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ELECTRO-BIOREMEDIATION AND EVALUATION OF BIOLOGICAL ACTIVITY IN SOILS CONTAMINATED BY HYDROCARBONS

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Abstract

The growing demand of actions for soil remediation has resulted in the development of alternative technologies, especially for applications of techniques in situ, caused for the many forms of contamination of soil and underground water with petroleum hydrocarbons. The present work proposed an electro-bioremediation technique to evaluate the capacity degradation of the pollutants through microorganisms present in the polluted soil. The monitoring of the degradation of hydrocarbons was accompanied through chemical analyses of Total Petroleum Hydrocarbons (TPH), Benzene, Toluene, Ethylbenzene, and Xylene (BTEX), Polycyclic Aromatic Hydrocarbons (PAH). The microbiological activity was monitored through analyses of Colony-Forming Unit (CFU). The results showed that rates of degradation of the xylene, toluene and ethylbenzene had an order of 69%, 60% and 10%. The microbiologic activity presented an increase of 12.5 times for the Mould and Yeasts, and of 178.5 times for aerobic microorganisms. The isolation and biodegradation analysis pointed the existence of 26 different morphotypes, of these, 20 morphotypes (77%) possess capacity to degrade hydrocarbons that, 60% were found in the areas of influence of the cathode and 40% of the anode. The yeast-like material was isolated and identified, where the predominant genus was Penicillium (50%), Paecilomyces 16.6%, Trichoderma 16.6 % and Cladosporium 16.6%.

Keywords: bioremediation, technologies, electrokinetic, microorganisms, petroleum.

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Introduction

The contamination of water and soil by hydrocarbons mostly occurs in places where the concentration of oil products is very large (gas stations) with very high working age. In many parts of world, soil and groundwater contamination by hydrocarbons has been related to accidental spills caused in the extraction, drilling sites, storage, accidents in fuel transportation and distribution of fuels, marine locations, improper waste disposal practices (Corseuil and Martins, 1997; Wang at al., 2013; Souza *et al*., 2014; Lahel *et al*., 2016). There are other sources of contamination, such as hydrocarbons released into the environment due to anthropogenic means, that impact the microorganisms residents (Wang *et al*. 2013). The Polycyclic Aromatic Hydrocarbons (PAHs) were identified in many emission sources such as exhausts from vehicles, power plants, chemical products, petroleum coke and shale industries, urban sewage, etc. In natural primary sources the PAHs can be found in forest fires and volcanic activity (Trapido, 1999). PAHs are ecologically important because some of their genotoxic activity can cause mutations and certain cancers (White *et al*., 1998).

Jackson *et al*. (1996) stated that there are currently more than 100 PAHs recognized by the IUPAC (International Union of Pure and Applied Chemistry). Nevertheless, only 16 PAHs are considered because of its industrial, environmental and toxicological importance. They are: acenaphthane, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, chrysene, dibenz(a,h)anthracene, phenanthrene, fluoranthene, fluorene, indeno (1,2,3-cd) pyrene, naphthalene and pyrene (Potin *et al*., 2004).

In Brazil, the large soil and water contamination problem by the oil and derivatives spills has gained special attention because of the increasing diagnosis of impacted areas, although by 2003, only the State of São Paulo presented an effective legislation that dealswith contamination of soil and groundwater contamination by hydrocarbons (Schneider *et al*., 2003). The main currently responsible for contamination are the gas stations, representing more than half of cases of contamination of soil and aquifers, largely due to the lack of effective monitoring and long lifetimes of underground storage tanks of about 25 years.

The problems caused by the contamination of soil and groundwater by hydrocarbons are numerous. Sanches (1998) points to three main problems: there are risks to the safety of persons and property; risks to public health and ecosystems; and restrictions on urban and real estate development. In addition, some of the organic compounds present in the composition of petrol and diesel oil are scientifically proven carcinogens.

In May 2002, Environmental Sanitation Technology Company - CETESB released for the first time the list of contaminated areas, recording the existence of 255 contaminated areas in the State of São Paulo. The register of contaminated sites is updated frequently, and, after the last update, were registered 5376 records in the Register of Contaminated and Rehabilitated Areas in the State of São Paulo (Cetesb, 2015).

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One of the alternatives to combat and treat contaminated areas is remediation, defined as the process of recovery of an area previously contaminated by polluting activities (Castro, 2010). The need to recover these contaminated areas in recent decades has led to the development of techniques *in situ* and *ex situ* for remediation of contaminated soil. But its remediation is linked to economic conditions and industrialization of each affected region and also by the current legislation in each country. The remediation needs biocatalysts to reduce the levels of contaminated, and the degradation rates of these contaminated depend the environmental conditions, bioavailability of pollutants (Militon *et al*., 2010; Larel *et al*., 2016). According Fernandes (2010), the electro-kinetic techniques are based on *in situ* application of an electric gradient between the electrodes for extraction and migration of the contaminants by electro-kinetic transport mechanisms. This electric field generates transport processes of ions, fluids in the pores and electrically charged particles toward electrodes, promoting, in the soil, extraction of contaminants or transport of nutrients and electron acceptors to improve the biodegradability conditions of organic compounds. These phenomena occur in soils due to the ability of interstitial fluids to conduct electric current.

The aim of this study was to analyze the influence of electro-kinetic remediation on the degradation of hydrocarbons in contaminated clayey soils and on the microbiological activity of the microorganisms in the soil.

Materials and methods

Contaminated soil

For the experiment, soil from a fuel supply site was used, where fuel storage tanks were removed and exchanged. In total, were collected approximately 35 kg of contaminated soil and mixed with oily sludge from fuel storage tanks at a ratio (soil / sediment) of 10:1 by weight.

Electro-bioremediation system

The electro-bioremediation system proposed had dimensions 60 x 40 x 10 cm, constructed in acrylic with a storage capacity of 24 L. It was composed of 6 alloy steel 1020 commercial electrodes, each 0.45 cm long, which were introduced into the soil in the reactor at equal distances (8.57cm) from each other, forming three pairs of anodes and cathodes connected to a source of direct current. The system had a metering pump which injected deionized water at a rate of 1 L/day in the system to promote saturation.

Assays were performed in two stages. The first step corresponds to the interval of time between 0 and 120 days, during which the electro-kinetic system worked with a tension of 5.0 volts direct current in the three pairs of electrodes in series. In this step, the applied current was 5.5 mA/cm². Also, it was added 10 mL of Omega Pro Safra 5-15-5 brand liquid fertilizer (30 and 60 days) with the following chemical composition: 5% w/w, N_2, 15% P_2O_5, 5% K_2O, 0.03% B, 0.05% Cu, 1% Mn and 0.05% Mo.

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In the second stage of the experiment (120 and 180 days) applied voltage was increased to 15.9 volts direct current, and also it was added 1 L of Tween 80 brand biosurfactantat 5% vol/vol ratio in order to improve the bioavailability of contaminants, and 10 g/L of NaCl (commercial purity), diluted to improve the system conductivity.

Colony forming units (CFU) evaluated and microorganism isolated

In order to evaluate the interference of electro-kinetic process on microorganisms, counting of Colony Forming Units (CFU) was made the in composite soil samples. Soil samples were taken from three different places, in the vicinity of the anode and cathode, homogenized and stored under refrigeration for use in these assays. The counting of CFU included the populations of fungi and yeast and viable mesophilic aerobic microorganisms, the technique used to count the microorganisms was the same used for food essays, which quantifies the colony forming units (CFU), *i.e.* those with a optimum multiplication temperature between 25 °C and 40 °C, minimum of 5 °C to 25 °C and maximum between 40 °C and 50 °C.

For the isolation of microorganisms, it was taken 10 g of soil (anode and cathode region) and suspended in sterile water at 1:10 proportion (w/vol), mixed for 1 minute, removing 1 mL of solution and dissolving in 9 mL of deionized water, repeating until reaching a solution between 10^{-3} and 10^{-4} (vol/vol). Of these solutions, it was taken aliquots and inoculated in triplicate by stretching technique into Petri dishes of 8 cm diameter containing 10 mL of the following culture media: Nutrient Agar; Sabouraud Agar and Caseinate Agar. The material was incubated at 30 °C in an oven for up to seven days. For the development of fungi and yeast it was used culture medium containing Sabouraund Agar, and for the isolation of bacteria and actinomycetes it was used Nutrient Agar and Caseinate Agar. Of the total strains obtained in this step, the most representatives were isolated and selected again to obtain pure strains. These plates were incubated for 15 days for further analysis and macroscopic identification of microorganisms. The identification of microorganisms is limited to the fungi and yeasts group due to the cost involved in the identification of bacteria.

Microorganism potencial degradation

The gasoline degradation potential by bacteria, yeasts and molds was evaluated according to Hanson *et al*. (1993). The technique involves the use of redox indicator 2,6-dichlorophenol indophenol (DCPIP) in mineral medium BH with an oil derived in a micro plate. Souza *et al*. (2010) used the same procedures for evaluating the hydrocarbon degradation by microorganisms of an area contaminated by commercial gasoline. For the experiment, it was used 25 µL of microbial suspension, standardized in 10^8 UFC/mL, and was added to the microplates containing 250 µL of BH medium, 4 µL of carbon source (diesel) and 5µl of the redox indicator DCPIP. Abiotic wells containing 25 µL of sterile water, replacing the microbial suspension, were used as negative control. The plates were incubated at 30 °C and visually monitored for a period of 4 days.

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To evaluate the degradation of contaminants (Benzene, Toluene, Ethylbenzene, Xylene, PAHs and TPH *finger-print*) samples were directed to chemical analysis in the Analytical Solutions laboratory located in Santana do Parnaiba/SP, essays being carried out according to the methodology USEPA 5021A - 8021B and 8015C / D for BTEX and TPH's and the USEPA 8270D method for PAHs**.**

Results and discussion

The results of the analysis of initial concentrations of soil contaminants are shown in Table 1. According to the values presented, all contaminants are shown below intervention values of CETESB list and the Netherlands list.

Table 1. Initial concentration of contaminants in contaminated soil (soil + sludge), with ratio (10:1) weight.

Contaminants	mg/kg	Intervention Limits CONAMA 420* resolution (mg/kg)	Intervention Limits Dutch Standards List (mg/kg)
n-Alkanes	145.06	-	-
HRP	386.05	-	-
UCM	1229.39	-	-
TPH-total	1615.44	-	5000.00
Benzene	0	0.15	1.00
Toluene	0.043	75	130.00
Ethylbenzene	0.02	95	50.00
m,p-Xylene	0.461	75	25.00
o-Xylene	0.559	75	25.00

* *Brazilian Regulation*

Microbiological characterization: electrodes influences in bacteria and fungi.

Microbiological characterization of the soil, performed using the technique of counting “viable mesophilic aerobic microorganisms” and “fungi and yeasts”, showed an initial value of 2.4 x 10^3 $CFUg^{-1}$ for fungi and yeasts and 1.4 x 10^5 $CFUg^{-1}$ for viable microorganisms. The results of the CFU counts are shown in Figure 1.

During the electro-bioremediation the microorganism population showed an increase of 12.5 times to fungi and yeast, and 178.5 times for aerobic microorganisms (bacteria). The most noticeable difference was observed between 30 and 180 days of trials, where aerobic microorganisms had 92.8 times increase over its initial concentration. Fungi and Yeasts showed a 66% reduction in its population in the first 30 days of testing, this result was observed for (Cupples, 2013) that decrease the bacterial species in saturated soil (32 to 13 species), the toxicity

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of certain hydrocarbons can be tease the reduction in first time of the bacterial population (Mansur *et al*., 2016) or same bacterial are not capable of competing for nutrients in contaminated soils (Makadia *et al*., 2011).

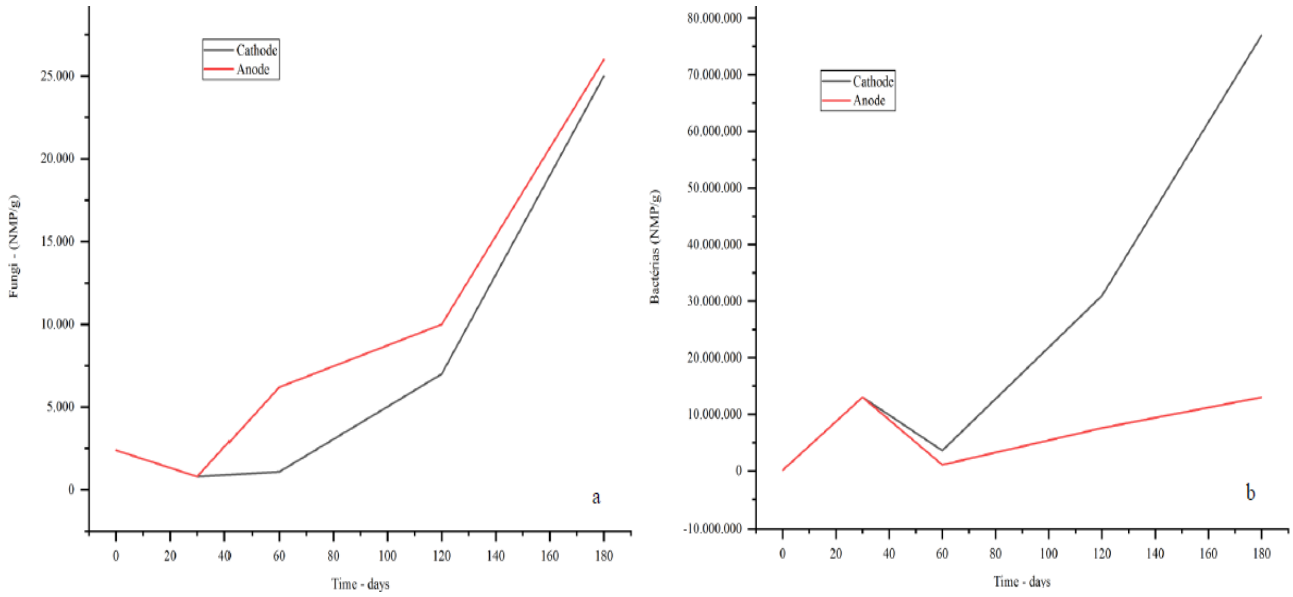

Figure 1. Development of bacteria (a) and fungi (b) during the electro-bioremediation process.

This reduction in bacterial population indicate that some bacteria cannot survive the toxicity of certain hydrocarbons or are not capable of competing for nutrients in the amended contaminated soils (Makadia *et al*., 2011). This reduction in bacterial diversity may, in part account for the reduction in TPH degradation in the system treatments. From an analysis of soil samples taken from the vicinity of the electrodes, it was possible to determine the effect of charges appliedon the populations of microorganisms present.

Figure 2 shows the results of the CFU counts of microorganisms in soil samples around the electrodes. The results show that the population of these microorganisms, around the electrode, did not presented great variations, and the CFU count of the same population is similar in both environments. This demonstrates the ability of microorganisms to withstand the conditions imposed by the presence of positive and negative charges. Another important factor is the increased population of these microorganisms after the increase of the applied voltage during the 120 day trial. Between the 120th and 180th day of testing the population of fungi and yeasts increased from 10.000 to 26.000 CFU/g and from 7.000 to 25.000 CFU/g at the anode and cathode, respectively. This increase indicates that applying a higher electric current stimulated

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microbial activity of fungi and yeasts. This observation is consistent with the work of Seong -Hye *et al*. (2010) who also observed the same effect. According to these researchers, the presence of electric current has improved the rate of substrate utilization and microbial metabolism through direct stimulation (electron transfer from the electrode to bacteria) and indirect (electron transfer through the hydrolysis of water).

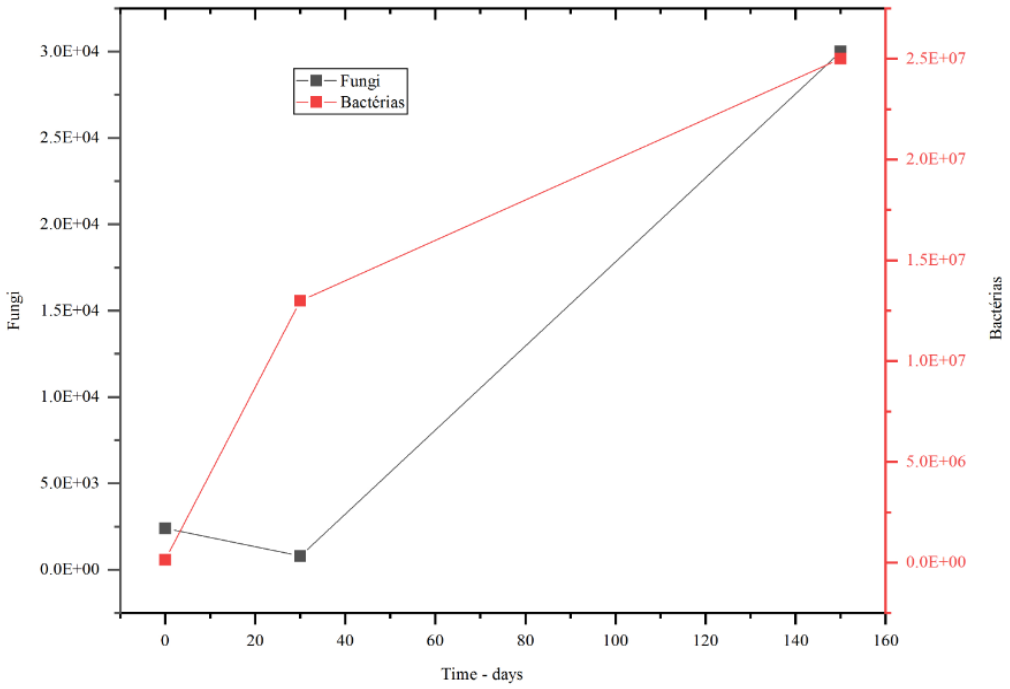

Figure 2. Development of bacteria and fungi in the electrodes (anode and cathode), during the electro-bioremediation process.

Another point to note is the increase of the bacterial population in the cathode region, which represents a more rapid kinetics between 120 and 180 days of testing, immediately after application of TWEEN 80 solution and the increase of the applied voltage. Murygina *et al*. (2016) noted that introduction of Rhoder solution in reduced contamination in until 72.6%. Despites this, Cerqueira *et al*. (2014) observed that the addition of nutrients don´t showed some influence in biodegradation rates. The application of a higher electric current can accelerate the effects of electro-osmosis and, associated with the addition of TWEEN 80 solution, there was obtained, as effect, greater desorption of contaminants from soil, increasing its bioavailability and also increasing the growth rate of the population of bacteria around the cathode. The same positive effect was not observed at the anode, thereby demonstrating that other phenomena were controlling bacterial growth in this region.

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Characterization of morphotypes in soil

With the isolation of microorganisms from the contaminated soil samples, twenty six morphotypes (strains) were obtained of the various microbial groups studied, being 20 bacteria and 6 fungi and yeasts. The morphological groups obtained were named M1 to M26, as shown in Table 2.

Table 2. Characterization of morphotypes in soil contaminated.

Morphotype	Culture medium	Microbial group	Cathode	Anode
M1	Nutrient	Bacteria	x	
M2	Nutrient	Bacteria	x	
M3	Nutrient	Bacteria		x
M4	Nutrient	Bacteria		x
M5	Caseinate	Bacteria		x
M6	Caseinate	Bacteria		x
M7	Caseinate	Bacteria	x	
M8	Caseinate	Bacteria	x	
M9	Sabouraud	Yeast	x	
M10	Sabouraud	Yeast		x
M11	Nutrient	Bacteria		x
M12	Nutrient	Bacteria		x
M13	Nutrient	Bacteria		x
M14	Nutrient	Bacteria	x	
M15	Nutrient	Bacteria	x	
M16	Nutrient	Bacteria	x	
M17	Caseinate	Bacteria		x
M18	Caseinate	Bacteria		x
M19	Caseinate	Bacteria		x
M20	Caseinate	Bacteria	x	
M21	Caseinate	Bacteria	x	
M22	Caseinate	Bacteria	x	
M23	Sabouraud	Yeast	x	
M24	Sabouraud	Yeast	x	
M25	Sabouraud	Yeast	x	
M26	Sabouraud	Yeast	x	

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The identification of bacteria in this work was not performed in this study.The results show that from the twenty-six morphotypes isolated, 57% were in the areas of influence of negative current (cathode) and 43% in the areas of anode (positive charges). From the morphotypes isolated in the area of influence of the cathode, 66% are bacteria and 34% are fungi and yeasts, and morphotypes isolated in the region of influence of the anode, 91% are bacteria and only 9% are fungi and yeasts. Of the 20 bacterial morphotypes found and separated, 10 were isolated from the anode and 10 from the cathode region. From the six morphotypes of fungi and yeast isolated, five were removed from the cathode region and only one from the anode region. Therefore, it can be seen that bacteria can have an affinity for both charges: positive and negative, but fungi and yeasts have greater affinity for the anode, where the positively charged particles are transported to.

In the literature there is no consensus on the number of bacteria present in the bioremediation system. Magalhães *et al*. (2004) isolated 75 colonies of a soil contaminated with hydrocarbons in the municipality of Guararema in Sao Paulo. Teixeira and Bento (2007) isolated and characterized 37 bacteria from soil contaminated with gasoline in Rio Grande do Sul. Nakamura *et al*. (2009) isolated 19 bacteria with potential for aromatic hydrocarbon biodegradation in Amazonian dark earth. Hughes (2007) isolated six fungi from soil samples contaminated with oil in the region of Adelaide Island, Antarctic Peninsula. Also from a contaminated soil, Potin *et al*. (2004) obtained 21 fungal isolates in MYEA medium.

Sarma *et al*. (2014) observed the presence of 39 respective strains, in crude oil contaminated soil, collected from five sites (Lakuwa, Geleky, Amguri of Sivasagar district, Borhola and Jorhat) urban area of Jorhat district, Assam, India.

Bisognin (2012) isolated three yeast strains, 19 fungi and 35 bacteria from the same contaminated soil used as basis for this work, using the same isolation technique performed in this study. However, the author used the biopiles process for the remediation of this soil.

A comparison of the results, obtained by Bisognin (2012) and this work, demonstrated that the type of process employed to promote bioremediation influences the number of populations of microorganisms active in the process. Apparently, the stress caused by the introduction of an electric current in the soil allows only certain types of fungi and bacteria to thrive and operate under these conditions.

From the serial dilutions and the sowing of the suspensions made in the Petri dish with solid culture medium, the colonies of microorganisms that began to appear approximately 3 days after incubation were visualized. The isolated colonies were those that presented visually distinct characteristics, with different colors and shapes, trying to achieve the greatest possible diversity among the species that have grown.

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After the isolation of fungi and yeast strains, cover slips were prepared for identification and analysis of microorganisms. The morphology of the strains was analyzed by light microscopy for characterization of the various microbial groups. Of the 6 originally isolated strains of fungi and yeasts, 4 species of fungi were identified.

Thus, by optical microscopy, four different kinds of filamentous fungi were identified. The genera were: three strains of *Penicillium sp*.; one strain of *Paecilomyces sp*.; one strain of *Trichoderma sp*.; and a strain of *Cladosporium sp*. Consequently, from the six isolated morphotypes, 50% are of the genus *Penicillium sp*., other genres are divided into: *Paecilomyces sp* 16.6%, *Trichoderma sp* 16.6% and *Cladosporium sp.* 16.6%.

In Araújo and Lemos research (2002), it was carried out the isolation and identification of filamentous fungi with oil degradation capacity. Starting from a soil contaminated with 5% w/w of oil, eighty strains were obtained, of which sixty showed the ability to degrade petroleum hydrocarbons. The identification of microorganisms grouped them into four genera of fungi (*Aspergillus sp*, *Penicillium sp*, *Paecilomyces* and *Fusarium sp*) subdivided into the following species: *Aspergillusterreus sp*, *Aspergillusfumigatus sp*, *Aspergillusversicolor sp*, *Aspergillusniveus sp*, *Aspergillusniger sp*, *Penicilliumcorylophilum sp*, *Parcilomycesvariotti sp*, *Paecilomycesniveus* and *Fusarium sp*.

Winquist *et al*. (2014) treat the contaminated soil in laboratory and field scales, were degraded 96% of total PAHs in three months, use the fungi *Phanerochaete velutina.* This same fungi demonstrated the efficiency degrade TNT in contaminated soil (Anasonye et. al., 2015). Scale up of the remediation was successfully implemented, as the fungus main- tained strong growth in non-sterile contaminated soil, tolerated high levels of TNT found in the soil and above all degraded the contaminant. Fungal treatment technology is effective and has potential to be use in full-scale remediation of TNT contaminated sites.

The finding of these fungi in this work is important, since they are capable of degrading hydrocarbon type environmental contaminants, particularly those with higher molecular weights. The bioremediation potential can be increased by optimizing environmental parameters, along with the selection of strains with desirable characteristics such as rapid growth and high survival rates at higher concentrations of contaminants (Bennet *et al*., 2001). The fungi can produced some trans-diols e potent carcinogens are formed Souza *et al*. (2014) so this capacity needs be studied more, and his metabolites too.

Biodegradation experiments with isolated microorganisms

The biodegradation experiments were performed with all strains isolated, with duplicate biodegradation tests, with 4 control cells (without addition of any kind of strains). The results showed that twenty of total isolated types presented diesel oil degradation capacity, being 15

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bacterial (M2, M4, M6, M7, M8, M11, M12, M13, M14, M17, M18, M19, M20, M21, and M22) and the five fungi-form (M9, M23, M24, M25, and M26). For morphotypes with biodegradability capacity, there was a temporal difference in the color change of the medium containing DCPIP. The complete discoloration after 24 hours was observed only in samples (wells) containing the M6 morphotype. On the other hand, the partial discoloration in 24 hours was observed in the wells inoculated with the morphotypes M2, M4, M7, M8, M9, M13, M17, M19, M22, M24 and M26, and almost complete discoloration occurred at the same time in wells M11, M12, M14, M18, M20, M21, M23, and M25. The morphotypes M1, M3, M5, M10, M15 and M16, did not obtained any discoloration in the tests.

Data from biodegradation tests indicate that the isolated M6 has the highest degradation capacity of commercial diesel, followed by M11, M12, M14, M18, M20, M21, M23, when compared to other isolates. From the fungi identified, all of them had the ability to degrade hydrocarbons compounds. These results coincide with those of Bisognin (2012), which also found the ability of these fungi to degrade hydrocarbon compounds. The presence of bacteria that did not showed direct hydrocarbon degradation capacity does not exclude them from the remediation process. These bacteria may be using the intermediate and/or waste products produced by other microorganisms that use the contaminants as a direct substrate.

According to the results, 77% of isolates had the potential to degrade diesel oil. Guisado *et al*. (2015), observed that the bacterial capacity was the result of use the carbon source to growth. Using DCPIP for the analysis of degradation, Gomes (2004) examined eight bacterial morphotypes isolated from samples contaminated with oil derivates, of these, four (50%) showed biodegradation potential. Souza *et al*. (2010) had 93% of the microbial strains isolated as being capable of degrading gasoline and Vieira *et al*. (2007) found 93.3% of bacterial isolates that showed biodiesel degradation potential. It is this adaptation that results in an increase in the proportion of hydrocarbon degradation. The presence of isolated microorganisms in the contaminated area indicates that perhaps they are able to metabolize existing chemical species, thereby decreasing the concentration of contaminants in the environment and therefore show promise for further studies involving the bioremediation process in this location. More studies

Benzene, Toluene, Ethylbenzene, m,p-Xylene and Xylene degradation

The BTEX compounds degradation results are shown in Figure 3 that indicated the behavior of contaminants changes throughout the process. The concentration of the compound m, p-Xylene increases from 0.461 mg/kg to 3.188 mg/kg after 90 days. This same pattern was observed with the other BTEX compounds in this time interval. However, after 90 days all compounds showed a decrease of their concentration up to 150 days of testing, where BTEXs were no longer detected in the chemical analysis or were analyzed close to the detection limit of analytical system. Apparently, the application of TWEEN 80 and the increase of the electric potential difference from the 120-day trial did not affect the degradation kinetics of the BTEX.

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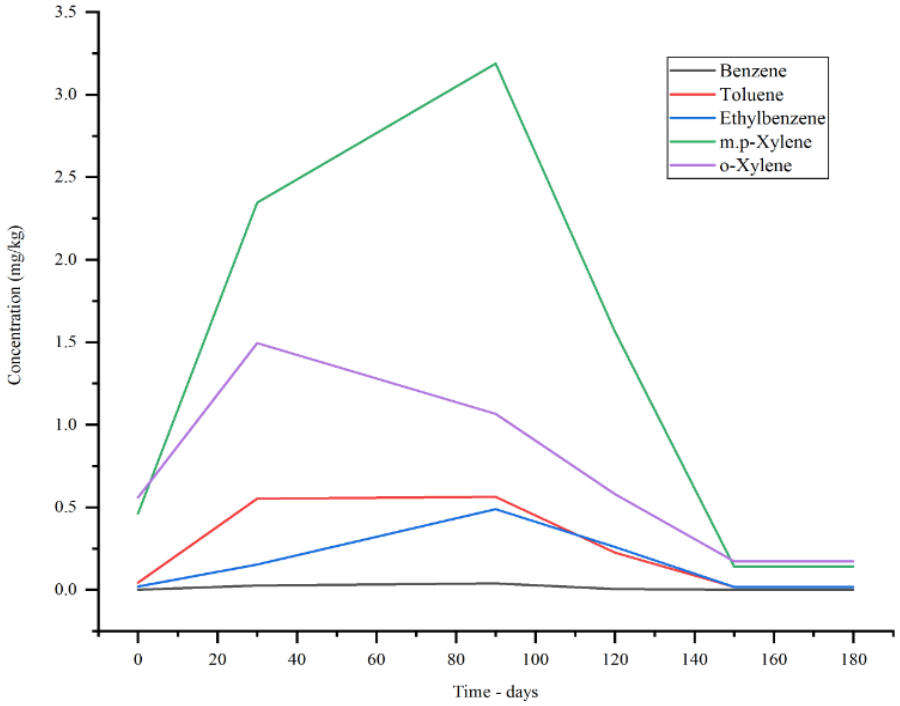

Figure 3. Degradation of BTEX compounds during the process.

The increase of the BTEX concentration in the samples may be associated with increased desorption of these compounds and transport of those by electro-osmosis process. This greater movement of these compounds to a particular area of concentration of contaminants (the area where soil samples were removed) may have influenced the results.

This higher desorption and transport of these compounds may have influenced the microbiological activity. If the same is compared with the increase of microbial populations, it can be seen that this coincides with the concentration increase of these elements in the soil. That is, the desorption and the electro-osmotic process increased the bioavailability of these compounds which, due to its physical-chemical characteristics (low molecular weight), are more biodegradable than other compounds of higher molecular weight, allowing the bioaugmentation of the studied microbiological populations. Rene *et al*. (2007) demonstrated that increasing the concentration of benzene and toluene in soil samples stimulates the biomass growth, increasing the rate of degradation of BTEXs compounds, Guisado *et al*. (2015) observed the phylum *Actinobacteria* that was capacity growth in contaminated sample with BTEX and damostrated significant degradation potential.

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Another factor that should be considered when analyzing the degradation of the BTEX, especially after 120 days, is the effect of the applied voltage on the generation of gas. The increase of this voltage increased gas production rate. This increased generation may have led to a greater removal by the process of BTEX "Stripping" since these compounds are quite volatile.

Degradation of TPH, UCM, HRP and total n-alkanes

The analysis of soil samples for compounds TPH, UCM, HRP and total n-alkanes are shown in Figure 4. The results showed that all compounds had an increase in their concentration with the lapse of test time. Thus, it was observed that the TPH increased from an initial concentration of 1.615,44 mg/kg to 4.418,78 mg/kg after 180 days of testing. The peak was reached at 120 days, reaching the concentration of 4725.5 mg/kg of TPH. The same behavior was observed with n-alkanes, HRP and UCM. Even with the increase of applied voltage from 5.0 VCC to 15.9 VCC and the addition of surfactant solution at 120 days of testing did not interrupted the trend of increasing concentration of compounds. On the contrary, the increase of contaminants concentration was enhanced after the addition of the surfactant solution and the increase of the applied voltage, as can be seen from the results of 120 and 180 days of the experiment. The contrast, was observed for Gomez and Sartaj (2014) with higher rates removal of TPH in contaminated soil by biopiles with combination of microbial consortium that was added. Cerqueira *et al*. (2014) observed that the addition of nutrients don´t showed some influence in biodegradation rates.

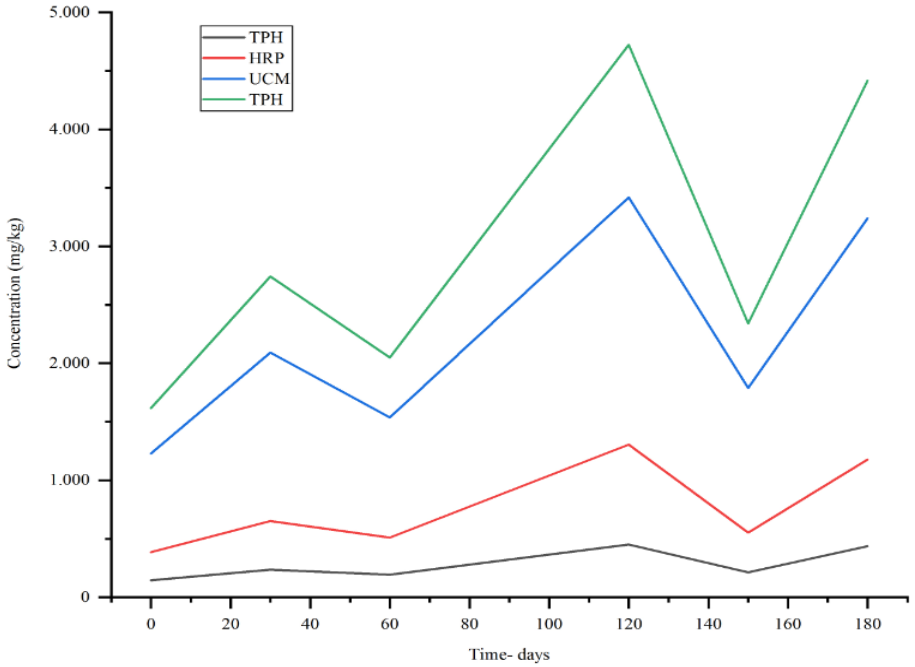

Figure 4. Hydrocarbon degradation behavior during the electro-bioremediation test.

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The higher increases observed between 120 and 180 days of testing could be explained by increase of desorption of the contaminants caused by the surfactant, generating more bubbles due to increase of the applied voltage, enhancing the flow caused by electro-osmosis and increased conductivity (Electrically -Migration) caused by addition of NaCl. This was observed of Suja *et al*. (2014) that use nutrients, and the activities was increase, and the TPH biodegradation process especially in the diesel range (C13 e C28). Another interpretation was observed for Cahan *et al*. (2013) that analyzed the biofilm produced can be by the bacterial activity that used the coal with source of carbon. The use of commercial surfactant more biofilm production on the test system can have direct influence on the reduction of the concentration of contaminants.

The behavior exhibited by these compounds can be associated with various factors derived from the effect of the application of electric current in the soil. As mentioned before, the electro-kinetic process may promote desorption of organic compounds from soil particles as well as enhancing its movement by means of electro-osmosis Gill *et al*. (2014). The electro-osmosis promotes transport by means of a hydraulic flow (advection) from the anode towards the cathode. These two processes would facilitate the concentration of hydrocarbons surrounding the cathodes in the process. The process can enhance the delivery of nutrients, electron acceptors and the electron donors (Wu *et al*., 2007; Lohner *et al*., 2008; Tiehm *et al*., 2010; Xu *et al*., 2010; Pazos *et al*., 2012) observed this phenomenon with the same samples: toluene, diesel and PAHs. Thus, during sampling, it may have been taken soil samples that were concentrated with these compounds. Kappel (2010), working with an electro-kinetic remediation process of oily sludge from fuel storage tanks (as used in this work) observed an increase in the concentration of hydrocarbon compounds with test time lapse. This researcher demonstrated that the electro-kinetic process was promoting phase separation (liquid/solid), causing the diesel oil to separate from the metallic phase of the sludge, concentrating in the top of the reactor, altering the quality of samples and results.

Another phenomenon that cannot be discarded within the electro-kinetic process is the electro-flotation of hydrophobic compounds, such as hydrocarbon compounds. If compared the increase of contaminants concentration with the population of microorganisms present in the soil, it can be observed that there is a direct relationship, especially with the number of bacteria in the system. However, the increase in present microbial population is not sufficient to biodegrade the hydrocarbons available by the electro-kinetic process (at least in the time interval used in this work). This observation is supported by the increase of UCM. The UCM represents partially degraded hydrocarbon compounds. Although their degradation is not exclusively one way biodegradation, increase in its concentration shows that the microorganisms are using hydrocarbons as substrate.

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PAHs concentration and biodegradation

In this study was investigated some 6 PAHs of 16 principals polycyclic aromatic hydrocarbons listed by EPA (United States Environmental Protection Agency) Simpanem *et al*. (2016). The results of the concentration analysis for PAHs are shown in Figure 5.

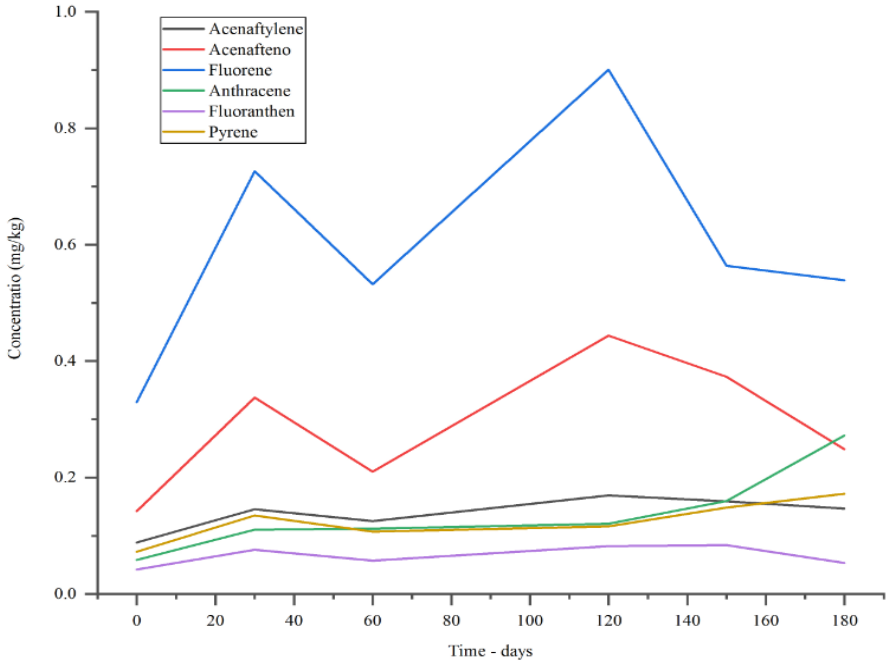

Figure 5. Behavior of the main polycyclic aromatic hydrocarbons (PAHS) during the electro-bioremediation process.

The reduction of the concentration of PAHs, only between 120 and 180 days of testing, may be associated with a characteristic of PAHs that are hydrophobic organic compounds with a low aqueous solubility Simpanem *et al*. (2016) and the PAHs compounds are resistant to the utilization by bacteria, that can degrade chemicals only in dissolved water. The increase of degraded PAHs in 120 – 180 days may be associated with higher rate of biodegradation of these components after addition of the surfactant and the increase of the applied voltage, Simpanem *et al*. (2016) observed the used the extractor enhanced the removal the PAHs with complex structures. Maturi *et al*. (2009), working with electro-kinetic remediation of PAHs contaminated soils found that these compounds migrate from the anode to the cathode by electro-osmosis. The results obtained in growth medium PAHs in the first 120 days of experiments can be associated with the desorption of these contaminants from the soil, and because of low activities of microorganism in initial days of experiment, Winquist *et al*. (2014) explain, the aerobic bacterial degradation of compounds is initiated by an oxygenation of tin his structure, after 120 – 180 days some PAHs

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declined, which may be interconnected with the increase of bacteria and fungi, as well as by the molecular weight of compounds initially being degraded by microorganisms. The variation of PAHs was also analyzed by (Juhasz; Aleer; Adetutu, 2014) that attribute this variation to high molecular weight and the recalcitrant nature of PAHs.

In his work, Winquist *et al*. (2014) observed that 3-ring PAHs was degraded 92%, and 4-ring PAHs 95% this can be explained by this study, observed an initial decay of the compounds acenaphthylene and fluoranthene. Furthermore, in accordance with Table 2 it is in the cathode that was verified greater number of isolated morphotypes, both for“bacteria” and “fungi and yeast”. Thus, these compounds would be degraded in this part of the reactor by present microorganisms, using them as a substrate.

Conclusions

The electro-kinetic process was positive for bacterial populations, as showed increases the fungi and yeasts and bacteria and note interfere in their growth. The bacterial communities were influenced by electro-chemical principal in the area of the cathodes where was detected the higher numbers of strains of fungi and yeasts. The bacteria showed no preference for any of the electrode areas, being distributed more homogeneously within the reactor. This work showed that about 77% of soil microorganisms (bacteria, fungi and yeasts) had capacity to degrade hydrocarbons.

The results of BTEX degradation showed that removal efficiency of these compounds depends on the applied current.

Regarding the compounds: TPH, UCM, HRP, PAHS and alkanes, it was not observed reduction in their concentration. The results indicated that the process mainly promoted a liquid-solid phase separationand further accumulation of the compounds by electro-osmosis in certain points of the reactor.

The electro-bioremediation process proved little efficiency during degradation tests, the parameters examined showed the actual process efficiency, thus requiring a better approach in the phenomena involved and seeking an elucidation of the effects which can be beneficial to the contaminant degradation process.

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