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Evaluation of the efficiency of the biological process in the treatment of effluents from automotive workshops

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Abstract: The activities of the oil production chain generate impacts on the environment justified in the name of development. However, the growing pressures for a management that can add to its growth, environmental, social and economic aspects have permeated the daily lives of companies potentially causing impacts. The objective of this study was to research alternative processes for the treatment of effluents, for this purpose it analyzed the efficiency of the biological process in the treatment of effluents originated from automotive maintenance workshops. In the treatment, he performed the bioremediation with the use of the "bacterial strain" with the potential for biodegradation of phenol. The results obtained in theprocessof 66% in thedegradation of the lubricating oil, the treatment can be applied in the treatment of the effluents of the automotive workshops.
Keywords: Automotive workshops, Bacterial strain, Biological process, BTEX, Effluent treatment.

I. INTRODUCTION

The oil industry has become fundamental to the development of a stable and solid economy in the pursuit of well-being for man. After the extraction, the oil by the refining process, where it performs the transformation of the raw material into several products, among which we have lubricating oil. Lubricating oils represent about 2% of petroleum products and are widely used for industrial purposes (SILVA, 2014).

According to Capelli (2010), the lubricating oil is used by the automotive industry as the main element in the reduction of friction of the engine components, resulting in less wear, thus increasing the life of the engine. The big problem is that this oil has an expiration date to be used after being placed in the engine, it has a durability between 6,000 and 8,000 kilometers, or 6 months, whichever comes first. According to Rodrigues (2013), the number of automobiles in Brazil increased from 24.5 million in 2001 to 50.2 million in 2012, which represents a 104.5% growth in that period.

With the huge fleet of vehicles running in Brazil and worldwide, there is also a large amount of used automotive lubricating oil that is discarded in several parts of the city at the same time. According to the National Petroleum Agency - ANP (2020), 1,408,931,436 litersof lubricating oils were sold in 2014.

According to data from DETRAN-CE (2020), thevehiclefleet in the municipality of Tabuleiro do Norte-CE is 15,801 vehicles, of which 884 are large vehicles, such as trucks and trailers, that is, 5.6% of the total fleet of the municipality. The estimated monthly consumption of lubricating oil for the fleet of large vehicles in the municipality is 12,376 liters.

According to Silva (2014), knowing that the disposal of automotive lubricating oils is a common reality in the daily work of servants in workshops and gas stations, some questions arise from this reality: what are the environmental impacts resulting from the improper disposal of used automotive lubricating oils? How do the employees of these establishments promote the disposal of this waste? What are the measures to reduce environmental impacts?

According to Soeiro (2014), simple activities such as washing a car can contaminate water with oil derivatives, considering possible leaks of fuel and / or oil, during washing this material can be dragged with water, migrating to rivers, seas and for underground water reserves. In view of this fact, mechanical workshops and jet washes are projects with high potential for water contamination.

Vehicle maintenance activities, such as vehicle washing, in addition to being a means of wasting water, generate large amounts of waste, since car wash waters may contain surfactants of various types, biodegradable or not, leftovers of dust, soot, grease, gasoline and all types of waste produced by automobiles (ASEVEDO; JERÔNIMO, 2012).

From this problem, some techniques can be used for the treatment of effluents produced in the process of maintenance of automobiles. Chemical and biological processes are presented as alternatives to minimize the environmental impacts caused.

II. BTEX

According to Heleno et al (2010), aromatic compounds, especially hydrocarbons called BTEX (benzene, toluene, ethylbenzene and xylene isomers), are among the main contaminants in water.

The presence of these compounds is commonly associated with atmospheric deposits, oil spills and some of their derivatives, chemical effluents, among others, due to high toxicity, representing a risk to human and animal health. Human exposure to these compounds can lead to the development of health problems, from irritation of the eyes, mucous membranes and skin, to weakening of the central nervous system, depression of the bone marrow, to the development of cancer, in the case of benzene, a compound classified by the World Health Organization as a potent carcinogen (HELENO et al, 2010).

According to Corseuil and Marins (1998), BTEX are dangerous chemical substances, as they are depressants of the central nervous system and cause leukemia in chronic exposures. Hydrocarbons by nonpolar molecules have little solubility in water. According to Tiburtius et al (2004), we cannot ignore this solubility, despite being very small, aromatic hydrocarbons are generally more toxic than aliphatic compounds with the same number of carbons and greater mobility in water, due to their solubility in water is about 3 to 5 times higher. In addition to migrating more quickly through waters than water supplies, aromatics can cause chronic toxicity more significantly than aliphatic hydrocarbons.

III. BIOREMEDIATION

The bioremediation process is carried out with the use of microorganisms, naturally occurring (native) or cultivated, to degrade or immobilize contaminants in groundwater and soil. Commonly, the microorganisms used are bacteria, filamentous fungi and yeasts. Of all microorganisms, bacteria are the most used and, therefore, are considered as the main element in works that involve the biodegradation of contaminants. They are defined as any class of single-celled microorganisms, usually aggregated in colonies, that live in different environmental compartments. They are important, due to their biochemical effects and for destroying or transforming potentially dangerous contaminants into compounds that are less harmful to humans and the environment (NRC, 1993).

With regard to the types of use of the technique, as for the treatment site, in-situ bioremediation is the most used in the world. However, regardless of the application site, bioremediation, as well as other chemical degradation techniques, has as its main objective the complete mineralization of contaminants, that is, transforming them into products with little or no toxicity (harmless), such as CO_2 and Water. In short, microorganisms metabolize organic substances, from which nutrients and energy are obtained. In order for this to occur, microorganisms must be active to perform their biodegradation task.

Microorganisms that originate in the subsoil can develop the ability to degrade contaminants after a long period of exposure. These microscopic beings adapt to low concentrations of contaminants and are located in regions external to the contamination plume and, rarely, will be present in the free phase (concentrated organic phase). Organic compounds are metabolized by fermentation, respiration or co-metabolism (CETESB, 2004). The bioremediation process can be aerobic or anaerobic, requiring oxygen or hydrogen, respectively. In most places, the subsoil is lacking in these species (oxygen or hydrogen), which prevents microorganisms from reproducing and completely degrading the target contaminant. In addition to these two processes, bioremediation can also occur in a co-metabolic way (VIDALI, 2001).

In "aerobic bioremediation", which requires an oxidizing medium, oxygen acts as an electron receptor and contaminants are used by microorganisms as carbon sources (electron donor), necessary to maintain their metabolic functions, including growth and reproduction . BTEX compounds fulfill this function as electron donors, if there are enough receptors (dissolved oxygen) for the reaction to occur. When oxygen is totally consumed, the microorganisms start to use other natural electron receptors available in the soil, and this consumption occurs in the following order of preference: nitrate (denitrification reaction), manganese, iron, sulfate and carbon dioxide, being this, converted into organic acids to generate methane (AELION; BRADLEY, 1991).

Anaerobic bioremediation occurs through the action of electron donor species, responsible for the degradation of halogenic pollutants. It is a process in which microorganisms, when metabolizing alternative carbon sources (other than the contaminants of interest), release hydrogenated inorganic compounds, hydrides (H-), which react with the contaminant's molecules and replace a chlorine atom (hydrogenolysis) or simultaneously remove two adjacent chlorine atoms creating a double bond between the carbon atoms. This process is ideal for use in places contaminated by organochlorine compounds, such as perchlorethylene (PCE), since the carbon source stimulates the reaction in bacteria called halorespiration or halo elimination. Although this principle, also called reductive dechlorination, is apparently simple, the difficulty of the technique lies in creating an ideal model of carbon source for a given microorganism. This carbon source must contain

compounds that are preferably and easily metabolized by microorganisms in the presence of contaminants (ACTON; BARKER, 1992).

According to Garnier et al (2000), the co-metabolic bioremediation process is one in which degradation occurs through the action of enzymes produced by microorganisms for other purposes. For co-metabolism, if there is no main substrate, that is, preferential sources of carbon, the degradation mediated by microorganisms does not occur for a given component, defined as a co-metabolized contaminant, and in the presence of a carbon source, the metabolization of the primary substrate may generate enzymes capable of degrading the contaminant of interest.

According to Embar et al (2006), some microorganisms survive in extremely adverse environmental conditions. Additionally, research shows that different microorganisms can degrade different substances, among them, recalcitrant substances, such as petroleum hydrocarbons. In some cases, certain microorganisms are more specialized in degrading specific contaminants.

The critical factor in defining whether bioremediation is the most appropriate technique for the treatment of the contaminated site is the biodegradability of the contaminant. Therefore, the detailed study of each parameter that affects biodegradation must be done cautiously by those responsible for the remediation project. This application has been studied as a promising alternative for the treatment of environments contaminated by oil and its derivatives.

IV. PROCEDURE WITH BACTERIA

The bacterial strains were isolated from environmental samples in Cubatão / SP, according to the methodology described by Pereira (1993) in the culture media presented below.

Culturemediums

The bacterial strains were grown in Plate Count Agar (PCA), Nutrient Broth and Minimal Mineral Medium, where all cultures were suitable for bacterial growth.

- Plate Count Agar (PCA
  Hydrolyzed Casein Peptone 0.50 g
  Yeast Extract 0.25 g
  Dextrose 0.10 g Agar 1.50 g
  Distilled water q.s.p. 100 mLpH 7.0 at 25ºC
  The medium was sterilized in an autoclave at1atm, 121ºC for 15 minutes.
- Nutrient broth
  Meat Peptone 0.50 g
  Yeast Extract 0.15 g
  Meat Extract 0.15 g
  Sodium Chloride 0.50 g
  Distilled water q.s.p. 100 mLpH 7.4 at 25ºC
  The medium was sterilized in an auto clave at1atm, 121ºC for 15 minutes.
- Minimal Mineral Medium
  K2HPO4 0.1 g
  MgSO4 7H2O 0.02 g
  NaCl 0.01 g CaCl2 0.01 g
  FeCl2 0.002 g
  (NH4) 2SO4 0.1 g
  Distilled water q.s.p. 1000 mLpHadjustedto 7.0
  The medium was filtered through a 0.45 μm Millipore membrane

Bacterial isolation

The microbial population, from these samples, was adapted to grow in crude oil to an initial concentration of 1,000 μg.L -1 . The adaptation process was carried out in reactors, submitted to room temperature with aeration. Crude oil was gradually added every 24 hours until reaching a concentration of 3,000 μg.L -1 , in the reactor. Plating of the samples was performed daily to ensure the viability of the microorganisms.

After 10 days, the samples were plated in PCA culture medium with incubation at 36ºC for 24h to obtain pure cultures.

Preservation of bactéria

The strains were preserved by the method of continuous raising in an inclined tube with PCA medium, in duplicate, kept under refrigeration at 10ºC, according to the methodology described by Torrezan et al. (2000).

Determination of the Minimum Inhibitory Concentration (MIC) of crude oil for the microorganisms present in the enrichment reactors.

To determine the Minimum Inhibitory Concentration (MIC), which represents the oil concentration necessary to inhibit bacterial growth, so that the lower the MIC, the greater the difficulty for the bacteria to develop, adaptation of the method proposed by Souza (2000): In 250 mL Erlenmeyer flasks, 1 mL of the bacterial strains isolated in the reactors was inoculated in 90 mL of minimal mineral medium, with varying volumes of oil solutions, so that each of the flasks remains at concentrations of 0; 100; 200; 400; 600; 800; 1,000; 3,000; 3,500 and 4,000 mg.L -1 of crude oil, adjusting the final volumes to 100 mL with minimal mineral medium.

Biodegradation test and quantification of phenol consumption

For the quantification of phenol consumption, the method: spectrophotometric with the Nanocolor kit (MACHEREY-NAGEL®) was used. For each bacterial strain and for the control, a bioreactor containing 90 mL of minimal mineral medium was used and crude oil was added for concentrations of 500 mg.L -1 , 1,000 mg.L -1 , 1,500 mg.L -1 . No bacterial suspension was added to the flask used as a control, and 1 mL of bacterial suspension from one of the isolated strains was added to the others. The flasks were incubated at 36ºC in an oven.

In the test tube of the kit, 4 mL of the study sample was added, diluted 1,000 times, together with the reagents of the kit (duly dosed by the trader), it was stirred and left to act for 5 minutes for measurement in the spectrophotometer, with ultraviolet detector. visible, at a wavelength of 520 nm. The spectrophotometer presents software for the quantification of phenol, when using the Nanocolor kit (MACHEREY-NAGEL®), eliminating the construction of a calibration curve or standard curve.

Quantification of Phenol Consumption

The biodegradation tests, showing the growth in mineral medium and phenol as the only carbon source, demonstrated that these strains were able to consume the oil in a period of 24 hours, decreasing the initial concentration. The quantification of phenol reduction in the culture medium was performed using spectrophotometer methods.

The phenol degradation tests by the bioremediation process, were carried out on average two experiments in triplicate, where they were supplemented with varying concentrations of phenol.

The results of Phenol in concentrations, between 500 and 1,500 mg.L -1 , after degradation. The blank indicates the initial Phenol (quantity at the beginning of the treatment).

Table 1: Phenolconsumption

Concentration ($mg.L^{-1}$)	Removal (%)
Blank	0
500	20
1000	55
1500	66

The bacterial strain was placed to degrade phenol in a periodof 24 hours, at a concentration of 1,500 mg.L-1 showed an efficiency of 66%.

V. CONCLUSION

The bacterial strain showed the capacity to consume phenol, with a percentage of 66%, using in the concentration of 1,500 mg.L -1 in the period of 24 hours.

Koehntopp (1998) states that the bacterial strain has the ability to degrade phenol present in effluents, consuming it with concentrations of up to 500 mg.L-1, as the only source of carbon and energy.

The results demonstrate that the consumption of higher concentrations becomes more efficient in the same period of time, since in both concentrations, 1 mL of bacterial suspension was used. Confirming Koehntopp (1998) that due to a higher concentration of phenol, it allows a greater growth of the colony.

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