Technical Bulletin 03
November 2019

INNOQUA is demonstrating how nature-based solutions can treat wastewater to a standard at which can be safely discharged back to the environment or used for irrigation purposes. This technical bulletin comprises a mini review of published data on the fundamental operating principles and performance of the Daphniafilter.

INTRODUCTION

Water fleas are small crustaceans in the Order Cladocera, the significant majority of which are found in lakes, rivers and other suitable freshwater habitats around the world. *Daphnia magna* is a common species that is widely used for ecotoxicological studies. It has a broad natural distribution, having been identified from Russia to India and South Africa. Other species occupy similar ecological niches in countries as diverse as Australia, Mexico and Sri Lanka (Teresa Serra et al., 2019). *Daphnia magna* favours a water temperature of around 20°C, although its distribution means that it can adapt to a broad temperature range (Burns, 1969; Elenbass, 2013). Schalau *et al.,* (2008) found that water temperatures above 6°C were required for reproduction, whilst in mesocosm experiments Serra & Colomer (2016) showed that populations experienced seasonal fluctuations, with maximum population densities achieved when water temperatures were between 15 and 25°C.

Figure 1 Daphnia magna (Ebert, 2005)

Daphnids are filter-feeders that consume small particles suspended in the water column (Ebert, 2005; Elenbass, 2013; Pau et al., 2013). This feeding mechanism does not discriminate between organic and inorganic particulate material. Pau et al. (2013) determined that *Daphnia magna* would filter particles of between 2 and 30 μm under experimental circumstances, which encompasses microalgae, fungal spores and bacteria (Ebert, 2005).

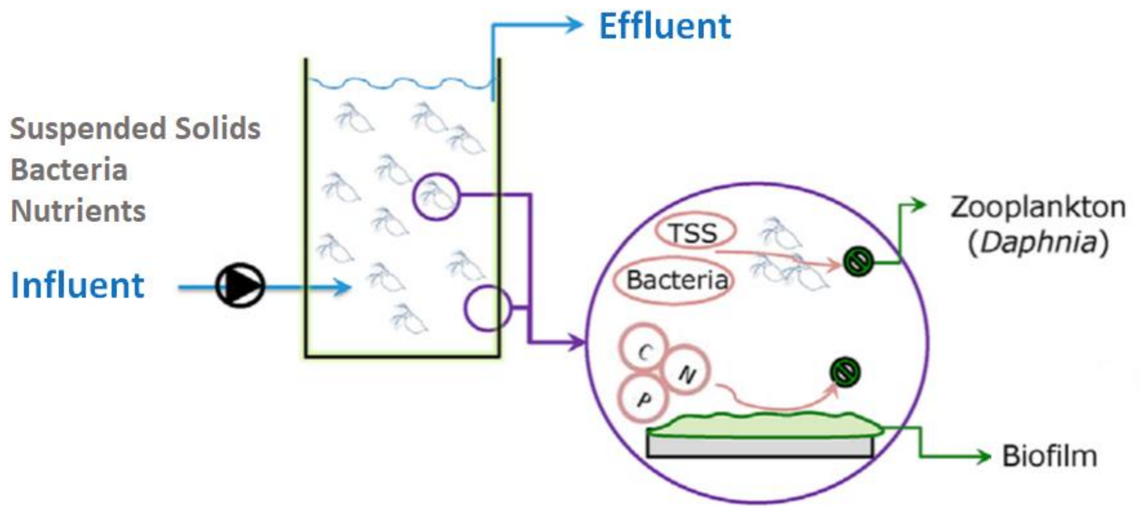
DESIGN CONCEPT

The natural filter-feeding characteristics of daphnids mean that they are able to remove suspended solids that may not normally settle during primary clarification of wastewater. This feeding mechanism also removes some bacteria - suggesting that Daphnia could be used both for clarification and disinfection of treated wastewater as a nature-based tertiary treatment (Pous et al., 2020). Daphnia are sensitive to numerous wastewater characteristics, including ammonium, nitrite and heavy metals - which means that any system using these organisms must follow initial primary and/or secondary wastewater treatment. Within the INNOQUA system, the Daphniafilter is always located downstream of a Lumbrifilter (see Technical Bulletin No. 2 for more information on this technology) whilst the treated effluent is either discharged or - where water re-use is intended - passed through a UV disinfection unit.

The INNOQUA Daphniafilter aims to couple the nutrient transformation and removal capabilities of microalgal/bacterial biofilms with the filtering capabilities of daphnids. Whilst not expected to provide the level of disinfection necessary for direct water re-use, reductions in turbidity resulting from removal of suspended solids will improve the efficacy of any final ultraviolet (UV) disinfection stage (Pau et al., 2013).

The Daphniafilter is configured as a tank inoculated with local species of Cladocera (usually *Daphnia magna*) which are free to move up and down the water column. Internal weir and baffle mechanisms (not shown) ensure that the daphnids remain within the reactor, whilst additional surface area is provided for the establishment of the microalagal/bacterial biofilm (Figure 2).

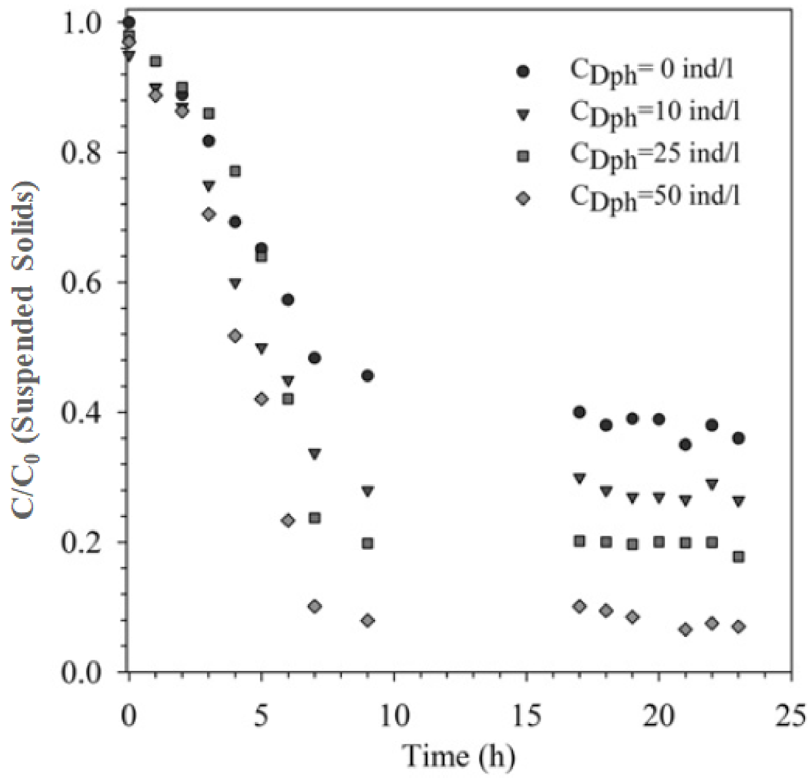
Figure 2 Daphniafilter concept and putative treatment mechanisms, adapted from Pous et al. (2020).



TREATMENT EFFICACY: SUSPENDED SOLIDS

Pau et al. (2013) conducted mesocosm experiments on a wastewater treatment plant (WWTP) in northeast Spain. A series of Daphniafilters was set up, each comprising a 1m^3 sedimentation tank followed by a biofilter tank of the same size, hosting the population of *Daphnia magna*. The system was loaded in such a way that each tank operated under a hydraulic retention time (HRT) of 1 day. The study found that the whole filter system delivered suspended solids' removal of up to 99% with *Daphnia magna* populations ranging from 10-50 organisms per litre. Pau et al. (2013) also reported that a laboratory-scale study with a similar arrangement could remove 92% of the suspended solids (shown in Figure 3), although ~60% of suspended solid reduction was attributed to sedimentation, with the remainder due to Daphniafiltration. These findings were reflected in subsequent research by Serra & Colomer (2016), who considered the interactions between HRT, solids' removal and Daphnia population density. They showed that particle removal efficiencies of >30% could be achieved under HRTs >12 hours, with daphnid populations of >50 individuals per litre. Reducing the HRT to 6 hours reduced solids' removal to 20%, with daphnid populations of >70 individuals per litre. Sediment layers can be siphoned from the bottom of the reactor without impact on its operation, and the frequency at which this might be necessary is being considered as part of the site-based demonstration programme.

Figure 3 Suspended Solids' ratios (C_0 = starting concentration; C = concentration at time of sampling) over time with different population densities of Daphnia magna (ind/l = individuals per litre) (Adapted from Pau et al., 2013).



TREATMENT EFFICACY: PATHOGENS

Shiny et al. (2005) studied the pathogen removal efficacy of *Daphnia magna*. They conducted a laboratory-scale test with a treatment period of 15 days. Sewage from an open sewer was diluted to four concentrations, from 5 to 20 ml of sewage per litre of de-chlorinated tap water. The results are summarised in Table 1. A 15 day residence time is extremely unlikely to be feasible at commercial scale, and INNOQUA demonstration facilities are experimenting with much shorter retention periods.

Table 1 Coliform populations (and Standard Errors) in diluted sewage treatments after 15 days of exposure to Daphnia magna at 30 individuals per 100ml of solution (experimental treatments) compared with Controls (no Daphnia). (Shiny et al., 2005).

Dilution	Experimental treatments		Controls	
	Day 1	Day 15	Day 1	Day 15
5ml/l	1100 (88.5)	19 (64.2)	1100 (74.6)	691 (100.5)
10ml/l	2300 (42.6)	353 (85.3)	2400 (26.4)	2000 (73.2)
15ml/l	6400 (91.5)	3605 (83.6)	4600 (78.2)	3900 (55.8)
20ml/l	7500 (72.6)	2521 (53.4)	7600 (52.0)	6400 (24.6)

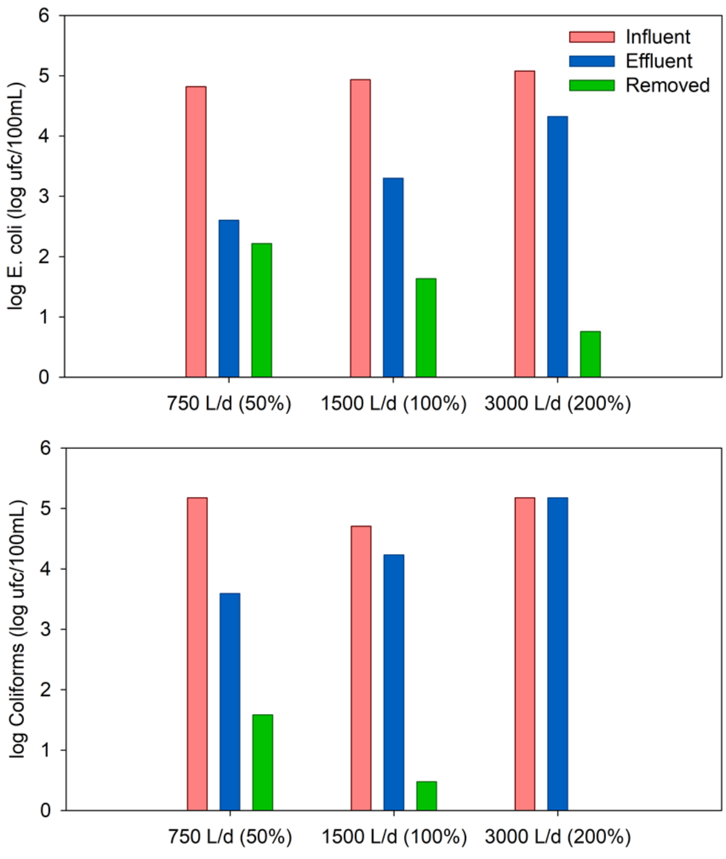
The introduction of *Daphnia magna* significantly increased coliform removal when compared with the experimental controls. Overall, mean reductions were ~1$\log_{10}$ although this ranged from 0.5 to 1.8$\log_{10}$. This compares with a study by Maceda-Veiga et al. (2015) that demonstrated a 2$\log_{10}$ (100-fold) reduction in total bacterial cell counts by the same species over a 30 day trial.

DAPHNIAFILTER DEMONSTRATION

Two pilot-scale Daphniafilters were installed by the University of Girona at the Quart WWTP in May, 2018. These are sized to treat wastewater from a nominal 10 population equivalent, with design daily flows of 1,500 litres. Secondary wastewater is used as the influent, and system performance has been monitored to understand its efficiency at solids' removal, nitrification / denitrification, and removal of pathogens. Results to date suggest that:

- There is higher removal of turbidity in autumn and winter due to the higher population densities of *Daphnia magna* at these times of year. This corresponds to water temperatures within the reactor of <25°C
- The removals of COD, nitrogen and pathogens are HRT-dependent, and likely to be HRT positive
- COD removal is ~30 mg/l
- Nitrification varied between 0.1 and 5.8 mg N/l across the two filters
- Denitrification varied between 0.0 and 3.9 mg N/l across the two filters
- *E. coli* and coliform removal varied between ~1 and 2 log_{10}, depending on flow rates, as shown in Figure 4

Figure 4 Pathogen removal in one of two pilot-scale Daphniafilters at Quart WWTP, under different daily flow rates



Demonstration-scale Daphniafilters have recently been installed at eight locations (Table 2). The performance of these installations is being tested for a range of common parameters, including:

- TSS (Total Suspended Solids) and Turbidity
- BOD, COD and Dissolved Oxygen
- Nitrogen: N-total, NH_4-N, NO_3-N, NO_2-N and Total Organic N
- Phosphorus: P-total and PO_4^{3-}
- pH and Conductivity
- *E. coli*

OPEN DAYS AND TRAINING

Daphniafilters are in operation at seven of the ten project demonstration sites. A series of open days and training events is planned for each site. If you would like to take part or arrange a visit, then please contact the relevant site manager. Further details of the INNOQUA project can be found at www.innoqua-project.eu.

Table 2 Locations of Daphniafilter demonstration facilities, and contact details for site managers

Country	Site manager	Contact details
France	Jean-Baptiste Dussaussois	jbdussaussois@nobatek.inef4.com
India	Tatjana Schellenberg	schellenberg@borda.org
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Romania	Costel Bumbac	costel.bumbac@incdecoind.ro
Tanzania	Evelyn Herrera Lopera	herrera@borda.org
Turkey	Serkan Naneci	serkan.naneci@ekodenge.com
Scotland	Anna Baran	Anna.Baran@scottishwater.co.uk

REFERENCES

Burns, C. W. (1969). Relation between filtering rate, temperature, and body size in four species of Daphnia. *Limnology and Oceanography*, *14*, 693–700. Retrieved from https://aslopubs.onlinelibrary.wiley.com/doi/pdf/10.4319/lo.1969.14.5.0693

Ebert, D. (2005). *Ecology, Epidemiology and Evolution of Parasitism in Daphnia*. Retrieved from https://www.ncbi.nlm.nih.gov/corehtml/pmc/homepages/bookshelf/pdf/daph_screenA4.pdf

Elenbass, M. (2013). Daphnia magna. Retrieved September 19, 2019, from Animal Diversity Web website: https://animaldiversity.org/accounts/Daphnia_magna/

Maceda-Veiga, A., Webster, G., Canals, O., Salvadó, H., Weightman, A. J., & Cable, J. (2015). Chronic effects of temperature and nitrate pollution on Daphnia magna: Is this cladoceran suitable for widespread use as a tertiary treatment? *Water Research*, *83*, 141–152. https://doi.org/10.1016/J.WATRES.2015.06.036

Pau, C., Serra, T., Colomer, J., Casamitjana, X., Sala, L., & Kamf, R. (2013). Filtering capacity of Daphnia magna on sludge particles in treated wastewater. *Water Research*, *47*(1), 181–186.

Pous, N., Hidalgo, M., Serra, T., Colomer, J., Colprim, J., & Salvadó, V. (2020). Assessment of zooplankton-based eco-sustainable wastewater treatment at laboratory scale. *Chemosphere*, *238*.

Schalau, K., Rinke, K., Straile, D., & Peeters, F. (2008). Temperature is the key factor explaining interannual variability of Daphnia development in spring: a modelling study. *Oecologia*, *157*(3), 531–543. https://doi.org/10.1007/s00442-008-1081-3

Serra, T., & Colomer, J. (2016). The hydraulic retention time on the particle removal efficiency by Daphnia magna filtration on treated wastewater. *International Journal of Environmental Science and Technology*, *13*(6), 1433–1442. https://doi.org/10.1007/s13762-016-0985-4

Serra, T., Müller, M. F., Barcelona, A., Salvadó, V., Pous, N., & Colomer, J. (2019). Optimal light conditions for Daphnia filtration. *Science of The Total Environment*, *686*, 151–157. https://doi.org/10.1016/J.SCITOTENV.2019.05.482

Shiny, K., Remani, K., Nirmala, E., Jalaja, T., & Sasidharan, V. (2005). Biotreatment of wastewater using aquatic invertebrates, Daphnia magna and Paramecium caudatum. *Bioresource Technology*, *96*(1), 55–58.