

REVIEW

UREASE ACTIVITY IN SOILS: A REVIEW

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Abstract: Extensive literature on the unique soil enzyme urease is reviewed and presented to create vigilance among local scientists, and to motivate them to conduct more research on urease activity under Sri Lankan conditions. Soil urease is mainly microbial in origin, and its activity in soils is due to accumulated urease. However, comparison of reported urease activity levels in different soils was extremely difficult as the assay procedures adopted in the various studies were different. Further, this enzyme is remarkably stable in organo-mineral complexes found in soil. Factors affecting urease activity and correlations between urease activity and soil properties are discussed. Problems associated with high urease activity, i.e. gaseous loss of N and plant damage due to ammonia gas and accumulated nitrite, and the effectiveness of most recently found compounds in overcoming these problems are also discussed.

Key Words: Soil, urea hydrolysis, urease activity, urease inhibition

1. INTRODUCTION

The requirement for nitrogenous fertilizers for increasing agricultural production is well known. Among the commercially available nitrogen fertilizers, urea is the most widely used source of N.¹ The use of urea is steadily increasing worldwide and this trend is likely to continue.² The situation in Sri Lanka is similar to the global scenario. Urea supplies > 63 % of total N consumed in Sri Lankan agriculture, especially in the paddy and field crop sectors.³ This may be attributed to several advantages of urea over other nitrogenous fertilizers, viz. (1) low cost per unit of N applied (2) low transport cost, and (3) less danger of increasing soil acidity.⁴

However, efficiency of urea rarely exceeds 50%, and N losses alone can be in the region of 50% when urea is applied to rice fields,⁵ or soil surfaces without incorporation.⁶ Nitrogen losses occur when urea is hydrolysed mainly due to the activity of the soil enzyme, urease.⁷⁻¹² In most arable soils, urea is rapidly converted to ammonia and CO₂ by soil urease and this leads to several problems including nitrite and ammonia toxicities to young seedling plants, and loss of ammonia through volatilization. Therefore, new technologies are needed to

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make urea a more effective fertilizer and this will not only help in increasing food production but will also minimize environmental pollution.

One approach to increase the efficacy of urea would be to control the hydrolysis of urea by soil urease and for this a thorough understanding of the urease activity within the soil is essential. Urease, (urea amidohydrolase EC 3.5.1.5) was first crystallized from jack bean (*Canavalia enciformis*) meal by Sumner in 1926 and for which he received the Nobel Prize.¹³ It was the first enzyme ever to be extracted and studied. Until recently most of the knowledge on urease has been derived from studies of the jack bean urease (see reviews of Kiss *et al.*,¹⁴ Bremner & Mulvaney,¹⁵ and Gould *et al.*¹⁶). However, in order to understand and control the urease activity in soil, studying its behaviour within the soil matrices is essential. Priority has been given to this aspect during the past several years¹⁷⁻²³ and this has enabled scientists to identify more effective compounds that inhibit urease activity in soil.^{13,16,24-26}

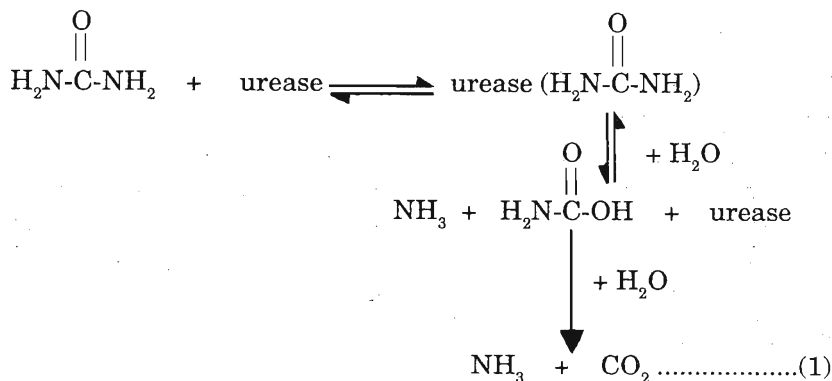
However, information on urease enzyme pertaining to Sri Lankan conditions is rather meagre.²⁷⁻³³ Therefore, further research on urease activity specific to local situations should be conducted.

2. UREA HYDROLYSIS AND SOIL UREASE

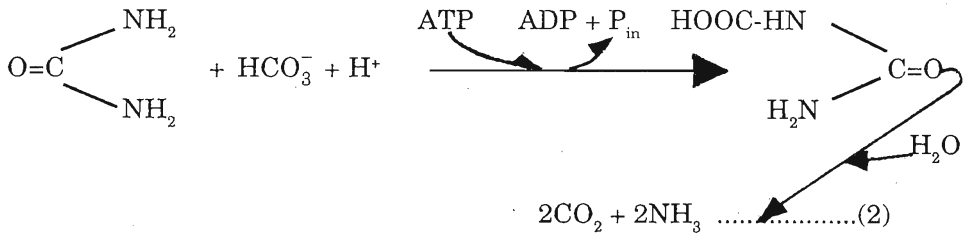
2.1. Urea hydrolysis

In soil, urea is decomposed enzymatically to CO₂ and NH₃. Effects of various treatments on urea decomposition activity in soil can often be related to those obtained with pure urease. Therefore, it has been assumed, probably correctly, that the reaction is catalyzed by soil urease. This enzyme was first examined by Rotini in 1935 as reported by Bremner & Mulvaney¹⁵; and pioneering work was done subsequently by Conrad in the early 1940s.⁷⁻¹²

The currently accepted reaction mechanism of enzymatic hydrolysis of urea is the enzymatic cleavage of urea to ammonia and carbamic acid, followed by the chemical hydrolysis of carbamic acid to ammonia and carbon dioxide.¹⁶ (equation 1)



However, an alternative pathway of hydrolysis of urea, not involving urease, has been demonstrated in yeast, algae, and in some fungi.³⁴ This is a two step reaction where urea is first converted to allophanate by the action of urea carboxylase (urea-CO₂ ligase, EC 6.3.4.6) and then allophanate hydrolase (allophanate amidohydrolase, EC 3.5.1.13) converts allophanate to CO₂ and NH₃ (equation 2).



Ladd & Jackson³⁴ believed that since the initial carboxylation steps require ATP, this pathway would be confined to live cells in the soil. The significance of this pathway for urea hydrolysis is therefore doubtful.

Urease hydrolysis, as in any enzymatic reaction, may only be needed to reduce activation energy for the formation of the intermediate product. Even in the absence of enzymes, urea can be hydrolysed physico-chemically. However, chemical hydrolysis is very slow compared to biochemical enzymatic hydrolysis.³⁵ Therefore, it can be concluded that urea hydrolysis in soils is mainly brought about by the action of the enzyme, urease.

2.2. Origin and locus of soil urease

2.2.1 Origin

Urease activity in soil may originate from plant residues, animal waste or soil microbes^{10,36} containing urease. Plants are rich sources of ureases.³⁷ However, there is no direct evidence for the production of urease by plant roots.³⁶ Urease is also reported to be present in animal intestines and excreta. Therefore, the addition of plant materials and animal wastes may supply urease to the soil. Skujins³⁸ reported that soil urease is of microbial origin. Sumner³⁹ also identified some species of bacteria, yeast and fungi which contained urease. Most of the nitrosomonas and nitrosospira isolated from soils in Scotland were capable of hydrolysing urea.⁴⁰ The ability to hydrolyse urea was found to vary from 17 to 77% for soil bacteria, and from 78 to 98% for soil fungi.^{41,42}

Urease has been purified from certain bacterial species and the factors affecting the production of urease by micro-organisms has also been studied. For

the production of urease within a microbial cell, N is essential. But certain forms of N for example ammonium ion, nitrate and amino acids have been reported to have an adverse effect on microbial urease production.⁴³ However, McCarty *et al.*⁴⁴ found that the microbial production of urease had not been directly repressed by NH_4^+ or NO_3^- , but by products formed by microbial assimilation of these forms of N. Therefore, it was suggested that urease synthesis by micro-organisms is controlled by the global N regulation within the soil.

2.2.2 Locus

It is generally assumed that micro-organisms are the chief agents in soils directly responsible for urea hydrolysis.⁴⁵ According to Conrad^{7,8} the reaction may be catalytic in part rather than completely biological. However, soil ureases are partly extracellular being liberated during microbial and plant root metabolism and death. They are also intracellular as part of the soil biomass. In the latter, urease exists as a component of the cytoplasm or is attached to the cell membrane. In the former case it would be attached to the soil colloids.⁴⁶ Urease as an endoenzyme will act more rapidly on urea if it enters cells in high concentrations. Similarly, if after lysis, the enzyme is released, it will act more intensely on the substrate.¹⁴

After reviewing the extensive literature available Kiss *et al.*¹⁴ concluded that ureolysis in soils is primarily due to accumulated urease. Paulson & Kurtz⁴⁷ stated that under steady state conditions, 79 to 89% of the urease activity of Drummer silty loam soil was due to urease adsorbed on soil colloids. Burns *et al.*⁴⁸ believed that for an enzyme to be persistent in soil it needs to be incorporated into organo-mineral complexes. Burns⁴⁹ postulated ten reasonably distinct categories of soil enzymes that are associated with different soil constituents (e.g. viable micro-organisms, cell debris, clay and humic colloids). He also reported that enzymes may change location with time. For example, intracellular urease in a viable cell will be associated with cell debris after subsequent cell death and lysis; this will then be released to aqueous phase as cell membranes are broken down and later be adsorbed to clay surfaces.

2.3 Biochemistry of soil urease

Urease (urea amidohydrolase, EC 3.5.1.5) hydrolyses non peptide C-N bonds in linear amides.¹⁵ Molecular weight of jack bean urease is 480,000.⁵⁰ There are forty seven -SH groups in the urease species of molecular weight 480,000, and it has been estimated that 4 to 8 of these groups are essential for activity.⁵¹

It has long been believed that jack bean urease is absolutely specific for urea, and is completely devoid of metals. But later findings confirmed that the enzyme can act upon hydroxy urea, dihydroxy urea and semicarbazide⁵² and that it

contains nickel, and is probably a metallo-enzyme.⁵³ Although it acts on substrates other than urea, the substrate specificity of urease is relatively high.³⁴ The specific role of the metal ion in the catalysis has yet to be defined. Ladd & Jackson³⁴ speculated that possibly Ni^{2+} is chelated to amino acid residues and is so positioned at the enzyme's active site, that on formation of the enzyme-substrate complex, the metal stabilises polarised positive charge on the carbonyl group of urea, thus promoting nucleophilic displacement of the nitrogen atom.

Maximum catalytic activity of jack bean urease occurs at 65°C ³⁹ and it is inactive at temperatures above 70°C .³⁷ The pH optimum for jack bean urease, usually lies between 6.0 and 7.0.^{17,20} Urease has an isoelectric point of 5.1.⁴⁶

2.3.1 Principles and biochemistry

Some authors reported urea hydrolysis to be a first order reaction with respect to the concentration of urea^{54,55} while others found zero order kinetics for urea.^{56,57} These different findings may be reconcilable in part on the basis of the method of urea application. Kumar & Wagenet⁵⁵ applied urea to the surface of soil and created a situation that may have resulted in high concentration of urea in the surface layer of the soil with limited contact with soil urease. It is conceivable that hydrolysis of surface applied urea was preceded by dilution through diffusion to deeper layers. The diffusion process is controlled by the urea gradient and thus may have caused urea hydrolysis to be apparently first order. In case of thorough mixing of urea with soil the urea diffusion step is eliminated, and the kinetics of urea hydrolysis become apparently zero order.⁵⁶ The behaviour of urea hydrolysis is substantially different when urea is heterogeneously distributed in the soil. Velk & Carter⁵⁷ observed that when prilled urea was placed in two calcareous soils, the hydrolysis of urea closely fitted first order kinetics initially followed by a shift to zero order kinetics. They attributed the first order phase to the limited contact between urea and soil urease or to possible inhibition of urease activity by urea at the site of placement⁵⁸ or to both.

2.3.2 Kinetics of urease activity

The activity of any particular enzyme in soil is a composite of activities associated with various biotic and abiotic components.⁴⁹ Enzymes within soluble and insoluble organic matrices, function in an environment completely different from that of enzymes in free solution. Therefore, the catalytic properties of urease varied widely in different soils and they differ significantly from those of crystalline jack bean urease with which they are frequently compared.

Soil urease, like free jack bean urease shows Michaelis-Menton kinetics.^{17,20} In a heterogeneous system like soil, urease is rapidly adsorbed to a matrix structured by different amounts and types of humic substances as well as clays,

and most K_m (Michaelis constant) values therefore vary over a range somewhere between 1.3 to 330mM, depending on the method of determination, and physico-chemical properties of the soil.^{17,20,59-62} Values for soil ureases in particle size fractions differed, ranging from 1.1 to 4.9mM; no trend was evident.⁶⁰ The K_m value for free urease (29.4mM) was lower than those of urease immobilized on kaolinite, montmorillonite and mineral constituents of a silty loam soil.¹⁷ Native soil urease has K_m values much lower than that of immobilized and free ureases. In contrary to this, others have found that the K_m of soil urease is 4 to 10 times higher than the average K_m values in the solution.⁶⁰⁻⁶² The difference in K_m value may be attributed to stable organo-mineral urease complexes or clay-enzyme associations. Makboul & Ottow⁶³ reported that montmorillonite and kaolinite enhanced the K_m values of urease considerably more than illite. Similar results have been reported elsewhere.¹⁷ According to Vaughan & Ord,⁶⁴ humic substances present in soils altered K_m and V_{max} (maximum velocity) values of a purified urease obtained from *Bacillus pasteurii*. Therefore, if great variations in the K_m of soil urease is to be explained, both the organic matter contents^{38,65} as well as the type and amount of clay minerals indigenous to the soil should be considered.⁶³

The differences in Michaelis-Menton constants may reflect varying rates of diffusion of urea substrate to other active sites of the bonded urease; in which case properties such as the nature and molecular size of the organic ligands may be important. Differences in K_m values could also be attributed to differences between ureases of different origin or to differences in the structure and charge distribution in the enzyme active site induced by complex formation.³⁴

2.3.3 Reaction energies of soil urease

The free energy of activation (ΔG_a) of soil urease is greater than that of free urease¹⁷ and ranged from 16.3 to 102.4 kJ/mole.^{17,55,66-68} ΔG_a and enthalpy of activation (ΔH_a) of urease immobilized on kaolinite and soil mineral constituents were similar to those of free urease but were lower than those of native soil urease.¹⁷ Immobilization of urease on 2:1 clay resulted in a lower ΔH_a value as compared with its immobilization on 1:1 clay.¹⁷ The enthalpy of activation (ΔH_a) of urease immobilized on the mineral constituents of soils were found to be similar to that of urease immobilized on kaolinite. A large ΔH_a indicates that a large perturbation or even breaking of chemical bonds is necessary for the formation of transition state.⁶⁹

The average activation energy (91.5 kJ/mole) of urease in 15 soils, assayed in the presence of toluene, was significantly higher than that (21.7 kJ/mole) of urease from the same soil.⁶⁸ They also observed that the free energies of activation of soil ureases, with or without toluene, were similar. The greater activation energy of soil urease in the presence of toluene is consistent with

greater energy requirement for formation of enzyme-substrate complexes in soils, where free intracellular ureases are eliminated and the active enzymes are those adsorbed or complexed with soil colloids.

2.3.4 Optimum pH

The optimum pH of soil urease activity has been reported to be 6.5 to 7.0⁵⁹ similar to that of jack bean urease.^{15,17,70} However, others have reported that the optimum pH for native urease is 8.8 to 9.0^{17,71-73} or even less than 5.87.²⁹ Nannipieri *et al.*⁶² observed a slightly alkaline pH optimum (pH=8.3) in soil extracts. The pH optimum of the enzyme is dependent on the buffer used.¹⁵ Pettit *et al.*⁶¹ using both phosphate and Tris buffers, found that the pH optimum for a urease active soil extract was 6.5 to 7.0, similar to that of the unextracted soil (6.5). In contrast, Nannipieri *et al.*⁶² using phosphate and borate buffers, showed that the pH optimum of a soil urease extract was 8.3.

3. UREASE ACTIVITY IN SOILS

3.1 Levels of urease activity in soils

Comparing the current information on the levels of urease activity is difficult due to the wide divergence in the methods adopted for assaying urease activity. Use of buffer and toluene is common in most of these assays, but the pH of buffer, incubation temperature, substrate concentration and the time of incubation varied greatly. Moreover, the results also have been expressed in a variety of units. Like other soil enzyme assays, the measured urease activity reflects only the activity of the soil, expressed under the chosen assay conditions. This may not accurately relate to the actual or potential urease activity in the natural soil environment.

Correlations between urease activity and various soil properties have been reported by several researchers. Sing *et al.*⁷⁴ found urease activity to be positively related to organic carbon (OC), cation exchange capacity (CEC), silt and clay, and negatively related to pH and sand irrespective of depth. Speir *et al.*⁷⁵ reported urease activity to be highly correlated to OC, total sulphur, total N (TN) and protease activity. Dash *et al.*⁷⁶ found a positive correlation between urease activity and TN, OC, silt plus clay and specific conductance. Urea hydrolysis rates of cultivated soils positively correlated with OC and TN.⁷⁷ Frankenberger and Dick⁷⁸ found urease activity to be significantly correlated to OC, TN, and CEC. McGarity & Myers⁷⁹ observed that urease activity in 100 Australian surface soils was highly correlated with OC and weakly correlated with pH. This was observed also in profile samples.⁸⁰ Using multiple regression analysis, Zantua *et al.*⁶⁵ showed that most of the variation in urease activity observed in soils was due to organic matter. Silva & Perera²⁹ found that urease

activity of rubber soils of Sri Lanka was significantly related to pH but was not significantly correlated with OC in two of six soil series studied. Wickramasinghe *et al.*³¹ found no relationship between urease activity and OC or texture in acid tea soils but urease activity was dependent on soil polyphenol content.

Soil urease activity is related to the vegetation of the soil.⁸¹ Higher urease activity in soils under vegetation compared to vegetation free soils was observed by Reddy *et al.*⁸² and they attributed it to the higher microbial proliferation and microbial activity at the rhizosphere. Urease activity was greater in the grassland soils and forest soils than in uncultivated soils from Himchal Pradesh, with urease activity being the greatest in the grassland soils.⁸³ Fenn *et al.*⁸⁴ also found that urease activity in non cultivated (40y without cultivation) was much lower than in the cultivated soils. Speir *et al.*⁷⁵ reported that urease activity under rye grass remained constant with time, but in fallow soil urease activity consistently decreased. Voets *et al.*⁸⁵ also observed a decrease in the soil urease activity when plant cover was removed with a herbicide.

Urease activity was found to be the most variable parameter when the spatial variability of several enzyme activities and chemical properties were tested.⁸⁶ However, in common with many enzymes, urease is distributed in decreasing amounts in deeper genetic horizons of soil profiles. The highest urease activity occurs in the surface horizons.^{41,79,80,87} Baligar *et al.*⁸⁸ observed a 1.6 times higher urease activity in surface horizons than in sub surface horizons in Appalachian soils. The major factor influencing the level and distribution of urease activity through the profile is the content of soil organic carbon,^{80,89} but this relationship is obviously modified within particular horizons by other soil properties such as reaction, gleying and soil texture.⁸⁰ Thormahlen and Preez⁹⁰ found that urease activity in sub soil taken from the central irrigation areas of South Africa is higher than that in surface soils.

According to Myers & McGarity,⁸⁰ urease activity in calcareous soils is lower than in non calcareous soils. They also reported that in saline soils urease activity was low. Skujins⁹¹ reported that in alkaline soils the activity is much less, and it is also much less in carbonate rich soils, apparently because of detrimental effect of Ca^{2+} on the urease producing organisms. Kumar & Wagenet⁵⁵ also found that urease activity is lowest in alkaline and saline soils. In a study of urease activity in low land rice soils of the Philippines, Sahrawat⁹² observed the highest urease activity in alkalized Maahas clay (Maahas clay+1.3% Na_2CO_3) and the least in the Malino acid-sulfate soils. He also observed that while the presence of 0.5 % Sodium chloride had hardly any effect on the rate of soil urease activity, alkali increased it.

Delaune & Patrick⁹³ studying the conditions of application of urea to water logged soils, demonstrated that urease activity was much more pronounced in the soil than in flood water overlying the soil. In general, the order of urea hydrolysis in the three main components of the wetland soil system was oxidized soil > reduced soil > flood water (without algae). Presence of algae increased urease activity in flood water.⁹⁴ Delaune & Patrick⁹³ did not detect appreciable urease activity in the flood water of rice soils incubated in the laboratory, but Sahrawat⁹² observed urease activity in flood waters of some rice growing soils and concluded that it may be enough to hydrolyse part of the urea applied on the surface water.

Tiunov⁹⁵ reported a 30-40% high urease activity in earthworm caprolites as compared with just ingested soils. The increased urease activity had remained in the caprolites for long periods after their release. Therefore, a high earthworm activity in soils may increase the risk of ammonia volatilization loss due to enhanced urease activity.

3.2 Factors affecting urease activity in soil

3.2.1 Temperature

Urease in soil is more resistant to high temperature than urease in pure preparations and solutions.¹⁸ Frankenberger & Tabatabai³⁷ reported that the inactivation temperature of plant ureases (70°C) are similar to those found in soils.⁹⁶

Numerous studies have shown that urease activity in soils increased with increase in temperature from 10° to 40°C.^{11,17,18,35,55,57,66-68,97,98} In some soils, urease activity has increased very markedly with the increase in temperature from 40° to 70°C; but decreased rapidly above this temperature range.^{61,98,99} In contrast, Dash *et al.*⁷⁶ reported an optimum temperature of 47°C for urease activity. There is no apparent reason for these markedly different reports. Possibly it may be due to the different assays that were used to measure activity. Dash *et al.*⁷⁶ used a buffer method for urease assay whereas Zantua & Bremner⁹⁸ used a non-buffer method. Sahrawat⁹⁹ showed that the temperature optima for urease activity in soils was nearly the same for both methods.

The findings of Zantua & Bremner⁹⁸ and Sahrawat⁹⁹ indicate that the urease activity in soils has a much higher optima of 60° to 70°C, and they suggest that soil urease is protected from inactivation by some mechanism even at higher temperatures. In general, immobilization of enzymes enhances their thermal stabilities. Persistence of immobilized enzymes to thermal denaturation may be related to the stabilization of the tertiary structure of proteins.¹⁸ Nannipieri *et al.*⁶² reported that humus-enzyme complexes with molecular weight >105 retained 70% of initial activity after pre-incubation at 70°C.

Although inactivation of enzymes has been detected between 65° and 70°C^{17,18,61} urease activity had not been completely destroyed when soils were heated to 75°C for 24h⁹⁸ or between 80° and 90°C for 48h.^{7,8} Urease activity in Indian alfisols and vertisols at 100°C was close to zero when a non-buffer method was used for the assay.⁹⁹ When soils were heated at 105°C for 24h, urease activity was inactivated completely.⁹⁸ This character, inactivation of soil urease at higher temperatures, can be used to minimize N losses following surface application of urea in high urease activity soils. Jackson & Burton¹⁰⁰ controlled gaseous loss of N from grass sods in Georgia (USA) by burning the soil surface before urea was applied to the surface. Similar investigations have been reported elsewhere.¹⁵

Marshall *et al.*¹⁰¹ estimated 26.8 and 1.9 $\mu\text{g N.g}^{-1}$ of soil.h⁻¹ of urea hydrolysis for L-H and B_f horizons respectively for British-Columbia forest soils at 0°C. They also estimated theoretically, that an operational application of urea (200kg N/ha) applied to snow could be hydrolysed within six days in the sites having lowest urease activity. Bremner & Zantua¹⁰² investigated the possibility that enzyme activity can occur in soils at sub-zero temperatures. They found that urease activity could be detected in soils at -10° or -20°C, but not in soils at -30°C. This activity was attributed to the enzyme-substrate interaction in unfrozen waters at the surface of soil particles.

3.2.2 pH

Many investigators have found that pH affects the activity of free urease,^{17,37} native urease,^{29,72,73,103} immobilized urease^{17,18} and urease in soil extracts.^{61,62,104} Generally, free urease is most active at neutral pH and soil urease is most active at slightly alkaline pH levels. But different pH optima values (ranging between 5.87 and 9.0) have been reported in the literature (also see pH optima in biochemistry of soil urease). This divergence may be related to the differences in the buffers and urea concentrations adopted in these investigations, in addition to the variability in soil types.

However, there are some reports which indicate that urease activity is unrelated to pH⁶⁵ or negatively related to pH⁷⁶.

3.2.3 Moisture content

Eventhough water plays an important and complex role in the urea hydrolysis, different results on the effect of water content on urease activity have been reported. In some studies urease activity was not appreciably affected by the soil water content.^{15,66,93,105} There are reports, however, that urease activity in soil is related either negatively^{67,76,106} or positively^{55,99,107} to the soil water content.

Urea hydrolysis increases with increasing soil water content up to near field capacity, followed by a decreasing trend thereafter.^{57,94,108} Sahrawat⁹⁹ observed a constant urease activity when the moisture content was increased further beyond field capacity. Savant *et al.*⁹⁴ observed a higher rate of urea hydrolysis in soils at field capacity than in water logged soils after 24h incubation. Urea hydrolysis rates decrease below the permanent wilting point⁵⁷ and in dry soils.⁹⁹

Reduced ammonia volatilization losses due to the inhibition of urea hydrolysis at low soil water potentials have been reported.¹⁰⁹⁻¹¹¹ Ferguson & Kissel¹¹² found that 3.1% more of applied urea was hydrolysed in 72h when soil moisture was maintained at levels adequate to support urea hydrolysis than when soil was dried. It appears therefore, that the sensitivity of soil urease to lack of soil moisture can be utilized advantageously through fertilizer management.

3.2.4 Urea concentration

As expected the rate of urea hydrolysis in soils treated with small amounts of urea is found to be much slower than that observed with large amounts of urea.^{54,66,97} Studies have shown that the rate of hydrolysis of urea by soil urease increases with increase in substrate (urea) concentration until the quantity of urea added is saturating and its activity becomes constant.^{15,67,71,103,113} Thormahlen *et al.*¹¹⁴ reported that urea concentration between 12.5 to 100mg N/kg soil was adequate to saturate the urease in soils of the central irrigation areas in South Africa.

Zantua *et al.*⁶⁵ reported urease activity to be positively related to the total N in soil. Several other workers have also confirmed the above observation.⁷⁵⁻⁷⁸ Kumar & Wagenet⁵⁵ reported that urease activity is independent of N concentration upto 1000 $\mu\text{g N/g soil}$; and that responses were similar for different Indian soils tested.

The hydrolysis of urea in soil generally follows Michaelis-Menton kinetics, eventhough soil is a highly heterogeneous system. This phenomenon, that is the increase in the rate of hydrolysis with the increasing substrate until enzyme is saturated, has been reported by several researchers.^{17,20,67,71} However, at very high urea concentrations the hydrolysis rate decreases probably due to either uncompetitive substrate inhibition of the enzyme¹¹⁵ or denaturation of enzyme at very high concentrations of urea.¹¹⁶

3.2.5 Oxygen

Ovarien (as reported by Bremner and Mulvaney¹⁵) found that oxygen had a significant effect on the rate of hydrolysis of urea added to an Indian soil, whereas Delaune & Patrick⁹³ found that oxygen had no effect on the rate of hydrolysis of

urea added to Crowley silt loam. Bremner & Mulvaney¹⁵ concluded that since the urea added to soil is hydrolysed largely, if not entirely, by native soil urease, there is no apparent reason why the activity of urease should be affected by O₂. Savant *et al.*⁹⁴ found that O₂ becomes a limiting factor in urea hydrolysis after 12h of submergence. Velk & Carter⁵⁷ observed substantial reduction of urease activity under flooded conditions of some soils. Delaune & Patrick⁹³ observed no difference in the rate of urea hydrolysis in soils under waterlogged and 0.33bar moisture conditions. The divergent findings concerning the effect of O₂ on soil urease activity may have resulted from differences in the amounts of readily decomposable organic materials in the soil.¹¹⁷

3.2.6 Organic matter

Many workers have found that urease activity in soils is positively correlated with organic C and total N,^{65,75-78} which are indices of organic matter content. Zantua *et al.*⁶⁵ suggested that organic matter content of a soil accounted for most of the variations in urease activity. Several workers have observed an increase in soil enzyme activities after incorporation of organic matter into the soil.^{19,118,119} Further, the constituents of the organic matter also determine the activity of urease in soil. These factors will be discussed at length under the topic, soil amendments.

3.2.7 Soil amendments

3.2.7.1 Organic materials

Incorporation of organic materials into soil promotes microbial activity and also soil urease activity.^{19,21,118-120} The increased levels of urease activity in the organic amended soil has generally been attributed to the increased microbial biomass although additional evidence has shown that plant materials and sludges may directly contribute enzyme to soil.^{55,119,121} Micro-organisms associated with the organic materials may also contribute to the urease in the soil enzyme pool.¹²² The urease activity in soil varies depending upon the type and amount of organic matter added. On addition of decomposed organic matter and farmyard manure (FYM) urease activity increased. However incorporation of undecomposed dried grass had no effect on urease activity.⁵⁵ Antil *et al.*¹⁰⁸ observed that the soil urease activity increased with the addition of FYM, sewage sludge and press mud but not with wheat straw. Urease activity increased significantly with application of organic matter to the soils and the stimulation was greater in salt amended soils.¹²⁰

The activity of soil enzyme may be inhibited by addition of certain organic amendments. Perrucci¹²³ found that the addition of organic residues (tomato, maize and wheat straw) inhibited several soil enzymes. The application of dried

sewage sludge and sludge effluents to agricultural lands is becoming a widespread practice in the world. Therefore, much attention has been paid to study its effects on soil enzymes. Frankenberger *et al.*¹²¹ found urease inhibition at lower sludge-loading rates (2.2 and 8.9mg sludge/g soil) and increased urease activity at higher sludge loading rates (22.2, 44.4 and 100 mg sludge/g soil). Reddy *et al.*⁸² observed that increasing concentration of sludge reduced urease activity in soils with no plants (no rhizosphere soil); but, increased the activity in soils with plants (rhizosphere soil). They also found that even in rhizosphere soils at higher rates of sludge (120 t/ha) urease activity was inhibited. The increase observed in urease activity after addition of sludge was attributed to the higher microbial proliferation and activity, while a reduction in urease activity was attributed to the higher concentration of available heavy metals.^{121,124}

According to Martens *et al.*¹²² urease activity increased after the first addition of organic matter while subsequent additions failed to sustain high enzyme activity. Burns⁴⁹ speculated that increasing urease activity after the addition of organic materials could be due to a trigger molecule or promoter released by the decay of organic amendments that stimulates soil organisms to secrete high level of enzymes. However, less response to subsequent additions may be due to a feedback mechanism that terminates the production of enzymes in a situation where adequate energy sources are available.¹²² They also proposed that in a soil receiving constant and regular additions, the process of promotion and suppression may be balanced resulting in a constant level of enzyme activity.

3.2.7.2 Liming

Kumar & Wagent⁵⁵ found that addition of amorphous CaCO_3 at rates of 2, 4 and 8 per cent decreased urease activity. Zantua *et al.*⁶⁵ also observed a negative relationship between natural CaCO_3 and soil urease activities. Kumar & Wagenet⁵⁵ concluded that this can be due to inactivation of urease by amorphous CaCO_3 . It can also be due to change in pH upon the addition of CaCO_3 ; or CO_3^{--} may directly influence the hydrolysis reaction. However, other workers have found that urease activity in soil can be increased by addition of liming materials.⁹⁸

3.2.7.3 Herbicides

Cervelli *et al.*¹²⁵ observed that certain substituted urea herbicides (Fenuron, Monuron, Diuron, Linuron and Neburon) inhibit urea hydrolysis by 10 to 30 per cent in some Italian soils. Wimaladasa & Wickramasinghe³³ studied the effect of Diuron, Paraquat, and Glyphosate on urea hydrolysis in Sri Lankan acid tea soils. None of the herbicides had any significant inhibiting effect on urea hydrolysis even at ten fold increase in the concentration of the herbicide.

However, Gianferda *et al.*¹²⁶ observed an enhanced urease activity in soils treated with glyphosate, paraquat, methanol and carbaryl and an inhibitory effect on immobilized and free urease by methanol, carbaryl and atrazine. Soil urease activity increased with application of monocrotophos, quinalphos, cypermethrin and fenvalerate at 5kg/h and reduced at higher levels (7.5 and 10kg/h).¹²⁷

3.3 Stability and persistence of soil urease

Soils exhibit a substantial proteolytic activity.¹²⁸ Therefore, rapid destruction of urease added to a soil can be expected. Several investigators have shown that jack bean urease is decomposed or inactivated very rapidly when added to the soil.^{48,61,98} Roberge¹²⁹ suggested that added urease is either inactivated or destroyed by proteolysis. A temporary increase in urea hydrolysis due to the addition of urease to the soil has been observed by Conrad⁷ and Moe.¹³⁰

The presence of a constant background level of enzyme activity, independent of microbial proliferation,⁴⁷ and its better correlation with organic matter and micro-organisms exists in soil. Bremner & Mulvaney¹⁵ reported that although most organic materials are metabolized rapidly by micro-organisms, both *in vitro* and *in vivo*, many enzyme-proteins persist as active moieties in the soil for very long periods of time. Zantua & Bremner^{98,119} showed that soil urease activity increased after the addition of organic substances, promoting microbial growth; but subsequently decreased and stabilized at the initial level. Similar observations have been made by Nannipieri *et al.*¹⁹ The stability of the enzyme within the soil matrix has invoked notions of the existence of two types of enzyme protection mechanisms: (1) as an adsorption reaction involving enzyme and clays;¹³¹ and (2) as an enzyme-organic matter interaction involving a physical or chemical binding mechanism.⁷ Stabilization of soil environment by soil organic matter rather than by soil inorganic components has been suggested by Ladd & Butler.¹²⁸ Burns⁴⁹ demonstrated that many enzymes are capable of becoming bound to the humic matter giving the enzyme a persistence they would not otherwise display in the hostile extracellular environment of the soil.

Proteolytic stability of the urease held on aluminium hydroxide was higher than that of enzyme free or adsorbed on montmorillonite and $\text{Al}(\text{OH})_x$ -montmorillonite complexes.¹³² Soil urease and organo-urease complexes extracted from soil are more stable than jack bean urease under comparable conditions.^{23,61} Burns *et al.*⁴⁸ and Pettit *et al.*⁶¹ have proposed that urease is immobilized within the organic matter of organo-mineral complexes and that the organic matter has pores large enough to allow the passage of substrates (urea and water) and products (NH_4^+ and CO_2) but not of proteolytic enzyme.

A possible relationship between micro-organisms, exogeneous substrates and extracellular immobilized enzymes in soil has been proposed.⁴⁹ According to this hypothesis, a close spatial relationship exists between immobilized enzymes and micro-organisms. Most of the activity of any enzyme may be localized in the "active" or "biological" space, that is the space resulting from the summation of those microhabitats where micro-organisms can survive or grow. The activity depends upon activities associated with extracellular immobilized enzymes, ephemeral enzymes free in the soil solution, enzymes attached to entire dead cells and cell debris, and enzymes present within living cells. It seems unlikely that significant amounts of extracellular free enzymes can evade the destructive (non-biological denaturation, adsorption and inactivation, and degradation and proteolytic micro-organisms) and non-destructive forces (adsorption without inactivation) present within and around the microhabitat.⁴⁹ Therefore, the total activity of any particular enzyme in soil may be related to the available "active" or "biological" space rather than total physical space.¹⁹

4. PROBLEMS ASSOCIATED WITH HIGH UREASE ACTIVITY

Although urea is considered as equivalent to other nitrogenous fertilizers,¹³³ poor crop responses to urea have frequently been observed.¹³⁴ The rapid hydrolysis of urea due to high urease activity can result in high soil pH values and high ammonium ion concentrations which are conducive to accumulation of ammonia. The major problems observed in urea fertilization are the loss of volatile ammonia gas and ammonia toxicity to germinating seedlings. Also the accumulation of nitrite in the soil following the hydrolysis of urea can result in toxicity and nitrogen losses.

4.1 Loss of ammonia and ammonia toxicity

Ammonia toxicity and the loss of N as volatile ammonia are the major problems encountered with fertilizer urea. An equilibrium between ammonium ion and ammonia gas occurs in aqueous solutions of ammonium salts.¹³⁵ When enzyme activity is high in soil, the rate of hydrolysis of urea increases, resulting in greater losses of gaseous ammonia. Gold *et al.*¹⁶ reported that ammonia volatilization losses from agricultural soils range from 0.4 to 80 per cent of the applied urea nitrogen, and from 3.5 to 24.9 per cent in forest soils depending on the soil conditions and assay conditions. Thus, losses of gaseous ammonia can be sufficiently great to reduce yields¹³⁴ and to reduce the efficiency of urea as a nitrogen fertilizer.

Although higher ammonium ion concentration in soil is non-toxic, ammonia gas is extremely toxic to the plant.¹⁶ Thenabadu¹³⁶ speculated that the lower effectiveness of urea over ammonium sulfate in some neutral to slightly alkaline

soils of Sri Lanka, was probably due to loss of urea N by volatilization and ammonia toxicity. Kiss *et al.*¹⁴ reported that subsequent production of ammonia after hydrolysis of urea inhibits the germination of seedling and growth of young plants.

4.2 Accumulation of nitrite

The alkaline pH and high ammonium ion concentrations resulting from urea hydrolysis in soils do not appreciably affect oxidation of ammonia to nitrite by *Nitrosomonas* species, but does inhibit the oxidation of nitrite to nitrate by *Nitrobacter* species.¹³⁷ Bezdicek *et al.*¹³⁸ compared the behaviour of urea, diammonium phosphate and ammonium sulfate in an alkaline soil. They found that the highest accumulation of nitrite (260ppm) occurred in the soil to which urea had been added. Toxicity to the plant from nitrite accumulation has been observed subsequent to the addition of urea fertilizer.¹³⁹ Also nitrite can be reduced to gaseous N either by biological reactions,¹⁴⁰ or by chemical reactions.^{133,141} Those reactions lead to formation of gaseous N which will evolve into the atmosphere, thus reducing the efficiency of urea.

5. UREASE INHIBITION IN SOILS

For improving fertilizer use efficiency new technologies are needed to retard the rapid hydrolysis of urea in soil. For this purpose investigations were conducted along three lines¹⁴; (a). production of urea fertilizer granules coated with a water resistant material to limit dissolving of urea, (b). production of urea derivatives that are more slowly hydrolyzed by the soil urease, (c). use of soil urease inhibitors in conjunction with urea. The third approach has received considerable attention in controlling gaseous loss of urea N as ammonia and damage to germinating seedlings and young plants by nitrite or ammonia.

A comprehensive review on urease inhibitors by Mulvaney & Bremner²⁴ has been published in 1981. Therefore, only the recent findings on the most effective urease inhibitors will be discussed here. Until late 1970s, the quinone class of compounds were the most effective inhibitors of soil urease.¹⁶ After the discovery of the ability of phenyl phosphorodiamidate (PPD) to inhibit urease activity, it emerged as a very effective urease inhibitor¹⁴²⁻¹⁵¹ and until 1985 was considered as the best known inhibitor.¹⁵² However, a phosphorotriamide, N-(n-butyl) thiophosphoric triamide (nBTPT), has been found to be more effective than PPD.¹⁵³⁻¹⁵⁷ Hendrickson²⁵ concluded that the most effective urease inhibitor to date is nBTPT. Considerable attention has also been paid to the effectiveness of ammonium thiosulphate (ATS); a sulfur fertilizer used in most liquid fertilizer formulations, as a potential inhibitor.^{19,158-160}

PPD applied at the rate of 2.2kg/ha with a 50% urea solution (95 kg N/ha) on untreated and oat-straw-amended soils extended the persistence of urea 2 to 3d.¹⁶¹ When PPD was applied to the flood water of wetland rice, urea persisted 8d longer than in plots without PPD.¹⁴³ PPD has effectively inhibited the urease activity, delayed urea hydrolysis, and reduced the build-up of ammonical N in flood water.^{142,162} Beyrouty *et al.*¹⁶³ found that PPD applied at a rate of 4kg/ha with 200kg N/ha as urea prills effectively inhibited urea hydrolysis reducing the initial rate of urea hydrolysis by 60-70%. There are also reports that PPD is ineffective as a urease inhibitor. Broadbent *et al.*¹⁶⁴ reported that addition of PPD to urea prills did not increase corn yields in a 1-yr field trial. According to Kucharski¹⁶⁵ PPD has no significant effect on wheat yield although it decreased ammonia losses in lab. experiments.

Several workers studied the effect of environmental and soil factors on the ability of PPD to inhibit soil urease activity. Wang *et al.*¹⁵⁷ found that PPD is better than HQ and nBTPT under anaerobic conditions. PPD was found to be less effective in soils with high pH and low titratable acidity^{163,166} and on soils with high bulk density.¹⁶⁷ In addition, PPD rapidly degrades in soils¹⁶⁸ or when incorporated into granules or fluid fertilizers¹, thereby limiting its potential usefulness.

nBTPT is currently the most effective urease inhibitor.²⁵ It has increased the yield significantly^{169,170} and also N recovery by plants.^{170,171} It has also reduced the delays in emergence of maize seeds caused by Urea Ammonia Nitrate (UAN) by 50%.¹⁷² Buresh *et al.*¹⁵¹ found that nBTPT was effective in reducing ammonia volatilization losses from urea broadcast into flood waters of rice fields. However, Phongpan & Byrnes^{173,174} observed neither a reduction in the N losses nor a increase in the grain yield, when urea was broadcast with nBTPT to flooded rice. Cai *et al.*¹⁷⁵ and Wang *et al.*¹⁵⁷ have found that nBTPT was more effective under aerobic conditions. The effectiveness of the nBTPT is highly dependent on soil type. For example, the percentage inhibition of ammonia loss at 0.28% level ranged from 99.4-54% in 16 grassland soils.¹⁷⁶

According to Byrnes & Christianson¹⁷⁷ nBTPT itself is a very poor inhibitor of jack bean urease and must be activated in the soil. The active form of nBTPT is its oxon analogue, N-(n-butyl) phosphoric triamide (nBPT)¹⁷⁸ and for this conversion it needs oxygen.¹⁷⁹ Therefore, in flooded soils, where an anaerobic condition prevails, oxon analogues of nBTPT are significantly better than nBTPT itself. In rice culture the conversion of nBTPT to its oxon analogue can be accelerated by bubbling O₂ into flood water or by adding H₂O₂.¹⁷⁹ Oxygen produced by photosynthetic algae present in flooded soils also facilitates this conversion.¹⁸⁰ However, the presence of photosynthetic algae reduced the effectiveness of nBPT. Therefore, Qui-Xiang *et al.*¹⁷⁹ suggested that a mixture of PPD and nBTPT could be more effective than either inhibitor alone in flooded

soils. Initially PPD inhibits soil urease allowing nBTPT to be converted to its oxon analogue.

nBPT was found to be a more effective inhibitor of jack bean urease¹⁸¹ and soil urease at very low concentrations¹⁸² than its thio form. The effectiveness of both nBTPT and nBPT was clearly related to the concentration of nBPT found in the soil.¹⁸³ Soil maintained a higher nBPT concentration after applying nBTPT than when nBPT is applied directly to the soil. Because of greater stability and resistance to degradation, the thio form provides longer sustained activity against soil urease.¹⁸⁴ However, at very low concentrations (0.01% w/w) the oxon analogue showed a better urease inhibition than the thio compound.¹⁸² This is partly as a result of their immediate effectiveness in the soil and partly due to their concentration in urea being higher on a molar basis.

The effectiveness of an inhibitor depends on its capacity to improve diffusion of $\text{NH}_4^+ - \text{N}$ away from the zone of high pH associated with urea hydrolysis. Christianson *et al.*¹⁸⁵ found that in a sandy soil (pH 5.2) nBTPT lowered soil pH and soil NH_4^+ concentration at the placement site compared to urea alone and allowed more diffusion of urea away from the fertilizer microsite. In a clay soil (pH 8.2), the effect of nBTPT was not pronounced, and high concentration of NH_4^+ developed in a high pH zone. Therefore, the effectiveness of nBTPT depends on the capacity of the soil to permit diffusion. Kumar *et al.*¹⁸⁶ reported that urease inhibitors will be more effective if they diffuse at approximately similar rates as does urea in soil. Christianson *et al.*¹⁸⁷ observed that nBPT and urea moved as compact spots at similar rates in an upland soil whereas nBTPT showed significant trailing.

ATS has recently been identified as a soil urease inhibitor.^{158,159} Graziano¹⁸⁸ reported that addition of ATS to UAN has tended to increase grain yield and ear-leaf N concentration at silking of maize, although the differences were not statistically significant. However, ATS activity was controversial as addition of up to 1,000ppm ATS to soil had no inhibitory effect on urease.¹⁸⁹ ATS had retarded urea hydrolysis only when applied at rates as high as 2,500 - 5,000 $\mu\text{g/g}$ of soil.¹⁹⁰ It has been found that ATS must be converted to tetrathionate, its active form, in the soil to inhibit urease activity.¹⁹¹ Even tetrathionate itself is needed at very high concentrations (2,500 - 5,000 $\mu\text{g/ml}$) to inhibit the activity of jack bean urease.¹⁹² Further, levels of urea hydrolysis inhibition reported for ATS has ranged from 10 - 50%¹⁵⁸⁻¹⁶⁰ which is considerably lower than levels reported for compounds especially made for urease inhibition such as PPD and nBTPT.^{190,193,194} Formation of tetrathionate, the active form of ATS is affected at high soil moisture conditions²⁶ which indicates its low efficiency in flooded soils. Considering these factors one could argue against the efficiency of ATS as a potential urease inhibitor. Eventhough it is a relatively weak inhibitor, it has

several advantages over other urease inhibitors *viz*: low cost, widespread availability, safety, easy storage and compatibility with fluid fertilizer materials.¹³ Therefore, only a small reduction in ammonia loss is required for economic benefits. According to Sullivan & Havlin,¹⁹ a grower applying a Urea Ammonium Nitrate (UAN) + 5% ATS (by volume) mixture to supply 100kg N/ha would have to reduce ammonia volatilization only by 4kg N/ha to break even. Therefore, the use of ATS as a urease inhibitor seems to be practical.

Although a variety of chemicals have been shown to inhibit urease activity, none has yet been marketed.¹⁵² The commercial viability of a potential inhibitor will depend on factors such as stability in urea, cost and suitability for incorporation into urea during manufacture. Studies in determining the compatibility of PPD under process conditions typically encountered during urea granulation,¹⁹⁵ in fluid fertilizer,¹⁹⁶ and in solid urea during long term bulk storage¹ showed that PPD can be granulated with urea, but decomposes rapidly in solid mixtures with urea and in fluid fertilizers. Raddel *et al.*¹⁵² speculated that stability of potential inhibitors was likely to increase if polymeric analogs of these inhibitors could be prepared. They found that the thiophosphoryl triamide (TPTA) and phosphoryl triamide (PTA) derived thermal polymers were significantly better than PTA and TPTA. However, PTA derived polymers have shown less apparent urease inhibition activity than PPD though their persistence in soil is higher. During the production of these polymers, the wide variation in the product mixtures was a problem. This may preclude their use as practical inhibitors.

The primary objective of using urease inhibitors is to increase the supply of N from soil more in accordance with the absorption of crop through the reduction of its volatile loss. Mismanagement of the use of urease inhibitors can create unnecessary problems to the farmer. There are reports that the inhibitor itself causes problems related to plant growth. HQ and BQ reduce the germination % of wheat, sorghum, barley, and maize seed significantly at concentrations of 50 - 2500 µg/g soil.¹⁹⁷ Seedling growth of wheat and maize also has been reported to be affected negatively by thiosulfate and tetrathionate.¹⁹² The persistence of urea in nBTPT amended high pH soils has been reported to be high.¹⁷⁶ This could have physiological implications for plants, because urea can be taken up by plant roots as intact molecules.¹⁹⁸

Therefore, the selection of an ideal inhibitor for a particular soil-crop combination is essential. It is also equally essential to determine the optimum application rate of the selected inhibitor. Xiaoyan *et al.*¹⁹⁸ found that a key factor affecting the efficient usage of HQ was the concentration of the inhibitor. A lower recovery of added N at higher rates of inhibitor can be expected due to the delayed urea hydrolysis and subsequent loss of free urea below the rooting zone following precipitation which could cause ground water pollution in areas where the soil is light textured and intensively cultivated.

6. CONCLUSIONS

The fate of urea in soil depends mainly on urease activity. An effort has therefore been made to investigate the activity of soil urease. The effectiveness of urea as a N fertilizer has been reduced because of high urease activity in most arable lands in the world.

It was very difficult to compare the levels of urease activity in different soils due to different assay procedures adopted, and also due to the fact that different units have been used to express urease activity. The authors therefore emphasize the need of formulating a common assay procedure for soil urease, which would help to compare and reproduce the results obtained under comparable conditions.

The unique feature of native soil urease is its remarkable stability against proteolytic activity and other processes leading to its inactivation. This stability is due to its immobilization as organo-mineral complexes. The total enzyme activity as well as its inhibition is therefore mainly dependent on the nature of the organo-mineral complexes found in soil. Despite the fact that substantial information on interactions between urease and soil mineral components and organic matter is available, there is still lack of information on the direct effect of humic substances on soil urease. This is particularly important when the current trend in the world is to use more and more organic materials as soil amendments in agriculture. Further, the production of effective inhibitors depends on the knowledge concerning the behaviour of urease in soil systems. Therefore, research should be carried out to investigate the orientation of active sites of urease in organo-mineral complexes.

Use of urease inhibitors has been identified as one of the most acceptable methods to control the rapid urea hydrolysis and to increase the efficiency of urea. Although nBTPT appears to be the most effective inhibitor to date, yet ATS will also receive a considerable interest in the near future as a potential inhibitor. Nevertheless, further research should be conducted to evaluate the effectiveness of the potential inhibitors in different soils under different management systems. Also, as crop response to inhibitors using measurements of yield or N uptake are probably small, it would be advisable to conduct field studies with direct measurements of ammonia loss or urea hydrolysis. Use of waste materials, (such as sewage sludge, black tea wastes, etc.) should also be evaluated as they are known to be environment friendly materials.

Although many compounds have been identified as effective and potential inhibitors, none except ATS has yet been marketed. Commercial viability of any urease inhibitor depends on such factors as stability in urea, cost, suitability for incorporation into urea during manufacture and impact on the environment, in

addition to their effectiveness in different soils under different management systems. Information available on the inhibitors currently considered as the most effective, is not sufficient to assess their commercial viability.

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