Loading Rates and Impacts of Substrate Delivery for Enhanced In Situ Anaerobic Bioremediation

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Effective application of enhanced *in situ* anaerobic bioremediation of chlorinated solvents in groundwater depends primarily on the delivery of appropriate levels of substrate amendments in the subsurface and the development of optimal geochemical conditions for anaerobic biodegradation processes to occur. An inadequate substrate loading rate (*i.e.*, the volume, concentration, and frequency of injection) may result in reducing conditions that are insufficient to support complete dechlorination of chlorinated solvents, thereby increasing the potential for accumulation of regulated intermediate dechlorination products. Conversely, excessive levels of organic substrate may lead to inefficient utilization of substrate for anaerobic dechlorination and the potential for long-term adverse impacts to secondary groundwater quality. Therefore, determining an appropriate substrate loading rate and delivery method are critical design and operational objectives for successful implementation of enhanced anaerobic bioremediation.

The Environmental Security Technology Certification Program (ESTCP) has funded a study to evaluate the design, application, and results of various approaches to determining substrate loading rates. The objectives of this study are to (1) better understand the effects that substrate loading rates have on substrate distribution and persistence, (2) determine how control of loading rates affects amendment reactivity and development of optimal geochemical conditions, (3) identify loading rates that have adverse impacts on secondary water quality, (4) evaluate the effect that differing substrates or loading rates may have on hydraulic conductivity, and (5) develop practical guidelines for designing and optimizing loading rates and injection scenarios for differing substrate types and for differing geochemical and hydrogeologic conditions.

Eighteen case studies were selected for a comparative review of the methods currently used to determine substrate loading rates. The sites include substrate applications using emulsified vegetable oil (EVO), combinations of EVO and sodium lactate, HRC®, molasses, ethanol, sodium lactate, and mulch and compost. Select study sites have been sampled to further evaluate substrate distribution and persistence, microbial sufficiency (*Dehalococcoides* species and reductase genes), changes in site geochemistry over periods of several years, and long-term impacts on secondary water quality.

Substrate determinations and subsequent field modifications for the cases studies provide practical insight into the utility of the theoretical substrate calculations or empirical methods employed. Results of this study are being used to develop guidance for designing appropriate substrate loading rates and implementing effective amendment strategies, with recommendations for the appropriate use of substrate demand calculations and site-specific design factors.