Journal of Water Process Engineering 42 (2021) 102103

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Influence of nutrients and pH on the efficiency of vertical flow constructed wetlands treating winery wastewater

M. Sánchez, O.G. Gonzalo, S. Yáñez, I. Ruiz, M. Soto *

Dept. of Chemistry, Faculty of Sciences & CICA, University of A Coruña, E-15071, A Coruña, Galiza, Spain

ARTICLE INFO

Keywords:
Constructed wetlands
Winery wastewater
High organic load
Low pH
Nutrient shortage

ABSTRACT

Winery wastewater is characterized by high organic content, low nutrient content and low pH at least during vintage periods. The effect of nutrient shortage and low pH on constructed wetlands (CWs) operation was scarcely studied, but early field studies indicate that some operational problems can arise. This work aims to determine the effect of nutrient shortage and acidic pH during the treatment of high organic load wastewater in one-step vertical subsurface flow (VF) CWs. Two lab scale VF units at hydraulic loading rates over 70 L/m^2·d and surface loading rate in the range of 110−170 g COD/m^2·d were operated for periods with and without nutrients in the influent as well as with influent pH of 7.0 and 4.5. The results showed that neither low nutrient nor low pH impair organic matter removal whilst low pH decreased nitrogen removal rates. At low pH, the effluent concentration of ammonia and nitrate increased, indicating deterioration in both nitrification and denitrification processes. The paper discuss the implications of these findings for a better strategy in the treatment of winery process wastewater, such as options for separate treatment or its combination with other nutrient-containing streams.

1. Introduction

In the last two decades, constructed wetlands (CWs) have been successfully applied to different kind of effluents, including wastewater from petrochemical industry, food industry (meat processing, dairy industry, fruit and vegetable canning), forestry, winery and distillery, textile, tanning, aquaculture, metal finishing and mining, among others [1,2].

The wine industry produces wastewater throughout the different processes of wine production. Winery wastewater comes mainly from the washing waters of equipment and bottles and from the cooling processes and represents a serious environmental problem in wine-producing countries. This requires the adoption of measures ensuring that their potential environmental impacts are minimal and within an acceptable range [3–5]. The characteristics of the effluents and the seasonal variability make the use of CW for the treatment of wastewater from wineries of special interest [3,4,6].

Winery wastewaters contain higher concentrations of soluble organic matter readily biodegradable and variable content of suspended solids. Chemical oxygen demand (COD) concentrations vary from 340 to 49.000 mg/L, total suspended solids (TSS) from 12 to 18.300 mg/L and biological oxygen demand (BOD_5) is about 0.4−0.9 of the COD value [3,7–9]. Even higher concentration values were recorded [4]. The concentration of nitrogen and phosphorus compounds in winery wastewaters is usually low and the pH is acidic. The recalcitrant constituents account for less than 10 % of total COD [7].

Conventional biological treatment systems as well as co-treatment in municipal wastewater treatment plants are not effective for effluents from the wine industry, particularly for small-scale wineries due to their cost and complexity [4,9,10]. This is because the wastewater from these facilities is highly concentrated in terms of COD and have shown highly variable flows and loadings (daily and seasonal). Nature-based on-site solutions like constructed wetlands (CWs) have been taken into consideration [3,6,7,11].

The surface loading rates (SLRs) applied to the 13 CW systems reviewed by Masi et al. [3] ranged from about 30 up to about 5000 g COD/m^2 d, with the 80th percentile of the reported values being below 297 g COD/m^2 d and the median at 164 g COD/m^2 d. The highest SLR values have in all cases been measured during the peak season (vintage) and often linked to lower surface removal rates (SRRs). Despite the high interest in recent years for this treatment alternative, percentage removal rate showed a high variability, ranging from 49 % to 99 %. Low

---

* Corresponding author.
E-mail address: m.soto@udc.gal (M. Soto).

https://doi.org/10.1016/j.jwpe.2021.102103
Received 2 February 2021; Received in revised form 11 April 2021; Accepted 26 April 2021

removal efficiencies can affect both the removal of organic matter and nitrogen [3,6].

Among the main problems in processing winery wastewater, Masi et al. [3] listed variable pH and low nutrient content and consequent unfavourable C/N ratio for the microbial growth. Johnson and Mehrvar [4] reported the addition of nutrients for the biological treatment of winery wastewater in conventional systems, while other authors [12–14] reported the addition of nutrients during the treatment of other types of wastewater in CWs. In the case of winery wastewater, this practice is based on theoretical considerations about bacterial growth in biological systems and not on empirical studies that demonstrate it [4]. Thus, considering the large variability of treatment efficiency and surface removal rates in CW systems treating winery wastewater and the sudden increase in applied loading rates during the vintage period, our first hypothesis is that a negative effect of nutrient shortage on the treatment efficiency of VF CW treating this kind of effluents may not be discarded.

On the other hand, Serrano et al. [15] indicated the possibility of a negative effect caused by the low pH values registered, which ranged from 3.3 to 7.2, and reported the use of sodium hydroxide addition to the influent. Arienzo et al. [16] found winery wastewater toxic to wetland plant species and other biological tests species and prescribed pH neutralization. In some cases, the combination of different types of effluents may increase the level of nutrients in the treated wastewater, but it is usually not enough to correct the pH [6,17]. Thus, our second hypothesis is that low pH values can affect not only the removal of organic matter, but also the processes of nitrogen transformation (i.e., nitrification and denitrification) whose microbial populations are generally more sensitive to unfavourable environmental conditions.

Despite the evidence already mentioned, no specific study has been found in the literature on the effect of nutrient level or pH on the performance of CWs treating wastewater from wineries. Thus, this work aims to determine the effect of nutrient shortage and acidic pH during the treatment of high organic load wastewater in VF CWs. To do this, two laboratory scale VF CW units were operated in parallel under high load rates and varying the pH and nutrient content of the influent.

2. Material and methods

2.1. Lab-scale VF CW units

Two lab columns (VF1 and VF2 units) of 13.9 cm diameter were used as VF CW systems (Fig. 1). The effective height was 50 cm, consisting of an upper layer of 5 cm of 0.25−2 mm sand, a main filtering layer of 35 cm of 4−8 mm gravel, and a 10 cm drainage layer at the bottom of 8−16 mm gravel. VF units were fed intermittently by pulses each 3 h for 4 days with rest periods of 3 days a week. Both columns were planted with *Phragmites australis* (5 young seedlings, about 30 cm tall) and monitored during subsequent operational periods with and without nutrients in the influent (VF1) as well as with an influent pH of 6.5 and 4.5 (VF2).

The influent entered the VF units over the filtering medium by means of a peristaltic pump (Dinko Instruments D-21 V) in an intermittent mode and drained by gravity to the bottom of the column and from there to a receiving tank. The units were operated without resting (12 pulses per day) during start-up period and, onwards, with a weekly feeding regimen of 4-day on and 3 days resting (8 pulses per day), following previously recommended feeding strategies [18].

2.2. Winery synthetic wastewater

To prepare the synthetic feed, red wine and white vinegar have been used as sources of organic matter, whose COD concentrations were 203 g/L and 71 g/L, respectively. These components as well as sources of macro and micro nutrients were added as indicate in Table 1 to prepare a

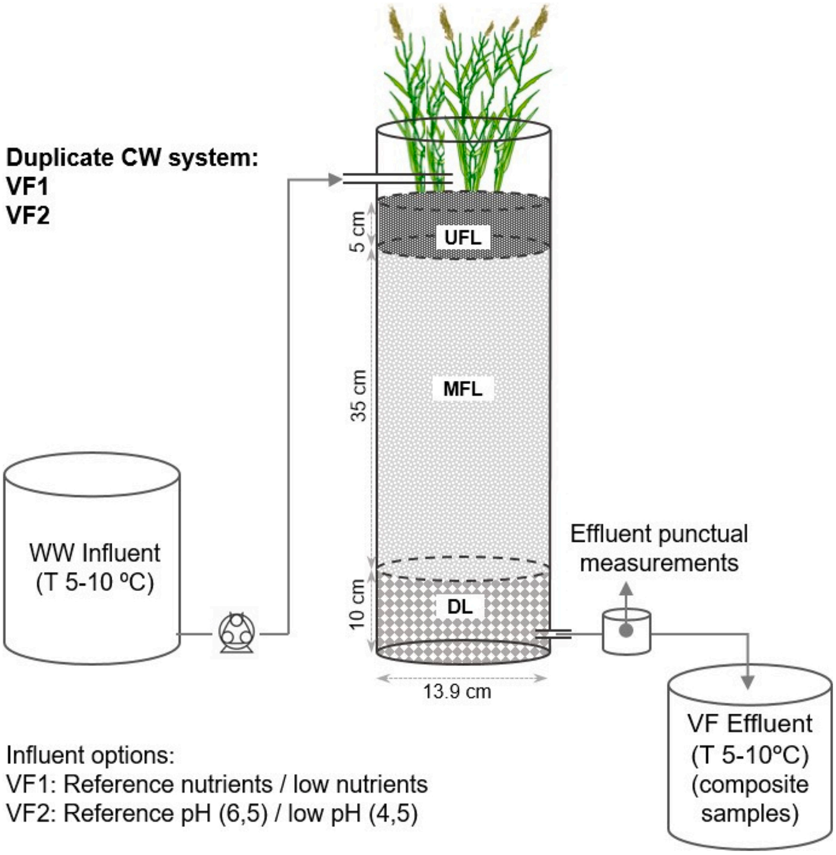

Fig. 1. Schematic representation of the laboratory-scale VF CW treatment system.

Table 1
Concentrate stock solution composition for synthetic wastewater preparation.

Compound	Added amount
Red wine (mL/L)	33
White vinegar (mL/L)	188
NH_4Cl (mg/L)	2140
$MgHPO_4 \cdot 3H_2O$ (mg/L)	220
$K_2HPO_4 \cdot 3H_2O$ (mg/L)	290
Peptone (mg/L)	300
$FeSO_4 \cdot 7H_2O$ (mg/L)	100
$CaCl_2$ (mg/L)	100
$Cr(NO_3) \cdot 9H_2O$ (mg/L)	15
$CuCl_2 \cdot 2H_2O$	10
$MnSO_4 \cdot H_2O$	2
$NiSO_4 \cdot 6H_2O$	5
$PbCl_2$	2
$ZnCl_2$	5

concentrate stock solution. From several considerations about the requirements of nitrogen (N) and phosphorus (P) for bacterial growth and typical biomass yields in CWs [13,19], a COD/N/P ratio of 250/7/1 was used as reference for non-limitation of nutrients. The concentrated was first diluted with distilled water to the required concentration and the pH was regulated to circa neutral (6.5–7.0) or acidic (4.5) values, as required, by adding NaOH or HCl. On the other hand, nutrients were not added to the VF1 unit during some operational periods.

2.3. Analysis and monitoring

TSS, COD, BOD_5, total nitrogen (TN), nitrate nitrogen NO_3^--N, ammonia nitrogen (NH_4^+-N) and phosphates (PO_4^{3-}-P) were determined in composite samples collected weekly (i.e. during the 4-day feeding period). Besides, in situ measurement of pH, dissolved oxygen (DO) and oxidation-reduction potential (ORP) was carried out. Allylthiourea was used as a nitrification inhibitor in BOD_5 assays. Analytical methods were carried out in duplicated as described in Standard Methods [20]. An integrated pH & redox 26 Crison electrode was used for pH and ORP determination, a selective electrode (Crison 9663) for ammonia and a YSI ProODO electrode for DO. The determination of NO_3^--N was made in a Biochrom Libra S6 spectrophotometer following the second derivative method of the UV absorption spectrum [20]. To analyze TN, samples were first digested with potassium persulfate to oxidize all the nitrogenous compounds to NO_3^- and after that the nitrates were determined by spectrophotometry. The viscosity of the effluents was measured using a Cannon Fenske viscometer with a glass capillary 0.42 mm in diameter.

The infiltration rate was periodically determined in both columns. For this, the effluent flow was measured for an entire dosing cycle in order to obtain the effluent flow profile and derived parameters, particularly the mean retention time (t_m), defined as the time required to drain off the 50 % of the wastewater volume added in a feeding pulse.

2.4. Operational characteristics and surface loading rates

The operational characteristics of the VF units are shown in Table 2. The start-up period lasted 43 days and was followed by a reference period of 2–3 weeks in which both columns operated in the same conditions. Subsequently, VF1 unit was subjected to nutrient shortage and VF2 unit to low pH for about 2 months of operation (Table 2). The influent COD concentration was established in the range of 1500–2100 mg/L, which was considered representative of winery wastewater during harvesting and wine making periods [6].

During the start-up period, both columns were fed with a high hydraulic loading rate and so with a high surface loading rate, which averaged 133 ± 13 and 172 ± 44 g COD/m^2·d for VF1 and VF2 CW units, respectively. After start-up, slightly lower but similar SLR were applied in both columns.

This type of down flow columns were used extensively to simulate actual high rate VF CWs [18]. VF CWs fed intermittently with large pulses (batch mode) lead to flooding of the bed surface and then wastewater is gradually percolated down through the bed, being collected by the drainage layer at the base [7,21]. One of the features of unsaturated VF CWs is that most of the pulse volume must be drained before the next pulse occurs, which necessarily leads to a short retention time. Thus, the very short retention time of discrete water pulses is typical of unsaturated vertical flow CWs [21–23], while biological processes improve due to complex phenomena such as sorption and the transfer of compounds to the retained interstitial water after the pulse, favoured by the large specific surface area of the filtering media. On the other hand, the rapid drainage of the water pulse allows oxygen to enter the filtering bed, which greatly increases biomass activity and degradation processes [21,22]. In the opposite situation, non-separation of drainage episodes and approach to continuous effluent flow would indicate hydraulic clogging of the VF CW and reduced oxygenation rates.

3. Results and discussion

3.1. Start-up and operation of VF units treating high strength winery wastewater

The effluent characteristics and removal efficiency of the VF units are shown in Table 3. The SLRs applied (Table 2) were approximately the same as the median reported by Masi et al. [3] for field systems that treat wastewater from wineries while surface removal rates were higher. In addition, the volumetric load rates (twice the SLR, due to the total bed height of 0.5 m and the resulting ratio of 0.5 m^3 bed/m^2 bed) were clearly higher than those applied by Welz and le Roes-Hill [24] and Holtman et al. [25] (i.e., 46 and 152 g COD/m^3·d, respectively) for biological sand filters treating winery wastewater. On the other hand, the higher SLR applied in VF2 in relation to VF1 can explain the higher effluent concentration in COD and BOD_5 and the slightly lower COD and BOD_5 percentage removal in VF2. In any case, COD removal was high, reaching 88 % in VF2 and 95 % in VF1, whilst BOD_5 removal was

Table 2
Characteristics of synthetic winery wastewater fed to CW units and surface loading rates applied.

		Concentration (mg/L)							SLR (g COD/m^2·d)	
Period (CW)	pH	TSS	COD	BOD_5	TN	NO_3^-N	NH_4-N	PO_4-P	VF1	VF2
I) Start-up (VF1, VF2)	6.9	23	2128	1290	64.4	3.8	64.4	9.3	133.2	172.3
II) Reference (VF1, VF2)	6.5	18	1573	954	47.6	2.9	47.6	6.9	111.6	104.8
III) Low nutrients (VF1)[a]	6.5	18	1573	954	**3.4**	**2.6**	**2.0**	**0.7**	111.3	–
IV) Low nutrients (VF1)[a]	6.5	5	1573	954	**1.6**	**2.0**	**2.0**	**0.6**	111.0	–
III) Low pH (VF2)	**4.5**	18	1573	954	47.6	2.9	47.6	6.9	–	110.2
IV) Reference pH (VF2)	6.5	18	1573	954	47.6	2.9	47.6	6.9	–	115.6

[a] Synthetic wastewater contained only wine, vinegar and peptone (Period III) or only wine and vinegar (Period IV). Low nutrient values and low pH highlighted in bold.

Table 3
Effluent concentration and removal efficiency of the VF1 and VF2 columns.

	VF1				VF2			
Period	I	II	III	IV	I	II	III	IV
Operation days	0–43	44–59	60–80	81–124	0–43	44–65	66–113	114–128
pH	7.37 ± 0.12	6.93 ± 0.26	7.0 ± 0.13	7.18 ± 0.14	7.36 ± 0.12	6.9 ± 0.24	5.64 ± 0.25	7.05 ± 0.07
DO (mg/L)	4.24 ± 2.25	7.35 ± 0.46	8.75 ± 0.57	8.01 ± 1.8	4.02 ± 2.62	6.81 ± 0.94	8.43 ± 1.06	7.26 ± 0.27
ORP (mV)	94.0 ± 50.9	151 ± 22	164 ± 23	206 ± 18	83 ± 57	131 ± 39	298 ± 22	193 ± 26
TSS (mg/L)	27 ± 10	11 ± 4	13 ± 2	48 ± 38	33 ± 9	18 ± 5	17 ± 4	101 ± 72
COD (mg/L)	102.2 ± 42.9 [b]	40 ± 16 [a]	47 ± 8 [a]	106 ± 58 [b]	261 ± 144 [c]	48 ± 22 [a]	37 ± 16 [a]	158 ± 75 [b,c]
BOD_5 (mg/L)	34.0 ± nd	15 ± 7	15 ± 5	27 ± 12	41 ± 8	10 ± 8	9 ± 7	32 ± nd
TN (mg/L)	9.2 ± 1.9	2.8±(nd)	1.4 ± 1.1	2.1 ± 1.7	8.3 ± 1.8	3.2 ± 1.6	28 ± 3.9 [d]	17.5 ± 11.3
NO_3^-N (mg/L)	1.2 ± 1.7	0.5 ± 0.6	0.4 ± 0.7	0.4 ± 0.6	nd	1.5 ± 0.6	3.5 ± 0.5	1.3 ± 0.6
NH_4^+-N (mg/L)	7.3 ± 2.2	5.8 ± 2.0	2.4 ± 1.4	3.1 ± 1.0	8.6 ± 4.9	4.9 ± 2.3	37.7 ± 0.8 [d]	5.6 ± 3.6
$PO_4^{3-}P$ (mg/L)	3.8 ± 3.3	1.7 ± 0.2	0.6 ± 0.4	0.7 ± 0.3	2.3 ± 1.4	2.7 ± 0.7	2.2 ± 0.4	6.3 ± 1.9
Removal								
COD (%)	95.2 ± 1.3	97.4 ± 1.0	97.0 ± 0.5	93.3 ± 3.7	87.7 ± 6.8	97.0 ± 1.4	97.7 ± 1.0	90.0 ± 4.8
BOD_5 (%)	97.4 ± 1.0	98.4 ± 0.7	98.4 ± 0.5	97.1 ± 1.3	96.8 ± 0.6	99.0 ± 0.9	99.1 ± 0.8	96.6±nd
TN (%)	85.7 ± 3.0	92.5 ± 6.2	58.8 ± 33.6	−32 ± 107	87.2 ± 6.8	93.2 ± 3.3	41.2 ± 8.2	63.3 ± 18.1
NH_4^+-N (%)	88.7 ± 3.4	87.7 ± 4.3	17.5 ± 10.6	−54 ± 52	86.6 ± 7.7	89.6 ± 4.7	20.8 ± 1.6	88.3 ± 21.1
PO_4^{3-}-P(%)	59.4 ± 34.8	75.8 ± 3.1	10.9 ± 66.2	−19.8 ± 54	75.9 ± 14.8	60.7 ± 10.4	68.4 ± 6.5	9.4 ± 33.4

[a,b,c]Statistical analysis of effluent COD (pair comparison through one-way ANOVA): different letters indicated statistically differences between mean values. [d] Steady state concentrations achieved 3 weeks since influent pH reduction.

approximately 97 % in both cases.

Nutrient removal was high during the start-up period, reaching 86–89 % removal of ammonia and TN, and 60 %–76 % of phosphorus. Taking into account the nitrogen SLR and the removal percentage, the columns removed about 3.5–4.5 g TN/m^2·d. Accounting up for COD and TN removal, the performance of both VF units is indicative of high oxygen transfer rates [18]. During start-up, a large part of the removed nutrients had probably been used for biomass growth, as suggested by estimation of biomass yields and required nutrient availability in similar conditions [14]. Nutrient limitations have not been observed because the effluent of both columns still contained sufficient nitrogen and phosphorus compounds (Table 3).

DO concentration and ORP was about 6 mg/L and 90 mV, respectively, at the end of the start-up period of both columns. Mean effluent BOD_5 ranged from 34 mg/L (VF1) to 42 mg/L (VF2), which are values above the discharge limit for municipal wastewater (i.e. 25 mg/L) or even for industrial wastewater (40 mg/L) [26]. This indicates that both columns were slightly overloaded. The flow profile of each feeding pulse showed a mean retention time ranging from 5 to 10 min (Fig. 2). However, VF1 column suddenly increased the pulse mean retention time to approximately 30 min (day 39 of operation), indicating a risk of flow clogging. To improve operation and reduce the risk of clogging, the feeding regime was changed by introducing resting periods, so the columns were fed 4 days a week, from Monday to Friday, remaining without feeding the other 3 days. In addition, the influent concentration was reduced as indicated in Table 2, reducing the SLR to 111 ± 6 g COD/m^2·d and 67 ± 4 g BOD_5/m^2·d as mean values for both columns since the end of the start-up period to the end of operation period. In this way, during periods II to IV both columns received similar and constant hydraulic loading rate (70.8 ± 4.1 L/m^2·d in VF1 and 70.1 L/m^2·d in VF2) and SLR (COD and BOD_5) which were only 1.0 % higher in VF1 in comparison to VF2 (Table 2).

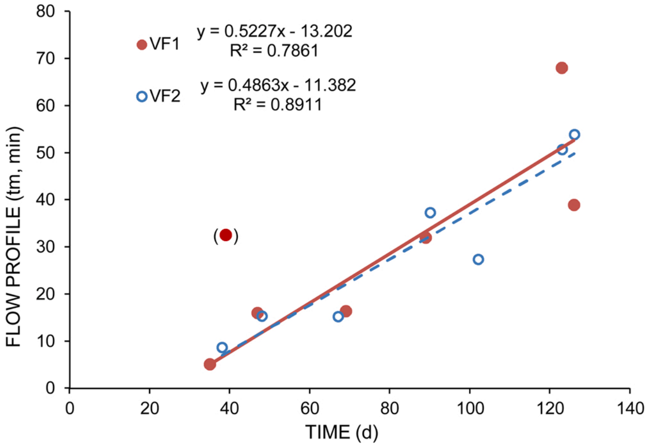

Fig. 2. Evolution of mean retention time (t_m) in both VF units over the operation time (the VF1 point into brackets was not included in the regression fit).

Effluent TSS concentration was similar in both columns during the first three months of operation but onwards were different (Fig. 3A). In overall, effluent concentration of TSS was higher than influent concentration and probably corresponded to the washout of active biomass. Washed TSS were mainly volatile and contributed to an increase in COD and BOD_5 in the effluent (Fig. 3B and C).

The flow profile was similar in both units throughout the entire period of operation (Fig. 2), but t_m increased steadily over the operation time. After approximately 100 d of operation, t_m reached 40 min and the columns began to experience clogging episodes revealed by more prolonged flooding of the surface after a feeding event. This situation coincided with a strong increase of suspended solids content in the effluent of both units (Fig. 3A). As shown in Fig. 3, a TSS washout episode more intense but for less time in VF2 compensated for the delay with respect to biomass washout in VF1. Thus, a similar amount of TSS was removed in both systems. In addition, a clear, although limited, decrease in DO content took place (Fig. 3D) during biomass washout episodes.

Biomass washing episodes are attributed to the short start-up period and high SLRs applied, along with scarce plant development due to the short duration of the experiments. This is due to the strategy followed for the study of the two objective factors (pH and nutrient effects) in laboratory-scale systems, but interference in this sense was not considered as both factors have a greater effect on microbial processes than on plant development. In the case of full-scale CWs, a low-load start-up is advisable that allows the development of the plants during almost a complete vegetative season prior to the periods of high load (harvesting season). In addition, full-scale systems use greater bed depth and larger particle size than those used in the laboratory simulation system. All of these factors reduce the risk of clogging, which is not usually a problem in wetlands treating winery effluents [27].

3.2. The effect of nutrient shortage on the VF1 unit performance

The shortage of nutrients in the influent reduced both the effluent

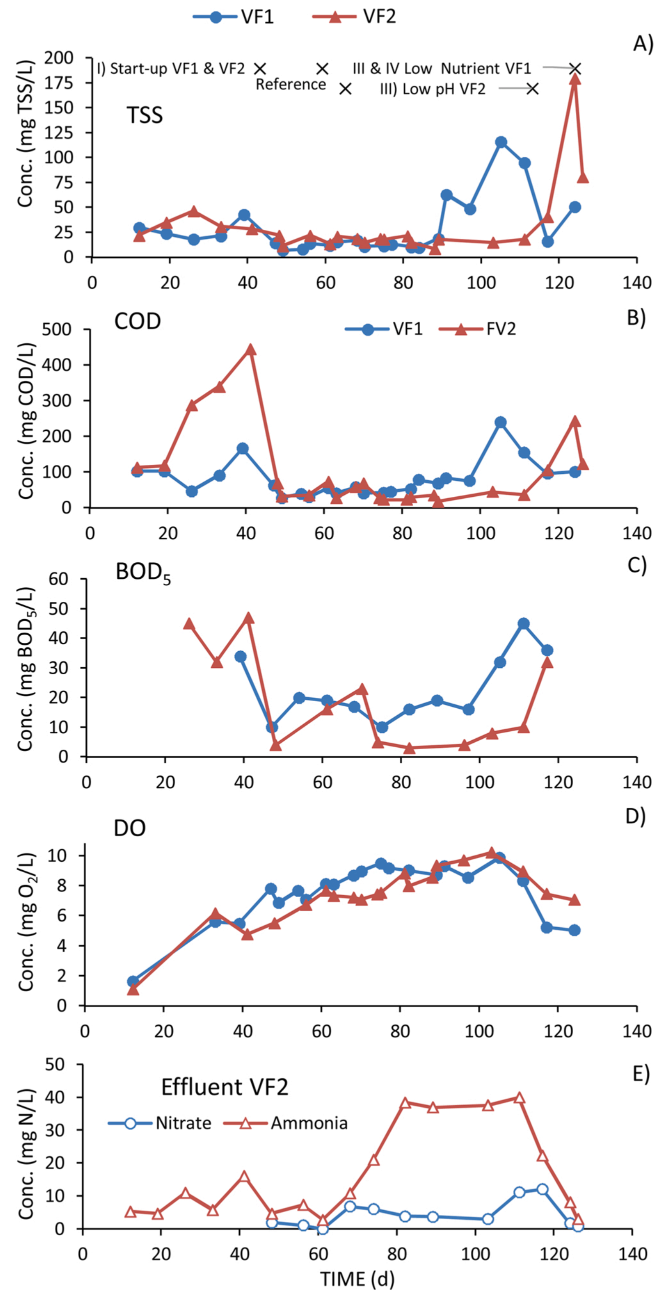

Fig. 3. Evolution of VF1 and VF2 effluent concentration of TSS (A), COD (B), BOD$_5$ (C), DO (D) and VF2 nitrate and ammonia concentration (E). (Note: The length of operational periods is indicated in Fig. 3A and their numerical values in Table 3).

concentration and the percentage removal of nitrogen and phosphorus, but the effect on COD and BOD$_5$ was negligible (Table 3). SS concentration in effluent ranged from 10 to 30 mg/L, being not affected by the changes in nutrient content or pH, but by the operation time as discussed in Section 3.1.

Significant differences were not found for effluent COD from VF1 at period III (without nutrient addition) with respect to period II of both VF1 and VF2 nor with respect to period III of VF2. Although effluent COD from VF1 at period III (also without nutrient addition) was higher than effluent COD from VF1 at period II, this can be attributed to the TSS washout due to partial clogging of filtering media (as discussed in Section 3.1) and not to the effect of the lack of nutrients. In fact, effluent COD from VF1 at period III was not statistically different from effluent COD of VF2 at period IV (with nutrient addition and circa neutral influent pH).

In this way, the mean COD and BOD$_5$ removal during an operation period of about 2 months with high organic load (1573 mg COD/L, 954 mg BOD$_5$/L; approximately the length of peak loading of winery wastewater) and very low nitrogen and phosphorus load (below 3 mg N/L and 1 mg P/L) remained above 93 and 97 %, respectively, and very close to those of reference period.

The change of the influent with nutrients to the influent without nutrients had a reduced effect on the effluent concentration of nitrogen and phosphorus compounds because this concentration was already very reduced due to the efficient removal in the reference period. The effluent concentration of nutrient compounds still decrease during period III and positive removal percentages were maintained. However, during period IV, mainly during the last days of this period, the increase in effluent TSS (Fig. 3A) was accompanied by a slight increase in nutrient concentration which led to negative removal percentages during period IV of VF1 (Table 3).

The positive effect of nutrient addition was demonstrated in two wetland systems treating a nutrient-deficient waste stream (aircraft deicing runoff) in which the treatment performance improved in response to nutrient addition and operational problems such as foaming and slime formation were eliminated [12–14]. These results are contradictory to those obtained in the present study and could be due to the different nature of the effluents treated in both studies. Murphy et al. [14] pointed out that the metabolic response of heterotrophic bacteria growing under nutrient-limited conditions is to produce an excess of polysaccharide slime, which can result in reduced hydraulic performance. In this situation, an increase of viscosity occurs. However, in our study, effluent viscosity was low, being at period IV only 7 % (VF1) and 3 % (VF2) higher than that of distilled water. Richard [28] stated that checking for nutrient deficiency is to be sure that some ammonia or nitrate and ortho-phosphate remain in the effluent at all times, and recommended effluent total inorganic nitrogen (ammonia plus nitrate) and ortho-phosphate concentrations of 1−2 mg/L to ensure sufficient nutrients. Following these criteria, nutrient deficiency did not occur in this study during the treatment of low nutrient wastewater. In fact, effluent concentration of inorganic nitrogen and ortho-phosphate still increased during the last days of period IV (VF1) probable due to nutrient recycling in the filtering bed.

On the other hand, Murphy et al. [14] found high yield ratios (0.77−0.90 kg bacteria per kg BOD$_5$) which were typical of technologies with rapid growth of bacteria, whilst technologies that rely on very stable and mature bacteria populations have lower yield ratios [13]. CWs are extensive systems in which the growth and accumulation of biomass should be low, and then the need of nutrients should also be low [3].

In this work, a COD/N/P ratio of 250/7/1 was used for the start-up and reference periods (I and II for VF1), which corresponded to a potential biomass yield of 0.66 g biomass/g BOD$_5$ (assuming BOD$_5$/COD ratio of 0.5 and 8.5 % N of cell composition). During low nutrient periods (III and IV), the COD/N/P ratio increased up to 7500/7/2.5 (potential yield of 0.02). Although the COD/TN ratio in the influent was very low, the small amounts of nutrients available in the synthetic winery wastewater in combination with nutrient recycling in VF1 CW appear to be sufficient for efficient treatment. On the other hand, if nutrients had to be added, the required amounts would be much lower than those proposed by Murphy et al. [14] for CWs treating aircraft deicing runoff, who indicate nutrient dosing for a yield of 1.0. The presence of nutrients in our study was even lower than that reported by Holtman et al. [25] for their long-term study (COD:N:P ratio of 1138/1.6/2.4) treating winery wastewater in biological sand filters without nutrient addition. To explain why COD removal efficiency was high despite nutrient limitation in the influencer, Holtman et al. [25] point out that bacterial fixation of N can take place in this type of systems.

3.3. The effect of low pH on the VF2 unit performance

The acidity of winery wastewater in particular during vintage can be considered as a factor influencing the performances, even though in most of the experiences examined by Masi et al. [3], the need for chemical neutralization has not been considered. This may be because the pH varies in a very wide range, depending on the specific operating conditions of the winery, due, for example, to the use or not of alkaline cleaning products. The reported pH ranges from 2.5 to 12.9 [4], while several authors have indicated mean values in the range of 4.4 to 6.8 [3, 11,29], which are not extremely acidic. However, very acidic pH values are also common, especially during harvest when the highest contaminant loads are generated. Ioannou et al. [29] reported that approximately half of the studies reviewed recorded pH values below 4.0.

In this study, the operation at low pH of 4.5 (period III, VF2) did not reduce the COD, BOD_5 and phosphate removal efficiency (Table 3). Significant differences were not found for effluent COD from VF2 at period III (influent pH of 4.5) with respect to period II (influent pH of 6.5) of both VF1 and VF2. High percentage removals of 97–98 % (COD), 99 % (BOD_5) and 61–68 % (PO_4^{3-}-P) were obtained at both influent pH values of 6.5 and 4.5.

These results are in accordance with the suggestion of Serrano et al. [6]. From multivariate analysis applied to a hybrid CW system treating winery wastewater on a field scale, these authors found that the long-term effect of low pH on the treatment efficiency (COD and BOD_5) was insignificant. pH effect on the performance of horizontal subsurface flow CWs was also discarded by de la Varga et al. [27].

However, low pH clearly reduces ammonia and TN removal (Fig. 3E). Effluent concentration of ammonia in VF2 showed a continuous increase during approximately 3 weeks after lowering the influent pH to 4.5 and then remained stable for the rest of period III. Ammonia accumulated in the effluent even though the pH of the effluent remained above 5.4 during this period. The large removal of COD and BOD_5 (98–99 %, Table 3) means the removal of most organic acids, but this effect was not sufficient to maintain the near-neutral effluent pH reached in previous periods.

TN followed the same trend of ammonia, whilst nitrate suffered an initial increase up to 7 mg N/L and progressively decreased to 3.5 mg N/L. In spite of the reduction of nitrification, effluent nitrate during period III was higher than during period II and period IV (Table 3). In addition, ammonia removal decrease from 90 % to 21 % and TN removal from 93 % to 41 %. Thus, low influent pH impaired both nitrification and denitrification process. Nevertheless, the effect on nitrification was stronger than the effect on denitrification. If nitrified ammonia is considered to have been converted to nitrate (i.e., 9.9 mg N / L on average for period III, Tables 2 and 3), the VF2 unit continued to remove 72.7 % of the nitrate available during the period III.

These effects of acidic influent pH occurred in spite that the effluent pH was kept above 5.4 (mean value of 5.64 ± 0.25 during period III of VF2 unit, Table 3). This behaviour partial neutralization is typical of CW systems treating winery wastewaters. CWs have the capacity to increase the winery wastewater pH in some extension. For example, Serrano et al. [6] reported a pH increase from 5.0 ± 1.2 to 6.0 ± 0.9 when treating winery wastewater at high SLR. Treating the wastewater from wineries in biological sand filters, an effective pH neutralization was achieved for a pH influent above 4.6 (average influential pH approximately 6.0) and volumetric loading rates of 152 g $COD/m^3 \cdot d$ [25], as well as for a pH influent of 3.5 and volumetric loading rates of 46 g $COD/m^3 \cdot d$ [24]. These findings suggest that the application of a lower SLR on VF CWs would offer greater neutralizing capacity and thus maintain good ammonia removal rates.

pH increasing or decreasing during wastewater treatment depends not only on the pH of the influent but also on the nature and extent of conversion processes in the CW. Treating domestic wastewater, VF CWs clearly enhance nitrification in comparison to horizontal flow CWs, but nitrate trend to accumulate in the final effluent and limit TN removal. Limited nitrogen removal was due to the pH decrease to approximately 4.5 which impaired both nitrification and denitrification in a two-step VF + VF CWs system [18]. This is because the activity of nitrifying bacteria decreases rapidly when the pH drops below 6.5 [30]. Torrijos et al. [18] reported that maintaining effluent pH above 6 through alkalis addition clearly increased nitrification and TN removal.

In the present study, the negative effects of low influent pH were progressively overcome in a short 10 day period after influent pH increase as indicated by the results of period IV VF2 (Table 3).

As indicated by Masi et al. [3], potential effects of acidic influent pH on the efficiency of CWs treating winery wastewater remained unknown. Reports on this subject for other kind high organic load wastewater are scarce. Similar to winery wastewater, wood waste leachate was characterized by high oxygen demand, tannin and lignin, and volatile fatty acids (VFAs), but low pH and nutrients. During wood waste leachate treatment in CWs, nutrient addition and pH adjustments helped to improve contaminant removal [31]. Closer to neutral pH circumstances, along with higher mean temperatures during the warmer period of year, favoured microbial activity. Besides, Lattrie et al. [32] reported that a CW treating multiple livestock wastewaters was not capable of reducing high levels of pollutants from the high strength silage leachate and its low pH. However, the results of the present study clearly indicate that an acidic influent of 4.5 pH did not impair the organic matter and phosphorus removal, whilst nitrogen removal rates appeared clearly reduced.

3.4. Reviewing the strategy for separate or combined treatment of winery wastewater and nitrogen-containing effluents

The application of constructed wetland systems for the treatment of winery wastewater started in around 2000s in the USA, France, Italy, Germany, Spain and South Africa. Nowadays, there are, for instance, about 100 CW systems operating in Northern and Central Italy for winery wastewater [3]. CWs were recently reported to be the most common type of on-site treatment in the Niagara region [4]. Due to the high acidity and lack of nutrients, the main trend was to combine the effluents from the wine production process with other effluents available in the vicinity that provided nutrients and contributed to the neutralization of the winery effluent. As highlighted by Masi et al. [3], this is a frequent situation in many small wineries in Europe. However, this may not be the best strategy, as the results of this study show, results that must be taken into account when designing and operating CW systems that treat combined winery and another type of wastewater containing large amounts of nitrogen.

According to de la Varga et al. [1], winery wastewater should not be diverted from other wastewater streams generated in the winery in order to guarantee a minimal level of nutrient availability. In fact, different kind of effluents can increase the level of nutrients in the treated wastewater [6,17]. However, the results of this research suggest the potential interest of a separate treatment of wastewater from the winery process when this is the main flow. In this situation, the incorporation of domestic wastewater and other nutrient-containing streams may not be sufficient to neutralize the pH, but may add a high level of nitrogen compounds, which in turn are difficult to remove if the influent is no further neutralized. Considering that pH discharge limits are not always required [4] or are less restrictive than limits in N compounds (pH range 5.5–9.5) [26], this study indicates that winery wastewater with very low nutrient organic load can be treated at high surface loading rates in VF CW to meet TSS, COD and BOD_5 discharge limits. The use of VF CW can avoid the addition of nutrients and pH adjustment needed for other biological treatment processes, as reported by Johnson and Mehrvar [4]. In addition, this option limits the presence of pathogenic contamination in the treated effluent, which is the main obstacle to meeting some reuse standards [11].

As an option, a separate pre-treatment of the nitrogen-rich effluent would be more successful. This nitrification pre-treatment is very effective in VF CW [33,34]. In this case, the pre-nitrified effluent can then be combined with the winery effluent for final treatment, including complete denitrification, as the results indicate a lower effect of pH on denitrification than on nitrification. Alternatively, nitrogen-rich effluent can be maintained in an always independent CWs treatment line, using a fraction of the winery raw wastewater as an external carbon source to increase the C/N ratio and favour complete denitrification [35].

When the aim is to treat the effluent for reuse in irrigation, it may always be of interest to keep the streams separate to avoid the presence of pathogens in the treated winery wastewater, or to mix them only after their advanced treatment (if the conditions of storage require it), as the lower content of organic matter in the treated effluents would limit the growth of pathogens [36].

4. Conclusions

One-step VF CW units receiving influent COD concentration in the range of 1500–2100 mg/L reached percentage removals of 88–98 % COD, 96–99 % BOD_5, 59–93 % TN, 87–90 % ammonia and 59–76 % phosphorus at high surface loading rate which ranged from 110 to 172 g $COD/m^2 \cdot d$. Typical nutrient shortage of winery effluents and low pH do not affect the removal efficiency of COD and BOD_5. The VF CW unit receiving an influent with a COD/N/P ratio 3500-7500/7/2.5 for a period of 2 months (approximately the length of peak loading of winery wastewater) maintained reduced but steady nutrient concentrations in the effluent due to the low biomass yield and nutrient recycling, which contributed to maintain the high treatment efficiency. However, low pH clearly produced high ammonia effluent concentrations if nitrogen is present in the influent. When the pH changed from 6.5 to 4.5, ammonia removal decreased from 90 % to 21 % and TN removal from 93 % to 41 %, increasing the effluent concentration of both ammonia and nitrate. Thus, low influent pH impaired both nitrification and denitrification processes in VF CWs. Joint treatment of winery wastewater with domestic wastewater and other nutrient-containing streams is an option to increase nutrient availability. However, this is not necessary for the efficient treatment of wastewater from wineries, while creating the risk of partial nitrogen removal due to the negative effect of low pH. The results indicate that the separate treatment of wastewater from the winery process can be carried out in VF CWs at a higher rate, without prior pH neutralization and more efficiently than in the case of its combination with other nutrient-containing streams.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors report no declarations of interest.

References

[1] D. de la Varga, M. Soto, C.A. Arias, D. van Oirschot, R. Kilian, A. Pascual, J. A. Álvarez, Constructed wetlands for industrial wastewater treatment and removal of nutrients, in: A. En Val, J.L. Campos, A. Mosquera (Eds.), Technologies for the Treatment and Recovery of Nutrients from Industrial Wastewater, IGI Global, Hershey PA (USA), 2016, pp. 202–230.

[2] T. Saeed, G. Sun, A comprehensive review on nutrients and organics removal from different wastewaters employing subsurface flow constructed wetlands, Crit. Rev. Environ. Sci. Technol. 47 (4) (2017) 203–288, https://doi.org/10.1080/10643389.2017.1318615.

[3] F. Masi, J. Rochereau, S. Troesch, I. Ruiz, M. Soto, Wineries wastewater treatment by constructed wetlands: a review, Water Sci. Technol. 71 (8) (2015) 1113–1127.

[4] M.B. Johnson, M. Mehrvar, Winery wastewater management and treatment in the Niagara Region of Ontario, Canada: a review and analysis of current regional practices and treatment performance, Can. J. Chem. Eng. 98 (1) (2020) 5–24.

[5] D. Bolzonella, D. Rosso, Winery wastewater characterization and biological treatment options, in: Proceedings of the Fifth International Specialized Conference on Sustainable Viticulture: Vinery Waste and Ecologic Impacts Management, Trento and Verona, Italy, 2009.

[6] L. Serrano, D. de la Varga, I. Ruiz, M. Soto, Winery wastewater treatment in a hybrid constructed wetland, Ecol. Eng. 37 (2011) 744–753.

[7] J. Vymazal, Constructed wetlands for treatment of industrial wastewater: a review, Ecol. Eng. 73 (2014) 724–751.

[8] H. Shepher, M.E. Grismer, G. Tchobanoglous, Treatment of high-strength winery wastewater using a subsurface-flow contructed wetand, Water Environ. Res. 73 (4) (2001) 394–403.

[9] R. Mosteo, P. Ormad, E. Mozas, J. Sarasa, J.L. Ovelleiro, Factorial experimental design of winery wastewaters treatment by heterogeneous photo-Fenton process, Water Res. 40 (2006) 1561–1568.

[10] K. Skornia, S.I. Safferman, L. Rodriguez-Gonzalez, S.J. Ergas, Treatment of winery wastewater using bench-scale columns simulating vertical flow constructed wetlands with adsorption media, Appl. Sci. 10 (3) (2020), 1063.

[11] M. Milani, S. Consoli, A. Marzo, A. Pino, C. Randazzo, S. Barbagallo, G.L. Cirelli, Treatment of winery wastewater with a multistage constructed wetland system for irrigation reuse, Water 12 (5) (2020), 1260.

[12] S. Wallace, M. Liner, Nutrient limitations in industrial treatment wetlands, in: 12th International Conference on Wetland Systems for Water Pollution Control, October 4–8, Venice: 1071–1074, 2010.

[13] S. Wallace, M. Liner, Design and Performance of the Wetland Treatment System at the Buffalo Niagara International Airport, Accessible at: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.715.5288&rep=rep1&type=pdf (last access: 03/01/2021), 2011.

[14] C. Murphy, S. Wallace, R. Knight, D. Cooper, T. Sellers, Treatment performance of an aerated constructed wetland treating glycol from deicing operations at a UK airport, Ecol. Eng. 80 (2014) 117–124.

[15] L. Serrano, D. de la Varga, M.A. Díaz, I. Ruiz, R. Bondelle, M. Soto, HUSB-CW treatment system for winery wastewater, in: Proceedings of the Fifth International Specialized Conference on Sustainable Viticulture: Vinery Waste and Ecologic Impacts Management, Trento and Verona, Italy, 2009, 317–234.

[16] M. Arienzo, E.W. Christen, W.C. Quayle, Phytotoxicity testing of winery wastewater for constructed wetland treatment, J. Hazard. Mater. 169 (1–3) (2009) 94–99.

[17] E.R. Rozema, L.R. Rozema, Y. Zheng, A vertical flow constructed wetland for the treatment of winery process water and domestic sewage in Ontario, Canada: six years of performance data, Ecol. Eng. 86 (2016) 262–268.

[18] V. Torrijos, O.G. Gonzalo, A. Trueba-Santiso, I. Ruiz, M. Soto, Effect of bypass and effluent recirculation on nitrogen removal in hybrid constructed wetlands for domestic and industrial wastewater treatment, Water Res. 103 (2016) 92–100.

[19] M. Henze, P. Harremoes, J. Jansens, E. Arvin, Wastewater Treatment, Springer-Verlag, New York, 2002.

[20] APHA, Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, 2005.

[21] A. Stefanakis, C. Akratos, V.A. Tsihrintzis, Vertical Flow Constructed Wetlands, 2014, Elsevier Science, Amsterdam, NL, 2014, p. 392, ISBN 9780124046122.

[22] K. Kayser, S. Kunst, Processes in vertical-flow reed beds: nitrification, oxygen transfer and soil clogging, Water Sci. Technol. 51 (9) (2005) 177–184.

[23] O.G. Gonzalo, I. Ruiz, M. Soto, Integrating pretreatment and denitrification in constructed wetland systems, Sci. Total Environ. 584–585 (2017) 1300–1309.

[24] P.J. Welz, M. le Roes-Hill, Biodegradation of organics and accumulation of metabolites in experimental biological sand filters used for the treatment of synthetic winery wastewater: a mesocosm study, J. Water Process Eng. 3C (2014) 155–163.

[25] G.A. Holtman, R. Haldenwang, P.J. Welz, Biological sand filter system treating winery effluent for effective reduction in organic load and pH neutralization, J. Water Process Eng. 25 (2018) 118–127.

[26] A. Rizzo, R. Bresciani, N. Martinuzzi, F. Masi, Online monitoring of a long-term full-scale constructed wetland for the treatment of winery wastewater in Italy, Appl. Sci. 10 (2) (2020), 555.

[27] D. de la Varga, I. Ruiz, M. Soto, Winery wastewater treatment in subsurface constructed wetlands with different bed depths, Water Air Soil Pollut. 224 (2013) 1485–1497.

[28] M. Richard, Activated sludge microbiology problems and their control, in: Presented at the 20th Annual USEPA National Operator Trainers Conference, Buffalo, NY, June 8, 2003.

[29] L.A. Ioannou, G. Li Puma, D. Fatta-Kassinos, Treatment of winery wastewater by physicochemical, biological and advanced processes: a review, J. Hazard. Mater. 286 (2015) (2015) 343–368.

[30] M. Henze, C.M. van Loosdrecht, G.E. Ekama, D. Brdjanovic, Biological Wastewater Treatment, IWA Publishing, London, 2008.

[31] A. Masbough, K. Frankowski, K.J. Hall, S.J.B. Duff, The effectiveness of constructed wetland for treatment of woodwaste leachate, Ecol. Eng. 25 (2005) 552–566.

[32] S. Lattrie, R. Gordon, A. Madani, G. Stratton, Performance evaluation of a constructed wetland treating multiple wastewaters, in: Paper Presented at the ASAE Annual International Meeting, 17–20 July 2005, American Society of Agricultural Engineers: Tampa, Florida, 2005.

[33] Y. Millot, S. Troesch, D. Esser, P. Molle, D.P.L. Rousseau, Effects of design and operational parameters on ammonium removal by single-stage French vertical flow filters treating raw domestic wastewater, Ecol. Eng. 97 (2016) 516–523.

[34] V. Torrijos, I. Ruiz, M. Soto, Microbial activities and process rates in two-step vertical and horizontal subsurface flow gravel and sand filters, Water Air Soil Pollut. 229 (2018) 290, https://doi.org/10.1007/s11270-018-3934-7.

[35] O.G. Gonzalo, I. Ruiz, M. Soto, Effect of different bypass rates and unit area ratio in hybrid constructed wetlands, Environ. Sci. Pollut. Res. 27 (2020) 40355–40369, https://doi.org/10.1007/s11356-020-09982-9.

[36] R.P. Shingare, P.R. Thawale, K. Raghunathan, A. Mishra, S. Kumar, Constructed wetland for wastewater reuse: role and efficiency in removing enteric pathogens. A review, J. Environ. Manage. 246 (2019) 444–461, https://doi.org/10.1016/j.jenvman.2019.05.157.