Journal of Applied Life Sciences and Environment
https://jurnalalse.com

USV 1842
Article
https://doi.org/10.46909/alse-564113
Vol. 56, Issue 4 (196) / 2023: 507-526

HORIZONTAL FLOW PILOT CONSTRUCTED WETLAND FOR DAIRY WASTEWATER PURIFICATION

Merima TOROMANOVIĆ[1*], Jasmina IBRAHIMPAŠIĆ[1] and Tibela LANDEKA DRAGIČEVIĆ[2]

[1]Biotechnical Faculty, University of Bihać, Bihać, Bosnia and Herzegovina;
e-mail: jasmina.ibrahimpasic@unbi.ba
[2]Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia;
e-mail: tlandekadragicevic@pbf.hr

*Correspondence: merima.toromanovic@unbi.ba

Received: Oct. 18, 2023. Revised: Dec. 06, 2023. Accepted: Dec. 11, 2023. Published online: Jan. 10, 2024

ABSTRACT. Due to the natural energy sources used in constructed wetlands, minimal energy and chemicals are required for wastewater treatment. For the successful wastewater treatment via constructed wetlands, adequate pretreatment and a sufficient amount of oxygen are crucial. Constructed wetlands can be used to treat industrial wastewater, such as wastewater from the dairy industry, with prior degreasing. This study was conducted on a horizontal flow pilot constructed wetland located on a plot of land used by the Biotechnical Faculty in Bihać. The constructed wetland consisted of two fields planted with rushes (*Typha latifolia*) and reeds (*Phragmites australis*). The substrate and plants were not changed during the experimental period. We monitored the efficiency of industrial wastewater treatment (wastewater from the dairy industry) by season, varying the flow rate as well as the hydraulic retention time of the water in the device. The quality of the influent and effluent was monitored by determining analytical parameters. The constructed wetland showed the highest efficiency in the summer period, with a hydraulic retention time of 6 days, with removal efficiencies of 98.03% for ammonia, 98.19% for total nitrogen, 95.27% for total phosphorous, 94.50% for COD and 97.73% for BOD_5. The organic substance removal efficiency across all four seasons was 94.68%.

Keywords: constructed wetland; dairy wastewater; hydraulic retention time; organic matter.

INTRODUCTION

Constructed wetlands (CWs) comprise a suite of recognised ecotechnologies that are designed and

Cite: Toromanović, M.; Ibrahimpašić, J.; Landeka Dragičević, T. Horizontal flow pilot constructed wetland for dairy wastewater purification. *Journal of Applied Life Sciences and Environment* **2023**, 56 (4), 507-526. https://doi.org/10.46909/alse-564113

constructed to mimic and manipulate the simultaneous physical, chemical, and biological processes occurring in natural wetlands. They can effectively remove dissolved organics and suspended solids (Gajewska *et al.*, 2020). Globally, they have been accepted as cost-effective, particularly for small communities (Shruthi and Shivashankara, 2022).

The removal of pollutants from wastewater using constructed wetlands is a complex process and depends on various mechanisms that take place during the process, including sedimentation, filtration, precipitation, evaporation, absorption, as well as various microbial processes (Wu *et al.*, 2014).

Constructed wetlands are systems that mainly consist of specific vegetation, substrate, soil, microorganisms and water, using complex processes that include physical, chemical and biological mechanisms to remove various pollutants or to improve water quality (Wua *et al.*, 2015). In constructed wetlands, microorganisms play a major role in the removal of pollutants (Faulwetter *et al.*, 2009; Stottmeister *et al.*, 2003).

Depending on the used plant species, a diverse efficiency of nutrient absorption is achieved. Plant root growth within the substrate helps in the decomposition of organic matter and prevents clogging. Plants serve as a habitat and source of oxygen for microorganisms, and the most frequently used ones include reeds (*Phragmites australis*), cattails (*Typha latifolia*), erect hedgehog (*Sparganium erectum*), common sedge (*Scirpus lacustris*), yellow iris (*Iris pseudacorus*) and sedges (*Carex sp.*) (Wu *et al.*, 2014). For the constructed wetland to successfully carry out wastewater treatmen, adequate pretreatment and a sufficient amount of oxygen must be ensured.

The dairy industry is one of the main sources of industrial wastewater in Europe (Demirel *et al.*, 2005). This industry is based on the production and processing of raw milk into products such as yogurt, ice cream, butter, cheese, and various types of sweets using various procedures such as pasteurisation, coagulation, filtration, centrifugation and cooling (Rivas *et al.*, 2010). Effluents from these factories are a source of different pollutants, resulting in not only economic losses to the industry (due to raw material wasted during the manufacturing process and treatment costs), but also damage to the environment. For each liter of milk processed in the dairy industry, 1–5 L of wastewater are generated (Schierano *et al.*, 2020).

The characteristics of wastewater from the dairy industry can vary significantly, depending on the final product and the methods used in production. This wastewater is a mixture of animal urine, spilled milk, floor and utensil washings, traces of animal dung, among others. It consists of small to medium levels of organic matter, phosphorous and nitrogen, responsible for the eutrophication of the receiving water bodies and the degradation of soil quality (Minakshi *et al.*, 2022).

Although constructed wetlands can be used for the treatment of wastewater from the dairy industry, preliminary degreasing is necessary (Comino *et al.*, 2011), or even degreasing via dilution with municipal wastewater (Farnet *et al.*, 2008).

This paper presents the results of a study conducted on the pilot plant constructed wetland located on the plot of the Biotechnical Faculty in Bihać, which was used for the purification of dairy wastewater.

Wastewater was sampled accordance with the standard norms for the sampling of wastewater, established by domestic legislation in this area. In recent years, the amount of wastewater generated as a result of human activities has increased because of improved living standards, in addition to industrialisation and urbanisation.

Although developed countries appropriately treat their wastewater, in developing countries such as Bosnia and Herzegovina, large amounts of untreated wastewater are still discharged into natural recipients, which affects the quality of the environment. As a result, the population faces various environmental and health problems, including water-borne diseases. Large amounts of organic matter in waterbodies can cause a process called eutrophication. This process is linked to an increase in aquatic plants and algae, called phytoplankton, reducing the amount of oxygen available in the water and making it difficult for fish and other aquatic organisms to survive. In eutrophication, the environment produces more organic matter than it is capable of consuming.

The decomposition of this organic matter, in turn, will intensify, reducing the amount of oxygen in the water and producing methane gas (CH_4) and hydrogen sulphide (or hydrogen sulphide, H_2S). Wastewater exposure has also been linked to viral, bacterial and protozoan diseases such as salmonellosis, shigellosis, cholera, giardiasis, amoebiasis, hepatitis A, viral enteritis and other diarrheal diseases (WHO, 2006).

One of the reasons for the construction of this pilot plant constructed wetland was also that its application would reduce the amount of wastewater discharged into the natural recipient. In this context, the aim of this research was to monitor the optimal parameters that affect the purification of dairy wastewater (flow rate, hydraulic retention time in the device, seasons) and to determine the efficiency of purification using a pilot plant constructed wetland. To obtain relevant data, it was necessary to monitor and analyse the physical and chemical parameters of the wastewater, such as pH value, ammonia, total nitrogen, phosphorus, COD and BOD.

This research serves as the basis for choosing the type and pollution degree of the wastewater that will later be treated at this constructed wetland. Also, this work aims to promote the application of constructed wetlands as an economically acceptable and efficient technology for wastewater treatment.

MATERIALS AND METHODS

This study was carried out on a pilot plant constructed wetland with a horizontal water flow and an area of 20 m^2, located on the plot that is used by the Biotechnical Faculty.

The pilot plant constructed wetland was composed of two fields, a filtering field and a cleaning field. The fields contained substrate (sand and gravel) of different fractions, with particle sizes from 0.2–36 mm, in different compositions. Cattail (*Typha latifolia*) was planted in the first field and reeds (*Phragmites australis*) in the second, with a density of at least 7 plants/m^2. After the constructed wetland, an additional research shaft was installed, which served to remove other pollutants, such as phosphorous, from the water, using substrates such as zeolites or calcites. The choice of substrate depends on the type of pollution.

We used wastewater from the dairy company "Milk-San" Sanski Most (Bosnia and Herzegovina); fat and oils were previously removed. Pretreatment, i.e., the removal of fats and oils, was done within the wastewater treatment device owned by the "Milk-San" dairy company, using the floating technology. Before the water could enter the constructed wetland, it needed to remain in the precipitation device, which enabled a 70% reduction in the TSS (total suspended solids). Wastewater sampling and analysis were carried out in accordance with domestic legislation.

During the planned research period, the flow rate varied, as well as the hydraulic retention time of the water in the device. The method for determining the flow rate is usually chosen depending on the conditions in which the flow is measured and the required accuracy.

In this case, a volumetric method was used to measure the flow rate of water. The inflow was regulated manually, whereas the outflow was regulated mechanically, depending on the planned HRT in the device. The water inflow into the constructed wetland (influent) at two different flow rates (higher and lower) was marked with I1a and I1b in the results. The retention time of the industrial wastewater in the device was 4, 5 and 6 days during all four seasons.

Given that the industrial wastewater was loaded with organic substances, as well as compounds with nitrogen and phosphorus, it was necessary to choose an extended water retention time in the device to achieve the best possible results that would be in accordance with the legal regulations (OGFBH, 2020). The effluent, depending on the flow rate and water retention time (4, 5 and 6 days), is represented in the graphs by E1a, E2a, E3a and E1b, E2b, E3b.

The study was conducted by seasons: in spring, summer, autumn and winter, with the aim to determine the season with the highest purification efficiency.

The stream Drobinica, which is approximately 10 m away from the location, received the purified wastewater.

The quality of the influent and effluent was monitored by determining analytical parameters according to the standard APHA methods (APHA, 2017) and ISO standards.

Analysis of the efficiency of industrial wastewater treatment using a horizontal flow pilot constructed wetland was performed using the non-parametric Kruskal–Wallis test (Kruskal and Wallis, 1952) at the significance level of 0.05 due to violation of the assumption of homogeneity of variances and deviations of residuals from normal distribution.

Table 1 – Parameters and analytical methods of water quality testing (OGFBH, 2007)

Parameter	Description of the method	Test method
pH value	Electrochemical, *in situ*	BAS EN ISO 10523:2013
Ammonia	Spectrophotometric	BAS ISO 7150-1:2002
Total nitrogen	Spectrophotometric	Analysis according to an internally developed method on the Spectrophotometer photoLab® 6600 UV-VIS and according to the instructions for the instrument and Merck kit Spectroquant 1.14763
Phosphorus	Spectrophotometric	BAS EN ISO 6878:2006
COD	Spectrophotometric	BAS ISO 6060:2000
BOD	Dilution method, incubation for 5 days at 20°C	BAS ISO 5815-1:2004

RESULTS AND DISCUSSION

This chapter shows some of the quality parameters of industrial wastewater at the inflow and outflow of the pilot plant constructed wetland during all four seasons, with hydraulic retention times of 4, 5 and 6 days and with flow adjustment. The aim was testing the efficiency of the purification of industrial wastewater using this horizontal flow constructed wetland.

The pH affects the biological activities occurring during the treatment in wetlands and potentially influences nutrient removal. Nitrifying bacteria are highly sensitive to pH, and the genus *Nitrosomonas* has an optimal pH between approximately 7.0 and 8.0. The optimum pH range for *Nitrobacterium* is approximately 7.5 to 8.0. Higher pH levels (greater than 9) may reduce nitrification, and low pH values can completely stop plant growth (Yazdani and Golestani, 2019).

The pH value in the influent during all four seasons ranged from 6.4 to 7.6. There were minor variations in the pH value, which are associated with the type of product, the production plan and the composition of the wastewater that is generated. The pH value of the effluent at a hydraulic retention time of 6 days for all four seasons ranged from 6.8 to 7.6. The official regulation (OGFBH, 2020) recommends a threshold pH value for industrial wastewater to be discharged into surface water bodies after treatment. Given that according to this regulation, the threshold pH value is in the range from 6.5 to 9, the pH values for all effluent samples obtained in this study met these requirements. The selected plant species, the water flow, the hydraulic retention time of 6 days and the seasons positively impacted the maintenance of the optimal pH value in the purified wastewater. The constructed wetland also controlled the pH within an acceptable range.

The results obtained for the pH values are in the range of those obtained by other authors. For example, Nyaki and Njau (2016) reported pH values of dairy wastewater ranging from 7.03 to 7.075. In a study conducted by Mantovi *et al.* (2003), the average pH value of dairy wastewater was 7.82. Yazdani and Golestani (2019) also conducted research on a constructed wetland with dairy wastewater. *Phragmites australis* and *Juncaeae* spp. were planted in the constructed wetland, and the study was conducted in the summer period. The pH remained in range of 7–9 during the treatment period, which is the optimum range for this purpose. In the research conducted by Drisya and Ashokan (2021) with a horizontal flow constructed wetland for the purification of the dairy wastewater, the pH in the influent was 6.85. The hydraulic retention times of the water in the device were 2, 4 and 6 days, and the average pH values for those days were 6.77, 6.63 and 6.49, respectively.

The most common processes for ammonia and nitrogen elimination in a horizontal flow built wetland are nitrification/ denitrification and plant/ microbial absorption. However, the availability of oxygen is critical to these activities. If the oxygen levels are low, ammonium nitrification is limited. The concentrations of ammonia and total nitrogen in the dairy wastewater used in this study were extremely high and exceeded the maximum allowed concentrations (MACs) established by the regulation (OGFBH, 2020) during all four seasons. *Figure 1*, *Figure 2*, *Figure 3* and *Figure 4* show the average values of ammonia, total nitrogen, and phosphorus in the influent in spring, summer, autumn and winter, as well as their concentrations in the effluent at hydraulic retention times of 4, 5 and 6 days in the pilot plant constructed wetland at two different flow rates.

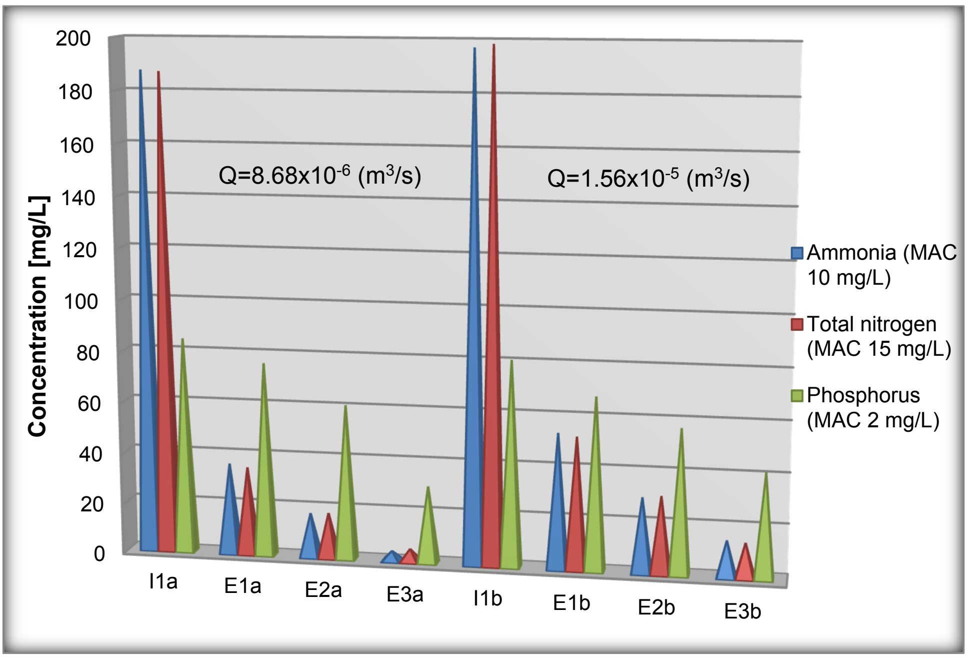

Figure 1 – Concentrations of ammonia, total nitrogen and phosphorus in the wastewater of the dairy industry (influent and effluent), depending on the flow and organic load, in the autumn of 2018 [mg/L]

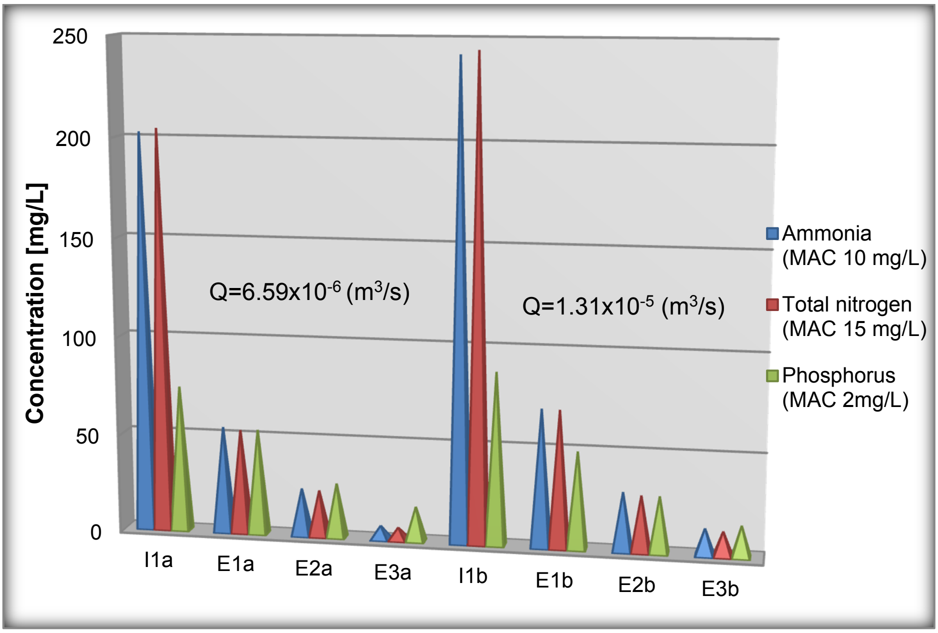

Figure 2 – Concentrations of ammonia, total nitrogen and phosphorus in the wastewater of the dairy industry (influent and effluent), depending on the flow and organic load, in the winter of 2018/2019 [mg/L]

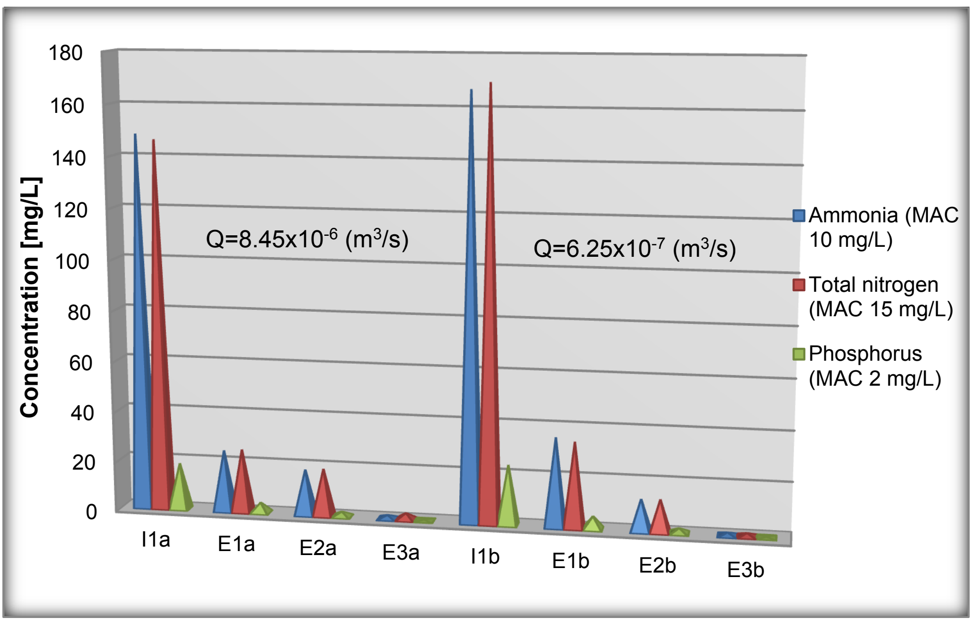

Figure 3 – Concentrations of ammonia, total nitrogen and phosphorus in the wastewater of the dairy industry (influent and effluent), depending on the flow and organic load, in the spring of 2019 [mg/L]

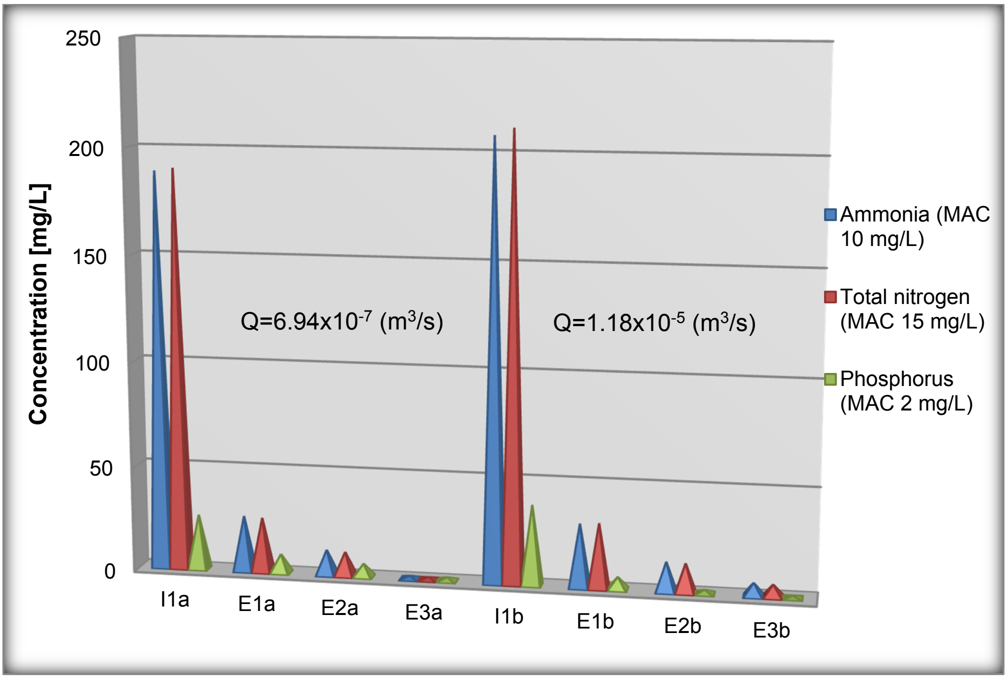

Figure 4 – Concentrations of ammonia, total nitrogen and phosphorus in the wastewater of the dairy industry (influent and effluent), depending on the flow and organic load, in the summer of 2019 [mg/L]

Legend: I1a and I1b – Influent on the first day (samples 1 and 2); E1a and E1b – Effluent on the fourth day; E2a and E2b – Effluent on the fifth day; E3a and E3b – Effluent on the sixth day (for samples 1 and 2)

The ammonia removal efficiency was higher in spring and summer, with higher temperatures and a lusher vegetation. In autumn and winter, the ammonia removal efficiency was slightly lower but still high. It varied depending on the flow rate and was generally higher at lower flow rates. The total nitrogen removal efficiency was also higher in spring and summer. Higher temperature may improve microbial activities, thereby increasing the nitrogen removal efficiency. The seasonal variation can be explained by the higher efficiency during the summer, which might in part be related to the better oxic conditions as more oxygen is released by the plant roots, promoting nitrification.

The concentrations of ammonia and total nitrogen in the effluent after an HRT of 6 days, during all four seasons, was in accordance with the legal regulations and did not exceed the MACs (10 mg/L for ammonia and 15 mg/L for total nitrogen). The only exceptions are the ammonia concentrations in autumn and winter at higher flow rates, which were slightly above the MAC (14.7 mg/L in autumn and 13.8 mg/L in winter). The efficiency of removing compounds with nitrogen is greater in the warmer seasons due to the higher activity in the root zone, which supports the decomposition of organic substances because the availability of oxygen is greater around the plant roots. A higher removal efficiency is achieved with longer water retention times in the device, with the maximum values at an HRT of 6 days.

Dairy wastewater and cleaning agents are the most common sources of phosphorous in water (Wang *et al.*, 2006). In the present study, the concentration of phosphorus in the wastewater of the “Milk-San” Sanski Most dairy company varied, but during all four seasons, it exceeded the MAC (OGFBH, 2020) (*Figure 1*, *Figure 2*, *Figure 3* and *Figure 4*). The phosphorus concentration in dairy wastewater was significantly higher in autumn and winter and lower in spring and summer, when the settling time before taking samples for initial analysis and passing through the device was prolonged. The highest phosphorus concentration in the influent in the autumn/winter period was 87.8 mg/L, whereas in the spring/summer period, it was 38.7 mg/L. The phosphorus removal efficiency was also higher in spring and summer. In all spring and summer effluent samples, the phosphorus concentration did not exceed the MAC (2 mg/L). In autumn and winter, when the phosphorus removal efficiency was lower, the phosphorus concentration in all effluent samples exceeded the MAC. The highest phosphorus concentration in the effluent was measured in autumn, with 41.9 mg/L. These results indicate that during the treatment of wastewater with a high concentration of phosphorus, especially in the autumn/winter period, a substrate for removing phosphorus (zeolites, calcites) must be used. In this case, the efficiency was higher in the warmer seasons, even with a lower flow rate. Total phosphorus and phosphates are mostly removed from wastewater by being absorbed by the plant and by adsorption on porous substrates (Kadlec and Knight, 1996). The efficacy of phosphorus removal is affected by a number of parameters, including the age of the created wetland, the adsorption qualities of the substrate, the gradual filling of sorption sites over time, and the presence of undecomposed plant material near the substrate surface. This efficiency appears to be high when the plants are young, the root length density per unit of substrate volume is low, and substrate adsorption is strong.

Comparing our findings with those of other studies, both similarities and differences were found. Constructed wetlands are highly effective in reducing nutrient concentrations in dairy wastewater (Hunt and Poach, 2000). In a survey of several constructed wetlands for the treatment of wastewater from the dairy industry in the United States, Hunt and Poach (2000) found that, although there was considerable variability among sites, all constructed wetlands could effectively remove nutrients, with removal efficiencies for total nitrogen and total phosphorus of 86% and 83%, respectively.

In the state of Maryland, a dairy wastewater treatment system was highly efficient in its first 4 years of operation, with a total nitrogen removal efficiency of 98% and a total phosphorus removal efficiency of 96% (Schaafsma *et al.*, 2000). Newman *et al.* (2000) found that in a Connecticut dairy wastewater treatment plant, the total nitrogen removal efficiency was 53% and the total phosphorus removal efficiency

68% during the first 3 years of operation.

A study on the treatment of wastewater from a dairy industry in New Zealand examined the effects of the nutrient loading rate on nitrogen and phosphorus removal efficiency. Tanner *et al.* (1995) determined that the total nitrogen removal ranged between 48% to 75% and the total phosphorus removal between 37% and 74%, at high and low loading rates, i.e., in the constructed wetland during the first 2 years of operation. In a study conducted by Crolla and Kinsley (2002) in Ontario, Canada, a constructed wetland with two plant species (*Typha latifolia* and *T. angustifolia*) was used to treat wastewater from the dairy industry. During the 4-year experimental period, the removal efficiency for total nitrogen was 72%, whereas that for total phosphorus was 58%.

Licata *et al.* (2021) used a constructed wetland for the purification of dairy wastewater in Italy. Tests were conducted in the 2 years from 2019 to 2020 on a horizontal flow constructed wetland. The two units were separately planted with giant reed *(Arundo donax L.*) and umbrella sedge (*Cyperus alternifolius L.*). Total ammonia removal in the first and second year was 45.05% and 51.51%, respectively. Total phosphorus removal in the first and second year was 39.86% and 38.88%, respectively.

Shruthi and Shivashankara (2022) also tested the effects of HRT and seasons on the performance of a pilot-scale horizontal subsurface flow constructed wetland. The authors used HRTs of 2,4,6,8 and 10 days, in winter, summer and the rainy seasons. With ammonia and nitrogen removal, the HRT plays a significant role. For ammonia, the result was significant within only 4 to 6 days. The nitrogen removal efficiency increased significantly for every increase in HRT from 2 to 4 days, 4 to 6 days and 6 to 8 days. The results showed that nitrogenous organic matter requires longer HRTs than carbonaceous matter. Total phosphorus showed a significant difference only when the HRT increased from 4 to 6 days ($p = 0.05$). The corresponding highest efficiency obtained for 8 days was 47.06%. Shruthi and Shivashankara (2022) also obtained higher ammonia, nitrogen and phosphorus removal efficiencies in the summer season. For ammonia and nitrogen, the removal efficiencies in winter were up to 59.39% and 52.69%, whereas in summer, they reached 64.23% and 55.44%. The values obtained for the rainy season were 59.89% and 52.90%, respectively. Phosphorus removal was also highest in summer, with up to 52.40% and 50.65%, followed by 50.21% and 48.23% in the rainy season and 48.50% and 48.70% in winter, respectively.

The presence of nutrients in high concentrations in water can cause excess weed and algae growth (Scheffer and Van-Nes, 2007). Regarding ammonia, total nitrogen, and phosphorus, several studies have investigated the removal of these compounds from dairy wastewater using constructed wetlands, and different results have been obtained. Whilst some authors obtained lower removal efficiencies, others reported removal efficiencies above 95%. These values depend on the vegetation, the composition of the wastewater, the flow rate and the HRT.

Better removal efficiencies for compounds with nitrogen and phosphorus in this research were achieved at a lower flow rate and at an HRT of 6 days. By increasing the HRT, better results can be achieved.

Given the high concentration of organic substances in dairy wastewater, they are some of the most significant indicators of the removal efficiency. In this study, the concentrations of organic substances in the influent and effluent were monitored through the COD and BOD_5. Regarding HRTs of 4, 5 and 6 days (at higher and lower flow rates for all days), these parameters were determined in the effluent for all mentioned days to monitor the efficiency of the decomposition of organic matter depending on the HRT. The MACs for COD and BOD_5 have been established by the Regulation (OGFBH, 2020).

The lowest COD value in the influent was measured in spring, with 842 mg/L and the highest one in summer, with 3,051 mg/L. The lowest BOD_5 value in the influent was also measured in spring, with 414 mg/L, and the highest in summer, with 1,240 mg/L.

Figure 6, *Figure 7*, *Figure 8* and *Figure 9* show that the best purification efficiency, expressed as COD, was achieved with an increase in the HRT in the device. The highest efficiency, i.e., the lowest concentration of organic substances in treated wastewater, was obtained at an HRT of 6 days, irrespective of the season. The BOD_5 values also decreased depending on the HRT. The highest organic matter removal efficiency, expressed as BOD_5, was also obtained for an HRT of 6 days, irrespective of the season. The COD and BOD_5 removal efficiencies were higher at a lower water inflow into the device. For water to be discharged into surface water bodies, the MAC for COD is 125 mg/L, whereas that for BOD_5 is 25mg/L. The COD values in all effluent samples, during all four seasons and for an HRT of 6 days, did not exceed the MAC. However, the BOD_5 values, in some cases, slightly exceeded the MAC, with the highest BOD_5 value in the effluent for an HRT of 6 days being measured in spring (31 mg/L). Given the initial high concentration of organic matter in the dairy wastewater, an extended HRT in the device, by at least 1 more day, would result in a BOD_5 value below the MAC.

Regarding the season as a factor affecting the efficiency of the purification of dairy wastewater using a horizontal flow pilot plant constructed wetland, it had a significant effect ($p \leq 0.05$) (*Figure 10*). Removal efficiency, expressed as COD, was highest in summer and autumn. According to the results of the Mann–Whitney U test (Mann and Whitney, 1947), the removal efficiency in summer and autumn was statistically significantly different compared to that in winter and spring ($p \leq 0.05$). No statistically significant differences were found in the efficiency of organic matter removal between winter and spring ($p > 0.05$).

Organic matter can be efficiently removed using horizontal constructed wetlands. Since purification processes take place in the soil, the efficiency of wastewater purification using constructed wetlands is high even in winter.

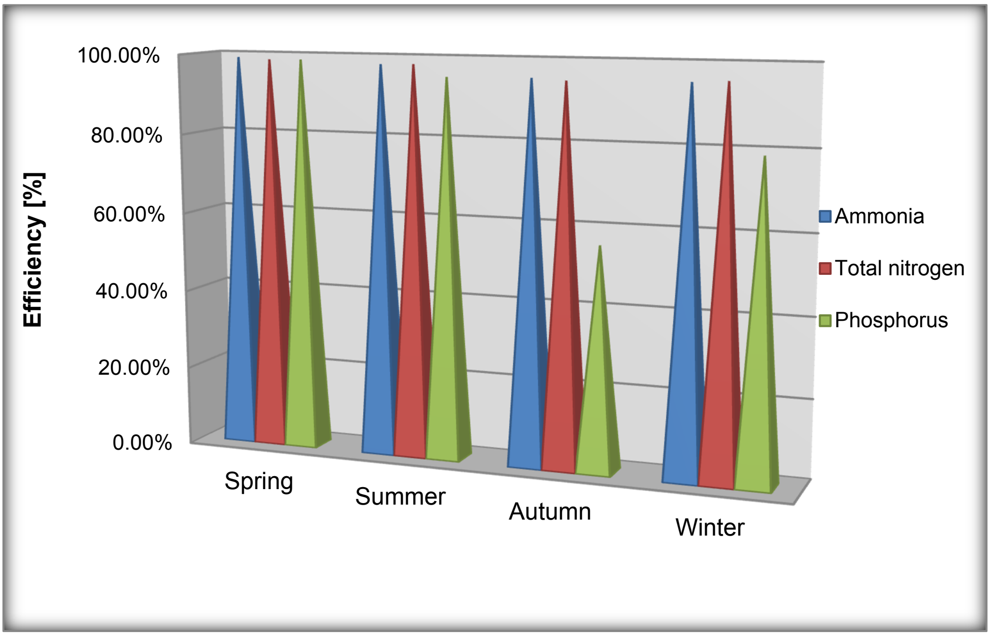

Figure 5 – Total removal efficiencies for ammonia, total nitrogen and phosphorus from dairy wastewater after a hydraulic retention time (HRT) of 6 days for all seasons

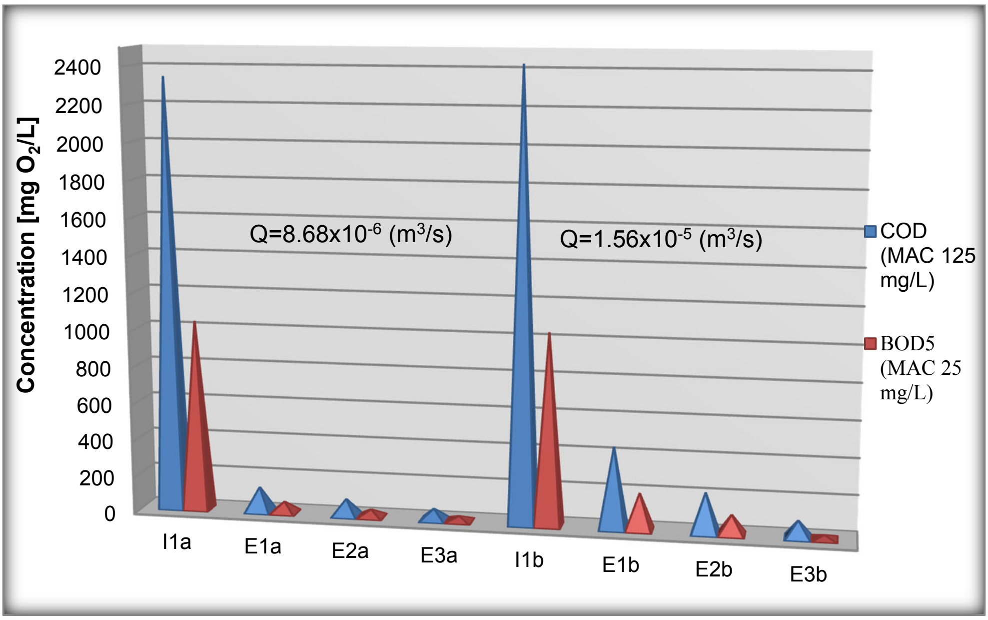

Figure 6 – Contents of organic substances expressed as COD and BOD5 in the wastewater of the dairy industry (influent and effluent), depending on the flow rate and the organic load, in the autumn of 2018 [mg/L]

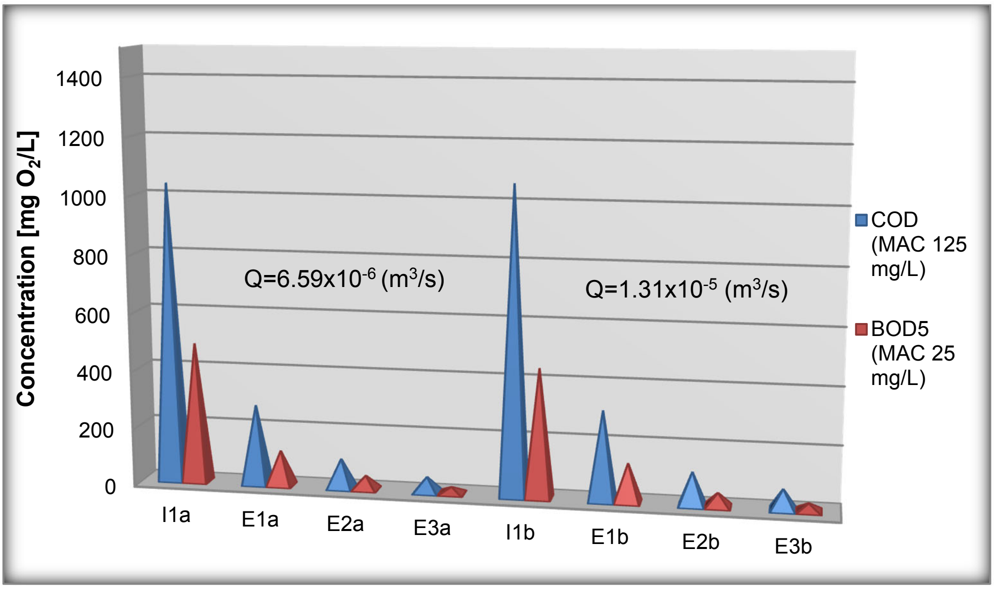

Figure 7 – Contents of organic substances expressed as COD and BOD5 in the wastewater of the dairy industry (influent and effluent), depending on the flow rate and the organic load, in the winter of 2018/2019 [mg/L]

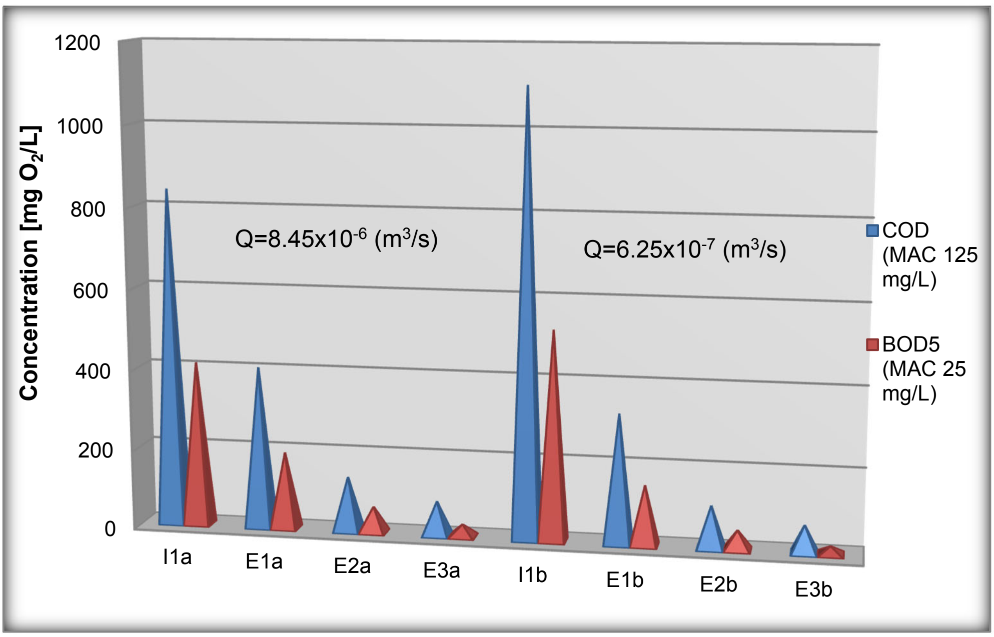

Figure 8 – Contents of organic substances expressed as COD and BOD5 in the wastewater of the dairy industry (influent and effluent), depending on the flow rate and the organic load, in the spring of 2019 [mg/L]

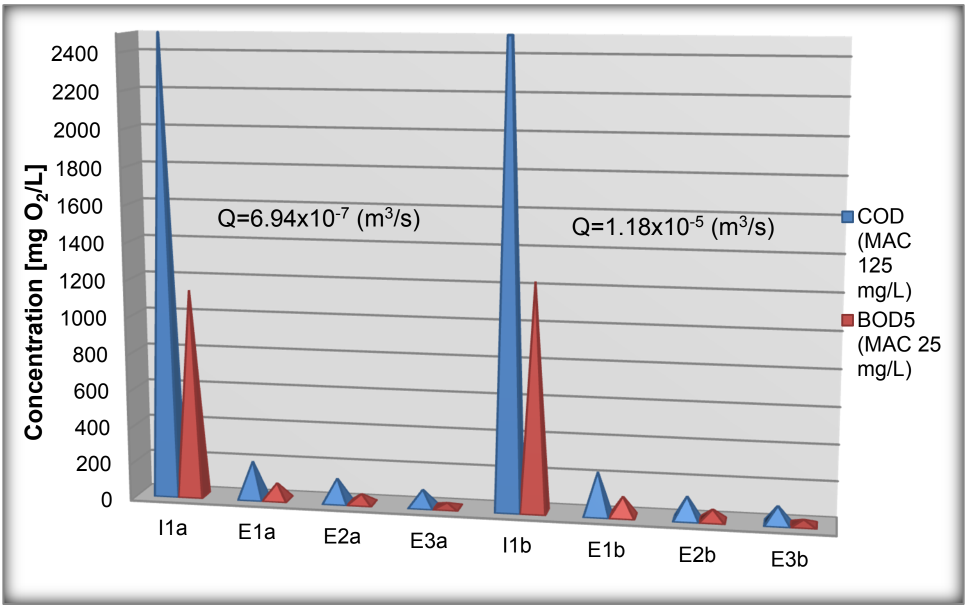

Figure 9 – Contents of organic substances expressed as COD and BOD5 in the wastewater of the dairy industry (influent and effluent), depending on the flow rate and the organic load, in the summer of 2019 [mg/L]

Legend: I1a and I1b – Influent on the first day (samples 1 and 2); E1a and E1b – Effluent on the fourth day; E2a and E2b – Effluent on the fifth day; E3a and E3b – Effluent on the sixth day (for samples 1 and 2)

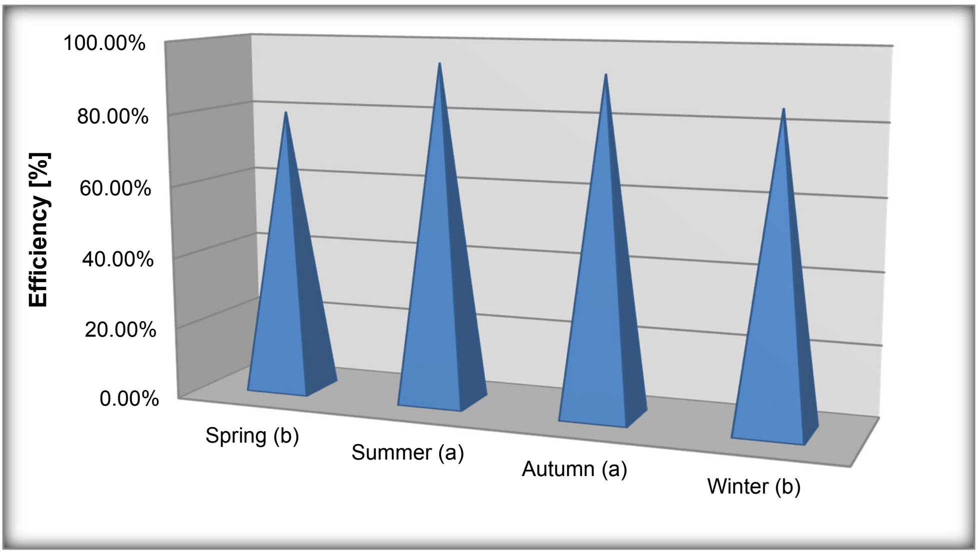

Figure 10 – Efficiency of wastewater treatment of the dairy industry using a constructed wetland by season in 2018/2019, expressed via the COD [%]

*Average values marked with the same letter are not significantly different according to the Mann–Whitney U test at the P ≤ 0.05 level

In summer, the efficiency is higher and reaches 90%–99%. Previous research has shown that aerobic and anaerobic bacterial activity play an important role in the degradation of carbonaceous organic materials; the bacteria involved in this process can survive and function to a temperature of 5°C. Based on the results of the present study, higher air temperatures are more suitable for organic matter removal. Regarding the influence of the seasons on the efficiency of organic matter removal, similar results were obtained by other authors. Shruthi and Shivashankara (2022), investigating a horizontal constructed wetland, reported COD and BOD removal efficiencies in the summer season of 88.34% and 94.93% respectively, whereas in winter, the values were 86.41% and 92.66%; in the rainy season, 87.23% and 93.32% were obtained.

The use of constructed wetlands for the treatment of dairy wastewater is a viable alternative depending on the amount of household wastewater, washing water, and whey produced. Farnet *et al.* (2009) reported that the COD removal efficiency in undiluted dairy wastewater was 76%. Pretreatment of dairy wastewater with anaerobic biodegradation can increase COD removal to 94% (Travis *et al.*, 2012).

Nyaki and Njau (2016) analysed wastewater from the milk processing industry and reported COD and BOD_5 values of 1,945 and 975 mg/L, respectively, in agreement with the findings of the present study.

Mantovi *et al.* (2003) investigated a horizontal constructed wetland with subsurface water flow, which was used for the treatment of dairy wastewater. Their experimental period was 2 years, and reeds (*Phragmites australis*) were planted in the two-phase plant device. The water flow in the device was 6.3 m^3/day, with an HRT of 3 days. The efficiency of the removal of organic substances, expressed as COD and BOD_5, was always above 90%.

In a study performed in the west of Greece, a horizontal constructed wetland, planted with *Phragmites australis*, was used for the treatment of wastewater from the milk processing industry. The experimental period was 2 years, and in the first year, the HRT was 4 days, whereas in the second year, it was 2 days. The COD value in the influent samples ranged from 120 to 6,135 mg/L. The efficiency of the removal of organic substances, expressed through the COD, was 83% throughout the study; the HRT did not significantly affect the efficiency of the constructed wetland (Akratos *et al.*, 2018).

Yazdani and Golestani (2019), treating dairy industrial wastewater using constructed wetlands, obtained excellent results regarding the efficiency of removing organic matter. In their research, the COD decreased from 2,100 mg/L to below 200 mg/L. The mean COD reduction values reported for *Phragmites australis* and *Juncaeae* were 93.62% and 92.33%, respectively.

In a study by Licata *et al.* (2021), the BOD_5 and COD values differed significantly between the influent and the effluent. The BOD_5 removal efficiency varied from 78.02% to 75.61% and the COD removal efficiency ranged from 62.67% to 61.12%.

Similar results were also obtained by Drisya and Ashokan (2021) for a horizontal flow constructed wetland for the purification of the dairy wastewater. After an HRT of 6 days, the COD removal efficiency was 69.44% and the BOD removal efficiency was 86.4%.

Our findings are in agreement with previously reported COD and BOD_5 removal efficiencies. The constructed wetland used in our study (*Figure 11*) could effectively remove organic substances during all four seasons (with and without vegetation) due to variations in water inflow, HRT and organic load, along with the selection of the specific plant species.

Table 2 – Differences between the COD removal efficiencies among the different seasons – results of the Mann–Whitney U test

Seasons	Spring	Summer	Autumn	Winter
Spring	0	0.005075	0.004998	0.0927
Summer	0.03045	0	1	0.03045
Autumn	0.02999	0.9361	0	0.02999
Winter	0.5562	0.005075	0.004998	0

Hi: 18.09; P: 0.0004205

Table 3 – Descriptive statistics for the overall efficiency of wastewater treatment of the dairy industry using a constructed wetland during all four seasons

Coefficients	Sample (dairy wastewater)
N	24
Min. value	89.43
Max. value	97.32
Sum	2,272.4
Mean	94.68333
Standard error	0.5112841
Variance	6.273875
Standard deviation	2.504771
Coefficients of variation (%)	2.645419

Figure 11 – Constructed wetland in the autumn of 2018 and the summer of 2019

At the end of each season, the aboveground part of the plant material from the constructed wetland was also analysed. We did not detect high concentrations of toxic compounds in the plant leaves, and therefore, in autumn, the plants were mown to enable the growth of new saplings in the spring. The precipitator located in front of the constructed wetland is emptied once a year by the utility company. The constructed wetland fits completely into the natural environment, and there are no issues with odour or insects.

CONCLUSIONS

Constructed wetlands with macrophytes in horizontal subsurface flow can be a good alternative for dairy wastewater treatment. The amount of wastewater discharged directly into natural recipients can be reduced by recycling and reusing the treated wastewater. The following conclusions were obtained in this study:

- The substrate, in this case, sand and gravel of different granulations (4–8 mm, 8–16 mm and 16–32 mm), as well as the selection of plant species (*Phragmites australis* and *Typha latifolia*), had a favourable effect on the efficiency of the plant device. The significance of vegetation in elimination processes was critical because plants influence microorganism activity through the release of oxygen in the root zone.

- The pilot plant constructed wetland can operate efficiently at different flow rates. By regulating the water inflow, optimal results can be obtained, complying with the standards and legal regulations.

- Due to the high concentration of phosphorus in dairy wastewater, especially during the winter period, it is recommended to use a substrate to remove the remaining phosphorus.

- Regarding the season as a factor affecting the efficiency of wastewater treatment, it had a significant ($p \leq 0.05$) effect on the purification efficiency, which was highest in summer, with 94.50%.

- Due to the high content of organic substances, but also phosphorus and compounds with nitrogen in the dairy wastewater, it is recommended to prolong the retention time in the device so that the physical and chemical parameters in the effluent are in accordance with the legal regulations. For dairy wastewater, the best efficiency was achieved for an HRT for 6 days and at a lower flow rate.

- The application of constructed wetlands for the treatment of different wastewater types, as a method of eco-remediation, represents an economically acceptable technology characterized by low costs and high efficiency.

Author Contributions: Conceptualisation, methodology, analysis, investigation, resources, data curation, writing, editing, MT, JI, TLD; review, supervision, TLD. All authors declare that they have read and approved the publication of the manuscript in the present form.

Funding: There was no external funding for this study.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

Akratos, C.S.; van Oirschot, D.; Tekerlekopoulou, A.G.; Vayenas, D.V.; Stefanakis, A. *Dairy Wastewater Treatment with Constructed Wetlands: Experiences from Belgium, the Netherlands and Greece. Constructed Wetlands for Industrial Wastewater Treatment*, First Edition. Edited by Alexandros Stefanakis. Published by John Wiley & Sons Ltd, 2018.

APHA. Standard Methods for the Examination of Wastewater and Wastewater Treatment. 23. Edition American Public Health Association. American Water Works Association and Water Environment Federation, Washington, D.C. 2017.

Comino, E.; Riggio, V.; Rosso, M. Mountain cheese factory wastewater treatment with the use of a hybrid con-struted wetland. *Ecological Engineering*. **2011**, 37, 1673-1680. https://doi.org/10.1016/j.ecoleng.2011.06.048.

Crolla, A.M.; Kinsley, C.B. Use of kinetic models to evaluate the performance of a free water surface con-structed wetland treating farmstead runoff. In: Proceedings of the 8th International Conference on Wetland Sys-tems for Water Pollution Control, Arusha, Tanzania, 2002.

Demirel, B.; Yenigun, O.; Onay, T.T. Anaerobic treatment of dairy wastewaters: a review. *Process Biochemistry*. **2005**, 40, 2583-2595. https://doi.org/10.1016/j.procbio.2004.12.015.

Drisya, C.G.; Varsha, A. Horizontal flow constructed wetland and macrophytes for waste water treatment. *International Research Journal of Engineering and Technology* (IRJET). **2021**, 08, 4028-4033.

Farnet, A.M.; Prudent, P.; Ziarelli, F.; Domeizel, M.; Gros, R. Solid state 13CNMR to assess organic matter transformation in a subsurface wetland under cheese-dairy farm effluents. *Bioresource Technology*. **2009**, 100, 4899-4902. https://doi.org/10.1016/j.biortech.2009.05.007.

Farnet, A.M; Prudent, P.; Cigna, M.; Gros, R. Soil microbial activities in a construted soil reed-bed under cheese-dairy farm effluents. *Bioresource Technology*. **2008**, 99, 6198-626. https://doi.org/10.1016/j.biortech.2007.12.026

Faulwetter, J.L.; Gagnon, V.; Sundberg, C.; Chazarenc, F.; Burr, M.D.; Brisson, J.; Campera, A.K.; Stein, O.R. Microbial processes influencing performance of treatment wetlands: a review. *Ecological Engineering*. **2009**, 35, 987-1004. https://doi.org/10.1016/j.ecoleng.2008.12.030.

Gajewska, M.; Skrzypiec, K.; Jóźwiakowski, K.; Mucha, Z.; Wójcik, W.; Karczmarczyk, A.; Bugajski, P. Kinetics of pollutants removal in vertical and horizontal flow constructed wetlands in temperate climate. *Science of the Total Environment*. **2020**, 718. https://doi.org/10.1016/j.scitotenv.2020.137371.

Hunt, P.G.; Poach, M.E. State of the art for animal wastewater treatment in constructed wetlands. *Water Science and Technology*. **2001**, 44, 19-25. https://doi.org/10.2166/wst.2001.0805.

Kadlec, R.H.; Knight, R.L. *Treatment Wetlands*. First Edition, CRC Press: Boca Raton, Florida, 1996.

Kruskal, H.W.; Wallis, W.A. Use of Ranks in One-Criterion Variance Analysis. *Journal of the American Statistical Association*. **1952**, 47, 583-621.

Licata, M.; Ruggeri, R.; Iacuzzi, N.; Virga, G.; Farruggia, D.; Rossini, F. Tuttolomondo, T. Treatment of Combined Dairy and Domestic Wastewater with Constructed Wetland System in Sicily (Italy). Pollutant Removal Efficiency and Effect of Vegetation. *Water*. **2021**, 13. https://doi.org/10.3390/w13081086.

Mann, H.B.; Whitney, D.R. On a test whether one of two random variables is stochastically larger than the other. *The Annals of Mathematical Statistics*. **1947**, 18, 50-60.

Mantovi, P.; Marmiroli, M.; Maestri, E.; Tagliavini, S.; Piccinini, S.; Marmiroli, N. Application of a horizontal subsurface flow constructed wetland on treatment of dairy parlor wastewater. *Bioresource Technology*. **2003**, 88, 85-94. https://doi.org/10.1016/S0960-8524(02)00291-2.

Minakshi, D.; Kumar Sharma, P.; Rani, A. Effect of filter media and hydraulic retention time on the per-formance of vertical constructed wetland system treating dairy farm wastewater. *Environmental Engineer-ing Research*. **2022**, 27. https://doi.org/10.4491/eer.2020.436.

Newman, J.M.; Clausen, J.C.; Neafsey, J.A. Seasonal performance of a wetland constructed to process dairy milkhouse wastewater in Connecticut. *Ecological Engineering*. **2000**, 14, 181-198. https://doi.org/10.1016/S0925-8574(99)00028-2.

Nyaki, A.; Njau, K. Assessment of dairy wastewater treatment and its potential for biogas production at Tanga fresh limited. *Tanzania Journal of Science*. **2016**, 42.

OGFBH. Parameters and analytical methods of water quality testing (in *Bosnian*). Official Gazette of the Federation of Bosnia and Her-zegovina, No 50/07, 2007.

OGFBH. Regulation on conditions and discharge of waste water into the environment and public sewage systems (in *Bosnian*). Official Gazette of the Federation of Bosnia and Herzegovina, No. 26/20, 2020.

Rivas, J.; Prazeres, AR; Carvalho, F.; Beltrán, F. Treatment of cheese whey wastewater: combined coagulation–flocculation and aerobic biodegradation.. *Journal of Agricultural and Food Chemistry*. **2010**, 58, 7871-7877. https://doi.org/10.1021/jf100602j.

Schaafsma, J.A.; Baldwin A.H.; Streb, C.A. An evaluation of a constructed wetland to treat wastewater from a diary farm in Maryland, USA. *Ecological Engineering*. **2000**, 14, 199-206. https://doi.org/10.1016/S0925-8574(99)00029-4.

Scheffer, M.; Van-Nes, E.H. Shallow lakes theory revisited: various alternative regimes driven by climate, nutrients, depth and lake size. *Hydrobiologia*. **2007**, 584, 455-466. https://doi.org/10.1007/s10750-007-0616-7.

Schierano, C.M.; Panigatti, M.C.; Maine, A.M.; Griffa, A.C.; Boglione, R. Horizontal subsurface flow con-structed wetland for tertiary treatment of dairy wastewater: Removal efficiencies and plant uptake. *Journal of Environmental Management*. **2020**, 272. https://doi.org/10.1016/j.jenvman.2020.111094.

Shruthi, R.; Shivashankara, G.P. Effect of HRT and seasons on the performance of pilot-scale hori-zontal subsurface flow constructed wetland to treat rural wastewater. *Water Practice & Technology*. **2022**, 17, 445-455. https://doi.org/10.2166/wpt.2021.104.

Stottmeister, U.; Wießner, A.; Kuschk, P.; Kappelmeyer, U.; Kastner, M.; Bederski, O.; Muller, R.A.;

Moormann, H. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. *Biotechnology Ad-vances*. **2003**, 22, 93-117. https://doi.org/10.1016/j.biotechadv.2003.08.010.

Tanner, C.C.; Clayton, J.S.; Upsdell, M.P. Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands—II. Removal of nitrogen and phosphorus. *Water Research*. **1995**, 29, 27-34. https://doi.org/10.1016/0043-1354(94)00140-3.

Travis, M.J.; Weisbrod, N.; Gross, A. Decentralized wetland-based treatment of oil rich farm wastewater for reuse in an arid environment. *Ecological Engineering*. **2012**, 39, 81-89. https://doi.org/10.1016/j.ecoleng.2011.11.008.

Wang, L.K.; Hung, Y.T.; Lo, H.H.; Yapijakis, C. *Waste treatment in the process industries*, First Edition.CRC Press, London, 2005. https://doi.org/10.1201/9781420037159.

WHO. (World Health Organization). Guidelines for the Safe Use of Wastewater, Excreta and Greywater. 2006, 2: Wastewater use in Agriculture.

Wu, S.; Kuschk, P.; Brix, H.; Vymazal, J.; Dong, R. Development of constructed wetlands in performance in-tensifications for wastewater treatment: A nitrogen and organic matter targeted review. *Water research*. **2014**, 57, 40-55. https://doi.org/10.1016/j.watres.2014.03.020.

Wua, H.; Zhang, J.; Ngo; H.H.; Guo, W.; Hub, Z.; Liang, S.; Fan, J.; Liu, H. A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation. *Bioresource Technology*. **2015**, 175, 594-601. https://doi.org/10.1016/j.biortech.2014.10.068.

Yazdani, V.; Golestani, H.A. Advanced treatment of dairy industrial wastewater using vertical flow con-structed wetlands. *Desalination and Water Treatment*. **2019**, 162, 149-155. http://dx.doi.org/10.5004/dwt.2019.24335.

Academic Editor: Dr. Isabela Maria Simion

Publisher Note: Regarding jurisdictional assertions in published maps and institutional affiliations ALSE maintain neutrality.