REMOVAL OF ORGANIC MATTER AND TOXICITY IN AN UPFLOW IMMOBILIZED BIOMASS ANAEROBIC REACTOR TREATING HOSPITAL WASTEWATER: PRELIMINARY EVALUATION

REMOCIÓN DE MATERIA ORGÁNICA Y TOXICIDAD EN UN REACTOR ANAEROBIO DE BIOMASA INMOVILIZADA Y FLUJO ASCENDENTE TRATANDO AGUA RESIDUAL HOSPITALARIA: EVALUACION PRELIMINAR

MÓNICA PORRAS TORRES

Ing. Civil, Universidad Militar Nueva Granada, Bogotá Colombia, monicaporras.t@gmail.com

JOSÉ CARDOZO LÓPEZ

Ing. Civil, Universidad Militar Nueva Granada, Bogotá Colombia, jose.cardozolpz@gmail.com

TATIANA R. CHAPARRO

Dra., Universidad Militar Nueva Granada, Bogotá Colombia, adela.rodriguez@unimilitar.edu.co

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ABSTRACT: The aim of this research was to evaluate the performance of an Upflow Immobilized Biomass Anaerobic Reactor (UIBAR) treating real hospital wastewater. The organic matter removal and the toxicity were assessed through COD, UV_{254nm} and the measure of the growth inhibition of onion roots (*Allium cepa L)*. The results show that the biological process was stable during the 287 days of continuous operation; the mean value of the IA/PA relation was 1.21±0.08, indicating that there was no acid accumulation in the system. However, the efficiency of reduction of COD, 56±15% and UV_{254nm}, 21±36%, is not quite representative. The toxicity was reduced in by 50%. Therefore, it is necessary to continue with the removal of organic matter to combine the anaerobic process with other processes such as an advanced oxidation process. The capacity of the anaerobic immobilized process to remove the compounds that produce toxicity was verified.

KEYWORDS: *Allium cepa L*, anaerobic digestion, hospital effluents, recalcitrance, toxicity.

RESUMEN: El objetivo de esta investigación consistió en evaluar el desempeño de un reactor anaerobio de flujo ascendente de biomasa inmovilizada (RAFABI) tratando un efluente hospitalario real. Se estudió la remoción de materia orgánica y toxicidad, por medio de análisis como UV_{254}, $DQO_{filtrada}$ y determinación del porcentaje de inhibición en el crecimiento de la raíz de la cebolla. Los resultados mostraron que el proceso biológico estuvo estable durante los 287 días de operación continua, el valor medio de la relación AI/AP fue de 1.21±0.08, indicando que no hubo acumulación de ácidos en el sistema. Sin embargo, los valores de la eficiencia de remoción de $DQO_{filtrada}$, 56±15% y $UV_{254,}$ 21±36%, no fueron representativos. La toxicidad se redujo en 50%. Con base en lo anterior, es necesario utilizar el reactor anaerobio en combinación con otros procesos como por ejemplo los procesos de oxidación avanzada, para continuar reduciendo la materia orgánica recalcitrante al proceso anaerobio. Se comprobó la capacidad que tienen los reactores anaerobios de biomasa inmovilizada para remover la toxicidad.

PALABRAS CLAVE: *Allium cepa D*igestión anaerobia efluentes hospitalarios, recalcitrancia, toxicidad.

1. INTRODUCTION

Hospitals require a significant quantity of water per day for different purposes and services, depending on the activities which take place there as indicated by Verlicchi et al. [1]. These wastewaters generally discharges in the urban sewage without treatment, and contain a high variety of toxic and persistent organic compounds, such as antibiotics, radionuclides, solvents and disinfectants used for medical purposes in a wide range of concentration due to: activities in laboratories, research, and excretion of patients. In most cases, these compounds correspond to the group called "emerging compounds". The control and management of hospital wastewater is considered to be of high importance, not only for public health, but also for the environment. According to Kummerer [2], several authors indicate that conventional wastewater treatment does not have the capacity to treat this type of compounds.

These substances correspond to unregulated pollutants, which may be candidates for future regulation depending on research on their potential health effects and their occurrence in the environment. The uncontrolled discharges of hospital effluents that treat patients with cholera have been widely associated with epidemics of cholera in some Latin American countries [1].

The anaerobic process of immobilized biomass is an attractive technology to treat hospital wastewaters, due not only to its recognized ability to tolerate toxic substances and high organic loads, but also because it requires a small area for operation, which would be advantageous for hospitals and health care centers. At present, the treatment of water that contains pharmaceutical compounds has been studied using anaerobic treatment in an investigation by Chelliapan et al. [3] and Carballa et al. [4. However, these studies show results only with specific compounds with pure cultures. Not much work has been carried out to investigate the effects of pharmaceutical compounds and real hospital wastewater in anaerobic environments.

Hospital wastewater urgently merits to be denominated a critical discharge which requires treatment before disposal. Based on this our objective was to evaluate the performance of an upflow immobilized biomass anaerobic reactor treating real hospital wastewater.

2. MATERIAL AND METHODS

2.1. Anaerobic reactor

The upflow immobilized biomass anaerobic reactor (UIBAR) was selected for this study as used by Fernandes [5]. The UIBAR bioreactor was comprised of a 700 mm long (L), 80 mm diameter (D) acrylic cylinder with a total volume of approximately 2262 mL. The bed porosity (ε) was determined to be 0.53, thus resulting in the net volume of 1200 mL. Polyurethane foam cylinders (23 mm, D – 58 mm, L) with an apparent density of 0.018 g/cm^3 were used as biomass immobilization support. The reactor was inoculated with granulated sludge from an Internal Circulation (IC) reactor used to treat effluent from a Brewing Industry (Bavaria S.A – Tocancipa, Colombia), this sludge presents important syntrophic relationships between the microorganisms, which are beneficial for the treatment of complex wastewaters. The UIBAR reactor was kept in a temperature controlled chamber, set at 30±5°C with a hydraulic retention time of 12±0.6 h for 287 days. The sludge was immobilized in the foam according to Hernandez [6]. The schematic diagram of bench-scale UIBAR reactor is shown in Figure 1.

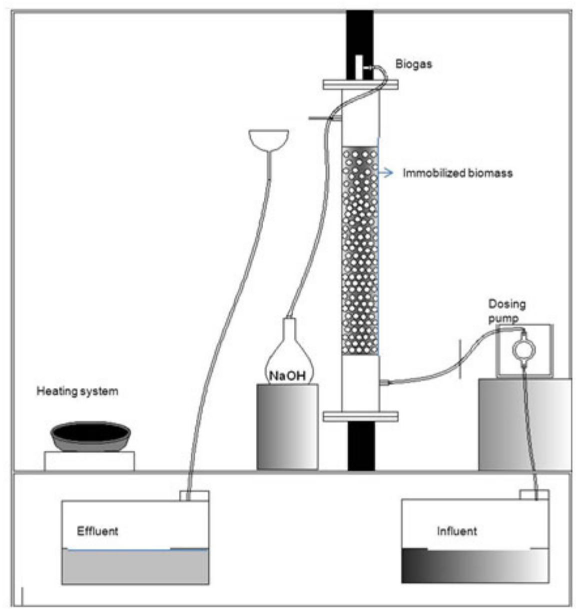

Figure 1. Schematic diagram of Upflow Immobilized Biomass Anaerobic Reactor. (***UIBAR).***

2.2. Wastewater

The hospital wastewater (HW) was obtained from the Military Hospital (MH) located in Bogotá (Colombia). The MH has a total of 110 beds. Wastewaters are released into the main sewer of the hospital where it joins the city`s sewerage system. Thus, the samples from this hospital were collected from the main sewer. The samples were taken four times during 2011 and fully characterized by chemical oxidation demand, total solids, total alkalinity, UV_{254}, faecal coliforms according to APHA. [7].

The hospital wastewater was diluted with synthetic wastewater composed of saccharose as the main carbon source with a chemical oxygen demand (COD) of 500 mg/L), prepared according to Souza et al.[8] (Table 1).

Table 1. Synthetic Wastewater.

Compound	Concentration (mg/L)
Meat extract	425
Starch ($C_6H_{10}O_5$)	111
Saccharose ($C_{12}H_{22}O_{11}$)	36
Sodium chloride ($NaCl^-$)	250
Magnesium chloride. ($MgCl_2$ $6H_2O$)	7
Calcium chloride ($CaCl_2$ $2H_2O$)	5
Potassium phosphate (KH_2PO_4)	6
Sodium sulfate (Na_2SO_4)	148
Sodium bicarbonate ($NaHCO_3$)	200

2.3. Reactor operation

Operation of the reactor began with the synthetic wastewater described in Table 1 from day 1 to day 16, after which the hospital wastewater was introduced gradually. The changes in organic load rate (OLR) were made according to the reactor performance in response to the COD initial concentration. The pH of influent was close to 7.2 and was supplied amounts of nitrogen and phosphorous to maintain the COD/N/P relation as recommended by Speece [9]. The procedure was described in Table 2.

2.4. Analytical methods

The COD was measured using the closed reflux colorimetric method described in methods 5220D [7]. The pH was monitored using the potentiometric method. The volatile acids and total alkalinity were determined according to the method described by Ripley et al. [10]. UV_{254} was measured as described in method 5910 [7]. All analyses were performed in duplicate and are reported as average values. The sample was filtered through 0.45 mm glass fiber membrane and stored at 4°C prior to analysis.

2.5. Bioassays

The capability of the UIBAR to reduce the toxicity of the Military Hospital wastewater was evaluated using the common onion (*Allium cepa L –Red creole variety*), this test was conducted when the UIBAR was operating with 100% HW. Ten small onion bulbs were exposed to raw hospital wastewater, UIBAR effluent, negative control (ultrapure water) and positive control (5 mg/L – $CuSO^{-2}{}_4$) for 72 h. The degree of toxicity was estimated by measuring the length of each of the root bundles for the treatments. A mean value of the growth was calculated for the test report according to Fiskesjo [11]. It is important to note that this method is easy, economical and a fast tool for measuring the total toxicity in polluted waters.

Table 2. Operation conditions of the UIBAR reactor .

Phases	Days	Relation (SW+HW)	OLR (kg COD/ m^3.day)
1	1-16	100% SW	4.84
2	17-82	70%SW+30%HW	4.14
3	83-97	50%SW+50%HW	7.08
4	98-200	30%SW+70%HW	0.84
5	201-287	100% HW	0.82

SW: synthetic wastewater
HW: Hospital Wastewater (Military Hospital)
OLR: Organic load rate.

3. RESULTS AND DISCUSSION

Hospitals consume an important volume of water per day. According to Rezaee et al. [12] the average requirement of water was estimated at 1000 L/hab. day, compared to the consumption of domestic water of on average 100 L/hab.day. This consumption gives significant volumes of wastewater load with microorganism, antibiotics, hormones and heavy metals. As a result one of the major characteristics of hospital wastewater is its wide range of concentrations of these substances. We can see this in Table 3. The Military hospital wastewater not only presents notable differences in its concentrations, but also the COD/ BOD_5 relation indicates that this type of effluent is inherently of recalcitrance nature.

Table 3. Hospital Wastewater Characterization

Parameter	Unit	Mean ±S.D
Total Alkalinity	mg $CaCO_3$/L	163±52
pH		7±0,5
Chlorides	mg Cl^-/L	153±40
Total Solids (TS)	mg/L	412±143
Total Suspended Solids (TSS)	mg/L	12±34
Volatile Solids (VS)	mg/L	102±64
Chemical Oxidation Demand (COD)	mg/L	310±123
Bichemical Oxidation Demand$(BOD)_5$	mg/L	46±37
COD/BOD_5	-	6,74±3,32
UV_{254}	cm^{-1}	0,7 ±0,4
COLOR (VIS_{436})	cm^{-1}	0,14 ±0,11
SO_4^{-2}	mg SO_4^{-2}/L	19 ±3
Total Kjeldahl Nitrogen	mg TKN/L	7 ±2
COD/SO_4^{-2}		16 ±4
Faecal Coliform	CFU/100mL	$3x10^6$±$2x10^6$

3.1. Effect on COD and UV_{254}

In anaerobic digestion, a delicate balance exists between the primary process, the hydrolysis and acidogenic step and the conversion of the acid products by acetogenic and *Archaea* methanogenic into methane and carbon dioxide as mentioned by Leitão et al. [13]. Strong variations in flow and toxic concentrations may adversely affect the efficiency of an anaerobic reactor.

As shown in Figure 2 during the phase 5, a decrease of removal efficiency of COD and the organic compounds measured as UV_{254} was observed. In phase 4 (ORL: 0.84 kg COD/m^3.day) the COD removal efficiency was 58% and started to drop to 29%. These results suggest the presence of possible inhibitors of the anaerobic process, since the efficiency was reduced by almost 50% when the reactor began to operate with a 100% hospital wastewater. Chelliapan et al. [3] studied the influence of the variation of organic load on the performance and the structure of the microbial community of an anaerobic reactor treating pharmaceutical wastewater. These authors found that an increase of organic load from 1.86 to 2.48 kg COD/m^3.day cause a decrease from 75% to 45% in the COD removal efficiency. One explanation for this response was that the drop in the removal efficiency was a consequence of the presence of toxic compounds, mainly hormones.

As can be seen during the period between the 211th and 235th day, the reactor suffers a thermal shock resulting from malfunctioning of the thermostat, causing the chamber's constant temperature of 30ºC to rise to 70-80ºC, where it remained for about 8h and then dropped to 15-20ºC. After this, the reactor becomes acidified, probably as a result of the high temperature that inhibited the methanogenic community at the same time that the acidogenic microorganisms continued to produce volatile acids. To recover the system, a solution of sodium bicarbonate (1000 mg/L), ethanol (0.025% v/v), sodium phosphate (3.5 mg/L) and urea (39.3 mg/L) was added to promote an increase in pH and, thus, to provide the conditions needed to recover the stability of the anaerobic process. According to [14] the increase in temperature may affect the solubility of the substrates and, hence the availability of nutrients in medium. As shown in Figure 2, after the thermal shock, the reactor remained unstable with relation to COD and UV_{254} reduction. UV_{254} indicates the aromaticity of the organic carbon present in the water; in this case, it could be an indirect indicator of the presence of recalcitrant compounds with double bonds in hospital wastewater. The absorbance in UV_{254} in phase 3 was 0.48±0.31 cm^{-1} and 0.36±0.30 cm^{-1} for influent and effluent, respectively, resulting in low values of removal efficiency (on average, 21%). Moreover, during this phase an increase of the absorbance in the anaerobic effluent was observed. We can infer from these results that the anaerobic process did not have a great ability to transform these compounds, but, to verify this hypothesis it is necessary to do additional studies that include the behavior inside the reactor. It may be, for example, that these compounds are not degraded, but instead, could be attached to the immobilization support.

Although, the analysis of UV_{254} for assessing the performance of an anaerobic reactor treating hospital wastewaters is a recent implementation, the results of this study suggest that this measurement provides additional information to compare with the conventional global parameters (i.e. COD, BOD). It is important to say that the measure of UV_{254} is a technique fully free of the harmful residues.

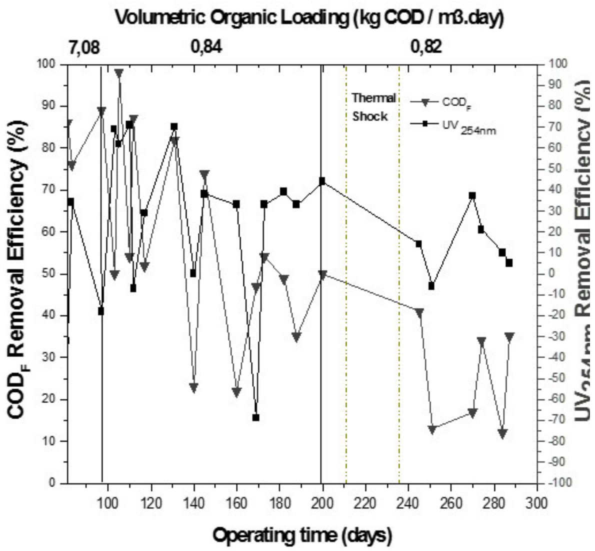

Figure 2. COD_F and UV_{254nm} removal efficiency (%)

3.3. Effect on pH, alcalinity and total volatile acids.

It is well known that methonogenic activity is more likely to proceed optimally in a pH value range between 6.3 and 7.9. The effect of drastic pH change in the effluent depends on the available alkalinity in the reactor. The pH during this study was 7.9±0.6 and 8.2±0.3 for influent and effluent, respectively. This result indicates that the system had a buffer capacity to maintain the reactor stable. According to Leitão et al. [13] it is possible to treat wastewater with high substrate and toxic compounds if enough buffer capacity is present. As indicated by Ripley et al. [10] the success of an anaerobic process depends on both maintenance of adequate bicarbonate buffering and avoidance of excessive volatile acid concentrations. These authors propose the monitoring of the following relations: Intermediary Alkalinity (IA)/Partial Alkalinity (PA) and Total Volatile Acids (TVA)/Total Alkalinity (TA), in order to evaluate the stability of the biological process. As can be seen in Figure 3, the mean value of IA/PA relation during the entire period was 1.21±0.08. According to Ripley et al. [10] values of this relation between 0.1 and 0.35 are considered typical of well-operated digesters. Based on this, one could conclude that the UIBAR reactor was unstable. Whilst, the TVA/TA relation was 0.036 and this value when compared with that suggested in the literature (TVA/TA < 0.1) for a well-operated and stable anaerobic digester, indicates that the UIBAR reactor was stable. Authors like Foresti [15] mentioned that these relations vary for each wastewater, because it depends on the initial wastewater quality. It is worth mentioning that the concentration of volatile acids before and after the thermal shock (see previous discussion) in the effluent was lower than the influent. According to the above we can say that the UIBAR reactor treating hospital wastewater was stable despite that the IA/PA was not close to the values suggested in the literature.

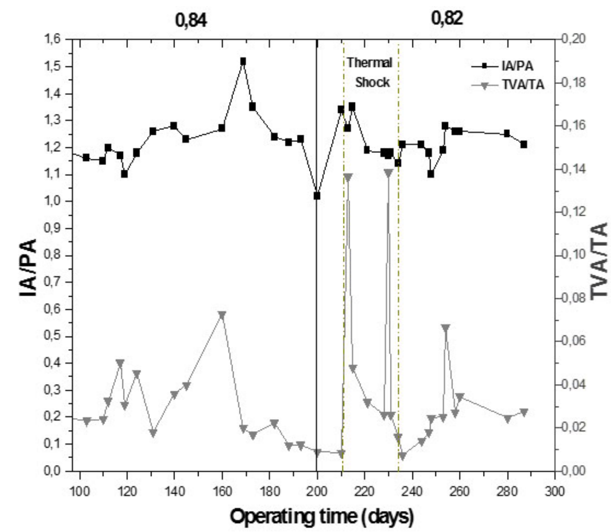

Figure 3. Variation of IA/PA and TVA/TA relations.

As indicated by Leitão et al.[13] the typical response to an anaerobic process disturbance is an incomplete methanogenesis, resulting in a certain accumulation of TVA, a drop in pH value and alkalinity. However, none of these responses where observed in our study. Hence, the results achieved in this work can be considered satisfactory and valuable. The knowledge of the performance of anaerobic reactors treating hospital wastewaters is quite limited.

3.4. Bioassays with Allium cepa L.

Higher plants are recognized as excellent bioindicators that detect environmental toxins and are frequently used in monitoring studies. The *Allium cepa* is characterized as being a low cost test, easy to handle and has advantages over other short term tests that requires previous preparations of samples. However, its application as an environmental tool to evaluate both the water quality and the performance of the treatments has not been well studied.

We observed that the UIBAR reactor treating 100% hospital wastewater removed 50% of total toxicity measured with *Allium cepa L.* This preliminary result confirms the capability of an anaerobic immobilized biomass reactor to tolerate toxic compounds. Similar results were reported by Chaparro et al. [16] treating pulp and paper effluents in a HAIB (Horizontal Anaerobic Immobilized Biomass Reactor). Authors like Gupta et al. [17] evaluated the genotoxicity of hospital wastewaters using the Ames test (*Salmonella typhimurium*). These authors applied different physicochemical processes (*e.g.* filtration, aeration and chlorination). They found that the HW was mutagenic and after the treatments this mutagenicity was notably reduced. From the results presented before, it was decided to start new studies to evaluate the genotoxicity using *Allium cepa L.* according to Fiskesjo [18], and Grisales et al. [19].

To date there is not much work on the biological treatment of HW and toxicity. In this sense, it is important to say that for this type of effluent it is necessary to do both the study of organic matter removal and the assessment of toxicity.

4. CONCLUSIONS

The performance and toxicity of an upflow anaerobic immobilized biomass reactor treating hospital wastewater was studied. The anaerobic process was stable, but the removal efficiency of organic matter and organic compounds measured by UV_{254} was quite low. The *Allium cepa* test appears to be a fast, inexpensive and easy to handle method to analyze complex mixtures. A 50% reduction in the toxicity of the hospital wastewater was observed. However, biological treatment alone is not sufficient to reduce the organic matter, for this reason it is necessary to integrate this process with others, such as an advanced oxidation process.

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