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Influence of bed media characteristics on ammonia and nitrate removal in shallow horizontal subsurface flow constructed wetlands

A. Albuquerque [a,*], J. Oliveira [b,1], S. Semitela [a], L. Amaral [c,2]

[a] Department of Civil Engineering and Architectural and C-MADE, University of Beira Interior, Bloco II das Engenharias, Calcada do Lameiro, 6201-001 Covilha, Portugal
[b] Municipality of Penamacor, Largo do Munícipio, 6090-543 Penamacor, Portugal
[c] Department of Sciences and Environmental Engineering, Faculty of Sciences and Technology, New University of Lisbon, Monte de Caparica, 2829-516 Caparica, Portugal

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ABSTRACT

Two bed media were tested (gravel and Filtralite) in shallow horizontal subsurface flow (HSSF) constructed wetlands in order to evaluate the removal of ammonia and nitrate for different types of wastewater (acetate-based and domestic wastewater) and different COD/N ratios. The use of Filtralite allowed both higher mass removal rates (1.1 g NH_4–N m^{-2} d^{-1} and 3 g NO_3–N m^{-2} d^{-1}) and removal efficiencies (>62% for ammonia, 90–100% for nitrate), in less than 2 weeks, when compared to the ones observed with gravel. The COD/N ratio seems to have no significant influence on nitrate removal and the removal of both ammonia and nitrate seems to have involved not only the conventional pathways of nitrification–denitrification. The nitrogen loading rate of both ammonia (0.8–2.4 g NH_4–N m^{-2} d^{-1}) and nitrate (0.6–3.2 g NO_3–N m^{-2} d^{-1}) seem to have influenced the respective removal rates.

1. Introduction

The use of HSSF reed beds for wastewater treatment (normally domestic, surface runoff, industrial and leachate from sanitary landfills) has received increasing attention in the last decade (EPA, 1999; IWA, 2000; Vymazal, 2003; Korkusuz, 2005; Wallace and Knight, 2006; Vymazal and Kropfelova, 2008; Kadlec and Wallace, 2008). Although it is considered a low-cost technology, it also consumes less energy and chemicals and uses less mechanical devices, when compared to conventional treatment technologies (e.g. activated sludge systems).

The media (substratum) of HSSF beds, usually based on gravel, normally present clogging problems, whose causes are related to both the variation of properties of the media and the characteristics of the wastewater. The gradual clogging of the bed media leads to resistance to flow, particularly near inlets, and, therefore, to the reduction of the volume available for treatment. In the last few years, alternative bed media (e.g. expanded clay aggregates or thermoplastics) have been developed in order to minimize the clogging problem or to increase the treatment capacity since they present both higher porosity and specific surface area, which allow a better biofilm adhesion (Grady et al., 1999; Metcalf and Eddy, 2003). Light-expanded clay aggregates (LECA) have shown both good water permeability and phosphorus removal capability (Wehrle-Werk, 2003; Vilpas et al., 2005; van Deun and van Dyck, 2008).

The direct atmospheric oxygen diffusion through the upper layer into the bed, which is the major supplier of oxygen to the subsurface environment, is normally low (EPA, 1999; IWA, 2000; Vymazal, 2003), which may compromise aerobic microbiological removal pathways such as respiration and nitrification. According to Caselles-Osorio (2006) at low-to-moderate organic loading rates (4–12 g COD m^{-2} d^{-1}) atmospheric oxygen diffusion may not be sufficient to supply adequate amounts of oxygen for nitrification. Therefore, HSSF reed beds may be seen as anoxic/anaerobic biofilm reactors and most of the biological removal pathways occur in the biofilm developed in the media bed, roots and rhizomes in the presence of low concentrations of dissolved oxygen.

However, Garcıa et al. (2004) have observed that shallow HSSF beds (0.27 m of water table) removed organic matter and nitrogen at very high rates and higher than deeper beds (0.5 m). The supporting data indicated that the shallower beds (0.2–0.4 m in depth) were more oxidized, possibly due to the shallow water and enhanced oxygen diffusion at the air–water interface.

The release of oxygen through roots and rhizomes (transported by the aerenchyma helophytes into the rhizosphere) contributes to a complex environment of aerobic, anoxic and anaerobic microzones in the subsurface of the HSSF (Randerson, 2006). The oxygen

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[*] Corresponding author. Tel.: +351 275329981; fax: +351 275329969.
E-mail address: ajca@ubi.pt (A. Albuquerque).
[1] Tel.: +351 277394106; fax: +351 277394196.
[2] Tel.: +351 212948397.

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supplied through this way may range from 0 to 12 g m^{-2} d^{-1} (Armstrong et al., 1990; IWA, 2000). However, the oxygen release in this source is rapidly consumed by aerobic microorganisms in the biofilm attached to roots and rhizomes, due to the presence of high oxygen demand compounds. Therefore, different organic and N transformation processes can occur simultaneously in the rhizosphere.

N is an important element in wetland biogeochemical cycles since it occurs in different oxidation states and is present in particulate and dissolved organic and inorganic forms (organic N, ammonia, nitrite and nitrate). N losses in the bed are related to biological removal pathways (e.g. nitrification and denitrification), plant uptake, volatilization, filtration, sedimentation, adsorption and microbial assimilation (IWA, 2000; Vymazal and Kropfelova, 2008; Kadlec and Wallace, 2008). The removal of ammonia and nitrate is of special interest because they can be toxic to aquatic species and vertebrates and may stimulate eutrophication.

Nitrification, a chemoautotrophic process, consists of the biological aerobic oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (nitritation) or nitrate (nitratation) by nitriteoxidizing bacteria, and is the most important ammonia removal pathway (Grady et al., 1999; Metcalf and Eddy, 2003) in biofilm reactors. However, in HSSF beds dissolved oxygen (DO) may not be enough for full nitrification to nitrate but only for partial nitrification to nitrite. Normally, DO levels below 2 mg L^{-1} in water substantially reduce nitrification (Grady et al., 1999; Metcalf and Eddy, 2003). However, some authors have reported the occurrence of nitrification for DO concentrations around 0.8 mg L^{-1} (Schmidt et al., 2003; Paredes et al., 2007). The ratio between influent chemical oxygen demand and N (COD/N) is one of the most critical parameters of the nitrification process, because it directly influences the growth competition between autotrophic and heterotrophic microorganisms and therefore defines the biofilm composition.

Although nitrification is widely believed to be an oxic process, investigations have shown that at least ammonia oxidizers are able to oxidize ammonia under anoxic conditions (Schmidt et al., 2003; Ahn, 2006; Paredes et al., 2007). Various heterotrophic and lithotrophic microorganisms, including bacteria (actinomycetes and planctomycetes), algae and fungi have also been reported to have nitrifying activity (Ahn, 2006). When pH is below 6.5 the free ammonia concentration becomes too low for sufficient growth of the ammonia oxidizers whilst a high pH (up to 9) benefits the ammonia oxidizers (Grady et al., 1999; IWA, 2000; Metcalf and Eddy, 2003). However, ammonia oxidizers have a low affinity for ammonia (affinity constant in the range 20–40 mg NH_4–N L^{-1}). In addition, HNO_2 inhibits the ammonia oxidizers, but they can tolerate high concentrations of nitrite (>0.5 g NO_2–N L^{-1}) at pH 7 (van Dongen et al., 2001).

The anaerobic/anoxic environment and the availability of organic compounds resulting either from the wastewater itself or by rhizodeposition products seem to be propitious for nitrite or nitrate reduction through denitrification (a stepwise anoxic reduction process in which those two anions are reduced to molecular N or N gases by chemoorganotrophic, lithoautotrophic, and phototrophic bacteria and fungi (Grady et al., 1999; Metcalf and Eddy, 2003; Ahn, 2006)).

Vymazal and Kropfelova (2008) reported that N removal in most CW is low when compared to organics and solids, varying between 40% and 55% and can rarely be higher because of their inability to provide oxic conditions for nitrification and anoxic conditions for denitrification simultaneously. In micro and mesocosm systems (Caselles-Osorio and García, 2007), as well as in wetland channels (El Refaie et al., 2004), both with shallow beds (water depths from 0.2 to 0.4 m), N removal efficiencies from 20% to 90% have been registered.

Plant uptake and matrix adsorption are other mechanisms involved in N cycling in wetland systems. The latter process has a finite storage capacity and depends on the characteristics of the substratum. For another side, the stored N can be re-mineralized back into solution or undergo desorption. Plants need nutrients for growth, which in rooted macrophytes are taken up primarily through their roots although some nutrients are taken through immersed stems and leaves from the surrounding water (Vymazal, 2003; IWA, 2000) and play an important role in evapotranspiration.

Currently, the importance of the different N removal pathways are well known (IWA, 2000; Wallace and Knight, 2006; Kadlec and Wallace, 2008), however, a growing interest is noted to better understand the role of non-conventional pathways in the subsurface environment such anaerobic ammonia oxidation and heterotrophic nitrification (Kuai and Verstraete, 1999; Schmidt et al., 2003; Paredes et al., 2007; Tao and Wang, 2009).

The operation of HSSF systems for domestic wastewater treatment (EPA, 1999; IWA, 2000; Korkusuz, 2005; Wallace and Knight, 2006; Vymazal and Kropfelova, 2008; Kadlec and Wallace, 2008) normally uses organic loading rates (OLR) from 5 to 25 g COD m^{-2} d^{-1}, N loading rates (NLR) from 1 to 5 g N m^{-2} d^{-1}, hydraulic loading rates (HLR) from 2 to 20 cm d^{-1} and hydraulic retention times (HRT) from 3 to 10 d.

The objective of this work was to study the influence of the characteristics of two bed media (gravel and LECA) on ammonia and nitrate losses in a shallow HSSF bed, using a mesocosm and both different COD/N ratios and wastewaters (acetate-based and primary treated domestic wastewater).

2. Methods

2.1. Experimental set-up

A pilot-scale mesocosm (2.0 × 0.8 × 0.7 m; length × width × height) was used and two different bed materials were tested: gravel (effective diameter of particle ranging from 40 to 70 mm and specific surface area of 700 m^2 m^{-3}) and a LECA with the commercial name of Filtralite NR (effective diameter of particle ranging from 4 to 8 mm and specific surface area of 1250 m^2 m^{-3}). The bed porosity with gravel was 0.40 and with Filtralite was 0.45. The water depth was kept at 0.2 m as in Renker and Albuquerque (2007) and Caselles-Osorio and Garcia (2006). The top bed was planted with common reed (*Phragmites australis*).

Nine sampling points (PI1–PI9) were provided along the bed to collect water samples for analytical measurements. However, only points PI2 (33 cm away from inlet), PI5 (67 cm away from inlet) and PI8 (90 cm away from inlet) were used in the experiments. The inlet device was composed of a “T” tube with several holes in order to enhance a homogeneous distribution of the influent flow into the bed. Three piezometers (PIEZ1, PIEZ2 and PIEZ3) were included in order to evaluate the evolution of head losses in three sections over time.

2.2. Operating conditions

The operation of the mesocosm involved three phases: colonization (development of biofilm in the media and the installation of plants), experimental assays for different ammonia and nitrate loads and wastewaters and change of the media bed. Four Series (G) for gravel (25 weeks long) and four Series (F) for Filtralite (23 weeks long) were performed as shown in Fig. 1.

For colonization the bed was inoculated with biomass collected in the recirculation line of and activated sludge system (i.e. form the settling tank to the activated sludge tank), which was stored

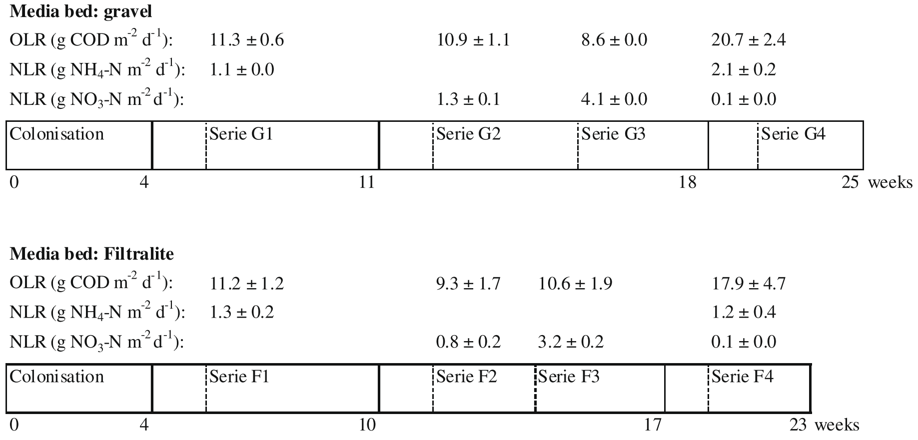

Fig. 1. Operating procedure.

in anoxic/anaerobic conditions. The colonization took approximately 4 weeks in a closed circuit (flow rate of 1 L h^{-1} and HLR of 4 cm d^{-1} (gravel) and 3.5 cm d^{-1} (Filtralite)) with discontinuous feeding (fed-batch mode) and a synthetic solution (0.5 mL L^{-1} of acetate and ammonia solutions were added to the bed every 24 h in order to ensure the concentrations of 100 g COD L^{-1} and 10 g NH_4–N L^{-1}). The measurements of DO, pH, temperature, COD and NH_4–N were performed at the end of each cycle before feeding (every day) in the influent, PI5 and the effluent. In the beginning of this stage, the bed was also rooted with 12 feet of *P. australis*.

The bed was, therefore, operated continuously as HSSF stage for different N loads and C/N ratio during 21 (Series G) and 19 (Series F) weeks. For each type of material 4 Series of assays were executed. Series G1 and F1 (approximately 300 mg COD L^{-1} and 30 mg NH_4–N L^{-1}), Series G2 and F2 (approximately 300 mg COD L^{-1} and 30 mg NO_3–N L^{-1}) and Series G3 and F3 (approximately 300 mg COD L^{-1} and 100 mg NO_3–N L^{-1}) with a synthetic wastewater. The COD and N concentrations were obtained by diluting the concentrated solutions of sodium acetate, ammonia chloride and potassium nitrate (Section 2.3 and Table 1). Series G4 and F4 (300–600 mg COD L^{-1} and 20–40 mg N L^{-1}) with primary treated domestic wastewater. This wastewater was produced in a small village with 500 inhabitants, without significant non-point sources, and was collected at the entrance of a real-scale HSSF bed, after primary treatment in a Imhoff tank. The flow rate was kept constant at 1 L h^{-1}, which gave a HLR of 4 cm d^{-1} (gravel) and 3.5 cm d^{-1} (Filtralite) and a HRT of 5.1 d (gravel) and 5.7 d (Filtralite) over the effective bed surface area.

Each Series included the operation of the bed to attain steady state conditions which was assessed through the stabilization of COD and NH_4–N removal in PI2, PI5 and PI8 based on weekly samples. In Fig. 1 is presented a scheme of the operating procedure with the values of OLR and NLR applied in each Series (i.e. average incoming concentration times flow rate over the total area of the bed).

For each Series weekly water samples were collected (6 and 5 in Series G and Series F, respectively) at the influent and sampling points PI2, PI5 and PI8 in order to determine following parameters: pH, DO, temperature, COD, NH_4–N, NO_2–N and NO_3–N. The hydrostatic pressure in the piezometers PIEZ1, PIEZ2 and PIZ3 was also measured weekly. The temperature in the laboratory was kept constant at 20 ± 0.4 °C.

2.3. Synthetic feeding solution

A synthetic feeding solution was used for bed colonization and for the experiments in Series G1 to G3 and Series F1 to F3. The medium included an organic carbon source (sodium acetate solution), ammonia source (ammonia chloride solution), nitrate source (potassium nitrate solution) and mineral source prepared as proposed by Dang et al. (1989) (buffer, magnesium sulphate, calcium chloride and iron chloride solutions), enriched with an oligoelements solution (according to the French standard NF EN 29439) as in Renker and Albuquerque (2007), whose composition is presented in Table 1.

The mineral medium was the same for colonization and experiments of Series G1 to G3 and F1 to F3 and was prepared by diluting the respective concentrated solutions. The desired concentrations of COD, NH_4–N and NO_3–N for colonization and the experiments were obtained by diluting the concentrated solutions of sodium acetate, ammonia chloride and potassium nitrate (the corresponding volumes are presented in Table 1). The feeding solution was kept in a storage tank with 72 L (ISCO FTD 220) at a

Table 1
Composition of solutions used to prepare the feeding solutions for Series G1 to G3 and F1 to F3.

Type of solution	Composition	Concentration (g L^{-1})	Volume used to prepare feeding solutions (mL L^{-1})
Buffer	KH_2PO_4	8.50	2
	K_2HPO_4	21.75	
	$Na_2HPO_4 \cdot 7H_20$	33.40	
	NH_4Cl	1.70	
Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	22.50	0.2
Calcium chloride	$CaCl_2 \cdot 2H_2O$	36.43	0.2
Iron chloride	$FeCl_3 \cdot 6H_2O$	0.250	0.2
Oligoelements	$MnSO_4 \cdot 4H_2O$	0.040	0.2
	H_3BO_3	0.060	
	$ZnSO_2 \cdot 7H_2O$	0.040	
	EDTA	0.0555	
	$FeCl_3 \cdot 6H_2O$	0.0445	
	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.032	
Ammonia chloride (20 g NH_4–N L^{-1})	NH_4Cl	76.41	0.5; 1.5[a]
Potassium nitrate (20 g NO_3–N L^{-1})	K_2NO_3	144.4	1.5; 5[b]
Sodium acetate (50 g COD L^{-1})	$CH_3COONa \cdot 3H_2O$	113.4	2; 6[c]

[a] 0.5 mL L^{-1} and 1.5 mL L^{-1} to get 10 mg NH_4–N L^{-1} and 30 mg NH_4–N L^{-1}, respectively.
[b] 1.5 mL L^{-1} and 5 mL L^{-1} to get 30 mg NO_3–N L^{-1} and 100 mg NO_3–N L^{-1}, respectively.
[c] 2 mL L^{-1} and 6 mL L^{-1} to get 100 mg COD L^{-1} and 300 mg COD L^{-1}, respectively.

constant temperature of 4 ± 0.5 °C and pumped to the bed through a peristaltic pump ISMATEC MCP CA4 (Switzerland).

2.4. Analytical methods

The measurements of DO, pH and temperature were carried out through probes SenTix 41 and CellOx 325 connected to a Multi 340i meter (WTW, Germany). The COD was evaluated by closed reflux digestion and titrimetric method (APHA-AWWA-WEF, 1999). Concentrations of N compounds (NH_4–N, NO_2–N and NO_3–N) were obtained using the cuvette-tests LCK 302 (47–130 mg NH_4–N L^{-1}), LCK 303 (2–47 mg NH_4–N L^{-1}), LCK 304 (0.015–2 mg NH_4–N L^{-1}), LCK 341 (0.015–0.6 mg NO_2–N L^{-1}), LCK 342 (0.6–6 mg NO_2–N L^{-1}), LCK 339 (0.23–13.5 mg NO_3–N L^{-1}) and LCK 340 (5–35 mg NO_3–N L^{-1}), following standards DIN 38406-E 5-1 (ammonia), DIN 38405 D10 (nitrite) and DIN 38405-9 (nitrate), and the CADAS 50 spectrophotometer UV–vis (HACH LANGE, Germany). For higher concentrations than the upper limits of the cuvette-tests the samples were previously diluted.

The results were treated with the SPSS statistical software (SPSS Inc., Chicago, USA), namely the tests for correlations between variables.

3. Results and discussion

3.1. Set-up operation

Steady-state conditions in each Series were, generally, achieved after one week operation, as it can be seen in Fig. 2 for NH_4–N removal (ΔNH_4–N), two different feeding conditions and two bed materials. The results for each Series are presented in Table 2. There was no detection of NO_2–N in the sampling points (all the values were below the detection limit).

There was no significant change in the hydrostatic pressure measured in the three piezometers and the head losses were minimal during the operating period. Therefore, it can be assumed that the bed clogging was insignificant and had no influence on the bed effectiveness.

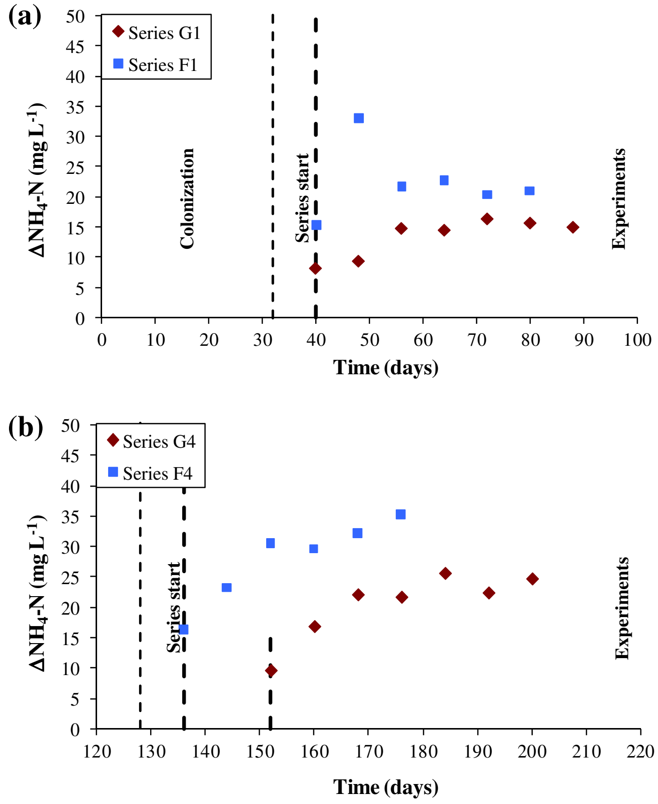

Fig. 2. Steady-state conditions in terms of ammonia nitrogen removal: (a) experiments with synthetic wastewater (Series G1 and F1) and (b) experiments with domestic wastewater (Series G4 and F4).

Table 2
Results for each Series.

Parameter	Point	G1	G2	G3	G4	F1	F2	F3	F4
COD/N[a]	Influent	10.1 ± 0.2	8.7 ± 0.8	2.5 ± 0	9.6 ± 0.8	8.9 ± 1.3	9.8 ± 1.2	2.7 ± 0.7	12.1 ± 2.4
pH	Influent	7.0–7.4	7.0–7.2	7.7	7.0–7.2	7.1–7.3	7.6–8.0	7.3–8.0	6.9–7.3
	PI2	7.5–8.0	8.6–9.0	9.1	7.1–7.3	7.3–7.9	8.3–8.9	9.0–9.2	7.2–7.8
	PI5	7.3–7.7	7.7–8.5	9.2	7.0–7.1	7.7–8.1	8.2–8.5	8.8–9.1	7.2–8.0
	PI8	7.3–7.9	7.5–8.2	8.2	6.9–7.0	7.5–8.0	8.2–8.2	8.2–8.3	7.1–7.7
DO (mg L^{-1})	Influent	2.1 ± 1.0	2.0 ± 1.0	2.2 ± 1.1	1.3 ± 0.1	2.4 ± 1.3	2.2 ± 1.1	2.5 ± 1.1	1.8 ± 1.0
	PI2	0.1 ± 0	0.3 ± 0	0.4 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0	0.2 ± 0	0.2 ± 0
	PI5	0.1 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.2 ± 0
	PI8	0.1 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
COD (mg L^{-1})	Influent	283 ± 14	285 ± 25	215 ± 0	518 ± 59	316 ± 34	301 ± 55	263 ± 76	507 ± 133
	PI2	151 ± 46	142 ± 24	50 ± 0	227 ± 20	203 ± 22	60 ± 14	118 ± 20	94 ± 14
	PI5	122 ± 46	158 ± 51	46 ± 0	202 ± 11	197 ± 13	67 ± 14	112 ± 17	57 ± 8
	PI8	91 ± 43	126 ± 33	30 ± 0	154 ± 20	94 ± 32	54 ± 5	97 ± 59	32 ± 16
NH_4–N (mg L^{-1})	Influent	28 ± 1	–	–	54 ± 5	36 ± 6	–	–	34 ± 12
	PI2	20 ± 1	–	–	39 ± 4	30 ± 3	–	–	23 ± 5
	PI5	18 ± 1	–	–	40 ± 2	29 ± 4	–	–	15 ± 5
	PI8	15 ± 2	–	–	31 ± 2	15 ± 8	–	–	4 ± 3
NO_3–N (mg L^{-1})	Influent	–	32 ± 1	103 ± 0	0.5 ± 0.1	–	22 ± 9	91 ± 4	0.3 ± 0
	PI2	–	<0.23[b]	51 ± 0	0.3 ± 0.1	–	<0.23[b]	11 ± 10	<0.23[b]
	PI5	–	<0.23[b]	50 ± 0	<0.23[b]	–	<0.23[b]	14 ± 14	<0.23[b]
	PI8	–	<0.23[b]	51 ± 0	<0.23[b]	–	<0.23[b]	9 ± 9	<0.23[b]

Note: average values and confidence interval (calculated for a confidence level of 95% and 6 values for Series G1 and G4, 5 values for F1 and F4 and 3 values for G2, G3, F3 and F4), except for pH, which is presented the range of values.

[a] For Series G4 and F4, N is the sum of NH_4–N and NO_3–N concentrations.

[b] Lower limit of the cuvette-test LCK 339.

The DO detected at the inlet point ranged from 2 to 2.5 mg O_2 L^{-1} (experiments with synthetic wastewater) and 1.3 to 1.8 mg O_2 L^{-1} (experiments with domestic wastewater). Although shallow beds are considered more favourable for oxygen diffusion from atmosphere into the subsurface (Caselles-Osorio and Garcia, 2006), in the present work DO detected in the three sampling points ranged from 0.1 to 0.4 mg O_2 L^{-1}. This circumstance is explained by the high levels of oxygen demand presents in the wastewaters and the DO was rapidly consumed through aerobic respiration and chemical oxidation. DO limitation is quite common in HSSF beds treating high organic or N load wastewaters either with low depths (Caselles-Osorio, 2006; Caselles-Osorio and Garcia, 2006) or high depths (IWA, 2000; Vymazal and Kropfelova, 2008).

The pH in the Series with ammonia as N source varied little and was generally below 8, despite the removal efficiency of NH_4–N having reached 45% and 62% for Series G1 and F2, respectively. This circumstance resulted from the buffer effect created by the simultaneous removal of acetate which, by generating alkalinity, compensated the consumption of alkalinity by nitrification. In Series with nitrate as N source (G2, G3, F2 and F3), the pH increased significantly (namely in Series G3 and F3 where it reached up to 9.2) due to the production of alkalinity through acetate oxidation and denitrification reactions.

3.2. Mass removal rates

Mass removal rates for COD (r_{COD}), ammonia (r_{NH_4-N}) and nitrate (r_{NO_3-N}) give a valuable information on the removal of organic matter and N per unit of area (EPA, 1999; IWA, 2000; Vymazal, 2003) and, therefore, may be compared with the correspondent applied loads (OLR and NLR). The mass removal rates were calculated through Eq. (1) and are presented in Table 3.

$$r_X = (C_i - C_e) \cdot Q/Au \qquad (1)$$

where r_X is the removal rate for the parameter X (g m^{-2} d^{-1}), C_i and C_e are the influent and effluent concentrations of the parameter (g m^{-3}), Q is the average flow rate (m^3 d^{-1}) and Au is the total effective area of the bed (m^2).

COD removal was similar in all the assays with the easily biodegradable organic substrate (acetate) and COD/N ratio around 10, with higher values for Filtralite (6.4–7.7 g COD m^{-2} d^{-1} for gravel; 6–8.3 g COD m^{-2} d^{-1} for Filtralite). However, when the bed was fed with synthetic wastewater and COD/N ratio around 3 or real wastewater (variety of complex organic substrates) and COD/N around 10, the r_{COD} were higher for Filtralite (9.1 and 16.8 g COD m^{-2} d^{-1}, respectively). The removal efficiency (RE) was quite similar in the first case (86% for gravel, 88.6% for Filtralite) and very different in the second case (70.5% for gravel, 94% for Filtralite). Therefore, in the presence of higher nitrate concentrations or complex organic substrates, the microorganism responsible for organics removal (both heterotrophic oxidizers and denitrifiers) seems to be better adapted over the surface of Filtralite, probably because the properties of this material (namely the micro-porosity) enhance a quick and stable biofilm development.

The r_{COD} and the corresponding RE for Series with gravel were lower than the ones found by other authors in shallow HSSF mesocosms (gravel-based, colonized with reed beds and similar OLR, NLR, HLR and HRT) with domestic wastewater (El Refaie et al., 2004; Caselles-Osorio and García, 2007) and glucose (according to Caselles-Osorio and Garcia (2006) and Camacho et al. (2007)), as presented in Table 3. Calheiros et al. (2008) observed lower RE (52%) in mesocosms with lower HRT (3.4 d) in the treatment of tannery wastewater.

The values obtained with Filtralite are higher than the ones reported by Vilpas et al. (2005) in two pilot HSSF beds treating domestic wastewater and by Scholz using a synthetic wastewater (Table 3), with similar operating conditions. Calheiros et al. (2007) observed a lower RE (58%) in similar mesocosms

Table 3

Variation of the overall applied and removed loads for both bed materials.

Bed material	Wastewater	OLR (g COD m^{-2} d^{-1})	r_{CQO} (g COD m^{-2} d^{-1})	RE (%)	NLR (g NH_4–N m^{-2} d^{-1})	r_{NH_4-N} (g NH_4–N m^{-2} d^{-1})	RE (%)	NLR (g NO_3–N m^{-2} d^{-1})	r_{NO_3-N} (g NO_3–N m^{-2} d^{-1})	RE (%)	Source
Gravel	Synthetic (G1)	11.3 ± 0.6	7.7 ± 1.5	68.1	1.1 ± 0.0	0.5 ± 0.1	45.5	–	–	–	This study
	Synthetic (G2)	11.4 ± 1.0	6.4 ± 1.6	56.1	–	–	–	1.3 ± 0.0	1.3 ± 0.6	100	
	Synthetic (G3)	8.6 ± 0.0	7.4 ± 0.0	86.0	–	–	–	4.1 ± 0.0	2.1 ± 0.0	51.2	
	Domestic (G4)	20.7 ± 2.4	14.6 ± 3.1	70.5	2.1 ± 0.2	0.9 ± 0.6	43.0	0.1 ± 0.0	0.1 ± 0.0	100	
Filtralite	Synthetic (F1)	11.2 ± 1.2	8.3 ± 1.7	74.1	1.3 ± 0.2	0.8 ± 0.6	61.5	–	–	–	This study
	Synthetic (F2)	9.3 ± 2.0	6.0 ± 0.8	64.5	–	–	–	0.8 ± 0.3	0.8 ± 0.3	100	
	Synthetic (F3)	10.6 ± 1.9	9.4 ± 1.9	88.6	–	–	–	3.2 ± 0.2	3.0 ± 0.4	93.4	
	Domestic (F4)	17.9 ± 4.7	16.8 ± 4.0	94.0	1.2 ± 0.6	1.1 ± 0.6	91.7	0.1 ± 0.0	0.1 ± 0.0	100	
Gravel	Synthetic	9.7	8.2	84.5	0.77	0.17	22	0.15	0.08	53.3	Camacho et al., 2007
Gravel	Synthetic	15	13.2	88	1.1	0.60	54.5	–	–	–	Caselles-Osorio and Garcia (2006)
Gravel	Domestic	25	21	84	0.56	0.13	23.2	1	0.34	34	El Refaie et al., 2004
Gravel	Domestic	18.5	15.5	83.8	1.7	1.44	84.7	–	–	–	Caselles-Osorio and Garcia (2007)
Filtralite	Synthetic	1	0.12	12	0.19	0.023	12	0.05	0.02	40	Scholz (2006)
Filtralite	Domestic	5.1–48	3.8–33	69–75	2–6.5	1.8–6.1	90–94	0.5–1.3	0.4–1	77–80	Vilpas et al. (2005)

with higher HRT (6.8 d), but for the treatment of tannery wastewater.

3.3. Nitrogen losses

Regardless of the type of wastewaters, the ammonia removal was low in the assays with gravel, reaching RE of only 45% (synthetic wastewater) and 43% (domestic wastewater).

Results obtained with synthetic wastewater and gravel are lower than the ones observed by Caselles-Osorio and Garcia (2006) in shallow HSSF beds with glucose but higher than the ones reported by Camacho et al. (2007). The results obtained with domestic wastewater and gravel are better than the ones found by El Refaie et al. (2004) but are worse than the values obtained by Caselles-Osorio and García (2007), in any case with COD/N ratios over 10 (Table 3). Calheiros et al. (2008) observed lower RE (33%) in mesocosms with lower HRT (3.4 d), but for the treatment of tannery wastewater.

The results obtained in the Series with Filtralite were significantly better for both wastewaters, reaching a higher r_{NH_4-N} and RE of 61.5% (synthetic wastewater) and 91.7% (domestic wastewater). These results are in the range of those reported by Vilpas et al. (2005) in two pilot HSSF beds treating domestic wastewater and higher than the ones reported by Scholz (2006) using synthetic wastewater (Table 3). Calheiros et al. (2007) observed lower RE (18%) in similar mesocosms with higher HRT (6.8 d), but for the treatment of tannery wastewater.

Therefore, it seems reasonable to assume that the microorganisms responsible for ammonia removal were more active in the presence of Filtralite. The characteristics of this material seem to be suitable for a stable development of autotrophic and heterotrophic microorganism communities, even knowing that they compete among themselves for the same electron acceptor (oxygen).

Regardless of the type of material or the type of wastewater, nitrate removal was complete for NLR below 1.3 g NO_3–N m^{-2} d^{-1} and COD/N ratio around 10 and occurred in the first section of the bed (Influent-PI2). In the assays with higher NLR and COD/N ratio around 3, the nitrate removal was higher with Filtralite, reaching RE of 93.4% (against 51.2% obtained with gravel). The results are better than the ones reported in the literature for shallow HSSF mesocosms with gravel, treating both glucose-based synthetic wastewaters (Camacho et al., 2007) and domestic wastewater (El Refaie et al., 2004), as well as with Filtralite, for synthetic wastewater (Scholz, 2006) and domestic wastewater (Vilpas et al., 2005), in any case with NLR below 1.3 g NO_3–N m^{-2} d^{-1} and COD/N ratios over 3, as showed in Table 3.

The results obtained with gravel in the treatment of domestic wastewater are also according to the values pointed out by EPA (1999), IWA (2000), Vymazal (2003), Wallace and Knight (2006) and Vymazal and Kropfelova (2008), which referred that HSSF gravel beds usually provide high removal of organic matter (BOD5 and COD) and suspended solids but lower N removal (lower than 50%). Therefore, the introduction of Filtralite seems to bring an increase in the removal of organic matter and, in particular, ammonia and nitrate.

The most common pathways for the removal of ammonia and nitrate are nitrification (ammonia), denitrification (nitrate), plant uptake (both), assimilation into biomass (both), ammonia volatilization (ammonia), filtration and sedimentation (IWA, 2000; Vymazal, 2003; Vymazal and Kropfelova, 2008). The contribution of the latter four mechanisms is very low, when compared to biological removal pathways, plant uptake and adsorption (IWA, 2000; Vymazal, 2003; Vymazal and Kropfelova, 2008). The loss of ammonia through volatilization is generally insignificant when compared to nitrification–denitrification, if the pH is below 8 (Vymazal and Kropfelova, 2008), as occurred in Series G1, G4, F1 and F4 (Series with ammonia). Plant uptake may contribute up to 15% of N removal (Tanner, 2001; Caselles-Osorio, 2006), especially in terms of NO_3–N (Vymazal and Kropfelova, 2008) since ammonia becomes toxic to plants by at high concentrations, especially over 100 mg L^{-1} (IWA, 2000; Horne, 2001).

It is possible that Filtralite enhanced the removal of organic carbon and ammonia and nitrate due to adsorption on the expanded clay. However, according to Calheiros et al. (2008) the adsorption of organic matter into Filtralite NR is negligible. Wehrle-Werk (2003) and van Deun and van Dyck (2008) have also concluded that ammonia and nitrate adsorption into Filtralite NR is an insignificant process in terms of the overall treatment performance of CW. Therefore, it seems reasonable to assume that microbiological pathways are the main mechanisms responsible for organic carbon, ammonia and nitrate removal.

The results suggest that replacing the source of N (ammonia by nitrate) from Series G1 to G2 (gravel) and from Series F1 to F2 (Filtralite), keeping the ratio COD/N around 10 and the same operating conditions, contrary to what would be expected, did not result in an increase of COD removal, despite the incoming nitrate concentration (approximately 30 mg NO_3–N L^{-1}) having been totally consumed. In other words, a higher COD removal was expected in those Series because it is presumed that, since the bed was in anoxic conditions, the removal of nitrate should have occurred mainly by denitrification. However, only when the incoming concentration of nitrate tripled (Series F3 and G3), was a higher removal of COD observed (a further 15% with gravel and a further 34% with Filtralite when compared to Series G2 and F2, respectively).

Therefore, in the presence of high concentrations of nitrate (and COD/N ratios around 3), Filtralite seems more favourable for the development of microorganisms capable of removing high nitrate concentrations, such as heterotrophic denitrifiers. This circumstance may be associated to its higher specific surface (1250 m^2 m^{-3} against 700 m^2 m^{-3} of gravel), which allows the adhesion of microorganisms with the ability to carry out different N removal pathways.

Decreasing the COD/N ratio does not seem to influence significantly the removal of nitrate, especially for Filtralite, since an increase was observed in the r_{NO_3-N} when that ratio decreased from 10 to 3, thus contradicting observations from other studies. Lin et al. (2002) observed NO_3–N removal efficiencies over 90% in HSSF beds treating groundwater with up to 47 mg NO_3–N L^{-1} and COD/N of 6.2. The RE declined to 70% when the COD/N ratio decreased to 3. RE from 70% to 100% for NO_3–N have been observed in HSSF for COD/N between 2 and 4 and HRT up to 10 d (Grasselly et al., 2005). These findings suggest that denitrifiers may use not only organic carbon from wastewater. This suggests a role for plant root exudates in supplying such substrates (Randerson, 2006).

The results from Series G1, G2, F1 and F2 better suggest that for a COD/N ratio of 10, the removal of nitrate may not have involved only the use of acetate as carbon source or energy. In other words, its removal may not have occurred only by conventional denitrification, but also through other alternative mechanisms. Ahn (2006) suggests that some autotrophic are able to denitrify, using hydrogen, sulphur compounds, ammonia, nitrite and nitrate as energy source and inorganic carbon sources. Some genera of microorganisms such as *Nitrosomonas* are chemolithoautotrophic able to nitrify and denitrified in the presence and absence of oxygen (Schmidt et al., 2003).

In Series G2 and F2 there was no ammonia in the bed and the COD removal was lower than in Series with ammonia (Series G1 and F1). The oxygen detected at the inlet point (2–2.2 mg L^{-1}) was presumably consumed at the entrance of the bed almost exclusively by aerobic heterotrophic microorganisms in the acetate oxidation, which did not have to compete for oxygen with aerobic autotrophic microorganisms. However, it can be reasonable assumed that there was availability of hydrogen (energy source), carbon dioxide and bicarbonate (carbon source), compounds released during the aerobic oxidation of acetate (Grady et al., 1999), which may have been used by autotrophic denitrifiers to remove nitrate (as suggested by Ahn, 2006). Several studies, carried out in small treatment plants (Kuai and Verstraete, 1999) and groundwater contaminated with nitrate (Moon et al., 2006), where the concentration of carbon was low, have reported the observation of autotrophic denitrification.

Regardless of the type of wastewater and bed material, the removal of COD and ammonia occurred mainly in the initial section of the bed (Influent-PI2), as shown in Fig. 3. Near the inlet point the removal of organic matter and ammonia would have occurred through aerobic microbiological pathways since the DO were higher (1.3–2.5 O_2 mg L^{-1}). It should be noted that section Influent-PI2 was not significantly colonized with plants, thus excluding the additional oxygenation of the medium through roots or the removal of N by plant uptake and, therefore, the N removal occurred mainly through microbiological pathways.

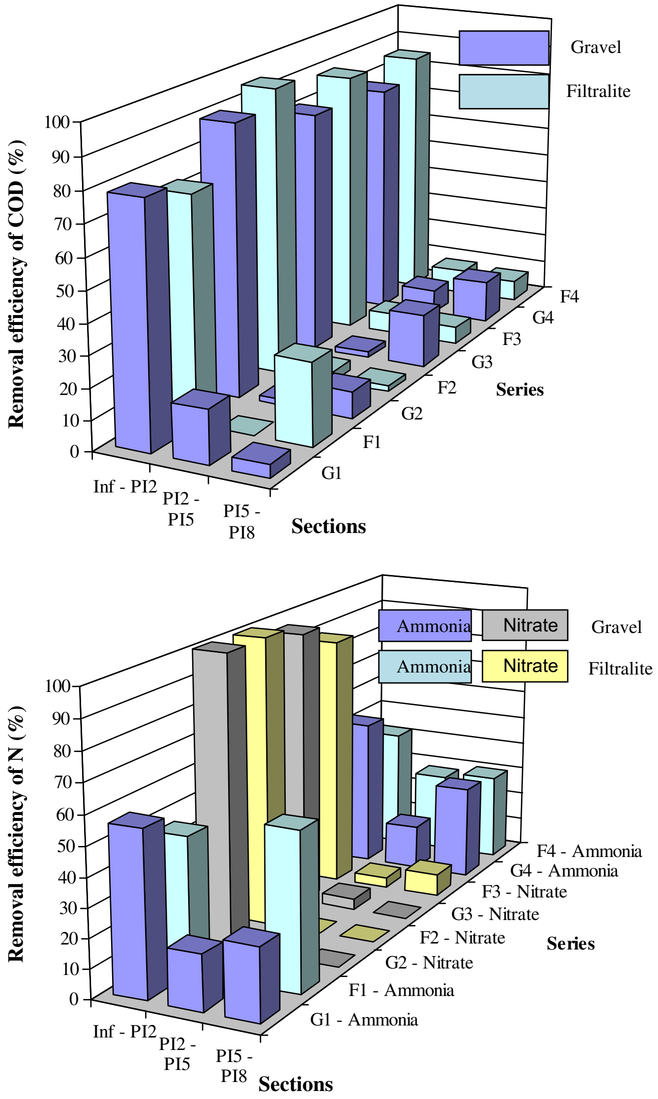

Fig. 3. Removal efficiencies along the bed for both materials: (a) COD and (b) NH_4–N and NO_3–N.

Ammonia removal with Filtralite was always superior to gravel. r_{NH_4-N} were always higher with Filtralite (Table 3) and mostly of the removal occurred in the section Inlet-PI2 (Fig. 3b).

Looking at the DO measured at the inlet point and the ammonia removed (ΔNH_4–N) in first section (Inlet-PI2) in the experiments with ammonia (G1, G4, F1 and F4), it seems there was no enough oxygen to justify that removal either by full nitrification (oxidation to nitrate) or partial nitrification (oxidation to nitrite). An oxygen balance based on the stoichiometric factors (Metcalf and Eddy, 2003; Ahn, 2006; Paredes et al., 2007) 4.57 mg O_2 per mg NH_4–N removed (full nitrification) and 1.71 mg O_2 per mg NH_4–N removed (partial nitrification) shows that the ammonia removed in the section Inlet-PI2 cannot only be explained by nitrification (Table 4) since for both mechanisms resulted a deficit in oxygen. The values are so high that cannot be explained by additional atmospheric oxygen diffusion into the bed.

For another side, considering that it had also organic carbon source in those experiments and, therefore, part of the available DO would have been consumed by heterotrophic microorganisms in the oxidation of organic matter, the deficit of oxygen for nitrification would be greater.

It seems therefore unlikely that there was sufficient oxygen flux to drive the apparent ammonia removal rates observed in the bed via the presumed mechanism of conventional nitrification. In other words, it can be assumed that other non-conventional ammonia removal pathways could had be present. Paredes et al. (2007) mentioned that mechanisms such as autotrophic anaerobic oxidation or heterotrophic nitrification have already been observed in HSSF beds.

Nitrate was present in all the experiments, with higher values in the section Influent-PI2, either introduced though the feeding solution (Series G2, G3, G4, F2, F3 and F4) or generated through ammonia oxidation (Series G1, G4, F1 and F4). However, it was only in Series G3 and F3 that it was experimentally detected in the first sampling point (PI2), when the incoming concentration was 100 mg NO_3–N L^{-1}. The ability of the first section to remove nitrate reached 52 mg NO_3–N L^{-1} in the presence of gravel, and 70 mg NO_3–N L^{-1} in the presence of Filtralite.

In most of the assays the pH ranged within values considered appropriate for organic carbon oxidation, nitrification, denitrification and other non-conventional removal pathways, such as anaerobic autotrophic ammonia oxidation and autotrophic denitrification (between 6.5 and 8.5, according to Grady et al. (1999), Metcalf and Eddy (2003), Ahn (2006), Paredes et al. (2007)). However, in Series with high concentrations of nitrate (G3 and F3), pH reached values above 9. This circumstance is associated with either high acetate oxidation or nitrate reduction, which generated alkalinity, and a lack of activity of the autotrophic nitrifiers (consumers of alkalinity) to stabilize the pH value. However, a pH

Table 4
Results of elementary mass balance calculations for section Inlet-PI2 (Series with ammonia).

Series	Concentrations (mg L^{-1})					
	ΔNH_4–N (Inlet-PI2)	DO available inlet	Full nitrification		Partial nitrification	
			DO requirements[a]	Deficit	DO requirements[b]	Deficit
G1	8	2.1	36.6	−34.5	13.7	−11.6
G4	15	1.3	68.6	−67.3	25.7	−24.4
F1	6	2.4	27.4	−25.0	10.3	−7.9
F4	11	1.8	50.3	−48.5	18.8	−17.0

[a] 4.57 mg O_2 per mg NH_4–N removed (Metcalf and Eddy, 2003; Ahn, 2006; Paredes et al., 2007).

[b] 1.71 mg O_2 per mg NH_4–N removed (Metcalf and Eddy, 2003; Ahn, 2006; Paredes et al., 2007).

above 9 is not inhibitive of both the denitrifying activity and the non-conventional mechanisms of nitrate removal (Metcalf and Eddy, 2003; Ahn, 2006; Paredes et al., 2007) and does not seem to have negatively influenced the removal of nitrate.

However, the absence of microbiological analysis does not allow further discussion on this point.

3.4. Correlations between NLR and nitrogen removal rates

A regression analysis between applied and removed N loads (Fig. 4), shows a linear relationship between NLR and r_{NH_4-N} in the experiments with domestic wastewater (Series G4 and F4) with statistical significance ($p < 0.05$), especially in the presence of Filtralite ($R^2 = 0.96$, $p = 0.002$), and between NLR and r_{NO_3-N} in the experiments with acetate, independently of the COD/N ratio (Series G2, G3, F2 and F3), also in the presence of Filtralite ($R^2 = 0.97$, $p = 0.003$).

Thus, except for experiments of Series G1 and F1, where the NLR did not vary significantly and the hypothesis of linear dependence could not be tested, there is statistical evidence to say that the hypothesis of the influence of the NLR on the removal of ammonia and nitrate cannot be rejected. Therefore, regardless of the type of material used, in the presence of complex substrates the ammonia load influences the respective removal rate, for NLR between 0.8 and 2.4 g NH_4–N m^{-2} d^{-1} and COD/N ratios around 10. Regardless of the type of bed material and wastewater, nitrate removal appears to be influenced by the nitrate load for NLR between 0.6 and 3.2 g NO_3–N m^{-2} d^{-1} and COD/N ratios from 3 to 10. A complete removal of nitrate was observed for NLR below 1.3 g NO_3–N m^{-2} d^{-1}.

There was no linear correlation between both the OLR and r_{COD} and the r_{NO_3-N} (the highest value of R^2 was 0.43, observed between r_{COD} and r_{NO_3-N} in Series F2, $p > 0.05$). In other words, there is no statistical evidence to say that the COD removal was influenced by nitrate removal or that the organic load has influence on nitrate removal, as would be expected if the nitrate removal had occurred only via denitrification. Therefore, these findings reinforce the conviction that nitrate removal may also have occurred through non-conventional mechanisms.

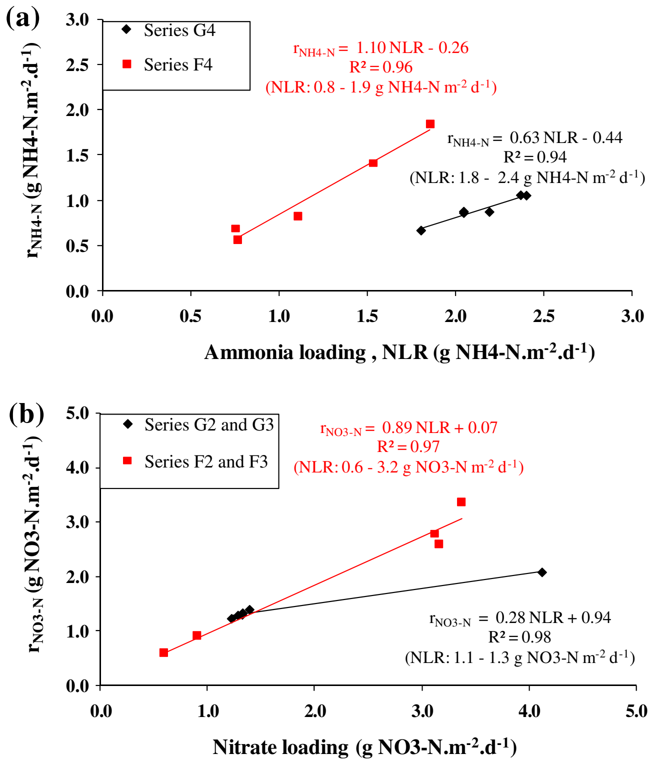

Fig. 4. Correlation between (a) NLR and r_{NH_4-N} and (b) NLR and r_{NO_3-N}.

The results obtained with Filtralite surpass those referred by Kadlec and Knight (1996) and Vymazal and Kropfelova (2008), which had pointed to a decrease in RE and low correlation coefficients between applied and removed loads for NLR below 3 g N m^{-2} d^{-1}. However, they are in accordance with the linear relationship found by Ayaz and Akca (2001) for NLR between 4 and 8 g N m^{-2} d^{-1} in gravel-based shallow HSSF mesocosms for the treatment of domestic wastewater.

4. Conclusions

The use of Filtralite seems to enable a quick development of biofilm with the capability of removal of organic matter, ammonia and nitrate at high rates. Regardless of the type of material or the type of wastewater, nitrate removal was complete for NLR below 1.3 g NO_3–N m^{-2} d^{-1} and occurred entirely in the section Inlet-PI2. The NLR of both ammonia (0.8 and 2.4 g NH_4–N m^{-2} d^{-1}) and nitrate (0.6 and 3.2 g NH_4–N m^{-2} d^{-1}) seem to have significantly influenced their removal rates. The removal of ammonia and nitrate did involve not only the conventional pathways of nitrification-denitrification.

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