

MicroSorb OW (Oxygenated Water)

A hydrated nitrogen liquid that destroys organic wastes and eliminates odors and grease

HOW IT WORKS

MicroSorb Oxygenated Water is a liquid chemical containing hydrated nitrogen and trace minerals. The hydrated nitrogen molecule provides an extremely high amount of oxygen. MicroSorb® OW combined with MicroSorb microbes will destroy organic wastes without the addition of air. MicroSorb® OW is normally used with MicroSorb microbes to break down the contaminant.

APPLICATIONS

MicroSorb® OW is used to:

- 1) Treat contaminated water and coated surfaces where the addition of air is not practical – such as catch basins, utility manholes, oil/water separators, waste collection systems and lagoons.
- 2) Destroy organic wastes and reduce odors in wastewater collection systems, manholes, catch basins, lift stations, and treatment works. (See projects C-1 and C-4)
- 3) Eliminate odors at landfills, transfer stations, kennels, latrines, dumpsters, grease pits, or any place organic odors present a nuisance.

ADDITIONAL PRODUCT INFORMATION

Use MicroSorb® OW in concert with MicroSorb microbes to treat contaminated water. One pound of MicroSorb® ER and one gallon of MicroSorb® OW will treat 1,000 gallons of water. If the surface area of contaminated water is small (such as a manhole), apply an ounce or two of MicroSorb® ER and OW to begin the hydrocarbon/organic waste degradation process.

Apply MicroSorb® OW and MicroSorb® ER, MicroSorb® IS, or MicroSorb® SC to wastewater collection systems to reduce odors, and begin the destruction of organic waste. It is suggested that two gallons of MicroSorb® OW and one pound of MicroSorb® SC be added to one million gallons of sewage. Odors will be eliminated in hours. Grease will be eliminated within days.

MicroSorb® Nutrients

Formulated to dissolve and be used with MicroSorb Microbes

HOW IT WORKS

MicroSorb® Nutrients are a custom blend of water soluble, inorganic nutrients and trace elements formulated to dissolve in water and to be immediately available for use by microbes.

APPLICATIONS

MicroSorb® Nutrients are utilized normally in conjunction with MicroSorb® SC in subsurface soil and groundwater contaminated areas. The nutrients provide needed nitrogen, phosphates and trace elements for the microbes to operate at peak efficiency. (See projects A-1 through A-9)

ANALYSIS

Total Nitrogen	21%
12.8% Ammoniacal Nitrogen	
8.2% Nitrate Nitrogen	
Available Phosphate	10%
Soluble Potash	10%
Sulphur	7%
Boron	0.02%
Copper	0.005%
Iron	0.10%
0.10% Chelated Iron	
Manganese	0.05%
0.05% Water soluble Manganese	
Molybdenum	0.001%
Zinc	0.05%

Derived from Ammonium Nitrate, Ammonium Phosphate, Ammonium Sulphate, Potassium Nitrate, Boric Acid, Iron EDTA, Sodium Molybdate, and the Sulphate form of Copper, Manganese, and Zinc. Potential acidity equivalent to 1101 lbs.

ADDITIONAL PRODUCT INFORMATION

Specific inorganic compounds have been selected for optimal use by MicroSorb microbes. Inorganic nutrients and trace elements are essential for all forms of life. Most soil remediation projects require a 20% ratio of nutrients to a pound of microbes. The substitution of common fertilizers used in gardening is not appropriate for bioremediation as some compounds often interfere with microbial degradation.

MicroBlue®

An emulsifier used with microbes (on surfaces or soils) to break up the contaminant

HOW IT WORKS

MicroBlue® is a biodegradable liquid cleaner designed to work on its own or with MicroSorb® ER. MicroBlue® is a non-contact food grade surfactant that emulsifies the hydrocarbon contaminant thus increasing surface area for the microbes to attack. It is nonflammable and can be used to clean any hard surface or soil media.

APPLICATIONS

MicroBlue® is used to:

- 1) Clean oil or grease-laden parts and equipment.
- 2) Clean sludge pits, tank bottoms, concrete surfaces, separators, other man-made surfaces, and grease traps.
(See project D-1)
- 3) Emulsify heavy oils in soil and reduce the hydrocarbons to water, carbon dioxide, and fatty acid.
- 4) Clean oil-soaked clothes and absorbents.
- 5) Clean filters, floors and other surfaces in restaurants where fats, oils, and grease accumulate.
- 6) Break down floating fats, oils, and greases in lift stations and sewage treatment plants.

ADDITIONAL PRODUCT INFORMATION

MicroBlue® emulsifies the hydrocarbon, thus increasing the surface area of the oil/water interface. The microbes, which live in water, are therefore presented with a greater hydrocarbon surface area providing more sites for microbial digestion.

To use MicroBlue®, add one teaspoon of MicroSorb® ER to a gallon of MicroBlue®, shake and let stand for 30 minutes. Shake again and spray the activated MicroBlue® onto the contaminated surface. Agitate the surface with a stiff broom or brush. The MicroSorb® ER microbes penetrate the pore space of the structure biodegrading the hydrocarbon contamination. Add a little more water and broom the surface again. After 15 minutes, flush with water. Activated MicroBlue® can also be used with power washing equipment.

Inject MicroBlue® into soils contaminated with heavy hydrocarbons such as #4 or #6 fuel oils. MicroBlue® will emulsify the heavy oils providing enhance bioremediation. The microbes in MicroSorb digest the hydrocarbon molecules and the MicroBlue® molecules.

MicroSorb® Natural Degreaser

A powerful surfactant from plant extracts

HOW IT WORKS

MicroSorb® Natural Degreaser dissolves grease into water. MicroSorb® microbes quickly digest the grease turning the grease into beneficial fatty acids. MicroSorb® Natural Degreaser separates the grease molecules and reduces the surface tension of the water. Odorous gases such as Hydrogen Sulfide and Ammonia are contained in the water and used by the microbes in waste reduction.

APPLICATIONS

- 1) Use in lift stations where grease accumulates and creates operational and maintenance problems.
- 2) Use in grease traps at restaurants and hotels to reduce the need for frequent pumping and to control odors.

ADDITIONAL PRODUCT INFORMATION

MicroSorb® Natural Degreaser is available in three strengths, **MicroSorb® Natural Degreaser**, **MicroSorb® Natural Degreaser, IS (Industrial Strength)** and **MicroSorb® Natural Degreaser SC (Super Concentrate)**.

MicroSorb® Natural Degreaser, IS and SC contain steroid saponins. Saponins are plant extracts. The difference between Natural Degreaser, Natural Degreaser IS and Natural Degreaser SC is the concentration of saponins.

For problem lift stations, use Natural Degreaser IS at a rate of 10 gallons per month for a flow of 75,000 gallons per day. Use MicroSorb® IS microbes at a rate of ½ Lb. per day for the first week and ½ Lb. per week thereafter. A good rule of thumb to the start of any application is a rate of MicroSorb® Natural Degreaser IS applied at 15ppm of flow.

For grease traps in restaurants and hotels, use Natural Degreaser IS at a rate commensurate with use. It is suggested that 5 gallons of Natural Degreaser be used per month along with 1.0 Lb. of MicroSorb® IS per month for a grease trap of 2,000 gallons. Adjust volumes and strength of products as required.

Please contact MicroSorb Environmental Products for engineering assistance for your particular application.

Material Safety Data Sheets

MicroSorb® (ER, IS, SC, and DC)

MicroSorb® Biocatalyst

MicroSorb® Nutrients

MicroSorb® OW (Oxygenated Water)

MicroBlue®

MATERIAL SAFETY DATA SHEET**SECTION I - IDENTITY****Product Name - MicroSorb****Manufacturers Name: Proprietary****Contact:****MicroSorb Environmental Products, Inc.****106 Longwater Drive****Norwell, MA 02061**

During regular business hours: (617) 878-0440

SECTION II - HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

MicroSorb - an enzymatic mixture of active hydrocarbon oxidizing natural single celled organisms. The enzymes and cells are contained in an inert preparation of natural clay. The mixture has no chemical impact. The mixture should be handled with the normal precautions of a hygroscopic powder. In enclosed areas protective eye and mask covering is recommended. This mixture has been tested by human contact for a 15 year period with no direct or indirect impact. **Warning** - This clay product contains a small amount of crystalline silica which may cause delayed respiratory disease if inhaled over a prolonged period of time, avoid breathing dust.

SECTION III - PHYSICAL/CHEMICAL PROPERTIES

Boiling Point	-not applicable	Melting point	-not applicable
Vapor Pressure	-not applicable	Solubility in Water	-insoluble, but may be water wet
Specific Gravity	-density of approximately 2.5		
Appearance and Odor	-gray to cream colored powder with a faint odor of hydrocarbons		

SECTION IV - FIRE AND EXPLOSION HAZARD DATA

Flash Point	-nonflammable	Special Fire Fighting Procedures	-not applicable
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SECTION V - REACTIVITY DATA

Stable	-avoid direct consumption or inhalation
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SECTION VI - HEALTH HAZARD DATA

Route of Entry	-inhalation
Health Hazards (acute and chronic)	-normal precautions for hygroscopic powder
Signs and Symptoms of Exposure	-reaction to hygroscopic powder-sneezing, etc..
Emergency and First Aid Procedures	-flush with water and remove to air

SECTION VII - PRECAUTIONS FOR SAFE HANDLING AND USE

Steps to be taken if material is released or spilled	- normal precautions for hygroscopic powder
Waste Disposal Methods	-swept or hosed into lawns, soil, flowerbeds, compost heaps, & septic systems
Precautions for Handling & Storage	-may cause delayed respiratory disease if the dust is inhaled over a prolonged period of time
Other Precautions	-may be slippery when wet

SECTION VIII - CONTROL MEASURES

Respiratory Protection	-in areas of concentration - mask and eye protection is recommended
Ventilation	-local exhaust - standard precaution
Protective Gloves	-not applicable
Eye Protection	-in areas of concentration or enclosed areas
Work/Hygienic Practices	-none
Other Protective Clothing/Equipment	-none

MATERIAL SAFETY DATA SHEET**SECTION I - IDENTITY:**

Product Name - MicroSorb Biocatalyst D.O.T. Class not required Formula: Proprietary
Manufactures Name:
MicroSorb Environmental Products Inc. Contact: Bruce Oborsky
106 Longwater Drive
Norwell, MA 02061
1-781-878-0440

SECTION II - HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

MicroSorb Biocatalyst - A water like solution containing grass extracts, neutral pH, slight discoloration, no turbidity, salts of normal ground water. Potable.

SECTION III - PHYSICAL AND CHEMICAL CHARACTERISTICS

Boiling Point - boiling point of water Melting Point - not applicable
Vapor Pressure - not applicable Solubility in Water - very soluble
Specific Gravity - density of approximately - 1.0
Appearance and Odor - slight discoloration, no odor

SECTION IV - FIRE AND EXPLOSION HAZARD DATA

Flash Point - non-flammable
Distinguishing Media - not applicable
Special Fire Fighting Procedures - not applicable

SECTION V - REACTIVITY DATA

Stable

SECTION VI - HEALTH HAZARDS

Route of Entry - ingestion
Health Hazards (Acute & Chronic) - not applicable
Signs and Symptoms of Exposure - not applicable
Emergency and First Aid Procedures - not applicable

SECTION VII - PRECAUTIONS FOR SAFE HANDLING AND USE

Steps to be taken in case material is released or spilled - none
Waste Disposal Method - Drain into lawns, soil, water, flowerbeds, compost heaps, storm drains and septic systems.
Precautions to be taken in handling and storing - none
Other Precautions - none

SECTION VIII - CONTROL MEASURES

Respiratory Protection - not applicable
Ventilation - not applicable
Protective Gloves - not applicable
Eye Protection - none required
Other Protective Clothing or Equipment - none

THE INFORMATION ON THIS MATERIAL SAFETY SHEET REFLECTS THE LATEST INFORMATION AND DATA THAT WE HAVE ON HAZARDS, PROPERTIES, AND HANDLING OF THIS PRODUCT UNDER THE RECOMMENDED CONDITIONS OF USE. THIS MATERIAL SAFETY DATA SHEET WAS PREPARED TO COMPLY WITH OSHA 24 CFR 1910.1200.

MATERIAL SAFETY DATA SHEET**SECTION I - IDENTITY****Product name - MicroSorb Nutrients Manufacturers name - Proprietary****Contact:****MicroSorb Environmental Products, Inc.****106 Longwater Drive, Norwell, MA 02061 -****During regular business hours: (781) 878-0440****SECTION II - PHYSICAL & CHEMICAL CHARACTERISTICS****Monoammonium Phosphate 11.5%****Ammonium Nitrate 11.5%****Potassium Sulfate 75.0%****Various Micronutrients 2.0%****SECTION III - PHYSICAL HAZARDS & DATA****Boiling Point - not applicable****Vapor Pressure - not applicable****Specific Gravity - not applicable****Solubility in Water - completely soluble****Appearance & Odor - white/gray, slight petroleum odor****SECTION IV - FIRE AND EXPLOSION HAZARD DATA****Flash Point - nonflammable****Flammable Limit Level - non applicable****Extinguishing Media - Water****Special Fire Fighting Procedures - firepersons must wear self-contained breathing apparatus. Heat can cause emission of highly toxic fumes. Heat can cause the nitrate present to act as an oxidizing agent which can support combustion or cause detonation of other products. Persons should be evacuated from down wind areas.****Unusual Fire & Explosion - nitrates may decompose violently when mixed with ammonium salts.****SECTION V - HEALTH & FIRST AID DATA****Threshold Limit Values - not applicable - non-toxic material****Effects Over Exposure - not under normal usage and conditions****Skin - If irritation occurs, wash with water and mild detergent. Remove contaminated clothing and wash before re-use. If irritation persists, seek medical attention.****Eyes - If eye irritation occurs, flush with flowing water for at least 30 minutes. Seek medical attention if irritation persists.****Inhalation - If breathing difficulty occurs due to dust inhalation, remove to fresh air. If discomfort continues, seek medical attention.****Ingestion - If person is conscious, give large amounts of water and induce vomiting. Seek medical attention. Oral ingestion of large amounts may cause nitrate poisoning resulting in dizziness, abdominal cramps, vomiting, bleeding diarrhea, weakness, convulsions and collapse.****SECTION VI - REACTIVITY DATA****Stability - yes****Incompatible Substance - corrosive to metals****Conditions to avoid if unstable-fire conditions and nitrates. Hazardous polymerization-will not occur.****Conditions to avoid uncontrolled polymerization - not applicable****Hazardous decomposition of products - highly toxic fumes of POX and oxides of nitrogen.****SECTION VII - SPILL & DISPOSAL PROCEDURES****Steps to be taken to handle spill or releases -****Pick up or sweep up material and store in plastic bags away from combustibles.****Waste disposal procedures - Dispose of in an approved landfill in accordance with state, federal and local regulations.****SECTION VIII - SPECIAL PROTECTION****Respiratory Protection****- in areas of concentration-mask & eye protection is recommended****Ventilation Required****- normal****Local Exhaust Required****- standard precaution****Protective Clothing/Equipment****- none****Work Hygienic/Practices****- gloves****THE INFORMATION ON THIS MATERIAL SAFETY SHEET REFLECTS THE LATEST INFORMATION AND DATA THAT WE HAVE ON HAZARDS, PROPERTIES, AND HANDLING OF THIS PRODUCT UNDER THE RECOMMENDED CONDITIONS OF USE. THIS MATERIAL SAFETY DATA SHEET WAS PREPARED TO COMPLY WITH OSHA 24 CFR 1910.1200.**

MATERIAL SAFETY DATA SHEET**MicroSorb® OW (Oxygenated Water)**

ENVIRONMENTAL DATA: 0 HMIS REACTIVITY
 0 HMIS HEALTH
 0 HMIS FLAMMABILITY
 0 PERSONAL PROTECTION

COMPANY NAME: MicroSorb Environmental Products, Inc.
 106 Longwater Drive, Norwell, MA 02061
 PHONE NUMBER: 781-878-0440

TRADE NAME: Microsorb® OW

CHEMICAL FAMILY: Inorganic Salts

CAS NUMBER: Blend

CHEMICAL FORMULA: Confidential

HAZARDOUS COMPONENTS: None

PHYSICAL DATA: BOILING POINT(F)	212 Degrees F
FREEZING POINT(F)	ND
VAPOR PRESSURE(mm Hg)	ND
VAPOR DENSITY(Air= 1)	ND
VOLATILITY/VOL(%)	ND
SOLUBILITY IN H2O	Soluble
APPEARANCE, ODOR	Clear, Slight Ammonia
SPECIFIC GRAVITY(H2O=1)	1.17
pH	7

FIRE & EXPLOSION HAZARD:

FLASH POINT	None
EXTINGUISH MEDIA	Will Not Burn
UNUSUAL FIRE HAZARD	Avoid Drying

HEALTH HAZARD DATA: THRESHOLD LIMIT VALUE No Data

ROUTES OF ENTRY:	INHALATION?	SKIN?	INGESTION?
	IRRITANT	MILD IRRITANT	IRRITANT

May irritate eyes, skin, respiratory tract. Ingestion of large amount - violent gastroenteritis

CARCINOGENICITY: No NTP: No IARC MONOGRAPHS: No OSHA REGULATED: No

FIRST AID PROCEDURES:

SKIN: Flush with soap/water.

EYES: Flush thoroughly with water 15 minutes, get immediate medical assistance.

MicroSorb® is the registered trademark of MicroSorb Environmental Products, Inc., 104 Longwater Drive, Norwell, MA 02061

MATERIAL SAFETY DATA SHEET

MANUFACTURERS NAME: MiroSorb Environmental Products Inc.
 106 Longwater Drive
 Norwell, MA 02061
 781-878-0440 / Fax 781-878-8004

IDENTITY: Micro Blue **D.O.T. Class not required** **FORMULA:** Proprietary

SECTION II - HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

(no hazardous ingredients)

SECTION III - PHYSICAL AND CHEMICAL CHARACTERISTICS

Boiling Point	- 100 deg C	Evaporating Rate	-<1
Specific Gravity	- 1.02	Melting Point	-NA
Percent Volatile	- NA	Vapor Pressure mm/Hg	-17.5 mg Hg @ 70 deg F
Flammable Limit	- NA	Vapor Density Air = 1	-624 @ 80 deg F
Reactivity with water	- NA	Solubility in Water	-Dispersible
Appearance	- Blue Liquid Emulsion	Odor	-Mild ammonia aroma
pH	- 7-8		

SECTION IV - FIRE AND EXPLOSION HAZARD DATA

Auto-ignite Temperature	- NA	Unusual Fire and Explosion Hazards	- None
Flash Point	- NA	Special Fire Fighting Procedure	- None
Minimum Ignition Temperature	- NA		

SECTION V - REACTIVITY DATA

Stability	- Stable	Incompatible Substance	- None Known
Polymerization	- No	Decomposition Byproducts	- Carbon Dioxide, water

SECTION VI - HEALTH HAZARDS

Route of Entry - Inhalation, Ingestion, Skin or Eye Contact
 Health Hazards, Acute and Chronic - None
 Conditions Aggravated by Exposure - Hypersensitive Individuals may develop allergic dermatitis
 Carcinogenicity - None
 Emergency First Aid Procedures - (Inhalation) Move to Fresh Air
 (Ingestion) If in large quantities, contact physician
 (Skin or Eye) Wash or irrigate with water

SECTION VII - PRECAUTIONS FOR SAFE HANDLING AND USE

Precautions to be taken in handling - None
 Precautions to be taken in case of spill - Floor may be slippery. Flush area with water
 Disposal procedures - In most areas may be flushed down drain with water. Consult agencies for special requirements in your area.
 Storage requirements - Closed container at temperature range of 40 to 120 degrees F

SECTION VIII - CONTROL MEASURES

Respiratory Protection	- None required in good ventilation
Ventilation Required	- Normal
Local Exhaust Required	- No
Protective Clothing/Equipment	- Rubber or plastic gloves and safety glasses/Eye wash facility
Work Hygienic/Practices	- Exercise reasonable personal cleanliness

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MicroSorb Correspondence

The following pages document the fact that MicroSorb microbes are safe and naturally occurring. This section also includes regulatory correspondence.

It is important to know that MicroSorb microbes are nonpathogenic and nonhazardous. They are naturally occurring and are harmless to living organisms. Accu Lab and The University of Padova's reports, which follow, verify that dangerous bacteria are not present in MicroSorb products.

This section also contains letters from various public agencies regarding MicroSorb products. Letters from regulatory agencies do not constitute an endorsement, warranty, or approval with respect to the benefit, effectiveness, or performance of any products.

Florida Department of Environmental Protection

Bureau of Petroleum Storage Systems

Massachusetts Water Resources Authority

Toxic Reduction and Control Department

Accu Lab, Newark, Delaware

Serenambiente / University of Padua (Universita Degli Studi Di Padova), Padova, Italy

BioTechnology Department Analysis Certificates



Department of Environmental Protection

Jeb Bush
Governor

Twin Towers Office Building
2600 Blair Stone Road
Tallahassee, Florida 32399-2400

David B. Struhs
Secretary

May 24, 2001

Mr. William E. Baird
MicroSorb Environmental Products, Inc.
106 Longwater Drive
Norwell, Massachusetts 02061

Re: **MicroSorb**

Dear Mr. Baird:

The Bureau of Petroleum Storage Systems hereby accepts the following MicroSorb products for both in situ and ex situ bioremediation of contaminated sites in Florida:

MicroSorb Super Concentrate (SC);
MicroSorb Biocatalyst;
MicroSorb Nutrient;
MicroSorb Emergency Response (ER);
MicroSorb De-Chlorinator Product (DC); and
MicroSorb Industrial Strength (IS).

Acceptance is for these six specific products, for applications within the jurisdiction of this bureau, which is generally the remediation of petroleum in subsurface soil and groundwater. There may be other uses for them in emergency response, surface cleaning, and the remediation of chlorinated organic hydrocarbons and other suitable compounds. This is not, however, an acceptance for the complete current line of MicroSorb products, future MicroSorb products, or any MicroSorb products for which this bureau has not yet received a complete chemical analysis.

MicroSorb ER, MicroSorb IS, MicroSorb SC, and MicroSorb DC products contain varying concentrations of non-pathogenic, naturally occurring, microorganisms which have been dehydrated and mixed with an inert preparation of natural clay. The microorganisms are cultivated in seawater with ammonia as the only nitrogen source and crude oil as the only source of carbon.

Per chemical analysis shown in enclosure 1, the MicroSorb Biocatalyst is a proprietary liquid of grass extracts and oxygen-enriching water that is used to supply the microbes with oxygen. MicroSorb Nutrient is a blended fertilizer containing nitrogen, phosphorous, potassium, sulfur, and trace nutrients. The bureau has no objection to the use of MicroSorb Nutrient for injection-type aquifer remediation projects provided that this material, which is shipped in dry form, is mixed with sufficient clean water to comply with the underground injection control requirements of

Mr. William E. Baird
May 24, 2001
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Chapter 62-528, Florida Administrative Code. If a Remedial Action Plan proposes the use of a MicroSorb nutrient/water ratio that does not meet the injection control requirements, then it will be necessary to obtain a variance for a temporary zone of discharge before the mixture is injected.

MicroSorb products augment the bioremediation of contaminants by the application of a mixture of microbes, nutrients, and the biocatalyst. The rapid biodegradation of pollutants is achieved through the injection of a varied species of microbes in high densities; for example, MicroSorb ER contains 5 billion microbes per gram, MicroSorb IS contains 20 billion microbes per gram, and MicroSorb SC contains 90 billion microbes per gram.

The in situ application of the MicroSorb mixture can take two forms. In the first form, if free product is present or soils in the vadose zone are contaminated, then an interceptor trench or series of recovery wells are installed. Contaminated water from them is then pumped to a bioreactor tank, the mixture is introduced, any free product is removed, and the treated fluid in the bioreactor drains back into the source area through leaching trenches or pits. The second form of application involves injecting the mixture of MicroSorb microbes, nutrients, and biocatalyst into a series of injection wells. This application is effective for the treatment of dissolved contamination. This MicroSorb mixture works by biochemically removing a carbon atom from the hydrocarbon and replacing it with two oxygen atoms to form a water-soluble fatty acid. The ultimate degradation products of hydrocarbon contaminants are carbon dioxide and water, and in the case of chlorinated hydrocarbon contaminants, chloride ions as well.

For in situ vadose remediation, when the underlying groundwater will not be affected by the leaching of this product, there are no special concerns beyond those which would normally need to be addressed in preparing a Remedial Action Plan and conducting a cleanup in accordance with the petroleum cleanup requirements of Chapter 62-770, Florida Administrative Code (F.A.C.). For ex situ groundwater treatment, when an aboveground treatment system produces effluent meeting the petroleum cleanup criteria referenced by Chapter 62-770, F.A.C., and the drinking water standards of Chapter 62-550, F.A.C., for disposal via recharge gallery or NPDES permit, there are no special concerns. But for in situ groundwater remediation via injection of the product into an aquifer, there are underground injection control (UIC) regulations that must be observed. Since in situ aquifer remediation via injection is likely to be the most common application of this product, the bulk of the regulatory requirements discussed in enclosure 2 will be directed to that topic.

The bureau recognizes the above-referenced MicroSorb products as a viable means for the bioremediation of petroleum contaminated sites in Florida. There are no objections to their use provided: (a) the considerations of this letter are taken into account; and (b) a Remedial Action Plan is approved by the Department.

Mr. William E. Baird

May 24, 2001

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environmental standpoint with respect to applicable rules and regulations, and the interests of public health, safety, and welfare. Vendors must then market the products and processes on their own merits regarding performance, cost, and safety in comparison to competing alternatives in the marketplace. In no way, however, shall this letter regarding regulatory acceptance (or the conditions of regulatory acceptance) be construed as certification of product or process performance.

Preparers of Remedial Action Plans are advised to include a copy of this letter in the appendix of plans they submit, and call attention to it in the text of their document. In this way, technical reviewers throughout the state will be informed that you have contacted the Department of Environmental Protection in regard to MicroSorb products. To aid those reviewers, the Bureau of Petroleum Storage Systems provides supplemental information as enclosure 3.

The Department reserves the right to revoke acceptance of any product or process if the nature or composition of either its ingredients or performance has been falsely represented. Additionally, Department acceptance of any product or process does not imply it has been deemed applicable for all cleanup situations, or that it is preferred over other treatment or cleanup techniques in any particular case. A site-specific evaluation of applicability and cost-effectiveness must be considered for any product or process, whether conventional or innovative, and adequate site-specific design details must be provided in Remedial Action Plans prescribing the product or process. Technical questions regarding acceptance of the MicroSorb products described in this letter may be directed to Ms. Donna Cline, P.E., at telephone 850/222-6446.

Sincerely,

Rick Ruscito

Rick Ruscito, P.E.

Bureau of Petroleum Storage Systems



MASSACHUSETTS WATER RESOURCES AUTHORITY

Charlestown Navy Yard
100 First Avenue
Boston, Massachusetts 02129

September 25, 1997

Telephone: (617) 242-8000
Facsimile: (617) 241-8070

Mr. William E. Baird, P.E.
President
MicroSorb Environmental Products
106 Longwater Avenue
Norwell, MA 02061

Subject: Approval to Use MicroSorb in Grease Traps and Grease Interceptors in the MWRA
Sewer District

Dear Mr. Baird:

We have reviewed the information that you sent us with your request for permission to discharge wastewater containing MicroSorb to the sewer. We understand that MicroSorb is a dry mixture containing microbes and inert powder. The information sent says that MicroSorb converts hydrocarbons, including fats, oils, and grease, to nontoxic fatty acids, carbon dioxide, and water. It also says that the microbes are not pathogens and that the mixture is not toxic. You provided evidence that the Massachusetts Department of Environmental Protection (DEP) has approved this product for use in septic systems.

The Massachusetts Water Resources Authority (MWRA) approves MicroSorb for use in grease traps and interceptors in the MWRA Sewer District, as authorized by 360 CMR 10.017, subject to the following conditions:

1. The approved use is for MicroSorb only, as described in the material gave to MWRA on August 12, 1997.
2. MicroSorb maintains its approval by DEP for use in septic systems.
3. Approval by the MWRA for use is not an endorsement of the product or an approval with respect to the benefit, effectiveness, or performance of the additive.
4. The resulting discharge will not cause or contribute to an obstruction or blockage in the sewer or otherwise violate MWRA discharge standards at 360 CMR 10.021 - 10.024.

If you have any questions or need further assistance please do not hesitate to call me at (617) 241-2375.

Very Truly Yours,

A handwritten signature in dark ink, appearing to read "David G. Drain".

David G. Drain
Manager, Technical Services
Toxic Reduction and Control Department

cc. K. McManus, C. Fiore, E. Benson, W. Lazaris, J. Auge, P. Yarossi,



MASSACHUSETTS WATER RESOURCES AUTHORITY

Charlestown Navy Yard
100 First Avenue
Boston, Massachusetts 02129

September 23, 1997

Telephone: (617) 242-6000
Facsimile: (617) 241-6070

Mr. William E. Baird, P.E.
President
MicroSorb Environmental Products
106 Longwater Avenue
Norwell, MA 02061

Subject: Request to Discharge MicroSorb

Dear Mr. Baird:

We have reviewed the information that you sent us with your request for permission to discharge wastewater containing MicroSorb to the sewer. We understand that MicroSorb is a dry mixture containing microbes and inert powder. The Authority does not grant approval of any products or pretreatment methods. Any product or system that produces an effluent complying with our local limits is acceptable to us.

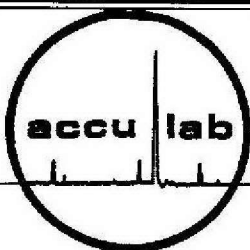
The material sent to us about MicroSorb does not give us any reason to object to its discharge, when it is used as directed. The information sent says that MicroSorb converts toxic hydrocarbons to nontoxic fatty acids, carbon dioxide, and water. The information sent also says that the microbes are not pathogens and that the mixture is not toxic. The discharge from your customer's facilities must meet our discharge limits for all regulated substances. A copy of our discharge limits is attached.

If you have any questions or need further assistance please do not hesitate to call me at (617) 241-2375.

Very Truly Yours,

David G. Drain
Manager, Technical Services
Toxic Reduction and Control Department

cc. K. McManus, C. Fiore, E. Benson, W. Lazaris, J. Auge, P. Yarossi,



700 Barksdale Road, Newark, Delaware 19711

December 5, 1996

William E. Baird, PE. LSP
Microsorb Environmental Products, Inc.
106 Longwater Drive
Norwell MA 02061

Dear Mr. Baird:

On November 12, 1996 we received a sample of your microbial product. We used recognized cellular fatty acid techniques to identify the various microbe species isolated from the sample (also using standard isolation techniques). Briefly, this involves extracting cellular fatty acids from sample cultures, creating fatty acid profiles for each using gas chromatography and, finally, comparing the resulting profiles to profiles of known species contained in a commercially available database (the Microbial Identification System or Sherlock System).

Although we were unable to obtain perfect matches to our database for all isolates, the matches we obtained were sufficient to enable me to state confidently that your sample contained no organisms that are considered pathogenic to animals or plants (where "pathogenic" is used to describe an organism that, when present, always or nearly always causes disease in its host):

As per your request regarding specific bacteria species, I can also state confidently that your sample contained none of the following:

Aeromonas spp.
Camylobacter spp
Clostridium botulinum
Legionella spp.
Salmonella spp
Staphylococcus aureus

Bacillus megaterium
Clostridium perfringens
E. coli
Pseudomonas marginalis
Shigella spp.
Yersinia enterocolitica

Please do not hesitate to contact me, if you have questions or would like additional information about our methods.

Sincerely,

C. Julius Meisel, Ph.D.
Executive Vice President

Telephone (302) 292-8888

FAX 302-292-8468



Zona Artigianale Lagarine, n 12 Scurelle (TN). Tel 0461-780122 - fax 0461-782760

Sede operativa di Belluno: tel 0437 291025 - fax 0437 292761
P.IVA 01705580221

William E. Baird, P.E. President
MicroSorb Environmental
Products, Inc.
106 Longwater Drive
Norwell, MA 02061

Scurelle, 29th July 2002

Dear Bill,

We are glad to send you the analysis certificates which the Agrarian Bio-technology Dept. of the University of Padua, in the person of the director, Prof. Sergio Casella, developed and regarding the certification that Microsorb products do not contain pathogen agents.

Italian law is very precise as regards the utilization of bacterial products: if they are employed in restrained spaces (bio-piles), there are no prescription for their utilization except their non-pathogeneity. Otherwise, if you want to use them in free spaces it is established that they shall be certified as non-pathogen and not genetically modified.

As you can well understand, for the utilization of bacteria in a free space, there is an obstacle to overcome, due to the fact that it must be an Italian laboratory, recognized by the Public Body, to certify that these micro-organisms are genetically unmodified, an action that, besides, only the manufacturer of these bacteria can do.

This is the reason why in the European Community the responsible persons are passing laws that provide that in the future all the imported biological products shall state exactly the type of bacteria they contain and they shall supply the instrument necessary for the verification of it.

At the moment the Italian law requires this kind of information, but it does not give any indication as for modalities; for this reason I thought the best way was that of involving Public Authorities (the Environment Dept. of the Province of Belluno, the Commune of Feltre and, above all, the University) in order to perform the monitoring of the bio-pile in Feltre. In this way, the Public Authorities, still suspicious in the utilizing of this technology, will have at their disposal data coming from official research, developed by authoritative organisms and this will permit us to utilize your bacteria in a free space.

In the document of approval of the land reclamation project, the Commune of Feltre and the Province of Belluno declare to approve the treatment in biopile and to interrupt the

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bioremediation exam in the open space for the verification of the results in biopile (see attached document).

When the University supply the result of its research (in September), Public Authorities (Commune and Province), on the basis of results, will express themselves for the utilization of micro-organisms in the free space.

In the research developed by the University, the monitoring system of the bacteria injected in the soil will be described, thus any problem will arise in their utilization. The question of genetically modified micro-organisms will stay suspended, and, in my opinion, it will be resolved by supplying some names of natural-originated bacteria that are present in the products and that are identifiable (for these technical aspects you will be contacted by Dr. Maurizio Paro).

Consequently, the certification to the utilization of your products could happen only when, once examined the report of the University, the Authorities of the Commune of Feltre and of the Province of Belluno give the authorization for the utilization of your products in a free space. At that time, we could utilize bioremediation products in site in all the Italian territories and thus in the European Community, without any problem.

This is the best way to progress in order to gain a sure result, to reassure authorities and to put them in the condition of take decisions positively, without distrusting private companies.

At the end of the research, that is supported by the Province of Belluno and by the Region of Veneto, it will take place a meeting to which all the operators in the sector, the authorities and the technicians of North Italy Public Bodies will be invited.

Your participation in the event will be decisive for the work of the meeting and will be particularly appreciated by me.

Sincerely

Nicola Cendron, Ph.D.

President

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UNIVERSITÀ DEGLI STUDI DI PADOVA
DIPARTIMENTO DI BIOTECNOLOGIE AGRARIE
AGRIPOLIS - STRADA ROMEA, 16 - 35020 LEGNARO (PADOVA)
TEL. (049) 8272905 - FAX 8272929

Legnaro, 29/04/02
Prot. N. 85

Con la presente si certifica che le analisi in allegato, eseguite presso l'Istituto Zooprofilattico Sperimentale delle Venezie, sono state eseguite sui prodotti denominati Microsorb DC (Farina Fossile-DC), Microsorb SC (Farina Fossile-SC), Microsorb IS (Farina Fossile-IS), Microsorb ER (Farina Fossile-ER), distribuiti dalla Ditta Serenambiente.

La D.ssa Marina Basaglia del Dipartimento di Biotecnologie Agrarie dell'Università di Padova ha personalmente eseguito il campionamento ed ha provveduto a consegnare il materiale all'Istituto Zooprofilattico Sperimentale delle Venezie.

Il Direttore
Prof. Sergio Casella

D.ssa Marina Basaglia

CERTIFICAZIONE DI AUTENTICITÀ

A seguito di controllo effettuato, si certifica che la copia del
Fiat IS, numero 2 del 1982, è una copia che ha la stessa copia
ottenuta dal procedimento fotografico e composta di

nr. T.N. fogli, e conforme all'originale

☐ qui depositato ☒ qui esistente ☐ qui annesso

Si rilascia ☐ in carta rosa legale ☐ in carta libera

per uso CAMPIONATO

Strigno, il 16.05.02 IL FUNZIONARIO INCARICATO



TISO 10A

(firma per estrazione)

(firma per estrazione)

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLE VENEZIE - AREA CONTROLLO ALIMENTI
 BATTERIOLOGIA - VIA ROMEA 14/A, 35020 LEGNARO PD - TEL 049/8830380 FAX 8830484

RAPPORTO DI PROVA

DIPARTIMENTO DI BIOTECNOLOGIE AGRARIE
 STRADA ROMEA 16
 35020 - LEGNARO - PD

laboratorio accreditato dal SINAL n°0155

N. REGISTRO = 756/A02 ACCETTAZIONE: 22/03/02 (inizio-fine analisi: 22/03/02-11/04/02)

VERBALE INVIO CAMPIONI : data: 22/03/02 n.riferimento: 756P firma: **
 ORIGINE CAMPIONI : DIP.BIOTECNOLOGIE AGRARIE
 LEGNARO - PD
 LUOGO PRELIEVO : (LUOGO PRELIEVO NON INDICATO)

PROSPETTO DEGLI ACCERTAMENTI E DELLE PROCEDURE DI PROVA <POP>

1-ANALISI MICROBIOLOGICA (N.CAMPIONI = 4)

include:	•COLIFORMI TOTALI	<PDP BAT25>(A) - REV. 4 (metodo interno)
	•YERSINIA ENTEROCOLITICA	<PDP BAT19>(N) - REV. 6 (metodo interno)
	•ESCHERICHIA COLI	<PDP BAT25>(A) - REV. 4 (metodo interno)
	•BACILLUS ANTHRACIS	<PDP NC>(N)
	•SALMONELLA SPP. (QUALITATIVA) SU 25 G	<PDP BAT07>(A) - REV. 7 (metodo interno)
	•LISTERIA MONOCYTOGENES (QUALITATIVA)	<PDP BAT30>(A) - REV. 6 (metodo interno)
	•VIBRIO CHOLERAE 01, 0139(FDA/BAM BED:95)	<PDP BAT32>(A) - FDA BAM Bed 95 cp.9,C1-C6 (a-d)

MATERIALE: (ALTRO)

METODICA ANALISI: METODICA MICROBIOLOGICA

SPECIE: (SPECIE ANIMALE NON ATTRIBUIBILE)

CAUSALE INVIO: ESAME A PAGAMENTO - CAMPIONE IN 1 ALIQUOTA

(A) = POP ACCREDITATA DAL SINAL

<PDP NC> = POP NON ANCORA CODIFICATA

(N) = POP NON ACCREDITATA DAL SINAL (non rientra nell'accreditamento SINAL di questo laboratorio)

ESITO DEGLI ACCERTAMENTI

N° CAMPIONE	IDENTIFICAZIONE	ESITO
(1-4)	(accertamento 1)	ANALISI MICROBIOLOGICA su (ALTRO) di (SPECIE ANIMALE NON ATTRIBUIBILE) (METODICA MICROBIO)
756/ 1	FARINA FOSSILE-DC	COLIFORMI TOTALI < 10 UFC/G YERSINIA ENTEROCOLITICA ASSENTE IN 25 G ESCHERICHIA COLI < 10 UFC/G BACILLUS ANTHRACIS NEGATIVO SALMONELLA SPP. (QUALITATIVA) SU 25 G ASSENTE IN 25 G LISTERIA MONOCYTOGENES (QUALITATIVA) ASSENTE IN 25 G VIBRIO CHOLERAE 01, 0139(FDA/BAM BED:95) ASSENTE IN 25 G
756/ 2	FARINA FOSSILE-SC	COLIFORMI TOTALI < 10 UFC/G YERSINIA ENTEROCOLITICA ASSENTE IN 25 G ESCHERICHIA COLI < 10 UFC/G BACILLUS ANTHRACIS NEGATIVO SALMONELLA SPP. (QUALITATIVA) SU 25 G ASSENTE IN 25 G LISTERIA MONOCYTOGENES (QUALITATIVA) ASSENTE IN 25 G VIBRIO CHOLERAE 01, 0139(FDA/BAM BED:95) ASSENTE IN 25 G

N° REGISTRO : 756/A02 DEL 22/03/02

N° CAMPIONE	IDENTIFICAZIONE	ESITO
(1-24)	(accertamento 1)	ANALISI MICROBIOLOGICA su (ALTRO) di (SPECIE ANIMALE NON ATTRIBUIBILE) (METODICA MICROBIO)
756/ 3	FARINA FOSSILE-IS	COLIFORMI TOTALI < 10 UFC/G YERSINIA ENTEROCOLITICA ASSENTE IN 25 G ESCHERICHIA COLI < 10 UFC/G BACILLUS ANTHRACIS NEGATIVO SALMONELLA SPP. (QUALITATIVA) SU 25 G ASSENTE IN 25 G LISTERIA MONOCYTOGENES (QUALITATIVA) ASSENTE IN 25 G VIBRIO CHOLERAE O1, O139(FDA/BAM BED:95) ASSENTE IN 25 G
756/ 4	FARINA FOSSILE-ER	COLIFORMI TOTALI < 10 UFC/G YERSINIA ENTEROCOLITICA ASSENTE IN 25 G ESCHERICHIA COLI < 10 UFC/G BACILLUS ANTHRACIS NEGATIVO SALMONELLA SPP. (QUALITATIVA) SU 25 G ASSENTE IN 25 G LISTERIA MONOCYTOGENES (QUALITATIVA) ASSENTE IN 25 G VIBRIO CHOLERAE O1, O139(FDA/BAM BED:95) ASSENTE IN 25 G

NOTE

Il presente referto riguarda solo i campioni sottoposti a prova.
Esso non può essere riprodotto parzialmente (salvo approvazione scritta da parte dell'Istituto).
I campioni vengono eliminati alla data di fine analisi, ad eccezione dei casi previsti da normative specifiche.

LEGHARD, li 11/04/02

Il laboratorista - Dr. G. PEZZOTI

Il direttore - Dr. F.M. CANCELLOTTI

PAGINA 2/2 - TERMINE STAMPA - COMUNICAZIONE DEFINITIVA

**University of Padova
AGRARIAN BIOTECHNOLOGIES DEPARTMENT
Agripolis-Strada Romea-Legnaro (PADOVA)**

Legnaro 29/04/02

This document certificate that analysis in enclosed are executed by the Zooprophyllactic experimental Institute of Padova University on Microsorb Product (Microsorb DC, Microsorb Sc, Microsorb IS, Microsorb ER) distributed by Serenambiente.

Dr.ssa Marina Basaglia (Agrarian Biotechnologies Department) has done sampling personally. She has given samples to Zooprophyllactic Institute Padova's University.

Reception: 22/03/02

PROSPECT ASSESMENT AND PROCEDURE TEST

Microbiological analysis (n° sample = 4) include:

- Total coliforms;
- Yersinia Enterocolitica;
- Escherichia Coli;
- Bacillus Anthracis;
- Salmonella SPP. On 25 grams;
- Listeria Monocytogenes;
- Vibrio Cholerae

		RESULTS	
N° sample (1 → 4)	Identification	Result	
1	Microsorb Dc	Total coliforms	< 10 UFC/g
		Yersinia Enterocolitica;	Absent in 25 gr.
		Escherichia Coli;	< 10 UFC/g
		Bacillus Anthracis;	Negative
		Salmonella SPP. On 25 grams;	Absent in 25 gr.
		Listeria Monocytogenes;	Absent in 25 gr.
		Vibrio Cholerae	Absent in 25 gr.
2	Microsorb Sc	Total coliforms	< 10 UFC/g
		Yersinia Enterocolitica;	Absent in 25 gr.
		Escherichia Coli;	< 10 UFC/g
		Bacillus Anthracis;	Negative

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		Salmonella SPP. On 25 grams;	Absent in 25 gr.
		Listeria Monocytogenes;	Absent in 25 gr.
		Vibrio Cholerae	Absent in 25 gr.
3	Microsorb IS	Total coliforms	< 10 UFC/g
		Yersinia Enterocolitica;	Absent in 25 gr.
		Escherichia Coli;	< 10 UFC/g
		Bacillus Anthracis;	Negative
		Salmonella SPP. On 25 grams;	Absent in 25 gr.
		Listeria Monocytogenes;	Absent in 25 gr.
		Vibrio Cholerae	Absent in 25 gr.
4	Microsorb ER	Total coliforms	< 10 UFC/g
		Yersinia Enterocolitica;	Absent in 25 gr.
		Escherichia Coli;	< 10 UFC/g
		Bacillus Anthracis;	Negative
		Salmonella SPP. On 25 grams;	Absent in 25 gr.
		Listeria Monocytogenes;	Absent in 25 gr.
		Vibrio Cholerae	Absent in 25 gr.

Legnaro 11/04/02

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MICROSORB ENVIRONMENTAL PRODUCTS, INC.

106 LONGWATER DRIVE, NORWELL, MASSACHUSETTS, USA 02061

781-878-0440 FAX 781-878-8004

Bioaugmentation & MicroSorb

Many articles have been written about enhancing the activity of indigenous microbes to destroy petroleum releases in situ (biostimulation). Applying oxygen in various forms to sites is widely accepted technology. There are many success stories and many failures. The success stories are well documented.

Much of the work in the area of biostimulation is at the university level or at sites where funds are provided by the USEPA, DOD or DOE. All of this work is informative in developing an understanding of the biological processes necessary to degrade or mineralize contaminants.

Environmental engineers and scientists confronted with numerous sites, limited budgets and the need to expedite remediation require certainty that sites will meet state standards for health, safety and environmental protection to achieve closure. It is well documented that physical and mechanical technologies such as "pump and treat, air sparging, vacuum extraction and dual phase vacuum extraction" may not remediate sites to levels required for closure in sensitive areas. After mechanical methods, including excavation, have been used to remove gross contamination, bioremediation is the only method by which sites will achieve closure. The use of bioaugmentation virtually guarantees that sites will achieve closure through bioremediation.

Approximately four years ago, the Massachusetts Department of Environmental Protection (MADEP) instituted a program to evaluate bioremediation. The results of the study were so positive that the Massachusetts Contingency Plan was revised to provide Licensed Site Professionals the regulatory method by which bioaugmentation could be applied to sites without prior MADEP approval. The MADEP audits approximately 10% of all site closures. Sites where MicroSorb products have been used have been audited and approved.

MicroSorb microbes are naturally occurring, archetypal microbes taken from hot springs, volcanoes and ocean vents. They are primitive microbes from very harsh environments where the carbon source is elemental. The microbes therefore have the capacity to adjust to many environments.

The microbes are cultivated in a laboratory on crude oil, seawater and ammonia. Therefore the only source of nitrogen is inorganic nitrogen. Pathogens are excluded by the fact that only inorganic nitrogen is supplied. The seawater provides other nutrients.

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Crude oil contains millions of different hydrocarbon compounds to which the consortium of microbes must adapt in order to survive.

MicroSorb microbes are certified non-pathogenic. The microbes have been applied for more than 10 years and no harmful effects have been identified.

In order for bioremediation to occur, the microbial population must achieve a density of 1 million microbes per gram. The microbes must develop a consortium capable of changing the many hydrocarbon compounds found in fuels to water soluble fatty acids. Fatty acids are a food source for fish and plants. The process of bioremediation is extremely complex and not well understood, however there is a basis of knowledge:

- At a minimum, a population of 1 million microbes per gram must be achieved.
- It requires a consortium of microbes to degrade hydrocarbons and other complex organic materials.
- Microbial populations only grow until the least nutrient is consumed.
- Aerobic degradation is much faster than anaerobic degradation.
- The best microbe wins. Microbes compete for food. MicroSorb microbes will out-compete pathogens for food.
- Microbes die when the food source is depleted.
- Microbes may exchange DNA in order to survive. After about 90 days, a microbial population will enter a death phase. Surviving microbes adjust to the environment and appear like indigenous populations. This process continuously occurs in ecosystems such as wetlands, oceans, etc.
- Fatty acid degrading microbes are slower than hydrocarbon degraders. If fatty acids build up, hydrocarbon degradation slows.

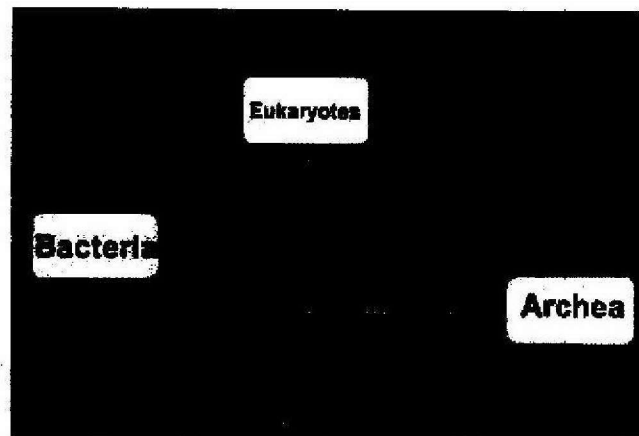
At any particular site, there may not be all the various species of hydrocarbon degrading microbes present, necessary to achieve bioremediation or the site professional may not be able to provide the correct nutrient balance in order to stimulate indigenous microbes to a population of 1 million microbes per gram. The use of MicroSorb microbial products assures the site professional that a satisfactory population of hydrocarbon degrading microbes is present at the site and that the proper nutrients and biocatalysts are applied to provide the microbes with the environment necessary for bioremediation.

This does not mean that the site professional simply adds microbes to a site and expects "miracles." Sites must be analyzed in order to determine the appropriate remediation technique to contain a plume. Sites must be monitored to be sure goals are being achieved. Microbes, generally, must be applied with some frequency to assure complete degradation. A measure of success is demonstrated by site closures and the use by environmental site professionals. In the five years, MicroSorb microbes have been marketed; there has not been one failure. Many sites are achieving closure within a year of inoculation.

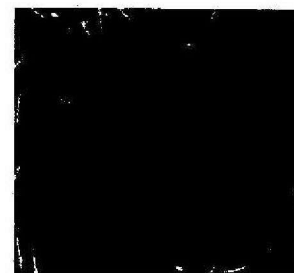


Archaea

The domain **Archae** had long been thought to exist by some microbiologists. They are similar in many respects to bacteria in that they are prokaryotes and by past convention they were classified amongst bacteria. As long ago as 1977, however, Dr Carl Woese suggested that the **archaea** were fundamentally different from bacteria and proposed that they constituted what he referred to as a separate and distinct **domain** of living organisms. In 1996 this view was generally accepted. Some microbiologists suggest that the archaea domain sits between that of the prokaryotes and that of the eukaryotes, but the current main-stream view is as shown below, which is based on ribosomal DNA analysis.



Archaea differ from bacteria primarily in that their cell wall does not contain a component called **peptidoglycan**. The plasma membranes in Archaea are different from those found in other organisms and are made from lipid-like materials. As with prokaryotes the genetic strands - DNA - of archaea float freely in the cell. The RNA polymerase and ribosomal proteins, however, are similar to that of eukaryotes.



Two views of archaea methanopyrus © University of Regensburg

Many Archaea live in extreme conditions and this has led to the name "extremophile" being given to them. According to Dr Carl Woese, there are four main types of archaea. Two can live in very hostile

environments, such as salt lakes, hot springs, or in mid-ocean thermal vents. Another can metabolize chemicals that we would consider dangerous, or at least unpleasant, and makes methane as a by-product. While the last type lives by reducing sulfates. Others can use simple elementary chemicals such as hydrogen and sulfur.

Some biologists and planetary scientists now think that if life is discovered elsewhere in the Solar System it will probably resemble the archaea kingdom.

Go to

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**BIOREMEDIATION OF BEDROCK GROUNDWATER
CONTAMINATED WITH
TETRACHLOROETHENE, TRICHLOROETHYLENE
TRICHLOROETHANE, TOLUENE AND HEXANE**

By

Jonathan A. Aisner, LSP

William E. Baird, PE, LSP

Michael J. Hudson

**Web Engineering Associates, Inc.
104 Longwater Drive
Norwell, MA 02061
Tel-781-878-7766
Fax-781-878-8004**

Summary

Contaminated groundwater contains a variety of chlorinated and hydrocarbon volatile organic compounds (VOCs). A bioreactor system consisting of four recovery wells, two 800-gallon plastic tanks, bag filters, granulated activated carbon (GAC) and an injection field began operation in the spring of 2001. The groundwater is circulated underground to the recovery wells. One advantage of this circulation system is that there is no offsite disposal of treated water and therefore no need for a NPDES permit.

After one year's operation, the aerial extent of contamination was reduced from 12,704 square feet to 6,417 square feet, a reduction of 45%. After two years the aerial extent of contamination greater than 50 mg/l was reduced from 5,095 square feet to 501 square feet, a reduction of 90%.

The site is glacial till over fractured bedrock. The primary transport of groundwater is in the bedrock to 40 feet below grade surface (BGS). The groundwater recovery, bio-treatment and re-injection system is operating in a stable and predictable manner. The bioreactors and granulated activated carbon (GAC) did not destroy the contaminants until microbes were added directly to the first bioreactor. The efficiency of the above ground bioreactor system is dependent upon water temperature. During the winter, the efficiency of the above ground system is 50%. During the summer, the destruction of VOCs may exceed 95%.

The recovery wells are causing contaminated groundwater under the building to flow into the recovery system. The groundwater mound at the leaching field has a total contaminant concentration of less than 10 mg/l.

The direct injection of microbes at the down gradient perimeter of the contamination has contained the migration of contaminated groundwater such that offsite monitoring wells meet the Massachusetts Department of Environmental Protection (MADEP) closure criteria.

The application of microbes to the groundwater has apparently had a positive effect upon the down gradient detention pond. Prior to the application of microbes, the detention pond destroyed about 35% of the Toluene and none of the chlorinated solvents entering the pond. Shortly after the application of microbes to the groundwater, 99.9% of the Toluene and 95% of the chlorinated solvent contamination entering the pond have been destroyed.

SITE DESCRIPTION

The site has been an adhesive manufacturing facility since 1970. Underground piping between the tank farm and the building leaked. Originally the tank farm had an earthen floor. Tank overfills are also responsible for subsurface contamination. A variety of chlorinated and non-chlorinated volatile organic

compounds (VOCs) have impacted the site soil and groundwater. Sediments and surface water in a nearby detention pond have also been contaminated.

The site contaminants include the chlorinated compounds: Tetrachloroethene (PCE), 1,1,1-Trichloroethene (TCE), Trichloroethane (TCA), and associated degradation compounds including 1,1-Dichloroethane (DCA), 1,1-Dichloroethene (DCE), *cis*-1,2-Dichloroethene (*cis*-DCE), Chloroethane (CE) and Vinyl Chloride (VC); the non-chlorinated compounds Toluene, Xylene and Methyl Ethyl Ketone and several light aliphatic textile spirit compounds including various hexanes and pentanes. At one time, LNAPL Toluene was present in the area of the leaking underground lines. DNAPL has not been identified. The groundwater has high iron content.

The site geology is 8 to 12 feet of dense sub-glacial till overlying granitic bedrock. The incompetent bedrock is highly fractured to depths of 40 feet and has good permeability. Groundwater fluctuates seasonally from 3 feet below grade surface (BGS) to 6 feet BGS. Numerous excavations exist across the site for water and storm water lines. Of particular note are a 24 inch and a 12 inch diameter reinforced concrete pipe storm drains that leak contaminated groundwater into a down gradient detention pond.

The aerial extent of the contamination measures about 180 feet by 240 feet. The down gradient storm water detention pond measures 100 feet by 300 feet.

Figure 1 is the site plan in May 2000.

TREATMENT SYSTEM

A groundwater recovery and treatment system has been operating since June 2001 at a pumping rate of 1 to 7 gpm. As of July 2003 the system has treated more than 1,000,000 gallons of contaminated groundwater. The treatment system consists of 3 recovery wells screened from 5 feet BGS to the bedrock and through the bedrock to 40 feet BGS. The contaminated groundwater is pumped from the recovery wells into a plastic 800-gallon aerated bioreactor tank. Biocatalyst, which is produced on site, is also pumped into this first bioreactor tank at a rate of 20 gallons per hour. Microbes and nutrient are added by hand to the first bioreactor. The water flows by gravity to an 800 gallon settling tank. The first tank is aerobic while the second tank settles out iron oxide and is anaerobic. The water is then pumped through bag filters and an 800-pound GAC vessel. From the GAC vessel, the water is drained by gravity to an up gradient leaching field. The GAC is also a bioreactor. It is intended that contaminated water containing microbes be discharged from the GAC to the leaching field.

The leaching field is located in the area of the original underground piping leaks and consists of perforated 4 inch diameter horizontal piping bedded in $\frac{3}{4}$ inch road stone. The leaching field area had previously had LNAPL Toluene present. The LNAPL Toluene was removed by a pump and treat system and excavation.

0 20' 40' 80'

SCALE

PROPERTY LINE

24" R.O.P. SUMP #1

24" R.O.P. SUMP #2

FORMER UNDERGROUND PIPING

TRENCH #1

TRENCH #2

REMEDATION TRAILER

EXISTING BUILDING

TO DETENTION POND

GROUNDWATER PLUME DELINEATION (TOTAL VOCs)

LESS THAN 10,000 PPB

10,000 TO 25,000 PPB

25,000 TO 50,000 PPB

GREATER THAN 50,000 PPB

(DATA COLLECTED SPRING, 2000)

AREA GREATER THAN 50,000 PPB = 4,981 SQ. FT.

AREA GREATER THAN 10,000 PPB = 10,214 SQ. FT.

MW-1035

MW-208

MW-1030

MW-1033

MW-207

MW-102

MW-206

MW-303

MW-204

MW-201

MW-203

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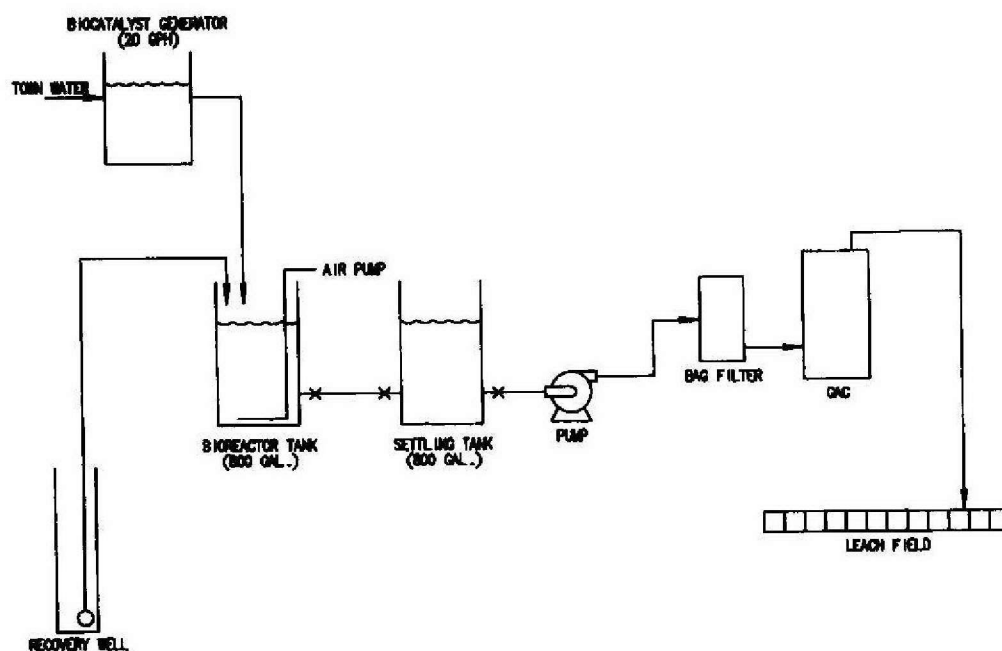
33

The objective of the current circulation system is to continuously apply treated water containing microbes, nutrients and biocatalyst to the source area and to capture and re-treat the contaminated water until the site meets the closure criteria. There is no discharge and therefore no NPDES permit is needed or required. The vapor space of the bioreactor tanks is connected to a 55-gallon vapor phase carbon drum. Figure 2 is a simplified process flow diagram.

Response Actions have also included in-situ bioremediation via direct injection of microbes, nutrients and biocatalyst to specific down gradient monitoring wells.

Microbes, nutrient and biocatalyst are supplied by MicroSorb Environmental Products, Inc. of Norwell, MA. The microbes are of the archea family. The hydrocarbon degrading microbes are cultivated aerobically in seawater with crude oil as the food source and ammonia as the nitrogen source. The dechlorinating microbes are cultivated aerobically in seawater with TCE as the food source and ammonia as the nitrogen source. The microbes are available in concentrations of 90 billion microbes per gram.

Figure 2



SYSTEM PERFORMANCE

The treatment system was started in June 2001 without the direct introduction of microbes. Microbes, biocatalyst and nutrients were however, being injected in-situ at perimeter monitoring wells and also into the leaching field. In the first three weeks of operation, the GAC experienced breakthrough and the reduction in contaminants was negligible. The reduction in contaminants remained

negligible for a period of 8 weeks. Microbes were then added to the first bioreactor and the treatment system began to reduce total VOCs by 50%.

Table 1 shows the analytical results of the treatment of the primary contaminants. The second row is the influent; the third row is after the two bioreactors and before the bag filters and GAC. The fourth row is the system effluent after the GAC. This sampling was conducted in October 2001. Concentrations are in ppb. The reactor treatment efficiency is calculated upon total VOCs removed and is 51%.

The bioreactor system efficiency is temperature dependent. The above sampling was performed in October. The trailer in which the bioreactor system is housed is not heated. During the winter months the temperature of the water in the bioreactors is about 42°F. In the summer the temperature of the water in the bioreactors is about 80°F. The efficiency of the bioreactors increases to 95% with the 40°F rise in bioreactor water temperature. Table 2 represents sampling in July 2002.

Figures 3, 4 and 5 delineate certain concentrations of the plume indicating the success of the bioremediation process. Figure 3 is the spring of 2001 before system startup. The aerial extent of contaminated groundwater with concentrations greater than 50 mg/l is 5,095 square feet. The total area of contamination greater than 10 mg/l is 12,704 square feet. Figure 4 is the spring of 2002 after one-year operation. The aerial extent of contamination greater than 50 mg/l is 729 square feet. The total area of contamination greater than 10 mg/l is 3,035. Figure 5 is the spring of 2003 after two years of operation. The aerial extent of the contamination greater than 50 mg/l is 501 square feet. The area of contamination greater than 10 mg/l is 7,034 square feet. There has been considerable reduction in the area of contamination greater than 50 mg/l. The area of contaminants greater than 10 mg/l increased between 2002 and 2003.

Figure 6 shows the groundwater contours in July 2003. Recovery wells 3 and 4 are operating. It is apparent that groundwater is being recovered from under the building. Figure 3 shows that contamination in the spring of 2001 is under the tank farm and under the building. Groundwater contamination greater than 10 mg/l is most likely migrating from under the building.

The contour plan also clearly shows the groundwater mounding at the leaching field injection area. Figure 5 indicates that the area of the leaching field has contamination less than 10 mg/l.

IN-SITU BIOREMEDIATION

With the start up of the system, the treated water was drained into the leaching field between the tanks and the building. A groundwater mound was created. Degradation products began to appear in the down gradient monitoring well MW-203.

Table 1
October 2001

Sample Location	T O L U E N E	P C E	T C E	T C A	C I S D C E	D C A	D C E	C E	V C
Influent	2,900	140	820	8,800	630	2,900	220	780	brl
After Bioreactors	2,500	brl	620	5,900	580	2,400	180	570	brl
Effluent	brl	brl	brl	1,500	1,400	brl	4,800	690	brl

(brl) Below Reportable Limits

Note the effect of the GAC. The GAC is a bioreactor. Cis-DCE, DCA, DCE, CE and Vinyl Chloride are degradation products of PCE, TCE and TCA.

Table 2
July 2002

T O L U E N E	P C E	T C E	T C A	C I S D C E	D C A	D C E	C E	V C
7,500	brl	1,500	23,000	brl	1,800	brl	950	brl
15	brl	15	500	58	430	15	460	28
brl	brl	brl	500	20	420	brl	590	35

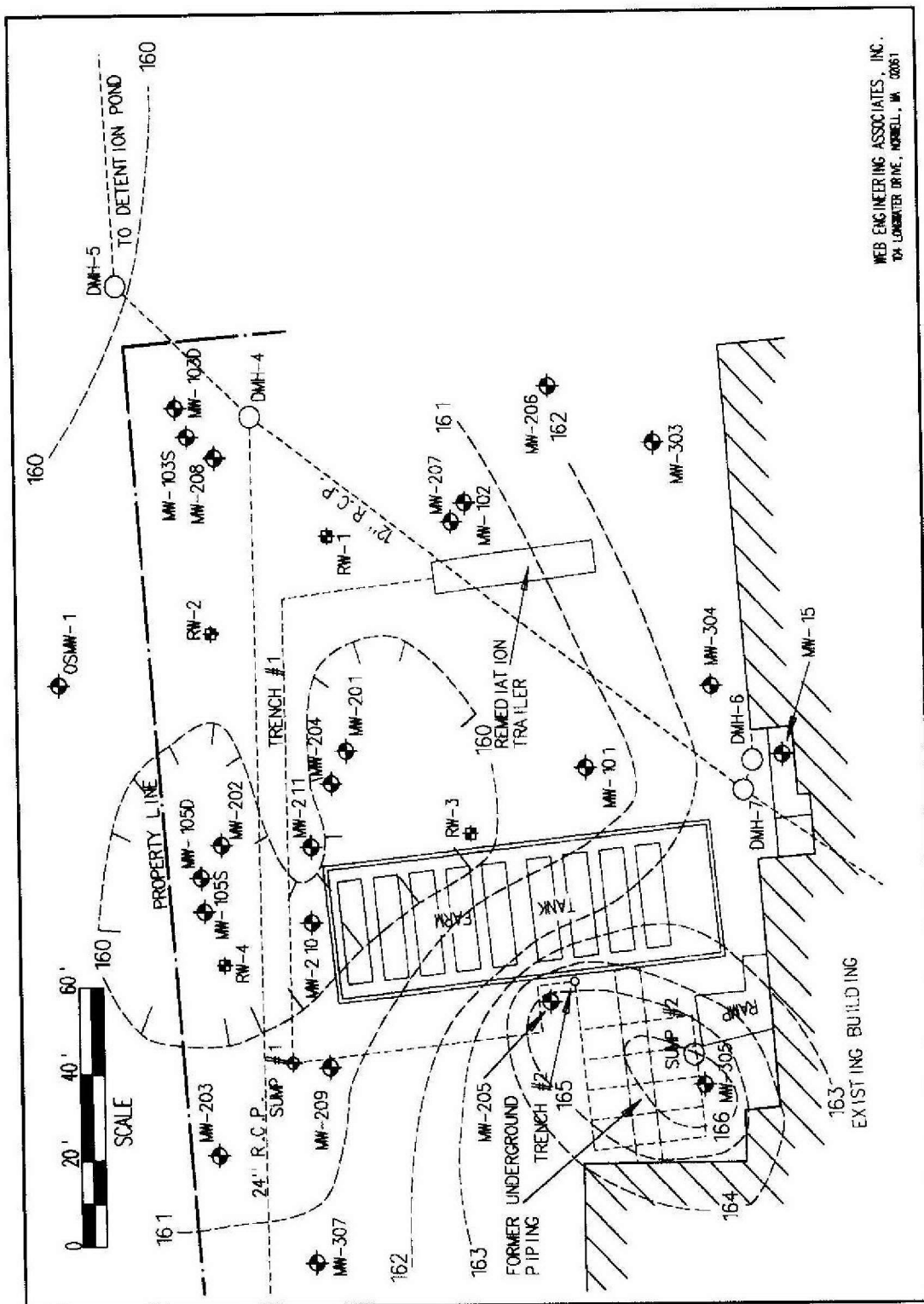
As a precaution against contaminated groundwater leaving the site, as a result of the mounding effect by the return of treated water to the source area, microbes, nutrient and biocatalyst were injected into monitoring wells at the down gradient property line. Vinyl Chloride reached the highest concentration in July, 2001, one month after startup, at 28 ppb. Since November 2002, the concentration of Vinyl Chloride in the down gradient wells has been BRL and the abutting property meets the closure criteria.

Figure 4 Area > 50ppm = 729 ft² Total area > 10ppm = 6,417 ft²



WEB ENGINEERING ASSOCIATES, INC.
BY LINDSEY GRIFF, KOWAL, & GRIFF

Figure 6



Detention Pond Treatment

Two concrete storm drains that transect the site leak. Thus contaminants flow into the detention pond. The detention pond is not really a pond but a wetland, completely overgrown. The pond measures 100 feet by 300 feet and is completely filled with silt such that the level of the inverts of the influent and effluent pipe set the elevation of water in the pond. The water flows diagonally through the pond from the SW corner to the NE corner.

Between January, 1999 and June, 2001, the influent and effluent of the pond were sampled 7 times. The average influent concentration of toluene was 274 ppb. The average effluent concentration of toluene was 177 ppb. The average influent concentration of chlorinated solvents during this time was 252 ppb and the average effluent concentration was 246 ppb.

Between July, 2001, (approximately two months after the application of microbes to the site) and July, 2003, the influent and effluent concentrations of the detention pond were sampled 18 times. The average concentration of toluene in the influent was 234 ppb and the average concentration in the effluent was 0.15 ppb, a 99.9% reduction.

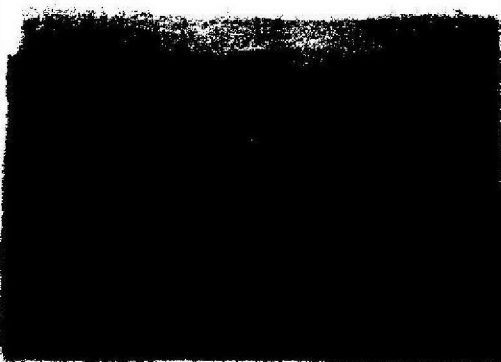
During the same time period, (July, 2001 to July, 2003) the influent and effluent were also sampled for chlorinated solvents. The average influent and effluent concentrations of chlorinated solvents was 240 ppb and 18 ppb respectively, a 92% reduction.

One explanation as to why the detention pond has begun degrading chlorinated solvents, thus becoming part of the treatment process for chlorinated solvents leaving the site in storm drains, is that the microbes added to the treatment system and injected into perimeter wells, have migrated to the detention pond. The microbes may be in the sediments, acting in the rhizome, and facilitating the plants in the destruction of the chlorinated solvents. Figure 7 is a picture of the detention pond.

Figure 7



CHEMICAL COMPANY: EASTERN MASSACHUSETTS



BIOREMEDIATION OF MIXED SOLVENTS IN BEDROCK

The Chemical Company discovered mixed hydrocarbons and chlorinated solvents in the soils and groundwater under and surrounding their buildings. MicroSorb Environmental Products, Inc. is treating the contamination with its innovative bioremediation technology. In just 18 months, MicroSorb® has been able to reduce the contamination in most areas by over 75% and contamination levels are at or approaching regulatory levels. This technology is significantly less expensive than traditional remediation options with vastly reduced monthly operating costs as well. Additionally, it does not require a NPDES permit.

Located in Eastern Massachusetts, the geology of the site is $\pm$ 10 feet of glacial till over granite. The groundwater elevation is 8 to 10 feet below grade surface. The aerial extent of the site is approximately 1 acre. Numerous releases had occurred at the site from leaking underground piping, tank overfills and leaking drums. The highest concentrations of contaminants detected in groundwater at the site are as follows: Trichloroethane-940 ppm, Trichloroethene-67 ppm, Tetrachloroethene-17 ppm, Toluene-14 ppm, Hexane-LNAPL. The groundwater has a high concentration of iron.

In June 2001, a pump and treat bioreactor system was installed. The system consists of pumping from two perimeter down-gradient recovery wells into a 500 gallon plastic tank seeded with MicroSorb® microbes and aerated by a small compressor followed by a 500 gallon plastic settling tank, two parallel bag filters and a 1,000 lb. carbon canister. The carbon canister, in effect, increases the holding time for microbial activity. The carbon adsorbs the contaminant molecule so that microbes residing in the carbon destroy the contaminant. The effluent is pumped into an up-gradient leaching field. Thus the entire site becomes a bioreactor as microbial treated water is circulated throughout the site.

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www.microsorb.com

BIOREMEDIATION OF MIXED SOLVENTS CONTINUED

The table on the right describes the performance of the treatment system operation during March 2003. The contaminated groundwater has been treated with an efficiency greater than 99.5% in two small 500 gallon bioreactors at a flow rate of 2 gpm. GAC was not employed during this period. The discharge water meets all regulatory standards. In addition, by returning the treated water to the subsurface, microbes are continually introduced to the groundwater, in effect making the whole site a bioreactor.

Contaminant	Influent (PPB)	Effluent (PPB)	Reduction
Methylene Chloride	660	BRL	100%
TCE	130	0.6	99.50%
cDCE	440	2	99.50%
1,1 DCE	110	BRL	100%
CA	2,700	11	99.60%
1,1,1 TCA	1,200	5	99.60%
1,1 DCA	2,600	13	99.50%
m&p Xylene	220	1	99.50%
Toluene	4,500	21	99.50%

To date, approximately 5 million gallons of groundwater have been treated at an average flow rate of 5 gpm. The average daily influent of contaminants is 559 mg/day. The average daily effluent of contaminants returned to the leaching field is 117.6 mg/day. The bioreactor efficiency or percentage removal of contaminants is 79%.

5 lbs. of MicroSorb®SC and 5 lbs. of MicroSorb®DC and 1 lb. of MicroSorb® Nutrient are added to the site each month. Biocatalyst is continually added to the first bioreactor. The capital cost of the system is less than \$5,000.00. Utility costs are approximately 160 kw/month. The monthly cost of microbes is \$1,300.00.

The activated carbon is used only during warm weather and only 2,000 pounds of carbon has been used since the system startup in June 2001. During the colder months, granular activated carbon (GAC) is not employed.

MicroSorb Environmental Products, Inc. manufactures and markets microbial products and related process equipment. For over 10 years, MicroSorb® has been providing superior product blends consisting of a consortium of naturally occurring, environmentally safe and non-pathogenic microbes. Because of their ultra-high concentration, MicroSorb® Microbes are the most powerful and fastest acting available.

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Generic Designs for Bioremediation

Typical Aboveground System

**Aboveground Treatment System
Air Diffuser and Base**

**Underground Treatment System
Collection/Separator Tank (1 of 2)**

**Underground Treatment System
Pump Chamber/Bioreactor Tank (2 of 2)**

Recovery Well

Groundwater Interception Trench

Pressure Injection Well

Injection Well

Injection Trenches

Generic Designs for Bioremediation

MicroSorb Environmental Products, Inc. has prepared the following generic designs of bioremediation systems which intercept and treat contaminated groundwater or simply treat the groundwater through direct injection.

Advantages of using an interceptor, treatment ,and injection bioremediation system:

- The plume is controlled.
- Free product is destroyed by microbes in the bioreactor, i.e., no waste disposal, no activated carbon usage.
- Microbes in the bioreactor adjust to the contamination.
- The injection of treated groundwater containing microbes saturates the soils above the water table, bioremediating contaminated soils.
- The injection of treated groundwater creates a groundwater mound which increases groundwater flow, reducing the time of remediation.

MicroSorb® SC (Super Concentrate) may be mixed with MicroSorb® Nutrients in a drum of MicroSorb® Biocatalyst. The mixture may then be pumped into a series of small diameter injection wells screened in the contaminated zone. The costs of the above bioreactor system are eliminated, however a number of injections may be necessary to achieve a satisfactory cleanup

MicroSorb provides solutions: products, services, or both

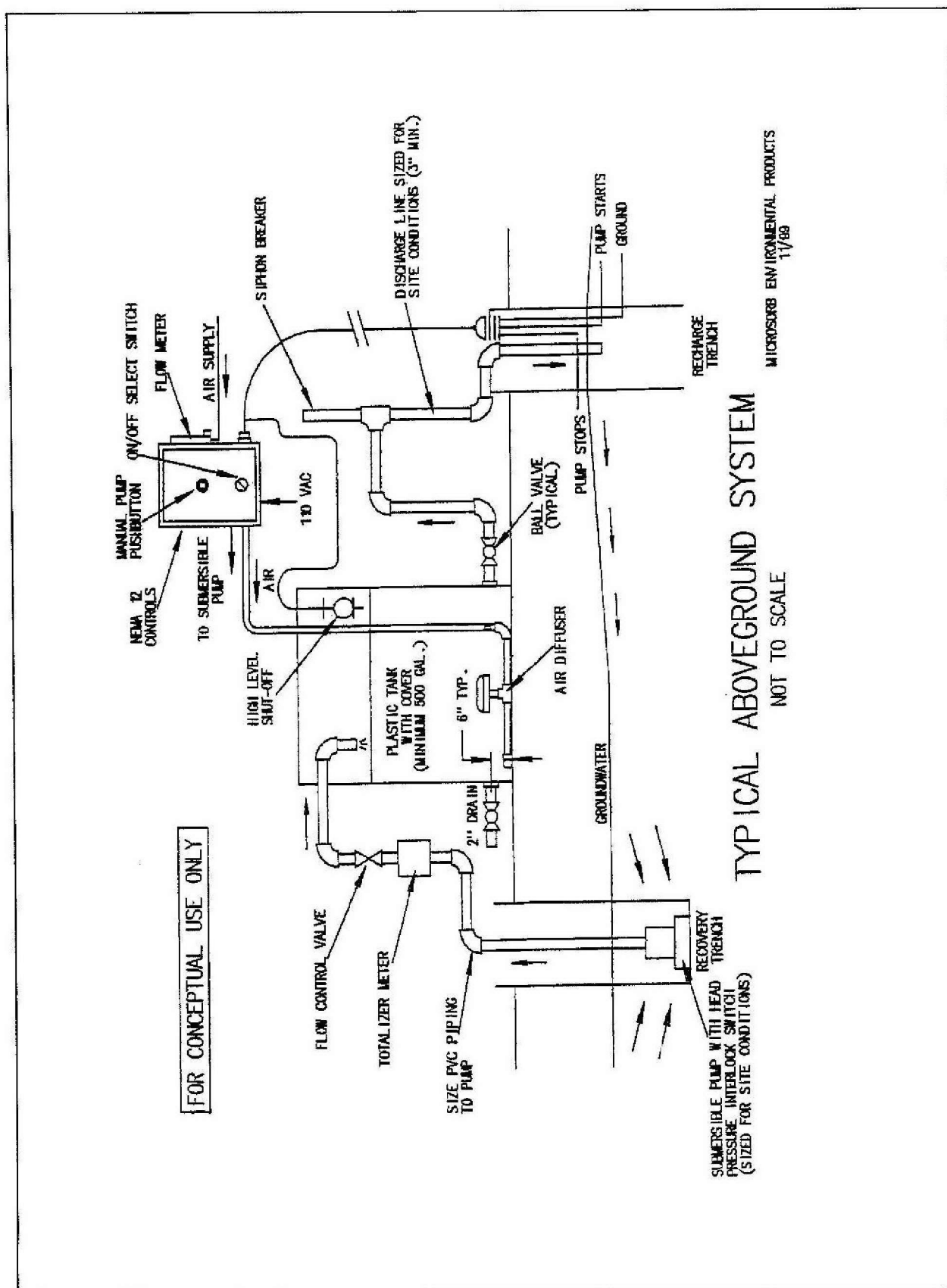
MicroSorb Environmental Products, Inc. will supply custom designed control systems for your next bioremediation project and will assist you in creating a bill of materials for equipment and supplies peculiar to your site.

MicroSorb personnel have the capacity to provide fully engineered solutions for site specific contamination conditions or we are prepared to simply provide microbial products to potential users.

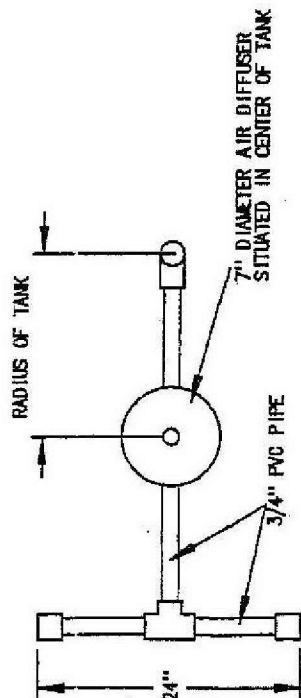
Using MicroSorb® microbial products assures you that the species of microbes necessary to destroy the contamination on your site will be present in quantities sufficient to get the job done.

Generic designs

The following generic designs are utilized to deploy MicroSorb products to the source of the contamination. Site specific conditions dictate the type of delivery system (design) used. The design schematics shown below are generic illustrations and are presented simply to provide the conceptual framework for more detailed site specific, engineered solutions.

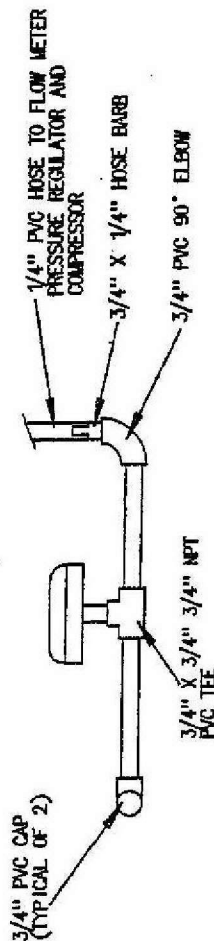


MicroSorb® is the registered trademark of MicroSorb Environmental Products, Inc., 104 Longwater Drive, Norwell, MA 02061



PLAN VIEW

FOR CONCEPTUAL USE ONLY



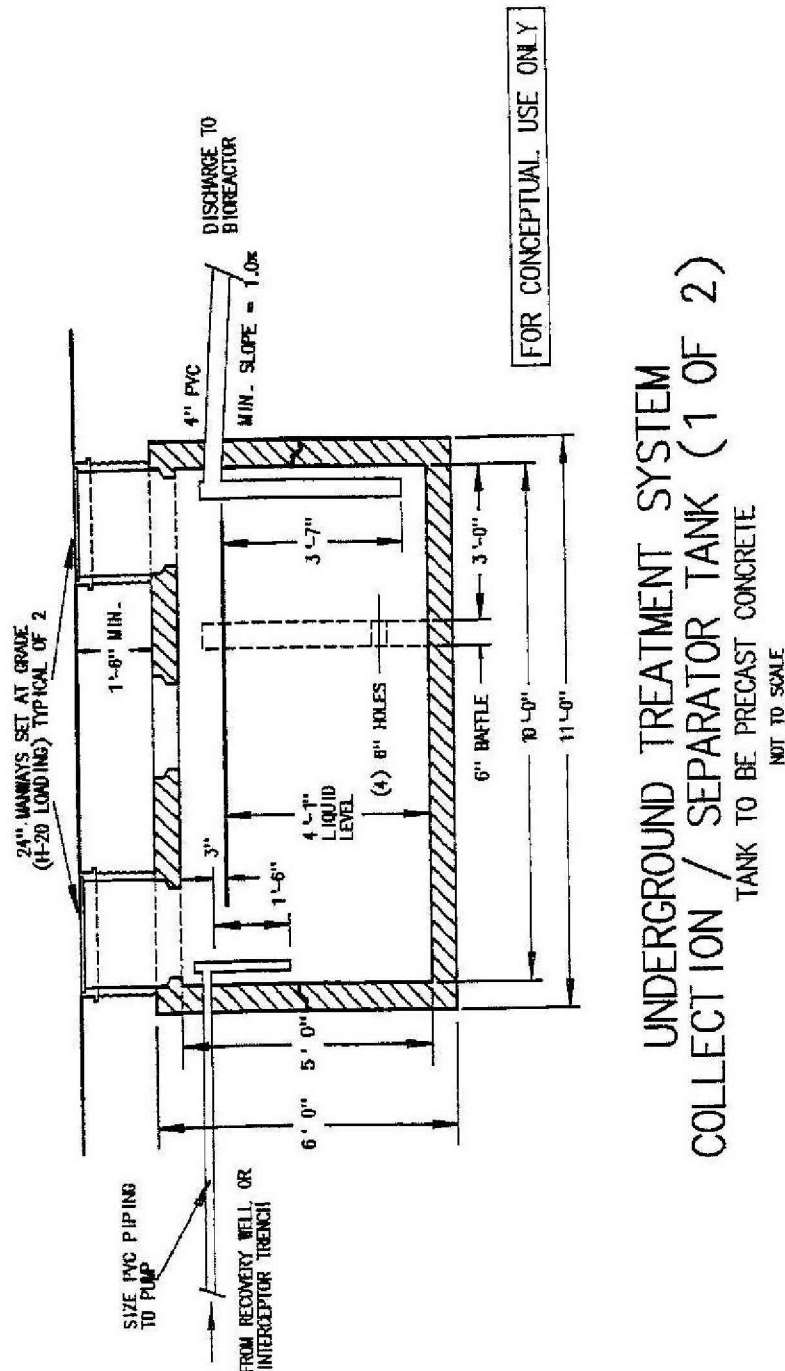
SIDE VIEW

ABOVEGROUND TREATMENT SYSTEM AIR DIFFUSER AND BASE

NOT TO SCALE

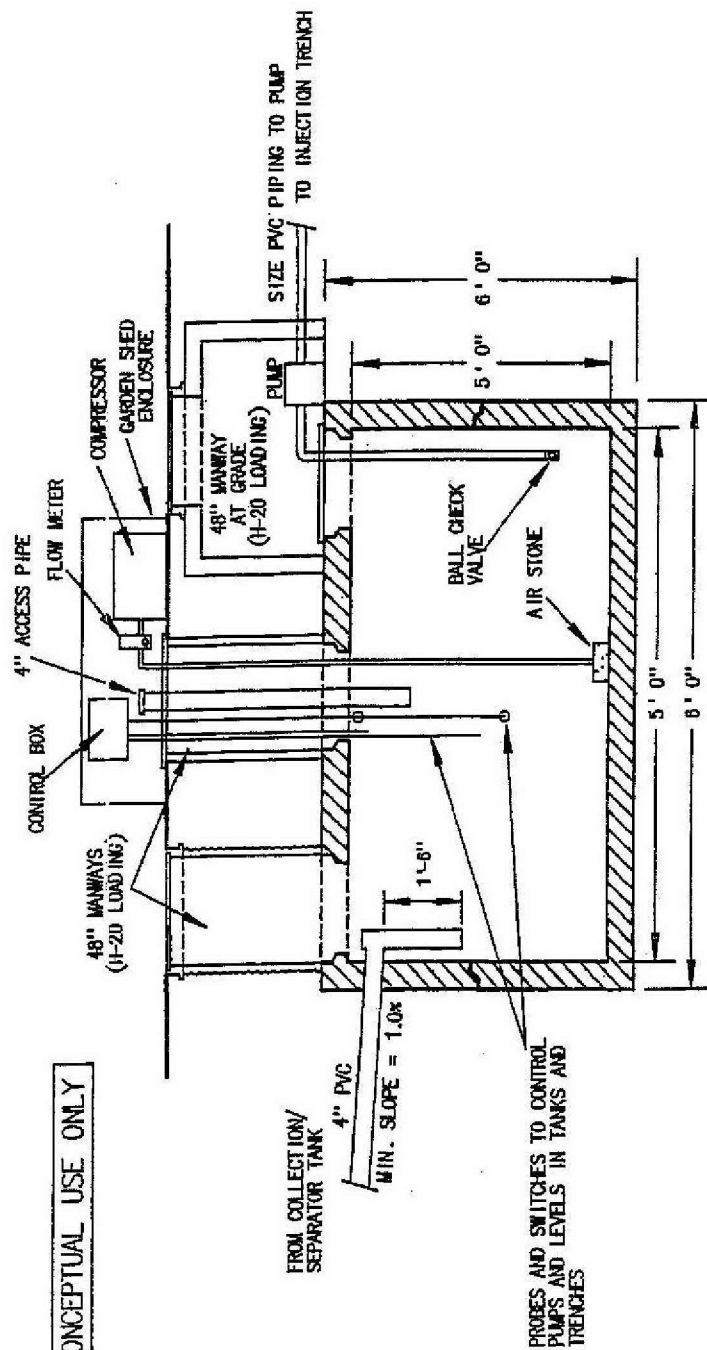
1 PSI = 2.31 FT. OF WATER:
ADJUST AIR PRESSURE TO CREATE
ACTIVE, SMALL BUBBLES

MICROSORB ENVIRONMENTAL PRODUCTS
1/99



MICROSORB ENVIRONMENTAL PRODUCTS
11/88

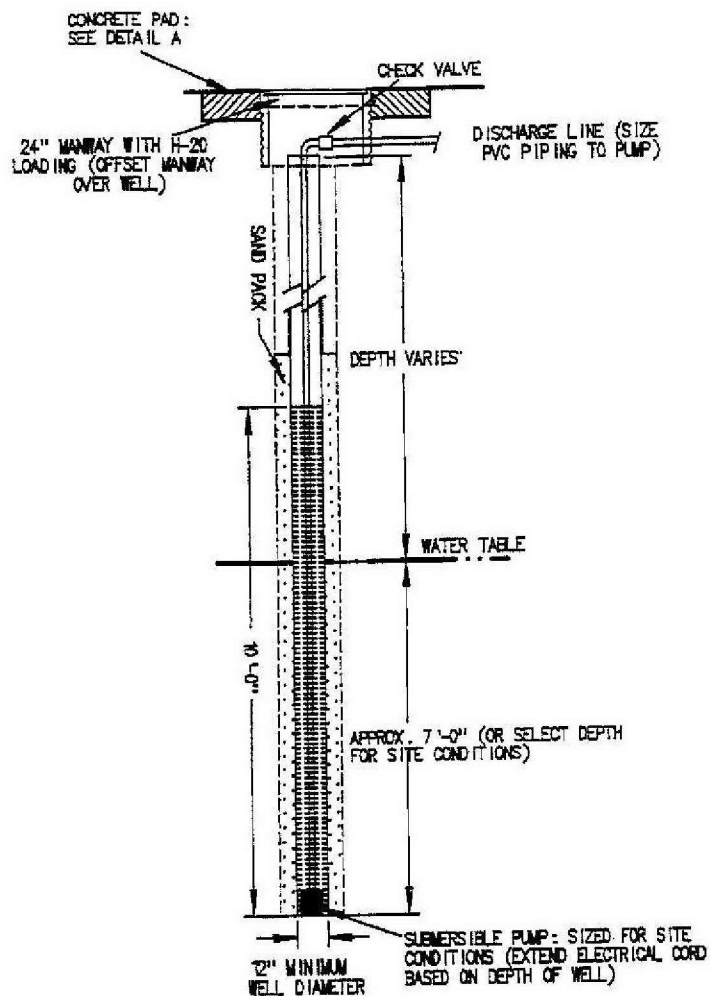
UNDERGROUND TREATMENT SYSTEM COLLECTION / SEPARATOR TANK (1 OF 2)



UNDERGROUND TREATMENT SYSTEM PUMP CHAMBER / BIOREACTOR TANK (2 OF 2)

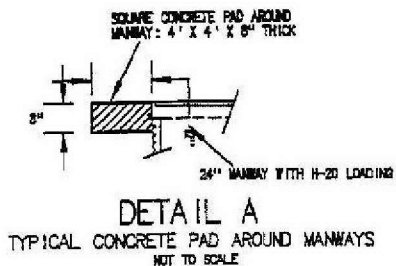
TANK TO BE PRECAST CONCRETE
NOT TO SCALE

MICROSORB ENVIRONMENTAL PRODUCTS
11/89



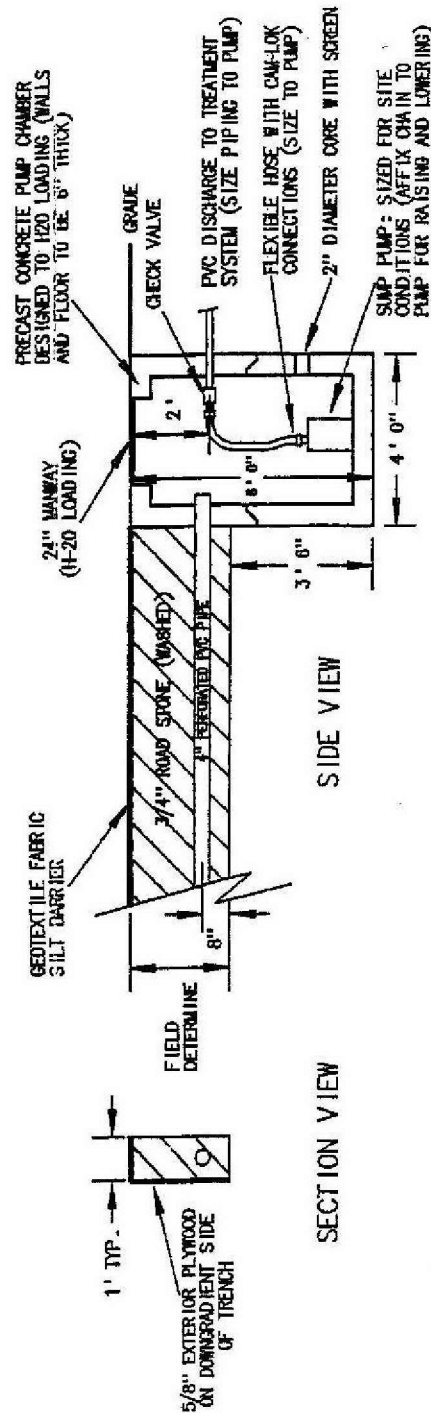
RECOVERY WELL

NOT TO SCALE



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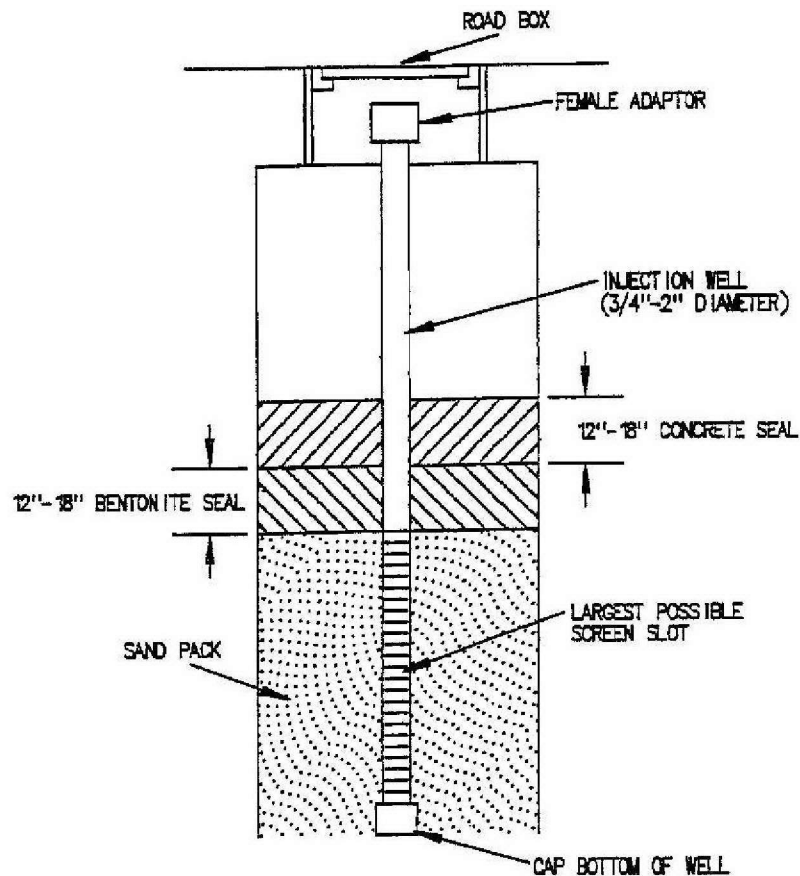
GROUNDWATER INTERCEPTION TRENCH

NOT TO SCALE

FOR CONCEPTUAL USE ONLY

NOTE: IF LIAPL PRESENT, ADD MICROSORB ER
OR REMOVE WITH PETROFORE BAILER

MICROSORB ENVIRONMENTAL PRODUCTS
11/00



PRESSURE INJECTION WELL NOT TO SCALE

TREATMENT OF SOIL:

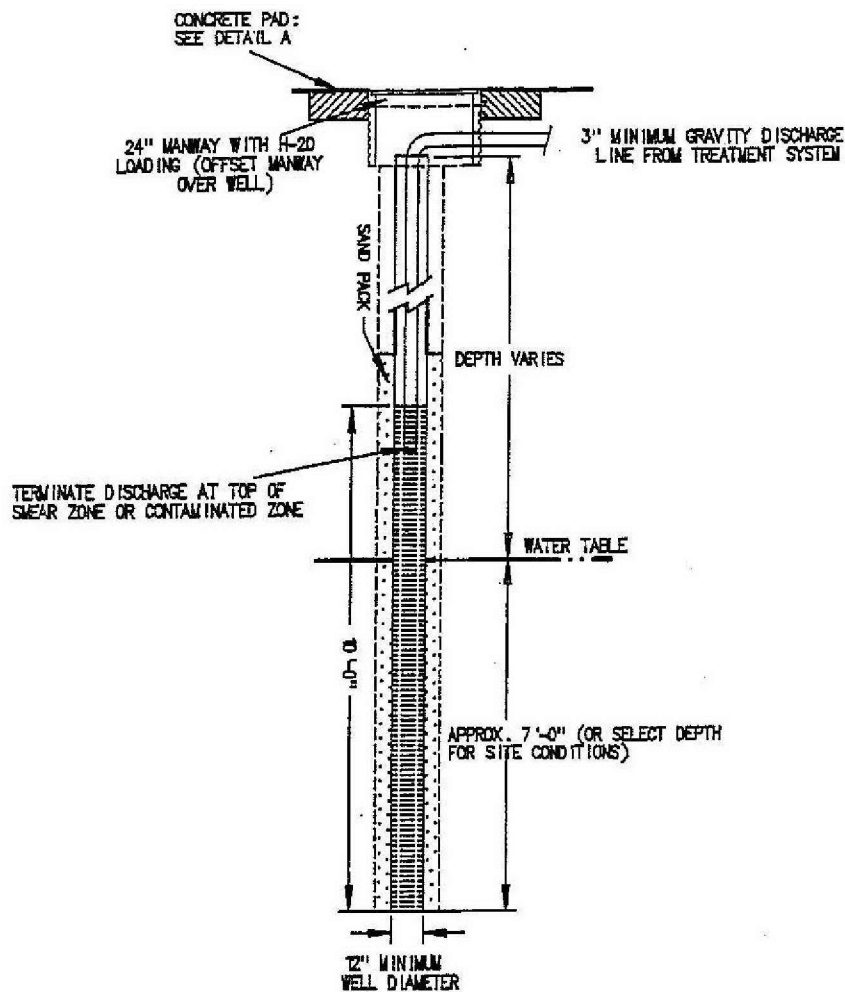
5' SCREEN WITH TOP OF SCREEN
SET 2' ABOVE CONTAMINATED SOIL

TREATMENT OF WATER:

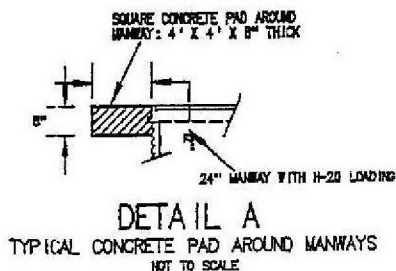
5' SCREEN WITH TOP OF SCREEN SET
AT HIGHEST GROUNDWATER ELEVATION

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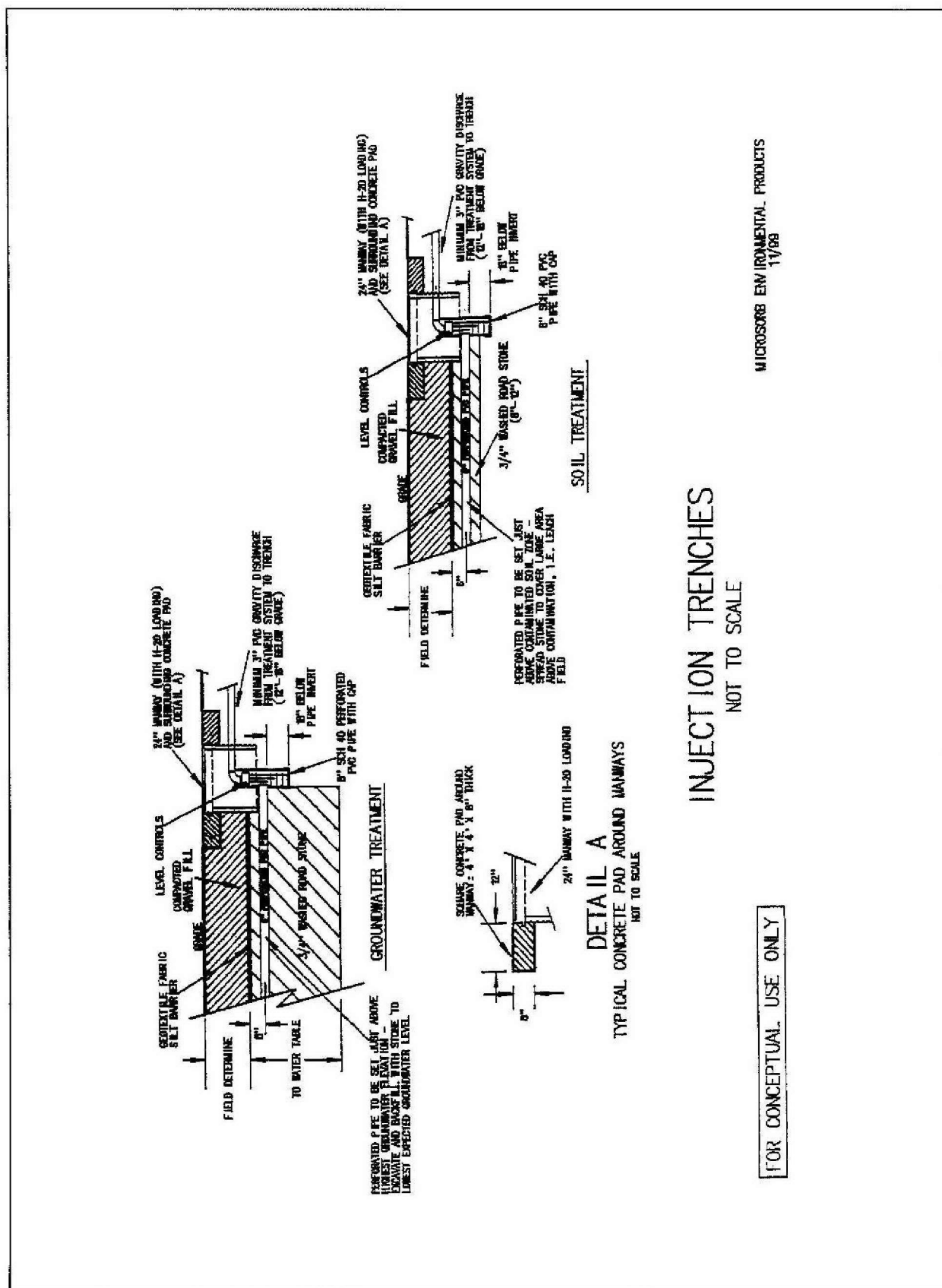


INJECTION WELL NOT TO SCALE



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INJECTION TRENCHES

NOT TO SCALE

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