BIOTECHNOLOGY APPROACH TO BIOREMEDIATION

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Introduction

The contamination of soil and water by wood-treating chemicals is a growing environmental concern in Mississippi and most of the southeastern United States. Two very common components of organic wood-treating wastes are polycyclic aromatic hydrocarbons (PAHs) and pentachlorophenol (PCP). From an economical standpoint, cleaning up the contaminated soil or water is very costly; however, from a biological standpoint, ignoring contamination could prove to be even more costly.

The application of adapted, microbial cultures to polluted areas is demonstrated to be an efficient and economical method for bioremediation of chemical wastes. Specific microorganisms decompose molecules such as phenol, naphthalene, anthracene, and other aromatic hydrocarbons (5, 6, 7). Apparently, the dominant microbial group concerned in the mineralization of compounds of these types is bacteria species such as Pseudomonas, Nocardia, Mycobacterium, Acinetobacter, Arthrobacter, and Bacillus (3, 4, 5, 6, 7, 9, 10, 12). Fungi and actinomycetes may also participate in the breakdown of polycyclic aromatic hydrocarbons (PAH) and chlorinated phenols. For example, Amorphotheca sp. or creosote fungus has attracted much attention in the last two decades because of its occurrence in petroleum products, aviation kerosene, creosote wood, wood treated with copper-chromium, or arsenic and similar substrates.

Objectives

The objectives of this study are three fold:

1) To use two different biological systems to evaluate the biodegradation rate of PAH and PCP in contaminated process water.

2) To use a bacterial system to determine the biodegradation rate of PCP and PAH in highly contaminated slurry condition.

3) To determine the effectiveness of a fungus in biodegradation of PAH in contaminated soil.

Procedures

Process water study

A bacterium isolated from Joplin processed water (JP-bacterium) was cultured in liquid medium (nutrient broth) (Difco Laboratories, Detroit, Michigan). Also, a fungus, Cladosporium sp., was isolated from contaminated soil in Weed, California, and was cultured in liquid medium (potato dextrose broth) (Difco Laboratories, Detroit, Michigan). Twelve 250-ml flasks with cheese cloth stoppers were sterilized and a solution of Joplin processed water and deionized water was prepared and homogenized in a blender (1:1 ratio).

Four treatments with three replications were used in this study. Treatments were as follows: checks (no additive), bacterial solution added, bacterial solution and inorganic fertilizer added, and fungal solutions added. For checks, in each of three flasks, 50 ml of Joplin process water and 65 ml of deionized water were added. For the bacterial treatment, 50 ml of Joplin process water, 50 ml of deionized water, and 15 ml of bacterial solution were added to the three flasks. For the next treatment, 50 ml of Joplin process water, 40 ml of deionized water, 15 ml of bacterial solution, and 10 ml inorganic fertilizer (hydroponic 1 g/liter) were added to each flask. The remaining three flasks each contained 50 ml of Joplin process water, 50 ml of deionized water, and 15 ml of fungal solution (Cladosporium sp.). The flasks were placed on a wrist-action shaker for a 5-day period at room temperature. At the end of that time, a 5-ml sample was removed from each flask for microcounts. The remaining solution in each flask was extracted using a liquid-liquid extraction procedure (EPA method 3520). The results were analyzed using gas chromatography (EPA 8040, 8100).

Slurry Study

Eight 250-ml flasks with cheese cloth stoppers were sterilized and a solution of slurry mixture from southern Mississippi sites was prepared. One hundred twenty-five ml of slurry sample and 25 ml of

JP-bacterium solution were put into each flask. Six of the flasks were placed on a wrist-action shaker for 1-, 2-, and 3-week periods. The remaining two flasks were used for day 0 analysis. Every week, two flasks were taken off and samples were removed for microcounts and chemical analysis. For chemical analysis, samples were air-dried and soxhlet extracted (EPA 3540). The results were analyzed by using the same gas chromatography method.

Soil study

Contaminated soil used in this study was from the California site. Soil was air-dried and screened with a 2-mm sieve. One thousand two hundred g of screened soil was mixed with 60 g of straw and 60 g. of chicken manure. The mixture was then homogenized for 2 hours in a clean glass jar using a ball mill with 50 revolutions/minute. Two treatments with three replications were used in this study. Treatments were checks (no fungal addition) and loaded (fungal solution added). For checks, in each of three brown leaded pans, 200 g of mixture and 25 ml of sterilized deionized water were added to provide 12.5% moisture content. For fungal treatment, 200 g of mixture and 25 ml of fungal solution were added to each pan. Soil in each pan was mixed with a glass rod. A 30-g soil sample for chemical and microbial analysis was taken from each pan for day 0. Pans were stored at 22°C. Samples were monitored weekly for moisture adjustment and soil aeration. Thirty g soil samples were taken from each pan at 30-day intervals for 90 days. Soil samples were extracted and analyzed for PAH using the same method as with slurry samples.

Media Preparation and Colony Count

The media used for this study were nutrient agar (NA), 23 g in one liter of deionized water, NA amended with 5 mg/L of technical-grade pentachlorophenol (P) (Vulcan Materials Company, Wichita, Kansas), NA amended with 20 mg/L of whole creosote (C), NA amended with a combination of 5 mg/L of pentachlorophenol and 20 mg/L of whole creosote (P + C), potato dextrose agar (PDA), 39 g in one liter of deionized water, PDA amended with antibiotics (PDAA), and 120 mg/L of streptomycin sulfate (Nutritional Biochemical, Cleveland, Ohio) and 30 mg/L of chlortetracycline (JCN Biochemicals, Cleveland, Ohio). The NA and PDA were autoclaved for 20 minutes at 15 psi and 121°C and then cooled to 55°C. Both creosote and pentachlorophenol were dissolved thoroughly in methyl alcohol and added to cooled NA. The antibiotics were added to the cooled liquid medium before pouring into petri dishes. The pH of the media was adjusted to 6.9 to 7.1 before autoclaving. Twenty-five ml of Na, C, P, C + P, and PDAA was poured into disposable petri plates and were allowed to solidify.

For colony counts, triplicate samples of both loaded and non-loaded soils were air-dried for 24 to 28 hours under a sterilized transfer hood. The air-dried soil was then screened with a 400-mesh sieve. Serial dilutions were made by using sterilized screened soil. Three 20-mg soil samples were weighed out from treated and non-treated soil for each medium at each sampling date. The modified Anderson sampler (1, 2, 11) was used to distribute the soil on the agar. Three 20-mg samples were distributed over each medium for each treatment. Colonies were counted after 24 to 48 hours of incubation at 28°C. A Darkfield Quebec Colony Counter (AO Scientific Instruments, Keene, New Hampshire) was used to count the number of colonies on each plate.

The number of counts recovered on NA and PDA plates provided an estimate of the total number of bacteria per gram of dry soil. On creosote-containing plates, it represented the approximate number of bacteria per gram of dry soil that was acclimated to creosote; on PCP-containing plates, it represented the approximate number of bacteria per gram of dry soil that was acclimated to pentachlorophenol; on C + P plates, it represented the approximate number of bacteria per gram of dry soil that was acclimated to both creosote and pentachlorophenol; and on PDAA plates it represented the approximate number of fungi per gram of dry soil. Dilution plate procedure was used to recover microorganisms from process water.

Results

The effect of each experimental treatment on the degradation rate of PAH and PCP is summarized in Table 1. The 92% reduction in PAH for bacterial treatment and bacteria with inorganic fertilizer are very similar. The fungus treatment showed no degradation during the five-day period. PCP results also showed that the bacteria and bacteria with amended fertilizer have similar significant reduction rates of PCP. Fungus showed no improvement in degradation of PCP over the checks.

The bacterial and fungal population of this study are given in Table 2. The high number of bacteria acclimated, as well as non-acclimated for treatment with bacterial solutions and also for inorganic fertilizer, is directly correlated with the rate of biodegradation of Joplin process water. Fungus treatments showed no degradation quality in Joplin process water.

The results of the slurry study are summarized in Figure 1. JP-isolate biodegraded 58% of PAH and 67% of PCP in three weeks. The most interesting aspect of the slurry study was biological breakdown under extremely high levels of PAH and PCP. This result showed the potential application of this isolate for highly contaminated areas.

The results of PAH's degradation in the soil study are summarized in Table 3. The fungus treatment showed a good degradation of 56%. Considering the high level of PAH in the soil, this degradation is even more significant. The fungal population of the soil study is given in Table 4. There is a high degradation rate in thirty days due to the large population of fungi. As the population declines, the rate of breakdown also declines.

Conclusion

Based on the results of this experiment, highly contaminated water, soil, and slurry could be effectively bioremediated by using proper microorganisms and correct methods of treatment. This study also showed the effectiveness of bioaugmentation in increasing the biodegradation rate of chemicals in contaminated soil and water.

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Table 1: Effect of bacteria, fungi, and inorganic fertilizer treatments on degradation rate of Joplin process water PCP and PAH over a five-day period.*

Treatments	PAH (ppm)	% PAH reduction vs. avg. check	PCP (ppm)	% PCP reduction vs. avg. check
Bacteria	13,257	92	97.5	58.65
Bacteria and inorganic fertilizer	14,329	91	87.6	62.88
Fungus	181,000	--	269.3	--
Checks	163,000	--	236.0	--

* Each figure represents an average of three replications.

Table 2: Microbial population of Joplin process water treated with bacteria, fungi, and inorganic fertilizer over a five-day period (colonies/ml).

	Types of media*				
Treatments	PDAA	C	P	C + P	NA
Bacteria	0	35×10^6	44×10^6	33×10^6	42×10^6
Bacteria and inorganic fertilizer	0	42×10^6	51×10^6	40×10^6	45×10^6
Fungus	30,000	315,000	83,500	253,000	382,000
Checks	0	900	674	620	1,200

* Each figure represents an average of three replications.

Table 3: Effects of fungal treatment on degradation rate of PAH in soil study over a ninety-day period.*

	Polycyclic Aromatic Hydrocarbons (ppm)				
Treatment	Day 0	Day 30	Day 60	Day 90	% reduction in 90 days
Fungus	34,941	18,985	22,898	15,364	56
Check	36,617	23,758	23,722	21,872	40

* Each figure for PAH represents an average of three replications.

Table 4: The fungal population of soil study over a ninety-day period.*

	Population (colonies/g of soil)			
Treatment	Day 0	Day 30	Day 60	Day 90
Fungus	3,500,000	300,000	70,000	40,000
Checks	28,000	64,666	63,000	17,000

* Each figure represents an average of three replications.

Fig 1. Effects of JP-isolate treatment on biological breakdown of PCP and PAH's of LSC slurry study