## Bioremediation of Coil Contaminated with Crude Oil by a Consortium Immobilized in Corn Stover

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Background/Objectives. Crude oil is considered as one of the main sources of energy in the world, and growth in population and industries increases the demand for more crude oil extraction. Crude oil spills resulting from processes like excavation and transportation, this can cause intensive damage to living organisms and result in changes in the environment. During the past decade, bioremediation of petroleum contaminated soil has been a hot issue in environmental research, and many bioremediation strategies have been developed and improved to clean up soils polluted with petroleum and its derivatives. Indigenous microorganisms in the soil can degrade a wide range of hydrocarbons, though the complexity of different environment can cause abiotic and biotic stresses and a decrease of microbial viability in a short time, mainly if microorganisms are applied as free cell suspensions<sup>2</sup>. Several studies have evidenced that different microbial immobilization strategies can provide a protector niche for microorganisms applied in bioremediation processes. Microbial immobilization favors a decrease of toxic compound concentrations in the cellular microenvironment, protects against depredation and competition, and improves the access to nutrients. In this work, we evaluate the contribution of a mixed microbial consortium immobilized in corn Stover and stored for 24 months in the degradation of TPHs in a contaminated soil with crude oil by bioaugmentation and biostimulation systems and its effect on the native soil microbiota.

**Approach/Activities.** Microbial consortium was formed by strains of *Aspergillus flavus*, *Aspergillus nomius, Thrichoderma sperellum, Bacillus cerus, Pseudomonas aeruginosa, Klebsiela pneumonia, Kelebsiela sp.*, and *Stenothrophomonas maltophilia*; these strains were immobilized by adsorption technique in corn stover 24 months ago. The evaluation was done at laboratory scale with biopiles of 1 kg of contaminated soil for 30 d. The treatments were: a) Bioestimulation + bioaugmentation of consortium (immobilized in corn stover), b) Bioestimulation + bioaugmentation (free cells), c) Bioestimulation of soil native microbiota, d) Natural Attenuation. The humidity was adjusted at 30% in all treatments, for bioestimulation+ bioaugmentation treatment was used sterile corn stover as a texturizer in a relation 95:3.5:1.5 (soil: sterile corn stover: immobilized consortium). This evaluation was in soil collected from a zone of crude oil extraction located in El Salitral, Veracruz, México. The measurement parameters were pH, colony forming units (CFU) and degradation of total petroleum hydrocarbons (TPHs).

**Results/Lessons Learned.** Growth of the immobilized consortium was obtained in biopiles with soil contaminated with crude oil, reaching a maximum viable account with a magnitude of  $10^8$  CFU/g initial wet material (IWM) during the degradation process and was maintained for 30 d. The bacteria (3.08 x10<sup>8</sup> CFU/g IWM) were more prevalent than fungi (5.5 x 10<sup>5</sup> CFU/g of IWM) in the kinetic with corn immobilized at 30 d. Respect to degradation, the most efficient treatment was bioestimulation + bioaugmentation (with immobilized consortium) because was it able to remove 48% of TPHs than bioestimulation + bioaugmentation treatment (inoculated with free cells) that only removed 22.9% and bioestimulation treatment (12%) in soil contaminated with crude oil. This shows that the immobilization processes increase the survival of microorganisms and favor the degradation of hydrocarbons.