TERRA STRYKE









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Cost-Effective Destruction of Petroleum Hydrocarbon Contaminants with Expedited Residual Mass

Smear Zone (LNAPL) Destruction under Anaerobic Conditions via Biostimulation

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What is Biostimulation

Biostimulation is the modification of the environment to stimulate existing bacteria capable of bioremediation.

This can be done by addition of various forms of rate limiting nutrients and electron acceptors including, but not limited to, phosphorus, nitrogen, oxygen, and/or carbon. Wikipedia – whenever...

We ask a new approach is needed as to how to view microbial performance and in-situ remediation; changing focus from what individual microbes do and/or produce, to examining and harnessing the ecosystem within which they live.



What is not Biostimulation





TPHenhanced[™]

Nitrate (NO₃) based additive combined with,

Proprietary macro-micro inorganic nutrient formulation.

Serves as alternative respiratory source for indigenous microbial populations (prokaryotic heterotrophs) under anerobic conditions.

Promotes anaerobic degradation of organic contaminants:

- ✓ Petroleum Hydrocarbon (PHC) Compounds
- ✓ Polyaromatic-Hydrocarbons (PAHs)
- \checkmark Non-Chlorinated and Chlorinated Hydrocarbons
- ✓ 1,2-DCB; 1,3,5-TMB; Naphthalene; Oils, Sludges







SITE 1 Chanute Air Force Base

Enhance Long-Term Compliance

TPHenhanced [®]	April 4 (µg/L)	April 18 (µg/L)	May 2 (µg/L)	May 21 (µg/L)	%	Reductio	'n
Benzene	606	1,780	8,350	24.6	\langle	99.7%	\mathbf{i}
Naphthalene	197	178	302	2.02		99.3%	
Toluene	2,360	3,620	8,370	13.4		99.8%	
1,2,4-TMB	282	224	843	4.13		99.5%	
рН	NT	5.7	5.3	6.1		NA	

Oxygen Based	April 4 (µg/L)	April 18 (µg/L)	May 2 (µg/L)	May 21 (μg/L)	%Reduction
Benzene	1,970	471	362	241	87.8%
Naphthalene	213	76.7	34.1	8.36	96.1%
Toluene	6,320	1,130	651	385	93.9%
1,2,4-TMB	349	80.7	37.8	17.1	95.1%
рН	NT	9.4	9.8	10.3	11.0

SITE 1 Chanute Air Force Base ORC µg/L TPHenhanced µg/L 9000 2500 8000 **Enhanced Solubilization** 2000 7000 **Enhanced Biotic Degradation** > 99.7% destruction of solubilized mass 6000 **Dissolve Phase Destruction** 1500 No Solubilization 5000 4000 >9x the molar mass -TPHenhanced 1000 **Benzene destroyed** 3000 ORC-Advanced 2000 500 1000 0 0 May 21 2012 June 7 2012 April 4 2012 April 18 2012 May 2 2012

Enhance Long-Term Compliance

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SITE 2

Mineral Oil Bioremediation Demonstration 122-day Change (%) in Soil Concentration from Baseline - OFBC Plot







Mineral Oil Bioremediation Demo 122-Day Change in Concentrations Diesel Reduction (%)







Mineral Oil Bioremediation Demonstration (soil) 122-day Change in Concentration from Baseline - TPH Reduction (%)



TPH





Mineral Oil Bioremediation Demonstration 2019 Soil Analytical Results - Diesel Concentrations







Mineral Oil Bioremediation Demonstration QuantArray-Petro Results - OFBC Plot







Mineral Oil Bioremediation Demonstration Total Biomass & PLFA Results - OFBC Plot







Research and Development

Battelle[®]

Exclusive collaboration.

Evaluating the combined effects of TPHenhanced[™]

Patented enzyme-based technology developed by Battelle.

Enhances breakdown of large chained hydrocarbons;

(TPH), (PAH) and (PCB) contaminants, and others.

Creating greater bioavailability for enhanced microbial populations to assimilate.

Laboratory evaluations on-going; field Proof-of-Concept evaluations scheduled.









SITE 3

Former Automobile Fuel-Service Station 1950-1982 Release(s) documented 2003 UST removal program. 2009 groundwater contamination extended off-site. Contaminants of Concern:

- ✓ Petroleum Hydrocarbon (PHC) Contaminants
- ✓ Remedial Driver Benzene, BTEX compounds
- Adversely impacting adjacent downgradient properties.







PURPOSE

One-year program – now full-scale

Evaluate biostimulation strategy to destroy residual LNAPL

- ✓ Expedited Source Zone Depletion
- ✓ Organically
- ✓ Sustainably

GOALS

Enhance respiration of indigenous microbial population Expedite residual Source Mass (LNAPL) Solubilization (flux) Realize sustainable dissolved-phase contaminant destruction

- ✓ Expedited Source Zone Depletion
- ✓ Maintain Anaerobic Conditions
- ✓ Sustainably







SITE CONDITIONS

No Reported Soil Exceedances Groundwater exceedances for:

Contaminants of Concern

- Benzene
- Total Volatile Petroleum Hydrocarbons (TVPH)
- Xylenes

Low-Plasticity Weathered Silty Claystone; overlying, 'Stiff' Weathered Claystone Bedrock Thin discontinuous sand layers throughout Acting as preferential pathways for dissolved-phase PHCs

Former UST Basin Source for GW dissolved-phase plumeProblemExtending > 500ft downgradient with \approx 300ft cross-gradientGW Elevation 17-27ft bgs (\approx 6-9m)Estimated effective porosity 2.11 x 10⁻² ft/day



BIOSTIMULATION STRATEGY

Seven (7) amending locations amongst network. Alternated locations per event. Groundwater amended via;

Additive-Filled Passive Release Socks (PRS) Bulk Additive-Solution (5-18 gallons per node)

Groundwater amended passively every 2-weeks Utilized existing network of 2-inch monitoring wells Monitoring locations for each 2-3m downgradient

Deployments maintained for 3-months Monitoring data discussed covers initial 7-months of program; delayed by COVID-19, restarting Fall 2020?





RESULTS Source Zone Location GP-1



TerraStryke[®] Safe, Sustainable, Expedited Source Mass Removal Organically Additive-solution deployed 11/2019 and <2-months later; Realized microbial expedited solubilization of LNAPL contaminants.

^e Result, [diss.phased] increased = flux of sorbed mass to GW. Specifically:

Benzene	+103% ↑
Toluene	+678% ↑
Ethyl Benzene	+456% ↑
Xylenes	+15,675% 1

[TVPH] increased +365% ↑



RESULTS Source Zone Location GP-1



TerraStryke[®] Safe, Sustainable, Expedited Source Mass Removal Organically As LNAPL removed,

Realize microbial enhanced utilization of dissolved-phase contaminants.

From *peak* bioavailability,

Benzene	-99.9%↓
Toluene	-87.1%↓
Ethyl Benzene	-82.0%↓
Xylenes	-99.4%↓
Total Volatile H	lvdrocarbo

Total Volatile Hydrocarbons decreased >99.7%↓



OBSERVATIONS



LNAPL removed by enhanced biosurfactant production from amended microbials

get the rebound out up-front

Dissolved-phase reductions due to enhanced microbes utilizing carbon source as electron donor

realize expedited PHC destruction

Remove low-perm residual mass organically and *sustainably* <u>Minimize</u> costs and site impacts <u>Maximize</u> long-term compliance objectives with biostimulation



MICROBIAL DATA – Heterotrophic Plate Count (HPC)



TerraStryke[®] Safe, Sustainable, Expedited Source Mass Removal Organically

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As additive is deployed and made available realized expedited solubilization of residual mass by biostimulated microbial populations.

get the rebound out up-front

TOC levels increase as carbon contaminant becomes bioavailable in groundwater.

However, microbial populations appear to plummet with increased additive availability; then,

As contaminant levels decrease the TOC rises over time until majority of contaminant has been destroyed/assimilated.

Biostimulated change in phenotype to allow biofilm generation and maximum performance.



SITE 4 Former UST Seattle WA











OBSERVATIONS

- Heterotrophic plate count densities decreased as contaminant solubilization/destruction performances at peak levels.
- Total Organic Carbon concentrations increased during periods of peak microbial activity; including, the assimilation of carbon-based contaminants.
- Total Volatile Petroleum Hydrocarbon contaminants, including Benzene, decreased >99.7% during 6-month period.
- Overall, 8 of 10 amended wells decreased >99.99% during program after periods of solubilization.
- Degradation and solubilization continue; anticipating 1 more year of amending to complete 18-month project.







HYPOTHESIS

- Additive enhanced microbial populations changed phenotypic characteristics from planktonic to sessile forms as environmental stresses to the subsurface ecosystem were removed.
- This allowed increased communication and establishment of biofilm.
- Within biofilm sessile microbial populations shared information and adapted to current site conditions.
- Were provided protection from predation, adverse conditions, and established 'in-situ bioreactor'.
- Microbes work collectively, as consortium of populations, similar to that of multi-cellular organisms.







CONCLUSIONS

- Prokaryotes are older, more organized and experienced than eukaryotic bacteria.
- Prokaryotes are ubiquitous throughout the Earth.
- Prokaryotes dominate under anaerobic conditions.
- Biofilm necessitates anaerobic conditions and is the most prevalent form of life on the planet.
- By enhancing the ecosystem of prokaryotic bacteria with TPHenhanced we expedite LNAPL solubilization from low-permeable and/or smear zone conditions.
- In doing so we maximize use of organic contaminants as electron donors.







WILL LEAVE YOU WITH THIS

- Many bacteria ferment organic compounds; however,
- Most cannot 'touch' Hydrogen (H₂).
- Many Archaea (methanogens) ferment H₂ and CO₂ but cant 'touch' organic compounds.
- TPHenhanced stimulates microbial respiration and enhances the syntrophic destruction of PHCs under anaerobic conditions.
- TPHenhanced maximizes the sustainable destruction of source zone and dissolved-phase contaminants to realize long-term compliance objectives the way Mother Nature intended.









Lots of options out there





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