# ACCELERATED DEPLETION OF 2′,3,4 PCB CONGENER USING COMBINATIONS OF THE ALFALFA PLANT AND BACTERIAL SPECIES

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## ABSTRACT

We have studied 2′,3,4 PCB (polychlorinated biphenyl) degradation capability of two bacterial species with the alfalfa plant. These bacterial species, *Commamonas sp*. and *Rhodococcus sp.*, were obtained from Dr. J. Tiedje (Michigan State University, East Lansing, MI). Five treatments were set up in duplicate and included an uninoculated control of sterile soil spiked with 100 ppm of 2′,3,4 PCB (pure congener) and autoclaved soil samples spiked with 100 ppm of 2′,3,4 PCB inoculated with *Rhodococcus sp*. or *Commamonas sp.* with and without alfalfa plants. Complete PCB depletion, measured by HPLC and GC-MS, was observed with both bacterial species after four weeks incubation period. The PCB depletion was proportional to the bacterial growth measured by CFUs count, indicating a possible use of the pollutant by these microorganisms as a source of carbon and energy. PCB depletion from the spiked soil was significantly accelerated by 50% on the second week and 100% by the third week when *Commamonas sp.* and *Rhodococcus sp.* were combined with alfalfa plants. Our study showed that concomitant use of microorganisms and plants could result in a tremendous quantitative improvement of *in situ* PCB cleanup from spiked soil in a time-saving manner.

***Key words:*** *alfalfa, Commamonas sp., Rhodococcus sp., PCB depletion*

## INTRODUCTION

PCBs are a mixture of 209 chlorinatedbiphenyl congeners (forms) which are fat-soluble stable compounds. PCBs are used as capacitors, hydraulics, transformer fluids, and plasticizers in paint. Manufacturing and industrial use has contributed to extensive soil and water contamination (found in the food chain, most notably fish). Various physical and chemical processes are already being used to remediate contaminated sites. Most of the physico-chemical processes to decontaminate PCBs are very expensive. Bioremediation on the other hand uses bacterial or fungal species to "clean up" contaminated soil, which has been proved in some instances to be efficient and cost effective. Phyto- (or green plant-based) remediation is an innovative technology that utilizes the natural properties of plants to remediate hazardous waste sites. Phytoremediation alone, however, takes a long time for complete depletion. In this study, we are combining phyto- and bacterial remediation to clean spiked soil samples. The bacterial strains used in this study, *Commamonas testosteroni* VP44 and *Rhodococcus sp.*, were recombinant variants carrying specific genes for dehalogenation of chlorobenzoates (Tiedje et al., 1999).

## MATERIALS AND METHODS

### *Cultures, Soil, and Plants*

The bacterial strains *C. testosteroni* and *Rhodococcus sp.* were obtained from Dr. James M. Tiedje at Michigan State University. The alfalfa seeds were obtained from Dr. Peter van Berkum,

Soybean and Alfalfa (*Medicago sativa* cv. ARC) Research Laboratory, Agricultural Research Center, USDA, Beltsville, MD. PCB-free loamy soil, which was further sterilized for these studies, was obtained from the same USDA source.

### *Culture Media*

The organisms were grown on the mineral medium K1 (Zaitsev and Karasevich, 1985). Plant tests were performed in Leonard jars (Leonard, 1943) filled with vermiculite and soil.

### *Analytical Methods*

PCB depletion from the culture medium was monitored by gas chromatography (GC). The gas chromatograph used was a 5890 Hewlett Packard (HP) Series II equipped with an electron capture detector (ECD). Soil samples (1 g) were removed from each treatment and the PCB was extracted by shaking with 2 ml of hexane: acetone (50:50) containing 1 ppm of hexachlorocylohexane (HCH) as the internal standard for overnight. The extract was passed through a 0.2 µ filter before injection in the GC.

### *Plant Studies*

Plant studies were done in Leonard jars filled with autoclaved vermiculite only or added to 2′,3,4 PCB-spiked soil. The alfalfa seeds were surface-sterilized and rinsed in sterile water and hydrogen peroxide. Seeds were inoculated with 10 ml of one-day-old cultures. The jars were inoculated with 10 ml of an overnight grown *Commamonas sp.* or *Rhodococcus sp.* All plant experiments were carried out in a controlled growth chamber set at 30°C with a 16 hr fluorescent and incandescent light period. The soils were spiked using pure 2′,3,4 PCB dissolved in a mixture of hexane: acetone (50:50) to make a final concentration of 1 ppm/g of soil.

### *Colony Forming Units (CFU)*

CFU count was determined by plating serial dilutions on relevant tryptone soy agar (TSA) media after 24-48 hrs incubation at 30°C. Enumeration of 2′,3,4 PCB-degrading bacteria was performed by plating on minimal media containing 100 ppm of the pollutant.

## RESULTS AND DISCUSSION

The CFU counts of bacterial cells in this study showed a pattern of increased viable count over the first week when used alone or in combination with alfalfa plants. Decrease in CFU counts coincided with the complete depletion of the 2′,3,4 PCB from the culture media. Data not shown.

Summary of plant/microbe tests using spiked soil are given in Figure 1. It shows that at initial time (zero time) no decrease in 2′,3,4 PCB was noticed. These data, as presented, were obtained by GC. Subsequent confirmation of these results was also done by high performance liquid chromatography (HPLC) studies. Both quantitation techniques gave similar results. Histograms of results clearly show that *Rhodococcus sp*. and *Commamonas sp*., without alfalfa plants, take four weeks

to completely degrade PCB. This degradation is significantly accelerated by two weeks when alfalfa plants are grown with bacterial cells.

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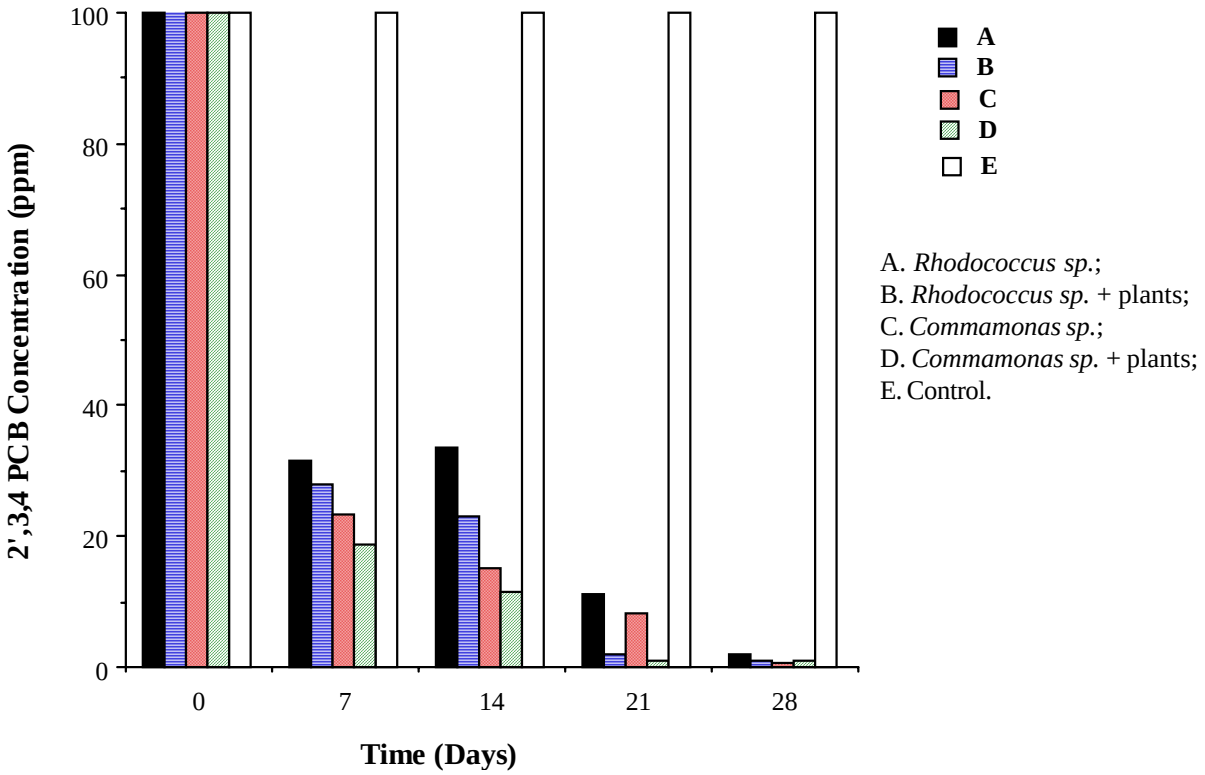


**Figure 1.** Composite data of several PCB-depletion tests shown as histograms.