Cometabolism of the Herbicide, 2,3,6-Trichlorobenzoate by Natural Microbial Populations

by R. S. HORVATH
Department of Biology
Bowling Green State University
Bowling Green, Ohio 43403

Cometabolism, the microbial oxidation of organic substances which fail to support growth of the microorganism, may be a means by which halogenated aromatic pesticides are degraded in nature. Oxidation of the herbicide, 2,3,6-trichlorobenzoate (2,3,6-TBA) by pure cultures of Brevibacterium sp. was shown to occur by a cometabolic process (1). Significantly, the product which accumulated as a result of this oxidation, 3,5-dichlorocatechol, was also subject to cometabolic attack by a pure culture of Achromobacter sp. (2,3).

These reports indicated that 2,3,6-TBA might undergo complete mineralization by a series of cometabolic reactions under mixed culture conditions. This study investigated the cometabolism of 2,3,6-TBA by microbial species present in water obtained from a small lake located on the campus of Bowling Green State University. In addition, enhancement of the rate of cometabolic oxidation of the herbicide was achieved by addition of a biodegradable analog of 2,3,6-TBA to the cometabolic system.

Materials and Methods

Cometabolism of the herbicide was allowed to proceed in 500 ml. lake water samples contained in 1-liter erlenmeyer flasks. Each study employed a sterilized water sample containing herbicide which served as an uninoculated control, a non-sterile sample containing 0.232 mg. herbicide per ml., and a water sample containing 0.232 mg. herbicide and 0.5 mg. sodium benzoate per ml. All flasks were incubated at ambient temperature without shaking.

Concentrations of 2,3,6-TBA were determined by gas chromatography. The gas chromatograph employed was a Varian Aerograph model 2740 equipped with hydrogen flame detector. A DC-200 column was used at a temperature of 200°C.

To prepare samples for analysis, 10 ml. aliquots were acidified with 2 ml. of 1 N HCl and extracted twice with 10 ml. ether. The combined ether phases were dried over anhydrous sodium sulfate, filtered and evaporated to dryness under a stream of nitrogen gas.

Bulletin of Environmental Contamination & Toxicology,
Vol. 7, No. 5, 1972, © by Springer-Verlag New York Inc.

One microliter of a solution containing the dried sample dissolved in 50 ul. BSA and 50 ul. CS_2 was injected into the gas chromatograph for analysis. Concentration of 2,3,6-TBA was computed by calculating peak area and referring this to known standards.

Thin layer chromatography employed Eastman Chromagram silica gel plastic sheets with fluorescent indicator. Benzene-dioxane-acetic acid (18:5:8) served as the solvent system. Chromatograms were developed to a height of 100 mm. and spots were detected under ultraviolet light.

Results

Because no significant differences were observed in either rate or total amount of oxidation of 2,3,6-TBA in three separate experiments, the average results of these three experiments are presented in Figure 1.

As seen in Figure 1, the water sample not enriched with benzoic acid yielded a total reduction in 2,3,6-TBA concentration of 35% in 18 days. No further oxidation of the herbicide occurred after this time.

Enrichment of the population for those microorganisms capable of oxidizing 2,3,6-TBA by addition of sodium benzoate resulted in this same reduction (35%) in only 4 days and a total oxidation of 80% in 14 days. Oxidation of the herbicide did not exceed this value with increased time.

Examination of samples by thin layer and gas chromatography did not reveal any chlorinated compounds other than 2,3,6-TBA indicating complete mineralization of the herbicide by the mixed culture system.

Enumeration of bacteria present showed no significant increase in numbers over the 20 day period when 2,3,6-TBA was supplied as the sole source of carbon and energy. Growth in flasks containing both the herbicide and benzoate did not exceed that occurring in flasks containing benzoate alone again showing an inability of bacteria present to grow at the expense of the chlorinated benzoate.

Discussion

The lack of microbial growth at the expense of 2,3,6-TBA might indicate a lack of microbial involvement in the oxidation of this compound. However, one important fact argues against this interpretation. The concentration of herbicide in the control flasks did not decrease during the course of the experiments establishing that breakdown of the compound did not occur by photochemical processes. In the absence of microbial life, oxidation of the herbicide did not occur.

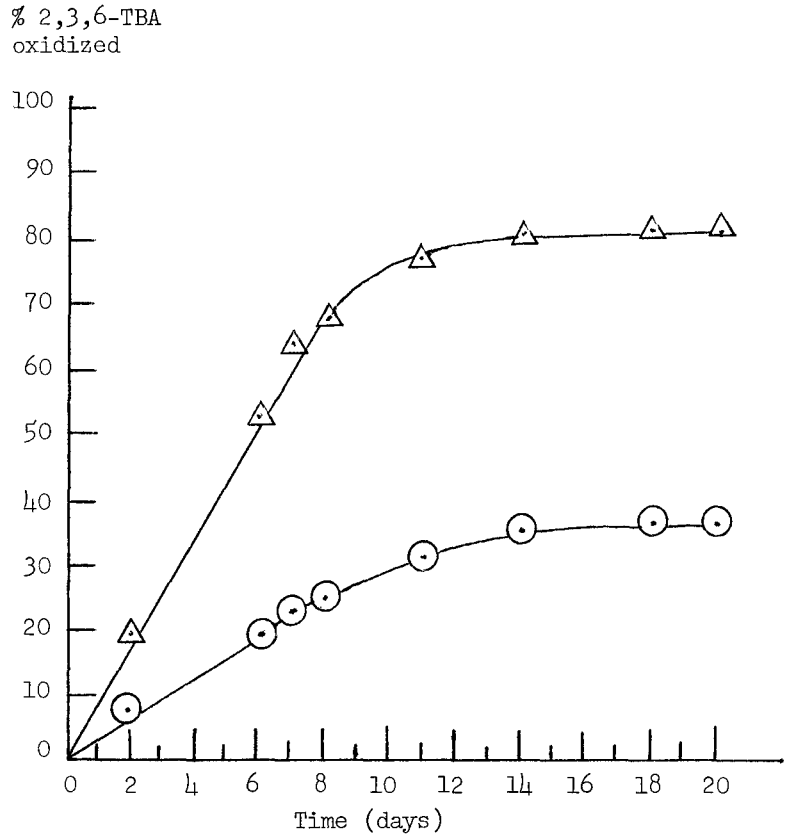

Figure 1. Cometabolism of 2,3,6-TBA in the presence of (△) and the absence of (⊙) sodium benzoate.

One is forced to conclude that this oxidation, in the absence of growth, occurred by the process of cometabolism. The fact that enrichment of the microbial population with sodium benzoate, a biodegradable simulant of the pesticide, increased both the rate and total amount of oxidation also indicated a cometabolic process. This is not surprising in view of the recent demonstration of cometabolism of this herbicide by pure cultures (1).

The inability to isolate any intermediate oxidation products is significant because it indicates that cometabolism can account for complete mineralization of 2,3,6-TBA under natural conditions. Dewey et al. (4) also noted oxidation of this herbicide by soil microorganisms but was unable to isolate intermediate products from the soil.

The failure of the microbial populations to oxidize 100% of the herbicide supplied may be the result of several factors. Undetected end products may have accumulated and produced a toxic environment to the cells. Also, depletion of some essential nutrient would account for the loss of activity. The system employed in this study did not truly simulate natural environmental conditions in that a closed system was used. A constant flow system, such as that employed in a chemostat, would more closely resemble a natural system and research in this area is necessary before definite conclusions can be drawn.

Nevertheless, this study does indicate that natural microbial populations are capable of degrading pesticides by the process of cometabolism. Furthermore, microorganisms capable of this cometabolic degradation can be enriched for by application of analogs of the pesticide to the microbial ecosystem.

This enrichment process may present a valuable technique to be used in the application of herbicides to natural ecosystems. Application of both the herbicide and a biodegradable analog of the herbicide may allow man to have both the benefit of action of the chlorinated aromatic pesticides and a rapid oxidation of the compound eliminating the environmental hazard which might otherwise accompany its use.

References

1. R. S. Horvath, J. Agr. Food Chem. 19, 291 (1971).

2. R. S. Horvath, Biochem. J. 119, 871 (1970).

3. R. S. Horvath, J. M. Duxbury, and M. Alexander, Bact. Proc., P. 9 (1970).

4. O. R. Dewey, R. V. Lindsay, and G. S. Hartley, Nature 195, 1232 (1962).