Bioremediation Treatment Optimization Study: Oilfields in the Persian Gulf Area

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Background/Objectives. The project takes place in portions of the desert in the Persian Gulf area, where the soil is contaminated by total petroleum hydrocarbons (TPH). The contamination is present as a consequence of oil spills and fires, and it is therefore aged and scorched. The bioremediation treatment optimization study has the objective to demonstrate that this technology can meet the required remediation targets (1%) and thus be used to remove TPH contamination between 1 and 5%. The study also aims at optimizing the remediation efficiency and designing the layout of the full-scale bioremediation plant. Metagenomic analysis combined with an innovative pilot plant are applied in this project to be able to identify the bacterial community of the soil from the site, pinpoint the species responsible for the biodegradation of TPH and follow their progress during the remediation and under different process conditions.

Approach/Activities. The project includes two phases: laboratory tests and field tests. The first phase consists of analyses on soil samples from the site and small-scale bioremediation tests (mesocosms). A physical-chemical characterization (micro- and macroaggregates, nutrients, salinity, carbon content) of the soils and of the contamination is performed to better understand the state of the aged contamination (mostly long chain hydrocarbons, >C40) and how it is bound to the soil particles. This information is particularly important in order to understand how to amend the soils with substances capable of making the contamination more bioavailable and thus biodegradable. The microbiological features of the soils are also analyzed, by means of metagenomics. Microorganisms colonizing the contaminated soils are identified and their capacity to transform the contaminants are predicted by functional genomic analysis. Mesocosms tests are set up to compare the degradation efficiency of biostimulation and bioaugmentation approaches, using both autochthonous inocula and commercially available products. Metagenomic analyses are performed on the samples taken at different time points along the duration of the mesocosms, to highlight the species and microbial communities responsible for the degradation processes. The mesocosm tests and the metagenomic analyses highlighted the most promising amendment protocols, the extent to which the contaminants can be biodegraded, and the microorganisms involved in the process.

The second phase of the project focuses on testing the most viable amendment protocols selected in lab scale at field scale (6 – 30 m^3 soil biopiles). For this phase, a mobile field laboratory for routine soil monitoring and microbiological analysis is installed at the treatment area, as well as a batch fermenter to produce the autochthonous culture needed within this test phase. Additionally, as the final part of the field testing phase, the selected treatment protocol is further examined and tested using an innovative pilot testing plant, named RoboNova®. This plant allows to further screen the efficacy of the selected treatment protocol and to adjust and modify in real time the settings of the process, if needed. The aim is to analyze all variables affecting the bioremediation efficacy at real scale, therefore optimizing the process and minimizing the risks of failure in full field scale remediation.

Finally, at the end of the optimization study, bioreactors for full-field scale are designed and commissioned in the treatment area for production of autochthonous inoculant for bioaugmentation purposes.

Results/Lessons Learned. Soil samples from the treatment area were analyzed for their physio-chemical properties (soil type, TPH, salinity, pH, nitrogen and phosphorus compounds, etc.), establishing the baseline conditions present at the site. Metagenomic analysis has produced data highlighting the dominant taxonomies in the soils as well as their contribution to the pool of functional genes coding for enzymes involved in the degradation pathways of hydrocarbons. The dominant bacteria species have been found to be *Pseudomonas* (39%), *Bacillus* (18%) and *Acinetobacter* (10%), known for their degradation abilities of hydrocarbons via enzymes such as Alkane 1-monoxygenase, Dye decolorizing peroxidase and catechol 1-2, dioxygenase.

An autochthonous culture was enriched from highly contaminated soil samples and used as inoculant in the mesocosms and field tests, along with two commercial formulations. Dominant species and enzymatic abilities of the enriched autochthonous culture were defined by metagenomic analysis. The same analyses were conducted on samples from different timepoints of the mesocosms tests, to better follow the evolution of the bacterial population after inoculation and during the degradation process. The inoculant culture was enriched for *Bacillus* (79%) and *Pseudomonas* (7%); the tests inoculated with this culture showed a faster and stronger speciation at T=2 months, shifting the bacterial population composition to actively degrade the hydrocarbons in the soil. RTC of 1% TPH was reached in 5 months, only in the test mesocosms inoculated with the autochthonous culture, with a final TPH (HEM) values of 0.3% from the initial concentration of 3.4% The successful treatment protocol was tested in field scale as well, obtaining similar results.

Finally, testing of the selected treatment protocol with the RoboNova® system is ongoing, while the full-scale treatment plant and bioreactors for inoculant preparation were designed and are in the process of being commissioned at the treatment area.