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Anaerobic-aerobic sequential biofilters packed with polyurethane/polypyrrole-co-polyaniline and constructed wetland for municipal wastewater treatment

Biofiltros secuenciales anaerobio-aerobio empacados con poliuretano/polipirrol-co-polianilina y humedal artificial para el tratamiento de aguas residuales municipales

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Resumen

Debido a la creciente demanda y a la problemática del agua que actualmente existe, se requieren sistemas de tratamiento de aguas residuales eficientes y económicos para fomentar el reúso de este vital líquido. Este trabajo tuvo como objetivo evaluar el desempeño de un sistema secuencial consistente de dos biofiltros anaerobio-aerobio y un humedal artificial a diferentes tiempos de retención hidráulica (TRH) en el tratamiento de agua residual municipal (ARM). Los biofiltros fueron empacados con compósitos de poliuretano/polipirrol-co-polianilina como soporte para la formación de biopelícula. El humedal artificial contenía tezontle como soporte para las plantas de *Canna indica*. Los parámetros analizados fueron la demanda química de oxígeno (DQO), la demanda bioquímica de oxígeno (DBO_5), las coliformes fecales (CF), los huevos de helmintos

(HH) y los sólidos totales (ST). Se observó que la remoción de estos parámetros se incrementó en proporción directa al TRH logrando hasta un 93% de eficiencia de remoción para la DQO, un 99% para la DBO, 28% de remoción en los ST, las CF disminuyeron de $1\times10^{6\pm1}$ hasta $1\times10^{1\pm1}$ MPN/100 mL y los HH de incontables a 3 ± 3 h/L, operando a un TRH de 9.9 días.

***Palabras clave*:** biofiltro, compósitos, *Canna indica*, humedal artificial, tratamiento de agua residual municipal.

Abstract

Due to the growing demand and the current water problems, efficient and economical wastewater treatment systems are required to promote the reuse of this resource. The objective of this work was to evaluate the performance of a sequential system consisting of two anaerobic-aerobic biofilters and a constructed wetland at different hydraulic retention times (HRT) in treating municipal wastewater (MWW). The biofilters were packed with polyurethane/polypyrrole-co-polyaniline composites as support for biofilm formation. The constructed wetland contained tezontle as substratum for *Canna indica* plants. The parameters analyzed were chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), fecal coliforms (FC), helminth eggs (HE) and total solids (TS). It was observed that the removal of these parameters increased in direct proportion to the HRT, achieving up to 93% removal efficiency for COD, 99% for BOD_5, 28% removal in TS, FC decreased from $1\times10^{6\pm1}$ to $1\times10^{1\pm1}$ MPN/100 mL and HEs decreased from being uncountable to 3 ± 3 HE/L, operating at a HRT of 9.9 days.

Keywords: biofilter, composites, *Canna indica*, constructed wetland, municipal wastewater treatment.

1. Introduction

Biofilters are an attractive alternative for wastewater treatment and are used for pollutant removal from air and water. Biofilters are biologically active biomass adhered to a support material forming a biofilm (immobilized biomass), in which organic compounds are degraded. Some of the most important parameters that govern the performance of a biofilter are the adherence of the biomass to the support (Chaudhary *et al.*, 2003; Zhang *et al.*, 2019), roughness, and wettability (hydrophobic/ hydrophilic balance) (Al-Amshawee *et al.* 2021). The materials used as support have been classified as natural or synthetic, the latter being biologically inactive. Various synthetic materials and composites have been previously reported for their use as support to develop biofilters, such as polyvinyl alcohol, polyethylene, waste tire (Al-Amshawee *et al*., 2020), polypropylene (Naz *et al.*, 2018), styrene (Portune *et al.*, 2020), basalt fibers (Gao *et al.*, 2021), polyurethane (Dacewicz and Grzybowska-Pietras, 2021), modified polyurethane (Feng *et al*., 2019) Polyurethane has advantages, such as mechanical strength, chemical resistance, porosity (Rastegar *et al*., 2022), low cost (Sandip and Kalyanraman, 2019) and a large surface area (Pi *et al.*, 2020).

Composites (hybrid materials) confer advantages such as high strength and stiffness (Campbell, 2010; Sundeep *et al*., 2023). Zhou *et al.* (2010) reported using a Fe_3O_4 -based composite/polyurethane foam as a support for a biofilter, detecting higher microbial colonization and high toluene removal efficiency compared to alone polyurethane foam. Chu *et al.* (2014) evaluated the performance of a moving bed biofilm reactor packed with cationic hydrophilic modified polyurethane foam (from a mixture of toluene diisocyanate, polyether polyol, foam stabilizer, dichloromethane, phosphoric acid, and acetic acid) for its use in the removal of synthetic municipal wastewater. The results indicated that the modified polyurethane foam improved the biofilm formation 1.3 times more than unmodified polyurethane foam. Feng *et al.* (2019) modified polyurethane foam with sodium alginate and polyvinyl alcohol to pack a trickling biofilter, obtaining a higher degradation of toluene, ethylbenzene and xylene, higher microbial diversity and stability when compared with a polyurethane foam control without modification.

The MWW contains organic matter, nitrogen, phosphorus, and other chemical pollutants and a high concentration of pathogenic organisms such as fecal coliforms and helminths, etc. Generally, the organic matter contained in the MWW is removed in high percentages through primary and secondary wastewater treatment; sometimes, it is necessary to implement a tertiary treatment, such as a constructed wetland, in such a way that it can be used to improve the chemical and microbiological quality (viruses, bacteria, protozoa and worms) of effluents that have been previously treated by traditional biological treatment processes such as oxidation lagoons or anaerobic or aerobic reactors (Wang *et al*., 2006; Desta *et al*., 2015; Muñoz-Nava and Baumann, 2017; Herrera-Lopez *et al*., 2021). Constructed wetlands have physical, chemical and biological mechanisms which can reduce solids, nitrogen, phosphorus and microbial pollutants concentration (Biswal and Balasubramanian, 2022). An essential part of a constructed wetland is the hydrophytic plants that can be floating, submerged and emergent (Selvaraj and Velvizhi, 2021). Among the most common emergent plants used in constructed wetlands are the genus *Canna*, *Iris*, *Heliconia* and *Zanteschia*, which are also ornamental plants (Sandoval *et al*., 2019). Constructed wetlands with *Canna indica* have shown high removal efficiency for COD, BOD, nitrogen, turbidity, *E. coli* and faecal coliforms (Chang *et al*., 2012; Sharma and Brighu, 2014; Rahmadyanti and Audina, 2020; Mittal *et al*., 2023). As a standalone treatment process, constructed wetlands can also be considered a low-cost alternative (Somprasert *et al*., 2021) and can be a highly efficient secondary or tertiary treatment (Ennabili and Radoux, 2021).

This work aimed to evaluate the performance of a system consisting of two anaerobic-aerobic biofilters packed with polyurethane/polypyrrole-co-polyaniline composites and a constructed wetland with *Canna indica* for the treatment of municipal wastewater at different HRTs.

2. Materials and Methods

This research was carried out in the Biology Laboratory of the Department of Botany of the Autonomous Agrarian University Antonio Narro, Saltillo Unit. The experiments were carried out over a year at room temperature (during the time of operation, the temperature varied from 5 to 29 ºC with an annual average temperature of 17 ºC), and the municipal wastewater used in these experiments was collected weekly from the reception module of the wastewater treatment plant (WWTP) of the Mexican Army Park (Bosque Urbano Ejército Mexicano) of the City of Saltillo, Coahuila. Table 1 shows the characterization of the municipal wastewater used in this study; the wastewater parameters varied over the course of the year.

Table 1. Chemical and microbiological characterization of the municipal wastewater used in the present paper.

Parameter	Average	Maximum	Minimum
COD (mg/L)	589	1070	53
pH	7.1	7.5	6.9
FC (MPN/100 mL)	2.2×10^6	1×10^8	4×10^5
HE (HE/L)	+	+	+
BOD_5 (mg/L)	95	175	78
TS (mg/L)	1340	2400	500

+ presence of helminth eggs (uncontable).

2.1 Sequential treatment system (anaerobic-aerobic-wetland)

The sequential treatment system was integrated by anaerobic (1) and aerobic biofilters (2), followed by a constructed wetland (3), as shown in Figure 1.

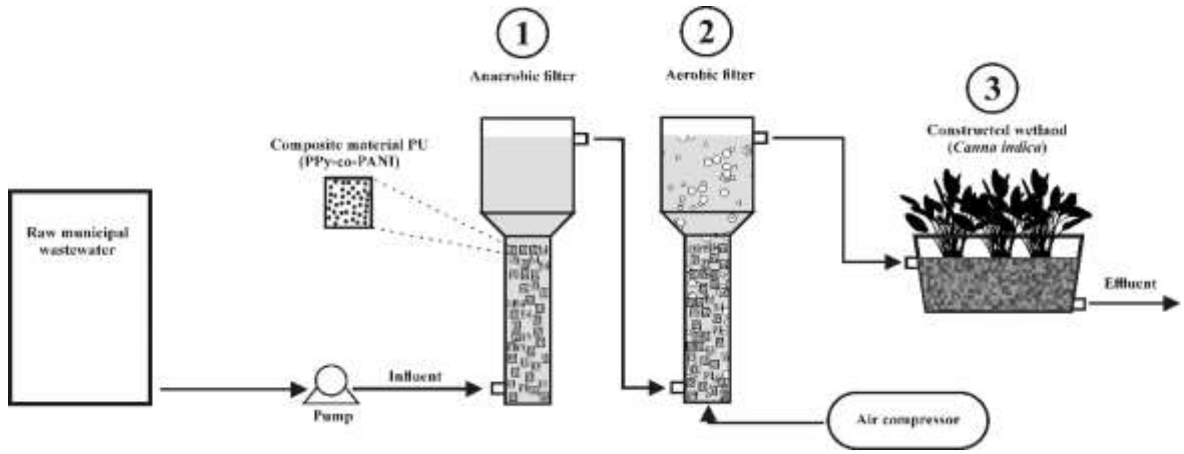

Figure 1. Sequential system.

The anaerobic biofilter was constructed with acrylic and had an effective working volume of 5.8 L. The biofilter was packed with 18 grams of polyurethane/polypyrrole-co-polyaniline composite. The composites were prepared according to the methodology used by Antonio-Carmona *et al*. (2015); the polyurethane foam was cut into cubes of approximately 1 x 1 x 1 cm, and these were submerged in one liter of an aqueous solution containing a mixture of two solutions (0.5 g of pyrrole /L with 0.5 g of aniline /L). The solution and the polyurethane foam were mixed with magnetic stirring for 2 hours to eliminate the air trapped in the foam; when this step is done, an oxidizing agent (ammonium persulfate) was added and mixed with a magnetic stirrer bar for 5 hours. Subsequently, the composites were washed with distilled water to remove unadhered residues and dried at 60 °C for 48 hours. The anaerobic sludge (obtained from a UASB reactor that treated wastewater from the brewing industry) was previously macerated to facilitate biofilm development on the surface of the support material.

The aerobic biofilter was also acrylic and had an effective working volume of 5.8 L. The biofilter was packed with composites prepared in the same way as those used in the anaerobic biofilter. An Elite model 799 aerator pump (1 L/min) was used to supply air to the biofilter. The sludge used in this biofilter also was provided by WWTP (activated sludge) Mexican Army Park.

The constructed wetland consisted of a plastic rectangular module with a volume of 27 L (73 L x 45 W x 35 H cm). Tezontle (volcanic rock of approximately 2 cm in diameter) was added to the plastic module (up to a height of 19 cm) as a substratum for the *Canna indica* plants. Nine *Canna indica* plants were planted with an approximate height of 30 cm in the plastic module (approximately 27 plants per m^2). The plants were collected from a constructed wetland that treated wastewater from a fishpond. The working volume of the constructed wetland was 16.2 L. An Elite model 799 aerator pump (1 L/min) was used to supply air to the constructed wetland.

2.2 Sampling and quantification

The influent and effluent of each stage (anaerobic, aerobic, and constructed wetland) were analyzed for the following parameters: pH, COD, BOD_5, TS, FC, and HE. The analysis of the parameters was carried out as follows: the pH using a pH meter (Thermo Scientific Brand, Model ORION STAR A215); TS was determined according to NMX-AA-034-SCFI-2015 (ARSA Brand Oven Model AR-290; US SOLID Brand Electronic Precision Balance Model USS-DBS 15-3); BOD_5 was determined according to NMX-AA-012-SCFI-2001 and NMX-AA-028-SCFI-2001 (HACH Model 205 Incubator); FC was determined according to NMX-AA-042-2015 (Vortex Brand SCILOGEX Model MX-S; Incubator Brand Boekel Scientific Model 133000); the determination of HE was carried out using the modified Bailenger Method described by Ayres and Mara, in 1996 (Solbat Brand Centrifuge Model J-600; McMaster Chamber); COD was determined according to NMX-AA-030/2-SCFI-2011 (Thermoreactor Brand HACH Model Digital Reactor Block 200; Spectrophotometer Brand HACH Model DR 5000).

2.3 Operation of the sequential treatment system

The operation of the sequential treatment system was carried out in three phases: the conditioning, the test, and the start-up phases. The conditioning or colonization phase of the biofilters was carried out for three months to promote the formation of a biofilm by anaerobic microorganisms (anaerobic biofilter), whereas the aerobic biofilm (aerobic biofilter) formation on the polyurethane / polypyrrole-co-polyaniline composites was carried out by recirculation at a low feed rate (48 h HRT) of activated sludge and with MWW, as shown in Figure 2. The duration of this phase was established according to the time mentioned by Espinoza *et al.* (2019) of 30 days.

The colonization phase was unnecessary for the constructed wetland because the soil and *Canna indica* plants were collected from a constructed wetland that treated water from a fishpond and it was used immediately. Subsequently, the test phase was carried out, in which the anaerobic, aerobic biofilters and the constructed wetland were connected sequentially to establish the treatment system, as shown in Figure 1. In this phase, the performance of the system was evaluated at three HRT (d = days): 9.9 d (36 h in the anaerobic biofilter, 36 h in the aerobic biofilter, and 167 h in the constructed wetland, equivalent to a total of 9.9 d), HRT 6.6 d (24 h in the anaerobic biofilter, 24 h in the aerobic biofilter, 112 h in a constructed wetland equivalent to a total of 6.6 d), and HRT 3.3 d (12 h in an anaerobic biofilter, 12 h in an aerobic biofilter, 56 h in a constructed wetland equivalent to a total of 3.3 d). Each treatment (HRT) was repeated five times, and the parameters analyzed are the average of these repetitions. At the end of this phase, the HRT was reduced to 8 h to eliminate the excess sludge that did not adhere to the composites; this HRT was kept for one day. In the start-up phase (6 months in which seven repetitions were performed), the sequential system was maintained at an HRT of 9.9 d; during this time, the best results were obtained.

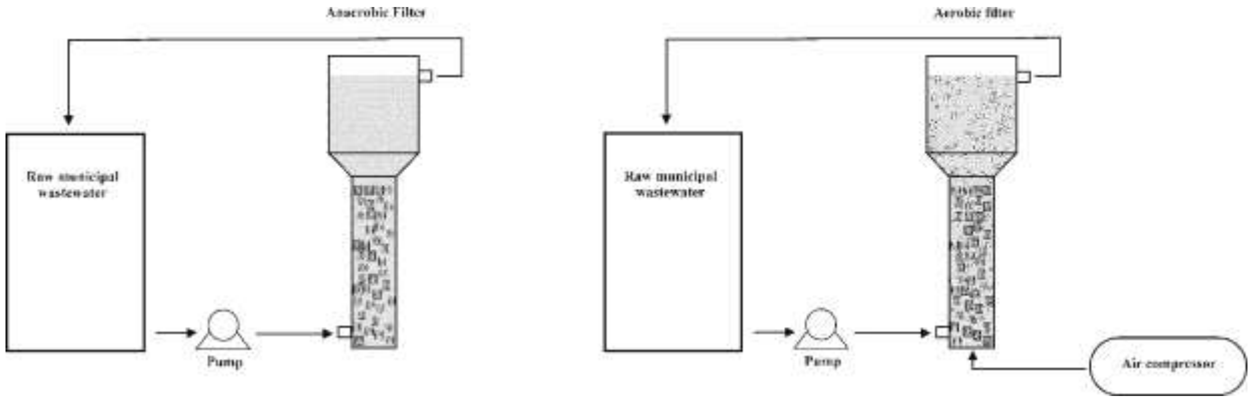

Figure 2. Anaerobic and aerobic biofilters in recirculation regime during the conditioning phase.

3. Results and Discussion

3.1 Test-evaluation phase at three different HRTs

At the end of the colonization phase, the biofilters and the wetland were connected in series. The HRT was established in 9.9 days, and previous tests were carried out for 15 days before evaluating the different HRT to know the operation of each part of the system and to solve possible failures in any of the parts of the system. Table 2 is presented below with the preliminary average results, where the performance of each part of the system, anaerobic filter, aerobic filter, and constructed wetland can be observed. The COD contained in municipal wastewater was removed in a 37% anaerobic biofilter, while up to 71% is removed in the aerobic biofilter (34% more in this part of the system) and up to 90% in the wetland (19% more in this stage), being the anaerobic biofilter where a greater removal occurred, these results agree with the work carried out by Jing *et al*. (2015) when treating contaminated surface water with a double layer biofilter (aerobic and anoxic) and a wetland and the one carried out by Rahmadyanti *et al*. (2020) that when treating residual water from a dyeing process (batik) with a biofilter and constructed wetland system, it was in the biofilters where a greater COD removal was detected. The average fecal coliforms present in the influent were 1 x 10^8 MPN/100 mL; when treated by the anaerobic biofilter, they decreased to 1 x 10^7 MPN/100 mL, resulting in a 90% removal, the effluent from the aerobic biofilter contained 1 x 10^6 MPN/100 mL removing 99.9% and the highest removal (99.99%) was detected in the constructed wetland effluent, reaching 1 x 10^3 MPN/100 mL. The MWW and the effluents from the biofilters contained a high number of helminth eggs that were not counted (uncountable), and the removal is not disclosed as there is no approximate number of HE, in the effluent of the constructed wetland the HE were lower or equal to 5 HE/L though. It is possible to appreciate the need for a tertiary treatment, with the results of microbiological contamination, such as constructed wetlands, which can greatly reduce the presence of pathogenic organisms (Wu *et al*., 2016). The BOD contained in the MWW was removed by 50% in the anaerobic biofilter, 82% in the aerobic biofilter (32% more) and 93% in the constructed wetland (11% more), for which the highest percentage of removal was in the anaerobic biofilter. Amiri *et al*. (2019) tested a system of biofilters and constructed wetlands in the treatment of domestic wastewater and detected that in the biofilters there was a BOD removal of 73.5%. TS was removed by 22% in the anaerobic biofilter and 40% in the aerobic biofilter (18%), while no removal was detected in the constructed wetland.

Table 2. Removal efficiency for each stage of the system.

Parameter	Anaerobic biofilter effluent	Aerobic biofilter effluent	Constructed wetland effluent
COD $_{\% RE}$	37	71	90
FC $_{\% RE}$	90	99.9	99.99
HE/L	+	+	≤ 5
$BOD_{5\% RE}$	50	82	93
TS $_{\% RE}$	22	40	40

$_{\% RE}$: percent removal efficiency; + uncountable.

After the 15 days of the preliminary phase, the effect of three different HRTs was evaluated: 9.9, 6.6 and 3.3 days (in this order), by quantifying the following parameters: COD, BOD_5, TS, FC, and HE (Table 3). The removal percentages of all the analyzed parameters depended on HRT: the higher HRT, the greater the removal. COD removal was 80% at an HRT of 3.3 days, 87% at an HRT of 6.6 days, and up to 94% at an HRT of 9.9 days. It is important to mention that, in the six months that this phase lasted, the COD of the MWW varied, and it was observed that when the COD was less than 100 mg/l, the removal percentage of this parameter drastically decreased to less than 50%. The COD removal was greater (93-94 %), when the MWW contained more than 770 mg COD/L, observing a lower removal when the MWW concentration was less than this value (84-88 % with a COD of 380-725 mg/L). The variation of the COD content of wastewater is common; therefore, some researchers suggest mixing this type of water with industrial wastewater (with a greater amount of organic matter) so that the COD remains stable before entering a biological treatment system (Kroiss *et al.*, 1992; LaPara and Alleman, 1999). This variability also affects the other parameters in the influent, which can be observed in Table 3. COD, FC, and TS are the parameters that vary in greater proportion, and the pH, the HE, and the BOD_5 vary in a lesser proportion. On average, the pH was higher in the influent, ranging between 8 and 8.87, with a decrease observed after passing through the sequential system (7.07 to 8.16).

Table 3. Results obtained at different HRTs in the test phase.

HRT d	3.3		6.6		9.9	
Parameter	Influent	Effluent	Influent	Effluent	Influent	Effluent
COD (mg/L)	523 ± 89	103 ± 50	444 ± 205	46 ± 0.71	642 ± 87	40 ± 5
pH	8.87 ± 0.25	8.16 ± 0.07	8 ± 0.19	7.07 ± 0.02	8.06 ± 1	7.71 ± 0.07
FC (MPN/100 mL)	$1 \times 10^{6 \pm 1}$	$1 \times 10^{5 \pm 1}$	$1 \times 10^{6 \pm 1}$	$1 \times 10^{3 \pm 1}$	$1 \times 10^{6 \pm 1}$	$1 \times 10^{1 \pm 1}$
HE (HE/L)	+	+	+	15 ± 5	+	3 ± 3
BOD_5 (mg O_2/L)	108.14 ± 17.51	24.3 ± 0.6	96.04 ± 18	15.27 ± 4.02	118.62 ± 19.08	8.04 ± 4.38
TS (mg/L)	1162 ± 286.11	1305 ± 128	992 ± 127.89	834.48 ± 110	1385 ± 225.3	984.12 ± 156.64

+ presence of helminth eggs (uncountable); influent (municipal wastewater without treatment); effluent (municipal wastewater treated by the sequential anaerobic-aerobic biofilters-constructed wetland system).

The FC detected in the influent were at $1\times10^{6\pm1}$ MPN / 100 mL, decreasing in the effluent to 1×10^5, 1×10^3 and 1×10^1, with HRT of 3.3, 6.6 and 9.9 days, respectively. The results show a fecal coliform removal efficiency of over 99% in all HRTs assessed. Ling *et al.* (2009) used a combined system of biofilters and constructed wetlands with

Syzygium campanulatum and *Ficus microcarpa* to treat a mix of grey and black water, obtaining a removal efficiency of 96% of FC at an HRT of 20 h. Fuentes and Vizcaíno (2018) reported a system that combined the use of biodigesters and biofilters (macrophytes) to treat domestic wastewater at different HRTs (6, 12 and 18 days), obtaining a high removal efficiency of FC. Khuntia *et al.* (2021) assessed the FC removal in greywater using a sequential multi-chambered up-flow anaerobic biofilm reactor, an up-flow aerobic reactor, and a vertical greenery system inhabited by *Sphagneticola trilobata*, obtaining a 99.95% removal efficiency at an HRT of 2.25 days. Beutel and Larson (2015) also reported the advantages of using a sequential treatment system comprised of biofilters and constructed wetlands to remove FC compared to only using biofilters.

Jenssen *et al*. (2010) reported using a filter bed system that consisted of aerobic biofilters and plants, obtaining a removal efficiency that complied with the Norwegian regulations for reuse in agriculture for heavy metals, fecal bacteria, and parasites. In the present work, a high HE removal was obtained at HRTs of 6.6 and 9.9 d, decreasing from a very high density (uncountable) to 15 and 3 HE/L, respectively. According to NOM-003-SEMARNAT-1997 (which establishes the maximum permissible limits of contaminants in wastewater treated for reuse for public services with direct contact), the wastewater treated by the sequential system at an HRT of 9.9 days complies with the regulation mentioned above for FC (240 MPN/100 mL), and almost complies with the allowed HE (≤1).

The influent had a BOD_5 that ranged from 118 to 96 mg/L, decreasing to 24, 15, and 8 mg/L with an HRT of 3.3, 6.6 and 9.9 days, respectively. Inamori *et al.* (1986) observed a greater removal of BOD by increasing the HRT and the temperature using a sequential system of anaerobic-aerobic biofilters for domestic wastewater treatment. Vigueras-Cortés *et al.* (2013) detected that the removal of BOD in municipal wastewater was greater when the temperature increased in aerobic biofilters packed with agave fibers.

In an HRT of 3.3 days, no significant removal of TS was observed, but with HRTs of 6.6 and 9.9 days, the removal was 16 and 29%, respectively.

3.2 Start-up phase-HRT 9.9 days

Based on the best results of the test phase, the HRT of 9.9 days was selected for the start-up phase, which lasted six months, to assess the stability of the sequential system. Table 4 shows that the initial concentration of the evaluated parameters had variations. The effluent of the sequential system presented removal efficiencies of 88 to 93, 99, ≥ 90, 90 to 96, and 24 to 41% for COD, FC, HE, BOD_5, and TS, respectively. The pH of the influent ranged from 6.68 to 7.58, and in the effluent ranged from 7.27 to 7.79. The effluent was visually colorless, transparent, and free of unpleasant odors. Vigueras-Cortés *et al.* (2013) evaluated municipal wastewater treatment using aerobic biofilters packed with agave fibers, obtaining a 92% removal efficiency for BOD, 79.7% for COD, 98% for HE and 99.9% for fecal coliforms. Ling *et al.* (2009), carried out a study on the treatment of gray wastewater through a sequential system of biofilters packed with expanded clays and a constructed wetland with *Syzygium campanulatum* and *Ficus microcarpa* plants, obtaining a removal efficiency of 99% for BOD, and 95% for COD, and reducing the FC in the effluent by two orders of magnitude. Ling *et al.*, (2009) reported that the BOD, COD, and FC were mainly removed during the treatment by the biofilters (aerobic). The removal efficiency in constructed wetlands will depend on the climatic conditions: a cold climate directly or indirectly will affect plant metabolism, plant absorption, rhizosphere oxygenation, substrate adsorption, and sedimentation capacity and metabolic rate (Kataki *et al.*, 2021); in addition, microbial diversity, and its activity, also undergo seasonal changes (Khouja *et al.*, 2020).

Table 4. Results obtained during the start-up phase of the sequential system at an HRT of 9.9 days.

Parameter	Influent	Effluent
COD (mg/L)	666 ± 251	64 ± 13
pH	7.13 ± 0.45	7.52 ± 0.25
FC (MPN/100 mL)	$10^{6 \pm 1}$	$10^{1 \pm 1}$
HE (HE/L)	+	8 ± 3
BOD_5 (mg/L)	137.04 ± 23.86	9.32 ± 3.82
TS (mg/L)	1480 ± 397.41	1002.14 ± 125.14

+ presence of helminth eggs (uncountable); * these parameters corresponds to the average of the cycles when stability was evaluated, considering only the wastewater without treatment (influent) and that of the wetland (effluent).

Figure 3 shows a visual comparison between the raw municipal wastewater and the effluents of each stage of the sequential treatment system. It can be visually observed that color, solids, and turbidity diminish greatly.

Figure 3. Visual comparison between the untreated wastewater (left), wastewater after treatment by biofilters (center), and wastewater after treatment by constructed wetland (right).

4. Conclusions

The material used as support to develop the biofilters (polyurethane foam coated with polypyrrole-co-polyaniline) and the whole system is a low-cost and highly efficient alternative to treat municipal or domestic wastewater that is similar in composition to municipal wastewater. *Canna indica* is a plant that, besides having beautiful flowers, has great potential to remove pathogens, as demonstrated in this research, while biofilters proved to be more efficient in removing COD, BOD_5, and TS. The sequential system also showed stability throughout the different phases of work. Future research will focus on reducing the HRT to 9.9 days, where the highest removal efficiencies were detected, which could be achieved by increasing the amount of support added to increase microbial density in each biofilter. In addition, the type of constructed wetland, in this case, a horizontal flow, can be replaced by one of vertical flow; another factor, such as the plant density in the constructed wetland, can also be increased, and the addition of other species with the potential for phytoremediation can also be assessed.

5. Acknowledgments

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