

BIOLOGICAL REMEDIATION OF SOIL POLLUTED WITH OIL PRODUCTS : AN OVERVIEW OF AVAILABLE TECHNOLOGIES

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Key words: oil hydrocarbons, bioremediation, microorganisms, polluted soil

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INTRODUCTION

In the extraction process, processing and utilization of petroleum or petroleum products may leak into the environment [68] raising the possibility of soil contamination. The steady expansion of areas polluted land makes the problem rectified their be current [32] due to the accumulation in the environment, particularly in soil organic chemicals which are of particular importance due to their toxicity, but also the ability to accumulate in systems live [25]. Petroleum based hydrocarbons, carbon multiple bonds which develops intense and complex structures increase when binding to other molecules specific to

[12]. In nature there is an effective system, which remove oil products through the vital activity of microorganisms capable of degrading organic matter in soil [68].

Bioremediation is a technique waste management means the use of biological organisms for cleaning polluted soil and groundwater. The technique consists of adding material in polluted environments in order to accelerate the natural process of biodegradation cause driving the growth of certain microorganisms using contaminants as a source of food and energy [8].

In Romania, following the political and economic changes, many industrial sites have not been

used or have been used only to a small extent. Moreover, existing infrastructure can no longer handle the technical standard requirements, labor and ecological Europe. These websites require their impulses from the reuse [15].

SOIL POLLUTION BY OIL PRODUCTS

Developing the oil industry, both the extractive how and the processing, including transportation of oil and oil products, is accompanied sometimes by the emergence of secondary phenomena, unforeseen effects more or less harmful on the environment and human life [54]. Besides this, in the areas of oil exploitation, large areas are taken out of crop production due to pipelines, wells out of operation, storage of materials and waste, plus roads and paths built legally and illegally, power lines and telephone [23].

Currently oil and natural gas is the main energy source of mankind. Since the mid-twentieth century,

oil consumption has risen almost continuously on average by 8-10% annually, from 510 million tons in 1950 to 1.98 billion tons in 1970, 4.5 billion tons in 1993 to over 6 billion tons in 2006. world production of crude oil also registered a continuous increase, from 20.9 million bpd in 1960 to 65.8 million bpd in 1999, reaching 81.7 million barrels per day in 2006 [43].

In Romania, the significant impact of the oil industry environment has the length extractive industry, attested by over 150 years, and the processing industry for over 145 years.

Statistically, pollution, oil infestations are due:

- Diversity of human activities, about 65%;
- Processing industry - distribution, 25%;
- Oil sites (sites), about 10%.

area of agricultural land polluted with oil and salt water was about 50 000 ha according to the report MAPM 2012 [30].

The largest areas with soils polluted with oil and salt water are in Teleorman, Braila, Bihor, Bacau, Dolj, Dambovita, Giurgiu and Gorj [54].

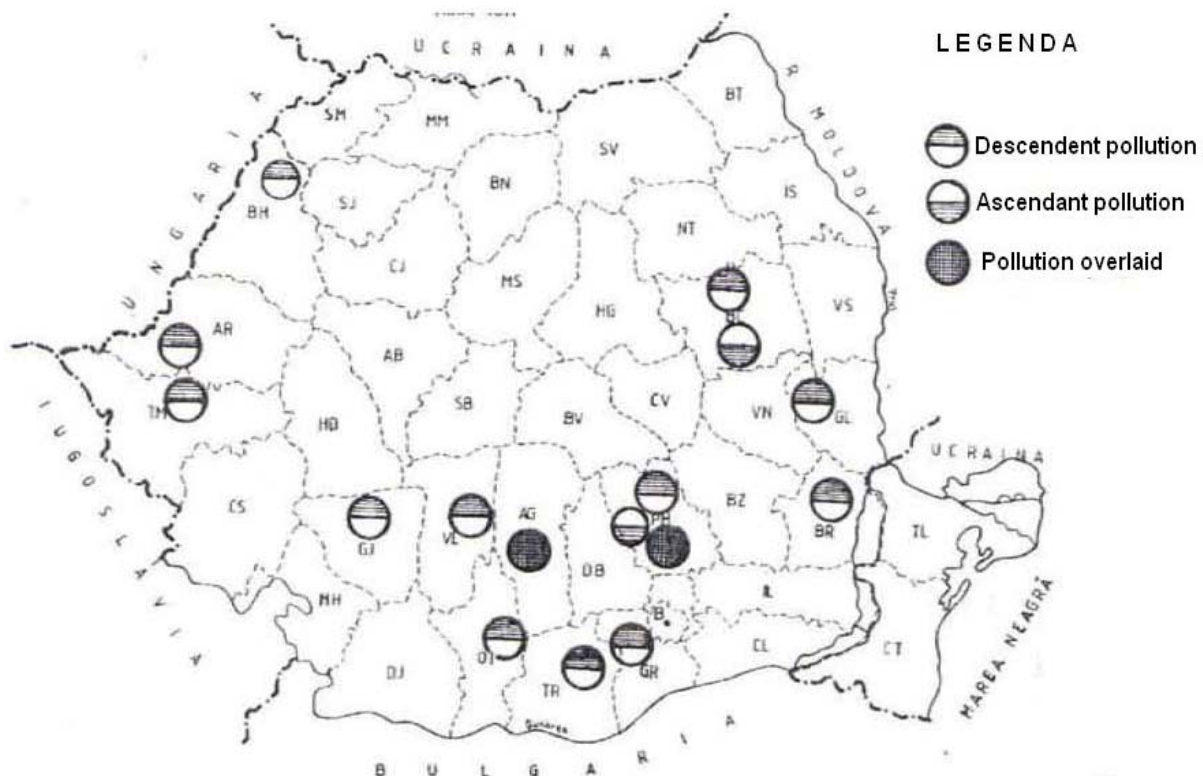


Fig. 1. Map of Romania - distribution main types of oil pollution [1]

BIOTECHNOLOGY = BIOREMEDIATION OF POLLUTED ENVIRONMENT

BIOREMEDIATION is a modern technology of treating pollutants that uses microorganisms [54] indigenous specifically designed and introduced into the polluted soil [29] to transform the chemicals into forms less harmful CO₂ and H₂O, which are nontoxic and are released free environment to substantially alter the balance of ecosystems [54]. Biotechnology plays an important role in the development of processes for the treatment of contaminated soils [25] with a number of advantages related to conditions in which the mild and relatively low costs. [24] As with any microbial process optimization in the bioremediation of environmental conditions is an important goal because microbiological activities, physiological and biochemical contaminants biodegradation are routed to the target [25].

Polycyclic aromatic hydrocarbons (PAH) attracted the attention of researchers because they have a high toxicity may be carcinogenic and / or mutagenic and bioremediation is a process by which living organisms break down or transform hazardous organic contaminants [55, 56].

CONDITIONS TO BE FULFILLED BY MICROORGANISMS USED FOR BIOREMEDIATION

Bioremediation techniques accelerate the degradation of natural biodegradation of organic compounds by optimizing conditions [52] and provides the benefits of using combinations of microorganisms selected for their synergistic capabilities to improve soil quality. Using these methods consist in optimizing the implantation and use of specialized living structure

(microorganisms and plants) for improving the soil quality. The latest research in the field provides information on the behavior of microorganisms extremely interesting, opening new insights into their use for decontaminating soils polluted with oil and petroleum products [2].

Requirements microorganisms used in bioremediation are:

- To come from nature, preferably subjected polluted site bioremediation.
- Not be the result of genetic manipulation activities.
- Do not pathogenic or toxic.
- To manifest metabolic flexibility.
- have equipment suitable enzyme.
- Develop the expense of polluting agent, disappearing with its total mineralization.
- To integrate the natural conditions of the habitat without affecting the biological balance.
- Do not produce unwanted effects contrary to the laws and regulations of environmental protection.

ADVANTAGES AND DISADVANTAGES OF BIOREMEDIATION

Bioremediation offers in many cases a permanent solution to the problem of soil contamination with petroleum products [3]. The remediation of soils polluted with hydrocarbons by biological processes are known as bioremediation processes being defined by Jelena Milić et al. (2009) as a method modern, where the natural ability of the microorganism is employed to reduce the concentration and / or toxicity of various chemicals, such as derivatives of petroleum - aliphatic and aromatic hydrocarbons, industrial solvents, pesticides, and metals [29].

Table 1. Advantages and disadvantages of bioremediation [Dobos Laura, Carmen Puia, 2010]

| Advantages | Disadvantages |
|---|---|
| Requires a moderate level of capital investment | Bioremediation is limited to biodegradable compounds |
| Safe for the environment | There are some concerns regarding the degradation products, which can be more toxic than the parent compound |
| Microorganisms have the ability to degrade a large number of contaminants, and the result remained after their work is harmless to the environment. | It is difficult to extrapolate from the laboratory scale (field) |
| Does not generate waste | Bioremediation is not going well on clay soils, compact, where oxygen or nutrients are difficult to insert in the treaty |
| Are self-sustaining | Process of bioremediation lasts much longer than other treatments, such as excavation, soil and incineration can be necessary to provide a control institutionalized for long-term protection |
| Can be made directly on the site, ecosystem disruption is minimal | Continue a migration is possible contaminant and its transfer via the environment |
| Eliminate transportation costs and operating | Hydrological and geochemical conditions may change over time, which could cause a remobilization of contaminants previously stabilized |
| It may be combined with other treatment technologies | Greater efforts are needed in education and training to win public acceptance for its monitored natural attenuation |

BASIC CONDITIONS OF BIOREMEDIATION

Soil conditions are often controlled to optimize the degradation of pollutants [25] according to contaminants situ. Bioremediation may be safer and less expensive than alternatives, such as incineration or disposal of contaminated materials [15, 30] and we need the presence of organisms with the ability to synthesize enzymes that can degrade the target pollutants. Therefore the second level of the pyramid shows that must be present adequate energy sources and electron acceptance. The third level shows the need for sufficient moisture and pH acceptable level fourth recalls the importance of avoiding extreme temperatures and ensuring the availability of inorganic nutrients such as nitrogen, phosphorus and trace metals. Finally at the bottom we have three environmental requirements that are important for the sustainability of bioremediation lack of high concentrations of substances that are toxic to microorganisms; invasive microbial metabolites inhibit specific activities and lack high concentrations of protozoa acting as predators on the bacteria responsible for the degradation of contaminants [15, 37].

Biological remediation methods in optimum conditions can be quite effective if they include the following:

- The existence of microorganisms that effectively summarizes the rate of degradation of oil and oil products

- The existence of sources of energy and nutrients to support microbial species
- Acidity / alkalinity of the soil to determine the need for fertilizers and aeration microbial species most suitable for decontamination of-situ [10, 12, 16].

IMPLEMENTATION STEPS OF APPROPRIATE BIOTECHNOLOGIES FOR REMEDIATION OF POLLUTED SOIL

Ability to degrade hydrocarbons is widespread in bacteria, yeasts, fungi and algae in a wide range of environmental conditions, microorganisms can synthesize a broad spectrum of enzymes that ensure individual hydrocarbon degradation. Also have developed conceptual models and theories applicable to bioremediation of soils contaminated with hydrocarbons [5].

During the implementation of the technology for bioremediation of contaminated soil amenable to follow the next steps:

- Polluted site description
- Soil characterization
 - physical
 - chemical
 - geological
 - microbiological
 - vegetation, etc.

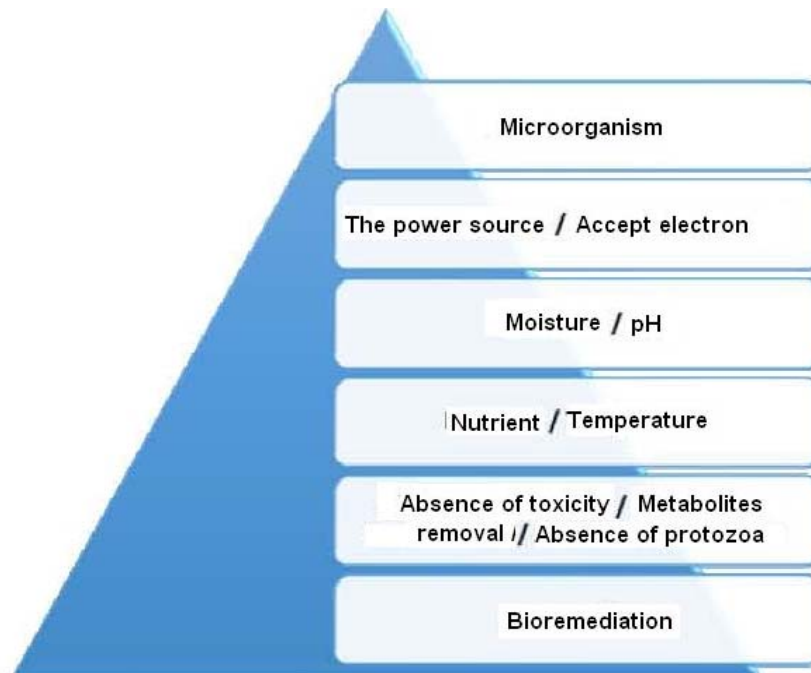


Fig. 2. Pyramid bioremediation

- Risk assessment
 - identification of pollution sources
 - identifying sources of pollution
 - assess the probability of consequences
 - high
 - average
 - low
 - negligible
 - assess the magnitude of the consequences
 - severe
 - moderate
 - bland
 - negligible
- Determining the objectives and means of remediation
 - Working decontamination using bioremediation techniques
 - Monitoring and restrictions on land use.

HYDROCARBON OXIDIZING MICROORGANISMS

The microorganisms capable of using petroleum hydrocarbons as sole carbon and energy source were termed Ahearn in 1973, hydrocarbon-oxidizing microorganisms or "hydrocarbonoclastice" [29]. For the remediation of environments contaminated by oil or oil residues are mainly used hydrocarbon-oxidizing

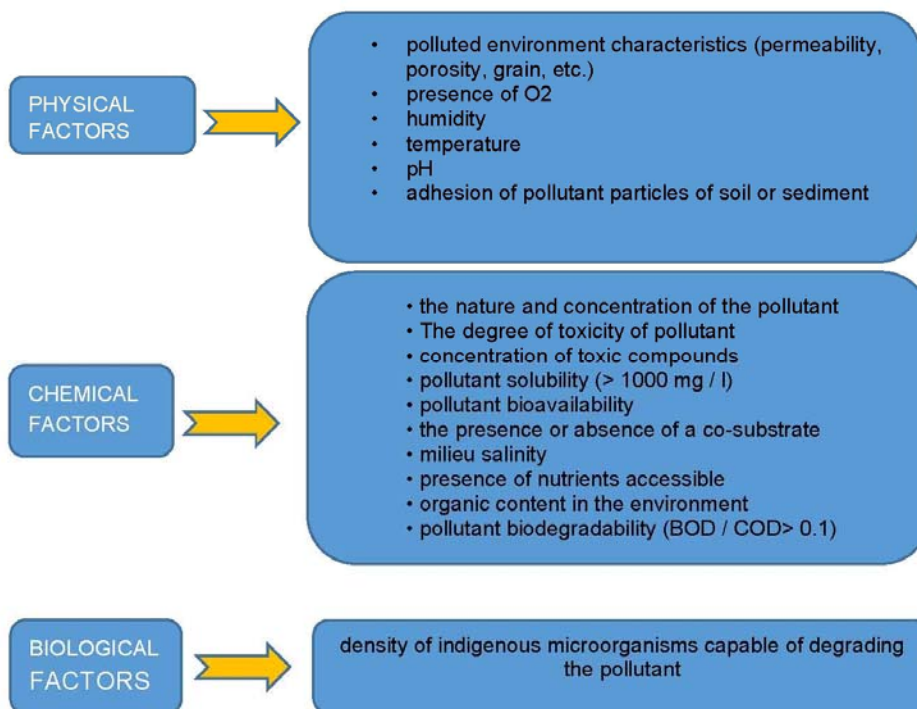
microorganisms, which are part of the five groups of microorganisms: bacteria (*Achromobacter*, *Acinetobacter*, *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Mycobacterium*, *Pseudomonas*, etc.); cyanobacteria (*Anabaena*, *Nostoc*, *Oscillatoria*); Yeast (*Candida*, *Rhodotorula*, *Sacharomyces*); filamentous fungi (*Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Trichoderma*) and algae (*Chlorella*, *Chlamydomonas*, *Dunaliella*, etc.).

The group most used in bioremediation processes is the hydrocarbon-oxidizing bacteria are usually mobile, able to move and easier to steer towards food sources contaminated sites that may be posed by pollutant respectively. Also, hydrocarbon-oxidizing bacteria have a high degree of hydrophobicity of the cell surface, which causes a high degree of affinity for the hydrophobic hydrocarbon type pollutant substrates [29, 44, 47].

Microorganisms have the ability to use both gaseous hydrocarbons, as well as liquid and solid aliphatic, aromatic and asphalt, which they use as a source of carbon and energy series [29]

FACTORS INFLUENCES THE BIOREMEDIATION OF SOILS POLLUTED WITH OIL RESIDUE

Factors influencing bioremediation of soils are abiotic (physical and chemical) and biotic factors [29, 32].



BIOREMEDIATION TECHNOLOGIES

Worldwide there is a tendency to develop simple methods, fast, cheap and efficient, ensuring their application in-situ blocking the migration of pollutants in the discharge of oil in underground or other neighborhoods, destroying pollutants and restoring the natural [15, 29]. Decontamination technologies biodegradation (bioremediation) can help the biodegradation process to be accelerated. The biodegradation of petroleum hydrocarbons existing in different environments, in particular in the ground, is based on one hand on the use of microorganisms indigenous existing in nature and adapted to the pollutant in question and, on the other hand the introduction of micro-organisms specific species [25, 51].

The concept of bioremediation is accepted as a summation of the processes of decomposition of constituents of natural or synthetic, by activating certain strains of microorganisms specialized resulting in end products useful or acceptable in terms of its environmental impact [52, 53, 60]. Bioremediation can be applied "in-situ" (the area substrate polluted place where there was contamination) or "ex-situ" (in systems / equipment specially designed, which bring substrate polluted that will be treated by methods biological) [78, 82, 83].

Tehnologiile de bioremediere se pot aplica **pe loc („in situ") sau „ex situ,, (prin transportarea substratului poluat la instalații speciale de tratare).**

Bioremediation technologies can be applied on the spot ("in situ") or "ex situ" (by transporting the polluted substrate handling special installations).

The technology used for treating a polluted site depends on the site and the pollutant.

Various bioremediation treatments "in situ" to bind to a number of technologies:

- Bioremediation in situ (ISB);
- In situ accelerated bioremediation;
- Monitored natural attenuation.

IN-SITU BIOREMEDIATION (ISB) is the use of microorganisms to degrade the contaminants in place ("in situ") in order to produce the final compounds harmless. In situ bioremediation has advantages such as:

- complete destruction of contaminants,
- lower risks for workers on site,
- lower costs for installation and operation.

Bioremediation "in situ" Accelerated used where desired growth rate of biotransformation of

contaminants, speed can be limited by a lack of nutrients needed, the lack of donors or acceptors of electrons. The purpose technology of bioremediation "in situ" Accelerated is to increase the amount of biomass within the aquifer contaminated and thereby achieving efficient biodegradation of contaminants dissolved and absorbed [25, 30, 42].

NATURAL ATTENUATION MONITORED

Natural attenuation implementation requires a good knowledge of the site and a long-term monitoring. Monitored natural attenuation (intrinsic bioremediation) is another method of application of bioremediation "in situ". A component of natural attenuation is the use of microorganisms to degrade contaminants indigenous implicatți, without human intervention (without added nutrients) [11].

SOIL TREATMENT TECHNOLOGIES BY BIOREMEDIATION

BIOVENTING

Introduces oxygen into the contaminated unsaturated soils through a forced air circulation (air extraction or injection) to increase the concentration of oxygen and stimulate biodegradation. It is a technology that **stimulates the natural in situ biodegradation** of any biodegradable compound by providing oxygen aerobic microorganisms existing in soil.

BIOAUGMENTATION

Bioaugmentation is a process in which local or inoculated microorganisms (such as fungi, bacteria and other microbes) degrade (metabolize) organic pollutants in soil and / or groundwater and neutralize their effect nociv.

Activitatea microbes that occur naturally stimulated by aqueous solutions flowing through contaminated soil and increase the level of *in situ* biological degradation of organic pollutants or immobilization of inorganic. Nutrients, oxygen and other amendments may be used to increase desorption and bioremediation of pollutants in groundwater materials (Valérie Guérin, Pierre Menger, 2010).

PHYTOREMEDIATION IN SOIL

Phytoremediation is a process of using plants to remove, transfer, stabilization and destroy pollutants in **soil and sediment**. Pollutants may be organic or inorganic. Phytoremediation is a process of using plants to remove, transfer, stabilization and destroy pollutants in soil and sediment. Fitoremedierii mechanisms include advanced rhizosphere biodegradation, fitoacumularea, fitodegradarea and fitostabilizarea (33, 55, 70, 71, 75, 80, 81).

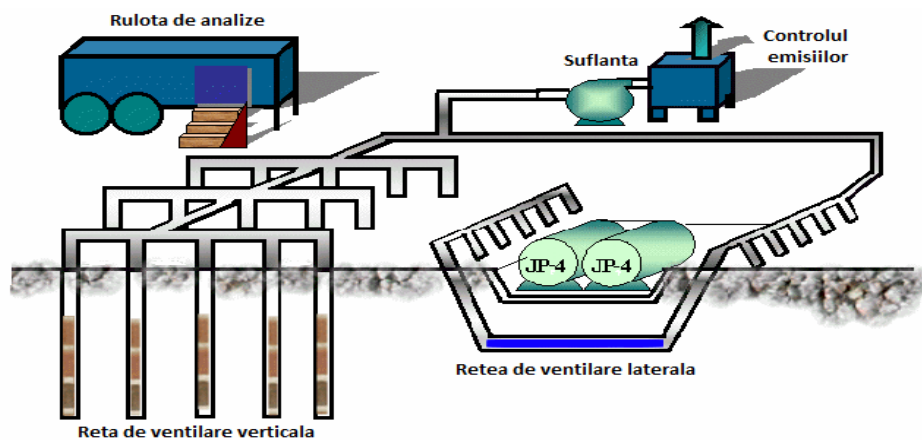


Fig. 4. Installation of bioventing

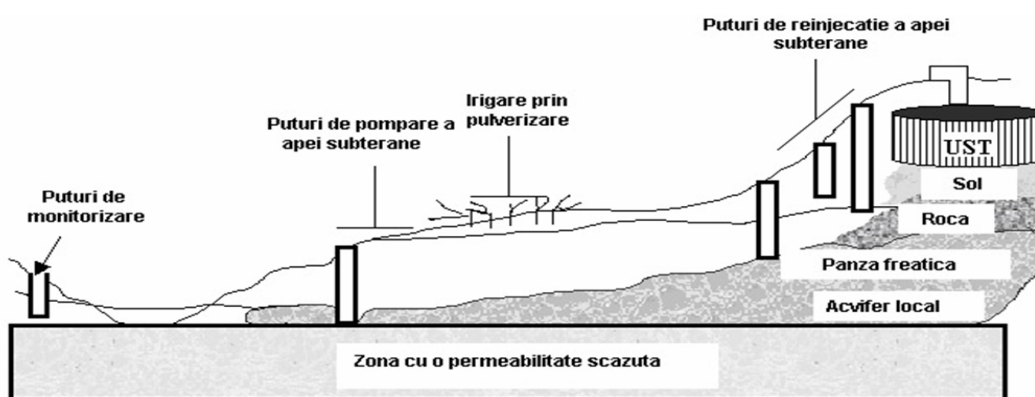


Fig. 5. Installation of bioaugmentation

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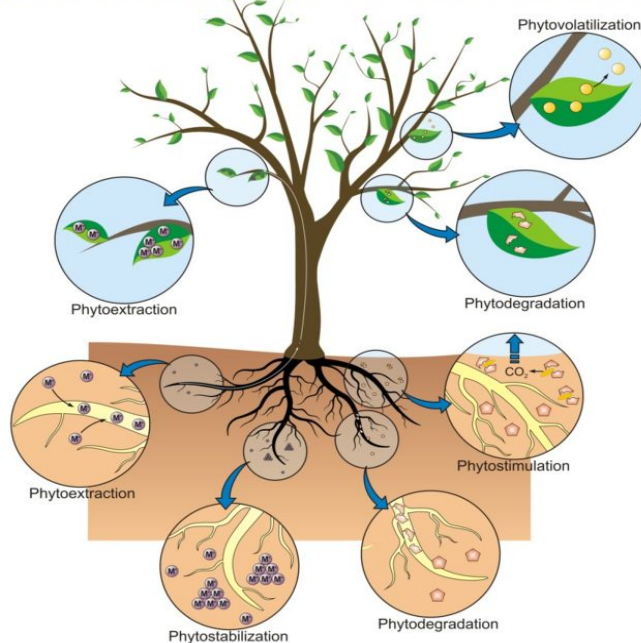


Fig. 6. Schematic representation of phytoremediation

BIOLOGICAL TREATMENT SITE OF POLLUTED SOILS

Ex-situ bioremediation is a biological process in which excavated soil decontamination area is lined above and aerated through various processes to enhance the degradation process by stimulating indigenous microorganisms [84].

BIOPILE

Excavated soil amendments are mixed and placed in enclosures on the surface. It is a process of composting mounds static aerated compost heaps is high and airy blower or vacuum pumps. Biopile treatment technology is widely excavated soils that are mixed with amendments and placed in treatment areas including water collection system infiltration and some forms of ventilation. It is used for reducing concentrations of petroleum constituents in excavated soils using biodegradation. Moisture, heat, nutrients, oxygen, and pH can be controlled to stimulate the biodegradation of [25].

COMPOSTING

The contaminated soil is excavated and mixed with extenders and organic amendments such as scrap wood, straw, manure and plant waste (for example

potatoes). Selecting suitable amendments provides sufficient porosity and provides a balance between carbon and nitrogen to promote thermophilic microbial activity.

Composting site is a controlled biological process by which organic pollutants (eg PAH) are transformed by microorganisms (aerobic and anaerobic conditions) in derivative products harmless stabilized. Normally, thermoelectric conditions (54-65°C) must be maintained to properly fertilize soil contaminated with hazardous organic pollutants.

Higher temperatures result of the heat produced by microorganisms during degradation of organic waste material. In most cases, this is achieved using microorganisms local pine.

The soils are excavated and mixed with fillers and organic amendments such as wood chips, animal and vegetable waste in order to increase the porosity of the mixture to be decomposed. Maximum efficiency degradation is achieved by maintaining oxygenation (such as return daily furrow) irrigation where appropriate, and careful monitoring of moisture in the soil and temperature (Valérie Guérin, Pierre Menger, 2010) [68, 75, 76 84,].

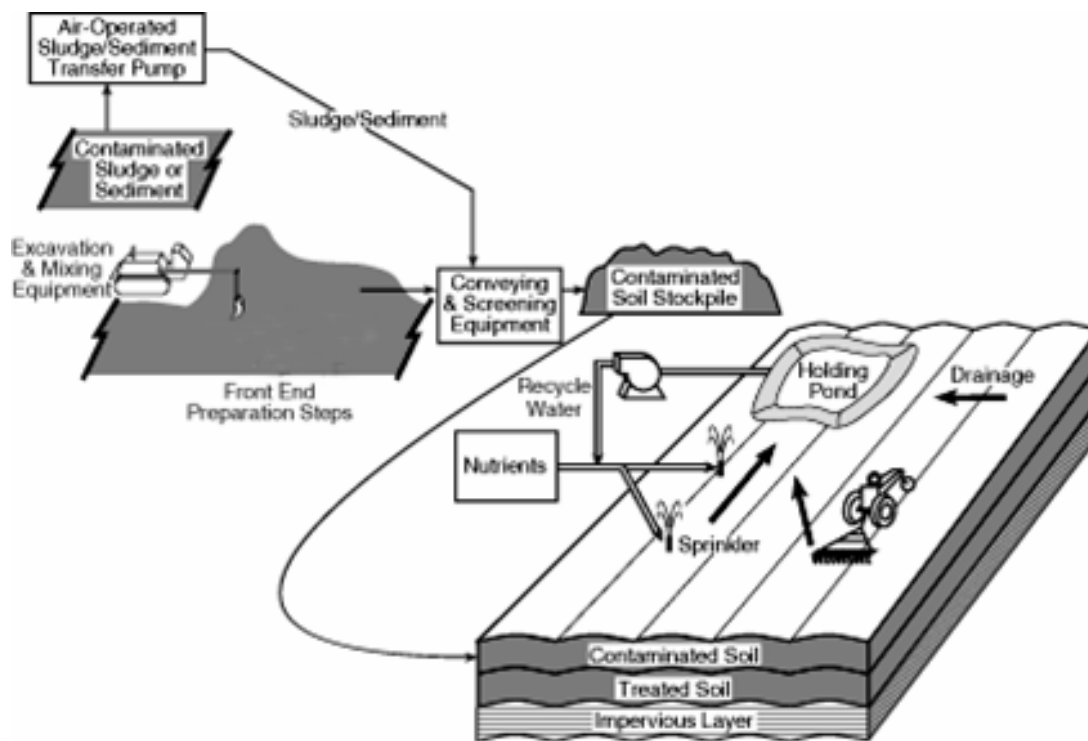


Fig. 7. Typical Biopile System for Solid Phase bioremediation (FRTR 2001)

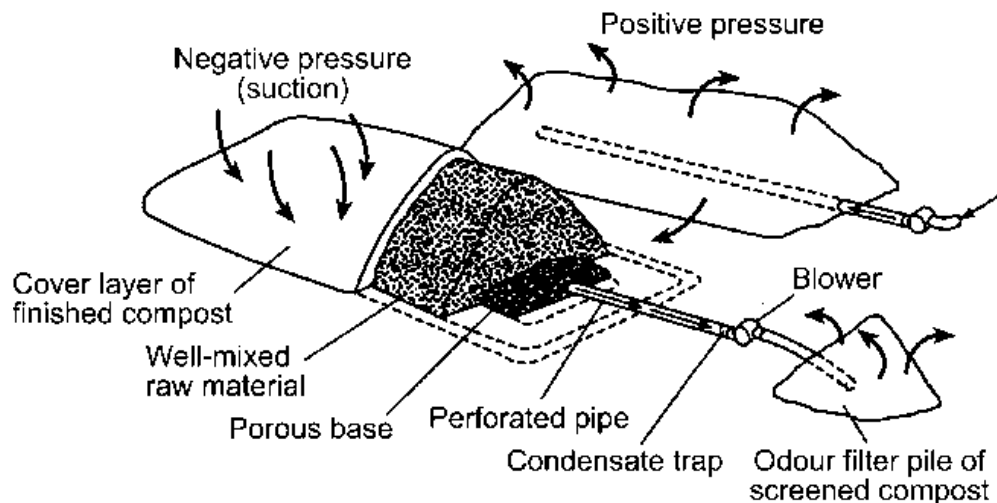


Fig. 8. Typical Windrow Composting Process Source: NRAES-114, 1999

FARMING

Contaminated soil, sediment or mud is excavated, is applied in layers aligned periodically returning or look for soil aeration. Land cultivation is a bioremediation technology used widely, typically involving the installation of pipelines and other methods for controlling leakage of pollutants, which require excavation of contaminated soil. Sits in polluted environments alignment layers returning regularly or is plowed for soil aeration. Soil conditions

are often controlled to optimize the degradation of pollutants [11,12, 15, 18, 23].

Normally controlled conditions include:

- The moisture content (usually through irrigation or spraying).
- Ventilation (at intervals determined by plowing, soil is mixed and airy).
- pH (limited around neutral pH by adding lime or crushed agrocalcar).
- Other amendments (such as fillers soil, nutrients, etc.).

Table 2. Advantages and disadvantages of bioremediation technologies

| Technologies | | Advantages | Disadvantages |
|--------------|------------------|--|--|
| In-situ | Bioventing | The contaminants can be fully rendered totally harmless substances (eg. Water, carbon dioxide, ethane). | Depending on the particular site, some contaminants may not be completely converted into harmless compounds |
| | Bioaugmentation | Bioremediation "in situ" often costs less than other options for remediation. | If the biotransformation process intermediates are formed, it may sometimes be more toxic than the parent compound or mobile |
| | Phytoremediation | Bioremediation "in situ" treatment volume can be achieved, which deals with both the dissolved and the sorbed contaminant. | Some contaminants may not be biodegradable (show recalcitrance to biodegradation). |
| Ex-situ | Biopile | Moisture, heat, nutrients, oxygen, and pH can be controlled to stimulate biodegradation. Generates a small number of waste. | Great length of time required for phytoremediation (usually several seasons) |
| | Composting | The soil is mixed with fillers and organic amendments such as scrap wood, hay, manure and plant wastes and organic pollutants are transformed by microorganisms (aerobic and anaerobic conditions) into harmless byproducts derived. | Limited groundwater depth (90-300 cm) |
| | Farming | | possibility of contaminant entering the food chain through the consumption of plants by animals. |

Polluted environments is usually treated in mounds up to 0,50m thick. Upon reaching the desired level of treatment, the ascent is removed and is building another. Removal is recommended only tip the relief and adding new remedied by adding more mounds of contaminated material from the remaining material and mixing them. This leads to immunization new material added with a microbial culture which actively degrade and reduce treatment times. Land treatment is a bioremediation technology used widely by the contaminated soils are turned (by plowing for example) and allowed to interact with the soil and climate of the site. The soil, climate and biological activity dynamically interact as a single system, degrading, transforming and immobilizing constituents pollution [31, 37, 41, 44, 63, 67].

BIOREMEDIATION AS A BUSINESS

Several companies have decided to develop and market technologies for biodegradation because of the explosive growth of traditional technologies treatment costs due to the growth of increasingly demanding regulations [25].

A private Romanian company that provides products, methods and equipment for solving environmental problems that occurred were caused by accidental or nature of various industrial and commercial sectors is of Ecological Solution Systems [6].

CONCLUSIONS

Soil Bioremediation is a method with a low cost effective decontamination of polluted soils and is one of the safest in terms of environmental impact.

Knowledge of microbial strains that can be used in the remediation of soils contaminated with hydrocarbons is very important because through this method of remediation degrades completely or partially pollutants, leading to the formation of compounds non-toxic for the environment and for human body.

ABSTRACT

The bioremediation of the soils which were contaminated with oil hydrocarbons constitutes an active preoccupation on the national and international level as well. In this paper is presented the situation of soils from Romania regarding their pollution with oil hydrocarbons and are enumerated the most important bioremediation technologies and the most important factors which can influence the treatment of oil hydrocarbons polluted soils.

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