

Characterization of Kinetics of Urea Hydrolysis in A Newly Reclaimed Tidal Soils

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It is imperative to study the hydrolysis of urea in high saline-sodic condition of a newly reclaimed tidal land in order to overcome the problems associated with use of urea fertilizer. The methodology adopted in this study tried to get a convenient way of estimating rate for N transformation needed in N fate and transport studies by reviewing pH and salt contents which can affect the microbial activity which is closely related to the rate of urea hydrolysis. The hydrolysis of urea over time follows first-order kinetics and soil urease activity in reclaimed soils will be represented by Michaelis-Menten-type kinetics. However, high pH and less micro-organisms may delay the hydrolysis of urea due to decrease in urease activity with increasing pH. Therefore, the rate of urea hydrolysis should adopt V_{\max} referring enzyme activity (E_0) accounting for urease concentration which is indicative for urea hydrolysis, especially in a high saline and sodic soils.

Key words: Urea, Hydrolysis, Reclaimed Tidal Soils, Salinity, Sodicity, Kinetics

Introduction

The use of urea, the most important solid fertilizer in world agriculture, as a nitrogen fertilizer has significantly increased over the past 20 years (Tomlinson, 1970; Engelstad and Hauck, 1974; Beaton, 1978). The conversion of nitrogen fertilizer is a crucial issue in agricultural production and environmental protection. However, Urea added to soil as fertilizer is enzymatically hydrolyzed by soil urease, resulting in adverse effect of ammonia toxicity on seed germination and seedling growth due to release of ammonia and rise in soil pH (Ouyang et al., 1998; Watson, 2000). Nitrite is highly toxic to plants (Camberato, 2001). Nitrite toxicity and volatilization of urea N as ammonia may cause air and water pollution problems (Gasser, 1964; Hutchinson and Viets, 1969). However, urea can be an inefficient N source due to rapid hydrolysis by soil urease leading to ammonia volatilization. The movement and transformation of urea depends on factors such as soil water holding capacity, temperature, salinity, pH, CaCO_3 and organic matter. The quantitative effects of these factors upon urea transformation are not clear how this data relates to dynamic field conditions. (Kumar and Wagnet, 1985)

A saline and sodic soil is one that is high in salt and sodium contents. Plants growing in saline soils may appear water stressed. This is because the high salt content of the soil hampers the ability of plants to take up water from the soil (Waskom et al., 2007). Sodium causes the clay in the soil to disperse, resulting in the loss of soil structure. As a result, internal drainage can be severely decreased. The rate of urea hydrolysis may be influenced by the ionic composition of the soil solution. Salts such as chlorides and sulfates of sodium have been reported to reduce urease activity and hence can be expected to affect the rate of urea hydrolysis (Frankenberger and Bingham, 1982; Galstyn, 1960; Singh and Bajwa, 1985).

In order to overcome the problems associated with use of urea fertilizer it is imperative to study the hydrolysis of urea in agricultural soils. Therefore, a greater understanding of the effects of salinity, sodicity and specific salt constituents on the rates of urea hydrolysis may provide guidance for urea management practices for newly developed reclaimed tidal soils.

The objectives of this study were to derive a simple kinetic expression for the enzymatic hydrolysis of urea to develop basic information about salt effects on urea and NH_4^+ transformation, and compare estimates of first-order rate coefficients under the saline sodic conditions of a reclaimed tidal soils.

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Theoretical Urea is a unique chemical nitrogen fertilizer in that its transformation to ammonium and use efficiency is controlled by the urease activity (Sahrawat, 1980). Transformation of urea refers to the conversion of urea to nitrate through ammonification and nitrification in soils (Fig. 1).

This conversion is a two step reaction performed by two distinct and specific microorganisms. The first step is the conversion of ammonium to nitrite and the second step is the conversion of nitrite to nitrate. This reaction is tightly coupled so that nitrite in soils rarely accumulates. Wagenet et al., (1977) proposed the two models that considers linear local equilibrium and reversible NH_4^+ sorption, and NH_4^+ sorption and desorption as independent kinetics processes (Fig. 2). Both models consider first-order kinetics for urea hydrolysis and pseudo-first order kinetics process for ammonia volatilization.

K_D is a distribution coefficient and k_V , k_N , k_{HU} , k_{ads} , k_{des} are rate coefficients (volatilization rate, nitrification

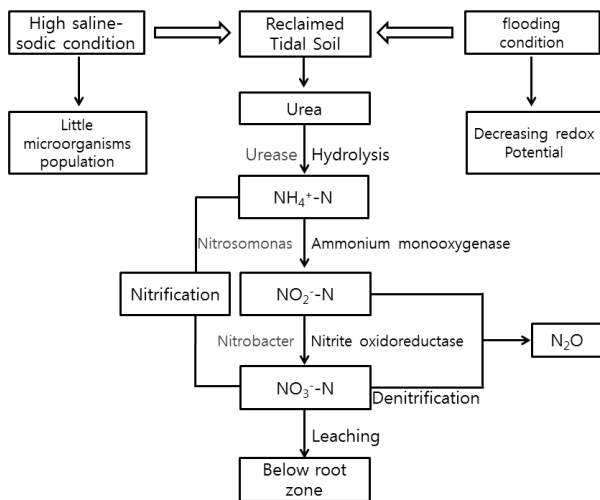


Fig. 1. Schematic of urea transformation processes in soil.

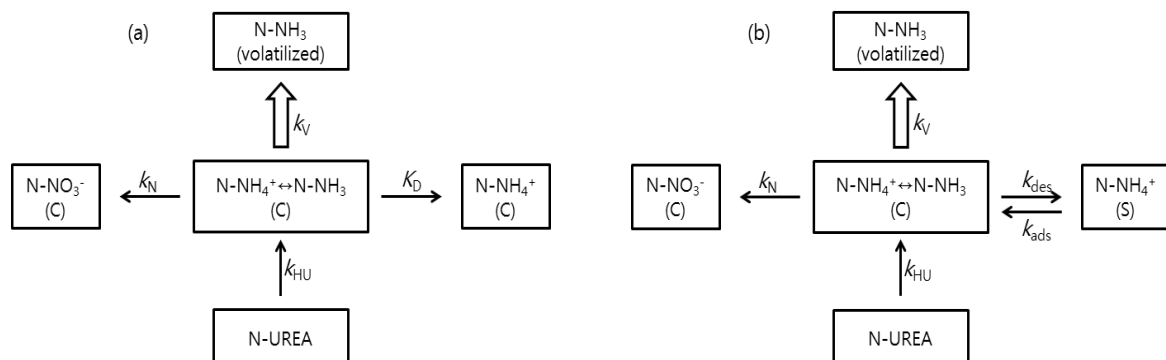


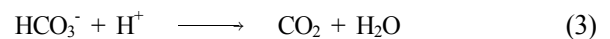
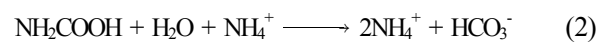
Fig. 2. Schematic of the two models describing conversion of urea in soils.

rate, urea hydrolysis rate, adsorption rate, and desorption rate, respectively)

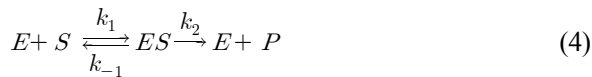
Many species of nitrifying bacteria which are chemoautotrophic or chemolithotrophic have key enzymes in nitrification: ammonia monoxygenase oxidizes ammonia to hydroxylamine, and nitrite oxidoreductase oxidizes nitrite to nitrate (Mancinelli, 1996). Nitrosomonas, chemoautotrophic bacteria, can get rid of excess ammonia by converting it to nitrite, and prefers an optimum pH of 6.0-9.0 and a temperature range of 20 to 30°C. Nitrobacter, chemoautotrophic bacteria, plays an important role in nitrogen cycle by oxidizing nitrite into nitrate in soil, and has an optimum pH between 7.3 and 7.5, and will die in temperatures exceeding 49°C or below 0°C.

Hydrolysis of Urea Hydrolysis is a chemical process during which molecules of water are split into hydrogen ion (H^+) and hydroxide anions (OH^-) in the process of a chemical mechanism (IUPAC, 2006). One fragment of the parent molecule gains a hydrogen ion (H^+) from the additional water molecule. The other group collects the remaining hydroxyl group (OH^-).

Urea hydrolysis by urease which is a naturally occurring enzyme that catalyzes the hydrolysis of urea to unstable carbamic acid proceeds rapidly in warm, moist soils, with most of the urea transformed to NH_4^+ in several days. Rapid decomposition of carbamic acid occurs without enzyme catalysis to form ammonium ion and carbon dioxide (Tisdale, 1985; Benini, 1999). Hydrolysis of urea in soil was assumed to follow the equation :



Kinetics of urea transformation by hydrolysis Most enzyme reactions concerned with catalysis occur at rates that are constant and do not change with substrate concentration or at rates proportional to the substrate concentration (Tabatabai, 1994). As seen Eq. (1), the simple conversion of urea as substrate(S) into ammonium as product(P) catalyzed by the urease as enzyme(E) is described as Eq. (4). The first step is substrate binding and the second step in the catalytic step.



The enzyme activity (ν) can be expressed as amount of urea-N hydrolyzed per hour in soil. A function of $S \nu^{-1}$ against S is plotted graphically to determine the intercept ($K_m V_{max}^{-1}$) and the slope ($1 V_{max}^{-1}$) of the linear transformation of the Michaelis-Menten equation, also known as the Hanes-Woolf transformation. Assuming a non-competitive mechanism for ammonium inhibition leads to the following rate expression (Eq. 5):

$$\nu = \frac{V_{max} \cdot [S]}{(K_m + [S])(1 + \frac{[P]}{k_p})} \quad (5)$$

where [S] and [P] are the substrate and ammonium ion concentrations, K_m is Michaelis constant, V_{max} is the maximum reaction rate for the enzyme. For enzymatic reactions which exhibit simple Michaelis-Menten kinetics, the Michaelis constant is defined as Eq. (6)

$$K_m = \frac{k_{-1} + k_2}{k_1} \quad (6)$$

where the unit of k_{-1} and k_2 is time^{-1} , while the unit of k_1 is $\text{concentration}^{-1} \text{times time}^{-1}$.

A Hanes-Woolf plot is a graphical representation of enzyme kinetics in which the ratio of the initial substrate concentration [S] to the reaction velocity ν is plotted against [S]. It is based on the rearrangement of the Michaelis-Menten equation shown below:

$$\frac{[S]}{\nu} = \frac{[S]}{V_{max}} + \frac{K_m}{V_{max}} \quad (7)$$

As is clear from the equation (7), perfect data will yield a straight line of slope $1/V_{max}$, a y-intercept of K_m/V_{max} and an x-intercept of $-K_m$.

Vlek And Carter (1983) reported that hydrolysis of

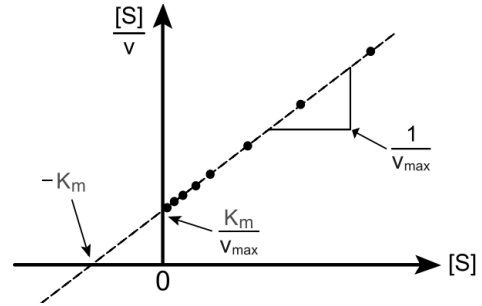


Fig. 3. A graphical representation of Hanes-Woolf plot.

urea uniformly distributed throughout the soil was adequately described by zero-order equations. However, urea hydrolysis was best described by first-order kinetics following a substantial lag phase in a heterogeneous system, followed by a rapid increase in hydrolysis rate, possibly due to a shift to zero-order kinetics. Also, they found that the hydrolysis rate decreased linearly with decreasing temperature, and moisture rapidly reduced the hydrolysis rate above the permanent wilting point. Moreover, hydrolysis rates were possibly affected by water-logging or excessive temperatures. Factors affecting the hydrolysis constant such as type of soil, soil moisture, and temperature should be maintained constant in order to avoid dependence on other experimental variables in Eq. (8). However, θ can be removed from both sides of Eq. (8) if the soil water content is constant. An increase in soil pH can be expected during relatively high rates of degradation because urea hydrolysis implies the net consumption of H^+ (Ferguson et al., 1984). Thus, hydrolysis has traditionally been described using first-order kinetics (Eq. 8) (Godwin and Jones, 1991; Vanclooster et al., 1996).

$$\frac{\theta \partial U}{\partial t} = -\nu \theta U \quad (8)$$

where U is the concentration of urea in soil solution, θ is the volumetric moisture content ($\text{cm}^3 \text{cm}^{-3}$), t is time, and ν is the kinetic rate constant for hydrolysis. The total amount of nitrogen in the system to satisfy Eq. (8) can be described as follows;

$$N_{total} = \theta U(t) + \theta C(t) + \rho S(t) + \theta N_{NO_3^-}(t) \quad (9)$$

where C is the concentration of $N-NH_4^+$ in the soil solution, S is the concentration of NH_4^+ adsorbed to the solid phase, ρ is the soil bulk density (g cm^{-3}), $N_{NO_3^-}$ is the concentration of NO_3^- in solution.

Discussions

In general, fine-textured soils possess higher urease activity because of a large fraction of the organic matter which act as a stabilizing agent for urease released into soil and also provide a potentially large number of sites for the preservation of urease activity (Pinck and Allison, 1961). However, a newly reclaimed tidal soils contain little organic matter and high salts, that can be categorized by saline-sodic or sodic soils.

Rodriguez et al., (2004) described evolution of the different nitrogen forms with the model assuming local equilibrium adsorption and the presence of an activation (lag) time for urea hydrolysis in the loamy sand. Overall changes of the different nitrogen forms schematically presented in Fig. 4 showed that decrease of urea was slightly rapid while volatilized-N gradually increased with time. They also indicated that the kinetic volatilization rate coefficient increased with temperature and decreased with soil moisture, being higher for the coarse-textured soil.

The saturation extract of saline-sodic soils have electrical conductivity greater than 4 dS m^{-1} , sodium adsorption ratio greater than 15, and pH less than 8.5, while pH of sodic soils is greater than 8.5 (Table 1). These pH and salt contents indicated as EC can be the factors to influence hydrolysis of urea in soils.

Fidaleo and Lavecchia (2003) reported that mathematical configuration of the urea hydrolysis yields the dependence of the apparent kinetic quantities, such as the Michaelis constant, K_m , and the enzymatic hydrolysis of urea in the pH range 4-9, and found a sharp dependence of both K_m and V_{max} on pH. Particularly, K_m displayed a minimum at pH 7, whereas V_{max} was maximal at the same pH. However, K_m was found to be practically independent on pH in buffer-free solutions. Also they observed that reaction rate was proportional to urease concentration as shown Fig. 5 as far as the specific enzyme activity remains constant within the urease concentration range.

Singh and Bajwa (1986) showed that the delay in urea hydrolysis was related to decrease in urease activity with increase in pH and seemed to follow first order reaction kinetics. Cabrera et al., (2002) found that the results of urea hydrolysis in soil depending urea concentration and soil pH were best described by a kinetic model involving two enzymatic reactions, both following simple Michaelis-Menten kinetics but

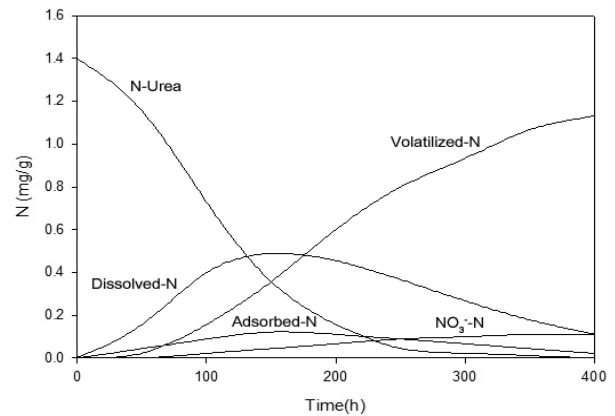


Fig. 4. Schematic picture of evolution of the different nitrogen forms described with the model assuming local equilibrium adsorption and the presence of an activation time for urea hydrolysis (Redrawn from Rodriguez, Gaite, and Benedi, 2004).

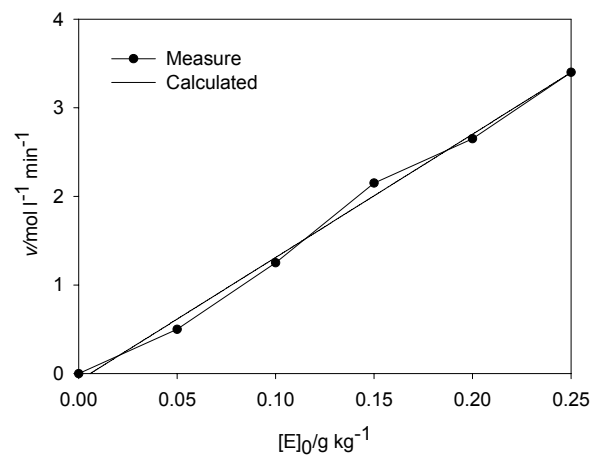


Fig. 5. Effect of urease concentration on the rate of urea hydrolysis at 25°C and pH 7 (Redrawn from Fidaleo and Lavecchia, 2003).

Table 1. Characteristics of saline, sodic, and saline-sodic soils.

Classification	Electrical conductivity dS m^{-1}	Soil pH	Sodium adsorption ratio (SAR)
Saline	>4.0	<8.5	<13
Sodic	<4.0	>8.5	≥ 13
Saline-Sodic	>4.0	<8.5	≥ 13

having different affinities for urea. The V_{\max} for the high affinity reaction showed a relatively small peak at pH 6.5, followed by a decline and then a sharp increase as the pH increased from 6.5 to 9.5. The V_{\max} for the low affinity reaction and the K_m values for both reactions showed maximum values at pH 6.5. The urea-N concentration at which both reactions contributed equally to the overall urease activity varied depending on soil type and pH.

The increasing amount of salts decreased the activity of urease and hence resulted in the maximum delay in urea hydrolysis. Urea hydrolysis was faster in recently reclaimed sodic soils than in unreclaimed soils (Fidaleo and Lavecchia, 2003). Increasing salt levels decreased the hydrolysis of urea in the two soils with decreasing first-order rate coefficients in both the fine sandy loam and a silty loam. Also, the nitrification rate decreased by 50% and 70% in the two soils as salinity increased.

Generally the term $[P]/K_p$ in Eq. (1) can be assumed to be much smaller than 1 if the kinetic data can be analysed by the initial-rate method. Therefore, Eq. (1) can be changed into Eq. (9)

$$\nu = \frac{V_{\max} \cdot [S]}{K_m + [S]} \quad (9)$$

However, high pH and low microbial activity of saline-sodic soils such as the newly reclaimed tidal soils can influence the rate of urea hydrolysis. The V_{\max} in Eq. (9) can be changed as follows;

$$V_{\max} = \frac{k \cdot [E]_0}{1 + \frac{[H^+]}{K_{ES1}} + \frac{K_{ES2}}{[H^+]}} \quad (10)$$

where $K_{ES,1}$ and $K_{ES,2}$ are the molecular dissociation constants for the free enzyme, and $K_{ES,1}$ and $K_{ES,2}$ are those for the enzyme-substrate complex. Shown in Eq. (10), V_{\max} can be decreased as $[E]_0$ decreased in soil due to high pH and salt contents, which can affect the microbial activity and concentration of $[H^+]$ in soil solution. The decrease in V_{\max} leads to decrease in the rate of urea hydrolysis in Eq. (1).

In estimating the quantities V_{\max} and K_m at each pH, the plot (Fig. 6) indicates that pH effects on K_m are much smaller than those on V_{\max} , at least over the pH range considered.

Experimental and predicted time course of urea

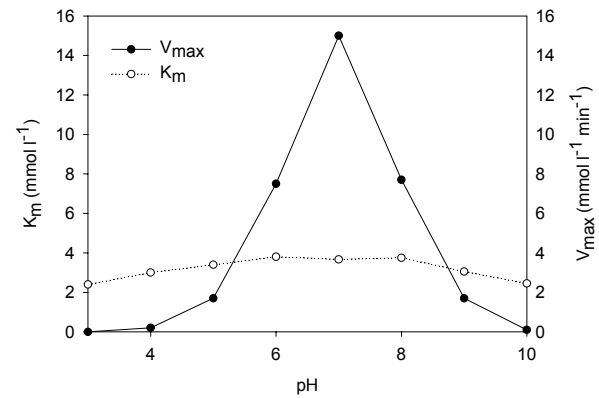


Fig. 6. Estimated V_{\max} and K_m at 25°C and various pH values.

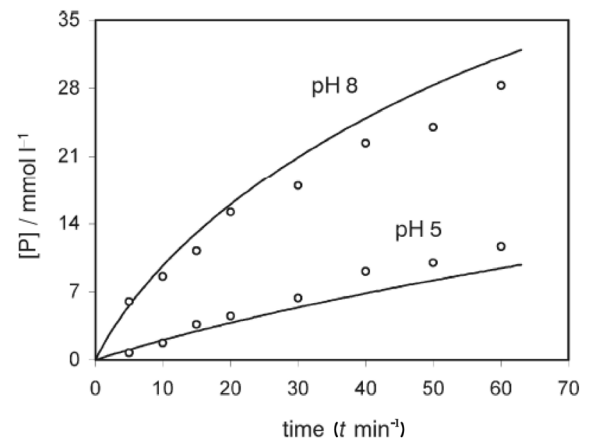


Fig. 7. Experimental and predicted time course of urea hydrolysis at 25°C and two different pH. Solid curves are calculated from eqns. [5], [10], and [11] using the given quantities.

hydrolysis at two different pH (Fidaleo and Lavecchia, 2003) revealed that the two equations can be obtained as:

$$-\frac{d[S]}{dt} = \nu([S], [P], [E]_0, T, pH) \quad (10)$$

$$-\frac{d[S]}{dt} = 2\nu([S], [P], [E]_0, T, pH) \quad (11)$$

As the initial conditions, $[S]$ and $[P]$ at time $t=0$ are greater than 0 and zero, respectively. An examination of Fig. 6 revealed that the kinetics of product formation at pH 5 and 8 was fairly well described by the appearance and accumulation of ammonium and carbonate ions due to active enzyme fraction in soil solution. The kinetics of product formation at different pH levels can describe the appearance and accumulation of ammonium and carbonate ions.

Conclusion

The methodology adopted in this study tried to get a convenient way of estimating rate for N transformation needed in N fate and transport studies by reviewing pH and salt contents which can affect the microbial activity which is closely related to the rate of urea hydrolysis. High saline-sodic condition of a newly reclaimed tidal soils affect the rate of urea hydrolysis as compared to that of normal soil. The hydrolysis of urea over time followed first-order kinetics and soil urease activity in reclaimed soils was represented by Michaelis-Menten-type kinetics. However, high pH and less microorganisms may delay the hydrolysis of urea due to decrease in urease activity with increase in pH. Therefore, the kinetics of urea hydrolysis may differ from Michaelis-Menten-type kinetics due to soils widely differing in salinity and sodicity. The hydrolysis of urea was represented using first-order kinetics with an activation or lag time to account for microbial dynamics. Thus, the rate of urea hydrolysis can be decreased with increasing soil pH and salt contents in a newly reclaimed tidal soils which have high H^+ and salt contents.

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