United States
Environmental Protection
Agency

Office of Research and
Development
Washington DC 20460

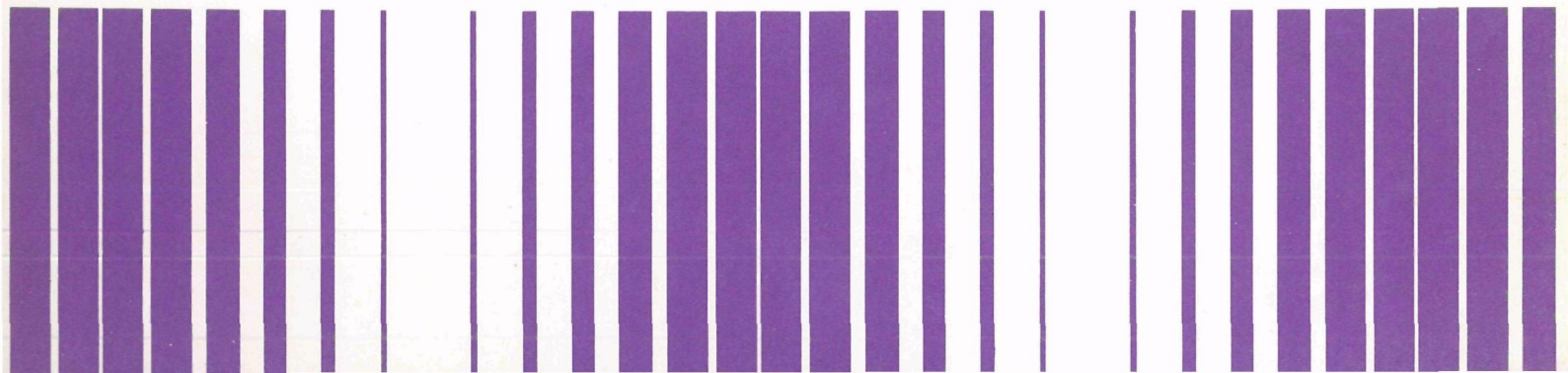
Center for Environmental
Research Information
Cincinnati OH 45268

Technology Transfer | February 1989 | CERI-89-11

EPA

Bioremediation of Hazardous Waste Sites Workshop

Speaker Slide Copies and Supporting Information

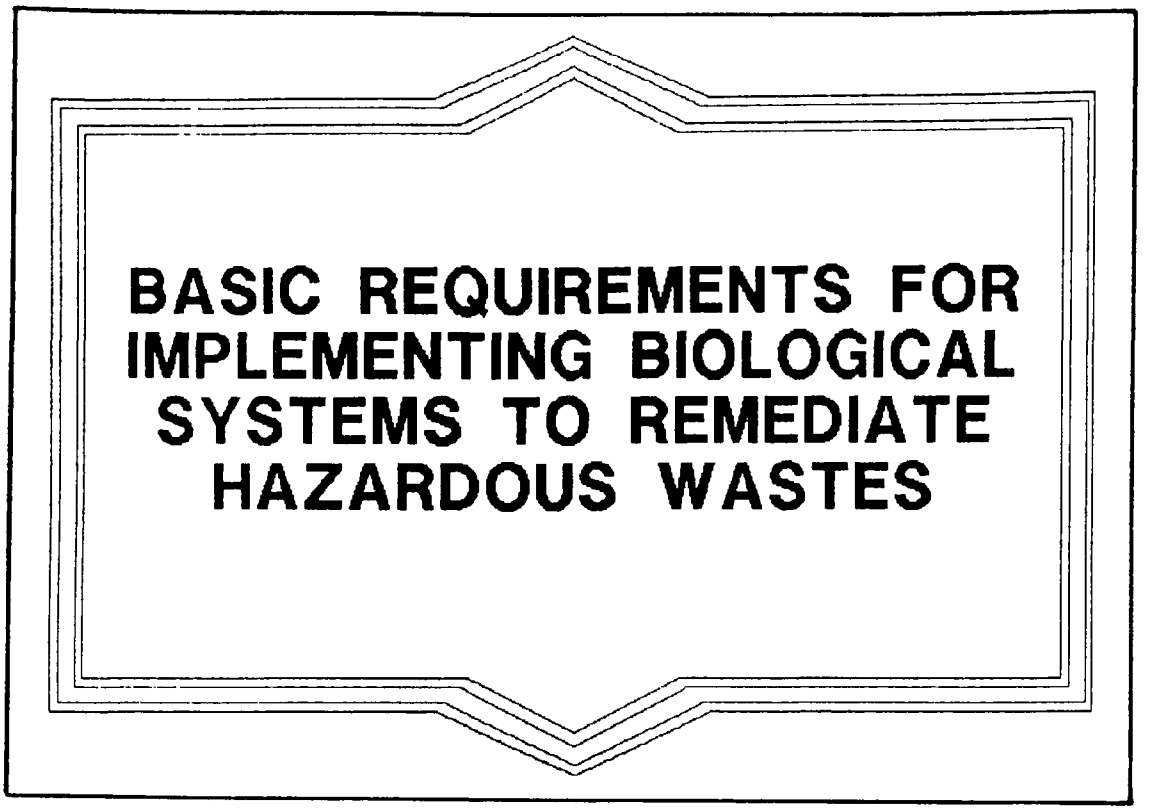


U.S. ENVIRONMENTAL PROTECTION AGENCY

WORKSHOP ON
BIOREMEDIATION OF HAZARDOUS WASTES

FEBRUARY 1989

Table of Contents



SECTION 1

BASIC REQUIREMENTS FOR IMPLEMENTING BIOSYSTEMS

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I. Introduction

The key to the assessment of the fate of organic chemicals in the environment is a realistic evaluation of their susceptibility to biological conversion. In order to make this evaluation rationally, it is important that the terminology used in the field is understood. The discussion presents some terms needed to understand the rest of the presentation. The following terms are defined: mineralization, biodegradation, recalcitrant compounds, persistent compounds, biogenic compounds, xenobiotic compounds, and biosystems.

Biological technology development is based on: (1) an adequate information base, which is derived from an understanding of microbiology, biochemistry, and genetics; (2) a basic understanding of the metabolic processes leading to the detoxification of hazardous wastes; and (3) an understanding and appreciation of the structure and function of natural microbial communities.

The key word above is "understanding." Without understanding the underlying microbiology, developing the technology becomes sheer guesswork. Thus, basic science research must be a part of any program concerned with biodegradation technology development.

II. The Carbon Cycle

Carbon plays a key role in the structural make-up of protoplasm and its essentiality in the energy metabolism of heterotrophs. The biogeochemistry of carbon is interesting because of the vast array of organic molecules that are involved and the cyclical nature of the interaction between these compounds and inorganic carbon, a cycle that describes the movement of carbon from the inorganic to the organic state and back to the inorganic again. Movement of organic carbon to the inorganic state is accomplished either through direct combustion or through the action of microbial biooxidation.

Biotransformation of organic pollutants is accomplished either aerobically or anaerobically.

A. Aerobic metabolism

1. Aerobic respiration: energy-yielding metabolism involving oxidation reactions in which hydrogen (electrons) is transferred to oxidized pyridine nucleotides (NAD and NADP)

resulting in reduced forms (NADH and NADPH) that either provide reducing power for biosynthetic reactions or can transfer the electrons to electron transport chains wherein high energy bonds of ATP are formed. The final electron acceptor is molecular oxygen.

2. Compounds devoid of oxygen atoms (alkanes, saturated ring structures, and unsubstituted benzenes) can still be acted upon by certain microorganisms by their unique ability to catalyze oxidations using molecular oxygen. They do this through the mediation of two types of enzymes, both of which activate oxygen from the triplet state to the singlet state.

   a. Monooxygenases: $R\text{-}H + NADH + H^+ + O_2 = R\text{-}OH + NAD^+ + H_2O$
   Monooxygenases yield hydroxyl groups, and all are extremely specific for their aromatic substrate.

   b. Dioxygenases: $R + O_2 = RO_2$

Dioxygenases are responsible for the fixation of the oxygen directly into organic compounds. A common use of dioxygenases is to cleave the benzene rings by inserting both atoms of the molecular oxygen. Before this can occur, however, the ring must contain two hydroxyl groups placed ortho or para to each other. Like the monooxygenases, the dioxygenases are highly specific for their substrates. Once the ring is cleaved, the product can enter more common degradative pathways.

B. Anaerobic metabolism. Many compounds can be mineralized anaerobically, yielding carbon dioxide and methane. The aromatic ring is first reduced to a cyclohexanone, then cleaved to an aliphatic acid. Reduced coenzymes must be available for such reactions.

   1. Anaerobic respiration: energy-yielding reactions in which the final electron acceptor is a compound other than molecular oxygen, such as sulfate or nitrate.

   2. Fermentation: anaerobic reactions in which the final product is partially oxidized organic compound such as organic acid.

C. Reactions involving organohalides. Organohalides have been around for millennia, and microorganisms have had a long time within which to develop methods for dealing with them.

   1. In aerobic environments, metabolism of haloaromatic compounds that contain only one or two halides generally leave the carbon-halogen bond intact until the aromatic ring has been cleaved by the oxygenases. Thereafter, dehalogenation usually

occurs by elimination of the halogen as the hydrogen halide, with subsequent double-bond formation in the aliphatic intermediate.

2. Dehalogenations have also been observed in anaerobic environments from both alkyl and aromatic halides. In both cases the halide is apparently replaced by hydrogen. Mechanisms have not been worked out yet but obviously require reducing power.

Some haloorganics appear to require anaerobic conditions for dehalogenation to occur whereas others require aerobic. This means that the environment within which biodegradation is attempted may well be a critical factor in the outcome.

III. Mechanisms for Attacking Xenobiotics

Bacteria can only do those things for which they have a genetic capability. If biodegradation requires the presence of enzymes, if enzymes are synthesized in response to the presence of a recognizable substrate, and if the genetic capability of a bacterium which allows it to synthesize those enzymes has evolved over time in response to its environment, how can biodegradation of xenobiotic compounds be achieved? The answer to those questions lies in the fact that the stereospecificity of enzymes is not exact.

A. Gratuitous biodegradation: reactions involving enzymes having high substrate specificity with respect to their catalytic function but low specificity with respect to substrate binding. It is not uncommon for enzymes to bind analogs of the natural substrate which contain xenobiotic functional groups. The success of gratuitous metabolism depends on:

1. Ability of xenobiotic to induce requisite enzymes.

2. Nature of product

   a. More toxic, either to organism or to other organisms.
   b. Less susceptible to further microbial attack, leading to persistence.
   c. More susceptible to bioaccumulation.
   d. Coordinate induction of many enzymes. May involve whole pathways through the combined efforts of many organisms within a community.

B. Cometabolism. In the situation in which an organism cannot extract energy and reducing power from metabolic reactions, the only way in which they can effect continual biodegradation of the xenobiotic compound is through the use of additional carbon and energy sources supplied externally or from the action of other organisms in a mixed

microbial community. Cometabolism is the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound. Two key concepts are involved here.

1. The non-growth substrate is one that will not support cell division.

2. There must be a growth substrate present in order for the transformation to occur.

C. Fate of products resulting from gratuitous metabolism and cometabolism.

1. If the transformed product is more toxic than the original compound, it will accumulate. If the transformed product is less toxic, the process may continue until it has been converted to a biogenic structure that fits into the normal metabolism of the cell. If the xenobiotic compound is cometabolized by a pure culture, then metabolic products will always accumulate. If it is cometabolized by an organism in a mixed culture, it may well not result in accumulation but rather be metabolized by other species in the consortium. Thus, it is possible that the compound may be completely degraded, even if there is no single organism in the community that can totally degrade it itself. THIS MEANS THAT THE CAPACITY TO SERVE AS THE SOLE CARBON AND ENERGY SOURCE FOR GROWTH OF A PURE (OR ANY) MICROBIAL CULTURE IS NOT AN APPROPRIATE CRITERION BY WHICH TO JUDGE THE BIODEGRADABILITY OF A XENOBIOTIC COMPOUND. BECAUSE OF THE SIGNIFICANCE OF COMETABOLISM AND MICROBIAL INTERACTIONS, BIODEGRADABILITY CAN ONLY BE ACCURATELY ASSESSED IN MIXED-CULTURE, MIXED SUBSTRATE SYSTEMS.

D. Requirements associated with the use of mixed-substrate systems.

1. Control of enzyme synthesis acts to conserve carbon and energy when the cell could not really benefit from having the enzyme present.

2. Control of enzyme activity is more rapid because it acts to influence the rates of enzymes that are already present. Classical batch studies place small inocula of bacteria into contact with high concentrations of substrate. Consideration of the above control mechanisms suggests that the presence of high concentrations of easily degradable substrates could well prevent the synthesis of the very enzymes needed to degrade a compound of interest.

3. The concentration of the compound being tested for biodegradability is another factor of importance. The concentration must be high enough to induce the enzymes needed for its transformation, but low enough either not to be toxic itself or its intermediates not to be toxic.

4. Importance of microbial communities: consortia. The complete mineralization of a compound may require the sequential metabolism of two or more organisms because no single species within the culture contains complete genetic complement of the whole culture.

   a. Typical interaction within communities. Organisms within microbial communities involved in the degradation of xenobiotics have been classified by some as falling into two groups: the primary utilizers and the secondary organisms. The primary utilizers are those species capable of metabolizing the sole or major carbon and energy substrate provided to the system. The secondary organisms cannot use the major substrate but, instead, rely on the utilization of products released by the primary utilizers.

   b. Importance of communities in adaptation. Mixed microbial communities have distinct advantages over pure cultures. This is because the biodegradative capacity of a community is much greater, both qualitatively and quantitatively, particularly where xenobiotic compounds are involved. Furthermore, the resistance of a community to toxic substances may be much greater because there is a greater likelihood that an organism that can detoxify them will be present. Finally, mineralization of xenobiotic compounds sometimes requires the concerted activity of multiple species.

      If a compound is degraded by the concerted action of several organisms, it is likely that the community will develop stepwise. That is, a product may accumulate until an organism that can degrade it becomes established. This suggests that development of the community will be expedited by continually seeding it rather than placing organisms into it at one time.

   c. The Ubiquity Principle states that "...all types of bacteria are available at all times everywhere..." Hence, natural population selection mechanisms will always result in the right biological culture for treatment of a given waste.

IV. Requirements for Successful Biodegradation

A. A capable organism or community must be present. With a single axenic culture, the chances of finding a capable organism are remote if substrate is the least bit peculiar. With a single mixed culture inoculum, chances are somewhat better because of the diverse genetic potential of the inoculum. Long-term continuous inoculation with organisms from diverse sources offer best potential for success.

B. Conditions must be adequate for enzyme induction. This is most likely to occur under carbon-limited conditions. Thus, batch shaker studies with multiple carbon sources are inappropriate. A supply of energy is needed for enzyme synthesis. This is best accomplished with continuous culture wherein the carbon source concentration is kept low and energy source is constantly provided.

   Induction may require an intracellular inducer, and entrance of the inducer may require energy. A steady, continuous supply of energy under carbon-limited conditions is best.

   Gratuitous or cometabolic biodegradation favors a supply of an auxiliary biogenic carbon source. The best course is to supply a diverse mix of compounds.

C. The concentration of test compound is important. Too high may be toxic. Too low may be inadequate for enzyme induction.

D. The proper aerobic or anaerobic environment must be provided for growth of the requisite organisms.

E. The physical-chemical characteristics of the compound must be considered, including such properties as volatility, absorbability, and solubility.

F. Methods to enhance biodegradation include: (1) applying physiological information (i.e., knowledge of the proper morphological and physiological state of the organism is essential to achieve enhanced activity); (2) adjusting environmental conditions; or (3) applying genetic engineering techniques. The mechanisms of gene transfer will be discussed here.

V. Reference Reading

The reader is referred to the following references for detailed discussions of the above information.

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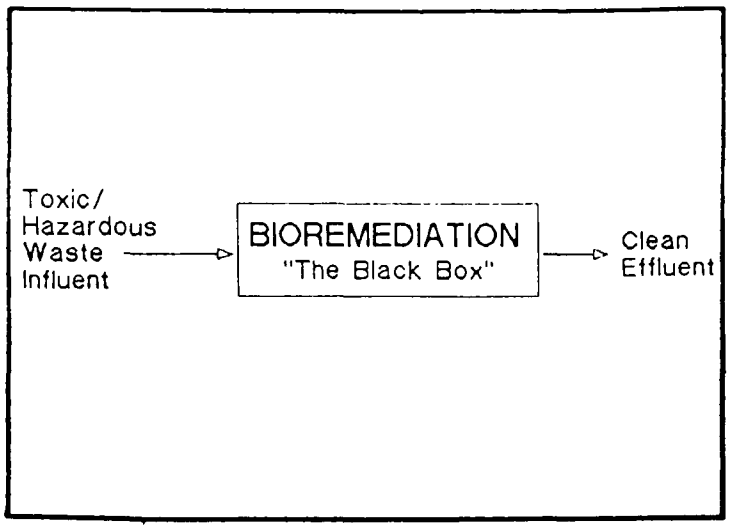

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OBJECTIVES

- Introduce concepts and terminology of Biodegradation/Bioremediation
- Discuss factors that influence biodegradation
- Discuss the benefits/limitations of this technology
- Generally provide an increased comfort level with this technology by delimiting the Black Box Concept

NOTES

ON-SITE TREATMENT AND REMEDIATION OF TOXIC AND HAZARDOUS MATERIAL

NOTES

SITE SPECIFIC SYSTEMS

- Biological
- Chemical
- Physical
- On-site engineering

NOTES

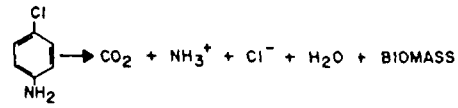
BENEFITS OF BIOREMEDIATION

- Terminal destruction
- On site
- Environmentally sound
- Cost effective

NOTES

MINERALIZATION

The conversion of organic chemicals to carbon dioxide and/or methane, water, and various inorganic forms.



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BIODEGRADATION

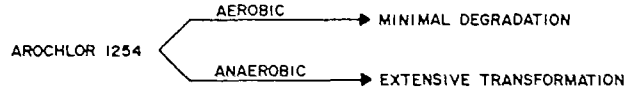
The biological transformation of an organic chemical to another form without regard to extent. Biologists, however, usually use biodegradation as a synonym for mineralization.

NOTES

PERSISTENT COMPOUND

A chemical that fails to undergo biodegradation under a specified set of conditions. A chemical may be inherently biodegradable yet persist in the environment.

PCBs IN HUDSON RIVER



NOTES

RECALCITRANT/REFRACTORY COMPOUND

A chemical that has an inherent resistance to any degree of biodegradation.

Toxaphene, Dieldrin, Endrin

NOTES

BIOGENIC COMPOUNDS

Naturally occurring compounds that have been present for millions of years. Thus, there are organisms somewhere in the biosphere that can initiate their biodegradation.

NOTES

XENOBIOTIC COMPOUNDS

Compounds that are "foreign" to the biosphere, having been present for only an instant on the evolutionary time scale. May or may not be biodegradable.

NOTES

ADAPTATION/ACCLIMATION

An increase in the biodegradation rate of a chemical after exposure of the microbial community to the chemical for some period of time.

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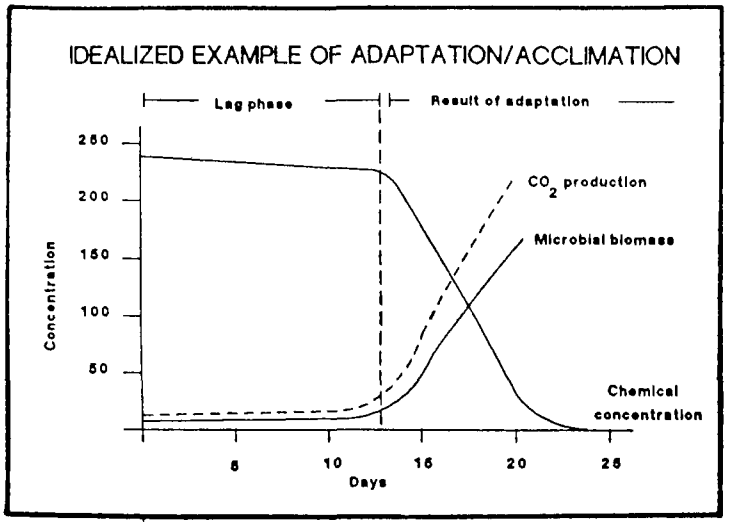


NOTES

BIOREMEDIATION

The manipulation of living systems to bring about desired chemical and physical changes in a confined and regulated environment.

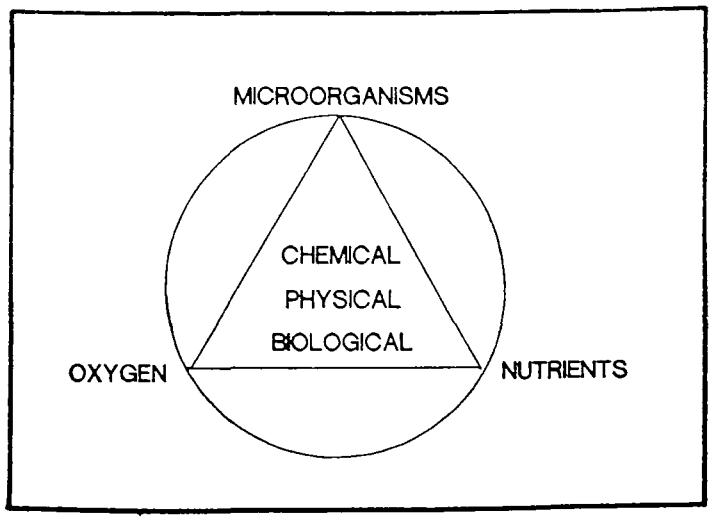
NOTES

BIOREMEDIATION

Hybrid Of:

- Microbiology
- Ecology
- Biochemistry
- Chemical engineering
- Environmental engineering
- In-situ technology (hydrogeology and soil science)
- Risk management

NOTES



NOTES

BASIC MICROBIOLOGY

Ecology

Physiology

Genetics

NOTES

BASIC MICROBIOLOGY

Ecology

Interaction of a microorganism
and its environment
(physical, chemical)

BASIC MICROBIOLOGY

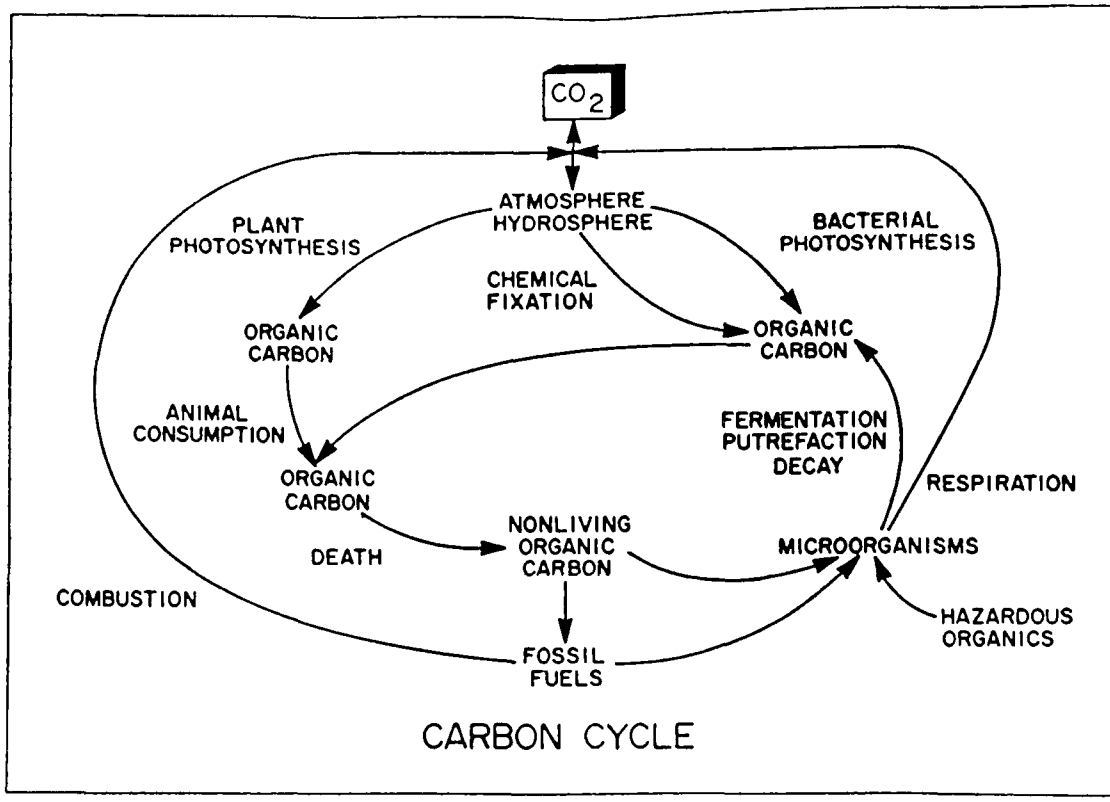
Physiology

Processes by which any organism obtains food and energy for biosynthesis and performing other work
(Chemical energy- ->Biological energy)
(proteins, enzymes, cell structural parts)

BASIC MICROBIOLOGY

Genetics

The equivalent of a computer program. Codes of information which control or dictate the physiology of an organism in response to its environment.
(DNA, genes)



CARBON CYCLE

BASIC PREMISES OF BIODEGRADATION

1. Organic compounds are converted to simpler structures by the action of microorganisms as part of the continual cycling of carbon in nature.

2. Microorganisms generally derive the nutritional and energy requirements necessary for growth from the compounds they degrade.

NOTES

BASIC PREMISES OF BIODEGRADATION
(Continued)

3. Biodegradation occurs in a wide variety of environments through the action of microorganisms using processes determined by environmental factors.

4. Enzymes evolved throughout time for the degradation of naturally occurring organics can be recruited to degrade man-made waste materials.

NOTES

BASICS OF PHYSIOLOGY

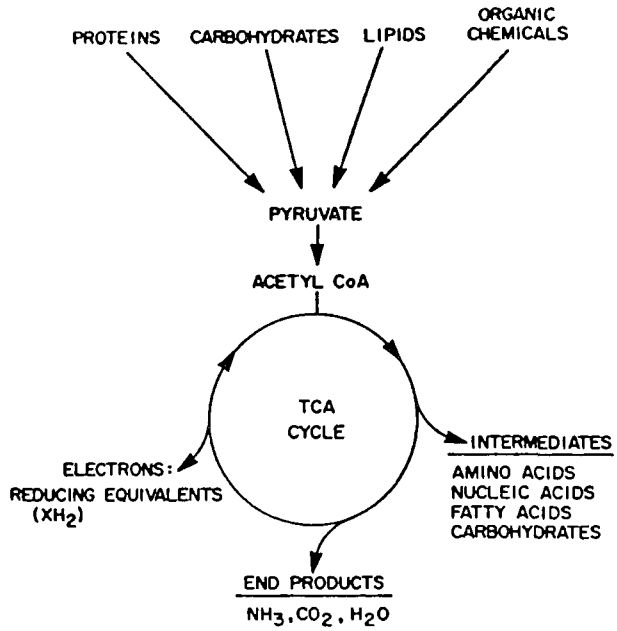
- Cell composed of macromolecules (proteins, polysaccharides, lipids, nucleic acids)
- Basic building blocks are amino acids, carbohydrates, fatty acids, nucleic acids

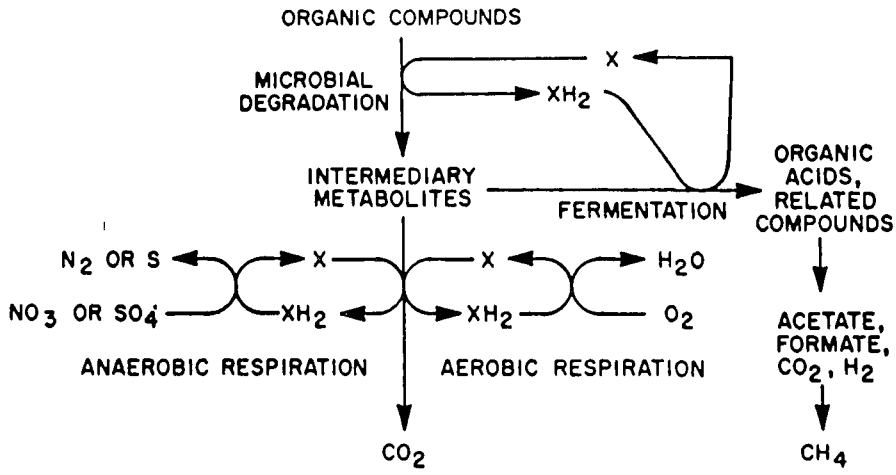
NOTES

BASICS OF PHYSIOLOGY
(Continued)

- Cells synthesize components from multitude of nutritional and energy sources
- Intermediary metabolism--central mechanism by which cells process and harness chemical energy to produce biomass and energy

INTERMEDIARY METABOLISM





CENTRAL REACTIONS IN MICROBIAL METABOLISM

BIODEGRADATION PATHWAYS

PATHWAY	EXAMPLE	END PRODUCTS	MICROBE
AEROBIC RESPIRATION	HEXANE	CO_2 , H_2O	PSEUDOMONAS
ANAEROBIC RESPIRATION	BENZOATE	ORGANIC ACIDS NO_2^-	PSEUDOMONAS
FERMENTATION	PHENOL	ORGANIC ACIDS CO_2 , CH_4	METHANOGENIC

NOTES

NOTES

AEROBIC RESPIRATION

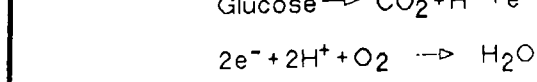
Energy-yielding metabolism in which the terminal electron acceptor for substrate oxidation is molecular oxygen.

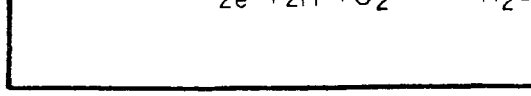
NOTES

AEROBIC BIODEGRADATION

Oxygen Involved In Two Ways

1. Acceptor of electrons produced from oxidation reaction resulting in reduction to water:



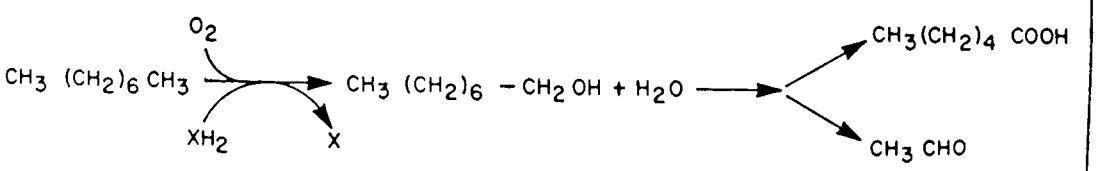


AEROBIC BIODEGRADATION

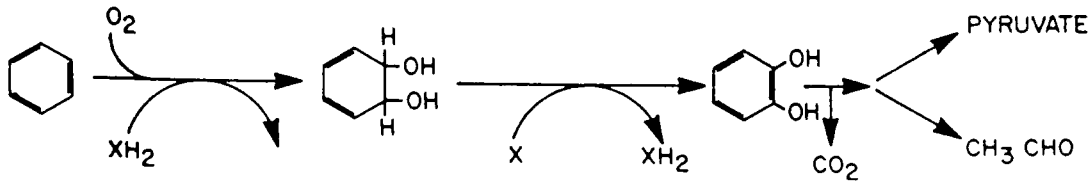
Oxygen Involved In Two Ways (Continued)

2. **Important substrate for oxygenase enzymes, which incorporate molecular oxygen into relatively unreactive compounds:**

EXAMPLES OF OXYGEN INVOLVEMENT IN AEROBIC BIODEGRADATION



EXAMPLES OF OXYGEN INVOLVEMENT IN AEROBIC BIODEGRADATION



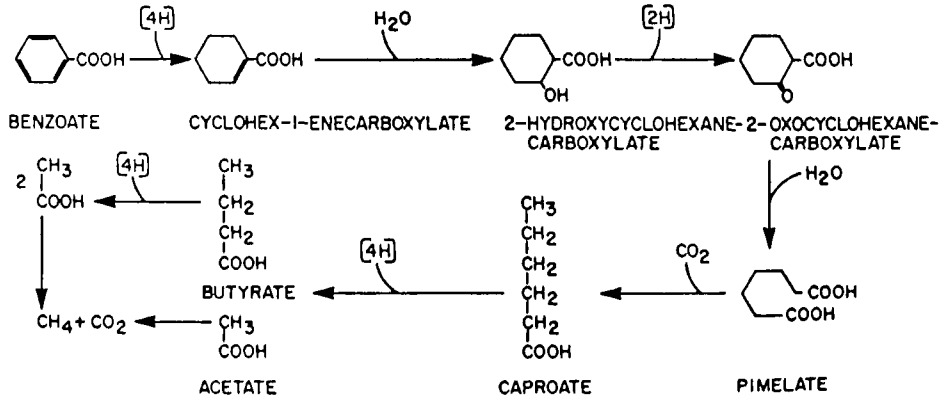
ANAEROBIC RESPIRATION

Energy-yielding metabolism in which the terminal electron acceptor for substrate oxidation is an inorganic compound other than molecular oxygen, such as sulfate or nitrate.

FERMENTATION

Energy-yielding metabolism that involves a sequence of oxidation-reduction reactions in which both the substrate (primary electron donor) and the terminal electron acceptor are <u>organic compounds.</u>

FERMENTATION OF BENZOATE UNDER METHANOGENIC CONDITIONS



ANAEROBIC BIODEGRADATION

Anaerobes Require Electron Acceptors Other Than Oxygen With Reduction To Characteristic Products:

CO_2	→ Methane	.....Methanogens
NO_3	→ N_2	.....Denitrifiers
SO_4	→ H_2S	.....Sulfate reducers
Glucose	→ Lactate Ethanol	.....Fermenters

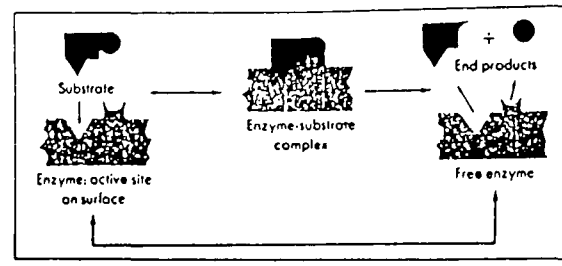
LIMITED DEGRADATIVE POTENTIAL BUT SEVERAL NOVEL REDUCTION REACTIONS (DEHALOGENATION, ETHER CLEAVAGE)

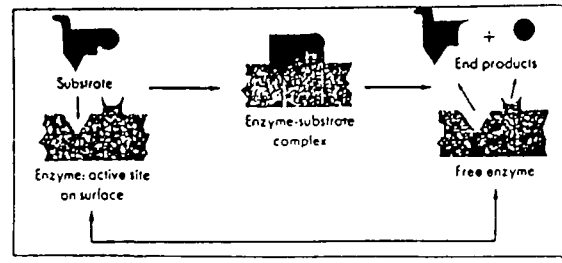
GRATUITOUS METABOLISM

- **Reactions involving enzymes having high substrate specificity with respect to catalytic function but low specificity with respect to substrate binding**

RELATIONSHIP BETWEEN ENZYME ACTION

AND GRATUITOUS METABOLISM



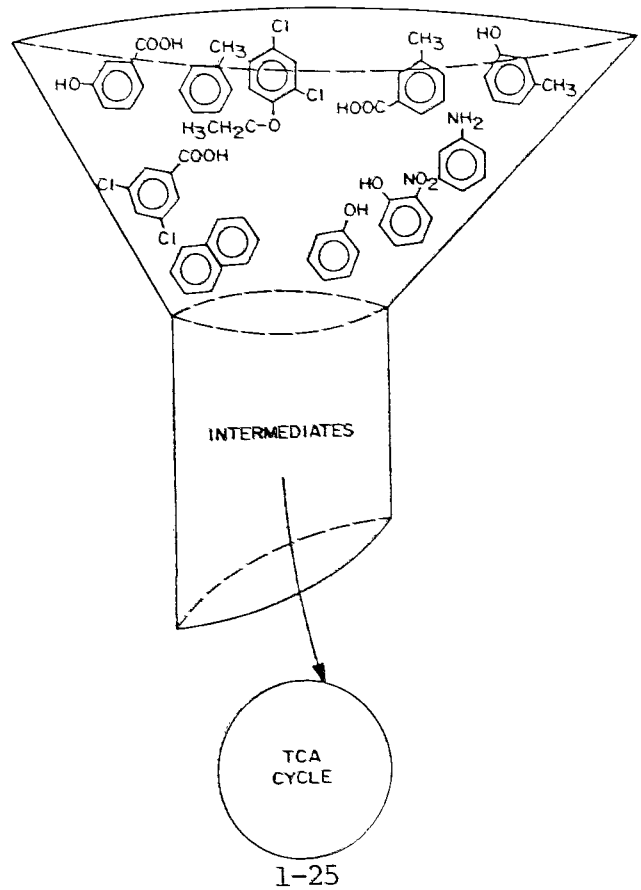


COMETABOLISM/COOXIDATION

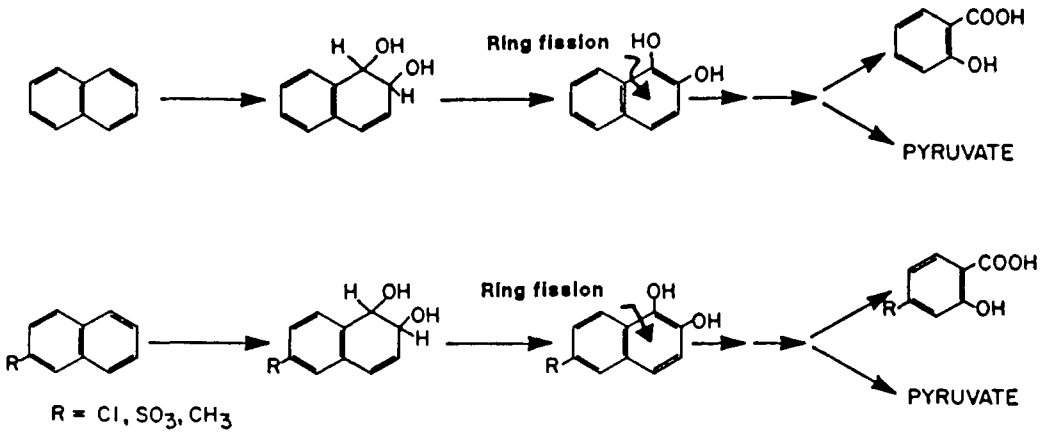
The transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound.

NON-GROWTH SUBSTRATE

A substrate that will not support cell division. There must be a growth substrate present in order for the transformation to occur.



COOXIDATION EXAMPLE



INDUCIBLE ENZYMES

Enzymes produced by a cell in response to a specific compound which is referred to as the inducer.

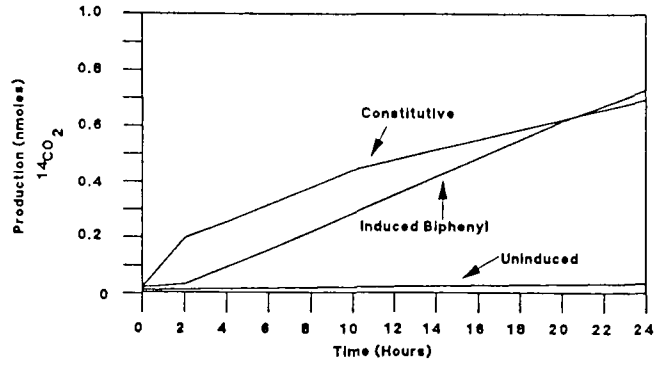
NOTES

CONSTITUTIVE ENZYMES

Enzyme(s) always produced by a cell regardless of the nature of the medium. An inducer compound is not required for the enzyme(s) formation.

NOTES

MINERALIZATION OF ^{14}C LABELLED BENZANTHRACENE BY BEIJERINCKIA B1



NOTES

ENVIRONMENTAL FACTORS LIMITING BIODEGRADATION

Biological

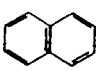
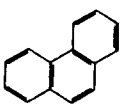
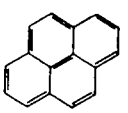
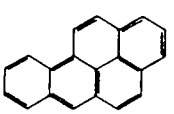
- Active viable biomass
- Physiological limitations
- Electron acceptors
- Predation

ENVIRONMENTAL FACTORS LIMITING BIODEGRADATION

Physical

- Temperature
- Availability of chemical
- Surface adhesion
- Access to substrate
- Light

Properties of Some PAH Compounds

COMPOUND	Aq. SOLUBILITY g/l	Log Kow	Log Koc
 NAPHTHALENE	31.7	3.37	3.11
 PHENANTHRENE	1.29	4.46	4.36
 PYRENE	0.135	5.32	4.92
 BENZO(a)PYRENE	0.0038	6.04	6.65

ENVIRONMENTAL FACTORS LIMITING BIODEGRADATION

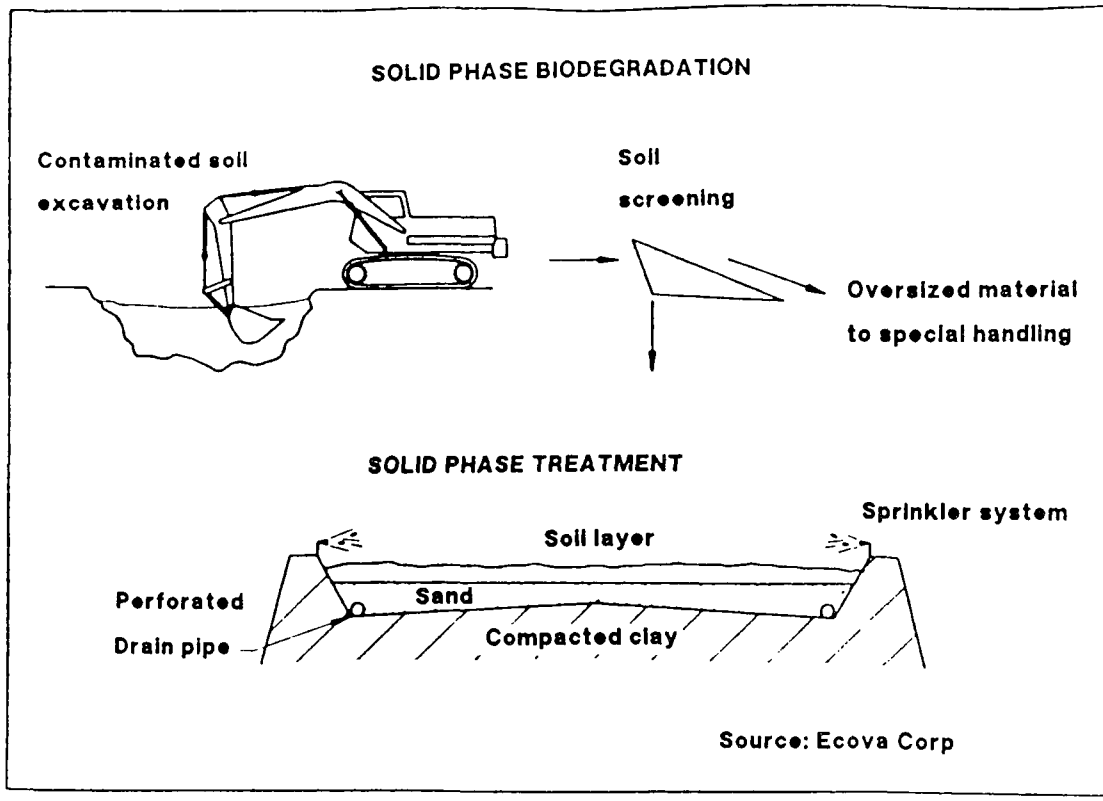
Chemical

- pH
- Salinity
- Organic nutrients (vitamins cofactors, substrates)
- Redox potential (O_2, NO_3^-, $SO_4^=$, CO_2)

ENVIRONMENTAL FACTORS LIMITING BIODEGRADATION

Chemical (Continued)

- Major inorganic nutrients (N, P, S, Mg, K, etc.)
- Trace elements (Fe, Zn, Mn, Mo, Co, Cu, Ca)
- Toxic chemicals
- Chemical mixtures


SOLID PHASE BIODEGRADATION
Contaminated soil
excavation
Soil
screening
Oversized material
to special handling
SOLID PHASE TREATMENT
Sprinkler system
Soil layer
Perforated
Drain pipe
Sand
Compacted clay
Source: Ecova Corp

FACTORS CONTROLLING BIODEGRADATION
(Liquids and Solids)

Factors	Effect	Data Needs
Variable waste composition	Inconsistent biodegradation caused by variation in biological activity.	Waste composition
Water solubility	Contaminants with low solubility are harder to biodegrade.	Solubility
Biodegradability	Low biodegradability inhibits process.	Chemical constituents, presence of metals/salts, bench-scale testing
Temperature outside 25-70°C range.	Larger, more diverse microbial population present in this range.	Temperature monitoring
Nutrient deficiency	Lack of adequate nutrients for biological activity (although nutrient supplements may be added).	C/N/S ratio
Oxygen deficiency	Oxygen depletion slows down the process.	Oxygen monitoring
Moisture content	A moisture content of greater than 79% affects bacterial activity and availability of oxygen. A moisture content below 40% severely inhibits bacterial activity.	Ratio of air to water in interstices, porosity of composting mass
pH outside 4.5-7.5 range	Inhibition of biological activity	Sludge pH testing
Microbial population	If indigenous microorganisms not present, cultured strains can be added.	Culture test
Presence of elevated levels of: • Heavy metals • Highly chlorinated organics	Can be highly toxic to microorganisms.	Analysis for contaminants

FACTORS CONTROLLING BIODEGRADATION
(Solids)

Factors	Effect	Data Needs
• Some pesticides, herbicides • Inorganic salts		
Water and air emissions and discharges (composting only)	Potential environmental and/or health impacts (control achieved through air scrubbing, carbon filtration, forced aeration, cement liner).	Concentrations of contaminants
Compaction of compost (composting only)	Particles tend to coalesce and form an amorphous mass that is not easily maintained in an aerobic environment (wood chips or shredded tires may be added as bulking agents).	Determine integrity, physical nature of material
Nonuniform particle (composting only)	Waste mixtures must be of uniform particle size.	Particle size distribution
Unfavorable soil characteristics		
• Low permeability	Hinders movement of water and nutrients through contaminated area.	Percolation testing
• Variable soil conditions	Inconsistent biodegradation due to variation in biological activity.	Soil mapping
• Low soil pH (< 5.5)	Inhibition of biological activity	Soil pH testing
• Low soil organic content	Lack of organic substrate for biological growth.	Soil humus content
• Low moisture content (< 10%)	Subsurface biological growth requires adequate moisture.	Soil moisture content
Unfavorable site hydrology	Groundwater flow patterns must permit pumping for extraction and reinjection.	Site hydrogeology must be well defined.

FACTORS CONTROLLING BIODEGRADATION
(Groundwater)

Factors	Effect	Data Needs
Unfavorable groundwater quality parameters		
• Low dissolved oxygen	Oxygen necessary for biological growth.	Dissolved oxygen in groundwater, determine amount of hydrogen peroxide needed to satisfy oxygen demand.
• Low pH, alkalinity	Inhibition of biological activity.	pH and alkalinity of groundwater

COMPARISON OF AVAILABLE TECHNOLOGIES FOR SOIL TREATMENT

Organic Contaminant	Technology: Rotary Kiln Incin.	In-Situ Chemical Treat.	Bio.	In-Situ Bio.
Halogenated volatiles	D	N	P	P
Halogenated semivolatiles	D	N	P	P
Nonhalogenated volatiles	D	N	P	P
Nonhalogenated semivolatiles	D	N	P	P
PCBs	D	N	P	P
Pesticides	D	N	P	P
Organic cyanides	D	P	P	P
Organic corrosives	D	P	X	X

D=demonstrated effectiveness; P=potential effectiveness; N=no effectiveness; X=potential adverse impacts to process or environment

COMPARISON OF AVAILABLE TECHNOLOGIES FOR SOIL TREATMENT

Organic Contaminant	Technology: Rotary Kiln Incin.	In-Situ Chemical Treat.	Bio.	In-Situ Bio.
Volatile metals	X	N	X	X
Nonvolatile metals	N	N	X	X
Asbestos	N	N	N	N
Radioactive materials	N	N	X	X
Inorganic corrosives	N	P	X	X
Inorganic cyanides	P	P	X	X

P=potential effectiveness; N= no effectiveness; X=potential adverse impacts to process or environment

COMPARISON OF AVAILABLE TECHNOLOGIES FOR SOIL TREATMENT

Organic Contaminant	Technology: Rotary Kiln Incin.	In-Situ Chemical Treat.	Bio.	In-Situ Bio.
Oxidizers	D	P	X	X
Reducers	D	P	X	X

D=demonstrated effectiveness; P=potential effectiveness;
X=potential adverse impacts to process or environment

EXAMPLES OF CONSTITUENTS WITHIN WASTE GROUPS

HALOGENATED VOLATILES
Bromodichloromethane
Bromoform
Bromomethane
Carbon tetrachloride
Chlorodibromomethane
Chlorobenzene
Chloroethane
Chloroform
Chloromethane
Chloropropane
Dibromomethane
Cis,1,3-dichloropropene
1,1-Dichloroethane
1,2-Dichloroethane
1,1-Dichloroethene
1,2-Dichloroethene
1,2-Dichloropropane
Fluorotrichloromethane
Methylene chloride
1,1,2,2-tetrachloroethane
Tetrachloroethene
1,1,1-Trichloroethane
1,1,2-Trichloroethane
1,2-Trans-dichloroethene
Trans-1,3-dichloropropene
1,1,2-trichloro-1,2,2-trifluoroethane
Trichloroethene
Vinyl chloride
Total chlorinated hydrocarbons
Hexachloroethane
Dichloromethane

HALOGENATED SEMIVOLATILES
2-chlorophenol
2,4-dichlorophenol
Hexachlorocyclopentadiene
p-chloro-m-cresol
Pentachlorophenol
Tetrachlorophenol
2,4,5-trichlorophenol
2,4,6-trichlorophenol
Bis-(2-chloroethoxy)methane
Bis(2-chloroethyl)ether
Bis(2-chloroisopropyl)ether
4-bromophenyl phenyl ether
4-chloroaniline
2-chloronapthalene
4-chlorophenyl phenylether

HALOGENATED SEMIVOLATILES (cont.)
Bis(2-chloroethoxy)phthalate
Bis(2-chloroethoxy)ether
1,2-bis(2-chloroethoxy)ethane

NONHALOGENATED VOLATILES
Acetone
Acrolein
Acrylonitrile
Benzene
2-butanone
Carbon disulfide
Cyclohexanone
Ethyl acetate
Ethyl ether
Ethyl benzene
2-hexanone
Isobutanol
Methanol
Methyl isobutyl ketone
4-methyl-2-pentanone
n-butyl alcohol
Styrene
Toluene
Trimethyl benzene
Vinyl acetate
Xylenes

NONHALOGENATED SEMIVOLATILES
Benzoic acid
Cresols
2,4-dimethylphenol
2,4-dinitrophenol
2-methylphenol
4-methylphenol
2-nitrophenol
4-nitrophenol
Phenol
Acenaphthene
Acenapthylene
Anthracene
Benzidine
Benzo(a)anthracene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(a)pyrene
Benzo(ghi)perylene
Benzyl alcohol
Bis(2-ethylhexyl)phthalate

EXAMPLES OF CONSTITUENTS WITHIN WASTE GROUPS (cont)

HALOGENATED SEMIVOLATILES (cont.)
1,2-dichlorobenzene
1,3-dichlorobenzene
1,4-dichlorobenzene
3,3-dichlorobenzidine
Hexachlorobenzene
Hexachlorobutadiene
1,2,4-trichlorobenzene

PESTICIDES
Aldrin
Bhc-alpha
Bhc-beta
Bhc-delta
Bhc-gamma
Chlordane
4,4'-DDD
4,4'-DDE
4,4'-DDt
Dieldrin
Endosulfan I
Endosulfan II
Endosulfan sulfate
Endrin
Endrin aldehyde
Ethion
Aluminum
Heptachlor
Heptachlor epoxide
Malathion
Methylparathion
Parathion
Toxaphene

NONHALOGENATED SEMIVOLATILES (cont)
4,6-dinitro-2-methylphenol
2,4-dinitrotoluene
2,6-dinitrotoluene
Di-n-octyl phthalate
1,2-diphenylhydrazine
Fluoranthene
Fluorene
Indeno(1,2,3-cd)pyrene
Isophorone
2-methylnapthalene
Napthalene
2-nitroaniline
3-nitroaniline
4-nitroaniline
Nitrobenzene
n-nitrosodimethylamine
n-nitrosodi-n-propylamine
n-nitrosodiphenylamine
Phenanthrene
Pyrene
Pyridine
2-methynaphthalene
Bis phthalate
Phenyl napthalene
Ethyl parathion
Butyl benzyl phthalate
Chrysene
Dibenzo(a,h)anthracene
Dibenzofuran
Diethyl phthalate
Dimethyl phthalate
Di-n-butyl phthalate

VOLATILE METALS
Arsenic
Bismuth
Lead
Mercury
Tin
Selenium

OTHER CATEGORIES
Asbestos

EXAMPLES OF CONSTITUENTS WITHIN WASTE GROUPS (cont)

INORGANIC CORROSIVES
Hydrochloric acid
Nitric acid
Hydrofluoric acid
Sulfuric acid
Sodium hydroxide
Calcium hydroxide
Calcium carbonate
Potassium carbonate
PCBs
PCB (Arochlor)-1016
PCB (Arochlor)-1221
PCB (Arochlor)-1232
PCB (Arochlor)-1242
PCB (Arochlor)-1248
PCB (Arochlor)-1254
PCB (Arochlor)-1260
PCB NOS (not otherwise specified)

ORGANIC CORROSIVES
Acetic Acid
Acetyl chloride
Aniline
Aeromatic Sulfonic acids
Cresylic acid
Formic acid

NONMETALLIC TOXIC ELEMENTS
Fluorine
Bismuth

NONVOLATILE METALS
Aluminum
Antimony
Barium
Beryllium
Bismuth
Cadmium
Calcium
Chromium
Copper
Cobalt
Iron
Magnesium
Manganese
Nickel
Potassium
Selenium
Sodium
Vanadium
Zinc

RADIOACTIVES
Radioactive isotopes of
iodine, barium, uranium
Radium
Gamma radioactivity

ORGANIC CYANIDES
Organonitriles

OXIDIZERS
Chlorates
Chromates

REDUCERS
Sulfides
Phosphides
Hydrazine

INORGANIC CYANIDES
Cyanide
Metallic cyanides
(e.g., ferricyanide,
sodium cyanide)

RELATIVE DEGRADABILITY

Classes of chemicals that are good candidates for treatment at hazardous waste sites

- Monochlorinated aromatic compounds (A)
- Benzene, toluene, xylene (A or AN)
- Phenolics (nonhalogenated) and cresols (A or AN)
- Polynuclear aromatic hydrocarbons (creosotes) (A)
- Alkanes and alkenes (fuel oil) (A)

(A) using aerobic biodegradation processes
(AN) using anaerobic biodegradation processes

NOTES

NOTES

RELATIVE DEGRADABILITY

Classes of chemicals that, with further research (short term), could be candidates for biological treatment at hazardous waste sites

- Polychlorinated biphenyls (A and AN)
- Pentachlorophenol (A or AN)
- Nitrogen heterocyclics (A)
- Chlorinated solvents (alkanes and alkenes) (A and AN)

(A) using aerobic biodegradation processes
(AN) using anaerobic biodegradation processes

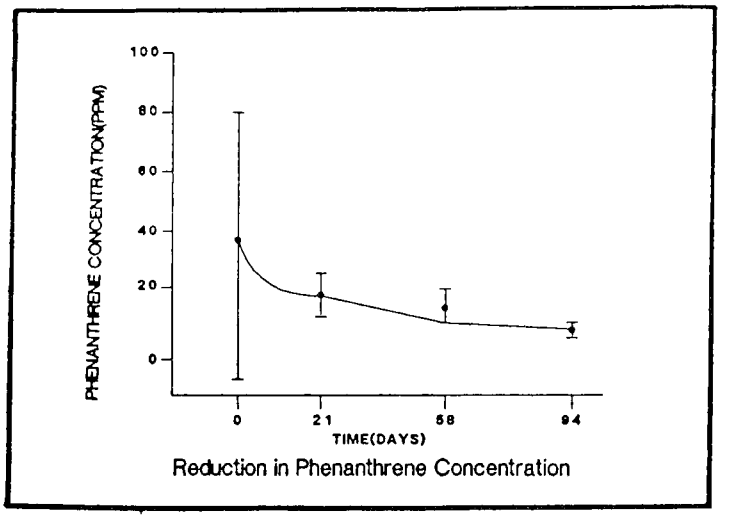
NOTES

Phenanthrene Degradation During Pilot-Scale Bioremediation of Styrene Tar Waste in Soils from a Refining Site

	Phenanthrene (PPB)			
Treatment	Initial (Day 0)	Final (Day 94)	Reduction	Half Life (Days)
Control	27,850	5,725	79.44%	40.8
Nutrient * Adjusted	19,400	2,712	86.02%	33.0
Single Inoculation	73,600	5,750	92.19%	25.7

* Nutrients: inorganic nitrogen & phosphorous

NOTES



Reduction in Phenanthrene Concentration

NOTES

Effect of Initial Concentration on Phenanthrene Degradation During Pilot-Scale Bioremediation of Styrene Tar Waste In Soils at a Refining Site

Initial Concentration, PPB	Average Reduction, %
1,000 4,999	27.4
5,000 - 9,999	33.4
10,000 - 49,999	67.2
50,000 - 100,000	94.0
greater than 100,000	96.7

NOTES

CONCENTRATIONS OF 2,4-D IN A SIMULATED SOLID-PHASE BIORECLAMATION SYSTEM
(mg/kg)

Sample	Day 0	Day 5	Day 10	Day 20
Sterile	19.7 (±5.0)	23	23	16
Covered	19.7	8.8 (±2.2)	8.4 (±3.1)	2.2 (±0.2)
Uncovered, uninoculated	19.7	7.3 (±0.5)	8.8 (±3.6)	2.1 (±0.2)
Uncovered + JMP 134 + TF-6	19.7	7.7 (±2.0)	6.0 (±1.1)	1.7 (±0.3)
Uncovered + ME-3 + TF-6	19.7	9.8 (±1.5)	4.0 (±0.2)	1.9 (±0.1)

NOTE: ND = Not detected at detection limit of 5.0 mg/kg
Numbers in parentheses indicate range of duplicate samples

NOTES

CONCENTRATIONS OF MCPA IN A SIMULATED SOLID-PHASE BIORECLAMATION SYSTEM (mg/kg)

Sample	Day 0	Day 5	Day 10	Day 20
Sterile	117 (±40)	121	115	18
Covered	117	71 (±22)	46 (±14)	ND
Uncovered, uninoculated	117	119 (±15)	44 (±17)	31 (±1)
Uncovered + JMP 134 + TF-6	117	82 (±44)	57 (±12)	16 (±3)
Uncovered + ME-3 + TF-6	117	86 (±13)	40 (±1)	24 (±9)

NOTE: ND = Not detected at detection limit of 5.0 mg/kg
Numbers in parentheses indicate range of duplicate samples

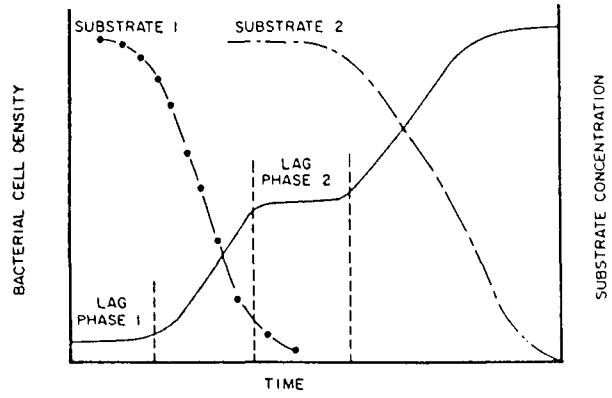
NOTES

DIAUXIE

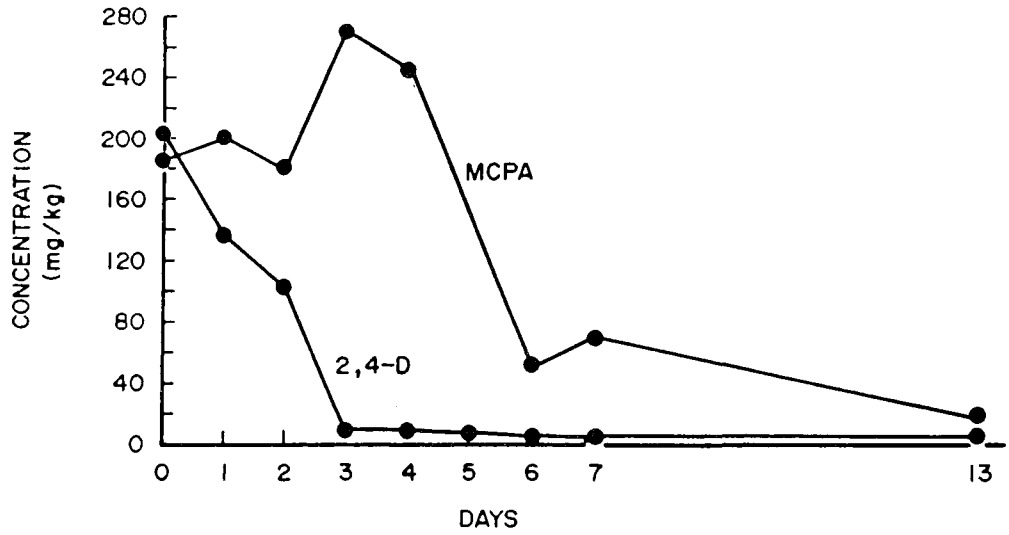
The response of microorganisms to the presence of mixed substrates in which preferential utilization of the substrates for carbon and energy is observed

NOTES

PHENOMENON OF DIAUXIC GROWTH WITH A BACTERIAL CULTURE GROWING ON MIXED SUBSTRATES



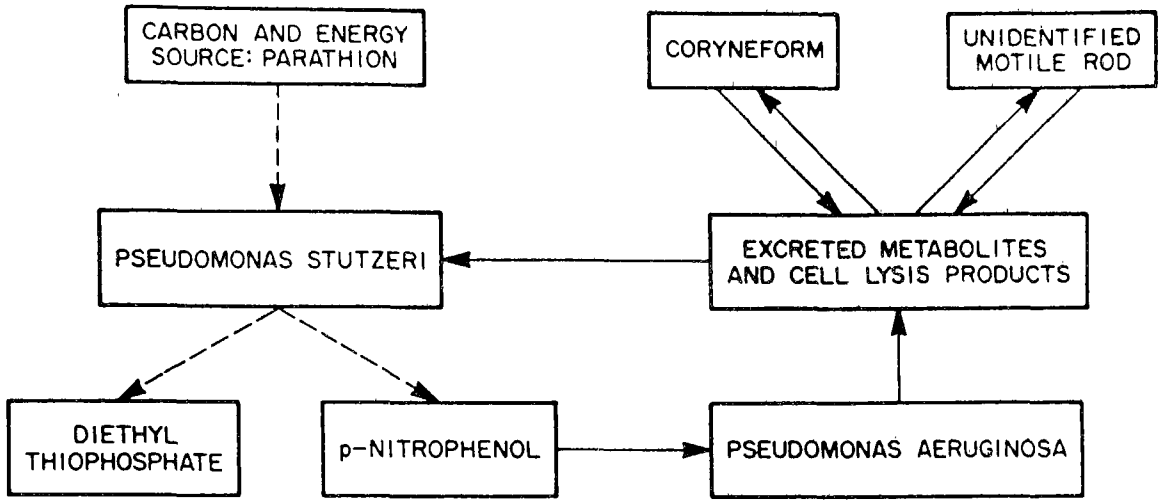
CONCENTRATIONS OF 2,4-D AND MCPA IN A SOIL SLURRY TREATMENT SYSTEM



Importance of Microbial Communities

CONSORTIA

- Typical interactions within communities
- Importance of communities in adaptation
- Changes in the genetic information or constitution of microorganisms
- The Ubiquity Principle

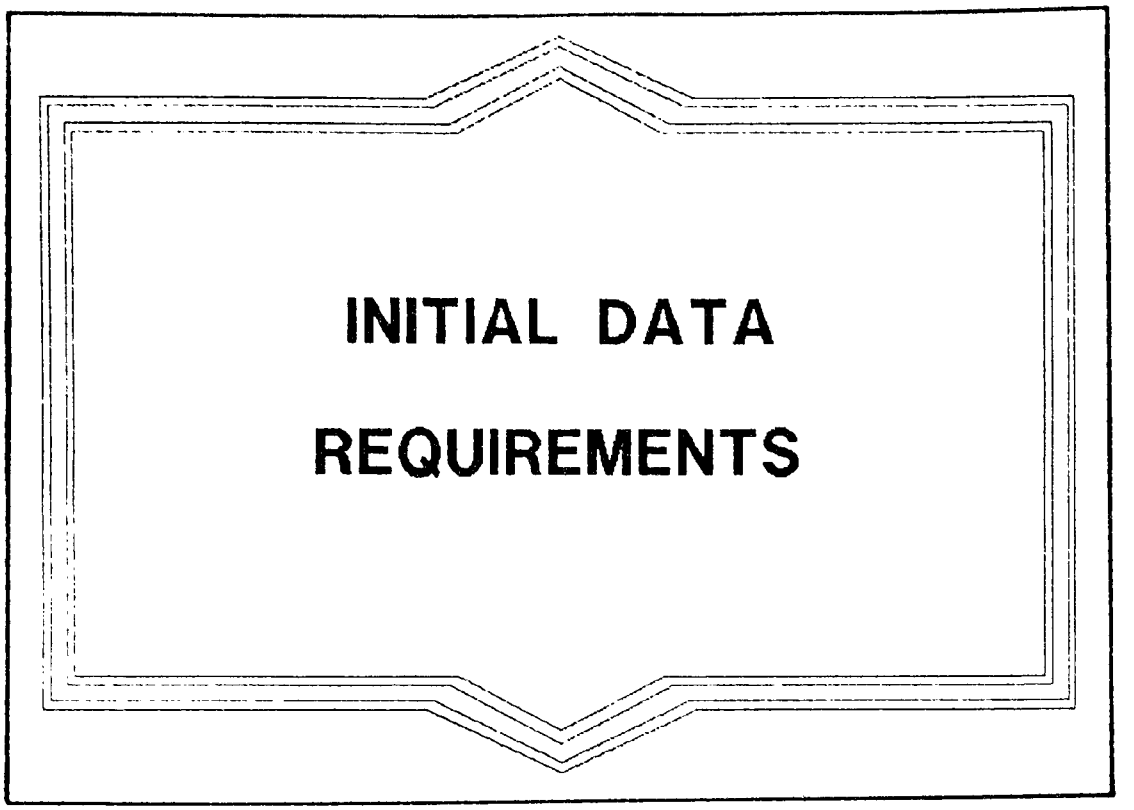


PARATHION MICROBIAL COMMUNITY BASED ON COMETABOLISM: ⟶, SUBSTRATE UTILIZATION ASSOCIATED WITH GROWTH; ----⟶, COMETABOLIC TRANSFORMATION NOT LINKED TO GROWTH.

(SOURCE: SLATER AND LOVATT)

GENETIC APPROACHES TO ENHANCE BIODEGRADATION

- Increase enzyme yields
- Overcome cell regulatory controls
- Engineer more efficient proteins
- Construct novel biodegradation pathways



SECTION 2

INITIAL DATA REQUIREMENTS

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U.S. EPA
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U.S. EPA
Gulf Breeze, Florida

Paul Flathman
OH Materials
Findlay, Ohio

Because of the tight time constraints in effecting the cleanup of Superfund hazardous waste sites it is imperative to make timely decisions in selecting the appropriate remediation technology. Such decisions, however, should be predicated on sound information about the site and some initial information about the individual remediation processes. Information on the site can be obtained from the initial site characterization. Information about the remediation process can be obtained from published literature as well as from simple laboratory feasibility studies. The purpose of this portion of the workshop is to describe what information should be collected during the initial site characterization to evaluate bioremediation processes and also to describe some simple feasibility studies that can be used to assist in the selection process.

At all sites, an initial site investigation is conducted to establish the identity of chemicals at the site, determine the nature and extent of the contamination, obtain a description of the environmental characteristics of the site, and to make an initial appraisal of the appropriate remediation technologies. This information is used to determine if the site is hazardous and, if necessary, what action should be taken to reduce the hazard to a safe level. The amount of information required to make these decisions is not insignificant. In this presentation and in these handouts only the information that is required to evaluate bioremediation has been emphasized.

To facilitate the data review a flow diagram is presented that can be used to walk through the data analysis. The diagram is divided into six major areas.

In the first area the problem is defined and the types of contaminants are identified. The physical and chemical properties of the compounds that can influence biodegradation are identified and the literature assessed for information concerning the degradation of the compounds.

In the second area the distribution of the chemicals within the site is determined. Examples of specific analytical procedures are presented in Appendix A. At this point the site is divided into a series of subsites for further evaluation. Compound concentration becomes important at this point because concentrations may be toxic and some pretreatment may be required before bioremediation can be considered. Pretreatment may consist of dilution of the contaminated area, e.g., mixing of wastes.

In the third area the contaminated environment is characterized. This characterization extends from gross characteristics such as soil, sediment, water or subsurface material to more specific characteristics such as permeability, redox conditions, pH and hydrology. The characteristic microbiological characteristics of the different environments are also identified. For example, anaerobic bacteria may predominate in sediments whereas aerobic organisms would predominate in unsaturated soils.

In the fourth area any adjustment of the environment that might be required to permit bioremediation is addressed directly. Such adjustments could include alteration in pH, preremoval of toxic metals, and changes in moisture content. In some cases the judgment may be that bioremediation is not possible because the environment cannot be adjusted

In the fifth area the microbiological needs of the sites are evaluated. At this point the concern becomes the availability of nutrients, the potential additions of bacteria with specific degradative characteristics, and whether the process should be conducted under anaerobic conditions or aerobic conditions.

In the sixth area a feasibility study is designed to test potential bioremediation scenarios.

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Protocol Development for the Prediction of the Fate of Organic Priority Pollutants in Biological Wastewater Treatment Systems. (AEROBIC AND ANAEROBIC MULTI-LEVEL BIODEGRADABILITY TESTING PROTOCOLS)

E.J. Kirsch, C.P.L. Grady Jr. and R.F. Wukasch, Purdue University, West Lafayette, Indiana 47507 and Henry H. Tabak, U.S. EPA, Water Engineering Research Laboratory, AWBERC, ORD, Cincinnati, Ohio 45268.

EPA/600/S2-85/141 February 1986

Protocol for Determination of Biodegradation Kinetics Through the Use of Electrolytic Respirometry

C.P.L. Grady, J.S. Dang, D.M. Harvey, A. Jobbagy and X.-L. Wang, Clemson University, Clemson, South Carolina 29634, and Henry H. Tabak, U.S. EPA, Risk Reduction Engineering Laboratory, AWBERC, ORD, Cincinnati, Ohio 45268.

Presented at the 14th Biennal Conference of International Association on Water Pollution Research and Control, Brighton, England 17-23 July 1988. To be published in the Water Science and Technology Journal, July, 1989.

Protocol for Evaluation of Biodegradation Kinetics with Respirometric Data

C.P.L. Grady, J.S. Dang, D.M. Harvey, A. Jobbagy, Clemson University, South Carolina, Clemson, South Carolina 29634, and Henry H. Tabak, U.S. EPA, Risk Reduction Engineering Laboratory, AWBERC, ORD, Cincinnati, Ohio 45268.

Presented at the 61st Annual Conference of the Water Pollution Control Federation, October 2-6, 1988, Dallas, Texas, and submitted for publication October, 1988 to the Journal of Water Pollution Control Federation.

Protocol for the Determination of Biodegradability and Biodegradation Kinetics of Toxic Organic Compounds with the use of Electrolytic Respirometry

Henry H. Tabak, Risk Reduction Engineering Laboratory, U.S. EPA, ORD, AWBERC, Cincinnati, Ohio 45268, Rakesh Govind and Sanjay Desai, University of Cincinnati, Cincinnati, Ohio 45221 and C.P.L. Grady, Clemson University, Clemson, South Carolina 29634.

Presented at the 61st Annual Conference of Water Pollution Control Federation, October 2-6, 1988, Dallas, Texas and submitted for publication in December 1988, to the Journal of Water Pollution Control Federation.

"Assessment of Bioaugmentation Technology and Evalution Studies on Bioaugmentation Products"

Henry H. Tabak, U.S. EPA, Wastewater Research Division, Water Engineering Research Laboratory, ORD, Cincinnati, Ohio 45268.

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Published in the Proceedings of the Tenth United States/Japan Conference on Sewage Treatment and NATO/Committee on the Challenges of Modern Society (NATO/CCMS) Conference on Sewage Treatment Technology, Volume I, Part B. United States Papers p. 431-499, 1986. EPA/600/9-86/015b, NTIS PB87-110631.

Screening Protocol for Assessing Toxicity of Organic Chemicals to Anaerobic Treatment Processes (MULTI-STEP SCREENING ANAEROBIC INHIBITION PROTOCOL)

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Presented at the AWMA/EPA International Symposium on Hazardous Waste Treatment: Biosystems for Pollution Control, February 20-23, Cincinnati, Ohio 45202 and accepted for publication in the Air & Waste Management Association Journal. 1989.

APPENDIX A

CHEMICAL ANALYSIS OF TEST CHEMICALS AND/OR WASTE SAMPLES

The selection of a suitable extraction procedure for a given combination of analyte(s) and soil matrix generally requires some method development (Coover et al. 1987). For example methods that successfully recover a compound from one medium may not adequately recover the same chemical from similar media (Albro 1979). Also, extraction recoveries from a given set of structurally similar media may vary (Albro 1979).

Where possible it is recommend that the existing and established analytical methods described in Test Methods for Evaluating Solid Waste (USEPA SW-846 3rd Edition November 1986) be used.

The recommended SW-846 methodology for selected analytes are:

Gas Phase Volatiles

Method 0010	Modified Method 5 Sampling Train
Method 0020	Source Assessment Sampling System (SSAS)
Method 0030	Volatile Organic Sampling Train (VOST)
Method 5040	Protocol for Analysis of Sorbent Cartridges from Volatile Organic Sampling Train.

Soil Phase Volatiles

Method 5030	Purge and Trap
Method 8010	Halogenated Volatile Organics
Method 8015	Non-Halogenated Volatile Organics
Method 8020	Aromatic Volatile Organics
Method 8030	Acrolein, Acrylonitrile, Acetonitrile

Selected Non-Volatiles

Method 8040	Phenols
Method 8060	Phythalate Esters
Method 8080	Organic Pesticides and PCB's
Method 8090	Nitroaromatics
Method 8100	Polynuclear Aromatic Hydrocarbons
Method 8120	Chlorinated Hydrocarbons
Method 8140	Organophosphorous Pesticides
Method 8150	Chlorinated Herbicides

Recommended extraction/concentration techniques (soils and sediments) are:

Method 3540	Soxhlet Extraction
Method 3550	Sonication Extraction

Other published methods for Soxhlet extraction (Anderson et al. 1985, Bossert et al. 1984, Coover et al. 1987, Eicemen et al. 1986, Kjolholt 1985, Grimalt et al. 1986), sonication extraction (de Leevw et al. 1986, Sims 1982) and homogenization and extraction (Coover et al. 1987, Fowlie and Bulman 1986, Lopez-Avila et al. 1983, Sims 1982, Stott and Tabatabai 1983, and U.S. EPA 1982a, and extraction of materials from treatability studies (Brunner et al. 1985, Russell and McDuffie 1983) are available for reference and special applications.

Soil spiking and recovery studies should be conducted to determine the effects of soil, test substance(s), and soil test substance(s) matrix on chemical extraction and recovery efficiency. Soil samples should be sterilized using a method such as mercuric chloride, causing minimal change in soil physical and chemical properties (Fowlie and Bulman 1986). The sterile soil should be spiked with the test substance(s) to achieve a range of initial oil concentrations (Coover et al. 1987). The range of concentration should include the highest concentration and less than one-half of the lowest initial concentration to be used in degradation evaluations. Extractions of the soil/test-substance(s) mixtures using the selected procedure will allow the evaluation of the effect of test substance(s) soil concentrations on recovery efficiency. The effect of soil concentration was evaluated and found to be significant for anthracene and benzo[a]pyrene by Fowlie and Bulman (1986).

Extracts of the soil and complex wastes should be spiked with test substance(s) of interest to evaluate the effect of these matrices on chemical identification and quantification. Interferences due to the extract matrix may be identified. Extraction procedures or instrumentation used for identification and quantification may then be changed if necessary.

Standard curves should be prepared using primary standards of the test substance(s), or chemicals in the test substance, dissolved in a suitable solvent that does not interfere with chemical identification and quantification. Standard curves should be generated using at least six points ranging from the highest concentration anticipated to the detection limit for the chemical.

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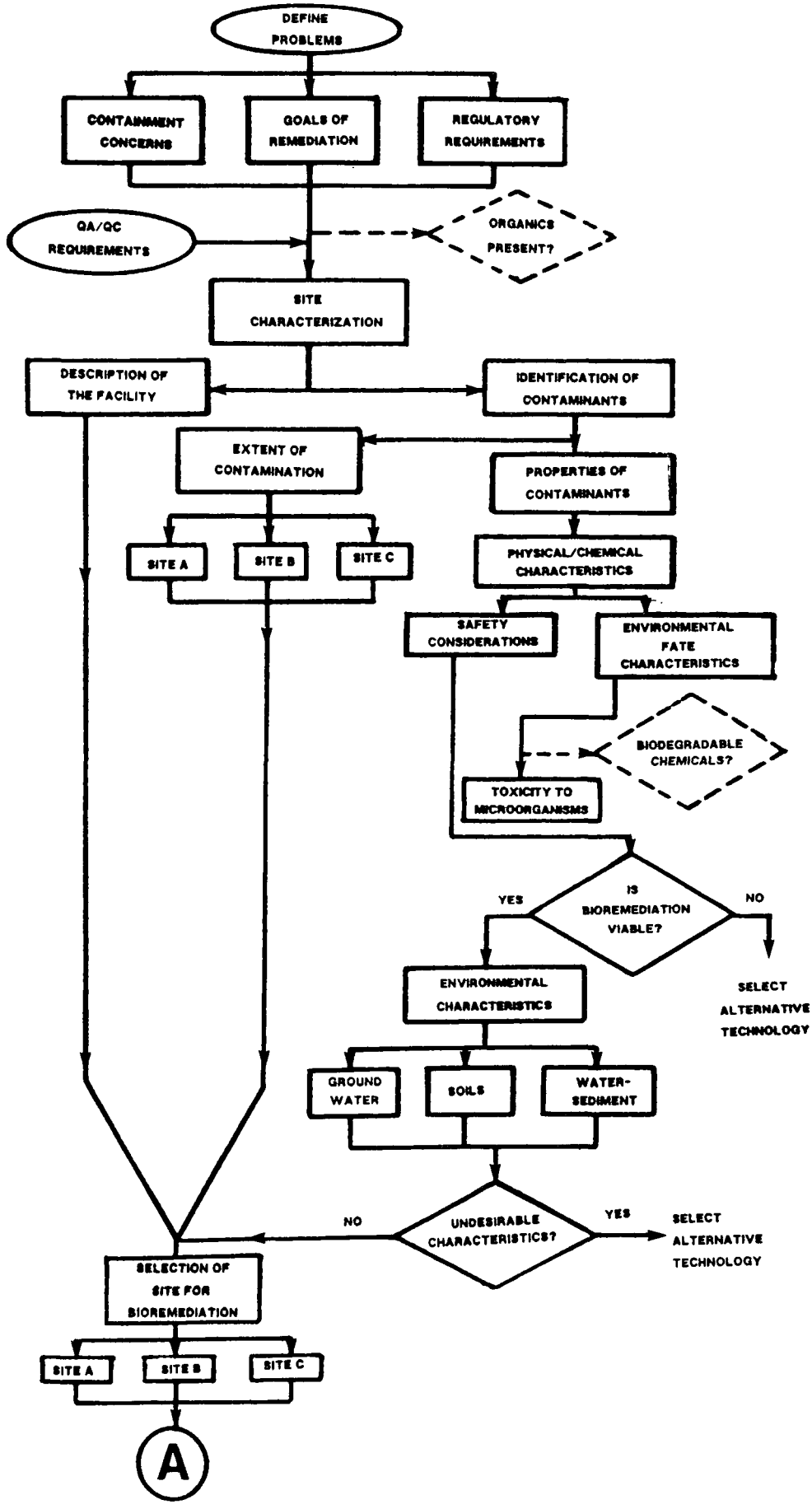
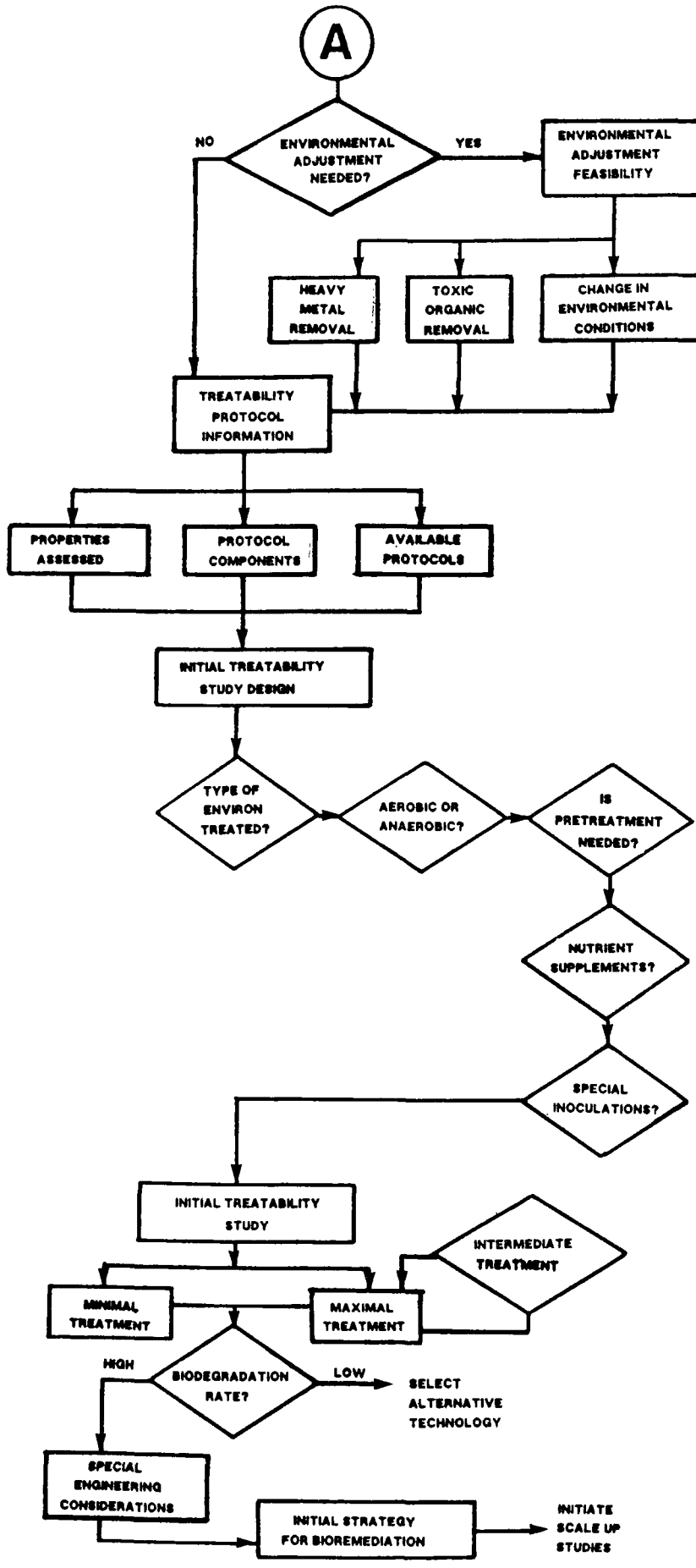
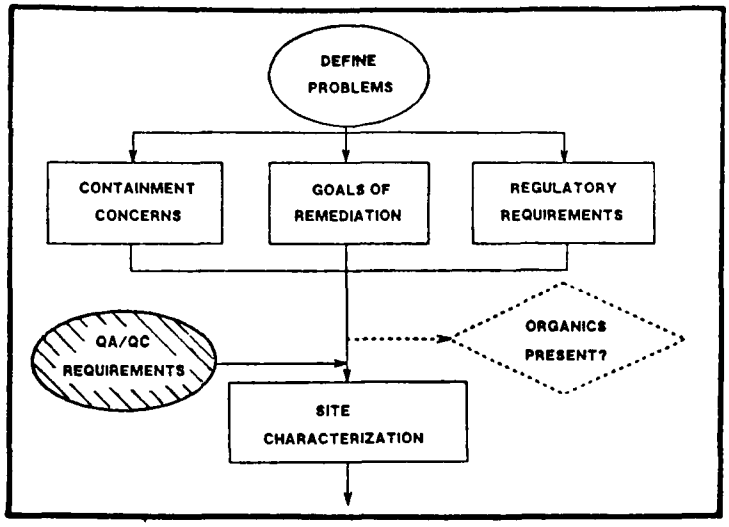

DEFINE PROBLEMS
CONTAINMENT CONCERNS
GOALS OF REMEDIATION
REGULATORY REQUIREMENTS
QA/QC REQUIREMENTS
ORGANICS PRESENT?
SITE CHARACTERIZATION
DESCRIPTION OF THE FACILITY
IDENTIFICATION OF CONTAMINANTS
EXTENT OF CONTAMINATION
PROPERTIES OF CONTAMINANTS
SITE A
SITE B
SITE C
PHYSICAL/CHEMICAL CHARACTERISTICS
SAFETY CONSIDERATIONS
ENVIRONMENTAL FATE CHARACTERISTICS
BIODEGRADABLE CHEMICALS?
TOXICITY TO MICROORGANISMS
IS BIOREMEDIATION VIABLE?
YES
NO
SELECT ALTERNATIVE TECHNOLOGY
ENVIRONMENTAL CHARACTERISTICS
GROUND WATER
SOILS
WATER-SEDIMENT
UNDESIRABLE CHARACTERISTICS?
NO
YES
SELECT ALTERNATIVE TECHNOLOGY
SELECTION OF SITE FOR BIOREMEDIATION
SITE A
SITE B
SITE C
A


A
ENVIRONMENTAL ADJUSTMENT NEEDED?
NO
YES
ENVIRONMENTAL ADJUSTMENT FEASIBILITY
HEAVY METAL REMOVAL
TOXIC ORGANIC REMOVAL
CHANGE IN ENVIRONMENTAL CONDITIONS
TREATABILITY PROTOCOL INFORMATION
PROPERTIES ASSESSED
PROTOCOL COMPONENTS
AVAILABLE PROTOCOLS
INITIAL TREATABILITY STUDY DESIGN
TYPE OF ENVIRON TREATED?
AEROBIC OR ANAEROBIC?
IS PRETREATMENT NEEDED?
NUTRIENT SUPPLEMENTS?
SPECIAL INOCULATIONS?
INITIAL TREATABILITY STUDY
INTERMEDIATE TREATMENT
MINIMAL TREATMENT
MAXIMAL TREATMENT
HIGH
BIODEGRADATION RATE?
LOW
SELECT ALTERNATIVE TECHNOLOGY
SPECIAL ENGINEERING CONSIDERATIONS
INITIAL STRATEGY FOR BIOREMEDIATION
INITIATE SCALE UP STUDIES



NOTES

NOTES

MINIMUM REQUIREMENTS FOR QA/QC

- Project description
- Project organization
- QA objectives
- Sample custody
- Internal QC checks
- Performance and system audits
- Preventative maintenance schedule

NOTES

MINIMUM REQUIREMENTS FOR QA/QC
(Continued)

- Data assessment procedures
- Corrective actions
- QA reports
- Sampling plan

NOTES

DOCUMENTATION REQUIREMENTS FOR QA

- Accepted sampling techniques
- Field actions contrary to QAPP
- All pre-field activities
- QC for field measurement data
- Field activities
- Post-field activities
- Quality control samples (generation & use)

NOTES

QA FOR ANALYTICAL PROCEDURES

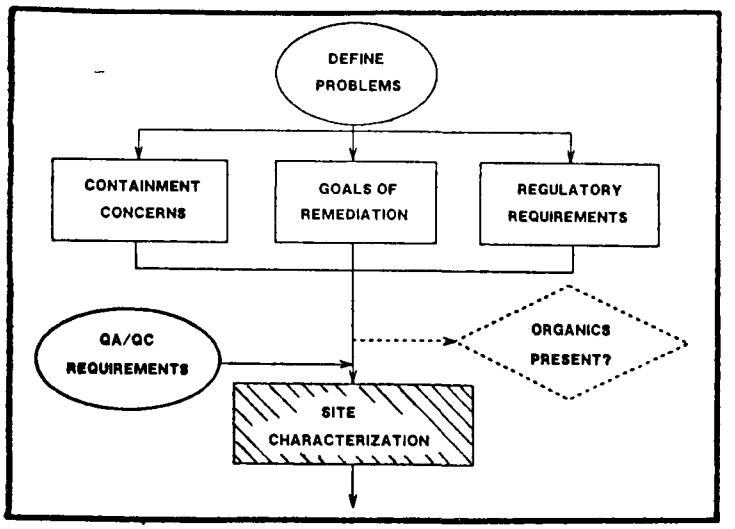
- Duplicate spike
- Reagent blank
- Documentation of fill samples
- Analytical procedures for surrogate compounds
- Recovery efficiency for columns
- Detection limits and data reduction

NOTES

QA FOR ANALYTICAL PROCEDURES (Continued)

- Internal QC checks
- Performance and system audits
- Equipment calibration
- Extraction and sample preparation procedures

NOTES



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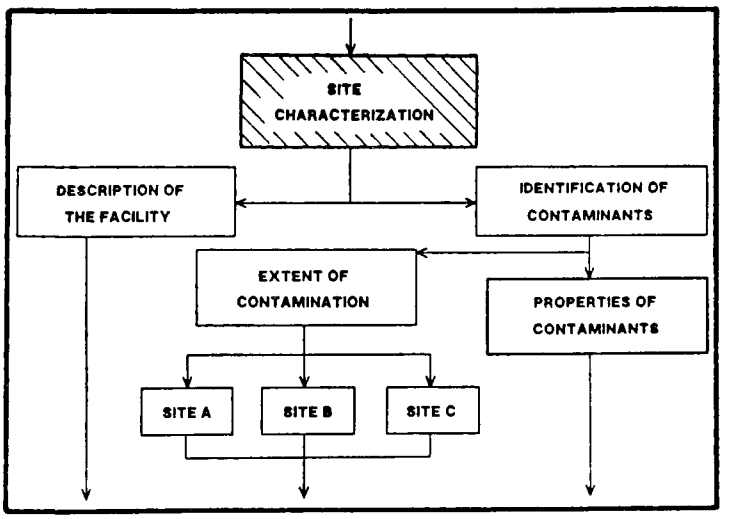


NOTES

SITE CHARACTERIZATION

- Description of facility
- Identification of contaminants
- Extent of contamination

NOTES

DESCRIPTION OF FACILITY

- Geographic location; property lines, topography and surface drainage
- Infrastructure present
- Description of hazardous waste treatment, storage, disposal and spill areas
- Surrounding land uses
- Production and groundwater monitoring wells

NOTES

IDENTIFICATION OF CONTAMINANTS

- Organic/inorganic
- Chemical classes (metals, halogenated volatiles, pesticides)
- Mixtures

NOTES

INITIAL MATERIAL CHARACTERIZATION

- Organics: GC or GC/MS, HPLC
- Group analysis: priority pollutants, fuels analysis, EP-Toxicity
- Metals: AA, ICP
- General chemistry: TOC, COD, BOD, TPH, Oil & Grease (IR or GC), TKN, NO_3, TP, PO_4, SO_4
- Optional radioisotope analysis: isotopically labeled substrate studies, $^{14}CO_2$

GENERAL CHEMISTRY

Analysis	Price Per Sample
Total Organic Carbon (TOC)	40
Total Kjeldahl Nitrogen (TKN)	50
Chromium VI	25
Cyanides	50
Phenols	50
Orthophoshates	20
Total Phosphorous	35
Nitrate	20
Sulfide	25
Oil and Grease	40
Total Suspended Solids (TSS)	15
Chemical Oxygen Demand (COD)	35
Ion Chromography (Bromide, Chloride, Fluoride, Nitrate, Nitrite, Phosphate, Sulfate)	65
Microtox	Price on Request
Radio Isotope Analysis (Liquid Scintillation)	Price on Request

NOTES

NOTES

ORGANICS

Analysis	Price For Sample Water	Price For Sample Solids	Method Water	Number Solids
GC/MS				
Volatile Organic Analysis	240	280	624	8240
Acid/Base Neutrals	420	475	625	8270
Confirmation by GC/MS	100	150		
GC				
Pesticides/PCBs	150	200	608	8080
PCBs in Oil	50			
Herbicides	200	250		8150
Phenols	100	100	604	8040
Pentachlorophenol (PCP)	90	90	604	
Polynuclear Aromatic Hydrocarbons (PNA)	115	130	610	8100
Hydrocarbon Fuels (gasoline/diesel)	110	130		
Creosote	90	90		

GROUP ANALYSES

Analysis	Price Per Sample Water	Price Per Sample Solids
Priority Pollutants	1195	1295
Acid/Base Neutrals (37)		
Volatile Organic Analysis (31)		
Pesticides & PCBs (28)		
Metals (13)		
Cyanides		
Phenols	450	450
EP-Toxicity		
Sample Prep and Extraction		
Metals		
(Ag, As, Ba, Cd, Hg, Pb, Se)		
Herbicides and Pesticides		
(2,4-D, 2,4,5-TP, Endrin, Lindane, Methoxy Chlor, Toxaphane)		
Fuels Analysis		
BTX (Benzene, Toluene, Xylene)	90	100
EDS (Ethyl Dibromide)	100	120
Tetraethyl Lead (total)	35	35
Characterization of Fuels by GC (Gasoline and Diesel)	110	130

NOTES

METALS

	Price Per Element
Method of Analysis	
Graphite Furnance	20
AAS	13
Hydride	30
Cold Vapor	30
	Price Per Sample
ICP Multi Element Analysis (Ag, Al, B, Ba, Be, Ca, Cd Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Se, Si, Sn Tl, V, Zn)	
1-12 Elements	90
13-24 Elements	115
Sample Preparation	**Price Per Sample**
Water	14
Soil/Water/Sludge	20
EP-Tox Extraction	95

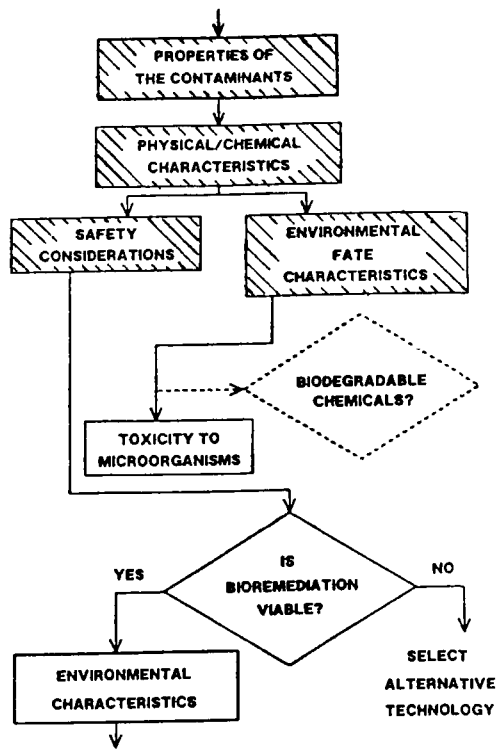
	Price Per Sample	
	Water	Solids
Group Metal Analysis		
Priority Pollutant Metals (Ag, As, Ba, Cd, Cr, Co, Hg Ni, Pb, Sb, Se, Tl, Zn)	160	199
RCRA Metals Analysis (Ag, As, Bs, Cd, Cr, Hg, Fe, Se)	130	130
Hazardous Substance Listed Metals (Non CLP) (Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Sb, Se, Tl, V, Zn	200	215

REPRESENTATIVE FIELD SAMPLES REQUIRED FOR BIOTREATABILITY STUDIES

- Evaluation of many samples to obtain a bioactivity site matrix
- Field composite to define any site bioactivity
- Field background samples essential for material characterization

EXTENT OF CONTAMINATION

- Groundwater
  - Plume size and movement
  - Contaminant concentration profiles
- Soil contamination
  - Distribution and concentration
- Surface water contamination
  - Horizontal and vertical distribution
- Sediment contamination
  - Horizontal and vertical distribution



NOTES

PROPERTIES OF CONTAMINANTS

Physical/Chemical Characteristics

- Solid, liquid or gas
- Powder, oily sludge
- Acid, base, valence or oxidation state
- Molecular weight
- Density
- Boiling point

NOTES

PROPERTIES OF CONTAMINANTS

Physical/Chemical Characteristics (Continued)

- Viscosity
- Solubility in water
- Cohesiveness
- Vapor pressure
- Flash point

NOTES

PROPERTIES OF CONTAMINANTS

Safety Considerations

- Toxicity (human, microorganisms)
- Flammability
- Reactivity
- Corrosiveness
- Oxidizing or reducing characteristics

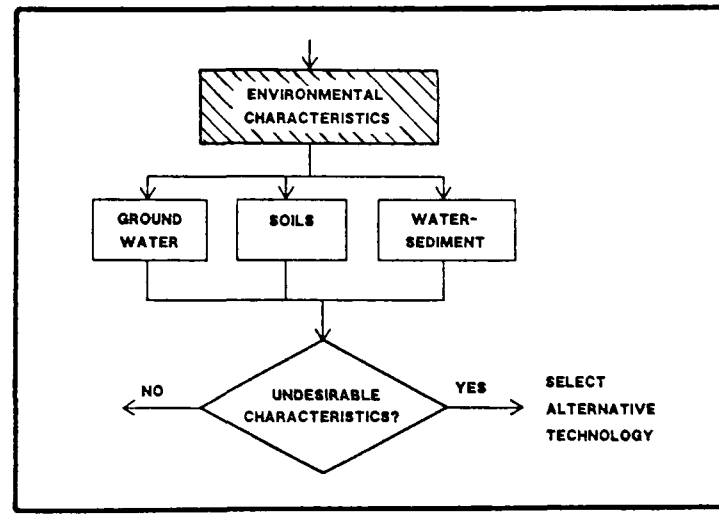
PROPERTIES OF CONTAMINANTS

Environmental Fate Characteristics

- Sorption
- Biodegradability
- Photodegradability
- Hydrolysis
- Chemical transformation

NOTES

NOTES



NOTES

ENVIRONMENTAL CHARACTERISTICS OF THE SITE

Groundwater

- Flow characteristics
- Hydrogeological units
- Water level and movement
- Man-made influences

NOTES

ENVIRONMENTAL CHARACTERISTICS OF THE SITE
Surface Water And Sediments

- Physical characteristics (location, velocity, depth, surface area, etc.)
- Seasonal fluctuations
- Temperature stratification
- Flooding tendencies
- Drainage patterns
- Evapotranspiration
- End use of water

NOTES

ENVIRONMENTAL CHARACTERISTICS OF THE SITE
Water/Sediment Chemistry

- pH
- Total dissolved solids
- Biological oxygen demand
- Alkalinity
- Conductivity

NOTES

ENVIRONMENTAL CHARACTERISTICS OF THE SITE
Water/Sediment Chemistry
(Continued)

- Dissolved oxygen profiles
- Nutrients NH_3, NO_3^-/NO_2^-, PO_4^{-3}
- Chemical oxygen demand
- Total organic carbon

NOTES

ENVIRONMENTAL CHARACTERISTICS OF THE SITE

Distribution And Soil Structure

- SCS soil classification
- Surface soil distribution
- Soil profile ASTM classification
- Depth to water table

NOTES

ENVIRONMENTAL CHARACTERISTICS OF THE SITE

Physical Characteristics Of Soils

- Hydraulic conductivity
- Relative permeability
- Bulk density
- Porosity
- Particle size distribution
- Moisture content
- Infiltration
- Vertical flow

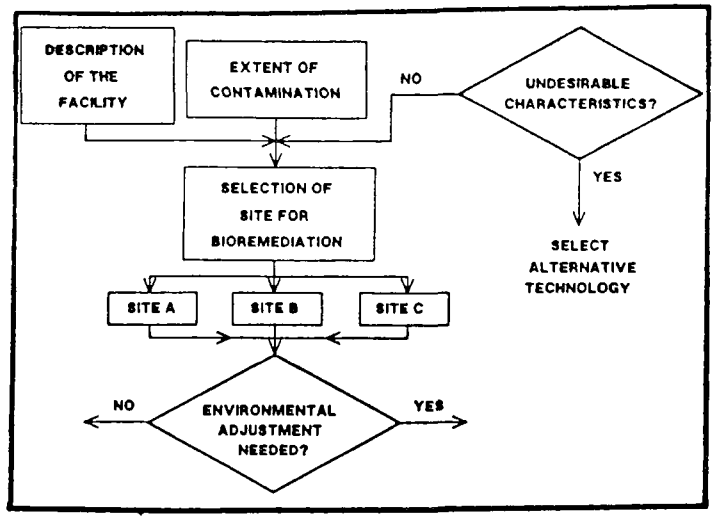
NOTES

ENVIRONMENTAL CHARACTERISTICS OF THE SITE

Chemical Characteristics Of Soils

- Soil stratigraphy
- Soil sorptive capacity
- Ion exchange capacity
- Soil organic content
- Soil pH
- Mineral content

NOTES



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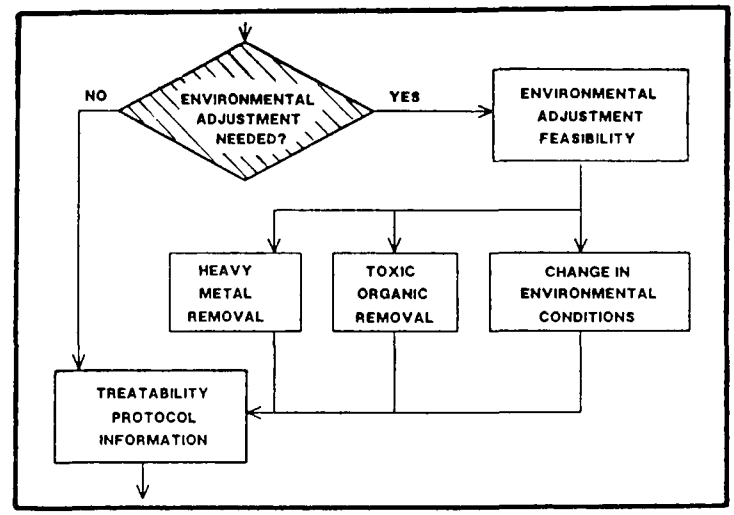

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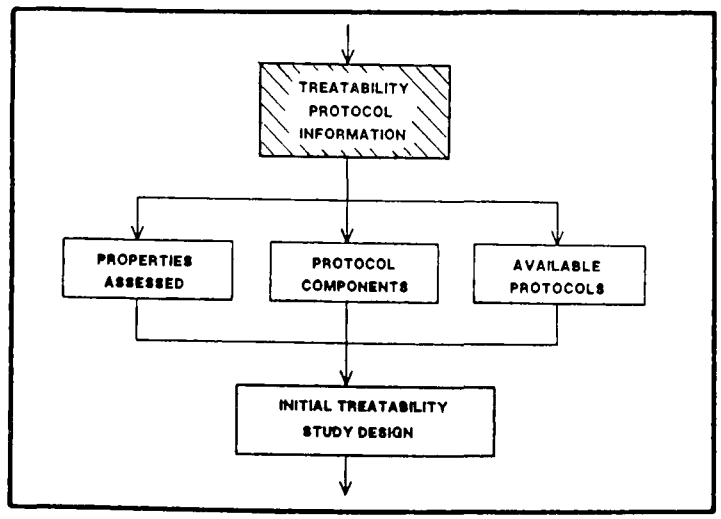


TREATABILITY PROTOCOLS

Properties Assessed

- Biodegradability of contaminants
  - aerobic
  - anaerobic
- Effectiveness of nutrient amendments
  - inorganic supplements (N, P, S)
  - electron acceptors
  - organic supplements

NOTES

NOTES

TREATABILITY PROTOCOLS

Properties Assessed
(Continued)

- Effectiveness of inocula
  - cultures of natural organisms
  - specific degraders
- Nondegradative losses
  - volatilization
  - sorption
  - leaching
- Genotoxicity of the waste

NOTES

PROTOCOL COMPONENTS

- Scope and approach
- Summary and method
- Collection and sampling of site materials
  - sample selection
  - sample collection
  - sample characterization
  - sample transportation
  - sample preservation
  - sample holding times

PROTOCOL COMPONENTS
(Continued)

- Apparatus and materials
  - reactor components
  - reactor design
- Procedures
  - reactor setup
  - reactor operation
  - analysis of reactor contents
  - reactor configurations
    - minimal treatment
    - intermediate treatments
    - complete treatment

NOTES

NOTES

PROTOCOL COMPONENTS
(Continued)

- Data recording and analysis
  - data to be reported
  - determination of degradation rates
- References
  - general
  - chemical analysis
  - sampling

NOTES

AVAILABLE TREATABILITY PROTOCOLS

NOTES

PROTOCOLS

SOILS
Aerobic

- Interim protocol for determining the aerobic degradation potential of hazardous organics in soil, September 1988, Biosystems Technology Development Program, U. S. EPA
- Uses four reactor configurations
  - no tillage
  - periodic tillage
  - forced aeration
  - soil slurry

NOTES

PROTOCOLS

SOILS
Aerobic
(Continued)

- Measures loss of target chemicals
- Corrects for volatile losses
- Requires psuedo-mass balance

NOTES

PROTOCOLS

SOILS
Anaerobic

- Pesticide assessment guidelines subdivision N chemistry: Environmental Fate, October 1982, Office of Pesticides and Toxic Substances, U.S. EPA, Washington, D.C. 20460
- Uses waterlogged soils (30 days)
- One reactor design
- Measures loss of product
- Strict anaerobic conditions optional

NOTES

PROTOCOLS

SUBSURFACE
Aerobic

- Not available

NOTES

PROTOCOLS

SUBSURFACE
Anaerobic

- 795.54 Anaerobic microbiological transformation rate data for chemicals in the subsurface environment, June 1988, Federal Register, Vol. 53, no. 115, 22320-22323
- Methanogenic
- Sulfate reducing
- Serum bottles for reaction vessels
- Requires strict anaerobic techniques

NOTES

PROTOCOLS

SUBSURFACE
Anaerobic
(Continued)

- Designed for subsurface materials
- Uses 20% (w/v) slurries
- Could be modified for denitrifying conditions
- Measures loss of hazardous compound

PROTOCOLS

SEDIMENTS

Aerobic

- Under development

NOTES

NOTES

PROTOCOLS

SEDIMENTS

Anaerobic

- 795.54 Anaerobic microbiological transformation rate data for chemicals in the subsurface environment, June 1988, Federal Register, Vol. 53, no. 115, 22320-22323
- Methanogenic
- Sulfate reducing
- Serum bottles for reaction vessels
- Requires strict anaerobic techniques

NOTES

PROTOCOLS

SEDIMENTS

Anaerobic (Continued)

- Designed for subsurface materials
- Uses 20% (w/v) slurries
- Could be modified for denitrifying conditions
- Measures loss of target chemicals

PROTOCOLS

WATER
Aerobic

- Under development

NOTES

NOTES

PROTOCOLS

WATER
Anaerobic

- Shelton, D.R. and J.M. Tiedje. 1984. General method for determining anaerobic biodegradation potential. Appl. Environ. Microbiol. 47: 850-857
- Methanogenic
- Serum bottles for reaction vessels
- Requires strict anaerobic techniques

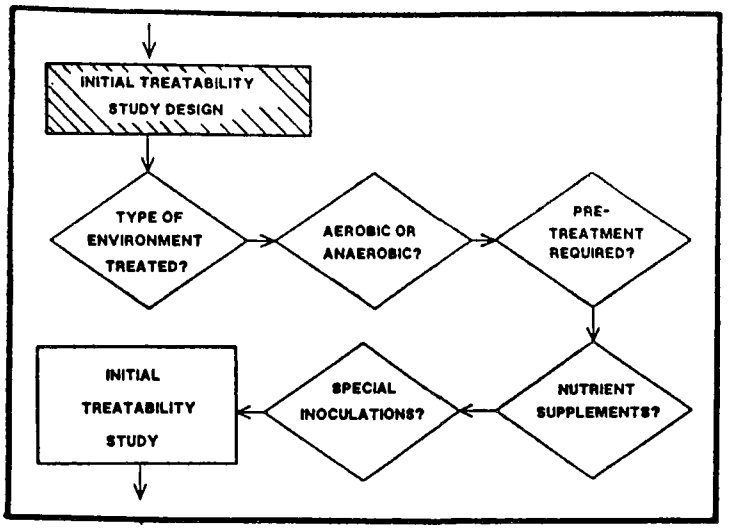
NOTES

PROTOCOLS

WATER
Anaerobic
(Continued)

- Measures gas production
- Sludge dependent
- Could be modified to include loss of hazardous chemical

NOTES



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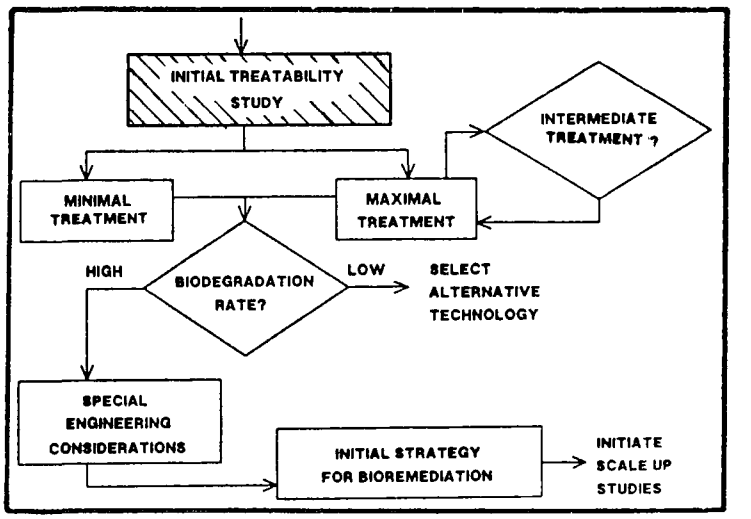

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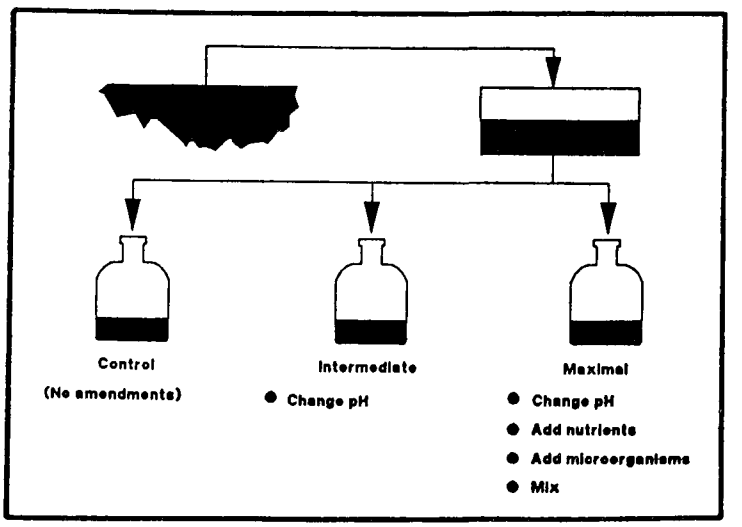

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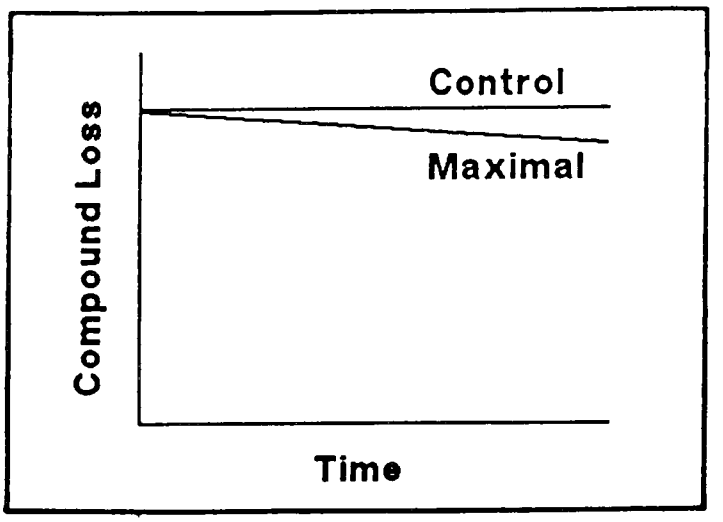

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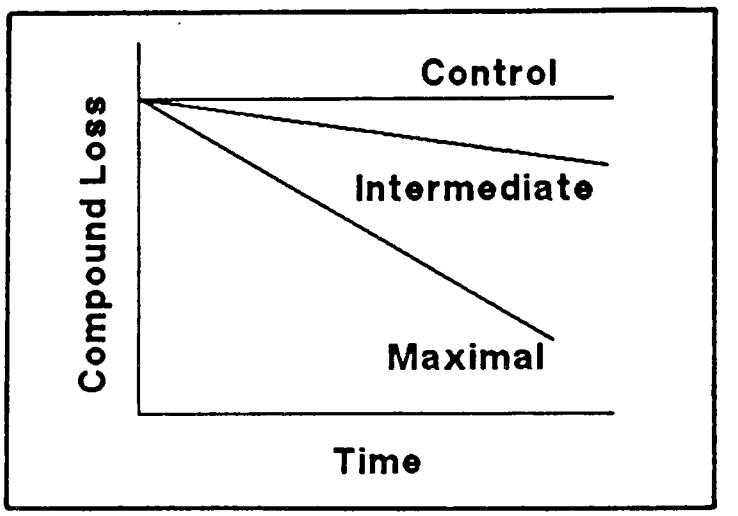


NOTES

EXPERIMENTAL DESIGN

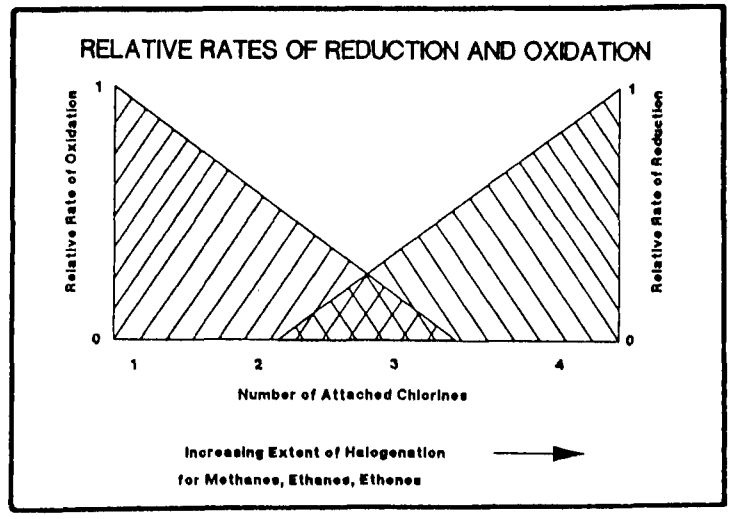
- Controls: sterile, no treatment, field background, number?
- Replicates: duplicate or triplicate? all time points? all controls?
- Treatments: what are the questions you want answered?
- How are you going to optimize the degradation process?

NOTES

EXPERIMENTAL DESIGN
(Continued)

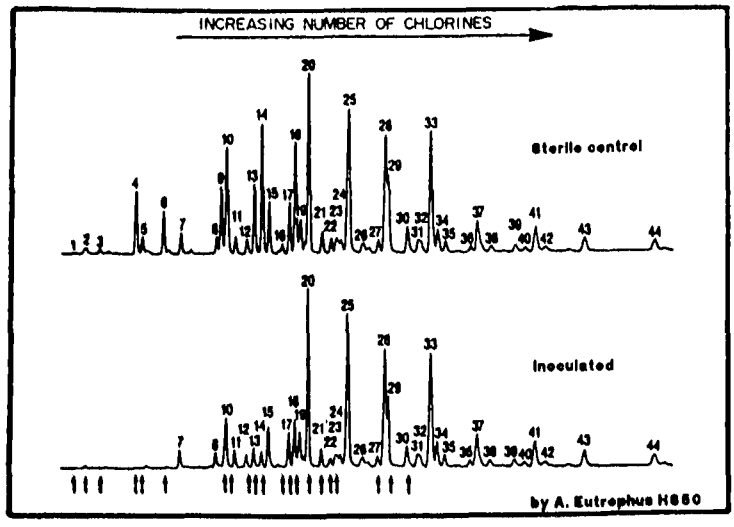
- Treatment time: how long should the study be performed?
- Types of analysis: bulk measurements? waste specific?
- Data reduction: raw data? massaged data? QC/QA?
- Cost considerations: how will it limit scope of test?

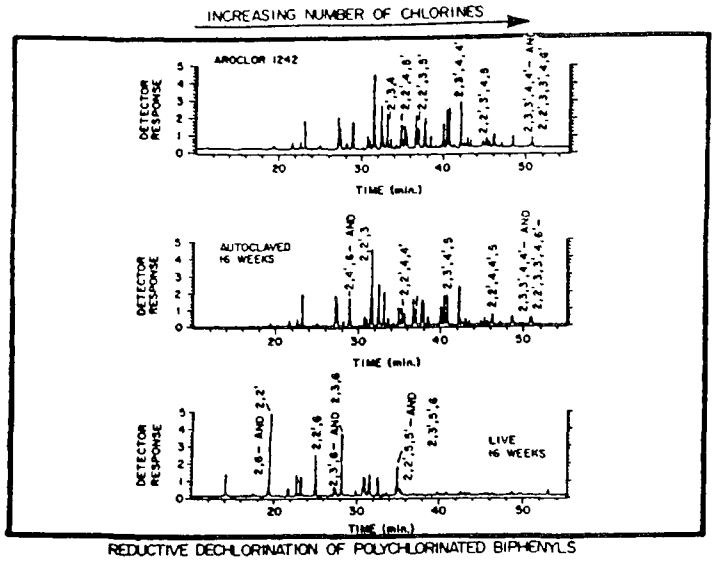
NOTES



AEROBIC BIODEGRADATION OF AROCLOR 1254

NOTES

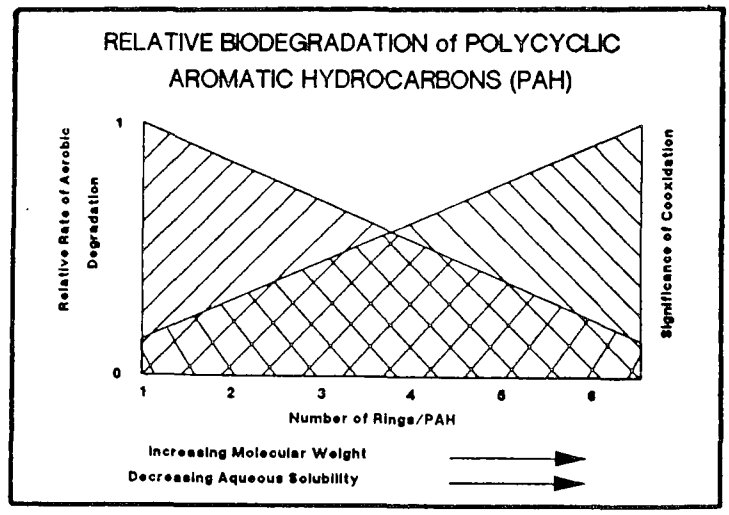




REDUCTIVE DECHLORINATION OF POLYCHLORINATED BIPHENYLS BY ANAEROBIC MICROORGANISMS FROM SEDIMENTS

NOTES

NOTES



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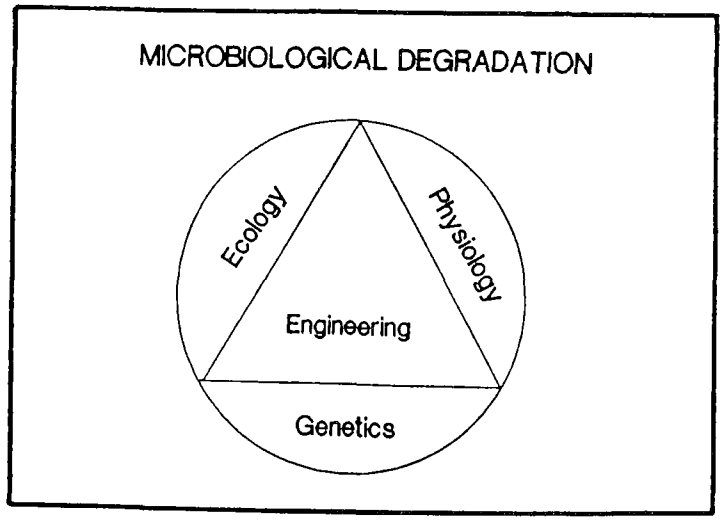


NOTES

FATE OF POLYNUCLEAR AROMATIC CONTAMINATES IN CREOSOTE WASTE DURING LAND TREATMENT
4 Month Study

PNA Class	% Reduction	Half-Life
2 Ring Structure (Naphthalene)	90	33 Days
3 Ring Structure (Phenaphthalene)	80	47 Days
4 Ring Structure (Pyrene)	25	235 Days
Total PNA	65	100 Days

NOTES

PHYSIOLOGICAL BARRIERS TO BIODEGRADATION

A contaminate will be a poor substrate if:

No active microorganism is present, therefore, no available enzymatic machinery

Microorganisms present, but...

- Substrate is a poor inducer
- Substrate concentration is too low
- Substrate fails to enter cells
- Cell lacks essential nutrients
- Inhibition/toxicity of enzymes by substrate or products
- Other necessary microbes are absent

NOTES

ENVIRONMENTAL BARRIERS TO BIODEGRADATION

Potentially Limiting Environmental Factors

- pH
- Salinity
- Other synthetic chemicals
- Heavy metals
- Osmotic pressure
- Hydrostatic pressure
- Free water limitations
- Radiation

NOTES

GENETIC BARRIERS TO BIODEGRADATION

- No genetic coding for contaminant degradation
- No genetic coding for transport into cell
- Genetics for biodegradation exist but not inducible or disbursed on genome
- Low level of expression

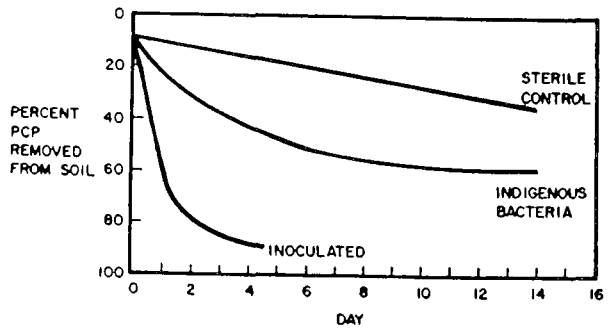
NOTES

BIODEGRADATION Requires

- Suitable electron acceptor
- Organic substrate
- Nutrients: nitrogen, phosphorous, others
- Trace metals

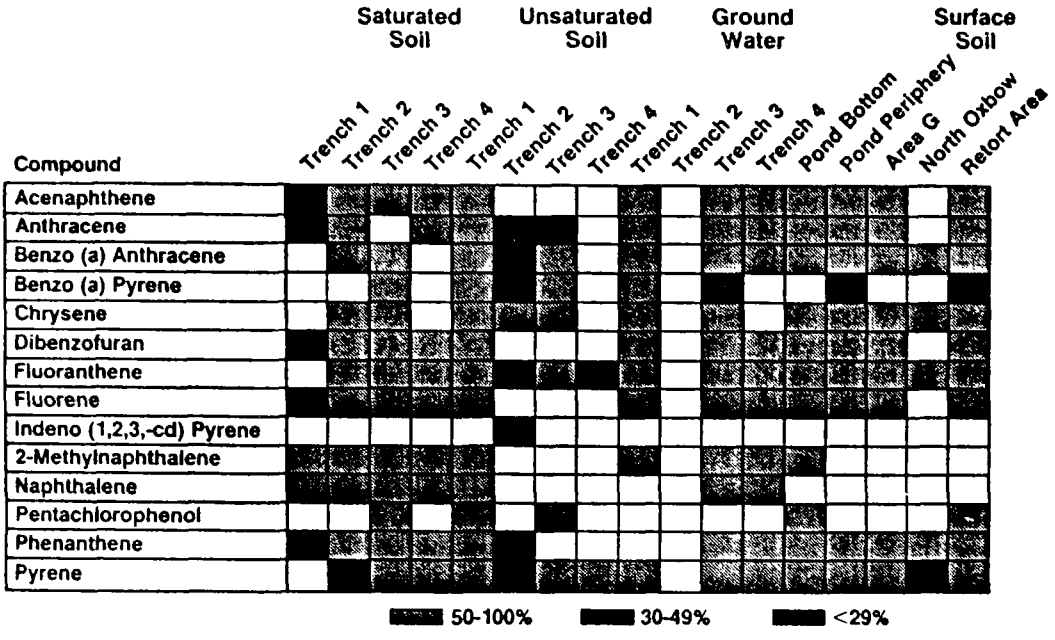
NOTES

BIODEGRADATION OF 20 ppm PCP IN SOIL UNDER LABORATORY CONDITIONS



MICROBIAL EVALUATION

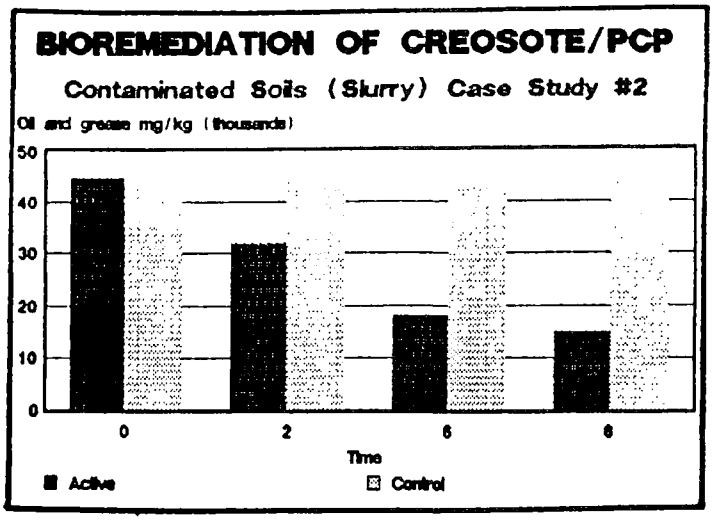
Reduction of Contaminants During a 4-Week Incubation of Nutrient Amended Site Samples



COST BREAKDOWN CASE # 1

	17	Field Samples
X	2	Replicates
	34	
X	2	Sample Times (0, 4 weeks)
	68	Samples for Analysis
X	$450	GC/MS BNA
	$30,600	Analytical Costs
+	4,000	Materials/Labor for Set up
	$34,600	Total Cost (est)*

*Note: No Administrative Charges; Data Evaluation; Report Preparation; QA/QC



NOTES

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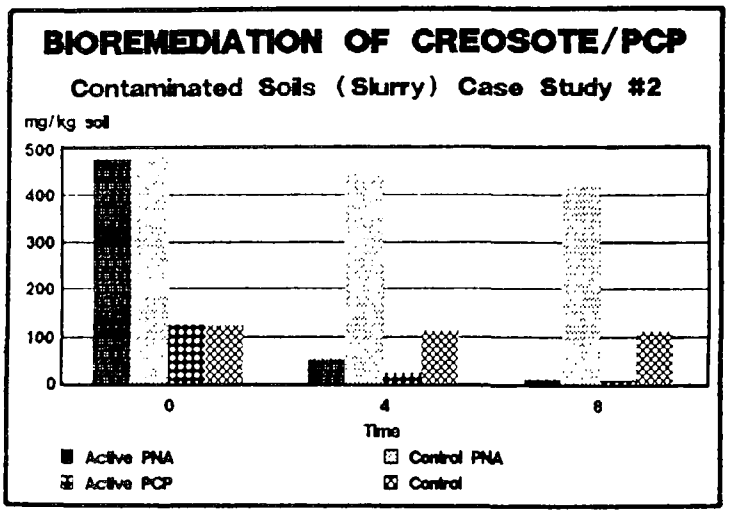


NOTES

CASE STUDY # 2

1 Single Soil Sample
3 Replicates
x2 Treatments (Active Amended/Control)
6
x4 Sample Times (0,2,6,8 wks)
24 Samples
$ 40 Oil/Grease (T.R.)
$960

6
x3 (0,4,8 wks)
18 Samples
x$450 GC/MS(BNA)
$8100

$960 + $8100 = $9060 Analytical Costs for Experimental Section Initial Material Characterization: TOC, TKN, O-PO_4, NO_3, NH_3

CASE STUDY # 2
(continued)

170	
x 2	Replicates
$340	
$9,400	Total Analytical Costs
$4,500	Labor/Materials
$13,900	Total Cost of Treatability*

* Note: No administrative charges; data evaluation, report preparation, QC/QA.

NOTES

NOTES

EFFECT OF SLURRY TREATMENT ON PAH AND PCP CONCENTRATIONS[a] IN CREOSOTE/PCP CONTAMINATED SOILS

Compound	Initial Concentration (mg/kg)	4 weeks (mg/kg)	8 weeks (mg/kg)
Acenaphthene	80 +/- 12	3.8W	3.8W
Acenaphthalene	3.4 +/- 0.1	0.8 +/- 0.1	2.1J
Dibenzofuran	17 +/- 3	3.8W	3.8W
Fluorene	37 +/- 6	3.8W	3.8W
Fluoranthene	167 +/- 38	3.9 +/- 0.8	3.6 +/- 0.3
Anthracene	30 +/- 3.5	2.2 +/- 0.6	6.7 +/- 1.2
Phenanthrene	130 +/- 17	0.5 +/- 0.1	0.7 +/- 0.1
Pyrene	177 +/- 38	26 +/- 18	10.6 +/- 1.5
Chyrsene	40 +/- 3	5.9 +/- 1.1	3.5J
Benzo(A)Anthracene	34 +/- 3	1.7 +/- 0.2	1.9 +/- 0.2
Benzo(A)Pyrene	19 +/- 1.3	9.8 +/- 1.3	10.6 +/- 2.1
Pentachlorophenol	127 +/- 12	24 +/- 2.0	31.6 +/- 5.0

a Average of triplicate analysis +/- variance.

W Undetected at the noted concentration.

J Estimated concentration. Sample data was less than the quantitation limit but greater than zero.

NOTES

PARAMETERS MONITORED DURING THE PILOT TEST OPERATION

Parameter	Range
Soil temperature	54 F to 82 F
Soil pH	7.0 to 8.9
Soil moisture content	11% to 14% by weight

TOTAL OIL AND GREASE CONCENTRATIONS IN SOIL MICROCOSMS (mg/kg)

Treatment	Sample Number	Week 0	2	4	8
CONTROL	1	510,000	410,000	510,000	530,000
	2	470,000	440,000	550,000	510,000
	3	460,000	450,000	510,000	460,000
Average		480,000	433,333	523,333	500,000
Standard Deviation		26,458	20,817	23,094	36,056
5% LOADING RATE	1	33,000	34,000	35,000	30,000
	2	33,000	26,000	28,000	32,000
	3	26,000	31,000	34,000	30,000
Average		30,667	30,333	32,333	30,667
Standard Deviation		4,041	4,041	3,786	1,155
5% LOADING RATE AND NUTRIENT-ADJUSTED	1	38,000	18,000	18,000	14,000
	2	43,000	19,000	18,000	16,000
	3	22,000	16,000	22,000	15,000
Average		34,333	17,667	19,333	15,000
Standard Deviation		10,970	1,528	2,309	1,000
5% LOADING RATE, NUTRIENT-ADJUSTED AND INOCULATED	1	22,000	26,000	37,000	18,000
	2	26,000	26,000	29,000	25,000
	3	28,000	59,000	21,000	18,000
Average		25,333	37,000	29,000	20,333
Standard Deviation		3,055	19,053	8,000	4,041
10% LOADING RATE	1	47,000	47,000	41,000	42,000
	2	66,000	87,000	43,000	31,000
	3	46,000	56,000	48,000	34,000
Average		53,000	63,333	44,000	35,667
Standard Deviation		11,269	20,984	3,606	5,686

TOTAL OIL AND GREASE CONCENTRATIONS (mg/kg) IN SOIL MICROCOSMS SIMULATING SOLID PHASE BIOREMEDIATION OF SLUDGE MATERIAL

Treatment	Time (weeks) 0	2	4	8
Control	480,000	433,333	523,333	500,000
5% Loading Rate +pH Adjust	30,667	30,333	32,333	30,667
5% Loading Rate + Nutrients + pH Adjust	34,333	17,667	19,333	15,000
5% Loading Rate + Nutrients + pH Adjust + Inoculated	25,333	37,000	29,000	20,333
10% Loading Rate + Nutrients + pH Adjust	53,000	63,333	44,000	35,667

NOTES

SUMMARY

- Clearly define the scope of work
- Look for well controlled studies
- Look for statistically valid experimental design
- Always look at the raw data and formulate your own opinion
- Beware of the limitations of standard methodologies
- Always seek expert opinion and independent evaluation

WORKSHEET FOR HAZARDOUS WASTE SITE CHARACTERIZATION

1. What information is important to the facilities description?

2. What are the most important aspects of the general site description?

3. What can the history of the ownership and operation tell us?

4. What site characteristics should be considered?

5. What chemicals are present at the site?

6. How many different contaminated areas are within the site? ____

7. Where is the contamination located?

   Site 1. _____

   Site 2. _____

   Site 3. _____

   Site 4. _____

   Site 5. _____

   Site 6. _____

   Site 7. _____

8. What is the extent (e.g. ppm) of the contamination at each site?

Site 1. _____

Site 2. _____

Site 3. _____

Site 4. _____

Site 5. _____

Site 6. _____

Site 7. _____

9. What do we need to know about the site to estimate the extent of contamination?

_____

10. What are the important hydrogeological aspects?

_____

11. Do you anticipate movement from these locations? If so, how could that impact treatment?

_____

12. What aspects of chemical identification should we be most concerned about?

_____

13. What are the important aspects of quality assurance?

14. What are the principal analytical tools used for the identification and quantification of hazardous organic chemicals and for which groups of compounds?

15. Where should you look for extraction and sample preparation procedures?

16. What do you need to know to ensure the validity of the analytical procedures?

17. Are the chemicals potentially biodegradable?

Site 1.

Site 2.

Site 3.

Site 4.

Site 5.

Site 6.

Site 7.

18. Are any of the contaminants potentially toxic to microbial degradation processes?

Site 1. _____

Site 2. _____

Site 3. _____

Site 4. _____

Site 5. _____

Site 6. _____

Site 7. _____

(Could you pretreat the waste so it could be degraded biological)

_____

19. Is the environment appropriate for biological treatment or can the environment conditions be adjusted to make it more appropriate for biological treatment?

Site 1. _____ Site 5. _____
Site 2. _____ Site 6. _____
Site 3. _____ Site 7. _____
Site 4. _____

20. Should aerobic or anaerobic biotreatment be considered?

Site 1. _____ Site 5. _____
Site 2. _____ Site 6. _____
Site 3. _____ Site 7. _____
Site 4. _____

21. How would you design a treatability study(ies) and what protocols would you use to encompass all of the contaminated areas?

_____

22. What information will be obtained from a treatability study and how will it be used?

23. What type of information should be sought before final technology selection?

EXAMPLE SITE FOR BIOREMEDIATION

HAZARDOUS WASTE SITE FOR BIOREMEDIATION

Background

The operations at a 25 acre industrial waste complex located near factories and various chemical processing plants have contributed to a seven acre hazardous waste disposal area located on site. Figure 1 represents the general layout of the industrial complex. To the north of the site a residential area has been developed. Over the past forty years, organics and inorganics generated from the on-site factory and other nearby industries have been dumped into the hazardous waste disposal site. During drought conditions, local water wells have been found to be contaminated by materials from the hazardous waste site. In response, a site investigation was completed to determine the contaminants present in each media, their approximate concentrations, and where each contaminant zone was located.

Site Description

The hazardous waste disposal area is approximately seven acres and is located in the southwest corner of the industrial complex as illustrated in Figure 1. It contains a one acre pit in which contaminated soils and sludges were deposited and a three acre pond containing miscellaneous liquid wastes. An underground storage tank containing diesel fuel, located between the pit and pond was abandoned when dredging of the pond was discontinued. An additional source of contamination identified was the tank farm area, where trucks had spilled their contents during loading and unloading operations.

The site geological setting, as determined from existing surveys of the area, is as follows. The surface soil layer at the site is a sandy soil with high permeability and a depth of 3-5 feet. The subsurface has been characterized as a silty and sandy clay that is moderately permeable and has a depth of approximately 30 feet.

Based on field investigations, a cross section of the site was developed as shown in Figure 2. The depth to groundwater from the surface averages 30 feet across the site, and the depth to bedrock is approximately 65 feet. The bedrock consists of an impermeable limestone. Table 1 lists additional information about each contaminated media.

The climate in this area is very humid and has an average temperature of 72°F and an annual precipitation of 53.4 inches. The high and low temperatures in Jaunary are 74°F and 49°F and in August are 92°F and 72°F, respectively.

Figure 1. Industrial Complex

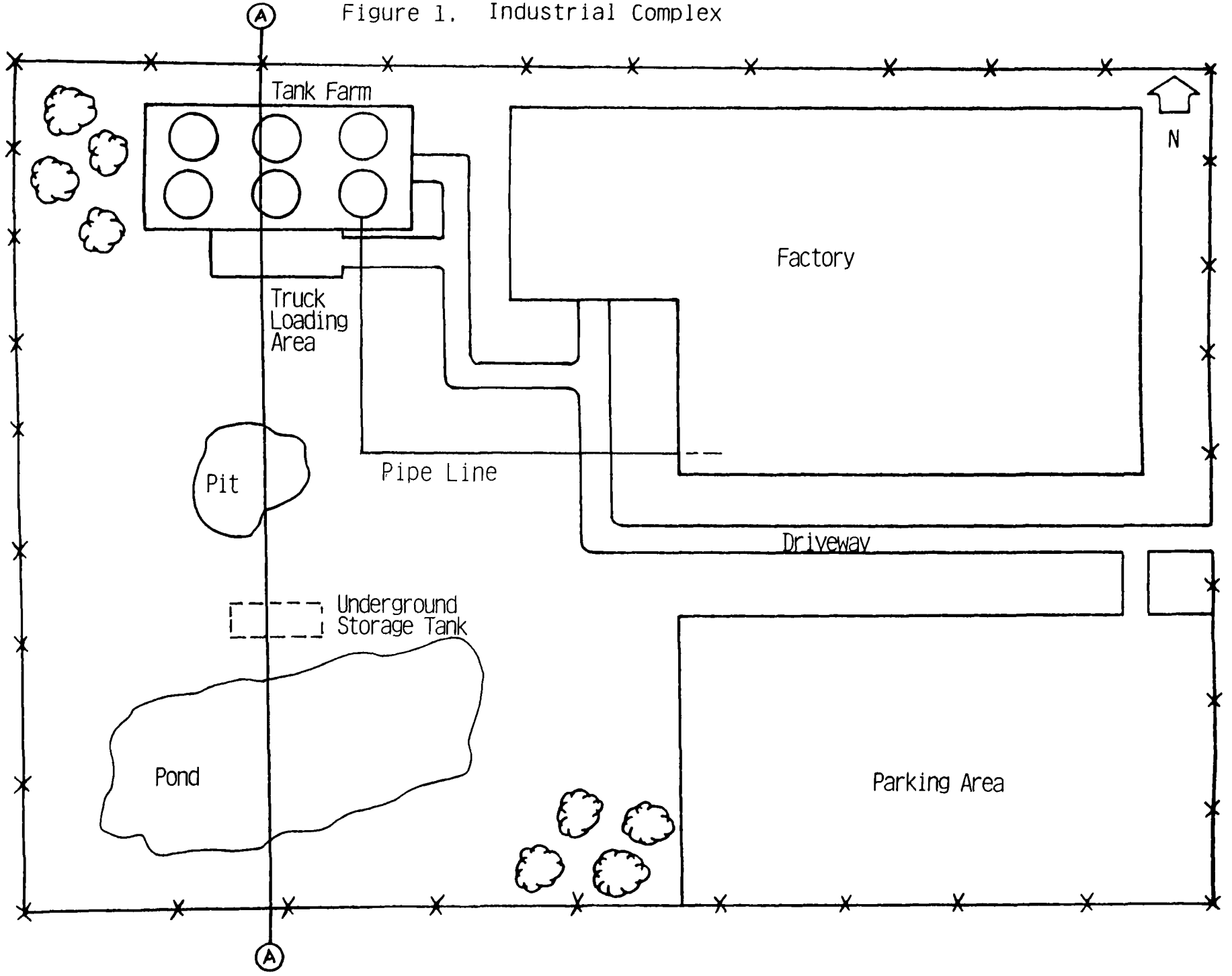


TABLE 1. ADDITIONAL SITE INFORMATION

System 1 -- Contaminated Surface Soil

Estimated Volume -- 2000 cubic yards
Estimated Size (50 ft x 200 ft x 5 ft)

System 2 -- Pit Containing Contaminated Sludges and Soils

	Surface Area	Depth	Volume
Pit Size (overall)	1 acre	5 feet	8000 cubic yards
Waste Volume	1 acre	4 feet	6400 cubic yards

System 3 -- Leaking Underground Storage Tank

Estimated Volume of Contaminated Soil Beneath the Tank -- 410 cubic yards (approximate size 45 ft x 25 ft x 10 ft)

Estimated Volume of Contaminated Groundwater -- 0.5 million gallons (approximate size 45 ft x 100 ft x 15 ft)

System 4 -- Pond

Estimated volume of contaminated water in the pond - 20 million gallons.

Estimated Volume of Contaminated Soil Beneath the Pond -- 91700 cubic yards (approximate size 660 ft x 250 ft x 15 ft)

Estimated Volume of Contaminated Groundwater -- 128 million gallons (approximate plume size 660 ft x 1300 ft x 20 ft)

System 5 -- Mixed Groundwaters - Tank and Pond

Estimated Volume -- 10,000 gallons

System 6 -- Broken Pipe Leakage

Estimated volume of contaminated soil -- 250 cubic yards

Estimated volume of contaminated groundwater -- 500,000 gallons (approximate size 125 ft x 25 ft x 20 ft)

System 7 -- Mixed Groundwater Pipe Leakage and Pond

Estimated volume of contaminated groundwater -- 75,000 gallon

Description of Contamination

During the field investigation, the hazardous waste site was found to contain a variety of organic contaminants as well as some inorganic contamination. The following is a general description of the contaminants found:

- Pit - The pit contains contaminated soils and sludges. The material is acidic and is contaminated with methyl ethyl ketone. In addition, an oil sludge was found at the bottom of the pit.

- Pond - The liquid in the pond contains water contaminated with coal tar and its by products including some cyanide.

- Underground Storage Tank - An undergound storage tank located between the pit and pond was found to be leaking diesel fuel.

- Tank Farm Area - The soil in the area of the loading dock is contaminated with pentachlorophenol, polychlorinated biphenols and trivalent chromium. A review of plant history indicated these spills resulted from loading and unloading operations prior to the construction of the concrete dock. Groundwater contaminated with trichloroethylene was identified during the field investigation. The source of this contamination was traced to a broken transfer line from the tank farm to the factory. The broken line was discovered and repairs made two years ago.

The contaminated leachate plumes from the various sources identified above are shown in Figures 2 and 3. Table 2 represents concentration levels for each contaminated system and media and other pertinent information.

Planning Site Response

The cleanup objectives for each contaminated media are also listed in Table 2. These objectives offer an end point for remediating the site when biological and other supporting technologies have been applied. These clean-up objectives are for the purposes of this workshop only.

Table 3 provides chemical and physical properties of the contaminants discovered at the industrial complex.

Figure 2. Cross-sectional View of Site

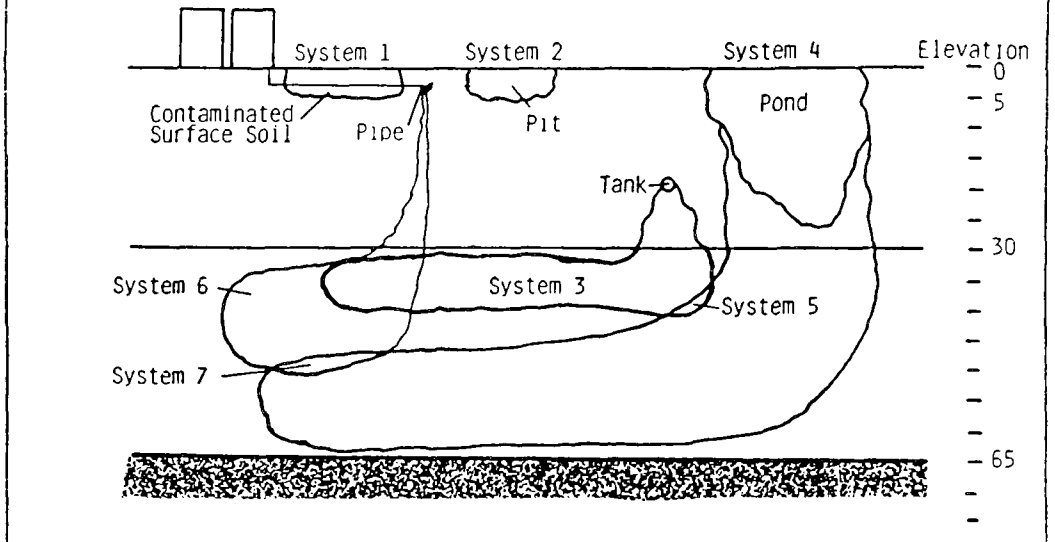


Figure 3. Industrial Complex Showing Plume Delineations

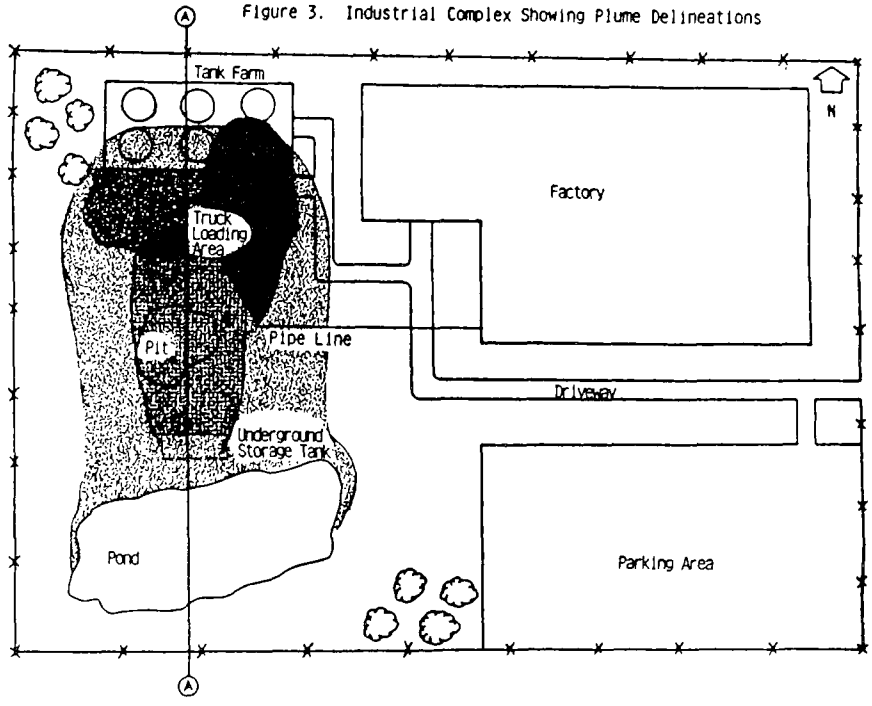


TABLE 2. CONTAMINATED SYSTEMS

Contaminant Concentrations and Clean Up Objectives

System 1 -- Contaminated Surface Soil

	Contaminant	Concentration	Clean-up Objectives
Soil	PCP	180 mg/kg	50 µg/kg
	PCB	300 mg/kg	50 mg/kg
	Cr^{+3}	900 mg/kg	170 mg/kg

System 2 -- Pit Containing Contaminated Sludges and Soils

	Contaminant	Concentration	Clean-up Objectives
Pit	MEK	400 mg/kg	1 mg/kg
	Oily sludge	900 mg/kg	45 mg/kg
	pH*	2.5	6-9
	Solids %	85	---

System 3 -- Leaking Underground Storage Tank and Related Contaminated Zones

	Contaminant	Concentration	Clean-up Objectives
Soil below tank (Soil - 3)	Diesel fuel	50 mg/kg	15 mg/kg
	Contaminant	Concentration	Clean-up Objectives
Groundwater (GW-3)	Diesel fuel	150 mg/ℓ	10 mg/ℓ
	Iron	25 mg/ℓ	NA
	pH*	6.5	6-9

System 4 -- Pond and Related Contaminated Zones

	Contaminant	Concentration	Clean-up Objectives
Pond	Cyanide	3 mg/ℓ	0.15 mg/ℓ
	Benzene	400 mg/ℓ	10 µg/ℓ
	Toluene	280 mg/ℓ	10 µg/ℓ
	Xylene	250 mg/ℓ	10 µg/ℓ
	Phenol	325 mg/ℓ	10 µg/ℓ
	Cresol	45 mg/ℓ	5 µg/ℓ
	Naphthalene	60 mg/ℓ	5 µg/ℓ
	Ammonia	39 mg/ℓ	2 mg/ℓ
	pH*	9.2	6-9
	TDS	500 mg/ℓ	---
	TSS	100 mg/ℓ	50 mg/ℓ
	TOC	298 mg/ℓ	15 mg/ℓ
	COD	950 mg/ℓ	50 mg/ℓ

TABLE 2. (continued)

	Contaminant	Concentration	Clean-up Objectives
Soil below pond (Soil-4)			
	Cyanide	1.7 mg/kg	0.09 mg/kg
	PCP	18 mg/kg	50 µg/kg
	PCB	50 mg/kg	50 µg/kg
	Benzene	250 mg/kg	10 µg/kg
	Toluene	160 mg/kg	10 µg/kg
	Xylene	110 mg/kg	10 µg/kg
	Phenol	190 mg/kg	10 µg/kg
	Ammonia	50 mg/kg	2 mg/kg
	Cr^{+3}	200 mg/kg	170 mg/kg
	Contaminant	Concentration	Clean-up Objectives
Groundwater (GW-4)			
	Cyanide	0.4 mg/ℓ	0.02 mg/ℓ
	Benzene	150 mg/ℓ	5 µg/ℓ
	Toluene	80 mg/ℓ	5 µg/ℓ
	Xylene	70 mg/ℓ	5 µg/ℓ
	Phenol	100 mg/ℓ	5 µg/ℓ
	Ammonia	80 mg/ℓ	2 mg/ℓ
	Iron	25 mg/ℓ	NA
	pH*	6.5	6-9

System 5 -- Groundwater Contaminated with Mixture of Pollutants from Tank and Pond

	Contaminant	Concentration	Clean-up Objectives
Groundwater (GW-5)			
	Diesel fuel	150 mg/ℓ	10 mg/ℓ
	Cyanide	0.4 mg/ℓ	0.02 mg/ℓ
	Benzene	150 mg/ℓ	5 µg/ℓ
	Toluene	80 mg/ℓ	5 µg/ℓ
	Xylene	70 mg/ℓ	5 µg/ℓ
	Phenol	100 mg/ℓ	5 µg/ℓ
	Ammonia	80 mg/ℓ	2 mg/ℓ
	Iron	25 mg/ℓ	NA
	pH*	6.5	6-9

TABLE 2. (continued)

System 6 -- Leaking Transfer Piping System

	Contaminant	Concentration	Clean-up Objectives
Soil below pipe (Soil - 6)	Trichloroethylene	2.50 mg/kg	10 µg/kg

	Contaminant	Concentration	Clean-up Objectives
Groundwater (GW-6)	Trichloroethylene	10 mg/ℓ	5 µg/ℓ
	Iron	25 mg/ℓ	NA
	pH*	6.5	6-9

System 7 -- Groundwater Contaminated With a Mixture of Pollutants From the Pipe Leakage and Pond

	Contaminant	Concentration	Clean-up Objectives
Groundwater (GW-4)	Cyanide	0.4 mg/ℓ	0.02 mg/ℓ
	Benzene	150 mg/ℓ	5 µg/ℓ
	Toluene	80 mg/ℓ	5 µg/ℓ
	Xylene	70 mg/ℓ	5 µg/ℓ
	Phenol	100 mg/ℓ	5 µg/ℓ
	Ammonia	80 mg/ℓ	2 mg/ℓ
	Trichoroethylene	10 mg/ℓ	5 µg/ℓ
	Iron	25 mg/ℓ	NA
	pH*	6.5	6-9

*standard units

TABLE 3. PROPERTIES OF CONTAMINANTS

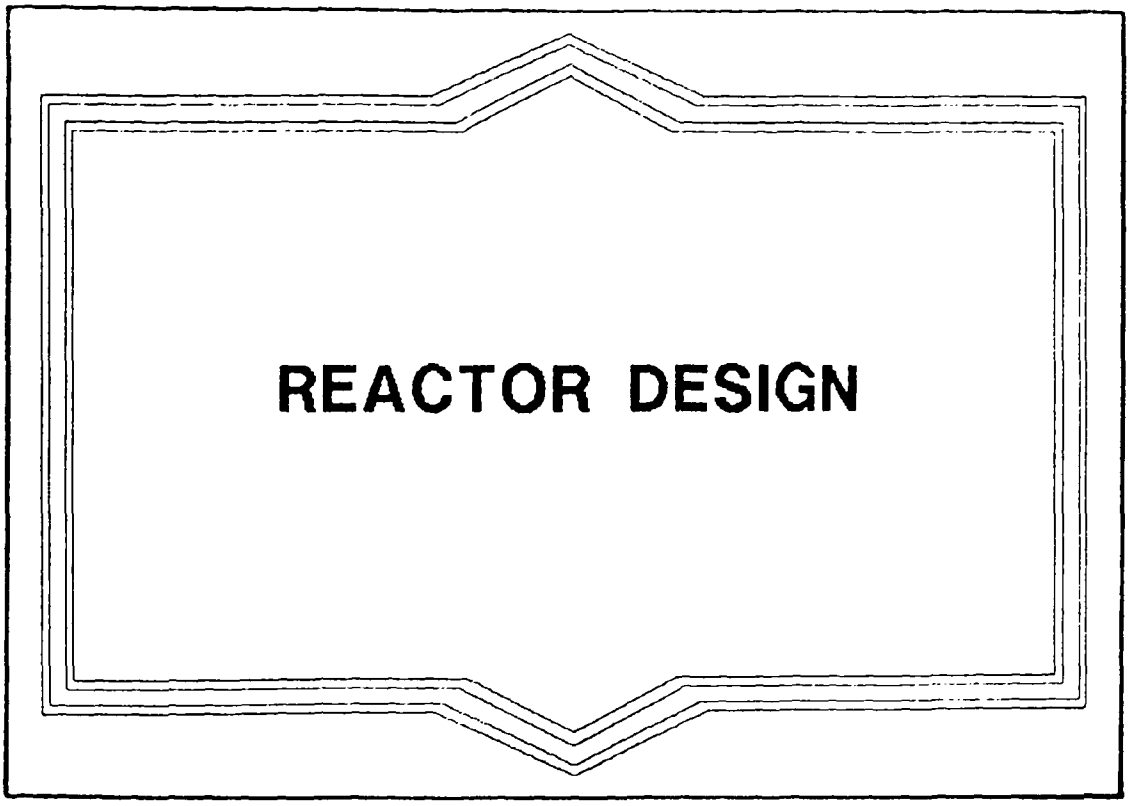
Chemical Class	Solubility in Water	Soluble in Solvents	Soil Adsorption	Henry's Constant (Volatility)	Biodegradability	Toxicity	Mobility
Halogenated Aliphatics • Trichloroethylene (TCE)	1000 mod.	alcohol, ether, acetone, chloroform	mod.	8.9×10^{3} high	R	toxic by inhalation	mod. - high in soil-water systems
Halogenated Polycyclic Aromatics • Polychlorinated biphenyls	3.1 low	alcohol, ether, acetone	high	1.7×10^{-3} high	D,R	highly toxic to ecology	low - v. low in soil-water systems, v. low-mod. in air
Monocyclic Aromatics • Benzene	low	alcohol, ether, acetone, carbon tetrachloride	high	high	D	highly toxic to ecology	mod. - high in soil-water systems
• Toluene	515 mod.	alcohol, ether, acetone, benzene	high	$6.6 \times 10^{-3+}$ high	D	toxic by ingestion and skin adsorption	mod. - high in soil-water systems
• Xylene	0.3 low	alcohol, ether, acetone, benzene	mod.-high	$6.3 \times 10^{-3+}$ high	D	toxic by ingestion and inhalation	mod. in soil-water systems, mod. - high in air
• Phenol	84,000 high	water, alcohol, ether, acetone, benzene, chloroform	low-mod.	7.0×10^{7} low	D	toxic by ingestion and inhalation and skin absorption	mod. - high in soil-water systems

(continued)

TABLE 3. PROPERTIES OF CONTAMINANTS (continued)

Chemical Class	Solubility in Water	Soluble in Solvents	Soil Adsorption	Henry's Constant (Volatility)	Biodegradability	Toxicity	Mobility
• Cresol	high	alcohol, glycol, water	low - mod.	low	D	toxic by skin absorption	mod. - high in soil-water systems
• Pentachlorophenol (PCP)	14 low	alcohol, ether, benzene	high	2.8×10^{-6} low - mod.	R,D	highly toxic to ecology	high - v. high in soil-water systems
Polycyclic Aromatics							
• Naphthalene	31.7 low	alcohol, ether, acetone, benzene	high	$4.8 \times 10^{-4+}$ mod. - low	D	toxic by inhalation	v. low - low in soil-water systems v. low - mod. in air
Alkylated Aliphatics							
• Methyl ethyl ketone	353,000* high	water, alcohol, benzene, ether, acetone	low	4.35×10^{-5} mod.	D	toxic by inhalation	mod. - high in soil-water systems
Metals							
• Chromium III	NA	hydrochloric acid, sulfuric acid	NA	NA	( )	low toxicity	negligible to v. low in air, v. low - v. high in soil and aqueous systems
Diesel Oil	low	benzene, toluene	mod.	low	D	environmental hazard	mod. - high in soil-water system
Ammonia	high	water, alcohol, ether	high	high	D	toxic by inhalation	high in air, mod. - high in soil-water systems

Solubility = mg/ℓ at 20°C (*at 10°C)
Henry's Constant = atm • m^3/mol at 20°C (+ at 25°C)
Biodegradability (D = degradable, R = refractory, N = non-degradable, ( ) = no information available)
NA = not applicable



SECTION 4

REACTOR TREATMENT DESIGN

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Biological processes have successfully transformed organic and inorganic materials on the earth for billions of years. Biological processes have been used extensively since the turn of the century to treat municipal and industrial wastewaters. The use of microorganisms to treat hazardous materials is a logical extention of applied microbiology. In the past few years, great progress has been made in isolating, characterizing, and modifying organisms able to metabolize materials considered to be hazardous. The successful application of these microorganisms to commercially available treatment systems falls within the engineering domain.

In many site remediation projects, it is difficult to determine if a waste stream (liquid or soil) is amenable to biological treatment, and if it is, what type of bioreactor design to use. Successful biological treatment of groundwater, leachate, or industrial process water requires the combined action of basic microbial processes and sound process engineering designs. Such a treatment system is then able to both efficiently and cost effectively remediate the contaminants present. The decision to consider and use bioremediation at hazardous waste sites, however, rests with site remediation project managers.

This presentation is designed to provide information about several subject areas critical to the success of any biological treatment project, including conceptual process design, basic bioengineering principles, a review of currently available biological unit processes, important pretreatment and postreatment factors, and case histories. While not being comprehensive in detail, the written material given below (coupled with the oral presentation) should provide class attendees with a base level of understanding of bioreactor selection and operation.

Conceptual Remediation Approach

One of the first steps to take in selecting remediation equipment is to define the treatment system needed. Specifically, this requires the project worker to identify all of the inputs and outputs to a treatment process. In all cases, the composition of the influent waste and required discharge standards for the waste stream must be considered. With a biological treatment system, consideration must also be directed to any anticipated air emissions and to proper biological and/or inorganic sludge disposal. Once the treatment parameters have been defined, attention can be given to the proper selection of remedial process designs.

"Life-Cycle Design" is a remediation approach that takes into account changes in site conditions throughout the duration of the project. Life-Cycle Design has three major facets:

- Time effect on parameters
- Capital equipment costs
- Operating expenses

The "time effect on parameters" considers that any process design must be flexible enough to overcome changes over time in the volume of materials to be treated (such as varying water flow rates), the appearance or disappearance of specific organic or inorganic contaminants, and changes in individual contaminant concentrations. A process designed only for present site conditions may become cost prohibitive or catastrophically fail at some point in the future.

Actual capital equipment costs reflect both the total dollar amount spent as well as the expected duration of equipment use. While most municipal projects are designed for 20 years or more of operation, many environmental projects will have a much shorter period of operation. Thus, the daily cost for equipment will tend to be higher for hazardous waste projects. To lower this cost, consideration should be given to using equipment that is portable and reuseable. Depending on the project, large permanent installations should be avoided if possible.

Lastly, Life-Cycle Design considers the affect of operating expenses on the remediation effort. Operating expenses consist of maintenance items, power costs, consumable supplies, and personnel costs. Personnel costs can be kept low by utilizing equipment that requires a minimal amount of operator attention or that is self operating. On many projects, personnel costs are the major operating expense, especially with complex treatment systems that require round the clock attention. High initial capital equipment costs can be quickly offset in many cases by lower annual operating expenses. The design engineer must consider operating as well as capital equipment costs when evaluating potential process equipment designs.

Bioprocess Engineering and Treatment Equipment

The design engineer must create an environment favorable for rapid microbial growth. In terms of overall treatment processes, bioreactors can be designed to handle either batch or continuous flows. Contaminants can be treated in:

- Batch mode with discontinuous flow
- Plug flow mode with continuous flow
- Partially mixed mode with continuous flow
- Completely mixed mode with continuous flow

Each of these treatment modes has advantages and disadvantages from both microbiological and operational perspectives. The microbial growth rate (and hence the specific compound removal rate) can be controlled by the design and operation of the specific bioreactor. For example, a fixed-film design may be superior to a dispersed growth design if the reactor needs to be populated with slow growing bacteria. The fixed-film design effectively separates the microbial residence time within the reactor from the hydraulic retention time of the water passing through the system.

Any bioreactor design must also ensure that proper pH, temperature, oxygen concentration, and inorganic nutrient concentrations (primarily nitrogen and phosphorus) are maintained. On a practical note, the hydraulic retention time needed for biodegradation to occur controls the size of the bioreactor. Suitable microbial populations must be maintained within the system to keep the hydraulic retention time (and hence the bioreactor size) to a minimum. Very large tanks are capital intensive and have greater operating costs due to power requirements in mixing and oxygen transfer.

Biological treatment equipment can take many forms, but all designs employ bacteria growing either dispersed in the bulk liquid or attached as films on some sort of inert support surface. Below are brief descriptions of several commercially available biological processes for water treatment:

Activated Sludge
- Suspended growth system
- Completely mixed mode
- Biomass captured in clarifier and recycled to reactor
- Contact time between waste and biomass controlled by wasting excess biomass

Aerated Lagoons
- Suspended growth system
- Completely mixed mode
- Contact time limited to hydraulic retention time
- Limited effluent quality

Extended Aeration
- Suspended growth system
- Completely mixed mode
- Biomass captured in clarifier recycled to reactor
- Long contact time created by enlarging aeration basin

Contact Stabilization
- Suspended growth system
- Completely mixed mode
- Waste quickly contacted with biomass in first aeration tank

- Contaminants adsorbed to clarified biomass are then digested in second aeration tank
- Total hydraulic residence time held to a minimum

Trickling Filter
- Fixed-film system
- Plug flow mode
- Design based on specific surface area
- Aeration provided by induced or forced draft

Rotating Biological Contactors
- Fixed-film system
- Plug flow mode
- Design based on specific surface area
- Aeration provided by rotating disks

Submerged Fixed-Film Reactors
- Fixed-film system
- Completely mixed or plug flow modes
- Design based on volume
- Aeration provided by air released below media

Powdered Activated Carbon Treatment (PACT)
- Hybrid suspended growth/fixed-film system
- Completely mixed mode
- Biomass suspended and fixed to carbon particles
- Carbon particles also adsorb organic contaminants
- Clarifier still controls bacterial residence time

Fluidized Bed
- Fixed-film system
- Completely mixed or plug flow modes
- Media fluidized in reactor

The nine treatment systems described above are designed for the aerobic biodegradation of contaminants. However, some chemicals are more readily biodegraded under anoxic (low oxygen) or strict anaerobic (no oxygen) conditions. With the proper engineering modifications, many of the above mentioned systems can be used for anoxic/anaerobic treatment of hazardous chemicals. Anaerobic digesters have been used for some time in combination with aerobic activated sludge to treat municipal waste. Combination anoxic/anaerobic treatment systems are also in use. Anaerobic fluidized beds, with and without activated carbon, have shown promise for use in the hazardous waste treatment field. While much is known from a microbiological standpoint about the anaoxic/anaerobic biodegradation of compounds, very few large scale applications of this technology exist today.

While the biological treatment of liquid wastes is a fairly well understood and straightforward process, the biological treatment of contaminated soils is more complex and difficult to put into practice. The same factors important to rapid microbial growth in above ground systems (pH, nutrients, oxygen concentration, etc.) are critical when treating soils. However, soils are typically quite heterogeneous, as opposed to the more homogeneous water matrix. It is more difficult for microorganisms (or physical/chemical reagents for that matter) to gain equal access to each and every soil particle present. In addition, soils treatment presents more difficult materials handling problems. Excavation of contaminated soils may reveal the presence of buried materials such as pipes or bricks, making it more difficult to homogenize the soils prior to treatment.

In spite of these difficulties, biological treatment of soils remains a valuable tool for the remediation specialist. In many cases, indigenous microorganisms possess the metabolic capability to metabolize the contaminants present. All that is needed is to further optimize growth conditions. In some cases, it may be necessary to inoculate the soils with microorganisms containing the desired metabolic activity. Two major forms of biological soils treatment are described below:

Contained Above Ground Soils Treatment

- Batch mode
- Contaminants treated in the heterogeneous soil matrix
- Nutrients, moisture and oxygen added as needed
- Leachate, runoff, and air emissions must be controlled
- Soil left on site when clean

Soil Slurry Reactors

- Batch or continuous flow mode
- Heterogeneous soils treated in a liquid slurry
- Nutrients and oxygen added as needed
- Water and soil must be separated after treatment
- Soil left on site when clean

Pre and Post Treatment Considerations

There are several factors that must be evaluated prior to and after using biological treatment. Pretreatment factors are concerned with creating a suitable microbial growth environment. Apart from the factors discussed earlier (pH, temperature, oxygen and nutrient concentrations), attention must be directed at the presence of high concentrations of toxic or inhibitory compounds. These materials may be organic or inorganic (such as metals) in nature. In many cases, toxic or inhibitory concentrations of materials can be effectively treated with the proper reactor design. For example, toxic concentrations of phenol, will cause process failure under batch treatment conditions, but may be easily

biodegraded in a continuous flow completely mixed bioreactor. The process engineer may need to consult with an environmental microbiologist when dealing with compounds of known microbial toxicity or inhibition.

Another pretreatment factor to consider is the presence of nuisance chemicals, such as high concentrations of iron. While iron would not adversely affect the biological processes taking place, it would oxidize and precipitate out of solution. This could cause fouling and degeneration of the biofilm or the production of excess metal-containing sludge.

Post-treatment factors which need to be evaluated include solids removal (both biological and inorganic precipitates) and pass through organics (those organics which cannot be biodegraded or remain as a result of process efficiency). Not every compound present in the waste stream may be completely metabolized during biological treatment under a defined set of conditions. Certain compounds (such as trichloroethylene or carbon tetrachloride) may pass through completely undegraded. Metabolic byproducts and cell lysis materials are also produced with any biological treatment process. These materials may have to meet certain discharge criteria (Total Organic Carbon, for example) before the treated water is suitable for disposal. Volatile compounds, especially those resistant to biodegradation, can be air stripped from biological treatment systems and may have to be controlled.

Once a decision has been made that a waste stream is amenable to biological treatment, conceptual process designs can be made. Several different types of biological treatment systems may be under consideration. At this point it is important to look at the overall economics of the project. This encompasses all capital, installation, and operating expenses (including disposal of any end-product materials). The expected duration of the project will have an obvious impact on the overall project costs. Changes in waste volume, contaminants, and concentrations over the life of the project will also impact the system design and project costs. It is important to have a realistic project time estimate and life cycle description in order to compare the costs associated with different biological treatment systems.

Lastly, there may be important benefits in combining the action of above ground and in situ biological treatment systems. This is especially true if treated water from the bioreactor (usually rich in nutrients, oxygen, and suitable bacteria) can be reinjected into the subsurface. The combined action of such treatment systems may considerably shorten the time required to complete a remediation as compared to above ground or in situ remediation used alone. However, care must be exercised to ensure that the subsurface injection of materials does not further solubilize and mobilize the contaminants present.

SELECTED ADDITIONAL READING

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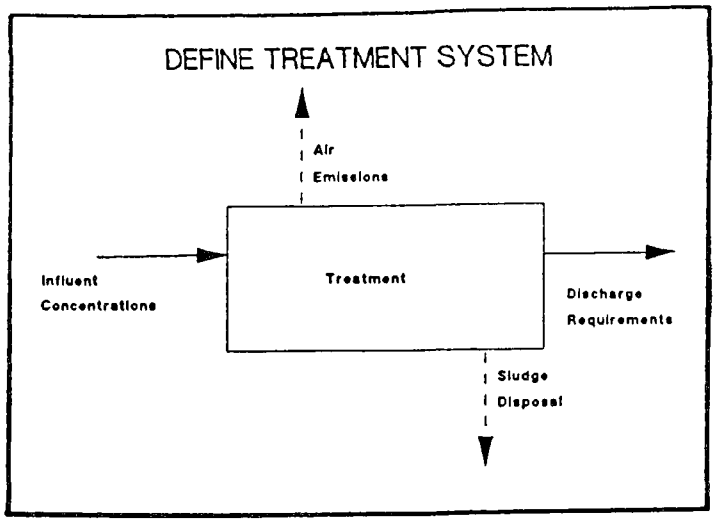
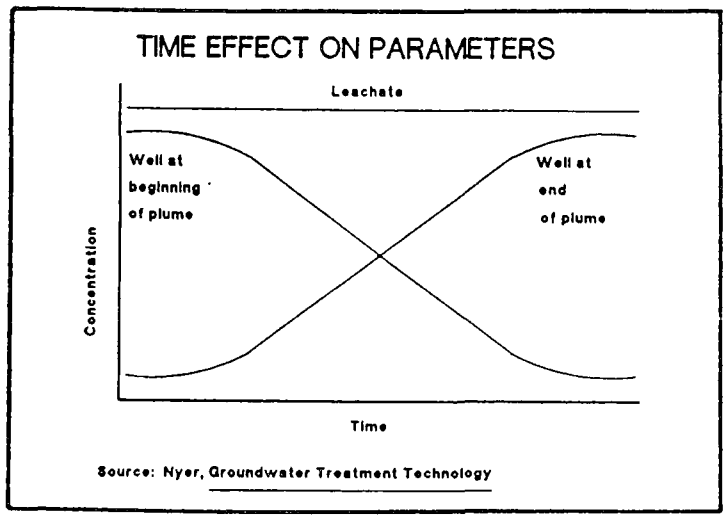


NOTES

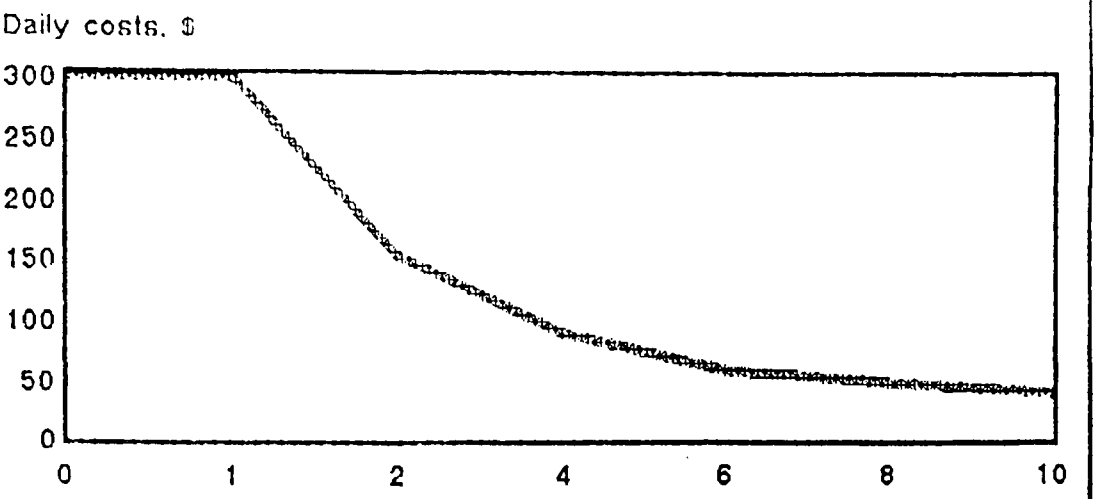
LIFE-CYCLE DESIGN

- Time effect on parameters
- Capital costs
- Operator expenses

NOTES



CAPITAL EQUIPMENT COSTS



Assume: $100,000 capital equipment costs and 12% interest rate

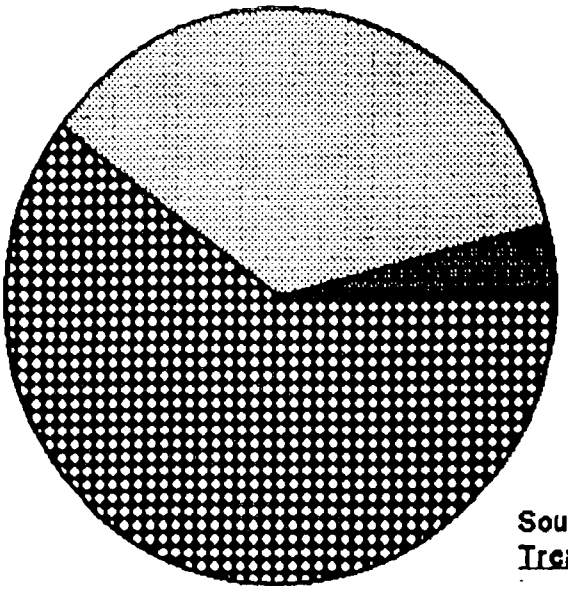
Source: Nyer, Groundwater Treatment Technology

OPERATIONAL EXPENSES

Assume:

- $100,000 capital cost
- 10 year life of equipment
- 12% interest rate
- 15 hp for power ($0.06/kWh)
- $3/day chemical cost
- $10/hour for operator

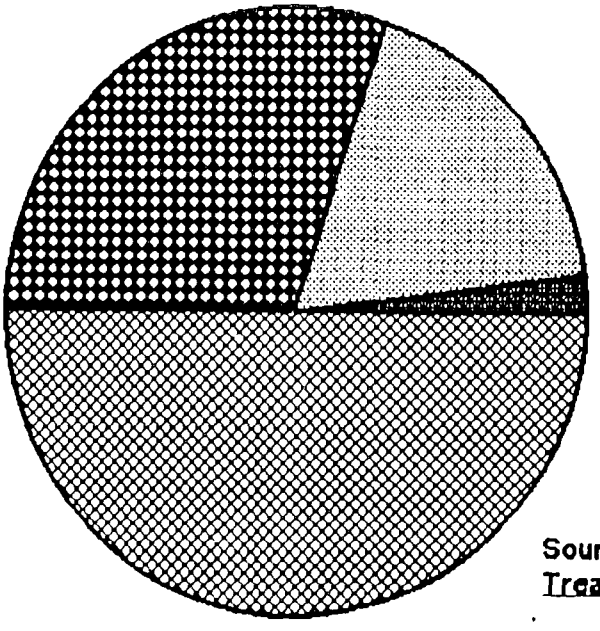
OPERATIONAL EXPENSES WITH NO OPERATOR ATTENTION



- Chemicals 4%
- Power 36%
- Equipment 60%

Source: Nyer, Groundwater Treatment Technology

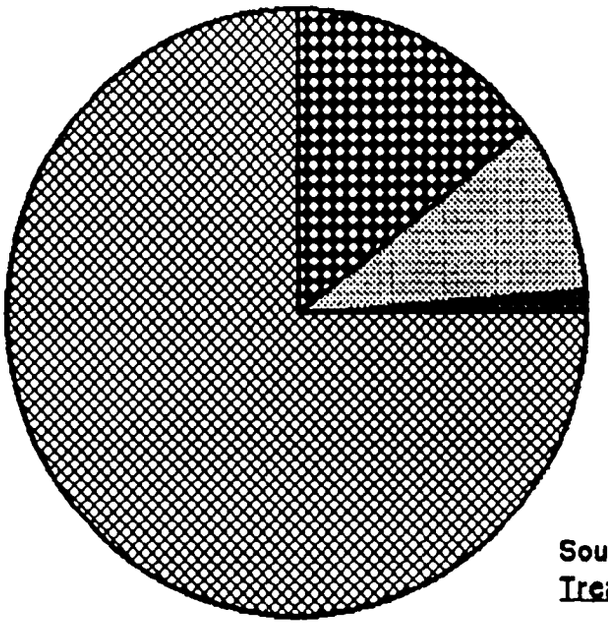
OPERATIONAL EXPENSES WITH 8 HOURS/DAY OPERATOR ATTENTION



- Chemicals 2%
- Power 18%
- Equipment 30%
- Operator 50%

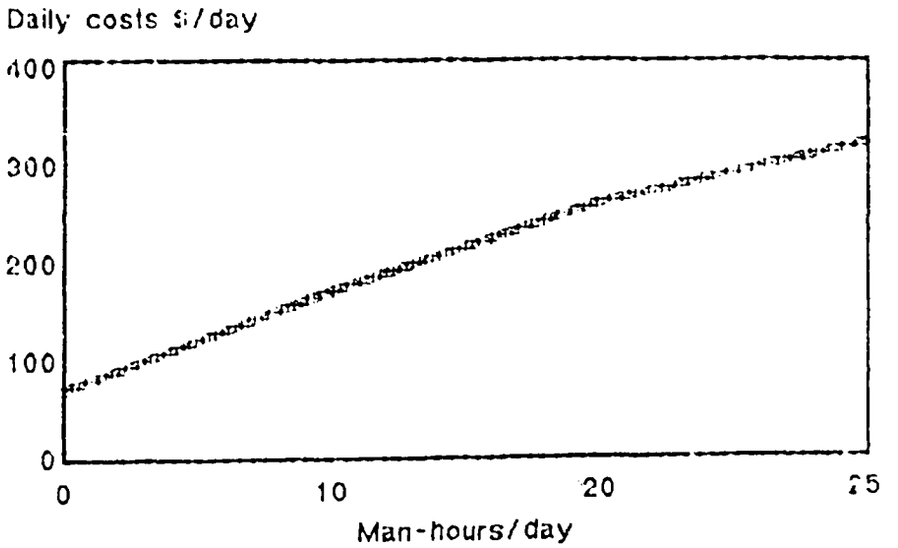
Source: Nyer, Groundwater Treatment Technology

OPERATIONAL EXPENSES WITH 24 HOURS/DAY OPERATOR ATTENTION



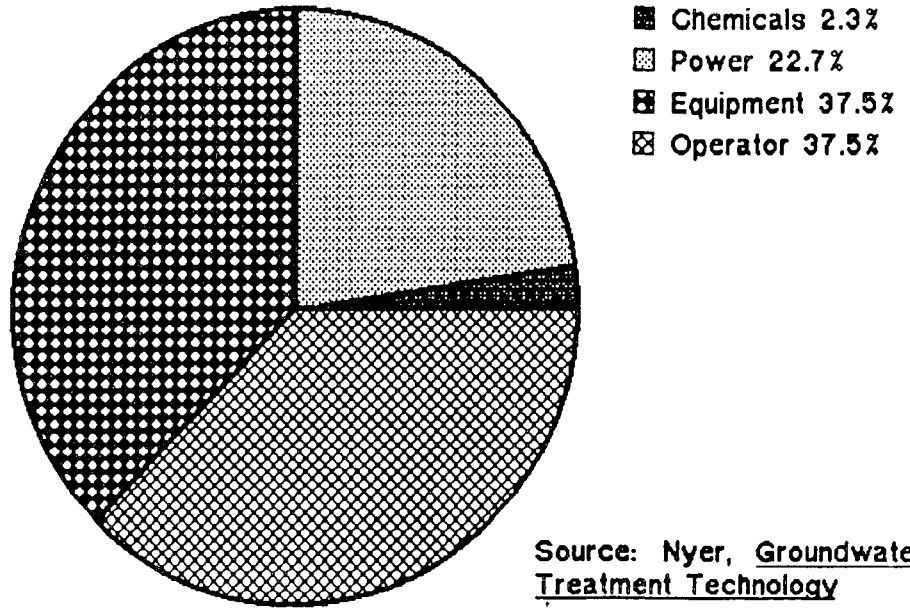
Source: Nyer, Groundwater Treatment Technology

OPERATIONAL EXPENSES SUMMARY



Source: Nyer, Groundwater Treatment Technology

OPERATIONAL EXPENSES WITH $500,000 CAPITAL EQUIPMENT AND 24 HOURS/DAY OPERATOR ATTENTION



Source: Nyer, Groundwater Treatment Technology

DIFFERENT DESIGN CONFIGURATIONS

Based On Practical Solution To Two Issues

- Microorganisms residence time and the relative effect on effluent concentration
- Oxygen transfer

NOTES

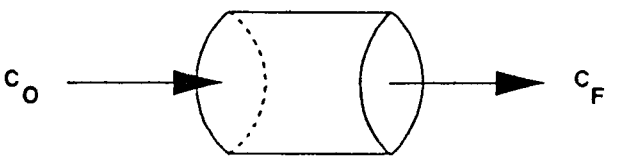
BIOREACTOR DESIGN

Flow Considerations

- Batch
- Plug flow
- Continuous flow completely mixed
- Continuous flow partially mixed

NOTES

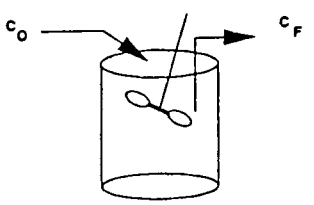
PLUG FLOW



- Ideally no mixing
- Equal treatment for all materials present
- Subject to shock load upsets
- Influent concentrations decrease with passage

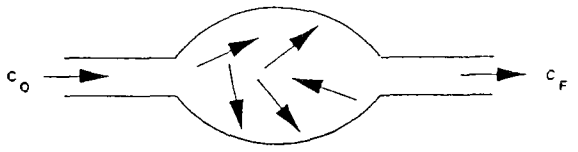
NOTES

CONTINUOUSLY STIRRED TANK REACTOR (CSTR)



- Evenness of treatment dependent upon reaction time within reactor
- Influent concentrations instantaneously diluted into bulk liquid
- Effluent concentration equals bulk liquid concentration
- Good with shock loads and with toxic/inhibitory concentrations of chemicals

ARBITRARY FLOW



- Somewhere between plug flow and CSTR
- Usually more representative of what actually happens

NOTES

NOTES

BIOREACTOR DESIGN

Environmental Conditions

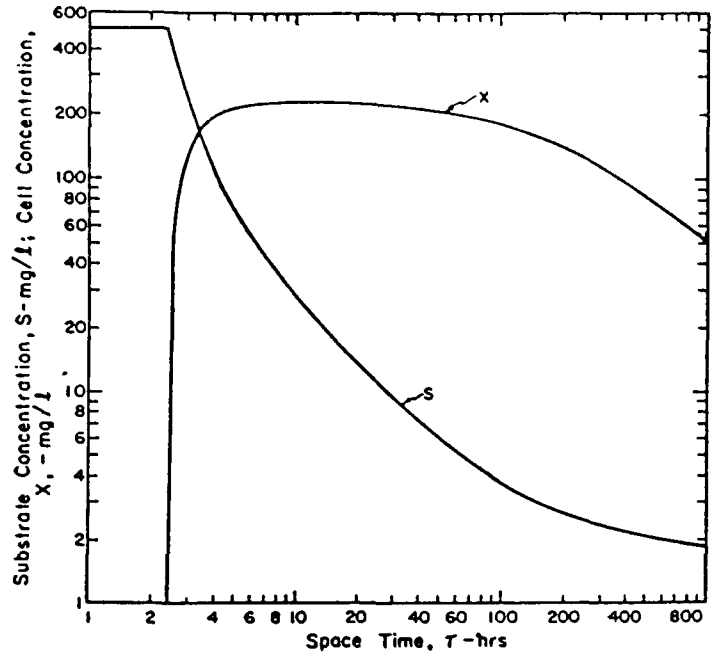
- Temperature
- pH
- Oxygen
- Inorganic nutrients
- Toxics

NOTES

REACTOR DESIGN

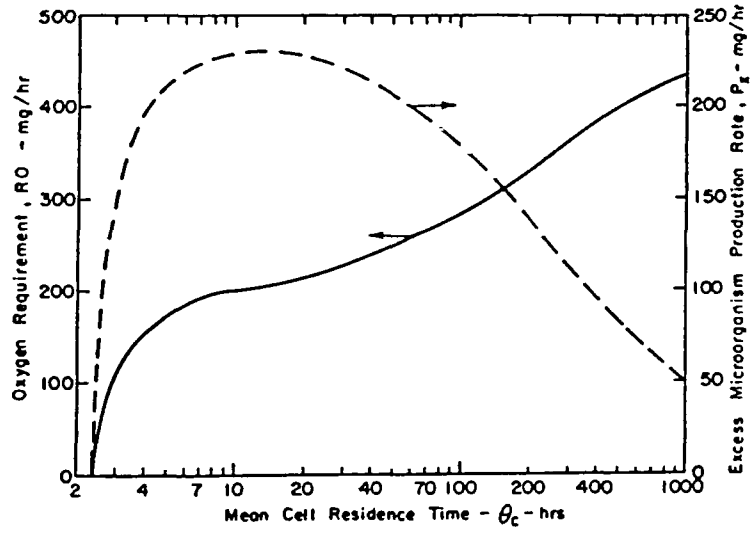
Practical Considerations

- Hydraulic residence time
- Bacterial residence time
- Mixing
- Oxygen transfer
- Bacteria/organics contact



Effect of Space Time on the Performance of a Single CSTR.

Source: Grady and Lim, Biological Wastewater Treatment



Effect of Mean Cell Residence Time on Oxygen Requirement and Excess Microorganism Production Rate in a Single CSTR with Cell Recycle.

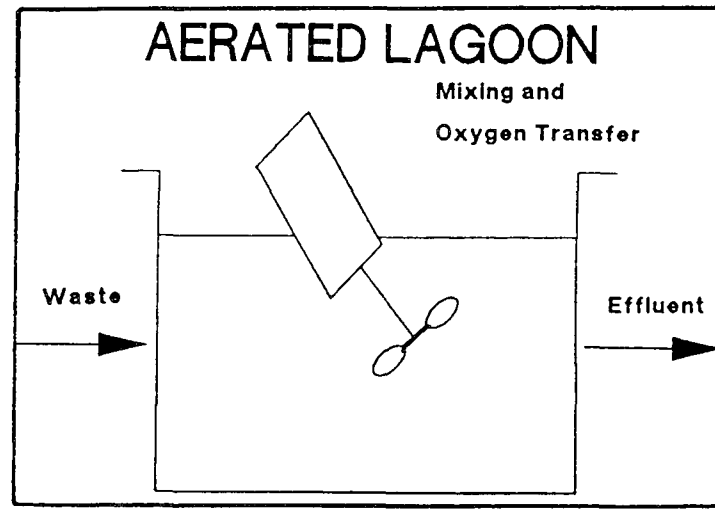
Source: Grady and Lim, Biological Wastewater Treatment

NOTES

AERATED LAGOON

- Biomass kept suspended in liquid
- Contact time limited to hydraulic residence time
- Limited effluent quality

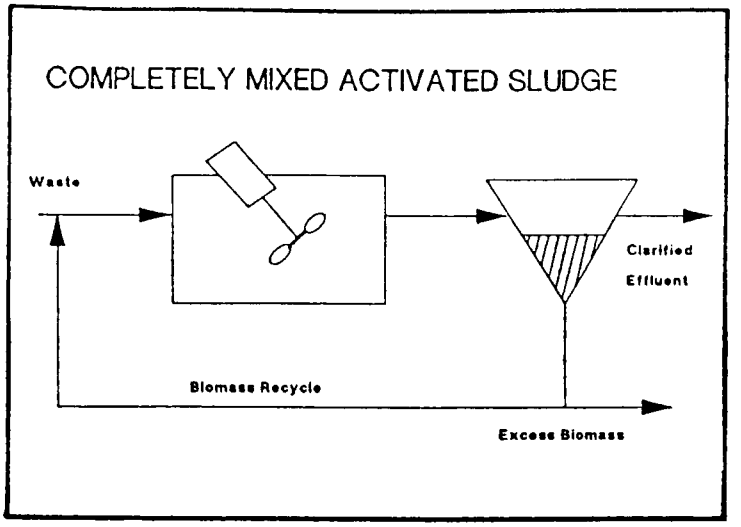
NOTES



NOTES

ACTIVATED SLUDGE

- Biomass kept suspended in liquid
- Biomass captured in clarifier recycled to reactor
- Contact time between waste and biomass controlled by wasting excess biomass



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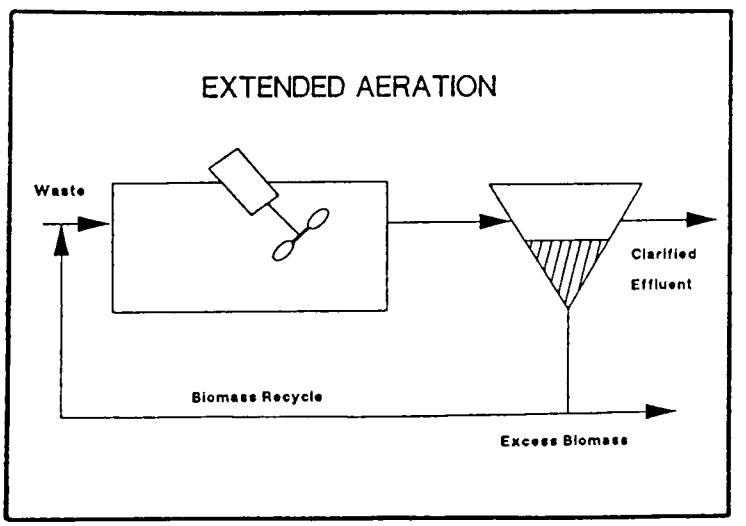
EXTENDED AERATION

- Biomass kept suspended in liquid
- Biomass captured in clarifier recycled to reactor
- Long contact time created by enlarging aeration basin



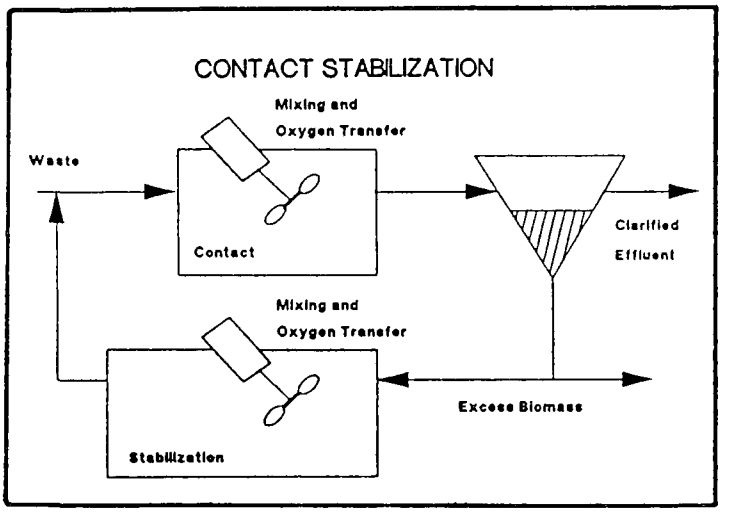
NOTES

NOTES

CONTACT STABILIZATION

- Biomass kept suspended in liquid
- Waste quickly contacted with biomass in first aeration tank
- Clarified biomass/waste is then stabilized in second aeration tank
- Total hydraulic residence time held to a minimum

NOTES



NOTES

SUSPENDED GROWTH REACTORS

Advantages

- Intimate contact between biomass and waste
- Several methods available for adjusting performance
- Very low concentrations of specific organics in effluent
- Large scale system relatively inexpensive

SUSPENDED GROWTH REACTORS

Disadvantages

- Relies on clarifier for performance
- Relative high operator attention

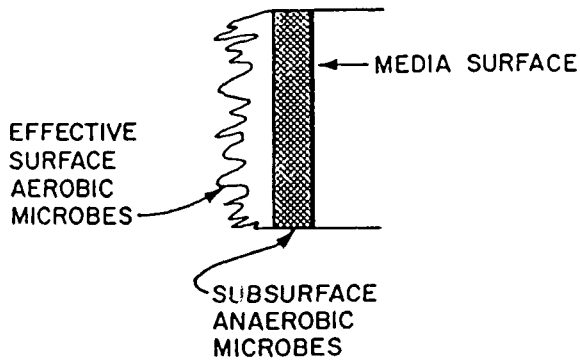
NOTES

NOTES

TRICKLING FILTER

- Biomass retained in reactor on inert support
- Design based on specific surface area
- Plug flow
- Aeration provided by induced or forced draft

NOTES



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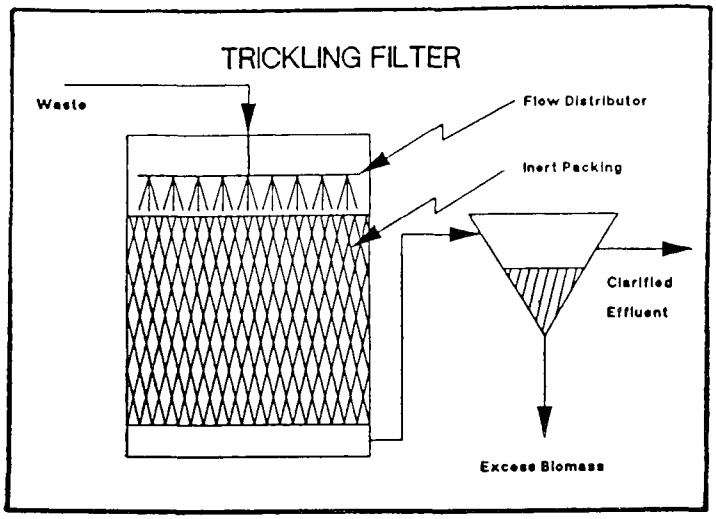
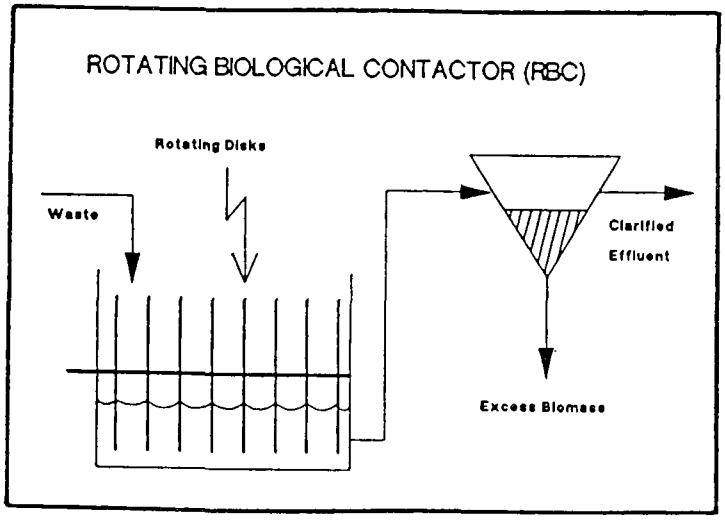


NOTES

ROTATING BIOLOGICAL CONTACTORS

- Fixed film keeps biomass in system
- Design based on specific surface area
- Aeration provided by rotating disks
- Plug flow

NOTES



FIXED FILM REACTORS

Advantages

- Low operator attention
- Retention of slow growing bacterial population
- Low cost oxygen transfer

NOTES

NOTES

FIXED FILM REACTORS

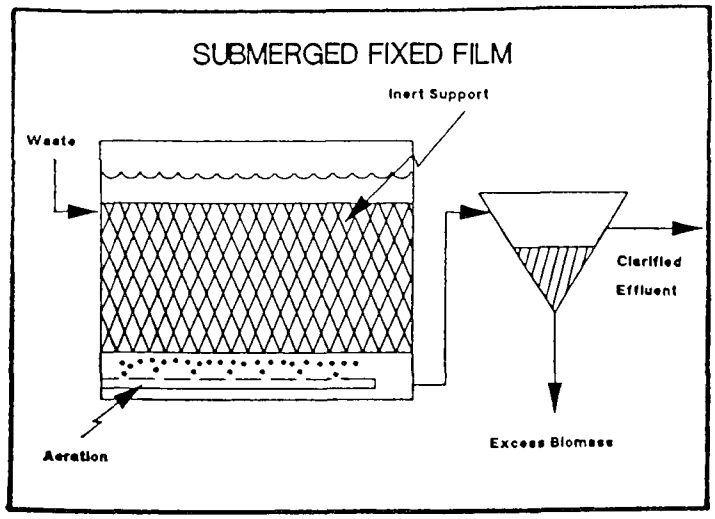
Disadvantages

- Plug flow
- Limited operation at high influent concentration
- Hard to adjust operation

NOTES

SUBMERGED FIXED FILM

- Biomass retained in reactor on inert support
- Design based on volume
- Completely mixed
- Aeration provided by air released below media



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NOTES

SUBMERGED FIXED FILM REACTORS

Advantages

- Combines advantages of suspended growth and fixed film systems
- Portable design possible
- Can be run in low-concentration mode

NOTES

SUBMERGED FIXED FILM REACTORS

Disadvantages

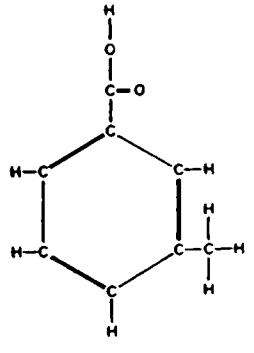
- Does not scale well - expensive for large scale system
- Relatively expensive for oxygen transfer

SUBMERGED FIXED FILM

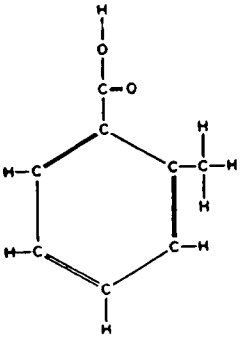
Case Study:

Industrial Landfill Leachate

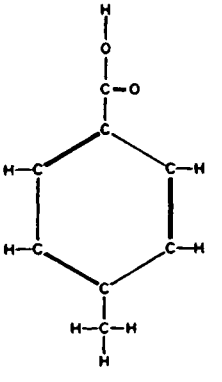
Source: DETOX, Inc. (Dayton, OH)



META-TOLUIC ACID
SOLUBILITY: 340 ppm



ORTHO-TOLUIC ACID
SOLUBILITY: 1180 ppm



PARA-TOLUIC ACID
SOLUBILITY: 850 ppm

TOLUIC ACID STRUCTURES

NOTES

TREATMENT OPTIONS

- Off-site disposal
  $0.20/gallon
- On-site activated carbon
  $0.08/gallon
- On-site biological treatment
  <$0.01/gallon

(Based on toluic acid concentrations of 300-400 ppm and flow rates of up to 5 gpm.)

NOTES

LABORATORY TREATABILITY STUDIES

Microbial Toxicity/ Growth Inhibition

- pH 6.6 and 8.7

NOTES

LABORATORY TREATABILITY STUDIES

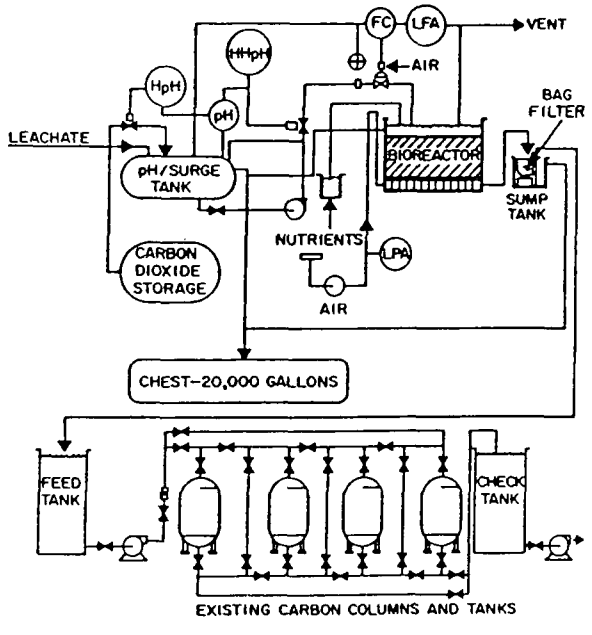
Aerobic Biodegradation Study

- 37 days
- 60 ppm toluic acids to <1.5 ppm
- Toluic acid plate counts

LABORATORY TREATABILITY STUDIES

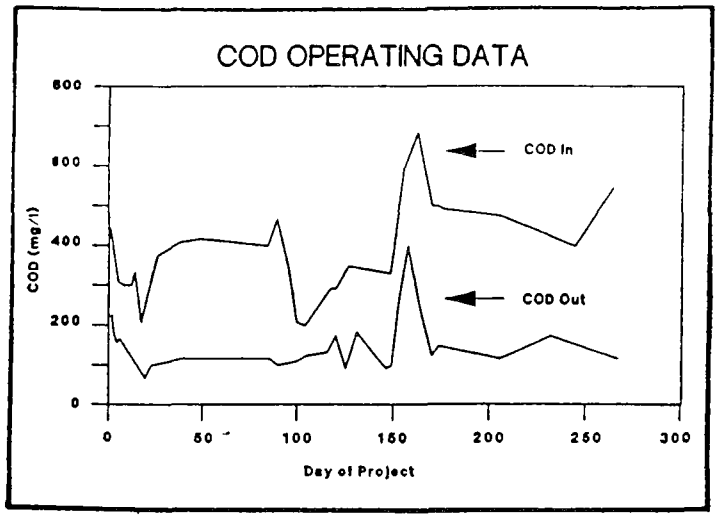
Anoxic Biodegradation Study

- 37 days
- pH from 7 to >9.5
- 60 ppm toluic acids to approximately 55.5 ppm



HpH: HIGH pH
HHpH: HIGH HIGH pH
FC: FLOW CONTROL
LFA: LOW FLOW ALARM
LPA: LOW PRESSURE ALARM
LTA: LOW TEMPERATURE ALARM

EQUIPMENT PROCESS DESIGN



NOTES

NOTES

TOLUIC ACID CONCENTRATIONS (ppm)				
Contaminant	8/20/87	1/18/88	4/13/88	7/25/88
Influent o-Toluic	43	79	71	13.8
Effluent o-Toluic	<0.5	<0.5	0.05	<0.01
Influent m & p-Toluic	1	25	45 (m) 3 (p)	6.11 (m) 0.64 (p)
Effluent m & p-Toluic	<0.5	<0.5	<0.078 (m) <0.052 (p)	<0.01 (m) <0.01 (p)

NOTES

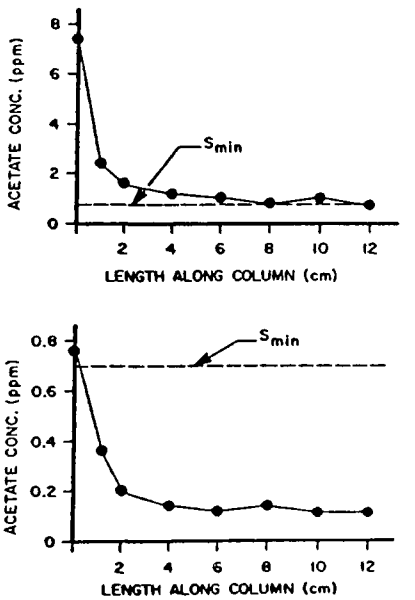
PROJECT COST SUMMARY

Bioreactor system	$21,800
Installation	$13,900
Winterization	$2,000
Total	$37,580
Daily operating	$5.40

LOW CONCENTRATION (<25 PPM) SUBMERGED FIXED-FILM BIOREACTOR

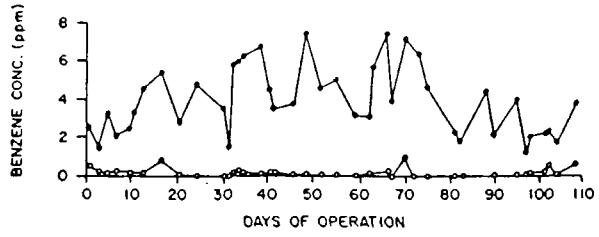
Case Study:

Source: DETOX, Inc. (Dayton, OH)



Examples of acetate removal under both steady-state and unsteady-state biofilm conditions.

NOTES



BENZENE REMOVAL DURING LABORATORY BIOREACTOR DEVELOPMENT.
KEY: INFLUENT BENZENE (•); EFFLUENT BENZENE (•).

NOTES

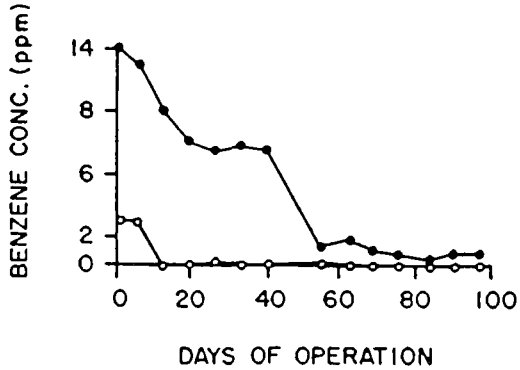
GASOLINE STATION

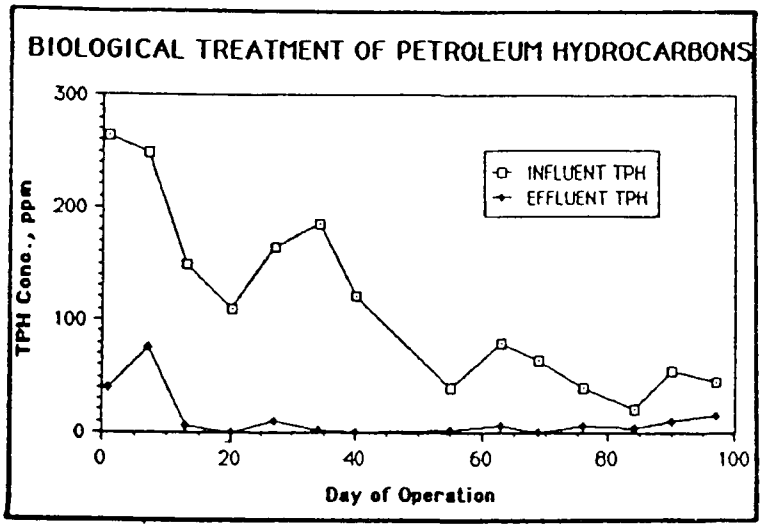
5 gpm

25 ppm total hydrocarbons

NOTES

BIOLOGICAL TREATMENT OF BENZENE FRACTION





NOTES

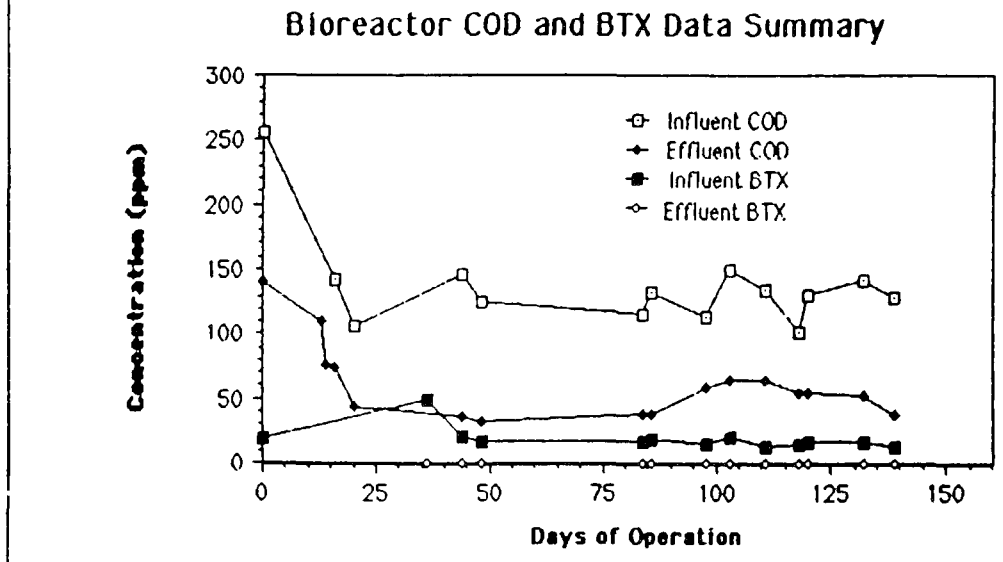
NOTES

Compound	Average Removal
Benzene	› 93%
Toluene	› 96%
Xylenes	› 91%

NOTES

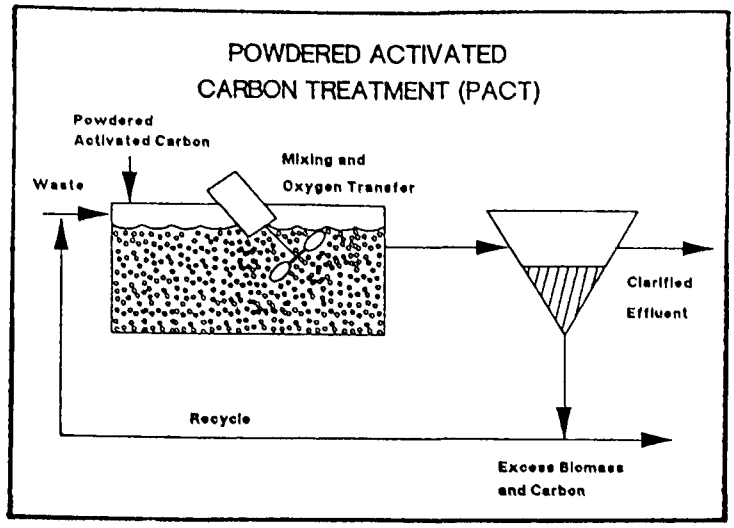
SERVICE STATION

- Flow: up to 6 gpm
- Influent BTX: 15-30 ppm



POWDERED ACTIVATED CARBON TREATMENT (PACT)

- Biomass suspended and fixed to carbon particles
- Carbon particles also adsorb organic material
- Clarifier still controls bacterial residence time
- Completely mixed



NOTES

NOTES

POWDERED ACTIVATED CARBON TREATMENT (PACT)

Case Study:

Bofors-Nobel, Inc.
Muskegon, MI

Source: Zimpro Passavant (Rothschild, WI)

NOTES

SITE BACKGROUND INFORMATION

- Herbicides and organic chemicals produced
- 1.2 mgd of groundwater from abandoned landfill
- 0.6 mgd of production process waters
- Wasted biomass and spent carbon treated onsite by wet air oxidation (WAO)

TREATMENT OPTIONS

- Biological treatment
- Liquid phase activated carbon
- Biological treatment followed by activated carbon
- Chemical oxidation
- Sorption onto bentonite/clay

NOTES

NOTES

IDENTIFIED GROUNDWATER CONTAMINANTS

Compound	Concentration (ppb)
ortho-Chloroanaline (OCA)	13,000
Benzene	4,900
Dichlorobenzene isomer	2,500
Toluene	1,500
1,2-Dichloroethane	420

NOTES

IDENTIFIED GROUNDWATER CONTAMINANTS (Continued)

Compounds	Concentration (ppb)
Ethyl benzene	220
Chlorobenzene	150
Bis (ethyl hexyl) phthalate	100
3,3-Dichlorobenzidine (DCB)*	86
3-Chloroanaline	68

IDENTIFIED GROUNDWATER CONTAMINANTS (Continued)

Compound	Concentration (ppb)
Benzidine isomer	65
Phenol	6
Cresol	5
Tetrachloroethylene	5
ortho-Chlorophenol	4

NOTES

NOTES

TREATABILITY STUDY RESULTS
(All concentrations are in ppm)

Parameter	Influent Conc.	Biological Treat.	Carbon Treat.	Combined Treat.
BOD	30 to 40	0 to 5	No Data	0 to 5
COD	70 to 80	5 to 10	No Data	5 to 10
TOC	20 to 30	5	No Data	<5
Suspended Solids	25	5 to 10	No Data	5

NOTES

TREATABILITY STUDY RESULTS
(All concentrations are in ppb)

Parameter	Influent Conc.	Biological Treat.	Carbon Treat.	Comb. Treat.
Dichlorobenzidine	100	75	<5	<5
ortho-Chloroanaline	30	ND*	300	ND
Benzidine	90	ND	15	ND
Ethylenedichloride	24	7	80	3
Toluene	130	12	30	12

*Indicates Non-Detected

TREATMENT SUMMARY

- Over 135 chemicals treated
- Over 780 million gallons of combined wastes treated to date(March 1983 to March 1987)
- COD reductions >98% (6,000 ppm to <100 ppm)
- Ortho-chloroanaline concentrations from 6,500-53,000 ppb to <100 ppb
- Dichlorobenzidine concentrations from 400-12,000* ppb to <2 ppb

*Soluble DCB only-system also receives DCB in solid form

NOTES

NOTES

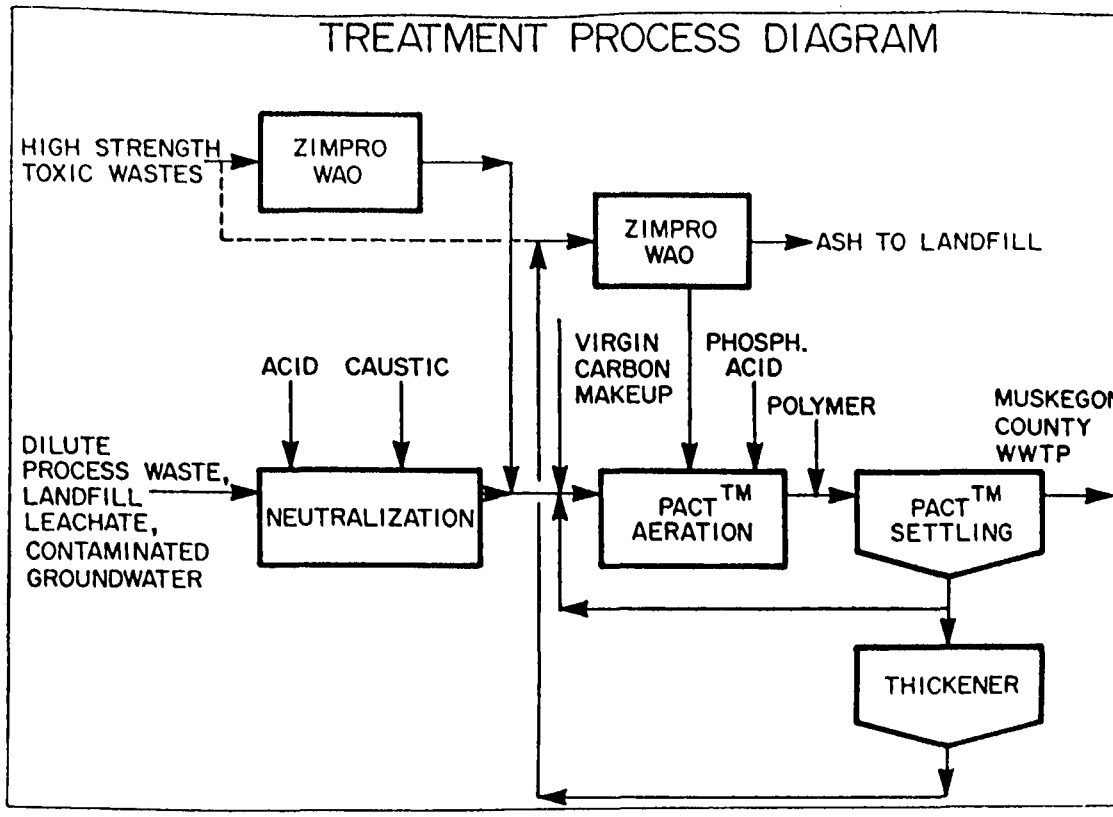
PACT SYSTEM OPERATION

- PAC concentration 4,000 to 12,000 mg/l
- Mixed liquor composition:
  - -PAC: 50%
  - -Biomass: 40%
  - -Ash: 10%

NOTES

SYSTEM OPERATING COSTS

- 1986 total operating costs(solids disposal, neutralization, ground water pumping, and county wastewater charges)were approximately $1,000,000
- $2.00 per 1,000 gallons treated
- <$0.10 per pound of COD treated
- Onsite carbon regeneration/solids disposal budgeted for $300,000 per year
- Offsite carbon disposal costs estimated to be over $1,000,000 - and liability would still exist



BIOLOGICAL SEQUENCING BATCH REACTOR (SBR)

Case Study:

Source: Occidental Chemical Corp. (Grand Island, NY)

NOTES

HYDE PARK LANDFILL

- Used from 1953 to 1975
- Contains 73,000 metric tons of chemical wastes
- Clay liner installed in 1978
- Tile leachate collection system installed in 1979
- Leachate trucked to Niagara plant and mixed with plant wastewaters

NOTES

ORIGINAL TREATMENT PROCESS

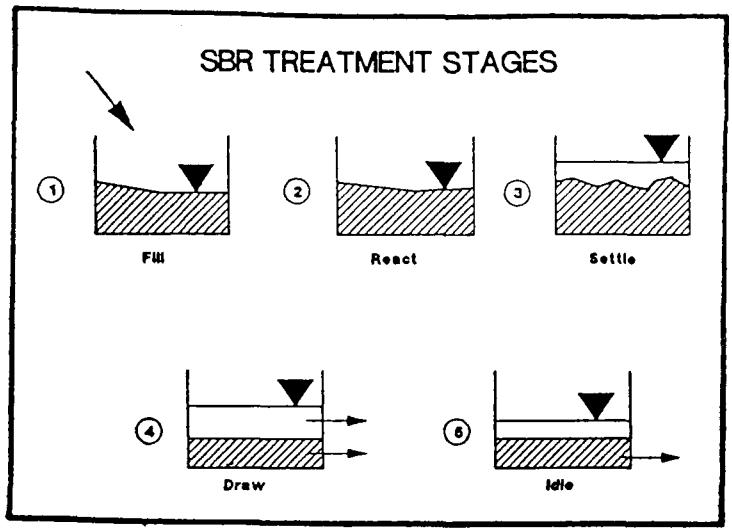
- pH adjustment
- Suspended solids settling
- Filtration through 50 micron bag
- Two-stage activated carbon

NOTES

RAW LEACHATE CHARACTERISTICS

pH	4.3
TOC	3,500
COD	10,040
BOD	7,500
SS	900
VSS	300
TDS	25,700

(Major organics include phenol, benzoic acid, and isomeric chlorobenzoic acids)



NOTES

NOTES

RESULTS OF 500 LITER PILOT SBRs

	TOC (mg/l)	COD (mg/l)	TOX (mg/l)
Influent Feed	2,000	5,300	325
Effluent A (5 day HRT & 5000 mg/l MLSS)	140 (93%)	510 (90%)	110 (66%)
Effluent B (5 day HRT & 10,000 mg/l MLSS)	120 (94%)	400 (92%)	105 (68%)
Effluent C (2 day HRT)	536 (73%)	1,700 (68%)	235 (28%)

NOTES

YEARLY TREATMENT EXPENSES SUMMARY
(Based On 1984 Dollars And 10 Years Operation)

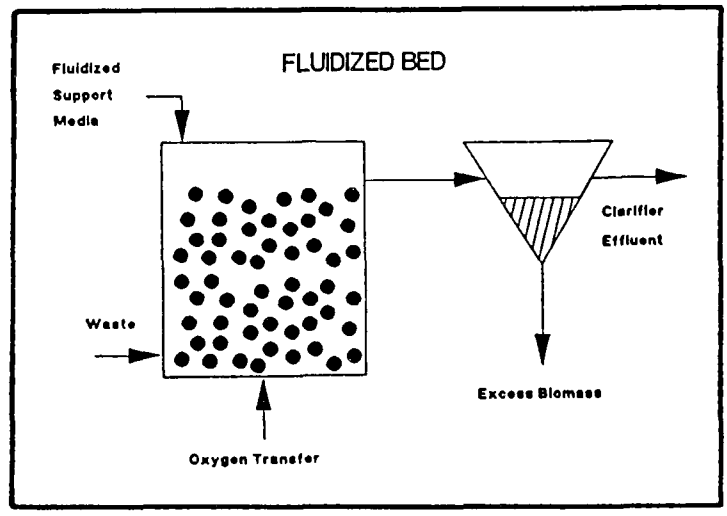
Activated Carbon Alone: ($1.65/kg)	$715,111
SBR Operation: (At 173 kg/day)	$116,900
Activated Carbon:	$71,511
Total:	$188,411
Net Savings Per Year:	$526,700

NOTES

FLUIDIZED BED

- Bacteria attached to support media
- Media fluidized in reactor
- Plug flow

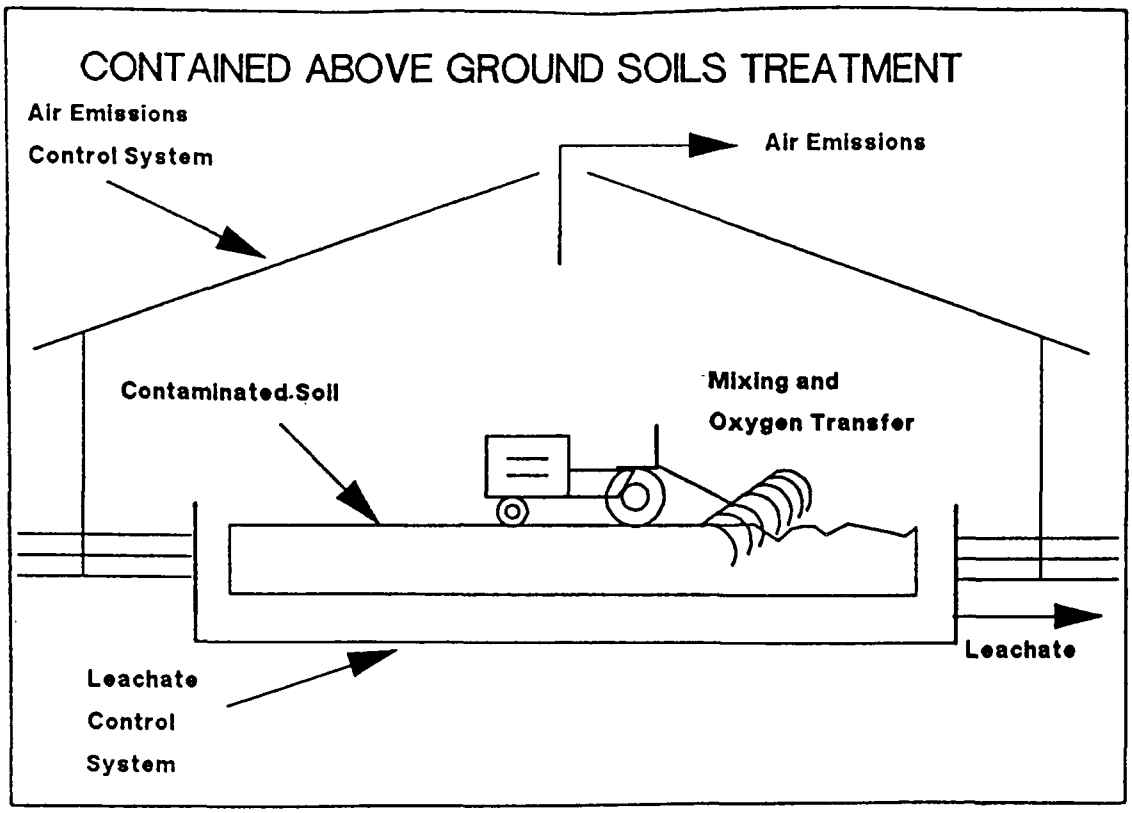
NOTES



NOTES

CONTAINED ABOVE GROUND SOILS TREATMENT

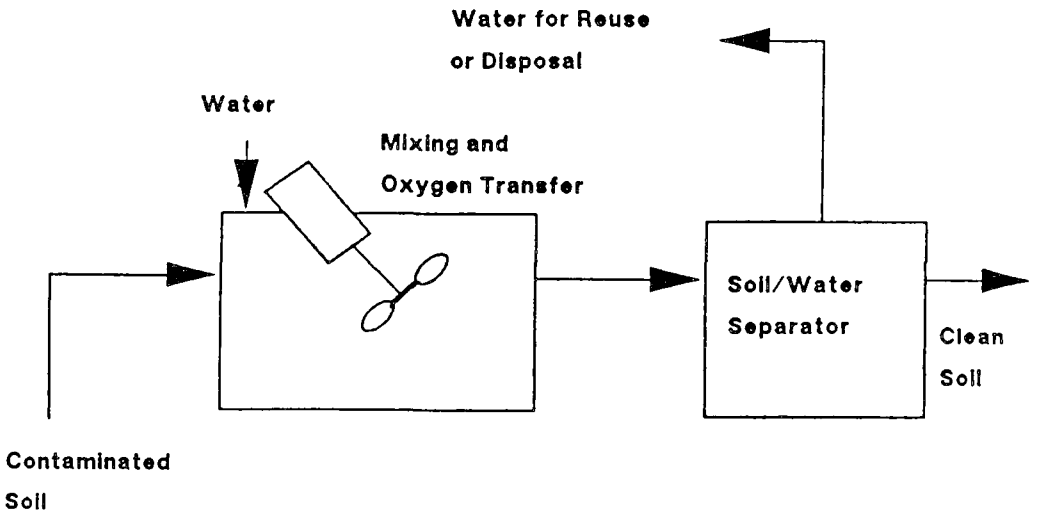
- Contaminants treated in the soil matrix
- Nutrients, moisture, and oxygen added as needed
- Leachate, runoff and air emissions must be controlled
- Soil left on site when clean



SOIL SLURRIES

- Contaminants treated in a soil slurry
- Nutrients and oxygen added as needed
- Water and soil must be separated after treatment
- Soil left on site when clean

SOIL SLURRIES



FIELD PILOT SOIL WASHING

Case Study:

NPL Wood Treating Facility
Minnesota
(Oct.-Nov. 1987)

Source: BioTrol (Chaska, MN)

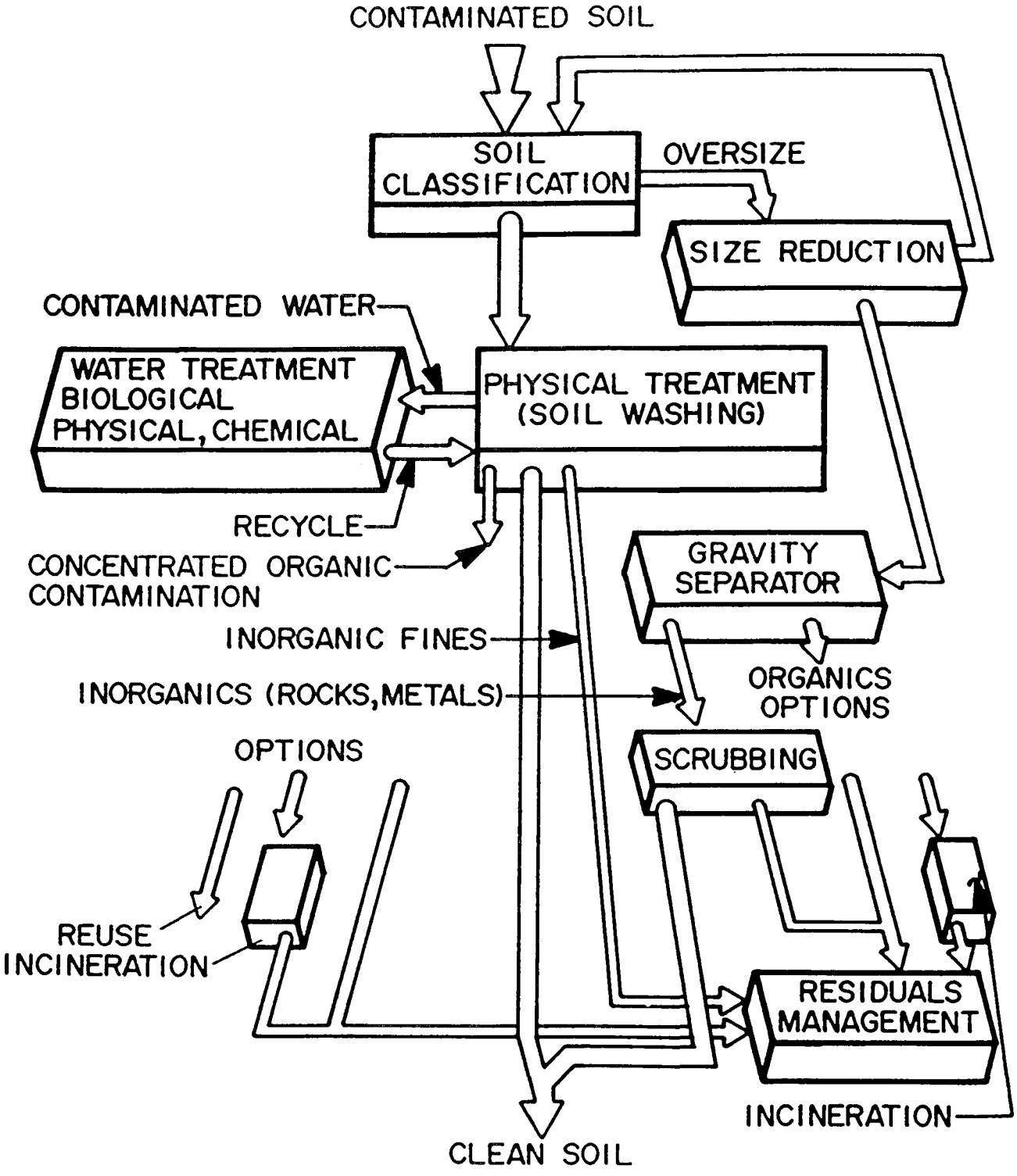
CONTAMINANTS

- Oil
- Creosote
- Pentachlorophenol
- Polynuclear aromatics

SITE SOIL CHARACTERISTICS

- Silty, fine to medium grained sands with intermediate and laterally discontinuous silt and sand lenses

BIOTROL SOIL TREATMENT SYSTEM (BSTS)



PROCESS DIAGRAM FOR SOIL WASHING SYSTEM

PILOT SOIL WASHING EQUIPMENT

- 42' semi-trailer
- Soil feed rate up to 500 pounds per hour (dry weight)
- Soils initially screened and classified
- Countercurrent soil washing using water

NOTES

NOTES

PILOT SOIL WASHING EQUIPMENT
(Continued)

- Contaminated water treated with aerobic biological treatment system
- Decontaminated water recycled to unit
- Sands and clays separated and treated
- Large debris treated separately

NOTES

PENTACHLOROPHENOL SOIL WASHING RESULTS
(All concentrations are in ppm)

Soil	# of Tests	Dry Feed (lbs/hr)	Influent Conc.	Treated Conc.	Percent Reduction
#1	4	282 (+/-77)	1,498 (+/-558)	80 (+/-37)	>94
#2	5	420 (+/-48)	160 (+/-26)	10 (+/-5)	>93
#3	5	443 (+/-51)	215 (+/-11)	24 (+/-4)	>88

NOTES

ESTIMATED TREATMENT COSTS

- $100 per cubic yard
- Final cost depends upon:
  - -volume of soil to be treated
  - -specific contaminants present
  - -composition of soils
  - -required effluent concentrations

NOTES

PRETREATMENT FACTORS

- Nonaqueous phase neat material removal
  - specific gravity <1
  - specific gravity >1
- pH
- Nutrients
- Toxicity
  - organic
  - inorganic
- Nuisance substances
  - iron
  - suspended solids

NOTES

POST TREATMENT FACTORS

- Solids removal and disposal
- Effluent organics
  - persistent compounds
  - metabolic by-products
- Air emissions

ECONOMICS

- Capital equipment
- Design/engineering
- Installation expenses
- Operational expenses

NOTES

NOTES

OPERATIONAL EXPENSES

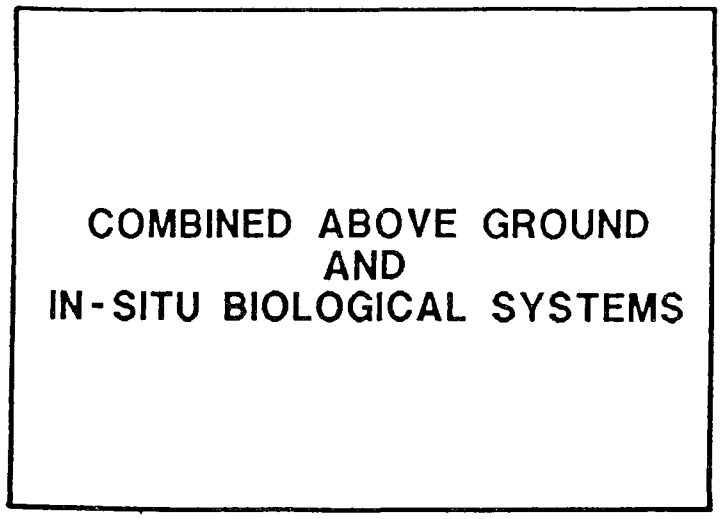
- Supplies/reagents
- Energy
- Operating personnel
- Disposal of end-products

NOTES

PROCESSES FOR SELECTING BIOREACTOR DESIGNS

- Applicability
- Technical/regulatory
- Cost effectiveness

NOTES



REACTOR TREATMENT DESIGN WORKSHEET

The following worksheet should be used to develop the information necessary for evaluating the suitability and design of biological treatment systems.

I. Waste Characterization

1. List the contaminants and the concentrations present.

_____
_____
_____
_____

2. List the required effluent concentration for each contaminant

_____
_____
_____
_____

3. Which contaminants are biodegradable (aerobic or anaerobic), inhibitory or toxic, or non-biodegradable?

_____
_____
_____
_____

4. What are the physical and chemical properties of the contaminants (density, solubility, etc)?

_____
_____
_____
_____

5. Are the observed contaminant concentrations and locations consistent with the properties of the chemicals?

_____
_____
_____
_____

II. Life-Cycle Design Considerations

6. Define the treatment system needed (include all inputs and outputs).

_____
_____
_____
_____

7. Will site conditions change during the life of the project? If so, how will these changes affect any proposed treatment system?

8. What is the expected duration of the project?

III. Conceptualized Process Design and Bioreactor Selection

9. Will the material be treated in place or moved to another location?

10. What method of collecting and conveying the wastes should be used?

11. What volumetric treatment rate will be required to process the wastes?

12. Will the waste stream be treated with a single unit process or several?

13. Do we need pretreatment to allow biological treatment to occur (adjust pH, remove toxics, addition of nutrients, etc.)?

14. What specific aerobic or anoxic/anaerobic biological processes are best for this situation?

15. What is the development status of the processes selected (demonstrated on similar site and situation, demonstrated in other applications, developmental, or conceptual)?

16. What organic/inorganic residues will be produced from the system? Are they hazardous? What equipment is required to remove the residues? What is the final disposal of these materials?

17. Draw process diagrams for the proposed treatment systems.

18. Will the proposed treatment systems meet or exceed all required effluent discharge requirements?

19. What are the overall advantages of the proposed treatment systems?

20. What are the overall disadvantages of the proposed treatment systems?

21. How do the biological unit processes interact with any needed non-biological unit processes?

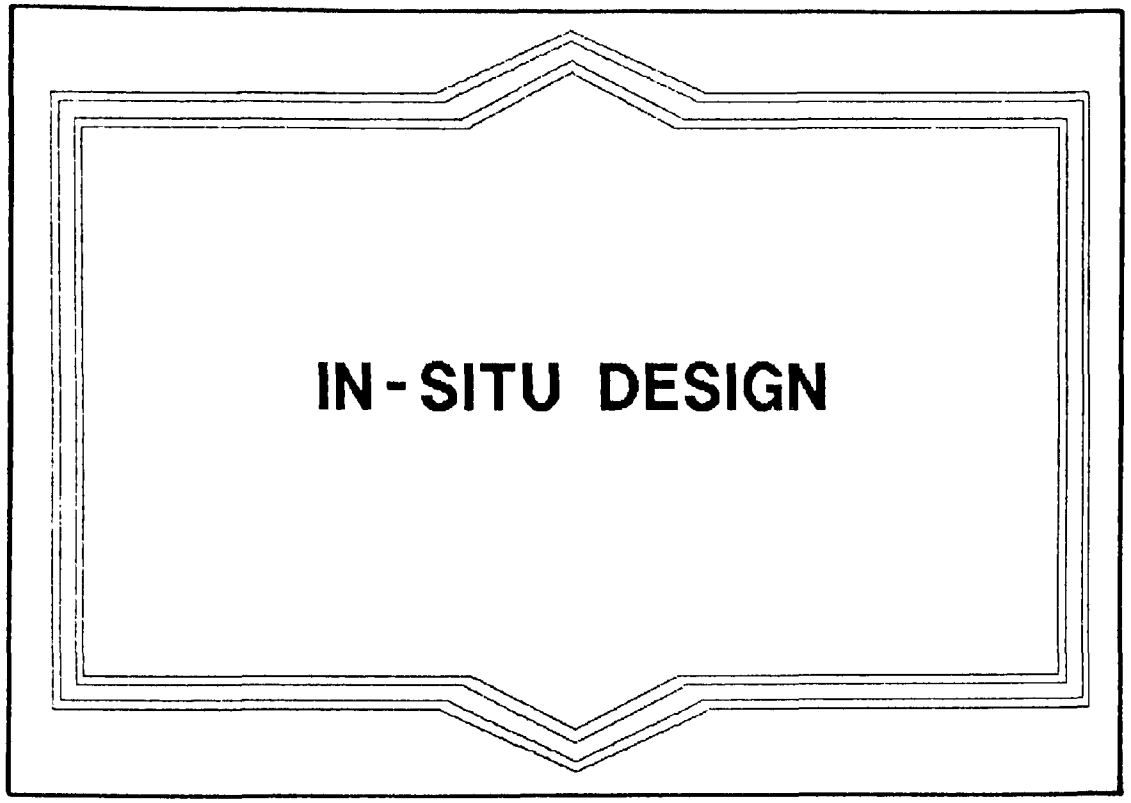
IV. Project Economics

22. List the site conditions needed for the proposed treatment systems (space requirements, power requirements, etc.).

23. Will laboratory and/or field pilot treatability work be required? How much should be budgeted?

24. What operating expenses will be incurred during treatment (consumables, maintenance, byproduct disposal costs, and operating personnel)?

25. Is it possible to reduce the manpower requirements for the proposed treatment systems?



SECTION 5

IN SITU TREATMENT DESIGN - SURFACE AND SUBSURFACE

John T. Wilson
U.S. EPA
Ada, Oklahoma

Ronald C. Sims
Utah State University
Logan, Utah

Surface Soil Treatment

Bioremediation of surface soils involves the use of naturally occurring microorganisms to treat specific chemicals associated with the soil environment at a site. The subject of bioremediation of contaminated soils, including applications and limitations of the technology, has been addressed at several recent scientific meetings and conferences identified in the references section. Three aspects that are important for consideration in order to accomplish in situ bioremediation include: (1) site-soil-waste characterization, (2) microbial activity, and (3) treatment system design and monitoring to evaluate treatment effectiveness. Information concerning mechanisms involved in vadose zone (soil) treatment and laboratory and field scale demonstration results provide a significant information base concerning the applications of this treatment approach. References are included to assist the reader in obtaining additional information. The goals of on-site bioremediation of contaminated soils are presented in Figure 1.

In situ treatment involves the controlled management and manipulation of soil microbial processes and of soil physical and chemical processes that affect natural soil microbial processes to achieve degradation and detoxification of waste chemicals. Successful application of in situ treatment requires information and understanding of site, soil and waste characteristics identified above. Specific waste, site, and soil characteristics that are important for determining the potential success for in situ treatment are summarized in Tables 1 and 2, and discussed in detail in the reference "Contaminated Surface Soils In-Place Treatment Techniques".

Table 3 identifies contaminated sites that are currently using bioremediation as the only remediation process or as one process in a "treatment train" to obtain the goals of on-site bioremediation identified in Figure 1. Management techniques that are currently being used for In Situ bioremediation of surface soils at the sites, identified in Table 3, involve the manipulation of factors influencing biological activity including: oxygen, nutrients, moisture, and pH, and addition of carbon and energy sources. Addition of amendments to surficial soils generally have fewer restrictions with regard to mass transfer than amendments applied to deeper soils, including microorganism inoculations.

With respect to microbial activity enhancement, when considering the potential application of on-site bioremediation of contaminated soils, there are several issues that should be considered as part of a

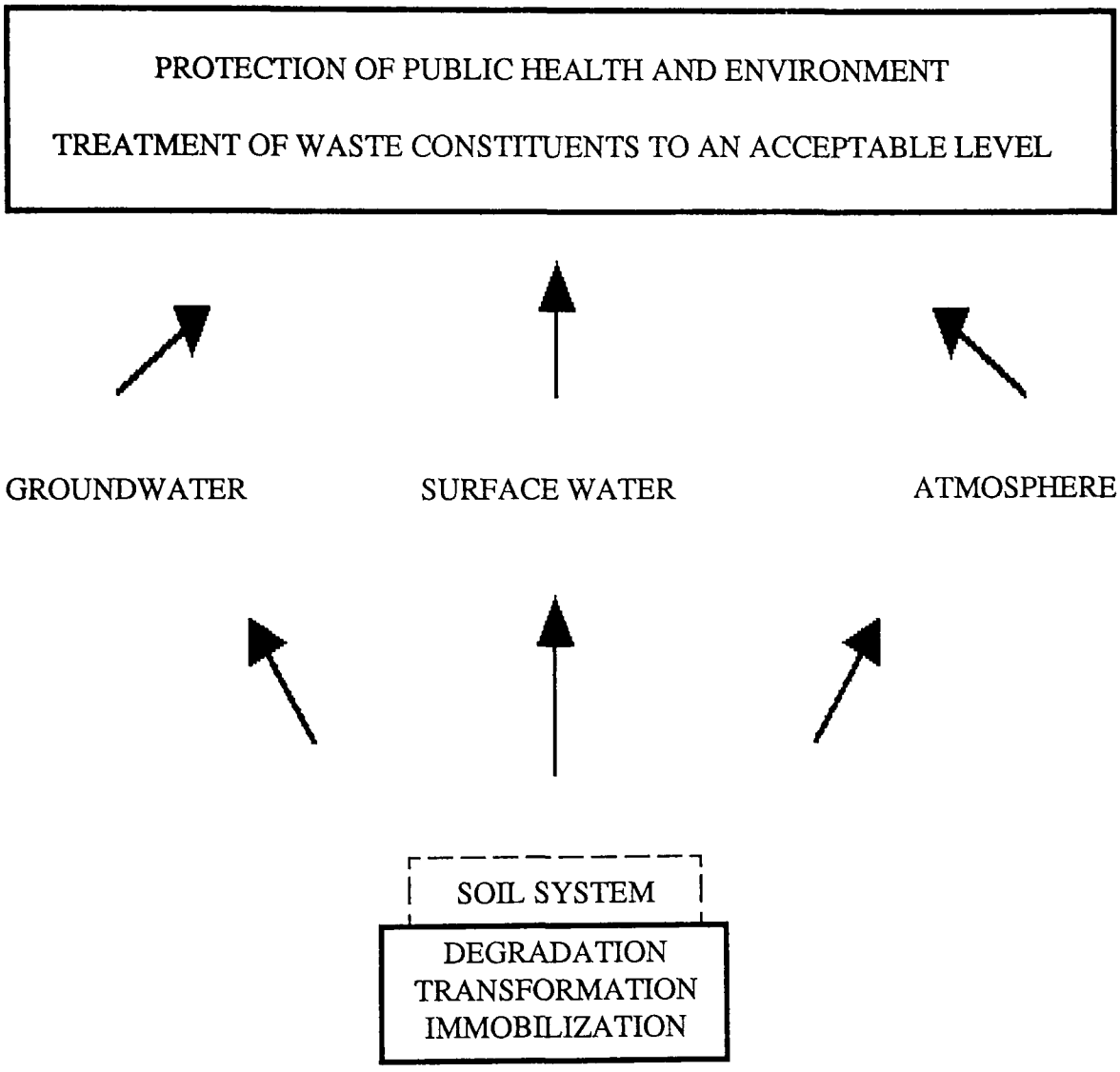


Figure 1. The Goals of Onsite Bioremediation of Contaminated Soils.

TABLE 1. SITE AND SOIL CHARACTERISTICS IDENTIFIED AS IMPORTANT IN IN SITU TREATMENT (Reference 5)

Site location/topography and slope

Soil type and extent

Soil profile properties

- boundary characteristics
- depth
- texture*
- amount and type of coarse fragments
- structure*
- color
- degree of mottling
- bulk density*
- clay content
- type of clay
- cation exchange capacity*
- organic matter content*
- pH*
- Eh*
- aeration status*

Hydraulic properties and conditions

- soil water characteristic curve
- field capacity/permanent wilting point
- water holding capacity*
- permeability* (under saturated and a range of unsaturated conditions)
- infiltration rates*
- depth to groundwater*, including seasonal variations
- flooding frequency
- runoff potential*

Geological and hydrogeological factors

- subsurface geological features
- groundwater flow patterns and characteristics

Meteorological and climatological data

- wind velocity and direction
- temperature
- precipitation
- water budget

*Factors that may be managed to enhance soil treatment

TABLE 2. SOIL-BASED WASTE CHARACTERIZATION (Reference 5)

Chemical class

- acid
- base
- polar neutral
- nonpolar neutral
- inorganic

Soil sorption parameters

- Freundlich sorption constants (K, N)
- sorption based on organic carbon content (K_{OC})
- octanol/water partition coefficient (K_{OW})

Soil degradation parameters

- half-life ($t_{1/2}$)
- rate-constant (first order)
- relative biodegradability

Chemical properties

- molecular weight
- melting point
- specific gravity
- structure
- water solubility

Volatilization parameters

- air/water partition coefficient (K_W)
- vapor pressure
- Henry's law constant ($1/K_W$)
- sorption based on organic carbon content (K_{OC})
- water solubility

Chemical reactivity

- oxidation
- reduction
- hydrolysis
- precipitation
- polymerization

Soil contamination parameters

- concentration in soil
- depth of contamination

TABLE 3. PROPOSED/ACTIVE BIOREMEDIATION SITES

Site Name	Region	Contaminant
1. L.A. Clark & Sons	3	1*
2. American Creosote	4	1
3. Brown Wood Preserving	4	1
4. Crosby	4	1
5. Wilmington	4	1
6. Burlington Northern	5	1
7. North Cavalcade Street	6	1
8. Old Inger	6	2**
9. Brio Refining	6	2
10. Joplin	7	1*
11. Baxter/Union Pacific	8	1
12. Burlington Northern	8	1
13. Libby	8	1
14. ARCO	8	3***
15. Koppers Company	9	1
16. J.H. Baxter	9	1

* Wood Preserving

*** Coal Gasification

preliminary evaluation. Bioremediation is often limited by factors that include: (1) distribution of the waste which may limit microorganism access to the waste, (2) supply of nutrients required for metabolism, (3) toxicity of the waste due to concentration and/or type of constituents present, (4) formation and accumulation of toxic byproducts, (5) inadequate population(s) of requisite microorganisms, (6) non-competitiveness of non-survivability of inoculated cultures, and (7) inadequate management of the system. Prior to the application of on site bioremediation, the factors identified above should be addressed.

The importance of conducting treatability experiments with appropriate controls and conducting a site characterization to identify environmental, soil, and ecological factors that will affect the process under field conditions cannot be overemphasized. Evaluation of commercial claims should involve side-by-side comparisons in time using appropriate and statistically rigorous control experiments that faithfully duplicate the commercial process but without inclusion of the commercial product.

Monitoring of treatment effectiveness in the vadose zone involves the evaluation of chemical and toxicity changes with time. Both soil core and soil-pore liquid samples are recommended, and in some cases, air monitoring is recommended. Monitoring strategies can be based upon information obtained in the characterization and treatability phases of the bioremediation of a site.

Subsurface Treatment

In general, biodegradation of hazardous organic chemicals in groundwater is not limited by the metabolic capability of microorganisms. However, the prospects for biodegradation is severely limited by the stoichiometry of microbial metabolism, and by mass transport limitations of the rate of supply of essential nutrients. These limitations determine the cost to remediate a site, the time required, and the level of remediation that can be attained. Practical application of biotechnology in the subsurface depends on an accurate three-dimensional understanding of the position and concentration of the contaminants, of the hydrology of the contaminated material, and an estimate of quantity of oxygen or other electron-acceptor required to remediate the site. This challenge is well illustrated in a demonstration project supported by the U.S. EPA and the U.S. Coast Guard on the *in situ* bioremediation of a fuel spill. Aviation gasoline was spilled from an underground storage tank at the Coast Guard Air Station at Traverse City, Michigan. The gasoline drained through unconsolidated sands until it reached the water table, then it spread laterally. Groundwater flows through the material contaminated with gasoline, and carries a plume of alkylbenzenes and other fuel hydrocarbons away from the original spill area. The Coast Guard and EPA plan to remediate the spill by perfusing it with oxygen and hydrogen peroxide. The alkylbenzenes are the object of the regulatory concern, and the

bioremediation will be finished when their concentration is brought to a level specified by the Michigan Department of Natural Resources.

The spill was cored to identify the depth interval that was contaminated, and the highest concentration of fuel hydrocarbons. The cores were extracted with methylene chloride, then analyzed by gas chromatography. The gasoline was confined to a narrow interval between 15 and 17 feet below the land surface. This interval corresponds closely with the seasonal high and low water table at the site. The concentration of fuel hydrocarbons in the most contaminated interval averages 7,500 mg/kg aquifer material. The porosity of the contaminated sand is 0.4, and its bulk density is 0.2 g/cm^3. Therefore, the water content of the aquifer is 0.2 liter/kg, and each liter of pore water is in contact with 37,500 mg of fuel hydrocarbons. The oxygen demand for microbial respiration of total fuel hydrocarbons was estimated assuming the following stoichiometry:

$$CH_2 + 1.5\ O_2 \longrightarrow CO_2 + H_2O$$

The oxygen demand of the alkylbenzene fraction alone was estimated from:

$$CH + 1.25\ O_2 \longrightarrow CO_2 + 0.5\ H_2O$$

Monitoring wells were installed 31 and 50 feet down gradient from the injection wells. Of the 31 feet between the injection wells and the first monitoring well, 15 feet was considered to be contaminated. Of the 50 feet to the next monitoring well, 35 feet was consider to be contaminated. The concentrations of hydrocarbons, the length of the contaminated portion of the flow path, and the assumed stoichiometry for microbial respiration were used to estimate the total oxygen required to remediate the flow paths to the two monitoring wells (Table 4). The spill was cored in August, 1987 to provide information to design the demonstration, then cored again in March, 1988, just before the demonstration began, to define the initial conditions. The concentration of alkylbenzenes in the spill declined dramatically over the time interval (Table 5). This was probably due to anaerobic microbial degradation.

For the first 140 days of the demonstration, the injected water contained 40 mg/liter oxygen. Then the oxygen was replaced with 80 mg/liter hydrogen peroxide for 20 days. Then the concentration of hydrogen peroxide was stepped up to 160 mg/liter for 50 days, and finally to 360 mg/liter for 80 days. Concentrations of alkylbenzenes and oxygen or hydrogen peroxide was monitored in the wells. The interval between the injected wells and the monitoring well at 31 feet was remediated after 220 days, and the interval to the monitoring well at 50 feet after 270 days.

TABLE 4. STOICHIOMETRY OF AEROBIC BIOREMEDIATION OF A FUEL SPILL

	Oxygen and Hydrogen Peroxide Demand along Flowpath to Monitoring wells at:	
	31 feet	50 feet
	--(mg oxygen/liter pore water)--	
Estimated demand based on:		
Total Fuel Hydrocarbons	62,212	90,000
Alkylbenzene content only, when sampled in 8/87	8,710	12,000
Alkylbenzene content only, when sampled in 3/88 just before the start of the demonstration	2,364	3,420
Actually required	2,989	2,952

TABLE 5. QUANTITIES OF ALKYLBENZENES AND TOTAL FUEL HYDROCARBONS REMAINING IN AN AQUIFER AFTER BIOREMEDIATION USING OXYGEN AND HYDROGEN PEROXIDE.

Parameter	Before Remediation 8/87	Just Before Remediation 3/88	After Remediation 10/88
	(mg/kg aquifer material)		
Total fuel hydrocarbons	6,500	1,200*	8,400
Toluene	544	37	<0.3
m+p-Xylene	58	<1	<0.3
o-Xylene	42	8.4	<0.3
Benzene	0.3	0.6	<0.3

*A composited sample containing clean as well as contaminated material.

It is not surprising that the non-aromatic fraction of the spill remained in the aquifer. A very minor fraction of their oxygen demand had been supplied when the aquifer was cleansed of alkylbenzenes.

A tracer test was done with chloride to determine the seepage velocity in the flow path from the injection wells to the monitoring wells. The velocity was multiplied by the concentration of oxygen or hydrogen peroxide along the flow path. The flux was multiplied by the time required for remediation to determine the actual oxygen demand for remediation (Table 4).

Aviation gasoline is composed primarily of branched chain alkanes. The material spilled at Traverse City was 38 percent 2,2,4-trimethylhexane, 7 percent 2,3-dimethylhexane, and 5 percent 2,4-dimethylpentane. Only 10 percent of the original spill was alkylbenzenes.

The aquifer was purged of alkylbenzenes very quickly. The quantity of oxygen and hydrogen peroxide required to remove alkylbenzenes from the wells agree closely with the projected oxygen demand of the alkylbenzenes alone. This selective removal of alkylbenzenes may result from their relatively high water solubility. Projected from Raoult's Law, the expected concentration of toluene in water in equilibrium with the fuel was 15 mg/liter. The expected concentration of 2,2,4-trimethylpentane is only 0.2 mg/liter.

Shortly after remediation, the area near the monitoring well at 31 feet was cored and analyzed for alkylbenzenes and total fuel hydrocarbons. Results were compared to earlier cores to determine whether the contaminants were removed from the aquifer material itself (Table 5).

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DISTINCTION BETWEEN SURFACE AND SUBSURFACE REMEDIATION

- **surface** treatment: dominant electron acceptor is oxygen supplied directly from the atmosphere
- **subsurface** treatment: electron acceptor is supplied by perfusing the contaminated material with water or air

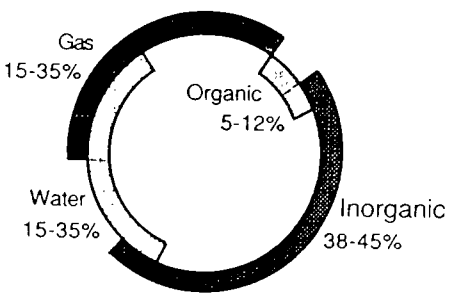
NOTES

NOTES

IN SITU TREATMENT OF CONTAMINATED SOIL

NOTES

Typical Volumetric Composition Of Soil



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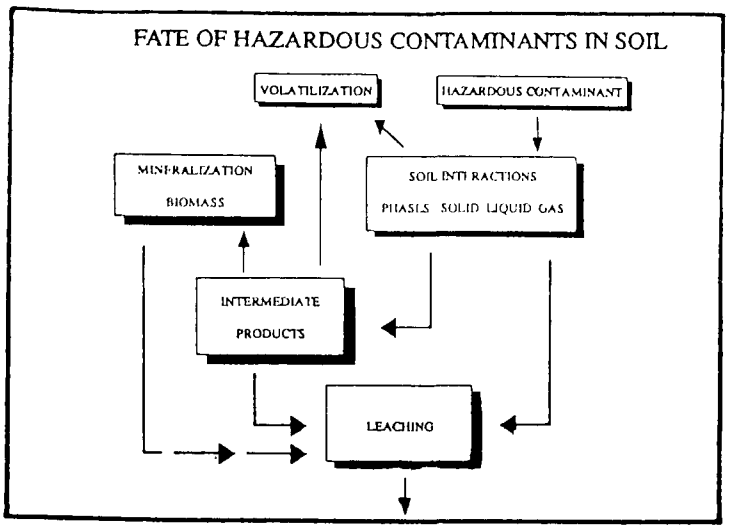

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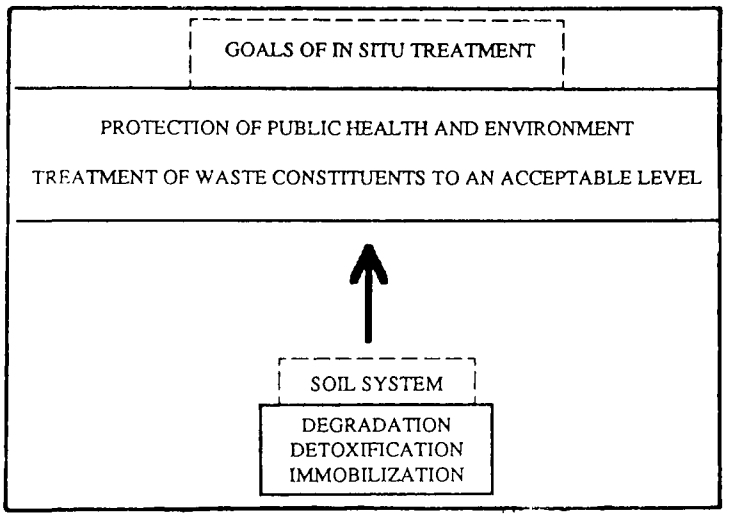


NOTES

PROPOSED/ACTIVE BIOREMEDIATION SITES

Site Name	Region	Contaminant
1. L.A. Clark & Sons	3	1*
2. American Creosote	4	1
3. Brown Wood Preserving	4	1
4. Crosby	4	1
5. Wilmington	4	1
6. Burlington Northern	5	1
7. North Cavalcade Street	6	1
8. Old Inger	6	2**
9. Brio Refining	6	2
10. Joplin	7	1*
11. Baxter/Union Pacific	8	1
12. Burlington Northern	8	1
13. Libby	8	1
14. ARCO	8	3***
15. Koppers Company	9	1
16. J.H. Baxter	9	1

* Wood Preserving
*** Coal Gasification

CHARACTERIZATION

NOTES

NOTES

SOIL-BASED WASTE CHARACTERIZATION

Chemical Class	Soil Sorption Parameters	Soil Degradation Parameters	Chemical Properties
Acid Base Polar Neutral Nonpolar Neutral Inorganic	Freundlich Sorption Constants (K,N) Sorption based on Organic Content (K_{oc}) Octanol water partition Coefficient (K_{ow})	Half-life ($t_{1/2}$) Rate Constant Relative bio-degradability	MolecularWeight Melting point Specific Gravity Structure Water Solubility

NOTES

SOIL-BASED WASTE CHARACTERIZATION

Volatilization Parameters	Chemical Reactivity	Soil Contamination Parameters
Air:water partition coefficient (K_w) Vapor pressure Henry's law constant ($1/K_w$) Sorption based on organic carbon content (Koc) Water solubility	Oxidation Reduction Hydrolysis Precipitation Polymerization	Concentration in soil Depth of Contamination

NOTES

BIOLOGICAL DEGRADATION

Half-life of a PAH Compound:

$$t_{1/2} = \frac{0.693}{k}$$

Where

$t_{1/2}$ = half-life of PAH compound in soil (time)

k = first-order rate constant ($time^{-1}$) for microbial degradation

NOTES

IMMOBILIZATION

$$R = 1 + \frac{\rho K_d}{\theta}$$

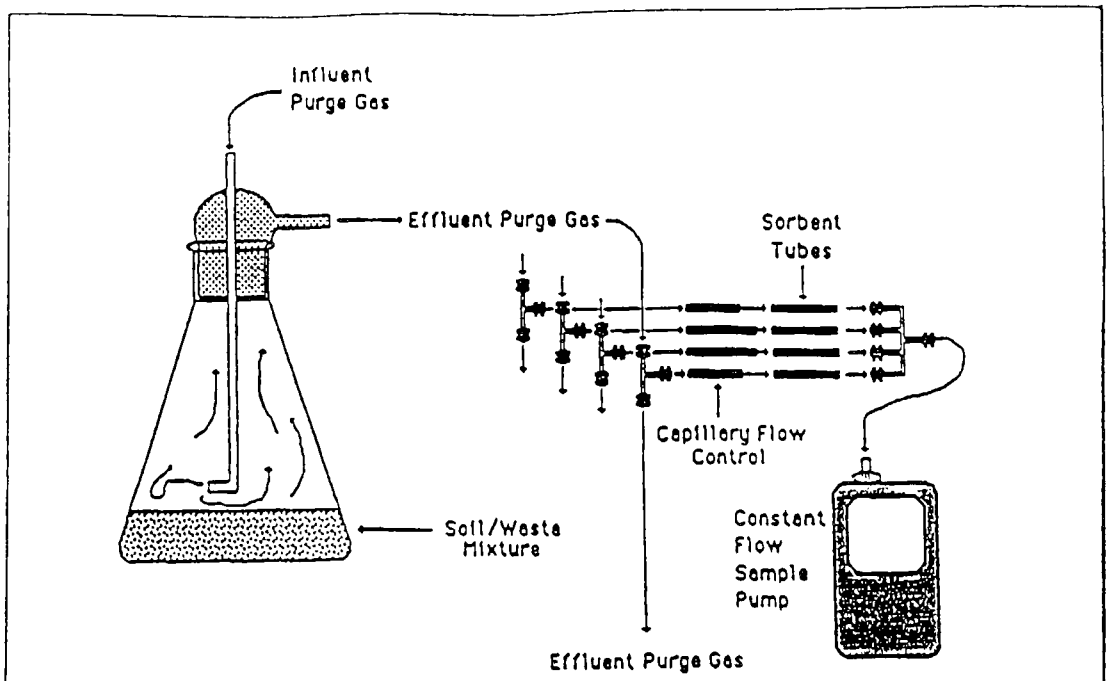
ρ = soil bulk density

K_d = partition coefficient

θ volumetric moisture content

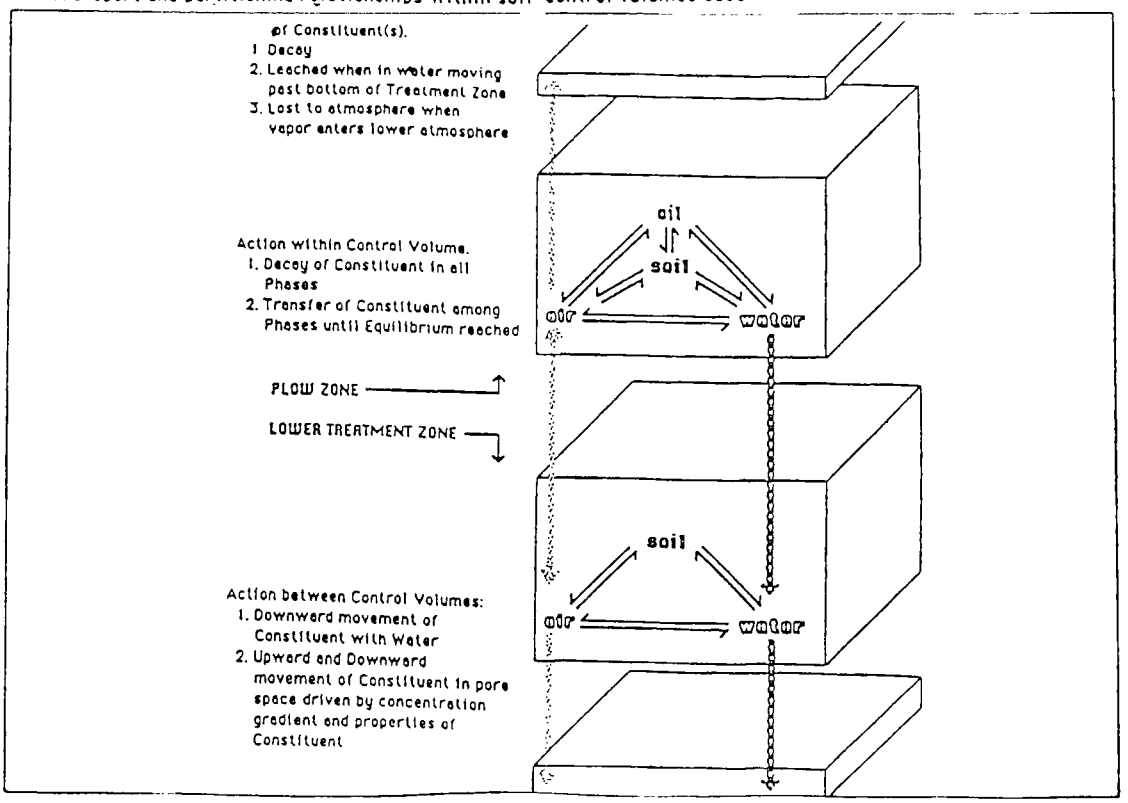
NOTES

INTERPHASE TRANSFER POTENTIAL



Laboratory flask apparatus used for mass balance measurements.

Transport and partitioning relationships within soil control volumes used in modified RITZ model

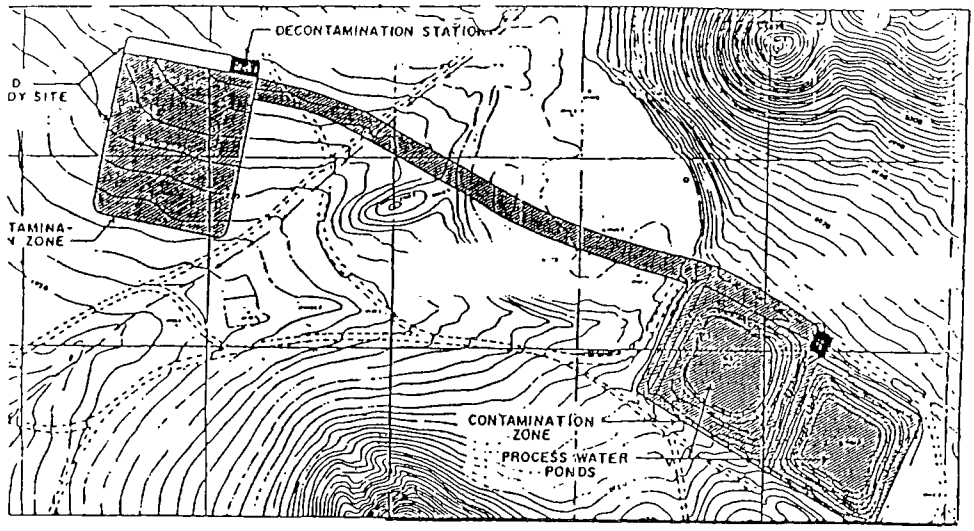


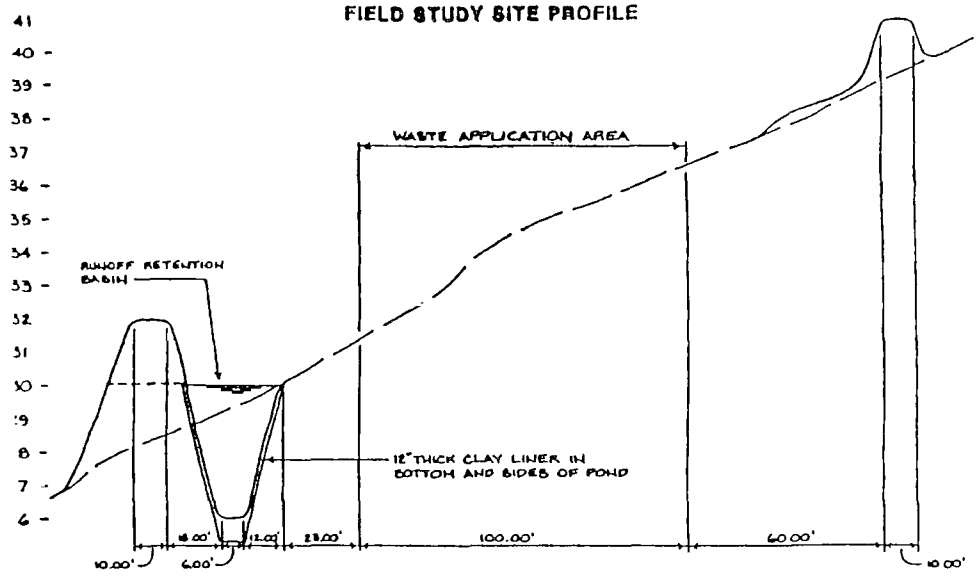
DETERMINATION OF CONTAINMENT REQUIREMENTS

PROBLEM FOR ASSESSMENT

If the rate of transport (leaching) is significant compared with the rate of biodegration, both factors must be considered (degradation and leaching)

The constituent(s) may reach a "critical depth" in the soil before being degraded


DECONTAMINATION STATION
CONTAMINATION ZONE
PROCESS WATER PONDS


FIELD STUDY SITE PROFILE
WASTE APPLICATION AREA
RUNOFF RETENTION BASIN
12" THICK CLAY LINER IN BOTTOM AND SIDES OF POND
100.00'
60.00'

NOTES

ENHANCEMENT OF MICROBIAL ACTIVITY

NOTES

REMEDIATION BASED ON ASSESSMENT

Increasing the degradation factor allows faster reduction in mass flow of the parent compound(s) and degradation products through the soil system toward ground water and surface water receiver systems.

NOTES

SOIL/SITE ASSIMILATIVE CAPACITY (SSAC)

Techniques

(1) Soil incorporation or mixing
(2) Aeration of the soil
(3) Addition of nutrients
(4) Addition of microbial carbon and energy sources
(5) Water addition (irrigation)
(6) Drainage
(7) Runon and Runoff Controls
(8) pH adjustment

NOTES

WAYS TO MAXIMIZE AVAILABLE SOIL OXYGEN

- Prevent Water Saturation
- Presence of Sand, Loam (Not Hvy Clay)
- Moderate Tilling
- Avoid Compaction
- Controlled Waste Loading

NOTES

EFFECT OF MANURE AND pH AMENDMENTS ON PAH DEGRADATION IN A COMPLEX WASTEINCORPORATED INTO SOIL

PAH Compound	Half-Life In Waste:Soil Mixture (Days)	
	Without Amendments	With Amendments
Acenaphthylene	78	14
Anthracene	28	17
Phenanthrene	69	23
Fluoranthene	104	29
Benz(a)antrhacene	123	52
Benz(a)pyrene	91	69
Dibenz(a,h)anthracene	179	70

NOTES

EFFECT OF SOIL MOISTURE ON PAH DEGRADATION

Moisture (Field Capacity)	Half-Life (Days)		
	Anthracene	Phenanthrene	Fluoranthene
20 - 40	43	61	559
60 - 80	37	54	231

TEMPERATURE EFFECT ON DEGRADATION RATE

Compound	Half-Life (days)[a] 10 C	20 C	30 C
Fluorene	60 (50-71)	47 (42-53)	32 (29-37)
Phenanthrene	200 (160-240)	<60	<60
Anthracene	460 (320-770)	260 (190-420)	200 (170-290)
Pyrene	f	1900 (1100-8100)	210 (150-370)
Benzo(a)pyrene	530 (300-2230)	290 (170-860)	220 (160-380)

a Half-life (95% confidence interval)
f Least squares slopes = zero with 95% confidence

NOTES

NOTES

ACCLIMATION OF SOIL TO COMPLEX FOSSIL FUEL WASTE

PNA Constituent	Unacclimated Soil Reduction in 40 days (%)	Acclimated Soil Reduction in 22 days (5)
Naphthalene	90	100
Phenanthrene	70	83
Anthracene	58	99
Fluoranthene	51	82
Pyrene	47	86
Benz(a)anthracene	42	70
Chrysene	25	61
Benz(a)pyrene	40	50

NOTES

EVALUATION OF TREATMENT

NOTES

PERFORMANCE EVALUATION-- MONITORING

- Soil Cores
- Soil-Pore Liquid
- Ground Water
- Runoff Water
- Air

NOTES

DEGRADATION IN CLAY LOAM SOIL

2 % Oil and Grease

Compound	C_o µg/g	$T_{1/2}$ days	R^2	95% Confidence Interval ($T_{1/2}$) (days) Lower	Upper
Fluoranthene	351	15	0.966	13	18
Pyrene	283	32	0.884	26	41
Benzo(a)anthracene	86	139	0.397	87	347
Benzo(g,h,i,)perylene	8	1661	0.006	139	ND
Indenopyrene	5	69	0.559	43	139

C_o - Initial Concentration

$T_{1/2}$ - Half-Life (first order kinetics)

NOTES

(^{14}C) 7,12-DIMETHYLBENZ(a)ANTHRACENE AND TRANSFORMATION PRODUCTS IN A SANDY LOAM SOIL

Time (days)	^{14}C in each fraction (%) Soil Extract: Parent Compound	Soil Extract: Transformation Products	Residue	CO_2	Total
0	62 (69)	4 (6)	12 (13)	0 (0)	78 (88)
14	26	43	16	0	85
28	20 (60)	53 (11)	17 (16)	0 (0)	90 (87)

Poisoned (control) data in parentheses

FIELD RESULTS FOR SOIL SAMPLES

Compound	C_o (µg/g)			91 days (µg/g)		
	AVG	SD	CV (%)	AVG	SD	CV(%)
Naphthalene	186	68	37	3	1.8	61
Acenaphthene	729	276	38	1	1.8	157
Phenanthrene	78	28	36	2.6	0.6	23
Benz(a) anthracene	86	42	49	2	0.8	38
Dibenz(a,h) anthracene	52	36	69	ND	--	--

C_o = Initial Soil Concentration

NOTES

NOTES

REMEDY SELECTION FACTORS

NOTES

SITE CONSTRAINTS

NOTES

COSTS

Scope	Current Dollars
● Laboratory Treatability Study	-- 50,000-100,000
● Pilot Scale Study	-- 150,000-200,000
● Full Scale Study	-- 400,000 +

NOTES

FIELD IMPLEMENTATION COSTS

- Land Area Requirements
- Site Preparation
- Amendments
- Equipment
- Maintenance
- Monitoring

DISTINCTION BETWEEN SURFACE AND SUBSURFACE REMEDIATION

For the purpose of this discussion, treatment will be considered **surface** treatment if the dominant electron acceptor is oxygen supplied directly from the atmosphere, and **subsurface** treatment if the electron acceptor is supplied by perfusing the contaminated material with water or air.

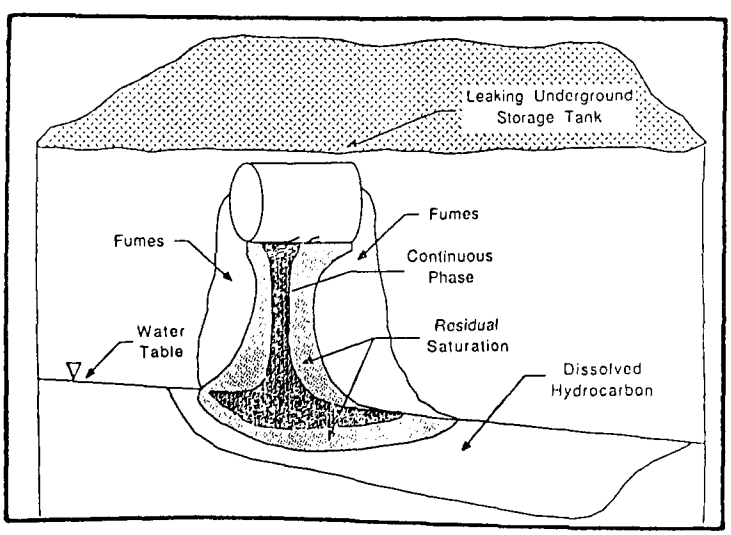
NOTES

NOTES

PRIMARY EMPHASIS IN SUBSURFACE REMEDIATION

Hazardous wastes that occur as a **discrete oily-phase** act as source areas for plumes of contamination in ground water. They also contaminate the soil air with hazardous fumes. The primary emphasis in subsurface bioremediation has been the **source areas**. Subsurface bioremediation of the plumes is often technically feasible, but it is usually easier to pump them out and treat them on the surface.

NOTES



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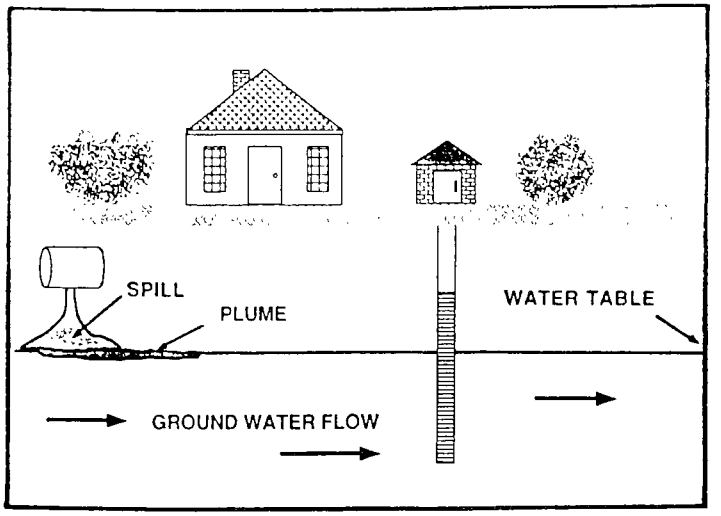

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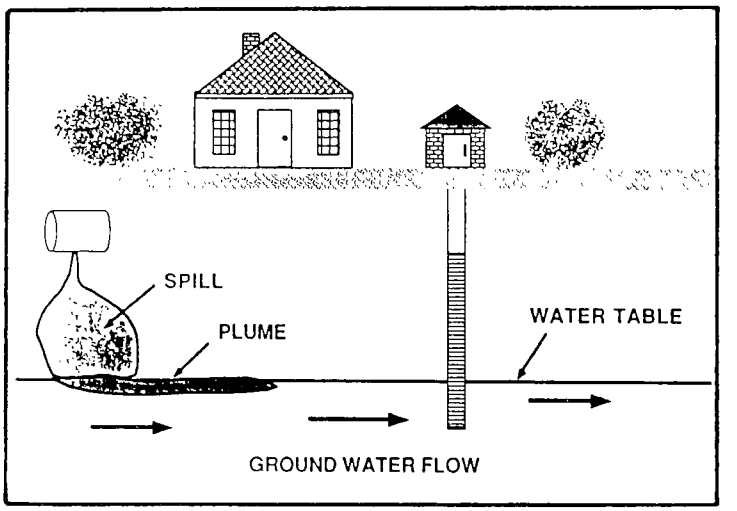

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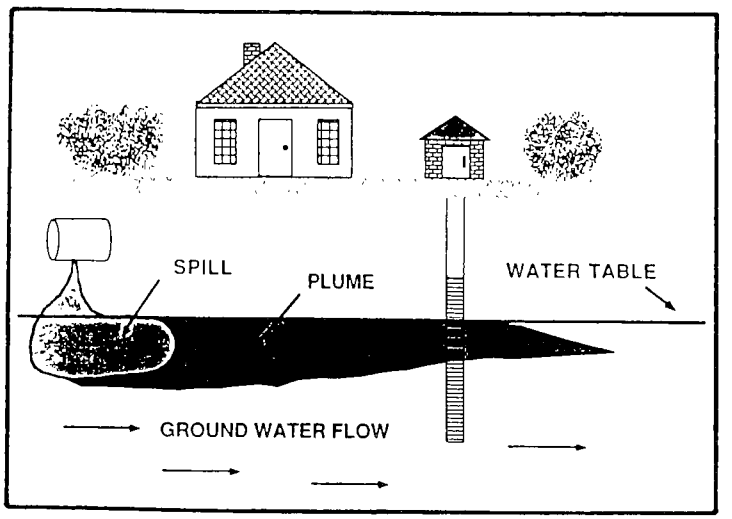

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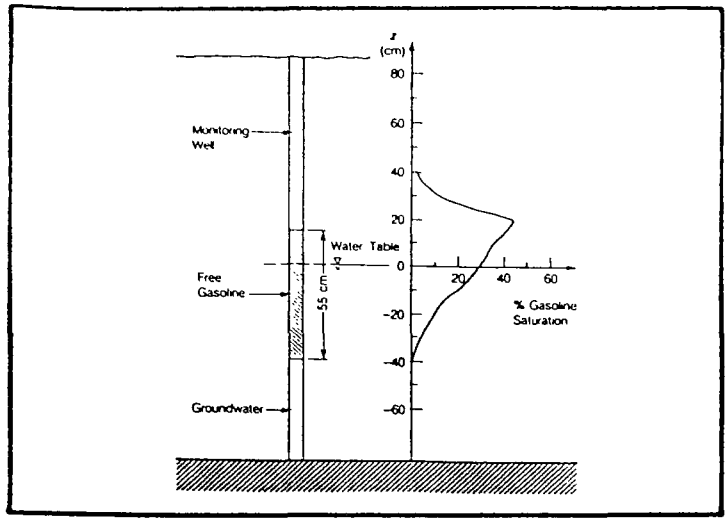

NOTES

IDENTIFY THE MOST CONTAMINATED FLOW PATH

Some regions of the source area will clean up faster than others. One flow path will **be the last to clean up.** If this flow path can be identified, then its properties can be used to determine how much effort is required to remediate the entire source area, and how long it will take.

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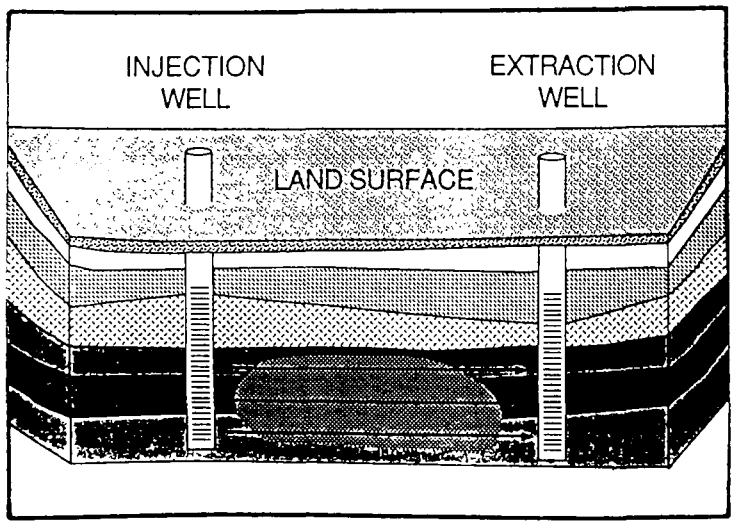

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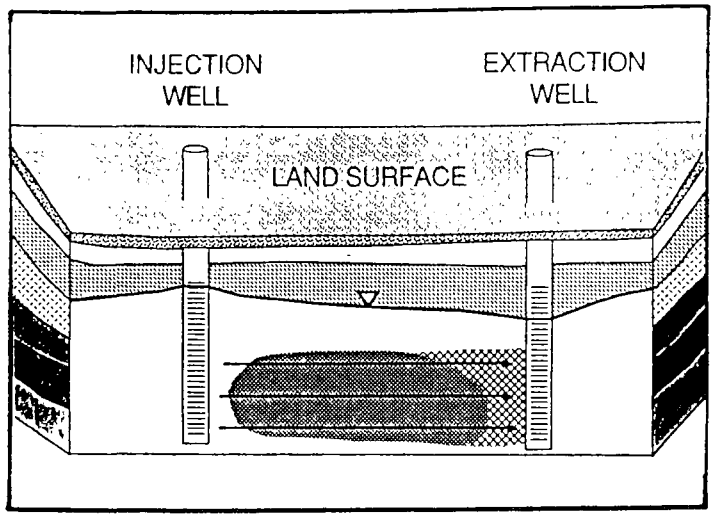

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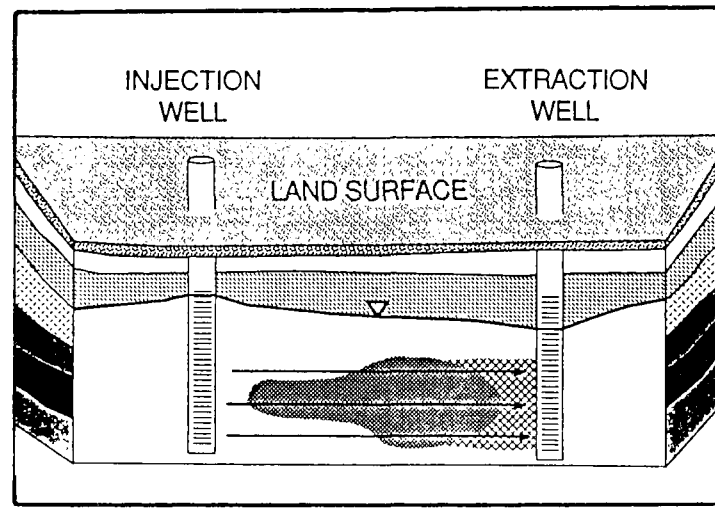

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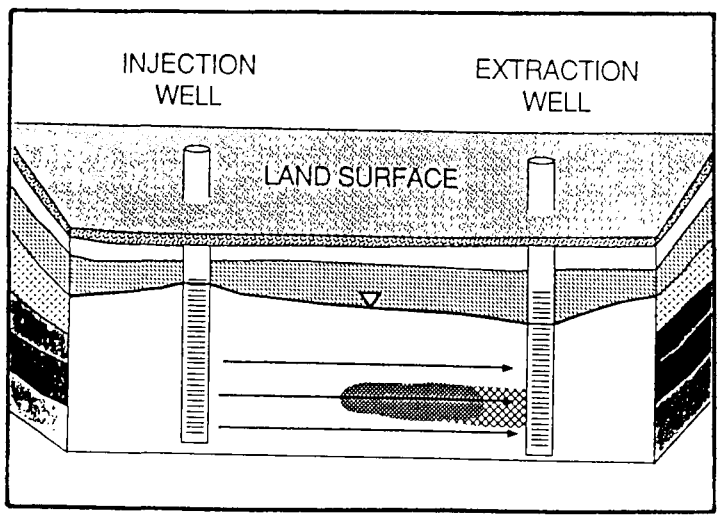

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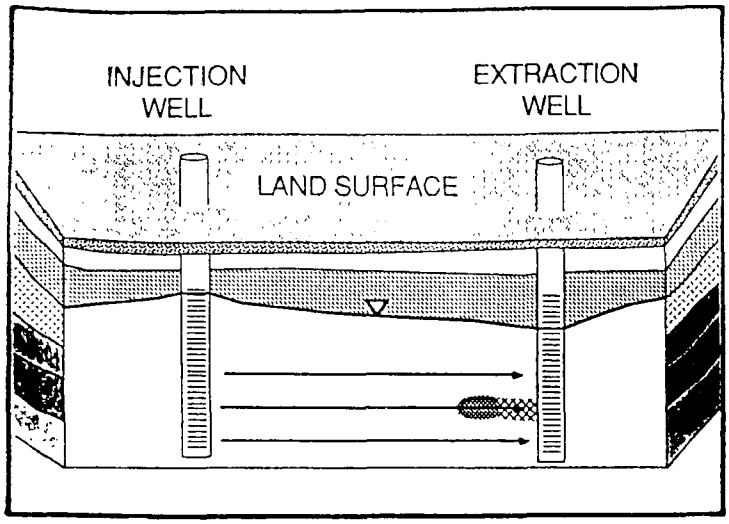


NOTES

If the supply of mineral nutrients is adequate, the rate of bioremediation is the rate of supply of electron acceptor. As a result, the rate of remediation is directly proportional to the **concentration of electron acceptor** in the injected water, and directly proportional to the **flow velocity of water** through the source area.

NOTES

CHARACTERIZATION OF THE MOST CONTAMINATED INTERVAL

$$\text{Time required to clean most contaminated flow path} \propto \frac{\text{Length of path through source area} \times \text{Concentration of contaminant along flow path}}{\text{Seepage velocity along the most contaminated flow path}}$$

NOTES

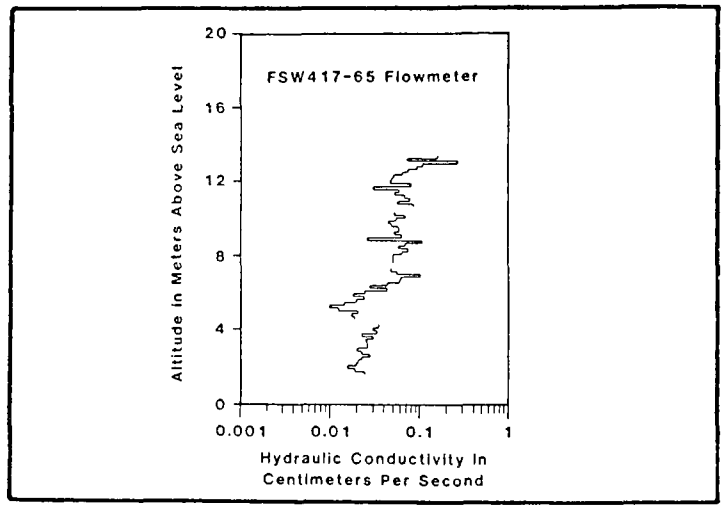
CONTROL OF HYDROLOGY ON THE RATE OF REMEDIATION

Seepage Velocity α Hydraulic Permeability x Hydraulic Gradient

Hydraulic permeability is an intrinsic property of the subsurface. It is difficult or impossible to improve it, but it is easily degraded.

The hydraulic gradient is controlled by the amount of water available for pumping, and by the difference in elevation between the source area and the land surface.

NOTES



NOTES

HOW TO PLUG UP AN INJECTION WELL

Add oxygen or hydrogen peroxide to water with Fe^{+2}

-> get $Fe(OH)_3$

Add oxygen or hydrogen peroxide to water with Mg/l of organics

-> get biofouling

Add phosphate to aquifer with Ca (Mg) CO_3 matrix

-> Ca (Mg) PO_4

NOTES

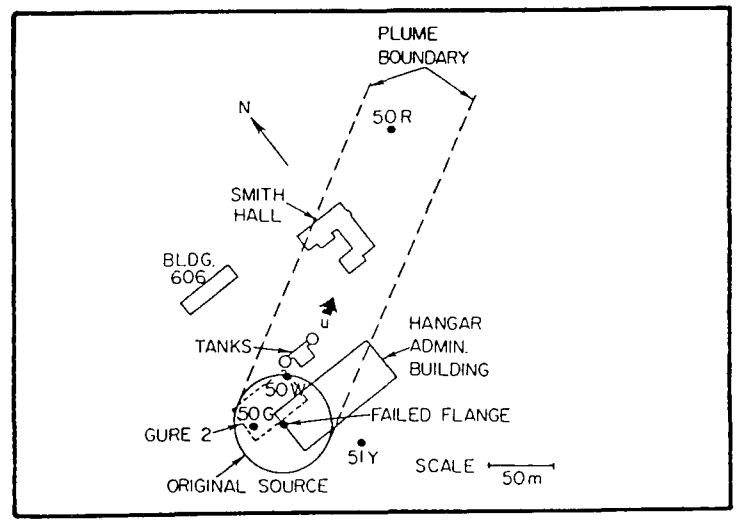
PROBLEMS WITH WELLS AS MONITORING TOOLS

Treatment can occur in the well itself. The water in the well may not be representative of the water in the aquifer.

A conventional monitoring well produces **a composited water sample.** Water from the most contaminated flow path is diluted by water from many other flow paths that are less contaminated.

A water sample from a well tells nothing about the amount of hazardous material that is **absorbed** to aquifer solids or is **trapped** as an oily phase.

NOTES



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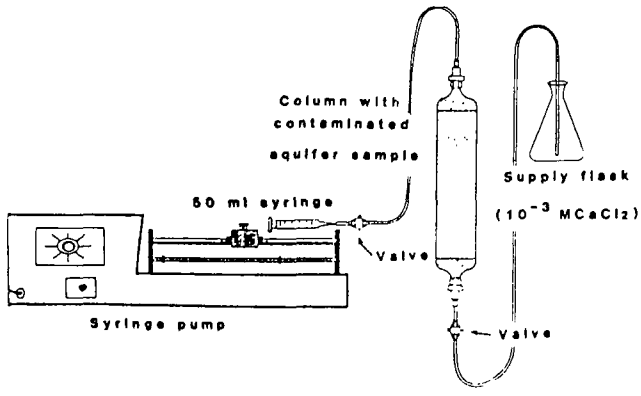
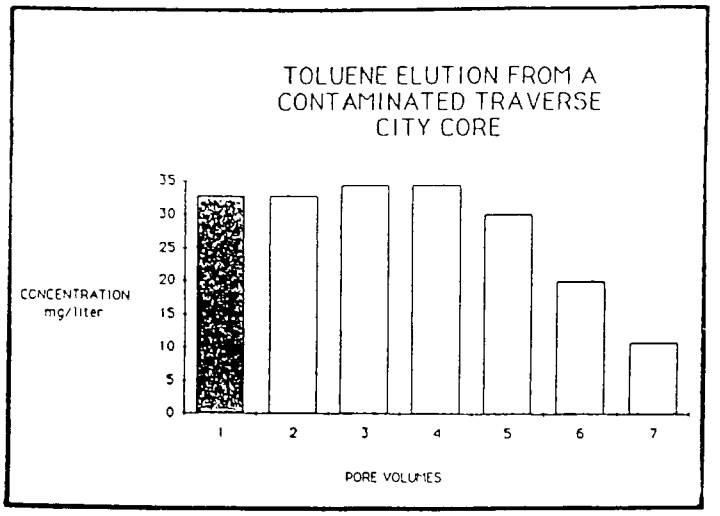


LEACHING COLUMN CONFIGURATION

NOTES



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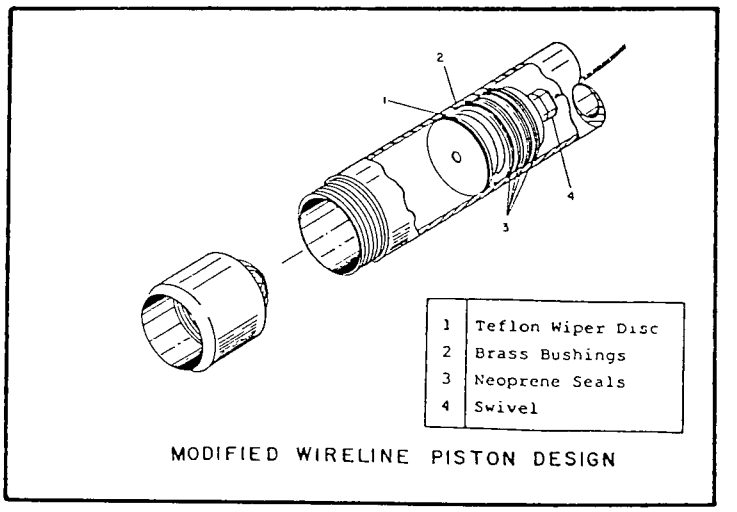

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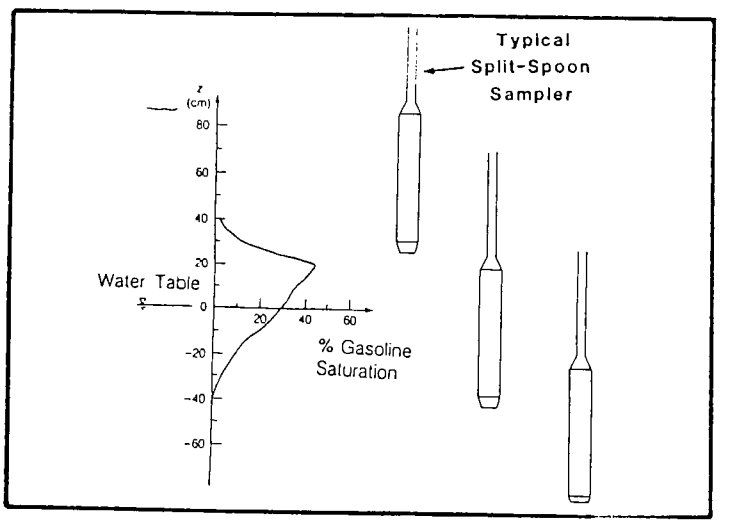


CO-DISTRIBUTION OF CONTAMINATION AND HYDRAULIC PERMEABILITY IN AN AQUIFER CONTAMINATED BY A FUEL SPILL

Depth Interval (feet below surface) Interval Cored or Screened Interval	Fuel Hydrocarbons (mg/kg aquifer)	Seepage Velocity (feet per day)
15.1 - 15.5	< 11	
15.5 - 15.8	39	
15.8 - 16.2	2370	
16.2 - 16.5	8400	7.2
16.5 - 17.2	624	
17.2 - 17.5	< 13	9.0
18.0 - 18.3	< 13	
19.4 - 19.6		15.6
20.9 - 21.4		19.7

NOTES

NOTES

In the most contaminated interval at Traverse City

The concentration of fuel hydrocarbons averages 7,500 mg/kg aquifer material, the porosity is 0.4, and the bulk density is 2.0 kg/dm^3.

Each kilogram of aquifer contains 0.2 liter of water, and each liter of pore water is exposed to 37,500 mg of fuel hydrocarbons.

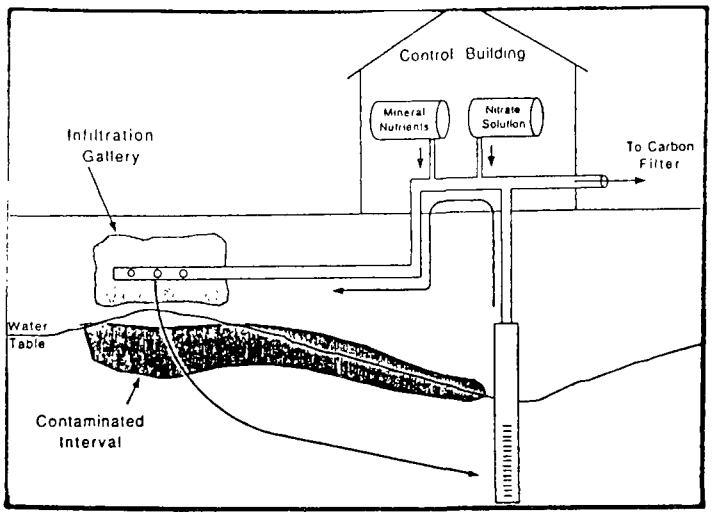
The oxygen demand of the hydrocarbons is 128,000 mg O_2 per liter pore water.

NOTES

HYDRAULIC CONTAINMENT

The migration of a plume away from its source area can often be prevented by capturing the plume with a purge well. The well must pump hard enough to overcome regional flow in the aquifer. The flow from purge wells that is necessary to capture a plume depends on the hydraulic permeability of the aquifer, the regional hydraulic gradient, and the size of the source area.

NOTES

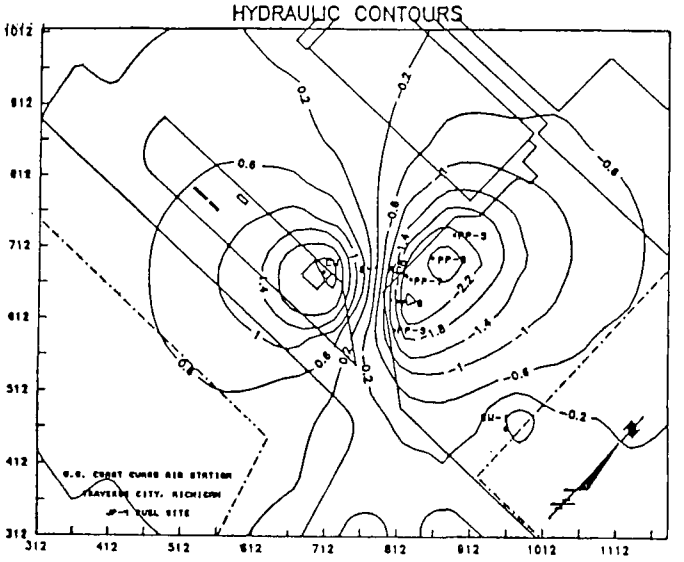


NOTES

HYDRAULIC CONTAINMENT OF SUBSURFACE REMEDIATION

Hydraulic containment of a source area can be achieved if **more water is extracted than injected.** If water is recirculated through the source area, a portion of the extracted water can be discharged to a sewer of surface drainage, resulting in a net extraction of water across the entire system.

HYDRAULIC CONTOURS



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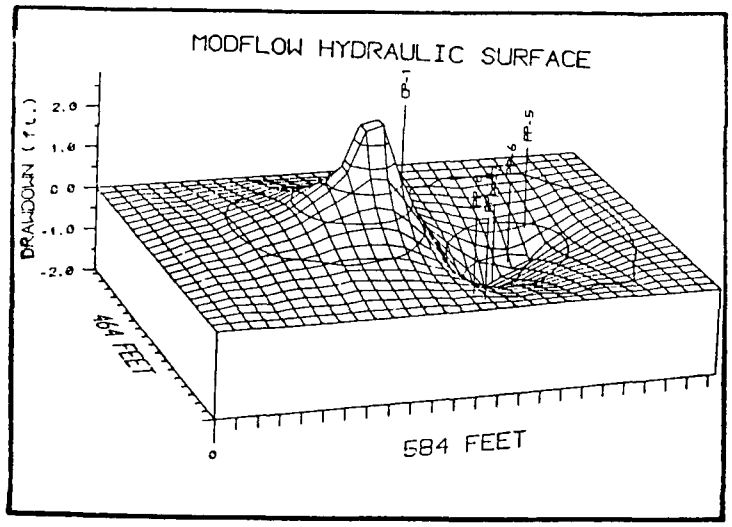


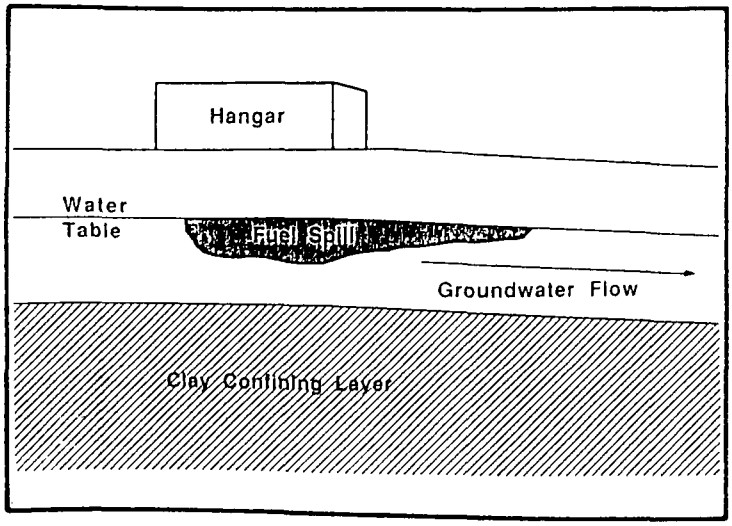
NOTES

NOTES

AQUIFERS AND NATURAL CONFINING LAYERS

Frequently, geological structures that readily yield water are layered above or between geological materials that do not readily transmit water. These **non-transmissive** layers can act as **natural containment** for subsurface bioremediation. Don't assume the bed rock is a confining layer; it is often fractured.

NOTES



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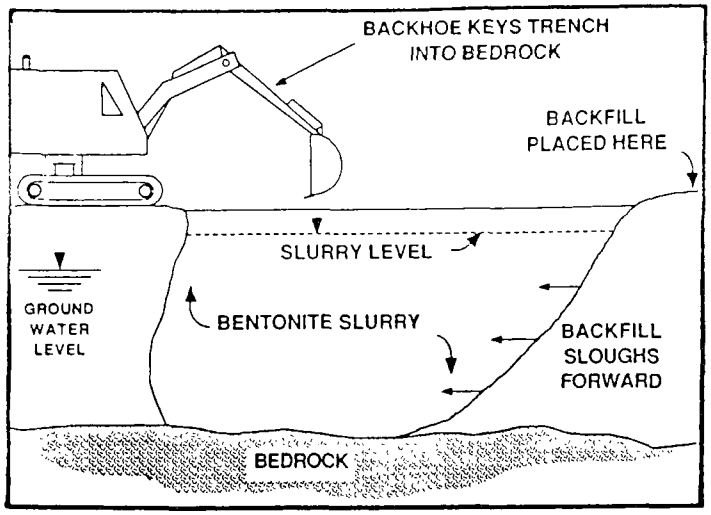

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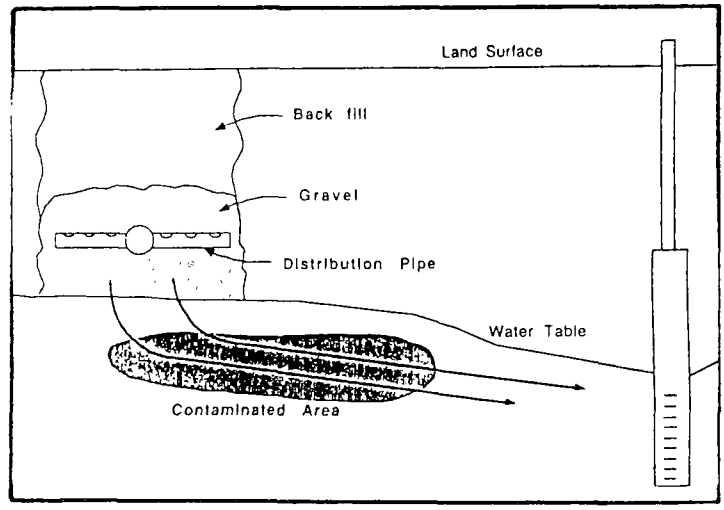

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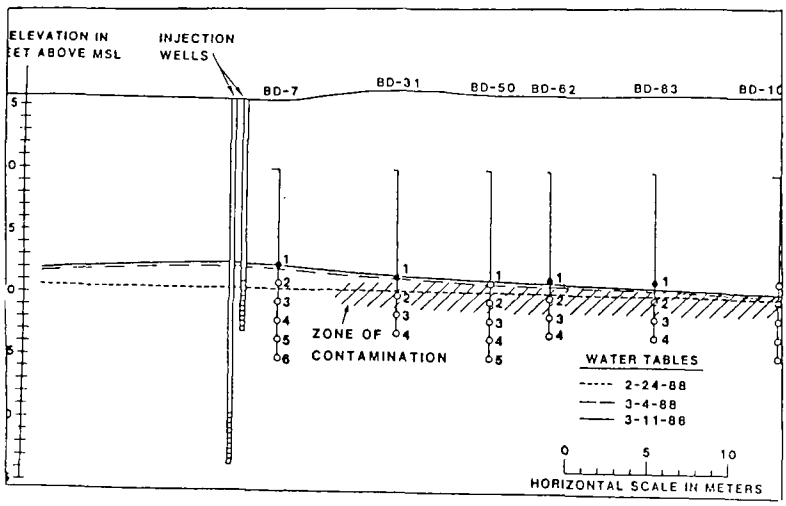

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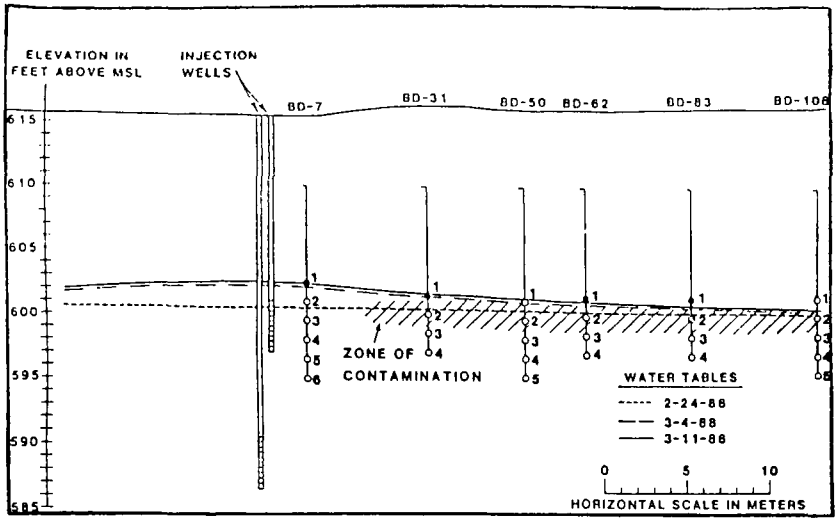


NOTES

FORMULATION OF NUTRIENT MIX

- Usually determined empirically
- Not related to C:N:P:S ratios
- Use high concentrations to project significant concentrations into the aquifer
- Should formulations be related to O:N:P:S ratios?

NOTES

PROPERTIES OF MOLECULAR OXYGEN

ADVANTAGES

- Low toxicity to acclimated organisms
- Supports removal of many organic compounds
- Inexpensive

DISADVANTAGES

- Low solubility in water
- Will precipitate iron hydroxide

NOTES

PROPERTIES OF HYDROGEN PEROXIDE

ADVANTAGES

- Miscible in water
- Supports bioremediation of many organic compounds
- Chemically oxidizes many organic and inorganic contaminants
- Removes biofouling

DISADVANTAGES

- Toxic at concentrations much above 500 mg/liter
- Will precipitate iron hydroxide
- Relatively expensive

NOTES

PROPERTIES OF NITRATE AS AN ELECTRON ACCEPTOR

ADVANTAGES

- Very soluble in water
- Low toxicity to microorganisms
- Does not cause precipitation of iron hydroxide
- Only aromatic compounds are removed
- Inexpensive

DISADVANTAGES

- A regulated substance
- Potential for accumulation of nitrite
- Only aromatic compounds are removed

NOTES

COST COMPARISON OF ELECTRON ACCEPTORS

Electron Acceptors	Bulk Cost (per kg)	Electrons Accepted (moles / kg)	Real Cost (per moles of electrons accepted)
Sodium Nitrate	$0.66	58.8	$1.12
Liquid Oxygen	$1.46	125.0	$1.17
Hydrogen Peroxide	$1.54	58.8	$2.62

NOTES

ADVANTAGES OF PULSING AMENDMENTS

If more than one amendment is required to promote subsurface bioremediation, they can be injected in alternating pulses. This **prevents undue production of biomass** near the injection system, which would otherwise plug the system.

High concentrations of hydrogen peroxide (>100,000 mg/liter) can **remove biofouling** and restore the efficiency in injection wells or injection galleries.

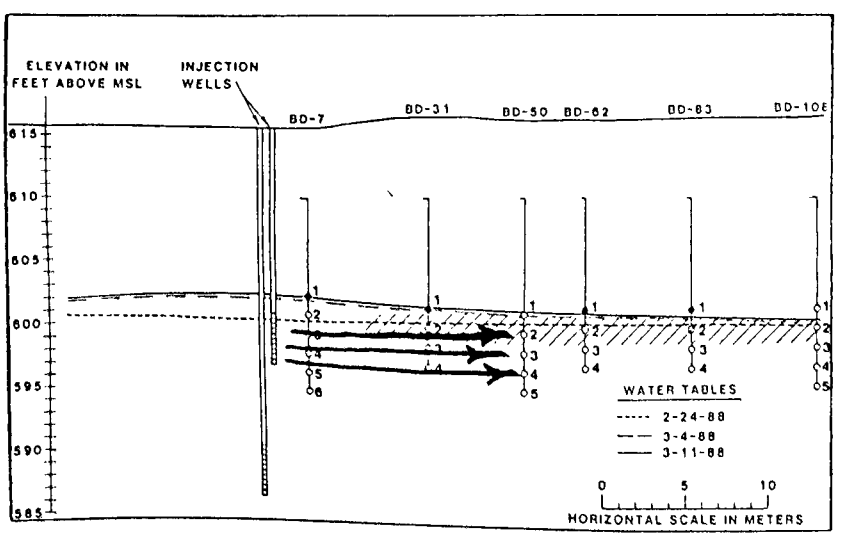
Pulses of hydrogen peroxide at high concentration can sterilize the aquifer and **destroy catalase activity**, preventing premature decomposition of the peroxide.

NOTES

MONITOR THE OPERATION OF THE SYSTEM AS WELL AS ITS PERFORMANCE

- Delivery of mineral nutrients
- Delivery of electron acceptor
- Position in the water table
- Effectiveness of containment

NOTES



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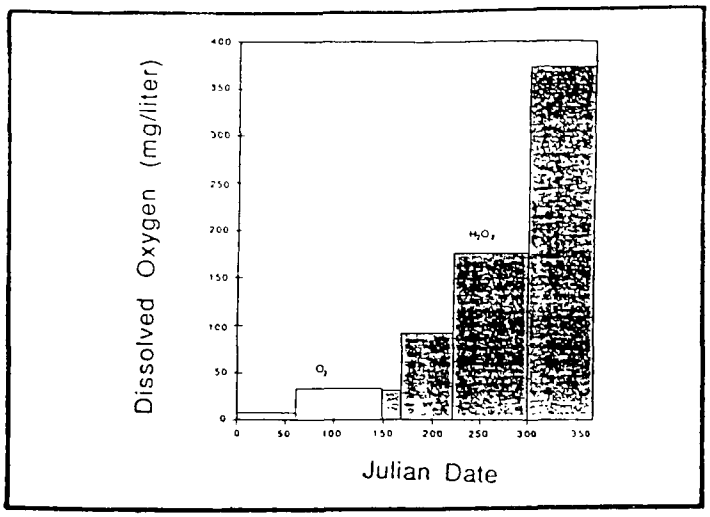

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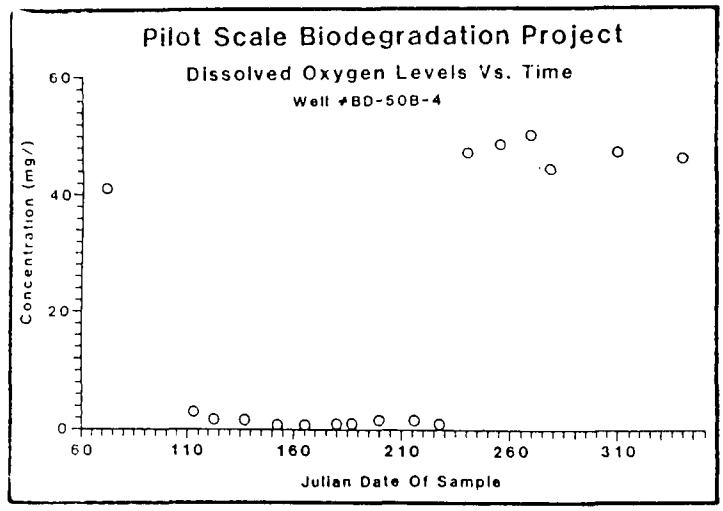

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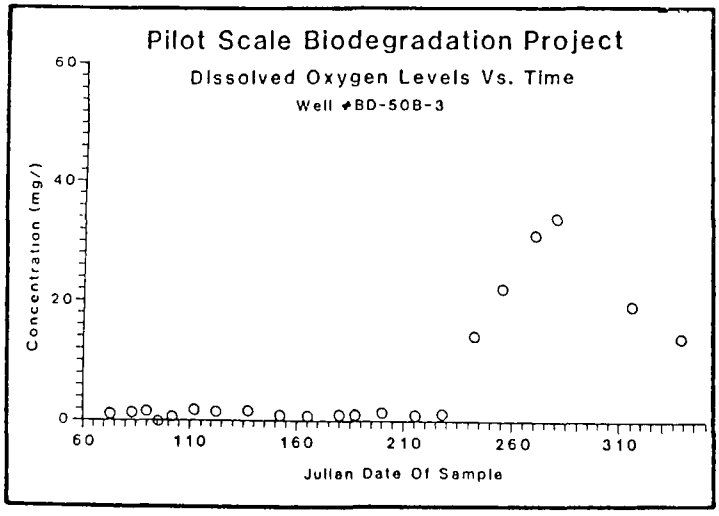

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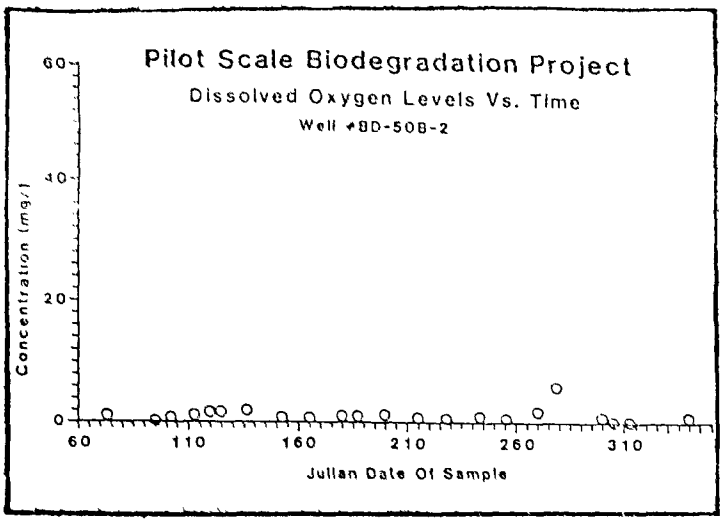

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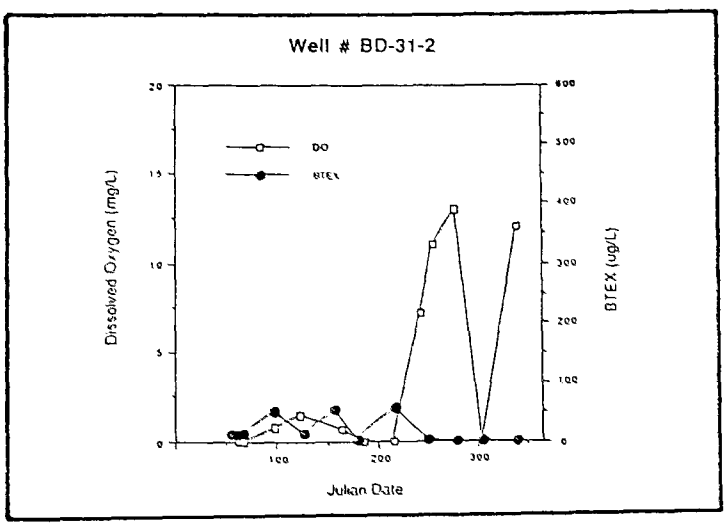

NOTES

PERFORMANCE OF BIORESTORATION NEAR BD 31

Parameter (mg/kg aquifer)	Before 8/87	Just Before 8/88	After 10/88
Total Fuel Hydrocarbon	6,500	1,220*	8,400
Toluene	544	37	<0.3
m + p Xylene	58	<1	<0.3
o - Xylene	42	8.4	<0.3
Benzene	0.3	0.6	<0.3

* Sample diluted with uncontaminated material.

NOTES

STOICHIOMETRY OF AEROBIC BIORESTORATION

Oxygen required	BD 31-2	BD 50B-2
	-----mg O_2 / liter pore water--	
Estimated based on:		
Total Fuel Hydrocarbons	62,212	90,000
BTX only (8/87)	8,710	12,000
BTX only (3/88)	2,364	3,420
Actually required	2,989	2,952

NOTES

HOW OFTEN SHOULD A MONITORING WELL BE SAMPLED?

The frequency of sampling should be related to the time expected for significant changes to occur along the most contaminated flow path.

IMPORTANT CONSIDERATIONS

- Time required for water to move from injection wells to the monitoring wells
- Seasonal variations in water-table elevation or hydraulic gradient.
- Changes in the concentration of electron acceptor.
- Cost of monitoring compared to day-to-day cost of operation.

NOTES

FACTORS CONTROLLING THE RATE AND EXTENT OF BIOREMEDIATION AT FIELD SCALE

- Rate of supply of essential nutrients, usually the electron acceptor
- Spatial variability in flow velocity
- Seclusion of the waste from the microorganisms

INTERPRETATION OF TREATABILITY STUDIES FOR SUBSURFACE REMEDIATION

A good treatability study determines whether bioremediation is **possible**, and whether there are any **biological barriers** to attaining the goal for clean-up. It can also provide an estimate on the rate of remediation that can be attained if the organisms are not limited by the rate of supply of some essential nutrient.

NOTES

NOTES

RATES OF OXYGEN CONSUMPTION IN THE MOST CONTAMINATED FLOW PATH AT TRAVERSE CITY

	Mg O_2 / Liter Day
Hydrogen Peroxide Injected	
7 feet from injection wells	60
Oxygen Injected	
7 feet from injection wells	≥20
31 feet from injection wells	≥ 8.1
50 feet from injection wells	≥ 7.3

NOTES

Rates and extent of treatment at field scale should be estimated with a comprehensive mathematical model that incorporates

- biological reaction rates
- stoichiometry of waste transformation
- mass-transport considerations
- spatial variability in treatment efficiency

COSTS ASSOCIATED WITH SUBSURFACE REMEDIATION

SITE CHARACTERIZATION

Wells, Soil Gas Survey, Coring and Core Analysis, Geological Section, Aquifer Tests, Tracer Tests

REMEDIAL DESIGN

Treatability Tests, Mathematical Modeling

SYSTEM DESIGN

Permits, Negotiating trade-offs between cost and time required

NOTES

NOTES

MORE COSTS ASSOCIATED WITH SUBSURFACE REMEDIATION

SYSTEM INSTALLATION

Wells, infiltration galleries, pumps, pipelines, tanks, control devices, treatment systems

MATERIALS AND OPERATING EXPENSES

Water, electron acceptor, fertilizer, inoculant, maintenance, power, sewer charges

MONITORING

Monitoring wells and pumps, cores and their analysis

SITE SECURITY AND OPERATIONAL OVERSIGHT

IN SITU TREATMENT DESIGN - SURFACE AND SUBSURFACE WORKSHEET

I. Site characterization

A. Surface

1. What are the important characteristics of the following elements for a "soil-based" characterization?

a) Soil factors

b) Engineering factors

c) Microbiology factors

2. What interphase transfer processes need characterization?

3. How do you use the information on interphase transfer processes for treatment and monitoring aspects in the vadose zone?

4. How can you characterize the following?

a) Potential for migration of chemicals at the site

b) Previous migration of chemicals at the site

B. Subsurface

1. What factors influence three dimensional distribution of oily phase material?

2. What factors influence three dimensional distribution of plume in solution?

3. What is the direction of groundwater flow?

4. What is the seasonal variation in direction of flow?

5. What is the seasonal variation in water table elevation?

6. What is the hydraulic conductivity in the most contaminated interval?

7. What is the frequency distribution of hydraulic conductivity across the contaminated interval?

8. What is the water filled porosity?

9. What is the concentration of oily phase contaminate along most contaminated flow line?

10. What is the relative concentration of regulated substances in the oily phase material?

II. Containment Requirements

A. Surface

1. Identify approaches for volatile chemicals

2. Identify approaches for leachable chemicals

3. How does one assess containment requirements?

B. Subsurface

1. Identify important boundaries in the flow field - rivers, pumping wells, impermeable layers

2. Determine if bed rock is fractured, or if it is a good confining layer

3. Can the system accept sheet piling?

4. Can the system accept a grout curtain?

5. Can the system accept a slurry wall?

6. Can the flow field be modelled as a steady state system?

7. Is there acceptable disposal for extracted water?

III. Appropriateness of in-situ treatment vs in-reactor treatment

A. Surface

1. Pros for in-situ treatment

2. Cons for in-situ treatment

3. Pros for in-reactor treatment

4. Cons for in-reactor treatment

B. Subsurface - Soils

1. Pros for in-situ treatment

2. Cons for in-situ treatment

3. Pros for in-reactor treatment

4. Cons for in-reactor treatment

C. Groundwater

1. Pros for in-situ treatment

2. Cons for in-situ treatment

3. Pros for in-reactor treatment

4. Cons for in-reactor treatment

IV. Enhancement of microbial activity

A. Surface

1. What factors affect the following biological processes?

a) Metabolism

b) Growth or reproduction

c) Activity

2. Identify important environmental factors

3. Identify important chemical factors

4. What factors affect the following processes?

   a) Rate and extent of "degradation" of a chemical

   b) Rate and extent of toxicity reduction

5. Identify approaches to evaluating the enhancement of microbial activity

B. Subsurface

1. How much electron acceptor is required to reclaim the most contaminated flow path?

2. What concentration of electron acceptor will the aquifer accept?

3. How soon must the site be reclaimed? How long can the interval be between injection and extraction well?

4. Is the nutrient mix compatible with the geochemistry of the groundwater and the aquifer matrix?
(Can this marriage be saved?)

5. How much water is available for injection? What is its quality?

6. Is inoculation required?

V. Evaluation of treatment

A. Surface

1. What types of information can treatability studies provide?

2. What types of information can be obtained from field monitoring?

3. How do you approach the following elements for evaluation of treatment?

a) Media to monitor

b) "Things" to monitor

c) When to monitor

4. Identify "target level" goals at a site

5. Identify factors affecting monitoring data variability

B. Subsurface

1. Does the nutrient mix adequately perfuse the source area?

2. Can the most contaminated interval be cored to evaluate performance?

3. Is sampling frequency related to flow velocity of water? To the expected rate of clean-up? To the distance from the injection wells?

4. Has reclamation left behind organic materials foreign to the aquifer?

VI. Remedy selection factors

A. Surface

1. How does the "pollutant pathways analysis" assist in identifying remedy selection factors?

2. How can time constraints affect remedy selection factors?

3. How can "site size" factors affect remedy selection factors?

4. Identify specific factors for remedy selection factors based on the following elements.

   a) Characterization of site

   b) Treatment evaluation (treatability studies)

   c) Constraints on filed implementation

B. Subsurface

1. Will the nutrient mix reduce hydraulic conductivity?

2. Is the treatability study an accurate description of the proposed technology?

3. What liability will be generated if containment fails?

4. Will variability in hydraulic permeability preclude reaching the target clean-up goals?

VII. Economics

A. Surface

1. What is the cost per unit volume of soil treated?

2. What is the cost comparison for treatment with other technologies?

3. What are the equipment needs at the site?

4. What are the monitoring costs?

5. Identify capital and operation and monitoring (O&M) costs

6. Identify "alternative" cost for different approaches based on in situ bioremediation

B. Subsurface

1. What is the most inexpensive electron acceptor?

2. What is the cost to identify and characterize the most contaminated flow path? How deep? What sort of material?

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