Dairy wastewater treatment in vertical flow constructed wetlands using a mixture of perlite and sponge carriers as substrate material

D. Kotsia[1], A.S. Stasinakis[1], M.S. Fountoulakis[1]
[1]Department of Environment, University of the Aegean, Mytilene, 81100, Greece,Tel. +30 6981579110/ +30 22510-36257/ +30 22510-36288, emails: envd21006@env.aegean.gr, astas@env.aegean.gr, fountoulakis@env.aegean.gr

ABSTRACT

The substrate is a critical component of constructed wetlands (CWs) for efficient removal of pollutants from wastewater, while the most used are gravel and sand mainly due to their relatively low cost. However, these materials sometimes fail to treat heavily polluted wastewater because of low sorption capacity and low specific surface area. The goal of this study was the investigation of an alternative substrate contained perlite and sponge biocarriers for dairy wastewater treatment in vertical flow constructed wetlands (VFCWs). The experiment was carried out at the outdoor research facility of the University of the Aegean in Mytilene, Greece. Two VFCWs were constructed using modified cylindrical plastic The VFCWs were filled with two layers of materials: a drainage layer (15 cm) filled with coarse gravel (20-40 mm) and a filter layer (60 cm) filled a) with fine gravel (named VFCW1) or b) with a mixture of perlite and sponge cubic form carriers (named VFCW2). The halophytic plant *Atriplex halimus* was used as wetlands vegetation in both cases. Results shown that the use of perlite and sponge carriers significantly improved the overall removal performance of VFCWs in comparison with fine gravel. The average COD concentration in the effluents of VFCW1 and VFCW2 was found 352.0 and 173.6 mg/L, respectively. Similar, the average turbidity removal in the system filled with perlite and sponge carriers was found 96 % while the average turbidity removal in the system filled with fine gravel was found 79 %.

Keywords: vertical flow constructed wetlands, dairy wastewater, sponge carriers as substrate material

1. INTRODUCTION

The dairy industry is a major value in the food industry, as it is one of the most consumed products in the world. In addition to their huge demand, the production of dairy products constitutes one of the driving sectors in the agricultural economy. Although it plays an important role in the economy, their production represents one of the largest sources of industrial wastewater.

During the daily production process, the dairy industry produces large volumes of wastewaters, which are characterized by high organic matter content (chemical oxygen demand (COD) ranging from 80 to 95 000mg/L) (Sultana et al., 2016), high concentrations total suspended solids (TSS), total nitrogen (TN) and total phosphorus (TP). The effects that the dairy wastewater created by liquid waste from cheese dairies cause serious impacts on the environment, but mainly appear more serious in water, where they cause deterioration of water quality, reduction of dissolved oxygen and, with the presence of high concentrations of phosphorus and nitrogen eutrophication, resulting in the deterioration or even destruction of the entire ecosystem (Carvalho et al., 2013). The basic regulations with which cheese factories must comply focus on avoiding environmental pollution and conserving natural resources (Schierano et al., 2020).

The Wastewater from the dairy production is a source of different pollutants, resulting in high energy and economic requirements for its treatment. One possible management practice for the treatment of dairy wastewater that has been used in recent years is CWs (Healey et al., 2007). CWs are innovative, low-cost and low-energy practices for managing dairy by-products (Tunçsiper et al., 2015). They are designed and constructed to use the processes that take place in plants and infill substrates as well as microbial communities to treat wastewater (Gorra et al., 2014). The infill substrates help to develop different microbial processes that contribute as catalysts for the removal of organic and inorganic components in wastewater (Gorra et al., 2014) while plants have been reported as one of the most important factors in the efficient removal of contaminants in CWs.

This technology is already being used successfully for the processing of cheese products in many countries such as Italy, Canada, Ireland, Japan, Australia, etc. (Schierano et al., 2018). Different types of CWs have been considered in the past, such as free water surface wetlands, constructed horizontal sub-surface flow wetlands (HSSF) and constructed vertical flow wetlands (VFCW). In all cases, substrates used as porous media are crucial for effective removal of pollutants from inputs, as most physical, chemical and biological reactions take place on substrates (Wang et al., 2020).

The typical substrates used in VFCW are sand and gravel. Gravel is a common substrate for constructed wetlands because it aids in the settling of suspended solids and provides surfaces for biofilm formation and ion exchange (Tanner et al., 1998) Another advantage of utilizing gravel as a substrate is that, because of the vast volume it takes up in these systems, an interstitial void area of 30 to 45% allows plant roots to develop between the gaps and provides more attachment sites for microorganisms to digest nutrients(Tanner et al., 1998). Although, the use of other substrate materials could improve the ability of VFCWs to process dairy waste. For example, a previous study in Greece showed that the use of zeolite and a high-density polyethylene (HDPE) plastic material in a HSSF could reduce the required footprint by up to 75% without altering the system performance (Tatoulis et al., 2017).

The aim of this research is to evaluate the effectiveness of contaminant removal and to test for the first time the use of a perlite and sponge carriers mixture in VFCWs as a filler substrate for the treatment of real low-strength dairy wastewater effluents. Perlite is a white, ultralightweight aggregate, inorganic, neutral in pH, biologically stable. It is used as a building material, insulation material and as an auxiliary filter. The sponges cubic form carriers are lightweight, highly porous and have a large surface area, making them ideal for supporting and protecting attached microbiological growth and leading to large bacterial populations (Dang et al., 2020).

2.Materials and methods

2.1 Experimental setup

The experiment was conducted from June 2021 until April 2022 on the outdoor facilities in the Department of Environment of University of the Aegean in Mytilene, Greece. The climatic conditions in the area during the experimental period (9 months) were as follows: mean daily temperature: 17.8 °C, maximum mean daily temperature: 21.1 °C, minimum mean daily temperature: 14 °C, total rainfall: 58 mm, average wind speed: 8.8 Kt. Two VFCWs were constructed using modified cylindrical plastic containers (diameter: 0.25 m, height: 0.80 m) as shown in Figure 1. The VFCWs were filled with two layers of materials: a drainage layer (15 cm) filled with coarse gravel (20-40 mm) and a filter layer (60 cm) filled a) with fine gravel (named VFCW1) or b) with a mixture of perlite (brand name: Geoflor (Perlite Hellas S.A) and sponge cubic form carriers ((Nisshinbo Chemical Inc.) (named VFCW2). The halophytic plant *Atriplex halimus* was used as wetlands vegetation in both cases. Dairy wastewater was obtained from a local cheese factory and stored at 4 ºC prior to use. Table 1 shows the physicochemical characteristics of the influent during the experimental period. Influents and effluents were sampled weekly for a period of about 9 months. During the first 50 days (named phase A) of the experiment the systems operated receiving dairy wastewater at an HLR of 40 mm/d. In the second phase (named phase B) the HLR was reduced to 20 mm/d.

Table 1: Chemical characteristics of dairy wastewater used in the experiment.

Parameter	Dairy wastewater phase a mean ± SD (number of samples)	Dairy wastewater phase a mean ± SD (number of samples)
pH	8.4 ± 0.3 (27)	7.9 ± 0.4 (42)
EC (μS/cm)	3539 ± 252 (27)	1688 ± 235 (33)
Turbidity (NTU)	342 ± 191 (27)	113 ± 64 (33)
COD (mg/L)	894 ± 384 (22)	1033 ± 283 (42)
BOD_5 (mg/L)	247 ± 89 (5)	197 ± 49 (3)
NH_4^+-N (mg/L)	25 ± 9 (24)	34 ±11 (39)
Total-P (mg/L)	10 ± 4 (22)	23 ±13 (32)

2.2 . Chemical analyses

The samples were analyzed for TSS, COD, TP and ammonium nitrogen (NH_4^+-N) , according to APHA(APHA et al., using gravimetric (dried at 105 ^{O}C), closed reflux method, persulfate digestion method & Ascorbic acid method and Kjeldahl /titrimetric method respectively. The pH was measured using a pH-meter (C932, Consort) and the Electrical conductivity (EC) using a portable conductimeter (LF95, WTW). Turbidity was monitored using a portable turbidimeter (2100Q, Hach). Biochemical oxygen demand (BOD) was determined using the closed respirometric method (OxiTop®, WTW).

Fig. 1.: a) Schematic presentation of VFCWs used in experimental studies, b) VFCW filled with the mixture perlite and sponge cubic form carriers.

3. Results and Discussion

The pH measurement showed no significant differences in both influent and effluents of the systems. Specifically, in the first phase the pH in the influent was 8.4 ± 0.3 while in the effluent of VFCW1 and VFCW2 was 8.6 ± 0.2 and 8.6 ± 0.3, respectively. Similar, in the second phase the pH in the influent was 7.9±0.4 while in the effluent of VFCW1 and VFCW2 was 8.0 ±0.3 and 8.1 ± 0.2, respectively.

The EC in the influent during first phase of the experiment was about 3539 ± 252 μS/cm while in the effluents of VFCW1 and VFCW2 was about 3361 ± 375 μS/cm and 3444 ± 382 μS/cm. During second phase of the experiment the influent had an EC average value of 1688±235μS/cm while the effluents of VFCW1 and VFCW2 had an EC value of 1781 ± 370 μS/cm and 1422 ± 288 μS/cm, respectively. There was no significant difference in the influent compared to the effluents. The differences in the EC values at the influent were due to the different dairy wastewater used during the experiment. In contrast to the turbidity which showed significant differences in the effluents.

Specifically, in the first phase of the experiment the turbidity in the influent was 342 ± 191 NTU while the average turbidity in the effluent of VFCW1 and VFCW2 was 51 ± 32 NTU and 11 ± 7 NTU, respectively. In the second phase of the experiment the influent had a turbidity value of 113 ± 64 NTU while the output was 62 ± 26 NTU for VFCW1 and 23 ± 13 NTU for VFCW2. Turbidity showed a decreasing trend in both the first and second phase of the experiment, however, in the first phase in the summer months the turbidity removal efficiency was better (VFCW1: 79%, VFCW2: 96%), compared to the second phase of the experiment where turbidity removal efficiency for the VFCW1 system showed a decreased performance.,

3.1 Organics

The dairy wastewater used in this experiment had a COD concentration of 894 ± 384 mg/L and a BOD_5 concentration of 247 ± 89 mg/L during first phase of the experiment. In the second phase of the experiment the COD concentration in the influent was 1033 ± 283 mg/L and BOD_5 concentration was 197 ± 49 mg/L. As shown in the fig.3, the COD concentration in the effluents of both systems decreases considerably in both phases. The average COD concentration ranged from 352 ±111 mg/L to 390 ±176 mg/L in the effluent of VFCW1 and from 174 ± 60 mg/L to 228 ±125 mg/L in the effluent of VFCW2.. The average COD removal in VFCW1 was 56% and 70% in phase A and phase B, respectively. In contrast the average COD removal in VFCW2 was 79% and 83% in phase A and phase B, respectively.

As shown in Fig.2, in the first phase of the experiment the influent for the experiment was made once in large quantity and placed next to the system and fed automatically however due to the warm conditions microalgae were created in the influent which consumed the organic matter of the dairy wastewater and as a result the COD concentration in the influent was reduced. It has been shown that temperature has an important role in the removal of organic matter in CWs as it affects both microbial activity and plant function. It is known that COD removal occurs through the process of phytoremediation and is highly dependent on the microbiological degradation of pollutants in the plant root degradation of organics (Yazdani & Golestani, 2019). Warmer conditions favor the increase of biological activities and consequently in wastewater treatment. While colder conditions can affect microbial production (Sultana et al., 2016b) .

During the second phase of the experiment the decreased HLR applied seems to have helped the systems to better degrade the organic matter as they had more time to interact the wastewater with the substrates and microorganisms. It is also known that mature wetland systems provide higher water quality (Thomaidi et al., 2022).

Throughout the experiment, the VFCW2 achieved higher COD removal efficiency may be due to a) the help of the sponges carriers in increasing microbial populations and removing organic matter s from the wastewater, and b) the smaller grain size of the perlite (1-5 mm) with the sponges compared to the gravel (2-6 mm). In one hand, it is known that materials with better grain size enhance natural filtration, provide a larger surface area for microbial growth and increase the contact time between the effluent and the porous media (Jayasooriya et al., 2017). On the other hand, the use of smaller grain sizes increases the risk of clogging (Jayasooriya et al., 2017). During the 9 months of the experiment there was no visible blockage of the systems.

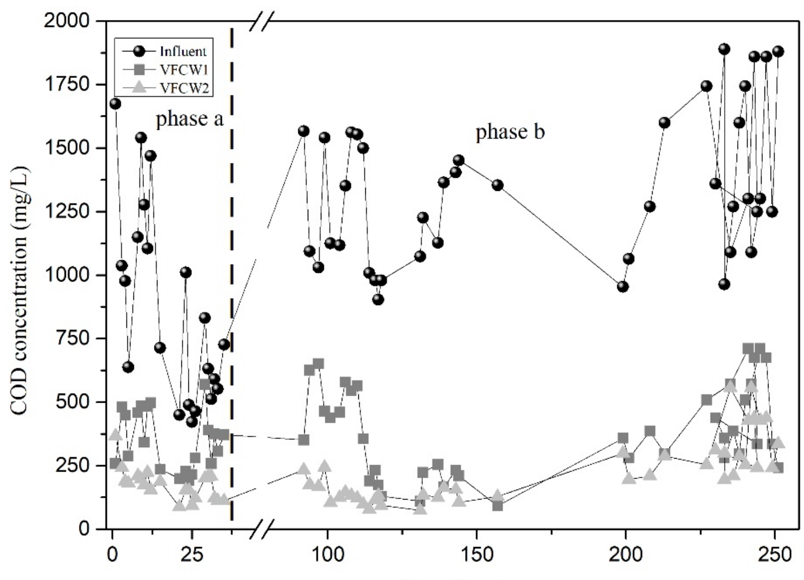

Fig.2: COD concentrations in the influent and the effluents of the VFCWs during the experimental period.

3.2 Ammonium nitrogen

The NH_4^+-N removal from the systems was satisfactory (Figure 3.); there were differences in the inlet and outlet of the systems. Removal rates were higher in the VFCW2 system in both the first phase of the experiment and the second phase. The removal of ammonia compounds from the systems depends on the following mechanisms (a) uptake by plants (2) biomass uptake by microorganisms and (3) adsorption by the filters used. (4) nitrification in aerobic microzones near the roots and (5) evaporation as NH3 is favoured by high PH (Schierano et al., 2018). The dairy wastewater used in this experiment had an NH_4^+-N concentration of 25 ± 9 mg/L in the first phase of the experiment, while in the second phase of the experiment it was 34 ± 11 mg/L. The average NH_4^+-N concentration in VFCW1 system was 7 ± 4 mg/L in both phases. The average NH_4^+-N concentration in the effluent of VFCW2 system was 5 ± 1 mg/L, and 3 ± 2 mg/L, in phase A and phase B, respectively. The removal efficiency in the systems were ranged from 69% to 75 % in VFCW1 and from 83% to 89% in VFCW2

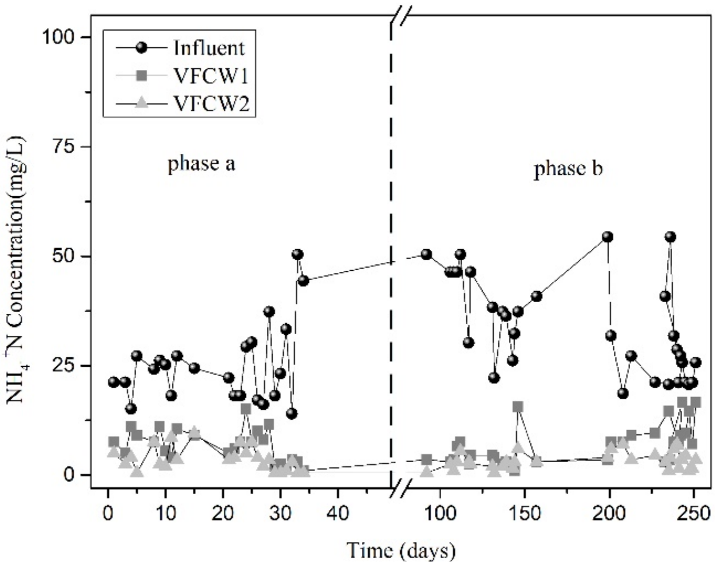

Fig. 3: NH_4^+-N concentrations in the influent and the effluents of the VFCWs during the experimental period

3.3 Hydraulic loading rate (HLR)

To evaluate the impact of HLR on pollutant removal, two different cases were tested. The concentration of all pollutants at the outlet of the two systems (except conductivity and pH) decreased with reduction of HLR (Fig. 4). The longer residence time of the waste in the system helped the systems to treat the wastewater.

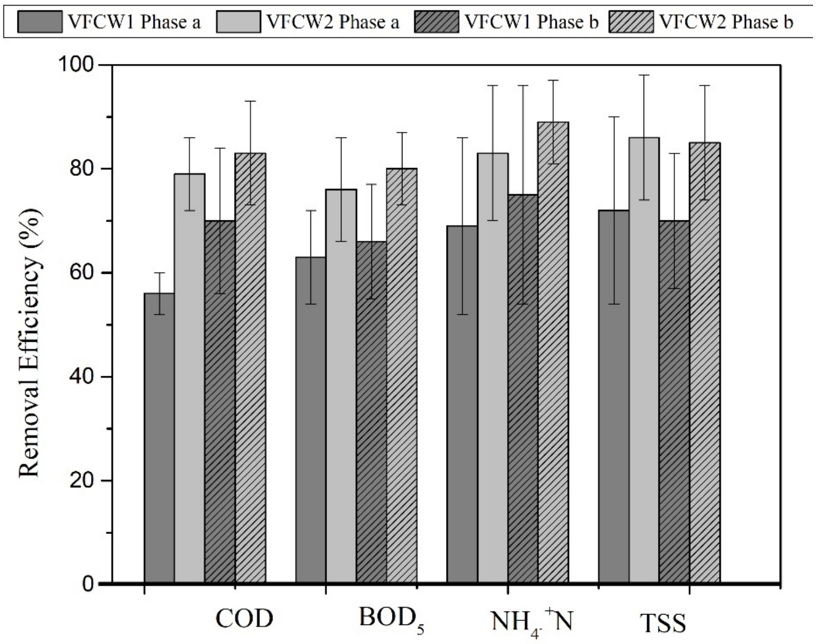

Fig.4: Average removal of COD, BOD, TSS, and N-NH4 in VFCWs during the experimental period. (Mean values and standard deviations were calculated from day 0 to day 251.

The application of lower HLR had as a result the decrease of Hydraulic retention time (HRT). HRT is essentially the time during which the pollutants are in contact with the substrate and plant rhizosphere and is a critical control factor in determining the effectiveness of contaminant removal (Stottmeister et al., 2003). In general, a long HRT allows for significant interaction between pollutants and wastewater, whereas a high HLR (or low HRT) permits wastewater to travel quickly to the outlet, limiting contact time between wastewater, rhizosphere, and microorganisms (Wang et al., 2017).

Table 2: Effluent quality of the VFCWs during the operation.

Parameter	Dairy wastewater (mean ± SD) Phase A		Dairy wastewater (mean ± SD) Phase B	
	VFCW1	VFCW2	VFCW1	VFCW2
pH	8.6 ± 0.2 (n=27)	8.6 ± 0.3 (n=27)	8.0 ± 0.3 (n=42)	8.1 ± 0.2 (n=42)
COD (mg/L)	352 ± 111 (n=22)	174 ± 60 (n=22)	390 ± 176173 (n=42)	228± 125 (n=42)
BOD (mg/l)	113.5 ± 10.5 (n=5)	26 ± 10 (n=5)	116 ± 4 (n=3)	33 ± 2.5 (n=3)
Turbidity (NTU)	51 ± 12 (n=27)	11 ± 2 (n=27)	61.5 ± 16.5 (n=27)	23 ± 3 (n=27)
NH_4^-N (mg/L)	7 ± 4 (n=24)	4 ± 2 (n=24)	7 ± 4 (n=39)	4 ± 2 (n=39)
Total-P (mg/L)	7.7 ± 2.5 (n=22)	5.0 ± 1.4 (n=22)	21.1± 2.4 (n=32)	16.8± 1.7 (n=32)
EC (μS/cm)	3361 ± 375 (n=27)	3444 ± 370 (n=27)	1781 ± 382 (n=33)	1422 ± 288 (n=33)

4. **Conclusions**

The objective of this study was to evaluate the effectiveness of contaminant removal and to test for the first time the use of a mixture of perlite and sponge cubic form carriers in a VFCWs as a filling substrate for the treatment of real low-strength dairy wastewater. Results shown that the use of VFCWs for dairy wastewater treatment was effective as they achieved significantly lower effluent quality in terms of physical (turbidity, total suspended solids) and chemical (organic matter, nitrogen, phosphorus) characteristics in comparison with the influent. The application of lower HLR had as a result a slightly increase of organic matter and nutrient removal in both examined systems. The system filled with the mixture of perlite and sponge cubic form carriers gave better performance in terms of pollutant's removal as their presence probably enhanced the development of microbial communities. In addition, perlite seems to promote sorption as a mechanism for the removal of nutrients from dairy wastewater in comparison with gravel.

Acknowledgements
We acknowledge support of this work by the project "Center of Sustainable and Circular Bioeconomy [Aegean_BIOECONOMY]" (MIS 5045851) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

Reference

1. APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA), AmericanWater Works Association (AWWA), and Water Environment Federation (WEF) (2005).
2. Sultana, M. Y. *et al.* Effect of hydraulic retention time, temperature, and organic load on a horizontal subsurface flow constructed wetland treating cheese whey wastewater. *Journal of Chemical Technology and Biotechnology* 91, 726–732 (2016).
3. Carvalho, F., Prazeres, A. R. & Rivas, J. Cheese whey wastewater: Characterization and treatment. *Science of the Total Environment* vols. 445–446 385–396 (2013).
4. Schierano, M. C., Panigatti, M. C., Maine, M. A., Griffa, C. A. & Boglione, R. Horizontal subsurface flow constructed wetland for tertiary treatment of dairy wastewater: Removal efficiencies and plant uptake. *Journal of Environmental Management* 272, (2020).
5. Tunçsiper, B., Drizo, A. & Twohig, E. Constructed wetlands as a potential management practice for cold climate dairy effluent treatment - VT, USA. *Catena (Amst)* 135, 184–192 (2015).
6. Gorra, R., Freppaz, M., Zanini, E. & Scalenghe, R. Mountain dairy wastewater treatment with the use of a "irregularly shaped" constructed wetland (Aosta Valley, Italy). *Ecological Engineering* 73, 176–183 (2014).
7. Schierano, M. C., Panigatti, M. C. & Maine, M. A. Horizontal subsurface flow constructed wetlands for tertiary treatment of dairy wastewater. *International Journal of Phytoremediation* 20, 895–900 (2018).
8. Tanner, C. C., Sukias, J. P. S. & Upsdell, M. P. *ORGANIC MATTER ACCUMULATION DURING MATURATION OF GRAVEL-BED CONSTRUCTED WETLANDS TREATING FARM DAIRY WASTEWATERS.*
9. Dang, H. T. T. *et al.* Loofah sponges as bio-carriers in a pilot-scale integrated fixed-film activated sludge system for municipal wastewater treatment. *Sustainability (Switzerland)* 12, (2020).
10. Sultana, M. Y. *et al.* Effect of hydraulic retention time, temperature, and organic load on a horizontal subsurface flow constructed wetland treating cheese whey wastewater. *Journal of Chemical Technology and Biotechnology* 91, 726–732 (2016).
11. Thomaidi, V., Petousi, I., Kotsia, D., Kalogerakis, N. & Fountoulakis, M. S. Use of green roofs for greywater treatment: Role of substrate, depth, plants, and recirculation. *Science of the Total Environment* 807, (2022).
12. Jayasooriya, V. M., Ng, A. W. M., Muthukumaran, S. & Perera, B. J. C. Green infrastructure practices for improvement of urban air quality. *Urban Forestry and Urban Greening* 21, 34–47 (2017).
13. Wang, M., Zhang, D. Q., Dong, J. W. & Tan, S. K. Constructed wetlands for wastewater treatment in cold climate — A review. *Journal of Environmental Sciences (China)* vol. 57 293–311 (2017).