J. Mycopathol, Res, ***50(2)*** : *235-241 (2012)*

Microbial urease activity of agricultural soil: enzymology and assessment of soil fertility

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Received : 17.12.2011 Accepted : 27.07.2012 Published : 29.10.2012

Use of nitrogen fertilizers, in particular urea, is common practice of farmers of all nations because it has much ability to supply nitrogen to crop plants. Even though, agricultural scientists suggest to use less amount of inorganic fertilizers because these molecules destruct the soil structure and texture as well as cause underground water pollution. Urea is highly soluble in water and decomposes by mostly microbial ureases present in a soil.

Without urease, break down of urea is not possible, therefore urease activity in the soil plays critical role in the utilization of the urea used as fertilizer in agricultural land. In this study, a simple procedure is developed to estimate urease activity in the soil sample collected from the agricultural lands of the district Burdwan, West Bengal, India. The technique is easy to implement and it will provide an important understanding regarding the amount of urea that will be used as a nitrogen fertilizer in a crop field. A standard assay procedure includes 0.5 ml of 100 mM sodium phosphate buffer, 100 mM urea and requisite amount of water. The assay temperature was 37°C and time of incubation was 60 min. Using the assay procedure the pH, temperature optima, substrate concentration, Km and Vmax were determined. Effects of metal ions on urease activity were also determined. It showed the optimum pH and temperature were pH 5.0, temperature 27°C respectively. The Km value was determined from the substrate concentrations assays. The apparent Km value was 1.5 mM^{-1}. Other parameters of urease of a soil sample were also determined. 10 various soil samples were collected from agricultural fields and their urease activity were determined. The procedure used in this study is simple and can be implemented in any field laboratory.

Key words: Biofertilizer, agricultural land, nitrogen fertilizer, urease, enzymology

INTRODUCTION

Use of fertilizer in an agricultural land is essential to obtain bumper crop. Nitrogen fertilizers of various types are used by farmers of all nations. Urea, ammonium sulfate, potassium nitrate, sodium nitrate etc are used heavily in agricultural lands (Gasser, 1964). However, crop plants unable to utilize all of them, therefore, mostly these molecules remain in the soil and transported to the underground and cause underground water causing pollution which is critical aspect of use of heavy nitrogen fertilizers and other chemical fertilizers in agricultural land (Hutchinson and Vlets , 1969). Urea is used in huge amounts by farmers because it has tremendous effects on the growth of crop plants. Whereas use of bio-fertilizers unable to produce such effects because of cumulative effects of bio-fertilizers that ensures good health of an agricultural land. Bio-fertilizers show very slow effects on growth and development of crop plants. The urea that we use in agricultural land should break into ammonia and carbon dioxide taking the help of microbial ureases. The ammonia further transforms into ammonium hydroxide which is easily absorbed by a crop plant. It is the available form of nitrogen. However, urea can be transported in plant by root system and even though it is very less and the conversions are occurred using intracellular urease into its components. The microbial ureases those are present in a soil break urea into ammonia and carbon dioxide in presence of water. The biochemical reaction is written as $NH_2–CO–NH_2 + H_20 = 2NH_3 + CO_2$ and the enzyme urease (EC 3.5.1.5) is the

mediator of this reaction. The ammonia further converts into ammonium hydroxide. The nitrogen is used to make amino acid using amino transferase activities in particular glutamine synthetase. These amino acids are used to biosynthesize cellular proteins. Therefore, urease is a critical enzyme that determines that amount of urea that will be used as fertilizer in an agricultural land. There are various methods of estimation of urease activity; however, all are complicated and used expensive chemicals (Conrad, 1940; Hofmann and Schmidt, 1953; McLaren *et al.*, 1957; Stoganovic, 1959; Hoffman and Teiusingcher, 1961; Porter, 1965; McGrarith and Myers, 1967; Simpson, 1968). The present study provides a simple procedure and it is easy to practice using less expensive equipments. Therefore, the procedure is very important and can be practiced in a field laboratory. Using the procedure enzymatic studies of urease of an agricultural soil and many other soil samples of various agricultural lands have been carried out.

MATERIALS AND METHODS

Soil samples were collected from rice, wheat, vegetable and post harvested agricultural fields of Manikara village at Rajbandh, Durgapur, West Bengal, India. Samples were collected using glass container and kept at room temperature with sufficient moisture in the laboratory for a few days. The following materials were purchased from the suppliers indicated: Urea, $HgCl_2$, KI, NaOH, NaH_2PO_4, Na_2HPO4, KCL, NaCl, $FeSO_4$, $CaCl_2$, $MgCl_2$, $MnSO_4$, were from the Merck, Co. ltd , India (Germany); non absorbent cotton, absorbent Cotton , Ethyl alcohol from Bengal Chemical and Pharmaceutical Works, India. Other chemicals were from Loba Co, Burdwan and Dayamoyee Scientific, Durgapur, West Bengal, India.

Assay of urease activity

The assay was a modified method using Nessler's reagent that detects ammonical nitrogen. In this assay urea was used as substrate and soil as the source of urease. 100 mg of soil sample was taken in a micro centrifuge tube. Then 500 µl of 100 mM phosphate buffer pH 7.0 was added and mixed. In the assay 10 mM urea was used as substrate. The final volume was made to 1.0 ml using requisite amount of water. The reaction mixture was incubated at 37 °C for 60 min. The assay mixture was centrifuged at 10,000 rpm for 5.0 min. The clear supernatant was taken in a test tube and amount of ammonia released due to the hydrolysis of urea was estimated using Nessler's reagent. 100 µl of Nessler's reagent was added in the clear supernatant. The final volume was adjusted to 3.0 ml with the addition of distilled water. The assay tube was kept for 10.0 min at room temperature. The reddish yellow colour which was produced in the reaction mixture was estimated colorimetrically at 430 nm. One unit of urease activity was 1 µg of nitrogen that was released from the substrate at standard assay conditions.

RESULTS

The primary assay was carried out using 0.5 g of soil sample to detect urease activity. After the detection of the urease activity , the assay were carried out using 100 mg of soil sample with similar amounts of substrate and buffer in all other assays. It was centrifuged and the clear supernatant was mixed with substrate and buffer and was incubated for 60 mins. at 37°C. After incubation it was mixed with Nessler's reagent. It showed presence of ammonia in the sample.

Effect of pH on urease activity of a soil sample

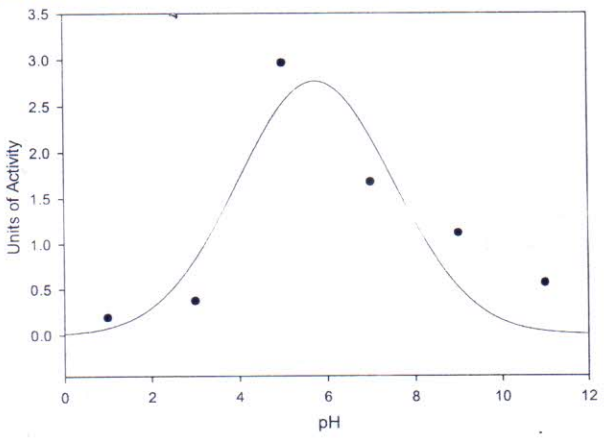

Fig. 1: Effect of pH on urease activity of soil a soil sample. The assay was carried out using various buffers with specific pH. Other parameters are same as described in materials and methods.

Using the standard protocol of assay the urease activity was carried out at various pH. The pH values were 1.0, 3.0, 5.0, 7.0, 9.0 and 11.0 respectively. 500 µl of 100 mM buffer was added in each assay. It showed that at pH 5.0 the urease activity was highest,

below or above this pH it showed reduction of enzyme activity (Fig.1). However, the reduction of enzyme activity was much in alkaline conditions in comparison with that of acidic ranges. At acidic pH the reduction of enzyme activity was not much.

Effect of temperature on urease activity of a soil sample

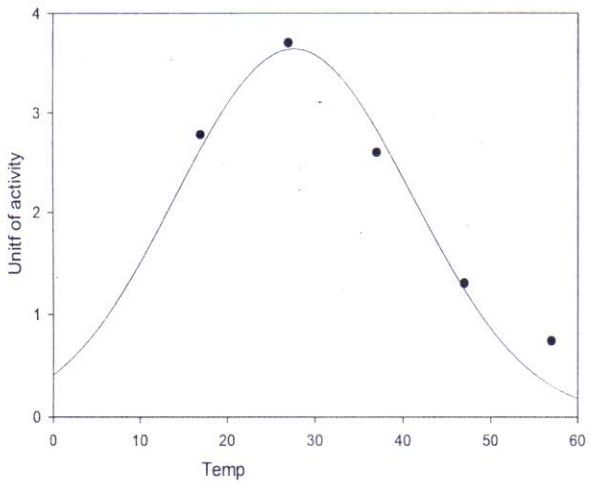

Fig. 2: Effect of temp. on urease activity of a soil sample. The assay was carried out using 100 mM buffer pH 5.0. Other parameters are same as described in materials and methods

Urease activity was estimated using a buffer with the pH 5.0. Other parameters were same as described. The incubation temperature was variable. The temperatures of incubation were 17.0, 27.0, 37.0, 47.0, 57.0 and 67.0 ^{0}C. The maximum urease activity was at 27.0 °C above or below this temperature showed reduced the enzyme activity

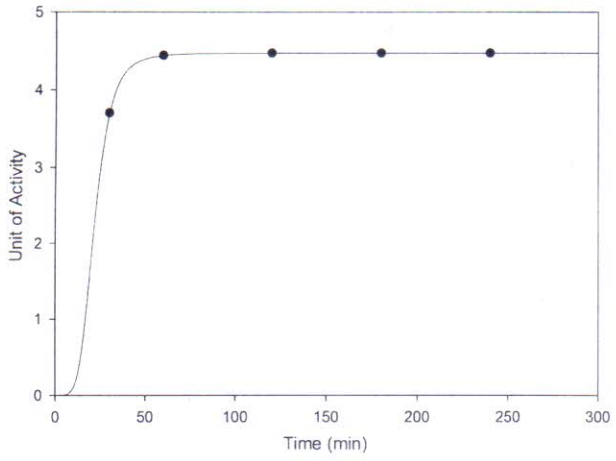

Fig. 3: Effect of time of incubation of urease activity of soil sample. The assay was carried out using 100 mM buffer pH 5.0, temp. 27^{0}C. Other parameters were same.

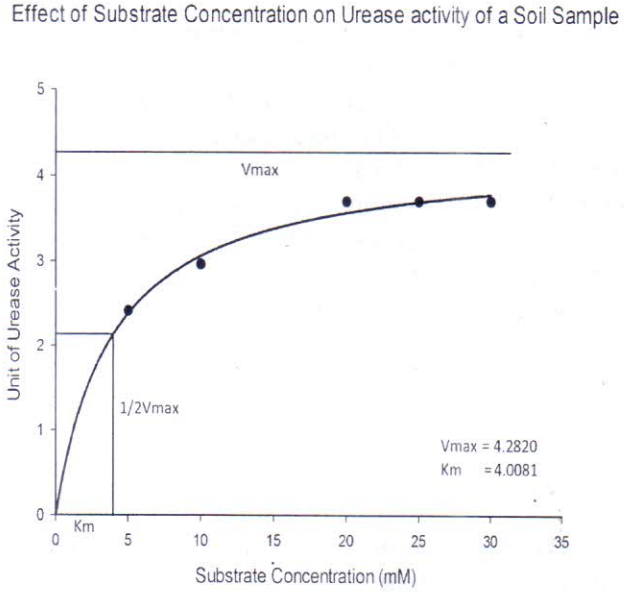

Fig. 4a: Effect of substrate concentrationon urease activity of soil sample. The assay was carried out using different conc. of substrate. Other parameters were same.

(Fig.2).

Effect of time of incubation

The assay of urease activity was carried out for several hours using standard assay conditions. The standardized conditions were pH 5.0 and incubation temperature 27.0 °C. Other parameters were remained same. It showed there was increase of urease activity up to 180.0 min. of incubation and further prolongation of time of incubation showed no change of enzyme activity (Fig.3).

Effect of substrate concentration on enzyme activity

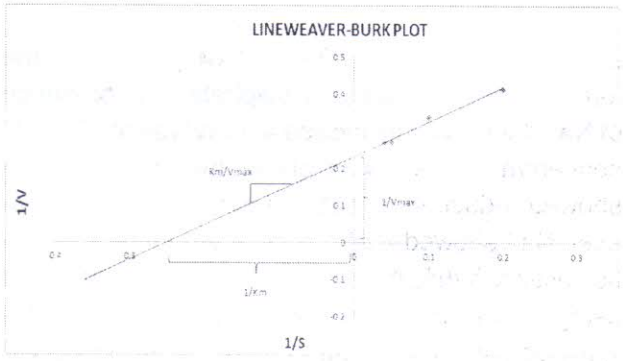

Fig. 4 (b): Effect of substrate concentration on velocity of urease activity of soil sample. The assay conditions were pH 5.0, temp. 27^{0}C. Other parameters are same as mentioned in materials and methods.

Using variable substrate concentration the urease activity was estimated. The concentrations were 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mM respectively. The enzyme activity was increased up to 40 mM concentration and it was the saturation point (Fig. 4a). The enzyme activity was increased as per the concentration of the substrate present in the reaction mixture. The data was plotted using 1/v and 1/s values following Lineweaver and Burk plot. It produced an apparent Km value of 1.5 mM which was corresponded to 1.5 millimole l^{-1} (Fig.4b).

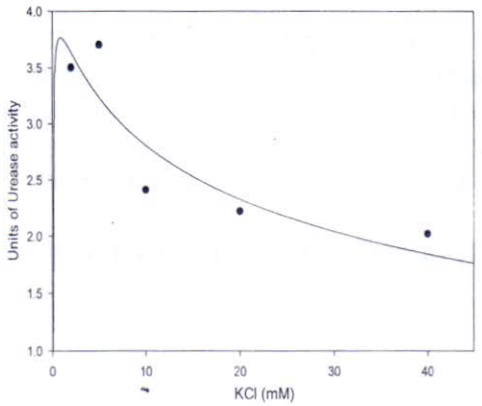

Fig. 6 : Effect of K^+ on urease activity.The assay was carried out using varia-ble concentration of K^+. Other para-meters are same.

Effect of various metal ions on the urease activity of a soil sample

Various divalent and univalent cations at variable concentrations were used in the reaction mixture to

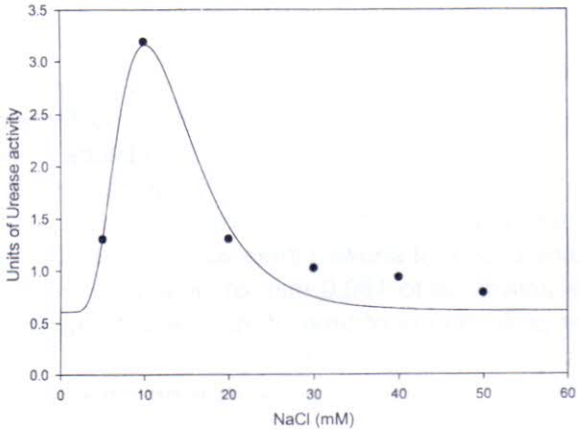

Fig. 5: Effect of Na^+ on urease activity. The assays were carried out at standard assay conditions. Variable concentrations of Na^+ were used separately in each assay.

assay urease activity. The ions were added in the form of salts of chloride or sulphate. The presence of Na^+, the maximum urease activity was at 10.0 mM concentration, below or above this concentration showed reduction of enzyme activity (Fig.5). However, Na^+ showed a little accelerating effect on urease activity. While the enzyme activity was estimated using K^+, it also showed a little influence on the enzyme activity. At 2.0 mM concentration the activity was maximum. It showed inhibitory effect above this concentration of K^+ (Fig. 6). Similarly, in presence of Ca^{2+} the maximum urease activity was at 2.0 mM

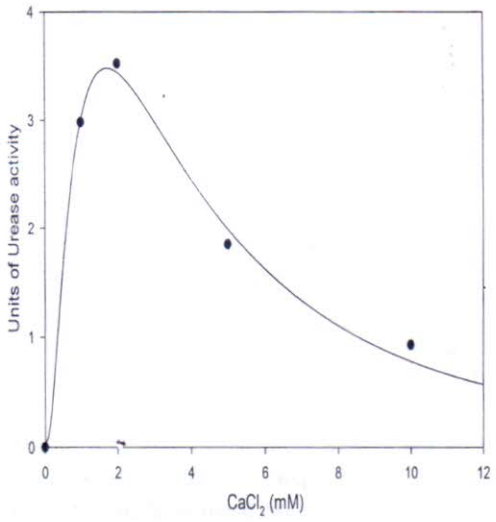

Fig. 7 : Effect of Ca^+ on urease activity of a soil sample. Variable concentrations of Ca^+ were added separately in each assay Other parameters were same as described.

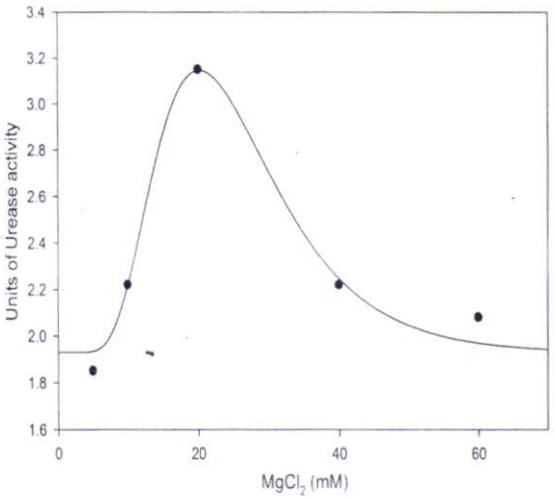

Fig. 8: Effect of Mg+ on urease activity of a soil sample. Variable concentra-tions of Mg+ were used in this assay. Other parameters are same.

concentration, below this concentration showed less activity and above this concentration showed inhibitory activity (Fig.7). When Mg^{2+} was used in the assay, it showed the optimum concentration was at 20.0 mM. Above or below this concentration the enzyme activity was inhibitory (Fig.8). Fe^{2+} and Mn^{2+} showed precipitation in the assay at even very less concentrations of these metals ions.

Urease activity of 10 soil samples collected from agricultural fields

Ten soil samples were collected from various agricultural lands. These samples were collected from the rice, wheat, vegetable fields where crops were under cultivation. One sample was collected from post harvested fields. The enzyme activity was measured at standard assay conditions. It showed variable amount of urease activity in all these soil samples used in this study. The maximum urease activity was obtained in the sample number 9 which was a wheat field. However, there was a little bit uniformity in the urease activity where rice was under cultivation. In post harvested land the enzyme activity was least. (Table 1).

Table 1: Urease activity of various soil samples collected from agricultural lands

Type of soil	name of the crop*	units of urease activity
Alluvial	Rice	10.74
Alluvial	Wheat	11.48
Alluvial	Open	5.93
Alluvial	Rice	10.93
Alluvial	Wheat	6.67
Alluvial	Wheat	7.60
Alluvial	Rice	9.60
Alluvial	Rice	12.41
Alluvial	Wheat	14.44
Alluvial	Vegetable	10.00

Rice, wheat and vegetable were under cultivation, open was post harvested field

DISCUSSION

Use of urea as a potent nitrogen fertilizer is practiced by all farmers in all nations because of its tremendous effects on the growth of crop plants (Gassrer, 1964; Zha and Chen, 2002), even though, urea has multiple harmful effects on soil environment (Hutchinson and Viets, 1969; Tabatabai and Bremner, 1972). However, in a soil environment urea must be converted into ammonia before it receives by a crop plant. However, urea can be incorporated in a plant system and the conversion is mediated by the enzyme urease which is also present in cytosol of a plant cell. The degradation of urea occur using ureases that come from soil microorganisms. As a matter of fact, transport of urea as such is very negligible in root system of a crop plant. The maximum conversion of urea into ammonia is associated with microbial activities. Soil microbes particularly bacteria play critical roles in this context. Microbial ureases break urea maximally and the free ammonia combines with water makes ammonium hydroxide (Delauve and Patrick, 1970). This molecule is easily available to the plant. Therefore, the amount of urea that will be used in an agricultural land depends on soil microbial urease activity. Heavy use of this fertilizer will cause underground water contamination. There are many methods of estimation of urease activity (Conard,1940, Conard,1942;Hofmann and Schmidt,1955; Hoffmann and Tercher,1961; Porter ,1965; Paulson and Kurtz,1969;Tabatabai andBremner,1972), however, this method which is described in this study is simple, applicable in field laboratory and reproducible. In this procedure estimation of urease activity was carried out using only 100 mg of soil sample. Many investigators used toluene treated soil samples that inhibits microbial activities to estimate urease activity (McGarity and Myers, 1967; Tabatabai and Bremer,1972). Toluene kills the microbes and also partly affects urease activity because topology of the enzyme substrate binding, conformational changes of the enzyme are important factors (Dalal, 1975). In this study such type of inactivation of microbial activities are not carried out. The present procedure uses soil samples and extracted the soluble ureases using a buffer that was used in the assay. Many investigators used procedure of ammonia estimation (Bremner and Edwards, 1965; Bremner and Keeney, 1966, Tabatabai and Bremer, 1972) which is also difficult. The urease activity is also estimated by measuring the release of CO_2 from

urea (Conard, 1940; Porter, 1965; Simpson, 1968; Skyjira and Mc Laren, 1969) which is also difficult. This process estimates ammonical nitrogen in the solution by Nesslerization which is also carried out by several investigators where to stabilize the colour complex use of KCN is suggested (Minari and Zilversmit, 1963). In this process the colour which is produced due to the use of Nessler's reagent is stable for many hours. However, if the concentration of nitrogen and Nessler's reagent are much then precipitation occurs. The buffers employed included phosphate , citrate ,THAM and other to estimate urease activity of soil samples (Hofmann and Schmidt,1953; Wall and Laider,1953; Briton 1955; Hoffmann and Teicher,1961; Wang et al., 1991) , however in this study several types of buffers were used to determine the optimum pH of soil urease , it was 5.0 using citrate buffer. The optimum temperature was 27°C which produced maximum enzyme activity. Similar data also reported by several investigators. Following the same optimized conditions various parameters were studied using an agricultural soil sample. It is the first report on the urease activity of the soil sample of agricultural land of the district Burdwan, West Bengal, India. The substrate concentration and apparent Km value were determined. The results also found similarities with other observations (Kumar and Wagenet, 1984; Juan, 2009). The effect of metal ions showed variable effect. The optimum concentrations were 10.0, 2.0, 20.0, 2.0 mM for Na, K, Mg and Ca respectively. The similar observations are also reported by others. However, the effect of Fe and Mn are difficult to study using the procedure used in this study. It showed precipitation at even in less concentration of these ions. However, variability of urease activity might be due to multiple known and unknown factors. Soil microbial content, temperature, pH, moisture and others are important factors. Using the same procedure 10 various soil samples were examined and it showed variability. The variability is mainly due to microbial concentrations in these samples. It is of great interest to study seasonal and moisture variation in agricultural land in relation to urease activity. There must not be any stability in the urease activity of an agricultural soil. However, one may assess quality of a soil and how much amount of urea might be used in an agricultural land. A large number of soil samples can be analyzed within a short period following this simple procedure. All the procedures appeared in various literatures are difficult to practice. This procedure is easy and reliable. It will also provide an important insight to understand the amount of urea that might be used in an agricultural land to increase soil fertility and yield of a crop.

ACKNOWLEDGEMENT

We thank Mr. R. N. Majumder for various supports to run the project at DCCS. We are thankful to all teaching and non teaching staffs of DCCS for many helps during the work. Thanks to Department of Science and Technology, Govt. of West Bengal for financial support.

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