Factors Affecting the Fate of Urea Peroxide Added to Soil

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Two important parameters which affect the degradation of petroleum hydrocarbons in soil are aeration and nutrients, particularly nitrogen (N). Urea peroxide provides both O and N upon catalysis by catalase and urease, respectively, in soil. Bryce et al. (1982) reported that soil aeration and plant growth were improved by the application of urea peroxide when added to the watering solution after flooding. Melsted et al. (1949) found that peroxide treatment or forced air improved soil aeration.

The combined application of one amendment for in situ bioremediation of hydrocarbons is favorable over several treatments. The addition of urea peroxide for in situ treatment has many advantages including the following: (i) \( \text{H}_2\text{NCONH}_4 \cdot \text{H}_2\text{O}_2 \) provides both aeration and N, (ii) urea peroxide is highly mobile, (iii) urea increases the stability of \( \text{H}_2\text{O}_2 \) (maintaining a uniform oxygen-releasing rate), (iv) urea peroxide is non-toxic at high concentrations, (v) it provides higher O concentrations compared to soil venting, (vi) it does not volatilize the pollutant and (vii) biofouling is not a problem as found in forced air systems.

Iron oxides and hydroxides can cause \( \text{H}_2\text{O}_2 \) to decompose before it reaches the intended plume of contamination. Urea serves as a stabilizer of \( \text{H}_2\text{O}_2 \) which is eventually released as \( \text{NH}_4^+ \). Urease (urea amidohydrolase, EC 3.5.1.5) catalyzes the hydrolysis of urea to carbon dioxide and ammonia (Florkin and Stotz, 1964). The enzyme is known to exist in both the intracellular and extracellular state in soil, protected by humus or clay colloids. It is well established that this enzyme exists deep within the soil profile. Urease is not significantly affected by the water level and can be active under air-dry conditions as well as under saturation (Bremner and Mulvaney, 1978). The enzymatic breakdown of urea peroxide is highly dependent on pH, substrate concentration, temperature and time. The objective of this investigation is to study these critical environmental factors which govern the fate of urea peroxide used in bioremediation of petroleum hydrocarbons.

MATERIALS AND METHODS

The soil used in this study was a surface (0 to 0.15 cm) sample contaminated with diesel fuel. The physical and chemical properties of this soil are as follows:
pH, 7.4; organic C, 1.2%; total petroleum hydrocarbons (EPA modified 8015), 2,200 mg kg\(^{-1}\); total N, 0.1%; NH\(_4\)-N, 12.2 mg kg\(^{-1}\); NO\(_3\)-N, 8.4 mg kg\(^{-1}\); clay, 21%, silt, 12% and sand, 67%. The field-moist soil sample (10 g on an oven dry basis) was placed in 8-oz (approximately 250-ml) French square bottles, treated with 2 ml of a 0.25% solution of urea peroxide (Sigma, St. Louis, MO) and incubated at 30°C for 24 h unless otherwise noted. The moisture content of the incubated soil was approximately 50% of the water holding capacity for all experiments except determination of pH\(_{opt}\), which was carried out under saturated conditions. Incubated soil samples were extracted with 100 mL of 2\(M\) KCl and the extracts thus obtained were analyzed for NH\(_4\)-N and NO\(_3\)-N (Keeney and Nelson, 1982). Nitrite-N was also analyzed using the method of Barnes and Folkard (1951) for each of the soil samples. However, NO\(_2\)-N was non-detected in this soil. Controls were performed on all soil samples to allow for determination of NH\(_4\)-N and NO\(_3\)-N not derived from the urea peroxide added. The procedure described for the urea peroxide-treated soil samples was followed to perform controls, but 2 mL of deionized water was added instead of the solution containing the urea peroxide.

Upon determination of the pH\(_{opt}\), 0.1 \(M\) THAM-H\(_2\)SO\(_4\) buffer was used at a ratio of 2:1 (buffer to soil). The buffer was made up by dissolving 12.2 g of tris (hydroxymethyl) aminomethane (THAM, Fisher certified reagent) in about 800 mL of water, adjusting the pH from 6.5 to 8.5 by titration with 0.2\(N\) H\(_2\)SO\(_4\) and diluting the solution with water to 1 liter.

The urea peroxide concentration was varied in another experiment to determine the dependence of NH\(_4\)-N and NO\(_3\)-N production. The stock solution of urea peroxide varied from 0, 0.010, 0.025, 0.050, 0.10, 0.25 and 0.50%.

Temperature of incubation was varied (10 to 50°C) to determine Q\(_{10}\) and temp\(_{opt}\) of the NH\(_4\)-N released. To determine temperature stability of soil urease in the breakdown of urea peroxide, soil samples were incubated at 40, 50, 60, 70, 80, 90 or 100°C for 48 h before the addition of urea peroxide by incubating the samples in the appropriate incubators. Afterwards, the soil sample was assayed for hydrolysis of urea cleaved from urea peroxide by the following assay: urea peroxide conc., 0.25%; temp, 30°C; time, 24 h.

All values reported are averages of triplicate determinations expressed on a moisture-free basis, moisture being determined from loss in weight after drying at 105°C for 24 hours.

The contaminated soil (25 g) was added to 8-oz French square bottles and subject to the following treatments: (i) sterilization (0.16 Mrad \(\gamma\)-irradiation from a \(^{60}\)Co source with 8 h of exposure); (ii) application of water to adjust the moisture level to 10% (wt/wt) (-33 kPa), and (iii) the application of urea peroxide (200 mg kg\(^{-1}\)) to the moist contaminated soil. The bottles were sealed with a screw cap and incubated at room temperature (23°C±2°C). At 2, 4, 6, and 8 weeks, 2 flasks per treatment (duplicates) were pulled from the pool of 24 flasks and analyzed for total petroleum hydrocarbons (TPH) by the method of EPA modified 8015.