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Escherichia coli removal in a treatment wetland - pond system: A mathematical modelling experience

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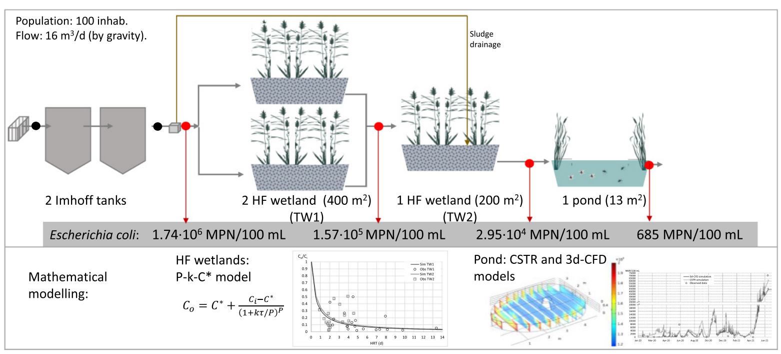
HIGHLIGHTS

- *E. coli* values suitable for water reuse without energy and reagent consumption.
- Small ponds at the end of a WWTP disinfect water and enhance biodiversity.
- Process-based models help to enhance the knowledge on *E. coli* decay.
- Solar disinfection and predation by daphnids are key for *E. coli* removal.

GRAPHICAL ABSTRACT



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ABSTRACT

A full-scale treatment wetland (TW) (100 inhabitants, 14 $m^3 \cdot d^{-1}$), composed of two horizontal subsurface flow wetlands (TW1–400 m^2 and TW2–200 m^2) and a small pond (13 m^2), has been evaluated for *Escherichia coli* (*E. coli*) removal. The results indicate a global removal from $1.74 \cdot 10^6$ to 685 MPN·100 mL^{-1} (3.41 log units), reducing *E. coli* sufficiently to reach values suitable for reuse purposes such as agricultural reuse, without energy and reagent consumption. The small pond at the end of the treatment train plays an important role in *E. coli* removal and biodiversity enhancement. Data from TW1 and TW2 have been fitted to the P-k-C* model, giving values of 134 and 100 $m \cdot yr^{-1}$ for the first-order kinetic reaction coefficient. For the pond, a process-based model using continuous stirred-tank reactor (CSTR) and a 3d-CFD model have been implemented and compared. The models indicate that solar disinfection and predation by daphnids are the most important mechanisms in the studied pond, representing 65% and 25% of the removal respectively. It can be concluded that CSTR can provide good results for small ponds and 3d-CFD model provides extra information, useful to enhance their design.

1. Introduction

Treatment wetlands (TWs) are cost-effective, sustainable, and affordable systems for wastewater treatment (Langergraber et al., 2019). This affordability is especially important in rural and remote communities where economic resources are scarce and where the access to sophisticated technologies is limited. This technology contributes to the long-term self-sufficiency and community empowerment, helping to achieve SDG 6 on water and sanitation for all.

In the current context of water scarcity, the reuse of reclaimed wastewater is an excellent opportunity to have a guaranteed water resource for agriculture or environmental uses (wetland maintenance, ecological flow, forestry, aquifer recharge, among others). Hence, assessing the ability of TWs to disinfect wastewater is crucial. There is a wide variety of pathogens, which can be divided into five groups: viruses, bacteria, fungi, protozoans, and helminths (Kadlec and Wallace,

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2009). It is common to select indicator organisms such as *Escherichia coli* (*E. coli*) (Headley et al., 2013; Wu et al., 2016). Water disinfection is important to provide reclaimed water safe for agricultural irrigation, to protect the environment and human and animal health (Regulation EU 2020/741). The use of non-disinfected water could cause different diseases because of the presence of pathogens (Kadlec and Wallace, 2009). Water reuse for agricultural irrigation or forestry also contributes to the circular economy by recovering nutrients from reclaimed water. This reduces the need of supplementary fertilizer application and restore nutrients to the natural biogeochemical cycles (Regulation EU 2020/741). This regulation has selected *E. coli* and intestinal nematodes eggs as indicators for reclaimed water quality, and *Legionella* spp. if there is a risk of aerosolization. It also foresees a validation monitoring based on different pathogen indicators (*E. coli* for bacteria, coliphages for pathogenic viruses, and *Clostridium perfringens* spores or spore-forming sulfate-reducing bacteria for protozoa) for new or renewed reclamation facilities.

Among the studies focused on pathogen removal in TWs and potential of effluent reuse, only 12 of 39 papers published from 2008 to 2019 are on full-scale systems (Nan et al., 2020). It is therefore important to improve the understanding of how full-scale systems work. Furthermore, the complexity and simultaneity of biotic and abiotic interactions hinders the identification of indicator bacteria removal mechanisms (Davies-Colley et al., 2000; Wu et al., 2016). Previous studies have shown high efficiencies of TWs for *E. coli* removal, especially in free water surface wetlands (FWS wetlands) (Ávila et al., 2015). Removal efficiencies vary between 94 and 99.999% for *E. coli* (2.7–5.4 log unit reduction) for the overall treatment systems, with hybrid and intensified systems as most efficient (Nan et al., 2020).

The aim of this study is to contribute with data from a full-scale TW-based WWTP and deepen the understanding of *E. coli* decay processes. Mathematical modelling of different *E. coli* removal mechanisms is an effective tool to enhance the understanding of the treatment system. This, in turn, facilitates the improvement of design and performance. Recent advances in pond modelling with computational fluid dynamics (CFD) have been done by previous authors, who generally simulate the *E. coli* decay through a unique reaction process (Sah et al., 2011; Gomes Passos et al., 2020; Allafchi et al., 2021; Dahl et al., 2021). In this study, a continuously stirred tank reactor (CSTR) and a three-dimensional CFD (3d-CFD) model, including several decay processes, are implemented and compared for the pond existing in the studied WWTP.

2. Overview of decay mechanisms and their mathematical modelling

The main removal processes and their kinetic rates (R_i) are described below.

- **Natural decay or die-off.** It is the death or inactivation of bacteria due to several processes. Starvation and exposure to physical and chemical stressors are common processes related to natural bacteria die-off (Wu et al., 2016). Predation is presumably included within this decay mechanism due to the difficulty of isolating both processes in aquatic environments (Davies-Colley et al., 2003; Craggs et al., 2004; Wu et al., 2016), but it can be considered an independent process (Kadlec and Wallace, 2009). Radiation damage is not considered to take part of this loss mechanism; indeed, it is commonly quantified through the dark death rate (Mayo, 1995; Davies-Colley et al., 2003; Craggs et al., 2004; Kadlec and Wallace, 2009). In general, a first-order kinetics is applied to simulate this decay process (Eq. (1)). Nevertheless, some authors have observed a two-phase pattern and accordingly proposed biphasic first-order kinetics for the process modelling (Easton et al., 2005; Hellweger et al., 2009).

$$R_{nd} = -k_{nd} \cdot C \tag{1}$$

where k_{nd} is the first-order rate constant in darkness (d^{-1}) and C is *E. coli* concentration (MPN or CFU/100 mL)

- **Solar disinfection**. Sunlight-mediated inactivation is a photoinactivation process that damage vital cellular components of bacteria (Curtis, 2003). It can be endogenous (direct or indirect) or exogenous (indirect), which likely occur simultaneously and interact, especially in bacteria (Nelson et al., 2018). Only a minor fraction, typically less than 1%, of the total solar irradiance causes disinfection, concretely UV wavelengths in the range 290–400 (Craggs et al., 2004; Kadlec and Wallace, 2009). However, total solar radiation is usually used to obtain the rate constant for pragmatism reasons, as then climate station records can be taken (Craggs et al., 2004; Davies-Colley et al., 2003; Mayo, 2004). Consequently, solar inactivation rates based on total solar radiation are much lower than those based on UV lamp sources (Kadlec and Wallace, 2009). Light extinction with depth can be modelled by the Beer-Lambert law, which indicates an exponential decay of light with depth. It can be integrated for the water depth (Eq. (2)).

$$S_H = \frac{S_0}{K_e \cdot H} \cdot \left(1 - e^{-K_e \cdot H}\right) \tag{2}$$

where (K_e, m^{-1}) is the extinction coefficient which can be related to suspended solids by the Eq. (3) (Chapra, 1997), S_0 is the total solar irradiation ($MJ \cdot m^{-2} \cdot d^{-1}$) and H is water depth (m). Furthermore, a correction factor to consider the ratio of the surface area exposed to solar intensity (not covered by plants or floating algae) can be applied (Kalibbala et al., 2008). Thus, the kinetic rate term could read as Eq. (4).

$$K_e = 0.55 \cdot TSS \tag{3}$$

$$R_S = -\frac{k_S \cdot S_0 \cdot \lambda_t}{K_e \cdot H} \cdot \left(1 - e^{-K_e \cdot H}\right) \cdot C \tag{4}$$

Where TSS are the total suspended solids ($mg \cdot L^{-1}$), k_S is the solar disinfection rate constant ($m^2 \cdot MJ^{-1}$), λ_t is the ratio of surface area exposed to solar irradiation (unitless) and C is *E. coli* concentration ($MPN \cdot 100\ mL^{-1}$).

- **Sedimentation.** Bacteria have a settling velocity too small to settle on their own, so they are only removed by sedimentation if they attach to larger particles or clump together to form aggregates with a sufficiently high settling velocity (Kadlec and Wallace, 2009; Jasper et al., 2013). Davies-Colley et al. (2003) estimated a removal of 40% of *E. coli* by settling attached to algal biomass. Therefore, a modelling approach for this process should be linked to the adsorption process. If the rate at which bacteria adsorb and desorb from particles is fast, a local equilibrium can be assumed, and the bacteria particulate fraction (attached to particles) can be estimated and used to calculate the sedimentation process through Eq. (5) (Chapra, 1997).

$$R_{sed} = -\frac{K_d \cdot TSS}{1 + K_d \cdot TSS} \cdot \frac{v_s}{H} \cdot C \tag{5}$$

where K_d is a partition coefficient ($m^3 \cdot g^{-1}$), v_s is the settling velocity of the particles ($m \cdot d^{-1}$), H is water depth (m) and C is *E. coli* concentration ($MPN \cdot 100\ mL^{-1}$).

- **Filtration**. Mechanical filtration plays an important role in pathogens removal, especially in subsurface flow TWs (Wu et al., 2016). It can be modelled through a first-order kinetics (Eq. (6)), where the filtration coefficient (λ) depends on properties of the filter bed (grain size and shape, porosity, depth), influent characteristics and operating conditions (Crittenden et al., 2012). The reviews by Wu et al. (2016) and Shingare et al. (2019) show that experimental studies do not always obtain a significant influence of grain size. In this sense, it is important to consider that the accumulation of intercepted solids and the biofilm growth can reduce the effective porosity. This reduction of porosity should be considered in subsurface flow TW modelling (Samsó and Garcia, 2013).

$$R_f = -\lambda \cdot C \tag{6}$$

where λ is the filtration coefficient.

- **Adsorption**. Adsorption of bacteria to different surfaces present in wetlands is determinant in filtration and sedimentation processes, as discussed above. These attaching surfaces are varied, including suspended inorganic or organic particles, such as minerals or algae, biofilms, submerged macrophyte and submerged parts of emergent and floating plants as well as their roots, or substrates, protozooplankton (Kadlec and Wallace, 2009; Wu et al., 2016; Shingare et al., 2019) or larger organisms. This process can also condition the efficiency of solar disinfection, as attached bacteria may be protected from radiation effects or against grazing (Nguyen et al., 2016).
- **Predation**. Grazing by filtering feeding organisms contribute to bacteria removal in TWs, ponds and natural aquatic systems (Kadlec and Wallace, 2009; Jasper et al., 2013). In particular, cladocerans have been proven to increase the *E. coli* loss rate, highlighting that predation pressure could be important, especially during seasonal peaks of *Daphnia* (Burnet et al., 2017). These authors performed experiments to assess both culturability and viability of *E. coli* over time and found absence of viable but non-culturale (VBNC) cells after exposure to *Daphnia*. Mathematical modelling is usually approached as a first-order kinetics, but by analogy with the modelling of the process of phytoplankton grazing (Chapra, 1997), it may be convenient to introduce the concentration of organisms (Eq. (7)).

$$R_p = -k_p \cdot P \cdot C \tag{7}$$

where k_p is the filtration rate (L·individual^{-1}·d^{-1}), P is the predator concentration (ind·L^{-1}). A Michaelis-Menten term for *E. coli* concentration (C) (Eq. (8)) could be considered to account for the fact that at high levels of substrate (C) the grazing rate levels off and, for low levels of substrate, it limits the grazers growth (Chapra, 1997).

$$\frac{C}{k_{sC} + C} \tag{8}$$

where k_{sC} (MPN·100 mL^{-1}) is the half-saturation constant for zooplankton grazing on *E. coli*.

Factors affecting the removal mechanisms are described below.

- **Dissolved oxygen (DO)** at high concentration promotes the photooxidation process (Davies-Colley et al., 2000). High concentrations of DO increase the probability to form toxic forms of oxygen (singlet oxygen, hydroxyl radicals, super-oxide radicals or hydrogen peroxide) (Curtis, 2003).
- Levels of **pH** above 8.5 also enhance *E. coli* inactivation, apparently due to exogenous photooxidation and overwhelming of homeostatic mechanism (Davies-Colley et al., 2000; Curtis, 2003). A synergistic effect was observed when both DO, and pH were elevated (Davies-Colley et al., 2000). This synergism might occur because the oxygen radicals damage the membrane, thus leaving the cells more vulnerable to high pH, or/and because of the prolongation of hydroxyl radicals' life (Curtis, 2003). Low values of pH, because of high nitrification rates, also lead to faster die-off of faecal coliforms (Wu et al., 2016). First-order kinetic rates or power functions have been proposed for introducing the influence of pH and DO in the bacteria loss rate (Mayo, 1995, 2004; Kalibbala et al., 2008).
- **Salinity** is apparently lethal for bacteria with membrane cell damaged by photooxidation (Davies-Colley et al., 2000) and a summand to the natural decay rate coefficient (Eq. (9)).

$$k_{nd} + 0.02 \cdot S \tag{9}$$

where S is salinity (g·L^{-1}), can be proposed for mathematical modelling (Chapra, 1997).

- **Temperature** rises lead to an acceleration of biological processes, however there is no clear consensus about the impact on faecal bacteria removal. Indeed, theta values of Arrhenius equation (Eq. (10)), for first-order rate constant correction varied between 0.952 and 1.073 for horizontal flow wetlands (HF wetlands) and from 0.951 to 1.030 for FWS wetlands (Kadlec and Wallace (2009). Recent reviews reported a positive influence of temperature on faecal bacteria removal (Wu et al., 2016; Shingare et al., 2019), such as the study by Elfanssi et al. (2018). Hernández-Crespo et al. (2022) found a significant positive correlation between removal efficiency of *E. coli* and temperature in a continuously saturated subsurface flow wetland with drinking water sludge as substrate, while this was not the case for a sequential operation wetland.

$$k = k_{20} \cdot \theta^{T-20} \tag{10}$$

where k_{20} is the rate constant at 20 °C, θ is the modified Arrhenius temperature factor, dimensionless.

- **Vegetation** can affect faecal bacteria removal for several reasons, including its influence on system hydraulics and oxygenation, exudation of antibiotic substances in the rhizosphere or increased surface area for biofilm development or adsorption (Kadlec and Wallace, 2009; Wu et al., 2016). However, there is not a universal conclusion about the significance of its influence in subsurface flow TWs (Headley et al., 2013; Wu et al., 2016; Nivala et al., 2019). In FWS wetlands, floating and emergent plants can influence negatively on bacteria removal because of the shadow effect or the reduced surface oxygen exchange, which in turn might decrease predator zooplankton populations (MacIntyre et al., 2006).
- **Depth** can influence faecal bacteria removal in different ways. In HF wetlands, a deeper bed can provide a longer hydraulic retention time (HRT), if there are not preferential paths. Morató et al. (2014) obtained lower efficiencies in shallower beds for total coliforms, *E. coli* or faecal enterococci while better efficiencies for *Clostridium* spores, compared to deeper HF wetlands. However, in general, enhanced removal is found in shallower bed depths (Kadlec and Wallace, 2009). A likely reason is that a larger fraction of water volume is in contact with rhizosphere, which can favour the microbial removal (Morató et al., 2014). In case of vertical flow wetlands (VF wetlands), an increase of depth implies a longer filtration path. Indeed, a linear rate of $\log_{10}$ concentration reduction with depth was found by Headley et al. (2013). In FWS and ponds, an increase of depth can also provide a longer HRT, however, as solar radiation is a major mechanism, the higher rates in shallow systems compensate for the shorter HRT (Davies-Colley et al., 2003).
- Lower **hydraulic loading rate (HLR)** and longer **HRT** generally enhance removal processes (Kadlec and Wallace, 2009). Hernández-Crespo et al. (2022) found a significant negative correlation between HLR and *E. coli* removal efficiency for a continuously saturated subsurface flow wetland with drinking water sludge as substrate, while this was not the case for a sequential operation wetland.
- **Filter media grain size** does affect removal efficiency, finer bed materials providing better performance (Headley et al., 2013; Morató et al., 2014), as long as they do not compromise the permeability of the system (Kadlec and Wallace, 2009).

3. Materials and methods

3.1. Study area, analytical procedures, and statistical analyses

3.1.1. Study area

The WWTP of Carricola, a small village (100 inhabitants, 113 PE) located in Valence (Spain) has been monitored for seven years. It is based on TW technology, consisting of two Imhoff tanks in series, two serial HF wetlands (TW1 composed of two wetland cells in parallel and TW2 with one single cell) and a small pond for renaturalization of treated wastewater (Fig. 1). Water flows by gravity throughout the system. The connecting pipe (0.3 m diameter) between IT and TW1 ends with an open vertical pipe, 1 m above the ground of the distribution chamber to TW1. Outflow is like a small waterfall (Fig. 1), aerating the sewage before its distribution to TW1. The consequence of this elevation is the flooding of part of the pipeline. It provokes a sedimentation of solids in the pipe connecting IT-eff and TW1-inf. This pipe is drained every month into TW2. The main design and operation parameters are indicated in Table 1. The difference in flow between the outflow of TW2 and the inflow to the pond is because part of the flow is directly discharged into a stream, and part is lost through evapotranspiration (which varied between 0 and 1.5 $m^3 \cdot d^{-1}$ for the study period). The pond has a small islet in the middle, for amphibians.

3.1.2. Sampling and analytical procedures

Grab samples for water quality analysis were taken in different sites since 2014 to the present with a monthly frequency. The following variables were analysed: Total Nitrogen (TN), ammonium, nitrites, nitrates, phosphate and total phosphorus, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total and Volatile Suspended Solids (TSS, VSS), turbidity, temperature, dissolved oxygen, electrical conductivity (EC) and pH. Methods are indicated in Table S1.

From February 2020 to January 2022 *E. coli* was analysed as indicator organism, as it is considered in European and Spanish water reuse regulations (EU regulation 2020/741 and RD1620/2007). It was analysed using the Colilert-18 Quanti-Tray™ method (IDEXX Laboratories) as specified by the manufacturer. Quality control was performed by analysing blanks, which were all negative, and certified reference material (*Escherichia coli* WDCM 00013 VT000136, 4100 CFU·100 mL^{-1}, range 2000–8400 CFU·100 mL^{-1}, Sigma-Aldrich), obtaining a recovery of 96% (3956 ± 500 MPN·100 mL^{-1}). Intestinal nematode eggs were determined by the Bailinger's modified method in five samplings, and none was found, neither at the inlet nor at any sampling point.

Aquatic invertebrates were sampled using a net (125 μm pore size) to sweep 1 m of the pond. The abundance was after assessed by counting the invertebrate under a stereomicroscope and dividing by the corresponding sampled volume.

Table 1
Design and operation parameters of Carrícola's WWTP.

Parameter	Value
Treatment capacity (PE)	150
Dimensions	
Total (m^2)	613
TW1 (m^2)	400
Length:width	2.75:1
TW2 (m^2)	200
Length:width	5.5:1
Pond (m^2)	13
Length:width	1.4:1
Plants	*Phragmites australis*
Harvesting frequency	1 per year
Grain size (mm) (TW1, TW2)	10–25
Start of operation	2014
Hydraulic loading rate (HLR)	
TW1 ($m^3 \cdot m^{-2} \cdot d$)	0.035
TW2 ($m^3 \cdot m^{-2} \cdot d$)	0.070
Pond ($m^3 \cdot m^{-2} \cdot d$)	0.508
Hydraulic retention time	
TW1 (d)	5.5
TW2 (d)	2.6
Pond (d)	0.8
Organic loading rate (BOD_5) (plan area)	
TW1 ($g \cdot m^{-2} \cdot d$)	3.3
TW2 ($g \cdot m^{-2} \cdot d$)	1.1
Pond ($g \cdot m^{-2} \cdot d$)	6.3
Organic loading rate (BOD_5) (cross-sectional area)	
TW1 ($g \cdot m^{-2} \cdot d$)	392
TW2 ($g \cdot m^{-2} \cdot d$)	155
Pond ($g \cdot m^{-2} \cdot d$)	58
Water level (in relation to media depth)	
TW1 (m)	0.28
TW2 (m)	0.24
Pond (m)	0.40

3.1.3. Statistical analyses

Statistical analyses were performed using Statgraphics XVII centurion. Water quality variables, including COD, BOD_5, TSS, TN and TP were compared in different sampling points. ANOVA and multiple range test (Fisher's Least Significant Difference) were used if normality was met, and Kruskal-Wallis test, otherwise. Statistical significance was indicated by a probability of type I error < 5% ($p < 0.05$).

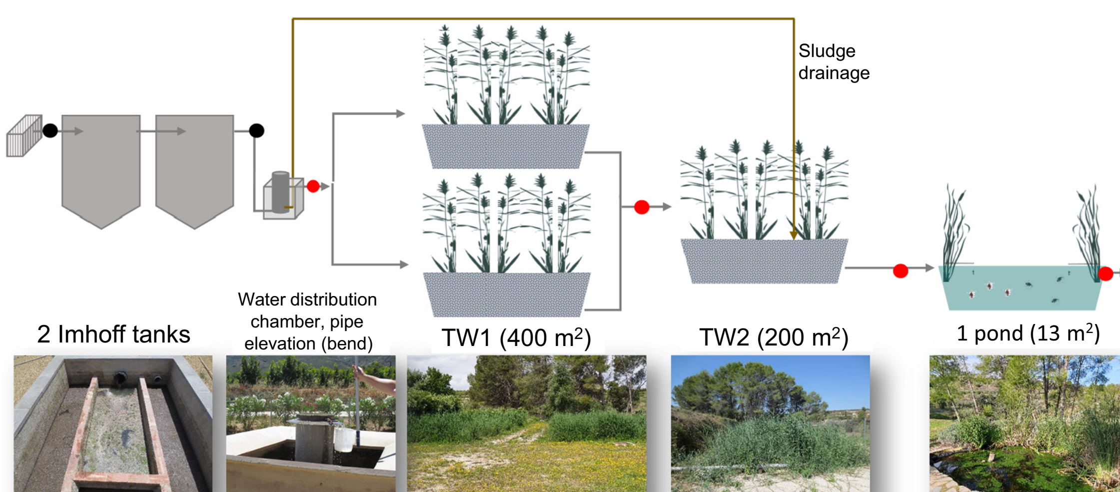

Fig. 1. Scheme of Carricola's WWTP, indicating sampling sites for water quality monitoring (black and red dots) and *E. coli* (red dots).

3.2. Mathematical modelling of E. coli

3.2.1. P-k-C* model for HSSF wetlands

The P-k-C* model developed by Kadlec and Wallace (2009) was implemented for *E. coli* modelling in TW1 and TW2. This model is a modified first-order equation with a non-zero background concentration (Eq. (11)).

$$C_o = C^* + \frac{C_i - C^*}{(1 + k\tau/P)^P} \tag{11}$$

where C_o is the outlet concentration, C_i is the inlet concentration, C* is the background concentration (was set equal to 100), k is the first-order reaction coefficient (1/d), τ is the nominal (theoretical) hydraulic retention time (d), P is the apparent number of tanks in series (dimensionless), $k = k_{20} \cdot \theta^{T-20}$, where T is the temperature (°C), P was estimated because a value calibrated with a tracer test was not available.

For calibration, first the procedure described by Ventura et al. (2022) was followed to obtain the parameters k_{20}, θ (Arrehnius temperature correction factor) and P (apparent number of tanks in series). Briefly, the Excel solver tool was used to estimate the values of k_{20} and θ that minimized the root-mean-square error (RMSE) and then P was varied to minimize RMSE, after that k_{20} and θ were estimated again and so on until no variation of the values was reached. The normalised to mean RMSE (%) obtained was high, around 80%. In a second phase, the relative error between means, observed and simulated, was also considered and constants were modified to minimize it, checking that RMSE (%) did not increase significantly.

3.2.2. Process-based models for pond: CSTR and 3d-CFD

3.2.2.1. Model description. A process-based model including natural decay, solar disinfection, predation, and sedimentation was implemented for the pond, considering the kinetic rates described in Eqs. (1)–(5), (7) and (10). A wide range of variation was observed in literature for the values of rate constants (Table 3). To reduce the uncertainty and support the calibration of rate constants, ancillary experiments described in Supplementary Information were carried out (S3.2.2.a).

Goodness-of-fit indicators, such as mean-normalised RMSE (%), Nash–Sutcliffe model efficiency coefficient (NSE), relative error between observed and simulated mean values, and observed versus simulated graph, were used to fit the model.

The model was implemented in the software COMSOL Multiphysics. A continuous stirred-tank reactor (CSTR) and a 3d-CFD model were built and compared to check the differences between them, and to assess the suitability of using the CSTR in small systems. Specific information on the 3d-CFD model (mesh characteristics and simplifications) is indicated in SI (S3.2.2.b).

3.2.2.2. Impulse tracer test. An impulse tracer test was performed to evaluate the hydrodynamic behaviour of the pond. A concentrated solution of sodium chloride (100 $g \cdot L^{-1}$) was slowly dosed and mixed with the influent to achieve a significant increase in the influent salinity. The dosage time (2.3 h) was estimated to provoke a raise in the influent salinity from 0.4 to 1.3 $g \cdot L^{-1}$ (1240 to 2600 $\mu S \cdot cm^{-1}$). This implied a density increase lower than 1% of the density of ambient wastewater in the pond, a limit recommended in literature to avoid a density-induced stratification (Headley and Kadlec, 2007; Kadlec and Wallace, 2009). Electrical conductivity (EC) was registered every 10 min in the outlet, with a probe placed at half the depth. The mean tracer retention time (τ) and the theoretical or nominal hydraulic retention time (nHRT) were calculated according to Eqs. (12) and (13) (Levenspiel, 1999; Headley and Kadlec, 2007).

$$\tau = \frac{\int_0^\infty t \cdot C(t) \cdot dt}{\int_0^\infty C(t) \cdot dt} \cong \frac{\sum_i t_i \cdot C_i \cdot \Delta t_i}{\sum_i C_i \cdot \Delta t_i} \tag{12}$$

where t is time since tracer addition, C (t) is the exit tracer concentration at time t since tracer addition.

$$\mathrm{nHRT} = \mathrm{V/Q} \tag{13}$$

where V is the volume (m^{-3}) of the pond and Q the inlet flow ($m^3 \cdot d^{-1}$).

3.2.2.3. Sensitivity analysis. The parameter perturbation method was used to assess the sensitivity of the model to each parameter. This method consists of varying each of the model parameters a fixed percentage while holding the others constant. In this study, a variation of 20% was applied, except for the temperature correction factors (θ_{nd}, θ_p, θ_S), which were varied between the minimum and the maximum found in literature (Table 3). The corresponding variations of the state variable, *E. coli*, reflect the model sensitivity to the varied parameter (Chapra, 1997). The variation can be quantified as Eq. (14).

$$\Delta C = \frac{C(k + \Delta k) - C(k - \Delta k)}{2} \tag{14}$$

where k is a parameter of the model.

Table 2
Water quality (WQ) results in different sites of Carricola's WWTP. Mean ± standard deviation values are presented ($n = 79$ for all sites except pond, $n = 22$ for pond). Inf: influent, Eff: effluent, IT: Imhoff tank. TW2 Inf is equal to TW1 effluent and Pond Inf is equal to TW2 effluent. In parenthesis the concentration reduction in primary treatment, TW1, TW2 and pond, respectively.

WQ variable	WWTP Inf	IT Eff	TW1 Inf	TW2 Inf	Pond Inf	Pond Eff
COD ($mg \cdot L^{-1}$)	794 ± 568	307 ± 138 (61%)	193 ± 136	57 ± 24 (70%)	42 ± 19 (26%)	49 ± 26 (−17%)
BOD_5 ($mg \cdot L^{-1}$)	485 ± 386	169 ± 81 (65%)	94 ± 83	15.9 ± 9.8 (83%)	12.4 ± 12.9 (22%)	13.7 ± 6.7 (−10%)
TSS ($mg \cdot L^{-1}$)	266 ± 434	76 ± 41 (71%)	38 ± 27	4.9 ± 3.3 (87%)	3.1 ± 3.7 (37%)	16.9 ± 15.6 (−445%)
TN ($mg \cdot L^{-1}$)	49.4 ± 27.4	53.4 ± 19.2 (−8%)	50.8 ± 20.5	38.7 ± 15.9 (24%)	31.3 ± 13.5 (19%)	29.7 ± 11.5 (5%)
NH_4^+-N ($mg \cdot L^{-1}$)	31.4 ± 17.1	44.7 ± 16.4 (−42%)	43.3 ± 16.8	35.9 ± 14.9 (17%)	28.5 ± 12.8 (21%)	23.9 ± 10.7 (16%)
NO_2^--N ($mg \cdot L^{-1}$)	0.15 ± 0.12	0.05 ± 0.02 (67%)	0.05 ± 0.02	0.04 ± 0.03 (20%)	0.05 ± 0.13 (−25%)	0.20 ± 0.12 (−300%)
NO_3^--N ($mg \cdot L^{-1}$)	0.65 ± 0.63	0.32 ± 0.31 (51%)	0.39 ± 0.40	0.38 ± 0.42 (3%)	1.47 ± 2.25 (−287%)	2.47 ± 2.55 (−68%)
TP ($mg \cdot L^{-1}$)	6.9 ± 4.0	6.6 ± 2.3 (4%)	5.9 ± 2.2	5.4 ± 1.9 (8%)	5.2 ± 1.8 (4%)	5.7 ± 1.3 (−10%)

4. Results and discussion

4.1. Water quality results

Results of water quality indicate a good global performance of Carricola's WWTP for COD (94%), BOD_5 (97%) and TSS (94%), complying with the requirements of the European regulation concerning urban wastewater treatment (Council Directive 91/271/EEC) (Table 2). Regarding nitrogen and phosphorus, it is noteworthy that the WWTP does not have discharge requirements because it does not discharge into a sensitive zone. Nitrogen removal is moderate (40%), because it does not have a combination of vertical and horizontal flow wetlands to promote nitrification-denitrification processes. An increase of nitrification has been observed during last two years, probably related to the lower depth fixed in this period (Fig. S3). Total phosphorus removal is also modest (17%) because it lacks a specific wetland cell or area with adsorbent material to remove phosphorus from water. Nevertheless, the decrease from the influent to the final effluent is statistically significant for COD, BOD_5, TSS, TN and TP ($p < 0.05$).

It is remarkable the buffering effect of wetland systems, i.e. the influent to the WWTP is highly variable in composition whereas the effluent from TW1 and TW2 is much more constant, as indicated by the standard deviations shown in Table 2.

Similarly, *E. coli* decreases gradually throughout the system (Fig. 2). In TW1 the concentration significantly decreased from an average of $1.74 \cdot 10^6$ to $1.57 \cdot 10^5$ MPN·100 mL^{-1} ($p < 0.05$), in other words a reduction of 1.1 logarithmic unit (log-unit) is achieved. This reduction is in the lower limit of the typical range of reductions obtained for HF wetlands (0.44–4.44 $\log_{10}$; Kadlec and Wallace, 2009). More recently, Nivala et al. (2019) obtained removals between 1.3 and 1.6 log units in HF pilot wetlands filled with 8–16 mm gravel. A likely reason for the lower reduction of TW1 is that the filter media in Carricola's WWTP (10–25 mm) is coarser than usual (3.5–10 mm, Morató et al., 2014; 4–12 mm, Ávila et al., 2015).

An additional average reduction of 0.7 log unit is observed in TW2 (non-significant, $p > 0.05$), from $1.57 \cdot 10^5$ to $2.95 \cdot 10^4$ MPN·100 mL^{-1}. This lower reduction may occur for two reasons: first, HRT in this wetland is approximately half that of TW1; second, TW2 periodically receives a load of settled solids at a bend in the pipe connecting the primary treatment effluent to the wetlands. This pipe is drained every month to prevent excessive solids accumulation.

In contrast, the small pond significantly reduces the *E. coli* concentration to an average of 685 MPN·100 mL^{-1} (excluding outliers) ($p < 0.05$), achieving a further reduction of 1.6 log units. This reduction is slightly lower than that obtained by Gomes Passos et al. (2020) or Dahl et al. (2021). This treatment unit presents a higher variability of data, with some high outlier values. These peaks are related to sampling events in which some of the following causes were observed: elevated cover of floating filamentous algae, elevated influent concentration probably coinciding with days after maintenance operations commented above, small population of daphnids or short hydraulic retention time, around half day.

Other important benefit provided by the pond is the enhancement of aquatic biodiversity, as it has become the habitat for amphibians and reptiles. Up to 18 different aquatic invertebrates have been found, daphnids being the most abundant. These filter-feeding cladocerans reached up to 235 individuals·L^{-1}, similar to levels measured in constructed wetlands within a natural park (Rodrigo et al., 2018), providing valuable ecosystem services, such as eutrophication control and contribution to water disinfection. Therefore, the entire wetland system meets the conditions to be considered a nature-based solution (Sowińska-Świerkosz and García, 2022).

Overall, the entire system reduces *E. coli* concentration 3.41 log-units, which is in line with other full-scale treatment wetlands. Šereš et al. (2021) found a significant decrease of *E. coli*, by three orders of magnitude, in a hybrid system composed of a septic tank, a HF wetland, a VF wetland, a partially saturated filter with recirculation and a stabilization pond with a total area of 467 m^2 and treating a similar flow (16.5 m^3·d^{-1}) and population equivalent (150 PE). Ávila et al. (2015) reported an overall *E. coli* removal of 5 log-units in a hybrid system composed of an Imhoff tank, a VF wetland, a HF wetland and a FWS wetland with a total area of 786 m^2 for treating an average flow of 14 m^3·d^{-1}.

In terms of areal load removal rate, on average the wetland units (TW1 and TW2) removed $5.5 \cdot 10^8$ and $8.8 \cdot 10^7$ MPN·m^{-2}·d^{-1} respectively, which are one and two orders of magnitude lower than those reported by Headley et al. (2013). The pond removed $1.7 \cdot 10^8$ MPN·m^{-2}·d^{-1}.

The final effluent could be reused in agriculture, as it meets the requirements for irrigation of crops belonging to quality class D (industrial, energy and seeded crops) defined in the European Regulation (2020/741). The requirements for uses with C quality class are not fully met, as few outliers exceed one log unit the limit established in the European Regulation. Class C includes 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, with drip irrigation or other irrigation method that avoids direct contact with the edible part of the crop (Regulation EU 2020/741). A recommendation to overcome this requirement could be to install a small sludge TW to manage the solids accumulated in the pipe bend commented before, instead of discharging them into TW2.

4.2. Mathematical modelling

4.2.1. PkC* model for HSSF wetlands

The parameters calibrated and simulation results are shown in Table S3 and Fig. 3. The first-order reaction coefficients were 2.14 d^{-1} (134 m·yr^{-1}) and 1.86 d^{-1} (100 m·yr^{-1}) for TW1 and TW 2 respectively. These results fall within the range reported by Kadlec and Wallace (2009) for faecal coliforms in HF wetlands, concretely between percentiles 50 and 60. Note that these authors set a P value equal to 6, whereas in this study $P = 1$ provided less error when the iterative method described in Section 3.2.1. was applied. This P-value indicated that TW1 and TW2 are far from an ideal plug-flow reactor. If a value of $P = 6$ were set, the value of the constants would decrease by about half.

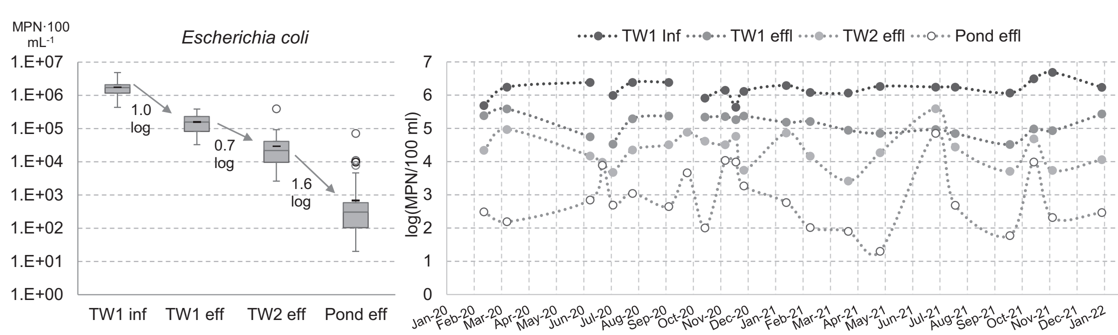

Fig. 2. Boxplot and temporal variation of *E. coli* at different sites of the WWTP.

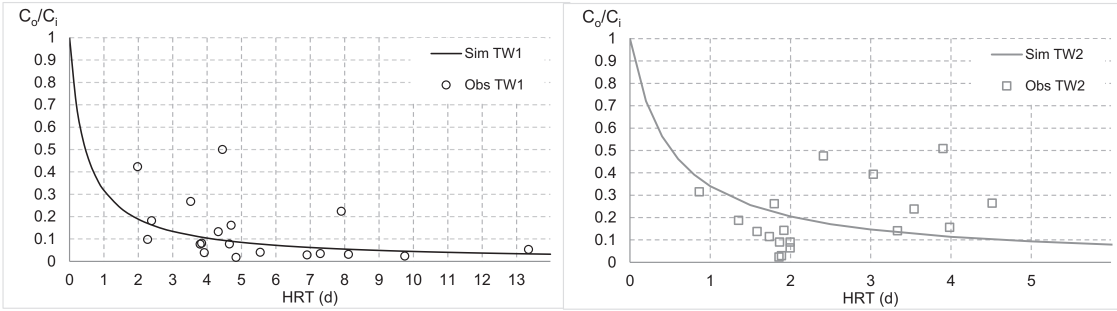

Fig. 3. P-k—C* model results. Dots are the observed data in each wetland, for HRT = 0 d Co/Ci is equal to 1. Continuous line represents the simulated concentration.

C* was set equal to zero in a first iteration, after which the own model stated that C_o tended to stabilize in a value around 100 MPN·100 mL^{-1} after a long period. Accordingly, C* was finally set at 100 MPN·100 mL^{-1}.

Theta (θ) best values resulted in 0.999 and 0.983 for TW1 and TW2 respectively, which are within the range of the literature (0.960–1.073; Kadlec and Wallace, 2009). These values indicated a low and negative influence of temperature on the removal of *E. coli*. Temperature can affect in both directions (positive and negative) for several reasons. On one hand, faecal bacteria originated in warm-blooded organisms have problems to survive outside them, so cold temperatures affect them negatively. On the other hand, protozoa predator activity increases with temperature, thus high temperatures promote faecal bacteria removal (Kadlec and Wallace, 2009). It would be recommendable to have more data to evaluate more deeply the effect of temperature.

4.2.2. Process-based models for pond: CSTR and 3d-CF

4.2.2.1. Impulse tracer test. The results obtained in the impulse tracer test are shown in Fig. 4. A recovery of 99% of the injected tracer was estimated from the EC measured in the outlet. The median retention time was estimated through the Eq. (12), giving a value of 0.77 day, which is a 95% of the theoretical or nominal retention time (0.82 d). Comparing the results obtained by the implemented models, it can be concluded that 3d-CFD approaches the tracer behaviour better than CSTR model. The higher differences are produced in the maximum reached in each case. Fig. 5 shows the distribution of the tracer in the pond simulated by the 3d-CFD model. It indicates that water is directed to the bottom because of the flow falling downwards and then it advances on two fronts, with a dead zone observed in the middle of the pond.

4.2.2.2. E. coli modelling and sensitivity analysis. For *E. coli* modelling, ancillary experiments were performed to approach the constant rate values (see SI S3.2.2.a). Natural-decay rate constant was fixed according to the

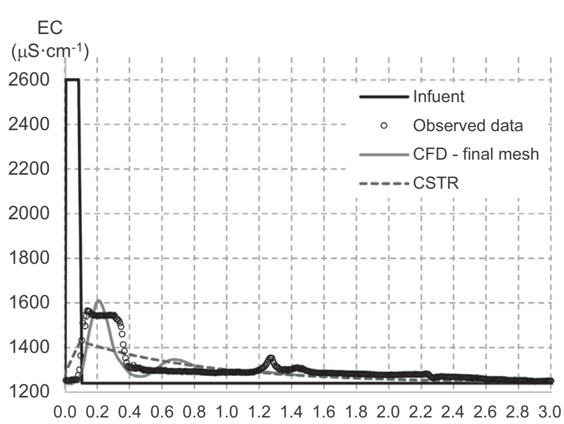

Fig. 4. Tracer response curve (raw residence time distribution, RTD) for sodium chloride impulse added to the pond.

values obtained in these experiments. For sedimentation velocity, the value obtained in the experiments could have been underestimated because it was done with water from TW2 effluent, which has lower TSS and COD concentrations than the pond, reason for which an intermediate value from the literature range was assigned. Photoinactivation and predation constant rates were fitted using several goodness-of-fit indicators. The final values adopted were calibrated to minimize the error between the average values, observed and simulated by 3d-CFD model (Tables 3, 4). This final adjustment did not consider the last peak value as it was very atypical and actions to prevent so high inlet concentration will be implemented (see Section 4.1). It was thought to be more reasonable to properly reproduce values within the interquartile range. The CSTR model was run with the same rate coefficients, to show the difference between models. Fig. 6 shows the *E. coli* concentration simulated with both models (CSTR and 3d-CFD) at the outlet of the pond. Both models reproduce relatively well the measured concentration, according to the indicators listed in Table 4. Additionally, working with CFD allows to better understand the hydrodynamic behaviour of the system, to check for dead zones and to propose appropriate design improvements. It is therefore considered a powerful tool for improving these aspects. Nevertheless, the CSTR model also gives reasonable and considerably good results. Therefore, considering the much shorter calculation time and its simplicity, it can be considered an appropriate tool to simulate *E. coli* decay in case of small systems such as the pond modelled in the present study. In larger systems, it may be more necessary to use CFD to correctly simulate the *E. coli* concentration (Dahl et al., 2021; Gomes Passos et al., 2020). Interestingly, with the CSTR model the magnitude of each decay process over time can be easily extracted from the software, whereas obtaining this information from the CFD is more laborious and complex. For this purpose, auxiliary variables can be defined to represent the terms associated with each process. Thus, it is possible to know how much *E. coli* has been removed by each process. This information complements that offered by the sensitivity analysis. Both, the magnitude of each process and the sensitivity analysis indicate that solar disinfection constant rate has the greatest effect on *E. coli* concentration, followed by predation and their temperature correction factors, while sedimentation and natural decay and its temperature correction have a considerably lower influence (Table 5). In terms of process magnitude, the CSTR model indicates the same trend, solar disinfection representing the most important removal process (65%, on average), followed by predation (25%), which becomes crucial in moments were the incident radiation is low, reaching values up to 70% (Fig. S4). A greater importance of solar disinfection (72.6% of removal) respect to other factors, such as pH, DO, T, filtration, adsorption or sedimentation, was also found in the model developed by Mayo for faecal coliform (2004). Kalibbala et al. (2008), concluded that solar intensity and root biofilm attachment contributed most to faecal streptococci removal (70.5%).

Therefore, it is important to keep these processes as much active as possible. Design and management recommendations can be proposed in this sense. For instance, creating small refuges with some gravel and planting few submerged plants could favour an enhanced colonization by aquatic invertebrate. Clearance rate by *Daphnia* is much higher than that by rotifers (up to 4 orders higher) (Ismail et al., 2019), so it is interesting to promote

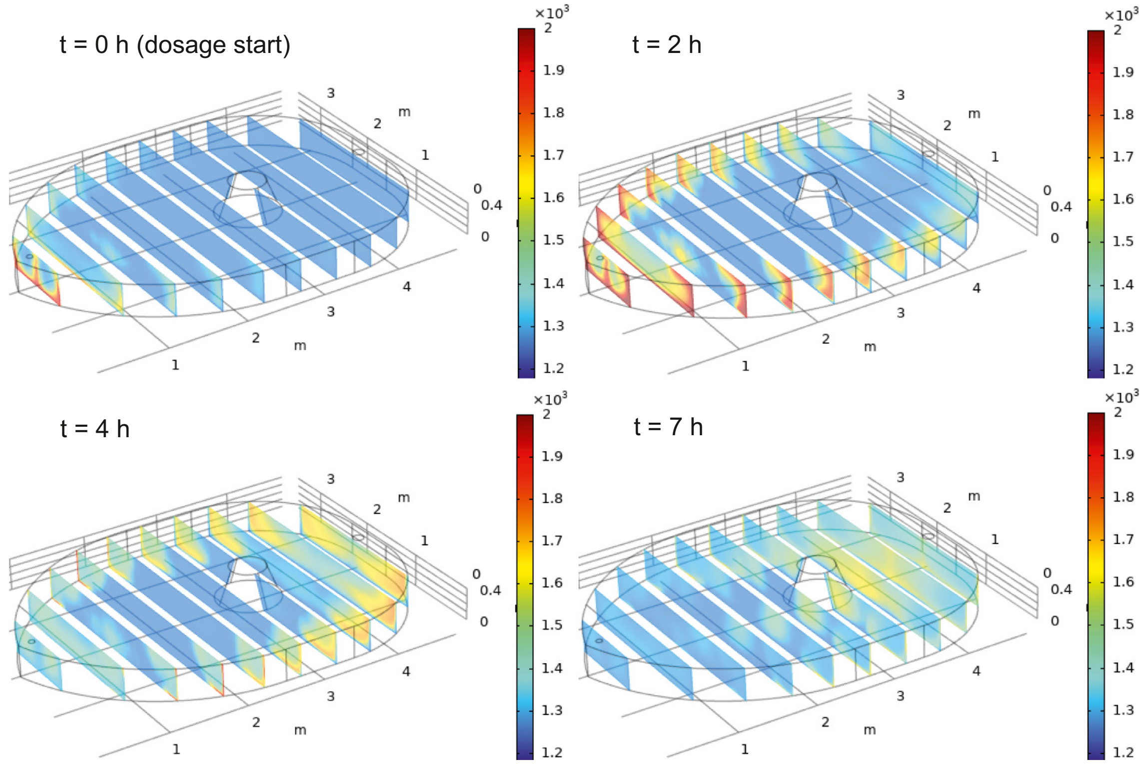

Fig. 5. Tracer test simulated in the 3d-CFD model at different times from the dosage beginning (Colour bar units: $\mu S \cdot cm^{-1}$).

their growth in ponds. Removing the filamentous floating algae periodically would help to promote the solar disinfection. The installation of a white waterproofing liner, instead of the black sheet commonly used, could favour reflection of solar radiation, thus intensifying solar disinfection.

After calibrating the model, the following simulations were performed:

- A periodic removal of the floating filamentous algae, e.g. once a week, could help to keep its cover at 15%. This condition (15% cover) was simulated with the model and the results indicated an average reduction around 35% (Fig. S5), so it represents a straightforward but efficient measure.
- A simple modification easily implementable was tested, by dividing the single inlet pipe in two inlets, which could be done by connecting a T-shaped pipe. This simulation produced a better water distribution, reducing dead zones and resulting in a significant reduction of *E. coli*, with an average outlet concentration of 1313 MPN·100 mL^{-1}, a 46% lower than the original simulation (see Fig. S6).
- The surface was increased to assess the surface necessary to achieve a target of 1000 MPN·100 mL^{-1}, equivalent to Class C in the EU Regulation. By increasing the surface to 21 m^2, 60% larger than current one, the average simulated *E. coli* would be 293 MPN·100 mL^{-1}. Less than 10% of simulated values would exceed 1000 MPN·100 mL^{-1} and no value would exceed the limit by more than one logarithmic unit (Fig. S7).

Some improvements to be introduced in the 3d-CFD model are related with the simplifications adopted (see SI S3.2.2.b), such as turbulence and heat transfer models, especially for larger ponds. Dahl et al. (2018, 2021) showed the convenience of considering turbulence closure models and buoyancy. Nevertheless, the model presented in this study already includes some suggestions of these authors, such as the inclusion of dark processes

Table 3
Process rate constants reported in the literature and values set in this study.

Symbol	Parameter	Literature range	This study
k_{nd}	Natural decay rate constant Darkness decay rate constant (presumably including grazing by protozoa).	0.12–1.0 d^{-1} (Burnet et al., 2017) 0.12–0.14 d^{-1} (Mayo, 1995) 0.48–0.55 d^{-1} (Craggs et al., 2004) 0.62 d^{-1} (Davies-Colley et al., 2003) 0.72–2.88 d^{-1} (20 °C) (Dias and von Sperling, 2018)	0.37 d^{-1}
k_S	Solar disinfection rate constant	0.0086–0.245 $m^2 \cdot MJ^{-1}$ (Mayo, 1995, 2004) 0.0675 $m^2 \cdot MJ^{-1}$ (Davies-Colley et al., 2003) 0.033–0.142 $m^2 \cdot MJ^{-1}$ (Craggs et al., 2004) 0.252 $m^2 \cdot MJ^{-1}$ (Kalibbala et al., 2008) 0.25–1.07 $m^2 \cdot MJ^{-1}$ (Maïga et al., 2009) 1.5 $m^2 \cdot MJ^{-1}$ (Salih, 2003)	1.80 $m^2 \cdot MJ^{-1}$
K_d	Partition coefficient	0.01 $m^3 \cdot g^{-1}$ (Bai and Lung, 2005)<0.25–1.9 L/g (Wu et al., 2019)	0.01 $m^3 \cdot g^{-1}$
v_s	Settling velocity	0.2–2.3 $m \cdot d^{-1}$ (particulate organic carbon; Chapra, 1997)	1.2 $m \cdot d^{-1}$
k_p	Predation rate constant	0.017 ± 0.002 $L \cdot ind^{-1} \cdot d^{-1}$ (Burnet et al., 2017) 0.005–0.011 $L \cdot ind^{-1} \cdot d^{-1}$ (Abtahi et al., 2021) 0.002–0.056 $L \cdot ind^{-1} \cdot d^{-1}$ (Ismail et al., 2019) 0.029–0.096 $L \cdot ind^{-1} \cdot d^{-1}$ (Serra et al., 2019)	0.020 $L \cdot ind^{-1} \cdot d^{-1}$
θ	Temperature correction coefficient	0.951–1.030 (for First-Order Plug Flow k-values; Kadlec and Wallace, 2009)	$\theta_{nd} = 0.951; \theta_S = 1; \theta_p = 1$

Table 4
Goodness-of-fit indicators. RMSE (%) is RMSE divided by the average observed concentration. NSE is Nash–Sutcliffe model efficiency coefficient. *wp means "without peak", i.e., the last peak value (Jul-21) has not been considered for the indicator calculation.

Indicator	3d-CFD	CSTR
RMSE (%)	101%	54%
RMSE (%) wp*	62%	105%
NSE (dimensionless)	0.84	0.96
NSE wp* (dimensionless)	0.82	0.49
R^2 (dimensionless)	0.98	0.96
R^2 wp* (dimensionless)	0.82	0.17
Relative error between means observed and simulated (%)	24%	-8%
Relative error between means observed simulated (%) wp*	0.2%	−48%

(Dahl et al., 2021). Indeed, predation has been shown to be an important removal process.

The model would be applicable to other pathogens considered in the EU Regulation, after a careful analysis of their behaviour and adapting the process rate coefficients. For instance, Gutiérrez-Alfaro et al. (2018) showed that *Clostridium perfringens* was highly resistant to solar disinfection (SODIS system, 0.9 log reduction, and high-rate algal ponds (HRAP), 0.1 log reduction), due to its spore-forming ability, whereas it was more efficiently removed by a Dissolved Air Flotation system (1.7 log reduction). Morató et al. (2014) found the maximum removal of *C. perfringens* (1.6 log reduction) for a shallow HF wetland (0.27 m depth) with fine granulometry (3.5 mm). García et al. (2008) obtained better performance for HF wetlands (2.0–3.6 log reduction) than for FWS wetlands (2.3–2.6 log reduction) or HARP (1.5–1.7 log reduction) and maturation ponds (1.1–1.5 log reduction). Thus, filtration seems to be an important process for the removal of this protozoan. In the case of coliphages, indigenous protozoa and ambient sunlight have been found to be important contributors to their decay (McMinn et al., 2020), as well as filtration process (Ruppelt et al., 2018).

5. Conclusions

TW-based WWTPs can reduce *E. coli* indicator bacteria sufficiently to reach values suitable for agricultural reuse or environmental uses, without energy and reagent consumption. The small pond at the end of the treatment train plays a very important role in *E. coli* removal and biodiversity enhancement. Therefore, it is highly recommendable to consider this unit in WWTP design. CSTR and 3d-CFD models provide valuable information to improve the knowledge about natural disinfection mechanisms. This study has proven that solar disinfection and predation by daphnids are the most important mechanisms in the studied pond. Hence, actions to promote these processes, such as those suggested in this study, are strongly recommended. Regarding mathematical modelling, it can be concluded that CSTR can provide good results for small ponds or wetlands and 3d-CFD model provide us with extra information, useful to enhance the design. Straightforward actions, such as periodically remove floating algae or dividing the influent in two inlets, can produce a significant enhancement of *E. coli* removal.

Table 5
Sensitivity analysis (|ΔC|: average absolute variation) and average magnitude of processes.

Process parameter	|ΔC|	Average process magnitude
k_S	803.9	64.7%
k_p	525.8	24.6%
θ_S	518.3	
θ_p	350.0	
v_s	82.0	5.8%
k_{nd}	71.1	5.0%
K_d	67.5	
θ_{nd}	48.4	

CRediT authorship contribution statement

Carmen Hernández-Crespo: Investigation, Software, Writing – original draft. **Miriam I. Fernández-Gonzalvo:** Software. **Rosa M. Miglio:** Supervision. **Miguel Martín:** Supervision.

Declaration of competing 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156237.

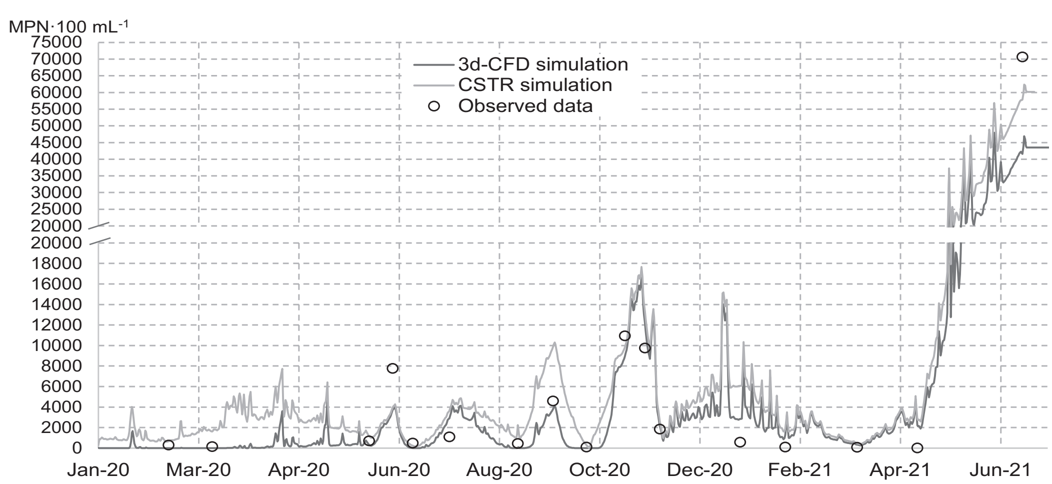

Fig. 6. *E. coli* concentration simulated by the 3d-CFD and CSRT models and observed data.

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