

# Assessment of natural biodegradation at petroleum release sites

## Petroleum Remediation Program

This guidance assesses the occurrence of natural biodegradation where a petroleum release has impacted a resource aquifer, along with providing guidance for data collection and sampling methods, and how to calculate an estimate of the contaminant reduction rate of which natural biodegradation plays into the breakdown of petroleum products in the subsurface. A contaminant reduction rate calculation is recommended for plumes in resource aquifers that are greater than 200 feet in length to help responsible parties choose between monitoring only or cleanup.

## Background

Various natural processes control the movement of a petroleum plume and act to limit the risk exposure. These processes include dispersion, sorption to soil particles, dilution, volatilization, natural biodegradation and natural chemical degradation. Of these processes, natural biodegradation through metabolism by naturally occurring microorganisms is the primary mechanism responsible for petroleum mass reduction.

For most petroleum releases, natural biodegradation will reduce toxic chemical compounds to non-toxic metabolic byproducts. The specific mechanism and the rate at which natural biodegradation takes place depends on the physical, chemical, and biological characteristics of the subsurface environment. Determining a precise rate of natural biodegradation is difficult due to the large variability of conditions found across a petroleum plume in the subsurface environment. Therefore, demonstrating the occurrence of natural biodegradation may be adequate for most sites.

## Evaluation of bioactivity

### 1. Inorganic parameters

Establishing the occurrence of natural biodegradation at petroleum release sites involves measuring the relative changes in the concentrations of electron acceptors and the presence of biodegradation byproducts both inside and outside a petroleum plume.

The mechanisms for natural biodegradation involve aerobic respiration and the anaerobic processes of denitrification, iron reduction, manganese reduction, sulfate reduction and methanogenesis. The terminal electron acceptors and their associated metabolic byproducts are listed in Table 1 below.

Table 1. Inorganic parameters

Terminal electron acceptors	Associated metabolic byproducts
Dissolved oxygen (DO)	CO <sub>2</sub> and water
Nitrate (NO <sub>3</sub> <sup>-</sup> )	Nitrogen gas (N <sub>2</sub> )
Manganese (Mn <sup>4+</sup> )	Manganese (Mn <sup>2+</sup> )
Ferric iron (Fe <sup>3+</sup> )	Ferrous iron (Fe <sup>2+</sup> )
Sulfate (SO <sub>4</sub> <sup>2-</sup> )	Sulfide (H <sub>2</sub> S, HS <sup>-</sup> )
Carbon dioxide (CO <sub>2</sub> )	Methane (CH <sub>4</sub> )

Source: From Rifai, H.S. and Hopkins L. 1995 (Draft), Natural Attenuation Toolbox, American Petroleum Institute

To evaluate the occurrence of natural biodegradation, measure concentrations of the following inorganic in the groundwater, both inside and outside the plume:

- Dissolved oxygen (O<sub>2</sub>)
- Nitrate (NO<sub>3</sub><sup>-</sup>)
- Soluble ferrous iron (Fe<sup>2+</sup>)
- Sulfate (SO<sub>4</sub><sup>2-</sup>) or sulfide (H<sub>2</sub>S, HS<sup>-</sup>)

## 2. Other parameters

Because other parameters listed below can affect the occurrence and the rate of natural biodegradation, they should also be collected to help understand the status of petroleum degradation at a resource aquifer release site.

**Temperature:** The rate of petroleum hydrocarbon biodegradation decreases by approximately half for every 10°C decrease in temperature between the range of 5°C to 25°C. Rates of biodegradation are greatly reduced at temperatures below 5°C.

**pH:** The pH of groundwater in Minnesota may vary substantially across the state. For example, the buffering capacity of the carbonate aquifers in southeastern Minnesota is typically much greater than that encountered in the volcanism of northeastern Minnesota. Since microbial populations tend to be pH sensitive, the pH of groundwater may have a pronounced effect on the presence and activity of microbes. The optimal pH range for degradation of petroleum hydrocarbons is between six and eight.

**Inorganic nutrients (not required):** Although nutrient availability has been shown to limit microbial biodegradation in laboratory scale studies, it is rarely a limiting factor in field studies. Therefore, the measurement of nutrients is not necessary for most sites.

**Microbial populations (not required):** Since evaluating the occurrence of petroleum degradation is based on comparing relative electron acceptor concentrations and metabolic byproduct concentrations within and outside the plume, enumeration studies of microbial populations are not required.

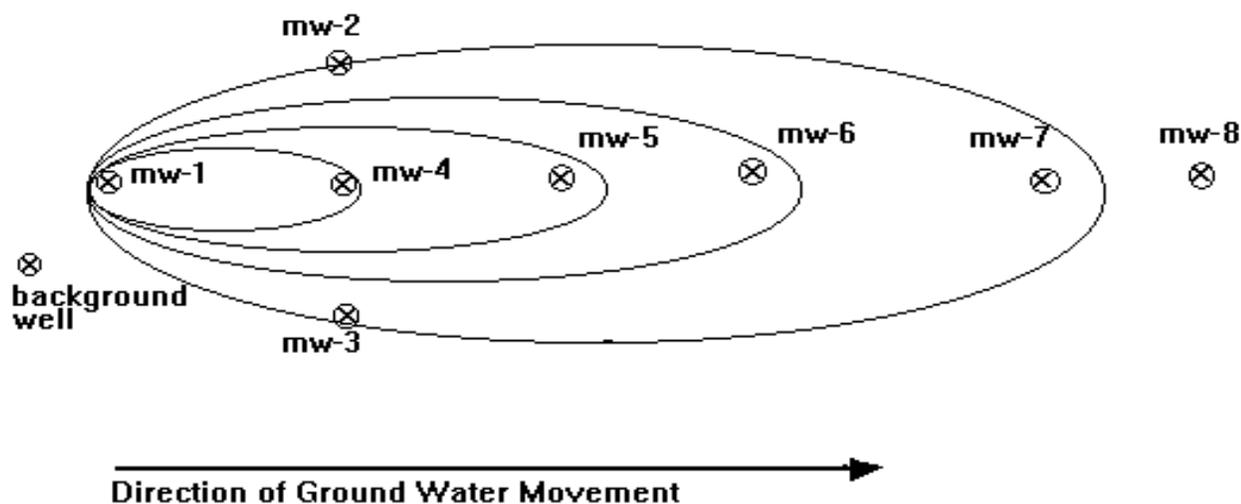
## 3. Monitoring well network<sup>1</sup>

The spacing and number of monitoring wells will depend on site-specific conditions. In general, adequate monitoring of natural attenuation will require:

- One well near the source of the release
- Several wells along the longitudinal axis of the contaminant plume
- One background well
- Several wells along the outer boundary of the contaminant plume

Monitoring wells with long well screens tend to draw water from over a larger area and may result in diluted samples. Therefore, to minimize dilution of samples, monitoring well screens should be limited to less than 5 feet where conditions allow. Nested monitoring wells are usually necessary to delineate the vertical extent of the petroleum contaminant plume.

Figure 1. Recommended monitoring well network for demonstrating natural attenuation



<sup>1</sup> From Rifai, H.S. and Hopkins L. 1995 (Draft).

#### 4. Data analysis

To assess the occurrence of natural biodegradation, analyze the required parameters listed above in addition to the required petroleum compounds during the first two rounds of groundwater sampling. Conduct subsequent natural biodegradation data collection only with the approval of the Minnesota Pollution Control Agency (MPCA). If the occurrence of natural biodegradation is not clear based on the initial data, additional data collection is recommended.

For a positive confirmation of natural biodegradation, the results of the data should show a relative difference of relative electron acceptor concentrations or metabolic byproduct concentrations inside versus outside the plume. For example, aerobic natural biodegradation is occurring if dissolved oxygen is present at five parts per million (ppm) outside the plume and at less than one ppm inside the plume, however, the reaction may be limited to the margins of the plume. If dissolved oxygen concentrations are similar both inside and outside the plume, aerobic natural biodegradation may not be occurring. Make similar comparisons for the other parameters.

If sufficient concentrations of oxygen are present, aerobic degradation may be effective at limiting the advancement of the contaminant plume. However, much of the actual total contaminant mass reduction is likely to be occurring anaerobically. Therefore, collection of both aerobic and anaerobic indicators will provide a good basis to assess the occurrence of natural biodegradation.

To document the results for the occurrence of natural biodegradation, iso-contour maps should be prepared for each of the critical parameters ( $O_2$ ,  $NO_3^-$ ,  $Fe^{2+}$ ,  $SO_4^{2-}$ ,  $CH_3$ ) showing the greatest concentration differences and the BTEX compounds.

Some expected concentration differences for specific parameters include:

- Dissolved oxygen concentrations decreasing below background concentrations during aerobic biodegradation
- Nitrate and sulfate concentrations decreasing below background concentrations during anaerobic biodegradation
- Soluble iron and sulfide concentrations increasing above background concentrations during anaerobic biodegradation

## Data collection and analytical methods

Table 2 contains the recommended data collection and analytical methods. All analysis must be conducted in the field as soon as possible after collecting the samples. If field tests show no signs of natural biodegradation, double check results for nitrate and sulfate using the Hach method (see footnote #3). In these cases, the environmental consultant should call the MPCA site hydrogeologist to discuss additional sampling parameters.

**Table 2. Data collection and analytical methods**

Analysis	Method description	Reference	Method number	Comments
Temperature	(field)	MCAWW <sup>1</sup>	170.1	
pH	pH meter (field) Colorimetric ampoules (field)	CHEMetrics <sup>2</sup>		Use manufacturer's instructions
Dissolved oxygen	AccuVac ampoules (field) Membrane electrode (field) Colorimetric ampoules (field)	Hach <sup>3</sup> MCAWW CHEMetrics	25150-25 360.1 K-7510	Unfiltered; analyze immediately for all methods. Flow cell recommended when using membrane electrode.
Nitrate (NO <sub>3</sub> -)	Anion chromatography (laboratory) Colorimetric (laboratory) Laboratory Colorimetric (field) Colorimetric ampoules (field)	SW-8464 MCAWW EPA method Hach CHEMetrics	9056 352.1, 353.2 353.2 8039 for high range, 8192 for low range K-69026	Filter, 0.45m 5 refrigerate; analyze within 48 hours.   See footnote #3.
Soluble iron (Fe II)	Colorimetric (field) Colorimetric (field) Colorimetric ampoules (field)	SMEWW <sup>7</sup> Hach CHEMetrics	3500-FeD 25140-25 K-6201	Filter, 0.45m and analyze as soon as collected.
Sulfate (SO <sub>4</sub> <sup>2-</sup> )	Anion chromatography (lab) Colorimetric (field)	SW-846 Hach	9056 8051	Filter, 0.45m refrigerate; analyze within 48 hours. See footnote #3.
Sulfide (H <sub>2</sub> S, HS <sup>-</sup> )	Color Chart (field) Colorimetric ampoules (field) Colorimetric (laboratory)	Hach CHEMetrics SMEWW	HS-C Test K-95108 4500-S2-D	Unfiltered; refrigerate; analyze within 48 hours.

<sup>1</sup> Methods for Chemical Analysis of Water and Wastes (MCAWW), 1983. USEPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH, EPA-600/4-79-020, Revised 1983.

<sup>2</sup> CHEMetrics Inc., Rt. 28, Calverton, Virginia 22016, 1996 Catalog.

<sup>3</sup> HACH Company, P.O. Box 389, Loveland Colorado, May 1, 1996 Catalog. Hach method numbers 8039, 8192, and 8051 refer to literature numbers provided by Hach. Hach methods, including catalog numbers 25150-25 and 25140-25 are for use with the DR2000 Spectrophotometer. Page 22 of the Catalog lists specific parameters and their corresponding catalog numbers.

<sup>4</sup> Test Methods for Evaluating Solid Waste, 1992. U.S. EPA, Office of Solid Waste and Emergency Response, Washington, DC, SW846.

<sup>5</sup> When using laboratory methods for NO<sub>3</sub><sup>-</sup>, Fe II and SO<sub>4</sub><sup>2-</sup> filter samples at the time of collection.

<sup>6</sup> The presence of nitrite can interfere with this method. However, background concentrations of nitrite are considered insignificant due to the instability of nitrite in groundwater.

<sup>7</sup> Standard Methods for the Examination of Water and Wastewater, 1992. American Public Health Assoc., American Water Works Assoc., Water Environment Assoc., 18th Edition

<sup>8</sup> Specific kit number will depend on the natural concentrations of sulfide in the groundwater.

Source: A Practical Approach to Evaluating Intrinsic Bioremediation of Petroleum Hydrocarbons in Groundwater, Mobil Oil Corporation, March 1995

## Estimation of contaminant reduction rate

A contaminant reduction rate calculation is optional, but is recommended for plumes in resource aquifers that are greater than 200 feet in length to help responsible parties choose between monitoring only or cleanup. Calculate using the concentration versus distance approach<sup>1</sup> described in this section.

The concentration versus distance approach uses groundwater monitoring data from benzene, toluene, ethylbenzene, and total xylenes (BTEX) concentrations to estimate the contaminant reduction rate. It requires data from two or more wells located close to the longitudinal axis of the plume, beyond the presence of any free phase product and far enough apart such that the BTEX concentrations differ by several fold. The method assumes a first order decay rate. A first order decay of BTEX has been estimated at several sites (Chiang et al. 1989; Buscheck et al. 1993; McAllister and Chiang 1994; Salanitro 1993; Wilson and Kampbell 1992; Wilson et al. 1994a,b).

The first order decay rate equation, as a function of distance and solving for the first order decay constant (k), can be written as follows:

$$k = \left( -\ln(C_z / C_y) \right) V_p / x \quad (\text{equation 1})$$

where:

C<sub>y</sub> = concentration of BTEX in a well near the source (mg/l)

C<sub>z</sub> = concentration of BTEX in a well further away from the source (mg/l)

k = first order decay constant (day<sup>-1</sup>)

V<sub>p</sub> = plume velocity (ft/day) (from equation 4 below)

x = distance between wells (ft)

Based on the data from the longitudinal axis of the wells, the approximate rate of contaminant reduction/degradation between well points can be determined, with the k value approximately equal to the percent reduced/degraded per day.

In order to determine the contaminant plume velocity, the following steps are necessary:

### Step 1. Calculate the groundwater velocity (V)

The equation to calculate ground water flow velocity (V) is based on Darcy's Law:

$$V = K i / n_e \quad (\text{equation 2})$$

where:

V = groundwater flow velocity (ft/day)

K = hydraulic conductivity (ft/day)

i = hydraulic gradient

n<sub>e</sub> = effective porosity (%)

## Step 2. Calculate the retardation factor (R)

Total organic carbon content and surface area of the soil matrix can have a partitioning effect on the petroleum hydrocarbons. This partitioning effect may retard the migration of the dissolved phase petroleum contaminant plume. Use the following equation to calculate the retardation factor (R):

$$R=1+(r_b/n_e)(K_{oc} \cdot f_{oc}) \quad \text{(equation 3)}$$

where:

R = retardation factor (unitless)

r<sub>b</sub> = aquifer material bulk density, (g/cm<sup>3</sup>)

n<sub>e</sub> = aquifer effective porosity (%)

K<sub>oc</sub> = organic carbon/water partition coefficient, (cm<sup>3</sup>/g)

f<sub>oc</sub> = fraction of organic carbon content of aquifer material. Take samples for organic carbon from the primary aquifer flow paths in an area not impacted by the petroleum release. Obtain site specific values for r<sub>b</sub>, n<sub>e</sub> and f<sub>oc</sub> from Table 4 below or from field investigation results. Obtain chemical specific K<sub>oc</sub> value from Table 3 below.

**Table 3. Chemical-specific organic carbon/water (K<sub>oc</sub>) partition coefficients (cm<sup>3</sup>/g)**

BTEX constituent	K <sub>oc</sub> value
Benzene	83
Toluene	300
Ethylbenzene	1100
Total Xylenes	240

**Table 4. Default values for bulk density, effective porosity, and fraction of organic carbon based on texture<sup>2</sup>**

Texture	Bulk density* (g/cm <sup>3</sup> )	Effective porosity*	Fraction of organic carbon*
Clay	1.8	0.20-0.22	0.01-0.1
Silty clay	1.8	0.25	0.01-0.1
Silty clay loam	1.8	0.27	0.01-0.1
Clay loam	1.55	0.30	0.01-0.1
Loam	1.55	0.30	0.01-0.1
Silt loam	1.55	0.35	0.01-0.1
Silt	1.55	0.27	0.01-0.1
Sandy clay	1.55	0.24	0.01-0.1
Sandy clay loam	1.4	0.26	0.001-0.0001
Sandy loam	1.4	0.25	0.001-0.0001
Loamy sand	1.4	0.28	0.001-0.0001
Sand	1.4	0.30	0.001-0.0001

\*Bulk density, porosity and fraction of organic carbon can also be determined on a site-specific basis by local soil test/agricultural laboratories.

Source: United States Department of Agriculture. Gleams User Manual, Version 1.8.53. March 1, 1990

### Step 3. Calculate the contaminant plume velocity ( $V_p$ )

Calculate the contaminant plume velocity by dividing the groundwater velocity ( $V$ ) by the retardation factor ( $R$ ).

$$V_p = V / R \quad \text{(equation 4)}$$

where:

$V_p$  = velocity of contaminant plume (ft/day)

$V$  = groundwater velocity (ft/day) (from equation 1)

$R$  = retardation factor (from equation 2)

### Step 4. Calculate contaminant reduction rate ( $k$ )

Calculate the contaminant reduction rate (percent per day) by solving Eq. 1 above.

<sup>1</sup> From A Practical Approach to Evaluating Intrinsic Bioremediation of Petroleum Hydrocarbons in Groundwater, Mobil Oil Corporation, March 1995.

<sup>2</sup> From Rifai, H.S. and Hopkins L. 1995 (Draft).

## Additional resources

Buscheck, T. E., P. E. Kirk, T. O'Reilly and S. Nelson. 1993. Evaluation of intrinsic bioremediation at field sites. Proceedings of the Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water; Prevention Detection and Restoration. National Ground Water Association/API, Houston, Texas, Nov. 4-6. pp. 509-515

Chiang, C. Y., J. P. Salanitro, E. Y. Chai, J. D. Colthart and C. L. Klein. 1989. Aerobic biodegradation of benzene, toluene, and xylene in a sandy aquifer - data analysis and computer modeling. Ground Water, 27: 823 - 834.

McAllister, P. M. and C. Y. Chiang. 1994. A practical approach to evaluating natural attenuation of contaminants in ground water. Ground Water Monitoring and Remediation, 14: 161-173.

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Wilson, J. and D. H. Kampbell. 1992. Innovative measures distinguish natural bioattenuation from dilution/sorption. Ground Water Currents, U.S. Environmental Protection Agency, Solid Waste and Emergency Response. EPA/542/N-92/006.

Wilson, J. T. and D. H. Kampbell and J. Armstrong. 1994a. Natural bioreclamation of alkylbenzenes (BTEX) from a gasoline spill in methanogenic ground water. In R. E. Hinchee, B. C. Alleman, R. E. Hoeppe and R. N. Miller (eds.) Hydrocarbon Bioremediation, Lewis Publishers, Ann Arbor, MI.

Wilson, J. T., F. M. Pfeffer, J. W. Weaver, D. H. Kampbell, T. H. Wiedemeier, J. E. Hansen and R. N. Miller. 1994b. Intrinsic bioremediation of JP-4 jet fuel. In Proceedings of the Symposium on Intrinsic Bioremediation of Ground Water. Denver, CO. August 30 - September 1.