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UREA HYDROLYSIS IN SATURATED LOAM SOIL

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ABSTRACT

In order to evaluate the extent of loss of applied fertilizer urea due to hydrolysis catalyzed by the enzyme urease and assist in the development of mathematical model for the spread of urea from the source of application, it is necessary to conduct hydrolysis studies in wet soils. Agricultural loam soil, with 13% clay content from west coast region of India, was taken up for this purpose. Maximum particle size of the soil was restricted to 2 mm in the study. Batch trials were conducted with different urea concentrations maintained in the soil which was previously incubated at 27^0C for 48 hours under saturated condition. Evolved gases were allowed to escape to prevent build up of alkali and the subsequent deactivation of the enzyme at high pH. Urea estimation was carried out by colorimetric method. Results indicate that for soil solution urea concentration up to 43.6 mg/mL, the rate of hydrolysis increased with increasing initial urea concentration. For concentrations in the range of 43.6 to 243 mg/mL, rate of hydrolysis decreased with increasing initial urea concentration. The urease induced hydrolysis was completely deactivated at concentration of 305 mg/mL and beyond, due to the substrate induced inhibition. The experimental data could be fitted to a substrate inhibition model.

Keywords: loam soils, enzyme, hydrolysis, urea.

INTRODUCTION

Most of the applied fertilizer urea in wet agricultural soils is lost by various mechanisms such as surface runoff, leaching, ammonia volatilization and nitrification – denitrification [1]. The first two of these losses can be curtailed by controlled irrigation in the wet fields. However, the remaining two types of losses occur due to hydrolysis of the applied fertilizer by the enzyme urease present in the agricultural soils and by the subsequent conversion of evolved ammonia respectively.

Urea hydrolyzes in wet soil under the action of urease enzyme into ammonia and carbon dioxide can be described by Equation 1.

$$NH_2\text{-}CO\text{-}NH_2 + H_2O \overset{\text{urease}}{\Leftrightarrow} [\text{urea-urease complex}] \overset{\text{irreversible}}{\rightarrow} 2NH_3 + CO_2 \quad (1)$$

Mobile urea present in soil solution diffuses and attaches to the immobilized urease enzyme, forming a complex and subsequently decomposing into ammonia and carbon dioxide irreversibly [2] as per Equation 1.

The study of hydrolysis is essential in the development of methods for combating urea loss due to volatilization. Parameters affecting the rate of hydrolysis are temperature, previous soil history [3], soil bulk density [2], moisture content [4], pH, organic content and rate of urea application [5]. Measurement of urease activity can be an indirect indication of organic content of a soil [6]. At lower concentrations of urea in wet soil, the kinetics of hydrolysis follows first order [7].

The present work was carried out with an aim of generating hydrolysis kinetic data required for modeling of urea diffusion from large sized applications such as briquettes. Utilization of urea in briquetted form gives rise to a zone of high concentration around the briquette as urea dissolves [1]. Concentration gradually decreases to zero at sufficient distance from the shrinking briquette. Understanding the kinetics of hydrolysis in these zones can help build mathematical models for diffusion coupled with simultaneous elimination by hydrolysis, as the concentration is likely to persist for a long duration if urea is transferred by diffusion with simultaneous adsorption, and convection effects are negligible.

MATERIALS AND METHODS

Loam soil was obtained from an agricultural field in western coastal region of India. The field was continuously subjected to rice cultivation for nearly four decades. Characterization of the soil was carried out as per the Indian Standards [8]. The soil has an organic content of 0.323%, is acidic and the clay content was estimated to be 13%. Both the factors, viz. acidic pH and presence of organic matter can be expected to stimulate urease activity in the soil.

The loam soil sample was dried in air since drying the soil in sunlight or heat can destroy residual enzyme left in the soil. Twenty five grams of dried soil, passing through 2mm sieve was mixed with 10g of distilled water so as to saturate the soil. This wet soil was spread in a Petri dish, covered with a cloth (kept wet by immersing ends in water) and incubated for 48 hours at 27^0C. Petri dish provides large aerated surface area. This in turn is required for the growth of aerobes generating enzyme urease. Porous cloth allows escape of evolved gases of hydrolysis viz. CO_2 and NH_3, since ammonia build up in the soil solution can hamper hydrolysis by virtue of high alkalinity generated. By maintaining cloth in wet condition, air inside the Petri dish was maintained at saturation humidity [9], thus preventing evaporation of water and drying out of the soil. Required quantities of urea was added to the soil after the incubation period and homogenized, spread in the Petri dish again, covered by wet wool cloth. Samples were withdrawn at regular

intervals of time, analyzed for urea concentration. Sketch of the experimental setup is illustrated in Figure-1.

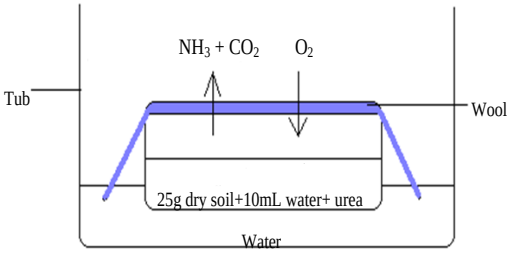

Figure-1. Schematic of the experimental setup.

Urea concentration in wet soil was estimated as per the colorimetric method reported by Marc Pansu and Jacques Gautheyrai [10]. Urea from the wet soil sample was extracted using 50 mL of 2M KCl containing 5ppm Phenyl Mercuric Acetate (inhibitor for urease). An aliquot (1 to10mL) of this filtered solution was taken up for assay such that urea content withdrawn was in the range of 0 to100µg. This solution was mixed with the coloring solution containing Thiosemicarbazide and Diacetyl Monoxime under acidic condition. Red color was developed by heating at 98^0C for 15 minutes. Absorbance of the solution with red color, measured at 520nm is directly proportional to urea concentration.

Most of the enzymes are very sensitive to pH and temperature variations. Hence while conducting trials, it is necessary to maintain pH and temperature. In agricultural fields, pH of the soils is not controlled and the *in situ* pH can vary depending upon the location and the source of applied urea. Since the present kinetic study was conducted with an aim of generating kinetic data that can closely describe the hydrolysis of diffusing urea in wet agricultural soils, pH of the soil sample taken up for the hydrolysis study was not controlled by adding pH buffers. Nevertheless, evolved gases were allowed to escape, thereby preventing buildup of inhibitory alkaline atmosphere.

RESULTS AND DISCUSSIONS

Urea concentration in soil solution (mg-urea/mL-solution) was computed by multiplying the weight fraction of urea in soil solution (mg-urea/g-solution) with the corresponding density of urea solution in g-solution/mL-solution [11]. Experiments were conducted with initial urea concentrations from 5mg/mL to 305mg/mL.

The concentrations of urea at different intervals of time were converted to non dimensional form by dividing it with the corresponding initial concentration of urea in soil solution, C_0. The variation of non dimensional concentrations with time for various initial concentrations of urea is shown in Figure-2.

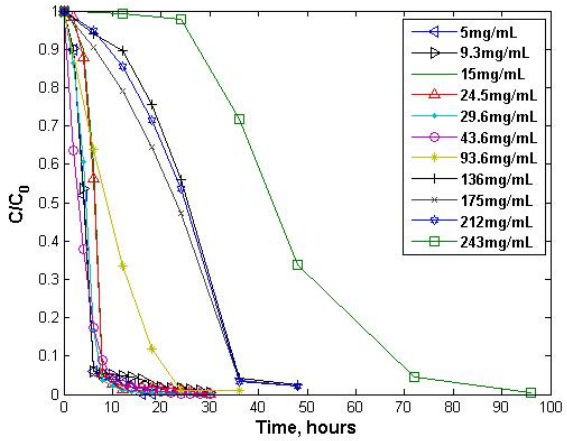

Figure-2. Non dimensional concentration(C/C_0) versus time for various initial soil solution urea concentrations (C_0) in the range of 5mg/mL to 243mg/mL

About 90% of soil urea was hydrolyzed in 5 to 8 hours with initial urea concentrations from 5 to 43.6 mg/mL (Figure-2). Time taken for the same quantum of hydrolysis increased from 19 hours with concentration of 93.6 mg/mL, to about 67 hours with concentration of 243 mg/mL. Hydrolysis practically ceased at initial urea concentration of 305 mg/mL and no change in urea concentration was observed with time at this initial urea concentration.

Initial rates of hydrolysis were computed manually by measuring initial slope at each of the initial concentration. Data of initial hydrolysis rate is plotted as a function of initial urea concentration in Figure-3.

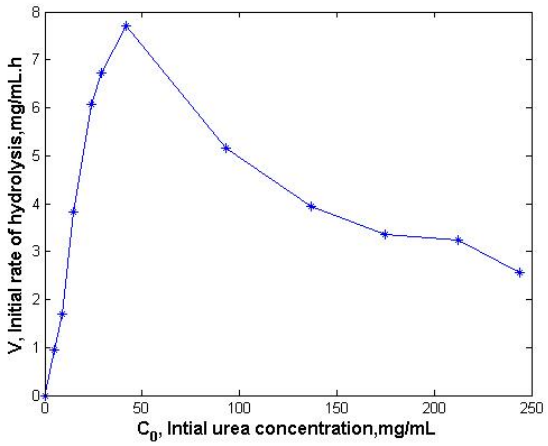

Figure-3. Variation of experimental initial hydrolysis reaction rates with initial urea concentrations

The initial rate of hydrolysis increased gradually for the initial urea concentration range of 5 mg/mL to 43.6 mg/mL (Figure-3). In this case, as the initial concentration increased, rate of reaction also increased signifying first order reaction. However, beyond initial urea concentration of 43.6 mg/mL, the rate of reaction decreased with initial

urea concentration due to increased inhibition by the high substrate concentration.

KINETICS

Two different kinetic models were considered for fitting the experimental rate data. They are Michaelis Menten model and Substrate Inhibition model. Michaelis Menten model [12] approximately describes kinetics of many enzymes in absence of various types of inhibitions and is represented by Equation 2.

$$V = \frac{V_{max}\, S}{K_S + S} \qquad (2)$$

For the model, V_{max} represents the maximum rate of reaction and K_s is the initial substrate concentration at which rate of reaction (V) is $\frac{V_{max}}{2}$.

Substrate Inhibition model, also referred to as Haldane model [12], is extensively used to describe enzyme kinetics and is represented by Equation 3.

$$V = \frac{V_{max}\, S}{K_S + S + (S^2/K_I)} \qquad (3)$$

Here, K_I measures the sensitivity of the enzyme to inhibition by inhibitory substances. When K_I is very large, the model equation simplifies to Michaelis Menten equation simultaneously signifying reduced or no inhibitory effect. Alternatively, higher values of $\frac{K_S}{K_I}$ indicates higher degree of inhibition and vice versa. If substrate is inhibitory, it is not possible to observe actual V_{max} and subsequently K_s takes a hypothetical meaning. Critical substrate concentration (S^*) occurs when $\frac{dV}{dS} = 0$. S^* and V^* (corresponding to S^*) can be calculated from Equations 4 and 5 respectively.

$$S^* = \sqrt{K_S K_I} \qquad (4)$$

$$V^* = \frac{V_{max}\, S^*}{K_S + S^* + (S^{*2}/K_I)} \qquad (5)$$

A peak in the rate of reaction (V^*) versus initial concentration at S^* can be observed for enzymatic reactions which follow the Substrate Inhibition model. Data were fitted using Matlab 2007b for both the models. Statistical and kinetic parameters of both the models are listed in the Table-1.

Table-1. Statistical and estimated kinetic parameters of Michaelis Menten and Substrate Inhibition models

Model	V_{max} mg/mL.h	K_S mg/mL	K_I mg/mL	V^* mg/mL.h	S^* mg/mL	RMSE %	Mean Absolute Error %	R^2
Michaelis Menten	6.0	22.9	----	----	----	53.24	43.54	0.050
Substrate Inhibition	32.8	95.14	22.09	6.368	45.84	29.28	18.13	0.9545

It can be observed from Table-1 that, in the case of Substrate Inhibition model, RMSE as well as Mean Absolute Error values are high, and the corresponding R^2 value is low. Initial hydrolysis reaction rates predicted from Michaelis Menten model and Substrate Inhibition model are compared with the corresponding experimental rate data. Predicted data from both models and the experimental data are plotted in Figure-4.

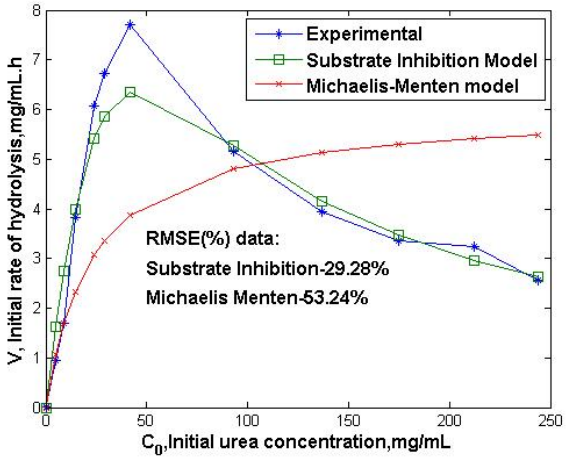

Figure-4. Variation of experimental hydrolysis rate, predicted rates from Michaelis Menten and Substrate Inhibition with initial urea concentration

From Table-1 and Figure-4, it is evident that Substrate Inhibition model provides a better representation of the experimental result in comparison with Michaelis Menten model. Hydrolysis of urea in presence of enzyme urease is highly susceptible to inhibition at high concentration of urea. Since Michaelis Menten model describes enzyme kinetics strictly in absence of various types of inhibitions, it is ineffective in explaining the peak observed in the initial rate of reaction, V^*. Peak observed in the initial rate is a characteristic of Substrate Inhibition model as mentioned previously.

For the fitted parameters of the Substrate Inhibition model, since the value of K_S is 4.3 times K_I and V_{max} is nearly five times V^*, it is possible to conclude that inhibition effect is strong. The observed peak V^* from Table-1 (6.368 mg/mL.h) approximately matches with the experimental peak of 7.7 mg/mL.h However, better agreement is observed for S^* from the Substrate Inhibition model (Table-1, 45.84 mg/mL) with the experimental S^* of 43.6 mg/mL.

CONCLUSIONS

Experiments were conducted in saturated loam soil with different initial urea concentrations. By measuring the initial slopes of unhydrolyzed quantum of urea vs. time, initial rates of reactions were estimated for different initial concentrations of urea. Initial rates of hydrolysis reactions increased up to an initial urea concentration of 43.6 mg/mL and subsequently the rate decreased with increase in initial urea concentration on account of inhibition caused by high substrate concentration. Substrate inhibition model provided a good fit for the experimental rate data in comparison with Michaelis Menten model.

REFERENCES

[1] Kesava Rao 1987. Large granules and slow release urea-A state of art report, Indian Institute of Science, Bangalore, India.

[2] Savant N.K., James A.F. and McClellan G.H. 1987. Effect of soil bulk density on hydrolysis of surface applied urea in unsaturated soils, Fertilizer Research. 11: 221-229.

[3] Xu J.G., Heeraman D.A. and Wang Y. 1993. Fertilizer and temperature effects on urea hydrolysis in undisturbed soil, Biology and Fertility of Soils. 16: 63-65.

[4] Sahrawat K.L. 1984. Effect of temperature and moisture on urease activity in semiarid tropical soils, Plant and Soil. 78:401-408.

[5] Overrein L.N. and Moe P.G. 1967.Factors affecting urea hydrolysis and ammonia volatilization in soil, Soil Sc. Soc. America Journal. 31: 57- 61.

[6] Omar A. Salman, George Hovakeemian and Nadia Khraish 1989. Polyethylene coated urea 2. Urea release as affected by coating material, soil type, and temperature, Ind. Eng. Chem. Res. 28: 633-638.

[7] Liwang Ma, Lindau C.W, Hongprayoon C., Burhan W., Jang B.C., Patrick W.H. and Selim H.M. 1999. Modeling urea, ammonium, and nitrate transport and transformations in flooded soil columns, Soil Science. 164: 123-132.

[8] Indian Standards 2720.1987. Method of test for soils, Bureau of Indian Standards (BIS), New Delhi, India.

[9] Yadav D.S., Vinod Kumar, Mahendra Singh and Relan P.S. 1987. Effect of Temperature and Moisture on Kinetics of Urea hydrolysis and Nitrification, Aus. J. Soil Res. 25:185-191.

[10] Mark Pansu and Jacques Gautheyrai 2006. Handbook of Soil Analysis, Mineralogical, Organic and Inorganic Methods, Springer-Verlag, p511.

[11] Urea and Urea Solution, Storage, Handling and Dilution. 2006. Terra Industries Inc., Sioux, USA, pp. 4 - 5.

[12] Bailey J.E. and Ollis D.F. 1986. Biochemical Engineering Fundamentals, 2nd Edition, Mc-Graw Hill Inc.

Notations

C	Concentration of urea in soil solution, mg/mL
C_0	Initial concentration of urea in soil solution, mg/mL
K_s	Half saturation constant, mg/mL
K_I	Inhibition constant, mg/mL
RMSE	Root Mean Square Error
S	Substrate (urea) initial concentration, mg/mL
S^*	Critical initial substrate concentration at which $\frac{dV}{dS} = 0$, mg/mL
V	Initial rate of reaction, mg/mL.h
V_{max}	Maximum initial rate of reaction, mg/mL.h
V^*	Initial rate of reaction corresponding to S^*,mg/mL