

Vertical flow constructed wetland as a green solution for low biodegradable and high nitrogen wastewater: A case study of explosives industry

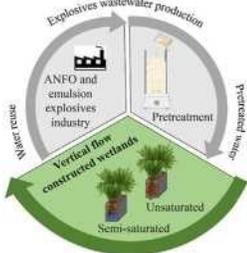
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HIGHLIGHTS

- VFCW with *Vetiveria zizanioides* for low biodegradable wastewaters is a treatment option.
- Nitrogen removal was worse in un-saturated conditions than saturated conditions.
- Nitrification, denitrification, and aerobic biodegradation can occur simultaneously.
- The highest nitrate removals (55%) were obtained at 30 g m⁻² d⁻¹ COD and low C/N.
- Denitrification was observed even at 10 mg L⁻¹ O₂ in influent.

GRAPHICAL ABSTRACT



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ABSTRACT

The removal of nitrogen compounds from a pretreated explosives wastewater in vertical flow constructed wetland planted with *Vetiveria zizanioides* (0.24 m² x 0.70 m), filled with light expanded clay aggregates (Leca@NR 10/20), was studied. Experiments under constant hydraulic load, 50 ± 4 L m⁻² d⁻¹ and 83 ± 5 L m⁻² d⁻¹ without and with flooding level (25%), respectively, were made at different ammonium (3e48 mg NH₄-N L⁻¹), nitrate (56e160 mg NO₃-N L⁻¹) and nitrite (0.3e1.1 mg NO₂-N L⁻¹) concentrations. Results indicate that without flooding level (unsaturated) the removal efficiencies obtained were 30 ± 9, 7 ± 1 and 96 ± 2%, respectively to NH₄-N, NO₃-N and NO₂-N. When using flooding level and an external carbon source (C/N ratio from 1.3 ± 0.19 to 2.5 ± 0.20), the organic matter (COD) removal efficiencies were above 90%, 75% for NH₄-N and 55% to NO₃-N. Increasing the C/N ratio from 2.9 ± 0.21 to 4 ± 0.22 did not contribute to upgrade the efficiencies of COD, NH₄-N and NO₃-N removal. The denitrification process was observed in aerobic conditions and nitrite production have been occurred, probably due to the presence of aerobic conditions that inhibited partially denitrification.

1. Introduction

Nitrogen compounds releases from human activities (domestic, industrial and agriculture) has a high impact on environmental nitrogen pollution and degrade water resources (OECD, 2017).

Reactive nitrogen compounds in the form of nitrites (NO₂), nitrates (NO₃) and ammonium (NH₄), and dissolved organic nitrogen affect soil pH and water quality through surface run-off and leaching into surface and groundwaters, and leading to water pollution (EU, 2013; Zhou et al., 2017; Holmes et al., 2019). Most of the nitrogen leached from agricultural soils is in the form of nitrate and come from synthetic fertilisers, as well as from livestock manure (Hussain et al., 2020; De Boer, 2017). In addition, localised peaks in pollution may be associated to run-off from urban spaces, from poorly treated sewage and industrial sources (Sutton and Billen, 2010; WRI, 2013). Recent estimative revealed an increase in nitrogen discharges of 35e46% in water resources between 2000 and 2050 (IFPRI and Veolia, 2015). According with EU (2013), in the absence of human influence, around 0.5 kg ha⁻¹ year⁻¹ of nitrogen is generally deposited to ecosystems, but in many areas of the world, the average nitrogen deposition rates exceed 10 kg nitrogen ha⁻¹ year⁻¹.

Tertiary wastewater treatment practices have been developed to avoid the deleterious effects of excess reactive nitrogen in the environment, ensuring the removal of reactive nitrogen species (Holmes et al., 2019). Many efficient technologies rely on physico-chemicals treatments (e.g. reverse osmosis membranes, ion exchange, air stripping, electro dialysis) to remove nitrogen compounds (Ahn, 2006; Metcalf and Eddy, 2014). However, biological nitrogen removal practices (e.g. modified activated sludge) are much more environmentally friendly and cost effective (Holmes et al., 2019). In this line, constructed wetlands (CWs) is an alternative to conventional treatments to remove nitrogen and organic matter. In CWs, removal and transformation mechanisms can occur such as filtration, sedimentation, adsorption, chemical precipitation, volatilization, bacterial metabolism (e.g. nitrification, ammonia, denitrification), and phytoremediation (phytoextraction, phytodegradation, phytostabilization, phytovolatilization, rhizodegradation and rhizofiltration) (Rajan et al., 2019).

Nitrogen (ammonium or nitrate) removals in CWs are dependent on the water flow regime, substrate matrix, plant, bed depth, applied

operational parameters (such as hydraulic load (HL), organic and nitrogen loads, C/N ratio, feeding regime and flooding level), and climatic conditions (e.g. temperature) (Almeida et al., 2018; Liu et al., 2018; Saeed and Sun, 2012; Wang et al., 2019). If effluents have low biodegradable organic matter, the nitrate reduction to molecular nitrogen can be compromised since the denitrification process involves the donation of electrons from biodegradable organic matter (Tjelliden et al., 2015). However, this may not be detrimental to certain plants since some plants may contribute to the denitrification process by releasing weak organic acid exudates through their root system and by producing enzymes that can transform complex organic matter into the simplest forms (Tjelliden et al., 2015). Some plants, such as *Phragmites australis*, *Typha latifolia*, *Cyperus papyrus*, *Typha domingensis*, are recognized for their great potential for wastewaters phytoremediation, but recently the interest in *Vetiveria zizanioides* plant has increased (Badejo et al., 2018; Almeida et al., 2019). This plant has wide tolerance to adverse conditions such as weather, soil, pH (between 3 and 10.5) and salinity (until 47.5 dS m⁻¹ although its productivity decreases from 8 dS m⁻¹) (Danh et al., 2009), and has high potential for the removal of total nitrogen, ammonia, total suspended solids (TSS) and chemical oxygen demand (COD) from wastewaters (Badejo et al., 2018). Wang et al. (2009) used *Vetiveria zizanioides* in subsurface vertical flow constructed wetland (VFCW) with gravel and coal slag as a substrate to treat domestic wastewater. These authors obtained high COD (71%), ammonium nitrogen (67%) and total nitrogen (80%) removals using 5 days of hydraulic retention time and HL of 600e800 L m⁻² d⁻¹. The use of subsurface VFCW has some advantages compared with other water flow regimes since it requires less space and provides higher levels of oxygen transfer for the occurrence of the nitrification process and degradation of organic matter (Stefanakis, 2019). However, it does not provide good conditions for the denitrification process since this process occurs in low oxygen conditions (Stefanakis, 2019). In turn, sub-surface horizontal flow constructed wetland (HFCW) is usually used for the denitrification process because of the low oxygen content provided in the system (Kadlec and Wallace, 2009). Xinshan et al. (2010) tested the combined systems VFCW-HFCW to treat sequentially an effluent containing ammonium and nitrate and low organic matter. VFCW allowed the conversion of ammonium to nitrate and then HFCW converted nitrate (the formed and the present in the effluent) to molecular nitrogen using additional organic matter source (Xinshan et al., 2010). However, if wastewater has initially high amounts of organic matter nitrification can be inhibited, so a combined system HFCW-VFCW with recirculation can be a good option (Cooper, 2005; Vymazal, 2005, 2013; bib_Vymazal_2013; bib_Vymazal_2005). Moreover, according to Kadlec (2010), nitrification and denitrification processes can occur simultaneously in the CWs bed. CWs have alternated zones with different oxygenation states in the proximities of the plant roots, where nitrification occurs and the produced nitrate is diffused to the soil anaerobic microzones to be denitrified (Kadlec, 2010). VFCW with flooding level (using *Vetiveria zizanioides*) also demonstrated good results in simultaneous nitrification and denitrification of wastewaters (Almeida et al., 2017; Almeida, 2012). In the flooding level, the dissolved oxygen (DO) content in the bed decreases when bed flooding level increases, providing anoxic conditions for the occurrence of denitrification.

Explosives wastewaters (EW), from the ammonium nitrate-fuel oil (ANFO) and emulsion industries, are industrial wastewaters with high amounts of nitrogen (in the form of nitrates and/or ammonium nitrogen) and high non-biodegradable organic matter. Due to these characteristics, these wastewaters are very difficult to treat (Madeira et al., 2020). The present work aims to: (i) evaluate the use of VFCW planted with *Vetiveria zizanioides* to remove ammonium and nitrate from a pretreated explosives wastewaters (PEW), using two different flooding conditions; (ii) analyze the increase of organic mass load in the nitrogen forms removal; and (iii) study the impact of the different C/N ratios on the removal of nitrogen compounds.

2. Material and methods

2.1. Experimental setup characteristics

Experiments were carried out in a pilot-scale VFCW (0.4 m x 0.6 m x 0.70 m), planted with *Vetiveria zizanioides* (plant density higher than 120 plants per m²) (Fig. 1), filled with light expanded clay aggregates (Leca® NR 10/20). A bottom slope of 2% was applied in the bed to enable the hydraulic collection of the effluent. A layer of gravel (diameter 10e50 mm) was placed around the outlet valve to prevent clogging by fine particles. The flooding levels were modified through a flexible siphon in the outlet. This VFCW was fed in continuous mode, through network sprinklers, equidistantly located over the whole VFCW using a submersible pump (Eheim 1250, Deizisan, Germany) in the feeding tank. The VFCW was acclimatized using PEW at same concentration that trials were done until the system being pseudo-stationary.

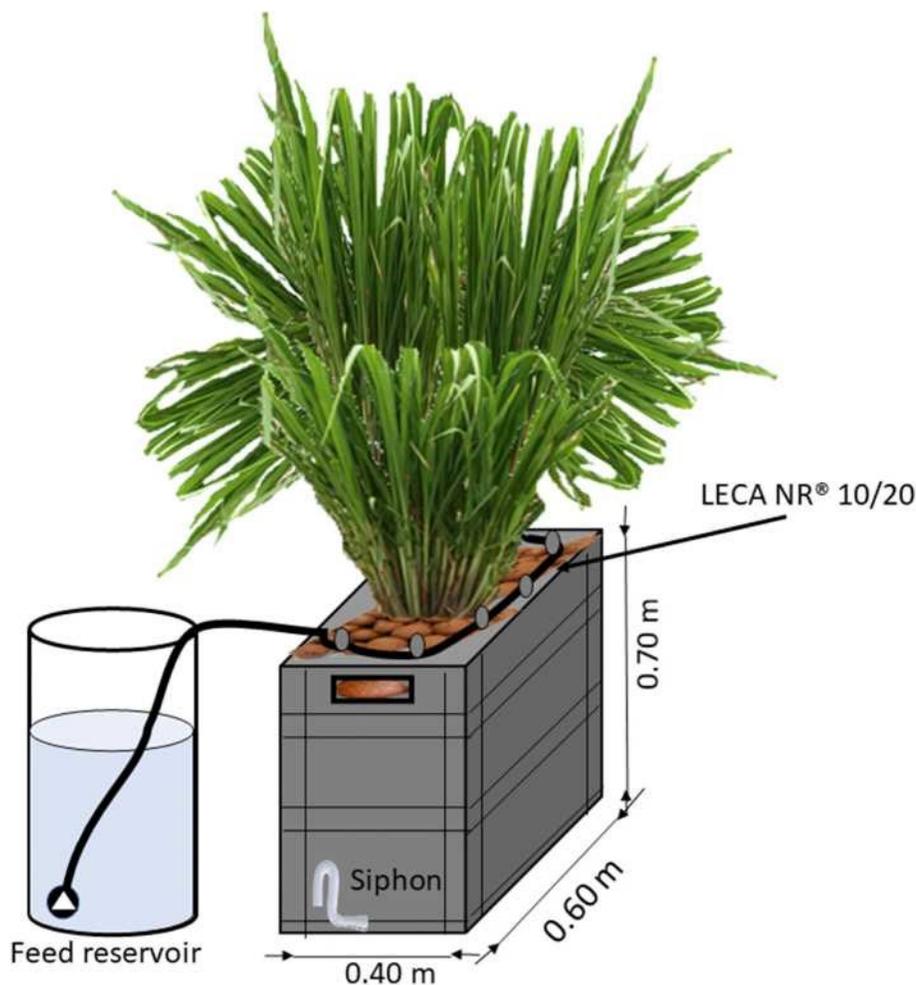


Fig. 1. Schematic representation (not at scale) of the vertical flow constructed wetland (VFCW) planted with *Vetiveria zizanioides*.

2.2. Experimental conditions

Explosives wastewater was collected from an international industry of ANFO and emulsion explosives, in Portugal. Then, EW was pretreated using a combined immediate one-step lime precipitation (IOSLM) and atmospheric CO₂ carbonation (AC) processes as presented in Madeira et al. (2020). IOSLM process was used to remove COD and consisted of applying an optimal hydrated lime dose (7.76 g L⁻¹) to the EW, under vigorous agitation for 1 min. After IOSLM process and sedimentation step, the obtained supernatant was pretreated by AC process to reduce supernatant pH to 9. Pretreated explosives wastewater (PEW) characteristics were: pH (9 ± 1.4), DO (6.8 ± 0.3 mg L⁻¹), conductivity (8.9 ± 0.1 mS cm⁻¹), COD (1226 ± 287 mg L⁻¹), BOD (<5 mg L⁻¹), NH₄-N (494 ± 464 mg L⁻¹), TKN (494 ± 464 mg L⁻¹), NO₃-N (2302 ± 372 mg L⁻¹) and NO₂-N (16 ± 3.2 mg L⁻¹). PEW was then used in VFCW experiments, after PEW dilution to desirable concentrations, as it is pointed out by some researchers that high nitrogen concentrations are toxic to plants (Almeida, 2012; Britto and Kronzucker, 2002).

The experimental trials were conducted in two phases (Table 1). In the first phase (phase I), the concentrations studied 45 ± 3 mg L⁻¹ for ammonium (NH₄-N), 107 ± 1 mg L⁻¹ for nitrate nitrogen (NO₃-N), 0.6 ± 0.0 mg L⁻¹ nitrite nitrogen (NO₂-N) and BOD/TKN <1 (since in PEW BOD <5 mg L⁻¹ and TKN 494 ± 464 mg L⁻¹), under constant H_L (50 ± 3 L m⁻² d⁻¹) without flooding level. In the second phase (phase II), the concentrations varied between 3 and 32 mg L⁻¹ for ammonium nitrogen (NH₄-N), 56e160 mg L⁻¹ for nitric nitrogen (NO₃-N), 0.3e1.1 mg L⁻¹ nitrite nitrogen (NO₂-N), 170e965 mg L⁻¹ for COD and H_L was kept again constant but at higher value (83 ± 5 L m⁻² d⁻¹) than phase I. In these trials (phase II) an external carbon source (sucrose) was added and the VFCW was operated with a flooding level of 25% to decreased DO into the bed (according to results obtained by Almeida et al. (2010) and Almeida (2012)). Throughout the experiments, the plants were assessed visually by identifying signs of toxicity (such as leaf curling, chlorosis, or death of the plant).

2.3. Sampling collection and analysis

The VFCW was monitored every weekday at 10:00 a.m., between April and September, until sample number >11. The flow rate at inlet and outlet was measured during all experimental period. Samples were collected in inlet (feed tank) and outlet of the VFCW and the following parameters were characterized: pH, electrical conductivity (EC), redox potential (Eh), dissolved oxygen (DO), ammonium (NH₄-N), nitrite (NO₂-N), nitrate (NO₃-N) and chemical oxygen demand (COD). The samples were stored at 5 °C, if it was not possible to analyze them immediately.

pH and Eh measurements were performed in a WTW InoLab level 1, using the electrodes SenTix 41 and WTW SenTix ORP, respectively. EC was evaluated in a Jenway 4510 m, using an

Table 1

Inlet experimental conditions in Phases I and II (n > 11).

Chemical composition		Applied operations									
		pH	EC (mS cm ⁻¹)	Eh (mV)	DO (mg L ⁻¹)	NH ₄ -N	NO ₃ -N	NO ₂ -N	COD	HL (L m ⁻² d ⁻¹)	Flooding level (%)
Phase I	Minimum	8.2	1.2	88	5.0	42	106	0.6		47	-
	Maximum	8.3	1.3	165	6.5	48	108	0.6	*	53	
	SD	0.0	0.1	54	1.0	4	1	0.0		4	
Phase II	Minimum	7.7	1.0	96	4.9	3	56	0.3	170	77	25
	Maximum	8.3	1.6	246	10.2	32	160	1.1	965	89	
	SD	0.2	0.2	52	1.5	14	44	0.3	232	5	

* < 25 mg O₂ L⁻¹ (detection limit).

electrode VWR CO 11. DO was determined by a modification of the Winkler method (APHA, 2012). Ammonium nitrogen was determined by the distillation method in BUCHI Distillation Unit B-316 and then by titration (APHA, 2012). Nitrite was determined by a colorimetric method (APHA, 2012) at 543 nm using a Pharmacia Biotech Ultrospec 2000 UV/Visible spectrophotometer model 802106-00. Nitrate was measured by the sodium salicylate method (Rodier, 1989) at 420 nm using a Pharmacia Biotech Ultrospec 2000 UV/Visible spectrophotometer model 80-2106-00. COD was determined by standard dichromate closed reflux method, using a digester WPA HC 6016 and quantified by Pharmacia Biotech Ultrospec (2000) UV/Visible spectrophotometer model 80-210600 at 600 nm (APHA, 2012). COD was used to indirectly quantify carbon content (Metcalf and Eddy, 2014).

2.4. Data processing and statistical analysis

The results obtained in each phase were statistically treated. Agglomerative Hierarchical Clustering (AHC) was determined by SPSS 20 (SPSS Inc.) software and performed by Euclidean distance, Ward's method, and standardization of variables. Cluster analysis was performed for phase II, applied to the affluent parameters (pH, Eh EC, DO, NO₂-N, NO₃-N, NH₄-N) and to the hydraulic load. The outliers were eliminated whenever they existed. According to AHC, it was identified two groups (A and B) in phase II (see Appendix A), both under HL constant (83 ± 5 L m² d⁻¹). Organic matter loads from 10 to 80 g m² d⁻¹ (COD) under constant loads of ammonium nitrogen (0.40 ± 0.1 g m² d⁻¹ NH₄-N), nitrate (12 ± 2 g m² d⁻¹ NO₃-N) and nitrite (0.08 ± 0.01 g m² d⁻¹ NO₂-N) were grouped in group A, and organic loads of 30e50 g m² d⁻¹ under constant loads of ammonium nitrogen (2.4 ± 0.4 g m² d⁻¹ NH₄-N), nitrate (5 ± 1 g m² d⁻¹ NO₃-N) and nitrite (0.04 ± 0.01 g m² d⁻¹ NO₂-N) were grouped in group B.

For the comparison between influent and effluent quality, ANOVA was used at 95% confidence level. Two hypotheses were tested against each other (in which the null-hypothesis H₀: "influent and effluent quality are identical", and the alternative hypothesis H₁: "influent and effluent quality are different"). Post-hoc (a posteriori) Turkey's test was used to determine differences between means of specific variables.

3. Results and discussion

3.1. Nitrogen removal in phase I

Ammonium (NH₄-N) and nitrate (NO₃-N) results under unsaturated conditions (without flooding level) (Table 1) are presented in Fig. 2. The ammonium nitrogen load in the VFCW outlet decreased significantly (p < 0.05) and ammonium nitrogen removal was 30 ± 16% (corresponding to 0.70 ± 0.5 g m² d⁻¹) (Fig. 2a). These results were not expected since the decrease in ammonium nitrogen observed in this work was lower than the data reported by Almeida et al. (2018), whose ammonia nitrogen removal efficiencies were around 60% using a synthetic effluent and a hydraulic retention time of 10 h identical that was used in this work (10 h ± 0.9). According to Cervantes et al. (2015) and Kadlec and Wallace (2009), NH₄-N transformation to NO₃-N consumes alkalinity, H⁺ ions are released, and dissolved oxygen is consumed too. These transformations can be observed by the pH decrease (Fig. 2f) and the DO consumption (Fig. 2e), both variations statistically significant (p < 0.05). However, it was observed that pH effluent showed values between 4.0 and 5.6, which can contribute to remove the ammonium efficiently, since according to Lee et al. (2009) when pH values decrease lower than 7.0 nitrification rate decrease too. In this study, the nitrification process was not inhibited by organic matter because PEW had a low concentration of organic matter (<25 mg O₂ L⁻¹) and the effluent contained 4 ± 0.1 mg O₂ L⁻¹. Moreover, the BOD/TKN ratio was less than 1, which was favorable for successful nitrification, as mentioned by Kadlec and Wallace (2009). Nitrification process had the ideal initial conditions (pH, redox, nitrogen, oxygen) to occur successfully, which did not happen, probably due to the presence of nitrate (106e108 mg L⁻¹ NO₃) in the influent, together with pH drop in effluent, which inhibit nitrification, although a nitrate removal (0.40 ± 0.1 g m² d⁻¹) (not significant, p > 0.05) with a removal efficiency of 7 ± 0.5% has been observed (Fig. 2b). Nitrate production and its removal are processes that can occur in sequential mode in CW since a low HL values can provide a greater removal of nitrate than formed.

Nitrite was not found in effluent (Fig. 2c), so considering the method detection limit a removal efficiency of 96 ± 2% was calculated. Usually, in biological treatment systems under stable operation, there is no accumulation of nitrites because the growth rate of *Nitrobacter* bacteria is significantly higher than the *Nitrosomonas* bacteria. As a result, the growth rate of the latter generally controls the overall nitrification rate (WEF et al., 2005).

An increase in the values of redox potential (from 126 ± 54 to 217 ± 7 mV) (Fig. 2d) in the effluent was observed. According to Reddy and DeLaune (2008), more oxidized substances raises the electric potential as the case of ammonium nitrogen transformation in nitrates. However, it did not happen in this work because there was no nitrate production in the effluent (Fig. 2b). Moreover, any eventual nitrate production from nitrification would be too little for that. This increase in the redox potential may be related to the decrease in the pH of the effluent (DeLaune and Reddy, 2005), caused by the interaction between PEW and CO₂ present inside the bed, releasing H⁺ ions (Madeira et al., 2020).

Electrical conductivity showed a significant increase (p < 0.05) (Fig. 2g), attributed to evapotranspiration. The electrical conductivity

Table 1

Inlet experimental conditions in Phases I and II (n > 11).

values in the effluent (about 1.5 dS m⁻¹) were always below the value (8 dS m⁻¹) from which plant *Vetiveria zizanioides* productivity decreases (Danh et al., 2009).

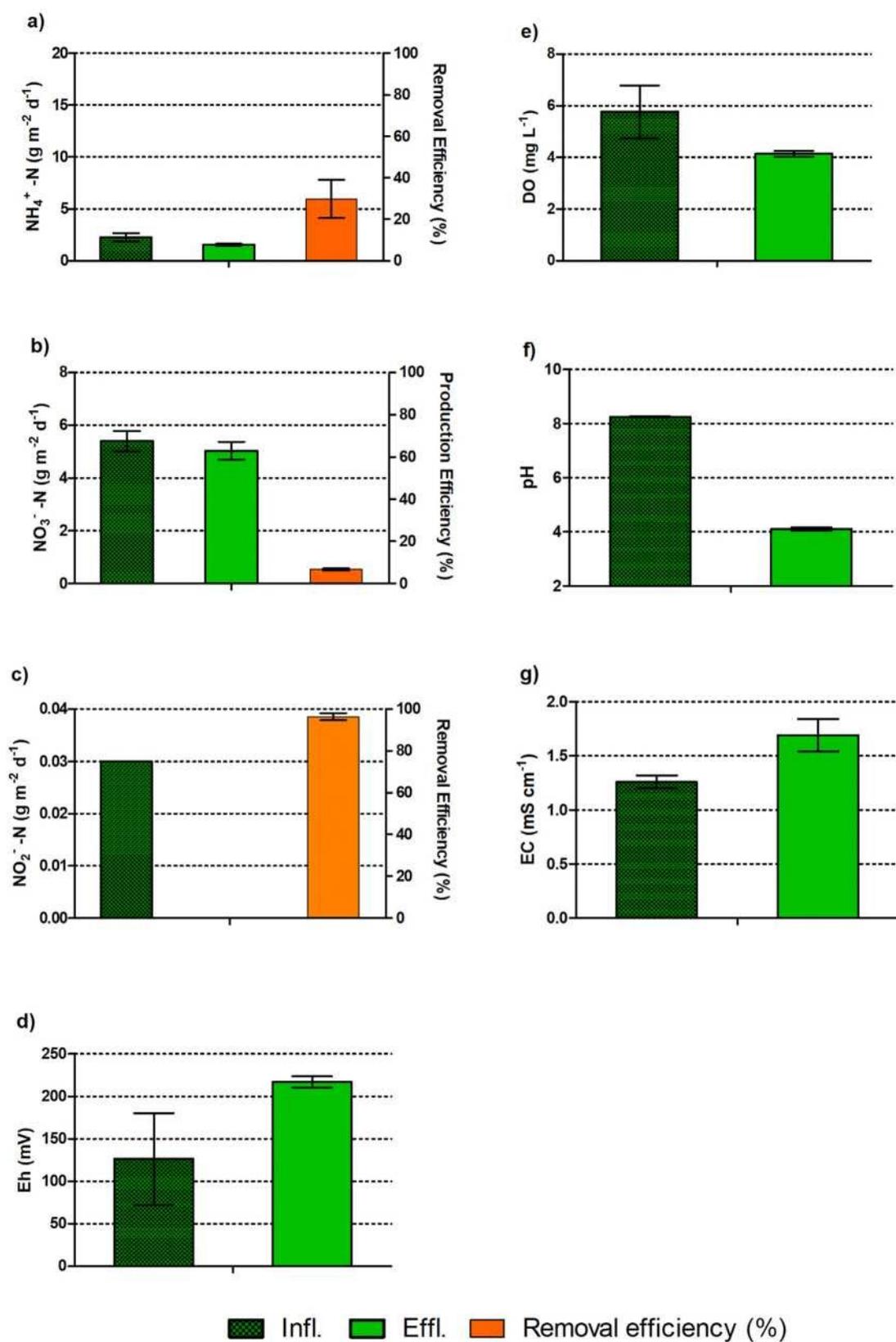


Fig. 2. Water quality in inlet (Infl.) and outlet (Effl.) of the VFCW for: **a)** ammonium applied load, **b)** nitrate applied load, **c)** nitrite applied load, **d)** redox potential, **e)** dissolved oxygen, **f)** pH and **g)** electrical conductivity. Removal efficiency for: **a)** ammonium, **b)** nitrate and **c)** nitrite. The hydraulic load was kept constant ($50 \pm 3 \text{ L m}^{-2} \text{ d}^{-1}$) and without flooding level. Bars represent standard deviation of the mean ($n > 11$).

3.2. Nitrogen removal in phase II

Ammonium nitrogen and nitrate removal in presence of organic matter was studied (Fig. 3). As PEW diluted had a low C/N ratio, it was necessary to add sucrose as an external source of carbon to ensure that all necessary conditions for denitrification were ensured.

Fig. 3a1 and 3a2 show the influence of the organic load applied in the C/N ratio in the influent and effluent. C/N ratio in influent varied between 0.4 to 2.5 and 2.9 to 4.3 for groups A and B, respectively. For both groups, the input C/N ratios reduced significantly to values around 0.2 ± 0.06 (Fig. 3a1) and 0.4 ± 0.08 (Fig. 3a2), for all applied organic loads. These reductions were due to the greatest removal of applied organic matter (COD) than nitrogen. Organic matter removal efficiencies remained above 90%, for organic matter loads applied above $30 \text{ g m}^{-2} \text{ d}^{-1}$ (Fig. 3b1 and 3b2).

Although the nitrate removal was observed along with the applied organic load range (Fig. 3c1 and 3c2), heterotrophic denitrification was not the main mechanism for organic matter removal in both groups because it was observed greater organic matter removal than would be expected by denitrification process. Schneider et al. (2008) referred that the degradation of 1 g of sucrose consumed only approximately 1.74 g of nitrate. Moreover, the organic matter removal was not proportional to the nitrate removal. So, most of the organic matter was due to aerobic biodegradation.

According to Fig. 3c1, a maximum nitrate removal from about $4.5 \text{ g m}^{-2} \text{ d}^{-1}$ (corresponding a maximum nitrate removal efficiency of about 55%) at $30 \text{ g m}^{-2} \text{ d}^{-1}$ COD was achieved, remaining constant for higher added organic loads. This maximum nitrate removal happened to C/N of 1.3 which is quite close to 1.25 where the denitrification process should be performed (Metcalf and Eddy, 2014). Xinshan et al. (2010) results showed that the excessive C/N ratio did not mean an increase in nitrogen removal efficiency. So, up to $30 \text{ g m}^{-2} \text{ d}^{-1}$ COD applied, the organic matter was the limiting agent in the denitrification process. From $30 \text{ g m}^{-2} \text{ d}^{-1}$ COD, the nitrate removal efficiency tended to decrease for values around 40%, which was possibly due to the increase in the dissolved oxygen concentration in the effluent (Fig. 4b1).

Nitrate removal efficiencies were lower in the group B than in the group A, for the same range of organic load applied. This difference can be due to greater conversion of ammonium nitrogen into nitrate in the group B (whose ammonium nitrogen removal ranged from 1.2 to 1.9 $\text{g m}^{-2} \text{ d}^{-1}$ NH₄-N) than in the group A (whose ammonium nitrogen removal ranged from 0.2 to 0.3 $\text{g m}^{-2} \text{ d}^{-1}$ NH₄-N) (Fig. 3d1 and 3d2), although the ammonium nitrogen removal efficiencies were the same (approx. 75% and 81%, for organic matter load range studied in both groups).

Nitrate removal happened in the presence of high concentrations of dissolved oxygen (about $5 \pm 12 \text{ mg O}_2 \text{ L}^{-1}$) in both groups (Fig. 4b1 and 4b2), and without significative differences in redox potential between influent and effluent (Fig. 4a1 and 4a2). Although not expected, because the presence of oxygen suppresses the enzyme required for denitrification (Cooper et al., 1996), the denitrification process was observed in CWs in the presence of considerable concentrations of dissolved oxygen: $6 \pm 2 \text{ mg O}_2 \text{ L}^{-1}$ by Kadlec (2010) and of $4.7 \pm 2.1 \text{ mg O}_2 \text{ L}^{-1}$ by Almeida et al. (2017). Kadlec (2010) referred that in the CWs there were alternating zones with different oxygenation states depending on the proximity to plant roots, which allowed nitrification and denitrification processes to occur simultaneously. In addition, the flooding level could determine aerobic/anaerobic activity (Moshiri, 1993).

An increase in oxygen concentration in the effluent (Fig. 4b1) was observed for organic matter loads above $50 \text{ g m}^{-2} \text{ d}^{-1}$ COD. Moreover, it would be expected a decrease in oxygen concentration, essentially due to the consumption of organic matter by aerobic biodegradation and to the nitrification process. HL was kept constant throughout the groups, so it is not responsible for the variation of oxygen in CW. The same behavior was observed by Bezbaruah and Zhang (2004). According to Bezbaruah and Zhang (2004), an increase in the concentration of organic matter in CW could promote the creation of areas with deficiencies of oxygen near the root, causing the plant to develop defense mechanisms, namely the formation of aerenchyma in the roots (large cavities in the interior of the plant filled with air). There are also other factors affecting oxygen released by the roots of macrophytes in the rhizosphere, namely some factors that affect the photosynthesis and oxygen transport through plants (Brix, 1994; Rehman et al., 2017).

Effluent pH decreased in the effluent in both groups (Fig. 4c1 and 4c2). The pH drop was the result of all the mechanisms occurred in the VFCW. As noted above, aerobic biodegradation of organic matter and nitrification process occurred in both groups and they were responsible for the pH drop due to the release of CO₂ and ions H⁺ to the matrix, respectively. Denitrification process also occurred in CW and it is responsible for an increase in pH, but this process did not prove to be predominant over the other processes to counteract the drop in pH.

Nitrite accumulation was observed throughout the organic load range applied (Fig. 3e1 and 3e2). Lu et al. (2009) also verified an increase of nitrite in the effluent when they supplemented the system with carbon. The accumulation of nitrite indicates that there were adverse conditions that inhibit the activity of *Nitro-bacter* and consequently the nitrite removal. However, nitrification was not the only mechanism that contributed to the production of nitrites (mainly for group A), since the removal of ammonium nitrogen was less than the nitrite produced. Thus, the accumulation of nitrite may be essentially due to the interruption of the denitrification process caused by the high levels of oxygen in the effluent (Fig. 4b1 and 4b2).

No significative oscillations of EC were found in both groups. The EC was around 1.5 dS m^{-1} (Fig. 4d1) and 1.0 dS m^{-1} (Fig. 4d2), throughout the organic load range applied. This means that the balance among evapotranspiration, salt precipitation or nitrate reduction allowed to maintain the EC. In both experiments, the EC values were below 8 dS m^{-1} indicating that the productivity of *Vetiveria zizanioides* was not affected by this parameter (Danh et al., 2009). No signs of toxicity and no symptoms of micro-nutrient deficiency (e.g. yellowing of the leaves) were detected in the plant by visual inspection.

4. Conclusion

In this work, a VFCW planted with *Vetiveria zizanioides* in LECA was used to remove nitrogen (ammonium and nitrate) from a PEW.

The experimental trials were conducted in two phases: in the first phase the ammonium nitrogen removal was evaluated under ammonium and nitrate nitrogen constant concentrations, constant HL ($50 \pm 3 \text{ L m}^{-2} \text{ d}^{-1}$) and without the presence of organic matter and flooding level. About 30 ± 9 and $7 \pm 1\%$ of ammonium and nitrate nitrogen removal were reached, respectively. According to the operational conditions applied (traces of organic matter and low hydraulic retention time), a greater removal of ammonium nitrogen would be expected. High nitrate concentration in the influent and the drop in pH to very low values in the effluent (between 4.0 and 5.6) would have been the main reasons to the inhibition of the nitrification process.

In the second phase, 55% of nitrate nitrogen removal was reached with addition of organic matter, constant HL ($83 \pm 5 \text{ L m}^{-2}$

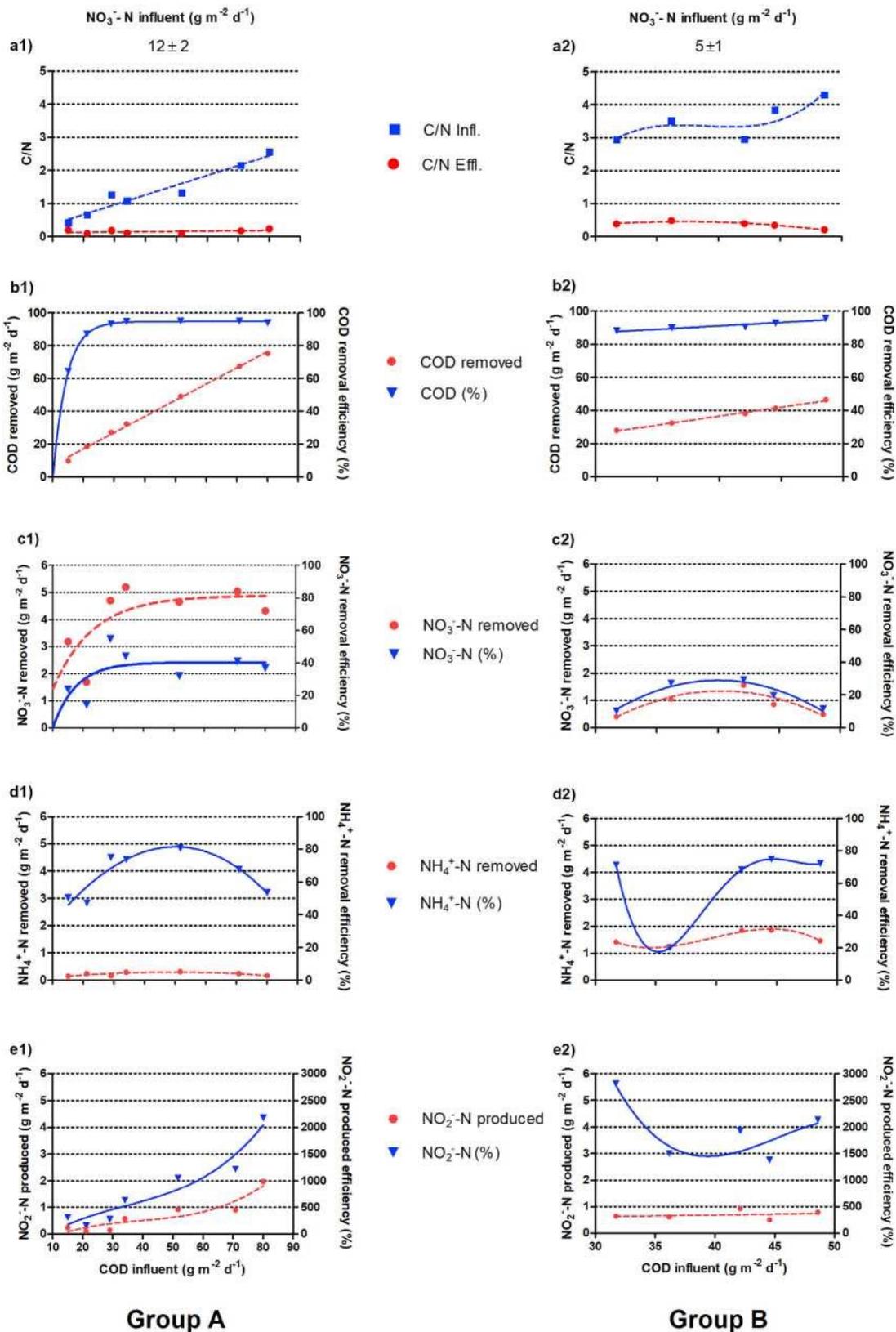
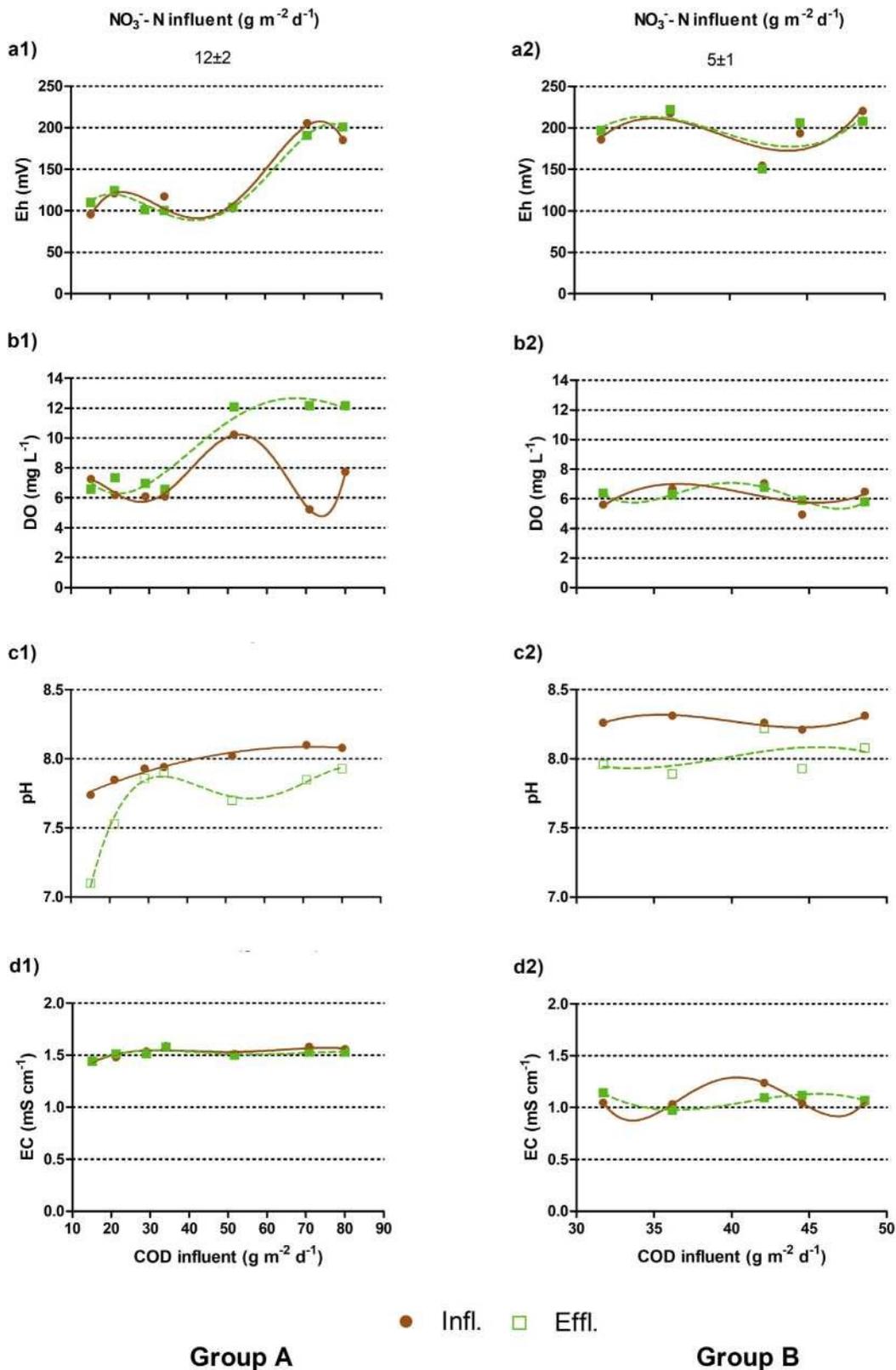


Fig. 3. Variation of the: C/N ratio in influent (Infl.) and effluent (Effl.) (a1 and a2); and removal load and removal efficiency for: COD (b1 and b2), nitrate (c1 and c2), ammonium (d1 and d2) and nitrite (e1 and e2), as a function of the applied organic matter load. Items a1) to e1) belong to group A (loads applied: $12 \pm 2 \text{ g m}^{-2} \text{ d}^{-1} \text{ NO}_3^- \text{-N}$, $0.40 \pm 0.1 \text{ g m}^{-2} \text{ d}^{-1} \text{ NH}_4^+ \text{-N}$ and $0.08 \pm 0.01 \text{ g m}^{-2} \text{ d}^{-1} \text{ NO}_2^- \text{-N}$) while items a2) to e2) belong to group B (loads applied: $5 \pm 1 \text{ g m}^{-2} \text{ d}^{-1} \text{ NO}_3^- \text{-N}$, $2.4 \pm 0.4 \text{ g m}^{-2} \text{ d}^{-1} \text{ NH}_4^+ \text{-N}$ and $0.04 \pm 0.01 \text{ g m}^{-2} \text{ d}^{-1} \text{ NO}_2^- \text{-N}$). The hydraulic load was kept constant ($83 \pm 5 \text{ L m}^{-2} \text{ d}^{-1}$) and with a flooding level of 25%.



Group A **Group B**

Fig. 4. Variation of the: redox potential (a1 and a2); dissolved oxygen (b1 and b2); pH (c1 and c2); electrical conductivity (d1 and d2); in influent (Infl.) and effluent (Effl.), as a function of the applied organic matter load. Items a1) to d1) belong to group A (loads applied: $12 \pm 2 \text{ g m}^{-2} \text{d}^{-1} \text{ NO}_3\text{-N}$, $0.40 \pm 0.1 \text{ g m}^{-2} \text{d}^{-1} \text{ NH}_4\text{-N}$ and $0.08 \pm 0.01 \text{ g m}^{-2} \text{d}^{-1} \text{ NO}_2\text{-N}$) while items a2) to d2) belong to group B (loads applied: $5 \pm 1 \text{ g m}^{-2} \text{d}^{-1} \text{ NO}_3\text{-N}$, $2.4 \pm 0.4 \text{ g m}^{-2} \text{d}^{-1} \text{ NH}_4\text{-N}$ and $0.04 \pm 0.01 \text{ g m}^{-2} \text{d}^{-1} \text{ NO}_2\text{-N}$). The hydraulic load was kept constant ($83 \pm 5 \text{ L m}^{-2} \text{d}^{-1}$) and with a flooding level of 25%.

d¹) and flooding level of 25%. Results showed that nitrate nitrogen removal was not dependent of the ratio C/N to values higher than 1.3 ± 0.19 . The denitrification process occurred in aerobic conditions. However, it was observed a nitrite production, which was probably due to partial inhibition of the denitrification process.

The results obtained show that it will be necessary to continue research related to the ammonium and nitrate nitrogen removal, using the VFCW in series and other flooding levels.

CRedit author statement

Luís Madeira: Investigation, Writing original draft, Writing review & editing, Fetima Carvalho: Methodology, Writing — review & editing, Margarida Ribau Teixeira: Methodology, Writing review & editing, Carlos Ribeiro: Conceptualization, Writing — review & editing, Adelaide Almeida: Conceptualization, Resources, Writing — review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2021.129871>.

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