Int. J. Environ. Sci. Technol. (2016) 13:1433–1442
DOI 10.1007/s13762-016-0985-4

ORIGINAL PAPER

The hydraulic retention time on the particle removal efficiency by *Daphnia magna* filtration on treated wastewater

T. Serra[1] **· J. Colomer**[1]

Received: 27 November 2015 / Revised: 15 March 2016 / Accepted: 15 March 2016 / Published online: 4 April 2016

Abstract The study of low-cost techniques for the tertiary treatment of wastewater is of global interest; above all low-energy techniques that do not require the use of chemicals. In this study, a wastewater treatment technology based on the filtration by a zooplanktonic population (*Daphnia magna*) is studied in controlled laboratory and mesocosm experiments for different hydraulic retention times (HRT). The efficiency of the treatment is evaluated in terms of particle removal efficiency. From laboratory experiments, HRT over 12 h and *Daphnia* concentrations above 50 individuals l^{-1} guarantee a particle removal efficiency greater than 30 %. However, low HRT of 6 h would require *Daphnia* concentrations above 70 individuals l^{-1} in order to obtain a particle removal efficiency of 20 %. The minimum removal efficiency of 2 % was for HRT = 3 h, independent of the *Daphnia* concentration. In the mesocosm, the growth of *Daphnia* individuals enhanced *Daphnia magna* filtering rates and higher removal efficiencies than those in the laboratory for the same HRT range. In the mesocosm experiments *E. coli* concentrations were reduced to a maximum of 2 logarithmic units. A balance equation model is proposed to predict particle removal efficiencies for varying HRT.

Keywords Tertiary treatment · *Daphnia magna* · Turbidity · *E. coli*

✉ T. Serra
Teresa.serra@udg.edu

[1] Department of Physics, University of Girona, Campus Montilivi, 17071 Girona, Spain

Introduction

Freshwater and energy demands have increased in pace with the world's population. Traditionally, wastewater treatment plants invested energy in removing pollutants from water, whereas they are now viewed as future water resource recovery facilities. Wastewater reuse represents a potential source of water, especially in water-deprived areas and also in the face of future scenarios that predict less available water due to climate change. The excess of sludge produced in wastewater treatment plants has become a serious problem since a significant amount of contaminants present in the water are transferred to the air and land (Fu et al. 2011). The use of artificial food web systems could be an efficient method for removing part of the pollutants and suspended solids from wastewater (Jung et al. 2009). As pointed out by Mujeriego and Asano (1999), the aim of water reuse is to achieve water quality standards with a reasonable cost-effective process. Low-cost methods for obtaining wastewater of sufficient quality to be used for non-potable purposes are of global interest, especially if these methods do not require the use of chemicals. Biologically based wastewater treatments are considered a sustainable alternative to conventional wastewater treatment. These biologically based systems have been used for wastewater treatment in small communities and found to be effective in removing emerging contaminants (Matamoros et al. 2012; Garcia-Rodríguez et al. 2014). Furthermore, Garcia-Rodríguez et al. (2014) mentioned that these systems can also work in combination with other conventional technologies in order to enhance removal efficiency for a low additional cost.

While large particles are easily removed from wastewater via settling tanks or filtering techniques, the settling velocity of small particles is too low and they are not easily retained. The use of particle size distribution to determine wastewater quality is widely accepted by a number of authors. Chavez et al. (2004) used particle size distribution to evaluate the microbial quality of wastewater. Particle size distribution was also used by Marquet et al. (2007) to characterize trickling filter effluent and by García-Mesa et al. (2010) to compare the water quality of effluent from different treatments: a submerged biofilm, a trickling filter and a rotating biological contactor. *Daphnia* filtration has also been found to be an efficient technique in removing emergent contaminants from wastewater (Matamoros et al. 2012).

Daphnia magna is an invertebrate zooplankton population from the Cladocera family and is known to prey on a wide variety of organisms provided these organisms are within a certain particle size range that coincides or overlaps with the organic particles that they regularly ingest (Arruda et al. 1983; Gliwicz 1990). Burns (1969) found that *Daphnia* prey on a population of particles with diameters below 35 µm. In her study, the filtration rate of each *Daphnia* individual depended on both water temperature and the body length of the *Daphnia*. DeMott (1982) found that *Daphnia* preyed on a population in the size range of 0.5–40 µm and proportional to the level of food concentration. Lin et al. (2014) also found that the minimum particle size ingested by Daphnia was 0.68 µm. The wide spectrum of particles ingested by *Daphnia* leads to a high individual filtration rate, which results in a high community filtration rate (Horn 1981). Several studies (Straile 2000, 2002; Berger et al. 2006; Schalau et al. 2008) demonstrated that during spring and early summer *Daphnia* are a major controller of the phytoplankton dynamics in lakes and are responsible for what it is known as the clear water phase of a lake. These studies demonstrate that the phytoplankton spring bloom in a lake is followed by the growth in the *Daphnia* population grazing on the phytoplankton. The phytoplankton population subsequently decreases to its minimum value, thus increasing the water clarity of the lake. Kasprzak et al. (1999) compared the effect of two different *Daphnia* populations (*pulicaria* versus *galeata mendotae*) in the clear water phase in Wisconsin lakes with similar climatic conditions. The larger biomass of *Daphnia pulicaria* compared to that of *Daphnia galeata mendotae* was the reason of the earlier clear water phase for lakes where *Daphnia pulicaria* was present. In such lakes, the clear water phase also lasted longer than in lakes with *Daphnia galeata mendotae*. Therefore, the presence of *Daphnia* in lakes is desirable because high *Daphnia* biomass may improve the self-purification potential of ecosystems and accelerate their recovery, especially in lakes with high levels of eutrophication.

As stated by Burns (1969), the *Daphnia* filtration rate is a function of the *Daphnia* body length and the water temperature. Schalau et al. (2008) found that water temperatures above 6 °C guaranteed *Daphnia* development. Serra et al. (2014) found an increase in the *Daphnia* filtration rate for temperatures above 20 °C, which also coincided with a greater food concentration in warmer periods. As mentioned by Burns (1969), in warmer environments *Daphnia* start to grow earlier than in colder environments (Berger et al. 2006). *Daphnia* is therefore considered the most powerful grazer among the zooplankton filter-feeders in lakes (Knisley and Geller 1986). Shiny et al. (2005) and Serra et al. (2014) from laboratory and mesocosm experiments, respectively, demonstrated that *Daphnia magna* are promising organisms in the area of water disinfection, preying directly on suspended sludge particles. Kim et al. (2003) used an artificial food web technology based on phytoplankton and *Daphnia magna* chambers to remove nutrients from domestic wastewaters. With this technology, nitrogen and phosphorous removal was 68 and 56 %, respectively. In this case, phytoplankton reduced the nitrogen and phosphorous from wastewater and *Daphnia* preyed on the phytoplankton population, thus decreasing the suspended solids. Jung et al. (2009) used an artificial food web system to mitigate the eutrophication of polluted stream waters. The artificial food web was based on phytoplankton and *Daphnia magna* sequential chambers and managed to achieve a removal of 69 and 73 % in total nitrogen and phosphorous, respectively. Lin et al. (2014) used an artificial food web system to decrease the excess of biomass in municipal wastewater. The artificial food web system was composed of bacteria, Chlorella and *Daphnia magna* working in sequential chambers. Serra et al. (2014) and Pau et al. (2013) studied the wastewater filtration by *Daphnia magna* in both laboratory and mesocosm experiments. In the experiments of both Serra et al. (2014) and Pau et al. (2013), *Daphnia* preyed directly on the suspended sludge particles. Serra et al. (2014) did not found any relationship between the removal of *E. coli* and the particle retention by conventional treatments, in the range of particle sizes from 2.5 to 500 µm. However, they did find that the *Daphnia* filtration method was more efficient in inactivating *E. coli* than the conventional particle retention methods, but less efficient than the inactivation attained by UV light. Pau et al. (2013) found that *Daphnia magna* prey on a sludge population of particles with diameters below 30 µm and that the greater the *Daphnia* concentration is, the greater the removal of suspended sludge particles. They found efficiencies in the range of 12–30 % for *Daphnia* concentrations in the range from 10 to 50 ind l^{-1}. Maceda-Veiga et al. (2015) studied the vulnerability of *Daphnia magna* for both water temperature and nitrate concentration. *Daphnia* vulnerability increased in temperatures above 26 °C and/or NO_3^- above 250 mg l^{-1}. Adamsson et al. (1998) identified ammonia (with concentrations above 280 mg l^{-1}) and copper (with concentrations of 800 mg l^{-1}) as the major toxicity agents in the *Daphnia magna*. In this case, the copper concentrations were 13 times the toxic copper concentration for *Daphnia magna* (Biesinger and Christensen 1972). Leung et al. (2011) demonstrated the toxic effect of unionized ammonia on a population of *Daphnia carinata* in digested piggery effluent water with a limit of 2.8 mg l^{-1} of NH_3. In general, the quality of secondary treated domestic wastewater is below the limits mentioned here for copper and ammonia.

Therefore, provided the wastewater to be treated is within the range of the water quality required, the main concern for this *Daphnia* filtration technology arises when a certain flow rate of treated wastewater is required to fulfill water reclamation. If the flow rate in the *Daphnia* water treatment reactor is too high, this may entail a decrease in the water quality of the outflow. Therefore, knowing how fast *Daphnia* can filter to produce water with the sufficient quality is required. Such information will provide engineers with the data needed to adapt the volume of the reactor to the target wastewater treatment plant.

In the present study, the filtration of wastewater sludge particles by a population of *Daphnia magna* will be quantified and modeled to test the effect of the hydraulic retention time in the efficiency of the treatment. *Daphnia magna* filtration efficiencies obtained from laboratory experiments are compared with those obtained from mesocosm experiments to obtain the optimal hydraulic retention times in terms of the removal of small sludge particle concentration, turbidity and *E. coli* concentration.

Materials and methods

Laboratory setup and methods

Laboratory experiments were carried out in five cylindrical containers, each $H = 30$ cm high and 14.5 cm in diameter (Fig. 1a). These containers were filled with 5 l of wastewater from the secondary treatment plant (Empuriabrava, NE Catalonia, Spain) and with five *Daphnia magna* concentrations ($C_{Dph} = 0$, 25, 35, 50 and 70 individuals/l), in accordance with the range of *Daphnia* concentrations obtained in the mesocosm. *Daphnia magna* individuals were collected from the Empuriabrava ponds situated in the wastewater treatment plant and stored in an open glass container (storage container) for 24 h before being used in the experiments. The storage container was previously filled with wastewater from the ponds and kept at a constant room temperature of 20 °C and placed in a location where plenty of diffused sunlight was available. Moreover, 0.5 l/min of air was pumped into the storage container. *Daphnia* individuals were pipetted from the storage container, counted and introduced in each 5 l container to obtain the *Daphnia magna* concentration ($N_{Dph} = 0$, 125, 175, 250 and 350 individuals) desired in each one. A 35-l container supplied wastewater to the *Daphnia* treatment containers. Five pipes connected the supply container to each treatment container with a peristaltic pump that allowed the hydraulic retention time of the wastewater in the *Daphnia* treatment to be set, thus ensuring the same hydraulic retention time in all the containers with different *Daphnia* concentrations. Four hydraulic retention times HRT = 3, 6, 12 and 24 h were tested. The hydraulic retention time was calculated as the time required to renew the water volume in the *Daphnia* treatment container. Each pipe injected the wastewater 15 cm from the base of the container. An overflow pipe was situated 30 cm from the base of the container.

From each container 80-ml water samples was taken at different times at $z = 25$ cm from the bottom of the container for being analyzed with a laser particle size analyzer (Lisst-100, Sequoia Inc.) to determine the time evolution of the particle volume concentration of the suspended solids (mainly composed of organic matter). The Lisst-100 has a measurable particle diameter range of 2.5–500 µm and has been found to perform well when determining particle size distribution and concentration of both organic (Serra et al. 2001) and inorganic particles (Serra et al. 2002, 2005) in water suspension. The particle concentration in the ingesting *Daphnia* particle size range was calculated by integrating the concentration within the size range. Once analyzed, samples were gently reintroduced back into the container to maintain a constant volume. The concentration of suspended particles with diameters below 30 µm (C) was calculated, and the ratio between this concentration and the initial concentration of the secondary effluent (C_0) was studied with time.

Mesocosm setup and methods

A mesocosm for the *Daphnia* wastewater treatment was installed after the secondary water effluent treatment in a wastewater plant (Empuriabrava, NE Catalonia, Spain). The wastewater treatment plant has a mean flow varying from 1500 m^3/day in winter to 8000 m^3/day in summer. The mesocosm consisted of 8 containers, each with a volume of 1 m^3 (Fig. 1b). These containers were distributed into four different lines (A, B, C and D, Fig. 1b), with two containers connected in series per line. Water from the secondary treatment was pumped to containers E, where the large particles settled. From container E water was then pumped to the second container where it was submitted to the *Daphnia* treatment. The mean flow through the containers was kept constant by means of four

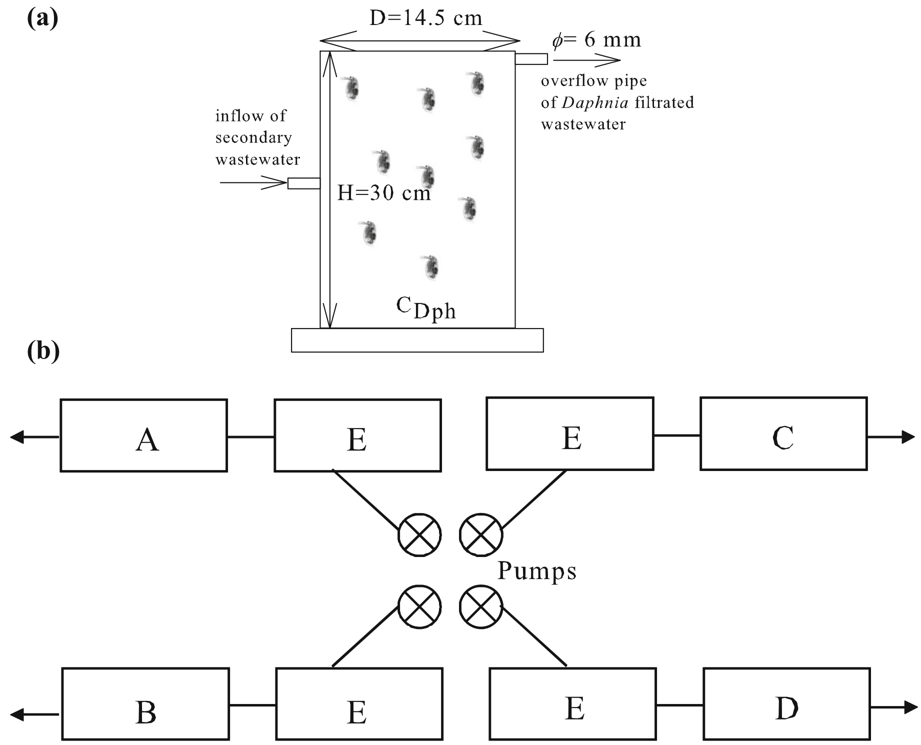

Fig. 1 Laboratory (**a**) and mesocosm (**b**) experimental setup

different pumps (one per line) also ensuring a constant retention time. The containers were inoculated with *Daphnia* and left to evolve with time. All the lines in the mesocosm worked with a hydraulic retention time (HRT) of 24 h for 11 months, and subsequently, over one month, two of the lines (C and B) were kept at a constant hydraulic retention time of 12 h (2 m^3/day) while lines A and D were kept at a constant hydraulic retention time of 24 h (1 m^3/day).

Daphnia magna was inoculated by taking some *Daphnia* individuals from the Empuriabrava wastewater ponds with the same concentration number in each container. For *Daphnia* counting, one water sample of 10 l was taken once per week from each tank, 1 l at ten evenly distributed locations along the container using a siphon tube. The 10-l water sample was filtered with a zooplankton net of 45 µm porous diameter. The filtered water sample was collected and then returned to its corresponding container in order to keep the water volume constant. *Daphnia* counting measurements produced a modification in the *Daphnia* concentration of 1 %. After this filtration, a second filtration with a mesh of 500 µm was performed in the laboratory in order to retain the *Daphnia magn*a. Once retained, the *Daphnia* were fixed with alcohol for being counted. Observations of samples under the optical microscope were carried out in order to count the *Daphnia* population in each mesocosm container. In the mesocosm, measurements of *E. coli*, turbidity, particle concentration and *Daphnia* counting were made at a monthly rate for the first 11 months and weekly for the last month, when the experiment with different HRTs was carried out. During this last month, the *Daphnia* body length was also measured by observations of samples under the microscope. The mean of the length of 25 individuals was considered for each sampling.

Turbidity was measured with a portable turbidimeter (Model 2100P, Hach Company), working in the range 0–1000 NTU. *E. coli* measurements were taken at the outflow of each container. Wastewater samples were collected and stored in sterile glass bottles for *E. coli* analysis; 100 ml samples was 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. 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.

Theoretical model

The time evolution of the concentration of suspended sludge particles in the containers can be described with a balance equation that takes into account the negative and positive fluxes of sludge particles as follows:

$$\frac{dC}{dt} = \frac{C_0}{\mathrm{HRT}} - K_s C - K_{\mathrm{Dph}} C - \frac{C}{\mathrm{HRT}} \quad (1)$$

where HRT is the hydraulic residence time, K_s is the rate at which particles settle through the water column of the container, and K_{Dph} is the rate at which *Daphnia* remove particles from the water column through filtration. The settling rate K_s, which has units of s^{-1}, can be considered to be proportional to the settling velocity of particles (v_s) divided by the height of the water column (H); i.e., $K_s = v_s/H$. The total *Daphnia* filtering rate (K_{Dph}) can be considered to be a linear function of the *Daphnia* concentration (C_{Dph}) and the individual *Daphnia* filtering rate (F) by following $K_{Dph} = FC_{Dph}$ (Pau et al. 2013). F is the individual filtering rate of each *Daphnia* and has dimensions of ml h^{-1} individual^{-1}. Burns (1969) found that F was a function of the *Daphnia* body length (L) and the water temperature (T). In the laboratory experiments of the present study, both L and T can be considered constant.

The first and the fourth terms on the right-hand side in Eq. 1 represent the inflow and the outflow of wastewater into the *Daphnia* treatment container, with concentrations C_0 and C, respectively. The rate at which this flow enters and exits the container is determined by the hydraulic retention time HRT. The second and third terms on the right-hand side account for the removal of particles by settling and the *Daphnia* filtration, respectively.

Merging all the removal agents in one term (settling, *Daphnia* filtration and the overflow), Eq. 1 can be written as follows:

$$\frac{dC}{dt} = \frac{C_0}{\text{HRT}} - \left(K_s + K_{\text{Dph}} + \frac{1}{\text{HRT}}\right)C \tag{2}$$

which has an exponential function as a solution that can be written as:

$$C = \frac{C_0/\text{HRT} - (C_0/\text{HRT} - KC_0)e^{-Kt}}{K} \tag{3}$$

where $K = K_s + K_{Dph} + K_{HRT}$, where $K_{HRT} = 1/\text{HRT}$. Then, Eq. 3 can be written as follows:

$$C/C_0 = \frac{K_{\text{HRT}} - (K_{\text{HRT}} - K)e^{-Kt}}{K} \tag{4}$$

In the steady state $dC/dt = 0$ and therefore,

$$(C/C_0)_{ss} = K_{\text{HRT}}/K \tag{5}$$

From Eq. (5), the ratio $(C/C_0)_{ss}$ (where ss denotes the value at the steady state) is defined as α and can be compared with the ratio $(C/C_0)_{ss}$ obtained only by sedimentation (defined as α_0) with the following equation,

$$\frac{\alpha_0 - \alpha}{\alpha_0} \tag{6}$$

This ratio will be denoted as the *Daphnia* removal efficiency for each *Daphnia* concentration.

Results and discussion

Mesocosm experiments

During the 11 months, the *Daphnia* concentration in the mesocosm varied in the range of 15 individuals l^{-1} to 55 individuals l^{-1}. The lowest concentrations corresponded to periods with the lowest water temperatures (winter), whereas the greatest *Daphnia* concentrations corresponded to spring, summer and autumn, when the water temperature was between 15 and 25 °C.

In the mesocosm, turbidity at the outflow of the *Daphnia* tertiary treatment (TB_{out}) was below the turbidity at the inflow of *Daphnia* tertiary treatment (TB_{in}) (Fig. 2a). However, TB_{out} increased with TB_{in}, indicating that the quality of the water treated depended on the inflow water quality. The best-fit line of the curve was $y = 0.35x + 0.28$ ($r^2 = 0.710$, 99 % of confidence), indicating that the mean efficiency of the *Daphnia* filtration was 65 %.

The *E. coli* colonies were also reduced in the mesocosm with the *Daphnia* tertiary treatment (Fig. 2b). For HRT = 24 h, the reduction was from 0.5 logarithmic units for the lowest number of *E. coli* colonies at the inflow to 2 logarithmic units for the greatest number of *E. coli* colonies at the inflow. Again, as was found with turbidity, the number of *E. coli* colonies at the outflow increased with the number of *E. coli* colonies at the inflow, indicating that the water disinfection of the outflow was a function of the water quality of the inflow.

In the second mesocosm experiment, *E. coli* reductions in line with HRT = 12 h and in line with HRT = 24 h were found to be within the same range (Fig. 2b). This is in accordance with the results found for the turbidity for the experiment at HRT = 12 h. In this experiment, turbidity for HRT = 12 h followed the same trend as found for HRT = 24 h (Fig. 2a).

Therefore, both the turbidity (Fig. 2a) and the *E. coli* (Fig. 2b) decreased after *Daphnia magna* wastewater treatment, showing the particle removal and disinfection potentials of this tertiary technology. The turbidity of the outflow always remained below 2 NTU, making it a potential treatment for wastewater reuse (RD 1620/2007). *E. coli* removal was up to 2 logarithmic units, which is slightly below what can be achieved with a UV light (Serra et al. 2014), but above what is achieved with mechanical filters (Serra et al. 2014). However, despite the high *E. coli* reduction, the observed *E. coli* colonies found in the outflow still remained high in some cases (the maximum *E. coli* was 4110 colonies/100 ml). In such eventual situations, the system may work in combination with other conventional systems to always ensure a safe water reuse (RD 1620/2007).

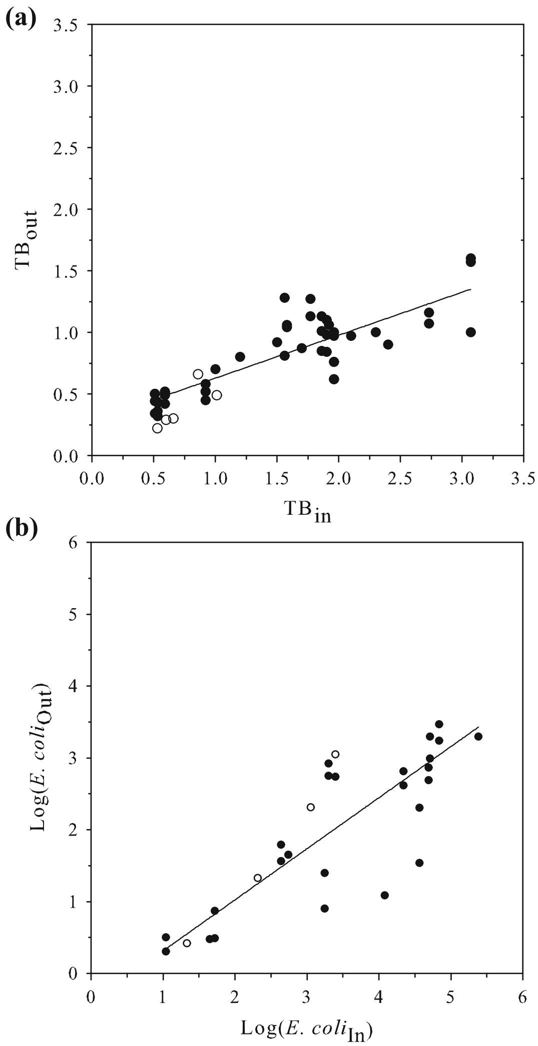

Fig. 2 **a** Turbidity at the outflow of the *Daphnia* treatment in the mesocosm (TB_{out}) versus the turbidity of the inflow (TB_{in}). *Solid symbols* represent experiments run at HRT = 24 h, whereas *open symbols* represent experiments run at HRT = 12 h. **b** Logarithm of the *E. coli* at the outflow of the *Daphnia* treatment in the mesocosm (Log(*E. coli*$_{out}$)) versus the logarithm of the *E. coli* at the inflow (Log(*E. coli*$_{in}$)). *Solid symbols* represent experiments run at HRT = 24 h, whereas *open symbols* represent experiments run at HRT = 12 h

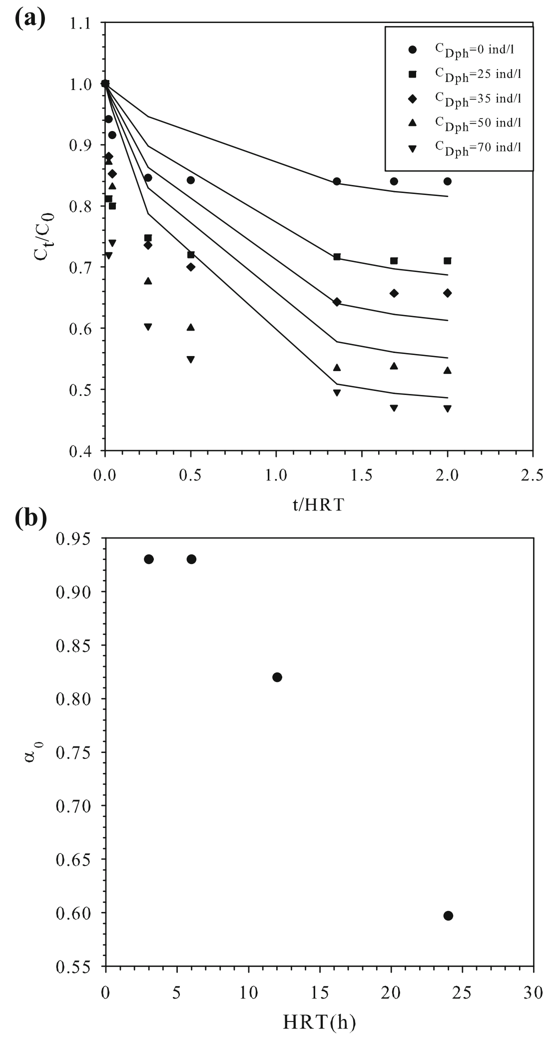

Fig. 3 **a** Behavior of the ratio of sludge concentration C/C_0 with the non-dimensional time t/HRT for the different *Daphnia* concentrations studied for HRT = 12 h. *Solid lines* represent the time evolution predicted by the model described in Eq. 4. **b** Relationship between the particle removal due to sedimentation (α_0) and HRT

Laboratory experiments

The ratio C_t/C_0 was calculated and represented versus the non-dimensional time t/HRT for the range of *Daphnia* concentrations studied, $C_{Dph} = 0$, 25, 35, 50 and 70 individuals l^{-1}, and for HRT = 12 h (Fig. 3a). C_t/C_0 decreased with t/HRT reaching a steady state, α. The greater the *Daphnia* concentration, the smaller α was. The case of $C_{Dph} = 0$ individuals l^{-1} represents the case of the particle removal by sedimentation, with α = 0.84. The minimum $C/C_0 = 0.46$ corresponded to the greatest C_{Dph} of 70 individuals l^{-1}.

The model described by Eq. 4 was found to predict the final steady state of C/C_0 for all *Daphnia* concentrations except for t/HRT ≤ 1, where experimental C/C_0 decreased faster than those predicted by the model (Fig. 3a). In the model, the settling velocity of particles was calculated from Stokes' law as $v_s = (2/9)R^2 g(\rho_{sludge} - \rho_{H2O})/\mu$, where R is the sludge particle radius, g is the gravity constant, ρ_{sludge} is the density of sludge, ρ_{H2O} is the water density, and μ is the water viscosity. The radius of the sludge was considered to be the mean of the particle size in the particle number distribution of the particle range studied with $d < 30$ μm. The particle number distribution was calculated dividing each particle volume concentration by the characteristic size of the particles to the fourth power (following the indications of the Lisst-100 manufacturer).

The gravity constant was considered as 9.8 ms^{-2}, the sludge density 1050 kg m^{-3} (Sperling 2007), the water density 1000 kg m^{-3} and the water viscosity 10^{-3} Pa s. With all these considerations, v_s was 0.0063 m h^{-1}. For calculating $K_s = v_s/H$, the settling depth H of the water column was considered to be 30 cm. All these assumptions resulted in $K_s = 0.021$ h^{-1}. For *Daphnia* cells of 2 mm in diameter and at a water temperature of 20 °C, the individual *Daphnia* filtration rate was considered to be ~1 ml ind^{-1} h^{-1} (Burns 1969). These considerations resulted in values of K_{Dph} in the range of 0–0.07 h^{-1} for the *Daphnia* concentration range of 0–70 ind l^{-1}. K_{HRT} varied in the range of 0.33–0.042 h^{-1} for the range of hydraulic retention times from 3 h to 24 h.

The steady state C/C_0 for the case with $C_{Dph} = 0$ individuals l^{-1}, i.e., with the removal only due to the sedimentation (α_0), is presented in Fig. 3b. Sedimentation removed suspended particles by 7 % for both HRT = 3 h and HRT = 6 h (with $\alpha_0 = 0.93$). For HRT > 6 h, α_0 decreased when HRT increased. The minimum $\alpha_0 = 0.60$ was found for HRT = 24 h, i.e., a particle removal of 40 %.

For each experimental run, the *Daphnia* removal efficiency calculated from Eq. 6 was plotted with the hydraulic retention time (HRT) (Fig. 4). For a fixed *Daphnia* concentration, the *Daphnia* removal efficiency increased with HRT with a nonlinear behavior. The greater the *Daphnia* concentration, the greater the *Daphnia* removal efficiency was (Fig. 4), except in the case of HRT = 3 h, where the efficiency was the same for the *Daphnia* concentrations studied. For the *Daphnia* concentrations from 35 individuals l^{-1} to 70 individuals l^{-1}, the change from HRT = 12–24 h produced an increase in the removal efficiency but this increase was smaller than for the change from HRT = 3 to HRT = 12 h. For the *Daphnia* concentration of 25 individuals l^{-1} and HRT of 12 and 24 h, the *Daphnia* removal efficiency remained constant.

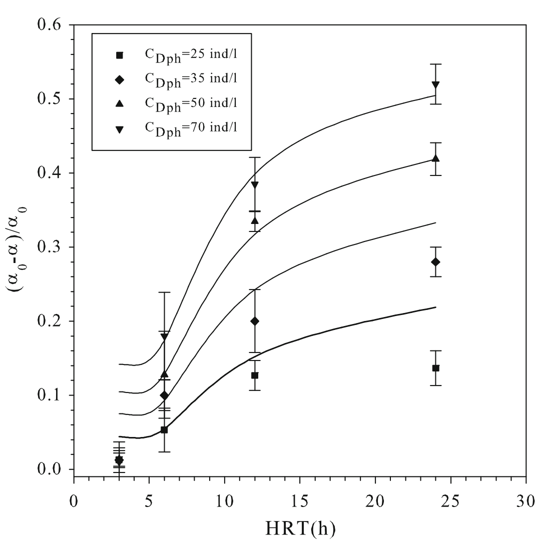

Fig. 4 Relationship between the *Daphnia* removal efficiency (α) compared with the sedimentation (α_0) and HRT for different *Daphnia* concentrations. *Solid lines* represent the removal efficiency predicted by the model in the steady state

The lines in Fig. 4 show the results for the removal efficiency obtained by the model described by Eq. 6. This model was able to satisfactorily predict the behavior of the efficiency for all HRTs, except for the lowest HRT = 3 h. At HRT = 3 h the model predicted a greater efficiency than that found experimentally. Furthermore, at this HRT the model predicted different removal efficiencies for the different *Daphnia* concentrations, with a greater efficiency for the highest *Daphnia* concentration. However, experimental data for HRT = 3 h did not show differences in the removal efficiency for the different *Daphnia* concentrations studied.

Both filtering of *Daphnia magna* on sludge particles and sedimentation of sludge particles reduced the sludge concentration of particles smaller than 30 µm in an amount that depended on the hydraulic retention time. For the sedimentation process and for the cases with HRT > 6 h, the removal efficiency depended on HRT, whereas for HRT ≤ 6 h the removal efficiency was constant, indicating that sedimentation was not dependent on HRT, i.e., that the hydraulic retention time was too low for small particles to settle. The *Daphnia* removal efficiency on sludge particles ranged from 0.02 (2 %) for the smallest HRT and for all *Daphnia* concentrations to 0.52 (52 %) for the highest HRT of 24 h and the highest *Daphnia* concentration of 70 individuals l^{-1} (Fig. 4). The greater both the *Daphnia* concentration and the HRT were, the greater the *Daphnia* removal efficiency was. *Daphnia* concentrations above 50 ind l^{-1} and HRT ≥ 12 h would guarantee a particle removal efficiency above 30 % than that found for sedimentation. Therefore, considering both removal due to sedimentation and removal due to *Daphnia* filtration, the total particle removal was from 9 % for HRT = 3 h to 92 % for HRT = 24 h.

Greater *Daphnia* concentrations than those studied in this work could guarantee higher removal efficiencies than those found for experiments working with low HRTs. However, the *Daphnia* survival is doubtful in situations where the water flow is high and with a high level of turbulence (Tóth et al. 2011). The proposed model could predict fairly well the removal efficiency for all the HRT studied except for the smallest HRT of 3 h. The reason for this could be due to the fact that the flow entering the container at HRT = 3 h generated a high level of turbulence in the system. It is known that turbulence may affect *Daphnia* swimming behavior (Seuront et al. 2004), *Daphnia* morphology (Laforsch and Torllrian 2004) and even cause their mortality (Tóth et al. 2011). Wickramarathna et al. (2014) found *Daphnia* swimming velocities of 1.54 cm s^{-1} for *Daphnia* cells 2 mm long. In the present study, the flow velocities at the entrance to the container in the laboratory were 0.07, 0.43, 0.82 and 1.64 cm s^{-1} for HRT = 3, 6, 12 and 24 h, respectively. Therefore, the *Daphnia* swimming velocity and the feeding capacity of *Daphnia* might be partially inhibited by the flow velocities at the inlet. To overcome this issue a modification to the inlet of water into the container minimizing the turbulence would prevent this effect over the *Daphnia* filtering efficiency at low HRT. Furthermore, the model predicted a slower temporal decrease in C_t/C_0 compared with the experimental results (Fig. 4). This difference might be due to the fact that in the experiments small sludge particles might be scavenged by larger particles while they settle (Honeyman and Santschi 1989), and which was not included in the model. This fact would account for a faster settling process than that described by the model. Despite this, the steady state was well predicted by the model. The model also overestimated the *Daphnia* removal efficiency when the *Daphnia* concentration was 35 individuals l^{-1} or lower and for experiments with HRT > 6 h. Degans et al. (2002) found that *Daphnia* concentrations below 30 individuals l^{-1} were not able to reduce the bacterial concentration in 24 h because the growth of bacteria during this period overcame the *Daphnia* filtration. The model did not consider the possible growth of bacteria, which may be an important fraction in the sludge composition. Since the bacterial growth was not incorporated in the model, the model may overestimate *Daphnia* removal efficiency for low *Daphnia* concentrations.

The removal efficiency, obtained experimentally in both the mesocosm and the laboratory, was plotted against the *Daphnia* concentration for the range of HRT studied (Fig. 5). The greater the *Daphnia* concentration, the greater the efficiency was for all HRT except in the case of HRT = 3 h, where removal efficiency did not vary with *Daphnia* concentration. The removal efficiencies obtained in the mesocosm for HRT = 12 h were close to those obtained for HRT = 24 h (Fig. 5). Furthermore, for *Daphnia* concentrations greater than 50 individuals l^{-1}, the *Daphnia* removal efficiencies in the mesocosm were close to those found in the laboratory, while for *Daphnia* concentrations below 50 individuals l^{-1}, the *Daphnia* removal efficiencies in the mesocosm were lower. Working in the same mesocosm, Serra et al. (2014) found a reduction of 25 % in the particle concentration, which was attributed to the sedimentation of particles in the containers. Therefore, a total particle reduction of 90 % (65 % + 25 %) was obtained for HRT = 12 and 24 h, which is close to that obtained in the laboratory.

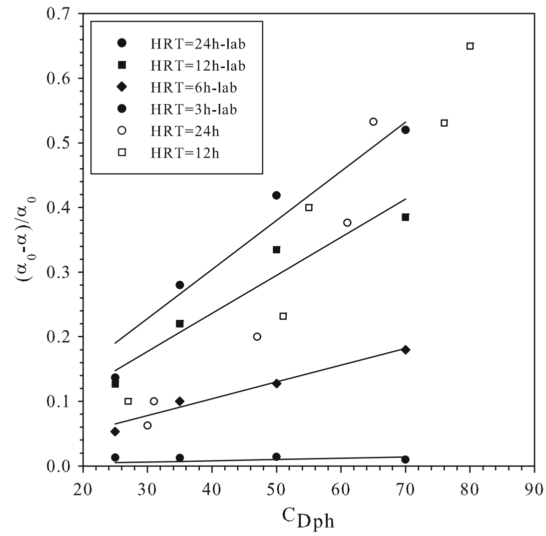

Fig. 5 Relationship between the *Daphnia* removal efficiency (α) compared with the sedimentation (α_0) and C_{Dph} for the different HRTs studied for laboratory experiments (*solid symbols*) and for mesocosm experiments (*open symbols*) and for the different HRTs. *Lines* represent the linear best-fit line from the results obtained in the laboratory. For HRT = 3 h, $y = 0.002x$ ($R^2 = -12.72$); for HRT = 6 h, $y = 0.0026x$ ($R^2 = 0.9734$); for HRT = 12 h, $y = 0.0059x$ ($R^2 = 0.9266$); for HRT = 24 h, $y = 0.0076x$ ($R^2 = 0.9440$)

The *Daphnia* body length observed in mesocosm experiments was found to change over time during the period of the study. For experiments with HRT = 12 h, *Daphnia* had greater growing rates than *Daphnia* in those experiments with HRT = 24 h (Fig. 6). For HRT = 12 h *Daphnia* reached lengths (L) up to 2.14 times the length they had had initially (L_0), while for HRT = 24 h *Daphnia* reached lengths up to 1.26 times the L_0.

The *Daphnia* removal efficiency obtained in the mesocosm experiments varied in the same range as that found for HRT = 12 and 24 h in the laboratory (Fig. 5). However, in the mesocosm, no differences were observed between the removal efficiencies at HRT = 12 h and HRT = 24 h, contrary to what was found in the laboratory where there were greater *Daphnia* removal efficiencies for higher HRTs. The reason for this might be in the fact that in the mesocosm *Daphnia* grew from time intervals between measurements. Larger *Daphnia* body sizes are expected to produce greater individual filtering rates (Burns 1969), therefore increasing removal efficiency. *Daphnia* growth was greater in the mesocosm for the case of HRT = 12 h, than it was for the case of HRT = 24 h (Fig. 6). *Daphnia* removal efficiency for smaller *Daphnia* concentrations ($C_{Dph} < 50$ individuals l^{-1}) in the mesocosm was slightly below that obtained in the laboratory for HRT = 12 h and HRT = 24 h. In contrast, the *Daphnia* removal efficiency for $C_{Dph} > 50$ individuals l^{-1} was equal

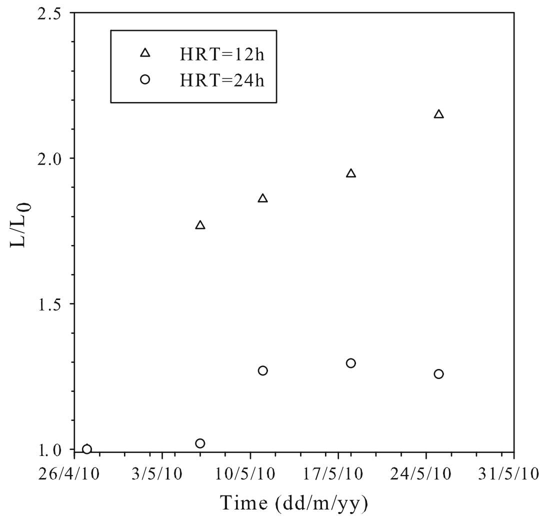

Fig. 6 Time evolution of L/L_0 in the mesocosm experiments for HRT = 12 h and HRT = 24 h. L_0 = 1.08 mm

to or slightly above that obtained in the laboratory for HRT = 12 h and HRT = 24 h. These slight differences between mesocosm and laboratory results at low *Daphnia* concentrations might be due to the different water temperature between the laboratory and the mesocosm experiments. The water temperature in the mesocosm was below that in the laboratory during the period of low *Daphnia* concentration at the beginning of the experiment. However, at the end of the mesocosm experiment temperatures rose, coinciding with higher *Daphnia* concentrations. Furthermore, since the *Daphnia* filtration also depends on the *Daphnia* concentration, the greater the *Daphnia* concentration, the higher the *Daphnia* filtration is. Although some studies have found that high *Daphnia* concentrations are possible (Jürgens et al. 1994) for short periods of time, the sustainability of such *Daphnia* concentrations for a long period of time still remains unknown.

Conclusion

Tertiary wastewater technology based on *Daphnia magna* filtration was found to be efficient at both removing suspended sludge particles and wastewater disinfection. Temperature had a positive effect on *Daphnia* development and feeding. The present study makes evident the important role hydraulic retention time plays, i.e., introducing a limitation for *Daphnia* filtration efficiency. Low flow rates (HRT > 6 h) increase the available preying time for *Daphnia* and therefore increases removal efficiency. However, low retention times produce water of low quality. Hydraulic retention times over 12 h and *Daphnia* concentrations above 50 ind l^{-1} ensure a particle removal above 30 % due to *Daphnia* filtration plus the removal by sedimentation (>18 %) resulting in a total particle removal above 68 %.

Acknowledgments We thank Concepció Pau and Joan Colom for their assistance in the field work. This work was funded by the Consorci de la Costa Brava (CCB). We also thank Lluis Sala for his encouragement during the work.

References

Adamsson M, Dave G, Forsberg L, Guterstam B (1998) Toxicity identification evaluation of ammonia, nitrite and heavy metals at the Stensund Wastewater Aquaculture Plant, Sweden

Arruda JA, Marzolf GR, Flauk RT (1983) The role of suspended sediments in the nutrition of zooplankton in turbid reservoirs. Ecology 64:1225–1235

Berger SA, Diehl S, Stibor H et al (2006) Water temperature and mixing depth affect timing and magnitude of events during spring succession of the plankton. Oecologia 150:643–654. doi:10.1007/s00442-006-0550-9

Biesinger KE, Christensen GM (1972) Effects of various metals on survival, growth, reproduction, and metabolism of daphnia magna. J Fish Res Board Canada 29:1691–1700. doi:10.1139/f72-269

Burns C (1969) The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. Limnol Oceanogr 14:693–700

Chavez A, Jiménez R, Maya C (2004) Particle size distribution as a useful tool for microvial detection. Water Sci Technol 50:179–186

Degans H, Zöllner E, Gucht K et al (2002) Rapid Daphnia-mediated changes in microbial community structure: an experimental study. FEMS Microbiol Ecol 42:137–149. doi:10.1111/j.1574-6941.2002.tb01003.x

DeMott WR (1982) Feeding selectivities and relative ingestion rates of Daphnia and Bosmina. Limnol Oceanogr 27:518–527

Fu T, Xiao Q, Huang X, Fu J (2011) Report on sludge treatment and disposal market in China. http://www2.h2o-china.com/report/2011/2011wunireport

García-Mesa JJ, Poyatos JM, Delgado-Ramos F et al (2010) Water quality characterization in real biofilm wastewater treatment systems by particle size distribution. Bioresour Technol 101:8038–8045. doi:10.1016/j.biortech.2010.05.071

Garcia-Rodríguez A, Matamoros V, Fontàs C, Salvadó V (2014) The ability of biologically based wastewater treatment systems to remove emerging organic contaminants—a review. Environ Sci Pollut Res 21:11708–11728. doi:10.1007/s11356-013-2448-5

Gliwicz ZM (1990) Food thresholds and body size in Cladocerans. Nature 343:638–640

Honeyman BD, Santschi PH (1989) A Brownian pumping model for oceanic trace-metal scavenging-evidence from TH-isotopes. J Mar Res 47:951–992

Horn W (1981) Phytoplankton losses due to zooplankton grazing in a drinking water reservoir. Int Rev der gesamten Hydrobiol und Hydrogr 66:787–810

Jung D, Cho A, Zo YG et al (2009) Nutrient removal from polluted stream water by artificial aquatic food web system. Hydrobiologia 630:149–159. doi:10.1007/s10750-009-9788-7

Jürgens K, Gasol JM, Massana R, Pedrós-Alió C (1994) Control of heterotrophic bacteria and protozoans by Daphnia pulex in the epilimnion of Lake Ciso. Arch fur Hydrobiol 131:55–78

Kasprzak P, Lathrop RC, Carpenter SR (1999) Influence of different sized Daphnia species on chlorophyll concentration and summer

phytoplankton community structure in eutrophic Wisconsin lakes. J Plankton Res 21:2161–2174. doi:10.1093/plankt/21.11.2161
Kim SR, Woo SS, Cheong EH, Ahn TS (2003) Nutrient removal from sewage by an artificial food web system composed of phytoplankton and Daphnia magna. Ecol Eng 21:249–258. doi:10.1016/j.ecoleng.2003.11.003
Knisley K, Geller W (1986) Selective feeding of four zooplankton species on natural lake phytoplankton. Oecologia 69:86–94
Laforsch C, Torllrian R (2004) Extreme helmet formation in Daphnia cucullata induced by small-scale turbulence. J Plankton Res 26:81–87
Leung J, Kumar M, Glatz P, Kind K (2011) Impacts of un-ionized ammonia in digested piggery effluent on reproductive performance and longevity of Daphnia carinata and Moina australiensis. Aquaculture 310:401–406. doi:10.1016/j.aquaculture.2010.11.015
Lin S, Jiang J, Yi X et al (2014) Decreasing excess biomass from municipal wastewater using an artificial food web system. J Environ Eng. doi:10.1061/(ASCE)EE.1943-7870.0000837
Maceda-Veiga A, Webster G, Canals O et al (2015) Chronic effects of temperature and nitrate pollution on Daphnia magna: is this cladoceran suitable for widespread use as a tertiary treatment? Water Res 83:141–152. doi:10.1016/j.watres.2015.06.036
Marquet R, Muhammad N, Vairavamoorthy K, Wheatley A (2007) Particle size distribution to assess the performance of trickling filters. Process Saf Environ Prot 85:99–103. doi:10.1205/psep.05194
Matamoros V, Sala L, Salvadó V (2012) Evaluation of a biologically-based filtration water reclamation plant for removing emerging contaminants: a pilot plant study. Bioresour Technol 104:243–249
Mujeriego R, Asano T (1999) The role of advanced treatment in wastewater reclamation and reuse. Water Sci Technol 40:1–9. doi:10.1016/S0273-1223(99)00479-5
Pau C, Serra T, Colomer J et al (2013) Filtering capacity of Daphnia magna on sludge particles in treated wastewater. Water Res 47:181–186. doi:10.1016/j.watres.2012.09.047
RD 1620/2007, de 7 de diciembre por el que se establece el régimen jurídico de la reutilización de las aguas depuradas [Spainsh Act of Parliament 1620/2007 of 7th December 2007 establishing the legal framework for reusing treated wastewater]. Boletín Ofic
Schalau K, Rinke K, Straile D, Peters F (2008) Temperature is the key factor explaining interannual variability of Daphnia development in spring: a modelling study. Glob Chang Biol 157:531–543
Serra T, Colomer J, Cristina X et al (2001) Evaluation of a laser in situ scattering instrument for measuring the concentration of phytoplankton, purple sulphur bacteria and suspended inorganic sediments in lakes. J Environ Eng 127:1023–1030
Serra T, Colomer J, Gacia E et al (2002) Effects of a turbid hydrothermal plume on the sedimentation rates in a karstic lake. Geophys Res Lett. doi:10.1029/2002GL015368
Serra T, Soler M, Julia R et al (2005) Behaviour and dynamics of a hydrothermal plume in Lake Banyoles, Catalonia, NE Spain. Sedimentology 52:795–808. doi:10.1111/j.1365-3091.2005.00611.x
Serra T, Colomer J, Pau C et al (2014) Tertiary treatment for wastewater reuse based on the Daphnia magna filtration—comparison with conventional tertiary treatments. Water Sci Technol 70:705–711. doi:10.2166/wst.2014.284
Seuront L, Yamazaki H, Souissi S (2004) Hydrodynamic disturbance and zooplankton swimming behavior. Zool Stud 43:376–387
Shiny K, Remani K, Nirmala E et al (2005) Biotreatment of wastewater using aquatic invertebrates, Daphnia magna and Paramecium caudatum. Bioresour Technol 96:55–58. doi:10.1016/j.biortech.2004.01.008
Straile D (2000) Meteorological forcing of plankton dynamics in a large and deep continental European lake. Oecologia 122:44–50. doi:10.1007/PL00008834
Straile D (2002) North Atlantic Oscillation synchronizes food-web interactions in central European lakes. Proc Biol Sci 269:391–395. doi:10.1098/rspb.2001.1907
Tóth G, Parpala L, Balogh C et al (2011) Zooplankton community response to enhanced turbulence generated by water-level decrease in Lake Balaton, the largest shallow lake in Central Europe. Limnol Oceanogr 56:2211–2222. doi:10.4319/lo.2011.56.6.2211
Von Sperling M (2007) Wastewater characteristics, treatment and disposal. IWA Publication, London, UK
Wickramarathna LN, Noss C, Lorke A (2014) Hydrodynamic trails produced by Daphnia: size and energetics. PLoS ONE. doi:10.1371/journal.pone.0092383