QuantArray Demonstrates Enhanced Biodegradation at Petroleum Impacted Sites and Confirms the Importance of Recently Elucidated Pathways

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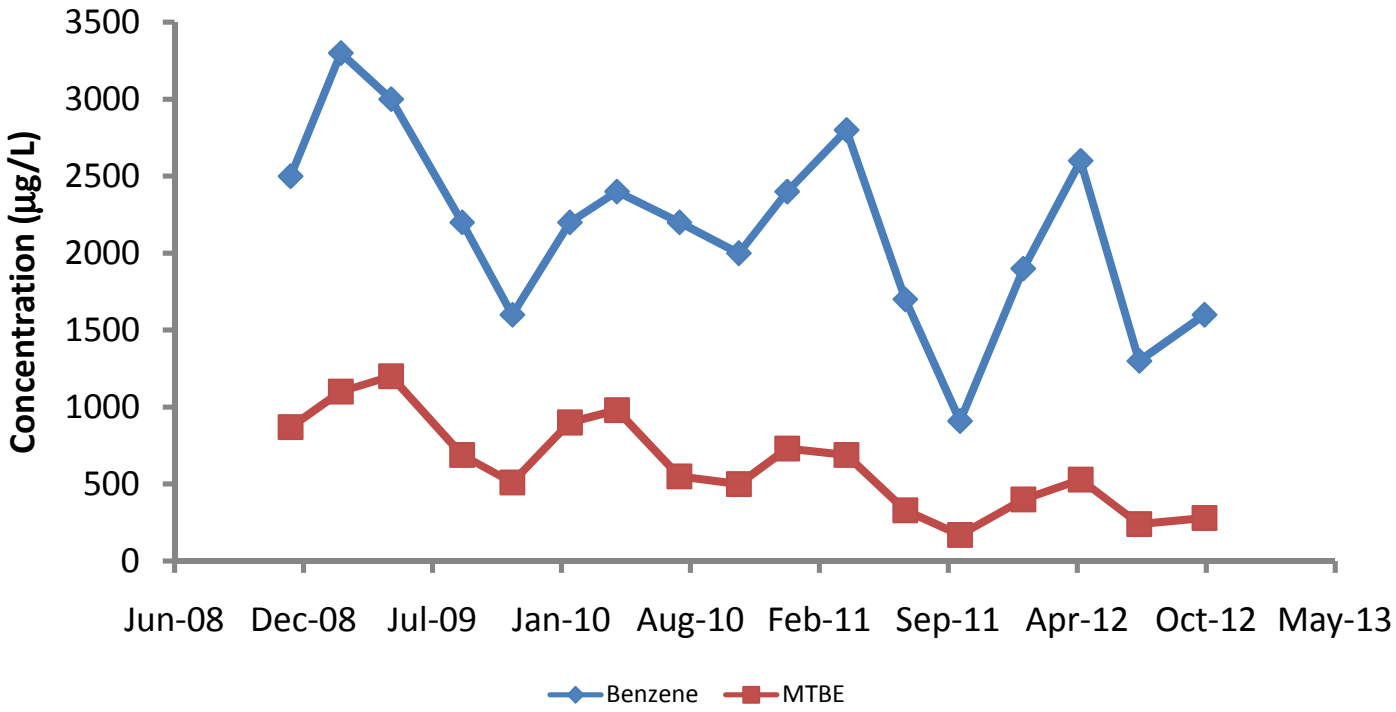
Evaluating Biodegradation

- Chemical lines of evidence



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Evaluating Biodegradation

- Geochemical lines of evidence

$$C_6H_6 + 7.5O_2 \longrightarrow 6CO_2 + 3H_2O$$

~ 3.1 mg O_2/ mg benzene

- Electron accepter concentrations in impacted vs non-impacted wells

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Evaluating Biodegradation

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

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qPCR

- Accurate and precise

- Sensitive and specific

- Rapid

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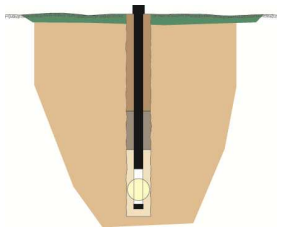
qPCR

Sample Collection

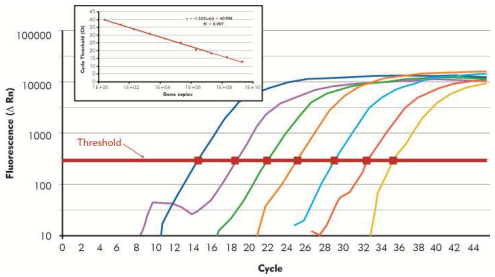


Groundwater, soil, or Bio-Trap samplers collected and shipped overnight on ice (4c)

Extraction

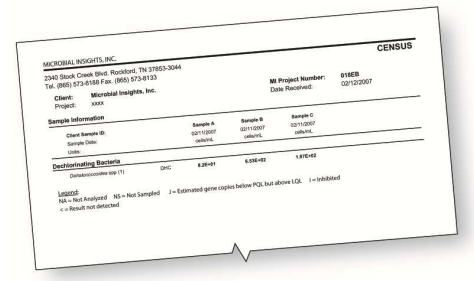
DNA or RNA is extracted from sample upon arrival

Amplification



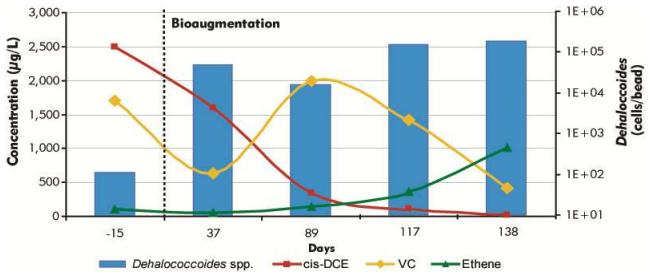
Quantitative Real-Time PCR is used to detect and quantify targets of interest (i.e.toluene monooxygenase)

Results



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

Assessment



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

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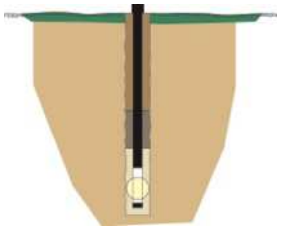
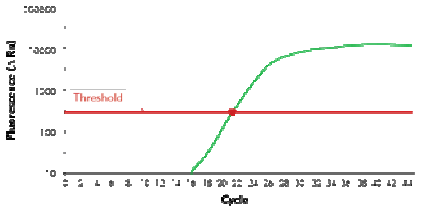
qPCR Approach

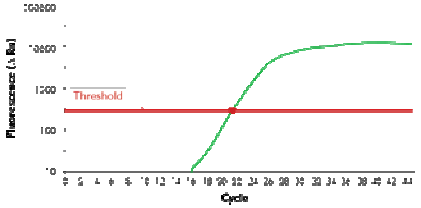
Sample Collection



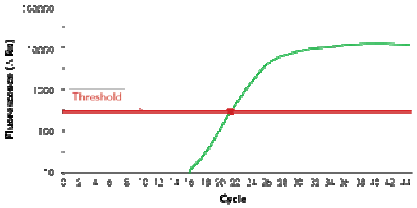
DNA Extraction



TOD



PHE



BSS

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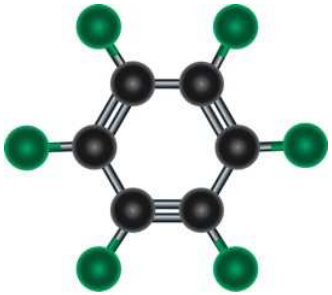
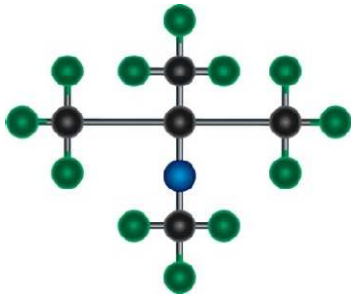
Mixtures of Contaminants

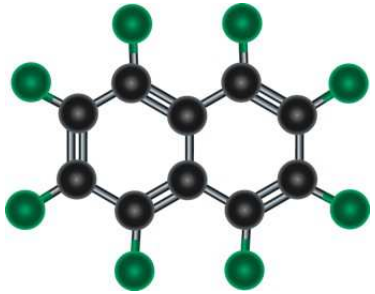
- Monoaromatics (BTEX)





- Fuel oxygenates (MTBE)

- Polycyclic aromatics hydrocarbons (PAHs)



- TPH and n-alkanes

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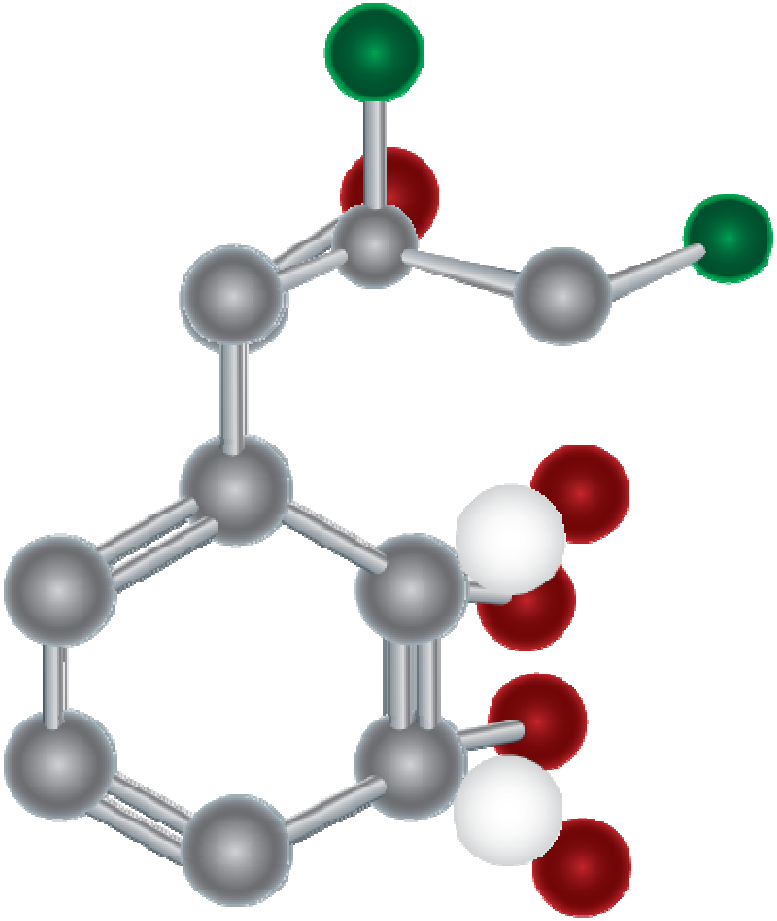
Anaerobic BTEX



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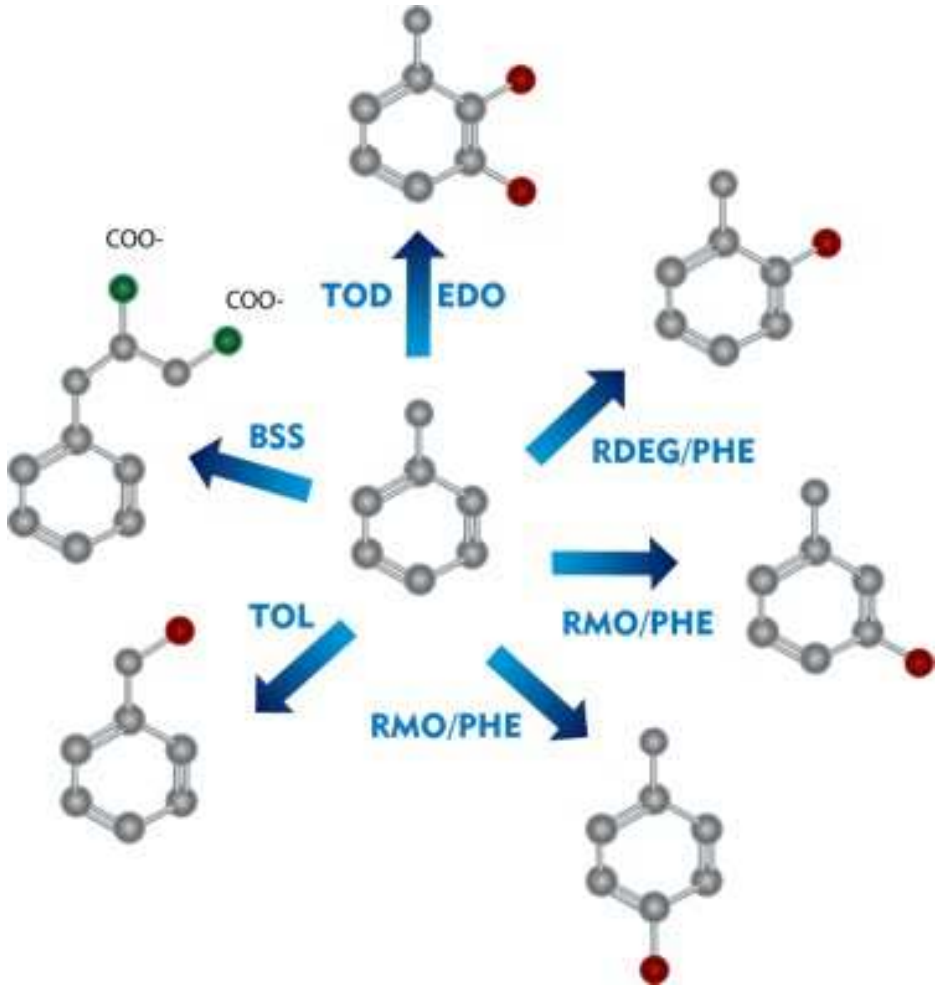
Multiple Pathways



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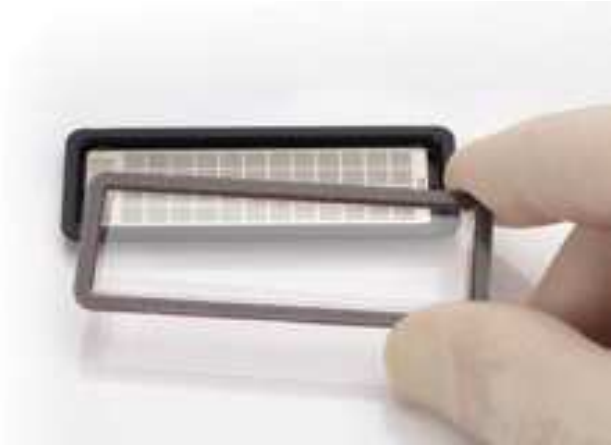
QuantArray™

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



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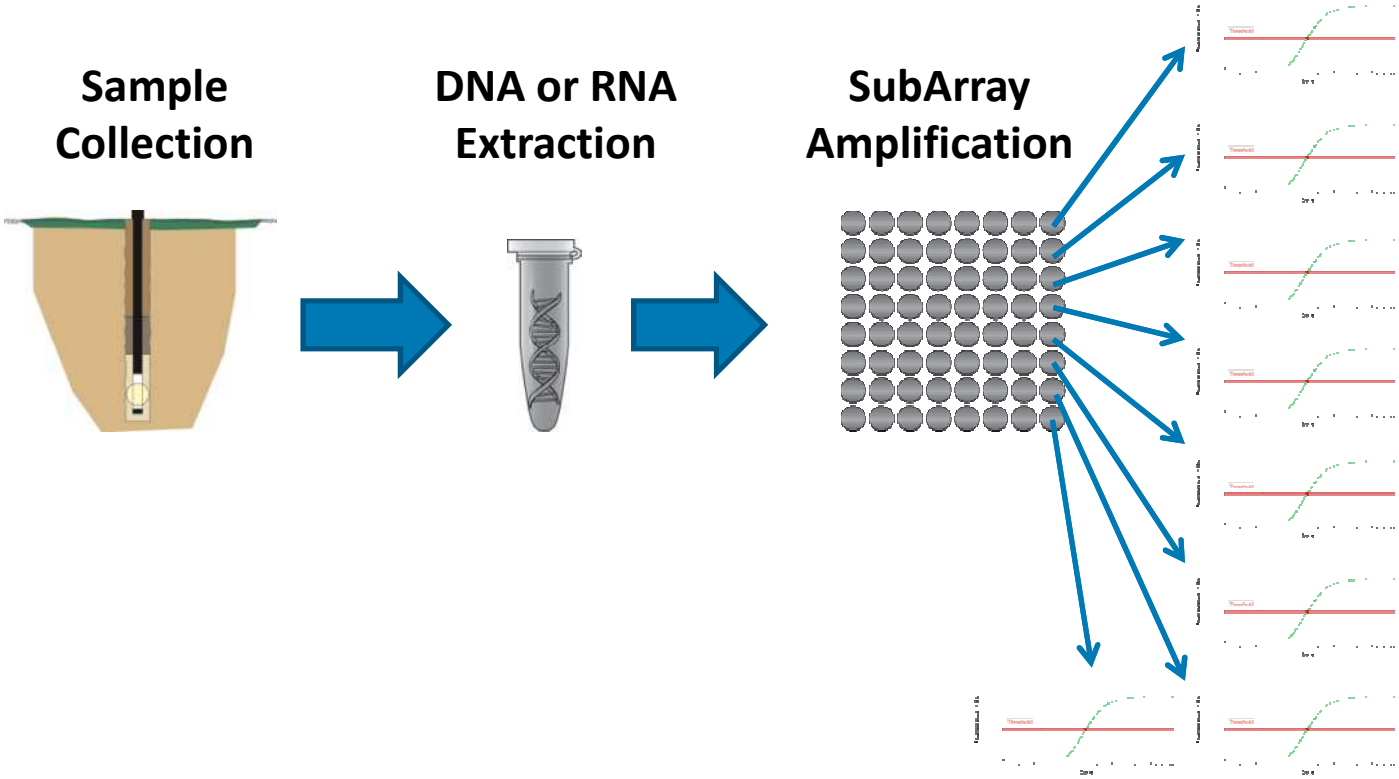
QuantArray Approach



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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
Methylibium petroliphilum 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
Alklysuccinate 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

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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)	**Aerobic BTEX**	
Xylene/Toluene Monooxygenase (TOL)		
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)		
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)		
Methylibium petroliphilum PM1 (PM1)	**Aerobic MTBE & TBA**	
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)		
Alklysuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

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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)		
Methylibium petroliphilum 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)		
Alklysuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

Anaerobic BTEX

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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)		
Methylibium petroliphilum 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 Benzene**	
Anaerobic PAHs and Alkanes (cells/mL)		
Benzoyl Coenzyme A Reductase (BCR)		
Naphthylmethylsuccinate Synthase (NMS)		
Naphthalene Carboxylase (ANC)		
Alklysuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

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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)		
Methylibium petroliphilum 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)		
Alklysuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

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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)		
Methylibium petroliphilum PM1 (PM1)		
TBA Monooxygenase (TBA)		
Aerobic PAHs and Alkanes (cells/mL)		
Naphthalene Dioxygenase (NAH)		
Phenanthrene Dioxygenase (PHN)		
Alkane Monooxygenase (ALK)		
Alkane Monooxygenase (ALMA)	**Aerobic Alkanes**	
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)		
Alklysuccinate Synthase (ASSA)	**Anaerobic Alkanes**	
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

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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)		
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Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)		
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)		
Methylibium petroliphilum 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)		
Alklysuccinate Synthase (ASSA)		
Other (cells/bead)		
Total Eubacteria (EBAC)		
Sulfate Reducing Bacteria (APS)		

Total Bacteria and SRBs

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Case Study Site

- Retail gasoline and diesel station

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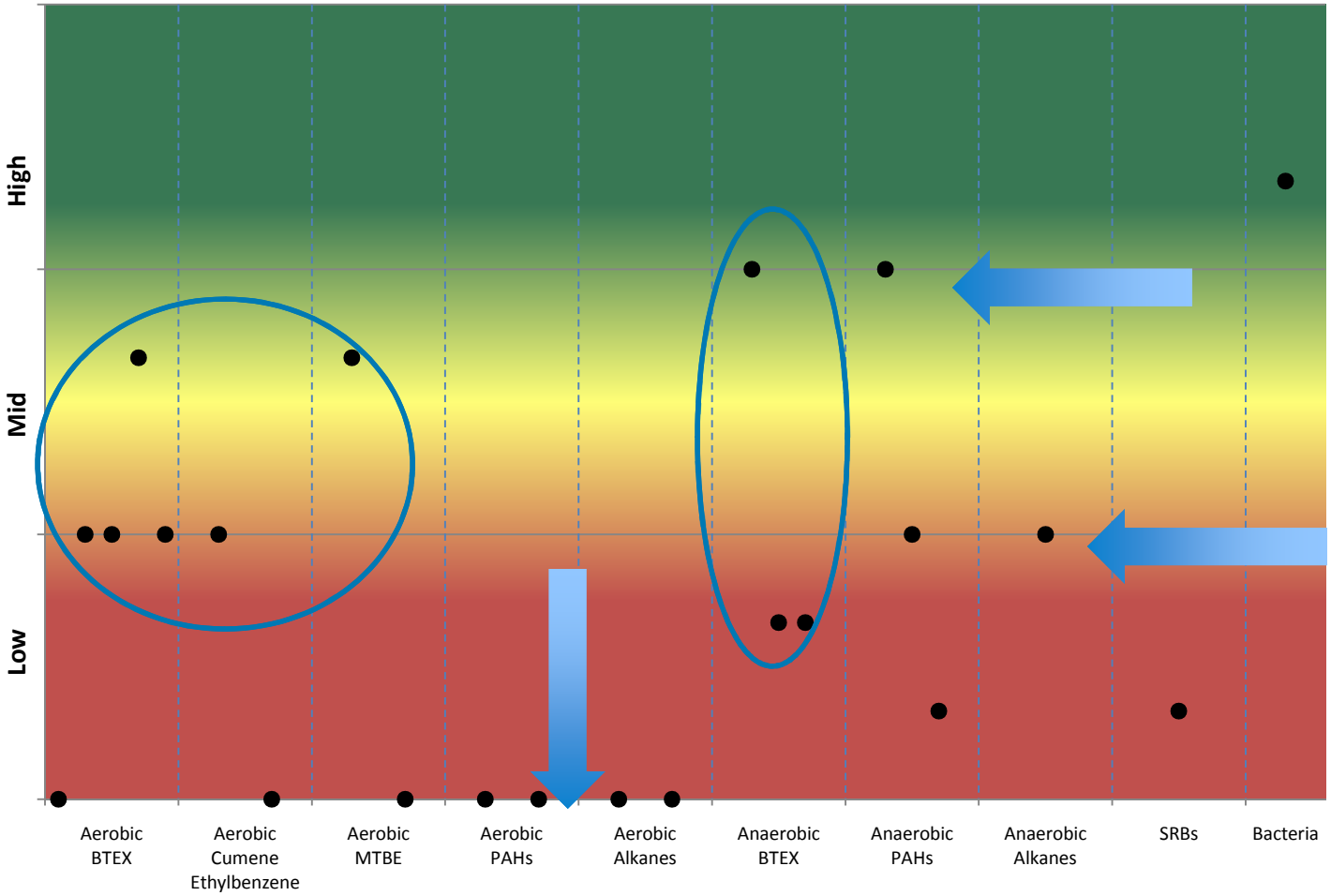
Microbial Populations - MNA Conditions



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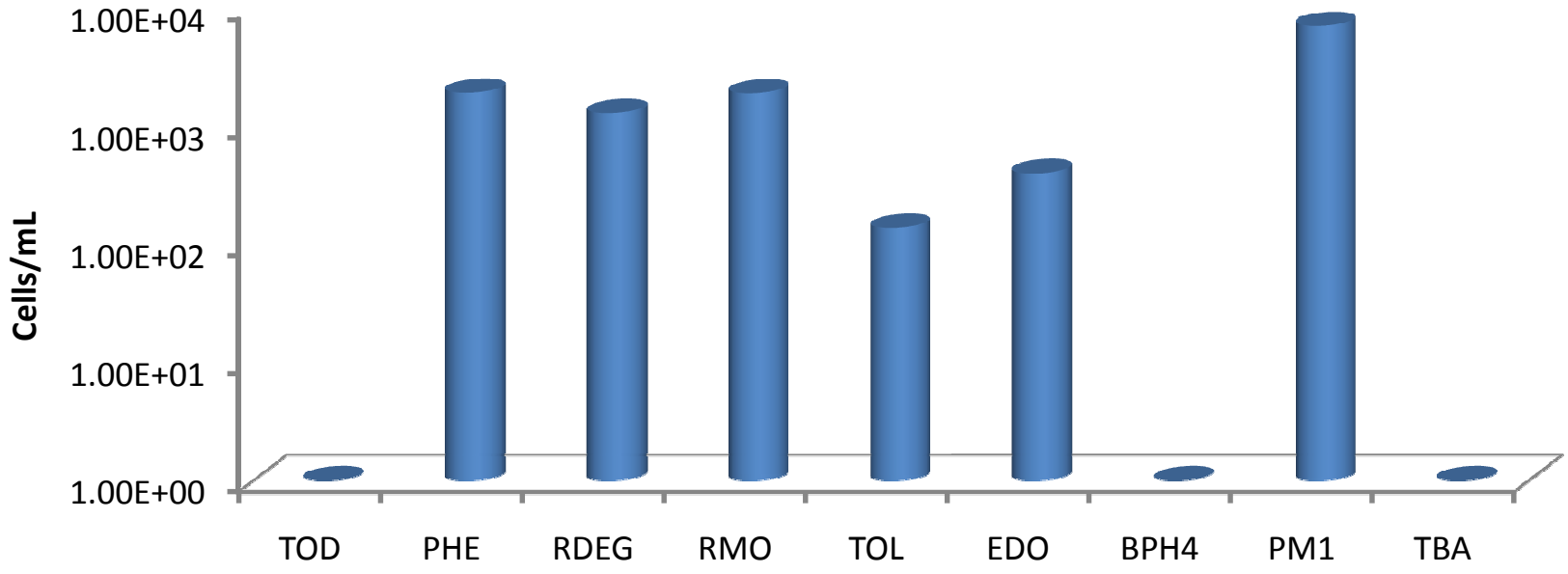
Aerobic Processes – BTEX and MTBE



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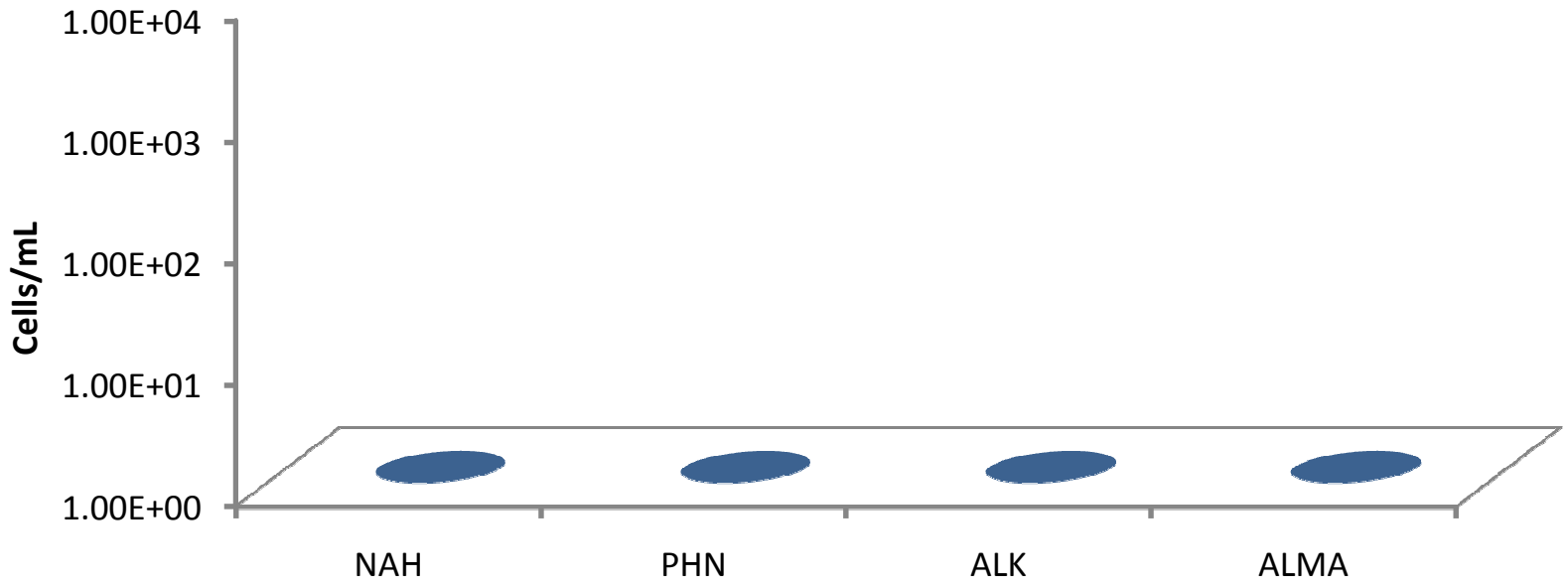
Aerobic Processes – PAHs and Alkanes



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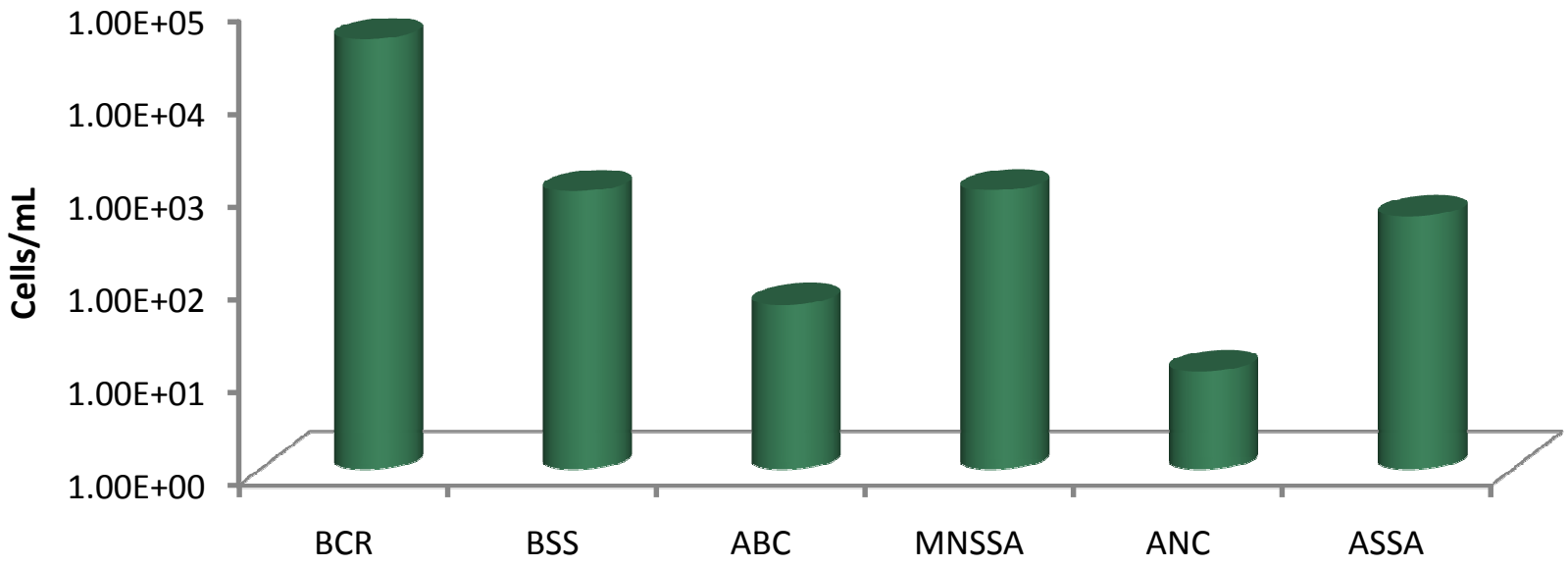
Anaerobic Processes – BTEX, PAHs and Alkanes



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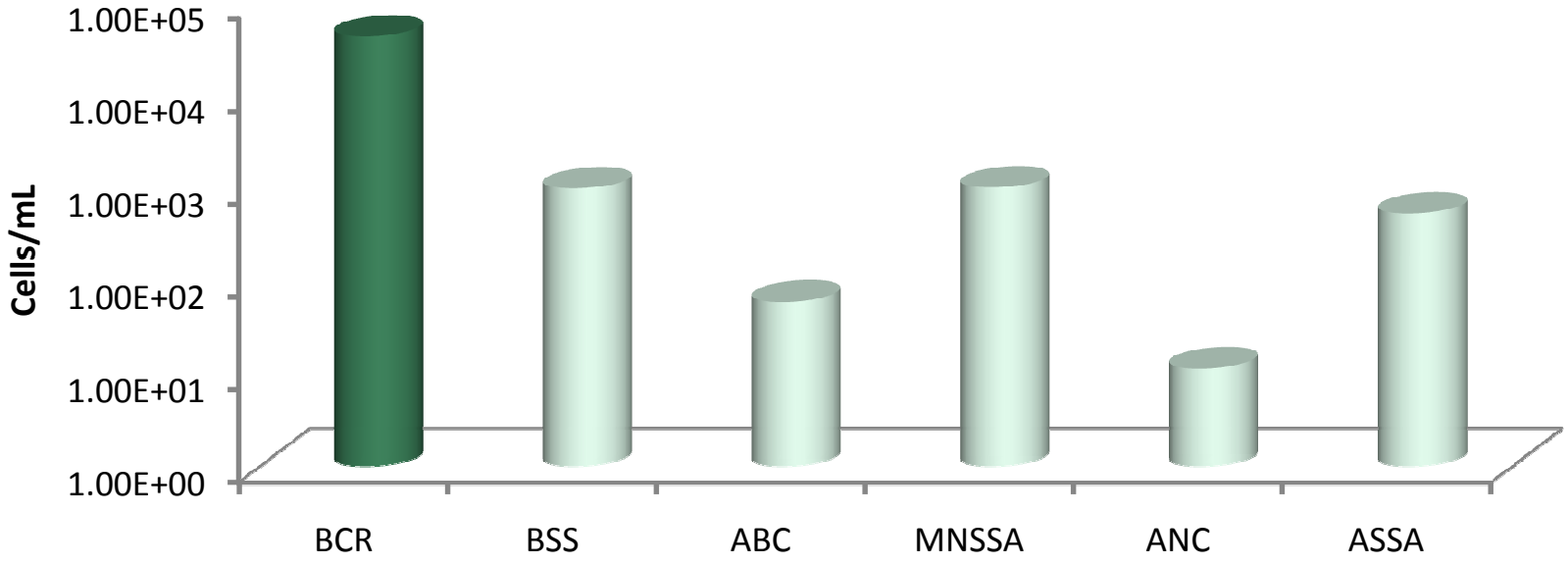
Anaerobic Processes – BTEX, PAHs and Alkanes



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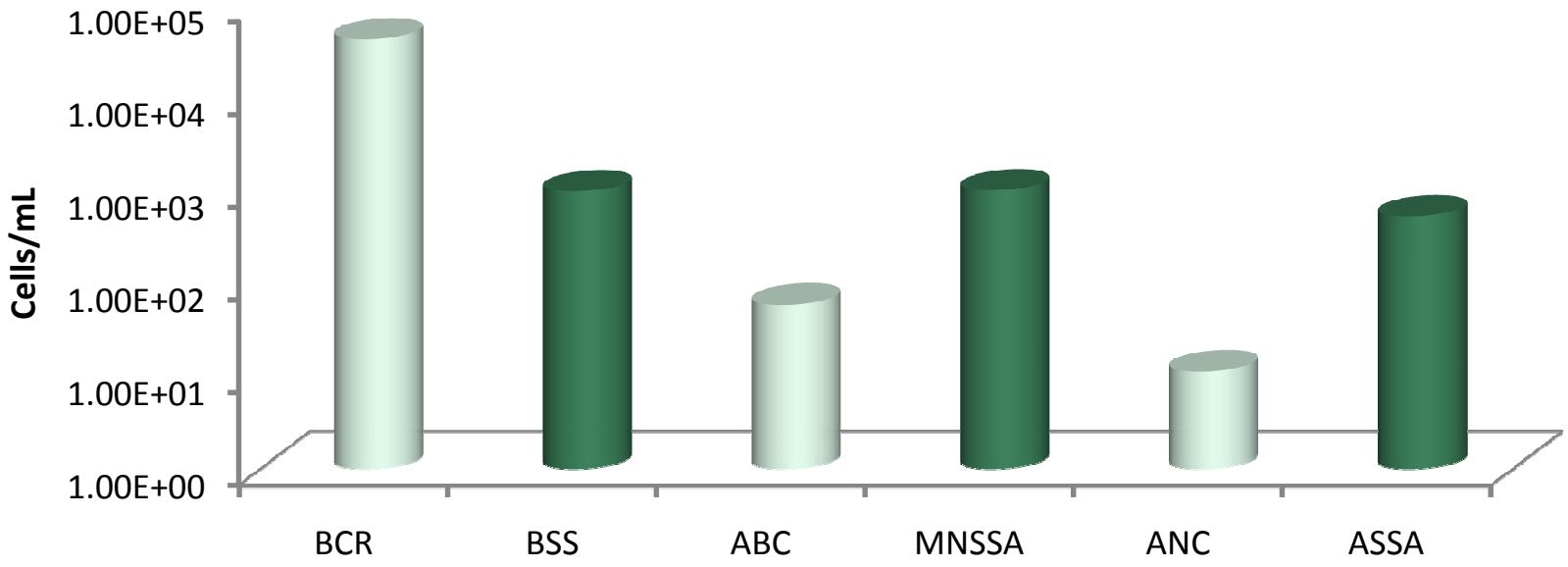
Anaerobic Processes – BTEX, PAHs and Alkanes



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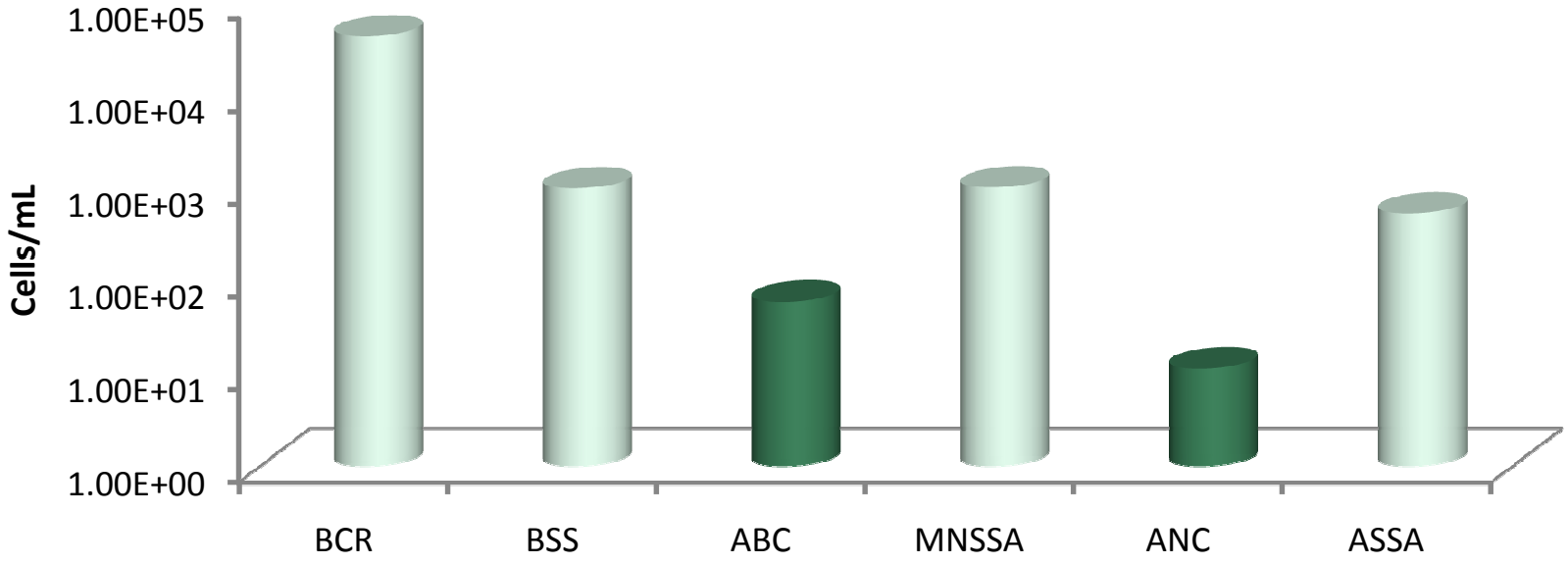
Anaerobic Processes – BTEX, PAHs and Alkanes



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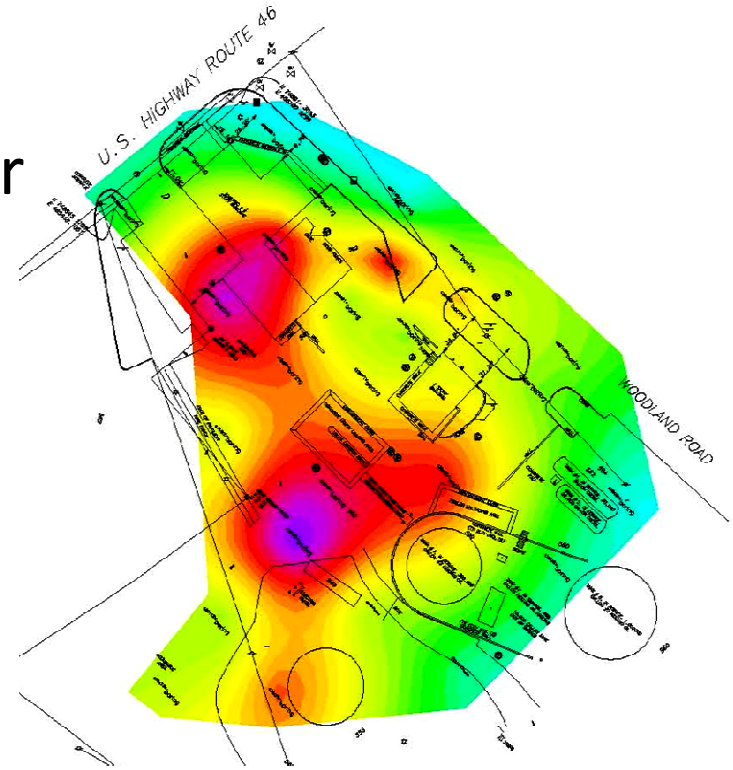
Mini-Case Study

- MNA
  - Aerobic pathways for BTEX and MTBE
  - Anaerobic pathways for BTEX, PAHs, and alkanes
- Biostimulation
  - Aerobic and anaerobic options

Study Site

- Petroleum distribution facility
- Gasoline impacted groundwater



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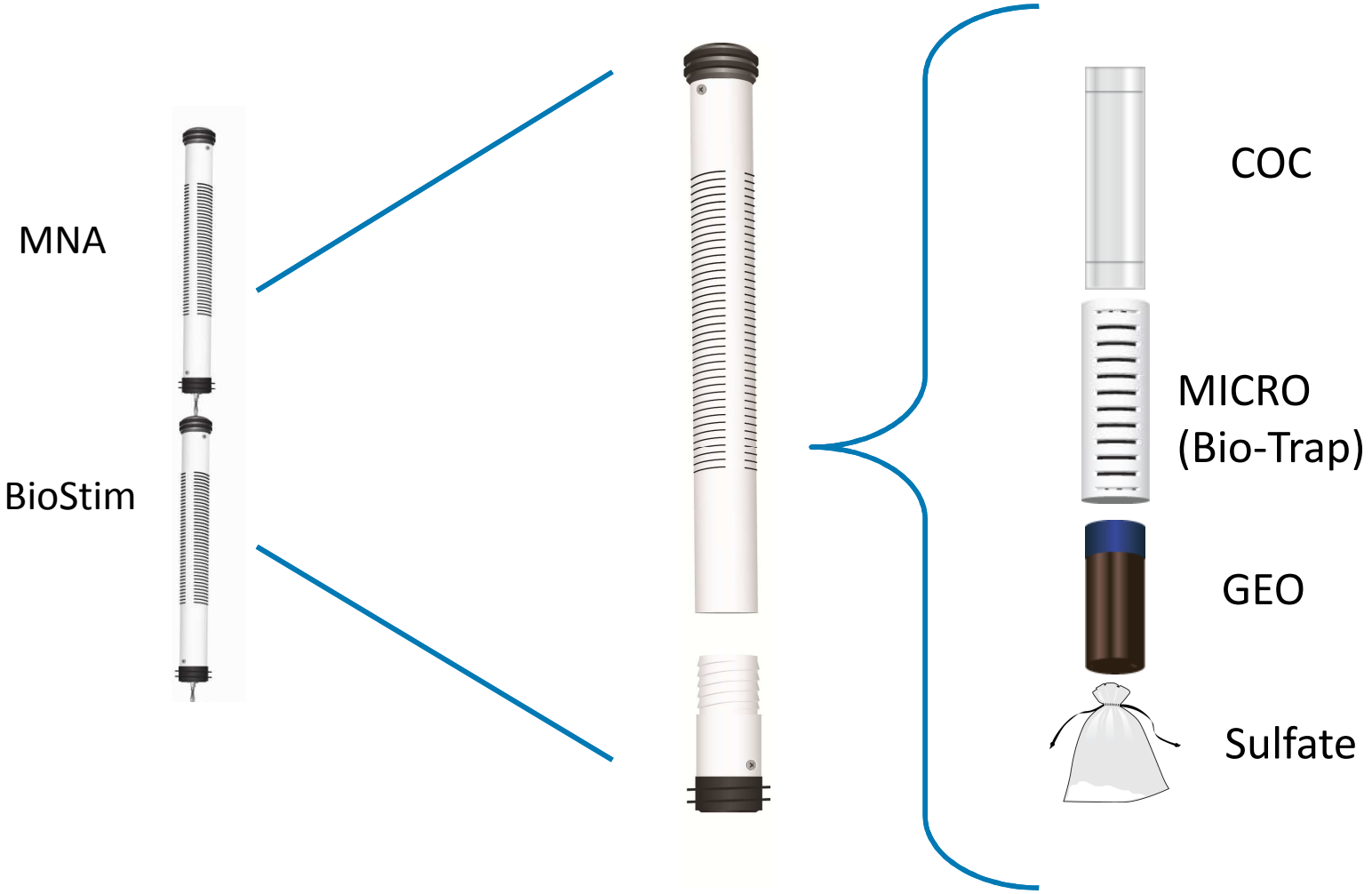
In Situ Microcosm Study



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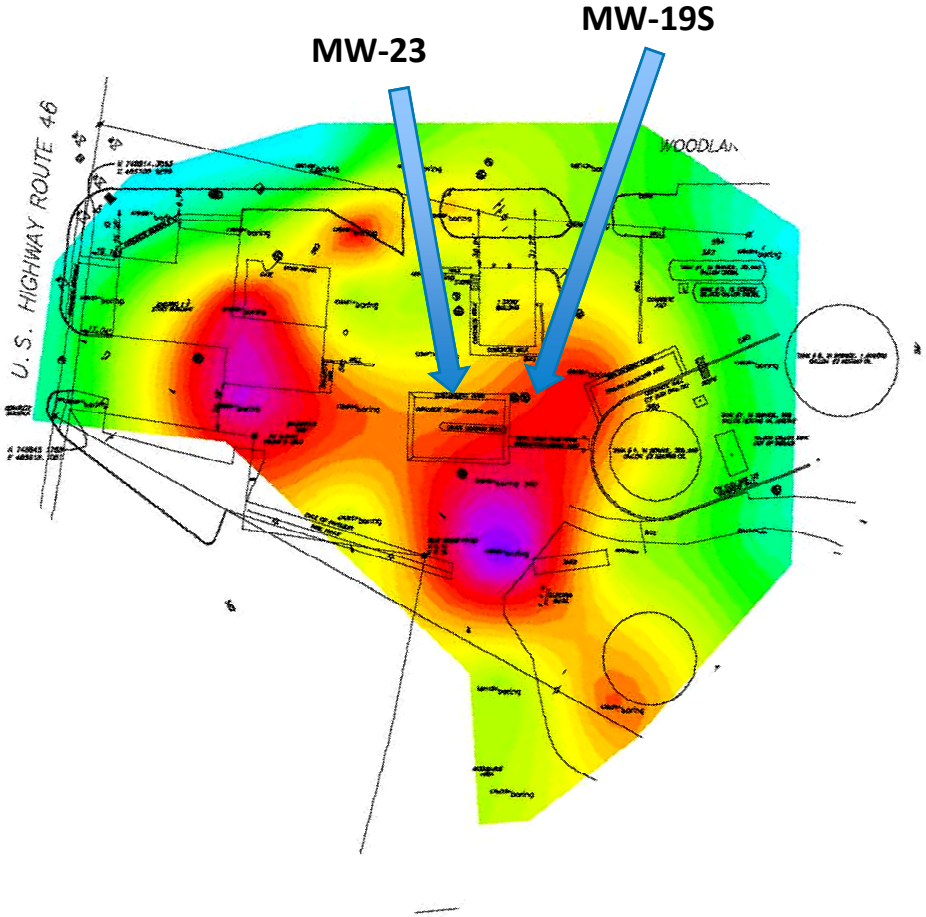
In Situ Microcosms – MNA and Sulfate



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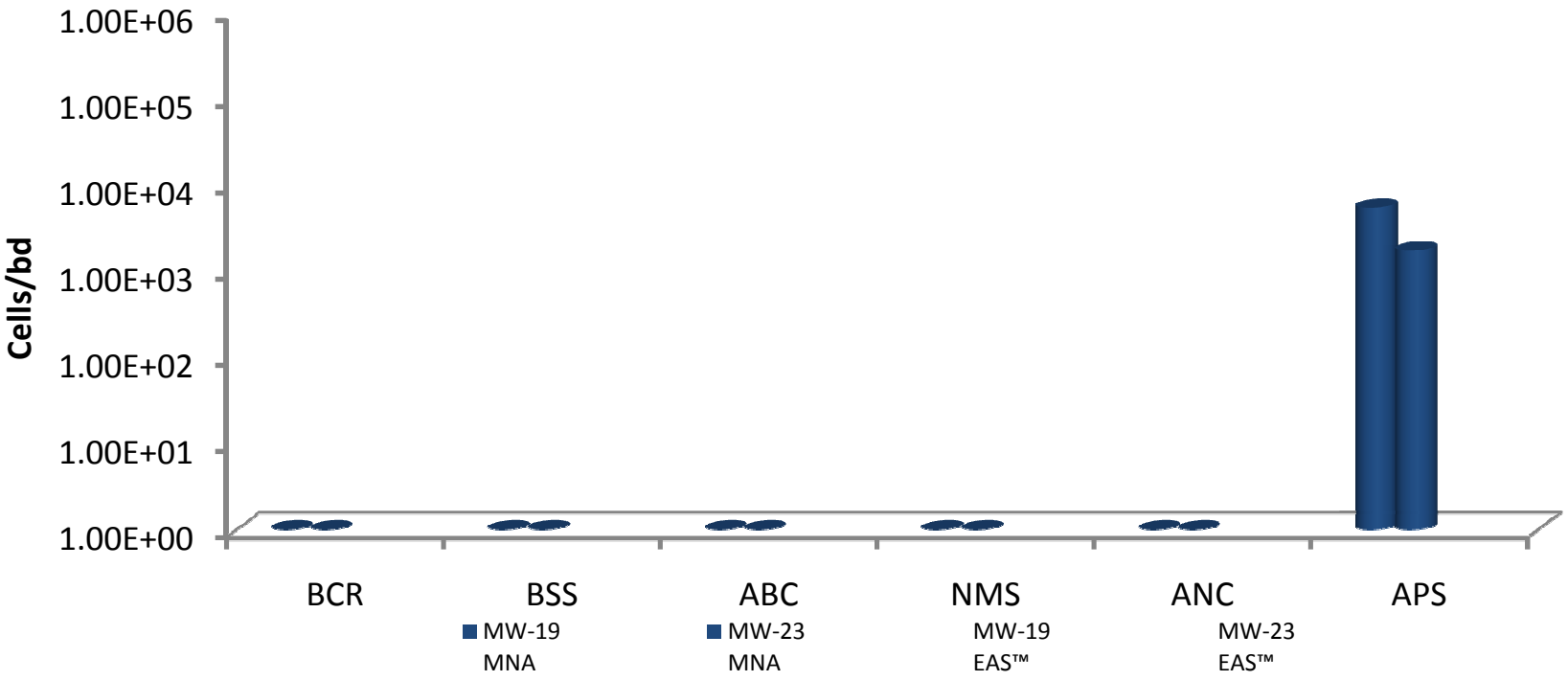
Anaerobic Processes – MNA Unit



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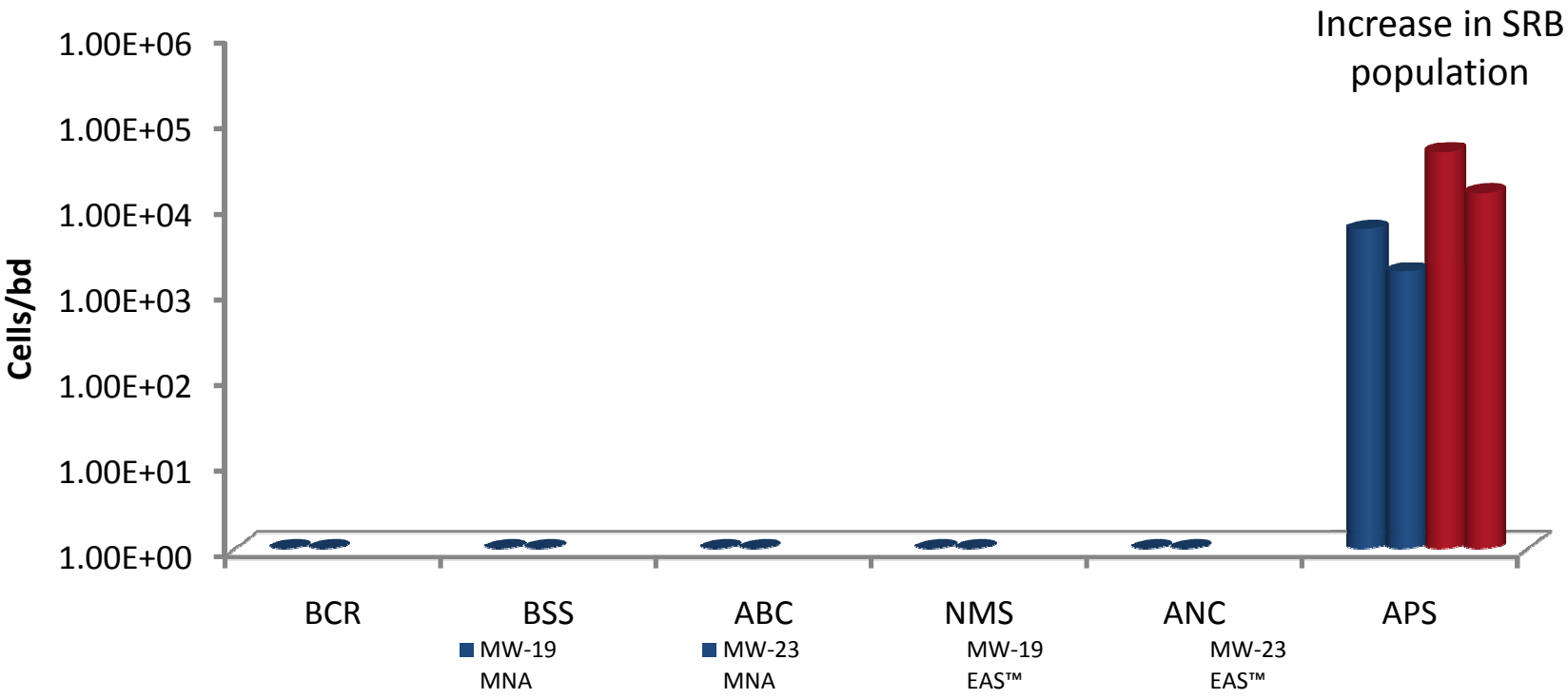
Anaerobic Processes – MNA vs BioStim



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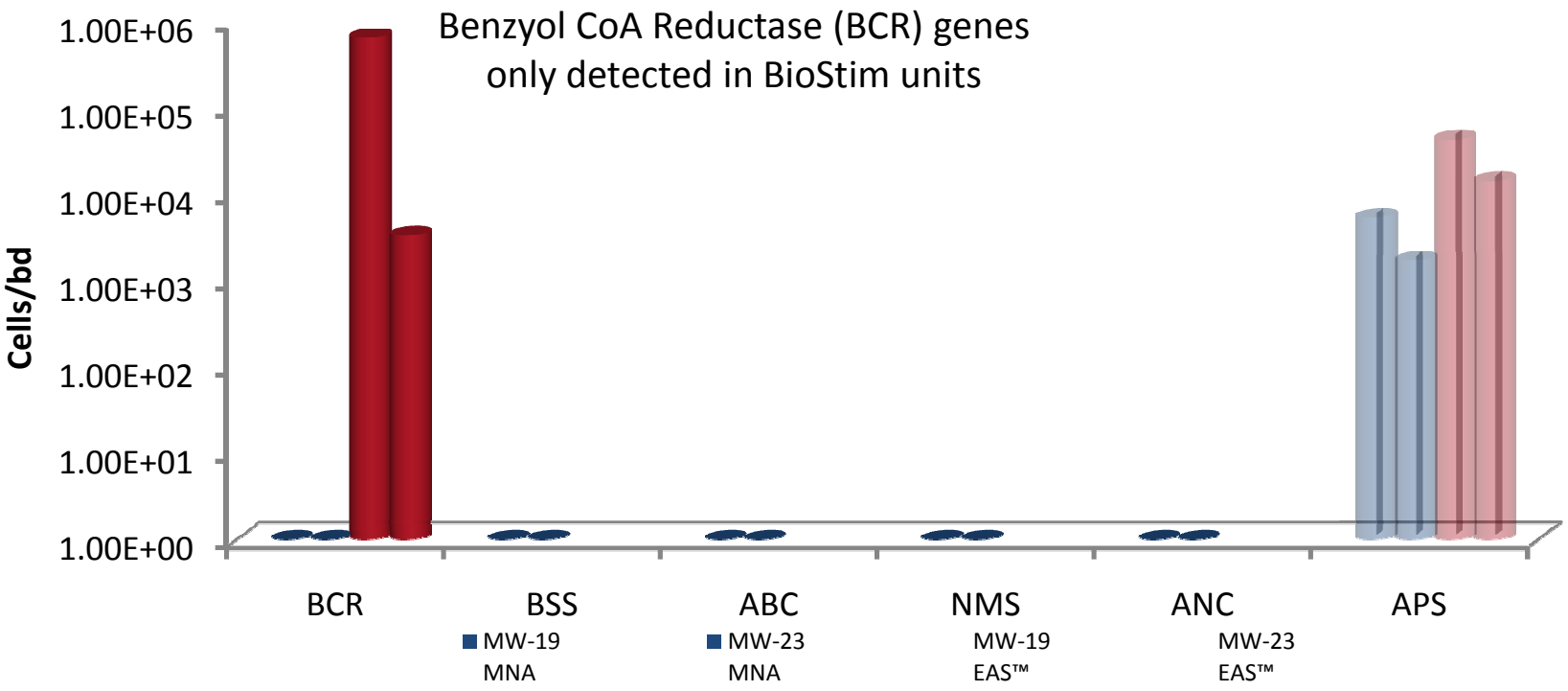
Anaerobic Processes – MNA vs BioStim



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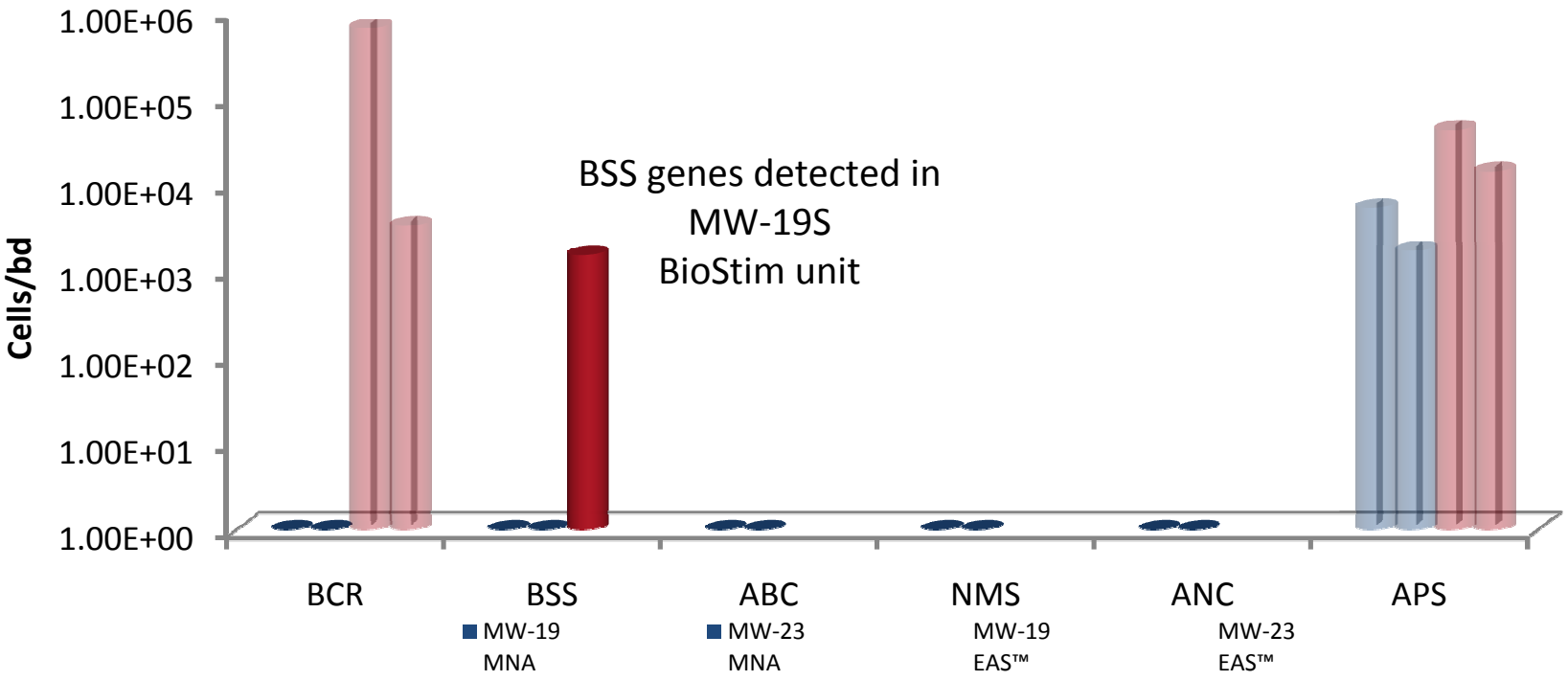
Anaerobic Processes – MNA vs BioStim



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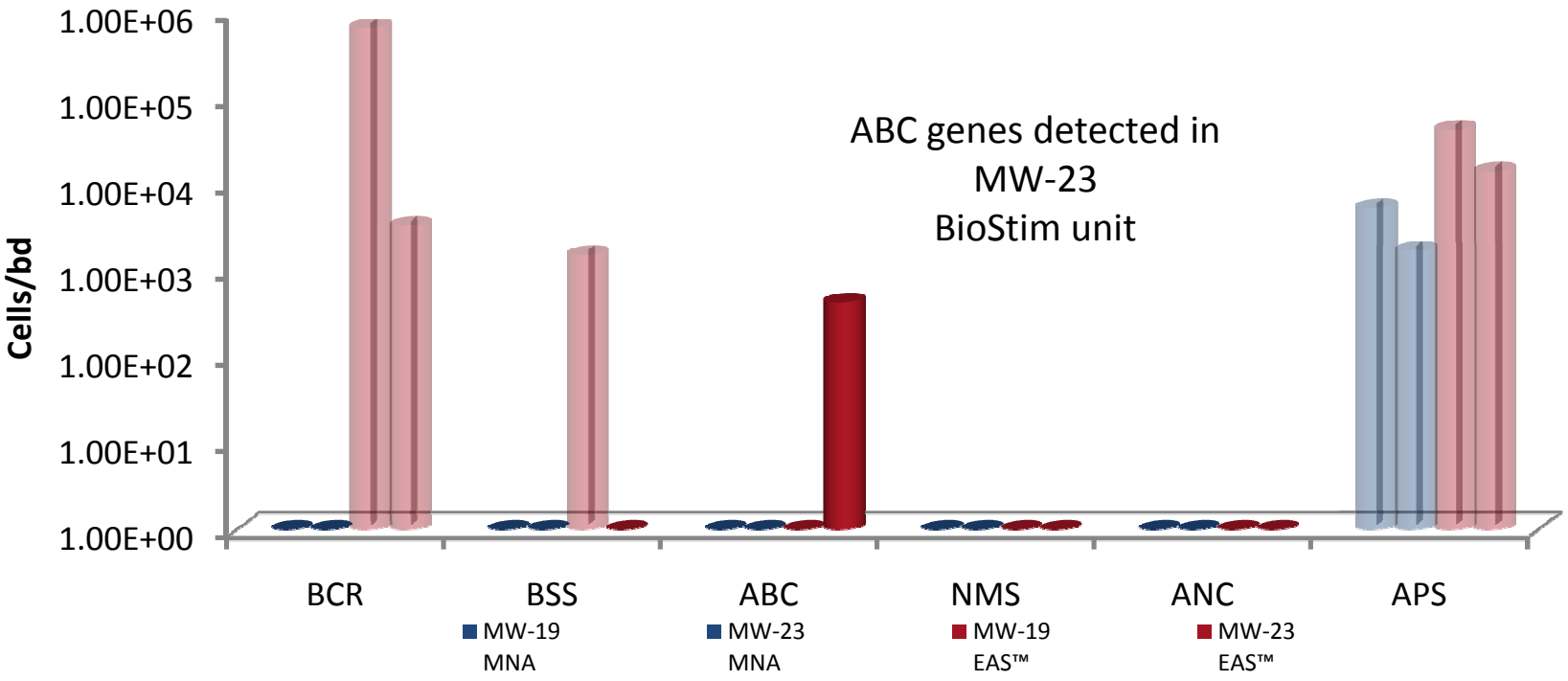
Anaerobic Processes – MNA vs BioStim



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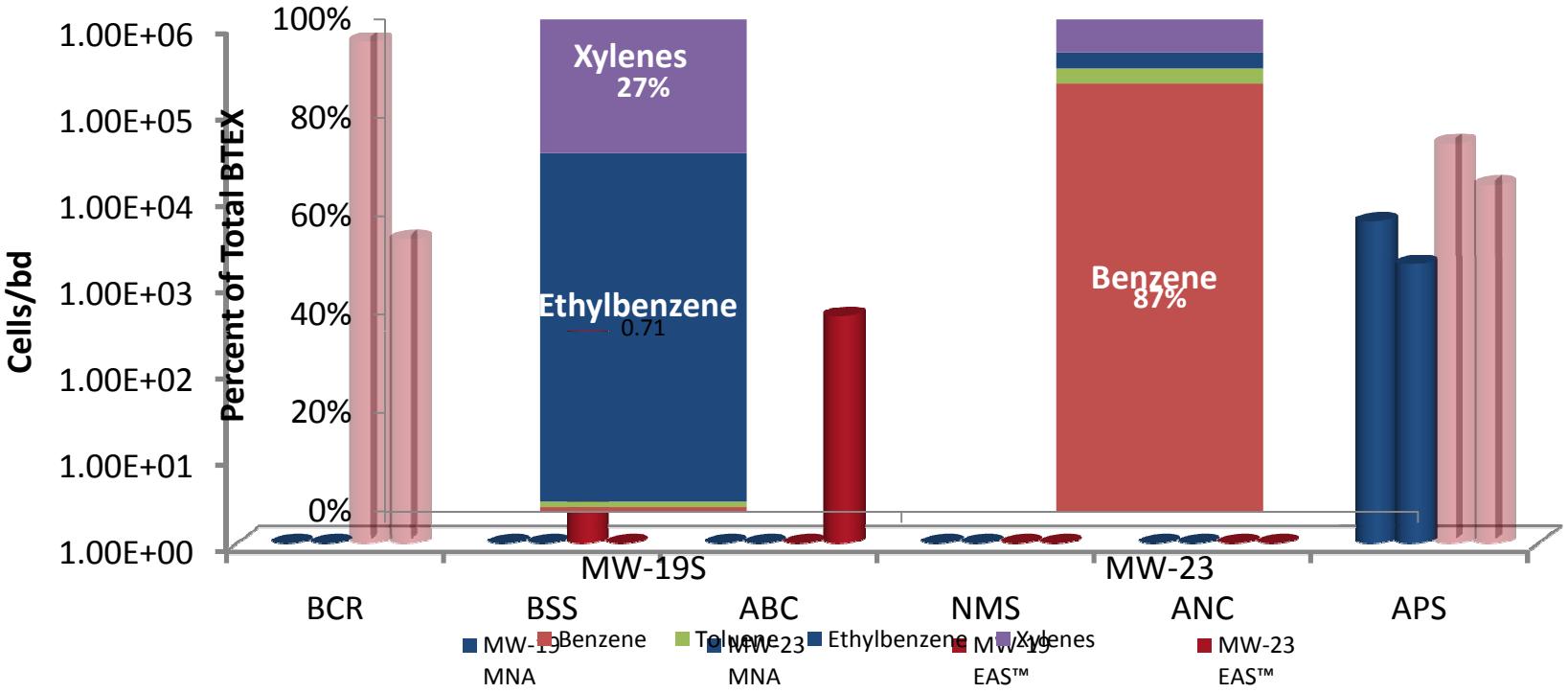
Anaerobic Processes - Sulfate



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In Situ Microcosm – Sulfate 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 - Sulfate

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

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Which tool to use?

qPCR

- "Simple" Sites
  - One or two parent compounds
  - Remedy selected or corrective action in place
  - Limited potential for competing electron accepting processes

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Which tool to use?

QuantArray

- Site Characterization/Remedy Selection
  - Simultaneous assessment of aerobic and anaerobic pathways
- Multiple contaminants
  - Petroleum hydrocarbons
  - Mixtures of chlorinated ethenes, ethanes, benzenes, etc.
  - Potential for competing electron accepting processes

Questions???

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