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Biodegradation of phenol at high organic loads in a newly configured reactor using an aerobic-anaerobic reactor design with UASB type at low dissolved oxygen rates

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Abstract

Among the aromatic compounds present in industrial effluents, phenols are considered as priority contaminants. Due to its great use, has caused, that are present in: air, food, drinking water and natural bodies of water. Industries such as: chemistry, pharmaceutical, paper, etc., discharge wastewater with concentrations between 35 y 400 mg/L of phenol. While the discharges from Mexican refineries show concentrations of 30,000 mg/L. Their effects are adverse in the short, medium and long term in human health and aquatic life, when these effluents without receiving any previous treatment, are discharged to the natural bodies of water.The objective of this work was to evaluate the performance of a reactor of new aerobic-anaerobic configuration at low rates of dissolved oxygen on the phenol biodegradation efficiency of an industrial effluent, varying the organic load and the hydraulic retention time (HRT) to 30±0.5ºC and dissolved oxygen 1.54±0.7 mg/L, without pH control, or recirculation. The results showed that the best rate of phenol removal (47%), was achieved with an organic load of 57.7±1.3 Kg COD/m^3·d and HRT of 0.5 days (experiment II). While the COD removal (67%), was achieved at lower organic load (36.9±0.68 Kg COD/m^3·d) and HRT of 0.75 days (experiment III).

Anaerobic-aerobic, Hybrid reactor, Phenols, Toxicity, Biodegradation

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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 and about 0.4% in soil and aquatic sediments (ATSDR, 2016). Discharges of wastewater from the chemical, pharmaceutical, paper, foundry, etc., provide concentrations between 35 and 400 mg/L of phenol (Lepik and Tenno, 2011; Pramparo et al., 2012). However, the presence of phenol in wastewater has been reported with concentrations up to 10,000 mg/L (Krastanov et al., 2013). And in extreme cases, with concentrations of the order of 30,000 until 80,000 mg/L in effluents from the petrochemical industry (Terreros et al., 2016). Most countries specify a maximum allowable concentration of phenol in wastewater to be less than 1 ppm (Maleki et al., 2005).

The exposure of phenol and its derivative compounds to human and animals causes liver and kidney damage, central nervous system impairment, diarrhea, and excretion of dark urine (Olujimi et al., 2010). Therefore, it is necessary to develop methods that allow one to detect, quantify and remove phenol from wastewater (Mahvi, 2008). Generating a major problem for its elimination, which because it is a compound of benzene origin, is highly toxic and recalcitrant. The problem is exacerbated when in addition to phenol, effluents contain other compounds of similar toxicity, such as formaldehyde.

A situation that arises when it comes to effluents from resin processing, further complicating its purification (Ortega-Méndez et al., 2015).

A wide variety of microorganisms are known to be capable of metabolising many of the organic pollutants or chemicals generated and discharged (Badia-Fabegrat et al., 2014). Metabolic processes are governed by the action of enzymes. Enzymes are specific for each type of reaction. The three major classes of these energy-yielding processes are: aerobic respiration, anaerobic respiration and fermentation. Many microbes are capable of completely metabolising or mineralising different environmental organic pollutants like phenol under aerobic and/or anaerobic conditions and the Pseudomonas species have demonstrated the ability to do this effectively, as Pseudomonas spp than tolerate concentrations of 10 to 25 g/L of phenol as Alcaligenes cepa TW1 (Essam et al., 2010), Rhodococcus opacus (Matera et al., 2010), etc.

The wide variety of microorganisms that can aerobically degrade phenol include pure bacterial cultures such as: Acinebacter calcoaceticus, Alcaligenes eutrophus (Leonard and Lindley, 1998), Bacillus stearothermophilus, Pseudomonas cepacia G4 also known as Burkholderia cepacia G4, Pseudomonas picketti, Pseudomonas putida are also capable of degrading phenol. Yeasts as Candida tropicalis which uses phenol under aerobic conditions as the only source of carbon and energy, with a potential for degradation up to 1700 mg/L (Yang et al., 2005), Rhodotorula rubra, Trichosporon cutaneum and algae as Ochromonas dánica (Komarkova et al., 2003).

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Amongst all the microorganisms listed as good degraders of phenol, the pure culture of Pseudomonads are the most utilized purposely for metabolic pathway studies and their ability to utilize or degrade many other aromatic compounds. In Pseudomonads, many of its induced enzymes are non-specific and its metabolic pathway contains a high degree of convergence, allow for the efficient utilization of a wide range of growth substrates while the non specificity of the induced enzymes allows for the simultaneous utilization of several similar substrates without an excess of redundant genetic coding for enzyme induction (Selesi et al., 2010). Other techniques include the encapsulation of microorganisms as an alternative to protect against the toxicity of effluents with phenol (Martínez-Trujillo et al., 2012). Its incorporation of free form in the treatment systems, as it is known, presents certain limitations due to the inherent toxicity of this type of contaminant, as well as to the competition between the native populations and the exogenous (Fantroussi and Agathos, 2005). To resolve this limitation, immobilized microorganisms have been used, with immobilization the cells are given a protection against the toxic effect of the toxic substances such as phenol present in the effluent to be treated and predation by other populations (Martínez-Trujillo et al., 2012). What has allowed to increase the overall rate of biodegradation, due to the high cellular densities reached, in addition to increasing the stability and tolerance of microorganisms to toxic compounds (Aneez Ahamad et al., 2011).

Biological processes have been poorly studied because it has been demonstrated that the presence of 10 mg/L of phenol in wastewater causes inhibition of microorganisms, consequently a low removal efficiency.

However, the study of the biodegradation of phenolic compounds via aerobics has shown that there is a common metabolic pathway for this type of compound and even for those not so close to the family of phenolic compounds as biphenyls. Under anoxic respiration conditions (anaerobic digestion), it uses different electron acceptors such as nitrate, sulfate, CO2, among others. With the purpose of produce reduced compounds of nitrogen, sulfur, methane and carbon dioxide. (Shalaby, 2003).

Aerobic biodegradation of phenol.- In microbial degradation of phenol under aerobic conditions, the degradation is initiated by oxygenation in which the aromatic ring is initially monohydroxylated by a mono oxygenase phenol hydroxylase at a position ortho to the pre-existing hydroxyl group to form catechol. This is the main intermediate resulting from metabolism of phenol by different microbial strains. Depending on the type of strain, the catechol then undergoes a ring cleavage that can occur either at the ortho position thus initiating the ortho pathway that leads to the formation of succinyl Co-A and acetyl Co-A or at the meta position thus initiating the meta pathway that leads to the formation of pyruvate and acetaldehyde. Leonard and Lindley (1998), described the biodegradation or metabolism of phenol by Pseudomonas putida, Pseudomonas cepacia, Pseudomonas picketti and Alcaligened eutrophus respectively via the meta cleavage pathway, while Paller et al. (1995) described the biodegradation of phenol by Trichosporon cutaneum, Rhodotorula rubura and Acinetobacter calcoacetium respectively via the ortho cleavage pathway.

The meta pathway for the biodegradation of phenol as presented by Nelson et al. (1987).

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The mono oxygenase phenol hydroxylase of the Trichosporon cutaneum, Pseudomonas pickett, Bacillus stearo thermo phylus BR219 and some species of acinetobacter and alcaligenes are monocomponent flavoproteins (Kim et al., 2002), while the mono oxygenase phenol hydroxylase of pseudomonas CF-600 and Acinetobacter radioresistens (Shingler et al., 1998) are multicomponent proteins. Multicomponent aromatic mono oxygenases contain at least two components. The former is an oligomeric protein while the latter is a monomeric iron transfer flavoprotein. Infact, the three-component toluene dioxygenase (TDO) from Pseudomonas putida uses dioxygenation followed by water elimination to convert phenol to catechol (Spain et al., 1989).

Anaerobic biodegradation of phenol .- Phenol can also be degraded in the absence of oxygen and it is less advanced than the aerobic process. It is based on the analogy with the anaerobic benzoate pathway proposed for Paracoccus denitrificansin (Williams and Evans, 1975). In this pathway phenol is carboxylated in the para position to 4 hydroxybenzoate which is the first step in the anaerobic pathway. Here the enzyme involved is the 4-hydroxy benezoate carboxylase. The anaerobic degradation of several other aromatic compounds has been shown to include a carboxylation reaction. Carboxylation of the aromatic ring in para position to the hydroxyl group of o-cresol resulting in 3-methyl 4-hydroxybenzoate has been reported for a denitrifying Paracoccus like organisms, as well as methogenic consortium was later shown to travel a varity of phenolic compounds including o-cresol, catechol and ortho halogenated phenols via para carboxylation followed by dehydroxylation.

The organisms capable of degrading phenol under anaerobic conditions were Thauera aromatica and Desulphobacterium phenolicum.

Aerobic or anaerobic studies for phenol biodegradation address the use of synthetic waste water and in some cases, industrial wastewater with hydraulic retention times (HRT) greater than 1 day, high rates of dissolved oxygen, low organic loads and in some cases, the use of co-substrates such as glucose (Godjevargova, 2003; Suarez et al., 2007; Bajaj, 2008; Farooqi, 2008; Donoso-Bravo, 2009; Tavares et al., 2009; Chérif, 2011; Almasi, 2012; Anushuya Ramakrishnan, 2013; Rosenkranz, 2013, Pradeep, 2015). His biodegradation by this type of processes, involves many factors such as; Organic loading speed (Liu et al., 2003a), temperature, pH, dissolved oxygen concentration, substrate concentration, dilution rate (Ba et al., 2014), thus as the acclimatization strategy (Terreros et al., 2016).

The objective of this work was to evaluate the performance of a reactor of new aerobic-anaerobic configuration at low rates of dissolved oxygen on the phenol biodegradation efficiency of an industrial effluent, varying the organic load and the hydraulic retention time (HRT) to 30±0.5oC, without pH control, or recirculation, taking advantage of the benefits of mixed microbial cultures. Through the acclimatization of biomass at a low organic load rate, with the aim of increasing over time its biodegradability at higher organic loading rates. The than is relevant and novel to have two microbial consortia in a single reactor, which in addition to being innovative, environmentally friendly, lowers the costs of treatment of this type of effluent, in relation to the technologies traditionally used.

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Materials and methods

Description of the UASB reactor

For the tests a hybrid reactor type UASB was used Upflow Anaerobic Sludge Blanket) at laboratory scale with design volume of 1.21L, useful volume of 1.04L, internal diameter of 4.5 cm and height of 53 cm (figure 1).

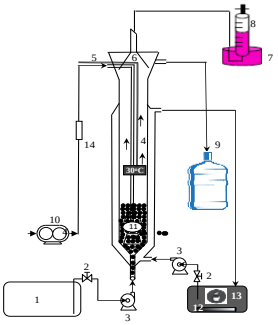

Figure 1 Description of the process. 1) Phenolic residual water, 2) Regulating valve, 3) Peristaltic pump, 4) Reactor UASB, 5) Temperature sensor, 6) Phase separation device, 7) Recipient of Saline solution pH 2, 8) Biogas measurement column, 9) Recipient of treated water, 10) Air pump, 11) Air conduction device, 12) thermostat, 13) Water container to 30±0.5oC, 14) Air flow meter

Inoculum.- The reactor UASB, was inoculated with 312 mL of sludge (total volume), of the which 94 mL correspond to aerobic sludge that was collected from an activated sludge reactor of the municipal wastewater treatment plant "Cerro de la Estrella", of Mexico City, with a concentration of 12.7 g/L de TSS and 9.7 g/L of VSS. And 218 mL of anaerobic granular sludge from a reactor UASB of the Autonomous Metropolitan University Iztapalapa unit that treats the wastewater of the academic unit, with a concentration of 67.9 g/L de TSS, 35.7 g/L of VSS and specific methanogenic activity (SMA) of 0.11 LCH4/gVSS·d.

Feed medium (Influent).- During the first week of operation, mineral medium was used RAMM (Shelton and Tiedje, 1984) with sodium acetate as the carbon source for the development of the methanogenic conditions of anaerobic biomass. And a dilution rate of 2% of phenolic residual water to acclimatize the biomass against this toxic. From the second week and throughout the experimental period, the reactor was fed with phenolic residual water. Calculating from Equation C1V1 = C2V2, the volume of phenolic residual water required to prepare the feed according to the fraction v/v of the 25 and 40% based on the characteristics of the industrial wastewater samples provided (table 1).

Parameter	Experiment		
	I X±S	II X±S	III X±S
Fraction v/v	25%	40%	40%
COD (g/L)	17.84±0.025	27.7±0.44	27.8±0.3
Phenol (g/L)	3.37±0.04	5.34±0.06	5.34±0.02
pH	6.82±0.13	5.25±0.34	5.15±0.2
TS	0.68±0.2	0.3±0.19	0.26±0.15
VS	0.45±0.05	0.23±0.04	0.14±0.08

Table 1 General characteristics of industrial residual water with phenol (influent)

Characterization of wastewater and evaluation of reactor performance.- For the characterization of the industrial effluent and evaluation of reactor performance (analysis of the difference of parameters) between the mixture of phenolic fed water and treated, the following parameters were analyzed (COD, phenol, pH, TS and VS) using the following analytical techniques; Chemical oxygen demand (COD), total solids (TS) and volatile solids (VS) were determined according to the standard method (APHA, 2016).

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The pH was evaluated by a potentiometer (Conductronic PC18). The phenol analysis was performed by the colorimetric method of 4-aminoantipyrine according to the Mexican standard (NMX-AA-050-SCFI-2001) using an equipment UV-VIS brand Perkin Elmer Spectrometer model Lambda XLS. The volume of biogas produced was quantified by inverted column in a vessel containing a saline solution at pH=2. Where, the volume of the displaced solution corresponded to the volume of biogas produced. The rate of dissolved oxygen supplied to the studied system was measured using a portable model YSI.

Operating conditions of the reactor.- For the operation of this type of reactor of new configuration, a device was used to separate both types of microbial consortium (aerobic-anaerobic) and to avoid their possible mixture. Feeding the reactor to continuous flow in the different runs in which the experiment was carried out (table 2), by means of a peristaltic pump. Furthermore, of a thermostat to maintain the working temperature of 30±0.5°C, and an air flow meter model "Dwyer", applying a hydraulic retention time (HRT) of 12 h, without pH control, agitation or recirculation.

Experiment	**I**	**II**	**III**
Fraction v/v of $RW_{Phenólic}$	25% X±S	40% X±S	40% X±S
Bv ($kgCOD/m^3 \cdot d$)	34.4±0.7	57.7±1.3	36.9±0.68
HRT (days)	0.5	0.5	0.75
Dissolved oxygen (mg/L)	1.54±0.7	1.54±0.7	1.54±0.7

Table 2 Operating conditions of the reactor

Results and Discussion

Characteristics of the treated phenolic residual water (effluent).- Before addressing the analysis of the results, it is presented in the table 3, the results of the averages of the main parameters evaluated to the treated wastewater samples taken throughout the experiment, to evaluate the performance of the newly configured reactor, on the phenol biodegradation rate of the industrial residual water. And there is a significant dispersion in the data, due to the variability that occurs during the treatment of phenolic industrial effluent from the polymer resins industry.

Parameter	**Experiment**		
	I X±S	**II** X±S	**III** X±S
COD (g/L)	7.42±0.36	17.6±0.6	9.27±1.2
Phenol (g/L)	2.56±0.18	2.84±0.47	3.18±0.34
pH	6.98±0.14	6.59±0.18	6.66±0.12
TS	0.73±0.25	0.29±0.14	0.26±0.15
VS	0.54±0.15	0.19±0.1	0.17±0.1

Table 3 General characteristics of treated wastewater (effluent)

In the figure 1, the behavior of pH during the development of the experiment is shown. The clear rhombuses represent the pH in the influent and the dark rhombuses, the pH of the effluent. And you can say in general terms, that the performance of the biological reactor throughout the experiment was adequate.

Despite having presented a variation pH in the 2 last samples of industrial phenolic residual water with which the hybrid system was fed during the experiments II y III, with an average pH value of 5.2±0.2, did not affect the metabolic activity of both bacterial consortia of the experimental system. The anaerobic digestion of organic matter is carried out in a range of pH between 6.2 and 7.8, with an optimum between 7 and 7.2 (Metcalf and Eddy, 2016).

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Variations of pH, affect the enzymatic activity of microorganisms by changes in the state of the ionizable groups, which causes alteration of the non-ionizable components of the system such as denaturation of the protein structure of the enzymes. High pH values favor the formation of free ammonia, inhibitor of the methanogenic phase which causes an imbalance between the production and the consumption of volatile fatty acids by accumulation of these, acidifying the reactor (Zeeman and Sanders, 2001). While extreme values of pH (less than 3 or greater than 9) may be inhibitory to the growth of microorganisms involved in phenol biodegradation (El-Naas et al., 2009). In this context, the behavior of pH in the reactor allowed the study, object of this research work, to be carried out adequately.

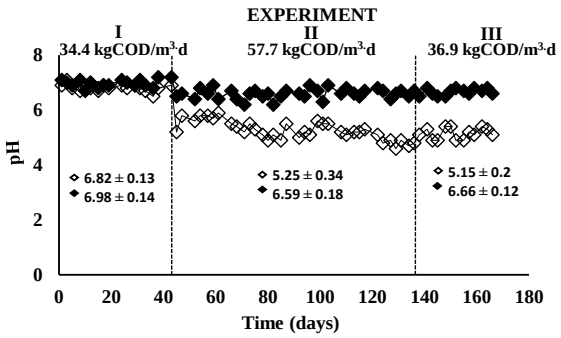

Figure 1 Variation of pH with time

In the figure 2, is shown the profile of volatile solids (VS). The clear rhombuses represent the VS in the influent and the dark rhombuses, the SV in the effluent. Most studies of phenol degradation and related compounds have been focused on microbial consortia that have previously been acclimated to the toxic compounds under study (Antizar-Ladislao and Galil, 2004). However, to date there is very little information on the dynamics and degree of adaptation achieved according to the strategy used to acclimate the microbial consortium to phenol.

In this context, during the development of the research, once industrial residual water was fed with a phenol concentration of 3.37±0.04 g/L, with organic load of 34.4±0.7 KgCOD/m3.d (experiment I), there is a slight biomass loss from the reactor. In the literature it is mentioned that due to the bactericidal effect of phenol, based on the ability of the compound to dissociate within the cells, it can cause the cytoplasmic membrane functions to be interrupted, which probably caused the death of some of the anaerobic cells (Tay et al., 2005), and consequently, the presence of solids in the reactor effluent. In addition, at high organic loading rates, a loss of cell integrity may occur and consequently, the disintegration of the granular structure (Quarmby and Forster, 1995).

Which apparently did not affect the aerobic consortium as in the case of aerobic granules, these may be formed in a wide range of organic loading, of 2.5 to 15 KgCOD/m3.d (Moy et al., 2002). Increasing the size of the aerobic granules of 1.6 to 1.9 mm with organic loads of 3 to 9 KgCOD/m3.d (Liu et al., 2003a). However, for the following runs, once the biomass of the new configuration hybrid reactor was adapted to the presence of phenol (Tay et al., 2005; Marrot et al., 2006; Vacca et al., 2008; Farooqi et al., 2008), presents stable conditions during the biodegradation of phenol, which allowed to reach that volatile solids in the effluent of the reactor, were in average of 0.19±0.1 and 0.17±0.1 g/L respectively (experiments II and III). Therefore, in addition to the factors involved during the biodegradation of phenol, in biological processes such as: temperature, pH, dissolved oxygen content, substrate concentration, among others (Nair, 2008; Agarry, 2008; Trigo, 2009), as well as organic loading (Moy et al., 2002; Liu et al., 2003a). It is very important to have a good biomass acclimatization strategy (Terreros et al., 2016).

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To achieve the maximum rate of biodegradation of phenol present in an industrial effluent, in such a way, that the biomass does not present any type of inhibition or affectation of its metabolism and much less, to cause its cell death, by exposure to this type of toxic compounds (Luo, 2009).

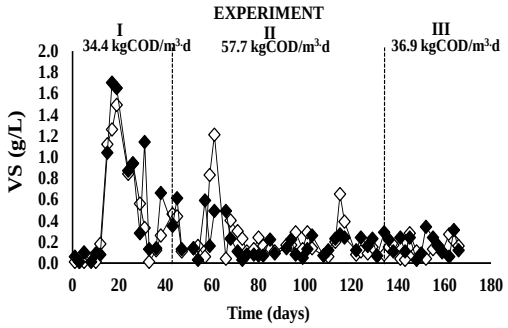

Figure 2 Variation of volatile solids during the biodegradation of phenol

The figure 3 shows the removal efficiency of chemical oxygen demand (COD) during the development of the experiment at different fractions v/v of residual water with phenol: 25 and 40% (table 2). The clear rhombuses represent the COD in the influent and the dark rhombuses, the COD remaining in the reactor effluent. And it is appreciated that once the reactor was fed with phenolic residual water with an organic load of 34.4±0.7 Kg COD/m3.d (experiment I) to 0.5 days of hydraulic retention time (HRT), Efficiency of removal of COD, from the 57%.

However, with the increase of the organic load in 57.7±1.3 Kg COD/m3.d during the experiment II, the efficiency of COD removal decreased significantly in one 29% on day 101 of operation. And it is observed that to the extent that the biomass acclimatizes to the presence of the phenol, the removal efficiency, is gradually recovered until reaching a 49%.

However, by varying both the organic load in 36.9±0.6 Kg COD/m3.d (organic load similar to the experiment I), as the hydraulic retention time in 0.75 days, during the experiment III, an improvement on the COD removal rate was observed in 67% based on the removal rate achieved during the experiment I.

In the literature it is mentioned that both aerobic and anaerobic granules can be formed over a wide range of organic charge rates ranging from 2.5 a 15 Kg COD/m3.d (Moy et al., 2002; Liu et al., 2003a), with an increase in size from 1.6 to 1.9 mm with organic loads over a range of 3 a 9 Kg COD/m3.d (Liu et al., 2003b), that in addition to factors such as pH, temperature, etc. that influence reactor performance during phenol degradation, it is important to acclimate to biomass (Terreros et al., 2016) to achieve the best results in order to avoid partial loss of its integrity, and consequently the disintegration of its granular structure, given the toxic effect of phenol, which causes inhibition of microbial growth according to what has been reported in other studies (Chen et al., 2008; Liu et al., 2008).

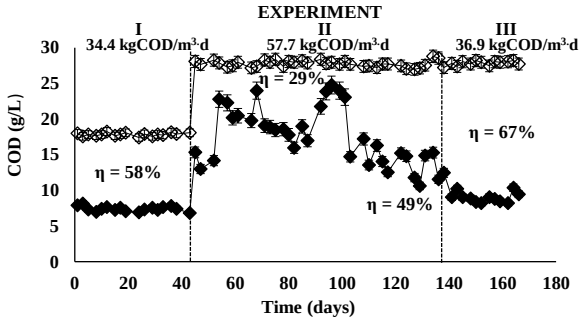

Figure 3 Variation of COD concentration with time

In the figure 4, is shown the rate of phenol biodegradation under the tested operating conditions (table 2). The clear rhombuses represent the phenol concentration in the influent and the dark rhombuses, the phenol concentration remaining in the reactor effluent.

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And it is observed that the best rate of phenol removal was achieved during the second run, with a fraction v/v phenolic residual water from the 40%, organic load of 57.7±1.3 Kg COD/m3.d, dissolved oxygen rate of 1.54±0.7 mg/L and 0.5 days of HRT.

And it is observed during the last run of the experiment, in which the reactor was fed with a fraction v/v similar to that of the second experiment (fraction v/v of 40%), but lower organic load (36.9±0.8 Kg COD/m3.d) similar to the experiment I. A higher phenol biodegradation rate (40%) with greater HRT of 0.75 days. Compared with the phenol biodegradation rate achieved during the first run. These results show that in order to achieve an adequate rate of biodegradation of phenol present in industrial wastewater by biological processes, it is necessary to make dilutions (Ba et al., 2014).

Since in the measure in which it increases its concentration, decreases the rate of phenol biodegradation by microorganisms. In this context, the reactor performed adequately in the study, without affecting the biomass at the phenol concentrations of the wastewater studied. Although at concentrations of phenol of more than 1300 mg/L, it causes an inhibitory effect (decrease of the metabolic activity of the biomass) and even, can lead to the total loss of its microbial activity (Busca et al., 2008).

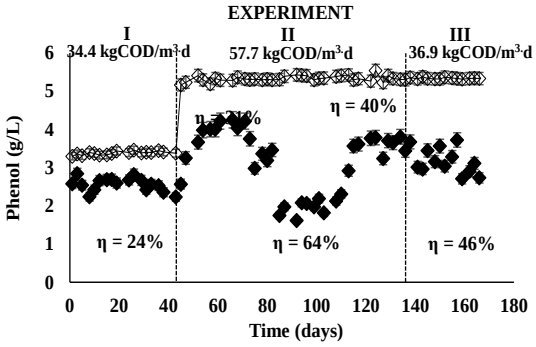

Figure 4 Variation of phenol concentration with time

In the figure 5, it is noted that the measure in that the organic load increases of 34.4±0.7 to 57.7±1.3 KgCOD/m3.d to 0.5 days of HRT, the rate of biogas production, decreases significantly from 19.2±1.5 mL (experiment I) to 5.4±1.1 mL (experiment II). And it is note that increasing the HRT 0.5 to 0.75 days during the last experiment, a slight reduction occurs place in biogas production, probably due to the concentration and exposure time of the biomass with phenol, causing that the metabolic activity of the anaerobic bacteria to be more sensitive to this toxic compound, present less methanogenic activity and, consequently, less production of biogas (Busca et al., 2008).

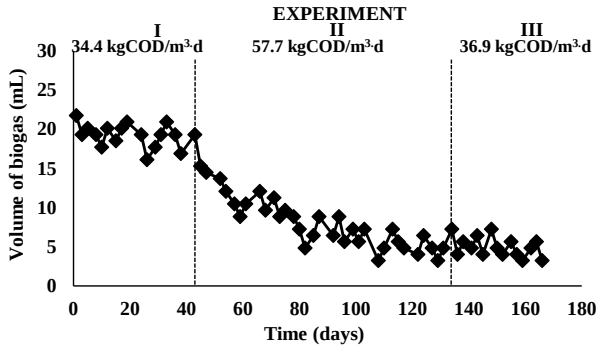

Figure 5 Biogas production with time

Conclusions

The strategy used to acclimate the aerobic and anaerobic microbial consortium used as reactor inoculum to study the biodegradation of phenol present in a real industrial effluent was the key factor in achieving the objective set out in this study.

With the results obtained, it has been demonstrated that it is possible to treat phenolic industrial effluents by the use of reactors of new configuration using an aerobic-anaerobic reactor design type UASB, at low rates of dissolved oxygen.

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The use of this new design of hybrid reactor of new configuration, Is an excellent alternative for the solution of real problems of environmental pollution, by discharge of industrial effluents with phenol, that besides being new, environmental friendly, is robust, by its capacity of biodegradation of phenol, at high rates of organic load, in relation to the technologies traditionally employed. With the use of this biotechnology, it can enhance the design and Construction of a new type of wastewater treatment plant With high concentration of toxic compounds as phenol, which in addition to lowering its construction costs, operation and maintenance, the surface for its construction it is less, significantly reducing investment costs.

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