



INDIANA DEPARTMENT OF ENVIRONMENTAL MANAGEMENT

**Bioremediation:
A General Outline**

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Notice

IDEM Technology Evaluation Group (TEG) completed this general outline for bioremediation based on review of items listed in the “References” section of this document. This evaluation does not approve this technology nor does it verify its effectiveness in conditions not identified here. Mention of trade names or commercial products does not constitute endorsement or recommendation by IDEM for use.

Background

This document provides a short overview with terms and notes as to applications and general effectiveness of aerobic (oxygen present) and anaerobic (oxygen absent) bioremediation. Bioremediation is a form of remediation resulting from microbial action. Normal soils and aquifers contain a wide range of naturally occurring microbes, which interact in a complex micro-ecosystem (or microcosm). Although concentrated contaminants (NAPL) may kill almost all microbes, some species will feed on and degrade many less concentrated contaminants. Aerobic bioremediation is the most active force in the natural destruction of non-chlorinated contamination, while anaerobic bioremediation is more effective for chlorinated compounds. Bioremediation is sometimes confused with natural attenuation. Natural attenuation is more encompassing and includes the degradation of contaminants through dilution, adsorption, biological activity, and chemical transformation.

Applied correctly under proper site conditions, some additives can improve the speed of bioremediation. Additives specified in a corrective action plan should be carefully evaluated to see if they will likely be cost- and time-effective improvements to the natural, subsurface conditions.

Corrective action plans using bioremediation should be evaluated on a site-by-site basis because microbial action is controlled by site conditions. However, there are general concepts that apply to all remedial scenarios. This document provides a basic overview



on the various processes and types of both aerobic and anaerobic bioremediation. This document also provides a limited overview of the more common forms of both aerobic and anaerobic bioremediation.

EX-SITU AEROBIC BIOREMEDIATION

Some natural attenuation via aerobic bioremediation occurs with every subsurface release, but the amount may not be of significance. Active or enhanced bioremediation methods involve some attempt at improving the speed or efficiency of natural bioremediation. This is accomplished by adding oxygen, microbes, nutrients, chemicals, etc.

Ex-situ refers to removal of contaminated material from its original position in the subsurface. This type of bioremediation takes place after contaminated soils are excavated and may take place immediately on-site or after transport off-site. Expenses are mainly from excavating and handling the soil. Excavation also disrupts the site and requires ample lateral space. However, because microbial and oxygen contact with the contamination can be controlled, ex-situ bioremediation is usually more successful than in-situ bioremediation (especially for soils). The act of excavation breaks up the natural soil packing and dramatically increases permeability and porosity. Air is naturally added as the soil is broken and more surface area exposed. This alone is usually enough to greatly stimulate microbial action.

EX-SITU ANAEROBIC BIOREMEDIATION

Ex-situ bioremediation occurs after contaminated soils are excavated. It can be expensive, mostly due to the cost of excavation and transportation of the soil. It can also disrupt the site and requires a good deal of space.

Ex-situ bioremediation is usually more successful than in-situ bioremediation. Excavation breaks soil, dramatically increasing permeability, porosity, and contaminant volatilization. Types of ex-situ anaerobic bio-remediation are similar to the aerobic units except the anaerobic units restrict oxygen flow.

IN-SITU BIOREMEDIATION

There are two broad categories of in-situ bioremediation: passive and active. Active bioremediation can be further divided into biostimulation and bioaugmentation. In-situ refers to in-place bioremediation, without excavation or removal. In-situ bioremediation is far more complex, is difficult to control, takes more time, and is less successful than ex-situ methods.

Passive In-situ Bioremediation

Natural contaminant reduction (e.g. biotransformation, dilution, dispersion, and adsorption) usually occurs at all sites, and is often the main factor limiting contaminant plume expansion. There are two ways that unaided bioremediation can be evaluated: plume behavior monitoring and monitored natural attenuation. Natural contaminant reduction can be used in both aerobic and anaerobic conditions.

- **Plume Behavior Monitoring**

Plume behavior monitoring evaluates trends in contaminant concentrations for each well and between wells using statistics. Section 4 of the 2012 Remediation Closure Guide (RCG) provides additional guidance on plume behavior monitoring.

There are three outcomes to using plume behavior monitoring; the plume can be:

- Increasing,
- Decreasing, or
- There is no discernable trend.

If a plume is increasing additional remedial measures will be needed. Site closure may occur if the plume is decreasing, under control, and no completed exposure pathways exist. A plume with *no discernable trend does not mean the plume is stable*. Closing a site where a plume has no discernable trend is possible, however additional information from the Conceptual Site Model (CSM) needs to be included in the evaluation (see RCG Section 4). Institutional and/or engineering controls may be needed to control exposure pathways.

- **Monitored Natural Attenuation (MNA)**

Natural attenuation may be exceedingly slow due to inherent site conditions (soil heterogeneity, permeability, pH, temperature, oxygen levels, other soil and groundwater chemistry, and type and amount of contamination). MNA differs from plume behavior because in addition to contaminants of concern (COCs) concentrations, MNA also evaluates subsurface geochemical conditions. Plume behavior evaluates only the changes in contaminant concentrations. Plume behavior is considered a line of evidence (LOE) to support site closure, while MNA is a remedial method that evaluates the geochemistry of the groundwater contaminant plume.

Natural attenuation is a general term used to describe all of the natural processes that can reduce contaminant concentrations. Using natural attenuation as a LOE involves evaluating the natural processes to show contaminant reduction is occurring. For more information on MNA see the references in the reference section at the end of the document.

Active In-situ Bioremediation

Due to inherent site conditions, using passive bioremediation may be exceedingly slow. Therefore, active in-situ bioremediation methods are attempts to overcome one or more of the limiting factors. Active in-situ bioremediation is classified as either biostimulation or bioaugmentation and can be used in both aerobic and anaerobic conditions, although different methods are used.

BIOSTIMULATION

- **Aerobic Biostimulation**
 - **Air sparge / Bioventing**
 - **Injection of oxygen releasing compounds**
 - **Recirculation**
 - **Carbon-based injectates and trap and treat**

- **Anaerobic Biostimulation**
 - **Carbon Source Addition**
 - Reductive dechlorination
 - Co-metabolism (e.g. degradation of cVOCs from enzymes produced in the presence of petroleum contamination)
 - **Anaerobic Bio-venting**
 - **Carbon Based Trap and Treat**
 - **Aquifer conditioning**
 - Emulsified Zero Valent Iron (ZVI) used in combination with a carbon source
 - Recirculation
 - **Permeable Reactive Barriers (PRBs) and Treatment Zones**

- **Bioaugmentation**

- **Aerobic Biostimulation**

Biostimulation attempts to increase the activity of the naturally occurring microbial population. This can range from the addition of nutrients to the increase of oxygen. This category includes bio-venting (or biosparging), the pumping of air through the soil and/or groundwater to increase oxygen levels. This process also includes chemical additives such as oxygen releasing compounds, nitrogen and fertilizer additions.

There are many additives and bioremediation systems on the market. Success usually depends on site conditions, and it is extremely difficult or impossible at many sites for a delivery system to reach all affected areas, particularly in fine-grained or highly stratified soils (USEPA 2000). Extra monitoring requirements are usually necessary to ensure that remediation is progressing.

All forms of biostimulation are dependent on site conditions and will not proceed in the same way at every site. A properly developed CSM is needed to effectively develop a biostimulation remedial measure that is successful. Detailed information pertaining to the form of remediation is needed for a complete and meaningful review of the proposed technology.

Aerobic biostimulation (addition of oxygen) should not be confused with in-situ chemical oxidation (ISCO) which involves physical destruction of the contaminants and not transformation through biological activity. For more information on ISCO see: http://www.in.gov/idem/cleanups/files/remediation_tech_guidance_in-situ.pdf

- **Air Sparge / Bioventing**

Air sparge and bioventing are processes that involve injecting atmospheric air into the subsurface. This can involve the use of blowers (air sparge) or can be the addition of a passive venting system (bioventing). This form of technology is not as effective on contamination in fine-grained materials as it is with coarse grained materials. With any engineered system, a good knowledge of the subsurface (fully developed CSM) is needed.

- **Injection of oxygen releasing compounds**

There are several products out on the market that release oxygen to the subsurface. There are two general types:

- Slow release, and
- Quick release.

Of these two types the slow release ones are more effective at reducing contamination. These products will release oxygen into the subsurface from six to twelve months after injection.

- **Recirculation**

Recirculation involves pumping groundwater out and then reinjecting it. The process can be as simple as pumping the groundwater out mixing it with atmospheric air, and then reinjecting it to treat the groundwater ex-situ and reinjecting to provide needed nutrients to the sub-surface.

- **Carbon-based injectates and trap and treat**

Trap and treat involves the use of activated carbon that has been treated with microbes collected from the site that can degrade specific contaminants. Trap and treat works as follows:

- The carbon will adsorb the contamination until equilibrium is reached,
- The microbes embedded in the carbon will degrade the COCs,
- The decreased concentration from the biologic degradation will allow the carbon to adsorb additional contaminants.

This cycle will continue until the contaminants are degraded or the process is interrupted due to other changes in site conditions. In most cases when dealing with aerobic bioremediation, oxygen levels have dropped low enough to interrupt the process. Microbes from other sources can be used but would be considered a form of bioaugmentation and not biostimulation.

- **Anaerobic Biostimulation**

Biostimulation is the addition or removal of something in an attempt to increase the activity of the naturally occurring microbial population. This can range from the addition of nutrients to the removal of oxygen. This category includes chemical additives such as molasses, vegetable oils (needed to deplete oxygen levels), nitrogen and other fertilizers (nutrients).

There are many additives and bioremediation systems on the market, but success usually depends on a proper assessment of site conditions, and it is sometimes extremely difficult or impossible for a delivery system to reach all affected areas, particularly in fine-grained or highly stratified soils (USEPA) 2000). Extra monitoring requirements are usually necessary to ensure that remediation is progressing.

Biostimulation methods are dependent on site conditions; none can be applied successfully at every site. Remediation work plans need a fully developed conceptual site model to provide the information needed for an effective design and method choice.

- **Carbon Source Addition**

- **Reductive Dechlorination (Bioremediation)**

In most cases reductive dechlorination involves injection of key nutrients needed by indigenous micro-organisms to effectively

degrade chlorinated solvents. Reductive dechlorination takes place in reducing conditions (i.e. oxygen poor conditions).

When using methods involving reductive dechlorination, in addition to sampling for PCE, TCE, dichloroethene (DCE), and vinyl chloride (VC) in soil, groundwater and vapor; vapor testing needs to include methane. The biodegradation of chlorinated volatile organic compounds (cVOCs) can be associated with increases in methane gas (Parsons 2004, USAF 2004, and ITRC 2008). Monitoring is needed to evaluate potential methane build-up and mitigation (see below).

- **Co-metabolism**

Co-metabolism is the process where a secondary contaminant source acts as the electron donor for the microbes degrading the primary contaminant source. This may occur when there are two different plumes intersecting. One plume is a petroleum based plume and the other is a cVOC based plume. This situation comes in non-residential settings where gas stations and drycleaners co-exist.

The petroleum plume is the primary substrate that supports growth of enzyme or cofactor to biodegrade the cVOC based plume. In these cases, biodegradation of the cVOC based plume does not yield any energy or growth benefit for the microbe mediating the reaction (USEPA 2000).

- **Anaerobic Bio-Venting**

Anaerobic bio-venting is used for soil contamination and uses nitrogen and electron donors, such as hydrogen and carbon dioxide. The nitrogen and electron donors displace the soil oxygen to facilitate microbial dehalogenation.

Anaerobic bio-venting may lead to the mobilization of volatile and semi-volatile organic compounds that are not anaerobically degradable (EPA 2006). Depending upon the type of bio-venting system employed, treatment of unaffected compounds may be needed.

- **Carbon-based injectates and trap and treat**

Trap and treat is similar to the aerobic method except the carbon is usually treated with microbes that will degrade cVOCs. This cycle will continue until the contaminants are degraded or the process is interrupted due to other changes in site conditions.

- **Aquifer Conditioning**

- **Emulsified zero valent iron (ZVI)**

- While not a bioremediation process itself, use of this method may be needed to provide suitable conditions for bioremediation. The limiting factor in reductive dechlorination is often elevated oxygen levels (i.e. reducing conditions are not present); therefore, it is necessary to artificially reduce oxygen levels. A common method to achieve reduced oxygen levels is through the introduction of ZVI. ZVI is usually introduced into the subsurface environment through injection, once oxygen levels are reduced; injections of key nutrients needed by indigenous micro-organisms are applied to the subsurface using the same injection technique. The reference list in this document provides additional details about this form of remediation.

- **Recirculation**

- This method is used to re-circulate substrate (i.e. vegetable oils, molasses) or other amendments (i.e. oxygen depletor, low levels of heat around 80° to 90° F) in contaminated groundwater. Contaminated groundwater from the site is extracted and amendments are added. The water is then re-injected into the subsurface, generally up-gradient of the target zone (ITRC 2008). The most common recirculation systems consist of a closed network of injection and extraction wells (Parsons 2004).

- **Permeable Reactive Barriers (PRBs) and Treatment Zones**

- PRBs and treatment zones contain a contaminant plume by treating only groundwater that passes through it (EPA 2000). This method involves creating an active bioremediation zone by methods such as backfilling a trench with nutrient-, oxidant-, or reductant-rich materials, or by creating a curtain of active bioremediation zone through direct injection or groundwater recirculation at the toe of a plume.

Use of Injected Materials and the Generation of Methane

Degradation of certain injected materials and contaminants could generate methane (Parsons 2004, USAF 2004, and ITRC 2008). While methane is not toxic it can be a vapor intrusion issue for three reasons:

- 1) Methane displaces oxygen which can lead to oxygen poor conditions in structures,
- 2) Methane is violently reactive with oxidizers, halogens, and some halogen-containing compounds,
- 3) Accumulations of methane can form explosive atmospheres.

Some remedial measures can generate methane as a result of the degradation of the injected materials. The methane generated from the degradation of the contaminants may be minor in comparison to the methane generated from the degradation of the injected materials. Not all remedial measures will generate methane; each method should be reviewed on a case by case basis.

To determine if methane mitigation should be considered, the TEG developed methane monitoring guidance that provides additional information on this subject; a copy can be found here: https://www.in.gov/idem/cleanups/files/remediation_tech_guidance_methane_mitigation.pdf

Familiarity with the added chemicals is important, especially if the materials degrade and generate methane. Whenever possible, case studies should be reviewed when determining if a chemical additive should be used at a site.

Biostimulation corrective measures that use materials that could generate methane should include monitoring plans for the build-up of methane. If injections are the proposed delivery method, the work plan should also address the standard issues that can occur with any type of subsurface injection, such as permitting requirements, the potential for displacement of the plume, increasing contaminant mobility, mobilizing vapors, negatively affecting utilities, health and safety issues, soil reactivity, heat generation, etc.

BIOAUGMENTATION

Bioaugmentation involves the addition of microbial cultures. Unless a site has been completely sterilized, there are usually microbial cultures already in place. There are complex interactions that occur within a native colony of microbial cultures. The activities of one species may provide nutrients for another. Some species often live on others, or their waste streams. Some species will degrade hydrocarbons much better at higher contaminant levels, while other microbes function at lower levels. A whole suite

of cultures is usually needed to ensure a complete progression to cleanup. The absence of one or two critical species can dramatically slow site remediation.

It is difficult to add microbial cultures. Microbes injected into a well rarely migrate beyond the well sand pack, potentially a foot or so into the formation. Adding extra microbes from the outside, even from cultures taken from the site, overburden the site population and change species proportions. In many cases, the lab-cultured microbes cannot survive in the foreign and possibly hostile environment (USEPA 1994). Also, the introduced microbes may prefer to eat other indigenous microbes rather than contaminants, so adding more microbes can lead to the destruction of some beneficial indigenous species.

Advances in bioremediation technology have made it possible to assess microbial populations in-situ. This allows testing of the microbial cultures under field conditions instead of in the lab.

For the reasons noted above, the USEPA does not recommend using bioaugmentation on sites which already have a viable microbial population (USEPA 1992) and also states "It is essential that independently-reviewed data be examined before employing a commercially-marketed microbial supplement"(USEPA 1994). Microbial cultures were applied on several sites in Indiana; conclusive data have yet to be submitted to IDEM for review. In addition there has been some success with the use of dehalocides bacteria for degrading cVOCs.

Injection of specialty microbes may facilitate MTBE remediation efforts. The indigenous microbes at most sites often have a limited capacity for MTBE ingestion, so cultivated microbes may be more successful.

Conclusion

Most of the successful applications of bioremediation involve combined remediation systems, where bioremediation was used along with another form of remediation, such as in-situ chemical oxidation, pump & treat, or source removal. Bioremediation by itself often cannot reduce contaminant levels below applicable screening levels, in a practical timeframe. Biostimulation is the most common and effective form of bioremediation. Additional bioaugmentation data were evaluated. There is no evidence to date (outside of very limited conditions) to show that bioaugmentation is an effective aerobic form of bioremediation.

Further Information

If you have any additional information regarding bioremediation or any questions about the evaluation, please contact the Office of Land Quality, Science Services Branch at (317) 232-3215. IDEM TEG will update this technical guidance document periodically or upon receipt of new information.

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