St. Lawrence TECHNOLOGIES

ABSTRACT

In view of the limitations of current methods to assess both the potential and the performance of environmental technologies, the National Research Council's Biotechnology Research Institute (BRI) has conducted in-field validity testing of a protocol to assess the biotreatability of soil contaminated with light hydrocarbons. This protocol monitors the chemical, microbiological, biochemical and toxicological effectiveness of the biotreatment. A characterization of the soil chemistry, the use of DNA probes, and the measurement of the mineralization rate of control pollutants and respiratory activity are the essential elements of this multi-disciplinary protocol. Using this approach, the processes involved in the biotreatment of a contaminated soil in concrete cells can be monitored.

ST. LAWRENCE ACTION PLAN

Environment Canada
Protection
Québec Region

Environnement Canada
Protection
Région du Québec

CONTAMINATED SOIL

A METHOD TO ASSESS SOIL BIOTREATABILITY AND TO MONITOR BIOPROCESS EFFECTIVENESS

MAIN FEATURES

- **Comprehensive methodology**
  - A series of analyses to monitor the chemical, microbiological, biochemical and ecotoxicological characteristics of a bioprocess.
- **Gene probes**
  - Innovative tools to quantify specific bacteria genetically capable of degrading certain aliphatic and aromatic hydrocarbons.
- **Mineralization tests**
  - Analysis to monitor the breakdown of representative pollutants labeled with carbon-14 to assess the specific activity of microorganisms indigenous to the contaminated soil.
- **Respirometer**
  - Tool to determine the overall respiratory activity of microorganisms in a soil sample.

National Research Council Canada
Biotechnology Research Institute

Conseil national de recherches Canada
Institut de recherche en biotechnologie

PROJECT OBJECTIVES

To help in the management of soil contaminated by light hydrocarbons, the Biotechnology Research Institute (BRI) has developed a protocol to assess biotreatability of soils and to monitor biotreatment. The protocol is used to determine the biodegradation potential of a soil contaminated with light hydrocarbons.

A series of analyses is performed to provide a comprehensive chemical characterization of the soil, and to monitor and quantify the biotreatment's microbiological, biochemical and ecotoxicological effectiveness.

The BRI developed the protocol in the laboratory, then validated the methodology's applicability during a full-scale demonstration project.

The demonstration project monitored the biotreatment of a clay soil contaminated with light hydrocarbons, in concrete cells, during the winter. The project also demonstrated that:

- the effectiveness of a biotreatment using biopiles can be monitored using this methodology.
- using biopile technology, oil and grease concentrations of less than 1000 mg/kg can be attained.

BACKGROUND

Although bioprocesses are commonly employed to restore contaminated sites, the parameters that influence light-hydrocarbon biodegradation are not completely understood and therefore not entirely controllable. This can be attributed in part to:

- the heterogeneity of sites, soil types, and pollutants
- the limited information on the factors controlling microbial activity in soil
- the lack of quality controls in chemical, biological and ecotoxicological analysis during pilot-scale and full-scale testing
- the absence of standard methods to assess soil biotreatability and to determine the amount of time required for complete pollutant biodegradation
- the absence of a method to adequately monitor the effectiveness of a biological soil treatment system.

TECHNOLOGY

As shown in the diagram below, there are six steps to the BRI protocol.

For each step, several physico-chemical, biochemical, microbiological and ecotoxicological parameters are analyzed, as shown in the following table.

BIOTREATABILITY PROTOCOL

STEPS	OBJECTIVES
Physical analysis of soil	To determine whether the physical caracteristics of the soil are favourable to an acceptable biological activity
Chemical characterization of soil	To determine whether the chemical properties of the soil are favourable to biodegradation and to determine the presence of other pollutants that could inhibit this process
Determination of the microbiological and genetic potential of soil ecosystem	To verify whether the microorganisms in the soil possess the genetic capacity for biodegrading pollutants
Mineralization of control pollutants	To verify whether the pollutants can be effectively degraded by the microorganisms
Determination of biological activity by respirometry	To mesure the overall respiratory activity of the contaminated soil microflora compared with a control soil
Ecotoxicological assessment	To determine whether the biotreatment successfully detoxified the soil and to assess the potential transfer of toxicity to the groundwater

RESULTS

The application of the BRI protocol to a biopile treatment allowed the biotreatability of light hydrocarbons to be determined, and to monitor the chemical, biochemical,microbiological and, to a lesser extent, ecotoxicological characteristics of the bioprocess.

The study showed that existing bio-assays need to be improved in order to be used for measuring the toxicity of contaminated and/or treated soil.

The respirometric analysis showed significant fluctuations in biological activity in soils at different sampling points, demonstrating soil heterogeneity in the treatment cells.

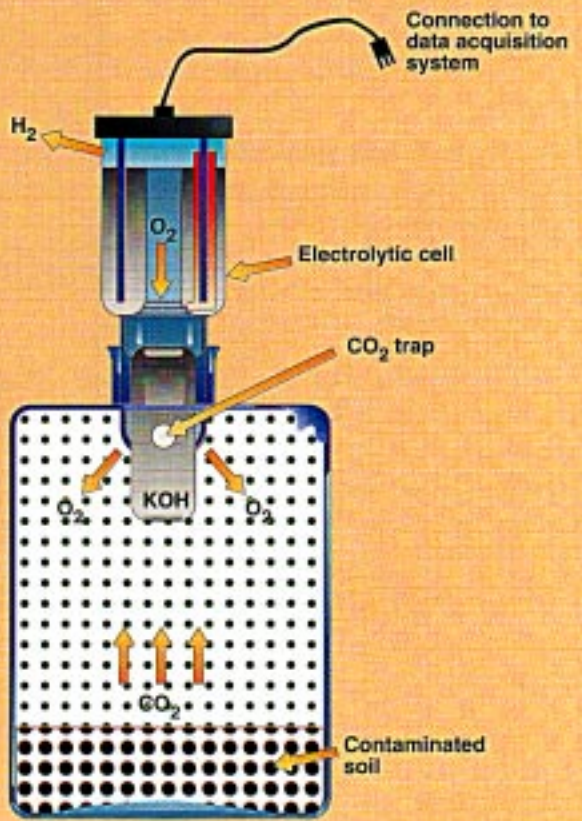
The use of genetic probes showed that, in this particular case, the heterotrophic bacteria responsible for the breakdown of hydrocarbons represented less than 1% of the viable soil bacteria, and they seemed to be more sensitive to fluctuations in temperature than the rest of the soil bacteria. Nevertheless, this sensitivity did not impede the biodegradation process during the winter.

The results also demonstrated that using a mass balance of polar and non-polar compounds, coupled with the quantification of target pollutants by GC/MS, permitted precise monitoring of oil and grease concentrations near the critical level of 1000 mg/kg.

PARAMETERS MEASURED

Physico-chemical	Biochemical
- Soil particle size distribution - Mineral oil and grease - Hydrocarbon components by GC/MS - Monitoring of target compounds by GC/MS - NH_4^+, NO_3^- and PO_4^{-3} ions - C/N ratio - pH - Moisture content	- Mineralization of carbon-14 labelled representative pollutants - Respiratory activity of microorganisms by electrolytic respirometer
Microbiological	**Ecotoxicological**
- Enumeration of mesophilic (optimal growth between 20°C and 45°C) and psychrophilic (optimal growth at about 15°C) microorganisms - Enumeration of specific hydrocarbon-degrading bacteria using catabolic gene probes	- Germination of seeds - Earthworm mortality - Microtox - Root growth - Inhibition of phytoplanktonic algae - growth, *Selastrum capricornutum* - Cladocera mortality

SCHEMATIC OF RESPIROMETRIC REACTOR



POTENTIAL AND LIMITATIONS

Potential

The innovative use of molecular tools, such as gene probes, demonstrates their potential application for determining the biotreatability of contaminated sites and for monitoring biotreatment. These tools are very versatile and may be applied to various pollutants, and used on different substrates such as soil, water and air.

This approach could lead to the further development of standard procedures for assessing the biotreatment of soils contaminated with hydrocarbons, and/or to regulations governing these projects.

Limitations

At present, few laboratories can perform all the analyses in the BRI protocol.

The methodology is currently limited to off-site treatment bioprocesses, although data on *in situ* applications will be available in the near future.

INFORMATION

This data sheet is based on the results of technology development and demonstration projects carried out by the Biotechnology Research Institute of the National Research Council of Canada, with financial assistance from Environment Canada. Documents having to do with these projects, are available:

Report entitled, *Demonstration of a new biotreatability protocol to monitor a bioprocess for the treatment of contaminated soils*, contains detailed information on the application of the BRI protocol under the demonstration project. It is available from the **Biotechnology Research Institute.**

Technical fact sheet published as part of the "St. Lawrence Technologies" series, and entitled, *The aerobic biodegradation of hydrocarbon- contaminated clay soil in winter conditions*, is available from **Environment Canada.**

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St. Lawrence Technologies data sheets are intended for all companies, industries, organizations and individuals interested in new environmental technologies. They are produced by the Technology Development Section, Environment Canada, as part of the St. Lawrence Action Plan. They serve to disseminate the results of technology development and demonstration projects conducted in the following four sectors: industrial wastewater; contaminated soil; hazardous wastes; contaminated sediment.

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