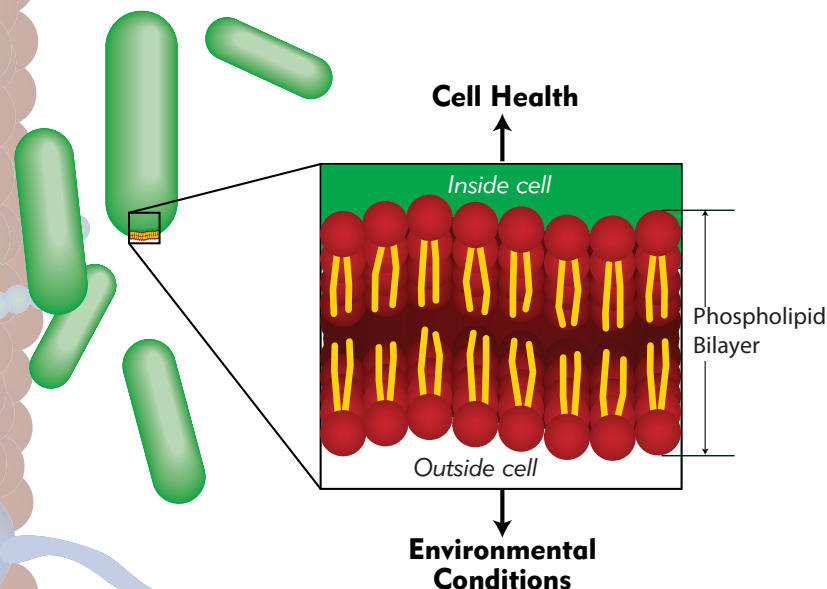




MOLECULAR BIOLOGICAL TOOL

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 <i>Proteobacteria</i> which includes a wide variety of aerobes and anaerobes	Many hydrocarbon utilizing bacteria are classified within <i>Proteobacteria</i>
Terminally Branched Saturated (TerBrSats)	Characteristic of <i>Firmicutes</i> and <i>Bacteroides</i>	<i>Firmicutes</i> 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 <i>Actinomycetes</i>	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

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)

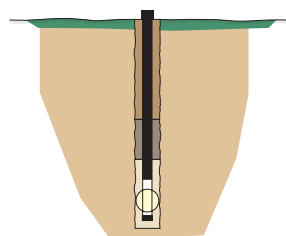
- Monitor growth in viable biomass following amendments.
- Monitor overall changes in the microbial "fingerprint" over time or in response to site activities.
- Assess shift in redox state after amendment.
- Electron donor injection (e.g. lactate) should increase the proportion of anaerobic PLFA biomarkers.
- 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

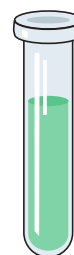
therefore can be used to generate a "fingerprint" of the microbial community composition. In principle, PLFA biomarker analysis is similar to the analysis of other chemical compounds: (1) PLFA biomarkers are extracted, (2) biomarkers are identified by gas chromatography with flame ionization detection (GC-FID), and (3) biomarkers can be confirmed by mass spectroscopy (MS), if necessary.

Sample Collection



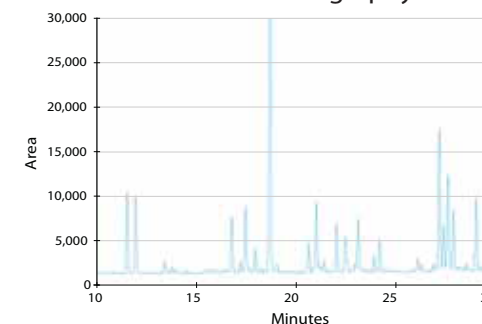
Groundwater, soil, or Bio-Trap Sampler collected and shipped overnight on ice (4°C)

PLFA Extraction



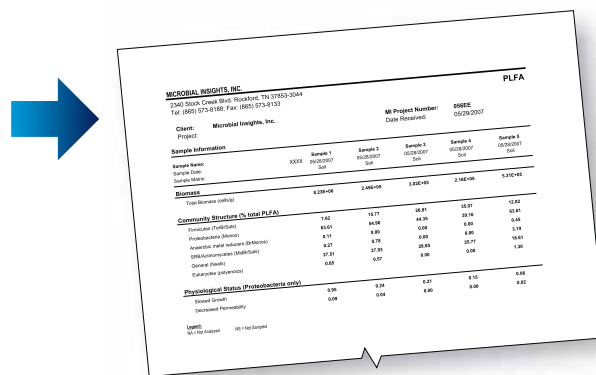
PLFA biomarkers are extracted from samples upon arrival

Gas Chromatography



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

Results



MICROBIAL INSIGHTS, INC.		PLFA	
2340 Black Creek Blvd, Moorabbin, VIC 3163-3004 Tel: (06) 939 5786 Fax: (06) 975 8153		MI Project Number: 0586 Date Received: 05/26/2007	
Client: Microbial Insights, Inc.	Sample Information	Sample 1	Sample 2
Sample Name: 3001	Sample Date: 05/26/2007	Sample 3	Sample 4
Sample ID: 3001	Sample Type: Soil	Sample 5	Sample 6
Sample ID: 3001	Sample Type: Soil	Sample 7	Sample 8
Sample ID: 3001	Sample Type: Soil	Sample 9	Sample 10
Sample ID: 3001	Sample Type: Soil	Sample 11	Sample 12
Sample ID: 3001	Sample Type: Soil	Sample 13	Sample 14
Sample ID: 3001	Sample Type: Soil	Sample 15	Sample 16
Sample ID: 3001	Sample Type: Soil	Sample 17	Sample 18
Sample ID: 3001	Sample Type: Soil	Sample 19	Sample 20
Sample ID: 3001	Sample Type: Soil	Sample 21	Sample 22
Sample ID: 3001	Sample Type: Soil	Sample 23	Sample 24
Sample ID: 3001	Sample Type: Soil	Sample 25	Sample 26
Sample ID: 3001	Sample Type: Soil	Sample 27	Sample 28
Sample ID: 3001	Sample Type: Soil	Sample 29	Sample 30
Sample ID: 3001	Sample Type: Soil	Sample 31	Sample 32
Sample ID: 3001	Sample Type: Soil	Sample 33	Sample 34
Sample ID: 3001	Sample Type: Soil	Sample 35	Sample 36
Sample ID: 3001	Sample Type: Soil	Sample 37	Sample 38
Sample ID: 3001	Sample Type: Soil	Sample 39	Sample 40
Sample ID: 3001	Sample Type: Soil	Sample 41	Sample 42
Sample ID: 3001	Sample Type: Soil	Sample 43	Sample 44
Sample ID: 3001	Sample Type: Soil	Sample 45	Sample 46
Sample ID: 3001	Sample Type: Soil	Sample 47	Sample 48
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Sample ID: 3001	Sample Type: Soil	Sample 73	Sample 74
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Sample ID: 3001	Sample Type: Soil	Sample 77	Sample 78
Sample ID: 3001	Sample Type: Soil	Sample 79	Sample 80
Sample ID: 3001	Sample Type: Soil	Sample 81	Sample 82
Sample ID: 3001	Sample Type: Soil	Sample 83	Sample 84
Sample ID: 3001	Sample Type: Soil	Sample 85	Sample 86
Sample ID: 3001	Sample Type: Soil	Sample 87	Sample 88
Sample ID: 3001	Sample Type: Soil	Sample 89	Sample 90
Sample ID: 3001	Sample Type: Soil	Sample 91	Sample 92
Sample ID: 3001	Sample Type: Soil	Sample 93	Sample 94
Sample ID: 3001	Sample Type: Soil	Sample 95	Sample 96
Sample ID: 3001	Sample Type: Soil	Sample 97	Sample 98
Sample ID: 3001	Sample Type: Soil	Sample 99	Sample 100

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

Assessment

