Developed by:

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Bioavailable Ferric Iron Assay

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BioAvailable Ferric Iron Assay

The Bioavailable Ferric Iron Assay measures the amount of ferric iron in soil or sediment that can be reduced to ferrous iron (Fe^{2+}) by iron-reducing bacteria. Bioavailable ferric iron is one indicator of natural attenuation and can be important for other in situ bioremediation technologies such as enhanced anaerobic bioremediation.

REAGENTS MATERIAL PROVIDED:

1. Foil Pouch:
   - A: 1ea: T_0 Sample Tube (25mL)
   - B: 1ea: T_{30} Sample Tube (25mL) containing Reagent "A".
   - C: 2ea: Filtered Sample Vial (4mL)
   - D. 2ea: Syringes, (3mL)
   - E. 2ea: Syringe Filters (0.45µm)

2. 1 ea: Bioassay Reagent "B" (Keep frozen until ready for use to ensure stability)

MATERIALS NOT PROVIDED:

1. Concentrated Hydrochlori
2. Sample Jar (2 or 4 oz)
3. Distilled Water
4. Gloves
5. Safety Glasses
6. Fe^{2+} analysis
7. Tube mixer
8. Work Station Rack
9. Portable Standard Balance

ACCESSORIES AVAILABLE:

1. Work Station Rack
2. Portable Standard Balance
3. Fe^{2+} analysis
4. Tube mixer

SAMPLE COLLECTION:

1. Collect saturated soil sample into Sample Jar. Try to get as homogenous and representative a sample as possible. Wet sieve sample to 3/16" minus if necessary.
2. Use the Scoop to weigh out 5 grams (± 0.5 grams) of soil into the weigh boat. Transfer the soil to the T_0 Sample Tube. Use of the provided funnel may be helpful. Mark date and site location onto label of tube. Weigh out another 5 grams of soil and put into the T_{30} Sample Tube. Mark date and site location on vial.

STEP 1: T_0 ASSAY PROCEDURE

1. Fill T_0 Sample Tube with distilled water, (~20 mL), leaving enough room for 1 mL of concentrated hydrochloric acid (HCl).
2. Add 1 mL concentrated HCl to sample. Top off with additional distilled water if necessary. Cap and invert to mix.
3. Place on tube mixer and mix for 48 hours.
4. Remove tube from tube mixer and allow soil to settle.
5. Remove cap and, with 3mL syringe, extract 3 mL of liquid, being careful not to disrupt the soil.
6. Attach Syringe Filter syringe and filter sample into T_0 Filtered Sample Vial (4mL).
7. Perform ferrous iron (Fe^{2+}) analysis on filtered sample.
8. Record results in mg/L.

STEP 2: T_{30} ASSAY PROCEDURE (with reagents):

1. Add contents of Bioassay Reagent vial "B" to Sample Tube T_{30} using the follow procedure. Gently tap vial "B" until the freeze-dried reagent moves freely. Remove the cap, invert the vial over Sample Tube T_{30}, and tap again to transfer the reagent. If the reagent does not move use a small spatula or other instrument to dislodge and transfer the reagent. If necessary, rinse vial "B" with distilled water (to ensure all reagents are collected) and pour rinsate into Sample Tube T_{30}.
2. Fill to the top of the neck of the T_{30} Sample Tube with distilled water. Tap tube as necessary to cause air bubble to rise to the surface and top off with additional distilled water. Invert the tube several times to mix soil, water, and reagents.
3. Store at room temperature for 30 days in the dark in an upright position.
4. After 30 days, remove cap and remove 1 mL of liquid. Dispose of this 1 mL.
5. Add 1 mL concentrated hydrochloric acid (HCl).
6. Place tube on tube mixer for 48 hours.
7. Remove tube from tube mixer and allow soil to settle.
8. Remove cap and, with 3-mL syringe, extract 3 mL of liquid, being careful not to disrupt soil.
9. Attach Syringe Filter syringe and filter sample into T_{30} Filtered Sample Vial (4mL).
10. Perform ferrous iron (Fe^{2+}) analysis on filtered sample.
11. Record results in mg/L.

FERROUS IRON (Fe^{2+}) ANALYSIS METHOD (RECOMMENDED):

1. Determine Fe^{2+} concentration range using Quantofix® Iron 1000 test strips (VWR Part No. 60787-724). Use the test strip directly and do NOT use the Iron 1 reagent which reduces Fe^{3+} to Fe^{2+}.
2. Dilute sample with distilled water so that Fe^{2+} is 3 mg/L or less. Dilute sample a minimum of 1:20 even if the test strip result is less than 30 mg/L in order to decrease the acidity which interferes with the Hach method.
3. Measure Fe^{2+} using the 1,10-phenanthroline method (Hach Color Disc Part No. 26672-00). Make sure the Hach test kit directions are followed carefully including the incubation time.

CALCULATE BIOAVAILABLE FERRIC (Fe^{3+}) IRON:

Results from T_0 samples (without reagents) indicate initial ferrous iron (Fe^{2+}) in the soil sample. Results from T_{30} samples (with reagents) indicate initial ferrous iron (Fe^{2+}) in the soil sample plus bioavailable ferric iron (Fe^{3+}) that has been reduced to ferrous iron (Fe^{2+}). Calculation gives grams of bioavailable ferric iron per kilogram of wet weight of soil. To convert to a dry weight basis, multiply answer by the percent solids in the sample used to conduct the assay.

Bioavailable Ferric Iron (g/kg Fe^{3+}) =
[T_{30} (mg/L Fe^{2+}) – T_0 (mg/L Fe^{2+})] ÷ 217

Background

Ferric iron (Fe III) is a widespread terminal electron acceptor used by iron-reducing bacteria under anaerobic conditions. These bacteria can oxidize various organic compounds and in turn reduce ferric iron (Fe III) to ferrous iron (Fe II). Some of the organic compounds that can be oxidized by certain iron-reducing bacteria include benzene, toluene, vinyl chloride (VC), *cis*-dichloroethene (cDCE), and methyl tertiary butyl ether (MTBE). Additionally, iron oxides play an important role in the immobilization of metals in aquifers and bacterial iron reduction is one factor affecting the transport of metals in aquifers.

Not all ferric iron can be biologically reduced. A definition of bioavailable ferric iron is:

Ferric iron (Fe III) that is capable of being reduced by microorganisms that oxidize another chemical species and derive energy from the electron transfer.

Prediction of the amount of bioavailable ferric iron is difficult because it is affected by many factors. Factors that can determine whether ferric iron is bioavailable include iron oxide crystallinity and surface area, groundwater pH and specific conductivity, concentrations of divalent cations, concentrations of electron shuttles such as humic acids, and adsorbed ferrous iron.

Assay Description

The assay is a bioassay that uses an iron-reducing bacterium to give an estimate of the maximum concentration of bioavailable ferric iron in soil or other solid materials. A five-gram soil sample is incubated in the assay medium along with the bacteria for a period of one month. During this time bioavailable ferric iron is reduced to ferrous iron. The newly formed ferrous iron plus the originally present ambient ferrous iron is extracted with weak acid (0.5 N HCl) at the end of the incubation period and measured using a Hach kit following dilution. The ambient ferrous iron concentration is measured by similarly extracting a soil sample that has not been incubated or exposed to the assay reagents. The ambient ferrous iron concentration is subtracted from the concentration in the incubated sample to obtain the bioavailable ferric iron concentration.

Assay Method

Soil samples are typically collected from the saturated zone. A four-ounce jar of soil is sufficient for the bioavailable ferric iron assay. Jars should be filled with water-saturated soil and kept refrigerated until analysis. Recommended holding times for soil samples have not been determined. Preferably, analyses should be initiated within one week of sample collection.

The sample is wet-sieved through a 3/16-inch sieve if necessary and two five-gram sub-samples of the sieved material are placed in each of two assay tubes labeled T_0 and T_{30}. The T_0 tube is filled with distilled water and one milliliter of concentrated HCl. The tube is capped and then placed on a tube rotator for 48 hours during which time the acid extracts weakly associated ferrous iron (Fe II) from the soil. Following the incubation period, the extract liquid is filtered if necessary and diluted prior to measurement of the ferrous iron concentration using the Hach phenanthroline method. The T_{30} tube, which also contains the assay reagent and lyophilized bacteria, is filled with distilled water, capped, mixed by hand, and then incubated in the dark at room temperature for 30 days. Following the incubation one milliliter of liquid is withdrawn, discarded, and replaced with one milliliter of concentrated hydrochloric acid. The tube is then

rotated for 48 hours and analyzed for ferrous iron. This concentration is the final ferrous iron concentration and is the sum of the ambient ferrous iron and the bioavailable ferric iron. The ambient ferrous iron and the bioavailable ferric iron concentrations on the soil are calculated as follows:

Ambient Fe II = (T_0 Fe II)/{(217)(solids fraction)}

Bioavailable Fe III = (T_{30} Fe II - T_0 Fe II)/{(217)(solids fraction)}

The terms in these equations are defined as follows:

Ambient Fe II – The concentration of Fe II in the soil sample (units of grams Fe per kilogram dry soil) prior to conducting the assay.

Bioavailable Fe III – The concentration of biologically reducible Fe III in the soil sample (units of grams Fe per kilogram dry soil) determined using the assay.

T_0 Fe II – The Fe II concentration measured in the T_0 tube (units of milligrams Fe II per liter) following acid extraction. Measured using a Hach phenanthroline kit.

T_{30} Fe II – The Fe II concentration measured in the T_{30} tube (units of milligrams Fe II per liter) following acid extraction. Measured using a Hach phenanthroline kit.

217 – A conversion factor to convert the liquid Fe II concentration to the soil concentration. It incorporates tube volume (25 milliliters), soil mass (5 grams), soil particle density (2.6 grams per milliliter), and unit conversions.

Solids fraction – Solids fraction in the soil sample (units of grams dry soil per gram wet soil). Measured separately and used to convert the ambient and bioavailable iron results from a wet-soil basis to a dry-soil basis. This term is optional if results expressed per kilogram of wet soil are acceptable.

Applications

The concentration of bioavailable ferric iron in soil is one parameter that may be used to determine the potential for oxidative degradation of organic chemicals and the transport of metals. It also may be used to determine the potential for inhibition of reductive dechlorination of chlorinated ethenes by maintenance of low dissolved hydrogen concentrations.

The assimilative capacity for oxidation of organic chemicals can be calculated by calculation of the electron equivalents that can potentially be accepted by the bioavailable ferric iron. This is calculated as follows:

Electron accepting equivalents = (Bioavailable Fe III)(eq./56 g) equiv./kg

As an example, vinyl chloride oxidation to carbon dioxide requires 10 electron equivalents/mole or 6 g/equiv. If the measured bioavailable ferric iron concentration is 1 g/kg, the assimilative capacity for vinyl chloride oxidation is:

0.11 g VC/kg soil = (1 g bioavailable Fe III/kg soil)(1 equiv. /56 g Fe III)(6 g VC/equiv.)

Another application is calculation of how much historical contaminant oxidation is attributable to iron reduction. This calculation requires comparison of background samples to samples in the contaminant plume. The difference in bioavailable ferric iron between these two samples can be used to calculate the amount contaminant that has been oxidized as shown in the following example. If the background sample contains 1 g/kg and the sample in the plume contains 0.5 g/kg bioavailable ferric iron, then the amount of vinyl chloride oxidation theoretically attributable to iron reduction is:

0.054 g VC/kg soil = {(1-0.5) g bioavailable Fe III/kg soil}(1 equiv. /56 g Fe III)(6 g VC/equiv.)

Note that oxidation of organic chemicals other than VC is also possible. The above calculations do not take into account oxidation of other chemicals and are provided for example only. They should be modified to meet the requirements of specific sites.

Disclaimer

The bioavailable ferric iron assay is an analytical method that was developed for the U.S. Air Force under the Small Business Innovative Research (SBIR) program. It is currently being evaluated by the Department of Defense under the Environmental Security Technology Certification Program (ESTCP) and by the EPA. It is not an EPA-approved test method. The results of the assay are only one of several types of analytical data that should be considered in assessing soil conditions and are intended to be used in combination with these other data. Users of the bioavailable ferric iron assay should not rely solely on the assay results and should exercise best professional judgment in determining the extent to which reliance on the assay results is appropriate in a particular instance. The user shall be solely responsible for inconsistent or erroneous assay results or

injuries that occur due to improper or negligent use or handling of the test kit or of any results of assays performed with the test kit.

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