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Enzymatic Hydrolysis of Concentrated Diazinon in Soil

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Organophosphate pesticides applied to agricultural environments at recommended rates are not extremely persistent. However, if spills occur as a result of handling or transportation accidents, the high pesticide level in the contaminated soil will not degrade rapidly (WOLFE et al. 1973). In these instances, the pesticide active ingredient in the soil may be in the range of 1% to 5% and therefore, detoxification and clean up procedures are required. Microbial enzymes may be an effective way to achieve rapid and complete decontamination of high concentrations of pesticides in soil.

In previous research, an enzyme was obtained from a Pseudomonas sp. which could hydrolyze parathion, methyl parathion, dursban, diazinon, paraoxon, aminoparathion and several other methoxy- or ethoxy-substituted organophosphates (MUNNECKE 1976, 1980). An extension of this work led to the examination of enzymatic hydrolysis of diazinon in soil. In these experiments, diazinon, either in 25% EC formulations or as a technical grade chemical was enzymatically hydrolyzed in an agricultural sandy soil when present at concentrations up to 1%. This paper will discuss the methodology of such an enzymatic hydrolysis of diazinon, the parameters required for detoxification, and the rates of hydrolysis.

MATERIALS AND METHODS

Enzyme. Parathion hydrolase (EC 3.1.3) used in this study was derived from a Pseudomonas sp. grown in a 2000 liter fermentor by Bayer A.G. Wupertal, Germany. The cells were harvested in log phase by centrifugation, freeze-dried and stored at room temperature in a dry, powdered form. Crude enzyme extract was prepared by dissolving 0.5 g of the cell powder in 100 ml of 10mM Tris buffer (pH 8.5) containing 10 mM cobalt chloride and sonicating the cell suspensions for 15 sec/ml of solution using a 150 watt sonicator (Blackstone Ultrasonics Inc., Sheffield, PA). The activity of the crude enzyme extract was assayed by adding 0.1 ml of a diluted extract to 5 ml of 10 ppm parathion in 10 mM

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Tris buffer (pH 8.5). The rate of parathion hydrolysis was monitored by measuring p-nitrophenol production at 410 nm using a UV-vis Perkin-Elmer spectrophotometer. The rate of diazinon hydrolysis was determined by following the disappearance of diazinon gas chromatographically. The specific activity of this enzyme preparation for diazinon hydrolysis in 10 mM Tris buffer (pH 8.5; 30°C.) was 0.4 μmol/mg protein/min (0.4 units).

Soil studies. Twenty-grams of a sandy loam soil were placed into 250 ml Erlenmeyer flask, autoclaved twice, and then air dried at 120° C. The initial pH of the soil was 6.6, and 8 ml of water was required to obtain 100% saturation. Various amounts of either technical or formulated (25% EC) diazinon in 4 ml of acetone were added to each flask and thoroughly mixed into the soil. The acetone was evaporated from the soil before enzyme experiments were initiated. Final diazinon concentrations in the soil ranged from 125 ppm to 10,000 ppm.

To determine the optimal buffer strength required for the complete hydrolysis of diazinon in soils, the crude enzyme extract was diluted with sodium carbonate/bicarbonate buffer to final buffer strengths of 10 to 200 mM. Enzyme concentrations used in these experiments ranged from 1 to 150 mg/10 ml. Controls used in the above procedures included no enzyme in the carbonate buffer. The experiments were run at 37°C.

Extraction and analysis of diazinon. The concentration of diazinon in the 20 g soil samples was determined by first extracting with 40 ml of a 1:1 acetone:methanol solution. The contents of the flasks were shaken for 1 h at 200 rpm. This extract was then filtered and diazinon was extracted into benzene (1:1, soil extract:benzene) and assayed by gas chromatography (Packard model 878) using a glass column (6' x 1/4" O.D.) packed with 10% SP-2250 on 100/120 Supelcoport. The operating conditions were as follows: nitrogen (carrier gas), 65 ml/min; hydrogen, 20 ml/min; air, 40 ml/min; injector temperature, 230° C; column temperature, 220° C; detector temperature, 240° C. Under these conditions, the retention time for diazinon was 4 min. The extraction efficiencies for diazinon ranged from 94% to 103%.

RESULTS AND DISCUSSION

Initial studies indicated that the optimal pH of enzymatic hydrolysis of diazinon was 9.0 and enzymatic activity decreased rapidly as the pH dropped to 6.5. The specific enzyme activity towards diazinon was 0.44 μmoles diazinon hydrolyzed/mg protein/min. This was approximately 21% of the activity observed when parathion was the substrate at equal concentrations (10 ppm) under similar conditions. Because the soil used in this experiment was not alkaline, a carbonate/bicarbonate buffer was required to maintain appropriate pH. The amount of buffer required was dependent on the natural soil pH and the initial concentration of diazinon since as diazinon is hydrolyzed, 1 mole of 2-isopropyl-6-methyl-4-hydroxy-pyrimidine and diethyl thiosphosphoric acid are

produced per mole of substrate degraded. Thus, the amount of carbonate buffer required for complete diazinon hydrolysis was dependent on the initial amount of diazinon present. The results of these experiments indicate that approximately 20 mmoles of carbonate buffer are required for enzymatic hydrolysis of 2500 ppm (8.2 mmoles) of diazinon in this slightly acidic soil.

The surface temperature of soils can vary greatly throughout the day, thus effecting enzyme activity. However, parathion hydrolase displayed good activity throughout the temperature range of 12° to 50° C. Optimal activity occurred between 35° and 47° C. while 50% of the maximal activity was observed at 12° C.

The ability of parathion hydrolase to hydrolyze both technical and formulated diazinon is shown in Figure 1. In this experiment,

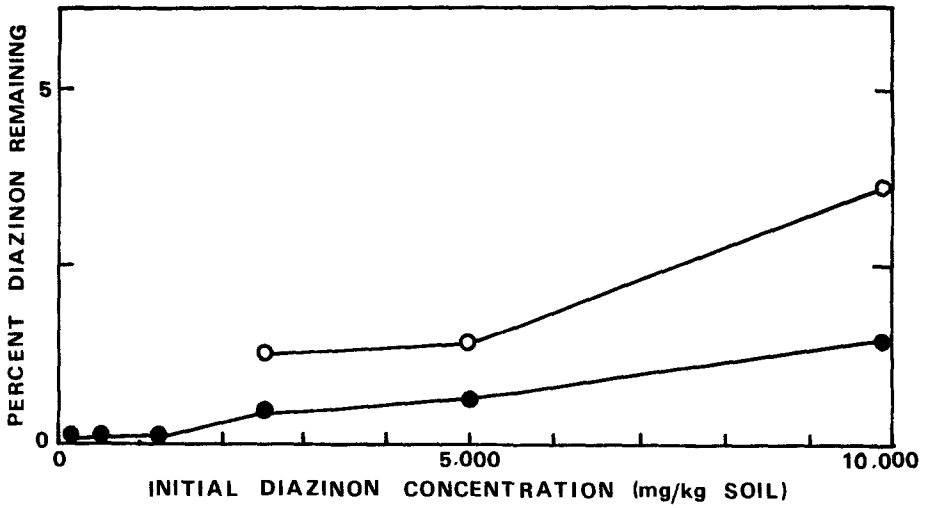

Figure 1. Enzymatic degradation of diazinon in soil after 24 hof incubation. (O) 25% E.C. formulated diazinon and (●) technical diazinon. Each flask contained 21 U of enzyme. Specific activity of the enzyme was 0.50 U/mg protein/min.

no effort was made to achieve complete enzymatic hydrolysis of diazinon in soil. Within 24 h, however, insignificant amounts (2 ppm or less) of pesticide remained in soils which initially contained less than 1250 ppm and 98% of the formulated diazinon was hydrolyzed in soils which initially contained 10,000 ppm. The hydrolysis of technical diazinon was only slightly slower (96% of 10,000 ppm degraded in 24 h). This residual amount could be further reduced if the experiments were run for a longer time before extraction. The time course of enzymatic hydrolysis of diazinon is shown in Figure 2. As the substrate concentration was reduced by hydrolysis, the rate of enzymatic reaction decreased. More than 75% of the initial 2500 ppm diazinon was degraded in the first hour of incubation, while the remaining

25% took 7 h to be degraded. This is typical of enzyme kinetics and does not indicate any enzyme decay, inhibition due to metabolite build-up, or increased soil-pesticide absorption.

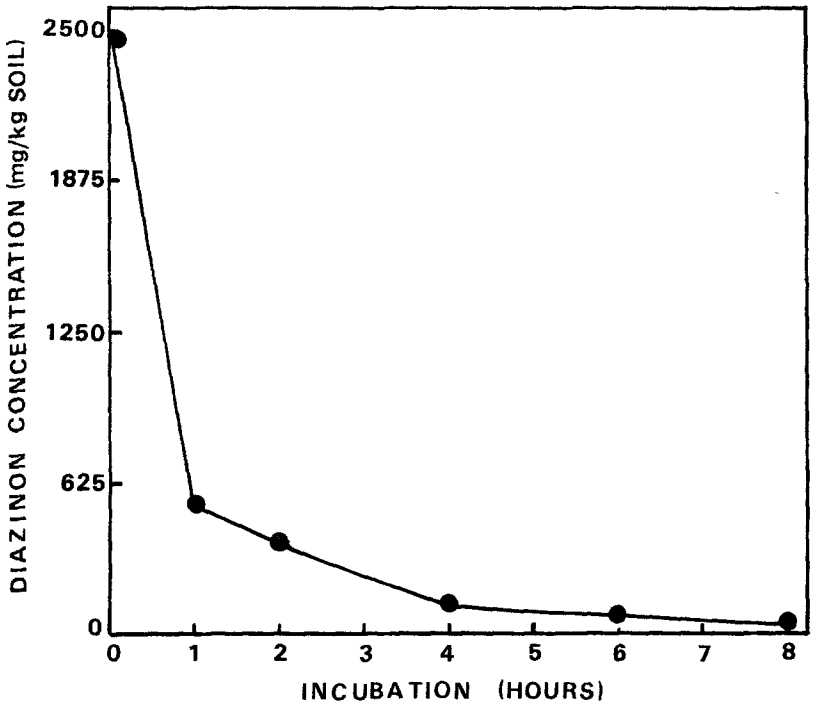

Figure 2. Kinetics of enzymatic degradation of diazinon in soil. Enzyme (150 mg) was added to 20 g soil samples and incubated at 37° C. The specific activity of the enzyme was 0.44 U/mg protein/min.

The effect of the enzyme concentration on diazinon hydrolysis in soil is shown in Figure 3. These experiments were allowed to run for 9 h and indicated that the degradation rate was approximately proportional to enzyme concentration up to 12 units per 20 g soil. This indicates that the initial rate of diazinon degradation is directly dependent on enzyme concentration and not on chemical or physical parameters of the soil-pesticide interactions. A kilogram of soil containing 2500 ppm diazinon would therefore require 500 units of enzyme activity (1 g of protein) for complete degradation within 24 h. For this Pseudomonas sp., approximately 2.5 g of protein can be produced in 1 liter of fermentation broth.

CONCLUSIONS

Our work demonstrates that a bacterial enzyme can hydrolyze diazinon in soil. Previous work has shown that parathion can also by hydrolyzed by this enzyme (FISCHER et al. 1980). More than 98% of 1% diazinon (4% diazinon, 25% EC) in soil can be removed within 24 h if sufficient buffer and enzyme are added to

the contaminated soil. This concentration of pesticide would normally remain in untreated soil for many years (WOLFE et al. 1973).

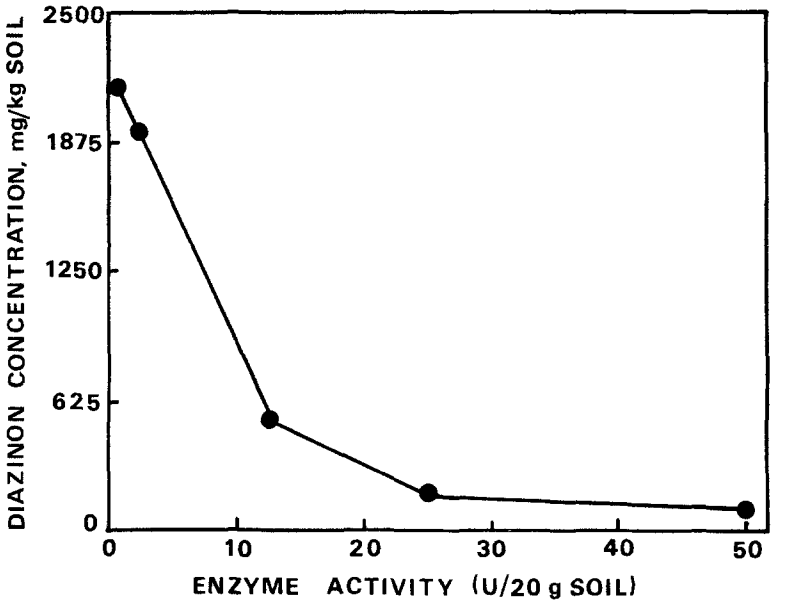

Figure 3. Effect of enzyme concentration on diazinon degradation in soil after 9 h of incubation at 37° C. Initial diazinon concentration was 2500 ppm and specific activity of the enzyme was 0.50 U/mg protein/min.

Although the enzyme was examined only in one soil, it is expected that it could also operate on cement or asphalt type surfaces as well as on synthetic polymers such as carpets. It is technically feasible to use parathion hydrolase to clean up spills of organophosphate pesticides in the environment and the cost of enzyme treatment seems realistic for treating emergency situations involving spills of concentrated pesticide solutions. Without strain improvement however, the treating of low levels of pesticides in agricultural soils would be cost prohibitive.

ACKNOWLEDGEMENTS

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REFERENCES

FISCHER, H.F., D.M. MUNNECKE and K.H. DOMSCH: Final Report, Mikrobielle bzw. enzymatische spaltung von parathion, BMFT. FB-T 80-046, Bonn. 54 (1980).

MUNNECKE, D.M.: Appl. Environ. Microbiol. 32, 7 (1976).

MUNNECKE, D.M.: J. Agr. Food Chem. 28, 105 (1980).

WOLFE, H.F., D.C. STAIFF, J.P. ARMSTRONG, and S.W. COMER: Bull. Environ. Contam. Toxicol. 10, 1 (1973).