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Use of bacterial Cepa for treatment of effluents contaminated with used lubricant oil

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Abstract: Seeking to research alternative processes for the treatment of effluents, this work studied the biological process to verify the efficiency in the treatment of effluents originating from automotive maintenance workshops and gas stations. In this treatment, he performed bioremediation using a "bacterial strain" with the potential to biodegrade phenol. The bacterial strain showed the ability to degrade the phenol present in effluents, consuming it at concentrations of up to 500 $mg.L^{-1}$, as the only source of carbon and energy. Based on the data obtained, the bacterial strain was placed to degrade the phenol in a period of 24 hours, at a concentration of 1,500 $mg.L^{-1}$ showed an efficiency of 60%, the consumption of higher concentrations became more efficient in the same period of time. The results obtained in the phenol degradation process, the treatment can be applied in the treatment of effluents from automotive maintenance workshops and gas stations.

Keywords: Bacterial Cepa, Phenol, Wastewater Treatment, Lubricant Oil, Biological Process.

I. INTRODUCTION

This article aims to analyze mitigation measures to reduce environmental impacts caused by improper disposal of used lubricating oil by automotive maintenance workshops and gas stations.

Lubricating oil is used by the automotive industry as the main element in reducing the friction of engine components, resulting in less wear, thus increasing engine life. The big problem is that this oil has an expiration date to be used after being placed in the engine, it lasts between 6,000 and 8,000 kilometers, or 6 months, whichever comes first.

The environmental impacts arising from the activities of changing automotive lubricating oils in workshops and gas stations, with regard to the inappropriate disposal of lubricating oil, are diverse. When used lubricating oils are released directly into the environment (in water, sewerage and soil) or when burned in an uncontrolled manner, they cause serious problems of soil, water and air pollution. When released into the ground, the used oils infiltrate together with rainwater, contaminating the soil they cross and, when they reach the underground water table, they also pollute the water from springs and wells. When released into wastewater drainage networks, they pollute the water receiving media and also cause significant damage to wastewater treatment plants.

The objective of this work is to identify measures that reduce the environmental impacts caused by the improper disposal of used lubricating oil, using the bioremediation process with the Bacterial strain.

II. COMMERCIAL ESTABLISHMENTS AND ENVIRONMENTAL RISKS

In Brazil, the current system for transporting goods and people is entirely on road transport, despite the country's continental dimensions, thus revealing, in the number of vehicles in circulation, the importance of this system for the country's economy.

Automotive maintenance workshops are commercial establishments that carry out various activities related to the maintenance and conservation of vehicles, such as changing lubricating oil, changing and cleaning parts, engine overhaul (PAULINO, 2009), gas stations, in addition to the activities performed by the workshops, they carry out vehicle supply and washing. All these activities carried out in these establishments generate different types of solid waste and effluents, which when discarded improperly, can pollute the environment and cause damage to public health. These establishments are one of the main sources of release of petroleum products into the environment, being enough to contaminate the consumption of drinking water.

The main sources of contamination in automotive maintenance workshops are characterized by:

- Spillage of "used lubricating oil" in the oil change operation;
- Unqualified professionals in handling used lubricating oil;

• Improper installation.

Several types of solid waste are associated with these activities, among the main ones are the disposal of tires, metal parts, grease, dirty cloths and rags, paints, solvents, packaging of parts and, mainly, lubricating oils that deserve attention special (GOMES FILHO, 2014) . According to CONAMA resolution nº 362 of 2005, in its 12th article, the disposal of used or contaminated oil in soil, subsoil, in springs, coastal region, in sewers or even in wastewater evacuation is totally prohibited.

A part of the automotive lubricants used in the world trade are derived from petroleum. This is composed in its natural state of a complex mixture of several different compounds, most of which are formed by Carbon and Hydrogen, which are called Hydrocarbons. There are also small amounts of other substances, such as nitrogen, sulfur, oxygen and some metals (DUARTE, 2003).

The main environmental impacts caused by the activities in these commercial establishments are (ROCHA; SILVA; MEDEIROS, 2004):

- Waste, the main and most dangerous types being used or contaminated oil, sand contaminated with oil and/or fuel, lubricating oil filters, vehicle fuel and air filters, used oil containers and used tows.
- Contamination of soil and groundwater, occurring through spills of used lubricating oil and/or fuels, from the level ofspillage and soil characteristics can reach the water table causing the contamination of the neighborhood through wells, which are most often used as a source of water supply for people. Also, vehicle washing activities cause contamination of soil and sewer systems.
- Human contamination, which can occur through the dermal, respiratory and oral routes. Employees in these workplaces are susceptible to skin diseases, which can even be cancerous.
- Fire, whose effects, when they occur, are quite harmful to employees, customers, owners and the neighborhood, potentially causing fatalities. In view of these dangers, some precautions must be taken when handling petroleum products, in order to avoid fires and risks to people.

In automotive maintenance workshops, these potential environmental impacts can be summarized in the table below, indicating that the main activities developed in these commercial establishments are related to preventive and corrective maintenance of motor vehicles handling various petroleum products, both when receiving these products. and its storage, when using vehicles, washing the vehicle, cleaning the environment and treating liquid effluents in the water and oil separator box. These activities are subject to a series of incidents, ranging from the simple emission of gases to fires.

Table 1 - Environmental Impacts by activities developed

ACTIVITIES	INCIDENT	CAUSES	IMPACTS
Product Receipt	Product Spill / Fire	Extravasation and presence of ignition source	Soil / water / air quality
Product storage	Product leakage	Inadequate facilities	Soil / water
Lubricating oil change	Product spill	Inappropriate operations	Soil / water
	Waste release	Inappropriate disposal of packaging and waste	
Preventive and corrective maintenance activities	Product spill	Inappropriate operations	Soil / water
	Liquid effluents: oily water	lack of treatment	
	Waste releases	Inappropriate disposal of packaging and waste	
vehicle wash	High water consumption	Absence of recycling process	Degradation of the Underground Water Basin
Office	Waste releases	Inappropriate waste disposal	Soil / water

It is necessary to highlight Reverse Logistics (RL) for this branch of activity, as the automotive maintenance workshops must make use of this environmental management practice to give the final destination to the waste generated in their respective activities. The concept of reverse logistics is related to the planning, operation and control of the return flow of after-sales and post-consumer goods to the production cycle, through reverse distribution channels, adding economic, ecological and competitive values to them, thus improving the corporate image (LEITE, 2006).

When it comes to Reverse Logistics (RL) it should immediately be configured in the inverse of business logistics, which consists of transport, material handling, storage, order processing and information management, involving the cycle of activities that accompany the product, from its raw material to the final consumer.

III. POLLUTING AGENTS

Oil, in its natural state, cannot be used in a practical way for purposes other than the supply of energy through combustion, but its chemical composition based on hydrocarbons of great molecular heterogeneity opens up possibilities for specialized and sophisticated industrial uses, as required by modern internal combustion machines. Thus, oil, also called black gold, is the main raw material used to produce several products such as fuels, lubricants and petrochemical products (FARAH, 2012).

Approximately 90% of the materials obtained from oil refining are used in combustion reactions, that is, they are burned to obtain energy for transportation, industrial and domestic heating, electricity production and lighting. The other 10% is used as raw material for the production of plastics, rubber and synthetic fibers, fertilizers, among others (USBERCO; SALVADOR, 2009).

The chemical composition and appearance of oil vary a lot, but its elemental composition varies little, as illustrated in Table 2, which can be explained by the fact that it consists of homologous series of hydrocarbons.

Table 2 - Average elementary composition of oil

Element	% in largescale
Carbon	83,0 a 87,0
Hydrogen	10,0 a 14,0
Sulfur	0,05 a 6,0
Nitrogen	0,1 a 2,0
Oxygen	0,05 a 1,5
Metals (Fe, Ni, V, etc.)	< 0,3

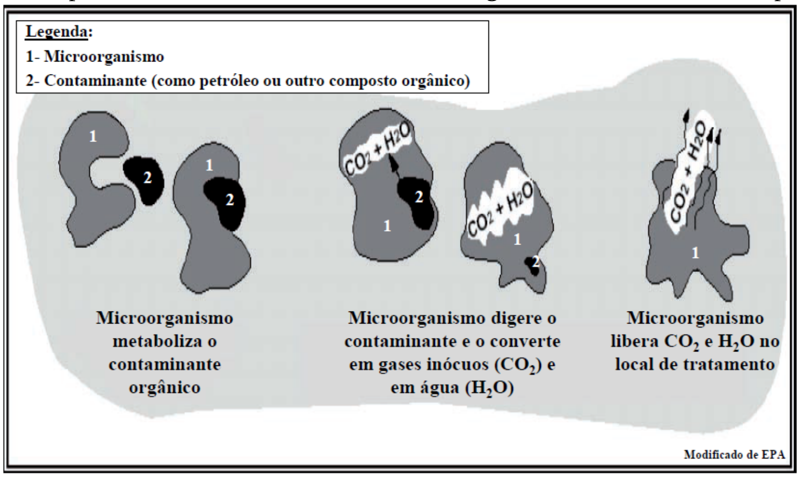
Refining basically consists of the fractionation of crude oil according to the boiling temperature ranges of each derivative which, in turn, may or may not be mixed with other fractions to compose the final derivatives. Fractions can also be subjected to chemical processes, in order to fit a derivative into current specifications or produce substances of industrial interest. Thus, petroleum derivatives are divided into two classes according to their purposes, they are: Fuels or Energy, for domestic, automotive, aviation, industrial and maritime use, and Non-fuel or non-energy, are lubricating oils, paraffins , fertilizers, solvents, asphalts, among others.

IV. BIOREMEDIATION

The bioremediation process is carried out using microorganisms, naturally occurring (native) or cultivated, to degrade or immobilize contaminants in groundwater and soils. Commonly, the microorganisms used are bacteria, filamentous fungi and yeasts. Of all microorganisms, bacteria are the most used and, therefore, are considered the main element in work involving the biodegradation of contaminants. They are defined as any class of unicellular microorganisms, usually aggregated in colonies, that live in different environmental compartments. They are important because of their biochemical effects and because they destroy or transform potentially dangerous contaminants into compounds that are less harmful to humans and the environment (NRC, 1993).

With regard to the types of technique used, regarding 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. For this to occur, microorganisms must be active to perform their biodegradation task. A general and simplified scheme of the action of microorganisms in bioremediation processes is shown in figure 1.

Figure 1 - Simplified scheme of the action of microorganisms in bioremediation processes.



Microorganisms that originate underground 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 will rarely 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 lacks 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 (VIDAL, 2001).

In "aerobic bioremediation", which requires an oxidizing medium, oxygen acts as an electron acceptor and the contaminants are used by microorganisms as carbon sources (electron donor), needed 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 take place. When oxygen is totally consumed, 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 halogen 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 molecules and replace a chlorine atom (hydrogenolysis) or simultaneously remove two adjacent chlorine atoms giving rise to a double bond between the carbon atoms. This process is ideal to be used in places contaminated by organochlorine compounds, such as perchlorethylene (PCE), since the carbon source stimulates the reaction in bacteria called halorespiration or haloelimination. 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 preferentially and easily metabolized by microorganisms in the presence of contaminants (ACTON; BARKER, 1992).

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, 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 acting in the degradation of the contaminant of interest (GARNIER; et al., 2000).

Some microorganisms survive in extremely adverse environmental conditions. Additionally, researches prove that different microorganisms can degrade different substances, among them, recalcitrant substances, such as petroleum hydrocarbons (EMBAR; FORGAS; SIVAN, 2006). 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 treating the contaminated site is the contaminant's biodegradability. Therefore, the detailed study of each parameter that affects biodegradation must be done carefully 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. Table 3 presents the main advantages and limitations inherent to soil bioremediation.

Table 3 - Advantages and limitations of bioremediation

Benefits	Limitations
The applicationinvolvesthe use ofequipmentthatiseasytoobtain, installandoperate.	Long-termcontinuousmonitoringand/ormaintenanceofthe site undergoingbioremediationmayberequired.
In in-situactivities, bioremediationgeneratesminimaldisturbancetoth eenvironment.	The techniqueisinefficient for organiccompoundsthat are adsorbedtothesoil, makingthemunavailable for biodegradation.
Underoptimaloperatingconditions, it haslowercostscomparedtoalternativeremediationt echniques.	It islessefficient in shorterperiodsof time comparedtootherremediationtechniquessuch as POA.
It canbecombinedwithothertechniques, such as EVS, tospeedupthedecontaminationprocess.	Lowsolubilitycontaminants in high concentrations, such as HTP, canbetoxictomicroorganismsand/or non-biodegradable, makingthetechniqueunfeasible.
In most cases, thistechnique does notproducetoxiccompounds, which must bedisposedofandtreatedelsewhere.	The physical, chemicalandmicrobiologicalpropertiesofsoilandclimaticcond itionscanalterthe rate ofbiodegradation.
It isveryefficient in biodegradingpetroleumand its derivatives in permeablesoils.	Difficultyof use onclayeysoilsorwithlowpermeability.

V. BACTERIAL CEPA

The bacterial strains were isolated from environmental samples in Cubatão/SP in the culture media:

- **Plate Count Agar (PCA)**
  Peptona de CaseínaHidrolizada 0.50 g
  Yeast extract 0.25 g
  Dextrose 0.10 g Ágar 1.50 g
  Distilled waterq.s.p. 100 mL pH 7,0 à 25ºC
  The medium was sterilized in an autoclave at 1 atm, 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 mL pH 7.4 at 25°C
  The medium was sterilized in an autoclave at 1 atm, 121ºC for 15 minutes.
- **Minimum Mineral Medium**
  K_2HPO_4 0.1 g
  $MgSO_4$ $7H_2O$ 0.02 g
  NaCl 0.01 g $CaCl_2$ 0.01 g
  $FeCl_2$ 0.002 g
  $(NH_4)_2SO_4$ 0.1 g
  Distilled water q.s.p. 1000 mL pH adjusted to 7.0
  The medium was filtered on Millipore 0.45 µm membrane

The microbial population from these samples was adapted to grow in crude oil at an initial concentration of 1,000 $\mu g.L^{-1}$. 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 $\mu g.L^{-1}$ in the reactor. Samples were plated daily to ensure the viability of the microorganisms.

The strains were preserved by the method of continuous subculture in an inclined tube with PCA medium, in duplicate, kept under refrigeration at 10ºC.

VI. BIODEGRADATION OF PHENOL

To quantify the consumption of crude oil, the method: spectrophotometric was used. For each bacterial strain and for the control, a bioreactor containing 90 mL of minimal mineral medium was used and the crude oil was added to concentrations of 500 $mg.L^{-1}$, 1,000 $mg.L^{-1}$, 1,500$mg.L^{-1}$. Bacterial suspension was not added to the flask used as control and to the others, 1 mL of bacterial suspension of one of the isolated strains was added. The flasks were incubated at 36ºC in an oven.

After bioremediation, a test was performed and it was observed that at the concentration of 1,500 $mg.L^{-1}$ there was a 60% reduction in phenol.

VII. FINAL CONSIDERATIONS

The oil industry, that is, the production, import, export, refining, processing, treatment, processing, transport, transfer, storage, storage, distribution, resale and marketing of oil products, is a factor causing pollution, whose consequences are large part are irreversible. The preventive maintenance activities of lubricating oil change by automotive maintenance workshops do not, therefore, escape this list.

The lack of environmental management of these establishments in the disposal of used lubricating oil, aggravating the environmental damage caused by domestic effluents, seeking to minimize the environmental impacts generated by the used lubricating oil, the bioremediation process was tested with the Bacterial strain to minimize the impacts caused by this pollutant. After bioremediation, a test was performed and it was observed that at the concentration of 1,500 $mg.L^{-1}$ there was a 60% reduction in phenol, the consumption of higher concentrations becoming more efficient in the same period of time.

Therefore, the results obtained indicate the feasibility of using the bacterial strain in the degradation of phenol, but more studies should be carried out to improve the use of the technique and reduce costs.

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