Tertiary treatment for wastewater reuse based on the *Daphnia magna* filtration – comparison with conventional tertiary treatments

Teresa Serra, Jordi Colomer, Conxi Pau, Maribel Marín and Lluís Sala

ABSTRACT

Tertiary treatments are required to permit safe reuse of wastewater. The performance of a new biological tertiary treatment based on the filtration by a population of *Daphnia magna* was studied and compared with the performance of other conventional tertiary treatments such as coagulation-flocculation, settling tank, disc filtration, sand filtering and ultraviolet (UV) light. The analysis was based on the efficiency in the particle removal and *Escherichia coli* inactivation. The *Daphnia magna* treatment reduced the concentration of particles with diameters below 30 μm by 35%, depending on abiotic parameters such as water temperature and the hydraulic retention time (HRT). The *Daphnia magna* filtration increased with water temperature for water temperatures >20 °C, while it remained constant for water temperatures <20 °C. Lower HRTs induced the growth of the *Daphnia magna* population, maintaining the same water quality. Furthermore, the *Daphnia magna* treatment inactivated *E. coli* in 1.2 log units. This inactivation was six times larger than that obtained by the conventional macrofiltration systems analyzed, although lower than the inactivation attained by UV light, which ranged between 1.5 and 4 log units.

Key words | *Daphnia magna*, disinfection, particle volume concentration, tertiary treatment, water reuse

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INTRODUCTION

Reintroducing wastewater into the environment with the minimum environmental impact, while ensuring public and environmental health, is the aim of wastewater reclamation and management. Reclaimed wastewater can be used for non-potable purposes that do not require high water quality indicators (i.e. below the quality standards of drinking water); for example, it can be used as a substitute for freshwater for agricultural irrigation, urban cleaning, fire fighting, recreational activities or aquifer recharge, among others. Chemicals and pathogens are the main parameters to be considered when assessing safe wastewater reuse. Although secondary effluents have low levels of suspended solids, pathogens and chemicals, there is still a risk when wastewater coming from the secondary effluent is used for non-potable purposes. Coagulation, flocculation, sedimentation tanks, sand filters and disc filtration, among others, are examples of different tertiary treatments that are used with the purpose of reducing suspended solids and afterwards ensuring proper water disinfection, together with chlorination or ultraviolet (UV) light disinfection. However, chlorination is known to generate non-desirable by-products (Minear & Amy 1996).

The particle content in wastewaters has been considered an important parameter for the evaluation of chemical, physical and biological treatments (Marquet *et al.* 2007). A large amount of suspended solids might prevent proper wastewater disinfection (Lazarova *et al.* 1999). Suspended solids are also recognized as carriers of both pathogens and chemicals, shielding them within their structure. Therefore, the removal of particles might provide an additional water disinfection method that would result in treatment plants requiring fewer dosages of chlorine. Wu *et al.* (2009) studied the relationship between particle size distribution and microorganisms and they proposed continuous analysis of the particle size distribution to evaluate the water quality of the effluent. Arevalo *et al.* (2009) and

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Gómez *et al.* (2007) also found that the quality of the effluent, characterized by the suspended solids in the water, was dependent on the quality of the influent. Urstun *et al.* (2011) found an 85% reduction in suspended solids, which coincided with a 5-log reduction in the total coliform for a tertiary treatment coupling coagulation, flocculation and disinfection. Ellis (1987) could not find differences in both particle concentration and *Escherichia coli* removal, when using different sand filters with different grain sizes. This method also inactivated *E. coli* with log unit reduction in the range of 1.9–3.0.

Daphnia magna has been found to ingest sludge particles if these particles are in the ingestible *Daphnia* particle size range (Pau *et al.* 2013). Burns (1968) found that the filtering rate of *Daphnia magna* decreased as the prey concentration decreased and that the maximum particle size ingestible was 35 μm. Filter feeders are also promising organisms in the area of water disinfection (Shiny *et al.* 2005) and the removal of emerging contaminants from wastewater (Matamoros *et al.* 2012).

While large particles are easily removed from wastewater via settling tanks or filtering techniques, small particles have a settling velocity too low to be trapped in sedimentation basins and they are also too small to be retained by filtering methods, becoming the most difficult particles to be removed from wastewater. Therefore, filtration of small sludge particles by *Daphnia magna* might result in a considerable improvement of the water quality when compared to other existing treatments. As indicated by Mujeriego & Asano (1999), the goal of water reuse is to achieve water quality standards with a reasonable cost-effective process.

In this study we present the efficiency in the particle removal of conventional tertiary treatments, such as coagulation–flocculation (CF), sedimentation (ST), sand filtration (SF), disk filtration (DF) and UV, and also the efficiency of a *Daphnia magna* treatment, working as a pilot scale plant. The efficiency of this treatment will be studied in terms of abiotic variability, especially water temperature and hydraulic residence time.

MATERIALS AND METHODS

Five wastewater treatment plants (WWTPs) were chosen with the purpose of covering a wide range of conventional tertiary treatments. In total, 38 surveys were carried out over periods of several days for a whole year in order to study the performance of each conventional tertiary treatment with different particle concentration loads in the secondary effluent. The WWTPs are situated along the Costa Brava (northeast Catalonia, Spain), an area of high tourist volume, thus resulting in a large variability throughout the year in the particle concentration from the secondary effluent wastewater.

Samples from the secondary effluent and from each section of the tertiary treatment method were taken and analysed with a laser particle size analyser (Lisst-100, Sequoia Inc.) in order to determine the evolution of the suspended particle concentration throughout the treatment. The Lisst-100 has been found to show good performance in the determination of particle size distribution in the range 2.5–200 μm for both organic and inorganic particles (Serra *et al.* 2002) in water suspension.

The new tertiary treatment method, consisting of the filtering of wastewater by a population of *Daphnia magna* (DM), was also under study and compared with other conventional methods, such as CF, ST, SF, DF and UV light (UV). Since DM filters particles with sizes below 30 μm, the concentration of particles with diameters of 2.5–30 μm (hereafter C) was studied. The DM pilot scale plant consisted of eight containers, each with a water capacity of 1 m^3 and distributed in four different lines with two containers per line connected in series (Figure 1). Each line represented different replicas of the experiment. The water entered from the secondary treatment effluent through containers E where large particles settled and after this first container the water was submitted to the DM treatment, after which it was discharged into the a constructed wetland. The mean flow through the containers was kept constant, ensuring a mean hydraulic retention time (HRT) of 24 h per container. Experiments with HRT of 12 h per container were carried out during a 1 month period. In this study, the HRT of two lines was reduced to 12 h, while the HRT of the other two lines was 24 h. Experiments with two lines without *Daphnia magna* were also carried out during a 1 month period in order to determine

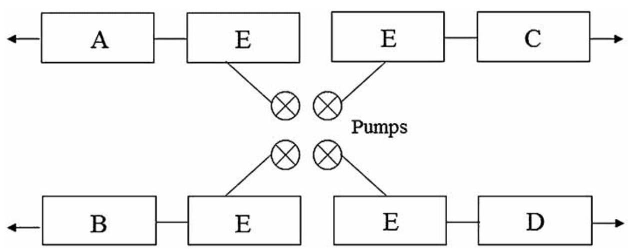

Figure 1 | Scheme of the mesocosm with the four replicas (A, B, C and D). Each box in the figure represents one container of 1 m^3 volume.

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the effect of particle sedimentation on the reduction of the particle concentration.

The *Daphnia magna* was inoculated in the containers in July 2007 and the present study started in June 2008 when the population in the mesocosm was well established. Both the evolution of the *Daphnia magna* concentration and the mean size of individuals were studied following the method described by Pau *et al.* (2013).

E. coli measurements were taken at the outflow of each tertiary treatment in order to compare the disinfection with the particle removal. Wastewater samples were collected and stored in sterile glass bottles for *E. coli* analysis. Samples of 100 ml were filtered through Millipore sterile membrane filters of 47 mm diameter and 0.45 μm pore size, placed on Petri discs containing a double layer of membrane lactose glucuronide agar. The Petri discs were then incubated at 44.5 °C for 24 h. The colony count was calculated from the arithmetic mean of three membrane filter counts.

RESULTS AND DISCUSSION

The size of the *Daphnia* population was found to vary with water temperature and two regions could be distinguished, a first region for water temperatures between 5 °C and 20 °C where *Daphnia* grew with temperature and a second region where the size reached a steady state. The ratio between the size of *Daphnia* population (L) and the mean size ($\bar{L}$) in each region was plotted (Figure 2(a)). The first region for water temperatures in the range 5–20 °C shows that $L/\bar{L}$ increased from 0.4 to 1.2. After this growth range a steady state was reached with a mean value of $L/\bar{L} = 1.2$, corresponding to water temperatures above 20 °C. This result is in agreement with those by Schalau *et al.* (2008) who found that a water temperature of 6 °C could be enough to allow growth rate to compensate for mortality and that the *Daphnia* growth rate was a function of the water temperature. In the present study, water temperature was always above 6 °C, indicating that *Daphnia* mortality did not dominate.

The concentration of particles in the outflow after the DM tertiary treatment (C_{Out}) was always smaller than the concentration in the inflow, the water from the secondary effluent (C_{Inf}) (Figure 2(b)), with values below the 1:1 ratio. It must be noticed that in some experiments C_{Out} was above the 1:1 ratio. These cases corresponded to C_{Inf} below ~10 μl l^{-1}. In contrast, for cases with C_{Inf} above 10 μl l^{-1}, C_{Out} was below the 1:1 ratio. In general, the DM

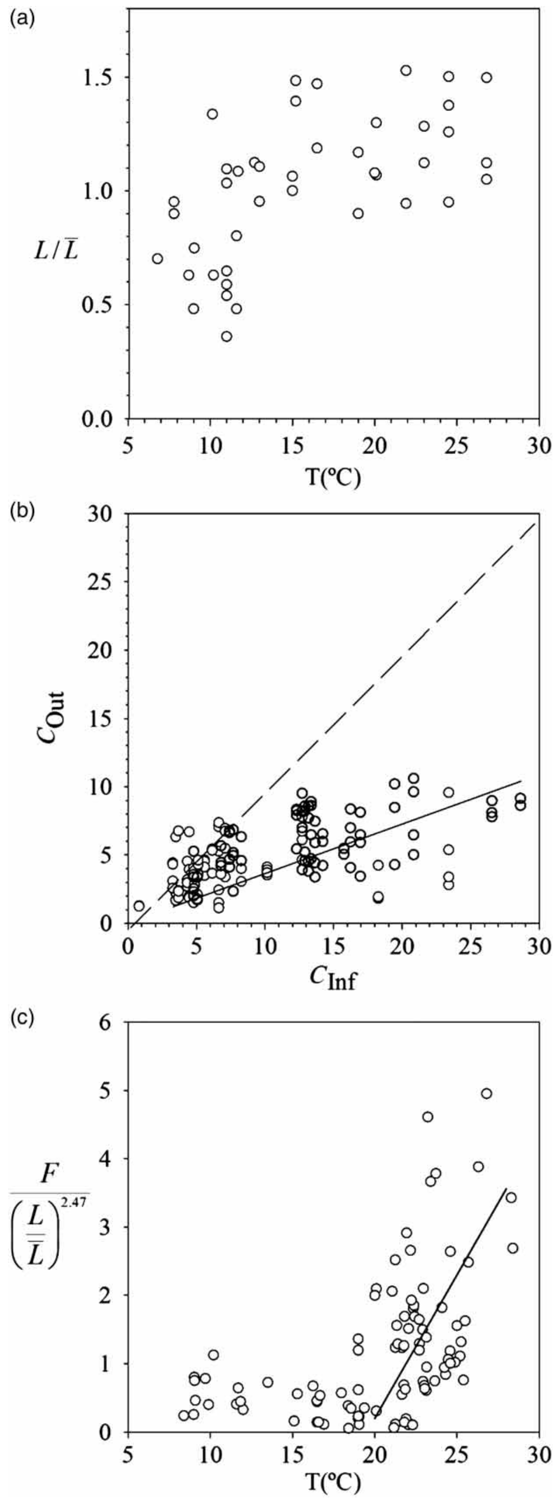

Figure 2 | (a) Relationship between the ratio $L/\bar{L}$ and the temperature for *Daphnia*. (b) Relationship between C_{Out} and C_{Inf}. The solid line represents the best fit line of the data (the slope of the line is 0.4, $r^2 = 0.79$, 99% confidence). The dashed line corresponds to the 1:1 ratio. (c) Evolution of $F/(L/\bar{L})^{2.47}$ versus temperature for *Daphnia*. The solid line represents the best fit line between $F/(L/\bar{L})^{2.47}$ and T for T > 20 °C.

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treatment showed a good performance in reducing the concentration of particles. The slope of 0.40 between C_{Out} and C_{Inf} indicates an overall particle removal of 60%. As shown by Pau *et al.* (2013) this reduction could be attributed to both the *Daphnia magna* filtration and to the settling of particles in the containers. From laboratory experiments, Pau *et al.* (2013) found that sedimentation was responsible for the 62.2% reduction in concentration, while the *Daphnia magna* filtration was responsible for a reduction in the range of 10.1–29.4% depending on the *Daphnia magna* concentration. The experiments in the mesocosm without *Daphnia magna* showed a reduction in the particle concentration of 25%, which was attributed to the sedimentation of particles in the containers. Therefore, the *Daphnia magna* filtration was responsible for the 35% reduction. This result is slightly above the reduction obtained by Pau *et al.* (2013) from laboratory experiments.

The filtration, F (in ml h^{-1} ind^{-1}) of each *Daphnia magna* individual was calculated following Burns (1968)

$$F = ln(C_{Out}/C_{Inf})/(HRTC_{Dph}),$$

where C_{Dph} was the *Daphnia magna* concentration in each container.

Following Pau *et al.* (2013), $F/(L/\bar{L})^{2.47}$ was calculated and plotted as a function of temperature (Figure 2(c)). Two regions were distinguished: for $T < 20\,^{\circ}C$, $F/(L/\bar{L})^{2.47}$ was nearly constant with a mean value of 0.5; for $T > 20\,^{\circ}C$, $F/(L/\bar{L})^{2.47}$ increased with temperature with a linear trend with a slope of 0.42 (99% confidence and $r^2 = 0.55$). The larger the temperature the larger $F/(L/\bar{L})^{2.47}$ was. Therefore, for water temperatures above 20 °C, coinciding with periods with a large food amount, *Daphnia magna* filtered at a larger rate. This result is in accordance with Burns (1968), who found that the *Daphnia* filtering rate decreased with a decrease in water temperature and a decrease in the prey concentration. This result is also in accordance with the fact that for low C_{Inf}, usually coinciding in low water temperatures, C_{Out} was sometimes above the 1:1 ratio (Figure 2(b)). However, large particle concentrations for the secondary effluent, corresponding to periods with large water temperatures, resulted in low C_{Out} due to the large filtration rates at these water temperatures.

The selected WWTPs have different secondary and primary treatments. They all comply with the treatment standards to reduce biochemical oxygen demand and the suspended solids below the limits of the Spanish regulations. Wastewater loads from the secondary treatment did not show any clear differences between the WWTPs. In contrast, the main variability in C_{Inf} was found with the period of the year under study, with the largest loads attained after holiday periods, coinciding with the increase in the number of tourists in the region under study (data not shown). The relationship between the C_{Inf} and the C_{Out} for all the conventional tertiary treatments considered is presented in Figure 3. C_{Out} was larger than C_{Inf} after the CF treatment (Figure 3(a)), indicating a large increase in the particle concentration by the processes of coagulation and flocculation. The best fit line between C_{Out} and C_{Inf} shows a slope of 2.67. Some measurements followed the 1:1 ratio (Figure 3(a)), coinciding with situations where no chemicals were added to the wastewater.

For the ST treatment, C_{Out} was smaller than the C_{Inf}, with all the points situated below the 1:1 line (Figure 3(b)). In this case, points followed a linear trend with a slope of 0.27, indicating that the ST treatment had an efficiency of 73%. Measurements that followed the 1:1 ratio also corresponded to cases where no chemicals were added to the system. Although small particles have low settling velocities, they were largely removed by ST, probably due to the scavenging of the very small particles by larger flocs as they settled through the water column, as was reported by Honeyman & Santschi (1989) in the clarification of the water column by large settling aggregates. In both CF and ST treatments, the C_{Out} depended on C_{Inf}, showing that the characteristics of the inflow determined the characteristics of the outflow. In contrast to the two previously mentioned treatments (CF and ST), the SF treatment presented a saturation tendency with a mean concentration of C_{Out} of ~12 μl l^{-1} (Figure 3(c)), corresponding to values of C_{Inf} above 15 μl l^{-1}. For values of C_{Inf} below 15 μl l^{-1}, C_{Out} increased linearly with C_{Inf} (Figure 3(c)). Therefore, the SF treatment showed a good performance at retaining small particles. Naddeo & Belgiorno (2007) found that SF treatments produced a minimum reduction of particles with diameters below 10 μm. In contrast, in the present study the concentration of particles below 10 μm for the SF outflow was found not to depend on the quality of the inflow. This result is in accordance with Kau & Lawler (1995), who found a good performance in the SF treatment for small particles, improving with the age of the filter.

The mean value of C_{Out} in the DF treatment was 10 μl l^{-1}; corresponding to values of C_{Inf} above 10 μl l^{-1}. Also, for values of C_{Inf} below 10 μl l^{-1}, C_{Out} increased with C_{Inf}. Therefore, C_{Out} after the DF treatment showed a similar behaviour to that found for the SF treatment (Figure 3(d)). These results seem to differ slightly from those obtained by Gómez *et al.* (2010), who found that the SF treatment reduced the total

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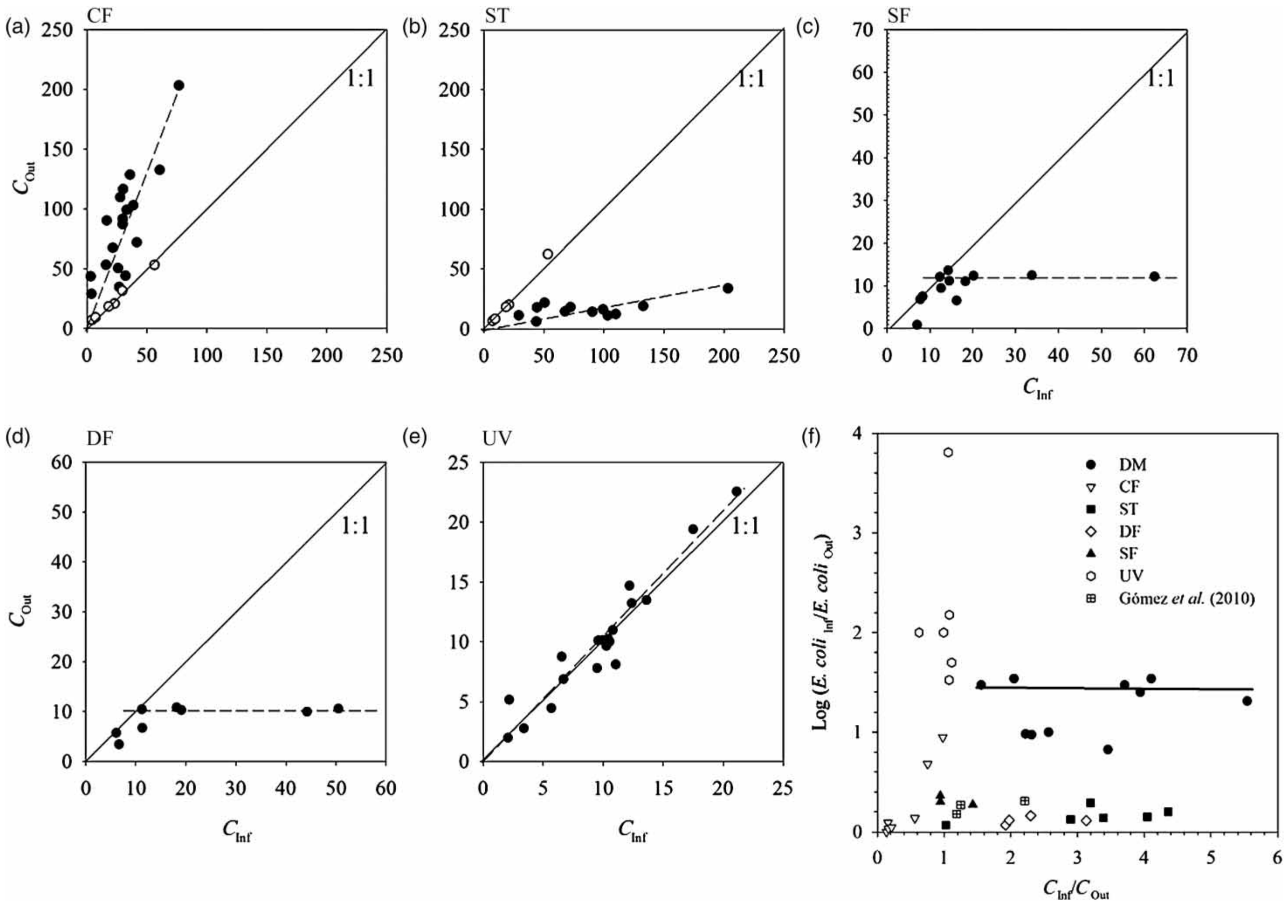

Figure 3 | C_{Out} versus C_{Inf} for the conventional tertiary treatments analyzed: (a) CF (the slope of the line is 2.67, $r^2 = 0.58$, 99% confidence), (b) ST (the slope of the line is 0.27, $r^2 = 0.68$, 99% confidence), (c) SF, (d) DF and (e) UV (the slope of the line is 1.07, $r^2 = 0.86$, 99% confidence). Open symbols represent days when no chemicals were added to the coagulation–flocculation system. Solid symbols correspond to days when chemicals were added to the CF system. (f) *E.coli* inactivation versus ratio of total particle removal (C_{Inf}/C_{Out}).

suspended solids by 45%, more than the DF treatment, which produced a 20% reduction of suspended solids. The lower pore size (10 μm) of the DF treatment used in the present work, compared to the 20 μm pore size of the DF used by Gómez *et al.* (2010), could explain the difference. The UV treatment did not play any role in the particle removal and C_{Out} followed a linear trend with C_{Inf} (Figure 3(e)).

The *E. coli* inactivation was found to range from 0 to 4 log units. The largest inactivation was found for the UV treatment, with values from 1.5 to 4 log units (Figure 3(f)). Low inactivation levels were attained by the CF, ST, DF and SF treatments, with mean values of 0.2 log units. Inactivation found for these macrofiltration systems is in accordance to that found by Gómez *et al.* (2010) for sand filters, disc filters and mesh filters. Results from Gómez *et al.* (2010) are also included in Figure 3(f) and also had mean values of 0.2 log units. Gómez *et al.* (2007) found that the disinfection level of the outflow was a function of the quality of the inflow for both SF and ultrafiltration tertiary treatments. Gómez *et al.* (2007) also found that macrofiltration showed low disinfection levels compared to those of ultrafiltration. *E. coli* inactivation was not found to depend on the ratio of C_{Inf}/C_{Out} (i.e. not dependent on the particle removal) in any of the treatments. The *E. coli* inactivation in the DM treatment remained constant at a value of 1.2 log units. It was larger than that obtained with the CF, ST, DF and SF treatments, although lower than that obtained for the UV treatment (Figure 3(f)), which ranged from 1.5 to 4 log units. The inactivation rates obtained are in agreement with the results of Gómez *et al.* (2007), who found inactivation to range between 2.7 and 5 log units. The *E. coli* inactivation in the DM treatment corresponded to a percentage of *E. coli* reduction in the range of 90–97%. This result is in accordance with that found by Shiny *et al.* (2005), who found percentages of *E. coli* reduction in the range of 60–100%, associated with the filtration by a population of

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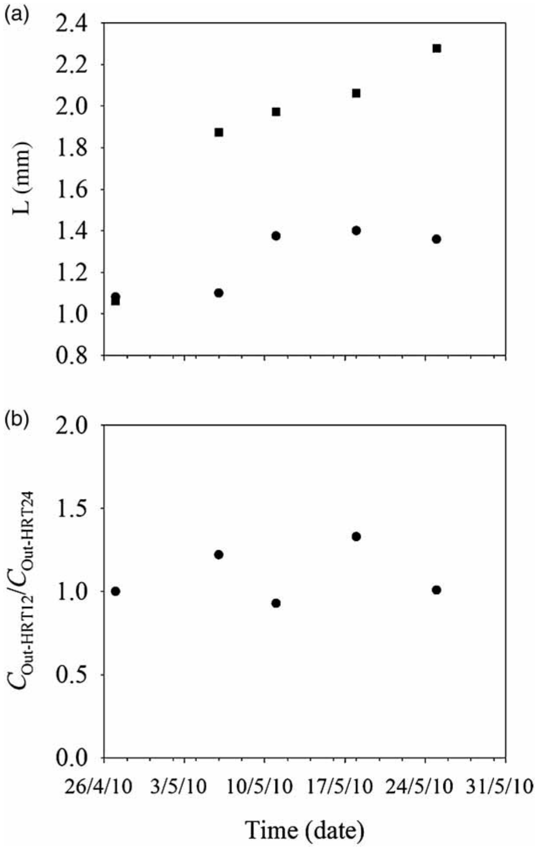

Figure 4 | Time evolution of the mean size L of *Daphnia* individuals (a) and the particle concentration ratio ($C_{Out\text{-}HRT12}$/ $C_{Out\text{-}HRT24}$) (b). In (a), squares correspond to experiments with HRT = 12 h and circles correspond to experiments with HRT = 24 h.

Daphnia magna. Ellis (1987) also found *E. coli* inactivation between 1 and 2 log units depending on both the filtration velocity and the grain media.

The change in the HRT in the DM treatment resulted in a larger growth in the mean size (L) of the *Daphnia* individuals for HRT = 12 h, reaching a value of L = 2.28 mm in 29 days, compared to L = 1.34 mm for the experiment with HRT = 24 h (Figure 4(a)). Furthermore, the ratio $C_{Out\text{-}HRT12}$/ $C_{Out\text{-}HRT24}$ was found to be ~1, showing that C_{Out} was approximately the same for the two different HRTs considered (Figure 4(b)). Therefore, lower residence times, that is, larger food availability, induced the growth of *Daphnia*, maintaining still the same water quality in terms of the suspended particle concentration.

CONCLUSIONS

The comparative analysis of six tertiary wastewater systems showed that a *Daphnia magna* treatment could be a potential and alternative tertiary treatment for wastewater reclamation, providing water with a quality comparable to current tertiary treatments, with low suspended particle content and without the need to use chemicals in the process. The *Daphnia magna* treatment provided wastewater with higher *E. coli* inactivation levels when compared to the inactivation attained by conventional macrofiltration systems but still lower than those obtained by UV light.

Furthermore, *Daphnia magna* adapted its population concentration and the size of their individuals, modulating the filtering capacity depending on both the water temperature and the HRT. In addition, *Daphnia magna* filtration was higher for periods when the water temperature was above 20 °C, corresponding to spring, summer and autumn seasons. Lower HRTs induced the growth of *Daphnia*, providing higher filtration rates and as a result maintaining the same particle removal compared to that for larger HRTs.

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