EFFECTS OF SOIL TEMPERATURE ON UREA HYDROLYSIS

by

CHRISTOPHER CHRISPIN MOYO

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Approved by:

David E. Kissel

Major Professor

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GENERAL INTRODUCTION

The use of urea as a nitrogen fertilizer has increased more rapidly than any other nitrogenous fertilizer. Between 1973 and 1983, world urea fertilizer production increased more than two fold while that of ammonium nitrate and ammonium sulfate remained almost constant (TVA, 1978). This increase has been attributed to a number of advantages urea has over other solid nitrogen fertilizers; these advantages include higher nitrogen content (46.6 % N), lower risk of fire or explosion hazard, lower tendency to coalesce and compact, and lower corrosivity. However, despite these advantages, there are some problems associated with the use of this fertilizer.

When applied to the soil, urea is rapidly hydrolyzed to ammonium and bicarbonate ions by soil urease. If the method of urea application results in high ammonium concentrations in soil (as with surface or band applications), high concentrations of ammonia and nitrite may result. Ammonia is toxic to germinating seedlings and may be lost as a volatile gas if present near or on the soil surface. Similarly, nitrite is toxic to plants and may result in gaseous loss of nitrogen through chemical denitrification.

The rate of urea hydrolysis in soil can be

influenced by soil water, soil pH, soil organic matter, soil temperature, and the urea concentration. Among these factors, temperature has been found to be one of the most important (Simpson and Melsted, 1963).

This study deals with the effect of soil temperature on the rate of urea hydrolysis. The first part examines this effect under laboratory conditions while the second part examines it under field conditions.

CHAPTER 1

LITERATURE REVIEW

When urea is applied to the soil it is rapidly hydrolyzed to ammonium and bicarbonate ions according to the following reaction,

$$CO(NH_2)_2 + 2H_2O + H^+ \xrightarrow{\text{Urease}} 2NH_4^+ + HCO_3^-$$

The enzyme that catalyzes this reaction in soil is urease. Conrad (1942) hypothesized that this enzyme is derived from many microorganisms and higher plants and is present in soil largely as extracellular complexes with soil colloids. However, MacGarity and Myers (1967) have demonstrated that urea could also be hydrolyzed by active soil microorganisms. Paulson and Kurtz (1969) considered soil urease to consist of two components: microbial urease, directly associated with soil microorganisms, and adsorbed urease, apparently adsorbed by soil colloids. These workers also found that under steady state conditions urea is predominantly hydrolyzed by adsorbed urease.

The rate at which urea hydrolysis takes place in soil depends on the number of active urease molecules and the factors that affect the activity of this enzyme.

Number of Active Urease Molecules

The number of active urease molecules in soil can be estimated as the soil's urease activity under standard conditions of temperature, pH, available water, and non-limiting substrate.

Several workers have correlated urease activity in soil with soil properties. Although different results have been obtained, soil organic carbon content and total nitrogen seem to correlate best with urease activity. For example, Zantua et al. (1977) found urease activity to be significantly correlated with soil organic carbon, total nitrogen, and cation exchange capacity in several Iowa soils. They further found urease activity to be significantly correlated with clay and surface area but not with pH. Speir et al. (1980), Dash et al. (1981), and Frankenberger et al. (1983) found similar high correlations between urease activity and organic carbon and total nitrogen.

Recently, Reynolds et al. (1985) studied the effects of soil properties on urease activity in pasture and cultivated soils. They found urease activity in cultivated soils to be positively correlated with total nitrogen, organic carbon, cation exchange capacity, and clay content. However, in pasture soils, urease activity was not as strongly correlated with organic carbon and

total nitrogen as in cultivated soils. This was attributed to variations in the amounts of decomposing organic matter and microbial populations.

Factors Affecting Urease Activity in Soil

pH

Studies on the effect of pH on urease activity in soil have shown that there is an optimum pH at which soil urease functions. At other pH values, there is a decrease in activity. Most of these studies have been conducted using pH buffers of different types and urea solutions of different concentrations. Consequently, the reported values of optimum pH for soil urease vary. For example, Pettit et al. (1976), using a phosphate buffer and a urea concentration of 1.5 M, found the maximum urease activity to be at pH 6.5 to 7.0 whereas Tabatabai and Bremner (1972) using THAM buffer and a urea concentration of 0.2 M found it to be at pH 8.8 to 9.0. These studies have described the effect of pH on urease activity primarily as a single effect regardless of urea concentration used.

Recent work on the effect of pH on urease activity in soil has shown that the pH effect and pH optimum vary with urea concentration. Rachhpal-Singh and Nye (1984) studied the effects of pH on urease activity in a Begbroke sandy loam at different urea concentrations and found that the reaction rate is more strongly influenced by pH at higher urea concentrations. However, the optimum pH for soil urease at 6.0 M urea N / L soil solution was around pH 6.0 whereas at 0.4 M urea N / L soil solution was

around pH 6.8. Thus, urea concentration influences not only urease activity but also the optimum pH.

Substrate (urea) concentration

Studies on the effects of urea concentration on urease activity in soil have indicated that the urea hydrolysis rate in soil increases with increasing urea concentration until the enzyme is saturated by the substrate (Dalal, 1975; Tabatabai and Bremner, 1972; Zantua and Bremner, 1977). Thereafter, the rate becomes independent of urea concentration. However, most of these studies have been conducted using concentrations of less than 1.0 M urea N.

Rachhpal-Singh and Nye (1984) studied the effects of urea concentration on urease activity in soil using concentrations of up to 10 M urea N. Their results indicated that the effect of urea concentration on urease activity can best be described by a model consisting of two Michaelis-Menten equations, one with uncompetitive substrate inhibition of the enzyme and the other without substrate inhibition. They found the maximum rate of urea hydrolysis occurred at a concentration of 4 to 6 M urea N. Cabrera and Kissel (1984) observed maximum hydrolysis rates at about 12 M

urea N with a sharp decrease at 16 M urea N concentration.

Soil water

Studies on the effects of water on the rate of urea hydrolysis in soil have produced differing results. In most cases, urease activity was not appreciably affected by the water content (Delaune and Patrick, 1970; Gould et al 1973; Skujins and McLaren, 1969; Zantua and Bremner, 1977). However, in some studies, the urea hydrolysis rate increased (Kumar and Wagenet, 1984; Rachinskiy and Pelttser, 1965) or decreased (Dalal, 1975; Simpson and Melsted, 1963) with increasing water content.

Recently, Kissel and Cabrera (1989), using data from Jones (1932), Vlek and Carter (1983), and their own unpublished work, found relative urease activity to be poorly related to gravimetric water content. However, when they converted the gravimetric water contents to water potentials, they found a better relationship between water potential and relative urease activity with the maximum activity occurring at a water potential of approximately -0.02 MPa. At decreasing water potentials, they observed only a slight decrease in urease activity; however, with an increase in water potential, this decrease was quite substantial.

Temperature

The effect of temperature on urea hydrolysis rate in soil has been studied by a number of workers. The literature discussed below indicates that the urea hydrolysis rate increases with increasing temperature up to 60 to 70° C and then decreases rapidly above that temperature range due to thermal inactivation of urease.

Fisher and Parks (1958) studied the effects of temperature on the urea hydrolysis rate in a Hermitage silt loam between 10 and 30° C without a pH buffer and containing urea at a concentration of either 50 or 100 mg N / kg soil. They found urea hydrolysis rates to increase with increasing temperature and concentration of urea in soil. The soil pH changed very little during the course of this study, indicating that pH did not affect hydrolysis rates. However, differences in the urea substrate concentration used may have affected the rates of hydrolysis that were measured. The urea concentrations used (50 and 100 mg N / kg soil) appear to have been too low and reaction times too long to avoid reaction rate changes due to changes in concentration of urea during incubation. At higher incubation temperatures, initial reaction rates would have been higher resulting in greater rate limitation due to insufficient substrate

concentration later in the incubation.

Simpson and Melsted (1963) compared urea hydrolysis rates at 1 and 25° C in several Illinois soils using urea solutions without a buffer at concentrations of 200 and 400 mg N / kg soil. Their results indicated that urea hydrolysis rates were 2 to 6 times greater at 25 than at 1° C, and that temperature caused a relatively greater increase in the hydrolysis rate in soils with low than in soils with high pH. The higher hydrolysis rates for low pH soils could be due to a greater increase in pH from increased temperature. Since no buffer was used to control changes in pH, higher pH from the higher incubation temperature would allow both pH and temperature to increase hydrolysis rates at the higher temperature. This effect would have been less for the high pH soil. Gibson (1930) found that an increase in pH on acid soils increased urea hydrolysis rates. The optimum pH for urea hydrolysis was found by Pettit et al (1976) to be around 6.5 to 7.0 whereas Tabatabai and Bremner (1972) found the optimum pH to be 8.8 to 9.0.

Chin and Kroontje (1963) compared urea hydrolysis rates at 4 and 25° C in a starch-treated Tatum silt loam without a buffer and containing urea solution equal to 4000 mg N / kg soil. They found urea hydrolysis rates to be much higher at 25 than at 4° C. Autoclaving the soil

at 127° C for 15 minutes completely stopped urea hydrolysis, indicating complete inactivation of the urease. Zantua and Bremner (1977) observed a similar loss of urease activity when several Iowa soils were dried at 105° C for 24 hours or autoclaved at 120° C for 2 hours.

Recently, the temperature dependence of urea hydrolysis in soil has been described (Gould et al., 1973; Dalal, 1975; and Kissel and Cabrera, 1989) using the Arrhenius equation,

$$k = A\ e^{-Ea\ /\ RT}$$

where, k is the rate constant (which could be Vmax from the Michaelis-Menten equation), A is a constant, Ea is the activation energy for the reaction, R is the universal gas constant, and T is the absolute temperature.

Gould et al. (1973) studied the effects of temperature on urea hydrolysis rates between 2 and 45° C in a Malmo silt loam (saline lucastrine material) without a buffer and containing urea solution at 200 mg N / kg soil. The plot of the log of urea hydrolysis rate with inverse temperature for this soil was linear in the above temperature range. The activation energy for soil urease was 9.8 kcal / mol, which was much lower than the value of 22.7 kcal / mol reported by Rachinskii and Pelttser (1967). The initial pH of the soil increased with urea hydrolysis during the course of the study by Gould et

al. since no buffer was used to control pH changes. This implies that urea hydrolysis was influenced not only by temperature but also by pH.

Gould et al. (1973) calculated urea hydrolysis rates based on the time it took for 50 % of the added urea to hydrolyze. Since urea hydrolysis appears to follow Michaelis-Menten enzyme kinetics, the hydrolysis rate should have been calculated based on the time that was required for 10 % of the initial urea substrate to hydrolyze; it is usually considered that the initial rate persists only when the substrate concentration is within 10 % of the initial value (Allison and Purich, 1979).

Dalal (1975) investigated the effects of temperature on urease activity in several Trindad soils in the presence and absence of toluene, a bacteriostatic agent. He found the mean activation energy for soil urease in the presence of toluene to be much higher, 21.9 kcal / mol, than in the absence of toluene, 5.2 kcal / mol. The latter value compares favorably with the activation energy of 4.0 kcal / mol for the Uvalde soil and 6.7 kcal / mol for the Vernon (Kissel and Cabrera, 1989). These values were lower than the 9.8 kcal / mol reported by Gould et. al. (1973) for a Malmo silt loam. The activation energy of soil urease in the presence of toluene was similar to the 22.7 kcal / mol reported by

Rachinskii and Pelttser (1967).

To summarize, the rate of urea hydrolysis in soil increases with increasing temperature, and the Arrhenius equation can be used to describe the temperature dependence of urea hydrolysis in the temperature range where no heat inactivation of urease occurs. However, the values reported in the literature for the activation energy for soil urease vary considerably. Theoretically, the activation energy should be the same in all soils since the reaction is the same. These differences could be due to different methods used in different studies. This may have resulted in confounding of the temperature effects with those of other rate controlling factors, such as pH, water content, and urea concentration.

CHAPTER 2

EFFECTS OF SOIL TEMPERATURE ON UREA HYDROLYSIS: LABORATORY STUDIES

INTRODUCTION

The effect of temperature on urea hydrolysis rates in soil has been reported by a number of workers (Fisher and Parks, 1958; Simpson and Melsted, 1963; Rachinskii and Pelttser, 1967; Gould et al., 1973; Dalal, 1975; and Vlek and Carter, 1983). In general, their results have indicated that the urea hydrolysis rate in soil increases with increasing temperature and that the effect of temperature on urea hydrolysis can be described quantitatively with the Arrhenius equation, provided heat inactivation of the enzyme does not occur in the temperature range studied.

To evaluate quantitatively the effects of temperature on the rate of urea hydrolysis in soil, it is important that other factors that affect urea hydrolysis rates, namely, pH, urea concentration, and water content, remain constant during the study. A review of the literature indicates that these variables have not been held constant in previous work. The effect of temperature on urea hydrolysis rates can be confounded by variation in

any of these factors. Such variation may have contributed to the large differences in the activation energy values previously reported for soil urease (4.0 to 22.7 kcal / mol). Theoretically, a specific chemical reaction should have a constant activation energy. Because of the wide range in temperature effects reported in the literature, a study was conducted to evaluate the effects of soil temperature on urea hydrolysis rate under carefully controlled laboratory conditions. To achieve this objective some preliminary work was carried out in the laboratory to:

1. select a pH buffer that keeps the pH stable during incubation,
2. insure that the buffer selected does not interfere with urea determination,
3. select a urea concentration around which the hydrolysis rate remains relatively constant,
4. estimate a suitable incubation time for adequate sensitivity of hydrolysis rate measurement while also meeting the requirement in (3), and
5. select a water content that is nonlimiting to the urea hydrolysis rate.

MATERIALS AND METHODS

Soils

The soils used in these studies were surface (0-15 cm) samples from areas mapped as Kahola (fine-silty, mixed, mesic Cumulic Hapludolls) and Smolan (fine, montmorillonitic, mesic Pachic Argiustolls) at the North Agronomy Farm, Kansas State University, Manhattan. The soils were collected in field-moist condition (170 to 190 g / kg), passed through a 2 mm sieve, thoroughly mixed in a Twin Shell blender, and stored in plastic containers at 4^{o} C.

Some of the properties for the two soils are presented in Table 1. Particle size distribution was determined by the hydrometer method after dispersion by sodium hexametaphosphate (Day, 1965) and soil pH (soil:water ratio, 1:2) was determined with a glass electrode (Peech, 1965). Organic carbon was determined by the photoelectric colorimeter (Graham, 1948).

Reagents

0.1 THAM buffer

THAM buffer, 0.1 M, was prepared by dissolving 6.0570 g of tris (hydroxymethyl) aminomethane buffer

(Fisher Certified reagent) in 350 mL of deionized water. The solution was adjusted to the desired pH at the desired temperature by addition of 0.2 M sulfuric acid, and then made to 500 mL volume at that temperature.

Urea solutions

To make urea solutions of 250, 500, 750, 1000, 1250, 1500, and 2000 mg N / L, urea (46.6 % N) weighing 0.1073, 0.2146, 0.3219, 0.4292, 0.5365, 0.6438, and 0.8584 g, respectively, was dissolved in 150 mL of water and made to 200 mL volume at the desired temperature. To make 1 M urea solution, 6.0050 g urea was dissolved in 75 mL distilled water and made to 100 mL volume at room temperature.

2 M KCl-PMA solution

To make 2 M KCl-PMA solution, 1500 g KCl was dissolved in 8 L of water and 0.05 g phenylmecuric-acetate (PMA) was dissolved in 1 L of water. The two solutions were then mixed and made to 10 L volume.

Reagents for colorimetric urea determination

These were prepared as described by Douglas and Bremner (1970).

PRELIMINARY EXPERIMENTS

1. Stability of pH during incubations

In assaying soil urease activity, it is important to have a high and constant pH at all temperatures. A constant pH would insure that pH does not confound the temperature effects on urea hydrolysis. In our study, tris (hydroxymethyl) aminomethane (THAM) buffer was used to stabilize pH during incubations. This buffer, unlike sodium or potassium phosphate buffers, has been found to have no activating or inhibiting effects on the activity of urease derived from jack bean (Wall and Laidler, 1953). High pH was considered important because THAM buffer has a poor buffering capacity below pH 7.5 and is often inhibitory at such values (Good et al., 1966). In addition, Tabatabai and Bremner (1972), working with 0.05 M THAM buffer, found maximum urease activity at pH 9.0. An experiment was therefore conducted to select a pH buffer that would keep the pH of the incubated samples high and stable throughout the incubation period.

An incubation pH was considered to be stable if the change in pH after incubation was not more than 0.1 pH unit. Preliminary work with unlimed soils, using pH 9 THAM buffer, showed that the pH dropped more than 0.3 of a pH

unit. On the other hand, work with soils limed to about pH 8.2 indicated that the change in pH after incubation was less than 0.3 of a pH unit. Because of the small changes in pH obtained with limed soils during incubations, we felt that even smaller changes in pH might result if the buffer pH was closer to the pH of the soil. To test this possibility, we incubated the samples with THAM buffer adjusted to either pH 8.8, 8.5, or 8.2 using the limed soils. The following procedure was used for each soil.

Moist soil equivalent to 5 g of oven-dry soil was weighed into nine 120 mL French square bottles. The bottles were then stoppered and, together with flasks containing the buffer and urea solution (500 mg N / L), were placed in a water bath at 45^{o} C. After equilibrating for 45 minutes, the bottles were removed from the water bath and 5 mL of the buffer and 5 mL of the urea solution were added. The bottles were then gently swirled and the pH of their contents was measured with an electrode using a 701A Orion digital pH meter. Immediately following pH measurements, the bottles were stoppered and placed back in the water bath. At intervals of 2, 4, and 6 h, a set of three bottles were removed from the water bath, gently swirled, and the pH of their contents measured again.

2. Effects of THAM buffer on urea determination

The colorimetric determination of urea is based on the reaction of diacetyl with urea when an aliquot of the extract is heated at 120° C for 30 minutes with diacetyl monoxime and thiosemicarbazide under acidic conditions (Douglas and Bremner, 1970). Absorbance of the resulting complex is then measured at 530 nm on a Technicon Autoanalyzer II.

A number of substances, such as ammonium sulfate, asparagine, alanine, glucosamine, creatine, lycine, glutamic acid, aspartic acid, creatinine, arginine, and potassium sulfate have been found not to interfere with urea determination (Douglas and Bremner, 1970). However, information regarding interference by THAM buffer was lacking. This study was therefore conducted to determine if THAM buffer interferes with colorimetric urea determination. Treatments, replicated four times, were designed to compare urea concentrations in unbuffered solutions and solutions buffered with THAM.

Buffered urea samples consisted of 1 mL of 1 M urea solution and 5 mL of pH 9 THAM buffer (0.1 M) solution in 100 mL volumetric flasks; unbuffered urea samples consisted of 1 mL of 1 M urea solution and 5 mL of distilled water. The samples were made to 100 mL volume with 2 M KCl solution and analyzed colorimetrically on the

Technicon Autoanalyzer II using the procedure of Douglas and Bremner (1970) described above.

3. Selection of urea concentration

Urease activity in soil is affected by urea concentration if the amount added is a limiting factor (Zantua and Bremner, 1975). The objective of this experiment was, therefore, to select a urea concentration that would not be limiting (i.e. the reaction rate would be on the plateau of the Michaelis-Menten curve).

For each soil, the following procedure was used: moist soil equivalent to 5 g of oven-dry soil was weighed into twenty-four 125 mL Erlenmeyer flasks. The flasks with soil and flasks containing the buffer, distilled water, and urea solutions were then placed in a constant temperature incubator at 45° C for thermal equilibration. After 45 minutes of equilibration, 5 mL of the pH 8.2 THAM buffer were added to the soil. Then 5 mL of urea solution at rates of 0, 250, 500, 750, 1000, 1250, 1500, and 2000 mg N / kg soil were added to respective flasks, in triplicate. The flasks were then stoppered and incubated at 45° C for 6 h. Following incubation, the samples were extracted with 2 M KCl-PMA solution and the urea remaining in samples determined colorimetrically (Douglas and

Bremner, 1970).

Soil extraction and urea determination

To extract urea from the soil, 30 mL of 2 M KCl-PMA solution was added to each flask. The flasks were then tightly stoppered and shaken on a mechanical shaker for 15 minutes. Immediately following shaking, the samples were filtered under vacuum on a Buchner funnel using Whatman No. 41 filter paper. This was followed by two rinses, 20 mL each, with 2 M KCl-PMA solution. The extracts were then transferred to 100 mL volumetric flasks and made to volume. After thoroughly mixing, the extracts were analyzed for urea using the procedure of Douglas and Bremner (1970).

MAIN EXPERIMENT

The effect of temperature on the urea hydrolysis rate was measured at 5, 15, 25, 35, and 45^{o} C. The soils used were limed to about pH 8.2 to insure a high and stable pH during incubations. At each temperature, the following procedure was used for each soil: moist soil equivalent to 5 g of oven-dry soil was weighed into six 125 mL Erlenmeyer flasks. The flasks containing soil and flasks containing distilled water, THAM buffer, and the

urea solution were then placed in the incubator at the selected temperature. After 45 minutes of thermal equilibration, three flasks were treated with 5 mL of THAM buffer and 5 mL of urea solution; the other three flasks were treated with 5 mL buffer and 5 mL distilled water. The flasks were then stoppered, gently swirled to mix the contents, and placed again in the incubator, in complete block arrangement, for periods of 57.07, 28.52, 14.26, 7.13, and 3.57 h for Kahola, and of 47.20, 23.60, 11.80, 5.90, and 2.95 h for Smolan at 5, 15, 25, 35, and 45° C, respectively.

The incubation periods were calculated based on a Q_{10} of 2 (Q_{10} is a factor by which a reaction increases with a 10° C increase in temperature), a urea hydrolysis rate of 28.1 and 33.9 mg N / kg soil h^{-1} at 45° C for Kahola and Smolan, and the time required for 10 % of the added urea to hydrolyze. The urea hydrolysis rates were determined from the plateau of the Michaelis-Menten curves of the urea concentration experiment for the two soils. Following incubation, the samples were extracted with 2 M KCl-PMA solution, and the urea concentration in the extracts was determined colorimetrically (Douglas and Bremner, 1970).

To estimate urea recovery, another series of samples were prepared in the same manner as for the

incubated samples except that they were extracted without incubation. These unincubated samples allowed a correction for zero time recovery. The urease activity (UA) at each incubation temperature was calculated as follows:

$$UA = [A - (c - d) / FR] / T$$

where, $FR = (a - b) / A$, is fractional recovery, a and b are urea in unincubated samples and blanks, respectively, c and d are urea in incubated samples and blanks, respectively, A is urea initially added, and T is the incubation time.

A nonlinear regression procedure, using the Marquardt iterative method (SAS Institute Inc., 1982), was used to fit the Arrhenius equation to urea hydrolysis data from the various incubation temperatures of the main experiment. The Arrhenius equation used is of the form:

$$k = A\, e^{-Ea / RT}$$

where, k is the reaction rate constant, R is the universal gas constant, A is a constant, T is absolute temperature, and Ea is the activation energy for the reaction. The data used in the regression analysis were the reaction rates calculated from the main experiment (an estimate of Vmax at each temperature) and the corresponding temperatures. The curve fitting parameters were Ea and A. The actual nonlinear equation used for fitting the data was: $k = e^{-B / T}$, where, $B = Ea / R$.

RESULTS AND DISCUSSION

PRELIMINARY EXPERIMENTS

1. Stability of pH during incubations

The pH of the reaction mixture remained high and nearly constant during incubations when both the Kahola and Smolan soils were limed to pH 8.2 (Table 2). Based on these findings, the soils used in all subsequent studies were limed to pH 8.2, and the buffer used was also adjusted to pH 8.2.

2. Effects of THAM buffer on urea determination

The THAM buffer (pH 9.0) used had no effect on colorimetric urea determination (Table 3). THAM buffer was therefore used to control pH changes during incubations in the main study, but its pH was adjusted to 8.2 following results of preliminary experiment No. 1.

3. Selection of urea concentration

Fig. 1 indicates that for the two soils used, a urea concentration of 1000 mg N / kg soil was in a concentration range around which the urea hydrolysis rate changed little. A urea concentration of 1000 mg N / kg soil was therefore used in the main experiment.

Selection of water content

The rate at which urea hydrolysis takes place in soil depends, among other factors, on the diffusion of urea to the urease enzyme. The rate of diffusion, in turn, depends on the amount of water in the soil. Rachhpal-Singh and Nye (1984) studied the effect of solution : soil ratio on urease activity and found that urease activity decreased significantly as the solution : soil ratio decreased from 1 to 0.5. However, increasing this ratio above 1 did not affect urease activity. Based on these findings, we used a solution : soil ratio of 2 : 1 in our study. This ratio does not limit the diffusion of urea to the enzyme and does not limit the rate of urea hydrolysis.

MAIN EXPERIMENT

The effects of temperature on the urea hydrolysis rate in Kahola and Smolan soils are presented in Fig. 2 and 3. Increasing the temperature from 5 to 45^{o} C greatly increased the urease activity. These findings are consistent with several other studies in the literature (Fisher and Parks, 1958; Simpson and Melsted, 1963; Gould

et al., 1973; Dalal, 1975; and Vlek and Carter, 1983) which indicate that the rate of urea hydrolysis in soil increases with increasing temperature, provided heat inactivation of urease does not occur in the temperature range studied.

In most chemical reactions, an increase in temperature imparts more kinetic energy to the reactant molecules resulting in more productive collisions per unit time. However, for enzyme catalyzed reactions, such as urea hydrolysis, this is true only up to a point. Too high temperatures can denature the enzyme by disrupting the tertiary structure, causing loss of catalytic ability. Thus, as temperature increases to high values, the expected increase in the reaction rate resulting from increased enzyme-substrate collisions may be offset by the increasing rate of enzyme denaturation. In soil, thermal inactivation of urease has been reported beginning at approximately 65 to 70^{o} C (Pettit et al, 1976 and Zantua and Bremner, 1977). The enzyme is completely destroyed at about 105 to 110^{o} C (Rotini, 1935 and Zantua and Bremner, 1977).

The temperature dependence of urease activity at lower temperatures covering the range of soil temperatures typically observed in the top 2 to 3 cm of soil can be described adequately using the Arrhenius equation,

$$k = A\, e^{-Ea / RT}$$

where, k is the reaction rate constant (Vmax at each temperature), A is a constant, R is a universal gas constant, T is absolute temperature, and Ea is the activation energy for the reaction. The activation energy can be determined from a plot of log k versus the inverse of temperature. However, in our study the activation energy for soil urease was determined from a nonlinear regression of the measured urease activities from the main experiment on temperature. The mean activation energy for soil urease in Kahola and Smolan soils determined by nonlinear regression were quite similar, at 11.8 and 12.8 kcal / mol, respectively.

The mean activation energy for enzyme catalyzed-reactions are around 10.0 kcal / mol (Gutfreund, 1972). Gould et al. (1973) found the activation energy for urease in a Malmo silt loam to be 9.8 kcal / mol. The mean activation energies of 11.8 and 12.8 kcal / mol for Kahola and Smolan soils compare favorably with these values. However, they were much higher than the 4.0 and 6.7 kcal / mol values reported by Kissel and Cabrera (1989) for the Uvalde and Vernon soils used by Vlek and Carter (1983) and were much lower than the 22.7 kcal / mol value reported by Rachinskii and Pelttser (1967). Theoretically, the

activation energy for a single chemical reaction should not vary. The observed differences could be due to differences in the techniques used in different studies.

It is known that different assay procedures could induce conditions which may influence energy requirements for the formation of the enzyme-substrate complex, and hence the activation energy, differently. The activation energies determined for our soils differs from those reported by other researchers probably because of differences in methodology.

First, we used a buffer to control pH changes during incubations whereas in other experiments (Fisher and Parks, 1958; Simpson and Melsted, 1963; Gould et al., 1973; and Vlek and Carter, 1983) no buffer was used. If pH is not held constant during incubations, it can also greatly influence the reaction rate and confound temperature effects on the urea hydrolysis rate. The greater the differences in initial pH between soils, the greater the differences in the average pH during the incubations. These differences will affect the energy requirements for the formation of the enzyme-substrate complexes for the soils being compared.

Secondly, the urea concentration used in our studies was 1000 mg N / kg soil, determined from the plateau of the Michaelis-Menten curves obtained for the

urea concentration experiment (Fig. 1). Tabatabai and Bremner (1972) used a urea concentration of 1120 mg N / kg soil in their work. Gould et al (1973) and Vlek and Carter (1983), used urea concentrations which were less than 1000 mg N / kg soil. The curves obtained for our two soils suggest that their urea concentration may have been in a concentration range where small differences in concentration greatly affect urease activity. In such a case, if reaction times are not adjusted to consume the same amount of substrate at all temperatures, error will result. It is likely therefore that the effect of temperature on the rate of urea hydrolysis in some of these studies may have been confounded by that of urea concentration.

Thirdly, most incubations reported in the literature were conducted at a solution : soil ratio of less than 1. For instance, Vlek and Carter (1983) incubated their samples at solution : soil ratio of 0.3. In our study, we conducted the incubations at a solution : soil ratio of 2 since low soil moisture levels (solution : soil ratios of less than 1) may slow the molecular diffusion of urea to urease (Rachhpal-Singh and Nye, 1984). Also, the relative hydrolysis rates at solution : soil ratios of less than 1 change with different urea concentrations, but do not at higher water contents. This

is because the rate of urea diffusion in soil depends on the amount of water present. On the other hand, solution : soil ratios of greater than 1 did not affect the relative urease activity.

Lastly, it appears that in many studies excessive incubation periods have been used, especially at higher temperatures, resulting in rate limiting substrate levels later in the incubation periods. For instance, Gould et al (1973) estimated their urease activity using incubation times that allowed half of the added urea to hydrolyze. Vlek and Carter (1983) incubated their samples for 6 and 16 h for Uvalde and Vernon soils, respectively, at all the incubation temperatures (10, 20, 30 and 40^{o} C). The incubation period in our study varied with incubation temperature and was calculated based on the hydrolysis rate at 45^{o} C, a Q_{10} of 2, and the time necessary for 10 % of the added urea to be hydrolyzed. For most enzyme studies, it is usually recommended that an incubation time be selected to insure that the substrate concentration is within 10 % of the initial value (Allison and Purich, 1979).

SUMMARY AND CONCLUSIONS

The objective of this study was to evaluate the effects of soil temperature on urea hydrolysis rates under carefully controlled laboratory conditions. To achieve this objective, preliminary work was conducted to minimize confounding temperature effects with those of other factors that affect urea hydrolysis. The following information from preliminary work was used in the main experiment.

1. In order to maintain a high and stable pH during incubations, the soils used were limed to pH 8.2 and the THAM buffer used was also adjusted to pH 8.2.
2. THAM buffer could be used in the study since it did not interfere with colorimetric urea determination.
3. A urea concentration of 1000 mg N / kg soil could be used since it was in a concentration range around which the hydrolysis rate changed little.
4. Incubation periods were based on a hydrolysis rate of urea at 45^{o} C, a Q_{10} of 2, and the time that was required for 10 % of the added urea to be hydrolyzed.
5. A solution : soil ratio of 2 : 1 was used to insure that the diffusion of urea to the urease was relatively insensitive to differences in water content and urea concentration.

The increasing rate of urea hydrolysis with increasing temperature from 5 to 45° C could be described with the Arrhenius equation. The mean activation energy for soil urease was quite similar, at 11.8 and 12.8 kcal / mol for Kahola and Smolan, respectively. However, the activation energy values for our soils were quite different from some of the values reported in the literature, probably because of different assay techniques used in those studies.

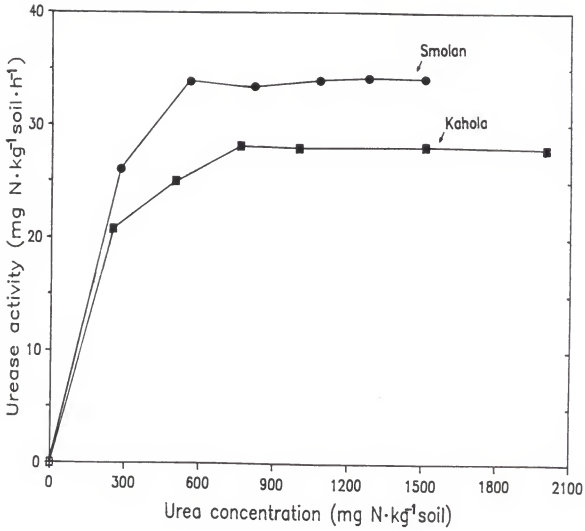

Fig. 1 Effect of urea concentration on urease activity in Smolan and Kahola soils.

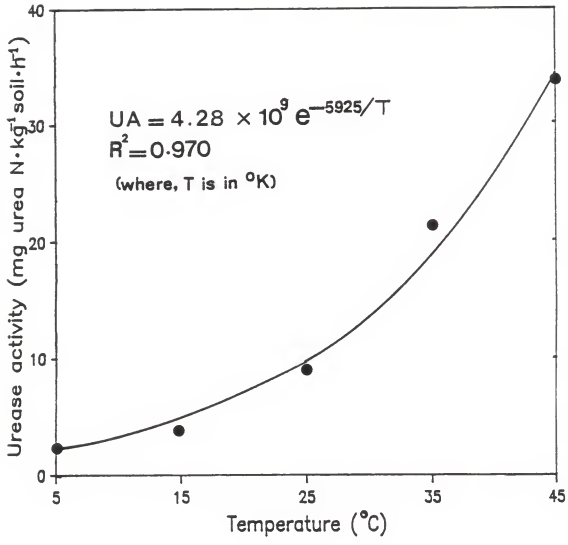

Fig. 2 Effect of temperature on urease activity in Kahola soil

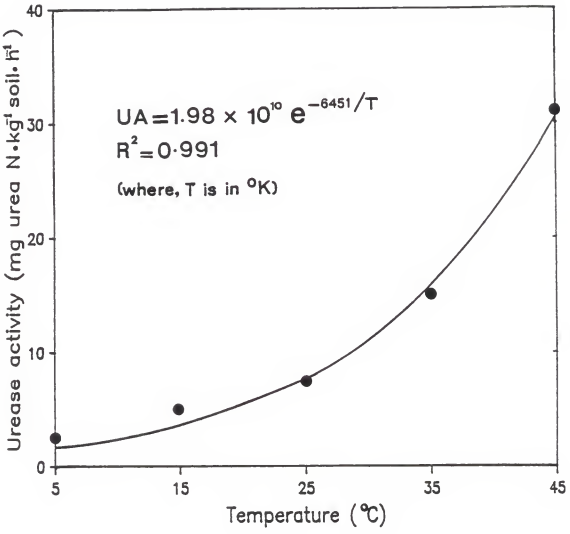

Fig. 3 Effect of temperature on urease activity in Smolan soil

Table 1. Chemical and physical properties of the soils used in the studies.

Soil series	pH	Organic carbon	Sand	Silt	Clay
		g / kg		%	
Kahola	5.46	15.0	3.2	76.1	20.7
Smolan	7.10	14.0	6.4	63.6	30.2

Table 2. Effect of 0.1 M THAM buffer (pH 8.2) on the pH of soil samples containing urea before and after incubation at 45° C.

Soil Series	Incubation Time	Before Incubation	After Incubation	Change
	h		pH	
Kahola	2.0	8.28	8.23	0.05
	4.0	8.29	8.24	0.05
	6.0	8.31	8.23	0.08
Smolan	2.0	8.32	8.26	0.06
	4.0	8.34	8.26	0.08
	6.0	8.33	8.25	0.08

Table 3. Effect of THAM buffer (pH 9.0) on colorimetric urea determination.

Treatment	Urea recovered
	mg N / kg soil
Urea with buffer	276.0
Urea without buffer	275.1
$LSD_{0.05}$	NS

CHAPTER 3

EFFECTS OF SOIL TEMPERATURE ON UREA HYDROLYSIS: FIELD STUDIES

INTRODUCTION

Several investigators have studied urea hydrolysis in soil and the factors which influence it. Some of these factors are soil pH, urea concentration, water content, and temperature. This study deals with temperature.

Most studies on the effects of temperature on urea hydrolysis in soil have been conducted in the laboratory under a controlled environment. Although these studies have greatly improved our understanding of temperature effects on the rate of urea hydrolysis, the laboratory environment is less variable than the field environment. Consequently, urea hydrolysis rates in the field may be different from those in the laboratory. There is a need for more field studies if there is to be a better understanding of urea hydrolysis once urea is applied in the field.

The objective of this study was to measure urea hydrolysis rates under field conditions in summer and winter.

MATERIALS AND METHODS

The field study was conducted at the North Agronomy Farm, Kansas State University, Manhattan. The study was conducted on areas mapped as Kahola (fine-silty, mixed, mesic Cumulic Hapludolls) and Smolan (fine, montmorillonitic, mesic Pachic Argiustolls) soils from where soil samples for the laboratory study were collected. Some of the soil properties in the surface (0-15 cm) layers are presented in Table 1. Soil pH was determined using a glass electrode (soil:water ratio, 1:2), organic carbon by the colorimetric method (Graham, 1948), and particle size distribution by the hydrometer method (Day, 1965).

Summer Study

The summer field study was conducted from August 30 to September 6, 1987. The experimental sites were rotor-tilled to a depth of 15 cm on August 5, 1987. Then the sites were leveled and thirty open-ended metal cylinders, 9.8 cm diameter by 10 cm long, were pushed into the soil to a depth of approximately 9.0 cm, in a complete block arrangement (Fig. 4), to provide microplots for the study.

In order to have a uniform soil surface at each site, the top 6 cm of soil from each cylinder was removed,

bulked, passed through a 2 mm sieve, mixed, and then equal weights uniformly packed back into the cylinders to a bulk density of 1.30 g / cm^3 for Kahola soil and 1.28 g / cm^3 for Smolan soil. The plots were then saturated with water three days prior to urea application to raise the soil water to near field capacity at the start of the experiment. Urea hydrolysis in soil is at maximum at water contents near field capacity (Kissel and Cabrera, 1989).

Urea solution (7.54 % N), was applied uniformly, at 200 kg N ha^{-1}, to the surface of bare soil within each microplot on August 30, 1987; 2 mL of this solution was applied per plot, using a 5 mL Gilson micropipette. Following urea application, the plots were covered with a movable 2.4 m by 1.2 m wooden shade (Fig. 4) to prevent rain water infiltration and excessive evaporation and hence maintain the soil water near field capacity. The shade was covered with plastic sheet to protect the wood from rain and was painted white to reflect sunlight and reduce heat build up and excessive evaporation. The roof was slightly slanted for easy drainage of water and its narrow sides were loosely covered with the plastic sheet to allow some air circulation. Water, in open pans, was placed at each corner of the experimental area to increase the humidity under the shade and hence reduce

water evaporation from the microplots.

Water contents were determined in the top 6 cm of soil by drying 3 cm^3 core samples, at 105^{o} C for 24 h, from plots designated for that purpose. The bulk density of the soil was calculated from oven-dried samples and a core volume of 3 cm^3. Soil temperatures were recorded in the top centimeter of the soil at 2 h intervals from the center of two plots provided for that purpose, using thermocouple sensors connected to data pod recorders. An earlier experiment at the Smolan site indicated that soil temperatures, at a depth of 1 cm, recorded at the center of the plots were always within 1^{o} C of those measured from the edge of the cylinders.

Three cylinders were removed from each site on days 0, 1, 2, 3, 4, 5, and 7 after urea application. Day zero was the time immediately after urea application. The holes left by the cylinders were immediately covered with soil to minimize temperature differences among the remaining cylinders. The cylinders were then taken to the laboratory and the soil from the 0-3 and 3-5 cm layers of each cylinder was removed, transferred to separate 1000 mL plastic bottles, shaken on a mechanical shaker for 15 minutes with 500 mL of 2 M KCl-PMA solution, and filtered through Whatman No. 2 filter paper. This was followed by two rinses, 100 mL each, with 2 M KCl-PMA solution. The

extracts were then transferred to 1000 mL volumetric flasks and made to volume with 2 M KCl-PMA solution.

Urea in the extracts was analyzed colorimetrically using the procedure of Douglas and Bremner (1970). The decrease in urea concentration in samples was assumed to be due to hydrolysis. Samples that could not be analyzed immediately were frozen until they could be analyzed.

Winter Study

The winter field study was conducted from February 27 to March 18, 1988, at the same sites where the summer study was conducted. The experimental procedure and layout were the same as the summer study except for some of the following changes.

The experimental areas were prepared in mid-November 1987, and the cylinders were pushed into the soil towards the end of the month. Immediately thereafter, the top 6 cm of the soil was removed from each cylinder, bulked, passed through a 2 mm sieve, mixed, and then stored in the cold room, at about 4^{o} C, until the end of February 1988, when the risk of soil freezing was reduced. Soil freezing was to be avoided because urea has to diffuse to the urease for it to be hydrolyzed, and this process could be significantly reduced in frozen soil.

Two days prior to urea application, 830 and 380 g

of water was uniformly added to 16 and 15 kg of Kahola and Smolan soils to raise their water contents from 160 and 190 g / kg soil, respectively, to near field capacity, 220 g / kg soil. Weighed soil, at field capacity, was then packed into the cylinders to a bulk density of 1.30 and 1.03 g / cm^3 for Kahola and Smolan, respectively. Urea solution, at 200 kg N ha^{-1}, was then uniformly applied to the surface of soil in each cylinder on February 27.

The cylinders were removed, in triplicate, on days 0, 2, 4, 6, 8, 10, 13, 16, and 20 after urea application. Day zero was the time immediately after urea application. The soil in the 0-3 and 3-5 cm layers was then extracted with 2 M KCl-PMA solution, and the urea in the extracts determined colorimetrically (Douglas and Bremner, 1970).

RESULTS AND DISCUSSION

The soil moisture content at field capacity for Kahola and Smolan is around 210 g / kg soil. In summer, the soil moisture in the top 6 cm layer was slightly higher than field capacity, averaging 260 g / kg soil for Kahola and 280 g / kg soil for Smolan. In winter, the soil moisture averaged 210 g / kg soil at both sites in the first 10 days but thereafter gradually decreased to 170 and 150 g / kg soil, respectively, by the end of the experiment. Air-dryness of the surface soil was observed in the Smolan soil towards the end of the winter study.

Daily mean soil temperatures, at 1 cm. depth, for the two soils are shown in Fig. 5. In summer, the temperatures averaged about 22^{o} C and in winter averaged about 3^{o} C. Unlike in summer when soil temperatures were almost constant throughout the study, in winter the first two weeks were warmer than the last week which was characterized by temperatures below freezing; this fluctuation in temperature was also reflected in a slowed rate of urea hydrolysis during this time (Fig. 6).

Urea hydrolysis was more rapid in summer than in winter (Fig 6). In summer, urea hydrolyzed at an average rate of 25 and 28 kg N / ha day^{-1} over the 7 day study period whereas in winter it hydrolyzed at an average rate of 8 and 11 kg N / ha day^{-1} during the first two weeks

and at an average rate of 5 and 6 kg N / ha day^{-1} during the last week, for Kahola and Smolan, respectively. The much reduced urea hydrolysis rates in the last week of the winter study was due to the freezing temperatures. These results are in agreement with many laboratory findings (Fisher and Parks, 1958; Chin and Kroontje, 1963; Simpson and Melsted, 1967; Gould et al., 1973; and Vlek and Carter, 1983) that the rate of urea hydrolysis in soil, among other factors, depends on temperature.

The practical significance of these results is that urea applied in summer to the surface of a moist soil will rapidly hydrolyze to ammonium and bicarbonate ions. This would make the urea nitrogen readily available to plants in the form of ammonium and possibly nitrate (through nitrification). However, the rapid hydrolysis of urea, especially where high rates have been used, can result in high pH values and high ammonium concentrations in soil which are conducive to the accumulation of ammonia and nitrite. Ammonia is toxic to germinating seedlings (Court et al., 1964) and can be lost to the atmosphere if present near or on the soil surface. Nitrite is also toxic to plants and can cause losses of nitrogen in gaseous form through chemical denitrification.

At both soil temperatures, urea hydrolysis was more rapid in the Smolan than in the Kahola soil. The slower

urea hydrolysis rates in the Kahola soil could be due to differences in soil pH (Table 1). The Smolan soil had a pH of 7.10 whereas the Kahola had a pH of 5.46. According to Pettit et al. (1976), the optimum pH for soil urease is pH 6.5-7.0. The measured Vmax in Chapter 2 was also approximately 20 % greater in Smolan than in Kahola.

SUMMARY AND CONCLUSIONS

The objective of this study was to measure urea hydrolysis from urea applied to the surface of Kahola and Smolan soils in summer and winter. Results indicate that urea hydrolysis was highly influenced by soil temperature. In summer, when soil temperatures were around 22° C, urea hydrolyzed at rates which were almost three times higher than those in winter when soil temperatures averaged 3° C or less. These results are in agreement with laboratory findings of this thesis which indicate that the rate of urea hydrolysis in soil is greatly influenced by soil temperature.

Fig. 4 Plot layout showing microplot cylinders, water pans, plywood cover, and plastic box holding soil temperature recorders.

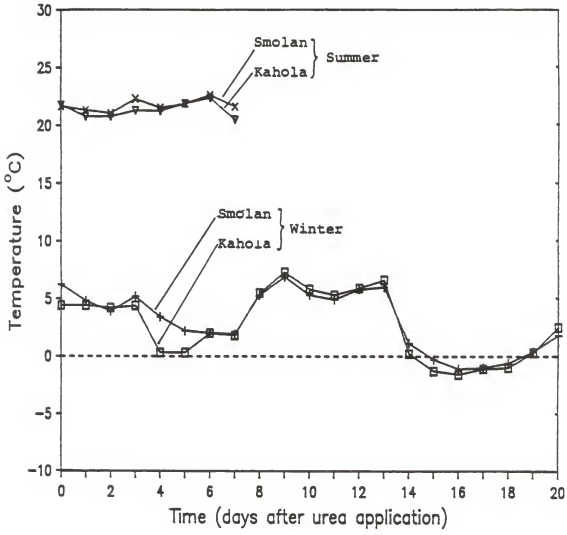

Fig. 5 Daily mean temperatures in the top centimeter of Kahola and Smolan soils during the summer and winter field studies.

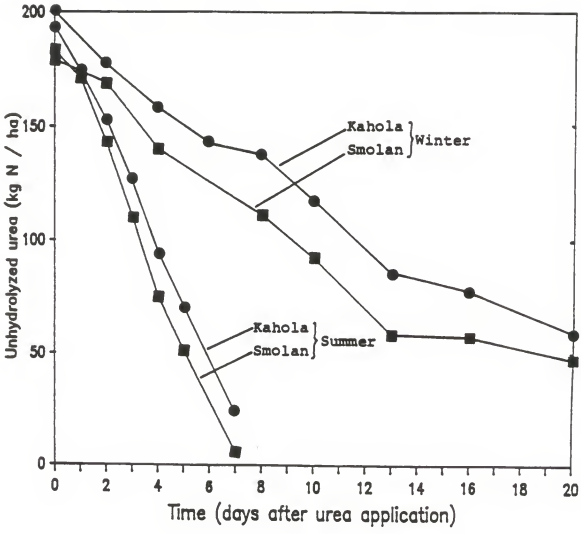

Fig. 6 Urea remaining in Kahola and Smolan soils with time after surface urea application in both summer and winter.

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EFFECTS OF SOIL TEMPERATURE ON UREA HYDROLYSIS

by

CHRISTOPHER CHRISPIN MOYO

B.S., Bunda College, University of Malawi, 1980

AN ABSTRACT OF A THESIS

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ABSTRACT

The first part of this study was conducted in the laboratory. Its objective was to evaluate the effects of temperature on the urea hydrolysis rate in Kahola and Smolan soils under carefully controlled conditions. To achieve this objective, other rate controlling factors were held constant or kept at nonlimiting levels by : 1) liming soils to pH 8.2 and and using THAM buffer adjusted to the same pH, 2) using a urea concentration of 1000 mg N / kg soil around which the hydrolysis rate changed little, 3) incubating for time periods that allowed only 10 % of the added urea to hydrolyze, and 4) using a solution : soil ratio of 2 : 1.

The effect of temperature on the urea hydrolysis rate was studied at 5, 15, 25, 35, and 45^{o} C. Results indicated that increasing temperature from 5 to 45^{o} C greatly increased the rate of urea hydrolysis. The mean activation energy for urease in Kahola and Smolan soils were 11.8 and 12.8 kcal / mol, respectively. However, these values were quite different from some of the values reported in the literature, probably because of the different assay techniques used.

The second part of this study was conducted in the field, on Kahola and Smolan soils, at locations where samples for the laboratory study were collected. The

objective of this study was to measure urea hydrolysis rates from urea solution applied in summer and winter. Results indicated that urea hydrolysis in the field was highly influenced by soil temperature. Urea hydrolyzed at rates almost three times higher in summer than in winter.