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Integrated Soil Bioremediation Using Selected *Pseudomonas spp.* Bacteria for the Cleanup of a Former Bulk Fuel Facility in an Urban Setting

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ABSTRACT: A former Department of Defense bulk fuel distribution facility (Site) located in an urban, residential setting required cleanup under California Regional Water Quality Control Board (RWQCB) oversight for redevelopment as a retail center and public park. Contaminants of concern (COCs) were total petroleum hydrocarbons (TPH) primarily in the diesel range (C13-C22) with lesser amounts in the C4-C12 and C23-C40 ranges. During the public participation process and in consultation with public officials, it was determined that an alternative to large-scale trucking through the area was desired. The excavation and off-Site disposal alternative would have resulted in ~8,000 truck trips transporting contaminated soil through city neighborhoods and returning with clean fill based on the estimated 100,000 tons of TPH-impacted soil requiring remediation. During the California Environmental Quality Act-required evaluation, it was also determined that transportation of the contaminated soil would result in approximately three million pounds of carbon dioxide emissions. Therefore, the project objectives were to identify an on-Site, sustainable remediation technology capable of meeting RWQCB cleanup objectives to allow for soil reuse at a competitive cost and within a timeframe consistent with property redevelopment goals.

Based on a feasibility study (FS) and technology screening process, an integrated bioremediation approach was selected for pilot testing. The approach integrates three technological elements including: (1) a proprietary blend of *Pseudomonas spp.* bacteria selected by culture methods to degrade petroleum hydrocarbons, (2) an Environmental Protection Agency (EPA) registered “bacteria-friendly” surfactant, and (3) a mobile, customized soil processing system (SPS). To evaluate bioremediation as the remedial approach for the Site, a pilot test was conducted on 300 tons of TPH-impacted soil. Post-treatment sampling and analysis confirmed a nearly 90% reduction in TPH C6-C40 and more than 95% reduction in TPH gasoline range organics (GRO) after one month of treatment. Near asymptotic reductions were documented during the second and third months of the pilot test. The pilot test results demonstrated that the bioremediation approach could meet the RWQCB cleanup criteria of 1,000 milligrams per kilogram (mg/kg) for TPH diesel and motor oil-range organics (DRO and ORO) and 100 mg/kg for TPH GRO over a short time period. Full-scale implementation included stockpiling the treated soil in biotreatment rows lined with 30-mil high-density polyethylene and covered with 6-mil plastic. The soil treatment stockpiles were engineered and constructed with a vapor collection and treatment system as required for the air permit. The air permit also required installation of a vapor mitigation system on the SPS during application of the bacteria treatment (or suspension).

Soil bioremediation treatment timeframes ranged from two to four months. Over 96% of the 94,000 tons of soil treated ultimately met the RWQCB cleanup criteria (soil TPH below 1,000 mg/kg for diesel and 100 mg/kg for gasoline fractions) after a single bacterial

application. The remaining 4% of the soil was retreated and 75% of this met the cleanup criteria. This effective and rapid soil treatment process implemented by Bulldog Green Remediation, Inc. (BGR) resulted in an estimated project savings of $1.3 million relative to off-Site disposal based on FS cost estimates and remediated the TPH at its source allowing soil reuse without landfilling. The goal of minimizing the noise impacts and diesel-trucking emissions to the local community was achieved and this approach also eliminated the safety risk and road deterioration related to trucking. The BGR sustainable "green" soil bioremediation approach successfully integrates the technological elements of using highly selected bacteria cultures and laboratory-based soil treatability studies combined with pragmatic and cost effective engineering. Lessons learned include: (1) air emission considerations for the treated biotreatment rows (aka. biopiles) and SPS, (2) maintaining optimal moisture conditions, (3) amendments such as rice hulls can improve oxygen circulation within the biopiles, and (4) operational efficiencies related to soil and storm water management.

FIGURE 1. Former Department of Defense bulk fuel distribution facility.

INTRODUCTION

The Site (Figure 1) is a former Department of Defense bulk fuel distribution facility located in an urban, residential setting required cleanup under California Regional Water Quality Control Board (RWQCB) for redevelopment as a retail center and public park. The Site previously contained ten 80,000-barrel and two 55,000-barrel aboveground storage tanks (ASTs) that were used to store and distribute jet propellants 5 and 8 (JP-5 and JP-8). Aviation gasoline and JP-4 was also reportedly stored in the ASTs. Contaminants were total petroleum hydrocarbons (TPH) primarily in the diesel range (C13-C22) with lesser amounts in the C4-C12 and C23-C40 ranges. During the public participation process and in consultation with city officials, it was determined that an alternative to large scale trucking through the city was desired. The excavation and off-Site disposal alternative would have resulted in ~8,000 truck trips transporting contaminated soil through city neighborhoods and returning with clean fill based on the approximately

100,000 tons of TPH-impacted soil requiring remediation. During the California Environmental Quality Act-required evaluation, it was also determined that transportation of the contaminated soil would have resulted in approximately three million pounds of carbon dioxide (CO_2) emissions. Therefore, the project objectives were to identify an on-Site, sustainable remediation technology capable of meeting RWQCB cleanup objectives to allow for soil reuse at a competitive cost and within a timeframe consistent with property redevelopment goals. The selected remediation approach included:

- Excavation and biotreatment of shallow soil (0 to 3 meters below ground surface [bgs]) and focused deeper soil (9.1 meters bgs) to meet numeric cleanup goals approved by the RWQCB;
- After meeting cleanup goals, treated soil would be reused on-Site in accordance with the conditions of a Waste Discharge Requirement (WDR) permit to be issued by the RWQCB;
- Extension of some excavations to groundwater to remove soils that contribute to the degradation of groundwater. Not all deeper soils with contaminant concentrations exceeding cleanup goals would be excavated; the residual concentrations of contaminants will be treated via traditional in situ methods (e.g., vapor extraction); and
- Treatment of shallow “oily sand” present near a former oil/water clarifier.

Regulatory and Permitting Framework. The lead oversight agency for the project is the RWQCB. The RWQCB is responsible for administering the Federal Clean Water Act and State Water Code. *Ex situ* biotreatment of the impacted soil was authorized under General WDR Order No. 90-148; General Waste Discharge Requirements for Land Treatment of Petroleum Hydrocarbon Contaminated Soil in Los Angeles and Santa Clara River Basins.

The Site is located within the Los Angeles air basin and required permitting through the South Coast Air Quality Management District (SCAQMD). The SCAQMD is responsible for administering the Clean Air Act and required permits for the excavation and handling of volatile organic compound (VOC)-contaminated soil in accordance with Rule 1166 Contaminated Soil Mitigation Plan (1166 Plan). In addition, a modification to an existing permit to operate the existing SVE system was required for the operation and maintenance of the soil treatment areas.

Soil Cleanup Goals. Soil cleanup goals (SCGs) were calculated using the procedures described in the *Interim Site Assessment & Cleanup Guidebook* (RWQCB, 1996), and are Site-specific goals calculated to be protective of groundwater quality. SCGs were calculated for two general depths including goals for shallow soil (<1.5 meters bgs) and deeper soil (>1.5 meters bgs), referred to as restricted use and un-restricted use, respectively. The RWQCB established cleanup criteria was 1,000 mg/kg for total petroleum hydrocarbons (TPH) diesel range organics and oil range organics (DRO and ORO) and 100 mg/kg for TPH gasoline range organics (GRO).

Pilot Study. Based on a detailed evaluation of the potential on-Site treatment approaches, BGR’s bioremediation technology was selected as the preferred remedial approach. The technology entails excavation of the soil, processing of the soil to add surfactants to reduce volatility and desorb hydrocarbons from the soil matrix, and the addition of a proprietary blend of *Pseudomonas spp.* bacteria to facilitate biotreatment. Once treated with the surfactants and bacteria, the soil is placed into biotreatment rows (biopiles) to provide adequate time (several weeks to a few months) for the bacteria to consume the hydrocarbons. A pilot scale test was conducted on approximately 300 tons of soil ("truck rack/water tank and oily sand soil") to evaluate the efficacy of BGR's bioremediation technology and soil processing system (SPS) to treat hydrocarbon impacted soil at the Site. After 16 days of treatment, progress soil samples indicated an 80% concentration reduction in the truck rack/water tank soils and a 65% concentration reduction of TPH in the oily sands. After 30 days of treatment, GROs were eliminated from both the truck rack/water tank and oily sand soil. After 90 days of treatment, DROs were reduced by more than 95%.

Full-Scale Bioremediation Treatment. The pilot test results were used to design a full-scale soil treatment process using BGR's SPS.

Soil Excavation and Preconditioning. Soil was excavated from previously delineated "target areas", stockpiled next to BGR's SPS, and conditioned with water prior to treatment. Excavated soil was required to be treated within 72 hours of being excavated as required by SCAQMD permits.

Soil Processing. BGR's patent-pending ex situ bioremediation technology combines a proprietary blend of highly concentrated non-pathogenic microbes (*Pseudomonas spp.* bacteria*)* and a compatible EPA-approve surfactant to degrade petroleum hydrocarbons and other organic compounds into carbon dioxide and water. The microbe and surfactant suspension is sprayed onto to contaminated soil as it is processed on the surface with BGR's custom-designed SPS. The SPS is engineered to mechanically introduce the bacterial suspension with the surfactant, oxygen, and macronutrients (as needed) through a one-time application process that can effectively degrade the TPH compounds to established cleanup requirements. The SPS can process up to 1,200 tons of soil per day (this rate may vary depending on soil and Site conditions).

BGR's custom treatment liquid contains a unique mixture of microbes and surfactant. The microbial treatment is derived from HC-selected strains of naturally occurring *Pseudomonas spp.* bacteria. The particular strains of *Pseudomonas spp.* were selected for their ability to metabolize the Site petroleum hydrocarbon contaminants as their sole carbon (food) and energy source. The inclusion of the non-toxic, biodegradable surfactant increases the solubility of the soil-bound hydrocarbons greatly increasing bioavailability to the bacteria; further the surfactant lowers the surface tension of water in the finer-grained soil pores and improves diffusion of atmospheric oxygen for more effective oxidative bioremediation. The surfactant consists of a non-ionic alcohol ethoxylate surfactant solution proven to be effective at solubilizing weathered petroleum product hydrocarbons. Alcohol ethoxylates have been recognized by the EPA to be safe and suitable surfactants for use in soil bioremediation processes.

The selected high density strains of bacteria used by BGR are cultured in a laboratory and then freeze-dried to stabilize the bacteria in an inactive, yet highly viable state. By freeze-drying the bacteria, they can be preserved for transport and reconstituted on Site. The bacteria were shipped to the Site as a dry powder in vacuum-sealed containers and kept frozen until blended with the surfactant solution for direct soil application.

The BGR custom liquid treatment was deployed using a mobile mixing trailer and the SPS. The mixing trailer was designed and configured for the storage, blending and delivery of the custom treatment suspension. Four 1,040-liter storage totes constructed of high-density polyethylene (HDPE) are located on the upper half of the trailer. Two 4,732-liter HDPE tanks are located on the lower half of the trailer. In the top storage totes, freeze-dried, powdered bacteria were re-hydrated with potable water. In the bottom tanks, the proprietary surfactant was combined with potable water.

The surfactant solution and bacteria suspension were combined and directed via flexible hoses to spray nozzles within the SPS for application to the soil at the initial rate of four gallons per ton of soil. The delivery rate could then be adjusted to reach an optimal final moisture content in the soil. Excavated soil was turned and pre-conditioned with water, then temporarily stockpiled next to the soil processing area. Soil was loaded into the SPS using a front-end loader that placed the pre-moistened soil onto a vibrating steel screen to remove stones and debris. Treated soil leaving the SPS was transported to the former AST basin areas using a front-end loader. The treated soil was turned and placed into discrete biopiles as detailed below.

Soil Biopile Construction. The design and construction of the biopiles incorporated requirements and permit conditions as specified by the RWQCB and SCAQMD. Due to volume of soil to be excavated and limitations of available space (within the basins), soil excavation, treatment, and backfilling operations were conducted in phases. HDPE liners were placed beneath each biopile. Clean soil was used as a working layer to cover the liner, protect the integrity of the liner, and to ensure that the liner remained secured in place. The perimeter of the buried liner was marked with wooden stakes and caution tape.

SPS-treated soil was turned and placed into a series of lifts that constitute the biopiles. At a center to center spacing of approximately 15.2 meters, approximately 0.6 meters above grade, lateral plastic perforated piping (constructed of schedule 40 polyvinyl chloride (PVC) was placed perpendicular to the long dimension of the biopile. The perforated lateral piping reduced the air pressure in the covered biopiles to enhance bioremediation with introduced atmospheric oxygen. The perforated lateral pipes also helped to mitigate VOC emissions as the vented air was directed to activated carbon canisters to capture volatile TPH by soil vapor extraction (SVE). Additional batches of treated soil from the SPS were carefully placed onto each subsequent lattice work of perforated pipe until the biopile was approximately 2.4 meters in height. Each lateral pipe was connected with a PVC ball valve to a main header and the header, in turn, was connected to the on-Site SVE treatment system. The soil vapors were treated through multiple granular activated carbon (GAC) vessels in series prior to being discharged to the atmosphere. The GAC vessels operated under restrictions authorized by a Site-specific SCAQMD permit and required regular monitoring of vented air.

Soaker hoses were placed on the biopile to augment and adjust the moisture content of the soil. A tensiometer was used to monitor moisture content; water was added if the soil moisture fell below the optimum range (12-16%) established in the laboratory. Each biopile was covered with 6-mil black HDPE plastic to reduce evaporation and diminish the escape of VOCs. The plastic sheeting was secured with sand bags and tie-down ropes. The overlapping seams were sealed with adhesive and tape to minimize VOC venting.

Biopile Monitoring and Treatment Progress Soil Sampling. Monitoring of the biopiles was performed regularly to track the effectiveness of treatment as well as to measure soil vapors from the biopile laterals using a photoionization detector (PID). Treatment progress samples of soil were collected at three to four week intervals after initial SPS treatment. Soil grab samples were collected for analysis at randomly selected locations in each biopile, roughly at one sample for every 382 meters^3 of soil from within the biopile per sampling event. Samples were collected from varying depths ranging from several inches to several feet into the treated biopile. Treatment progress soil samples were analyzed for:

- TPH (C13-C44 hydrocarbon range) using EPA Method 8015 (CA modified)
- TPH GRO and VOCs including fuel oxygenates using EPA Method 8260B
- Heterotrophic plate count (HPC) using Standard Method 9215C

- Moisture using AOAC Method 950.46

Final Treatment Confirmation Soil Sampling. Biotreatment was judged to be complete once PID levels of the vented air leaving the laterals were less than 50 parts per million by volume (ppmv) and the treatment progress sample TPH and VOC concentrations met the SCG requirements for closure. Final confirmation samples were collected at random locations at a frequency of 35 samples per biopile. Similar to treatment progress sampling, final confirmation sampling was accomplished by collecting grab samples of soil from varying depths ranging from several inches to several feet in each biopile.

Treatment confirmation soil samples were analyzed for:

- TPH (C13-C44 hydrocarbon range) using EPA Method 8015 (CA modified),
- TPH GRO and VOCs including fuel oxygenates using EPA Method 8260B

Biopile Dismantling. Following receipt of final confirmation sample results, biopiles were dismantled. The dismantling process included segregating (extracting) any soil that did not meet SCGs and any soil with significant petroleum odors (identified as "operations" soil) for subsequent retreatment. Treated soil that met the defined cleanup criteria was used to backfill the excavations. Less than 1% of the total excavated soil was disposed of off Site.

RESULTS

Pre-treatment and final confirmation results for select treatment basins are presented on Figures 2 and 3.

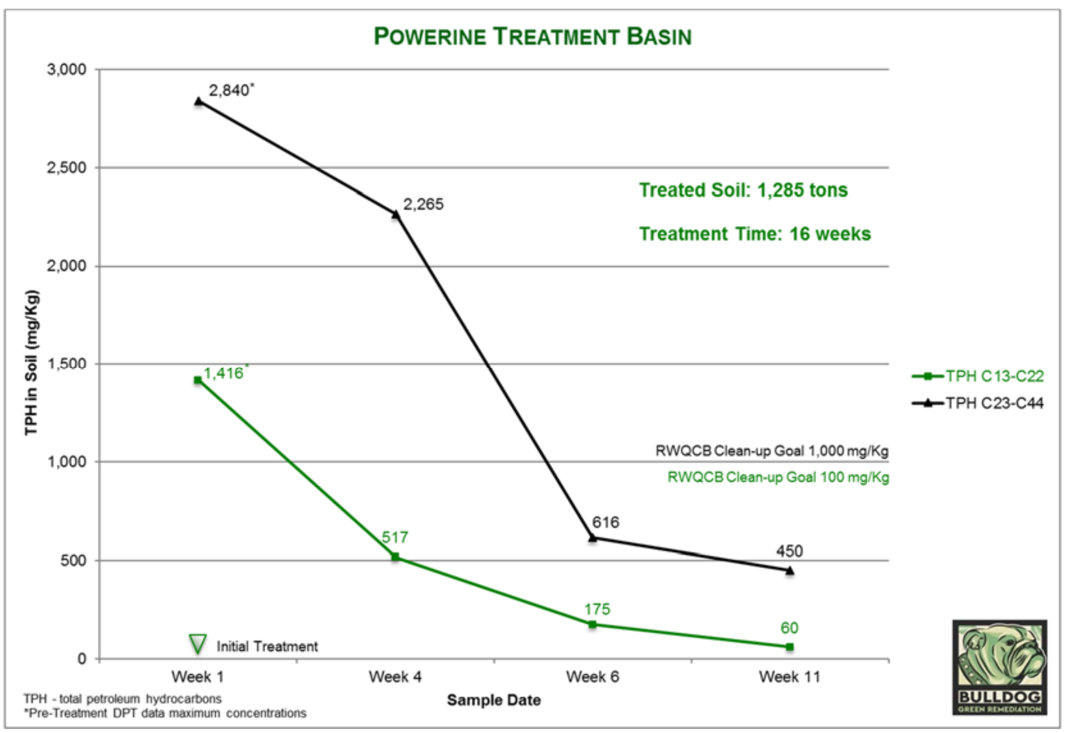

FIGURE 2. Powerine Basin time versus concentration results.

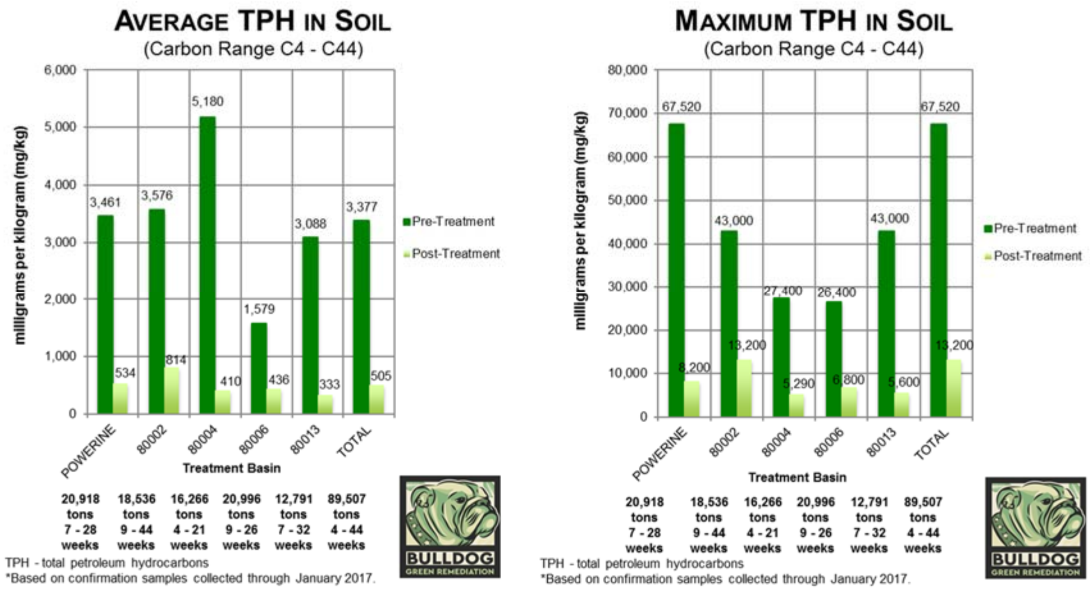

FIGURE 3. TPH levels in soil, pre-treatment versus post-treatment.

A summary of treatment results with respect to cleanup goals is presented in Figure 4.

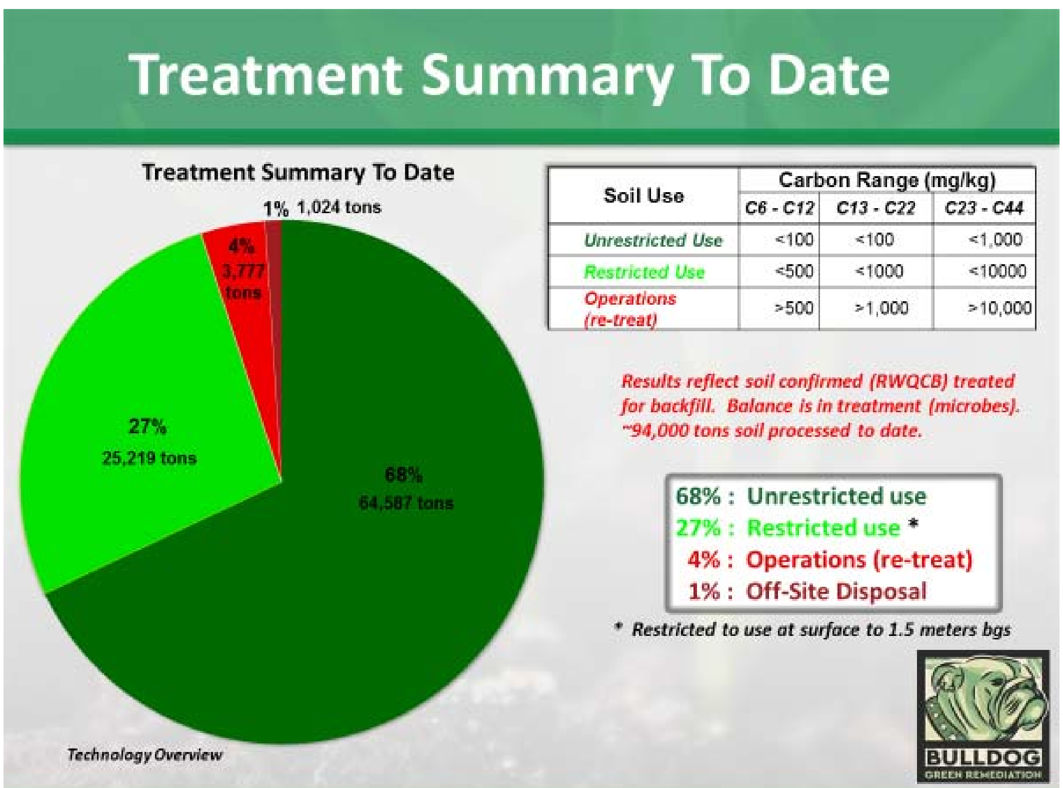

FIGURE 4. Treatment summary to date.

Challenges and Solutions. Challenges encountered during the project included maintaining optimum moisture and adequate aeration conditions in the covered biopiles.

In addition, areas of oily sand (clods) were occasionally found in the treated biopiles associated with the oil/water clarifier and were re-processed. Since uncovered soil tilling in the open atmosphere is not permitted in the Los Angeles air basin, another option that was considered was blending the soil with a dry biodegradable composting material to improve air flow and create millions of tiny air pockets in the soil that would encourage the penetration of atmospheric oxygen. The overall challenge of any solid phase soil treatment is to achieve some degree of homogeneity and good mass transfer among the critical reactants: TPH bound to soil particles, bacteria, nutrients, moisture and water.

BGR engaged with California State University (CSU) Chico's Center for Water and the Environment to perform a preliminary laboratory study to assess the use of soil amendments to improve the oxidative bioremediation process. One of the materials CSU Chico suggested as a soil amendment to expand and aerate the excavated soil was dry rice hulls, a waste product of rice processing in the northern central valley of California. Rice hulls are abundant in this region and inexpensive to obtain and transport by truck. Rice hulls have a very low specific gravity of approximately 0.14 kilograms per liter and an ability to absorb and retain moisture for the surrounding soil. A preliminary study was performed in the CSU Chico laboratory to determine what ratio of dry rice hulls to moist soil would be most effective in terms of expanding the soil and improving aeration.

In the preliminary trial, a challenging reference soil was used to test the technique and establish baseline ratios of rice hulls to soil. This reference soil was stiffer and had higher clay content but less moisture than the other three samples from the Site. The reference soil test results indicated that blending dry rice hulls into the soil appeared to greatly improve the 'workability' of the soil and allowed ample aeration as the soil quickly expanded. Blending in even small amounts of rice hulls helped to expand the soil volume (as sifted, not compressed) even in the amendment range of 5 to 10% total rice hulls (by weight). At 5% rice hulls, the damp clay soil expanded by 60% in total volume. At 10% rice hulls, the clay soil more than doubled (120% increase) in total expanded volume (again, without compacting the soil, only with sifting and shaking in a plastic beaker to mimic turning and mixing in the field).

The expansion of the blended soil and rice hulls was influenced by the water retained in the soil. One of four laboratory soil samples analyzed (sample 1) had the lowest expansion of net volume after blending with rice hulls but it was a dense clay soil that also had the highest moisture content. Even with a higher level of amendment, 10% rice hulls, soil sample 1 (27% moisture content) had expanded by 80% in net volume in contrast to more expansion with the drier reference soil also containing 10% rice hulls (net 120% expansion). The other two Site soil samples were intermediate with net volume expansions of approximately 110% when amended with 10% rice hulls

The CSU Chico laboratory data suggest that if soil moisture levels are controlled in the field to the near-optimal range of 12-16% water content, amending the contaminated soil with even 2.5% to 5% rice hulls could expand the soil and improve the circulation of oxygen dramatically.

At 5% rice hulls, soil samples 2 and 3 contained less moisture (17-18%) and, as a result, higher net volume expansions of 50 to 60%. to improve circulation of fresh air in the biopile soil – even in static soil piles lacking perforated pipe aeration. By reducing anaerobic zones in the stockpiled soil, the rice hulls amendment should increase the rate and extent of aerobic biodegradation of soil TPH contaminants. The effectiveness of the rice hulls amendments and soil expansion in the field would be magnified by reducing soil compression simply by constructing lower biopiles (e.g., 0.9-1.5 meters rather than 2.4 meters in height). By improving the passive aeration in combination with active flow of air from perforated pipe laterals, these biopiles can be engineered to optimize oxygen distribution in the biopiles, resulting in dramatically shorter biotreatment times.

Numerous laboratory soil microcosm studies performed by CytoCulture and the CSU Chico environmental microbiology laboratory have documented rapid biodegradation rates for high levels of non-volatile TPH contaminants once the soil condition, air penetration, moisture content and mass transfer blending have been optimized.

In August 2016, previously treated biopiles associated with the oil/water separator excavation (identified as Excavation 7) that still contained oily sands were re-treated using a combination of tilling, application of the bacteria/surfactant/water solution, and the amendment with dry rice hulls (approximately 10% rice hulls by volume). The amendment and tilling combination was very effective. Final confirmation results indicated treatment successes with TPH (diesel and oil) reductions ranging from 43% to 100% in less than 14 weeks.

Lessons learned from this case study include: (1) air emission considerations are critical for constructing the biopiles and SPS, (2) sufficient soil mixing and maintaining optimal moisture conditions for the inoculated soil is critical, (3) soil amendments such as rice hulls can improve oxygen circulation within the biopile, particularly if care is taken to avoid soil compression and (4) overall operational efficiencies related to soil handling and management can facilitate biotreatment rates, reduce costs and allow up to 99% of the excavated soil to be re-used as backfill on Site.

CONCLUSIONS

Aerobic soil bioremediation treatment times were reduced by improved mass transfer, optimized moisture content and distribution of air, and the introduction of selected strains of HC-degrading *Pseudomonas spp*. bacteria and a biodegradable non-ionic surfactant. Bioagumentation treatment times for heavily contaminated ex situ soils ranged from two to four months. Up to 96% of the 94,000 tons of soil that were treated by our SPS process ultimately met RWQCB the SCG's after a single treatment application. Most of the remaining 4% of the treated soil that did not initially achieve cleanup goals was successfully retreated to meet the SCG's and reuse on-Site. Less than 1% of the treated soil consisting of oil tar soil and clods of oily clay required off-Site disposal. BGR's on-Site, ex situ soil bioremediation process reduced TPH levels to meet RWQCB closure criteria at its source allowing for soil reuse at a significantly lower cost than traditional transportation and disposal (T&D) at a landfill while eliminating liabilities associated with T&D.

There was a significant community benefit of this approach: the number of trucks hauling contaminated soil from, and clean backfill to the Site was reduced by 99%, achieving the publicly-stated goal of minimizing impacts on the local neighborhoods that included noise, air emission, road degradation and safety concerns. BGR's patent-pending, sustainable "green" bioremediation process successfully integrates the technological elements of using highly selected hydrocarbon-degrading bacteria cultures with a compatible surfactant in conjunction with laboratory-based soil treatability studies and a pragmatic, cost effective engineering approach for field implementation.

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