In-situ Bioremediation of Oil Contaminated Soil - Practical Experiences from Denmark

Anne Louise Gimsing, Jan Boeg Hansen, Erik Permild, Gry Schwarz & Erik Hansen
Cleanfield, Mesterlodden 36, DK-2820 Gentofte, Denmark. E-mail: alg@cleanfield.com

Abstract
Contaminated soil poses a serious threat to human health and ecosystems. Remediation of contaminated soils is therefore required and an alternative to the conventional method of excavating and depositing the soil is needed. For a decade Cleanfield has remediated oil polluted soils by a combination of bioaugmentation and biostimulation. The technology is continuously optimized by taking the latest scientific results into account to ensure faster remediation. To optimize remediation conditions we add electronacceptors and nutrients and we add a detergent to enhance bioavailability. Introduction of bacteria and nutrients, electronacceptors and detergent takes place either through permanent wells or by lance depending on the site. To get a flow through the soil we pump groundwater from the soil, which is biologically treated before the water is discharged to the sewer. Practical experience from several remediation projects show that bioremediation does work in real life and that oil contaminated soil can be remediated in-situ within 1½ - 2 years.

INTRODUCTION

The last decade has seen the emergence of new and promising biological in-situ techniques, which remediate the soil on-site at a markedly lower cost than excavation, while at the same time being more environmentally sustainable. But despite convincing results, still only a few sites are remediated this way.
For the last decade Cleanfield has remediated oil polluted soils in-situ by a combination of bioaugmentation and biostimulation.

INPUTS TO EFFICIENT BIOREMEDIATION

In in-situ bioremediation of oil contaminated soil by bioaugmentation oil degrading bacteria are injected into the soil matrix and the conditions for degradation are optimized by adding nutrients and electronacceptors and a detergent to desorb the hydrocarbons.

Bacteria
Many bacteria are capable of degrading the constituents of oil /1, 2/, and the oil degrading bacteria are the most important input in our bioremediation technique. Bacteria for oil hydrocarbon degradation are commercially available as freezedried bacteria, which after propagation to a minimum of $2 \cdot 10^8$ CFU/ml can be used in bioremediation. We use a consortium of degraders with *Pseudomonas sp*. and *Rhodococcus sp*. as the most predominant once.

Nutrients
The bacteria degrade the oil to get energy and building blocks for their biomass, but the bacteria will also need the essential nutrients as the hydrocarbons only contain the elements hydrogen and carbon. Especially the macronutrients nitrogen and phosphorus are important to

ensure a fast and complete degradation of the oil /3, 4/. It is important that the nutrients are supplied at the right ratio in order to ensure optimal growth conditions for the bacteria. Alexander et al. /3/ finds that a C:N:P ratio of 100:10:2 is the optimal, Zhang et al. /5/ finds that the ratio 100:5:1 is the optimal and Vieira et al. /6/ finds the ratio 100:20:2,7. Even if the different studies do not reach exactly the same ratio it is within the same range.
Adding the nutrients will not only stimulate the added bacteria, but also the bacteria already present in the soil and they will also degrade the oil.

Electronacceptors
Under aerobic conditions the oil hydrocarbons are degraded according to the following reaction:

$$C_{16}H_{34} + 24\tfrac{1}{2}\, O_2 \rightarrow 16\, CO_2 + 17\, H_2O$$

It can be seen that a complete degradation (mineralization) takes place.
If large quantities of oil are present in the soil the oxygen in the soil will be depleted very fast /7, 8/. When the oxygen has been depleted the anaerobic bacteria will take over and they will use other electronacceptors /7, 9/. Depending on the redoxpotential nitrate, iron or sulphate can be used as electronacceptors:

$$C_{16}H_{34} + 19{,}6\, NO_3^- + 3{,}6\, H^+ \rightarrow 16\, HCO_3^- + 9{,}8\, N_2 + 10{,}8\, H_2O$$

$$C_{16}H_{34} + 98\, Fe(OH)_3 + 82\, HCO_3^- + 82\, H^+ \rightarrow 98\, FeCO_3 + 246\, H_2O$$

$$C_{16}H_{34} + 12{,}25\, SO_4^{2-} + 8{,}5\, H^+ \rightarrow 16\, HCO_3^- + 12{,}25\, H_2S + H_2O$$

Under very anaerobic conditions methanogenesis will take place:

$$C_{16}H_{34} + 11{,}25\, H_2O \rightarrow 3{,}75\, HCO_3^- + 12{,}25\, CH_4 + 3{,}75\, H^+$$

Oil can hence be degraded even under very anaerobic conditions, but the energy yield for the bacteria is less than if oxygen is used as the electronacceptor. If nitrate is used as an electronacceptor the bacteria will get approximately 95 % of the energy they get by aerobic degradation, if iron is used the yield is approx. 44% and if sulphate is used the yield is approx. 28% of aerobic degradation. If methanogenesis takes place the yield is less than 2% of the yield by aerobic degradation /7, 9/. The lower energy yield results in slower degradation and hence a longer remediation time.
To make remediation fast it is therefore preferable to have aerobic conditions. An efficient way to get oxygen into the soil is by adding a dilute hydrogen peroxide solution to the soil. When the hydrogen peroxide gets in contact with the soil iron and manganese and microbial catalases will catalyse the decomposition of hydrogen peroxide and oxygen and water will be formed /8, 10/:

$$H_2O_2 \rightarrow \tfrac{1}{2}\, O_2 + H_2O$$

Detergent

Hydrocarbons are very hydrophobic compounds and therefore they are strongly sorbed to the soil, especially to the soil organic matter /11/. The consequence of the strong sorption is that the bioavailability of the hydrocarbons is limited and hence the degradation slows down /12/. Adding detergents to the soil help desorbing the hydrocarbons and hence speed up remediation. The detergents desorb the hydrocarbons by a number of mechanisms. They can form micelles if the concentration is above the critical micelle formation concentration, and the hydrocarbons dissolves in the hydrophobic center of the micelle /13/. They also work by lowering the surface tension of water and hence also the tension between the surface of the soil particles and the soil water and this enhance bioavailability /12/. Finally they can make the surface of the bacteria more hydrophobic which help the bacteria absorb the hydrocarbons /14, 12/.

We use microbiologically produced detergents called rhamnolipids, characterized by having to rhamnose moeities and a fatty acid tail /12/. Rhamnolipids are biodegradable and have a low toxicity /15/.

CASES

Case 1

In this project we remediate a minor recent heating oil spill mixed with an older contamination with heavier oil at a site with surface near groundwater, which is also contaminated.

We have installed six injection wells and five pumps. The groundwater moves from the injection points to the pumps.

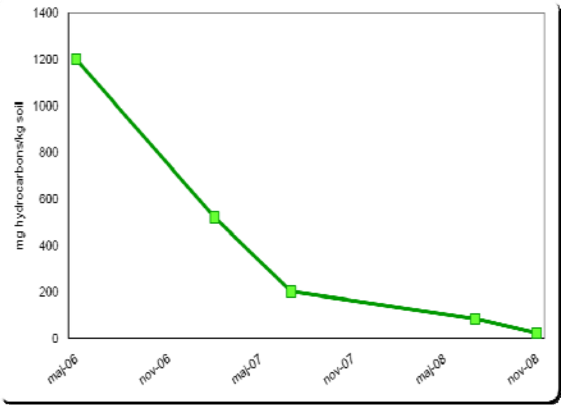
In the injection wells we injected diluted hydrogen peroxide, detergent, bacteria and nutrients. The groundwater is pumped from the soil to a biocube where bacteria degrade the oil so that the outlet water can be discharged to the sewer.

Within four months we have achieved more than a 50% reduction in the amount of oil present in the soil. The concentration in the water has increased demonstrating the desorption by the detergent.

Case 2

Remediation at a site with two heating oil spills. Before the remediation was initiated the first spill covered 120 m^3 with a max.concentration of TPH of 3400 mg/kg. The other spill covered 240 m^3 with max. 7500 mg/kg TPH.

The contamination has been treated by injecting diluted hydrogenperoxide, detergent, bacteria and nutrients through 13 injection wells since july 2006. Groundwater, which was also contaminated, was pumped from the ground and treated before dicharging to the sewer.



The project is now finished, and the above shows results from one of the monitoring points.

Conclusions

Based on the scientific literature and experience from actual remediation projects efficient biodegradation requires input of:

- Bacteria capable of degrading oil. These can be added, as in bioaugmentation, or the naturally present bacteria can be stimulated.
- Nutrients for the metabolic processes of the bacteria, especially nitrogen and phosphorus is important for the bacteria to perform optima
- Electronacceptors, preferably oxygen, for the degradation to proceed as fast as possible
- Detergent to enhance bioavailability and increase the degradation rate

Our remediation projects have demonstrated that bioremediation does work in real life and that bioremediation typically takes 1½ - 2 years.

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