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Variation of the feeding/resting period in modified vertical treatment wetlands (depth, zeolite as medium) employed for treating rural domestic wastewater in tourist areas

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ABSTRACT

This work aimed to evaluate the performance of modified vertical flow treatment wetlands (VF-TWs) in terms of depth and medium to assess the effect of the feeding/resting periods and footprint (FP). The modifications were proposed for treating domestic wastewater in rural areas with flow variations such as tourist sites. The experimental setup included six laboratory-scale VF-TWs: (a) normal (VF-N), bed depth 1.0 m, filled with sand and (b) modified (VF-M), bed depth 0.5 m, filled with sand (upper) and zeolite (bottom, saturated). The operation was divided into three phases (3 months each), varying the feeding/resting period and FP: phase I, 5 d/10 d, 2.6 m^2/person-equivalent (PE); phase II, 3.5 d/3.5 d, 1.7 m^2/PE; and phase III, only feeding no resting, 0.85 m^2/PE. Influent and effluent grab samples were taken every 2 weeks. The results showed effective removal (above 60%) of total solids, organic matter, and pathogens for both VF-N and VF-M. Regarding nutrients, VF-M showed a phosphate removal below 60%, but no consistent removal (15–60%) of total nitrogen. Thus, the results suggest that proposed modifications can be an option to be established in tourist sites, but further work should be conducted to improve and optimize total nitrogen removal.

Key words: domestic wastewater, feeding/resting periods, nature-based solution, rural tourist areas, treatment wetlands, vertical flow

HIGHLIGHTS

- VF-TWs with the proposed modifications (bed depth, feeding/resting period; footprint reduction; zeolite as medium) had effective removal of total solids, organic matter, and pathogens.
- VF-TWs with the proposed modifications were shown to be robust and able to cope with flow variations in tourist facilities of rural areas.
- Further work should be conducted for consistent nitrogen removal in VF-TWs with the proposed modifications.

1. INTRODUCTION

Vacation centers and dwellings in rural tourist areas increase the population during vacation periods (Goulding 2006). This implies a seasonal increase in wastewater flow that must be treated. Therefore, the design of wastewater treatment systems must be robust to cope with flow variations. Treatment wetlands (TWs), a type of nature-based solution (NBS), have the potential to be an effective solution for wastewater treatment in rural tourist facilities precisely for their capacity to support flow changes, maintaining treated water of good quality throughout the year (Calheiros *et al.* 2015). In addition, TWs provide a wide range of ecological benefits and ecosystemic services that include water quality improvement, climate regulation, nutrient processing, and carbon sequestration, as well as recreation and habitat improvement (Arias & Brown 2009; Cross *et al.* 2021). These are important ancillary benefits that are appealing, can be easily part of the local landscape, and can be used to promote rural tourism.

Among the different types of TWs, vertical subsurface flow (VF) is one of the alternatives to traditional horizontal subsurface flow (HF), which is the most commonly used TW worldwide (Moreira & Dias 2020). VF-TWs are becoming more common than HF-TWs because of their potential to cope with higher organic loadings (>6 g 5-day Biological Oxygen Demand (BOD_5)/m^2-d) (Dotro *et al.* 2017), capacity to nitrify (ammonium removal above 90%; Brix & Arias 2005), and smaller footprint demand (VF-TWs, 2–4 m^2/PE (person-equivalent); HF-TWs, 5 m^2/PE) (Dotro *et al.* 2017). In addition, VF-TWs have more design and operational options (surface area, bed depth, filter medium, loading mode, and plant species; Vera-Puerto *et al.* 2021a) than HF-TWs; therefore, their adaptability to operational conditions makes VF-TWs more suitable as a sustainable sanitation system for rural areas (Vera-Puerto *et al.* 2021b).

VF-TWs with sequential loading patterns are the most commonly employed and recommended by the few existing design guidelines (Brix & Arias 2005; Vera-Puerto *et al.* 2021a). This kind of TW has been shown to be effective for total suspended solids (TSS) and organic matter (chemical oxygen demand (COD)) removal, exhibiting efficiencies of between 35 and 95% when they are applied in domestic or municipal wastewater treatment (Stefanakis *et al.* 2014). In the case of total nitrogen (TN) and phosphorus (total or phosphate), the removal efficiency is lower, usually below 60% (Tsihrintzis 2017). One way to increase the nitrogen but mainly the phosphorus removal is the inclusion of special media in the bed or as external filters after TWs (Arias *et al.* 2005). Zeolite is one of these special media that have been employed (Andrés *et al.* 2018). Natural zeolites stand out among the different adsorbent media for their abundance, low price, and regenerative capabilities (Vera-Puerto *et al.* 2021a). Natural zeolites as medium in TWs have contributed to an increase of between 20 and 50% phosphorus removal in comparison to traditional media employed in TWs (Andrés *et al.* 2018). For this reason, natural zeolites represent an alternative to the traditional media that can be considered to be employed in VF-TWs.

In the case of operational parameters, one of them that can be changed during the operational life of the VF-TWs with sequential loading patterns is the feeding/resting periods, if more than one bed is established. According to Stefanakis *et al.* (2014), feeding periods between 2 and 6 days have been recommended, and resting periods should be double the loading period for a good adaptation and operation of VF-TWs. However, the design parameters and operation schemes of VF-TWs must be selected so that they fit with the environmental conditions of the site (as tourist facilities in rural areas), the discharge limits, and the characteristics of the influent water (Vera-Puerto *et al.* 2021b). Therefore, the implementation of VF-TWs and their adaptation to new operational conditions must be assessed.

Based on this information, this work aimed to evaluate an experimental VF-TW for treating domestic wastewater in Central Chile, where modifications on the bed depth, medium, feeding/resting period, and associated modification in the footprint (FP) are introduced, so that they can be established in rural tourist areas. For the modification in the feeding/resting period, this is a strategy employed to feed several beds of TWs with raw wastewater (to treat solids in TW's bed). However, this research studied several beds of VF-TWs, because rural tourist places have flow variations throughout the year, and the results can be useful to establish a recommendation on whether a treatment system based on several VF-TW's beds can work with or without rotation of these beds.

2. METHODS

2.1. Experimental setup

Six experimental units of VF-TW mesocosm were constructed and operated. They were isolated from the rain in a room without temperature control and covered by a translucent roof to allow natural light; this location was near the city of Talca (Maule Region, Central Valley, Chile, 5°27′50″S, 71°37′15″W; average annual temperature, 14.2 °C; average annual precipitation, 643 mm; Köppen–Geiger climate classification, CSa, hot summer Temperate Mediterranean; DGA 2016; Vera-Puerto *et al.* 2021b). The VF-TWs were built using Ø 0.2 m PVC pipes and planted with *Schoenoplectus californicus*. *S. californicus* was selected because it is a native plant from Chile and the American Pacific region and has been employed successfully in previous experiments of TWs in the country (López *et al.* 2015; Vera-Puerto *et al.* 2021b). The plants were transplanted from a local channel near the experimental site and put first in single pots for 1 month previous to being planted in the experimental units. After that, a commissioning period of 3 months was employed. The experimental setup was divided into two treatment lines: (a) normal, three VF-TWs with a total bed depth of 1.0 m (VF-N) and (b) modified, three VF-TWs with a total bed depth of 0.5 m (VF-M). The bed depth was reduced in VF-M, assuming that the highest bacterial activity for processing different pollutants in wastewater occurs in the first 0.2 m (Arias *et al.* 2022). All the VF-TWs were filled with gravel layer (Ø, 5–19 mm) at both the top and bottom. For the VF-N, a layer of sand (Ø, 0.08–5.0 mm) was used as the support medium with a depth of 0.8 m. For VF-M, the support medium was divided into two layers: a 0.15-m sand (Ø, 0.08–5.0 mm) layer and a 0.15-m zeolite (Ø, 3–5 mm) layer. The sand was used in all VF-TWs with a d_{10} of 0.25 mm and d_{60} of 1.2 mm and a uniformity coefficient ($U = d_{60}/d_{10}$) of 4.8. This sand was in agreement with the proposed values of sand rate percolation (SRP), between 45 and 75 s (Weedon *et al.* 2016). Zeolite was supplied by Zeolitas del Maule, a local supplier. Zeolite was employed in VF-M to evaluate its performance for water quality in effluents, taking into account its adsorption capacity (Vera-Puerto *et al.* 2020). Figure 1 presents the characteristics of the mesocosm VF-TWs.

2.2. Operational and monitoring strategy

The hydraulic loading rate (HLR) was selected by assuming a flow of 100 L/PE-d. An HLR of 120 mm/d was applied. The HLR was applied in 12 pulses per day according to recommendations by Stefanakis *et al.* (2014) and Brix & Arias (2005). For each pulse, a total of 10 mm was applied similar to Nivala *et al.* (2018). The operation was divided into three phases varying the feeding/resting period and the associated FP: phase I, 5 d/10 d, 2.6 m^2/PE; phase II, 3.5 d/3.5 d, 1.7 m^2/PE; phase III, only feeding no resting, 0.85 m^2/PE. Each phase was operated on for 3 months. The variations in the feeding/resting periods were proposed to evaluate the possibility of working with several beds of VF-TWs, taking into account the flow variations along the year in rural tourist places, and to make a potential recommendation about whether a treatment system based on several VF-TW's beds can work with or without rotation during the year.

The zeolite layer in VF-M was saturated to evaluate whether the adsorption capacity of the material could be enhanced by increasing contact time with the wastewater or whether the bottom saturation modifies the oxidative conditions. Both cases aimed at studying the potential improvement of effluent quality (mainly nitrogen and phosphorus removal). VF-N was not bottom saturated to represent a typical downflow VF-TW.

Domestic wastewater was used as feeding influent for the experimental systems. Primary-treated wastewater was pumped from effluents to a septic tank (primary treatment), serving a single household of six inhabitants. The influent was pumped into a well and subsequently into the experimental system. Table 1 shows the physical and chemical influent characteristics for each operational phase.

The concentrations shown in Table 1 for each of the phases are typical, expected from primary-treated domestic wastewater (Vera-Puerto *et al.* 2021b). However, a reduction of around 50% in COD and TN during phases II and III and an increase in temperature (at least 5 °C on average) for phases II and III should be highlighted. The temperature increase can be explained because phase I was developed during austral winter, while phases II and III were developed during spring and summer, respectively. The other water quality changes (i.e., COD, TN, TSS) can be related to water use by the family, because

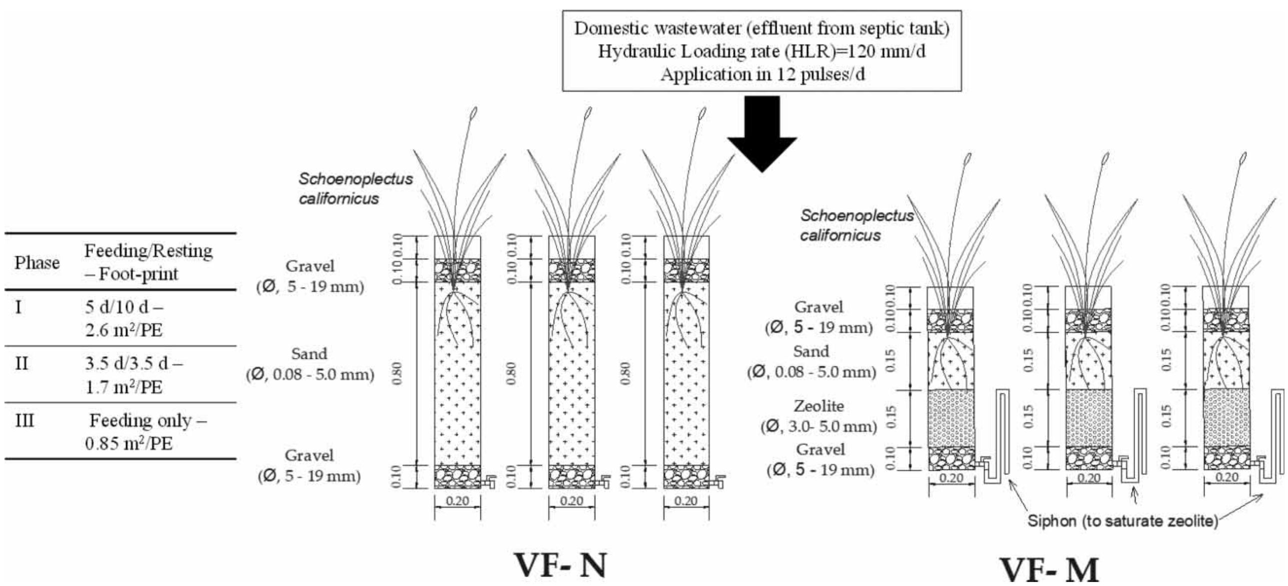

Figure 1 | Schematic view of the VF-TWs experimental units.

Table 1 | Influent wastewater characteristics

Water quality parameter	Unit	Phase I	Phase II	Phase III
T	°C	10.7 ± 1.7	16.4 ± 2.7	20.6 ± 1.7
pH	Uni.	7.9 ± 0.1	7.8 ± 0.3	7.2 ± 0.5
ORP	mV	-133.2 ± 135.5	$+74.1 \pm 150.54$	-123.1 ± 188.0
TSS	mg/L	194.0 ± 225.2	112.6 ± 76.4	115.01 ± 99.4
COD	mg/L	210.4 ± 70.8	90.3 ± 33.3	123.0 ± 62.9
NH_4^+-N	mg/L	44.5 ± 5.8	26.9 ± 14.2	27.6 ± 8.8
NO_3^--N	mg/L	1.0 ± 0.4	0.1 ± 0.3	0.0 ± 0.0
TN	mg/L	46.1 ± 9.6	28.8 ± 17.0	27.1 ± 8.0
PO_4^{-3}-P	mg/L	5.6 ± 3.1	4.5 ± 2.1	4.8 ± 1.3
E. coli	Log_{10} (MPN/100 mL)	5.9 ± 0.6	5.0 ± 0.6	6.0 ± 1.1

T, temperature; ORP, oxidation–reduction potential; TSS, total suspended solids; COD, chemical oxygen demand; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; TN, total nitrogen; PO_4^{-3}-P, phosphate phosphorus; MPN, Most Probable Number.
$n = 6$ for each phase.

during warm seasons (spring and summer) that match with phases II and III, the water consumption increases with a dilutive effect on domestic wastewater.

Grab samples of influents and effluents were analyzed for temperature (T), pH, oxidation–reduction potential (ORP), TSS, COD, ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), TN, phosphate (PO_4^{-3}-P), and *Escherichia coli*. The physicochemical parameters were monitored every 2 weeks. Grab samples in effluents were taken from the collection tank (dark color to prevent algae formation) to each TW at the ending of the dosing period. The collection tank was sealed during the dosing period to prevent oxygen interchange and it was only opened to take samples. Only one sample was sent to the laboratory. This sample was integrated from three parts extracted from the collection tank.

2.3. Analytical methods

The influent and effluent samples were filtered using a fiber glass filter with a 0.7 μm pore size. The physicochemical parameters COD, NH_4^+-N, NO_3^--N, TN, and PO_4^{-3}-P were measured photometrically by a HI83399 multiparameter photometer (Hanna, Woonsocket, RI, USA) using reagent test kits as follows: COD, HI-93754B (medium range), NH_4^+-N, HI-93715 (medium range), NO_3^--N, HI-93728 (medium range), TN, HI-93767 (low range), and PO_4^{-3}-P, HI-93713 (low range) and HI-93717 (high range). These determinations are modifications of standard procedures from APHA-AWWA-WEF (2017). TSS were analyzed gravimetrically according to the procedures in APHA-AWWA-WEF (2017). pH, T, and ORP were measured *in situ* when grab samples were collected employing specific electrodes in a multiparameter portable Hanna HI 98194 (Hanna, Woonsocket, RI, USA) calibrated instrument following the procedure described in APHA-AWWA-WEF (2017). The pathogens, *E. coli,* were analyzed using the Colilert simplified method (Dichter 2011).

2.4. Statistical analysis

Statistical analysis was performed using INFOSTAT with a significant level $\alpha = 0.05$ (Di Rienzo *et al.* 2019) to determine the differences between VF-N and VF-M for the three phases, as well as the differences between phases for VF-N and VF-M. For response variables, pH, T, ORP, TSS, COD, NH_4^+-N, NO_3^--N, TN, PO_4^{-3}-P, and *E. coli* effluent concentrations from the VF-TWs were used. The data were subjected to the Shapiro–Wilk normality test to determine the statistical test for comparison. Then, to determine the difference between VF-N and VF-M, the data were compared for each phase using a *t*-test for data with normal distribution or the Wilcoxon test for data without normal distribution. To determine the difference between phases for each VF-TW, the data were compared using an analysis of variance (ANOVA) test for data with normal distribution or the Kruskal–Wallis test for data without normal distribution. A par comparison was made to determine similarity between phases; for ANOVA, the least significant difference (LSD)-Fisher was employed, and for the Kruskal–Wallis test, a comparison test was employed.

3. RESULTS AND DISCUSSION

3.1. Operational conditions

Table 2 shows the real average HLR applied to each TW for each phase. The real HLR was adjusted to a theoretical HLR of 120 mm/d with deviations below 10%, showing stability in the wastewater application to the experimental VF-TWs during operation time.

Table 3 shows the operational conditions for each operational phase and TW. The average pH for all phases ranges between 6.8 and 7.2 (nonsignificant differences ($p > 0.05$) between phases for the VF-N and VF-M, neither significant difference ($p > 0.05$) between VF-M and VF-N for each phase). pH values between 6.0 and 8.0, similar to those found in this study, have been reported by other authors for VF-TWs (Brix & Arias 2005; Perdana *et al.* 2018).

Average temperature increases from phase I to phase III by 5 °C and from phase II to phase III by 3 °C (significant differences ($p < 0.05$) between phases for the VF-N and VF-M, but nonsignificant ($p > 0.05$) differences between VF-M and VF-N for each phase). The temperature increases can be explained by the season in which the experiment took place, which was between austral winter (phase I) and summer (phase III), and are comparable with Mediterranean climatic conditions.

ORP showed mean values above +150 mV for all the phases in VF-N and VF-M, suggesting predominantly aerobic conditions, despite the bottom saturation in VF-M (nonsignificant differences ($p > 0.05$) between phases for the VF-N and VF-M, neither significant difference ($p > 0.05$) between VF-M and VF-N for each phase). These ORP mean values (>+150 mV) are in accordance with results from other VF-TWs operated with sequential feeding schemes (fed by pulses) (Pandey *et al.* 2013) and indicate that aerobic conditions were present during all phases despite the reduction of FP (phase I, 2.6 m^2/PE; phase III, 0.85 m^2/PE).

3.2. Removal efficiency

3.2.1. Solids and organic matter removal

Table 4 shows the effluent concentration for TSS and COD as well as the associated removal efficiency.

Effluent TSS for all phases was similar between VF-N and VF-M with mean values of below 10 mg/L, despite the variations between phases at influent concentrations (Table 1) (only significant differences ($p < 0.05$) in phase I between VF-N and VF-M; for VF-M, only phases I and III showed significant differences ($p < 0.05$)), suggesting that bed depth reduction and bottom saturation in VF-M did not have influence and even showing that the FP reduction for both TWs did not influence the performance. Removal efficiency was for all phases and both TWs above 90%. This removal efficiency has been reported for VF-TWs with bed depths of 1.0 and 0.5 m, similar to this study (Vera-Puerto *et al.* 2021a; Arias *et al.* 2022). TSS removal in TWs is explained by the physical filtration developed in the bed (Vera-Puerto *et al.* 2021b), which was maintained despite FP reduction.

Table 2 | Hydraulic loading rate (phase I, $n = 18$; phases II and III, $n = 26$)

Parameter	Unit	Phase	VF-N	VF-M
Hydraulic loading rate (HLR)	mm/d	I	121.6 ± 6.7	114.1 ± 5.3
		II	117.5 ± 9.5	109.0 ± 8.6
		III	116.2 ± 10.3	108.7 ± 10.7

Table 3 | pH, temperature (T) and oxidation–reduction potential (ORP), for each phase ($n = 6$ in each phase)

Parameter	Unit	Phase	VF-N	VF-M
pH	Un.	I	6.9 ± 0.3	7.0 ± 0.1
		II	6.9 ± 0.2	7.1 ± 0.3
		III	7.2 ± 0.6	6.9 ± 0.6
T	°C	I	10.8 ± 1.6	10.6 ± 1.5
		II	17.2 ± 2.6	17.1 ± 2.6
		III	20.5 ± 1.1	20.2 ± 1.3
ORP	mV	I	+195.2 ± 71.4	+160.8 ± 107.0
		II	+234.7 ± 29.6	+229.0 ± 38.4
		III	+197.1 ± 55.8	+205.0 ± 64.7

Table 4 | Mean effluent concentrations and removal efficiency for TSS and COD ($n = 6$ in each phase)

		Effluent concentrations (mg/L)		Removal efficiency (%)	
Parameter	**Phase**	**VF-N**	**VF-M**	**VF-N**	**VF-M**
TSS	I	2.4 ± 2.3	6.5 ± 3.7	98.2 ± 1.0	93.5 ± 3.6
	II	2.8 ± 1.6	3.1 ± 1.2	95.8 ± 3.2	95.3 ± 3.2
	III	3.0 ± 3.8	1.4 ± 0.5	97.8 ± 2.9	97.3 ± 2.8
COD	I	68.5 ± 46.5	74.5 ± 28.4	65.1 ± 21.1	60.9 ± 14.5
	II	22.2 ± 12.8	30.2 ± 16.5	74.4 ± 13.7	65.0 ± 13.3
	III	30.5 ± 20.6	31.0 ± 14.8	75.9 ± 8.0	72.7 ± 9.8

Effluent COD in phase I showed the highest mean concentration above 60 mg/L for both TWs (significantly different ($p < 0.05$) for the VF-N and VF-M when compared to the other two phases), which correlates with the highest influent concentration (Table 1, above 200 mg/L). For phases II and III, mean concentrations were below 40 mg/L (nonsignificant differences ($p > 0.05$) between these two phases for the two VF-TWs), with an influent concentration of around 120 mg/L (Table 1). These results suggest a potential effect of influent reduction (phases II and III, Table 1) on the effluent concentration. However, these effluent concentrations show removal efficiency above 60% (nonsignificant difference ($p > 0.05$) between VF-N and VF-M for the three phases). This removal efficiency has been reported for VF-TWs with a bed depth of 1.0, 0.7, and 0.5 m, and even VF-TWs with bottom saturation (Oliveira *et al.* 2016; Tsihrintzis 2017; Vera-Puerto *et al.* 2021a). The COD removal capacity of the two types of TWs is explained by the high oxidizing conditions (>+ 150 mV, Table 3) that are maintained during the three phases and can cope with organic matter removal. In addition, the results in Table 4 show that the change of operational feeding/resting and related FP reduction proposed in this work does not affect the COD removal capacity. Thus, the previous results show the potential application of VF-TWs for removing TSS and COD from wastewater when flow variations along the year are present, the condition of the rural tourist areas.

Figure 2 shows the relationship between the applied and removed organic loading rate (OLR). This OLR is calculated as an instantaneous OLR. The OLR applied varies from 5 to 40 gCOD/(m^2-d). These OLR values are in agreement with several international recommendations: below 30 gCOD/(m^2-d) (exception only two values) (Dotro *et al.* 2017). Furthermore, OLR in Figure 2 shows that VF-M with the change in the feeding strategy, the reduction of footprint (up to 0.85 m^2/PE), and the change in bed depth and media (zeolite layer) can cope with TSS and COD removal from wastewater produced in rural areas. In addition, Figure 2 shows the highest OLR during phase I and the lowest OLR during phases II and III. The highest OLR in phase I is explained by the highest COD concentration in the influent during this phase (Table 1, above 200 mg/L). Furthermore, Figure 2 shows that for applied OLR below 15 gCOD/(m^2-d), the removal efficiency in terms of load is above 95%. Finally, the relationship between applied and removed OLR is similar for the two VF-TWs

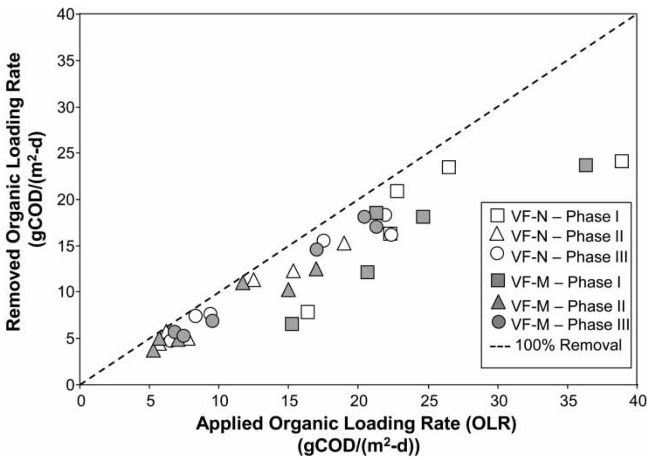

Figure 2 | Relationship between the applied and removed organic loading rates.

evaluated, showing similar behavior in the effluents coming from a VF with traditional design (VF-N) and VF with modifications (VF-M).

3.2.2. Nutrients

Table 5 shows effluent concentrations for different forms of nitrogen (NH_4^+-N, NO_3^--N, TN) and phosphate phosphorus (PO_4^{-3}-P).

NH_4^+-N was effectively transformed to NO_3^--N in the two VF-TWs, achieving NH_4^+-N removal efficiencies above 95% and average effluent concentrations below 4 mg/L for all phases (only phase I was significantly different ($p < 0.05$) for VF-N and VF-M, and nonsignificant differences ($p < 0.05$) between VF-N and VF-M could be established for the three phases). The NH_4^+-N removal by both VF-TWs is explained by the aerobic conditions inside the bed (ORP $> +150$ mV, both VF-TWs), which are necessary for the nitrification process. This is an expected behavior of classical download pulse-fed VF-TWs (Brix & Arias 2005; Vera-Puerto *et al.* 2021b). In addition, the similarity in NH_4^+-N effluent concentrations shows that bed depth reduction does not affect NH_4^+-N transformation, similar to findings by Arias *et al.* (2022), who established that the main nitrogen transformation occurs in the first 0.2 m of bed depth.

TN showed the lowest effluent mean concentration in phase I for VF-M: below 20 mg/L (significant difference ($p < 0.05$) with phase II), despite that influent concentration was the highest in comparison to the other two phases (Table 1). However, during phases II and III, the effluent mean concentrations correspond to influent concentration (in addition, nonsignificant difference ($p > 0.05$) between these two phases for VF-N and VF-M); thus, for these two phases, the results suggest no effect on TN removal by reductions on influent concentration, and also showing that the two strategies, bottom saturation and zeolite inclusion in VF-M, did not improve the TN removal. This result is opposite to the findings of Vera-Puerto *et al.* (2021a), who showed that the inclusion of bottom saturation in VF-TWs with modifications in bed depth and the inclusion of a zeolite layer could increase the TN removal in a significant way ($p < 0.05$). In the same way, Pelissari *et al.* (2017) showed removal efficiencies of 58% for TN in VF-TWs of a 0.70 m bed depth and a partial saturation of 0.20 m. Furthermore, the results suggested for TN showed that despite the modifications, the FP reduction negatively affected its removal. In Table 5, the results also suggest that NH_4^+-N was transformed into NO_3^--N previously to achieve the saturated part in the VF-M TW, limiting the possibility of zeolite contributing to TN removal by adsorption of NH_4^+-N (Vera-Puerto *et al.* 2020). In a complementary way, the results for different nitrogen forms in Table 5 also show that in the saturated part, the denitrification is not completely developed, suggesting two things, a lack of organic sources for this process (Arias *et al.* 2022), and the affectation to this process due to the short retention time of 0.5 d (Vera-Puerto *et al.* 2021a). Therefore, the results for nitrogen forms suggest that they have to be followed carefully in VF-M TW because the limitations in nitrogen transformations and contribution of inorganic N to effluent TN (around 50% of NO_3^--N, Table 3, phases II and III, VF-N and VF-M) could be aspects to be taken into account for a better understanding and improvement in removal of this water quality parameter.

Table 5 | Mean effluent concentrations and removal efficiency for nutrients ($n = 6$ in each phase)

		Effluent concentrations (mg/L)		Removal efficiency (%)	
Parameter	**Phase**	**VF-N**	**VF-M**	**VF-N**	**VF-M**
NH_4^+-N	I	3.5 ± 4.4	2.3 ± 2.7	96.2 ± 2.8	96.9 ± 3.8
	II	0.5 ± 0.2	0.5 ± 0.2	97.8 ± 1.4	97.7 ± 1.3
	III	0.2 ± 0.4	0.1 ± 0.2	99.1 ± 1.6	99.8 ± 0.5
NO_3^--N	I	46.8 ± 16.5	19.5 ± 17.9	–[a]	–
	II	13.4 ± 5.9	11.5 ± 4.0	–	–
	III	13.3 ± 3.1	13.0 ± 3.9	–	–
TN	I	35.3 ± 10.6	15.5 ± 13.6	35.8 ± 24.4	63.3 ± 33.1
	II	37.2 ± 13.8	36.7 ± 12.9	15.8 ± 9.1	18.3 ± 9.1
	III	28.0 ± 4.4	26.6 ± 10.1	19.2 ± 2.2	19.8 ± 14.6
PO_4^{-3}-P	I	3.3 ± 0.8	4.7 ± 1.3	51.4 ± 20.2	33.3 ± 22.3
	II	2.0 ± 1.2	3.0 ± 2.3	60.5 ± 22.2	42.2 ± 41.9
	III	2.0 ± 1.0	2.5 ± 2.1	44.7 ± 32.2	49.0 ± 33.6

[a]Removal efficiencies were not reported because NO_3^--N was produced during the treatment process.

The phosphate concentration of the effluent water was below 3.5 mg/L during phases II and III, which is a reduction of around 40% in comparison to the influent concentrations (nonsignificant ($p > 0.05$) differences between the three phases for VF-N and VF-M, and neither significant difference ($p > 0.05$) between VF-N and VF-M for each of the three phases). This PO_4^{-3}-P removal was in agreement with VF-TWs with traditional bed depth (1.0 m) and medium (sand), which typically vary between 30 and 60% (Brix & Arias 2005; Tsihrintzis 2017). However, this range of percentage can be reduced during the lifespan of the TW system (Tsihrintzis 2017). Furthermore, Table 5 suggests for phases II and III that vegetation development, which occurred during the growing season of spring and summer during these two phases, contributes to the PO_4^{-3}-P removal and therefore helps to modify the feeding/resting period and associated FP reduction proposed in this work. Plants grew from an average height of 0.9 m in phase I up to an average of 1.5 m at the ending of phase II. Then, for space reasons, the plants were cut to an average height of 0.5 m at the beginning of phase III, to achieve at the ending of this phase, an average height of 0.6 m. At this moment, ending phase III, the number of leaves (around 35) was double in comparison to phase I (around 17). This growth was the same for all VF-TWs.

3.2.3. Pathogens

Table 6 shows the removal of pathogens measured as *E. coli* during each experimental phase. VF-TWs can provide some pathogen removal (Adrados *et al.* 2018). Pathogen removal in VF-TWs can be explained by the fact that the most frequent and well-validated removal mechanisms include natural die-off due to starvation or predation, sedimentation, filtration, and adsorption, but filtration would be the main pathway (Vera-Puerto *et al.* 2021a, 2021b).

The results in Table 6 show that *E. coli* strains in effluents for VF-M were below 3 Log MPN/100 mL for phases II and III in a proportion above 80% of the gathered data, similar to the results of VF-N TW (nonsignificant difference ($p > 0.05$) between VF-N and VF-M for each of the three phases). This result suggests that bed depth reduction and bottom saturation do not affect the pathogen removal in the VF-M TWs. In the case of modification in the feeding/resting period, only phase I was significantly different ($p < 0.05$) in VF-N and VF-M but not the best, suggesting no influence by this modification and associated FP reduction on pathogen removal. Despite that, an effect of water temperature could be associated with *E. coli* removal, because in phase I the lowest average water temperature (10 °C, Table 1) and lowest removal were recorded, while in phases II and III with higher and similar *E. coli* removals, a higher average water temperature (above 15 °C, Table 1) was recorded. However, Alufasi *et al.* (2017) mentioned that no conclusive results are available on the effects of seasonal fluctuations on pathogen removal by TWs, and only suggest that an increase in temperature helps to increase the activity of nonpathogenic organisms such as grazing protozoa, increasing the possibility of pathogen removal rates. Thus, the pathogen removal results show the potential application of the proposed modifications for treating wastewater from rural tourist areas. In addition, the difference (despite being nonsignificant ($p < 0.05$)) between VF-N and VF-M for phase I), suggests that the VF-M needs an acclimation period (especially if the first operative part is in winter) to improve pathogen removal and suggests that the other mechanisms different from sedimentation (previously commented) would also be important in VF with the modifications proposed in this work. Although the *E. coli* concentration in effluents to VF-N and VF-M can comply with international regulations (below 1000 MPN/100 mL) (Vera-Puerto *et al.* 2022), a disinfection system has to be included in the treatment process to increase microbiological water quality, if the users wish to reuse treated wastewater.

Table 6 | *E. coli* removal by the operational phase ($n = 6$ in each phase)

	VF-N			VF-M		
Phase	Average Log units removed	Data $\leq 1.0 \times 10^3$ MPN/100 mL (3 Log units) (%)	Min–Max	Average Log units removed	Data $\leq 1.0 \times 10^3$ MPN/100 mL (3 Log units) (%)	Min–Max
I	2.4	80	1.0×10^3–2.3×10^5	1.1	0.0	1.2×10^4–2.4×10^6
II	2.2	100	1.0×10^2–1.0×10^3	2.2	100.0	1.0×10^2–1.0×10^3
III	3.7	100	1.0×10^1–1.0×10^3	3.7	83.3	1.0×10^1–1.3×10^3

4. CONCLUSIONS

The results of the measured water quality parameters at the effluent suggest that the proposed modifications for VF-TWs can be an option to be established in the vacation places of rural tourist areas. The change in feeding strategy and the associated footprint reduction (up to 0.85 m^2/PE) besides the change in bed depth and medium (zeolite layer) were shown to be robust and able to cope with flow variations producing water quality with the potential to meet discharge limits. The change in the feeding/resting period will be a strategy with a high participation of the owners of the treatment system; thus, the capacitation will be an important part of implementing this proposed modification. Furthermore, the inclusion of zeolite as medium could be expensive in comparison to sand. However, zeolite as medium in a VF-TW is cheaper in comparison to other adsorbent materials, especially if the extraction site is located near to VF-TW implementation site (similar to this work). Thus, zeolite use has to be carefully evaluated during the design process. Finally, the results achieved here due to the experiment scale (laboratory, potential wall effects) should be verified in experiments at the pilot scale, to provide more information for potential real-scale applications. In future experiments, it is important to follow up on nitrogen removal since adjustments may be needed due to the potential lack of organic carbon to sustain denitrification or the necessity to improve the zeolite adsorption capacity for improving TN removal. Thus, the VF-TWs (modified in depth and medium) as a Nature-Based Solution (NBS) will provide additional benefits to water quality improvement, such as esthetics and habitats for wildlife, which can contribute to the promotion of rural tourist places.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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