(ITRC, 2005)

Status Report on Innovative *In Situ* Remediation Technologies Available to Treat Perchlorate-Contaminated Groundwater

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ABSTRACT

Advancing low-cost, effective *in situ* treatment technologies is a proactive way to address perchlorate contamination in our nation's groundwater. EPA's Technology Innovation and Field Services Division provided a grant through the National Network for Environmental Management Studies (NNEMS) for an environmental science student to research and write a status report on innovative *in situ* treatment technologies available to remediate perchlorate-contaminated groundwater. This report was prepared during the summer of 2006 by Jennifer Hoponick, a 3rd year graduate student at Indiana University earning a joint degree in Environmental Science and Policy. It has been reproduced to help provide federal agencies, states, consulting engineering firms, private industries, and technology developers with information on the current status of *in situ* treatment technologies for perchlorate.

ACKNOWLEDGMENTS

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LIST OF ABBREVIATIONS

AFCEE	Air Force Center for Environmental Excellence
ATSDR	Agency for Toxic Substances and Disease Registry
bgs	below ground surface
CAH	Chlorinated Aliphatic Hydrocarbon
DCA	Dichloroacetic Acid
DCE	Dichloroethylene
DNAPL	Dense Non-Aqueous Phase Liquid
DoD	Department of Defense
DO	Dissolved Oxygen
DPRB	Dissimilatory Perchlorate-Reducing Bacteria
DWEL	Drinking Water Equivalent Level
EOS®	Edible Oil-in-Water Substrate
EPA	United States Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FFRRO	EPA's Federal Facilities Restoration and Reuse Office
gpm	gallons per minute
HRC®	Hydrogen Release Compound
IC	Ion Chromatography
IHDIV	Indian Head Division, NSWC
ISB	*In Situ* Bioremediation
kg	kilograms
LEL	Lower Explosive Limit
LHAAP	Longhorn Army Ammunition Plant
LLNL	Lawrence Livermore National Laboratory
MassDEP	Massachusetts Department of Environmental Protection
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MD	Maryland
MS	Mass Spectrometer
µg	micrograms
uS/cm	microsiemens per centimeter
mg	milligrams
mV	millivolts
NASA	National Aeronautics and Space Administration
NC	North Carolina
NIS	Sodium-Iodide Symporter
NSWC	Naval Surface Warfare Center
NWIRP	Naval Weapons Industrial Reserve Plant
O&M	Operation & Maintenance
ORP	Oxidation-Reduction Potential

PCE	Perchloroethylene
PRB	Permeable Reactive Barrier
ppm	parts per million
ppb	parts per billion
Psi	pounds per square inch
RfD	Reference Dose
SDWA	Safe Drinking Water Act
SERDP	Strategic Environmental Research and Development Program
TCA	Trichloroacetic Acid
TCE	Trichloroethylene
TNT	2,4,6-trinitrotoluene
TOC	Total Organic Carbon
TRH	Thyrotropin-Releasing Hormone
TSH	Thyroid-Stimulating Hormone
T3	Triiododythyronine
T4	Thyroxine
TX	Texas
US	United States
ww	wet weight
dw	dry weight

PURPOSE

This report is intended to provide individuals and organizations information regarding the *in situ* remediation of perchlorate-contaminated groundwater. The discussion will focus on, but will not be limited to, using *in situ* bioremediation as a low-cost treatment technology shown to be effective in treating perchlorate under multiple configurations and different site types. The case studies are designed to serve as examples of successful *in situ* bioremediation projects that were designed differently and located at separate sites. There are differences in the design and outcome of each case study, and potential improvements will be outlined in an effort to further advance *in situ* treatment technologies to treat perchlorate-contaminated groundwater throughout the United States.

1. INTRODUCTION

Perchlorate, ClO_4^-, is a powerful oxidizing agent and thyroid toxicant that has extensively contaminated groundwater throughout the United States due to high volume usage during the last 60 years. Perchlorate has been occasionally found naturally in soils. A small amount of contamination emanates from the application of naturally contaminated fertilizer, most notably from Chile. Nevertheless, perchlorate is most prevalent in surface water and groundwater in the United States (US) due to its use in the defense and aerospace industry. Approximately 90% of human-made perchlorate is used as ammonium perchlorate in the manufacturing of solid rocket and missile propellant. The perchlorate anion is ionically bonded to an ammonium (NH_4^+) cation to form a solid ammonium perchlorate salt. Perchlorate is also used in nuclear reactors and electronic tubes, the production of paint and enamel, the tanning of leather, the manufacturing of rubber, aluminum, and electroplating, a mordant for fabrics and dyes, an additive in lubricating oil; and is a major component of air bag inflators, pyrotechnics (including explosives, fireworks, flares, gun powder, and matches). Ammonium perchlorate is incredibly useful in solid rocket/missile fuel and other applications because it can act as a powerful oxidant, but under normal environmental conditions it is kinetically stable. (Motzer, 2001; ITRC, 2005a)

Applications containing perchlorate are tested and disposed of at Department of Defense (DoD) and National Aeronautics and Space Administration (NASA) facilities. Perchlorate has an inherently limited shelf life as an oxidizer – perhaps only 15 years before perchlorate-based applications become ineffective (Hatzinger, Pers. comm, 2006). Expired perchlorate is typically removed from munitions at onsite production, storage, and testing facilities using a high-pressure water washout, and then replaced with newer perchlorate. The washout releases ammonium perchlorate onto the ground onsite. The large size and weak charge of the perchlorate anion enhance its water solubility. Once ammonium perchlorate enters the environment, it completely dissociates in water and becomes very mobile in the subsurface environment (ATSDR, 2005). As a result, the past and present utilization of perchlorate has created widespread groundwater contamination that affects humans and surrounding ecosystems in at least 36 states throughout the U.S. Perchlorate has contaminated more than 153 public drinking water systems and can be detected in many items purchased from the grocery store – including fruits, vegetables, dairy, wine, and meat. (Motzer, 2001; FFRRO, 2005, ITRC, 2005a)

In recent years, the presence of perchlorate in groundwater aquifers has become a major environmental concern, due to newer studies indicating that perchlorate acts as a thyroid toxicant at exposure levels previously thought to be safe – and also because better detection procedures, developed in 1997, have revealed that perchlorate contamination is pervasive. Recent studies suggest that exposure to environmentally relevant levels of perchlorate adversely affects humans and wildlife in the U.S. Once perchlorate-contaminated food or water is ingested, it competitively inhibits iodide uptake by the thyroid gland, which normally synthesizes thyroid hormones that control the body's metabolism, growth and development. Prolonged exposure to perchlorate can lead to many problems, including hypothyroidism, neurological impairment and abnormal fetal development, particularly during sensitive stages in fetal and childhood development. (Greer et al., 2002; Lawrence et al., 2000; Braverman et al., 2005)

Due to perchlorate's toxicity and pervasiveness in the US, a significant amount of research has been devoted to advancing effective, low-cost treatment technologies to remediate perchlorate-contaminated groundwater. *Ex situ*, "pump-n-treat" technologies, most notably ion exchange, are frequently used to remediate perchlorate-contaminated groundwater. Unfortunately, these technologies can be quite costly due to the large volume of water that needs to be pumped from the sub-surface environment for treatment. Furthermore, effective *ex situ* technologies for perchlorate are physical removal processes that do not actually degrade the perchlorate, so further treatment or land-filling is required. (EPA, 2005; ITRC, 2005a; ITRC, 2006)

More focus has been placed on utilizing *in situ* treatment technologies, also known as in place or sub-surface treatment, as a way of destroying perchlorate without pumping massive quantities of water out of the ground and building major above-ground treatment facilities. Enhanced anaerobic *in situ* bioremediation (ISB) is the most developed *in situ* treatment technology to remediate perchlorate-contaminated groundwater to date. Other promising *in situ* treatment technologies for perchlorate-contaminated groundwater include phytoremediation and wetlands remediation. These three *in situ* remediation technologies can be highly effective, less costly alternatives to the more traditional *ex situ* technologies. (EPA, 2005; ITRC, 2005a; ITRC, 2006)

This report is focused on *in situ* remediation of groundwater contaminated with perchlorate previously associated with ammonium perchlorate salts. The purpose of this report is to briefly summarize the occurrence, toxicity, and regulation of perchlorate; discuss the types of *in situ* remedial technologies that are available to treat perchlorate-contaminated groundwater at different site types; outline the major *in situ* design considerations; and use three detailed case studies to highlight the use of *in situ* bioremediation to treat perchlorate-contaminated groundwater. Data was collected from many sources, including available peer-reviewed scientific literature, governmental information, project data and reports, as well as applicable environmental scientists and engineers.

2. Occurrence in the Environment

Before 1997, perchlorate was not regulated anywhere in the United States. In 1997, improved perchlorate detection limits (EPA Method 314.0) spurred concern over newly detected perchlorate contamination found in groundwater aquifers throughout the country, supported new research on the toxicity of perchlorate, and sparked debate over whether perchlorate should be

regulated and at what level. To date, the limit of detection for perchlorate is between 0.01 ppb and 4 ppb, depending on the detection procedure used. Ion Chromatography (IC) can be used alone or paired with a single or tandem mass spectrometer (MS) to detect perchlorate. The highest level of perchlorate ever detected in the United States to date is 3,700,000 ppb, found in groundwater near Las Vegas, Nevada. The map below illustrates locations that have detected perchlorate in groundwater at levels that warrant further studies and, most likely, remediation. (ITRC, 2006; EPA, 2005, FFRRO, 2005)
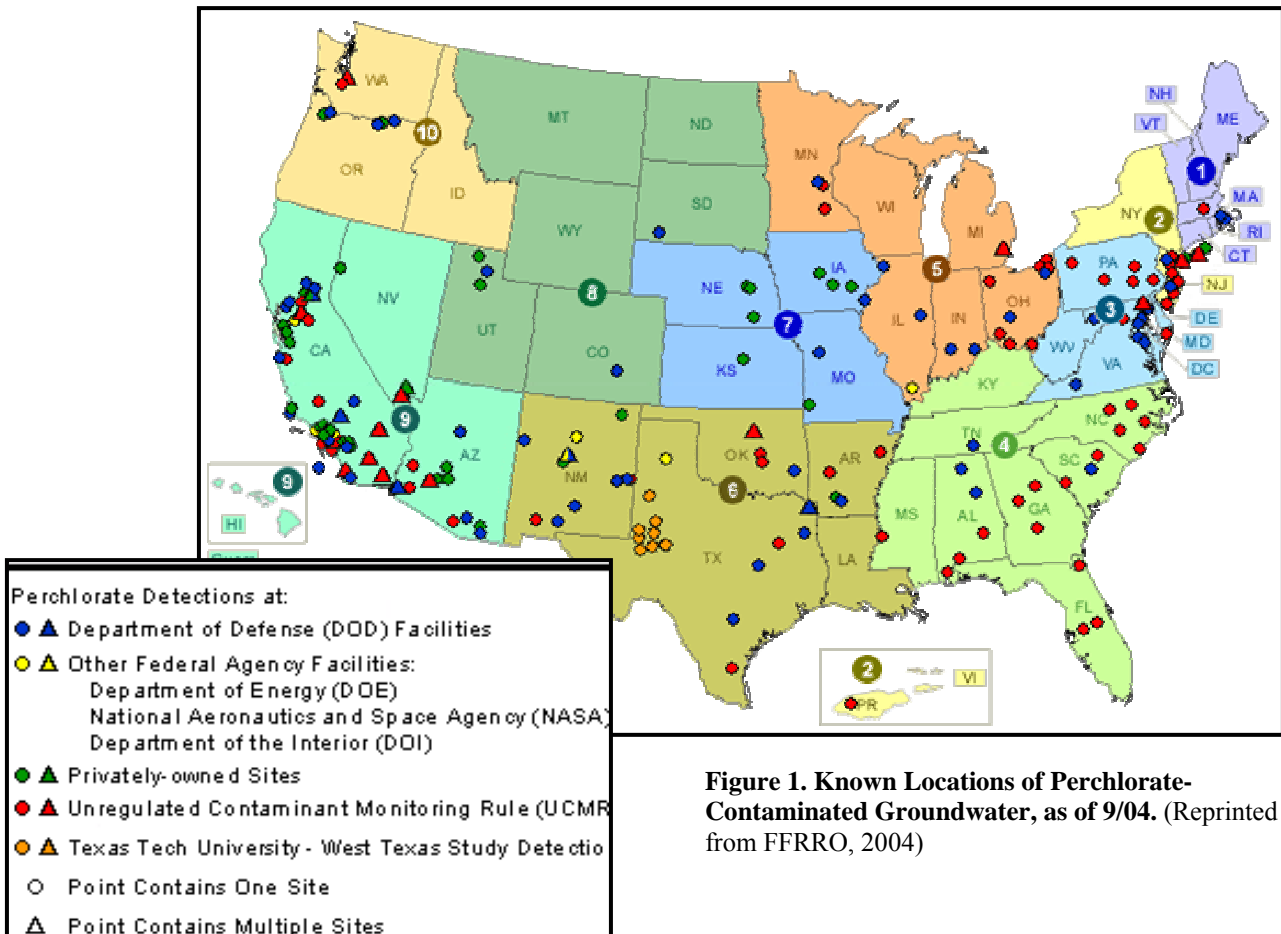
Figure 1. Known Locations of Perchlorate-Contaminated Groundwater, as of 9/04. (Reprinted from FFRRO, 2004)

Perchlorate contamination has been documented in at least 153 public drinking water systems across the U.S., based on data from January of 2005 from the EPA's Federal Facilities Restoration and Reuse Office (FFRRO). In California, more than 350 drinking water sources (wells and surface water) have identified perchlorate contamination at levels exceeding currently the accepted 6 ppb safe level of perchlorate in California drinking water. Furthermore, perchlorate contamination is pervasive in the lower Colorado River and Lake Mead, which causes concern since this surface water is used for both drinking and irrigation of the nation's crops in the southwest. It is estimated that more than 500 pounds of perchlorate flows into Lake Mead daily. (FFRRO, 2005; ITRC, 2006)

The perchlorate-contaminated Colorado River serves as the major source of water for farms throughout the southwest (including California, Nevada and Arizona), which supply much of the nation's fruits and vegetables and almost 90% of the nation's winter lettuce. In 1997, Lockheed

Martin tested farm vegetables, including lettuce, irrigated from the Colorado River at Lucky Farms in the Redlands area of San Bernardino County, California. Lawyers have since subpoenaed the results, which show that the vegetables contained more than 2,600 ppb of perchlorate, on average (OCA, 2003). Other studies indicate that not all produce contains such high levels of perchlorate, but many samples still contain more perchlorate than EPA's estimated safe dose level. A more recent Food and Drug Administration (FDA) study detected perchlorate in 217 out of 232 samples, or almost 94% of milk and lettuce chosen from 15 states. Perchlorate averaged 5.76 ppb in milk, with 38% of samples exceeding 6 ppb. In lettuce, perchlorate concentrations averaged 10.49 ppb, with almost 60% of samples exceeding 6 ppb. (OCA, 2004; Jackson et al., 2005)

Surprisingly, researchers recently found that perchlorate contamination is even present in prenatal vitamins (and regular vitamins) at levels up to 18 micrograms (μg), which is 37% of the estimated safe daily intake for an average 70 kilogram (kg) adult. Scientists believe that the perchlorate stems from seaweed, which normally is used as a source of iodide in vitamins, but now may also be another exposure route for perchlorate (Renner, 2006). A recent study conducted at Texas Tech University found that American women drinking from an uncontaminated water system contained up to 92 ppb of perchlorate in breast milk, and 63 ppb on average. This study indicates that women must be ingesting perchlorate-contaminated food or supplements regularly (Renner, 2005). As time progresses and additional locations are tested, perchlorate will likely be detected in more groundwater aquifers and food sources.

3. Chemical and Physical Properties

Perchlorate is a uniquely powerful oxidant. Ammonium perchlorate, more specifically, is thermodynamically highly reactive when its activation energy is reached, but the perchlorate anion itself is kinetically sluggish. Perchlorate's stability arises from the tendency for the chlorine atom to require considerable energy to be slowly reduced from a +7 oxidation state to a -1 oxidation state. As a result, perchlorate compounds are quite stable at room temperature. Ammonium perchlorate is typically heated to overcome the activation energy required to evoke an explosive reaction between the oxidizer and fuel. (OSHA, 1991; ATSDR, 2005)

The perchlorate anion is very soluble, and thus mobile, in the subsurface environment because its kinetic sluggishness makes it non-reactive, and its large molecular volume and small charge give perchlorate a low affinity to cations (and therefore complexation and precipitation). Under a neutral pH range and typical groundwater characteristics, perchlorate sorbs weakly to soil and sediment (negligible K_d, K_{oc}). Perchlorate is non-volatile, so it does not partition into the air phase either.

CAS Number	7790-98-9
Molecular Mass	117.49 g/mol
Color	Colorless/White
Form	Crystal Salts
Taste	Salty, Bitter

Odor	Odorless
Density	1.952 g/cm^3
Water Solubility	217-220 g/L @ 20$^)$C
Vapor Pressure	Non-volatile
pH	5.5 – 6.5
Octanol-Water Partition Coefficient (LogK$_{ow}$)	-5.84
Organic Carbon Partition Coefficient (K$_{oc}$)	n/a, Very Low
Distribution Partition Coefficient (K$_d$)	n/a, Very Low
Bioconcentration Factor (BCF)	215-380 (Wheat, Alfalfa)
Decomposition Temperature	322$^)$C

Table I. Chemical and Physical Properties of
Ammonium Perchlorate, NH$_4$ClO$_4$ (ATSDR, 2005)

Once ammonium perchlorate enters the environment, it completely dissociates in water, often infiltrates into groundwater, and then travels with the surrounding groundwater plume. The center of the contaminant plume travels via advection at the same speed as groundwater flow, but as the center of the plume diffuses outwardly, the contaminant mass moves slightly more quickly than average groundwater flow. Without a catalyst (i.e. enhanced microbial activation), perchlorate can persist in groundwater aquifers for decades without being degraded, even when a reducing environment is present. Furthermore, pure product – concentrated perchlorate – is denser than water, so it can act as a dense non-aqueous phase liquid (DNAPL). (Solomon, 2004; Sowinski et al., 2003; Urbansky et al., 2000)

Since perchlorate is not lipophilic, it does not biomagnify in exposed organisms. However, plants have been shown to bioconcentrate notable amounts of perchlorate via water uptake. Fruit and vegetable produce that normally contains lots of water can uptake an appreciable amount of perchlorate, based on the concentration of perchlorate in groundwater and the degree of water uptake. Studies show that perchlorate might accumulate in wheat and alfalfa up to 380 times more perchlorate than the level that is present in water. Similarly, perchlorate can be bioconcentrated in wildlife that is exposed to perchlorate-contaminated groundwater (and corresponding food), but studies have not shown any biomagnification of perchlorate up the food chain. (Jackson et al., 2005)

4.   TOXICITY OF PERCHLORATE

4.1 Disposition

Due to perchlorate's physical and chemical properties, dermal absorption and inhalation is not an exposure concern. After perchlorate is ingested, it is quickly absorbed through the gastrointestinal tract and distributed to the thyroid gland through the bloodstream. In the thyroid gland, perchlorate competes with and inhibits iodide uptake. As the concentration of perchlorate increases, there is a corresponding decrease in iodide uptake. Once the concentration of perchlorate reaches a certain threshold, iodide uptake is fully inhibited, and the thyroid's iodine

reserve is depleted. If the iodide uptake continues to be completely inhibited for a sufficient time period, a variety of adverse effects can materialize. (Amdur et al., 1991)

Most perchlorate is not metabolized in the body and it is excreted in urine quickly after absorption. The estimated half-life of perchlorate for humans is 6-9 hours. In one study, nine healthy men who ingested 10 mg of potassium perchlorate per day for 14 days excreted 7.7 mg of perchlorate per day, on average. (Lawrence et al., 2000; CADTSC, nd; Amdur et al., 1991)

4.2 Mechanism of Action

Exposure to perchlorate inhibits thyroid hormone synthesis and can lead to hypothyroidism, neurological impairments, and developmental deficits, especially among developing fetuses and young children. Thyroid hormones are produced when iodide is absorbed into the thyroid and then united with the amino acid tyrosine. The thyroid hormones then travel into the bloodstream and are distributed throughout the body – via the hormone-transporting protein, transthyretin – where they regulate metabolism and play a significant role in protein synthesis, fetal organogenesis, and neurodevelopment. Since perchlorate has the same ionic charge and a similar radius as the iodide ion, perchlorate inhibits iodide uptake into the thyroid by the sodium-iodide symporter (NIS), which stops the production of thyroid hormones – triiododythyronine (T3) and thyroxine (T4). (Wu et al., 2006; Greer et al., 2002)

Thyroid hormone depletion triggers a negative feedback mechanism to produce more T3 and T4. The hypothalamus in the brain produces thyrotropin releasing hormone (TRH) to stimulate the pituitary gland to release the thyroid stimulating hormone, thyrotropin (TSH). The release of TSH signals the thyroid to produce more thyroid hormones – T3 and T4. When perchlorate shuts down the uptake of iodide, the release of TSH signals the thyroid in the body to use its storage first to secrete more T3 and T4. After the storage is used and the thyroid hormones in the bloodstream become low again, more TSH is released, but the normal feedback mechanism is no longer functional. T3 and T4 are no longer secreted into the bloodstream due to perchlorate's inhibition of iodide into the thyroid. Thyroid hyperplasia can result, followed by hypothyroidism and a variety of corresponding effects. (Braverman et al., 2005; Braverman & Utiger, 2000; Amdur et al., 1991).

Typical effects of perchlorate exposure include a decreased IQ, mental retardation, delayed myelination, loss of hearing, vision or speech, a decrease in motor skills, slower metabolism, retarded growth, abnormal fetal organogenesis, cognitive deficits, abnormal testicular development, impaired balance, and shortened attention span. (Smith et al., 2002, Wu et al., 2006)
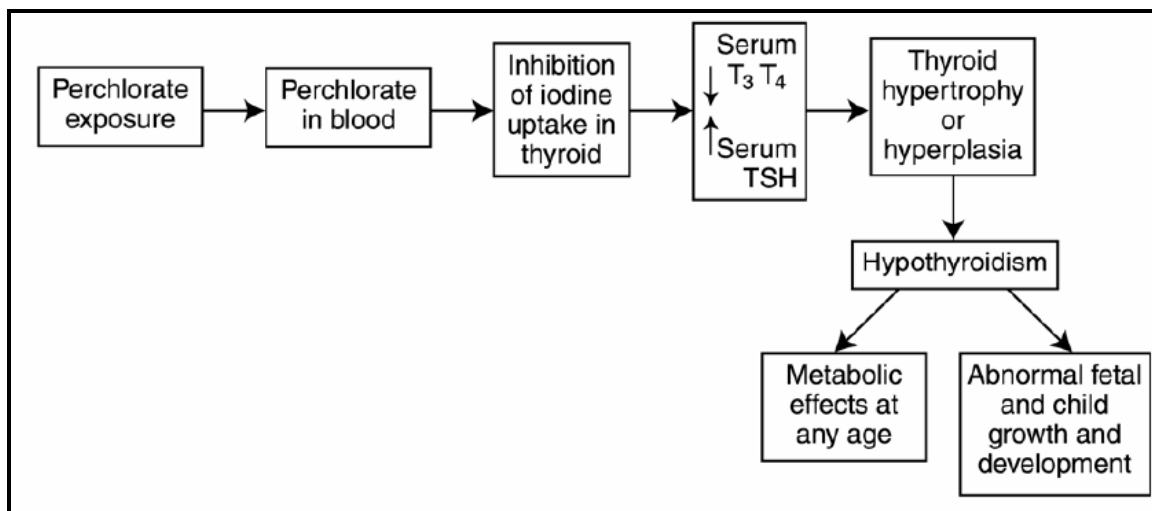
Figure 2. Diagram of the Mechanism of Action for Perchlorate Toxicity. (Reprinted from ITRC, 2005a)

A decrease in thyroid hormones during embryonic development increases the risk of congenital malformations such as low birth weight due to bone growth retardation. Before the 12th week of pregnancy, the fetus has no thyroid hormone production and thus relies solely on the mother for thyroid hormones essential for cerebral neurogenesis. After the 20th week, the fetus has an active thyroid gland, but does not produce enough thyroid hormones – the mother usually provides 30% of the fetus' thyroid hormones (Solomon, 2004). When both the mother and fetus are exposed to perchlorate at levels that inhibit any iodide uptake into the thyroid, both individuals develop hypothyroidism. Pregnant mothers, children and fetuses are most susceptible to developing adverse effects as a result of perchlorate exposure, but other population groups can be negatively affected in the same manner. (Wu et al., 2006; Greer et al., 2002))

If a large amount of iodine is stored in the thyroid, it could take months of iodide inhibition before hypothyroidism occurs in healthy adults. However, babies store only enough iodine to last for one day, and it is estimated that 15% of adult pre-menopausal women are deficient in iodine and therefore more susceptible to the adverse effects of perchlorate exposure (Hollowell et al., 2002). One study found that pregnant women in the 10th percentile for thyroid hormone levels were at least two and a half times more likely to bear a child with an IQ less than 85, and five times more likely to bear a child with an IQ less than 70 (Pop et al., 1999). Furthermore, the mammary gland also utilizes a sodium-iodide symporter, so not only does perchlorate bioconcentrate in human and cow milk, it also inhibits uptake of iodine into milk, which doubly harms breast-feeding infants.

Two more common contaminants also inhibit iodide uptake – nitrate, which is found in processed meat and water contaminated with the agricultural additive – and thiocyanate, which is found in vegetables and cigarette smoke. Exposure to these contaminants, in addition to perchlorate, could cause more deleterious effects. (Braverman et al., 2005)

5. REGULATORY STATUS

Due to its toxicity and prevalence, perchlorate is in the process of becoming regulated in the US. In 2005, the EPA set a non-regulatory toxicological reference dose (RfD) of 0.0007 mg/kg-d for perchlorate, based on biochemical changes. An RfD is a scientific estimate of a safe daily dose level, for a particular chemical and a particular endpoint. It is designed such that someone exposed to the RfD of perchlorate over a lifetime should not develop a potentially adverse effect, in this case, biochemical changes. This RfD corresponds to a drinking water equivalent (DWEL) of 24.5 µg /L (ppb). (Bodine, 2006)

While most state advisory levels for perchlorate in drinking water range from 1 ppb to 6 ppb, the EPA and the DoD have differed over the proposed Maximum Contaminant Level (MCL) since perchlorate became a public health concern. In 2002, the EPA initially supported an RfD corresponding to an MCL of 1 ppb. However, the DoD has lobbied to make the drinking water standard as high as 200 ppb. The RfD was set to the current standard of 0.0007 mg/kg-d in 2005, which is 23 times higher than the initial recommendation of 0.00003 mg/kg-d (Renner, 2005; OCA, 2003). There are a number of concerns regarding the recently approved RfD's applicability and protection for sensitive subpopulations. The RfD assumes that 100% of human exposure to perchlorate is due to ingestion of contaminated drinking water only. This major assumption is unlikely to be met, as most Americans ingest food contaminated with at least small amounts of perchlorate. Furthermore, the study used less than 10 healthy adults to calculate the RfD, based on an acute exposure duration (Bodine, 2006). Most Americans are chronically exposed to perchlorate, and the individuals that are most susceptible to adverse effects of perchlorate exposure are developing fetuses and children.

Currently, no federally enforceable MCL has been set for perchlorate under the EPA's Safe Drinking Water Act (SDWA). The goal of the SDWA is to protect the quality of the nation's drinking water. The National Primary Drinking Water Regulations (primary water quality standards) legally regulate the amount of certain contaminants that can be present in drinking water. The California EPA (CalEPA) has set a state voluntary MCL Goal (MCLG) of 6 ppb, and the Massachusetts Department of Environmental Protection (MassDEP) recently set the first state enforceable MCL of 2 ppb for perchlorate in drinking water (EPA, 2006; MassDEP, 2006). Overall, it is clear that widespread groundwater contamination of perchlorate poses a problem and must be addressed through remedial action in many cases.

6. TREATMENT TECHNOLOGIES

6.1 *Ex Situ* Treatment Technologies

In situ treatment technologies degrade perchlorate in the sub-surface environment, while *ex situ* treatment technologies remove and/or degrade the contaminant above ground. Many typical water treatment technologies are not effective in removing and/or degrading perchlorate from groundwater. Common treatment technologies that cannot be used to remediate perchlorate-contaminated groundwater include:

- *Air Partitioning* – i.e. Air Sparging and Vapor Extraction Technology
  - Designed to partition the contaminant from the water to the air phase,

then remove the volatilized contaminant
  o Perchlorate is non-volatile
- *Sorption* – i.e. Activated Carbon or Sedimentation Technologies
  o Sorb contaminant to soil or sediment, remove from groundwater
  o Perchlorate has a high solubility and low affinity to sorb to soil
- *Precipitation or Complexation*– i.e. Immobilization Technologies
  o Perchlorate will not precipitate at any pH
  o Perchlorate remains mobile, does not readily complex
- *Chemical Reduction*
  o Add a chemical to enhance contaminant degradation
  o Perchlorate is nonlabile, cannot overcome activation energy

None of these popular treatment technologies are suitable for perchlorate degradation due to perchlorate's unique chemical characteristics and stability.

There are some *ex situ* technologies that have proven to be effective at degrading perchlorate, however. Ion-exchange is the most common *ex situ* treatment technology used to treat perchlorate-contaminated groundwater. It is a physical treatment process that utilizes synthetic resins to switch the perchlorate anion in groundwater with another, non-toxic ion – in the process capturing the perchlorate ion onto the resin. However, this *ex situ* "pump-n-treat" treatment technology can be costly as it requires that groundwater first be pumped to the surface and then contacted with the synthetic resin. Additionally, further treatment or land-filling must occur once the perchlorate is sorbed to the resin, because the perchlorate is not actually destroyed. Reverse osmosis, ozonation, and fixed film bioreactors are additional *ex situ* technologies that can treat perchlorate contamination, but are less commonly used, due to cost concerns.

Nearly all *ex situ*, pump-n-treat technologies are very costly, when compared to *in situ* technologies. *Ex situ* technologies require constant operation and maintenance (O&M) costs due to continual water pumping, above ground technologies needs, mass transfer limitations, and employing onsite workers. *Ex situ* treatment usually takes a longer period of time as well, and creates the burden of additional treatment or disposal, once perchlorate is removed from the groundwater.

6.2 *In Situ* Treatment Technologies

6.2.1 Advantages & Disadvantages

Due to the limitations of *ex situ* treatment technologies, it is beneficial to consider *in situ* treatment technologies when treating perchlorate-contaminated groundwater. *In situ* treatment usually costs substantially less than *ex situ* treatment. Capital costs are lower because little to no infrastructure is required, while O&M costs are lower because less labor is required, perchlorate is fully degraded, treatment is completed much more quickly, substrate is fairly inexpensive, and groundwater does not need to be pumped to the surface. Furthermore, dissimilatory perchlorate-reducing bacteria (DPRB) are indigenous in most environments and locations, and only need to be activated to reduce perchlorate. *In situ* treatment is also quite flexible to various site types and levels of perchlorate contamination. There are various design configurations that can be used to treat and contain the perchlorate contamination at a particular site, while still maintaining natural groundwater flow patterns and preexisting land use requirements/plans. Furthermore, certain co-

contaminants, such as solvents, can utilize the same treatment technology, and more than one *in situ* technology can be used in site remediation at the same time. ISB is flexible and adaptable to changing conditions.

In Situ Advantages	In Situ Disadvantages
Lower costs (capital and O&M)	Site-specific variability
Land above treatment site can be utilized	Substrate distribution
GW treated without pumping	Hydrogeochemical conditions
Can maintain GW flow patterns	Suitability requirements
More flexible to variation	Biofouling (with ISB)
Can treat certain co-contaminants	Degradation of other water quality parameters
Can combine *in situ* technologies	Difficult to implement with increased depth to GW
Perchlorate-Reducing Bacteria are Ubiquitous	Odor concerns from nearby residences
Can maintain groundwater flow patterns	Potential reduction in aquifer permeability

Table II. Advantages and Disadvantages of In Situ Treatment of Perchlorate-Contaminated Groundwater
(AFCEE/ERT, 2002)

There are possible disadvantages when using *in situ* treatment to reduce perchlorate in groundwater. A site's hydrogeochemical characteristics have a large influence over whether a prospective treatment site has the potential to be treated *in situ*. I*n situ* treatment may be more costly and less effective for sites that have a long depth to groundwater or a deep groundwater aquifer. Furthermore, treatment design and results are more variable than when compared to *ex situ* treatment, because there can be major site-specific variability. Thus, site-specific characteristics are much more important to consider for subsurface treatment. Potential complications during treatment may include biofouling, incomplete substrate distribution, reduction of primary and/or secondary water quality characteristics, a reduction in aquifer permeability, odor concerns, and an incomplete treatment zone. It is best to keep the treatment zone 100 feet away from any surface water bodies or residences, due to possible odor and short-term water quality concerns. As the scale of a remediation project increases, *ex situ* and *in situ* costs would both increase, but generally *in situ* costs increase less rapidly. Thus, *in situ* treatment may still be the best option for a large project, although it would likely be more challenging to complete. (AFCEE/ERT, 2002; ITRC, 2005; EPA, 2005)

Proper design can preempt many potential problems when using *in situ* treatment. As *in situ* technologies are developed further, additional strategies will be discovered to minimize potential problems.

6.2.2 Types of *In Situ* Treatment

To date, there are three types of *in situ* technologies that may be used to treat perchlorate-contaminated groundwater:

1. Enhanced Anaerobic *In Situ* Bioremediation (ISB)
   a. Mobile Injection of Amendments
   b. Permeable Reactive Barrier (PRB)

     2.Phytoremediation
     3.Wetlands Remediation

Of these technologies, ISB has been used much more frequently and has been successful under a myriad of environmental conditions. Phytoremediation and wetlands remediation are both promising *in situ* technologies, but there is not a plethora of information detailing their design and effectiveness. As a result, this report focuses on ISB, in general, and in the case studies below. Many of the principles and advantages of ISB are applicable for phytoremediation and wetlands remediation as well.

7. ENHANCED ANAEROBIC *IN SITU* BIOREMEDIATION (ISB)

Enhanced anaerobic *in situ* bioremediation (ISB) is a process that utilizes microbes to catalyze biochemical reactions to ultimately reduce perchlorate to chloride and oxygen. An electron donor (substrate) is added to the contaminated groundwater plume to provide an energy, growth and carbon source for the indigenous dissimilatory perchlorate-reducing bacteria (DPRB). Occasionally additional amendments are added to activate the indigenous DPRB and enhance ISB (i.e. carbonate buffer). It is important to note that phytoremediation and wetlands remediation also rely on indigenous DPRB to help degrade perchlorate present in groundwater via rhizodegradation, but the use of an amendment addition may vary. ISB is most effective in treating perchlorate-impacted groundwater under the following conditions:

1) Indigenous DPRB active
2) Geochemical conditions are favorable
    a. Bioavailable molybdenum present
    b. Competing electron acceptors easily reduced
        1. Dissolved oxygen < 1 ppm
        2. Nitrate < 1 ppm
    c. Reducing environment present
    d. Neutral pH (6-8) to support optimal microbial growth
    e. High alkalinity to buffer acidic compounds formed in reactions
3) Optimal hydrogeological conditions
    a. Medium groundwater flow velocity
    b. Medium permeability
    c. Small amount of aquifer heterogeneity
    d. Shallow-medium groundwater depth and thickness
4) Critical receptors not closely downgradient from prospective site
5) Sufficient space before encountering a larger body of water

The table below provides some general numerical guidelines to follow when considering the suitability of ISB at a prospective site. (ITRC, 2006; EPA, 2005)

Site Characteristic	Suitable	Suitability Uncertain	Further Evaluation Needed
Plume size	Small; 0-2 acres	Medium-large; 2+ acres	Large; many acres
Contaminant Migration Risk	> 100 ft from receptor	Target treatment zone > 50 ft from receptor	Target treatment zone < 50 ft from critical receptor
Depth to Groundwater	<50 ft	>100 ft	> 100 ft for GW & ClO_4^-
Hydraulic Conductivity	> 1 ft/day	0.01 – 1 ft/day	< 0.01 ft/day
Groundwater Velocity	1 – 5 ft/d	5 – 10 ft/d	< 1 ft/d, > 10 ft/d
pH	6 – 8	5 – 6, 8 – 9	< 5, > 9
Sulfate Concentration	< 500 ppm	500 to 5,000 ppm	>5,000 ppm or gypsum present

Table III. Important Site Suitability Characteristics to Consider Before *In Situ* Bioremediation (ISB) of Perchlorate-Contaminated Groundwater (Adapted from ITRC, 2006)

A prospective site that is less than two acres with a shallow groundwater depth, average groundwater velocity, neutral pH, and low sulfate level is usually easiest to remediate via ISB. Due to odor and water quality concerns, sites that are at least 100 feet from a critical receptor or surface water body are ideal. As prospective sites veer from the above characteristics, the ease with which ISB can be implemented typically decreases, as does the potential for effective perchlorate reduction.

8. ISB DESIGN CONSIDERATIONS

There are many important considerations that must be taken into account before designing an ISB project to remediate perchlorate-contaminated groundwater. The following section covers:

- 8.1 Microbiology of Dissimilatory Perchlorate-Reducing Bacteria (DPRB) – Although DPRB are ubiquitous in the environment, without enhancement, these microbes have not been known to degrade perchlorate in an appreciable way. Thus, understanding the microbiology behind perchlorate reduction is critical before undertaking any bioremediation study. The sections that will be covered include:
  o Perchlorate Reduction Pathway
  o Types of DPRB
  o Identifying DPRB
  o Utilization of Electron Acceptors
  o Biofouling
  o Bioaugmentation

- 8.2 Recirculation – There are three types of recirculation systems that can be used during ISB. Each system has advantages and disadvantages that should be considered before ISB implementation. The system types are:
    - o Active
    - o Semi-Passive
    - o Passive
- 8.3 Mobile or Immobile Treatment – Depending on the above ground and subsurface characteristics of a potential site, it may be preferable to distribute the substrate in one of two ways:
    - o Mobile Substrate Injection –soluble substrate follows contaminant plume
    - o Immobile Substrate Formation – creation of a Permeable Reactive Barrier (PRB)
        - ▪ Injection
        - ▪ Excavation
- 8.4 Substrate Type – Different substrate types are available to meet various ISB designs. There are three main types of substrate that can be used:
    - o Soluble Quick-Release Substrate
        - ▪ Typically used for mobile ISB
    - o Slow-release Substrate
        - ▪ Liquid substrate
            - • Used for formation of a PRB via injection
        - ▪ Solid substrate
            - • Used for excavation of a PRB

This section will also consider the following:
    - o Combinations of Substrate Types
    - o Experimental Substrates
    - o Minimizing Substrate Costs
- 8.5 Environmental Factors Affecting ISB Effectiveness
    - o pH/Alkalinity
    - o Microbial Nutrients
    - o Salinity
    - o Hydrogeology
    - o Geochemistry
- Laws and Regulations

There are many combinations of ISB that can be effectively used to treat perchlorate in groundwater. Different site conditions often dictate the type of integrated ISB system that will be used. These variations in ISB will be discussed in more detail below.

8.1 Microbiology of Perchlorate-Reducing Bacteria

8.1.1 Perchlorate Reduction Pathway

Dissimilatory perchlorate-reducing bacteria (DPRB) contain enzymes that reduce the perchlorate through a three step process, shown below. DPRB use perchlorate as the terminal electron acceptor. The reaction also produces biomass, CO_2 and H_2O.

(Eq. 1)

$$ClO_4^- \qquad\qquad ClO_3^- \qquad\qquad ClO_2^- \qquad\qquad Cl^- + O_2$$

$$Perchlorate \xrightarrow[perchlorate\ reductase]{} Chlorate \xrightarrow[perchlorate\ reductase]{} Chlorite \xrightarrow[chlorite\ dismutase]{} Chloride + Oxygen$$
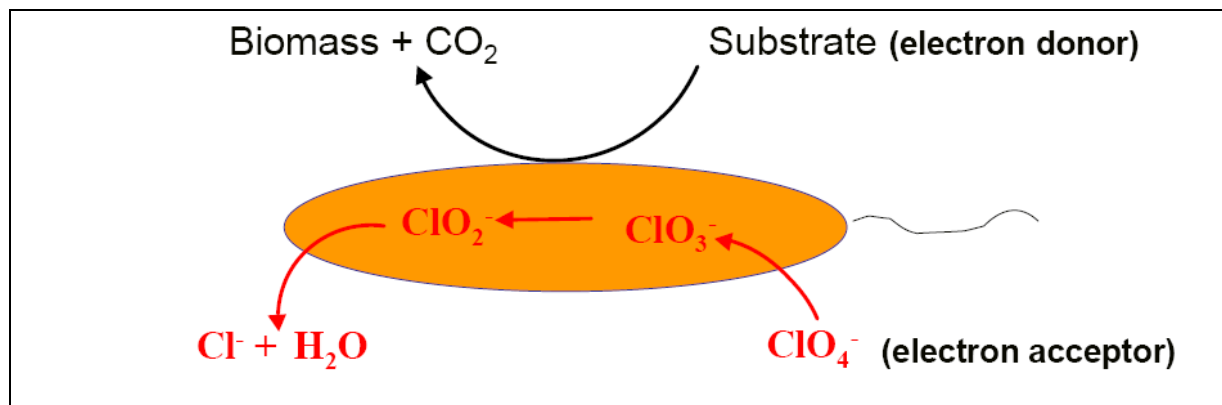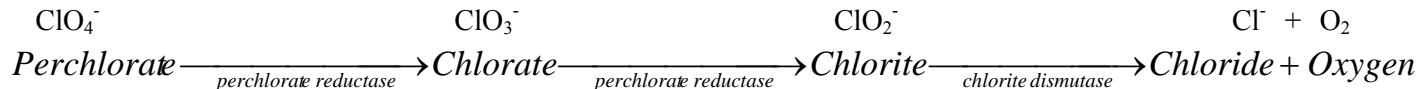
Figure 3. Visual Depiction of Enhanced Microbial Perchlorate Reduction.
(Reprinted from Hatzinger, 2000)

DPRB consume substrate while degrading perchlorate into chlorate, then chlorite, via perchlorate reductase. The process of degrading chlorite into chloride is a non-energy yielding step carried out by the chlorite dismutase enzyme in DPRB.

8.1.2 Types of DPRB

More than 50 types of DPRB have been identified to date. Interestingly, DPRB have been found in nearly every type of environment on earth, from the poles to the tropics, and in every type of environment, from contaminated urban landscapes to uninhabited wilderness areas. (Coates & Achenbach, 2005)

All known perchlorate-reducing bacteria are part of a subclass of Proteobacteria. The various DPRB are phylogenetically diverse, as indicated by the number of nucleotide sequences (genes) the different subclasses of DPRB have in common. The majority of perchlorate-reducing microbes are in the beta (β) subclass. DPRB within the Proteobacteria include the:

- Alpha (α) Subclass
  - *Dechlorospirillum* species
    - WD-type
- Beta (β) Subclass
  - *Dechloromonas* species
    - CKB-type
    - RCB-type
  - *Azospira* species (formerly known as Dechlorosoma)
    - PS-type
- Epsilon (ϵ) Subclass
  - *Wolinella Succinogenes* species

Some of the critical features of the various subclasses of DPRB include the following:

- DPRB are facultative anaerobes
- DPRB cannot utilize complex substrate (without further degradation by other microbes)
- Most DPRB reduce nitrate also
- DPRB use perchlorate or chlorate as terminal electron acceptors

The next figure visually depicts the various types of DPRB that are indigenous in many locations throughout the U.S. It is common to have a few types of indigenous DPRB present at any given location. (Coates & Achenbach, 2005)
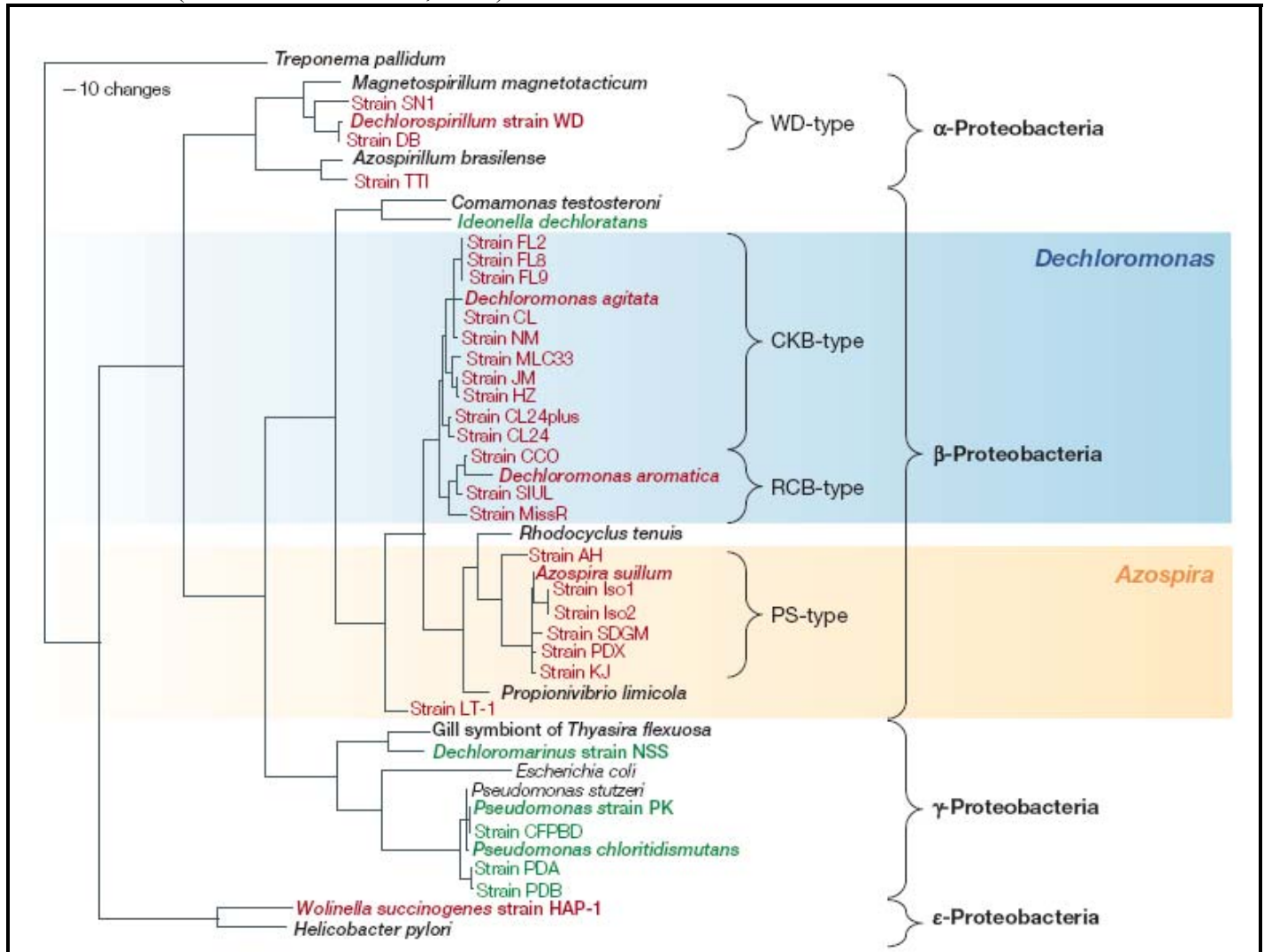
Figure 4. Phylogenetic Tree of Proteobacteria. Dissimilatory Perchlorate-Reducing Bacteria (DPRB), Listed in Red, Include the *Dechloromonas* and *Azospira* Species of β -Proteobacteria, the *Dechlorospirillum* Species of the α-Proteobacteria, and the *Wolinella Succinogenes* Species of ε-Proteobacteria. Chlorate-Reducing Bacteria are listed in Green. (Reprinted from Coates & Achenbach, 2004)

8.1.3 Identification of DPRB

By developing specific molecular probes that search for signature nucleotide sequences within a specific ribosomal RNA gene (16S rDNA), the Dechloromonas and Azospira species of the BetaProteobacteria can be further classified by type. Since these species compose the majority of DPRB present in the environment, this molecular probe is extremely valuable in determining

whether or not DPRB are indigenous at a prospective ISB site. Isotope-signature tracing is another way of identifying microbial perchlorate reduction as the mechanism behind decreasing levels of perchlorate (instead of dilution, contaminant migration, or phytoextraction). (Coates & Achenbach, 2004)

For more information on DPRB identification and enumeration, please refer to articles published by John D. Coates or Laurie A. Achenbach, experts in perchlorate microbiology, or contact them directly for assistance.

8.1.4 Utilization of Electron Acceptors

Microbes can be quickly stimulated to grow and become active when a substrate is added to groundwater in the surrounding area. While consuming an electron donor, DPRB will degrade electron acceptors in a specific order, starting with the most favorable redox reactions, when oxidation-reduction potential (ORP) is at 800 millivolts (mV). Electron acceptors are degraded in the following order:

+800

ORP (mV)

-250

$O_2 \rightarrow H_2O$ — Aerobic Respiration: +800 → +50 mV

$NO_3^- \rightarrow N_2$ — Denitrification: +50 → -50 mV

$ClO_4^- \rightarrow Cl^-$ — Perchlorate Reduction: 0 → 100 mV

$SO_4^{--} \rightarrow HS^-$ — Sulfate Reduction: -200 → -240 mV

$CCl_4 \rightarrow Cl^-$ — Reductive Dechlorination: > -240 mV

$CO_2 \rightarrow CH_4$ — Methanogenesis: > -240 mV
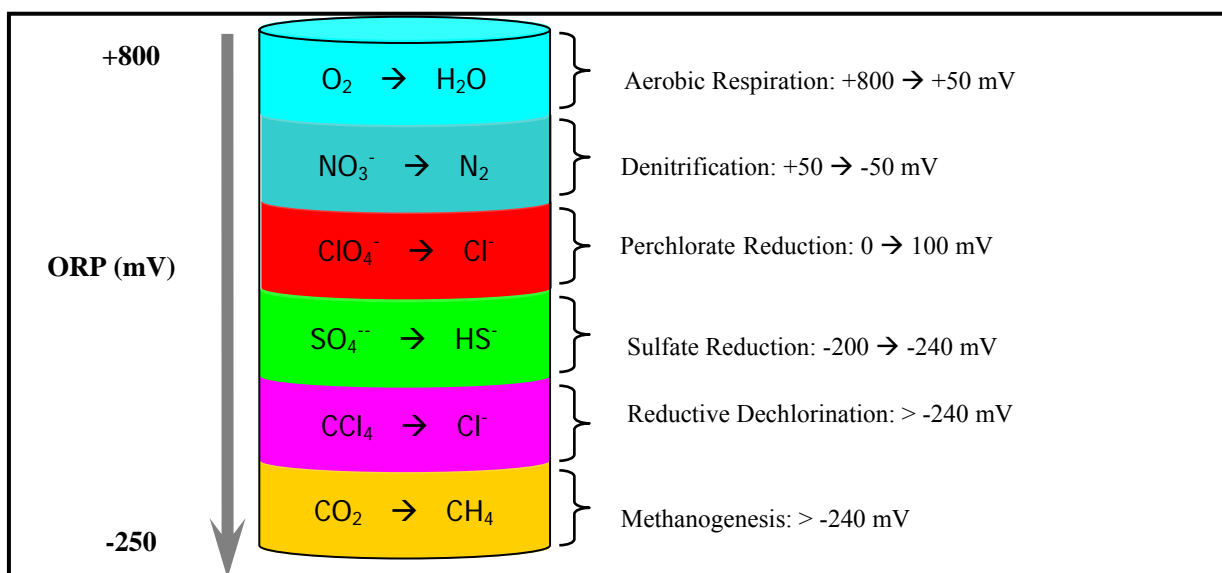
Figure 5. Microbial Depletion of Electron Acceptors in Amended Groundwater under Ideal Redox (ORP) Conditions. (Adapted from ITRC, 2005c)

Dissolved oxygen (DO) and nitrate are both competing electron acceptors that must be taken into account during ISB of perchlorate-contaminated groundwater. DO is the first electron acceptor used by microbes. DO must be degraded before any substrate can be used for perchlorate biodegradation. Anaerobic perchlorate biodegradation may begin when hypoxic conditions form at DO levels of 2-3 ppm, but is more likely to take place once anoxic conditions form at DO levels less than 1 ppm. It is ideal to have a DO concentration less than 0.5 ppm for microbial perchlorate degradation to be most effective. (Song et al., 2003; ITRC, 2005; ITRC, 2006)

Nitrate (NO_3^-) is the second competing electron acceptor that must be considered before perchlorate ISB. Microbial nitrate reduction usually occurs before perchlorate reduction, or at least simultaneously. If DO or NO_3^- is present, the microbes will preferentially degrade these

compounds first and use some or all of the available substrate (electron donor). Consequently, it is important that oxygen and nitrate are depleted in order to carry out effective microbial perchlorate reduction. The substrate quantity that is required for perchlorate reduction includes the amount necessary for oxygen, nitrate, and perchlorate reduction, as well as a safety factor accounts for substrate migration out of the treatment zone, variable distribution, and use by other microbial populations. (Song et al., 2003; ITRC, 2005; ITRC, 2006)

After oxygen and nitrate are depleted, perchlorate is used as the terminal electron acceptor. DPRB consume the electron donor while degrading perchlorate. Any excess substrate may be used to microbially reduce the next favorable electron acceptor, sulfate, as long as reducing conditions are still present in the treatment zone. Sulfate reduction is undesirable because it produces hydrogen sulfide, an odorous, toxic and corrosive chemical. Monitoring sulfate degradation throughout treatment is an important indicator of hydrogen sulfide production. Sulfate concentrations greater than 1,000 ppm are considered high and can impair secondary water quality characteristics. Sulfate reduction can be minimized by precisely estimating the appropriate amount of substrate necessary for perchlorate reduction. If most substrate is exhausted shortly after the perchlorate treatment goal is met, sulfide production will not become a concern, as excess substrate will not be available and oxidizing conditions will resume shortly. However, a much larger quantity of substrate is purposely added to account for substrate loss and uncertainty. According to Paul Hatzinger, a senior scientist at the Princeton Research Center of Shaw Environmental and an expert on enhanced ISB for perchlorate, "you can never mix so thoroughly that you get perchlorate to non-detect, yet have no sulfate reduction" (Hatzinger, pers. comm., 2006; ITRC, 2005; ITRC, 2006).

Solvents may undergo reductive dechlorination after sulfate reduction, when strong reducing conditions are present. The ability to degrade solvents in the same treatment zone as perchlorate makes ISB even more appealing as a treatment technology, as solvent contamination is often present as a co-contaminant. Costs can be reduced by treating both contaminants using ISB. Unfortunately, to reduce solvents, conditions also become ideal for methanogenesis, metals mobilization and sulfide production. Methane production is highly undesirable, as it is an explosion hazard and an extremely potent greenhouse gas. An increase in dissolved metals also poses water quality concerns and can corrode pipes. Thus, adding excess substrate to reduce perchlorate can decrease other water quality parameters, by producing sulfides and methane and mobilizing metals. (ITRC, 2005; ITRC, 2006)

8.1.5 Biofouling

Excess substrate can also stimulate the growth and activity of non-perchlorate reducing microbial populations that clog the subsurface environment near wells. Biofouling can decrease and even completely inhibit perchlorate reduction by using up available substrate, decreasing hydraulic conductivity in the treatment zone, adversely altering subsurface water quality, and ruining injection well systems. Significant biofouling can devastate an ISB project and become cost-prohibitive if new wells have to be installed. (Coates & Achenbach, 2005; Coates & Achenbach, 2004)

Biofouling occurs for two main reasons: indirect substrate utilization and excess substrate utilization. Indigenous DPRB can effectively reduce perchlorate when a variety of substrates are added, but they are actually only able to biodegrade simple substances, including monocarboxylic and dicarboxylic acids, simple alcohols, and monoaromatics. Substrates that indirectly stimulate indigenous DPRB include molasses and edible oils, and require that the electron donor is first biotransformed by non-perchlorate reducing bacteria into simpler substrate. Thus, most substrates used for perchlorate reduction indirectly support indigenous DPRB, which can encourage rapid growth of non-DPRB fermentative microbes. (Coates & Achenbach, 2005; Coates & Achenbach, 2004)

After perchlorate reduction, excess substrate supports reduction of the next favorable electron acceptors (sulfate, solvents, and carbon dioxide). Thus, excess substrate also encourages biofouling and impairs both primary and secondary water quality characteristics by supporting sulfide and methane production, as well as metals mobilization. (Coates & Achenbach, 2005; Coates & Achenbach, 2004)

There are specific ways to reduce the possibility of biofouling by choosing a substrate that is:
- Simple, non-fermentable, and directly utilized by indigenous DPRB
- Biocidal to other non-DPRB so the perchlorate treatment zone may increase
- Not effective at degrading other electron acceptors or changing aquifer geochemistry
- Thus, it is important to realize that while complex substrate can effectively degrade perchlorate, it also increases the chance of biofouling and degradation of other water quality parameters. Steps should be taken to decrease the possibility of biofouling before treatment. Alternatively, a biocide such as chlorine dioxide can be periodically injected during treatment. (Coates & Achenbach, 2005; Coates & Achenbach, 2004)

8.1.6 Bioaugmentation

It is highly unlikely that bioaugmentation will be necessary for any prospective ISB site to remediate perchlorate-contaminated groundwater. Perchlorate-reducing microbes are ubiquitous throughout the subsurface environment. If there is not a sufficient number of active DPRB, the substrate addition will likely increase the population to levels that are necessary for effective perchlorate reduction. (Hatzinger, pers. comm., 2006)

In the extremely rare case that no DPRB are indigenous, bioaugmentation would be necessary for ISB – adding substrate and other amendments would not degrade perchlorate without perchlorate-reducers. Paul Hatzinger, a senior research scientist at Shaw Environmental, indicated that their facility has a 1,000 gallon fermentor to grow DPRB. However, bioaugmentation would create higher remediation costs. (Hatzinger, pers. comm., 2006)

8.2 Recirculation

The success of enhanced anaerobic ISB depends on the distribution of the amendments in the treatment zone. The amendments that are added to the treatment zone are already naturally distributed via advection and dispersion processes. As a site's hydrogeological characteristics vary, however, so does the extent of substrate mixing and distribution. If the amendments are not

evenly distributed throughout the entire treatment area, there will be variability in the results, a higher likelihood that perchlorate is not effectively degraded, and a greater possibility of remediation difficulties. (EPA, 2005; ITRC, 2005a; ITRC, 2005c; ITRC, 2006)

There are three types of recirculation approaches that can be used when treating perchlorate-contaminated groundwater *in situ*:
1) Passive
    a. Natural distribution, no active recirculation
    b. One-time injection of amendments planned for project
2) Semi-passive
    a. Integration of active and passive approach
    b. Intermittent periods of active recirculation
    c. Re-injection of amendments during active period
3) Active
    a. Continual recirculation
        i. Encourages horizontal flow
    b. Continual injection of amendments
        i. Improves mixing in the treatment zone
    c. Extraction/injection wells constructed

There are a number of differences that must be considered before choosing which type of recirculation system to choose when using ISB to treat perchlorate-contaminated groundwater. In every category, a semi-passive system ranks in the middle – it is a safe choice to use when trying to reduce the disadvantages associated with both the active and the passive system. (EPA, 2005)

Certain hydrogeological characteristics may make it difficult to uniformly distribute substrate into the targeted treatment zone without any mechanical recirculation. Difficulties in implementing ISB without recirculation may arise when there is:
- Low Permeability
- Low Hydraulic Gradient
- Low Hydraulic Conductivity
- Aquifer Heterogeneity

In these cases, the substrate does not travel well by advection. Diffusion processes take considerably more time to distribute the substrate so that enhanced bioremediation can occur. To enhance substrate mixing in the treatment zone, the amended water can be mechanically recirculated across the center section of the contaminant plume. (ITRC, 2005a; ITRC, 2006)

Not surprisingly, there can be major differences in the requirements, costs, and results of each recirculation system (Table IV.). The injection wells must be spaced tightly when using a passive injection system, to optimize substrate distribution. Injection wells can be spaced further apart when using an active recirculation system. The amount of substrate that must be added also depends on the type of recirculation system used. A larger quantity of substrate must be injected for a passive system's one-time injection. It is important to inject an appropriate amount of substrate with a passive system, because the system is less flexible to changes (especially if an excavated PRB is created). The substrate quantity injected into an active system can be adapted as needs change. (EPA, 2005; ITRC, 2005a; ITRC, 2005c; ITRC, 2006)

The success of a recirculation system can be gauged by the extent of substrate distribution, control and flexibility, and maintenance of other primary and secondary water quality characteristics. In general, an active recirculation system is best in these three categories of effectiveness – substrate can be distributed most effectively, the amount of substrate injected can be controlled easily, and other water quality parameters are less likely to be degraded. An active pumping system that recirculates the amended water should distribute the amendments more uniformly and encourage more effective perchlorate reduction. Active recirculation may also reduce sulfate reduction, metals mobilization and methanogenesis by making it less likely that certain locations will contain large amounts of excess substrate. Larger substrate additions occur with a passive system, which increases the probability of biofouling and degradation of other water quality parameters. (Krug et al., 2005).

Overall, capital and operation and maintenance (O&M) costs are highest in an active system and lowest in a passive system (without biofouling). Sites that have greater advection, dispersion, and/or geological heterogeneity have the potential cost advantage of not needing to use an active treatment system for substrate to travel throughout the treatment zone. It is important to realize that, with a passive system, the substrate may only be well-mixed if there is sufficient advection and dispersion.(EPA, 2005; ITRC, 2005a; ITRC, 2005c; ITRC, 2006)

	Active	Semi-Passive	Passive
Injection Spacing	far [a]	~ [b]	tight
# Wells Needed	↓	~	↑
Substrate Injection Quantity	↓	~	↑
Effectiveness			
Substrate Distribution	↑	~	↓
Control/Flexibility	↑	~	↓
Water Quality Maintenance	↑	~	↓
Costs			
Infrastructure	↑	~	↓
Injection Well Installation	↓	~	~ / ↑ [c]
O& M	↑	~	↓

[a] Highlighted boxes indicate best choice for that category

[b] ~ Indicates Average

[c] Can use direct push probes; Biofouling may increase costs

Table IV. Comparison of the Advantages and Disadvantages of Active, Passive, and Semi-Passive ISB in Treatment Zone. (ITRC, 2005; EPA, 2005)

The major advantages of a passive system are its lower overall costs. Personnel are not required to be present at the site, other than during installation and monitoring. A passive system can encourage a high perchlorate reduction rate, if permeability is not reduced in the treatment zone. Though methanogenesis, sulfide production, and metals mobilization may be a concern, many experts believe that such degradation of other water quality parameters is only temporary, and the advantages of a passive system can often outweigh the disadvantages. (Borden, pers. comm., 2006)

A semi-passive system retains some of the advantages of each system, without retaining the extreme disadvantages of either system. (ITRC, 2005; ITRC 2006c; EPA, 2005)

8.3 Mobile or Immobile Design

When implementing *in situ* bioremediation, substrate can be added to the subsurface treatment zone using a mobile or immobile biobarrier design. In a mobile injection, the substrate promotes perchlorate reduction while following the contaminant plume. For an immobile biobarrier, a Permeable Reactive Barrier (PRB) can be implemented in the treatment zone.

A PRB is placed downgradient of the contaminated groundwater plume and allows groundwater to flow unimpeded in the direction of groundwater flow. Substrate comprising the barrier supports DPRB. Perchlorate salts are degraded as contaminated groundwater reaches the barrier.

Two barrier configurations are available when using solid substrate: a Funnel and Gate barrier or a continuous trench barrier. The continuous trench barrier requires that trenches are dug perpendicular to the contaminated groundwater plume, which flows naturally towards the

Figure 6. Schematic of a continuous trench PRB Treating a Contaminated Groundwater Plume. (Reprinted from Powell & Associates, 2006)

PRB. The trenches are then filled with solid substrate and other amendments to maintain permeability. Alternatively, a continuous trench PRB can be formed by injecting slow-release liquid substrate perpendicular to the flow of groundwater to form a reactive wall. Some of the major drawbacks of using PRBs that are excavated, as opposed to injected, include:
1) Increased capital costs
2) Must remove above ground infrastructure
3) Excavating bedrock may cause difficulties
4) Impractical at sites with a long depth to groundwater

Completely unfractured bedrock may make it difficult to excavate the trench, while completely fractured sediment will make the PRB unstable. Both of these scenarios make it difficult to create a deep trench PRB; successful excavation requires temporary bracing on the sides of the trench. It is less expensive and easier to construct a PRB via injection rather than excavation, but solid substrate is expected to last longer.

Figure 8. Depiction of a Funnel and Gate PRB.
(Reprinted from Powell & Associates, 2006)

Figure 7. Continous PRB Trench Excavated to Add Solid Substrate. (Reprinted from DNEP, 2001)

The funnel and gate barrier is designed by building solid walls around the ground water plume to funnel the groundwater into a gate of reactive material and necessary amendments.

Overall, both injected and excavated PRBs have shown to be a useful *in situ* technology for the reduction of perchlorate contamination. The main problems that may arise during implementation of any type of PRB design (excavation or injection) include:

1) *Substrate Depletion* – PRB must be replaced once reactive capacity lost
2) *Biofouling* – Increases construction costs
3) *Complex Hydrology* – Not suitable for some groundwater regimes
   a. Medium groundwater flow
   b. Small-medium depth to groundwater
4) *Placement* – Cannot treat contamination plume already downgradient of PRB
5) *Permeability of PRB* – Potential reduction in permeability reduces effectiveness

If the permeability of the PRB is decreased during construction or due to the substrate addition, groundwater will flow around the PRB and perchlorate will not be reduced. Thus, immobile PRBs are commonly effective, but not always ideal when using ISB to reduce perchlorate-contaminated groundwater. (ITRC, 2005b; ITRC 2006; EPA, 2005)

A mobile system is another way to implement ISB. The amendments are injected into the treatment zone and allowed to flow with the groundwater plume. A mobile ISB injection may be ideal when the prospective site has the following characteristics:

- Increased depth to groundwater
- Deep groundwater table
- Completely unfractured bedrock
- Extremely fractured bedrock
- Above ground infrastructure
  o Costly to remove (excavated PRB)
  o Difficult to install many injection points (injected PRB)
- Concerned citizens do not want excavation of a PRB
- Flexibility is considered important

Using an active or semi-passive recirculation system can provide some control over the amendment distribution. Since the amendments are mobile, it is more important to provide a soluble substrate and inject the substrate more frequently. Mobile ISB usually has higher O&M costs, but can also adapt to change more easily. (ITRC, 2005b; ITRC 2006; EPA, 2005)

8.4 Substrate Types

To activate perchlorate-reducing bacteria, an electron donor is added to the treatment zone. The electron donor (substrate) provides an energy, carbon, and growth source for the DPRB, which encourages perchlorate-reduction. The substrate also provides an electron donor for perchlorate's competing electron acceptors to be depleted. There are three categories of substrate:

1) Soluble substrate
2) Slow-release liquid substrate
    a. Low viscosity
    b. High viscosity
3) Slow-release solid substrate

The most commonly used substrates are sodium lactate (lactic acid sodium salt), HRC® (Hydrogen-Release Compound) and vegetable oil emulsions. Solid substrates such as mulch and compost are becoming more popular. ISB studies have also used a number of other substrates, including alcohol, starch, and pecan shells. The classes of potential substrates are as follows:

1) Organic acids, i.e. lactic acid
2) Carbohydrates i.e. glucose from sugar, starch from potato
3) Alcohols, i.e. ethanol, methanol
4) Protein, i.e. cottonseed, brewer's yeast, whey
5) Vegetable oil
6) Plant matter i.e. mulch, compost, chitin
7) Hydrogen i.e. HRC®

Zero-valent iron has also been used as an *in situ* technology to reduce perchlorate in groundwater, but it is typically more expensive than many of the available biological substrates, so it is not discussed here. (ITRC, 2005a; ITRC 2006; EPA, 2005; Strietelmeier, nd; Okeke & Frankenberger, 2004)

The amount of substrate needed to reduce perchlorate is stoichiometrically related to the amount of perchlorate. In actuality, substrate requirements are greater than the stoichiometric relationship between perchlorate as an electron acceptor and a specified substrate as an electron donor. Substrate utilization needs are dependent on the amount of perchlorate present, in addition to competing electron acceptors (dissolved oxygen, nitrate), and general demand of the substrate by other non-perchlorate reducing microbes. A lack of substrate will create an incomplete treatment zone and may not create reducing conditions. A safety factor is used to account for the small rate of substrate utilization. A higher substrate loading rate increases the potential that perchlorate biodegradation occurs before substrate is depleted. Ultimately, the substrate loading rate is dependent upon the substrate type, volume, and concentration of active ingredients; injection frequency; groundwater flow in the treatment zone; the amount of perchlorate; and the amount of competing electron acceptors and microbes. (ITRC, 2005b; ITRC 2006; EPA, 2005; Hatzinger, pers. comm., 2006)

When substrate demand is higher, more frequent injections and/or a larger substrate quantity is required, especially when using soluble substrates. Hydrogen gas is by far the most rapidly used and depleted substrate (not to be confused with HRC®, which is designed to be a slow-release substrate), followed by organic acids then carbohydrates. If a project manager wishes to decrease the frequency of substrate injection throughout the life of the project, a less soluble substrate should be considered. (ITRC, 2005a; ITRC 2006; EPA, 2005)

The three substrate categories have differing characteristics. Many design decisions, such as recirculation, delivery, system type, targeted substrate concentration, injection frequency, and treatment configuration are dependent on the substrate chosen. The table below provides the general difference between soluble, slow-release, and solid substrate. (ITRC, 2006)

Substrate Type	Soluble	Slow-release Liquid	Slow-release Solid
Examples of Substrate	Sodium Lactate, Ethanol	EOS®, HRC®	Mulch, Compost
Active Recirculation?	Usually	Seldom	Seldom
Typical Delivery	Injection	Injection	Excavation
Mobile System or PRB?	Usually Mobile	PRB	PRB
Targeted Substrate Concentration	50 - 300 ppm	100 - 500 ppm	100 - 1,000 ppm
Potential Injection Frequency	Frequently - Continuous	Once - Infrequently	Once – Very Infrequently
Configuration: Partial Plume Treatment	Linear injection pts perpendicular flow	Linear injection pts perpendicular flow	Linear trenches perpendicular flow
Configuration: Plume-Wide Treatment	Grid or multiple linear rows of wells	Grid	Widen PRB
Typical Lifespan	0 - 3 months	1 - 5 years	Estimated > 5 years

Table V. Characteristics of the Different Substrate Types That Can Be Added During *In Situ* Bioremediation of Perchlorate-Contaminated Groundwater. (Adapted from ITRC, 2006)

An ideal substrate for a specific site depends on a variety of site considerations, discussed below.

8.4.1 Soluble Substrate

Soluble substrate is the most mobile of the substrates available, due to its low viscosity and extremely high solubility. These substrates, which include carbohydrates, organic acids and alcohols, are transported throughout the treatment zone via advection and dispersion processes (and potentially active or semi-passive recirculation). The soluble substrates used in field-scale ISB include sodium lactate, molasses and ethanol. The mobility of soluble substrates allows for easy travel throughout the treatment zone, which is advantageous when perchlorate contamination is located in deep groundwater aquifers. Using soluble substrate may also reduce drilling costs, because the frequency and depth of injection points can be minimized. (ITRC, 2005a; ITRC 2006; EPA, 2005)

Due to the high solubility of soluble substrates, frequent additions are required throughout treatment. The substrate can be injected into the groundwater aquifer through a gravity feed or pressure injection, with the pressure injection being preferable as it will provide a more consistent and expedient distribution. The injection time period can be continuous or periodically pulsed. (ITRC, 2005a; ITRC 2006; EPA, 2005)

Overall, the disadvantages of using soluble substrate, instead of a slow-release substrate are:
- More frequent injections
- Higher costs
  - Capital costs include mixing tanks, etc.
  - O&M costs include labor, additional substrate demand, recirculation
- It is more difficult for soluble substrate to reach the outside of larger plumes

Increased operation and maintenance costs stem from using an active or semi-passive injection system to consistently distribute substrate into the treatment zone. It is not advised to use soluble substrate when there is an unusually low or high groundwater flow velocity, because in either case, the substrate will not be distributed sufficiently and consistently throughout the treatment zone before being degraded or flowing away. (ITRC, 2005a; ITRC 2006; EPA, 2005)

On a positive note, active injection systems do allow for greater flexibility than the passive systems, making it easier to quickly adapt to changes in environmental conditions within the treatment zone. Soluble substrates are a popular choice for many ISB projects due to their flexibility, effectiveness in deep groundwater tables, and extreme mobility. (ITRC, 2005a; ITRC 2006; EPA, 2005)

8.4.2 Slow-Release Liquid Substrate

Slow-release liquid substrate can be used in the creation of an immobile barrier that slowly becomes a more mobile reactive zone (< 30 feet) due to advection and dispersion. The reactive zone typically contains organic carbon concentrations over 100 mg/L. The most common types of low viscosity, slow-release substrates are HRC® (Hydrogen Release Compound) and edible vegetable oil emulsions like EOS®. Meanwhile, the most common high viscosity, slow-release substrate is vegetable oil. (IES, 2006; ITRC, 2005a; ITRC 2006; EPA, 2005)

Injecting a non-aqueous phase liquid (NAPL) substrate immobilizes the substrate near the injection points, thus creating a PRB that is effective for a longer period of time. The oils are inexpensive, non-toxic and have high energy content. Microbes will break down edible oils into shorter chain fatty acids that are readily used as substrate. There are two methods of oil injection:

1) Direct, low-pressure injection
   a. Inject oil only
   b. Most common
   c. Harder to Distribute
2) Direct, pressurized injection
   a. Inject oil, then chase with water
   b. Used to distribute oil better

When conducting pressurized injections, keeping overburden pressure less than one psi is important to avoid hydraulic fracturing and therefore inconsistent distribution of substrate. (Hunter, 2005; ITRC, 2005, ITRC, 2006, EPA, 2005)

The main advantage to using slow-release liquid substrate is that excavation is not required and generally only one injection period is needed throughout the life-cycle of the remediation project. As a result, there are no major O&M needs, as long as biofouling does not occur. On the downside, slow-release liquid substrate tends to encourage increased sulfide and methane production, as well as metals mobilization (Hatzinger, pers. comm., 2006). Decreased aquifer permeability is another major disadvantage of slow-release liquid substrate. One way to mitigate this problem is by using a low viscosity oil emulsion, which has been shown to distribute into water more easily. The typical emulsifiers used are food-grade lecithin, polysorbates, monoglycerides, diglycerides, glycerol and mono-oleate. Emulsifiers composed of soybean oil and lecithin have been shown to effectively sorb in sandy aquifers with a small amount of organic or clay material. Meanwhile, studies show that polysorbate and glycerol mono-oleate is better suited for aquifers with higher organic or clay materials. In very fine-grained sandy or silty soils, oil emulsion droplets less than 1 μm are necessary for effective distribution. Similar to other substrates, emulsion retention is dependent on the rate of degradation, sorption capacity, groundwater flow velocity, and competing electron acceptors. (ITRC, 2005; ITRC, 2006; EPA, 2005)

HRC® can be purchased from Regenesis Bioremediation Products. It is most suitable in shallow aquifers and contaminated plumes, utilizing direct-push technology to distribute the substrate over a larger treatment zone or barrier. Studies that have utilized HRC® in deep groundwater aquifers injected it through a screened well with a glycerin chaser to distribute the substrate further. (ITRC, 2005; ITRC, 2006; EPA, 2005)

8.4.3 Slow-Release Solid Substrate

Slow-release solid substrate can be used when excavating a PRB is a viable option. This substrate type is a good long-term option, as it can last for an extended period of time without any renewal. Solid substrate lasts longer than slow-release liquid substrates because it takes longer for the electron donors to dissolve into the groundwater in the treatment zone. Studies indicate that solid substrates such as mulch and compost can potentially last for 5-10 years, with

little to no O&M costs, whereas a slow-release liquid substrate may need to be reinjected every few years. (ITRC, 2005a; ITRC, 2005b; ITRC 2006; EPA, 2005)

A variety of solid substrates have been tested recently for their effectiveness in stimulating perchlorate reduction, including pecan shell, potato waste and more. The most popular solid substrates are currently mulch and compost. Slow-release solid substrates are recommended most when the perchlorate contamination lies within a:

1) Shallow groundwater aquifer
2) Lower permeability
3) Non-continuous geological formations

Low permeability retards groundwater flow from entering a trench PRB. The subsurface environment can be recreated to provide a more permeable path for groundwater to flow through without excavating very deep into the subsurface. Slightly increasing the permeability of the PRB, relative to the surrounding geological formations in the groundwater aquifer, encourages contaminated groundwater to preferentially pass through the PRB to be treated. Coarse sand or gravel can be added in a 20-60% mixture with the substrate, depending on site characteristics, to ameliorate a low permeability aquifer or prevent decreased permeability caused by compaction during excavation. (ITRC, 2005a; ITRC, 2005b; ITRC 2006; EPA, 2005)

Solid PRBs work well for heterogeneous aquifers because a long biowall barrier does not allow contaminated groundwater flow to easily escape the treatment zone. The main disadvantage to solid substrate is the fact that, to date, it has only been successful at shallow depths (up to 35 feet) in geological formations that are neither completely unfractured nor fractured. An insufficient residence time for perchlorate reduction, stemming from a high perchlorate concentration or high groundwater flow velocity can be another disadvantage. To prevent this potential problem, a larger reactive zone can be created or a line of parallel trenches can be constructed. Solid substrate is also not an ideal option if excavation will be difficult or costly, which often occurs when above-ground infrastructure would need to be removed and people are living close by. In this case, it may be best to create a PRB by using an injectable slow-release liquid substrate. (ITRC, 2005a; ITRC, 2005b; ITRC 2006; EPA, 2005)

Major considerations that must be taken into account before using a slow-release solid substrate includes substrate composition, the width of the reactive zone, and retention time. There can be significant variability in substrate composition of mulch for example; the density of electron donors may vary throughout the mulch. Inconsistency in the amount of electron donors present in the PRB will create less effective perchlorate reduction and more variable results. (ITRC, 2005a; ITRC 2006; EPA, 2005)

8.4.4 Combinations of Substrate Types

Slow-release solid substrate provides long-term substrate suitability but can make it difficult to initially start active perchlorate reduction. Combining a slow-release low viscosity liquid substrate (which is more soluble) with a slow-release solid substrate will provide a higher loading rate in contaminated aquifers. In trench PRBs, installing wells or perforated pipe can assist in adding a fluid substrate. The Navy has combined vegetable oil substrate with solid substrate (mulch and compost) in an effort to enhance the rate of available organic carbon in the

contaminated zone of a groundwater aquifer under the Naval Weapons Industrial Reserve Plant (NWIRP) in McGregor, TX. A soluble substrate could also be injected along with a slow-release substrate to meet short and long-term requirements. Occasionally soluble substrates are also added to oil-in-water emulsion mixtures, such as EOS®, to kickstart perchlorate reduction. (ITRC, 2005a; ITRC 2006; EPA, 2005)

8.4.5 Experimental Substrates

There are many newer substrates that have been used in laboratory and pilot studies successfully, but have not been extensively used for field-scale ISB. These experimental substrates include:
- Potato Starch
  - Amylolytic organisms convert starch to sugar
  - Sugar shown to be effective substrate for DPRB
  - 20 – 59% of potato wasted during food processing
  - Purchase cheap potato waste for substrate
- Pecan Shells
  - Used to create PRB at Lose Alamos National Laboratory (LANL)
  - Multi-contaminant groundwater plume, including perchlorate
  - Composed of cellulose and lignin
  - High porosity, slow-release substrate
  - Use could mitigate waste problem
  - Shown to be effective at reducing perchlorate
- Cheese whey
  - Soluble, but more chemically complex than other soluble substrates in use
  - Waste product of dairy industry, so inexpensive to obtain
  - Powdered whey is more expensive, but easier to purchase and transport
  - Longer substrate retention time than other soluble substrates → lower O&M costs
  - Lifespan ~ one year
- Bulk chitin
  - Slow-release solid substrate
  - Similar to other solid substrates: mulch and compost
  - More consistent, constant degradation than mulch or compost
  - Much more costly than other solid substrates
  - Shorter lifespan than other solid substrates → higher O&M costs
- Chitin Mixture
  - Use during hydraulic fracturing applications
  - Mixture composed of chitin, sand, guar gum, and water slurry
  - Fracturing distributes mixture into more impermeable subsurface areas (silt, clay)
  - Substrate is retained in impermeable areas due to the sand's porosity
- Pure hydrogen
  - Best electron donor and stimulator of DPRB, but highly combustible
  - Degrades more quickly than any other soluble substrate
  - Inexpensive however, and easy to transport safely due to available technologies
  - Need to provide carbon source for microbial growth though in many cases
  - Sow-release hydrogen substrates more widely used (i.e. HRC®)

Further research should be conducted to verify the efficacy and cost feasibility of these experimental substrates. However, current data suggests that these substrates may become more prevalent for ISB as long as legal standards are met. (ITRC, 2006; Okeke & Frankenberger, 2004; Strietelmeier, nd)

8.4.6 Minimizing Substrate Costs

Microbes need carbon-based substrates for energy, cellular maintenance and growth. Without a substrate, DPRB will not reduce perchlorate. Since substrate is one of the main technology inputs for *in situ* bioremediation, the cost of substrate is a major factor in deciding whether or not the benefits of using this treatment technology outweigh the overall costs.

Utilizing food waste from the agricultural, industrial, and commercial sectors is one way to cut down on the costs of ISB. Since 30-70% of food waste is composed of non-cellulosic carbohydrates (i.e. sugar, starch), such waste can be both an ideal substrate for DPRB and a way to divert the materials from the waste stream. In potato food-processing, 20-59% of the potato is actually thrown away. A recent laboratory study found that the starch in potato peel waste (and potentially wheat, rice, and corn) could be used as an effective substrate, at least for the perchlorate-reducing bacteria *Azospira* genre, when an addition of amylolytic organisms were also added to convert the potato starches to sugar that the perchlorate-reducing bacteria could utilize. The study determined that the degree of perchlorate reduction was directly related to the amount of potato-based substrate added, with up to 93.4% of the perchlorate being treated within 4 days (Okeke & Frankenberger, 2004).

At Los Alamos National Laboratory, pecan shells were used to create a solid trench PRB due to the large amount of waste that is produced from harvesting pecans. Current information indicates that the PRB was inexpensive to create and effective at reducing perchlorate. (Strietelmeier, nd)

8.5 Environmental Factors Affecting ISB Effectiveness

There are many environmental factors that affect the effectiveness of ISB. In some cases, amendments can be added or the type of treatment can be changed to mitigate an unfavorable site condition. In other cases, it may be difficult to change the hydrogeochemical characteristics onsite to make ISB more suitable. Factors that can inhibit effective ISB include:

1) Low pH/alkalinity
2) Lack of bioavailable molybdenum
3) Competing electron acceptors
4) High ORP
5) High salinity
6) Low aquifer permeability
7) Extreme aquifer heterogeneity
8) Long depth to groundwater table
9) Deep groundwater table
10) Extreme groundwater flow

Indigenous DPRB usually require a substrate addition to grow into an active, robust microbial population. A low pH can inhibit initial microbial growth however. A short lag period is not uncommon once the ISB system is implemented. Competing electron acceptors must be depleted, a reducing environment must be created and bioavailable molybdenum must be present for perchlorate reduction to occur. (EPA, 2005, Coates & Achenbach, 2004; ITRC, 2005a; CLU-IN, 2006; AFCEE-ERT, 2002)

Important factors that affect the effectiveness of ISB will be discussed below, such as high salinity, difficult hydrogeological features (i.e. extreme flow conditions, large distance to groundwater) and large contaminant plumes.

8.5.1 pH

A contaminated groundwater plume that has a low pH and/or low alkalinity may make it difficult for DPRB to actively reduce perchlorate. A low pH can inhibit microbial perchlorate reduction in a number of ways:

Figure 9. Diagram of Three Important Environmental Factors Affecting Perchlorate-Reducing Bacteria (DPRB). (Reprinted from Coates & Achenbach, 2004)

1) Acidity can make DPRB inactive or less active
2) Lack of bioavailable molybdenum (molybdate)
   a. Present at low quantities in most aquifers
   b. Trace nutrient required for the DPRB to reduce perchlorate
   c. Less soluble at low pH, so not available for DPRB
3) Bioavailability of toxic metal compounds at low pH
   a. Many metals become mobile at lower pH, such as:
      i. Manganese
      ii. Iron
      iii. Arsenic
      iv. Selenium
   b. These metals may retard DPRB from activity

DPRB are most active within a pH range of 6-8; while they may be able to reduce perchlorate at a pH from 5-9, the microbes are less effective. If indigenous DPRB are discovered via enumeration studies, but microcosms simulating perchlorate degradation are not effective, the pH should be tested. In cases where pH is low, adding a carbonate/bicarbonate buffer would create more ideal conditions for perchlorate reduction by increasing the pH, alkalinity, and bioavailable molybdenum. (Cramer et al., 2004; Hatzinger, pers. comm., 2006; EPA, 2005, Coates & Achenbach, 2004; ITRC, 2005a)

8.5.2 Microbial Nutrients

Molybdenum is a trace nutrient that is required for DPRB to reduce perchlorate. Most subsurface environments contain an adequate amount of bioavailable molybdenum (molybdate). However, if molybdenum is not present, it may need to be added along with the substrate injections. Alternately, if molybdenum is present, but not bioavailable due to a low pH, a buffer must be added to allow DPRB to use the molybdenum in the treatment zone, as stated above.

Nutrients such as nitrogen (N) and phosphorus (P) are typically present at levels that support small indigenous microbial populations located in groundwater aquifers. Nitrate must be reduced before perchlorate reduction occurs, so adding nitrate for ISB of perchlorate would likely decrease the treatment's effectiveness. (Coates & Achenbach, 2004; Coates & Achenbach, 2005)

8.5.3 Salinity

Highly saline groundwater can decrease and even completely inhibit microbial growth for DPRB. Studies indicate that most DPRB cannot reduce perchlorate when salinity exceeds 3% in water. However, a study that obtained microbes from naturally high salinity environments found that DPRB were adapted to the salinity, and therefore were effective at degrading perchlorate when exposed to higher salt concentrations. The study found that certain DPRB grew quickest at a salinity of 5%, at which point growth rates markedly decreased until microbial death occurred at a salinity of 13%. Still, only half of the microbial samples reduced perchlorate with salinity greater than 3%. Thus, it is possible that certain site-specific DPRB adapted to highly saline conditions will fare better at perchlorate reduction than is expected. Additionally, such DPRB can be used for bioaugmentation at other sites that do not have salt-resistant perchlorate reducers. (Logan, 2001)

8.5.4 Hydrogeology

Unfavorable hydrogeological site conditions, such as a long depth to groundwater and extreme groundwater flow rate (high or low), cannot be changed. Any *in situ* treatment technology must work around these site characteristics to develop a treatment design that will be effective.

There are also some adverse hydrogeological changes that can occur during ISB. Sometimes, a substrate addition can decrease aquifer permeability and hydraulic conductivity, thereby reducing groundwater flow through the treatment zone and the effectiveness of remediation. As a result, it is important to carefully choose a substrate that will meet each specific site's characteristics, and monitor the ISB site for any changes once remediation begins. In general, viscous oil substrates, which tend to sorb to sediment and reduce porosity, cause more problematic hydrogeological changes. However, certain soil types are naturally less permeable. Clay is the least permeable soil type, followed by silt.

Soil	Permeability Coefficient (cm/sec)	Relative Permeability
Coarse gravel	$>10^{-1}$	High
Sand	$10^{-1} - 10^{-5}$	Low - Medium
Silt	$10^{-5} - 10^{-7}$	Very Low
Clay	$<10^{-7}$	Impervious

**Table VI. Affect of Soil Type on Pre-Treatment Permeability
of a Groundwater Aquifer** (Adapted from Scott, 2006)

It is very important to characterize the subsurface environment well before choosing a substrate for this reason. Low permeability soil types may be better suited by less viscous, more soluble and permeable substrate types. (Chaudhuri et al., 2002; Song and Logan, 2003)

8.5.5 Geochemistry

During ISB, some geochemical changes are clearly positive and expected to occur before perchlorate reduction can take place. These favorable changes include:
- Decrease in DO to < 1 ppm
- Decrease in ORP to < 50 mV (preferably 0 to – 100 mV)
- Decrease in nitrate to < 1 ppm

There are also some unfavorable changes in geochemistry that may indirectly occur as a result of enhanced anaerobic ISB. These include:
- Sulfate reduction → hydrogen sulfide production
- Carbon dioxide reduction → methanogenesis
- Metals mobilization
  - Manganese
  - Dissolved iron
  - Arsenic
  - Selenium

ISB technologies aim to encourage positive changes that support perchlorate reduction and minimize negative changes that make it difficult to achieve treatment goals and decrease other water quality characteristics. However, passive ISB systems usually encounter negative changes in water quality more than active ISB systems, which can add less substrate at any given time and easily adapt to changes in site conditions. (EPA, 2005, ITRC, 2005a; ITRC, 2006)

8.6 Legal Concerns

As required by the Safe Drinking Water Act (SDWA), the EPA has established primary and secondary water quality standards that apply to many groundwater aquifers that are contaminated with perchlorate. Primary water quality standards are designed to protect human health and are enforceable by law. The standards for primary pollutants include Maximum Contaminant Levels (MCLs), as well as Maximum Contaminant Level Goals (MCLGs). Meanwhile, secondary water quality standards are based on taste and odor considerations, but ingesting higher concentrations of these contaminants may also cause adverse health effects. (EPA, 2006)

There are multiple legal concerns that must be addressed before choosing a substrate for remediation of perchlorate-contaminated groundwater.   First, the substrate chosen may not

include known hazardous waste. Second, before injecting substrate underground, an underground injection control permit might be necessary, according to the SDWA. Third, the substrate's influence on creating potentially harmful intermediate by-products, degradation of primary and/or secondary water quality standards, and creation of explosive or noxious gases must be considered. The table below lists common contaminants that are found at ISB projects, pre-treatment and/or post-treatment. (EPA, 2005; ITRC, 2005a)

Contaminant	MCL (ppm)	MCLG (ppm)	Secondary Standard (ppm)	Health Effect (s)
Nitrate	10	10	n/a	blue-baby syndrome
Sulfate	n/a	n/a	250	laxative
Selenium	0.05	0.05	n/a	neurological/reproductive
Manganese	n/a	n/a	0.05	neurological difficulties
Arsenic	0.01	0	n/a	carcinogen; organ failure
Dissolved Iron	n/a	n/a	0.3	liver & kidney damage
Chloride	n/a	n/a	250	taste/odor

Table VII. List of Maximum Contaminant Levels (MCLs), Maximum Contaminant Level Goals (MCLGs), and Secondary Standard for Contaminants Often Found in Groundwater at Perchlorate Treatment Sites
(EPA, 2006)

These contaminants should be monitored and considered when designing an ISB project. Nitrate levels should decrease during ISB to allow for perchlorate reduction, so water quality improves in this regard. However, ISB often increases the concentration of bioavailable heavy metals (arsenic, selenium, manganese, iron). Hydrogen sulfide is not listed as a primary or secondary contaminant by the EPA, although people can detect a rotten egg odor at just 0.5 ppm of hydrogen sulfide and it can also corrode pipes. As such, decreasing sulfate levels during ISB is also unfavorable, because it may correspond with increasing sulfide levels. Perchlorate reduction can increase levels of chloride, which can sometimes be detected in drinking water wells.

In general, project managers agree that potential decreases in short-term water quality parameters are overshadowed by remediation of perchlorate. Additionally, the other water quality parameters improve once oxidizing conditions return (when groundwater flows outside of the treatment zone, and after the end of the project inside the treatment zone). (Cox, pers. comm., 2006; Hatzinger, pers. comm., 2006)

CASE STUDIES OF *IN SITU* BIOREMEDIATION

The following case studies highlight differences among *in situ* bioremediation applications, note the effectiveness of each project, and comment on suggestions to improve future projects with a similar site design. The cases should exemplify the similarities and differences in, as well as the advantages and disadvantages of, different ISB approaches that are available to treat perchlorate-contaminated groundwater.

9. ISB CASE STUDY 1: NAVAL SURFACE WARFARE CENTER, INDIAN HEAD, MD

Key ISB Features:
- **Study Scale: Pilot**
- **ISB Type: Mobile injection**
- **System Type: Active**
- **Substrate: Sodium Lactate**
- **Project Duration: 7/25/02 – 11/13/02**
- **Monitoring: 1/22/02 – 12/12/02**
- **Notable Point: low pre-treatment pH**
- **Average Perchlorate Reduction: 93.06%**

The purpose of the Indian Head Division of the Naval Surface Warfare Center (IHDIV), Indian Head, MD, is to "support the nation's warfighter" by "developing, testing, and manufacturing the newest generation of explosives and propellants" (IHDIV, 2006). In doing so, significant amounts of perchlorate have contaminated the groundwater under the Superfund site. The Strategic Environmental Research and Development Program (SERDP) funded the IHDIV cleanup project, CU-1163. To remediate the perchlorate onsite a laboratory study and pilot scale application of ISB was conducted using an active recirculation system to encourage uniform distribution of the mobile, soluble amendments. The purpose of this study was to determine whether perchlorate-contamination in an acidic, shallow aquifer could be effectively degraded in less than four months, using a mobile injection of soluble substrate and a buffer, along with recirculation. The main questions that this study aims to answer are:
- Is ISB of perchlorate-contaminated groundwater a viable treatment option on the East Coast of the U.S., which frequently has shallow, acidic aquifers?
- Can ISB degrade over 250,000 ppb of perchlorate *in situ*, in a matter of months?
- Is sodium lactate a good soluble substrate choice for a mobile injection of amendments?
- Does recirculation optimize perchlorate reduction and maintenance of other water quality parameters?
- What improvements could be made for future projects similar to this one?

In the following sections, IHDIV's site characteristics will be discussed, along with the study's design and outcome. (Cramer et al., 2004)

9.1 Site Characterization

9.1.1 Source of Contamination

Ammonium perchlorate was used as the solid propellant in building military defense systems, such as rockets and explosives, at IHDIV. Building 1419 was used to clean (also known as "hog out") solid propellant out of military defense systems. This cleaning caused ammonium perchlorate releases onsite, which led to groundwater contamination under and around the site. (Cramer et al., 2004)

9.1.2 Site Hydrogeology

The geological features of the area IHDIV originate from Pleistocene lowland deposits from historic coastal lowlands, and Cretaceous Potomac Group formations created by ancient rivers that deposited interbedded layers of sand, silt and clay. The field study was conducted just 300 feet from Mattawoman Creek and adjacent to the Potomac River.

Figure 10. Aerial Photograph of the Land Composing the Naval Surface Warfare Center in Indian Head, MD, located near the Potomac River (on the left) and Mattawoman Creek (on the right). Arrow Points to the ISB Project Site. (Reprinted from Hatzinger, 2003)

Geological soil samples indicated that the top 2-4 foot layer of soil includes organic matter, gravel and silty sand, followed by approximately 12 feet of clay and sandy silt, then a 1-1.5 feet thick sand and gravel layer, and finally, a clay layer. There is a very shallow groundwater table in the area. The groundwater monitoring wells showed that the depth to groundwater in the field study treatment zone ranged from 6.5-10.25 feet below ground surface (bgs). The average hydraulic gradient was 0.023 ft/ft. (Cramer et al., 2004)

9.2 Laboratory Study Findings

Laboratory studies were initially conducted to determine whether a pilot-scale enhanced anaerobic ISB project would be successful in remediating the perchlorate-contaminated groundwater plume at IHDIV. Samples of the groundwater under Building 1419 were taken to evaluate the presence of dissimilatory perchlorate-reducing bacteria (DPRB), geochemical characteristics, and appropriate substrate in this location. The laboratory tests indicated that there were in fact indigenous DPRB located in the groundwater aquifer under IHDIV. An enumeration

study, conducted by contaminant microbiologist John Coates, revealed that three types of indigenous DPRB were present in different soil and water locations on the IHDIV site:

- *Dechloromonas agitatus* CKB type
- *Dechloromonas aromatica* RCB type
- *Azospira suilla* PS type

Nevertheless, the initial microcosm studies showed that the perchlorate reducers were not actively degrading perchlorate. There was no notable perchlorate degradation, regardless of the substrate type. Project Managers working on the site correctly inferred that the low pH was the primary reason that there was a lack of effective perchlorate reduction in the laboratory studies. The groundwater pH was 4.2-5.0 in most places, and less than 5.5 in all water samples tested. In order to effectively biodegrade the perchlorate, the pH had to be increased and buffered using a carbonate/bicarbonate mixture. (Hatzinger, pers. comm., 2006; Cramer et al., 2004)

Different types of soluble substrate were tested to determine the most suitable substrate, considering the site's indigenous DPRB and hydrogeochemical characteristics. The soluble substrates that were tested included hydrogen, propane, ethanol, methanol, acetate, benzoate, lactate, molasses, sucrose, and a yeast/ethanol mixture. According to Paul Hatzinger, one of the project managers, "all of the substrates tested supported denitrification, but only lactate and acetate supported perchlorate reduction, and only after buffering." (Hatzinger, pers. comm., 2006)

9.3 Pilot Study Design

The IHDIV study was designed to create a mobile, bioactive treatment zone through continual amendment injection and recirculation (Figure 11).
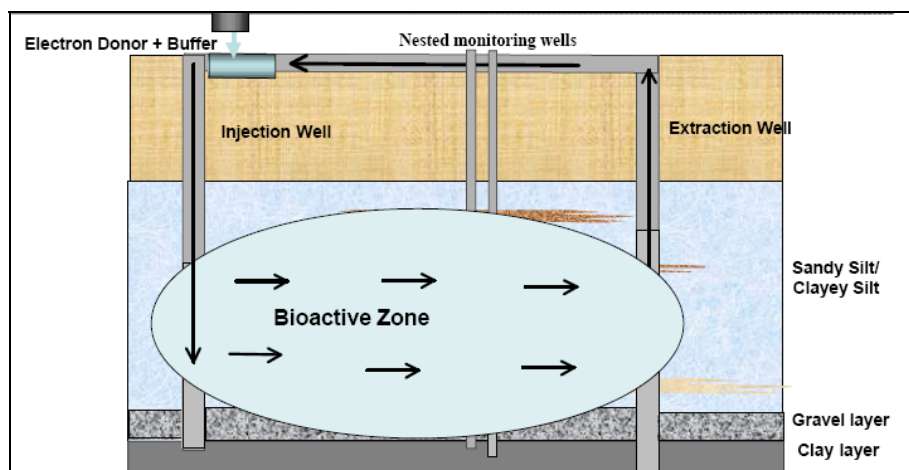

Figure 11. Depiction of Active, Mobile *In Situ* Bioremediation at the Naval Surface Warfare Center, IHDIV. (Hatzinger, 2003)

This diagram visually depicts the IHDIV ISB project design, which will be discussed more thoroughly below.

9.3.1 Substrate Addition

The substrate used for the field study was food-grade sodium lactate, a soluble substrate. Although both lactate and acetate allowed for effective perchlorate reduction, lactate was chosen over acetate because it was available as a food-grade substrate (Hatzinger, Personal Communication, 2006). The solution (60% wet weight syrup) was purchased from Purac America Inc., based in Lincolnshire, Illinois. The substrate's pH was neutral, which helps to maintain pH levels for effective perchlorate reduction.

In sum, 24 gallons of the substrate solution was added into the treatment zone during the 111 day period. During week three and five, three gallons of the substrate was added directly to the aquifer, along with an extra injection of buffer, to stimulate ISB further. The extra addition was then recirculated for one week without adding more substrate. Otherwise, the substrate was continually reinjected into the treatment zone. Each tank contained approximately 380 mg/L of substrate when it was reinjected into the treatment zone. This concentration of substrate was sufficient to allow depletion of oxygen, nitrate, and perchlorate within a margin of safety, given the site's hydrogeochemical characteristics. (Cramer et al., 2004)

9.3.2 Buffer Addition

The buffer was added to the treatment zone by filling a tank with groundwater taken from the extraction well on the site. The sodium bicarbonate/sodium carbonate mixture was added as a 6.67% buffer mixture. The food-grade product was purchased from Seidler Chemical Company, based in Newark, New Jersey.

During week three and five when extra substrate was added, 250 gallons of the buffer mixture was also added to the treatment zone and recirculated without further addition for one week. This large injection of the buffer mixture was added to more quickly increase alkalinity and the pH. Otherwise, a constant addition of the buffer mixture was added into the treatment zone. A buffer pump was used to transfer the buffer mixture from the buffer-holding tank into the water holding tank. Every batch of water contained approximately 2,500 ppm of the buffer solution. The buffer was injected back into the treatment zone with the water and substrate amendment via the two injection wells. (Cramer et al., 2004)

9.3.3 Recirculation

An active pumping system was used to distribute the amendments more uniformly, encourage more effective perchlorate reduction, and minimize sulfate reduction.

To recirculate the water, two injection wells and two recovery wells were constructed within 12 feet of each other. The two injection wells were installed downgradient of each other, while the two extraction wells were installed cross-gradient of the injection wells to facilitate lateral mixing of the substrate and buffer. During the demonstration, approximately 20,000 gallons of groundwater was recirculated. To ensure that the recharge rate exceeded the extraction rate, the site managers determined that the injection pump would pump approximately 1 gallon/minute, while the extraction pumps would extract approximately 0.25 gallon/minute.

Figure 12. Photograph of the Above Ground Infrastructure: The Injection Skid, Lactate Tank and Buffer Tank. (Reprinted from Hatzinger, 2004)

To constantly recirculate the amended water, two holding tanks were used to temporarily store 40 gallons of water extracted from the treatment zone. The substrate and buffer was added to the holding tanks and mixed, and then both injection wells would reinject the amended water at 1 gallon/minute. Each 40 gallon tank of amended water took 20 minutes to reinject. (Cramer et al., 2004)

9.3.4 Control Plot

A control plot was used to ensure that ISB occurred as a result of the amendment addition and recirculation, not other factors. The control plot was situated near the treatment plot in order to maintain similar hydrogeochemical conditions. Water was recirculated in the same way, but no amendments were added. (Cramer et al., 2004)

9.4 Pilot Study Results

Unless otherwise specified, pre-treatment versus post-treatment results below emanate from the following time periods and wells.
- Pre-treatment Samples:
  - 7days before the study began, on July 18, 2002
  - 9 test plot monitoring wells
- Post-treatment Samples:
  - 105 days after beginning treatment, on November 7, 2002
  - 9 test plot monitoring wells

Results of the geochemical characteristics that were tested are below. To provide a conservative estimation, samples below the limit of detection were assumed to be at the detection limit for calculating averages. It should be noted that a number of characteristics were not tested in the IHDIV study, including methanogenesis, oxidation-reduction potential (ORP), and metals bioavailability. The following table summarizes the pre-treatment versus post-treatment ranges, averages, and percent change for the various geochemical parameters that were tested during the study. (Cramer et al., 2004)

Geochemical Parameter	Pre-Treatment	Post-Treatment	% Change, on Average
Perchlorate			
Range (ppb)	72,000 - 250,000	<4 - 89,900	
Average (ppb)	173,778	12,053.84	**-93**
Dissolved Oxygen			*2 recalibration errors not included
Range (ppm)	<0.1 - 1.64*	n/a	
Average (ppm)	0.74	n/a	**n/a**
Nitrate			
Range (ppm as N)	0.88 - 4.3	0.2 - 0.82	
Average (ppm as N)	2.09	0.32	**-85**
Sulfate			
Range (ppm)	79 - 320	21 - 540	
Average (ppm)	173.78	221.11	**27**
pH			
Range	3.82 - 5.99	5.83 - 6.8	
Average	4.73	6.3	**33**
Alkalinity			
Range (ppm as CaCO3)	<2 - 92	250 - 1,600	
Average (ppm as CaCO3)	21.82	698.89	**3102**
Lactate			*After Injection **After study's end
Range (ppm)	<0.5 - 376*	<0.5 - 230**	
Average (ppm)	121.22	38.17	**-68**

Table VIII. Geochemical Characteristics in Groundwater at the Active, Mobile ISB Project at IHDIV, Pre-treatment Versus Post-treatment. (Cramer et al., 2004)

These values are discussed in further detail below.

9.4.1 pH and Alkalinity

The pH was fairly acidic before the study began; it ranged from 3.82 – 5.99 in the targeted treatment zone, and averaged only 4.73. The carbonate/bicarbonate buffer was added throughout the study's duration to increase the pH and alkalinity, as a way of activating the indigenous DPRB. After the buffer was added, the pH ranged from 5.83 – 6.8, and averaged 6.3. There was a 33.19% average increase in the pH.

The buffer addition also drastically increased alkalinity in the treatment zone. Pre-treatment, alkalinity ranged from less than 2 – 92 ppm as $CaCO_3$, whereas after treatment alkalinity ranged from 250 – 1,600 ppm as $CaCO_3$. The pre-treatment average alkalinity of 21.82 ppm as $CaCO_3$ markedly contrasts the post-treatment average alkalinity of 698.89 ppm as $CaCO_3$ – a 3,102% average increase. The alkalinity helped to stabilize the pH during ISB (Cramer et al., 2004)

9.4.2 Depletion of Electron Acceptors

9.4.2.1 Dissolved Oxygen

Pre-treatment, DO levels were already in the hypoxic to anoxic range (<0.1 – 1.64 ppm), with an average DO level of 0.74 ppm. Since low DO levels persisted before treatment, post-treatment levels were not evaluated.

One monitoring well accidentally received air exchange with the injection well. Further investigation revealed that increased DO levels competed with perchlorate as an electron acceptor, and used an appreciable amount of substrate. (Cramer et al., 2004)

9.4.2.2 Nitrate

It is clear that microbes effectively reduced nitrate once substrate and a buffer were added to the treatment zone. Nitrate levels were well under the EPA's MCL standard before treatment, ranging from 0.88 – 4.3 ppm and averaging 2.09 ppm. After treatment, nitrate levels decreased further, ranging from 0.2 – 0.82, and averaging 0.32 ppm. There was an 84.69% decrease in nitrate, on average. (Cramer et al., 2004)

9.4.2.3 Perchlorate

Once competing electron acceptors were reduced, and amendments continued to be added, perchlorate reduction became favorable in the treatment zone. Overall, there was a marked decrease in perchlorate levels within less than 4 months of active treatment, considering the average pre-treatment perchlorate concentration was 173,778 ppb, whereas the average post-treatment perchlorate concentration was 12,053.84 ppb; a 93.06% average decrease. Before treatment, perchlorate levels were detected as high as 250,000 ppb in the groundwater under IHDIV's Building 1419; the highest concentration of perchlorate found of the case studies in this report. After treatment, perchlorate concentrations on the site ranged from less than 4 ppb, the limit of detection, to 89,900 ppb.

Only one monitoring well showed a lower decrease in perchlorate (43% reduction), due to accidental air exposure. Depleting the oxygen in this well used up most of the available lactate very quickly and therefore inhibited effective perchlorate reduction in that area, when compared to the other wells that were situated farther away from the injection wells. Excluding this well, every other monitoring well experienced at least a 95% decrease (and up to a 99% decrease) in the amount of perchlorate present in the treatment zone, pre-treatment versus post-treatment. (Hatzinger, pers. comm., 2006; Cramer et al., 2004).

9.4.2.4 Sulfate

Excess substrate left over from perchlorate reduction may be used to reduce sulfate, the next favorable electron acceptor. Sulfate concentrations ranged from 79 – 320 ppm before treatment, with an average sulfate level of 173.78 ppm. Post-treatment, sulfate concentrations ranged from 21 – 540 ppm, and averaged 221.11 ppm. There was a 27% average increase in sulfate, which

suggested that hydrogen sulfide production was not a concern. A hydrogen sulfide smell was detected during the study, but tests indicated H_2S only slightly increased from 174 ppm to 240 ppm, pre-treatment versus post-treatment. (Cramer et al., 2004)

9.4.3 Control Plot

There was not a notable decrease in perchlorate levels in the control plot, which simulated recirculation of groundwater but no amendment addition. There were no other significant changes in the control plot's geochemical characteristics either, which suggests that the substrate and buffer addition significantly enhanced ISB. (Cramer et al., 2004)

9.4.4 Substrate Utilization

Distribution of the substrate is very important for effective ISB. Tests were conducted six times throughout the study to determine whether or not the substrate was being distributed throughout the treatment zone and at what concentration. The tests indicated that lactate was present in seven of nine monitoring wells within 14 days. Eight of nine wells exceeded 100 ppm of lactate during the study. The well with air exchange had lactate levels ranging from 6 – 21 ppm. After the 111th day, no more lactic acid was added; 29 days later, seven of nine monitoring wells could not detect lactate at the detection level of 0.5 ppm. The supply was exhausted in most monitoring wells, as expected. The small amount of residual lactate suggest that there was a sufficient amount of substrate present to effectively reduce competing electron acceptors, without encouraging excessive hydrogen sulfide production, methanogenesis, or metals mobilization. (Cramer et al., 2004)

9.5 IHDIV Summary

This was the first enhanced anaerobic ISB field study for perchlorate on the East Coast of the U.S., the first study that took place in an acidic groundwater aquifer, and the first study that confirmed that enhanced anaerobic ISB can effectively degrade over 250,000 ppb of perchlorate *in situ*. This field study confirmed that higher concentrations of perchlorate could be quickly degraded at IHDIV by adding a buffer to increase the pH and alkalinity, injecting sodium lactate as an electron donor, and actively recirculating the mobile amendments. Perchlorate levels underwent a 95% reduction in eight of nine wells in less then four months.

The active recirculation system mixed the amendments effectively throughout the treatment zone. Both the substrate and the buffer played a critical role in stimulating the indigenous DPRB to actively reduce perchlorate. The buffer amendments were essential, as they increased the acidic pH to fairly neutral in the entire treatment zone, buffered acidic compounds, and activated DPRB.

The amount of sodium lactate added proved to be effective as an electron donor and in the amount added, as oxygen, nitrate and perchlorate levels were reduced. Low DO levels created a reducing environment conducive to perchlorate reduction. Nitrate levels were fully depleted (below detection levels) in most of the monitoring well tests. Furthermore, there was not a substantial increase in the amount of hydrogen sulfide, indicating that there was not an

abundance of lactate left. After perchlorate was degraded, reducing conditions no longer remained to further sulfate reduction. Biofouling did not pose a problem.

A 93.06% average reduction in perchlorate in less than four months supports that ISB at this site was incredibly successful. However, post-treatment average perchlorate levels were still much higher than EPA's non-enforceable DWEL of 24.5 ppb (which would be comparable to a proposed MCL). Further ISB projects on the site could reduce perchlorate below the limit of detection by conducting treatment for a longer time period and spacing injection wells farther away from monitoring wells (to minimize DO entering into the treatment zone. (Cramer et al., 2004)

10. ISB CASE STUDY 2: CONFIDENTIAL DOD SITE, ELKTON, MD

<u>**Key ISB Features:**</u>
- **Study Scale: Pilot**
- **ISB Type: PRB**
- **System Type: Passive**
- **Substrate: Slow-release oil emulsion, EOS®**
- **Project Duration: 10/03 – 4/05**
- **Monitoring Duration: 9/03 – 4/05**
- **Notable Point: TCA degraded as well**
- **Average Perchlorate Reduction: 99.90%**
- **Average TCA Reduction: 96.05%**

In this pilot-scale study, a passive PRB was created in northeastern Elkton, MD during October of 2003. This confidential DoD site contained perchlorate and 1, 1, 1-trichloroacetic acid (TCA) groundwater contamination. The study was sponsored by the Environmental Security Technology Certification Program (ESTCP). The purpose of the study was to determine the remedial successfulness, cost effectiveness and simplicity of employing a passive PRB using a slow-release emulsified oil substrate, EOS®, to microbially degrade perchlorate and TCA simultaneously. The questions that this study aims to answer include:

- Can an EOS®-created PRB form conditions conducive to both perchlorate and TCA reduction?
- Does a passive system allow for effective perchlorate degradation and cost savings, without degrading secondary water quality characteristics?
- Are emulsified oil substrates a good electron donor choice? When might this substrate not be ideal?

In the following case study, the qualitative and quantitative aspects of the remediation project will be summarized, and the advantages and disadvantages of using a passive system with slow-release substrate will be considered. (IES, 2006)

10.1 Site Characterization

10.1.1 Site Contamination History

The Maryland DoD site was previously used to research, manufacture, and test rocket engines containing ammonium perchlorate. Manufacturing included fireworks and munitions production. To contain spent perchlorate and waste solvent, which was also prevalent onsite, a surface impoundment was created in 1976. Onsite testing revealed that the rubber liner containing the waste chemicals was leaking in 1983; it was replaced with a plastic liner, and impoundment use halted in 1988. The leak caused migration of the perchlorate and TCA, and subsequent groundwater contamination under and around the site. (IES, 2006)

10.1.2 Site Hydrogeology

The major hydrological parameters in the contaminated area onsite are as follows:
- Geologic formations: silty sand and gravel to 15 ft. bgs, then silty clay

- o Water table: 1-8 ft bgs
- o Groundwater depth: 15 ft bgs
- o Groundwater velocity: 400 ft/yr avg.
- o Groundwater flow: 36,135 ft^3/yr
- o Hydraulic conductivity: 14,600 ft/yr; 40 ft/d (pre-injection avg.)
- o Hydraulic Gradient: 0.003 ft/ft (pre-injection avg.)
- o Effective Porosity: 0.18

Further information pertaining to hydrogeology was not available. (IES, 2006)

10.2 Laboratory Study Design

The pilot study was preceded by a microcosm study for two main reasons – first, to find an oil that would assist microbes in completely degrading both perchlorate and TCA, and second, to determine if bioaugmentation would be necessary to fully degrade TCA.

The microcosms indicated that the edible soybean oil-in-water emulsion, EOS®, was an excellent slow-release substrate choice. Perchlorate was fully and quickly degraded in the microcosms containing EOS® substrate – in less than 14 days, perchlorate decreased from 50 ppm to less than 0.008 ppm. EOS® was also an effective electron donor for TCA reduction, but the microcosms suggested that TCA biodegradation would take substantially longer than perchlorate. Most TCA degraded within 70 days, and its metabolite, 1,1-DCA, mostly degraded within 110 days. The microcosms indicated that indigenous DPRB were present, but enumeration studies were not conducted to determine the types of indigenous DPRB present. The laboratory study also indicated that bioaugmentation would not be necessary for TCA reduction.

After EOS® was chosen for the oil substrate, column tests were used to verify that it would successfully distribute throughout the aquifer's sand and gravel geology. EOS® was injected into columns simulating the subsurface environment at the site. Results indicated that the substrate was able to distribute throughout the entire column, and there were higher concentrations in the shallower area of the column, as expected. (IES, 2006)

10.3 Pilot Study Design

For the PRB to be successful, a number of requirements must be met. EOS® must be distributed across the treatment zone to form the PRB. DPRB must be able to use EOS® as an energy, carbon and growth source. There must be a sufficient contact time so that perchlorate is degraded to the target goal after passing through the PRB.

The microcosm studies suggested that this PRB could, in fact, succeed in these criteria. Four study design elements were considered before constructing the EOS® PRB:
- Injection well spacing
  - o Tighter spacing distributes substrate more uniformly and effectively
- Screen intervals for injection wells
  - o Enlarging the screen interval increases radius of influence

- Substrate quantity
  - Adding excess substrate encourages more effective reduction, but increased likelihood of sulfide production, methanogenesis, and metals mobilization
- Injection volume (diluted substrate and chaser)
  - Chasing the oil substrate with water assists in distributing the substrate into the targeted treatment zone

A pre-treatment bromide tracer test was performed in July of 2003, to characterize baseline flow parameters. Another tracer test was carried out nine months after the EOS® injection, in July of 2004, to determine if the oil barrier decreased permeability along the PRB. (IES, 2006)

10.3.1 Substrate Utilization

10.3.1.1 Substrate Biodegradation

DPRB use the EOS® for microbial growth, a carbon source, and energy. The emulsified oil is biodegraded into shorter chain fatty acids, and ultimately degraded into H_2 and CO_2. Dehalorespiring microbes then use H_2 to degrade the TCA via reductive dechlorination. As a result, EOS® provides electron donors to both perchlorate-reducing and TCA-reducing microbes.

EOS® substrate must be soluble enough to continually supply a sufficient electron donor source to maintain reducing conditions and support microbial perchlorate reduction. If the biodegradation rate is too slow, groundwater will pass through the PRB with residual perchlorate contamination. The PRB will need to be widened to increase contact time; additional EOS® could be added, or soluble substrate could supplement EOS®. If EOS® biodegrades more quickly than anticipated, more will have to be reinjected, which increases costs. (IES, 2006)

10.3.1.2 Substrate Distribution

EOS® sorbs strongly to soil, which creates residual substrate that dissolves into the groundwater plume slowly over time and facilitated extended microbial perchlorate reduction. Slow-release oil substrates can be directly injected into the subsurface environment in two ways, either by distributing oil in its non-aqueous phase, or by using an oil-in-water emulsion. An emulsified oil substrate is less viscous, more mobile and more effective at distribution than non-aqueous phase oil.

There are several factors that must be considered for optimal EOS® distribution and biodegradation. To create a continuous PRB that encourages contaminated groundwater to flow through, the EOS® substrate must be uniformly and continuously distributed horizontally (to the planned width of the PRB) and vertically (to the planned depth of the PRB). Secondly, permeability cannot be reduced in the treatment zone – if it is, the contaminated groundwater will travel to the path of least resistance – around the PRB – and bypass treatment.

Studies using an oil substrate without an emulsion have not been effective because of difficulties in distributing the substrate due to its oil:water density (specific gravity), repulsion to water, and capillary forces. EOS® is fully miscible with water, has a similar specific gravity to water, and

reduced viscosity, making emulsified oil a more conducive substrate for this study and in general. Water can be used as a chaser to push oil or oil emulsions further into the treatment zone. (IES, 2006)

10.3.1.3 Design Considerations

A number of important parameters were considered before the injection delivery took place. Substrate demand was calculated considering two factors:
1) Amount of available oil needed to complete perchlorate and TCA reduction
    a. Oil demand by competing electron acceptors
        i. Oxygen
        ii. Nitrate
    b. Perchlorate treatment goal (pre vs. post-treatment concentration)
    c. TCA treatment goal (pre vs. post-treatment concentration)
2) Amount of carbon released from PRB
    a. Oil retention by sediment
    b. Solubility of substrate

The amount of oil required for the PRB to be effective, according to hydrogeochemical characteristics and design plans, is determined by considering substrate demand, design life, and water flux (Appendix Eq. 1). A lifespan of three years was chosen so that the project could evaluate the rate of oil depletion and PRB characteristics as the project reaches the end of the lifespan. To determine the groundwater flow rate, the aquifer's porosity, hydraulic conductivity, and hydraulic gradient must be estimated, along with the PRB's estimated width and height (Appendix Eq. 2). The amount of substrate required was confirmed by calculating the site's oil retention capability, which is vital to maintaining a PRB (Appendix Eq. 3). (IES, 2006)

The emulsified oil was purchased from EOS® Remediation, Inc., which is located in Raleigh, NC. EOS® consists of a mixture of 60% food-grade soybean oil, 24% water, 2% yeast extract, 10% emulsifier, 1% lactic acid, and 3% sodium lactate. Once the two EOS® barrels were obtained, it was mixed with water onsite using a 1:4 EOS® to water ratio. This ratio was determined by considering the total volume of water and emulsion mixture necessary to fully distribute the substrate to the PRB design specifications. (IES, 2006)

The chosen PRB site was located in a field 150 feet downgradient from the former surface impoundment. The PRB was designed to be 50 feet wide, with an effective height of 10 feet (but expecting only five feet of increased permeability). The PRB length of five feet was planned by estimating the contact time necessary to meet treatment goals. Two barrels of EOS® were chosen for the substrate quantity. Ten injection wells were constructed five feet apart from each other, perpendicular to the flow of groundwater. The wells consisted of screened PVC piping and no above ground infrastructure was needed. (IES, 2006)

10.3.2 Injection Delivery

Diluted EOS® was injected under low pressure into each injection point along a 50 foot line perpendicular to the flow of contaminated groundwater. The total amount of substrate injected is equal to 110 gallons of concentrated EOS®. The 10 EOS® injections were each promptly chased

with 165 gallons of water along the injection path to create more uniform distribution. This one-time process took place using only two site workers during a two day period, and only required a mixing/holding tank, injection hoses, transfer pump, flow meters, pressure gauges, valves, and water.

Creating strong reducing conditions could indirectly decrease downgradient water quality by encouraging sulfide production, methanogenesis, heavy metals mobilization, and taste and odor concerns. To degrade TCA via reductive chlorination, however, strongly reducing conditions are necessary, so an overabundance of EOS® was purposefully added to ensure that the substrate created sufficient contact time and a strongly reducing environment along the PRB. According to Bob Borden, a consultant for EOS® and Industrial & Environmental Solutions, adverse effects on secondary water quality characteristics usually are not a problem once groundwater travels just 50-100 feet downgradient of the PRB, as oxidizing conditions outside of the treatment zone degrade methane and sulfate, and immobilize metals once again. (IES, 2006)

10.4 Pilot Study Results

Thirty locations around the PRB were tested for various geochemical characteristics using a Geoprobe Screen-Point Sampler®. Perchlorate concentrations were monitored at the upgradient and downgradient monitoring wells, in addition to the injection wells, seven times throughout the 18 month lifespan of the study. The various monitoring results, pre-treatment versus post-treatment, are discussed below.

The ranges and averages were compiled for important geochemical characteristics, using the five downgradient monitoring wells (7.5, 12.5, 20, 20, 20 ft away). Note that the pre-treatment values are from September 29-30 2003, and post-treatment values are taken from the April, 21 2005, unless otherwise specified. To provide a conservative estimation, samples that were below the limit of detection were assumed to be at the detection limit for calculating averages. The following table summarizes the pre-treatment versus post-treatment ranges, averages, and percent change for the various geochemical parameters.

Geochemical Parameter	Pre-Treatment	Post-Treatment	% Change, on Average
Perchlorate			
Range (ppb)	3,100 - 20,000	<4 - 40	
Average (ppb)	8,600	8.5	**-99.90**
Dissolved Oxygen			
Range (ppm)	1.27 - 5.83	0.39 - 1.69	
Average (ppm)	3	1.23	**-59**
Nitrate			
Range (ppm as N)	<0.5 - 13.9	<0.5	
Average (ppm as N)	9.5	<0.5	**-95**
Sulfate			
Range (ppm)	18.3 - 34.4	0.9 - 8.7	
Average (ppm)	27.8	6.5	**-77**
Methanogenesis			
Range (ppb)	<0.2 - 0.4	1,463.8 - 3,551.5	

Average (ppb)	0.26	2,737	**1,052,592**
Dissolved Manganese			
Range (ppm)	0.05 - 0.6	<0.05 - 9.7	
Average (ppm)	0.23	6.27	**2,626**
Dissolved Iron			
Range (ppm)	<0.5	<0.5 - 23	
Average (ppm)	< 0.5	11	**2,100**
Dissolved Arsenic			*measured at higher detection limit – cannot compare changes
Range (ppm)	<0.01*	0.0049 - 0.014	
Average (ppm)	< 0.01*	0.0095	**n/a**
ORP			
Range (mV)	102 - 154	-7.8 to -53.1	
Average (mV)	132	-31.26	**-124**
pH			
Range	5.39 – 5.79	6.59 - 6.84	
Average	5.67	6.7	**18**
TCA			
Range (ppb)	5,700 - 25,000	220 - 1,000	
Average (ppb)	14,540	574	**-96.05**
Total CAHs			
Range (ppb)	6,038 - 26,652	1,500 -2,925	
Average (ppb)	15,474	1,916	**-87.62**
TOC			
Range (ppm)	<1 - 1.4	31.9 - 51.9	
Average (ppm)	1.2	44.24	3,586.67

Table IX. Geochemical Characteristics in Groundwater at the Passive EOS® Permeable Reactive Barrier (PRB) in Elkton, MD, Pre-treatment Versus Post-treatment (IES, 2006)

The geochemical values are discussed in further depth below. (IES, 2006)

10.4.1 Oxidation-Reduction Potential (ORP)

Oxidation reduction potential (ORP) – which describes whether an environment is reducing (or oxidizing) and the likelihood of a reducing (or oxidizing) chemical reaction – is determined using an ORP electrode to detect the ratio of reduced ions to oxidized ions. Perchlorate degradation can take place effectively under slightly reducing conditions, but to degrade TCA via reductive chlorination, strongly reducing conditions must be present in the treatment zone – an ORP less than – 100 mV is ideal. Strong reducing conditions also promote adverse secondary water quality changes, but usually these changes are only temporary and are not considered major, relative to the TCA contamination. (Borden, pers. comm., 2006; IES, 2006)

ORP ranged from 102 – 154 mV before treatment, with an average of 132 mV. These oxidizing conditions quickly changed once the EOS® PRB was formed. Post-treatment, ORP ranged from -7.8 to -53.1 mV, with an average ORP of -31.26. There was a 123.68% decrease in ORP, on average, indicating that solid reducing conditions were created in the treatment zone around the

PRB. Perchlorate reduction is ideal in this type of reducing environment. However, an even lower ORP (< - 100 mV) would have created better conditions for TCA degradation. (IES, 2006)

10.4.2 pH

The pH increased by 18.17%, on average, over the course of the study. Before treatment, the pH ranged from 5.39 – 5.79, with a slightly acidic average pH of 5.67. After treatment, the pH ranged from 6.59 – 6.84, with an average pH of 6.7. No buffer was added during the study. It is customary for the pH to increase when reducing conditions are present. (IES, 2006)

10.4.3 Depletion of Electron Acceptors

10.4.3.1 Dissolved Oxygen (DO)

DO levels decreased enough to permit effective perchlorate and TCA reduction. On average, DO decreased from 3 ppm to 1.23 ppm in the five downgradient monitoring wells, pre-treatment to post-treatment. Pre-treatment DO ranged from 1.27 – 5.83 ppm, while post-treatment DO levels ranged from 0.39 – 1.69 ppm. Some of the wells had ideal anaerobic conditions, with DO less than 0.5 ppm, while other wells contained borderline hypoxic conditions (i.e. DO at one well was 1.69 ppm). On average, there was a 59% reduction in DO levels in the treatment zone, after the PRB was formed. (IES, 2006)

10.4.3.2 Nitrate

Pre-treatment, average nitrate levels at the site remained just below the 10 ppm MCL standard (9.5 ppm), while a few locations contained slightly higher concentrations of nitrate (13.9 ppm). Nitrate was completely eliminated in the site's subsurface environment (below detection limit of 0.5 ppm) shortly after the electron donor was injected. On average, there was a 95% reduction in nitrate, pre-treatment versus post-treatment. Quick depletion of nitrate encouraged more expedient perchlorate reduction, with the added benefit of eliminating the less toxic, but potentially problematic groundwater contaminant. Low levels of nitrate (< 1 ppm) were detected in some of the injection wells 18 months after injection, indicating that PRB efficiency was beginning to decrease as EOS® was being depleted. (IES, 2006)

10.4.3.3 Perchlorate Degradation

The PRB exceeded the project goal of reducing perchlorate by 90% in groundwater downgradient of the old surface impoundment. Groundwater perchlorate concentrations ranged from 3,100 – 20,000 ppb before treatment, with an average perchlorate concentration of 8,600 ppb. Post-treatment, perchlorate levels ranged from below the detection limit of 4 ppb – 40 ppb, with an average of 8.5 ppb. On average, there was a 99.9% decrease in the amount of perchlorate contamination located in the groundwater downgradient of the old surface impoundment. Furthermore, perchlorate concentrations were reduced at all locations downgradient of the PRB, indicating that the substrate was distributed uniformly and continuously. Three of the five monitoring wells were below the limit of detection, met the DWEL for perchlorate of 24.5 ppb, and would most likely meet any MCL that will be enacted. The two monitoring wells that

contain detectable levels of perchlorate – 13 ppb and 40 ppb – still experienced more than a 99.58% and 99.7% reduction, respectively, from pre-treatment concentrations in those wells. (IES, 2006)

10.4.3.4 Trichloroacetic Acid

Chlorinated aliphatic hydrocarbons (CAHs), such as TCA and its metabolites, have been used extensively as solvents at DoD facilities. Chlorinated solvents depress central nervous system functioning and damage the liver and kidneys. The drinking water MCL for 1,2-TCA and 1,1-TCA, which are both present onsite, is 5 ppb and 200 ppb, respectively (EPA, 2006). Prior to treatment, TCA concentrations were as high as 25,000 ppb. Reducing CAH contamination in groundwater is important to reduce human exposure to these chemical compounds. As CAHs are degraded, less chlorinated, less toxic metabolites form. CAHs containing only one chlorine are the least toxic of the CAHs. (IES, 2006)

There are a number of ways to confirm that CAHs, especially the more prevalent TCA onsite, are degraded via ISB. Groundwater samples were taken to check for decreases in TCA, decreases in total CAHs, and fluctuations in CAH metabolites (which reveals the extent of degradation). TCA concentrations decreased markedly in all monitoring wells after treatment. On average, TCA was reduced from 14,540 ppb to 574 ppb, corresponding in a 96.05% reduction in downgradient TCA concentrations. The monitoring wells located 20 ft downgradient of the PRB experienced a 98% reduction in TCA. (IES, 2006)

In general, there was a slight increase in the TCA metabolites, dichloroacetic acid (DCA) and monochloroacetic acid. This indicates that TCA was degraded to intermediate compounds. Increased contact time in the PRB could fully degrade all of TCA's metabolites. (IES, 2006)

Other CAHs that were monitored included dichloroethylene (DCE), PCE, TCE and VC. There was a significant decrease in total CAHs in every downgradient monitoring well. On average, CAH concentrations decreased from 15,474 ppb to 1,916 ppb, which corresponds to an 88% reduction. Considering pre-treatment concentrations ranged as high as 26,652 ppb, this is a major reduction. (IES, 2006)

10.4.3.5 Sulfate Depletion

The PRB's reducing environment created favorable conditions for hydrogen sulfide production. The amount of sulfide production was not a major concern however, because initial sulfate levels were low. Prior to treatment, sulfate averaged 27.8 ppm, with a high concentration of 34.4 ppm. Post-treatment, sulfate concentrations were 6.5 ppm, on average, and ranged from 0.9 – 8.7 ppm. There was a 76.62% average decrease in sulfate levels due to formation of the PRB, but soil-gas monitoring results indicated that no hydrogen sulfide was detected at any of the upgradient, downgradient, or injection wells during the three times it was tested. (IES, 2006)

10.4.3.6 Methanogenesis

The EOS® addition created solid reducing conditions along the PRB. Such reducing conditions, paired with excess EOS®, encourage methanogenesis. Before the PRB was created, methane concentrations in the treatment zone ranged from below the detection level of 0.2 ppb – 0.4 ppb. Post-treatment, methane concentrations skyrocketed to 1,463.8 – 3,551.5 ppb, with an average concentration of 2,737 ppb. Methane was detected in every downgradient monitoring well four months after creation of the PRB. There was more than a one million percent increase in the amount of methane present in the treatment zone 18 months after EOS® was added. Meanwhile, methane concentrations never reached more than 8 ppb in any of the upgradient monitoring wells during the pilot study. Soil gas monitoring tests suggested that the methane was being consumed in the vadose zone, before reaching the surface, so it did not become an explosion hazard. Future studies should consider capturing the methane produced in the subsurface environment, for safety reasons, and, if feasibly possible, use the energy onsite. (IES, 2006)

10.4.4 Metals Mobilization

EOS® is very effective at providing an electron donor for indigenous DPRB and TCA-reducing bacteria. However, the same reducing conditions that allow for effective perchlorate and TCA degradation encourage certain types of metals mobilization.

Dissolved manganese concentrations increased in all injection and downgradient wells after the creation of the PRB. Before treatment, manganese concentrations ranged from 0.05 – 0.6 ppm, with an average of 0.23 ppm. After treatment, manganese concentrations ranged from 1,463.8 – 3,551.5 ppm and averaged 2,737 ppm. Dissolved manganese levels in the treatment zone increased by 2,626%, on average.

Dissolved iron concentrations also increased significantly after the PRB was created. Before treatment, dissolved iron levels were less than 0.5 ppm. After treatment, iron levels ranged from 13 – 23 ppm, and averaged 18.33 ppm. Dissolved iron levels in the treatment zone increased by 2,955%, on average.

Dissolved arsenic levels were below the standard of 10 ppb before treatment. After treatment, arsenic levels ranged from 4.9 ppb to 14 ppb, with an average of 9.5 ppb. The pre-treatment and post-treatment averages could not be compared because different detection limits were used. (IES, 2006)

10.4.5 Soil Permeability

Hydraulic conductivity is a measure used to consider the ease with which groundwater can flow through certain subsurface soil or rock formations. Since EOS® sorbs strongly to soil, it can decrease hydraulic conductivity along the PRB, making it more difficult for contaminated groundwater to flow through the PRB and allow for perchlorate reduction. In the downgradient monitoring wells, hydraulic conductivity decreased by approximately 50% after 18 months since the EOS® injection, although conductivity remained nearly the same just 4 months after the PRB was formed. There were even more drastic decreases in hydraulic conductivity in the injection

wells, located at the center of the PRB, while there was not any appreciable change in the upgradient wells. Therefore, EOS® likely decreased hydraulic conductivity. (IES, 2006)
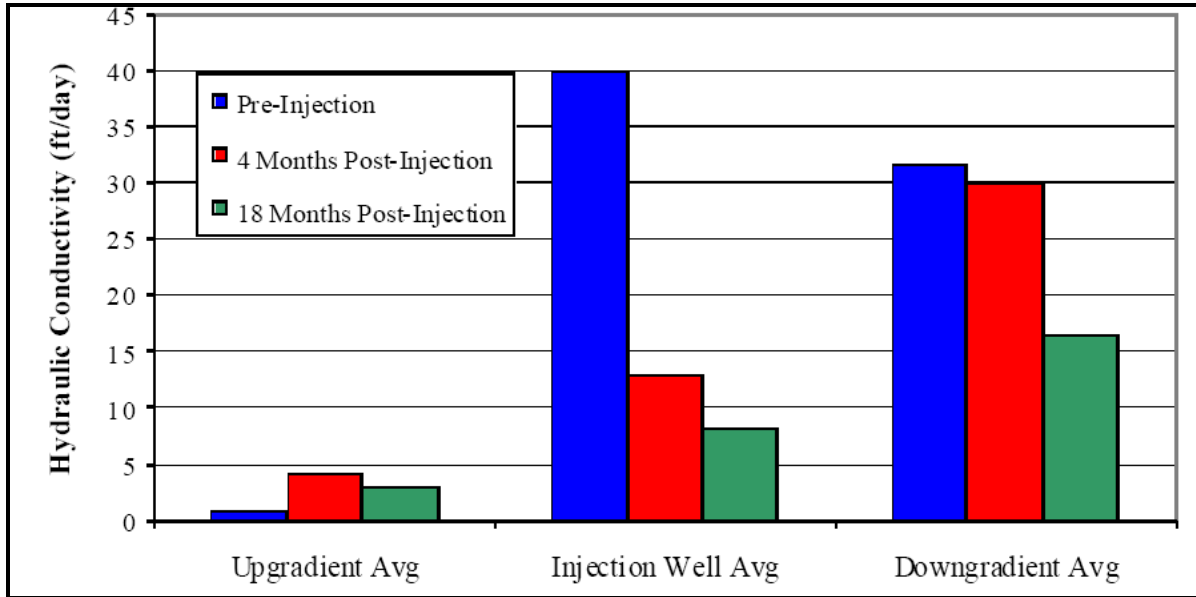

Figure 13. Average Hydraulic Conductivity Changes in the Monitoring Wells, Pre-treatment versus Post-treatment. (Reprinted from IES, 2006)

However, the bromide tracer tests suggested that groundwater flow into the PRB was not substantially affected. Overall, the pre-treatment versus post-treatment bromide tracer tests are comparable. There was some flow around the edge of the PRB, which likely caused perchlorate detection in the downgradient monitoring wells located on the outside of the PRB. The decrease in hydraulic conductivity did not seem to inhibit effectiveness of the PRB and the success of the study though. (IES, 2006)

10.4.6 Odor Concerns

It is important that an EOS® barrier, or any other ISB technique, is not implemented just upgradient of an inhabited area, as smell can become an issue for residents. Biodegradation of EOS® creates a faint soybean oil odor, due to the formation of shorter chain fatty acids. This odor mostly dissipated within 20 ft of the PRB. (Borden, pers. comm., 2006)

10.4.7 Substrate Utilization

Monitoring the amount of total organic carbon (TOC) present in the wells gauges the amount of EOS® substrate that has dissolved into the groundwater and is available for microbial reduction. After formation of the PRB, there was nearly a five-fold increase in the amount of TOC present in soil located 5-10 ft bgs, and nearly a two-fold increase in the amount of TOC present in soil 10-15 ft bgs. Pre-treatment, the average TOC concentration in groundwater was 1.2 ppm, and ranged from less than 1 – 1.4 ppm. Post-treatment, average TOC present in the groundwater in the treatment zone was 44.24 ppm, and ranged from 31.9 – 51.9 ppm. There was a 3,587% increase, on average, in dissolved TOC after EOS® was added, which indicated that EOS® was slowly releasing into the groundwater in the treatment zone for indigenous DPRB, as expected.

Small amounts of the EOS® injection spread to the well 12.5 ft downgradient. However, most of the substrate quickly sorbed to soil and was slowly released into groundwater near the injection delivery points, as expected. (IES, 2006)

10.5 Costs

Capital costs are usually much lower when an *in situ*, rather than *ex situ* technology is used, because infrastructure installation is minimal. For the EOS® PRB, initial costs include construction of injection points, purchasing substrate, and injecting the substrate. These capital costs are dependent on the mass of substrate necessary for the targeted amount of perchlorate reduction, the treatment zone's geochemical characteristics, subsurface lithology, the injection system design, and the degree of oil sorption.

The cost of injecting the substrate was minimal once the injection points were set and the substrate was purchased. In two days, two site workers created the PRB. The largest potential "life cycle" cost (capital plus O&M costs) for the project would have been an unplanned substrate reinjection. However, since the PRB stayed reactive, O&M costs only included monitoring for changing geochemical conditions.

The costs associated with this pilot scale, *in situ* EOS PRB were estimated against the costs of alternatively installing an ion exchange system on the site (using an already constructed pump-n-treat system).
- 30 year Life Cycle Costs
  - *EOS PRB:* $161,400
  - *Ion Exchange:* $383,600
- Capital Costs – Installation of Technology
  - *EOS PRB*: $38,000
    - Equivalent: $19 ft^2 barrier, $0.02/gallon treated
    - Assume 1,000 gallons treated H$_2$O /d, 200 ft. long PRB
  - *Ion Exchange:* $50,000
    - Without installing pump-n-treat system to extract groundwater
- O&M Costs
  - *EOS PRB:* none/minimal
    - Subject to substrate longevity
    - Given no unforeseen problems
  - *Ion Exchange:* $17,000

The costs for the pilot study PRB differ somewhat from the above estimate, which is based on a full-scale EOS® PRB, because the pilot focused on in-depth monitoring. The pilot study's infrastructure cost approximately $23,200 to install ($0.13/gallon, $46/ft^2), with a probable life of 1.5-2 years. (IES, 2006)

10.6 Summary

This EOS® PRB was extremely successful at degrading perchlorate by 99.9% and TCA by more than 96%. The results of this case study indicate the value of using ISB as a multi-contaminant groundwater remediation option. This study confirms that an EOS®-created PRB can effectively degrade perchlorate and chlorinated solvents simultaneously, using different reduction pathways.

Furthermore, this study indicates that emulsified oil-in-water substrates can be cost-effective and efficient in creating long-term PRBs to degrade perchlorate and solvent contamination. EOS® has fairly low solubility, but the emulsion allowed it to distribute well across the PRB and provide a consistent electron donor. Additionally, the small amount of lactic acid in the EOS® formulation immediately provided some soluble substrate to kickstart biodegradation. The passive treatment system minimized costs and the substrate distributed well without any reinjection or recirculation. No maintenance was required throughout the 18 month project and substrate was only added once, which makes the slow-release substrate very appealing.

It is very beneficial to have a treatment technology that can remediate perchlorate and solvents at the same time. On many DoD sites, perchlorate is used along with solvents such as TCA, which was used at the site in this case study. Often, these solvents contaminate the same groundwater plume as perchlorate. In some instances, co-contaminants must be treated using different methods, so treating one can make the other more prevalent. Having a technology available that will simultaneously treat both contaminants minimizes costs and makes remediation easier.

While the passive treatment was successful and cost-effective, there are still some improvements that can be made. Adding additional substrate could have improved TCA and CAH reduction further, as CAH reduction is optimal in strong reducing conditions. Unfortunately, increased substrate additions could further increase sulfide and methane production, as well as metals mobilization. Since EOS® is usually injected only once during the lifetime of a PRB project, a much larger quantity is added when compared to ISB projects that use multiple injections to create a mobile treatment zone. As EOS® releases slowly, it is more likely that electron donors are still dissolving into the groundwater after perchlorate reduction is over – which can create the potential for increased sulfide production, methanogenesis, and heavy metal mobilization. Increasing the amount of substrate added would promote even more degradation of these other water quality characteristics.

Metals mobilization and methanogenesis did rapidly increase after formation of the passive PRB. The methane was evidently being degraded in the vadose zone close to the PRB though, and it is speculated that most metals recomplexed with soil once oxidizing conditions were present. Sulfide levels were not a concern because of low levels of sulfate present in the aquifer before treatment. At a site that has naturally high levels of sulfate, using a slow-release substrate, such as EOS® could encourage a considerable amount of hydrogen sulfide production. Such high sulfate levels at a prospective passive EOS® PRB could be a concern that warrants consideration of another substrate or ISB application.

Another common complaint about EOS® substrate is its negative taste and odor effect on groundwater within 50 feet of the injection, which is caused by the formation of fatty acids from breakdown of the soybean oil. Using this substrate for treatment of a drinking water aquifer may not be recommended if residents and/or drinking water wells are located within 50 feet downgradient of the EOS® PRB.

A decrease in the permeability of the PRB is also a concern. Although EOS® reduced hydraulic conductivity in this study, the tracer tests suggested that the reduction in permeability did not compromise the PRB. However, when aquifers already have low permeability, slow-release

substrates may aggravate the problem. Even emulsified oils like EOS® will not be adequately distributed in aquifers that contain a low permeability and low hydraulic gradient, and thus a low velocity. EOS® addition can decrease permeability even more by strongly sorbing to soil.

Another major limitation to EOS® is depth to groundwater. EOS® has never successfully injected more than 65 feet bgs, because it is difficult to distribute oil so far vertically and drilling costs become prohibitive (since injection points must be very tightly spaced for an oil-based PRB).

This type of PRB is less suitable at sites with low groundwater flow, long depth to groundwater, and critical receptors nearby. In many cases though, utilizing an EOS®-based PRB would be a cost-effective, easy ISB technology to efficiently degrade perchlorate. (IES, 2006; Borden, pers. comm., 2006)

11. ISB CASE STUDY # 3: LONGHORN ARMY AMMUNITION PLANT, TX

Key ISB Features:
- **Study Scale: Full**
- **ISB Type: PRB**
- **System Type: Semi-Passive**
- **Substrate: Sodium Lactate**
- **Project Duration: 3/04 – ongoing**
- **Monitoring: 6/03 – ongoing (data until 3/05)**
- **Notable Point: Issue with Substrate Depletion**
- **Average Perchlorate Reduction: 87.87%**

Longhorn Army Ammunition Plant (LHAAP) is located in east central Karnack, TX. LHAAP began producing munitions for the military in 1942. Ammonium perchlorate was used for a variety of onsite production activities, among other chemicals. LHAAP was placed on the EPA's National Priority List (NPL) in 1990, due to widespread contamination. Since that time, the Superfund site has undergone multiple types of remedial action. In the 1990's, *ex situ* treatment was used to remediate perchlorate-contaminated groundwater located under Landfill 16. In this full scale project, an *in situ*, semi-passive PRB was created at LHAAP's Landfill 16 in March of 2004, under sponsorship by the Environmental Security Technology Certification Program (ESTCP). The purpose of this ISB project is to control the perchlorate-contaminated groundwater plume, effectively remediate the entire plume below detection levels using an *in situ* technology, minimize adverse effects on groundwater parameters, evaluate the effectiveness of the substrate type and delivery approach, and minimize costs in comparison to *ex situ* treatment technologies (Geosyntec, 2005). The main questions that this study aims to answer are:
- Can this treatment technology be used to degrade perchlorate on a full scale?
- Can a semi-passive PRB using soluble substrate create conditions suitable for perchlorate reduction and maintain these conditions throughout the study?
- Does a semi-passive system provide a good balance between cost-minimization and substrate distribution?
- Are soluble substrates a good electron donor choice for a PRB? When might this substrate not be ideal?
- What improvements could be made for future projects similar to this one?

Please note that the latest available results are from March of 2005, but further data should soon be available. Check the contact list and literature cited below as a starting point to finding more up-to-date information.

11.1 Site Characterization

LHAAP consists of 8,943 acres and is located in rural east central TX, on the Louisiana-Texas border. It borders State Road 43 and 134 on the west of the plant and Caddo Lake State Park to the east of the plant boundary. Landfill 16 is 16 acres and is located in the southeast corner of LHAAP.

The land nearby is primarily used as farmland or is forested. There are no major cities close to the site. The largest town is Marshall, located 15 miles southwest, and Shreveport LA, located 30 miles east of the plant. (Muckelrath, 2001)

11.1.1 Source of Contamination

Onsite munitions contained ammonium perchlorate as an oxizider. Perchlorate contamination emanated from solid rocket fuel production, missile firing, munitions burning, and adding munitions waste (such as burned rocket casings) to onsite landfills. At Landfill 16, rocket motor casings containing ammonium perchlorate were burned and buried during the 1950's. (CES, 2002)
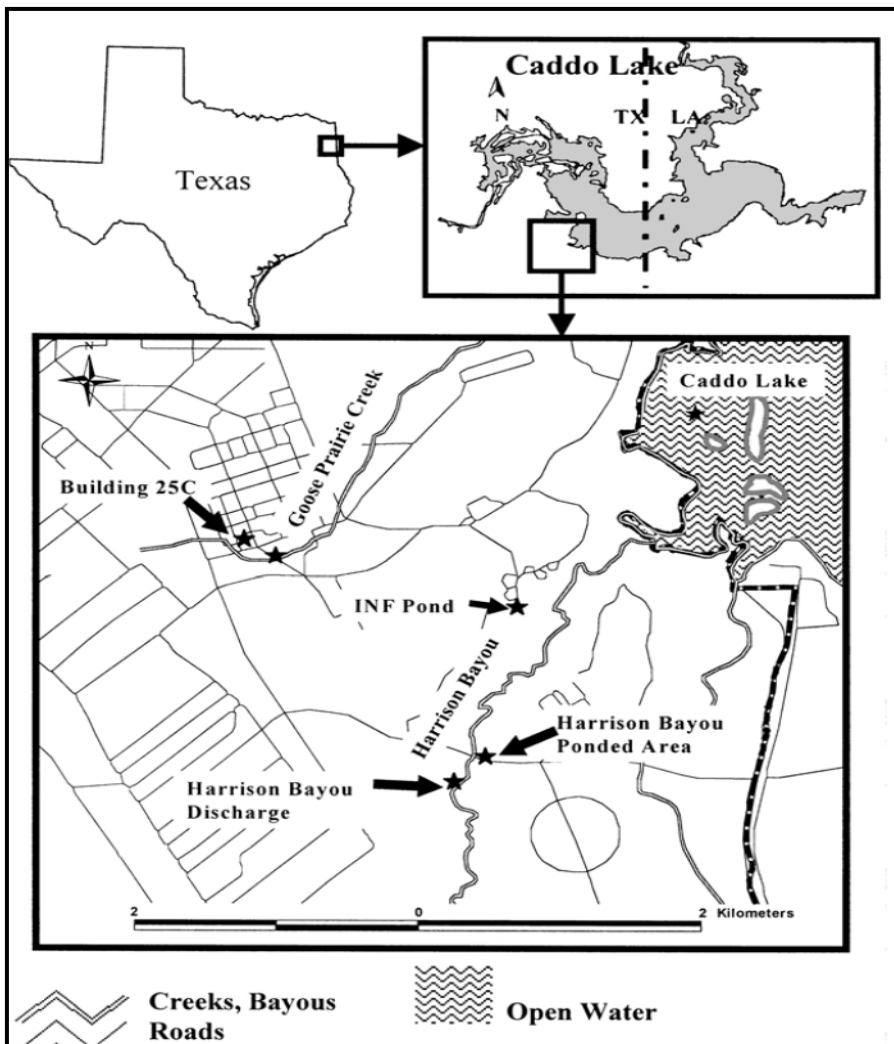
Figure 14. Map of LHAAP and the Surrounding Area.
(Reprinted from Smith, 2001)

11.1.2 Site Operations History

LHAAP began operations in 1942 by manufacturing 2,4,6-trinitrotoluene (TNT) during World War II (WWII). TNT productions ceased after WWII ended in 1945 and LHAAP transferred to standby status. In 1952, LHAAP was reopened to manufacture pyrotechnics, such as flares, photoflash bombs and ground signals. Pyrotechnics production peaked during the Vietnam War, but continued even after the war ended. From 1953-1971, LHAAP also produced solid rocket fuel motors. In 1989, LHAAP was chosen to destroy Pershing IA and II missiles left over from the Cold War, on account of the Intermediate Nuclear Forces Treaty. The missiles were fired on site and destroyed until the end of the project in 1991. In 1997, the plant was rendered inactive.

In 2000, during remedial action, the Army agreed to transfer 7,100 acres of the plant into a wildlife refuge that would back up to Caddo Lake State Park. In 2005, the establishment of the refuge was halted to consider constructing an industrial park at LHAAP instead. (HTO, 1991; Muckelrath, 2001).

Perchlorate contamination from Landfill 16 was initially addressed in 1998 via construction of a landfill cap and development of an onsite *ex situ* pump-n-treat system. Eight extraction wells removed perchlorate-contaminated leachate and sent it to the site's groundwater treatment plant. However, continued water extraction and *ex situ* treatment costs (up to $1.35M/year) encouraged consideration of ISB for perchlorate-contaminated groundwater emanating from landfill leachate. (Muckelrath, 2001; CES, 2002)

11.1.3 Regulatory Status

LHAAP was listed on the NPL in 1990, and a Federal Facility Agreement was reached in 1991 to remediate contamination on the site under a Resource Conservation and Recovery Act (RCRA) permit. A revised RCRA Facility Assessment list indicates that there are 47 sites of concern located on LHAAP property, which includes Landfill 16. To date, numerous types of remedial action have taken place, including smaller perchlorate treatment pilot studies. (CES, 2002)

11.1.4 Potentially Exposed Populations

The perchlorate contamination at Landfill 16 is located next to environmentally sensitive areas, including Harrison Bayou, Goose Prairie Creek, Central Creek, Caddo Lake, and supporting wetlands. Caddo Lake State Park is home to many flora and fauna species that could be adversely affected by perchlorate contamination. Second, Caddo Lake is used as a drinking water supply for residents in the surrounding area. Contamination of drinking water wells and Caddo Lake would expose residents drinking this water to perchlorate. (CES, 2002)

A 2001 study found varying concentrations of perchlorate in both flora and fauna at and around the LHAAP site, as follows:
- Vegetation: 555 – 5,557,000 ppb
- Aquatic insects: 811 – 2,038 ppb
- Fish: < 4 – 207 ppb
- Frogs: <4 – 580 ppb
- Mammals: <4 – 2,328 ppb

Such high levels of perchlorate in flora and fauna is a cause for concern for multiple reasons. First, survival rates decrease for wildlife that contain perchlorate at such high concentrations. Second, humans ingesting any contaminated plants or animals are exposed to perchlorate (Smith, 2001). The EPA-specified RfD for humans is currently set at 0.0007 mg/kg-d. A 70 kg (154 lb) person eating 1 kilogram of meat containing 2,000 ppb of perchlorate exceeds the safe daily dose level for perchlorate more than 40 times.

11.1.5 Site Hydrogeology

The landfill was placed in a high risk area for groundwater and surface water contamination. Landfill 16 is located on Wilcox Group strata, which consists of sandstone and mudstone that was deposited during the Paleocene epoch when eastern Texas was a shallow, marine region. The Wilcox Group is the basal part of the Carrizo-Wilcox aquifer, so contaminants leaching

from Landfill 16 can contaminate this aquifer. More specifically, the site contains fine to medium grained sands with clay and lignite seams in the Wilcox Group. In the Midway Group, which underlies Wilcox strata, calcareous clay is present. (CES, 2002)

Harrison Bayou, a tributary to Caddo Lake, backs up to the south-southeast edge of the landfill. The southeast end of the landfill also sits in the 100-year floodplain for the bayou. Furthermore, Caddo Lake is only 3.6 miles away from the eastern portion of the plant. As a result, leachate migration from the landfill has the potential to contaminate both groundwater and surface water in the surrounding area. (CES, 2002)

The groundwater aquifer under LHAAP is unconfined. Depth to groundwater varies with rainfall, but is usually 5-10 feet bgs under Landfill 16, and never greater than 30 feet. The site is geologically heterogeneous and has layers of low permeability clays, along with higher permeability silty sands. Some areas on the site have less than one foot layers of sand, while other locations have thicker layers of sand that are greater than four feet. Major groundwater characteristics onsite include:

- Groundwater depth: < 35 ft bgs, usually 5-10 feet bgs
- Hydraulic Gradient: 0.003 – 0.007 ft/ft
- Hydraulic conductivity: 10-2 to 10-3 cm/sec

Historic data indicate that groundwater flow is directed 10 degrees north and south of due east, towards Harrison Bayou. The groundwater elevation onsite ranged from 72-75 feet mean sea level. (CES, 2002)

11.2 Laboratory Study

John Coates, an expert in perchlorate microbiology, performed a microbial characterization of the LHAAP project site to determine the type and amount of DPRB present. Enumeration studies were conducted using Most Probable Number (MPNs) counts. In the laboratory, a reducing environment was created, and acetate and perchlorate was added as an electron donor and an electron acceptor, respectively. There was no visually identifiable increase (< 10 cells/g) in the density of the microbes in the enumeration study. Ion chromatography indicated that the indigenous DPRB were present at very low levels however. Genomic DNA was extracted and 16S rDNA probes were used in a molecular analysis that identified *Dechloromonas* sp. CKB and RCB in the groundwater at LHAAP. (Geosyntec, 2005)

11.3 Field Study Design

11.3.1 System Configuration

The PRB in this study is considered to be semi-passive because substrate delivery and recirculation occurs semi-annually. Five recirculation wells were constructed perpendicular to groundwater flow. Fifteen foot well screens were used to discourage interference of sand in the well. The groundwater extraction wells were designed to pump 1-2 gpm and inject the water back into the treatment zone via piping to three injection wells.
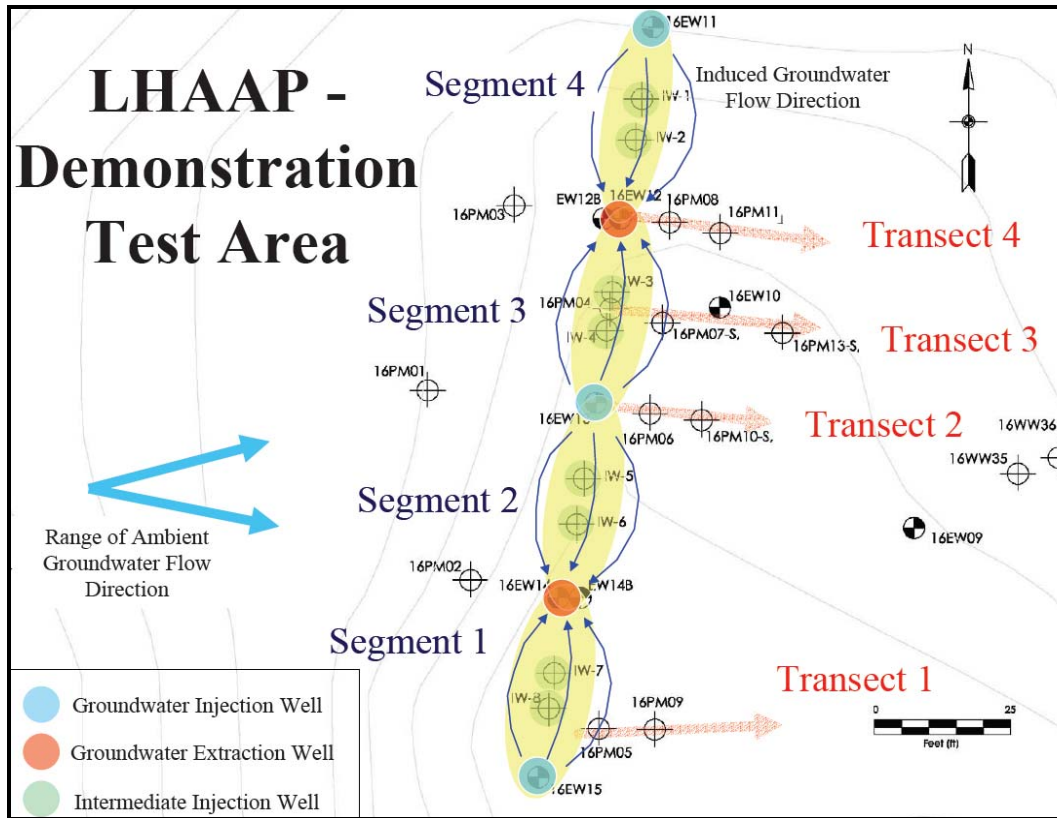
Figure 15. Diagram of the Semi-Passive PRB at LHAAP.
(Reprinted from Krug et al., 2005)

Semi-annual injections of soluble sodium lactate were added perpendicular to the contaminant plume, so as to create a biologically active treatment zone. The substrate addition was recirculated for approximately 3 weeks after injection, via extraction and injection of amended groundwater. The substrate additions occurred in April of 2004, December of 2004, and October of 2005, respectively. (Geosyntec, 2005)

11.3.2 Tracer Tests

Tracer tests indicated that the recirculation system successfully distributed water laterally across the treatment zone and back into the extraction system. The tracer was detected in the monitoring wells throughout the treatment zone for nearly two months, suggesting that the semi-passive system would allow for effective distribution of the substrate. (Geosyntec, 2005)

11.3.3 System Delivery

A semi-annual substrate addition and active recirculation period began on March 25, 2004 and lasted for approximately three weeks. The 60% sodium lactate solution was added in a batch addition three times a week. The substrate was injected into 13 wells to promote more extensive distribution of the electron donor in the targeted treatment zone, given the temporary water recirculation. The PRB remained passive until another active phase began on December 3, 2004, when the substrate solution was added over the next 25 days. The third active recirculation and substrate delivery phase occurred in October of 2005. (Geosyntec, 2005)

11.4 Field Study Results

The following table summarizes the pre-treatment versus post-treatment ranges, averages, and percent change for the various geochemical parameters. Pre-treatment values are taken from March of 2004, while post-treatment values are taken from the March 2005 samples – unless otherwise specified. (Geosyntec, 2005)

Geochemical Parameter	Pre-Trtmt	Post-Trtmt	% Change, on Avg.
Perchlorate			
Range (ppb)	<4-1,040	<4-373	
Average (ppb)	433.89	52.64	**-87.87**
Dissolved Oxygen			
Range (ppm)	0.62-2.78	0.44-5.0 *	*9 mo. post-trtmt, 12/04
Average (ppm)	1.47	2.51	**70.75**
Nitrate			*post-trtmt 4/04
Range (ppm)	<0.02-7.83 *	<4-9.4 **	**post-trtmt 1/05
Average (ppm)	1.22	4.05	**231.97**
Sulfate			
Range (ppm)	206-4,790	190-3,320*	*1 mo. post-trtmt 5/04
Average (ppm)	2,061.62	1,553.35	**-24.65**
Methanogenesis			
Range (ppb)	<650	<650 - 7,750	
Average (ppb)	<650	1,410	**116.92**
Dissolved Manganese			
Range (ppm)	0.83-9.4	0.83-38*	*1 mo. post-trtmt 5/04
Average (ppm)	3.25	4.84	**48.92**
Dissolved Iron			
Range (ppm)	<0.4-30	0.57-126*	*1 mo. post-trtmt 5/04
Average (ppm)	6.26	12.67	**102.40**
ORP			
Range (mV)	8-250	-199 – 136	
Average (mV)	223.73	42.05	**-81.21**
pH			
Range	5-6.3	5.6-7.1	
Average	5.99	6.95	**18.53**
Acetate (umol/L)~	~Sodium lactate substrate degrades into acetate		*During Inj. 3/24/04
Range (ppm)	<12.5 – 144*	<12.5 - 12,100	** 2 months later: 5/20/04
Average (ppm)	26.1	728.42	**2,690.88**

Table X. Geochemical Characteristics for the Semi-Passive PRB at LHAAP using Sodium Lactate Substrate, Pre-treatment Versus Post-treatment. (Geosyntec, 2005)

To provide a conservative estimation, samples that were below the limit of detection were assumed to be at the detection limit for calculating averages. As expected, there were

considerable differences in pre-treatment versus post-treatment geochemical characteristics – along with variability within samples – when groundwater at LHAAP's Landfill 16 was tested. Overall, perchlorate, DO, and nitrate concentrations decreased over time, which was intended. The monitoring wells that displayed higher concentrations of perchlorate, DO, and nitrate before treatment account for the highest concentrations found during post-treatment samples. There may not have been as effective recirculation and distribution of the substrate in these monitoring wells, or competing electron acceptors could have entered the treatment zone in this region and decreased perchlorate degradation. Sulfate concentrations also tended to decrease, which suggests that some unfavorable H_2S production may have occurred during treatment. (Gesoyntec, 2005)

11.4.1 Depletion of Electron Acceptors

11.4.1.1 Dissolved Oxygen (DO)

Pre-treatment, DO levels were already in the anoxic to hypoxic range (0.62 – 2.78 ppm), with an average DO level of 1.51 ppm. When the wells were tested for DO content in May of 2004, less than one month after the first active phase, DO levels decreased to 1 ppm or less in 16 of the 21 monitoring wells. However, nine months after the PRB was formed, in December of 2004, DO levels ranged from 0.44 – 5 ppm, with an average of 2.59 ppm. There was an unexpected 76.52% increase in DO, on average. DO concentrations most likely increased within nine months because more substrate needed to be added to deplete DO entering the treatment zone from upgradient flow. (Geosyntec, 2005)

11.4.1.2 Nitrate

No pre-treatment nitrate samples were taken. Just one month after creation of the PRB, in April of 2004, nitrate levels ranged from below the detection limit of 0.02 ppm to 7.83 ppm, with an average nitrate level of 1.22 ppm. In January of 2005, 10 months after treatment began, nitrate levels ranged from below the detection limit of 4 ppm to 9.4 ppm, with an average nitrate level of 4.05 ppm. Interestingly, there was a 231.97% increase in nitrate levels during this time period, on average. Microbes initially degraded most nitrate present in the treatment zone, using the available substrate; but as the soluble substrate was quickly depleted, nitrate levels began to increase again (until another active phase began). The main problem with increased nitrate levels is its effect on perchlorate levels. Less effective nitrate reduction over time often corresponds with less effective perchlorate reduction over time. Adding additional substrate in the next active phase would encourage DO and nitrate depletion again, and promote more effective perchlorate reduction (until substrate was depleted once again). (Geosyntec, 2005)

11.4.1.3 Perchlorate

Before treatment, perchlorate levels ranged from below the 4 ppb detection limit to 1,040 ppb, with an average perchlorate concentration of 433.89 in the targeted treatment zone. One year after treatment, perchlorate concentrations ranged from below the detection level of 4 ppb to 373 ppb, with an average perchlorate concentration of 52.64 ppb. On average, there was an 87.87% reduction in perchlorate within one year of the creation of the PRB.

Overall, perchlorate concentrations were significantly reduced at locations downgradient of the PRB. There is still room for improvement however, as only 11 of the 21 monitoring wells (or 52.38 %), would meet the non-enforceable, but safety relevant DWEL of 24.5 ppb. The monitoring wells located furthest from the injection wells experienced less effective perchlorate reduction because much of the substrate was depleted before it reached the outer portion of the treatment zone. (Geosyntec, 2005)

11.4.1.4 Sulfate Depletion

Monitoring sulfate degradation throughout treatment was an important indicator of hydrogen sulfide production. Sulfate concentrations greater than 1,000 ppm are considered high and can impair secondary water quality characteristics. The secondary standard for sulfate is 250 ppm.

Pre-treatment, the sulfate concentrations ranged from 206 – 4,790 ppm, with an average of 2,061.62 ppm. Only eight of 20 wells had sulfate levels lower than 1,000 ppm. Meanwhile, seven wells had sulfate concentrations more than 4,000 ppm. One month after the end of the first active phase, sulfate concentrations ranged from 190-3,320 ppm, with an average of 1,553.35 ppm.

Thus, it appears that some hydrogen sulfide production occurred closely after the first injection – sulfate concentrations most likely decreased more in wells with the highest pre-treatment concentrations. The three monitoring wells that exhibited marked decreases in sulfate are located near the PRB, where higher quantities of excess substrate are present that could promote sulfide production. The semi-passive system generated more methane than the active system used at IHDIV (Case 1), and less methane than the passive system used in Elkton, MD (Case 2). (Geosyntec, 2005)

11.4.1.5 Methanogenesis

Pre-treatment, methane levels were less than the 650 ppb limit of detection. Post-treatment, methane levels in the treatment zone varied from less than the 650 ppb limit of detection to 7,750 ppb, with an average of 1,410 ppb. Thus, there was a 116.92% increase in methane levels, which is undesirable, but not a major concern. At the DoD site in Elkton, MD using the slow-release EOS® substrate, methane levels around the PRB increased by more than one million percent. This study indicates that using a semi-passive PRB with soluble substrate may minimize methanogenesis, when compared to a passive PRB utilizing slow-release substrate.

11.4.2 Metals Mobilization

Before treatment, dissolved manganese levels ranged from 0.83-9.4 ppm, with an average of 3.25 ppm. After treatment, dissolved manganese levels ranged from 0.83-38 ppm, with a slightly higher average of 4.84 ppm. There was a 48.92% average increase in dissolved manganese within one month after the first active period ended. The secondary standard for manganese is 0.05 ppm. Dissolved manganese levels were higher after treatment, but exceeded the voluntary standard before treatment as well. According to consultants at Geosyntec, there was not a positive correlation between manganese concentrations in groundwater and the amount of

substrate present in the same location. There was an increase of manganese in Transect 1, but not a marked difference in other wells located near the PRB, which suggests that geochemical conditions may vary some along the PRB. (Cox, pers. comm., 2006)

Dissolved iron levels ranged from below the 0.4 ppm detection limit to 30 ppm before treatment, with an average of 6.26 ppm. Post-treatment, dissolved iron levels increased by 102.4%, range from 0.57-126 ppm and averaged 12.67 ppm in the treatment zone. The secondary standard for dissolved iron is 0.3 ppm. Levels in the treatment zone exceeded the secondary standard both pre-treatment and post-treatment. (Geosyntec, 2005)

11.4.3 Other Geochemical Characteristics

11.4.3.1 pH

Pre-treatment, the pH ranged from 5-6.3, with an average of 5.99. Post-treatment, the pH ranged from 5.6-7.1, with a near neutral average of 6.95. The starting pH was slightly acidic in some places, but DPRB seemed to be active once substrate was added, so a buffer was not needed. The pH became more neutral during treatment, as expected. (Geosyntec, 2005)

11.4.3.2 Oxidation-Reduction Potential (ORP)

In this field study, the perchlorate-contaminated groundwater plume was initially located in an oxidizing environment. Pre-treatment, the 12 monitoring wells tested for ORP ranged from 8 – 250 mV, with an average of 223.73 mV. In March of 2005, after 1 year of treatment, the ORP levels found at 12 monitoring wells ranged from -199 to 136, with an average ORP of 42.05. Overall, every monitoring well exhibited decreases in ORP, but only 4 wells reached an ORP less than 0 mV, which is most favorable for microbial perchlorate reduction. There was an 81.21% decrease in ORP, on average, which indicates that a much more reducing environment was created around the PRB. The monitoring wells that had lower ORP values tended to have more significant decreases in perchlorate as well. (Geosyntec, 2005)

11.4.4 Substrate Utilization

Sodium lactate dissociates into a sodium and lactate ion and then degrades into acetate. Acetate levels were tested to determine what levels of the substrate were present in the treatment zone throughout the study. During the first active injection phase, acetate levels ranged from below the 12.5 ppm detection limit to 144 ppm, with an average of 26.1 ppm. Two months after the first active phase, acetate levels ranged from below the 12.5 ppm detection limit to 12,100 ppm, with an average of 728.42 ppm. Thus, there was a 2,690.88 % average increase in acetate levels throughout the treatment zone two months after the substrate injection. This marked increase suggests that enough substrate was present to degrade competing electron acceptors and promote effective perchlorate reduction. However, substrate levels decreased before the additional active phase, as was evident by increases in DO and nitrate months into each passive phase. A lack of continually available substrate decreased the effectiveness of the PRB. (Geosyntec, 2005)

11.5 Summary

Results from the field-scale LHAAP site indicate that a semi-passive PRB can be a viable, effective option for *in situ* perchlorate bioremediation. Microbial reduction began quickly after the PRB was formed and perchlorate levels decreased more than 87% after one year of treatment. Furthermore, the treatment zone covered the entire contaminant plume and markedly decreased the risk to the surrounding environment.

This semi-passive PRB project is unusual because it is more common to use slow-release liquid or solid substrate to create a PRB. Project managers at LHAAP chose to reinject the soluble substrate more often than a slow-release substrate, and employ active phases periodically to facilitate distribution and formation of the PRB. The soluble substrate is easily distributed throughout the PRB, provides project managers with adaptability, and may minimize degradation of other water quality parameters. Indeed, much less methanogenesis and metals mobilization occurred during this study than during the EOS®-created PRB. Sulfate levels were very high before the PRB was installed, but sulfide production did not become a major issue after treatment. Using a slower-release substrate without any recirculation may have created more sulfide production. Biofouling did not pose a problem either.

The semi-passive system balances the advantages and disadvantages of both the active and the passive system. The benefits to using a semi-passive approach are three-fold:
- Adding smaller quantities of substrate (than passive treatment) decreases the possibility of adversely affecting secondary water quality
- Periodic recirculation facilitates distribution of substrate across the contaminant plume (as opposed to passive treatment)
- O&M costs are reduced by not continuously recirculating the amended groundwater (as with active treatment)

The possible degradation of a soluble substrate before distribution to the outside of the targeted treatment zone makes effective circulation important. Costs also play a large role in making ISB a feasible remediation approach, so a semi-passive system can be an ideal way to balance substrate and water quality concerns with cost constraints. This project shows that a semi-passive system can be used to minimize costs and distribute substrate into the PRB efficiently.

On the down side, the average perchlorate concentration of 52.64 ppb exceeds the non-enforceable DWEL of 24.5 ppb. The semi-passive PRB did create reducing conditions conducive to perchlorate reduction during the active phase of the system (when substrate was added). DO and ORP levels decreased immediately after the first active phase when sodium lactate was first added. The reducing conditions were not maintained for the entire duration of the passive phase because the substrate was degraded too quickly due to its solubility. Consequently, DO, ORP and nitrate levels increased. Once the next active phase began and more sodium lactate was added, a reducing environment was recreated.

Using soluble substrate to create a PRB is not ideal in a very heterogeneous subsurface environment with a large amount of competing electron acceptors, or at a location with high groundwater flow. In these cases, the soluble substrate needs to be reinjected even more frequently, which increases costs.

There are several improvements that can be made to the semi-passive PRB at LHAAP. Creating additional monitoring wells further downgradient of the PRB allows project managers to determine whether another PRB should be established, additional substrate should be added, or the current PRB should be widened to increase contact time. Modifying the system delivery to provide a larger, wider distribution of substrate would encourage more consistent perchlorate reduction. Alternately, a less soluble substrate could be used to create the PRB. Increases in DO and nitrate months into the passive phase of the treatment system indicate that more substrate needs to be added for a semi-passive PRB using soluble substrate to be effective. Since sodium lactate degrades more quickly than slow-release and solid substrates often used to construct PRBs, it is important to inject the right quantity and have more frequent and longer active phases. If DO and nitrate levels had stayed low throughout the study, perchlorate reduction would have been more consistent and more effective. Increasing the duration and frequency of the active phase, along with the substrate loading rate, would improve results. (Geosyntec, 2005)

The duration of the active phase was increased from three weeks to four-six weeks during the third active phase in November of 2005. Additionally, up to three times as much substrate is now added during each delivery. Results are not yet compiled for the latest active phase, but improvements to the ISB system likely decreased perchlorate levels further. (Cox, pers. comm., 2006; Krug, pers. comm., 2006)

12. PHYTOREMEDIATION AND WETLANDS REMEDIATION

Phytoremediation utilizes terrestrial plants and trees to remediate contaminated soil or water via removal, biodegradation, and/or containment. The concept of wetlands remediation is very similar to phytoremediation – aquatic plant species are used to remediate perchlorate-contaminated groundwater instead of terrestrial species. Both phytoremediation and wetlands remediation are emerging as promising *in situ* treatment technologies for low to moderate levels of perchlorate contamination. The applications are being considered at perchlorate-contaminated groundwater sites that have shallow groundwater aquifers, or alternatively, contaminated sites where groundwater is irrigated over a plot of land. These phytotechnologies hold most of the advantages of *in situ* bioremediation (ISB). They are becoming more popular as *in situ* treatment technologies due to their low-costs, reduction efficiency and aesthetically-pleasing design that can easily work in combination with other remedial technologies and contaminants. Perchlorate phytotechnology takes advantage of a number of natural processes:

1) Phytoextraction
2) Phytodegradation
3) Rhizodegradation
4) Hydraulic Control

Dozens of studies indicate that the most effective plant process to remediate perchlorate is rhizodegradation, followed by phytoextraction and phytodegradation. Phytoextraction, also known as rhizofiltration for groundwater contaminants, relies on plants' uptake of contaminated ground water into the plant tissue. Plants have consistently been shown to uptake perchlorate in this manner, but perchlorate can be re-released into the environment through exudation and deciduous leaves falling off trees in the fall. Phytodegradation also has a small application for perchlorate contamination. Studies show that biodegradation occurs in the leaves of plants, but it is a slow process estimated to account for roughly 11% of perchlorate remediation (Nzengung et al., 1999). The most important phytotechnology for perchlorate is rhizosphere biodegradation, also known as rhizodegradation, whereby the nutrients released from plant roots support indigenous perchlorate-reducing bacteria (DPRB) that can reduce perchlorate located near the rhizosphere, the soil surrounding the root zone of plants. DPRB can be stimulated to reduce a large amount of perchlorate present in water located in the vadose zone and in shallow aquifers. Studies also suggest that phytotechnology can provide some hydraulic control over perchlorate migration due to the sheer amount of water that can be transpired by trees and plants when established root systems are present. Up to 300 gallons of water can be transpired per day by the dense root network of a tree, which can help to naturally retain perchlorate near the point of release. (Tan et al., 2006; McCutcheon & Schnoor, 2003)

Halophytes (i.e. salt cedar trees) and phreatophytes (i.e. willow trees) are especially useful for phytoremediation of perchlorate-contaminated groundwater. Halophytes can survive in salty conditions, while phreatophytes often uptake water from the saturated zone of the groundwater table. Wetlands plants, such as smartweed, can often build deeper roots that can uptake shallow groundwater. (McCutcheon & Schnoor, 2003)

The extent of perchlorate phytoremediation and wetlands remediation is dependent on the depth of the plant/tree roots, species tolerance, hydrogeology, water uptake rates, exposure duration,

geochemical parameters, nutrients, climate, and individual variation among trees. (Tan et al., 2006; McCutcheon & Schnoor, 2003)

Just as nitrate and DO are competing electron acceptors in ISB, they play a similarly important role in the effectiveness of phytoremediation. Anaerobic conditions must be present for rhizodegradation of perchlorate to occur. More than 1-5 ppm of nitrate may decrease and even fully inhibit perchlorate rhizodegradation. In a study that simulated anaerobic wetlands remediation of perchlorate in a bioreactor, rhizodegradation was much more effective without nitrate as the nitrogen source (96.1% average perchlorate reduction versus 76% average perchlorate reduction with nitrate present at low levels). Ammonia or urea are more suitable nitrogen sources to add for phytoremediation. (Tan et al., 2004a; Tan et al., 2004b; Nzengung et al., 2004; Shrout et al., 2006; Krauter et al., 2005).

Phytotechnologies take more time to develop than other technologies, because root systems must be developed. Phytoremediation of contaminated groundwater becomes more effective as trees and plants become bigger, grow longer roots, and can uptake more water. The falling leaves are generally collected and the trees and plants are harvested at the end of the project to avoid a re-release of perchlorate into the environment. Furthermore, it is appropriate to build fences around the site to inhibit animals from eating leaves (which can destroy the plants and harm the animals due to concentrated levels of perchlorate). (Tan et al., 2006; McCutcheon & Schnoor, 2003)

Many studies indicate that plants located in areas with contaminated groundwater concentrate appreciable amounts of perchlorate in the leaves. Naturally occurring aquatic and terrestrial plants located around the perimeter of the Naval Weapons Industrial Reserve Plant (NWIRP) were monitored for accumulation of perchlorate in plant tissue. The highest level of perchlorate in groundwater sampled just offsite was 536 ppb. Meanwhile, wetlands and terrestrial plants concentrated much higher levels of perchlorate. In smartweed, a common wetlands plant, the average perchlorate concentration was 40,600 ppb dry weight at one site. The study found a statistically significant linear relationship between perchlorate levels in water and corresponding concentrations in smartweed ($P<0.0001$). Terrestrial willow trees contained average perchlorate concentrations of 1,580 ppb dry weight at one site, and 6,590 ppb dry weight at another site. The other terrestrial tree species, which include hackberry, elm, mulberry and china-berry, also accumulated a large amount of perchlorate (average ranged from 467 – 5,043 ppb dry weight). The highest accumulation of perchlorate occurred towards the end of the growing cycle, which logically indicates that accumulation increases with exposure duration. Furthermore, perchlorate concentrations decreased in leaves that fell off of trees, possibly due to leaching, rainfall, or microbial degradation. (Tan et al., 2004a)

A myriad of laboratory studies have detailed the effectiveness of phytoremediation (Nzengung et al., 1999a; Nzengung et al., 1999b; Nzengung et al., 2000; Nzengung et al., 2003; Nzengung et al., 2004). There are also studies focusing solely on wetlands remediation (Tan et al., 2004b; Tan et al., 2006). However, there are only limited phytotechnology field-scale applications that have been installed to date.

A pilot-scale phytoremediation project was installed at LHAAP (ISB was also applied onsite – see Case Study 3). In 2003, 425 hybrid poplar trees were planted on a 0.7 acre area onsite.

Current estimates indicate that perchlorate concentrations decreased from 100,000 ppb to 10,000 ppb in the irrigated groundwater. (Schnoor et al., 2004; Shrout et al., 2006)

In Las Vegas, NV, studies have examined the effect of well-established salt cedar trees on perchlorate levels. The invasive tree species is incredibly salt tolerant and can uptake approximately 300 micrograms of perchlorate per gram of tissue. (Urbansky et al., 2000).

A wetlands bioreactor was installed at Lawrence Livermore National Laboratory (LLNL) in California, to remediate perchlorate-contaminated groundwater. Perchlorate degradation has been highly successful thus far (Krauter et al., 2005).

There is potential for phytotechnologies to effectively and feasibly remediate perchlorate-contaminated groundwater, but not every site and plant species are suitable. According to Alan Jacobs, a consultant associated with NWIRP remediation, phytoremediation was attempted twice onsite and was unsuccessful both times. Rabbit and insect infestation, as well as the dry climate, made it difficult for the saplings to survive. (Jacobs, pers. comm., 2006)

To obtain more information covering phytoremediation and wetlands remediation, please refer to the articles referenced in this section.

13. CONCLUSION

Current studies suggest that *in situ* treatment technologies are a feasible way to effectively treat perchlorate-contaminated groundwater. Project managers are increasingly focusing on the benefits of using *in situ* technologies. *In situ* technologies are superior to *ex situ* treatment technologies in critical areas:

1. **Effectiveness:** Up to 99.9% reduction efficiency to date
2. **Feasible:** lower O&M and capital costs
3. **Flexible:** many design configurations to meet site-specific variation
4. **Adaptable:** technology can adapt to changes that occur during treatment
5. **Project Duration:** *in situ* treatment much quicker than pump-n-treat
6. **Aesthetics:** no major infrastructure, plants can beautify surroundings
7. **Ability to Mix & Match:** technologies can be combined
8. **Solvent Degradation:** effectively degrades some common co-contaminants
9. **Waste Minimization:** food waste can become useful substrate

To date, *in situ* bioremediation (ISB) is more widely used than wetlands remediation or phytoremediation. Data pertaining to phytoremediation and wetlands remediation efficacy would be especially valuable. Additional ISB research is also critical in affirming the benefits of using bioremediation to treat perchlorate-contaminated groundwater. To further advance the use of *in situ* technologies, future projects should place a larger emphasis on obtaining feasibility data.

To more easily determine the benefits of ISB, future studies should focus on obtaining more standardized data, so results are comparable and different *in situ* design configurations can be analyzed. Monitoring data that includes the following geochemical parameters would be especially helpful: perchlorate, DO, nitrate, TOC, pH, ORP, alkalinity, sulfate, dissolved manganese, dissolved iron, dissolved arsenic, dissolved selenium and solvents. The drawbacks of *in situ* technologies must be thoroughly studied to show that in situ technologies can easily adapt to site-specific variation, as well as short-term degradation of certain primary and secondary water quality parameters. (Borden, pers. comm., 2006; ITRC, 2006; EPA, 2005)

14. REFERENCES

AFCEE/TTD – Air Force Center for Environmental Excellence Technology Transfer Division. 2002. Perchlorate Treatment Technology Fact Sheet. In situ Anaerobic Bioremediation.

Amdur M., Doull J., and Klassen C. 1991. Casarett and Doull's Toxicology: The Basic Science of Poisons. 4th ed., Mcgraw-Hill, New York.

ATSDR – Agency for Toxic Substances and Disease Registry. 2005. Perchlorate Chemical and Physical Properties. Center for Disease Control.

Bodine, S. 2006. Assessment Guidance for Perchlorate. Memorandum. Assistant Administrator. EPA Office of Solid Waste and Emergency Response.

Borden, R.C. 2006. Personal Communication. Industrial & Environmental Solutions Consultant for EOS® Permeable Reactive Barriers.

Braverman, L.E., Utiger, R.D. 2000. Werner and Ingbar's the Thyroid: A Fundamental and Clinical Text. 8th ed. Philadelphia, PA. Lippincott Williams & Wilkins, pp.593-666, 744-832.

Braverman, L.E., He, X.M., Pino, S., et al. 2005. The Effect of Perchlorate, Thiocyanate, and Nitrate on Thyroid Function in Workers Exposed to Perchlorate Long-term. Clinical Endocrinology and Metabolism, Vol. 90, No. 2, pp. 700-706.

CADTSC – California Department of Toxic Substances Control. nd. Chemistry and Toxicology of Perchlorate. Section 2. Workshop.

CES – Complete Environmental Service. 2002. Final Five Year Review Report for Longhorn Army Ammunition Plant.

Chaudhuri, S.K., O'Connor, S.M., Gustavson, R.L., Achenbach, L.A. and Coates, J.D. 2002. Environmental Factors That Control Microbial Perchlorate Reduction. Applied and Environmental Microbiology, Vol. 68, No. 9, pp. 4425-4430.

CLU-IN – Hazardous Waste Clean-Up Information. 2006. Contaminant Focus: Perchlorate. U.S. EPA, Technology Innovation and Field Services Division. Retrieved in 5/06 from http://clu-in.org/contaminantfocus/default.focus/sec/perchlorate/

Coates, J.D., and Achenbach, L.A. 2004. Microbial Perchlorate Reduction: Rocket-Fueled Metabolism. Nature Reviews, Vol. 2, pp.569-580.

Coates, J.D., and Achenbach, L.A. 2005. The Microbiology of Perchlorate Reduction and Its Bioremediative Application. Chapter 11. University of California, Berkeley, CA, and Southern Illinois University, Carbondale, IL.

Cox, E. 2006. Personal Communication. Geosyntec Consultant for LHAAP.

Cramer, R.J., Yates, C., Hatzinger, P., and Diebold, J. 2004. Field Demonstration of In situ Perchlorate Bioremediation at Building 1419. Naval Surface Warfare Center, Indian Head Division & Shaw Environmental, Inc.

DNEP – Department of the Navy Environmental Program. 2001. Perchlorate. Remediation Innovative Technology Seminar.

EPA – Environmental Protection Agency. 2005. Perchlorate Treatment Technology Update. Federal Facilities Forum Issue Paper. Prepared by the Office of Solid Waste and Emergency Response.

EPA – Environmental Protection Agency. 2006. List of Contaminants and Their MCLs. Retrieved in 7/06 from http://www.epa.gov/safewater/mcl.html#mcls

FFRRO – Federal Facilities Restoration and Reuse Office. 2004. National Perchlorate Detections as of September 23, 2004 (Map). EPA. Retrieved in 6/06 from http://www.epa.gov/fedfac/documents/perchlorate_map/nationalmap.htm

FFRRO – Federal Facilities Restoration and Reuse Office. 2005. Known Perchlorate Releases in the U.S. (as of March 25, 2005). EPA. Retrieved in 6/06 from http://www.epa.gov/swerffrr/pdf/detect0305.pdf

Geosyntec Consultants. 2005. Data Analysis White Paper for Remediation of Perchlorate through Semi-Passive Bioremediation at the Longhorn Army Ammunition Plant. Environmental Security Technology Certification Program (ESTCP) Project 200219.

Greer, M., Goodman, G., Pleus, R., and Greer, S. 2002. Health Effects Assessment for Environmental Perchlorate Contamination: The Dose Response Assessment for Inhibition of Thyroidal Radioiodine Uptake in Humans. Environmental Health Perspectives, Vol. 110, pp. 27-37.

Hatzinger, P.B. 2000. In situ Bioremediation of Perchlorate in Groundwater. Envirogen Presentation.

Hatzinger, P.B. 2003. In situ Perchlorate Bioremediation at IHDIV NSWC. NDIA Symposium. Envirogen, Inc.

Hatzinger, P.B. 2006. Personal Communication. Envirogen Consultant for IHDIV.

Hollowell, J.G., Staehling, N.W., Flanders, W.D., Hannon, W.H., Gunter, E.W. 2002. Serum TSH, T4, and Thyroid Antibodies in the United States Population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). Clinical Endocrinology & Metabolism, Vol. 87, No. 2, pp. 489-99.

HTO – Handbook of Texas Online. 1991. Longhorn Army Ammunition Plant. Retrieved in 6/06 from www.tsha.utexas.edu/handbook/online/articles/LL/dml3.html

Hunter, W.J. 2005. Injection of Innocuous Oils to Create Reactive Barriers for Bioremediation: Laboratory Studies. Journal of Contaminant Hydrology, Vol. 80, pp. 31-48.

IHDIV – Indian Head Division, Naval Surface Warfare Center. 2006. About Us. Retrieved in 6/06 from http://www.ih.navy.mil/about_us/about_us.html

IES – Industrial & Environmental Services. 2006. Edible Oil Barriers for Treatment of Perchlorate-Contaminated Groundwater. ESTCP – Environmental Security Technology Program.

ITRC – Interstate Technology and Regulatory Council. 2005a. Perchlorate: Overview of Issues, Status, and Remedial Options. Technology Overview.

ITRC – Interstate Technology and Regulatory Council. 2005b. Permeable Reactive Barriers: Lessons Learned/New Directions. PRB-4. Washington D.C.

ITRC – Interstate Technology and Regulatory Council. 2005c. Perchlorate: Overview of Issues, Status, and Remedial Options. Web-based Training Program. Co-sponsored with EPA's Office of Superfund Remediation and Technology Innovation.

ITRC – Interstate Technology and Regulatory Council. 2006. ITRC Draft Perchlorate Technology and Regulations Document.

Jackson, A.W., Preethi, J., Laxman, P., Tan, K., Smith, P.N., Yu, L., and Anderson, T.A. 2005. Perchlorate Accumulation in Forage and Edible Vegetation. Journal of Agricultural and Food Chemistry, Vol. 53, pp. 369-373.

Jacobs, A. 2006. Personal Communication. Consultant for NWIRP.

Krauter, P., Daily, B. Jr., Dibley, V., Pinkart, H., and Legler, T. 2005. Perchlorate and Nitrate remediation Efficiency and Microbial Diversity in a Containerized Wetland Bioreactor. International Journal of Phytoremediation, Vol. 7, No. 2, pp. 113 – 128.

Krug, T. 2006. Personal Communication. Geosyntec Consultant for LHAAP.

Krug, T., Cox, E., and Bertrand, D., Harre, B., and Leeson, A. 2005. In situ Bioremediation of Perchlorate in Groundwater. U.S. EPA Technical Support Project. Presentation. Semi-Annual Meeting. San Antonio, Texas.

Lawrence, J.E., Lamm, S.H., Pino, S., Richman, K., and Braverman,m L.E. 2000. The Effect of Short-Term Low-Dose Perchlorate On Various Aspects of Thyroid Function. Thyroid Journal, Vol. 10, pp. 659-663.

Logan, B. Wu, J., and Unz, R.F. 2001. Biological Perchlorate Reduction in High-Salinity Solutions. Water Resources, Vol. 35, No. 12, pp. 3034-3038.

MassDEP – Massachusetts Department of Environmental Protection. 2006. Massachusetts' First-In-The-Nation Perchlorate Standards Take Effect Today. Drinking Water, Cleanup Standards of 2 ppb Are Set to Protect Public Health. Retrieved in 8/06 from http://www.mass.gov/dep/public/press/pchl0706.htm

Motzer, W.E. 2001. Perchlorate: Problems, Detection and Solutions. Environmental Forensics, Vol. 2, pp. 301-311.

Muckelrath, L. 2001. Installation Action Plan for Longhorn Army Ammunition Plant. Prepared by Remedial Project Manager.

Nzengung, V.A.., and McCutcheon, S.C. 2003. Phytoremediation: Transformation and Control of Contaminants. Edited by McCutcheon, S.C., and Schnoor, J.L. John Wiley & Sons, pp. 863 -885.

Nzengung, V.A., Penning, H., and O'Niell, W. 2004. Mechanistic Changes During Phytoremediation of Perchlorate Under Different Root-Zone Conditions. International Journal of Phytoremediation, Vol. 6, No. 1, pp. 63 – 83.

Nzengung, V.A.., and Wang, C. 2000. Influences on Phytoremediation of Perchlorate Contaminated Water. American Chemical Society (ACS) Special Symposium Series: Perchlorate in the Environment. Editor: Urbansky. Kluwer Academic/Plenum Publishers, New York. Chapter 21, pp 219 - 229.

Nzengung, V.A., Wang, C., Harvey, G., and McCutcheon, S.C. 1999a. Plant-Mediated Transformation of Perchlorate into Chloride. Environmental Science and Technology, Vol. 33, pp.1470-1478.

Nzengung, V.A.., Wang, C., and Harvey, G., McCutcheon, S.C., and Wolfe, N.L. 1999b. Phytoremediation of Perchlorate Contaminated Water: Laboratory Studies. Symposium Series: Fifth International Symposium on In situ and On-Site Bioremediation: Phytoremediation. Edited by Leeson, A. and Alleman, B.C. Battelle Press, pp 239-244.

OCA – Organic Consumers Association. 2003. Rocket Fuel Toxic Found in Lettuce. Retrieved in 6/06 from http://www.organicconsumers.org/Toxic/030503_toxic_lettuce.cfm

OCA – Organic Consumers Association. 2004. Rocket Fuel Contaminates Lettuce and Milk. Retrieved in 6/06 from http://www.organicconsumers.org/foodsafety/lettuce120104.cfm

Okeke, B.C., and Frankenberger, W.T. Jr. 2004. Use of Starch and Potato Peel Waste for Perchlorate Bioreduction in Water. Sciences of the Total Environment, Vol. 347, pp.35-45.

OSHA. 1991. Hazard Information Bulletin on the Classification of Ammonium Perchlorate. http://www.osha.gov/dts/hib/hib_data/hib19910925.html

Office of Solid Waste and Emergency Response (OSWER). 2005. Perchlorate Treatment Technology Update. Federal Facilities Forum Issue Paper. United States Environmental Protection Agency.

O¹Niell, L.W., Nzengung, V.A., and Adesida, A. 2000. Treatment of Perchlorate Contaminated Water in Microbial Mat, Algae, and Ebb-and-Flow Hydroponic Bioreactors. Symposium Series: Case Studies in the Remediation of Chlorinated and Recalcitrant Compounds. Editors: Godage B. Wickramanayake, Arun R. Gavaskar, James T. Gibbs, and Jeffrey L. Means. Battelle Press, Columbus, Ohio. 2(7), Pp 101-106

Pop, V.J., Kuijpens, J.L., van Baar, A.L., Verkerk, G., van Son, M.M., de Vijlder, J.J., Vulsma, T., Wiersinga, W.M., Drexhage, H.A., and Vader, H.L. 1999. Low Maternal Free Thyroxine Concentrations During Early Pregnancy Are Associated With Impaired Psychomotor Development In Infancy. Clinical Endocrinology, Vol. 50, pp. 149-155.

Porterfield, S.P. 1994. Vulnerability of the Developing Brain to Thyroid Abnormalities: Environmental Insults to the Thyroid System. Environmental Health Perspectives, Vol. 102, pp. 125-130.

Powell & Associates. 2006. Contamination Remediation Using Permeable Reactive Barriers.

Renner, R. 2005. High Levels of Perchlorate Found in U.S. Mother's Milk. Science News. Environmental Science and Technology, Vol. 38, pp.120.

Renner, R. 2006. Perchlorate Found in Vitamins and Elsewhere. Science News. Environmental Science and Technology, online.

Schnoor, J.L., Seppanen, M.L., Shrout, J.D., Parkin, G.F., Just, C.L., and Beyer, P. 2004. Demonstration Project of Phytoremediation and Rhizodegradation of Perchlorate in Groundwater at the Longhorn Army Ammunition Plant. Final Report. The University of Iowa Department of Civil and Environmental Engineering.

Scott, A. 2006. Geology and Soil Mechanics. University of Wisconsin. Retrieved in 6/06 from http://physics.uwstout.edu/geo/perm_dewat.htm

Shrout, J.D., Struckhoff, G.C., Parkin, G.F., and Schnoor, J.L. 2006. Stimulation and Molecular Characterization of Bacterial Perchlorate Degradation by Plant-Produced Electron Donors. Environmental Science and Technology, Vol. 40, pp. 310 -317.

Smith, P.N., Theodorakis, C.W., Anderson, T.A., and Kendall, R.J. 2001. Preliminary Assessment of Perchlorate in Ecological Receptors at the Longhorn Army Ammunition Plant (LHAAP), Karnack, Texas. The Institute of Environmental and Human Health, Texas Tech University & Health Sciences Center.

Smith, J.W., Evans, A.T., Costall, B., Smythe, I.W. 2002. Thyroid Hormones, Brain Function and Cognition: A Brief Review. Neuroscience and Behavior Review, Vol. 26, pp. 45-60.

Solomon, G. 2004. Perchlorate Fact Sheet. Eleventh Symposium in GRA's Series on Groundwater Contaminants: Perchlorate in California's Groundwater. University of California, San Fransisco.

Song, Y., and Logan. B.E. 2003. Effect of O2 Exposure on Perchlorate Reduction by Dechlorosoma sp. KJ. Water Research, Vol. 38, pp. 1626-1632.

Sowinski, J.M., Emily, A.V., and Morrison, R.D. 2003. Facing the Growing Challenges of Perchlorate Contamination: Detection, Source Assessment, and Cleanup. DPRA.

Strietelmeier, B.A., Espinosa, M.L., Adams, J.D., Leonard, P.A., and Hodge, E.M. nd. Use of a Unique Biobarrier to Remediation Nitrate and Perchlorate in Groundwater.

Tan, K., Anderson, T.A., Jackson, W.A. 2006. Uptake and Exudation Behavior of Perchlorate in Smartweed. International Journal of Phytoremediation, Vol. 8, pp. 13-24.

Tan, K., Anderson, T.A., Jones, M.W., Smith, P.N., and Jackson, W.A. 2004a. Bioremediation and Biodegradation – Accumulation of Perchlorate in Aquatic and Terrestrial Plants at a Field Scale. Journal of Environmental Quality, Vol. 33; pp. 1638 -1646.

Tan, K., Jackson, W.A., Anderson, T.A., Pardue, J.H. 2004b. Fate of Perchlorate-Contaminated Water in Upflow Wetlands. Water Research, Vol. 38, pp. 4173-4185.

Urbansky, E.T., Magnuson, M.L., Kelty, C.A., and Brown, S.K. 2000. Perchlorate Uptake by Salt Cedar (Tamarix Ramosissima) in the Las Vegas Wash Riparian Ecosystem. Science of the Total Environment, Vol. 256, pp. 227-232.

Urbansky, E.T. 2002. Perchlorate as an Environmental Contaminant. Review Article. Environmental Science and Pollution Restoration, Vol. 9 (3), pp.187-192.

Wu, T., Flowers, J.W., Tudiver, F., Wilson, J.L., and Natavut, P. 2006. Subclinical Thyroid Disorders and Cognitive Performance among Adolescents in the United States. Biomedcentral Pediatrics, Vol. 6, pp. 12.

15. APPENDIX

15.1 Equations

The following equations are based on ISB Case Study 2, the EOS® PRB at the confidential DoD site in Elkton, MD. These equations can be generalized for other sites and ISB applications.

To determine the groundwater flow rate, the aquifer's porosity, hydraulic conductivity, and hydraulic gradient must be estimated, along with the PRB's estimated width and height Appendix Eq. 1):

$$Q = y * z * n_e * K * I \qquad \textbf{(Eq.1)}$$

Where: Q= flow rate (ft^3/yr) = 394.2 ft^3/yr = 2,880.67 gal/yr
     y = PRB width (ft) = 5 ft
    z = PRB height (ft) = 10 ft
    n_e = effective porosity = 0.18
    K = hydraulic conductivity (ft/yr) = 14,600 ft/yr (pre-injection avg.)
   I = hydraulic gradient (ft/ft) = 0.003 ft/ft (pre-injection avg.)

The amount of substrate required for ISB to be effective, according to hydrogeochemical characteristics and design plans, is determined by considering substrate demand, design life, and groundwater flow (Appendix Eq. 2).

$$S = Q * T * C \qquad \textbf{(Eq. 2)}$$

Where: S = oil substrate required (mg)
     Q = flow rate (L/yr)
     T = design life (yrs)
     C = oil substrate demand (mg/L)

The amount of substrate required was confirmed by calculating the site's oil retention capability, which is vital to maintaining a PRB. The equation for the amount of oil required when considering oil retention is:

$$S = x * y * z * \circledR_ß * O_R \qquad \textbf{(Eq. 3)}$$

Where: S = oil quantity required for retention (lbs) = 150 – 600 lbs
    x = PRB length (ft) = 50 ft
    y = PRB width (ft) = 5 ft
    z = PRB height (ft) = 10 ft
    $\circledR_ß$= sediment bulk density (lb/ft^3) = 120 lb/ft^3
    O_R = effective oil retention (lb oil/lb sediment) = 0.001-0.002

The total volume of water and emulsion injected is based on the dimensions of the PRB and porosity of the soil in the treatment zone (Appendix Eq. 5).

$$V = x * y * z * n_e \qquad \textbf{(Eq. 4)}$$

Where: V = total volume of water and emulsion (ft^3) = 225 - 450 ft^3
        x = PRB length (ft) = 50 ft
        y = PRB width (ft) = 5 ft
        z = PRB height (ft) = 5 -10 ft
        n_e = effective porosity = 0.18

The following equations and values are derived from Industrial & Environmental Solutions (IES, 2006).

15.2 Contact List

Organization	Name	Number	Email	Expertise
Shaw Environmental	Paul Hatzinger	609-895-5356	paul.hatzinger@shawgrp.com	IHDIV
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IES	Bob Borden	919-515-1625	rborden@solutions-ies.com	EOS® PRB
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University of California - Berkeley	John Coates	n/a	jcoates@nature.berkeley.edu	microbiology
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	Ellen Rubin	703-603-0141	rubin.ellen@epa.gov	Phytoremediation

Appendix Table I. Contact List for Information on Treatment Technologies and Case Studies