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Potential for denitrification in sequencing batch constructed wetlands cultivated with *T. latifolia* and *C. zizanioides*

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ABSTRACT

Denitrification and uptake by plants in constructed wetlands (CWs) were studied. Nitrate was applied in CWs operated in batch mode. The systems received 50 g m^{-3} NO_3^-–N, and among six units, three received ethanol as carbon source. The experiment consisted of two main stages, with each one cycle time (t_c) of 3 and 1 d. In an extra stage, the decay values of water variables were assessed. The range of nitrate-nitrogen removal (stage I) was 11.7–54.8% for CWs without ethanol and 98.0–99.9% for CWs receiving the external carbon source. During stage II, NO_3^-–Nremovals were 3.6–15.7% for CWs without ethanol and 94.7–97.5% for CWs with ethanol addition. CWs were effective for removing nitrate, especially the planted systems. CWs cultivated with vetiver showed the best results in nitrogen removal. The addition of ethanol increased the denitrification efficiency, but increasing nitrite concentrations in the CWs should also be considered.

Keywords: Nitrate; Ecotechnology; Vetiver; Cattail; Constructed wetlands

1. Introduction

Nitrogen is an element present in large amounts in the environment. A wide variety of wastewaters contain nitrogen concentrations. In developing countries, municipal sewage, wastewaters from industries and intensive livestock production, as well as agricultural run-off are increasingly becoming important sources of nutrients (nitrogen and phosphorus), negatively affecting water resources. When present as nitrate-nitrogen (NO_3^-–N), its most oxidized form, nitrogen may cause health problems such as digestive tract illness and poor blood oxygen transport [1,2].

Constructed wetlands (CWs) are systems characterized as those presenting advantages such as moderate implantation costs, very low energy consumption, and low maintenance requirements [3–5]. These natural treatment systems have been used to treat a wide range of pollutants. Nitrate is only one of the pollutants which can be removed by CWs [6]. Baker [7] and Lin et al. [8] stated that CWs could represent a viable alternative to remediate nitrate-contaminated groundwater.

Sirivedhin and Gray [9] highlighted the ability of CWs to reduce nitrate levels via biological

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denitrification and emphasize the importance of the presence of organic matter (electron donor). In CWs, carbon for denitrification can be provided by the vegetation (exudates) and used as a carbon source and energy by heterotrophic bacteria, such as nitrate-reducing bacteria (NRB).

Besides biological denitrification, nutrient removal by plants is one of the main factors responsible for the recycling of minerals in CWs. Moreover, these plants can remove substances containing heavy metals and toxic organic compounds [5].

Cultivated CWs tend to be more effective in removing nitrate when compared with non-planted systems. Moreover, certain plants remove nitrate more efficiently than others [6,10]. For instance, Lin et al. [10] observed better performance of CWs cultivated with *Pennisetum purpureum* than the other cultivated CWs. Zhu and Sikora [6] also reported that CWs planted with *Phalaris arundinacea* and *Phragmites communis* provided higher nitrate removing rates than systems cultivated with *Scirpus atrovirens* and *Typha latifolia*.

In light of these facts, it was proposed to evaluate separately the role of micro-organisms and plants on denitrification in CWs. So, this work was aimed to study biological denitrification in sequencing batch CW systems cultivated with different plant species: *Chrysopogon zizanioides* (vetiver grass) and *T. latifolia* (common cattail).

2. Materials and methods

Six CW cells were used. These CWs were operated in batch mode where the cells were filled and emptied via a vertical pathway. The dimensions of each cylindrical CW were 55 cm (diameter) and 90 cm (height), corresponding to 0.24 m^2 in superficial area and 214 dm^3 in total volume. Drain tubes were installed at the bottom of each cell.

Pea gravel was used as the support media. Upon filling of the units, each CW presented a working volume equal to 80 dm^3. Regarding the cultivated CWs, two cells were planted with *T. latifolia* (cattail), two with *C. zizanioides* (vetiver grass, formely *Vetiveria zizanioides*), and the other two were left unplanted (control cells).

For planting and initial development of the plants selected, each cell was saturated with raw sewage and water (25/55 dm^3 ratio). In order to maintain the same conditions, the same procedure was utilized in the control cells.

During five months, small amounts of sewage were applied to the systems for maturation of the plants. After this period, the plants reached stable conditions so as to begin the main step of the investigation with the application of a synthetic nitrogenated solution.

For preparing the synthetic influent solution, 17.15 g of $NaNO_3$, 4.04 g of KNO_3, and 3.28 g of $Ca(NO_3)_2$ were diluted in tap water, achieving a theoretical concentration of 50 mg dm^{-3} of nitrate-nitrogen (NO_3^-–N) and providing equilibrated levels of Na, K, and Ca. To supply the nutritional demand of plants and micro-organisms in CWs, 10 g of superphosphate was added as well.

Ten milliliters of fuel ethanol (CH_3CH_2OH) was added as an external carbon source to medium of the influent solutions. After this addition, the chemical oxygen demand (COD) in these solutions was nearly 200 g m^{-3}. Thus, a COD/NO_3^-–N ratio equal to four was obtained.

The CWs were labeled according to the cultivated species and according to with or without ethanol addition to the influent solution:

(1) NC—Control treatment (non-cultivated), without ethanol addition to the influent,
(2) NC*—Control treatment (non-cultivated), with ethanol addition to the influent,
(3) TL—Cultivated with *T. latifolia*, without ethanol addition to the influent,
(4) TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent,
(5) VZ—Cultivated with *C. zizanioides*, without ethanol addition to the influent,
(6) VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

The CWs were operated in an intermittent filling and drawing system also known as sequencing batch mode. The influent solution was applied from the top until the reservoir was filled, at which the liquid level reached the gravel layer. This condition was maintained during a cycle time (t_c) which was indicated by emptying of the reactor, featuring a vertical downflow in the discharge.

The influent volume added to the CWs was measured so that after each batch, observing the t_c, the CWs were emptied and the entire effluent volume was quantified. No "resting" intervals were adopted between batches. Influent and effluent samples for each CW were then sent to the laboratory. The volumetric difference between the influent and effluent represented evapotranspiration (ET) in cultivated CWs and evaporation in non-cultivated CWs. It should also be specified that during this study there

was no rainfall in the area in which the CWs were installed.

The first stage of the experiment (stage I) lasted for 40 d. During this stage, the nitrate-nitrogen solution was applied in batches with t_c of 72 h (3 d). Upon completion of this stage, the shoots (aerial portion) of the plants were cut in order to perform tissue analysis.

In the second stage (stage II), the batch cycles were reduced to 24 h (1 d). This stage lasted for 34 d. The last stage (stage III) was conducted for 3 d. During this stage, analyses were performed every few hours for observation of nitrogen removal and other variables of interest along the t_c of 3 d in the CWs. At the end of this stage, the plants were completely removed from the CWs for analysis of aerial and subterranean portions (shoots and roots).

Monitored concentration values were corrected based on the volumes lost (due to ET or evaporation), in order to obtain the actual pollutant removal efficiency (on mass basis). The analyses of COD, total ammonia nitrogen (TAN), nitrate-nitrogen, nitrite–nitrogen, pH, and redox potential (Eh) were performed according to the *Standard Methods for the Examination of Water and Wastewater* [11]. Alkalinity was measured according to the titrimetric method.

Regarding the plant analyses, samples were dried in an oven with recirculating air at a temperature of 65˚C for 72 h, after which they were submitted to trituration in a Wiley mill. Then, total nitrogen in the foliar tissue was quantified using the semimicro Kjeldahl method with the addition of salicylic acid.

The nitrogen standing stock (NSS) was calculated, where absorption is defined as the total nitrogen concentration (TN) multiplied by mass production (MP), and this absorption divided by the surface area (A) is the NSS (Eq. (1)):

$$\mathrm{NSS} = \mathrm{TN} \times \mathrm{MP}/A \quad (1)$$

At the end of the experiment, samples were collected via the support media in the middle region of CWs for biofilm analyses. The biofilm was extracted physically by submerging the gravel in water and stirring until the biofilm was removed.

The experiment was conducted in a randomized block design (RBD) and analyzed according to a 3 × 2 factorial with the following factors: plant (2 species and 1 control) and ethanol (present or absent). The Tukey's test at 5% level was utilized for analyses of the means. For each stage, means of the CWs receiving the external carbon source were analyzed separately from CWs without ethanol addition.

3. Results and discussion

3.1. Nitrogen removal in batch CWs

Measurement of the water lost by ET is crucial to accurately determine the mass balance in CWs. The average ET in CWs cultivated with cattail and vetiver were calculated as 9.1 and 9.5 mm d^{-1}, respectively. On the other hand, in the uncultivated CWs the average evaporation value was 4.0 mm d^{-1}.

Fig. 1 shows the values of nitrate-nitrogen concentrations in the effluent from the six CWs monitored. It is noteworthy that the superficial NO_3^-–N loading rates (L_s) were 5.8 and 18.1 g m^{-2} d^{-1} for stages I and II, respectively. Regarding the volumetric NO_3^-–N loading rates (L_v), the values applied were 6.8 g m^{-3} d^{-1} (stage I) and 18.1 g m^{-3} d^{-1} (stage II).

During stage I, the CWs which did not receive the external carbon source showed average nitrate removal on the order of 11.8% (CW control) and 45.8% (CWs vegetated). In stage II, the average nitrate removals were 2.0% and 11.5% for the uncultivated and cultivated CWs, respectively. Moreover, note that for the t_c of 1 d (stage II), nitrate removal was small in systems without ethanol addition. However, the NO_3^-–N removal can be considered satisfactory in cultivated CWs with t_c of 3 d (stage I), even without addition of an external carbon source.

With the addition of ethanol, removal efficiency in the CWs was far superior compared to those obtained in CWs receiving only nitrate. In stage I, with the addition of ethanol, the NO_3^-–N removals were 98.0% and 99.8% for uncultivated and cultivated CWs, respectively. In stage II, the removals were 94.7% and 96.5% for uncultivated and cultivated CWs,

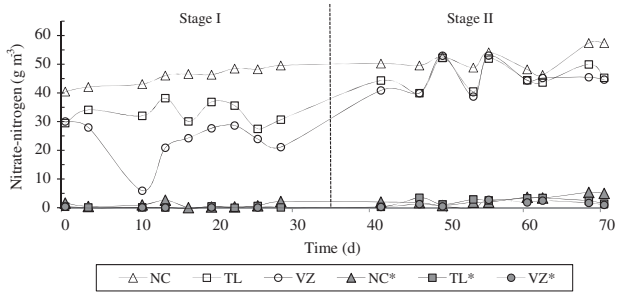

Fig. 1. NO_3^-–N effluent concentrations in CWs.
Notes: NC—Control treatment (non-cultivated), without ethanol addition to the influent; TL—Cultivated with *T. latifolia*, without ethanol addition to the influent; VZ -Cultivated with *C. zizanioides*, without ethanol addition to the influent; NC*—Control treatment (non-cultivated), with ethanol addition to the influent; TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

respectively. Based on these results, it was noted that the addition of an external organic carbon source was essential for efficient denitrification, particularly for waters devoid of dissolved organic carbon. This is in accordance with Lu et al. [12] that showed that the addition of glucose remarkably improved the nitrate removal ability of CWs studied.

Table 1 shows the data obtained. Effluent concentrations were calculated taking into account both ET and evaporation.

The nitrate concentration in the effluents of CWs receiving the external carbon source remained close to zero, even for stage II, when t_c was reduced to 1 d. The TL* significantly differed from NC* in stage I only, whereas the nitrate removal efficiency in VZ* was statistically different from the control in both stages.

For systems that received no external carbon source, the nitrate concentrations in the effluents of all CWs were statistically different in stage I, indicating differences in NO_3^-–N removals. However, in stage II the average nitrate concentration in the VZ* effluent showed no significant differences compared to those obtained in the TL* effluent.

In order to determine the amount of nitrogen removed by plants, a foliar analysis was performed. These values were determined as 0.23 g m^{-2} d^{-1} (cattail) and 0.26 g m^{-2} d^{-1} (vetiver) during stage II. These values are expressive, but smaller than the total removal of 2.7 g m^{-2} d^{-1} (TL) and 3.0 g m^{-2} d^{-1} (VZ). It is noteworthy that, even in CWs without ethanol addition, most of the nitrogen removal was due to denitrification. In these specific cases, the carbon source probably consisted of the exudates liberated by the plants. Nitrogen absorbed by the plants during stage II was on average 9.7% of the nitrogen removed by cultivated CWs without ethanol addition, and only 1.4% of the nitrogen removed by cultivated CWs with ethanol addition.

Table 1
Nitrogen–nitrate concentrations (mean ± standard deviation) corrected in function of ET and mass removal efficiencies of the CWs

	Stage I (t_c of 3 d)		Stage II (t_c of 1 d)	
Nitrogen–nitrate	(g m^{-3})	ε (%)	(g m^{-3})	ε (%)
Influent	51.7 ± 3.5	–	53.8 ± 6.8	–
NC	45.7 ± 3.1	11.1 C	51.6 ± 4.0	3.6 B
TL	32.7 ± 3.6	36.4 B	45.8 ± 4.6	14.4 A
VZ	23.4 ± 7.3	54.9 A	45.0 ± 5.1	15.7 A
NC*	1.01 ± 0.9	98.0 b	2.86 ± 1.6	94.7 b
TL*	0.19 ± 0.3	99.6 a	2.33 ± 1.1	95.5 ab
VZ*	0.02 ± 0.2	99.9 a	1.33 ± 1.0	97.5 a

Notes: Means followed by the same upper-case letter (for the CWs which did not receive the external carbon source) in the column do not differ and the means followed by the same lower-case letter (for the CWs which received the external carbon source) in the column do not statistically differ at the probability level of 5% by the Tukey's test.

NC—Control treatment (non-cultivated), without ethanol addition to the influent; TL—Cultivated with *T. latifolia*, without ethanol addition to the influent; VZ—Cultivated with *C. zizanioides*, without ethanol addition to the influent; NC*—Control treatment (non-cultivated), with ethanol addition to the influent; TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

Lin et al. [10] used unplanted and planted CWs with five macrophytes (*P. australis, C. communis, P. purpureum, I. aquatica, and P. stratiotes*) to treat groundwater with NO_3^-–N concentrations between 21 and 47 g m^{-3}. It was reported that a percentage from 4% up to 11% in the CWs were due to uptake by plants and denitrification was responsible for 89–96% of total removal. According to Wen et al. [13], different nitrate removal rates can be greatly affected by the composition of the plant biomass. Table 2 shows the global values of nitrogen–nitrate removed per surface area.

As observed in Table 2, the daily values of nitrate removal based the on surface area, in CWs which received the external carbon source, were greater in stage II when the t_c was only one day. These variations in results between stages for CWs receiving ethanol were due to the fact that the entire nitrate fraction was degraded in virtually a single day, which means that the daily removal values in stage I are smaller compared to those obtained in stage II.

Nitrogen removal rates in CWs without ethanol addition were similar to those found in literature. Bachand and Horne [14] studied the effect of different plants for nitrogen removal in CWs used to treat agricultural run-off. They found statistical differences among plant species and reported removals of 0.57 g m^{-2} d^{-1}(*Scirpus* spp.), 0.26 g m^{-2} d^{-1} (*Typha* spp.), and 0.84 g m^{-2} d^{-1} (multiple species).

Analysis of nitrite was performed to determine whether denitrification was taking place. A high conversion of nitrate to nitrite was observed in CWs receiving ethanol, whereas in CWs receiving no ethanol, the amount of nitrite accumulated in the reactor was practically zero.

Analyses of the decay profiles were performed in which the results were obtained at intervals of a few hours, for a total period of 3 d (stage III). The added concentration in this stage was (48.4 ± 0.7) g m^{-3} of NO_3^-–N. In the analyses, it was observed that nitrate was consumed rapidly in systems in which ethanol was added. Focusing on the decay profile after the first hour of the reaction, behavior of the NO_3^-–N

Table 2
Nitrate-nitrogen removed by superficial area (removed L_s). The applied NO_3^-–N loading rates were 5.8 g m^{-2} d^{-1} (stage I) and 18.1 g m^{-2} d^{-1} (stage II)

Removed L_s (g m^{-2} d^{-1})		Stage I (t_c of 3 d)			Stage II (t_c of 1 d)		
		Control	Cattail	Vetiver	Control	Cattail	Vetiver
NO_3^-–N	Without ethanol	0.7	2.1	3.2	0.7	2.7	3.0
	With ethanol	5.7	5.8	5.8	17.2	17.3	17.7

concentration in the residing solution can be better observed, as shown in Fig. 2. Note that the presence of plants aids in better nitrate removal efficiency in CWs, after 36 h.

Comparing the results of the nitrite analysis, it is noticed that the peak concentration of this variable coincides with the moment of greatest nitrate consumption by the micro-organisms in denitrification. The analysis of nitrite is shown in Fig. 3.

After the first hour of reaction, the nitrite concentration continued to decrease until the values reached very close to zero. The uncultivated wetland (NC*) presented poorer ability to remove nitrite present in the medium than the cultivated wetlands (TL* and VZ*), reaching values obtained in the cultivated CWs only after about 24 h. The improved nitrite removal efficiency by cultivated units may be attributed to greater denitrification rate due to the release of exudates from the plant roots.

3.2. Other water quality variables

Regarding organic material (measured as COD), in CWs without an external carbon source the average COD of the influent was 4.8 g m^{-3}, while for CWs in which ethanol was added the average was 202.6 g m^{-3} COD. COD values of the effluents did not tend toward a constant value and no statistical difference among the means was observed, presenting a high standard deviation. The high COD resultant of ethanol addition to the system was rapidly consumed by denitrifying for nitrate reduction.

During stages I and II, a wide variation in the values of redox potential (Eh) were observed for the same treatments between different days, and no definite pattern was observed. In analyses of decay (stage III), in Fig. 4, note the change in redox potential for CWs receiving the external carbon source.

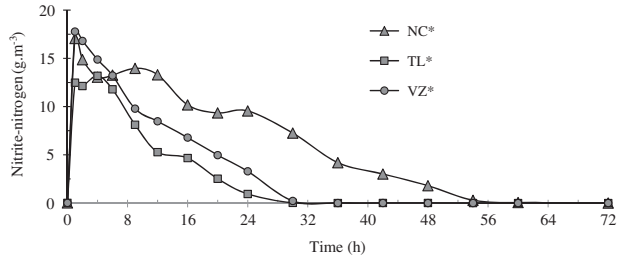

Fig. 3. Nitrite–nitrogen (NO_2^-–N) concentrations obtained in stage III (decay study).
Notes: NC*—Control treatment (non-cultivated), with ethanol addition to the influent; TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

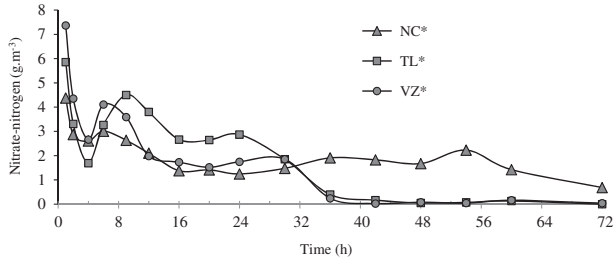

Fig. 2. NO_3^-–N concentrations obtained after the first hour of the reaction in stage III (decay study).
Notes: NC*—Control treatment (non-cultivated), with ethanol addition to the influent; TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

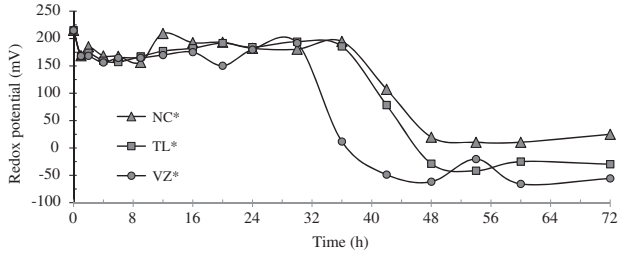

Fig. 4. Redox potential behavior in stage III (decay study).
Notes: NC*—Control treatment (non-cultivated), with ethanol addition to the influent; TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

Note that Eh values were above 150 mV for much of the time, only decreasing significantly after more than 30 h of monitoring the VZ*, and after more than 36 h for the TL* and NC*. Under the tested conditions, electron acceptors suffered the reducing activity of microorganisms, and thus the Eh tended to decrease. The lowest values of Eh suggest that oxidizing agents are consumed in larger amounts, leaving only the less energetic elements. Nitrate is a good oxidizing agent and tends to increase the value of Eh.

The declining trend of Eh in the planted systems can be attributed to deposition or release of exudates from plant roots in a liquid medium, serving as a substrate and source of organic matter for nitrate-reducing microorganisms. Flessa [15], in an experiment on the ability of plants to remediate an eutrophic aquatic environment, concluded that the Eh was affected by oxygen transport through the aerenchyma of plants, reporting an increase in redox potential values. This increase was also proportional to solar radiation. Moreover, Dušek et al. [16] found higher values of Eh overnight and assigned the lower values observed during the day to root exudates released by the plants. These exudates accelerate microbial processes that use oxygen and other electron acceptors.

Regarding pH, Fig. 5 shows the behavior of the effluent solutions from the CWs.

As can be seen in Fig. 5, the pH values are quite similar among the systems. However, note that (i) NC* effluents showed high pH values and (ii) in stage II, the effluents from CWs with the addition of ethanol were significantly higher. According to Kadlec and Wallace [17], the pH range in which denitrifying bacteria exhibit their best performance is from 6.5 to 7.5.

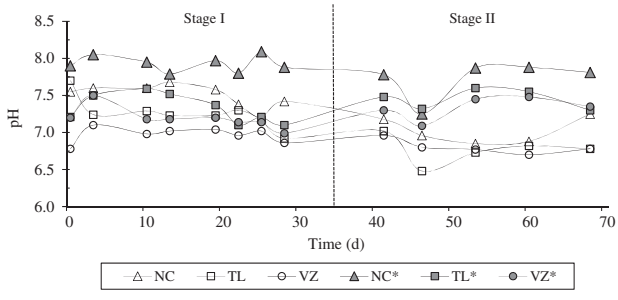

Fig. 5. pH values in stages I (t_c of 3 d) and II (t_c of 1 d).
Notes: NC—Non-cultivated CW, without ethanol addition to the influent; TL—Cultivated with *T. latifolia*, without ethanol addition to the influent; VZ—Cultivated with *C. zizanioides*, without ethanol addition to the influent; NC*—Non-cultivated CW, without ethanol addition to the influent; TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

None of CWs showed an average pH of lower than 6.5, yet the effluent of NC had a mean pH of 7.5 during stage I, and NC* presented pH values of 7.9 and 7.7 in stages I and II, respectively.

One factor that may have contributed to the decrease in pH of the effluent from cultivated CWs was the presence of organic substances generated by growth cycles, death, and decomposition of plants. Changes in pH may also result from the interaction of the biofilm with the substrates. Authors such as Theis and Young [18] found that the biofilm is a major buffer of pH to the point that the same pH values may be observed in cultivated and uncultivated CWs.

As a result, it is noted that even with production of alkalinity by denitrification, the wetland systems tend to be extremely effective in maintaining the pH of the medium. This buffering capacity was best evident in systems planted, which is slightly modified in relation to the influent pH of 6.9.

3.3. Volatile solids in biofilm from support media

After concluding the experimental stage for treatment of water enriched with nitrate-nitrogen, analyses of total volatile solids (TVS) were performed on the biofilm removed from the support. The concentration of TVS in this case can be related to the microorganisms present. Table 3 shows the amount of TVS found in each CW system.

Based on the data presented in Table 3, there are large amounts of biofilm (TVS) formed on CWs receiving the external carbon source when compared to that obtained in CWs receiving no external carbon source. Higher values were expected in systems supplemented with the additional carbon source, since in these CWs many microbiological reactions were reported, mainly due to denitrification. It was also observed that in the cultivated CWs without addition

Table 3
TVS mass added to support media (gravel) and TVS by gravel mass in studied CWs

TVS	NC	TL	VZ	NC*	TL*	VZ*
g	22.8	31.9	32.6	60.6	56.2	55.2
mg g^{-1}	0.32	0.44	0.45	0.84	0.78	0.77

Notes: NC—Control treatment (non-cultivated), without ethanol addition to the influent; TL—Cultivated with *T. latifolia*, without ethanol addition to the influent; VZ—Cultivated with *C. zizanioides*, without ethanol addition to the influent; NC*—Control treatment (non-cultivated), with ethanol addition to the influent; TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

of ethanol, the TVS mass was 40% (cattail) and 43% (vetiver) higher than those obtained in the control CW.

3.4. Plants

The first cut corresponded to the dry matter produced during a 194 d period, which consisted of the 154 d adaptation phase and 40 d of experimental stage I. The second cut was performed after the next 40 d of plant development, corresponding to stages II and III (decay study). At the beginning of stage II when the first cutting was performed, faster vegetative growth was observed, indicating the positive effect of periodic cutting of the plants. Fig. 6(a) shows the results for dry matter production by CWs planted with the different species.

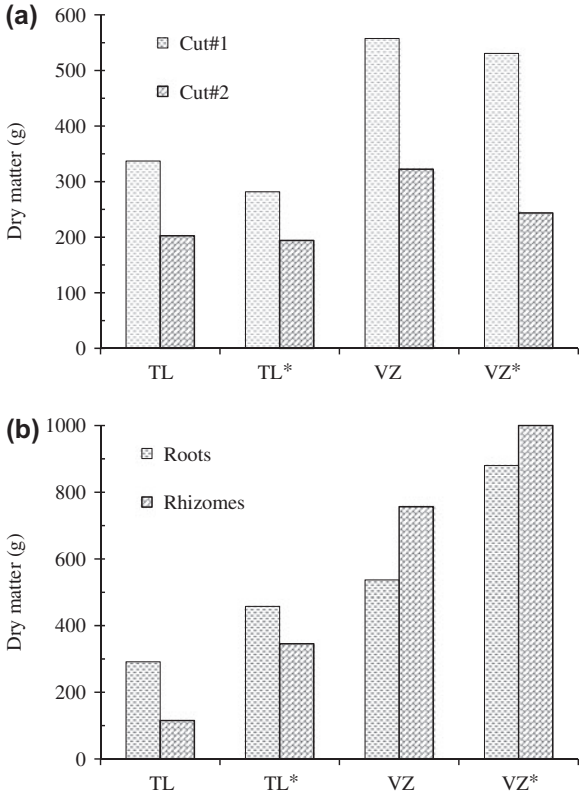

Fig. 6. Dry matter production by planted CWs: (a) in aerial compartments, considering two cuts performed and (b) in underground portion, after completing the experiments.
Notes: TL—Cultivated with *T. latifolia*, without ethanol addition to the influent; TL*—Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ—Cultivated with *C. zizanioides*, without ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

After completing the experiment and dismantling the CWs, all plant tissues were collected that were not removed during the cuttings and these samples were sent to the laboratory. The predominant root depth of cattail was roughly 0.15 m, with few roots reaching 0.30 m in length. Roots of vetiver reached up to 0.90 m in the CWs. Dry matter yields with respect to the underground portion (roots and rhizomes) are presented in Fig. 6(b).

The nitrogen concentrations in the dry cattail mass were higher than the concentrations found for vetiver, but the last one presented a larger production of dry plant mass which means that there is a relationship between values of nitrogen removed from the system and absorption by plants (Table 4). It is also noted that the percentage of foliar nitrogen was higher in the second cut. Nitrogen is a mobile element in the plant and can easily migrate from the old leaves to the new leaves that have greatest need of the element due to the development stage. Therefore, there should be a set time for cutting to optimize the removal of nutrients such as nitrogen, by concentration in the leaf.

The percentages of nitrogen removed by plant uptake, considering the total nitrogen applied daily to the CWs were 1.54, 1.36, 1.51, and 1.05% in the TL, TL*, VZ, and VZ*, respectively. These values correspond to a nitrogen uptake of 0.28, 0.24, 0.27, and 0.19 g m^{-2} d^{-1} in TL, TL*, VZ, and VZ*, respectively. To complement the data comparison an analysis on cattail and vetiver leaf nitrogen was performed, where samples were collected at the sites where the seedlings were removed. From the analysis of foliar nitrogen values of 0.9 dag kg^{-1} of N in the dry mass of cattail and 0.25 dag kg^{-1} of N in the dry mass of vetiver were obtained. Note that in wetland systems the amount of nitrogen in plants was much higher when compared to that obtained for non-intensive cultivations, for instance.

Table 4
Nitrogen per dry matter contents from the underground (roots and rhizomes) and aerial parts (two cuts) for each cultivated CW

dag kg^{-1}	TL	TL*	VZ	VL*
Cut#1	1.23	1.20	0.67	0.68
Cut#2	1.31	1.21	0.81	0.74

Notes: TL—Cultivated with *T. latifolia*, without ethanol addition to the influent; VZ—Cultivated with *C. zizanioides*, without ethanol addition to the influent; TL* -Cultivated with *T. latifolia*, with ethanol addition to the influent; VZ*—Cultivated with *C. zizanioides*, with ethanol addition to the influent.

CWs planted with vetiver grass showed better results for nitrogen removal. The plant likely provided a more favorable environment for denitrification due to the quality or quantity of exudates released in the medium. It was due to root distribution throughout the whole system, since the systems planted with cattail had an average root depth of only 0.15 m, while roots of vetiver achieved depths up to 0.90 m.

4. Conclusions

The CWs showed high denitrification rates in both the stages. By adding ethanol, it was possible to achieve high nitrate removal efficiencies, even in the control cells, with means equivalent to 99.2% (stage I) and 96.0% (stage II).

The plants contributed positively in getting better results in nitrogen removal in CWs, being nitrogen uptake by plants accounted for 13.3 and 10.4% of the N removal efficiency in the TL and VZ, respectively. For systems with ethanol addition, these values corresponded to 1.6 and 1.4% in the TL*and VZ*, respectively.

Results showed that CWs operating in batch mode can be used for the denitrification of nitrate-contaminated water.

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List of symbols and abbreviations

A — area
COD — chemical oxygen demand
CWs — constructed wetlands
Eh — redox potential
ET — evapotranspiration
MP — mass production
NC — non-cultivated constructed wetland, without ethanol addition to the influent
NC* — non-cultivated constructed wetland, with ethanol addition to the influent
NO_3^-–N — nitrate-nitrogen
NRB — nitrate-reducing bacteria
NSS — nitrogen standing stock
RBD — randomized block design
TAN — total ammonia nitrogen
t_c — cycle time
TL — constructed wetland cultivated with *Typha latifolia*, without ethanol addition to the influent
TL* — constructed wetland cultivated with *Typha latifolia*, with ethanol addition to the influent
TN — total nitrogen
TVS — total volatile solids
VZ — constructed wetland cultivated with *Chrysopogon zizanioides*, without ethanol addition to the influent
VZ* — constructed wetland cultivated with *Chrysopogon zizanioides*, with ethanoladdition to the influent

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