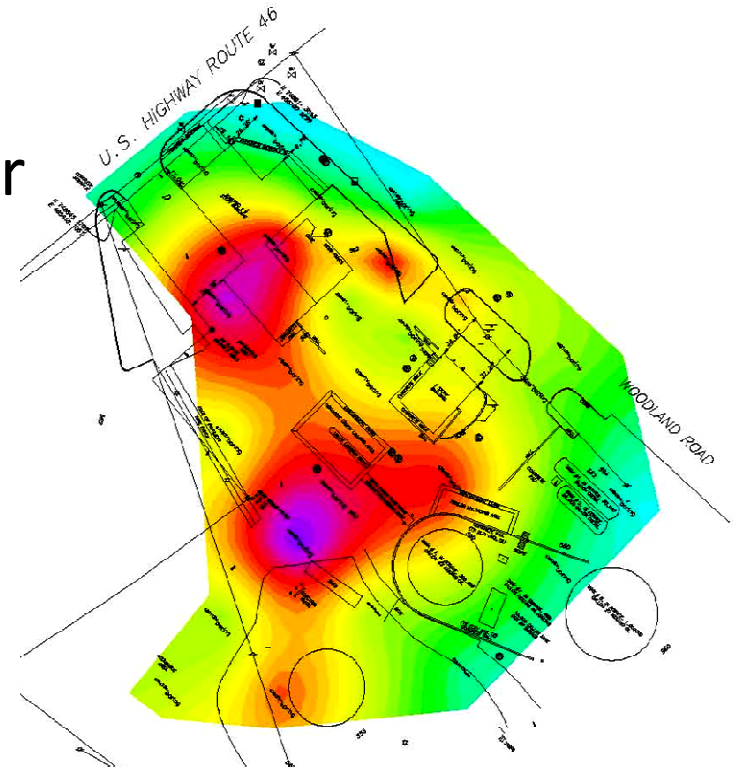




A qPCR Array (QuantArray™) Demonstrates Enhanced Biodegradation

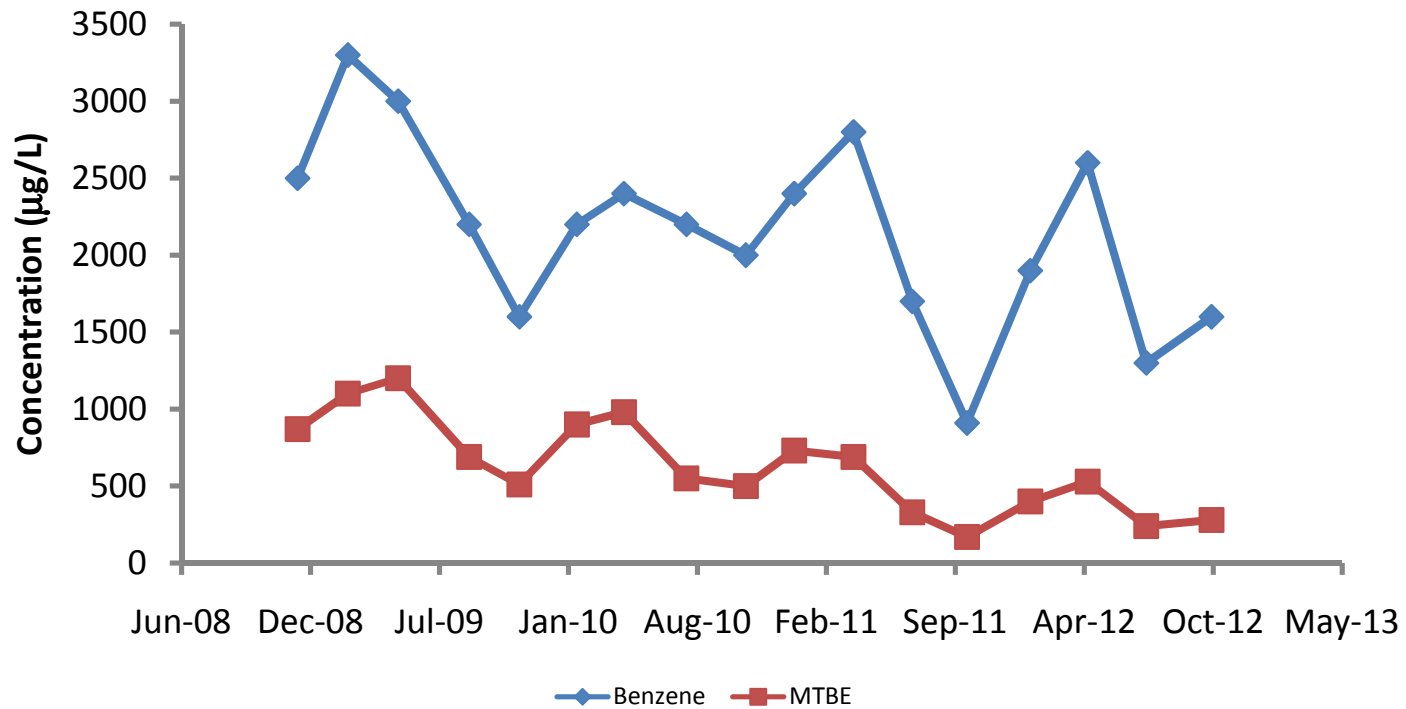
Study Site

- Petroleum distribution facility
- Gasoline impacted groundwater



Evaluating Biodegradation

- Chemical lines of evidence



Evaluating Biodegradation

- Geochemical lines of evidence



~ 3.1 mg O₂/ mg benzene

- Electron acceptor concentrations in impacted vs non-impacted wells

Evaluating Biodegradation

- Microbiological lines of evidence
- Plate counts ?
 - <1% of bacteria can be cultured
 - Vastly underestimate populations



qPCR

- Accurate and precise



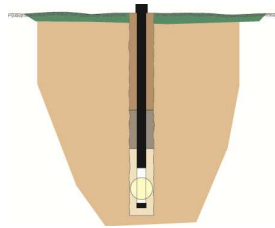
- Sensitive and specific

- Rapid



qPCR

Sample Collection



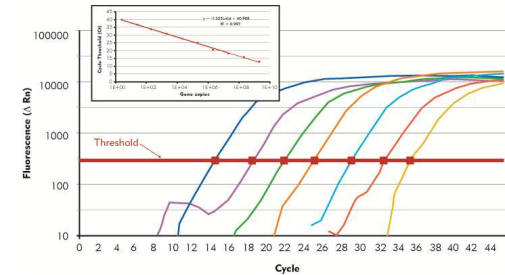
Groundwater, soil, or Bio-Trap samplers collected and shipped overnight on ice (4c)

Extraction



DNA or RNA is extracted from sample upon arrival

Amplification



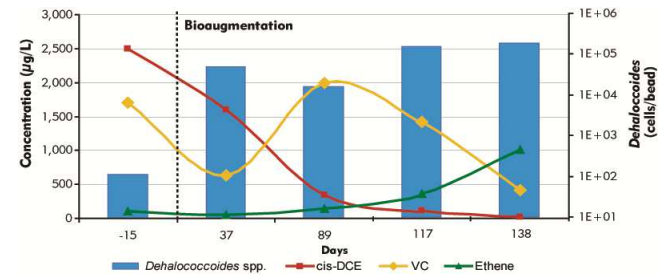
Quantitative Real-Time PCR is used to detect and quantify targets of interest (i.e. toluene monooxygenase)

Results

MICROBIAL INSIGHTS, INC.		CENSUS		
2340 Stock Creek Blvd. Nashville, TN 37203-2044		MI Project Number: 01888		
Tel: (615) 875-8188 Fax: (615) 875-8133		Date Received: 02/12/2007		
Client: Microbial Insights, Inc.				
Project: 2340				
Sample Information				
Client Sample ID:	Sample A:	Sample B:	Sample C:	
2340	02110207	02110207	02110207	
Sample Date:	02/07/07	02/07/07	02/07/07	
Dehalococcal Bacteria				
Dehalococcales spp (%)	DHC	8.2E+01	6.5E+02	1.8E+02
Legend: NS = Not Analyzed, E = Estimated gene copies below PCR, but above LOD, I = Inhibited, - = Result not detected				

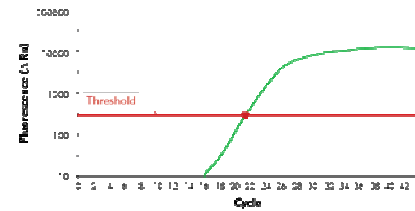
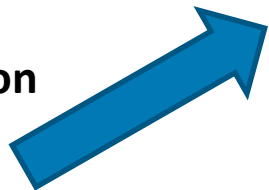
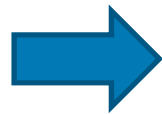
Results are emailed to project contact (7 to 10 day TAT)

Assessment

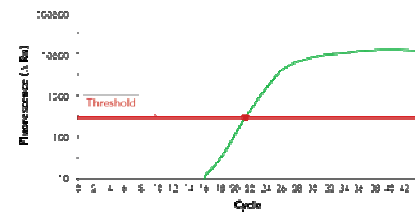


Results are integrated with other site parameters to evaluate site management decisions

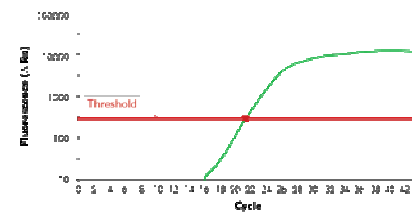
qPCR Approach



TOD



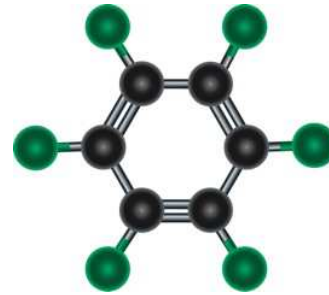
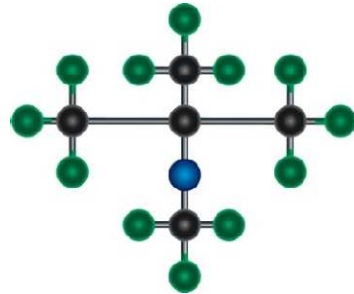
PHE



BSS

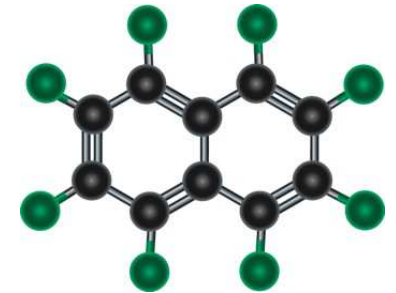
Mixtures of Contaminants

- Monoaromatics (BTEX)



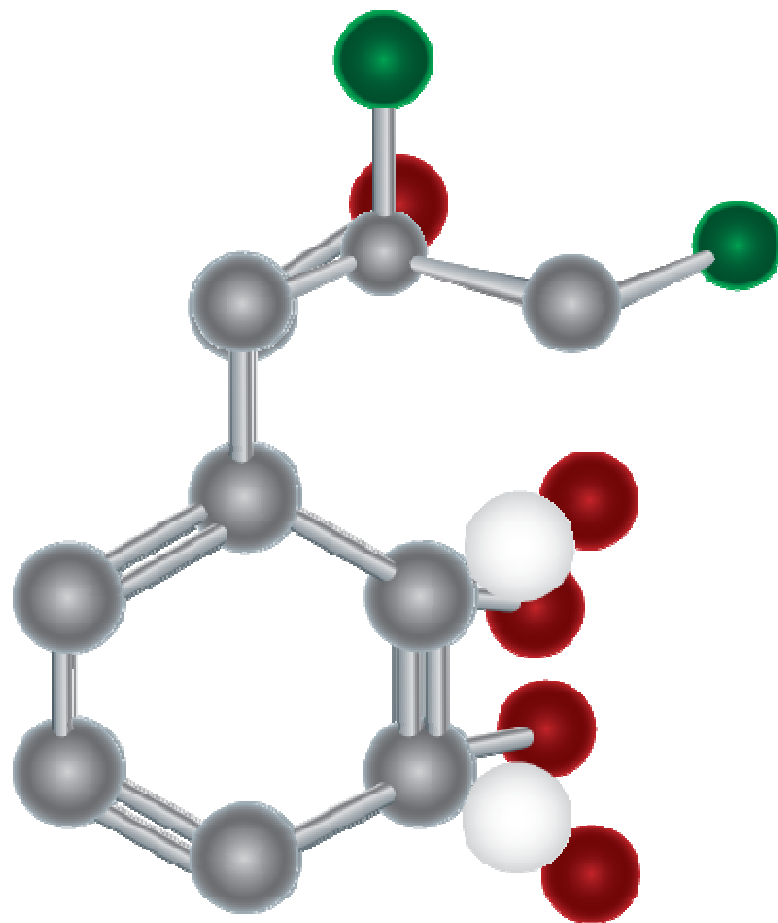
- Fuel oxygenates (MTBE)

- Polycyclic aromatics hydrocarbons (PAHs)

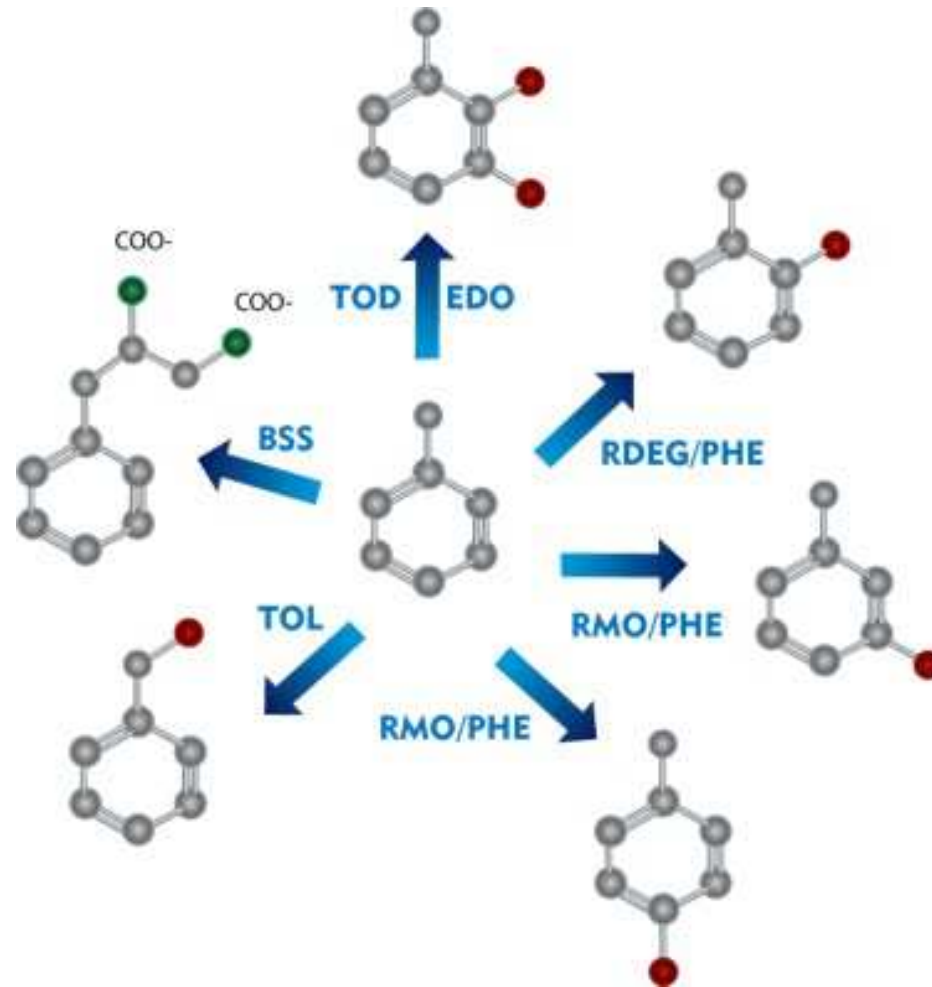


- TPH and n-alkanes

Anaerobic BTXEX



Multiple Pathways

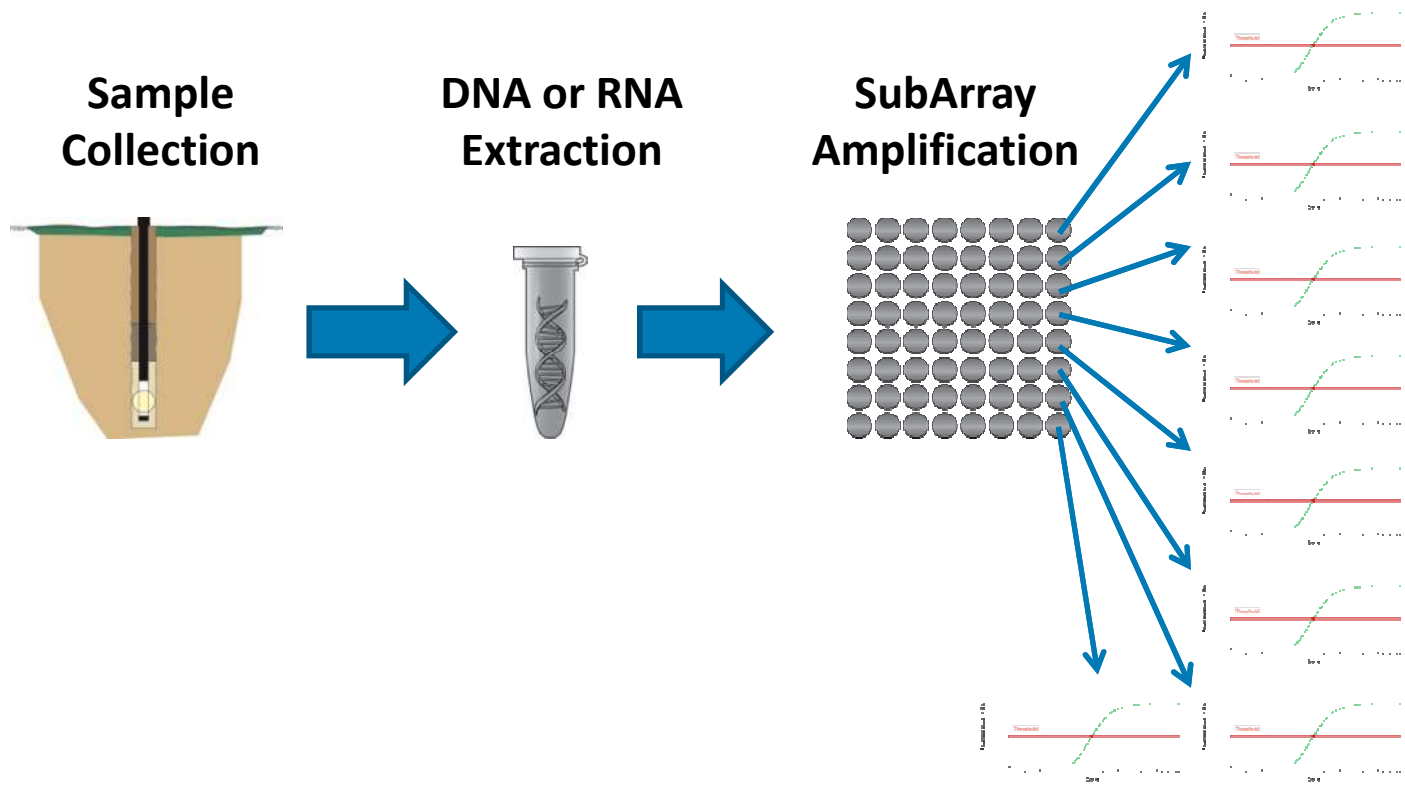


QuantArray™

- Array platform for simultaneous analysis of numerous targets
- Accurate quantification of all targets from a single analysis



QuantArray Approach



QuantArray™-Petro

Sample Information	MW-1	MW-2
Aerobic BTEX and MTBE (cells/mL)		
Toluene 3- and 4-Monooxygenases (RMO)	1.98E+03	7.30E+03
Toluene 2 Monooxygenase (RDEG)	2.50E+02	4.61E+03
Phenol Hydroxylase (PHE)	2.16E+04	2.17E+04
Toluene/Benzene Dioxygenase (TOD)	<2.50E+02	<2.50E+02
Xylene/Toluene Monooxygenase (TOL)	<2.50E+02	<2.50E+02
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)	1.38E+04	<2.50E+02
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)	<2.50E+02	<2.50E+02
<i>Methylbium petroliphilum</i> PM1 (PM1)	5.65E+05	1.92E+05
TBA Monooxygenase (TBA)	<2.50E+02	<2.50E+02
Aerobic PAHs and Alkanes (cells/mL)		
Naphthalene Dioxygenase (NAH)	3.49E+05	<2.50E+02
Phenanthrene Dioxygenase (PHN)	<2.50E+02	<2.50E+02
Alkane Monooxygenase (ALK)	<2.50E+02	<2.50E+02
Alkane Monooxygenase (ALMA)	<2.50E+02	<2.50E+02
Anaerobic BTEX (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)	2.16E+05	3.33E+03
Benzylsuccinate synthase (BSS)	<2.50E+02	<2.50E+02
Benzene Carboxylase (ABC)	<2.50E+02	4.31E+02
Anaerobic PAHs and Alkanes (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)	2.16E+05	3.33E+03
Naphthylmethylsuccinate Synthase (NMS)	<2.50E+02	<2.50E+02
Naphthalene Carboxylase (ANC)	<2.50E+02	<2.50E+02
Alkylsuccinate Synthase (ASSA)	<2.50E+02	<2.50E+02
Other (cells/bead)		
Total Eubacteria (EBAC)	1.72E+07	1.72E+07
Sulfate Reducing Bacteria (APS)	<2.50E+02	<2.50E+02

QuantArray™-Petro

Sample Information	MW-1	MW-2
Aerobic BTEX and MTBE (cells/mL)		
Toluene 3- and 4-Monooxygenases (RMO)		
Toluene 2 Monooxygenase (RDEG)		
Phenol Hydroxylase (PHE)		
Toluene/Benzene Dioxygenase (TOD)		
Xylene/Toluene Monooxygenase (TOL)		
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)		
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)		
<i>Methylibium petroliphilum</i> PM1 (PM1)		
TBA Monooxygenase (TBA)		
	Aerobic BTEX	
		Aerobic MTBE & TBA
Aerobic PAHs and Alkanes (cells/mL)		
Naphthalene Dioxygenase (NAH)		
Phenanthrene Dioxygenase (PHN)		
Alkane Monooxygenase (ALK)		
Alkane Monooxygenase (ALMA)		
Anaerobic BTEX (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Benzylsuccinate synthase (BSS)		
Benzene Carboxylase (ABC)		
Anaerobic PAHs and Alkanes (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Naphthylmethylsuccinate Synthase (NMS)		
Naphthalene Carboxylase (ANC)		
Alkylsuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

QuantArray™-Petro

Sample Information	MW-1	MW-2
Aerobic BTEX and MTBE (cells/mL)		
Toluene 3- and 4-Monooxygenases (RMO)		
Toluene 2 Monooxygenase (RDEG)		
Phenol Hydroxylase (PHE)		
Toluene/Benzene Dioxygenase (TOD)		
Xylene/Toluene Monooxygenase (TOL)		
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)		
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)		
<i>Methylbium petroliphilum</i> PM1 (PM1)		
TBA Monooxygenase (TBA)		
Aerobic PAHs and Alkanes (cells/mL)		
Naphthalene Dioxygenase (NAH)		
Phenanthrene Dioxygenase (PHN)		
Alkane Monooxygenase (ALK)		
Alkane Monooxygenase (ALMA)		
Anaerobic BTEX (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Benzylsuccinate synthase (BSS)		
Benzene Carboxylase (ABC)		
Anaerobic PAHs and Alkanes (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Naphthylmethylsuccinate Synthase (NMS)		
Naphthalene Carboxylase (ANC)		
Alkylsuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

Anaerobic BTEX

QuantArray™-Petro

Sample Information	MW-1	MW-2
Aerobic BTEX and MTBE (cells/mL)		
Toluene 3- and 4-Monooxygenases (RMO)		
Toluene 2 Monooxygenase (RDEG)		
Phenol Hydroxylase (PHE)		
Toluene/Benzene Dioxygenase (TOD)		
Xylene/Toluene Monooxygenase (TOL)		
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)		
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)		
<i>Methylbium petroliphilum</i> PM1 (PM1)		
TBA Monooxygenase (TBA)		
Aerobic PAHs and Alkanes (cells/mL)		
Naphthalene Dioxygenase (NAH)		
Phenanthrene Dioxygenase (PHN)		
Alkane Monooxygenase (ALK)		
Alkane Monooxygenase (ALMA)		
Anaerobic BTEX (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Benzylsuccinate synthase (BSS)		
Benzene Carboxylase (ABC)		
Anaerobic PAHs and Alkanes (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Naphthylmethylsuccinate Synthase (NMS)		
Naphthalene Carboxylase (ANC)		
Alkylsuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

Anaerobic Benzene

QuantArray™-Petro

Sample Information	MW-1	MW-2
Aerobic BTEX and MTBE (cells/mL)		
Toluene 3- and 4-Monooxygenases (RMO)		
Toluene 2 Monooxygenase (RDEG)		
Phenol Hydroxylase (PHE)		
Toluene/Benzene Dioxygenase (TOD)		
Xylene/Toluene Monooxygenase (TOL)		
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)		
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)		
<i>Methylibium petroliphilum</i> PM1 (PM1)		
TBA Monooxygenase (TBA)		
Aerobic PAHs and Alkanes (cells/mL)		
Naphthalene Dioxygenase (NAH)		Aerobic PAHs
Phenanthrene Dioxygenase (PHN)		
Alkane Monooxygenase (ALK)		
Alkane Monooxygenase (ALMA)		
Anaerobic BTEX (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Benzylsuccinate synthase (BSS)		
Benzene Carboxylase (ABC)		
Anaerobic PAHs and Alkanes (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		Anaerobic PAHs
Naphthylmethylsuccinate Synthase (NMS)		
Naphthalene Carboxylase (ANC)		
Alkylsuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

QuantArray™-Petro

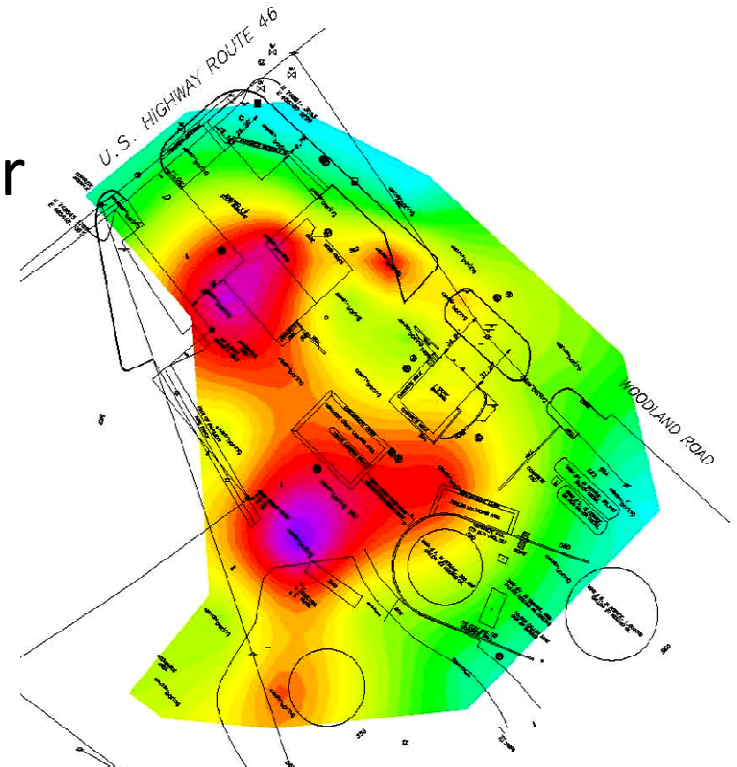
Sample Information	MW-1	MW-2
Aerobic BTEX and MTBE (cells/mL)		
Toluene 3- and 4-Monooxygenases (RMO)		
Toluene 2 Monooxygenase (RDEG)		
Phenol Hydroxylase (PHE)		
Toluene/Benzene Dioxygenase (TOD)		
Xylene/Toluene Monooxygenase (TOL)		
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)		
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)		
<i>Methylbium petroliphilum</i> PM1 (PM1)		
TBA Monooxygenase (TBA)		
Aerobic PAHs and Alkanes (cells/mL)		
Naphthalene Dioxygenase (NAH)		
Phenanthrene Dioxygenase (PHN)		
Alkane Monooxygenase (ALK)		Aerobic Alkanes
Alkane Monooxygenase (ALMA)		
Anaerobic BTEX (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Benzylsuccinate synthase (BSS)		
Benzene Carboxylase (ABC)		
Anaerobic PAHs and Alkanes (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Naphthylmethylsuccinate Synthase (NMS)		
Naphthalene Carboxylase (ANC)		
Alkylsuccinate Synthase (ASSA)		Anaerobic Alkanes
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

QuantArray™-Petro

Sample Information	MW-1	MW-2
Aerobic BTEX and MTBE (cells/mL)		
Toluene 3- and 4-Monooxygenases (RMO)		
Toluene 2 Monooxygenase (RDEG)		
Phenol Hydroxylase (PHE)		
Toluene/Benzene Dioxygenase (TOD)		
Xylene/Toluene Monooxygenase (TOL)		
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)		
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)		
<i>Methylibium petroliphilum</i> PM1 (PM1)		
TBA Monooxygenase (TBA)		
Aerobic PAHs and Alkanes (cells/mL)		
Naphthalene Dioxygenase (NAH)		
Phenanthrene Dioxygenase (PHN)		
Alkane Monooxygenase (ALK)		
Alkane Monooxygenase (ALMA)		
Anaerobic BTEX (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Benzylsuccinate synthase (BSS)		
Benzene Carboxylase (ABC)		
Anaerobic PAHs and Alkanes (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Naphthylmethylsuccinate Synthase (NMS)		
Naphthalene Carboxylase (ANC)		
Alkylsuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		
		Total Bacteria and SRBs

Study Site

- Petroleum distribution facility
- Gasoline impacted groundwater



In Situ Microcosm Study

MNA



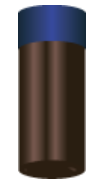
BioStim



COC



MICRO
(Bio-Trap)

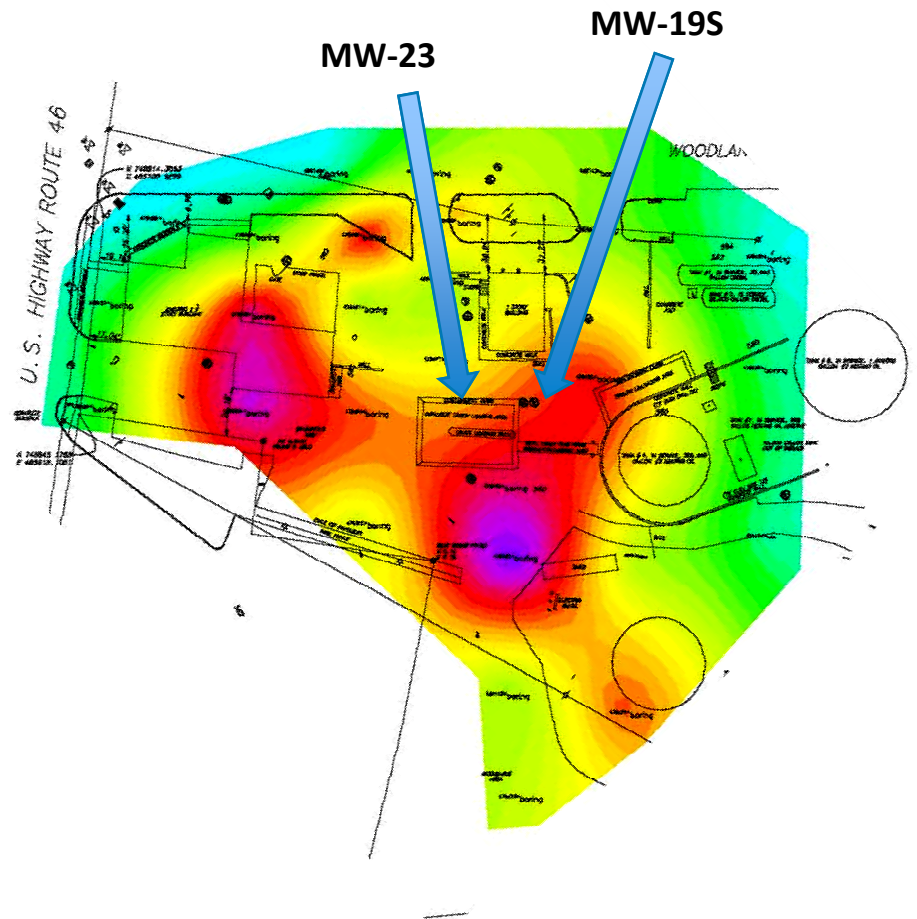


GEO

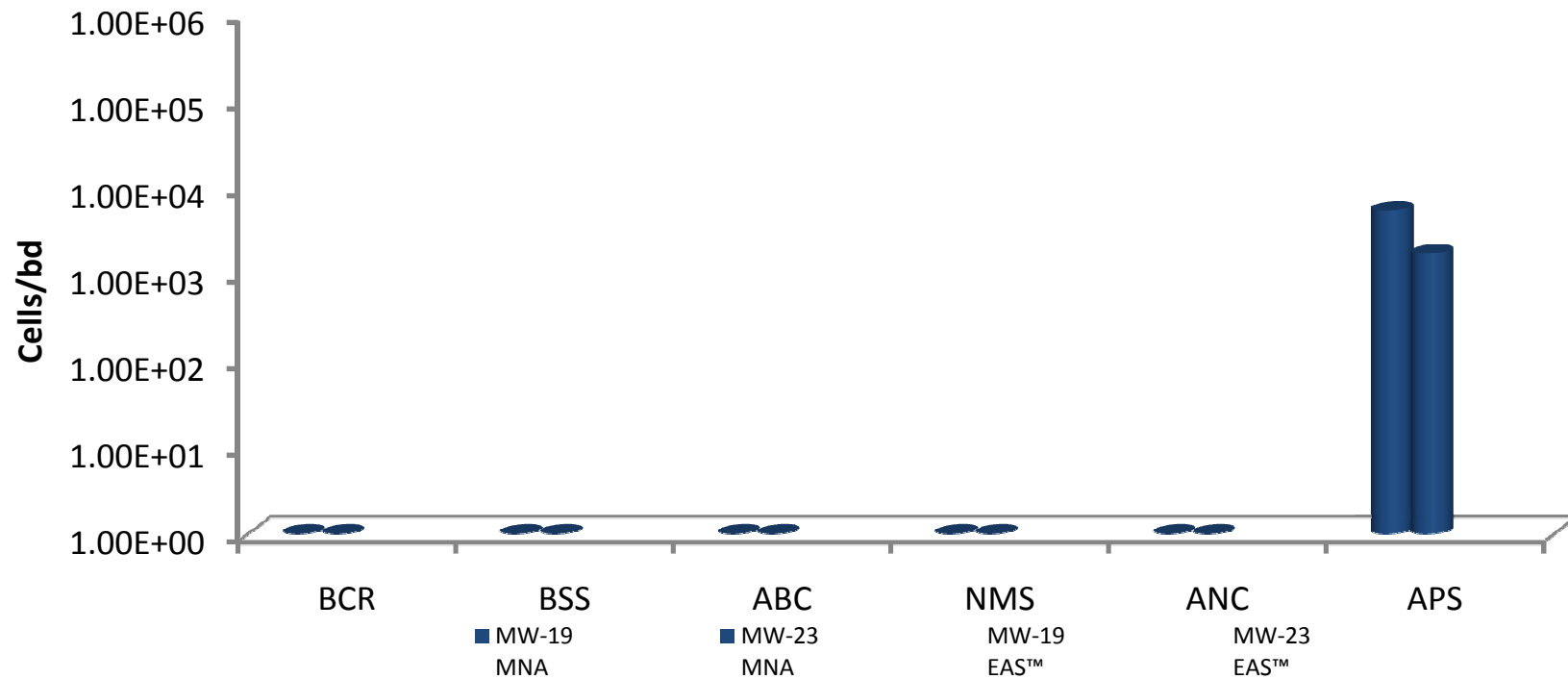


EAS™

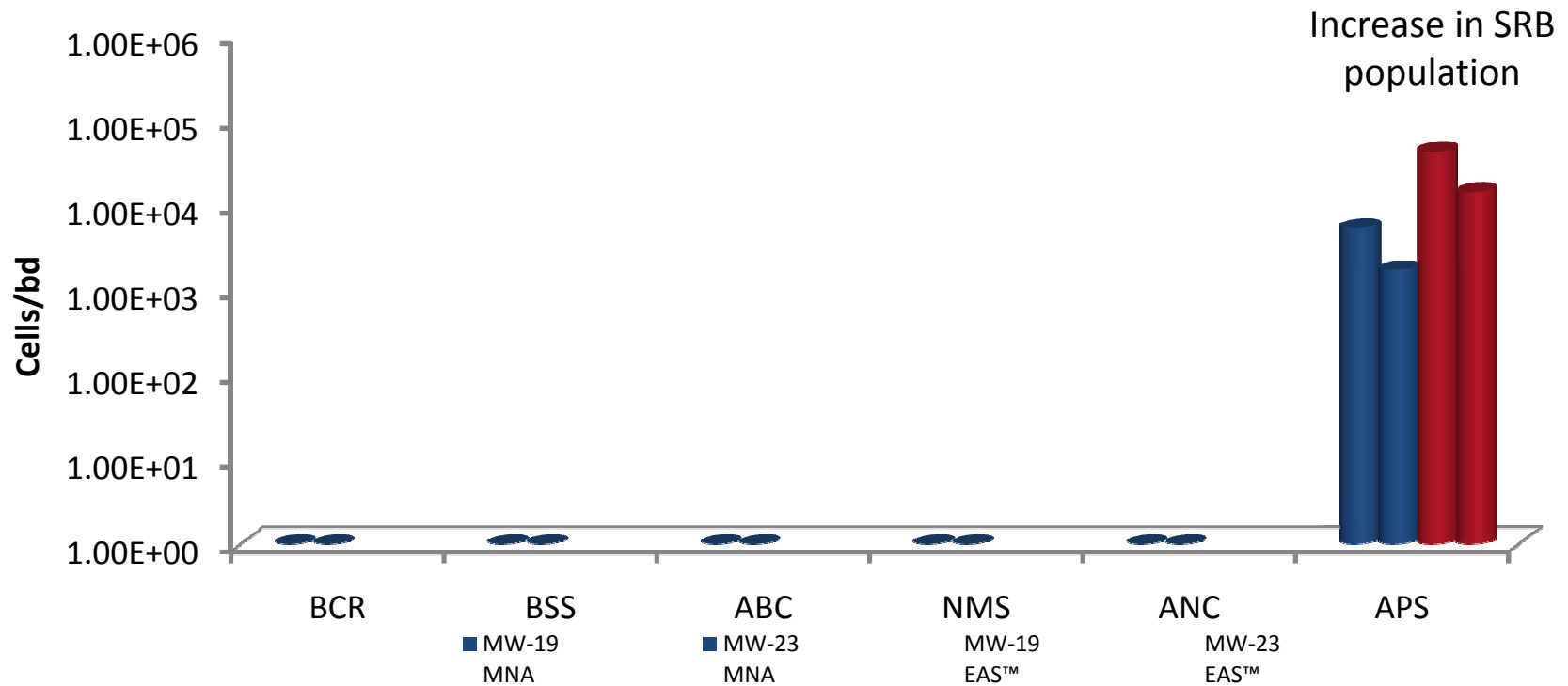
In Situ Microcosms – MNA and EAS™ Amended



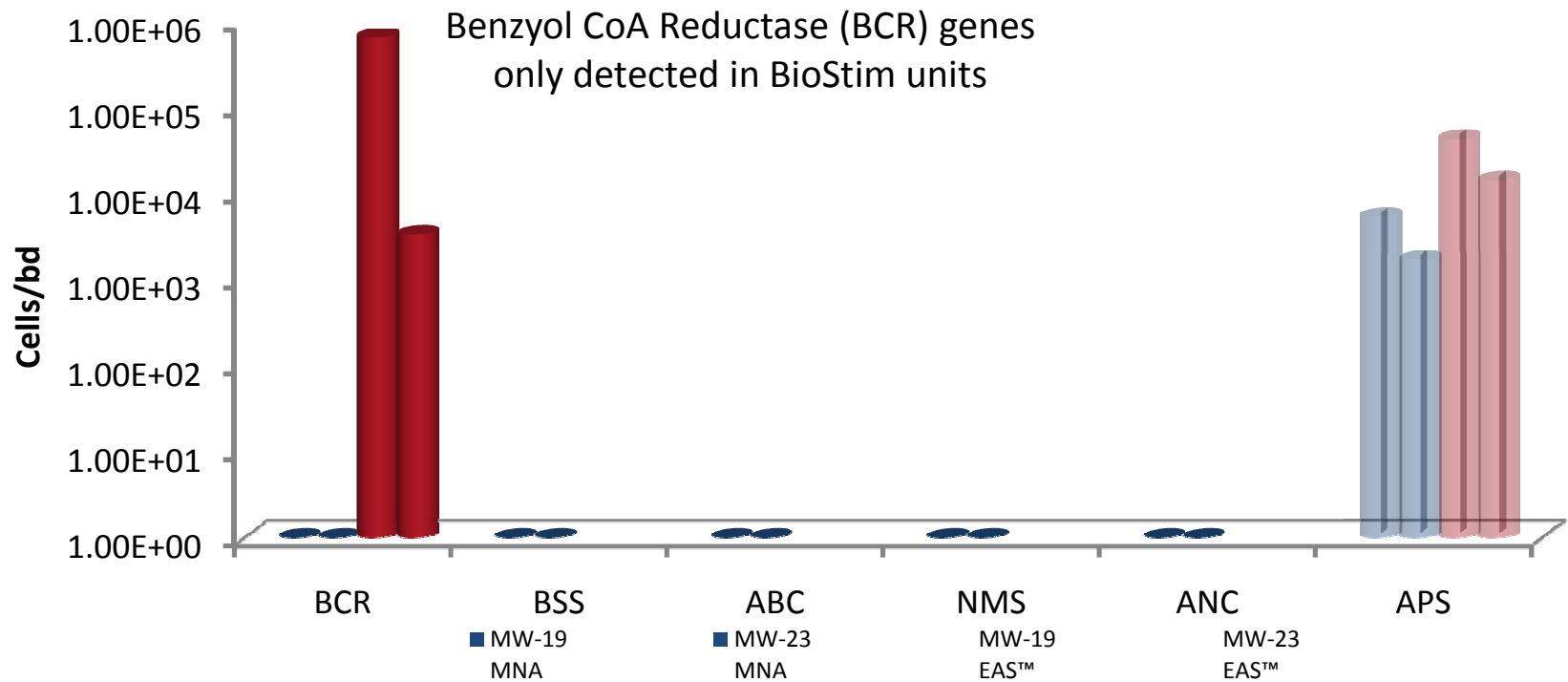
Anaerobic Processes – MNA Unit



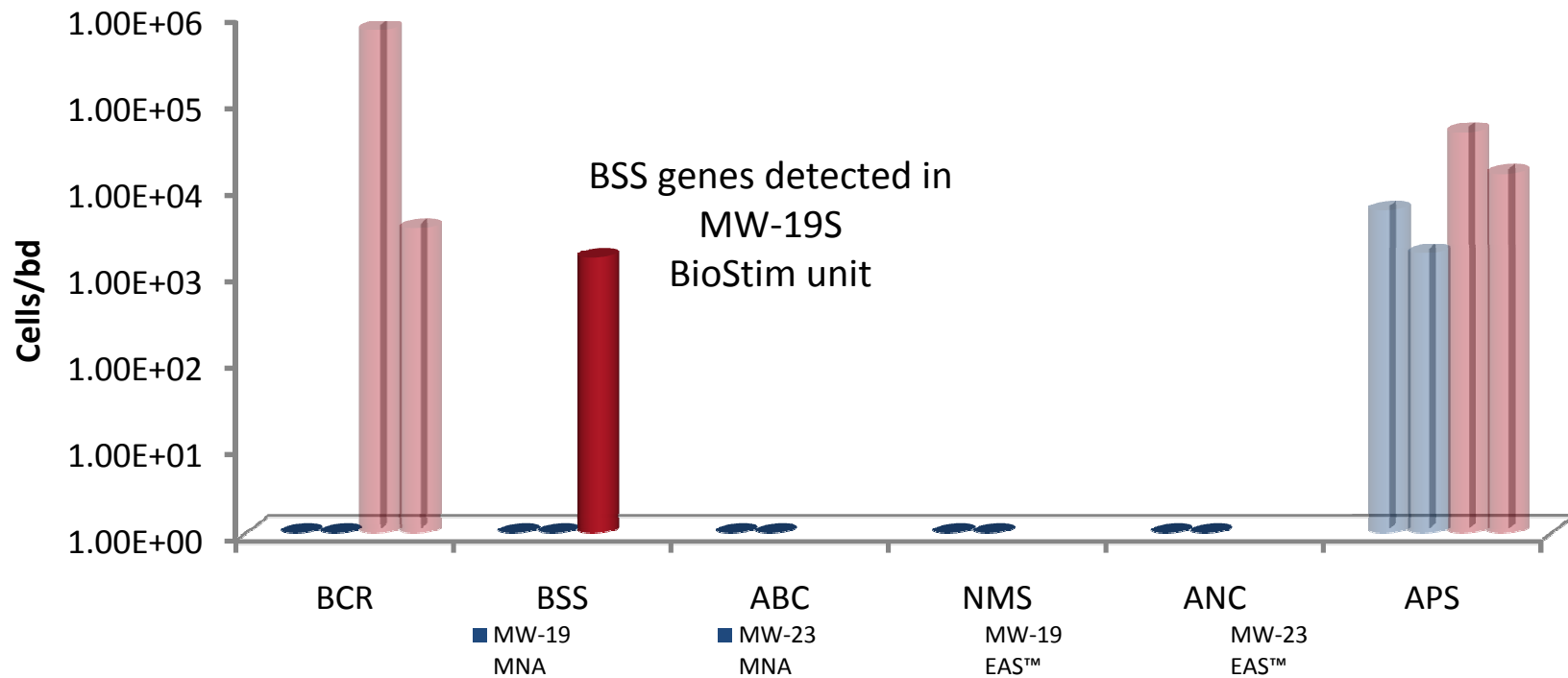
Anaerobic Processes – MNA vs BioStim



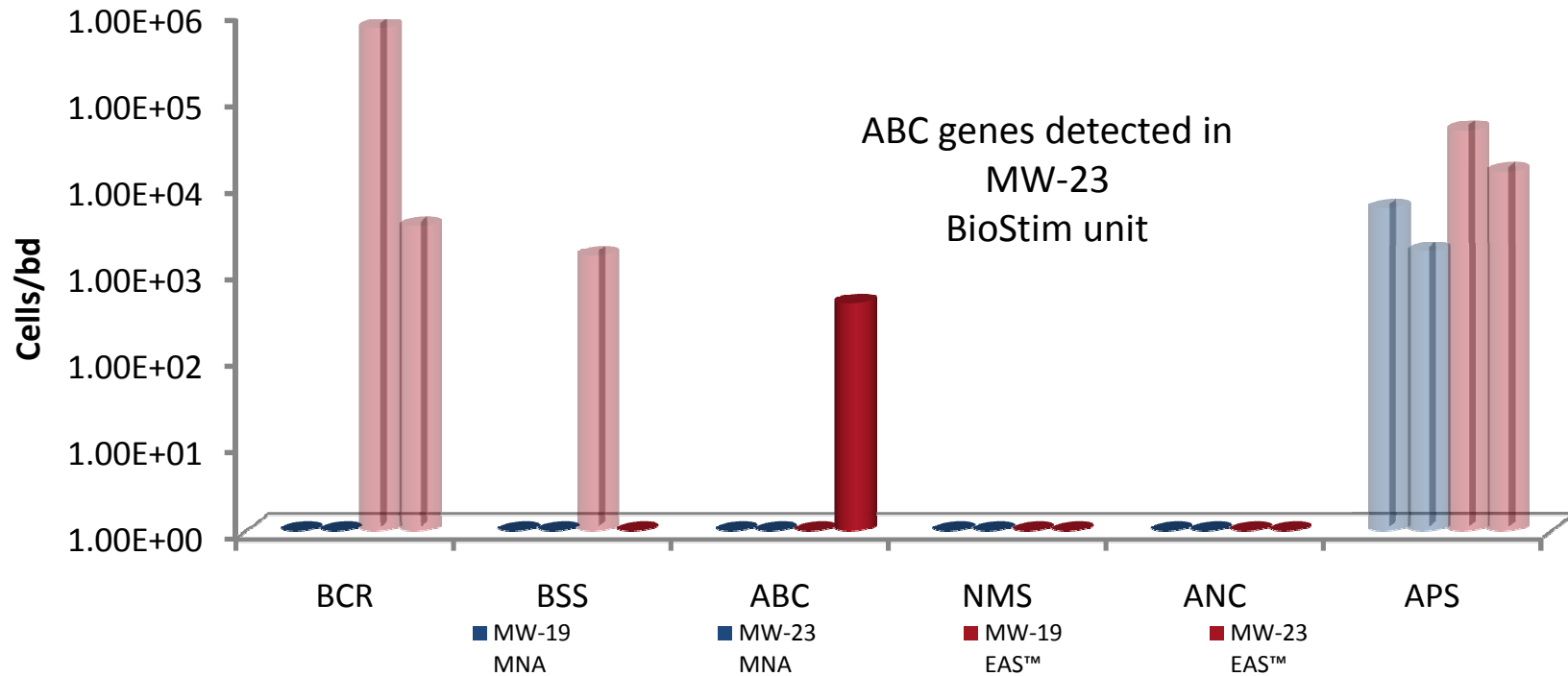
Anaerobic Processes – MNA vs BioStim



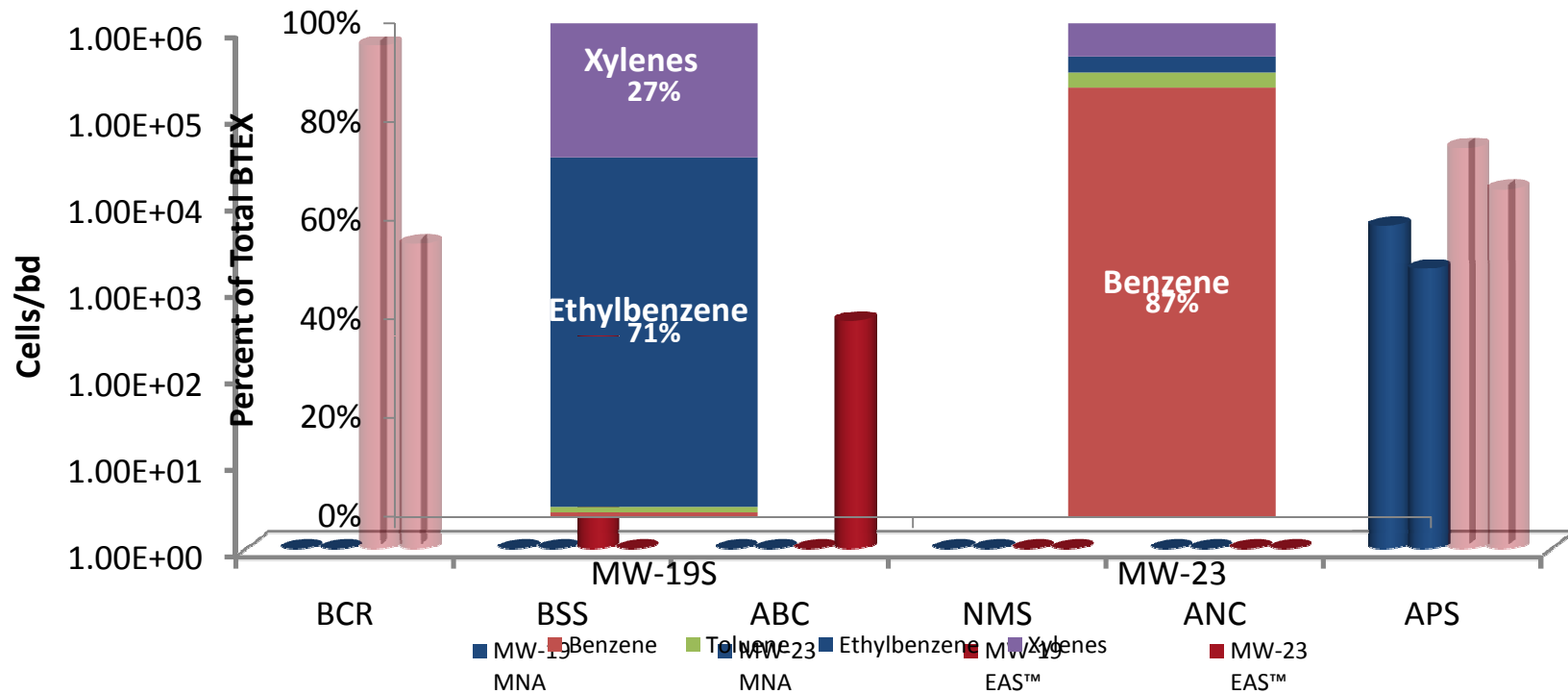
Anaerobic Processes – MNA vs BioStim



Anaerobic Processes – MNA vs BioStim



Anaerobic Processes - EAS™



In Situ Microcosm - EAS™ Addition

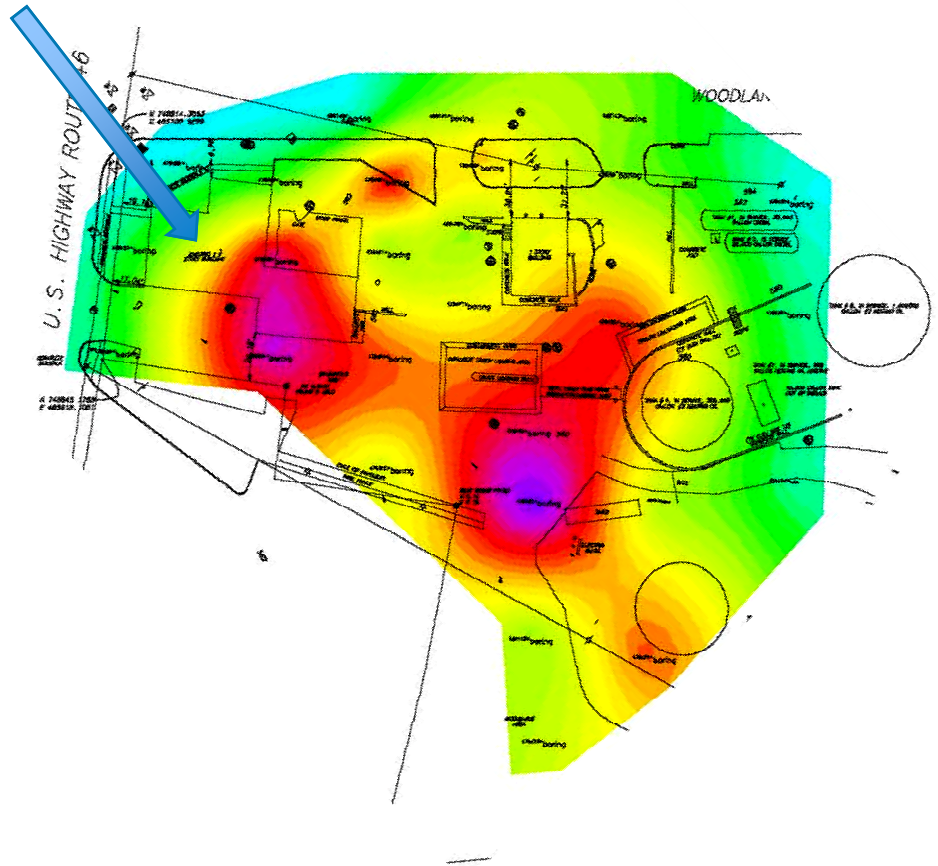
- Stimulated increases in
 - Sulfate reducing bacteria (APS)
 - Anaerobic bacteria capable of aromatic hydrocarbon utilization (BCR)
 - Benzylsuccinate synthase (BSS) genes for anaerobic biodegradation of TEX
 - Anaerobic benzene carboxylase genes (ABC)

In Situ Microcosm - EAS™ Addition

- Enhanced potential for anaerobic BTEX biodegradation
- No evidence of anaerobic PAH and alkane degraders
 - Limited deployment time
 - Low PAH concentrations
 - TPH data not available

In Situ Microcosms – MNA and EOx™ Amended

MW-7



In Situ Microcosm Study

MNA



BioStim



COC



MICRO
(Bio-Trap)



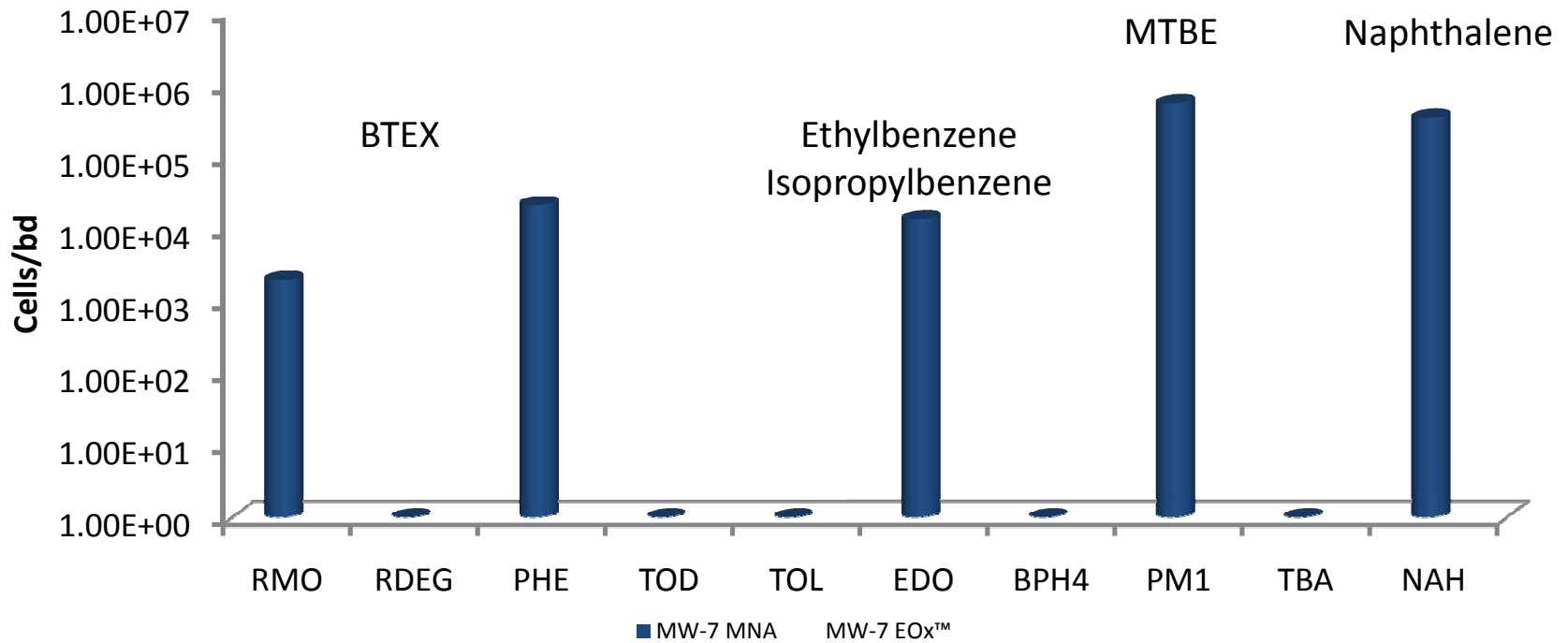
GEO



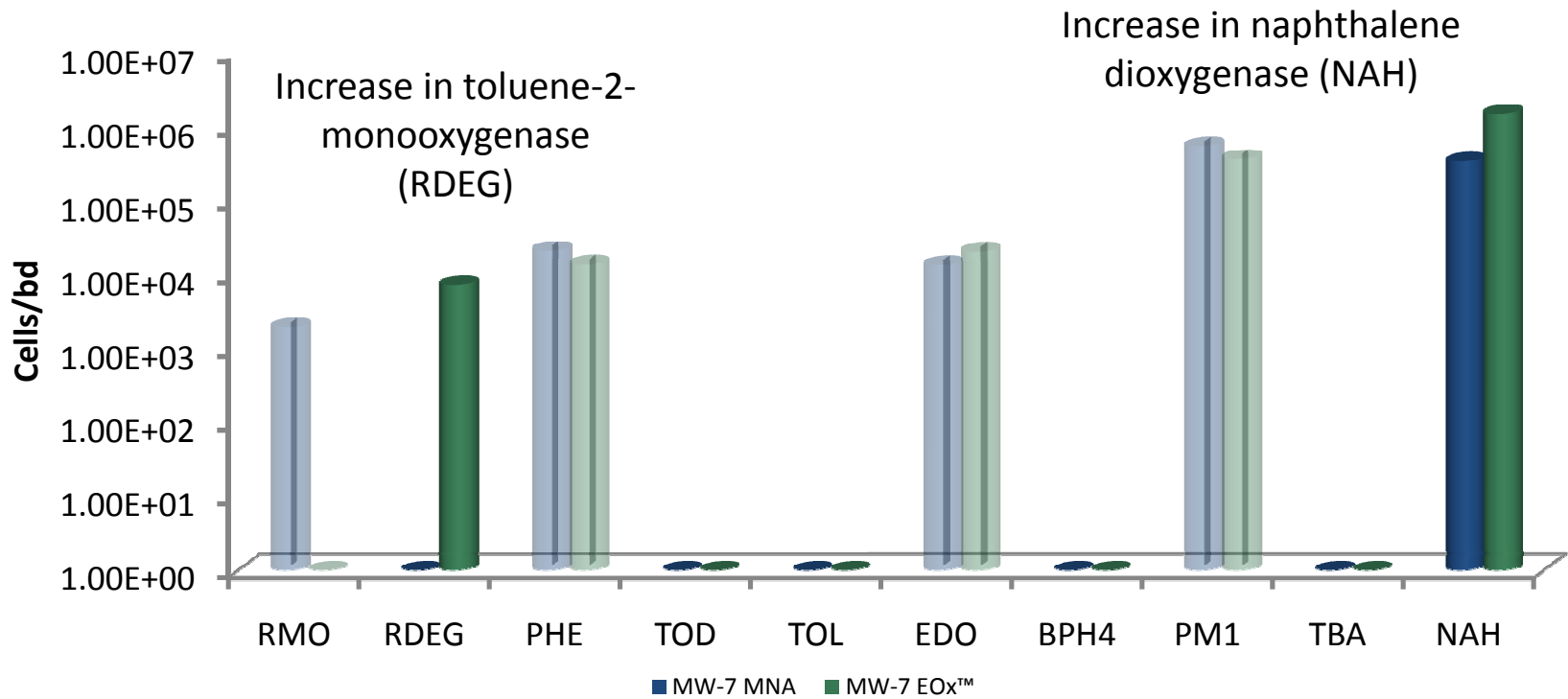
EOx™



Aerobic Processes – MNA



Aerobic Processes – MNA vs BioStim



In Situ Microcosm - MNA

- QuantArray – Petro Summary
 - Benzene/toluene monooxygenases (RMO, RDEG, PHE)
 - Ethylbenzene dioxygenases
 - MTBE utilizing strain PM1
 - TBA monooxygenase
 - Naphthalene dioxygenase (NAH)
- Substantial populations of aerobic BTEX, MTBE, and PAH utilizing bacteria

In Situ Microcosm - EOx™ Addition

- Aerobic biodegradation
 - Substantial increase in toluene-2-monoxygenase genes (RDEG) which is not a frequently requested gene target
 - Increase in naphthalene dioxygenase genes (NAH)
 - Comparable concentrations of other target genes

Site Management

- Anaerobic ISM Studies (MW-19S and MW-23)
 - EAS addition stimulated growth of anaerobic BTEX utilizing bacteria closer to the source zone
- Aerobic ISM Study (MW-7)
 - Approved MNA site
 - Upgradient well with intermittent detection of BTEX
 - Baseline degrader populations significant
 - Some increases with oxygen addition
 - Possible increase in activity – Future studies with RT-qPCR?

Special Thanks

- Eric Raes
 - Engineering and Land Planning
- EOS Remediation
- Dr. Kerry Sublette
 - University of Tulsa

Questions???

