SiREM
Leading Science · Lasting Solutions

Getting the Most Value from Your Site's Molecular Biology Testing Program

Phil Dennis, SiREM

Online Short Course: Petroleum Hydrocarbon Site Data Management and Performance Monitoring Strategies -5 November 2020

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SiREM Service Areas

treatability studies

genetrac

KB-1

DGGB

Passive Samplers

WATERLOO MEMBRANE SAMPLER

SP3

Introduction to gene-trac® Testing

- Gene-Trac = DNA based tests
  - Quantitative (q)PCR tests for specific microbes & functional genes for bioremediation/other biological processes
  - Gene-Trac NGS for microbial community analysis

- Over 35,000 tests performed from sites worldwide
- 40 specific qPCR targets available,

You can now use our online form to order your sampling supplies.

CLICK HERE TO ORDER SAMPLING SUPPLIES

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Gene-Trac Test Targets

Over Forty Gene-Trac Tests for various compound classes:

- Chlorinated solvents (anaerobic and aerobic pathways)
- 1,4-dioxane (metabolic and cometabolic)
- BTEX degradation (anaerobic and aerobic)
- MTBE
- Nitrogen compounds (e.g., anammox/denitrification)
- Perchlorate
- Microbial groups (e.g. SRB/SOB/Archaea/)
- Microbial community characterization
- SARS CoV-2 environmental testing
- And more…

Visit www.siremlab.com for Gene-Trac targets list

Gene-Trac Tests for PHC Degraders

Anaerobic Pathways

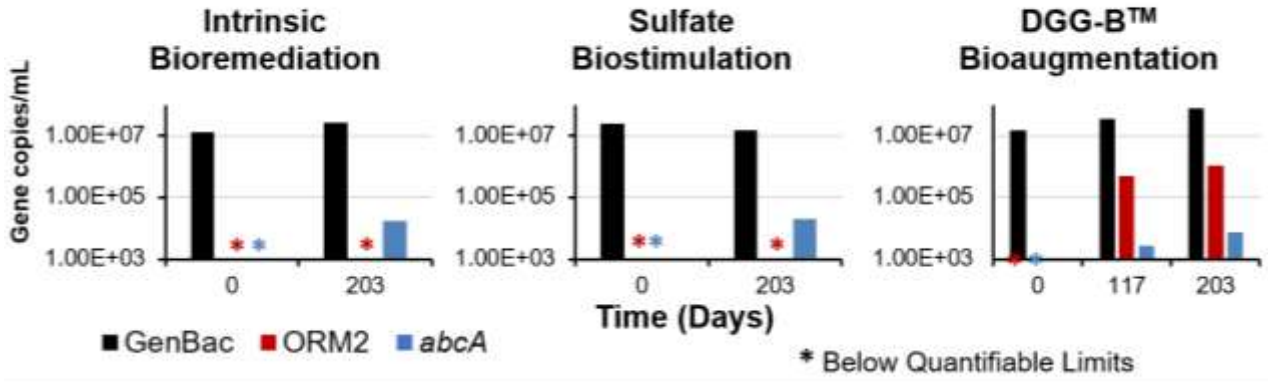
- Benzene – ORM-2/*abcA* *Peptococcaceae*
- Toluene-*bssA*
- *Geobacter* – aromatics
- Sulfate/Nitrate Reducers synthophs to anaerobic pathways

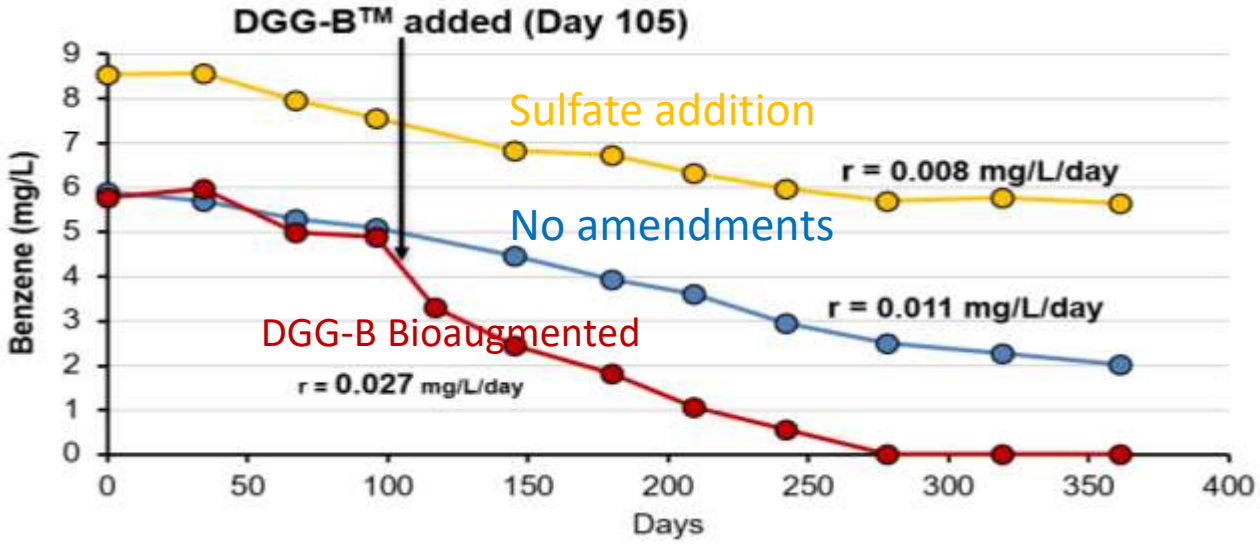
Aerobic Pathways

- Methane/Propane Monooxygenases
- Naphthalene Dioxygenase
- Toluene Monooxygenase
- Toluene Dioxygenase
- Phenol Monooxygenase
- Xylene Monooxygenase
- MTBE/TBA degradation

ORM2 Anaerobic Benzene Degrader

- ORM2 is a *Deltaproteobacterium*
- Benzene specialist from an oil refinery site
- Produces enzymes that ferment benzene
- Gene-Trac ORM2 test is used to quantify





Molecular Testing Data From a Gasoline Site Study

Increases in ORM2 after DGG-B bioaugmentation corelated with higher rates of benzene degradation

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MICROBIAL COMMUNITY CHARACTERIZATION BY NEXT GENERATION SEQUENCING

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What is Next Generation Sequencing (NGS)?

High throughput DNA sequencing technologies that provide massive amounts of sequence data –used for microbial community characterization

Most Common Platform:

- Illumina “MySeq”
  - Millions of sequence reads/run
  - Tens of thousands of sequences per sample
  - Has reduced sequencing costs dramatically

Some Uses of Gene-Trac NGS Microbial Community Analysis

Make better informed decisions manage bioremediation systems:

- Understand existing and potential microbial processes (e.g., MNA)
- Visualize microbial community spatial and temporal variability
- Determine impact of amendments electron donors/acceptors, nutrients, pH buffers, bioaugmentation etc.

Understanding Microbial Communities using NGS

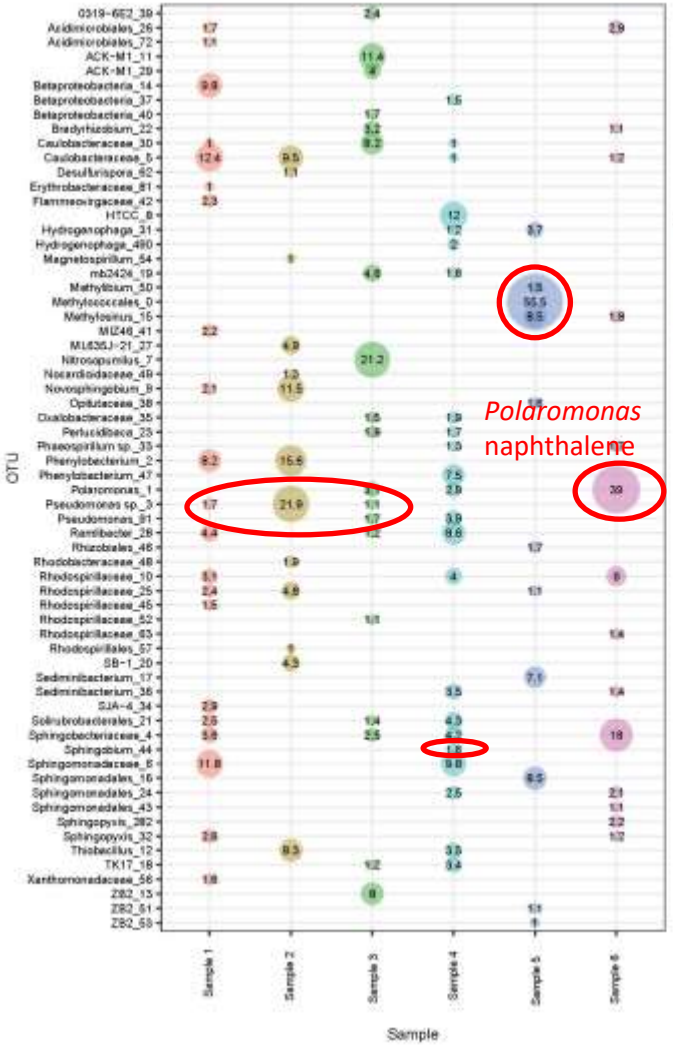
- Gene-Trac NGS provides detailed microbial community characterization, reports include easy to interpret figures including:

  - Composition bubble plots
  - Semi-quantitative enumeration of dominant microbes
  - Functional analysis
  - Cluster analyses to relate microbial community similarity/difference

Methylococcales
Methane degrader

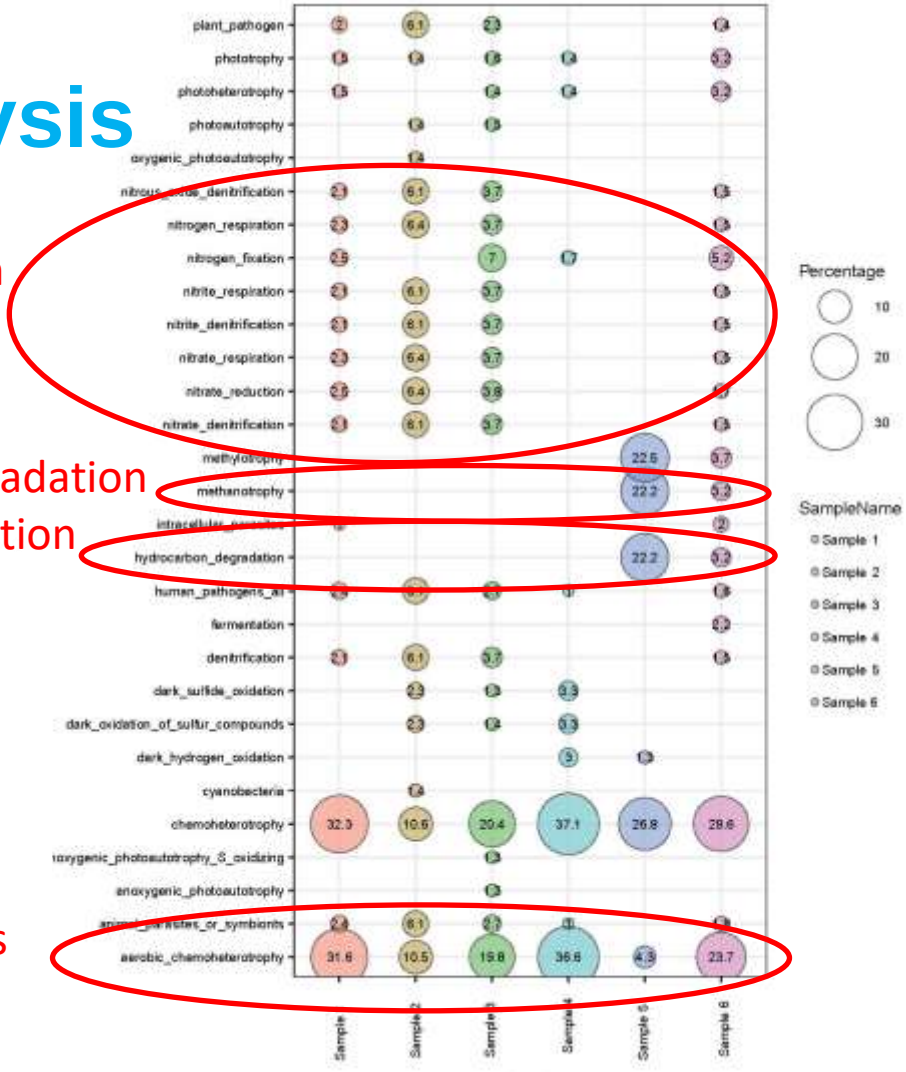
Pseudomonas
alkanes/ aromatics

Sphingobium
polyaromatics



Functional Analysis

- Converts microbial names to metabolic functions
- Over 80 metabolic functions covered
- Makes data Interpretation easier



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Enumeration

- Gene-Trac NGS provides estimated enumeration for dominant microbes

- While not as quantitative as qPCR, provides lots of data at ~$10/enumeration

Taxonomic Designation	OTU ID	Estimated Enumeration/L					
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
p__Armatimonadetes; c__0319-6E2	39	3.E+02	0.E+00	1.E+06	0.E+00	0.E+00	0.E+00
o__Acidimicrobiales	26	2.E+05	3.E+04	3.E+04	2.E+05	4.E+05	2.E+06
o__Acidimicrobiales	72	1.E+05	0.E+00	0.E+00	4.E+03	0.E+00	1.E+03
o__Actinomycetales; f__ACK-M1	11	0.E+00	0.E+00	7.E+06	4.E+02	0.E+00	0.E+00
o__Actinomycetales; f__ACK-M1	29	0.E+00	0.E+00	2.E+06	0.E+00	4.E+04	3.E+03
c__Betaproteobacteria	14	1.E+06	0.E+00	0.E+00	2.E+04	1.E+05	3.E+04
c__Betaproteobacteria	37	2.E+03	0.E+00	0.E+00	7.E+05	4.E+04	3.E+05
c__Betaproteobacteria	40	0.E+00	0.E+00	1.E+06	3.E+04	4.E+04	5.E+02
g__Bradyrhizobium	22	8.E+04	0.E+00	2.E+06	1.E+05	4.E+04	7.E+05
f__Caulobacteraceae	30	1.E+05	9.E+02	5.E+06	5.E+05	2.E+06	3.E+04
f__Caulobacteraceae	5	1.E+06	4.E+06	1.E+04	5.E+05	1.E+07	8.E+05
g__Desulfurispora	62	0.E+00	4.E+05	0.E+00	0.E+00	0.E+00	0.E+00
f__Erythrobacteraceae	81	1.E+05	5.E+04	0.E+00	8.E+03	1.E+05	2.E+04
f__Flammeovirgaceae	42	3.E+05	6.E+03	0.E+00	0.E+00	4.E+04	0.E+00
o__Alteromonadales; f__HTCC2188; g__HTCC	8	0.E+00	1.E+05	2.E+05	6.E+06	5.E+05	3.E+05
g__Hydrogenophaga	31	3.E+02	3.E+03	7.E+04	6.E+05	2.E+08	2.E+04
g__Hydrogenophaga	490	2.E+02	0.E+00	0.E+00	1.E+06	4.E+04	1.E+03
g__Magnetospirillum	54	0.E+00	4.E+05	0.E+00	0.E+00	0.E+00	0.E+00
c__Acidobacteria-6; o__Ill1-15; f__mb2424	19	2.E+04	0.E+00	3.E+06	8.E+05	1.E+06	6.E+05
g__Methylibium	50	8.E+01	0.E+00	1.E+04	1.E+03	8.E+07	8.E+03
o__Methylococcales	0	2.E+02	1.E+03	4.E+02	1.E+03	3.E+09	0.E+00
g__Methylosinus	15	0.E+00	0.E+00	9.E+02	0.E+00	4.E+08	1.E+06
c__Deltaproteobacteria; o__MIZ46	41	2.E+05	0.E+00	0.E+00	0.E+00	0.E+00	0.E+00
p__Cyanobacteria; c__ML635J-21	27	0.E+00	2.E+06	0.E+00	0.E+00	4.E+04	0.E+00
g__Nitrosopumilus	7	0.E+00	0.E+00	1.E+07	0.E+00	2.E+07	0.E+00
f__Nocardioidaceae	49	5.E+03	5.E+05	1.E+04	2.E+03	0.E+00	1.E+04
g__Novosphingobium	9	2.E+05	4.E+06	4.E+02	2.E+04	4.E+04	6.E+04
f__Opitutaceae	38	0.E+00	0.E+00	2.E+05	4.E+02	8.E+07	5.E+04
f__Oxalobacteraceae	35	1.E+04	2.E+04	9.E+05	9.E+05	0.E+00	1.E+05
g__Perlucidibaca	23	5.E+02	0.E+00	1.E+06	8.E+05	3.E+06	4.E+05
g__Phaeospirillum; s__fulvum	33	4.E+04	0.E+00	0.E+00	6.E+05	4.E+04	1.E+06
g__Phenylobacterium	2	9.E+05	6.E+06	3.E+03	7.E+04	4.E+04	6.E+05
g__Phenylobacterium	47	5.E+03	3.E+04	9.E+03	4.E+06	1.E+05	5.E+03
g__Polaromonas	1	4.E+03	1.E+03	2.E+06	1.E+06	2.E+06	3.E+07
g__Pseudomonas; s__stutzeri	3	2.E+05	9.E+06	6.E+05	4.E+05	2.E+06	5.E+05
g__Pseudomonas	91	6.E+04	9.E+03	1.E+06	2.E+06	5.E+05	6.E+05
g__Ramlibacter	28	5.E+05	6.E+02	7.E+05	4.E+06	3.E+06	6.E+05
o__Rhizobiales	46	3.E+03	0.E+00	4.E+03	4.E+02	8.E+07	0.E+00
f__Rhodobacteraceae	48	2.E+03	8.E+05	0.E+00	0.E+00	0.E+00	0.E+00
f__Rhodospirillaceae	10	3.E+05	3.E+02	4.E+05	2.E+06	2.E+06	4.E+06
f__Rhodospirillaceae	25	3.E+05	2.E+06	0.E+00	0.E+00	6.E+07	6.E+04
f__Rhodospirillaceae	45	2.E+05	1.E+05	0.E+00	0.E+00	6.E+05	0.E+00
f__Rhodospirillaceae	52	0.E+00	0.E+00	6.E+05	0.E+00	5.E+05	0.E+00
f__Rhodospirillaceae	63	7.E+03	0.E+00	5.E+05	5.E+04	6.E+05	9.E+05
o__Rhodospirillales	57	0.E+00	4.E+05	0.E+00	0.E+00	0.E+00	0.E+00
o__Bacteroidales; f__SB-1	20	0.E+00	2.E+06	4.E+02	4.E+02	0.E+00	0.E+00
g__Sediminibacterium	17	3.E+03	0.E+00	0.E+00	2.E+05	4.E+08	3.E+04
g__Sediminibacterium	36	0.E+00	3.E+02	4.E+05	2.E+06	9.E+05	9.E+05
k__Bacteria; p__TM6; c__SJA-4	34	3.E+05	0.E+00	9.E+04	2.E+04	4.E+05	2.E+05
o__Solirubrobacterales	21	3.E+05	0.E+00	8.E+05	2.E+06	2.E+06	3.E+05
f__Sphingobacteriaceae	4	4.E+05	0.E+00	2.E+06	2.E+06	8.E+06	1.E+07
g__Sphingobium	44	0.E+00	2.E+03	0.E+00	8.E+05	0.E+00	0.E+00
f__Sphingomonadaceae	6	1.E+06	9.E+03	2.E+05	5.E+06	2.E+07	1.E+05
o__Sphingomonadales	16	4.E+04	5.E+03	4.E+05	1.E+05	4.E+08	5.E+02
o__Sphingomonadales	24	3.E+04	9.E+02	0.E+00	1.E+06	0.E+00	1.E+06
o__Sphingomonadales	43	0.E+00	2.E+03	2.E+05	3.E+05	0.E+00	7.E+05
g__Sphingopyxis	282	0.E+00	2.E+05	2.E+04	9.E+04	0.E+00	1.E+06
g__Sphingopyxis	32	3.E+05	3.E+05	1.E+03	2.E+04	4.E+06	8.E+05
g__Thiobacillus	12	7.E+04	3.E+06	4.E+05	2.E+06	3.E+05	7.E+04
p__Chloroflexi; c__TK17	18	0.E+00	0.E+00	7.E+05	2.E+06	1.E+05	2.E+05
f__Xanthomonadaceae	56	2.E+05	7.E+03	0.E+00	0.E+00	7.E+06	0.E+00
k__Bacteria; p__OD1; c__ZB2	13	0.E+00	0.E+00	5.E+06	0.E+00	0.E+00	0.E+00
k__Bacteria; p__OD1; c__ZB2	51	0.E+00	0.E+00	0.E+00	0.E+00	6.E+07	0.E+00
k__Bacteria; p__OD1; c__ZB2	53	0.E+00	0.E+00	0.E+00	4.E+04	5.E+07	0.E+00

Notes: k=kingdom, p=phylum, f=family, o=order, c=class, g=genus, s=species

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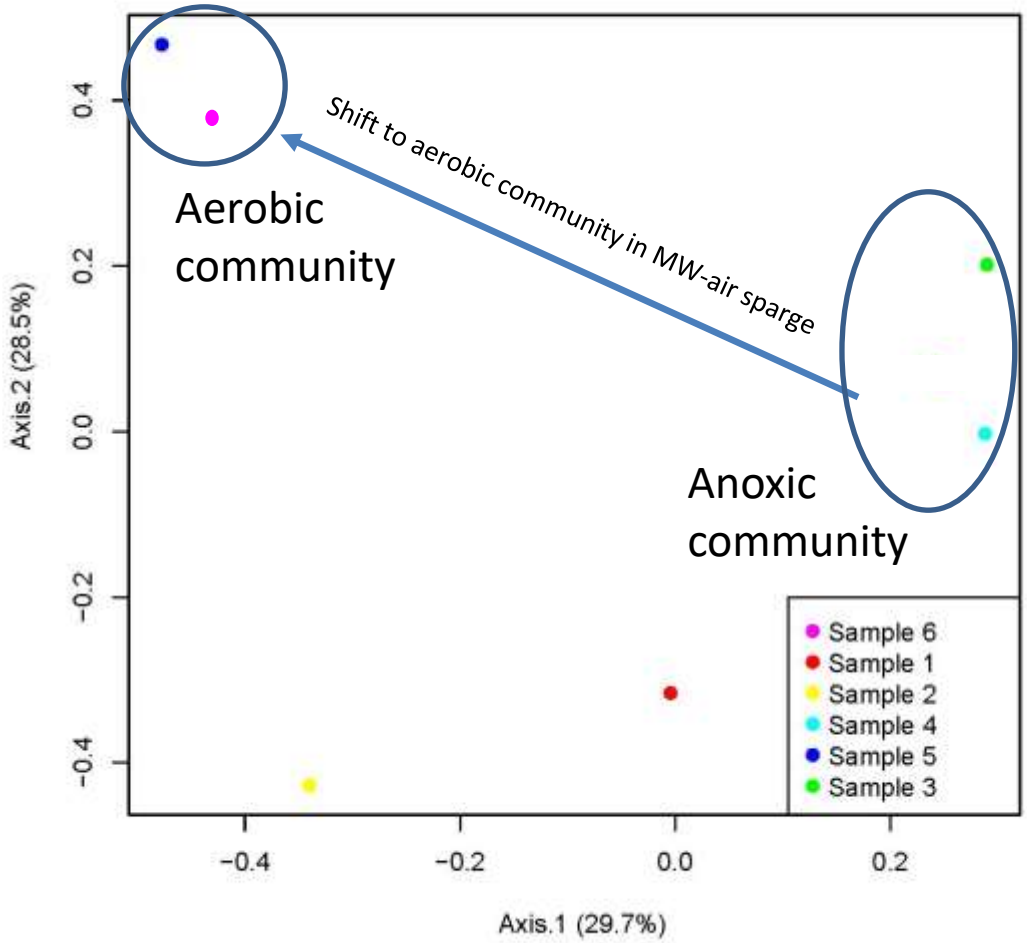
NGS Principal Coordinates Analysis

relates similarity of microbial communities



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MAXIMIZING VALUE OF YOUR MOLECULAR TESTING PROGRAM

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Value of Molecular Genetic Testing

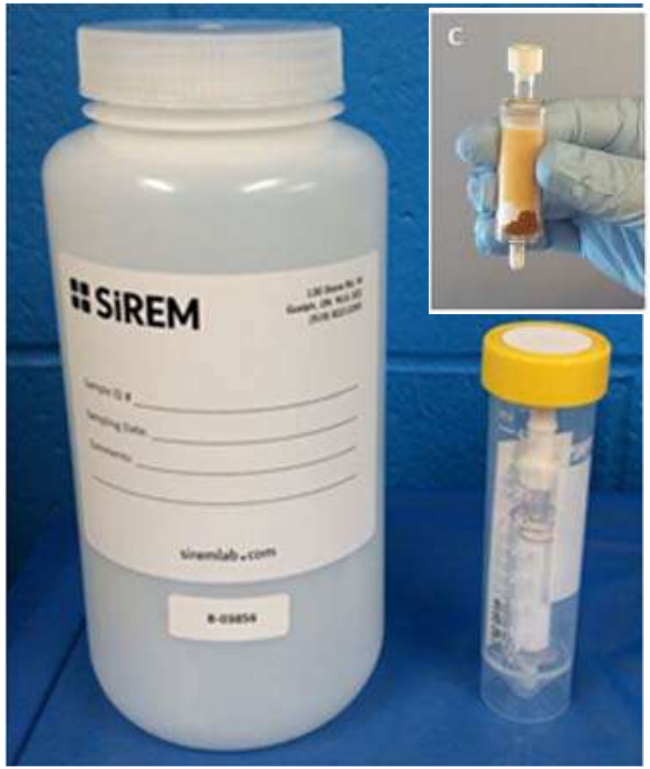
- **Establish Causation:** Molecular tests provide information on why changes are occurring-Proof bioremediation is occurring

- **Parallel Lines of Evidence:** provide additional evidence of site changes and progress – for LNAPL sites, may not see decreases in aqueous concentrations but may see increases in hydrocarbon degraders

- **Very Sensitive:** detect changes in microbiology before geochemical changes observed (e.g., *Dhc* increases months before ethene detected)- Sensitivity especially useful for MNA sites where microbial abundance is low

- **Spatially Discrete:** microbes are more localized than their metabolic products (e.g., methanogens vs. methane)-can help locate problem areas

Sampling Methodologies



- Samples can be either field filters or 1L groundwater samples
- Groundwater samples weigh 1 kg (2.2 lbs), filter weighs <25 grams, ships in Styrofoam 1/40th weight
- =Substantial shipping $ saving with filter samples

Maximizing Value with your Sampling Strategy

Maximize Interpretable Data

- You only have one chance to get a baseline sample- get these even if you expect it to be negative
- Sample source zone, upgradient /downgradient/locations with varying geochemistry- gives context
- Blind duplicates increase confidence in data/provide replication

$ Savings

- Move testing outward from injection locations-impact of injections often takes time to spread
- Focus on zones where changes are occurring-or are likely to occur
- Can pool samples e.g., to determine if certain biodegrader present or absent at site - impact detection limit
- You can sample, extract and archive for $100/sample-test later as needed based on other data etc.

Choosing the Right Testing Regime

- Bioaugmentation typically tracking only 1 or 2 key microbes e.g., ORM2 and SRB-for benzene bioremediation

- MNA–often requires using more test targets to confirm /rule out multiple/rare degradation pathways–NGS good tool

- Chlorinated solvents *Dehalococcoides* and *Dehalobacter* and *Dehalogenimonas often* key players- main functional genes well characterized

- Petroleum hydrocarbons-79 microbial genera degrade/multitude of functional genes–NGS good tool to find diversity individual qPCR (or even arrays) may be insufficient

Getting the Most from your Data

- **Look at trends-** are key microbes increasing? stationary? declining?

- **Use doubling times of key microbes-** as a performance measure for different locations across a site “inverse half lives”
  q2 =microbe concentration at t2 q1=microbe abundance at t1

$$T_d = (t_2 - t_1) \times \frac{\log(2)}{\log(\frac{q2}{q1})}$$

- **Dig deeper-** NGS provides detailed spreadsheets of rare site microbes = identify potential biodegraders to biostimulate

- **Use microbes as biomonitors-** to better understand site changes e.g., in DO/ORP/pH/toxicity/salinity etc.

Data Interpretation Documents

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SiREM Technical Note 2.0:
Interpretation of Gene-Trac®-SRB
(Sulfate Reducing Bacteria) Assay

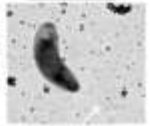
Background

This technical note provides background information and guidelines for interpretation of the Gene-Trac® SRB, sulfate reducing bacteria Assay. SiREM Technical Note 1.4 - *Quantitative Gene-Trac® Assay Test Procedure and Reporting Overview* provides detailed information on general aspects Gene-Trac® test procedures and reporting including data qualifiers and commonly used notes.

Gene-Trac®-SRB is a quantitative polymerase chain reaction (qPCR) test targeting gene sequences unique to SRB, specifically the *dsrA* gene. The *dsrA* gene is ubiquitous in all known sulfate reducing bacteria and is a highly conserved nucleotide sequence making it an excellent gene target for tracking SRB (Rodriguez-Mora et al. 2016).

Sulfate Reducing Bacteria

Sulfate reducing bacteria (SRB) are strict anaerobes that vary significantly in terms of morphology including vibrio and rod-shaped cells (Rabus et al. 2006). SRB can survive in harsh environments including metal contaminated sediments and acid mine drainage ponds. A primary metabolic pathway for SRB is the reduction of sulfate to more reduced forms primarily odiferous hydrogen sulfide (H_2S).



Transmission-electron microscopy image of a vibrio shaped SRB (Tippkötter et al. 2012).

SRB gain energy by coupling the reduction of sulfate to sulfide with the oxidation of various organic compounds (e.g., acetate) or elemental hydrogen (Rabus et al. 2006). The reduction of sulfate to sulfide relies upon several enzymes (Figure 1) including dissimilatory sulfite reductase (DSR) which consists of several subunits including *dsrA* (Figure 1) (Rabus et al. 2006). DSR catalyzes the conversion of sulfite (SO_3^{2-}) to hydrogen sulfide and is found in all sulfate reducers, the presence or absence of the *dsrA* subunit gene is therefore diagnostic for sulfate reducing activity and SRB.

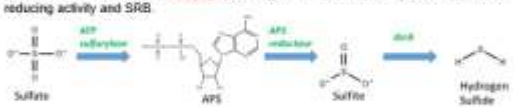


Figure 1: Pathway for the reduction of sulfate indicating the role of ATP Sulfurylase, APS Reductase and Dissimilatory Sulfite Reductase (DSR) the *dsrA* subunit gene codes for the alpha subunit of the DSR enzyme.

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Role of SRB in Petroleum Hydrocarbon Biodegradation

The biodegradation of petroleum hydrocarbons (PH) by SRB is well documented and SRB are able to degrade various (PH) compounds including:

a) Cycloalkanes including the complete degradation of cyclohexane (Jaekel et al. 2015).
b) *N*-alkanes including the gaseous alkane n-butane (Jaekel et al. 2015).
c) Polycyclic aromatic hydrocarbons (such as fluorene, anthracene and pyrene) (Chang et al. 2002).
d) SRB play important roles in the biodegradation of benzene, toluene, ethylbenzene, xylene (BTEX). They do this through the synergistic removal of fermentation products of BTEX degradation including hydrogen and volatile fatty acids such as acetate, allowing the fermentation reactions to proceed (Phelps et al. 1998; Weelink et al. 2010). Furthermore, some species of SRB are needed for the initiation of toluene degradation by other microbial taxa, this may be because the presence of sulfate impedes the degradation of toluene (Da Silva and Alvarez 2004).

SRB Role in Biogeochemical Biodegradation of Chlorinated Solvents and Metals

SRB play an important role in the process of biogeochemical reduction of chlorinated solvents (Brown et al. 2009). SRB reduce sulfate to sulfide which can then combine with iron (Fe II or Fe III) to form iron sulfides (FeS) which abiotically dechlorinate some chlorinated solvents (Brown et al. 2009). Furthermore, after this abiotic process sulfate is regenerated allowing SRB to reduce it back into sulfide to continue the cyclical process (Brown et al. 2009).

Figure 2: Biogeochemical reduction of chlorinated solvents. SRB reduce sulfate (SO_4^{2-}) to hydrogen sulfide anion (HS^-) which reacts with Fe^{2+} producing ferrous sulfide (FeS). FeS reacts with chlorinated compounds including PCE, TCE and carbon tetrachloride regenerating SO_4^{2-} which is reused by SRB to produce HS^- allowing the process to continue.

SRB are able to reduce the highly soluble and toxic metal hexavalent chromium Cr(VI) to insoluble trivalent chromium Cr (III) thereby reducing its toxicity and mobility in groundwater (Cheung and Gu 2003).

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Conclusions

The growing range of molecular testing tools to better manage petroleum bioremediation remediation sites:

- Choose tests wisely
- Sample wisely
- Interpret well
- Speak with your lab - we can help you plan and make the most of your data

Thank you for Joining!

Course Code

“WBTP”

Further Information

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