APPENDIX B
In Situ Soil Bioflushing

1.0 Technology Background

In situ soil bioflushing takes advantage of the ability of bacteria to utilize certain contaminants as terminal electron acceptors for respiration under anaerobic conditions. Contaminants that can be treated through in situ bioflushing include soluble salts that can be treated through reduction (e.g., perchlorate, chlorate, and nitrate), metals that can be treated through reduction (e.g., hexavalent chromium, uranium), and chlorinated organic compounds such as beta-BHC.

This process transforms perchlorate into chloride and is a promising method for the treatment of perchlorate contaminated soils. In situ soil bioflushing involves the addition of amendments containing water, an electron donor, and nutrients essential for bacterial growth. Examples of electron donor include ethanol, acetates, butyrate, lactate, and molasses. Amendments are typically applied in aqueous form via surface infiltration, delivery trenches, or subsurface injection (ITRC, 2008). The amendment can also be tilled into the upper few feet of soil and allowed to leach into the target zone during water infiltration. Perchlorate is a highly soluble salt that dissolves as the amendment infiltrates through the soil. Microorganisms capable of reducing perchlorate to chlorate, chlorite, and ultimately to the chloride anion have been shown to occur ubiquitously when particular environmental conditions at met (i.e., anaerobic conditions; Coates et al., 1999; Wu et al., 2001). The presence of nitrate and oxygen inhibits the perchlorate reduction since these are more energetically favorable electron acceptors than perchlorate.

In situ bioflushing is generally considered to be more sustainable and economically viable than excavation for remediation of vadose zone contamination. The type and application rate of amendment, soil permeability and field capacity (i.e., the ability of soil to retain water), soil chemistry, and degradation kinetics are key factors that control the rate of contaminant removal (O’Niell and Nzengung, 2003a-b). Bioflushing also has a potential advantage over soil flushing with water alone in that leaching of perchlorate to groundwater may be minimized. There are several limitations and issues to consider during implementation of the technology. For example, the electron donor may be completely consumed near injection zones and/or preferential flow paths. This may limit the delivery of amendment throughout the treatment zone such that homogeneous distribution is not achieved. There may also be problems with biofouling of the amendment delivery system; this was an issue with implementation at a site in California.

Perchlorate may become liberated in the aqueous phase and mobilize into areas outside of the treatment zone which are not conducive to perchlorate degradation, ultimately leaching into groundwater. Additionally, reducing conditions may mobilize some metals and non-metals, for example arsenic and manganese.

2.0 Technology Implementability

In situ bioflushing relies upon infiltration of amendment for delivery of electron donor to stimulate biological degradation. Subsurface conditions that are important considerations include the geology, heterogeneity of soils, contaminant spatial distribution, soil permeability, and ability to attain environmental conditions appropriate for degradation. This technology is most applicable to relatively shallow and highly permeable soils. The effective depth range is dependent on the groundwater potentiometric surface, perchlorate contamination and co-contaminant distribution, and hydraulic conductivity of the soil. The presence and concentration of co-contaminants such as arsenic and manganese, and their impact on the efficacy of treatment, as well as attainment of site-wide remediation goals should be assessed as a part of the treatment technology evaluation.

Samples from the Tronox site were used to conduct bench-scale studies related to a potential in situ bioflushing demonstration (Diebold et al., 2010). The primary objective of this project was to demonstrate and validate the treatment of perchlorate within vadose zone soils through bioremediation and flushing via two electron donor delivery methods: Treatment #1, the infiltration of liquid electron donor using an engineered infiltration gallery; and Treatment #2, the addition of a electron donor source to the upper soil column and periodic watering to promote vertical distribution within the vadose zone. The results from the laboratory microcosm study showed that three liquid amendments (emulsified vegetable oil substrate [EOS], ethanol, and citrate) were effective for promoting biological degradation of nitrate and perchlorate in unsaturated soils (~75 – 85 % of water holding capacity). Among these amendments, EOS resulted in the fastest and most consistent biodegradation of the target anions. Perchlorate concentrations in the EOS-treated samples declined from > 1,400 mg/kg to < 0.3 mg/kg in 18 weeks. Several solid (or solid/liquid combination) amendments were also effective for stimulating perchlorate biodegradation in the vadose soils, including soybean oil with peat moss, bioreactor sludge with acetate, and cheese whey. Among these substrates, the former two mixtures resulted in the most rapid and consistent perchlorate biodegradation. Based on the laboratory results, EOS is likely to be the most effective substrate for promoting perchlorate biodegradation in an infiltration gallery design (Treatment #1) in which the amendment is diluted with water and percolated through the formation, and a mixture of soybean oil and peat moss is suggested in the surface amendment design (Treatment # 2) in which the substrate is mixed into the soil surface, which is then watered to promote distribution of the carbon to deeper regions of the soil. The laboratory studies suggest that, if amendments can be well-distributed in the vadose soil matrix, bioremediation of perchlorate from >1,400 mg/kg to <1 mg/kg is feasible.

Prior to full-scale implementation of the remedy, additional laboratory bench-scale studies are warranted to optimize design parameters (e.g., water volumes and flow rates, electron donor concentrations, nutrient requirements and concentrations, and degradation kinetics). Additionally, pilot-scale infiltration tests and focused geologic characterization of the treatment zone should be conducted for design purposes, such as injection system flow rates, pump sizes, electricity requirements, and plume capture requirements. Infrastructure for in situ bioflushing includes the infiltration system and controls (i.e., irrigation and/or subsurface injection wells), amendment throughput requirements (e.g., water, electron donor, and nutrients), subsurface monitoring equipment (e.g., soil moisture content, sampling of pore water, soil gas, and soil), extraction and treatment system (i.e. extraction wells, *ex situ* treatment and treatment media, storage for off-site disposal), and down-stream groundwater monitoring wells.

3.0 Technology Performance

A field-scale study was conducted at the Longhorn Army Ammunition Plant (LHAAP) in Karnack, Texas (Borch, 2001). This study focused on the upper few feet of soil, where wet cow manure was applied to the surface of undisturbed soil and a total of 26 inches of seasonal rainfall over a period of five months allowed the bacteria, moisture, and organic material to infiltrate from the manure to the subsurface. Over 90% of perchlorate in the high concentration areas was destroyed within the first 30 days. The initial concentration of perchlorate was 600 to 1,400 mg/kg and concentrations were reduced to 12 to 95 mg/kg after 30 days. However, no perchlorate degradation was observed in the dry underlying soil (> 1 foot below ground surface).

A subsequent pilot study at LHAAP tested application of tilled amendment (horse or chicken manure) and liquid amendment (ethanol and ethyl acetate) in other plots to one foot below ground surface (O’Niell and Nzengung, 2003a). Initial concentrations of perchlorate were heterogeneous and ranged from 8.2 to 480 mg/kg. After 10 months, greater than 95% reduction was observed to three feet below ground surface, with the highest reductions observed in the most saturated soils. Ethanol was the most effective at stimulating biodegradation at greater depths than the solid amendments, with 100 percent removal of perchlorate.

In situ bioflushing was used at the Olin site in Santa Clara Valley, California to treat 40,000 cubic yards of soil in the vadose zone for remedial action. The average initial concentration was 215 µg/kg of perchlorate. After 10 months, perchlorate concentrations were reduced to 11 µg/kg. The half-life for biodegradation of perchlorate based on laboratory treatability tests was approximately 1.0 to 1.7 days.

4.0 Case Study

The utility of this technology has been explored in multiple bench- and pilot-scale studies (ITRC, 2008; Borch, 2001; O'Niell and Nzengung, 2003a-b; Deitsch et al., 2005; Frankel and Wuerl, 2005), and has also been demonstrated at full scale (GeoSyntec, 2006; Deitsch et al., 2005). The Olin site in Santa Clara Valley, California used in situ bioflushing for full scale remedial action to treat approximately 40,000 cubic yards of soil from 0 to 16-feet below ground surface (GeoSyntec, 2006, Deitsch et al., 2005). The average initial concentration was 215 µg/kg of perchlorate and the site-specific soil screening level was specified by the Central Coast Regional Water Quality Control Board (CC RWQCB) as 50 µg/kg of perchlorate. The CC RWQCB approved use of the technology, in conjunction with excavation and *ex situ* bioremediation of surficial soils.

A bench-scale laboratory treatability study found that all electron donors investigated, including calcium magnesium acetate (CMA), ethanol, potassium oleate (vegetable soap), and methyl soyate (biodiesel), were suitable for bioremediation of perchlorate. Average perchlorate concentrations were reduced from approximately 1,000 µg/kg to 50 µg/kg within the 13-day study. The half-life for biodegradation of perchlorate in the treatability study microcosms was calculated as 1.0 to 1.7 days (Deitsch et al., 2005).

The full-scale implementation involved tilling the upper two feet of soil using agricultural equipment and amending with a total of 11,000 pounds of CMA, 800 pounds of potassium bromide (tracer), and 3,600 pounds of gypsum (increases soil hydrophilicity) over the period of 10 months (GeoSyntec, 2006). Initial tests indicated the geometric mean of the infiltration rate was $1.3X10^{-4}$ cm/s and ranged over two orders of magnitude (Deitsch et al., 2005). To enhance percolation through the top 4 feet, the soil was loosened with a bulldozer equipped with ripper teeth. Drip irrigation was applied using groundwater treated by anion exchange to leach amendment to the target zone. A total of 20,000 pounds of citric acid was added to 6.4 million gallons of water (13 to 31 gallons per minute [gpm] on average) during the study over 2.4 acres.

Assuming an effective pore volume of 0.25, two pore volumes of water were added to the vadose zone.

Performance monitoring was conducted to assess the lateral and vertical extents of amendment infiltration. Thirty soil suction lysimeters were used to sample pore water and monitor amendment delivery as well as performance of perchlorate degradation on a monthly basis. The lysimeters were co-located with 30 soil moisture sensors that continuously monitored moisture content and data were stored in data loggers. Two pairs of monitoring wells were placed between the target zone and the groundwater potentiometric surface and 17 on-site monitoring wells were sampled outside of the target zone. Extraction wells were placed downstream to recover and treat recharge water by anion exchange. Quarterly groundwater monitoring was conducted downstream of the treatment zone. After approximately 10 months of amendment application, 24 soil samples were collected and the concentration of perchlorate was reduced from an initial geometric mean of 215 to 11 µg/kg. The upper 95% confidence limit was reduced from 1,020 µg/kg to 15 µg/kg. The treatment system was successful in meeting the CC RWQCB remediation goal of 50 µg/kg: Additionally, the remedial action did not mobilize co-contaminants (metals) to the groundwater.

5.0 Regulatory Acceptance

Biological degradation of perchlorate is an approved method for treatment of drinking water in the State of California (Min, 2004). At the Olin site in California, the technology was accepted by the California CC RWQCB as one of the remedial actions at the site. The remedial goals were attained within one year of implementation.

6.0 Costs

The primary cost drivers are chemicals and water used for amendment; sampling and analysis for monitoring; and groundwater extraction, treatment, and disposal costs. The pilot scale tests conducted at the LHAAP site in Karnack, Texas estimated treatment costs were in the range of $22 to $67 per cubic yard (O’Niell and Nzengung, 2003b). Another study found treatment costs ranged from $75 during the pilot study to a projected $40 per cubic yard for full scale operations (Frankel and Wuerl, 2005). Costs are highly site specific.

7.0 References

Borch, R., 2001. Case study: bioremediation of perchlorate in soils by the surface application of wet cow manure. Association for Environmental Health and Sciences, 11th Annual West

Coast Conference on Contaminated Soils, Sediments and Water, San Diego, California. March 21, 2001.

Coates, J. D., U. Michaelidou, R. A. Bruce, S. M. O'Connor, J. N. Crespi, and L. A. Achenbach, 1999. Ubiquity and diversity of dissimilatory (per)chlorate-reducing bacteria. *Applied Environmental Microbiology*. **65**(12), 5234-41.

Deitsch, J., E. Cox, L. Griffin, C. Gokmen, R. Borch, M. Monteleone, and R. W. McClure, 2005. In-situ bioremediation of perchlorate in soil. American Society of Civil Engineering Water Containment and Remediation Conference Proceedings.

Diebold, J. B., P. Hatzinger, S. Streger, 2009. In situ bioremediation of perchlorate in vadose zone source areas. Final Summary Report (DRAFT), ESTCP Project ER-0435. July 2010.

Frankel, A. J. and B. J. Wuerl, 2005. In situ anaerobic remediation of perchlorate-impacted soils. National Groundwater Association Conference on MTBE and Perchlorate: Assessment, Remediation, and Public Policy. May 2005.

GeoSyntec Consultants, 2006. Soil in situ bioremediation system closure report, Olin/Standard Fusee site, Morgan Hill, California. July 18, 2006.

Interstate Technology Resource Council (ITRC), 2008. Remediation technologies per perchlorate contamination in water and soil. Technical/Regulatory Guidance. March 2008.

ITRC, 2005. Perchlorate: Overview of Issues, Status, and Remedial Options. Technology Overview. September 2005.

Min, B., P. J. Evans, A. K. Chu, and B. E. Logan, 2004. Perchlorate removal in sand and plastic media bioreactors. *Water Research*. **38**, 47-60.

O'Niell, W. L., and V. A. Nzengung, 2003a. Field Demonstration of In Situ Bioremediation of Perchlorate-Contaminated Soils and Groundwater. *Proceedings, 96th Annual Conference, Air Waste Management Association*.

O'Niell, W. L., and V. A. Nzengung, 2003b. In Situ Bioremediation of Perchlorate. *Hazardous Waste Consultant* **21**(6): 1.12–1.12.

Wu, J., R. F. Unz, and B. E. Logan, 2001. Persistence of perchlorate and the relative numbers of perchlorate- and chlorate-respiring microorganisms in natural waters, soils and wastewater. *Bioremediation Journal*. **5**, 119-130.