

## Bioremediation Accelerates Brownfield Cleanup

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Bioremediation, using CL-out microbes, clears the obstacles to Brownfield redevelopment by reducing wells contaminant concentrations in groundwater and removing the environmental stigma from the property. The CL-out bacteria were selected for their ability to degrade a wide range of organic chemicals under aerobic conditions. CL-out bioremediation of chlorinated solvents does not produce harmful breakdown products such as vinyl chloride.

### *Background*

CL-out is comprised of a patented microbial species, which were isolated from a contaminated site in northern United States. These species have the ability to reduce chlorinated organic contaminants, such as tetrachloroethylene (PCE) and trichloroethene (TCE) under aerobic conditions. Breakdown of PCE under aerobic conditions has been previously thought impossible to achieve, but lab studies and field applications have proven differently. PCE and TCE are broken down completely under aerobic conditions, without harmful byproducts.

The theoretical pathway for chemical break down is based on the anaerobic pathways that have already been established. CL-out oxidizes chlorinated chemicals using a cometabolic metabolism, which requires the addition of dextrose to trigger the reactions. A dehalogenating enzyme first removes one chlorine atom from PCE, which has four chlorine atoms total, to produce TCE. This allows oxygenases enzymes, probably monooxygenases (MMO), to oxidize TCE forming an epoxide, which is further oxidized to 1,2-dihydroxy-TCE. Then 1,2-dihydroxy-TCE is oxidized completely to carbon dioxide, fatty acids, and water.

CL-out is produced in a freeze-dried or liquid form. Once the freeze-dried CL-out is hydrated, then applied under light pressure through one-inch injection wells (vertical or horizontal), within 24 hours of adding water to the bag. The liquid form is ready-to-use.

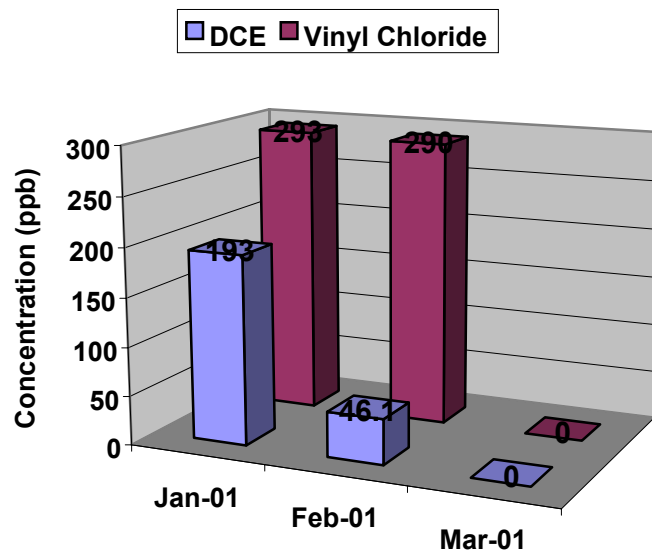


Figure 1



## Field Applications

### *Enhanced Natural Attenuation*

The suite of chlorinated contaminants found in groundwater at an engraving facility suggested the partial anaerobic degradation of tetrachloroethylene (PCE) and trichloroethene (TCE). The solvents were historically used in the 1970s to clean equipment in the facility and may have been released to the environment during handling. Once in the soil and ground water, the solvents were partially degraded to cis-1, 2-dichloroethene (cis-1, 2-DCE) and vinyl chloride. The relative concentrations are shown on [figure 1](#). The natural degradation appeared to have stalled at cis-1,2-DCE and vinyl chloride, which is typical in anaerobic degradation. Studies have shown that vinyl chloride and possibly cis-1, 2-DCE more readily degrade under aerobic conditions, which the aquifer may have been unable to sustain.

The risk-based closure criteria, required significant reduction of the cis 1,2-DCE and vinyl chloride concentrations. Combined aeration and bioaugmentation were implemented to treat the groundwater. The combination of these technologies was used to provide aerobic microbes shown to be capable of degrading the target chemicals and an associated oxygen-rich environment suitable for this bioremediation pathway.

CL-out microbes were added initially without aeration in January 2001. After the initial inoculation, the cis-1,2-DCE concentrations decreased from 193 to 46 ppb with little change in the vinyl chloride concentration. Aeration was added in February and the aquifer was reinoculated with CL-out microbes. The following monthly samples showed both the cis-1, 2-DCE and vinyl chloride concentrations decreased to less than detection limits (2 ug/L).

This project demonstrates that CL-out bioremediation combined with air sparging may be used to polish off the products of natural anaerobic biodegradation. The total cost for implementing this bioremediation project was less than \$20,000.

### *Dry Cleaning Facility Achieves Clean Closure*

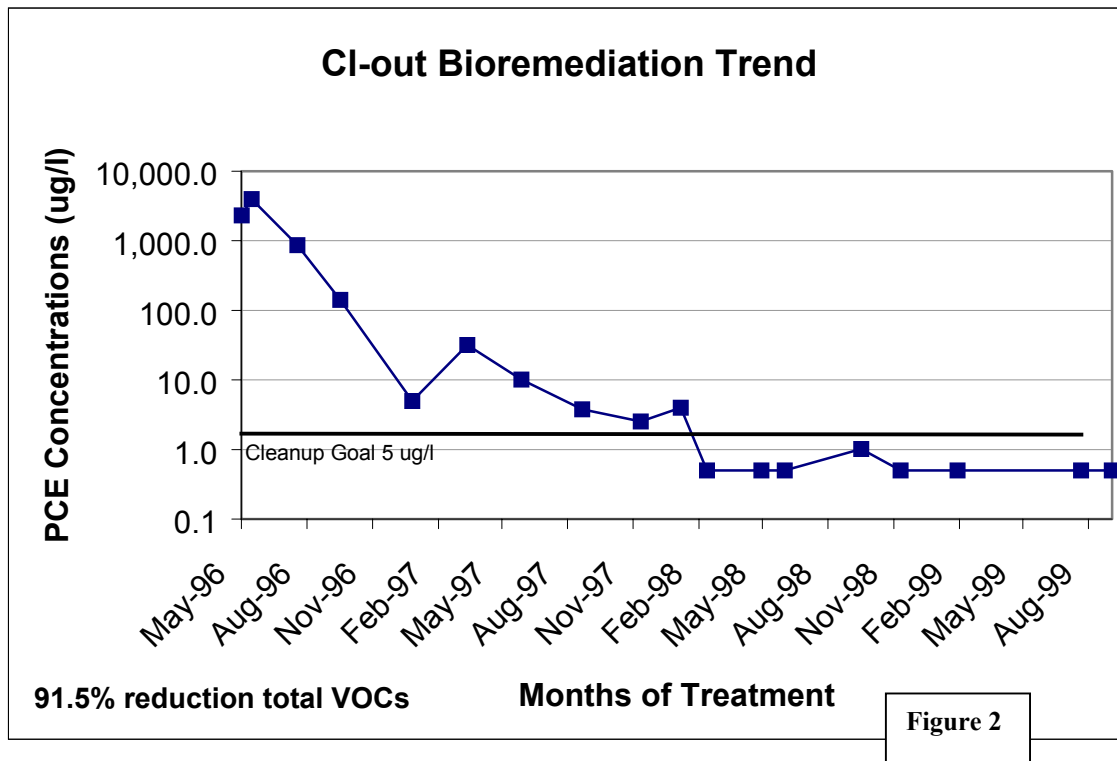
Soil and groundwater contamination resulted from improper solvent handling at a 50-year old dry cleaning facility. After the initial site investigation, the accessible contaminated soil was excavated, but there was residual contamination in the bottom and sides of the cavity that could not be excavated due to proximity to the building. Several groundwater samples taken from monitoring wells beneath and adjacent to the cavity contained PCE concentrations up to 14,000 ug/l.

A pump and treatment groundwater re-circulating system was installed in 1994 to mitigate contamination migration and flush residual contamination from the soil for capture and treatment. After two years of pump and treat with a carbon filter, there was no significant reduction in the contaminant concentrations in groundwater samples.

CL-out bioremediation was initiated in 1996. The gravel backfill in the excavation UST cavity was used as an injection gallery. An active CL-out bacteria population was maintained in the soil around the cavity by periodic CL-out additions to the gravel backfill.

After less than one year of CL-out bioremediation, the groundwater concentrations decreased by 90% to 99%. After two years of CL-out bioremediation the PCE and other VOC levels in all monitoring wells decreased to less than the drinking water MCL. [Figure 2](#) shows the cleanup trend in one of the monitoring wells.

The Kentucky Department for Environmental Protection has given the property No Further Action (NFA) status. This status removes the environmental stigma from the property and restores it's economic value.



*Treatment of Groundwater Under Dry Cleaning Facility*

Soil and groundwater contamination by chlorinated solvents was discovered at a former self-service dry cleaning facility in southwestern Ohio. The contamination had leached through the soil beneath the dry cleaners into the groundwater. The PCE concentrations in the groundwater were as high as 13,000 ppb. The typical anaerobic byproducts were detected in lower concentrations, including TCE, 1,2-DCE, and vinyl chloride (190, 120, and 125 ppb, respectively).

The groundwater at the property is perched at the bedrock surface approximately 16 to 18 feet deep beneath the property. The groundwater flow direction is largely controlled by the fracture pattern in the bedrock. The groundwater contamination traveled through the fractures in the bedrock in three directions from the property. The extent of groundwater contamination covered approximately one acre.



Freeze-dried CL-out Culture



CL-out Bottle Bag



CL-out Hydrated and Ready to Inject



Inoculation in Progress

CL-out was introduced through one-inch diameter piezometers that were installed using a direct push sampler. The small diameter injection points made it possible to install injection points inside the cleaners without disrupting the facility operations. Eight injection points were installed to cover the area of the groundwater plume. The first CL-out inoculation on August 18, 2000 was a high dose of five drums to establish the CL-out microbe population within the treatment zone. a smaller inoculation, of three drums, was introduced after one and two months to maintain the CL-out microbial activity.

The monthly groundwater sampling results are summarized on [table 1](#). The monthly samplings show a continuous decrease in the PCE concentrations from 13,00 to 650 ppb in three months. While there was an apparent slight increase in the TCE and DCE concentration, vinyl chloride was not detected after the first inoculation. The table summarizes the data taken during the treatment period. Figure 3 demonstrates the extent of the VOC plume concentrations over time.

CL-out bioremediation has achieved site-specific remediation goals for this site in less than three months, allowing the property to be marketed without environmental stigma. The total remediation and monitoring cost was less than \$25,000. The CL-out bioremediation continues at this facility with the goal of reaching drinking water standards.

Table 1

Sampling Date	Concentration of Contaminants Detected (ug/l)				
	PCE	TCE	DCE	VC	Total VOCs
7/16/99	13,000	190	120	125	13,435
9/19/00	6,000	170	93	0	6,263
10/5/00	4,600	1,800	960	0	7,360
11/17/00	650	400	370	0	1,420
Percent Reduction	99.95	97.89	96.92	100.00	99.89

### Accelerated Site Remediation

CL-out bioremediation was used along with excavation to cost-effectively treat soil and groundwater contamination in less than two months. During a pre-acquisition due diligence of a chemical manufacturing facility, soil and groundwater contamination was found beneath a drum storage area. The contamination consisted of more than 20 volatile organic compounds. A primary contaminant was PCE, which was detected in groundwater as high as 470 ug/l. Although the contaminant levels were relatively low, the concentrations exceeded applicable regulatory limits and impacted the potential property transaction.

To facilitate the property transaction, the drum storage pad was removed and the contaminated soil was excavated for off-site disposal. CL-out bioremediation was then implemented in conjunction with air sparging and groundwater recirculation to accelerate the groundwater remediation. The combination of these technologies was used to accelerate the circulation of CL-out microbes in the subsurface and to augment the microbial activity by boosting the dissolved oxygen concentration in the groundwater.

Remediation progress was monitored by analysis of samples from the groundwater circulation system. After less than one month, the PCE concentration decreased from 470 to 3.5 ug/l. The concentrations of its potential breakdown products, TCE, DCE, also decreased significantly. Overall, the concentrations of all contaminants were decreased by 90 to 99%. The groundwater sampling results are summarized in [table 2](#).

Contaminants	Pre-Treatment	Post-Treatment	
	9/15/00	9/29/00	10/3/00
cis-1,2-Dichloroethylene	160	17	15
trans-1,2-Dichloroethylene	5.3	ND	ND
Benzene	100	3.6	1.6
Chloroform	150	2.8	2.4
Methylene Chloride	150	6.3	2.1
Ethylbenzene	140	8.2	2.7
Methyl-ethyl ketone-(2-Butanone)	55	130	150
Tetrachloroethylene	470	6.2	3.5
Toluene	970	57	18
Trichloroethylene	73	ND	ND
Vinyl Chloride	40	3.7	6.1
o-Xylene	130	12	3.4
p+m-Xylene	270	30	11



*Conclusions*

These various field applications have proven that bioremediation with CL-out can achieve fast and economical results. In many cases the environmental stigma was removed from the property, restoring the value within a few months. This technology can be used at a variety of different properties, including contaminated dry cleaning, industrial, or commercial sites. The accelerated approach may be used to complete site remediation much faster than traditional techniques and in a timeframe that facilitates a property transfer.

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