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Development of a process configuration using a new design anaerobic reactor at low rates of dissolved oxygen, for biodegradation of phenol in an industrial effluent

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Abstract

The wastewater from the chemical, pharmaceutical, paper, etc., present typical phenol concentrations between 35 and 400 mg/L, however, there are extreme cases as some Mexican refineries, which produce on average 14 m^3/d of wastewater with phenol concentrations of about 30,000 mg/L. Their presence in industrial effluents has adverse effects on health and aquatic life in the short and long term, when they receive no prior treatment are discharged into natural bodies of water (rivers, lakes and seas). Among the methods commonly studied for disposal and/or recovery are; the use of hot gas, steam distillation, adsorption, ion exchange, solvent extraction, oxidation, phase transfer catalysis, photo-decomposition, volatilization, biological methods, polymerization, electrocoagulation, advanced oxidation and ion exchange. Its high concentration in industrial effluents makes impractical the use of biological processes for treatment because there is inhibition of microorganisms. In this research, the biodegradation of phenol of an industrial effluent was evaluated in a new configuration process using an anaerobic reactor design at low rate of dissolved oxygen and hydraulic retention time (HRT). 2 tested organic loads: 3.2 and 13.9 kg COD/m^3·d, HRT of 0.5 days, dissolved oxygen rate of 0.78±0.18 mg/L and 30±0.5ºC controlled temperature. The results showed low load, removal efficiency of phenol and COD of the order of 74 and 64%, while at higher load, both the phenol removal efficiency as COD, decreased by 54 and 60% respectively. Based on the results obtained in this investigation, it is demonstrated that the new reactor configuration employed, it was possible to phenol biodegrade industrial effluent.

UASB, dissolved oxygen rate, biodegradation, phenol, Industrial effluent

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Introduction

The presence of phenols in the environment is a result of both natural and anthropogenic actions contribution mainly agricultural and industrial character. The production processes of industries; pharmaceutical, perfumery, explosives, phenolic resins, plastics, textiles, oil, dyes, leather, paper, coking plants, distilleries tar and pesticides, among others, expelled about 26.3% of phenols air, 73.3% in their wastewater discharges (ATSDR 2016), and about 0.4% in soil and aquatic sediments (Mohan et al, 2004). Discharges of wastewater from the chemical, pharmaceutical, paper, foundry, etc., provide concentrations between 35 and 400 mg/L of phenol (Chen et al, 1997). Those from petrochemical, reach values of the order of 30,000 to 50,000 mg/L (Olguín et al, 2003). In chemical structure, the phenol has a benzene ring, and a hydroxyl ($^{-}$OH) group instead of one of the hydrogen atoms own benzene (C_6H_6).

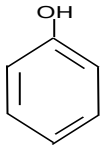

Figure 1 Chemical structure of phenol

The Environmental Protection Agency under section 313 of Title III (EPA, 2008) EPA, for its acronym in English, classified as highly toxic substance and determined as limit, less than 1 part per billion (ppb) in surface water, unchlorinated water 0.1 mg/L (100 ppb), and in chlorinated water in order from 0.001 to 0.002 mg/L (1-2 ppb).

Acute exposure to phenol causes adverse effects; skin irritation, headache, bitter mouth, diarrhea, vision problems, dark urine excretion, because it is easily absorbed by the skin and mucosa.

It affects the nervous system, causes severe damage; kidneys, liver, lungs, heart, and has a lethal effect in blood of 150 mg/100 mL (Wang et al, 2011), also inhibits DNA synthesis and replication in cells. A study revealed that phenol stopped DNA synthesis in human diploid fibroblasts (Michalowicz and Duda, 2007). Toxic levels usually range at concentrations of 1024 mg/L for humans, from 9-25 mg/L for fish (Ahmed et al, 2012), affects both flora and fauna of the environment (Lika and Papadakis, 2009). Therefore, industrial effluents should be pretreated for the download at any receiving body, present a final concentration of less phenol than 0.5 mg/L and thus be safe to the environment (Veereshet al, 2005). Moreover, their presence in natural waters can lead to the formation of substituted compounds during the disinfection and oxidation processes (Rappoport, 2003).

Many technologies have been studied for the treatment of industrial effluents with phenol, but only a few have proven to be really efficient. These technologies are classified into three groups; 1) Recovery technologies that addresses all processes or unit operations that attempt to separate and/or recover the phenol wastewater for reuse. Within these conventional recovery technologies are; Distillation, extraction, absorption, adsorption (activated carbon, zeolites, clays, bio-adsorbents), 2) Technology degradation (oxidation or mineralization), which contemplates; biological processes through a biochemical mechanism, the microorganisms used to phenol as a carbon source to transform it into less hazardous products. But, due to its high toxicity (200 mg/L), it is not possible to reduce the total organic carbon (TOC) of the wastewater to harmless levels for the environment and/or their environment (Abu-Hamed et al, 2004).

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Processes or Advanced Oxidation Technologies (AOP or AOT) based on physicochemical processes capable of producing changes in the chemical structure of the contaminants. Involve the generation and use of oxidants transient species mainly hydroxyl radical ($^{-}$OH). Which can be generated by photochemical means (sunlight) or other sources of energy, and possesses highly effective for the oxidation of organic matter. Some AOP resort to chemical inducers (electron donors) which allow transfers toxic contaminants little susceptible to oxidation, such as metal ions or halogen compounds. Within the AOP, they are not photochemical technologies (chlorination, ozonation, ozone/H_2O_2, Fenton's reagent, Electrochemical oxidation, oxidation in water), and photochemical Technologies (photolysis of water in the vacuum ultraviolet (UV), UV/H_2O_2, UV/Ozone, photo-Fenton, heterogeneous catalysis-Photo) that do not involve the use of light for pollutant degradation. And 3) new technologies or hybrid processes that include; the per-evaporation, solvent extraction using membranes and membrane systems for the recovery of aromatic (Ten et al, 2000). The study of biological processes for biodegradation of phenol in waste water has been very disappointing, at concentrations of about 10 mg/L, causes inhibition of microorganisms, and therefore low efficiency of removal. Because its toxicity, is the factor preventing or delaying the metabolic reactions, which depends on the type of microorganisms and toxic concentration of this specific compound. Why, these processes are limited by the concentration of phenol present in industrial effluents as required dilutions of the contaminant, using large volumes of water to degrade and avoid inhibiting the growth of microorganisms (Luo et al, 2009). The use of mixed cultures, allows to take place faster degradation of phenol with a pure culture (Gerrard et al, 2006).

However, there is evidence showing that degradation of phenolic compounds can be carried out by prokaryotic and eukaryotic organisms. Under aerobic respiration, oxygen used as the electron acceptor and carbon molecules to produce CO_2, H_2O and energy as ATP. Biodegradation studies phenolics this way, they have shown that there is a common metabolic pathway for this type of compound and even for those not so close family of phenolic compounds, such as biphenyls (Autenrieth et al, 1991). During aerobic respiration, oxygen is consumed, and by action of the enzyme phenol monooxygenase, $^{-}$OH the resulting phenolic ring group is added, the formation of catechol be broken in two ways, and therefore two possible routes biodegradation, the *ortho* and *meta* route path (Shalaby, 2003). In the *ortho*-break, 1-2 catechol bond is broken to produce the muconic acid. While ring opening by the target route occurs between carbons 2-3, to result in the formation of 2-semialdehyde hidroximuconico and is very common in the metabolism of bacteria, fungi and microalgae of different types (Heesche-Wagner et al, 1999). These conditions (aerobic way), are distributed in various bacteria, yeasts and fungi (Harwood and Parales, 1996). Despite its wide taxonomic distribution, it has only been identified him soil microorganisms, particularly in groups of bacteria associated with plants and specifically, taking place in bacteria that have the goal via encoded in plasmids (Ramirez, 2005).

Under anoxic conditions respiration (anaerobic digestion), different electron acceptors are used as: nitrate, sulfate, metal ions or carbon dioxide in order to produce compounds reduced nitrogen, sulfur, methane and carbon dioxide (Lovley and Lonergan, 1990).

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In this way, the biodegradation of phenol is carried out, when the rate of oxygen consumption by the microorganisms exceeds its diffusion rate in the medium or when it is zero. The fermentation and anaerobic respiration are the two basic mechanisms of anaerobic catabolism of organic compounds. In this context, the metabolism of aromatic rings can be carried out in anaerobiosis allowing the break ring in the absence of oxygen. Although the benzene ring is a very stable chemical structure, can be reduced by hydrogenation and hydroxylated complete dehydration for subsequently broken by action of a non-oxidative enzymatic process (Borraccino, 1997). Catechol, muconate *cis-cis*, *β*-ketoadipate, succinate and acetate, are some of the intermediates in the biodegradation of phenol (Rodriguez, 2003).

Among the most studied for the degradation of phenol microorganisms are; Bacteria of the genera *Bacillus spp*., *Micrococcus spp*. and *Pseudomonas spp*. that tolerate concentrations of 10 to 25 g/L of phenol and yeast *Candida tropicalis* using the phenol under aerobic conditions as sole source of carbon and energy with a potential degradation of this compound up to 1700 mg/L (Fialova, 2004;. Yang, 2005). Other microorganisms studied are; *Phanerochaete Chrysosporium*, *Bacillus pumilus* and *Asomopergillus terreus* (Gallego, 2003). For the case of *Pseudomona putida* has a phenol degradation capacity of 500 to 600 mg/L in 48 hours of incubation. The use of isolated bacteria can lead to a considerable decrease of treatment time and improve the rate of elimination of phenol (Tziotzios, 2005). Biodegradation involves many factors (Wheat, 2009). Among them; temperature, pH, oxygen content and concentration of substrate (Nair, 2008; Agarry, 2008).

Extreme pH values of wastewater (less than 3 or greater than 9) may be inhibitory to the growth of microorganisms. In sequential batch reactors (SBR) mixed cultures of activated sludge, it has been reported that concentrations greater than 1200 mg/L phenol, occurs a strong inhibitory effect on microorganisms. In the case of a peak concentration of 1850 mg of phenol/L, biomass is inhibited and requires a cycle over 300 h to degrade the inhibitory compound. Yoong (2000) found a similar behavior, they found that at concentrations greater than 1300 mg/L of phenol in activated sludge treatment, resulted in complete inhibition of the system. Under this scenario, phenol can be degraded both aerobic and anaerobic conditions. In general, laboratory studies on the biodegradation of phenol are carried out at pH values close to neutral (pH=7.0). Each microorganism has a specific temperature range for growth. *Bacillus stearothermophiles* is capable of efficiently degrading phenol 50ºC (Naas, 2009). Studied exposure to temperatures above 35ºC has a detrimental effect on bacterial enzymes that are responsible for the breakdown of the benzene ring. While exposure to temperatures below 30ºC, decreases the bacterial activity. Bevilaqua (2002) studied a conventional aerobic system coupled batch to an enzymatic treatment using tyrosinase as enzyme, and observed a degradation efficiency of 75% with a remainder of 420 mg/L of phenol in a reaction time of 4 hours with 46 Um/L tyrosinase and 50 mg/L of chitosan (as coagulant). Silva (2002) used a SBR type reactor, reaching a phenol biodegradation of the order of 99%. Hossein (2006) in a bioreactor packed bubble for treatment of phenolic residues, 100% found deletion at a load rate of 33120 $mg/m^2 \cdot hr$. Marrot (2006) studied the biodegradation of high concentration of phenol by activated sludge membrane bioreactor.

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For his part, Bajaj (2008) in a reactor with a cycle of 360 minutes operation (260 minutes under aerobic and 100 minutes under anoxic conditions conditions), they found a removal of 50% phenol present in synthetic waste water with an initial concentration of 5.17 g/L phenol. Donoso-Bravo (2009) in a study with phenol synthetic water at a concentration of 5 gCOD/L and content; 10, 25 and 40% phenol as a carbon source from a concentration of 400 mg/L of phenol, in ASBR reactors, biodegradation found an efficiency of about 30%. For his part, Almasi (2012) using an anaerobic lagoon dimensions; 1.2×0.6×0.55 m, HRT of 10 days, hydraulic load rate of 43.5 L/d and organic load rate of 150 kg/h·d with initial phenol concentrations in the influent: 0-28, 3080, 90-130 and 150-200 mg/L respectively under conditions of warm temperatures and: 100-140 and 200-260 mg/L under cold temperature conditions, reported a phenol removal efficiency of 71.8% at warm temperatures and 14.66% to cold temperatures.

In this research process new configuration using a design of anaerobic reactor UASB type at low rate of dissolved oxygen and hydraulic retention time (HRT), to biodegrade the phenol present in an industrial effluent, varying the volumetric organic load we were assessed (Bv) two different ratios; 2 and 10% (v/v), in order to achieve maximum removal.

Materials and methods

Sampling. Was counted with a batch of 10 L of phenolic wastewater from a resin industry. The method used for sampling is described in the standard (NMX-AA-003-1980). Analytical techniques. For the evaluation of the main parameters in the industrial effluent phenol feed (influent) of processed water (effluent) and control system studied, the following analytical techniques were used:

The pH was evaluated by a potentiometer (Conductronic PC18). Dissolved oxygen was measured using a portable YSI model. COD, total solids (TS), total suspended solids (TSS), volatile solids (VS) and volatile suspended solids (VSS) were determined according to standard method (APHA, 2005). The determination of phenol, was performed by the colorimetric method of the 4-aminoantipyrine according to the Mexican standard NMX-AA-050-SCFI-2001 using a UV-VIS Spectrometer Perkin Elmer Lambda XLS model computer. Measuring biogas, it was effected by means of an inverted column in a container with saline (pH=2). The volume of the displaced solution corresponds to the volume of biogas produced. The analysis of volatile fatty acids (VFA), was determined by gas chromatography using a gas chromatograph (Hewlett Packard Model 5890 series II) equipped with FID detector and capillary column Superox FA, AT 1000 under the following operating conditions; column temperature of 120ºC to 140ºC, with a increase of 10ºC/minute, 130ºC injector temperature, 150°C detector temperature and N_2 as carrier gas at 3 mL/min.

Experimental design. In this research, an anaerobic UASB reactor was used for its acronym in English Upflow Anaerobic Sludge Blanket) at low rate of dissolved oxygen with a volume of 1.21L design, useful volume of 1.04L, internal diameter of 4.5 cm, height 53 cm, operated at a temperature of 30±0.5°C, without pH control, agitation or recirculation.

Inoculum. The biomass used as inoculum was collected of a UASB reactor of the Autonomous Metropolitan University of Iztapalapa unit that treats wastewater from the academic unit, with a concentration of 67.9 g/L of TSS, 35.7 g/L of VSS and methanogenic activity specified (MAE) of 0.11 LCH_4/gVSS·d.

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During the starting period and stabilization, it was used RAMM mineral medium (Shelton and Tiedje, 1984), with sodium acetate as carbon source for developing the inoculum methanogenic conditions.

Feed preparation (influent). From equation $C_1V_1 = C_2V_2$ it was calculated the volume of the sample taking phenolic wastewater previously characterized phenolic wastewater, making a series of dilutions according to the proportion of 2 and 10% to test in the reactor of new configuration.

Operating conditions of the reactor. Table 1 shows the operating conditions of the reactor in the various stages in the experiment was conducted.

Stage	I	II
Proportion of Phenolic Wastewater	2 %	10%
Bv ($kgCOD/m^3 \cdot d$)	3.2	13.9
HRT (days)	0.5	0.5
Dissolved oxygen (mg/L)	0.78±0.18	0.78±0.18

Table 1 Conditions of reactor operation

Results and discussion

Characteristics phenolic wastewater. Before addressing the analysis of the results in Table 2 an estimate of the average of the main parameters (pH, turbidity, electrical conductivity, total solids, total suspended solids, volatile solids and volatile suspended solids), the chemical oxygen demand (COD) and total phenol, evaluated through calibration curves.

Parameters	Units	value
pH		6.5±0.15
Turbidity		142
Electric conductivity	/L	128
Total solids (TS)		3.25±0.02
Total suspended solids (TSS)		3.04±0.01
Volatile solids (VS)	/L	3.21±0.04
Volatile suspended solids (VSS)	/L	3.18±0.32
COD	/L	71.8±0.51
Phenol	/L	13.07±0.023

Table 2 General characteristics of industrial effluent phenol

Table 3 shows the characteristics of feeding two dilution rates (2 and 10%) from the original sample (Table 2) that the UASB reactor was fed.

Parameter / Stage	I	II
Proportion (V/V)	2%	10%
Bv ($kgCOD/m^3 \cdot d$)	3.2	13.9
HRT (d)	0.5	0.5
pH	7.04±0.1	7.0±0.13
TS (g/L)	0.12±0.07	0.11±0.11
VS (g/L)	0.03±0.01	0.04±0.03
COD (g/L)	1.43±0.05	7.3±0.13
Phenol (g/L)	0.48±0.025	1.49±0.04

Table 3 General characteristics of the influent (UASB reactor feed)

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Biological degradation of phenol in a reactor UASB new configuration

During the experiment continuously for biodegradation peak concentration of phenol and COD according to the rate corresponding dilution influent biological reactor based on the characteristics of phenolic wastewater they are presented in Table 2, from 0.48±0.025 g/L of phenol and COD of 1.43±0.05 g/L corresponding to a dilution rate of 2% over 36 days (stage I) and 1.49±0.04 g/L of phenol with COD of 7.3±0.13 g/L from day 37 until day 71 of operation, corresponding to a dilution rate of 10% (stage II), the reactor showed a stable pH during the course of the experiment (figure 2), with a in influent pH 7±0.1 and a pH in the effluent of 7.2±0.13. Triangles represent the influent pH and dark rhombs the effluent pH. It reported in the literature that extreme pH values (less than 3 or greater than 9) may be inhibitory to the growth of microorganisms involved in the biodegradation of phenol (Naas, 2009). However, given the behavior of pH in the reactor throughout the experiment, biomass reactor presented no inhibitory effect, which allowed the biodegradation process of phenol was carried out properly under the operating conditions tested in the study.

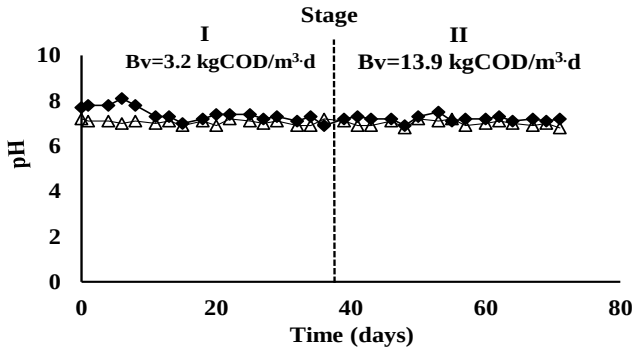

Figure 1 Performance of pH in the reactor: Influent ( ) and effluent ( )

The figure 3 shows the volatile solids (VS) in the reactor effluent (stage I and II). The triangles represent the SV in the influent and the dark rhombus, the SV in the effluent. And it is seen that during the acclimatization phase biomass reactor at a concentration of phenol 0.48±0.025 g/L (stage I) over 36 days to loss of biomass is results (0.24±0.1 g/L of VS) in effluent. Similar behavior to that reported by (Buitrón, 2005) SBR reactors with biomass losses at concentrations of phenol of 7000 mg/L and reported by Tziotzios (2005) in sequential batch reactors (SBR) at concentrations of 1200 mg/L of phenol. However, it is noted that once the biomass is acclimated to the presence of phenol as sole carbon source at a concentration of Phenol of 1.49±0.04 g/L and 13.9 Bv kgCOD/m^3·d (stage II), results in a decrease of volatile solids (VS) in the reactor effluent, 50%. In the literature mentioned that to carry out biodegradation involves many factors (Trigo, 2009). Among them; temperature, pH, oxygen content and concentration of substrate (Nair, 2008; Agarry, 2008). Each of these factors needs to be optimized for maximum degradation. In this vein, optimizing the concentration of phenol as a substrate for biodegradation by microorganisms, it may be inhibitory, especially at very high concentrations causing toxicity and death of microorganisms resulting in a loss of biomass and solids in the reactor outlet stream (Luo, 2009).

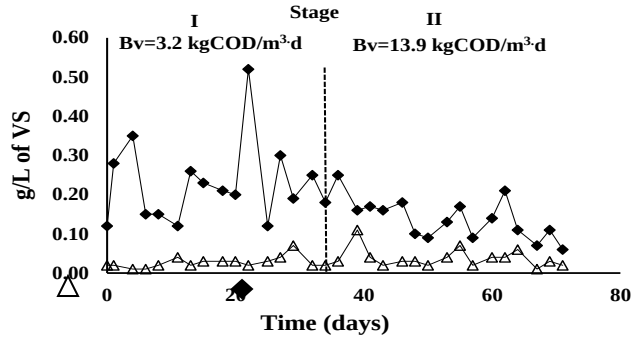

Figure 2 Performance of the volatile solids in the reactor: Influent ( ) and effluent ( )

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In the figure 4, the efficiency of COD removal is shown, the dark rhombus represent the COD present in the influent (2%) based on the COD of the phenolic Industrial effluent (Table 2) and triangles represent the COD remaining in the reactor effluent. This figure shows that by increasing the volumetric organic load of 3.2 and 13.9 kgCOD/m^3·d, the COD removal efficiency decreases moving from a 64 to 60%, due to the toxic nature of phenol, which causes inhibition biomass reactor and consequently, low efficiency of removal to prevent or retard the metabolic reactions of microorganisms for biodegradation (Abu-Hamed, 2004;. Luo, 2009). However, despite a decrease in the efficiency of COD removal the results achieved under the operating conditions tested, are better based on what is reported in the literature on biological processes for the biodegradation of phenol (Use of synthetic water, low concentrations of phenol, use of co-substrates, support means, HRT long, dissolved oxygen concentrations greater than 1 mg/L and independent systems).

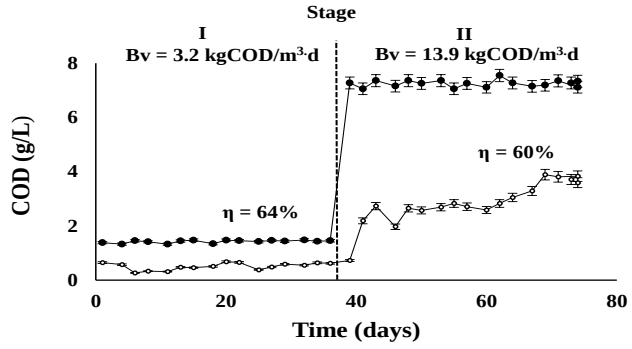

Figure 3 Elimination of COD: Influent ( ) and effluent ( )

For his part, Figure 5 shows the behavior of the reactor on the degradation of phenol, dark diamonds represent the phenol concentration in the influent and triangles, the phenol remaining in the reactor effluent.

And it is seen that, to the extent that their concentration increases of 0.48±0.025 g/L (stage I) to 1.49±0.04 g/L (stage II) in feed (influent) of the studied system, the different organic loading rates (3.2 y 13.9 Kg/m^3·d) respectively, the degradation efficiency of phenol decreases moving from 74% to 54%. Similar behavior to that shown by the reactor on the COD removal. This probably be attributed to the rate of dissolved oxygen for limiting oxidation of the phenol molecule factor, aerobically, reducing the possibility of contributing fully to the anaerobic consortium phenol degradation and consequently inhibition and involvement of both the activity of cellular metabolism of the microbial consortium. In the literature it is reported that a phenol concentrations greater than 1300 mg / L, inhibition of biomass and including total loss of activity of the microorganisms in biological processes (Yoong, 2000) is raised. However, despite the results, the new configuration of anaerobic reactor UASB type used in research, showed biodegradability of such toxic compound, which is an excellent sustainable alternative treatment of industrial effluents with phenol.

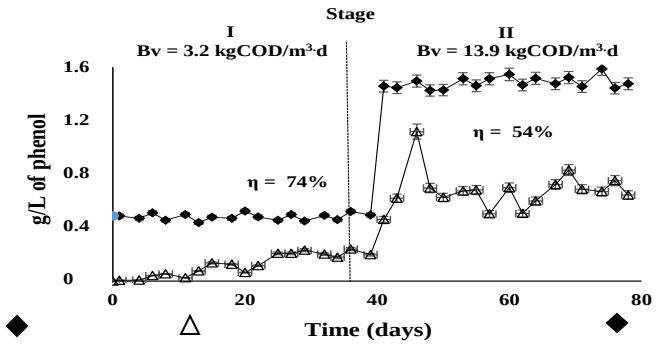

Figure 4 Biodegradation of phenol: Influent ( ) and effluent ( )

From the figures 2, 3, 4 y 5 is constructed the table 4, what represents the average days of operation when the reactor reached stationary phase in each stage.

Parameter	Stage		
	I		
	II		
Operation period (d)	0-36		37-
pH	7.39±0.33	71	
TS (g/L)	0.59±0.21		
VS (g/L)	0.24±0.1	7.2±0.13	
COD (g/L)	0.51±0.13		
η_{COD} (%)	64	0.26±0.15	
Phenol (g/L)	0.12±0.08		
η_{Phenol} (%)	74	0.13±0.04	
Acetate (g/L)	0.048±0.07		
Propionate (g/L)	0.017±0.04	2.9±0.79	
Butirate (g/L)	0.014±0.03		60
VFA (g/L)	0.026±0.05		
Biogás$_{STP}$ (L/L_{R}·d)	0.06±0.2	0.68±0.15	
			54
		0.075±0.08	
		0.06±0.05	
		0.04±0.02	
		0.058±0.05	
		3.1±0.9	

STP: standard temperature and pressure; COD: chemical oxygen demand, VFA: volatile fatty acids, η_{COD}: COD removal efficiency, η_{Phenol}: phenol removal efficiency, $L/L_{R\text{-}d}$: liters of biogas by liter of reactor per day.

Table 4 General characteristics of UASB reactor effluent

Conclusions

The low rate of dissolved oxygen in the reactor type applied UASB new configuration favored the oxidation of phenol (Aerobic way) contributing in mineralization to CH_4 and CO_2 (anaerobic way), allowing achieve removal efficiencies phenol and COD present in the wastewater of the order of 74 y 64% an organic load 3.2 kgCOD/m^3·d and of the 54 y 60% respectively, to a load of 13.9 kgCOD/m^3·d under the operating conditions described above. The results obtained with this new configuration unconventional reactor, showed their biodegradability, based on those reported in the literature, besides being novel, it turns out to be an excellent alternative biological treatment of phenolic wastewater. Contributing to this research, the generation of new scientific knowledge, have 2 types of consortia microbial (anaerobic-aerobic) in a single biological reactor upflow UASB, without variants pH destabilizing development, which in addition to solution a real problem of environmental pollution, proves to be robust and friendly to the environment.

Despite having a major drawback in the study, the use of large volumes of water for conducting respective dilutions (2 y 10%), to avoid involvement and inhibition of biomass biological reactor for phenol degradation of industrial effluent. However, this disadvantage in a wastewater treatment plant at the industrial level, resolved, with the volume fraction of exchange that is returned to power processing system, which significantly reduces the volume of water to be used for biodegradation, and therefore , generating good quality treated water for reuse.

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