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Vermifilter and zooplankton-based reactor integration as a nature-based system for wastewater treatment and reuse

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ABSTRACT

The performance of an innovative, decentralized, nature-based wastewater treatment system composed of modular units (vermifilters and zooplankton-based reactors) on a pilot scale level (10 p.e.) is presented here. The efficiency of this system was evaluated over a 10-months period under controlled conditions at different flow-rates (750, 1,500 and 3,000 L·d-1). Vermifiltration alone delivered average removals of 88 ± 7%, 89 ± 8%, 91 ± 8% and 85 ± 19% for COD, TSS, NH4+ and BOD5, respectively. Zooplanktonbased reactor provided an important polishing stage that allowed the achievement of removal efficiencies above 95% for TSS, NH4+ and 91% for COD and BOD5, respectively. As the main function of zooplankton-based reactor is to filter fine particles (<30 μm), removal efficiencies of E.coli and turbidity were improved showing the good performance of this reactor as a nature-based tertiary system. The system showed promising results at flow-rates between 750 and 1,500 L·d-1 and only for short periods of time (less than fifteen days) at 3,000 L·d-1. The effluent water obtained was suitable for reuse for different purposes such as agricultural irrigation and process water in conformity with Spanish legislation. These results demonstrate that the integrated system presented here can be used as an eco-sustainable wastewater treatment and also to provide an effluent suitable for reuse.

1. Introduction

Intensive wastewater treatments such as activated sludge can provide good sanitation, but their high installation and operational costs make these systems unaffordable for small communities. It is necessary to develop sustainable technologies that can improve both wastewater treatment and water reuse with affordable initial capital outlays and operational costs. Nature-based solutions (NBS) could play a crucial role in this, but the maintenance requirements, electricity consumption, large area needed and meeting discharge criteria all year round can still be problematic. Most of these systems are based on the use of bacteria, plants and algae [1,2]. Only a few have explored the use of other biological organisms. One example is vermifiltration, in which the combined action of earthworms and bacteria, supported by a solid matrix that also acts as a filter unit, reduces the organic matter, solid and ammonium content [3–5], although the quality of the effluent sometimes falls short of the reuse criteria [6]. An innovative zooplankton reactor based on the filtration capacity of *Daphnia magna* (Cladocera order) combined with microbial/algae biofilm was developed to regenerate secondary wastewaters from an activated sludge system [7,8]. The reactor proved to be effective for the removal of solids and pathogens due to the *D. Magna* activity, producing effluent water suitable to be reused in accordance with Spanish water reuse legislation [9]. *D. magna* activity is affected by the concentration levels of organic matter [8], ammonium and nitrite [10] of raw wastewater, hence, their use is limited as a tertiary biological filter [11–13].

This study evaluates the integration of vermifilter and zooplankton-based reactors to develop an innovative, decentralized NBS that is able to regenerate wastewater in small communities. This low maintenance integrated system has the potential to efficiently remove organic matter, ammonium, solids and pathogens, giving an effluent water that meets quality criteria for agricultural irrigation and other uses.

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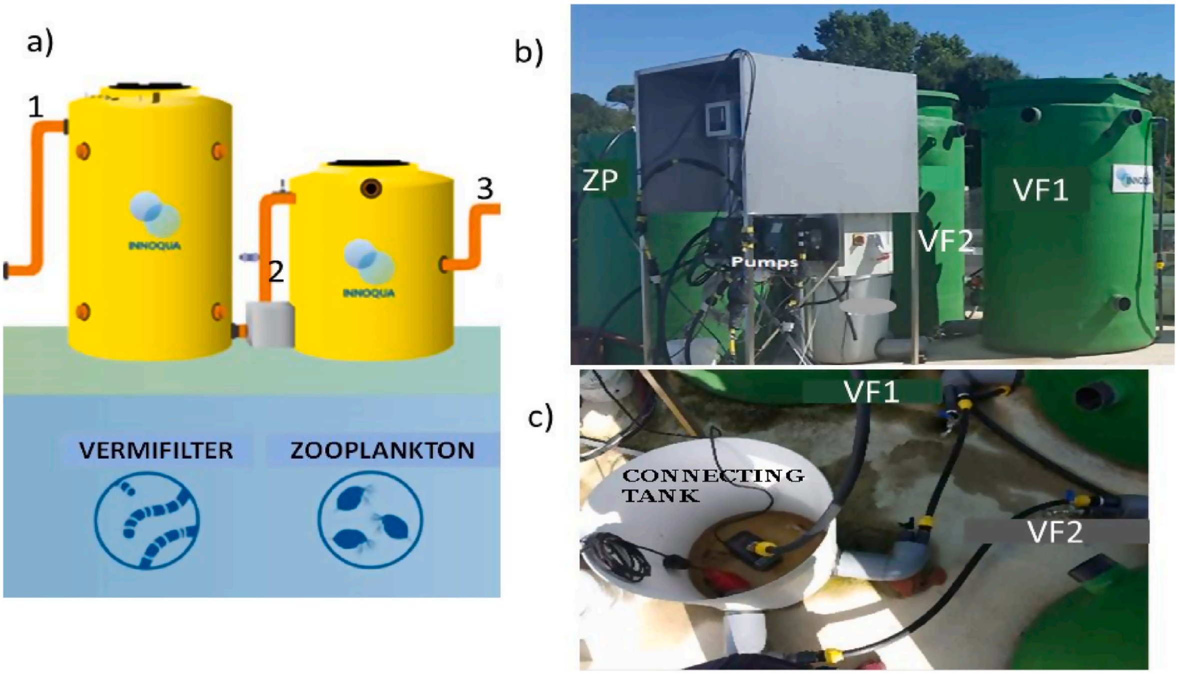

Fig. 1. Pilot at WWTP of Quart (41.96674 °N, 2, 2.844997 °E, Spain) (a) Scheme of the integrated system (1,2,3 indicate the sampling points), b) picture of the pilot plant: VF1 and VF2 are the two Vermifilters and ZP refers to the Zooplankton-based reactor, and c) detail of the connecting tank.

2. Materials and methods

2.1. *Pilot plant*

The integrated pilot plant was set-up and installed at the sewage treatment plant of Quart (41.966742 °N, 2.844997 °E, Spain) (Fig. 1). Influent wastewater was directly taken from the WWTP inlet, passed through a hydraulic circuit to separate coarse solids and grease, and then pumped directly to the vermifilters. The wastewater characteristics were pH 7.4 ± 0.2, 1.5 ± 0.2 mS·cm^{-1}, 706 ± 407 mgO$_2$·L^{-1}, 30.9 ± 11.5 mgN-NH$_4$$^{+}$·L^{-1}, 0.5 ± 1.2 mgN-NO$_3$$^{-}$·L^{-1}, 0.1 ± 0.1 mgN-NO$_2$$^{-}$·L^{-1}, 5.5 ± 3.6 mgP-PO$_4$$^{3-}$·L^{-1}, and 549 ± 866 mgTSS·L^{-1}. The pathogens load was 2.3·10^{+6} ± 0.9·10^{+6} CFU·100mL^{-1} E. *coli*, 5.7·10^{+6} ± 0.8·10^{+6} CFU·100mL^{-1} total coliforms and 0.5·10^{+6} ± 0.4·10^{+6} CFU·100mL^{-1} Enterococcus.

2.1.1. *Vermifilters*

Vermifiltration was performed in two identical cylindrical reactors

Table 1
Wastewater flow-rates used for the integrated Vermifilter-Zooplankton-based system.

		Days of operation and season						
		Summer				**Autumn**		
		0 - 11	12-16	17-22	23-77	82-91	92-100	101-107
Wastewater flow-rate (L·d^{-1})	1,500	🟩			🟩		🟩	
	750		🟩			🟩		
	3,000			🟩				🟩
		Winter					**Spring**	
		108-156	157-170	171-183	184-265	266-275	276-282	
Wastewater flow-rate (L·d^{-1})	1,500		🟩		🟩		🟩	
	750	🟩				🟩		
	3,000			🟩				

Table 2

Results of the system operating under nominal load of 1500 L d^{-1}. Removal at the Vermifilter effluent accounts for the removal of the Vermifilter reactors. Removal at the Zooplankton effluent accounts for the removal of the integrated system (Vermifilter and Zooplankton).

		Influent	Vermifilter effluent	Zooplankton effluent
TSS	**Content** (mgTSS·L^{-1})	549 ± 866	27 ± 14	13 ± 6
	Removal (%)		89 ± 8	95 ± 4
Suspended particle concentration d < 30 μm	**Content** (μL·L^{-1})	138 ± 91	11 ± 9	2 ± 2
	Removal (%)		83 ± 27	98 ± 1
Suspended particle concentration d < 100 μm	**Content** (μL·L^{-1})	300 ± 192	27 ± 22	7 ± 3
	Removal (%)		87 ± 13	97 ± 1
COD	**Content** (mgO$_2$·L^{-1})	706 ± 407	58 ± 16	43 ± 9
	Removal (%)		88 ± 7	91 ± 7
BOD$_5$	**Content** (mgO$_2$·L^{-1})	392 ± 262	60 ± 25	38 ± 13
	Removal (%)		85 ± 9	91 ± 5
N–NH$_4^+$	**Content** (mgN·L^{-1})	30.9 ± 11.5	2.5 ± 2.3	1.6 ± 1.8
	Removal (%)		91 ± 8	94 ± 8
N-TN	**Content** (mgN·L^{-1})	31 ± 11	34 ± 6	30 ± 9
	Removal (%)		-	-
P-PO$_4^{3-}$	**Content** (mgP·L^{-1})	5.4 ± 4.5	5.1 ± 1.1	4.6 ± 1.4
	Removal (%)		-	-
E. coli	**Content** (CFU·100mL^{-1})	2.2·10^{+6} ±1.0·10^{+6}	1.5·10^{+5} ±1.4·10^{+5}	3.1·10^{+4} ±5.4·10^{+4}
	Removal (%)		93 ± 7	98 ± 3
Total coliforms	**Content** (CFU·100mL^{-1})	5.8·10^{+6} ±3.5·10^{+6}	1.5·10^{+6} ±1.3·10^{+6}	2.7·10^{+5} ±2.9·10^{+5}
	Removal (%)		74 ± 21	95 ± 5
Enterococcus	**Content** (CFU·100mL^{-1})	5.4·10^{+5} ±6.2·10^{+5}	3.1·10^{+4} ±3.4·10^{+4}	9.5·10^{+3} ±2.0·10^{+4}
	Removal (%)		91 ± 10	98 ± 3
NTU	**Content** (NTU)	511 ± 592	38 ± 44	10 ± 6
	Removal (%)		89 ± 13	97 ± 3

working in parallel (1.2 m diameter and 1.7 m height) (Fig. 1). The filling media was composed, from bottom to top, of river pebbles 40/60 mm (0–0.2 m), pozzolana 15/20 mm (0.2–0.4 m) and woodchip 15/25 mm (0.4–1.4 m height). About 15,000 earthworms (*Eisenia fetida*, Lombriventa, Spain) were added. The inlet was located at the upper side of the reactor and wastewater flowed through the media filling to the effluent, located at the bottom of the reactors. Reactors were operated in fed-batch mode, with 5 min feeding and 25 min drawing. After a start-up period that lasted three months, the effluent quality required to connect the two vermifilters with the zooplankton-based reactor was achieved [8,10].

2.1.2. Zooplankton-based reactor

The zooplankton-based reactor (1,500 L) was fed with the vermifilter effluent and inoculated with *D. magna* (0.1 ind·L^{-1}) collected from a laboratory aquarium culture [7,13]. After a period of 30 days the population was found to be stable and the experimental period started.

2.2. Evaluation of the water-flow effect

The integrated system was designed to operate normally at 1,500 L·d^{-1} (3.6 days HRT). Short stress-tests (Table 1) were performed to evaluate the effect of a decrease in the influent flow-rate (750 L·d^{-1}) and of an overflow (3,000 L·d^{-1}).

2.3. Chemical and microbiological analyses

Appropriate volumes of influent and effluent samples, collected at the inlet/outlet of the vermifilters and at the outlet of the zooplankton-based reactor, were regularly taken to analyse the *E. coli*, Total coliforms and Enterococcus content, pH, conductivity, chemical oxygen demand (COD; Merck, Germany), biological oxygen demand (BOD$_5$; OxiTop®, ThermoFisher, USA), total suspended solids (TSS), turbidity (DR3900, Hach Lange, Germany) and nitrites (N–NO$_2^-$), nitrates (N–NO$_3^-$), ammonium (N–NH$_4^+$), and ortophosphates (P-PO$_4^{3-}$) (Dionex, ThermoFisher, USA), in accordance with American Public Health Association (APHA) standards [14]. The wastewater particle size distribution was measured with the Lisst-100x particle size analyser (Sequoia Inc., USA). Since *Daphnia* feed on particles that are less than 30 μm in diameter, the volume concentration of particles within the range of 2.5 to 30 μm was calculated [10] and used as a proxy to evaluate particle removal.

3. Results and discussion

3.1. Suspended solids, organic matter and nitrogen removal

The integrated system worked at 1,500 L·d^{-1} under normal conditions (Table 2). Influent wastewater contained 549 ± 866 mgTSS·L^{-1}. Good vermifilter performance was achieved removing suspended solids (89 ± 8% TSS removal), providing an effluent concentration of 27 ± 14 mgTSS·L^{-1}, which is below the discharge limit (35 mgTSS·L^{-1}) [15]. The zooplankton-based reactor then further reduced the TSS content to 13 ± 6 mgTSS·L^{-1}. This corresponds to 95 ± 4% TSS removal overall, well above rates typically observed for other low-cost wastewater treatments such as waste stabilization ponds (67%) [2], and similar to the ranges of more costly membrane bioreactors (85–99%) [16].

No significant differences were observed in the removal efficiencies of particles of different sizes (one-way ANOVA test, *p-value*>0.05). In the vermifilters, the removal efficiencies of particles with a diameter <100 μm and <30 μm were 87 ± 13% and 83 ± 27%, respectively, due to the combination of the different filtering materials and the earthworm activity. Particle removal was further increased to 97 ± 1 and 98 ± 1% for particles <100 μm and <30 μm, respectively, due to the *Daphnia* filtration. Thus, particles of different sizes were removed in the integrated system by the combined action of earthworms, filtering materials and *Daphnia magna* individuals (mean number concentrations of 321 ± 215 ind·L^{-1}) [17].

In spite of the great variability in the influent organic matter content (706 ± 407 mgO$_2$.L^{-1}), the vermifilter effluent constantly presented low COD content (58 ± 16 mgO$_2$·L^{-1}), corresponding to COD removal of 88 ± 7%, which is in the top range of performances reported in the literature (67–90%) [18,19]. The zooplankton reactor further decreased the effluent COD content to 43 ± 9 mgO$_2$·L^{-1} [7]. Effluent COD values for the integrated system met the discharge standards (<125 mgO$_2$·L^{-1}). The overall integrated system achieved higher COD removals (91 ± 7%) than waste stabilizations pond (62%) [2], and similar to membrane bioreactors and activated sludge systems (70–90%) [16]. With regards to BOD$_5$, the integrated system achieved a decrease from 392 ± 262 mgO$_2$·L^{-1} to 38 ± 13 mgO$_2$·L^{-1}, obtaining higher removals (91 ± 5%) than minimum standards (70–90%) [15]. Particulate organic matter was removed by the filtering activity of the different reactors, whereas dissolved COD was probably removed by the action of aerobic bacteria in the two systems.

Ammonium content decreased from 30.9 ± 11.5 mgN-NH$_4^+$·L^{-1} to 2.5 ± 2.3 mgN-NH$_4^+$·L^{-1} in the vermifilter, and then to 1.6 ± 1.8 mgN-NH$_4^+$·L^{-1} in the zooplankton reactor. Hence, total ammonium removal (94 ± 8%) presented values which were similar to those obtained using vermifiltration alone (92%) [18], and higher than membrane bioreactors and activated sludge (70–90%) [16]. The reduction in the COD

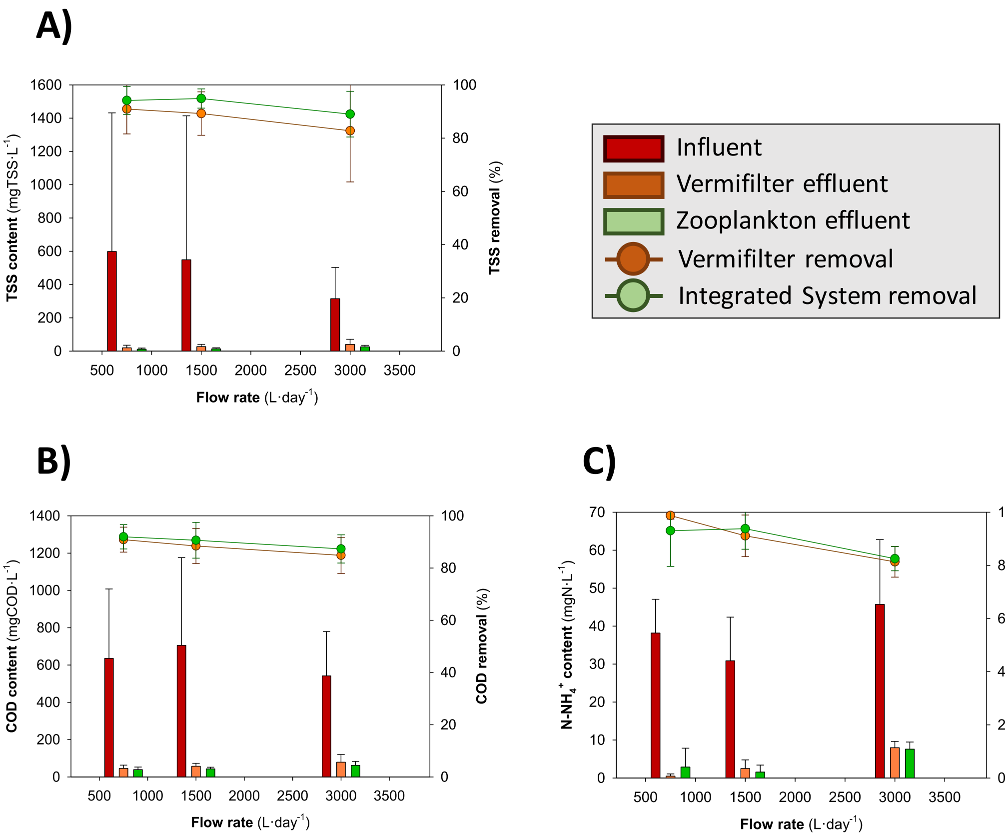

Fig. 2. Evolution of content and removal at different influent wastewater flow-rates of: A) Total suspended solids (TSS); B) Organic matter (COD); C) Ammonium ($N–NH_4^+$).

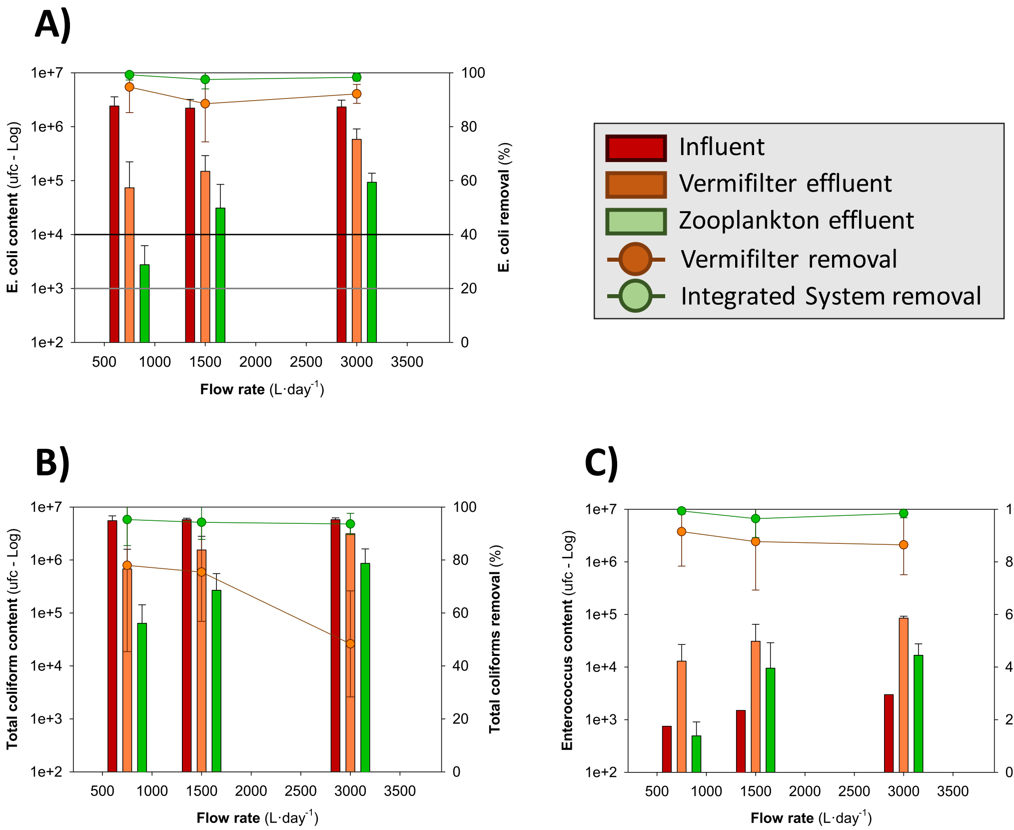

Fig. 3. Evolution of content and removal at different influent wastewater flow-rates of: A) *E. coli*; B) Total coliforms; C) Enterococcus.

and ammonium content achieved by the vermifilter permitted its successful coupling to the zooplankton reactor, avoiding *Daphnia* inhibition [8,10]. However, ammonium was accumulated as nitrate at the vermifilter and zooplankton effluents (99 ± 21% and 91 ± 28%, respectively), resulting in an effluent total nitrogen content (TN), which is unregulated in the case of small communities, of 30 ± 9 mgN·L^{-1}. The aerobic environment of the vermifilter and zooplankton reactors allowed the successful conversion of ammonium into nitrate by nitrifying bacteria, but the lack of electron donors limited denitrifying activity. No significant variation was observed in ortophosphate content.

The reduction of the flow to 750 L·d^{-1} did not have a significant impact on the system performance (Fig. 2). However, when overflow conditions (3,000 L·d^{-1}) were applied, the impact on the system was greater. For instance, NH_4^+ removal was 94 ± 8% at 1,500 L·d^{-1} but only 83 ± 5% at 3,000 L·d^{-1}. Nevertheless, the integrated system still met the standard effluent quality criteria in terms of TSS (26 ± 10 mgTSS·L^{-1}), COD (62 ± 21 mgO_2·L^{-1}) and BOD_5 (78 ± 11% removal) when overflow conditions were applied [15]. It is worth noting that *Daphnia*, which is particularly sensitive to flow-rate changes, remained mostly stable since particle removal was maintained above 97% under the different flow rates tested.

3.2. Turbidity and pathogen removal

The presence of *Daphnia* in the zooplankton-based reactor was expected to decrease both turbidity and the pathogen load, permitting water reuse (Table 2).

Vermifilters permitted a significant decrease in the turbidity from 511 ± 592 to 38 ± 94 NTU (89 ± 13% removal). However, these values were still above those required for stricter water reuse purposes (<15 NTU, [9]). The coupling of a zooplankton reactor resulted in these values being improved to 10 ± 6 NTU (97 ± 3% removal), meeting the turbidity requirements for broader water reuse applications.

Pathogen removal was observed in both the vermifilters and the zooplankton-based reactor. The aerobic characteristics and the filter activity of the vermifilter allowed a decrease of around 1 logarithmic unit of the different microbiological parameters. For example, *E. coli* was removed at 93 ± 7%. The removal of pathogens becomes harder as their concentration decreases, which is when the contribution of a zooplankton reactor becomes important. The *Daphnia* population achieved a decrease in the pathogen content by an additional logarithmic unit, resulting in an increase of the overall removal to values above 95% in *E. coli*, Total coliforms and Enterococcus. It has been previously reported that *E. coli* removal in vermifiltration can present seasonal fluctuations from 33.9 to 96.8% [6]. In the present study, *E. coli* removal fluctuated only between 78.9 and 99.3% in the vermifilter, and between 89.4 and 99.9% with the full integrated system.

The application of different wastewater flow-rates was expected to affect the performance in removing particles and pathogens [7,8]. However, the NTU at the effluent of the system fluctuated from 17 ± 21 to 5 ± 3 NTU from 750 to 3,000 L·d^{-1} (Fig. 3). The lengthening of the operational time in the zooplankton reactor provided greater robustness to the system in terms of particle removal [7]. On the other hand, the *E. coli* content at the effluent of the integrated system was $2.62 \cdot 10^{+3} \pm 3.30 \cdot 10^{+3}$ at 750 L·d^{-1} and $8.25 \cdot 10^{+4} \pm 3.98 \cdot 10^{+4}$ at 3000 L·d^{-1}. This represents an increase of 1 logarithmic unit, which decreases the options for water reuse applications.

3.3. Valuing effluent water

The integrated system produced an effluent that met the standards required for treated wastewater in terms of organic matter content (COD <125 mgO_2·L^{-1}, 70–90% BOD_5 removal) and solids (<35 mgTSS·L^{-1}). In the case of small communities (<10,000 p.e.), such as the ones targeted with the current nature-based solution, the effluent wastewater would meet all the standards required [15]. Further values from effluent water can be found in reuse applications [9]. At a nominal load of 1,500 L·d^{-1}, outlet could be safely used for applications requiring $<1 \cdot 10^{+4}$ CFU·100mL^{-1} and/or <35 mgTSS·L^{-1}, such as forest and agricultural irrigation of non-food products and to refill recreational lakes. At 750 L·d^{-1}, the uses could be extended to those requiring $<1 \cdot 10^{+3}$ CFU·100mL^{-1} and <35 mgTSS·L^{-1}. Under these conditions, effluent water could also be reused for agricultural irrigation if direct contact with edible parts is avoided and as process water in food industries. In addition, the full conversion of ammonium into nitrate could be presented as an opportunity to replace the nitrogen and phosphorus currently used in agriculture [20], given that the irrigating water already contains the fertilizer.

4. Conclusions

This case study demonstrates that vermifilters and zooplankton-based reactors coupled together are a sustainable approach for providing sanitation and water reuse with low maintenance costs. Wastewater was successfully treated in terms of TSS (95 ± 4% removal), COD (91 ± 7% removal), ammonium (94 ± 8% removal) and pathogens (98 ± 3% *E. coli* removal) at a nominal load of 1,500 L·d^{-1} (3.6 days HRT). The effluent was suitable for water reuse in four different categories (e.g. agricultural irrigation and process water) according to Spanish water reuse legislation, and it contained nitrate that could be used as fertilizer. Further studies are required to improve the robustness of the system at overload conditions (3,000 L·d^{-1}). The nature-based solution developed in the current study is a promising alternative for low income communities, supporting local water and nutrient recovery initiatives.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M.A. Rodriguez-Dominguez, D. Konnerup, H. Brix, C.A. Arias, Constructed wetlands in Latin America and the Caribbean: a review of experiences during the last decade, Water 12 (6) (2020) 1744, https://doi.org/10.3390/w12061744.

[2] N. Mburu, S.M. Tebitendwa, J.J.A. van Bruggen, D.P.L. Rousseau, P.N.L. Lens, Performance comparison and economics analysis of waste stabilization ponds and horizontal subsurface flow constructed wetlands treating domestic wastewater: a case study of the Juja sewage treatment Works, J. Environ. Manag. 128 (2013) 220–225, https://doi.org/10.1016/j.jenvman.2013.05.031.

[3] L. Jiang, Y. Liu, X. Hu, G. Zeng, H. Wang, L. Zhou, X. Tan, B. Huang, S. Liu, S. Liu, The use of microbial-earthworm ecofilters for wastewater treatment with special attention to influencing factors in performance: a review, Bioresour. Technol. 200 (2016) 999–1007, https://doi.org/10.1016/j.biortech.2015.11.011.

[4] S. Ghasemi, M. Mirzaie, A. Hasan-Zadeh, M. Ashrafnejad, S.J. Hashemian, S. R. Shahnemati SR, Design, operation, performance evaluation and mathematical optimization of a vermifiltration pilot plan for domestic wastewater treatment, J. Environ. Chem. Eng. 8 (2020), https://doi.org/10.1016/j.jece.2019.103587.

[5] A. Ndiaye, H.A. Andrianisa, S.S.Y. Saapi, O.A. Changotade, A.T. Adugna, Y. Konate, A.H. Maiga, Assessment on overall efficiency of urban greywater treatment by vermifiltration in hot climate: enhanced pollutants removal, Environ. Technol. 41 (2020) 2219–2228, https://doi.org/10.1080/09593330.2018.1561755.

[6] S. Arora, A.A. Kazmi, The effect of seasonal temperature on pathogen removal efficacy of vermifilter for wastewater treatment, Water Res. 74 (2015) 88–99, https://doi.org/10.1016/j.watres.2015.02.001.

[7] N. Pous, A. Barcelona, L. Sbardella, M. Hidalgo, J. Colomer, T. Serra, V. Salvadó, Zooplankton-based reactors for tertiary wastewater treatment: a pilot-scale case

study, J. Environ. Manag. 278 (2021), https://doi.org/10.1016/j.jenvman.2020.111538.

[8] N. Pous, M. Hidalgo, T. Serra, J. Colomer, J. Colprim, V. Salvadó, Assessment of zooplankton-based eco-sustainable wastewater treatment at laboratory scale, Chemosphere 238 (2020), https://doi.org/10.1016/j.chemosphere.2019.124683.

[9] Spanish Royal decree for water reuse - R.D.1620/2007 - "Reusage of Treated Waters".

[10] T. Serra, M. Soler, N. Pous, J. Colomer, *Daphnia magna* filtration, swimming and mortality under ammonium, nitrite, nitrate and phosphate, Sci. Total Environ. 656 (2019) 331–337, https://doi.org/10.1016/j.scitotenv.2018.11.382.

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

[12] A. Goździejewska, S. Tucholski, Zooplankton of fish culture ponds periodically fed with treated wastewater, Polish, J. Environ. Stud. 20 (2011) 67–79.

[13] T. Serra, J. Colomer, C. Pau, M. Marin, L. Sala, Tertiary treatment for wastewater reuse based on the *Daphnia magna* filtration - comparison with conventional tertiary treatments, Water Sci. Technol. 70 (2014) 705–711, https://doi.org/10.2166/wst.2014.284.

[14] APHA – American Public Health Association, Standard Methods for the Examination of Water and Wastewater, nineteenth ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA, 2005.

[15] European Commission (EU) council directive 91/271/EEC - Urban Wastewater Treatment. 1991.

[16] M. Molinos-Senante, M. Garrido-Baserba, R. Reif, F. Hernández-Sancho, M. Poch, Assessment of wastewater treatment plant design for small communities: environmental and economic aspects, Sci. Total Environ. 427–428 (2012) 11–18, https://doi.org/10.1016/j.scitotenv.2012.04.023.

[17] C.W. Burns, Relation between filtering rate, temperature, and body size in four species of *Daphnia*, Limnol. Oceanogr. 14 (1969) 693–700.

[18] D.-b. Wang, Z.-y. Zhang, Z.-m. Li, W. Zheng, Q. Yang, Y. Ding, T.-j. Zeng, J.-b. Cao, X. Yue, T.-t. Shen, G.-m. Zeng, J.-h. Deng, A full-scale treatment of freeway toll-gate domestic sewage using ecology filter integrated constructed rapid infiltration, Ecol. Eng. 36 (2010) 827–831, https://doi.org/10.1016/j.ecoleng.2010.03.005.

[19] S. Arora, A. Rajpal, T. Kumar, R. Bhargava, A.A. Kazmi, A comparative study for pathogen removal using different filter media during vermifiltration, Water Sci. Technol. 70 (2014) 996–1003, https://doi.org/10.2166/wst.2014.318.

[20] J. Mateo-Sagasta, L. Raschid-Sally, A. Thebo, in: P. Drechsel, M. Qadir, D. Wichelns (Eds.), "Global Wastewater and Sludge Production, Treatment and Use" in Wastewater: Economic Asset in an Urbanizing World, Springer, 2015, https://doi.org/10.1007/978-94-017-9545-6_2.