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EARTHWORM'S ENHANCEMENT POTENTIAL OF CONSTRUCTED WETLAND WASTEWATER TREATMENT PROCESS IN A REUSE PERSPECTIVE

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ABSTRACT

Introduction

Many studies exist about constructed wetlands (CWs) performance optimization, especially for organic compounds and nutrients removal, and describe optimal sizing, design and operation of CWs depending on the inlet wastewater (WW) to be treated. Co-benefits of CW make this type of system quite challenging with classical activated sludge process but performance still needs demonstration for adoption with reuse objectives. Specially the water quality provided may orientate the type of water reuse. CWs still face issues such as clogging and poor nutrient removal. Earthworms (EWs) are macro-invertebrates inhabiting soil ecosystems. Due to their high influence on soil physics, chemistry and biology, they are nicknamed "ecosystem engineers". They are known for their positive influence on the ecological service of water purification in natural ecosystems (Liu *et al.*, 2017) and their ability to thrive in CWs has been noticed (Li *et al.*, 2011; Lavrnić *et al.*, 2019; Gilibert *et al.*, 2022). Vertical subsurface flow constructed wetlands (VSSF-CWs) have been identified as viable habitat for EWs (Li *et al.*, 2011). A quite abundant literature exists on enhancing CWs or similar WW treatment technologies such as vermifilters (VF) or macrophyte-assisted vermifilters (MAVF) (Li *et al.*, 2011). Nevertheless, among all this literature, most of the studies are carried out at lab scale, while studies carried out at larger scale and in open-air systems (mesocosms) are scarce. They also do not feature clear results and oftenly do not use replicates which do not allow to be conclusive on EW potential for CWs enhancement. This work studies the effect of EWs' inoculation in mesocosm (0.2 m^2) and pilot (5 m^2) scale systems on the campus of Toulouse University. This experimental system allows replicates and parallel experiments with filters (10 mesocosms and 3 pilots) for testing research hypotheses with statistical analyses. A combined approach of hydraulic tests and water quality assessment is intended to evidence EWs effect on the process.

Materials and methods

Experimental conditions

Three experiments were carried out on this VSSF-CW platform. Raw WW came from a building dedicated to teaching and administrative work producing 10-50 PE effluents. Details on experimental conditions, design and operational parameters are provided in Table 1.

Physico-chemical analysis

Inlet and outlet water sampling was carried out every 2-3 weeks and was composed of four subsamples collected on a 24 h cycle.

Hydraulic test

Tracer tests were performed using fluorescein WT. Outlet fluorescein concentration was then measured with an IN-SITU Aquatroll 600 probe equipped with Fluorescein WT sensor. Flow rate was assessed with a flow rate sensor designed in a cooperative work between CRBE and IRIT laboratories. Hydraulic conductivity was measured thanks to falling head permeametry techniques using 125 mm diameter PVC tubes.

Table 1. Experimental conditions, design and operational parameters (HLR = hydraulic loading rate; OLR = organic loading rate).

Experiment	**Experimental conditions**		**Design**		**Operational parameters**					
	Replicates	OLR ($gBOD_5/m^2/day$)	Sand-gravel substrate (from top to bottom)	Saturation level (m)	HLR (m/day)	Volume of water treated (L/week)	Feeding :resting time ratio	Feeding duration (h/week)	Batches No. per feeding day	Batch water depth (m)
Meso 1 (Sept. 2023-Jan. 2024)	3 mesocosms + plants* (M1-OLR40-plant) - control	40	1) Φ 0-4 mm: 30 cm 2) Φ 4-14 mm: 10 cm 3) Φ 20-40 mm: 15 cm	0.15	0.3	140	1:3	56	2	0.15
	3 mesocosms + plants* + EWs** (M1-OLR40-plant + EWs)									
	3 mesocosms + plants* + EWs** (M1-OLR20-plant + EWs)	20			0.15	70			1	
Meso 2 (May-July 2024)	3 mesocosms + plants* (M2-OLR40-plant) - control	40		0.15	0.3	140	1:3	56	12	0.025
	3 mesocosms + plants* + EWs** (M2-OLR40-plant + EWs)									
Pilot (April-June 2024)	1 pilot + plants* (P-OLR40-plant) -control	40	1) Φ 0-4 mm: 30 cm 2) Φ 4-14 mm: 10 cm 3) Φ 20-40 mm: 40 cm	0.3	0.3	3 500	1:3	56	12	0.025
	1 pilot + EWs** (P-OLR40-EWs)									
	1 pilot + plants* + EWs** (P-OLR40-plant + EWs)									

*10 plants/m² of *Phragmites australis*; **0.5 kg/m² of EWs (mix of *Eisenia fetida*, *E. hortensis* and *E. andrei*)

Results and discussion

Macro-contaminant removal performances

No statistically significant differences existed between mesocosm and pilot inlet, except for $P\text{-}PO_4^{3-}$ concentrations, showing that the different feeding systems between the mesocosms and pilots did not modify inlet physico-chemical properties (Table 2). Outlet concentrations of pilots and mesocosms were significantly different for only some parameters (TSS and COD_p) where pilots showed better removal performances. Differences also existed between mesocosm and pilot controls for $P\text{-}PO_4^{3-}$ and also for EWs-inoculated CWs for BOD_5 where pilots were more efficient. But except this, mesocosm and pilot experiments showed similar trends.

Then, we could see that statistically significant removal of BOD_5, COD_p, TSS and $N\text{-}NH_4^+$ can be evidenced at both scales. COD_d removal was significant for all conditions except mesocosm scale with inoculated EWs. But, TN and TN_d removal were not significant. Particulate contaminants (TSS, COD_p and BOD_5) were well removed, with removal above 85% at both pilot and mesocosm scales. Pilot experiment demonstrated higher removal rates above 98, 98 and 94% for TSS, COD_p and BOD_5 respectively. These removal rates are comparable to full-scale system literature data (Morvannou *et al.,* 2017). Then, nitrogen was quite well nitrified with ammonium removal rates averaging between 64% and 75%, but denitrification processes were quite limited, without significant removal of TN. What is interesting to notice is that despite a big difference in substrate depth and saturation level between mesocosms and pilots (Table 1), no significant differences in TN removal could be observed. It is likely that a limiting factor avoids efficient denitrification. On the other hand, phosphates were not removed at all, as we could even see a release of phosphate probably due to sludge mineralization (from -44% to 7% removal rates).

Finally, even if it was difficult to get numbers of samples to always evidence statistically significant differences, some can be shown and trends can be evidenced. Considering EWs effect on these macro-contaminant removal, no statistically significant differences, with a level of significance of 95%, existed between CW inoculated or not with EWs, and this, at both scales.

Table 2. Physico-chemical properties of inlet and outlet water and removal rates at mesocosm and pilot scales, compact letters display (a,b,c) on the basis of p-value < 0.05 calculated with a Dunn test [H_0 = *the two group distributions are equal*] adjusted with Benjamini-Hochberg procedure. Each column (variable) shows comparison tests results independent from the other columns. In case of outlet concentrations the test compares +/- EWs conditions or +/- plants in the pilots. [(n) is the number of samples analyzed; [1]Morvannou *et al.* (2017); [2]WW discharge: Arrêté du 21 juillet 2015; Reuse: Arrêté du 25 juin 2014].

Treatment	**BOD$_5$ (mg/L)**	**TSS (mg/L)**	**COD$_p$ (mg/L)**	**COD$_{tot}$ (mg/L)**	**COD$_d$ (mg/L)**	**TN (mg/L)**	**TN$_d$ (mg/L)**	**N-NH$_4^+$ (mg/L)**	**N-NO$_3^-$ (mg/L)**	**P-PO$_4^{3-}$ (mg/L)**
Meso 2 : Inlet	411 ± 43 (6) **c**	401 ± 54 (6) **c**	709 ±138 (6) **c**	977 ± 142 (6)	130 ± 31 (6) **b**	71.5 ± 7.7 (6) **a**	60.6 ± 6.8 (6) **a**	54.9 ± 8.5 (6) **b**	2.3 ± 0.4 (6) **b**	4.1 ± 0.3 (6) **b**
Meso 2 : Outlet *+plant*	37.0 ± 5.6 (18) **ab**	36.8 ± 3.1 (18) **a**	43.0 ± 3.8 (18) **a**	113.5 ± 5.5 (18)	66.7 ± 3.9 (18) **ab**	56.7 ± 3.3 (18) **a**	53.9 ± 3.2 (18) **a**	13.9 ± 1.6 (18) **a**	33.9 ± 2.0 (18) **a**	5.9 ± 0.4 (18) **a**
Removal (%)	91.0	90.8	94.0	88.4	48.9	11.1	20.7	74.7	-	-43.7
Meso 2 : Outlet *+plant + EWs*	46.1 ± 7.4 (18) **a**	36.8 ± 3.1 (18) **a**	53.0 ± 5.1 (18) **a**	116.9 ± 7.0 (18)	58.8 ± 4.7 (18) **ac**	56.6 ± 3.9 (18) **a**	53.2 ± 3.8 (18) **a**	16.8 ± 2.2 (18) **a**	32.0 ± 2.8 (18) **a**	4.8 ± 0.2 (18) **ab**
Removal (%)	88.8	88.9	92.5	88.0	55.0	12.18	20.8	69.3	-	-17.1
Pilot : Inlet	432 ± 107 (5) **c**	475.0 ± 86.6 (5) **c**	493.0 ± 42.4 (5) **c**	630 ± 44 (5)	94.4 ± 10.4 (5) **bc**	78.0 ± 8.1 (5) **a**	69.2 ± 8.3 (5) **a**	46.6 ± 6.3 (5) **b**	9.6 ± 2.3 (5) **b**	5.01 ± 0.6 (5) **a**
Pilot : Outlet *+plant*	24.4 ± 10.7 (5) **ab**	8.4 ± 1.0 (5) **b**	8.8 ± 2.5 (5) **b**	69.0 ± 4.7 (5)	57.7 ± 3.9 (5) **a**	61.9 ± 2.9 (5) **a**	61.3 ± 2.9 (5) **a**	13.2 ± 1.8 (5) **a**	40.3 ± 3.2 (5) **a**	4.6 ± 0.2 (5) **b**
Removal (%)	94.4	98.2	98.3	89.0	38.9	11.5	20.7	71.7	-	7.4
Pilot : Outlet *+ EWs*	15.8 ± 6.8 (5) **ab**	9.0 ± 0.5 (5) **b**	8.2 ± 1.1 (5) **b**	64.0 ± 4.4 (5)	54.7 ± 4.2 (5) **a**	62.4 ± 2.4 (5) **a**	61.7 ± 2.4 (5) **a**	16.3 ± 3.3 (5) **a**	33.3 ± 2.7 (5) **a**	5.5 ± 0.5 (5) **ab**
Removal (%)	96.3	98.1	98,5	89.9	42.0	10.9	20.0	64.9	-	-9.3
Pilot : Outlet *+plant + EWs*	14.6 ± 8.5 (5) **b**	7.2 ± 1.1 (5) **b**	6.96 ± 2.0 (5) **b**	66.6 ± 4.1 (5)	57.6 ± 3.5 (5) **a**	58.6 ± 2.9 (5) **a**	58.1 ± 3.0 (5) **a**	16.9 ± 6.3 (5) **a**	35.1 ± 1.4 (5) **a**	5.5 ± 0.3 (5) **ab**
Removal (%)	96.6 %	98.5 %	98.7 %	89.4	39.0	16.1	24.8	63.8	-	-9.5
Full scale VSSF-CW partially saturated removal[1] (%)	***98 ± 2***	***95 ± 3***	**-**	***97 ± 2***	**-**	**-**	**-**	**-**	***84 ± 12***	**-**
Regulation minimum removal for reclaimed WW[2] (%)	***60 or 80***	***50 or 90***	**-**	***60 or 75***	**-**	**-**	***70***	**-**	**-**	***80***
REUSE regulation targets – class A (classes B,C,D)[2]	**< 35 (<35)**	**< 15 (<35)**	**-**	**<60 (<200)**	**-**	**-**	**10-15**	**-**	**-**	**P_{tot} < 1-2**

Hydraulic performances

The first mesocosm experiment showed a statistically significant effect of EWs introduction in CWs (Fig. 1.A). CWs inoculated with EWs had a saturated hydraulic conductivity (K_s) of 7.8 ± 3.0 $.10^{-2}$ m/s, while the control was at an average of 9.0 ± 3.1 $.10^{-5}$ m/s. In the second mesocosm experiment, after modification of the feeding system, no statistically significant differences in hydraulic conductivity could be observed, nevertheless a slight increase in K_s was observed from an average of 4.4 ± 1.7 $.10^{-5}$ to 6.1 ± 1.7 $.10^{-5}$ m/s (+28%). Pilot scale experiment did not show any significant differences in K_s. Even the effect of the reeds on K_s was not significant.

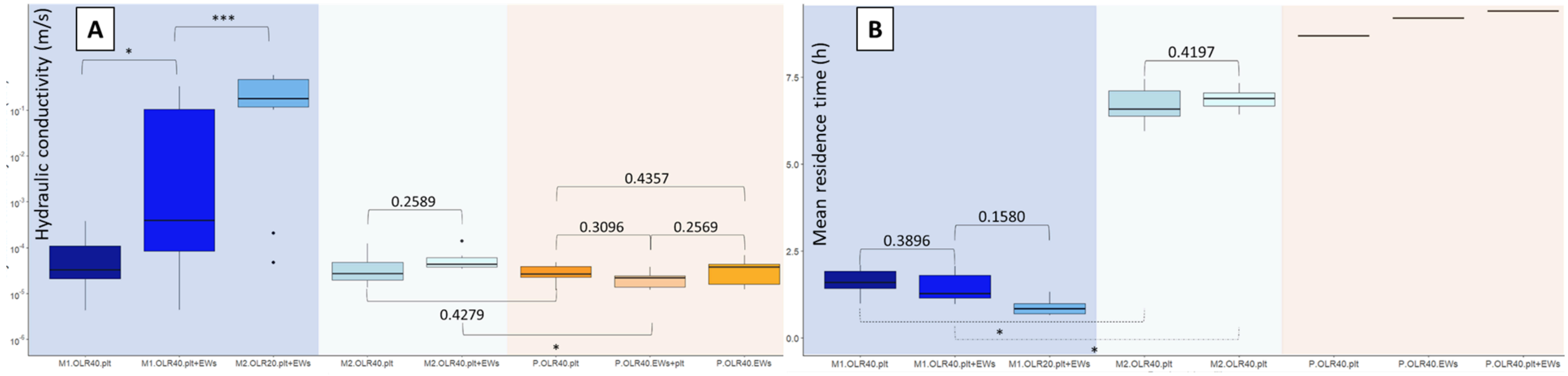

Fig 1. Hydraulic conductivity measured at mesocosm and pilot scales (A) and mean residence time for the two mesocosm experiments and for the pilot experiment after earthworm inoculation (B)p-value of Dunn test adjusted with Benjamini-Hochberg procedure are shown with solid line [H_0=*The two group distribution are equal*)], p-value > 0.05 are directly reported p-value < 0.05 with *. Dashed lines show the results of the Wilcoxon matched test (H_0=*The two independent groups come from the same population, meaning their distributions are identical*), p-value results for the matched groups

Considering EWs effect, we even saw a slight decrease of K_s in presence of EWs, from 3.0 ± 0.5 $.10^{-5}$ to 2.2 ± 0.4 $.10^{-5}$ m/s (-26%). Then, we can infer that the feeding mode is impacting EWs influence on hydraulic conductivity of the media. Indeed, K_s largely decreased in the experimental condition with EWs when the automatic feeding mode was applied. At this stage, we can infer that the increase in K_s in the first experiment can be attributed to EW activity of deposit layer destruction at the CW surface. The absence of K_s increase in the second experiment is probably due to the fact that the deposit layer is not anymore the limiting factor of K_s but the substrate beneath where interstitial biofilm grows. The decrease in K_s observed during this second experiment in presence of EWs could be caused by bioclogging due to preferable conditions for biofilm growth with the automatic feeding. Mean residence time (MRT) of the first mesocosm experiment was between 0.90 and 1.62 hours (Fig. 1.B). No statistically significant differences were observed, neither in the presence of EWs nor by changing the organic loading rate (OLR). Nevertheless, a higher influence of the OLR was evidenced with a 0.72 hour decrease of average MRT. After implementing the automatic feeding system, a statistically significant improvement of the MRT was observed, reaching 6.69-6.88 hours. Still no statistically significant differences were observed due to EWs inoculation. Nevertheless, a slight tendency of MRT increase was observed with EWs (+2.8%). Tracer test results from the pilot-scale experiment showed higher MRT from 8.7 to 9.4 hours. EWs presence induce an increase of 0.5h in CW planted with reeds.

Conclusion

For this experimental bioinspired CW system with a single stage of treatment, pilot performances were close to that of full-scale similar systems and with compliance of the French standards for discharge in the receiving environment. This study demonstrated that VFSSF-CW are able to handle a first position in the treatment chain and could be coupled with green or grey technologies of tertiary treatment. In this perspective, pathogens removal still needs to be assessed in reclaimed water coming out of this CW. In general, these CWs perform well regarding solids and COD removal, but less toward N and P removal which make this type of treatment a good candidate for water reuse in fertigation. On one hand, EWs influence is more on hydrological conditions, even though EWs effect on hydraulic conductivity is not obvious, MRT seems to be slightly improved by EWs. On the other hand, it should be more evident on macro-contaminant removal with more extreme experimental conditions [*i.e.*, higher OLR, lower resting time or even no resting time, or by inoculating EWs in older CWs (>2 years) with a better developed deposit layer]. EWs effect on hydrology and removal rates are slightly in agreement with literature or at the level of trends that will be confirmed (or not) in longer term surveys carried out on this new experimental platform.

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