Biotreatment of Winery Wastewater Using a Hybrid System Combining Biological Trickling Filters and Constructed Wetlands

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Abstract: The objective of this work was to determine the ability of a pilot-scale hybrid system to treat real (non-synthetic) winery wastewater. The experimental treatment system consisted of two stages: An attached growth pilot-scale bioreactor (biological trickling filter with plastic support material) was initially used to remove a significant amount of dissolved chemical oxygen demand (d-COD) from winery wastewater, and then a pilot-scale, horizontal subsurface flow constructed wetland (CW) was examined as a post-treatment step for further d-COD removal. Results from the biofilter revealed that the recirculation rate of 1.0 L/min lead to higher d-COD removal rates than that of 0.5 L/min for all feed d-COD concentrations tested (3500, 7500, 9000 and 18,000 mg d-COD/L). Experiments in the CW were performed using feed d-COD concentrations of about 1500 mg/L (equivalent to biofilter effluent when initial filter feed d-COD concentrations are 18,000 mg/L). The wetland polishing stage managed to further remove d-COD and produced effluent concentrations below current legislation limits for safe disposal. Furthermore, the presence of zeolite in CW (one third of the length of CW) enhanced ammonium removal. The experimental results indicate that the combination of a biological trickling filter and a constructed wetland could effectively treat effluents originating from small wineries typical of the Mediterranean region.

Keywords: winery wastewater; trickling biofilter; constructed wetland; d-COD removal

1. Introduction

Agro-industries are major contributors to the worldwide industrial pollution problem, since they produce large quantities of wastewater that are very often left untreated and eventually end up in the environment. Among these agro-industries, wineries are important pillars of the local economy in Mediterranean countries, including Greece [1]. The amount and complexity of wastes generated by wineries and the management of these wastes is very problematic [2,3]. Additionally, most of these wineries are usually small-sized, scattered throughout the country, and produce byproducts and wastes seasonally. Financial resources for proper waste disposal are not usually available, and therefore, in order for these industries to survive and remain competitive in the market, in many cases legislative standards for wastes and wastewaters are not adhered to.

The wine making process comprises two main periods: harvest and post-harvest, and waste production is considerably higher in the harvest season [4]. Solid waste and sludge may be produced during several procedures, such as destemming, pressing, decanting, clarifying, etc., and these wastes include solid grape residues (grape stalks, etc.) and lees (winery sludge, or dregs). In addition, wine production generates large quantities of wastewater that originate from grape processing and washing.
operations such as grape crushing and pressing, and the rinsing of fermentation tanks, barrels and other equipment and surfaces [5]. Winery wastewater (WW) is characterized by fluctuations in both quality and quantity during the year [2,3,6,7], causing environmental concern in wine-producing countries. WW is characterized by low pH values (in the range of 3–6 (acidic), with the exception of some winemaking stages such as bottling, where pH values increase), low nutrient content [8–10], and high organic content, which according the literature is readily biodegradable [2,4,11–14].

Although several technologies have been proposed for winery wastewater treatment in Greece and other countries, [2,10,13,15–19] (e.g., chemical precipitation, coagulation/flocculation processes, aerobic and anaerobic biological treatment, constructed wetlands (CWs), advanced oxidation processes, etc.), unpretreated WW is usually discharged directly into the septic system or irrigated onto agricultural or other land [20].

A literature review reveals that most research concentrates on WW treatment using a single treatment method, while only recent research works have demonstrated a hybrid approach combining two or more treatment methods. Pipolo et al. [21] showed that mild Fenton’s treatment with biofiltration by Asian clams as a secondary treatment step can completely remove the Chemical Oxygen Demand (COD) of WWs, the concentration of which however was rather low (about 1267 ± 101 mg/L). A similar treatment system was also examined by Ferreira et al. [22], who achieved COD removal near 100% from a WW with a relatively low initial COD concentration (up to 2280 mg/L). The use of solar Fenton as a pretreatment step before an immobilized biological reactor or activated sludge was studied by Souza et al. [23] and Mosteo et al. [24], respectively. They both achieved up to 96% COD removal with initial COD concentrations of up to 3300 mg/L. Benitez et al. [25–27] examined the ozonation of an aerobically pretreated WW (with an initial COD concentration of raw WW 27,000–29,000 mg/L) and found that the combination of these two processes leads to a COD removal efficiency of up to 67%. An aerobic biological process followed by a chemical oxidation process using Fenton’s reagent (as a final polishing step) was examined by Lucas et al. [28], Anastasiou et al. [29] and Beltran-Heredia et al. [30] using WW with a high COD concentration (up to 20,000 mg/L), and achieving a COD removal efficiency of 80%–99%. A similar hybrid system (combined membrane bioreactor and solar Fenton oxidation) with significant COD removal efficiencies (69–85%) was also examined by Ioannou et al. [31–33], but for lower initial COD concentrations (120–210 mg/L). Braz et al. [34] examined the use of long-term, aerated storage combined with coagulation/flocculation and recorded a COD-soluble removal performance of about 87.9%, using WW with an initial COD concentration of over 25,000 mg/L. The combination of activated sludge and chemical processes with significant COD and polyphenol abatements (above 60% and 95%, respectively) was examined by Solís et al., [35] who treated WW with a high initial COD concentration (about 139.25 g/L). Photocatalysis as a post-treatment step after adsorption on bentonites was tested by Rodríguez et al. [18]. In this study, COD removal reached 58.2% for initial total COD values of 12,400 ± 700 mg/L. Finally, Amaral-Silva et al. [36] studied three treatment stages comprising coagulation, a Fenton-like process and biological treatment using activated sludge. The final COD reduction was 74% (145 mg/L) when applying WW with an initial COD concentration of about 5180 mg/L.

Although aerobic biological treatment of WW is considered a low-cost method [3], it cannot be used as a single treatment technique, since it leads to inefficient COD removals, especially when treating WW with high initial COD concentrations [16,29,35]. For this reason, biological treatment has been examined in combination with other methods, mainly advanced oxidation processes, as pre-treatment or post-treatment steps [16,57]. CWs have been used for the treatment of WW [38–43], but their use as an integrated treatment solution for all winery wastes (wastewater, slurry sludge, solid wastes, etc.) has not been thoroughly studied or implemented. According to Masi et al. [41], WW treatment is mainly approached by using extensive aerated ponds, sequencing batch reactors (SBRs) or activated sludge plants, and then adding a tertiary treatment stage comprising CWs for sludge treatment and polishing. To the best of our knowledge, biological trickling filters have not been examined in combination with CWs for the treatment of real WW. Only Kim et al. [44] examined the
performance of a vertical-flow-constructed wetland with a trickling filter; however, they applied a mixture of domestic sludge and WW, and also used a further step of chemical precipitation.

The objective of this study was to examine the efficiency of a hybrid system comprising a biological trickling filter and a horizontal subsurface flow (HSF)-constructed wetland to treat real winery wastewater originating from a local winery. Experiments in a pilot-scale trickling filter were carried out using different WW feed concentrations (3500–18,000 mg/L) and two different recirculation rates (0.5 and 1.0 L/min). In addition, the possibility of pairing biological treatment in attached growth systems with CWs was investigated for the complete mineralization of WW.

2. Materials and Methods

2.1. Winery Wastewater (WW) Composition and Analytical Procedures

The WW used in this research was obtained from the local Grivas winery located in Agrinio city (western Greece), and derived from the washing of equipment and winery surfaces during the production of white wine. Following 3 hours of sedimentation (to remove large particles) the effluent was transferred into 4-liter bottles and stored at 4 °C in the dark for a maximum of three days before use. The effluent presented pH values of between 4–5, conductivity of about 300–700 µS/cm, dissolved COD (d-COD) of 50,000–70,000 mg/L, and BOD₅ (Biological Oxygen Demand after five days) in the range of 22,000–31,000 mg/L. The raw wastewater was diluted with tap water at different rates leading to four initial WW concentrations of about 3500, 7500, 9000 and 18,000 mg d-COD/L (C³500, C⁷500, C⁹000, C¹₈000, respectively).

d-COD and BOD₅ concentrations were determined following the methods described in ‘Standard Methods for the Examination of Water and Wastewater’ [45]. To determine d-COD the samples were initially filtered through 0.45 µm-Millipore filters (GN-6 Metricel Grid 47 mm, Pall Corporation) and then measured following the closed reflux, colorimetric method using a Multiparameter Bench Photometer (HANNA C99). Controlled conditions of temperature, agitation, and light absence were applied for BOD₅ determination following the respirometric method (WTW OxiTop). Values of pH, temperature, conductivity and Dissolved Oxygen (DO), were measured using a HANNA HI9828 instrument. Inorganic ions (NH₄⁺–N) were quantified by ionic chromatography using a Thermo Dionex ICS-5000DC instrument coupled to a conductivity detector AERS 500 (4 mm) employing an IonPac AS23 (4 mm x 250 mm) column for anions (the eluent comprised 4.5 mM Na₂CO₃/0.8 mM NaHCO₃ with a flow rate of 1.0 mL/min, a temperature of 30 °C, and an injection volume of 25 µL), and an IonPac CS12A (2 mm x 250 mm) column for cations (the eluent comprised 20 mM H₂SO₄ with a flow rate of 1.0 mL/min, an ambient temperature and an injection volume of 25 µL). Kinetic experiments in the trickling filters were performed in triplicate, and the results presented in all of the figures display the mean value ± standard deviation (SD). To investigate statistically significant differences, a one-way between groups analysis of variance (ANOVA) at the 95% significance level was used to examine the effect of the design and operational parameters on the CWs. ANOVA was applied to compare the effluent concentrations of each unit. Post Hoc pair comparisons were also performed to test equal variations, using Tukey’s honestly significant difference test. Homogeneity of variance tests (Levene) were by-passed, since the number of data points for each group was the same. Statistical analyses were performed using SPSS Statistics 23.0 for Windows.

2.2. Biological Treatment Using the Pilot-Scale Trickling Filter

The pilot-scale trickling filter used for the biological treatment of WW has been described in detail in Tatoulis et al. [46]. The working volume of filter was 7 L, and the support material was plastic material of a 500 m²/m³ specific surface area and 0.8 filter porosity (Figure 1). Initially the filter was operated under batch operation to ensure microorganism growth and attachment onto support material. The set-up of the filter for d-COD removal was achieved by using WW and an enriched
culture (containing predominant indigenous species from WW able to biodegrade WW), so that the initial d-COD in the filter was recorded at 18,000 mg/L.

The procedure for the establishment of this enrichment culture was performed in 1L Erlenmeyer flasks. Specifically, 500 mL winery wastewater of concentration 18,000 mg d-COD/L (C$_{18000}$) was kept under aerobic conditions (DO > 4 mg/L) and constant mixing (500 rpm). As soon as d-COD degradation ceased, 450 mL liquid volume was discarded and the flask content was brought back to 500 mL by adding tap water and WW to achieve the desired final concentration of 18,000 mg/L. In this way, the predominance of indigenous microorganisms able to biodegrade WW was achieved.

![Trickling Filter Diagram](image)

Figure 1. Scheme of the hybrid system combining biological trickling filter and constructed wetland for the treatment of winery wastewater.

A start-up time of about six weeks was necessary to reach maximum d-COD degradation rates. Inoculum of the established enriched culture (500 mL) was added to set up the trickling filter with WW as our substrate, so that the initial d-COD concentration in the filter was 18,000 mg/L. As microorganism attachment was observed on the support material, the inoculum volume was reduced gradually, until no further inoculum addition was required. The filter was then operated as a sequencing batch reactor with recirculation in order to achieve a completely mixed flow pattern within the bioreactor. After about one month the filter reached a steady performance state and the experiments could commence. Three different volumetric flow rates of 0.5, 1.0 and 2.0 L/min were tested for the recirculation stream for all the initial WW concentrations of C$_{37500}$, C$_{7500}$, C$_{9000}$ and C$_{18000}$. For each initial WW, the concentration cycles were repeated until the maximum degradation rate of d-COD was recorded for at least three cycles. The filter backwashing frequency depended on the WW feed concentration and was determined by observing the loading time (less than 1 min) of the working volume of polluted water into the filter. Values over 1 min indicated pore clogging.

### 2.3. Post-Treatment Using Horizontal Subsurface Flow (HSF) Constructed Wetlands (CWs)

Two identical pilot-scale HSF CWs were constructed and placed outdoors on the rooftop of the Department of Environmental Engineering, University of Patras, located in Agrinio, western Greece.
Each unit was a high-density polyethylene (HDPE) tank with inner dimensions of 72 cm length, 33 cm width (surface area = 0.24 m$^2$) and 35 cm depth.

Two thirds of the length of each unit (i.e., 48 cm) were filled with igneous fine gravel obtained from a local river bed (D50 = 6 mm, specific surface area = 3075 m$^2$/m$^3$, porosity = 35%) and the remaining one third of their length (i.e., 24 cm) was filled with natural Bulgarian zeolite (D50 = 4 mm, specific surface area = 4370 m$^2$/m$^3$, porosity = 25%) (Figure 1). One unit (CW-P) was planted with common reed ($Phragmites australis$) obtained from local streams and the other unit (CW-U) was kept unplanted as the control. To achieve rapid vegetation growth in the planted unit, and ensure quick plant adjustment and a minimal commissioning phase, three reed stems (i.e., 12 reeds/m$^2$) were planted in CW-P. Both CW units were equipped with inlet and outlet hydraulic structures similar to those used in full-scale systems to control the water level. Wastewater inflowed through a perforated plastic pipe (diffuser) placed across the entire upstream width side of the tank, just above the surface of the substrate layer. These inlet diffusers were fastened horizontally onto the inner tank wall to ensure the uniform distribution of wastewater across the tank width. The outflow was collected through an orifice (1/4 inch diameter) placed at the bottom of the downstream width side of the tank, connected to a U-pipe. The elevation of the U-pipe from which water overflowed was adjustable, thus allowing control of the water level within the tank. The water level was maintained at the same height as the substrate media (i.e., at about 35 cm). Wastewater overflow from the U-pipe was collected in a 35 L plastic tank for proper disposal. Void volume was estimated by filling and draining both tanks and measuring the volume of the water collected. At the end of the experimental water volume and mean porosity values in both units were 14 L and 17%, respectively. The CWs units were operated for a 2-year period, in which two hydraulic residence times (HRTs) were applied (i.e. 4 and 2 days). Plant biomass was removed from CW-P unit on January, due to reed biomass decay.

3. Results and Discussion

3.1. Pilot-Scale Trickling Filter Experiments

Although the biological treatment of WW has been tested mainly in suspension processes [2,9,47,48], promising results have recently been demonstrated when using attached growth systems [2,13,14,16,49]. The pilot-scale trickling filter was initially inoculated with enriched microorganisms and operated as a batch reactor until their attachment onto the support material. The operating mode with 0.5 L/min recirculation was then examined. The application of this mode provided DO concentrations constantly above 4.0 mg/L without an external oxygen supply, thus reducing drastically the operational cost of the system. The first experiments were performed with an initial WW feed concentration of 3500 mg d-COD/L and a recirculation rate of 0.5 L/min. A series of operating cycles was performed until the system reached a constant, efficient performance and recorded a maximum degradation rate of d-COD for at least three cycles. After these operating cycles, a minimum period of 24 h was needed to reach the maximum degradation rate of C$_{3500}$ (Figure 2a). The same procedure was followed for the feed concentrations of 7500, 9000 and 18,000 mg d-COD/L (Figure 2b–d) and minimum operating cycle durations of 48, 54 and 64 hours, respectively, were achieved. The effect of volumetric flow rate on the system’s performance was also studied, by examining the values of 1 and 2 L/min. It should be noted that with a recirculation rate of 2 L/min the filter was not able to operate sufficiently due to detachment of biofilm from the support material and very low d-COD removal rates (data not shown). Data from the three last cycles of the set of experiments with 0.5 and 1.0 L/min are presented in Figure 3.

The data presented in Figure 3 clearly show that the trickling filter operated successfully for both recirculation rates (0.5 and 1.0 L/min) as high d-COD removal (above 90%) was achieved for all initial d-COD concentrations tested. Figure 3 also depicts the positive effect that increasing the recirculation rate from 0.5 to 1.0 L/min had on the filter’s performance for all feed d-COD concentrations tested. Specifically, for the volumetric flow rate of 0.5 L/min and concentrations of C$_{3500}$, C$_{7500}$, C$_{9000}$ and C$_{18,000}$, percentage removals of 90.06%, 90.85%, 90.13% and 90.89% were achieved, respectively, while
for the higher volumetric flow rate of 1.0 L/min and the same initial d-COD concentrations, increased percentage removals were recorded: 94.68%, 93.72%, 93.22% and 94.17%, respectively (Figure 3).

For the volumetric flow rate of 0.5 L/min the maximum degradation rate of 256 mg d-COD/(L-h) was achieved for C<sub>18,000</sub>, while for C<sub>3500</sub>, C<sub>7500</sub> and C<sub>9000</sub>, the recorded degradation rates were 138.96, 139.45 and 148.77 mg d-COD/(L-h).

Figure 2. Operating cycles of the trickling filter under batch operating mode with a recirculation rate of 0.5 L/min for initial dissolved chemical oxygen demand (d-COD) concentration of: (a) 3500 mg/L (b) 7500 mg/L (c) 9000 mg/L and (d) 18,000 mg/L. Recorded duration of minimum stable cycles: (a) 24 h, (b) 48 h, (c) 54 h, and (d) 64 h.

For the volumetric flow rate of 1.0 L/min, the maximum degradation rate was also reported for the high feed concentration of C<sub>18,000</sub> (284.61 mg d-COD/(L-h)), while for C<sub>3500</sub>, C<sub>7500</sub> and C<sub>9000</sub> the degradation rates were 142.41, 152.32 and 155.64 mg d-COD/(L-h), i.e., higher than those observed with the volumetric flow rate of 0.5 L/min. The positive effect of the recirculation rate on d-COD removal in similar packed bed reactors was also reported by Michailides et al. [50] and Tatoulis et al. [51]. It is possible that higher recirculation provides a more favorable environment within the trickling filter by ensuring a better distribution of microorganisms, dissolved oxygen and nutrients.

A literature review reveals that the percentage removal of COD (or removal rates) achieved in this study are among the highest reported compared to previous studies where aerobic microbiological treatment processes were used in different suspended or attached systems (conventional activated sludge: up to 92.2% [48] and 50% [9], sequencing batch reactor: up to 95% [32], jet-loop-activated sludge reactor: 80%–90% [47,53], rotating biological contactor: 23%–43% [54,55], aerobic lagoons: 91% [56], fixed-bed biofilm reactor: up to 91% [14], biological sand filter: up to 98% [57], membranes: up to 97% [2,58–61]). Although the most efficient aerobic biological process appears to be membrane application (where higher COD removal rates are achieved at shorter hydraulic retention times,
and simultaneously exhibit stable operation under various organic loadings), these reactors present significant disadvantages such as high capital and operating costs, membrane fouling and difficulty in dewatering the produced waste sludge [16].

On the other hand, suspended growth systems may lead to relatively significant percentage removals, but only when they operate at optimum conditions (mainly at low feed COD concentrations). Additionally they are strongly influenced by the toxicity of some compounds naturally present in WW (e.g., polyphenols), and cannot cope with intense variations in influent loadings [3]. Attached growth systems, in turn, can be considered very effective aerobic biological processes that present various advantages (e.g., stable operation under high hydraulic and organic loadings, tolerance to toxicity effects, low hydraulic retention time) that render these systems a viable solution for small industrial plants [51].

![Figure 3. d-COD concentration in the trickling filter under batch operating mode with recirculation rates of 0.5 and 1.0 L/min for initial d-COD concentrations of: (a) 3500 mg/L (b) 7500 mg/L (c) 9000 mg/L and (d) 18,000 mg/L.](image)

The trickling filter examined in this research work provided a high biomass concentration that ensured stability and high performance (92.22%), even at the high initial organic loading of 18,000 mg d-COD/L, which is the highest initial d-COD concentration tested at attached growth systems. It should be mentioned that directly comparable values for percentage removals (removal rates) are only those derived from attached growth systems treating real WW. Specifically, Andreottola et al, [14] used
fixed-bed biofilm reactors to examine lower feed COD concentrations (7130 ± 3533 mg/L), and achieved a percentage removal of about 91% and removal rates up to about 5 Kg COD/(m²d) (corresponding to about 167 mg d-COD/(L·h)).

Ramond et al. [57] (used a biological sand filter for real WW treatment, and reported higher COD removals (98%) than those of the present work (90.1–94.7%), however the authors tested a very low influent COD concentration (2304 mg/L) and a longer retention time (112 d), leading to significant lower COD removal rates (about 20.57 mg d-COD/(L·d)).

During the experiments (for all initial d-COD concentrations and the two volumetric flow rates of 0.5 and 1.0 L/min) the pH and temperature evolution was monitored (Figure 4). Healthy pH and temperature values are key factors for optimum filter operation and microbial growth. Experiments were performed using WW diluted with tap water at five different dilution ratios, leading to different initial pH and d-COD concentrations. Since the pH of undiluted WW was between 4 and 5 (slightly acidic due to the presence of organic acids [20]), experiments with high dilution ratios presented higher initial pH values (up to 5.96).

![Figure 4](image)

Figure 4. pH and temperature variations recorded in the trickling filter under batch operating mode with recirculation rates of 0.5 and 1.0 L/min for initial d-COD concentrations of: (a) 3500 mg/L, (b) 7500 mg/L, (c) 9000 mg/L and (d) 18,000 mg/L.

It can be concluded from Figure 4 that pH values increase (up to 7.01) during biological d-COD degradation. This is consistent with the observations of Moreira et al. [37] and Lucas et al. [62], the
latter stating that “this happens due to the adaptation and type of microorganisms naturally occurring along time, which clearly prefer higher pH values”. According to Litaor et al. [63] a decrease in dissolved organic compounds is accompanied by an increase of dissolved inorganic carbon (during the oxidation of organic matter, CO$_2$ is released) that result in an increase of pH to more alkaline values.

Concerning the temperature of the treated water, this increased up to 30 °C, mainly due to the recirculation applied via the recirculation pump. Although the d-COD removal levels reported using the trickling filter were significantly high, the final effluent showed d-COD values over the permissible limits for municipal and industrial effluents (125 mg/L) (Government Gazette (GR) 2011/354B [64]). Specifically, for the highest concentration of C$_{18000}$ and the volumetric flow rate of 0.5 L/min, the effluent’s d-COD concentration was about 1500 mg/L. Therefore, a suitable post-treatment step is necessary to improve the quality of the final effluent.

### 3.2. Post-Treatment Using CW

Influent pH values of the filter-treated WW presented a mean average of 7.01 and increased slightly to 7.30 during the post-treatment process in both CW units after the gravel layer (2/3 of the unit’s length). This increase suggests further biological degradation and the adaptation of specific microorganisms, as described above. The pH values were observed to remain stable at 7.30 after the zeolite layer. Influent EC values presented a mean average of 433 µS/cm, and were observed to increase during treatment in the CW units to 814 µS/cm after the gravel layer, and to 930 µS/cm after the zeolite layer, most likely due to wastewater concentration caused by high evapotranspiration (ET) rates [65].

Figure 5a,b present time series charts for the removal of d-COD as recorded in the CW-P and CW-U units, respectively. Concentrations at the 2/3 point of unit length and effluent points were appropriately corrected to take into account the precipitation and evapotranspiration volumes. Influent d-COD concentrations varied from 788 to 2985 mg/L as the quality of the pre-treated WW was not constant, meaning that the CWs were tested under real operating conditions. Mean d-COD removal efficiencies for the two-year operation period were 91.7% and 81.0% for the CW-P and CW-U units, respectively. As shown in Figure 5, d-COD removal efficiency predictably fluctuated during the commissioning phase, after plant biomass harvesting (end of January), and after HRT alterations [65–67]. As reported in previous studies [67–70], most of the organic load was removed within the first two thirds of the length of the pilot-units (mean removal efficiencies measured at the 2/3rd length point were 75.5% and 64.7% in CW-P and CW-U, respectively), indicating that organic matter is mainly removed in the first sector of a CW. On the other hand, additional organic matter removal was observed to occur in the zeolite layer, as final COD removal rates averaged 92% in CW-P and 82% in CW-U, thus confirming the ability of zeolite to remove organic substances [66,67,71].

![Figure 5. Time series charts for d-COD concentrations in the: (a) CW-P, and (b) CW-U unit.](image-url)
In contrast to d-COD, NH$_4^+$-N removal from the pre-treated WW was significant, as removal rates of 78% and 75% were recorded from the zeolite layers of CW-P and CW-U, respectively. In CW-P, 57% of the initial ammonium concentration was removed in the gravel layer, while in the CW-U unit this value was limited to 30% (Figure 6).

![Figure 6. Mean ammonium concentrations along the length of the CW-P and CW-U units.](image)

The relatively low removal rates observed in the gravel layer, especially in CW-U, could be attributed to the low HRT values, which were not sufficient to remove NH$_4^+$-N [68], and to the inability of the experimental units to remove nitrogen when treating effluents with high organic matter loads [67]. On the other hand, NH$_4^+$-N was sufficiently removed on the zeolite layer, mainly through adsorption onto the zeolite grains, which are known to be very efficient at ammonium removal [66,67,71–76].

3.2.1. Effect of HRT

The pilot-scale CWs were operated under two hydraulic residence times (HRTs) of 4 and 2 days. These two HRTs were selected based on previous experiments with other agro-industrial wastewaters [65,67], containing similar COD concentrations as the WW. Under 4-day HRT, mean d-COD removal rates were 91% for CW-P and 78% for CW-U, while under 2-day HRT, the mean d-COD removal rates were 92% and 82% for CW-P and CW-U, respectively. The effect of HRT was statistically assessed by applying one-way ANOVA to the data obtained under 4- and 2-day HRT operation. To exclude the temperature effect, temperature values from these two different operational periods were also tested using one-way ANOVA. In both cases, no statistically significant differences were found (p > 0.05). ANOVA results showed that COD removal rates were not significantly affected by HRT values in either unit (p > 0.05 for both units), thus confirming previous studies [65,67], which also indicate an HRT of 2 days as sufficient for organic matter removal.

3.2.2. Effects of Vegetation

Although CWs are distinguished by the macrophytes they contain, the exact contribution of plants and their effect on pollutant removal is still under discussion [65,67,68,76–78]. It is well-known that vegetation provides favorable conditions for microbial growth in the root zones of CWs, and simultaneously transfers oxygen via its roots to within the CW body [66]. To examine the effect of vegetation, a one-way ANOVA was performed. Results showed that the vegetation did not significantly affect d-COD removal (p > 0.05).

4. Conclusions

A hybrid system comprising a pilot-scale biological trickling filter and constructed wetland was designed and tested for the treatment of real winery wastewater. The main goal of the present work...
was to reduce the organic load of the specific wastewater, so that the biologically-treated effluent could be disposed into a municipal treatment plant. The trickling filter tested significantly decreased the dissolved chemical oxygen demand (d-COD) content from winery wastewater, and achieved removal efficiencies up to 94.2%, even for the maximum initial feed concentration of 18,000 mg/L. The maximum loading was about 6.5 Kg COD/(m^3·d), and is relatively high compared to other more expensive bioreactors. The removal of remaining organics was accomplished using horizontal subsurface flow constructed wetland as a final polishing step. The horizontal subsurface flow constructed wetland units managed to remove the remaining organic load, as final dissolved chemical oxygen demand effluent concentrations were on average below legislation limits, showing mean removal rates of 92% and 81% for planted and unplanted constructed wetland, respectively. A hydraulic residence time of 2 days was found sufficient for organic matter removal, and the constructed wetland’s zeolite layer enhanced NH_4^+–N removal. The hybrid winery wastewater treatment system presented and tested here has the potential to effectively treat effluents originating from small wineries typical of the Mediterranean region. Additionally, both the filter and the constructed wetland present low capital/operating costs and operational simplicity.

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