



Leading Science · Lasting Solutions



HEALTH, SAFETY AND PERFORMANCE... SiREM BIOAUGMENTATION CULTURES

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To: LARWQCB, WDR Working
Group
Los Angeles, CA
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SiREM Bioremediation Focused Service Areas

Molecular Genetic Testing

gene & trac[®]

Biotreatability Studies

treatability
studies

Bioaugmentation Products

KB-1[®]





Where we are Located

SiREM is located in the University of Guelph Research Park in Ontario, Canada 45 minutes west of Toronto International Airport, allowing efficient overnight shipping to/from international destinations



University of Guelph Research Park





Bioaugmentation



Injection of KB-1® at a site in Florida



Premeasured KB-1® ready for application at Coastal site in Southern California

- **Bioaugmentation:**
the addition of beneficial microorganisms to improve the rate or extent of biodegradation
- **KB-1® and KB-1® Plus:**
commercial bioaugmentation cultures used to introduce beneficial organisms to sites where they are absent or at low concentrations/poorly distributed





SiREM Bioaugmentation Cultures

KB-1[®]

- Chlorinated ethenes (PCE, TCE, cDCE and VC) degrading culture + 1,2-DCA
Primarily *Dehalococcoides (Dhc)*

KB-1^{plus}[®]

Custom blended from source cultures degrades:

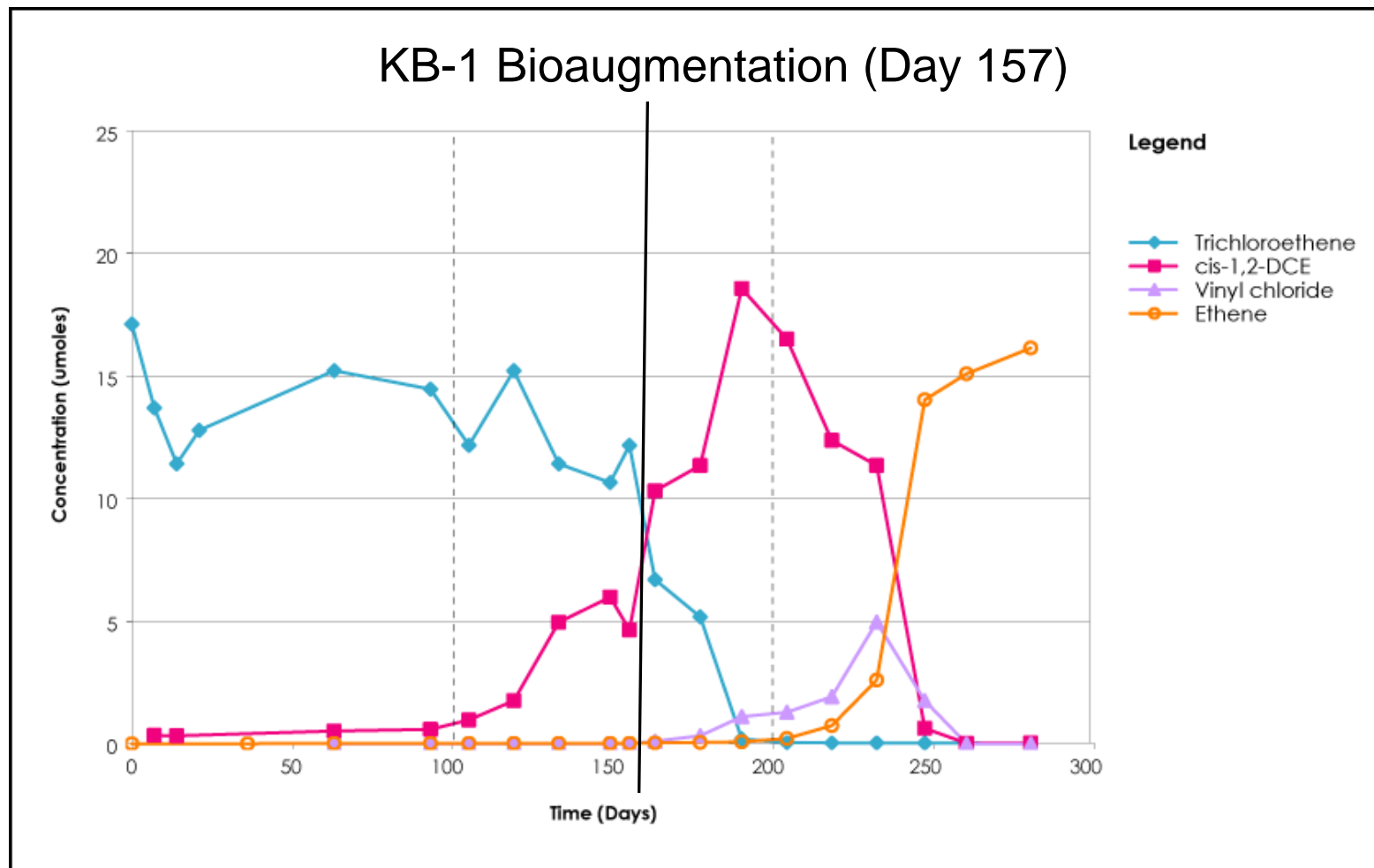
- Chlorinated ethanes (tetrachloroethane, 1,1,2-TCA and 1,1,1-TCA, 1,1-DCA)
- Chlorinated methanes (CT/CF/DCM)

**Primarily *Dehalobacter (Dhb)* and
*Dehalogenimonas (Dhg)***





Effect of KB-1 At Site Northern California





SiREM Bioaugmentation: Safe/Dependable/Approved/Guaranteed

- SiREM cultures are proven performers used for over 10 years
- Regulatory approval obtained in many jurisdictions based on excellent quality control and extensive culture characterization
- Delivered in high quality stainless steel pressure vessels
- SiREM has the only cultures with performance guarantees
- High level of technical support through the planning, injection and data analysis stages





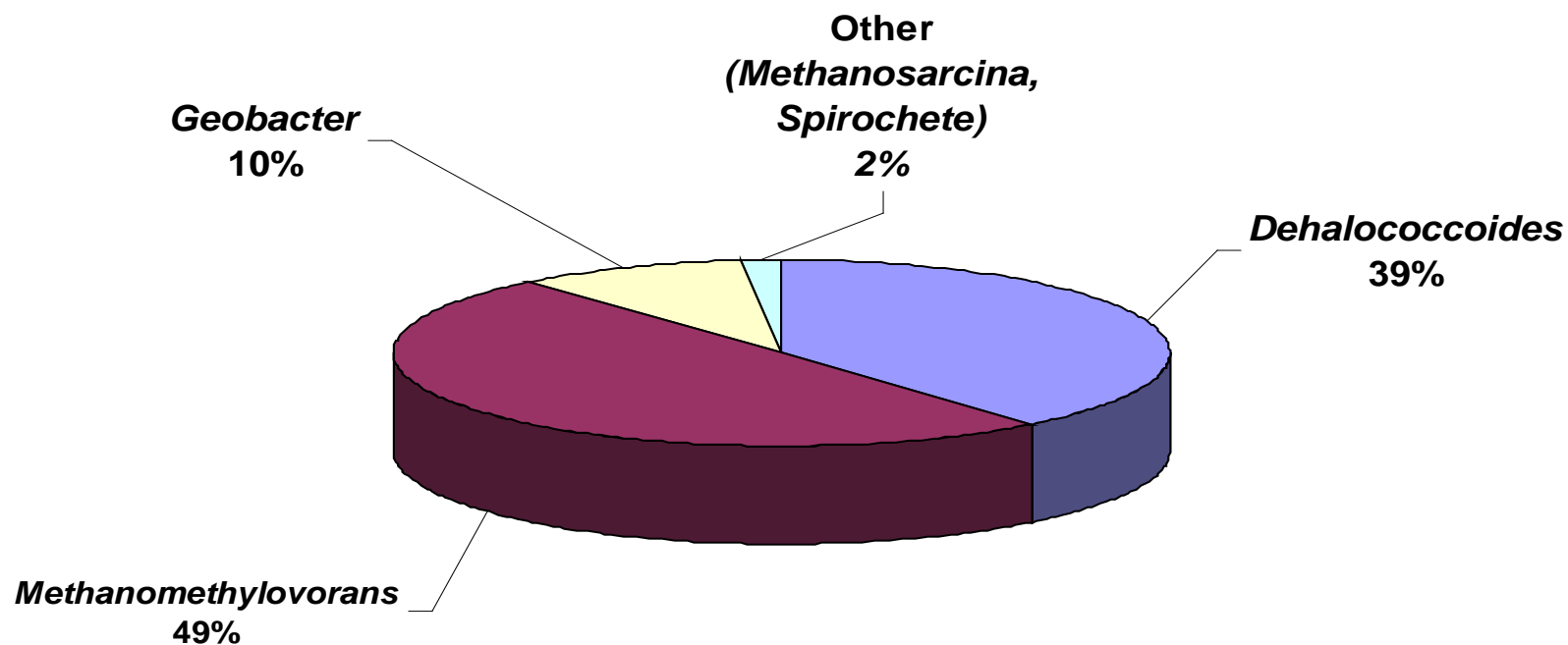
KB-1[®] (101)

- Anaerobic liquid bioaugmentation culture enriched from TCE site
- Contains > 100 billion *Dhc*/Liter
- Not genetically engineered
- Pathogen free





Microbial Characterization of KB-1 by qPCR





Media Composition

Chemical Name	Formula	CAS#	Concentration grams/Liter
Potassium Phosphate Dibasic	KH_2PO_4	7758-11-4	0.27
Potassium Phosphate Monobasic	K_2HPO_4	7778-77-0	0.34
Ammonium Chloride	NH_4Cl	12125-02-9	0.535
Calcium Chloride	CaCl_2	10035-04-8	0.07
Magnesium Sulfate	MgSO_4	10034-99-8	0.125
Ferrous Chloride	FeCl_2	13478	0.02
Sodium bicarbonate	NaHCO_3	144-55-8	2.0
Ferrous Ammonium Sulfate	$(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$	7783-85-9	0.4
Sodium sulfide	Na_2S	1313-84-4	0.12
Resazurin	$\text{C}_{12}\text{H}_6\text{NNaO}_4$	62758-13-8	0.001
Boric Acid	H_3BO_3	10043-35-3	0.0006
Zinc Chloride	ZnCl	7646-85-7	0.0002
Sodium Molybdate	Na_2MoO_4	10102-40-6	0.0002
Nickel II Chloride	NiCl_2	7791-20-0	0.0015
Manganese Chloride	MnCl_2	13446-34-9	0.002
Copper II Chloride	CuCl_2	10125-13-0	0.0002
Cobalt Chloride	CoCl_2	7791-13-1	0.003
Disodium Selenite	Na_2SeO_3	10102-18-8	0.00004
Aluminum Trisulfate	$\text{Al}_2(\text{SO}_4)_3$	10043-01-3	0.0002
Vitamins	Various	Various	0.01 maximum



Bioaugmentation Culture Production



Steam in place methods used for growth media and vessel sterilization

SiREM has facilities for growing thousands of liters of bioaugmentation cultures

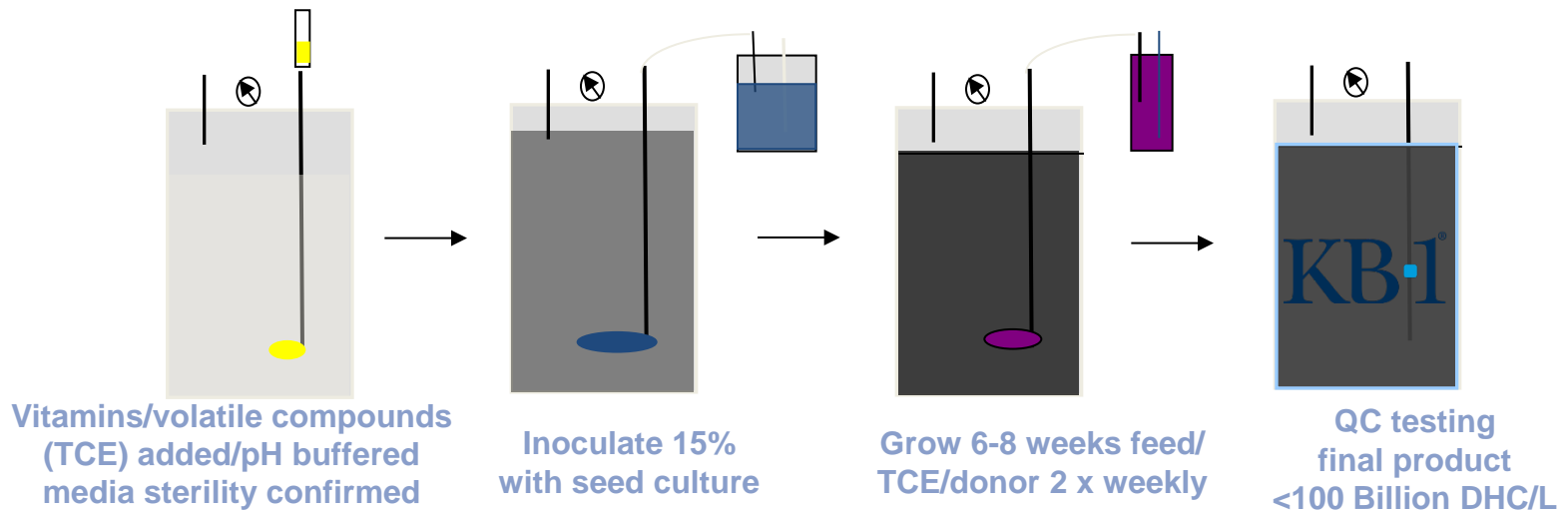
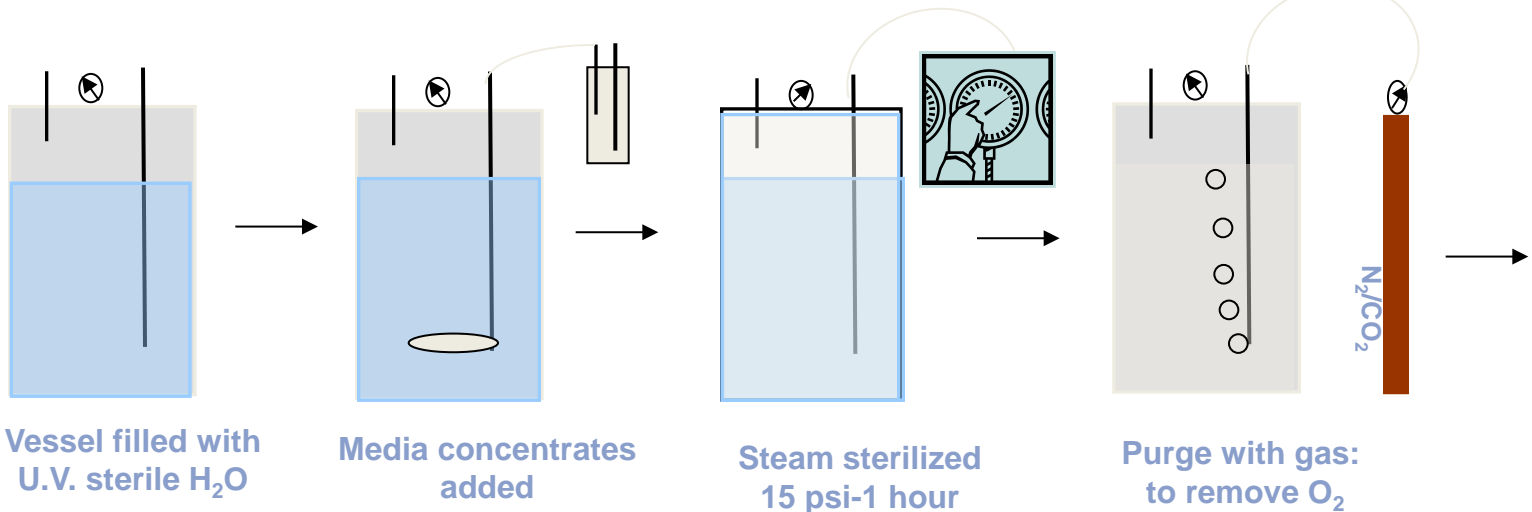


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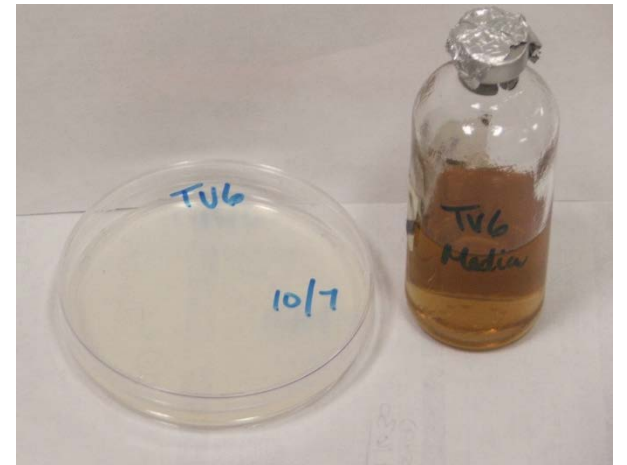


Bioaugmentation Culture Production



■ ■ Preventing and Detecting Pathogens

- Pathogen free initial inoculum
- Sterile production methods
- Pre-sterilization of growth vessels
 - Filtration of purge gases
 - Sterilized hoses etc.
- Media Sterility Checks
(e.g., plate counts)
- Regular stability and pathogen screening
- High quality stainless steel delivery vessels



■ ■ KB-1/KB-1 Plus Non-Detect for Pathogenic Microorganisms
■ ■ in over 10 years of Commercial Production

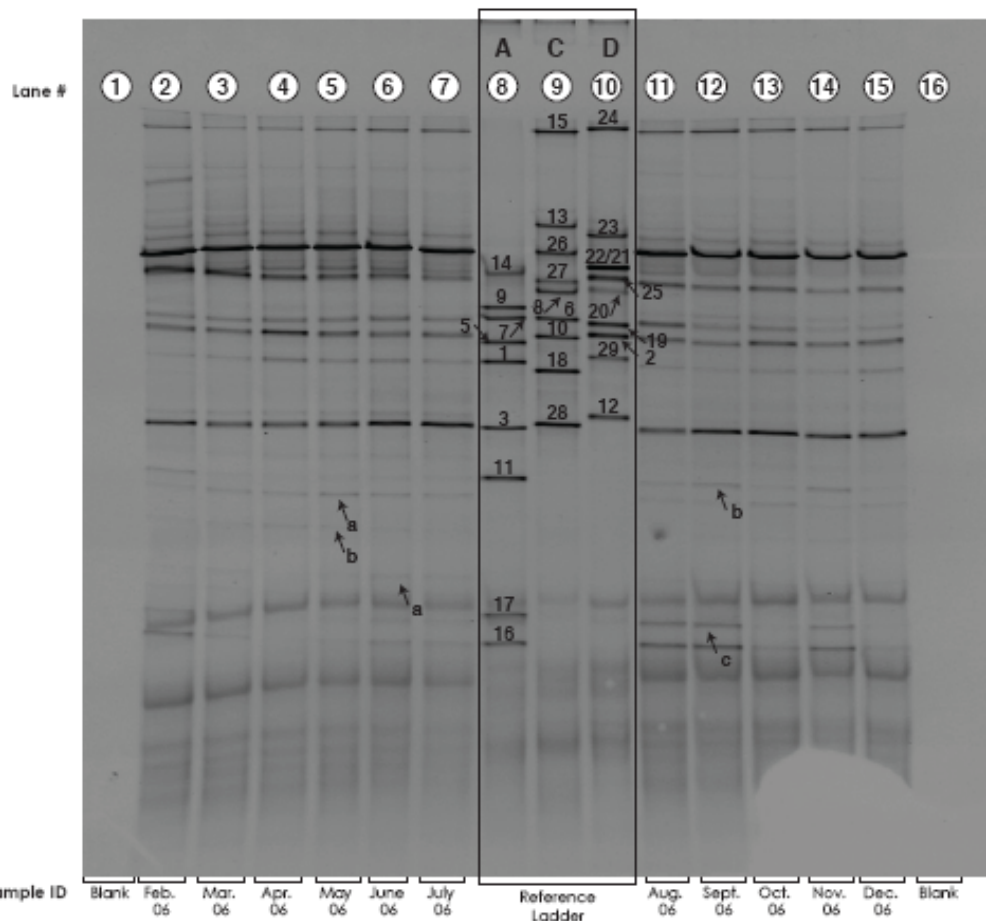
Organism*	Status in KB-1 / KB-1 Plus
<i>Salmonella sp.</i>	Not Detected
<i>Listeria monocytogenes</i>	Not Detected
<i>Vibrio sp.</i>	Not Detected
<i>Campylobacter sp.</i>	Not Detected
<i>Hemolytic Clostridia sp.</i>	Not Detected
<i>Bacillus anthracis</i>	Not Detected
<i>Pseudomonas aeruginosa</i>	Not Detected
<i>Yersinia sp.</i>	Not Detected
<i>Pathogenic Yeast and Mold</i>	Not Detected
<i>Fecal coliforms</i>	Not Detected
<i>Enterococci</i>	Not Detected

*Environment /Health Canada-Recommendations for Testing of Microbial Consortia under New Substances Notification Guidelines





Assessing Stability and Composition of Bioaugmentation Culture KB-1 using DGGE



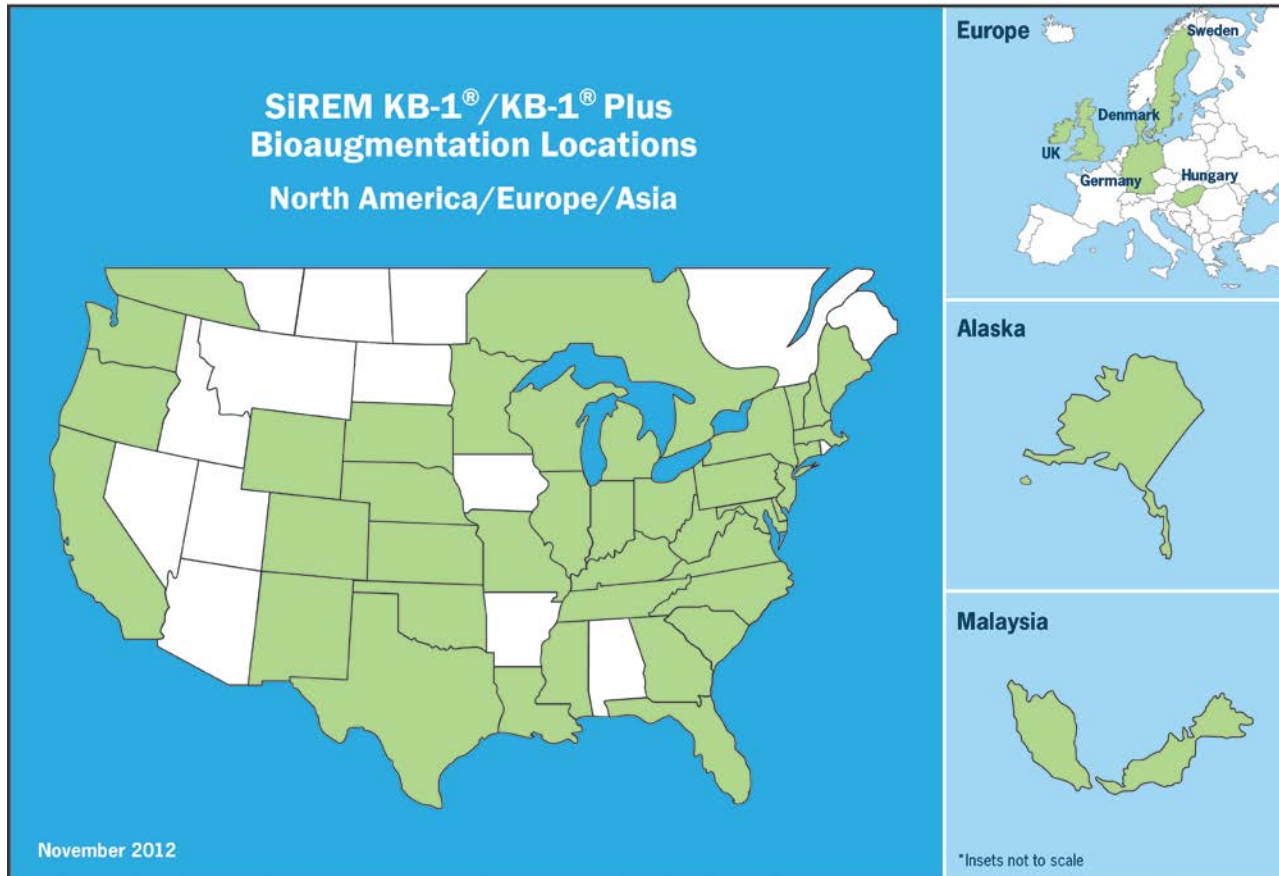
SiREM Cultures: Regulatory Approvals

- SiREM cultures have been applied in ~50 jurisdictions in North America, Europe and Asia
- Permission to apply SiREM cultures has never been denied by a regulator
- KB-1 is approved for:
 - Use in Canada under Environment Canada NSN
 - Mobile Injection Certificate Ontario Ministry of Environment for all Ontario, Canada
 - North Carolina DWQ approved injectables list
 - Approved for import into Australia





KB-1[®]/KB-1[®] Plus Bioaugmentation Locations



With over 300 hundred sites bioaugmented with KB-1[®] & KB-1[®] Plus, our ability to understand the performance and conditions under which these cultures are effective continues to increase





SiREM Cultures: Bioaugmentation In California



GREEN OC Living green in Orange County



- KB-1 and KB-1 Plus applied at over 60 sites in California over past 10 years
- Over 5,000 liters of KB-1 applied in California
- 7 of 9 RWQCB regions have granted WDRs for SiREM cultures
- Largest volume injection ~800 injection locations

Pollution-gobbling bacteria set loose in Seal Beach
December 16th, 2008, 4:38 pm · 2 Comments · posted by Pat Brennan,
green living, environment editor

The creatures released this week on the [Seal Beach Naval Weapons Station](#) were chosen for a special talent: their ability to breathe chlorine, and to make harmful chemicals harmless.



Naval contractor Sean Gardner adjusts machinery



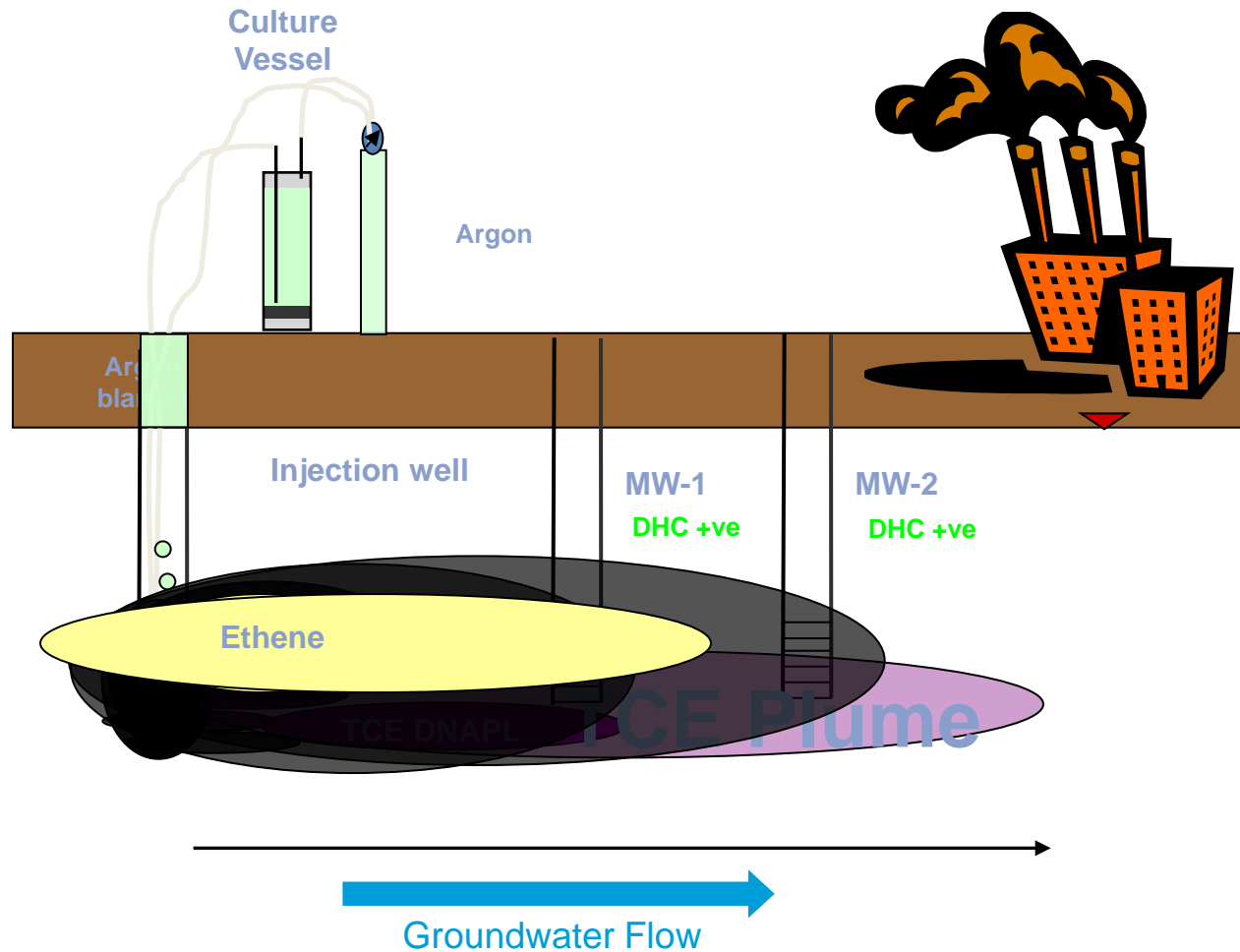
Bioaugmentation Field Kit



Materials Shipped to Site



Bioaugmentation Culture Field Application



Minimizing any Potential Risks Associated with Bioaugmentation

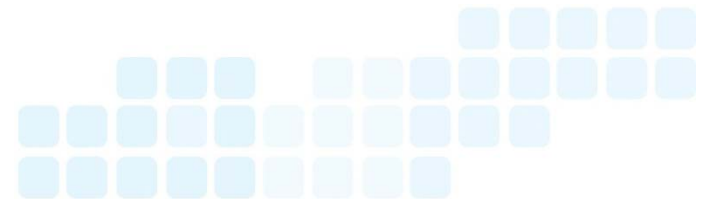
- Culture source with low likelihood of pathogens (e.g., groundwater)
- Sterile production methods-prevent pathogen introduction
- Contained growth and application process
- Pathogen screening of seed cultures





Conclusions

- KB-1 and KB-1 Plus are produced from naturally occurring North American microorganisms that are pathogen free and not GMO
- QA/QC protocols and high quality injection vessels ensure the safety and performance of each batch
- KB-1 and KB-1 Plus have been approved for injection in 50 jurisdictions
- KB-1 and KB-1 Plus have history of safe use at over 60 sites in California





Thank you!

Questions? Comments!

Further Information

siremlab.com

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