Bioremediation of PCB & pesticide contaminated soils utilising Gene Expression Factors - A new cost effective site remediation treatment to eliminate Persistent Organic Pollutants -

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Introduction

Historically soils contaminated by persistent organic compounds such as pesticides and PCBs were not considered good candidates for bioremediation due to the simple fact that the chemical industry designed these compounds to withstand the destructive effects of natural attenuation through weathering and microbial activity. Although the chemical industry has long appreciated the benefits of persistence afforded by chlorinating a chemical product, the industry failed to appreciate the long-term environmental and potential human health consequences of producing millions of tonnes of toxic materials that were essentially immune to natural breakdown. Today PCBs and pesticides are ubiquitous in the environment and while there is little qualified dispute as to the harmful effects of these toxins, there is also a growing body of scientific and medical research that point to a causal relationship between these persistent toxins and human disease.

Early in 1992, Dr. Ellis L. Kline expanded his research in microbiology to include genetic studies of indigenous soil microorganisms. In his study of soils contaminated by PCBs and pesticides, Dr. Kline found that viable populations of soil microorganisms are able to survive in contaminated soil but that the organisms are incapable of biologically degrading the pollutants. Additional studies revealed that the presence of chlorinated compounds in the soil has the effect of inhibiting or repressing the microbial genes responsible for enzyme production. As an essential element in the biological reduction of organics for mineralisation and reproduction, it became clear that it is the inability of soil microorganisms to produce reductive enzymes that permit PCBs and pesticides to resist biological breakdown.

For the first time, the physiological mechanisms of designed persistence were becoming known. With this knowledge Dr. Kline and his research team launched a focused effort to develop a method by which chemically repressed genes could be *de-repressed* thereby promoting microbial enzyme production and the destruction of the target pollutant. In 1994, the research team succeeded in developing the first Microbial Gene Expression Factor designed to negate chemically induced gene repression and to promote rapid microbial destruction of persistent organic chemicals in treated soils.

This report provides a brief synopsis of four laboratory bench scale and field studies that illustrate the effectiveness of *Factor Treatments* in biologically reducing and eliminating PCBs and pesticides. In addition to the effectiveness of the treatments, the principals of RTE believe that the economics of Factor Treatments will motivate owners of contaminated sites to take a pro-active position relative to site remediation. Initial modelling suggests potential savings of up to 75% over conventional disposal methods.

Factor Treatment Performance

The first study of a Factor Treatment for an industry client was initiated for a chemical company in 1998. At the company's Brunswick Georgia plant site, the pesticide toxaphene ($C_{10}H_{10}Cl_8$) (CAS # 8001-35-2) had been manufactured for more than twenty years until EPA ordered a halt to production in 1980. Across the 350-acre plant site, toxaphene concentrations range from an average 600 ppm to more than 100,000 ppm. Off site transport of toxaphene by wind and water action has resulted in contamination of adjacent properties including private properties and community common areas. Responding to community and regulatory concerns the chemical company funded a study to identify a Factor Treatment alternative to a proposed $300 million clean-up utilising conventional site remediation methods (soil incineration & haz-waste landfill). The toxaphene-contaminated test soil was collected at a former production site where toxaphene concentrations averaged 5,000 ppm.

The second Factor Treatment study was initiated in January 2000 for Amtrak Rail. A problem common to may rail carriers is the presence of PCBs in rail facilities arising from operations. At one of Amtrak's East Coast switching facilities, PCB contamination of soil and the potential for off site contamination convinced management that

a remediation effort would be justified. From RTE's perspective, this site was a challenge as the target PCB (aroclor 1260), is the most highly chlorinated of the aroclor series (1013-1260). PCB concentrations in the test soils averaged 860 ppm.

The third test commenced in February 2000 for a West Virginia utility company that had experienced a transformer leak of PCBs (aroclor 1254). This study presents a unique challenge as the contaminated site encompasses a saturated marsh area and any future remedial action would necessitate a treatment with the capacity to degrade PCBs under in-situ anaerobic conditions. From a performance perspective, the ability of a Factor Treatment to eliminate PCBs in an anaerobic condition would allow treatment of contaminated soils at depths too great for excavation and allow treatment of soils below permanent structures and in marine sediments. PCB concentrations in the test composite averaged 200 ppm.

The fourth test commenced in July 2000 for the US Air Force. At the Air Force Base, soils with an average PCB (aroclor 1260) concentration of less than 5 ppm were collected. The focus of this study was to determine if a modified Factor Treatment could reduce the PCB levels to less than 1 ppm in 60 to 90 days. As many PCB sites are only nominally contaminated (<25 ppm), owners of these sites are faced with costs that are equal to clean-up costs associated with more highly contaminated sites. A less costly and timely clean-up option would allow owners of marginally contaminated sites to clean-up their sites quickly and at a fraction of the cost of conventional clean-up.

Chlorodiphenyl (60% Cl) [11096-82-5] Synonyms: Aroclor 1260; Clophen A 60; Arochlor 1260; pcb-1260; polychlorinated biphenyl 1260;

The Table illustrates the results of the four Factor Treatment tests. Each test involved a single Factor Treatment incorporated into the test soil as a dry powder. The test soils were amended to increase organic carbon content and an average moisture level of 23% was maintained during the course of testing. Except in the anaerobic studies, soils were turned every ten days and monitored for microbial activity, pH, moisture content and chloride ion production. In the anaerobic studies, flooding the test chambers with de-gassed water maintained an anaerobic condition. The target contaminants were degraded in a two-step process, the first of which was microbial dechlorination of the compound followed by rapid destruction of the residual constituent compounds. Because dechlorination occurs as a first step in the treatment process *(confirmed in chloride ion production studies)*, no problematic intermediate compounds were created during the treatment process.

Test	Target	Initial Level	Final Level	Time Period	% Reduction
Chemical Co. / aerobic	Toxaphene	5,000 ppm	Non Detect	23 weeks	100
Amtrak / aerobic	PCB / 1260	860 ppm	<20 ppm	24 weeks	98
Columbia Power /anaerobic	PCB / 1254	200 ppm	<10 ppm	12 weeks	95
Tyndall AFB / aerobic	PCB /1260	5 ppm	< .4 ppm	8 weeks	92

Conclusions

The tests conducted by Resource Technologies Environmental clearly illustrate that persistent soil borne organic pollutants once thought to be incapable of biological destruction are in fact, excellent candidates for bioremediation. The tests confirm that Factor Treatments are successful in reversing the inhibiting effects of certain chemicals on the microbial genes necessary for reductive enzyme production. Analysis of the microbial populations in Factor treated soils also suggests that both aerobic and anaerobic microbial populations respond positively to Factor Treatments, although anaerobic populations respond at a slightly slower rate. Aerobic studies suggest that a modified on site land farming treatment could eliminate the necessity for off site disposal and ultimately result in a more complete clean-up as treated soils may be used to "seed" other nominally contaminated areas.

The implications of Gene Expression Factors extend beyond a new site remediation or clean-up option. For years researchers have attempted to create "super bugs" or microorganisms genetically altered to perform functions not inherent to their natural genetic composition. The wisdom of constructing a *potential* pathogen to eliminate a toxin is questionable wisdom at best. However, in the absence of an understanding of chemically induced gene repression, it's understandable that research has moved in this direction. There is a critical distinction to be made between *Gene Expression Factors* and genetic engineering or genetic manipulation. Where genetic engineering and manipulation

endeavour to attach a capability not inherent to the microorganism, ***Gene Expression Factors simply restore to the microorganism the natural and inherent capabilities that have been inhibited or repressed in the presence of man-made pollutants***.

And finally, there is a cautionary analogy to be drawn from this research that should be considered. Microorganisms are in some respects sentinel species that alert us to potential danger. At the risk of over simplification, there is little difference between human gene function and gene function in microorganisms. The simple fact is that our genes and those of our microscopic friends both operate through the expression of chemical messages that trigger specific functions within our bodies. If, as our research indicates, chemical compounds are capable of repressing essential gene functions in microorganisms, is it then such a far reach to suppose that the same chemical compounds might also act to repress important human genes such as those which support our immune system and our ability to ward off opportunistic disease?

As Resource Technologies Environmental expands its research, we expect to uncover more answers to questions pertaining to the interrelationship of organic chemicals to a variety of ecosystems and the inhabitants. While past Factor Treatment studies have been performed utilising powdered formulations, RTE plans to conduct future tests utilising liquid treatments for subsurface injection into less accessible anaerobic zones of contamination. Studies are also planned to monitor the reductive effects of Factor Treatments in contaminated marine sediments.

It's appropriate and timely that at the beginning of a new millennium a new and better method of eliminating environmental pollutants is at hand. The antiquated practice of burning or burying our environmental problems must be reexamined in light of our knowledge that burn/bury practices are ultimately destructive and merely displace or transfer the inevitable resolution of the problem to future generations. While it's safe to assume that an industrialised society will generate hazardous wastes, it should also be assumed that advances in dealing with these wastes would be made and adopted.

Biotechnology and Gene Expression Factor Treatments are not a panacea for all environmental problems and certainly site conditions exist where Factor Treatments may not perform well. But, at thousands of sites across the United States this simple yet effective remediation technology has the potential of significantly reducing site clean-up costs. As cost considerations remain the principal factor delaying site clean-up, it stands to reason that a less costly treatment would serve to motivate responsible parties to become more proactive in cleaning-up their polluted sites and returning their sites to productivity.

In order to make Factor Treatments available to the environmental industry, RTE is planning an expansion of its Pendleton, South Carolina laboratory and the company is negotiating the acquisition of an additional research and production facility where Factor Treatments will be manufactured and warehoused. Negotiations on licensing agreements have been initiated with environmental engineering and remediation companies and the requisite regulatory approvals for broad use of Factor Treatments have been granted.

Study principals

1. Christopher W. Young - President
2. Dr. Ellis L. Kline - Director of R&D
3. Dr. Val Paynter - Protocol and Laboratory Director
4. Dr. Paulette Langfort - Analytical Services - Environmental Science Corp. Mt. Joliet, TN