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Constructed wetland performance and potential for microbial removal

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ABSTRACT

Wastewater treatment plants serving low flows require special attention in many countries, where a significant part of the total population lives in small agglomerations. This may be especially relevant in interior regions, where agglomerations are generally scattered and there is no economy of scale in centralised systems, thus suggesting local and decentralized solutions. Additionally, compliance with national laws regarding wastewater discharge is often the same for smaller plants as for large systems, especially when the receiving water bodies are bathing waters or the effluent is intended to be reused for agricultural purposes. In these cases, a very important quality standard of the final effluent is the level of concentrations of pathogenic microorganisms. Two horizontal subsurface flow constructed wetland treatment plants located in the Alentejo, in the south of Portugal, were monitored over an extended period period for microbiological parameters, total suspended solids and transmittance. Removal efficiencies were observed to be consistent with values reported in the literature although an event of increasing flow, due to an annual summer festival nearby, lead to a decrease of the effluent quality. During the monitoring campaigns microorganism concentrations in the effluent did not reach significant levels, but for some uses further disinfection might be required. The application of UV disinfection to an effluent from constructed wetlands was evaluated with reference to the characteristics of the wetland system itself. The effluent of Fataca WWTP was subject to a collimated beam test in order to evaluate the response of faecal coliforms and Escherichia coli to different doses of UV radiation. Additionally, the repair rate, a phenomenon originated by photo reactivation or dark repair and associated with further multiplication of non- inactivated bacteria by previous UV irradiation, was evaluated.

Keywords: Constructed wetlands; Microbial removal; Small agglomerations; Wastewater treatment

1. Introduction

Wastewater treatment plants (WWTPs) serving low flows require special attention in many countries, where a significant part of the total population live in small agglomerations. This may be especially relevant in interior regions, where agglomerations are scattered.

The use of constructed wetlands for wastewater treatment relies on the treatment capacities of natural systems, with low or even zero energy inputs to the system, which reduces the use of electromechanical equipment. The use of natural marshes for effluent treatment dates back to the beginning of the 20th century [1], and nowadays it is well established that constructed wetlands provide a biological treatment level consistent with most legislative requirements. Research regarding constructed wetlands started in the 50's, with the work of Kate Seidel [2], but only later did the microbial removal capacity of these systems start to be studied more intensively [3– 11].

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In Portugal, the extensive coastline along with its Mediterranean-type climate allowed the development of the tourist industry. This industry is mostly concentrated close to bathing areas (including inland areas), and, therefore the concentration of pathogenic microorganisms or corresponding indicators is a very important quality standard of the final effluent discharging to those areas.

The reduction in the concentration of indicator organisms such as total and faecal coliforms, Enterococcus or *Escherichia coli* may be a relevant aspect of effluent quality even in small agglomerations, since the compliance with national laws demanded for small wastewater treatment plants is often the same as for larger systems, especially when the receiving water bodies are bathing waters or the effluent is intended to be reused for agricultural purposes.

When tertiary treatment for microbial removal is required due to the uses of the receiving waters and when there is no more space economically available for maturation ponds, the application of ultra-violet radiation (UV) has often been considered a viable technology for microbiological wastewater disinfection. UV efficiency depends on the quality of the wastewater, namely its transmittance, and also on the UV dose, which can be calculated from the average intensity and the time of exposure. This situation is becoming usual in countries like Portugal.

The use of UV radiation for the disinfection of the effluent from a constructed wetland system may be evaluated considering the characteristics of the system itself, which are seldom analyzed and reported.

This paper presents the results of sampling campaigns performed in two horizontal subsurface flow constructed wetlands WWTPs located in the Alentejo, in the south of Portugal, from June to September 2005 and from May 2007 to January 2008. Microbiological parameters were tested on a weekly basis, in order to assess constructed wetland performance regarding bacterial removal and to promote discussion as to whether the concentration reached in the effluent might allow reuse for different purposes.

The suitability of the effluent for UV disinfection was addressed, through the analysis of total suspended solids (TSS) and transmittance. Additionally, a collimated beam test was performed to determine the response of faecal coliforms and *Escherichia coli* to different doses of UV radiation.

2. Materials and methods

2.1. Location and characteristics of the study sites

The WWTPs of Fataca and Malavado are located in the south of Portugal, near the city of Odemira (latitude 37°35′ N, longitude 8°38′W), at an altitude of 35 m and 70 m, respectively. The climate in the region is Mediterranean, with a mean annual temperature of 15°C and mean annual precipitation of 600 mm. The occupation is mainly individual houses with few commercial establishments, and the surrounding areas are rural, agriculture being one of the main activities.

Each plant is a full-scale WWTP serving a small population: Fataca WWTP was designed to serve 200 inhabitants and Malavado WWTP was designed to serve 350 inhabitants. Nowadays there are approximately 80 inhabitants in Fataca and 100 in Malavado, thus the two WWTPs are operating below their full capacity.

Each plant has a grid chamber followed by a septic tank and one horizontal subsurface flow constructed wetland. Fataca wetland bed has a surface area of 400 m² and Malavado wetland bed has a surface area of 690 m². Each wetland is 0.7 m deep (mean value) with an average slope of 0.005 m/m. Both CW are planted with *Phragmites australis,* with the peak growth being reached usually between June and July, and presenting a total height over 2 m.

2.2. Flow measurement and evapotranspiration rates

Flows through the WWTPs were monitored in three sections: before the septic tank, after the septic tank (flow entering the constructed wetland) and after the constructed wetland (final effluent from the WWTPs). Flow measurements were performed with an ultrasound transducer (PULSAR Oracle 3.0) associated with a V-notch weir, and data was recorded every 5 min in a data logger. The average flow entering each constructed wetland for the two sampling periods is presented in Table 1.

During the summer of 2005, from 3 to 7 of August, both WWTPs received additional flows from a summer festival nearby, which gives rise to a significant temporary population. The total additional volume discharged to Fataca WWTP during the festival was about 20 m³, being 48 m³ for Malavado WWTP. In 2007, only Malavado treatment plant received additional flows from the summer festival, between 2 and 5 of August, with a total additional volume discharged of about 64 m³. These discharges were delivered once or twice a day, causing a peak inflow of around 3 l/s at the entrance of the WWTP.

Evapotranspiration rates were estimated from the difference between the total volume entering the wetland

Table 1

Average daily flow of Fataca and Malavado constructed wetlands

CW	Average flow (m ³ /d)		
	2005	2007	
Fataca	5.9	6.5	
Malavado	7.1	7.6	

and the total volume leaving the wetland, on a monthly basis. Precipitation was recorded in a weather station located 10 km from the WWTPs, and days with precipitation were excluded from the evapotranspiration calculation, due to the uncertainty of the exact intensity of each rainy period in the WWTP areas. The evapotranspiration values where only calculated for Fataca constructed wetland (CW), and ranged from 10 mm/d in July to 1 mm/d in December, resulting in a flow reduction through the beds between 30% and 50% during the summer months. Zero flow discharges were observed at least during some periods, which reflects the high intensity of the evapotranspiration rate.

2.3. Sampling and analysis

Grab samples were collected upstream and downstream of each constructed wetland, once a week, from July to September 2005 and from May 2007 to January 2008. Interruptions occurred during the month of October 2007 with only one sample being collected. Regular sampling was resumed in the middle of November 2007. Sampling was also interrupted between the middle of December 2007 and the middle of January 2008.

The parameters analyzed included total suspended solids (TSS), total coliforms (TC), faecal coliforms (FC) and Enterococcus (Ent.). Escherichia coli was also analysed in 2007 and 2008, showing similar concentrations as faecal coliforms for both influent and effluent samples in almost all the samples, which is a characteristic of domestic wastewaters. All analyses were performed according to Standard Methods [12]. Transmittance of the effluent was measured with a spectrophotometer with UV reading for a wavelength of 254 nm.

Seasonal differences were analyzed by verifying the normality of the variables to support the use of parametric or nonparametric tests, by means of the Mann-Whitney U test (p < 0.05), using the statistical package SPSS 14.0.

2.4. Collimated beam test

UV dose-response curves were obtained by performing a collimated beam test in the laboratory. The equipment (UV Trojan System) is presented in Fig. 1, and consists of a mercury low vapor pressure lamp emitting UV radiation mainly at 254 nm, mounted over a collimating tube (Fig. 1a). A Petri dish containing a magnetic stirring bar and 50 milliliters of the sample to be tested was placed on a magnetic stirrer under the collimated beam lamp (Fig. 1b). Incident radiation intensity was measured at different points, before and after the exposure using a radiometer (International Light Model 1700). An example of the UV radiation intensity distribution is presented in Fig. 2. The average intensity in the suspension was calculated by measuring the UV absorbance of the suspension at 254 nm and the intensity profile at which the solution was exposed.

Microorganisms were exposed to UV radiation with an incident intensity of approximately 0.1 mW/cm². The dose was calculated as the product of the average intensity with the exposure time. The irradiation time required to obtain the predetermined dose was calculated according to Eq. (1):

$$D = I \cdot t \tag{1}$$

where D is the dose (mWs/cm²), I is the incident radiation intensity (mW/cm^2) and *t* is the exposure time (s). Different time exposures were selected in order to obtain different doses, with the time exposure being controlled by using an opaque shutter placed between the end of the collimator and the Petri dish.

Before the collimated beam test, part of the total sample was analyzed for the concentration of faecal coliforms and Escherichia coli. After the exposure to UV radiation, the tested sample was divided in two and put into sterilized recipients. One of the recipients was opaque and taken immediately to the laboratory to be analyzed for the concentration of faecal coliforms and







Fig. 1. Photos of the collimated beam equipment: a) global view; b) Petri dish under the collimated beam tube.



Fig. 2. Measured UV radiation intensity distribution.

Escherichia coli and the other was exposed to one hour of sunlight before the microbiological analysis.

3. Results and discussion

3.1. Fataca constructed wetland

Fig. 3 presents the results for total coliforms, faecal coliforms and Entecococus concentrations obtained in Fataca constructed wetland (CW). The results for *Escherichia coli* are very close to those of the faecal coliforms, and, therefore are not displayed.

Concentrations of TC and FC in wastewater entering the wetland were in the order of 10⁶ CFU/100 ml, while Enterococcus were present in lower concentrations, in the order of 10³ CFU/100 ml, with concentrations in 2007 and 2008 slightly higher than in 2005. Enterococcus concentrations also presented a larger variation than TC and FC. Average concentrations in the effluent from the CW were in the range of 10³–10⁴ CFU/100 ml for TC and FC, while Enterococcus showed an increase in the effluent



Fig. 3. Microorganism concentrations (TC, FC and Ent.) in Fataca CW.

concentrations in the 2007/2008 campaigns compared to that of 2005. In fact, all samples collected in 2005 where compliant with the "Excellent Quality" level defined in the new EU Bathing Water Directive 2006/07/EEC for Enterococcus, in respect to inland sites (<200 CFU/100 ml) as well as for costal and transition waters (< 100 CFU/ 100 ml). In 2007 during 19 of the 25 weeks sampled, Enterococos concentrations were below 400 mg/l, which is the limit set for a "Good quality" of inland waters. As for *E. coli*, the other indicator considered in the new EU Bathing Water Directive, none of the samples complied.

In 2005, the additional discharges from the summer festival did not seem to significantly affect the perfomance of Fataca constructed wetland, since only FC showed an increase of about 1 log in the effluent concentrations. However, the low number of samples taken prior to the additional discharges (n = 4) is not considered representative enough to describe the wetlands' performance during standard operating conditions.

In mid-September 2007, a peak in the effluent concentrations of TC, FC and Enterococcus was observed, which might have been caused by a high intensity precipitation event (35 mm/h recorded by the nearby weather station) directly affecting the wetland hydraulic residence time.

Table 2 presents the average concentrations and log₁₀ reduction for total coliforms, faecal coliforms, Enterococcus and *Escherichia coli* obtained in the 2005 and 2007/2008 Fataca CW campaigns. Distinction was made regarding summer (from May to September) and winter (from October to January) in the 2007/2008 campaign.

Fig. 1 and the average values presented in Table 1 suggest that during the 2007/2008 winter, the effluent concentrations of TC and FC seemed to increase when compared to the summer ones, suggesting a lower wetland

Table 2

Average concentrations of TC, FC, Ent. and *E. coli* in the influent (in) and in the effluent (out) of Fataca CW

		2005	2007/2008	
			summer	winter
TC	in	3.7E+06	2.3E+06	3.8E+06
	out	1.5E+04	3.2E+04	3.1E+04
	log10 reduction	2.4	2.4	2.2
FC	in	1.6E+06	1.3E+06	2.3E+06
	out	1.2E+04	6.1E+03	1.6E+04
	log10 reduction	2.5	2.5	2.2
Ent.	in	3.8E+03	1.9E+04	9.9E+03
	out	1.7E+01	2.4E+02	5.5E+02
	log10 reduction	2.1	1.4	1.2
E. coli	in	_	1.2E+06	2.1E+06
	out	_	6.1E+03	1.6E+04
	log10 reduction	_	2.5	2.2

performance. A possible seasonal effect was analyzed with the Mann-Whitney U test, showing no significant differences (p < 0.05) between summer and winter, for all parameters.

The existence of seasonal differences in constructed wetlands performance regarding the removal of microbiological microorganisms is not consistently reported. In fact, Karathanasis et al. [13] reported a larger removal efficiency regarding faecal coliforms in summer than in winter, while in the results presented by García et al. [11] no clear seasonal differences were observed.

3.2. Malavado constructed wetland

Fig. 4 presents the results regarding total coliforms, faecal coliforms and Entecococus concentrations obtained in Malavado constructed wetland. *Escherichia coli* data are not displayed since they are very similar to faecal coliforms data. Fig. 4 shows that the effect of additional discharges was noticeable in Malavado CW, both in 2005 and 2007/2008 campaigns, possibly due to the larger additional volume delivered to the WWTP during the summer festival. Although TC and FC influent concentrations did not show a relevant increase during that period, the corresponding effluent concentrations showed an increase of up to 2 log, followed by a smooth decrease. Concentration values in the range of those obtained before the additional discharges were only observed again in mid-September.

On the other hand, in the 2005 campaign, Enterococcus concentrations at the inlet of the constructed wetland showed an increase of up to 2 log that lasted for 3 weeks after the discharges. This situation could indicate that the septic tank conditions, upstream the wetland bed, might have suffered internal mixing due to the additional discharges, releasing previously accumulated material (sludge).

Once the effluent concentrations reached the initial levels, the performance of the Malavado constructed wetland remained fairly stable, even in mid-September and November 2007, when high intensity precipitation events occurred (with peak rainfall intensities up to 35 mm/h). Although the two wetlands are only 1.5 km apart, the effect of a significant precipitation event mentioned for Fataca might not have been reflected in Malavado due to spatial variations of the precipitation distribution, which are typical of that area.

In a global analysis, neither Enterococos effluent concentrations nor E. coli complied with the new EU Bathing Water Directive, even in weeks without the influence of the additional discharges from the summer festival.

Table 3 presents the average concentrations and log₁₀ reduction for total coliforms, faecal coliforms, Enterococcus and *Escherichia coli* obtained in Malavado CW in 2005 and 2007/2008 campaigns. Distinction was made regarding summer (from May to September) and winter (from



Fig. 4. Microorganism concentrations (TC, FC and Ent.) in Malavado CW.

Table 3 Average concentrations of TC, FC, Ent. and *E. coli* in the influent (in) and effluent (out) of Malavado CW

		2005	2007/2008	
			summer	winter
TC	in	1.0E+07	4.2E+06	4.9E+06
	out	5.4E+06	2.6E+05	4.2E+04
	log10 reduction	1.7	1.4	2.1
FC	in	5.0E+06	2.4E+06	2.4E+06
	out	2.4E+06	8.3E+04	1.5E+04
	log10 reduction	1.7	1.5	2.2
Ent.	in	2.6E+06	2.5E+06	1.3E+05
	out	2.8E+05	1.4E+06	2.2E+03
	log10 reduction	0.9	0.8	1.6
E. coli	in	-	2.3E+06	2.3E+06
	out	-	8.3E+04	1.5E+04
	log10 reduction	-	1.5	2.2

October to January) in 2007/2008. Based on the data presented in Fig. 4 and the previous analysis it can be considered that winter data was not influenced by the additional discharges from the summer festival.

Additional discharges had a significant impact on Malavado CW, decreasing the average concentration reduction in the summer by approximately 0.7 log. Winter data obtained at Malavado is similar to the winter data presented for the Fataca constructed wetland.

3.3. Effluent conditions for UV disinfection

The global analysis of the results presented in the previous sections shows that the average removal efficiencies are consistent with data presented in other studies of constructed wetlands fed with primary effluents [5– 8,11].

The concentrations obtained for the effluent indicate that additional disinfection is needed (even under normal operating conditions) if discharging directly to bathing waters, in order to meet the levels defined by the new EU Bathing Water Directive 2006/07/EEC. In Portugal, in the last few years, a strong investment has been made in the construction and rehabilitation of a large number of WWTPs, in order to improve wastewater treatment standards. In sensitive areas, like bathing waters or where water is used for irrigation, the demand for wastewater disinfection has become more relevant. Despite being energy-consuming, UV disinfection systems have been considered a viable option for WWTPs serving small agglomerations in specific situations, particularly when there is no available area to implement simpler solutions with smaller operation costs, such as maturation ponds. The disinfection is provided by UV lamps that emit radiation with a wavelength of 254 nm, which causes microorganism inactivation.

The performance of the disinfection systems depends strongly on the effluent transmittance at the operational wavelength, with required minimum values, in general, around 40–50%. This condition demands low TSS concentrations, since particles reflect UV light causing a lower transmittance. One way of achieving lower TSS concentrations and higher transmittance values is by introducing a filtration stage immediately upstream the disinfection system.

Constructed wetlands usually present good TSS removal, which is the case for Malavado, with average TSS concentrations in the effluent lower than 10 mg/l (during normal operation), and also for Fataca constructed wetland, with an average concentration of 16 mg/l during the study period. This performance could suggest that there is no need for additional filtration after the constructed wetland, if UV disinfection is to be installed.

The conditions for further disinfection with UV light where analysed for Fataca and Malavado constructed wetlands. When sampling started, a dark colouration was noticed in both effluents, which prompted transmittance readings. The results presented in Fig. 5 showed that although TSS concentrations were low (80% of the samples were below 25 mg/l), transmittance was not higher than 40%, even for TSS concentrations as low as 5 mg/l. The samples collected in the 4 weeks after the additional discharges from the summer festival had transmittance values below 10%.

In order to assess the causes of the low transmittance values, samples collected after October 2007 were analysed for heavy metal concentration, namely iron, since it can absorb UV light directly [14]. The effluent from Fataca CW presented concentrations in the range of 0.25–4 mg/l, which are above the typical values usually found in raw wastewater and these concentrations were not present in the wastewater entering the wetland, while the effluent from Malavado CW showed iron concentrations lower than 0.25 mg/l on most samples. Iron concentration and the transmittance for each sample are presented in Fig. 6.

Although iron concentrations, especially for Fataca CW, might explain, at least partially, the low transmittance values, there seems to be no direct correlation between these two variables. Other substances that can lower transmittance are humic substances, which are major components of the natural organic matter present in soil and water and which also absorb UV radiation [14]. The dark colour imparted by these substances when dissolved is consistent with the coloration observed in the constructed wetlands effluent. It was also observed that when the outlet structure discharges with a free-fall drop, foam is formed, which is also consistent with the detergent character of humic substances. This suggests the presence of humic substances in the effluent resulting from the decay and transformation of plant and microorganisms remaining inside the wetland, as is characteristic of a natural system. It is considered that the impact of humic substances on transmittance values of the effluents can only be verified with further investigation.



Fig. 5. Effluent TSS concentration and corresponding transmittance at 254 nm for Fataca and Malavado CW.



Fig. 6. Iron concentration and corresponding transmittance at 254 nm for Fataca and Malavado CW.

3.4. Results of the UV collimated beam test

In order to assess whether the effluent might have been disinfected with UV radiation, in the light of the low transmittance levels obtained, UV dose-response curves were obtained using a collimated beam test. The test was performed on the effluent from Fataca CW collected on the 11th April 2007, with TSS concentration of 3 mg/l and transmittance at 254 nm of 19%.

The UV dose-response behavior of wastewater microorganisms was examined for UV doses ranging from 0 to 48 mWs/cm². Fig. 7 presents the dose-response curves for the tests without sunlight exposure and with 1 h of sunlight exposure after the UV irradiation.

If sunlight exposure is not considered, the UV dose required to achieve concentration values lower than 2000 CFU/100 mL (of both faecal coliforms and *E. coli*) was found to be 6 mWs/cm². As for sunlight exposure after radiation, to achieve a concentration lower than 2000 CFU/100 mL a dose over 10 mWs/cm² was needed. If disinfection requirements are more stringent and if the target of microorganism concentrations less than 100 CFU/100 mL is to be met, the UV dose required, taking into consideration sunlight exposure, was found to be close

to 18 mWs/cm², for *E. coli* concentration. For the same dose, the obtained average concentration of faecal coliforms was approximately 138 CFU/100 mL.

As expected, the UV inactivation dose-response for faecal coliforms and *E. coli* showed a linear decline for doses up to 18 mWs/cm².

The microorganism recovery concentration of both *E. coli* and faecal coliforms observed in the samples submitted to sunlight is associated with photoreactivation, with possible multiplication of non-inactivated bacteria after the exposure. Recovery was not verified with a 48 mWs/cm² dose.

4. Summary and conclusions

Wastewater treatment for small agglomerations often presents a challenge in areas of low income and without specialized human resources to operate complex technologies due to the reduced economy of scale resulting from scattered systems, as well as due to the need for reliable and simple to operate WWTPs.

Constructed wetlands are being recognized as an efficient means of meeting these needs. Although many studies have been developed for the removal of biochemi-



Fig. 7. Faecal coliforms and *Escherichia coli* UV dose-response curves.

cal pollutants, microorganism removal studies are still few and the scientific knowledge is still limited.

Results from two full-scale subsurface flow horizontal constructed wetlands operating under normal conditions in Portugal showed average bacterial reduction rates around 2 log for total coliforms, faecal coliforms and *Escherichia coli*, and in the range 1.2–2.1 for Enterococcus. Seasonal differences were not statistically significant. The impact of additional discharges from an annual nearby festival (4–5 days) on microorganisms removal was especially significant for the CW that received the larger wastewater flows, with some of the samples demonstrating a very limited treatment at that constructed wetland.

The analysis under normal operating conditions showed that the wetland's removal efficiencies regarding microorganism concentrations are similar to the ones obtained in previous studies [5–8,11], and are generally higher than those obtained in conventional systems, like trickling filters or activated sludge WWTPs. However, effluent concentrations from the monitoried constructed wetlands did not consistently comply with European Directives for direct discharges in bathing areas or for direct reuse for agriculture. In such situations further disinfection might be required.

The use of UV disinfection systems is being considered a viable option for treating the effluent of small agglomerations in Portugal under specific circumstances. Its application has been selected when there is no available area for simpler solutions such as maturation ponds, despite the fact that this technology is more complex and requires specialized operating personal. The transmittance of effluents with low TSS values from two constructed wetlands in Portugal was measured at 254 nm, to assess the conditions for UV disinfection. Results showed transmittance values below 40%.

The effluent from the constructed wetlands could be filtrated to further lower suspended solids and improve transmittance, but this may not be totally efficient. The dark colouration of the effluent suggest the presence of dissolved or colloidal substances like humic substances, but further investigation is needed to support this hypothesis.

Knowledge of responses of the microorganisms to disinfectant exposure is also critical for predictions of process behavior. A collimated beam test was carried out to analyze UV intensity needs for the Fataca CW effluent and the effect of subsequent sunlight exposure.

Microbial recovery of both *Escherichia coli* and faecal coliforms was observed in samples submitted to sunlight.

Considering sunlight exposure after radiation, to achieve a concentration lower than 2000 CFU/100 mL (both for faecal coliforms and *E. coli*) a dose over 10 mWs/cm² was needed. The UV dose required to achieve concentrations below100CFU/mL wasfound to be close to 18mWs/cm², for *E. coli*. For the same dose, the obtained average concentration of faecal coliforms was 138 CFU/100 mL.

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