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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_2 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) $H_2NCONH_4H_2O_2$ provides both aeration and N, (ii) urea peroxide is highly mobile, (iii) urea increases the stability of H_2O_2 (maintaining a uniform oxygen-releasing rate), (iv) urea peroxide is non-toxic at high concentrations, (v) it provides higher O_2 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 H_2O_2 to decompose before it reaches the intended plume of contamination. Urea serves as a stabilizer of H_2O_2 which is eventually released as 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_2SO_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_2SO_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 5O°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 γ-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.

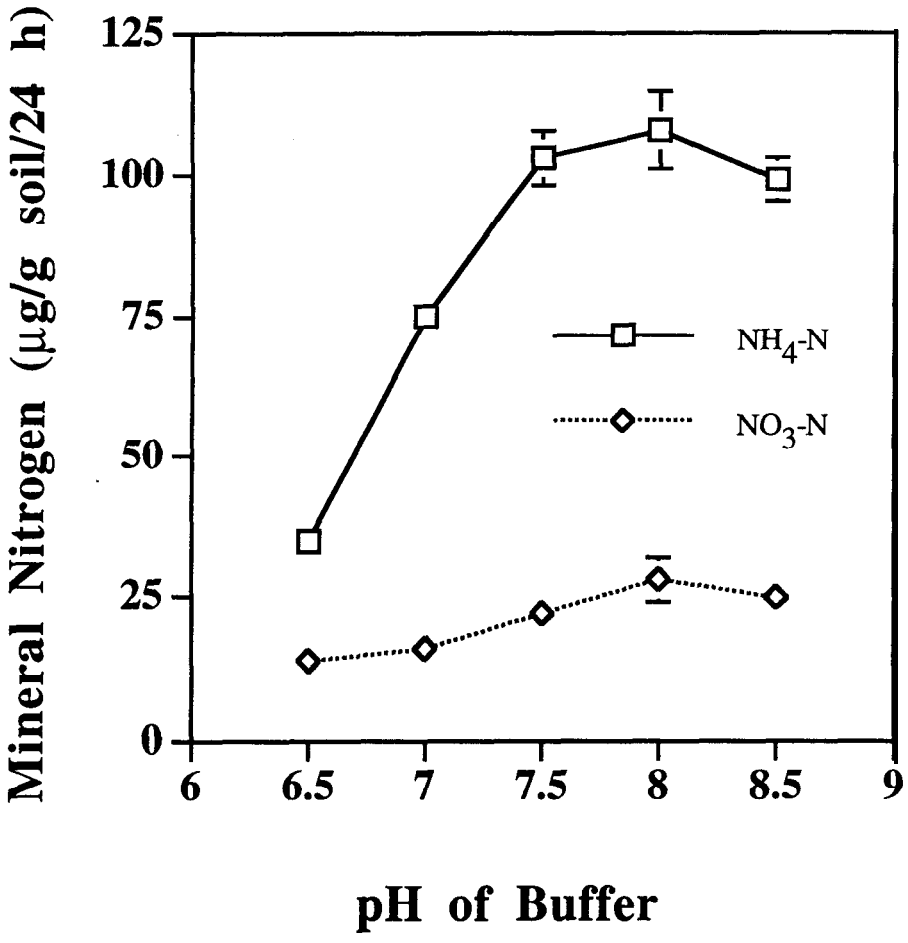

Figure 1. Effect of pH of buffer on the release of mineral nitrogen from urea peroxide added to soil.

RESULTS AND DISCUSSION

The pH_{opt} for hydrolysis of urea derived from urea peroxide was observed at pH 8.0 (Fig. 1). The release of NH_4-N was greatest at pH 7.5 to 8.5 over the pH range of 6.5 to 8.5. The pH of the reaction system affects the ionization groups of the enzyme-protein and can influence the ionization state of the substrate; thus, the affinity constant of the enzyme is influenced by the pH of the incubation medium (Frankenberger and Tabatabai, 1980). The reported pH_{opt} for NH_4-N derived from urea peroxide via soil urease is in good agreement wtth the Wall and Laidler (1953) study on the activity of jackbean urease in the presence of THAM (tris-H_2SO_4) buffer. Delaune and Patrick (1970) also noted the rate of conversion of urea into NH_4-N in a waterlogged soil adjusted at different pH values was highest at pH 8.0. However, Tabatabai and Bremner (1972) showed higher soil urease activity at pH 9.0 when assaying three Iowa soils.

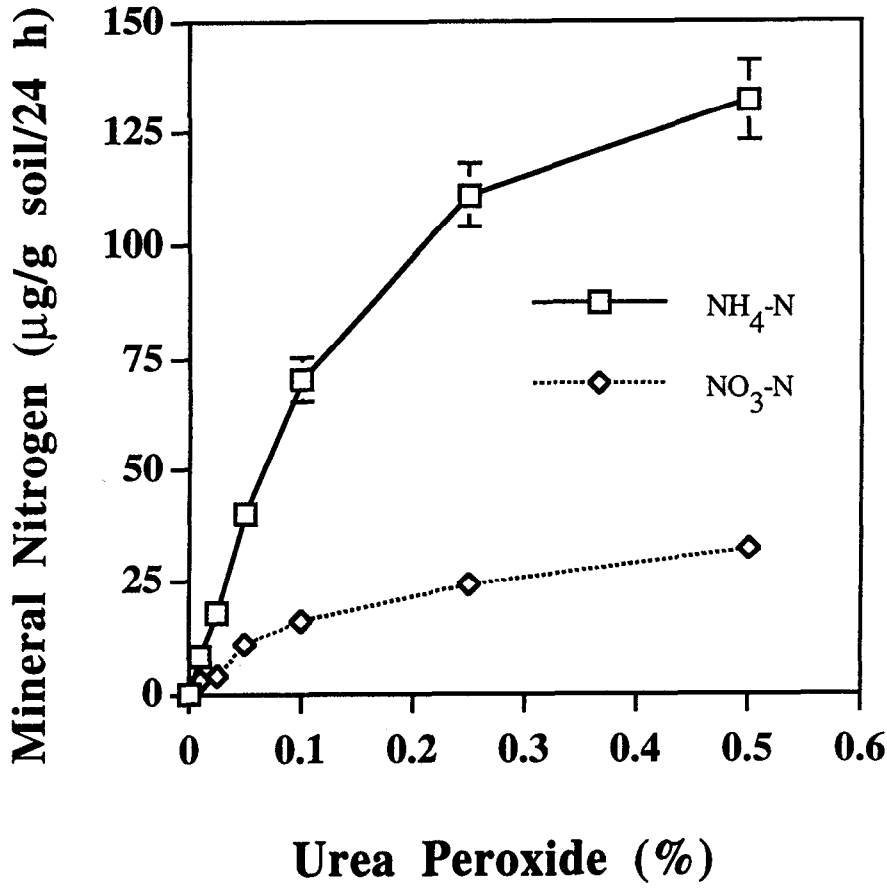

Figure 2. Effect of urea peroxide concentration on the release of mineral nitrogen in soil.

The buffer employed in this study was chosen based upon previous findings that WH_4-N released by soil N hydrolasis is considerably higher in the presence in tris-H_2SO_4 than in the presence of phosphate, citrate and modified universal buffer (Frankenberger and Tabatabai, 1980). Another reason for choosing this buffer is that no ammonium fixation in soils occurs in the presence of tris-H_2SO_4 buffer (Frankenberger and Tabatabai, 1980). Wall and Laidler (1953) reported that THAM buffer has no activating or inhibitory affect on the hydrolysis of urea by jackbean urease.

The dependence of NH_4-N and NO_3-N production upon the concentration of urea peroxide is shown in Fig. 2. At 0.25% urea peroxide, the NH_4-N released was approaching steady state, indicating that unease was saturated with the substrate, urea. It was evident that with increasing urea peroxide some of the NH_4^+ was oxidized to NO_3^-, particularly at the higher concentrations of urea peroxide.

The $temperature_{opt}$ for urea hydrolysis derived from urea peroxide was observed at 40°C with inhibition occurring at >40°C (Fig. 3). The temperature corresponds to

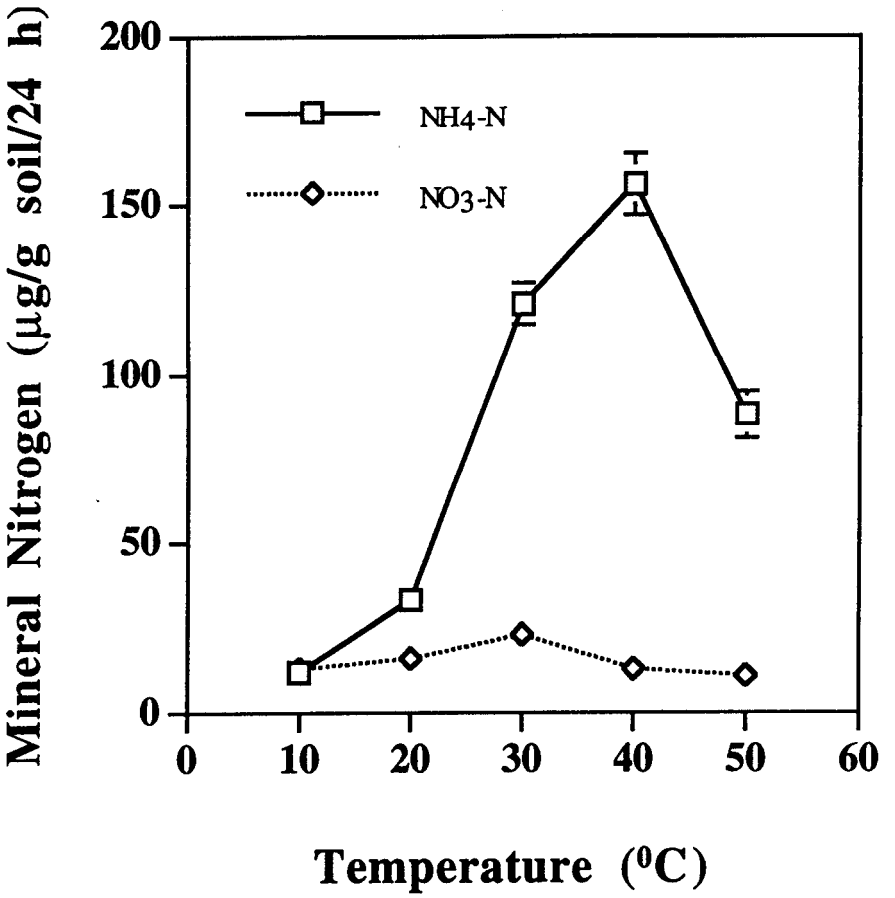

Figure 3. Effect of temperature on the release of mineral nitrogen from urea peroxide added to soil.

the standardized incubation temperature used to assay urease activity in Iowa soils (37°C) (Tabatabai and Bremner, 1972). The Q_{10} (temperature coefficient), a factor by which the rate constant is increased by raising the temperature 10°C was calculated at 2.56. Other studies have shown that urease activity in soils increases with increasing temperature from 10 to 40°C (Simpson and Melsted, 1963; Dalal, 1975).

The effect of temperature on the stability of urease is shown in Fig. 4. In this work, soil samples were exposed to different temperatures for 48 h and then brought to room temperature (23°C) and assayed according to the procedure previously described. The enzyme, urease appears to be stable from 40 to 60°C, but is irreversibly inactivated at >60°C. The NH_4-N released at 70°C is approximately 50% of that observed at 40°C. There was very little NO_3-N produced at >40°C. Nitrifying bacteria are sensitive to elevated temperatures,

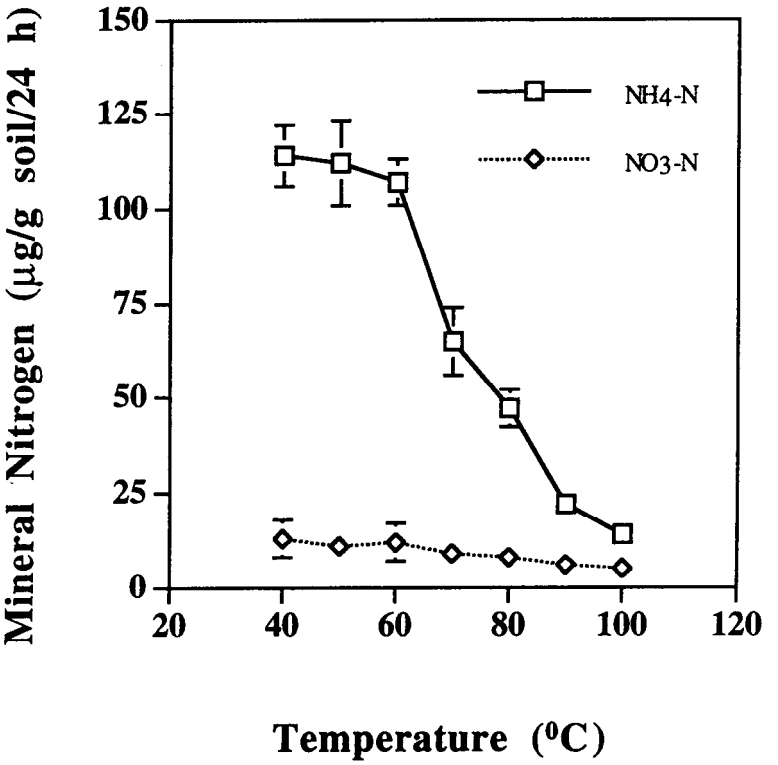

Figure 4. Effect of temperature on urease stability in soil.

particularly >40°C. This study indicates that remediation of soil subject to low temperature thermal desorption will inactivate enzymes (urease and catalase) responsible for the breakdown of urea peroxide.

Figure 5 shows the results obtained by varying the time of incubation in the assay described. The observed linear relationship up to 24 h incubation, is evidence that this assay is not complicated by microbial growth or assimilation of the mineral nitrogen released by microorganisms. There was no significant difference in the amount of NH_4-N released from 24 to 120 hours. However, the NO_3-N released over time continued to increase up to 120 hours of the assay.

The TPH content of the contaminated soil ranged from 2,100 to 2,250 (avg. 2,200) mg kg^{-1} soil at the onset of this study. After 8 weeks of incubation, the sterile treatment declined to 1,680 mg kg^{-1}, indicating that abiotic factors contributed slightly to the decrease in TPH over time (Fig. 6). The moist treatment in which only water was added promoted a 50% decline in TPH. The application of urea peroxide resulted in the greatest decline from 2,180 to 170 mg kg^{-1}, resulting in a 92% decline in TPH after 8 weeks of incubation. The addition of supplemental N (urea) and O_2 (H_2O_2) as urea peroxide promoted the biodegradation of diesel fuel in this study.

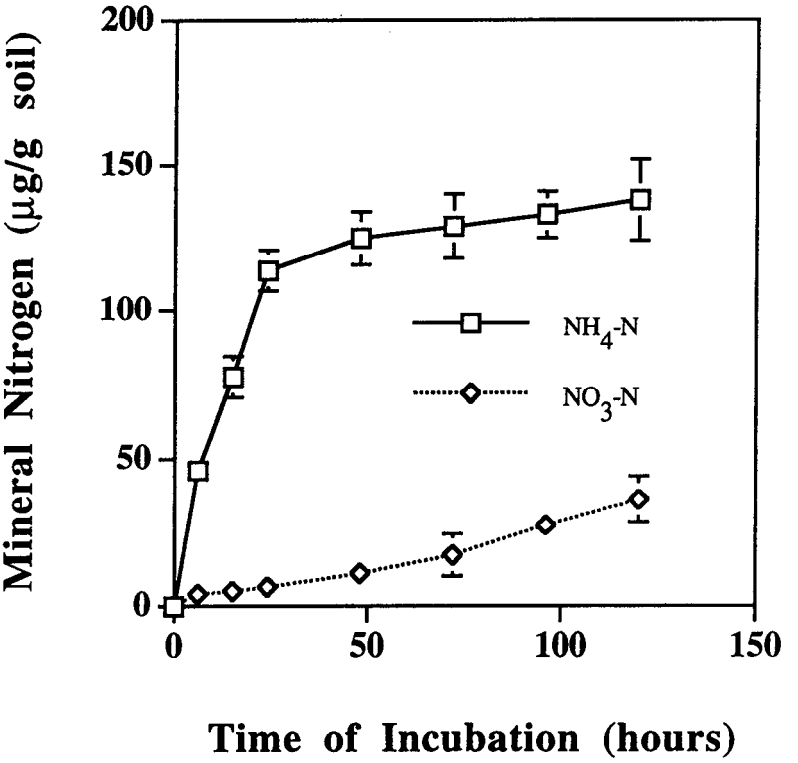

Figure 5. Effect of time of incubation on the release of mineral nitrogen from urea peroxide added to soil.

This study indicates that urea peroxide can be used successfully in bioremediation of petroleum hydrocarbons to promote aeration and nitrogen in soil. Environmental factors shown to promote the release of NH_4-N from urea peroxide were a pH_{opt} of 7.5 to 8.5; urea peroxide concentration of 0.25%; and a $temp_{opt}$ of 30 to 40°C. This study also indicates that thermal treatment of the soil may inactivate urease responsible for the breakdown of urea peroxide, thus bioremediation with the use of urea peroxide will only be successful at temperatures below 60°C.

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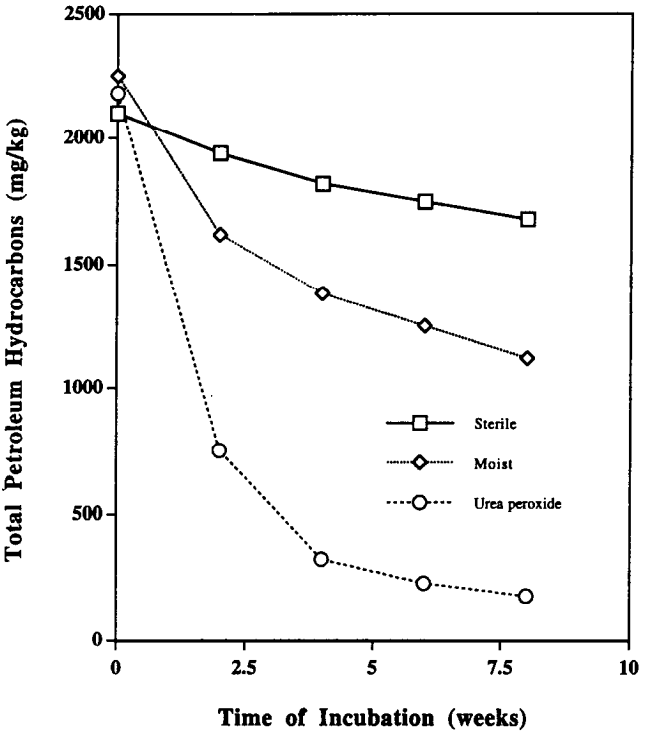

Figure 6. Decline in total petroleum hydrocarbons upon sterilization, the addition of moisture, and urea peroxide.

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