Studies concerning the decontamination of hydrocarbons-polluted soil areas using bioremediation techniques

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Abstract. The accidental or historic contamination of soils with hydrocarbons, in areas crossed by oil pipelines or where oil- or gas-extraction installations are located, is a major concern and has significant financial and ecological consequences, both for the owners of those areas and for the oil transportation or exploitation companies. Therefore it is very important to find the optimal method for removing the pollution. The current paper presents measures, mainly involving bioremediation, recommended and applied for the depollution of a contaminated area in Romania. While the topic of dealing with polluted soils is well-established in the Romanian speciality literature, bioremediation is a relatively novel approach and this paper presents important considerations in this regard. Contaminated soil samples were taken from 10 different locations within the targeted area and subjected to a thorough physical and chemical analysis, which led to determining a specific scoring table for assessing the bioremediation potential of the various samples. This has allowed the authors to establish for each of the sampled areas the best mix of factors such as nutrients (nitrogen, phosphorus, potassium), gypsum, microelements etc., that would lead to obtaining the best results in terms of the contaminants' biodegradation.

1. Introduction

The contamination of soils with oil-derived products is a very important problem nowadays, because the presence of hydrocarbons in the soil tends to affect the normal biological circuits, blocking the development of plants [1, 2].

This type of pollution can have several sources, among which can be mentioned:

- Accidental failures of hydrocarbons pipelines;
- Exploitation of research and exploitation wells;
- Leakage from hydrocarbons storages or losses during their transportation;
- Road and railway accidents which involve oil tanks;
- Marine pollution due to the activity of oil rigs;
- Pollution of water courses and their shores by intentional or accidental spilling. [1]

Pipeline failures alone have accounted for the spilling of around 6.4 million liters of oil on land, in 2001. [3]

While there have been proposed and developed several types of solutions for the depollution of soils contaminated with hydrocarbons – among others chemical methods, thermal methods, pedological methods etc. - bioremediation is a relatively new approach that is gaining more and more attention and appreciation due to its benefits.
Bioremediation is a technique that uses live microorganisms to generate enzymes that would attack and degrade environmental contaminants into less toxic forms. The microorganisms can originate from the polluted environment itself, or they can be brought there from other areas. The process often involves manipulating the environmental parameters so that the microbial growth is encouraged and the degradation is accelerated. [1, 4]

Bioremediation has several advantages over the „conventional” depollution technologies. Firstly, its application is relatively cheap. Also, it involves reducing oils to mineral compounds, while the chemical methods, for example, merely transfer the contaminant from one environmental factor to another one. Also, it can be considered as a „green” technology, being based on a natural depollution process.

Currently there can be identified two main directions for the bioremediation of soils:
- bioaugmentation, that involves adding bacteria known to efficiently contribute to the degradation of oil are added to the already existing microbial population;
- biostimulation, that involves stimulating the growth of indigenous oil-degrading microorganisms by adding nutrients and other materials. Recent field studies have proven that biostimulation is more efficient that bioaugmentation because adding more microorganisms does not seem to significantly improve the degradation of hydrocarbons more than the simple addition of nutrients [5, 6]

The main steps in selecting the bioremediation treatment and the response plan include:
- Pre-treatment assessment; this step analyses whether bioremediation is a viable option, based on the type and concentration of contaminating hydrocarbons, on the presence of biodegrading microorganisms, on the type of affected soil and not least on environmental factors such as the pH and the temperature;
- Setting up a plan for treatment and monitoring; this step involves selecting agents that would limit the treatment’s speed (for example nutrients) and determining the strategies for applying these agents;
- Assessing the treatment’s evolution and ending it; after implementing the treatment, analyses have to be carried out periodically to assess its efficiency and to determine the optimal moment for ending the treatment. [7]

Bioremediation can be applied in-situ, to the soil directly on the pollution site, or ex-situ, on soil that has been removed from the polluted area by excavation.

While the in-situ bioremediation techniques are generally more desirable because they imply less costs and less disturbance to the environment, they are limited by the depth of the soil that can be treated efficiently. Often, an optimal diffusion of oxygen in soil, a very important factor in the successful development of biodegrading microorganisms is possible only for depths up to 0.3 m. Also, on-site treatments require a maximal content of 8% TPH in the soil.

Ex-situ techniques involve excavating the contaminated soil and placing it on an impermeable barrier. They require airing equipment, equipment for providing nutrients and generally a large volume of work. The main applicable ex-situ treatment types are landfarming, composting and bio-stacking.

Starting from analyzing the various factors influencing the biodegradation rate of contaminants and thus the success of bioremediation, in this paper the authors present an attempt at a systematic approach to applying a bioremediation treatment, based on an actual pollution case.

2. Assessment of the importance of the main factors influencing the selection of bioremediation treatments. Development of an assessment matrix

Based on earlier studies and on the speciality literature [3, 4, 6, 8, 9], the authors have determined that the main factors that need to be taken into account for selecting the bioremediation approach include:
- The type of contaminating oil, the total petroleum hydrocarbons (TPH) in the soil and the “age” of pollution (expressed using the ratio between hydrocarbons and phytane as reference);
- The type of soil, its salinity, its pH, temperature and humidity;
- The presence of nutrients (N, P, K), of oxygen and of microelements in the soil;
- The existence of a microbial population capable of degrading the pollutants.

The microbial development and activity are affected by the environment’s pH, temperature and humidity. The bacteria usually associated with quick biodegradation of organic matter require a pH-neutral environment, so it is important to create optimal conditions in this regard. For example, if the soil is too acid, the pH value can be increased by adding lime.[8]

The hydrocarbons are more easily degraded in aerobic conditions, so providing enough oxygen in the soil is another important factor. This is why bioremediation is usually applied together with soil loosening methods.

The soil’s structure can decide the efficiency of supplying the air, water and nutrients that are needed by the microorganisms. Therefore, among other materials, straw or sawdust can be added to the soil to improve its structure.

An important factor is also the so-called pollution aging. This refers to the fact that oils tend to change their composition over time due to chemical reactions, biotransformation, volatilization etc. However, some hydrocarbon molecules such as phytane or pristine are more resistant to the microbial degradation than for example C18-hydrocarbons, so over time the ratio between C18 hydrocarbons and phytane will decrease as the oil is biodegraded by the microorganisms.

The soil’s salinity can be expressed by means of the electrical conductivity. The balance between the content of Na and Ca+Mg in the soil can be corrected by adding gypsum. Gypsum, however, cannot be added in amounts larger than 10 t/ha every 6 months, so if the soil’s salinity would require a larger amount of gypsum, it needs to be added in several batches over this amount of time.

Starting from the characteristic values for each of the factors described above and on previous researches in this regard [4], the authors have developed an assessment matrix, presented in table 1. This assessment matrix also allows to define global biodegradability ranges based on the total number of points for each of the cases mentioned above and, in correlation with the initial TPH values, there can be determined the optimal duration of bioremediation treatments.

### Table 1. Pollutant biodegradability assessment matrix

| Parameter            | Very easily biodegradable (4 points) | Easily biodegradable (3 points) | Average biodegradability (2 points) | Difficultly biodegradable (1 point) | Very difficultly biodegradable (0 points) |
|----------------------|--------------------------------------|---------------------------------|------------------------------------|-------------------------------------|------------------------------------------|
| TPH (%)              | 1-3                                  | 3-5                             | 5-10                               | 10-15                               | >15                                      |
| EC, μS/cm            | <2000                                | 2000-4000                       | 4000-8000                         | 8000-14000                         | >14000                                   |
| Microorganisms (MPN/g soil) | ≥ 107                              | ≥ 105                           | ≥ 104                             | ≥ 103                              | < 103                                    |
| Clays (%)            | < 10                                 | 10 - 20                         | 20 - 40                           | 40 - 60                            | > 60                                     |
| API density          | > 30                                 | 25 - 30                         | 20 - 25                           | 15 - 20                            | < 15                                     |
| Pollution age (C18/phytane) oil | soil ≈ (C18/phytane) oil            | (C18/phytane) oil ≈ 75% (C18/phytane) oil | (C18/phytane) oil ≈ 50% (C18/phytane) oil | (C18/phytane) oil ≈ 25% (C18/phytane) oil | (C18/phytane) soil almost insignificant |
| pH                   | 6.81-7.20                           | 5.81-6.80                       | 5.41-5.80                        | 5.01-5.40                         | <5.00                                    |
|                      | 7.21-7.80                           | 7.21-7.80                       | 7.81-8.40                        | 8.41-9.00                         | >9.00                                    |
| Global biodegradability | 28 points                          | 21-27 points                    | 14-20 points                     | 7-13 points                        | 0-6 points                               |
3. Target area. Analyses carried out on the contaminated soil
In order to check the applicability of the assessment matrix presented above, the authors have focused their research on an area that has been subjected to an accidental pollution with hydrocarbons near Poarta Alba, Constanța county, Romania.

From this area, in order to better assess the pollution and to be able to provide specific bioremediation solutions for different degrees of contamination, there have been analysed soil samples from 10 different locations within the visibly polluted area.

These soil samples were analysed from a physical, chemical and microbiological point of view.

The physical analyses targeted elements such as the texture, porosity, hydraulic conductivity and apparent density of the soil.

The chemical analyses in turn referred to determining the pH, the content of nutrients (N, P, K), the content of clays, the total petroleum hydrocarbons (TPH), the API gravity of the contaminating oil etc.

Microbiological analyses were carried out in order to determine the amount of hydrocarbons-oxidating bacteria and to isolate efficient strains that can be grown and multiplied ex situ and then reintroduced in the contaminated soil in order to carry out the actual biodegradation processes.

4. Results and discussion
The main results of the chemical and microbiological analyses for the 10 locations are presented in table 2.

Table 2. Results of the chemical and microbiological analyses

| Soil sample no. | TPH-IR, mg/kg | Bacteria, MPN/g soil | pH | Electrical conductivity μS/cm | Fe, mg/kg | Mn, mg/kg | Clays, % | API gravity of the oil | C18/phytane oil | C18/phytane soil |
|----------------|---------------|----------------------|----|-------------------------------|-----------|-----------|---------|-----------------------|----------------|------------------|
| A1             | 13900         | ≥10⁷                 | 8.2| 2060                          | 50        | 280       | 1.9     | 45                    | 2.5            | 2.5              |
| A2             | 21000         | ≥10⁷                 | 8.31| 2910                          | 1000      | 360       | 7.8     | 34                    | 2.31           | 2.3              |
| A3             | 119600        | ≥10⁷                 | 8.45| 590                           | 2900      | 480       | 0.2     | 34                    | 2.31           | 1.68             |
| A4             | 100900        | ≥10⁷                 | 8.06| 11200                         | 3200      | 270       | 0.4     | 34                    | 2.31           | 2.26             |
| A5             | 55300         | ≥10⁷                 | 8.49| 5960                          | 120       | 120       | 3.6     | 31                    | 2.07           | 1.14             |
| A6             | 203900        | ≥10⁷                 | 8.37| 1300                          | 550       | 160       | 1.6     | 31                    | 2.07           | 1.16             |
| A7             | 142000        | ≥10⁷                 | 8.28| 7980                          | 910       | 210       | 5.7     | 28.5                  | 2.25           | 1.64             |
| A8             | 65000         | ≥10⁷                 | 8.36| 10360                         | 18        | 230       | 6.9     | 34                    | -              | -                |
| A9             | 9700          | ≥10⁷                 | 7.62| 17100                         | 2420      | 440       | 10.5    | 28.8                  | 5.3            | 3.05             |
| A10            | 19300         | ≥10⁷                 | 7.81| 11200                         | 1220      | 490       | 9.6     | 28.8                  | 5.3            | 1.88             |

These values were then compared to those comprised in the global assessment matrix described above and the resulting matrix is presented in table 3.

Based on this matrix, there have been then designed specific bioremediation treatments for each of the 10 locations, as shown in table 4.
Table 3. Assessment matrix applied to the 10 analysed locations

| Soil sample no. | Score for TPH | Score for EC | Score for bacteria | Score for clays | Score for API density | Score for pollution age (C18/Phytane) | Score for pH | Total score | Assessment of global biodegradability |
|-----------------|---------------|--------------|--------------------|-----------------|-----------------------|----------------------------------------|--------------|------------|-------------------------------------|
| A1              | 4             | 3            | 4                  | 4               | 4                     | 4                                      | 2            | 25         | Easy                                |
| A2              | 4             | 3            | 4                  | 4               | 4                     | 4                                      | 2            | 25         | Easy                                |
| A3              | 1             | 4            | 4                  | 4               | 4                     | 3                                      | 1            | 21         | Easy                                |
| A4              | 1             | 1            | 4                  | 4               | 4                     | 4                                      | 2            | 20         | Average                             |
| A5              | 2             | 2            | 4                  | 4               | 4                     | 2                                      | 1            | 19         | Average                             |
| A6              | 0             | 4            | 4                  | 4               | 4                     | 2                                      | 2            | 20         | Average                             |
| A7              | 1             | 2            | 4                  | 4               | 4                     | 3                                      | 2            | 19         | Average                             |
| A8              | 2             | 1            | 4                  | 4               | 4                     | 0                                      | 2            | 17         | Average                             |
| A9              | 4             | 0            | 4                  | 3               | 3                     | 2                                      | 3            | 19         | Average                             |
| A10             | 4             | 1            | 4                  | 3               | 3                     | 1                                      | 2            | 18         | Average                             |

Table 4. The bioremediation treatments prescribed for each of the 10 locations in the targeted area

| Sample no. | Total treatment duration [months] | NPK treatment duration [months] | N [mg/kg soil] | P [mg/kg soil] | K [mg/kg soil] | Gypsum, stage I [g/m²] | Gypsum, stage II [g/m²] | Gypsum, stage III [g/m²] | Straw [kg/m²] | Surfactant [l/m²] |
|------------|----------------------------------|---------------------------------|----------------|---------------|-----------------|------------------------|------------------------|------------------------|---------------|-----------------|
| A1         | 24                               | 6                               | 596            | 119           | 119             | 0                      | 0                      | 0                      | 10            | 3               |
| A2         | 24                               | 9                               | 900            | 180           | 180             | 0                      | 0                      | 0                      | 10            | 3               |
| A3         | 84                               | 51                              | 5126           | 1025          | 1025            | 0                      | 0                      | 0                      | 10            | 3               |
| A4         | 96                               | 43                              | 4324           | 865           | 865             | 1000                   | 170                    | 0                      | 10            | 3               |
| A5         | 72                               | 24                              | 2370           | 474           | 474             | 590                    | 0                      | 0                      | 10            | 3               |
| A6         | 108                              | 87                              | 8739           | 1748          | 1748            | 0                      | 0                      | 0                      | 10            | 3               |
| A7         | 96                               | 60                              | 6086           | 1217          | 1217            | 470                    | 0                      | 0                      | 10            | 3               |
| A8         | 72                               | 28                              | 2786           | 557           | 557             | 1000                   | 170                    | 0                      | 10            | 3               |
| A9         | 24                               | 4                               | 416            | 83            | 83              | 1000                   | 1000                   | 900                    | 10            | 3               |
| A10        | 24                               | 8                               | 827            | 165           | 165             | 1000                   | 560                    | 0                      | 10            | 3               |

As can be seen from table 4, the total treatment duration depends both on the global biodegradability and on the initial TPH value of the contaminated soil. Even if the global biodegradability index points to an easy biodegradation, a large initial TPH value (e.g. 119600 mg/kg for A3) can impose a long total treatment duration (up to 7 years), whereas if the initial TPH value is very large and the global biodegradability is only average, the treatment duration may surpass 8 years.

The addition of a surfactant is necessary in order to increase the oil’s mobility [4] and thus to facilitate a better contact between the microorganisms and the contaminant.

The duration of the treatment with nutrients depends on the contents in nitrogen, phosphorus and potassium already present in the soil, on the requirement to achieve an optimal recommended C:N:P:K ratio that is comprised between 100:10:1:1 and 100:5:1:1 [10] and on following recommended monthly doses:

- N - 30 g/m²;
- P₂O₅ - 12 g/m²;
- K - 7 g/m².

In all cases, when the TPH value drops below 3% (30000 mg/kg), there can be applied a phytoremediation treatment to complement bioremediation, by planting specific plant species for the respective terrain type (willow and poplar for wooded areas or alfalfa and oat for agricultural lands.) [1]
5. Conclusions

The current paper has presented a scientific, methodical approach to applying a bioremediation treatment in order to eliminate or at least reduce the contamination of soils with hydrocarbons.

The assessment matrix developed by the authors can, in correlation with other considerations and factors, taken as a fundament for unfolding a successful bioremediation treatment.

Also, an area accidentally polluted with hydrocarbons has been assessed from a physical, chemical and biological point of view and the assessment matrix was applied to it for determining the optimal response.

In future, the authors intend to continue their researches and if possible refine the assessment matrix.

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