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Removal of nitrogen and phosphorus from cattle farming wastewater using constructed wetland system

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The confined dairy cattle system employs the most modern production techniques with respect to the genetic standards of the herds. The success found in these systems promoted an increase in the number of confined animals and, consequently, an increase in the produced volume of waste, causing the waste from dairy cattle farming to be one of the largest problems in intensive management systems. This effluent has high amount of phosphorus and nitrogen, and the accumulation of these nutrients in surface waters can cause eutrophication of water courses deteriorating their quality. This study is aimed to evaluate the efficiency of a constructed wetland system, cultivated with vetiver grass (*Vetiveria zizanioides*) in the removal of important environmental pollutants nitrogen (N) and phosphorus (P) from dairy cattle farming wastewater. The effluent received pre-treatment and before passing through the constructed wetland system which was built in a trapezoidal shape. The wastewater from dairy cattle farming, at the inlet and outlet of the constructed wetland system, was biweekly analyzed for different forms of N (ammonia, total Kjeldahl N, nitrate and nitrite) and P. The constructed wetland system proposed cultivated with vetiver grass showed good removal pollutants with greater mean efficiency in the removal of nitrite (43.6%), the total Kjeldahl nitrogen (32.0%) and ammonia (31.0%). Vetiver grass cultivation showed good adaptation to the constructed wetland system, with satisfactory development and no visual symptoms of nutrients deficiency.

**Key words:** Cultivated beds, water resources, wastes, reuse, biological treatment.

INTRODUCTION

Agricultural activity plays an important role in the national economy, besides being one of the first economic activities to be developed in the country. With the increase in food demand, agriculture has used more
areas to intensify production.

In Brazilian agriculture, dairy farming has stood out and milk has gained space in the market, being among the most important products of this activity. The confined dairy cattle system employs the most modern production techniques regarding the genetic standards of the herds. The success found in these production systems promoted an increase in the number of confined animals and, consequently, an increase in the produced volume of waste, making it one of the largest problems in intensive management systems and a challenge for farmers and specialists, because it involves technical, sanitary and economic aspects.

Thus, the organic effluents originated from confined dairy production systems, when disposed in a receiving body, cause physical and chemical alterations in the water sources, besides posing risks to public health (Silva and Roston, 2010).

Among the technologies used in the treatment of effluents, Constructed Wetland Systems (CWSs) have stood out and shown high potential of usage, because they have been used in the treatment of effluents (Vymazal, 2011). These are systems with moderate costs of installation, reduced energy consumption and maintenance, besides landscape aesthetics. Through the use of natural processes involving vegetation, supporting medium and microorganisms, these systems are projected to be able to promote, at least partly, the treatment of effluents or other types of low-quality waters.

One of the greatest concerns in effluent treatment systems is their capacity to remove phosphorus (P) and nitrogen (N). The excessive accumulation of N in surface waters may cause eutrophication of watercourses and lead to ecological imbalance, promoting exaggerated growth of plants and animals, deteriorating the quality of the water. P, in turn, for being one of the nutrients that are essential for algae growth in watercourses, is considered as the main element involved in the process of eutrophication, which makes its removal very important in effluent treatment systems (Prochaska and Zouboulis, 2006).

Various plant species have been used and indicated for cultivation in CWSs, because they naturally occur under conditions similar to those in the CWSs. The plants used in CWSs must be perennial, with high tolerance to excess water, easy harvest and management, and high capacity to remove nutrients and pollutants (Paganini, 1997). It is noteworthy that the CWS configuration and type of substrate can influence the removal efficiency of system pollutants. Moreover, the various pollutants are not removed of the CWS in the same proportion.

Therefore, the species Vetiveria zizanioides, known as vetiver grass, very resistant to climatic variations and tolerant to contaminants (Ucker et al., 2012), is used in Brazil as vegetation on slopes, with the objective of controlling erosive processes (Andrade et al., 2011). The vetiver grass, since it has a deep and abundant root system, can be an alternative in constructed wetland systems (Barbosa and Lima, 2013).

Given the above, the aim of this study was to evaluate the capacity of removal of important environmental pollutants P and N using the constructed wetland system cultivated with vetiver grass in the post-treatment of wastewater from dairy farming.

**MATERIALS AND METHODS**

**Characterization of the area**

The study was carried out in the Agroecological Production Integrated System (SIPA) area, also known as “Fazendinha Agroecológica do km 47” (Figure 1), located in the municipality of Seropédica-RJ, Brazil (22°48’00”S; 43°41’00”W; 33 m), from June to November 2014. According to Köppen's classification, the climate of the region is Aw, with rains concentrated from November to March, mean annual rainfall of 1,213 mm and mean annual temperature of 24.5°C (Carvalho et al., 2006).

**Wastewater treatment system**

The treatment system of wastewater from dairy cattle farming (WDCF) comprised a pilot treatment unit consisting of: compost box, sedimentation tank, crushed stone filter, organic filter and constructed wetland system (CWS) with horizontal subsurface flow which was sized according to methodology presented by Marques (1999). The WDCF passed through the treatment system following the order of the previously mentioned components (Figure 2).

The CWS had a superficial area of 5.2 m² and was built in masonry and waterproofed with a 0.5-mm-thick PVC canvas; its inside was filled with crushed stone n° 1 (24 mm mesh) until the height of 40 cm and a 5-cm-thick layer of sand was in place to fix the crop (Figure 3). The CWS was built in a trapezoidal shape and its lower and upper sides were 0.5 and 1.3 m long, respectively. The hydraulic retention time (HRT) of the CWS was equal to 1.4 days (33.6 hours), considering the pore volume of the crushed stone n° 1 as equal to 50%. The wastewater from dairy cattle passed through continuously CWS.

The CWS was cultivated with vetiver grass (Vetiveria zizanioides), and has the following characteristics: very efficient in slope stabilization, aromatic plant that reaches height of 2 m and its roots can penetrate with a depth of 3 m (Barbosa and Lima, 2013).

Vetiver seedlings were obtained in the SIPA and planted in the CWS using a spacing of 0.12 x 0.2 m, making a total of 200 seedlings. A spacing smaller than that recommended in Andrade et al. (2011) was used in order to allow a higher number of plants for the removal of nutrients. The quantification of nutrients absorbed the culture was not carried out. The visually performance grass vetiver and average plant height after the conclusion of the experiment using measuring tape was evaluated. Were selected 20 plants randomly along the CWS. The vetiver grass height from the base to the apex of the plant was determined.
There are several shapes, sizes and substrates used in CWS. Therefore, it is extremely important to evaluate the efficiency of pollutant removal in the CWS proposed in this work and check if the results are consistent with those observed in the literature.

**Monitoring and evaluation of the treatment system**

The performance of the constructed wetland system was evaluated biweekly through the collection of 500 mL of WDCF at the inlet and the outlet of the CWS cultivated with vetiver. The collection of WDCF was started in August 2014. After collection, the WDCF was sent to the Laboratory of Environmental Monitoring I – Water and Effluents, of the Engineering Department in UFRJ, for the following analyses: phosphorus, ammonia, nitrate, nitrite and total Kjeldahl nitrogen (TKN), according to the recommendations contained in APHA (1999).

The P removal efficiency of the CWS was analyzed based on the phosphate ion ($\text{PO}_4^{3-}$). It is one of the main forms of P in waters and is considered as an orthophosphate.

The main residues from dairy cattle farming are feces and urine. However, the effluent also showed water from washing, fur, milk (colostrum) and fat from the body animals and milk.

It should be pointed out that the WDCF came from the washing of the SIPA pen and there was no standardization about the amount of water used or the frequency of washings.

Before receiving the treatment, the WDCF showed mean values of 75.9, 229.9, 120.9, 46.8 and 2.2 mg L$^{-1}$ of phosphorus, ammonia, nitrate...
Table 1. Inlet and outlet values and standard deviation of the analyzed parameters in the constructed wetland system cultivated with vetiver grass.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inlet</th>
<th>Outlet</th>
<th>Average removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus (mg L(^{-1}))</td>
<td>59.7 ± 26.9</td>
<td>44.1 ± 20.5</td>
<td>25.2</td>
</tr>
<tr>
<td>Ammonia (mg L(^{-1}))</td>
<td>51.3 ± 12.4</td>
<td>35.4 ± 9.6</td>
<td>31.0</td>
</tr>
<tr>
<td>TKN (mg L(^{-1}))</td>
<td>72.9 ± 33.4</td>
<td>49.6 ± 26.6</td>
<td>32.0</td>
</tr>
<tr>
<td>Nitrate (mg L(^{-1}))</td>
<td>9.9 ± 1.3</td>
<td>8.9 ± 2.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Nitrite (mg L(^{-1}))</td>
<td>0.6 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>43.6</td>
</tr>
</tbody>
</table>

TKN: Total Kjeldahl nitrogen.

RESULTS AND DISCUSSION

The results referring to the mean values of the parameters phosphorus, ammonia, total Kjeldahl nitrogen, nitrate and nitrite at the inlet and outlet of the horizontal subsurface-flow CWS cultivated with vetiver grass are shown in Table 1.

The concentrations of all parameters analyzed in the effluent, at the CWS outlet, were lower than those at the inlet, demonstrating that there was a reduction of these parameters in the system. The reduction of pollutants has been observed in various studies with different types of wastewater using constructed wetland system cultivated or uncultivated (Tee et al., 2009; Fia et al., 2012; Chagas et al., 2011; Colares and Sandri, 2013).

The high values of standard deviation can be explained by the fact that there was no control over the wastewater that came to the SIPA's compost box, which was pumped directly into the treatment system from the cattle pen. This represented variation in the concentrations of elements and pollutants in the utilized WDCF. High values of standard deviation were also observed by Wu et al. (2014) and in various studies that used CWSs of different types for the removal of N and organic matter in wastewaters.

There were low mean concentrations of nitrate and nitrite at the outlet of the CWS cultivated with vetiver grass, which were respectively equal to 8.9 and 0.4 mg L\(^{-1}\) (INEA, 2014). Nitrite is an ion that has intermediate conditions of reaction, since it is formed from the nitrification process, which is the first oxidation of ammonia in the nitrification pathway (Nunes, 2012). For this reason, it is commonly found at low concentrations in constructed wetland systems.

However, the mean concentrations of total Kjeldahl nitrogen, phosphate and ammonia in the effluents at the outlet of the horizontal subsurface-flow CWS cultivated with vetiver grass were 44.1, 49.6 and 35.4 mg L\(^{-1}\), respectively. This indicates that the effluent cannot be disposed in water resources (INEA, 2014), even after passing through the treatment. Hence, potential forms of reuse of WDCF, especially agricultural reuse, must be stimulated provided that agronomic and environmental criteria are considered.

It should be pointed out that the mean concentrations of the parameters phosphorus, ammonia, total Kjeldahl nitrogen, nitrate and nitrite in the effluent at the CWS outlet were dependent on their concentrations in the total Kjeldahl nitrogen (TKN), nitrate and nitrite, respectively. The mean pH value of the analyzed samples before passing through the CWS was 6.7.

**Figure 3.** Constructed wetland system cultivated with vetiver grass; A: Transplantation of seedlings; B: Established vetiver.
Figure 4. Removal efficiency and standard deviation of the parameters phosphorus, ammonia, total Kjeldahl nitrogen (TKN), nitrate and nitrite using a constructed wetland system (CWS) cultivated with vetiver grass.

Phosphorus

In constructed wetland systems, P removal mainly occurs through processes of adsorption, precipitation and absorption by plants (with subsequent harvest) (Vymazal, 2007).

According to the author, P removal varies between 40 and 60% among all types of wetland systems; however, results lower than 40% were found by Brasil et al. (2007), Pelissari (2013) and Stone et al. (2004). Constructed wetland systems have limited capacity for P removal, because there is no permanent loss of the nutrient in these systems, which occurs, for example, in the removal of N compounds (Ucker et al., 2012).

The mean CWS efficiency in P removal was 25.2%, which is considered good according to some literatures (Matos et al., 2010; Pelissari, 2013; Stone et al., 2004). According to Vymazal (2007), P removal in all types of CWS is low, unless special substrates with high capacity of absorption of this nutrient are used. This depends on the physical and chemical properties of the materials (Vymazal, 2014). The use of alternative substrates, such as lightweight clay aggregates and industrial slags, among others, can increase P removal efficiency in constructed wetland systems. Calcareous materials can promote the precipitation of calcium phosphate.

It should be pointed out that the filtering medium used in the experiment (crushed stone) has low retention capacity for cations to promote P adsorption (Vymazal, 2014). The observed value of efficiency was lower than those reported by Brasil et al. (2007), who obtained mean total P removal efficiency of 31 to 48%. However, it was greater than that obtained by Pelissari (2013), who observed P removal efficiency of 10% in the CWS. Most of the P is removed through the accumulation of organic P in the supporting medium and its immobilization by microorganisms (Matos et al., 2010). These authors obtained P removal efficiency of 33 to 55%, but highlighted that there are studies in the literature in which the P concentration in the wastewater increased after treatment with the CWS, i.e., there was no efficiency in the system to remove this nutrient. These authors pointed out that a possible cause for the low P removal or increase in its concentration at the outlet of the CWS is the loss of water from the system through evapotranspiration, underestimating or canceling the efficiency obtained in the CWS.

The phosphate removal efficiency showed wide variation, from 12 to 42%. Stone et al. (2004) observed P removal efficiency from 13 to 29% while working with CWSs cultivated with southern cattail for the treatment of swine farming wastewater. The release of P to the
effluent may occur due to the formation of organic acids, nitrates or sulfates in the environment, which may cause oscillations in the redox potential and reduction in pH, thus releasing P to the environment (Headley et al., 2005).

Probably, in order to obtain greater P removal efficiency, it would be necessary, among other factors, to adopt a longer hydraulic retention time (Matos et al., 2010; Vymazal, 2009; Silva and Roston, 2010) so that the effluent has a longer time of contact with the supporting medium of the treatment system and consequently improving its efficiency.

Although phosphate ion (PO$_4^{3-}$) is one of the main forms of P in water, other forms of P should be monitored in order to detect if there is removal of P in other forms available in the wastewater.

Nitrogen

The total nitrogen expressed based on the total Kjeldahl nitrogen, corresponds to the sum of organic N, ammonia and ammonium. Hence, part of the observed removal is due to the decrease in the concentration of ammonia and mineralization of organic matter.

On average, the values of efficiency of removal of total Kjeldahl nitrogen, ammonia, nitrate and nitrite from the WDCF using the horizontal subsurface-flow CWS were 32.0, 31.0, 10.2 and 43.6%, respectively. The lowest and the highest efficiency of the system occurred in the removal of nitrate and nitrite, respectively. Also, the adopted CWS promoted reasonable performance in the removal of pollutants (Tanner et al., 2005; Vymazal, 2007).

 Constructed wetland systems remove pollutants through sedimentation, adsorption, accumulation of organic material, microbial assimilation, nitrification-denitrification, ammonia volatilization and removal by plants (Vymazal, 2011). Probably, for a higher adsorption to occur, it would be necessary for another type of filtering medium, instead of crushed stone, which has low capacity of retention for cations (Vymazal, 2014), or an increment in the hydraulic retention time (Matos et al., 2010).

N is an ion that is present in different forms in the effluent. N removal in CWSs starts after the transformation of organic N into inorganic N, a process called ammonification. Organic N can be converted into ammonia (NH$_3$) or ammonium (NH$_4^+$), and environments with pH close to or lower than neutrality lead to the predominance of NH$_4^+$. The ammonium ion can be absorbed by the crop and/or oxidized to nitrate through the process of nitrification (Saeed et al., 2012). Since the pH observed in the WDCF was close to or lower than neutrality, the ammonia may have been transformed into the ammonium ion (Nunes, 2012) and absorbed by the vetiver grass.

The observed removal of ammonia may also have occurred through the process of ammonia volatilization; however, this is not a desirable process, because a transfer of pollution would be occurring, since the NH$_3$ is an atmospheric pollutant and can contaminate aquatic and terrestrial environments, through dry deposition in soils or precipitation, reaching aquatic systems, causing acidification and eutrophication, and significantly altering natural ecosystems (Zhou et al., 2008).

There are different processes of removal of ammonia from the liquid fraction in CWSs, among which nitrification stands out, followed by denitrification. Nitrification is the biological process in which ammonia is oxidized to nitrite and the latter to nitrate, mostly by autotrophic bacteria called Nitrosomonas and Nitrobacter, respectively (Nunes, 2012). During the process of nitrification, nitrifying bacteria utilize the oxygen transferred from the atmosphere to the substrate through plant roots to oxidize the ammonia.

The nitrification process is highly influenced by environmental conditions; the pH and the presence of organic compounds, inorganic compounds and toxic substances are some of these influencing factors (Nunes, 2012). According to Nunes (2012), the pH considered as optimal for nitrification is between 7.2 and 8.0. The pH of the WDCF observed during the experimental period was, on average, 6.7, that is, below the optimal range for the occurrence of nitrification. This may have contributed to the occurrence of reduction in the ammonia removal efficiency. Additionally, the CWS used in the present study has horizontal subsurface flow, which shows anaerobic conditions compromising the process of nitrification because had no root system in the bottom of the CWS. Vymazal (2014) analyzed various studies conducted using CWSs and observed that, in one of the analyzed studies, the concentration of ammonia at the outlet of the CWS was superior to that at the inlet, which was attributed to the limited capacity of nitrification of the horizontal subsurface-flow CWS.

Therefore, ammonia may also be removed through anaerobic ammonia oxidation (ANAMOX), which occurs in environments with deficit of oxygen. Under these conditions, ammonium reacts with nitrite, generating molecular N (N$_2$) as the product (Saeed et al., 2012; Scheeren et al., 2011). This process plays an important role in constructed wetland systems where NH$_4^+$ and NO$_2^-$ coexist, thus stimulating the increment in N removal rates. It is also possible that part of the N has been immobilized and/or mineralized in the medium by microorganisms through biogeochemical pathways.

Matos et al. (2010), using constructed wetland systems in the treatment of swine farming wastewater (PFW), obtained total N removal efficiency of 64% in systems cultivated with Tifton 85, and 61% for systems cultivated with Alternanthera.

Ucker et al. (2012) obtained good efficiency in the removal of ammonia in CWSs cultivated with vetiver.
These authors observed removal efficiency values of 93 and 73% for CWSs cultivated with this species, varying the effluent depth level, and 42 and 43% in non-vegetated systems.

A higher efficiency of removal of N compounds by the CWS cultivated with vetiver grass, which has rapid and high capacity to remove nutrients such as N and P, besides large amounts of agrochemicals and heavy metals was expected (Ucker et al., 2012). Cheng et al. (2008) evaluated the performance of constructed wetland systems for an effluent that is disposed in one of the most polluted rivers of Taiwan, the Wu-Luo River, and obtained results of N removal from the effluent of about 93%.

According to Wu et al. (2014), traditional wetland systems constructed with horizontal subsurface flow often have low efficiency in the treatment of wastewaters, in comparison to other types of CWS, because the horizontal subsurface-flow CWS has a small amount of oxygen.

Despite the good removal of N, on average, for ammonia, TKN and nitrate, the effluent at the outlet of the CWS still had pollutant power. An important factor that may have promoted this result is the hydraulic retention time used in the present study, which was 33.6 h, because the lack of contact time necessary between the effluent and the substrate of the CWS can reduce its efficiency (Fia et al., 2010). Tanner et al. (2005) used cultivated beds in the treatment of wastewater from dairy cattle farming and obtained a total N removal of 79% in the first year and only 21% the second year.

According to Vymazal (2007), simple CWSs cannot achieve a high removal of total N, due to their inability to provide aerobic and anaerobic conditions, at the same time. This is because vertical-flow CWSs are able to successfully remove ammonia, but the denitrification is limited in these systems. On the other hand, according to the same author, horizontal-flow CWSs provide good conditions for denitrification, but the capacity of these systems for ammonia nitrification is very limited. Therefore, it is recommended to use more than one type of CWS to obtain better efficiency in the removal of this pollutant.

It should be highlighted that, along the present study, the CWS showed different values of efficiency, causing high amplitude in the removal of pollutants. In the samplings with the best results, it was observed that the treatment using horizontal subsurface-flow CWS was able to remove 76.5, 50.5, 37.4 and 64.4% of TKN, ammonia, nitrate and nitrile, respectively. These results are similar to those observed by many authors (Ucker et al., 2012; Matos et al., 2010; Tanner et al., 2005). It is important to point out that no chemical treatment was performed in the present study, only biological treatment for the removal of pollutants.

It was observed that the standard deviation of nitrate removal efficiency was superior to the mean of this parameter. This occurred because the nitrate concentration at the inlet; therefore, nitrate removal did not occur in some samples. Similar results were observed in the study conducted by Matos et al. (2010), who used CWSs in the treatment of swine farming wastewater. These authors suggest that the increase in nitrate concentration at the outlet of the CWS may have been due to the oxidation of ammoniacal forms, which probably caused the inefficiency of the system in the removal of nitrate.

The biological process that effectively removes the N from the treatment system is called denitrification. This reaction may occur in anoxic environments or environments with restricted oxygen, where nitrate will be reduced to nitrous oxide, which is released to the atmosphere. Nitrate can also be removed through absorption by the plant. Probably, in some samples, the vetiver grass does not absorb the nitrate present in the WDCF in an amount necessary for the occurrence of higher removal efficiency in the system, because its consumption by the plant is limited. The evapotranspiration of the system may also have contributed to this result (Ucker et al., 2012), besides the low retention of nitrate in the supporting medium.

According to Vymazal (2009), satisfactory results in wastewater treatment using CWS with horizontal subsurface flow have been reported by various authors. It should be pointed out that, although the CWS did not promote high efficiency in the removal of pollutants, it showed results similar to those observed by Stone et al. (2004) and Tanner et al. (2005). Therefore, the CWS with horizontal subsurface flow can be used in the post-treatment of wastewaters.

The vetiver grass used in the CWS showed good development in comparison to the data reported in the literature (Barbosa and Lima, 2013). The vetiver grass of average height was 1.4 m. Value above that observed by Andrade et al. (2011). The good performance of this crop, besides demonstrating its good adaptation to the CWS, shows that the WDCF probably provided the nutrients necessary to its development. In addition, no symptom of toxicity or deficiency of nutrients was observed in the vetiver grass. Ucker et al. (2012) used vertical- and horizontal-flow CWSs cultivated with vetiver grass and CWS without cultivation and observed higher N and P removal efficiency in CWSs cultivated with vetiver grass.

Studies have demonstrated that the CWSs with plants show higher efficiency of treatment, especially in the removal of organic matter and nutrients (Vymazal, 2011). According to Tee et al. (2009), this occurs due to the creation of microaerobic regions in the root zone of the plants in the CWS, which allows a faster rate of biodegradation and uptake of nutrients by plants.

It is emphasized that although the vetiver grass assist in the removal of pollutants, the plant can sometimes cause an increase in organic matter in the effluent due to the replacement of culture roots and thus increase the
concentration of pollutants. This can reduce system efficiency.

Conclusions

Based on data observed in the literature, the constructed wetland system proposed cultivated with vetiver grass showed adequate efficiency in the removal of pollutants, with higher efficiency in the removal of nitrate, total Kjeldahl nitrogen and ammonia.

The vetiver grass exhibited good adaptation to the constructed wetland system, satisfactory development and there were no visual symptoms of nutrients deficiency.

For the same configuration of the constructed wetland system proposed in this work, it is recommended to use a larger hydraulic retention time for greater efficiency of the treatment system in removing pollutants.

Conflict of Interests

The authors have not declared any conflict of interests.

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