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Molecular Responses of Soil Microbial Communities under Bioaugmentation Process

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Extended Abstract

The aim of study was to assess the changes in the structure of total and hydrocarbon-degrading bacterial communities in soil contaminated with hydrocarbons subjected to bioaugmentation process. Additionally, the changes in the total bacterial number and number of bacteria with potential to degrade aliphatic hydrocarbons in soil during bioaugmentation experiment were studied. The petroleum polluted soil used in the study was obtained from an industrial area located around refinery in Czechowice-Dziedzice (Southern Poland). The bioaugmentation experiment was carried out under laboratory conditions using bacterial strains isolated from hydrocarbon-contaminated soil. The experiment had a completely randomized block design with three replications that had four treatments: (1) soil inoculated with strain *Bacillus subtilis* T'-1, (2) soil inoculated with strain *Pseudomonas* sp. P-1, (3) soil inoculated with mixture of T'-1 and P-1 strains and (4) control soil treated with sterile saline solution instead of bacterial suspension. Contaminated soil was placed into pots and then the bacterial solutions of T'-1, P-1 or T'-1+P-1 strains were poured into soil to reach the number of 10^7 bacterial cells g^{-1} dry weight of soil. The pots were incubated for 91 days at room temperature. The total petroleum hydrocarbon concentration in soil was quantified before and after bioremediation study as hydrocarbons with carbon number between 10 and 40 (TPH_{C10-40}) following ISO 16703:2011 standard. The impact of introduced bacteria on the structure of total and hydrocarbon degrading bacterial communities in bioaugmented soil was determined using denaturing gradient gel electrophoresis (DGGE) method. For the quantification of 16S rRNA and *alkB* gene copies real-time PCR was used.

After bioaugmentation process a significant ($P<0.05$) decrease in the TPH content was reported in all soils inoculated with bacteria. Inoculation of soil with the consortium of strains resulted in 3 times greater TPH removal compared to soils inoculated with single strains. Analysis of 16S rRNA and *alkB* genes-based DGGE fingerprints showed that profiles of soils bioaugmented with T'-1, P-1 or their mixture were similar to patterns obtained for non-inoculated soil at 1, 7, 42 and 91 day of the experiment. Quantification of the 16S rRNA gene copies showed significantly ($P<0.05$) higher content of this gene in soils inoculated with bacteria (T'-1, P-1, T'-1+P-1) on days 1 and 7. In turn, the number of copies of *alkB* gene was statistically ($P<0.05$) higher in bioaugmented soil, compared to control soil, during whole experimental period.

Results obtained in this study showed that introduction of T'-1, P-1 and T'-1+P-1 strains into soil contaminated with hydrocarbons did not change the genetic diversity of tested communities however affected number of total bacteria and bacteria with potential to degrade aliphatic hydrocarbons

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