



Petroleum Remediation Program

Minnesota Pollution Control Agency

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Assessment of Natural Biodegradation at Petroleum Release Sites

Guidance Document 4-03

This document explains how to assess the occurrence of natural biodegradation where a petroleum release has impacted a resource aquifer. Also included is a section on recommended data collection and analytical methods. Another section explains how to calculate an estimate of the “contaminant reduction rate” (of which natural biodegradation plays a significant part). Determining this rate is optional, yet it may give a general sense of how long the petroleum contamination might remain in place if the responsible party is interested in this information. 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 by products 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 ₂ and water
nitrate (NO ₃ ⁻)	nitrogen gas (N ₂)
manganese (Mn ⁴⁺)	manganese (Mn ²⁺)
ferric iron (Fe ³⁺)	ferrous iron (Fe ²⁺)
sulfate (SO ₄ ²⁻)	sulfide (H ₂ S, HS ⁻)
carbon dioxide (CO ₂)	methane (CH ₄)

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

To evaluate the occurrence of natural biodegradation, concentrations of the following inorganic parameters should be measured in the ground water, both inside and outside the plume:

- dissolved oxygen (O₂)
- nitrate (NO₃⁻)
- soluble ferrous iron (Fe²⁺)
- sulfate (SO₄²⁻) or sulfide (H₂S, HS⁻)

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 one half for every 10°C decrease in temperature between the range of 5-25°C. Rates of biodegradation are greatly reduced at temperatures below 5°C.

pH: The pH of ground water 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 volcanics of northeastern Minnesota.. Since microbial populations tend to be pH sensitive, the pH of ground water may have a pronounced effect on the presence and activity of microbes. A pH range of 6-8 is generally considered optimal for degradation of petroleum hydrocarbons.

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.

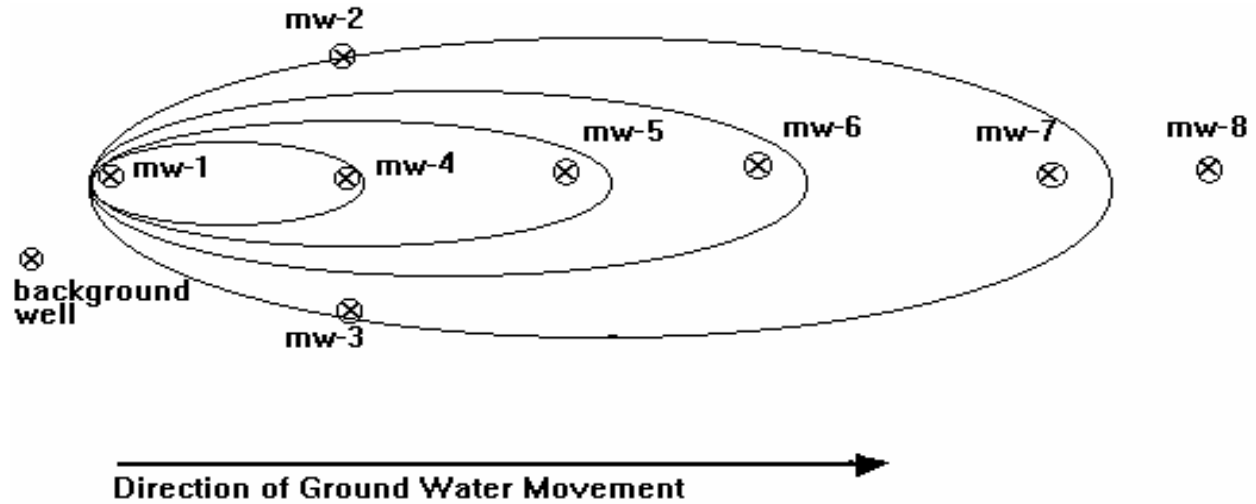
3. Monitoring Well Network¹

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 results in diluted samples. Therefore, to minimize dilution of samples monitoring well screens should be limited to less than five 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



¹ From Rifai, H.S. and Hopkins L. 1995 (Draft).

4. Data Analysis

To assess the occurrence of natural biodegradation, the required parameters listed above should be analyzed in addition to the required petroleum compounds during the first two rounds of ground water sampling. Subsequent natural biodegradation data collection should be conducted only with the concurrence of the MPCA staff hydrogeologist. Additional data collection should be recommended if the occurrence of natural biodegradation is not clear, based on the initial data.

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, if dissolved oxygen is present at five parts per million (ppm) outside the plume and at less than one ppm inside the plume, then aerobic natural biodegradation is occurring (however, the reaction may be limited to the margins of the plume). Conversely, if dissolved oxygen concentrations are similar both inside and outside the plume, aerobic natural biodegradation may not be occurring. Similar comparisons should be made for the other parameters.

Aerobic degradation may be effective at limiting the advancement of the contaminant plume if sufficient concentrations of oxygen are present. 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

The recommended data collection and analytical methods are presented in Table 2. It is recommended that all analysis be conducted in the field as soon as possible after collecting the samples. The field methods by Hach and CHEMetrics are fairly simple to use. Laboratory methods are listed for nitrate and sulfate to double check the results if all the field tests show no signs of natural biodegradation. 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 ¹	170.1	
pH	pH meter (field)			Use manufacturers instructions
	Colorimetric ampoules (field)	CHEMetrics ²		

Analysis	Method Description	Reference	Method Number	Comments
Dissolved Oxygen	AccuVac ampoules (field)	Hach ³	25150-25	unfiltered; analyze immediately for all methods. Flow cell recommended when using membrane electrode.
	Membrane electrode (field)	MCAWW	360.1	
	Colorimetric ampoules (field)	CHEMetrics	K-7510	
Nitrate (NO ₃ ⁻)	Anion chromatography (laboratory)	SW-846 ⁴	9056	filter, 0.45μ; ⁵ refrigerate; analyze within 48 hours
	Colorimetric (laboratory)	MCAWW	352.1, 353.2	
	laboratory	EPA method	353.2	see foot note #3
	Colorimetric (field)	Hach	8039 for high range, 8192 for low range	
	Colorimetric ampoules (field)	CHEMetrics	K-6902 ⁶	
Soluble Iron (Fe II)	Colorimetric (field)	SMEWW ⁷	3500-FeD	filter, 0.45μ; and analyze as soon as collected
	Colorimetric (field)	Hach	25140-25	
	Colorimetric ampoules (field)	CHEMetrics	K-6201	

Analysis	Method Description	Reference	Method Number	Comments
Sulfate (SO ₄ ²⁻)	Anion chromatography (lab)	SW-846	9056	filter, 0.45μ; refrigerate; analyze within 48 hours
	Colorimetric (field)	Hach	8051	see footnote #3
Sulfide (H ₂ S, HS ⁻)	Color Chart (field)	Hach	HS-C Test	unfiltered; refrigerate; analyze within 48 hours
	Colorimetric ampoules (field)	CHEMetrics	K-9510 ⁸	
	Colorimetric (laboratory)	SMEWW	4500-S2-D	

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

² CHEMetrics Inc., Rt. 28, Calverton, Virginia 22016, 1996 Catalog.

³ 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. Specific parameters and their corresponding catalog numbers are listed on page 22 of the Catalog.

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

⁵ When using laboratory methods for NO₃⁻, Fe II and SO₄²⁻ samples should be filtered at the time of collection.

⁶ The presence of nitrite can interfere with this method. However, due to the instability of nitrite in ground water, background concentrations of nitrite are generally considered insignificant.

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

⁸ Specific kit number will depend on the natural concentrations of sulfide in the ground water.

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. This can be calculated using the concentration vs. distance approach¹ described in this section.

The concentration vs. distance approach uses ground water monitoring data--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_y = concentration of BTEX in a well near the source ($\mu\text{g/l}$)

C_z = concentration of BTEX in a well further away from the source ($\mu\text{g/l}$)

k = first order decay constant (day^{-1})

V_p = 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 Ground Water 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 = ground water flow velocity (ft/day)

K = hydraulic conductivity (ft/day)

i = hydraulic gradient

n_e = 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. To calculate the retardation factor (R) the following equation may be used:

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

where:

R = retardation factor (unitless)

r_b = aquifer material bulk density, (g/cm³)

n_e = aquifer effective porosity (%)

K_{oc} = organic carbon/water partition coefficient, (cm³/g)

f_{oc} = fraction of organic carbon content of aquifer material. Samples for organic carbon should be taken from the primary aquifer flow paths in an area not impacted by the petroleum release.

r_b , n_e and f_{oc} are site specific values which may be obtained from table 4 below or from field investigation results. K_{oc} is chemical specific and may be obtained from table 3 below.

Table 3. Chemical-Specific Organic Carbon/Water (K_{oc}) Partition Coefficients (cm³/g).

BETX CONSTITUENT	K_{oc} VALUE
Benzene	83
Ethylbenzene	1100
Toluene	300
Total Xylenes	240

Table 4. Default Values for Bulk Density, Effective Porosity, and Fraction of Organic Carbon Based on Texture²

TEXTURE	BULK DENSITY* (g/cm ³)	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)

The contaminant plume velocity is calculated by dividing the ground water 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 = ground water velocity (ft/day) (from equation 1)

R = retardation factor (from equation 2)

Step 4. Calculate Contaminant Reduction Rate (k)

The contaminant reduction rate (percent per day) is calculated by solving Eq. 1 above.

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

² From Rifai, H.S. and Hopkins L. 1995 (Draft).

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