading rate for horizontal subsurface flow TWs for several authors is 4–6 g/m^2 day (Pedescoll et al. 2011), thus it can be considered that the values applied in this study were remarkably high. Nevertheless, no sign of clogging was observed in this unit during the study period. In fact, the HF is still in operation at the time of writing this paper without any clogging symptoms. The average removals achieved by the HF in this study were 62, 66, 84 and 89 % for BOD, COD, SS and turbidity, respectively (Table 1). Despite of the high load of the influent, removals achieved by the OVF and HF were high enough to greatly reduce the risk of clogging in the VF.

Correlations of surface organic loading with removal, surface organic loading removed and HLR were studied for the HF and VF. Similarly to that observed with the OVF, no correlation (R^2 <0.1) was observed for the HF and VF between removal and HLR for any of the parameters analyzed. However, removal was positively correlated with surface loading rate for the OVF (Fig. 3) but not for the HF and VF (data not shown) with the exception of that of turbidity for the HF which was positively and significantly correlated (log fit) with HLR (R^2: 0.2683, $p < 0.01$) and with surface loading rate (R^2: 0.3858, $p < 0.01$).

Figure 5 shows the efficiency of the HF and VF in terms of surface organic loading removed versus surface organic loading for BOD. As for the OVF, good correlations were obtained in all cases indicating high predictability of the behavior of the HF and VF. In the case of the VF for SS and turbidity (data not shown), most points are located quite close to the $x = y$ line, indicating that the reactor was performing at the highest levels possible for these parameters.

The European and national limits for organic matter and SS concentrations in treated wastewaters intended to be discarded into the environment are set at BOD (25 mg/L), COD (125 mg/L) and SS (35 mg/L) by the EU Directive 91/271 (Council of the European Union 1991) and the Spanish Royal Decree 509/1996 (RD 509). In Spain, the reuse of the treated wastewater is legally regulated by Royal Decree 1620/2007 which considers four basic parameters for water reuse: turbidity, SS, *E. coli*, and intestinal nematode eggs.

The average BOD concentration of the HF effluent was 99 mg/L (Table 1), still too high if the limit of 25 mg/L is taken as a reference, while that of COD (134 mg/L) was quite close to the permitted maximum. The average concentration of SS (14 mg/L) of the HF effluent is well below the maximum permitted value of 35 mg/L for discarding the treated water to the environment and close to the second most stringent limit of SS (10 mg/L) used for various reuses included in the RD 1620 such as ponds, ornamental water bodies and circulating water without public access, irrigation of forests and green areas with no public access and silviculture.

At the average BOD loading rate applied to the HF (18 g/m^2 day, Table 1), the surface used for this unit was 3.3 m^2/he, a reduced value considering the high concentrations of BOD of the influent and the high BOD loading rate applied to this unit.

Efficiency of the VF The average HLR applied to the VF was 344 L/m^2 day while those of BOD, COD and SS were 35, 47 and 39 g/m^2 day, respectively (Table 1). For this unit, the obtained average removals for BOD, COD, SS and turbidity were 84, 31, 68 and 69 %, respectively (Table 1). With the exception of COD, the obtained removals can be considered to be quite high considering the short HRT determined (87 min) for this unit.

Although the VF was continuously fed with the HF effluent, its high performance can be attributed at least partially to the fact that the substrate was not completely saturated and it contained enough oxygen to provide an oxidizing environment (Kadlec and Wallace 2009). In this sense, Pedescoll et al. (2011) observed that the COD removal in shallow subsurface flow HFs could be improved if operated with filling–resting–drain phases which allowed a better aeration of the substrate.

Figure 6 shows the concentrations of BOD, COD, SS and turbidity of the VF effluent versus the concentrations of the OVF influent. Dotted lines show the reference concentrations of BOD (25 mg/L) and COD (125 mg/L) for the effluent according to the EU Directive 91/271 and the RD 509. Regarding SS and turbidity, the RD 1620 for treated wastewater reuse was used as reference. For SS, the

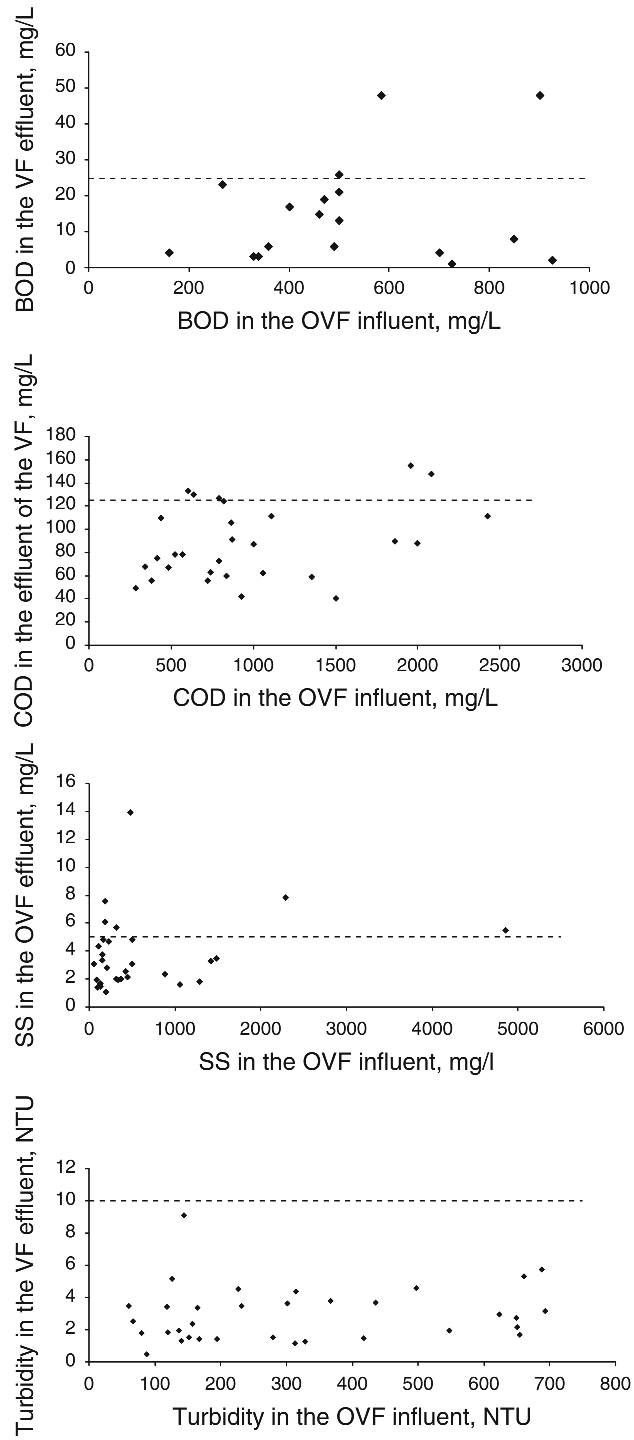

Fig. 6 Concentrations of BOD, COD, SS and turbidity of the VF effluent versus the concentrations of the OVF influent. *Dotted lines* show the legal limits taken as reference

lowest limit was selected (5 mg/L), and for turbidity (10 NTU), the value that allowed most uses.

As Fig. 6 illustrates, most of the concentrations measured in the VF effluent were below the legal references considered. The average effluent concentrations of BOD (16 mg/L), COD (87 mg/L) and SS (4 mg/L) obtained by the system are below the European limits (BOD: 25 mg/L, COD: 125 mg/L and SS: 35 mg/L). Note that the inclusion of the VF allowed meeting the standard of 25 mg/L for BOD as the HF alone could not do it. Regarding the reuse of treated water, the low average concentration of SS in the effluent of the VF (4 mg/L) is below the most stringent limit for this parameter (industrial use, quality 3.2, refrigeration towers and evaporative condensers: 5 mg/L) included in the RD 1620. In the case of turbidity, the inclusion of the VF reduced the HF effluent value down to 3 NTU (Table 1), well below the limit of 10 NTU considered for various uses of the treated wastewater according to the mentioned law.

Considering that the average BOD loading rate applied to the VF was 35 g/m^2 day (Table 1), the surface area used with this unit was 1.7 and 2.2 m^2/he for the complete system (OVF + HF + VF). Nevertheless, improving the OVF performance for instance by incrementing its depth would also increment the HRT and eventually would result in reducing the necessary surface area for the system.

Removal of fecal indicators

Many different variables (temperature, pH, presence of plants or UV radiation) and mechanisms (predation, natural die off, sedimentation or adsorption) can help to remove fecal bacteria in TWs. Boutilier et al. (2009) found that inactivation rather than sedimentation or adsorption, was the main removal mechanism for *E. coli* in a surface flow HF. Removal efficiencies of fecal indicator bacteria in TWs has been reported in the literature to range from 52 to >99.9 % (Boutilier et al. 2009). In dry weather regions, treated wastewater reuse is of paramount importance. Hence, achieving a high level of pathogen removal should be one of the most important goals of treatment.

Removal of fecal coliforms and E. coli The concentration of fecal coliforms (FC) was measured from March 2010 to May 2011 (Fig. 7). The average concentration in the influent was 2.1 × 10^7 CFU/100 mL and in the HF effluent was 4.9x10^5 CFU/100 mL (Table 1), 92 % being the removal achieved by this unit. The VF effluent average concentration was 2.6 × 10^4 CFU/100 mL with a removal of 87 %. The overall removal achieved by the hybrid TW (OVF, HF and VF) was 99.9 %. Figure 7 shows the concentrations of *E. coli* and FC in the influent of the OVF and effluent of the VF.

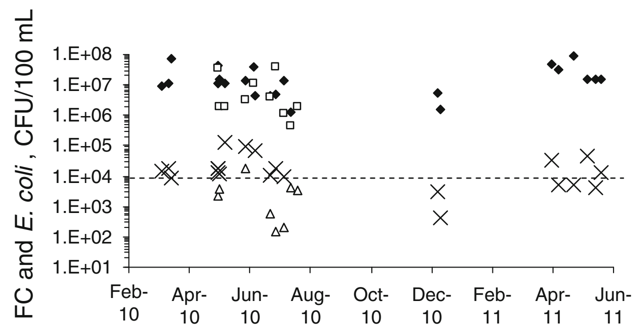

Fig. 7 Concentrations (log scale) of *E. coli* (influent: *square*, effluent: *triangle*) and FC (influent: *diamond*, effluent: X) in the influent of the OVF and effluent of the VF. The *dotted line* indicates the limit of *E. coli* (10^4 CFU/100 mL) for irrigating industrial crops and fruit trees without contact between water and fruit (quality 2.3 RD 1620)

E. coli is considered to be one of the best fecal contamination indicators because of its high concentration in the human digestive tract and its absence in other environments (Molleda et al. 2008). The concentration of *E. coli* was measured during a short sampling campaign between May and July 2010 (Fig. 7). The average concentration in the influent was 9.9 × 10^6 CFU/100 mL while in the HF effluent, it was 3 × 10^5 CFU/100 mL. Hence, the HF achieved a removal of 94.6 %. The concentration in the VF effluent was reduced down to 4 × 10^3 CFU/100 mL yielding this unit a removal of 94.9 %. The overall *E. coli* removal achieved by the complete system was 99.9 %. According to our results, about 52 % of the FC measured were *E. coli*. The RD 1620 uses *E. coli* as a reference parameter to determine the possible reuse (urban, agriculture, industrial, recreational and environmental) of treated wastewaters. In the case of agricultural reuse, the maximum permitted concentration of *E. coli* varies between 2 × 10^2 CFU/100 mL for crops, for which contact between water and the edible part of the product aimed at human consumption in fresh is allowed (quality 2.1) up to 10^4 CFU/100 mL for irrigating industrial crops and fruit trees without contact between water and fruit (quality 2.3). Although the number of results for *E. coli* was scarce, it can be observed in Fig. 7 that most samples (7 out of 8) were below the limit of quality 2.3. In spite of the good disinfection results obtained, this is still an aspect to be improved with TWs. In our case, longer HRTs, i.e., deeper VF, should be studied to achieve the required levels for agricultural reuse.

Removal of fecal enterococci The use of fecal enterococci (FE) has been proposed as an alternative to coliforms because of their greater resistance and inability to grow in environments such as soil or water (Molleda et al. 2008). The concentration of FE was determined from November

2009 to May 2011. The average concentration of FE in the influent was 1.7×10^6 CFU/100 mL while that of the HF effluent was 1×10^5 CFU/100 mL. This unit achieved a removal of 88.6 %. The concentration in the VF effluent was reduced down to 1.7×10^4 CFU/100 mL, i.e., an 85 % removal. For the hybrid TW, FE removal was 99 %, which is clearly lower than those achieved for coliforms and *E. coli*, most probably because of the higher resistance of FE.

The HF and VF were treating different waters and cannot be fairly compared, but it must be underlined that they achieved similar removals even though the nominal HRT of the VF (1.42 h) was remarkably shorter than that of the HF (2.8 days). The higher relative efficiency of the VF could be explained, among other reasons by the higher concentrations of dissolved oxygen and possibly higher number of active protozoa (Ausland et al. 2002) in its substrate.

Removal of hormones

The hormones studied in this work were sexual hormones, both natural and synthetic. Among the steroid ones, several female hormones were analyzed: estrone (E1, natural), 17β-estradriol (E2, natural), estriol (E3, natural), and 17β-ethynyl estradiol (EE2, synthetic); one androgen: testosterone (TET, natural) and a progestagene: norethisterone (NOR, synthetic). Only one nonsteroid hormone, diethylestylbestrol (DES, synthetic) was analyzed.

The system was sampled seven times at four sampling points (Fig. 1): inflow of the OVF, outflow of the OVF, outflow of the HF and outflow of the VF, at 12 h during April and May 2011, and other two times in July 2012 and September 2013.

The concentrations found in 2011 in the influent (Table 2) were notably variable and higher than those reported in the literature for standard urban wastewaters (Hamid and Eskicioglu 2012). However, Froehner et al. (2011) found average concentrations of E1, 17α-ethynyl estradiol and E2 of 1.5 μg/L in the influent of different wastewater treatment plants in Brazil and claimed that these concentration levels coincided with those found by other authors. Nevertheless, to confirm that the concentrations levels determined with PDA and fluorescence detectors were correct the samples of May 24, 2011 and September 2013 were analyzed by a different analyst with a LC/MS/MS. As can be observed, also in this case, the concentration level of the hormones detected in the influent was μg/L. These levels are higher than those found in the literature for sewage treatment plants although these results are in agreement with the high concentrations of the other parameters measured, particularly BOD_5, COD and turbidity. Such high concentrations can be attributed to the low dilution of the wastewater generated in the Campus due to the almost complete lack of rain during the whole year. In a Campus, toilets are frequently used, but the volume of other less contaminated wastewaters such as those of laundries or showers which would dilute the final wastewater is much lower. Additionally, the presence of a small farm within the limits of the Campus may increase the concentration of hormone residues in wastewater since estrogenic hormones are frequently administered to livestock as growth promoters although limited data are available with regard to the daily excretion rates of estrogens from various animal types (Khanal et al. 2006). In addition to this, as stated above the influent was pumped from a pump pit tank in which SS have been progressively concentrated under anaerobic conditions. Considering the low volatility and hydrophobic character of the hormones and thus their ability to adsorb onto particulate matter (Lai et al. 2000) and the fact that under anaerobic conditions estrogens biodegradation is strongly hampered (Ying et al.

Table 2 Concentrations (μg/L) of hormone residues in the influent of the OVF before and after cleaning the pump pit (November 2011)

Before cleaning						After cleaning	
Compound/date	Apr 28, 2011	May 3, 2011	May 6, 2011	May 10, 2011	May* 24, 2011	July 15, 2012	Sep* 23, 2013
Estriol	9.2 ± 1.0	18.8 ± 3.15	25.5 ± 1.3	25.8 ± 3.1	17.1 ± 0.8	2.6 ± 0.2	0.424 ± 0.05
Estrone	2.9 ± 1.0	10.0 ± 1.1	14.4 ± 9.4	20.0 ± 1.4	17.2 ± 1.6	n.d	0.241 ± 0.02
17β estradiol	3.4 ± 1.3	5.2 ± 1.3	10.7 ± 1.0	7.0 ± 5.3	18.6 ± 1.0	0.07 ± 0.00	n.d
17β-ethynyl estradiol	1.4 ± 0.2	2.8 ± 1.1	3.7 ± 2.1	3.8 ± 2.1	21.7 ± 1.8	n.d	n.d
Norethisterone	n.d	n.d	n.d	2.4 ± 1.6	11.4 ± 2.1	n.d	n.d
Testosterone	n.d	5.4 ± 1.8	11.2 ± 0.6	12.1 ± 1.8	11.2 ± 1.1	n.d	0.118 ± 0.04
Diethylstilbestrol	n.d	n.d	5.0 ± 1.4	6.1 ± 0.1	13.3 ± 2.1	n.d	n.d

Values are the average of three replicates ± standard deviation

* The samples of May 24, 2011 and September 23, 2013 were measured with LC/MS/MS for comparison purposes

n.d stands for not detected

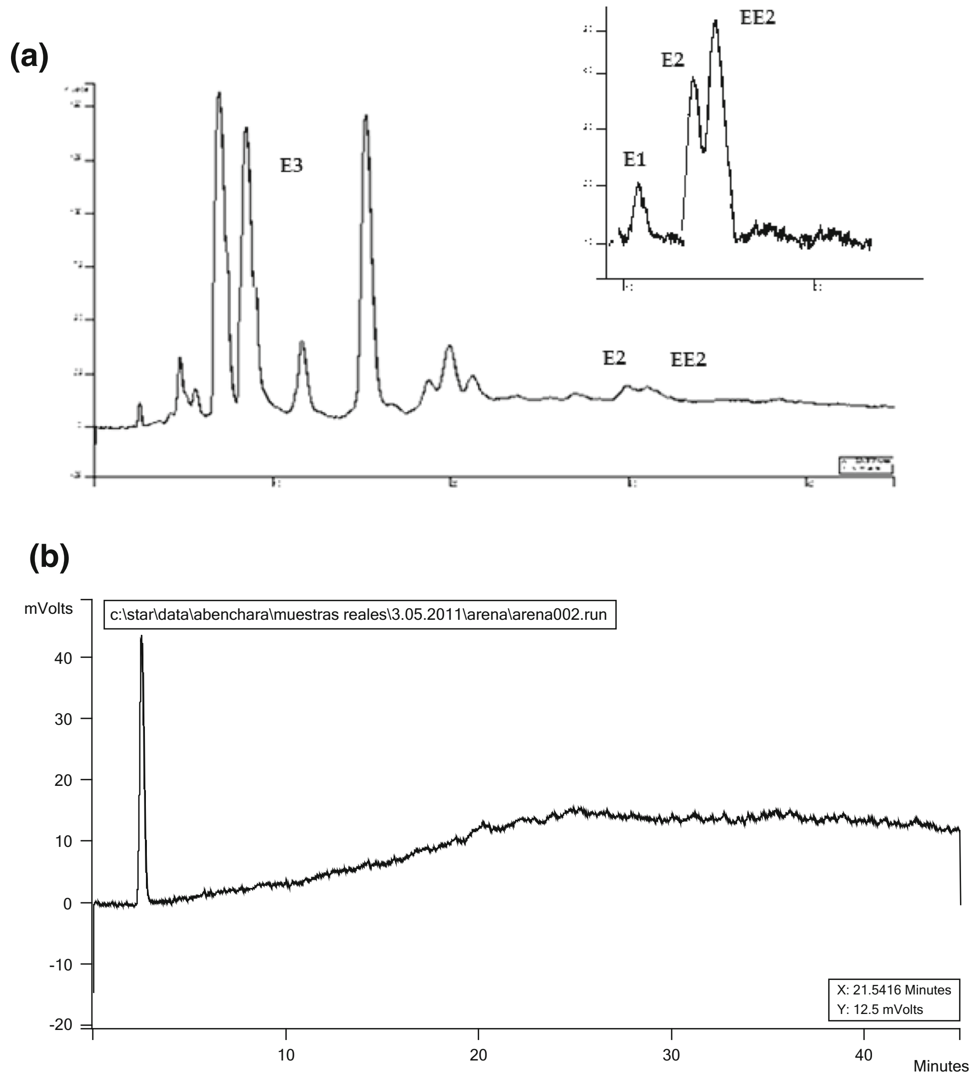

Fig. 8 Chromatograms from the influent just before the OVF (**a**) and after the OVF (**b**) showing the peaks corresponding to E1, E2, E3 and EE2

2003), we can conclude that the pump pit could concentrate not only organic matter and SS but also hormone residues. To determine whether this concentration effect of the pump pit was real, on November 2011, it was emptied and cleaned. After this, the measured concentrations of organic matter (BOD and COD), SS and turbidity became reduced and hormone residues were measured again in 2012 and 2013 (Table 2). With the exception of estriol (E3) in July 2012, the concentration levels found were notably lower than those obtained before cleaning the pump pit. These results help to confirm the hormone accumulation effect of the pump pit.

Even though the concentrations of hormones in the influent were remarkably high, their concentrations in the other sampling points were not detected.

Figure 8 shows the chromatograms obtained with the fluorescence detector of a sample of the influent (a) and the effluent of the OVF (b). As can be observed, the chromatogram from the effluent is very clean in comparison with that of the influent where many other peaks appear in addition to those of the hormones analyzed. Considering the low HRT of the OVF (<3 h) and the high adsorption affinity of the hormones, it is probable that adsorption was the main removal mechanism. However, the role of biodegradation should not be underestimated as the fill-and-drain hydraulic regime employed might have favored aerobic conditions. These results highlight the interest of investigating the use of organic-based substrates to improve the sorption and biodegradation of toxic organic compounds in TW.

Conclusion

A laboratory-scale, organic-based vertical flow filter was used as primary treatment of raw, high-strength, urban wastewater. At extremely high hydraulic and organic loading rates, the filter achieved significant removals of SS, turbidity, COD and BOD, performing best for the most concentrated influent samples. Thus, an organic-based TW could be used as a primary treatment for a conventional, gravel-based TW to reduce the risk of clogging in the latter. The organic-based filter also showed a remarkable capacity for removing natural and synthetic hormones suggesting the great potential of this sort of substrate to improve the removal of emerging pollutants.

Although the results obtained in this study must be considered with great care because of the scale used, they suggest that by combining the OVF (0.11 m^2/he) with a HF (3.3 m^2/he) and a VF (1.7 m^2/he), the legal limits for BOD, COD, SS, turbidity and *E. coli* can be met. According to the Spanish legislation, the effluent could be reused for irrigating industrial crops and fruit trees without contact between water and fruit.

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