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Bioremediation in Canada: Research Directions

By:

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Management Perspective

This report will be published as a chapter in a book entitled "Bioremediation: Principle and Practice", Subhas Sikdar and Robert Irvine, editors, as part of an international section discussing bioremediation research activities in countries other than the U.S.

As everywhere else in the world, the Canadian research community has refocussed its attention in the last few years to bioremediation as an environmentally acceptable, hopefully cost effective means of restoring contaminated soils. Is the work done here different than in other countries? The reader will certainly be able to judge by comparing the numerous chapters in this book.

The level of research effort has probably quadrupled in the past five years and ranges from the very fundamentals of bioremediation at the molecular level, with groups studying gene probes as techniques of finding appropriate enzymic activities within a bacterial population to applied research where nutrients and soil texture modifiers are mixed to a soil to enhance the process of degradation. Some of these techniques will be tested at the National Water Research Institute within the newly created Aquatic Ecosystem Remediation Evaluation Facility (AQUEREF). This facility provides near field scale conditions while allowing the scientific control of conditions only available within a laboratory.

In Canada, research is done not only within government sponsored institutes and universities, but also by the consulting industry, who typically have the final say in what they can sell to the clients. The technologies to be applied must also fit within the realms of regulation, and in that area, Canada seems to be amongst the most progressive countries in allowing engineered organisms an equal chance to that of indigenous population, at least as far as legal constraints are concerned.

BIOREMEDIATION IN CANADA: RESEARCH DIRECTIONS

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INTRODUCTION

The Canadian research scene for the bioremediation of contaminated soils and groundwater has evolved in the past five years from the research efforts of a few selected individuals to being the major focus of remediation research. More than half of the proposals submitted for funding for the development of novel remedial technologies are based on biological processes, shifting from the physico-chemical methods, such as soil venting or pump-and-treat. In this respect, the Canadian research community seems to follow the world-wide trend towards natural, less invasive and maybe less expensive, technologies for remediation.

The research activities themselves range from studies of the fundamental processes at the genetic level, to large field scale comparisons of technologies. The rate of acceptance of the biotechnologies as they are developed has been staggeringly rapid, such that some have moved from the laboratory, to the field, to commercial application within a few years. This is very Canadian. The willingness to move to full scale and being given the permission to do so by regulators has been the trademark of the remediation successes in Canada. Biopiles are now accepted practice for the remediation of petroleum contaminated shallow soils.

This chapter will give an overview of the research efforts in Canada. Most of the information is from reports and conference proceedings, which are not widely distributed outside the country. Hopefully this account will give the reader enough information such that he will not hesitate to contact the individual researchers for more information.

1. Fundamental processes:

Research on the fundamental processes of bioremediation is being conducted mainly at the universities of Waterloo and Guelph, in Ontario, and at the École Polytechnique and the Biotechnology Research Institute (BRI) of the National Research Council of Canada, both located in Montreal, Quebec.

Genetics

One of the most innovative basic technique that has been developed in recent years is the use of gene probes to find whether bacteria have the genetic ability to degrade certain compounds. A research group under the direction of Dr. Charles Greer (Greer and Beaumier, 1993) at BRI worked on the application of catabolic gene probes to survey and monitor soils contaminated with petroleum hydrocarbons using the *alkB, xylE* and *ndoB* gene probes, which are specific for aliphatic and aromatic degradation. The *alkB* gene codes for the enzyme alkane hydroxylase whereas the *xylE* gene codes for catechol dioxygenase and the *ndoB* probe is one of the three genes encoding for naphthalene dioxygenase. They found that the bacterial populations present in soils generally contained the appropriate genetic potential to degrade the hydrocarbons and that failure to do so must then be attributed to other environmental factors. The technique was also applied to PCB contaminated soils, where it was found that the bacterial population could generally effect the initial step of the degradation from chlorobiphenyl to chlorocatechol, but that the occurrence of bacteria capable of complete PCB mineralization was very low.

The technique can also be used as a tool to enrich bacteria with specific catabolic abilities (Gamati, 1993; Greer, Masson et al., 1993). A bacterium that has shown a specific catabolic activity can be isolated, cultured and reintroduced to enhance the overall degradation rate. The survival and activity of bacteria introduced into contaminated environments as bioremediation agents are essential, if this technique is to be used successfully. Reporter genes were introduced into a 2,4-dichlorophenoxyacetic acid degrading bacterium. The *lac*ZY and *lux*AB genes confer the ability to survive on lactose as a sole carbon source and bioluminescence, respectively. This allows to monitor the presence and viability of the bacteria amongst the much more prevalent native populations (Godbout et al., 1993).

A similar genetic probe system was used to characterize the bacterial population in an above ground reactor, searching for chlorobenzoate degradability (Wyndham et al, 1993). It was found that bacteria capable of degrading the 2-chloro isomers were generally unable to degrade the 3- and 4-chloro isomers. The genetic ability was found to be encoded within a transposable element (Tn5271) which means that the degradative genes would be highly mobile within and between a variety of bacterial hosts. This group has also been involved in cloning of the transposable chlorobenzoate-3,4-dioxygenase gene (Nakatsu et al., 1993).

Metabolism

The number of potential contaminants in the environment is such that the bacterial metabolism for all of them is far from being known. For example, in the petroleum sector, most of the attention has been focused on polynuclear aromatic hydrocarbons, while petroleum is composed of hundreds of compounds, which are just as likely to be toxic, or whose metabolites have the potential to be toxic. One such group of compounds are the benzothiophenes. Researchers at the University of Alberta have been studying the fate of the benzothiophenes and the methylbenzothiophenes, from petroleum and coal tar. They have synthesized metabolites, looked for their presence in the environment and studied their toxicity (Fedorak et al., 1993). They found that the sulfur heterocycles are oxidized to hydroxyformylbenzothiophene and the corresponding sulfone and sulfoxides, and that the metabolites identified in pure cultures can also be found in sediments contaminated with crude oil (Kropp et al., 1994).

Another important, yet seldom studied phenomenon, is the possibility of substrate interaction during bioremediation of complex mixtures. A very comprehensive study was done for a mixture of compounds present in creosote, using a factorial designed experiment (Millette et al., 1993) and a dynamic system of soil columns (Millette et al., 1994). The group studied the effect of phenanthrene, fluorene and p-cresol on the aerobic biodegradation of carbazole in groundwater. The results obtained in the columns were different from those in the batch studies. p-Cresol caused an increase in the lag phase for the degradation of fluorene and carbazole, but eventually allowed for complete mineralization, which did not occur in its absence. One important fact to note is the necessity to run dynamic experiments, not only batch studies, to describe accurately in-situ bioremediation and interaction between substrates.

Other researchers are interested in manipulating bacterial metabolism, not to degrade specific compounds, but to produce compounds that may assist in bioremediation. The group under the direction of J. Trevors at the University of Guelph, have been isolating biosurfactants from bacteria isolated from oil-contaminated soils. The strain *Pseudomonas aeruginosa* UG2 was found to excrete a rhamnolipid biosurfactant that was found to enhance the bioavailablity of chlorinated biphenyls (Trevors et al., 1992) and of aliphatic hydrocarbons (Jain et al., 1992). The biosurfactants were found to be effective in enhancing the removal of selected hydrocarbons from soils and to be as effective as most synthetic surfactants (Scheibenbogen et al., 1994)

Microcosm studies

In a microcosm study, the rhamnolipid surfactant from*Pseudomonas aeruginosa* UG2 was found to be more effective than sodium dodecyl sulfate for the solubilization of PAHs, but both surfactants seemed to be preferential substrates and hindered rather than helped the biodegradation of PAHs (Deschenes et al. 1994).

Many researchers report on the biodegradability of aromatic hydrocarbons, but few rigorous studies have been done on the kinetics of the transformation in soil and on the effect of the availability of nutrients on the rate. In their paper, Allen-King et al. (1994) suggest that, where oxygen is in ample supply, such as in the vadose zone, the observed variations in rates are due to the differing amount of biomass of the organisms responsible for BTEX degradation, to prevailing redox conditions and to the availability of nutrients. Hawari et al. (1994) studied the correlation between biodegradation and sorption/desorption of nitrogen-containing PAHs. An initially rapid degradation rate was followed by a much slower phase. This was attributed to the degradation of contaminants present in the dissolved and sorbed states respectively. The authors also identified different sorption mechanisms for the nitro- and amino-naphthalenes, naphthalene and quinoline.

Chlorofluorocarbons (CFCs) have been the target of much environmental research in recent years, but mostly because of their adverse effects on the ozone layer. CFCs are also know as groundwater contaminants, but have received less attention probably because they are not on anyone's list of priority toxic chemicals. CFC-113 (1,1,2-trichloro-1,2,2-trifluoroethane) has been used extensively in the electronic industry and in the analysis of oil and grease content in the petroleum and waste sectors. CFCs are a problem in the stratosphere because of their recalcitrance to chemical and biological degradation. For years, these compounds were thought to be totally non-biodegradable. Because of their similarity to the chlorocarbons, however, there was a distinct possibility for their biodegradation under anaerobic conditions. In microcosm experiments using landfill leachate as a biological inoculum, a half-life of 5 days for the reductive dechlorination of CFC-113 to HCFC-123a, and 5.6 days for the removal of one more chlorine atom were measured (Lesage et al., 1992). The products HCFC-133 and HCFC-133b were identified by GC/MS.

Because landfill leachate is a major source of groundwater contamination, secure landfills are now required to have leachate collection and recirculation or treatment. Municipal landfills often contain chlorinated and aromatic organic solvents present in waste from either households or small industries. Treatment can be costly and exceed the budget of municipalities administering the facilities. The use of waste stabilization ponds for the leachate has been studied as a cost effective treatment method. The fate of the organic solvents in such systems was studied both in microcosm and in pilot field scale (Lesage et al. 1993). It was found that during storage in closed, but not airtight containers, most of the chlorinated solvents would be biodegraded within two to three months without the addition of any nutrients. The aromatic hydrocarbons were not significantly lost during the same period. The preferred alternative would therefore to follow the storage in closed containers by an active aeration period, prior to discharge.

One of the major problem in the pollution of groundwater due to petroleum spills, is that while most of the components of gasoline are easily biodegraded under aerobic conditions and many aromatic compounds can also be degraded under anaerobic conditions, benzene, the most toxic component, has been recalcitrant in most anaerobic aquifers. The search however continues for the appropriate organism or growth conditions (Edwards, 1994). Some success was achieved in laboratory microcosms where it was shown that nitrate was not essential to this activity but sulfate reduction seemed to be involved.

While biological processes have been shown to be of great advantage in the remediation of contaminated groundwater and soil, some difficulty in keeping the microbes alive in highly contaminated environments has hampered its widespread acceptance. One means to circumvent the problem is to use the isolated enzymes that are responsible for the transformation. This task is far from simple for many enzyme systems, but in the case of reductive dechlorination, it has been found that relatively simple molecules such as porphyrins (e.g., hematin) or corrinoids (e.g., vitamin B12) could effect the transformation, even in the absence of the protein that surrounds them in living organisms. This led to the possibility to apply vitamin B12, itself a product of microbial fermentation, to the dechlorination of tetrachloroethylene present as residual DNAPL (Lesage et al. 1993). The vitamin, with titanium citrate as a reducing agent, can be used either alone or in conjunction with methanogenic bacteria (Millar et al. 1994).

2. Bench and pilot scale

Bioreactors

The native biomass at a site may be insufficient to degrade the contaminant at the desired rate. In such a case, it may be preferable to isolate the bacteria and enrich them in a batch reactor under ideal growth conditions before reinserting them in the soil. One such experiment was conducted with a soil contaminated with a mixture of pentachlorophenol (PCP) and creosote (Otte et al., 1993). In the reactor, the rate of PCP degradation was increased ten-fold and the rate of phenanthrene and pyrene were increased thirty and eighty-fold respectively. The use of bioaugmentation has the advantage of reducing the lag-phase by reintroducing an acclimated bacterial population.

A similar technique was used to produce an inoculum acclimated to the presence of phthalate esters (Ward et al. 1993). This group carried out first a laboratory feasibility study followed by a pilot field application. Conditions were established which enabled soils contaminated with greater than 5000 µg/kg of plasticizer to be remediated to below 15 µg/kg in less than 80 days in plots with soil depths between 6 and 18".

The performance of a combined anaerobic/aerobic reactor was found to be similar to that of the conventional upflow anaerobic sludge bed reactor for the degradation of tetrachloroethylene (Kuang et al., 1994), except that the coupled reactor demonstrated higher specific perchloroethylene degradation rates.

Bioreactors were designed to treat diesel invert mud residue (DIMR) in a high brine soil (Johnson et al., 1994). The experiments were aimed at optimizing reactor conditions and at comparing the rate of degradation of this petroleum waste with other residues. It was found that while nitrogen was essential, it could be applied in any form and that a single application was needed. The pattern of microbial respiration of DIMR was similar to that of other petroleum wastes.

Fungi

The use of white rot fungi for the biodegradation of PAHs in soils contaminated with creosote is being investigated by a group in New Brunswick (Boyle et al. 1993, 1994). Although field application is envisaged as part of the project, the only results reported so far are the bench microcosm experiments. One interesting feature of the work is the use of the ability of the fungi to decolorize a polymeric dye Poly-R as a visual mean of assessing the viability of the fungal population. Several fungi were isolated and their PAH degradation ability assessed. *Trametes versicolor* was found to give the best results for phenanthrene, fluoranthene and benzo-a-pyrene, but none of the fungi were able to degrade chrysene. Fungi's growth in soil required the addition of lignin-rich amendments and ground alfalfa gave the best results. The problem of population survival and competition with other species in soil remain to be addressed.

Coal tar/creosote

One of the major sources of soil contamination in Canada is coal tar/creosote, often with pentachlorophenol, widely used in the wood preservation industry. Coal tar and creosote have a very high content of PAHs, much higher than any petroleum product. A group of companies specialized in remediation were asked to do a bench-scale treatability study of soils from an industrial area called Pacific Place, in Vancouver, British Columbia (Whiting and Hayes, 1993). The groups used either solid-phase and slurry-phase bioreactors. All results were very disappointing, because most of the remediation criteria were not achieved. Where PAHs did disappear in the bioreactors, there was no difference with the control, thus indicating that bioremediation wasn't occurring. Most vendors agreed that the levels of contaminants were probably inhibitory to bacterial growth. The consultants found it difficult to assess the progress of treatment, because the laboratory analytical data wasn't sufficiently reliable. This illustrates the fact that those who apply remediation technologies often underestimate the difficulty in analysing PAHs in highly contaminated soils.

Air/Biofilters

Soil and groundwater remediation technologies such as soil venting and air sparging result in off-gases that require treatment in most jurisdictions. The use of biofilters has been sought as an alternative to the current expensive technologies of carbon sorption, catalytic oxidation and wet scrubbing (Lei et al, 1994; Rho et al., 1994; Parker et al. 1994). The contaminants to be treated here are benzene, toluene and xylenes (BTEX). As with any bioreactors, the biofilter must be inoculated with appropriate strains and the suitable moisture and nutrient concentration maintained to achieve optimal performance.

In situ treatment

A research facility to bridge the gap between laboratory and field scale, was developed in 1993-4 at the National Water Research Institute of Environment Canada in Burlington Ontario. The controls necessary to produce scientifically defensible results are only possible when the experiments are conducted in an area when variables such as light, temperature and precipitation can be regulated. The Aquatic Ecosystem Remediation Evaluation Facility (AQUEREF) will provide an area where pilot scale experiments for the remediation of contaminated sediments, soils and groundwater will be conducted such as to minimize impact to the environment. The groundwater component houses a stainless steel tank (8ft x 6ft x 20ft) filled with aquifer solids and equipped with a very dense monitoring well system (more than 300 sampling points). The initial experiments will focus on the use of humic acids to enhance the solubility and rate of bioremediation of petroleum products.

3. Field

The number of full scale application that can still be considered research projects is somewhat limited. One such application involves the use of a soil treatment adjuvant registered under the trade name Daramend™. Its use on soils was found to increase the effectiveness of the biodegradation of chlorinated phenols, PAHs and petroleum hydrocarbons (Seech et al., 1993, 1994). The soil amendment is a mixture of nutrients and bulking agents that help create the ideal conditions for bioremediation. They were able to treat soils contaminated with up to 700 mg/kg of pentachlorophenol to below CCME guideline levels. Total PAHs were reduced to below guidelines whereas total petroleum hydrocarbons were reduced from 6000 to less than 50 mg.kg $^{-1}$. The treatment was also used successfully at another site in British Columbia (Herbert et al., 1994).

Amendments can also be used advantageously for the biodegradation of heavier fuels (Paquin, 1994). Both mechanical shearing and additives made of mixtures of biosurfactants and enzymes were used successfully to increase the accessibility of the microbial population and of nutrients to the oil and grease residues, thus greatly increasing the rate of biodegradation.

Some Canadian researchers conduct their field experiments outside the country. Cox and Major (1993) reported on an in situ biodegradation field experiment carried out in Merced California. This site was unusual because trichloroethylene did not seem to be naturally biodegrading. The research team installed in situ microcosms (ISM, Gillham et al, 1992) and studied the microbial population present. The major product of transformation of [1,2-^{14}C]-TCE was not the reductive dechlorination products, cis- and trans-dichloroethene and vinyl chloride, but rather $^{14}CO2$. The addition of methanol was successful in generating anaerobic conditions and the biotransformation was attributed to a consortium of both acetogenic and methanogenic bacteria.

The ISMs were also used at a site in North Toronto where tetrachloroethene degradation was observed to occur naturally (Major, 1994). The group did in-situ injections of PCE, bromide and methanol and found that the reductive dechlorination to ethylene and ethane was accompanied by the production of acetate and methane, thus providing evidence of the presence of an active methanogenic/acetogenic consortium.

Biopiles in a Cold Climate

One of the Canadian realities is the nature of its climate. A full scale demonstration of soil bioremediation using biopiles was done on the site of a former refinery in Montreal (Desrochers et al., 1993). In addition to the adverse climatic conditions, the soil to be treated had a large clay content. Therefore, the soils were subjected to a pretreatment consisting of sorting and mixing with a bulking agent such as sawdust or gypsum, before being placed in the treatment cells. The group found that the major factor in successful remediation was the efficient aeration of the pile, more than moisture or the availability of nutrients. Intensive monitoring using gene probes showed that the bacteria specifically responsible for petroleum degradation (*xyl*E and *alk*B positive) were much more sensitive to cold conditions than the general heterotrophic population present in the soil (Samson, 1993). In less than a year, the criteria of less than 1000 ppm oil and grease was achieved. In spite of a slightly higher initial cost for set-up, this technology is considerably less expensive than the alternatives of excavation and landfilling, landfarming or incineration.

A similar type of bioreactor was used for gasoline and diesel contaminated soils at the Canadian Forces Base Petawawa (McNicoll et al. 1993, 1994). The climate conditions were similar to those found in the Montreal area (winter -20 to -10°C, summer 20 to 30°C), but the soil was a fine to medium grained sand of deltaic origin. Attempts were made to control the summer solar heat by covering the soil pile with a white instead of a black membrane. One of the biopiles did not receive any added nutrients as a control. In spite of the more permeable soil type, there was a considerable difference in the distribution of contaminants between bioreactors. No significant difference in soil temperature was observed between the white and the black covered cells. The total petroleum hydrocarbon concentration was reduced by 97% after six months.

Two additional projects were carried out on poorly permeable soils, one targeting transformer oil, the other a soil highly contaminated with diesel (exceeding 50,000 mg/kg oil and grease)(Pouliot and Sansregret, 1993). In these cases, the operators periodically mixed the soils in the biopiles, while adding nutrients and amendments such as peat moss and sawdust. A 15 kW heater was also used in the winter to keep the temperature optimal. The addition of bacteria and surfactants was also assessed and found to be of no advantage. The treatment of the transformer oil was very successful, but the target criteria were not achieved for the highly contaminated soil. One of the reason was that an excessive temperature (50°C) was reached, thereby destroying some of the hydrocarbon degrading bacteria. The authors also comment that the older the spill, the more difficult it is to treat, because of the accumulation of the more recalcitrant higher molecular weight hydrocarbons. The same group was able to apply this technology successfully on the east coast of James Bay (latitude 53° 12') for diesel contaminated soil (Faucher et al., 1994). Poorly permeable soils required special treatment in a site demonstration in the Toronto area (Itamunoala, 1994). The group studied the use of a high flow/high pressure pump to improve clay dissolution, and a vortex agitator to reduce clay lump size. Bioventing was also found to compare advantageously to soil vapour extraction in a northern climate (Armstrong et al., 1994).

Passive and active biopiles were compared for effectiveness in treating contaminated soils at a polystyrene plant (Benazon et al., 1994). Both systems were capable of treating ethylbenzene, styrene and semi-polymerized material, although the passive system required more time and space and was more difficult to monitor.

<u>Nitrate</u>

The addition of nitrate in agricultural areas can lead to groundwater contamination in zones of high soil permeability. To prevent infiltration, in many areas, drainage tiles are installed in agricultural fields to channel the run-off water into the surrounding drainage channels and streams, then posing a threat to the surface water quality. Fixed-bed bioreactors were used on-site to treat this run-off water (Blowes et al., 1994). Three reactors were made of 200-L barrels containing a mixture of sand, grow-bark, wood chips and composted leaf mulch, in varying proportion. The organic matter was added as a source of organic carbon to support the growth of denitrifying bacteria. After an acclimation period of two weeks, the effluents from the barrels were consistently below 0.02 mg/L, in spite of the fluctuation in water flow. The best results were obtained where a mixture of different types of organic carbon was used. These reactors are very simple in design and could be used widely in agricultural areas at a low cost.

Metals

Mine tailings impoundments generate leachates that are characterized by low pH and high concentration of sulfate, iron and other reduced metals. A group at the university of Waterloo is involved in in-situ bioremediation of the plumes generated downstream of these installations (Blowes et al., 1994b). A reactive wall was built across the zone to be remediated and filled with a variety of organic wastes easily accessible in a northern environment, such as leaf compost, sheep manure, sawdust, wood chips and sewage sludge. Anaerobic, sulfate reducing bacteria were from a nearby creek. The biological activity caused the reduction of sulfate to sulfide, which causes precipitation of most metals. Preliminary results from the field experiments show that neutralization and removal of Al, Ni and Zn to below drinking water standard are possible.

Pesticides

Pentachlorophenol is one of the most used pesticide in Canada. The addition of sawdust was investigated as a soil amendment for its biodegradation (Trudell, 1994) and found to reduce the half life of PCP to 160 from 190 days for the unamended soil. Both cells received nutrients.

Atrazine, bromacil, tebuthiuron and diuron have been used as soil sterilants in Alberta. When a formerly industrial site is to be rehabilitated, or if the herbicides have moved off site, it is desirable to neutralize the effect of these chemicals and allow revegetation to occur. Of a host of experimental adjuvants (activated charcoal, sawdust, manure, fly ash, commercial humates, commercial microbes, peat, and fertilizer), activated charcoal was the only truly effective compound (Cotton et al. 1994).

Delivery techniques:

The major hurdle in effectively implementing any in situ treatment is the effective delivery of the treatment to all affected areas. This is why it is now well established that there are ideal sites for in situ bioremediation: those sites where air and water can be delivered rapidly an efficiently, coarse sand and gravel aquifer. Unfortunately, it is not only the overall permeability that must be considered, but its distribution. Indeed very permeable zones within less permeable ones cause channelling, which impedes the even distribution of nutrients and air. For this reason, a group at the University of Waterloo has been investigating alternate delivery systems. A permeable wall system was applied to a BTEX plume at the CFB Borden (Barker et al., 1993). The efficiency of the system was limited by the amount of oxygen that can be introduced in water. The funnel and gate scenario channels the water to be treated through a remedial zone (Smyth et al., 1994) in which an air sparging can be installed or in which an oxygen-releasing compound can be added (Bianchi-Mosquera et al., 1994). The compound, a peroxide salt, was added either as a powder or in a briquette of concrete. The latter form seemed to be the most effective and oxygen was still being released 9 weeks after the installation (Weber and Barker, 1994).

Surface application

Remediation of groundwater contaminated by gasoline is often accomplished by pumping and treating above ground using either air stripping or adsorption on charcoal. However, in the vadose zone where ample oxygen supply exists, biodegradation has been found to occur naturally. This suggested that surface application, often termed trickle irrigation, would be a potential cost-effective technique for groundwater treatment (O'Leary et al., 1993). In a controlled field study conducted at the Canadian Forces Base Borden, they found that with nitrogen addition, a 25 m^2 plot of sandy soil could remediate 18 m^3 per day of water containing 11 mg/L of BTEX to below drinking water standards. Bioremediation was the major source of removal with volatilization accounting for only 6% of the losses.

Air induction

At many sites where contamination is extensive and hydrogeologic conditions varied, no single system is likely to be able to remediate the site effectively. A combination of treatment, which they collectively termed "air induction" was devised by a consulting group in the Atlantic region (Elder et al. 1993). The system combines the sequential injection of air and/or water with nutrients, dewatering through the same wells, induction of air through the ground surface by extraction at the wells to enhance bioremediation. The operations are repeated on a cyclical basis where it appears that nutrients may have become rate limiting in the ground. This was applied successfully at bulk fuel distribution facility in Sydney Nova

Scotia, where the overall cost of $20./m^3 was half of the estimated cost for excavation and off-site treatment.

4. Monitoring, Regulations and Funding

Monitoring

A systematic study was done at seven gasoline service stations to determine the most useful monitoring parameters for bioremediation (Samson et al., 1994). The group found no link between the extent of contamination and the presence of bacteria capable of degrading hydrocarbons. While gene probes were interesting tools, the most useful biodegradation parameter was the mineralization rate of target compounds. Amongst the chemical tests, total petroleum hydrocarbons (TPH) and BTEX provided the most information. When more than one laboratory is used, it is however important to standardized the methodology used for TPH.

Most of the assessments of bioremediation success are based on the chemical analysis of a selected group of target priority chemicals. However, the real goal of bioremediation is the return of a soil or water to its "before" state, that is to a soil typical of a forest or of an agricultural soil, able to sustain the growth of food suitable for human consumption. The preferable option, then, is clearly to use a bioassay that would measure the soil's toxicity (Renoux et al., 1993). One of the difficulties is that most soils, even when taken from pristine areas, do show a positive response in the bioassays that were mostly developed for water. Therefore, for every contaminated soil, a control soil must be obtained from a nearby source. The research group introduces the concept of AELS (Acceptable Ecotoxicological Level for Soils) to be used as the baseline for comparison. In assessing a petroleum contaminated site, no correlation could be found between the reduced level of target chemicals and the toxicity of the soils. This shows that the ideal approach requires a battery of tests, probably combining ecotoxicological and chemical assays, and that some commonly used assays, such as Microtox, are not suited to soil testing.

Alternative testing for toxicity in soil contaminated with sour gas processing plants was proposed (Hamilton et al., 1994). The group studied the usefulness of seed germination and root development for lettuce radishes and barley in addition to earthworm survival, as parameters that are more indicative of soil ecosystems rather than human health. For groundwater, bacterial luminescence, cerodaphnia dubia survival and SOS-chromotest were added. The soil tests gave excellent results, but the tests were found to lack sensitivity for groundwater. A similar battery of tests was applied to a crude oil and diesel invert mud residues (DIMR) contaminated soils (Visser et al., 1994) to assess the efficacy of bioremediation. The results did not correlate completely with the current TPH guidelines and, at the same TPH concentration, DIMR contaminated soil was more toxic that crude contaminated soils. The exact reason for this difference could not be confirmed, although the higher concentration of PAHs, was identified as a possible cause.

Regulations

The Canadian Environmental Protection Act encompasses all the new products being introduced to the Canadian market. As such, the products of biotechnologies are also targeted by the Act. However, because of the complexity of the issues of biotechnology, the government has placed a tremendous effort in the development of regulations for the application of the Act, and at the time of writing, these regulations are not in force (McIntyre, 1993, 1994). The regulations are being developed, not only in consultation with stakeholders in industry, academia, governments and environmental groups, but also in collaboration with the U.S. E.P.A., in an effort to be scientifically relevant and to avoid duplication of efforts. One of the most interesting features of the proposed regulations is to regulate based on effects, and not on the origin of the product. In that frame of reference, a genetically engineered bacterium would be assessed in the same way as a strain isolated from natural environments. The products of bacteria, such as enzymes or surfactants, are also included. The proposed regulations would also require notification of a new biotechnology product, prior to importation and manufacture.

Mackay (1993) and Cherry (1992) have begun to reflect on the increasing number of new technologies being developed and on the realities of the goals to be achieved in groundwater remediation. Most of the full scale applications are done without a complete bench and pilot scale study to back them up. Monitoring of field applications is driven by regulatory concerns, not scientific ones. This means that we may not learn as much from these expensive exercises than we would if they were conducted as large scale, but nonetheless experimental treatments. Considering the mitigated successes of most remedial operations, it is useful to reassess the goals to be achieved. The likelihood of success is better if we can decide whether plume containment, plume removal, or full aquifer restoration is what we really want, or what we can achieve given our current knowledge and the available resources.

Funding

In Canada, all university-based scientific research is generally funded through the Natural Sciences and

Engineering Research Council of Canada (NSERC), which also administers research funds for industry and joint governement-industry sponsored chairs. For example, a chair for research in Bioremediation of Contaminated sites was awarded at l'École Polytechnique in Montréal. There are very few research funds that are specific to site remediation. There are only two national programs of that nature: the Groundwater And Soil Remediation Program (GASReP) and Demonstration of Site Remediation Technologies (DESRT).

GASReP is a program funded almost entirely through the Panel on Energy Research and Development (PERD), a fund of the federal government, and addresses primarily the remediation problems of the petroleum sector. It is available to all researchers from industry, government and universities, but the proponents must have co-sponsors. Its purpose is to fund either fundamental or applied research at the bench or pilot scale. On the other hand DSERT targets the demonstration of technologies in the field. It is approved and funded jointly by the federal government and the government of the province in which the project is conducted.

Some other research activities are funded regionally or provincially, but these are generally in response to specific problems or needs and are not for generic research. The Canadian government scientists also conduct some remediation research as part of the mandate of Environment Canada. Canadian researchers have also been able to access funds from either U.S. or international funds.

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