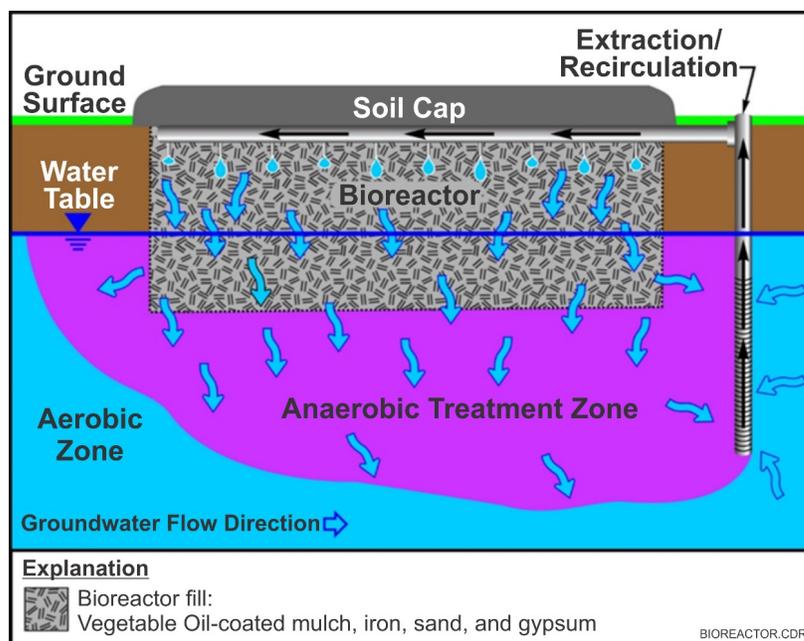


# In Situ Anaerobic Bioreactors

On this page:

- [Schematic](#)
- [Introduction](#)
- [Other Technology Names](#)
- [Description](#)
- [Development Status](#)
- [Applicability](#)
- [Cost](#)
- [Duration](#)
- [Implementability Considerations](#)
- [Resources](#)

## Schematic



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In Situ Anaerobic Bioreactor Schematic

# Introduction

An in situ bioreactor is a form of treatment that relies on biological processes to remediate groundwater. A treatment cell, consisting of organic material and other amendments (see Description section), is constructed within the source area or contaminated plume. The organic material is used as an energy source by naturally occurring or augmented microorganisms, creating a highly reducing and anaerobic environment in which contaminants of concern (COCs) such as chlorinated ethenes, munitions constituents, metals, and other contaminants (see Applicability section) can be degraded or immobilized. A bioreactor functions like a [biowall](#); however, bioreactors are constructed much wider and often are placed within a source area, whereas biowalls typically are designed to intersect and treat the dissolved phase contaminant plume.

## Other Technology Names

Mulch Reactor  
Biological Activated Filter

## Description

Anaerobic bioreactors are constructed using backhoes and other types of excavation equipment to remove soil and create a pit into which the bioreactor materials are placed. It is common to install bioreactors into the saturated zone to maximize groundwater treatment. Hence, they typically are most effective at sites where the groundwater table is relatively shallow since typical excavation equipment can achieve depths of 45 feet or less, although more specialized (and costly) equipment can achieve depths as great as 200 feet below ground surface (bgs).

Anaerobic bioreactors often are designed to include a network of wells beneath or adjacent to the reactor as well as downgradient extraction wells or trenches and associated piping, through which groundwater is pumped and recirculated through the reactor. The advantages of this approach are two-fold. First, recirculation allows a greater footprint of the plume to be treated than only

treating the groundwater that passes through the bioreactor. Second, groundwater that is pumped through the reactor will become enriched with soluble carbon (electron donor), nutrients, and microorganisms that can promote additional treatment as the groundwater flows downward and outward from the reactor into the contaminated plume. If water is extracted from wells and trenches and introduced into the bioreactor at a point near ground surface, it is common practice to construct a portion of the reactor cell in the vadose zone to allow additional residence time for degradation to occur as the water percolates through the reactor.

Reactors typically are constructed with a low permeability cap to prevent infiltration of precipitation. Precipitation can adversely impact operation of the reactor by introducing oxygen, flushing amendments and contaminants from the reactor, and creating a mounded water surface at the reactor, potentially impacting capture by the extraction wells.

Depending on site conditions, bioreactors may require multiple amendments to stimulate and maintain biodegradation. Commonly used amendments include:

- **Carbon Source.** Common carbon sources include mulch and compost, although other solid substrates containing carbon including woodchips, chitin, straw, and others can be used based on availability and cost. Liquid substrates including vegetable oil, emulsified oil amendments, whey-containing solutions, molasses, and other persistent liquid carbon sources can be combined with solid substrates and other support materials to provide an easily degradable readily available source of carbon. Liquid carbon sources also may be added after the bioreactor has been in operation for several years to improve performance and extend the life of the reactor. For treatment of chlorinated ethenes, the resulting concentration of total organic carbon in groundwater should range between 50 and 100 mg/L (ESTCP, 2010).
- **Support Material.** Sand and gravel can be used to provide a support matrix for the carbon materials to minimize consolidation of the materials and improve permeability to promote groundwater flow through the reactor. These materials generally comprise 40 to 60% of the reactor (AFCEC, 2008).
- **Microorganisms.** Microorganisms that use carbon as an energy source and create an anaerobic and reducing environment required for degradation of many COCs are ubiquitous in the environment. However, microorganisms that degrade the COCs themselves may not be present in sufficient quantities for biodegradation to proceed. Hence, it may be necessary to bioaugment the reactor with a culture that contains the appropriate bacteria (e.g.,

*Dehalococcoides*, *Dehalobactor* and *Dehalogenimonas* to degrade chlorinated ethenes and ethanes).

- **Nutrients.** Generally, the carbon source (e.g., lactate or vegetable oil) should be high in nutrients including nitrogen and phosphorous. Nonetheless, analyses can be performed prior to installation of the bioreactor and at various times during operation to determine the concentrations of nutrients. Additional nutrients can be added as necessary during construction or amended during operation through injection of a liquid.
- **Buffers.** Biodegradation generates metabolic acids, which lower the pH of the aquifer. The buffering capacity of the reactor material and aquifer should be determined. Limestone can be included as part of the reactor during construction to buffer changes in pH. Limestone offers an added advantage in that it provides support and improves permeability of the bioreactor. Alternatively, the reactor can be amended periodically with liquid buffers (e.g., solution of sodium bicarbonate) during operation if needed to improve its buffering capacity and increase groundwater pH.
- **Others.** Other types of amendments may be required based on the types of COCs present in groundwater and site-specific conditions and objectives. For instance, it may be desirable to amend the bioreactor with iron and sulfate to enhance [biogeochemical transformation](#) processes of chlorinated solvents, by which chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethylene (TCE) are completely dechlorinated to non-toxic products such as acetylene without the production of (toxic) vinyl chloride.

Amendments may be used individually or together, depending on the application and site-specific remedial goals and objectives. For example, mulch could be coated with emulsified vegetable oil (EVO) to form a system to treat multiple contaminants in an anaerobic environment over a long treatment period. During operation of the bioreactor, if additional amendments are needed, they generally are introduced in liquid form and under pressure with the goal of achieving a continuous reaction zone. Additional guidance to inject and distribute aqueous amendments can be found [here](#).

The recirculation system may either be operated continuously or intermittently. During the early stages of remedy implementation, it may be possible to achieve relatively rapid decreases in contaminant levels via continuous recirculation. However, during later stages of remedial action operations, as contaminant concentrations approach asymptotic limits, there may be little or no advantage to be gained via continuous recirculation. A data-driven, adaptive

management approach, can be used to determine when to transition from continuous to intermittent recirculation operations.

There are no specific requirements for the length, width, or overall shape of a bioreactor; however, reactors should be designed with sufficient dimensions to ensure adequate residence time for degradation to occur within and/or immediately downgradient of it. Key design parameters include the anticipated combined groundwater and recirculation flowrate, the biodegradation rate of COCs, and the maximum COC concentration expected in the groundwater. Using this information, it is possible to estimate the length of the reaction zone that is needed to sufficiently degrade the COCs to achieve remedial goals.

## Development Status and Availability

The following checklist provides a summary of the development and implementation status of bioreactors:

- At the laboratory/bench scale and shows promise
- In pilot studies
- At full scale
- To remediate an entire site (source and plume)
- To remediate a source only
- As part of a technology train
- As the final remedy at multiple sites
- To successfully attain cleanup goals in multiple sites

Bioreactors are Commercially available nationwide through the following vendors:

- Commercially available nationwide

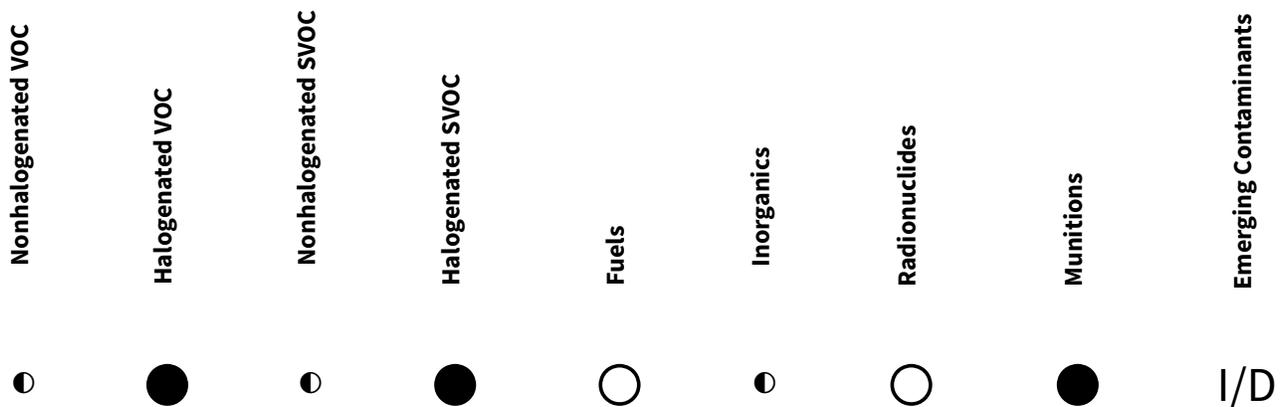
Commercially available through limited vendors because of licensing or specialized equipment

Research organizations and academia

## Applicability

**Contaminant Class Applicability Rating for Bioreactors**

(Rating codes: ● Demonstrated Effectiveness, ◐ Limited Effectiveness, ○ No Demonstrated Effectiveness,  
◊ Level of Effectiveness dependent upon specific contaminant and its application/design, I/D Insufficient Data)



Bioreactors can be used to treat any contaminant that can be degraded anaerobically. Hence, contaminants, including chlorinated ethenes and polycyclic aromatic hydrocarbons (PAHs), and munitions constituents such as trinitrotoluene (TNT) and 1,3,5-trinitro-1,3,5-triazine (Royal Demolition Explosive [RDX]), oxidizers such as perchlorate and nitrate, and dissolved metals such as chromium (VI) that can be reduced to form a precipitate can be treated. If groundwater at a site is naturally aerobic, an aerobic zone will be present at some distance from the bioreactor, which can 1) promote biodegradation of other constituents, such as vinyl chloride, which might be created and slow to degrade in the anaerobic zone but will more rapidly degrade in the aerobic portion of the site, and 2) reprecipitate metals that are mobilized under reducing conditions, e.g. arsenic, manganese, and iron. In addition, semi-volatile organic compounds (SVOCs) and volatile organic compounds (VOCs) that are amenable to anaerobic oxidation, such as benzene, can be degraded.

It is particularly cost effective to apply anaerobic bioreactors at sites that have a shallow groundwater table and/or where excavation is required for other reasons. The excavated area is easily backfilled with the necessary materials to create the bioreactor. The successful application of bioreactors in landfills and other source area locations can be found in Air Force Civil Engineer Center (AFCEC) guidance (AFCEC, 2008)

## Cost

Cost drivers for bioreactors include the type and quantity of media required and the emplacement methods needed. Major cost drivers include:

### Upfront Costs

- The size of the treatment cell, which impacts construction and treatment costs (ESTCP, 2008) and the volume of media required. Area and depth of the bioreactor impact the type of equipment required, the time to excavate the soil, and cost for its disposal. Bioreactor construction requires the use of heavy equipment to install the system, which can be costly.
- Installation of groundwater extraction wells and/or downgradient recovery trench. Systems designed to recover deeper contamination require deeper wells and increase the cost of the application. Although installation of recovery wells increases the upfront costs, wells allow for a greater portion of the plume to be treated, which can help reduce the total life-cycle cost of the remedy.
- Expected groundwater flow rate and mass flux of contaminants through the bioreactor, which determines the necessary thickness, and therefore quantity of bioreactor media required, as well as impacts the expected longevity of the media.
- Nature of COCs and degradation pathways, which determine the bioreactor media that should be used, and impact the expected longevity of the media. Mulch, gravel, and sand are relatively inexpensive; however, cost of amendments such as pH buffer-amendments, bioaugmentation cultures and other specialized carbon substrates can be greater.
- Costs to dispose of investigation-derived waste (IDW) during drilling and/or excavation activities. Depending on the type of contamination present, soil could require disposal at a facility that accepts hazardous waste.

### Operation and Maintenance Costs

- Labor for operation, maintenance, and monitoring.

- Longevity of media, which is dependent on the types of media used and contaminants, contaminant concentrations, and site characteristics. Re-application of amendments (carbon source, buffers, etc.) may be required to maintain bioreactor performance.
- Remedial goals and performance criteria, which influence how long the bioreactor must remain operational.
- Number of monitoring wells and other monitoring requirements.
- Rehabilitation of fouled extraction wells, pumps, injection system, and related treatment.

The list above highlights those cost dependencies specific to bioreactors, and does not consider the dependencies that are general to most in situ remediation technologies. Click [here](#) for a general discussion on costing which includes definitions and repetitive costs for remediation technologies. A project-specific cost estimate can be obtained using an integrated cost-estimating application such as RACER® or consulting with a subject matter expert.

## Duration

Bioreactors rely on biodegradation to reduce concentrations of COCs to low levels. Furthermore, they rely on passive flow through the reactor and, in some cases, hydraulically pumping groundwater from other locations through the barrier. Hence, they typically require longer times to achieve cleanup goals than do other more aggressive remedial technologies. At some sites, bioreactors are designed as part of a plume containment system, the operation of which can last for decades. In general, bioreactors are designed and constructed to operate for multiple years and can be operated for a decade or longer. It may be possible to reduce the duration of operations by increasing the recirculation rates, increasing the number of extraction / injection wells, and reducing the spacing between extraction / injection wells.

The longevity of bioreactors is dependent on many factors, including the following conditions.

- Groundwater quality and waterborne inhibitors that poison biowall catalysis or microflora
- Groundwater flowrate and mass flux of contaminants

- Native and anthropogenic electron acceptor demand impacting the media use rate
- Iron and sulfate availability and usage (determines biogeochemical transformation process rates)
- Initial quantity and reactivity of the media used in the bioreactor

Bioreactors can last several years without requiring replenishment. However, at some point, it may be necessary to re-apply a carbon substrate to ensure that the reactor performs as was originally designed. In addition, it may be necessary to periodically add other amendments including nutrients, microorganisms, and buffers to ensure optimum performance. It is recommended that during design, performance parameters are selected, and their monitoring locations and schedule be established to maintain optimum performance.

## Implementability Considerations

The following are key considerations associated with implementing bioreactors:

- Very high contaminant concentrations may be toxic to microorganisms.
- Performance may decrease over time due to fouling (biological or chemical).
- Depletion of the carbon source over time will occur, which can contribute to decreased performance. However, the bioreactor can be amended with liquid carbon substrates to prolong its longevity.
- An injection permit may be required if extracted groundwater is recirculated into the reactor. The Safe Drinking Water Act (SDWA) regulates injection wells under the Underground Injection Control (UIC) program. The UIC program requires that injection cannot violate primary drinking water standards nor have adverse health effects. Injection wells used for in situ remediation are designated as Class V under the UIC program. These wells are not authorized by rule and do not require a separate UIC permit; however, Class V wells regulated by a state UIC program may require a permit. Modeling of the contaminant plume and monitoring during and after the application may be required to demonstrate that the application achieves treatment and does not result in migration of COCs.
- If groundwater is recirculated into the barrier, the biodegradation rate of the COCs and flowrate of the recirculated groundwater must be known to design an appropriate reactor volume that will ensure destruction of COCs.

- Formation of metabolic acids during degradation of contaminants can lower the pH within and immediately downgradient of the bioreactor. Limestone can be included in the biobarrier during construction to buffer changes in pH. Alternatively, liquid buffer amendments can be added during operation if needed.
- Toxic byproducts (e.g., vinyl chloride) can be created, which can travel downgradient outside of the bioreactor. In addition, changes in geochemical conditions can mobilize naturally-occurring minerals that may be toxic (e.g., arsenic) or result in exceedances of secondary groundwater quality standards (e.g., pH, total dissolved solids, odors). Also, there is a possibility of vapor intrusion impacts to nearby buildings due to potential generation of methane, hydrogen sulfide, and/or vinyl chloride. It is necessary to ensure that any byproducts will attenuate naturally and not pose risk to any downgradient receptors.
- Care must be taken when emplacing multiple materials (e.g., sand and mulch) to ensure that materials are placed uniformly, especially if the bioreactor is installed below the water table, which can facilitate separation of the lighter organic material from the solids (AFCEE, 2008).
- Bioreactors are advantageous in areas where source removal by excavation is planned. The excavated area is easily backfilled with a carbon substrate that can promote long-term treatment of remaining groundwater contamination. Downgradient or deep wells may be installed to extract contaminated groundwater, which is then percolated through the barrier, achieving a much larger treatment area than the bioreactor itself.
- Bioreactors are designed to operate anaerobically. In some instances, excessively high dissolved oxygen and/or high oxidation-reduction potential in groundwater could preclude effective operation of the barrier.
- Excavation generally is only practical to a depth of about 45 feet. Hence, many times bioreactors are installed at sites at which groundwater contamination is shallow. However, bioreactors can be designed to include extraction and recirculation through the reactor to treat contaminated water deeper than the bioreactor.

## Resources

**AFCEC (Formerly AFCEE). [Technical Protocol for Enhanced Anaerobic Bioremediation Using Permeable Mulch Biowalls and Bioreactors \(May 2008\)](#). (PDF) (302 pp, 5.56 MB)**

This protocol recognizes potential biowall/bioreactor sites and assists in appropriate design and application of the remedy.

**Department of Energy (DOE). Dayvault, et. al. [Water Treatment for Uranium at the U.S. Department of Energy's Legacy Management Sites – 9438. WM2009 Conference, March 1-5, Phoenix \(2009\) \(PDF\)](#)** (12 pp, 843 KB)

This paper describes several case studies that involve treatment of uranium in contaminated groundwater, including biological treatment in a constructed bioreactor.

**ESTCP. [Impact of Landfill Closure Designs on Long-Term Natural Attenuation of Chlorinated Hydrocarbons \(2008\) \(PDF\)](#)** (108 pp, 677 KB)

Cost and performance report for a pilot-scale demonstration of a recirculation biobarrier at the Altus Air Force Base Landfill 3 site.

**ESTCP. [Addendum to Principles and Practices Manual – Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation. ESTCP Project ER-200627 \(2010\) \(PDF\)](#)** (39 pp, 473 KB)

Enhanced in situ anaerobic bioremediation involves the delivery of organic substrates into the subsurface to stimulate anaerobic degradation of contaminants in groundwater. Effective application of the technology depends primarily on the delivery of appropriate levels of organic substrate in the subsurface and the development of optimal geochemical and oxidation-reduction (redox) conditions for anaerobic degradation processes to occur. Determining an appropriate substrate loading rate and an effective distribution method for the various substrate types commonly applied is a critical design and operational objective.

**EPA. [Introduction to In Situ Bioremediation of Groundwater \(542-R-13-018\) \(2013\) \(PDF\)](#)** (86 pp, 2.23 MB)

This document provides an overview of in situ bioremediation and serves as a reference for designers and practitioners.

**EPA. [CLU-IN Bioremediation of Chlorinated Solvents Web Site \(2016\)](#)**

This web page is part of the CLU-IN Technology Focus area which consolidates information for specific technologies (in this case bioremediation of chlorinated solvents) in the following categories: Overview, Guidance, Application, Training, and Additional Resources.