Evaluation of different substrates to support the growth of *Typha latifolia* in constructed wetlands treating tannery wastewater over long-term operation

Cristina S.C. Calheiros, António O.S.S. Rangel, Paula M.L. Castro *

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

Keywords: Constructed wetlands; Phytoremediation; Tannery wastewater; *Typha latifolia*; Expanded clay

Abstract

The aim of this study was to investigate the performance of horizontal subsurface flow constructed wetlands planted with *Typha latifolia* treating tannery wastewater under long-term operation. Two expanded clay aggregates (Filtralite®MR3–8-FMR and Filtralite®NR3–8-FNR) and a fine gravel-FG were used as substrate for the constructed wetland units plus one unit with FMR was left as an unvegetated control. The systems were subject to three hydraulic loadings, 18, 8 and 6 cm d^{-1}, and to periods of interruption in the feed. The relationship between the substrate, plant development and removal efficiency, especially of organic matter, was investigated. Organic loadings up to 1800 kg BOD_5 ha^{-1} d^{-1} and 3849 kg COD ha^{-1} d^{-1} were applied leading to mass removals of up to 652 kg BOD_5 ha^{-1} d^{-1} and 1869 kg COD ha^{-1} d^{-1}, respectively. The three different substrates were adequate for the establishment of *T. latifolia*, although the clay aggregates allowed for higher plant propagation levels. The units with FNR and FMR achieved significantly higher COD and BOD_5 removal when compared to the FG and to the unplanted units. The systems proved to be tolerant to high organic loadings and to interruptions in feed suggesting this technology as a viable option for the biological treatment of tannery wastewater.

Introduction

According to INETI (2000) the wastewater originated from the tannery industry is the main environmental problem in this sector in Portugal, due to the complex effluent composition, which is characterized by high organic and inorganic contents. An integrated water management is crucial for the sustainable development of the sector due to its high water consumption level.

Constructed wetlands (CWs) are a feasible technology for the treatment of tannery wastewater as an alternative to conventional biological systems (Daniels, 2001; Kucuk et al., 2003; Calheiros et al., 2007). When designing and effectively applying CWs in the field, several good practice guidelines should be taken into account (Korkusuz, 2005). A CW, in a simplified way, consists of a properly designed basin that contains water, a substrate and plants, which may be manipulated to some extent to provide an adequate performance and treatment efficiency (USEPA, 1995). The materials, such as the substrate, liner and plant species, should be chosen in a way that the costs of the system are kept low, and the use of local products should be encouraged. Using indigenous plant species prevents undesired impacts, and selecting a plant species that has proven tolerance to a certain wastewater will be a cautious option. *Typha latifolia* and *Phragmites australis* have been established effectively in CWs receiving tannery wastewater (Calheiros et al., 2007).

* Corresponding author. Tel.: +351 22 5580059; fax: +351 22 5090351.
E-mail addresses: mccalheiros@mail.esb.ucp.pt (C.S.C. Calheiros), aorangel@esb.ucp.pt (A.O.S.S. Rangel), plcastro@esb.ucp.pt (P.M.L. Castro).

The choice of substrate in CWs is of major importance as it serves as the support of the living organisms and provides storage for many contaminants. Its permeability affects the wastewater flow through the CW, and it is where chemical and biological transformations, by microorganisms and plants, occur (USEPA, 1995). The substrates can be natural, such as gravel, sand and organic materials including compost and waste material (USEPA, 1995; Korkusuz, 2005). Typical effective sizes of the media for subsurface flow CWs vary between 2 and 128 mm and porosity varies between 28% and 45% (USEPA, 2000). A porous media may be an interesting option since it provides greater surface area for treatment contact and for biofilm development.

In this study the treatment of tannery wastewater by *T. latifolia* CWs, established in different substrates, Filtralite®NR3–8, Filtralite®MR3–8 and fine gravel, was evaluated. The systems were subject to different hydraulic conditions and interruptions in feed. The enzymatic and physiological response of *T. latifolia* to the tannery wastewater was assessed through its peroxidase activity (POD) and chlorophyll content. The relation between substrate, plant development and the constructed wetland pilot units (CWUs) performance was investigated.

Methods

Constructed wetlands set-up

The monitoring of three planted CWUs and one unvegetated unit (Uc) was undertaken in a site located at the wastewater treatment plant of a leather company in the North of Portugal. The set up conditions for these units were similar to what was described previously in Calheiros et al. (2007). The macrophyte used was *T. latifolia* (in a range of 10 plants m^{-2}), which was transplanted from an industrial polluted site in Estarreja, Portugal (Oliveira et al., 2001). The aggregates used to fill the CWUs were: Filtralite®MR3–8 (FMR) and Filtralite®NR3–8 (FNR) with particle size ranging from 3 to 8 mm (from maxit – Argilas Expandidas, SA – Portugal), and fine gravel – AGH 4–8 (FG) with particle size ranging from 4 to 8 mm (from Areipor – Areias Portuguesas, Lda – Portugal). The substrates used in the units were analyzed for porosity (Tan, 1995).

The unit with FMR (U1) and the unvegetated unit (Uc) had been in operation for 17 months under two hydraulic regimes, 3 and 6 cm d^{-1} (Calheiros et al., 2007). The units with FNR (U3) and FG (U2) were filled with water and after three weeks the tannery wastewater was applied. After that, the systems operated for 31 months under different hydraulic conditions and interruptions in feed. During 2 months (corresponding to days 1–61), the systems were monitored under a hydraulic loading rate (HLR) of 18 cm d^{-1}. By the 3rd month (between days 62 and 85), the systems were not fed due to the shutdown of the tannery production plant. A second period of operation occurred subsequently during 23 months (corresponding to days 86–760) under a HLR of 6 cm d^{-1}. The wastewater supply was stopped twice, corresponding to the periods between 372 and 399 days and between 428 and 455 days. By day 479 a prune was made leaving around 10 cm of aboveground plant material. A third period of operation occurred during 6 months (corresponding to days 761–928) under a HLR of 8 cm d^{-1}, and the systems were not fed due to the shutdown of the tannery production plant by the second month within that period (corresponding to days 792 and 826).

Adsorption studies

Equilibrium adsorption isotherms were performed on the three substrates used to fill the units. The substrates FMR, FNR and FG were previously washed with deionised water and dried in an oven at 40 °C for 4 days. The adsorption of organic matter to the substrates was determined by incubating a series of 100 ml acid-washed glass bottles containing 10 g of substrate with 50 ml of tannery wastewater at different concentrations. The wastewater concentration ranged from 50 to 903 mg COD L^{-1}, corresponding to 5%, 20%, 50%, 75% and 100% tannery wastewater content. Dilutions were made with deionised water. Three replicates were carried out for each concentration. A control containing the substrate and a volume of deionised water equal to the test solutions was used. The chemical oxygen demand (COD) was determined based on Standard Methods (APHA, 1998) using the Colorimetric Method – Closed Reflux. The bottles were sealed with rubber caps and placed in a rotatory shaker at 150 rpm and 25 °C. The adsorption of organic matter to the substrate was monitored after a 72 h period, since preliminary studies indicated that this contact time period was sufficient to achieve adsorption equilibrium.

Plant parameters

Macrophyte number and height (starting from the substrate level) were registered periodically. For that the CWUs were divided in four zones – A and C corresponded to the inlet and outlet zone, respectively, and B and D corresponded to the middle-left and middle-right side of the CWUs (inlet reference point) – and three plants were marked in each zone. They were visually inspected, on a periodical basis, for toxicity signs, such as chlorosis, necrosis and malformed plants.

POD determination

POD was determined on root samples of *T. latifolia*, collected at the inlet and outlet of the CWUs and immediately frozen in liquid nitrogen. The experimental procedure described in USEPA (1994) for plant peroxidase activity determination was followed with three replicates. One gram of fresh plant tissue was ground with a calcium chloride solution. The crude extract was added to an assay mixture and absorbance readings were done in a spectrophotometer (Novaspec II, Visible spectrophotometer) at 510 nm.

Chlorophyll content

For chlorophyll analysis, fresh circular discs from mature leafs of plants collected at the inlet and outlet of the CWUs were cut with a 10.5 mm corer, and were extracted in *N*,*N*′-dimethylformamide. Chlorophyll *a* and *b* content was determined according to Wellburn (1994).

Physico-chemical analysis

Wastewater samples were periodically taken at the inlet and outlet of the CWUs and the following parameters were determined based on Standard Methods (APHA, 1998): pH, color (spectrophotometric method), COD (closed reflux, Titrimetric method), biochemical oxygen demand (BOD_5; 5-day BOD test), total suspended solids (TSS; total solids dried at 103–105 °C method), Kjeldahl nitrogen (TKN; Kjeldahl Method), nitrate nitrogen (NO_3^-–N; nitrate electrode method), ammonia nitrogen (NH_3–N; phenate method), total phosphorus (total P; manual digestion and flow injection analysis for total phosphorus), total chromium (total Cr; nitric acid digestion followed by the colorimetric method) and hexavalent chromium (Cr(VI); colorimetric method). The sulfates determination (SO_4^{2-}; turbidimetric method) was done based on Association of Official Analytical Chemists (AOAC, 1995). The analysis were done immediately after sample collection, otherwise were properly stored. The temperature was measured at the inlet, outlet and 15 cm below the surface of the CWUs, using a thermometer. The dissolved oxygen (DO), conductivity and salinity (Sal.) were registered with a WTW handheld multi-parameter instrument 340i at the inlet and outlet of the units.

Data analysis

Statistical analysis was performed using the software SPSS (SPSS Inc., Chicago, IL, USA; Version 12.0). The data were analyzed through one-way analysis of variance (ANOVA) to compare the performance of each bed concerning the removal of COD, BOD_5, TSS, color, TKN, NH_3 and SO_4^{2-}. The plant parameters were also analyzed through ANOVA to compare inlet and outlet CWUs material. Student's *t*-test was applied in the chlorophyll analyses. To detect the statistical significance of differences ($p < 0.05$) between means of observation, the Duncan test was performed. When applicable, values were presented as the mean ± standard error.

Results

In this study, three CWUs planted with *T. latifolia* in different substrates and an unvegetated bed were subject to real tannery wastewater under three different HLRs.

The development of the plants was monitored and the CWUs performance in terms of removal of contaminants was evaluated.

Adsorption isotherms

Data on the adsorption of organic matter, represented by COD, on the three substrates, were generated from the equilibrium concentration of the adsorbate that ranged from 0 to 750 mg L^{-1}. The amount of adsorbate adsorbed per unit of the adsorbent was up to 2.26 mg g^{-1} for substrate FNR, 1.20 mg g^{-1} for FMR and 0.74 mg $g^{-1}$for FG.

The adsorption equilibrium data were correlated with three adsorption isotherm models. The two parameter models, Freundlich and Langmuir (Doran, 1995), and the three parameter model, Redlich–Peterson (Redlich and Peterson, 1959), were analyzed using the Experimental Design package of STATISTICATM v 6.0 (Statsoft®, Tulsa, OK, USA). The Levenberg–Marquardt algorithm for the least squares function minimization was used. The Freundlich model was the one that fitted better the data concerning the organic matter adsorption capacity by each substrate. The statistical indicators of the quality of the regression, coefficient of determination (R^2) and the mean square error (MSE, i.e. the sum of squares of residuals divided by the corresponding degrees of freedom) were obtained directly from the software. The precision of the estimated parameters was also evaluated by the standardized half width (SHW), which was defined as the ratio between the 95% standard error and the value of the estimate (Table 1).

The model is described by the following equation:

$$q_e = K * C_e^{1/n} \quad (1)$$

where C_e is the equilibrium liquid phase solute concentration (mg L^{-1}) and q_e is the amount of adsorbate adsorbed per unit weight of adsorbent (mg g^{-1}). Parameters K and n are Freundlich constants, whereby K is a measure of the adsorption capacity and n a measure of the adsorption intensity. Values of n higher than 1 show, in most cases, beneficial adsorption, if $n < 1$ then adsorption is unfavorable (Doran, 1995; Annadurai et al., 2000). The higher Freundlich n constant obtained was for FNR substrate with 1.09, followed by FMR and FG, although these values were lower than 1. The K parameter value, which is an indi-

Table 1
Isotherm constants for Freundlich model concerning the adsorption of organic matter onto three substrates at 25 °C

Substrate	MSE	R^2	K (mg g^{-1})	SHW (%)	n	SHW (%)
FNR	1.27E−02	0.990	7.23E−03	160	1.06	29
FMR	5.74E−04	0.998	1.15E−03	84	0.93	12
FG	5.96E−04	0.996	1.96E−04	167	0.80	21

Evaluation of the quality of the regression on the basis of mean square error (MSE) and R^2. Precision of the estimated parameters evaluated using half with (SHW).

cator of the sorption capacity (Annadurai et al., 2000), was higher for FNR, followed by FMR and FG.

The porosity is an important characteristic of the substrate and may affect several phenomena such adsorption of substances and movement of water and air within the medium. In this study, the substrates porosity is 37% for FMR, 43% for FNR and 29% for FG.

Development and characteristics of T. latifolia plants

The growth of the plants was monitored during the operation of the CWUs. The plant propagation in terms of the total number of shoots is shown in Fig. 1. When the tannery wastewater was initially supplied to the CWUs, at a HLR of 18 cm d^{-1}, the total number of shoots was 28 for U1, 16 for U3 and 16 for U2. By the end of the experiment, at day 928, the total number of shoots was 56 in U1, 51 in U3 and 13 in U2.

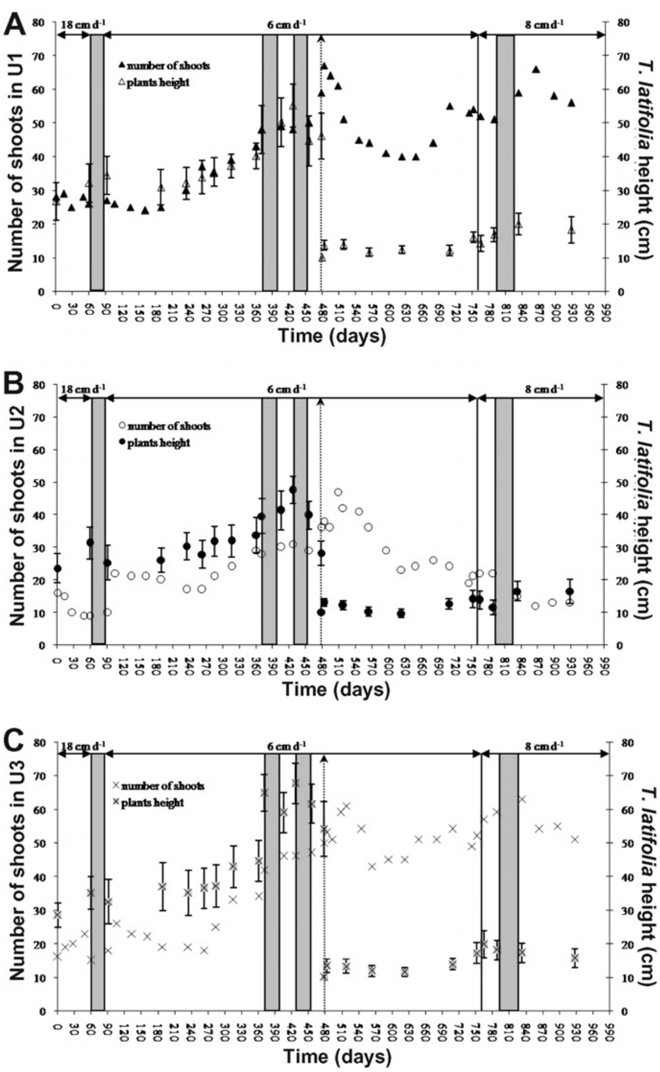

Fig. 1. Variation in number of shoots and height of *T. latifolia* in the pilot units during the system operation. (A) U1, (B) U2 and (C) U3 ▬ No loading occurred, …… prune of the plants at the day 479.

ANOVA one-way was performed to compare the number of shoots between units before each HLR regime (the last five countings before the HLR alteration were considered). For an HLR of 8 and 6 cm d^{-1} a significantly lower number of shoots was registered for the gravel unit U2 compared with the clay aggregate units U1 and U3.

The differences in the number of *T. latifolia* shoots between zones A, B, C and D, along the whole operation of the CWUs, were notorious. In zone A, corresponding to the inlet of the CWUs, lower shoot countings were observed. In the FNR and FG units, at the beginning of the experiment, the shoots were equally divided in each zone while at the end zone A, B, C and D presented 8, 12, 17 and 14 shoots, respectively, in U3 and 0, 2, 5 and 6, respectively, in U2. The U1 unit presented 2, 23, 19 and 12 for zone A, B, C and D respectively, having started with 3, 8, 8 and 6 respectively.

The height of the plants along the CWUs operation is shown in Fig. 1. The decrease in the height of some plants corresponds to the decay of dry leaves. Oscillations concerning the number of shoots and height of the plants were registered during the long-term operation of the systems.

The plants did not visually show signs of phytotoxicity in the leaves and shoots, although their height was lower than plants occurring in the origin environment. In the warmer periods (spring/summer) the presence of aphids and plant insects that secret a white frothy as a result of sucking sap and other nutrients from the plant was observed, although no control measure was necessary to take. Occasionally garden spiders (*Araneus diadematus*) were seen.

The POD of *T. latifolia* roots growing on the three substrates is presented in Table 2. No significant differences between the units and between the inlet and outlet of each unit were detected.

No significant differences were observed for chlorophyll pigments extracted from the leaves of *T. latifolia*, by the 12th month of operation, between units and between the inlet and outlet of FMR, FNR and FG CWUs (Table 2). The second determination, at the 25th month of operation, was also undertaken in summer time but most of the plant leaves were dry. Only a comparison between the inlet and outlet of U3 was possible. There were no significant differences between inlet and outlet for chlorophyll *a* and *b*.

CWUs operation

The characteristics of the tannery wastewater corresponding to the three different HLRs are presented in Tables 3–5, showing its variability in the inlet and outlet of the CWUs and between the different hydraulic conditions tested.

Wastewater inlet pH throughout the CWUs operation varied between 4.43 and 8.46 and at the outlet ranged from 7.00 and 8.93. Concerning conductivity, the inlet values varied between 4.74 and 10.05 mS cm^{-1}. At the outlet, conductivity varied between 4.04 and 10.12 mS cm^{-1}. The

Table 2
Chlorophyllous pigments and POD activity of *T. latifolia*

Units	Date	June 2005			June 2006			POD activity
	CW site	Chlorophyll (mg g^{-1} FW)			Chlorophyll (mg g^{-1}FW)			
		a	*b*	*a/b*	*a*	*b*	*a/b*	(ΔA min^{-1} g^{-1} fine root FW)
Ul[a]	Inlet	0.67 ± 0.12	0.32 ± 0.03	2.06 ($n = 5$)	nd	nd		0.43 ± 0.19 ($n = 3$)
	Outlet	0.68 ± 0.07	0.31 ± 0.02	2.21 ($n = 5$)	0.53 ± 0.02	0.28 ± 0.07	1.90 ($n = 5$)	0.45 ± 0.12 ($n = 3$)
U2[a]	Inlet	0.67 ± 0.16	0.31 ± 0.07	2.16 ($n = 5$)	nd	nd		0.40 ± 0.10 ($n = 3$)
	Outlet	0.65 ± 0.07	0.31 ± 0.02	2.10 ($n = 4$)	nd	nd		0.33 ± 0.05 ($n = 3$)
U3[a]	Inlet	0.66 ± 0.15	0.30 ± 0.07	2.22 ($n = 5$)	0.93 ± 0.14	0.39 ± 0.13	2.36 ($n = 4$)	0.82 ± 0.22 ($n = 3$)
	Outlet	0.23 ± 0.10	0.11 ± 0.05	2.17 ($n = 5$)	1.08 ± 0.36	0.35 ± 0.04	3.12 ($n = 5$)	0.62 ± 0.25 ($n = 3$)

[a] Units: U1: *T. latifolia* + Filtralite®MR3–8, U2: *T. latifolia* + fine gravel, U3: *T. latifolia* + Filtralite®NR3–8; nd: not determined.

Table 3
Mean composition of the inlet and outlet of the pilot units for a HLR of 18 cm d^{-1}

Parameters	Average results (min.–max.) for each unit outlet				
	Inlet	U1[a]	Uc[a]	U2[a]	U3[a]
pH	7.82 (7.66–8.10)	8.27 (8.09–8.65)	8.24 (8.06–8.55)	8.23 (8.02–8.59)	8.43 (8.21–8.92)
Conductivity (mS cm^{-1})	7.39 (6.64–8.31)	6.15 (5.04–7.64)	6.55 (5.44–7.87)	6.68 (6.18–7.50)	6.79 (5.98–7.42)
Salinity (%)	0.41 (0.36–0.46)	0.37 (0.31–0.44)	0.36 (0.29–0.44)	0.37 (0.34–0.41)	0.37 (0.34–0.41)
DO (mg O_2 L^{-1})	nd	nd	nd	nd	nd
COD (mg O_2 L^{-1})	1629 (1354–2138)	883 (710–1100)	1131 (890–1420)	1131 (919–1439)	981 (765–1250)
BOD_5 (mg L^{-1})	826 (720–1000)	554 (487–660)	647 (570–760)	612 (550–730)	573 (500–680)
TSS (mg L^{-1})	168 (98–324)	30 (21–43)	39 (28–50)	27 (16–42)	25 (19–32)
Total P (mg L^{-1})	0.31 (0.21–0.43)	0.24 (0.17–0.31)	0.25 (0.17–0.33)	0.26 (0.18–0.35)	0.25 (0.16–0.31)
Color (Pt/Co)	nd	nd	nd	nd	nd
TKN (mgTKN–N L^{-1})	136 (110–150)	97 (81–110)	102 (79–115)	93 (75–115)	87 (67–100)
NH_3 (mgNH_3–N L^{-1})	76 (63–87)	46 (38–53)	52 (44–60)	49 (40–56)	48 (39–57)
NO_3^- (mg NO_3^-–N L^{-1})	47 (37–54)	31 (23–35)	35 (30–40)	31 (23–38)	31 (26–36)
SO_4^{2-} (mg SO_4^{2-} L^{-1})	1531 (934–2206)	1087 (745–1479)	1243 (804–1717)	1212 (743–1890)	1144 (718–1630)
Total Cr (mg L^{-1})	0.370 (0.021–0.885)	0.110 (0.006–0.322)	0.219 (0.087–0.412)	0.167 (0.006–0.383)	0.128 (0.006–0.287)
Cr(VI) (mg L^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001

Minimum and maximum values are indicated in brackets ($n = 7$).
[a] Units: U1: *T. latifolia* + Filtralite®MR3–8, Uc: Filtralite®MR3–8, U2: *T. latifolia* + fine gravel, U3: *T. latifolia* + Filtralite®NR3–8; nd: not determined.

Table 4
Mean composition of the inlet and outlet of the pilot units for a HLR of 8 cm d^{-1}

Parameters	Inlet	Average results (min.–max.) for each unit outlet			
		U1[a]	Uc[a]	U2[a]	U3[a]
pH	6.36 (5.84–7.37)	7.82 (7.16–8.19)	8.15 (8.12–8.20)	8.15 (8.03–8.21)	8.23 (8.13–8.36)
Conductivity (mS cm^{-1})[b]	8.01 (6.95–9.54)	7.89 (5.21–9.27)	7.07 (6.22–8.97)	7.16 (5.54–9.57)	7.14 (5.52–9.48)
Salinity (%)[b]	0.45 (0.38–0.54)	0.44 (0.28–0.52)	0.39 (0.34–0.50)	0.40 (0.30–0.54)	0.39 (0.30–0.53)
DO (mg O_2 L^{-1})[b]	0.5 (0.2–1.0)	0.4 (0.1–0.7)	0.2 (0.1–0.5)	0.2 (0.1–0.6)	0.3 (0.2–0.6)
COD (mg O_2 L^{-1})	1908 (1751–2100)	921 (819–984)	1123 (1054–1210)	1089 (976–1212)	881 (798–981)
BOD_5 (mg L^{-1})	728 (620–860)	406 (330–450)	483 (440–550)	452 (410–500)	420 (380–490)
TSS (mg L^{-1})[b]	107 (98–121)	33 (30–36)	41 (38–44)	28 (25–32)	31 (27–37)
Total P (mg L^{-1})	0.21 (0.13–0.31)	0.17 (0.11–0.27)	0.15 (0.10–0.28)	0.16 (0.12–0.24)	0.14 (0.11–0.22)
Color (Pt/Co)	242 (221–280)	141 (112–163)	150 (123–169)	148 (125–162)	136 (103–156)
TKN (mgTKN–N L^{-1})	128 (121–134)	79 (73–87)	82 (69–88)	80 (74–88)	80 (73–88)
NH_3 (mg NH_3–N L^{-1})	83 (76–90)	48 (43–53)	51 (48–56)	53 (49–58)	46 (44–52)
NO_3^- (mg NO_3^-–N L^{-1})	46 (43–55)	35 (33–38)	37 (34–42)	34 (32–40)	35 (32–39)
SO_4^{2-} (mg SO_4^{2-} L^{-1})	215 (177–295)	137 (103–184)	140 (112–181)	140 (110–182)	133 (101–172)
Total Cr (mg L^{-1})	0.043 (0.010–0.120)	<0.001	<0.001	<0.001	<0.001
Cr(VI) (mg L^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001

Minimum and maximum values are indicated in brackets ($n = 6$).
[a] Units: U1: *T. latifolia* + Filtralite®MR3–8, Uc: Filtralite®MR3–8, U2: *T. latifolia* + fine gravel, U3: *T. latifolia* + Filtralite®NR3–8.
[b] $n = 7$.

Table 5
Mean composition of the inlet and outlet of the pilot units for a HLR of 6 cm d^{-1}

Parameters	Inlet	Average results (min.–max.) for each unit outlet			
		U1[a]	Uc[a]	U2[a]	U3[a]
pH[b]	6.61 (4.43–8.46)	8.07 (7.42–8.93)	8.15 (7.50–8.41)	7.96 (7.00–8.55)	8.12 (7.36–8.68)
Conductivity (mS cm^{-1})[c]	7.29 (4.74–10.05)	6.47 (4.31–10.12)	6.23 (4.04–8.30)	6.45 (4.64–9.03)	6.54 (4.82–9.61)
Salinity (%)[c]	0.39 (0.25–0.56)	0.35 (0.23–0.57)	0.34 (0.21–0.46)	0.35 (0.25–0.51)	0.35 (0.26–0.55)
DO (mg O_2 L^{-1})[d]	0.7 (0.1–1.9)	0.4 (0.1–1.1)	0.4 (0.1–1.1)	0.4 (0.1–1.7)	0.4 (0.1–1.2)
COD (mg O_2 L^{-1})	1598 (808–2449)	550 (270–883)	826 (490–1300)	766 (431–1226)	581 (285–1004)
BOD_5 (mg L^{-1})	706 (420–860)	314 (190–455)	434 (270–550)	416 (240–570)	337 (225–510)
TSS (mg L^{-1})	80 (32–134)	24 (10–40)	33 (12–51)	23 (10–34)	22 (11–37)
Total P (mg L^{-1})	0.41 (0.10–0.95)	0.32 (0.07–0.80)	0.31 (0.06–0.72)	0.32 (0.07–0.77)	0.31 (0.03–0.70)
Color (Pt/Co)[e]	304 (132–610)	122 (78–231)	164 (109–253)	147 (101–245)	133 (96–212)
TKN (mgTKN–NL^{-1})	122 (87–160)	84 (56–100)	86 (55–95)	80 (51–101)	74 (44–90)
NH_3 (mg NH_3–NL^{-1})	81 (60–98)	54 (35–64)	58 (44–65)	54 (40–67)	51 (38–60)
NO_3^- (mg NO_3^-–N L^{-1})	43 (17–59)	30 (14–45)	34 (12–47)	30 (12–44)	30 (9–44)
SO_4^{2-} (mg $(SO_4^{2-}$ $L^{-1})$[b]	297 (78–1231)	189 (37–775)	203 (42–879)	189 (40–844)	186 (32–863)
Total Cr (mg L^{-1})[b]	0.366 (0.010–2.500)	0.164 (<0.001–0.725)	0.179 (<0.001–0.750)	0.159 (<0.001–0.700)	0.164 (<0.001–0.725)
Cr(VI) (mg L^{-1})[b]	<0 001	<0 001	<0 001	<0 001	<0 001

Minimum and maximum values are indicated in brackets ($n = 27$).
[a] Units: U1: *T. latifolia* + Filtralite® MR3–8, Uc: Filtralite® MR3–8, U2: *T. latifolia* + fine gravel, U3: *T. latifolia* + Filtralite® NR3–8.
[b] $n = 28$.
[c] $n = 24$.
[d] $n = 16$.
[e] $n = 10$.

salinity at the inlet was in average 0.41%, 0.45% and 0.39%, for 18 cm d^{-1}, 8 cm d^{-1} and 6 cm d^{-1}, respectively. For the outlet, the values ranged between 0.21% and 0.57%. DO concentrations were low at the inlet and outlet of all beds for 6 and 8 cm d^{-1} HLRs, varying between 0.1 and 1.9 mg O_2 L^{-1} for inlet and 0.1 to 1.7 mg O_2 L^{-1} for outlet.

In terms of COD and BOD_5 removal, considering the long-term operation of the systems, the expanded clay units with FMR (U1) and FNR (U3), outperformed significantly the unvegetated unit (Uc) and the FG unit (U2).

The COD inlet concentration for the three HLRs varied between 808 and 2449 mg L^{-1}. The COD inlet variation is due to the fact that real tannery wastewater was used, and consequently that varies along time, according to the production process. The removal efficiency in U1 varied between 41% and 49% for 18 cm d^{-1}, between 48% and 56% for 8 cm d^{-1} and between 46% and 81% for 6 cm d^{-1}. In the corresponding unvegetated unit, Uc, removal efficiencies were lower, varying between 27% and 34% for 18 cm d^{-1}, between 34% and 47% for 8 cm d^{-1} and between 29% and 64% for 6 cm d^{-1}. For U2 efficiencies ranged between 28% and 33% for 18 cm d^{-1}, between 39% and 48% for 8 cm d^{-1} and between 33% and 71% for 6 cm d^{-1}. Finally, for the U3 the efficiencies approached those of the U1 unit, varying between 36% and 45% for 18 cm d^{-1}, between 46% and 62% for 8 cm d^{-1} and between 44% and 82% for 6 cm d^{-1}. For each HLR applied there was a significantly higher COD percentage removal for U1 and U3 in relation to U2 and Uc, although for 18 cm d^{-1} the U1 had a significantly higher percentage removal than U3. Fig. 2 shows, as an example, the pattern of COD removal in the U1 unit during the system operation at three HLRs and through the interruption in the feed. The COD removal efficiency was always resumed after the starvation periods. No significant differences between the COD removal efficiency in Spring/Summer (April–September) and Autumn/Winter (October–March) periods were found. Concerning the temperatures registered in the center of the units, 15 cm below the surface, when the samples collection took place, it varied between 3.1 and 29.0 °C. The inlet and outlet wastewater temperature varied between 5.0 and 28.9 °C. In Fig. 2 the air temperature is presented.

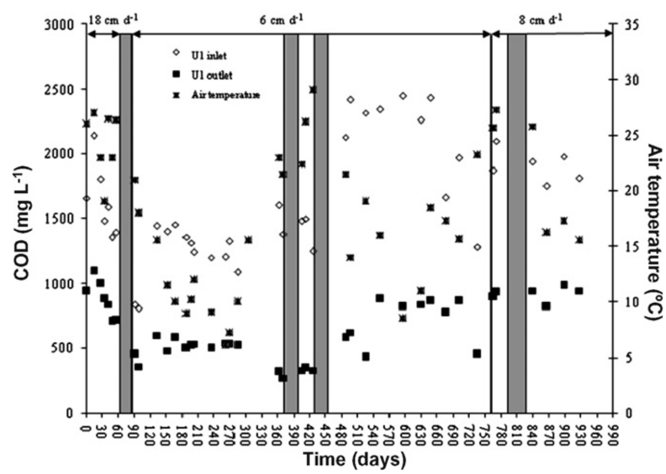

Fig. 2. COD at the inlet and outlet of the FMR unit and air temperature, during the time of operation, for the three HLRs applied. ▬ No loading occurred.

The BOD_5 inlet concentration for the three HLRs varied between 420 and 1000 mg L^{-1}. The removal efficiency in the U1 varied between 28% and 36% for 18 cm d^{-1}, between 40% and 48% for 8 cm d^{-1} and between 44% and 69% for 6 cm d^{-1}. Concerning the Uc it varied between 20% and 24% for 18 cm d^{-1}, between 26% and 37% for 8 cm d^{-1} and between 30% and 48% for 6 cm d^{-1}. For U2 it varied between 22% and 30% for 18 cm d^{-1}, between 28% and 44% for 8 cm d^{-1} and between 33% and 51% for 6 cm d^{-1}. Finally for U3 it varied between 27% and 34% for 18 cm d^{-1}, between 36% and 50% for 8 cm d^{-1} and between 39% and 63% for 6 cm d^{-1}.

For each HLR applied there was a significantly higher BOD percentage removal for the expanded clay units compared to the unvegetated unit (Uc) and U2, except for 8 cm d^{-1}, where U2 was not significantly different from U3. Besides this, U2 had significantly higher removal efficiency than Uc for 18 cm d^{-1} and U1 had significantly higher removal efficiency than U3 for 6 cm d^{-1}.

Concerning the organic mass loadings they varied between 485 and 3849 kg $ha^{-1}d^{-1}$ for COD and between 252 and 1800 kg ha^{-1} d^{-1} for BOD_5 (Fig. 3). A linear correlation was established between mass loading and mass removal. For the three HLRs the unvegetated unit had always a significantly lower COD mass removal when compared to the expanded clay units (U1 and U3) and showed no significant differences when compared to the FG unit (U2). When comparing the FG unit with the expanded clay units (U1 and U3) the mass removal was always lower except for the HLR of 6 cm d^{-1}, for which no significant differences were found between the FG unit and that of U3. For BOD_5 the unvegetated unit showed, as for COD, a significant lower mass removal when compared to the expanded clay aggregates (U1 and U3), except for the HLR of 8 cm d^{-1} for which no significant differences. When comparing the FG unit (U2) with the expanded clay units (U1 and U3) the mass removal was significantly lower for a HLR of 6 cm d^{-1}. For a HLR of 18 cm d^{-1} there were no significant differences between U2 and U3.

Concerning TSS removal, significant differences were found between the Uc and the other CWUs. The TSS inlet concentration for the three HLRs varied between 32 and 324 mg L^{-1}. The removal efficiency for Uc varied between 60% and 87% for 18 cm d^{-1}, between 56% and 64% for 8 cm d^{-1} and between 42% and 75% for 6 cm d^{-1}. In the other units removal efficiencies were higher, varying between 73% and 91% for 18 cm d^{-1}, between 65% and 77% for 8 cm d^{-1} and between 41% and 93% for 6 cm d^{-1}.

The color at CWUs inlet varied between 132 and 610 Pt/Co being the removal efficiency between 14% and 84%. No significant differences were found between the units.

The TKN, NH_3 and NO_3^-, inlet concentrations varied between 87 and 160 mg TKN–N L^{-1}, 60 and 98 mg NH_3–N L^{-1} and 17 and 59 mg NO_3^-–N L^{-1}. The TKN removal efficiency varied between 20% and 44% for U1, between 12% and 45% for Uc, between 20% and 55% for U2 and between 20% and 52% for U3. When comparing this parameter between units there were significant differences between U3 and the other units. There were no significant differences between U2 and U1 and between Uc and U1. The NH_3 removal efficiency varied between 24% and 51% for U1, between 14% and 42% for Uc, between 17% and 49% for U2 and between 21% and 51% for U3. Significant differences were found between Uc, U1 and U3. U2 was not significant different from Uc and from U1. U3 was not significant different from U1.

For SO_4^{2-} removal no significant differences were found between the CWUs. The SO_4^{2-} inlet concentration for the three HLRs varied between 78 and 2206 mg L^{-1}. The removal efficiency varied between 6% and 37% for 18 cm d^{-1}, between 28% and 48% for 8 cm d^{-1}, and between 9% and 67% for 6 cm d^{-1}.

Phosphorus and chromium (total and hexavalent) were only detected at lower concentrations at the inlet and outlet of the CWUs.

Discussion

Adsorption isotherms

In this study, the capacity of two expanded clay aggregates and a fine gravel to adsorb organic matter were evaluated through the determination of the respective isotherm. The equilibrium adsorption isotherm is important because the adsorption capacity of an adsorbent may play a relevant role in a wastewater treatment system. The Freundlich isotherm was the best fit model, for the three substrates, when compared to Langmuir and Redlich–Peterson models. The Freundlich isotherm is used in liquid–solid systems for heterogeneous surface energies systems and different particle sizes (Doran, 1995; Annadurai et al., 2000). The low adsorption capacity of the substrates may indicate that the organic reduction in the wastewater passing through the CWUs is due, in a substantial part, to biological degradation.

In the present study, removal of organic matter through adsorption onto these substrates was evaluated, however adsorption studies for nitrogen (Namorado et al., 2004) and phosphorus (Lisboa et al., 2004) have already been undertaken. Namorado et al. (2004) concluded that for FMR and FNR there was no removal by adsorption or other physico-chemical process when a solution of ammonium nitrate (with a equivalent concentration of 112 mg L^{-1} of nitrates and 50.6 mg L^{-1} of total nitrogen) was used and Lisboa et al. (2004) concluded that for FNR there was no significant removal of phosphorus but for FMR the average removal was 1040 mg P kg^{-1} of clay, when a solution of phosphate (200 ppm) was used.

Each substrate has different characteristics in terms of porosity. According to FAO (2006) classification it is considered that FMR and FG have a high porosity, because is between 15% and 40%, and FNR is considered to have a very high porosity (higher than 40%).

Development and characteristics of T. latifolia plants

T. latifolia developed and propagated in all the three substrates tested, although with greater success in the

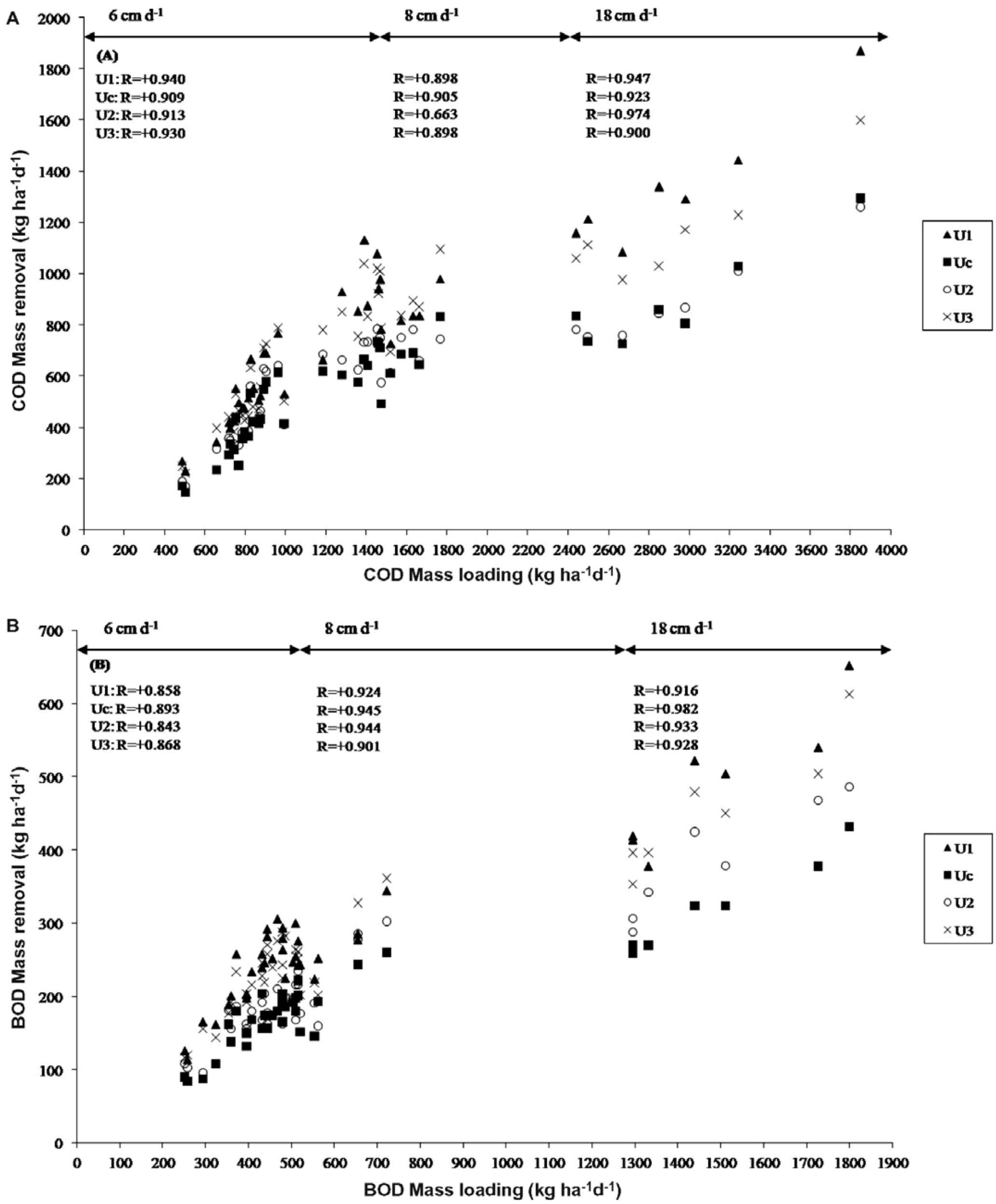

Fig. 3. Removal of organics during system operation. (A) COD mass loading vs. COD mass removal and (B) BOD_5 mass loading vs. BOD_5 mass removal. The coefficients of the linear correlation are represented all with $p < 0.05$.

expanded clay aggregates. In general in the winter months a slightly decrease in the number of shoots was verified and an increase in the growth of the plants was more notorious in spring/summer period. This plant has been found to be tolerant to water level fluctuations, perennial flooding, anaerobic conditions and has been shown to adapt to different substrates, such as fine medium and coarse soil (Kirkpatrick, 2004; USDA, 2006). Calheiros et al. (2007) reported that this plant species together with *P. australis* were the plants better established in FMR, in terms of survival and propagation, when compared to *Iris pseudacorus*, *Canna indica* and *Stenotaphrum secundatum*. The plants near the inlet of CWUs, where the organic loading was higher, were reduced in number when compared to the whole area. Shepherd et al. (2001) described the same phenomenon when treating high-strength winery wastewater with CWs.

Peroxidases are a family of isozymes found in plants and its activity may be affected by several abiotic and biotic stressors (Yoshida et al., 2003). A large group of peroxidase enzymes are considered to be part of the general defense mechanisms in plants (Markkola et al., 2002) and thus regarded as a defense metabolic activity against stress (Klar et al., 2006), the roots of *T. latifolia* in each CWU were analyzed for POD activity. The fact that no significant differences were found between the CWUs suggests that they were facing similar stress conditions, regardless of the substrate used in the units. However, it would have been interesting to follow its variations when the hydraulic conditions were changed.

Chlorophyll content is useful to study the effects of environmental stresses on plant since this pigment is linked to the visual symptoms and photosynthetic plant productivity (Gupta and Sinha, 2007). In this study there were no significant differences between units and from the inlet to the outlet of the U1, U2 and U3, suggesting that *T. latifolia* plants were reacting similarly to the imposed conditions.

In general plants are relatively tolerant during germination but become more sensitive during emergence and early seedling stages of growth. The excess salinity within the plant root zone has a general deleterious effect on plant growth (Rhoades et al., 1992). Moreover, the conductivity of a substrate affects the ability of plants and microbes to process the waste material flowing in to the CW (USEPA, 1995). In this study, the inlet and outlet wastewater from the CWUs is considered moderately saline, based on Rhoades et al. (1992), and the three substrates are considered not salty (FAO, 2006). *T. latifolia* is characterized to have a low to high salinity tolerance (range that encompasses <4 mS cm^{-1} to <9 mS cm^{-1}) (Kirkpatrick, 2004; USDA, 2006). These two parameters do not alone contribute to inhibition or restriction of the plant development. Concerning the inlet wastewater pH it is within the optimum range (3.0–8.5) for *T. latifolia* (USEPA, 1995). According to USEPA (1995) the pH of the substrate affects the availability and retention of heavy metals and nutrients and should be generally between 6.5 and 8.5. In this case the pH of the substrate acquired was 9.75 for FMR (Calheiros et al., 2007), 8.90 for FNR and 6.97 for FG (data not shown).

CWUs operation

The DO in this tannery wastewater was consistently low, as was the DO of the wastewater coming from the outlet of the CWUs. Since the influent has high levels of oxygen demand this scenario was expected. Vymazal and Kröpfelová (2006) stated that the outlet DO concentration of horizontal CWs provided little information about the processes occurring within the plant beds and the amount of DO entering in the beds forms only a negligible portion of the DO consumed in the beds. The low DO at the CWUs outlet can be explained by the fact that the horizontal subsurface flow system contain virtually no free oxygen as they are considered as anoxic or anaerobic systems if operating in a continuous mode (Kadlec et al., 2000; Vymazal and Kröpfelová, 2006). Also, when the substrate is flooded the oxygen present will be promptly consumed by microbial respiration and chemical oxidation (Kadlec et al., 2000). The oxygen required for aerobic degradation is supplied from atmosphere by diffusion into the vegetated beds and by oxygen release from the macrophyte roots (Kadlec et al., 2000; Vymazal, 2001; Stein and Hook, 2005; Vymazal and Kröpfelová, 2006). Bendix et al. (1994) reported an effective aeration system based on internal gas transport in *T. latifolia* that occurs mainly via pressurized convective throughflow of gases.

The high color removal achieved in the CWUs (up to 84% from a range between 132 and 610 Pt/Co) is comparable with the performance of a cattail unit used for dye-rich wastewater treatment (laboratory prepared) where an average of 72% removal was achieved for mean inlet concentration of 100 Pt/Co (Mbuligwe, 2005). Although in the present study there were no significant differences for planted and unplanted units, Mbuligwe (2005) reported statistically better performance in color removal for planted units when compared to unplanted units, filled with river sand. Yoo et al. (2001) inferred about the effectiveness of decolorization through sulfate reducing bacteria (SRB) in anaerobic bacterial systems with sulfate. This may happen, according to this author, since the sulfide produced via sulfate respiration by SRB chemically decolorizes azo dyes. In the present study, the inlet sulfate concentration is considered high, up to 2206 mg L^{-1}, and the reduction occurred up to 67%. Under anaerobic conditions the reduction of sulfate produces hydrogen sulfide that can be detected by "rotten egg" smell (Mbuligwe, 2005). During the trial this smell was often noticed when the sample collection was made.

The CWUs with FNR and FMR presented significant higher COD percentage removal when compared to the FG and to the unplanted unit, Uc, for the three hydraulic regimes. This difference was registered from the beginning and was maintained through the long-term operation. The fact that in the first hydraulic regime the percentage removal in the U1 was significant higher than in the U3 is most probably due to the fact that the U1 unit had been subject to tannery wastewater previously and was a more mature bed. The U1 and U3 outperformed the unplanted unit, in terms of COD removal, by 4–34% and 6–28%, respectively. Calheiros et al. (2007) had found no significant differences in COD removal between planted (*I. pseudocarus*, *S. secundatum*, *T. latifolia* and *P. australis*) and unplanted units in the first 17 months of operation, when the systems were subject to the same wastewater at HLRs of 3 and 6 cm d^{-1}. It is interesting to note that although the FMR (U1) and the unvegetated control (Uc) units had been in operation for 17 months previous to this study without showing significant differences in terms of COD removal, the FNR unit (U3) showed significant differences from the unplanted unit from the start of operation. However U3 has a different type of substrate, which can also contribute to this fact.

Mbuligwe (2005) stated that planted units with cattail and cocoyam outperformed a control unit, in terms of COD removal, by 17.5–21.7%, although the organic loadings (105 kg ha^{-1} d^{-1}) and time of operation were much lower than in the present study. The removal efficiencies presented in this study may be compared with the results of Calheiros et al. (2007), who reported percentages from 54% up to 73% for a HLR of 6 cm d^{-1} using tannery wastewater from the same origin.

Kucuk et al. (2003) applied real tannery wastewater to a horizontal subsurface flow constructed wetland and reported that the higher removal performance achieved was 30% (COD effluent = 210 mg L^{-1}) with a hydraulic retention time of 8 days.

When operating at a HLR 6 cm d^{-1} (during approximately 23 months) the season factor did not provoke significant differences in COD removal. It can be inferred that the temperature variation between seasons, at this location, does not affect the performance of the CWUs in terms of COD removal. These results agree with those of Vymazal (2001) who refer to the fact that removal of organics (COD and BOD_5) was not influenced by the season, based on operational systems in Czech Republic. Calheiros et al. (2007) reported that for five CWUs and one unvegetated control COD removal was not significant affected by the time of the year when a HLR of 3 cm d^{-1} was applied. Stein and Hook (2005) refer that high carbon loading and continuous flow operation likely restrain seasonal and plant effects through maintenance of a continuously high oxygen demand in the root zone and limiting development of oxidizing conditions. Also, the consequences of seasonal and plant-species effects depend on wastewater composition. Moreover, physiological responses to temperature differ among plant species as does the temperature dependence of growth (Madsen and Brix, 1997). Concerning the starvation periods, the treatment efficiency was resumed when the systems restarted, effect also reported by other authors (Daniels, 2001).

BOD_5 removal may be compared with that achieved in Calheiros et al. (2007), who reported removals ranging between 41% and 58% for a HLR of 6 cm d^{-1}. Vymazal (2001) reported an average BOD_5 removal efficiency from domestic wastewater (considering 23 systems) of $83 \pm 14\%$ with a final effluent concentration of 11 ± 10 mg L^{-1}. IULTCS (2004) refers a typical performance for the CWs applied to tannery wastewater treatment, after primary treatment, of 70–80% (300–400 mg L^{-1}) in terms of COD and of 85–95% (60–100 mg L^{-1}) for BOD_5.

In horizontal subsurface flow wetlands the settable organics are removed from the wastewater by filtration and deposition (Vymazal, 2001). The removal efficiencies may range between 80% and 90% (Kadlec et al., 2000). In the present study the removal achieved was between 41% and 93%, varying to some extent with the mass loading. Particulate matter constitute a significant fraction of the organic matter entering in wastewater treatment plants and the removal processes will not only reduce overall amount of organic matter but also modify the chemical composition of the remaining organic matter (Sophonsiri and Morgenroth, 2004).

The variation in NH_3 and NO_3^- suggests that the nitrification and denitrification process would occur simultaneously. The oxygenation in the ryzosphere is not sufficient for a complete nitrification to take place causing a limitation for nitrogen removal (Kadlec et al., 2000). Although horizontal subsurface flow is considered adequate for organic removal, nutrient removal is lower but comparable to the treatment efficiency of conventional treatments without a special regime for nutrient removal (Kadlec et al., 2000; USEPA, 2000). The phosphorus removal was low but in subsurface wetlands this happens unless the substrate chosen has a high phosphorus binding capacity (Kadlec et al., 2000).

The low chromium level in the wastewater is due to the fact that the facility that supplies the tannery wastewater treatment plant does not use chromium in high amounts and when enters the CWUs that has been removed in the previous physico-chemical operation.

Organic loadings

High organic removals were achieved with all the CWUs along time for the different HLRs. A maximum of 1800 kg BOD_5 ha^{-1} d^{-1} was applied for an HLR of 18 cm d^{-1}, 722 kg BOD_5 ha^{-1} d^{-1} for HLR of 8 cm d^{-1} and 516 kg BOD_5 ha^{-1} d^{-1} for HLR of 6 cm d^{-1}, which means 11.5, 4.6 and 3.3 times, respectively, higher loading than the maximum suggested by USEPA (2000) (157 kg BOD_5 ha^{-1} d^{-1}). COD loading was also high, varying between 485 and 3849 kg COD ha^{-1} d^{-1}. Shepherd et al. (2001) evaluated the potential of CWs to treat high strength winery wastewater, and loaded CW systems with 345 and 1640 kg COD ha^{-1} d^{-1} and obtained removal efficiencies up to 99%. Masi et al. (2002) also achieved high removal efficiencies (for BOD_5 87.5% and for COD 91.6%) for high organic content winery wastewaters (352 kg COD ha^{-1} d^{-1}).

Vymazal (2001) reported steady and high removal of organics (COD and BOD_5) in HSF CWs in long-term operation basis, where the horizontal subsurface flow (BOD_5) loading of 18 CWs varied between 2.6 and 99.6 kg ha^{-1} d^{-1} and the outflow between 0.32 and 21.7 kg ha^{-1} d^{-1}.

The tendency of the organic removal increase with higher loading is a behavior reported in other studies (Vymazal, 2001; Mbuligwe, 2005; Calheiros et al., 2007). The wetland performance is commonly summarized by regression equations and first-order models (Kadlec et al., 2000).

According to the data from this study the three substrates provided a good platform for *T. latifolia* establishment being the expanded clay aggregates more successful than sand, and allowing a higher removal efficiency especially for organics. Scholz and Xu (2002) compared several substrates packed in different order (gravel, sand, granular activated carbon, charcoal and Filtralite), in vertical flow wetlands, and reported no significant performance increases in lead, copper and BOD_5 reduction, during the set-up phase of 10 months. Furthermore, the presence of macrophytes did not lead to a higher removal performance for the same parameters tested at a laboratory scale. Khatiwada and Pradhan (2006) reported that no significant effect of media size was verified for BOD removal but a significant relation between media size (sand, pebbles and gravel) and the performance of CW in removing COD,

NH_4^+–N, NO_2^-–N and NO_3^-–N, both in horizontal and vertical flow wetlands, was found. However besides the media size, the bed depth and the root depth were also found to have significant effect on performance of CW.

The experimental results indicate that an HLR of 6 cm d^{-1} ensures a quite stable and considerably high removal of organics during different seasons. As the HLR increased the removal efficiency decreased, although the organic mass removal at an HLR of 6 cm d^{-1} was similar to that at an HLR of 8 cm d^{-1}, which indicates that HLRs of up to 8 cm d^{-1} may be adequate for this system. However, the fact that the system operated for different time periods for each HLR counteracts for a fair comparison.

This study further suggests constructed wetlands as a feasible option for tannery wastewater treatment especially when organics removal is set as target, although there are still needs for proper data on field scale studies applied to tannery industry with physico-chemical and microbiological evaluations.

Conclusions

- The Freundlich isotherm model was found to provide a good prediction for the sorption of organic matter into the three substrates (Filtralite®MR3–8, Filtralite®NR3–8, and fine gravel), although their adsorption capacity is low.
- The three substrates were adequate for *T. latifolia* development although there was higher propagation and organic removal for the expanded clay aggregates when compared to the fine gravel. COD and BOD_5 removals up to 82% (789 kg ha^{-1} d^{-1}) and 69% (258 kg ha^{-1} d^{-1}), respectively, were achieved in the expanded clay units.
- Overall, the expanded clay aggregates planted with *T. latifolia* demonstrated significant higher percentage removals in terms of COD and BOD_5 when compared with the unplanted unit after long-term operation.
- The treatment efficiency, especially for organics, was considered satisfactory, having in consideration the complexity of the wastewater tested, since it was possible to load the constructed wetlands at higher rates than the recommended in the literature. Mass removals up to 1869 kg COD ha^{-1} d^{-1} and 652 BOD_5 kg ha^{-1} d^{-1} were achieved.

Acknowledgements

The authors thank Dias Ruivo, Curtumes e Produtos Industriais, Lda for making possible the establishment of this project and to maxit - Argilas Expandidas, SA that kindly offered Filtralite®. Cristina S.C. Calheiros wishes to thank a research grant from Fundação para a Ciência e Tecnologia (FCT), Portugal (SFRH/BDE/15507/2004). The work was supported by the Project POCI/AMB/60126/2004. The authors are grateful to Dr. Mafalda Quintas that provided support on statistical analysis for the adsorption isotherms studies.

References

Annadurai, G., Rajesh Babu, S., Mahesh, K.P.O., Murugesan, T., 2000. Adsorption and bio-degradation of phenol by chitosan-immobilized *Pseudomonas putida* (NICM 2174). Bioprocess. Eng. 22, 493–501.

AOAC, 1995. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists Inc., Arlington, VA, USA.

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

Bendix, M., Tornbjerg, T., Brix, H., 1994. Internal gas transport in *Typha latifolia* L. and *Typha angustifolia* L. 1. Humidity-induced pressurization and convective throughflow. Aquat. Bot. 49, 75–89.

Calheiros, C.S.C., Rangel, A.O.S.S., Castro, P.M.L., 2007. Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater. Water Res. 41 (8), 1790–1798.

Daniels, R., 2001. Enter the root-zone: green technology for the leather manufacturer, part 2. World Leather. 14 (5), 52–54.

Doran, P.M., 1995. Adsorption. In: Bioprocess Engineering Principles. Academic Press, UK.

FAO, 2006. Guidelines for Soil Description, fourth ed. Food and Agriculture Organization of the United Nations, Rome, Italy.

Gupta, A.K., Sinha, S., 2007. Phytoextraction capacity of the *Chenopodium album* L. grown on soil amended with tannery sludge. Bioresour. Technol. 98, 442–446.

INETI, 2000. Guia Técnico do Sector dos Curtumes. Instituto Nacional de Engenharia e Tecnologia Industrial, Lisboa, Portugal (in Portuguese).

IULTCS, 2004. Typical performance for tannery waste water treatment (IUE5). In: Technical Guidelines for Environmental Protection Aspects for the World Leather Industry. International Union of Environment (IUE) Commission of International Union of Leather Technologists and Chemists Societies.

Kadlec, R.H., Knight, R.L., Vymazal, J., Brix, H., Cooper, P., Haberl, R., 2000. Constructed wetlands for pollution control – processes, performance, design and operation. IWA Scientific and Technical Report No. 8. IWA Publishing, London, UK.

Khatiwada, N.R., Pradhan, B.B.N., 2006. Media effects on performance of constructed wetlands. In: 10th International Conference on Wetland Systems for water pollution control, Lisbon, Portugal, pp. 1169–1176.

Kirkpatrick, A., 2004. Selected plant species characteristics and suitability for use in constructed wetlands. Montana State University Extension Water Quality Program, USA.

Klar, A.E., Jadoski, S.O., Lima, G.P.P., 2006. Peroxidase activity as an indicator of water stress in sweet pepper plants. Irriga. Botucatu. 11 (4), 441–447.

Korkusuz, E.A., 2005. Manual of Practice on Constructed Wetlands for Wastewater Treatment and Reuse in Mediterranean Countries. Report, MED-REUNET II Support Programme (EC Project No: INCO-CT-2003-502453). Agbar Foundation, Spain. </http://med-reunet.org//>.

Kucuk, O.S., Sengul, F., Kapdan, I.K., 2003. Removal of ammonia from tannery effluents in a reed bed constructed wetland. Water Sci. Technol. 48 (11–12), 179–186.

Lisboa, C.S., Rosa, C.L.G., Barbosa, T.M.C., Pinho, H.J.O., Mateus, D.M.R., 2004. Remoção de fósforo de efluentes líquidos. Tecnologia da água, Edição I, pp. 14–18 (in Portuguese).

Madsen, T.V., Brix, H., 1997. Growth, photosynthesis and acclimation by two submerged macrophytes in relation to temperature. Oecologia 110, 320–327.

Markkola, A.M., Tarvainen, O., Ahonen-Jonnarth, U., Strömmer, R., 2002. Urban polluted forest soils induce elevated root peroxidase activity in Scots pine (*Pinus sylvestris* L.) seedlings. Environ. Pollut. 116, 273–278.

Masi, F., Conte, G., Martinuzzi, N., Pucci, B., 2002. Winery high organic content wastewaters treated by constructed wetlands in Mediterranean climate. In: 8th International Conference on Wetland Systems for Water Pollution Control, Arusha, Tanzania, pp. 274–281.

Mbuligwe, S.E., 2005. Comparative treatment of dye-rich wastewater in engineered wetland systems (EWSs) vegetated with different plants. Water Res. 39, 271–280.

Namorado, A.M.N., Figueiredo, A.L., Cunha, S.L., Mateus, D.M.R., Pinho, H.J.O., 2004. Utilização de zonas húmidas artificiais na remoção de azoto de efluentes urbanos. Tecnologia da água, Edição I, pp. 30–35 (in Portuguese).

Oliveira, R.S., Dodd, J.C., Castro, P.M.L., 2001. The mycorrhizal status of *Phragmites australis* in several polluted soils and sediments of an industrialised region of Northern Portugal. Mycorrhiza 10, 241–247.

Redlich, O., Peterson, D.L., 1959. A useful adsorption isotherm. J. Phys. Chem. 63, 1024–1026.

Rhoades, J.D., Kandiah, A., Mashali, A.M., 1992. The Use of Saline Waters for Crop Production – FAO Irrigation and Drainage Paper 48. Food and Agriculture Organization of the United Nations, Rome, Italy.

Shepherd, H.L., Grismer, M.E., Tchobanoglous, 2001. Treatment of high-strength winery wastewater using a subsurface-flow constructed wetland. Water Environ. Res. 73 (4), 394–403.

Scholz, M., Xu, J., 2002. Performance of experimental constructed wetlands with different filter media and macrophytes treating industrial wastewater contaminated with lead and copper. Bioresour. Technol. 83, 71–79.

Sophonsiri, C., Morgenroth, E., 2004. Chemical composition associated with different particle size fractions in municipal, industrial, and agricultural wastewater. Chemosphere 55, 691–703.

Stein, O.R., Hook, P.B., 2005. Temperature, plants and oxygen: how does season affect constructed wetland performance? J. Environ. Sci. Health 40, 1331–1342.

Tan, K.H., 1995. Soil Sampling, Preparation and Analysis. The University of Georgia Athens, GA, USA.

USDA, 2006. Plant guide: broad-leaved cattail – *Typha latifolia* L. The Plants Database, Version 3.5. United States Department of Agriculture – Natural Resources Conservation Service, National Plant Data Center, LA, USA.

USEPA, 2000. Wastewater Technology Fact Sheet – Wetlands: Subsurface Flow. EPA: 832-F-00-023. United Sates Environmental Protection Agency, USA.

USEPA, 1995. A Handbook of Constructed Wetlands, A Guide To Creating Wetlands For: Agricultural Wastewater, Domestic Wastewater, Coal Mine Drainage, Stormwater In The Mid-Atlantic Region, Volume1, General Considerations. EPA: 903b95004a. United Sates Environmental Protection Agency, USA.

USEPA, 1994. Plant Peroxidase Activity Determination. EPA, SOP#: 2035, 28/11/94. United Sates Environmental Protection Agency, USA.

Vymazal, J., 2001. Removal of organics in Czech constructed wetlands with horizontal sub-surface flow. In: Vymazal, Jan (Ed.), Transformations of Nutrients in Natural And Constructed Wetlands. Backhuys Publishers, Leiden, The Netherlands, pp. 305–327.

Vymazal, J., Kröpfelová, L., 2006. Can we predict processes in the filtration bed of horizontal-flow constructed wetlands according to dissolved oxygen concentrations at the outflow? In 10th International Conference on Wetland Systems for water pollution control, Lisbon, Portugal, pp. 549–557.

Wellburn, A.R., 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant. Physiol. 144, 307–313.

Yoo, E.S., Libra, J., Adrian, L., 2001. Mechanism of decolorization of azo dyes in anaerobic mixed culture. J. Environ. Eng. 127 (9), 844–849.

Yoshida, K., Kaothien, P., Matsui, T., Kawaoka, A., Shinmyo, A., 2003. Molecular biology and application of plant peroxidase genes. Appl Microbiol. Biotechnol. 60, 665–670.