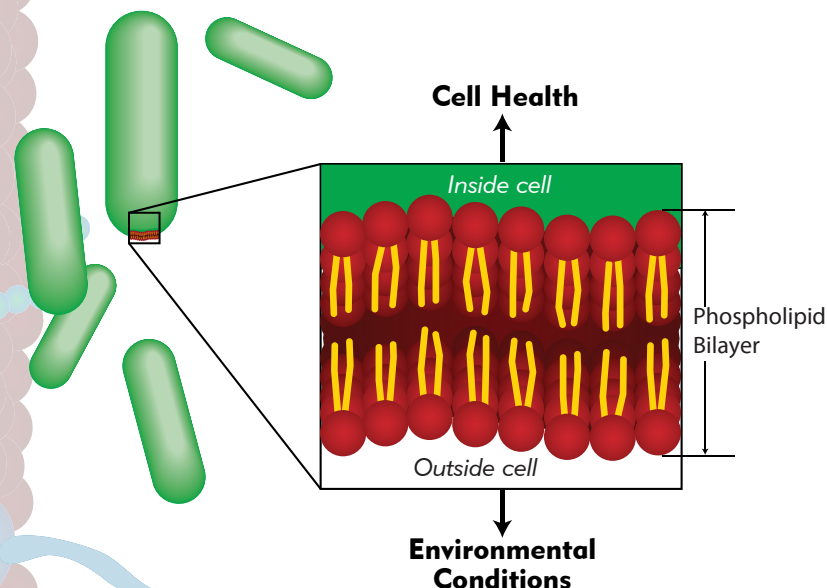# mi PLFA
MOLECULAR BIOLOGICAL TOOL

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.

Cell Health

Inside cell

Phospholipid Bilayer

Outside cell

Environmental Conditions

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 MIC
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* are typically anaerobes and fermenters which produce organic acids and can also support growth of other MIC associated microorganisms
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)	Organic acids produced by fungi have also been linked to pitting corrosion

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MI PLFA
MOLECULAR BIOLOGICAL TOOL

PLFA applications include:

Routine Monitoring

- Track viable biomass in fluid or coupon samples.
- MIC potential increases with increases in biomass and biofilm formation.
- Assess redox state (aerobic vs. anaerobic) and gain insight into potential MIC mechanisms.

Biocide Assessment

- Determine whether biocide dose reduced viable biomass.
- Examine changes in microbial community following biocide application.
- Investigate microbial recovery and re-establishment of biofilms following biocide application.

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 (corrosion coupon, water, soil, Bio-Trap®, etc.) 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.
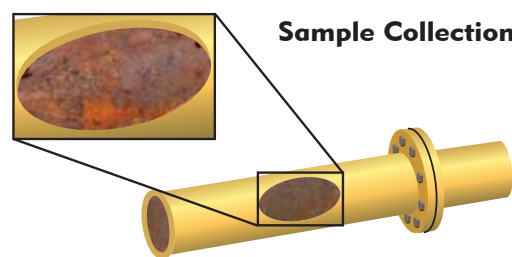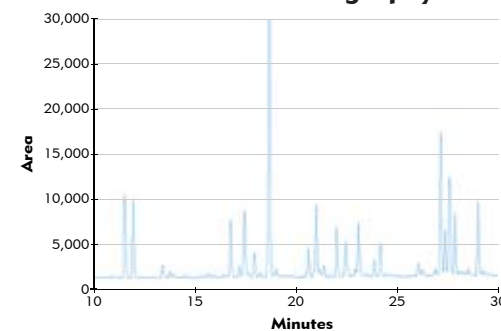
Sample Collection

Samples (corrosion coupon, water, soil, Bio-Trap, etc.) are collected and shipped overnight on ice (4°C).
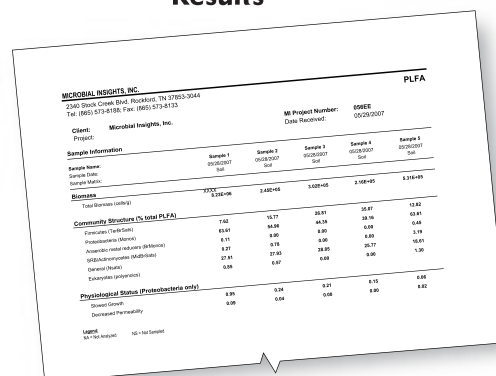
PLFA Extraction

PLFA biomarkers are extracted from samples upon arrival

Gas Chromatography

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

Results

Assessment
Community Structure

Legend:
- Firmicutes (TerBrSats)
- Proteobacteria (Monos)
- Anaerobic metal reducers (BrMonos)
- Actinomycetes (MidBrSats)
- General (Nsats)
- Eukaryotes (polyenoics)

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

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