December 2019 Vol.5 No.16 17-25

Effectiveness of Humic Acids, Fulvic Acids and lechuguilla extract (A*gave lechuguilla*), as biosurfactants in the Remediation of soils contaminated with Hydrocarbons

Efectividad de los Ácidos Húmicos, Fúlvicos y extracto de lechuguilla (*Agave lechuguilla*), como agentes biosurfactantes en la Remediación de suelos contaminados con Hidrocarburos

RESENDIZ-VEGA, Marisol†* & GARCÍA-MELO, José Alberto

Universidad Tecnológica Tula – Tepejí, Chemistry Academic Corps

ID 1st Author: *Marisol, Resendiz-Vega* / **ORC ID**: 0000-0001-8199-6548, **Researcher ID Thomson**: T-7946-2018, **CVU CONACYT ID:** 819939

ID 1st Coauthor: *José Alberto, García-Melo* / **ORC ID**: 0000-003-0340-0272, **CVU CONACYUT ID**: 285374

DOI: 10.35429/JESN.2019.16.5.17.25 Received June 26, 2019; Accepted December 30, 2019

Abstract

The environmental pollution caused by oil and its derivatives is recognized as one of the most serious problems. Once spilled on the ground, most aliphatic compounds volatilize, while other hydrocarbons such as polycyclics persist on the surface causing an impact on the environment and living beings. There are a variety of techniques for treatment, but they have the disadvantage of being expensive, so viable alternatives have been sought such as bioremediation, which consist of making use of microorganisms and constitutes a very competitive technology, capable of achieving the biodegradation of hydrocarbons contained in the soils. Depending on the characteristics of the soil and the content of organic matter, the hydrocarbons of higher molecular weight and lower solubility can adsorb in the micropores of the soil particles, resulting in this being inaccessible as carbon and energy sources for the microorganisms. Therefore, surfactant agents that act by increasing bioavailability through the parallel action of the desorption and solubilization of the contaminant are required. This article compares 3 surfactant agents of natural origin, which analyze and discuss the effectiveness of each of them for the treatment of soils contaminated with hydrocarbons. Once the lechuguilla extract is obtained in cold and hot, the soil is washed, fats and oils are determined by soxhlet method and the Chemical Oxygen Demand (COD) is determined, finding a marked effectiveness of the cold lechuguilla extract (Agave lechuguilla).

Bioremediation, Hydrocarbons, Surfactant Agent

Resumen

La contaminación ambiental ocasionada por el petróleo y sus derivados se reconoce como uno de los más graves problemas. Una vez derramado en el suelo, la mayoría de los compuestos alifáticos se volatilizan, mientras que otros hidrocarburos como los policíclicos persisten en la superficie causando un impacto al ambiente y los seres vivos. Existe una diversidad de técnicas para el tratamiento, pero tienen la desventaja de ser costosas, por lo que se han buscado alternativas viables como la biorremediación, que consisten en hacer uso de microorganismos y constituye una tecnología muy competitiva, capaz de conseguir la biodegradación de los hidrocarburos contenidos en los suelos. Dependiendo de las características del suelo y del contenido de materia orgánica, los hidrocarburos de mayor peso molecular y menor solubilidad pueden adsorberse en los microporos de las partículas del suelo, resultando con esto ser inaccesibles como fuentes de carbono y energía para los microorganismos. Por lo que se requieren agentes surfactantes que actúan logrando incrementar la biodisponibilidad mediante la acción paralela de la desorción y solubilización del contaminante. En este artículo se comparan 3 agentes surfactantes de origen natural, que analizan y discuten la efectividad de cada uno de ellos para el tratamiento de suelos contaminados con hidrocarburos. Una vez obtenido el extracto de lechuguilla en frío y caliente, se realiza el lavado de suelo, se determinan grasas y aceites por método soxhlet y determina la Demanda Química de Oxígeno (DQO), Encontrando una marcada efectividad del extracto de lechuguilla frío (*Agave lechuguilla*).

Biorremediación, Hidrocarburos, Agente Surfactante

Citation: RESENDIZ-VEGA, Marisol & GARCÍA-MELO, José Alberto. Effectiveness of Humic Acids, Fulvic Acids and lechuguilla extract (A*gave lechuguilla*), as biosurfactants in the Remediation of soils contaminated with Hydrocarbons. Journal of Environmental Sciences and Natural Resources. 2019, 5-16: 17-25

* Correspondence to Author (email: marisol.resendiz@uttt.edu.mx)
† Researcher contributing first author.

Introduction

Petroleum products are currently the most demanded for power generation, this has resulted in an increasing number of contaminated soils due to spills. Rojas Molina, N. A. (2019) points out that the soils have been turned into heavy metal sinks and other pollutants. This, together with the growth of the population worldwide, causes great pressure on the availability of resources and land (Vreysen and Maes, 2005).

Environmental pollution caused by oil and its derivatives is recognized as one of the most serious problems, especially when associated with large-scale accidental spills (Plohl K, and Leskovsek H., 2002).

Once spilled on the ground, most aliphatic compounds volatilize, while other hydrocarbons such as polycyclics persist on the surface (Jiménez DJ, Medina SA, Gracida JN., 2010), causing an impact on the environment considering their toxic effects and for living beings (Tissot B and Welte DH., 1984). The most studied hydrocarbons are benzene, toluene, ethylbenzene and xylene also grouped under the BTEX and polyaromatic (HAP) appeal, naphthalene diaromatic and phenanthrene, anthracene and fluorene triaromatics (Kästner M and Mahro B.1996).

According to Paul EA and Clark FE. (1998), the extraction of hydrocarbons by vacuum, washing of soil contaminated with water, incineration and electrokinetic recovery are among others some of the most used techniques for soil treatment. These techniques have the disadvantage of being expensive, so viable, correct, simple and economical alternatives have been sought; and bioremediation techniques have been used, which consist of making use of microorganisms or plants (Bollag, J.-M. 1992), and constitutes a very competitive technology, capable of achieving the biodegradation of hydrocarbons contained in soils.

It is important to have mechanisms and techniques economically and environmentally sustainable soil remediation since pollution decreases arable and productive land, thus hindering sustainable economic development.

The contamination of the soil leads to the loss of the economic, cultural and environmental values associated with the use of the land, for example, if the soil of a property is contaminated, the monetary value of these sites, the local competitiveness of the area where they are located decreases, as does the quality of life and economic activity and sustainable development are inhibited.

Not protecting the soil also means the disappearance of environmental services, for example, to constitute the filter for the recharge of aquifers (the supply of drinking water is jeopardized) or to allow recreational activities (loss of social and cultural value), (Cubero-Mata, AV (2019).

One of the problems presented in the methods of remediation of soils contaminated with hydrocarbons is related to the hydrophobic nature of hydrocarbons, their low water solubility and high adhesion to soil molecules, are two reasons why it is necessary to increase the bioavailability of these so that they are more accessible to the microorganisms responsible for their degradation (Petro et al., 2014). Surfactants, which are amphipathic molecules that have the ability to interact with hydrophobic and hydrophilic compounds, being located at their interface.

However, the use of chemical surfactants adds an extra contaminant (Bognolo, 1999), so the use of biosurfactants would be more effective, since they have low toxicity, biodegradability, biocompatibility, they are environmentally friendly and equally effective bioproducts in the solubilization of pollutants and being biodegradable the changes in the structure of the treated soil are minor (Raiger et al., 2009).

Among the microorganisms that have most commonly been isolated in soils contaminated with hydrocarbons are the genera: *Arthrobacter, Bacillus, Pseudomonas, Agrobacterium, Alcaligenes, Flavobacterium, Corynebacterium, Micrococcus, Taphylococcus, Xanthomonas and Mycobacterium*, with a total population of 1% approximately 104 to 106 cells per gram of soil. Soils contaminated with hydrocarbons contain more microorganisms than unpolluted soils, but their diversity is reduced (Messarch M and Nies L. (1997).

Contaminants present in the non-aqueous liquid phase (NAPL) or absorbed in the soil matrix are usually not bioavailable, so the degradation rate is often limited (Aronstein BN and Alexander M., 1992). Depending on the characteristics of the soil and the content of organic matter, hydrocarbons of greater molecular weight and lower solubility can be absorbed in the micropores of soil particles, resulting in being inaccessible as sources of carbon and energy for microorganisms.

Therefore, in most cases, surfactants are required that act by increasing the bioavailability through the parallel action of desorption and solubilization of the contaminant (Singh A, et. Al. 2007, Helmy Q., et. Al. 2009) . To choose a surfactant for bioremediation, the type of pollutant to be remediated, the properties of the soil, as well as the properties of the surfactant itself must be taken into account, and finally the existence of microorganisms that degrade the pollutant to be remedied (Volkering F, 1998).

Surfactants are essential for the bioremediation process. Some microorganisms produce their own surfactant to solubilize hydrophobic organic compounds (Lange S and Warger F., 1987)). This article compares 3 surfactants of natural origin, which analyze and discuss the effectiveness of each of them for the treatment of soils contaminated with hydrocarbons.

Hypothesis

The working hypothesis states that the biosurfactant extracted from Agave lechuguilla Torrey, is as effective as humic and fulvic acids.

Objectives

General

To evaluate the effect of the extract of Agave lechuguilla torrey, humic acids and fulvic acids as biosurfactant agents, in the washing of soils contaminated with hydrocarbons

Specific:

1. Obtain soil from the surroundings of the Miguel Hidalgo Refinery and determine its fat and oil content.
2. Obtain Humic Acids (AHs) from humus, obtained from vermicompost.
3. Obtain Fulvic Acids (AF) from humus, obtained from vermicompost.
4. Obtain extract of Agave lechuguilla Torrey by cold method (Ambient temperature 20 ° C) and hot (50 ° C).
5. Wash contaminated soil with hydrocarbons.
6. Monitor the effectiveness of removal by comparing the fat and oil content, before and after washing.
7. Monitor the effectiveness of the removal by measuring the Chemical Oxygen Demand (COD) of the biosurfactant solutions used in the washing of each soil sample.
8. Analyze advantages and disadvantages of each surfactant.

Theoretical framework

Soil is defined as that unconsolidated material composed of inorganic particles, organic matter, water, air and organisms, which comprises from the upper layer of the earth's surface to different levels of depth (NOM-138-SEMARNAT / SS-2003). Maintains ecological systems, since they provide chemical and mineral components (as a result of biodegradation); and organic complexes such as humic and fulvic acids, enzymes, vitamins, hormones and antibiotics; They also have a rich genetic reserve. The soil constitutes a natural resource that performs various functions on the Earth's surface, providing mechanical support, as well as nutrients for the growth of plants and micro-organisms.

A contaminated soil can be defined as that in which hydrocarbons or foreign materials are present that, due to their quantities and characteristics, affect the nature of the soil. Land degradation can be defined as any process that decreases the current and potential capacity of the soil to produce, quantitatively and qualitatively, goods and services. Although it can be caused by natural causes, soil degradation is primarily the direct consequence of man-made anthropogenic activities. The soil represents an ecosystem where, currently, a wide variety of toxic compounds can be found, including hydrocarbons derived from oil activities. It is considered a soil that should be remedied that exceeds the limits established in NOM-038-SEMARNAT-2003 (See table 1).

ISSN 2444-4936

December 2019 Vol.5 No.16 17-25

Table 1. Maximum permissible limits for hydrocarbon fractions in soil.

Hydrocarbon Fraction	Land use predominant (mg / kg dry base)		
	Agricultural	Residential	Industrial
Light	200	200	500
Median	1200	1200	5000
Heavy	3000	3000	6000

Table 1 Maximum limits of hydrocarbons in soil according to their use to define if they need to be remedied. Source NOM-138-SEMARNAT / SS-2003

Remediation. It is the action of remedying or working on the site, including digging in the mud or mud and underlying soils. Soil remediation can be divided into two large groups:

a) *Ex situ:* When the soil is excavated and washed outside the site where it was contaminated.
b) *In situ:* The term in situ comes from Latin and means "in its original location".

The treatment is carried out below the surface. When digging is not required. It consists of the injection of washing solutions (surfactants) to the subsoil, under controlled conditions in order to remove contaminants adsorbed to the soil matrix. The washing of soils in situ is a treatment that can be in situ which consists of flooding the contaminated soils with a solution that transports the contaminants to a determined and localized area where they can be eliminated.

Thus, pollutants are extracted from the soil by passing water or other solutions (surfactants) through an injection or infiltration system. Surfactants are substances whose molecules have both a polar group and a nonpolar group. The polar group is in general a functional group that contains heterotherms such as O, S, N or P, if in the surfactant the polar group is ionic with negative charge (anion) and a metal cation is called an anionic surfactant, if on the contrary it has positive charge then it is a cationic, there are those that do not have ionic charge to the latter are known as non-ionic surfactant (Volkering et al, 1998). Therefore, the differences between the types of surfactants are related to the ionic charge.

The surfactants are preferably located on a surface or interface, which constitutes a hydrophilic part that makes the surfactant water soluble and another hydrophobic part (Volkering et al, 1998).

Humus:

Conceptually the organic component of the soil can be defined as a set of living and dead organic matter. In the dead organic matter the unaltered material can be differentiated, in which the morphology of the original material still exists, while the alteration of the transformed products is also called humus. Generally soil humus is defined as a mixture of dark, colloidal organic polydispersed compounds with high molecular weight and relatively resistant to decomposition (Ayuso et al., 1996).

In general, humus distinguishes between non-humic and humic substances. Non-humic substances comprise compounds that belong to biochemistry classes, such as amino acids, proteins, carbohydrates, lipids, lignin, nucleic acids, hormones, pigments and A variety of organic acids. Humic substances are subdivided into fulvic acids (See image 2), humic acids (See image 1) and residual humines; humines are the insoluble fraction of humic substances, and humic acids the soluble fraction in alkaline media and acidic media at pH> 2 although not in strong acidic conditions pH <2 and fulvic acids is the soluble part in alkaline conditions and acids (Hayes et al., 1989).

Schulten and Leinweber in 1991, proposed that Humic Acids (AHs) consist of isolated aromatic rings covalently linked by aliphatic chains. Oxygen is present in the form of carboxyls, phenolic and alcoholic hydroxyls, esters and ketones, while nitrogen is produced in nitriles and heterocyclic structures. The resulting carbon skeleton shows high microporosity with holes of various dimensions, which can trap and join other organic and inorganic soil elements, as well as water. According to Cruz-Narvaez Y (2019), the degradation of hydrocarbons improves with the use of humic acids in treatment with bacteria.

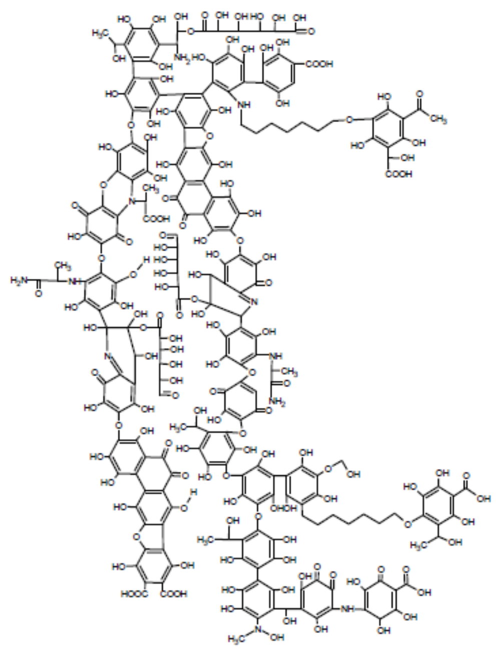


Figure 1 Hypothetical 2D representation of humic acids. López-Salazar R., (2014)

Fulvic Acids

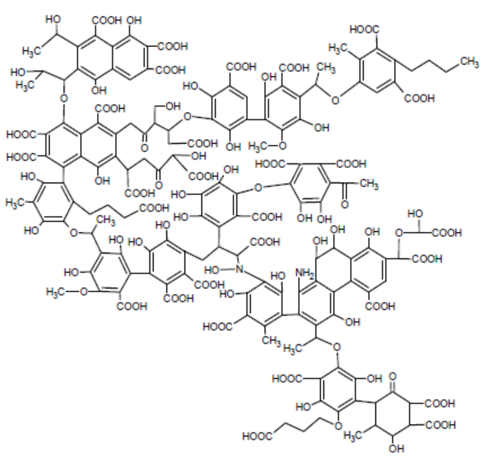


Figure 2 Hypothetical 2D representation of fulvic acids. López-Salazar R., (2014)

Saponins

Agave lechuguilla Torrey is a plant that belongs to the Agavaceae family, grows in arid areas, in limestone and rocky terrain. It is exploited to obtain fiber, known in Mexico as ixtle, which is widely used in the brush manufacturing and construction industries due to its abrasive characteristics and high water retention rate (65 percent). The fiber is obtained by carving the sheet consisting of 15 percent fiber and 85 percent pulp. The pulp contains bioactive compounds among which saponins with several properties stand out.

Saponosides, also known as saponins, are heterosides that consist of a glycosidic part with one or more sugars being more frequent glucose, xylose, arabinose, rhamnosa or glucoronic acid that bind to saponin (also called genic aglyconeo) through bonding glycosidic Depending on its chemical nature, sapogenin can be steroidal, steroid or triphenic glycoalkaloid. Hernández ´ et al. They report the extraction of saponins from agave extracts among them (Agave lechuguilla), from 0.1 to 1.3% dry base, establishing for this species, that only the temperature factor influences the performance of saponin extraction from foliar material. Saponins constitute a diverse group of compounds widely distributed in the plant kingdom, which are characterized by their physicochemical properties (surfactants). Obtaining extracts from ´ foliar material of some agaváceas (Agave lechuguilla Torrey), for which the presence of esmilagenina (steroidal sapogenin) is reported, in addition to 8 ´ saponins plus: yucagenine, phytogenin, hecogenin, ´ tigogenin, diosgenin, gentrogenin, chlorogenin and ruizgenin (Hernández ´ et al., 2005).

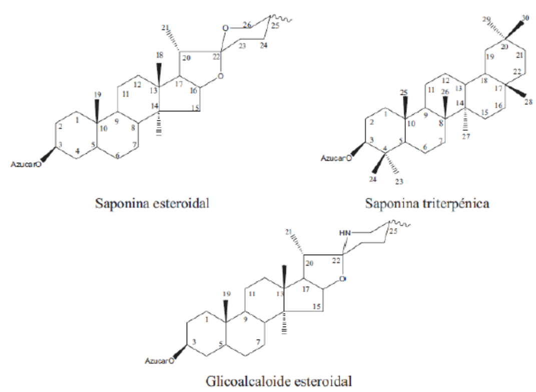


Figure 3 Classification of saponins
Recovered from: Monterrosa-Brisson N. (2013)

Materials and methods

The study approach is quantitative experimental descriptive since it is sought to compare and analyze advantages and disadvantages of 3 surfactants of natural origin.

Origin and sampling of the soil simple

The samples were taken from soils contaminated with hydrocarbons in the surroundings of the Miguel Hidalgo Refinery, which were preserved in sterile amber bottles and processed within 24 hours of their collection / stored in refrigeration.

ISSN 2444-4936

Theoretical Methods

The methods used were those described in the Official Mexican Standards: Determination of pH in water (NMX-AA-008-SCFI-2011), and for COD the EPA 5220 D method; APHA, 1989. NMX-AA-005-SCFI-2013-Water analysis - measurement of fats and oils

Obtaining hot and cold extract of lechuguilla (Agave lechuguilla), extraction of humic and fulvic acids and washing of soil contaminated with hydrocarbons

At this stage the following materials were used: 250ml Pyrex brand graduated cylinder, knob, 500ml beakers (Kimax), Potentiometer, Blender, Conductometer, heaters with stirring, handles, Refrigerants. And the following procedures were followed:

Cold extract

A fraction of lettuce of 220 grams is cut, the thorns are washed and removed, cut into small segments and ground in a blender adding 240ml of de-ionized water, passed through a sieve to remove solid waste

Hot extraction

In the flat bottom ball flask 250gr of lettuce and 240 ml of de-ionized water are added. It is placed in a closed reflux system for 2hr. The extract is passed through a sieve to remove waste.

Obtaining Humic and Fulvic Acids:

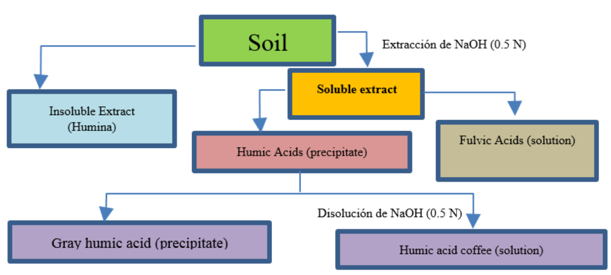


Figure 1 Method for obtaining humic and fulvic acids
Source: Self Made

Figure 4 Lechuguilla extract (*Agave lechuguilla*), obtained cold (left) and hot (right)

Soil wash:

4 triplicate washes were performed:

Washing with humic acids, fulvic acids, lechiguilla extract obtained by room temperature method (cold method) and hot obtained lechuguilla extract

Sieve contaminated soil with sieve number 18 and 10, Weigh 10 grams of the sample for each beaker, 50 ml of the corresponding surfactant solution was added, it is stirred for a period of 2 hours. The washed soil and the residual solution are filtered and recovered separately, labeled for fat and oil determination and Chemical Oxygen Demand (COD).

Likewise, a triplicate series was used in each case of a wash target with uncontaminated soil and a wash with deionized water.

Determination of fats and oils in the soil; before and after washing

Bring the flask to constant weight. Weigh the sample and place it in the cartridge, Install the Soxhlet, add 185ml of hexane, perform the extraction for 4hr, once it is finished, recover the hexane by distillation, let it cool inside a desiccator and obtain the amount of fats and oils by difference of weight.

December 2019 Vol.5 No.16 17-25



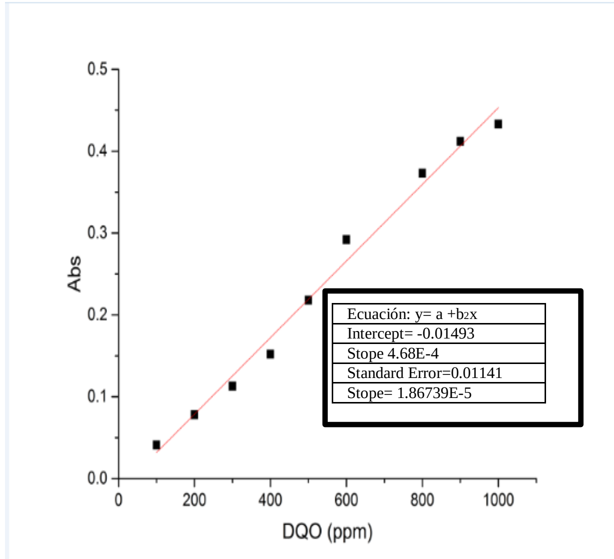
Figure 5 Fats and oils extraction, soxhlet method**, Figure 6** Hexane recovery**, Figure 7** Extracted hydrocarbons

Teflon cap glass tubes were used, to which 2.5ml of sample, 1.5ml of digester solution (10.216g of $K_2Cr_2O_7$ + 33.3 $HgSO_4$ and 167ml of H_2SO_4) and 3.5ml of sulfuric acid solution (10.142g of H_2SO_4 Ag_2SO_4), once prepared the samples are digested within a heating block at 150 ° C for 2 hours. Subsequently, allow the samples to cool to room temperature and the absorbance is read at 600 nm. (EPA 5220 D; APHA, 1989).

Results

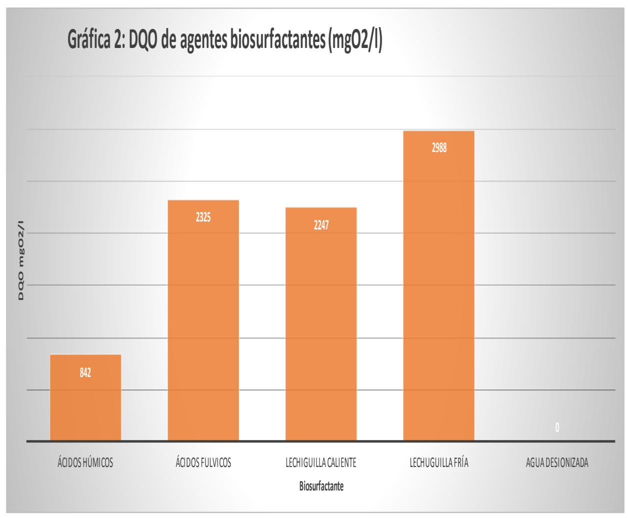
COD values are obtained from a calibration curve performed with potassium biftalate (0-681mg / l, equivalent to 0-800mgO2 / l). (See Graph 1). The COD values of biosurfactant agents are shown in graph 2, in which we can observe that humic acid is the one with the lowest content of organic matter 842 mgO2 / l and cold lettuce extract is the one that contains the highest amount 2988mgO2 / l, this is because the extract is still filtered; remains of lechuguilla itself pass to it; however, it is within the range of hot lettuce and fulvic acids, water was used as white.

Once the soil was washed with the surfactants, we see in graph 3 that, although the 4 agents tested work properly, there is a greater removal of fats and oils in the case of the soil washed with cold lettuce extract, every time that a greater amount of organic matter passed to the aqueous extract. We could prove it by observing that the COD increased considerably from 2988mgO2 / l to 10612mgO2 / l (See graph 4). It is also the simplest and most economical process since in this case obtaining lechuguilla extract does not require heating.

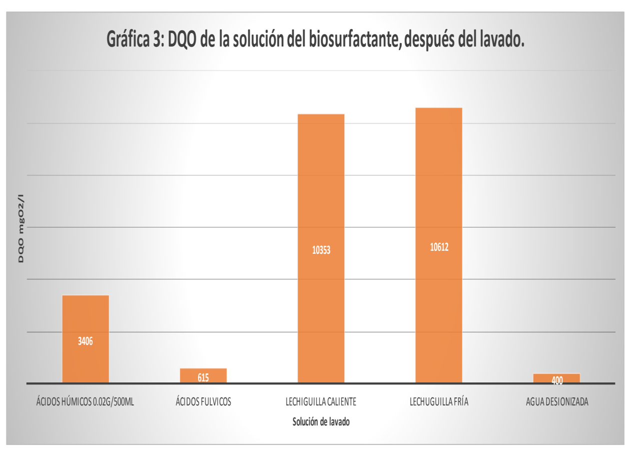


Graphic 1 COD Calibration Curve
Source: Own Elaboration

From the graph the formula was defined to determine the concentration where:

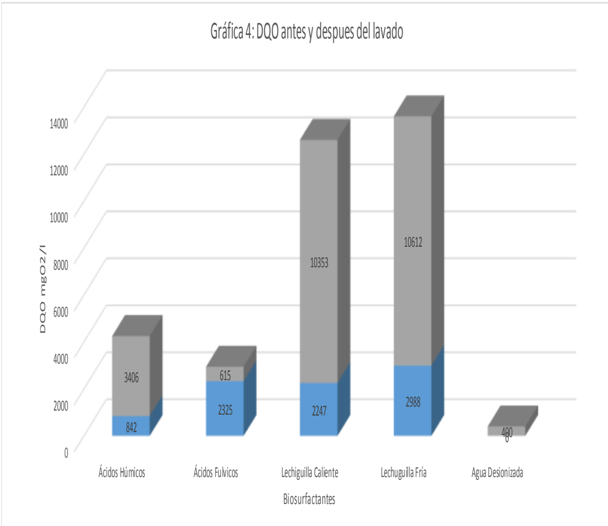


Graphic 2 Chemical demand of the biosurfactants obtained
Source: Self Made



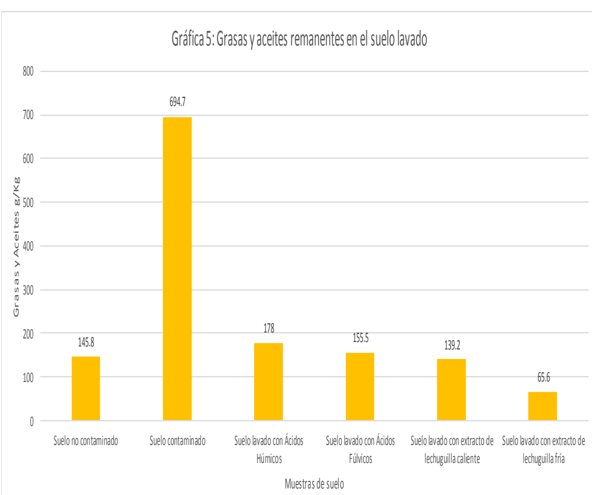
Graphic 3 Chemical demand of the biosurfactant solution: after washing the soil contaminated with hydrocarbons
Source: Self Made

ISSN 2444-4936



Graphic 4

Once the soil was washed, fats and oils were determined by finding a lower amount in the soil washed with lechuguilla extract obtained at room temperature (See graph 4). The amount of fat decreased in the case of the soil washed with a humic acid solution from 694.7 g / kg to 178g / kg, in the case of the soil washed with a solution of fulvic acids it decreased from 694.7 g / kg to 155.5 g / kg , in the case of hot lettuce extract the decrease was from 694.7 g / kg to 139.2g / kg and in the case of washing with the extract of lettuce obtained at room temperature (cold lettuce) the decrease was from 694.7g / kg to 65.6g / kg A soil sample was also taken that was considered uncontaminated within the University campus and yet we found that it contained 145.8 g / kg, this may be due to herbicides that are used in garden areas or contaminated irrigation waters (See Graphic 5)



Graphic 5 Fat remaining in the soil washed with the different surfactants: Humic acids, Fulvic acids, Hot and cold Lechuguilla extract

Conclusions

Agave lechuguilla Torrey contains in the pulp bioactive compounds among which saponins with surfactant properties stand out, which can be used in soil bioremediation processes contaminated with hydrocarbons.

The extract of Agave lechuguilla Torrey obtained at room temperature turned out to have a better surfactant effect than the extract obtained with heating. According to Hernández et al. (2005), Agave lechuguilla Torrey contains 9 saponins (esmilagenina, yucagenina, gitogenina, hecogenina, tigogenina, diosgenina, gentrogenina, chlorogenina and ruizgenina), that have surfactant effect.

A better option for the washing of soils contaminated with hydrocarbons was the extract of Agave lechuguilla Torrey, compared to the hot extract and the solution of humic and fulvic acids. The extract obtained at room temperature was able to remove the hydrocarbons since the COD of the extract passed from 2988mgO2 / l to 10612mgO2 / l when washing the soil and the soil was finally with 65.6 g / Kg of fats and oils.

Finally, the treatment of the extract will be necessary to degrade the hydrocarbons that were removed from the soil.

References

Aronstein BN and Alexander M. (1992). "Surfactants at Low Concentrations Stimulate Biodegradation of Sorbed Hydrocarbons in Samples of Aquifer Sands and Soil Slurries,"*Environ. Toxicol. Chem. 11*, 1227-1233.

Bollag, J.M. (1992). Decontaminating soil with enzymes: An in situ method using phenolic and anilinic compounds. *Environ. Sci. Technol. 26*:1876–1881.

Cubero-Mata, A. V. (2019). Identificación del peligro por contaminación de metales pesados y bacterias patógenas en los sedimentos del embalse de Central Hidroeléctrica Platanar de COOPELESCA RL, Costa Rica.

Cruz Narváez, Y. (2019). Construcción de un biorreactor con bacterias inmovilizadas para la biodegradación de hidrocarburos.

Helmy Q, Kardena E, Wisjnuprapto. (2009). Performance of petrofilic consortia and effect of Surfactant tween 80 addition in the oil sludge removal Process. *J. of Applied Sciences in Environ. Sanitation*, *4* (3): 207-218.

Jiménez DJ, Medina SA, Gracida JN. (2010). Propiedades, aplicaciones y producción de biotensoactivos. *Int. Contam. Ambient. 26* (1) 65-84.

Kästner M and Mahro B. (1996). Microbial degradation of polycyclic aromatic hydrocarbons in soils affected by the organic matrix of compost. *Appl Microbiol Biotechnol*. *44*:668–675.

Lange S and Warger F. (1987). Structure and Properties of Biosurfactants. *In Biosurfactants and Biotechnology*, Marcel Dekker Inc., N.Y

López-Salazar R., (2014). *Efecto de un humato de calcio y fulvato de hierro en la calidad y producción de chile jalapeño (capsicum annum l.) y piquin (capsicum annum l. aviculare) y en la porosidad en un suelo calcisol*. (Tesis Doctoral). Universidad Autónoma de Nuevo León, Nuevo León Monterrey.

Messarch M and Nies L. (1997). Modification of heterothropic plate counts for assessing the bioremediation potencial of petroleum contaminated soils. *Environ. Technol. 18*, 639-646.

Monterrosa-Brisson N. (2013), Evaluación de especies de Agavesobre los procesos fisiopatológicos asociados al Alzheimer, (Tesis Doctoral), Instituto politécnico Nacional, Ciudad de México.

Paul, E.A. and Clark, F.E. (1989) Soil Microbiology and Biochemistry, Academic Press, San Diego.

Paul, E.A. and Clark, F.E. (1989) Soil Microbiology and Biochemistry, Academic Press, San Diego.

Paul EA, Clark FE. (1998), *Soil microbiology and biochemistry*, Academic Press, San Diego.
Plohl K, and Leskovsek H. (2002). Biological degradation of motor oil in water. *Acta Chim. Slov.*, *49*, 279-289

Rojas Molina, N. A. (2019). Validación de una metodología para la determinación de benceno en suelos mediante HS-GC-FID y su aplicación en biorremediación en suelos co-contaminados con Hg (ii).

Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT), 2012, Norma Oficial Mexicana que establece los límites máximos permisibles de hidrocarburos en suelos y las especificaciones para su caracterización y remediación (NOM-138-SEMARNAT/SS-2003). México, D.F. Diario Oficial de la Federación, 29 de marzo de 2003, 21 Recuperado de http://www.dof.gob.mx/nota_detalle.php?codigo=5313544&fecha=10/09/2013

Singh A, Van Hamme JD, Ward O. (2007). Surfactants in microbiology and biotechnology: Part 2. *Biotechnology Advances*, *25*. 99 – 121.
Tissot B, Welte DH. (1984). *Petroleum formation and occurrence*. New York USA: Sppringer-Verlang.

Volkering F, Breure AM, Rulkens WH. (1998). Microbiological aspects of surfactant use for biological soil remediation. *Biodegradation, 8*. 401–417.