USE OF KENGRO BIOSORB WITH AND WITHOUT FUNGUS FOR REMEDIATION OF CRUDE OIL CONTAMINATION

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ABSTRACT

A ninety-day laboratory study was conducted to determine if KenGro Biosorb with and without an oil degrading fungus, Cladosporium sp., could enhance the degradation of Total Petroleum Hydrocarbons (TPH) in sand contaminated with 2% crude oil. The study consisted of contaminated sand with no additives (control), contaminated sand amended with 3% KenGro Biosorb, and contaminated sand amended with 3% KenGro Biosorb inoculated with Cladosporium sp. The three replicates of each treatment were monitored twice a week for aeration and moisture adjustment and maintained at 24∞C. Samples were taken at 30-day intervals for TPH analysis, microorganism counts, and toxicity characteristic leaching potential. A significant reduction of TPH occurred for all the treatments by 30 days. By the end of the study, KenGro Biosorb and KenGro Biosorb inoculated treatments contained 50% less TPH than controls. Microorganism populations increased significantly by day 60 for all treatments. No significant reduction of Toxicity Characteristic Leaching Procedure (TCLP) occurred by 90 days.

INTRODUCTION

Bioremediation has been accepted as a viable soil remediation technology for many classes of organic pollutants. It has been shown that biological degradation of pollutants such as crude oil in the environment could be limited by the availability of nutrients and that degradation of crude oil could be stimulated by addition of soil additives to oil contaminated beaches (1,4,5). KenGro Biosorb, an experimental absorbent, has shown promise as a soil amendment (7). It is a specialty fiber crop with excellent oil sorbency capabilities, high protein content, and large populations of indigenous microorganisms. The porous nature of KenGro Biosorb not only enhances adsorption, but also provides greater contact between target pollutants and microorganisms. In some cases, microorganisms capable of degrading pollutants are not present in soil. Addition of Cladosporium sp., a fungus shown to be effective in degrading organic contaminants in field and laboratory studies, could be a logical choice for use to remediate petroleum contaminated soil (2,7). The objective of this study was to determine if KenGro Biosorb with and without the oil degrading fungus Cladosporium sp. can enhance the degradation of total petroleum hydrocarbons.

METHODS

Eighteen-hundred g of clean sand was contaminated with 36 g of Iranian crude oil to a 2% concentration level. The contaminated sand was thoroughly hand-mixed then put in a 3-L jar and placed on a rolling ball mill for two hours. The mixed sand then was placed in nine 1.5 L amber glass dishes (200 g/dish). Three of the dishes were amended with 3% (6 g) KenGro Biosorb, three were amended with 3% (6 g) KenGro Biosorb and 30 mL of Cladosporium sp, and the remaining three dishes with no additives were used as controls. Dishes containing KenGro Biosorb, and inoculated KenGro Biosorb were thoroughly hand-mixed again. Thirty mL of deionized water was added to the control and KenGro Biosorb dishes to provide the same level of moisture (15%) as fungal inoculated dishes. The fungus, Cladosporium sp., was isolated from a superfund site in Weed, California. The fungus was grown on potato dextrose broth for two weeks before it was filtered and suspended in sterile deionized water for use. Dishes were monitored twice a week for moisture adjustment and aeration. Fungal suspension was added to fungus treatment dishes at sixty days. Samples were taken at 30-day intervals for TPH and microcount measurements. TCLP analysis was done on days 0 and 90 only. Sand samples were extracted by EPA Method 3540 (soxhlet extraction) (8). TPH concentrations were measured by modified standard Method 5520-F (3). The TCLP was determined using a modified version of EPA TCLP procedure (6). Dilution plate technique was used to estimate bacterial and fungal populations. Nutrient agar (Difco Lab., Detroit, Michigan) media for bacteria was made by adding 23 g of nutrient agar to one L of deionized water. The solution was autoclaved at 120∞C for 20 minutes then allowed to cool to 60∞C. Seventeen mL of media solution was added to each plate by a Wheaton Unispense II (Wheaton Instruments, Millville, NJ) and placed under a laminar flow hood to solidify. The media used for fungal counts was potato dextrose agar with antibiotics (Difco Lab., Detroit, Michigan). Thirty-nine g of potato dextrose agar was added to one L of deionized water, autoclaved, 30 mg chlorotetracycline and 120 mg of streptomycin sulfate dissolved in water was sterile filtered into cooled media, and then plates were poured as previously described.

RESULTS AND DISCUSSION

Total petroleum hydrocarbon (TPH) results are summarized in Figure I. All treatments showed a significant reduction of TPH after 30 days, however, by day 60 KenGro Biosorb and KenGro Biosorb inoculated fungus treatments showed significantly lower TPH concentration than control. By the end of the study this reduction of TPH was more than 50% in comparison with control. Bacterial and fungal enumerations are summarized in Tables I and II. A significant increase of bacterial and fungal populations occurred after 30 days for all treatments due to increased aeration and moisture. Populations of microorganisms decreased by the end of the study probably due to depletion of the simple compounds as well as no fungal additions after day 60. No significant reductions of TLCP among treatments were observed by 90 days (Figure II). This could be due to the high level of TPH still left in the sand and also the sandy nature of the contaminated materials.

Results of this study revealed that addition of KenGro Biosorb to sandy soil contaminated with crude oil with or without added microorganisms could enhance biodegradation of total petroleum hydrocarbons in laboratory studies. However, a pilot-scale study will be needed to further confirm these results.

REFERENCES

1. Atlas, R.M., and R. Bartha. 1972. Degradation and mineralization of petroleum in seawater: Limitation by nitrogen and phosphorus. Biotechnol. Bioeng. 14:309-317.

2. Borazjani, H., D.A. Strobel, C. Templeton, J. Wagner, M. Hannigan, F. Hendrix, M. Welch, and L. Prewitt. 1991. Application of four bacterial and fungal species for bioremediation of selected polycyclic aromatic hydrocarbons and pentachlorophenol. In: Proc., 1st Annual Hazmat Conference South, October 2-4, 1991. Atlanta, GA., pp. 217-240.

3. Clesceri, L.S., A.E. Greenburg, and R.R. Trussell. 1989. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC, pp. 5-47.

4. Colwell, R.R., and J.D. Walker. 1977. Ecological aspects of microbial degradation of petroleum in the marine environment. Critical Reviews Microbiology 5:423-445.

5. Croft, B.C., R.P.J. Swannell, A.L. Grant, and K. Lee. 1995. Effect of bioremediation agents on oil biodegradation in medium-fine sand. In: Applied Bioremediation of Petroleum Hydrocans. R.E. Hinchee and J.A. Kittle (eds.), Battelle Press, pp. 423-434.

6. Federal Register. 1986. Appendix II - Toxicity Characteristic Leaching Procedure (TCLP). 51(114):21685-21688.

7. Hurt, K., H. Borazjani, S.V. Diehl, M. Hannigan. 1998. Biopiling of creosote contaminated soil. In: Global Environmental Biotechnology. D.L. Wise (ed.), Elsevier Press. pp.677-687.

8. Prince, R.C. 1993. Petroleum spill bioremediation in marine environments. Critical Reviews Microbiology 19:217-242.

9. U.S. Environmental Protection Agency. 1986. Test methods for evaluation solid wastes. SW-846, 3rd Edition.

Table I. Bacterial Population in colonies/g.

Treatment	Day 0	Day 30	Day 60	Day 90
Control	23×10^3	1.5×10^6	2.5×10^6	33×10^4
KenGro Biosorb	40×10^3	15×10^6	30×10^6	3.5×10^6
Biosorb + Fungi	78×10^4	33×10^6	19×10^6	1.1×10^6

Each figure represents an average of 3 replicates.

Table II. Fungal Population in colonies/g.

Treatment	Day 0	Day 30	Day 60	Day 90
Control	20×10^3	70×10^3	63×10^3	9×10^3
KenGro Biosorb	3×10^3	33×10^4	1.2×10^6	42×10^3
Biosorb + Fungi	47×10^4	2.6×10^6	50×10^3	20×10^3

Each figure represents an average of 3 replicates.

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