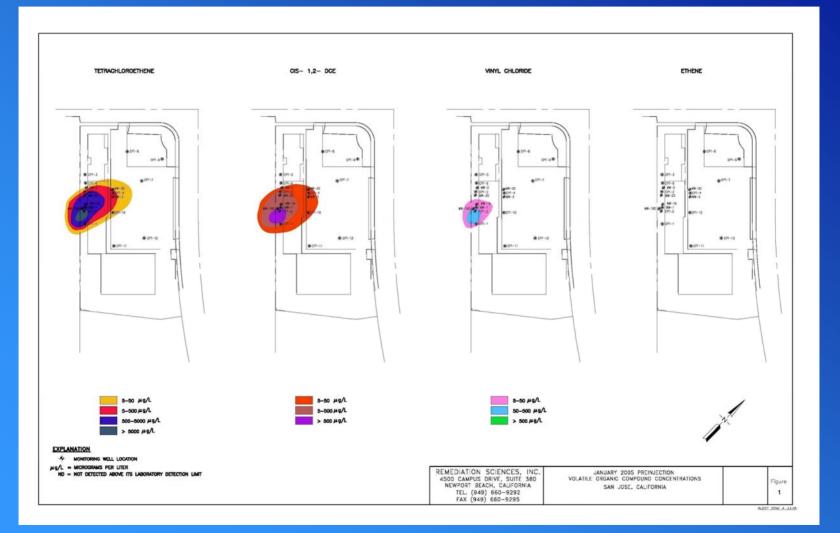
Anaerobic Bioremediation of Chlorinated Solvents in Groundwater Using Edible Oil Substrate EOS®

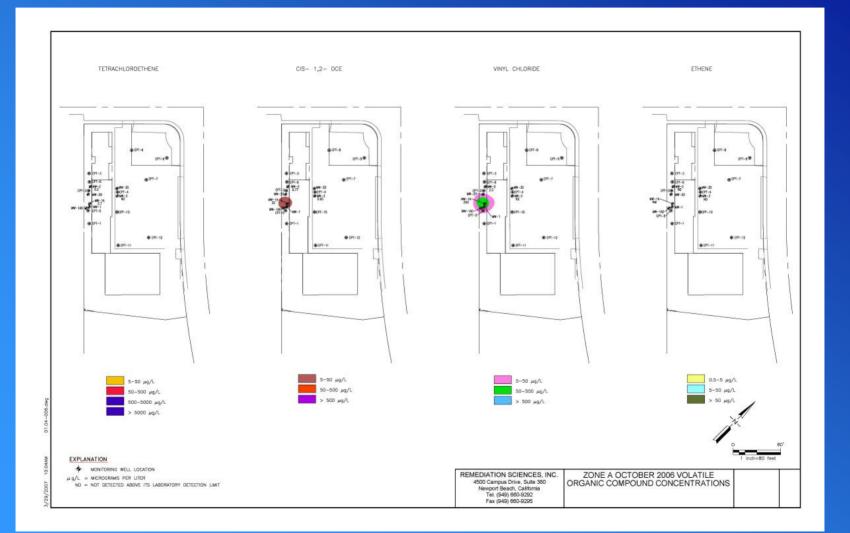
- *Matt Sedor, M.S.*, Yonathon Yoseph, P.G., C.H.G. (Remediation Sciences, Inc.)
- Jeff Baker (Vironex, Inc.),
- John Sankey, P.Eng. (True Blue Technologies Inc.).

Given by John Sankey, P.Eng., True Blue Technologies

From this...



...to this in 18 months!



Anaerobic Bioremediation of Chlorinated Solvents in Groundwater Using Edible Oil Substrate EOS®

Site Intro

Anaerobic Bioremediation– Few slides

Options: What ferments to hydrogen?

Designing the Project

Preparing and Injecting Substrate

Results

Ground Water Characterization--What to Monitor?



Site Intro: Dry Cleaners Site Located in San Jose, California

Highest PCE and TCE concentrations in the January 2005 were 8,500 µg/L.

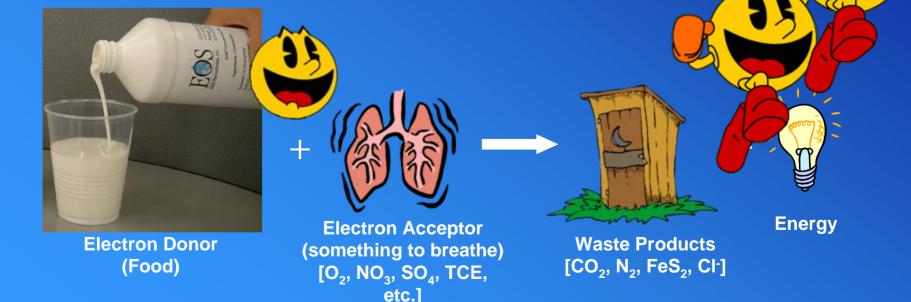
After evaluating several alternatives, *in situ* bioremediation was selected.

The goal was to find a substrate that was long lasting and easily distributed into the saturated soils.



How Does Anaerobic Bio Work?

Growth-Promoting Biological Reduction



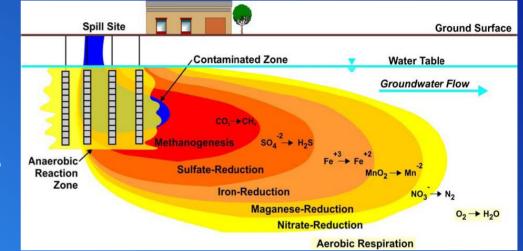
(Drawing Modified from AFCEE and Wiedemeier)

What is Needed for Effective Anaerobic Bioremediation?

- Organic substrates that ferment to:
 - Acetate
 - Hydrogen (H₂)
- Strongly reducing conditions (Sulfate Reducing or Methanogenic)
- Right halorespiring bacteria (*Dehalococcoides* for DCE / VC)
- Nutrients

В

 Vitamins and trace minerals to stimulate *Dehalococcoides* growth



Source: AFCEE, Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents, August 2004



What quickly ferments to hydro

Soluble substrates (e.g., lactate, butyrate, propionate, acetate, molasses, and sugars).

Solid substrates (e.g., bark mulch, compost, chitin and peat).

Slow release substrates such as vegetable oil.

Soybean oil

C₅₆H₁₀₀O₆ (soybean oil¹) + 50 H₂O -<u>B</u>->

-<u>B</u>-> 28 CH₃COOH (acetic acid) + 44 H₂

¹Represents weighted average of constituent fatty acids and glycerol.



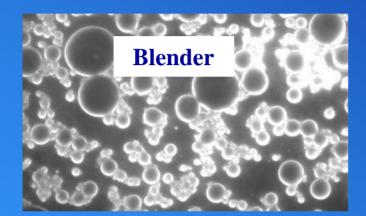
Slide 9

How Many Electrons Can We Pump into the Ground?

	e ⁻ Released				
	per mole	per lb			
Acetate	8	0.13			
Lactate	12	0.13			
Glucose	24	0.13			
Soybean Oil	313	0.36			
Canola Oil	319	0.36			
Lard	311	0.36			

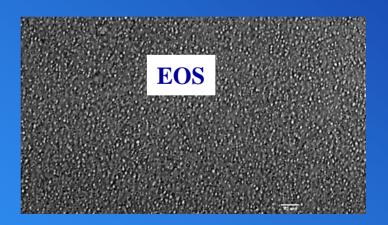
Soy/Lactate Emulsions



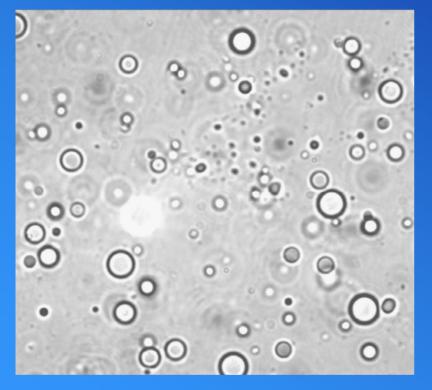


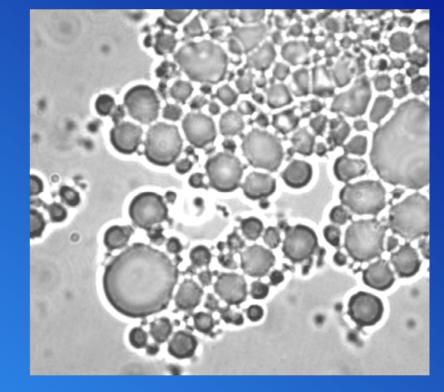






The Secret of Good Oil Distribution "Emulsions that do NOT Flocculate"

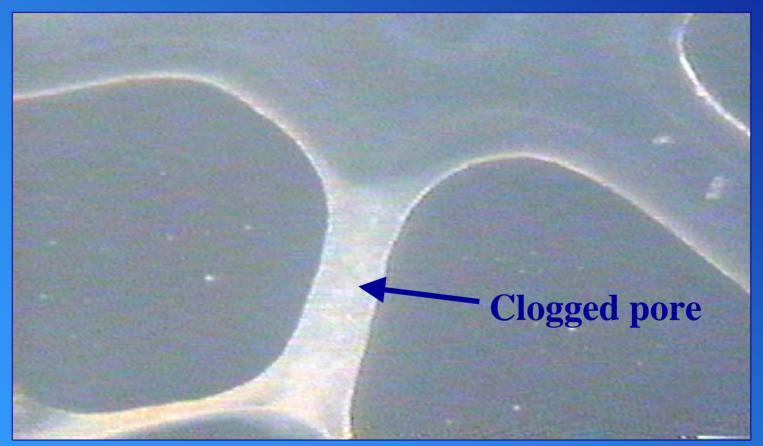




Dispersed Oil Droplets

Flocculated Oil Droplets

Secrets of Good Emulsion Distribution "Use Emulsions that do NOT Flocculate"



Technology Choice--EOS®

- Why?
- Slow release substrate
 - Emulsified soybean oil (GRAS)
 - Small, uniform droplets
 - Negative surface charge
- Easily biodegradable substrate
 - Lactate
- Micronutrients
 - Amino acids, Trace nutrients, Vitamins
- Easy to inject and distributed throughout treatment area
- Solid reputation
- Cost



Copyright © 2006 EOS Remediation, Inc.

Technology Choice—Proposed Cost EOS598 B42 Cost-6600 lbs • \$19,000

Drilling 12 points, injected 4,400 gallons of EOS mix and 22,700 gallons of flush water over a period of 6 days.

• \$30,000

Plus monitoring and engineering

Designing the Injection at The Dry Cleaner Site "You need to make contact...with the contaminant



Radius of Influence? 10ft to 100ft with EOS







.....To This



True Blue Technologies Inc. 🗸

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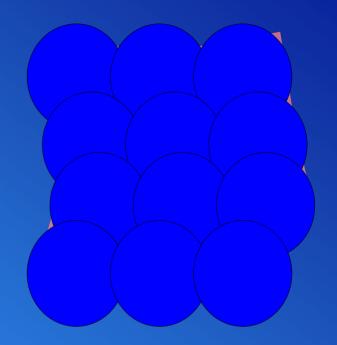
Source Treatment with Barriers

• Barriers 0.5 to 1 year apart

- Advantages
 - Low cost
 - Fewer injection points
 - Less oil and water
 - Release of TOC enhances downgradient biodegradation
 - Aquifer remains permeable
- Disadvantage
 - Longer clean-up time

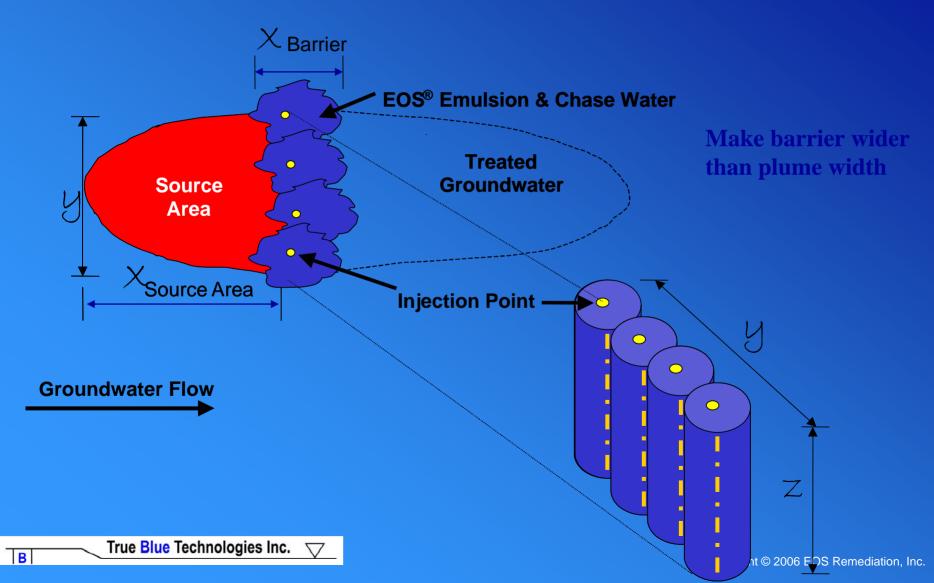
Groundwater Flow

Source Area Treatment



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How much do we inject?



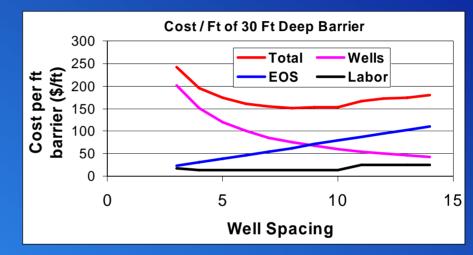
Design Tool Used

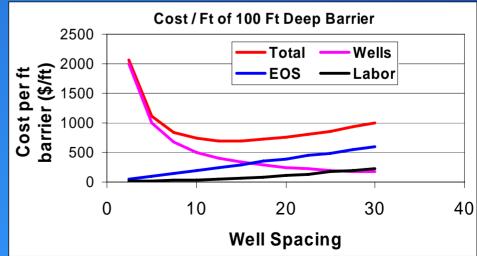
- Substrate needed for biodegradation
 - Flow rate through barrier
 - Pollutant concentrations
 - Competing electron acceptors (O₂, NO₃, SO₄)
 - Theoretical substrate life (5 to 10 years)
- Oil retention by aquifer
 Higher retention with fine grained materials

EOS	Emulsified Edible Oil Source Design Software Beta Version 1.1 www.eostermediation.com						
EOS Remediation, Inc.	Site Name:						
	Location: Project No.:						
	i lojost no						
Section A: Treatment Area Dimensions Length of source area parallel to groundwater flow. "."	50	1.	15.2		Groundwater Flo	w .	
Width of source area perpendicular to groundwater flow, "x" Width of source area perpendicular to groundwater flow, "u"	50	ft ft		m m		OS® Emulsion &	Charo Wa
Minimum depth to contamination	5	ft					Chase wa
Maximum depth of contamination	50	ft	15.2	m u 🖍 s	ource Treats	ad Groundwater	
Treatment thickness, "z"	45	ft			Area 🦕	マフ	
Treatment zone cross-sectional area = g * z	2,250	ft ²	209	m² 🛨			
Groundwater Flow Rate/ Site Data				+			
Groundwater Flow Rate/ Site Data Soil Characteristics				' ×	Source Area	10	
Nominal Soil Type (enter clay, silt, silty sand, or sand)	sand	1		Injectio	in Point	TC.	7/0
Hydraulic Characteristics				.,			<u> </u>
Total Porosity (accept default or enter n)	0.38	(decimal)					
Effective Porosity (accept default or n _e)	0.29	(decimal)					•
Hydraulic Conductivity (accept default or enter K)	28.5	ft/day ft/ft	1.0E-02	cm/se	•		
Hydraulic Gradient (accept default or enter i) Non-reactive Transport Velocity (V _s)	0.005	ft/ft ft/day	0.149772414	an falan i		1	<u> </u>
Groundwater flowrate through treatment zone (Q)	2398.275	tt/day gallons/day	0.149772414 9079.088939	L/day			
Calculated Contact Length (\mathbf{x}) = C _t * V _x			-				
Contact time (C ₁) between oil and contaminants (accept default or enti-	er C _i)	60	typical values 3	10 to 90 days	, see comment		
Calculated Contact Lenght (x) = Ct * V _x		29.483	lu l				
Treatment zone volume	135.000	ft ²	3.186	m ²			
Treatment zone groundwater volume (volume * effective porosity)	292,842	gallons	923,837	L .			
	_						
Design Lifespan For One Application Total groundwater volume treated over design life	10 9,046,546	year(s) gallons	typical values 5 34,062,512				
-	5,540,540	Banoug	34,002,012	-			
Electron Acceptors					0	11-1	
					Stoichmetry	Hydrogen	
Inputs	Typical Value	GW Conc. (mg/L)	MW (g/mole)	e' equiv./ mole	Contaminant/H ₂ (wt/wt H ₂)	Demand (g H ₂)	
Inputs Dissolved Oxygen (DO)	0 to 8	(mg/L) 5	(g/mole) 32.0	mole 4	Contaminant/H ₂ (wt/wt H ₂) 7.94	Demand (g H ₂) 21458.05817	
Inputs Dissolved Oxygen (DO) Ntrate Nitrogen (NO ₂ ' N)	0 to 8 1 to 10	(mg/L) 5 10	(g/mole) 32.0 62.0	mole 4 5	Contaminant/H ₂ (wt/wt H ₂) 7.94 12.30	Demand (g H ₂) 21458.05817 27684.59072	
Inputs Dissolved Oxygen (DO) Wrate Nirogen (NO ₂ - N) State (SO ₄ ²)	0 to 8	(mg/L) 5 10 50	(g/mole) 32.0 62.0 96.1	4 5 8	Contaminant/H ₂ (wt/wt H ₂) 7.94 12.30 11.91	Demand (g H ₂) 21458.05817 27684.59072 142962.5791	
Inputs Dissolved Oxygen (DO) Winter Nitrogen (NO,` N) Suffats (SO,^`) Tetrachioneshmere (PCE), C,Cl,	0 to 8 1 to 10	(mg/L) 5 10 50 1	(g/mole) 32.0 62.0	mole 4 5	Contaminant/H ₂ (wt/wt H ₂) 7.94 12.30	Demand (g H ₂) 21458.05817 27684.59072	
Inputs Distributed Gragem (DO) Nimate Nimogen (NQ) - Ni Suitate (SO, -) Tetrachoreshmer (PCE), C,CLa Trichoreshmer (PCE), C,CLa	0 to 8 1 to 10	(mg/L) 5 10 50	(g/mole) 32.0 62.0 96.1 165.8	4 5 8 8	Contaminant/H ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914	
Inputs Descrived Oxyget (DO) Nitimis Nitimiser, (NO, - N) Satura (SO, -) Terachiconethere (PCE), G, CA, Terachiconethere (PCE), G, CA, Solid Articlonethere (PCE), CA, CA, Solid Articlonethere (PCE), CA, CA, Nity (Pothods (VC, CA, CA, CA))	0 to 8 1 to 10	(mg/L) 5 10 50 1 13	(g/mole) 32.0 62.0 96.1 165.8 131.4	4 5 8 8 6	Contaminant/H ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136	
Inputs Distributed Oxygen (DO) Whitele Nitrogan (ND) - N) Subtan (SOA ²) Terandarosethere (PCB), C,C/L Terandarosethere (PCB), C,C/L Terandarosethere (PCB), C,C/L Terandarosethere (PCB), C,C/L Stationardister (PC), C,C/L Stat	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8	mole 4 5 8 8 6 4 2 8	Contaminant/H ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Dissolved Oxygen (DO) Water (MO) Suffex (DO) Suffex (DO) Suffex (DO) Suffex (DO) Suffex (DO) Suffex (DO) October (DO) Suffex (DO) Su	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4	mole 4 5 8 8 6 4 2 8 6	Contaminant/H ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Insuration of Control	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8	mole 4 5 8 8 6 4 2 8 6 8 6 8	ContaminantiH ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.62	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Discolved Oxygen (DO) Nitrate Nitrogen (NO, * N) Sufface (DO, 1) Suffac	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4	mole 4 5 8 8 6 4 2 8 6 8 6 8 6	ContaminantiH ₂ (wt/wt H ₃) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Discoled Cargen (IDC) Writes Kingen (ND- N) Suites (SO-7) Tetrachorostene (PCD, C,CL) Tetrachorostene (PCD, C,CL) Tetrachorostene (PCD, C,CL) Conv Internationality, C,CL,CL) Calcon tetrachorostene (C,CL) Calcon tetrachorostene (C,CL) To-Chardene (C,CL) To-Chardene (C,CL) C,CL)	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4 99.0	mole 4 5 8 8 6 4 2 8 6 8 6 8 6 4	ContaminantiH ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06 24.55	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Districted Oxygen (DO) White Bit (No) Tean-strongen (ND) - N) Subte (SO, 1) Tean-strongen (SO, EOL) Tean-strongen (SO, EOL) Subte (SO, EOL) Su	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4	mole 4 5 8 8 6 4 2 8 6 8 6 8 6	ContaminantiH ₂ (wt/wt H ₃) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Disched Grapen IDO Minnes Wingen (NO: N) Suites (SA): Tereachronotheme (PCE), C,CIA, Trichocenteme (TCE), C,CIA, Sinforcenteme (TCE), C,CIA, Sinforcenteme, C,CIA, Pereformer, C,GIA, Pereformer, C,GIA, Pereformer, C,GIA,	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4 99.0 64.9	mole 4 5 8 8 6 4 2 8 6 8 6 8 6 4 2	ContaminantiH ₂ (wt/wt H ₂) 7.94 1.200 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06 24.55 32.18	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Desched Oxyper (IO) Nitrais Nitrages (IV), N) Satura (SO, ²) Ternachisorechner (PCE), C, CA, Techocenterer (ICC), C, CA, Techocenterer (ICC), C, CA, Carlonationation, C, CA, Carlonation, CA,	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4 99.0 64.9 99.4	mole 4 5 8 8 6 4 2 8 6 8 6 8 6 8 6 8 6 4 2 8 8 6 8 6 8 8 6 8 8 6 8 8 8 8 8 8 8 8	ContaminantiH ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06 24.55 32.18 12.33	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Discoluted Corgans (IDC) White Is Wingson (NG) - N) Subtase (SG), T Tetrachorocheme (PCE), C,CLa, Teinocheme (PCE), Teinocheme (PCE)	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4 99.0 64.9 99.4	mole 4 5 8 8 6 4 2 8 6 8 6 8 6 8 6 8 6 4 2 8 8 6 8 6 8 8 6 8 8 6 8 8 8 8 8 8 8 8	ContaminantiH ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06 24.55 32.18 12.33	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Desched Organ (IO) Nines Ningsen (NO), Ni Sahar (SOA) Terinschorechner (PCE), C,CA, Teinschorechner (PCE), C,CA, Teinschorechner (PCE), C,CA, Service (C,CA, Service (C,CA	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4 99.0 64.9 99.4	mole 4 5 8 8 6 4 2 8 6 8 6 8 6 8 6 8 6 4 2 8 8 6 8 6 8 8 6 8 8 6 8 8 8 8 8 8 8 8	ContaminantiH ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06 24.55 32.18 12.33	Demand (g H ₂) 21458.05817 27684.59072 142962.5791 1656.1914 20381.2136 1416.55704	
Inputs Disched Organ IDD Winde Kingen (ND, N) States (SO, ²) Tetrachtonethere (PCG), C,C4, Tetrachtonethere, C,C4, Discretardance (CA), C,C4,C5, 11,1-Trichtonethane, C,C4,C4, Discretardance (CA), C,C4,C5, 11,1-Tetrachtonethane, C,4,C4, Descretardance (CA), Descretardance (C	0 to 8 1 to 10	(mg/L) 5 10 50 1 13 13	(g/mole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4 99.0 64.9 99.4	mole 4 5 8 8 6 4 2 8 6 8 6 8 6 8 6 8 6 4 2 8 8 6 8 6 8 8 6 8 8 6 8 8 8 8 8 8 8 8	ContaminantiH ₂ (wt/wt H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06 24.55 32.18 12.33	Demand (g Hz) 21458:05817 27684:59072 12692:5781 1856:1914 20381:52136 1416:5703 1416:5703 1998:63423	
Inputs Disclosed Organs (IOO) Nitraes Nitrogen (NOC) N) Salata (SOA, 7) Terinschoordene (PCE), C, CA, Terinschoordene (PCE), C, CA, Trichoordene (PCE), C, CA, Trichoordene (PCE), C, CA, Salardone, CA, Vary Cholder (VC, CA, CA, Vary Cholder	0 to 8 1 to 10 10 to 500	(mg/L) 5 10 50 1 13 1 1 1 1	(g/mde) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 167.8 133.4 99.0 64.9 99.4 4 52.0	mole 4 5 8 6 4 2 8 6 6 4 2 8 6 4 2 8 3	Contaminant/H (wtw H ₂) 7.94 12.30 11.91 20.57 21.73 24.05 31.00 19.08 19.74 20.82 22.06 24.55 32.18 12.33 17.20	Demand (g Hz) 21458.05817 27684.58072 142962.5791 142962.5791 1416.5704 1098.63423 H16.5704 H98.63423	DOC
Inputs Disched Oxypen IDO Nimes Ningen (ND) - Ni States (SA) - Discher Constant (SA) - SA -	0 to 8 1 to 10	(mgL) 5 10 50 1 1 13 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	(gmole) 32.0 62.0 96.1 165.8 131.4 96.9 62.5 153.8 119.4 153.8 119.4 167.8 133.4 99.4 52.0 64.9 99.4	mole 4 5 8 6 4 2 8 6 8 6 4 2 8 8 6 4 2 8 3	Contaminant/H ₂ (wth H ₂) 7.94 12.30 12.30 12.057 21.73 24.05 31.00 19.08 19.74 20.57 22.06 24.05 31.00 19.08 19.74 20.62 22.06 24.55 32.18 12.33 17.20	Demand (g Hz) 21458:05817 27684:59072 12692:5781 1856:1914 20381:52136 1416:5703 1416:5703 1998:63423	

Determining Injection Well Spacing

- Tradeoff between
 - Well installation cost
 - Labor cost for injection
 - Material cost for emulsion





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Enhanced Anaerobic Bioremediation Using Emulsified Edible Oils

• Preparing and Injecting Emulsions

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Injection System Design Options Direct-push technology - Using pressure Injection wells - Gravity feed - Low pressure

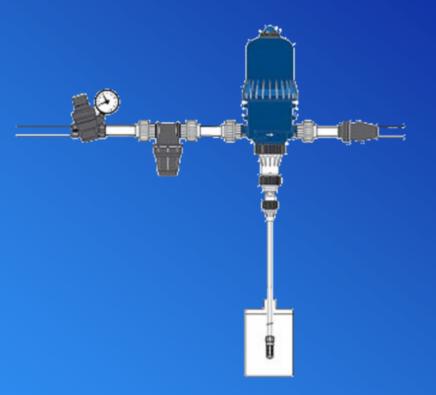
- Continuous injection of dilute emulsion without chase water
 Dilution ratios range from 1:10 to 1:30
 Depends on effective porosity
- Injected 4,400 gallons of EOS mix and 22,700 gallons of chase water
- Chase water used to distribute emulsion out into the formation

Slide 27

Emulsion Dilution

- In-line metering system
 - Eliminates labor and equipment for field blending
 - Adjustable dilution ratio

Continuous Metering System

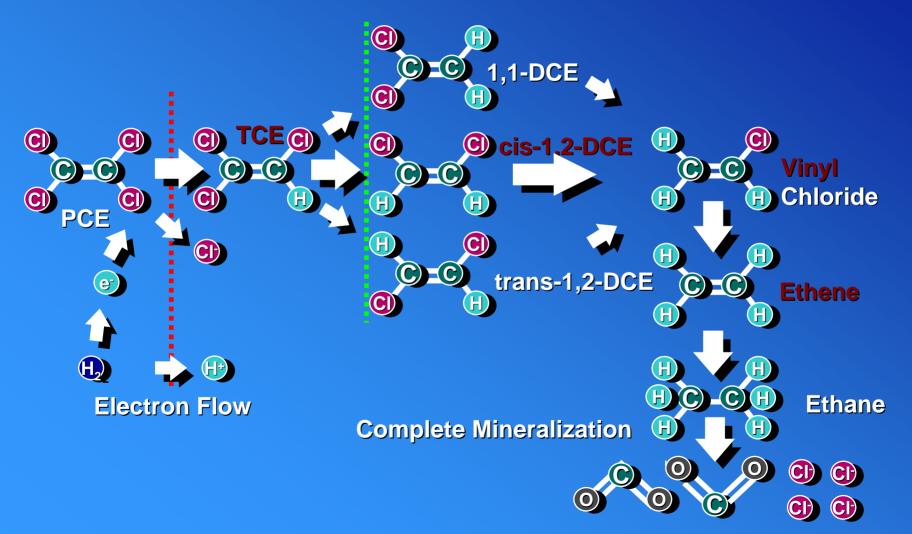


Ground Water Characterization What to Monitor

Indicator Parameters

- Electron acceptors (O_2, NO_3, SO_4)
 - Low levels of O₂ are not a major problem
 - High levels of SO₄ increase substrate demand
- Electron donors (Mn, Fe, CH₄, TOC)
- ORP, PH
- Degradation products
- See EPA / AFCEE protocol for MNA of Chlorinated Solvents

Degradation products



(Drawing Modified from AFCEE, Technology Transfer Division)

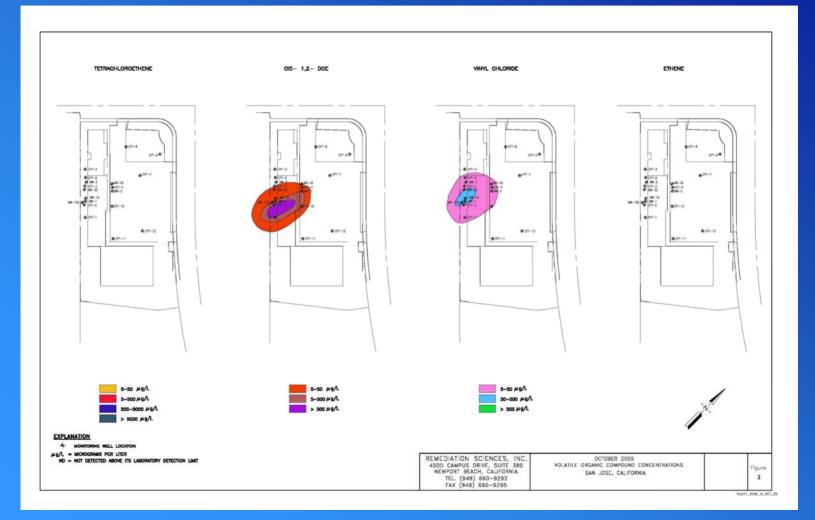
Anaerobic Bioremediation of Chlorinated Solvents in Groundwater Using Edible Oil Substrate EOS®

• Results

PLUME OF THE MAJOR CONTAMINANTS Pre-Injection (injection April 2005)



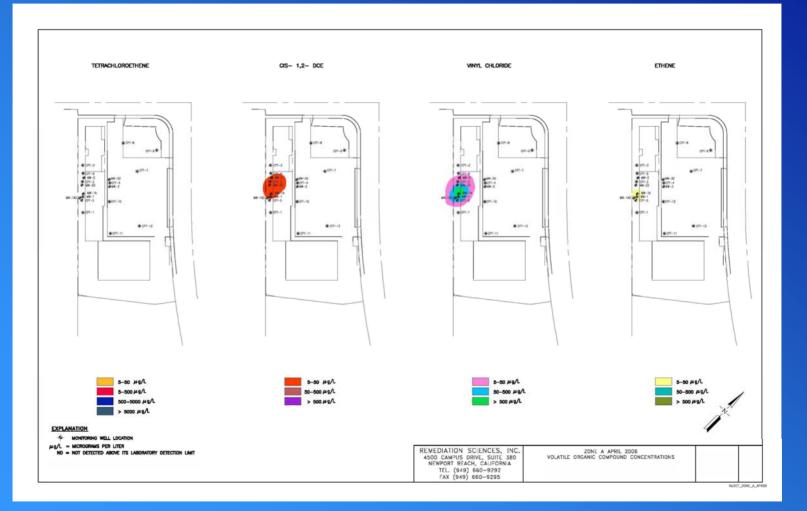
PLUME OF THE MAJOR CONTAMINANTS 6-months post-injection



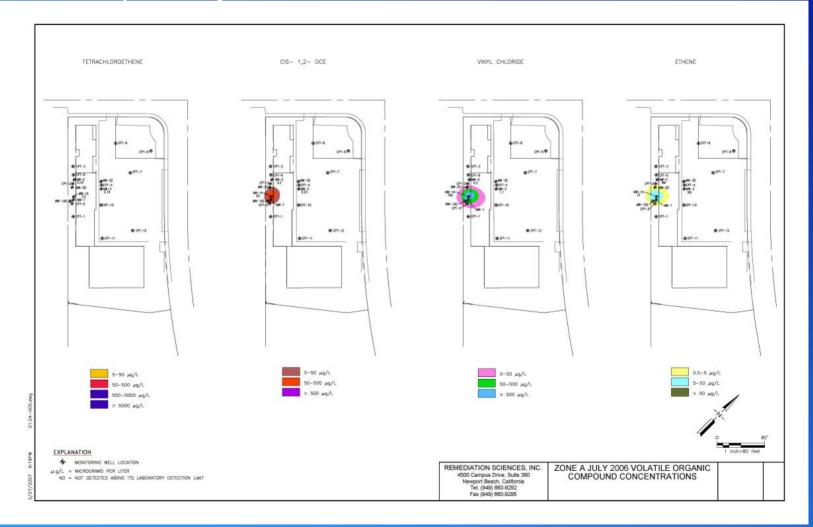
PLUME OF THE MAJOR CONTAMINANTS 9-months post-injection



PLUME OF THE MAJOR CONTAMINANTS 12-months post-injection

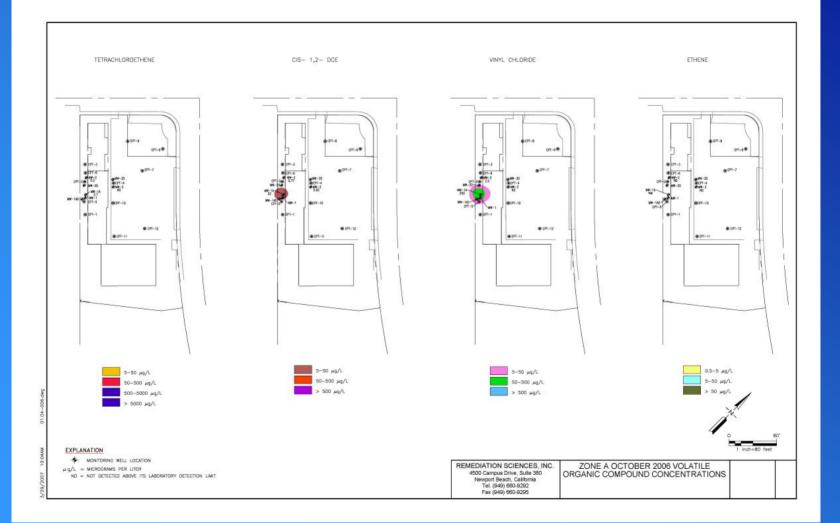


PLUME OF THE MAJOR CONTAMINANTS 15-months post-injection



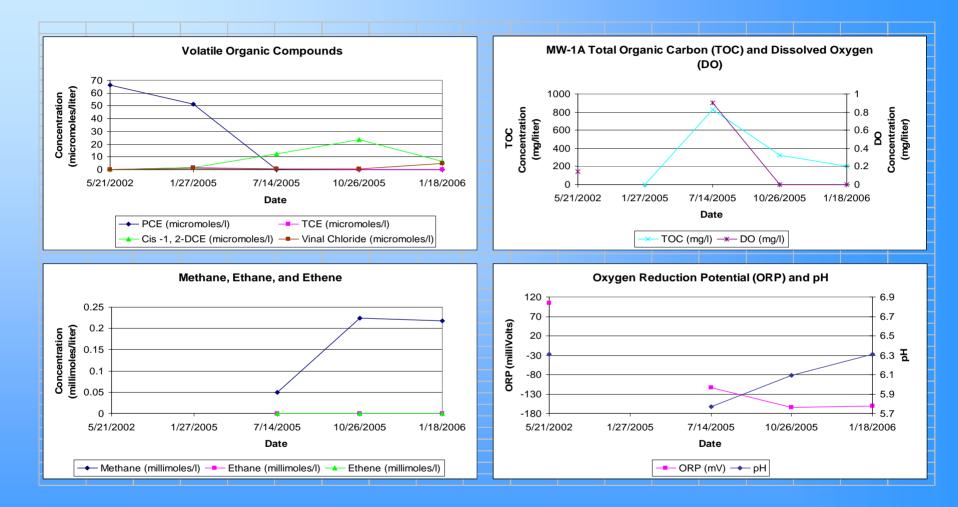
Тв

PLUME OF THE MAJOR CONTAMINANTS 18-months post-injection



Slide 37

RESULTS: CHART 1: MW-1A ANALYTICAL RESULTS VERSES TIME



Project Conclusions

- EOS[®] effectively distributed throughout treatment area
- Quickly established favorable geochemistry for reductive dechlorination.
- Dramatic improvements in groundwater conditions compared to prior technologies
- Substantial reductions in TCE observed.
- Apply for decrease monitoring to every 6 months.
- Apply for closure this year.

B

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Robert C. Borden and Christie Zawtocki, Michael D. Lee, Erica S Becvar, Patrick E. Haas, Bruce M. Henry, AFCEE Protocol For Enhanced Anaerobic Bioremediation Using Edible Oils

For More Information

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- Visit www.eosremediation.com
- Design Tool, Case Studies
- Complete Product Line
 - Chlorinated Solvent Site Remediation
 - EOS ® , emulsified soybean oil, for enhanced in situ bioremediation
 - BAC 9, microbes for bioaugmentation
 - Petroleum Site Cleanup Remediation
 - EOx[™], a calcium-based oxygen releasing substrate for aerobic bioremediation