Microbiological evaluation of constructed wetlands and solar disinfection in wastewater treatment and reuse

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ABSTRACT

The objective of this study was to evaluate the removal of fecal indicator bacteria and select bacterial pathogens (total coliforms, *Escherichia coli*, *Shigella* spp., *Salmonella* spp. and *Pseudomonas aeruginosa*) in vertical flow constructed wetlands (VF-CWs) with earthworms and solar disinfection (SODIS) as post-treatment of effluent from a septic tank. There was no significant difference between them in removing bacteria using a VF-CW with earthworms (VF-CW W/E) and without earthworms (VF-CW N/E). Both VF-CWs did not completely remove pathogens; however, with the SODIS technology receiving the effluent from VF-CW W/E, for *E. coli*, *Shigella* spp., *Salmonella* spp. and *P. aeruginosa*, the values were reduced below the limit of detection and 4.3 log unit average reduction for total coliforms, with 6 h in SODIS.

Key words | *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp., solar disinfection, treatment wetlands

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HIGHLIGHTS

- Earthworm presence showed no difference in pathogen removal.
- 6 h sufficient time for reduced pathogens below the limit of detection.
- 4 h to agricultural reuse and crops irrigation.
- CWs provide enough pathogen removal for reuse with restrictions.

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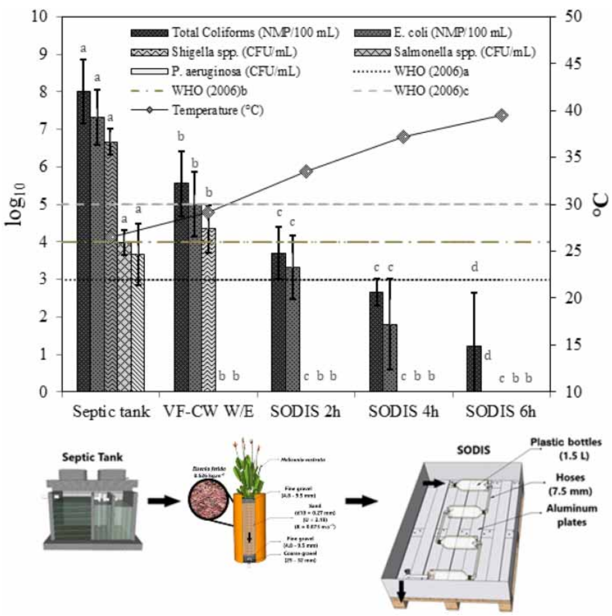
GRAPHICAL ABSTRACT



INTRODUCTION

Human pathogens are a common component of domestic sewage and are potentially infectious; their control is one of the fundamental reasons for wastewater treatment. Constructed wetlands (CWs) are robust wastewater treatment systems as post-treatment of anaerobic systems such as septic tank (ST). According to Arias *et al.* (2003), in CWs, pathogens are removed by factors such as natural die-off, low temperatures, ultraviolet radiation, unfavorable water chemistry, predation and sedimentation. However, few studies are using CWs with a focus on pathogen removal, total and thermotolerant coliforms, fecal streptococci (Arias *et al.* 2003) and *Escherichia coli* (Adrados *et al.* 2018). Moreover, they have shown removal results of about 1.5 log units and 4.0 log units, which are inefficient for the total and complete removal of these microorganisms.

De Oliveira *et al.* (2019) suggest the use of CWs with advanced oxidative processes. It is an advantageous solution, but operation and maintenance are more expensive than UV disinfection. The UV disinfection is effective for inactivating virus, spores and cysts. It does not cause a formation of by-products, odor and does not pose any danger of overdosing, but it is recommended to study other fecal indicator bacteria (Hijnen *et al.* 2006).

SODIS (solar disinfection) is a simple and natural water treatment method that improves microbiological water quality where other means are unavailable (EAWAG 2016), especially in countries that use chlorine for disinfection. Because the effects associated with the formation of organochlorine compounds, such as trihalomethanes, their possible carcinogenic properties are concerns when looking for sustainable and safe solutions (Montao & Kramer 2018). This method uses ultraviolet, visible and infrared radiations that, by reaching the earth's surface, inactivate microorganisms. While the UVB radiation fraction is responsible for modifying the microorganism's DNA or RNA, the UVA fraction causes reactive oxygen species, which react and damage the microorganism's DNA or proteins, and the infrared radiation is responsible for the elevation of water temperature. Therefore, sunlight inactivates, and finally, the death of the irradiated microorganism cells occurs.

Water turbidity can interfere with SODIS by limiting the amount of light penetration, and for an effective SODIS, it should be less than 30 NTU (EAWAG 2016).

CWs are nature-based solutions with high potential for removing carbonaceous and nitrogenous organic matter (Haddis *et al.* 2019) and can reduce suspended solids and remove nutrients that could hinder the operational performance of SODIS with algae and turbidity excess. In this sense, CWs can produce biomass and make integration with SODIS even more attractive if there is interest in agricultural reuse. An operational problem of CWs is the substrate clogging due to high levels of organic matter in the wastewater that reduces the system's efficiency, accelerates the accumulation of solids in the upper layer of the bed matrix and decreases hydraulic conductivity. A natural alternative to increase the useful life of CWs is using earthworms, which restore clogged substrates and prevent clogging, yet help digest solids associated with the clog, improving the N and P absorption. Additionally, the existing chain of microbial metabolism is amplified by the introduction of earthworms into CWs (Xu *et al.* 2013). Under these conditions, Arora *et al.* (2014) found significant removal of total coliforms and *E. coli* in effluent with earthworms when compared to that without earthworms, but was not effective for *Salmonella* spp. removal. In a study carried out by Atalla *et al.* (2020), VF-CW with earthworm addition did not show a more efficient treatment than systems without earthworms. Nevertheless, the study showed that the use of earthworms in CWs can be considered, above all, a preventive measure against clogging.

Thus, the objective of the present work was to evaluate the removal of total coliforms (CT), *E. coli*, *Salmonella* spp., *Shigella* spp. and *P. aeruginosa* from VF-CW W/E and VF-CW N/E as post-treatment of a ST and SODIS as post-treatment of constructed wetlands towards agricultural reuse.

MATERIALS AND METHODS

The set-up system is located at the Center of Technology and Agribusiness (CeTeAgro), Brazil (latitude 20°23′17.77″S and longitude 54°50′52.36″W), which generates daily 38.4 m^3 of domestic wastewater with an ST as the primary treatment.

Description of a pilot design

The VF-CW system, with a total height of 80 and 720 cm^2 of the superficial area, was used. This system was planted with *Heliconia rostrata*, using earthworms (*Eisenia fetida*), 0.526 kg/m^2, as a measure to reduce clogging. It had 60-cm filter media (sand), a 5-cm free superficial layer to apply the effluent and prevent overflow due to possible clogging, a 5-cm fine gravel layer on the surface to prevent filter media erosion, and a 5-cm layer of medium gravel (4.8–9.5 mm) and 5 cm of coarse gravel (25–32 mm) at the bottom operating in the downflow (Figure 1). The SODIS system was built with four plastic bottles of 1.5 L each, connected by 7.5 mm diameter hoses. Aluminum plates were placed behind the plastic bottles to improve heat absorption and to reflect solar radiation (Figure 1).

Operational aspects and sampling

The VF-CW W/E and VF-CW N/E operated initially with hydraulic loads between 160 and 180 mm d^{-1} of post-treated sewage from an ST for 300 days. During the study phase, receiving a 200-mm d^{-1} hydraulic load for 112 days, samples were collected after passing through the VF-CW system. The average of organic load was 7 $g{\cdot}N{\cdot}m^{-2}{\cdot}d^{-1}$, 105 $g{\cdot}COD{\cdot}m^{-2}{\cdot}d^{-1}$, 27 $g{\cdot}BOD{\cdot}m^{-2}{\cdot}d^{-1}$, 0.8 $g{\cdot}P{\cdot}m^{-2}{\cdot}d^{-1}$ and 27 $g{\cdot}SS{\cdot}m^{-2}{\cdot}d^{-1}$ distributed in three batches per day. Wastewater coming from the ST has concentrations according to Table 1.

After the VF-CW W/E treatment, the effluent was sent to SODIS for a 6-h exposure to solar radiation between 9:00 a.m. and 3:00 p.m. (in operation for 100 days). Samples were collected every 2 h at 11:00 a.m., 1:00 p.m. and 3:00 p.m., resulting in three daily samples. All experiments were performed in triplicate and were performed every 2 weeks, and a total of 64 samples were analyzed.

Microbiological analysis

Samples (100 mL) were analyzed for microorganisms and pathogens, such as total coliforms and *E. coli* (method

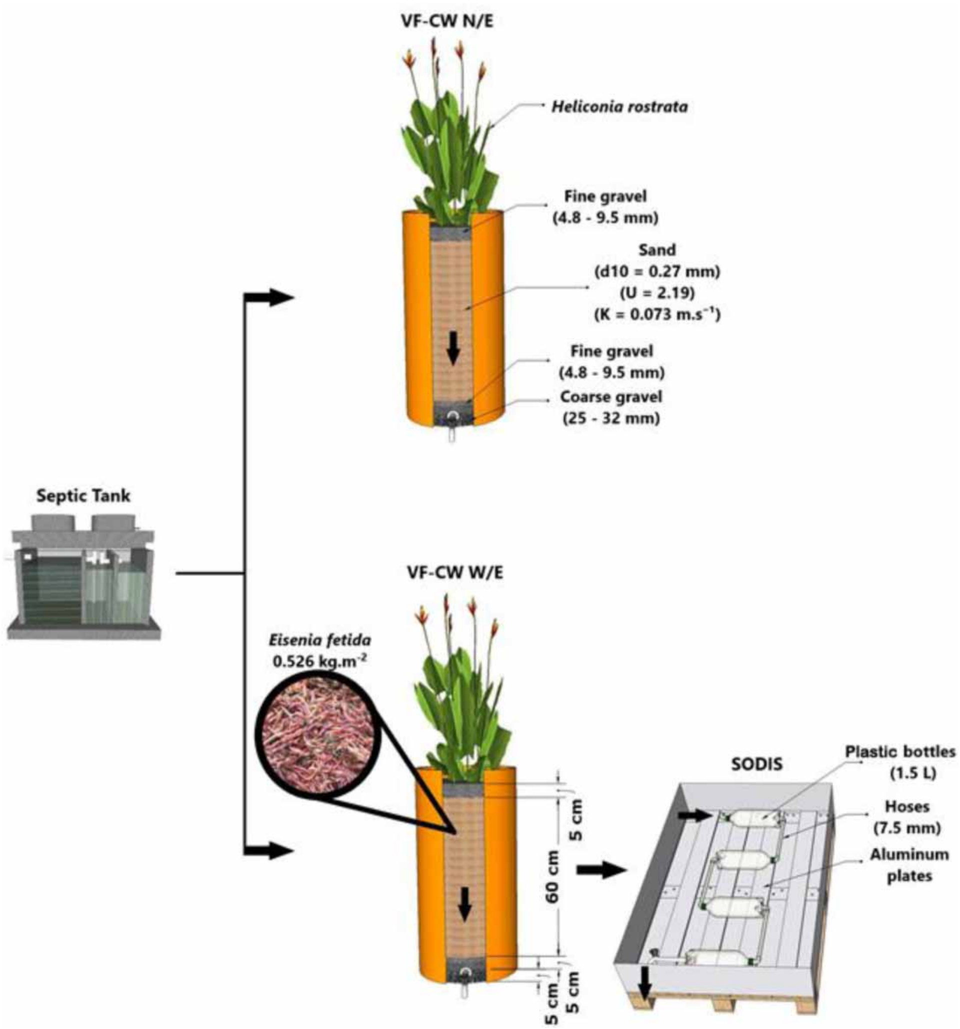


Figure 1 | System set-up.

9221B, C, F, 9223B) using the most probable number (MPN) method, *Salmonella* spp. (method ISO 6579:2002), *Shigella* spp. (method ISO 6579:2002) and *P. aeruginosa* (method ISO 16266:2006) by the pour plate method and by the colony forming unit (CFU), according to *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.* 2012). The limit of detection was <2.0 MPN for coliforms and *E. coli*; <25 CFU for *Salmonella* spp.; <25.0 CFU for *Shigella* spp. and <5.0 CFU for *P. aeruginosa* (da Silva *et al.* 2013). Results were analyzed in SISVAR. Variance analysis was performed to find statistical differences ($P \leq 0.05$) between influents and effluents of the systems.

RESULTS AND DISCUSSION

Vertical flow constructed wetlands

The CWs showed statistically similar removal results according to the Tukey test. The VF-CW W/E system obtained a 2.5 log removal for total coliforms, 2.3 for *E. coli*, 4.0 for *Salmonella* spp., 2.3 for *Shigella* spp. and 3.7 for *P. aeruginosa*. The VF-CW N/E system obtained a 2.5 log removal for total coliforms, 2.3 for *E. coli*, 4.0 for *Salmonella* spp., 2.4 for *Shigella* spp. and 3.7 for *P. aeruginosa*. There was total removal (reduced below the limit of detection) for *Salmonella* spp. and *P. aeruginosa* in the two CWs (Figure 2).

Table 1 | Characterization of the ST and CW effluent

Parameters	ST measured values ± SD	CW measured values ± SD
Temperature, °C	28 ± 3	27 ± 3
pH	5.5 ± 0.6	5.7 ± 0.4
Redox potential, mV	133.3 ± 33.7	93.9 ± 25.5
Turbidity, NTU	35.2 ± 2.5	1.9 ± 0.8
COD (chemical oxygen demand), mg·L^{-1}	525.7 ± 174.1	190.9 ± 70.9
BOD (biological oxygen demand), mg·L^{-1}	134.0 ± 61.1	65.2 ± 29.4
TN (total nitrogen), mg·L^{-1}	32.9 ± 22.5	0.9 ± 0.6
NO_2^- (nitrite), mg·L^{-1}	0.3 ± 0.3	0.1 ± 0.1
NO_3^- (nitrate), mg·L^{-1}	0.4 ± 0.2	0.1 ± 0.1
NH_4^+ (ammoniacal nitrogen), mg·L^{-1}	16.3 ± 5.5	0.3 ± 0.1
TP (total phosphorus), mg·L^{-1}	4.0 ± 0.8	0.9 ± 0.8
TDS (total dissolved solids), mg·L^{-1}	134 ± 40	91 ± 39
TSS (total suspended solids), mg·L^{-1}	98 ± 41	69 ± 43

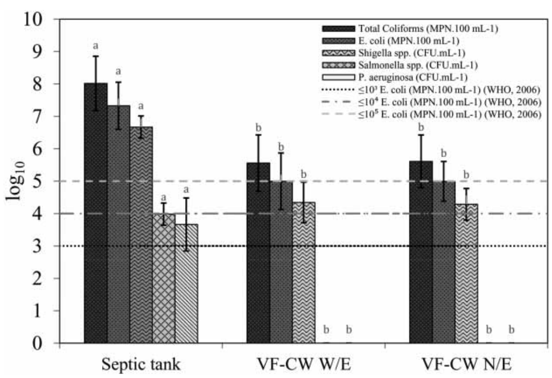


Figure 2 | Log_{10} output concentrations of each technology. WHO: World Health Organization. Mean values followed by different letters are statistically different by Tukey's test at 5% probability.

Removal rates were higher than those in the study of Arias *et al.* (2003), which obtained the removal of 1.5 log units for total coliforms. However, the mean temperature was 7.2 °C in the influent and 5.9 °C in the effluent, which is below that in the present study. Removal rates were lower than the ones found in Adrados *et al.* (2018), which obtained the removal of 2.4–3.4 log units for total coliforms and *E. coli*, with temperatures lower than the present study, ranging from 0 to 16 °C. Nonetheless, the VF-CW systems had effluent recirculation favoring the removal of microorganisms. Tuncsiper *et al.* (2012) obtained the mean removal of 1.03 CFU·100 mL^{-1} for total coliforms and 0.92 CFU·100 mL^{-1} for fecal coliforms and results lower than those in the present study. This is probably because they used coarse gravel (15–40 mm) with a depth of 0.10 m and gravel (5–25 mm) with a depth of 0.22 m. The coarse sand used in our study allows better removal although there is a greater risk of clogging.

Pramanik (2010) reported that worms ingest microorganisms with organic substrates, but not all microorganisms are killed during their passing through the intestine. The literature presents little information about the influence of worms on pathogen removal in treatment wetlands. Atalla *et al.* (2020), by adding 0.526 kg m^{-2} of earthworms, found that the method was efficient in reducing the risk of clogging compared with the CW without earthworm, but organic matter (BOD, COD and TN) removal performance was similar, no influence of earthworm presence in this process.

SODIS + vertical flow constructed wetland

By using VF-CW W/E + SODIS systems, mean log removal results for CW-VF W/E were 2.3 for total coliforms, 2.2 for *E. coli*, 3.8 for *Salmonella* spp., 1.9 for *Shigella* spp. and 3.8 for *Pseudomonas*. There was the total removal of *Salmonella* spp. and *P. aeruginosa* (Figure 3).

Mean results at a 2 h sun exposure were 1.9 log unit for total coliforms, 2.1 for *E. coli* and 5.0 for *Shigella* spp., while *Salmonella* spp. and *P. aeruginosa* remained inactive. With 4 h of sun exposure, the average removal was 1.0 log units for total coliforms and 1.5 for *E. coli*; *Shigella* spp., *Salmonella* spp. and *P. aeruginosa* remained inactive. After 6-h sun exposure, *E. coli*, *Shigella* spp., *Salmonella* spp. and *P. aeruginosa* were reduced below the limit of detection.

The use of SODIS as VF-CW post-treatment was efficient in removing pathogens. A decline according to the time of exposure to the sun was observed in the microorganisms analyzed. Although solar radiation was more intense at the beginning of solar exposure, the temperature increased according to the time of exposure. However, when considering logarithmic units, the period from 09:00 a.m. to 11:00

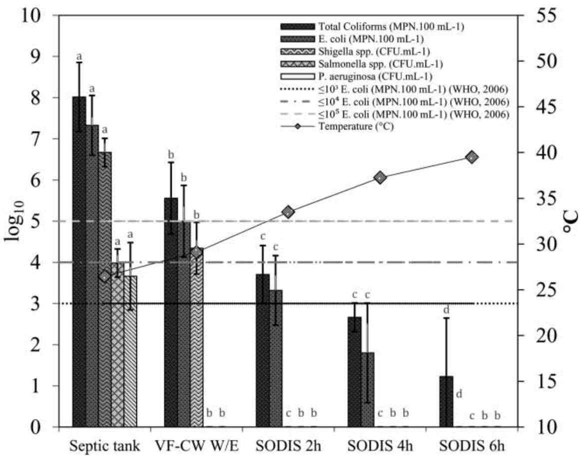


Figure 3 | Log_{10} output concentrations of each technology. WHO: World Health Organization. Mean values followed by different letters are statistically different by Tukey's test at 5% probability. SODIS 2 h: ~625 $W{\cdot}m^{-2}$; SODIS 4 h: ~500 $W{\cdot}m^{-2}$; SODIS 6 h: ~525 $W{\cdot}m^{-2}$.

a.m. was more efficient for the removal of fecal indicator bacteria. The values fit the microbiological water quality standard for some reuses in all studied microorganisms' indicators, except for total coliforms.

Removal rates were smaller than those found in Caslake *et al.* (2004), which obtained 4.0 log units for total coliforms in 44 min of exposure, with 500 $W{\cdot}m^{-2}{\cdot}d^{-1}$ average solar radiation reaching a temperature of 55 °C and turbidity values between 0.09 and 0.32 NTU. Turbidity is an important factor, as evidenced by EAWAG/SANDEC (2016); particles suspended in water absorb and disperse UV radiation, reducing the effectiveness of disinfection. A CW was effective in reducing turbidity and reduced from ~35 to ~2 NTU.

Using SODIS as lake water and secondary effluent treatment, Al-Gheethi *et al.* (2013) reduced *Salmonella* spp. by more than 4.0 log_{10} $CFU{\cdot}100\,mL^{-1}$ after 6 h, while after 7 and 8 h they were undetectable (<1 $CFU{\cdot}100\,mL^{-1}$), and showed that the values were reduced below the limit of detection after 6 h of exposure, similar to our data. The authors concluded that there was no recrudescence of *Salmonella* spp. in samples treated by SODIS with 8 h of sun exposure. Wegelin *et al.* (1994) had a 3.0 log reduction of *E. coli* in 5 h of sun exposure. The authors concluded that water temperature around 50 °C increased the pathogen removal rate considerably and increased with temperatures in the range of 20–50 °C.

Safe reuse of treated wastewater for agriculture

The CWs provide enough pathogen removal for reuse with restrictions (drip irrigation, mechanized agriculture and tall crops). CWs remove turbidity which makes SODIS more efficient and compact. We obtained *E. coli* values lower than 5,000 $MPN{\cdot}100\,mL^{-1}$ after 2-h sun exposure with SODIS, which is suitable for irrigating orchards, cereals, fodder and cattle pasture according to the Brazilian standard (ABNT 1997). For the irrigation of crops likely to be eaten uncooked, sports fields and public parks, *E. coli* values lower than or equal to 1,000 $MPN{\cdot}100\,mL^{-1}$ were required (WHO 2006), and thus, lower mean values were obtained after SODIS treatment with 4 h of exposure. For agricultural reuse, food crops not commercially processed, surface irrigation of orchards and vineyards, non-food crops pasture for dairy animals, fodder, fiber, golf courses, parks and cemeteries, *E. coli* values of less than or equal to 200 $MPN{\cdot}100\,mL^{-1}$ (USEPA 2012) are required, and thus, lower mean values were obtained after SODIS treatment with 4 h of exposure. Von Sperling (1999), evaluating coliform die-off in (sub)tropical stabilization ponds, verified removals of 2–3 log units, although the hydraulic detention time ranged from 3 to 100 days. While the CWs with SODIS enabled higher removal values in a considerably shorter time, it can be applied on a real scale.

CONCLUSIONS

The VF-CWs with (*Eisenia fetida* 0.526 $kg{\cdot}m^{-2}$) and without earthworms showed no difference in pathogen removal. The VF-CW with earthworms and SODIS, when receiving post-treated sewage, was efficient in pathogen removal, and the values were reduced below the limit of detection for *E. coli*, *Salmonella* spp., *Shigella* spp. and *P. aeruginosa*, which fit the microbiological water quality standard for some types of reuse and disposal. Thereby, the longer the sun exposure was, the greater the removal rates of microorganisms, requiring 4 h to agricultural reuse and crops irrigation, and 6 h was sufficient time for reduction below the limit of detection.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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