OUTLINE OF METHODOLOGY FOR BIOLOGICAL REMEDIATION OF UNDERGROUND HYDROCARBON CONTAMINATION

BACKGROUND

During the last few decades the scientific and regulatory communities have been interested in the potential of microorganisms to more quickly and economically address the issues of environmental damage. As the technology has slowly emerged a greater understanding of the microbe's abilities and limitations became available.

In underground environments the collection and confirmation of microbial data proved especially challenging. This data evaluation required the expertise of many fields of study. Currently there is ample published data to evaluate and implement biological remediation efforts either singularly or in conjunction with other technologies (SVE, sparging, injection, etc).

A major question throughout the emergence of in situ bioremediation has been how best to stimulate the indigenous microbes at the site. The goal is for the indigenous microbes to increase their population (reproduction) several orders of magnitude and consume the targeted contamination. Many types and combinations of nutrients, oxygen, and electron acceptors have been tried in order to increase the bacterial population below the surface. Difficulties encountered include inhibition, rate limitation, toxicity reactions, precipitation of solids, and clogging of wells/formations. These and other difficulties arose due to an attempt to provide the necessary metabolic requirements for cell growth from aboveground. By attempting to completely distribute chemicals through a chemically reactive zone, to an exponentially small population of microbes capable of contaminant reduction, variable results can be expected. An additional problem, which plagues traditional indigenous species stimulation techniques, is a subsurface temperature which is unsuitable for rapid cell reproduction causing a lengthy lag phase of several months for significant population (above 10^6/ml) increase.

The following proposal details an alternative method of increasing the sites microbial population by introducing large populations (10^9/ml) of laboratory grown fermentation products consisting of bacteria selected for their ability to consume specific compounds such as BTEX.

BIOREMEDIATION OBJECTIVE

The objective of this method is fivefold:

1. The promise of delivering selected species in situ at high concentrations fully acclimated to the sites specific water chemistry.
2. Simple two variable decision making for calculation of injection volume by water movement per day and hydraulic conductivity.
3. Minimization of nutrient additions. Cellular N-P-K and micronutrients are provided during lab growth.
4. Gain better control of the bioremediation process by providing required nutrients to the cells rather than the soil and groundwater.
5. Confirm biological activity by sampling, data collection, and field results.

SERVICE AND TYPE OF MATERIAL

The focus of this service is to provide the sampling, bacteria, and microbial knowledge necessary to confirm and quantify the degradation of the sites contaminants using many different microbial species. These various microbes are delivered as a concentrated liquid (inoculum) containing not less than 1.5 x 10^9 cells/ml of microbes selected for their ability to consume BTEX. All microbial blends are grown at Advanced Microbial Services Laboratory (Tulsa, OK). The concentration of cell mass is achieved under controlled laboratory conditions within one of several 50 gallon reactors.

SAMPLING STATEGY AND SITE MONITORING

To confirm and quantify the degradation of the site's contaminants, a sampling strategy is followed which monitors the conditions at the site throughout the project. In a typical underground project the background microbial population will be in the range of thousands to tens of thousands per milliliter of water or gram of soil. By sampling the wells at the site, an immediate population increase is experienced once biological products are administered in situ. The goal of 1,000,000 cells/ml is indicative of an average healthy remediation project. By sampling the sites wells over time a pattern of biological expansion throughout the site is evaluated. Calculation of water movement and zones of communication can be evaluated from population data as the biomass spreads downgradient.

Although the initial bloom of population indicates a project moving in a positive direction, monitoring the population decline is important to project success.

Because the most difficult fraction of an in situ project is the last 10 to 20% of contamination, having population data is critical to decision making for those last ppm's. By changing treatment variables a population sampling program allows quick evaluation of which methods are most able to stimulate the biological consumption of BTEX.

TRADITIONAL METHOD ENHANCEMENT

Conventional SVE and air sparge treatment methods can be used successfully with biological products by inducing a "push-pull" tidal effect underground. Using an air sparge "push" and an SVE "pull" the movement of microbe/ nutrient laden products through the zone of contamination is accelerated. By alternating the sparge and SVE schedule a smear zone can be inoculated with species capable of consuming the contaminant allowing for quicker remediation times and site closures. Progress is monitored by quarterly analysis to determine Bacterial counts, pH and Dissolved Oxygen as well as periodic BTEX analysis. Observation of this data allows us to optimize the growing conditions in the affected area by adjusting for pH and electron acceptors, as well as track the reduction in contaminate.

OUTLINE OF METHODS AND SERVICE

SECTION 1

COLLECTION AND TRANSPORTATION OF INDIGENOUS POPULATION

Advanced Microbial Services personnel will collect and transport a determined volume of groundwater pumped from the area of contamination. This volume of water will contain a population of indigenous

bacteria and other microbes, which have been able to live in, and make metabolic use of the contaminants targeted for remediation.

SECTION 2

SECTION 3

GROWTH OF INOCULUM

Upon receipt at the A.M.S Lab, the collected site liquid will undergo the following:

- Sample for microbial counts (Serial dilution plate counts).
- Adjustment of pH if necessary.
- Addition of microbial nutrient solution.
- Relocation into biological reactor for 72-84 hours. Or until cell mass reaches 1.5 x 10^9 cell/ml as confirmed by photometric methods in terms of turbidity. Turbidity readings of high cell concentrations (10-100's of millions and above) are confirmed to be accurate via standardization plate counts.
- Storage of inoculum in cold storage (45^o F) until delivery date.

SECTION 4

ON-SITE SAMPLING SCHEDULE

Initial testing to determine the background or indigenous microbial population is to be measured at wells BMW # 15, #18, #20, and BSB #35. These specific wells, of the many involved, are in the direct gradient path of the contamination plume. Sampling these wells throughout the remediation project will provide data to confirm and quantify the increase in microbial population (cell count) as compared to a decrease in contaminant levels. The length of migration time and magnitudes of population increase will also be recorded. These values will be useful in the management and monitoring of this project particularly as the contaminant levels decrease to near closure levels.

Sampling Locations and Sample Method- Wells BMW # 15, #18, #20, and BSB #35- plate count sampling of each well for bacterial population and wet mount observation of microbial predators.

Sampling Schedule- once per quarter, four wells, eight samples total.

SECTION 5

DELIVERY OF INOCULUM

Inoculum delivery is to be scheduled once per week on a particular day of the week. The volume delivered will be 1/10^{th} of the weeks calculated biological product injection volume (ex. 100gal.for 1000 gal. weekly injection) and will be added to a 9/10^{th}-volume groundwater pumped from the location. Typical deliveries consist of 5-gallon pails or 30-gallon plastic drums of a specific number.

The weekly biological product injection volume is from 1 to 5% of the weekly fluid movement. In tight or loose soil structures a 1-5% range respectively, is observed to address well plugging concerns. Note: In situ methods become difficult when hydraulic conductivity is below 10^{-4} cm/sec

Following this volume delivery schedule guarantees a 150 x 10^6 cell/ml population once diluted with groundwater and allows some measure of control of the cell mass injection concentration. An additional benefit of cell acclimation is achieved during this phase before injection. The acclimation tank is to be aerated, temperature regulated, used as biological product storage only, and purged of $1/7^{th}$ of its liquid volume each day. The second of the dual tank design should receive $1/7^{th}$ of its liquid volume per day from a selected down gradient well or wells.

At the time of delivery an identical volume of newly collected groundwater from the area of contamination will be taken back to the Advanced Microbial Services Laboratory where the process will be repeated as detailed above.

SECTION 6

LABOR, MATERIALS AND COST

Transportation and Shipping-

- 150 miles one way or 300 miles total at .32 cents per mile

Sampling per quarter-

- 4 Plate counts- $75.00 each
- 4 Wet Mounts- $20.00 each

Production cost for inoculum ($/gal)

Nutrient for injection tank ($/gal)

Biological Product Storage-Dual Tank/month