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Dairy farm wastewater treatment using horizontal subsurface flow wetlands with *Typha domingensis* and different substrates

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ABSTRACT

The aim of this work was to evaluate the influence of different substrates in the performance of a horizontal flow constructed wetland employed in dairy farm wastewater treatment. *Typha domingensis* was chosen for this study due to its high productivity and efficiency in nutrient removal. Fifteen microcosm-scale reactors simulating horizontal flow constructed wetlands were disposed in a greenhouse in triplicate. Five substrates (river gravel, gravel, LECA, river gravel + zeolite and gravel + zeolite) were evaluated. Real effluent with previous treatment was used. Dairy farm effluents favoured *T. domingensis* growth, probably due to their high nutrient concentrations. The treatments with the different substrates studied were efficient in the treatment of the dairy farm effluent obtaining ammonium (NH₄⁺) and total phosphorus (TP) removals between 88–99% and 86–99%, respectively. Removal efficiencies were significantly higher in treatments using LECA and combined substrate (gravel + zeolite). After treatment, the quality of the final effluent was significantly improved. Outlet effluent complied with regulations and could be discharged into the environment.

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substrates; wetland

1. Introduction

In Argentina, specifically in the Pampas region, milk production increased remarkably some years ago, generating a growing demand of water and increasing effluent production in milking activities. Improper handling of effluents is one of the main sources of pollution of groundwater as well as surface water. Wastewater from agricultural operations has been a long-standing concern with respect to contamination of water resources, particularly in terms of nutrient pollution. Contaminants from livestock wastes can enter the environment through pathways such as leakage from poorly constructed manure lagoons, or during major precipitation events resulting in either overflow of lagoons and runoff from recent applications of waste to farm fields, or atmospheric deposition followed by dry or wet fallout.

In Argentina, dairy farm wastewaters are either poorly treated or not treated at all, and are directly discharged into surface water courses, especially low flow, causing their pollution by eutrophication. Nosetti et al. [1] studied 65 dairy farms in Buenos Aires, Argentina, where 50% of such farms eliminated the untreated effluent directly into nearby streams. In order to reduce the

economic and environmental impact of those activities, two actions are considered necessary: (1) recycling of water - in order to decrease its total consumption - separating the relatively clean fractions from the heavily contaminated ones; and (2) a final treatment of wastewater, in order to adopt a simple depuration strategy for the less-contaminated fraction which might allow its discharge into surface waters. The use of constructed wetlands (CWs) could be a viable option to treat dairy farm wastewater, due to their relatively low construction and operating cost and their demonstrated effectiveness. In the last years, scientific research into the effectiveness of CWs in breaking down and treating animal wastewater and removing contaminants has intensified [2–4]. In the area under study, there is a growing interest in the potential of alternative treatment methods such as CWs due to an increasing trend to intensive farming and thus larger amount of wastewater to be treated.

Horizontal subsurface flow wetlands (HSSFWs) have been successfully tested for dairy wastewater treatment worldwide [5–9]. Macrophytes and media play an important role in the removal of pollutants [10]. The type of substrate influences plant growth, the species richness and the structure of bacterial communities in wetland

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systems, as well as the treatment performance [11,12]. It has been shown that selection of substrate is also very important for the lifespan of the system because a very fine media will clog and thus surface runoff will occur [13].

Cattails very often invade natural wetlands and displace other species. Also, when planted in CWs, they tend to out-compete other species planted in the wetland [14]. *Typha domingensis* was chosen for this study due to its high productivity and efficiency in nutrient removal [15,16] and because it is widely distributed in natural wetlands in Argentina.

The aim of this work was to evaluate the influence of different substrates in the performance of a horizontal flow constructed wetland employed in dairy farm wastewater treatment.

2. Materials and methods

2.1. Experimental design and substrate properties

Microcosm-scale reactors simulating HSSFWs were disposed in a greenhouse. Reactors $(0.35 \times 0.30 \times 0.35 \text{ m};$ length x width x depth) containing two plants of *T. domingensis* and different substrates were disposed in triplicate. Studied substrates are indicated in Table 1. Reactors operated with horizontal subsurface flow. The hydraulic load applied was low, 10 mm.d⁻¹, corresponding to a nominal hydraulic residence time (HRT) of about 7 days. Organic load applied was 0.7 g m⁻² day⁻¹.

River gravel and gravel are substrates widely used in CWs mainly due to their low cost and porosity. LECA is initial letters for 'Light Expanded Clay Aggregate' and it consists of small, lightweight, bloated particles of burnt clay. Small air-filled cavities give LECA its strength and thermal insulation properties. The base material is plastic clay, which is extensively pretreated and then heated and expanded in a rotary kiln. Finally, the product is burned at about 1100°C to form the finished product.

The use of natural zeolites in environmental applications is spreading due to their properties and significant worldwide occurrence. They are natural minerals mined in various deposits and can also be produced

Table 1. Experimental design.

Treatment	Substrate	Diameter (mm)	Substrate height (cm)
T ₁	River gravel	10–30	30
T ₂	Gravel	6–19	30
T_3	LECA	5–19	30
T ₄	River gravel + zeolite	10–30 and 0.6–5	10 + 20
T ₅	Gravel + zeolite	6–19 and 0.6–5	10 + 20

synthetically. Natural zeolites are crystalline, hydrated alumino-silicates of alkali and earth metals that possess infinite, strong, open, one or three-dimensional crystal structure [17,18]. Natural zeolite was chosen for this study due to its capacity to increase NH⁺₄ nitrogen abatement performance.

2.2. Plant growth monitoring

Macrophytes were collected from a natural environment, pruned to 30 cm, disposed in the reactors and acclimatized prior to the experiment. The acclimatization period lasted 150 days. During the first 60 days, tap water was added to the reactors. Then, diluted real dairy farm effluent was added during 90 days. Growth was monitored by measuring the plant height in each reactor. When the acclimatization period finished, macrophytes were pruned to a uniform height of 30 cm and the experimental period began.

2.3. Experiment and chemical analysis

Reactors were filled with real dairy farm effluent with previous treatment (anaerobic and facultative ponds). HRT in the reactors was 7 days. The experiment lasted 35 days. In order to evaluate contaminant removal efficiencies in each reactor, pH, electrical conductivity, suspended solids (SSs), total Kjeldhal nitrogen (TKN), ammonium (NH₄⁺), nitrate (NO₃⁻), total phosphorus (TP) and chemical oxygen demand (COD) were analyzed according to APHA [19]. All these measurements were carried out before and after the treatment in each reactor. Analytical determinations were carried out in triplicate. Temperatures ranged between 20 and 34°C during the development of the experiment.

2.4. Statistical analysis

Analysis of variance (ANOVA) was used to determine whether significant differences existed in contaminant removal efficiencies among treatments with completely randomized blocks.

The normality of residuals was analyzed graphically and homogeneity of variances was checked using Bartlett's test. When necessary, data were transformed (log) to achieve homogeneity of variances and normality. Duncan's test was used to differentiate means where appropriate. A level of p < .05 was used for all comparisons. Calculations were performed using the Statgraphics Plus 3.0 software.

Table 2. Chemical composition of the inlet effluent.

Parameters	Value ranges	Mean
pH	7.87-8.44	8.18 ± 0.23
Electrical conductivity (mS cm ⁻)	3.48-4.33	3.88 ± 0.30
SSs (mg L ⁻)	140-245	208 ± 48.4
TKN (mg N L^{-1})	54.7-67.7	62.8 ± 5.8
Ammonium (mg $NH_4^+ L^{-1}$)	42.8-53.9	48.8 ± 4.7
Nitrate (mg $NO_3^{-}L^{-1}$)	15.3-45.0	24.0 ± 11.9
TP (mg P L^{-1})	10.8-18.7	14.6 ± 3.7
$COD (mg O_2 L^{-1})$	194–412	271 ± 98.6

3. Results and discussion

3.1. Inlet effluent characterization

Means and ranges of the parameters analyzed are indicated in Table 2. The chemical composition of the effluent to be treated presented high variability, a common characteristic of dairy farm effluents treated by ponds. Electrical conductivity remained between 3.48 and 4.33 mS cm⁻¹. TKN varied between 54.7 and 67.7 mg L⁻¹, being NH₄⁺ the main nitrogen species (42.8–53.9 mg L⁻¹). Nitrate varied between 15.3 and 45.0 mg L⁻¹. COD ranged widely between 194 and 412 mg L⁻¹.

3.2. Growth monitoring

Growth monitoring was carried out in three stages: adaptation with tap water, adaptation with effluent and experimental stage. Figure 1 shows growth evolution during the three stages mentioned. Maximum plant growth rates were observed after day 60, when the reactors were filled with wastewater for the first time. This is due to an increased nutrient supply form dairy farm effluent. During the experimental stage, plants grew faster after pruning than in the acclimatization



Figure 1. Macrophytes growth evolution. (a) In reactors using river gravel. (b) In reactors using gravel. (c) In reactors using LECA. (d) In reactors using river gravel + zeolite. (e) In reactors using gravel + zeolite.



Figure 2. Macrophyte's growth evolution.

stages. Figure 2 shows macrophytes height and biomass evolution at the beginning and the end of the experiment.

The highest growth rates were registered in macrophytes of T₃ (LECA), T₄ (river gravel + Zeolite) and T₅ (gravel + zeolite) reactors, proving to be suitable substrates (Figure 1). Plants did not fully grow in reactors filled with river gravel and gravel. High temperature and weight of these substrates might have been the cause of lower macrophytes growth. When dismantled, plants of reactors with gravel had few roots if compared with other substrates.

3.3. Removal efficiencies

pH was uniform in all experiments, varying between 7.71 and 8.93. These values are similar to pH range of the inlet effluents used for the experiments.

Electrical conductivity did not present significant difference before and after the treatment. This parameter ranged between 3.12 and 3.94 mS cm⁻¹ through the experiment.

Removal ranges and mean values of the different parameters measured for each treatment under study can be observed in Figures 3–8. The concentration of SSs in the wastewater after treatment (Figure 3) decreased around 55% (49.4–58.9%) in all studied systems. There were no statistically significant differences among the treatments (p > .05). Effluent depuration was also checked visually since turbidity and color of samples decreased notoriously after treatments. The contribution of emergent plants on total SSs removal is attributed to the growth of their stems, roots and rhizomes that enhance SSs removal efficiency by reducing water velocity and reinforcing settling and filtration in the root network [20]. Besides, SSs removal efficiency is mostly related to



Figure 3. Removal efficiency of SSs.



Figure 4. Removal efficiency of TKN.



Figure 5. Removal efficiency of ammonium.



Figure 6. Removal efficiency of nitrate.



Figure 7. Removal efficiency of TP.



Figure 8. Removal efficiency of COD.

the sedimentation and filtration processes within the wetland media [21].

The removal percentages for TKN were significantly different for all treatments (p < .05). The greatest TKN removal efficiency was registered in T₅ (gravel + zeolite) reaching a mean removal of this contaminant of 85.1% (Figure 4), probably due to NH₄⁺ adsorption by zeolite. Reactors with river gravel and gravel also had good results. Treatments T₃ (LECA) and T₄ (zeolite + river gravel) presented a significantly lower performance than the other treatments. In inlet effluent, total nitrogen is approximately 61% in the form of ammonia. In consequence, TKN removal efficiencies reached could be due mainly to high NH₄⁺ removal efficiencies.

nitrogen removal mechanism is achieved by biological processes that convert the organic and ammonia nitrogen to nitrate in an aerobic environment (nitrification) and then reduce the nitrate to nitrogen gas in an anoxic environment (denitrification) [22]. Volatilization, absorption and plant uptake play a much less important role in CWs [23].

Ammonium removal rate varied between 92 and 97% in all treatments and the statistical analysis showed no significant differences (p > .05) (Figure 5). The decrease of NH₄⁺ concentration in wastewater could be explained by the transformation of NH₄⁺ into NO₃⁻ (nitrification). It is probably that nitrification could be produced in aerobic microzones near roots plus a subsequent denitrification. NH₄⁺ removal could also be produced by anaerobic NH₄⁺ oxidation (ANAMMOX).

In reactors containing zeolite, the main removal mechanism could be adsorption by the filtration media since the main zeolite property exploited in wastewater treatment processes is its NH_4^+ exchange ability [18]. Furthermore, ammonia nitrogen loss through volatilization was negligible since it generally requires a pH of 9.3 [24].

Removal percentages for nitrate were significantly different for all treatments (p < .05). Reactors containing zeolite performed lower than reactors containing river gravel, gravel and LECA. T₃ (LECA) had a good performance, with a mean removal efficiency of 53%. Reactors containing river gravel and gravel (T₁ and T₂) presented similar efficiencies, 59% and 56%, respectively (Figure 6). These low removal percentages may be due to an incomplete denitrification process.

The concentration of TP in wastewater decreased along the experiment in all treatments (Figure 7). In all reactors efficiency was over 86%. There are several mechanisms by which phosphorus can be retained in wetlands systems: uptake by plant roots or absorption through plant leaves in submerged species, adsorption to soils and sediments and uptake by microbiota. According to Vymazal [25], adsorption and precipitation of phosphorus are effective in systems where wastewater gets in contact with filtration substrate. Removal percentages did not differ significantly from one treatment to another (p > .05), indicating that substrates are not responsible for phosphorus removal. Macrophytes uptake is probably the main mechanism for P removal. HSSFWs have high potential for P removal, as the substrate is constantly flooded and there is no significant fluctuation in redox potential in the bed.

The results for removal efficiencies obtained for main parameters are consistent with other experiments

reported in the literature. Case studies of dairy and swine wastewater treatment wetlands have shown nitrogen and phosphorus removals between 48–98% and 35–96% respectively, depending on nutrient loading conditions and wetland ages [7,8,26–29].

COD removal efficiency shows significant differences among the microcosms (p < .05). The highest COD removal (78–80%) was registered in T₃ (LECA), T₄ and T₅ (treatments using zeolite). T₁ and T₂ were the least efficient treatments, with mean removal efficiencies ranged between 67 and 71% (Figure 8), probably due to a lower macrophytes growth, as it was said previously. A positive correlation coefficient ($R^2 = 0.899$) was observed between COD removal efficiencies and macrophyte biomass development. A more developed root-rhizome system will allow more aerobic microzones affecting organic matter oxidation. In CWs, COD removal is mainly related to microbiological degradation attached to the matrix and the plants roots [30].

4. Conclusions

The studied substrates were efficient in the effluent treatment, showing high but not significant different NH_4^+ and TP removal efficiencies (88–99% and 86–99%, respectively). Treatments with combined substrate gravel + zeolite presented significantly higher TKN removal efficiencies while reactors containing LECA presented the best performance in NO_3^- and COD removal. LECA and gravel + zeolite are the most suitable substrates to be used in HSSFW for dairy farm wastewater tertiary treatment. The best option will depend on cost.

T. domingensis grew satisfactorily in HSSFWs treating dairy farm wastewater and was efficient in the removal of the contaminants studied.

Currently, in Argentina, dairy farm effluents are dumped into the environment untreated or treated with inefficient traditional methods. It is necessary to find a final low-cost treatment to comply with regulation. The use of CWs to treat these effluents could be a viable option because of its low construction and operation costs and demonstrated depuration efficiency. HSSFWs demonstrated to be efficient in the final treatment of dairy farm effluents. After treatment, the quality of the final effluent was significantly improved. Outlet effluent complied with regulations and could be discharged into the environment.

Disclosure statement

No potential conflict of interest was reported by the authors.

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