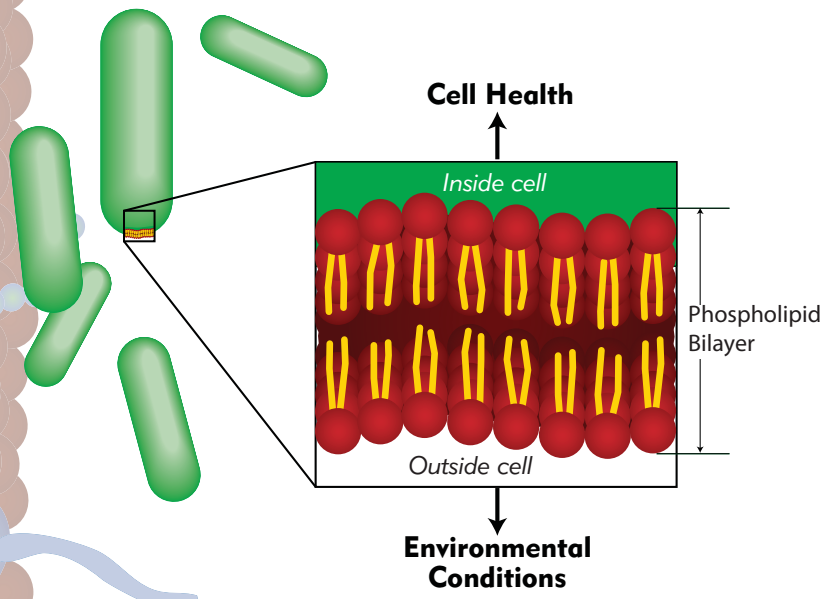
PLFA

MOLECULAR BIOLOGICAL TOOL

Quantify total biomass and assess the entire microbial population

Phospholipid fatty acids (PLFA) are a main component of the membrane (essentially the skin) of all microbes.



PLFA analysis provides direct information on the entire microbial community in three key areas:

- **Biomass** — PLFA decomposes quickly upon cell death, so the total PLFA biomarkers in a sample represent all living cells.
- **Population "Fingerprint"** — Some organisms produce specific or signature types of PLFA biomarkers allowing quantification of important microbial functional groups (e.g. iron reducers, sulfate reducers, or fermenters). The relative proportions of these groups of PLFA biomarkers provide a fingerprint of the microbial community.
- **Microbial Activity** — Some microbes, most notably *Proteobacteria*, modify specific PLFA biomarkers during periods of slow growth or in response to environmental stress providing an index of their health and metabolic activity.

PLFA Type	Bacterial Group	Potential Relevance to Bioremediation
Monoenoic (Monos)	Abundant in Proteobacteria which includes a wide variety of aerobes and anaerobes	Many hydrocarbon utilizing bacteria are classified within Proteobacteria
Terminally Branched Saturated (TerBrSats)	Characteristic of Firmicutes and *Bacteroides*	Firmicutes include anaerobic fermenting bacteria which produce the H_2 necessary for reductive dechlorination
Branched Monoenoic (BrMonos)	Anaerobes and micro-aerophiles such as sulfate- or iron-reducing bacteria	High proportions are often associated with anaerobic sulfate and iron reducing bacteria
Mid-Chain Branched Saturated (MidBrSats)	Common in sulfate reducing bacteria and also *Actinomycetes*	High proportions are often associated with anaerobic sulfate and iron reducing bacteria
Normal Saturated (Nsats)	Found in all organisms	High proportions often indicate less diverse populations
Polyenoic (Polys)	Found in eukaryotes (fungi, algae, protozoa, plants and animals)	Eukaryotic scavengers often prey on contaminant utilizing bacteria

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PLFA applications include:

Monitored Natural Attenuation (MNA)

- Determine whether bacterial biomass is sufficient for bioremediation.
- Determine the microbial community composition "fingerprint".
- Evaluate microbial populations indicative of dominant redox status (aerobic vs. anaerobic).
- Monitor microbial activity under site conditions.

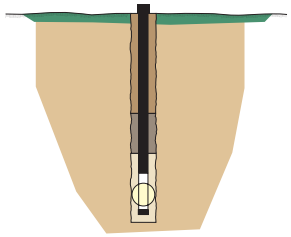
Biostimulation (Enhanced Bioremediation)

- Monitor growth in viable biomass following amendments.
- Monitor overall changes in the microbial "fingerprint" over time or in response to site activities.
- Assess shift in redox state after amendment.
- Electron donor injection (e.g. lactate) should increase the proportion of anaerobic PLFA biomarkers.
- Oxygen amendment should decrease anaerobic PLFA biomarkers.
- Monitor the metabolic health of Proteobacteria (several key degraders are classified within Proteobacteria).

How does PLFA analysis work?

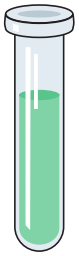
All cells have membranes which consist mainly of phospholipid fatty acids (PLFA). PLFA biomarkers break down quickly when a cell dies, so intact PLFA extracted from an environmental sample (groundwater, soil, sediment or Bio-Trap ®) is only from living (viable) organisms and is expressed as cells per unit of sample. The chemical composition of the PLFA biomarkers differs depending on the type of organism and therefore can be used to generate a "fingerprint" of the microbial community composition. In principle, PLFA biomarker analysis is similar to the analysis of other chemical compounds: (1) PLFA biomarkers are extracted, (2) biomarkers are identified by gas chromatography with flame ionization detection (GC-FID), and (3) biomarkers can be confirmed by mass spectroscopy (MS), if necessary.

Sample Collection



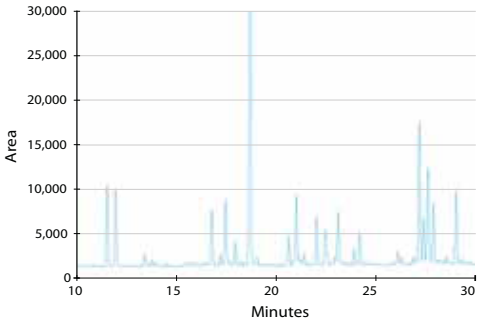
Groundwater, soil, or Bio-Trap Sampler collected and shipped overnight on ice (4°c)

PLFA Extraction



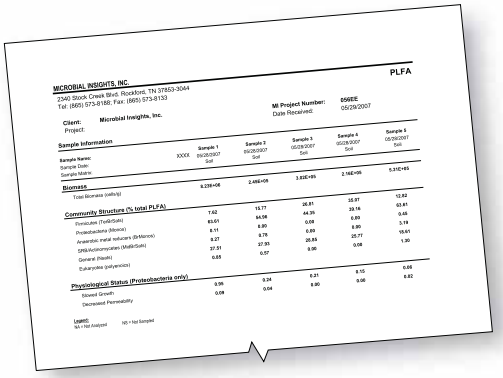
PLFA biomarkers are extracted from samples upon arrival

Gas Chromatography

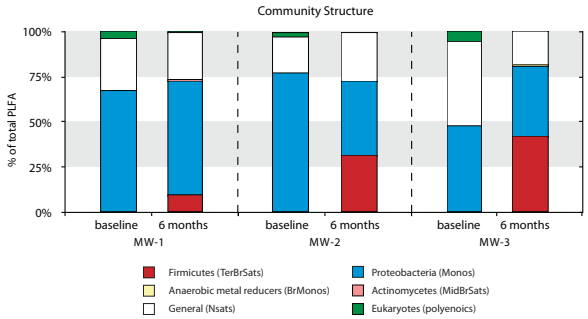


Biomarkers are identified by gas chromatography with flame ionization detection (GC-FID)

Results



Assessment



Results are integrated with other site parameters to evaluate site management decisions

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