VŠB – TECHNICAL UNIVERSITY OF OSTRAVA
FACULTY OF MINING AND GEOLOGY
Institute of environmental engeenering

REMEDIATION OF SOILS AND GROUNDWATER CONTAMINETED WITH HIDROCARBONS IN AREA DE OPERATION OF ECOPETROL IN PALAGUA OIL FIELD, COLOMBIA

Dissertation work

Author:	**MSc. Ing. Maria Kopytko**
Supervisor:	**doc. Ing. Vladimir Čablík, Ph.D.**
Study programmes:	**P3904 Engineering Ecology**
Field:	**3904V012 Environment Protection within Industry**

Ostrava 2013

I am very thankful to my family and without their love and comprehension this would not have been possible. I would like to deeply express my profound gratitude for my first supervisor at Technical University of Ostrava prof. Ing. Peter Fečko, CSc. for his affording opportunity to do this work in Colombia and constructive suggestions and valuable guidance for the writing of the thesis. I am very grateful to doc. Ing. Vladimír Čablík, Ph.D. for supervision, his valuable ideas, guidance and useful comments that greatly improved my thesis. I express the deepest gratitude and appreciation to Ing. Liliana Rodriguez for her encouragement and unconditional help, which made possible to finish this work.

Abstract

The soils of the Station 1 in Palagua oil field and bordering property indicated similar characteristics of both, classifying them like sandy, which coincides with the high permeability and rapid infiltration, together with low relative thickness and low retention of moisture. Also both present a capacity of cationic exchange and granular structure. The underground water contains phenols and metals as the Barium, Lead, Arsenic, hexavalent Chromium and Cadmium that they overcome the limit allowed by the Colombian current environment policy in several points of sampling. The bioaugmentation process represent better results for biodegradation of TPH in 83,7% in average, as well as for biodegradation of oil and grease (58,2%) and PAHs(31,7%). Combination of two treatments is more convenient for elimination of hydrocarbons than bioremediation solo. The flushing with surfactant and washing with hydrogen peroxide solution as the pre-treatments, presented similar efficiency for elimination of TPH, oil and grease but elimination of PAHs it is better to handle with peroxide pre-treatment, where can be achieved more than 80%.The results of the modeling of groundwater flow in the zone of interest, indicate a predominant direction northwest - south-west (NW to SW) and a maximum speed of flow 0.37 m/d of groundwater. The direction of displacement of the underground water together with the pen is towards the Marsh Palagua. The calibration of model lives average error -0,007m and absolute error 0,346m. After one month of biostimulation and two weeks flushing with surfactant as pre-treatment in Palagua oil field, was achieved elimination of TPH (56,3%) oil and grease(44,8%) and PAHs (49,2%).

Key words: natural attenuation, biostimulation, bioaugmentation, biodegradation of soil, hydrocarbon treatments

.

I declare that the work undertaken during period 2008-2013 was carried out by myself, with guidance from my supervisors and support from my colleagues. I declare that this is the only course for which I have been registered to study for the period from 01.09.2008 to 30.01.2013. In accordance with Section 47a of the Act (c) 111/1998 Sb., on universities and change and complement to the next law I agree with publishing the text of their work on the Web page of the HGF VŠB-Technical University of Ostrava.

Ostrava 22 of April 2013 MSc. Ing. Maria Kopytko

Motivation

The development of petroleum industry also the extensive use of petroleum products, leads to inevitable spillages which nowadays have been recognized as the most significant contamination problem.

Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. At the same way petroleum causes organic pollution of groundwater which limits its use, decreases the agricultural productivity of the soil and makes economic loss as well as all kind of environmental problems.

Physical, mechanical and chemical treatment strategies to recover affected lands have limited effectiveness and can be expensive.

Many indigenous microorganisms in water and soil are capable of degrading hydrocarbon contaminants so bioremediation of petroleum hydrocarbons has gained increased attention and application during the last decades.

In the present work were analyzed natural attenuation, biostimulation and bioaugmentation as possible methods for remediation of soil contaminated with hydrocarbons.

In order to find the best method, was analyzed possibility to recombination of pre-treatments, as flushing with surfactants or hydrogen peroxide, with biodegradation process.

Content

List of figures

List of tables

1 SCOPE OF INVESTIGATION

The reason for this investigation in remediation of soils and groundwater contaminated with hydrocarbons was created by environmental responsibility of Ecopetrol S.A.

The requirement of this company has been to find economical, efficiently and environmental friendly method, to reduce final total petroleum hydrocarbon (TPH) to 3% content, accordance with the Louisiana 29B Regulation (USA), and restoring contaminated soil in the zone of interest. The method which it expires with the previous expectations is bioremediation, where is used the physiological potentials of indigenous hydrocarbon-degrading microorganisms. Taking into account, long time required to clean up the contaminated soils by natural attenuation, in the present work was analyzed possibility to enhance biodegradation by biostimulation or bioaugmentation. Hence, the inorganic nutrients addition, with electron acceptors such as oxygen, was one of the strategies in this work, and addition of enriched microbial inoculum degrading hydrocarbons, was the other one, to enhanced bioremediation process.

In the other way, as the effectiveness of biodegradation process can be limited by toxicity of petroleum compounds and its bioavailability in soil, to identifying the main factor that impairs the biodegradation (toxicity or bioavailability), were carried out before biodegradation process, tests in pretreatment with hydrogen peroxide and flush with surfactants. Finally to assess the groundwater flow, particle tracking, and hydrocarbon plume transport simulations, was used software Visual MODFLOW applied to the real case in the zone of interest.

The objectives of this study are to:

- Investigate bioremediation of soil contaminated with hydrocarbons by natural attenuation without and with pretreatment in situ.
- Investigate bioremediation of soil contaminated with hydrocarbons by biostimulation without and with pretreatment in situ.
- Assess bioremediation of soil contaminated with hydrocarbons by the bioaugmentation, without and with pretreatment in situ..
- Investigate the behavior of groundwater flow, and the trajectory of the pollutant pen
- Apply, the selected alternative physicist - chemistry and/or microbiological method.

2 LITERATURE REVIEW

2.1 GROUNDWATER

When rain falls to the ground, the water moves downward through empty spaces or cracks in the soil, sand, until it reaches a layer of rock through which water cannot easily move. In this case the water fills the empty spaces and cracks above that layer. So, the term of groundwater apply for subsurface water that occurs beneath the water table in soils and geologic formation that are fully saturated. [1]

2.1.1 Groundwater as resource

The study of groundwater has an interdisciplinary nature. It can integrate geologists, ecologists, hydrologists, soil scientists, chemical engineers, agricultural engineers, foresters, geographers, geotechnical engineers, mining engineers, sanitary engineers, petroleum reservoir analysts, and more others.

The principal motivation for the study of groundwater has traditionally been its importance as a global resource. Morris et al. [2] notes that groundwater supplies about 2 billion people with drinking water, and that 40% of the world's food is produced through irrigated agriculture that relies strongly on groundwater. It is possible to do because groundwater is reliable and it may require relatively less treatment, capital and organizational resources.

The National Groundwater Association (NGWA) estimates that about 75%, 47%, 32%, 29%,and 15% of the population in Europe, the US, the Asia-pacific region, Latin America and Australia, respectively, depend on groundwater for their drinking water supply. [3] The same way it has been estimated that 80% of world's population depends on groundwater for survival. [4]

Unfortunately every day is more and more contaminated this source of water. Between different kinds of pollutants hydrocarbons are frequents, because of significance of oil and natural gas in modern civilization as is well known.

Crude oil is a complex mixture of hydrocarbons. Once a liquid petroleum product is released into the ground it partitions into three separate phases: dissolved, liquid and gas. One small part of the hydrocarbons is dissolved in the soil moisture, and can be dragged or transported to lakes, rivers and groundwater. Major part remains is adhered to soil particles in pore space in its pure liquid form as residual saturation, and some of it can also become or volatilized into air (light compounds).

Pure phase liquid that do not readily dissolve in water are called non-aqueous phase liquid (NAPL). In generally NAPLs are subdivided into two classes: those that are lighter than water (LNAPLs) and those with a density greater than water (DNAPLs). [5]

2.2 HYDROCARBONS TOXICOLOGY

Hydrocarbons are type of organic substances that are basically composed of carbon and hydrogen molecules and can be derived from petroleum. Petroleum is classified as a hazardous material. [6]

The toxicity of hydrocarbons is directly related to their physical properties like volatility, surface tension, viscosity, solubility and chemical activity. For example hydrocarbons with high volatility are directly related with the risk of aspiration but the surface tension and the viscosity is inversely related with this risk. [7]

Toxicity from hydrocarbon exposure can be thought of as different syndromes, depending on which organ system is predominately involved. Organ systems that can be affected by hydrocarbons include the pulmonary which is the most commonly, the neurologic, cardiac, hepatic, renal gastrointestinal, hematologic and dermatologic systems.

Also is important the route of ingestion (ex. oral, dermal, inhalational) the amount and the time of the substance ingested and age of person. More fatalities are associated with children younger than 5 years. [8,9]

Pulmonary complications are reported as the result of hydrocarbon aspiration and can create a severe pneumonitis. [10] Many hydrocarbons affect the central nervous system including direct injury to the brain because of their highly lipophilic character. [11,12]

At the same way prolonged abuse of hydrocarbons can result in white matter degeneration (leukoencephalopathy) and atrophy. Prolonged exposure to n-hexane or methyl-n-butyl ketone can result in peripheral neuropathy, blurred vision, muscle atrophy and Parkinsonism [13] Many authors reported that exposure to hydrocarbons can result cardio toxicity and Carbon tetrachloride and others chlorinated hydrocarbons are hepatotoxic. [14]

Following the acute ingestion of different kind of hydrocarbons, hemolysis has been reported. In addition prolonged exposure to certain aromatic hydrocarbons can lead to an increase of anemia, leukemia and multiple myeloma. [15,16]

3 REMEDIATION METHODS OF SOILS AND GROUNDWATER CONTAMINATED WITH HYDROCARBONS

To facilitate decision making for investigation, and cleanup of petroleum contamination in soils and groundwater, the American Petroleum Institute (API) developed a petroleum decision framework. [17] Environmental Solution, Inc. for the Western States Petroleum Institute some information about technologies for treating petroleum hydrocarbon contaminated soils was compiled. This manual has technology description and an overview of the technology screening process. [18]

There are two basic approaches to the cleanup of contaminated groundwater plumes: active and passive remediation. [19,20]

Passive remediation is when the nature takes its course, like biodegradation with sorption and dispersion processes.

In contrast to passive remediation, active remediation involves a range of intervention technologies. These technologies may be subdivided into:

- Liquid-phase hydrocarbon (LPH) removal technologies.
- Vadose zone treatment technologies.
- Saturated zone treatment technologies.
- Treatment technologies for recovered groundwater.
- Off-gas treatment technologies.

Following the identification and delineation of plume, on the first tasks undertaken is to contain the plume if possible, that is, to prevent it from traveling any farther. The next step is usually to clean up any residual materials remaining at the source in order to prevent any more contaminants from entering the groundwater. [21]

Some physical and chemical treatment strategies to recover affected lands are: thermal treatment, soil vapor extraction, chemical oxidation and reduction processes, air sparging, permeable reactive barrier, flushing in situ and the others. [22]

The efficiency of these methods depends on several factors, such the type of oil and polluted soil, the age and degree of contamination, the amount of spilled oil and the penetration depth of the oil into the soil and behavior of contaminants in the subsurface environment. [23]

The following physical and chemical processes are some of the techniques that might be employed to treat the petroleum contaminated soil and groundwater. Especial interest for this project has in situ remediation methods.

3.1 Physical and chemical processes

Although more of these strategies are efficient for contaminant removal, they are generally conceived as temporary solutions and often, the contamination is transferred to other medium and required additional treatment. That’s why cleaning up contaminated groundwater plumes and soils can be exceedingly expensive.

3.1.1 Thermal Treatment

Thermal treatment technologies can be grouped into two categories. The first group uses incineration (e.g., rotary kilns, cement kilns) to destroy hydrocarbons ex situ by heating them to very high temperatures in the presence of air. The second one uses thermal desorption
(ex situ or in situ), in which heat is applied to vaporize volatile and semi volatile components.

In this process petroleum contaminated soil is heats under an inert atmosphere to increase the vapor pressure of the organic volatile contaminants, transferring them from the solid to the gaseous phase. Many different methods and combinations of techniques can be used to apply heat to polluted soil and/or groundwater in situ. This technology uses electrical resistance/electromagnetic/fiber optic/radio frequency heating or hot-air/steam injection to increase the volatilization rate of semi-volatiles and facilitate extraction. [24,25] The heat can destroy or volatilize organic chemicals. As the chemicals change into gases, they can be extracted via collection wells for capture and cleanup in an additional ex situ treatment unit.

Thermal treatment offers quick cleanup times, but it is generally the most costly treatment group. Thermal methods can be particularly useful for dense or light non aqueous phase liquids (DNAPLs or LNAPLs). Some researches in thermal treatment ensure the efficiency of above 99%, for example Joong Kee Lee *et al* reported a novel type of fluidized- bed desorber at low temperature with high efficiency and they make certain, that operation at 300°C of that fluidized-bed desorber exhibited 99.9% desorption efficiency. [26]

In situ treatment often requires longer time periods for the reason that here is less confidence about the uniformity of treatment because of the variability in soil and aquifer characteristics and the efficacy of this kind of process is more difficult to verify. [27]

Nevertheless one significant advantage of thermal treatment over other in situ remediation techniques is that these methods do not require chemicals as part of the remediation effort.

Many different methods can be used to apply heat to polluted soil and/or groundwater in situ which is very important issue for efficiency and the time treatment.

Electrical resistance heating uses arrays of electrodes installed around a central neutral base to make a concentrated flow of current in the direction of the study point. The temperature which flow from resistance to the soil is about 100ºC and can produce steam and readily mobile contaminants that are recovered and processed at the surface. This technology is extremely rapid form of remediation, some authors reported effective treatment in less than 40 days. [28]

Three-phase heating and six-phase soil heating are varieties of this technology. The following figure 1 shows typical six phase heating system.

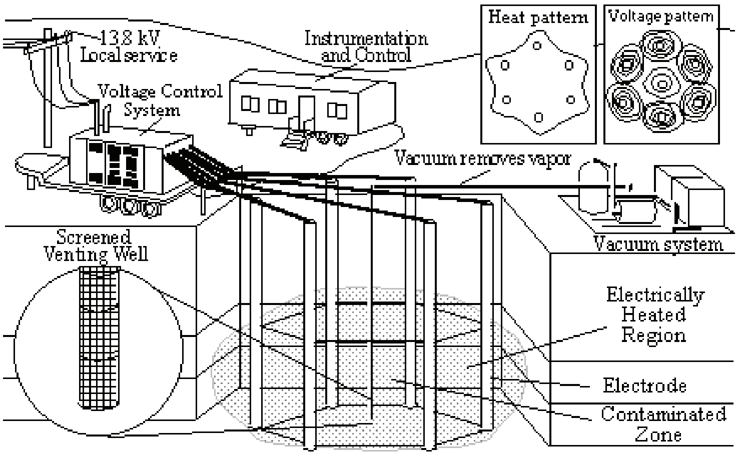


Figure 1. *Typical Six-phase heating system [29]*

Microwave heating was use the first time in 1992 for the decontamination of soil by Windgasse and Dauerman, which yielded very promising results. [30]

Dawei Li *et al* used microwave-induced thermal treatment of the soil contaminated with petroleum hydrocarbon and employ granular activated carbon as microwave absorber for achieving an appropriate temperature to decontaminate the soil. They confirm that granular activated carbon enhance microwave energy dissipation. The authors show that more than 99% contaminant removal within 10 minutes. Additionally the analysis performed on the recovered oil indicates that is does not change noticeably in chemical composition and physical properties, compares with the original oil. [31]

Radio frequency(RF) heating uses electromagnetic energy to heat soil, after the same principle as the microwave oven and can be used in situ, follow by enhance soil vapor extraction.

Heating materials using radio frequency to recover petroleum produces was initially explored during the 1970-s oil crisis to remove oil from tar sands.

Basically RF heating interacts with polar molecules in the subsurface, causing them to rotate and generate heat within the soil. Water is the primary molecule involved in the generation of heat but also the contaminants are affected by the introduction of RF energy into the sub-surface. The rate of the rotation of each molecule that is affected depends upon the dipole moment of the molecule; in addition the frequency of reversal of the RF energy emitted into the soil will create optimal results when it matches the rate at which the molecules can rotate.

Applying the low-frequent RF waves of 3 to 50 MHz, can be utilized at temperatures between -20°C and 400°C. In this technique can be treated larger volumes of contaminated soil.

The big advantage of the RF treatment technology is that even soils with lower porosity such as clays and chalk can be treated by this method. [32]

Thermal conduction (also referred to as electrical conductive heating or in situ thermal desorption) supplies heat to the soil through steel wells or a blanket that covers the ground surface. [33] As the polluted area is heated, the contaminants are destroyed or evaporated. Steel wells are used when the polluted soil is deep. The blanket is used where the polluted soil is shallow. Disadvantage of this method is that the temperatures decrease sharply with distance from the source. [34] The thermal conductivity values of a large range of soil types. Normally, a carrier gas or vacuum system transports the volatilized water and organics to a treatment system. [35]

Injection of steam heats was first developed by the petroleum industry for the enhanced recovery of oils from reservoirs the soil and groundwater by decreasing viscosity and accelerating volatilization. This method has been applied to recovery high viscosity oil reservoirs. Castianier and Gadelle reported that in 1988 due to steam injection, 72% of the total enhanced oil was recovery for the U.S.A. [36]

Steam injection may also destroy some contaminants. The steam might be injected above the water table and has also been successfully carried out below the water table for remediation purposes. [37]

Many studies have been conducted to explain steam-flood mechanisms associated with the complex oil displacement process. Basically, as steam is injected through a series of wells within and around a source area, the steam zone grows radially around each injection well. Initially, the steam will heat the well bore, and the formation around the injection zone of the well. Then the steam condenses and the formed water loses temperature. As more steam is injected, increase the temperature and water moves into the formation further into the porous media. When the porous media has absorbed enough heat to reach the temperature of the injection stream, steam itself enters the media, pushing the cold water and the bank of condensed steam (hot water) in front of it.

As these flowing fluids approach a region that contains the volatile hydrocarbons, the steam front drives the contamination to a system of ground-water pumping wells in the saturated zone and soil vapor extraction wells in the vadose zone. [38,39]

Willman et al. demonstrated that high oil recovery by steam is much better than by water flood. They reported that the recovery by steam is greater for lighter oil because they contain a larger fraction of steam-distillable components. [40]

In general the amount of residual saturation remaining after the cold and hot water flush is depend on the capillary proprieties of the porous media, the interfacial properties of the contaminant, increasing the temperature, and the pressure gradient in the water which causing the displacement. [41,42]

Steam extraction can be used also in mobile systems. The mobile system has a unit that volatilizes contaminants in small areas by injecting steam and hot air through rotating cutter blades that pass through contaminated medium.

Advantages of steam injection over other remediation techniques include the fact that excavation is not required however is very important to characterize the site adequately to determine the horizontal and vertical distribution of the contaminant, and the preferred flow paths for the injected stream.

Injection of hot air can volatilize fuel hydrocarbons in soils or sediments. With deeper subsurface applications, hot air is introduced at high pressure through wells or soil fractures. In surface soils, hot air is usually applied in combination with soil mixing or tilling, either in situ or ex situ.

Injection of hot water via injection wells heats the soil and ground water and enhances contaminant release. Hot water injection also displaces fluids (including LNAPL and DNAPL free product) and decreases contaminant viscosity in the subsurface to accelerate remediation through enhanced recovery. Although hot water and steam injection is recommended to recover nonvolatile, viscous oil, hot water injection is better method because equipment and facilities for generating and handing hot water are relatively simple and inexpensive. [43] Typical hot water/steam flushing system is illustrated in Figure 2 below.

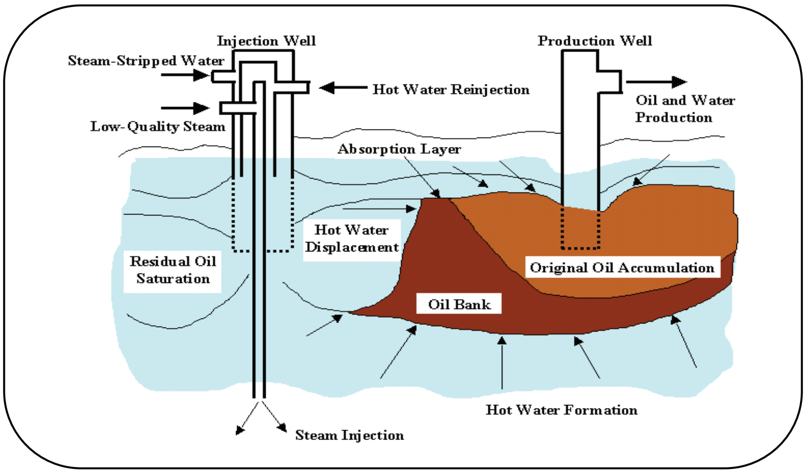


Figure 2. *Typical hot water/steam flushing system [29]*

Plasma system can be also use as an energy source in thermal treatment to recovering or remediating process in soils or groundwater contaminated with hydrocarbons.

Plasma is an ionized gas at high temperature produced by an electric arc which strikes between two metallic electrodes, inside en equipment called plasma torch. The common gas such as air, nitrogen, steam and others are injected into the plasma torch, the molecules or atoms of the gas collided with the electrons present in the electric arc and as a result, generated heating and ionization gas that reaches very high temperatures. The plasma process is working at approximately 15.000°C and operating in an inert atmosphere. The plasma gas can be recycled in the process.

The industrial plasma process can volatilizes the organic compounds initially present in the contaminated soil, in en ambient that does not contain oxygen. In subsequent stages, these hydrocarbons are condensed, and most are reclaimed as clean oil and returned to process stream. The resulting soil are inert and contain less than 0,01% hydrocarbons.

Advantage of the process is that thermal plasma volatilization is transferred as heat 85% of the energy but a disadvantage is the potentially high cost of the process. [44,45]

3.1.2 Soil vapor extraction

It is one of the most utilized techniques applicable for soil with VOC and/or fuels contamination, also known as "soil venting" or "vacuum extraction", is an *in situ* remedial technology that reduces concentrations of volatile constituents in petroleum products adsorbed to soils in the unsaturated (vadose) zone which is simple and relatively economic. Before arriving at the atmosphere, the extracted vapor is then treated with the purpose of protecting the quality of the air. The increased air flow through the subsurface can also stimulate biodegradation of certain contaminants that are less volatile. [46]

Soil vapor extraction (SVE) shows the best results in subsoil stratum when the characteristics of soil are uniform with high porosity and low moisture content and air stream follow a horizontal path. [47] In the same way high concentration of organic matter in soil can adsorb VOCs and may retard their volatilization process. [47] In the Figure 3 below we can see typical in situ soil vapor extraction technology.

In figure 3 is showed that soil vapor extraction requires drilling extraction wells within the contaminated area. These wells are drilled into the soil, but not the groundwater. Attached to the wells is equipment that creates a vacuum, which pulls air and vapors through the soil and up to the surface to off-gas treatments.

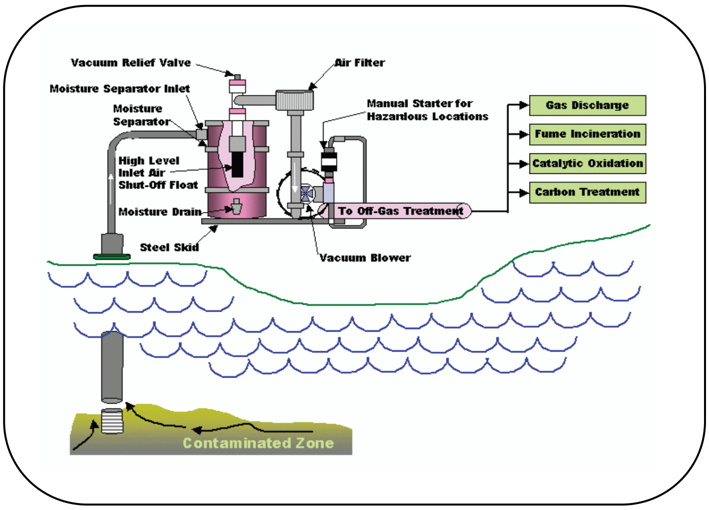


Figure 3. *Typical in situ soil vapor extraction system [29]*

Air injection wells can be drilled to help the cleanup. The air causes the contaminants to evaporate faster. Sometimes air vents are used instead of air injection wells. Air vents don`t pump air, but provide a passage for fresh air to enter the ground to provide only enough oxygen to sustain microbial activity. Using in situ Bioventing minimize soil vapor extraction cost. [48]

Advantage of soil vapor extraction over traditional VOC treatment technologies such as thermal incineration, catalytic incineration, carbon adsorption, liquid scrubbing, and condensation, include lower operating costs and fewer secondary waste stream problems. [49]

Limitations which this technique has are following:

Soil with high moisture and organic content or extremely dry requires higher vacuums, hindering the operation of the SVE system, because of a high VOCs sorption capacity. In the same way the soil with low permeability also limit the effectiveness of SVE technique.

As a result of vacuum pressure the groundwater can be rising into the unsaturated zone and could show increases in contamination levels. In this case will be necessary pump and treat water in special units. [49]

The biggest problem encountered with SVE is the need for off-gas treatment, which may exceed 3 to 5 times the basic costs of soil venting equipment. [50]

3.1.3 Chemical oxidation

In recent years there has been interest in using chemical treatment to remediate soils contaminated with hydrocarbons and overcome some of the problems associated with bioremediation. These techniques can offer a rapid and effective alternative that is not as sensitive to the type and concentration of contaminant. [51]

It is based on the delivery of chemical oxidants to contaminated soils or groundwater in order to convert contaminants into more biodegradable compounds.

This process typically applies redox reactions that chemically transform hazardous pollutants components into other, commonly found in the nature or less toxic compounds that are inert, more stable and less mobile.

The Chemical oxidants most commonly employed include ozone, peroxide, Fenton’s Reagent, permanganate, hypochlorite and chlorine and others.

These oxidants have been able to cause the rapid and complete chemical destruction of many toxic organic chemicals; other organics are probable to partial degradation as an aid to subsequent bioremediation. [52]

It is important to taking into account that conditions inside or the immediate vicinity of oxidant injection locations may be alters by chemical oxidant so data from these locations are not representative of the subsurface conditions that exist beneath most of the sides.

To understand the effect the chemical oxidation system is having on the subsurface conditions as a measure of its performance, samples of soil and groundwater should be collected from alternate locations. [53]

Ozone is highly reactive and one of the strongest oxidants accessible for chemical soil treatment in situ. Ozone can be applying in either the gas or aqueous phase and in the gaseous phase can be delivered via vertical or horizontal wells.

It may oxidize organic pollutants either directly or by generation of free radical intermediates. Due big instability, ozone must be generated on-side then during discomposed provides oxygen, which can improve bioremediation. Since early 80-ties ozone was used in soil system decontamination and in the stimulation of biological activity. [54]

Another benefit of using ozone is that after a short period of time ozone that has not reacted reverts back to atmospheric oxygen and therefore no toxic residues of the oxidant remain in the soil. That's why there has been extensive interest in using ozone to remediate contaminated soils, especially sites covering low or non-volatile organic compounds as PAHs, which are not removed by conventional soil venting. [55]

Ozone reacts with organic substances by two main mechanisms in aqueous solution. At neutral to acidic pH, an electrophilic addition of molecular ozone takes place at the electron rich parts of organic molecules like C–C double bonds. At alkaline conditions, ozone decays mostly to hydroxyl radicals (OH*) and by chain reactions to other radicals, which cause an unspecific radical reaction with organic substances. Generally PAH-ozonation can take place by both direct and radical reactions at neutral pH. [56]

Kyoungphile Nam and Jerome j. Kukor reported that ozonation is very efficient method in the removal of naphthalene, Fluorene, phenanthrene, and Antracene, but not for pyrene, chrysene, and benzo pyrene from soil freshly spiked with the hydrocarbons. Additionally they confirm adverse effect of organic carbon in the soil on the efficiency of ozone treatment. [57]

Mark M. O'Mahony and collaborates shows that the greater the water content of the soil and higher levels of clay, the less effective the ozone treatment for reduction of phenanthrene levels. [58]

The results of many researchers suggest that a combined treatment like ozonation and biodegradation may be a promising bioremediation technology in soil contaminated with mixtures of polycyclic aromatic hydrocarbons. However, ozone in high concentration is a sterilizing agent, so the time of contact and the concentration must be carefully controlled before bioremediation is encouraged. The dosage should not be greater than 1 mg/l of ozone per mg/l total organic carbon (TOC) so at many sites, this may limit the use of ozone as a pretreatment method to oxidize refractory organics, making them more amenable to biological oxidation. [59]

Peroxide Hydrogen is considerably more stable in water than ozone. Meanwhile the half-life of ozone in groundwater is less than 15 min, the peroxide hydrogen have approximately 1 hour [60,61]. Stability of the hydrogen peroxide solution inserted into the groundwater is particularly important. Peroxide disintegration can result from either homogeneous or heterogeneous catalysis. Iron and copper are the most common catalysts, but other metal species can also serve to decrease the stability of the peroxide.

The following reactions show how different iron salts affect the decomposition of H_2O_2 [62, 63]

$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH^- + OH^\cdot$ (Hydroxyl radical)

$OH^\cdot + H_2O_2 \rightarrow H_2O + H^+ + O_2^{\cdot -}$ (Superoxide radical)

$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + OH^- + OH^\cdot$

$Fe^{+++} + H_2O_2 \rightarrow Fe^{++} + 2\ H^+ + O_2^{\cdot -}$

$Fe^{+++} + O_2^{\cdot -} \rightarrow Fe^{++} + O_2$

In the same way enzymatic catalysts, such as catalase, also has negatively affect in peroxide stability. The enzymatic decomposition reactions are:

$2H_2O_2 \rightarrow 2\ H_2O$

$H_2O_2 + XH_2 \rightarrow 2\ H_2O + X$

where X can be: NADH, glutathione or other biological reductants.

If peroxide decomposes too quickly into oxygen and water, the oxygen may not get far enough downgrading to serve as an oxygen source for the microorganisms. Also with poor stability, the amount of hydrogen peroxide necessary for a remediation project can increase dramatically, thus increasing the project cost. For this reason, the stability of hydrogen peroxide must be considered for each and every system.

If peroxide stability is a problem, stabilizers can be added most easily by modifying the nutrient formulation. For example, adding phosphate to hydrogen peroxide solutions decreases the catalytic action of iron but practical experience shows that excessive use of phosphate as a stabilizer leads to aquifer plugging, due to precipitation of the phosphates. This plugging problem can be avoided by using polyphosphates because of their higher solubility. [64]

To reduce decomposition from enzymatic catalysts, several options have been reported; however, the only stabilizer reportedly used under field conditions is citrate. The additions of small amounts of sodium silicate increases peroxide stability and can also improve soil permeability. Other peroxygen compounds, such as calcium peroxide and magnesium peroxide, demonstrate good stability during in-situ bioremediation. [65]

Hydrogen peroxide is reasonably inexpensive and produced by different methods as: the anthraquinone oxidation based on an optimized distribution of isomers of 2-amyl anthraquinone, the direct synthesis of H_2O_2 from O_2 and H_2 using a variety of catalysts as gold-palladium nanoparticles and electrolytic reduction of oxygen in a dilute sodium hydroxide solution. [66, 67, 68]

Technical and Regulatory Guidance for In situ Chemical Oxidation of Contaminated soil and groundwater reported that the efficacy of hydrogen peroxide oxidation may be limited by incomplete site delineation, subsurface heterogeneities, low soil permeability and highly alkaline soil where carbonate ions are free radicals scavengers. Other disadvantage of hydrogen peroxide oxidation as remediation method includes the need for pH control and difficulties controlling in situ heat and gas production. In the other way is reported that 100 mg/l H_2O_2 provides 50 mg/l oxygen and hydrogen peroxide can be added in-line along with nutrient solution which keeping the well free of heavy bio-growth. However, the concentration of H_2O_2 in the infiltration water shall not go beyond 1000 m/l as otherwise the micro flora may be damaged. Also, too high concentrations of H_2O_2 may result in creation of O_2-gas bubbles and thus in dropping the hydraulic permeability with a subsequent deficient supply with nutrient salts and oxygen in the areas hydraulically not reached. Apart from that, loss of O_2 may occur by outgassing into the unsaturated soil zone. [69]

There is an ongoing debate as to what oxidant is the best. Some believe that hydrogen peroxide is the most effective oxidizing agent and can supply improved oxygen levels. In the same way less equipment is required to oxygenate the subsurface.

Anna Goi at al. have reported that in combined chemical treatment (Fenton-like and ozonation) efficiency strongly dependent on the soil matrix, in this way the organic-rich soil required higher addition of chemicals than sand matrix soil. They also analyzed the influence of soil pH and find very useful decreasing pH when emergency response action must be taken, but in situ remediation natural soil pH is recommended. Finely they concluded that application of combined Fenton-like pre-treatment and subsequent biotreatment is the most effective technology for oil contaminated soil remediation. In this case moderate doses of hydrogen peroxide should be applied in order to support the following biodegradation. [70]

Other Electron acceptors as hypochlorite is used in the treatment of drinking water but is not recommended the use of this substance for in situ treatment of organic wastes because undesirable by-products production.

Brown, Mahaffey and Norris have been investigated use of nitrate as an electron acceptor for degradation of monoaromatic (except benzene) and polyaromatic compounds and they found that nitrate does not result in degradation. [71]

3.1.4 Air sparing

Air sparging was introduced in the mid-1980s [72] as a remediation technique used by injecting air into contaminated ground water. The main purpose of the air sparging system is volatilizing organic compounds (VOCs) and to provide biodegradation in the groundwater passing through the treatment zone. Injected air traverses horizontally and vertically in channels through the soil column, creating an underground stripper that take away contaminants by volatilization into the unsaturated zone, where a vapor extraction system is usually employed to remove the generated vapor phase contamination. Together they are called an AS/SVE. The VOCs in petroleum are drastically reduced by the process but air sparging has a medium to long duration which may last, generally, up to a few years. [73,74]

The use of air sparging has increased rapidly since the early 1990's because in recognition of pump and ex-situ treatment limitation. It is now likely to be the most practiced engineered in situ remediation option when targeting the treatment of hydrocarbon-impacted aquifers. [75,76] The following figure 4 shows typical air sparing technology.

Air sparging may be used for different application such as:

1. Treat saturated zone in an area, where hydrocarbons have been releases into the subsurface.
2. Treat dissolved phase plume.
3. Contain a dissolved phase plume.
4. Immobilize contaminants through chemical changes.

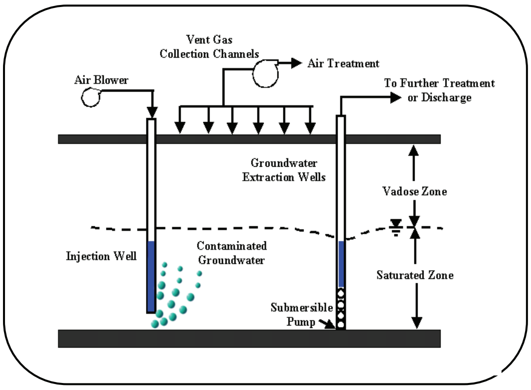


Figure 4. *Air sparging technology [29]*

In the first case is important to identify physical limitation on the effectiveness of air sparging for dense non aqueous phase liquid (DNAPL) treating, because of limitation of interphase mass transfer. Modeling performed by McCray and Falta suggest that DNAPL can be remediated in situ by air sparging in homogeneous media but in heterogeneous media in situ air sparging is not favored. [77]

Another common use of in situ air sparging is for the treatment of dissolved phase contamination in plume, downgradient of source area like light non aqueous phase liquid (LNAPL). In this case in situ air sparging points can be conveniently located just below the plume to get the desired coverage. In a survey of 32 in situ air sparging case studies, Bass and Brown determined that performance of this technology was generally better in system treating dissolved-phase plumes than in systems treating adsorbed contaminants. [78]

To contain a dissolved phase plume is require the installation of series points with overlapping zones of influence in a manner similar to the placement of a permeable barrier or reactive wall. The objective of this approach is to halt contaminant migration. [79]

Finally an air sparging technology can be used to immobilize contaminants through chemical changes. Basically aeration increases dissolved oxygen concentration in the groundwater and consequently increases oxidation-reduction potential and redox reactions can occur at or near in situ air sparging wells.

The technology of spraying of standard air has been modified in 90-s, testing with the steam injection, warm air and gases other than air as pure oxygen, ozone, pure nitrogen, methane, butane, propane or nitrous oxide. Recent laboratory-scale studies strongly suggested an advantage to operating air-sparing systems in a pulsed mode. [80,81,82]

During the early years of developing the air sparging technology was commonly assumed that physical process may be as the “aquarium model” with small air bubbles that rise within the aquifer. [83] It was later demonstrated in bench –scale research that bubble flow can take place, but only in porous media having relatively large soil grains (more than 2 mm diameter) like sands or gravels. [84]

In finer-grained soils, saturated-zone airflow resulting from air injection presents discrete pore-scale or large-scale channels, rather than as uniform bubbles.

Leeson et al in a field experiment shows air channel distribution during air sparging, where the introduction of air into a water saturated formation in first step displaces some water and then air makes its way to the water table surface, creating a transient groundwater mound. [85]

Each displacement of water represents more vertical (and horizontal) mixing than is normally seen in ground water. This is significant issue for cleanup because most subsurface processes are intrinsically mixed-limited.

Lundegard et al using geophysical visualization tools have observed a tendency in uniform sands for some portion of the initially dewatered zone to resaturate while stable airflow patterns become established. [86]

More recently, Peterson et al have described a third airflow geometry, termed “Chamber flow”, that they have observed in soils with grain size of approximately 0,2 mm. Chamber flow is characterized by much higher air-filled volumes than would be expected from channel flow. [87] In any case flow paths and density of air depends of both, soil stratigraphy and heterogeneity.

In the other way, addition of oxygen to the soil and groundwater by air sparging technology encourages biodegradation process. Dissolved oxygen is often the factor that limits biodegradation in the saturated zone but the solubility of oxygen from air is quite low at normal ground water temperatures so, the rate that oxygen can be dissolved into groundwater is often slower than the rate that microbes consume the oxygen. [88]

In 1995 Mohr presents an analytical solution for estimating the rate of biodegradation associated with air sparging. It was known that typically, approximately 3g of oxygen are necessary to biodegrade 1 g of petroleum hydrocarbon so a comparison of the mass of oxygen necessary for biodegradation and an estimate of rate of oxygen dissolution into groundwater during biosparging can be used to check design parameters of biosparging system. [89,90]

The primary advantage of air sparging applies in situ over alternate remediation technologies is low cost, especially if the treatment do not requires soil vapor extraction for waste stream remediation. The same way advantage of air sparging is simplicity of this technology. [91]

Disadvantages of air sparging over alternate remediation technologies are mainly related to side physical or chemical characteristics that either preclude contaminant removal or change contaminant mobility to threaten potential receptors.

Other disadvantage is when NAPL present in amounts considerably greater than residual saturation and may constitute infinite source of dissolved VOCs, so that may come only limited contact with injected air. This is especially likely to be a concern relative to DNAPLs that will generally be present farther below the water table than LNAPLs, and thus will tend to have even less contact with spared air.

Finely a single air sparging well has a limited a real coverage and for big area a significant number of injection wells are commonly requires. [92,93]

3.1.5 Bioslurping

It is one of the multi-phase extraction (MPE) methods that is employed adaptation and use of vacuum-enhanced dewatering technologies to remediate hydrocarbon-contaminated sites. [94] During the remediation of hydrocarbon-contaminated areas, the usual first step, if free product is present, is its recovery by direct pumping from extraction wells or trenches. However, such methods cannot overcome the capillary forces that retain the remaining NAPL within soil or fractured rock pores.

Unlike other LNAPL recovery technologies, bioslurping systems treat two separate geologic media simultaneously. Bioslurping pumps are designed to extract free-phase LNAPL from the water table and to aerate vadose zone soils through soil gas vapor extraction.

A simple bioslurping installation consists of a well into which an adjustable length other tube is installed. This “slurp tube”, connected to a vacuum pump, is lowered into the LNAPL layer, and pumping begins to recover free product along with some groundwater. The depth of the “slurp” tube can be adjusted to minimize water uptake. [95]

The vacuum-induced negative pressure area in the well encourages LNAPL flow toward the well and also pulls LNAPL trapped in small pore spaces above the water table. When the LNAPL level debilities slightly in response to pumping, the slurp tube begins to draw in and extract vapors. This removal of vapors promotes air movement through the unsaturated zone, increasing oxygen content and enhancing aerobic bioremediation.

The typical bioslurping technology is depicted in figure 5.

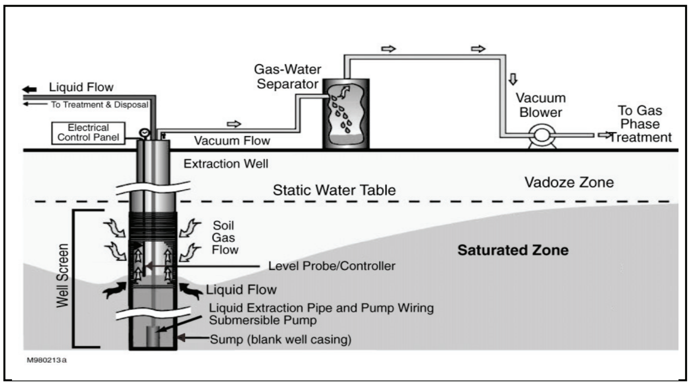


***Figure 5**. Bioslurping technology [96]*

A comparison of bioslurping to conventional methods of LNAPL recovery reported that bioslurping achieved the greater recovery rates than either skimming or dual pump methods. [97,98]

The U.S. Air force Center for Environmental Excellence (AFCEE) Technology Transfer Division Bioslurper developed procedure for evaluating the potential for recovering free-phase LNAPLs from petroleum-contaminated areas. There are factors that need to be considered when using this process that may limit the applicability and effectiveness of this technology like:

- Bioslurping is less effective in low-permeability soils.
- Biodegradation may be limit in low soil moisture because bioventing tends to dry out the soils.
- Frequently, the off-gas from the Bioslurper system requires treatment before discharge. However, treatment of the off-gas may only be required shortly after the startup of the system as fuel rates decrease.
- At some sites, Bioslurper systems can extract large volumes of water that may need to be treated prior to discharge depending on the concentration of contaminants in the process water.
- Emulsions can be formed by the high-velocity pump systems; however, pre-pump separation or a de-emulsification unit can solve this problem
- Since the fuel, water and air are removed from the subsurface in one stream, mixing of the phases occurs. These mixtures may require special oil/water separators or treatment before the process water can be discharged. [99]

Depending on specific site conditions operation and maintenance duration for bioslurping varies from a few months to years.

E.Gidarakos and M.Aivalioti reported results of a 4-year application of bioslurping technology on the subsurface of a Greek petroleum refinery where was significant presence of LNAPL in 1,000,000m^2 area. About 55 wells are connected to the central bioslurping unit and 120 additional wells for the monitoring. They observed a significant reduction of the free phase layer thickness within the study area. [100]

Bioslurping, designed to address floating LNAPL layers, is applied most often at sites with fine to medium-grained overburden materials, but it is also used successfully at sites with medium to coarse grained materials and even in fractured rock. [101]

In the same way it can be used at area with shallow groundwater as well as place with the groundwater below 30m. [102]

As compared to most other LNAPL remediation systems, bioslurping is considered to be cost-effective but few applications have been reported.

3.1.6 Permeable Reactive Barrier

A permeable reactive barrier is a wall built below ground, to perform in situ remediation of contaminated groundwater. The wall is designed to be more penetrable than the surrounding aquifer materials so that pollutants are treated as groundwater readily flows through, without considerably modifying groundwater hydrogeology. The filled trench or funnel is covered with soil, so it usually cannot be seen above ground. [103]

The distinguishing feature about this technology is that it is a passive system that requires no pumping and therefore more cost-effective than standard remediation techniques. [104,105,106]

A permeable reactive barrier material, containing of permanent, semi-permanent or replaceable reactive media, is located in subsurface transversely of path of the flow of contaminated groundwater. As the contaminant transports through the material, reaction occur that convert the pollutant into less harmful or immobile class by precipitation or sorption. A wide variety of materials are being proposed for use in permeable reactive barriers for in situ groundwater cleanup. [107]

Organic compounds tend to be undergoing sorption due to hydrophobic expulsion from the surrounding water. Metals, however, tend to sorbs through electrostatic attraction or surface complexation reactions. Sorption and precipitation are potentially reversible and may thus require eliminate of the reactive medium and collected products in order to continue with remediation. Different situation is presented when the contaminants are transformed in to a less harmful or nontoxic form. Through chemical or biological response this process most commonly takes the form of an irreversible redox reaction and the medium may directly supply electrons for reduction or stimulate microorganisms to facilitate electron transfer. [108]

In order to make possible elimination or transformation of contaminants is necessary to use reactive material which interacts with them. A study by USEPA [107], classified the permeable reactive barrier materials according to the target pollutant and the mechanism of removal.

The most common reactive material is zero-valent iron which tend to be oxidized, passing its electron to contaminants. [109]

J.A.Sacre evaluated 124 permeable reactive barrier projects in the USA and reported that in majority of projects (45%), zero valent iron was the material used as reactive barrier materials. [110]

Another study conducted by USEPA, on around 50 permeable reactive barrier, across Europe, US and Canada, informed that zero-valent iron is used in more number of cases. [111]

Zero-valent iron was the first material to be use in permeable reactive barrier for groundwater remediation which is applied mainly to treat chlorinated solvents. The mechanism of this degradation is given in part 1 of Meggyes's T. and Simon's F.G. publication. [112]

Many studies indicate that during the oxidation of Fe^0 to Fe^{+2} can increase pH which in time leads precipitation of number of minerals. In order to overcome this problem, iron sulfide or pyrite are added (oxidation process of pyrite produced acid) which lowers the pH and increases the organic removal efficiency but in this condition higher levels of dissolved iron could be presented in water downstream after the reactive barrier. [113,114,115]

In the other hand, product of iron corrosion under anaerobic conditions which is hydrogen gas, can make the iron surface temporarily passive. In this case, venting of hydrogen gas or alternatively, using microorganism that can utilize hydrogen as an energy source in anaerobic environment may help. [116]

Additionally zero valent iron is not efficient on all types of organic compounds especially dense non-aqueous phase liquid compounds like 1,2-dichloroethane and dichloromethane. [117]

Significant increase of the surface area and a minor total iron mass is requires in the treatment zone with granular sized iron medium, like colloidal (1-3 µm) or nanosize (1-100 nm). Per mole of iron, ferra-gels reduced 20-30 times more contaminant than commercial iron filings. [118,119]

Greater performance compared to having zero valent iron along have shown bimetallic systems like Fe-Cu, Fe-Pd, and Fe-Ni. Although initial laboratory-and pilot-scale studies of bimetallic systems look promising because of the fast reduction rates, there is still a great deal of uncertainty regarding the reaction mechanism, product formation, and long term efficiency. Additionally, significant problem with bimetallic couples is the observed loss of reactivity over time. [120,121,122,123]

Recent studies show a synergetic interaction between microbial activity and zero valent iron which can increase degradation efficiency of hydrocarbon pollutants. Under anaerobic conditions, nitrate, ferric ion, sulfate, and carbon dioxide can serve as electron acceptors. [124]

Novak et. al. demonstrated by experiments with pure cultures of methanogens, including hydrogenotrophic species that could grow on hydrogen, therefore cathodic hydrogen could enhance microbial reduction of chlorinated solvents [125].

In other publication the author is showed Fe^o stimulated effect over *M.thermophila* to excrete an extracellular factor with protein-like characteristics that degraded both, carbon tetrachloride and chloroform, which suggest that some microbial–Fe^o interactions can enhance significantly the efficacy of Fe° barriers [126].

This notion is supported by field sampling of a pilot test after 6 months of operation, which revealed the presence of methanogens, sulfate reducers, metal reducers, and trichloroethylene-degrading bacteria [127,128].

Apart from iron based materials, many natural resources such as activated carbon, leaf, peat, sewage, sludge, sawdust [129] also alkaline-complexion agents like hydrated lime, ferrous sulphate [130], phosphate minerals, fish bone [131], zeolites [132,133] and clay [133,134]. In the same way have being evaluated different polymers and polymer membrane containing Fe^0 [135].

The selection of the reactive media is based on the following criteria [137]

- Reactivity – evaluated by the reaction rate constant or residence time. In order to keep the barrier's thickness is desirable to have low residence times and high reaction rates.
- Stability - replacement of the reactive materials is not easy to do, that's why is expected to remain it active for long time. Also result essential stability in changes of pH, temperature, pressure and antagonistic factors.
- Availability and cost – Due to large amount of reactive material required for the construction is important to have considerable quantities of it, in low prices.
- Hydraulic performance – To make hydraulic conductivity greater or equal of the surrounding soil, the reactive material must provide appropriate permeability.
- Environmental compatibility – To avoid a new source of contamination the reactive media cannot form any byproducts or solubility by itself.
- Safety- Handing of the material should not generate any risks for the workers' health.

The following materials are most common used to remove halogenated hydrocarbons and aromatic compounds from contaminated groundwater: zero valent Iron, zero valent iron pellets or mixture concrete, sand and iron, surface modified zeolite, iron with oxygen, activated carbon and bimetallic systems Fe-Cu, Fe-Pd, and Fe-Ni. [130]

Permeable reactive barrier use two structures for plume capture. There are Continuous and Funnel-and-Gate systems used in field application, where hydrogeological characteristics of the sides and the reactive material cost make difference to choice.

Figure 6 depicted general scheme for both systems. [137]

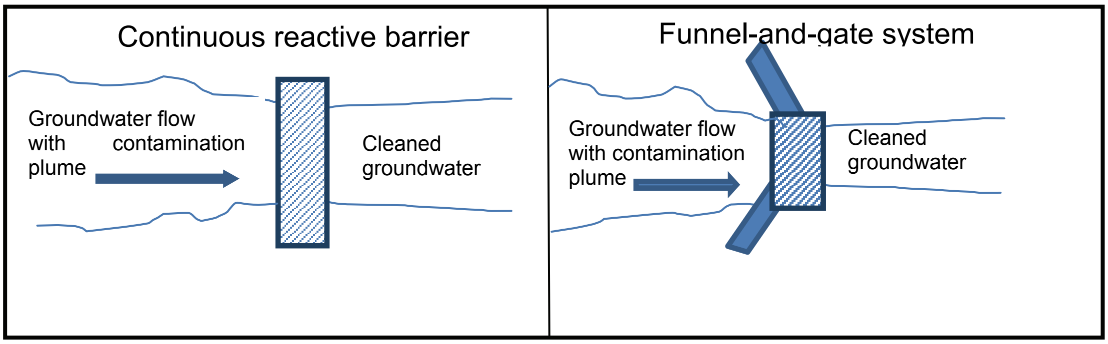


Figure 6. *Configuration of the continuous reactive barrier and the funnel-and-gate system*

Although funnel-and gate configuration requires less material, the construction cost of continuous type barrier is much cheaper. [138]

The continuous systems are installed underground using special foundation techniques. They are filled with reactive or adsorbing material, which was described above, e.g. iron sponge, to intercept the contaminants from the groundwater. The treatment process takes place along the entire surface of the barrier. Because this technology is passive, complete process only occur after the total plume has passed through the wall. This may take many years, so groundwater monitoring system should be put in place to monitor whether the technology is still working over the long term. [139]

The major disadvantage of the continuous reactive barriers is that in case of large aquifers great amounts of costly reactive material have to be installed reducing the economic efficiency of the remediation. Besides, over dimensioning of the reactive zone may cause insufficient treatment performance. [140] To avoid that, funnel-and-gate systems have been developed.

Funnel and gate system is used to channel the contaminant plume into a gate which contains the reactive material. The funnels are non-permeable, and the simplest design consists of the a single gate with walls extending from both sides. The main advantage of this system is, that a minor reactive region can be used for treating the plume, resulting in a lower cost. Besides, if the reactive media needs to be replaced, it is much easier to do so because of the small gate. [141]

Funnel-and gate setup can be installed in series or succession. Alternatively a less expensive method is multiple caisson gates.

Turlough et al. reported positive result an application of a funnel and gate permeable reactive barrier in treatment of petroleum hydrocarbon contaminated groundwater. They achieved from 63 to 96% of removal efficiencies for the monocyclic aromatic hydrocarbons during the 10 month period. [142]

Usually, the conventional permeable reactive barrier installation techniques need some degree of excavation, which limits this treatment to fairly shallow depths of 20 m. [143]

However, application of new construction techniques, such as slurry injection and hydro-fracturing allows to overcome this depth limitation. In this system is no necessary construct a trench and possible aquifer access at greater depths. [143]

In injection system, the reactive material (chemical/particulate mixture) is injecting into the treatment zone by series of bore holes or wells. Nevertheless, it has to be made sure that the contaminant plume is efficiently taken care of and no by-passing, which may impair the remediation effect. [144]

Although the technology of the reactive permeable barriers is continuously progressing, there is still certain disadvantage such as: [145]

- Mineral precipitation may limit the permeability and require replacement of the reactive media.
- The wall system can generate byproducts in the surrounding environment.
- Large and deep plumes are more difficult to remediate than small and shallow plumes.
- It is limited to subsurface lithology that has a continuous aquitard at a depth that is within the vertical limits of the trenching equipment.
- Limited long term field testing–data

Even if treatment walls are often used for groundwater contaminated with volatile organic compouns (VOCs), semi- volatile organic compouns (SVOCa) and inorganics pollutants this tecnology is ineffective in treating other fuel hydrocarbons. [146,147]

3.1.7 Flushing in situ

This treatment use large volumes of water, at times supplemented with surfactants, co solvents, or treatment compounds, which are applied to the soil or injected into the groundwater to raise the water table into the contaminated soil zone. Injected water and treatment agents are isolated within the underlying aquifer and recovered together with flushed contaminants. This helps flush the harmful chemicals from the ground by moving them toward wells that pump the chemicals out of the ground. [148]

Local soil conditions affect the process. If the soil has a high percentage of silt or clay, the flushing solution cannot easily move through the soil and make contact with the contaminants. Soil with greater than 1% organic matter and high clay content hinders movement of the flushing solution. [149]

Khalladi et al. has found that the surfactant to be efficient, have to take enough contact time with contaminated soil. [150]

Soil flushing methods remove contaminants not only by dissolving the liquid but also sorbet, or vapor phase or by mobilizing contaminants existing as free product in the soil pores and adsorbed to the soil. [151]

The former processes are controlled by the solubility of the contaminants and Henry's law constants, while the latter are removed by the pressure gradient of the flushing water and are controlled by the viscosity and density of the contaminants. [152]

Solutions with the greatest potential for use in soil flushing would be water, acidic aqueous solution, basic solution, complexion and chelating agents, surfactants, and reducing agents.

Water is applicable for flushing water soluble compound such as phenols or water mobile organics and inorganics. Organics docile to water flushing can be identified according to their soil/water partition coefficient, or valued using the octane/water partition coefficient, K_{ow}. Inorganics that can be flushed from the soil with water are soluble salts, such as the carbonates of nickel, Zink and copper. [153]

The removal of PHSs from soil matrices with pure water, by ex situ soil washing or in situ soil flushing, is quite unsuccessful because of their low solubility and hydrophobicity. [154]

Adjusting the pH with dilute solution of acid or base will enhance inorganic solubility and removal, also could be used if the soil contains sufficiency alkalinity or acidity to neutralize but this addition require careful management and knowledge of possible reactions that can undesirably affect the soil system. For example addition of sodium hydroxide may adversely affect soil permeability by disturbing the soil sodium absorption ratio. Complexing and chelating agents can mobilize metals strongly adsorbed in soil. [155]

Surfactants are Surface Active Agents with large ranging properties including the lowering of surface and interfacial tensions of liquids. Surfactants have both hydrophilic and hydrophobic portions in the molecule. The hydrophilic (polar) part of a surfactant is usually referred to as the "head", whereas the nonpolar hydrophobic portion in known as the "tail". Surfactant are classified according to the ionic charge residing in the polar part of the molecule. Hence anionic, cationic, nonionic and zwitterionic (combined presence of anionic and cationic charges) surfactants exist. The presence of surfactants can lead to an increase in the concentration of hydrophobic compounds in the water phase. This is achieved through the formation of oil/water emulsions and solubility, where, above the Critical Micelle Concentration (CMC), surfactants molecules aggregate to form micelles. Micelles arise when the lipophilic part of the surfactants molecule, that is unable to form hydrogen bonding in an aqueous phase, causes an increase free energy of the system. One way to alleviate this free energy is for the hydrocarbon tail to be isolated from water by adsorption onto surfaces, absorption into an organic matrix or the formation of micelles vesicles. [156]

In this way surfactants can get better the solvent property of the restore water emulsify non soluble organics, and enhance elimination of hydrophobic organics entrapped onto soil pore. That's why they have been applied in oil washing for in the secondary oil recovery and to clean oil pipes and oil. The efficiency of this technique is directly related to types and amount of flushing agents added. [157,158]

An important characteristic of surfactants relates to the relative abundance of hydrophilic and hydrophobic constituents. Surfactants can be classified according to their Hydrophile-Lipophile Balance (HLB) that affects their physicist-chemical properties. [159]

The HLB classification can be used to define the suitability of use of surfactants.

In general, a surfactants with low HLB is lipophilic whereas a high HLB confers better water solubility [160].

In desorption of hydrocarbons from the contaminated soils, both synthetic and natural (biological) surfactants have been used. Synthetic surfactant most de time used for soil flushing is Sodium dodecyl sulfate (SDS), an anionic surfactant. The maximum SDS adsorption and precipitation occur when SDS concentration is in the range of the critical micelle concentration. [161]

Ouyang et al. used sodium lauryl sulfate to remove gasoline and tetra-ethyl lead entrapped in the soil pores. They detected that saline solution displaced only limited amounts of mobile gasoline and associated tetra-ethyl-lead(TEL) components from soil: However, 95% of the immobile or residual gasoline and 90% of the associated TEL captured in the soil pores can be removed by a surfactant/co-surfactant/water solution.

They established that 1 g of surfactant (sodium lauryl sulfate) can removed 0.6 g immobile gasoline and 2 mg immobile lead from the soil. [162]

Abdul et al. reported a result of the field test of the surfactant-washing method applied to a site contaminated with PCBs and oil. During 70 days nearly 10% of the initial mass of the contaminants was washed from the plot during the test. They used 5375 gal of an 0,75% aqueous surfactant solution at an advantage rate of 77gal/day, and 10981 gal of leachate were recovered at an average of 157 gal/day. [163]

After the treatment is complete, the remaining surfactant can be separated and reused. Numerous alternatives for removing surfactants and contaminants from leachate have been considered: Flocculation-coagulation-sedimentation, sorbent adsorption, ultrafiltration, foam fractionation, surfactant hydrolysis and phase separation, centrifugation, and solvent extraction. Ang and Abdul showed, in an in situ washing of contaminated soil that ultrafiltration unit with an XM50 membrane can recover 46% of the surfactant while the membrane retains 89% of contaminant oils. A PM500 membrane recovers 67% of the surfactant and retains more than 83% of the oil. This is make soil washing technique more economical. [164]

Edwards et al. determined that treating soil with successive surfactant washing result in greater removal of hydrocarbons than a single washings with the same amount of surfactant. [165]

Synthetic surfactants have been shown to remove nonpolar compounds from surfaces but problem can be associated with their use, such as reduced availability of compounds sequestered into micelles, their toxicity and ultimate resistance to biodegradation leading to increased pollution. [166,167]

When compared with synthetic surfactant, biosurfactants in general exhibit greater environmental capability, better surface activity, lower toxicity, and higher biodegradability. [168.169] The problem is the high production costs which currently prohibits their large scale utilization, although organism involved in their production can use a wide range of cheap substrates including industrial such us oils, fats and agricultural waste products[170,171]

Bacterial surfactant-producing organisms include *Pseudomonas aeruginosa* (mono- and di-rhamnolipid biosurfactant), *Corynebscterium,Nocardia* and *Rhodococcus,* spp. (phospholipids, trehalose dimycolates/dicorynomycolates, glycolipids, etc.),*Bacillus subtilis* (surfactin), *Bailluslicheniformis* (lipopeptide similar to surfactin), *Arthrobacter paraffineus* (trehalose and sucrose lipids) and others. [172,173]

A method of incorporating surfactants in soil washing followed by surfactants-aided bioremediation is being developed in France. Over 80% of diesel oil was recovered by flushing the soil with a RESOL 30 solution containing nonionic and anionic biodegradable surfactants at a concentration of 10g/l and 1,5 g/l sodium chloride. This was followed by pumping air through the porous soil and adding an aqueous solution containing nutrients (nitrates and phosphates) and an 0,05 wt% solution of RESOL 30. [174]

Finally observations related to soil washing performance are:

- Soil washing is cost-effective method but the flushing solution must be compatible with the in situ environment.
- Low permeability and subsurface heterogeneity can interfere with uniform distribution of flushing solution.
- This technique is not feasible when complex wastes containing a range of contaminants with different solubility characteristics are involved
- Intensive washing may deteriorate the soil.
- Surfactants can adhere to soil and reduce effective soil porosity.
- Synthetic surfactant decrees organic degradation.
- Wash water must be recovered and treated before are final disposal.

Desirable features of this technique are:

- This method can provide for recovery of chemicals in cases involving spill of individual substances.
- A benefit of this method is that is removes contaminants more quickly without disturbing the soil.
- This remediation can be combined with others such as biodegradation or bioventing applied into groundwater.

3.2 BIOTECHNOLOGY PROCESS

The development of petroleum industry also the extensive use of petroleum products leads to inevitable spillages that occur during operation and registers of severe accidents during transportation, has called for new studies into oil pollution problems, which nowadays has been recognized as the most significant contamination problem on the continent. [175]

These pollution problems often result in huge disturbances of both the biotic and abiotic components of the ecosystem. The currently used physical and chemical treatments are effective for the degradation of petroleum products, but they are expensive and need high technology, also they frequently produce various hazardous compounds, which are strong immunotoxicants a carcinogenic for living beings. In contrast bioremediation is effective treatment in terms of efficacy, safety on long terms use, cost and simplicity on administration.

3.2.1 Bioremediation of petroleum oil contaminates soil and water

Bioremediation has been defined by Madsen as "a managed or spontaneous process in which biological, especially microbial catalysis act on pollutant compounds thereby remedying or eliminating environmental contamination". [176]

Microbial remediation of an oil contaminated site is possible with the help of a diverse group of microorganisms, mainly the indigenous bacteria present in soil.

Claude U.Sable had as far back as 1946, documented that numerous microorganisms, widely distributed in nature, have the ability to utilize hydrocarbons as the sole source of carbon energy. The author further recognized that this process depends of chemical structure of the compound within the petroleum mixture and environmental circumstances. [177] [178]

Since beginning 1970s, bioremediation of petroleum hydrocarbons has become an known technology and has gained increased attention and application during the last decades. [179] It has been shown that, given adequate time, bioremediation is the most cost-effective method to clean up hydrocarbon contaminated soils and groundwater.

Soils considered suitable for bioremediation, are typically contaminated with hydrocarbons at levels of 0,2-55% by volume of soil concentration and technologies can be generally classified as ex situ or in situ. [180]

Ex situ treatments remove pollutants at a separate treatment and in situ involve biodegradation in the place itself.

In situ remediation is a worthy on many occasions, especially because it is costly to excavate the whole mass and transport it for remediation, but a major challenge of this method is to achieve uniform remediation throughout the treatment area [181].

Most of reported bioremediation techniques have been tested in laboratories, performed under optimal conditions, which do not correlate well with real condition in the field, for this reason, application of data from laboratory experiments to the field, has often lead to unsatisfactory result [182],[183],[184],[185].

The rate of biodegradation in soils is a function of the availability of the chemicals to the microorganisms that can degrade them. It is a key factor in the efficient biodegradation of pollutants. Chemotaxsis or the direct movement of organisms towards or away from chemicals may contribute to effective catabolism of molecules in the surroundings. Additionally, mechanisms for the intracellular accumulation of aromatic molecules using diverse transport mechanisms are important too. [186]

To make more efficient these processes, the application of genetically engineered microorganisms for bioremediation in situ has also been considered. [187]

Petroleum oil is a heterogeneous liquid of hydrocarbons contained of the elements hydrogen and carbon but also nitrogen, sulphur and oxygen which constitute less than 3% (v/v). There are also trace ingredients, containing less than 1% (v/v), including phosphorus and heavy metals such as vanadium and nickel. Due to big variety of compounds, biodegradation of petroleum is managed by consortia of bacteria which produce specific enzymes for this purpose and take place under both oxic and anoxic condition. [188]

Oxigenase enzymes are requires to carry out metabolism of hydrocarbons in oxygenic condition. Normally with aliphatic hydrocarbons, alcohols are initially formed; these are oxidized sequentially via dehydrogenases to carboxylic acids, which then undergo β-oxidation. In aromatic substrates as well as Polycyclic Aromatic Hydrocarbons (PAHs) hydroxylation of a ring occurs via mono or diocygenase enzymes in eukaryotes and prokaryotes. After diol creation the ring is cut, then further degraded but no strains have yet been found to utilize PAHs with more than four rings. [189],[190],[191]

Some of the common microbial genera degrading hydrocarbons in soil are: *Achromabacter, Aerobacillus, Alcaligenes, Arthrobacter, Bacillus, Bacterium, Beijerinckia, Botritis, Citrobacter, Clostridium, Corynebacterium, Desulfovibrio, Enterobacter, Escherichia, Flavobacterium, Gaffkya, Methanobacterium, Microroccus, Micromonospora, Mycobacterium, Pseudomonas, Sarcina, Serratia, Spirilum, Thibacilus*. [192],[193]

Other petroleum hydrocarbon degraders include: *Acinetobacter sp., Capnocy-tophaga sp., Moraxella sp., Providencia sp.,Rosemonas sp., Stenotrophomonas sp., Streptococcus sp., Sphingobacterium sp., Yokenella sp.,* [194],[195],[196]

Also organisms such a fungi, are able of degrading the hydrocarbons; however, they take longer periods of time to grow than bacterial counterparts [197]

It is important to take into account that different microorganisms exhibit different group specificities. For example, some of them grow on alkanes of six to ten carbons in chain length, whereas, others develop on long chain alkanes. In the same way some of the oxigenases are encoded on plasmids and others on chromosomal genes and sub-terminal oxidation apparently take place in some bacterial species. [198]

Hydrocarbon biodegradation under anaerobic condition also follows an oxidative strategy.

This process can take place under Fe(III)-reducing, denitrifying and sulfate-reducing conditions, by anoxigenic photosynthetic microbes, or in synotrophic consortia of proton-reducing and methanogenic bacteria. Other terminal electron acceptors shown to be used during anaerobic hydrocarbon metabolism include soil humid acids, manganese oxides, and fumarate in a fermentative oxidation route. [199],[200],[201].

Nevertheless the anaerobic pathway od aliphatic hydrocarbon metabolism has not been described in great detail.

The first study of anaerobic degradation of petroleum hydrocarbons in natural locations by microorganisms has been shown to take place only at negligible rates and its ecological implication has been usually considered to be minor. More newly mixed-culture work continues as enhanced bioremediation tactics are tested and new metabolites are defined. [202],[203],[204].

3.2.2 Physical, chemical and environmental factors affecting the biodegradation of hydrocarbons

It is important take into account that abiotic losses, play a important role in decontamination of oil spill environments due to evaporation, photo-oxidation, and dispersion. [205]

In order to adopt appropriate methodology to optimize the process of biodegradation it is necessary to understand the factors limiting microbial degradation. Several reports show different rate degradation in various conditions and factors [206],[207].

Temperature plays significant role in biodegradation of petroleum hydrocarbons because affects both the property of spilled oil and activity as well as diversity of microorganisms. Temperature variously affects the solubility of hydrocarbons. [208] At low temperatures, the viscosity of the oil increases, while the volatility is reduced which can delaying the onset of biodegradation and the rate of this process generally decreases with decreasing temperature. [209]

Highest degradation rate usually take place in range of 30-40°C in soil surroundings, 20-30°C in some freshwater locations and 15-20°C in marine environments. [210]

Applicability of bioremediation technologies for petroleum-contaminated soil under cold climate conditions have generally focused on anoxic biodegradation processes by cold adapted microorganisms. [211],[212]

In contrast to soil, groundwater deficiencies air phase and thus the accessibility of oxygen as an electron acceptor is significantly diminished. Hence the unassisted biodegradation of petroleum hydrocarbons in groundwater may be controlled by anaerobic processes However, some anaerobic bacteria can degrade hydrocarbons using other electron acceptors, such as sulfate, nitrate or ferric iron. [213]

Nutrients has direct impacts on microbial activity and biodegradation process, especially nitrogen, phosphorus and in some cases low concentration of iron, amino acids and vitamins. [214]

Okolo et al. reported effectiveness of poultry manure as organic fertilizer in biodegradation of contaminated soils but the extent of biodegradation was influenced by the incorporation of alternate carbon substrates or surfactants. [215]

Roldan et al. studied the effect of mixing low amounts of orange peel (*Citrus reticulata*) with soil contaminated with hydrocarbons for stimulating the soil bioremediation. They were reported an increase in microbial respiration activity and TPH removal(69%) after 15 days in the treatment with a soil to orange peel ratio of 92:8. [216]

Several authors have reported, that excessive nutrient concentrations (high NPK levels) can inhibit the biodegradation of hydrocarbons and more specifically on the aromatics. [217],[218][219]

Effect of chemical composition of petroleum hydrocarbons plays also very important role in biodegradability process. Hydrocarbons susceptibility to microbial attack and it possible to organize in following order of decreasing vulnerability: n-alkanes>branched alkanes>low molecular weight aromatics>cyclic alkanes>polynuclear aromatics>asphaltenes. [220]

Solubility of hydrocarbons in water is low, which increases their sorption to soil particles and limits their accessibility to biodegrading microbes.

Bioavailability is a key factor in the efficient biodegradation of hydrocarbons. [221] It has been shown that hydrocarbons as other hydrophobic contaminants, with increasing age of contamination, become adsorbed in the soil matrix via partitioning into soil organic matter or diffusion into nonporous, thus becoming less extractable and bioavailable to microorganisms and that bioavailability limitation is responsible for slow biodegradation. [222]

Introduction of external nonionic surfactants can disperse the main components of oil spill and influence the alkanes degradation rate. [223]

Experience so far indicated that the use of surfactants in areas of oil contamination may have a stimulatory, inhibitory or neutral influence on the microbial degradation of the oil hydrocarbons. [224] That's why is necessary to accurately characterize the role of chemical and biological surfactants in order to predict performance of this process. [225]

Physiochemical properties of the soil represent other very essential parameters for biodegradation process. Is important taking into account, that the soil differ widely with regard to geology, fertility, climate, hydrology and other physical characteristics.

Oxygen is one of the most important parameter in determining the rate of biodegradation of petroleum compounds. In the initial attack, molecular oxygen is used as electron sink. In the absence of molecular oxygen may be supported by nitrate or sulphate reduction. [226]

The flow of oxygen into the system is controllers by oxygen concentration in the carrier and the permeability of the geological material to the carrier. [227]

The amount of oxygen accessible for biodegradation of hydrocarbons differs considerably with the particular electron acceptor and its carrier medium. With water as a carrier, oxygen in air will supply 8,0 mgO_2/l water and 400,000 kg water would be requires to degrade 1 kg hydrocarbon. [228] If the oxygen comes from H_2O_2 , a level of 100 mgO_2 /l H_2O_2 in water would require 65,000 kg carrier/kg hydrocarbon. [229]

In the same way the soil moisture is limiting parameter, because microbes live in the interstitial water of soil pores. It has been observed that degradation is considerably greater at 80% than at 40% of soil field capacity. [230]

Acidity or alkalinity of soils may have a negative influence on the ability of microbial populations to degrade hydrocarbons if takes extremes pH. Verstraete et al. reported that a doubling rate of biodegradation of gasoline in an acid (pH 4,5) soil by correcting the pH to 7,4. Rates decreases considerably, however, when the pH was further elevated to 8,5. [231]

Texture and structure of soil along with water content may affect biodegradation of hydrocarbons with time because of the formation of clay-humic acid complexes that would raise the adsorption of substrate and nutrients on soil particles, making them less bioavailable.

A „release“ as original pollutant does not seem to take place given natural circumstances, the bound remainders are rather mineralized in the course of a slow natural transformation of the humic substances. Humic substances may have an age of up to 500 years, caused, among others, by their macromolecular structure preventing specific processes of degradation to keep a quasi-stable state. [232][233]

3.2.3 Bioremediation systems and process

In situ bioremediation processes currently used in the field are classified into the following three categories:

- **Bioatenuation.** This is the method of monitoring the natural process of degradation to insure that contaminant concentration decreases with time at appropriate sampling points.

Barker et al. used the term “natural attenuation” to describe the combined dilution, dispersion, sorption, and biodegradation processes that caused contaminant concentrations to decline. [234]

In 1993 after enormous information and effort to assess and monitor petroleum hydrocarbon pollution, a committee of the National Research Council (NRC) first made the distinction between engineer’s bioremediation, where human intervention was used to enhance microbial process in soil and ground water systems, and *intrinsic bioremediation*, where natural microbial processes alone could renovate contaminated sites. In their definition of *intrinsic bioremediation*, the NRC committee also made a distinction between natural attenuation, which happens ubiquitously, and intrinsic bioremediation, where these natural processes look after human health and the environment. [235] To demonstrate that natural attenuation processes could prevent contaminant migration to sensitive receptors

such a nearby wetlands, rivers or marsh, intrinsic bioremediation required a rigorous site evaluation. [236]
Wilson J.T of the U.S.EPA and the U.S. Air Force Center for Environmental Excellence (AFCEE) developed methods for assessing intrinsic bioremediation, relied on three independent lines of evidence to establish the efficiency of intrinsic bioremediation. This approach was codifies into a protocol for use by Air Force personnel at field sites and used widely in the United States. [237]
Bioatenuation is widely used as a cleanup method for underground storage tanks sites with petroleum-contaminated soils and groundwater. [238] Is understood that Bioatenuation is the least invasive approach to bioremediation, because requires no intervention other, than by the indigenous microbial population, but its efficacy remains controversial. The growth and metabolism of microorganisms in natural environments is always limited by the availability of electron donors, as well as electron acceptors such as oxygen, nitrate, sulfate or Fe(III), and basics nutrients. [239]

The field studies have shown that hydrocarbons can be oxidized under anaerobic condition. Serrano demonstrated that methanogenic consortia could potentially biodegrade fuel hydrocarbons using CO_2 as the electron acceptor after the reduction od sulfate. The presence of raised methane in groundwater relative to background methane concentration is a good indicator of methane formation in anaerobic condition. [240],[241]

Biodegradation of groundwater contaminated with hydrocarbon is more complicated than biodegradation of soil, because ground water systems are inherently inaccessible, usually heterogeneous, and difficulty to accurately characterize. Experience has shown that satisfactory hydrologic conditions, and a perfect understanding of these conditions, are critical to the achievement of bioremediation process. [242] The natural processes as Bioatenuation are slow and there is a requirement to increase the rate of biodegradation. This can be accomplished by one of two main techniques as bioaugmentation and biostimulation.

- **Biostimulation.** If natural biodegradation does not progress, or this process is too slow, he environment side has to be stimulated. The measures to be taken, called biostimulation, include supplying the environment with nutrients such as phosphorus and nitrogen, with electron acceptors such as oxygen or other electron acceptors.

Sometimes is used chemical additives as methane or phenol or toluene but the concentration of these substances should be carefully monitored. [243],[244]

Nutrient addition to the contaminated soils has special importance. It is generally accepted that nutrient availability is the most common limiting factor. The microorganisms utilize these nutrients, as nitrogen, phosphorous and smaller quantities of other elements, (magnesium, potassium and iron) that stimulated the enzymes and effectively degrade the pollutants to harmless products. [245],[246],[247].

The effect of the fertilizer on the diesel biodegradation process is not consistent in the literature. Acceleration of the diesel biodegradation process was detected by using fertilizer to stimulate microbial growth that were in charge for clean up the marine environment and in alpine glacier skiing area. [248],[249].Marqesin et al found that the higher the initial concentration of the hydrocarbons and incubation time, the more marked was the effect of fertilization, during bioremediation of diesel-contaminated soil. [250] Singh and Lin evaluated the nutrient addition to diesel contaminated soils, and they found the nutrient stimulated the abilities of indigenous microbial population, but only during the initial stages of degradation and the levels of effect in biostimulation are based on the soil type, environmental factors and the structure of the microbial community present in the soil. The authors observed that as the carbon length increased, the % of degradation were also increased. [251]Several authors reported that the chain compounds shorter than C_9 are more difficult to degrade than longer chains, because the short chain alkanes are toxic for many microorganisms. [252],[253],[254].

- **Bioaugmentation.** Bioaugmentation can be defined as the addition of pre-grown in a laboratory microbial cultures, to enhanced microbial populations at a site to improve contaminant clean up, and reduce treatment time and cost. These degradation strains may be taken also before from site samples isolated or as collections of microorganisms. [255] Seeding microorganisms onto soil was first tried around 1968. [256]since then, the seeding of microorganisms has been used in a number different environments with varying success. Maxwell and Baqai have had unsuccessful experience with bioaugmentation process applied in site contaminated by gasoline from an underground tank. [257] It appears, that the laboratory culture microorganisms (*Bacillus, Pseudomonas, Serratia and Azobacter)*did not survive in the soil. Probably because of the nutrient limitations or contact with toxic level of contaminants.

Turco and Sadowski have indicated that the indigenous and applied microorganisms in soils may not be able to compete because of several factors as type and number of indigenous microorganisms, resistance time in soil, bacteriocin and antibiotic production, mobility in soil, and time in laboratory culture before introduction into soils, nutrient limitations, moisture requirements, soil texture and porosity, temperature, pH, and organic matter. In the same way the microorganisms may be susceptible to toxins or predators such as protozoa, in the environment. [258]

Several new methodologies have enabled recent studies on the microbial biodegradation mechanisms of organic pollutant. Especially study of the genetics of hydrocarbon utilizing micro-organisms has been stepped up within the last decade. These studies have focused mainly on aerobic pathways but also have presented detailed and comprehensive reviews, on anaerobic biodegradation of hydrocarbons. [259], [260], [261]

Genetic factors play significant roles in conferring biodegradation potentials on microorganisms and plasmids probably play leading role in this feature.

Leathy et al. have assured that exposures of a microbial community to hydrocarbons have been shown to result in an increase in number of bacterial plasmids types, which provide a metabolic versatility not normally present in the cell. Such genetic potential allows evolution of integrated and regulated path ways for the degradation of hydrocarbons. [262]

All microbiological remediation techniques may be combined with the so-called bioaugmentation. This is considered to be an effective approach in the case of very recalcitrant chemicals, where Bioatenuation or biostimulation does not work.

However, is vital to pay much attention to the application of bioaugmentation because of its unknown effects on the ecosystem. Moreover, it needs to be established that the inoculated bacteria have perished after the bioremediation and thus do not disturb the indigenous microbial community for a long period. Basically, bioaugmentation is disputed as the mass of bacteria is immobilized in subsoil within some centimeters during an infiltration into subsoil. Also the infiltration of nutrients ensures usually that the microorganisms added are overgrown by the autochthones micro flora and thus the microorganisms added do not bring an advantage as to the speed or quality of degradation of contaminants.

It is well known, that mixture of contaminated soils with clean soil improve the physical and chemical conditions of the affected soils, and reduce the contaminant concentration. In the same way is possible make more efficient bioremediation, when different soils, contaminated with hydrocarbons are mixed, because of increase numbers and versatility of indigenous bacteria. [263]

In this kind of studies, the most probable number of microbial cell must be estimated (MPN) and the method which is applied was described by Braddock and Catternall in 1999. [264]

3.3 MODFLOW

Visual MODFLOW is the software used for modeling groundwater flow, and contaminant transport simulations. This program combines MODFLOW, MODFLOWSURFACT, MODPATH, ZoneBudget, MT3Dxx/RT3D, MGO, and WinPEST with graphical interface available. The model input parameters and results can be visualized in 2D (cross-section and plan view) or 3D at any time during the development of the model, or the displaying of the results. The techniques mentioned previously, will also be utilized like support to predict the movement of the plume contaminant in the zone, the Visual software Modflow is utilized for shape the environment, with practical applications in three dimensions of the flow of groundwater and transportation of contaminants.

The Software Visual MODFLOW utilizes the mathematical method of finite differences of netting centered for the approach of the groundwater flow network.

The data at the outset that delivers the program Visual MODFLOW are: water balance, elevations, velocities and directions of flow. It is as well as the behavior of the flow of the groundwater can be observed and the transportation of the contaminants of the subsoil. In the same way is possible assess distribution of control wells. [265]

4 EXPERIMENTAL PART

Experimental part of work was carried out in the Laboratory of Colombian Petroleum Institute and some of them in the Laboratory of Environmental Department at the Pontificia Bolivarian University, Bucaramanga-Colombia.

The most important petroleum company in Colombia is Ecopetrol, fourth principal in Latin America and one of the 35 largest in the world. The oil fields Palagua of Ecopetrol S.A is located in Boyacá, occupying an area of about $800m^2$ and is supplied the needs of oil, stored in four tanks with total capacity of 42.000 BLS, being one of the key producers of the company.

In the outskirts of the Station 1, can be found to a distance in straight line of approximately 500m the houses-estates Acapulco and Alliance Palagua; to 700m the houses-estates The Mangos and The Revenge, which they are found at present inhabited, to 600m the Administrative Headquarters of Field Palagua and a School. Finally to 1.2Km approximately the Marsh Palagua is found. In the Figure 7 the picture of the Station is presented and the adjoining ground affected.

***Figure** 7. Station 1 and adjoining ground, Field Palagua (zone of interest)*

The works of exploitation of the resource of petroleum date from 1986, and since that has come causing an environmental damage not only to the soil in the area of the field, but also in the groundwater. In the 1997, a discharge of hydrocarbons by inadequate operational practices, presented spill of hydrocarbons in surface.

All the previous thing caused a problems of extensive contamination, in which was visualized the soils impregnated with hydrocarbons in the next area to the tanks of storage and the water table was affected in the side south westerner of said station. This subterranean flow is the responsible for feeding the Marsh and a part is stored and remains static.

4.1 ANALYTICAL PROCEDURES

Physicist-chemical, microbiological and topographical characterization of soil, was made in situ or in ICP laboratory, taking the simple in area where the presence of hydrocarbons has been registered during last three years, and groundwater was made in the control wells in the Station 1 (PM-10) and adding land (PM-17,18 and 19).

4.1.1 Physicist-chemical, microbiological and topographic characteristics of soil contaminated with hydrocarbons

Initially, were selected for the sample the points, taking as the criterion the place of the spill and his possible displacement indicated by the aspect of the soil (see figure 8). Each one of the sample was extracted in approximate distance of 5 m from the monitoring wells and to 30 cm of depth.

All of samples were labeled and taken to laboratory of ICP for analysis of the texture, structure, infiltration, permeability, temperature, porosity, real and apparent density, pH, gravimetric and volumetric moisture, the cation exchange and organic matter and the total petroleum hydrocarbons (TPH) to identify initial condition of soil.

***Figure 8**. Aspect of analyzed soil, measure of depth, and preservation of sample*

Texture

Texture was determined by Bouyoucos Method. The dispersed soil sample was mixed in a tall glass cylinder with water and, once settled, the density of the suspension was measured with the hydrometer. The time, which each particle takes to fall below an imaginary set plane on the cylinder, was taken after 40 seconds to measure settling sand and, after two hours to measure silt particles. All readings and hydrometer as well, were corrected for variations in temperature due the alteration of viscosity and density of the water. (Table1)

Table 1. *Correction for variation of temperature, in hydrometer measure*

°C	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
15	-1.6	-1.56	-1.53	-1.49	-1.46	-1.42	-1.38	-1.35	-1.31	-1.28
16	-1.24	-1.20	-1.17	-1.13	-1.10	-1.06	-1.02	-0.99	-0.95	-0.92
17	-0.88	-0.84	-0.81	-0.77	-0.74	-0.70	-0.66	-0.63	-0.59	-0.56
18	-0.52	-0.48	-0.45	-0.41	-0.38	-0.34	-0.30	-0.27	-0.23	-0.20
19	-0.16	-0.12	-0.09	-0.05	-0.02	-0.02	0.06	0.09	0.13	0.16
20	0.2	0.24	0.27	0.31	0.34	0.38	0.42	0.45	0.49	0.52
21	0.56	0.60	0.63	0.67	0.70	0.74	0.78	0.81	0.85	0.88
22	0.92	0.96	0.99	1.03	1.06	1.10	1.14	1.17	1.21	1.24
23	1.28	1.32	1.35	1.39	1.42	1.46	1.50	1.53	1.57	1.60
24	1.64	1.68	1.71	1.75	1.78	1.82	1.86	1.89	1.93	1.96
25	2.00	2.04	2.07	2.11	2.14	2.18	2.22	2.25	2.29	2.32
26	2.36	2.40	2.43	2.47	2.50	2.54	2.58	2.61	2.65	2.68
27	2.72	2.76	2.79	2.83	2.86	2.90	2.94	2.97	3.01	3.04
28	3.08	3.12	3.15	3.19	3.22	3.26	3.30	3.33	3.37	3.40
29	3.44	3.48	3.51	3.55	3.58	3.62	3.66	3.69	3.73	3.76
30	3.8	3.84	3.87	3.91	3.94	3.98	4.02	4.05	4.09	4.12
31	4.16	4.20	4.23	4.27	4.30	4.34	4.38	4.41	4.45	4.48
32	4.52	4.56	4.59	4.63	4.66	4.70	4.74	4.77	4.81	4.84
33	4.88	4.92	4.95	4.99	5.02	5.06	5.10	5.13	5.17	5.20

The hydrometer reading at 40 sec represents silt and clay in the suspension. Hence, to obtain the amount of sand, it was subtracted from the sample mass, the previous readings.

The following equations were used to calculate the percentage of sand, clay and silt in the soil sample:

$$\boxed{\%\,\mathrm{Sand} = 100 - \frac{\text{The hydrometer reading at } 40'' \times 100}{\text{sample mass}}}$$

$$\boxed{\%\,\mathrm{Clay} = \frac{\text{The hydrometer reading at } 2\,\mathrm{h} \times 100}{\text{sample mass}}}$$

$$\boxed{\%\,\mathrm{Silt} = 100 - (\%\,\mathrm{Sand} + \%\,\mathrm{Clay})}$$

Finally the Soil Texture Triangle was applied to determine the textural class of the soil sample. (Figure9)

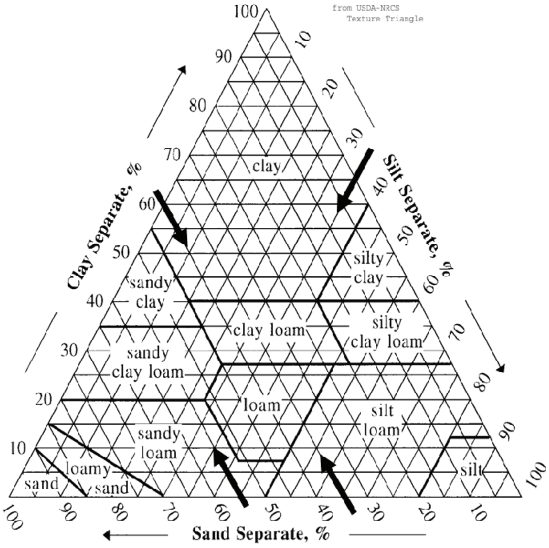


Figure 9. Soil Texture Triangle

As complementary analysis was performed Sieve Test. A soil sample of known weight is passed through a set of sieves of known mesh sizes. The sieves are arranged in downward decreasing mesh diameters. The sieves are vibrated for a fixed period of time. The weight of sediment retained on each sieve is measured and converted into a percentage of the total sediment sample. First of all a wash of the soil was washed with detergent, in order to withdraw residues of hydrocarbon of the soil to avoid the formation of masses that might alter the results. Then the sample spent to itself for a sifter 200 to eliminate thin or clayish material and avoiding the loss of thick material. Then the sample was surrendered to dried in oven for 24 hours to 105°C and later it passed for sifters number ¾ ", ½ ", 3/8 ", 4, 10, 20, 40, 60, 80, 100, and 200. Finally it was weighed the soil retained in every sifter and was prepared a texture curve.

Structure

Soil structure is the shape that the soil takes on based on its physical and chemical properties. Usually the structure of the soil is following: granular, crumb, blocky, platy, prismatic, columnar, single-grained, or massive.

To determine the structure of soil in the study zone, a sample of soil that has not been disturbed, was taken and was looked closely at its shape, then was compared with structural types normally established in the literature.

Infiltration (permeability)

Infiltration rate is a measure of how fast water passes in the soil. Usually this test is performed in situ.

These parameter was determined in accordance with the specifications standard, where there was realized the burial of a pipe of PVC of 6 " of diameter up to the half of his height (6 " of entire height), remaining like that 3 free inches of pipe on the level of the soil .Then was covered the soil inside the ring with plastic wrap and was added water (444ml), as is shown in figure 10. Finally the plastic wrap was removed by gently pulling it out, leaving the water in the ring and it was noted the amount of time (in minutes) it takes for the 1" of water to infiltrate the soil. When the surface was just glistening the time was stopped.

Figure 10. *Measurement of soil infiltration in situ*

The mm/hour value obtained was interpreted from the information in the table 2 below.

Table 2. *Relation between infiltration, and structure of soil*

Rate of water entry (mm/h)	**Soil structural assessment**
0–10	Very poor structure quality
10–30	Poor structure quality
30–70	Moderate structure quality
>70	Good quality of structure

Source: After Geeves et al. 1995

Apparent (bulk) density

The ability of the soil to hold and transmit water and air is impacted by the amount of pore space in the soil and pore size distribution.

Unlike the measurement of particle density, the bulk density measurement accounts for the spaces between the soil particles (pore space) as well as the soil solids.

The sample of soil was extracted with help of a cylinder of PVC of 3 " of diameter and 15 cm high, with a beveled end, being careful to preserve the sample without losses up to taking them to the laboratory, where it was weighed and later he surrendered to dried in the oven to 105°C for 24 hours. To avoid significant losses of material and moisture, during the transport to the laboratory, the pipes with sample of soil in his interior were sealed in his ends. (Figure 11)

Figure 11. *Sampling of soil for the apparent density analysis*

Then the dry soil was weighed again and was used to calculate apparent (bulk) density by following equation:

$$\boxed{D_a \frac{\text{Weigh of dry sample [g]}}{\text{Volume of solids \& pore spaces [ml]}}}$$

Where: D_a=apparent (Bulk) density [g/ml]

Apparent density is closely related to the soil porosity through the following relationship:

$$\boxed{\text{Porosity} = 1 - \frac{D_a}{D_r}}$$

Where: D_r= Real density

The results were compared with following table 3 which related la porosity with bulk density.

Table 3. *Relation between bulk density and percentage of soils porosity*

Bulk density (g/ml)	**Porosity %**	**Characteristic of soil**
< 1.0	> 63	Typical clay soil
1.0 – 1.2	55 – 62	
1.3 – 1.4	47 – 54	
1.5 – 1.6	40 – 46	Typical sandy soil
1.6 – 1.8	32 – 39	
> 1.8	< 31	

Real Density

Real density is defined as the ratio of the dry specimen mass to the volume of its solid part. About 20 g of the soil, previously drying at 105°C during 24 hours, was introduced into a graduated cylinder filled with 25 ml of deionized water. The real density was expressed as the ratio, of the mass of the ground dry specimen to the volume of liquid displaced by this mass, by the following equation:

$$\boxed{D = \frac{M}{V} [\frac{g}{mL}]}$$

Porosity

Soil porosity refers to that part of a soil volume that is not occupied by soil particles or organic matter. The value of porosity was calculated by following equation:

$$\boxed{\%P = \left[1 - \left(\frac{Da}{Dr}\right)\right] \times 100}$$

Where: P= total porosity; D_a= apparent density; D_r=real density.

Gravimetric and volumetric moisture

Gravimetric moisture was obtained from the field sample, previously drying at 105°C during 24 hours. This sample was weighed before and after drying and percentage of moisture was calculated used following equation:

$$\boxed{\%M_G = \frac{\text{Moisture sample weight - Dry sample weight}}{\text{Dry sample weight}} \times 1000}$$

Where: M_G=Gravimetric moisture

Volumetric moisture was calculated taking into account apparent density and gravimetric moisture and was applied following equation:

$$\boxed{\%M_V = \%M_G \times Da}$$

Where: M_v= volumetric moisture; M_G=gravimetric moisture; D_a=apparent density.

Cation exchange capacity and organic matter

The total number of cation which soil can hold--or its total negative charge--is the soil's cation exchange capacity. This, in turn, can have a significant effect on the fertility management of the soil. CEC is measured in millequivalents per 100 grams of soil (meq/100g).

The value of Cation Exchange Capacity (CEC) was obtained by titration of sample with 0,1M NaOH. The sample was previously washed with ammonium acetate, then the excess of ammonium salt, was washed with 95% ethanol and was filtered. Finally, the sample was washed with 10% NaCl and it was added formaldehyde to proceeded to titration by 0,1 M NaOH. Cation Exchange Capacity was calculated by following equation:

$$\boxed{CIC\left(\frac{meq}{100gr}\right) = 2(V - B)}$$

Where: V=Volume of NaOH used in titration of the sample(ml); B=Volume of NaOH used in blank titration (mL).

Soil organic carbon and organic matter

Soil Organic Carbon is one of the most important constituents of the soil. A direct effect of poor Soil Organic Carbon is reduced microbial biomass, activity, and nutrient mineralization due to a shortage of energy sources. Estimates of total organic carbon (OC expressed as C) are used to assess the amount of organic matter in soils.

The quantification of these parameters was realized by titration with ferrous sulphate 0.5N. Previously the sample of soil was dried off to 45°C for 24 hours, then was added dicromato of potassium 1N and sulphuric acid concentrated and due to the warming, the sample was rested during half an hour and 85 %phosphoric acid was added. Finally was added diphenylamine as indicator to detect the change of blue color to brilliant green in the titration with ferrous sulphate 0.5N. The following equations were used:

$$N = \frac{\text{Volume } K_2Cr_2O_7 \times \text{Normality of } K_2Cr_2O_7}{\text{Volume of ferrous solphate solution}}$$

$$\%C = \frac{(B - S) \times N \times 0.003 \times 1.3 \times 100}{W_s}$$

$$\%OM = \%C \times 1.724$$

Where: %C=percentage of organic carbon

B = Volume of $FeSO_4$ used in blank titration (mL)

S = Volume of $FeSO_4$ used in sample titration (mL)

N = Normality of ferrous sulphate solution

1,3= efficiency of reaction , which is 77%(100/77)

W_S = Oven-dry sample weight (g)

%OM=percentage of organic matter

Total petroleum hydrocarbons (TPH)

The analysis of TPH's was done in laboratory, credited by environmental authorities, of Colombian Petroleum Institute (ICP), applying the method standardized for not polar hydrocarbons, oil and grease, and gas chromatography brand Agilent 6890 and additionally system for analysis de PAHS-s system coupled to a mass spectrophotometer HP6890 with detector 5975.

Analysis condition:

Column: HP-5MS, 30m x 0.25mm x 0.25µm ; helium carrier gas at a flow of 1.2 ml/min

Gas Makeup: Nitrogen at a flow of 18.8 ml/min; the temperature program used was: 70 °C (2 min),6 °C/min, 300°C (15 min); temperature of Injector 250 ºC, temperature of detector

320 ºC, volume of injection: 1 µL, Splitless, detector FID with air at a flow of 390 ml/min and hydrogen at a flow of 39 ml/min.

Percentage of degradation = [TPH control-TPH treatment/TPH control]x 100.

Temperature

The temperature of soil was measured directly by thermometer, approximately to 5cm of depth, in every point of sampling, to obtain an average value.

pH

Value of pH was measured by calibrated (buffer pH=4,0; pH=7, pH=10) pH meter, Brand Hach Hq40d, where was used dilution of sample in proportion 1:1 soil and deionized water. This dilution was waved during 5 minutes and after one hour, was measured pH value. Additionally the same procedure was realized but with KCl 1N instead of water and finally the following formula was applied:

$$\boxed{\Delta \text{pH} = \text{pH in KCl} - \text{pH in water}}$$

Analysis of Microbiological characteristics in soils of interest

Autochthonous microorganisms in Palagua field (adjoining ground), with the ability to biodegrade TPHs were successfully isolated at the beginning. For their identification was used, prepared with water the modified cultures, a portion of the soil sample and the corresponding broth: nutritive, brain heart for bacteria identification and Bengal rose and yeast extract for fungi. Inoculation was done in water-soil solutions that were incubated for 24 hours at 37 ºC. The microorganisms that grew on the Petri dishes were classified according to their observed macroscopic and microscopic morphological characteristics by optical microscope. During biodegradation process, the microbiological cell numbers were estimated using the method PTE 120023, enunciated by Journal Microbiological Methods and acquaintance as Inventory of entire microorganisms in drop (Forming Units of Colony / milliliter) in three different mediums: Nourishing Agar, Agar Cetrimide and Agar MBS-HC. The last one medium was specially developed for petroleum-degrading microorganism in Colombian Petroleum Institute (ICP).

Assessment of Topographic characteristics of contaminated side

The topographic plane which delimits the area and coordinated with the place of interest it was prepared and provided by the division of Underground Waters of the Colombian Institute of the Oil (ICP) using the software AutoCAD.

4.2.2 Physicist-chemical characteristics of groundwater contaminated with hydrocarbons

The physic-chemical characterization of groundwater was carried out in the wells of monitoring that have presented touches of hydrocarbons during the pursuit realized in the last three years. The following wells were selection: in the Station1 (PM-10) and in the bordering property (PM-17, 18 and 19).

Dissolved oxygen concentration

This parameter was measured in situ, by Winkler test, using field Kit, brand Merck.

Water Conductivity

Water conductivity is defined as '*the ability to conduct or transmit heat, electricity, or sound*'. It is a measure of the ionic activity of a solution in term of its capacity to transmit current that's why in diluted solution; values of total dissolved solids are comparable with water conductivity. It unit are Siemens per meter [S/m] in SI and millimhos per centimeter [mmho/cm] in U.S. customary units.

The measure of water conductivity was took using the conductimeter Horiba DS-12 calibrated with solution of KCl for high status (1412 micromhos/cm) and low status (146.9 micromhos/cm).

Water alkalinity

Alkalinity is the measure of a solution's capacity to react with a strong acid. The alkalinity of a solution is usually made up of carbonate, bicarbonate, and hydroxides.

Alkalinity was determined by titration with sulfuric acid until 4.5 pH, using pH meter test and the following formula was applied:

$$\boxed{\text{mg CaCO}_3\ /\text{l} = \frac{V_a\, N_a \times 50\,000}{V_s}}$$

Where: V_a is a acid volume; N_a is a normality of acid solution; V_s is a volume of sample

Phenol compounds

The phenol compounds were analyzed by gas chromatography (GC) with a detector of ionization of flame (FID).

Oil and grease

The value of oil and grease in water was measure by gravimetric method, using n-hexane as solvent to extraction process. (EPA - Method 1664A)

Non-polar hydrocarbons

Non-polar hydrocarbons were analyses using a silica gel column, where polar compounds are retained and the solvent (n-hexane) with the non-polar hydrocarbons are gathered to quantify by gravimetric method.

Total dissolved solids

Dissolved solids refer to any minerals, salts, metals, cation or anions dissolved in water. The method applied was gravimetric, where the fraction of solid presents in a sample and that pass across a filter of fiberglass of 1.5 microns, was drying to 180ºC up to constant weight.

Chlorides, nitrites, nitrates

The concentrations of chlorides were tested by ionic chromatography (Environmental testing-EPA 300.0; whereas the concentration of nitrates and nitrites was calculated by the detector UV to 210 nm. (Figure 12)

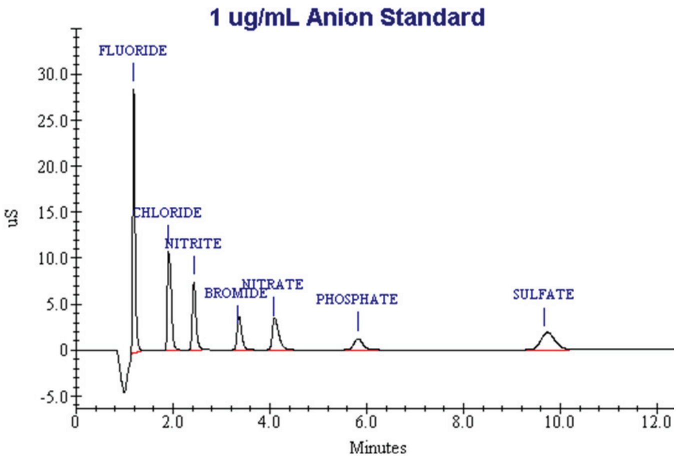


Figure 12. *Anion Standard used to chloride test*

Sulphates

Measurement of sulphates was by turbidimetric method.

Polyaromatic hydrocarbons (PAH's)

For measure of polyaromatic hydrocarbons, Gas Chromatographic method was applied with FID detector. (US EPA 610 method)

Metals

The Magnesium, Sodium, Potassium, Iron, Aluminum, Manganese, Boron, Chromium, Copper, Cadmium, Zinc, Lead, Barium, Nickel, Vanadium, Molybdenum, Cobalt, Silver, Beryllium, Selenium, Arsenic and Lithium, were measured by Inductively coupled plasma atomic *absorption* spectroscopy. (US EPA 1620 method). The calcium was measure by the same method applied Inductively coupled plasma atomic *emission* spectroscopy and value of concentration of mercury was obtained by atomic absorption spectroscopy with hydride vapor generator unit.

4.2.3 Hydraulic behavior of groundwater

To describe hydraulic behavior of groundwater was necessary measure the following features:

Static levels of groundwater

The methodology applied to measure the static levels was given by the internal protocol of the company, in which has been used the wells sounding device Geotech, continued by the extraction of oil and underground water by means of a bailer that is a pipe of PVC with a valve of water entry (Figure13). In the first image of figure 18, it was emphasized the sounding device, used to measure the static level of water into control wells. All the taken samples were analyzed in laboratory of the Colombian Institute of the Oil (ICP).

Figure 13. *Measurement of the static levels in field*

Hydraulic conductivity

In order to calculate the magnitude of the hydraulic conductivity of the aquifer was carried out the execution of tests of Slug or of rapid change of pressure in each of the monitoring wells. In the procedure, was removed a measured quantity of water from a well , following by making a rapid series of water-level measurements, to assess the rate of water-level recovery (either rising-head).

Using the Hvorslev method (Figure 14), which constitutes a rapid means of characterizing the hydraulic conductivity of shallow ground-water systems, was applied following equation:

$$\boxed{K = \frac{r^2 \ln\left(\frac{L}{R}\right)}{2 L_e T_o}}$$

Where:
K = hydraulic conductivity (L/T) (ft/d)
r = radius of well casing (ft)
R = radius of well screen (ft)
Le = length of well screen (ft)
To = time it takes for water level to rise or fall 37% of initial change (d)

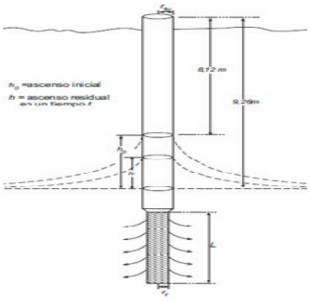


Figure 14. Typical well configuration for a slug test in an unconfined aquifer
Source: Sánchez, San Román F. Javier. Medidas puntuales de permeabilidad "Slug test".

The following figure 15 shows oil recover and measurement of water conductivity into control Wells.

***Figure 15.** Oil recovers and measurement of water conductivity into control wells*

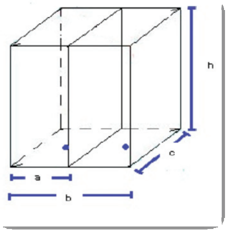
4.3 EXPERIMENTAL PROCEDURES

The sample of soil, object of this investigation, was extracted from a petroleum field located in jurisdiction of the municipality of Port Boyacá, in the department of Boyacá, Colombia. Initially, were selected for the sample the points, taking as the criterion the place of the spill and his possible displacement indicated by the aspect of the soil. Each one of the sample was extracted in approximate distance of 5 m from the monitoring wells and to 30 cm of depth. These separated samples were analyzed to have physicist-chemistry characteristic, in real place. To realize the experimental assembly was carried out the homogenization of the sample contaminated with hydrocarbon. For this reason was removed vegetable material and stone. After this the sample was mixed and sifted with size of mesh of 2mm. (Figure 16)

Figure 16. *The sample homogenization*

4.3.1 Bioreactors description

All of the experiments were realized in the bioreactors without control of the hydraulic behavior in the inside of the reactor, because there was no continued flow in his interior but they have had drain hollow. Figure 17 shows design of the bioreactors.



Dimensions:
a = 10 cm
b = 20 cm
c = 10 cm
h = 35 cm
● 1 cm (diameter of drain hollow)

Figure 17. *Design of bioreactor used for laboratory experiments*

The Figure 18 show the pipes of PVC adapted as control wells, and the covering in the walls of the reactors, to avoid the step of light, and to simulate analogous conditions which the native microorganisms can find in field. Each bioreactor had two, independents, compartments to be able to realize the same essay twice.

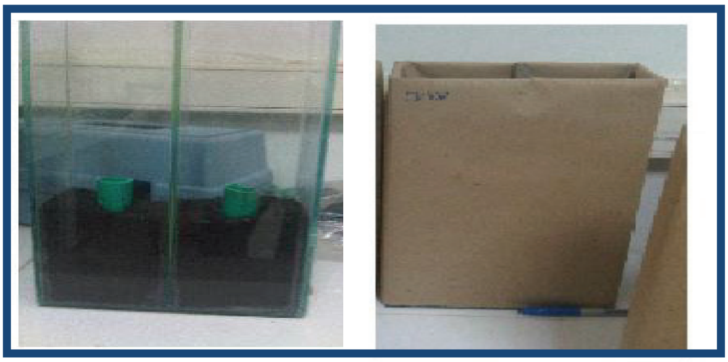


Figure 18. *Preparation of bioreactor for experiments*

The top part of the bioreactors it was opened to facilitate emission of carbon dioxide, produced in the biodegradation process, to have condition similar as natural in the field.

4.3.2 Systems description

Aeration

To test the effect of aeration in biostimulation process, the soil in respective bioreactor was oxygenated with air, by air pump, where diffuser of the aeration pump was put directly into the pipes of PVC adapted as control wells. Air supply of the pump was 1,2l of air per minute with pressure 0,010 Mpa.

Nutrients

The soil was biostimulated by adding 250 mgkg^{-1} of $(NH_4)_2SO_4$ and 100 mgkg-1 of K_2HPO_4.

3 Soil moistening

During 16 weeks, the samples remained in treatment. Throughout this time were assured conditions of moisture, by means of the addition of 40 milliliters of distilled water every 48 hours. In a such way the native microorganisms could to have correct growth, but it was prevented a formation of leached, which could to diminish the values of not polar hydrocarbons as consequence of a dragging.

Homogenized sample

The anionic surfactant used in this treatment was developed in ICP and called Emulgrosson®.

Two weeks preliminary assays, have allowed determining the best concentration of the surfactant solution. The analyzed concentration was 0.0% (control–wash with water), 2%, 5%; 8% of surfactant. Microbiological growth and TPH analysis were controlled during this time. After that, throughout two weeks was applied flushing the soil with Emulgrosson. Every two days was added 40 ml of surfactant solution into soil, used for this action, control wells simulation in bioreactor. During this time, the soil did not receive watering and other treatments as well. The dragging solution was flowing by the hollow drainage. The washed soil was sampled at 0 and 2 weeks for TPH and microbiological analyses.

Once, the pretreatment by flushing with surfactant was finished, was added into the soil an aqueous solution which contained nutrients (nitrates and phosphates). After that, the soil was divided in equal portions (1,5kg) and was continued by the three independent processes of biodegradation: natural extenuation, biostimulation and bioaugmentation.

Pretreatment using hydrogen peroxide solution or surfactant solution in contaminated soil

Pretreatment using hydrogen peroxide lasted two weeks. To achieve the stability of the peroxide of hydrogen was added sales of phosphate, as the recommendations of literature. Previous assays have allowed determining the best concentration of the hydrogen peroxide solution. The analyzed concentration was: 0,0F (control-wash with water), 0,01F; 0,05F; 0,1F. Microbiological growth and TPH analysis were controlled during this time. After that, throughout two weeks was applied flushing the soil with hydrogen peroxide solution. Every two days was added 40 ml of hydrogen peroxide solution into soil, used for this action, control wells simulation in bioreactor. During this time, the soil did not receive watering and other treatments as well. The dragging solution was flowing by the hollow drainage. The washed soil was sampled at 0 and 2 weeks for TPH, oil and grease, and microbiological analyses. Once, the pretreatment by flushing with hydrogen peroxide solution was finished, was added into the soil an aqueous solution which contained nutrients (nitrates and phosphates). After that, the soil was divided in equal portions (1,5kg) and was continued by the three independent processes of biodegradation: natural attenuation, biostimulation and bioaugmentation.

The same conditions were applied for pretreatment with anionic surfactant solution, (Emulgrosson), used in the company as dispersant agent and emulsifying agent. In previous assays were analyzed the following concentration 2% 5% and 8%. Control was only water applied for wash. The company assures that based upon chemical analysis Emulsifier Emulgrosson® contains no known EPA priority pollutants, heavy metals, or chemicals listed under RCRA, CERCLA, or CWA. Analysis by TCLP (Toxicity Characteristic Leaching Procedure) according to RCRA revealed no toxic organic or inorganic constituents.

The pretreatment process was realized in the same way as the flushing with hydrogen peroxide and after that, the biodegradation processes were continued in the independent way.

Bioremediation treatments

All bioremediation treatments were carried out in equal bioreactors, with 1,5 kg of the sample of soil, duplicate each one, placed in separated compartments and in equal conditions of temperature, moisture and pH, using sterile soils as control. The treatment were :(a) natural attenuation (NA);(b) biostimulation (BS);(c) Bioaugmentation(BA).

The soil used as the control, was sterilized by the addition of hydrochloric acid solution 2M (2 liters for sample), repeated after 48 hours. During the bioremediación tests, just distilled water was added to the sample of control. The temperature in laboratory was 28°C in average. (Figure 19)

<u>Natural attenuation process-</u>This process which is soil's natural ability to degrade the contaminant was analyzed in three independent conditions: (a) without pretreatment; (b)

with pretreatment, using flushing of contaminated soil with surfactant; (c) with pretreatment using hydrogen peroxide solution in contaminated soil.

Biostimulation process- In this process was added nutrients and air to improve the natural biodegradation rate. The same conditions were analyzed, in three independent situations: (a) without pretreatment; (b) with pretreatment, using flushing of contaminated soil with surfactant; (c) with pretreatment using hydrogen peroxide solution in contaminated soil.

Figure 19. *Reactors applied for biostimulation process*

Bioaugmentation process- The same conditions provided in the biostimulation treatment were used in the bioaugmentation treatment plus the addition of 40 ml of 2,6,x10^{8} cells ml^{-1} of microbial consortium called ECOMAXBACT, whose procedure of application is guarded due to policy of privacy of the ICP. Again, were analyzed three independent situations: (a) without pretreatment; (b) with pretreatment, using flushing of contaminated soil with surfactant; (c) with pretreatment using hydrogen peroxide solution in contaminated soil. Figure 20 shows all treatments performed in laboratory.

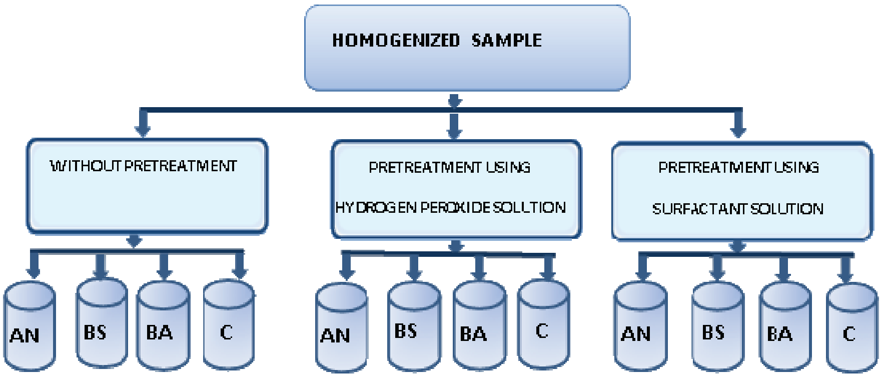


Figure 20. *Summary of the treatments realized in laboratory*
Where: AN- natural attenuation; BS-biostimulation; BA- bioaugmentation: C- control

4.4 VISUAL SOFTWARE MODFLOW APPLICATION FOR INVESTIGATE THE BEHAVIOR OF GROUNDWATER FLOW

The modeling of flow of underground water and the extension of the pollutant pen, was taken in in the zone of bordering property to the Station1, of Field Palagua. In the Station 1, monitoring wells did not register presence of the pollutant.

To prepare the model of flow of underground water of Field Palagua, the use of three software became necessary: Autocad 2D, Surfer and Visual ModFlow (VMODFLOW).

4.4.1 Delimitation of area and coordinated topographic of the location of interest

The topographic plane, which delimits the area and coordinated with the place of interest, it was provided by the division of Underground Waters of the Colombian Petroleum Institute (ICP) using the software AutoCAD.

4.4.2 Profile of level, modeling by Surfer

To get profile of level, was introduced the information of the topographic leveling out, realized in field and a value of Z, corresponding to the level of the area. After that, was generated the Model grid in areas of interest, next step was refining the Model Grid to get more detailed simulation results in areas of interest.

4.4.3 Simulation in Visual Modflow for flow of underground water and extension of the pen pollutant

In order to obtain information about the extension and direction of the pen pollutant was necessary the use of a mathematical model ModFlow, which used additional information like: Number of wells of monitoring and recovery (trench of interception) and the information obtained in each of these and static current levels and levels of wells.

4.4.4 Simulation stage

The model can develop in two states: stationary and transitory, the first one make reference to the natural state of flow without presenting external perturbations to the aquifer, whereas the second one alludes to stages of simulation where the system presents changes in the time (pumping). In this work the stationary state was simulated, because interventions are not had in the system (aquifer).

4.4.5 Layers of the model and type of aquifer

For the modeling of the flow of underground water and extension of the pen pollutant, in the adjoining ground of Field Palagua, it was considered layer of type 2, which admits the possibility of having a free or confined aquifer.

4.4.6 Discretization of the model

A general mesh of 20 lines was used by 20 columns, refining for four (4), the zone where the wells of monitoring PM1, PM14 and Pm17 located to PM19, where there was had a final mesh of 5X5. The underground topography was assumed parallel to the superficial one with a difference of 10 meters of thickness.

4.4.7 The topography

The topography realized in field was defined by leveling out, which got in the model to interpolate the obtained values of coordinated and levels for Krigging method and this way to have a better distribution in the whole area of interest.

4.4.8 Hydraulic parameters

The hydraulic parameters were taken in by slug test and equal values are assigned for the X and Y directions, whereas for the direction Z, is took the tenth part of the previous value. The model recognized interpolation for Krigging, to distribute the value of conductivity in the whole zone of interest.

4.4.9 Initial conditions

The model runs for stationary state, using a value of constant head of 154m, which was average of values of piezometer levels.

4.4.10 Limits of flow

As limits of flow of the underground water, values of constant heads were assigned, in accordance with the information from the year 2008 to 2010 taken in field. For this it was considered: the levels of area and the static levels. To have the value of piezometer level in every well, was extracted from area level the static level. Later these values assigned to themselves, with denomination of constant head to the south of the bordering property, between 152m to 154m in direction of the Marsh Palagua, and other one defined towards the Station 1 (North-East of the bordering property) between 154.5m to 156m.

4.4.11 Application of PATHLINES and MODPAHT for evaluation of the trajectory of particles

The simulation of the extension of the pollutant pen in the area of inters, was carried out by software PATHLINES and MODPAHT (systems integrated to MODFLOW). These two systems allow adding particles to the model to project the way that they will have in a estimated time of one, two and three years, after which a treatment of recovery was projected.

4.4.12 Selection of the best treatment or recombination of them, which will be based on experiments results, regulatory acceptance, technology applicability, cost and treatment time

The decision about selection of the best method for remediation was taking in Petroleum Company and was applied in first semester of 2012 in zone of interest.

The result of experiments as elimination of oil and grease in 80,2% TPH in 62,15% and 76,2% of PAH, as well as easy application in field by direct supply into monitoring wells, and low cost of treatment, were issues which company took into account in time to decide.

5 RESULTS AND DISSCUSION

The samples of soil and groundwater was obtained from the Station 1 and adjoining property of Field Palagua, located in the municipality of Puerto Boyaca in the Department of Boyacá, which belongs to Ecopetrol S.A. This field is located at a height of 130 m above sea level, with precipitations average of 6.0mm per year, and average temperature between 32°C and 27°C. The figure 21 indicates the place of the municipality.

Figure 21. *Side of interest location*
Source: http://maps.google.es/

5.1 PHYSICIST-CHEMICAL, MICROBIOLOGICAL AND TOPOGRAPHIC CHARACTERISTICS OF SOIL CONTAMINATED WITH HYDROCARBONS

Initially were realized the physicist – chemical, microbiological and topographic tests of soil in Palagua Field (adjoining ground) and some of them in Station1.

5.1.1 Texture

Two Methods were applied: Bouyoucos and sieve test for determination of soil texture in Station 1 and Palagua Field, site of interest. Table 4 and 5 shows results of Bouyoucos method and table 6 and 7 results of sieve test. The corresponding calculations are indicated next:

- ***Bouyoucos method***

First the value of the temperature and hydrometer measure was fitted, according to table 1 indicated in the methodology. After that, the manual calculations for sand, clay and silt percentage had applied values from table 4 in following equations:

40 second Reading = 0,50; measure of the temperature= 26°C

Correction of temperature from the table 4 = 26+2,54=**28,54°C**
Temperature correction: (°C) =(°t x 0,36)-7 ; °C= (28,54 x 0,36)-7= **3,27**
Measure of corrected hydrometer = (°C) + sample hydrometer reader
Measurement of corrected hydrometer= 3,27+0,50 =**3,77**

Weigh of dry screened soil = **50 g.**

$$\boxed{\%\,\text{Sand} = 100 - \frac{\text{The hydrometer reading at } 40'' \times 100}{\text{sample mass}}}$$

$$\%\,\text{Sand} = 100 - \frac{3{,}77 \text{ x } 100}{50} = 92{,}5\%$$

<u>2 Hour Reading = 0,25; measure of the temperature= 25°C</u>

Correction of temperature from the table 4 = 25+2,07=**27,07°C**
Temperature correction: (°C) =(°t x 0,36)-7 ; °C= (27,07 x 0,36)-7= **2,74**
Measure of corrected hydrometer = (°C) + sample hydrometer reader
Measurement of corrected hydrometer= 2.74+0,0,25 =**3,0**
Weigh of dry screened soil = **50 g.**

$$\boxed{\%\,\text{Clay} = \frac{\text{The hydrometer reading at } 2\,\text{h} \times 100}{\text{sample mass}}}$$

$$\%\,\text{Clay} = \frac{3{,}0 \text{ x } 100}{50} = 6\%$$

<u>Determination of Silt</u>

$$\boxed{\%\,\text{Silt} = 100 - (\%\,\text{Sand} + \%\,\text{Clay})}$$

% Silt = 100-(92,5+6) =1,5%

In the same way was calculated, percentage of sand, clay and silt, for other samples

Table 4. *Texture for the soil in Palagua field (adjoining ground)*

Sample	40 second Reading			2 Hour Reading			% sand	% clay	% silt
	reading	°C	Correction	Reading	°C	Correction			
1	0.50	26.00	3.77	0.25	25.00	3.00	92,5	6	1,5
2	0.60	26.00	3.89	0.40	26.00	3.66	92.2	7.3	0.5
3	0.70	28.00	4.98	0.30	26.00	3.55	90.0	7.1	2.9

Table 5. *Texture for the soil in Station 1*

Sample	40 second Reading			2 Hour Reading			% sand	% clay	% silt
	reading	°C	Correction	Reading	°C	Correction			
1	0.60	27.00	4.38	0.30	26.00	3.55	91.2	7.1	1.7
2	0.60	27.00	4.38	0.40	27.00	4.15	91.2	8.3	0.5
3	0.70	26.00	4.00	0.30	26.00	3.68	92.0	7.4	0.6

The obtained results were applied in the Soil Texture Triangle to determine tipe of soil. Both soils were qualified as sand, which coincides with the high permeability and rapid infiltration, together with low relative density and low retention of moisture which are related next.

- ***Sieve test***

First of all the soil was washed to eliminate interferences caused by hydrocarbon incidence. The weight of soil before washing was 1113.6g and after washing and sieved by 200 mesh size, was 922,.0 g. Loss on Sieving and Washing was equal 191.6 g.

Then the soil was sifted with different mesh size. The results of this test are shown in Table 6 for the soil in Palagua field, and Table 7 for the soil in Station 1.Corresponding figures are 22 and 23.

Table 6. *Weight and percentage of soil retained in specific sifter in Palagua field (adjoining ground)*

Sifter	Openings (mm)	Weight retained (g)	Weight retained (%)	Total weight passing (%)
¾	19.05	0	0	100
½	12.7	82	7.36	92.636
3/8	9.52	69.2	6.21	86.422
4	4.75	128.4	11.53	74.892
10	2	78.6	7.06	67.834
20	0.85	57.4	5.15	62.680
40	0.42	123.6	11.10	51.580
60	0.25	189	16.97	34.608
80	0.18	74.6	6.70	27.909
100	1.15	33.8	3.04	24.874
200	0.074	74.8	6.72	18.157
	Loss weigh	10.6	0.95	17.205
	TOTAL	**922,0**		

The calculation which was applied for determination of the sand, clay and gravel percentage, for determination of type of texture by Sieve method, was the following:

Calculation formula:

Percentage Retained =(Mass Retained/W_{Total}) x 100%;

Where W_{Total} = Weight of dried soil sample.

% Gravel = 100%- % weight passing sifter 4
% Gravel=100%-74,892**%= 25,108%**

% Sand= 100 - Silt – weight passing sifter 200
% Sand= 100% - 25.108% - 18.157%= **56,735%**

% Clay = Weight passing sifter 200
% Clay = **18.157%**

In the same way was calculated, the percentage of material in other samples.

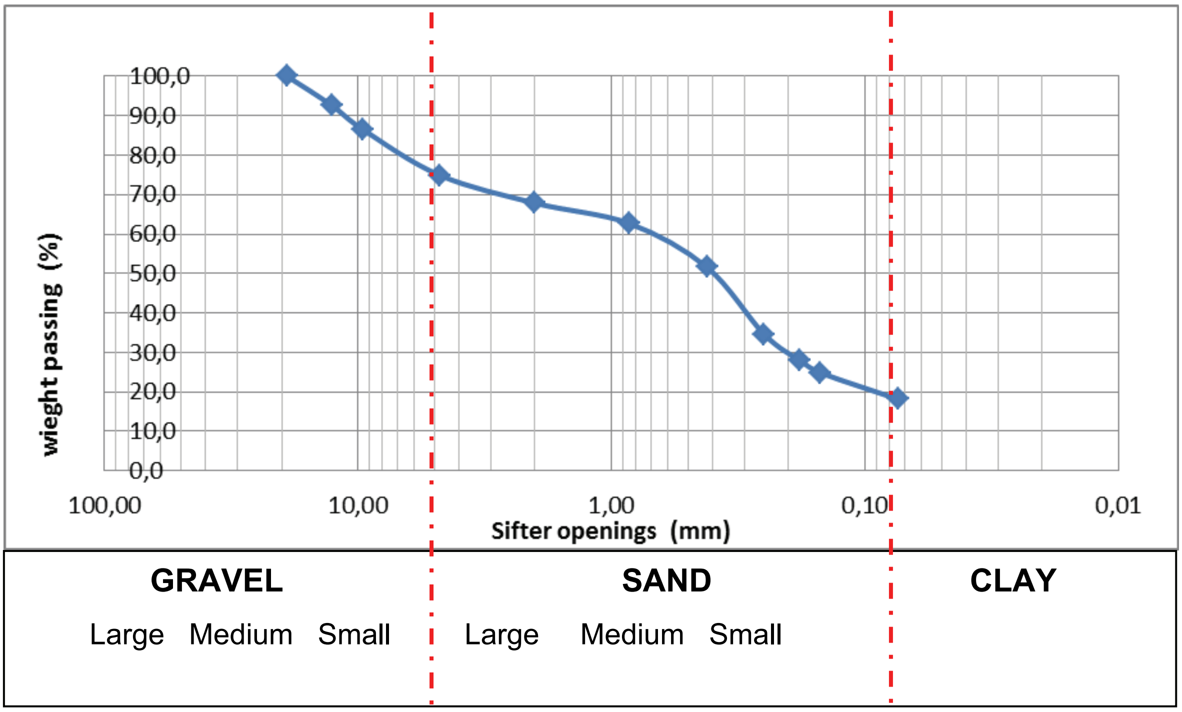


Figure 22.*Grading curve for soil in Palagua Field (adjoining ground)*

Taking into account that 56,735% of texture represented large and medium size, this agrees with Bouyoucos test, which classifies this sample as sand.

The same treatment was applied for the sample from the station1 and the following results

were obtained: The weight of soil before washing was 1110,4 g, and the weight after washing and sieved by 200 mesh size was 922,9 g. Loss of the weight after Sieving and Washing was equal 187,5 g.

% Gravel=100%-75,2%= **24,8%**

% Sand= 100% - 24.8% - 18.4%= **56,8%**

% Clay = Weight passing sifter 200 =**18,4%**

The gravel, sand and clay percentage shows very similar result for this sample and the Palagua field one, leaving the same classification of soil as sandy.

Table 7. *Weight and percentage of soil retained in specific sifter in Station1 sample*

Sifter	Openings (mm)	Weight retained (g)	Weight retained (%)	Total weight passing (%)
¾	19.05	0	0	100
½	12.7	80	7.2	92.8
3/8	9.52	70	6.3	86.5
4	4.75	125.3	11.3	75.2
10	2	77.4	7.0	68.2
20	0.85	58.1	5.2	63.0
40	0.42	125.2	11.3	51.7
60	0.25	192	17.3	34.4
80	0.18	73.5	6.6	27.8
100	1.15	36.3	3.3	24.6
200	0.074	70.2	6.3	18.4
	Loss weigh	14.9	1.3	17.1
	TOTAL	**922.9**		

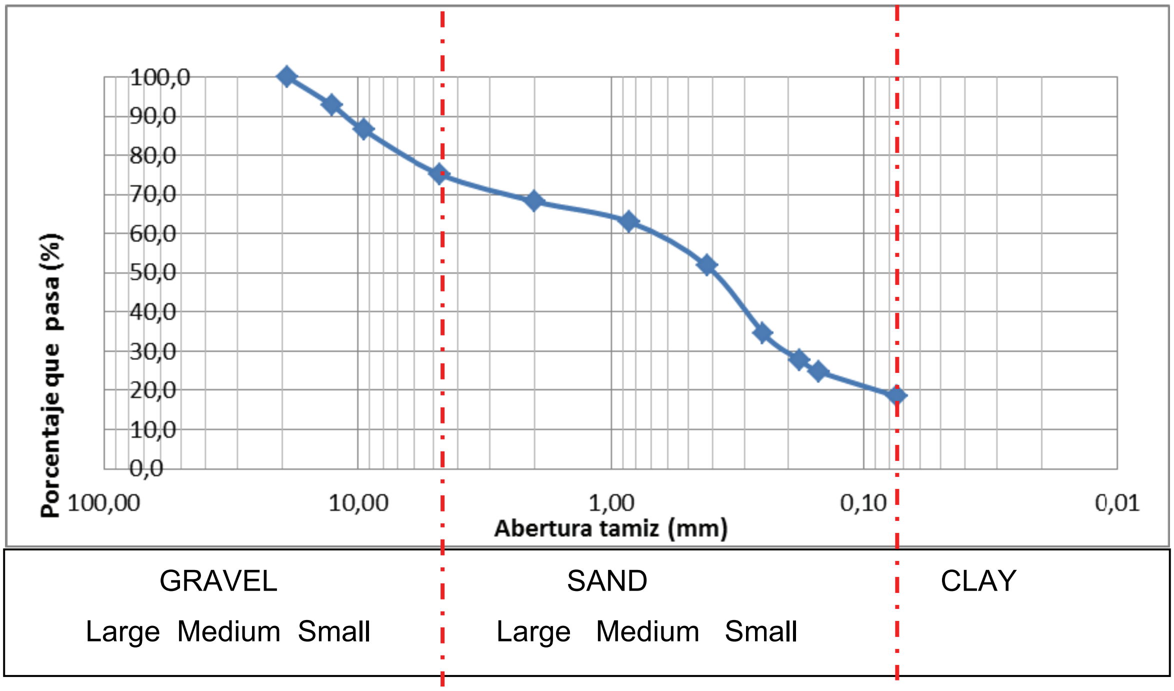


Figure 23. *Grading curve for soil in Station 1*

5.1.2 Structure

The figure 24 shows the structure of the soil in the adjoining ground (Palagua field) and of the Station 1 respectively. In spite of the rock is appreciated a granular structure in both of samples, characterized by the formation of imperfect spheres of similar size. This type of structure is characteristic for, relatively high infiltration, involving at the same time has a low retention of humidity.

Figure 24. *Structure of soil in the Adjoining ground and Station 1*

5.1.3 Permeability e infiltration

The measure of permeability and infiltration was taken in Station1 and Palagua field (Adjoining ground).The results show the similar soil conditions of permeability and infiltration, for two sides. Also, these values classify both soils as Sandy Loam (SL), of high permeability with an implication of low retention of moisture, and possibility to erosion problem. (Table 8) This indicated that during the hydrocarbon biodegradation process the irrigation must be frequent in order to maintain optimum humidity conditions.

Table 8. *Permeability and infiltration of soils in Station 1 and Palagua field*

SITE	WATER VOLUME	TIME (min)	Speed of Infiltration (cm/h)	Soil classification	Permeability classification
Station 1	444ml	7.625	19.98	SL	Fast
Palagua f.	444ml	7.4162	20.55	SL	fast

5.1.4 Apparent (bulk) density

The obtained results are shown in table 5, where the average of apparent density in soil of Station 1 was 1.49 g/cc and for the adjoining ground, was 1,41g/cc.

Beside, taking into account the indication in literature reports about fluctuation of values between 1,0 and 1,8 g/cc, is observed expected value of measure for these samples.

The calculations of the apparent density have applied data from table 9 and are illustrated next:

$$D_a = \frac{\text{Weigh of dry sample [g]}}{\text{Volume of solids \& pore spaces [ml]}}$$

$$D_a = \frac{463{,}8\text{g}}{306{,}05\text{cc}} = 1{,}52\,\frac{\text{g}}{\text{cc}}$$

In the same way was calculated, the others measures of the apparent density.

Table 9. *Apparent density measures for in Palagua field (adjoining ground) and Station1*

Measures	Palagua field Sample1	Palagua field Sample2	Station1 Sample1	Station1 Sample2
Cylinder weight (g)	117.6	120.3	118.2	117.5
Cylinder weight + wet sample (g)	580.9	547.4	592.2	576.9
Cylinder weight + dry sample (g)	570.8	538.0	582	567.3
Weight of dry simple (g)	453.2	417.7	463.8	449.8
Cylinder diameter (cm)	5.08	5.08	5.08	5.08
Cylinder Length (cm)	15.3	15.2	15.1	15.2
Cylinder Volume (cc)	310.10	308.08	306.05	308.08
Apparent density (g/cc)	1.46	1.36	1.52	1.46
Average of apparent density (g/cc)	**1.41**		**1.49**	

5.1.5 Real density

The real density is relating the weight of the dry soil sample (M), and the displaced volume of the liquid in the test cylinder (V). In table 10 and table 11 are shown results of real density measure in soil samples of Palagua field (adjoining ground) and Station1. The calculations of the apparent density have applied data from table 6 and are illustrated next:

$$D = \frac{M}{V} \quad \rightarrow \quad D = \frac{19.62\text{g}}{20\text{mL}} \quad \rightarrow \quad D = 0.98\frac{\text{g}}{\text{mL}}$$

Table 10. *Real density measure in soil sample in Palagua field (adjoining ground)*

Measures	*Palagua Field (adjoining ground)*		
	Sample 1	**Sample 2**	**Sample 3**
Cylinder weight (g)	71.2	71.3	85.4
Cylinder weight + dry sample (g)	88.76	89.26	104.15
Weight of dry simple (g)	17.56	17.96	18.75
Displaced volume of liquid (ml)	18	20	19
Real density in each sample (g/ml)	0.98	0.90	0.99
Average of real density (g/ml)		**0.95**	

Table 11. *Real density measure in soil simple in Station1*

Mesures	Station 1		
	Sample 1	**Sample 2**	**Sample 3**
Cylinder weight (g)	69.8	72.3	87.3
Cylinder weight + dry sample (g)	89.42	90.2	105.31
Weight of dry simple (g)	19.62	17.9	18.01
Displaced volume of liquid (ml)	20	18	19
Real density in each sample (g/ml)	0.98	0.99	0.95
Average of real density (g/ml)		**0.97g/ml**	

Analysis of the real density allows evaluate mineral content of the soils. According to the reports in literature, the real density can fluctuate between 1,1 g/ml and 2,65 g/ml, which indicates that the analyzed samples have very low real density. It can be because of high quantity of minerals, especially gypsum, present in this soils.

5.1.6 Porosity

Following table 12 shows the results of porosity in soil sample from the Palagua field (adjoining ground) and Station1.

The porosity was expressed as percentage, and for the calculation, was applied to following equation: $\%P = [1-(\frac{D_a}{D_r)]})]x100$ $\%P = [1-(\frac{1,52}{0,98})]x100 = 54,48\%$

Where: D_a- apparent density; D_r- Real density

The data for the calculation were taken from table 8. In the same way, was calculated the porosity for the others samples. Both of soils have similar porosity. Beside, more that 50% of porosity indicated that this soil has unrestricted void spaces, characteristic for the sandy texture, and coincides with high permeability of this sample.

Table 12. *Porosity results of the soil sample from Palagua field (interest side) and Station1*

The Measure	**Palagua field (adjoining ground)**		**Station 1**	
	Sample 1	Sample 2	Sample 1	Sample 2
Apparent density (g/ml)	1.46	1.36	1.52	1.46
Real density (g/ml)	0.98	0.90	0.98	0.99
Total porosity (%)	49.81%	50.98%	54.48%	46.82%
Average of porosity(%)	**50.4%**		**50.65%**	

5.1.7 Gravimetric and volumetric moisture

Gravimetric moisture for Palagua field (adjoining ground) was 4.74%, which is larger water content in soil than Station1 with 3.32%. However, both of samples have low capacity to storage of water into the inter-basal spacing between the pores, which it was expected in sandy soil. The percentage of gravimetric moisture was calculated by difference of wet weight of soil sample and dry weight of soil sample related with dry weight.

The following equation applied data from Table 13 to illustrate the calculations:

$$\boxed{\%M_G = \frac{\text{Moisture sample weight - Dry sample weight}}{\text{Dry sample weight}} \times 1000}$$

$$\%M_o = \frac{100,039 - 95,508}{95,508} \text{ x } 100 = 4,744\%$$

Table 13. *Gravimetric moisture of soil in Palagua field (adjoining groud) and Station 1*

Measure	**Palagua field (adjoining ground)**		**Station1**	
	Sample1	**Sample 2**	**Sample1**	**Sample 2**
Plate weight (g)	126.802	127.012	128.090	127.382
Plate weight + wet sample (g)	226.841	227.034	228.102	227.472
Weight of wet simple (g)	100.039	100.022	100.012	100.09
Plate weight + dry sample	222.31	222.508	224.980	224.162
Weight of dry simple (g)	95.508	95.496	96.89	96.78
% gravimetric moisture (%)	4.744%	4.74%	3.22%	3.42%
Average of measure (%)	**4,74 %**		**3,32 %**	

Average of volumetric moisture of soil for Palagua field (adjoining ground) was 6,7% and for station1 was 4,94%, which indicated low quantity of water that is stored to 15 cm of depth. Volumetric moisture was calculated taking into account apparent density and gravimetric moisture, and was applied following equation:

$$\boxed{\%Mv = \%MG \times Da}$$

Where: Mv= volumetric moisture; MG=gravimetric moisture; Da=apparent density.

To show the calculation were taken data from table 14. The same mathematical procedure was applied for other samples.

$$\%HV = \%HG \times Da \quad \rightarrow \quad \%HV = 4.744\% \times 1.46 \quad \rightarrow \quad \%HV = 6.93\%$$

Table 14. *Volumetric moisture of soil in Palagua field(adjoining ground) and Station1*

Measure	**Palagua field (adjoining ground)**		**Station1**	
	Sample 1	**Sample 2**	**Sample1**	**Sample 2**
Gravimetric moisture (%)	4.74%	4.74%	3.22%	3.42%
Apparent density (g/ml)	1.46	1.36	1.52	1.46
Volumetric moisture (%)	6.93%	6.44%	4.89%	4.99%
Average of volumetric moisture (%)	**6.7%**		**4.94%**	

5.1.8 Cation exchange capacity and organic matter

The quantity of cationic interchangeable for the two samples of soil are found located in a medium rank, indicating calcium reserves moderates, magnesium, potassium and sodium.

Taking into account data from table 15 was applied following equation to calculate cation exchange for sample of Palagua field (adjoining ground) and Station1.

$$\boxed{CIC\left(\frac{meq}{100gr}\right) = 2(V - B)}$$

Where: V=Volume of NaOH used in titration of the sample(mL);
B=Volume of NaOH used in blank titration (mL).

$$\mathrm{CIC}\,(\frac{\mathrm{meg}}{100\mathrm{g}}) = 2(6{,}6\mathrm{ml} - 0{,}3\mathrm{ml}) = 12{,}6$$

Table 15.*Cation Exchange Capacity (CIC) for soil in Palagua field and Station1*

Measure	**Palagua field (adjoining ground)**		**Station1**	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**
Volume of NaOH used in simple titration (ml)	6.6 ml	6.1 ml	8.2 ml	7.8 ml
Volume of NaOH used in blank titration (ml)	0.3 ml			
Normality of soda solution (N°eq-g/l)	0.1	0.1	0.1	0.1
CIC (meg/100g)	12.6	11.6	15.8	15

5.1.9 Organic Matter

Initially there was standardized of ammonium ferrous sulfate solution (FAS) by titration with potassium dichromate solution. The following equation allows calculate normality (N) of ammonium ferrous sulfate solution.

$$\mathrm{N} = \frac{\mathrm{V}_{\mathrm{K_2Cr_2O_7}}\ \mathrm{x}\ \mathrm{N}_{\mathrm{K_2Cr_2O_7}}}{\mathrm{V}_{\mathrm{FAS}}} = \frac{5\mathrm{ml}\ \mathrm{X}\ 1\mathrm{N}}{5\mathrm{ml}} = 1\mathrm{N}$$

After that, by titration of sample and the blank, with ammonium ferrous sulfate solution was the carbon percentage calculated, and the organic matter. The following calculate applied the values of table 16, to illustrate the others results, which was found in the same way.

$$\%\,\mathrm{C} = \frac{(\mathrm{S}-\mathrm{B})\ \mathrm{x}\ 1\mathrm{Nx}0{,}003\ \mathrm{x}\ 1{,}3\ \mathrm{x}\ 100}{\mathrm{SW}} = \frac{(5\mathrm{ml} - 1{,}2\mathrm{ml})\ \mathrm{x}\ 0{,}003\ \mathrm{x}\ 1{,}3\ \mathrm{x}\ 100}{0{,}3\mathrm{g}} = 4{,}94\%$$

$$\%\,\mathrm{OM} = \%\mathrm{C}\ \mathrm{x}\ 1{,}724 \qquad \%\,\mathrm{OM} = 4{,}94\%\ \mathrm{x}\ 1{,}724 = 8{,}52\%$$

Where: S- volume of ammonium ferrous sulfate solution spent in titration of sample.
B - Volume of ammonium ferrous sulfate solution spent in titration of blank (ml).
SW- weight of simple (g); OM - organic matter (%).

Table 16. *Measure of organic matter in Palagua field (adjoining ground) and Station1*

Sample	**Ferrous sulfate solution concentration**	**% C**	**% OM**
Palagua field, sample 1	1N	4.94	8.52
Palagua field, sample 2		3.77	6.50
Station 1, Sample 1		9.36	16.14
Station1, sample2		8.32	14.34

The content of organic matter is inferior of 20% which is according with high content of minerals.

5.1.10 Total Petroleum hydrocarbons (TPH)

The table 17 shows the significant difference between TPH content in soil from Palagua field and Station 1. Clearly the initial checkup indicate zone with contamination problem.

***Table 17.** Measure of TPH in Palagua field*

Sample	TPH (%w/w)	TPH (mg/kg of soil)
Station 1	0.02	200
Palagua field, sample 1	0.174	1740
Palagua field, sample 2	0.161	1610

5.1.11 Measure of pH of soil

Both samples of soils have neutral values of pH and negative ΔpH (table 18), which classifies to the soil as an cationic exchanger. Example of the calculation is illustrated next:

***Table 18.** Measure of pH in Palagua field and Station1 soil sample*

Sample	pH of sample	pH of KCL 1N	Δ pH	Average
Palagua field, sample 1	7.28	6.46	-0.82	-0.79
Palagua field, sample 2	7.31	6.55	-0.76	
STACION 1, sample 1	7.12	5.24	-1.88	-1.885
STACION 1, sample2	7.20	5.31	-1.89	

5.1.12 Temperature

Both soils in their interior, registered 28°C in average, what is agree with temperature in the zone, and is considered to be adequate for microbial growth.

5.1.13 Analysis of Microbiological characteristics in soil of interest

After 4 days of growth for bacteria and of one week for fungi, the plates of bacteria were applying Gram staining method, to differentiating bacterial species into two large groups (Gram-positive and Gram-negative). (Figure25) In the plates of fungi were applied the methylene blue, as the contrast for microscopic observation.

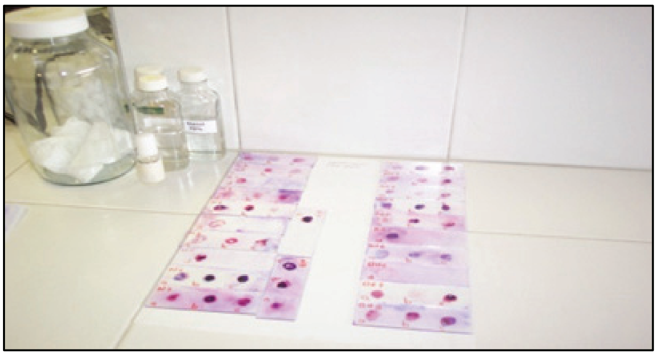


***Figure 25.** The slides with Gram staining*

The bacterial genus which appeared most frequently in this soil was the gram negative coco bacillus, followed by the gram positive and gram negative bacillus, respectively.

Around 90% of the observed bacillus samples had endospore, which provide higher resistance for desiccation events, intense solar radiation and contaminant presence. (Figure 26)

The fungal species found were: *aspergillus spp., fusarium spp., penicillium spp., stenphylium spp., cladosporium spp., helmitosphorium spp., hormodemdrum spp.;* nonetheless, some *aspergillus spp* and *cladosporium spp.* These were considered of great interest for the hydrocarbon biodegradation process. (Figure27)

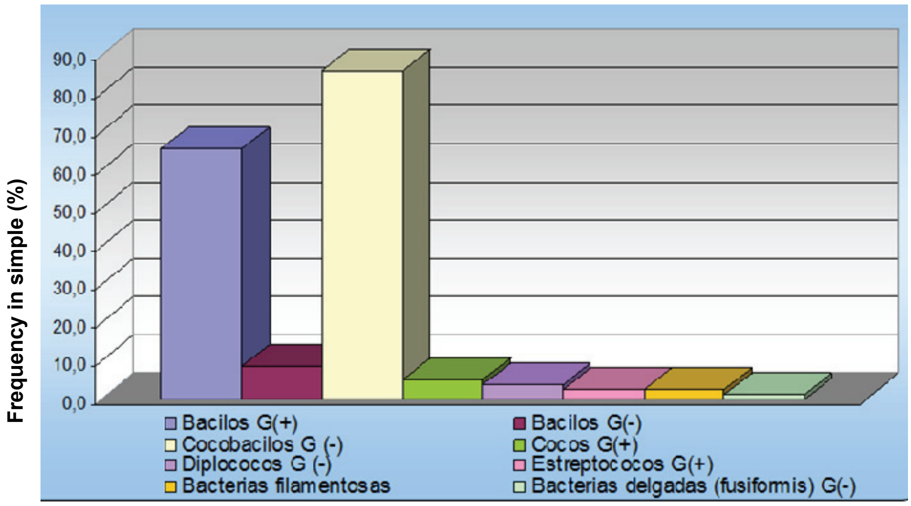


Figure 26. *Percentage of frequency in Palagua soil sample of bacterial genus*

The most frequently species of fungi was *Cladosporium, Aspergillus* and *Fusarium* in Palagua field soil sample. (Table 19) The literature reports show *Aspergillus* and *Penicillium,* as the most efficient, metabolizers of hydrocarbons species.

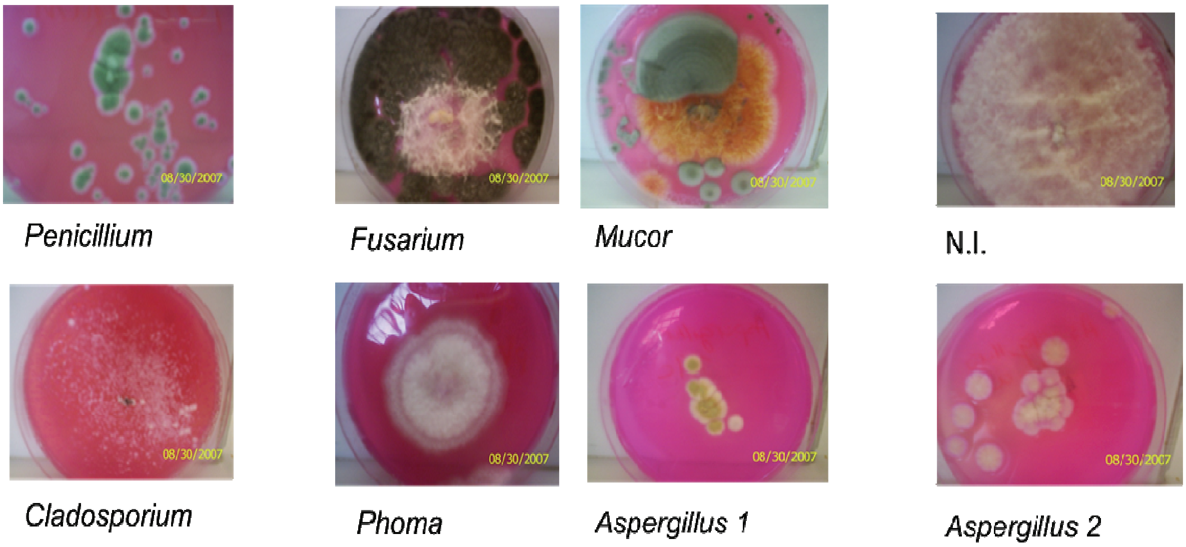


Figure 27. *Some of fungal species, found in the soil of interest*

Table 19. *Frequency of fungal species found in Palagua field soil (adjoining ground)*

Fungi	Frequency	%	% Accumulated
Phoma	1	1,52	100
Yeasts	1	1,52	98,48
Mucor	3	4,55	96,97
Penicillium	4	6,06	92,42
Aspergillus	5	7,58	86,36
Fusarium	8	12,12	78,79
Cladosporium	44	66,67	66,67
Total	66	100	0,00

The variety of genders of fungi and of bacteria found, demonstrates the biological wealth of the analyzed samples. Thus there are evidences of big microbiological potential for bioremediation process, where hydrocarbons can be using, as a carbon source in microorganisms biological functions.

5.1.14 Assessment of Topographic characteristics of contaminated side

The topographic plane shows different zones as Station1 and adjoining ground. In the area of Station 1, four storage tanks stand out. Next to tanks it is observed API separator, where is removed gross quantities of oil and suspended solids from refinery wastewater and tank treatment. In the tank treatment, crude that contains water is called "wet", and the water can then be bound in an emulsion in the crude to allow pumping on a pipeline. There are also pump station, office and laboratory. In the area are located monitoring wells and control wells (apiques) (Figure 28). Using GPS Data with Surfer and Grapher, it was possible to visualize that the area of Station1 is located in higher part than adjoining ground.

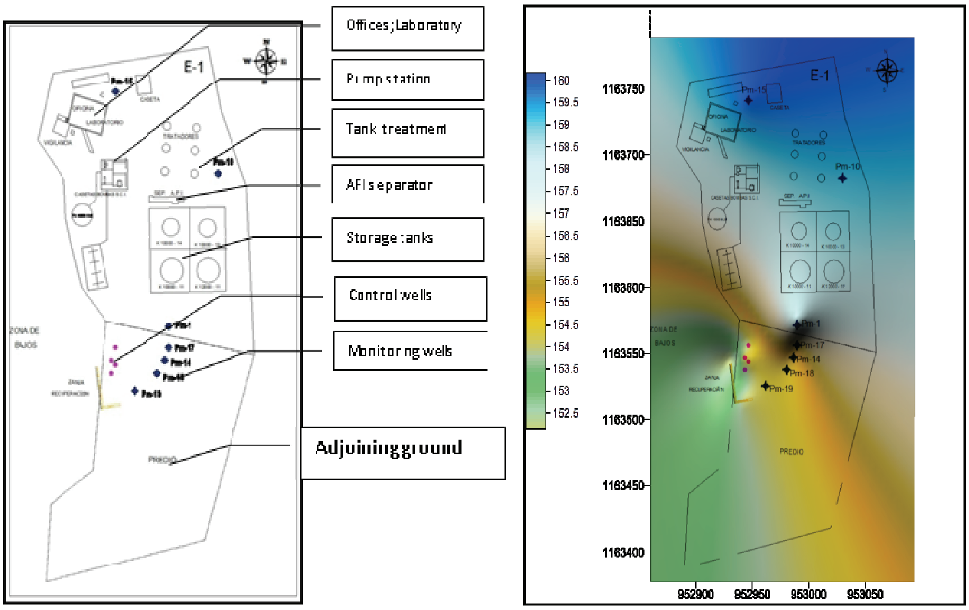


Figure 28. *Topographic plane of Station 1 and adjoining ground in Palagua field.*

5.2 PHYSICIST-CHEMICAL CHARACTERISTICS OF GROUNDWATER CONTAMINATED WITH HYDROCARBONS

In the table 20 are summarized results of physicist-chemical characteristics of groundwater taken in monitoring wells (PM10; PM17; PM18; PM 19)in the study area.

Table 20. *Physicist-chemical characteristics of groundwater*

MEASURE PARAMETER		Station 1	Adjoining ground		
	UNID	PM 10	PM 17	PM 18	PM 19
DEPHT OF SAMPLE	m	4,45	1,3	0,7	0,7
DISSOLVED OXIGEN	mg 0_2/L	0.5	0.0	0.0	0.0
pH/T (°C)	Uni. pH	6.62/33.0	6.68/34.8	6.11/34.3	6.13/36.2
CONDUCTIVity/T	Micromhos/c	487/22.1	564/21.9	13630/21.7	4280/21.9
TOTAL ALCALINITY	mg $CaCO_3$/L	236	218	177	206
BICARBONATES	mg HCO_3/L	288	266	216	252
CARBONATES	mg CO_3/L	0.0	0.0	0.0	0.0
SULPHATES	mg SO_4^{-2}/L	7.94	<1.00	<1.00	28.3
NITRATES	mg NO_3/L	0.71	<0.080	NM	248
NITRITES	mg NO_2/L	0.29	0.057	0.20	0.12
CHLORIDES	mg/L	5.16	69.9	4706	1237
DISSOLVED SOLIDS	mg/L	347	413	6871	2780
AMMONIATED NITROGEN	mg NH_3/L	<0.59	3.97	38.0	11.6
OIL AND GREASE	mg/L	1.84	207	64.8	828
TOTAL HIDROCARBONS	mg/L	<1.07	32.7	20.5	251
FENOLES	mg/L	<0.030	<0.030	<0.030	<0.030
IONIC BALANCE	NA	16.3	1.6	0.9	0.8
TDS/CONDUCTIVIDAD	TDS/CE	3.0	0.7	0.5	0.5
TDS MED/TDS CAL	NA	0.2	1.1	0.9	1.4
BTEX ANALYSIS					
BENZENE	microg/L	<2.76	<2.76	<2.76	<2.76
TOLUENE	microg/L	<2.23	<2.23	<2.23	<2.23
ETHYLBENZENE	microg/L	<1.82	<1.82	0.23	<1.82
M+P-XYLENE	microg/L	<1.85	<1.85	4.2649	<1.85
O-XYLENE	microg/L	<2.30	<2.30	1.2893	<2.30
HIDROCARBONS HCs					
OCTANE	mg/L	<9.00E-05	<9.00E-05	<9.00E-05	<9.00E-05
NONANE	mg/L	<8.00E-05	<8.00E-05	<8.00E-05	<8.00E-05
DECANO	mg/L	<8.00E-05	6.00E-05	4.00E-04	5.00E-04
UNDECANE	mg/L	<9.00E-05	8.00E-04	2.00E-04	7.00E-04
DUDECANE	mg/L	<9.00E-05	3.60E-03	8.00E-04	4.20E-03
TRIDECANE	mg/L	<9.00E-05	1.27E-03	2.70E-03	1.02E-02
TETRADECANE	mg/L	<9.00E-05	6.80E-03	3.50E-03	4.80E-03
PENTADECANE	mg/L	<9.00E-05	6.60E-03	2.20E-03	3.10E-03
HEXADECANE	mg/L	<9.00E-05	2.52E-02	1.13E-02	1.64E-02
HEPTADECANE	mg/L	<9.00E-05	2.04E-02	6.80E-03	1.23E-02
PRISTANE (2,6,10,14-Tetramethylpentadecane)	mg/L	<9.00E-05	5.03E-02	1.88E-02	2.86E-02
OCTADECANE	mg/L	<9.00E-05	1.48E-02	8.10E-03	8.50E-03
FITANE (2,6,10,14-Tetramethylhexadecane)	mg/L	<9.00E-05	3.23E-02	1.33E-02	2.03E-02
NONADECANE	mg/L	<9.00E-05	3.69E-02	3.10E-03	4.10E-03
EICOSANE	mg/L	<9.00E-05	2.25E-02	2.20E-03	7.20E-03
HEINECOSANE	mg/L	<8.00E-05	2.38E-02	9.50E-03	2.76E-02

Sequel table 20 Physicist-chemical characteristics of groundwater

MEASURE PARAMETER		Station 1	Adjoining	ground	
		PM 10	PM 17	PM 18	PM 19
TETRACOSANE	mg/L	<8.00E-05	1.23E-02	4.10E-03	8.50E-03
PENTACOSANe	mg/L	<8.00E-05	2.43E-02	9.00E-03	8.90E-03
HEXACOSANE	mg/L	<8.00E-05	1.45E-02	6.40E-03	9.70E-03
HEPTACOSANE	mg/L	<8.00E-05	2.06E-02	5.00E-03	1.66E-02
OCTACOSANE	mg/L	<8.00E-05	8.10E-03	5.40E-03	1.48E-02
NONACOSANE	mg/L	<8.00E-05	8.90E-03	6.00E-03	6.80E-03
TRIANCONTANE	mg/L	<9.00E-05	1.53E-02	3.80E-03	4.90E-03
HENTRIACONTANE	mg/L	<9.00E-05	1.21E-02	4.70E-03	2.02E-03
DOTRIACONTANO	mg/L	<1.00E-04	1.25E-02	3.60E-03	6.50E-03
TRITRIACONTANO	mg/L	<1.00E-04	2.58E-02	1.15E-02	1.17E-02
TETRATRIACONTANO	mg/L	<1.10E-04	4.40E-03	4.10E-03	8.20E-03
PENTATRIACONTANO	mg/L	<1.20E-04	3.70E-03	5.60E-03	7.20E-03
HEXATRIACONTANO	mg/L	<1.30E-04	3.40E-03	4.50E-03	5.70E-03
HEPTATRIACONTANO	mg/L	<1.40E-04	3.30E-03	1.10E-03	5.00E-03
OCTATRIACONTANO	mg/L	<1.70E-04	1.30E-03	2.50E-03	2.00E-03
TETRACONTANO	mg/L	<2.50E-04	6.00E-04	<2.50E-04	<2.50E-04
Polycyclic Aromatic hydroarbons (PAH’s)					
NAFTALENE	mg/L	<1.60E-4	<1.60E-4	<1.60E-4	<1.60E-4
ACENAFTILENE	mg/L	<1.90E-4	<1.90E-4	<1.90E-4	<1.90E-4
ACENAFTENE	mg/L	<1.90E-4	<1.90E-4	<1.90E-4	0.0299
FLUORENE	mg/L	<2.00E-4	4.43E-02	1.64E-02	7.35E-02
FENANTRENE	mg/L	<2.20E-4	5.69E-02	2.26E-02	8.86E-02
ANTRACENE	mg/L	<2.20E-4	<2.20E-4	<2.20E-4	<2.20E-4
FLUORANTENE	mg/L	<2.20E-4	<2.20E-4	<2.20E-4	<2.20E-4
PIRENE	mg/L	<2.20E-4	<2.20E-4	<2.20E-4	<2.20E-4
BENZO (A) ANTRACENE	mg/L	<2.50E-4	<2.50E-4	<2.50E-4	<2.50E-4
CRISENE	mg/L	<2.50E-4	0.0587	0.0072	0.0494
BENZO (B)FLUORANTENE	mg/L	<2.60E-4	<2.60E-4	<2.60E-4	<2.60E-4
BENZO (K) FLUORANTENE	mg/L	<2.80E-4	<2.80E-4	<2.80E-4	<2.80E-4
BENZO(A) PIRENE	mg/L	<2.80E-4	<2.80E-4	<2.80E-4	<2.80E-4
DIBENZE (A,H) ANTRACENE	mg/L	<4.10E-4	<4.10E-4	<4.10E-4	<4.10E-4
BENZO (G,H,I) PERILENE	mg/L	<4.00E-4	<4.00E-4	<4.00E-4	<4.00E-4
INDENE (1,2,3-CD) PIRENE	mg/L	<3.60E-4	<3.60E-4	<3.60E-4	<3.60E-4
METALS ANALYSIS					
CALCIUM	mg/L	661	36.15	528.2	159.9
BARIUM	microg/L	1743	1338	13110	2772
MAGNESIUM	mg/L	18.53	13.64	197	33.12
SODIUM	mg/L	1481	60	1451	396
POTASSIUM	mg/L	3.795	2.585	14.49	11.78
MANGANESE	mg/L	2.671	1.580	11.55	1.869
IRON	mg/L	249	42.81	26,9	32.03
ALUMINUM	mg/L	344	22.51	12.12	8.611
CADMIUM	microg/L	33.1	0.967	0.906	3.60
CHROMIUM	microg/L	154	26.5	16.8	11.1
COPPER	microg/L	535	53.3	24.6	15.0
LEAD	microg/L	77.8	32.1	16.4	9.21
NICKEL	microg/L	237	24.2	29.4	13.6
VANADIUM	microg/L	468	88.6	38.2	31.5
ZINC	microg/L	4818	118	85.4	36.8
ARSENIC	microg/L	35.5	16.4	18.4	5.36
SELENIUM	microg/L	<0.860	0.880	23.1	4.76
MOLIBDENUM	microg/L	0.779	1.53	1.18	2.01
MERCURY	microg/L	<0.123	<0.123	<0.123	0.255
NM – no mesurable					

The analyzed samples are situated in monitoring Wells PM-10 in Starion1 and the other three (PM17, PM18 and PM19) are located in adjoining ground (site of interest). All of them presented typical behavior for groundwater sample, which is absence of dissolved oxygen.

The temperature is about 35°C which is high, so pH of water is nearly neutral and varies within small ranges. Significant conductivity, especially in PM18 and PM19 is related with high present of dissolved solid as chlorides. The low concentration of ammoniated nitrogen and nitrates as well as nitrites in Station 1, and very high in adjoining ground, is related with cattle activity practiced in the contaminated zone of interest. (Figure 29).

Figure 29. *Cattle activity practiced in contaminated zone*

The alkalinity values were range between 177mg/l $CaCO_3$ and 236 mg/$CaCO_3$ which have relationship with concentration of bicarbonates. The Highest level of alkalinity in PM10 (288 mg/l) corresponding with the highest value of bicarbonates in this point (288 mg/L)

Ionic balance presented decreasing of value on having increased the distance from Station 1 to adjoining ground. This can be result of soil texture change, because of hydrocarbon contamination, which makes the soil more compact and less permeable. Whereas the soil in Station 1 presented low total hydrocarbon concentration (<1,07mg/l) the monitoring wells in adjoining ground had had until 251 mg/l (PM19). In the same way analysis of oil and grease presented the highest value (828 mg/l) in this point. This measure indicated existence of large quantity of lineal hydrocarbons. The simple observation allows seeing abundance of crude in this site. (Figure 30)

Figure 30. *Presence of hydrocarbons into monitoring Well (PM19)*

Although the concentration of phenol is low (<0,03 mg/l) it overcomes values, allowed by regulatory in Colombia which is 0,002 mg/l. Beside, having into account the cattle activity in the zone, worries his toxic character. The highest value of BTEX's analysis was reported in PM18 (4.2649 µg/l) for M+P-Xylene. The others BTX's presented lower values between 1.82 to 2.76 µg/l. Taking into account that BTX's are sorbed predominately by capillary forces in moist or wet soil, is expected the small quantity of this compounds in groundwater. More nevertheless this kind of water soluble components, can cause long-term pollutant plumes in groundwater, and extend their toxicity for long time. The lineal hydrocarbons as well as PAHSs, had low concentration in groundwater, for the same reason exposed previously.

Very worrying turned out the high concentration of metals as: Barium, Cadmium, hexavalent Chromium, Lead and Arsenic; all of them very toxics. The presence of Barium in underground waters it's related with the contamination by the hydrocarbons. This metal is added in the hydrocarbons extraction phase so appears in storage in the zone of monitoring. The values found in the bordering property are 1338microgram/L, 13110microg/L and 2772microgram/L for PM-17, PM-18 and PM-19 respectively.. Recognized poisonous effects that this metal presents as: block of the nervous system, muscular, cardiovascular alterations and renal damages is very important control of his concentration.

The Cadmium is related with the catalytic processes in the oil industry, his presence in water represents a risk to the health, since it can go so far as to cause hypertension, bony fragility and illness of "Itai-Itai". The concentration 33.1 microgram/l of Cadmium found in PM10 near of treatment tank, it overcomes more than three times the value, accepted in Colombia regulatory policy (10 microgram/l). In the same way the concentration of hexavalent Chromium and Lead, exceeds the value allowed by Colombian regulatory, in the PM 10 (154microgram/L and 77.8microgram/L respectively at the Station. These two metals are used by the oil industry in processes of cracking and increase of octane number in the production of commercial gasoline respectively. It is necessary to remember carcinogenic character of chromium and bioaccumulative of lead.

Although Arsenic presents value (35.5 microgram/l) below the Colombia regulation (50microgram/L), nevertheless, it is important to take into account his carcinogenic and teratogenic character. In the oil industry these metals are used in catalyst process, that's why their concentration raised next to the tanks of treatment.

The presence of calcium in underground waters could be result of the dissolution of the lime rock and the infiltrations in the soil. The high concentration of calcium is reflected in the important value of ionic balance; especially in the PM-10, where value of calcium is 661 mg/l and the corresponding ionic balance is 16.3. In the same point, the concentration of sodium, potassium and magnesium reached their highest value. This sample is located in Station 1, where the soil has sandy texture, with good permeability and characteristic of be cationic exchanger. Hence some of the metals as calcium, magnesium could be primary source from weathering, and then to be stored as a cation on soil exchange to reach finally groundwater.

Concentration of Mercury analyzed in groundwater, in all monitoring Wells it is below of Colombian regulatory value 2 microgram/l. This metal is related with natural deposits of crude oil and is bioaccumulative.

5.3 BIODEGRADATION OF SOIL CONTAMINANTED WITH HYDROCARBONS WITHOUT PRE-TREATMENT

5.3.1 Assessment of natural attenuation of soil contaminated with hydrocarbons without the pretreatment

During 4 month was analyzed the process of biodegradation of soil contaminated with hydrocarbons from adjoining ground in Field Palagua by natural attenuation. The assessment of the laboratory experiments, was realized by measure of variation of concentration of no polar hydrocarbons (TPH), oil and grease and change in the population of native bacteria applied three type of medium (nutritive, Cetride and MBS-HC). The contaminated soil was sampled at 0,1,2,3, and 4 months for chemical and microbiological analyses. The soil, used like the control, was sterilized by the addition of a solution of hydrochloric acid 2M (2 liters for sample), repeated after 48 hours. In the Table 21 are listed the results of this experimentation.

Table 21. *Biodegradation of soil contaminated with hydrocarbons by natural attenuation*

Sample	TPH (%W)	Percentage of degradation * TPH	Oil and grease (%W)	Percentage of degradation Oil and grease	Microbial enumeration (UFC)/ml		
					nutritive Agar 8900*	Cetride agar 6400*	MBS-HC agar 2100*
0C	3,52	Control	8,14	control	0	0	0
1N	2.36	32,9	7.30	10,3	26.000	64.000	21.000
1DN	2.36	32,9	7.30	10,3	26.000	64.000	21.000
2N	2,07	41,2	7,28	10,6	300.000	120.000	280.000
2DN	2,04	42,0	7,23	11,2	320.000	130.000	270.000
3N	1,868	46,9	6,71	17,6	220.000	180.000	320.000
3DN	1,821	48,3	6,75	17,1	200.000	175.000	318.000
4N	1,609	54,3	5,51	32,3	110.000	25.000	150.000
4DN	1,569	55,4	5,58	31,4	130.000	27.000	150.000

*degradation accumulated %of degradation = [(TPHcontrol –TPH treatment)/TPH control] x 100

** Initial enumeration

Biodegradation process of TPH, by natural attenuation, shows increase from the first month, because the number of all heterotrophic microorganisms present in soil, contaminated with petroleum crude oil, upon the bioremediation treatment was significant.

Taking into account that the contamination event in this soil was long time ago, the soil has had great quantity of adapted native microorganisms. Biodegradation of TPH's was continual and at the end of forth month reached in average 55.4% meanwhile that the bioremediation of oil and grease by natural attenuation achieve 31,4% in the same time.

Chromatographic analysis allowed to estimate the degradation of total PAH's, as well as individual ones. (Table 22) The result achieved was 10,32% which shows, that natural attenuation is very slow process, so there are need to enhance biodegradation to eliminate these compounds from contaminated soil.

Table 22. *Biodegradation of PAH's by natural attenuation*

Sample	Acenaftilene mg/kg	Fluorene mg/kg	Fenantrene mg/kg	Antracene mg/kg	Total mg/kg	Biodegradation %
Initial	0,1041	0,1586	0,8177	0,2486	1,3289	
Final	0,1040	0,1477	0,7021	0,2380	1,1918	**10,32%**

5.3.2 Investigation of biostimulation of indigenous microorganisms for the bioremediation of soil contaminated with hydrocarbons, without pretreatment

In order to compare percentage of biodegradation in soil contaminated with hydrocarbons, the same conditions were applied for natural attenuation as biostimulation. The difference was addition of nutrients and air to enhance the process. The results of corresponding analysis shows table 23.

Table 23. *Biodegradation of soil contaminated with hydrocarbons by biostimulation process*

Sample	TPH (%W)	Percentage of degradation * TPH	Oil and grease (%W)	Percentage of degradation Oil and grease	Microbial enumeration (UFC)/ml		
					nutritive Agar 8900*	Cetride agar 6400*	MBS-HC agar 2100*
0C	3,52	Control	8,14	control	0	0	0
1S	2.66	24,4	7,12	12,1	56.000	66.400	61.800
1DS	2.70	23,3	7,10	12,3	56.000	76.100	62.000
2S	1,87	46,9	6,80	16,0	300.000	170.000	280000
2DS	1,88	46,6	6,78	16,3	310.000	166.000	260000
3S	1,34	61,9	5,63	30,5	410.000	260.000	300.000
3DS	1,29	63,4	5,59	31,0	450.000	270.000	360.000
4S	0,89	74,7	4,89	39,6	316.000	98.000	280.000
4DS	0,90	74,4	4,80	40,7	320.000	90.000	270.000

*degradation accumulated %of degradation = [(TPHcontrol –TPH treatment)/TPH control] x 100

** Initial enumeration

Biostimulation process has had better results in percentage of biodegradation of TPH as well as oil and grease with average values 74.6 and 40.2 respectively. The effect of nutrient addition and the air to the soil, promoted significantly degradation of the hydrocarbons. The growth of bacteria in all of three media was similar, where nutritive media presented better results, indicating good nutritional conditions and oxygen sufficiency. From the third month the growth of microorganisms droop in all media. Cetride agar and MBC-HC presented minor values that the nutritive agar in the end of forth month, which can be explained by low concentration of hydrocarbons in biodegraded soil, so insufficiency of the carbon source for their development. Chromatographic analysis shows mayor percentage of PAH's biodegradation, that by natural attenuation, but it still turns out a slow process. (Table 24)

Table 24. *Biodegradation of PAH's by biostimulation of native microorganisms*

Sample	**Acenaftilene mg/kg**	**Fluorene mg/kg**	**Phenanthrene mg/kg**	**Antracene mg/kg**	**Total mg/kg**	**Biodegradation %**
Initial	0,1041	0,1586	0,8177	0,2486	1,3289	
Final	0,0989	0,1387	0,6821	0,1780	1,0977	**17,4%**

5.3.3 Examination of bioremediation of soil contaminated with hydrocarbons by the bioaugmentation

In the same way like in the natural attenuation and biostimulation experiments, all conditions were the identical. To carry out process of bioaugmentation it was added 40 ml of 2,6,x10^8 cells/ ml of microbial consortium called ECOMAXBACT, whose procedure of application is guarded due to policy of privacy of the ICP. At the beginning of the process, the nutrients were added, like in the biostimulation process, and was vented the soil in the reactors, all the time for four months. The results of the experiments in bioaugmentation process shows table 25. In the bioaugmentation treatment was interesting to see low level of biodegradation during first month, especially for oil and grease. In spite of this, at the end of process was achieved 83.7% of biodegradation of TPH in average and 58.2% of oil and grease in average. Adding up specific microbial community and nutrients with ventilation of soil, it has generated stimulated conditions to enhanced process. An increase population of microorganisms in the contaminated soil might cause at first the competition, between existed native bacteria and added the microbial consortium, which it can be related to the low percentage of biodegradation in first month. In the same way that specialized microorganisms were adjusting to the changing substrate conditions, but after one month their growth overcame the others.

The biodegradation of PAH's in analyzed soil was 31,7%, better than in natural attenuation and biostimulation process, which has correlation with a presence of specialized in degrading the hydrocarbons, microorganisms.

Table 25. *Biodegradation of soil contaminated with hydrocarbons by bioaugmentation process*

Sample	**TPH (%W)**	**Percentage of degradation * TPH**	**Oil and grease (%W)**	**Percentage of degradation Oil and grease**	**Microbial enumeration (UFC)/ml**		
					nutritive Agar 8900*	Cetride agar 6400*	MBS-HC agar 2100*
0C	3,52	Control	8,14	control	0	0	0
1A	2,96	15,9	7,90	2,9	66.000	60.000	65.000
1DA	2,80	20,5	7,88	3,2	65.000	59.000	62.000
2A	1,97	44,0	5,82	28,5	73.500	68.000	150.900
2DA	1,94	44,9	5,67	30,3	70.000	65.800	159.400
3A	1,10	68,8	4,09	49,8	140.200	103.000	370.000
3DA	1,06	69,9	4,11	49,5	142.000	115.000	384.000
4A	0,55	84,4	3,42	58,0	99.000	100.000	280.800
4DA	0,6	83,0	3,38	58,5	101.000	87.800	290.000

*degradation accumulated %of degradation = [(TPHcontrol –TPH treatment)/TPH control] x 100

** Initial enumeration

Table 26. *Biodegradation of PAH's by bioaugmentation process*

Sample	**Acenaftilene mg/kg**	**Fluorene mg/kg**	**Phenanthrene mg/kg**	**Antracene mg/kg**	**Total mg/kg**	**Biodegradation %**
Initial	0,1041	0,1586	0,8177	0,2486	1,3289	
Final	0,0889	0,0987	0,5821	0,1380	0,9077	**31,7%**

5.3.4 Discussion: biodegradation process without pre-treatment

All the biodegradation processes presented increasing tendency of degradation of the analyzed hydrocarbons. Among biodegradation treatments, the highest degradation rate (83,7%) of TPH and 58,2% of oil and grease, 31,7% of PAH's was observed in the Bioaugmentation process, when a microbial consortium called ECOMAXBACT, specialized in degrading the hydrocarbons was added. The microorganisms were stimulated by labile hydrocarbon sources that induced a high percentage of biodegradation, as those forms decreases microbial population had to use more recalcitrant hydrocarbons(with higher molecular weight) less efficiently. This can be observed in the decrease of UFC/ml for agar nutritive and agar Cetride, but increase of bacteria cultivated in agar MSB-HC, especially to degrade hydrocarbons. The same tendency presented biodegradation of oil and grease. In the first month the biodegradation of TPH as well as oil and grease achieved greater result than following month, except in bioaugmentation experiment for oil and grease. The competition of native bacteria with added microbial consortium has generated possible stress at the beginning, but in the second month their performance improved. In general the growth of microorganisms presented into the soil, decrease after four months. Taking into account that the contamination event in this soil was years ago, the hydrocarbons become adsorbed in the soil matrix via partitioning, into soil organic matter or by diffusion into porous space. Thus becoming less extractable to microorganisms and that poor bioavailability limits the growth of the bacteria. (Figure 31 and Figure 32).

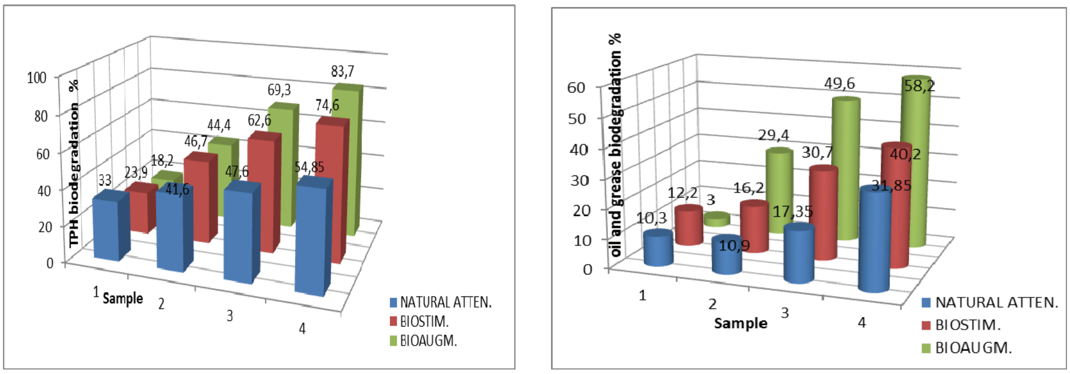


Figure 31. *TPH, oil and grease biodegradation percentage presented in biodegradation processes without pretreatment.*

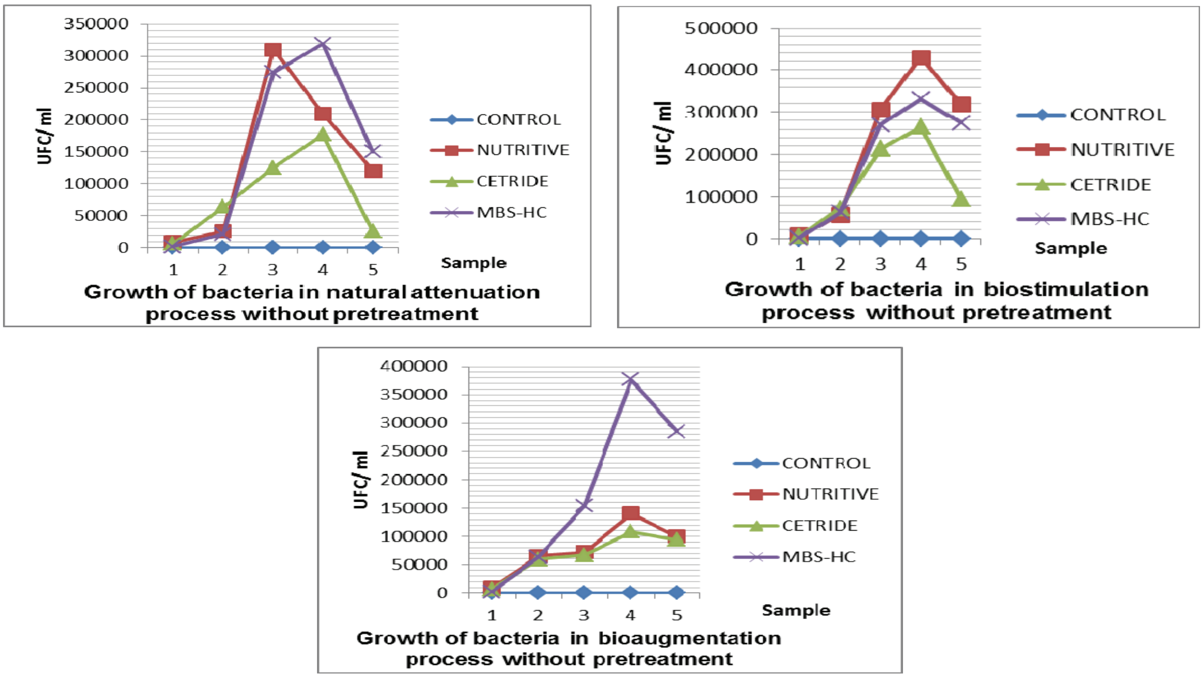


Figure 32. *Bacteria growth in biodegradation process without pre-treatment.*

5.4 BIODEGRADATION OF SOIL CONTAMINANTED WITH HYDROCARBONS WITH SURFACTANT FLUSHING AS PRE-TREATMENT

5.4.1 Recombination of treatments, using flushing of contaminated soil with surfactant as pre-treatment, continued of natural attenuation

The characteristics of surfactant applied for pretreatment are specified next. (Table 27)

Table 27. *Physical and chemical properties of Emulgrosson.*

Appearance	Yellow-brown liquid	Viscosity, centipoises (25°C)	95
Odor:	Detergent	Boiling Point:	100.5°C
Specific Gravity:	1.025+/- 0.020	Water Solubility	100%
pH:	8-9	CMC (*Critical Miscelar Concentration*)	670 ppm

Based on bio-toxicology experiments carried on in Biotechnology Laboratory of Colombian Petroleum Institute is readily decomposed by naturally occurring microorganisms. The biological oxygen demand (BOD), as a percentage of the chemical oxygen demand (COD), will approximate 85% after 8 days, in a standard biodegradation test with soils from three different lands of Colombia, Venezuela and Trinidad & Tobago. Butyl Cellosolve reached 50% degradation in 5 to 23 days, depending upon soil type, and exceeded the rate of degradation for glucose, which was used as a control for comparison. Emulgrosson is considered practically non-toxic per EPA’s Aquatic toxicity scale.

After previous experiments, was selected 5% concentration of solution of Emulgrosson, as the better for flushing treatment. (Figure33) The decision was based in analysis of bacteria growth and release of hydrocarbons linked the soil. Once was determined concentration of surfactant was initiated pretreatment, as an aid to subsequent bioremediation. (Table 28) The sample was analyzed in 0 week and after two weeks in first part (pretreatment) and every month during bioremediation, which lasted four month.

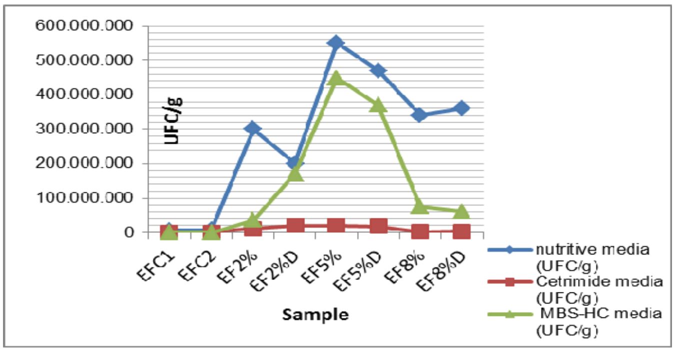


Figure 33. *Emulgrosson concentration pre-selection*

Release of Hydrocarbons from contaminated soil, was provide the source of the carbon for bacteria so, during washing process was observed great growth of microorganisms into the contaminated soil. It worthwhile emphasizing the significant growth of the bacteria, specialized in degradation of hydrocarbons. In the end of pretreatment the number of microorganisms was dropped drastically.

Table 28. *Flushing of soil with 5% solution of Emulgrosson*

Sample	TPH (%W)	**(%W) Elimination TPH**	Oil and grease (%W)	**(%w) Elimination oil and grease**	**MICROBIAL ENUMERATION (UFC)/ml**		
					Nutritive Agar	Cetride agar	MBS-HC agar
Inicial	3,52		8,14		8900	6400	2100
Final	1,16	67%	3,90	52,1%	3000	2400	1.200

The flushing of soil with surfactants leaves the greatest results of elimination of TPH (67%), oil and grease (52.1%). After this process the same soil was continued with biodegradation by natural attenuation. (Table 29)

Table 29. *Biodegradation of soil contaminated with hydrocarbons by natural attenuation after pretreatment with surfactant*

Sample	**TPH (%W)**	**Percentage of degradation* TPH after pretreatment**	**Oil and grease (%W)**	**Percentage of degradation Oil and grease after pre-treatment**	**Microbial enumeration (UFC)/ml**		
					nutritive agar 3000*	Cetride agar 2400*	MBS-HC agar 1200*
0CS	1,16	Control	3,90	control	0		0
1SN	0.96	17,2	3.30	15,4	30.000	14.000	27.000
1DSN	0,98	15,5	3.33	14,6	26.000	14.000	25.000
2SN	0,90	22,4	3,28	15,9	80.000	18.000	80.000
2DSN	0,92	20,7	3,23	17,2	70.000	17.800	70.000
3SN	0,868	25,2	2,71	30,5	100.000	21.000	120.000
3DSN	0,821	29,2	2,75	29,5	92.000	25.000	118.000
4SN	0,709	38,9	1,91	51,0	71.000	12.000	80.000
4DSN	0,769	33,7	1,88	51,8	73.000	17.000	85.000

*degradation accumulated %of degradation = [(TPHcontrol –TPH treatment)/TPH control] x 100

** Initial enumeration

The biodegradation total of TPH, taking into account the initial concentration (3.52%W for TPH and 8.14%W for oil and grease) was 79% and oil and grease 76.8%. After pretreatment with surfactant solution, the biodegradation process by natural attenuation presented 36.3% in average of TPH degradation and 51.4% of oil and grease. It is possible that after flushing with surfactant were still release hydrocarbons, when the soil was dampening during experimentation time which can explain better degradation of oil and grease.

After pretreatment was observed elimination of 63.4% of total PAH, but during natural attenuation was not registered additional degradation. (Table30)

Table 30. *Elimination of PAH's by flashing with surfactant solution pretreatment*

Sample	Acenaftilene mg/kg	Fluorene mg/kg	Fenantrene mg/kg	Antracene mg/kg	Total mg/kg	Biodegradation %
Initial	0,1041	0,1586	0,8177	0,2486	1,3289	
Final	0,0480	0,0887	0,2621	0,0880	0,4868	**63,4%**

5.4.2 Recombination of treatments, using flushing of contaminated soil with surfactant as pre-treatment, continued of biostimulation process

In this experimentation the same soil after pretreatment with surfactants, was used to observe biostimulation process during four month. The condition and procedure was the same, with difference that the soil was provided continuously with air and nutrients, for the period of experimentation time. The results of this assessment show table 31.

The biodegradation of TPH and oil and grease in biostimulation process was: 62,15% and 80,25 in average respectively. The biodegradation total of TPH and oil and grease, taking into account the initial concentration (3,52%W for TPH and 8,14%W for oil and grease), was 86,7% and 90,5% respectively. Biodegradation of PAH after flushing, during biostimulation process was 34, 9%. Throughout total remediation process (Flushing and biostimulation) was eliminated from the soil 76, 2% of PAH. (Table 32)

The growth of microorganisms were great, especially in MBS-HC media, but after third month was observed decreasing process.

Table 31. *Biodegradation of soil contaminated with hydrocarbons by biostimulation after pretreatment with surfactant*

Sample	TPH (%W)	Percentage of degradation* TPH after pretreatment	Oil and grease (%W)	Percentage of degradation Oil and grease after pre-treatment	Microbial enumeration (UFC)/ml		
					nutritive Agar 3000*	Cetride agar 2400*	MBS-HC agar 1200*
0CS	1,16	Control	3,9	Control	0	0	0
1SS	0,96	17,2	3,1	20,5	35.000	24.000	37.000
1DSS	0,97	16,4	3,15	19,2	36.000	24.000	35.000
2SS	0,80	31,0	1,64	57,9	99.000	48.000	90.000
2DSS	0,82	29,3	1,55	60,3S	100.000	47.800	87.000
3SS	0,668	42,4	0,99	74,6	160.000	124.000	229.000
3DSS	0,621	46,5	0,8	79,5	182.000	127.000	226.000
4S	0,409	64,7	0,75	80,8	170.000	115.000	180.000
4DSS	0,469	59,6	0,79	79,7	173.000	117.000	185.000

*degradation accumulated %of degradation = [(TPHcontrol –TPH treatment)/TPH control] x 100

** Initial enumeration

Table 32. *Elimination of PAH's after flashing with surfactant solution pretreatment in biostimulation process*

Sample	Acenaftilene mg/kg	Fluorene mg/kg	Phenanthrene mg/kg	Antracene mg/kg	Total mg/kg	Biodegradation %
Initial	0,0480	0,0887	0,2621	0,0880	0,4868	
Final	0,0280	0,0387	0,1621	0,0880	0,3168	**34,9%**

5.4.3 Recombination of treatments, using flushing of contaminated soil with surfactant as pre-treatment, continued of bioaugmentation process

After flushing the soil with Emulgrosson (5% solution) was carried out process of bioaugmentation, so it was added 40 ml of 2,6,×10^8 cells/ ml of microbial consortium called ECOMAXBACT, nutrients and air. Every month was sampling, to analyze TPH and oil and grease concentration, and growth of microorganisms. In the table 33 are reported result of this experimentation process.

Table 33. *Biodegradation of soil contaminated with hydrocarbons by biostimulation after pretreatment with surfactant*

Sample	TPH (%W)	Percentage of degradation * TPH after pretreatment	Oil and grease (%W)	Percentage of degradation Oil and grease after pretreatment	Microbial enumeration (UFC)/ml		
					nutritive Agar 3000*	Cetride agar 2400*	MBS-HC agar 1200*
0CS	1,16	Control	3,9	Control	0	0	0
1SS	1,06	8.6	3,78	3.1	55.000	34.000	27.000
1DSS	0,99	14.7	3,88	3.1	56.000	34.000	25.000
2SS	0,86	25.9	2.89	23.6	149.000	58.000	70.000
2DSS	0,82	29.3	2,99	23.3	150.000	59200	77.000
3SS	0,78	32.8	2.19	43.8	360.000	264.000	199.000
3DSS	0,77	33.6	2.18	44.1	372.000	267.000	196.000
4S	0,69	40.5	1,55	60.3	280.000	105.000	118.000
4DSS	0,66	43.1	1,70	56.4	293.000	107.000	115.000

*degradation accumulated %of degradation = [(TPHcontrol –TPH treatment)/TPH control] x 100

** Initial enumeration

Bioaugmentation process carried out after pretreatment with surfactant solution presented 41.8% of TPH biodegradation in average and 58.35% of oil and grease degradation in average. The low biodegradation process presented in the first month might be explained by competition process developed in great quantity of microorganisms (there were an extra of bacteria) and low concentration of hydrocarbons as carbon source. The total degradation percentage in two treatments (flushing with surfactant and bioaugmentation) were: 80.80% in average for TPH and 80.03% for oil and grease.

Biodegradation of PAH shows Table 34. In this process was achieving 35.33%, which is similar result as in biostimulation process after pretreatment with surfactant. Total degradation of PAH, in flushing with surfactant and subsequent biodegradation, was 76.3%.

Table 34. *Elimination of PAH's after flashing with surfactant solution pretreatment in bioaugmentation process*

Sample	Acenaftilene mg/kg	Fluorene mg/kg	Phenanthrene mg/kg	Antracene mg/kg	Total mg/kg	Biodegradation %
Initial	0,0480	0,0887	0,2621	0,0880	0,4868	
Final	0,0290	0,0487	0,1521	0,0850	0.3148	**35.33%**

5.4.4 Discussion: biodegradation process with flushing with surfactant pre-treatment

Comparison of natural attenuation, biostimulation and bioaugmentation with surfactant pretreatment is summarized in Figure 34 and growth of microorganisms in each of the process, shows Figure 35. Probably the low concentration of hydrocarbons needs stimulation for the microorganism to eliminate remaining quantity of the hydrocarbons from the soil. Due to high microbial density, low hydrocarbons concentration in the soil, and the needs to adaptation in new condition for additional microbial consortium, the bioaugmentation process after flushing with surfactant, presented initial problem in removing of hydrocarbons, so at the end the removal was lower, than in biostimulation process. As the growth of microorganism after pre-treatment with surfactant solution was better in biostimulation process, probably is more convenient to give stimulating condition, instead to add of specialized microorganisms, when concentration of hydrocarbon is low, due to previous treatment. The native micrococsmos are the big variety of microorganisms, which can grow, if his nutritional conditions are supported all the time. Hence, is observed major growth of bacteria in nutritive media, than in Cetride or MBS-HC, especially because of low hydrocarbon concentration in soil. (Figure 34 and Figure35)

After pre-treatment with surfactant, the biostimulation process left better results than natural attenuation and bioaugmentation for TPH biodegradation as well as oil and grease. (Figure 36) Comparing total treatments (biodegradation without and with pretreatment) the biostimulation process achieved better results if is combined with surfactant pre-treatment than without it, but previous flushing with surfactant is no convenient for bioaugmentation process in analyzed soil. (Figure 37)

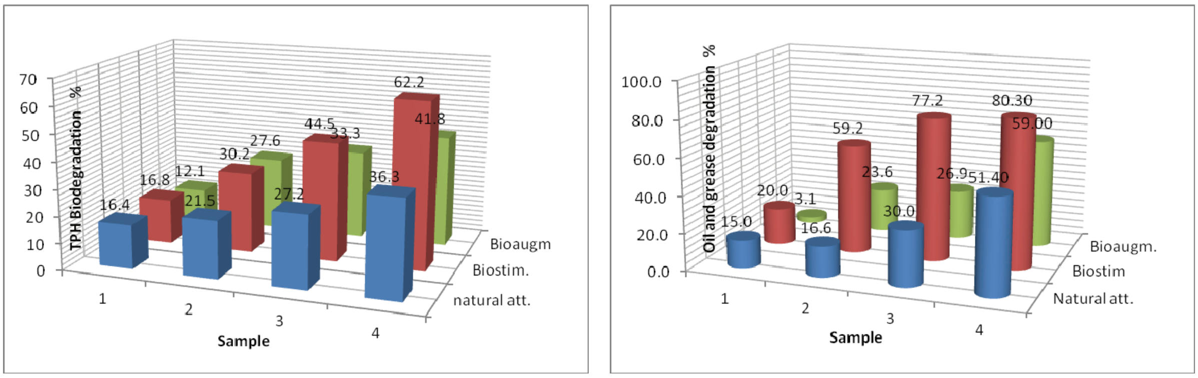


Figure 34. *Comparison of TPH, oil and grease biodegradation after surfactant pretreatment*

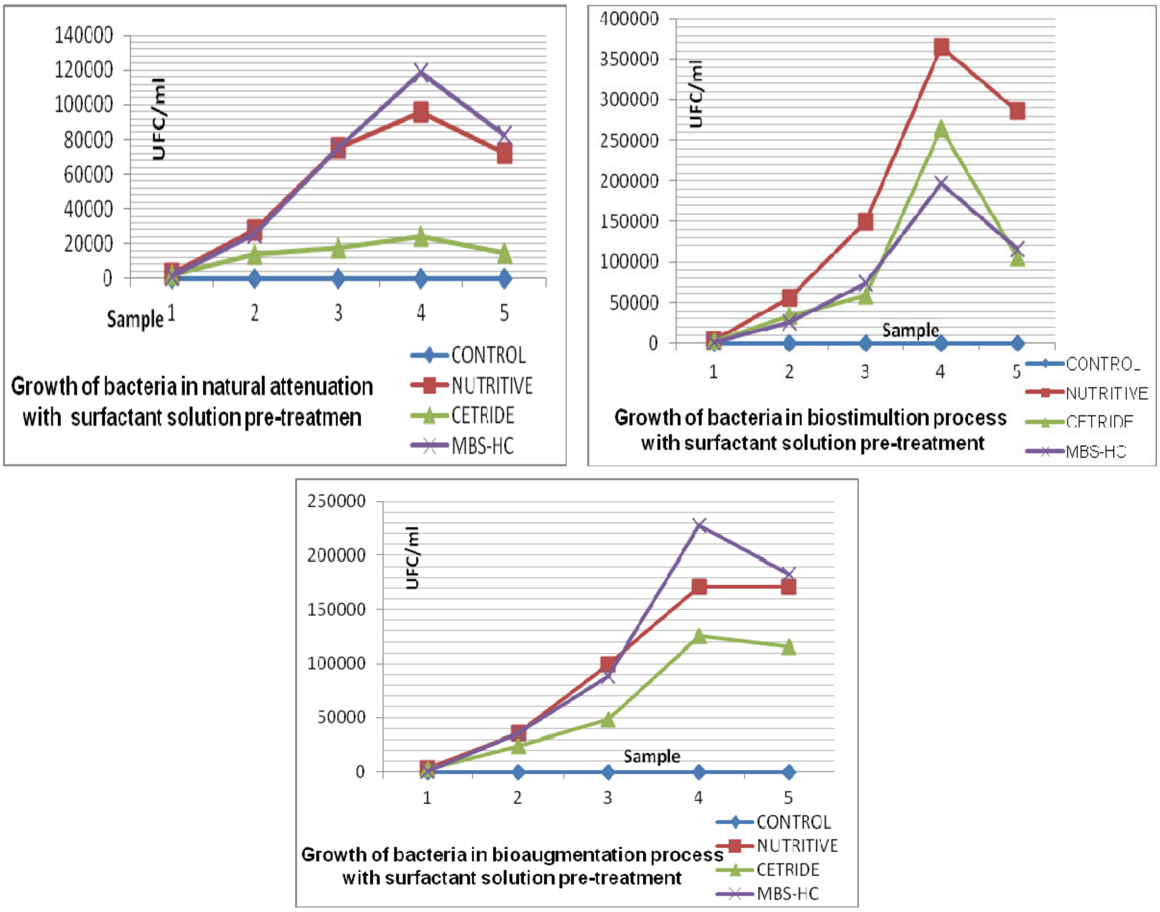


Figure 35. *Growth of bacteria in biodegradation process, after surfactant solution as pre-treatment*

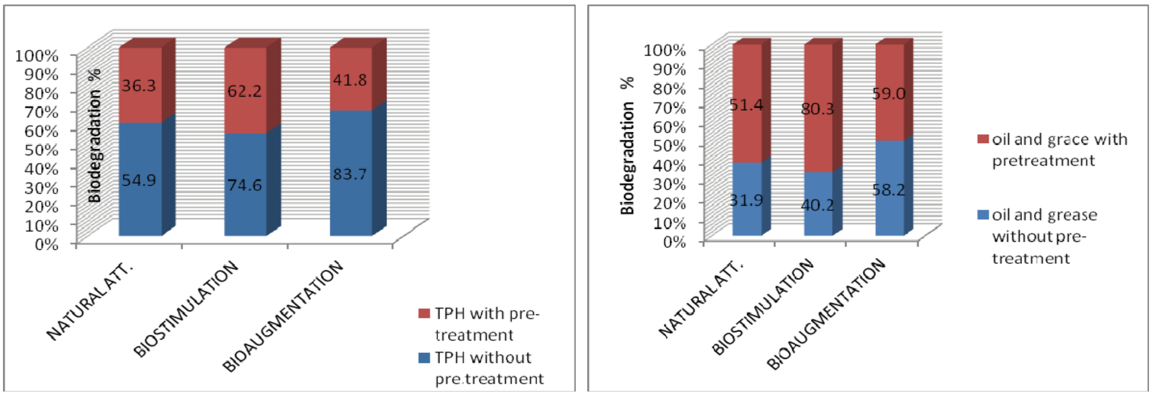


Figure 36. TPH and oil and grease biodegradation, process with and without pre-treatment

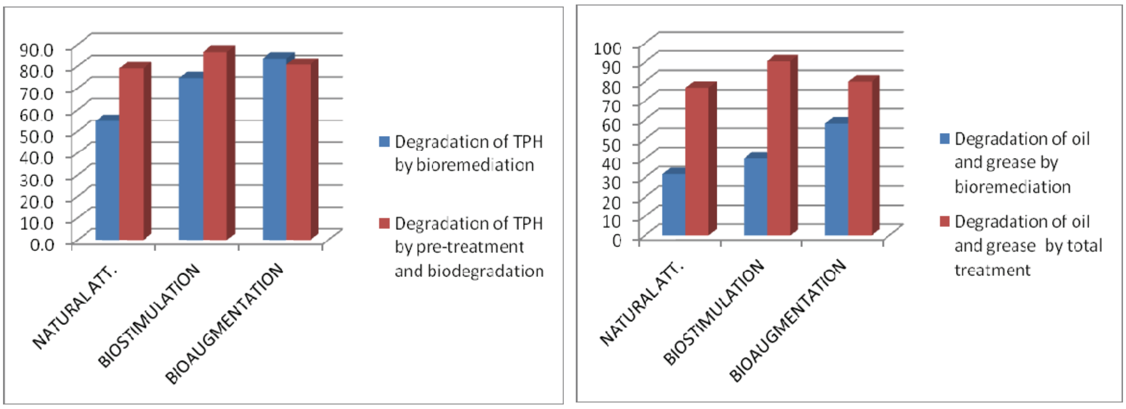


Figure 37. Biodegradation process compared with total combined treatment (flushing with surfactant and bioremediation), for TPH and oil and grease remediation

5.5 BIODEGRADATION OF SOIL CONTAMINANTED WITH HYDROCARBONS WITH HYDROGEN PEROXIDE AS PRE-TREATMENT

5.5.1 Recombination of treatments using hydrogen peroxide solution as pre-treatment continued of natural attenuation

Pretreatment using hydrogen peroxide lasted two weeks, as in earlier pre-treatment with surfactant. Previous assays have allowed determining the best concentration of the hydrogen peroxide solution. The analyzed concentration was: 0,0F (control, wash with water), 0,01F; 0,05F; 0,1F. The decision was based in analysis of bacteria's growth and release of hydrocarbons linked the soil. As in preceding pre-treatment, once concentration of the hydrogen peroxide determined, was initiated pretreatment as an aid to subsequent bioremediation. (Table 35 and Table 36) The sample was analyzed in 0 week and after two weeks in first part (pretreatment) and every month during bioremediation, which lasted four month. Are depicted in the Figure 38 microbiological growth results, for determination of concentration for hydrogen peroxide solution As it is possible to see in the figure 39, the best growth of the bacteria allows the 0,05F solution of hydrogen peroxide, so this one was applied for pre-treatment process.

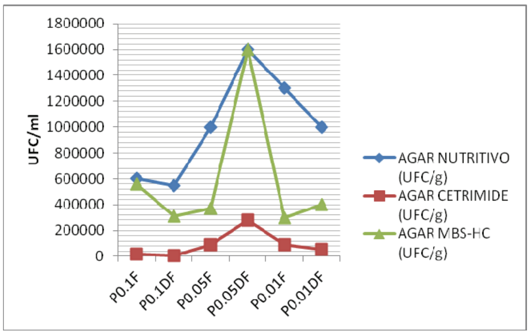


Figure 38. *Determination of the best concentration of hydrogen peroxide solution, for pre-treatment*

Table 35. *Results of two weeks pre-treatment; flushing with 0,05F solution of hydrogen peroxide*

Sample	TPH (%W)	**(%W) Elimination TPH**	Oil and grease (%W)	**(%W) Elimination oil and grease**	**MICROBIAL ENUMERATION (UFC)/ml**		
					Nutritive Agar	Cetride agar	MBS-HC agar
Inicial	3,52		8,14		8900	6400	2100
Final	1,02	71,0%	2,73	66.4%	2800	2600	800

Biodegradation of TPH by natural attenuation, after a pre-treatment with 0,05F hydrogen peroxide solution, was en average: 57,8% of TPH and 43,4% of oil and grease. After two treatments was achieved 86,9% degradation of TPH and 80,6% of oil and grease. Chromatographic analysis of PAH shows elimination of 79,17% of this compounds from soil after pre-treatment with hydrogen peroxide, and additional 5,78% in subsequent, natural attenuation process. (Table 37) Based in initial concentration of PAH (1.3289 mg/kg), total degradation of two processes leaves the result of 80,37% .

Table 36. *Biodegradation of soil contaminated with hydrocarbons by natural attenuation with pre-treatment with hydrogen peroxide solution*

Sample	**TPH (%W)**	**Percentage of degradation* TPH**	**Oil and grease (%W)**	**Percentage of degradation Oil and grease**	**Microbial enumeration (UFC)/ml**		
					nutritive agar 2800*	Cetride agar 2600*	MBS-HC agar 800*
0C	1.02		2.73	control	0	0	0
1NP	0.98	3.9	2.50	8.4	26.000	44.000	31.000
1DNP	0.99	2.9	2.38	12.8	26.000	44.000	32.000
2NP	0,87	14.7	2,28	16.5	38.000	60.000	80.000
2DNP	0,84	17.6	2,23	18.3	37.000	63.000	70.000
3NP	0,68	33.3	1,79	34.4	129.000	150.000	120.000
3DNP	0,69	32.4	1,75	35.9	130.000	155.000	118.000
4NP	0,40	60.8	1,51	44.7	98.000	85.000	50.000
4DNP	0,46	45.1	1,58	42.1	100.000	97.000	50.000

*degradation accumulated where:%of degradation = [(TPHcontrol –TPH treatment)/TPH control]x 100

** Initial enumeration (after pre-treatment)

Table 37. *Elimination of PAH's after flashing with hydrogen peroxide solution by natural attenuation process*

Sample	Acenaftilene mg/kg	Fluorene mg/kg	Phenanthrene mg/kg	Antracene mg/kg	Total mg/kg	Biodegradation %
Initial	0.0394	0,0677	0,1621	0,0389	0,2768	**5.78%**
Final	0,0280	0,0497	0,1491	0,0340	0,2608	

The pretreatment with the hydrogen peroxide, turns out to be a rapid and effective alternative for the elimination of hydrocarbons and persistent in the soil PAH, but during the process the temperature of the soil increase. This might be a cause of low biodegradation for natural attenuation due to the inhibition of some bacteria to major temperature.

5.5.2 Recombination of pretreatment using hydrogen peroxide solution as pre-treatment continued of biostimulation process

Biostimulation process used the same soil like was applied in the previous treatment with hydrogen peroxide, as well as the conditions managed in others biostimulation processes.

The elimination of TPH, oil and grease and growth of microorganisms are illustrated in Table 38. The chromatographic result of PAH biodegradation in biostimulation process showing Table 39. The percentage of TPH degradation was 60.3 in average, which represented really great process. In total biodegradation of TPH in two treatments (flushing with hydrogen peroxide and biostimulation) was 88.64%. Degradation of oil and grease in biostimulation process was 52.2% in average and total 83.4%. The growth of microorganisms were satisfactory, so it makes to think, that after chemical oxidation should be deliver nutrients and air, to generate suitable condition for bacteria and biodegradation process. The growth of bacteria in all media was similar although nutritive agar has presented better results, because the nutrient condition in biostimulation process encouraged all kind of microorganisms and air refresh soil and stimulate grow of bacteria. Biodegradation of PAH presented 30.24% in biostimulation process and total 85.47%. These results show good combination of treatments and possibility of elimination from analyzed soil persistent compounds as polynuclear aromatic hydrocarbons (PAHs).

Table 38. *Biodegradation of soil contaminated with hydrocarbons by biostimulation with hydrogen peroxide solution pre-treatment*

Sample	**TPH (%W)**	**Percentage of degradation * TPH**	**Oil and grease (%W)**	**Percentage of degradation Oil and grease**	**Microbial enumeration (UFC)/ml**		
					nutritive Agar 2800**	Cetride agar 2600*	MBS-HC agar 800*
0C	1.02		2.73	control	0	0	0
1SP	0.88	13.7	2.30	15.8	96.000	73.000	61.000
1DSP	0.79	22.5	2.28	16.5	98.000	73.000	62.000
2SP	0,77	24.5	1,88	31.1	128.000	90.000	87.000
2DSP	0,74	27.5	1,83	33.0	124.000	93.000	90.000
3SP	0,58	43.1	1,69	38.1	329.000	180.000	169.000
3DSP	0,59	42.2	1,65	39.6	333.000	185.000	168.000
4SP	0,41	59.8	1,31	52.0	198.000	95.000	80.000
4DSP	0,40	60.8	1,30	52.4	188.000	97.000	75.00

*degradation accumulated   where:%of degradation = [(TPHcontrol –TPH treatment)/TPH control]x 100

** Initial enumeration (after pre-treatment)

Table 39. *Elimination of PAH's after flashing with hydrogen peroxide solution by biostimulation process*

Sample	**Acenaftilene mg/kg**	**Fluorene mg/kg**	**Phenanthrene mg/kg**	**Antracene mg/kg**	**Total mg/kg**	**Biodegradation %**
Initial	0.0394	0,0677	0,1621	0,0389	0,2768	
Final	0,0143	0,0407	0,1161	0,0220	0.1931	**30.24%**

5.5.3 Recombination of pretreatment using hydrogen peroxide solution as pre-treatment continued of bioaugmentation

The addition of microorganisms after hydrogen peroxide solution treatment, generated additional degradation of 33,85% for TPH in average and 41.2 percent for oil and grease. The low remaining concentration of hydrocarbon in soil and increase density of microorganisms has produced competition for primary source of carbon, and this made less efficient biodegradation process. (Table 40) Total treatment achieved 80,82% elimination of TPH and 80,28% of oil and grease in average. This result is very similar that was obtained in total treatment with surfactant flushing as the pre-treatment. The biodegradation of PAH presented 27,46% and total degradation of this compounds was 84,88% in the combined processes (pre-treatment and bioaugmentation). (Table 41) During pre-treatment with hydrogen peroxide was developed oxidizing process of PAH and others compounds which might blocks PAHs in the soil. After pre-treatment with hydrogen peroxide, the biodegradation of both, TPH and oil and grease was increased, in every monthly measure. Besides, the biostimulation process was more effective than natural attenuation and bioaugmentation. (Figure 39)

Microbiological growth presents effective increase during three months which goes in accordance with biodegradation process. After fourth month the bacterial population was decreased, which can be caused by low concentration of source of carbon, presents in the soil, and low bioavailability of them. In natural attenuation process the bacteria which presents better growth was hydrocarbon degrading, due to the existence of different by-products as a result of hydrocarbons oxidizing process, after pre-treatment in the soil.

Table 40. *Biodegradation of soil contaminated with hydrocarbons by bioaugmentation after flushing with hydrogen peroxide solution as pre-treatment*

Sample	**TPH (%W)**	**Percentage of degradation * TPH**	**Oil and grease (%W)**	**Percentage of degradation Oil and grease**	**Microbial enumeration (UFC)/ml**		
					nutritive Agar 2800*	Cetride agar 2600*	MBS-HC agar 800*
0C	1.02		2.73	control	0	0	0
1AP	0.91	10.8	2.58	5.5	96.000	55.000	75.000
1DAP	0.94	7.8	2.58	5.5	95.000	59.000	72.000
2AP	0,87	14.7	1,88	31.1	113.500	88.000	138.000
2DAP	0,84	17.6	1,90	30.4	117.000	86.800	140.000
3AP	0,78	23.5	1,79	34.4	132.200	103.000	162.000
3DAP	0,76	25.5	1,75	35.9	138.000	115.000	160.000
4AP	0,69	32.4	1,61	41.0	112000	90.000	130.800
4DAP	0,66	35.3	1,60	41.4	100.000	87.800	128.000

*degradation accumulated where: %of degradation = [(TPHcontrol –TPH treatment)/TPH control]x 100
** Initial enumeration (after pre-treatment)

Table 41. *Elimination of PAH's after flashing with hydrogen peroxide solution by bioaugmentation process*

Sample	**Acenaftilene mg/kg**	**Fluorene mg/kg**	**Fenantrene mg/kg**	**Antracene mg/kg**	**Total mg/kg**	**Biodegradation %**
Initial	0.0394	0,0677	0,1621	0,0389	0,2768	**27.46%**
Final	0,0140	0,0317	0,1281	0,0270	0.2008	

5.5.4 Discussion: biodegradation process with hydrogen peroxide pre-treatment

The nutrient addition in biostimulation process increase population of different microorganisms presented in the soil. In Natural attenuation process the microorganisms have grown very similar although in third day the *Pseudomonas* which grow in Cetride media has achieved better performance. In bioaugmentation process, the addition of hydrocarbon degrading bacteria, makes major growth of this kind of microbes. (Figure 40)

After pre-treatment with hydrogen peroxide, the biodegradation process with better performance presented was biostimulation which according to the best bacterial growth.

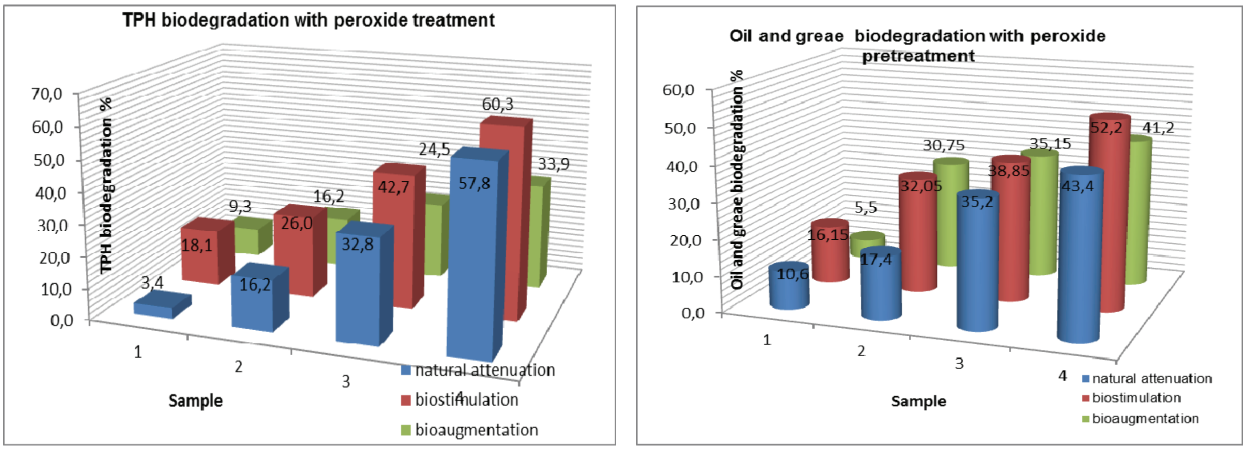


Figure 39. *Comparison of TPH, oil and grease elimination, by biodegradation processes after hydrogen peroxide pre-treatment*

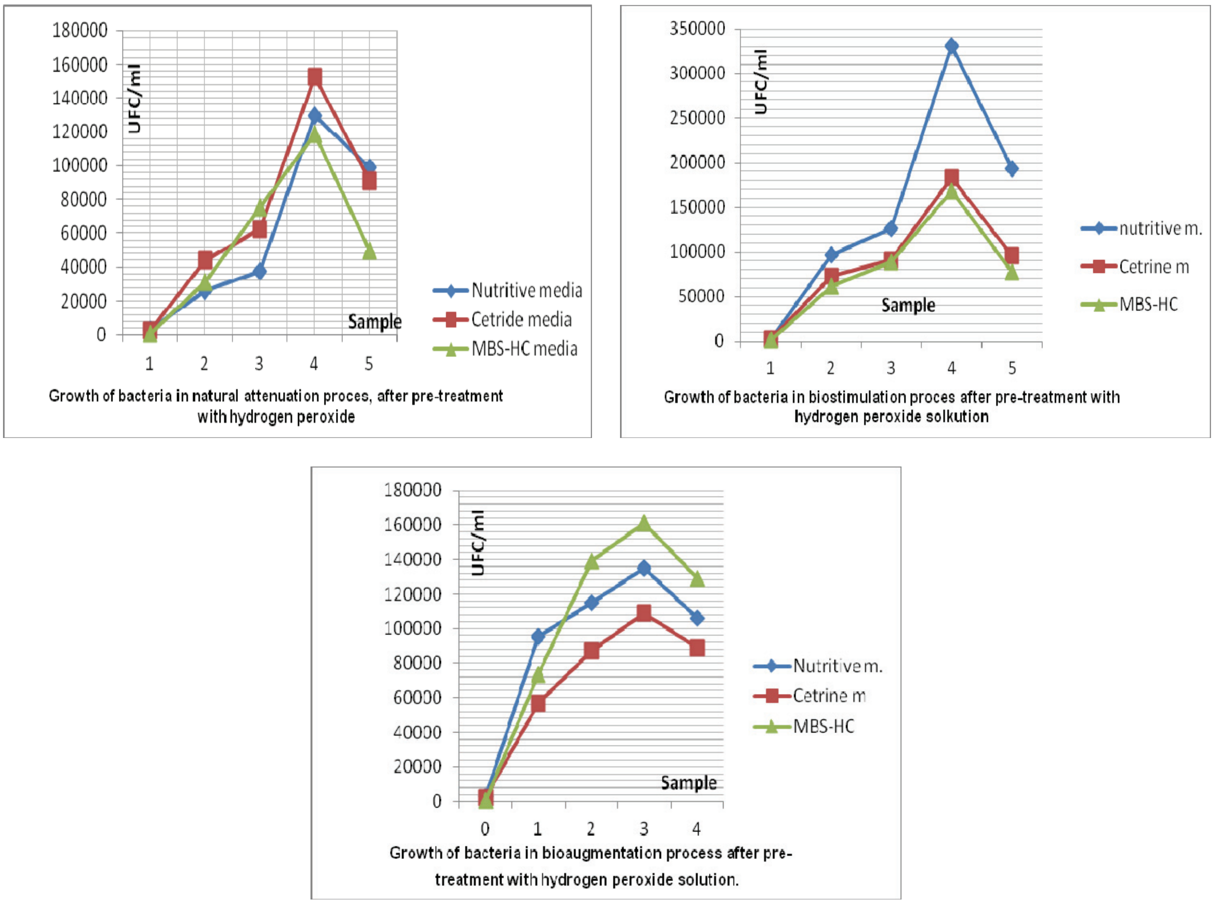


Figure 40. *Bacteria growth in the soil, after pre-treatment with hydrogen peroxide solution, during natural attenuation, biostimulation and bioaugmentation process*

In general the biodegradation process, especially for oil and grease, performed better with peroxide as pre-treatment, except Bioaugmentation. The low results of bioaugmentation, show possible competition of microorganisms for scarce carbon source. (Figure 41)

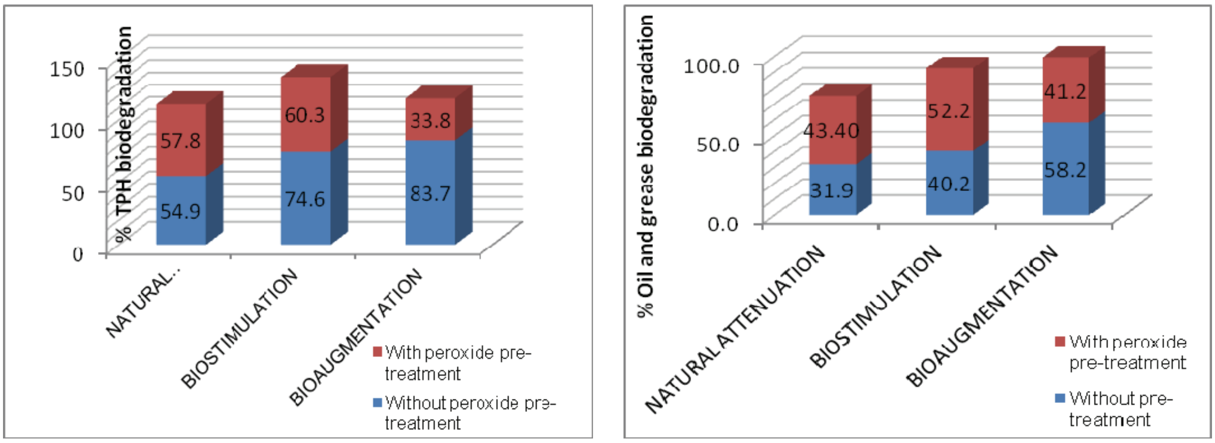


Figure 41. *TPH and oil and grease biodegradation, process with and without pre-treatment*

Total percentage of TPH degradation as well as the oil and grease in combined treatment, (peroxide pre-treatment and later biodegradation) presents better result if after pre-treatment was applied biostimulation process.

Bioaugmentation process applied after pre-treatment with hydrogen peroxide, presented lower results than the others biodegradation processes, due to the low concentration of source of carbon presented after oxidizing process and high bacterial population. (Figure42)

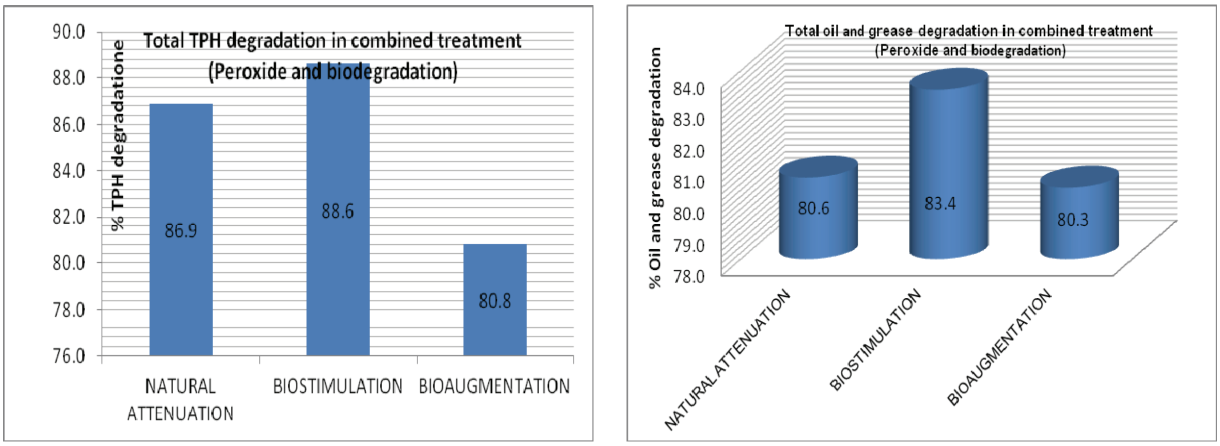


Figure 42. *Total percentage of TPH and oil and grease degradation, in combined treatment (peroxide pre-treatment and later biodegradation)*

5.6 DISCUSSION: ALL TREATMENTS APPLIED FOR REMEDIATION OF SOIL CONTAMINATED WITH HYDROCARBONS

Figure 43 shows comparison of biodegradation process of TPH and oil and grease, taking into account all treatments and recombination of them.

After the pre-treatment the biodegradation process is lower than without them, due to the decrease of the concentration of degraded hydrocarbons. The biodegradation of TPH is better in bioaugmentation process without pre-treatment. The best result of biodegradation of TPH after pre-treatment was for biostimulation process in both, surfactant flushing and oxidizing process with hydrogen peroxide.

To eliminate by biodegradation oil and grease, from analyzed soil is favorable apply pre-treatments, especially the surfactant flushing and later biostimulation.

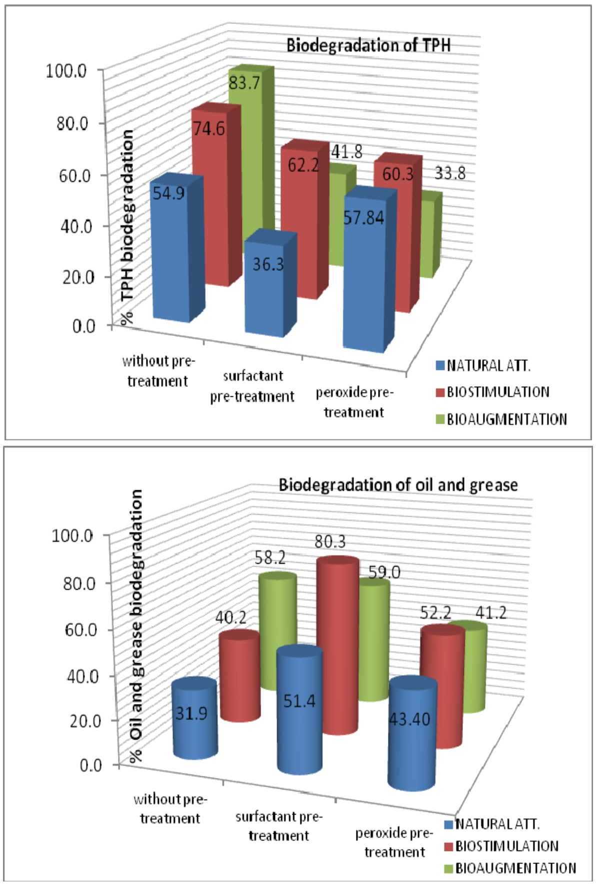


Figure 43. *Comparison of percentage of TPH, oil and grease biodegradation in applied treatments*

The best result for remediation of TPH offered biostimulation process with peroxide pre-treatment, (total efficiency of 88.64%), which is similar as surfactant flushing combined with biostimulation pretreatment (86.7%). For oil and grease elimination is convenient recombination of pre-treatment with surfactant and biostimulation process which leaves 90,5% of efficiency. (Figure 44)

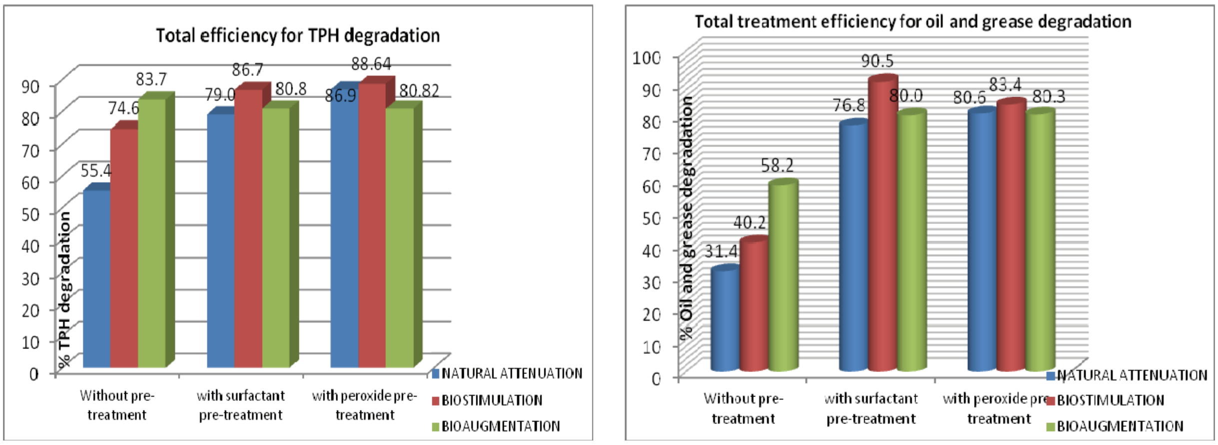


Figure 44. *Comparison of efficiency of treatments, for TPH and oil and grease degradation*

The PAHs degradation presented better performance in combined treatment, especially in peroxide pre-treatment and later biostimulation process, where was obtained 85,47% of efficiency. (Figure 45) The difference between removal of PAHs in biodegradation process and recombined treatments is quite large. Taking into account, that pre-treatment with surfactant, removed 63,4% of PAHs and pre-treatment with peroxide eliminated 79,17% of those compounds, the results of total efficiency in combined treatments, as well as only biodegradation shows difficulty in biodegradation of this kind of compounds.

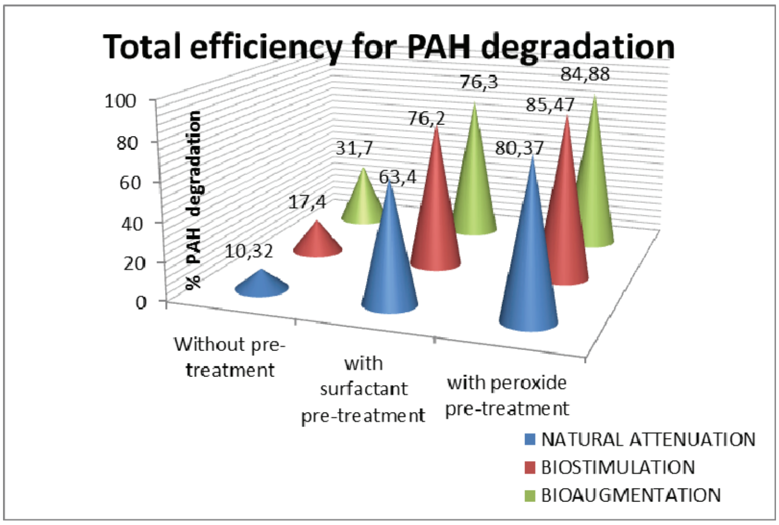


Figure 45. *Total efficiency for PAH degradation*

5.7 VISUAL SOFTWARE MODFLOW APPLICATION FOR INVESTIGATE THE BEHAVIOR OF GROUNDWATER FLOW IN FIELD PALAGUA

The values applied for the modeling shows table 42 where coordinates, cota, current depth and hydrometric characteristics are specified for each one Wells in station 1 and adjoining ground.

Table 42. *The values used for modeling*

Monitoring wells / APIQUE	COORDENATES		COTA (m)	Depth designe (m)	Current depth measure (m)	STATIC LEVEL (m)						PIEZOMETRIC LEVEL(m)					
	NORTH	EAST															
PM-1	1163571.5	952989.2	158.0	7.7	7.2	3.9	4.2	4.4	4.4	5.0	4.6	154.1	153.8	153.6	153.6	153.0	153.4
PM-10	1163682.1	953029.3	159.2	6.3	6.0	NR	NR	3.7	3.7	4.5	3.6	NR	NR	155.5	155.5	154.7	155.6
PM-14	1163547.0	952986.1	154.7	6.3	5.7	0.0	0.0	0.2	0.2	0.8	0.2	154.7	154.7	154.5	154.6	153.9	154.6
PM-15	1163740.5	952946.3	160.0	6.3	6.2	NR	NR	5.4	5.4	5.7	5.9	NR	NR	154.6	154.6	154.3	154.1
PM-17	1163556.2	952989.2	155.5	6.0	3.7	0.1	0.3	0.6	0.5	1.4	0.3	155.4	155.3	154.9	155.0	154.1	155.2
PM-18	1163537.7	952979.9	154.5	6.0	4.3	0.9	0.3	0.5	0.3	0.8	0.5	153.6	154.2	154.1	154.2	153.8	154.1
PM-19	1163525.5	952961.5	154.2	6.0	4.4	0.1	0.2	0.3	0.2	0.6	0.1	154.1	154.0	153.9	154.0	153.6	154.1
AP-21	1163543.9	952946.1	154.3	-	2.5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
AP-22	1163547.0	952943.0	154.2	-	2.0	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
AP-23	1163556.2	952946.1	153.9	-	1.2	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
PR-1	1163515.0	952944.0	154.0	3.0	2.8	NR	NR	NR	NR	NR	0.6	NR	NR	NR	NR	NR	153.4
PR-2	1163521.0	952938.0	153.5	3.0	2.8	NR	NR	NR	NR	NR	0.3	NR	NR	NR	NR	NR	153.2
PR-3	1163529.0	952938.0	153.0	3.0	2.2	NR	NR	NR	NR	NR	0.4	NR	NR	NR	NR	NR	152.6
PR-4	1163538.0	952936.0	153.0	3.0	2.0	NR	NR	NR	NR	NR	0.2	NR	NR	NR	NR	NR	152.8
PR-5	1163545.0	952933.0	152.0	3.0	1.5	NR	NR	NR	NR	NR	0.2	NR	NR	NR	NR	NR	151.8

NR (no reported) *The apique number 20 was obstructed. ** Current minor depth to that of design, for the sediments formed at the bottom of the wells. The piezometric level was calculated by reducing cota of area the corresponding static level.

5.7.1 Delimitation of area and topography coordinate in area of interest

The figure 46 shows the topographic plane used for the simulation in Surfer and Visual Modflow. The coordinated were: 952859.8492E and 1163377.7525N as coordinated minims and 953093.1318E and 1163787.8477N as maxims. (Coordinated was gotten from the low left point up to the top right point of the plane). In the plane can be see the closeness of the tanks of storage of hydrocarbons to the bordering property and a sequence of the wells of monitoring and control wells nearby at the edge of Station 1, located in this way to support the control of the displacement of the pollutant.

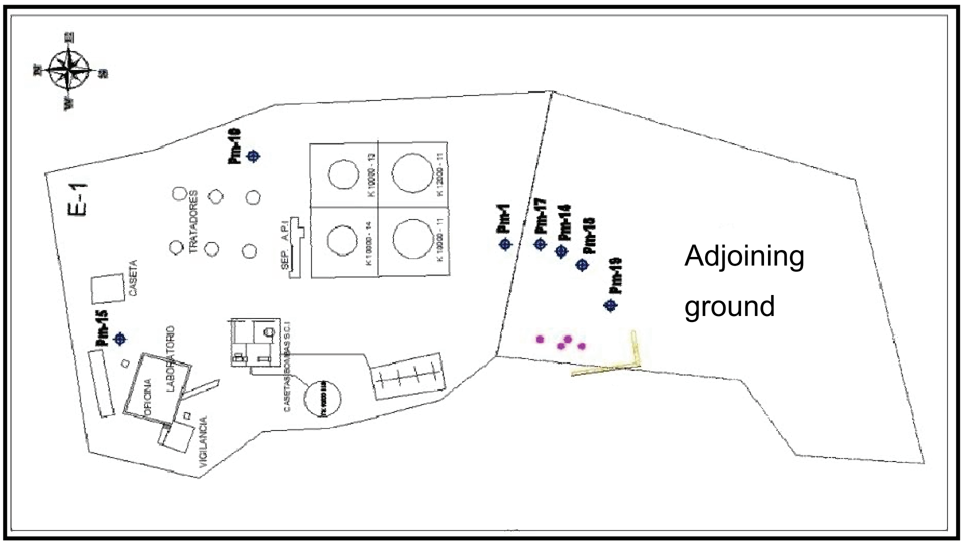


Figure 46. *Topographic plane for Station 1 and adjoining ground*

5.7.2 Profile of level, modeling by Surfer

Figure 47 highlights the system of coordinated and corresponding levels obtained across the superposition of maps with AutoCAD and Surfer, as well as the place of the wells of monitoring located in the zone of major interest (adjoining property). In the same way it can be see the change of heights of the area.

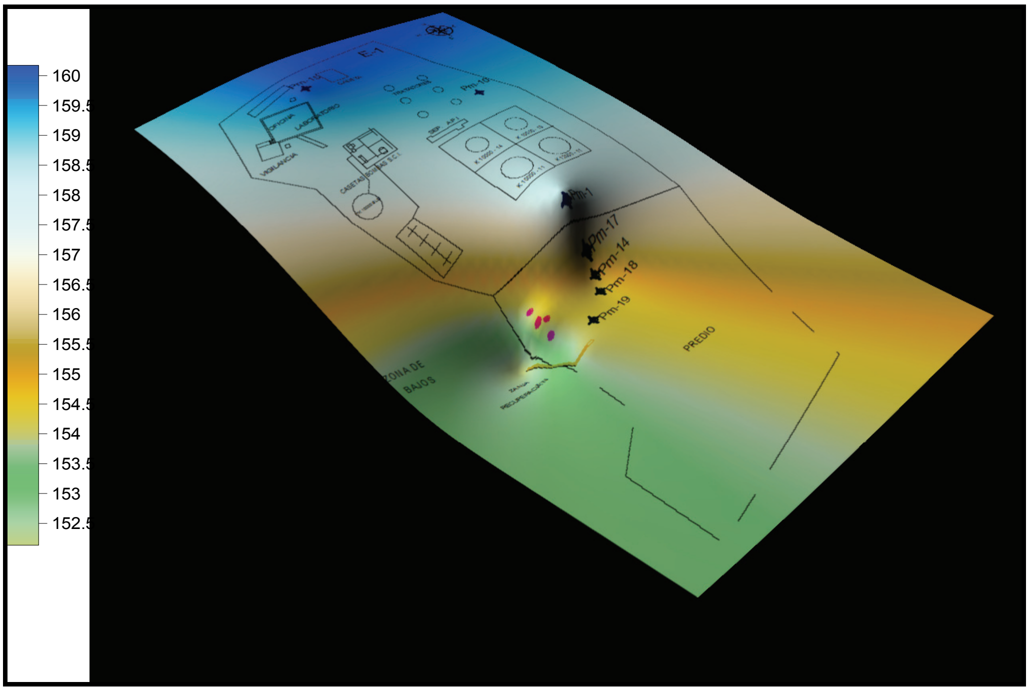


Figure 47. *Profile of level, modeling by Surfer*

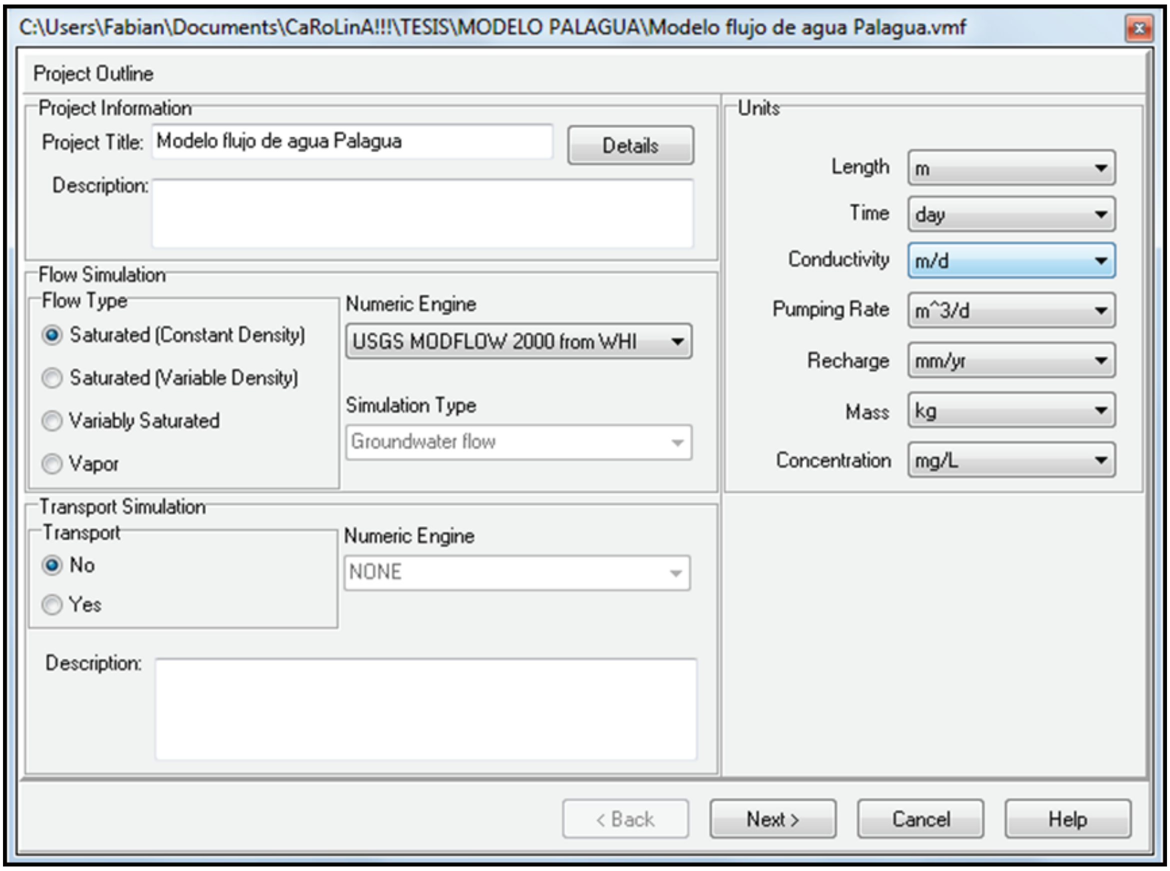
In the scale of colors, the blue coloration represents the biggest height or level (160m), diminishing up to greenish coloration which represents the lowest levels (about 152.5m).

It makes clear that the wells of monitoring 17, 14, 18 and 19 are in an area of a level lower than the PM-10 of the Station 1 and the nearby tanks of storage so, the pollutant (TPH's) concentration found in the wells 14, 17 and 19, has major concentration as the possible result of displacement of the pen.

5.7.3 Simulation in Visual Modflow for flow of underground water and extension of the pen pollutant

Initially the model needed the primary information as a project title, flow type and corresponding units. Simulation of flow was a saturated type (constant thickness) and the negative election of simulation of transport (because it is a stationary model). The window of installation of the model shows Figure 48.

Next was introduced information as: hydraulic conductivity in –x-, -y- and -z- direction (K_X=8,64m/d, K_Y=8,64m/d, K_Z=0,864) specific storage (S_s= 1E-5 1/m) specific yield (S_y= 0,20), effective porosity (Effective Porosity=0,3) and total porosity (Total Porosity=0,505).



C:\Users\Fabian\Documents\CaRoLinA!!!\TESIS\MODELO PALAGUA\Modelo flujo de agua Palagua.vmf

Project Outline

Project Information

Project Title: Modelo flujo de agua Palagua

Description:

Flow Simulation

Flow Type

● Saturated (Constant Density)
○ Saturated (Variable Density)
○ Variably Saturated
○ Vapor

Numeric Engine: USGS MODFLOW 2000 from WHI

Simulation Type: Groundwater flow

Transport Simulation

Transport

● No
○ Yes

Numeric Engine: NONE

Description:

Units

Length: m
Time: day
Conductivity: m/d
Pumping Rate: m^3/d
Recharge: mm/yr
Mass: kg
Concentration: mg/L

Figure 48. *Initial information required to start software*

5.7.4 Discretization of the model

The Figure 49 shows refinement that was realized for the zones of interest, which understand the wells of monitoring, trench of interception and apiques. The final mesh of

5×5 was applied in central zone, in order to get more detailed simulation results in areas of interest.

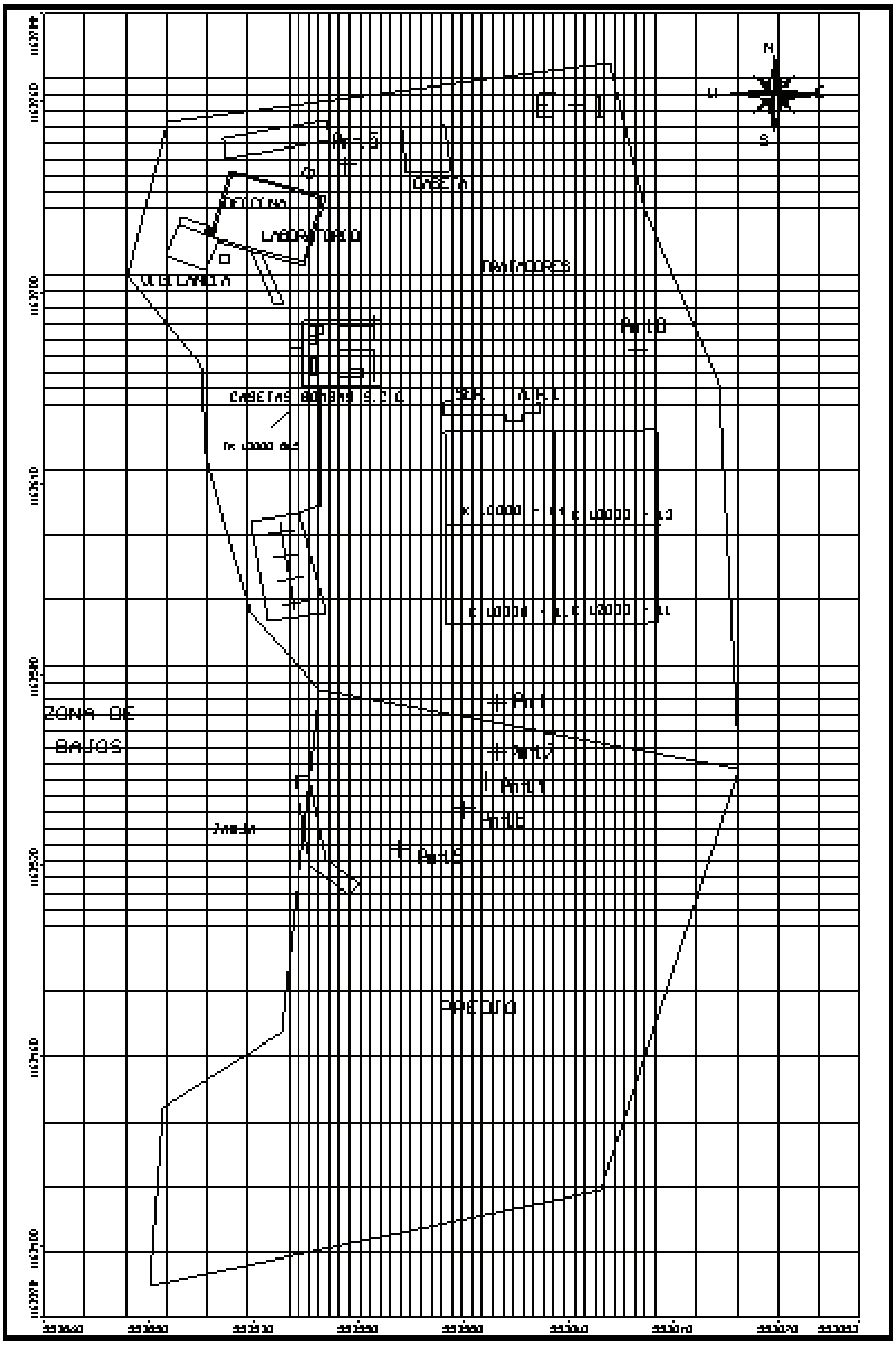


Figure 49. *Refining the Model Grid in zone of major interest*

5.7.5 The topography of the area

For the modeling program was necessary to include both surface topography and the subsoil topography where was assumed that the subsoil behaves likewise as the surface. Applied the method of interpolation (Krigging) chosen to obtain better distribution of the features, it allowed to illustrate the topography of area, emphasizing the leveling out (Figure 50) and subsoil and surface topography. (Figure 51)

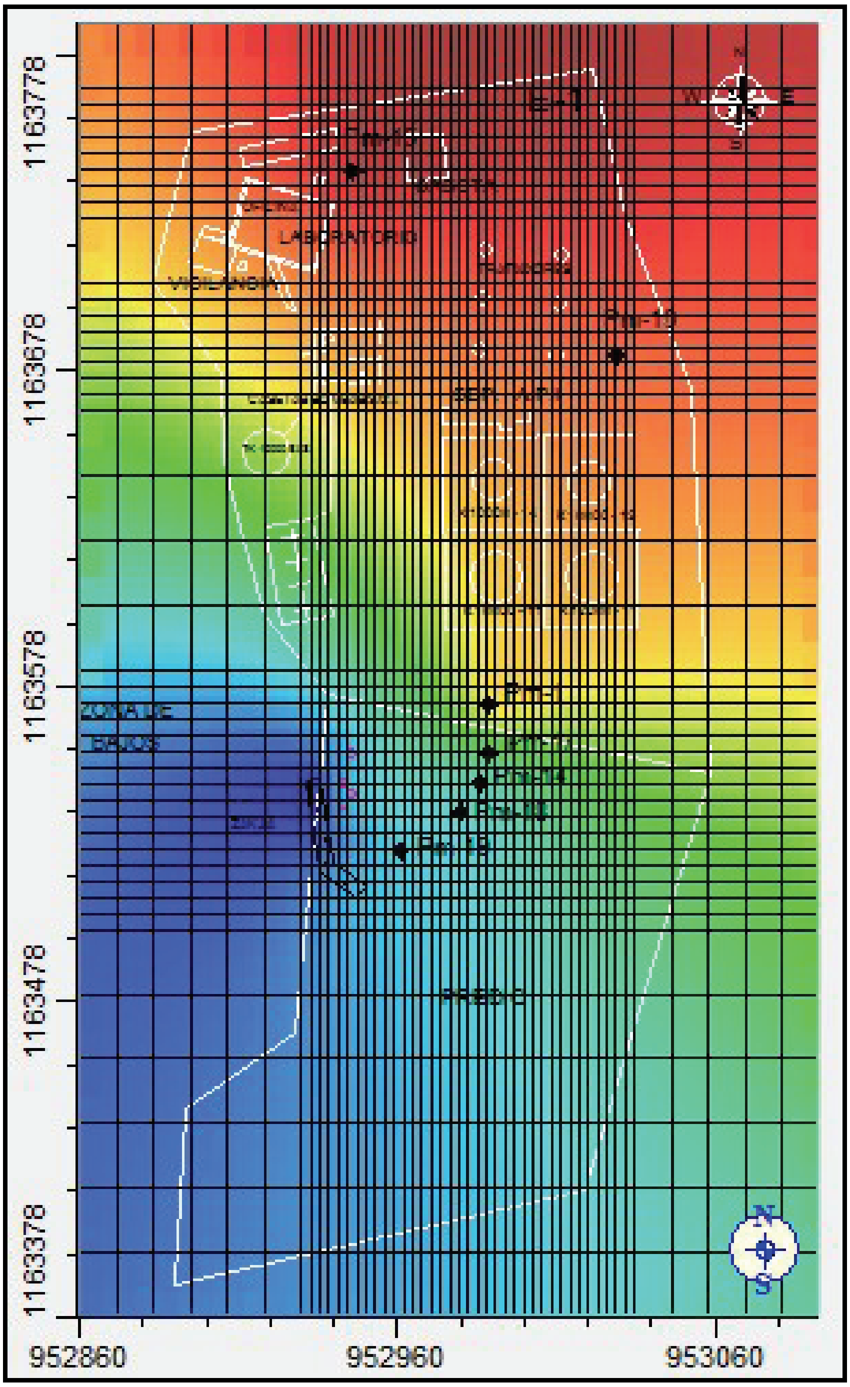
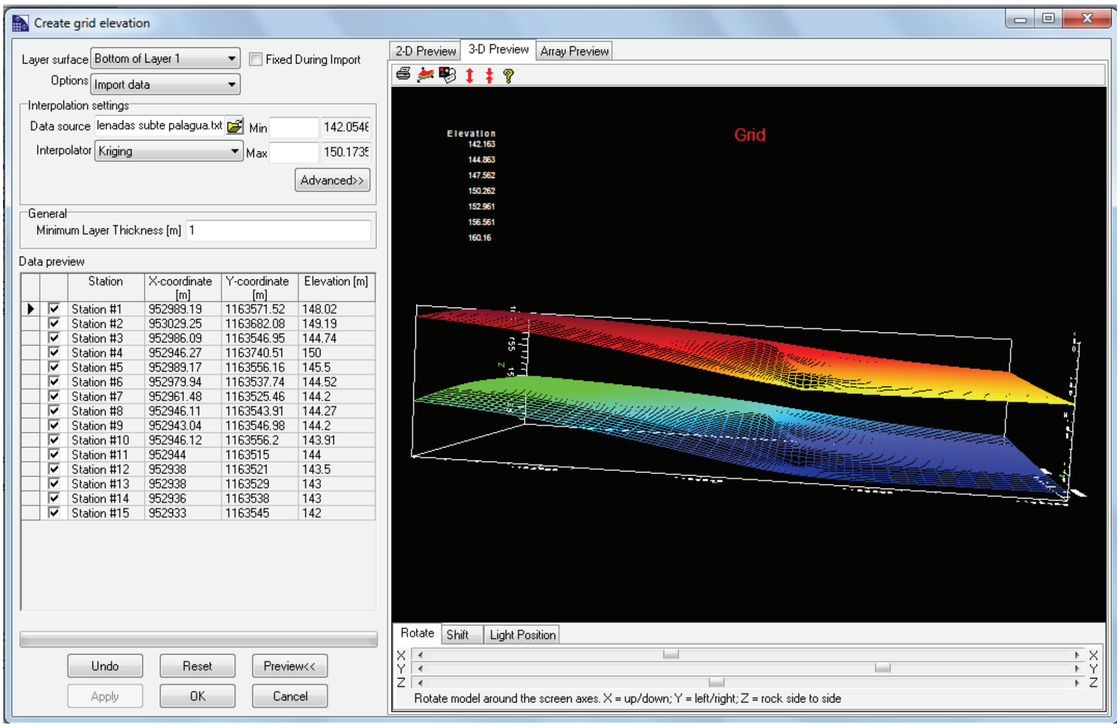


Figure 50. Surface topography in the zone of interest



Layer surface: Bottom of Layer 1

☐ Fixed During Import

Options: Import data

Interpolation settings

Data source: lenadas subte palagua.txt

Min: 142.0546

Interpolator: Kriging

Max: 150.1735

General

Minimum Layer Thickness [m]: 1

Data preview

		Station	X-coordinate [m]	Y-coordinate [m]	Elevation [m]
▶	☑	Station #1	952989.19	1163571.52	148.02
	☑	Station #2	953029.25	1163682.08	149.19
	☑	Station #3	952986.09	1163546.95	144.74
	☑	Station #4	952946.27	1163740.51	150
	☑	Station #5	952989.17	1163556.16	145.5
	☑	Station #6	952979.94	1163537.74	144.52
	☑	Station #7	952961.48	1163525.46	144.2
	☑	Station #8	952946.11	1163543.91	144.27
	☑	Station #9	952943.04	1163546.98	144.2
	☑	Station #10	952946.12	1163556.2	143.91
	☑	Station #11	952944	1163515	144
	☑	Station #12	952938	1163521	143.5
	☑	Station #13	952938	1163529	143
	☑	Station #14	952936	1163538	143
	☑	Station #15	952933	1163545	142

Rotate model around the screen axes. X = up/down; Y = left/right; Z = rock side to side

Figure 51. *Subsoil and surface topography in the zone of interest*

5.7.6 Hydraulic parameters

In the table 43 below are registered values of hydraulic parameters in each of control and monitoring wells, necessary for groundwater flow modeling in zone of interest.

Table 43. *Hydraulic conductivity of monitoring and control Wells in the zone of interest*

WELLS	**HYDRAULIC CONDUCTIVITY (m/día)**		
	Kx	**Ky**	**Kz**
PM-1	1.03E-01	1.03E-01	1.03E-02
PM-10	1.04E-01	1.04E-01	1.04E-02
PM-14	9.91E-02	9.91E-02	9.91E-03
PM-15	7.27E-02	7.27E-02	7.27E-03
PM-17	1.01E-01	1.01E-01	1.01E-02
PM-18	3.38E-02	3.38E-02	3.38E-03
PM-19	1.99E-02	1.99E-02	1.99E-03
AP-21	0.00	0.00	0.00
AP-22	0.00	0.00	0.00
AP-23	0.00	0.00	0.00
PR-1	1.77E-01	1.77E-01	1.77E-02
PR-2	2.30E-01	2.30E-01	2.30E-02
PR-3	1.31E-02	1.31E-02	1.31E-03
PR-4	0.00	0.00	0.00
PR-5	0.00	0.00	0.00

In the previous table it is possible to appreciate big values of hydraulic conductivity for PR-1 and 2. In these situ did not find hydrocarbons, so the recovery of the water in these wells was very rapid.

Otherwise in the wells PM-14, 15, 18 and 19 the present hydrocarbon in the soil braked the conductivity, which demonstrated in minor values of this one. In the apiques (AP) information is not brought because they are excavations of exploration, realized to detect the presence of hydrocarbon, that's why information of construction is not had as depth of design and information of monitoring as static levels.

On the other hand, it was not obtained information for the recovery wells 4 and 5, because during monitoring it was found hydrocarbons into these wells, which made difficult the procedure of slug test.

In general the conductivity is increasing in direction south in adjoining ground. It is possible to appreciate that the high hydraulic conductivity of the soil appears in the closeness of the interception trench, constructed by the company for the recovery of free product, spreading widely towards the southeast and south-west, which worries for the possible displacement of the remaining pollutant. (Figure 52)

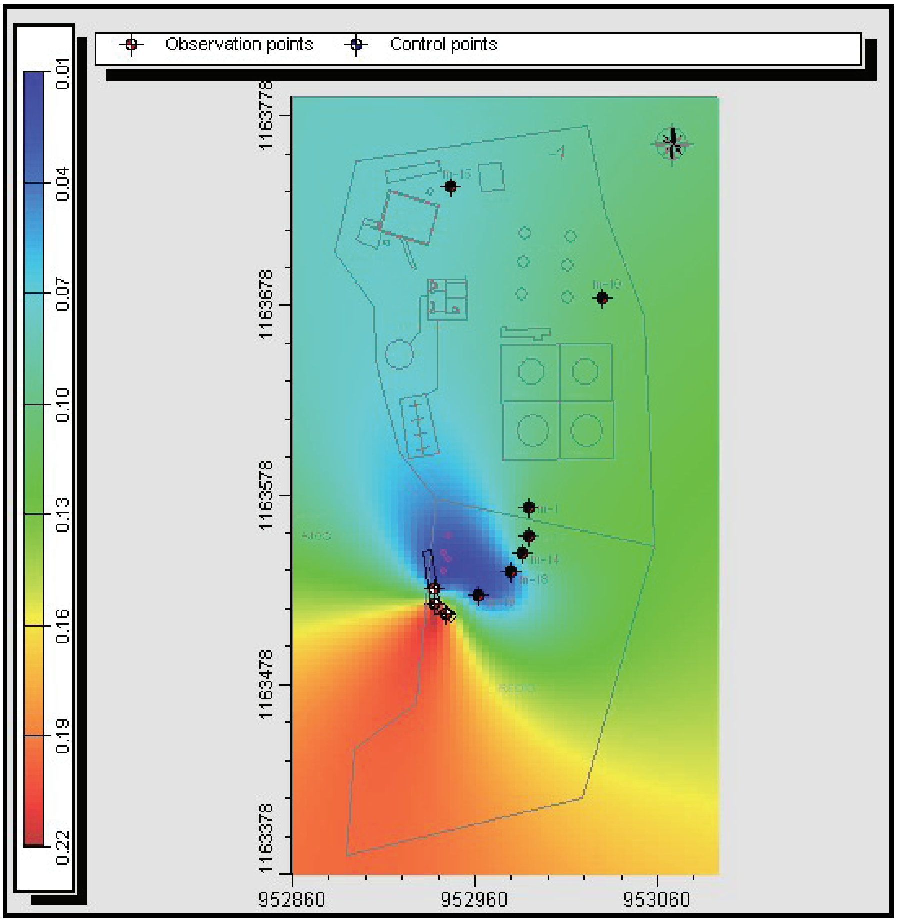


Figure 52. *Change of hydraulic conductivity in zone of interest*

5.7.7 Limits of flow and modeling

To initiate model of groundwater flow in zone of interest was necessary have values of initial head and limits of flow.

In the figure 53 can be see two limits (shading area) assigned to the model. In the right end, there stipulated a value of constant head of 154.5m to 156m in direction North-East of the bordering property and to the south of the property, in direction of the Marsh Palagua of 152m to 154m. As the initial conditions, these values took of the piezometric levels calculated from the level of area and the static levels measured in field.

The results of the modeling indicate a predominant direction Northwest - south-west (NW to SW) and a maximum speed of flow of groundwater 0.37 m/d. In the Figure 54, the arrows indicate the direction of the flow and with this possible displacement of the pen pollutant.
The direction of displacement of the underground water together with the pen is towards the Marsh Palagua, which was confirmed in the field by the presence of hydrocarbons in this zone. The calibration of model lives average error -0,007m and absolute error 0,346m.

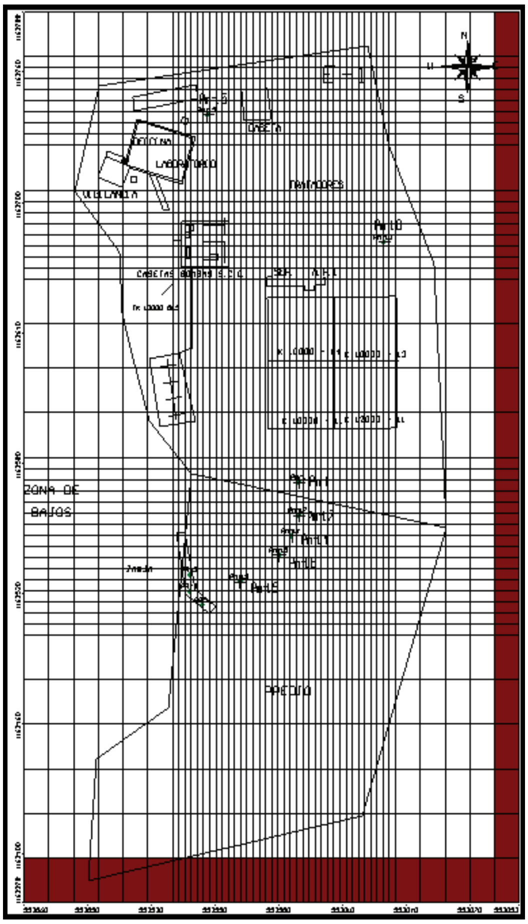


__Figure 53.__ Limits of flow assigned to the model Modflow in zone of interest

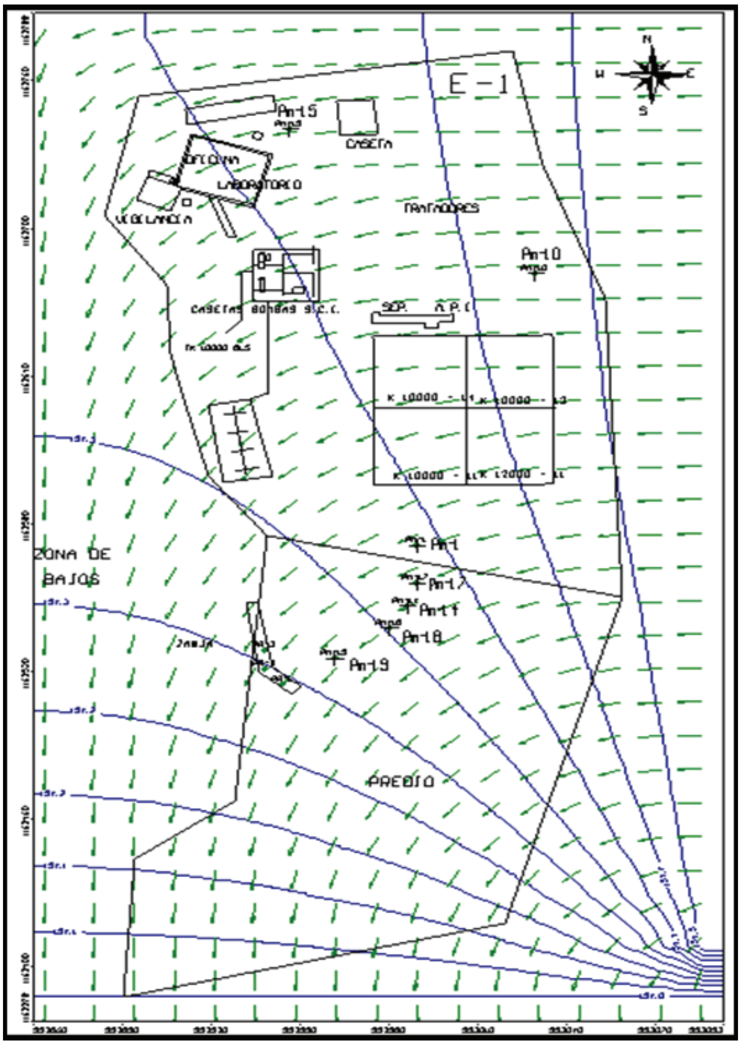


__Figure 54.__ Result of the modeling of the groundwater flow in zone of interest

5.7.8 Application of PATHLINES and MODPAHT for evaluation of the trajectory of particles

The figure 55 shows the projection of trajectory, that the particles of contaminant would take, in one, two and three years (1, 2, 3, years), considering that these move in the same groundwater direction which was modeling and the same speed of flow.

It can be see that for a one year that the displacement of the particles is significant and almost reach the trench of interception constructed as measurement of recovery of free product.

On the other hand, the trajectory projected for the particles proceeding from the PM-19 suggests a route next to the trench in such a way that this pollutant does not manage to be retained. From the second year the pollutant's particle from PM-19 manage to pass the retention trench. The particles from the others wells presented very extensive trajectory in direction Northwest - south-west (NW to SW). These projections, leave worry on the fast contamination extension, and suggest the achievement of a rapid treatment.

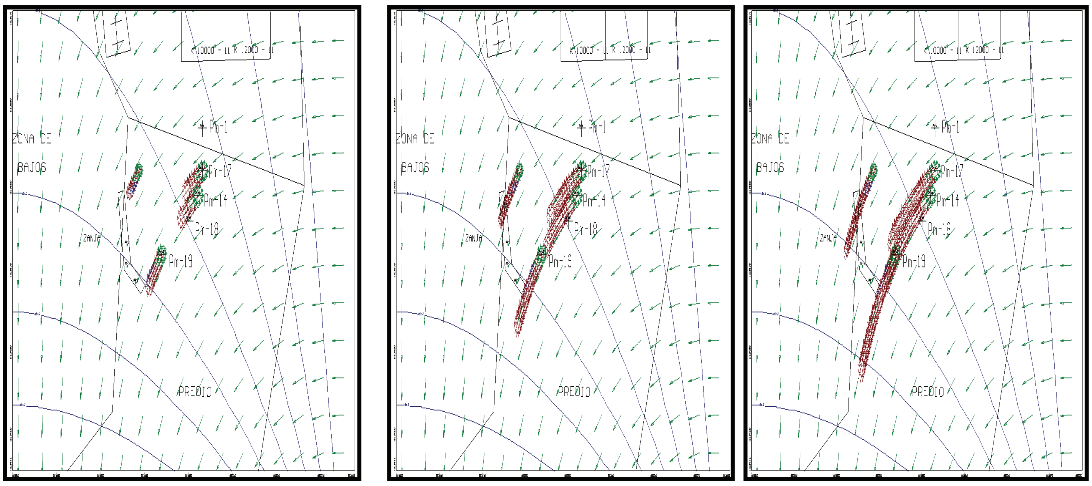


Figure 55. *Projection of particle trajectory in one, two and three years respectively*

5.8 SELECTION OF THE BEST TREATMENT OR RECOMBINATION OF THEM, AND APPLICATION ON ZONE OF INTEREST

Remediation of contaminated zone in Field Palagua treatment was selected by Ecopetrol, which was flushing with surfactant solution during two weeks and later biostimulation process. The flushing process with the Emulgrosson solution in concentration 5%, was applied direct into monitoring wells, during 2 weeks and simultaneously was pumped the ground water for external treatment. (figure 55)   After that, has started biostimulation process during few months, managed in the same way (solution dosage into the monitoring wells).Finally the same treatment was applied for contaminated soil.

Figure 56. *Selected treatment application, for remediation of contaminated zone*

The results, after two weeks of flushing and one month of biostimulation are reported in table 44.

Table 44. *Elimination of hydrocarbons from de soil by selected remediation in Field Palagua*

Total % degradation TPH	Total % degradation Oil and grease	Total % degradation PAH
56,3	44,8	49,2

After two weeks of flushing and one month of biostimulation process, the results of field application show significant progress in hydrocarbon degradation process. Taking into account climatic condition in the zone, where average of temperature is 30°C and relative humidity 78% with average of monthly precipitation 172,5 mm, the conditions for biodegradation process are excellent .

Hydrocarbon spill since 1997 has made more difficult recuperation process due to stabilization of hydrocarbons into the soil, and extension of pollutant on the area.

6 CONCLUSIONS

Presented doctoral thesis was separated into three parts. In the first one Physicist-chemical and microbiological characteristics of soil and groundwater contaminated with hydrocarbons was analyzed as well as topographic description of the zone of interest.

In the second part was carried out the assessment of bioremediation, where is used the physiological potentials of indigenous hydrocarbon-degrading microorganisms and recombination of pretreatment as: flushing with synthetic surfactant comparing with chemical pretreatment with hydrogen peroxide and later bioremediation process.

The bioremediation of soil contaminated with hydrocarbons was analyzed by natural attenuation, biostimulation and bioaugmentation in each experiment.

Finally was investigated behavior of groundwater flow, and the trajectory of the pollutant pen in an area of Ecopetrol S.A., using the Visual Software Modflow, for the recovery of groundwater contaminated with hydrocarbons and applied , the selected alternative for remediation of soil and underground water contaminated with hydrocarbons in the zone of interest.

6.1 PHYSICIST-CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF SOIL AND GROUNDWATER CONTAMINATED WITH HYDROCARBONS

- Physicist-chemical analysis of the soils of the Station 1 and bordering property indicated similar characteristic of both, classifying them like sandy, which coincides with the high permeability and rapid infiltration, together with low relative thickness and low retention of moisture. Also both present a capacity of cationic exchange and granular structure.
- The underground water analyzed in the points of monitoring of the Station 1 of Field Palagua and bordering property contains phenols and metals as the Barium, Lead, Arsenic, hexavalent Chromium and Cadmium that they overcome the limit allowed by the Colombian current environment policy in several points of sampling.
- Big difference in values of electrical conductivity, dissolved solids and present chlorides in underground waters of the wells PM-10 (Station 1) and PM-18 and 19 (bordering Property) is the result of the displacement of the water towards the low parts of the area and the cattle activity that is established in this zone.

6.2 ASSESMENT OF BIOREMEDIATION PROCESSES AS NATURAL ATENUATION, BIOSTIMULATION AND BIOAUGMENTATION, OF SOIL CONTAMINATED WITH HYDROCARBONS

- Biodegradation of TPH's by natural attenuation after forth month reached in average 55.4% meanwhile that the bioremediation of oil and grease achieve 31,4% and PAHs 10,32%in the same time. The grade of biodegradation by natural attenuation is related with molecular weight of hydrocarbons where higher molecular weight, less efficiently process.

- The biodegradation process by natural attenuation proceeds in an organized way, using different kind of compounds by specific microorganisms, which changing microbial population.
- Biostimulation process has had percentage of biodegradation of TPH, oil and grease as well as TPHs with average values 74,6; 40,2 and 17,4% respectively. The effect of nutrient and the oxygen sufficiency in the soil is positive for degradation of the hydrocarbons and growth of microorganisms.
- Bioaugmentation process represent better results for biodegradation of TPH in 83,7% in average, as well as for biodegradation of oil and grease (58,2%) and PAHs(31,7%). With the increase of a specific microbial community, air and nutrient addition can be supported biodegradation process, especially for recalcitrant hydrocarbons, makes it more efficient.

6.3 ASSESMENT OF RECOMBINATION OF TREATMENTS

Pre- treatment with surfactant and later bioremediation

- Flushing with surfactant eliminated 67% of TPH and 52,1% oil and grease. The low concentration of remaining hydrocarbon as the carbon source, bears consequently poor performance in natural attenuation (36,3%) as the later process
- After flushing with surfactant the best performance (62.2%) presented biostimulation process, where microorganisms supported with air and nutrients can grow and release of hydrocarbons linked into the soil.
- Bioaugmentation process applied after flushing with surfactant is better solution for bioremediation as natural attenuation but the efficient (41,8%) is lower than biostimulation due competition of greater population of microorganisms for carbon source.
- Combination of treatments (flushing surfactant and later biodegradation) have better performance than biodegradation process without pre-treatment, where flushing with surfactant and later biostimulation represented total elimination of 86,7% TPH, 90,5% oil and grease and 76,2% of PAHs.

Hydrogen peroxide solution applied as pretreatment and later bioremediation

- Biodegradation process after pre-treatment with hydrogen peroxide solution, presented similar behavior as the flushing with surfactant, where it can be see lower biodegradation, compared with the same process without pre-treatment..
- The biostimulation process after pre-treatment with hydrogen peroxide solution presented better results in elimination of TPH (60,3%), oil and grease (52,2%)and PAHs (85,5%).
- The grow of microorganism before pre-treatment with hydrogen peroxide solution presented increase comportment but decrease concentration of hydrocarbons could be

related with decline of microbial population , hence bioavailability of carbon source is very important issue in biodegradation process.

- Combination of two treatments is more convenient for elimination of hydrocarbons than bioremediation solo. The flushing with surfactant and washing with hydrogen peroxide solution as the pre-treatments, presented similar efficiency for elimination of TPH, oil and grease but elimination of PAHs it is better to handle with peroxide pre-treatment, where can be achieved more than 80% efficiency.

6.4 VISUAL SOFTWARE MODFLOW APPLICATION AND APPLICATION SELECTED TREATMENT IN THE ZONE OF INTEREST

- The results of the modeling indicate a predominant direction Northwest - south-west (NW to SW) and a maximum speed of flow 0.37 m/d of groundwater.
- The direction of displacement of the underground water together with the pen is towards the Marsh Palagua, which was confirmed in the field by the presence of hydrocarbons in this zone. The calibration of model lives average error -0,007m and absolute error 0,346m.
- The projection of trajectory of particle of contaminant in one, two and three years, projected route next to the trench, in such a way that this pollutant does not manage to be retained if a treatment is not realized for his elimination .
- The soil environments are comparatively more harsh than laboratory condition that's why was achieved lower efficiency in elimination of TPH (56,3%) oil and grease(44,8%) and PAHs (49,2%) after one month of biostimulation and two weeks flushing with surfactant as pre-treatment.

REFERENCES

[1] Wanielista,M., Kersten,R., Eaglin,R., Hydrology-Water Quantity and Quality Control 1997 pp.4-6.

[2] Morris, B.L., Lawrence, A.R.L., Chilton, P.J.C., Adams, B., Calowand, R.C., Klinck, B.A., Groundwater and its susceptibility to degradation: a global assessment of the problem and options for management. Early Warning and Assessment Report Series, RS. 03-3. United Nations Environment Programme, Nairobi, Kenya. *Science* 2003

[3] National Groundwater Association (NGWA),2005. The Groundwater Supply and its Usde,< http:// www.wellownenorg/agroundwater/gwsupplyanduse.shtml>. (access February 2011)

[4] Moore,W., 1989. Balancing the Needs of water use. Springer-Verlag,New York

[5] Hansen B.R.,Davies, S.H,Reviev of potential technologies for the removal of dissolved compounds from produced water. *Chem.Eng.Res.Des.*72(1994) 176-188.

[6] Bronstein AC, Spyker DA, Cantilena LR Jr, Green J, Rumack BH, Heard SE. 2006 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS). *Clin Toxicol (Phila)*. Dec 2007;45(8):815-917.

[7] Seymour FK, Henry JA. Assessment and management of acute poisoning by petroleum products. *Hum Exp Toxicol*. Nov 2001;20(11):551-62.

[8] Lifshitz M, Sofer S, Gorodischer R. Hydrocarbon poisoning in children: a 5-year retrospective study. *Wilderness Environ Med*. 2003;14(2):78-82.

[9] Gross P, McNerney JM, Babyak MA. Kerosene pneumonitis: an experimental study with small doses. *Am Rev Respir Dis*. Nov 1963;88:656-63.

[10] Rodricks A, Satyanarayana M, D'Souza GA, Ramachandran P. Turpentine-induced chemical pneumonitis with broncho-pleural fistula. *J Assoc Physicians India*. Jul 2003;51:729-30.

[11] Borne J, Riascos R, Cuellar H, Vargas D, Rojas R. Neuroimaging in drug and substance abuse part II: opioids and solvents. *Top Magn Reson Imaging*. Jun 2005;16(3):239-45.

[12] Filley CM, Halliday W, Kleinschmidt-DeMasters BK. The effects of toluene on the central nervous system. *J Neuropathol Exp Neurol*. Jan 2004;63(1):1-12.

[13] Garrettson LK. n-Hexane and 2-Hexanone. In: Sullivan JB, Krieger GR, eds. *Clinical Environmental Health and Toxic Exposure*. 2nd ed. Philadelphia, Pa: Lippincott Williams and Wilkins; 2001:1211-14; chap110.

[14] Klein BL, Simon JE. Hydrocarbon poisonings. *Pediatr Clin North Am*. Apr 1986; 33(2):411-9.

[15] Algren JT, Rodgers GC Jr. Intravascular hemolysis associated with hydrocarbon poisoning. *Pediatr Emerg Care*. Feb 1992;8(1):34-5.

[16] Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Heard SE. 2007 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 25th Annual Report. *Clin Toxicol (Phila)*. Dec 2008;46(10):927-1057.

[17] Tam E. K.L., Byer P.H., Remediation of Contaminated lands. A decision methodology for site owners. *J.of Environmental Management April 2002.64 (4): 387-400.*

[18] Environmental Solution, Inc.,Irvine, California, prepared for Western States Petroleum Association, March 1990

[19] USEPA: Technology Screening Guide for Treatment of CERCLA Soils and Sludges EPA/540/2-88/004 (September 1988)

[20] USEPA Alternative treatment technology information center (ATTC) Research and Development (RD-681) EPA/600/M-91/049 (November 1991)

[21] Angell,K. Selection Methodology for Environmental Remediation Planning. Presented at Mid Atlantic Environmental Exposition, Baltimore, MD (April 9-11,1990).

[22] Rao,P.S.C.,DavisG.B., and Johnston,C.D.1996.Tecnologgies for enhanced remediation of contaminated soils and aquifers: overviews, analysis and case studies. *Contaminants and the soil environment in Australasia-Pacific Region,Kluwer Academic Publ., Dordrecht, Chapt.12, 361-410.*

[23] Gingrich T., Groundwater remediation: the evolution of technology. *Pollu*tion Engineering, August, 2007:39(8):24-28

[24] WESTON, IIT Research Institute, November 1992. *Final Rocky Mountain Arsenal in Situ Radio Frequency Heating/Vapor Extraction Pilot Test Report, Vol. I*, U.S. Army Report 5300-01-12-AFP.

[25] Guía para el Ciudadano sobre Métodos de Tratamiento Térmico in Situ. EPA 542-F-01-012S, 2001 http:// clu-in.org/download/citizens/thermal_sp.pdf (Access February 2011)

[26] Lee J.K.,Park D.,Kim B.,Dong J. Lee S., Remediation of petroleum-contaminated soils by fluidized thermal desorption. Waste Management Oct.1998; 18 (6-8):503-507.

[27] LightyJ.S.,Silcox GD,PershingDW. Investigation of rate processes in the thermal treatment of contaminated soils.GRI-90/0112. Gas Research Institute.

[28] Buettner,H.M., Daily,W.D., Cleaning contaminated soil using electrical heating and air stripping *.J.Envirin.Ing.* **121**, 580-589

[29] FRTR Remediation Technologies Screening Matrix and Reference Guide, Version 4,0 on line. (access February 2011)

[30] Windgasse,G. and Dauerman,L.1992 Microwave treatment of hazardous wastes: removal of volatile an semi-volatile contaminants from soil. J. Microwave Power Electromag. Energy **27,** 23-32

[31] Dawei Li,Xie Quan,Yaobin Zhang, Yazhi Zhao., Microwave-Induced thermal treatment of petroleum- hydrocarbon contaminated soil. *Soil & Sediment Contamination.* 2008:**17** 486-496.

[32] Beieski,R. J., Jarosch T. R.,and. Faust,D. 1993 FinalReport: In Situ Radio Frequency Heating Demonstration, Volume I,93-9TDO-RFHET-R3

[33] Iben,I.E.T., et al. (1996) Thermal blanket for in-situ remediation of surficial contamination – a pilot test.*Envir.Sci and Tecnology.,*30(11), 3144-3154.

[34] Lighty,JS., Silcox,G.D., Pershing,D.W., Cundy,V.A., and Linz,D.G.,(1990) Fundamentals for the thermal remediation of contaminated soils- particle and bed desorption models. *Envir. Sci. and Technol.*, 24(5), 750-757

[35] Gilot,P.,Howard,J.B.,and Peters,W.W. (1997) Evaporation Phenomena during thermal decontamination of soils. *Envir.Sci and Technology,* 31(12), 461-466.

[36] Castanier,L.M., and Gadelle,C., 1991. Hit injection Basic concepts in enhanced oil recovery process. Elsevier Applied Science. Cri.Rep.Appl.Chem., 33:271-312.

[37] Newmark, R.L.,and Aines R.D., Summary of the LLNL gasoline spill demonstration-Dynamic Underground Stripping Project, Lawrence Livermore National Laboratory, Berkeley Environmental Restoration Center,UCRL-ID-120416,April2,1995.

[38] Lighty,J.S. Persing,V.A., Cundy, and LinzD.G., Characterization of thermal desorption phenomena for the cleanup of contaminated soil, Nucl. Chem. Waste Manage, 8:225-237, 1988.

[39] Boberg,T.C., Thermal Methods of oil Recovery, John Wiley & Sons, New York,1988.

[40] Willman,B.T., Valleroy,V.V., Runberg,G.W.,Cornelius,A.J., and Pwers,L.W., 1961 Laboratory studies f oil recovery by steam injection. J.Pet.Technol.,Trans.AIME (Am.Inst.Min.Metall.),222:681-690.

[41] Environmental Protection Agency, In situ steam enhanced recovery process Huges Environmental Systems, Inc. Innovate Technology Evaluation Report , EPA/540/R-94/510 National Risk Management Research Laboratory, Cincinnati,OH, July 1995b.

[42] Jabbour, C., Quintard, M., Bertin, H., and M. Robin (1996).”Oil Recovery by Steam Injection:Three-phase Flow Effects” J. of Pet. Science and Engineering, 1999 (16) pp.109- 130

[43] Harmsen,G.J., Oil recovery by hot water and steam injection, Eighth World Petroleum Congress,3:243-251,1971.

[44] Yamazaki Z., Hashimoto H., First International EPRI Plasma Symposium, California, USA, 1990.

[45] Shente,R.N., Industrial applications of thermal plasmas, AAIP Conf. Proceedings,n.345 p.487-494.,1995.

[46] Beckett, G.D. and D. Huntley. 1994. Characterization of Flow Parameters Controlling Soil Vapor Extraction. Groundwater. Vol. 32, No. 2, pp. 239-247.

[47] BHI-00720, 2002, Performance Evaluation Report for Soil Vapor Extraction Operations at the Carbon Tetrachloride Site, February 1992 - September 2001, Rev. 6, Bechtel Hanford,

[48] Downey, D.C., Frishmuth,R.A., Archabal, S.R., Pluhar,C.J., Blystone,P.G., Miller,R.N.,1995. Using in situ Bioventing to minimize soil vapor extraction costs. In: Hinchee,R.E., Miller,R.N., Johnson,P.C. (Eds), In Situ Aeration: Air sparging, Bioventing and related Remediation Processes, vol. 3. Battelle Press, pp.247-276.

[49] Lu, C., Chu, W., Lin, M., 2000.Removal of BTEX vapor from waste gases by a trickle bed biofilter. J. Air Waste Manage. Assoc.50, 411-417.

[50] Malina, G., Grotenhuis,J.T.C.,Rulkens,W.H.,1997. Effective use of air during bioventing of model hydrocarbons. In: Alleman, B.C., Leeson,A. (Eds.) In situ and On-site bioremediation, vol.4.Battelle Press, Columbus, OH, pp 367-372.

[51] Kim J., Choi, H. Modelling in situ ozonation for the remediation of non-volatile PAH-contaminated unsaturated soils, *J. Contam. Hydrol.* **55** (2002), pp. 261–285.

[52] Riser-Roberts E., Remediation of petroleum contaminated soils. Lewis Publishers, 1998, pp10-11.

[53] Technical/Regulatory Guidelines, 2001.Technical and Regulatory Guidance for in situ Chemical Oxidation of Contaminated Soil and Groundwater. Prepared by Interstate Technology and Regulatory Cooperation Work Group In Situ Chemical Oxidation Work Team

[54] Choi H., Y.-Y. Kim, H. Lim, J. Cho, J.-W. Kang and K.-S. Kim, Oxidation of polycyclic aromatic hydrocarbons by ozone in the presence of sand. *Water Sci. Technol.* **43** (2001) (5), pp. 349–356.

[55] Hsu M.I., Masten, S.J., The kinetics of the reaction of ozone with phenanthrene in unsaturated soils. *Environ. Eng. Sci.* **14** (1997), pp. 207–218.

[56] Bailey PS. The reactions of ozone with organic compounds. Chem. Rev. 1958; 58: 925–1010.

[57] Kyoungphile N., Kukor J.,J., Combined ozonation and biodegradation for remediation of mixtures of polycyclic aromatic hydrocarbons in soil. Bioremediation **11** (2000), pp. 1-9.

[58] O'Mahony M., M., Dobson, A., D., W., The use of ozone in the remediation of polycyclic aromatic hydrocarbon contaminated soil. Chemosphere 63 (2006) 307–314

[59] U.S. EPA Handbook for Remedial Action at Waste Disposal Sites. (1985a.) Revised. EPA/625/6-85/006.

[60] Sung-Ho Kong, Choong-Il Kwon Moo-Hoon Kim, Ozone Kinetics and Diesel Decomposition by Ozonation in Groundwater. *Korean J. Chem. Eng*., **20**(2), (2003) pp.293-299

[61] Holm,,T.R., et al, (1987) Fluorometric determination of hydrogen peroxide in groundwater.Anal.Chem,59,pp582-586

[62] Haber, F. and Weiss,J.1934.Proc.R.Soc.Ser.A. 147:332

[63] Ho.C.L.,Shebl, M.A.-A., and Watts,R.J.1995. Development of an injection system for in situ catalyzed peroxide remediation of contaminated soil. Haz.Wasre Haz. Mater. 12(1):15-25

[64] HERA., Human & Environmental Risk Assessment of Ingredients of European Household Cleaning Products: Phosphonates (2004)

[65] ThermoRetec Consulting Corporation, In Situ Oxidation, Technology Status Review, performed by ThermoRetec Consulting Corporation with assistance from HydroGeoLogic, Inc. and Coleman Research Corporation – Energy &Environmental Group, Environmental Security Certification Program, USA, November 1999,42p.

[66] "Gold-palladium Nanoparticles Achieve Greener, Smarter Production Of Hydrogen Peroxide". Sciencedaily.com. 2009-03-03.

http://www.sciencedaily.com/releases/2009/02/090219141507.htm. (access May2011).

[67] Jennifer K. Edwards, Benjamin Solsona, Edwin Ntainjua N, Albert F. Carley (Feb 2009). Switching off hydrogen peroxide hydrogenation in the direct synthesis process. *Science* **323** (5917): 1037–41. doi:10.1126/science.1168980

[68] Hydrogen Peroxide 07/08-03 Report, ChemSystems, May 2009. (Access May 2011).

[69] Technical and Regulatory Guidance for in Situ Chemical Oxidation of Contaminated Soil and Groundwater, Technical/Regulatory Guidelines, prepared by Interstate Technology and Regulatory Cooperation Work Group, In Situ Chemical Oxidation Work Team, ed. 2005,25p.

[70] Go,i A.,Kulik, N.,Trapido,M., 2006,Combinmed chemical and biological treatment of oil contaminated soil. Chemosphere 63 1754-1763

[71] Brown, R.A. Mahaffley, W., and Norris,R.D.1993.In situ bioremediation: The state of the practice. In situ remediation When Does it Work? National Research Council. Water Science and Technology Board, Com. Eng. Tech. System. National Academy of Science. National Academy Press, Washington, D.C. p121-135.

[72] Middleton,A.C., Hiller,D.H.,1990.In situ aeration of groundwater- a technology overview.In a Conference on Prevention and treatment of Soil and Groundwater Contamination in the Petroleum Refining and Distribution Industry,13,0-13.14.Montreal, Quebec.Alnerta, Canada

[73] USEPA,1992. A citizen’s guide to air sparging. EPA/542/F-92/010; U.S.Environ.Prot.Agency, Off.Solid Waste Emergency Response: Washington D. C.

[74] Acomb,L.J.,McKay,P.Currier,S.T.Berglund.T.V.Sherhart,Benediktsson,C.V., 1995.In situ Aireation:Air sparging,Bioventing,and Related Remediation Processes, ed. R.E.Hinchee, R.N.Miller and P.C. Johnson, 47-61.Columbus, Ohio: Battelle Press.

[75] Brown,R.A., Jasiulewicz F., Air sparging: A new model for remediation. Pollut.Eng. 1992,(jul)52-55

[76] Marley,M.C.,Hazebrouck,D.J., WalshM.T., The Application In Situ Air Sparging as an Innovative Soil and GroundWater Remediation Technology,]Ground Water Monitoring Review, National Ground Water Association, (1992) Vol.XII N° 2, p 137

[77] McCray,J.E.,Falta,R.W., 1997. Numerical simulation of air sparging for remediation of NAPL contamination:J.Ground Water, 35:99-110.

[78] Bass,D., Brown,R., 1996.Air sparging case database update. In Proceedings of the First International Symposium on In Situ Air Sparging for Site Remediation. Las Vegas, Nevada: International Network for Environmental Training Inc.

[79] Wade, A., G.W. Wallace, S.F. Siegwald, W.A. Lee, and K.C.McKinney. 1996. Performance Comparison Between a Horizontal and a Vertical Air Sparging Well: A Full-Scale One-Year Pilot Study. Proc. National Outdoor Action Conference Expo., 10th. pp. 189-206.

[80] Xiaomin,Y.,Beckmann, D.,Florenza,S., Niedermeier,C., Field Study of Pulsed Air Sparging for Remediation of Petroleum Hydrocarbon Contaminated Soil and Groundwater S*ci.* Environ. S Technol*.,* 2005**,** 39 *(18), pp 7279–7286*

[81] Johnson,P.C.,Das,A.,Bruce,C., Efect of flow rate changes and pulsing on the treatment of sources zones by in situ air sparging. Environ. Sci. Technol. 199, 33(10), 1726-1731.

[82] Reddy, K.R., and Adams, J.A., "Effect of Pulsed Air Injection During In-Situ Air Sparging for Groundwater Remediation," Proceedings of the ASCE Conference pp.68-82.

[83] Brown,R.A.,Fraxedas,R.1991.Air sparging-Extending volatilization to contaminated aquifers. In proceedings of the First International Symposium on Soil Venting. EPA/600/R-92/174. P249-269.

[84] Ji,W., Dahmani A., Ahlfeld,D.P.,Lin,J.D.,Hill E.1993 Laboratory study of air sparging:Air flow visualization. Ground qater Monitoring and Remediation 13,no.4: p.115-126

[85] Leeson, A., Hinchee R.E, Headington G.L.,Vogel C.M.,1995. Air channel distribution during air sparging: A field experiment. In situ Aeration: Air Sparging, Bioventing and related Remediation Processes, 214-222.In Proceedings of the Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection and Restoration. Dublin, Ohio: National Ground Water Association.191-206

[86] Lundegard,P.D.,Andersen,G.,1993. Numerical simulation of air sparging performance. In Proceeding of the Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water:Prevention,Detection and Restoration, Dublin, Ohio281-296.

[87] Peterson,J.W.,Murray,K.S.,TuluI.Y.,PeulerD.A.,2001.Air-Flow Geometry in air sparging of Fine-grained sands. Hydrology Journal ,9:168-176.

[88] R.N. Miller, D.C. Downey, V.A. Carmen, R.E. Hinchee and A. Leeson, Petroleum Hydrocarbons and Organic Chemicals in Groundwater: Prevention, Detection, and Restoration**,** Houston, Texas, National Water Well Association, Dublin, OH, 1993, pp. 397-411.

[89] Mohr,D., 1995. Mass Transfer Concepts Applied to In Situ Air Spargigng., presented at the Third International Symposium on On Site and In Situ Bioreclamation.

[90] Leeson, A., Johnson, P.C. Johnson, R.L., Vogel, C. M.,. Hinchee, R.E., Marley M., Peargin,T., Bruce,C.L., Amerson,I.L., Coonfare,Ch.t., Gillespie,R.D., and McWhorter D.B., 2002. Air Sparging Design Paradigm. Sponsored by the U.S. Air Force Research Laboratory, Airbase and Environmental Technology Division, Tyndall Air Force Base, Florida

[91] Aivalioti,M.,Gidarakos,E.,Air Sparging, Demostration in contaminates petroleum refinery site, Interanational Conference on Bioremediation ofd Soil and Groundwater, Krakow,Poland,2004.

[92] Wilson,J.L.,Conrad,S.H.,1984.Is physical displacement of residual Hydrocarbons a realistic possibility in aquifer restoration? In Proceedings of the NWWA/API Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water – Prevention Detection and Restoration. Dublin, Ohio. p274-298.

[93] Ardito,C.P.,Billings,J.F 1990.Alternative Remediation strategies: The subsurface volatilization and ventilation system. In Proceedings of the Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water:Prevention,Detection and Restoration, Dublin,Ohio.P 281-296.

[94] Kittel,J.A.,Hoeppel,R.E.,Hinchee,R.E.,Zwick,T.C., and Watts,R.J., 1994 In situ Remediation of Low-Volatility Fuels Using Bioventing Technology.Hydrocarbon Contaminates Soils, Vol.IV,ASP,Amherst, Massachusetts.

[95] Keet,B.A.,Hinchee,R.E.,Kittel,J.A.,1995.Bioslurping state of the art.In Aplied Bioremediatioin of Petroleum Hydrocarbons.pp329-334

[96] Department of the Army US Army Corps of Engineers, 1999 Engineering and design Multi- phasae Extraction http://140.194.76.129/publications/eng-manuals/em1110-1-4010/S2.pdf (access September 2011).

[97] Safrin,T., 1996, Dual-phase Treatment Reduces Cleanup Time and Cost, Environmental Technology, Vol.6, N°3, May/June, pp60-62.

[98] Khan,F.I., Husain,T.,Hejazi,R.,„ An overview and analysis of site remediation technologies,J.Environ.Manage.71(2004) 95-122.

[99] Air Force Center for Environmental Excellence Technology Transfer Division Bioslurper Initiative, Test Plan and Technical Protocol for Bioslurping, 1995

[100] Gidarakos,E.,Aivalioti,M.,Large Scale and Long term application of bioslurping:The case of Greek Petroleum Refinery Side..J. of Hazardous Material. (2007) 574-581.

[101] GWRTAC,1996b.Bioslurping.Groundwater Remediation TechnologiesAnalysis Center.Publication #GWRTAC-TO-96-05 Sixth avenue, Regional Enterprise Tower, Pttsburgh,PA.

[102] Yen,H.K.,Chang,N.B.,Lin,T.F.,2003.Bioslurping Model for assessing Light Hydrocarbon Recovery in Contaminated Unconfined Aquifer.I: Simulation Analysis.Practice Periodical of Hazaedous,Toxic, and Radioactive Waste Management 7(2),114-130.

[103] A Citizen's Guide to Permeable Reactive Barriers EPA 542-F-01-005 2001.

[104] National Research Council (NRC), Alternatives for Groundwater Cleanup, National Academy Press, Washington,DC,1994.

[105] Manz, C. and Quinn, K. (1997) Permeable treatment wall design and cost analysis. International Containment Technology Conference, St. Petersburg, Florida, USA.

[106] Mitchell, P. (1997) Reactive Walls. Industrial Waste Water, 7/8, 33-37.

[107] Gavaskar,A.R., Gupta,N.,Sass,B.M.,Janosy,R.J.,O'Sulivan,D.,Permeable Barrers for Groundwater Remediation:Design,Construction and Monitoring,Battelle Press, Columbus, Ohio,1998.

[108] Scherer,M.M.,Richter,S.,Valentine,R.L.,Alvarez,P.J.J.,(2000). Chemistry and microbiology of permeable reactive barriers for in situ groundwater clean up. Critical Reviews in Environmental Science and Technology.30(3):363-411.

[109] USEPA,Permeable Reactive Barrier Technologies for Contaminant Remediation, Report number EPA/600/R-98/125, Washington,DC, 1998.

[110] Tratnyek,P.G.,Scherer,M.M.,Johnson,T.L.,Matheson,L.J.,Chemical Degradation Methods for Waste and Pollutants:Environmental and Industial Application,2003,p.371

[111] Sacre ,J.A.,Treatment Walls:A Status Update,Groundwater Remediation Technology Analysis Center,TP-97-02, Pitdsburg,PA,1997.

[112] Meggyes T.,Simon,F.G.,Removal of Organic and Inorganic Pollutants from Groundwater Using Permeable Reactive Barrier.,Land Contam.&Reclam. J. 8(2)(2000) part 1.

[113] Burris D.R., Campbell T.J. and Manoranjan V.S. **1995** Sorption of Trichloroethylene and Tetrachloethylene in a Batch Reactive Metallic Iron-water System, Environmental Science and Technology, 29, 2850-2855.

[114] Holser,R.A.,McCutcheon,S.C.,Wolfe,N.L.,Mass transfer Effect on the Dehalogenation of Trichloroethene br Iron/pirite Mixtures.Extended Abstracts from the 209th ACS National Meeting,Anaheim,CA 35(1) p.788,Division of Environmental Chemistry, Am.chem.Society, Washington, DC, 1995.

[115] Johnson, T.L., Fish, W., Gorby, Y.A. and Tratnyek, P.G. 1998 Degradation of Carbon Tetrachloride by Iron Metal: Complexation Effects on the Oxide Surface, *Journal of Contaminated Hydrology*, *29*, 379-398.

[116] Vidic,R.D., Permeable Reactive Barriers:Casae study Reviev, Technology Evaluation Report TE01-01, Groundwater Remediation Technologies Analysis Center, 2001.

[117] Gillham,R. W.,and O'Hannesin.S.F.,1994.Enhanced degradation of halogenated aliphatics by zero-valent iron.Ground Water,32(6):958-967.

[118] Sherman, M.Ponder,J.G.,Mallouk,T.E.,2000 Sufface Chemistry and Electrochemistry of Supported Zerovalent Iron Nanoparticles in the Remediation of Acuous Metal Contaminants. Chem.Mater.13,,479-486

[119] Krug,T.,O'Hara,S.,Watling,M.,QuinnJ., Emulsifield Zero-Valient Nano-Scale Iron Treatment of Chlorinated Solven DNAPL Source Areas, ESTCP Proyect ER-0431,763 pp, 2010

[120] Zhang, W.X., Wang, C.B. and Lien, H.L. 1998 Treatment of Chlorinated Organic Contaminants with Nanoscale Bimetallic Particles, *Catalyst Today*, *40*, 387-395.

[121] Fennelly, J.P. and Roberts, A.L. 1998 Reaction of 1,1,1-Trichloroethane with Zerovalent Metals and Bimetallic Reductants, *Environmental Science and Technology*, *32*,1980-1988.

[122] Lui, Y., Yang, F., Yue, P.L. and Chen, G. 2001 Catalytic Dechlorination of Chlorophenols in Water by Palladium/ Iron, *Water Research*, *35*, 1887-1890.

[123] Schrick, B., Blough, J.L., Jones, A.D. and Mallouk, T.E. 2002 Hydrodechlorination of Trichloroethylene to Hydrocarbons using Bimetallic Nickel-Iron Nanoparticles, *Chemical Materials*, *14*, 5140-5147.

[124] Wrenn H.,Enhanced Reductive Dechlorination through Biological Interaction with zero-valent Iron,Federal Remediation Technologies Roundtable Meeting, Arlington, Virginia, USA, 2004.

[125] Novak, P.L. Daniels, and Parkin, G., 1998a. Enhanced dechlorination of carbon tetrachloride and chloroform in presence of elemental iron and *Methanosarcina*

barkeri, Methanosarcina thermophile and *Metanosaeta concilli.* Environm. Sci. Technol. 32:1438-1443.

[126] Novak,P.L. Daniels, and Parkin, G., 1998b. Rapid dechlorination of carbon tetrachloride and chloroform by extracellular agents in culture *Methanosarcina thermophile.* Environm.Sci.Technol. 32:3132-3136.

[127] Liang,L., West,N.Korte,J.D.,Goodlaxson,D.Pickering,J.Zutman.F.Anderson,C. Welch and M.Pelfrey. 1997b. The X-625 Groundwater Treatment Facility: A Field Scale Test of Trichloroethylene Dechlorination Using Iron Filings for the X-120/X749 Groundwater Plume.Oak Ridge National Laboratory: Environmental Science Divition Publication No.4667.

[128] KorteN.,O.West,L.Liang,M.Pelfrey, and T.Houk. 1997. A field-scale test facility of permeable reactive barriers at the Portsmouth gaseous diffusion plant federal facilities.Environ.J.Autumn:105-114.

[129] Benner,S.G.,D.W.Blowes,andC.J.Ptacek.1997.A full-scale reactive wall for prevention of acid mine drainage. Ground Wat. Monitor.Remed. Fall 1997:99-107.

[130] PEREBAR,5th Framework Programe Research and Technology Development Proyect on Long-term Performance of Permeable Reactive Barriers Used for the Remediation of Contaminated Groundwater. PEREBAR EVK1-CT-1999-00035,National Technical University of Athens, Greece,2002.

[131] Admassu, W.; Breese, T. Feasibility of using natural fishbone apatite as a substitute for hydroxyapatite in remediating aqueous heavy metals. J Hazardous Mater 1999, B69, 187–196.

[132] Rose,J.A., Roy, S.J.,Burt,T,Johnson,R.L.,Pilot test of a surfactant-modified zeolite permeable barrier for groundwater remediation, in: Smith, J.A., Burns,S.E., Phisicochemical Groundwater Remediation, Kluwer Academic/Plenum Publishers, Dordrecht,2001, pp161-185.

[133] Browman R.S.,Li,Z.,Roy,S.J.,Burt,T.,Johnson,T.L.,Johnson,R.L., Surface-altered Zeolites as Permeable Barriers for In-situ Treatment of Contaminated Groundwater, Phase II, Work Performed Under Contract#DE-AR21-95MC321008,Agust, New Mexico Institute of Mining and Technology, Socorro,NM,1999 pp58

[134] MinatoH.,Yoshida,M., Schbute,Y., New use of natural zeolites and clay for environmental protection and remediation of toxic metals contaminated sites, in: paper submitted to the EUROCLAY 1999, Kraków,5-9

[135] Shimotori.T.,Nuxoll,E.E., Cussler,E.L., and Arnold,W.A., (2004), A Polymer Membrane Containing Fe(0) as a Contaminant Barier, Environmental Science and Technology 38(7):2264-2270.

[136] USEPA, Field Applications of in Situ Remediation Technologies:Permeable Reactiva Barriers,Office of Solid Waste and Emergency Response Technology Innovation Office, Washington,DC,2002

[137] Gavaskar,A.R.,Gupta,N.,Sass,B.,Fox,T.,Janosy,R.,Cantrell,K., and Olfenbuttel,R., (1997),Design guidance for application of permeable barriers to remediate dissolved chlorinated solvents.AL/EQ-TR-1997-0014, <united Stated Air Force, Amstrong Laboratory,Battelle,p.104

[138] Gavaskar,A.R.,(1999), Design and Construction Techniques for Permeable Reactive Barriers., Journal of Hazardous Materials,68,41-71.

[139] Cushing,R.,Gravelding,D., McMahon,P.B., 1996, Overview of the Denver Federal Center. Remediation Technologies Development Forum, Permeable Barriers Action Team meeting. Denver, Colorado.

[140] Vidic,R.D.,Pohland,F.G.,Treatment wells, Technology Evaluation Report TE 96-01.GWRTAC,Pittsburg,USA,1996.

[141] Sutherson,S.S.,(1997).In situ reactive wall.., Remediation Engineering: Design Concept. CRC Press,Newtown,Papp 187-213

[142] Turlough F.,Guerin,Stuart Horner,Mz Govern,T.,Davey,B, An application of permeable reactive barrier technology to petroleum hydrocarbon contaminated groundwater, Water Research 36 (2002) 15-24.

[142] Meiggs,T. (1999) High pressure jetting and reactive slurry injection techniques to create deep permeable barriers.Subsurface Barrier Technology,Scottsdale, Arizona, USA.

[143] Warner, S., Yamane,C.,Gallinatti,J.,and Szerdy,F. (1995), Assessing the feasibility of permeable reactive barriers for treating VOC-affected groundwater in situ: Experience for the first full-scale commercial application in California. International Containment Technology Workshop, Baltimore,MD.

[144] Chang,P.R.,Jet-Assisted Injection of Nano-Scale, Zero-Valent Iron to Treat TCE in a Deap Alluvial Aquifer.,Pantaleoni,A.D.Shenk Proceedings of the Seventh International Conference on Remediation of Chlorinated and Recalcitrant Compounds (Monterey,CA, 2010)

[145] FRTR,1999c.Passive/reactive treatment walls.Federal Remediation Technologies RoundTable, USEPA,Washington DC, http://www.frtr.gov/matrix2/section4/4-41.html (access November2011)

[146] Baciocchi,R.,Boni,M.R.,D'Aprile,L.,2003.Characterization and performance of granular iron as reactive media for TCE degradation by permeable reactive barriers.Water,Air and Soil Pollution 149(1-4),211-226.

[147] Birke,V.,Burmeier,H.,Rosenau,D.,2003. Design,construction,and operation of tailored permeable reactive barrier.Practice Periodical of Hazardous,Toxic, and Radioactive Wadte Management 7(4),264-280.

[148] Johns,F.J., Nyer,E.K.,1996 Miscelaneous in situ treatment technologies.In situ treatment technology. Nyer,E.K., Kiddd,D.F., Palmer,P.L., Crossman,T.L., Fam,S., Johns,F.J.,II. Boettcher,G., and Suthersan,S.S.,Eds.CRCPress,Boca Raton,Fl. Pp 289-319.

[149] EPA (1996) A citizen's gide to in situ soil flushing Tecnology Fact Sheet EPA 542-F-96-006.

[150] Khalladi R.,BenhabilesO., Bentahar F.,Moulai-Mostefa N.,Surfactant remediation of diesel fuel polluted soil; Journal of Hazardous Materials 164 (2009) 1179–1184;

[151] Lyman,W.J.,Noonan,D.C.,Reidy,P.G.,1990.Cleanup of petroleum contaminated soils at anderground storage tanks.Pollut.Technmol.Rev.No195

[152] Liu,Z.,Laha,S., and Luthy,R.G.,1991.Surfactant solubilization of polycyclic aromatic hydrocarbon compounds in soil water suspensions.Water Sci.Technol.23:475.

[153] Amdurer, M.,Fellman,R., and Abdelhamid,S.,1985.In situ treatment technologies and superfund. In Proc. Int. Conf. on New Frontiers for Haz.Waste Mgmt.EPA Report No EPA-600/9-85/025.

[154] Joshi,M.M., and Lee,S.,1996.Optimization of surfactant-aided of industrially contaminated soils.Energ.Sourc.18(3):291-301

[155] U.S.EPA.1985a.Handbook for Remedial Action at Waste Disposal Sites.Revised EPA/625/6-85/006.

[156] Haigh,S.D. (1996) A review of the interaction of surfactants with organic contaminants in soil.Science of the Total Environment 185,161-170.

[157] Zhou,QX.Research progress on in-situ remediation of polluted land.Pollut Control Technology 1998;11(4):207-211.

[158] Bruell,C.J., Laboratory evaluation of a biodegradable surfactant for in-situ soil flushing.J.Soil Contam. 1997;6(5):509-523.

[159] Tiehm,A (1994) Degradation of polycyclic aromatic hydrocarbons in the precence of syntethic surfactants. Applied an Environmental Microbiology 60,258-263.

[160] Sabatini,D.A.,Knox,R.C., and Harwell,J.H., (1995) Surfactant enhanced subsurface Remediation:Emerging Technologies.ACSSymposium Series 594.Washington D.C:American Chemical Society.

[161] Liu,M. and Roy,D.1995.Surfctant-induced interactions and hydraulic conductivity changes in soil. Waste management. 15/(7):463-470.

[162] Ouyang,Y.,Mansell,R.S., and Rhue,R.D.1996. A microemulsification approach for removing organolead and gasoline from contaminated soil.J.Haz.Mater.46 (1):23-35

[163] Abdul,A.S., Gibson,T.L.,Ang,C.C., Smith,J.C., and Sobczynski,R.E.,1992.In situ surfactand washing of polychlorinated biphenyls and oil from a contaminated site. Ground Water 30 (2):219-231.

[164] Ang,C.C.,and Abdul,A.S.,1994.Evaluation of an ultrafiltration method for surfactant recovery and reuse during in situ washing of contaminated sites: laboratory and field studies.Ground Water Monit.Rem.14(3):160-171.

[165] Edwards,D.A., Liu,Z.,and Luthy,R.G. 1994b.Experimental data and modeling for surfactand micelles,HOCs and soil.J.Environ.Eng. (N.Y.)120(1):23-41.

[166] Cort,T.L.,Song,M.S.,and Bielefeldt,A.R. (2002). Non ionic surfactant effects on pentachlorophenol degradation .Water Research 36,1253-1261.

[167] Mulligan,C.N.Yong,R.N. and Gibbs,B.F.,(2001b)Surfactant remediaion of contaminated soil:a review. Enginering Geology 60,371-380

[168] Ducreux,J., Bviere,M., SeabraP., Razakarisoa,O., Shaefer,G. and Arnaud,C. 1995. Surfactand-aidede recovery/in bioremediation for oil contaminated sites. In Applied Bioremediation of Petroleum Hydrocarbons Hinchee,R.E., Kittel,J.A., and Reisinger,H.J., Eds.Battelle Press,Columbus,OH.pp.435-443.

[169] Mulligan,C.N., Yong,R.N., Gibbs,B.F., Surfactant-enhanced remediation of contaminated soil: a review, Eng.Geol.60(2001)271-380.

[170] Haba,E.,Espuny.M.J., Busquets,M. and Manresa,A. (2000)Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils.Journal of Applied Microbiology 8,379-387.

[171] Veenanadig,N.K., Gowthman,M.K., and Karath,N.G.K. (2000) Scale up studies for the production of biosurfactant in packed column bioreactor.Bioprocess Engineering 22, 95-99.

[172] Vardar-Sucar,F and Kosaric,N. (2000) Biosurfactants.In Encyclopedia of Microbiology 2nd edn,Vol1, ed.Lederberg,J.pp. 618-635 San Diego Academic Press.

[173] Lang,S.,(2002) Biological amphiphiles (microbial surfactants).Current Opinion in Colloid and Interface Science /,12-20.

[174] Christofi,N.,Ivshina,I.B., Areview: Microbial surfactants and their use in field studies of soil remediation.J.Appl.Microbiol.93(2002)915-929.

[175] Snape,I.,Riddle,M.J.,Stark,J.S.,Cole,C.M.,King,CK.,Duquesne,S.,Gore,D.B.,2001 Management andremediation of contaminated sites at Casey Station,Antarctica.Polar Rec.37:199-214.

[176] Madsen,E.L.,1991.Determining in situ biodegradation: fact and challenges. EnvironmentalSci. Technol.25(10):1663-1673.

[177] Atlas,R.M.,1981.Microbial Degradation of petroleum hydrocarbons:an environmental perspective.Mirob.Rev.45:180-209.

[178] Ghazali, F.M., Zaliha, R.N.R., Salleh, A.B., Basri, M., 2004. Biodegradation of hydrocarbons in soil by microbial consortium. Int. Biodeg., 54:61-67.

[179] Glass,D.J.,Raphael,R.Valo,and Van Eyk,J.,1995.International activites in bioremediation: Growing markets and opportunities. In Applied Biorremediation of Petroleum Hydrocarbon ed.R.E.Hinchee,J.A.Kittel and H.J.Reisenger,11-34 Columbus,Ohio:Battelle Press.

[180] Huesemann, M.H., Huesemann,T.S.,Fortman,T.J.,2002 Microbial factors rather than bioavailability limit the rate and extent of PAH biodegradation in aged crude oil contaminated model soils. Bioremed,J.6(4):321-336

[181] Federal Remediation Technologies Roundtable, 2000.Remediation Technologies screening matrix and reference guide,version 4.0. Engineerred Approaches to in situ Bioremediation of Chlorinates Solvents:Fundamentals and Field Applications, EPA-542-R-00-008.

[182] Davis,C.,Cort,T.,Dai,D.,Illangasekare,T.,Munakata-Marr,J.,2003. Effects of heterogeneity and experimental scale on the biodegradation of diesel. Biodegradation, 14:373-384.

[183] Goltz,M.,Boiuwer,E.,Huang,J., 2001.Transport issues and bioremediation modeling for the in situ aerobic co-metabolism of chlorinated solvents. Biodegradation, 12:127-140.

[184] Schirmer,M.,Molson,J.,Frind,E.,Barker,J.,2000Biodegradation modeling of dissolved gasoline plume applying independent laboratory and field parameters. J.Contam. Hydrol.,46:39-374.

[185] Allard,A.S.,Neilson,A.H.,1997.Bioremendiation of organic wadte sities: A critical review of microbiological aspects.Int.Biodeterioration Biodegradation,39:253-285.

[186] Parales,R.E.,2008.Bioavailability,Chemotaxis and Transport of Organic Pollutants, Microbial Biodegradation:Genomics and Molecular Biology.Caister Academic Press,UK

[187] Garibsu,C., Alcorta,I., 1999. Utilization of generically engineered microorganisms (GEMS) for bioremediation.J.Chem.Technol.Biotechnol.74:599-606.

[188] Shailubhai,K. 1986,Treatment of petroleum oil sludge in soil.Trends Biotechnol.4:337-343.

[189] Van Beilen,J.M.,Wubbolts,M.G.,Withold,B.,1994.Genetics of alkane oxidation by *Pseudomonas oleovorans.*Biodegradation 5:161-174.

[190] Van Beilen,J.M., Panke,S., Lucchini,S., Franchini,A.G., Röthlisberger,M., Witholt, B., 2001. Analysis of *Pseudomonasputida* alkane degradation gene clusters and flanking insertion sequences:evolution and regulation of the *alk* genes.Microbiology 147:1621-1230.

[191] Cerniga.CE., 1992.Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3:351-368.

[192] Dojika,M.A.,Hugenholtz,P.,Haack,S.K., and Pace,N.R.,1998.Microbial diversity in a Hydrocarbon and chlorinated solvent contaminated aquifer undergoing intrinsic bioremediation.Appl.Environ.Microbiol.,64,3869-3877.

[193] Brito,E.M.S.,Guyoneaud,R.,Goni-Urriza,M.,Ranchou-Peyruse,A.,VerbaereA., 2006.Characterization of hydrocarbonclastic bacterial communietes from mangrove sediments in Guanabara Bay ,Brazil.Res.Microbiology.,157:752-762

[194] Antai,S.P.,1990.Biodegradation of Bony light crude oil by *Bacillus sp.* and *Pseudomonas sp.*Waste manag., 10:61-64.

[195] Bhattacharya,D.,Sarma,P.M.,Krishnan,S.,Lal,B.,2002.Evaluation of genetic diversity among *Pseudomonas citronellolis* stains isolated from oily sludge contaminated sides. Applied Environ.Microbiol.,663:1435-1441.

[196] Jain,P.K.,Gupta,V.K.,Pathak,H.,Lowry,M. and Jaroli,D.P.,2010.Characterizzzation of 2T engine oil degrading indigenous bacteria,isolated from high altitude (Mussoorie) India.World J.Microbiol.Biotechnol.,26:1419-1426.

[197] Garrett,R.M.,Pickering,III.J.,Haith,C.E.,and Price,R.C.,1998.Photooxidation of crude oils.Environ.Sci.Technol.32:3719-3723.

[198] Markovetz,A.J.,Kallio,R.E.,Subterminal oxidation of aliphatic hydrocarbons by microorganisms.Cri.Rev.Microbiol.1:225-237.

[199] Langenhoff,A.A.M.,Zehnder,A.J.B.,Schraa,G., 1996.Behavior of toluene, benzene and naphthalene under anaerobic conditions in sediment columns.Biodegradation 7:267-274

[200] Langenhoff,A.A.M., Brouwers-Ceiler,D.L.,Engelberting,H.H.L.,Quist,J.J., Wolkenfelt, J.G.P.N., Zehnder, A.J.B., Schraa,G., 1997. Microbial reuction of manganese coupled to toluene oxidation. FEMS Microbiol.Ecol. 22:119-127.

[201] Meckenstock,R.U., 1999.Fermentative toluene degradation in anaerobic defined syntrophic cocultures FEMS Microbiol.Lett.177:67-73.

[202] Anderson.R.T.,Lovely,D.R., 2000.Anaerobic bioremediation of benzene under sulfate-reducing conditions in a petroleum-contaminated aquifer. Envirin. Sci. Technol. 34:2261-2266.

[203] Annweiler,E.,Michaelis,W.,Meckenstock,R.U.,2002.Identical ring cleavage products during anaerobic degradation of naphthalene,2-methyl-naphtalene,and tetralin indicate a new metabolic pathway. Appl.Environ.Microbiol.68:852-858.

[204] Elshahed,M.S., Gieg,L.M., McInerney,M.J.,Suflita,J.M.,2001.Signature metabolites attesting to the in situ attenuation of alkylbenzenes in anaerobic environemnts. Environ.Sci.Technol.35:632-689.

[205] Salanitro,J.P.,2001 Bioremediation of petroleum hydrocarbons in soil.Adv.Agron. 72:53-105.

[206] Owabor,C.N.,Ogunbor,O.F.,2007.Naphthalene and pyrene degradation in contaminated soil as a function of the variation of particle size and percent organic matter.Afr.J.Biotechnol.,6:436-440.

[207] Oleszczuk,P.,Baran,S.,2003.Degradtion of individual polycyclic aromatic hydrocarbons PAHs in soil polluted with aircraft fuel.Polish J.Environ.Res., 45:113-125.

[208] Foght,J.M.,Westlake,D.,Johnson,W.M.,Ridgway,H.F.1996.Environmental gasoline-utilising isolates and clinical isolates of *Pseudomonas aeruginosa* are taxonomically indistinguishable by chemotaxonomic and molecular techniques. Microbiol.142:1333-2340.

[209] Venosa,A.D.,Zhu,X.,Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands.Spill Sci.Tech.Bull.,82:163-178

[210] Bossert,I.,Bartha,R.,1984.In:The Fate of Petroleum in Soil EcosistemsPetroleum Microbiology,Atlas,R.M.,(EDS). Macmillan,New York,pp:453-473.

[211] Magesin,R.,Schinner,F.,1999.Biological decontamination of oil spills in cold environments.Journal of Chemical Technology and Biotechnology 74,381-389.

[212] Filler,D.M.,Reynolds,C.M.,Snape,I.,Daugulis,A.J.,Barnes,D.L.,Wiliams,P.J.,2006. Advances in engineered remediation for use in the Arctic and Antarctica.Polar Record 42 (221),11-120.

[213] Lovley,D.R.,1997. Potential for anaerobic bioremediation of BTEX in petroleum-contaminated aquifers.Journal of Industrial Microbiology and Biotechnology 18, 75-81.

[214] Choi, S.C.,Kwon,K.K.,SohnJ.H.,KimS.J.,2002. Evaluation of fertizer additions to simulate oil biodegradation in sand seashore mescocosms. J.Microbiol. Biotechnol. 12:431-436.

[215] Okolo,J.C.,Amadi,E.N.,Odu,C.T.I.,2005.Effects of soil treatments containing poultry manure on oil degradation in sandy loam soil.Appl.Ecol.Environm.Res.3(1):47-53.

[216] Roldan-Martin,A.,Esparza-Garcia,F.,Calva-Calva,G., Rodriguez-Vazquez,R., 2006. effects of mixing low amounts of orange peel(citrus reticulata) with hydrocarbon-contaminated soil in solid culture to promote remediation.

[217] Challain,F.,Chaineau,C.H.,Point,V,Saliot,A.,Oudot,J.,2006.Factors inhibiting bioremediation of soil contaminates with weathered oils and drill cuttings. Environmental Pollution. 144: 255-265.

[218] Chayneau, C.H., G. Rougeux, C. Yepremian and J. Oudot, 2005. Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. Soil. Biol. Biochem., 37: 1490-1497.

[219] Carmichael, L.M. and F.K. Pfaender, 1997. The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. Biodegradation, 8: 1-13.

[220] Perry,J.J., 1984.Microbial Metabolism of cyclic Alkanes.In: Petroleum Microbiology, Atlas,R.M.,(Eds).Macmillan Publishing,New York,pp:61-97.

[221] Uraizee,F.A.,Venosa,A.D.,Suidan,M.T., 1998.A model for diffusion controlled bioavailability of crude oil components. Biodegradation.8:287-296.

[222] Alexander,M.,2000.Aging,bioavailabililty and overestimation of risk from environmental pollutants.Environ.Sci.Technol. 34(2):4259-4265.

[223] Liste,H.H.,Alexander,M.,2002.Butanol extraction to predict bioavailability of PAHs in soil.Chemosphere 46(7):1011-1017.

[224] Rahman,K.S.M., Rahman,T.J.,Kourkoutas,Y.,Petsas,I.,Banat,I.M., 2003. Enhanced bioremediation of n-alkane in petroleum sludge using consortium amended with rhamnolipid and nutrients. Bioresour. Technol.,90:159-168.

[225] Braddock,J.F.,Ruth,M.L.,Catterall,P.H.,Walworth,J.L.,McCarthy,K.A.,1997. Enhancement and inhibition of microbial activity in hydrocarbon contaminates Arctic soils:Implications for nutrient-amended bioremediation. Environ.Sci.Tech.,31: 2078-2084.

[226] Atlas, R. M. 1991. Microbial Hydrocarbon Degradation – Bioremediation of oil spills J.Chem. Technol – Biotechenol 52: 149 – 156.

[227] Hinchee,R.E.,Miler,R.N.,1990.Bioreclamation of hydrocarbons in the unsaturated zone.In Hazardous Waste Management of Contaminated Side and Industrial Risk Assessment.Pillman,W. and Zirm,K., Eds.Vienna pp.641-650.

[228] Doupont,R.R.,Doucette,W.J.,Hinchee,R.E.,1991.Assessment of in situ bioremediation potential and the application of bioventing at a fuel-contaminated side.In In Situ Boreclamation. Applications and Investigations for Hydrocarbon and Contaminated Site Remediation.Hinchee,R.E., and Olfenbuttel,R.F., Eds. Butterworth-Heinsemann, Stoneham, MA.pp 262-282.

[229] Holman,H.Y.,Tsang,Y.W.1995.Influence of soil moisture on biodegradation of petroleum hydrocarbons. In R.E. Hinchee,R.N.Miller and P.C.Johnson (Eds.), In situ aeration:air sparging, bioventing and related remediation processes (pp 323-332. Colombus:Battelle.

[230] Verstraete,W.,Vanloocke,R.,DeBorger,R.,Varlindne, A.,1075.Modeling of the breakdown and the mobilization of hydrocarbons in unsaturated soil layers.In: Proceedings of 3er International Biodegradation Symposium,Sharpley,J.M., and Kalpan,A.M., (Eds)Applied Science Publisher,London,pp98-112.

[231] Godbout,J.G.,Gomeau,Y.,Greer,C.W.,1995.Soil characteristics effects on introduced bacterial survival and activity.In Bioaugmentation for Site Remediation.Pap.3rd Int.In Situ On-Site Bioreclam. Symp. Hinchee, R.E., Fredrickson, J., and Alleman,B.C., Eds.Battelle Press,Columbus,OH,pp115-120.

[232] Dineen,D.,Slater,J.P.,Hicks,P,Holland,J.,and Clendening,L.D., 1993.In situ biological remediation of petroleum hydrocarbons in unsaturated soils.In Princ.Pract.Petrol.Contam.Soils.Calabrese,E.J.,Ed.pp.453-463.

[233] Hughes,J.B.,Neale,C.N.,Ward,C.H.,2000.Bioremediation,p.587-610.In J.F.Lederberg (ed.), Encyclopedia of microbiology,2nd ed.,vol.1.Academic Press, San Diego,Alif.

[234] Barker,J.F.,Patrick,G.C.,Major,D.,1987.Natural attenuation of aromatic hydrocarbons in a shallow sand aquifer. Ground Water Monitoring Review 7,No1:64-71.

[235] National Research Council.1997.Innovations in Ground Water and Soil Cleanup.Washington D.C. National Academy Press.

[236] Salanitro,J.P.,1993.The role of bioattenuation in the mamagement of aromatic hydrocarbon plumes in aguifers.Ground Water Monitoring and Remediation 13: 150-161.

[237] Wiedemeier,T.H.,Wilson,R.N.,Miller,R.N.,Kampbell,D.H., Hansen,JE., 1995a. Technical protocol for implementing intrinsic remediation with long-term monitoring for natural attenuation of fuel contamination dissolved in groundwater.VolumesI and II.Prepared for Air Force Center for Environmental Excellence,Books Air Force Bases,Texas.

[238] Mahro, B.; Schaefer, G. (1998): Bioverfügbarkeit als limitierender Faktor des mikrobiellen Abbaus von PAK im Boden - Ursachen des Problems und Lösungsstrategien. (Bioavailability as the limiting factor of microbial degradation of PAHs in soil – causesof the problems and strategies of solution) altlasten spektrum (3/98), 127-134.

[239] Holba,A.G.,Dzou,I.L.,Hickey,J.J.,Franks,S.G.,May,S.J., Lenney,T. 1996.Reservoir Geochemistry of South Pass 61 Field,Gulf of Mexico:Compositional Heterogeneiteies Reflecting Filling History and Biodegradation: Org.Geochem. (24):1179-1198.

[240] Serrano,A.,Gallego,M.,Gonzalez,J.L.,2006.Assessment of natural attenuation of volatile aromatic hydrocarbons in agricultural soil contaminated with diesel fuel. Environ.Pollut.,144,203-209.

[241] Seagren,E.,Becker,J.2002.reviev of natural attenuation of BTEX and MTBE in groundwater. Pract.Period.Hazard.Toxic Radioact.Waste Manage.,6,156-172.

[242] National Research Council.1993.In situ Bioremediation:When Does It Work?Washington D.C. National Academy Press.

[243] Yagi,O and Nishimura,M.,1997. Envinronmental biotechnology,The Japan perspective.p.201-207.In Sayler,G.S. (ed.) Biotechnology in the sustainable environment.Plenum Press,New York.

[244] Hanada, S.,Shigematsu, T.,Shibuya,K., Eguchi,M.,Hasegawa,T.,Suda,F., Kamagata,Y., Kanagawa,T., Kurane,R.,1998. Phylogenetic analysis of Trichloroethylene- degrading bacteria newly isolated from soil polluted with this contaminant. J.Ferment.Bioeng.,86,539-544.

[245] Dzantor,E.K., 1999. Bioremediation of contaminated soils-what it is and how to do it.University of Agrriculture and Natural Resourses,pp.1007-1019.

[246] Ausma,S.,Edwards.G.C.,Fitzgerald-Hubble,C.R.,Halfpenny-Mitchell,L.,2002.Volatile hydrocarbon emissions from a diesel fuel-contaminated soil bioremediation facility.Air Waste Manag.Association,52:769-780.

[247] Kim,S.,Choi,D.H.,Sim,D.S.,Oh,Y.,2004. Evaluation of bioremediation effectiveness on crude oil-contaminated sand. Chemosphere,59(6):845-852.

[248] Rosa,A.P.,Triquis,J.A.,2007. Bioremediation process on Brazil shoreline.Laboratory experiments.Environ.Sci.Pollut.Res.Int. 14(7):470-476

[249] Margesin,R,Schinner,F.,2001.Bioremediation (natural attenuation and biostimulation) of diesel-oil contaminated soil in an Alpine glacier akiing area.Appl.Environ.Microbiol. 67(7):3127-3133.

[250] Margesin,R.,Hämmerle,M.,Tscherko,D.,2007.Microbial activity and community composition during bioremediation of diesel-oil-contaminated soil:effects of hydrocarbon concentration,fertilizers,and incubation time.Microb.Ecol.53(2):259-269.

[251] Singh,C.,Lin,J.,2009.evaluation of nutrient addition to diesel biodegradation in contaminated soils.African J.of Biotechn.8(14):3286-3293.

[252] Del´Arco,P.J., de Franca,FP.,2001.Influence of oil contamination levels on hydrocarbon biodegradation in sandy sediments. Environmental Pollution,110:515-519.

[253] Mehrashi,M.R.,Haghighi,B.,Shariat,M.,Naseri,S.,Naddafi,K.,2003.Biodegradation of petroleum hydrocarbons in soil:iranian J.Public Helth.32(3):28-32.

[254] Naidoo,E.,2007.Astudy on the substrate specificity of oil degrading Acetobacter isolates,Honours dissertation, University of KwaZuluNatal,South Africa.

[255] Nakamura,K.,Ishida,H.,Iizumi,T.,Shibuya,K., Okamura,K.,2000.Quantitative PCR-detection of a phenol-utilizing bacterium,*Ralstonia eutropha*KT-1,injected to a trichloroethylene-contaminated side.Environ.Eng.Res.,37,267-278.

[256] Gutnick,D.L.,Rosenberg,E.,1979.Oil tankers and pollution:a microbiological approach.Annu.Rev.Microbiol.31:379-396.

[257] Maxwell,C.R.,Baqai,H.A.,1995.Remediation of petroleum hydrocarbons by inoculation with laboratory-cultured microorganisms.In Bioaugmentation for Site Remediation.Pap.3ed Int.In situ on-site Bioreclam. Symp. Hinchee, R.E.,Fredrickson, J.,Alleman,B.C.,Eds.Battelle Press,Columbus,OH.pp129-128.

[258] Turco,R.F.,Sadowsky,M.1995.The microflora of bioremediation.In Bioremediation:Science and Applications.SSSA Special Pub.43.Soil Science Society of American Siciety of Agronomy and Crop Science Society,Madison,WI.pp87-102.

[259] Widdel,F.,Rabus,R.2001.Anaerobic biodegradation of saturated and aromatic hydrocarbons.Curr.Opin.Biotech.12:259-276.

[260] Heider,J.,Spormann.A.M.,Beller,H.R.,Widdel,F.,1998.Anaerobic bacterial metabolism of hydrocarbons.FEMS Microb.Rev.22 (5):459-465.

[261] Spormann,a.M.,Widdel,F.2000.Metabolism of alkylbenzenes,alkenes,and other hydrocarbons in anaerobic bacteria.Biodegrad.11:85-105.

[262] Leahy,J.G.,Somerville,C.C.,Cunningham, K.A, Adamantiales, G.A., Byrd J.J.,Colwell,R.R.,1990.Hydrocarbon mineralization in sediments and plasmid incidence in sediment bacteria from the Campeche Bank.Applied Environ.Microbiol.,56:1565-1570.

[263] Kopytko, M., Ibarra, D., 2010. Biodegradation potential of total petroleum hydrocarbons in oil industry contaminated soils. Journal of the P. Mineral Engineering Society. V.X Nº 2(24): 1640-4920.

[264] Braddock,J.F.,Catterall,P.H.,1999.A simple method for numerating gasoline and diesel-degrading microorganisms. Bioremed.J.3,81-84.

[265] Schlumberger Water Service; Visual ModFlow Premium Demo Tutorial. http://trials.swstechnology.com/software/Visual_MODFLOW/2011/Manuals_and_Guides/VMOD-2011.1_Demo_Tutorial.pdf (access February 2011).