

Comparing Microbial Profiles from Five Service Station Sites

Battelle Bioremediation Symposium Wednesday – May 24, 2017









Characterizing the Site





BioTrap[®] Samplers

- 30 days of in-well time
- Place at level other samples are collected







Bio-flo Filter[®] Sampling

• Real time sample collection

• Low budget

Comparable accuracy







-0



-0







Four CENSUS Targets



- Phenol Hydroxylase (qPHE) Catalyzes further oxidation of BTEX compounds, indicates the potential for aerobic BTEX biodegradation.
- Naphthalene dioxygenase (qNAH) Catalyzes aerobic biodegradation of naphthalene and other PAHs by incorporation of oxygen into the aromatic ring.
- MTBE utilizing PM1 (qPM1) Targets *Methylibium petroleiphilum* PM1, one of the few bacteria isolated that is capable of growth on MTBE, indicates potential for aerobic MTBE biodegradation.
- Total Eubacteria (qEBAC) total biomass estimate.





Observations from the 5 Sites

- Total biomass averaged E+06 cells/ml or cell/bead regardless of soil type
- Little difference between BioTrap[®] and Bio-flo[®] filter
- PHE gene function most prevalent at four sites/PHE increases possible
- NAH gene function prevalent at only one site/NAH increase possible
- PM1 organism typically at E+02 at sites where it was present
- PM 1 increases found
- Unusual conditions can exist







Observations Compared to 15 Additional Sites

- Total biomass averaged E+07 cells/ml or cells/bead regardless of soil type
- PHE gene function most prevalent at 11 sites
- NAH gene function most prevalent at 4 sites
- PM1 organism typically at E+02 at sites where it was present.
- Unusual conditions can exist







Optimal Response

	Date													
Well I.D.		рН	ORP	DO	TPH-g	Benzene	мтве	ТВА	Methane	Sulfate	Manganese (Dissolved)	Manganese (Total)	lron (Total)	Iron (II) Ferrous
		(units)	(millivolts)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
MW-1	3/16/2012	6.6	(208.4)	0.59	2,200	56	13	2,800	3530	110	2.77	3.00	9.95	0.24
	8/1/2012	11.66	(57.8)	28.48	400	17	11	470	52.9	780	<0.00500	0.0777	0.866	<0.100
	10/18/2012	9.9	48	32.39	240	7.4	16	49	81.8	680	<0.00500	0.0190	0.220	<0.100
	12/18/2012	8.35	106.9	15.2	53	0.73	2.1	19	22.2	650	0.0198	0.0905	0.714	<0.100
	2/18/2013	8.04	129.8	10.84	52	<0.50	<1.0	<10	4.05	600	0.0147	0.0852	0.314	<0.100



Microbial Profile near MW-1

- PHE baseline was E+04
- PHE increased to E+07
- NAH baseline was E+02
- NAH increased to E+05
- EBAC baseline was E+07
- EBAC increased to E+09

PM1 baseline was E+02





What's up here?



Well I.D.	Date													
		рН	ORP	DO	TPH-g	Benzene	МТВЕ	ТВА	Methane	Sulfate	Manganese (Dissolved)	Manganese (Total)	lron (Total)	Iron (II) Ferrous
		(units)	(millivolts)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
B2-A	3/15/2012	6.75	224.7	3.68	68	<0.50	3.9	38	10.9	270	0.553	0.946	0.995	<0.10
	8/17/2012	6.63	(25.8)	1.33	120	0.60	1.6	1300	408	260	2.62	2.76	5.16	<0.100
	10/18/2012	7.03	(6.7)	1.68	230	1.6	<2.0	1700	1090	250	2.81	2.99	8.07	7.14
	12/18/2012	7.18	68.6	1.17	270	<1.0	<2.0	1700	950	280	2.53	2.88	11.4	5.26
	2/18/2013	7.21	47.2	0.92	230	<0.50	1.9	1400	754	270	2.40	2.40	7.43	5.63





Microbial Profile near B2-A

- PHE baseline was E+03
 PM1 baseline was E+02
- PHE decreased to E+02
- NAH baseline was E+02
- NAH decreased to E+01
- EBAC baseline was E+06
- EBAC decreased to E+04

PM1 decreased to E+00





Case Study – Inactive Service Station

- MTBE/TBA remediation
- (20 μg/L/6000 μg/L)
- Lesser concentrations of
- benzene and TPHg
- Full suite of analytical and
- field parameters
- No degradation observed
- No apparent response in geochemical or field data







Disappointing Performance

Time Series Graph 9 2705663 Huntington Beach TBA Concentrations During DPI Remedy Implementation







Case Study – The cause?

- Methane and carbon dioxide showed dramatic fluctuation across the site.
- Possibly a microbial issue?
- Investigation undertaken to evaluate microbial population
 - Total bacteria
 - MTBE/TBA degrader
 - Methanotrophs bacteria that consume methane as a source of energy
 - Methanogens bacteria that produce methane while degrading organic matter under anaerobic conditions





Remediation Troubleshooting Data

• CENSUS – upgradient, treatment area, downgradient

Client: Antea Group Project: MI Project Number: 005JB Date Received: 002/02/2012 Sample Information MW-6_2012020 DFE-3_2012020 B-17_20120201 MW-3_2012020 Sample Date: 02/01/2012 02/01/2	CENSUS				3044	i, TN 37853- 73-8133	reek Blvd. Rockford 3-8188 Fax. (865) 5	340 Stock Cr el. (865) 573
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Client Sample ID: MW-6_2012020 DPE-3_2012020 B-17_20120201 MW-3_2012020 Sample Date: 02/01/2012 0							mation	ample Inforr
1 1 1 1 1 1 Sample Date: 02/01/2012 02/01/2012 02/01/2012 02/01/2012 02/01/2012 Units: Analyst: RW RW RW RW RW Other Genera RW RW RW RW RW RW Methylibium petroleiphilum PM1 PM1 3.05E+02 8.49E+01 4.28E+01 1.12E+01 Phylogenetic Group Total Eubacteria EBAC 2.15E+04 7.82E+03 5.60E+03 2.47E+03 Methanogen MGN 2.47E+06 1.86E+06 2.02E+06 5.44E+05 Methano Oxidizing Bacteria MOB 7.41E+07 6.28E+07 5.35E+07 4.01E+07		3_2012020	B-17_20120201 MW-3	DPE-3_2012020	MW-6_2012020		mple ID:	Client Sar
Sample Date.O210 h2012O210 h2012O210 h2012O210 h2012O210 h2012Units:cells/mLcells/mLcells/mLcells/mLcells/mLAnalyst:RWRWRWRWOther GeneraMethyliblum petroleiphilum PM1PM13.05E+028.49E+014.28E+011.12E+01Phylogenetic GroupTotal EubacteriaEBAC2.15E+047.82E+035.60E+032.47E+03MethanogenMGN2.47E+061.86E+062.02E+065.44E+05Methano Oxidizing BacteriaMOB7.41E+076.28E+075.35E+074.01E+07		1/2012	1	1	1) etc.	Comple D
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		E+07	5.35E+07 4.01E	6.28E+07	7.41E+07	МОВ	xidizing Bacteria	Methane Ox
IOGEN								OGEN [°]



Case Study - Optimization

- Evidence of Deeply Reduced Aquifer
- Bio-Geochemically methanogenic
- Raise REDOX (climb the REDOX ladder)
 - Satisfy O₂ debt
- Used a quick release O₂ material to
 - rapidly satisfy REDOX demands and
 - reduce non-target degrader populations
 - improve target degrader populations
- Use ORC Advanced for long-term O₂ release
 - Maintain beneficial REDOX condition
 - Provide support for biodegradation of target COC's







Multi- parameter Tracking





Remediation Troubleshooting Data

MICROBIAL INSIGHTS, INC.

10515 Research Dr., Knoxville, TN 37932 Tel. (865) 573-8188 Fax. (865) 573-8133									
Client: Antea Group Project: ELT 5663				MI Project Number: Date Received:					
Sample Infor	mation								
Client Sa	mple ID:		DPE-1-20140212	DPE-3-2014021 2	B-17-20140212				
Sample E Units: Analyst:	Date:		02/12/2014 cells/mL RW	- 02/12/2014 cells/mL RW	02/12/2014 cells/mL RW				
Functional G	enes								
Methylibiun	n petroleiphilum	PM1	<5.70E+00	<1.00E+01	<1.11E+01				
Phylogenetic	: Group								
Total Euba Methane O Methanoge	cteria xidizing Bacteria n	EBAC MOB MGN	1.27E+06 3.14E+07 1.00E-01 (J)	1.31E+06 5.91E+07 1.13E+02	3.12E+06 5.28E+07 4.00E+01				
<u>Legend:</u> NA = Not Ar < = Result n	nalyzed NS = No not detected	ot Sampled	J = Estimated ge	ne copies below	PQL but above LQL	l = Inhibite	ed		





SUMMARY

Microbial data establish a baseline.

Microbial data allow tracking of progress.

Microbial data can help troubleshoot a site.







Questions and Answers

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Acknowledgement – Jeff Friedman, Antea Group-Long Beach

