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Disinfection and particle removal by a nature-based *Daphnia* filtration system for wastewater treatment

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ABSTRACT

Nature-based solutions (NBS) to treat wastewater are ecological treatments that work to mitigate the impact of conventional systems in wastewater treatment processes. In this study, the efficiency of an innovative wastewater tertiary treatment based on *Daphnia* filtration in removing suspended solids and *E. coli* is evaluated in combination with either vermifiltration or conventional secondary treatments. *Daphnia* filtration NBS was found to increase particle removal and *E. coli* inactivation over a wide range of water temperatures, solar radiation intensities, and hydraulic residence times. Moreover, two models to predict the quality of the treated wastewater, one that describes the removal of particles and another for *E. coli* inactivation, are developed and presented here. Both models depend on water temperature, the exposure to solar radiation and the hydraulic residence time in the reactor. The results using these models align with the experimental results in all cases. Hydraulic residence times above 24 h allowed suspended particle concentrations to be reduced by >75 % with water temperatures in the range of 10 °C to 27 °C and for *E. coli* to be inactivated by 1–3 log units in water temperature ranging from 8 °C to 27 °C. The developed models can also be used to provide information regarding the operating conditions (i.e. hydraulic residence times) required to obtain the desired regenerated water quality in accordance with reuse purposes and the regulations of different countries.

1. Introduction

Increased demand for water, together with increased water scarcity, drives the need to improve wastewater treatment technologies, which require investment in supply, sanitation and water management [1]. The implementation of tertiary wastewater treatments makes it possible to significantly improve the quality of treated wastewater and, hence, both increase the variety of reuse applications [2] and permit its reintroduction into the environment in better conditions [3]. However, the widespread adoption of tertiary treatments has been limited by the high energy and chemical dosage requirements [4].

Nature-based solutions (NBS) are based on the concept of exploiting ecosystem services found in nature to obtain efficient and sustainable technologies that are able to replace conventional energy- and resource-demanding technologies. NBS are innovative solutions that can improve the health and resilience of ecosystems and foster more sustainable, low-carbon and climate-resilient societies [5], providing both sustainability and economic growth [6]. Access to NBS by vulnerable communities reduces inequalities between societies (SDG10, [7]) and this, in turn, results in improved health and well-being and safer and more sustainable cities (SDG3 and SDG11, respectively). Implementing NBS requires finding the correct balance between a series of benefits and trade-offs [8]. Since NBS are treatments that have low operating costs and capital outlays, they are fast becoming an alternative in producing reclaimed wastewater that can reduce pressure on water bodies, which is of particular importance in arid and semi-arid areas and during periods of water scarcity.

However, before wastewater can be reused it has to fulfil certain water quality conditions that depend on the legislation of each country and that differ depending on the end-usage [9]. For example, in Spain aquifer recharge through land percolation can be performed with treated wastewater with a maximum of 1000 CFU/100 mL of *E. coli* and 35 mg L^{-1} of suspended solids. In contrast, direct aquifer recharge can only be performed when treated wastewater fulfil 0 CFU/100 mL of *E. coli* and 10 mg L^{-1} of suspended solids [10].

Different tertiary treatments have already been studied to evaluate

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their ability to meet national targets. For instance, slow sand filtration working together with anaerobic biofilters produce an *E. coli* inactivation of 1.35 log units, which is slightly above that obtained for sand filters alone [11]. Coagulation and filtration are also used for their simplicity and effectiveness [12]. Wang et al. [12] found that the removal of suspended solids ranged from 50 % to 74 %, depending on the dosage of the coagulating agent. Ultrafiltration of wastewater by membranes removed as much as 94 % of suspended solids and completely eliminated *E. coli* [13], although the high cost of this technology may be prohibitive [14]. Constructed wetlands are a type of NBS in which aquatic plants are used to filter wastewater. Despite being useful in many settings, the large land area required poses practical limitations in their implementation although, multilayer constructed wetlands can provide a compact version that allows them to be used in urban areas [15]. A pilot project on constructed wetlands by Schierano et al. [16] succeeded in removing 78 % of suspended solids and reduced *E. coli* by one log unit. Bai et al. [17] studied the performance of a constructed wetland and found a 90 % reduction in suspended solids that did not increase when the hydraulic retention time (HRT) was raised from 1 to 3 days. In natural wetlands, high proportions of *E. coli* isolates can survive in low-anthropized environments [18] while wetland lagoons with floodplains populated with different types of vegetation can both improve the retention times of incoming polluted waters and boost the retention of pollutants that would otherwise reach coastal seawaters. Artificial food web systems using a combination of phytoplankton and zooplankton (with *Daphnia*) reactors have also been used [19] and are another example of NBS for wastewater treatment. In this case, a first phytoplankton reactor was used to reduce nutrients and a second reactor with *Daphnia* had the role of biomass regulator. A similar combination of phytoplankton and zooplankton reactors has been also applied to treat polluted stream waters [20].

The presence of small particles (<30 μm), which can go through meshes, filters or settling tanks, reduces water clarity, affecting the efficiency of disinfection by UV-based technologies. The genus *Daphnia*, a zooplanktonic population of Cladocera, has shown its ability to disinfect wastewater [21–23], to remove emerging contaminants [24] and, hence, have been postulated as filtration organisms for biologically-based tertiary wastewater treatments [22,23,25–28]. Pous et al. [26,28] developed a tertiary treatment based on *Daphnia* filtration that was evaluated both by connecting the system to a conventional secondary treatment and a vermifiltration system. In both cases the reclaimed water obtained had the required quality to be used for agricultural irrigation.

Furthermore, nature-based solutions make daily operations easier although tools to optimize the operating conditions are required. For this reason, the present study aims to develop two models to evaluate the effectiveness of a *Daphnia*-based tertiary system in disinfecting and removing particles under different working conditions. For this aim, two different real case studies have been considered: i) a *Daphnia* reactor downstream of a conventional secondary treatment (i.e., activated sludge), ii) a *Daphnia* reactor downstream of a vermifiltration reactor. The experimental data obtained will be applied to set and test prediction models for particle removal and *E. coli* disinfection under a set of abiotic variables, particularly water temperature and solar radiation, *Daphnia* concentrations and operating conditions. The great advantage of these models for both particle removal and *E. coli* inactivation is that they can be used as tools to provide information on the water quality of the treated wastewater and its possible reuses, which vary depending on each country's specific regulations. Furthermore, they can be used to calculate the operating conditions that are needed to achieve the wastewater quality objectives.

2. Materials and methods

2.1. Description of the NBS Daphnia magna tertiary treatment

The tertiary treatment based on *Daphnia* filtration has been tested on a pilot-plant scale using two different set-ups.

The first one consisted of four reactors of 1 m^3 each connected sequentially to an activated sludge secondary wastewater treatment with a nitrogen and phosphorus removal unit situated in Empuriabrava, NE of Spain (conventional secondary system A, CA, Fig. 1). Four replicas of this configuration were used in this analysis for this CA site. The residence time of wastewater in each reactor was one day and so the *Daphnia* treatment accounted for a total residence time of HRT = 24 h in the effluent of the first reactor, HRT = 48 h in the effluent of the second, HRT = 72 h in the effluent of the third and HRT = 96 h in the fourth reactor (Fig. 1). The water entered from above and flowed out from the bottom of the reactor. The reactors were square-shaped and had one vertical lamellae from the surface all the way down to the bottom of the reactor, dividing it into two parts, containing 3 cm diameter holes to permit water to flow from one side to the other. The secondary water entered into the system presented 8.9 ± 4.3 $mgO_2 \cdot L^{-1}$ (Chemical Oxygen Demand, COD), 9.1 ± 8.5 $mgN \cdot L^{-1}$, 4.2 ± 2.5 $mgP \cdot L^{-1}$, TSS = 8.2 ± 7.1 mg L^{-1} and *E. coli* = 792.8 ± 3007.2 CFU mL^{-1}. The standard deviation given represents the variation over three years. Large seasonal increases in the local population as a result of tourism explain the greater values corresponding to late spring and summer and the lower values in the winter period. This *Daphnia* filtration system was operated for three years to monitor its functioning over a large range of water temperatures and light conditions.

The knowledge generated at the CA set-up was used to redesign the square-shaped reactors used in CA into a cylindrical reactor according to *Daphnia* requirements in terms of water circulation inside the reactor and solar radiation [29–31]. The second set-up (conventional secondary system B, CB, Fig. 1) was located at the WWTP of Quart, NE of Spain and consisted of a cylindrical reactor (1.5 m^3 capacity) with two 30 × 30 cm^2 lamellae situated halfway down the reactor at an angle of 45° to enhance the sedimentation of big sludge particles [26,27]. The CB influent contained 68 ± 59 $mgO_2 \cdot L^{-1}$, 29 ± 15 $mgN \cdot L^{-1}$, and 4 ± 7 $mgP \cdot L^{-1}$ (mean values and standard deviations over one year). In this case, the secondary system consisted of a conventional activated sludge system treating urban wastewater for <10,000 p.e. Three residence times were considered (HRT = 12 h, 24 h, 48 h). The *Daphnia* filtration system was operated for one full year.

The same *Daphnia* reactor was connected to a vermifiltration system at the same site (vermifiltration, V, Fig. 1). *Daphnia* influent in this case contained 58 ± 16 $mgO_2 \cdot L^{-1}$, 2 ± 2 $mgN \cdot L^{-1}$, 5 ± 1 $mgP \cdot L^{-1}$ (mean values and standard deviations over one year). Four residence times were considered (HRT = 12 h, 16 h, 24 h and 48 h). The *Daphnia* filtration reactor connected to the vermifiltration unit was operated for one year.

2.2. Daphnia magna inoculation in each set-up

D. magna were collected from Empuriabrava WWTP ponds [22], which receive inputs of secondary wastewaters, and were kept for two years in 50 L aquariums in the laboratory with a continuous air flow. *Daphnia* were fed twice a week with a mixture of *Spirulina* sp. and yeast, and 1/3 of the water was renewed every 15 days. This *D. magna* culture was used for inoculating the different reactors.

Reactors were fed with treated wastewater for a month in the CA and for 19 days in both the CB and V systems to allow bacterial and algal biofilm growth. In the CB set-up, approximately 1000 *D. magna* individuals from a laboratory aquarium were added, resulting in a *Daphnia* concentration of 0.67 individuals L^{-1}. In the V set-up, the initial *Daphnia* concentration was 0.1 individuals L^{-1}. The systems were left to stabilise for a period of three months before starting the study. Reactors in the CA

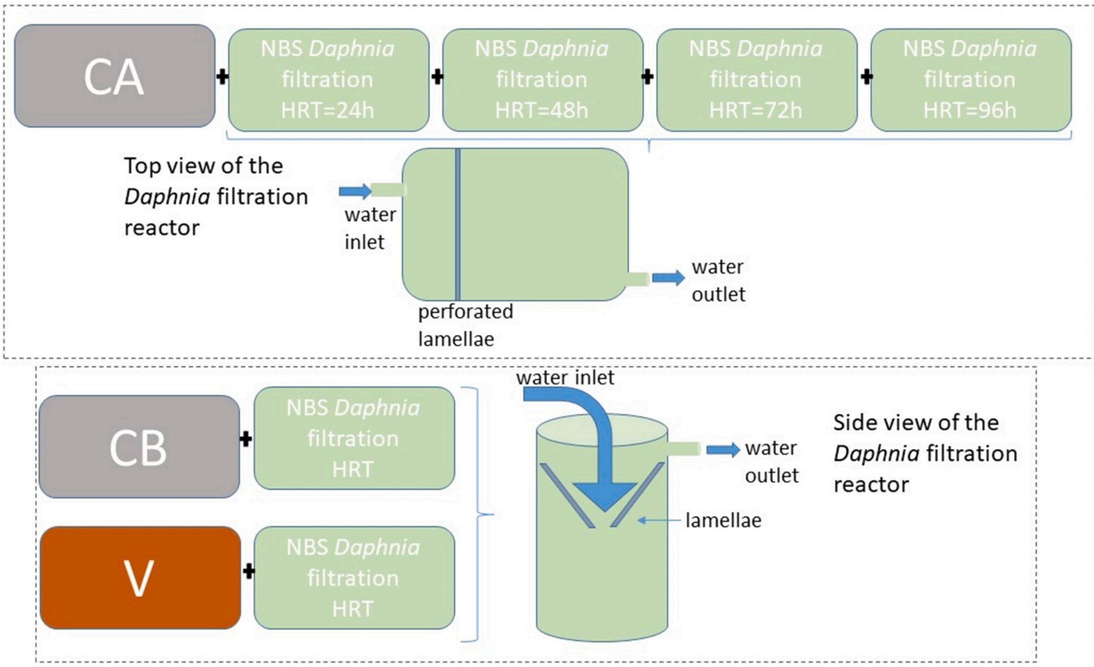

Fig. 1. Scheme of the different wastewater tertiary treatments based on *Daphnia* filtration. The first set-up: four *Daphnia* filtration reactors were connected to the secondary treatment outlet CA (top). The second set-up: a single *Daphnia* filtration reactor was connected to the secondary treatment outlet CB (middle) or to the vermifiltration treatment V (bottom). The scheme below CA represents the top view of each *Daphnia* reactor in the first set-up. In the right bottom panel, the cylinder shows the side view of the *Daphnia* reactor corresponding to the second set-up in CB and V.

system were fed directly with *Daphnia* from the Empuriabrava ponds with an initial concentration of approximately 1 individuals L^{-1} and the system was left to stabilise for a period of one year before the study begin.

In the case of the CB and V set ups, where high flow rates were studied, a Venturi system was added to the inlet of the *Daphnia* reactor to oxygenate the influent wastewater.

2.3. Measurement of suspended particles

In each *Daphnia* reactor, five samples of 100 mL were taken from both the secondary and the tertiary outlets to measure the concentration of suspended particles by means of a laser particle size analyser (Lisst-100×, Sequoia Inc.). The device measures the particle volume concentrations in the size range of 2.5–500 μm using a procedure based on light diffraction theory. It can determine the particle size distribution and concentration of either phytoplankton organisms [32,33] or inorganic suspended particles [34].

Since Cladocerans ingest particles within sizes up to 30 μm [35,36], the volumetric concentration of particles within the range of 2.5 to 30 μm was integrated and used as a proxy to evaluate the concentration of suspended particles. The particle removal (c/c_0) was calculated as the ratio between the concentrations of particles at the outlet of the *Daphnia* filtration reactor (c) versus the concentration of particles at the inlet of the *Daphnia* reactor, i.e., the outlet of the secondary treatment (c_0). The measurement of particle removal was carried out with and without *Daphnia* individuals in all set-ups.

2.4. Measurement of the *E. coli* colonies

1-L water samples were collected at both the outlet of the secondary and the *Daphnia* reactor during the performance of the three set-ups (Fig. 1). 100 mL samples were filtered through Millipore sterile membrane filters of 47 mm-diameter and 0.45 μm-pore size, placed on Petri dishes containing a double layer of Mb lactose glucuronide agar. The Petri dishes were then incubated at 44.5 °C for 24 h. The colony count was calculated from the arithmetic mean of three membrane filter counts to determine *E. coli* colonies at both the inlet ($Ecoli_0$) and the outlet (*Ecoli*) of the *Daphnia* reactor. Measurements of water disinfection were carried out with and without *Daphnia* individuals in all set-ups.

2.5. Measurement of the water temperature and solar radiation

In the CA configuration, a water temperature sensor (SEB-39, Seabird Electronics Inc.) was deployed mid-depth in each *Daphnia* reactor. Water temperature was recorded in the *Daphnia* reactors at hourly intervals. In the second set-up CB and V, a water temperature sensor (with a measuring frequency of 1 Hz) was submerged in the cylindrical *Daphnia* filtration reactor (Oxymax COS61D, Endress-Hauser, Germany). Daily mean water temperature was considered as the characteristic temperature of the wastewater in the *Daphnia* reactors (in CA, CB and V set-ups).

Solar radiation was measured at the closest meteorological stations (L'Estartit meteorological station for the CA set-up and the University of Girona meteorological station for the CB and V set-ups).

2.6. Measurement of the concentration of *Daphnia* in the reactors

In the CA configuration, the *Daphnia* concentration was measured monthly. For this purpose, 1-L water samples were taken at eight positions across the square reactor by means of a syphon tube, submerged at a depth of 1 m. Once a volume of 1 L had entered the tube a valve closed the inlet. This was repeated at each of the eight positions and so 8 L of water were collected. This water volume was then filtered through a 45 μm mesh size net to retain *Daphnia* to be counted. The measurements produced a modification in the *Daphnia* concentration of 1 %. For the period studied, the concentration of *Daphnia* in the reactor varied with the water temperature (Fig. 2a) following two different trends depending on whether the water temperature was above or below 20 °C:

$$C_{Dph} = 5 \times 10^{12} T_w^{-8.14}, R^2 = 0.7851, \text{for } T_w > 20^{\circ}C \quad (1)$$

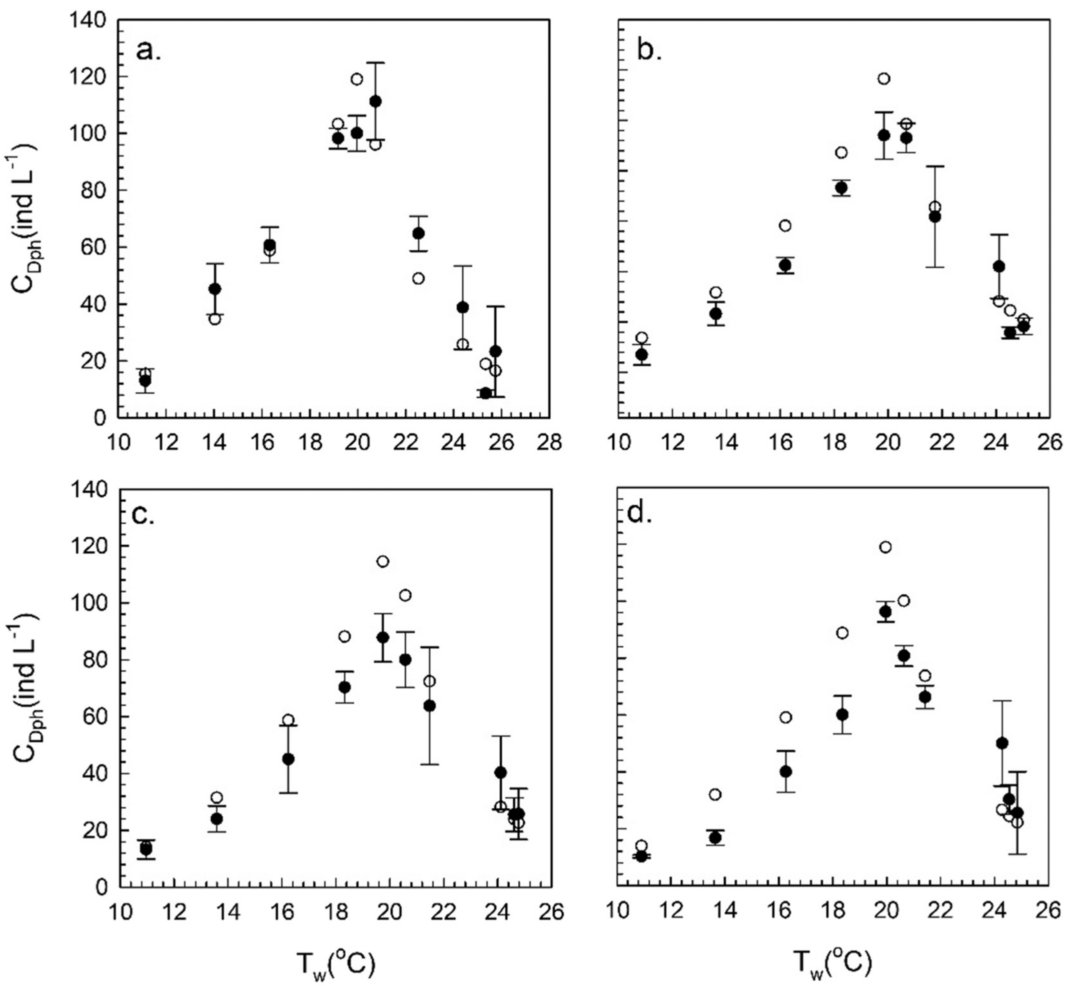

Fig. 2. *Daphnia* concentration (C_{Dph}, in ind mL^{-1}) in CA vs. water temperature (T_w, in °C) for different hydraulic residence times (HRT): a) HRT = 24 h, b) HRT = 48 h, c) HRT = 72 h, d) HRT = 96 h. Experimental results are represented by black symbols whereas predictions by the model are unshaded.

$$C_{Dph} = 3\times10^{-3}{T_w}^{3.51}, R^2 = 0.9628, \text{for } T_w < 20°C \quad (2)$$

In the case of the cylindrical *Daphnia* reactor, the lamellae only allowed two sampling points situated across the surface of the reactor. Measurements were repeated 1 h after the first sample, at the same points. The *Daphnia* concentration was taken from the mean of the four measurements.

2.7. Model for the removal of suspended particles

The removal of the suspended particles in the *Daphnia* reactors could be attributed to both the particle sedimentation and the *Daphnia* filtration activity through an exponential decrease with a constant rate of decay k and can be represented as $k = k_s + k_{Dph}$, where k_s and k_{Dph} correspond to the contributions of both sedimentation (k_s) and *Daphnia* filtration (k_{Dph}) processes [25]. k_s was determined from the experiments performed without *Daphnia* in the reactor (in which $k_{Dph} = 0$). In this case, $k_s = 0.05$ h^{-1} in both *Daphnia* reactors connected to the CB and V systems, and $k_s = 0.007$ h^{-1} in the *Daphnia* reactor connected to the CA. The *Daphnia* filtration rate was assumed to depend on the concentration of *Daphnia*, C_{Dph}, therefore $k_{DPh} = F \times C_{Dph}$, from which F, the filtration rate, was calculated from the *Daphnia* filtration and the *Daphnia* concentration [25]. The *Daphnia* filtration was found to be a function of the water temperature [37], following different trends depending on whether the water temperature was below 20 °C or above 20 °C:

$$F = 2\times10^{-3}{T_w}^{2.46}, \text{for } T_w < 20°C \quad (3)$$

$$F = 3\times10^{8}{T_w}^{-6.07}, \text{for } T_w > 20°C \quad (4)$$

Using Eqs. (3) and (4) to calculate the *Daphnia* filtration rate and Eqs. (1) and (2) to calculate the *Daphnia* concentration (Methods 2.6), a model (Eq. (5)) to predict the removal of particles was obtained:

$$\left(\frac{c}{c_0}\right)_{model} = e^{-Kt} \quad (5)$$

where c is the concentration at the outlet of the *Daphnia* treatment, c_0 was the concentration at the inlet of the *Daphnia* treatment, k is the rate of decay defined in Section 2.5 and t is the time during which *Daphnia* individuals undergo filtration, i.e. the HRT.

Daphnia filtration was a function of the hours of irradiance [31] and follows a linear decrease as the hours of light increase. In the current study, the hours of irradiance for both sites ranged from 9.7 h to 12.5 h. Experimental studies by Serra et al. [31] determined that the most efficient photoperiod for *Daphnia* filtration 8 h of light and 16 h of darkness. Therefore, considering the range of light hours that reached the surface of the experimental reactors, a linear trend was fitted between the ratio F/F_{max} versus the hours of light:

$$F/F_{max} = -0.013t_{light} + 1.106, \quad (6)$$

where F_{max} is the maximum filtration rate and t_{light} is the hours of light during the experimental conditions. The results predicted by the model for the predicted particle removal $(c/c_0)_{model}$ were compared with those obtained experimentally $(c/c_0)_{exp}$.

2.8. *E. coli* inactivation model

The *E. coli* in the *Daphnia* reactors was inactivated by three mechanisms: solar radiation, water temperature and filtration by *Daphnia* individuals. Therefore, *E. coli* inactivation could be calculated as the sum of the three processes as:

$$I_{Ecoli-model} = I_{Ecoli\_Dph} + I_{Ecoli\_light} + I_{Ecoli\_Temp} \tag{7}$$

First, the model considers that *E. coli* is inactivated by the *Daphnia* individuals following an exponential decay, as has been observed by Ismail et al. [38]:

$$Ecoli = Ecoli_0 e^{-K_{Dph}t}. \tag{8}$$

In the current study, the *E. coli* inactivation by *Daphnia* was considered to depend on both the *Daphnia* filtration (F) and concentration (C_{Dph}) through the rate of decay K_{DPh}:

$$I_{Ecoli\_Dph} = log\left(\frac{Ecoli_0}{Ecoli}\right) = log\left(e^{K_{Dph}\ t}\right) \tag{9}$$

Second, *E. coli* inactivation by solar radiation was investigated by Mosteo et al. [39], Nguyen et al. [40] and Šolić and Krstulović [41]. Šolić and Krstulović [41] demonstrated that the time required for the solar radiation to reduce the *E. coli* population by a 90 % (T_{90}) was:

$$T_{90} = e^{3.905-0.0047\ R} \tag{10}$$

where R is the solar radiation, in W m^{-2}. The model considered an exponential reduction of *E. coli* with time due to the solar radiation as:

$$Ecoli = Ecoli_0 e^{-K_{light}\ t} \tag{11}$$

where t is the time in hours. The rate of decay k_{light} was calculated from T_{90} as:

$$k_{light} = \frac{1}{T_{90}} ln(10) \tag{12}$$

Then, the *E. coli* inactivation due to the solar radiation was defined as:

$$I_{Ecoli\_light} = log\left(\frac{Ecoli0}{Ecoli}\right) = log\left(e^{K_{light}\ t}\right) \tag{13}$$

Third, water temperature has been found to inactivate *E. coli* but its effect is below the inactivation produced by solar radiation [41,42]. Šolić and Krstulović [41] demonstrated that the time required for the water temperature (T_W) to reduce the *E. coli* population in a 90 % (T_{90}) was:

$$T_{90} = e^{5.93-0.0837\ T_W} \tag{14}$$

The model considered an exponential reduction of *E. coli* with time due to the temperature as:

$$Ecoli = Ecoli_0 e^{-K_{Tw}\ t} \tag{15}$$

where t is the time in hours. The rate k_{TW} can be calculated from T_{90} as:

$$k_{Tw} = \frac{1}{T_{90}} ln(10) \tag{16}$$

The *E. coli* inactivation due to the temperature can be defined as:

$$I_{Ecoli\_Temp} = log\left(\frac{Ecoli0}{Ecoli}\right) = log\left(e^{K_{Tw}\ t}\right) \tag{17}$$

The experimental *E. coli* inactivation by the *Daphnia* treatment in the three set-ups was calculated as:

$$I_{Ecoli-exp} = log\left(\frac{Ecoli_0}{Ecoli}\right) \tag{18}$$

The inactivation predicted by the model ($I_{Ecoli-model}$, Eq. (7)) might result in values above those obtained experimentally ($I_{Ecoli-exp}$). This result is maintained at the *Daphnia* optimal conditions (water temperatures $T_W \sim 20$ °C), resulting in maximum *Daphnia* filtration rates and a maximum *Daphnia* concentration. Since *Daphnia* will inactivate *E. coli* as long as they find them to feed on, the maximum inactivation achievable in each experiment was calculated as:

$$I_{max} = log\left(\frac{Ecoli_0}{Ecoli_{min}}\right) \tag{19}$$

where $Ecoli_{min}$ is the minimum concentration of *E. coli* colonies (=1) and $Ecoli_0$ is the measured concentration of *E. coli* at the inlet of the *Daphnia* reactor, i.e. at the secondary effluent. A constriction to the model was applied for the I_{Ecoli_model} to not surpass I_{max}.

3. Results

3.1. *Daphnia* concentration

The *Daphnia* concentration (in ind L^{-1}) increased with water temperature from 10 °C to 20 °C (Fig. 2). For water temperatures above 20 °C the *Daphnia* concentration decreased as the temperature increased (Fig. 2a). A power trend for $T_W < 20$ °C and another for $T_W > 20$ °C were fitted to data in the CA set-up for HRT = 24 h (Fig. 2a, Section 2.4) and was used to predict the *Daphnia* concentration for the other HRTs and for the whole range of water temperatures (Fig. 2b, c and d). For all the HRT studied, the *Daphnia* concentration also showed an increase with temperature for $T_W < 20$ °C. In contrast, for $T_W > 20$ °C, the *Daphnia* concentration decreased with water temperature. The model results for C_{Dph} versus T_W were close to those obtained experimentally for the CA set-up (Fig. 2b, c and d). The agreement between the values predicted by the model and the experimental ones confirms that the *Daphnia* concentration is mainly dependent on the water temperature. Therefore, the model for CA was used to predict the *Daphnia* concentration in the cylindrical reactor in the two configurations (connected to CB or V units) and the predicted concentrations were compared to the experimental ones (Fig. 3).

3.2. Removal of suspended particles due to *Daphnia* filtration

In all the set-ups, the removal of suspended particles (c/c_0) in the *Daphnia* reactors presented a non-linear relationship with the water temperature (Fig. 4). The removal increased with T_W below 20 °C (i.e. c/c_0 decreased with T_W) and it decreased with temperature above 20 °C (i.e. c/c_0 increased with T_W). The particle removal also depended on HRT. The higher the HRT, the more particles were removed. The particle removal in the cylindrical reactor in both CB and V set-ups was always greater than in the CA set-up (with lower values of c/c_0).

3.3. *E. coli* inactivation

In the CA set-up, *E. coli* inactivation had a non-linear relationship with water temperature. For water temperatures below 20 °C, *E. coli* inactivation increased as the water temperature increased, decreasing afterwards for water temperatures above 20 °C. The *E. coli* inactivation also depended on HRT. The lowest inactivation was achieved for HRT = 24 h, which was the lowest HRT tested in this set-up (Fig. 5). Inactivation increased with HRT with maximum inactivation values of 4.7 log units for high HRT and water temperatures of ~20 °C. The results of the *E. coli* inactivation predicted by the model (Eq. (7)) present a similar evolution versus the water temperature for the CA system to the experimental values of *E. coli* inactivation.

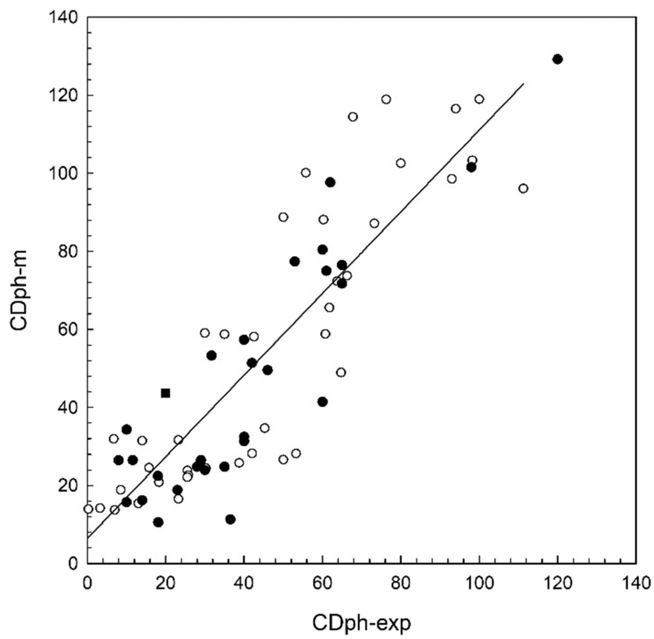

Fig. 3. The *Daphnia* concentration calculated from the model ($C_{Dph\text{-}m}$, model equations in Section 2.4) vs. the experimental *Daphnia* concentration ($C_{Dph\text{-}exp}$) measured in the reactors in the three set-ups (CA, CB and V) for all the HRT studied. $C_{Dph\text{-}m}$ follows a linear relationship with $C_{Dph\text{-}exp}$ as, $C_{Dph\text{-}m} = 1.05C_{Dph\text{-}exp} + 6.45$ ($R^2 = 0.748$, *p-value* > 0.01), where the slope is close to 1. Open circles correspond to the CA set-up, solid squares correspond to CB set-up, and solid circles correspond to the V unit.

3.4. Validation of the models for particle removal and E. coli inactivation

The models developed in this study for the removal of suspended particles and *E. coli* inactivation were validated by comparing the prediction values calculated by each model with the experimental ones. A linear relationship with the experimental values of the particle removal $(c/c_0)_{exp}$ in a log-log plot ($(c/c_0)_{model} = 1.00(c/c_0)_{exp} - 0.20$, $R^2 = 0.7589$, *p*-value > 0.01, Fig. 6a) was found when both sedimentation and *Daphnia* filtration were considered (Eq. (5)).

The model of *E. coli* inactivation in the CA set-up has been used to predict the *E. coli* inactivation in the CB and V set-ups (Eqs. (7)–(19)). A linear relationship between the model predictions and the experimental values of *E. coli* inactivation in all the three set-ups studied was found ($I_{Ecoli\text{-}model} = 0.84I_{Ecoli\text{-}exp} + 0.50$, $R^2 = 0.8554$, *p-value* > 0.01, Fig. 6b).

The particle removal effectiveness (calculated as 1-c/c$_0$) increased with HRT for all the temperatures in the range T_w from 10 °C to 25 °C (Fig. 7a). For $T_w = 20$ °C the system had the maximum effectiveness for all the HRT studied. However, for temperatures of 15 °C, the system gave effectiveness above 90 % for HRT equal or above 24 h whereas for temperatures of 10 °C or 25 °C, effectiveness >90 % were achieved for HRT ≥ 48 h. The *E. coli* inactivation increased with HRT for all the water temperatures (Fig. 7b). Inactivation above 3 log units were obtained for all HRT for a water temperature of 20 °C. However, for a water temperature of 15 °C, *E. coli* inactivation above 3 log units was achieved for HRT > 24 h, for $T_w = 25$ °C at HRT > 36 h and for $T_w = 10$ °C at HRT > 96 h (Fig. 7b).

4. Discussion

The innovative *Daphnia*-filtration tertiary wastewater system presented has been shown to significantly improve the quality of secondary wastewater by both increasing particle removal and inactivating *E. coli*. The effectiveness of the system is based on the fact that *Daphnia* are filter feeders that uptake particles and *E. coli* entering from the secondary effluent. The removal of particles depends on the hydraulic residence time and the *Daphnia* concentration and its filtration capacity, which in turn depend on the water temperature and the degree of light exposure both in terms of time and irradiance. Long-term conditions in the conventional and nature-based pilot scale treatments were tracked on a weekly time scale to better observe the reactor response to changing conditions. Few general differences were noted in the three set-ups that we tested on a pilot-scale. The results obtained in the CA set-up were used to optimize the design of a cylindrical *Daphnia* reactor for the CB and V set-ups. Hence, a greater particle removal was observed in the

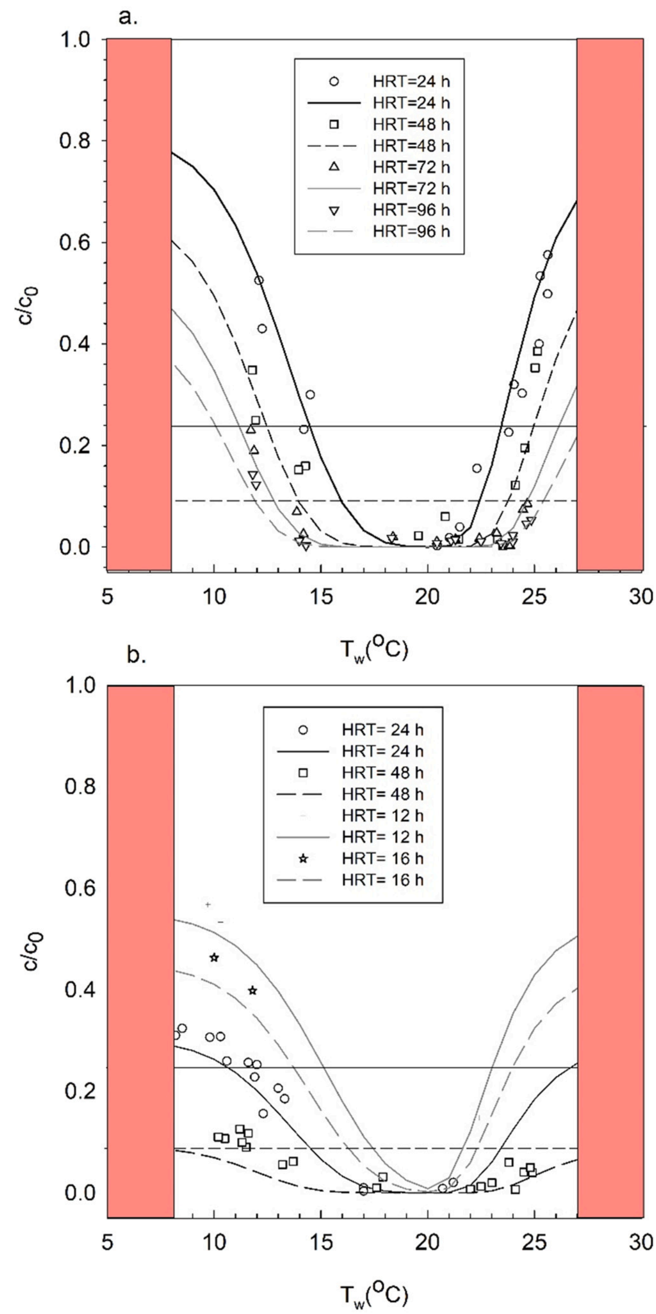

Fig. 4. c/c$_0$ versus water temperature (T_w) for the CA set-up. (a) and cylindrical reactor in CB and V set-ups (b) for the different HRTs studied. Circles, squares, triangles and stars represent experimental measurements and continuous lines represent the output predictions of the model (Eq. (5)). Horizontal lines at c/c$_o$ of 0.1 and 0.25 represent removal effectiveness of 90 and 75 %. Vertical red squares for T_w < 8 °C and >27 °C represent the range of water temperatures that threaten the development of *Daphnia* [37,43].

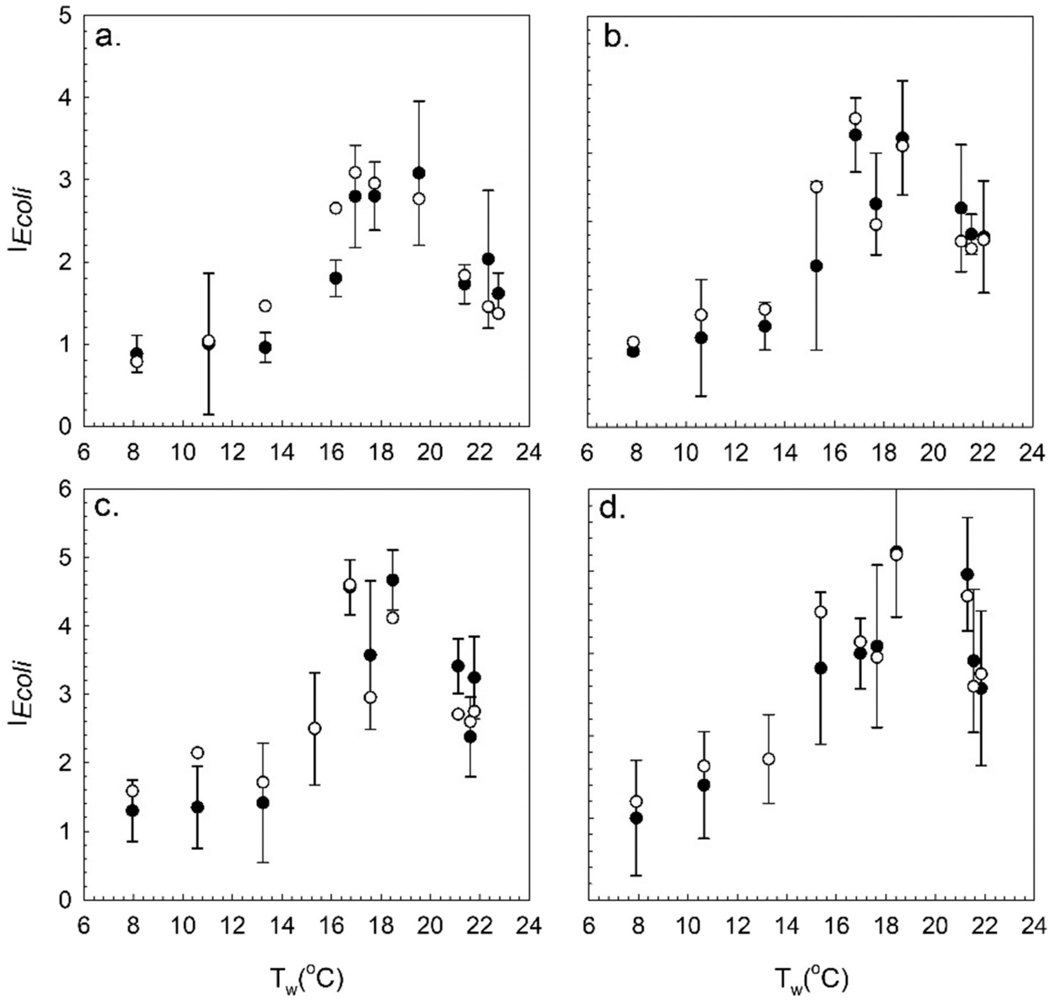

Fig. 5. *Ecoli* inactivation (I_{Ecoli}) vs. water temperature (T_w) in the CA set-up for the different HRT studied: a) HRT = 24 h, (b) HRT = 48 h, (c) HRT = 72 h and (d) HRT = 96 h. Experimental results are represented by black symbols whereas predictions by the model are unshaded.

improved reactor. The efficiency of the tertiary system based on *Daphnia* filtration can be predicted in terms of three outputs: modelled *Daphnia* concentration, *E. coli* inactivation and the removal of particles.

4.1. Daphnia development in the wastewater reactor

Daphnia concentration increased with water temperatures <20 °C, decreasing afterwards as water temperature increased independently of the upstream treatment used (i.e. conventional activated sludge with or without nitrogen and phosphorus treatment, or a nature-based solution based on vermifiltration). Müller et al. [37] also examined the *Daphnia* filtration performance in laboratory controlled tests, finding that at water temperatures around 20 °C, *Daphnia* individuals showed a considerable rise in their metabolic rate, greater particle uptake rates, and faster growth rates. The optimum thermal tolerance window for *Daphnia* filtration has been found to be centred at water temperatures of 24 °C [44] or between 20 and 21 °C [23,37,44–47]. Water temperatures >27 °C in combination with high nitrate concentrations have been found to have a negative impact on the survival of *Daphnia* individuals [46]. Schalau et al. [43] found that water temperature was the key parameter driving the evolution of *Daphnia* populations, finding that *Daphnia* concentrations decreased when water temperatures fell below 6 °C. In the current study, water temperature was also found to be a key parameter in determining the evolution of the *Daphnia* population in the reactors and water temperatures of 11 °C were less efficient in inactivating *E. coli*, presumably due to their low feeding rates at these water temperatures [37].

4.2. Particle removal by Daphnia filtration

Increased particle removal was found to be dependent on increased HRT in all set-ups. Both particle sedimentation and *Daphnia* filtration in the *Daphnia* reactor improved the removal of particles with diameters below 30 μm. This result agrees with our previous findings [29] in which the removal of particles remained low with HRT = 3 h and 12 h and increased progressively until reaching a plateau at HRT = 24 h. However, such a plateau was not found in the current investigation, since higher HRT always improved the particle removal. At the wastewater treatment plants where the *Daphnia* filtration solution was installed, the water temperature ranged between 8 °C and 27 °C all year round. The particle removal model at HRT = 24 h predicted a removal rate > 75 % and >90 % for the rectangular reactors in CA and cylindrical reactors (in CB and V) set-ups, respectively. Therefore, greater particle removal was obtained with both the CB and V set-ups at HRT = 24 h, probably due to the fact that the sedimentation in the optimized reactor was greater than with the rectangular reactors. For the CB and V set-ups, and for an HRT = 48 h, the model predicts a particle removal >90 % in the water temperature range of 8 °C to 27 °C, in contrast to the particle removal >75 % predicted for the same set-up and the same water temperature range but for a smaller HRT of 24 h.

Moreover, the removal of suspended solids depended on the *Daphnia* concentration, the *Daphnia* filtration and the hydraulic residence time

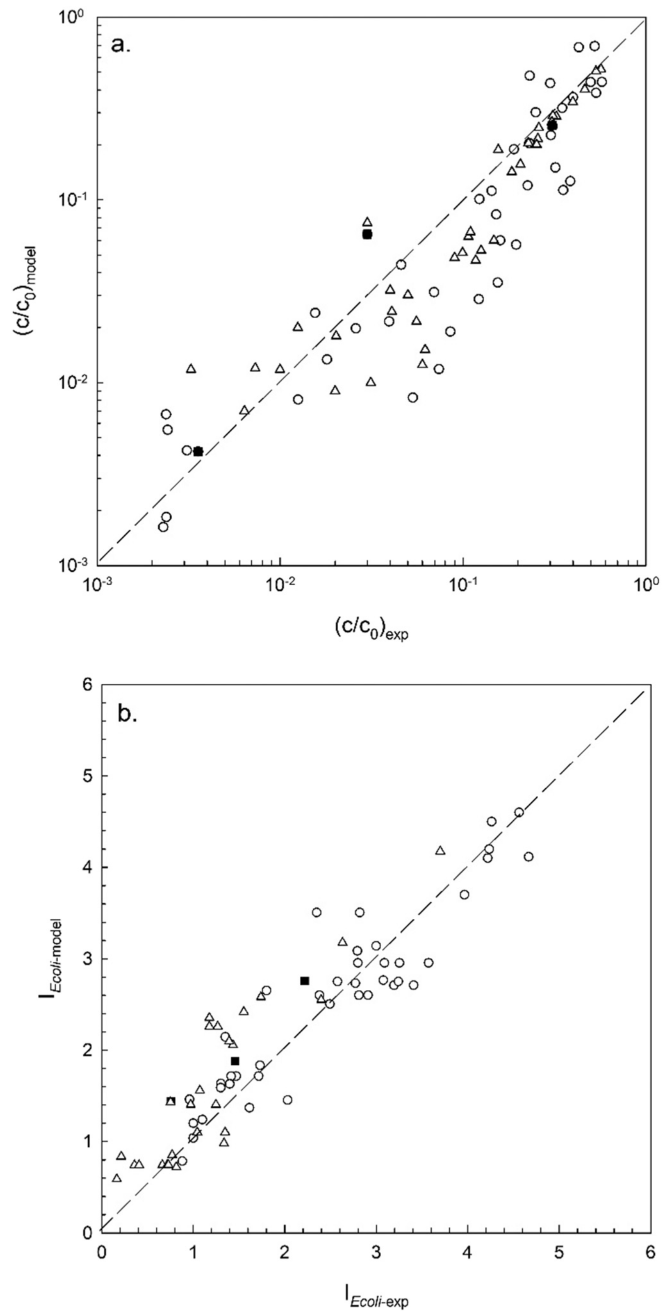

Fig. 6. $c/c_{0\text{-model}}$ vs. $c/c_{0\text{-exp}}$ (a) and $I_{Ecoli\text{-model}}$ vs. $I_{Ecoli\text{-exp}}$ (b) for all the three set-ups and HRT considered. Unshaded circles correspond to the CA set-up, black squares correspond to the CB set-up and unshaded triangles correspond to the V set-up. Dashed lines represent the 1:1 relationship.

and ranged between $c/c_0 = 0.6$ (40 % of particle removal) to $c/c_0 = 0.002$ (99.8 % of particle removal). When the cylindrical reactor was connected to the vermifiltration unit with HRT = 48 h, a particle removal >90 % was achieved for all the water temperatures. These percentages are similar to those obtained by subsurface constructed wetlands [17] and by ultrafiltration membranes [13]. Using the same set-up at HRT = 24 h, particle removal was always above 70 %, which is higher than that obtained in coagulation and filtration [12]. Effectiveness above 75 % was found for the range of water temperatures T_w = 10 °C to 25 °C for HRT > 24 h. For the same range of water temperatures, particle removal effectiveness was ≥90 % for HRT ≥ 48 h.

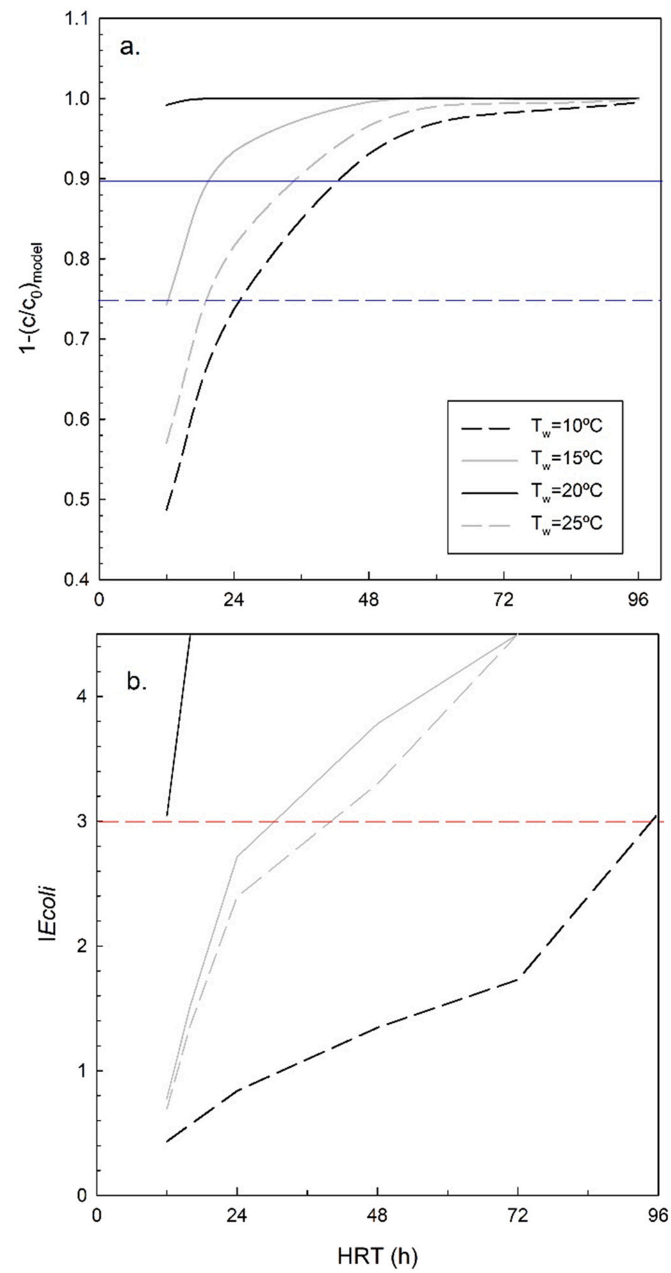

Fig. 7. Particle removal effectiveness (1-c/c_0) versus HRT for different water temperatures (a) and *E. coli* inactivation vs. HRT for different water temperatures (b). Horizontal continuous and dashed blue lines represent particle removal effectiveness of 90 % and 75 %, respectively. The horizontal dashed red line represents an *E. coli* inactivation of 3 log units.

4.3. E. coli disinfection by Daphnia filtration

HRT fine-tuning boosted *E. coli* inactivation up to HRT = 24 h, after which a further rise in HRT led to no discernible further improvement. For HRT = 24 h the *E. coli* inactivation by *Daphnia* filtration ranged from 1 log unit to 3 log units for the range of temperatures studied (8 °C to 27 °C). This inactivation is in agreement with the *E. coli* inactivation by *Daphnia* of 1.5–3 log units in 24 h found by Ismail et al. [38] in ponds and synthetic freshwater, respectively. *E. coli* inactivation increased with HRT, and for HRT = 72 h this ranged from 1 log unit to 5 log units for the water temperatures studied. However, for HRT > 72 h a further increase in HRT did not noticeably affect the *E. coli* inactivation. The levels of *E. coli* inactivation obtained in the present study are similar to those found with conventional systems such as UV, which are in the range of 3 log unit for a UV dose of 10 mJ cm^{-2} to 6 log units for a UV dose of 30 mJ cm^{-2} [48]. Wei et al. [49] found an *E. coli* inactivation of 3.5 log units with a UV dose of 7.5 mJ cm^{-2}, increasing by one more logarithmic unit when UV was combined with other treatments (ozone or chlorine).

In the current study, the *E. coli* inactivation increased with the *Daphnia* concentration aligning with the results of Ismail et al. [38]. Therefore, the *E. coli* inactivation in the *Daphnia* reactor was higher than that obtained by sand filtration [11]. For HRT > 24 h and warm temperatures (T_w = 20 °C), the *E. coli* inactivation reached its maximum value and complete water disinfection was achieved. Given this, under optimal conditions, the tertiary treatment based on *Daphnia* filtration produced regenerated wastewater with a reduction of *E. coli* that was similar to that obtained with ultrafiltration membranes [13]. The *E. coli* inactivation depended on water temperature. Between 11 °C and 18 °C, disinfection increased progressively with water temperature, finally reaching 2 log units. For water temperatures above 16 °C, the system showed a faster increase in disinfection up to 20 °C, decreasing afterwards with a further increase in water temperature. The low disinfection levels observed at water temperatures below 16 °C might be attributed to the fact that *Daphnia* grow more slowly at these water temperatures. These results are in accordance with the findings by Ismail et al. [38] who found that at low water temperatures filter feeding by *Daphnia* was reduced. In addition, periods of low water temperatures typically go together with fewer hours of solar radiation. However, for water temperatures above 16 °C, *Daphnia* growth is enhanced and their filtration activity increases, coinciding with longer periods of solar radiation. Low water temperatures combined with low solar radiation, corresponding to the end of the summer, are likely to favour *E. coli* development [41]. Besides this seasonal effect, in all of the samples analysed in the CA set-up and for all the HRT studied, *E. coli* remained below 16 CFU/100 mL. Based on the *E. coli* inactivation, disinfections above 3 log units were achieved for HRT > 12 h for a water temperature close to 20 °C. For water temperatures <15 °C and >20 °C, disinfection was below 3 log units for HRT < 48 h.

5. Conclusions

The nature-based solution presented in this study successfully develops a wastewater tertiary treatment based on *Daphnia* filtration as a polishing system. The *Daphnia* filtration system can be used connected to either conventional wastewater treatment set-ups (i.e. activated sludge with and without nitrogen and phosphorus treatment) or to nature-based solutions such as a vermifiltration system. The *Daphnia* filtration system, which does not require the use of chemicals, has no negative impact on the environment and is highly efficient in removing suspended particles while reducing the production of sludge.

The deterministic models presented here predict the particle removal and the *E. coli* inactivation in the three systems studied for the different seasons of the year. A set of water temperatures and solar radiation and hydraulic residence times have been used to calibrate the models, to best predict particle removal and *E. coli* disinfection by the *Daphnia* filtration system. Hydraulic residence times above 24 h reduced the concentration of suspended particles by >75 % with water temperatures in the range of 10 °C to 27 °C and produced *E. coli* inactivations of 1–3 log units in water temperature ranges from 8 °C to 27 °C. T These models have provided essential data in understanding how to optimize *Daphnia* filtration tertiary treatment, informing us, for example, when the best time to inoculate the *Daphnia* is and determining which operating conditions are the most efficient. They also reveal the *Daphnia* filtration and particle sedimentation parameters required to achieve the desired wastewater quality in terms of both particle removal and disinfection. The results of the models also allow us to identify the best operating conditions to obtain wastewater of the quality required to meet specific country's regulations for the different reuse applications.

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.

Data availability

Data will be made available on request.

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