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RESEARCH ARTICLE

Elimination of faecal indicator microorganisms from wastewater by combining constructed wetlands and heterogeneous photocatalysis: from laboratory to pilot-scale implementation

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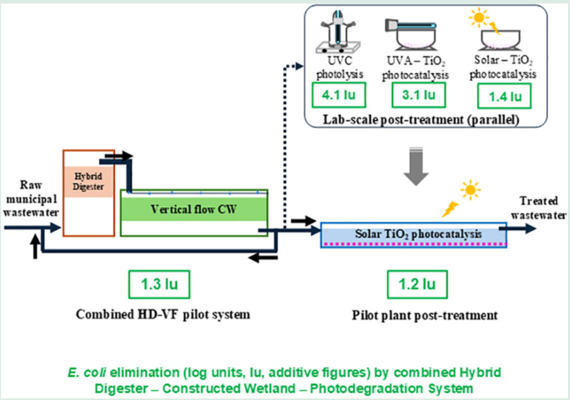
HIGHLIGHTS

- Total coliforms, *E. coli* and *C. perfringens* were evaluated at pilot scale system.
- Constructed wetland effluent post-treatment with sunlight and TiO_2 photocatalysis.
- The combined CW-TiO_2 sunlight system required 1.1 m^2 per equivalent inhabitant.
- *E. coli* elimination reached 2.5 log units in the pilot CW-TiO_2 sunlight system.
- The photocatalysis pond improved CW effluent quality widening water reuse potential.



ABSTRACT: A combined system comprising a hybrid anaerobic digester (HD), a vertical subsurface flow constructed wetland (VF), and a heterogeneous photocatalysis unit was evaluated at pilot-scale for the elimination of faecal indicator microorganisms—total coliforms, *Escherichia coli* and *Clostridium perfringens*. The VF effluent was subjected to laboratory-scale experiments using different photodegradation post-treatments: UVC photolysis, heterogeneous photocatalysis with ultraviolet light (UVA/TiO_2), and sunlight-driven heterogeneous photocatalysis (Sol/TiO_2). Subsequently, the Sol/TiO_2 system was scaled up and implemented at pilot-scale (p.Sol/TiO_2). The total footprint of the combined HD+VF+p.Sol/TiO_2 system was 4.4 m^2. Under continuous operation, the combined HD+VF system was able to remove approximately 1.0, 1.3 and 1.1 log units for total coliforms, *E. coli* and *C. perfringens*, respectively, with the VF unit accounting for more than 80% of the overall elimination during biological

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treatment. Laboratory-scale experiments showed high removal efficiency, following the order UVC > UVA/TiO_2 > Sol/TiO_2. In contrast, the p.Sol/TiO_2 post-treatment (after 2 h of exposure) achieved lower removals of approximately 0.5, 1.2 and 0.1 log units for total coliforms, *E. coli* and *C. perfringens*, respectively. To our knowledge, this is the first study on the combination of VF constructed wetlands and photodegradation processes with the aim of improving the quality of reclaimed water for potential reuse. As a general conclusion, the photocatalysis pond employed in the present study improved the quality of the VF effluent, widening the possibilities for reuse of the reclaimed water.

KEYWORDS: Constructed wetlands, Faecal indicators, Photocatalytic disinfection, Solar disinfection, Municipal wastewater reuse

1 Introduction

Continued population growth and the risks associated with climate change (e.g., droughts and heat-waves) will lead to unpredictable increases in global demands for water, energy and food. Increased water demand (for agriculture, industry, or domestic use), the risk of drinking water scarcity and water stress are already a current problem and is expected to worsen over time. In this context, the treatment and subsequent reuse of reclaimed wastewater for agricultural or environmental uses is a valuable opportunity to redress water balance and alleviate drinking water consumption (Santos et al., 2023).

An important aspect of reclaimed water reuse is the presence of pathogenic microorganisms. Pathogen contamination in water is a significant global problem that poses serious risks to human and animal health, as well as environment protection. Waterborne pathogens, which include viruses, bacteria, fungi, protozoa and helminths, can cause a variety of diseases when ingested via contaminated water sources, often resulting from untreated or poorly treated wastewater discharges. Therefore, specific threshold values for physico-chemical, biological, and microbiological parameters have been established, which have to be fulfilled before reuse of reclaimed water. These threshold values have been included in different regulations or standards depending on the country or area concerned. In most guidelines, total and faecal coliforms (FC) are used as indicator microorganisms for water quality (de Campos and Soto, 2024). At European level, a recent guideline on minimum water quality requirements for water reuse in agricultural irrigation has been published, named as Regulation (EU) 2020/741, which included a new classification by crop categories and irrigation methods according to the quality achieved by the reclaimed water. In Spain, reclaimed water reuse is regulated by Royal Decree 1620/2007. Both regulations set different maximum acceptable values of *Escherichia coli* (*E. coli*) as a mandatory microbiological requirement for each reuse category. In addition, these regulations have encouraged the monitoring of other microorganisms for a more comprehensive assessment of water quality. For example, *Clostridium perfringens* (*C. perfringens*) was proposed as bioindicator of human faecal contamination, including viruses and protozoan cysts (Payment and Franco, 1993; Ferguson et al., 1996; Suzuki et al., 2021). Different wastewater treatment technologies have been developed to achieve reclaimed water suitable for reuse (Castellar et al., 2022; Al-Hazmi et al., 2023; Santos et al., 2023). Nature-based solutions have emerged as green alternatives to conventional systems. Among them, constructed wetlands (CWs) are defined as robust, environmentally friendly, cost-effective, and easy-to-operate wastewater treatment technologies with minimal maintenance requirements (Nan et al., 2020; Kataki et al., 2021). However, treating raw wastewater in single-stage CWs leads to a large area footprint and possible clogging of the granular bed. To alleviate their drawbacks, certain types of anaerobic digesters, such as an up-flow anaerobic sludge bed digester or an hydrolytic up-flow sludge bed digester, are employed as a pre-treatment step before CWs (Álvarez et al., 2008; Ruiz et al., 2010; de la Varga et al., 2013; Pascual et al., 2017). Anaerobic digesters are capable of removing between 60%–90% of suspended solids and between 30%–70% of organic matter, reducing the contaminant load entering the CW (Álvarez and Soto, 2011; Fernández del Castillo et al., 2022).

Recently, the combination of anaerobic digesters with vertical subsurface flow CWs (VF) has been investigated for advanced removal of nitrogen and emerging organic pollutant, showing promising results with a total required surface area of 1.4 m^2/inhabitant equivalent (Sánchez et al., 2022, 2023). Regarding the elimination of pathogens, anaerobic digesters have little impact on most indicators, except on helminth eggs, for which it seems very effective. On the other hand, the type of CW used to treat domestic or urban wastewater determines to some extent its average elimination of pathogenic microorganisms (Wu et al., 2016; Shingare et al., 2019). In single stage CWs, for both horizontal subsurface flow CW and surface flow CW, the reduction potential of faecal bacteria was potentially higher than for VF. However, the treatment efficiency of hybrid or multi-stage CWs is greater than that of single-stage CWs (López et al., 2019). According to the recent review by de Campos and Soto (2024), pathogen removal in CWs ranges from 0.5 to 2.3 log units, and can reach up to 3 log units of removal (i.e., 99.9%). Shingare et al. (2019) reported a mean removal of 1.5 ± 0.9 log units of *E. coli* in VF systems. The higher efficiency of hybrid CWs is explained by the synergy of the different removal mechanisms specific to each CW type (Shingare et al., 2019; Nan et al., 2020). Mechanisms involved in pathogen removal in CWs include sedimentation, filtration, secretion of antibiotics or biocides, phytoremediation, inactivation or death due to natural causes, temperature effects, unfavourable water chemistry, oxidation, predation, and exposure to sunlight or UV radiation (Wu et al., 2016; Alufasi et al., 2017; Shingare et al., 2019). On the other hand, factors such as CW design (substrate type, granular bed particle size, and vegetation type), operational parameters (hydraulic loading rate and hydraulic retention time), seasonal fluctuation, and wastewater composition are key to the efficiency of the above-mentioned removal mechanisms (Wu et al., 2016). López et al. (2019) concluded that particle size of the granular media, organic matter removal and pH conditions were the main factors affecting pathogen removal in CWs. Nevertheless, de Campos and Soto (2024) found that pathogen removal was not related to hydraulic retention time or hydraulic loading rate, due to the wide diversity of CW types, designs and operating parameters. In addition, these authors noted the presence of a potential short-circuit that could cause the actual hydraulic retention time to be less than the design retention time, thus leading to biased results.

So far, the effective elimination of pathogenic microorganisms for reuse of reclaimed wastewater can be achieved by combining CWs with a disinfection post-treatment. Chlorination disinfection has generally been the most widely tertiary treatment due to its low cost and simple maintenance. Nevertheless, the release of chlorinated by-products is still harmful to public health (Armesto et al., 1998; Kali et al., 2021), so other disinfection technologies have been investigated. Among them, advanced oxidation processes (e.g., ultraviolet photolysis or heterogeneous photocatalysis) can been integrated as post-treatment to CWs to polish the final effluent and improve its quality for reuse. Ultraviolet treatment (usually referred to apply UVC light, i.e., radiation of wavelengths between 100 and 280 nm) has been considered as an effective photo-degradation post-treatment for several pathogens without generating toxic by-products (Canle et al., 2012; Castellar et al., 2022). However, UVC post-treatment requires high energy consumption, which increases the operating and maintenance costs of decentralized treatment systems. As a more cost-effective alternative, heterogeneous photocatalysis is emerging as a promising photodegradation post-treatment. Titanium dioxide (TiO_2) has been widely used as a catalyst in heterogeneous photocatalytic processes due to its abundance, low cost, photoactivity, non-toxicity and insolubility in water. TiO_2, when suspended in water, can be irradiated with near UV light at wavelengths below 385 nm to form reactive oxygen species, among which the hydroxyl radical, $HO^•$, is the most reactive and the one with highest oxidizing power (Ma et al., 2021; Canle et al., 2022). The energy source can be artificial (e.g. lamps emitting UVA light, i.e., radiation with wavelengths between 315 and 400 nm) or natural (e.g. sunlight as a clean, safe, free, and abundant energy source) (Malato et al., 2009). The scientific literature on pathogen removal efficiency during the treatment of real wastewater at full-scale or pilot-scale using a combination of CWs and photodegradation processes is scare. For example, Azaizeh et al. (2013) treated domestic wastewater using horizontal subsurface CW followed by a UV system and achieved *E. coli* removals of about 0.7 log units in the horizontal CW and 3.7 log units in the UV system. Russo et al. (2019) treated secondary effluent from a wastewater treatment plant using a horizontal sub-surface CW followed by UVC irradiation. These authors achieved log removals of 1.4 ± 0.7, 1.3 ± 0.9 and 1.3 ± 1.0 for total coliforms (TC), *E. coli* and *C. perfringens* in their horizontal CW while the UVC treatment removed 3.1 ± 0.9, 3.0 ± 0.7 and 0.1 ± 0.4 for TC, *E. coli* and *C. perfringens*. To the best of our knowledge, no studies are available on the efficacy of the combination of VFs and photodegradation processes for reuse of the final treated effluent. Further, there is a need for research on heterogeneous photocatalysis based on TiO_2 catalyst as a post-treatment to VFs, since this technology can be integrated as an improvement to an intensified VF, following a model that emulates hybrid CWs. In addition, decentralized systems are currently very relevant due to the requirements of new legislation on water reuse. Nevertheless, the use of suspended photocatalysts requires a subsequent separation process to prevent the release of semiconductor particles in the effluent. Furthermore, suspended catalysts are frequently nanoparticulate, thereby impeding their effective recovery. Consequently, the use of immobilized photocatalysts seems indispensable to avoid costly filtration procedures and ensure the effective reuse of the photocatalyst.

Therefore, this study aims to improve the quality of the final effluent of a combined anaerobic digester–VF system through heterogeneous photocatalysis treating raw municipal wastewater. The main objective was to address the integration of CWs and heterogeneous photocatalysis using sunlight and TiO_2, since sunlight is a clean, safe, free and abundant energy source. This post-treatment system, which operates without chemicals or electricity and requires minimal maintenance, has the potential to be fully integrated into the semi-natural environment of constructed wetlands. To enable comparison of results, the VF effluent was treated on a lab-scale by three different photodegradation post-treatments: UVC photolysis, UVA/TiO_2 photocatalysis (i.e., UVA light as energy source and TiO_2 as photocatalyst) and Sol/TiO_2 photocatalysis (i.e., sunlight as energy source and TiO_2 as photocatalyst). Finally, the post-treatment based on heterogeneous photocatalysis using sunlight and TiO_2 was tested on a pilot-scale (p.Sol/TiO_2). Thus, the VF effluent can receive an advanced treatment to expand the possible reuse applications for reclaimed water, employing an environmentally friendly and low-cost technology.

2 Material and methods

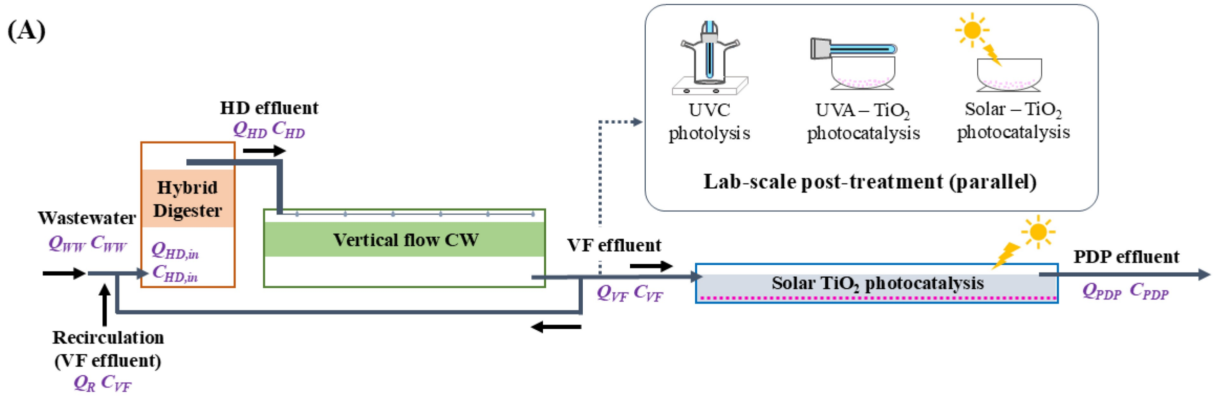
2.1 Experimental units

The study of the elimination of faecal indicator microorganisms was carried out during the treatment of municipal wastewater using a two-stage anaerobic digester–VF system at pilot plant scale, followed by a third stage based on photodegradation processes as post-treatments. The raw wastewater was generated at a university site (mainly effluents from the cafeteria and toilets), mixed with frequent rainwater and runoff.

The combined anaerobic digester–VF system was evaluated by Sánchez et al. (2023) in terms of conventional water quality parameters (i.e., organic matter, suspended solids and nitrogen compounds). Briefly, the treatment line consisted of a grid for coarse solids, a hybrid digester (HD) as the pre-treatment, and an unsaturated vertical subsurface flow constructed wetland (VF) as a second stage. The combined HD + VF system operated in series, with recirculation of the VF effluent to the HD inlet (Fig. 1). As for the HD, its distinctive feature was the superimposition of a planted anaerobic filter on the top of the sludge bed of a typical up-flow anaerobic sludge bed digester. The HD operated in up-flow mode with short hydraulic retention time to limit the methanogenic step while facilitating the retention and hydrolysis of solids. The surface area of the HD was 0.38 m^2 (volume 0.222 m^3) and the VF surface area was 3 m^2. The bed particles used in the different filter materials were coarse gravel (particle size 0.5–16 mm) in the anaerobic filter, coarse sand in the VF main filter media (particle size 1–3 mm) and fine sand in the VF surface layer (particle size 0.5–2 mm). Further details on the design of both units are given by Sánchez et al. (2023).

Several photodegradation post-treatment (PDP) options have been studied for the HD + VF system effluent, leading to the different options for the three-step HD + VF + PDP system. A first post-treatment evaluation was performed at lab-scale applying three different technologies: direct photolysis with UVC light, and heterogeneous photocatalysis with both UVA light and sunlight. The UVC post-treatment system consisted of an 8-cm-diameter glass photoreactor containing a Heraeus TNN 15/32 low-pressure Hg-vapor lamp (wavelength: 254 nm) with a quartz jacket (Fig. 1). The heterogeneous photocatalysis was carried out using a newly patented photocatalyst (Ramos et al., 2023), based on 10% local clay and 90% Degussa Aeroxide P25 TiO_2 in the form of pellets. The employed clay, designated BP50, and obtained from Terras de Buño, S.L. (A Coruña, Spain), is a mixture of two naturally mined clays, containing 63.0% SiO_2, 19.5% Al_2O_3, 8.0% Fe_2O_3, and other oxides at much lower concentrations. The photocatalytic composite was prepared according to Aguilar et al. (2023b) as follows. A mixture of 10 wt% clay and 90 wt% TiO_2 was prepared by thoroughly blending both powders. Then, organic-free bidistilled water was gradually added drop by drop under mild stirring to form a thick slurry. The resulting material was shaped into thin cylindrical strands using a syringe with a 2 mm inner diameter nozzle. These pieces were dried at 90 °C for 12 h, subsequently cut into 0.5 cm long pellets, and calcined at 600 °C for 3 h, applying a heating rate of 5 °C/min. Once cooled, the pellets were thoroughly rinsed with water and dried again at 90 °C for 12 h.

The high Si and Al content of the clay is essential to prevent the anatase to rutile transition during the calcination step (Hanaor and Sorrell, 2011; Dlamini et al., 2021), enabling the production of hard pieces that



(B) Combined HD-VF pilot system

Pilot plant post-treatment

Fig. 1 (A) Scheme of wastewater treatment line through the two-step HD+VF system and the different post-treatments evaluated (acronyms: Q = flow, C = concentration, WW = raw wastewater, HD = hybrid digester, VF = subsurface vertical flow CW, PDP = photodegradation post-treatment). (B) Photographs of the three main elements of the pilot plant.

are easy to manipulate while retaining their photocatalytic activity. In addition, the density (ca. 4.0 g/mL) and size of these pellets allow them to be used at the bottom of a flow reactor, from which they can be directly recovered for reuse. This arrangement allows for much easier reusing of the photocatalyst than suspended particles, which have the potential to contaminate discharge water. However, it is more susceptible to radiation loss, which depends on the depth and turbidity of the water layer.

This new photocatalyst has already been tested in the removal of emerging pollutants (Sánchez et al., 2022). The heterogeneous photocatalysis experiments were tested with two different energy sources: UVA light (using a medium-pressure Heraeus TQ 150 Hg-vapor lamp at a wavelength of 365 nm) and sunlight. These two systems, named UVA/TiO_2 and Sol/TiO_2 respectively, were tested in borosilicate vessels with a flat round base of 14 cm in diameter (Fig. 1).

Finally, the Sol/TiO_2 alternative was scaled up and implemented in the pilot plant, being named p.Sol/TiO_2. For this purpose, a 1-m^2 surface-flow methacrylate basin was built to hold a layer of photocatalyst and facilitate the contact of the wastewater with the photocatalyst and sunlight. The capacity of the combined HD + VF + p.Sol/TiO_2 system (total area of 4.38 m^2) was estimated to treat the wastewater from a single-family house (up to 4 equivalent inhabitants) and to achieve the secondary treatment and advanced nitrogen removal targets (Sánchez et al., 2023). The objective of the third photodegradation stage was to improve the elimination of emerging pollutants (Sánchez et al., 2022) and pathogens (this study). Both laboratory and pilot plant photodegradation experiments were conducted in batch mode.

2.2 Operational conditions

Three successive campaigns, carried out between June and September, were conducted to study the elimination of faecal indicator microorganisms. When these experiments began, the combined HD + VF system had already accumulated 275 d of prior operation, including the start-up period. The combined HD + VF system was fed on 4 consecutive days per week, keeping the other 3 d at rest. Table 1 shows the operational parameters applied to the HD + VF system in each campaign and the recirculation ratios applied. The HD operated with a hydraulic retention time ranging from 6.7 to 13.6 h, while the VF unit received a hydraulic loading rate of 130 to 263 mm/d.

Different amounts of the VF effluent were used for

Table 1 Operational conditions applied to the HD+VF system in each campaign

Parameter	Campaign I (*n* = 2)	Campaign II (*n* = 2)	Campaign III (*n* = 1)
Flow rate $_{\text{INFLUENT}}$ (Q_{WW}, L/d)	607 ± 13	791 ± 1	391 ± n.d.
Recirculation ratio ($Q_{\text{R}}/Q_{\text{WW}}$)	0.85 ± 0.01	0.67 ± 0.01	1.38 ± n.d.
Temperature $_{\text{INFLUENT}}$ (ºC)	20.2 ± 0.7	22.0 ± 0.1	21.5 ± n.d.
Solar radiation (W/m^2)	456 ± 152	804 ± 110	678 ± n.d.
Organic loading rate $_{\text{HD}}$ (kg COD/m^3·d)	1.6 ± 0.4	1.8 ± 0.4	1.0 ± n.d.
Hydraulic retention time $_{\text{HD}}$ (h)	8.77 ± 0.19	6.72 ± 0.19	13.61 ± n.d.
Hydraulic loading rate $_{\text{VF}}$ (mm/d)	202.4 ± 4.3	263.8 ± 0.3	130.3 ± n.d.
Organic loading rate $_{\text{VF}}$ (g COD/m^2·d)	57.4 ± 3.0	49.3 ± 13.6	15.5 ± n.d.
Organic loading rate $_{\text{HD+VF}}$ (g COD/m^2·d)	88.8 ± 8.6	125.9 ± 14.6	66.0 ± n.d.

Acronyms: COD = chemical oxygen demand; other acronyms: see Fig. 1. n.d. = not determined

the photodegradation tests, both at laboratory and pilot plant scale. In the UVC photoreactor, 500 mL of VF effluent were treated for 30 min under continuous stirring. In both UVA/TiO_2 and Sol/TiO_2, 20 g of TiO_2-based photocatalyst were placed at the base of the vessel and 500 mL of VF effluent were added, reaching a water layer height of 2.5 cm. In the two heterogeneous photocatalysis treatments, the batch tests lasted 2 h. At the pilot plant, 2 kg of TiO_2-based photocatalyst and 50 L of effluent (water layer height of 5 cm) were introduced into the photocatalysis pond and processed for 2 h. Sol/TiO_2 and p.Sol/TiO_2 experiments were carried out in the central hours of the day (13.30 – 15.30 UTC+2) to work at the peak of maximum solar irradiance.

2.3 Sampling, analysis, and calculations

The combined HD+VF system was kept in continuous operation throughout the study, with three campaigns differentiated by their operating conditions, as shown in Table 1. For the study of faecal indicator microorganisms, samples were taken at three different points (influent, HD effluent and VF effluent) throughout the three campaigns. For the same three campaigns, the HD + VF effluent was subjected to the different post-treatments at lab-scale, obtaining samples after 30 min of UVC, and after 1 and 2 h of UVA/TiO_2 and Sol/TiO_2. Other three sampling campaigns were carried out for the photocatalysis pilot pond, obtaining samples at 0, 0.5, 1, and 2 h. A total of 42 samples were analysed: 15 from the combined HD+VF system, 15 from the photodegradation lab-tests, and 12 from the photocatalysis pilot pond.

The three faecal indicator microorganisms studied were TC, *E. coli* and *C. perfringens*. A sample volume of approximately 250 mL was taken at each indicated sampling point in sterile bottles. Samples were kept refrigerated and analysed on the same day. TC and *E. coli* analyses were performed by the membrane filtration method using sterile 0.45 µm pore size cellulose acetate filters. Prior to filtration, the samples were serially diluted up to 10^{-3}. 50 mL of the undiluted sample and of each dilution were filtered in triplicate. The filters were placed on culture media suitable for the enumeration of each group of microorganisms analysed. After incubation, the plates with typical colony counts between 30 and 300 colony forming units (CFU) were selected, and used in the determinations. Thus, the counts were used to determine the CFU per unit volume of the original sample taking the corresponding dilution into account. The culture media used were m-Endo LES agar medium for TC counts (incubation at 37 ± 0.2 ºC, 24 h) and mFC medium for *E. coli* counts (incubation at 44 ± 0.2 ºC, 24 h). To confirm *E. coli*, the blue colony was coated with a drop of Kovacs’ reagent. The enumeration of spores of *C. perfringens* was carried out using SPS agar medium by tube seeding. Kimax tubes, containing 20 mL of the culture medium at double concentration, were mixed with 20 mL of sample from the serial dilutions. Previously, the sample was heated at 80 ºC for 5 min to remove vegetative forms. The tubes were covered with 0.5 cm of liquid petroleum jelly to achieve anaerobiosis and incubated under appropriate conditions (37 ± 0.2 ºC, 24 h). All dilutions were seeded in triplicate. Counts were made for the presence of black colonies and those tubes from the dilution that allowed an adequate count were selected for counting.

The data from the microorganism counts were expressed in both concentration (CFU/100 mL) and logarithmic (log) units (log (CFU/100 mL)). Although in the literature mentioned in the discussion of the results log units were found referring to both concentrations per 100 mL and per mL, we verified that the elimination values in logarithmic units did not vary. Therefore, it was not considered necessary to specify the reference volume for each cited study.

For the calculation of the overall elimination efficiency in percentage (% EE), Eqs. 1 and 2 were applied for the HD+VF system and for the combined HD+VF+PDP, respectively. The contribution of the biological treatment unit to the global elimination efficiency was calculated using Eqs. 3–5. The effect of recirculation (R) was considered in Eq. 6, defining the calculation of the concentration influent to the HD ($C_{\mathrm{HD,in}}$).

$$EE_{(\mathrm{HD+VF})}(\%) = \frac{C_{\mathrm{WW}} - C_{\mathrm{VF}}}{C_{\mathrm{WW}}} \times 100, \tag{1}$$

$$EE_{(\mathrm{HD+VF+PDP})}(\%) = \frac{C_{\mathrm{WW}} - C_{\mathrm{PDP}}}{C_{\mathrm{WW}}} \times 100, \tag{2}$$

$$EE_{\mathrm{PDP}}(\%) = \frac{C_{\mathrm{VF}} - C_{\mathrm{PDP}}}{C_{\mathrm{WW}}} \times 100, \tag{3}$$

$$EE_{\mathrm{HD}}(\%) = \frac{Q_{\mathrm{HD,in}} \times (C_{\mathrm{HD,in}} - C_{\mathrm{HD}})}{Q_{\mathrm{WW}} \times C_{\mathrm{WW}}} \times 100, \tag{4}$$

$$EE_{\mathrm{VF}}(\%) = \%EE_{(\mathrm{HD+VF})} - \%\mathrm{EE}_{\mathrm{HD}}, \tag{5}$$

$$C_{\mathrm{HD,in}} = \frac{Q_{\mathrm{WW}} \times \mathrm{C}_{\mathrm{WW}} + Q_{\mathrm{R}} \times \mathrm{C}_{\mathrm{VF}}}{Q_{\mathrm{WW}} + Q_{\mathrm{R}}}, \tag{6}$$

where C = concentration, Q = flow rate, R = recirculation, WW = raw wastewater, the subscript “HD,in” refers to the influent to the HD unit, and the subscripts “HD”, “VF” and “PDP” refer to the effluent concentration from the respective system unit.

Likewise, the reduction in log units for HD + VF system was obtained by applying Eq. 7, while the reduction in each biological unit is equal to the total reduction in the HD+VF system multiplied by the corresponding reduction fraction per step (Eqs. 8 and 9). Eq. 10 gave the elimination at the PDP step.

$$\log_{10}\mathrm{reduction}_{\mathrm{HD+VF}} = \log_{10}C_{\mathrm{WW}} - \log_{10}C_{\mathrm{VF}}, \tag{7}$$

$$\log_{10}\mathrm{reduction}_{\mathrm{HD}} = (\log_{10}C_{\mathrm{WW}} - \log_{10}C_{\mathrm{VF}}) \times \frac{\log_{10}C_{\mathrm{HD,in}} - \log_{10}C_{\mathrm{HD}}}{\log_{10}C_{\mathrm{HD,in}} - \log_{10}C_{\mathrm{VF}}}, \tag{8}$$

$$\log_{10}\mathrm{reduction}_{\mathrm{VF}} = (\log_{10}C_{\mathrm{WW}} - \log_{10}C_{\mathrm{VF}}) \times \frac{\log_{10}C_{\mathrm{HD}} - \log_{10}C_{\mathrm{VF}}}{\log_{10}C_{\mathrm{HD,in}} - \log_{10}C_{\mathrm{VF}}}, \tag{9}$$

$$\log_{10}\mathrm{reduction}_{\mathrm{PDP}} = \log_{10}\mathrm{C}_{\mathrm{VF}} - \log_{10}C_{\mathrm{PDP}}. \tag{10}$$

2.4 Statistical analysis

SPSS 27 (IBM, Spain) was used for statistical analyses. All data were expressed as the mean ± standard deviation. Student’s *t* test (two groups), one-way ANOVA and Tukey’s test (more than two groups) were used, when necessary, for the statistical comparison of the means obtained for the different treatments ($\alpha = 0.05$). Previously, the data were checked to determine that they met the postulates required by these methods.

2.5 Legal requirements on reclaimed water quality

Both European and Spanish legislations use *E. coli* as an indicator for pathogenic bacteria (Ministerio de Medio Ambiente, 2007; European Union, 2020). Spanish regulations set criteria for various reuse applications, including urban, agricultural, industrial, recreational and environmental uses, while EU regulation criteria are limited to agricultural reuse. The maximum acceptable values for alternative reclaimed water reuse options are presented in Section 3 (Results and discussion) and used to evaluate compliance or non-compliance of the final effluent with the established requirements.

In addition, these EU and Spanish regulations also encouraged the control of other microorganisms for a more comprehensive assessment of water quality. Among them, *C. perfringens* spores or spore-forming sulphate-reducing bacteria were proposed as an indicator for protozoa (Ministerio de Medio Ambiente y Medio Rural y Marino, 2010). In this sense, for certain industrial and agricultural uses, Spanish legislation obliges to conduct detection tests for presence-absence of *C. perfringens* (or equivalent indicators). According to European legislation, the monitoring of *C. perfringens* is mandatory for the most stringent reclaimed water quality class. In consideration of these legal requirements, *E. coli* and *C. perfringens* were selected as pathogen indicators for the present study. The TC content was also determined, as this parameter is used in other regulations, such as those of China or Cyprus (de Campos and Soto, 2024).

It should be noted that, both at European and Spanish level, the minimum requirements for reclaimed water are fulfilled when the analyses performed satisfy the following criteria: i) the values indicated for the microbiological indicator are fulfilled in a percentage equal to or greater than 90% of the samples; and ii) none of the sample values exceeds the maximum deviation limit (i.e., 1 log unit for *E. coli*).

3 Results and discussion

3.1 Elimination of faecal indicator microorganisms by the combined HD + VF system

The biological units (HD and VF) that made up the combined system, were evaluated individually and jointly (HD + VF) during the three operating campaigns for the concentration of TC, *E. coli* and *C. perfringens*. The mean concentrations of these faecal indicator microorganisms at the inlet and outlet of the biological units are shown in Table 2, while the corresponding mean removal efficiencies are shown in Fig. 2.

During the three study campaigns, the wastewater showed a high load of indicator microorganisms, with mean concentrations over the whole period of 6.27 ± 0.35 log units for TC, 5.97 ± 0.44 log units for *E. coli*, and 4.21 ± 0.54 log units for *C. perfringens*. Across all samples, the concentration trend followed the order TC > *E. coli* > *C. perfringens*. Since all three campaigns occurred during the dry season (from June to September), the concentrations were not significantly affected by dilution due to rainfall. The combined HD+VF system eliminated on average 1.0 ± 0.1 log units for TC, 1.3 ± 0.1 log units for *E. coli* and 1.1 ± 0.2 log units for *C. perfringens*. However, no statistical differences were found between them (Fig. 2), so the three faecal indicators showed similar elimination efficiency in the overall biological HD + VF system.

Table 2 Concentration (log units) of indicator microorganisms in wastewater (inlet), HD effluent and VF effluent (outlet) in each operating campaign

Campaign	TC	*Escherichia coli*	*Clostridium perfringens*
Campaign I (*n* = 2)			
Raw wastewater	5.93 ± 0.23	5.51 ± 0.23	3.68 ± 0.69
Influent to HD [a]	5.71 ± 0.28	5.27 ± 0.26	3.46 ± 0.68
HD effluent	5.72 ± 0.45	4.94 ± 0.59	3.60 ± 0.75
VF effluent	4.96 ± 0.65	4.16 ± 0.79	2.83 ± 0.60
Campaign II (*n* = 2)			
Raw wastewater	6.25 ± 0.08	6.03 ± 0.16	4.75 ± 0.35
Influent to HD [a]	6.06 ± 0.09	5.84 ± 0.14	4.51 ± 0.30
HD effluent	5.73 ± 0.11	5.64 ± 0.18	4.27 ± 0.05
VF effluent	5.25 ± 0.28	4.83 ± 0.31	3.59 ± 0.04
Campaign III (*n* = 1)			
Raw wastewater	6.63 ± n.d.	6.38 ± n.d.	4.20 ± n.d.
Influent to HD [a]	6.29 ± n.d.	6.01 ± n.d.	3.86 ± n.d.
HD effluent	6.29 ± n.d.	5.89 ± n.d.	3.54 ± n.d.
VF effluent	5.51 ± n.d.	4.92 ± n.d.	3.02 ± n.d.
Overall period [b]			
Raw wastewater	6.27 ± 0.35	5.97 ± 0.43	4.21 ± 0.54
Influent to HD	6.02 ± 0.29	5.71 ± 0.39	3.95 ± 0.53
HD effluent	5.92 ± 0.32	5.49 ± 0.49	3.80 ± 0.40
VF effluent	5.24 ± 0.27	4.63 ± 0.42	3.14 ± 0.40

[a] The concentration at the HD inlet was calculated taking into account both wastewater and recirculation flow according to Eq. 6. [b] The overall period data corresponded to the mean ± SD of the three campaigns; n.d.= not determined.

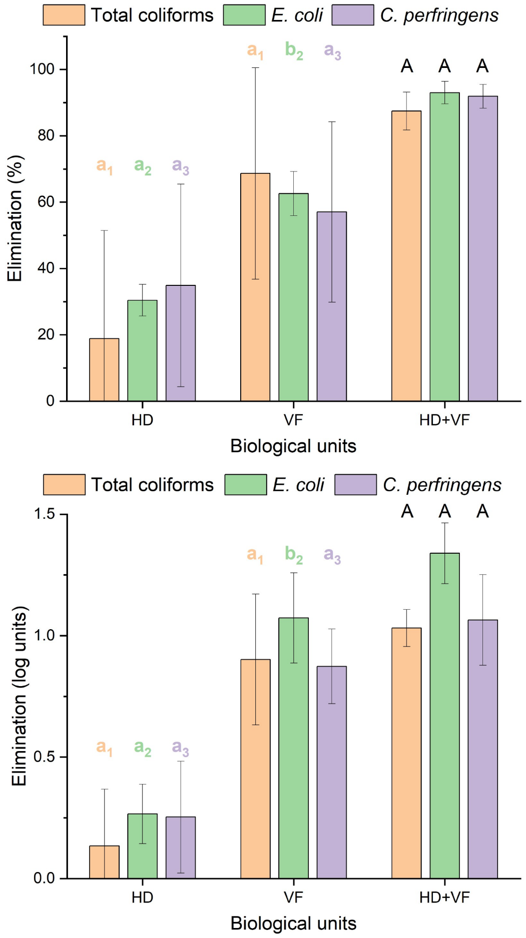


Fig. 2 Elimination efficiency (%) and reduction in log units of the three groups of faecal indicator microorganisms studied in the biological units for the overall period (mean ± SD of the three campaigns, *n* = 3). Different letters indicate significant differences between the HD and VF units for each faecal indicator (lowercase letters; note that subscripts 1, 2, 3 refer to total coliforms, *E. coli*, and *C. perfringens*, respectively) and between the three faecal indicators for the HD + VF system (uppercase letters).

A slight reduction in pathogen concentration was observed at the outlet of HD compared to the inlet to that unit. This behaviour was also reported by Carballeira (2016). The greatest contribution to the overall elimination efficiency of the combined HD +

VF system for the three faecal indicator microorganisms studied corresponded to the VF (Fig. 2). The removal efficiency of *E. coli* by the VF unit was significantly higher than that achieved by the HD unit, both in percentage and logarithmic units. VF also showed higher removal efficiencies for TC and *C. perfringens*, but the differences were not statistically significant. The VF eliminated from 0.6 to 1.1 log units for TC, from 0.9 to 1.3 log units for *E. coli* and between 0.7 to 1.0 log units for *C. perfringens*. The eliminations obtained are not high, but they are in the ranges defined by recent studies, which are very variable, as we analyse below (Bohórquez et al., 2017; Wu et al., 2017; Carballeira et al., 2021; Hernández-Crespo et al., 2022).

The high standard deviation values observed in Fig. 2 are partly due to variations in influent concentrations during operation under field conditions. However, the main reason appears to be the behaviour of the HD unit, which exhibited a lower removal efficiency for these indicators. In fact, the HD effluent concentrations of TC and *C. perfringens* were higher than those of the influent in some campaigns (Table 2). This also caused an increase in the standard deviations observed for the VF removal indicators, although these variations were more attenuated, especially when logarithmic units were used (Fig. 2). Finally, the combined HD + VF system showed a good capacity to buffer these internal variations, resulting in reduced standard deviations for the removal indicators.

Carballeira et al. (2021) studied the removal of FC (where approximately 99% of them belong to *E. coli* (Carballeira, 2016)) and *C. perfringens* in two VF with different particle size as filtering material (coarse sand 1–3 mm and fine gravel 2–6 mm). These authors observed better removal of FC in the VF using a smaller bed particle size (1.0 log unit removal using sand vs. 0.7 log unit using fine gravel). In our VF unit, similar removals for *E. coli* were achieved by applying higher hydraulic loading rates (130–263 mm/d) than Carballeira et al. (2021) (between 80–105 mm/d), while the main filtering material was also coarse sand (1–3 mm particle size). Furthermore, Carballeira et al. (2021) reported elimination efficiencies of *C. perfringens* in the order of approximately 0.8 log units with both sand and gravel. The effect of particle size was also observed by Bohórquez et al. (2017) who tested different particle sizes as substrate for various VF configurations with 150 mm/d of hydraulic loading rate. These authors obtained practically zero removal of *E. coli* in VFs with gravel (d_{10} = 5 mm, d_{60} = 12 mm, Cu = 2.4) as the main filter material while VFs with sand (d_{10} = 0.34, d_{60} = 0.90 mm, d_{10}/d_{60} = 2.6) achieved removals ranged from 1 to 3 log units. However, higher pathogen removal was observed in horizontal subsurface flow CW in the range of 0.4 to 4.4 log units (Kadlec and Wallace, 2008). For example, Carballeira et al. (2016) achieved removals between 2–2.9 log units of FC and between 1–1.5 log units of *C. perfringens* when treating diluted municipal wastewaters using horizontal subsurface flow CW. Hernández-Crespo et al. (2022) obtained 1.6 log units for *E. coli* reduction in the first stage of CWs, which were two parallel units of horizontal subsurface flow CW (400 m^2 total surface area). The authors justified the low treatment efficiency due to the bed particle size, as the horizontal subsurface flow CW consisted of a grain size of 10–25 mm. In fact, Morató et al. (2014) reported a significant effect due to the use of finer filter material in horizontal subsurface flow CW to remove *E. coli*. However, Nivala et al. (2019) achieved removals above 1.5 log units by treating septic tank effluent using horizontal subsurface flow CW with particle size from 8–16 mm.

Wu et al. (2017) reported the treatment of a mixture of sanitary and industrial wastewater in a full-scale system consisting of a VF, a surface flow CW, and a horizontal subsurface flow CW in series. The integrated wetland system aimed to provide advanced treatment, achieving an overall *E. coli* removal of 98%. By stages, the removals of *E. coli* were 92%, 12% and 72% through VF, free water surface CW and horizontal flow CW, respectively (Wu et al., 2017). Ávila et al. (2015) treated municipal wastewater using a hybrid CW system (VF + horizontal subsurface flow CW + free water surface CW) at full scale and reported an overall *E. coli* removal of approximately 5 log units.

3.2 Photodegradation post-treatment at laboratory scale

Table 3 shows the mean concentrations for the influent to the post-treatment units (i.e., VF effluent) and for the different effluents collected at different times for the UVC, UVA/TiO_2 and Sol/TiO_2 post-treatments. The elimination efficiency (in percentage) and the reduction in log units, through each post-treatment, are shown in Fig. 3.

UVC disinfection (treatment duration of 30 min) proved to be the most effective post-treatment, removing from 4.3–5.2 log units for TC, 3.4–5.1 of *E. coli*, and 2.1–3.3 of *C. perfringens*. Therefore, the UVC post-treatment achieved an overall elimination of 99.99% considering the initial concentrations in the VF effluent (Table 3, Fig. 3).

The second most effective post-treatment was UVA/TiO_2 photocatalysis, reducing between 2.7–

Table 3 Mean initial concentration and mean final concentration (CFU/100 mL) after each post-treatment applied (mean ± standard deviation, $n = 3$)

Concentration	Total coliforms	*Escherichia coli*	*Clostridium perfringens*
Initial (VF effluent)	163333 ± 157665	37167 ± 33750	2003 ± 1704
Final (after post-treatment by)			
UVC	5.3 ± 8.7	2.2 ± 3.8	0.0 ± 0.0
UVA/TiO_2 1 h	69.7 ± 30.7	33.3 ± 39.2	11 ± 2.0
UVA/TiO_2 2 h	67.0 ± 58.8	29.7 ± 25.1	0.0 ± 0.0
Sol/TiO_2 1h	29933 ± 15768	8702 ± 12625	1008 ± 234
Sol/TiO_2 2h	11335 ± 12050	1364 ± 1375	888 ± 214

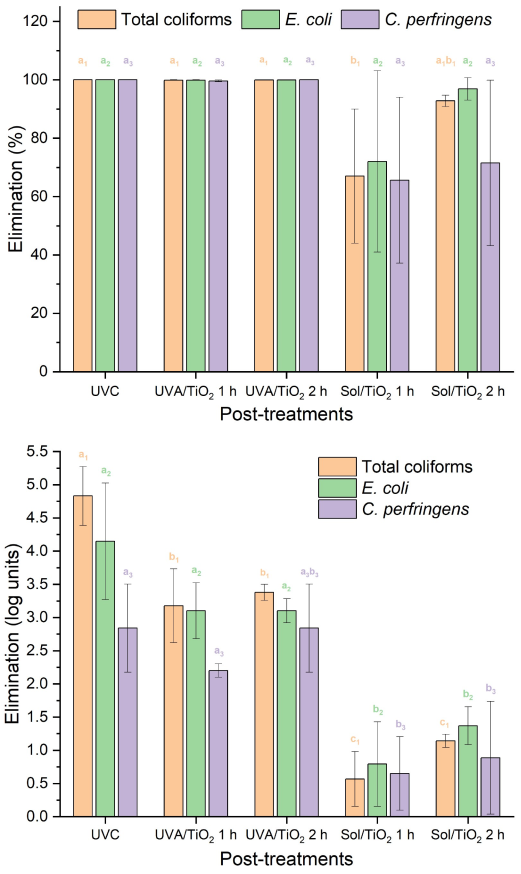


Fig. 3 Elimination of total coliform, *Escherichia coli* and *Clostridium perfringens* through each post-treatment. For each faecal indicator, different letters indicate significant differences between treatment systems (note that subscripts 1, 2, 3 refer to total coliforms, *E. coli*, and *C. perfringens*, respectively).

3.8 log units, 2.7–3.5 log units and 2.1–2.3 log units for TC, *E. coli* and *C. perfringens*, respectively, for the two treatment times applied. These eliminations are equivalent to percentages higher than 99.93%. Final concentrations were slightly lower after 2 h of treatment than after 1 h (Table 3).

Finally, the Sol/TiO_2 photocatalysis post-treatment was able to reduce the influent concentrations from 1.0–1.2 log units for TC, 1.1–1.7 log units for *E. coli* and 0.6–1.8 log units for *C. perfringens* after 2 h of treatment. In this case, the removal efficiencies improved during the second hour of treatment, as can be seen in Fig. 3.

Statistical analysis using ANOVA indicated that there were significant differences in the elimination of the different groups of faecal indicators depending on the post-treatment. In terms of removal percentage, UVC and UVA/TiO_2 treatments showed a significantly higher removal of TC than the Sol/TiO_2 1 h treatment. However, for *E. coli*, although the Sol/TiO_2 1 h treatment obtained a lower elimination percentage, it could not be shown that this value was statistically significant. The same occurred for *C. perfringens*, where no statistically significant differences were obtained despite the fact that the Sol/TiO_2 1 h treatment obtained a lower elimination percentage. This was due to the strong variation in influent concentrations that translates into a high standard deviation of the removal percentages, as can be seen in Fig. 3.

The differences in the elimination efficiency between treatment systems were clearer when the elimination was expressed in log units than in percentage. In log units, the three types of treatment presented statistically different efficiencies in the elimination of TC, while the process time in the TiO_2 treatments did not show significant differences (Fig. 3). Therefore, UVC treatment was the most effective in removing TC. However, in the case of both *E. coli* and *C. perfringens*, no significant differences were achieved between UVC and UVA/TiO_2 (both at 1 and 2 h), while the efficiency with Sol/TiO_2 was significantly lower (with the exception of *C. perfringens* with UVA/TiO_2 2 h). Although the Sol/TiO_2 was not as effective as the other two post-treatments, the eliminations obtained were of interest as the energy source was sunlight (abundant, renewable, safe, and clean) and the reduction of indicator microorganisms was on the order of those achieved in the VF unit.

The disinfecting effect of the radiation itself must be considered when interpreting these results. Exposure to UVA has been demonstrated to cause DNA damage and oxidative stress to cells through direct action of light (Giannakis et al., 2016). The metabolic cycle components may be particularly vulnerable to UVA radiation, and the potential contribution of singlet oxygen to this damage should be noted (Giannakis et al., 2022). However, UVA light has minimal impact on bacteria under these conditions, and significant germicidal effects require substantially higher exposure times and UVA doses than those examined in this study (Rodríguez-Chueca et al., 2017). Sunlight possesses a broader emission spectrum, incorporating a minimal percentage of UVB radiation, which is the principal agent responsible for DNA damage, thereby expediting the disinfection process (Coohill and Sagripanti, 2009). It is evident that both radiation types require the assistance of a photocatalyst to achieve sufficient bactericidal efficiencies. However, it is pertinent to consider the toxicity of TiO_2 in this context. The semiconductor possesses antimicrobial properties, attributable to its interaction with molecules within the cell wall (Leung et al., 2016). The inactivation of bacteria during photocatalytic disinfection is a process involving direct contact of the photocatalyst with the cell wall through electrostatic interaction, as well as damage to bacterial cells induced by reactive oxygen species (ROS) generated by the photocatalyst activity (Ganguly et al., 2018). However, while suspended photocatalysts can easily interact with present microorganisms and the inherent toxicity of nanoparticles, immobilized TiO_2 pellets, as used in this study, have shown low bactericidal rates attributed to the direct interaction of the catalyst with the bacterial cells (Aguilar et al., 2023a). A third possible mechanism for bacterial removal during the post-treatment is the adsorption of bacteria onto the surface of the photocatalyst. Although it was not specifically investigated in the present study, previous findings (Aguilar et al., 2023a) have shown that the disinfection of *E. coli* and *S. aureus* in the presence of photocatalytic pellets under dark conditions is negligible compared to the effect of sunlight alone. This observation rules out any significant contribution from both the toxicity of TiO_2 (as previously discussed) and bacterial adsorption onto the photocatalyst in the elimination of pathogenic microorganisms during post-treatment. Evidently, the inactivation observed in this study is predominantly attributable to $HO^\bullet$ and other ROS. However, the underlying mechanisms remain beyond the scope of the present study, although the bactericidal activity of this radical has been previously investigated, and it has been established that it is capable of damaging both the cell wall and membrane (Cho et al., 2004; Hou et al., 2012).

The wavelengths with the highest bactericidal effect are those between 250–270 nm, due to their capacity to damage their genetic material. The peak germicidal wavelength, which occurs at approximately 260 nm, corresponds to the maximum absorbance of nucleic acids, thereby inducing the dimerization of pyrimidine bases. The disruption of the DNA structure can impede accurate replication, leading to defective replication products and compromising the viability of the microorganism (Dai et al., 2012; Beck et al., 2015; Choi et al., 2020). Consequently, UVC at a wavelength of 254 nm as an external energy source was a key factor in microbial inactivation. In fact, the UVC post-treatment applied in this study had the highest efficiency in reducing the concentration of the three pathogens analysed, despite its significantly shorter application time. Adequate disinfection can also be achieved by heterogeneous photocatalysis using UVA or sunlight as an external energy source although longer exposure times may be necessary. For example, Bernabeu et al. (2011) removed 99% of *E. coli* in 100 min of treatment by solar photocatalysis using TiO_2. Furthermore, these authors found that after 2 h of treatment with UVA but without catalyst, some regrowth of *E. coli* appeared and concluded that the presence of TiO_2 was essential for complete faecal removal. Teodoro et al. (2017) evaluated the disinfection of greywater by heterogeneous photocatalysis using TiO_2 and a medium-pressure lamp emitting wavelengths between 300–700 nm. These authors compared the UV and UV/TiO_2 processes and found that the photocatalytic process was more efficient (0.4 log units, NMP/100 mL) although neither process achieved complete inactivation of TC.

In the same vein, Aguilar et al. (2023a) also tested the effect of a very similar TiO_2-based photocatalyst, but containing a different clay, under UVA or sunlight irradiation and concluded that the presence of photocatalyst considerably improved the inactivation of *E. coli*. In fact, single UVA irradiation removed about 2 log units of *E. coli* after 300 min exposure, while UVA irradiation with photocatalyst achieved removals in the range of 4 to 6.5 log units of *E. coli* for the same exposure time (Aguilar et al., 2023a). When Aguilar et al. (2023a) used UVA irradiation with 20 g/L of 80% TiO_2-based photocatalyst, slightly more than 5 log units of *E. coli* were eliminated after 2 h of treatment.

In our study, UVA irradiation with 40 g/L of 90% TiO_2-based photocatalyst eliminated 3.1 ± 0.2 log units of *E. coli* in 2 h. In the case of the experiments with 40 g/L of 80% TiO_2-based photocatalyst and sunlight, Aguilar et al. (2023a) removed 6 log units of *E. coli* in 30 min of treatment, while 1.4 ± 0.3 log units of *E. coli* were eliminated in our study using 40 g/L of 90% TiO_2-based photocatalyst and sunlight. The better performance obtained by Aguilar et al. (2023a) can be largely attributed to the use of synthetic waters in their study, as real wastewater/effluent contains organic and inorganic matter that can compete with bacteria for both radiation and photocatalyst (Mecha et al., 2019). Nonetheless, the effect of the slightly different formulation of the composite, both in terms of TiO_2 content and clay type and composition, on its photocatalytic activity cannot be dismissed. On the other hand, the differences in the efficiencies achieved in the study by Aguilar et al. (2023a) and the present study using sunlight may be due to the different configuration and scale of the system used, in the first case a laboratory device and in the present study a first adaptation to a pilot plant in real (outdoor) conditions, with a volumetric scale-up factor of 1282. This is precisely one of the challenges that require additional research, to transfer the laboratory results to pilot-scale and finally to full scale.

In addition, with regard to the synergies between the biological system and the photodegradation process, Sánchez et al. (2022) highlight the existence of several research gaps. In particular, they refer to the position of the PD unit within the combined system and the implementation of recirculation, which influences the concentration of radical scavengers and photosensitizers. These aspects are complex to assess and were not addressed in the present study. However, the placement of the PD unit downstream of the biological treatment guarantees a lower content of organic matter, especially suspended solids and turbidity, which certainly favours the efficiency of the photodegradation stage. In fact, a decrease in the efficiency of the photocatalytic treatment was observed at higher organic matter concentrations (Aguilar et al., 2023a).

3.3 First results for the faecal indicator microorganisms elimination through a combined HD + VF + p.Sol/TiO_2 system at pilot plant scale

Faecal indicator microorganisms content in samples obtained at 0, 0.5, 1, and 2 h of post-treatment in three batch trials carried out in the p.Sol/TiO_2 pilot system is shown in Fig. 4. For both TC and *E. coli*, the concentration decreased as the treatment time increased. In fact, the best elimination efficiency was obtained at 2 h of treatment, in agreement with the results obtained al lab-scale (Fig. 3). However, the efficiencies obtained at lab-scale were better than the efficiencies at pilot plant scale, both at 1 and 2 h. This discrepancy most probably arises from the different water layer heights in both studies, 5 cm in p.Sol/TiO_2 vs. 2.5 cm in Sol/TiO_2. The photocatalyst load employed was the same (40 g/L), but the upper surface exposed to sunlight in Sol/TiO_2 was double per wastewater volume thus also doubling the radiation received. Consequently, the inactivation of microorganisms caused by solar photolysis in laboratory experiments should also be higher. Additionally, the turbidity of VF effluent, although low, has a more significant impact on the light availability for the pellets situated at a deeper level (p.Sol/TiO_2). These factors contribute to the diminished efficiencies observed at the pilot plant scale.

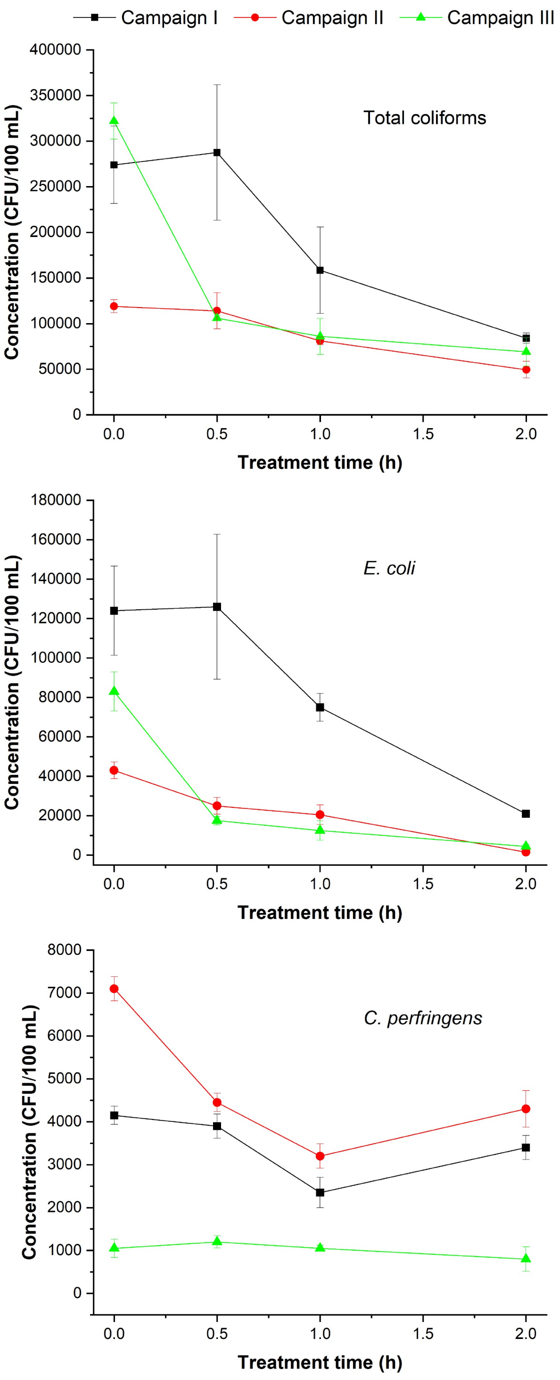


Fig. 4 Evolution in the concentration of total coliforms, *Escherichia coli* and *Clostridium perfringens* during 2 h of treatment under sunlight and TiO_2 photocatalysis of VF effluent in the p.Sol/TiO_2 pilot system.

As for *C. perfringens*, a different behaviour was observed. During the first hour of treatment, the concentration decreased over time. However, in two of the three campaigns, the concentration increased slightly in the last hour of treatment (Fig. 4). A clear explanation for the observed increase in *C. perfringens* concentrations between 1 and 2 h of post-treatment could not be determined. Although this could be due to possible regrowth or release of spores retained in the system, this possibility has not been proven. The limited overall reduction in *C. perfringens* concentrations could be added to the variability in the microbiological sampling itself. However, Figure 4 shows an initial reduction in *C. perfringens* concentration that diminished over time, suggesting a threshold effect in the potential elimination of *C. perfringens* by this process. The low efficiency of *C. perfringens* disinfection with solar treatment time was also observed by Gutiérrez-Alfaro et al. (2018), who used a parabolic solar collector as a solar water disinfection system and achieved a reduction of 0.9 ± 0.4 log units of *C. perfringens* by treating a flow rate of 27 L/min for more than 3 h. Gutiérrez-Alfaro et al. (2018) highlighted the difficulty of inactivating *C. perfringens* by solar disinfection due to its spore-forming capacity.

In addition, the fact that *E. coli* was removed to a greater extent than *C. perfringens* in all post-treatments (Figs. 3 and 5) is consistent with the premise that Gram-negative bacteria (i.e., *E. coli*) are more easily inactivated than Gram-positive bacteria (i.e., *C. perfringens*) as Gram-positive bacteria have a thicker cell wall (Venieri et al., 2020). However, some studies reported a higher disinfection rate for Gram-positive bacteria than Gram-negative bacteria (He et al., 2021; Aguilar et al., 2023a). This could be related to the ζ-potential on the surface of the photocatalyst, which, in this case, is largely influenced by the clay characteristics (Aguilar et al., 2023a). Therefore, more research is needed on the disinfection of real wastewater by solar photocatalysis.

Figure 5 clearly shows that the elimination percentages reached similar values despite varying influent or initial concentrations, which in turn led to highly variable removals in log units. The mean removal after 2 h of treatment was 68.8% ± 10.1% of TC, 91.3% ± 7.2% of *E. coli* and 27.1% ± 11.1% of *C. perfringens* (Fig. 5). This means that the photocatalysis pond reduced the concentration of TC, *E. coli* and *C. perfringens* by approximately 0.5 ± 0.1, 1.2 ± 0.3, and 0.1 ± 0.1 log units, respectively. The *C. perfringens* elimination efficiency was significantly lower than that of TC and *E. coli*, while the differences between the latter two were not significant.

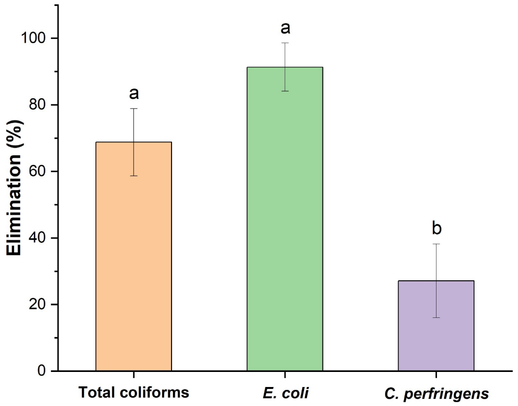


Fig. 5 Elimination of the three faecal indicator microorganisms studied during post-treatment of the VF effluent in the pilot-scale photocatalysis pond after 2 h. Different letters indicate significant differences between the three faecal indicators.

Although no literature was found on a photocatalysis pond as a tertiary treatment similar to the present study, some authors used other CW typologies based on solar radiation to disinfect their effluent. For example, García et al. (2008) treated domestic wastewater using a free water surface CW, which consisted of three connected ponds with a total capacity of 1.8 m^3, a surface area of 3.3 m^2, a hydraulic retention time of 3 d and a water depth of 20 cm. The non-planted free water surface CW unit used by García et al. (2008) removed 2.3 log units of *C. perfringens*. The higher efficiency of the system used by García et al. (2008), which did not have a photocatalyst, could be due to the longer hydraulic retention time (72 h) compared to that of this study (2 h). Ávila et al. (2015) removed approximately 3.5 log units of *E. coli* in a free water surface CW, which had been planted and operated for years, with a surface area of 240 m^2, 30 cm depth and a hydraulic retention time of 5.1 d. Hernández-Crespo et al. (2022) used a small pond of 13 m^2 with a hydraulic retention time of 19.2 h and a water depth of 40 cm to re-naturalise effluent from a wastewater treatment plant based on Imhoff tanks and horizontal subsurface flow CWs. These authors were able to achieve 1.6 log unit removal for *E. coli* in its pond system and highlighted the improvement of biodiversity due to the treatment pond. Overall, the photocatalysis pond employed in the present study improved the quality of the VF effluent, widening the possibilities for reuse of the reclaimed water as will be discussed in Section 3.4.

3.4 The quality of the final effluent

The final effluent quality was evaluated for possible reuse according to the requirements established in the

Spanish legislation (Ministerio de Medio Ambiente, 2007) and in the recent European regulation (European Union, 2020). In the present study, the parameter to be discussed is the concentration of *E. coli*, as this is the basic indicator of faecal contamination in both pieces of legislation. These regulations include different maximum acceptable values of *E. coli* for the different possibilities of reuse of reclaimed water. Tables 4 and 5 show the compliance or non-compliance of the final effluent obtained by each of the processes applied in this research according to the mentioned Spanish and EU regulations, respectively.

The concentration of *E. coli* in the effluent of the combined HD + VF system was 66916 ± 25722 CFU/100 mL (n = 3). Consequently, the HD + VF system was not able to comply with the maximum acceptable values of *E. coli* for most types of reuses specified in the Spanish legislation (Table 4). In this way, the effluent from the combined HD+VF system can only comply with environmental uses #5.3 (i.e., irrigation of forests, green areas and other non-public areas, and silviculture activities), where no limit is established for *E. coli*. Regarding environmental uses #5.4 (i.e., maintenance of wetlands, minimum flows and similar), no limit is established; however, the required minimum quality must be studied for each particular case. As far as the European regulation for agricultural irrigation is concerned (Table 5), the combined HD + VF system also does not comply with the maximum acceptable values for any quality class according to the concentration of *E. coli*.

Regarding the different post-treatments applied to the effluent from the combined HD + VF system, UVC and UVA/TiO_2 were effective in reducing the concentration of *E. coli* to values lower than those required by Spanish legislation for urban uses (#1.2), agricultural uses (#2.1 and #2.2) and environmental uses (#5.1) (Table 4). The lab-scale Sol/TiO_2 (2 h) post-treatment

Table 4 Compliance or non-compliance of the different technologies studied with the maximum acceptable value (CFU/100 mL) of *E. coli* for certain reuses of reclaimed water according to Spanish legislation (i.e., RD 1620/2007)

Type of use Quality type	MAV[a] for *E. coli*	HD+VF	Post-treatments			
			UVC	UVA/TiO_2	Sol/TiO_2	p.Sol/TiO_2
Urban uses						
#1.1. Residential (irrigation of private gardens; discharge of sanitary appliances)	0	No	No	No	No	No
#1.2. Service (urban green area irrigation; street washing; industrial vehicle washing; fire-fighting systems)	200	No	Yes	Yes	No	No
Agricultural uses						
#2.1. Irrigation of crops for obtaining fresh products prior to human consumption	100	No	Yes	Yes	No	No
#2.2. Irrigation of crops for obtaining products with subsequent industrial treatment prior to human consumption; irrigation of pastures for the consumption of animals producing milk or meat; aquaculture	1000	No	Yes	Yes	No	No
#2.3. Localised irrigation of woody crops; irrigation of ornamental flower crops, nurseries and derivates; irrigation of non-food industrial crops, nurseries and derivates	10000	No	Yes	Yes	Yes	Yes
Industrial uses						
#3.1. Process and cleaning water except in the food industry; other industrial uses	10000	No	Yes	Yes	Yes	Yes
#3.2. Evaporative condensers and cooling towers	Absence	No	No	No	No	No
Recreational uses						
#4.1. Irrigation of golf courses	200	No	Yes	Yes	No	No
#4.2. Ponds, bodies of water and ornamental flowing streams, where public access to the water is prevented	10000	No	Yes	Yes	Yes	Yes
Environmental uses						
#5.1. Aquifer recharge (localized percolation through the ground)	1000	No	Yes	Yes	No	No
#5.2. Aquifer recharge (direct injection)	0	No	No	No	No	No
#5.3. Irrigation of forests, green areas and other areas not accessible to the public; silviculture	No limit	Yes	Yes	Yes	Yes	Yes
#5.4. Other environmental uses (maintenance of wetlands)	Minimum quality requirements will be set on a case-by-case basis					

[a] Maximum acceptable value; The # symbol does not appear in the RD 1620/2007, but we added it here to facilitate identification and reference in the text of specific regulated uses.

Table 5 Compliance or non-compliance of the different technologies studied with the maximum acceptable value (CFU/100 mL) of *E. coli* for the reuse of reclaimed water in agricultural irrigation according to Regulation (EU) 2020/741

Reclaimed water quality class Crop category	MAV[a] for *E. coli*	HD+VF	Post-treatments			
			UVC	UVA/TiO_2	Sol/TiO_2	p.Sol/TiO_2
A. All food crops consumed raw where the edible part is in direct contact with reclaimed water and root crops consumed raw	10	No	No	No	No	No
B. Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals	100	No	Yes	Yes	No	No
C. Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops used to feed milk- or meat-producing animals [b]	1000	No	Yes	Yes	No	No
D. Industrial, energy and seeded crops	10000	No	Yes	Yes	Yes	Yes

Note: [a] Maximum acceptable value, [b] drip irrigation or other irrigation method.

reached mean concentrations of 1364 ± 1375 CFU/100 mL of *E. coli*, which means that the effluent obtained after 2 h of treatment by heterogeneous photocatalysis with sunlight could be potentially reused for agricultural uses (#2.3), for industrial uses (#3.1) or for recreational uses (#4.2) (Table 4), according to Spanish legislation. The p.Sol/TiO_2 post-treatment achieved mean concentrations of 9017 ± 10482 CFU/100 mL of *E. coli* after 2 h of treatment. Therefore, both at laboratory and pilot-scale, the heterogeneous photocatalysis process achieved the same potential reuse (i.e., uses #2.3, #3.1 or #4.2). However, the results of the p.Sol/TiO_2 post-treatment did not meet the requirement that 90% of the samples must have values lower than the maximum acceptable value.

As for the European regulation, the UVC and UVA/TiO_2 post-treatments achieved a reclaimed water quality class B (i.e., ⩽ 100 CFU/100 mL of *E. coli*, Table 5) while Sol/TiO_2 achieved reclaimed water quality class D (⩽ 10000 CFU/100 mL of *E. coli*). As with Spanish legislation, the effluent from the pilot-scale photocatalysis pond did not fully meet all the required monitoring criteria (as defined in Section 2.4). However, two of three analysed effluent samples from the photocatalysis pond reached *E. coli* concentrations below 10000 CFU/100 mL, suggesting a potential treatment capacity to achieve more restrictive uses or water quality classes (e.g., uses #2.3, #3.1 or #4.2 of the Spanish legislation and class D of the European regulation).

Other parameters regulated by both EU and Spanish legislation that limit the options for reuse of recovered wastewater are TSS, turbidity and helminth eggs (de Campos and Soto, 2024). The strictest limit regarding TSS is 10 mg/L for uses #1.1, #3.2 and #5.2 of the Spanish regulations (described in Table 4), being also the same value for all agricultural uses in the EU (Table 5). The average concentration of the HD+VF effluent was 5 ± 3 mg TSS/L (Sánchez et al., 2023), thus observing the required standard of TSS without additional treatment. However, neither turbidity nor helminth egg content was measured in that study. As pointed out by Gonzalez-Flo et al. (2023), although CWs can achieve very low TSS values, turbidity can be high due to dissolved organic matter produced in the CW, which is difficult to remove, although not considered a serious threat to public health (Gonzalez-Flo et al., 2023).

Regarding the elimination of helminth eggs in CW, studies are less frequent than those on TC, FC and *E. coli* (de Campos and Soto, 2024). However, some available studies indicate that helminth egg removal in CW and other natural, decentralized treatment systems is greater than in conventional, centralized systems. Also, in CWs, helminth eggs are often easier to remove than FC or *E. coli*. Amoah et al. (2018) found that decentralized wastewater treatment systems, consisting of anaerobic reactors with baffles and planted gravel filters, removed helminth eggs more efficiently than centralized sewage treatment plants. Decentralized wastewater treatment systems achieved 95%–100% removal of helminth eggs, which was due to filtration and sedimentation mechanisms (Amoah et al. 2018). The greatest reduction was shown by the anaerobic treatment step. Torrens et al. (2020) reported that raw wastewater had a high content in helminth eggs (mainly *Ascaris* spp.), but which were almost completely eliminated in pretreatment and the first stage of the CW. Zacharia et al. (2020) also found that natural wastewater treatment systems, such as sedimentation ponds and CWs, were effective in removing helminth eggs from wastewater.

The complete elimination of helminth eggs has also been reported by other authors. Darvishmotevalli et al. (2019) investigated the treatment of domestic wastewater in a system consisting of Imhoff tanks and horizontal flow CWs in series. These authors reported that despite achieving complete removal of helminth eggs, the treatment failed to produce effluent suitable for agricultural reuse according to EU guidelines, due to FC content. Gonzalez-Flo et al. (2023) reported that most microbial removal occurs in the CW, which played a fundamental role in improving water quality. However, even if *E. coli* and *Enterococcus* had achieved a 3 log unit removal in the hybrid CW, the effluent still required chlorine disinfection for the intended reuses. On the other hand, Gonzalez-Flo et al. (2023) reported that helminth eggs were usually not detected in the CW hybrid effluent after the first few years of operation. The authors speculated whether the high removal of helminth eggs was due to the development of vegetation and the maturation of the system, which would be in agreement with the results of Darvishmotevalli et al. (2019) when comparing the performance of the planted CW unit with the unplanted control.

3.5 Future directions and integration of photocatalysis in the CWs system

CWs, as decentralised wastewater treatment systems, succeed in reducing the concentration of pathogenic microorganisms. However, the reuse of their effluent for different purposes is regulated by a maximum acceptable value of concentration of different microbiological parameters, among others. So far, studies on pathogen removal in CWs have shown wide-ranging efficiencies. In terms of reuse of reclaimed water, CWs still need a post-stage to polish their effluent. In fact, in the present study, the CW effluent would not be suitable for any type of reuse according to Spanish and EU regulations due to the residual concentration of *E. coli*.

In this context, the present study treated municipal wastewater using a HD and a VF as biological units, which operated in series and in continuous operation. In order to reuse the reclaimed water, a photocatalysis pond (i.e., p.Sol/TiO_2) was tested in batch mode to treat the VF effluent. This photocatalysis pond was designed to simulate an intensified surface flow CW. The intensification was provided by heterogeneous photocatalysis processes, which were carried out by reactions between a novel TiO_2-based photocatalyst, sunlight (used as an energy source) and the VF effluent.

To compare results, the VF effluent was also treated on a lab-scale by three different photodegradation post-treatments: UVC photolysis, UVA/TiO_2 photocatalysis and Sol/TiO_2 photocatalysis, the latter two employing the same TiO_2-based photocatalyst. Despite the interest in TiO_2 as a photocatalyst, most of the studies on pathogen removal were on a lab-scale, so here we address a first research to move from laboratory scale to pilot-scale. Although the tested Sol/TiO_2 post-treatment, at both laboratory and pilot-scale, was not as effective as the other two post-treatments, the removals obtained were of interest as the energy source was sunlight, which is abundant, renewable, safe and clean. In addition, *E. coli* reduction by p.Sol/TiO_2 post-treatment was on the order of those achieved in the VF unit, thus doubling the treatment efficiency.

It is true that in order to polish the effluent of large wastewater treatment plants, heterogeneous photocatalysis requires unacceptable retention times. However, in a decentralised wastewater treatment (e.g. from a single-family house, group of houses, small communities, etc.), photocatalysis as a polishing technology in combination with CWs represents a very attractive option, so further research in pilot and full-scale systems is needed.

Photocatalytic disinfection efficiency is influenced by numerous factors and is highly dependent on the intensity and availability of incident light, which varies with both weather conditions and seasonal changes. Consequently, performance fluctuations are expected, and the system should be designed to operate effectively under the least favourable conditions. This may require increasing the illuminated surface area and/or extending the hydraulic retention time to ensure the effluent meets the required quality standards. Additionally, the recovery and reuse of the photocatalyst are commonly cited as major limitations of this technology in water treatment. While nano- and microparticulate catalysts are difficult and costly to recover, and supported materials can easily lose their catalytic surface, the pellets used in this study offer a practical and a cost-effective alternative, as they remain stationary at the bottom of the reactor while the contaminated water flows over them. Furthermore, we have observed that TiO_2 pellets exhibit good reusability: they were used in fifteen 7-h solar photodegradation cycles of phenol without any noticeable loss of activity (based on unpublished preliminary results).

The application of the photocatalysis pond as post-treatment to the combined HD + VF system had the capacity to reach certain reuse types (#2.3, #3.1 and #4.2) of the Spanish legislation and class D quality of reclaimed water for agricultural irrigation according to the European legislation, which were not achieved with CW treatment as a single system. This fact means an important improvement in the quality of the final effluent of a system that combines green technologies, low operation and maintenance costs and easy operation. However, the implementation of the photocatalysis pond in continuous operation with the combined HD + VF system, and a routine control according to the legislation, is necessary to conclude the feasibility of this post-treatment to develop into a fully operational advanced treatment.

Regarding the potential of CWs in combination with photodegradation post-treatments, the results of the present study can be considered preliminary. First, high variability was observed in the elimination figures. This is largely due to the high variability in the concentrations of microorganisms (in this case faecal indicators) in real wastewater from one moment to the next, which makes statistical comparisons between treatments and operating conditions difficult. On the other hand, the p.Sol/TiO_2 post-treatment was performed in batches, and it is advisable to experiment with configurations that allow continuous operation, integrating the post-treatment unit into the flow line of the overall system. An important variable in this regard is the depth of the water layer, which conditions the retention time and the transmission of solar radiation to the catalyst. Furthermore, these configurations must take into account the variation throughout the day of solar radiation, and in particular between the day and night periods, and between seasons. Therefore, more research is needed in this aspect.

The proposed combined system consists of 3 sequential treatment stages: HD + VF + p.Sol/TiO_2. As demonstrated in previous research, the three technologies contribute significantly to the removal of different conventional and emerging pollutants (Sánchez et al., 2022, 2023). HD ensures the removal of particulate organic matter, avoiding VF clogging, and the removal of nitric nitrogen (in the case of VF effluent recirculation, which is optional depending on the reuse objective). The VF unit performs nitrification and the removal of residual organic matter that is more difficult to biodegrade. Both units contribute to the removal of different emerging compounds, which is completed in the photocatalysis post-treatment. The elimination of pathogens is one of the main requirements for most options of treated water reuse. The three combined technologies also contribute to this objective. Although in terms of the parameters studied here, HD shows a lower contribution to the elimination of pathogens, its role in the elimination of helminth eggs should not be forgotten, as corroborated in the reviewed literature. VF and p.Sol/TiO_2 present a similar contribution to the elimination of pathogenic microorganisms, although the surface area used for the p.Sol/TiO_2 phase was only one third of that of VF.

In this relative sizing of the different stages, it was taken into account that the HD+VF system is an intensified system in relation to the typical sizing of CW systems. In fact, the VF unit used requires approximately 1 m^2 per equivalent inhabitant (1.7 m^2 per equivalent inhabitant for the 3-stage system, in summer conditions, including HD and p.Sol/TiO_2 2 h post-treatment). In this way, an intensive photodegradation post-treatment was sought, in which photocatalysis with TiO_2 leads to a very intensive and compact hybrid system. Optimizing the system for seasons with lower solar radiation and daytime periods is expected to require a larger footprint, but could still be economically viable for decentralized applications. CWs are considered a low-cost technology compared to traditional wastewater treatment systems. Recent evaluations have confirmed this aspect and estimated that the overall costs of wastewater treatment in CWs are two to three times lower than those of conventional intensive technologies (San Miguel et al., 2023). As an initial approximation, the catalyst production cost was estimated at €1 to €1.5 per kilogram. Thus, the cost of the catalyst, as a new element added, should not alter the classification of the 3-step system as a low-cost technology.

4 Conclusions

The combined HD + VF system removed approximately 1.0, 1.3 and 1.1 log units for TC, *E. coli* and *C. perfringens*, respectively, with the VF being the primary contributor to this removal. However, the combined HD + VF system could not comply with the maximum acceptable values of *E. coli* for any type of reuse with limitation of *E. coli* specified in Spanish and EU regulations.

At lab-scale, UVC photolysis was the most effective post-treatment, removing 4.8 ± 0.4, 4.1 ± 0.9, and 2.8 ± 0.7 log units for TC, *E. coli*, and *C. perfringens*, respectively. The second most effective post-treatment was UVA/TiO_2 photocatalysis after 2 h, which reduced 3.4 ± 0.1, 3.1 ± 0.2, and 2.8 ± 0.7 log units for TC, *E. coli*, and *C. perfringens*, respectively. These two treatments achieved similar results with regard to compliance with the *E. coli* reuse limit, as the treated effluent was compatible with various types of reuse in Spanish (8 out of 11 regulated uses) and EU (3 out of 4 regulated uses) legislation.

The Sol/TiO_2 photocatalysis post-treatment was able to reduce influent concentrations by 1.1 ± 0.1, 1.4 ± 0.3 and 0.9 ± 0.9 log units for TC, *E. coli* and *C. perfringens* after 2 h of treatment at lab-scale. An even lower removal efficiency was obtained for the p.Sol/TiO_2 post-treatment tested at pilot plant scale, which reached removals of approximately 0.5 ± 0.1, 1.2 ± 0.3 and 0.1 ± 0.1 log units for TC, *E. coli* and

C. perfringens, respectively. Regarding the *E. coli* limits, both the laboratory and pilot plant scale Sol/TiO_2 photocatalysis post-treatments showed similar and clearly lower results than those achieved by UVC and UVA/TiO_2 post-treatments. The treated p.Sol/TiO_2 effluent was compatible with only 3 out of 11 (Spanish regulation) and 1 out of 4 (EU regulation) regulated uses. However, this also represents a significant improvement in the final effluent quality with respect to the CW effluent, with a clear expansion of potential reuses.

CRediT Authorship Contribution Statement

M. Sánchez: Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing-original draft; Writing-review & editing. **E. Torres:** Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing-original draft; Writing-review & editing. **D. R. Ramos:** Data curation; Formal analysis; Methodology; Validation; Writing-review & editing. **S. Aguilar:** Data curation; Formal analysis; Validation. **M. Isabel Fernández:** Formal analysis; Methodology; Supervision; Validation. **I. Ruiz:** Conceptualization; Data curation; Investigation; Supervision; Validation; **M. Canle:** Conceptualization; Formal analysis; Methodology; Supervision; Funding acquisition; Project administration; Resources; Writing-review & editing. **M. Soto:** Conceptualization; Formal analysis; Methodology; Supervision; Visualization; Writing-original draft; Writing-review & editing.

Conflicts of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aguilar S, Guerrero B, Benítez Á, Ramos D R, Santaballa J A, Canle M, Rosado D, Moreno-Andrés J (2023a). Inactivation of *E. coli* and *S. aureus* by novel binary clay/semiconductor photocatalytic macrocomposites under UVA and sunlight irradiation. Journal of Environmental Chemical Engineering, 11(5): 110813

Aguilar S D, Ramos D R, Santaballa J A, Canle M (2023b). Preparation, characterization and testing of a bulky non-supported photocatalyst for water pollution abatement. Catalysis Today, 413–415: 113992

Al-Hazmi H E, Mohammadi A, Hejna A, Majtacz J, Esmaeili A, Habibzadeh S, Saeb M R, Badawi M, Lima E C, Mąkinia J (2023). Wastewater reuse in agriculture: prospects and challenges. Environmental Research, 236: 116711

Alufasi R, Gere J, Chakauya E, Lebea P, Parawira W, Chingwaru W (2017). Mechanisms of pathogen removal by macrophytes in constructed wetlands. Environmental Technology Reviews, 6(1): 135–144

Álvarez J A, Ruíz I, Soto M (2008). Anaerobic digesters as a pretreatment for constructed wetlands. Ecological Engineering, 33(1): 54–67

Álvarez J A, Soto M (2011). Anaerobic treatment of domestic wastewater. In: Caruana D J, ed. Anaerobic Digestion: Processes, Products and Applications. New York: Nova Science Publishers, 1–47

Amoah I D, Reddy P, Seidu R, Stenström T A (2018). Removal of helminth eggs by centralized and decentralized wastewater treatment plants in south Africa and Lesotho: health implications for direct and indirect exposure to the effluents. Environmental Science and Pollution Research, 25(13): 12883–12895

Armesto X L, Canle L M, García M V, Santaballa J A (1998). Aqueous chemistry of N-halo-compounds. Chemical Society Reviews, 27(6): 453–460

Ávila C, Bayona J M, Martín I, Salas J J, García J (2015). Emerging organic contaminant removal in a full-scale hybrid constructed wetland system for wastewater treatment and reuse. Ecological Engineering, 80: 108–116

Azaizeh H, Linden K G, Barstow C, Kalbouneh S, Tellawi A, Albalawneh A, Gerchman Y (2013). Constructed wetlands combined with UV disinfection systems for removal of enteric pathogens and wastewater contaminants. Water Science and Technology, 67(3): 651–657

Beck S E, Wright H B, Hargy T M, Larason T C, Linden K G (2015). Action spectra for validation of pathogen disinfection in medium-pressure ultraviolet (UV) systems. Water Research, 70: 27–37

Bernabeu A, Vercher R F, Santos-Juanes L, Simón P J, Lardín C, Martínez M A, Vicente J A, González R, Llosá C, Arques A, et al. (2011). Solar photocatalysis as a tertiary treatment to remove emerging pollutants from wastewater treatment plant effluents. Catalysis Today, 161(1): 235–240

Bohórquez E, Paredes D, Arias C A (2017). Vertical flow-constructed wetlands for domestic wastewater treatment under tropical conditions: effect of different design and operational parameters. Environmental Technology, 38(2): 199–208

Canle L M, Fernández M I, Martínez C, Santaballa J A (2012). (Re)greening photochemistry: using light for degrading persistent organic pollutants. Reviews in Environmental Science and Bio/Technology, 11(3): 213–221

Canle M, Fernández M I, Santaballa J A (2022). Applications of nanomaterials in environmental remediation. In: El-Fetouh Barakat M A, Kumar R, eds. Nanomaterials for Environmental Applications. Boca Raton: CRC Press, 1–22

Carballeira T, Ruiz I, Soto M (2016). Effect of plants and surface loading rate on the treatment efficiency of shallow subsurface constructed wetlands. Ecological Engineering, 90: 203–214

Carballeira T, Ruiz I, Soto M (2021). Improving the performance of vertical flow constructed wetlands by modifying the filtering media structure. Environmental Science and Pollution Research, 28(40): 56852–56864

Castellar J A C, Torrens A, Buttiglieri G, Monclús H, Arias C A, Carvalho P N, Galvao A, Comas J (2022). Nature-based solutions coupled with advanced technologies: an opportunity for decentralized water reuse in cities. Journal of Cleaner Production, 340: 130660

Cho M, Chung H, Choi W, Yoon J (2004). Linear correlation between inactivation of *E. coli* and OH radical concentration in TiO_2 photocatalytic disinfection. Water Research, 38(4): 1069–1077

Choi S W, Shahbaz H M, Kim J U, Kim D H, Yoon S, Jeong S H, Park J, Lee D U (2020). Photolysis and TiO_2 photocatalytic treatment under UVC/VUV irradiation for simultaneous degradation of pesticides and microorganisms. Applied Sciences, 10(13): 4493

Coohill T P, Sagripanti J L (2009). Bacterial inactivation by solar ultraviolet radiation compared with sensitivity to 254 nm radiation. Photochemistry and Photobiology, 85(5): 1043–1052

Dai T H, Vrahas M S, Murray C K, Hamblin M R (2012). Ultraviolet C irradiation: an alternative antimicrobial approach to localized infections? Expert Review of Anti-Infective Therapy, 10(2): 185-195

Darvishmotevalli M, Moradnia M, Asgari A, Noorisepehr M, Mohammadi H (2019). Reduction of pathogenic microorganisms in an Imhoff tank-constructed wetland system. Desalination and Water Treatment, 154: 283–288

de Campos S X, Soto M (2024). The use of constructed wetlands to treat effluents for water reuse. Environments, 11(2): 35

de la Varga D, Díaz M A, Ruiz I, Soto M (2013). Avoiding clogging in constructed wetlands by using anaerobic digesters as pre-treatment. Ecological Engineering, 52: 262–269

Dlamini M C, Maubane-Nkadimeng M S, Moma J A (2021). The use of TiO_2/clay heterostructures in the photocatalytic remediation of water containing organic pollutants: a review. Journal of Environmental Chemical Engineering, 9(6): 106546

European Union (2020). Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 May 2020 on Minimum Requirements for Water Reuse (Text with EEA Relevance). Brussels: European Union

Ferguson C M, Coote B G, Ashbolt N J, Stevenson I M (1996). Relationships between indicators, pathogens and water quality in an estuarine system. Water Research, 30(9): 2045–2054

Fernández del Castillo A, Garibay M V, Senés-Guerrero C, Orozco-Nunnelly D A, de Anda J, Gradilla-Hernández M S (2022). A review of the sustainability of anaerobic reactors combined with constructed wetlands for decentralized wastewater treatment. Journal of Cleaner Production, 371: 133428

Ganguly P, Byrne C, Breen A, Pillai S C (2018). Antimicrobial activity of photocatalysts: fundamentals, mechanisms, kinetics and recent advances. Applied Catalysis B: Environmental, 225: 51–75

García M, Soto F, González J M, Bécares E (2008). A comparison of bacterial removal efficiencies in constructed wetlands and algae-based systems. Ecological Engineering, 32(3): 238–243

Giannakis S, Gupta A, Pulgarin C, Imlay J (2022). Identifying the mediators of intracellular *E. coli* inactivation under UVA light: the (photo) Fenton process and singlet oxygen. Water Research, 221: 118740

Giannakis S, Polo López M I, Spuhler D, Sánchez Pérez J A, Fernández Ibáñez P, Pulgarin C (2016). Solar disinfection is an augmentable, *in situ*-generated photo-Fenton reaction. Part 1: A review of the mechanisms and the fundamental aspects of the process. Applied Catalysis B: Environmental, 199: 199–223

Gonzalez-Flo E, Romero X, García J (2023). Nature based-solutions for water reuse: 20 years of performance evaluation of a full-scale constructed wetland system. Ecological Engineering, 188: 106876

Gutiérrez-Alfaro S, Rueda-Márquez J J, Perales J A, Manzano M A (2018). Combining sun-based technologies (microalgae and solar disinfection) for urban wastewater regeneration. Science of the Total Environment, 619–620: 1049–1057

Hanaor D A H, Sorrell C C (2011). Review of the anatase to rutile phase transformation. Journal of Materials Science, 46(4): 855–874

He J H, Zheng Z X, Lo I M C (2021). Different responses of gram-negative and gram-positive bacteria to photocatalytic disinfection using solar-light-driven magnetic TiO_2-based material, and disinfection of real sewage. Water Research, 207: 117816

Hernández-Crespo C, Fernández-Gonzalvo M I, Miglio R M, Martín M (2022). *Escherichia coli* removal in a treatment wetland - pond system: a mathematical modelling experience. Science of the Total Environment, 839: 156237

Hou Y, Li X Y, Zhao Q D, Chen G H, Raston C L (2012). Role of hydroxyl radicals and mechanism of *Escherichia coli* inactivation on Ag/AgBr/TiO_2 nanotube array electrode under visible light irradiation. Environmental Science & Technology, 46(7): 4042–4050

Kadlec R H, Wallace S D (2008). Treatment Wetlands. 2nd ed. Boca Raton: CRC Press

Kali S, Khan M, Ghaffar M S, Rasheed S, Waseem A, Iqbal M M, Bilal khan Niazi M, Zafar M I (2021). Occurrence, influencing factors, toxicity, regulations, and abatement approaches for disinfection by-products in chlorinated drinking water: a comprehensive review. Environmental Pollution, 281: 116950

Kataki S, Chatterjee S, Vairale M G, Dwivedi S K, Gupta D K (2021). Constructed wetland, an eco-technology for wastewater treatment: a review on types of wastewater treated and components of the technology (macrophyte, biolfilm and substrate). Journal of Environmental Management, 283: 111986

Leung Y H, Xu X Y, Ma A P Y, Liu F Z, Ng A M C, Shen Z Y, Gethings L A, Guo M Y, Djurišić A B, Lee P K H, et al. (2016). Toxicity of ZnO and TiO_2 to *Escherichia coli* cells. Scientific Reports, 6: 35243

López D, Leiva A M, Arismendi W, Vidal G (2019). Influence of design and operational parameters on the pathogens reduction in constructed wetland under the climate change scenario. Reviews in Environmental Science and Bio/Technology, 18(1): 101–125

Ma D S, Yi H, Lai C, Liu X G, Huo X Q, An Z W, Li L, Fu Y K, Li B S, Zhang M M, et al. (2021). Critical review of advanced oxidation processes in organic wastewater treatment. Chemosphere, 275: 130104

Malato S, Fernández-Ibáñez P, Maldonado M I, Blanco J, Gernjak W (2009). Decontamination and disinfection of water by solar photocatalysis: recent overview and trends. Catalysis Today, 147(1): 1–59

Mecha A C, Onyango M S, Ochieng A, Momba M N B (2019). UV and solar photocatalytic disinfection of municipal wastewater: inactivation, reactivation and regrowth of bacterial pathogens. International Journal of Environmental Science and Technology, 16(7): 3687–3696

Ministerio de Medio Ambiente (2007). Real Decreto 1620/2007, de 7 de diciembre, por el que se establece el régimen jurídico de la reutilización de las aguas depuradas. Boletín Oficial del Estado, 294: 50639-50661 (in Spanish)

Ministerio de Medio Ambiente y Medio Rural y Marino (2010). .Guía para la Aplicación del R.D. 1620/2007 por el que se establece el Régimen Jurídico de la Reutilización de las Aguas Depuradas (in Spanish)

Morató J, Codony F, Sánchez O, Pérez L M, García J, Mas J (2014). Key design factors affecting microbial community composition and pathogenic organism removal in horizontal subsurface flow constructed wetlands. Science of the Total Environment, 481: 81–89

Nan X, Lavrnić S, Toscano A (2020). Potential of constructed wetland treatment systems for agricultural wastewater reuse under the EU framework. Journal of Environmental Management, 275: 111219

Nivala J, Boog J, Headley T, Aubron T, Wallace S, Brix H, Mothes S, van Afferden M, Müller R A (2019). Side-by-side comparison of 15 pilot-scale conventional and intensified subsurface flow wetlands for treatment of domestic wastewater. Science of the Total Environment, 658: 1500–1513

Pascual A, de la Varga D, Arias C A, Van Oirschot D, Kilian R, Álvarez J A, Soto M (2017). Hydrolytic anaerobic reactor and aerated constructed wetland systems for municipal wastewater treatment – HIGHWET project. Environmental Technology, 38(2): 209–219

Payment P, Franco E (1993). Clostridium perfringens and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. Applied and Environmental Microbiology, 59(8): 2418–2424

Rodríguez-Chueca J, Silva T, Fernandes J R, Lucas M S, Puma G L, Peres J A, Sampaio A (2017). Inactivation of pathogenic microorganisms in freshwater using HSO_5^-/UV-A LED and HSO_5^-/M^{n+}/UV-A LED oxidation processes. Water Research, 123: 113–123

Ramos D R, Canle M, Santaballa J A, Aguilar S D (2023). Photocatalyst element for fluid decontamination. Access from the website of consultas2.oepm.es

Ruiz I, Díaz M A, Crujeiras B, García J, Soto M (2010). Solids hydrolysis and accumulation in a hybrid anaerobic digester-constructed wetlands system. Ecological Engineering, 36(8): 1007–1016

Russo N, Marzo A, Randazzo C, Caggia C, Toscano A, Cirelli G L (2019). Constructed wetlands combined with disinfection systems for removal of urban wastewater contaminants. Science of the Total Environment, 656: 558–566

Sánchez M, Ramos D R, Fernández M I, Aguilar S, Ruiz I, Canle M, Soto M (2022). Removal of emerging pollutants by a 3-step system: hybrid digester, vertical flow constructed wetland and photodegradation post-treatments. Science of the Total Environment, 842: 156750

Sánchez M, Ruiz I, Soto M (2023). Sustainable wastewater treatment using a new combined hybrid digester–constructed wetland system. Journal of Environmental Chemical Engineering, 11(5): 110861

San Miguel G, Martín-Girela I, Ruiz D, Rocha G, Curt M D, Aguado P L, Fernández J (2023). Environmental and economic assessment of a floating constructed wetland to rehabilitate eutrophicated waterways. Science of the Total Environment, 884: 163817

Santos A F, Alvarenga P, Gando-Ferreira L M, Quina M J (2023). Urban wastewater as a source of reclaimed water for irrigation: barriers and future possibilities. Environments, 10(2): 17

Shingare R P, Thawale P R, Raghunathan K, Mishra A, Kumar S (2019). Constructed wetland for wastewater reuse: role and efficiency in removing enteric pathogens. Journal of Environmental Management, 246: 444–461

Suzuki H, Oonaka K, Hashimoto A (2021). Evaluation of *C. perfringens cpe*-positive strain as a source tracking indicator of human contamination in freshwater environments. Journal of Water and Environment Technology, 19(1): 1–12

Teodoro A, Boncz M Á, Paulo P L, Junior A M (2017). Desinfecção de água cinza por fotocatálise heterogênea. Engenharia Sanitaria e Ambiental, 22(5): 1017–1026

Torrens A, de la Varga D, Ndiaye A K, Folch M, Coly A (2020). Innovative multistage constructed wetland for municipal wastewater treatment and reuse for agriculture in Senegal. Water, 12(11): 3139

Venieri D, Mantzavinos D, Binas V (2020). Solar photocatalysis for emerging micro-pollutants abatement and water disinfection: a mini-review. Sustainability, 12(23): 10047

Wu S B, Carvalho P N, Müller J A, Manoj V R, Dong R J (2016). Sanitation in constructed wetlands: a review on the removal of human pathogens and fecal indicators. Science of the total environment, 541: 8–22

Wu Y H, Han R, Yang X N, Zhang Y K, Zhang R D (2017). Long-term performance of an integrated constructed wetland for advanced treatment of mixed wastewater. Ecological Engineering, 99: 91–98

Zacharia A, Outwater A H, Van Deun R. (2020). Natural wastewater treatment systems for prevention and control of soil-transmitted helminths. In: Summers J K, ed. Water Quality-Science, Assessments and Policy. London: IntechOpen