True Blue Technologies
Remediation and Characterization Products and Support

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Alkane Gases

Cometabolic bioremediation is an emerging remediation practice area to address groundwater impacts and large dilute plumes. This approach has been used on some of the most recalcitrant contaminants, e.g., trichloroethene, dichloroethene, vinyl chloride, 1,4-dioxane. Cometabolism is the simultaneous degradation of two compounds, in which the degradation of the second compound (the secondary substrate) depends on the presence of the first compound (the primary substrate).

In aerobic cometabolic bioremediation indigenous bacteria are stimulated by adding oxygen and a cometabolic growth substrate to trigger the production of enzymes. These enzymes then oxidize or degrade the target pollutant via cometabolism. Alkane gases such as **methane**, **propane** or **ethane** are commonly used as the primary substrate. Indigenous mircroorganisms while oxidizing the primary substrate for energy and growth express a monooxygenase enzyme that fortuitously degrades the contaminant (the secondary substrate). The enzyme is a protein-like substance that acts as a catalyst for degradation of the contaminant. Contaminant degradation provides no apparent benefit to the microorganism involved. The biodegrader is not dependent on the contaminant for carbon or energy therefore can perform at low levels of contamination as in the case of meeting vinyl chloride groundwater standards.

This method is most useful for bioremediation of pollutants that are not themselves good aerobic growth substrates for bacteria. Bioremediation strategies that employ cometabolism have the advantage of being able to degrade contaminants to trace concentrations, very low parts per billion levels and actually to parts per trillion.

A source of oxygen must be introduced for cometabolism to occur. Gas inFusion technology is currently deployed at a site in the Pacific Northwest for the *in situ* cometabolic bioremediation of a mixed 1,4-dioxane and chlorinated solvent plume. A vinyl chloride case study published in

Ground Water Monitoring & Remediation 32, no. 1/ Winter 2012/pages 99–105 is attached for your review.

Papers with field data are attached.

Please call if you have any questions.

Regards,
John Sankey,
True Blue Technologies

Material Safety Data Sheet

Methane

Section 1. Chemical product and company identification

Product name : Methane

Supplier : AIRGAS INC., on behalf of its subsidiaries
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

Product use : Synthetic/Analytical chemistry.

Synonym : fire damp; marsh gas; methane (dot); methyl hydride

MSDS # : 001033

Date of Preparation/Revision : **4/1/2013.**

In case of emergency : 1-866-734-3438

Section 2. Hazards identification

Physical state : Gas. [COLORLESS GAS; MAY BE A LIQUID UNDER PRESSURE OR REFRIGERATION.]

Emergency overview : WARNING!

GAS:
CONTENTS UNDER PRESURE.
Extremely flammable.
May cause flash fire.
Do not puncture or incinerate container.
Can cause rapid suffocation.
May cause severe frostbite.
LIQUID:
Extremely flammable.
Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.
May cause severe frostbite.

Keep away from heat, sparks and flame. Do not puncture or incinerate container. Use only with adequate ventilation. Keep container closed.

Contact with rapidly expanding gases or liquids can cause frostbite.

Routes of entry : Inhalation

Potential acute health effects

Eyes : Contact with rapidly expanding gas may cause burns or frostbite. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Skin : Contact with rapidly expanding gas may cause burns or frostbite. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Inhalation : Acts as a simple asphyxiant.

Ingestion : Ingestion is not a normal route of exposure for gases. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Medical conditions aggravated by over-exposure : Acute or chronic respiratory conditions may be aggravated by overexposure to this gas.

See toxicological information (Section 11)

Section 3. Composition, Information on Ingredients

Name	CAS number	% Volume	Exposure limits
Methane	74-82-8	100	**ACGIH TLV (United States, 1/2009).** TWA: 1000 ppm 8 hour(s).

Section 4. First aid measures

No action shall be taken involving any personal risk or without suitable training.If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus.It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

Eye contact : Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention immediately.

Skin contact : In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. To avoid the risk of static discharges and gas ignition, soak contaminated clothing thoroughly with water before removing it. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention immediately.

Frostbite : Try to warm up the frozen tissues and seek medical attention.

Inhalation : Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Ingestion : As this product is a gas, refer to the inhalation section.

Section 5. Fire-fighting measures

Flammability of the product : Flammable.

Auto-ignition temperature : 539.85°C (1003.7°F)

Flash point : Closed cup: -188.15°C (-306.7°F).

Flammable limits : Lower: 5% Upper: 15%

Products of combustion : Decomposition products may include the following materials:
carbon dioxide
carbon monoxide

Fire hazards in the presence of various substances : Extremely flammable in the presence of the following materials or conditions: open flames, sparks and static discharge and oxidizing materials.

Fire-fighting media and instructions : In case of fire, use water spray (fog), foam or dry chemical.

In case of fire, allow gas to burn if flow cannot be shut off immediately. Apply water from a safe distance to cool container and protect surrounding area. If involved in fire, shut off flow immediately if it can be done without risk.

Contains gas under pressure. Flammable gas. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion.

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions : Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment (section 8). Shut off gas supply if this can be done safely. Isolate area until gas has dispersed.

Environmental precautions : Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Methods for cleaning up : Immediately contact emergency personnel. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment. Note: see section 1 for emergency contact information and section 13 for waste disposal.

Section 7. Handling and storage

Handling : Use only with adequate ventilation. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. High pressure gas. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Keep container closed. Keep away from heat, sparks and flame. To avoid fire, eliminate ignition sources. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.
Never allow any unprotected part of the body to touch uninsulated pipes or vessels that contain cryogenic liquids. Prevent entrapment of liquid in closed systems or piping without pressure relief devices. Some materials may become brittle at low temperatures and will easily fracture.

Storage : Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame). Segregate from oxidizing materials. Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F).
For additional information concerning storage and handling refer to Compressed Gas Association pamphlets P-1 Safe Handling of Compressed Gases in Containers and P-12 Safe Handling of Cryogenic Liquids available from the Compressed Gas Association, Inc.

Section 8. Exposure controls/personal protection

Engineering controls : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.

When working with cryogenic liquids, wear a full face shield.

Skin : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

The applicable standards are (US) 29 CFR 1910.134 and (Canada) Z94.4-93

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Insulated gloves suitable for low temperatures

Personal protection in case of a large spill : Self-contained breathing apparatus (SCBA) should be used to avoid inhalation of the product.

Product name

methane

ACGIH TLV (United States, 1/2009).
TWA: 1000 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Section 9. Physical and chemical properties

Molecular weight : 16.05 g/mole

Molecular formula : C-H4

Boiling/condensation point : -161.6°C (-258.9°F)

Melting/freezing point : -182.6°C (-296.7°F)

Critical temperature : -82.4°C (-116.3°F)

Vapor density : 0.55 (Air = 1) Liquid Density@BP: 26.5 lb/ft3 (424.5 kg/m3)

Specific Volume (ft ^{3}/lb) : 23.6128

Gas Density (lb/ft ^{3}) : 0.04235

Section 10. Stability and reactivity

Stability and reactivity : The product is stable.

Incompatibility with various substances : Extremely reactive or incompatible with the following materials: oxidizing materials.

Hazardous decomposition products : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Hazardous polymerization : Under normal conditions of storage and use, hazardous polymerization will not occur.

Section 11. Toxicological information

Toxicity data

Other toxic effects on humans : No specific information is available in our database regarding the other toxic effects of this material to humans.

Specific effects

Carcinogenic effects : No known significant effects or critical hazards.

Mutagenic effects : No known significant effects or critical hazards.

Reproduction toxicity : No known significant effects or critical hazards.

Section 12. Ecological information

Aquatic ecotoxicity

Not available.

Products of degradation : Products of degradation: carbon oxides (CO, CO_2) and water.

Environmental fate : Not available.

Environmental hazards : No known significant effects or critical hazards.

Toxicity to the environment : Not available.

Section 13. Disposal considerations

Product removed from the cylinder must be disposed of in accordance with appropriate Federal, State, local regulation.Return cylinders with residual product to Airgas, Inc.Do not dispose of locally.

Section 14. Transport information

Regulatory information	UN number	Proper shipping name	Class	Packing group	Label	Additional information
DOT Classification	UN1971	Methane, compressed or Methane or Natural gas, compressed (with high methane content)(Methane)	2.1	Not applicable (gas).		-
	UN1972	Methane, refrigerated liquid				

TDG Classification	UN1971 UN1972	(Methane)Methane, compressed or Methane or Natural gas, compressed (with high methane content) Methane, refrigerated liquid	2.1	Not applicable (gas).		**Explosive Limit and Limited Quantity Index** 0.125 **ERAP Index** 3000 **Passenger Carrying Ship Index** Forbidden **Passenger Carrying Road or Rail Index** Forbidden
Mexico Classification	UN1971 UN1972	(Methane)Methane, compressed or Methane or Natural gas, compressed (with high methane content) Methane, refrigerated liquid	2.1	Not applicable (gas).		-

"Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product."

Section 15. Regulatory information

United States

U.S. Federal regulations : **United States inventory (TSCA 8b):** This material is listed or exempted.

SARA 302/304/311/312 extremely hazardous substances: No products were found.
SARA 302/304 emergency planning and notification: No products were found.
SARA 302/304/311/312 hazardous chemicals: methane
SARA 311/312 MSDS distribution - chemical inventory - hazard identification: methane: Fire hazard, Sudden release of pressure

Clean Water Act (CWA) 307: No products were found.

Clean Water Act (CWA) 311: No products were found.

Clean Air Act (CAA) 112 regulated flammable substances: methane

Clean Air Act (CAA) 112 regulated toxic substances: No products were found.

State regulations : **Connecticut Carcinogen Reporting:** This material is not listed.
Connecticut Hazardous Material Survey: This material is not listed.
Florida substances: This material is not listed.
Illinois Chemical Safety Act: This material is not listed.
Illinois Toxic Substances Disclosure to Employee Act: This material is not listed.
Louisiana Reporting: This material is not listed.
Louisiana Spill: This material is not listed.
Massachusetts Spill: This material is not listed.
Massachusetts Substances: This material is listed.
Michigan Critical Material: This material is not listed.
Minnesota Hazardous Substances: This material is not listed.
New Jersey Hazardous Substances: This material is listed.

New Jersey Spill: This material is not listed.
New Jersey Toxic Catastrophe Prevention Act: This material is not listed.
New York Acutely Hazardous Substances: This material is not listed.
New York Toxic Chemical Release Reporting: This material is not listed.
Pennsylvania RTK Hazardous Substances: This material is listed.
Rhode Island Hazardous Substances: This material is not listed.

Canada

WHMIS (Canada) : Class A: Compressed gas.
Class B-1: Flammable gas.

CEPA Toxic substances: This material is listed.
Canadian ARET: This material is not listed.
Canadian NPRI: This material is listed.
Alberta Designated Substances: This material is not listed.
Ontario Designated Substances: This material is not listed.
Quebec Designated Substances: This material is not listed.

Section 16. Other information

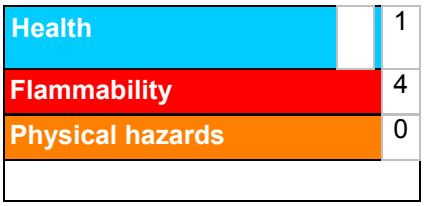
United States

Label requirements : GAS:
CONTENTS UNDER PRESURE.
Extremely flammable.
May cause flash fire.
Do not puncture or incinerate container.
Can cause rapid suffocation.
May cause severe frostbite.
LIQUID:
Extremely flammable.
Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.
May cause severe frostbite.

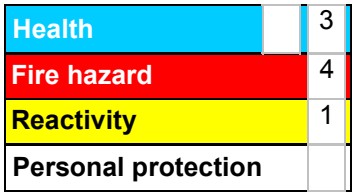
Canada

Label requirements : Class A: Compressed gas.
Class B-1: Flammable gas.

Hazardous Material Information System (U.S.A.) :

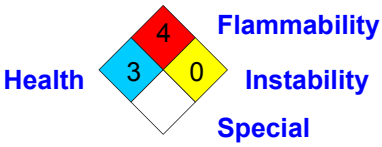


liquid:



National Fire Protection Association (U.S.A.) :

liquid:



Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Material Safety Data Sheet

Propane

Section 1. Chemical product and company identification

Product name : Propane

Supplier : AIRGAS INC., on behalf of its subsidiaries
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

Product use : Synthetic/Analytical chemistry.

Synonym : n-Propane; Dimethylmethane; Freon 290; Liquefied petroleum gas; Lpg; Propyl hydride; R 290; C3H8; UN 1075; UN 1978; A-108; Hydrocarbon propellant.

MSDS # : 001045

Date of Preparation/Revision : **8/19/2013.**

In case of emergency : 1-866-734-3438

Section 2. Hazards identification

Physical state : Gas. [COLORLESS LIQUEFIED COMPRESSED GAS; ODORLESS BUT MAY HAVE SKUNK ODOR ADDED.]

Emergency overview : WARNING!

FLAMMABLE GAS.
MAY CAUSE FLASH FIRE.
MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
CONTENTS UNDER PRESSURE.

Keep away from heat, sparks and flame. Do not puncture or incinerate container. May cause target organ damage, based on animal data. Use only with adequate ventilation. Keep container closed.

Contact with rapidly expanding gases can cause frostbite.

Target organs : May cause damage to the following organs: the nervous system, heart, central nervous system (CNS).

Routes of entry : Inhalation

Potential acute health effects

Eyes : Contact with rapidly expanding gas may cause burns or frostbite.

Skin : Contact with rapidly expanding gas may cause burns or frostbite.

Inhalation : Acts as a simple asphyxiant.

Ingestion : Ingestion is not a normal route of exposure for gases

Potential chronic health effects

Chronic effects : May cause target organ damage, based on animal data.

Target organs : May cause damage to the following organs: the nervous system, heart, central nervous system (CNS).

Medical conditions aggravated by over-exposure : Pre-existing disorders involving any target organs mentioned in this MSDS as being at risk may be aggravated by over-exposure to this product.

See toxicological information (Section 11)

Section 3. Composition, Information on Ingredients

Name	CAS number	% Volume	Exposure limits
Propane	74-98-6	100	**ACGIH TLV (United States, 3/2012).** TWA: 1000 ppm 8 hour(s). **NIOSH REL (United States, 1/2013).** TWA: 1800 mg/m³ 10 hour(s). TWA: 1000 ppm 10 hour(s). **OSHA PEL (United States, 6/2010).** TWA: 1800 mg/m³ 8 hour(s). TWA: 1000 ppm 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** TWA: 1800 mg/m³ 8 hour(s). TWA: 1000 ppm 8 hour(s).

Section 4. First aid measures

No action shall be taken involving any personal risk or without suitable training.If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus.It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

Eye contact : Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention immediately.

Skin contact : In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. To avoid the risk of static discharges and gas ignition, soak contaminated clothing thoroughly with water before removing it. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention immediately.

Frostbite : Try to warm up the frozen tissues and seek medical attention.

Inhalation : Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Ingestion : As this product is a gas, refer to the inhalation section.

Section 5. Fire-fighting measures

Flammability of the product : Flammable.

Auto-ignition temperature : 450°C (842°F)

Flash point : Closed cup: -104°C (-155.2°F). Open cup: -104°C (-155.2°F).

Flammable limits : Lower: 2.1% Upper: 9.5%

Products of combustion : Decomposition products may include the following materials:
carbon dioxide
carbon monoxide

Fire hazards in the presence of various substances : Extremely flammable in the presence of the following materials or conditions: open flames, sparks and static discharge and oxidizing materials.

Fire-fighting media and instructions : In case of fire, use water spray (fog), foam or dry chemical.

In case of fire, allow gas to burn if flow cannot be shut off immediately. Apply water from a safe distance to cool container and protect surrounding area. If involved in fire, shut off flow immediately if it can be done without risk.

Contains gas under pressure. Flammable gas. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion.

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions : Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment (section 8). Shut off gas supply if this can be done safely. Isolate area until gas has dispersed.

Environmental precautions : Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Methods for cleaning up : Immediately contact emergency personnel. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment. Note: see section 1 for emergency contact information and section 13 for waste disposal.

Section 7. Handling and storage

Handling : Use only with adequate ventilation. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. High pressure gas. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Keep container closed. Keep away from heat, sparks and flame. To avoid fire, eliminate ignition sources. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.

Storage : Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame). Segregate from oxidizing materials. Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F).

Section 8. Exposure controls/personal protection

Engineering controls : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.

Skin : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

The applicable standards are (US) 29 CFR 1910.134 and (Canada) Z94.4-93

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Personal protection in case of a large spill : Self-contained breathing apparatus (SCBA) should be used to avoid inhalation of the product.

Product name

propane

ACGIH TLV (United States, 3/2012).
TWA: 1000 ppm 8 hour(s).
NIOSH REL (United States, 1/2013).
TWA: 1800 mg/m³ 10 hour(s).
TWA: 1000 ppm 10 hour(s).
OSHA PEL (United States, 6/2010).
TWA: 1800 mg/m³ 8 hour(s).
TWA: 1000 ppm 8 hour(s).
OSHA PEL 1989 (United States, 3/1989).
TWA: 1800 mg/m³ 8 hour(s).

Propane

TWA: 1000 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Section 9. Physical and chemical properties

Molecular weight : 44.11 g/mole

Molecular formula : C3-H8

Boiling/condensation point : -42°C (-43.6°F)

Melting/freezing point : -189.7°C (-309.5°F)

Critical temperature : 96.6°C (205.9°F)

Vapor pressure : 109 (psig)

Vapor density : 1.6 (Air = 1)

Specific Volume (ft^3/lb) : 8.6206

Gas Density (lb/ft^3) : 0.116

Section 10. Stability and reactivity

Stability and reactivity : The product is stable.

Incompatibility with various substances : Extremely reactive or incompatible with the following materials: oxidizing materials.

Hazardous decomposition products : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Hazardous polymerization : Under normal conditions of storage and use, hazardous polymerization will not occur.

Section 11. Toxicological information

Toxicity data

Product/ingredient name	Result	Species	Dose	Exposure
propane	LC50 Inhalation Gas.	Rat	>800000 ppm	15 minutes

IDLH : 2100 ppm

Chronic effects on humans : May cause damage to the following organs: the nervous system, heart, central nervous system (CNS).

Other toxic effects on humans : No specific information is available in our database regarding the other toxic effects of this material to humans.

Specific effects

Carcinogenic effects : No known significant effects or critical hazards.

Mutagenic effects : No known significant effects or critical hazards.

Reproduction toxicity : No known significant effects or critical hazards.

Section 12. Ecological information

Aquatic ecotoxicity

Not available.

Products of degradation : Products of degradation: carbon oxides (CO, CO_2) and water.

Environmental fate : Not available.

Environmental hazards : This product shows a low bioaccumulation potential.

Toxicity to the environment : Not available.

Section 13. Disposal considerations

Product removed from the cylinder must be disposed of in accordance with appropriate Federal, State, local regulation.Return cylinders with residual product to Airgas, Inc.Do not dispose of locally.

Section 14. Transport information

Regulatory information	UN number	Proper shipping name	Class	Packing group	Label	Additional information
DOT Classification	UN1978	PROPANE	2.1	Not applicable (gas).		**Limited quantity** Yes. **Packaging instruction** **Passenger aircraft** Quantity limitation: Forbidden. **Cargo aircraft** Quantity limitation: 150 kg **Special provisions** 19, T50
TDG Classification	UN1978	PROPANE	2.1	Not applicable (gas).		**Explosive Limit and Limited Quantity Index** 0.125 **ERAP Index** 3000 **Passenger Carrying Ship Index** 65 **Passenger Carrying Road or Rail Index** Forbidden **Special provisions** 29, 42
Mexico Classification	UN1978	PROPANE	2.1	Not applicable (gas).		-

"Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product."

Section 15. Regulatory information

United States

U.S. Federal regulations :

TSCA 8(a) IUR: Not determined
United States inventory (TSCA 8b): This material is listed or exempted.

SARA 302/304/311/312 extremely hazardous substances: No products were found.
SARA 302/304 emergency planning and notification: No products were found.
SARA 302/304/311/312 hazardous chemicals: propane
SARA 311/312 MSDS distribution - chemical inventory - hazard identification: propane: Fire hazard, Sudden release of pressure

Clean Air Act (CAA) 112 accidental release prevention - Flammable Substances: Propane

Clean Air Act (CAA) 112 regulated flammable substances: propane

State regulations :

Connecticut Carcinogen Reporting: This material is not listed.
Connecticut Hazardous Material Survey: This material is not listed.
Florida substances: This material is not listed.
Illinois Chemical Safety Act: This material is not listed.
Illinois Toxic Substances Disclosure to Employee Act: This material is not listed.
Louisiana Reporting: This material is not listed.
Louisiana Spill: This material is not listed.
Massachusetts Spill: This material is not listed.
Massachusetts Substances: This material is listed.
Michigan Critical Material: This material is not listed.
Minnesota Hazardous Substances: This material is not listed.
New Jersey Hazardous Substances: This material is listed.
New Jersey Spill: This material is not listed.
New Jersey Toxic Catastrophe Prevention Act: This material is not listed.
New York Acutely Hazardous Substances: This material is not listed.
New York Toxic Chemical Release Reporting: This material is not listed.
Pennsylvania RTK Hazardous Substances: This material is listed.
Rhode Island Hazardous Substances: This material is not listed.

Canada

WHMIS (Canada) :

Class A: Compressed gas.
Class B-1: Flammable gas.

CEPA Toxic substances: This material is not listed.
Canadian ARET: This material is not listed.
Canadian NPRI: This material is listed.
Alberta Designated Substances: This material is not listed.
Ontario Designated Substances: This material is not listed.
Quebec Designated Substances: This material is not listed.

Section 16. Other information

United States

Label requirements :

FLAMMABLE GAS.
MAY CAUSE FLASH FIRE.
MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
CONTENTS UNDER PRESSURE.

Canada

Label requirements :

Class A: Compressed gas.
Class B-1: Flammable gas.

Hazardous Material Information System (U.S.A.) :

Health	*	1
Flammability		4
Physical hazards		2

National Fire Protection Association (U.S.A.) :

Notice to reader

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Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Material Safety Data Sheet

Ethane

Section 1. Chemical product and company identification

Product name : Ethane

Supplier : AIRGAS INC., on behalf of its subsidiaries
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

Product use : Synthetic/Analytical chemistry.

Synonym : Bimethyl; Dimethyl; Ethyl hydride; Methylmethane; C2H6; UN 1035; UN 1961, R170

MSDS # : 001024

Date of Preparation/Revision : **10/23/2012.**

In case of emergency : 1-866-734-3438

Section 2. Hazards identification

Physical state : Gas. [Compressed gas.]

Emergency overview : WARNING!

GAS:
CONTENTS UNDER PRESURE.
Extremely flammable.
May cause flash fire.
Do not puncture or incinerate container.
Can cause rapid suffocation.
May cause severe frostbite.
LIQUID:
Extremely flammable.
Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.
May cause severe frostbite.

Keep away from heat, sparks and flame. Do not puncture or incinerate container. May cause target organ damage, based on animal data. Use only with adequate ventilation. Keep container closed.

Contact with rapidly expanding gases or liquids can cause frostbite.

Target organs : May cause damage to the following organs: heart, central nervous system (CNS).

Routes of entry : Inhalation

Potential acute health effects

Eyes : Contact with rapidly expanding gas may cause burns or frostbite. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Skin : Contact with rapidly expanding gas may cause burns or frostbite. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Inhalation : Acts as a simple asphyxiant.

Ingestion : Ingestion is not a normal route of exposure for gases. Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Potential chronic health effects

Chronic effects : May cause target organ damage, based on animal data.

Target organs : May cause damage to the following organs: heart, central nervous system (CNS).

Medical conditions aggravated by over-exposure : Pre-existing disorders involving any target organs mentioned in this MSDS as being at risk may be aggravated by over-exposure to this product.

See toxicological information (Section 11)

Section 3. Composition, Information on Ingredients

Name	CAS number	% Volume	Exposure limits
Ethane	74-84-0	100	**ACGIH TLV (United States, 2/2010).** TWA: 1000 ppm 8 hour(s).

Section 4. First aid measures

No action shall be taken involving any personal risk or without suitable training.If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus.It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

Eye contact : Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention immediately.

Skin contact : In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. To avoid the risk of static discharges and gas ignition, soak contaminated clothing thoroughly with water before removing it. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention immediately.

Frostbite : Try to warm up the frozen tissues and seek medical attention.

Inhalation : Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Ingestion : As this product is a gas, refer to the inhalation section.

Section 5. Fire-fighting measures

Flammability of the product : Flammable.

Auto-ignition temperature : 472°C (881.6°F)

Flash point : Closed cup: -135.15°C (-211.3°F).

Flammable limits : Lower: 3% Upper: 12.5%

Products of combustion : Decomposition products may include the following materials:
carbon dioxide
carbon monoxide

Fire hazards in the presence of various substances : Extremely flammable in the presence of the following materials or conditions: oxidizing materials.

Fire-fighting media and instructions : In case of fire, use water spray (fog), foam or dry chemical.

In case of fire, allow gas to burn if flow cannot be shut off immediately. Apply water from a safe distance to cool container and protect surrounding area. If involved in fire, shut off flow immediately if it can be done without risk.

Contains gas under pressure. Flammable gas. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion.

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions : Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment (section 8). Shut off gas supply if this can be done safely. Isolate area until gas has dispersed.

Environmental precautions : Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Methods for cleaning up : Immediately contact emergency personnel. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment. Note: see section 1 for emergency contact information and section 13 for waste disposal.

Section 7. Handling and storage

Handling : Use only with adequate ventilation. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. High pressure gas. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Keep container closed. Keep away from heat, sparks and flame. To avoid fire, eliminate ignition sources. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.
Never allow any unprotected part of the body to touch uninsulated pipes or vessels that contain cryogenic liquids. Prevent entrapment of liquid in closed systems or piping without pressure relief devices. Some materials may become brittle at low temperatures and will easily fracture.

Storage : Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame). Segregate from oxidizing materials. Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F).
For additional information concerning storage and handling refer to Compressed Gas Association pamphlets P-1 Safe Handling of Compressed Gases in Containers and P-12 Safe Handling of Cryogenic Liquids available from the Compressed Gas Association, Inc.

Section 8. Exposure controls/personal protection

Engineering controls : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.

When working with cryogenic liquids, wear a full face shield.

Skin : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

The applicable standards are (US) 29 CFR 1910.134 and (Canada) Z94.4-93

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Insulated gloves suitable for low temperatures

Personal protection in case of a large spill : Self-contained breathing apparatus (SCBA) should be used to avoid inhalation of the product.

Product name

ethane

ACGIH TLV (United States, 2/2010).
TWA: 1000 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Section 9. Physical and chemical properties

Molecular weight : 30.08 g/mole

Molecular formula : C2-H6

Boiling/condensation point : -89°C (-128.2°F)

Melting/freezing point : -183°C (-297.4°F)

Critical temperature : 32.4°C (90.3°F)

Vapor pressure : 543 (psig)

Vapor density : 1.1 (Air = 1) Liquid Density: BP@34.1 lb/ft3 (546 kg/m3)

Specific Volume (ft ^{3}/lb) : 12.6582

Gas Density (lb/ft ^{3}) : 0.079

Section 10. Stability and reactivity

Stability and reactivity : The product is stable.

Incompatibility with various substances : Extremely reactive or incompatible with the following materials: oxidizing materials.

Hazardous decomposition products : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Hazardous polymerization : Under normal conditions of storage and use, hazardous polymerization will not occur.

Section 11. Toxicological information

Toxicity data

Chronic effects on humans : May cause damage to the following organs: heart, central nervous system (CNS).

Other toxic effects on humans : No specific information is available in our database regarding the other toxic effects of this material to humans.

Specific effects

Carcinogenic effects : No known significant effects or critical hazards.

Mutagenic effects : No known significant effects or critical hazards.

Reproduction toxicity : No known significant effects or critical hazards.

Section 12. Ecological information

Aquatic ecotoxicity

Not available.

Products of degradation : Products of degradation: carbon oxides (CO, CO_2) and water.

Environmental fate : Not available.

Environmental hazards : This product shows a low bioaccumulation potential.

Toxicity to the environment : Not available.

Section 13. Disposal considerations

Product removed from the cylinder must be disposed of in accordance with appropriate Federal, State, local regulation.Return cylinders with residual product to Airgas, Inc.Do not dispose of locally.

Section 14. Transport information

Regulatory information	UN number	Proper shipping name	Class	Packing group	Label	Additional information

Ethane						
DOT Classification	UN1035 UN1961	ETHANE Ethane, refrigerated liquid	2.1	Not applicable (gas).		**Limited quantity** Yes. **Packaging instruction** **Passenger aircraft** Quantity limitation: Forbidden. **Cargo aircraft** Quantity limitation: 150 kg
TDG Classification	UN1035 UN1961	ETHANE Ethane, refrigerated liquid	2.1	Not applicable (gas).		**Explosive Limit and Limited Quantity Index** 0.125 **ERAP Index** 3000 **Passenger Carrying Ship Index** Forbidden **Passenger Carrying Road or Rail Index** Forbidden
Mexico Classification	UN1035 UN1961	ETHANE Ethane, refrigerated liquid	2.1	Not applicable (gas).		-

"Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product."

Section 15. Regulatory information

United States

U.S. Federal regulations : **TSCA 8(a) IUR**: Not determined
United States inventory (TSCA 8b): This material is listed or exempted.

SARA 302/304/311/312 extremely hazardous substances: No products were found.
SARA 302/304 emergency planning and notification: No products were found.
SARA 302/304/311/312 hazardous chemicals: ethane
SARA 311/312 MSDS distribution - chemical inventory - hazard identification: ethane: Fire hazard, Sudden release of pressure, Immediate (acute) health hazard

Clean Air Act (CAA) 112 accidental release prevention - Flammable Substances: Ethane

Clean Air Act (CAA) 112 regulated flammable substances: ethane

State regulations :

Connecticut Carcinogen Reporting: This material is not listed.
Connecticut Hazardous Material Survey: This material is not listed.
Florida substances: This material is not listed.
Illinois Chemical Safety Act: This material is not listed.
Illinois Toxic Substances Disclosure to Employee Act: This material is not listed.
Louisiana Reporting: This material is not listed.
Louisiana Spill: This material is not listed.
Massachusetts Spill: This material is not listed.
Massachusetts Substances: This material is listed.
Michigan Critical Material: This material is not listed.
Minnesota Hazardous Substances: This material is not listed.
New Jersey Hazardous Substances: This material is listed.
New Jersey Spill: This material is not listed.
New Jersey Toxic Catastrophe Prevention Act: This material is not listed.
New York Acutely Hazardous Substances: This material is not listed.
New York Toxic Chemical Release Reporting: This material is not listed.
Pennsylvania RTK Hazardous Substances: This material is listed.
Rhode Island Hazardous Substances: This material is not listed.

Canada

WHMIS (Canada) :

Class A: Compressed gas.
Class B-1: Flammable gas.

CEPA Toxic substances: This material is listed.
Canadian ARET: This material is not listed.
Canadian NPRI: This material is listed.
Alberta Designated Substances: This material is not listed.
Ontario Designated Substances: This material is not listed.
Quebec Designated Substances: This material is not listed.

Section 16. Other information

United States

Label requirements :

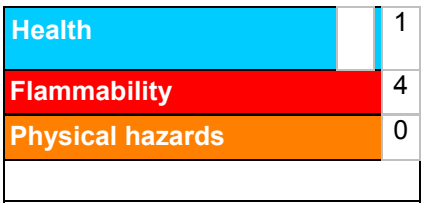
GAS:
CONTENTS UNDER PRESURE.
Extremely flammable.
May cause flash fire.
Do not puncture or incinerate container.
Can cause rapid suffocation.
May cause severe frostbite.
LIQUID:
Extremely flammable.
Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.
May cause severe frostbite.

Canada

Label requirements :

Class A: Compressed gas.
Class B-1: Flammable gas.

Hazardous Material Information System (U.S.A.) :

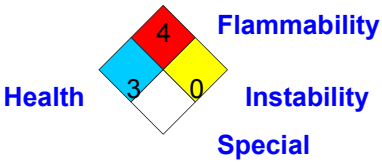


liquid:

Fire hazard	4
Reactivity	0
Personal protection	

National Fire Protection Association (U.S.A.) :

liquid:



Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Material Safety Data Sheet

Ethylene

Section 1. Chemical product and company identification

Product name : Ethylene

Supplier : AIRGAS INC., on behalf of its subsidiaries
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

Product use : Synthetic/Analytical chemistry.

Synonym : ACETENE ;ATHYLEN (GERMAN) ; BICARBURRETTED HYDROGEN ; ELAYL ; ETHENE ; ETHYLENE (ACGIH, DOT, OSHA) ; ETHYLENE, REFRIGERATED LIQUID (CRYOGENIC LIQUID) (UN1038) (DOT) ; LIQUID ETHYLENE ; OLEFIANT GAS UN1038 (DOT) ; UN1962 (DOT)

MSDS # : 001022

Date of Preparation/Revision : **5/11/2011.**

In case of emergency : 1-866-734-3438

Section 2. Hazards identification

Physical state : Gas or Liquid.

Emergency overview : WARNING!

GAS:
CONTENTS UNDER PRESURE.
Extremely flammable
Do not puncture or incinerate container.
Can cause rapid suffocation.
May cause severe frostbite.
LIQUID:
Extremely flammable
Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.
May cause severe frostbite.

Keep away from heat, sparks and flame. Do not puncture or incinerate container. May cause target organ damage, based on animal data. Use only with adequate ventilation. Keep container closed.

Contact with rapidly expanding gases can cause frostbite.

Target organs : May cause damage to the following organs: lungs, heart, muscle tissue.

Routes of entry : Inhalation

Potential acute health effects

Eyes : Contact with rapidly expanding gas may cause burns or frostbite.Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Skin : Contact with rapidly expanding gas may cause burns or frostbite.Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Inhalation : Acts as a simple asphyxiant.

Ingestion : Ingestion is not a normal route of exposure for gases.Contact with cryogenic liquid can cause frostbite and cryogenic burns.

Potential chronic health effects

Chronic effects : May cause target organ damage, based on animal data.

Target organs : May cause damage to the following organs: lungs, heart, muscle tissue.

Medical conditions aggravated by over-exposure : Pre-existing disorders involving any target organs mentioned in this MSDS as being at risk may be aggravated by over-exposure to this product.

See toxicological information (Section 11)

Section 3. Composition, Information on Ingredients

Name	CAS number	% Volume	Exposure limits
Ethylene	74-85-1	100	**ACGIH TLV (United States, 2/2010).** TWA: 200 ppm 8 hour(s).

Section 4. First aid measures

No action shall be taken involving any personal risk or without suitable training.If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus.It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

Eye contact : Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention immediately.

Skin contact : In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. To avoid the risk of static discharges and gas ignition, soak contaminated clothing thoroughly with water before removing it. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention immediately.

Frostbite : Try to warm up the frozen tissues and seek medical attention.

Inhalation : Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Ingestion : As this product is a gas, refer to the inhalation section.

Section 5. Fire-fighting measures

Flammability of the product : Flammable.

Auto-ignition temperature : 490°C (914°F)

Flash point : Closed cup: -135.85°C (-212.5°F).

Flammable limits : Lower: 2.7% Upper: 36%

Products of combustion : Decomposition products may include the following materials:
carbon dioxide
carbon monoxide

Fire hazards in the presence of various substances : Extremely flammable in the presence of the following materials or conditions: oxidizing materials.

Fire-fighting media and instructions : In case of fire, use water spray (fog), foam or dry chemical.

In case of fire, allow gas to burn if flow cannot be shut off immediately. Apply water from a safe distance to cool container and protect surrounding area. If involved in fire, shut off flow immediately if it can be done without risk.

Contains gas under pressure. Flammable gas. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion.

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions : Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment (section 8). Shut off gas supply if this can be done safely. Isolate area until gas has dispersed.

Environmental precautions : Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Methods for cleaning up : Immediately contact emergency personnel. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment. Note: see section 1 for emergency contact information and section 13 for waste disposal.

Section 7. Handling and storage

Handling : Use only with adequate ventilation. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. High pressure gas. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Keep container closed. Keep away from heat, sparks and flame. To avoid fire, eliminate ignition sources. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.

Storage : Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame). Segregate from oxidizing materials. Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F).

Section 8. Exposure controls/personal protection

Engineering controls : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.

Skin : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

The applicable standards are (US) 29 CFR 1910.134 and (Canada) Z94.4-93

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Personal protection in case of a large spill : Self-contained breathing apparatus (SCBA) should be used to avoid inhalation of the product.

Product name

Ethylene

ACGIH TLV (United States, 2/2010).
TWA: 200 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Section 9. Physical and chemical properties

Molecular weight : 28.06 g/mole

Molecular formula : C2-H4

Boiling/condensation point : -104°C (-155.2°F)

Melting/freezing point : -169.2°C (-272.6°F)

Critical temperature : 10°C (50°F)

Vapor density : 1 (Air = 1) Liquid Density@BP: 35.3 lb/ft3 (566 kg/m3)

Specific Volume (ft^3/lb) : 13.8007

Gas Density (lb/ft^3) : 0.07246

Section 10. Stability and reactivity

Stability and reactivity : The product is stable.

Incompatibility with various substances : Extremely reactive or incompatible with the following materials: oxidizing materials.

Hazardous decomposition products : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Hazardous polymerization : Under normal conditions of storage and use, hazardous polymerization will not occur.

Section 11. Toxicological information

Toxicity data

Chronic effects on humans : **CARCINOGENIC EFFECTS**: A4 (Not classifiable for humans or animals.) by ACGIH, 3 (Not classifiable for humans.) by IARC.
May cause damage to the following organs: lungs, heart, muscle tissue.

Other toxic effects on humans : No specific information is available in our database regarding the other toxic effects of this material to humans.

Specific effects

Carcinogenic effects : No known significant effects or critical hazards.

Mutagenic effects : No known significant effects or critical hazards.

Reproduction toxicity : No known significant effects or critical hazards.

Section 12. Ecological information

Aquatic ecotoxicity

Not available.

Products of degradation : Products of degradation: carbon oxides (CO, CO_2) and water.

Environmental fate : Not available.

Environmental hazards : No known significant effects or critical hazards.

Toxicity to the environment : Not available.

Section 13. Disposal considerations

Product removed from the cylinder must be disposed of in accordance with appropriate Federal, State, local regulation.Return cylinders with residual product to Airgas, Inc.Do not dispose of locally.

Section 14. Transport information

Regulatory information	UN number	Proper shipping name	Class	Packing group	Label	Additional information
DOT Classification	UN1962	ETHYLENE, COMPRESSED	2.1	Not applicable (gas).		**Limited quantity** Yes. **Packaging instruction** **Passenger aircraft** Quantity limitation: Forbidden. **Cargo aircraft** Quantity limitation: Forbidden. **Special provisions**
	UN1038	ETHYLENE, REFRIGERATED LIQUID	2.1			

						T75, TP5
TDG Classification	UN1962 UN1038	ETHYLENE, COMPRESSED ETHYLENE, REFRIGERATED LIQUID	2.1 2.1	Not applicable (gas).		**Explosive Limit and Limited Quantity Index** 0.125 **ERAP Index** 3000 **Passenger Carrying Ship Index** Forbidden **Passenger Carrying Road or Rail Index** Forbidden
Mexico Classification	UN1962 UN1038	ETHYLENE, COMPRESSED ETHYLENE, REFRIGERATED LIQUID	2.1 2.1	Not applicable (gas).		-

"Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product."

Section 15. Regulatory information

United States

U.S. Federal regulations : **TSCA 8(a) IUR**: Partial exemption
United States inventory (TSCA 8b): This material is listed or exempted.

SARA 302/304/311/312 extremely hazardous substances: No products were found.
SARA 302/304 emergency planning and notification: No products were found.
SARA 302/304/311/312 hazardous chemicals: Ethylene
SARA 311/312 MSDS distribution - chemical inventory - hazard identification: Ethylene: Fire hazard, reactive, Sudden release of pressure, Delayed (chronic) health hazard

Clean Air Act (CAA) 112 accidental release prevention - Flammable Substances: Ethylene

Clean Air Act (CAA) 112 regulated flammable substances: Ethylene

SARA 313

		Product name	CAS number	Concentration
Form R - Reporting requirements	:	Ethylene	74-85-1	100
Supplier notification	:	Ethylene	74-85-1	100

SARA 313 notifications must not be detached from the MSDS and any copying and redistribution of the MSDS shall include copying and redistribution of the notice attached to copies of the MSDS subsequently redistributed.

State regulations : **Connecticut Carcinogen Reporting**: This material is not listed.
Connecticut Hazardous Material Survey: This material is not listed.
Florida substances: This material is not listed.
Illinois Chemical Safety Act: This material is not listed.
Illinois Toxic Substances Disclosure to Employee Act: This material is not listed.
Louisiana Reporting: This material is not listed.
Louisiana Spill: This material is not listed.
Massachusetts Spill: This material is not listed.
Massachusetts Substances: This material is listed.
Michigan Critical Material: This material is not listed.
Minnesota Hazardous Substances: This material is not listed.
New Jersey Hazardous Substances: This material is listed.
New Jersey Spill: This material is not listed.
New Jersey Toxic Catastrophe Prevention Act: This material is not listed.
New York Acutely Hazardous Substances: This material is not listed.
New York Toxic Chemical Release Reporting: This material is not listed.
Pennsylvania RTK Hazardous Substances: This material is listed.
Rhode Island Hazardous Substances: This material is not listed.

Canada

WHMIS (Canada) : Class A: Compressed gas.
Class B-1: Flammable gas.
Class D-2B: Material causing other toxic effects (Toxic).

CEPA Toxic substances: This material is not listed.
Canadian ARET: This material is not listed.
Canadian NPRI: This material is listed.
Alberta Designated Substances: This material is not listed.
Ontario Designated Substances: This material is not listed.
Quebec Designated Substances: This material is not listed.

Section 16. Other information

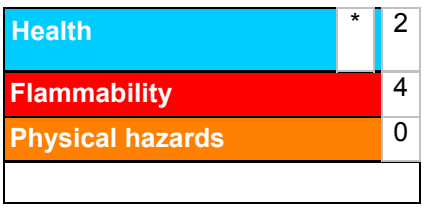
United States

Label requirements : GAS:
CONTENTS UNDER PRESURE.
Extremely flammable
Do not puncture or incinerate container.
Can cause rapid suffocation.
May cause severe frostbite.
LIQUID:
Extremely flammable
Extremely cold liquid and gas under pressure.
Can cause rapid suffocation.
May cause severe frostbite.

Canada

Label requirements : Class A: Compressed gas.
Class B-1: Flammable gas.
Class D-2B: Material causing other toxic effects (Toxic).

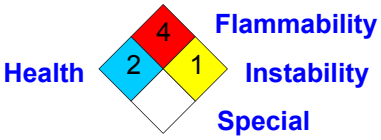
Hazardous Material Information System (U.S.A.) :



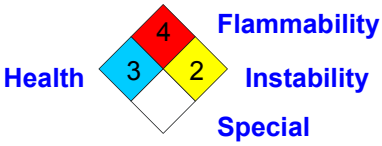
liquid:

Fire hazard	4
Reactivity	1
Personal protection	

National Fire Protection Association (U.S.A.) :



liquid:



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Ground Water Monitoring&Remediation

Oxygen and Ethene Biostimulation for a Persistent Dilute Vinyl Chloride Plume

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Abstract

Contamination of groundwater with chlorinated ethenes is common and represents a threat to drinking water sources. Standard anaerobic bioremediation methods for the highly chlorinated ethenes PCE and TCE are not always effective in promoting complete degradation. In these cases, the target contaminants are degraded to the daughter products DCE and/or vinyl chloride. This creates an additional health risk, as vinyl chloride is even more toxic and carcinogenic than its precursors. New treatment modalities are needed to deal with this widespread environmental problem. We describe successful bioremediation of a large, migrating, dilute vinyl chloride plume in Massachusetts with an aerobic biostimulation treatment approach utilizing both oxygen and ethene. Initial microcosm studies showed that adding ethene under aerobic conditions stimulated the rapid degradation of VC in site groundwater. Deployment of a full-scale treatment system resulted in plume migration cutoff and nearly complete elimination of above-standard VC concentrations.

Introduction

The chlorinated ethene vinyl chloride (VC) is a toxin and human carcinogen (EPA 2000) that has been found at over 37% of National Priority List sites tested by the US Environmental Protection Agency as of 2003 (ATSDR 2006). It is also highly mobile in groundwater, thus posing a threat to drinking water supplies and complicating remediation efforts. VC often persists in groundwater after direct contamination of a site with VC-containing wastes or following in situ generation of VC as a daughter product of the anaerobic degradation of more highly chlorinated ethenes such as tetrachloroethene (PCE), trichloroethene (TCE), and dichloroethene (DCE) (Smith and Dragun 1984; Kielhorn et al. 2000).

A variety of bacteria can attenuate chlorinated ethenes anaerobically using these contaminants as terminal electron acceptors for anaerobic respiration, but only *Dehalococcoides ethogenes* has been shown to completely dehalogenate PCE and TCE to ethene, (Holliger et al. 1993; Maymo-Gatell et al. 1999). At sites contaminated with chlorinated ethenes, the design of remediation systems, generally based on these anaerobic pathways, may involve biostimulation with electron donors and/or bioaugmentation with cultures of anaerobic dehalogenating bacteria.

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However, such remediation strategies may not be effective at sites with high concentrations of competing electron acceptors, low concentrations of VC, or conditions that are otherwise not conducive to maintaining the growth and complete dehalogenation activity of *D. ethogenes* (EPA 2000; OPPTD 2006; ITRC 2008). This may lead to a condition often referred to by remediation practitioners as stall. In VC stall, the more highly chlorinated ethenes (PCE, TCE, and DCE) are degraded to VC, but VC reduction slows and concentrations stabilize at levels above the regulatory limit for drinking water sources. This is due to the thermodynamics of reductive dehalogenation (Cupples et al. 2004). Consequently, while one environmental problem has been solved, another one has been created: generation and persistence of the more toxic and carcinogenic daughter product—VC.

Bioremediation strategies that utilize aerobic bacteria may afford a solution to this problem. A variety of bacteria are able to cometabolically degrade VC under aerobic conditions using a wide range of substrates for growth, including alkanes, alkenes, and aromatic hydrocarbons (reviewed in Arp et al. 2001 and Mattes et al. 2010). Even more promising than cometabolism, certain bacteria have been shown to directly degrade VC, using it as a growth substrate (Hartmans and De Bont 1992; Verce et al. 2000; Verce et al. 2001; Coleman and Spain 2003; Singh et al. 2004; Mattes et al. 2005; Jin and Mattes 2008). These bacteria, called ethene-assimilators or ethenotrophs, which can directly metabolize VC through their catabolic pathway for ethene, have been isolated from numerous VC-contaminated

sites (Hartmans and De Bont 1992; Verce et al. 2000, 2001; Mattes et al. 2005; Hartmans et al. 1985; Coleman et al. 2002; Danko et al. 2004). Isolates that have been described thus far belong to several different phyla, suggesting the possibility that this metabolic activity may be widespread.

Here we describe the implementation of a full-scale biostimulation treatment for a large dilute groundwater plume with persistent low levels of VC. Oxygen was added to provide aerobic conditions for VC oxidation and ethene was added to stimulate the growth of ethenotrophic bacteria in order to maximize direct aerobic VC degradation. In addition to this direct aerobic pathway where VC serves as a carbon and energy source for bacterial growth, addition of ethene as well as oxygen to groundwater may also stimulate cometabolic degradation of VC (Freedman and Herz 1996; Freedman et al. 2001).

The remediation project described here was implemented in response to a release of PCE at a landfill resulting in a dilute groundwater plume with persistent low levels of VC (2 to 27 μg/L). The PCE leached into groundwater along with unresolved dissolved organic matter that contained electron donors, resulting in anaerobic conditions and reductive dechlorination of PCE to VC. However, there were also significant concentrations of competing electron acceptors for anaerobic respiration and conditions less favorable to anaerobic dechlorination downgradient of the landfill. The plume migrated away from the source and detached following landfill capping. Risk management actions at the site included the implementation of in situ aerobic biobarriers designed to deliver a constant supply of oxygen with initial periodic pulsing of ethene to stimulate the growth of aerobic ethenotrophs in the groundwater. This treatment option was chosen in part because initial VC concentrations were too low to stimulate efficient aerobic degradation using oxygen alone (Verce et al. 2001; Coleman et al. 2002).

Dissolved oxygen and ethene were delivered to groundwater primarily using in situ mass transfer devices deployed in treatment wells arranged in lines in order to cut off plume migration. We used biochemical, genetic, and microbiological tools to establish feasibility and to monitor treatment progress. Together with field data showing diminution of VC concentrations in site groundwater, these results suggest that aerobic biostimulation can provide an efficient remediation strategy for low-level VC plumes where complete anaerobic treatment is not feasible.

Materials and Methods

Site Description

The study site was contaminated by disposal of material containing PCE at a demolition debris landfill in 1986. PCE and other dissolved organic compounds leached into the groundwater where reducing conditions resulted in incomplete reductive dechlorination of PCE to VC. This gave rise to a migrating dilute VC plume in the groundwater.

When the site was assessed in 2002, there was a detached plume of VC at concentrations >2 μg/L extending over 3000 feet. The plume was approximately >400 feet wide and 30 feet thick and located 50 feet below the water table in the downgradient area. The maximum VC concentration measured was 27 μg/L, which is >13 times the applicable Massachusetts standard of 2 μg/L.

The VC plume was found to be migrating at the base of a glacial outwash aquifer in fine sand with silt that includes lenses of coarse sand, gravel, silt and clay. The VC was expected to continue migrating at close to groundwater velocity, which was estimated at 0.5 ft/d.

The groundwater within the core of the plume was characterized by elevated dissolved organic carbon (up to 30 mg/L), sulfate (up to 180 mg/L), dissolved iron (up to 16 mg/L), and manganese (up to 1 mg/L). The initial concentration of methane was around 1 mg/L and ethene was approximately 0.001 mg/L. Mildly reducing conditions were observed in the plume area where dissolved oxygen and oxidation-reduction potential were initially around 0.5 mg/L and −50 mV, respectively. Consequently the methanogenic conditions necessary for reductive dechlorination of VC to ethene were unlikely to be achievable.

Treatment System Design

Baseline site assessment was conducted in 2002 and field treatment pilots, adding oxygen to the aquifer, began in 2003. Microcosm studies evaluating ethene addition were completed in 2004. Because the half-saturation (K_S) constants for direct aerobic metabolism (Verce et al. 2001; Coleman et al. 2002) and aerobic cometabolism of VC (Chang and Alvarez-Cohen 1996), 62 and 56 μg/L, respectively, are higher than the VC concentrations throughout the plume and more than 30-fold higher than the lowest VC concentrations of concern, additional substrate was deemed necessary to accelerate degradation to achieve remediation goals.

Because ethene is assimilated by the same pathway as VC (Hartmans and De Bont 1992; Verce et al. 2000; Coleman et al. 2002; Coleman and Spain 2003), ethene addition was incorporated into the treatment design to overcome the substrate limitation problem and accelerate degradation. In addition to stimulating the growth of ethenotrophs capable of using ethene as a carbon and energy source (i.e., direct metabolism), ethene can also stimulate aerobic cometabolism of chlorinated ethenes, (Freedman and Herz 1996; Freedman et al. 2001), leading to fortuitous VC degradation.

The pilot scale treatment tested two oxygen delivery systems: bio-sparging and in situ mass transfer of pure oxygen. The oxygen delivery via iSOC in situ gas infusion technology (InVentures Technologies, Fredericton, Canada) was selected for full-scale treatment. This technology provided the flexibility to deliver ethene as well as oxygen and was less likely to be negatively impacted by the presence of impermeable clay layers at the site. The treatment system was designed to promote plume cut-off by stimulating the growth of aerobic, ethenotrophic bacteria at treatment lines. The initial treatment schedule alternated gas supplies with one week of ethene delivery followed by one month of oxygen delivery.

The full-scale treatment system was comprised of the original pilot bio-sparging line plus six iSOC treatment lines. Gas cylinders supplied oxygen and a dilute mixture of ethene and nitrogen gas and no power was required to operate the iSOC systems. The iSOC treatment lines included

four cut-off lines, with treatment wells set across the entire width of the plume, and two shorter lines focused on the plume core. Treatment wells were spaced a distance of 20 to 25 feet cross-gradient and screened along the full 30 feet thickness of the plume (see Figure 1).

Groundwater Sampling and Chemical Analysis

Periodic groundwater monitoring events were used to assess and optimize treatment system performance. The monitoring program included, at minimum, semiannual measurements of field parameters including dissolved oxygen (DO), oxidation-reduction potential (ORP), pH, temperature, specific conductance, and laboratory analyses for volatile organic carbon (VOC), inorganics, and bacteria. Selected wells in the immediate vicinity of the treatment systems were monitored on a more frequent basis to optimize treatment efficiency.

Groundwater samples were obtained using low-flow sampling (EPA 1996) and transported in sterile bottles on ice to commercial laboratories for analysis. All containers, preservatives, and shipping requirements were consistent with Massachusetts DEP Standard References. Appropriate quality control samples were collected and trip blanks accompanied the project samples. Groundwater laboratory test data were reviewed for compliance with the Compendium of Quality Assurance and Quality Control Requirements in accordance with the Massachusetts Contingency Plan.

Microcosm Studies

Equal volumes of groundwater were combined from six monitoring wells and 100 mL of this mixed groundwater sample were added to each of six 160-mL serum bottles, which were sealed with Teflon-lined butyl rubber septa affixed with crimped caps. Amendments were prepared and added to microcosms by syringe from stock solutions or pure compressed gas cylinders. All microcosms were amended with VC to 29 ppb. Ethene gas (Scott Gas) and/or mineral salts medium (2.2 mM K_2HPO_4, 1.7 mM KH_2PO_4, and 0.4 mM KNO_3 plus trace minerals) were added to microcosms as indicated. One milliliter of 100% O_2 was added to all microcosms on day 1. Microcosm bottles were wrapped in aluminum foil and slowly shaken in a rotary shaker. Dissolved gases and VC in the microcosms were analyzed according to EPA Method 5021A.

Biofilm Assays

Biofilm formation was measured by a modified crystal violet dye-binding assay (O'Toole and Kolter 1998). Briefly, 1 mL samples of groundwater were incubated at room temperature with aeration in 24-well microtiter plates for 1 to 2 weeks. No media or supplements were added. Following incubation, the groundwater was decanted and the wells were washed with sterile distilled water. One milliliter of crystal violet was added to each well and allowed to bind for 5 min. The crystal violet was decanted and the wells were washed three times with water. The bound dye

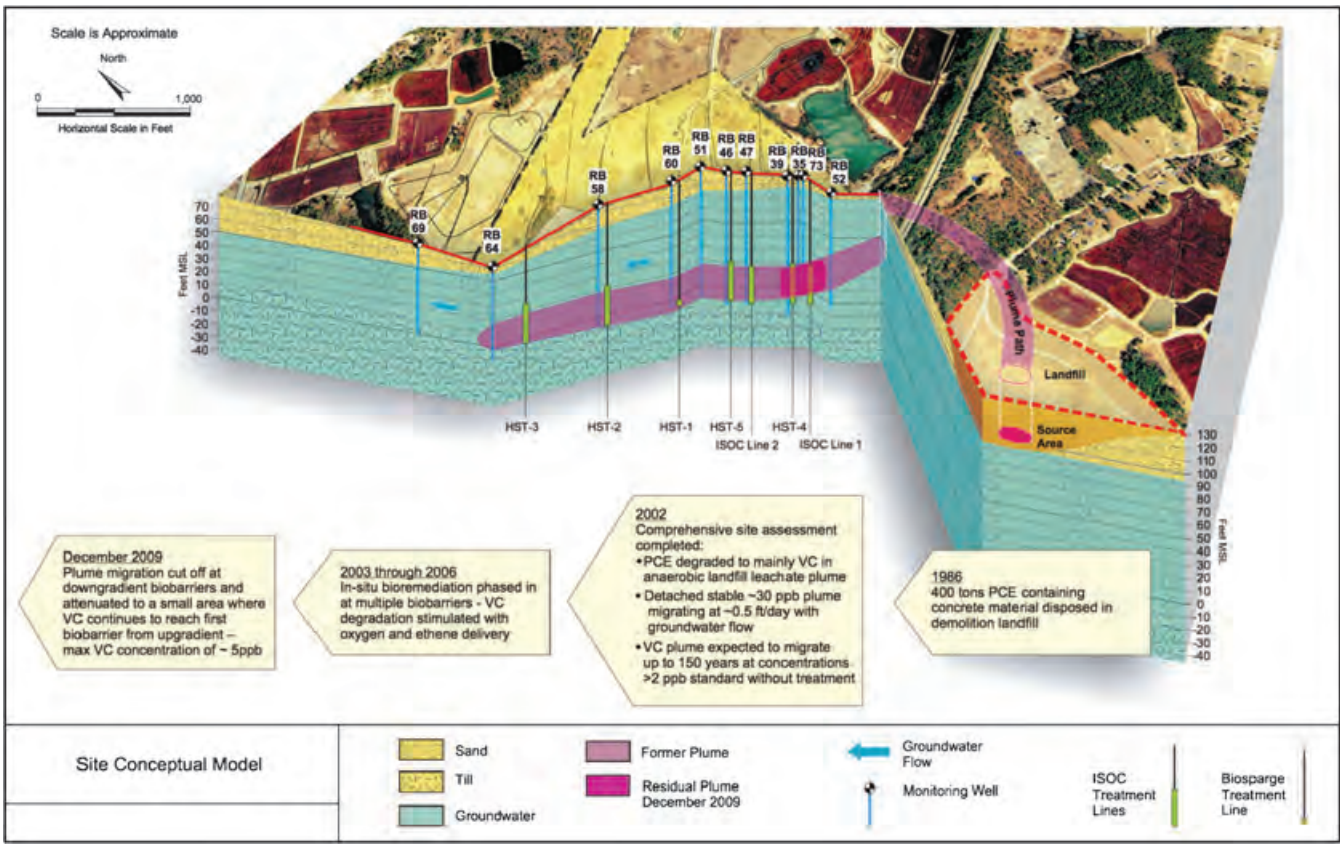

Figure 1. Site model. The light pink shading shows the plume path and the dark pink shading indicates the only remaining area with VC concentrations above 2 µg/L (iSOC treatment lines include iSOC Lines 1 and 2 plus HST-2 through HST-5).

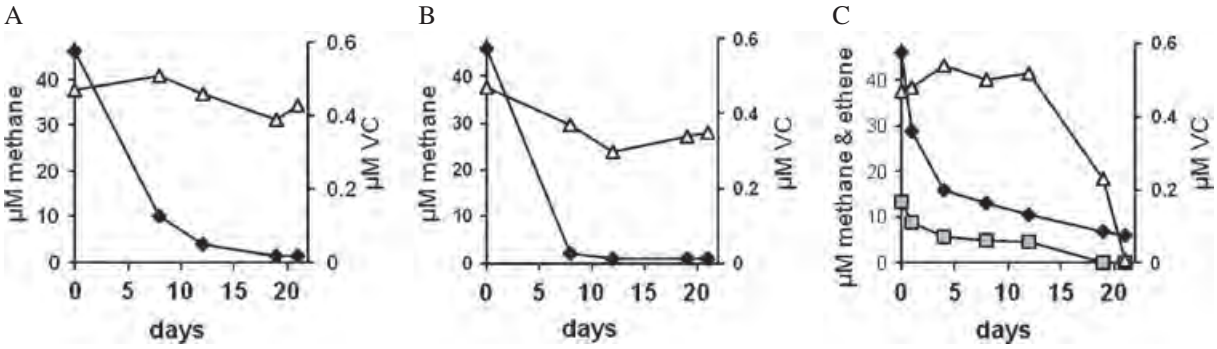

Figure 2. (A) Microcosm with no amendments; (B) microcosm with added mineral nutrients; (C) microcosm with added mineral nutrients and ethene. (♦) VC; (Δ) methane; (□) ethene.

was eluted with 1 mL of 95% ethanol and the absorbance at 600 nm was measured in a spectrophotometer.

Microscopy

Microscopic observations of live biofilm formation were performed using 5 mL of groundwater incubated with aeration in sterile petri plates. No media or supplements were added. The plates were marked so as to allow the same field of view to be observed at each time point. Images were captured under phase contrast with an inverted microscope at 800×.

PCR Analysis

Colonies grown from groundwater on 1:10 R2A medium were screened for the epoxyalkane coenzyme M transferase (EaCoMT) gene. A 431 bp fragment of EaCoMT was amplified by polymerase chain reaction using degenerate primers based on published EaCoMT sequences as previously described (Begley et al. 2009).

Results and Discussion

Laboratory Scale Biodegradation

In order to establish whether aerobic biostimulation might be feasible at the study site, a mixed site groundwater sample was used to generate a series of microcosms to test for aerobic VC degrading activity. Unamended groundwater showed rapid degradation of methane consistent with the activities of native methane-oxidizing bacteria, but only 9% of the VC was degraded after 21 days (Figure 2A). Groundwater amended with mineral nitrogen, phosphorus, and trace elements showed rapid degradation of methane, but only slow degradation of VC, amounting to 25% after 21 days (Figure 2B). However, when groundwater was amended with minerals and 13 µM ethene, degradation of VC was complete in 21 days (Figure 2C). The concentration of VC in the ethene-supplemented microcosm remained unchanged for the first 12 days, but was degraded to nondetectable levels by day 21, suggesting that VC degradation occurred after ethene consumption. No change in the concentration of VC and ethene occurred over a period of 21 days in the autoclaved control microcosm (not shown). The microcosm studies indicated that aerobic microbial VC-degrading activity was present in site groundwater and that it could be stimulated by ethene. Although cometabolic degradation of VC can occur under methanotrophic conditions (Fogel et al. 1986), the metabolism observed in site groundwater, which contains methane, was limited. In contrast complete degradation of VC was observed after addition of ethene to microcosms. So although methane is present, adding oxygen or oxygen and nutrients alone to the microcosms did not stimulate methanotrophic bacteria to degrade VC. These results suggested that methane-stimulated cometabolism would not be the optimal treatment design for this site.

Effect of Biostimulation Treatment on Biofilm Forming Ability

Oxygen treatment of anoxic groundwater should stimulate the growth of aerobic bacteria. Because bacteria primarily function in the environment in surface-attached communities, we focused our growth studies on biofilm formation (Peacock et al. 2004). We obtained groundwater samples from study site wells that were either within or outside of the predicted treatment zones and compared growth in a dye binding biofilm assay. This assay, which did not involve any growth media or addition of supplements, indicated approximately 50% greater biofilm formation, on average, in groundwater from treatment zone wells compared to groundwater sampled outside of the treatment zone (Figure 3).

We also directly visualized surface-associated growth in groundwater from a control well and a treatment zone well over a three-week period under aerobic conditions by incubating 5-mL groundwater samples in petri plates and monitoring

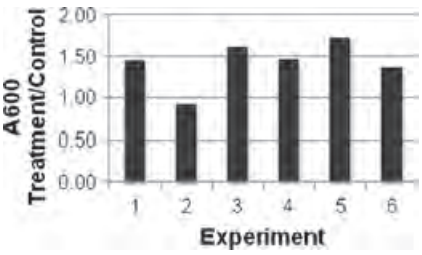

Figure 3. Groundwater samples were taken from a well inside the predicted treatment zone (treatment) and a well outside the predicted treatment zone (control). Biofilm assay data are shown as average growth (A_{600}) of the treatment zone sample relative to average growth of the control sample.

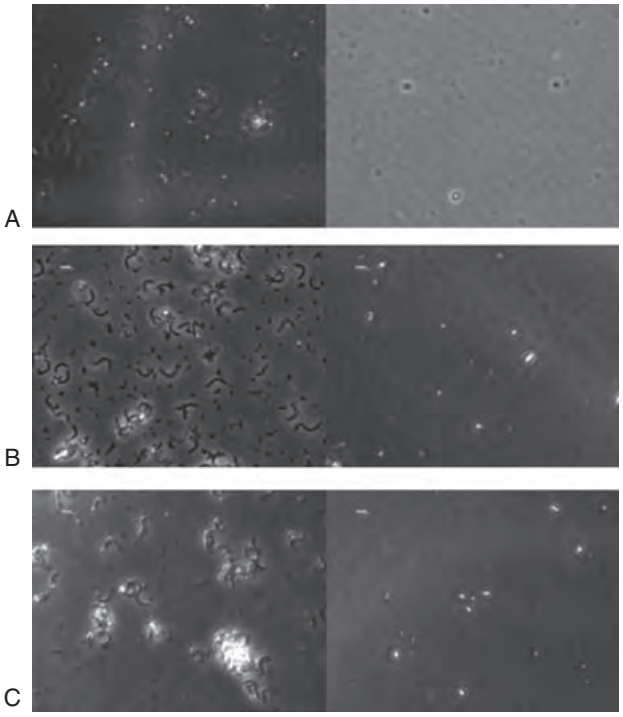

Figure 4. Micrographs of surface-associated growth in groundwater taken from a well within the predicted treatment zone (left) and outside of the treatment zone (right), incubated for one (A), two (B), or three (C) weeks.

growth with a phase contrast inverted microscope. We observed greater biomass in the groundwater from the treatment zone as well as a greater tendency to form surface associated microcolonies (Figure 4). A dye binding assay performed on these samples after the observed incubation period yielded absorbances of 0.167 for the treatment sample and 0.076 for the control sample, consistent with the biofilm assay data.

These results suggest that the remediation treatment employed at the study site stimulates growth of biofilm-forming aerobic bacteria, as predicted. However, biomass measurements are insufficient to demonstrate stimulation of the targeted aerobic VC degradation pathway.

Detection of a Gene Involved in Aerobic Ethene Assimilation

Stimulation of the growth of aerobic bacteria in situ would not be sufficient to affect VC removal if aerobes capable of VC assimilation were not represented in the aerobic community, consequently biomass measures alone may be misleading. Our initial microcosm studies demonstrated ethene-stimulated VC-metabolizing activity in mixed site groundwater. We also used a semiquantitative whole-cell PCR assay (Begley et al. 2009) to detect the presence of the epoxyalkane coenzyme M transferase gene, EaCoMT, which has been implicated in aerobic growth on both ethene and VC (Coleman and Spain 2003). The PCR test was applied to groundwater samples obtained from the site in June of 2007 and 2008, as previously reported (Begley et al. 2009). EaCoMT positive colonies were found in each well, with wells undergoing active treatment having higher numbers of positive colonies, consistent with microcosm and biomass results. Screening of the same wells the following year revealed an interesting pattern: the percentage of EaCoMT positive colonies appeared to track the stage of treatment in these three wells sampled over a three-year period. The wells were at different stages of treatment, based on treatment line startup times and distances downgradient from treatment (Figure 5). This suggests a pattern in which ethenotrophs represent a small, but measurable percentage of the culturable bacteria before treatment, increase to a peak after approximately three years in treatment, and then decline toward the background level. VC concentrations also tended to drop off to low or nondetectable levels over the same time frame.

Groundwater Monitoring

The results of field measurements indicate changes in groundwater chemistry and biological activity resulting from oxygen addition, as well as associated reductions in VC and methane concentrations. Figure 6 shows VC and methane data for three representative monitoring wells within the plume. Well 39I (panel A) is located in the most upgradient plume area immediately downgradient of the first treatment lines and had the highest initial VC concentration before treatment began. Methane concentrations were initially elevated but have increased or pulsed to higher concentrations over time. Well 47D (panel B) is in the mid-plume area and had intermediate initial VC and high methane concentrations. Well 58I (panel C) is in the downgradient plume area and had lower initial VC and methane concentrations. VC concentrations have been reduced to below the regulatory standard of 2 µg/L at wells 58I and 47D and the concentration at 39I has been reduced ~85%. In general monitoring wells located downgradient of treatment lines closer to the source of groundwater contamination showed little change in dissolved oxygen concentration. Increases in dissolved oxygen were noted in the more downgradient part of the plume where monitoring at well 58I showed an increase from less than 0.5 mg/L to up to 3 mg/L. Small transient increases in ethene concentration were noted, increasing from less than 0.001 mg/L to approximately 0.010 mg/L at several locations. The most upgradient treatment area of this detached

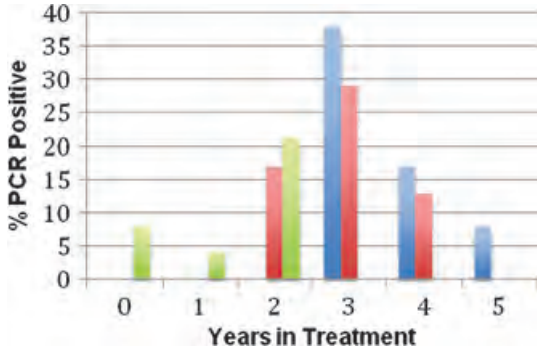

Figure 5. Groundwater was sampled from three wells (green, 51I; red, 58I; blue, 47D) over the same three-year period and colonies were screened for the EaCoMT gene. The percentage of colonies testing positive for the EaCoMT gene is shown for each well relative to the predicted time that the well had been within the treatment zone.

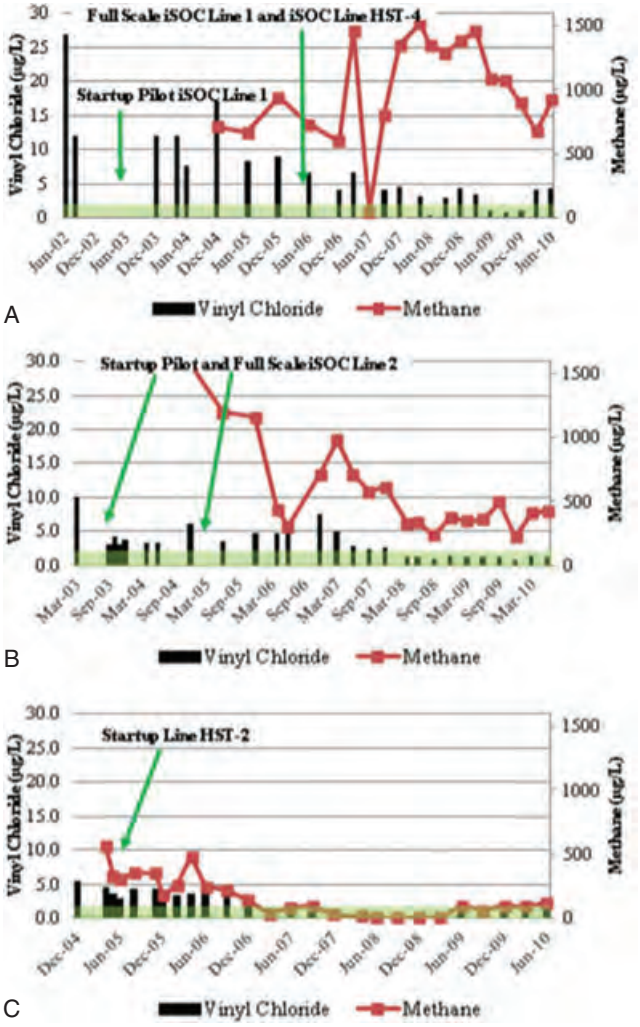

Figure 6. Vinyl chloride and methane concentrations in groundwater from selected monitoring wells. Panels A to C show monitoring data from wells where VC concentrations were initially higher (well 39I), moderate (well 47D), and low (well 58I), respectively. Monitoring wells 47D and 58I are now below the GW-1 groundwater standard for VC (shown in green).

plume near well 39I is approximately 2000 feet downgradient of the original source and contaminated groundwater continues to migrate into the treatment area. Methane is more persistent at higher concentration at well 39I indicating significant oxygen demand in arriving groundwater. It appears that groundwater at treatment lines further downgradient (e.g., well 58I) had lower initial oxygen demand allowing for faster attainment of remediation goals.

Treatment Outcomes and General Applicability

At the time of the initial site assessment, modeling suggested that the plume would continue to migrate passing through areas with private drinking water wells. The plume was projected to discharge to downgradient surface waters in approximately 50 years at VC levels still above the regulatory standard. The bioremediation treatment reported here resulted in cut-off of plume migration and full attenuation of most of the plume only three to four years after full-scale implementation. Three downgradient treatment lines have now been discontinued and the area is undergoing continued monitoring. Residual VC greater than the 2 µg/L GW-1 standard continues to migrate into the area of the first line of treatment from an upgradient source. Groundwater treatment is continuing in this area while the persistent source is the subject of further investigation.

In summary, we have used aerobic biostimulation with oxygen and ethene to treat groundwater at a PCE release site where low-level vinyl chloride has been persistent. In situ delivery of oxygen and ethene gas to the groundwater has resulted in cessation of plume migration, decreased vinyl chloride levels at all monitoring wells, and reduced contaminant concentrations to below the regulatory target throughout most of the plume. Given the low initial VC concentrations, the observed aerobic VC degradation in microcosms supplemented with ethene and detection of temporal changes in a gene involved in ethene/VC degradation suggest that ethene-stimulated aerobic VC degradation led to VC removal observed in the treatment area. At contaminated sites where VC concentrations are higher, oxygen alone may stimulate effective biodegradation, however microcosm studies are recommended to assess the utility of added substrates, as site-specific conditions will vary.

This example of a successful full-scale aerobic treatment of a dilute vinyl chloride plume with oxygen and ethene suggests the possibility of rapid, efficient containment and enhanced attenuation of VC at similar sites where conditions preclude complete anaerobic treatment.

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LOAN DOCUMENT

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IRP, AEROBIC COMETABOLIC IN SITU BIOREMEDIATION TECHNOLOGY GUIDANCE MANUAL AND SCREENING SOFTWARE USER'S GUIDE

EARTH TECH, INC.
1420 KING ST., SUITE 600
ALEXANDRIA VA 22314

JUNE 1998

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AIR FORCE RESEARCH LABORATORY
MATERIALS & MANUFACTURING DIRECTORATE
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This Guidance Manual and Screening Software User's Guide has been prepared for the US Air Force by Earth Tech, Inc., under the US Air Force Installation Restoration Program (IRP). The limited objectives of this document and the ongoing nature of the IRP, along with the evolving knowledge of site conditions and chemical effects on the environment and health must be considered when using this document to help determine whether this treatment technology can be applied at a particular site. The results presented by the software are based on a limited number of actual field implementation results. As the technology becomes more widely used, the screening software will be adjusted accordingly. Acceptance of this document in performance of the contract under which it was prepared does not mean that the US Air Force adopts the conclusions, recommendations, or other views expressed herein, which are those of the contractor only and do not necessarily reflect the official position of the US Air Force.

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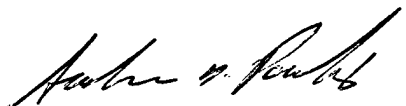

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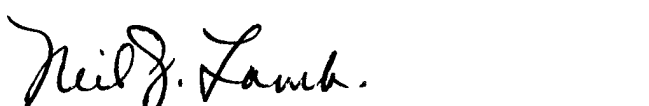

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13. ABSTRACT (Maximum 200 words)

Trichloroethene (TCE) is the most commonly detected contaminant at Superfund sites in the US. It has been widely used for degreasing and cleaning activities. TCE was commonly discharged to the environment for disposal purposes. In recent years, the hazards of exposure to TCE and other chlorinated aliphatic hydrocarbons (CAHs) have been documented. These compounds can pose a serious threat to human health or the environment. Aerobic cometabolic in situ bioremediation is an innovative technology being used for treatment of groundwater contaminated with CAHs, especially TCE. This document presents the principles of aerobic cometabolic in situ bioremediation as well as mathematical models used describe the technology and a discussion of its applicability and limitations. A section of this document describes a software program that can help determine if this technology is appropriate for implementation. The steps needed to design and implement the technology, based upon results of a full-scale evaluation at Edwards AFB, CA, are also presented. The document concludes with a discussion surrounding regulatory acceptance the technology and a description of case studies where the technology has been implemented in the field. This document is designed for use by project managers not necessarily experienced in treatment technology design who are exploring potential technology alternatives for groundwater treatment.

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PREFACE

This report was prepared by Earth Tech, Inc., 1420 King St., Suite 600, Alexandria, VA 22314, for the US Air Force Research Laboratory, Materials and Manufacturing Directorate, Airbase and Environmental Technology Division (AFRL/MLQE), Suite 2, 139 Barnes Drive, Tyndall Air Force Base, Florida 32403-5323.

Trichloroethene (TCE) is the most commonly detected contaminant at Superfund sites in the US. It has been widely used for degreasing and cleaning activities. TCE was commonly discharged to the environment for disposal purposes. In recent years, the hazards of exposure to TCE and other chlorinated aliphatic hydrocarbons (CAHs) have been documented. These compounds can pose a serious threat to human health or the environment. Aerobic cometabolic in situ bioremediation is an innovative technology being used for treatment of groundwater contaminated with CAHs, especially TCE. This document presents the principles of aerobic cometabolic in situ bioremediation as well as mathematical models used describe the technology and a discussion of its applicability and limitations. A section of this document describes a software program that can help determine if this technology is appropriate for implementation. The steps needed to design and implement the technology, based upon results of a full-scale evaluation at Edwards AFB, CA, are also presented. The document concludes with a discussion surrounding regulatory acceptance the technology and a description of case studies where the technology has been implemented in the field. This document is designed for use by project managers not necessarily experienced in treatment technology design who are exploring potential technology alternatives for groundwater treatment.

This Technology Guidance Manual and Screening Software User's Guide provides information on the use of aerobic cometabolic in situ bioremediation as a technology for treatment of groundwater contaminated with CAHs. This document has been prepared according to the requirements of Contract No. F41624-94-D-8055, Delivery Order No. 2 between the US Air Force and Earth Tech, Inc. The AFRL/MLQE project officer was Ms. Alison Thomas.

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This document was produced under the direction of Mr. Ray Sugiura, Earth Tech's Program Manager for this contract. Ms. Tara MacHarg served as the Task Order Manager. The principal authors are Ms. Judy Gallagher, Mr. Robert Sandoli, and Mr. Brian Woodbury. The word processing and graphics support was provided by Ms. Pamela Anderson and Mr. Peter Sandberg, respectively.

1. INTRODUCTION

Chlorinated aliphatic hydrocarbons (CAHs) are the most prevalent contaminants found at Superfund sites, with trichloroethylene (TCE) being the most common constituent. Aerobic cometabolic bioremediation is an innovative technology currently being studied to treat groundwater contaminated with TCE and other CAHs such as 1,2-dichloroethylene (1,2-DCE) and vinyl chloride; the technology does not work for tetrachloroethylene (aka, perchloroethylene, or PCE), 1,1,1-trichloroethane (1,1,1-TCA), 1,1-dichloroethylene (1,1-DCE), carbon tetrachloride, chloroform, or methylene chloride. The technology was developed to work *in situ*, or in place, and in some cases, pumping contaminated water to the surface may be avoided. The remediation of the groundwater occurs when indigenous microorganisms oxidize a compound (primary substrate) by producing a nonspecific enzyme that fortuitously oxidizes the contaminant. The primary substrate, as well as a source of dissolved oxygen to serve as an electron acceptor, are introduced into the groundwater via injection wells.

Studies involving the use of aerobic cometabolic *in situ* bioremediation are ongoing to determine which compounds may best serve as the primary substrate and the electron acceptor. Toluene, methane and phenol are several of the compounds that may be used as the primary substrate, with toluene appearing to be the most effective. The dissolved oxygen source alternatives to be used as the electron acceptor are air, pure oxygen, and hydrogen peroxide. Pure oxygen seems to be the most efficient and least expensive choice, but periodic use of hydrogen peroxide offers benefits as well (e.g., it helps prevent bioclogging). Selection of the primary substrate and electron acceptor is also dependent on the CAH concentrations in the groundwater at a particular site.

This implementation guide and user's manual presents the principles of aerobic cometabolic *in situ* bioremediation as well as the mathematical models used to describe the technology and a discussion of the applicability and limitations of the technology. A section of this document describes a software program that may be used to help determine whether this technology is appropriate for implementation at a user-defined site. The steps needed to design and implement the technology, based upon results of a full-scale evaluation at Edwards AFB Air Force Base (AFB), California, are also presented. The document concludes with a discussion surrounding the regulatory acceptance of the technology and a description of case studies where the technology has been implemented in the field.

This document is designed for use by project managers not necessarily experienced in treatment technology design, and who are exploring potential technology alternatives for groundwater treatment. Section 5 of this document presents the details of the technology implementation at a particular site, and may be useful only to those managers having a higher level of design experience.

2. OBJECTIVES

The objectives of this manual are:

(1) To introduce the process of aerobic cometabolic *in situ* bioremediation as an innovative technology for removing CAHs from groundwater;

(2) To provide information on when to consider using this technology at hazardous waste sites;

(3) To describe how to use a software program developed to assist in technology selection at a user-defined site;

(4) To describe steps involved in implementing the technology based on a full-scale demonstration at Site 19, Edwards AFB, California;

(5) To discuss regulatory considerations associated with the selection and implementation of this technology, and;

(6) To compare and contrast site conditions, system design and operation, and performance data from a series of case studies.

3. OVERVIEW

This section provides a brief description of the technology (Section 3.1); a historical synopsis of how the technology progressed to its current state (Section 3.2); a description of controls used and parameters measured to demonstrate usefulness of the technology (Section 3.3); a list of the advantages and disadvantages of implementing the technology (Section 3.4); and a discussion of a decision tree designed to assist in determining whether the use of this technology should be considered at a site (Section 3.5).

3.1 Technology Description and Definitions

A brief description of the technology is provided below, followed by generalized descriptions of some of the terms and processes pertinent to the technology.

Aerobic cometabolic *in situ* bioremediation is an environmental remediation technology that is primarily focused on the cleanup of groundwater contaminated with CAHs. The technology uses indigenous microorganisms at a site to destroy dissolved contaminants. Using this technology, the cleanup process occurs *in situ*, or in place. In some cases, no contaminated groundwater need be pumped to the surface, eliminating the need for costly disposal of hazardous waste, as well as treatment of air emissions and other treatment wastes. The process is cometabolic, meaning the indigenous microorganisms, while oxidizing one chemical compound (the primary substrate) for energy and growth, produce an enzyme that fortuitously degrades the target contaminant. A source of oxygen (electron acceptor) must be introduced to the system for the cometabolism to occur. The enzyme produced by the microorganisms to oxidize the primary substrate is a protein-like substance that acts as a catalyst for the degradation of the contaminant. Contaminant degradation provides no benefit to the microorganism (McCarty and Semprini, 1993).

Bioclogging. In general, bioclogging describes a condition of excessive microbial growth near the point of introduction of nutrients, thereby clogging the introduction point and limiting flow in a dynamic bioremediation system (Taylor *et al.*, 1993). During aerobic *in situ* cometabolism, bioclogging can occur around injection wells where the primary substrate and electron acceptor (i.e., some source of oxygen) are introduced into the subsurface.

Cometabolic Enzyme. The cometabolic enzyme is the protein-like substance that is directly responsible for the cometabolism of contaminant compounds. Cometabolic enzymes differ depending on the organism that produces them, or more accurately, depending on the primary substrate to which the microorganisms are exposed. Exposure of the microorganisms to the primary substrate of the cometabolic enzyme typically induces production of more of the enzyme by the microorganism. In the case of aerobic cometabolism of CAHs, numerous studies have demonstrated that the process is mediated by the action of non-specific oxygenase enzymes (e.g., Zylstra *et al.*, 1989; Wackett and Gibson, 1988; Anderson *et al.*, 1996; Fox *et al.*, 1990; Oldenhuis *et al.*, 1989). Discussion of the enzymes and their mode of action is beyond the scope of this document.

Cometabolism. Cometabolism describes the process whereby an organic compound (e.g., a contaminant such as TCE) is fortuitously degraded by an enzyme expressed for the metabolism of a different compound (i.e., a primary substrate such as methane or toluene). Cometabolism produces no energy for the host organism, and the compound(s) subject to cometabolism often compete with the primary substrate for the active site of the cometabolic enzyme (see Competitive Inhibition).

Competitive Inhibition. This term describes the phenomenon whereby the primary substrate inhibits oxidation of the target contaminant(s) for cometabolism by competing for the active site of the cometabolic enzyme in a mutually exclusive fashion (Zubay, 1988).

Deactivation. The term describes the decrease in the rate and extent of cometabolism that is observed when the primary substrate concentration is reduced (Semprini and McCarty, 1992). While the presence of the primary substrate typically induces production of more of the cometabolic enzyme and allows microbial growth under appropriate conditions, its absence results in deactivation, regardless of the concentration of the contaminant(s) targeted for cometabolism.

Electron Acceptor. An electron acceptor is a substance that accepts electrons during an oxidation-reduction action. The application of the aerobic *in situ* cometabolism technology depends upon the oxidation of an organic compound in which oxygen serves as the electron acceptor that becomes reduced to H_2O. How oxygen is delivered *in situ* (i.e., as pure oxygen gas, as a natural component of air, or as a degradation product of hydrogen peroxide) is an important decision factor in terms of cost and for dealing with bioclogging.

Microbial Growth. Microbial growth occurs when the primary substrate and electron acceptor are in abundance and there are no other factors limiting growth (e.g., low nitrogen or phosphorus, extreme temperature or pH conditions, etc.)

Primary Substrate. The primary substrate refers to the organic compound that the cometabolic enzyme is designed to oxidize. This organic compound typically serves as the carbon and energy source for the microorganism that produces the cometabolic enzyme; when this is the case the compound may be referred to as the electron donor. Wilson and Wilson (1985) first demonstrated cometabolism of TCE in soil columns through the addition of natural gas (i.e., methane) as the primary substrate to stimulate the growth of indigenous microorganisms. Since then, other compounds have been shown to serve as primary substrates. These compounds include propane (Wackett *et al.*, 1989); ethylene and propane (Hartmans *et al.*, 1992; Ensign *et al.*, 1992; Roberts *et al.*, 1989), cresol, phenol, and toluene (Nelson *et al.*, 1988); ammonia (Arciero *et al.*, 1989, Rasche *et al.*, 1991); isoprene (Ewers *et al.*, 1991); and isopropyl benzene (Dabrock *et al.*, 1992).

3.2 Technology Progression Through Field Demonstrations

While the basic science of cometabolism has been intensively studied in the laboratory from the molecular to the microcosm level, field demonstration of the technology has been limited. Peer reviewed publications from field demonstration projects are primarily from three study sites: Savannah River Site (SRS), Moffett Federal Airfield, and Edwards AFB Site 19. Researchers at Stanford University have led the research efforts at the latter two sites. The technology progression discussed in this section is based on research and experience at both of these sites. Moreover, the system design discussed in Section 5 and the software accompanying this manual are based on the design implemented at the Edwards AFB site.

The first field studies to evaluate the efficacy of aerobic cometabolic *in situ* bioremediation were conducted at the Moffett Federal Airfield (formerly the Moffett Naval Air Station) in Mountain View, California. The results of the field studies were published in 1990, five years after the original publication by Wilson and Wilson (1985) that documented the cometabolism of TCE in soil columns (Semprini *et al.*, 1990). These studies used methane as the primary substrate and oxygen gas as the source of oxygen for aerobic cometabolism of TCE, cis-1,2-, and trans-1,2-dichloroethylene (c-DCE, and t-DCE), and vinyl chloride. Results indicated that the methane

consuming consortium developed was highly effective at degrading t-DCE and vinyl chloride, but removal of TCE and c-DCE was not as successful (Semprini *et al.*, 1990).

The low removal efficiency of TCE and c-DCE using methane as the primary substrate led to the exploration of other potential primary substrates. The studies were again conducted at the Moffett Federal Airfield and focused on the utility of phenol as a primary substrate, again using oxygen gas as the source of oxygen. The studies, conducted over two field seasons with results published in 1993, found phenol to be superior to methane for aerobic cometabolic *in situ* degradation of TCE and c-DCE (Hopkins *et al.*, 1993b).

Toluene was also evaluated at the Moffett Federal Airfield site for use as a primary substrate for the cometabolic degradation of TCE. Additionally, hydrogen peroxide was evaluated as an oxygen source. The studies concluded that toluene is an effective primary substrate with performance levels very similar to that of phenol. Hydrogen peroxide was also found to be a good source of oxygen, achieving TCE removals similar to those achieved when using oxygen gas. Finally, the studies verified a laboratory study (Dolan and McCarty, 1995) that the presence of 1,1-dichloroethylene (1,1-DCE) as a co-contaminant would significantly reduce TCE removal efficiencies (Hopkins and McCarty, 1995).

A summary of the Moffett Federal Airfield studies is provided in Table 3-1 (Hopkins and McCarty, 1995).

Table 3-1. Efficiency of Chlorinated Aliphatic Hydrocarbon Removal Obtained at the Moffett Federal Airfield Site with Different Primary Substrates (after Hopkins and McCarty, 1995)

Primary Substrate	Substrate Concentration (mg/L)	%Removal				
		TCE	1,1-DCE	c-DCE	t-DCE	Vinyl Chloride
Methane	6.6	19	NE	43	90	95
Phenol	12.5	94	54	92	73	>98
Toluene	9	93	NE	>98	75	NE

Key: TCE = Trichloroethylene; t-DCE = trans-1,2-Dichloroethylene; c-DCE = cis-1,2-Dichoroethylene; 1,1-DCE = 1,1-Dichloroethylene; NE = Not Evaluated; mg/L = Milligrams per liter

With the lessons learned from the initial field evaluations at the Moffett Federal Airfield, the next logical technology development step was a full-scale technology demonstration. Full-scale technology demonstrations play an important role in the transfer of an environmental remediation technology for commercialization and use at additional sites. The data gathered during these demonstrations are essential to design and implement the technology at other sites, as well as to better understand technology limitations.

The site selected for the demonstration of aerobic cometabolic *in situ* bioremediation was Site 19 at Edwards AFB. Edwards AFB is located on the western portion of the Mojave Desert, California. Site selection was critical to the full-scale demonstration. Like any environmental technology, aerobic cometabolic *in situ* bioremediation is best suited for use at some sites while inappropriate at others. The results at the Moffett Federal Airfield studies were used to select a site that was most appropriate for the full-scale technology demonstration (McCarty *et al.*, 1998).

In addition to the technical criteria used to select a site, the researchers were interested in finding a site that would be overseen by regulators and project management personnel supportive of the evaluation of an innovative technology. This was particularly important in the case of aerobic cometabolic *in situ* bioremediation because the chosen primary substrate for the demonstration, toluene, is a moderately hazardous regulated chemical. Regulators would have to approve the injection of the chemical into the subsurface for the full-scale technology demonstration to become a reality. The Moffett Federal Airfield field studies conducted over the previous decade with peer reviewed results were a factor in the acceptance of the demonstration by Edwards AFB remedial project managers and federal, state, and local officials tasked with overseeing the cleanup at Site 19. Additionally, a near real-time monitoring system was proposed for immediate notification of unacceptable toluene concentrations migrating from the site and a contingency plan for toluene removal was prepared should it be needed (McCarty *et al.*, 1998).

Once the site was selected, the system design had to be conceived. One challenge was to design a system to efficiently mix the contaminant, primary substrate, and electron acceptor, and to deliver this mixture to indigenous microorganisms. All components must be brought together at the same place and time for biodegradation to occur. Traditionally, delivery systems have been devised either to infiltrate nutrients into the contaminated zone (by flooding, ponding, surface spraying, infiltration galleries/beds, etc.) or to pump the nutrients into the zone. However, such delivery systems do not promote optimal mixing, as the water which carries the nutrients and oxygen to the zone of contamination tends to displace contaminated water, and mixing is then restricted to the boundaries of the nutrient and contaminant plumes, where diffusion/dispersion processes play a role (McCarty and Semprini, 1993).

Several innovative solutions to this mixing problem have recently been presented. Horizontal wells have been used to bubble gaseous nutrients into contaminated groundwater (SRS, 1998). *In situ* passive systems, such as filters (Taylor *et al.*, 1993), trenches (Woods *et al.*, 1995), and funnels and gates (Starr and Cherry, 1994), where organisms and nutrients are localized, and contaminated groundwater flows into the bioactive zone, have also been proposed to enhance mixing of contaminants, nutrients, oxygen, and microorganisms. Another approach involves use of a groundwater circulation well, where contaminated groundwater is pumped through the well, mixed in the well bore with nutrients, and the mixture is reintroduced into the aquifer. This was the design selected for the Edwards AFB site and discussed throughout this manual[1].

The field demonstration at Edwards AFB, Site 19, verified that aerobic cometabolic *in situ* bioremediation is an effective environmental remediation technology. The demonstration yielded TCE removal efficiencies of 95 to 97 percent. Toluene degradation was 99.98 percent, leaving an average of 1.2 to 1.3 micrograms per liter (µg/L) at the boundaries of the treatment zone, well below the maximum goal of 20 µg/L set by regulatory agencies (McCarty *et al.*, 1998).

The field demonstration at Edwards AFB also achieved an important objective of any full-scale technology demonstration, which is compiling and documenting information that is relevant to site managers and regulators considering the use of the technology. For example, methods to alleviate the phenomena known as bioclogging were evaluated during implementation of the technology at Edwards AFB. Bioclogging is the preferential growth of microorganisms near the injection well where nutrients are most abundant (Taylor *et al.*, 1993). This preferential growth

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[1] Groundwater circulation wells were designed by Stanford University researchers specifically for the Edwards AFB site. The U.S. Air Force does not endorse any particular vendor supplying groundwater circulation well parts or services.

can cause the injection well to become partially clogged with biomass. One solution to this problem is the injection of hydrogen peroxide as an oxygen source. The injected hydrogen peroxide enters the subsurface in the bioclogged zone in concentrations high enough to be toxic to the microorganisms responsible for the bioclogging. As the hydrogen peroxide migrates away from the well, it hydrolyzes, providing valuable oxygen to the *in situ* system. Pulse injection of the primary substrate, which limits microbial growth in the region close to the injection well, is another way to prevent bioclogging. Finally, well-redevelopment is a strategy that has been shown to alleviate not only problems associated with bioclogging, but also those associated with the presence of fines, which tend to clog pumping and injection wells (McCarty *et al.*, 1998).

Competitive inhibition is another phenomenon that had to be dealt with in implementing the technology. Competitive inhibition describes the process whereby the primary substrate inhibits oxidation of the target contaminant(s) for cometabolism by competing for the active site of the cometabolic enzyme in a mutually exclusive fashion. Thus, when the primary substrate and the target contaminant(s) are simultaneously present, biodegradation of the target contaminant(s) is inhibited. The field demonstration showed that a pulse addition of primary substrate was an effective means to balance the maintenance of a healthy microbial population with the cometabolic degradation of contaminants. Conveniently, this pulsing strategy also served to prevent bioclogging, as noted above.

The above discussion of bioclogging and competitive inhibition is offered to illustrate the sort of phenomena that may be elucidated through full-scale demonstrations. These phenomena may not be adequately evaluated or possibly not even apparent in laboratory or other preliminary studies. The discussion highlights the importance of full-scale demonstrations to fully understand the rate limiting processes and practical applications of a particular environmental remediation technology.

3.3 How Do I Know It Works?

In situ bioremediation is difficult to prove, for not only do decreases in contaminant concentrations have to be documented, but the decreases must be linked to microbial action. Without evidence of microbial involvement, there is no way to verify that contaminants did not volatilize, migrate off-site, sorb to subsurface solids, or change form via abiotic chemical reactions (National Research Council [NRC], 1993).

The NRC (1993) reported that three lines of evidence should be presented in order to "prove" *in situ* bioremediation:

1. Documented loss of contaminant from the site,

2. Laboratory assays showing that microorganisms in site samples have the potential to transform the contaminants under the expected site conditions, and

3. One or more pieces of evidence showing that the biotransformation potential is actually realized in the field.

The first line of evidence may be achieved via standard sampling techniques. The second line of evidence is relatively simple to demonstrate using customary laboratory methods and should be considered a necessary precursor to implementing aerobic cometabolic *in situ* bioremediation (see Section 5). The final piece of evidence is the most difficult to generate, but there are many methods available to reach this end. These methods are summarized in Table 3-2.

Many of the techniques identified in Table 3-2 may be innovative and interesting from an academic standpoint, but they may not be applicable or cost-effective in terms of implementing a bioremediation technology such as *in situ* aerobic cometabolism. Depending on the regulatory agency representatives involved, only one or a few of the techniques may have to be used. For example, at Moffett Federal Airfield and Edwards AFB Site 19 (see Sections 3.2 and 7.0 for a summary of the work conducted at these sites), researchers at Stanford University successfully proved *in situ* bioremediation to the satisfaction of both regulatory agency representatives and academic peers (Hopkins and McCarty, 1995; McCarty *et al.*, 1998). Table 3-3 lists evidence indicating *in situ* bioremediation at these sites was occurring according to each of the three lines of evidence required to "prove" *in situ* biodegradation as described above.

While it has been proven that the technology ***can*** work at these and other sites, proving that the technology ***is*** working should be considered a type of "performance monitoring" required of any remedial technology. Accordingly, techniques for demonstrating that the technology is working in the field (i.e., that contaminant loss is due to bioremediation as opposed volatilization, dilution, etc.) should be considered an essential component of the remedial work plan for a site.

3.4 Advantages and Disadvantages

Many authors cite the advantages of aerobic cometabolic *in situ* bioremediation over traditional pump-and-treat technology (McCarty *et al.*, 1998, Saaty *et al.*, 1995, Taylor *et al.*, 1993). These advantages include: the avoidance of costs and health risks associated with pumping groundwater to the surface to be treated; the avoidance of an above ground treatment system to treat groundwater contaminants; TCE is destroyed in the process and not simply concentrated in another medium for disposal; disposal of treated groundwater is not an issue; and uncontaminated groundwater is not polluted by bringing it into the contaminated zone as generally occurs in pump-and-treat systems (McCarty *et al.*, 1998). Additionally, Saaty *et al.* (1995) indicates that *in situ* bioremediation has been shown to require less time for remediation than pump-and-treat technology. Taylor *et al.* (1993) explains that this is due to the highly heterogeneous nature of most subsurface media, which creates preferential flowpaths for the extracted groundwater.

One disadvantage of *in situ* bioremediation for chlorinated aliphatics is that it is commonly a cometabolic process requiring a regulated chemical, such as toluene, to be supplied as the primary substrate (McCarty *et al.*, 1998). Regulatory approval for injection of a regulated chemical into the subsurface presents obvious obstacles. Experiences at Site 19 suggest that as long as sufficient oxygen is present and the aquifer has sufficient nutrient mass to support biological growth, residual concentrations (of toluene) acceptable to regulatory agencies can be achieved through aerobic biodegradation (McCarty *et al.*, 1998). Additionally, toluene is also biodegradable anaerobically so that even if sufficient oxygen were not available, toluene would still be degraded to below levels of regulatory concern (McCarty *et al.*, 1998).

Table 3-2. Techniques for demonstrating biodegradation in the field (after NRC, 1993)	
Technique	**Notes**
Measurements of Field Samples	
Number of Bacteria	• Increase in number of bacteria and corresponding loss of contaminant may indicate bacteria are using the contaminant for carbon and energy.
Direct microscopic counting	• Provides data on total bacterial counts but does not give information on cell types or metabolic activity. • Tedious.
The INT activity test	• Identifies metabolically active bacteria. • Used to enhance direct microscopic counting.
Plate counts	• Counts only bacteria capable of growing on selected media. • Commonly underestimates the number and diversity of bacteria. • Easy to isolate bacteria for study of factors affecting biodegradation potential.
The most probable number (MPN) technique	• Dilution method that uses statistics to estimate bacterial numbers. • Counts only bacteria capable of growing in selected media and may underestimate the number of bacteria.
Oligonucleotide probes	• Small pieces of DNA that bind to the DNA of target cells. • Highly specific. • Requires knowledge of cell's genetic sequence. • Only semiquantitative in its current state.
Fatty acid analysis	• Identifies bacteria by characteristic "signature" of fatty acids present in cell membranes. • Use in quantification not well established. • Limited sensitivity.
Number of Protozoans	• Increase in protozoans suggests an increase in the number of bacteria because protozoans prey on bacteria.
Rates of Bacterial Activity	• Can measure microcosms accurately in laboratory using radiolabeled contaminants. • Uncertainty in extrapolating laboratory results to the field.
Bacterial Adaptation	• Indicated by an increase over time in the rate at which microorganisms transform contaminants in a microcosm test.
Inorganic Carbon Concentration	• Increase in CO_2 or HCO_3^- indicates metabolism of organic compounds. • Not accurate in conditions of high background bicarbonate or dissolution of calcareous minerals.
Carbon Isotope Ratios	• Microorganisms preferentially degrade ^{12}C versus ^{13}C containing compounds. • A $^{13}C/^{12}C$ ratio in inorganic carbon from site samples lower than the $^{13}C/^{12}C$ ratio from mineral sources at the site indicates biodegradation.

Table 3-2. Techniques for demonstrating biodegradation in the field (after NRC, 1993)	
Technique	**Notes**
Measurements of Field Samples (Continued)	
Electron Acceptor Concentration	• Decrease in electron acceptor concentration (e.g., oxygen, nitrate, sulfate) compared to adjacent uncontaminated areas indicates biodegradation.
Byproducts of Anaerobic Activity	• Increase in byproducts of anaerobic activity (e.g., methane, sulfides, reduced forms of iron and manganese, nitrogen gas) compared to adjacent uncontaminated areas indicates biodegradation.
Intermediary Metabolite Formation	• Identification of compounds unique in microbial metabolism of a contaminant indicates biodegradation. • Many intermediary metabolites are short-lived and cannot be detected.
Ratio of Nondegradable to Degradable Substances	• Increase in ratio of nondegradable to degradable substances compared to the ratio in the source of the contaminants indicates biodegradation. • Also useful for single contaminants having different forms, one of which is biodegradable and the other of which resists biodegradation.
Experiments Run in the Field	
Stimulating Bacteria Within Subsites	• Contaminant loss in areas where stimulants of bacterial activity (e.g., electron donors, electron acceptors, nutrients) are added indicates biodegradation. • Requires a setting uniform enough to have comparable subsites.
Measuring the Electron Acceptor Uptake Rate	• Relatively rapid loss of an added supply of electron acceptor (e.g., oxygen) in a contaminated versus an uncontaminated area indicates biodegradation.
Monitoring Conservative Tracers	• Chemical tracers have chemical and transport properties similar to those of microbiologically reactive chemicals but are not microbiologically reactive themselves. • Used to distinguish abiotic chemical changes (e.g., volatilization, sorption, dilution) from chemical changes caused by microorganisms.
Labeling Contaminants	• Biodegradation indicated if the expected metabolic byproducts (e.g., inorganic carbon and intermediary metabolites) carry the same relative amounts of radioisotope (e.g., ^{13}C or ^{3}H) as the labeled contaminants added to a site.

Table 3-3. Proving *In situ* Bioremediation at Moffett Federal Airfield and Edwards AFB (after NRC, 1997; Hopkins and McCarty, 1995; McCarty *et al.*, 1998)[1]	
Data Objective	**Type of Data**
1. Document reduction in quantity of TCE	• Reduction in TCE concentrations determined from spatial and temporal sampling and comparison with tracer concentrations. • Reduction in TCE mass determined from spatial integration of concentration measurements (at Edwards AFB); comparison of steady-state TCE concentrations upgradient and downgradient of the treatment system (at Edwards AFB); comparison of breakthrough of organic contaminants before and after biostimulation (at Moffett Federal Airfield); and a mass balance comparing mass of contaminant injected to mass removed (at Moffett Federal Airfield).
2. Show microorganisms from the site can cometabolize TCE	• Microcosm studies using aquifer material from the site showed evidence of cometabolism. Microbes isolated from the microcosms showed the same cometabolic activity.
3. Link TCE disappearance to the technology	• Decrease of TCE concentrations coinciding with methane or toluene utilization. • Increase in TCE concentrations when addition of primary substrate stopped. • Rate and extent of field biodegradation consistent with laboratory microcosm studies. • No degradation of TCE observed in zone where no methane was present to support bacterial growth (Moffett Federal Airfield). • No evidence of anaerobic conditions (i.e., no intermediate products of anaerobic degradation). • Presence of indigenous methanotrophic bacteria (Moffett Federal Airfield) and correlation of inferred biomass distribution with zones of TCE biodegradation (Edwards AFB).

(1) In the Moffett Federal Airfield experiments, controlled contaminant injections, conservative tracers, untreated test areas, systematic variation of operating parameters, and start-and-stop testing were used as controls.

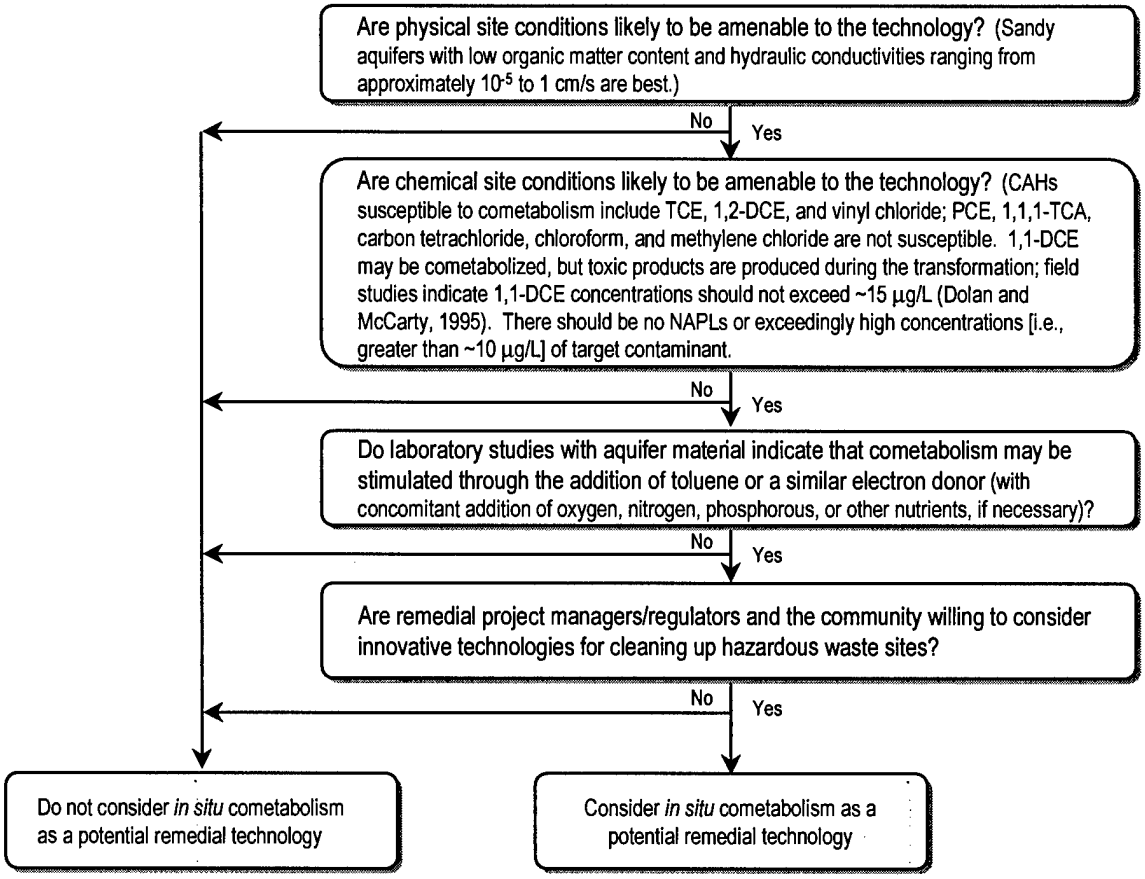
An additional disadvantage associated with aerobic cometabolic *in situ* bioremediation are problems associated with bioclogging. The Edwards AFB Site 19 experienced difficulties associated with bioclogging at the injection well screen. The injection of hydrogen peroxide and well-redevelopment was employed to alleviate the problem. However, at a cost of $4.00 per kg ($1.80 per pound), hydrogen peroxide is quite expensive. For comparison, the primary substrate used at Site 19, toluene, costs only $0.20 per kg ($0.09 per pound). Well-redevelopment was employed three times at Site 19, once due to a sudden head increase and twice as a routine procedure. On average, well-redevelopment costs about $4,000 per redevelopment. The use of hydrogen peroxide and periodic well-redevelopment may significantly contribute to the cost of a remediation project (McCarty *et al.*, 1998). Other innovative approaches to reduce this potential problem are thus worth pursuing.

3.5 When Should I Consider Using This Technology At My Site?

Before selecting any groundwater remedial technology, information on site conditions must be gathered. This includes characterizing physical, chemical, geological, and hydrogeological parameters. When considering aerobic *in situ* cometabolism, the capacity of intrinsic microbial populations to degrade the compound(s) of interest given the appropriate stimulating conditions must also be evaluated.

A decision tree that indicates when to consider the use of aerobic cometabolic *in situ* bioremediation for cleaning up CAHs in groundwater is given in Figure 3-1. The first few steps involve assessing whether site conditions are amenable to the technology. Ideal physical site conditions are sandy aquifers with low organic matter content and hydraulic conductivities greater than 10^{-3} cm/s. Generally, bedrock aquifers are excluded from the use of this technology. Because of the sensitivity of pumping costs to aquifer depth, it should be noted that this technology would be more cost-effective in anisotropic deeper aquifers since groundwater extraction is not necessary. Chemical conditions should include CAH concentrations not exceeding ~10 mg/L; no dense or light non-aqueous phase liquids (DNAPLs or LNAPLs); and low concentrations (i.e., less than ~15 µg/L) of 1,1-DCE, which can inhibit cometabolism of other CAHs. In addition, note that the technology works only for TCE, 1,2-DCE, and vinyl chloride. Carbon tetrachloride, 1,1,1-TCA, chloroform, and methylene chloride are not degraded using this technology. Finally, laboratory microcosm studies should be available to indicate that indigenous microbial populations have the ability to cometabolize CAHs upon addition of toluene (or similar electron donor) and, if necessary, oxygen and other nutrients (e.g., nitrogen, phosphorus, etc.).

Figure 3-1 When to Consider Using *In situ* Cometabolism as a Potential Remedial Technology for Cleaning Up CAHs in Groundwater



The next step is to obtain approval from regulators and the community (if applicable). Special consideration is required for two reasons. First, the technology is an "innovative technology," meaning that little information exists on its cost and performance because of its limited application. Some regulators and communities may not want to consider innovative technologies, despite the potential for faster cleanup and cost savings. Second, the technology requires the injection of a moderately hazardous substance (i.e., toluene) into the groundwater. Even with a sound contingency plan covering all possible system malfunctions, regulators and communities may not accept a technology that involves injecting one toxic substance to clean up another. In addition, some states require review of injection technology proposals by state implemented underground injection control (UIC) programs, and a few states require a UIC permit for the implementation of such technologies (USEPA, 1996a).

Once it has been established that the technology may be used, it should be included as an option along with other remedial technologies to determine the best one to use. This process typically involves consultation and negotiation among parties responsible for the cleanup, their contractors, and various regulatory agencies. Regulatory considerations concerning the implementation and performance of this technology are presented in Section 6.

The software included in this manual uses models to determine whether site conditions are appropriate for use of the technology. While these models use some user-defined site-specific parameters, the use of more complex models that account for sorption, dissolution, and competitive inhibition may be prudent when designing the system. Such models developed for the Moffett Federal Airfield site (Semprini and McCarty, 1991) and the Edwards AFB site (McCarty *et al.*, 1998) successfully predicted performance of the technology and are suggested for use as reference.

4. SCREENING SOFTWARE: IS THIS TECHNOLOGY A POTENTIALLY COST-EFFECTIVE SOLUTION FOR MY SITE?

This section describes the use of the Aerobic Cometabolic *In Situ* Bioremediation Screening Software, which was designed to help the user determine whether aerobic cometabolic *in situ* bioremediation is a feasible and cost-effective solution for implementation at a site with contaminated groundwater. The software was developed at the Air Force Institute of Technology (AFIT), Wright-Patterson AFB, Dayton, Ohio, by Dr. Mark N. Goltz, Capt. Glenn C. Mandalas, Lt. John A. Christ, and Mr. Hugh Goltz. Further information about the software can be obtained by contacting Capt. Mandalas or Lt. Christ. Copies of the software may be obtained by contacting Erica Becvar of the Air Force Research Laboratory's Airbase and Environmental Technology Division. Contact information is provided below.

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The software was developed after a successful field demonstration of the technology at Edwards AFB. The field demonstration was designed and implemented by the Western Region Hazardous Substance Research Center (at Stanford University) with funding provided by the Headquarters, U.S. Air Force Environmental Quality Division, the Air Force Flight Test Center at Edwards AFB, the USEPA, and the Air Force Research Laboratories. Earth Tech, Inc., assisted with the implementation of the technology under contract with the Air Force Center for Environmental Excellence. The software and this section make frequent reference to the Edwards AFB site, the results from which have been published in peer-reviewed literature (McCarty *et al.*, 1998). It should be noted that the technology has been studied extensively in the laboratory and demonstrated at other field sites as well (see Section 7).

This section describes the essential elements of the software. Two goals of this section are (1) to describe the principles behind the calculations that the program executes to determine feasibility and cost of the technology, and (2) to explain the main user interface screens to help the user navigate through the program.

4.1 What This Program Does

The purpose of this software is to give the user an opportunity to determine whether aerobic cometabolic *in situ* bioremediation may be appropriate for use at a site with groundwater

contamination. This program evaluates the effectiveness of the technology, under user-defined site conditions, to contain and treat a migrating contaminant plume so that downgradient contaminant concentrations will meet acceptable regulatory levels.

This software will calculate, for a specific user-defined site, the number of injection and extraction wells required, the amount of primary substrate (microbial food source/toluene), the amount of electron acceptor (oxygen source for microbial stimulation), the in-well flowrates required for plume capture, and the approximate capital and operating costs to ensure regulatory specified concentrations are met downgradient of the treatment well system. In addition, the software contains brief explanations of the history and regulatory acceptance of the technology. These portions of the program will not be discussed in this section as they are discussed elsewhere in this guidance manual.

4.2 What Information Do I Need To Know About My Site To Use This Program?

In order to use this program, the following information about a particular site is required:

- Contaminants present
- Contaminant present at the highest concentration
- Whether the site is isotropic or anisotropic[1] or if a confining layer is present
- Aquifer saturated thickness
- Regional hydraulic gradient
- Plume width
- Influent concentration of the contaminant (i.e., contaminant concentration upgradient of the treatment well system)
- Desired effluent concentration of the contaminant (i.e., regulatory cleanup concentration downgradient of the treatment well system)
- Depth of the water table
- Hydraulic conductivity (or at least the geological composition of the aquifer material)

[1]Anisotropic: vertical hydraulic conductivity ÷ horizontal hydraulic conductivity < 0.1

4.3 Models and Assumptions

To simulate aerobic cometabolic *in situ* bioremediation, models have been developed. These models describe the physical and biochemical fate and transport processes occurring during technology implementation. The models also help assess whether this particular groundwater treatment option is feasible at a specific site. Additionally, models may be used to help optimize designs to control costs. Two basic models were employed in the development of the Aerobic Cometabolic *In situ* Bioremediation Screening Software. These models are the Biological and Flow Models. The models and assumptions associated with the use of each are discussed in Appendix A. The remainder of this section describes how the software calculates costs.

The models and assumptions used have been adapted from Christ (1997), and have been included in this manual for background information purposes only. The software program uses these models in making calculations of a plume contaminated with CAHs. It should also be noted that in order for this program to be useful, several other conditions regarding the site are assumed. Many of these conditions are outlined in Section 3.5 of this manual, "When Should I Consider Using This Technology at My Site?"

COST CALCULATIONS. From the calculations shown in Appendix A, the capital and operating costs associated with the treatment system design can be determined.

Capital Costs. The capital costs for implementing the treatment system are those costs associated with installation and start-up of the injection and extraction wells. The software estimates these costs based on the number of wells, N, determined necessary for complete capture of the contaminant plume, as calculated in equation (A.11) of Appendix A.2, Flow Model. The program assumes each well costs $10,000 for installation, and provides annual capital costs amortized over a user specified duration (in years) and anticipated interest rate.

(1) Annual Capital Costs = ($installation/well)*N*y

y = factor for determining annual costs based on number of years and interest rate

Operating Costs. Operating costs are defined as the costs required to maintain the system at the desired efficiency. These include pumping costs, primary substrate costs, and oxygen source costs. These costs are a function of the aquifer characteristics; the number of wells required to capture the entire contaminant plume and reduce the contaminant concentrations to regulatory levels; and, the calculated design efficiency. Operating costs do not include monitoring costs, such as sampling and analysis.

To arrive at the annual operating costs, the program makes several assumptions, all of which are discussed below. One important assumption, inherent in the flow model, is that the aquifer is isotropic, so that extracted water must be pumped to the ground surface for amendment with primary substrate and oxygen source prior to reinjection. If, in fact, the aquifer is anisotropic (as was the case at Edwards AFB Site 19) it may be possible to design a system with subsurface nutrient amendment (see Chapter 5). This would allow for significant savings in pumping costs. Due to potential clogging at the injection well, it is likely that periodic well redevelopment will be required. This cost is assumed to be $4,000/year/injection well. The software assumes the primary substrate being used is toluene, and using equation (A.7), Appendix A.1, calculates the substrate and oxygen concentrations for each of the three potential dissolved oxygen sources (air, oxygen gas, and hydrogen peroxide). The program then evaluates annual costs for primary substrate and oxygen, as discussed below.

Pumping Costs. Pumping costs are determined by the distance water must be lifted from the aquifer and the efficiency of the pump used:

(2) Annual pumping costs = $(P*N*Cost_P)/\eta + N*Cost_R$

where

P	=	Power needed to lift water ($P = \gamma*H*Q$) [ML^2T^{-3}]
Q	=	Annual flow in each well [L^3/well-yr]
γ	=	Specific gravity of water [$ML^{-2}T^{-2}$]
H	=	Distance to water table or distance the water is lifted [L]
N	=	Number of wells
$Cost_p$	=	Pumping Costs [$\$M^{-1}L^{-2}T^2$]

$Cost_R$ = Well redevelopment costs [$/well-yr]
η = Pump wire-to-water efficiency (Christ, 1997).

Primary Substrate Costs. Annual primary substrate costs are calculated based on the flow rate and the desired substrate concentration. Note that primary substrate is only introduced into N/2 injection wells.

(3) Annual primary substrate costs = $(N/2)*Q*C_D*Cost_{ps}$

where
$Cost_{ps}$ = Cost of Primary substrate [$/M]
Q = Annual flow in each well [L^3/well-yr]
C_D = Primary substrate concentration [ML^{-3}]

Electron Acceptor Costs. Annual electron acceptor costs are determined similarly:

(4) Annual electron acceptor costs = $(N/2)*Q*C_A*Cost_{EA}$

where
$Cost_{EA}$ = Cost of oxygen source [$/M]
Q = Annual flow in each well [L^3/well-yr]
C_A = Oxygen source concentration [ML^{-3}]

The total costs (TC) of implementing the treatment system design is equal to the sum of the capital and operating costs calculated above:

(5) TC = Capital Costs + Pumping Costs + Primary Substrate Costs + Electron Acceptor Costs

The assumptions and basic costs implanted in the software package are listed in Table 4-1.

Table 4-1. Aerobic cometabolic *in situ* bioremediation Screening Software Assumptions and Standards

CAPITAL COSTS	
Well Installation ($/well)	10,000[1]
Blower Cost (when air is selected oxygen source) ($/unit)	250
Number of Blowers Purchased (when air is selected)	2
OPERATING COSTS	
Well Redevelopment ($/injection well-yr)	4,000[1][2]
Electricity ($/Kw-hr)	0.20[2]
Wire-to-Water Efficiency (%)	63
Primary Substrate (toluene) ($/kg)	0.2[3]
Electron Acceptor Air/O_2/H_2O_2 ($/Kg)	2.77[4]/1.74[5]/4.0[2]
Energy costs for blower operation (air)	Negligible
Monitoring Costs	Not considered

(1) Tessier, 1998
(2) Hopkins, 1996
(3) McCarty *et al.*, 1997
(4) Pool Products Inc., 1997 cost data
(5) Based on Ohio distributor cost

4.4 Running the Program

In order to use this program, a minimum of a 486, 33 MHz processor, 16 MB RAM, Microsoft Windows 95, and Microsoft Excel 95 or later must be available. There must be at least 1.3 MB free disk space on the hard drive. The software is user friendly and operates on a "point and click" basis using a standard mouse. This section describes how to navigate through the program to obtain feasibility and cost data for implementation of aerobic cometabolic *in situ* bioremediation at a particular site. It should be noted that the program also provides background information and references for the technology, discusses regulatory acceptability, and has other information similar to material found elsewhere in this manual. Access to these portions of the program is obtained by simple "point and click" procedures. These portions of the program are not discussed below.

Figure 4-1 shows the first screen (Welcome screen) that appears upon opening the software. The Welcome screen allows you to review the license agreement, start the program, or close the program by clicking on the appropriate box. Additionally, the user may choose to resize the viewing area if the default size is larger than the monitor screen size. Click on <START>, and the Software Description screen appears (Figure 4-2). This screen provides a brief overview of the technology and the program. The only option from this screen is to click on <MAIN MENU>. Upon doing so, the Main Menu screen appears (Figure 4-3).

Figure 4-1. Welcome Screen

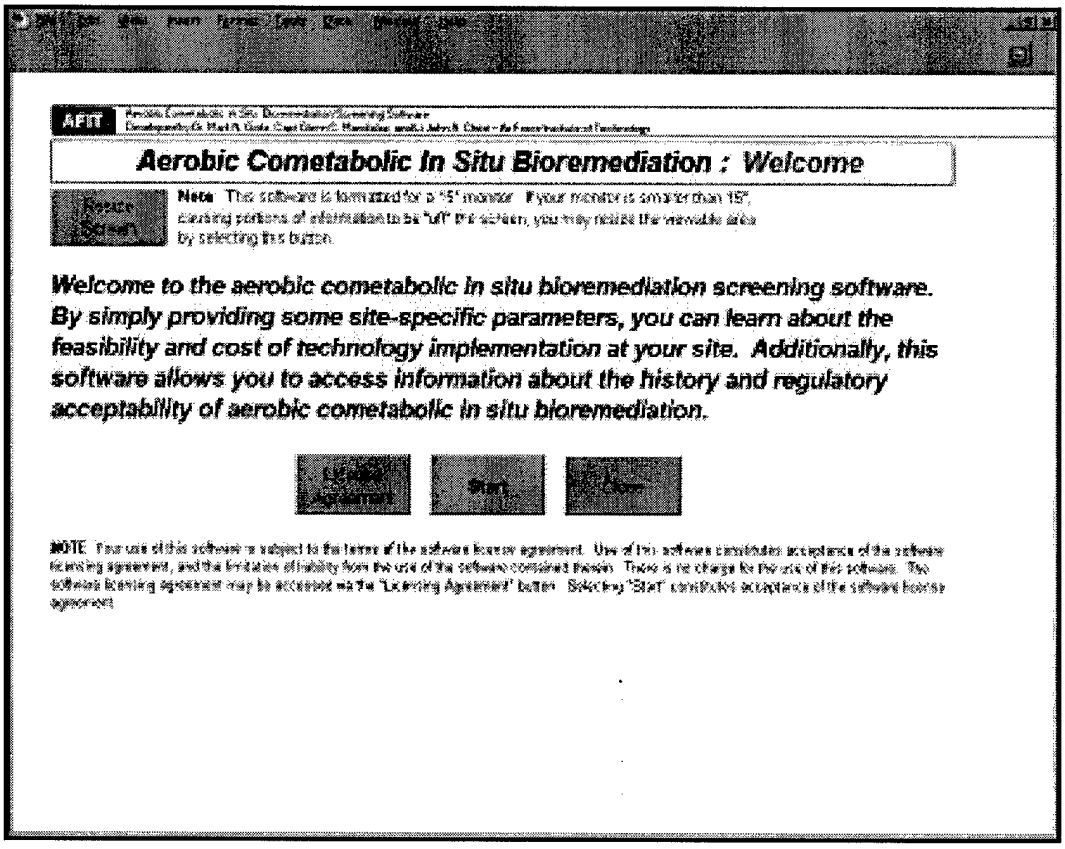


Figure 4-2. Software Description Screen

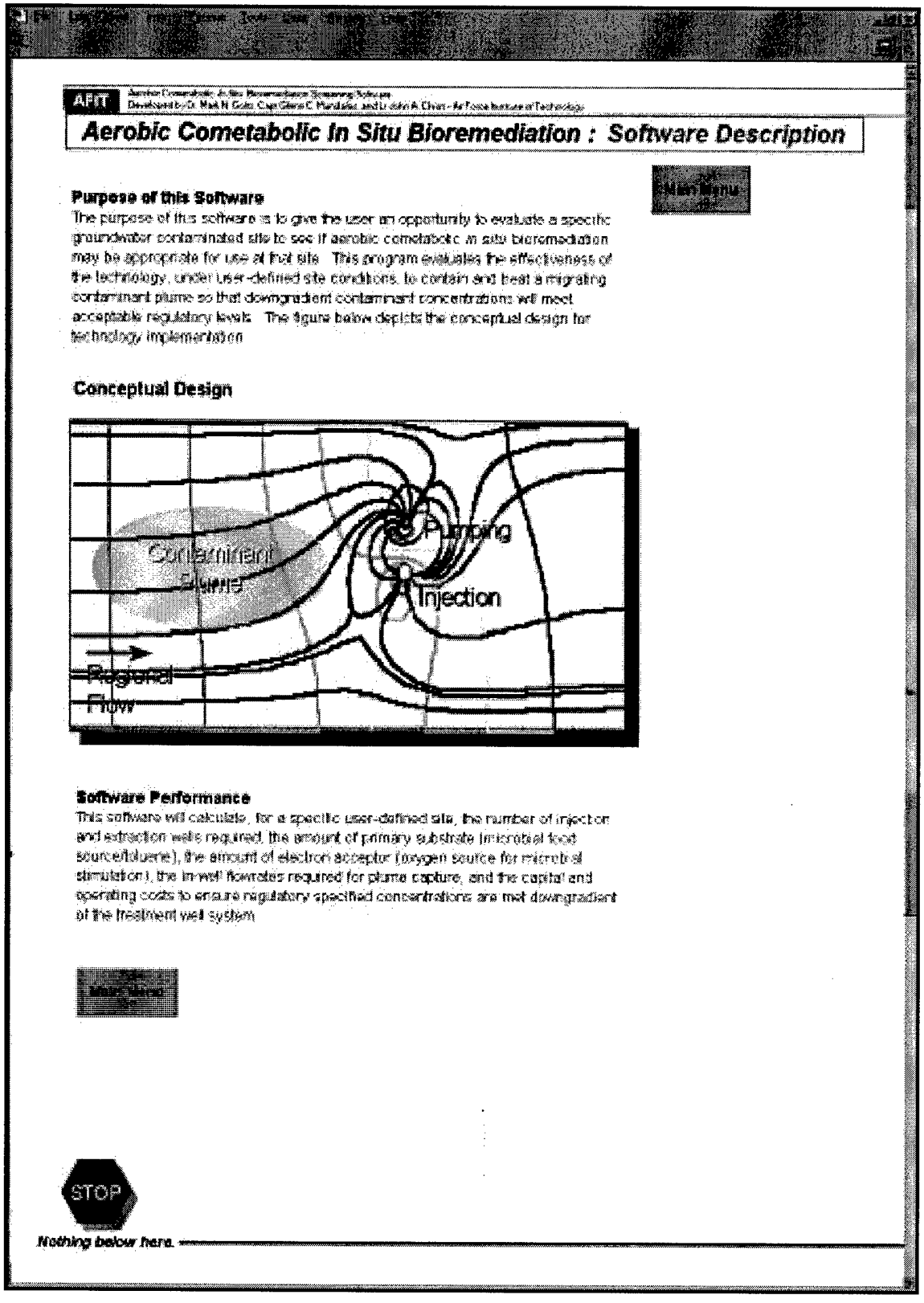
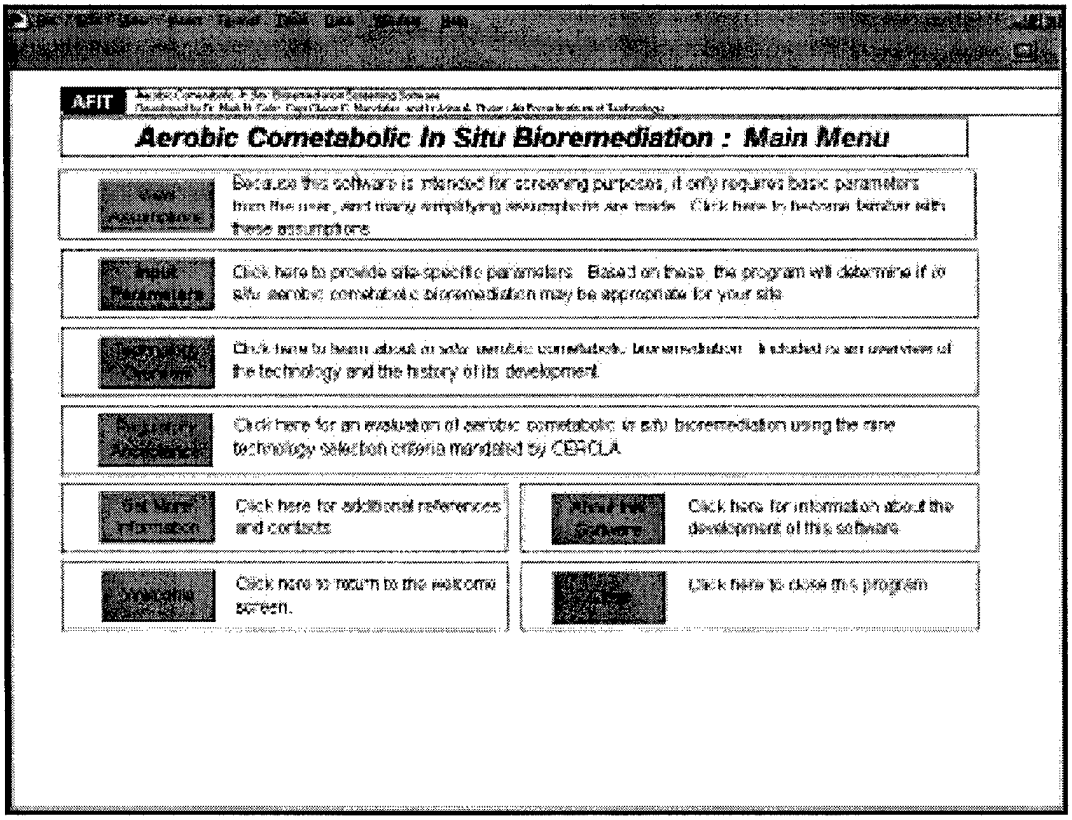


Figure 4-3. Main Menu Screen



There are several choices on the Main Menu, each of which is briefly described on the Main Menu screen. The first listed option is to view the basic assumptions made regarding the contaminated site and corresponding plume. The assumptions are discussed in Section 4.3 and Appendix A, which covers the mathematical models developed for the software.

To begin entering site-specific data so that the program can perform its calculations, choose <INPUT PARAMETERS>. The first Parameter Input screen queries you as to whether the site is isotropic or anisotropic, and allows selection from a list of the contaminants present on site (Figure 4-4). The user is also prompted to indicate which one of the contaminants is present in the highest concentration. Once finished making these selections, the user should click <OK>. (To exit this part of the program and return to the main menu, click the <MAIN MENU> button).

Clicking <OK> will bring the user to the next Parameter Input screen (Figure 4-5). On this screen, the user enters the remediation project life (in years), and the interest rate to which funds borrowed to complete the project would be subjected. In addition, the following information about the contaminated aquifer must be entered:

- Aquifer thickness (m)
- Regional gradient (m/m)
- Plume width (m)
- Influent concentration (of the chlorinated aliphatic hydrocarbon having the highest concentration in the plume) (mg/L)

Figure 4-4. Parameter Input Screen 1

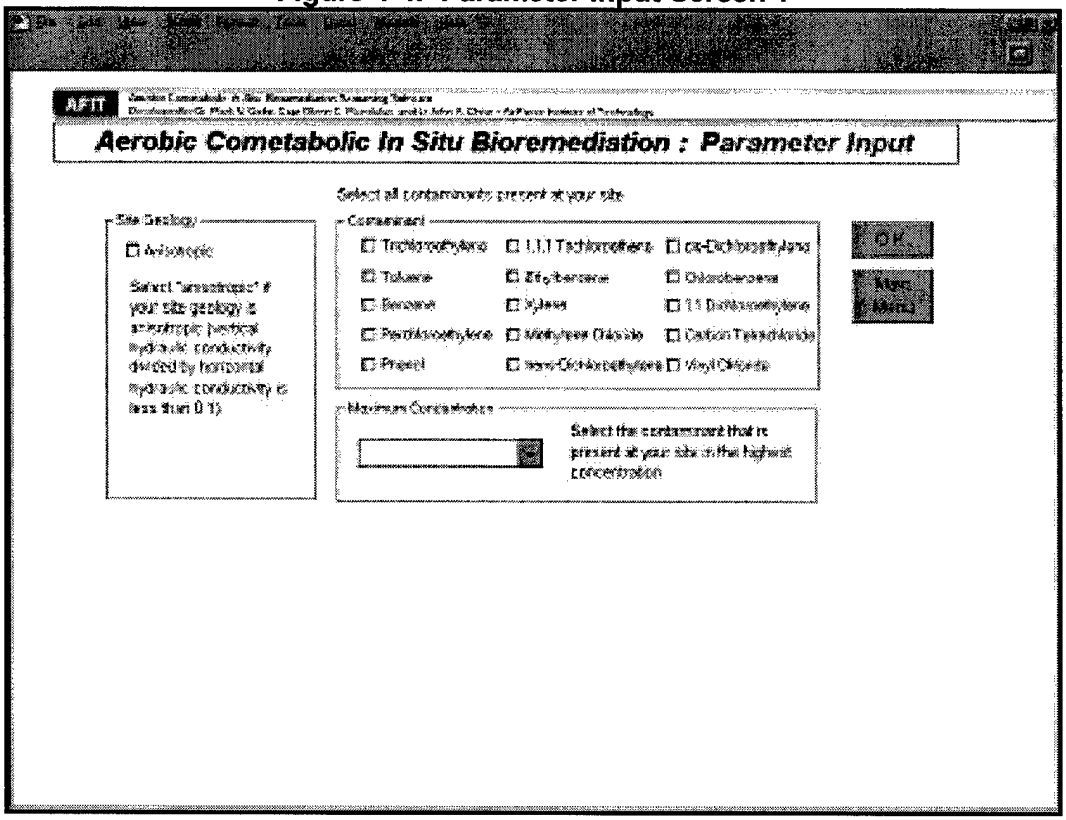
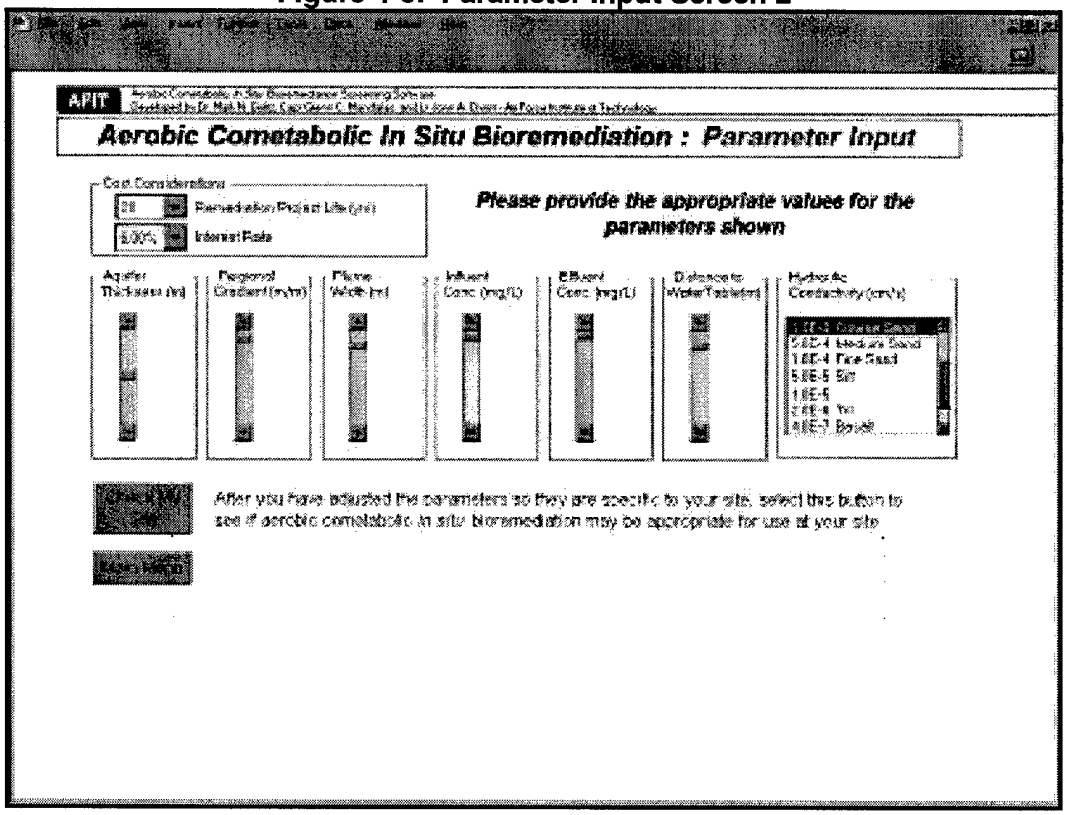


Figure 4-5. Parameter Input Screen 2

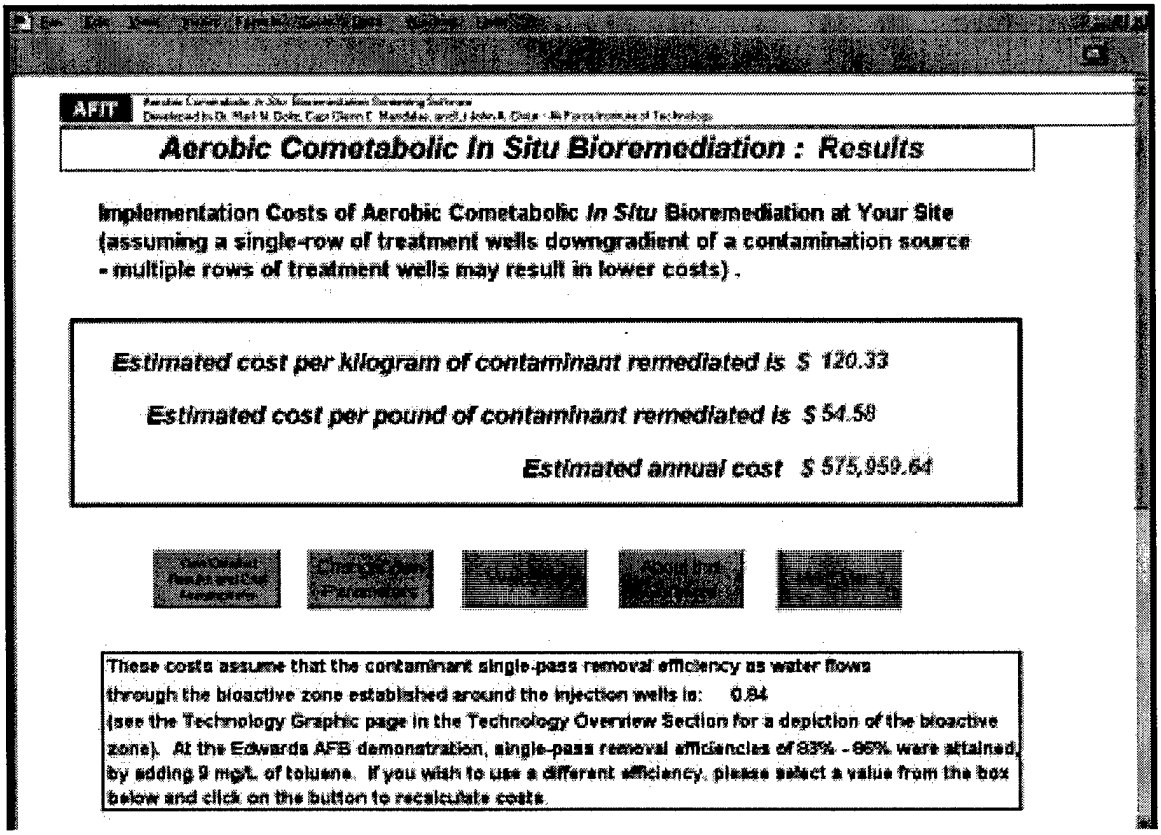


- Desired effluent concentration (of the chlorinated aliphatic hydrocarbon having the highest concentration in the plume) (i.e., regulatory cleanup concentration) (mg/L)
- Distance to the water table (m)
- Hydraulic conductivity (cm/s) (or at least the geological composition of the aquifer material)

Each entry of a parameter value is made by adjusting a sliding scale of values using the mouse. When finished adjusting the input parameters for your site, click <CHECK MY SITE>. This will connect the user to the Results screen. The program is designed to notify the user if an entered parameter is beyond the range of the software. If this occurs, a message appears on the screen stating such. To abort the operation and return to the main menu, click <MAIN MENU>.

The Results screen provides cost estimates for remediating the specified site in terms of dollars per kilogram of contaminant removed, dollars per pound of contaminant removed, and dollars per year (i.e., annual cost) (Figure 4-6). To get more information about how the cost was calculated, click <VIEW DETAILED RESULTS AND COST ASSUMPTIONS>, and the Details Screen will appear. Other options available from this screen include <CHANGE INPUT PARAMETERS>, <GET MORE INFORMATION>, <WELCOME>, <ABOUT THIS SOFTWARE>, and <MAIN MENU>. All of these options have been described or are self-explanatory. The user may also select a new single-pass treatment efficiency and recalculate costs based on the new efficiency.

Figure 4-6. Results Screen



The Details screen contains a wide range of information and requires the user to scroll down to see all of the costs (Figure 4-7). Information provided includes the following:

- a description of the method used to calculate the cost
- a pie chart and table showing the distribution of cost
- a list of assumptions made when calculating cost
- the program's selected oxygen source and cost associated with alternative oxygen sources
- the number of treatment wells required using the selected and alternative oxygen sources
- the well flow rate (cubic meters/day)
- the water table draw down due to pumping (m)

Costs are calculated based on the least expensive of the three potential dissolved oxygen sources: air, oxygen gas, and hydrogen peroxide. Following the detailed costs for the selected alternative, all three dissolved oxygen sources are compared for feasibility. The comparison lists the number of wells required for each alternative; the concentration of primary substrate and electron acceptor required per liter of water; and the costs for contaminant removal on a per pound or kilogram basis. This feature allows the user to compare the costs of the other two dissolved oxygen sources with the least expensive alternative.

At the top and bottom of the Details screen are button bars enabling the user to return to the Main Menu screen (<MAIN MENU>) or the Results screen (<BACK TO COST>). The user can step back through previous screens in the program or connect to text screens not described here using the button bars present on each screen.

Though based upon many simplifying assumptions, the screening software allows a user to learn more about the technology, as well as helping to determine whether *in situ* aerobic cometabolic bioremediation is appropriate under specified site conditions. If it appears that the technology may be appropriate, further study and more detailed design is required. This is discussed in Chapter 5.

Figure 4-7. Details Screen

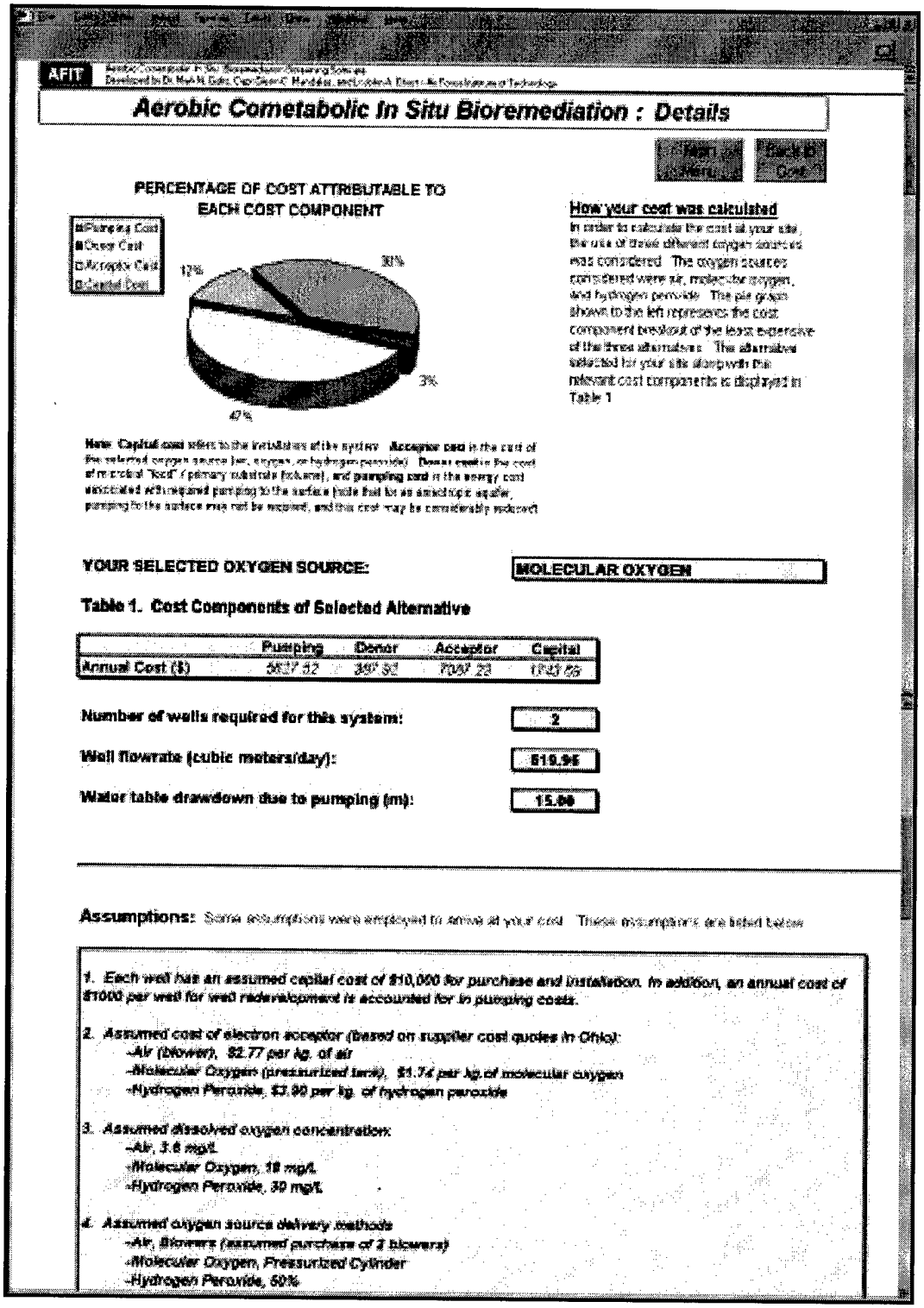
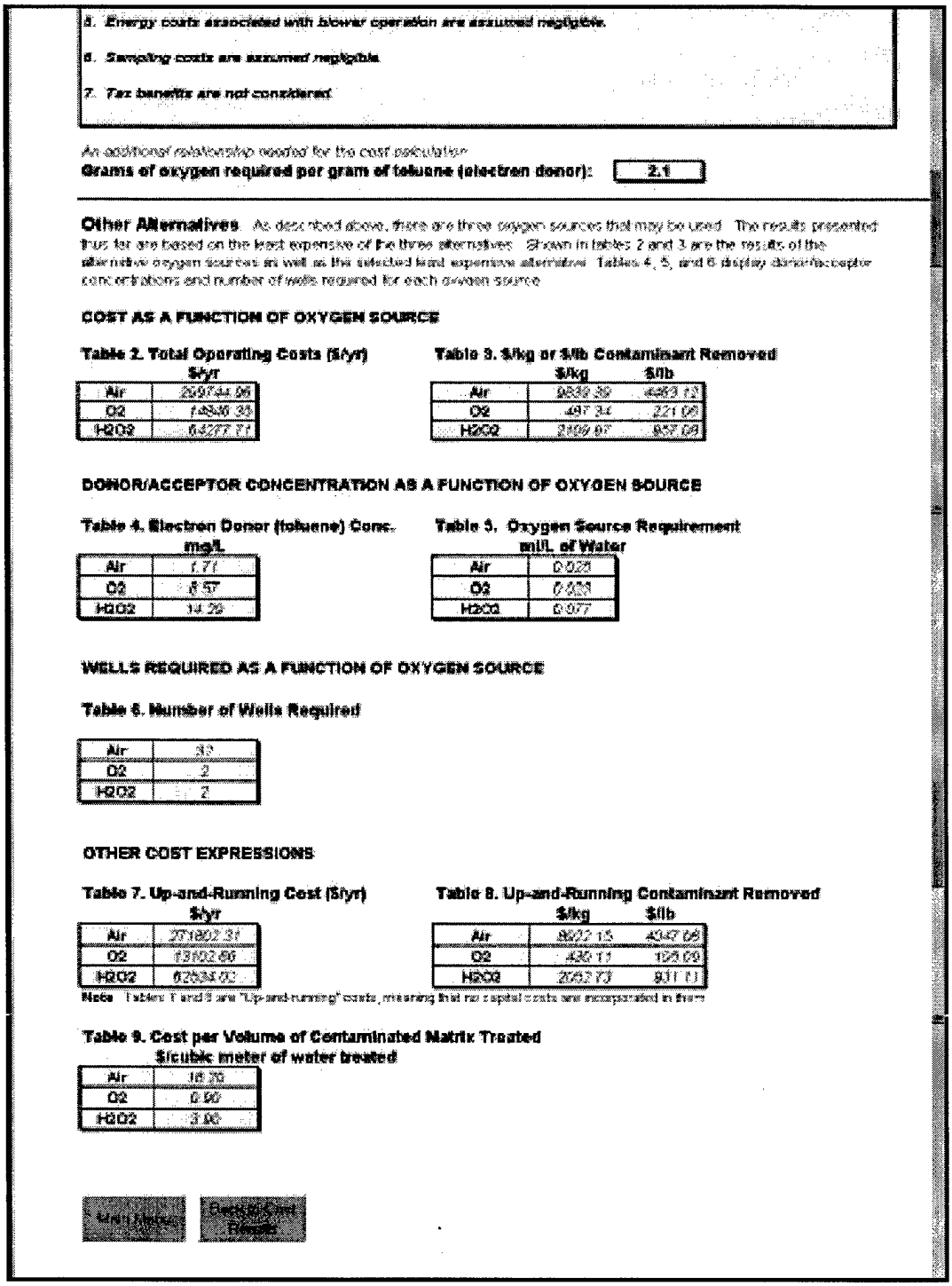


Figure 4-7. Details Screen (Continued)



5. *Energy costs associated with blower operation are assumed negligible.*

6. *Sampling costs are assumed negligible.*

7. *Tax benefits are not considered.*

An additional relationship needed for the cost calculation

Grams of oxygen required per gram of toluene (electron donor): 2.1

Other Alternatives. As described above, there are three oxygen sources that may be used. The results presented thus far are based on the least expensive of the three alternatives. Shown in tables 2 and 3 are the results of the alternative oxygen sources as well as the selected least expensive alternative. Tables 4, 5, and 6 display donor/acceptor concentrations and number of wells required for each oxygen source.

COST AS A FUNCTION OF OXYGEN SOURCE

Table 2. Total Operating Costs ($/yr)

	$/yr
Air	259734.56
O2	14536.33
H2O2	64277.71

Table 3. $/kg or $/lb Contaminant Removed

	$/kg	$/lb
Air	9830.39	4463.13
O2	497.34	221.06
H2O2	2309.87	957.69

DONOR/ACCEPTOR CONCENTRATION AS A FUNCTION OF OXYGEN SOURCE

Table 4. Electron Donor (toluene) Conc.

	mg/L
Air	1.71
O2	8.57
H2O2	14.29

Table 5. Oxygen Source Requirement

	ml/L of Water
Air	0.023
O2	0.023
H2O2	0.077

WELLS REQUIRED AS A FUNCTION OF OXYGEN SOURCE

Table 6. Number of Wells Required

Air	32
O2	2
H2O2	2

OTHER COST EXPRESSIONS

Table 7. Up-and-Running Cost ($/yr)

	$/yr
Air	271802.31
O2	13102.66
H2O2	62534.02

Table 8. Up-and-Running Contaminant Removed

	$/kg	$/lb
Air	8622.15	4047.06
O2	435.11	193.09
H2O2	2063.73	931.11

Note: Tables 7 and 8 are "Up-and-running" costs, meaning that no capital costs are incorporated in them.

Table 9. Cost per Volume of Contaminated Matrix Treated

	$/cubic meter of water treated
Air	16.20
O2	0.90
H2O2	3.90

5. TECHNOLOGY IMPLEMENTATION

This section describes how to implement an aerobic cometabolic *in situ* bioremediation system based on the full-scale demonstration at Edwards AFB Site 19. Considerations for each step of implementation are presented, and sample results from the Edwards AFB site are provided. This Section is divided into six subsections: Microcosm Studies (Section 5.1), System Design (Section 5.2), System Monitoring and Testing (Section 5.3), System Start Up (Section 5.4), System Operation (Section 5.5) and Cost (Section 5.6).

5.1 Microcosm Studies

Once it has been determined through application of the screening software that aerobic cometabolic *in situ* bioremediation may be an appropriate technology for the site, further investigation is necessary to verify the feasibility of the technology. Along with physico-chemical, geological, and hydrological parameters, the feasibility of a given contaminated site to undergo *in situ* bioremediation is dependent on the capacity of the indigenous microbial population to degrade the compound(s) of interest given the appropriate stimulating conditions. **The biodegradation potential of a specific organic contaminant at a given site needs to be determined prior to design and construction of a treatment system, generally through laboratory studies.** Such studies have shown that depending on site geochemistry, nutrients (e.g. nitrate and phosphate) may need to be supplied to the indigenous microorganisms so that they can metabolize the primary substrate. When considering which laboratory studies to apply in order to simulate biodegradation potential, it is critical to ask whether laboratory test conditions are applicable and comparable to processes that will occur in the field.

A commonly selected lab microcosm for simulating aquifer natural processes is a glass column containing aquifer material. However, over the past several years at Stanford University, column microcosms were found to have several limitations. First, disturbed aquifer material when placed in laboratory columns exerts an oxygen demand much greater than experienced in the field, thus limiting the concentration of primary substrate that can be added because of oxygen limitations. Second, small columns were found most desirable for use because of limited aquifer material availability, the desire to test many different conditions at a time, and the necessity to operate columns under aseptic conditions. However, the desire for small microcosms limits the amount of sample available for analysis. Third, sorption of chlorinated solvents to aquifer material is a significant phenomena, and because of the high solids to water ratio in columns, a very long time is then needed to reach steady-state operation where biodegradation potential can best be evaluated (Jenal-Wanner and McCarty, 1997).

In order to solve the above problems and yet maintain the advantage of a small microcosm, it is useful to develop a semi-continuous slurry microcosm system. Stanford University researchers demonstrated the value of this method by developing a slurry microcosm using uncontaminated soil from the Moffett Federal Airfield test site, where TCE was efficiently biodegraded *in situ* by aerobic cometabolism in the presence of phenol or toluene. Removal efficiencies of TCE and the primary substrate consumption ratios of oxygen to the primary substrate and the oxygenase enzyme induced in the slurry microcosm were similar to field results found during pilot scale testing of the technology at Moffett Federal Airfield (Hopkins and McCarty, 1995). The slurry method was then applied to evaluate the TCE degradation potential for aquifer material and groundwater from the Edwards AFB where full-scale application was contemplated. One main difference between the slurry microcosm method and both the column approach and field conditions is the lower solids to liquid ratio in the slurry microcosm. Jenal-Wanner and McCarty (1997) studied the effect of this difference using duplicate microcosms with 2 millimeter (mm) glass beads for increased surface area for microbial attachment and microcosms with increased aquifer material. The results of this comparison revealed no differences in TCE

removal, suggesting that the low solids/liquid ratio of the slurry approach had no negative impact on TCE removal. Another advantage of the slurry approach over the column microcosm is that slurry microcosms can readily be sampled for total biomass concentration determinations, an analysis that is exceedingly difficult with column microcosms. The application of the slurry method to the Moffett Federal Airfield test site and the Edwards AFB site for preliminary evaluation of the technology's applicability will serve as the primary model for development of a semi-continuous slurry microcosm system. Because this method is a specialized procedure not typically run in a commercial laboratory, refer to university laboratories such as Oregon State University to have a slurry microcosm study conducted.

The first step in developing the slurry microcosm is to obtain aquifer material from the site. A soil boring should be performed in a representative region of the aquifer. At the Edwards AFB site, cores of 15 cm length and 5 cm diameter were collected from four different depths: 10.4 m, 12.2 m, 14.3 m, and 20m. Each core cap is removed and the top 20 mm of aquifer material is removed aseptically. Then a 4 cm diameter sterile steel cylinder is driven into the remaining core. The aquifer material is scraped from the cylinder into a sterile glass bottle. Aquifer materials are manually mixed with 100 mL of filter-sterilized groundwater to achieve uniformity. The mixture is then equally distributed between eight sterile, 65 mL screw cap glass bottles, which are then filled with filter-sterilized oxygen-saturated groundwater. The microcosms are capped with Teflon-lined silicon septa and open-hole screw caps.

Eight microcosms are prepared with aquifer material from each of four depths for a total of 32 microcosms. Triplicate microcosms from each depth are fed phenol, a second triplicate set receives toluene, duplicate microcosms are not fed a primary substrate, and two control microcosms contain no aquifer material and are exchanged with groundwater containing only TCE and dissolved oxygen. Additional triplicate sets can be used to evaluate other potential primary substrates. Differences in TCE removal between triplicate microcosms for each substrate and aquifer depth should also be evaluated. Edwards AFB microcosms showed significant variation between depths, which may reflect differences in TCE removal ability of dominant organisms at each depth.

The next step in the slurry development process is incubation of the microcosms. Both phenol and toluene should be used as primary substrates for comparison. It should be noted that in both Moffett Federal Airfield and Edwards AFB microcosms, toluene ortho-monooxygenase was the dominant TCE-oxidizing enzyme present. However, due to varying site conditions, either substrate may be optimal at a given site. At the beginning of each incubation period, 300 µL of a 19 millimole (mM) phenol stock solution and 700 µL of a 6 mM toluene stock solution (toluene-saturated Milli-Q-water) are added to the respective microcosms. In order to avoid possible substrate toxicity, both spikes should be added in three separate pulses within the first 12 h of an incubation period, i.e., 3x100 µL for phenol, and 1x300 µL and 2x200 µL for toluene. This pulse approach was found to be critical in the Moffett Federal Airfield microcosm, where TCE removal declined from 90% to 40% when the total mass of phenol was added in a single spike. The necessity of three successive pulses is a disadvantage of the slurry approach and may be related to the toxicity of the primary substrates. Such a toxicity problem does not exist significantly in the field or in column microcosms, perhaps because bacteria in micropores are not exposed to the higher concentrations. All microcosms are spiked at each period with 20 µL of a 1 mM TCE stock solution (TCE-saturated Milli-Q-water). Microcosms are inverted in the dark at 20°C on a rotating shaker at 180 rpm.

During incubation microcosms are sampled for measurement of dissolved oxygen, primary substrate, and TCE analyses before and after each incubation period. In the Edwards AFB microcosm, dissolved oxygen concentration was above air saturation, which is the likely result of the slurry method. Because TCE and toluene are volatile, a special procedure is used to spike and sample the microcosms. The open-hole screw caps are removed from microcosms

without displacing the septa. The amount of sample needed is pulled from the bottles by inserting the needle of a precision syringe between the septum and the rim of the bottles into the culture liquid. Sample volumes removed are carefully replaced immediately with sterile groundwater in order to prevent formation of a head space. Toluene, phenol, and TCE are added the same way, except that prior to their addition, the liquid volume of the microcosms is reduced by the volume of the primary substrate spikes to be added in order to prevent overflowing.

There are three primary rate coefficients needed for the biological model discussed earlier. These include the bacterial yield coefficient from primary substrate utilization Y (mg/mg), the organism decay rate b (d^{-1}), and the contaminant utilization rate K' (L/mg cell-d). The yield value Y can be taken from the biomass yield estimates from oxygen consumption during microcosm start-up, which for Edwards AFB were 0.77 and 0.59 g biomass/g substrate for toluene and phenol, respectively. No information is available from these analyses for the decay coefficient b, but typically it lies between 0.1/d and 0.2/d (Lang, 1995). The contaminant utilization rate K' (L/mg cell-d) is estimated as the ratio of the maximum contaminant consumption rate (M contaminant/M Cell - T) to the contaminant half-saturation constant (M/L^3) ($K' = K_2/K_{s2}$). These values are based upon an estimate of active biomass concentration (X_a). Examples of contaminant utilization rates for several contaminants is given in Table 5-1.

Table 5-1. Contaminant Utilization Rates

Contaminant	Contaminant Utilization Rate K' (L/mg cell-d)
trichloroethylene (TCE)	0.07[a]
trans-dichloroethylene (trans-DCE)	0.25[b]
cis-dichloroethylene (cis-DCE)	0.035[b]
vinyl chloride (VC)	0.25[b]

a. Jenal-Warner and McCarty, 1997
b. Semprini and McCarty, 1991

A number of inter-relationships should be evaluated based on the results of the slurry microcosm system to determine the physiological requirements of the microbial population in the aquifer material and groundwater, including oxygen availability, substrate addition and concentration, nutrient sufficiency, and the necessity for physical support. The effect of dissolved oxygen on phenol/toluene consumption rate can be studied with duplicate microcosms amended with groundwater containing 0.12 mM phenol and four different initial dissolved oxygen concentrations (0.3, 0.6, 0.8, and 1 mM). The Moffett Federal Airfield microcosm dissolved oxygen requirement for phenol was found to be 0.59± 0.04mM or 4.9 mol dissolved oxygen per mol phenol. Because some substrate is diverted for organism synthesis, the oxygen demand of control microcosms not containing phenol was first subtracted for this evaluation. Higher dissolved oxygen concentrations had no inhibiting effect on phenol degradation. However, when dissolved oxygen dropped below air-saturation (0.29 mM), phenol degradation rate decreased.

To evaluate the effect of phenol/toluene concentration, duplicate microcosms with oxygen saturated groundwater (1 mM) are incubated at four different initial phenol/toluene concentrations (0.06, 0.10, 0.17, and 0.22 mM). Oxygen-saturated conditions in the Moffett Federal Airfield microcosm were insufficient for oxidation of 0.22 mM phenol, thus 0.17 mM phenol was the highest concentration used in subsequent studies. To evaluate nutrient sufficiency in aquifer material and groundwater, it is suggested that nitrate, phosphate, magnesium sulfate and a mixture of trace elements be added to separate microcosms,

respectively. With the Moffett Federal Airfield field system, no nutrient or buffer addition was necessary since these supplements did not increase TCE removal or dissolved oxygen consumption rates compared to non-amended microcosms. However, this might not always be the case and must be evaluated for each site of interest. The lack of sufficient nitrogen in soil has been shown to impair biodegradation of toluene (Hopkins, *et al.*, 1993) and phenol (Roberts, *et al.*, 1989).

Aerobic TCE biodegradation was successfully stimulated in the semi-continuous batch microcosms by addition of either toluene or phenol as primary substrates in both Moffett Federal Airfield and Edwards AFB microcosms. TCE and primary substrate removal efficiencies, oxygen to primary substrate ratios, and oxygenase enzyme expression were all reasonably similar between the two studies. The average percentage of TCE removed correlated well with primary substrate concentrations. Oxygen demand was essentially identical, and the dominant oxygenase appeared to be toluene ortho-monooxygenase in both studies.

It should be noted that there were some noticeable differences in performance with Edwards AFB aquifer material taken from different depths. The shallowest sample (10.4 m) and deepest sample (20.0 m) approached steady-state faster and provided higher TCE removals (98 to 100 percent) with both phenol and toluene than samples from the intermediate depths (12.2 m and 14.3 m), which provided TCE removals in the 87 to 94 percent range. These differences likely result from differences in the bacteria that come into dominance at the respective depths. The actual TCE degrading capability of a given mixed population that happens to develop in one aquifer sample is thus likely to differ greatly from another. This should reinforce the importance of taking samples at multiple depths for evaluation, and is why microcosm studies are highly desirable before instigating aquifer remediation by cometabolism.

5.2 System Design

5.2.1 Primary Substrate Selection

The choice of primary substrate to use depends first upon results from preliminary laboratory studies specific to the site. Prior studies (Hopkins and McCarty, 1995; Jenal-Wanner and McCarty, 1997) have shown that efficient TCE cometabolism can be obtained with either phenol or toluene. Methane, the original substrate used in the Moffett Federal Airfield studies, was demonstrated to be far less effective in degrading both TCE and *c*-DCE (this is why it is not recommended as a possible alternative). (Note: Although methane is used in alternative system designs such as the one developed at the Savannah River Site [SRS] [see Section 7.3], that design combines cometabolism with air stripping, and cometabolism accounts for only about 25.5% of contaminant loss [SRS, 1998].) Other compounds shown to promote cometabolism by microorganisms include propane (Wackett *et al.*, 1989), ethylene and propene (Hartmans *et al.*, 1992; Ensign *et al.*, 1992; Roberts *et al.* 1989), cresol (Nelson *et al.*, 1988), ammonia (Arciero *et al.* 1989; Rasche *et al.*, 1991), isoprene (Ewers *et al.*, 1991) and isopropyl benzene (Dabrock *et al.*, 1992). However, the effectiveness of these compounds for promoting cometabolism has not been demonstrated in the field. The optimal substrate may vary based on hydrogeology, indigenous microbial populations, and other site-specific characteristics. It is therefore important to carefully consider results from the slurry microcosm studies for your site in order to weigh the relative effectiveness of each substrate.

Recent microbiological studies have given cause for carefully considering another factor when choosing a primary substrate. Mars *et al.* (1998) point out that, because cometabolism of CAHs provides no energy for microorganisms and may produce cytotoxic byproducts, those organisms that degrade primary substrate but do not cometabolize TCE will be selected for

under substrate limiting conditions (i.e., conditions used during operation of a treatment system). Indeed, their laboratory study demonstrated the negative effect of cometabolic degradation of TCE on the competitive behavior of toluene-degrading microorganisms that transform TCE. Moreover, Munakata-Marr *et al.* (1997) reported a gradual decline in the breakdown of TCE in phenol-fed microcosms containing aquifer material from the Moffett Federal Airfield site, while degradation of phenol remained complete. Although Fries *et al.* (1997) found a large variety in the capacity of toluene- and phenol-degrading microorganisms isolated from the Moffett Federal Airfield site to transform TCE, they anticipated that organisms that do not degrade TCE will take over the population. This information led Mars *et al.* (1998) to propose *o*-cresol as an alternative to toluene and phenol, because it can be degraded only by enzymes that also convert TCE and there are no known alternative pathways. (There are at least five pathways for the degradation of toluene, each involving a different oxygenase enzyme). *o*-Cresol is degraded by the same toluene monooxygenase found in organisms that dominated the TCE-contaminated Moffett Federal Airfield (Shields *et al.*, 1991).

For the purposes of this manual, we will consider only the use of either phenol or toluene. If microcosm studies demonstrate comparable effectiveness of both phenol and toluene, there are several factors to consider when deciding between the two. Both phenol and toluene are relatively inexpensive chemicals. In addition to cost, toluene offers several advantages over phenol as a primary substrate. Toluene, a component of gasoline and a naturally produced organic chemical, is a common groundwater contaminant and already is present at many contaminated sites. Based on toxicological data, a maximum contaminant level (MCL) for toluene in drinking water of 14.3 mg/L was recommended (Lederer, 1995). The USEPA, taking a more conservative approach, has promulgated both a health-based MCL and a maximum contaminant level goal (MCLG) for toluene of 1 mg/L (Pontius, 1993). Phenol, which is rarely found in water at levels where health effects would be anticipated, has had no MCLG established, though toxicological data suggest that to avoid health risks, drinking water concentrations should not exceed 3.5 mg/L (Lederer, 1995). Considering aesthetics, toluene has an odor threshold of 24 µg/L and a taste threshold of 120 to 160 µg/L (Alexander *et al.*, 1982). Phenol has odor and taste thresholds of 1,000 and 100 µg/L, respectively (World Health Organization, 1984). However, phenol is a particular problem since it reacts during chlorination to form chlorophenols which have extremely low taste and odor thresholds below 1 µg/L. In addition, pentachlorophenol and 2,4,6-trichlorophenol are probable human carcinogens (USEPA, 1993). Because of this, the World Health Organization recommends that the total phenol content be below 1 µg/L for water to be chlorinated, and the European Drinking Water Standard for phenol is set at 0.5 µg/L (World Health Organization, 1984). Thus, although phenol and toluene are similar from a health-effects perspective, the facts that toluene has an established MCLG, does not create taste and odor problems at concentrations below 24 µg/L, and is not a precursor to probable carcinogens when chlorinated, make it more attractive as a primary substrate. Another factor of importance is that toluene can be air stripped from groundwater while phenol cannot. This property could be important if it is found, after adding the primary substrate to the groundwater, its degradation is inadequate and it has to be removed. Additionally, toluene is a liquid which can be pump-fed neat to the treatment system.

Both field and laboratory studies at Edwards AFB have indicated that as long as adequate dissolved oxygen was present, toluene concentrations near 1 µg/L could be obtained from toluene biodegradation in the treatment system (McCarty *et al.*, 1998). This concentration is more than an order of magnitude below the taste and odor threshold, and several orders of magnitude below the drinking water MCLG. Again, this may or may not be the case at the site under consideration and should be determined based on studies specific to your site.

5.2.2 Primary Substrate Concentration

The primary substrate concentration is the amount of "food" provided to the microorganisms. There is a minimum concentration of primary substrate required to stimulate growth. Beyond this minimum concentration an increase in primary substrate increases the "single pass" treatment efficiency up to a point. However, due to competition between the primary substrate and the target contaminant for the active site on the enzyme, too much primary substrate can decrease the efficiency of the system. One should note that the simplified biological model presented in this document (see equations (A.4)-(A.6) in Appendix A.1) does not account for competitive inhibition (Christ, 1997), though the model may be used to approximate the bounds of the single-pass treatment efficiency for a given primary substrate concentration. Microcosm studies, such as those described in Section 5.1, should be used to determine the optimum primary substrate concentration. Recall from Section 5.1 that duplicate microcosms with oxygen saturated groundwater are incubated at four different phenol/toluene concentrations to determine the effect of substrate concentration. Based upon the Moffett Federal Airfield results (Hopkins and McCarty, 1995) and microcosm studies (Jenal-Wanner and McCarty, 1997), a time-averaged toluene concentration of 7 to 15 mg/L was proposed at Edwards AFB to achieve on the order of 90 percent TCE destruction with each pass through a circulation well.

A strategy of injecting in pure toluene for 30 minute pulses every 8 hours was used in the Moffett Federal Airfield and Edwards AFB studies. It should be emphasized that the primary substrate concentration discussed in the previous paragraph and in the biological model of Appendix A.1 is a time-averaged concentration. Thus, the actual concentration of primary substrate introduced for 30 minutes every eight hours is 16 times greater than the time-averaged concentration. This pulsing strategy helped distribute the toluene more uniformly through the aquifer, thereby reducing microbial clogging potential at the well screens. It also helped reduce the effects of competitive inhibition, where TCE degradation rate is reduced when primary substrate and TCE are simultaneously present. The design for the Edwards AFB evaluation also incorporated the toluene concentration and pulsing strategies used at Moffett Federal Airfield, with allowance for changes to compensate for site-specific conditions.

5.2.3 Oxygen Introduction

As noted previously, oxygen is the electron acceptor needed to stimulate microbial growth. There are three sources of dissolved oxygen that may be used: (1) air; (2) molecular oxygen; (3) hydrogen peroxide. The screening program discussed in Section 4 has been run for various "typical" scenarios and has shown that oxygen gas is most often the best (least expensive) source of dissolved oxygen. Air is rarely selected as the oxygen source since it supplies such low concentrations of electron acceptor. Hydrogen peroxide, due to its cost ($4.00 per kg or $1.80 per lb), is normally only selected when the effluent concentration is specified at a very low level so that high "single-pass" efficiencies are required. However, hydrogen peroxide may be needed to alleviate the problem of bioclogging, the preferential growth of microorganisms near the injection well where nutrients are most abundant (Taylor *et al.*, 1993). This preferential growth can cause the injection well to become partially clogged with biomass. Injected hydrogen peroxide enters the subsurface in the bioclogged zone in concentrations high enough to be toxic to the microorganisms responsible for the bioclogging. As the hydrogen peroxide migrates away from the well it hydrolyzes, providing valuable oxygen to the *in situ* system (Hopkins and McCarty, 1995; McCarty *et al.*, 1998).

Recall from the discussion in Section 5.2.2 that based upon the Moffett Federal Airfield results (Hopkins and McCarty, 1995) and microcosm studies (Jenal-Wanner and McCarty, 1997), a time-averaged toluene concentration of 7 to 15 mg/L was proposed at Edwards AFB to achieve on the order of 90 percent TCE destruction with each pass through the BAZ. Theoretically, 9 moles of oxygen are required for complete oxidation of a mole of toluene, with actual oxygen

requirements being about 6 mol/mol as a portion of the toluene is synthesized into bacterial cells. This translates into a minimum requirement of about 2.1 g oxygen per g toluene or up to 31 mg/L dissolved oxygen for 15 mg/L toluene using equation (A.7), Appendix A.1.

Some additional oxygen is needed beyond this to ensure aerobic conditions are maintained and to satisfy potential background oxygen demanding materials in the aquifer. Thus, 30-40 mg/L of dissolved oxygen was required at Edwards AFB to support the planned aerobic cometabolism (Hopkins and McCarty, 1995; McCarty *et al.,* 1998), and this was substantiated through the microcosm studies (Jenal-Wanner and McCarty, 1997). Either oxygen gas or hydrogen peroxide had been used as an oxygen source at Moffett Federal Airfield. It may be advantageous initially to add only oxygen gas because of the potential bactericidal properties of hydrogen peroxide. Once an active population is developed, hydrogen peroxide might then be used as an additional source of oxygen, which could also later help to suppress potential microbial clogging near the well screens due to these same bactericidal properties (Hopkins and McCarty, 1995). Additionally, due to hydrogen peroxide's solubility in water, higher dissolved concentrations could be achieved than through use of pure oxygen alone.

5.2.4 Treatment Well Number, Locations, and Pumping Rates

Making certain simplifying assumptions, Christ (1997) derived an analytical solution which permits determination of an overall treatment efficiency and capture zone width for a system of N injection and extraction wells arranged in a line on the x-axis of a coordinate system. N is an even number, and the well with the smallest (most negative) x-value is an extraction well. Injection and extraction wells alternate, each separated by a distance 2d from its neighbor. Steady groundwater flow with a Darcy velocity U in a homogeneous isotropic aquifer with a constant thickness (B) is assumed. The regional flow makes an angle α with the positive x-axis (measured counterclockwise from the x-axis). If we define the total interflow ratio (I_T) as the flow through all extraction wells that originated in injection wells divided by the flow through a single extraction well (note from the definition of I_{AVG} in Appendix A that $I_T=I_{AVG}*N/2$), Christ (1997) derives the following equation:

(6)
$$I_T = \frac{N}{2} - \frac{\psi_1 - \psi_2}{Q / B}$$

where

Q	=	Extraction/injection rate [L^3T^{-1}]
ψ_1	=	Stream function evaluated at stagnation point associated with left most extraction well (extraction well at location with the minimum x-value) [L^2T^{-1}]
ψ_2	=	Stream function evaluated at stagnation point associated with right most injection well (injection well at location with the maximum x-value) [L^2T^{-1}]
B	=	Aquifer thickness [L]
N	=	Total number of treatment wells (injection plus extraction)

Stagnation point locations and the value of the stream function at the stagnation points may be determined using standard methods (e.g., Christ, 1997; Javandel *et al.*, 1984). The values of ψ_1 and ψ_2 are a function of the distance between wells (2d), the Darcy velocity of uniform regional flow (U), well pumping rates (Q), aquifer thickness (B), the angle between the direction of regional flow and the x-axis (α), and the total number of wells (N).

Once the total interflow ratio for the treatment system is known, the capture zone width can be calculated using the following equation (Christ, 1997):

(7) $$CZW = \frac{Q}{UB}\left[\frac{N}{2} - I_T\right]$$

and by mass balance, the efficiency of the entire N-well treatment system (η) can also be calculated:

(8) $$\eta = \frac{\eta_{SP}}{1 - I_{AVG}(1-\eta_{SP})} \qquad \frac{C_{out}}{C_{in}} = \frac{(1-\eta_{SP})(1-I_{AVG})}{1 - I_{AVG}(1-\eta_{SP})}$$

where

I_{AVG}	=	the average interflow ratio = 2* I_T/N
η_{SP}	=	the single-pass treatment efficiency (calculated from equations (5)-(6))
C_{out}	=	contaminant concentration downgradient of the treatment system [ML^{-3}]
C_{in}	=	contaminant concentration upgradient of the treatment system [ML^{-3}]
η	=	overall treatment efficiency = 1 - C_{out}/ C_{in}

Note that this equation applies only to an N co-linear well system of single screened injection and extraction wells operating in a single confined aquifer. For a system of dual screened groundwater circulation wells (GCW) operating in two aquifers as in the Edwards AFB demonstration, or for a GCW system operating in a single aquifer where short circuiting between well screens is possible, more complex equations are required (see Christ, 1997: Chapter 3, equations 35a through 36b). Even more complex scenarios (wells that are not co-linear, heterogeneous conditions, etc.) must be modeled numerically.

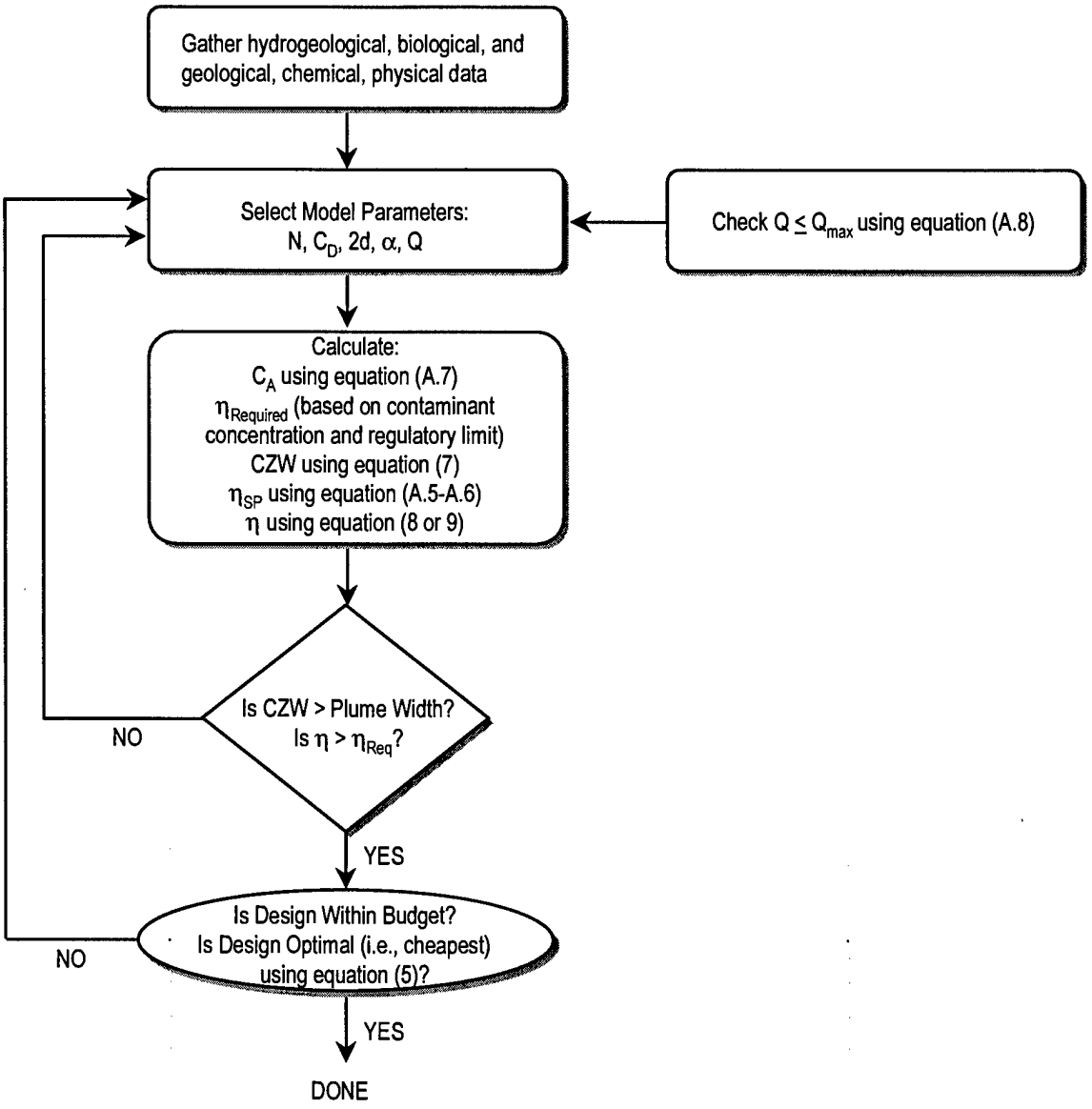
One specific scenario that may be worth discussing is the case where multiple rows of treatment wells in series are emplaced. In this case, if we assume all treatment well rows reduce contaminant concentration equally, the overall treatment efficiency for M treatment well rows in series will be:

(9) $$\eta = 1 - \left(\frac{C_u}{C_d}\right)^M$$

where C_u and C_d are the contaminant concentrations upgradient and downgradient, respectively, of a single treatment well row. As may be seen from equation (9), it is possible to obtain very high overall treatment efficiencies using treatment well rows in series.

The goal of the flow modeling is to calculate a value for the capture zone width (CZW) and the average interflow ratio (I_{AVG}). I_{AVG} can then be used, in conjunction with the value of the single-pass treatment efficiency (η_{SP}) calculated using the biological model and/or microcosm study results, to determine an overall treatment efficiency (η). Then, knowing the plume width (PW) and the required overall treatment efficiency of the bioremediation system (η_{Req}), it is possible to determine whether the specified treatment system attains design goals. Figure 5-1 shows how a system can be designed following this procedure.

Figure 5-1. Flow Diagram for System Design



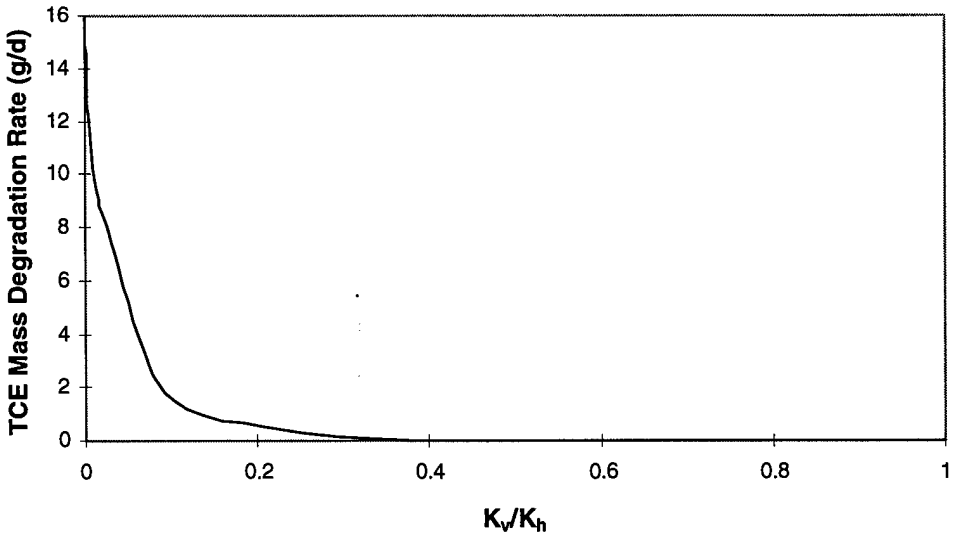
After initial data have been collected through a site investigation and microcosm study, reasonable values for engineering parameters (decision variables) can be selected and the variables which depend on these parameters can be calculated. These calculated variables can then be used to determine whether the current design meets the design constraints. Once a parameter set which meets the design constraints is found, this represents a solution to the problem (Christ 1997). It should be recognized that while there may be several solutions to the problem, the true optimal point is obtained when both the desired overall treatment efficiency is obtained *and* the cost of the treatment system is minimized. Calculation of cost is discussed in Section 5.6.

5.2.5 Impact of Hydraulic Conductivity Anisotropy

The design scheme outlined above is intended primarily for remediating a single isotropic aquifer with conventional injection/extraction treatment wells screened throughout the thickness of the aquifer. However, the two-dimensional analytical model was also shown to agree quite well with field data from the Edwards AFB site (Christ, 1997), where a GCW system in two aquifers was installed. This was in large part due to the anisotropic conditions at the site preventing flow in the vertical direction, which allowed each aquifer to be treated as an individual unit. However, vertical flow can be a significant factor if a dual-screen well design is to be implemented at sites where there is no aquitard separating the two screened sections of the treatment wells. Graphical presentations of the different ways in which a treatment system may be implemented under isotropic and anisotropic conditions are given in Section 5.2.6.

Christ (1997) investigated the implications of flow in the vertical direction on treatment efficiency using a numerical model (MODFLOW) which allows for anisotropic conditions and multiple screened wells in a single aquifer. The Edwards AFB scenario was modeled with a vertical hydraulic conductivity (K_v) that varied from 0 cm/s (aquitard present - two dimensional flow assumed) to 3.4×10^{-3} cm/s (isotropic conditions). Thus, the anisotropy ratio (K_v/K_h) was varied to determine how vertical flow affected the degree of treatment. Figure 5-2 shows the mass of TCE degraded per day as a function of the anisotropy.

Figure 5-2. TCE Degradation Versus Anisotropy Ratio



Notice that where $K_v/K_h = 0$ (aquiclude present), the TCE degradation rate is the highest. The amount of TCE degraded decreases as the system becomes more isotropic. As vertical flow

becomes easier, there is more short-circuiting between screens in a single well, and less treatment. In fact, when $K_v/K_h = 0.3$ all of the water being extracted by the pumping well is either originating in the injection screen of the same well or in the injection screen of the second treatment well. This means the system has become closed and the capture zone width has become zero. All of the water in the system is being treated to 0 µg/L, but no new water is being brought into the system. Thus, for the Edwards AFB parameters, the horizontal hydraulic conductivity must be at least about 10 times greater than the vertical hydraulic conductivity for the system to work effectively.

Before selecting a circulation well system for a single aquifer, then, it is important to consider the anisotropy ratio. The modeling of the Edwards AFB site above indicated that an anisotropy ratio greater than 0.1 may render the GCW system ineffective. For sites with higher anisotropy ratios, it is advisable to use a single-screened well, conventional injection/extraction system. If a GCW system is selected for a relatively isotropic aquifer, increasing the distance between screens in the treatment wells or decreasing the distance between wells can help to improve the effectiveness of treatment (Christ, 1997).

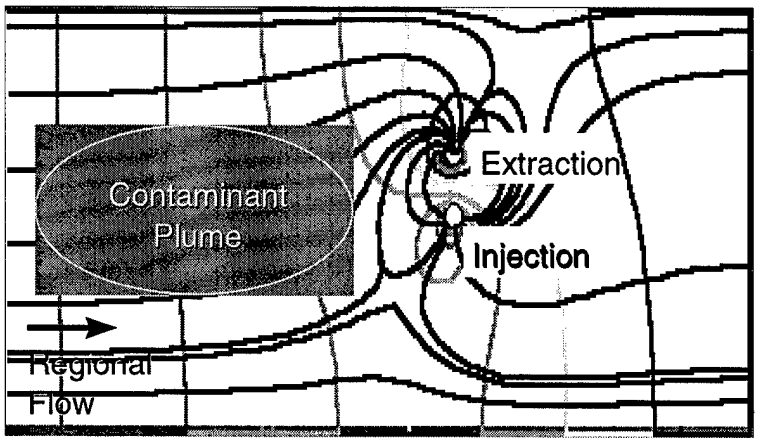
5.2.6 Technology Design and Construction

Construction of the bioremediation system will start with treatment and monitoring well installation. The treatment well type (circulation or injection/extraction), the orientation of treatment wells with respect to regional groundwater flow (regional flow angle), the number of wells, and the well spacing should have already been determined during the design phase. In addition to the treatment wells, a system of monitoring wells must also be installed, which serve to evaluate the ongoing effectiveness of the treatment system. The study at Edwards AFB utilized a well diameter of 20 cm for the treatment wells, while a 5 cm diameter was used for the surrounding monitoring wells. The required number and placement of monitoring wells will depend on site hydrogeology and on regulatory concerns. The governing regulatory authority may require monitoring wells along the boundary of the site, both up gradient and down gradient of regional flow to ensure that primary substrate (e.g. toluene) concentrations do not exceed some minimum threshold and to evaluate treatment effectiveness.

Figure 5-3 shows a conceptual depiction of the technology in plan view. The system consists of at least two treatment wells (one pumping and one injection) situated perpendicular to regional groundwater flow. The pumping well draws contaminated groundwater out of the aquifer. The injection well introduces the drawn groundwater, primary substrate, and electron acceptor to the aquifer. The treatment system is implemented down gradient of a contaminant plume and acts to intercept dissolved phase CAHs, degrading the compounds *in situ* as well as preventing migration further downgradient.

Figures 5-4 and 5-5 display methods of implementing the technology based on site geology. Details on the operation of these systems are presented in Section 5-5. Figure 5-4 represents a site that has an semi-confining layer present as is the case at Edwards AFB. The figure depicts a system of two GCW, each screened in both the upper and lower aquifers. The first treatment well withdraws contaminated groundwater from the upper aquifer and discharges it into the lower aquifer after mixing (inside the well with static mixers) with primary substrate and oxygen. The second treatment well does the reverse, withdrawing from the lower aquifer and discharging into the upper. Thus, water circulates between the two aquifers. Highly anisotropic conditions can use this design. (Anisotropic conditions will be considered to exist when the

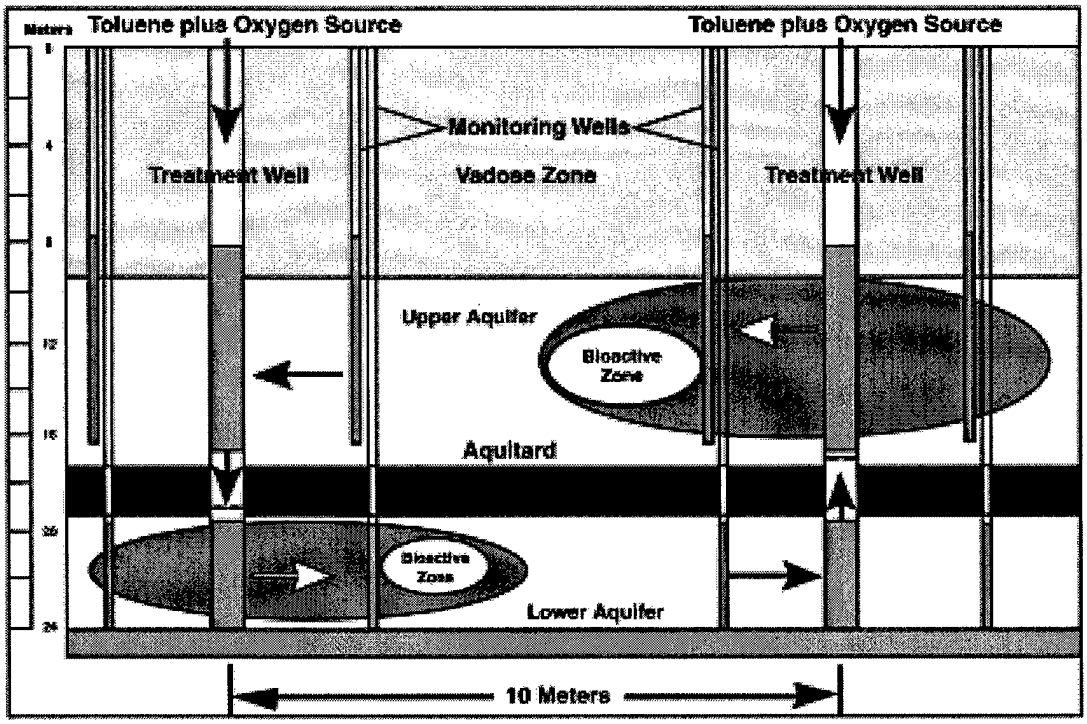
Figure 5-3. Conceptual Plan View of Aerobic Cometabolic *In Situ* Bioremediation



vertical hydraulic conductivity divided by horizontal hydraulic conductivity is less than 0.1). Figure 5-5 represents a GCW system at a site that does not have an aquitard and is isotropic. Some short-circuiting may occur between screens in a single well using this design in a single aquifer. The design shown in Figure 5-6 is more appropriate for single aquifers where relatively isotropic conditions exist. This figure presents conventional, single-screened injection/extraction wells. The extraction well pumps the contaminated water to the surface for amendment with primary substrate and oxygen, and the injection well discharges the mixture back into the aquifer. Note that short-circuiting is eliminated through this design.

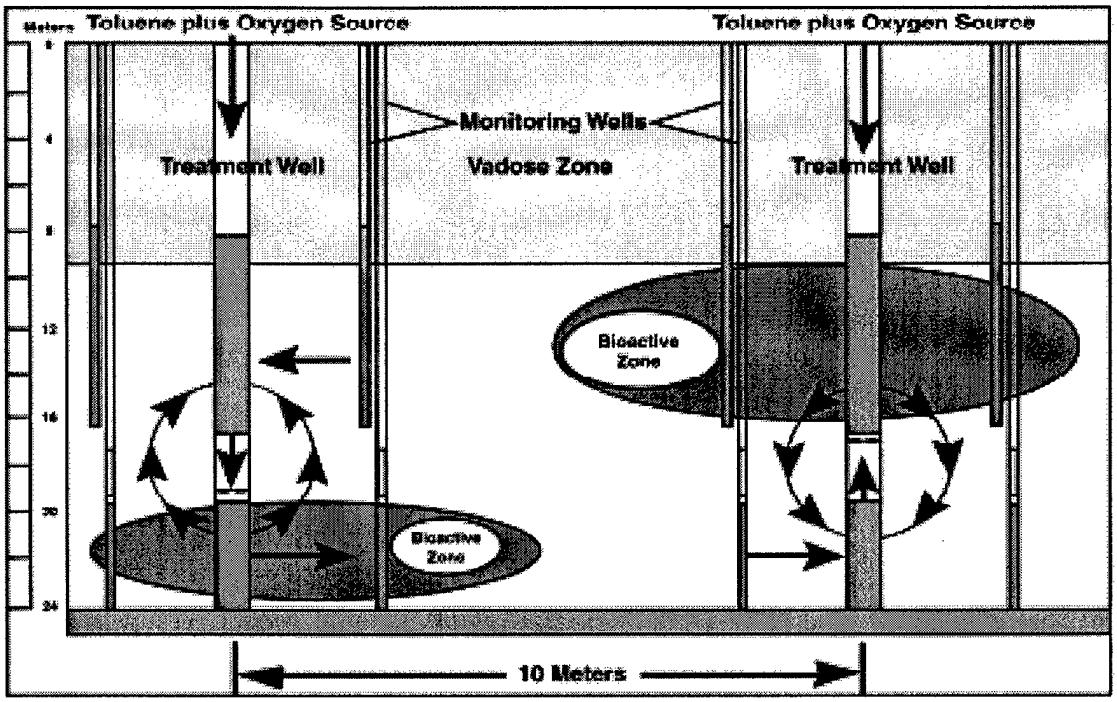
The treatment and monitoring well system layout used at Edwards AFB in the field demonstration is presented in Figure 5-7. T1 and T2 are dual-screened treatment wells operating in the downflow and upflow modes, respectively (see Figure 5-4). The extensive monitoring system used here was to obtain sufficient data that would permit definitive conclusions about system effectiveness, and would generally not be needed in the typical treatment system. Each of the nested monitoring locations (N1 through N14) has two 5-cm diameter monitoring wells, each screened in a different aquifer. At the center of the site, C-U is a 10 cm diameter monitoring well screened in the upper aquifer, while C-L is a 10 cm well screened in the lower aquifer. Surrounding the site are four 10 cm diameter "compass point" wells, located 15 m from the site center, approximately to the north, south, east, and west. The north, south, and west wells each allow groundwater samples to be obtained from both the upper and lower aquifers, while the east well, which was the first well constructed at the site, can only be sampled in the upper aquifer. These compass point wells were installed to meet regulatory concerns and to provide further monitoring information for evaluating treatment effectiveness. It was proposed that toluene concentrations not exceed 20 µg/L at these locations, a concentration below the taste and odor threshold. Thus, altogether at the site, there are 41 sampling locations: the two treatment wells, which can each be sampled in the upper and lower aquifers, the 14 nested monitoring locations, each of which can be sampled in both aquifers, three compass point wells that can be sampled in both aquifers, a fourth compass point well that can only be sampled in the upper aquifer, and the two wells at the center of the site (McCarty *et al.*, 1998).

Figure 5-4. Aerobic Cometabolic *In Situ* Bioremediation – Implementation Under Anisotropic Conditions



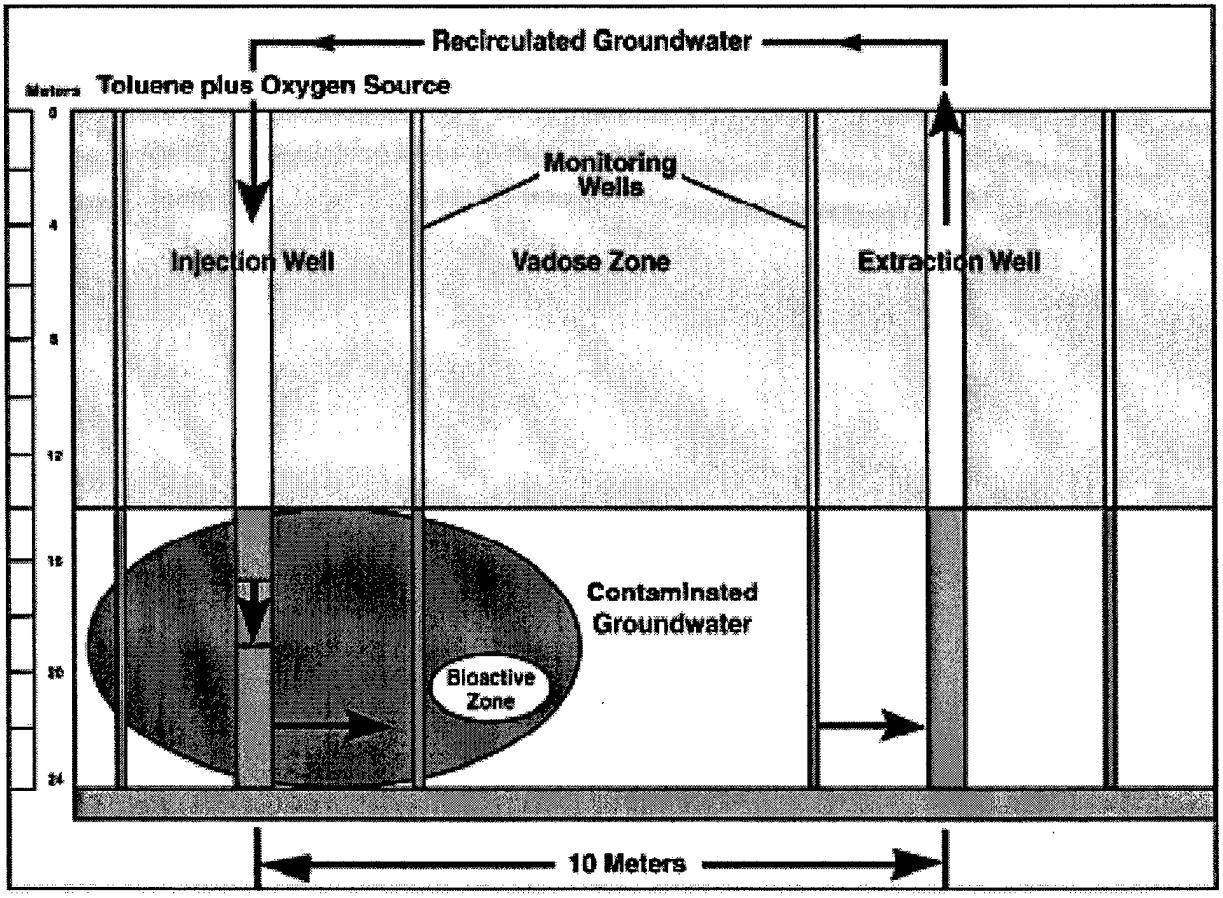
Note 1: Direction of groundwater flow is "into" the page.
Note 2: This figure represents a site that has a low-permeability layer present. Highly anisotropic conditions can use this design.

Figure 5-5. Aerobic Cometabolic *In Situ* Bioremediation - Implementation Under Isotropic Conditions



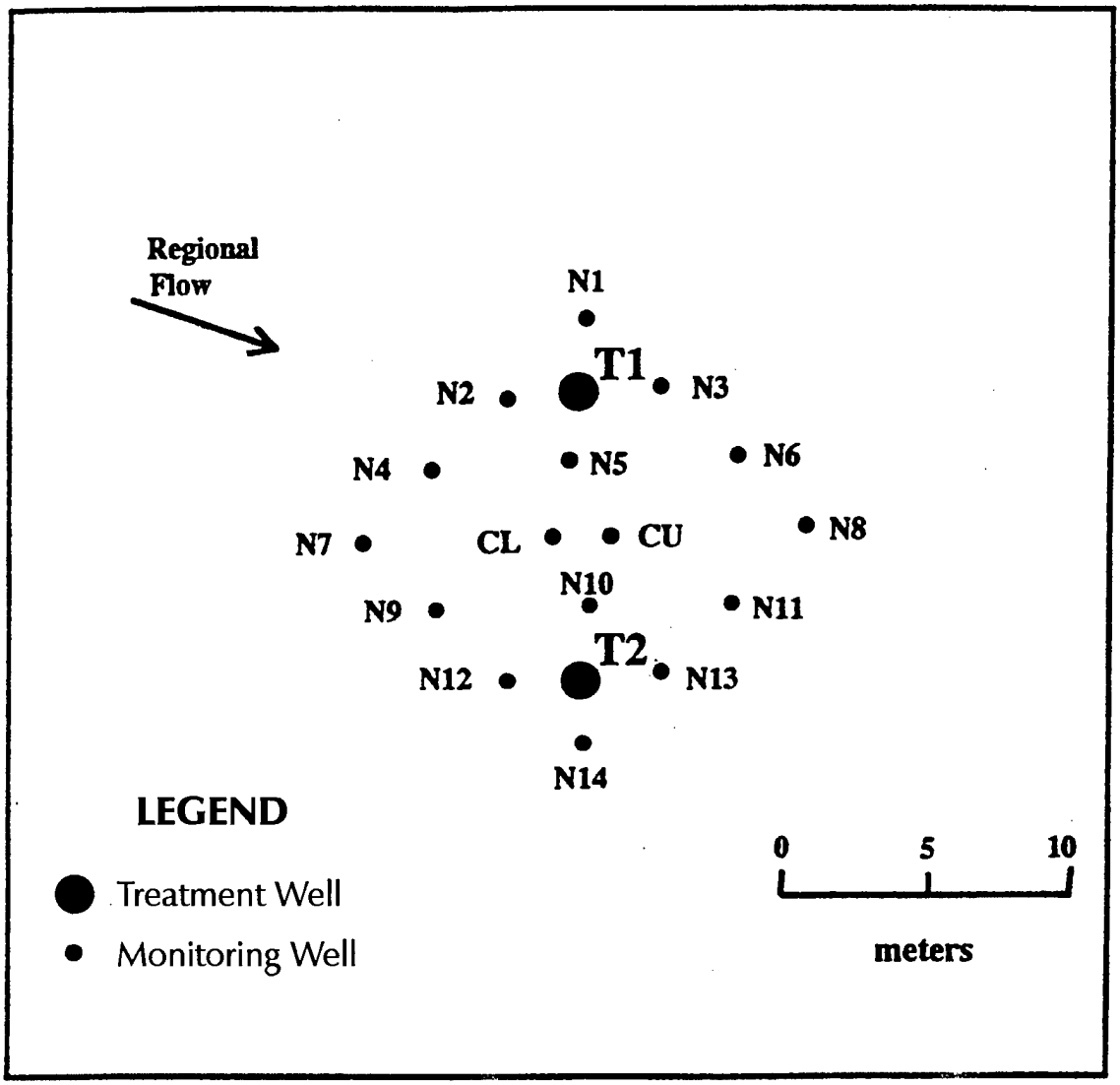
Note: **Direction of groundwater flow is "into" the page.**

Figure 5-6. Aerobic Cometabolic *In Situ* Bioremediation - Implementation Under Isotropic Conditions with Injection/Extraction Wells



Note: Direction of groundwater flow is "into" the page.

Figure 5-7. Plan View of Treatment Area with Locations of Treatment and Monitoring Wells at Edwards AFB



The subsurface recirculation system at Edwards AFB serves as a good example of treatment well construction. The GCW system, consisting of two treatment wells as illustrated in Figure 5-4, was constructed after the circulation wells described by Herrling *et al.* (1991) and McCarty and Semprini (1993). Each treatment well was screened at two depths. Based upon the results of the aquifer testing and model studies, a flowrate of 38 L/min (10 gpm) at each treatment well was selected because this should be obtainable without excessive drawdown in the upper aquifer or pressure change in the lower aquifer (no more than 5 m total hydraulic head change). A submersible pump that could deliver this flowrate was installed between the two screens of each well to draw TCE contaminated water into the well at one of the screened intervals. The primary substrate and oxygen source were introduced into the well through feed lines, and mixed into the TCE-contaminated water using two 2.54-cm diameter Model 100 812 static mixers placed in series in the treatment well (TAH Industries, Inc., Robinsville, NJ). The groundwater, containing a mixture of TCE, primary substrate, and oxygen, was discharged into the aquifer from the second screened interval. An *in situ* bioactive treatment zone was thus created in the aquifer around the discharge screen of each treatment well. One treatment well

withdraws groundwater from the upper aquifer and discharges it into the lower aquifer, while the other treatment well does the reverse. Thus, water is caused to circulate between the two aquifers. Water is never brought to the surface, with the attendant savings in pumping cost and treatment and disposal requirements (McCarty *et al.*, 1998).

If it is determined that a single-screened injection/extraction system is more appropriate for the site, then the treatment wells will have only one screen per well, with one well extracting contaminated water while the other well injects the water after mixture with primary substrate and oxygen source. In this case, the groundwater must be brought to the surface for amendment with primary substrate and oxygen. The pump and static mixers will remain aboveground, and feed lines into the well for primary substrate and oxygen introduction are unnecessary (see Figure 5-6).

5.3 System Monitoring and Testing

5.3.1 Monitoring System

The following system monitoring equipment, as described by McCarty *et al.* (1998) was used at Edwards AFB for the two-well groundwater circulation system to fully demonstrate system performance. Such extensive monitoring with near real-time data analysis can be expensive, but would not generally be required for a normal treatment system. Probably only a few monitoring wells strategically placed with weekly to monthly or even less frequent sampling and analysis would generally be sufficient.

An automated sampling and analysis platform (ASAP, Analytical and Remedial Technology, Inc., Milpitas, California) with 28 sample ports was connected directly to submersible Grundfos Rediflo-2 pumps placed in the treatment wells and in selected monitoring wells that were to be monitored most frequently. The remaining 13 monitoring well locations (the compass point wells, N1 to N3 upper aquifer wells, and N12 to N14 lower aquifer wells) were manually connected to the ASAP system with Grundfos pumps as needed. The ASAP incorporates an interface module used to control the Grundfos pumps for purging a total of approximately 200 liters from the sample wells and providing a representative groundwater sample to the ASAP system. The excess extracted groundwater was filtered and returned to the subsurface through treatment well T1.

The ASAP allows continuous, near real-time sampling of approximately 30 samples per day using modules to process aqueous sample aliquots for introduction into attached analytical instrumentation (USEPA, 1993). Analytical results are automatically stored in a computer data base for both local and remote access and analysis including graphic display. The ASAP allows remote control of sampling activities and provides automated calibrations and quality assurance/quality control (QA/QC) analysis of known standards (USEPA, 1993). Purgeable hydrocarbons (chlorinated aliphatic hydrocarbons and aromatic hydrocarbons) are analyzed by gas chromatography (GC), inorganic ions through single column ion chromatography, and dissolved oxygen and pH with probes.

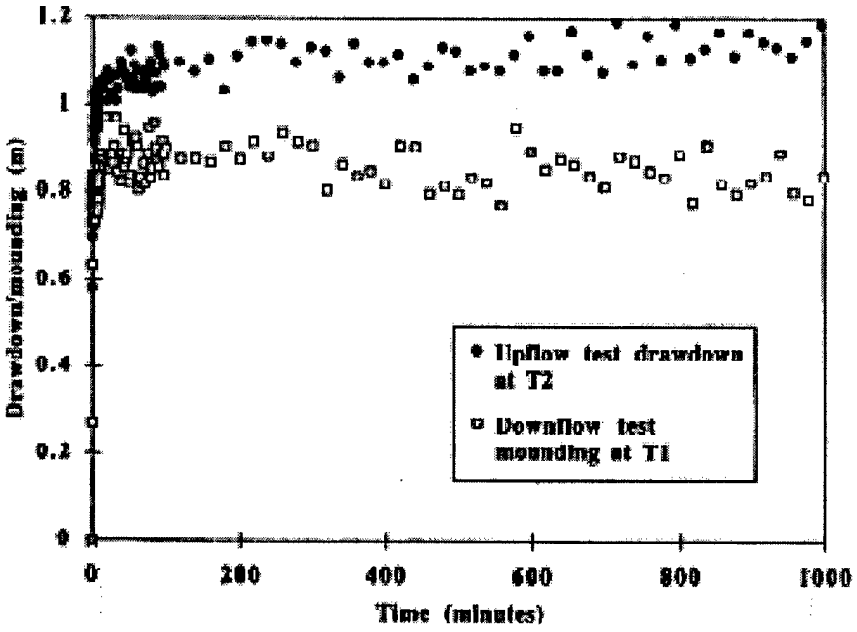
A multi-port sample loop valve provides samples for purge and trap GC analysis with selectable volumes from 0.2 mL to 10 mL. A 30 m thick film DB-5 mega bore GC column in series with a 15 m thick film DB-624 mega bore column (J&W Scientific, Folsom, California) provide good resolution for the compounds of interest. The GC (Finnigan-Tremetrics, Model 9000, San Jose, California) is equipped with tandem photo ionization and flame ionization detectors for TCE and toluene analyses. Chromjet integrators (Thermal Separation Products, San Jose, California) provide integration of the detector signals and communicate with the ASAP computer for data storage and retrieval.

Inorganic ions (bromide) are processed by the ASAP high performance liquid chromatography (HPLC) module using fixed loop injection, a standard anion column and conductivity detector (Model 350) (both from Alltech Associates, Deerfield, Illinois), and a binary gradient HPLC pump (Thermal Separations Products, model P2000, San Jose, California). The fluent is 4 mM potassium acid phthalate. The ASAP system also collects data for dissolved oxygen and pH using probes and associated meters (Orion Research, Inc., models 860 and 520A, respectively, Beverly, Massachusetts).

5.3.2 System Testing

Once the system has been constructed, pump tests should be initiated. The following describes the pump tests that were used prior to the Edwards AFB demonstration, to determine whether homogeneous hydrogeological conditions existed at the site. At the Edwards AFB site, 24-hour pump tests were conducted at each treatment well using a flowrate of 38 L/min. Under homogeneous conditions and equal flow rates, the lower (confined) aquifer drawdown response to the upflow test was anticipated to be identical to the mounding response due to the downflow test. The drawdown and mounding of the lower aquifer during the upflow and downflow tests are shown in Figure 5-8. As is seen in Figure 5-8, the mounding during the downflow test was about 70% of the drawdown during the upflow test. While this may have been due to site hydrogeology, another possible explanation is that the net flowrate at T1 was less than that at T2 by about 30%. This may have been caused by damage to the bentonite slurry seal which separates the lower and upper aquifers at T1.

Figure 5-8. Lower Aquifer Response During the Downflow (T1) and Upflow (T2) Treatment Well Pump Tests



An additional verification of homogeneity comes from head responses at various monitoring wells during the T1 and T2 24-hour pump tests. Assuming homogeneity and equal pumping rates during the two pump tests, the lower aquifer's drawdown response to the T2 upflow pump test should equal the mounding due to the T1 downflow pump test. The head data can be analyzed quantitatively using the following expression which, for two monitoring wells in a homogeneous confined aquifer, relates observed drawdown (or mounding) at the two wells (s1 and s2), the distance from each monitoring well to the extraction (or injection) well (r1 and r2),

the aquifer transmissivity (T), and the pumping rate (Q) of the extraction (or injection) well (Kitanidis, 1997):

(10) $$s_1 - s_2 = \frac{2.3Q}{2\pi T} Log \frac{r2}{r1}$$

Assuming T is equal for each of the pump tests , a ratio of pumping rates for the upflow and downflow tests can be derived by comparing the responses of monitoring well pairs. Applying equation (20) to all pairs of the aquifer monitoring wells (and discarding those pairs where s1≈s2 and/or r1≈r2) and averaging the results, the flow through T1 in the downflow test can be calculated relative to the flow through T2 in the upflow test (McCarty, *et al.*, 1998). Using this method, it was determined that the flow through T1 in the downflow test was 62% of the flow through T2 in the upflow test or about 24 L/min.

Another test that can be used during the system testing phase is a tracer test. During a tracer test, a conservative solute (one which will not degrade readily in the aquifer) is injected at a constant rate into a treatment well. Tracer measurements are then taken at the surrounding monitoring wells. Tracer tests can serve one of two purposes. First, the resulting tracer concentrations at the monitoring wells can be used to estimate the flowrate in the injection well through mass balance. This information can than be used to verify pumping test results. Second, tracer test results can be used to verify that flow from the treatment wells reaches all selected monitoring well locations. Such verification not only confirms homogeneous aquifer properties, it also assures that the fate and transport of the substrate to be injected into the upper and lower aquifers can be adequately monitored. At Edwards AFB, a sodium bromide solution containing 50 g/L bromide was continuously pumped into the T1 well at a flowrate of 34 mL/min for five days, and bromide measurements were made at the lower aquifer monitoring wells nearest T1 (N1, N2, N3, and N5). The resulting increased bromide concentration at these wells was 65 to 68 mg/L. From mass balance on these values, the T1 flowrate is estimated in this manner to be 25 to 26 L/min, values which are similar to those estimated from the pumping tests (McCarty, *et al.*, 1998). To further characterize flow at Edwards AFB, as well as to assure the regulatory agencies that the toluene that would be injected during the evaluation could be tracked by the monitoring network, a second tracer test was conducted. As the aquifer was oxygen deprived, methane could serve here as a conservative tracer. The tracer test consisted of continuously adding methane to the circulating groundwater at both treatment wells at a concentration of 25 to 30 mg/L over a 14-day period. Methane was found to arrive at all the monitoring locations. Based on an analysis of 50% breakthrough times for methane at the monitoring wells, methane travel time contours were constructed for the upper and lower aquifers. These results indicated the aquifers are relatively homogeneous, with groundwater flow from the treatment wells reaching all the monitoring wells. This homogeneity allows evaluation of system efficiency based upon TCE reduction at the monitoring locations. Additionally, the methane tracer test results provided assurance that the fate and transport of the toluene to be injected into the upper and lower aquifers for the evaluation could be adequately monitored (McCarty, *et al.*, 1998).

5.4 System Start Up: Establishing a Cometabolizing Consortium

The purpose of this phase of the operation is to establish a viable substrate-degrading (e.g. toluene degrading) microbial population in the aquifer. This objective is achieved through an incremental increase of substrate and oxygen injection through the treatment wells. Each injection stage is followed by a cessation of substrate and oxygen introduction during which sampling is conducted at various monitoring wells. A rapid decline in substrate and oxygen

concentrations at the monitoring wells is indicative of an adequate substrate-degrading population. When substrate concentrations approach zero, the next stage of substrate and oxygen injection is initiated at double the preceding substrate concentration. Once the time lag between substrate injection and a near zero substrate concentration at the monitoring wells is sufficiently short, a viable substrate-degrading consortium has been established. The following is a description of the toluene-degrading consortium established at the Edwards AFB site. A similar procedure could be used with a single aquifer. If using single-screened wells, then only one consortium per well pair is used at the injection well.

In order to establish a toluene-degrading consortium in the two aquifers at Edwards AFB, the pumping rates were established and on day 34, pure oxygen was added for 4 days in order to provide an aerobic environment for the toluene injection. Then, while oxygen addition was continued, sufficient neat toluene was added in pulses, one per hour, to provide time-averaged concentrations of 2.7 and 18 mg/L at T1 and T2, respectively. Such toluene injection was continued for two days (days 38 - 40) and then toluene and oxygen injection was stopped and the pumps were turned off. Samples from various monitoring wells were analyzed for toluene and oxygen decrease, which would provide evidence for the growth of an indigenous toluene oxidizing population. Toluene reached near zero concentration within ten days. Pumping and oxygen and toluene injection were reinstituted for a two-day period, this time with about double the initial toluene concentration. As a toluene-degrading population had now been established, toluene utilization was quicker, and reached near zero within five days. This procedure was repeated once again, but with another doubling of the toluene concentration. A subsequent rapid depletion of toluene by day 56 indicated that an adequate population had been established to proceed to the next stage (McCarty, *et al.*, 1998).

5.5 System Operation

5.5.1 Pre-Steady-State

The objective of pre-steady state operation is to achieve optimal contaminant removal efficiency by adjusting operational parameters, including substrate and oxygen addition rates and oxygen source. For instance, the primary substrate addition pulsing rate should be adjusted such that a viable TCE degrading population is maintained. Too much substrate can result in organism growth near the treatment well (bioclogging) and can cause competitive inhibition, where TCE degradation rates are reduced due to the substrate competing for the active site on the TCE degrading enzyme. Organism growth near the treatment well can be inhibited by use of hydrogen peroxide as an oxygen source. Hydrogen peroxide also provides elevated dissolved oxygen levels necessary for increased substrate concentrations. TCE removal through treatment is represented at any given time by concentration differences between a treatment well and a downgradient monitoring well. By sampling from successive wells along the groundwater flow path between injection and extraction wells, it can be determined where the maximum amount of treatment is occurring. Monitoring well sampling should also be conducted during this phase to check whether substrate concentration levels are below regulatory limits. Below is a discussion of the pre-steady state phase at Edwards AFB which may provide insights into the process of system optimization. Operational results are summarized in Table 5-2.

Table 5-2. Operational Summary for Days 38 through 444

	Days 38-136 Pre Steady State		Days 142-204 Steady State		Days 209-271 Steady State		Days 317-444 Steady State		Totals	
	Upper Aquifer	Lower Aquifer	Upper Aquifer	Lower Aquifer	Upper Aquifer	Lower Aquifer	Upper Aquifer	Lower Aquifer	Upper Aquifer	Lower Aquifer
Treatment Well	T2	T1	T2	T1	T2	T1	T2	T1		

Pumping Rate - liters/min	0 - 38	0 - 25	38	25	38	25	25	25		
TCE, µg/L										
Upgradient	1212±108	617±59	1080±49	649±60	1147±25	770±63	1207±89	554±36		
At Treatment Well	679 to 338	597 to 124	304±17	80±26	254±12	63±7	171±12	107±13		
After Treatment	668 to 71	631 to 15	46±19	17±8	29±7	26±11	24±3	18±6		
Groundwater Treated - m^3	4706	3096	3393	2232	3393	2232	4572	4572	16,063	12,132
Toluene Added - kg	41	31	45	22	45	18	41	41	173	112
Oxygen Added - kg	136	136	98	149	98	149	201	201	534	636
Peroxide Added - kg	141	91	283	186	159	133	215	201	798	611
TCE Removed - g	834	326	875	214	763	126	672	407	3145	1072

In this phase from days 56 to 136 at Edwards AFB, operation of the treatment system with pumping was continuous, as was the introduction of dissolved oxygen. Toluene was added initially at 12 pulses per day, but this gradually decreased to 3 pulses per day by day 62 as a steady-state population became established. This pulsing strategy was designed to reduce organism growth near the treatment well and to reduce the effects of competitive inhibition. Initially, toluene was added to provide a continuous low time-averaged concentration. Then the concentration was slowly increased with time to a maximum of 11.6 and 13.4 mg/L at wells T1 and T2, respectively, as field evidence of adequate toluene degradation was obtained, together with evidence that toluene did not exceed regulatory levels at the compass point wells. TCE concentration decreased as water passed from T2 to N10 and N5, which are located 2.5 and 7.5 m, respectively, from T2 on the path between T2 and T1. The TCE removal from water emanating from the treatment wells is represented by the TCE concentration differences at any given time between T2 and N5 in the upper aquifer and T1 and N10 in the lower aquifer. In both aquifers, TCE removal generally increased with time, in-line with the increase in toluene concentration and the resulting buildup of a toluene-consuming TCE-degrading population.

By the end of the pre-steady state period, TCE removal evidenced in this manner was over 80% in both aquifers. However, the overall TCE removal performance differed markedly between the upper and lower aquifers. In the upper aquifer, TCE removal increased gradually with time. Up to about day 105, most of the removal occurred between T2 and N10, after that, most occurred between N10 and N5. This progression of the treatment zone away from T2 was desired and resulted from planned operational modifications. One modification involved increasing the time between pulses, a strategy designed to move the toluene out further into the aquifer before complete degradation could occur. A second modification was the addition of hydrogen peroxide after day 80 to increase the dissolved oxygen supply as needed for degradation of higher toluene concentrations. This also tended to inhibit bacterial growth near the treatment well. The combination of these two strategies thus was successful.

The above strategies were not as successful initially for TCE removal in the lower aquifer. Although at the beginning, the mass rates of addition of toluene, oxygen, and hydrogen peroxide were the same at the two treatment wells, the concentration at T1 was higher because the net flowrate was lower. Once this was recognized, the mass of toluene added was reduced. Hydrogen peroxide addition should also have been reduced because of the excess inhibition that it caused, as found later. In spite of this problem, TCE removal up to day 80 was better in the lower aquifer than in the upper aquifer, perhaps because the actual concentration of toluene then at T1 was 1.33 times that at T2. When hydrogen peroxide was added on day 81 (at a concentration 1.33 times higher than at T2 as the difference in net flowrates as the two

wells was then not known), inhibition appeared to be very high, and TCE removal stopped. On day 89, toluene concentration was dropped somewhat and on day 99, hydrogen peroxide addition was stopped. By day 100, TCE removal began again. On day 103, pulsing was changed from 3 times per day to once per day, and this appeared to help spread TCE removal beyond N5. TCE removal increased to about 80% by day 120. However, a sudden increase in pumping head at T1 around day 130 suggested clogging may have become a problem. A small amount of hydrogen peroxide was added in an attempt to control this clogging, but this was insufficient and the well field was shut down on day 136 to redevelop T1 as preventive maintenance. This ended the period of pre-steady-state operation (McCarty *et al.*, 1998).

5.5.2 Steady-state Operation and Trouble Shooting

Once the optimal parameter settings have been determined during pre-steady state operation, the system is ready for steady state operation. While there should not be a need for significant operational changes to the system during steady state operation, continued well sampling and system monitoring should take place to be able to quickly detect any problems that may arise. One problem that may occur, is increasing pumping head at each well due to biomass buildup in the aquifer. At Edwards AFB, the system was temporarily shut down for redevelopment of wells T1 and T2 to alleviate this problem. Another problem that must be avoided is excessive hydrogen peroxide addition. Hydrogen peroxide may be attractive as an oxygen source because of its ability to deliver higher dissolved oxygen levels than molecular oxygen or air and its tendency to reduce bioclogging because of its toxicity to microorganisms at high concentrations near the treatment well. However, too much hydrogen peroxide will result in a net loss of contaminant removal due to losses of biomass. Note that elevated hydrogen peroxide concentrations may result from either too much addition or from an overestimation of the net flow rate at the treatment well (this occurred at Edwards AFB). Below is a discussion of the steady state operation at Edwards AFB which will help in understanding the possible problems encountered during system operation and how they were addressed. An operational summary is provided in Table 5-2.

Steady-state operation at Edwards AFB occurred between days 142 and 444. The upgradient TCE concentration in the regional groundwater flow entering the treatment zone remained relatively constant in the two aquifers over the course of the study (see Table 5-2). Here it averaged between 1,080 and 1,212 µg/L in the upper aquifer and between 554 and 770 µg/L in the lower aquifer. The flows at the treatment wells were a mixture of regional flow entering the treatment zone and already-treated interflow water. TCE concentrations at the two treatment wells were thus lower than in the upgradient regional flow water and indeed decreased with time as treatment progressed. The TCE concentrations after treatment are much less, the values in Table 5-2 here were taken from concentrations measured at N5 upper for the upper aquifer and N10 lower for the lower aquifer. The measured concentration at N5 upper during the last two steady-state periods averaged 24 to 29 µg/L. About two-thirds of the removal took place between the N10 and N5 monitoring wells, or further out in the aquifer as desired. TCE concentration varied more markedly at the near monitoring well (N10) than at the distant monitoring well (N5), a phenomenon observed previously at Moffett Federal Airfield. This is a direct result of the once per day pulsing of toluene and resulting competitive inhibition. As the pulse moved through the aquifer, TCE removal momentarily decreased due to competition between toluene and TCE for the oxygenase. Such TCE oscillations were attenuated by the time the circulating groundwater reached N5 due to the lower toluene concentration near there as well as TCE sorption/desorption effects. The only operational change here, occurred from day 204 to 209 when the system was shut down for routine redevelopment of wells T1 and T2 to reduce the pumping head, which was slowly increasing at both wells due to biomass buildup in the aquifer. Upon restarting, pumping pressures were as at the beginning of the study, and efficient TCE removal resumed almost immediately upon restarting.

The steady-state period results for the lower aquifer initially had much greater variation than in the upper aquifer. Over the steady-state period, the T1 TCE concentration decreased from about 150 μg/L to about 50 μg/L due to the unbalanced net flowrates between the two treatment wells and the increasing efficiency of TCE removal in the upper aquifer. TCE removal occurred more between T1 and N5 than between N5 and N10, indicating that biodegradation remained for the most part near the treatment well. Overall removal varied considerably as well, often exceeding 90 percent, but at times decreasing to less than 50 percent. A great deal of the variation in TCE removal was related to the operational changes made in toluene and peroxide additions. A major problem was the unreliability in the hydrogen peroxide feeding system to the lower aquifer which resulted in excessive feeding of peroxide at times and underfeeding at others. This problem was resolved on day 317 with a reduction in the hydrogen peroxide concentration at T1 to 47 mg/L. This resulted in dramatic improvement in the single-pass (η_{SP}) removal efficiency over the last 137 days of the study to 83% in the lower aquifer. This is comparable to the 85% to 89% single-pass efficiency that was maintained in the upper aquifer over the 339 day steady-state period of the study (McCarty *et al.*, 1998).

The pumping head required to pump at the given rates was measured and found to be 2 to 2.5 m in both aquifers with a flowrate of 25 L/min and about 3 m at 38 L/min during initial startup and after the wells had been redeveloped. The pumping heads then slowly increased due to aquifer clogging to maximums of 5.5 to 6 m at 38 L/min and 4.5 m at 25 L/min during the first three operational periods. However, during the last steady-state four-month operational period when toluene pulsing and hydrogen peroxide addition were optimized, the head increase was minimal (McCarty et al., 1998). Routine redevelopment would not then have been required. The pumping heads at all times with this *in situ* treatment system were well below the heads that would be required to pump the groundwater for treatment at the ground surface.

5.6 Costs

This section provides actual quantities and costs of well installation, drilling, equipment, chemicals and other materials expended during implementation of the treatability study at Edwards AFB, Site 19. Section 5.6.1 briefly presents the data used to arrive at the quantities of water treated and the amount of TCE removed from each aquifer at Site 19. Sections 5.6.2 and 5.6.3 present capital and annual operating cost information, respectively. The costs incurred at Edwards AFB may serve as a baseline for calculating cost of implementation. However, most costs incurred were related to the extensive monitoring system used to thoroughly evaluate system operation and effectiveness, which would not normally be required.

5.6.1 Basis of Cost

Table 5-2 contains an operational summary of results over the 444 day period when toluene was injected in to the subsurface system at Edwards AFB. The volume of groundwater within the 22 m square test site in the upper 8 m deep aquifer is 1,160 m^3, assuming a porosity of 0.3. During the 444 days of this study, 12,132 m^3 were pumped from the upper to the lower aquifer at T1 or about 10.5 times the total amount of water in that aquifer. In the lower 20 m deep aquifer, the quantity of water in the test zone is 726 m^3, while 16,063 m^3 or almost 22 times that volume was pumped into the upper aquifer through T2. Thus, a much greater volume of water was treated over the time of this study than was present within the test zone.

Modeling studies were conducted to estimate the portion of the contaminated plume that was being treated by the system and the amount of interflow for conditions during the last steady-state period where balanced flows of 25 L/min were maintained at both treatment wells. These studies indicated the width of the capture zones to be 62 m and 53 m in the upper and lower

aquifers, respectively. This simulation also indicated that the T1 flow was comprised of 71% treated interflow water coming from the T2 well and 29% from the upgradient untreated regional flow. The T2 flow was comprised of 85% treated interflow from the T1 well and 15% from the upgradient untreated regional flow. Based upon data compiled in Table 5-2 for the last steady-state period of balanced flow, a mass balance for TCE concentrations in the upgradient and after treatment locations and in the treatment wells themselves can provide another estimate of interflow percentage. Such calculations indicate the amount of interflow reaching the T1 well was 93%, while that reaching the T2 well was much less or only 72%. These interflow values are much higher for the T1 well and much lower for the T2 well than indicated by model simulations. The differences may be due to any one or more of a number of factors, such as inadequate time to reach a true steady-state, or errors in modeling assumptions made concerning such factors as aquifer thickness, regional velocity, or homogeneity. A more detailed site characterization would be needed to determine the factor or factors involved. These comparative results do indicate that simple model calculations must be used with caution and that actual designs for such things as treatment well spacing should perhaps be more conservative than that estimated from models where homogeneity is assumed.

5.6.2 Capital Costs

Capital costs are those costs incurred during the set-up and installation of the treatment system. They include drilling, well installation, and system equipment necessary to implement the remedial design. The capital costs incurred during the full-scale evaluation of the treatment system at Edwards AFB are given in Table 5-3. The table shows that the major capital costs incurred were for the monitoring systems, which were much more extensive than would normally be required in a treatment system, but were used here to fully evaluate system effectiveness. Capital costs for the analytical equipment used are not included in the table. The cost for sodium bromide, which was purchased for proposed extensive tracer studies but was mostly not used, is also not included in the table.

Table 5-3. Capital and Operational Costs for Aerobic Cometabolic *In situ* Bioremediation at Site 19, Edwards AFB, California (2 wells)	
CAPITAL COSTS	
Treatment Costs	
Treatment Wells (80 feet, 8-inch Schedule 80 PVC)	$30,000.00
Flow Sensors and Controllers	$2,790.00
Static Mixers	$1,076.00
Packer Assembly	$9,338.00
Deionized Water System	$6,847.00
Pumps and Ancillary Equipment(1)	$10,000.00
Tubing & Connectors(2)	$1,789.00
Valves & Fittings(2)	$867.00
Total Treatment Costs	**$62,707.00**
Monitoring Costs	
Nested Monitoring Wells (80 feet, 2-inch Schedule 80 PVC) (19 wells)	$190,000.00
Pumps & Ancillary Equipment(1)	$36,923.00
Tubes & Connectors(2)	$16,096.00
Values & Fittings(2)	$7,808.00
Miscellaneous Supplies	$9,919.00
Total Monitoring Costs	**$260,746.00**
TOTAL CAPITAL COSTS	**$323,453.00**
ANNUAL OPERATING COSTS (extrapolated from actual costs for 444 days of operation)	
Well Redevelopment ($/well-year) x 2 wells	$8,000.00
Hydrogen Peroxide, 30%	$4,633.00
Toluene	$47.00
Oxygen	$1,674.00
TOTAL ANNUAL OPERATING COSTS	**$14,354.00**

(1) Estimated that $10,000 of the $46,923 spent on pumps and ancillary equipment was for treatment costs, the remainder for monitoring costs (Tessier, 1998).

(2) Estimated that 10% of the cost of these items was for treatment costs, 90% for monitoring costs (Tessier, 1998).

5.6.3 Annual Operating Costs

The operating costs incurred at Site 19 are given in Table 5-3. The costs have been annualized based on the recorded costs for operating the system for 444 days. Toluene is a relatively inexpensive chemical (on the order of $0.20 per kg for technical grade); molecular oxygen is also relatively inexpensive at $1.74 per kg, but the cost of hydrogen peroxide is high (approximately $4.00 per kg). Thus, it is the hydrogen peroxide that represents the major chemical cost. The amount used in the Site 19 demonstration is believed to have been more than actually required to prevent bioclogging. This amount might be reduced in a normal design with an aquifer medium similar to that at Site 19, but the actual amount necessary at any given site would depend upon hydraulic conductivity and hence plugging potential of the aquifer.

Based upon microcosm studies, about 2.1 kg oxygen per kg toluene is required at steady-state. The pure oxygen added alone would satisfy this need. However, not all oxygen added was successfully transferred to the groundwater with the static mixers; some portion escaped at the treatment wells. The added peroxide helped satisfy the demand and also helped add an excess of dissolved oxygen to the water leaving the 22 m square treatment zone. Note these costs do not include installation of monitoring wells and monitoring costs, such as sampling and analysis costs, nor power costs, as electricity was provided free at the Edwards AFB site.

6. REGULATORY ACCEPTANCE

Remediation technologies are generally selected under one of two environmental regulatory frameworks: the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) or the Resource Conservation and Recovery Act (RCRA). The selection phase under CERCLA is called the feasibility study, while the analogous phase under RCRA is called the corrective measure study. This Section describes regulatory issues concerning the selection of aerobic cometabolic *in situ* bioremediation under CERCLA (Section 6.1) and RCRA (Section 6.2).

6.1 CERCLA

Aerobic cometabolic *in situ* bioremediation is considered an "innovative technology" under CERCLA. Technologies are classified as innovative if they are developed fully but lack sufficient cost or performance data for routine use at CERCLA sites. Innovative technologies may be used in treatability studies and taken through the screening phase of a feasibility study if there were reason to believe that the innovative technology would offer significant advantages. These advantages may be in the form of better treatment performance or implementability, fewer adverse impacts than other available approaches, or lower costs for similar levels of performance (USEPA,1988). Regulators responsible for overseeing cleanup of hazardous waste sites may or may not be supportive of the use of innovative technologies.

If a regulator is willing to consider the use of this innovative technology, its predicted performance must still be scrutinized. CERCLA stipulates that remediation technologies selected during a feasibility study must be evaluated with respect to nine performance criteria (40 CFR 300.430(e)(9)(iii)). The criteria are given in Table 6-1.

Table 6-1. CERCLA Technology Performance Criteria

Criterion Number	Criterion Type	Criterion
1	Threshold	Overall protection of human health and the environment
2	Threshold	Compliance with all other applicable or relevant and appropriate requirements (ARARs)
3	Balancing	Long-term effectiveness and permanence
4	Balancing	Reduction of toxicity, mobility, or volume through treatment
5	Balancing	Short-term effectiveness
6	Balancing	Implementability
7	Balancing	Cost
8	Modifying	State acceptance
9	Modifying	Community acceptance

The first two criteria are threshold criteria, meaning that a technology must fully satisfy these criteria to be considered for implementation at a contaminated site. Criteria three through seven are balancing criteria. The performance of a technology does not have to fully satisfy each of these criteria. Instead, the criteria are used as overall performance indicators, meaning that for a particular technology to be selected for implementation at a site, it must demonstrate the best overall performance relative to these criteria when compared to alternative environmental remediation technologies (Skumanich, 1994). The final two criteria are modifying criteria. The purpose of modifying criteria is to ensure that state and local issues not directly addressed in the threshold and balancing criteria are given adequate attention.

Below, the nine CERCLA technology performance criteria are discussed with respect to how aerobic cometabolic *in situ* bioremediation fulfills each one.

Overall Protection of Human Health and the Environment. As a final assessment to determine whether the technology will function in a safe manner that will provide adequate protection to human health and the environment, this criterion is closely related to other criteria, especially long-term effectiveness and permanence, short-term effectiveness, and compliance with applicable or relevant and appropriate requirements (ARARs). Because of this relationship, to the degree that aerobic cometabolic *in situ* bioremediation can satisfy these other criteria, it should be able to satisfy this encompassing criterion of overall protection of human health and the environment (Skumanich, 1994; Mandalas, 1997).

Compliance with ARARs. This criterion is used to determine how well a proposed environmental remediation technology complies with federal, state, and local environmental laws. Although compliance with ARARs is a relatively straightforward process, there are some ARARs that may pose particular issues for the feasibility of technology implementation. For example, aerobic cometabolic *in situ* bioremediation usually requires the injection of a regulated chemical to the subsurface to achieve the highest removal efficiencies. The use of injectants for *in situ* groundwater remediation is a relatively new concept and although it has been allowed in nearly two-thirds of the States, a lengthy process is required to obtain the necessary State permit, an Underground Injection Control permit, for their use (USEPA, 1996). This might affect the feasibility of implementation if the permitted allowable level is set below the level for proper stimulation of the microbes or if expedient cleanup start-time is critical (Skumanich, 1994). Results from Edwards AFB Site 19 suggest that as long as sufficient oxygen is present and the aquifer has sufficient nutrients to support biological growth, concentrations (of toluene) well below levels of regulatory concern can be achieved. Additionally, toluene is also biodegradable anaerobically so that even if sufficient oxygen were not available, regulatory concerns may not pose any substantial problems (McCarty *et al.*, 1998). With the knowledge gained and documented during the Moffett Federal Airfield studies and the full-scale evaluation at Site 19, parties wishing to implement this technology are equipped with hard data supporting the ability of the technology to comply with ARARs. The degree to which state and local officials charged with overseeing a remediation project accept the data will determine how well aerobic cometabolic *in situ* bioremediation satisfies this criterion (Mandalas, 1997).

Long-Term Effectiveness and Permanence. This criterion is used to evaluate the ability of aerobic cometabolic *in situ* bioremediation to reliably protect human health and the environment, after the cleanup is completed. Under this criterion USEPA has generally favored permanent treatment technologies (destruction) over technologies that pose the possibility of contaminants being re-released to the environment (containment). USEPA also favors

technologies that treat contaminants at the site rather that those that require removal to off-site locations (Skumanich, 1994).

Because aerobic cometabolic *in situ* bioremediation is a permanent treatment process that fully degrades contaminants into innocuous substances, it should be preferred under this criterion. The *in situ* nature of the technology avoids risks associated with transferring contaminants to another site or media and because of the permanence of the process, little will be required in terms of long-term maintenance after cleanup is complete (Mandalas, 1997).

Reduction of Toxicity, Mobility, and Volume through Treatment. The objective of this criterion is to measure the degree to which aerobic cometabolic *in situ* bioremediation includes destruction of the contamination, as opposed to containment or disposal elsewhere. Again, because aerobic cometabolic *in situ* bioremediation is a process that leads to complete degradation of a contaminant to harmless substances, it should be favored under this criterion (Skumanich, 1994; Mandalas, 1997).

Short-Term Effectiveness. Under this criterion, technologies favored are those that require a relatively short and uncomplicated construction period and a relatively short time to implement. Additionally, those technologies that pose the least disruption to the environment are preferred as are those whose impacts to the environment can be easily monitored. Aerobic cometabolic *in situ* bioremediation may receive mixed evaluation under this criterion. In favor of the technology, little disruption to the environment is posed (certainly no more than other treatment technologies) and although workers are exposed to moderately hazardous chemicals (toluene and hydrogen peroxide), short-term risks to workers are minimal. If rapid reduction in contaminant is top priority for a particular site, aerobic cometabolic *in situ* bioremediation will likely score poorly under this criterion (Skumanich, 1994), as will most other existing technologies.

An additional concern under this criterion is the injection of toluene or some other regulated chemical as the primary substrate. Obviously, this technique presents some short-term disruption to the environment. However, as demonstrated at Site 19, this disruption is localized and does not extend beyond the treatment zone (Mandalas, 1997).

Implementability. The objective of this criterion is to measure the technical and administrative feasibility of a proposed remedy. Implementation of aerobic cometabolic *in situ* bioremediation is relatively straightforward, requiring little in the way of material or labor. In terms of general availability of goods and materials, the technology should receive favorable evaluations under this criterion. However, a critical aspect of this criterion is how well the technology has been demonstrated for use, and how reliable the technology will be once it is fully operational. Until aerobic cometabolic *in situ* bioremediation is generally recognized as an accepted technology, regulator acceptance barriers may delay implementation of the technology at particular sites. While it is true that all innovative environmental remediation technologies face regulatory barriers, it is particularly true for aerobic cometabolic *in situ* bioremediation because of the need to introduce hazardous substances to the subsurface. Additionally, monitoring of the *in situ* system presents some short-term implementation challenges as the monitoring system would likely have to be demonstrated before full system operation could begin (Skumanich, 1994). Although monitoring an *in situ* process is problematic, the full-scale demonstration at Site 19 confirmed that monitoring technology is available and able to perform well. The peer reviewed documentation of the Moffett Federal Airfield studies combined with the extensive full-scale technology demonstration at Site 19 and its accompanying peer reviewed literature should

prove to be important factors in the overall ability of aerobic cometabolic *in situ* bioremediation to satisfy this criterion.

Cost. The objective of this criterion is to identify technologies that have reasonable costs, not necessarily the technology with the lowest cost. One of the strongest arguments for bioremediation technology is that it can present significant cost savings over more conventional treatment technologies (Skumanich, 1994). Some discussion shall be presented here exploring the cost of aerobic cometabolic *in situ* bioremediation.

A comparison of costs for implementation, maintenance and operation of an aerobic cometabolic *in situ* bioremediation system versus an extraction system utilizing an air stripper and carbon absorption can be made using Site 19, Operable Unit (OU) 1, Edwards AFB, California as an example. The actual costs for installation of two treatment wells for bioremediation as well as the annual costs for continued treatment were compiled and are presented in Table 6-2. These costs are discussed in further detail in Section 5.6.1 of this manual. Table 6-2 also includes estimated costs for implementation of groundwater extraction system combined with air stripping and granular-activated carbon absorption. According to the Draft Final OU1 Feasibility Study, this treatment system was the preferred remedial alternative for overall treatment of the Site 19 groundwater plume. The costs in Table 6-2 are on a per-well basis.

Table 6-2. Comparison of Groundwater Treatment Costs at Site 19 ($/well)

	***In Situ* Bioremediation**	**Extraction with Air Stripping**
Capital Costs		
Treatment/Extraction Well	$15,000	$15,000
Supplemental Costs*	$6,500	$15,670
Groundwater Treatment System	$0	$31,000
Annual Costs	**$7,200**	**$3,100**

*Supplemental capital costs include mobilization, labor, geophysical surveys, etc.

The costs in Table 6-2 do not include monitoring or sampling and analysis costs since they would be approximately equal for each treatment alternative, thus canceling each other out.

In comparing the costs for the two alternatives, the major differences are associated with supplemental costs and treatment system costs. Supplemental costs are those costs incurred during the installation of the treatment system. Examples of supplemental costs include geophysical surveys, mobilization and demobilization of heavy equipment, labor for installation of the system, and miscellaneous parts, supplies, and equipment necessary for implementation of the treatment design. The main reason for the vast difference in costs per well is due to the extensive amount of equipment and supplies required for electrical and other utility hook-ups for extraction of the groundwater to the air stripper and reinjection of the treated water. Because the groundwater is not extracted or reinjected when treatment via in situ bioremediation is used, these costs are not incurred. Costs for the groundwater treatment system are only incurred for the extraction and air stripping alternative since these costs involve trenching and piping, the air stripper system, and all electrical instrumentation for controlling the groundwater flow rates. The other major difference in costs on a per well basis is in annual costs. For in situ bioremediation, annual costs incurred are for well redevelopment, which is required to prevent clogging of the screens; and the substrates and oxygen source required for the treatment

process. Annual costs incurred during operation of the extraction and air stripping process include utility costs for operation of the pumps and blowers, and transport and regeneration of carbon. Routine costs for monitoring and sampling and analysis are not included in annual costs for either alternative since they would be approximately equal. As shown in Table 6-2, annual costs are the only group of costs which are higher for in situ bioremediation than extraction and air stripping. This is mainly due to the necessary redevelopment of the treatment wells to prevent the well screens from clogging due to growth resulting from injection of the primary substrate, toluene.

Based on studies such as the Site 19, Edwards AFB site evaluation, aerobic cometabolic *in situ* bioremediation may be less expensive to treat TCE-contaminated groundwater than traditional pump-and-treat remediation, especially in deep anisotropic aquifers, where savings from not having to pump groundwater to the surface for treatment may be significant. Additionally, costs associated with pumping groundwater to the surface are avoided as are hazardous waste disposal fees (McCarty *et al.*, 1998).

State Acceptance. This criterion is used to assess the degree to which aerobic cometabolic *in situ* bioremediation addresses any policy or administrative issues that the state may have. In general, state concerns tend to be with issues similar to those addressed in other areas of the performance criteria. It is likely that the performance of aerobic cometabolic *in situ* bioremediation under this criterion will be a reflection of its reliability, permanence, ease of implementation, cost, and ability to meet ARARs (Skumanich, 1994). In the case of the full-scale evaluation of aerobic cometabolic *in situ* bioremediation of TCE at Site 19, use of the technology was strongly supported by State of California regulators.

It is important to note that, as discussed under the "Compliance with ARARs" criterion, the technology requires the injection of a regulated chemical to the subsurface. Most states have policies regarding the injection of nutrients, co-solvents, surfactants, and other compounds into groundwater. USEPA's Technology Innovation Office (TIO) has compiled a summary table of state policy and experience with *in situ* groundwater remediation (see USEPA, 1996a). This document also contains a list of state regulatory agency contacts. The reader is referred to this document and to Rich Steimle ([703] 308-8800) of USEPA's TIO for more information regarding this issue.

Community Acceptance. This criterion is used to measure the acceptance of aerobic cometabolic *in situ* bioremediation by the local community. As in the previous criterion, community concerns tend to be with issues similar to those addressed in other areas of the performance criteria. It is likely that the performance of aerobic cometabolic *in situ* bioremediation under this criterion will be favorable if it performs well in other areas of the performance criteria. Note, however, that community concerns are sometimes "emotional." For example, stimulating microbial growth in the subsurface may lead some community members to be concerned about the ramifications of an increased population of "mutant" microorganisms. Additionally, community members may become concerned over the introduction of toluene as primary substrate and the idea that the introduction of this compound will eventually cause cancer or some other disease in members of the community. Department of Energy (DOE), through its VOC-Arid Integrated Demonstration Program has published a report (Peterson and McCabe, 1994) that defines many of the community concerns that are specific to *in situ* bioremediation. These concerns can usually be allayed through community education and communication. Skumanich (1994) reports that, overall, the public generally has a favorable opinion of bioremediation technology. In the case of the full-scale evaluation of aerobic cometabolic *in situ* bioremediation of TCE at Edwards AFB Site 19, the local community, after

being briefed on the site remedial plans, offered no objections to the use of the technology. It is important to note, however, that the community consists largely of transient military personnel, and the groundwater is not used for drinking water in the area.

6.2 RCRA

Performance criteria for remedial technologies have not been promulgated under RCRA as they have been under CERCLA. Thus, remedial project mangers (i.e., regulators at either state environmental agencies or USEPA regional offices) responsible for overseeing cleanup of sites under RCRA often have a great deal of leeway when selecting a remedial technology. Because CERCLA guidance is commonly used for direction when appropriate RCRA guidance is unavailable, the analysis of how aerobic cometabolic *in situ* bioremediation fulfills the CERCLA performance criteria (see Section 6.1) may be used to justify selection of this technology for RCRA sites. However, as for CERCLA sites, the utility and acceptance of the technology will vary, and even if site conditions are amenable to the use of the technology, it may not always be the best technology for implementation.

7. CASE STUDIES

This Section describes the backgrounds, site conditions, and results of field tests where aerobic cometabolic *in situ* bioremediation of CAHs has been conducted. The conditions and results at these sites are summarized in Table 7-1. Sites mentioned in this table are the best studied sites for which data are publicly available.

Note that the method of implementation of this technology (see System Design Summary column in Table 7-1) is different at each site. The implementation strategies used at Moffett Federal Airfield and Edwards AFB were unique to those sites (although the Edwards AFB work was an extension of what was done at Moffett Federal Airfield) and have not, to the best of our knowledge, been implemented elsewhere. Conversely, implementation of the SRS approach (which combines cometabolism with some form of air stripping technology) is underway at sites throughout the U.S. SRS has patented its approach, and commercial firms seeking to use this approach must obtain licenses; several firms have already done so (SRS, 1998).

It is important to reiterate that this guidance manual describes how to implement an aerobic cometabolic *in situ* bioremediation system similar in design to the one implemented at Edwards AFB. The other designs presented in the case studies below are provided for comparison purposes only and are not discussed elsewhere in this manual. In addition, the Edwards AFB technology is designed to serve as a barrier for plume migration, whereas the SRS technology may potentially be used to cleanup source areas.

7.1 Moffett Federal Airfield

Moffett Federal Airfield (formerly Moffett Naval Air Station) is located in Mountain View, California. The site is approximately three kilometers south of the southwest extremity of the San Francisco Bay. This site was selected for the first pilot scale application of *in situ* cometabolic bioremediation in the field because it had several favorable site characteristics: a shallow, semi-confined aquifer consisting of sands and gravels; high permeability; and an indigenous community of methanotrophic bacteria (Roberts *et al.*, 1990). A test zone was selected where groundwater contaminants consisted primarily of chlorinated organic compounds, mainly 1,1,1-TCA, but no chlorinated ethenes were present (TCE, DCE, vinyl chloride) (Semprini *et al.*, 1992). Chlorinated ethenes were added in the test zone in controlled biotransformation experiments, which allowed mass balances to be performed (Semprini *et al.*, 1990).

The design of the Moffett Federal Airfield well field is shown in Figure 7-1. It consisted of two injection wells and an extraction well with monitoring wells interspersed between them. Induced gradient conditions were created by extracting groundwater at a rate approximately seven to eight times greater than the injection rate, to dominate the regional groundwater flow (Roberts *et al.*, 1990). Once the wells were installed and preliminary pump and tracer tests were performed, a series of stimulus-response experiments were conducted. The stimulus was the continuous injection of measured concentrations of the chemical of interest in the test zone, and the response was the concentration history of the chemicals in the groundwater sampled from the monitoring wells and the extraction well (Semprini *et al.*, 1990).

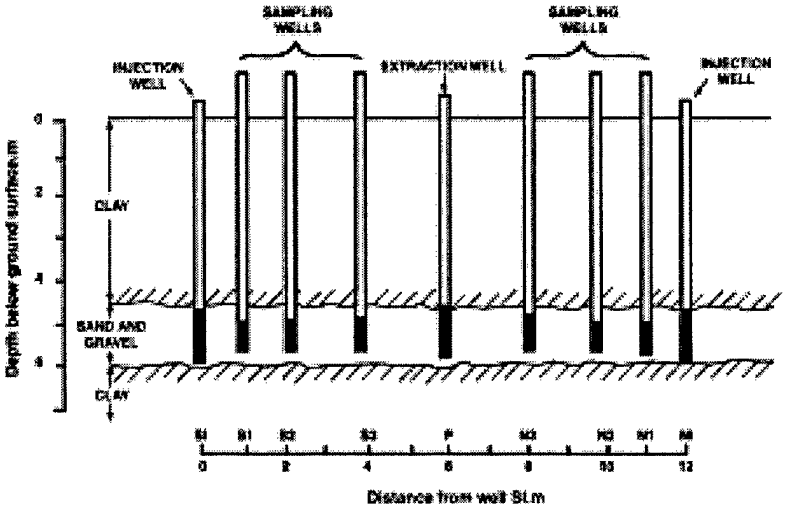
Table 7-1. Summary of Site Conditions and Results Where Aerobic *In Situ* Cometabolism of CAHs has been Implemented

Site	Depth to Ground-water	Aquifer Material	Organic Carbon Fraction (Foc)	Hydraulic Conduc-tivity	Primary CAH Contaminant for co-Metabolism (initial conc.)	Primary Substrate Added to Stimulate Co-Metabolism	Oxygen Sources Used	System Design Summary	Removal Efficiencies	Comments	References
Moffett Air Field, Califomia[1]	5 m (confined)	Fine to course grain sand and gravel	~0.11%	~0.1 cm/s	TCE (45 µg/L); trans-DCE (52 µg/L); cis-DCE (100 µg/L); VC (44 µg/L)	Methane	O_2 gas	Circulatory system in which groundwater is extracted to surface, augmented with substrate, O_2 and CAHs, and reinjected	TCE (20%); trans-DCE (90%); cis-DCE (50%); VC (95%)	1,1,1-TCA was initially present. TCE, DCE, and VC were added to test the technology	Semprini *et al.*, 1990, 1992
Moffett Air Field, Califomia[1]	5 m (confined)	Fine to course grain sand and gravel	~0.11%	~0.1 cm/s	TCE (250 µg/L); cis-DCE (125 µg/L); trans-DCE 125 (µg/L); VC (60 µg/L); 1,1-DCE (65 µg/L)	Phenol, Toluene	O_2 gas, H_2O_2	Circulatory system in which groundwater is extracted to surface, augmented with substrate, O_2 and CAHs, and reinjected	TCE (>90%), cis-DCE >90%); trans-DCE (74%); VC (<90%), 1,1-DCE (50%)	1,1,1-TCA was initially present. TCE, DCE, and VC were added to test the technology Presence of 1,1-DCE as a co-contaminant significantly reduced TCE cometabolism	Hopkins and McCarty, 1995; Fries *et al.*, 1997
Edwards AFB, California	Upper Unconfined Aquifer: 8 m Lower Confined Aquifer: 20 m	Fine to medium size sand with some silt	0.0001-0.0004%	1.5 to 5.5 x 10^{-3} cm/sec	TCE (500 to 1,200 µg/L)	Toluene	O_2 gas, H_2O_2	Circulate groundwater between upper and lower aquifers and augment with periodic pulses of substrate and oxygen	TCE (95-97%) (upper aquifer) lower aquifer slightly less	99.98% of toluene added was degraded	McCarty *et al.*, 1998
Savannah River Site, South Carolina	40 m	Upper: fine to medium sand, 23% silt/clay or silt/clay beds Lower: medium sand, 22% silt/clay or silt/clay beds	NA	NA	TCE (10-1031 µg/L); PCE (3-124 µg/L)	Methane	Air	Methane, nitrous oxide, triethyl phosphate, and air injected in horizontal well below water table; VOCs not cometabolized extracted from a horizontal well in vadose zone and subjected to air stripping	PCE and TCE (95%), cometabolism accounts for only ~25% of contaminant loss	Technology also removes VOCs in vadose zone.	CCEM, 1995; SRS, 1998

Key:
CAH = Chlorinated Aliphatic Hydrocarbon
DCE = Dichloroethylene
NA = Not Available
TCE = Trichloroethylene
VC = Vinyl Chloride

(1) This site has been studied extensively and therefore requires two entries. For the purpose of this table, the experiments are divided according to the primary substrate used.

Figure 7-1. Cross Section of the Test Zone and Wellfield Used in Moffett Federal Airfield Experiments



The initial experiments conducted at Moffett Federal Airfield used methane as the primary substrate and oxygen gas as the source of oxygen for aerobic cometabolism of TCE, *c*-DCE, *t*-DCE, and vinyl chloride. Results indicated that the methane consuming consortium developed was highly effective at degrading *t*-DCE and vinyl chloride, but removal of TCE and *c*-DCE was not as successful (Semprini *et al.*, 1990).

The low removal efficiency of TCE and *c*-DCE using methane as the primary substrate led to the exploration of other potential primary substrates. The studies were again conducted at the Moffett Federal Airfield and focused on the utility of phenol as a primary substrate, again using oxygen gas as the source of oxygen. The studies, conducted over two field seasons with results published in 1993, found phenol to be superior to methane for *in situ* aerobic cometabolic degradation of TCE and *c*-DCE (Hopkins *et al.*, 1993b).

Toluene was also evaluated at the Moffett Federal Airfield site for use as a primary substrate for the cometabolic degradation of TCE. Additionally, hydrogen peroxide was evaluated as an oxygen source. The studies concluded that toluene is an effective primary substrate with performance levels very similar to that of phenol. Hydrogen peroxide was also found to be a good source of oxygen, achieving TCE removals similar to those achieved when using oxygen gas. Finally, the studies suggested that the presence of 1,1-DCE as a co-contaminant would significantly reduce TCE removal efficiencies (Hopkins and McCarty, 1995).

A pulsing strategy for addition of methane and oxygen was developed at Moffett Federal Airfield. Delivering the nutrients in alternating pulses prevented bioclogging near the injection wells and allowed the nutrients to be better distributed downgradient. Pulsing also served to lessen the impact of competitive inhibition.

A summary of the Moffett Federal Airfield studies is provided in Table 7-2 (Hopkins and McCarty, 1995).

Table 7-2. Efficiency of Chlorinated Aliphatic Hydrocarbon Removal Obtained at the Moffett Federal Airfield Site with Different Primary Substrates (after Hopkins and McCarty, 1995)						
Primary Substrate	Substrate Concentration (mg/L)	% Removal				
		TCE	1,1-DCE	c-DCE	t-DCE	Vinyl Chloride
Methane	6.6	19	NE	43	90	95
Phenol	12.5	94	54	92	73	>98
Toluene	9	93	NE	>98	75	NE

Key: TCE = Trichloroethylene
1,1-DCE = 1,1-Dichloroethylene
c-DCE = cis-1,2-Dichloroethylene
t-DCE = trans-1,2-Dichloroethylene
NE = Not Evaluated

7.2 Edwards AFB, California

Edwards AFB is located in the western portion of the Mojave Desert, about 60 miles north of Los Angeles. Site 19 is an area of about 53 acres on the west side of Rogers Dry Lake. From 1958 through 1967, engines for the X-15 rocket plane were maintained in facilities at the site. Approximately one 55-gallon drum of TCE was used each month to clean the engines. Disposal of the TCE-contaminated wastewater into the nearby desert created a large groundwater contaminant plume (Earth Tech, 1996). Researchers who pioneered the application of cometabolism for *in situ* bioremediation at Moffett Federal Airfield and were seeking a site for full-scale demonstration of this technology selected an area within Site 19 that lies about 400 meters east of the contamination source (McCarty *et al.*, 1998). The goal of the researchers was to prove the ability of the technology to perform at an actual site and to define the conditions that are most appropriate for technology implementation.

The plan view and profile view of the treatment system used at Edwards AFB are shown in Figure 7-2 and Figure 7-3, respectively. Figure 7-2 shows model simulations of upper aquifer stream tubes entering the treatment zone, recirculating between the two treatment wells, and then leaving the treatment zone. The system was set up approximately perpendicular to regional groundwater flow to intercept the migrating plume of TCE. The presence of a low-permeability layer separating two contaminated zones allowed a subsurface circulatory system to be established (Figure 7-3). Toluene was injected as the primary substrate, and both pure oxygen and hydrogen peroxide oxygen sources were tested.

Figure 7-2. Plan View of the Cometabolic Bioremediation System Implemented at Edwards AFB Site 19

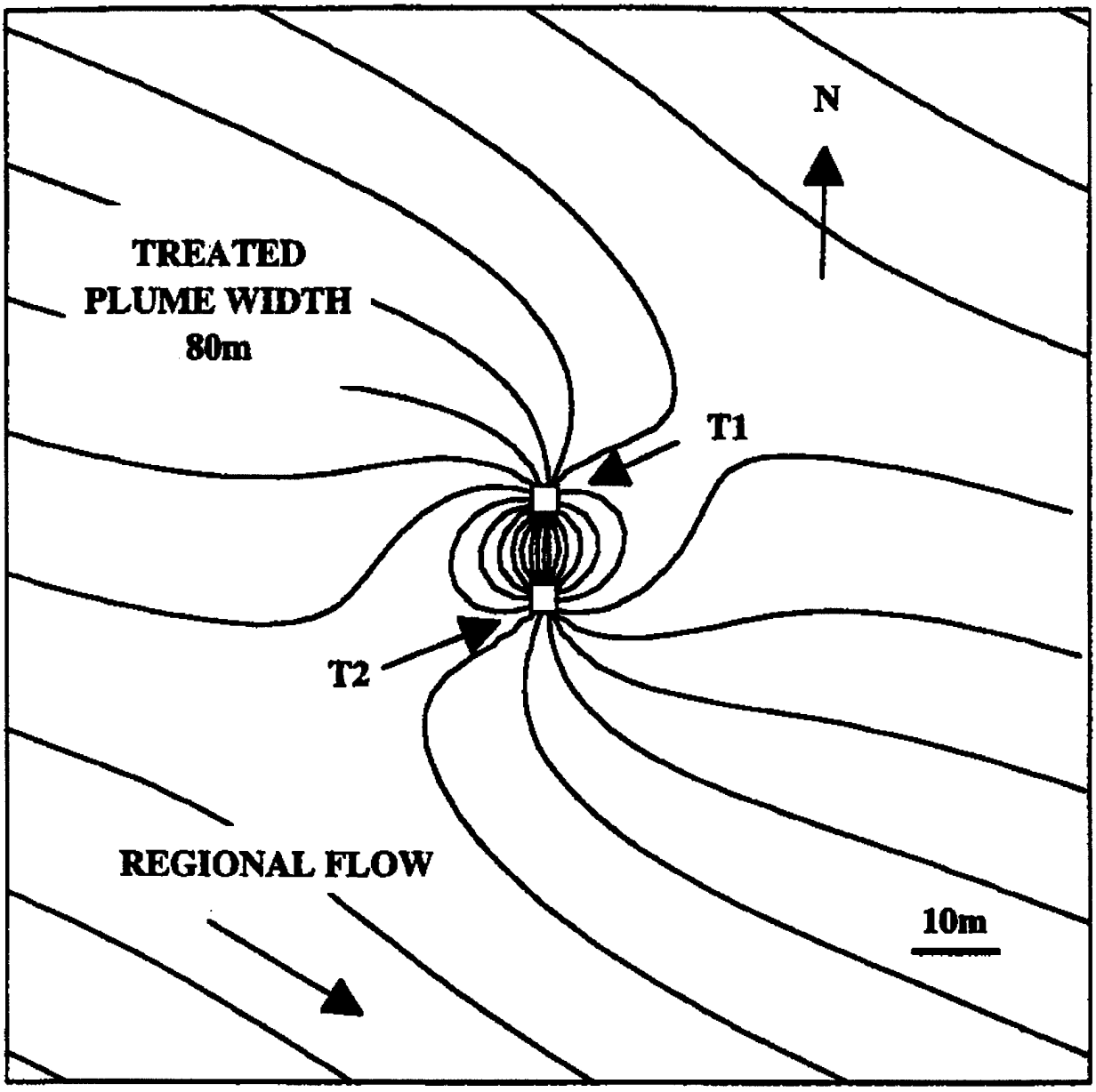
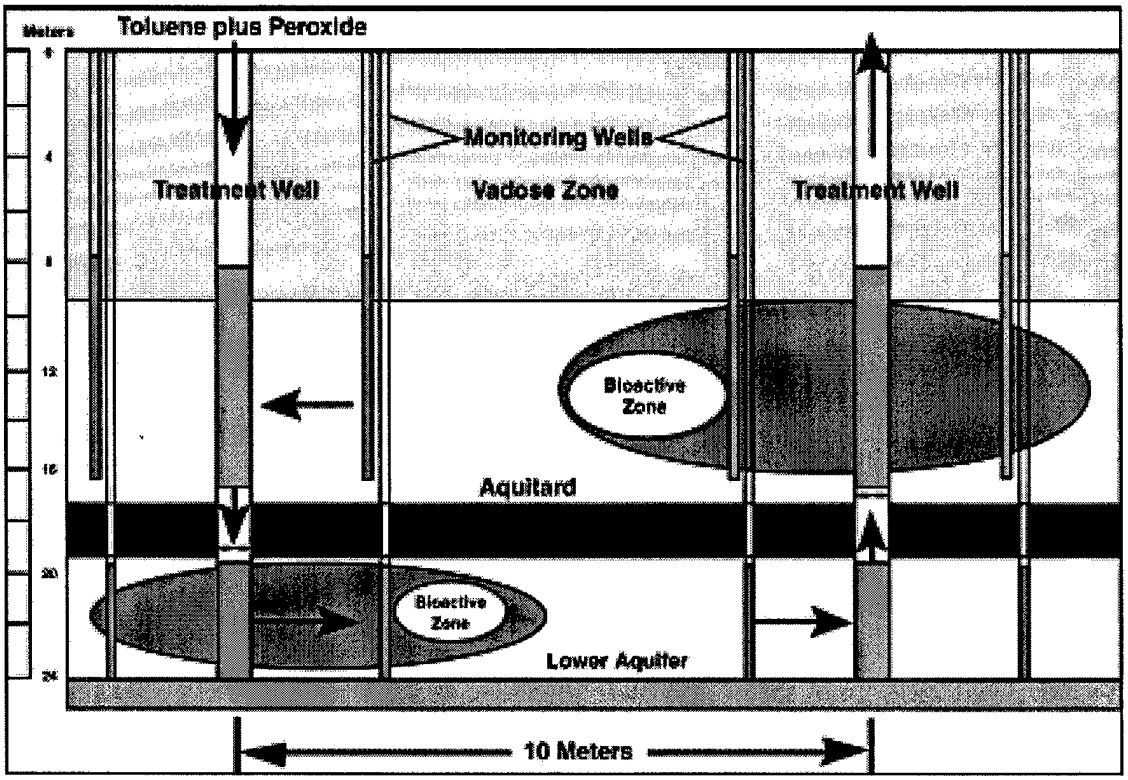


Figure 7-3. Profile View of the Cometabolic Bioremediation System Implemented at Edwards AFB Site 19



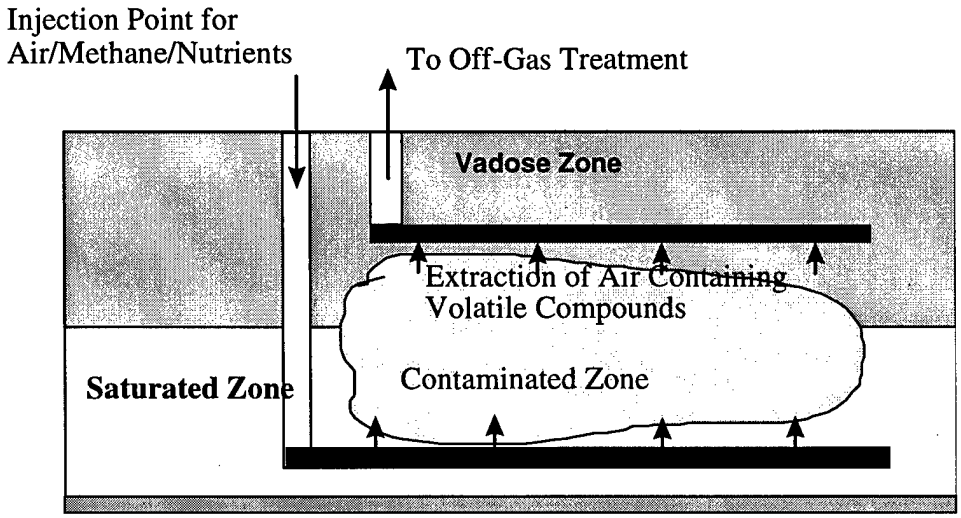
Once operating parameters were optimized and steady-state operation was underway, the full-scale demonstration at Edwards AFB, Site 19 verified that aerobic cometabolic *in situ* bioremediation is an effective environmental remediation technology. The demonstration yielded TCE removal efficiencies of 95 to 97 percent. Toluene degradation was 99.98 percent, leaving an average of 1.2 to 1.3 µg/L at the boundaries of the treatment zone, well below the maximum goal of 20 µg/L set by regulatory personnel (McCarty *et al.*, 1998). The full-scale demonstration at Edwards AFB also achieved an important objective of any full-scale technology demonstrations, which is compiling and documenting information that should be relevant to site managers and regulators considering the use of the technology.

7.3 Savannah River Site (SRS)

The SRS is a 300 square mile facility owned by the U.S. DOE and operated under contract by the Westinghouse Savannah River Company. The site is near Aiken, South Carolina. The site has been operated as a nuclear production facility for DOE since 1950. The production processes carried out over the past 40 years have generated considerable waste and numerous waste sites. One area at SRS, the 300-M Area, consists of a former leaky sewer line and settling basin that received primarily TCE, tetrachloroethylene (PCE), and 1,1,1-TCA (Hazen, 1992). This area has been used to investigate groundwater remediation strategies, including *in situ* aerobic cometabolism.

Methanotrophs (methane-oxidizing bacteria) were stimulated by injecting natural gas (methane) and air into an aquifer using horizontal wells (see Figure 7-4). One horizontal well acted as an injection well and was placed below the water table while a second horizontal well acted as an extraction well and was placed above the water table. This well configuration allowed methanotrophs to biodegrade TCE cometabolically, while contaminants that were not aerobically biodegradable (such as PCE) were removed by air stripping.

Figure 7-4. Profile View of the Savannah River Site Horizontal Well *In situ* Bioremediation Demonstration (after SRS, 1998)



A characterization study at the SRS showed the presence of a methanotrophic bacterial community which had the ability to degrade the TCE-contaminated groundwater (Bowman *et al.*, 1993). After methane injection in the study area, a seven order of magnitude increase in the microbial population was observed (SRS, 1998). It is reported that biostimulation was immediate and resulted in PCE/TCE dissolved concentration reductions of 95% and vapor phase PCE/TCE reductions of 99%. This is 42% more than would have been removed by air stripping alone (SRS, 1998).

Interestingly, the investigators reported that PCE was biodegraded. Because PCE has not been shown to degrade aerobically researchers speculated that anaerobic zones formed in the subsurface to allow the anaerobic reductive dechlorination of the PCE to TCE which was then aerobically cometabolized. The researchers estimated that the cometabolic treatment system reduced cleanup time by greater than 50% (SRS, 1998).

To simulate the degradation found at the site a model was developed. Model simulations allowed the researchers to deduce that microbial predation had a significant impact on the TCE degradation efficiency of the remediation system (Travis and Rosenberg, 1997). Similar to the Moffett Federal Airfield work, the model studies also showed that pulsing of nutrients could be useful in enhancing removals, by enlarging the area where the removal rates are high.

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Appendix A

Biological Model and Flow Model

A.1 Biological Model

The biological model used in this software estimates the extent to which the target contaminant is biologically degraded in the groundwater as it passes through the area of the aquifer where treatment is ongoing, known as the bioactive zone (BAZ). The model is derived based on Monod kinetics. Monod kinetics accounts for the first order microbial growth that occurs at low concentrations, followed by zero order growth at high concentrations, when the substrate is not limiting (Criddle, 1993). Following simplification of the governing equations developed by Semprini and McCarty (1991) using the assumptions presented below, the equation describing contaminant reduction ratio in the BAZ is written as follows:

(A.1)
$$\frac{C_e}{C_i} = e^{-XK't}$$

where

C_e = the effluent contaminant concentration leaving the BAZ [ML^{-3}]
C_i = the influent contaminant concentration entering the BAZ [ML^{-3}]
X = the biomass concentration in the BAZ [ML^{-3}]
K' = K_2/K_{s2}
  K_2 = maximum utilization rate of cometabolism [T^{-1}]
  K_{s2} = contaminant half-saturation coefficient [ML^{-3}]
t = the time the contaminant is in the bioactive zone [T]

The assumptions upon which equation (A.1) are based are:

- No deactivation of the biomass in the BAZ
- The BAZ can be modeled as a plug flow reactor (PFR)
- No competitive inhibition effects. During field implementation, inhibition of contaminant degradation by the simultaneous presence of primary substrate is combated by pulsing in the primary substrate
- The influent contaminant concentration (C_i) is much less than the contaminant saturation coefficient (K_{s2}). This assumption is reasonable since this technology will be employed at the edge of a plume as a treatment/containment barrier
- The primary substrate is completely consumed
- The dissolved oxygen concentration is not limiting
- Steady-state conditions exist with regard to the flow field and constituent concentrations. Numerical modeling studies, in conjunction with the field data obtained from the Edwards AFB demonstration, show that steady-state conditions occur relatively quickly
- All microbial growth is due to the primary substrate only. Any co-contaminants in the plume are not being utilized as a carbon and energy source (Christ, 1997).

Because we can assume microbial growth and contaminant concentrations are at steady-state, we can calculate the microorganism concentrations in the BAZ by equating the rate of microorganism production with the rate of microorganism decay (Jenal-Wanner and McCarty, 1997):

(A.2) $$YC_DQ = XVb$$

where

Y = yield coefficient, based on the substrate and electron acceptor selected [-]
C_D = primary substrate concentration [ML^{-3}]
b = cell decay coefficient [T^{-1}]
Q = flow rate of the well [$L^3 T^{-1}$]
X = steady-state microbial concentration in the BAZ [ML^{-3}]
V = volume of the BAZ [L^3]

Rearranging the above equation to solve for X, the steady-state microbial concentration in the BAZ, we have:

(A.3) $$X = \frac{YQC_D}{Vb}$$

Since we have assumed that the BAZ acts as a plug flow reactor (PFR), the time in the BAZ (t) can be determined by dividing the bioactive zone volume (V) by the flow rate (Q). Substituting this value of t into equation (A.1), and substituting equation (A.3) into equation (A.1), results in the following expression for the contaminant-reduction ratio:

(A.4) (Jenal-Wanner and McCarty, 1997) $$\frac{C_e}{C_i} = e^{\frac{-YK'C_D}{b}}$$

From the Edwards AFB demonstration, it was observed that rather than behaving like a PFR, the BAZ could better be modeled as two continuously mixed flow reactors (CMFRs) in series (McCarty, 1998). In this case, the contaminant-reduction ratio may be expressed as follows:

(A.5) $$\frac{C_e}{C_i} = \frac{1}{(1+\frac{YK'C_D}{2b})^2}$$

Since equation (A.5) is based upon filed observations, the screening software uses it to calculate the single-pass contaminant-reduction ratio. Using the single-pass contaminant-reduction ratio, we can then calculate the single-pass treatment efficiency (η_{SP}) as follows:

(A.6) $$\eta_{SP} = 1 - \frac{C_e}{C_i}$$

Note that based on laboratory studies and the work at Moffett Federal Airfield and Edwards AFB, equations (A.5) and (A.6) apply only when $C_i < 1$ mg/L (McCarty, 1998). Parameter values needed in equations (A.5) and (A.6) are obtained for use in the screening software as follows. Previous studies (McCarty *et al.*, 1998) showed that the primary substrate concentration (C_D) is dependent upon the dissolved oxygen concentration (C_A) in accordance with the following expression:

(A.7) $$C_D = C_A/F$$

where

C_D = primary substrate concentration [ML^{-3}]
C_A = dissolved oxygen concentration [ML^{-3}]
= 3.6 mg/L for air as the oxygen source,
= 18 mg/L for oxygen gas as the oxygen source
= 30 mg/L for hydrogen peroxide as the oxygen source
F = dissolved oxygen mass required per mass of primary substrate (in this case F=2.1 mg dissolved oxygen per mg toluene)

Note that the values used for dissolved oxygen concentrations for the three potential oxygen sources were estimated based on results obtained during the evaluation of aerobic cometabolic *in situ* bioremediation at Edwards AFB Site 19 (Christ, 1997). Applying equation (A.7) results in C_D = 1.7 mg/L when air is used as the oxygen source, 8.6 mg/L when oxygen gas is used, and 14.3 mg/L when hydrogen peroxide is used. Knowing the primary substrate concentration, and given the following parameter values

b = 0.15 /d (Jenal-Wanner and McCarty, 1997)
Y = 0.77 mg/mg (Jenal-Wanner and McCarty, 1997)
K' = 0.07 L/mg cell-d for TCE (Jenal-Wanner and McCarty, 1997)
= 0.25 L/mg cell-d for trans-dichloroethylene (Semprini and McCarty, 1991)
= 0.035 L/mg cell-d for cis-dichloroethylene (Semprini and McCarty, 1991)
= 0.25 L/mg cell-d for vinyl chloride (Semprini and McCarty, 1991)

the program solves equations (A.5) through (A.7) for the single-pass treatment efficiency that would be obtained by using each of the three potential oxygen sources. Also note that the screening software allows the user to select a value for the single-pass treatment efficiency (η_{sp}) independently. In that instance, the software determines the oxygen source that is required to obtain the selected value of η_{sp}.

A.2 Flow Model

Figure 4-2 in Section 4 depicts how an *in situ* treatment system may be implemented as a barrier to contaminant plume migration, using a series of injection and extraction wells. Contaminated upgradient water is captured by the extraction well, pumped to the injection well, and then injected through the BAZ into the aquifer. A certain fraction of injected water is recycled back to the extraction well, with the remainder flowing downgradient. The contaminant concentration in the water flowing downgradient is constrained by regulation. Thus, for a given upgradient concentration and single-pass treatment efficiency (calculated using the biological model described in Section A.1), the fraction of water which must be recycled to meet specified downgradient limits is determined. In this section, the submodel which is used in the screening software to determine the number and flowrates of extraction/injection well pairs needed to capture the contaminant plume and attain necessary recycle is described. Assumptions made for this model are:

- The groundwater flow is constant over a homogeneous, continuous, isotropic, confined aquifer having a constant thickness
- The extraction rates are constant
- The maximum allowable drawdown is 30% of the aquifer thickness

- Well locations are co-linear with the same number of extraction and injection wells (Christ, 1997).

The user first enters the following aquifer information: aquifer thickness (B), horizontal hydraulic conductivity (K_h), and Darcy velocity of uniform regional flow (U). Then, using equation (A.8), the program determines the maximum flowrate (Q_{max}) in a well (Bear, 1979).

(A.8)
$$Q_{\max} = \frac{2\pi TS_w}{\ln\left(R/r_w\right)}$$

where

T = transmissivity [L^2/T]
r_W = radius of well (assumed to be 0.2 m)
R = radius of influence =3000*S_W*$(K_h)^{0.5}$
S_W = maximum allowable drawdown in meters (assumed to be 30 % of aquifer thickness (B))
K_h = horizontal hydraulic conductivity in m/s (note: these units must be used since R is determined empirically)

The user also enters the contaminant concentration to be treated (upgradient of the treatment system, (C_{in}), the required downgradient concentration (C_{out}), and the contaminant plume width (PW). Note that C_{in} is restricted to values less than 20 mg/L, as the technology is not appropriate for treating higher contaminant concentrations. From these values, the required overall treatment efficiency of the bioremediation system (η_{Req}) is calculated using equation (A.9).

(A.9)
$$\eta_{Req} = 1 - \frac{C_{out}}{C_{in}}$$

Let us define a new parameter, average interflow ratio (I_{AVG}), as the fraction of flow through all the extraction wells in the treatment system that originated in injection wells. For chemical and environmental engineers more familiar with the concept of recycle ratio (f), where f is defined as the ratio of recycled flow in a treatment system to total influent flow, I_{AVG} is equivalent to f/(1+f). By mass balance, the following relationship may be derived:

(A.10)
$$I_{AVG} = \frac{\eta_{Req} - \eta_{SP}}{\eta_{Req}(1-\eta_{SP})}$$

Note that the value for single-pass treatment efficiency (η_{SP}) used in equation (A.10) comes from the biological model (equation (A.6)). Now, using input parameters and the parameters calculated in equations (A.8) and (A.10), the program applies equation (A.11) below to determine the number of wells (N) pumping at flow rate Q_{max} that are required to capture a contaminant plume of width PW, and treat the contaminated water at the specified overall efficiency (η_{Req}).

(A.11)
$$N = \frac{2UB(PW)}{Q_{Max}(1-I_{AVG})}$$

where

PW	=	plume width [L]
B	=	aquifer thickness [L]
U	=	Darcy velocity of uniform regional flow [LT^{-1}]
Q_{max}	=	Maximum pumping rate [L^3T^{-1}] (see equation (A.8))
I_{AVG}	=	Average interflow ratio [-] (see equation (A.10)

Note that based on the assumption of an equal number of extraction and injection wells, N will be an even number, and there will be N/2 extraction wells, N/2 injection wells.

2011 Battelle Symposium

Aerobic Cometabolism Revisited Lessons Learned

Reno, Nevada

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Brian Timmins

ETEC Environmental Technologies

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Agenda

1. Introduction
2. Site background
3. Approach
4. Results
5. Questions

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Site Background

- 25-acre former industrial facility
- In operation for over 80 years
- Manufacturing of color bases, pigments, and resins
- Groundwater impacted by a mixture of solvents generated by cleaning of equipment
- In 1985 the facility was sold triggering ECRA which is the predecessor to NJ's Industrial Site Recovery Act (ISRA)

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Site Background (cont'd)

Environmental investigations since the late 1970's have primarily identified :

- Trichloroethene (TCE)
- Perchloroethene (PCE)
- Cis-1,2-dichloroethene (cis-1,2 DCE)
- 1,2-dichloroethane (1,2-DCA)
- Chlorobenzene
- Chloroform in the shallow fractured bedrock

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Site Geology

- Site location: the Newark Basin in New Jersey

- Overburden:
  - Approximately 5 to10 feet of anthropogenic fill
  - Residual soil
  - Highly weathered bedrock

- Underlying the overburden:
  - Mudstone facies of the Passaic Formation
  - Strikes at approximately N 70 degrees E and dips 10 degrees to the NW approximate to groundwater flow

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Site Hydrogeology

- Fractured bedrock beneath the Site:
  - Multi-unit groundwater system
  - Groundwater flows primarily in larger aperature bedding plane fractures
  - Cross cutting joints and other extensional fractures
- Groundwater system beneath the Site:
  - Shallow fractured bedrock (up to 80 feet) and
  - Deeper fractured bedrock (80 to 130 feet)
- Shallow bedrock zone : approximately 1 gpm or less
- Deeper bedrock zone: 7 to 15 gpm
- Groundwater flow in shallow bedrock is predominantly to the south/southwest towards the local river
- Local flow components in the central part of the Site are to the east and west and may be indicative of a recharge zone

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Aerial View

Deep Groundwater Contours

Area of aerobic cometabolism treatment

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The General Approach

- Microorganisms naturally occur in the groundwater:
  - Add a hydrocarbon (e.g., propane)
  - Organisms oxidize it for their energy needs
- Microorganisms produce enzymes that degrade the chloroethenes and chloroethanes
- Continual / effective delivery of the hydrocarbon = oxygen supporting the enzyme activity

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Aerobic Cometabolism Diagram

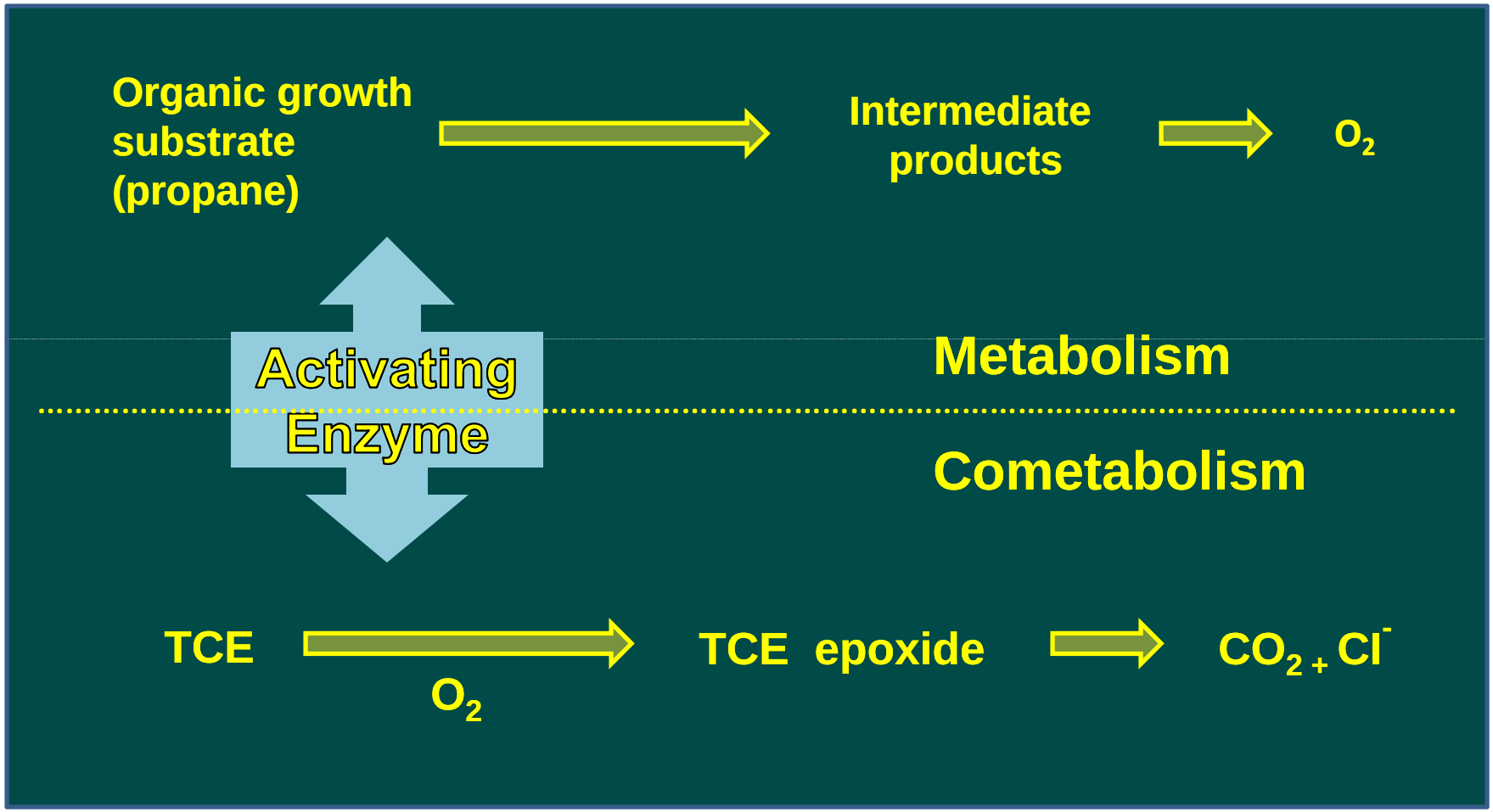

Diagram adapted from Brief #8, *Aerobic Cometabolism with Butane-Grown Microorganisms*, Western Region Hazardous Substance Research Center (WRHSRC), Oregon State University, August 2005.

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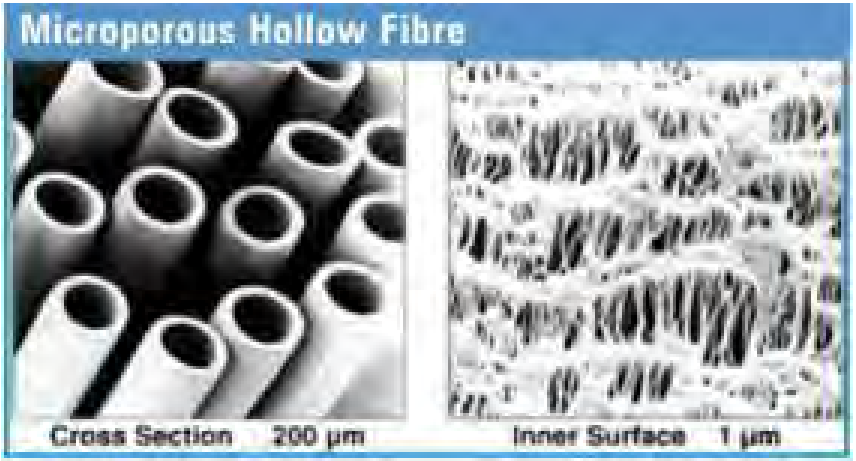
Gas Infusion Technology

iSOC™ gas infusion technology:

- A mass-transfer technology developed by InVentures Technologies, Inc.
- Capable of delivering long term, high concentrations of dissolved gases



Dissolved Gas Concentrations in a Water Column

Gas Type	Water Column Depth (ft)				
	5'	10'	15'	20'	50'
Oxygen	42	55	62	69	111
Methane	22	30	33	37	59
Propane	66	88	99	110	175
Hydrogen	2	2	3	3	5
Ethane	57	75	85	95	150

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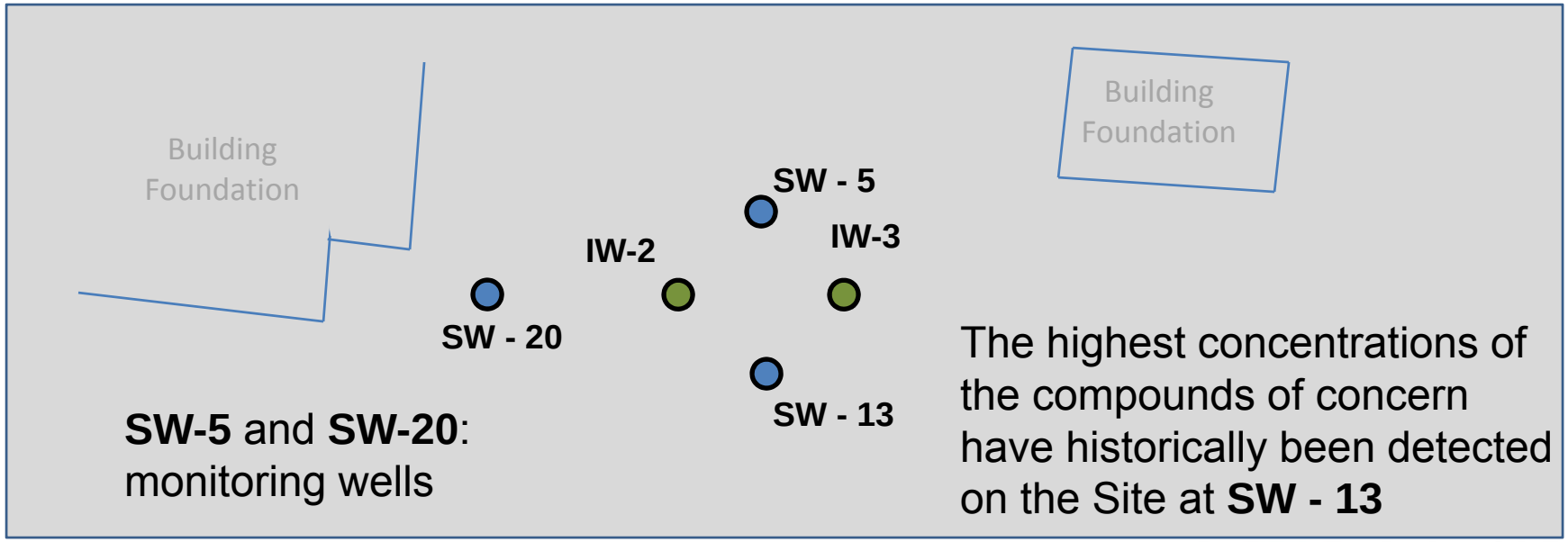
Site Pilot Test

The aerobic cometabolism system:

- Cylinders of industrial grade oxygen and propane (housed separately in portable garden sheds)
- Two-stage regulators to control the line pressure in the gas tubing and iSOC™ units



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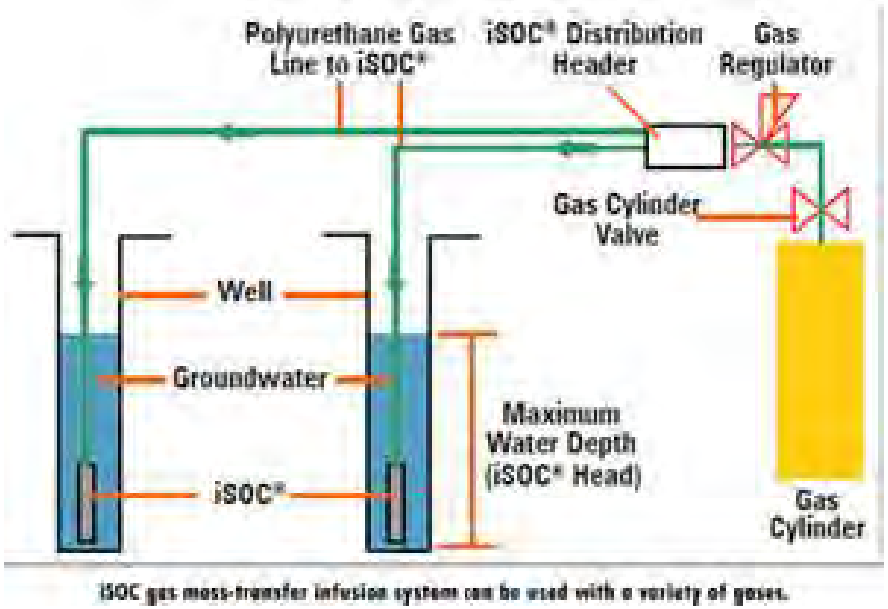
Timeline

2004	2005	2006	2007	2008	2009	2010

Initiated cometabolic pilot test



iSOC gas mass-transfer infusion system can be used with a variety of gases.

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Timeline

2004	2005	2006	2007	2008	2009	2010

Initiated cometabolic pilot test

O_2 and nitrogen/ propane mixture alternated in each well

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Timeline

2004	2005	2006	2007	2008	2009	2010

Initiated cometabolic pilot test

O_2 and nitrogen/ propane mixture alternated in each well

Field testing indicated nitrogen/ propane mixture infusion sup-pressed the O_2 infusion

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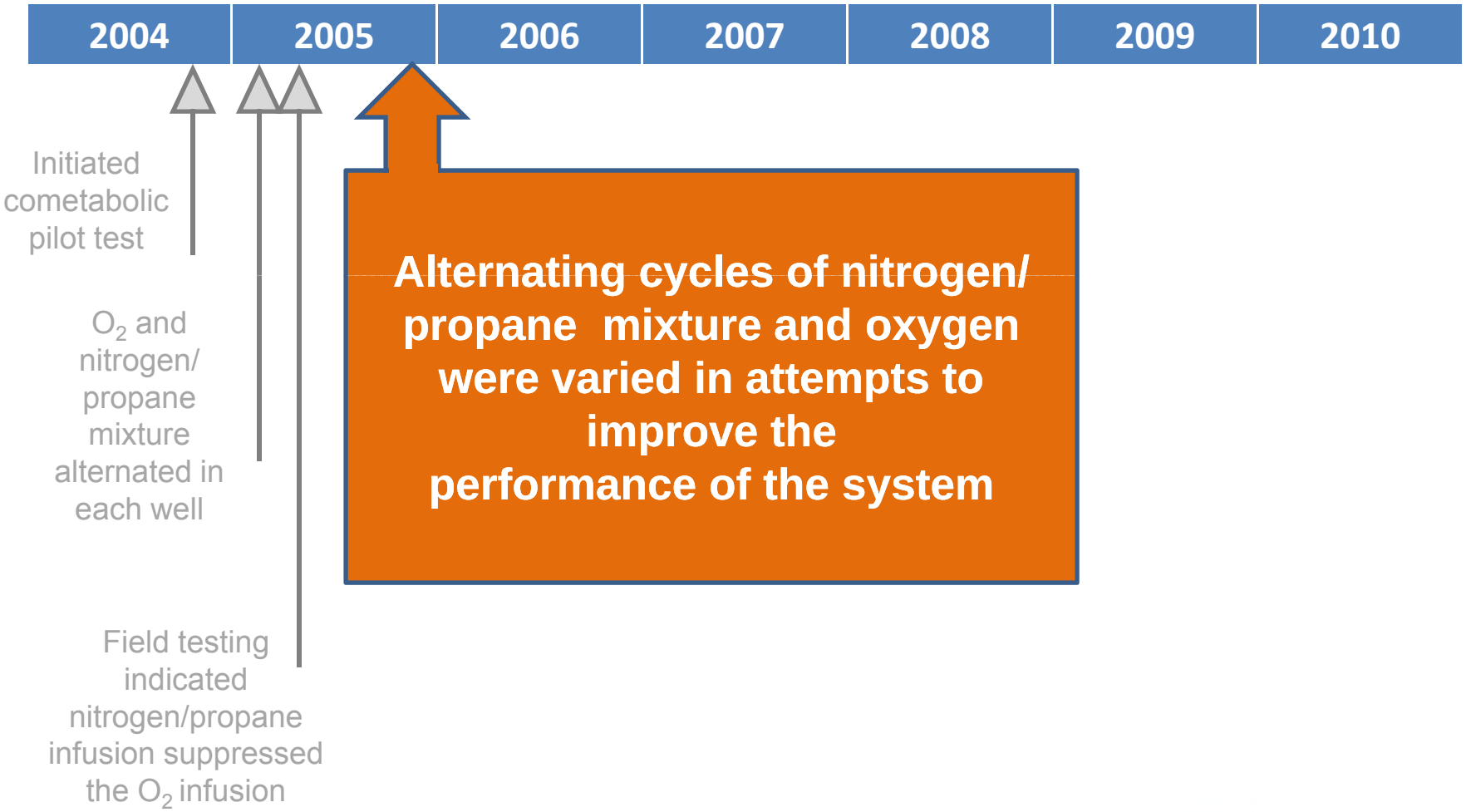
Timeline



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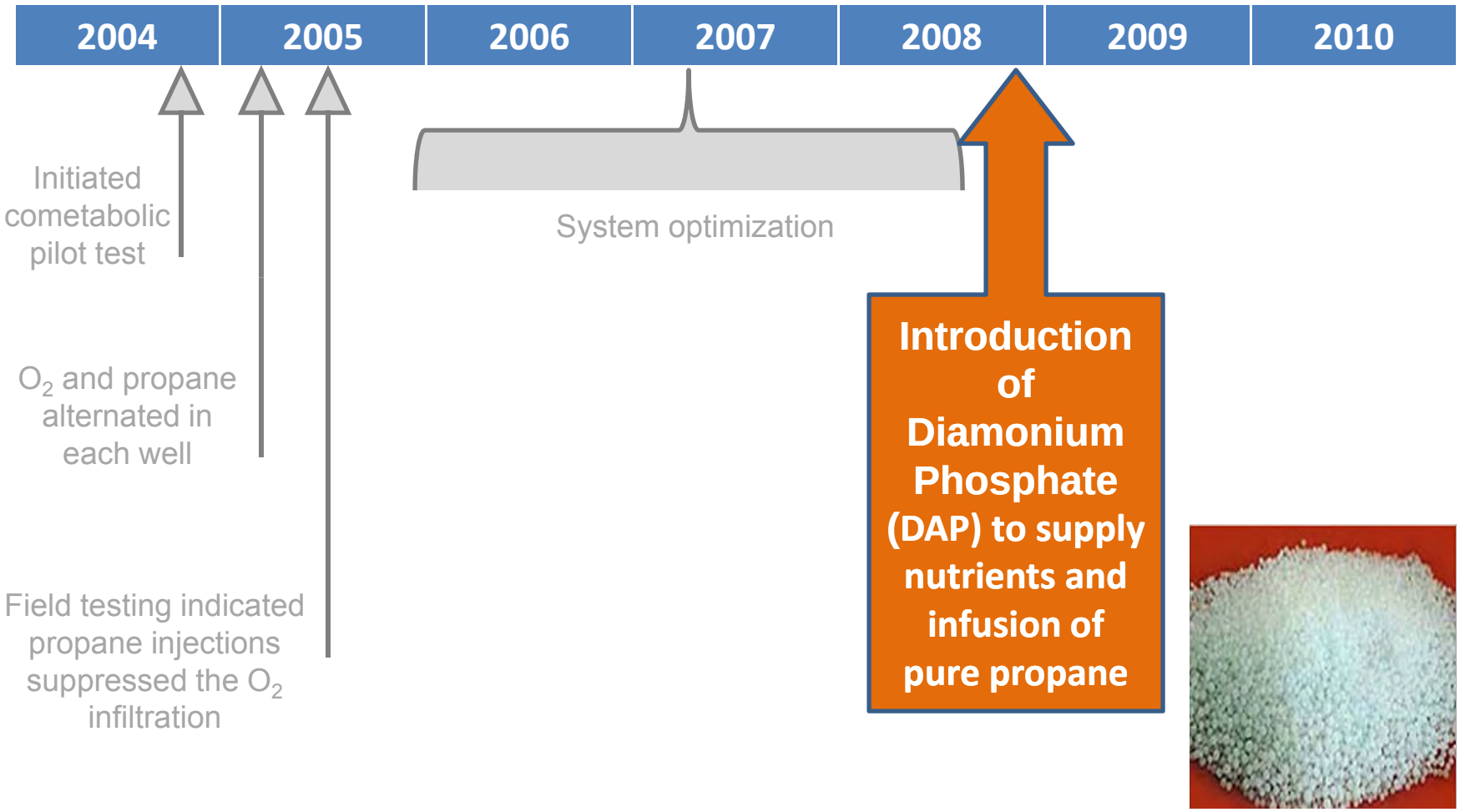
Timeline



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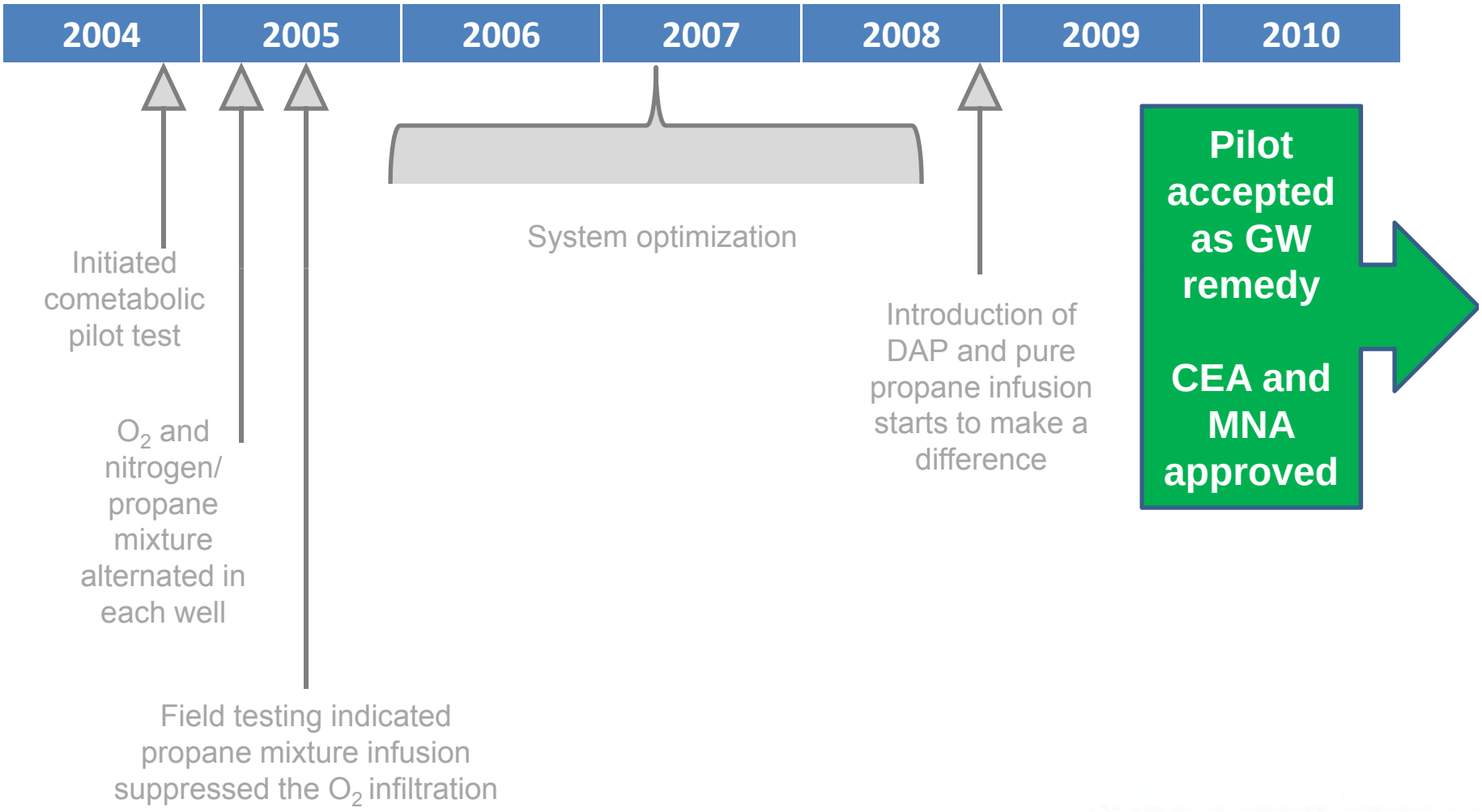
Timeline



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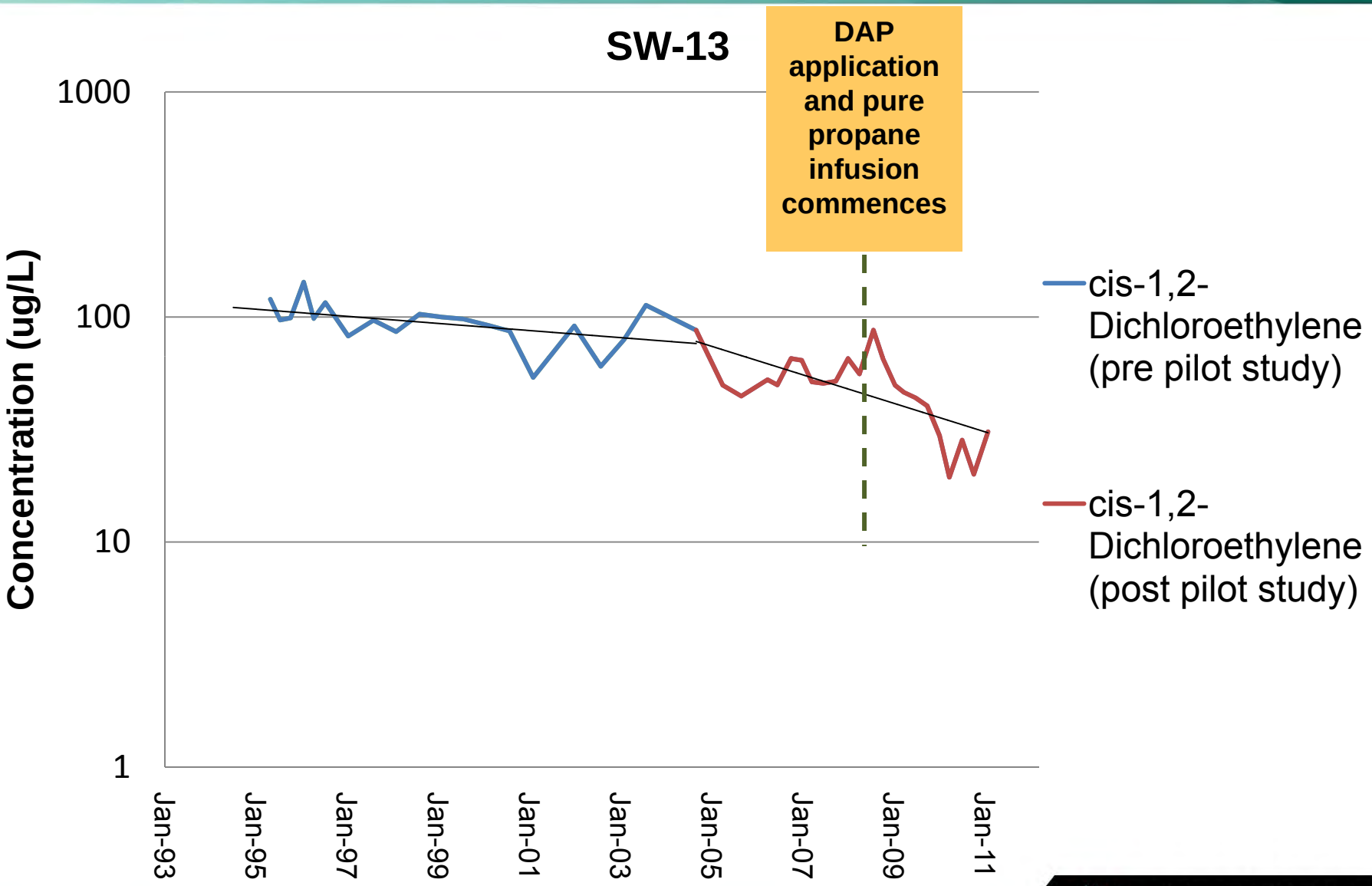
Evaluation of Results of SW-13



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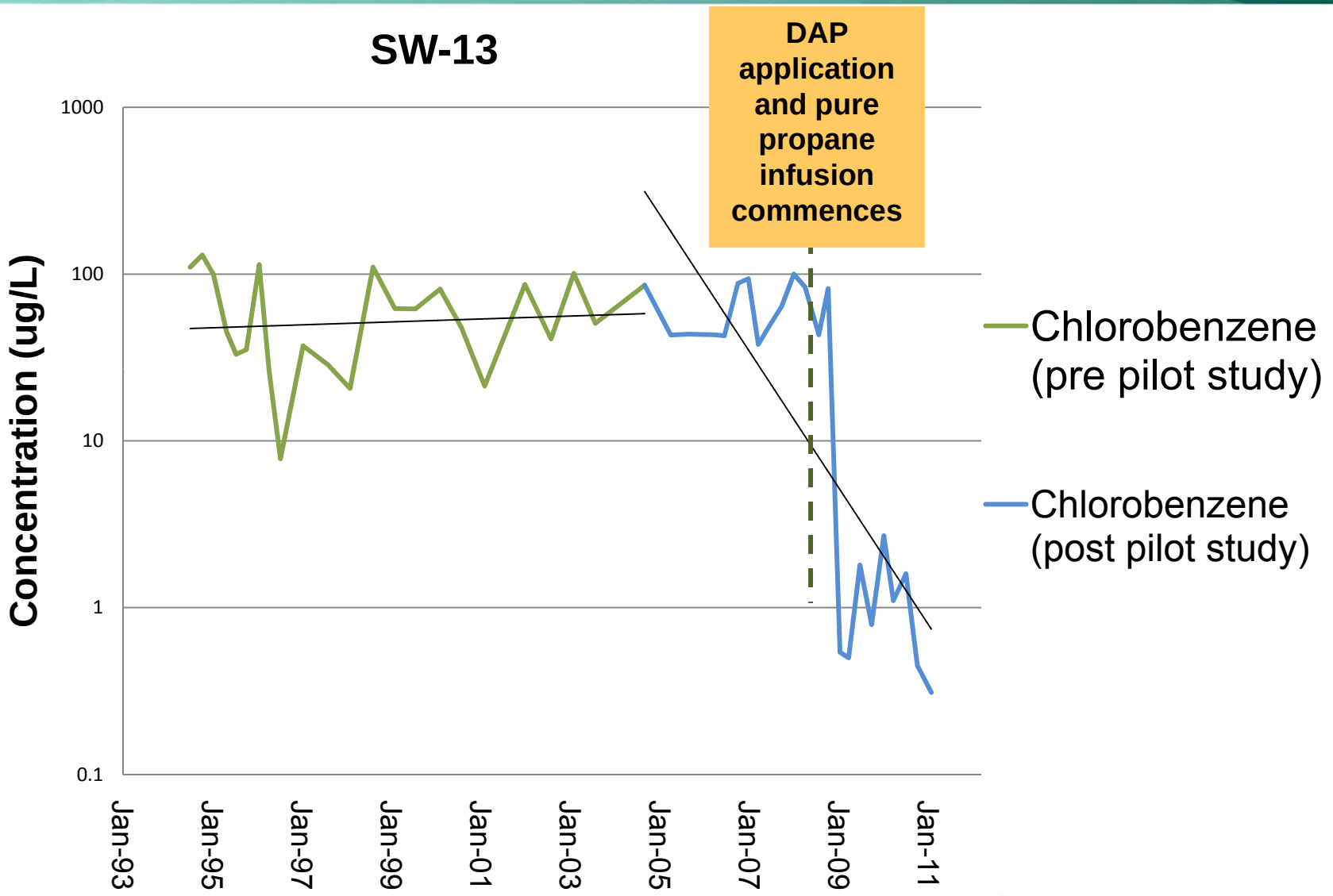
Evaluation of Results of SW-13



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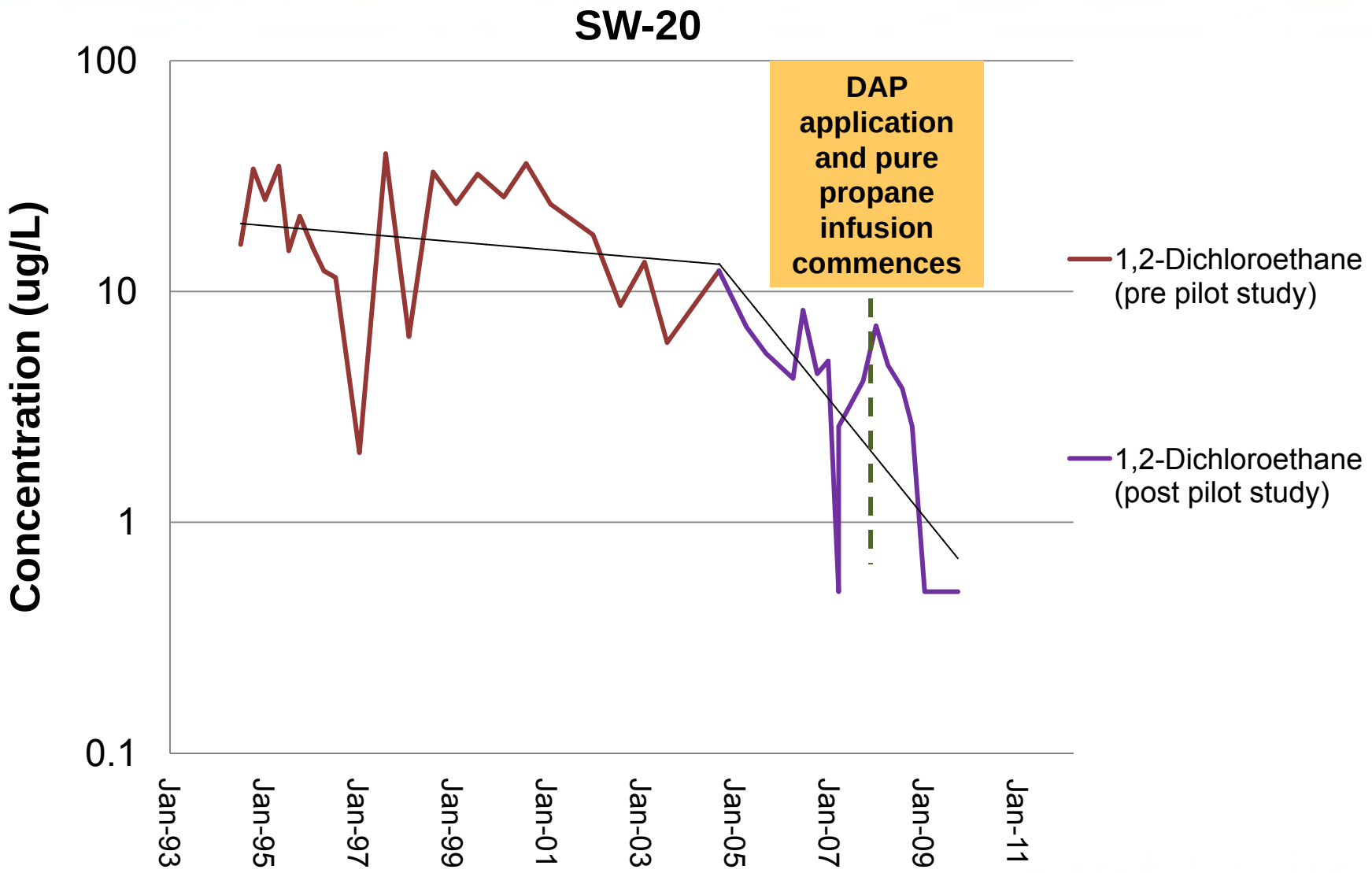
Evaluation of Results of SW-20



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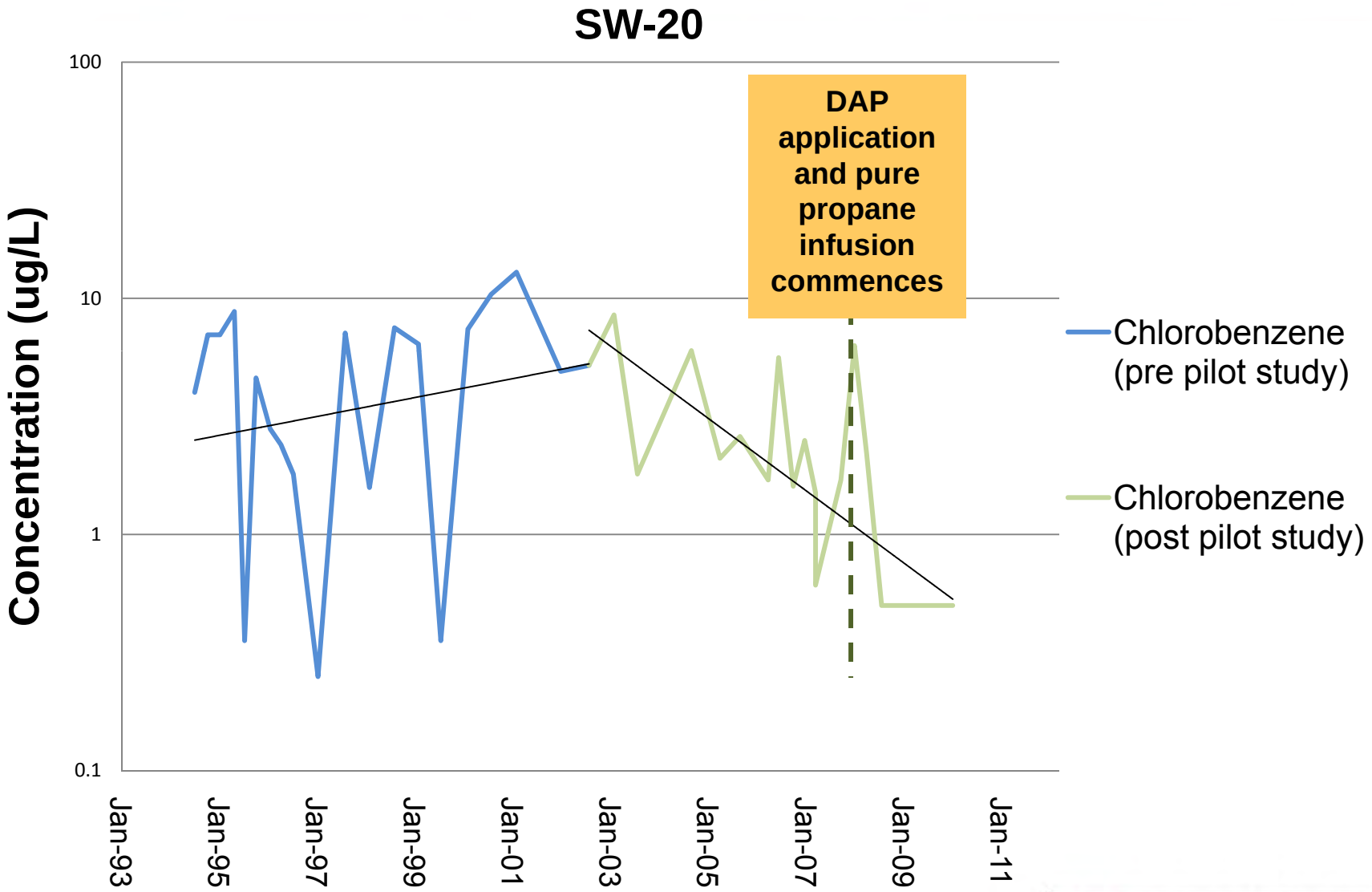
Evaluation of Results of SW-20



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IW-2	Jul-06	Oct-06	Jan-07	Apr-07	Jul-07	Oct-07	Jan-08	Apr-08	Aug-08	Oct-08	Feb-09	Apr-09	Jul-09	Oct-09	Jan-10	Apr-10
Total Organic Carbon	8.8	3.7	3.8	4.2	10.7	15.3	3.8	8.4	6.9	5.4	3.6	11.4	16.4	9.4	5.7	8.5
Inorganic Carbon	25.7	27.2	25.2	24.2	26.1	29.5	24.2	32.7	41.1	15	16.9	62.1	15.6	27.6	14.8	51.7
Total Carbon	34.5	30.9	29.0	28.4	36.8	44.8	28.0	41.1	48	20.4	20.5	73.5	32	37	20.5	60.2
Nitrate	0.56	<0.11	0.57	0.55	<0.21	<0.11	1.2	0.72	<0.11	0.6	0.94	0.35	0.17	0.19	0.42	0.51
Nitrite	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.14	<0.010	0.01	0.048	<0.010	<0.010	<0.010
Nitrogen	0.56	<0.10	0.57	0.55	<0.21	<0.10	1.2	0.72	<0.10	0.74	0.94	0.36	0.22	0.19	0.42	0.51
Ferrous Iron	<0.20	<0.10	<0.10	<0.10	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	0.42	<.20	<0.20	<0.20	0.41
BOD	<2.0	<2.0	2.7	<10	2.9	<3.4	<2.0	<2.0	<2.0	<2.0	<2.0	50.8	5.5	2.4	<3.4	<5.0
COD	42.5	<20	<20	<20	41.6	143	33.6	20.8	23.9	26.3	20.8	121	57.6	23.6	<20	244
Phosphorous	0.062	0.058	<0.050	<0.050	0.085	0.17	0.12	0.063	0.065	0.79	0.49	1.3	0.32	0.33	0.66	0.62
Heterotrophic Plate Count	2812075	23900	48500	20900	10000	26500	16500	17850	1550	>2	17400	95000	32700	66000	31500	270000
Dissolved Propane ug/L	0.19	0.11	0.016 J	0.035 J	1100	0.670		0.22		0.056	0.17	42000	18	67		
DO	19.99	5.63	6.81	9.89	8.79	0.99	6.05	--	19.1	19.91	7.08	2.53	0	7.16		NA
ORP	265	91	65	154	160	-31	131	--	80	80	-142	148	140	121		116

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IW-3	Oct-06	Jan-07	Apr-07	Jul-07	Oct-07	Jan-08	Apr-08	Aug-08	Oct-08	Feb-09	Apr-09	Jul-09	Oct-09	Jan-10	Apr-10
Total Organic Carbon	1.9	1.5	<2.0	2	1.7	2.1	2.6	1.8	1.7	3.9	1.7	2.6	1.8	1.7	2.3
Inorganic Carbon	51.8	50.0	49.0	53.2	46.6	38.3	49.4	51.5	50.2	27.6	51.3	52.4	80.4	42.5	27
Total Carbon	53.7	51.5	50.9	55.2	48.3	40.4	52	53.3	51.9	31.5	53	55	82.2	44.2	29.3
Nitrate	0.29	0.13	0.2	<0.21	0.39	<0.11	<0.11	<0.11	<0.11	8.4	<.11	<.11	<0.11	<0.11	1.7
Nitrite	<0.010	<0.010	<0.010	<0.20	<0.010	<0.010	<0.010	<0.010	0.019	0.042	<0.010	<0.010	<0.010	<0.010	<0.010
Nitrogen	0.29	0.13	0.2	<0.010	0.39	<0.10	<0.10	<0.10	0.11	8.4	<0.10	<.10	<0.10	<0.10	1.7
Ferrous Iron	<0.10	<0.10	<0.10	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<.20	<.20	<0.20	<0.20	1
BOD	<3.4	<2.0	<10	<2.0	<3.4	<2.0	2.6	<2.0	<2.0	3.6	41.8	<2.0	9.2	2.3	<2.0
COD	<20	<20	73.4	<20	<20	<20	<20	<20	<20	<20	57.9	<20	<20	<20	21.9
Phosphorous	0.18	<0.050	0.1	0.074	0.13	<0.050	<0.050	<0.050	4.2	1.3	0.56	3	1.4	0.66	56.3
Heterotrophic Plate Count	134000	6450	25900	4400	8000	8050	1000	<100	1145	18350	2313	4100	2000	40000	52000
Dissolved Propane ug/L	0.04	0.32	0.040 J	550	0.280		2700		0.13	0.18	55000	28	71000		
DO	7	7.01	16.4	14.32	0.59	8.63	--	15.32	10.8	8.54	4.43	0.09	4.93		41
ORP	99	139	169	172	30	97	--	102	147	192	161	107	69		88

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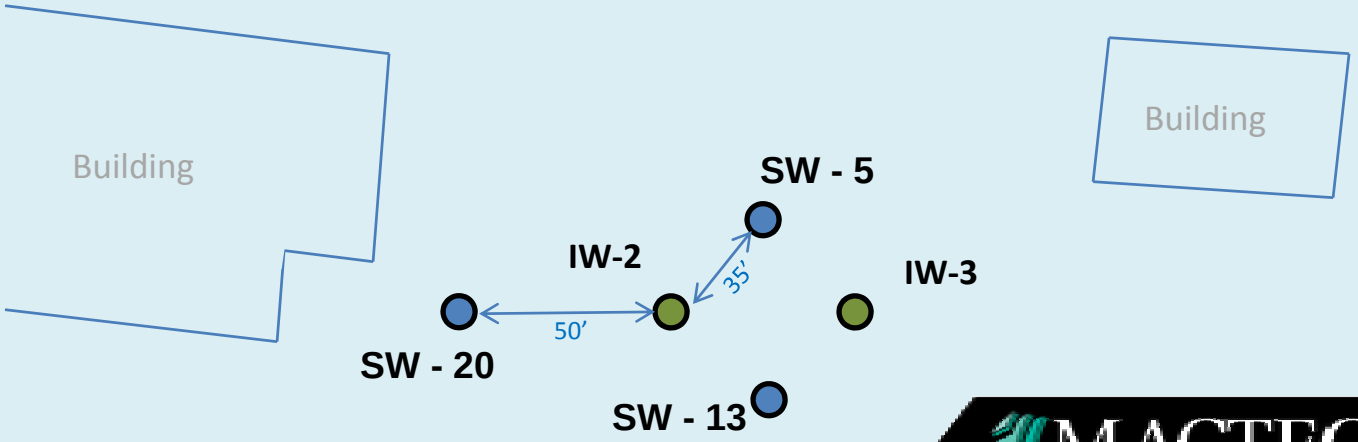
Results

- DO measurements in SW-5, SW-13, and SW-20 generally indicated enrichment and availability of DO and its distribution away from the infusion wells toward these wells
- ORP measurements in SW-5, SW-13, and SW-20 were generally positive and indicative of aerobic enzymatic reactions, but were occasionally negative
- DAP successfully supplied depleted nutrients
- HPC measurements indicated a well developed population of microorganisms
- Dissolved propane concentrations in SW-5, SW-13, and SW-20 (especially over the last 18 months) indicate abundant substrate to support enzyme activity and its distribution away from the infusion wells toward these wells
- For IW-2 the extent of the system's effectiveness was approximately 35 feet north and 50 feet west



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Conclusions

- Bioenhancement was optimized by:
  - The infusion of pure propane
  - The rotation of propane and oxygen infusion from one well to the other
  - The periodic infusion of propane-only and oxygen-only
  - The addition of DAP

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Conclusions

- Aerobic metabolism/cometabolism and reductive dechlorination processes:
  - Bioenhanced the shallow fractured bedrock groundwater system
  - Reduced COCs; and
  - Established downward trends in COC concentrations allowing approval of MNA and monitoring
  - Successfully established reductions of low level COCs to gain closure for groundwater

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I CAN'T STOP VOICING MY HONEST OPINIONS. IT'S CAUSING PROBLEMS AT WORK.
ACCORDING TO YOUR MEDICAL RECORDS, YOU'RE AN ENGINEER. WE CLASSIFY THAT AS A DISEASE NOW.
GAAA!!! YOU HAVEN'T HAD YOUR PON FARR VACCINATION.
IS IT WARM IN HERE?
Dilbert.com DilbertCartoonist@gmail.com
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1-11-11

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Questions

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