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Biodegradation of Diclofenac and Paracetamol Present in Wastewater through an Integrated System of Anaerobic Reactor and Constructed Wetland

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ABSTRACT

The objective of this work, that belongs to the field of alternative options for drug pollutants degradation, is to design an integrated system of anaerobic reactor and constructed wetland to eliminate paracetamol (N-acetyl-p-aminophenol) and diclofenac (2-[2-(2,6-dichloroanilino)phenyl]acetic acid) residues contained in contaminated waters. The integrated system of anaerobic reactor and constructed wetland consisted of a water tank supplying wastewater to a biodigester through a feed tank, a constructed wetland home to two species of plants Cyperus alternifolius and Typha angustifolia, and a treated water collection tank. The parameters were analyzed pursuant to NOM-001-SEMARNAT-1996, while diclofenac and paracetamol were analyzed through capillary electrophoresis. The results showed a 90% biodegradation of the organic material in the effluent. In the biodigester, the average biodegradation reaction speed was Kt = 4.9 days-1 ± 0.870; with $p<0.05$. The drugs were not detected in the effluent from the wetland, and thus the integrated system of anaerobic reactor and constructed wetland represents an alternative for removing pollutants from municipal wastewaters.

Keywords: Wastewater; Drugs pollutants; Wetlands; Water treatment

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INTRODUCTION

Health care requires the consumption of various drug products, and these products, once administered to the people, reach the environment through excretion and metabolization [1,2]. The occurrence of active pharmaceutical substances in the environment is of growing concern. Many drugs are released into the environment, which makes them pollutants, through industrial waste and sub-products, animal and human excretions, household garbage, etc. [1,3,4]. The administered drug product can be excreted without change, as glucuronides or sulphate conjugates, as main metabolite, or as a mixture of various metabolites. In the organism, drug products are metabolized through various mechanisms such as: oxidation, reduction, hydrolysis, conjugations, among others, and then excreted as more polar and water soluble derivatives having reduced pharmacological activity compared to the original compound [4,5]. It has been shown that drug residues are disseminated in the environment and have been detected in water supply sources, underground waters and even in drinking water, and thus it is a cause of concern because the consumption of drug products without medical prescription may lead to alterations in human beings. Conventional wastewater and drinking water treatment plants are not designed to remove these pollutants. Many of pharmaceuticals are not effectively removed by conventional treatments. The non-planned reutilization of wastewater has had negative effects on the health of the local population and on the ecosystem in general. Both farmers and their families as well as consumers are exposed to gastrointestinal diseases caused by microorganisms present in irrigation water, besides other pollutants such as drugs, or emerging organic pollutants including pharmaceuticals [6,7]. Drug product concentrations in wastewaters (in the order of 5 g/L to 50 g/L), mostly from hospitals, health centers, and population treated at home, are complex and in some cases persistent compounds, and their constant disposal in bodies of water leads to their presence in the influents to the Wastewater Treatment Plants (WWTPs) that are not designed to eliminate them, causing their accumulation in the ecosystems [4,8]. The drug products most frequently used by the Mexican population are analgesics and anti-inflammatories that are taken with or without medical prescription. Among them, diclofenac and paracetamol stand out because of their potential hepatotoxicity [4,9]. In some countries, the growing concern for the environmental impact of pharmaceutical products has led the regulators to recommend ways for healthcare waste handling and disposal. However, domestic wastewaters are not generally appropriately treated to eliminate drug products and thus it is necessary to look for efficient and economically viable procedures to remove this type of pollutants. It is also necessary to design standards as regards pharmaceutical waste disposal in bodies of water [10] and thus the objective of this research was to evidence the removal of drug products residues contained in wastewaters through an integrated system of anaerobic reactor and constructed wetland (ISARCW).

EXPERIMENTAL SECTION

Method

The design of the ISARCW is as follows: a feed tank supplies wastewater to a biodigester sealed with a concrete lid and equipped with a polymeric washer; said biodigester has one synthetic wastewater (SWW) inlet located at the bottom and a corresponding outlet located in the opposite upper part and is in turn fed to the constructed wetland. The materials used as substrate for the constructed wetland were: ¾" gravel, sand, 10% fertile soil and river sand. Since gravel was the most abundant material in the constructed wetland, its porosity was determined. The porosity of the substrate consisting of ¾" gravel was determined by the law of fluid displacement (Archimedes Law) according to [11]. The Archimedes principle indicates that "the upward buoyant force that is exerted on a body immersed in a fluid is equal to the weight of the fluid that the body displaces". A 20 L container was gauged with tap water at environment temperature = 33.5°C and atmospheric pressure of 785 mmHg. Gravel was then gradually added. The displaced water was collected and quantified in liters. Then, porosity percentage was determined as follows: porosity % = (20 L water - L of displaced water / 20 L) × 100.

Calculations for the Design and Building of the ISARCW and Route followed by the SWW in the Biodigester and the Constructed Wetland

The site is located on loam soil. A Rotoplas water tank was installed at the beginning of the processing path, and then two already existing concrete tanks were outfitted to form the feed tank supplying wastewater to the biodigester, and the biodigester. The floor supporting the feed tank and the biodigester was built, and inside finishing was applied to seal the biodigester. A wire mesh fence was placed around the constructed wetland for protection purposes. A treated water collection tank was built at the end of the processing path. The system of affluent and effluent conduction pipes of the integrated system of anaerobic reactor and constructed wetland was set up according to the [12]. The 450 L Rotoplas water tank was supplied with SWW, which was fed to the 350 L feed tank, that in turn was fed to the biodigester tank of a capacity of 275 L; equipped with a polymeric washer, and having a contact surface of 2.673 m^2 equivalent to 40 L, in the central part of the biodigester, with the following dimensions: Length = 2.43 m, Width = 0.55 m and Thickness = 0.000108 m, obtaining thus a working surface of 2.673 m^2. The reactor (biodigester) dimensions were: real working volume = 0.040095 m^3, depth= 0.60 m, width= 0.50 m and length= 1.05 m, V_{Total}= 0.315 m^3. The average working temperature was (28.23°C ± 2.86). After passing through the biodigester, wastewater is directed to the sub-surface wetland (SSWL) having a hydraulic residence time (HRT) of 10.33 days, with a rate of flow of 131.96 L/d (Figure 1). The sub-surface wetland with a volume of 2840 L was built on solid concrete floor, with fine finishing inside, and a slope of approximately 1%. A concrete floor was built around it, as well as a mesh booth with polycarbonate cover to allow the passage of light and offer protection against rain. A treated water collection tank was built at the end of the processing path (Figures 1 and 2).

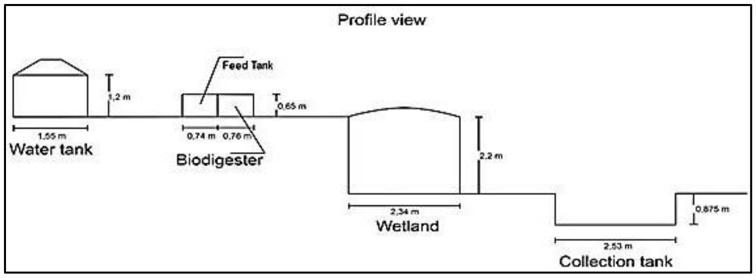

Figure 1: Integrated system of anaerobic reactor and constructed wetland with water tank, feed tank, biodigester, wetland and treated water collection tank

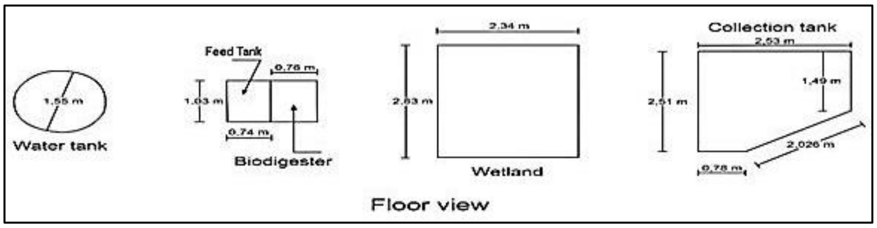

Figure 2: Plan view of the integrated system of anaerobic reactor and constructed wetland showing the dimensions of the water tank, feed tank, biodigester, wetland and treated water collection tank

SWW Preparation

Synthetic Wastewater (SWW) was prepared according to a formulation suggested by some authors such as [13], as shown in Table 1.

Table 1: Formulation of synthetic wastewater

Component	Quantity (mg/L)
Peptone	160
Meat extract	110
Urea	30
KH_2PO4	28
$MgSO_4.7H_2O$	2
$CaCl_2.H_2O$	4
NaCl	7

BOD_5 was adjusted at 180 mg/L according to the proposal of [14]. Diclofenac concentrations were kept in a range from 0.1 µg/L to 28.30 mg/L while paracetamol concentrations were kept in a range from 6.0 mg/L to 53.0 mg/L, as reported by various authors [8,15] in WWTP effluents. In adherence to these criteria, 2.40 mg/L of diclofenac and 4.8 mg/L of paracetamol were added to the SWW formulation as working concentrations for the integrated system of anaerobic reactor and constructed wetland. During the operation of the combined system at steady state during 90 days, the following parameters were analyzed weekly according to APHA, Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), and Biochemical Oxygen Demand (BOD). The parameters were measured at steady state over a period of eight weeks; samples were taken from the biodigester affluent and effluent. The results obtained were compared with the values permissible according to NOM-001-SEMARNAT. The species cultivated in the ISARCW were selected according to their availability in zones close to the site to ensure their proper adaptation to this specific environment. They consist of the following aquatic plants: common cattail, *Family: Typhaceae*. *Scientific name: Typha angustifolia* and umbrella papyrus, *Family: Cyperaceae*, *Scientific name: Cyperus alternifolius.* Figure 3 shows the constructed wetland, with the cultivated plants *Typhas* and *Cyperaceae*, part of the integrated system of anaerobic reactor and constructed wetland.

Registrations of Parameters BOD_5, COD, DO, Temperature, pH of the Experimental ISARCW during Operation

Once the system was operating at steady state, (8 months after the start) weekly samples were taken during eight consecutive weeks, at the three following places: feed tank of synthetic wastewater plus drugs, biodigester outlet and constructed wetland outlet. Wastewater sampling at each one of the points was performed according to conditions appropriate to ensure sample preservation pursuant to MEXICAN STANDARD NMX-AA-003-1980. The wastewater samples were transferred in rows at 4°C to the CONAGUA laboratory in Tuxtla Gutiérrez, Chiapas where BOD_5, COD, DO, and pH were determined.

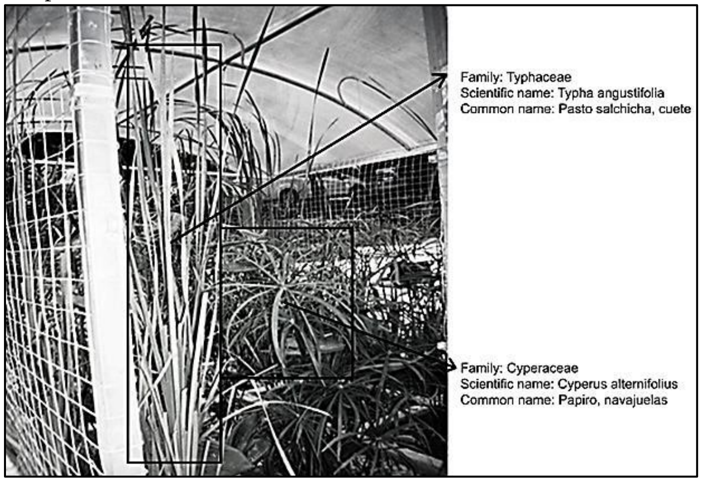

Figure 3: Constructed wetland of the SWW treatment system

Operation and Analysis of Drug Residues in the SWW Treated in the ISARCW: Diclofenac and Paracetamol

After a three-month steady state operating period, sampling was performed to quantify drug concentrations in the system. Samples were taken at the three above mentioned points to determine diclofenac and paracetamol contents. The purification of the drugs present in the synthetic wastewater was performed through C18 solid phase cartridges according to the EPA 1694 method (quoted by EPA). Diclofenac and paracetamol were quantified through the capillary zone electrophoresis technique using Beckham model MDQ equipment, with a 10 cm long fused-silica column having an internal diameter of 75 µm. The supporting electrolyte was a 30 mM phosphate solution, pH 8. The sample was injected during 5 seconds at 0.5 psi, with a detection limit of 40 ng/L. Equation 1 was used to determine the drug degradation constant. Calculations for the design and construction of the integrated system of anaerobic reactor and constructed wetland.

Equation 1

$$\frac{\text{Ln Ci} - \text{Ln Ce}}{T} = K_t$$

ISARCW Maintenance

The routine operation and maintenance of the constructed wetland included: control of water supply and water depth, cleaning of the inlet and outlet structures and revision of their integrity, handling of the constructed wetland vegetation, and routing monitoring. Since the species *Tipha* and *Cyperus* grew at an approximate rate of 4-5 cm/month, pruning was performed every 3-4 months [7,16,17]. Water levels were always kept below the constructed wetland substrate. In the System, SWW was sampled at the inlet and oulet of each unit (biodigester and constructed wetland) in order to analyze the biological parameters and drug concentrations.

RESULTS AND DISCUSSION

The operation temperatures observed in the integrated system of anaerobic reactor and constructed wetland were as follows: the average environment temperature (exposed to the sun) was 35.7°C ± 5.65, the average plant root temperature was 27.7°C ± 4.36 while the average gravel temperature was 34.5°C ± 6.20; the average temperature of the biodigester was 28.23°C ± 2.86 and the average temperature of the constructed wetland was 27.77°C ± 4.36 (Figure 4). As regards porosity calculations, the results were: displaced water = 10.40 L; porosity% = (20L – 10.40 L/ 20 L) × 100 = 48%.

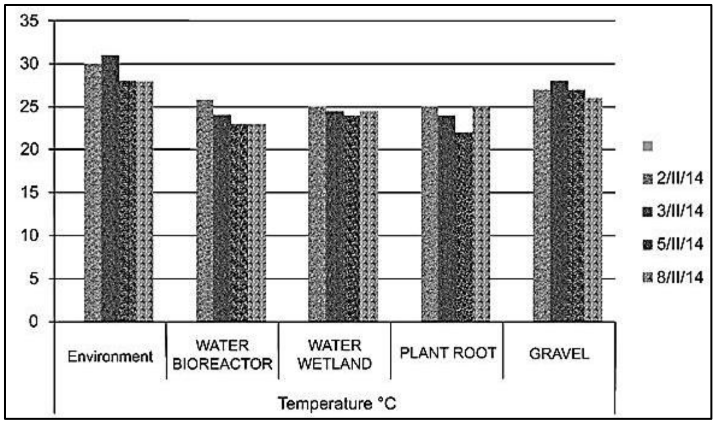

Figure 4: Comparative graph of the temperatures in the system: environment, biodigester water, wetland water, plant root, gravel

ISARCW Operation and Maintenance

The biodigester operated with an efficiency of 98% as regards drug elimination. Paracetamol was totally degraded and was not detected anymore. According to Russo 5% of the total quantity consumed is converted in active metabolite by the cytochrome P-450 oxidation system found in hepatic cells, leading to N-acetyl-para-benzoquinoneimine (NAPBQ). According to Russo, at normal paracetamol doses, the small quantity of active metabolite produced is detoxified through conjugation, preferably with reduced glutathione and excreted in urine as non-toxic conjugates of cysteine and mercapturic acid, residues that were eliminated by the biodigester. Table 2 shows the absence of drug products at the constructed wetland outlet. Probably some diclofenac metabolites were left, according to Table 2 shows the results obtained in the biodigester where $K_{t\ average}$ reaction speed = 4.905 days^{-1} ± 0.870.

Table 2: Concentrations: diclofenac Ci initial and Ce final concentrations in the ISARCW biodigester and calculation of the reaction speed constant, HRT = 24 h

Sample	Untreated water mg/L, Ci	Water from biodigester outlet mg/L, Ce	Ln Ci	Ln Ce	T(días)	Kt = LnCi – LnCe/T
1	2.2	0.02189938	0.78845736	-3.82129689	1	4.609754245
2	2.177262178	0.02554278	0.77806821	-3.66740043	1	4.445468635
3	2.185571976	0.00185003	0.78187757	-6.29255505	1	7.074432614
4	2.315141762	0.03335523	0.83947092	-3.40054079	1	4.240011709
5	2.26622879	0.03648969	0.81811712	-3.31072547	1	4.128842593
6	2.132798073	0.01687239	0.75743477	-4.08207672	1	4.839511488
7	2.246993384	0.01777578	0.80959305	-4.02991841	1	4.839511458
8	2.175117935	0.01565403	0.77708289	-4.15702689	1	4.934109773
9	2.297223501	0.0165328	0.83170122	-4.10240899	1	4.934110213
Average	2.221815289	0.02066357				4.893972525
Standard deviation=	0.062130806	0.01034226				0.870243111

Discussion of the Analyzed Parameters: BOD_5, COD, DO, in the ISARCW

The average BOD_5 (mg/L) results obtained in synthetic wastewater (SWW) at the biodigester inlet are 154.16 mg/L ± 17.042, while said values at the biodigester outlet are 57 mg/L ± 31.412; moreover, an important reduction is observed at the wetland outlet, at 13.01 mg/L ± 5.59, as shown in Figure 5. The results obtained as regards the chemical oxygen demand COD (mg/L) of the Synthetic Wastewater (SWW) were as follows: 131.54 mg/L ± 10.859 at the biodigester inlet, 71.56 mg/L ± 12.42 at biodigester outlet, while an important 68% reduction was observed at the constructed wetland outlet, leaving only 41.93 mg/L ± 4.582 (Figure 6). The average results obtained as regards dissolved oxygen (DO) (mg/L) of the synthetic wastewater (SWW) were as follows: 7.28 mg/L ± 1.492 at the biodigester inlet, down to 0.38 mg/L ± 0.142 at the biodigester outlet, and up a little to 3.99 mg/L ± 1.613 at the wetland outlet (Figure 7).

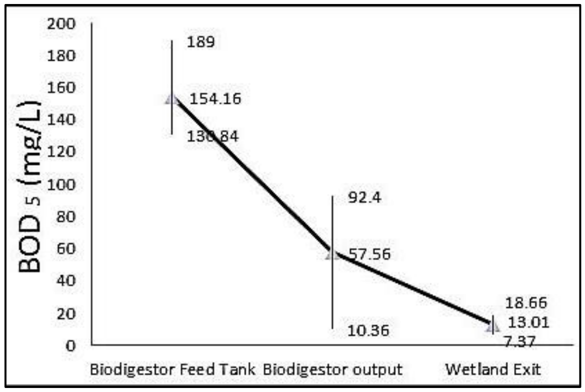

Figure 5: Results of the biochemical oxygen demand of the SWW treated in the ISARCW

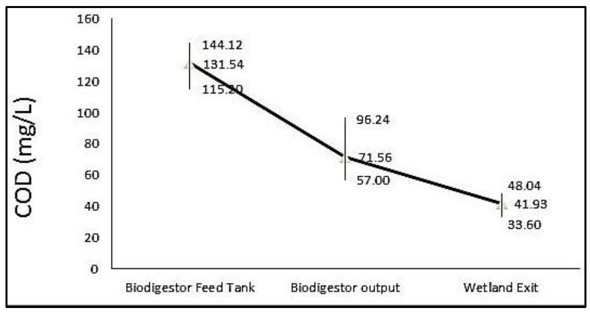

Figure 6: Graph of the chemical oxygen demand of the SWW treated in the ISARCW

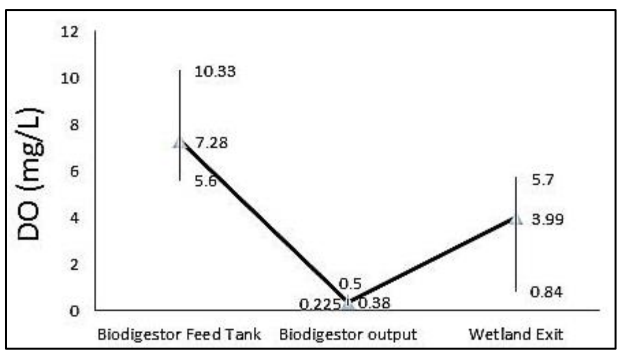

Figure 7: Results of the contents of dissolved oxygen of the SWW treated in the ISARCW

Discussion of the Results of the Analysis of Drugs Treated in the ISARCW

Figure 8 shows the results of untreated water, obtaining values that are very close to the ones initially fed to the system. At the biodigester outlet, the presence of a residue that does not correspond to diclofenac was observed, possibly some of the metabolites generated by the diclofenac molecule in its biological degradation. At the constructed wetland outlet, no presence of diclofenac was detected, but traces of residues (probably metabolites) were observed, that must be identified through other analytical methods. Figure 8 shows the results of the paracetamol and diclofenac removal from the ISARCW.

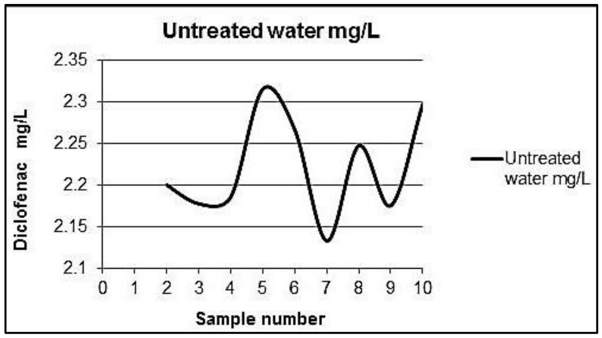

Figure 8: Graph of diclofenac concentration in the SWW fed to the biodigester of the ISARCW

Figure 9 shows the concentrations of diclofenac residues in the SWW treated by the biodigester, with a 98% removal rate.

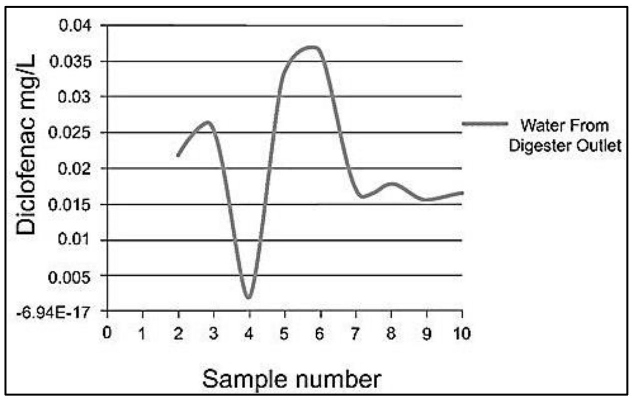

Figure 9: Diclofenac concentrations at biodigester outlet

Figure 10 shows the compared concentrations at the ISARCW; it can be seen that the concentration at the constructed wetland outlet is nil.

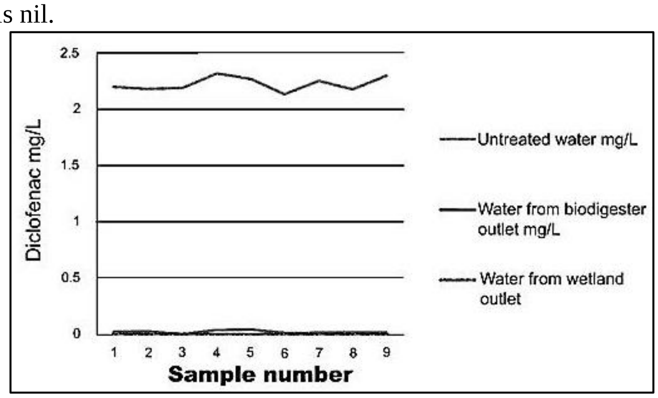

Figure 10: Diclofenac concentrations in untreated SWW, at the biodigester outlet, and at the constructed wetland outlet of the ISARCW

With the information shown in Table 1 and Equation 1, diclofenac degradation constant K_t was determined, averaging 4.905436668 days^{-1} ± 0.8702 at an average temperature of 28.2°C ± 2.86. On the other hand, as reference to the typical values of the kinetic parameters reported in works performed by other authors such as [17,18], no Kt values have been especially referred to for diclofenac or paracetamol or for any specific drug. A repetitive pattern was observed as regards diclofenac concentrations. In the electropherogram, peaks of the diclofenac sample in untreated water were observed, migrating at 9 minutes according to diclofenac standard sample. In the electropherogram of effluent water from the biodigester, the peak was observed migrating at 12 minutes, the 3-minute difference possibly corresponding to another compound. There is no quantitative recording of wetland effluent because no characteristic peak was observed (Figure 11).

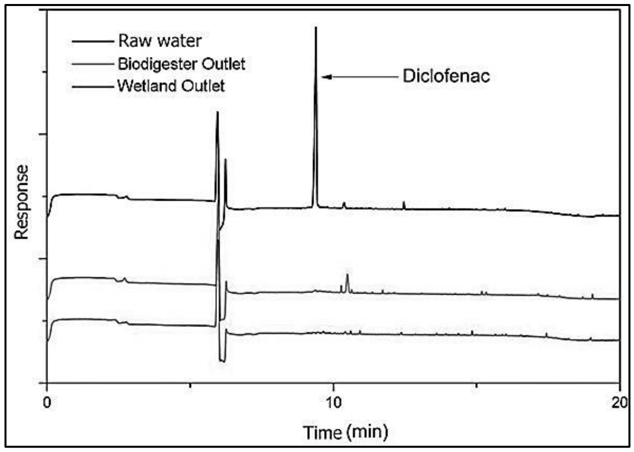

Figure 11: Electropherogram of diclofenac degradation in the ISARCW

Paracetamol may have been eliminated by the solvents during the extraction process because of its solubility in them, [19] and could not be detected. As control, an analysis was conducted without extracting the untreated water, SWW, in order to check whether it was present, and, once verified, a Spiking (standard paracetamol aliquot) was added to confirm its presence in the sample, which was corroborated (Figure 12). It is not possible to run the samples in these conditions, because the equipment would be damaged if the analyses were performed directly without the extraction requested by the technique. Moreover, paracetamol presents some difficulty because of its water solubility due to the possible instability of its polarity [20] (Figure 12).

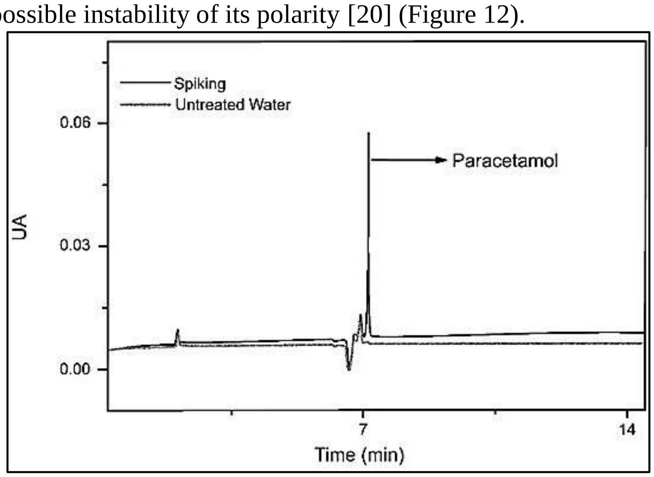

Figure 12: Electropherogram of paracetamol in untreated SWW of the ISARCW

For the above reasons regarding paracetamol, a statistical analysis was performed through the One-way anova method on the data obtained with diclofenac [21-23]. As regards the results of the diclofenac concentrations in untreated water (prior to treatment in the biodigester), an X means of 2.2 and a standard deviation, $\sigma = 6.21308E^{-02}$ were obtained. The statistical report showed calculated T (Tc) = 107.28 with p = 0.0000; in this case, a critical T was calculated (Tcr) = 2.31, according to the decision Law that if Tc > Tcr, $p<0.05$, 95% removal was recorded. The diclofenac concentration at the biodigester outlet was obtained for diclofenac having an average $X = 2.066357E^{02}$ and a standard deviation, $\sigma = 1.034226E^{02}$ [24]. In statistical analysis, a calculated T (Tc) = 5.9939 with p = 0.000326 was reported; in this case, critical T (Tcr) = 1.86.

SWW plus drugs without treatment through the ISARCW:
Graphs of the statistical analysis through the One-way anova method.

SWW plus drugs at the biodigester outlet:
Graphs of the statistical analysis through the one-way anova method (Figures 13 and 14).

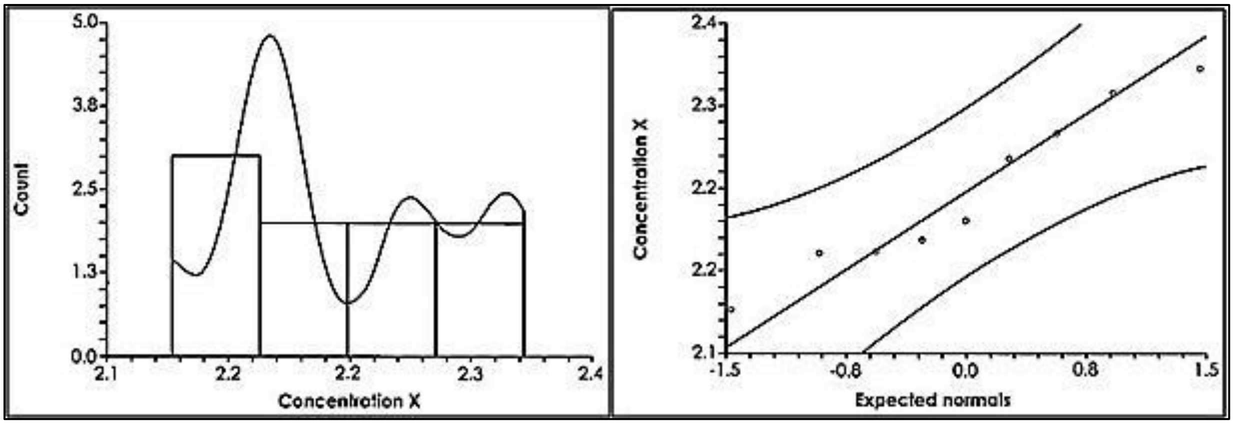

Figure 13: (A) Concentration histogram, (B) Normal distribution of diclofenac concentration in SWW plus drugs without treatment through the integrated system of anaerobic reactor and constructed wetland

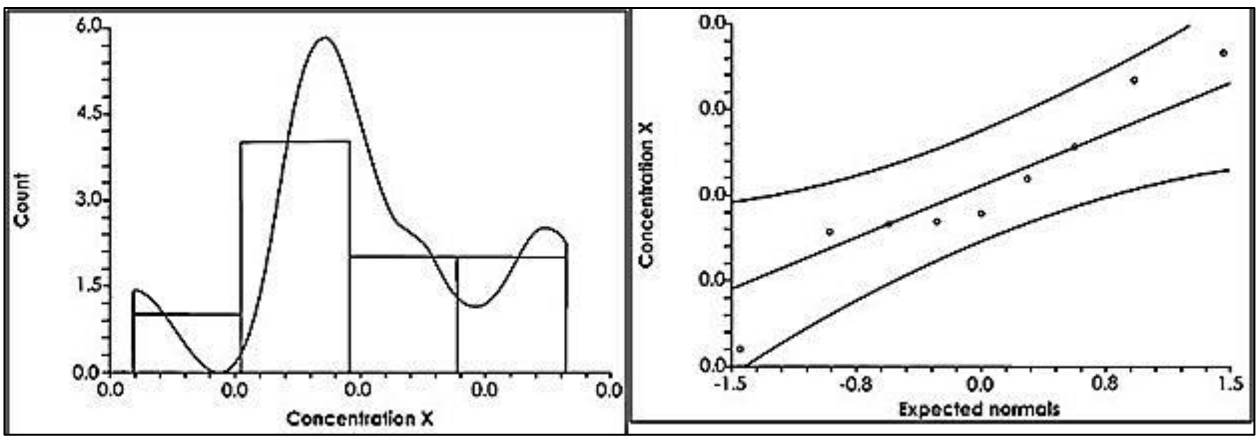

Figure 14: (A) Concentration histogram, (B) Normal distribution of diclofenac concentration in SWW plus drugs treated in the ISARCW

CONCLUSION

The integrated system of anaerobic reactor and constructed wetland adequately built and operated eliminates drugs. No presence of diclofenac or paracetamol was analytically detected from the constructed wetland effluent. A biodegradation constant in the biodigester of $K_{t\ average}$ reaction speed = 4.905 $days^{-1}$ ± 0.870 was obtained**;** with $p<0.05$. The typical values of the anaerobic process constant k_{tdel}, in wastewater were: for acetates [25,26], from 3.6 to 4.8 mg/mg d; for propionates, 9.8 mg/mg d; for palmitates, 3.85 mg/mg d, resulting from an anaerobic biodegradation. With the wetland expressly built for this study, 98% efficiency was obtained as regards anti-inflammatory and analgesic drugs from wastewaters having passed through the biodigester. Plants of the species *Typha spp*. and *Cyperus spp.* used in the wetland of this study were extremely well adapted to the environment, and generated excellent results. Thus, this depuration technique for all types of contaminants is valid, economical, requires low maintenance and no energy, is effective and should be taken into account as an alternative or complement to conventional WWTPs.

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