

Microbial Toluene Utilization Capability in Petroleum Waste Contaminated Soil

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BTEX (Benzene, toluene, ethylbenzene, and xylene) compounds are volatile monoaromatic hydrocarbons common in crude petroleum and petroleum products. Petroleum hydrocarbon contaminated soil microflora was qualitatively and quantitatively examined for its toluene removal and degradation capability. Petroleum hydrocarbon contaminated soil was collected from a fuel filling station in Kiribathgoda, Sri Lanka. The soil suspension was prepared. Flask with Bushnell Haas broth (9.00 mL) with toluene 20% (v/v) was incubated for one day to get equilibrated with toluene, and soil suspension (1.00 mL) was transferred to the flask. Flasks were sealed with rubber silicone septa and paraffin tapes and incubated at 100 rpm, 30 °C. Soil suspension was enriched in 3 stages as 1st, 2nd and 3rd by providing toluene as the whole carbon source. Headspace air (1.00 mL) of each enrichment sample was withdrawn after one, four, and seven days and manually dissolved in dichloromethane (2.00 mL) and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). After seven days, the number of toluene degrading bacteria in each enrichment was isolated using BHA, and plates were incubated at 30 °C for seven days with a toluene containing filter pad. The toluene reduction percentages were calculated compared to the initial toluene level in the headspace of the 1st, 2nd, and 3rd enrichments; it revealed 54.88%, 54.42%, and 79.36% toluene reduction within three days. After six days, 1.76×10^3 , 6.68×10^5 , 1.57×10^6 CFU/mL of bacteria from 1st, 2nd, and 3rd enrichments reduced the initial toluene amount by 67.87%, 45.70%, and 80.94%, respectively. During the enrichment, the number of bacteria present in the suspensions and the toluene utilization amount by bacteria increased. Five morphologically distinct bacterial colonies were isolated. According to the results, headspace toluene reduction pattern is obtained, which may be helpful for the development of novel environmentally friendly VOC bioremediation methodologies.

Keywords: Toluene, BETX, Bacterial degradation, Petroleum contaminants

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