UREASE ACTIVITY IN RICE SOILS OF KERALA

By

P. SARASWATHI

THESIS

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DECLARATION

I hereby declare that this thesis entitled "Urease Activity in Rice Soils of Kerala" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship of any other similar title, of any other University or Society.

Vellanikkara,

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P. SARASWATHI

CERTIFICATE

Certified that the thesis entitled "Urease Activity in Rice Soils of Kerala" is a record of research work done by Smt. P. Saraswathi under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellanikkara,

1 -8-1989.

Dr. P. V. BALACHANDRAN,
Chairman, Advisory Committee,
Assistant Professor,
Department of Agronomy,
College of Horticulture,
Vellanikkara, Trichur.

CERTIFICATE

We, the undersigned members of the Advisory Committee of Smt. P. Saraswathi, a candidate for the degree of Master of Science in Agriculture with major in Agronomy, agree that the thesis entitled "Urease Activity in Rice Soils of Kerala" may be submitted by Smt. P. Saraswathi, in partial fulfilment of the requirement for the degree.

CHAIRMAN — **Dr. P.V. Balachandran**

MEMBERS — **Dr. C. Sreedharan**

Dr. R. Vikraman Nair

Dr. P.A. Wahid

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Introduction

INTRODUCTION

Urease (urea amidohydrolase, EC 3.5.1.5) catalyses the hydrolysis of urea to carbon dioxide and ammonia. Urease is unique among soil enzymes as it greatly affects the fate and performance of applied urea, one of the most important nitrogeneous fertilizer. Urea as a fertilizer is being increasingly used in world as well as in Indian agriculture and it is important to know the urea hydrolysing power of the soil. A rapid hydrolysis of urea leads to accumulation of ammmonia and nitrite in soil which may be toxic to germinating seedlings and young plants. It may also lead to serious volatilization loss of ammonia from soil. On the other hand, a slow rate of hydrolysis is likely to increase the leaching losses of urea. The recovery of fertilizer N by low land rice crop is extremely low, rarely exceeding 40 per cent under field condition. A thorough evaluation of the urea hydrolysing power of rice soils is thus essential for proper management of urea in rice culture.

Urease activity is known to vary widely in different soils. Total urease activity can be considered to be due to urease located intracellularly or extracellularly (free or bound to soil colloids). Usually urease activity refers to the activity of extracellular urease in the soil and excludes the activity of metabolizing microorganisms. Soil urease activity is influenced by several factors such as substrate concentration, pH,

soil submergence, organic matter etc. Among these factors soil submergence is found to have a profound effect on urease activity. This needs further investigation as the reports on effects of soil water levels and especially those of submergence on the urea hydrolysis have not been consistent. The rhizosphere also is another important factor influencing urease activity. Greatest activity of urease is reported to occur in the rhizosphere where microbial activity is high and where it can be excreted from plant roots.

The kinetics of urease have been found to vary with the soil type. The Michaelis constant (K_m value) and the maximum reaction velocity (V_{max}) are the two kinetic properties of the enzyme. The wide divergence in the kinetic data reported for urease can be partly attributed to the wide variety of conditions adopted in different kinetic studies. Many methods are used for the assay of urease activity in soil. Two of the common methods are buffer method (Tabatabai and Bremner, 1972) and non-buffer method (Zantua and Bremner, 1975a). The values of urease activity obtained employing these two methods have been found to differ significantly for the same soil type. Urease activity is also estimated employing ^{14}C - labelled urea. Many of the divergent findings in research on soil urease can be attributed to the difference in the methods employed. Hence the need for evaluation of methods used to assay urease activity in soils deserves emphasis.

Considering all these aspects an investigation was undertaken with the following objectives.

1. To evaluate urease activity in different rice soils of Kerala.
2. To examine the factors affecting urease activity.
3. To study the effect of soil submergence on urea hydrolysis.
4. To look into the role of rice rhizosphere on urease activity.
5. To compare the methods of assaying urease activity in soils.

Review of Literature

REVIEW OF LITERATURE

A brief review on soil urease activity is presented in this chapter. The literature reviewed are classified under the following heads.

1. Origin and location of soil urease
2. Methods of assay of urease activity in soils
3. Kinetics of urease activity
4. Factors affecting urease activity
5. Molecular absorption of urea

2.1 Origin and Location of Soil Urease

The presence of urease in soil was first indicated by Rotini (1935). Later Conard (1940, 1942 a,b) hypothesized that urea hydrolysis was catalyzed by extracellular urease derived from dead and ruptured cells of ureolytic microorganisms and plant organs adsorbed onto soil fractions. Later McGarity and Myers (1967) reported that urea could be hydrolysed by urease produced by active soil microorganisms. Paulson and Kurtz (1969) demonstrated that urease activity of soil could be divided into two components : microbial urease, directly associated with microorganisms and adsorbed urease, apparently adsorbed on soil colloids. But it was generally assumed that the urease in soils is essentially a microbial extracellular enzyme accumulated through release of urease from living and disintegrated microbial cells (Skujins, 1976). Therefore urease activity refers to the activity of

extracellular urease in the soil and excludes the urease activity of metabolizing microorganisms.

Burns et al. (1972 a, b) reported that urease in soil was protected by humus or clay colloids and this protection arose through immobilization of the enzyme within organic colloids during humus formation. Colloids, both organic and inorganic, apparently acted as stabilizing agents for urease released into the soil and high contents of colloids might provide a potentially large number of sites for preservation of urease activity. Because of this reason fine textured soils appeared to have an inherently higher potential for retention of this activity than the coarse textured soils (McGarity and Myers, 1967).

Although some of the urease activity produced on treatment of soil with organic material persisted for several weeks, the urease activity of soil amended with organic materials eventually becomes identical to that of the unamended soil (Zantua and Bremner, 1976, 1977). This indicates that the urease activity of unamended soils reflects their capacity for protection of urease and that urease in excess of their capacity is decomposed or inactivated. Zantua and Bremner (1976, 1977) concluded that native urease in soils was remarkably stable and different soils had different levels of urease activity determined by the ability of their constituents to protect urease against microbial digradation and other processes leading to inactivation of the enzyme.

According to Burns (1982) the location of urease enzyme was at least partially determined by such factors as the size and solubility of its substrate, the species of microorganisms and the physical and chemical nature of the soil colloids. He also found that enzyme bound to clay and humic colloids had a residual activity not found in enzymes free in the soil-aqueous phase. The results of the studies by Tiwari et al. (1988) demonstrated that there were at least three different loci of enzyme activity in soil: inside viable cells, on the surfaces of clay-humic colloids and in the soil solution.

2.2 Methods of Assay of Urease Activity in Soils

Numerous methods had been used for assay of urease activity in soils. Most of these methods involved estimation of the ammonia released on incubation of toluene-treated soil with buffered urea solution (Hoffman and Teicher, 1961; McGarity and Myers, 1967; Tabatabai and Bremner, 1972). Others involved estimation of the urea decomposed or the carbon dioxide released on incubation of soil with urea (Porter, 1965; Skujins and McLaren, 1969; Douglas and Bremner, 1971; Norstadt et al. 1973; Zantua and Bremner, 1975 a). Several methods adopted did not involve the use of a buffer to control pH (Porter, 1965; Douglas and Bremner, 1971; Zantua and Bremner, 1975a) or toluene to inhibit microbial activity (Paulson and Kurtz, 1969; Skujins and McLaren, 1969; Zantua and Bremner, 1975a).

Most of the methods proposed must be empirical because no studies to evaluate them have been reported. The methods which had been thoroughly evaluated were the buffer method proposed by Tabatabai and Bremner (1972) and the non-buffer method proposed by Zantua and Bremner (1975a). The buffer method involved determination of the ammonium released on incubation of the soil sample with THAM (tris-H_2SO_4) buffer (pH 9.0), urea and toluene at 37°C for 2h, ammonium release being determined by shaking the incubated soil sample with 2.5*M* KCl containing a urease inhibitor (Silver sulphate) and by steam distilling an aliquot of the resulting soil suspension with magnesium oxide. The non-buffer method cited was essentially a scaled-down version of the method proposed by Douglas and Bremner (1971), the only significant difference being that toluene was omitted. It involved determination of the amount of urea hydrolysed on incubation of the soil sample with urea at 37°C for 5h, urea hydrolysis being estimated by colorimetric determination of urea in the extract (Douglas and Bremner, 1970) obtained by shaking the incubated soil sample with 2 *M* KCl containing a urease inhibitor (PMA) and filtering the resulting suspension. According to Zantua and Bremner (1975 a), although both methods gave precise results, the buffer method gave markedly higher values than the non-buffer method and detected urease activity that did not occur when soils were treated with urea in the absence of buffer. He also noticed that the nonbuffer method provided a very good index of the ability of soils to hydrolyze urea under natural conditions and its results were not affected by inclusion of toluene.

Although addition of toluene or irradiation with γ-rays or with an electric beam to inhibit microbial growth was commonly used in assay of enzyme activity in soils, the extensive literature indicated that they caused more problem than they solved (Kiss et al., 1975). Many workers had found that toluene could significantly affect the results obtained by buffer or non-buffer methods of assaying soil urease activity. McGarity and Myers (1967) found that the urease activity, following buffer method, was substantially reduced by addition of toluene. Dalal (1975) found that addition of this reagent greatly reduced the values obtained by a non-buffer method of assaying urease activity. McGarity and Myers (1967) concluded that the urease activity measured in the presence of toluene was derived from extracellular urease adsorbed on soil colloids whereas the activity measured in the absence of toluene included activity derived from metabolizing ureolytic microorganisms. However, Tabatabai and Bremner (1972) found that the results obtained by the buffer method of assaying urease activity in soils were increased by the addition of toluene. Several workers have suggested that toluene released urease from microorganisms and increased the activity (Kiss and Boaru, 1965; Skujins, 1976; Thente, 1970).

Persistence of urease activity in radiation sterilized soils had been reported by Roberge (1968) and Skujins and McLaren (1968). Irradiation also led to drastic changes in soil urease activity. The type of change was a function of soil and treatment dose (Thente, 1970). He observed that regardless of whether the soil was treated with toluene or γ-radiation a linear urea decomposition rate occurred during the first 20 hours of

incubation. This indicated that urea hydrolysis was catalyzed by a fixed amount of accumulated urease. The fact that toluene treatment or irradiation increased urease activity in some soils and decreased it in others and that the degree of increase or decrease varied with different soils, reflected the presence of different ratios of cell free and cell bound quantities of urease.

Several workers had assayed soil urease activity or studied urea decomposition in soils by estimating the $^{14}CO_2$ released through hydrolysis of ^{14}C- labelled urea (Skujins and McLaren, 1969; Peltser, 1972 ; Norstadt et al., 1973). Skujins and McLaren (1969) observed a linear rate of $^{14}CO_2$ evolution in all urea-amended soils for several hours, after which time an increase of rate indicating microbial proliferation. Thus an intrinsic urease activity might be distinguished from enzymatic activity due to microbial proliferation. One problem recognised by Skujins and McLaren (1968, 69) was that the $^{14}CO_2$ produced by hydrolysis of ^{14}C- labelled urea in soils was not evolved quantitatively. They proposed use of an acidic (pH 5.5) buffer for assay of urease activity in soils by determination of the $^{14}CO_2$ released from ^{14}C - labelled urea. Another problem in use of ^{14}C-labelled urea was the possibility of isotope effects (Rabinowitz et al., 1956).

Most assays of soil enzymes activity have been performed on air-dried soils and this holds for assay of soil urease activity. However, there are reports that air drying and air dry storage of soil can lead to an increase or decrease in urease activity (McGarity and Myers, 1967; Gould et al.,

1973). According to Zantua and Bremner (1975b), the divergent findings were at least partly due to differences in the techniques used to study those effects or due to the difference in amount and type of plant residues in the soil samples studied. They found no change in urease activity when air dried soils were stored at 21 - 23°C for times ranging from one week to one year.

2.3 Kinetics of Urease Activity

Dalal (1975) studied the effect of varying the period of incubation on the urease activity and found that urea hydrolysis followed a zero order kinetics. The substrate concentration was not a limiting factor in the assay of enzyme activity for periods of incubation extending upto 16h. Sankhayan and Shukla (1976) found that most of the urea added to five Indian soils was hydrolysed within 24h and the average half time values ranged from 3.7 to 7.9h. Hydrolysis was found to follow a first order reaction. Sahrawat (1980 a) observed in a non-buffer method study with three alluvial soils of India that urea hydrolysis followed a zero order kinetics at least upto 12h and the urea hydrolysis rate coefficient (*Ko*) of the soils ranged from 0.083 to 0.167 μ moles g^{-1} soil h^{-1}. The urease activity of the soils varied from 5.1 to 10 μg urea hydrolyzed g^{-1} soil h^{-1}. Kumar and Wagenet (1984) reported that urea transformation followed first order kinetics and the rates of urea hydrolysis increased with temperature in the three soils studied. Singh and Bajwa (1986) found that urea hydrolysis seemed to follow first order kinetics in salt affected soils and

the average time for one half of the hydrolyhsis to occur (t 1/2) ranged from 0.51 to 4.55 days. They also found that urea hydrolysis was faster in recently reclaimed sodic soils than in unreclaimed soils.

Paulson and Kurtz (1970) reported that the Michaelis constant (K_m) for adsorbed urease to be higher than microbial urease. They obtained K_m values of 0.213 M for the total urease activity and 0.057 M and 0.252 M for microbial and adsorbed forms of soil urease respectively. Tabatabai (1973) reported K_m values ranging from 1.3 - 7 mM urea in the presence of THAM buffer and found that values of K_m and V_{max} for urease activity were different for different soils. Pettit et al. (1976) in a similar study obtained a higher K_m value of 52.3 mM urea in Tris-HCl buffer while a K_m of 62.5 mM urea was recorded in the presence of phosphate buffer. Beri et al. (1978) found that the integrated form of the Michaelis-Menten equation was more suitable for enzyme kinetic studies in soil system where the K_m and V_{max} values bore close relationship. They obtained K_m values ranging from 10.4 to 22.2 mM urea and V_{max} ranging from 2.0 to 6.2 mM urea Ng^{-1} soil for soil urease. In a study by Nor (1982) the computed K_m and V_{max} values for 3 Malaysian soils according to the Hanes equation were 2.06, 1.73 and 1.04 mM urea and 0.4, 0.22 and 0.14 mM urea hydrolyzed g^{-1} soil h^{-1} respectively. The correlation coefficient between K_m and V_{max} was 0.96. The t 1/2 values calculated by him were 13.7, 39.9 and 132.5h respectively for the above 3 soils.

Pal and Chhonkar (1979) evaluated the influence of different soil characteristics on enzyme kinetics and found that Michaelis constant (K_m)

for the enzyme varied in different soils and was significantly and positively correlated with soluble salt content. From the kinetic studies of urea hydrolysis Ioppolo (1979) concluded that the inhibition of urease appeared to be effected by the simultaneous operation of the two mechanisms : one, the non-competitive inhibition which was more marked at higher temperature and the other which was competitive, increased with decreasing humus content.

2.4 Factors Affecting Urease Activity

2.4.1 Organic matter and CEC .

Several studies have indicated that urease activity tends to increase with organic matter content and that sandy or calcareous soils tend to have a lower urease activity than heavy textured or noncalcareous soils (McGarity and Myers, 1967; Skuins and McLaren, 1969).

McGarity and Myers (1967) found that urease activity in 100 Australian surface soils was highly correlated with organic carbon whereas Silva and Perera (1971) found that urease activity in rubber soils of Srilanka was not significantly correlated with organic carbon. According to Pancholy and Rice (1973), urease activity in nine Oklahoma surface soils was not significantly correlated with organic carbon. Gould et al. (1973) observed a significant relationship between urease activity and organic carbon in profile samples of an Alberta soil, and Tabatabai (1977) found that urease activity in surface and profile samples of Iowa soils was significantly

correlated with organic carbon. Sahrawat (1980 a) reported that urease activity increased with increase in the organic carbon content of soil.

Cation exchange capacity may be an indicator of the ability of a soil to complex and retain urease (Gould et al., 1973). Dalai (1975) observed a highly significant correlation between urease activity and organic carbon or cation exchange capacity. According to Zantua et al. (1977) urease activity in Iowa soils was very significantly correlated with organic carbon, total N and CEC. It was also significantly correlated with clay, sand and surface area but not with silt. According to Dash et al. (1981) urease activity was positively correlated with silt + clay content but negatively with sand content.

In a study conducted by Sahrawat (1983) in tropical wetland rice soils, simple correlation analyses of urease activity with soil properties indicated that urease activity was correlated highly significantly with total N and organic carbon, but was not significantly correlated with CEC and clay. Multiple regression analyses showed that organic matter content of soils measured by organic carbon and total N accounted for most of the variation in urease activity.

2.4.2 Effect of pH.

McGarity and Myers (1967) found a weak correlation between urease activity and pH. Silva and Perera (1971) found that urease activity in rubber soils of Srilanka was significantly related to pH whereas Pancholy

and Rice (1973) and Zantua et al. (1977) found that urease activity was not significantly correlated with pH.

From the studies involving use of buffers to determine the effect of pH on soil urease activity, Pettit et al. (1976) found that optimum pH for soil urease was 6.5 - 7.0, whereas Tabatabai and Bremner (1972) and May and Douglas (1976) found that it was 8.8 - 9.0. Frankenberger and Johanson (1982) reported that the pH stability of soil enzymes was highly dependent on the type of soils being assayed. Singh and Nye (1984) observed that V_{max} of the enzyme urease increased with rise in pH, reached a maximum value at pH 6.0 and then declined at higher pH. They also found that pH for optimum activity varied slightly with concentration of urea.

Beri and Brar (1978) found that urea was completely hydrolyzed within 20 - 50h in alkaline, sub tropical soils of Punjab. Acid sulphate soils were found to have lower urease activity than organic soils and other mineral soils having near neutral or alkaline pH (Sahrawat, 1980 b). These results imply that in alkaline soils the rate of urea hydrolysis is more rapid which increases the possibility for N loss through NH_3 volatilization.

2.4.3 Effect of soil moisture .

There are divergent findings of the effect of soil moisture or water level on urease activity. Several workers had found that urease activity in soil was not significantly affected by the water level (Skujins and

McLaren, 1969; Delaune and Patrick, 1970; Gould et al., 1973). Skujins and McLaren (1969) found that activity in an air dried soil equilibriated at 100 per cent relative humidity was close to that observed when this soil contained 50 per cent water. Dalal (1975) found that urease activity increased with moisture content from 25 to 50 per cent WHC beyond which it decreased. According to Vlek and Carter (1983), soil urease is sensitive to the lack of soil moisture and an increased hydrolysis of urea occurs with increasing soil water content upto near field capacity followed by a decrease thereafter. Kumar and Wagenet (1984) also reported a linear increase in urease activity with moisture contents upto field capacity. Sahrawat (1984) observed that urease activity increased with increasing moisture content from air dried state to field capacity and then remained constant with further increase in moisture content. However urease activity was not detected in soil samples collected in late summer when the soil moisture content was below -15 bar atm.

According to Sahrawat (1980 b) flood water of tropical lowland rice soils might have measurable amounts of urease activity though its contribution was far less than that of soil urease. Vlek et al. (1980) reported that urease activity in flooded soils was not constant but was dynamic and changed with the duration of flooding. According to them the flood water urea was hydrolyzed largely at the soil-flood water interface. Savant et al. (1985) observed that in a submerged soil system incubated for < 24h, the depletion of O_2 seemed to retard the hydrolysis and with a longer submergence time, soil Eh decreased and soil urease activity

also decreased to a stabilized value. However, on reoxidation of the reduced soil under a continuous 1 cm of flood water, the soil urease activity showed a marked increase. In general the order of the urea hydrolysis in the main three components of the wetland soil system was oxidised soil > reduced soil > flood water (without algae). Thus urea hydrolysis in a wetland soil system showed temporal and spacial variation.

Work with soil urease has shown that this enzyme is strongly inhibited by metal ions and perhaps the decrease observed upon waterlogging of soils is due to the increase in solubility of the reduced metals formed in the waterlogged soils (Tabatabai, 1977; Gototh and Patrick, 1974). Pulford and Tabatabai (1988) reported that urease activity decreased after waterlogging of soils for 7 days. He also found that activity was significantly correlated with Eh.

2.4.4 Effect of substrate concentration.

The rate of hydrolysis of urea by soil urease increased with increase in urea concentration until the amount of urea added was sufficient to saturate the enzyme with substrate (Douglas and Bremner, 1971; Tabatabai and Bremner, 1972; Dalal, 1975). Gould et al. (1973) found that there was an approximately linear increase in hydrolysis with increased initial concentration of urea in the soil. The rate of hydrolysis of urea in a buffered system increases with increase in substrate concentration and this hydrolytic reaction of urea attains maximum velocity at the critical concentration beyond which there is no increase (Patra and Jain, 1984).

According to Singh and Nye (1984) urease activity increased with increase in substrate concentration reached a maximum and then declined at higher urea concentration. Savant et al. (1987) suggested that it was necessary to consider effective urea concentration and effective urease activity for adequate understanding of insitu hydrolysis of broadcast fertilizer urea in unsaturated soil.

2.4.5 Effect of rhizosphere .

Rice rhizosphere was found to have a positive influence on urease (Tisdale et al., 1985). Greatest activity of urease was reported to occur in the rhizosphere where microbial activity is high and where it can be excreted from plant roots. Extracellular urease activity had been reported to be associated with rice roots (Mahapatra et al., 1977).

Rhizosphere urease varies depending on plant species (Tisdale et al., 1985). Khan (1971) observed that urease activity in the rice rhizosphere was highest on 50th day after sowing. In a study with mesta crop, Tarafdar and Roy (1981) observed that maximum rhizosphere contribution was between 10 and 30 days after sowing the crop and thereafter it declined.

Sites where urease is produced in a wheat soil ecosystem was identified by growing wheat seedlings under sterilized and unsterilized soil environment by Chhonkar and Pal (1987). The amount of urease in the root exudates of aseptically grown wheat seedlings measured was always

higher in unsterilized soil than in sterilized soil irrespective of the age of wheat seedlings, indicating thereby significant microbial contribution towards the build up of urease in soil.

2.5 Molecular Absorption of Urea

Urea can enter the plants not only after its decomposition but also as a whole molecule and this phenomenon has been confirmed by several workers (Freiberg and Payne, 1957; Dilley and Walker, 1961; Mitsui and Kurihara, 1962a, b)

It was shown that urea can be absorbed by an organism in which no active urease is to be found. Consequently it is not necessarily subjected to enzymatic hydrolysis inside the cells. Freiberg and Payne (1957) noted the absorption of undecomposed urea by the leaves of banana, and Japanese researchers (Mitsui et al., 1960; Mitsui and Kurihara, 1962a, b) by the roots of rice. No activity of urease was found in the tissues of roots of rice or in the assimilatory tissues of banana. Molecules of urea were found in the juice of the crushed leaves and in the guttation exuded by corn grown in a solution of urea, which proved the absorption of urea by corn plants (Koren'kov, 1966).

Materials and Methods

MATERIALS AND METHODS

The details of the materials used and the techniques adopted during this study are presented in this chapter.

Six major types of rice soils of Kerala were used for the study. These were laterite soil of Mannuthy, karappadam, kari and kayal soils of Kuttanad, kole soil of Trichur district and black soil of Palghat district.

The following experiments were undertaken during the course of this investigation.

i) Kinetics and levels of soil urease activity in the six soil types under two different moisture regimes viz:, soil at 60% WHC and under submergence.

ii) Effect of the following factors on soil urease activity.

   a) Soil submergence

   b) Organic and inorganic reducing substances

   c) pH

   d) Rice rhizosphere

iii) Comparison of the three different methods for the assay of the urease activity - buffer method, non-buffer method and isotope method.

iv) Molecular absorption of urea by rice plant

Surface soil samples (0-15 cm) of the six soil types which were air dried and ground to pass through a 2 mm sieve were used for the studies.

3.1 Physico-chemical Properties of Soil

The physico-chemical properties of laterite, karappadam, kari, kayal, kole and black soils are presented in Table 1.

Table 1. Physico-chemical properties of laterite, karappadam, kari, kayal, kole and black soils.

1. Mechanical composition

Soil type	Fraction (per cent composition) Sand	Silt	Clay	Procedure adopted
Laterite	85	12.5	2.5	Hydrometer method
Karappadam	60	31.25	8.75	
Kari	66.25	26.25	7.5	(Bouyoucos, 1962)
Kayal	75	8.75	16.25	
Kole	55	23.75	21.25	
Black	73.75	10	16.25	

2. Physical constants

Soil type	Maximum water holding capacity (Keen Raczhowski Box method, Piper, 1950)	Field capacity (Gravimetric method)
Laterite	34 per cent	11.67 per cent
Karappadam	56.1 "	22.05 "
Kari	59.4 "	25.71 "
Kayal	74.1 "	37.77 "
Kole	67.4 "	31.3 "
Black	72.86 "	35.05 "

3. Chemical properties

Soil characteristic	Soil type						Method used
	Laterite	Karappadam	Kari	Kayal	Kole	Black	
Organic carbon (per cent)	0.38	1.62	3.96	1.83	1.57	0.63	Walkley and Black method (Piper, 1950)
Total N (per cent)	0.154	0.182	0.35	0.29	0.26	0.14	Micro-Kjeldahl method (Jackson, 1958)
Available P (ppm.)	19.0	4.5	5.0	1.5	2.5	7.25	Chlorostannous reduced molybdo phosphoric blue colour method (Jackson, 1958)
Available K (ppm)	70.0	120.0	25	140	140	350	Flame photometric method (Jackson, 1958)
pH	4.7	4.6	2.5	4.4	4.7	8.0	Elico pH meter (Jackson, 1958)
Eh (mV)	$^{+}320$	$^{+}365$	$^{+}480$	$^{+}420$	$^{+}415$	$^{+}310$	Model RM-1K oxidation reduction potential meter of TOA Electronics Ltd., Japan.
CEC (me 100 g^{-1} soil)	3.8	12.7	33.3	16.4	21.3	52.4	Ammonium acetate method (Jackson, 1958)

3.2 Kinetics and Levels of Soil Urease Activity

3.2.1 Urea hydrolysis as influenced by moisture regimes.

Urea hydrolysis was studied at two different moisture regimes viz., soil at 60% WHC and under submergence.

Five gram samples each of the six soil types were treated with urea (2000 $\mu g\ g^{-1}$ soil) and incubated for different intervals of time namely, 2, 5, 10, 15, 20, 24, 34, 48, 120, 240, 360, 480, 600 and 720hat the above mentioned two moisture regimes. In the first case (ie. moisture at 60% WHC), incubation was performed in plastic bottles (200 ml). In the other case, incubation was performed in glass tubes (30mm x 120mm, 30ml capacity) after submerging the soil with 5 ml of water. Sufficient number of replications were kept to allow the removal of samples in duplicate for each soil at different intervals. At the end of each incubation period urease activity was estimated by the non-buffer method (Zantua and Bremner, 1975a). In the case of soil samples incubated under submergence, Eh and pH were also measured at each interval, before estimating the urease activity.

3.2.2 Determination of Michaelis constant.

To find out K_m (Michaelis constant) and V_{max} (maximum reaction velocity) values for soil urease, the following experiment was conducted. Five gram soil samples were treated separately with 250, 500, 1000, 1500

and 2500 µg of urea (substrate) and these concentrations were equal to 4.16, 8.33, 16.66, 25 and 41.67 µ moles of urea. Another set of the soil samples were treated with urea solutions buffered with Tris-HCl buffer (pH 7.2) to give similar substrate concentrations. Soil samples were then incubated for 10h. The urease activity was determined following the non-buffer method (Zantua and Bremner, 1975 a). The three soils used in this study were karappadam, kari and black soil.

The values of $\frac{1}{V_o}$ (V_o is the initial velocity) were then plotted against $\frac{1}{S}$ values (S is substrate concentration) to obtain the double reciprocal Lineweaver-Burk plot represented by the equation

$$\frac{1}{V_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \times \frac{1}{S}$$

where $\frac{K_m}{V_{max}}$ is the slope. The intercept on $\frac{1}{V_o}$ ordinate is equal to $\frac{1}{V_{max}}$ while the intercept on the negative side of the $\frac{1}{S}$ abscissa equals $\frac{-1}{K_m}$.

S is substrate concentration expressed in µ moles; V_o is initial velocity expressed in µ moles of urea hydrolysed g^{-1} soil h^{-1}; V_{max} is maximum reaction velocity and K_m is Michaelis constant.

3.3 Effect of Soil Anaerobiosis on Urea Hydrolysis

To study the effect of soil anaerobiosis on urea hydrolysis, the following experiment was undertaken. Five gram samples of each soil were weighed into flat bottomed glass tubes (30mm x 120mm, 30ml capacity) and flooded with 5ml distilled water. The tubes were closed with screw caps and kept undisturbed at room temperature for different periods, namely, 0, 5, 10, 15, 20, 24, 48, 120, 240, 360, 480, 600 and 720h to allow soil reduction

to take place. A thin polythene sheet cut to proper size was also sandwitched for each tube between the screw cap and the mouth of the tube to prevent adhering of soils on to the screw cap while shaking during subsequent analysis. In this way sufficient number of tubes were kept with flooded soils to allow the removal of two tubes for each soil at different intervals. At the end of the respective incubation period, Eh and pH were measured and the soil sample was treated with urea (2000 µg urea g^{-1} soil) and incubated for 5h. After 5h, the contents of the tube were completely transferred into 200 ml plastic bottle and urea hydrolysis was estimated by the non-buffer method.

3.4 Effect of Organic and Inorganic Reducing Substances on Urease Activity

The effect on urea hydrolysis of some of the organic reducing substances produced under submerged paddy soils, namely, formaldehyde, ethanol, acetic acid and acetone was studied. Three soils, namely, kari, karappadam and black soils were selected for the study as these soils were found to have the lowest, medium and highest urease activities respectively. The concentrations of the reducing substances used were 100 ppm and 500 ppm. Five gram soil samples were flooded with 5ml solution of these reducing substances in glass tubes and incubated with urea (2000 µg urea g^{-1} soil) for 5h. Urease activity was assayed by the non-buffer method. Duplicate samples were used for each assay.

The effect of ferrous iron, one of the important inorganic reducing substances formed in flooded soils, on urease activity of kari, karappadam and black soils was studied. The Fe^{2+} concentrations of the soil (5 g sample) produced following flooding for 0, 1, 2, 3, 4, 5 and 6 days was estimated by extracting the soil with 1*M* sodium acetate (pH 2.8) solution (Motomura and Yokoi, 1969) and by analysing the extract spectrophotometrically using O-phenanthroline (Black, 1965). Urease activity of the soils for the corresponding periods was also estimated separate sets to study the influence of Fe^{2+} concentration on urease activity.

3.5 Effect of pH on Urease Activity

To determine the influence of pH on urease activity, laterite soil which is having a pH of 4.7 was used. The pH of the soil was varied using 0.05 *M* THAM H_2SO_4 buffers of pH 6, 7, 8 and 9. To 5g portions of the soils 5ml each of the buffer solutions were added and a control was also kept in which 5ml of water were added. Soil samples were then treated with urea (2000 μg urea g^{-1} soil), incubated for 5h and analysed for urease activity using the method of Zantua and Bremner (1975 a). Each treatment was replicated thrice. The pH of the soil buffer solutions were also taken just before incubation with urea.

3.6 Influence of Rice Rhizosphere on Urease Activity

A pot culture experiment using the six soils was conducted during the period from 25-1-1989 to 5-5-1989 using the rice variety Jyothi to examine

the role of rhizosphere on urease activity of the six soil types. The meteorological data for the crop period are presented as weekly average in Appendix I.

Plastic pots of capacity 4 l were filled with 2kg of each soil and two rice seedlings (20 days old) were planted in each pot and grown under flooded condition. There were three replications for each soil. Three pots containing soil but without growing rice were also kept for each soil type. Fertilizers were applied as per the Package of Practices Recommendations (KAU, 1986). Nitrogen was applied as ammonium sulphate, phosphorus as superphosphate and potassium as Muriate of potash. Urease activity of different soils, with and without gorwing rice, was estimated by drawing soil samples from the rhizosphere at the time of planting, 30 DAP, 60 DAP and at harvest by the non-buffer method (Zantua and Bremner, 1975 a). The study could not be completed, however, with kari soil as the rice seedlings wilted a few days after planting.

3.7 Methods of Assaying Urease Activity in Soils

Three methods were used for comparison. These were non-buffer method (Zantua and Bremner, 1975 a), buffer method (Tabatabai and Bremner, 1972) and isotope method in which ^{14}C - labelled urea was used.

3.7.1 Non-buffer method.

Urease activity was assayed by the non-buffer method described by Zantua and Bremner (1975 a). The method used in this study involved

determination of the amount of urea hydrolysed by incubation of the soil sample (5 g on oven dry basis) at room temperature with 1ml of urea solution containing 10mg urea (2000 μg urea g^{-1} soil) for 5h at 60% WHC. Incubation was performed in a 200ml plastic bottle after the water content was adjusted to 60% WHC. After an incubation period of 5h soil samples were shaken for 60min with 50ml of 2*M* KCl containing 5 μg ml^{-1} of phenyl mercuric acetate (PMA) and the extracts obtained after filtering (Whatman No.42 filter paper) were analysed for urea colorimetrically as described by Douglas and Bremner (1970). Suitable aliquot of the extract was pipetted into a 25ml volumetric flask, made up the volume to 5ml with 2*M* KCl-PMA solution and 15ml of the colour reagent (Diacetyl monoxime and thiosemicarbazide in acetic acid) were added to it. The flask was then swirled for a few seconds and placed in an oven ajusted to 120°C for colour development. After 30 min the flask was removed from the oven and cooled immediately by placing them in deep tray containing cold water for 15 min. The volume was made upto 25 ml with distilled water and mixed thoroughly. The intensity of the red colour was measured spectrophotometrically at a wavelength of 525nm within one hour.

The urea content of the extract analysed was calculated by reference to a calibration graph. Urease activity was expressed as micrograms of urea hydrolysed per gram of soil.

3.7.2 Buffer method.

The buffer method (Tabatabai and Bremner, 1972) involved determination of the ammonium released by incubation of the 5g soil sample with 9ml trishydroxymethyl amino methane (THAM) H_2SO_4 buffer (pH-9.0), 1ml 0.2*M* urea solution (12 mg of urea ml^{-1}) and 0.2 ml toluene at room temperature for 5h in a 50ml volumetric flask. Ammonium release was determined by shaking the incubated soil sample with 2.5*M* KCl containing a urease inhibitor (Ag_2SO_4) for a few seconds and then steam distilling an aliquot of the resulting soil suspension with MgO. A control was also performed to allow for NH_4^+-N not derived from urea through urease activity. To perform this, the same procedure was followed excepting that the addition of 1ml of 0.2 M urea solution (12 mg urea ml^{-1}) was done after the addition of 35 ml of KCl-Ag_2SO_4 solution. The experiment was done with all the six soils in duplicate.

3.7.3 Isotope method.

3.7.3.1 Estimation of urease activity.

Two methods were tried both of which involved the use of ^{14}C-labelled urea.

The first method involved the estimation of urease activity through the measurement of $^{14}CO_2$ released (Skujins and McLaren, 1969). Laterite and karappadam soils were used for the study. To five gram portion of the soil sample taken in a 100ml conical flask were added 2ml distilled

water and 1ml ^{14}C-labelled urea solution (2000 μg urea g^{-1} soil) to give 31cpm ^{14}C μg^{-1} urea (specific activity). The soil was then incubated for 5h after suspending a tube containing 3ml of 1*N* KOH into the flask to trap $^{14}CO_2$ releasing during urea hydrolysis and closing the flask air tight with a rubber cork. (Fig. 1) After 5h, 1ml of KOH from the suspended tube into the flask was transferred into a liquid scintillation vial containing 15ml of dioxan-based liquid scintillator and $^{14}CO_2$ trapped in KOH was measured using a liquid scintillation system. The determination was done in duplicate for each soil.

The second method involved the estimation of urease activity through the determination of ^{14}C-urea remaining after the incubation period. The soils used were kari, karappadam and black soils as these found to have the lowest, medium and highest urease activities. To five gram portion of soil taken in a 100ml conical flask were added 2ml of 0.05*M* potassium acetate buffer (pH 5.5) and 1ml of ^{14}C-labelled urea solution (2000 μg urea g^{-1} soil) of specific activity 32 cpm μg^{-1} urea. The flasks were then closed with rubber cork and incubated for different intervals, namely, 5h, 10h, 15h, 20h and 24h. Sufficient number of replications were kept for the removal of samples in duplicate at different intervals for each soil. At each interval soil samples were shaken with 50ml of 2*M* KCl-PMA solution for 60 min and the resulting soil suspension was filtered through Whatman No. 42 filter paper. An aliquot of the extract was taken in a liquid scintillation vial containing 15ml of dioxan based liquid scintillator and the radio-activity was determined using a liquid scintillation system. From the radioactive content of ^{14}C-urea solution initially added and

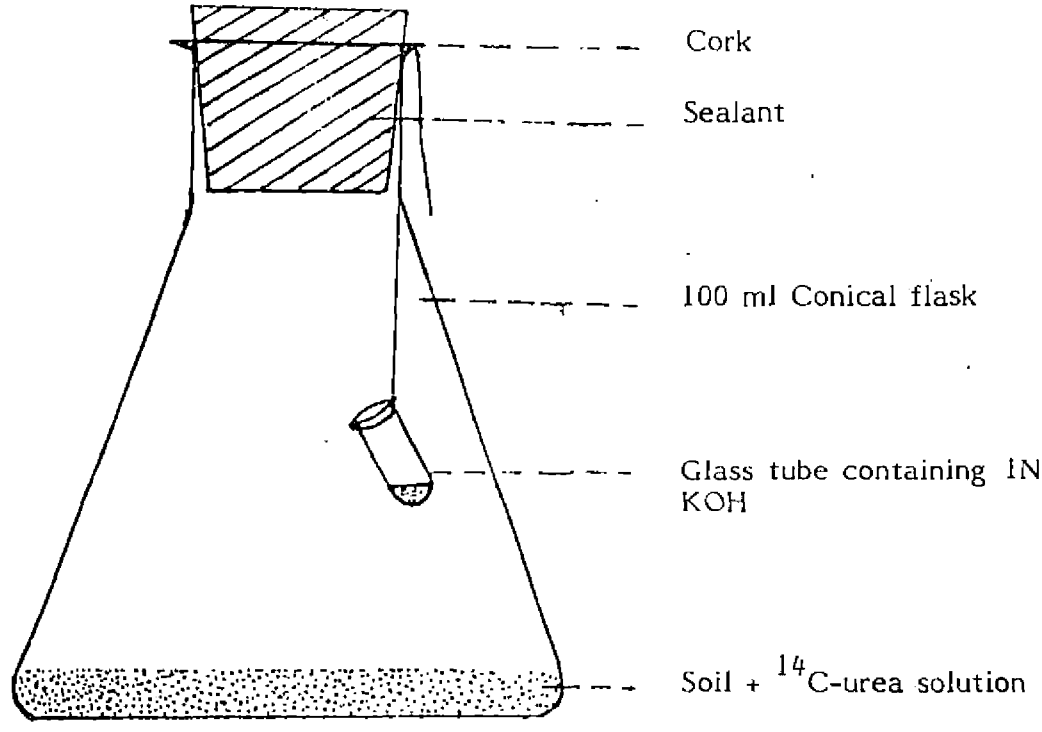


FIG.1. EXPERIMENTAL SET-UP USED FOR TRAPPING $^{14}CO_2$ LIBERATED DURING THE HYDROLYSIS LABELLED UREA IN SOIL

the count rates obtained for the KCl extract, the per cent of urea hydrolysis was calculated.

3.7.3.2 Isotope effect on urea hydrolysis.

To examine if there is any isotope effect on urea hydrolysis, three solutions of ^{14}C-labelled urea (10 mg urea ml^{-1}) were used to give three different specific activities of 32 cpm μg^{-1} urea, 58 cpm μg^{-1} urea and 113 cpm μg^{-1} urea. Five gram portions of kari, karappadam and black soils were incubated with 2ml *0.05M* potassium acetate (pH *5.5*) and 1ml ^{14}C- labelled urea solution for 5h and radioactivity of ^{14}C- urea remaining after incubation was determined by the same method as described above. The determinations were made in triplicate for each soil and for each specific activity.

3.7.3.3 Radiochemical purity of ^{14}C- labelled urea.

Two microlitres of the ^{14}C- urea stock solution was spotted on pre-coated cellulose F thin layer chromatographic plate (Camag). Alongside, 2 µl of unlabelled urea (2000 ppm) solution was also spotted as authentic compound. The chromatograms were developed in butanol-acetic acid water (60:15:25) solvent as per the method of (Touchstone and Dobbins, 1978). The unlabelled urea was identified as yellow spot by spraying on the plate, Ehrlich's reagent. From this, R_f value was obtained as 0.5. The radioactive urea spot based on the R_f value was then scraped off from the TLC plate and transferred into a liquid scintillation vial containing

15 ml of dioxan-based liquid scintillator. The radioactivity was determined using a liquid scintillation system. From the radioactive content of 2 µl ^{14}C-urea stock solution estimated by the liquid scintillation technique and the count rates obtained for the TLC spot for ^{14}C-labelled urea, the radiochemical purity of the substance was calculated as follows :

$$\text{Radiochemical purity (\%)} = \frac{\text{The count rate observed for the TLC spot of } ^{14}\text{C-urea}}{\text{Count rate of the applied quantity}} \times 100$$

Radiochemical purity of the ^{14}C - urea stock solution was found to be 95.4%.

3.7.3.4 Radioassay :

The liquid scintillator used in the studies consisted of 4g PPO, 0.2g POPOP, 60g naphthalene, 100ml methanol and 20ml ethylene glycol diluted to 1000ml with dioxan. The radioactivity was determined in a micro computer controlled liquid scintillation system (Rackbeta 1215 of Wallac OY, Finland).

3.8 Molecular Absorption of Urea by Rice Plant

The study was conducted with ^{14}C-labelled urea. A few rice plants (55 days old) were allowed to absorb for 3 and 4h ^{14}C- urea from a ^{14}C-urea solution (specific activity, 31 cpm μg^{-1} urea) in 100ml conical flasks. A 5g sample of plants which were allowed to absorb ^{14}C- urea for 3h was extracted with water containing 5ppm PMA and was made upto 35ml. A 5g sample from plants which were allowed to absorb ^{14}C-urea for 4h was extracted with boiling ethanol (80%) and was made up to 25ml. After

centrifuging at 10000 rpm for 10min, 1ml of these extracts were analysed for radioactivity using liquid scintillation technique.

Counting efficiency of the two assays were found out using internal standard method. The internal standard used here was 10 µl of ^{14}C- urea stock solution the dpm of which is known and equals to 669155.56 dpm. Radioactivity of 1ml extract was taken first and after adding 10µl of ^{14}C- urea stock (internal standard) counts were taken again. The counting efficiency was then calculated as follows :

$$\text{Counting efficiency} = \frac{(cpm_2 - cpm_1) \times 100}{dpm_i}$$

Where cpm_1 is the counts of 1ml extract above

cpm_2 is the counts of 1ml extract and internal standard together

dpm_i is the radioactivity of internal standard in terms of disintegration per minute

Autoradiograph of a rice plant which was allowed to absorb ^{14}C-urea for 3h was also prepared. For this, the specimen was pressed using a herbarium press and dried at 70°C in an oven for 30min. The specimen was then kept in contact with X-ray film in the dark. After an exposure period of one month, the X-ray film was developed using Agil X-ray developer and Agil X-ray fixer and positive prints were taken.

3.9 Statistical Analyses

The statistical analyses were done adopting the method suggested by Panse and Sukhatme (1985).

Results

RESULTS

The results of the experiment conducted to study the urease activity of six major rice soils of Kerala are presented in this chapter under the following heads.

1. Kinetics and levels of urease activity in the different soil types.
2. Changes in urease activity, Eh and pH in anaerobic soils following flooding.
3. Effect of organic and inorganic reducing substances on urease activity.
4. Effect of pH on urease activity.
5. Influence of rice rhizosphere on urease activity.
6. Methods of assaying urease activity in soils.
7. Molecular absorption of urea by rice plant.

4.1 Kinetics and Levels of Urease Activity in the Different Soil Types

4.1.1 Urea hydrolysis at different moisture regimes.

The data on the urease activity assayed by the non-buffer method (Zantua and Bremner, 1975 a) are presented in Table 2.

Table 2. Levels of urease activity in the six soil types at 60% WHC and under submergence for different incubation periods

Incubation period (h)	Urease activity (µg urea hydrolysed g^{-1} soil)											
	Laterite		Karappadam		Kari		Kayal		Kole		Black	
	60 % WHC	Submer-gence	60 % WHC	Submer-gence	60 % WHC	Submer-gence	60 % WHC	Submer-gence	60 % WHC	Submer-gence	60 % WHC	Submer-gence
2	0	0	0	0	0	0	29	0	11	0	66	0
5	248	212	285	248	175	139	230	175	285	175	321	212
10	613	759	686	978	212	248	759	1197	668	431	905	431
15	1544	1069	1526	1142	248	139	1763	1489	832	449	1015	540
20	1872	1788	1745	1531	248	66	1836	1785	942	686	1343	942
24	1939	1796	1919	1679	285	175	1925	1905	1170	1124	1124	1015
34	-	-	-	-	-	321	-	-	1894	-	1555	-
48	1971	1920	1993	1927	467	175	1989	1920	1932	1905	1558	1613
120	2000	2000	2000	2000	850	467	2000	2000	1954	2000	1945	2000
240	2000	2000	2000	2000	668	686	2000	2000	2000	2000	2000	2000
360	2000	2000	2000	2000	887	1088	2000	2000	2000	2000	2000	2000
480	2000	2000	2000	2000	1270	1737	2000	2000	2000	2000	2000	2000
600	2000	2000	2000	2000	1900	1869	2000	2000	2000	2000	2000	2000
720	2000	2000	2000	2000	1967	1971	2000	2000	2000	2000	2000	2000

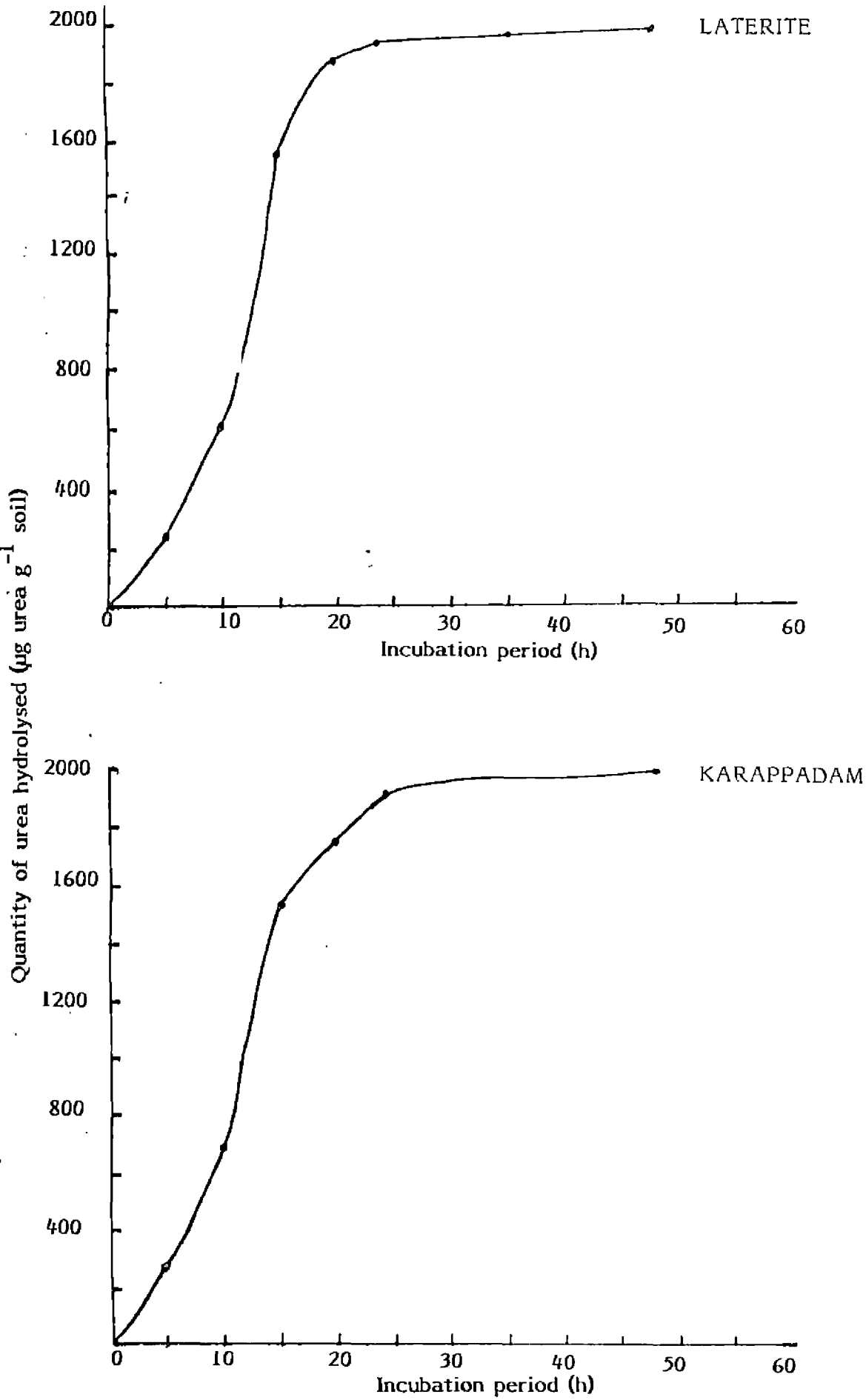


FIG. 2a. PATTERN OF UREA HYDROLYSIS IN LATERITE AND KARAPPADAM SOILS AT 60% WHC.

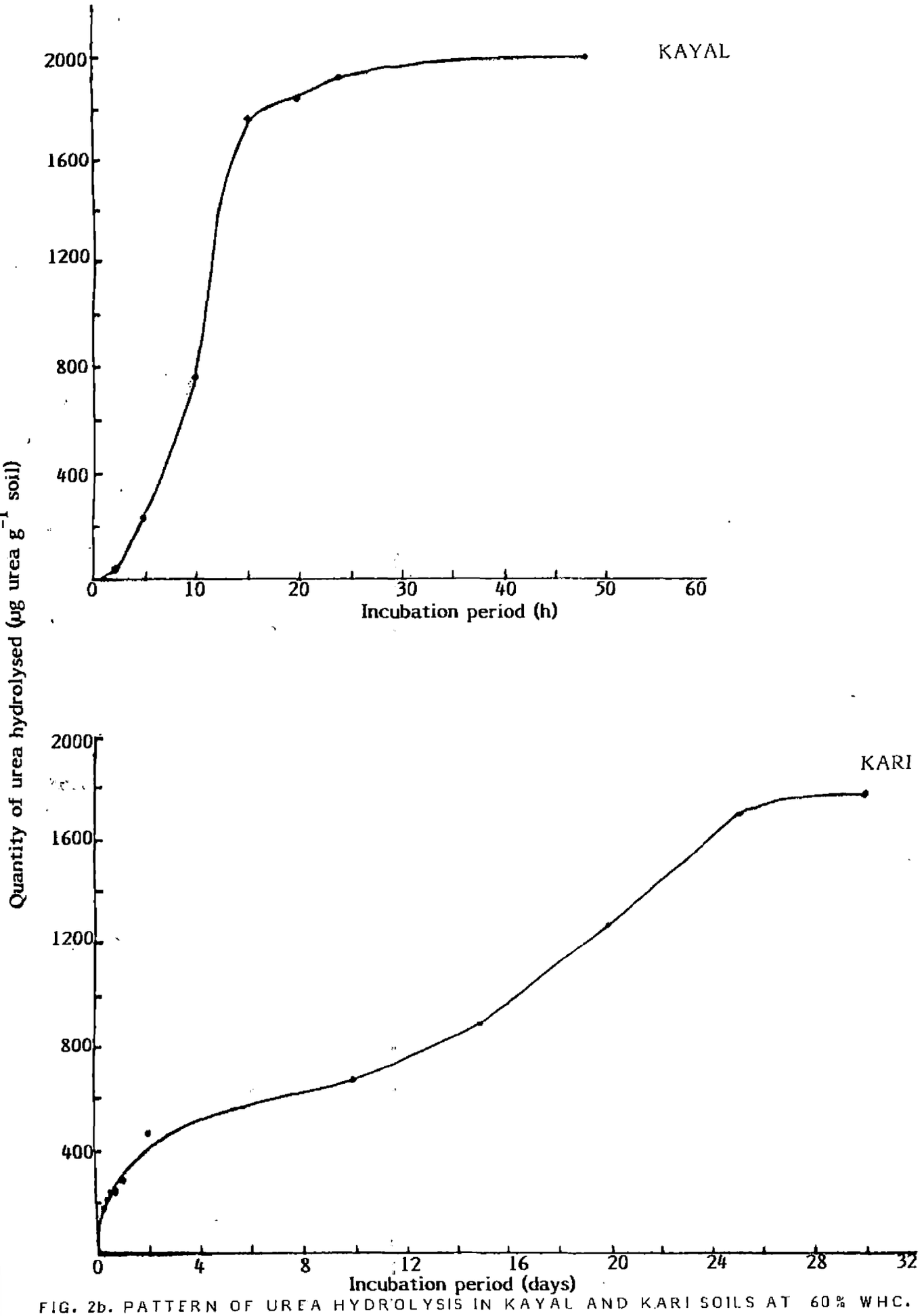


FIG. 2b. PATTERN OF UREA HYDROLYSIS IN KAYAL AND KARI SOILS AT 60% WHC.

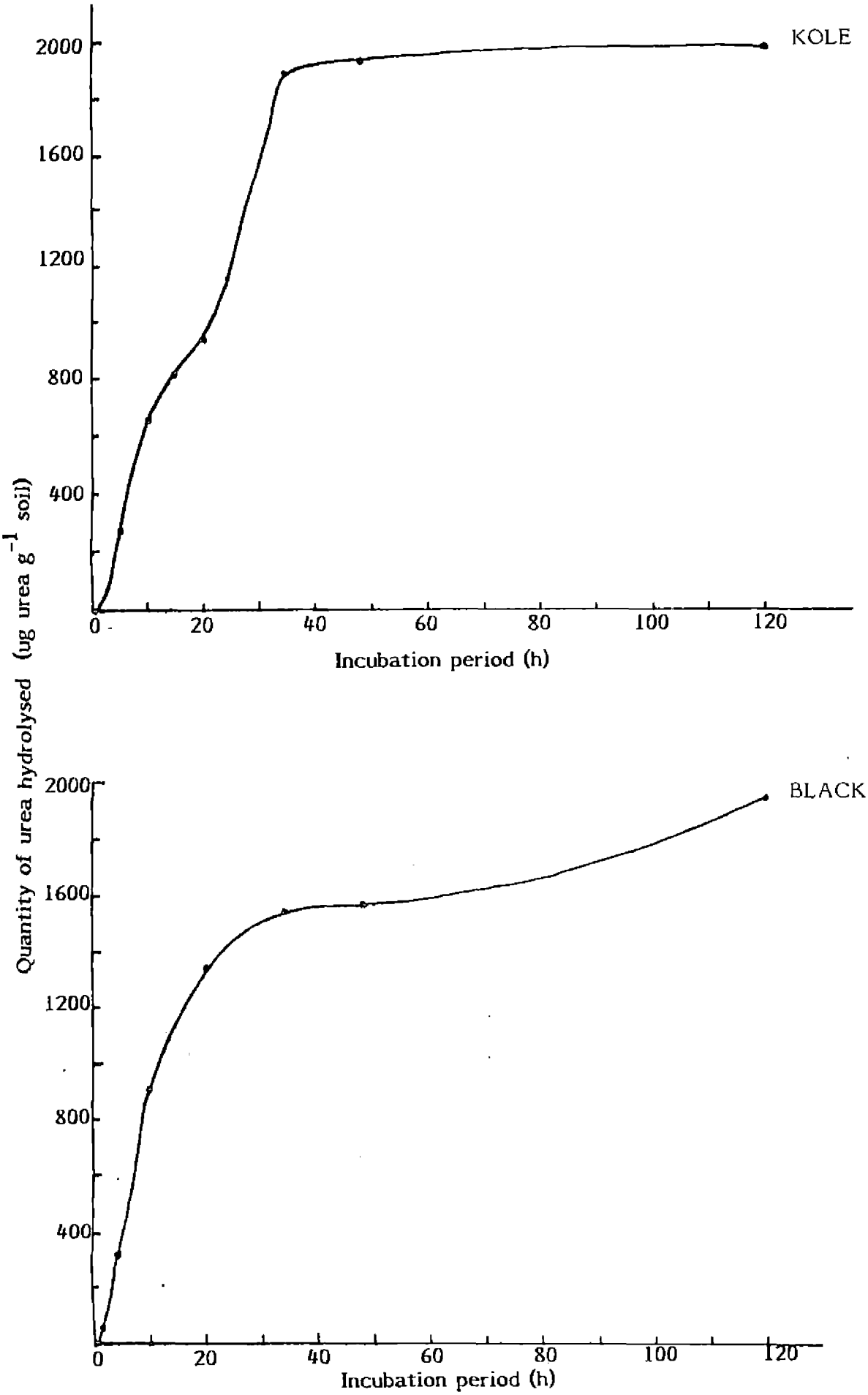
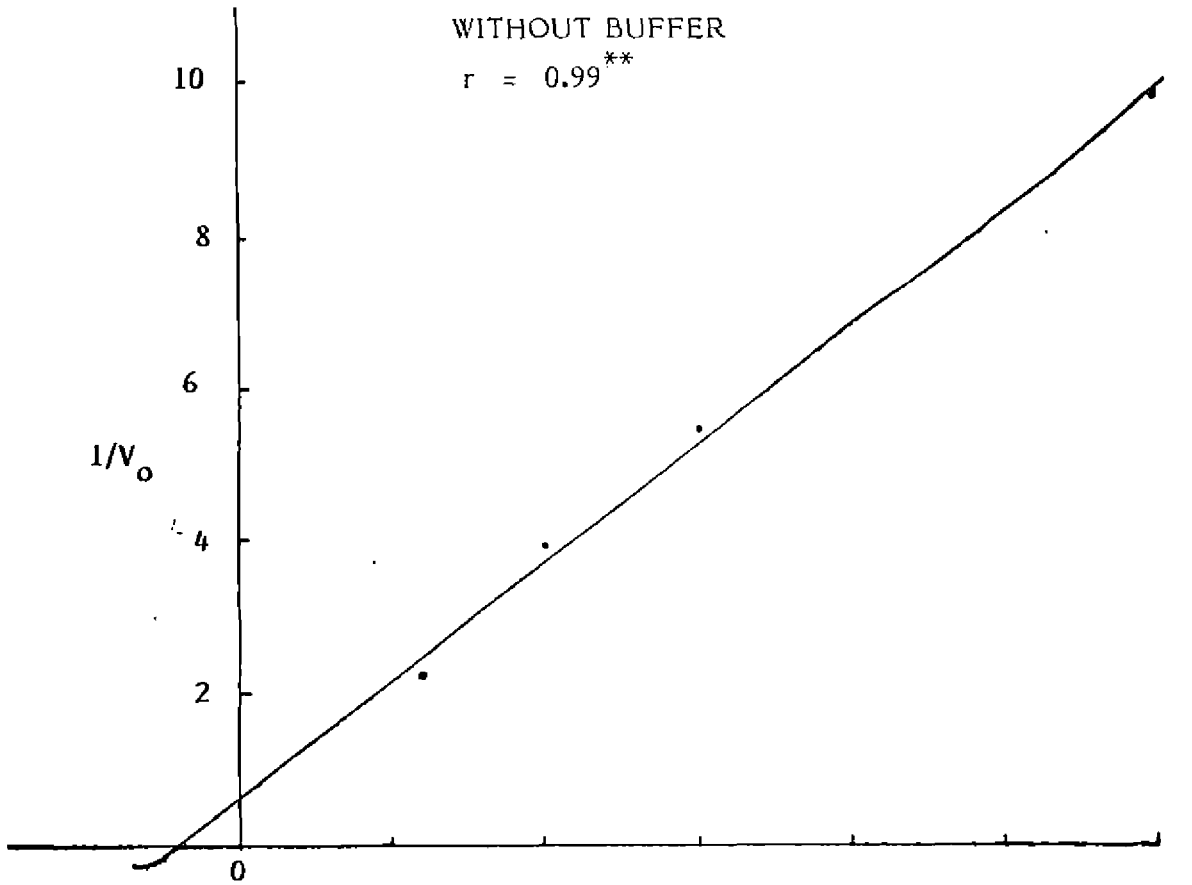


FIG. 2c. PATTERN OF UREA HYDROLYSIS IN KOLE AND BLACK SOILS AT 60% WHC.



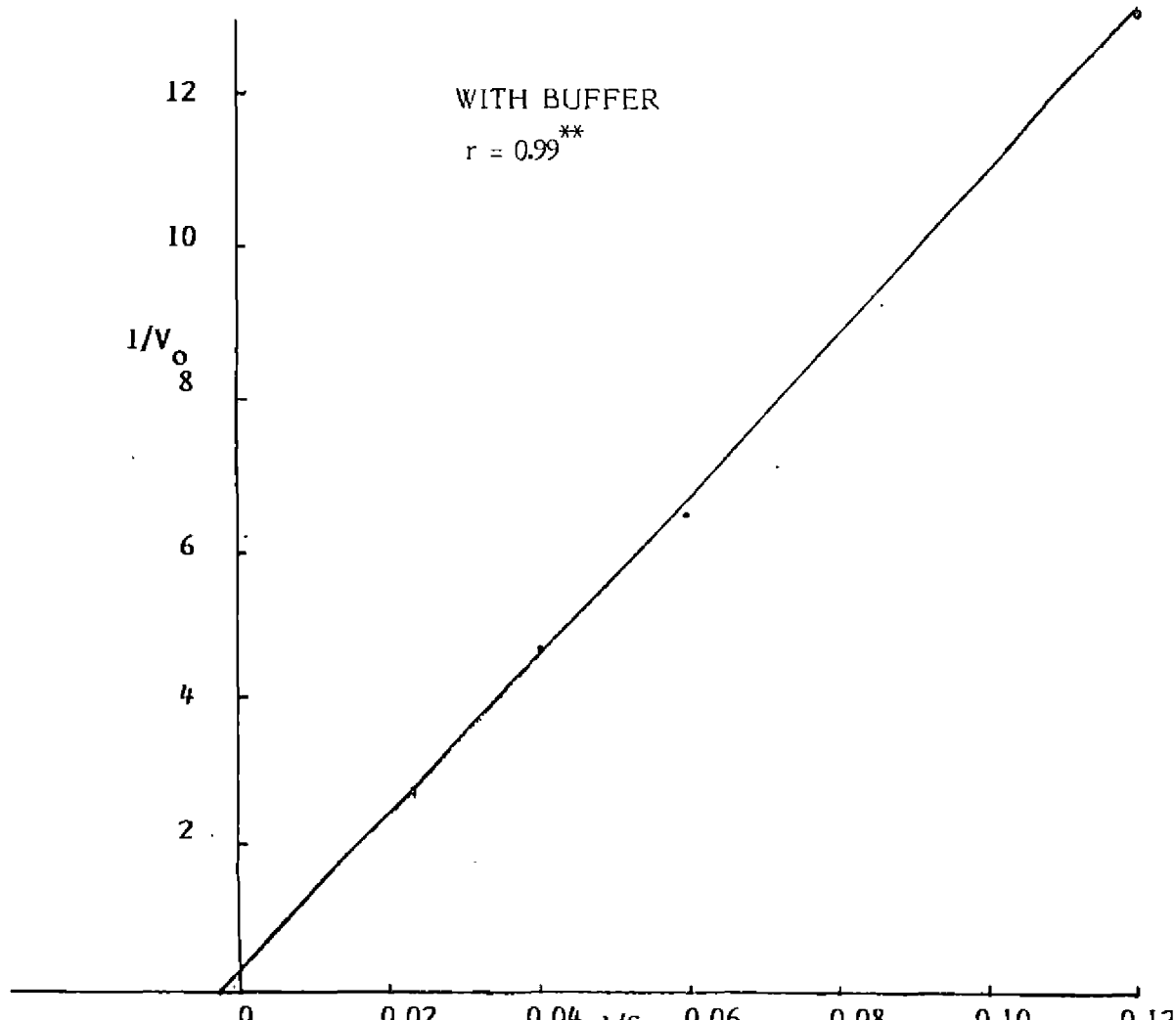
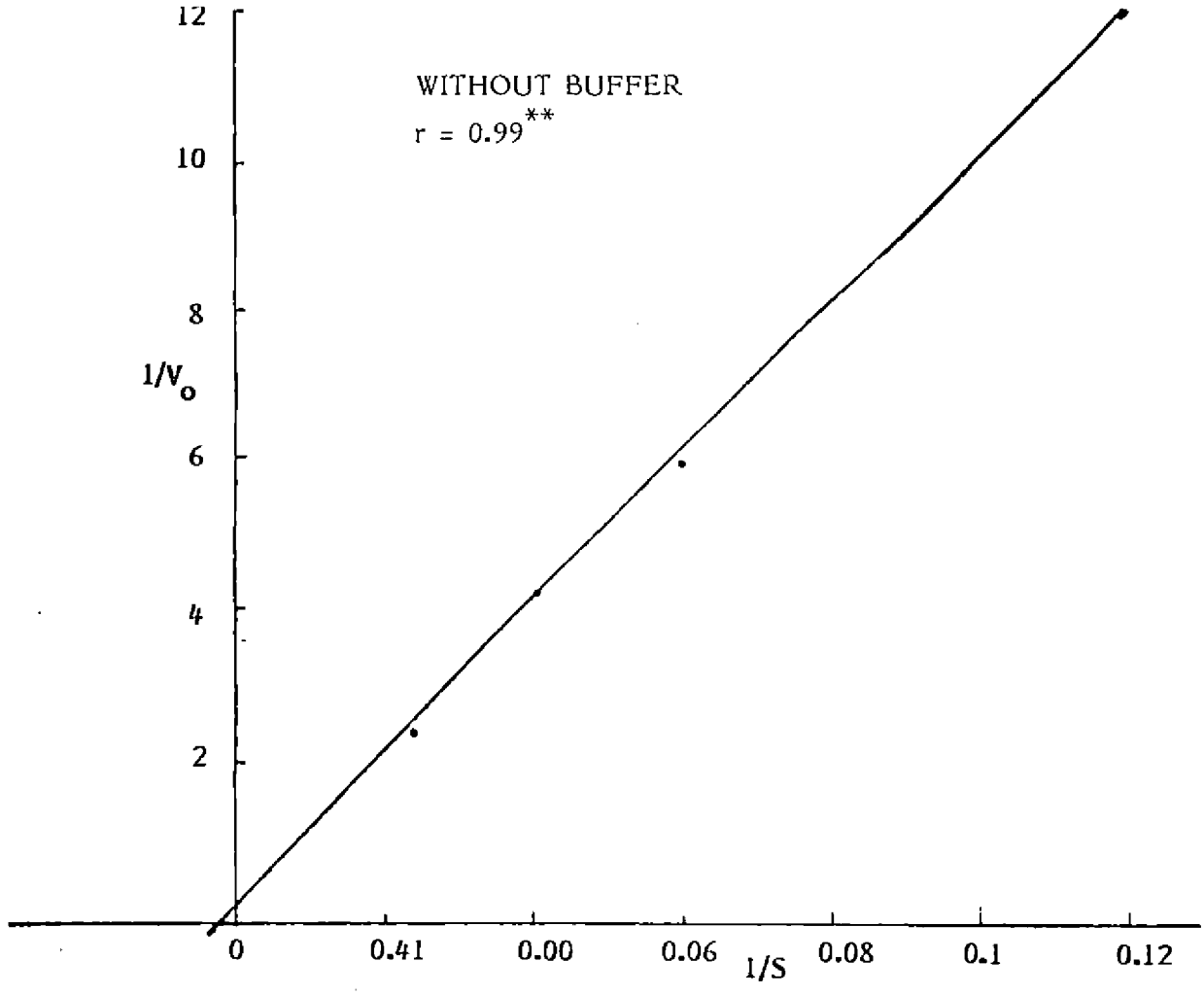


FIG. 3a. LINEWEAVER-BURK PLOT OF UREASE ACTIVITY OF KARAPPADAM SOIL (WITHOUT AND WITH BUFFER)



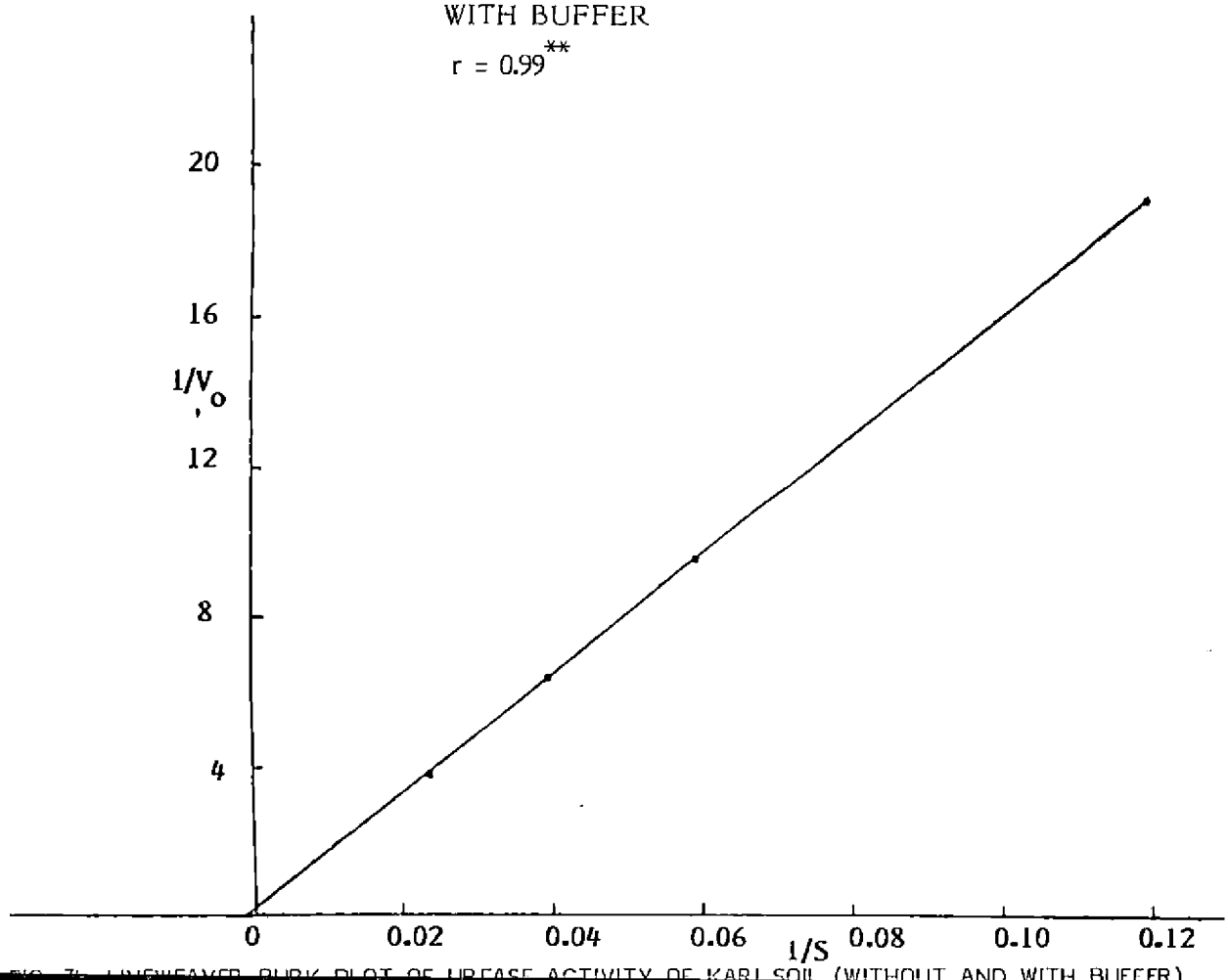
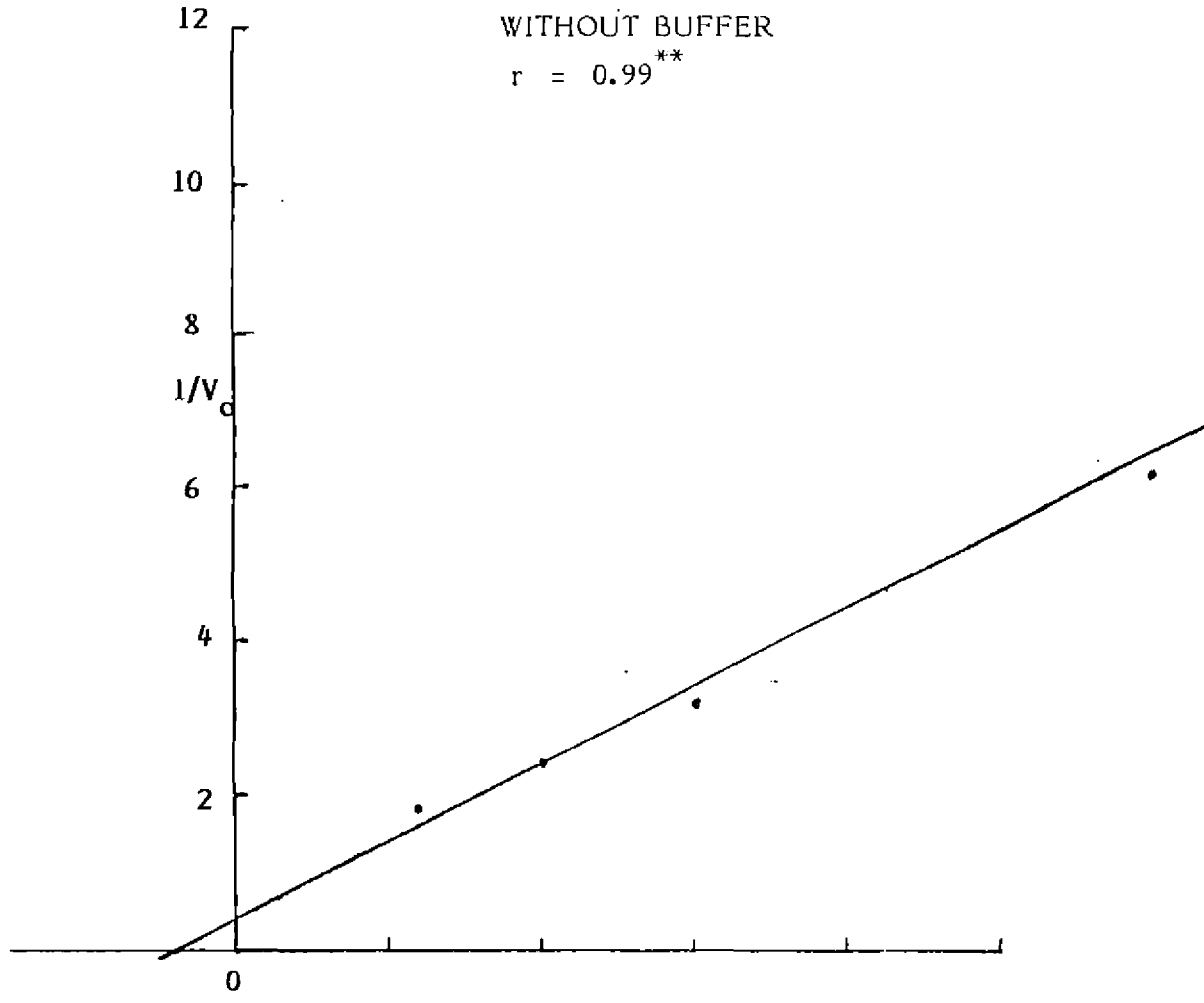


FIG. 7: LINEWEAVER-BURK PLOT OF UREASE ACTIVITY OF KARI SOIL (WITHOUT AND WITH BUFFER)



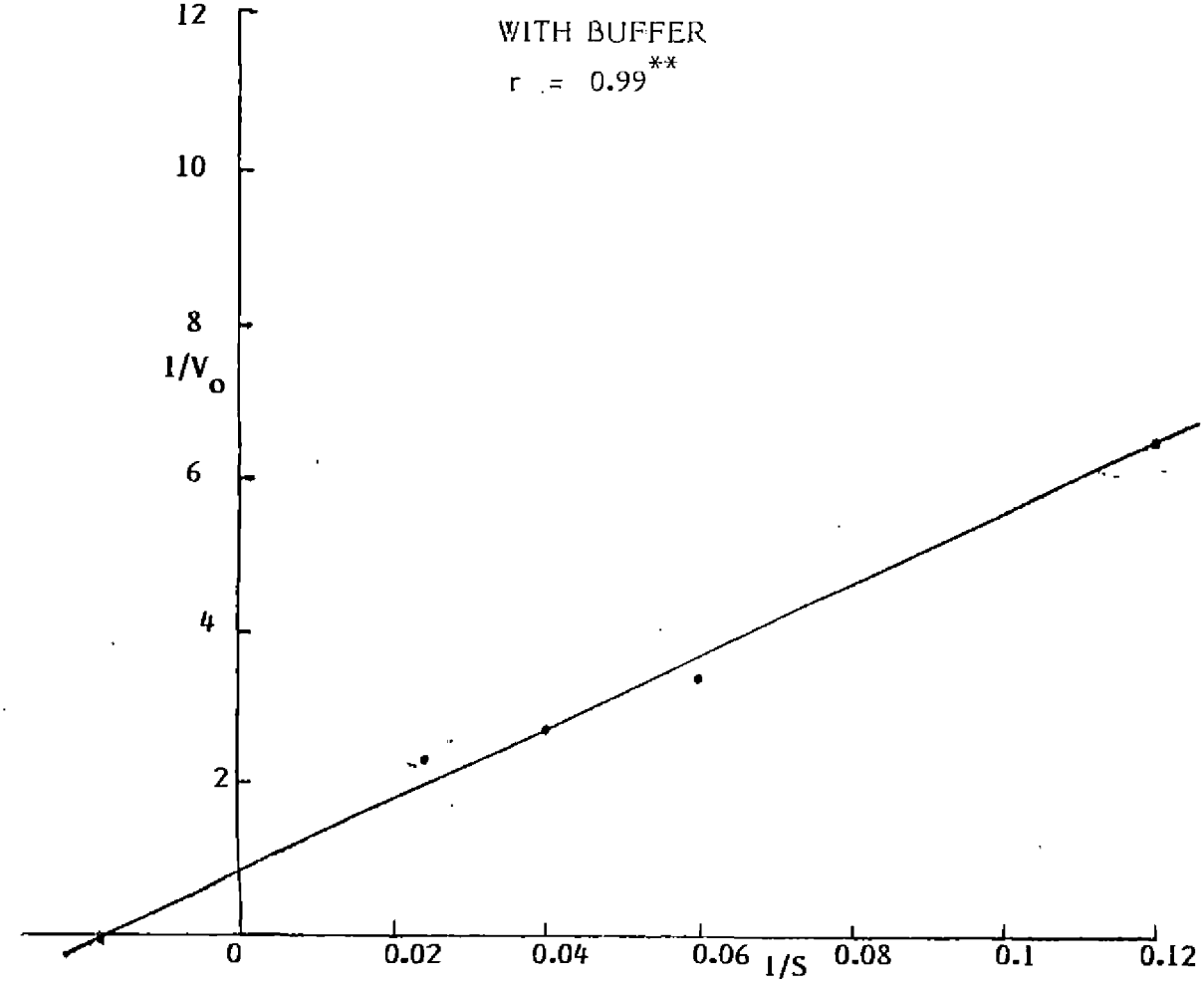


FIG. 3c. LINEWEAVER-BURK PLOT OF UREASE ACTIVITY OI BLACK SOIL (WHITHOUT AND WITH BUFFER)

At 60% WHC and for the 5h incubation period, black soil registered the highest urease activity (321 μg urea hydrolysed g^{-1} soil) and kari soil the lowest (175 μg urea hydrolysed g^{-1} soil). The urease activity of other soils did not show much variation and it ranged from 250 to 285 μg urea hydrolysed g^{-1} soil. For laterite, karappadam and kayal, the hydrolysis of urea was complete within 48h (two days). It took 120 h (five days) for the completion of urea hydrolysis in kole and black soils and 30 days in kari soil (Fig. 2).

The soils incubated with urea under submergence for different intervals showed the same pattern of urea hydrolysis as those kept at 60% WHC (Table 2). In these cases also it took two days for laterite, karappadam and kayal, five days for kole and black and 30 days for kari soil for the complete hydrolysis of urea.

4.1.2 Determination of Michaelis constant of soil urease

The kinetics of urease activity was worked out using urea concentrations in the range of 250 ppm to 2500 ppm. The best fitted straight line, double reciprocal plot of $1/V_o$ against 1/S was plotted by statistically analysing the data using the principle of least squares. Employing the Lineweaver-Burk equation, which represents the 'double reciprocal' plot, V_{max} and K_m values were computed (Fig. 3).

The calculated V_{max} and K_m values were 2.6 μ moles of urea hydrolysed g^{-1} soil h^{-1} and 130.2 μ moles of urea for black soil without buffer and with buffer the corresponding values were 1.2 μ moles of urea hydrolysed g^{-1} soil h^{-1} and 55.1 μ moles of urea respectively (Table 3). The V_{max} and K_m values obtained for karappadam soil were 1.5 μ moles of urea hydrolysed g^{-1} soil h^{-1} and 116 μ moles of urea without buffer and 4.2 μ moles of urea hydrolysed g^{-1} soil h^{-1} and 445.3 μ moles of urea with buffer respectively. The corresponding values for kari soil wer 7.3 μ moles of urea hydrolysed g^{-1} soil h^{-1} and 714.3 μ moles of urea without buffer and 16.7 μ moles of urea hydrolysed g^{-1} soil h^{-1} and 2646.6 μ moles of urea with buffer respectively.

4.2 Changes in Urease Activity, Eh and pH in Anaerobic soils Following Flooding

The data on urea hydrolysis during 5 h incubation following flooding the soils for different intervals are presented in Table 4. In this experiment urea was added to the submerged soil in the reduced condition following flooding for periods ranging from zero to 30 days (0 to 720 h). There was a steady decrease in the urease activity with increasing time of submergence. Though laterite, kari, kayal and black soil did not show much decrease in urease activity after 24 h of submergence, they exhibited 50 per cent decrease in the activity at 48 h of submergence. Excepting for black soil, urease activity could not be detected in other soils for

Table 3. Kinetic constants of soil urease employing Lineweaver - Burk equation

Soiltype	With buffer		Without buffer	
	K_m (μmoles of urea)	V_{max} (μmoles of urea hydrolysed g^{-1} Soil h^{-1})	K_m (μmoles of urea)	V_{max} (μmoles of urea hydrolysed g^{-1} soil h^{-1})
Karappadam	445.3	4.2	116.0	1.5
Kari	2646.6	16.7	714.3	7.3
Black	55.1	1.2	130.2	2.6

Table 4. Urease activity in six rice soils under anaerobic condition following flooding for different intervals

Flooding period (h)	Urease activity (μg urea hydrolysed g^{-1} soil 5 h^{-1})					
	Laterite	Karappadam	Kari	Kayal	Kole	Black
0	212	285	212	321	255	386
5	212	321	175	285	321	431
10	285	321	321	248	212	394
15	285	358	321	358	285	467
20	321	285	212	303	248	394
24	248	139	248	212	139	358
48	102	102	102	102	84	66
120	-	-	-	-	-	29
240	-	-	-	-	-	-

the subsequent periods of submergence. In the case of black soil urease activity (10%) could be detected upto five days after submergence. Karappadam and kole soils registered 50 per cent reduction in the enzyme activity upon 24 h of submergence itself. Though the same level of activity was maintained upto 48 h after submergence, no activity could be detected for the subsequent submergence periods.

The changes in redox potential (Eh) of the soils following flooding are presented in Table 5. Kari soil registered a highest initial redox level of $^{+}480$ mV and black soil, the lowest of $^{+}310$ mV. A gradual decrease in Eh was observed following flooding from first day onwards except in kari soil. While the decrease in redox potential of laterite, karappadam, kayal and kole soils was rapid, the drop in Eh in black soil was slow. The redox potential of kari soil was more or less static till about five days and was higher than all other soils at all intervals upto 30 days.

Follwing flooding the pH of all the soils generally increased with the exception of black soil (Table 5). The extent of rise in pH was less in kari soil. The increase in pH of laterite, karappadam, kayal and kole soils was more marked from 5th day onwards. On the other hand, the pH of black soil decreased following flooding.

4.3 Effect of Organic and Inorganic Reducing Substances on Urease Activity

The study was aimed to find out whether the organic and inorganic reducing substances produced in submerged paddy soils have any inhibitory

Table 5. Changes in Eh and pH in the six soil types following flooding for different intervals

Flooding period (h)	Laterite		Karappadam		Kari		Kayal		Kole		Black	
	Eh (mV)	pH	Eh (mV)	pH	Eh (mV)	pH	Eh (mV)	pH	Eh (mV)	pH	Eh (mV)	pH
0	+320	4.70	+365	4.6	+480	2.5	+420	4.45	+415	4.7	+310	8.0
5	+385	4.60	+365	4.4	+460	2.5	+410	4.75	+395	4.65	+275	8.05
10	+265	4.55	+305	4.65	+460	2.65	+400	4.75	+365	4.5	+240	8.0
15	+280	4.65	+255	4.4	+460	2.55	+340	4.15	+350	4.25	+280	7.9
20	+310	4.3	+230	4.65	+460	2.45	+370	4.4	+215	4.4	+240	7.65
24	+230	4.8	+195	4.55	+440	2.2	+390	4.15	+330	4.4	+260	7.45
48	+205	5.15	+85	5.55	+445	2.1	+290	4.5	+300	4.55	+255	7.75
120	-50	6.2	-80	6.3	+440	2.7	0	5.55	+100	5.35	+190	7.6
240	-25	7.2	-25	7.1	+310	3.0	-15	5.5	-55	5.9	-	7.75
360	-70	6.3	-120	6.4	+330	2.45	-70	5.75	-70	5.45	+145	7.4
480	-50	6.55	-75	6.75	+345	2.8	+50	5.5	-85	5.7	+135	7.65
600	-10	6.4	-40	6.8	+320	2.5	+25	5.7	0	6.05	+140	7.55
720	-30	6.4	-30	6.4	+250	2.55	-10	5.5	-40	5.45	+105	7.35

effect on soil urease activity. Three soils (karappadam, kari and black) were used to study the effect of organic reducing substances, namely, formaldehyde, ethanol, acetic acid and acetone on urease activity (Table 6). There was no effect for these substances on the urease activity at the two concentrations tried. The urease activities recorded were more or less same for the untreated and treated soils with these substances.

The ferrous iron content of the soils following flooding from zero to five days was found to increase in karappadam and kari soils (Table 7). Fe^{2+} was not detected in black soil at any of the intervals following flooding upto five days. The urease activities were also found to decrease upon flooding. Among the three soils used in this study, urease activity could be detected upto two days after flooding in the case of karappadam and kari and upto five days for black soil.

4.4 Effect of pH on Urease Activity

Correlation was worked out between the pH of the different soil types and urease activity. A significant positive correlation (0·87*) was observed between pH and urease activity.

Using buffers, effect of pH on urease activity was studied in laterite soil which had an original pH of 4.7 (Table 8). An increase in urease activity was noticed in soil samples buffered at higher pH than the control. An almost stable mean activity of around 400 µg of urea hydrolysed g^{-1} soil 5 h^{-1} was obtained from pH 6 to 9.

Table 6. Effect of some organic reducing substances on urea hydrolysis in karappadam, kari and black soil

Organic substance	Urea hydrolysis (μg urea hydrolysed g^{-1} soil 5 h^{-1}		
	Karappadam	Kari	Black
Formaldehyde			
a. 100 ppm	285	248	285
b. 500 ppm	212	248	285
Ethanol			
a. 100 ppm	230	303	339
b. 500 ppm	230	175	285
Acetic acid			
a. 100 ppm	212	248	266
b. 500 ppm	248	230	266
Acetone			
a. 100 ppm	212	230	230
b. 500 ppm	230	194	248
Control	252	212	312

Table 7. Ferous iron content and urea hydrolysis in karappadam, kari and black soil upon flooding for different periods

Flooding period (d)	Karappadam		Kari		Black	
	Fe^{2+} content (ppm)	Urea hydrolysis *	Fe^{2+} content (ppm)	Urea hydrolysis *	Fe^{2+} content (ppm)	Urea hydrolysis *
0	38.5	285	302.5	212	0	386
1	481.2	139	391.8	248	0	358
2	756	102	619	102	0	66
5	3300	0	756	0	0	29

* μg urea hydrolysed g^{-1} soil 5 h^{-1}

Table 8. Effect of pH on urease hydrolysis in laterite soil

pH of buffer	pH of soil buffer mixture	Quantity of urea hydrolysed (μg urea g^{-1} soil $5\ h^{-1}$)
Control	4.7	226
6	5.3	394
7	6.1	430
8	7.7	394
9	8.7	406

4.5 Influence of Rice Rhizosphere on Urease Activity

The data on the urease activity of different soil types with and without growing rice are presented in Table 9. The comparison by paired t-test between urease activities of soils with and without growing rice at each of the three stages of growth, 30 DAP, 60 DAP and at harvest, shows a higher activity in soils in which rice was grown (Appendix II). Urease activity in the rice rhizosphere was found to increase with the growth of rice plants upto 60 days and thereafter it declined. From the comparison of urease activities between the three stages of crop growth by paired t-test it was seen that urease activity in the rhizosphere was highest at 60th day after planting (Appendix II). Urease activities in the rice rhizosphere at 30th day after planting and at harvest were at par. This study could not be conducted in kari soil as the plants completely failed to grow after transplanting perhaps due to the soil acidity produced upon floodidng the soil.

4.6 Methods of Assaying Urease Activity in Soils

4.6.1 Compaprison of the methods.

The three methods employed in the estimation of urease activity were buffer method (Tabatabai and Bremner, 1972), non-buffer method (Zantua and Bremner, 1975a) and isotope method (by determining the remaining ^{14}C-labelled urea). The soil urease activity determined using these three methods are presented in Table 10. The buffer method gave

Table 9. Effect of rhizosphere on urease activity of five soil type at different growth stages of rice

Soil Type	Urease activity (μg urea hydrolysed g^{-1} soil 5 h^{-1})							
	At the time of planting		30 DAP		60 DAP		At harvest	
	Without rice	with rice	Without rice	With rice	Without rice	With rice	Without rice	With rice
Laterite	175	139	90	248	175	394	139	285
Karappadam	226	153	175	273	200	321	187	309
Kayal	226	153	224	309	236	467	224	321
Kole	54	66	151	273	236	423	212	321
Black	200	248	273	394	248	492	285	394

Table 10. Urease activity of different soil type following buffer, nonbuffer and isotope method of assay for 5h incubation period

Soil type	Urease activity (μg urea hydrolysed g^{-1} soil 5 h^{-1})		
	Non-buffer method	Buffer method	Isotope method*
Laterite	248	285	--
Karappadam	285	315	236
Kari	175	285	202
Kayal	230	465	--
Kole	285	480	--
Black	321	600	259

(*By determining the remaining labelled urea)

higher values for all soil types. For karappadam soil rease activity (μg urea hydrolysed g^{-1} soil) obtained using buffer method was 315 and for non-buffer and isotope methods the values were 285 and 236 respectively. Almost the same pattern was observed for other soils also. For black soil, urease activity using the buffer method (600 μg urea hydrolysed g^{-1} soil) was much higher than that obtained using non-buffer 321 μg urea hydrolysed g^{-1} soil) and isotope methods (259 μg urea hydrolysed g^{-1} soil).

4.6.2 Comparison of isotope methods.

Two different methods were compared using the ^{14}C - labelled urea for the estimation of urease activity. In the first method, $^{14}CO_2$ released was measured and in the second method, ^{14}C - urea remaining was determined after the incubation period for the estimation of urease activity. By the first method, urease activity recorded in laterite and karappadam soils were only 2.9 and 3.4 μg urea hydrolysed g^{-1} soil $5\ h^{-1}$ respectively. The corresponding values obtained using the non-buffer method for these soil types were 248 and 285 μg urea hydrolysed g^{-1} soil $5\ h^{-1}$ respectively.

In the second method which involved the determination of ^{14}C - urea remained after 5 h incubation period, three soil types were used. The urease activity recorded for karappadam, kari and black soils using this method were 236, 202, and 259 μg urea hydrolysed g^{-1} soil respectively. As these values were comparable with those of non-buffer method, the study was continued with longer incubation intervals of 10, 15, 20 and 24 h

also (Table 11). For karappadam and kari soils, the isotope method gave slightly higher values than the non-buffer method for all the incubation periods. For karappadam soil, the values ranged from 285 to 1919 μg urea hydrolysed g^{-1} soil for non-buffer method and 236 to 1919 μg urea hydrolysed g^{-1} soil for isotope method. However for black soil, the isotope method gave markedly lower values than the non-buffer method for incubation periods longer than 5 h. The values ranged from 321 to 1124 μg urea hydrolysed g^{-1} soil for non-buffer method and 259 to 623 μg urea hydrolysed g^{-1} soil for isotope method.

4.6.3 Isotope effect.

To study the isotope effect on urea hydrolysis in karappadam, kari and black soils, three solution of ^{14}C - labelled urea were used to give three different specific activities of 32, 58 and 113 cpm ^{14}C μg^{-1} urea. The results are presented in Table 12. In karappadam soil there was a slight decrease in urea hydrolysis with increase in the specific activity. The urea hydrolysis decreased from 236 (at lowest specific activity of 32 cpm ^{14}C μg^{-1} urea) to 150 μg urea hydrolysed g^{-1} soil 5 h^{-1} (at highest specific activity of 113 cpm ^{14}C μg^{-1} urea). However there was not much variation in hydrolysis of kari and black soil at the three different specific activities.

Table 11. Urease activity in karappadam, kari and black soil following isotope and nonbuffer method for different incubation periods

Incubation period (h)	Urease activity (μg urea hydrolysed g^{-1} soil)					
	Karappadam		Kari		Black	
	Nonbuffer	Isotope	Nonbuffer	Isotope	Nonbuffer	Isotope
5	285	236	175	202	321	259
10	686	738	212	285	905	335
15	1526	1847	248	289	1015	422
20	1745	1876	248	362	1343	604
24	1919	1919	285	460	1124	623

(*By determining the remaining labelled urea)

Table 12. Effect of three different specific activities of ^{14}C- on the hydrolysis of labelled urea in karappadam, kari and black soils

Specific activity (cpm μg^{-1} urea)	Urease activity (μg urea hydrolysed g^{-1} soil)		
	Karappadam	Kari	Black
32	236	202	259
58	203	236	315
113	151	202	238

4.7 Molecular Absorption of Urea by Rice Plant

In this study two rice plants each (55 days old) were allowed to absorb ^{14}C - urea from ^{14}C - urea solution for 3 h and 4 h respectively.

Plants which were allowed to absorb ^{14}C - urea for 3 h were extracted with water containing 5ppm PMA and then centrifuged at 10000 rpm for 10 min. Aliquots of this were then analysed for radioactivity. The mean radioactivity obtained was 2065 cpm h^{-1} g^{-1} of fresh tissue. Counting efficiency of this assay was tested and was found to be 56.6 per cent. The samples of plants which were allowed to absorb ^{14}C - urea for 4 h were extracted with boiling ethanol (80%). The extracts were then centrifuged and analysed for radioactivity. The mean radioactivity recorded was 1596 cpm h^{-1} g^{-1} of fresh tissue. The counting efficiency of this assay was 58.7 per cent.

Autoradiograph of a rice plant which was allowed to absorb ^{14}C - urea for 3 h is given in Plate 1. ^{14}C - urea was found to be absorbed by the rice plant and it was seen concentrated more in the basal part of the culm and lower leaves.

Plate 1a. Photograph of the rice plant which absorbed ^{14}C-urea.

Plate 1b. Autoradiograph of the rice plant showing the distribution of ^{14}C-urea.

Discussion

DISCUSSION

5.1 Kinetics and Levels of Urease Activity in Rice Soils

5.1.1 Urea hydrolysis at different moisture regimes.

There was marked variation in urease activity of the different soil types at 60% WHC (Table 2). While the black soil exhibited the highest urease activity (for 5 h incubation) the kari soil registered the lowest activity. The urease activities of other soils did not show much variation after 5 h incubation period and the values ranged from 250 to 285 μg urea hydrolysed g^{-1} soil. Many studies have releated soil urease activity with soil properties like organic carbon, total N, pH, CEC and sand, silt and clay content (McGarity and Myers, 1967; Gould et al., 1973; Zantua et al., 1977; Dash et al., 1981; Sahrawat, 1983). While some workers reported a positive relation, some did not get any relationship at all. In the present study, though kari soil which gave the lowest activity was having fairly high organic carbon content it was low in silt and clay content. The pH of this soil was as low as 2.5 even after submergence for a long period. This could be one of the main reason for the low activity exhibited by this soil. Many workers have observed the optimum pH for urease activity to range from 6 to 9 (Tabatabai and Bremner, 1972; May and Douglas, 1976; Pettit et al., 1976). The black soil which gave the highest activity was having a high pH of around 8 and CEC of 52.4 me 100 g^{-1} soil. CEC is believed to have the capacity to complex and retain urease and many have reported a highly significant positive correlation between

urease activity and CEC (Dalai, 1975; Zantua et al., 1977). Laterite, karappadam, kayal and kole soils which had a medium level of activity were having a pH of around 4.7 and medium CEC.

The pattern of urea hydrolysis of the soil when submerged for 5 h interval was almost the same as that at 60% WHC (Table 2). In this case soil was kept submerged only after the addition of urea. Hence at the time of incubation with urea the soil was aerobic. Several workers have also found that urease activity in soil was not affected by the water level (Skujins and McLaren, 1969; Gould et al., 1973).

Remarkable difference in urea hydrolysis of the soil types was exhibited when it was incubated for intervals longer than 5 h (both at 60% WHC and under submergence). The urea hydrolysis was complete within two days in the case of laterite, karappadam and kayal soil. For black and kole soils, it took five days and for kari soil it took 30 days for the completion of urea hydrolysis (Fig. 2). The very slow rate of hydrolysis of urea in kari soil should be expected as the soil is strongly acidic (pH 2.5) and acidity is known to inhibit urea hydrolysis (Bremner and Mulvaney, 1978). Sahrawat (1980b) also observed that acid sulphate soils had a lower urease activity than soils with near neutral or alkaline pH.

5.1.2 Michaelis constant of soil urease.

The V_{max} values for the three soils, karappadam, kari and black soils were 1.5, 7.3 and 2.6 μ moles of urea hydrolysed g^{-1} soil h^{-1} when non-buffer method was used (Table 3, Fig. 3). The K_m values for these

soils were 116, 714.3 and 130.2 μ moles of urea respectively. The K_m and V_{max} values for urease activity have been found to differ for different soils (Pal and Chhonkar, 1979). Beri et al., (1978) obtained K_m values ranging from 10.4 to 22.2 mM urea and V_{max} ranging from 2.0 to 6.2 mM urea N g^{-1} soil for urease. In a study by Nor (1982) in three Malaysian soils, the K_m and V_{max} values ranged from 1.0 to 2.0 mM urea and 0.15 to 0.4 mM urea hydrolysed g^{-1} soil h^{-1} respectively.

Using the Tris - HCl buffer (pH 7.2) for the assay, the V_{max} values of the three soils studied ranged from 1.2 to 16.7 μ moles of urea hydrolysed g^{-1} soil h^{-1}. The K_m values ranged from 55 to 2647 μ moles of urea (Fig. 3). It could be seen that the K_m and V_{max} values differed considerably when buffer and non-buffer methods were used. Although the K_m value is usually considered a constant for a particular enzyme it is recognised that all environmental conditions like pH, buffer system, temperature etc., can influence it (Paulson and Kurtz, 1970).

5.2 Changes in Urease Activity in Anaerobic Soil

In this experiment urea was added to soils pre-reduced by flooding for different intervals. Submergence had a retarding effect on urea hydrolysis in all soils which was marked after 20 h in karappadam and kole soils and after 24 h in laterite, kari, kayal and black soils. No activity could be noticed for submergence periods of more than 48 h (Table 4). Savant et al. (1985) also noticed a retarding effect for soil submergence

on urea hydrolysis after 12 h.

The immediate consequence of soil submergence is the rapid depletion of O_2 because of the activity of aerobic microorganisms that eventually leads to the anaerobic microbial activity and development of reduced soil condition (Ponnamperuma, 1972). The depletion of O_2 may have resulted in retarded hydrolysis of urea when soil was under submergence. With longer submergence time, there was a decrease in Eh and a corresponding increase in pH (Table 5). Pulford and Tabatabai (1988) also found that urease activity decreased after waterlogging of soils and was significantly correlated with Eh.

The enzyme urease was found to be strongly inhibited by metal ions and the decrease observed upon waterlogging of soils could be due to the increase in solubility of the reduced metals formed in the water-logged soils (Tabatabai, 1977, Gototh and Patrick, 1974). In this investigation an attempt was made to know the effect of some of the reducing substances produced in submerged paddy soils. The Fe^{2+} content (Table 7) of karappadam and kari soils showed an increase upon submergence. However, Fe^{2+} could not be detected in black soil at all, which continued to exhibit urease activity upto five days after submergence. Similarly some of the organic substances like formaldehyde, ethanol, acetic acid and acetone which commonly occur in lowland paddy soils did not inhibit urease activity (Tabie 6). Thus these organic and inorganic reducing substances did not seem to inhibit the urea hydrolysis upon submergence of soil. Hence it can be concluded that the main reason for reduction

in the activity of urease may be the depletion of O_2 upon flooding and this is in conformity with the findings of several workers (Savant et al., 1985; Pulford and Tabatabai, 1988).

5.3 Effect of pH on Urease Activity

A significant positive correlation was obtained between pH and soil urease activity which is in conformity with the findings of Silva and Perera (1971).

In the study using buffers to determine the effect of pH on urease activity of laterite soil, maximum activity was found to be at around pH 7 (Table 8). The activity was found to be stable from pH 6 to 9. Segel (1975) reported that the pH stability of enzymes depends on many factors includidng temperature, ionic strength, chemical nature of buffer, contaminating metal ions, concentration of substrate and co-factors and enzyme concentration. Singh and Nye (1984) obtained a weak positive correlation between soil reaction in the range of pH 4.8 - 6.7 and urease activity. According to them, it seems most likely that more than one of the ionizing groups together confer catalytic activity on the enzyme of which one group could be required in the form of its conjugate acid and the other as conjugate base. Thus the rise in activity on the lowerside of the optimum pH could be due to regeneration of the conjugate base by deprotonation as the pH rises and the decline in activity on the otherside of optimum pH represents the loss in activity of the conjugate acid.

5.4 Influence of Rhizosphere on Urease Activity

The higher activity of urease recorded in the rice rhizosphere indicates that rhizosphere has a positive influence on urease activity (Table 9). In rhizosphere, the microbial activity is high and this in turn is a source of soil enzymes. The activity of soil enzymes has been reported to be correlated with the microbial activity (Frankenberger and Dick, 1983; Tiwari et al., 1988). It was also observed from the present study that maximum rhizosphere contribution of urease was on 60th day after planting. This is in conformity with the result obtained by Khan (1971) who reported highest activity in the rice rhizosphere on 50th day after sowiwng. This may be due to the highest root activity at this stage. Extracellular urease had been reported to be associated with rice roots (Mahapatra et al., 1977). In addition, rice rhizosphere is relatively oxidised because of diffusion of O_2 from the rice roots (Armstrong, 1971). The presence of rice roots in the wetland soil system is thus likely to influence the urea hydrolysis.

5.5 Methods of Assaying Urease Activity

5.5.1 Comparison of the methods.

Among the three methods employed, the buffer method gave markdely higher values of urease activity for all the six soil types studied after the 5 h incubation period (Table 10). The non-buffer method and the isotope method resulted in more or less comparable values of urease activity in the three soils studied (karappadam, kari and black). Many

workers have reported higher urease activity when buffer method was employed (Zantua and Bremner, 1975a). It is believed that the buffer method gives higher values as it detects urease activity not detected by the non-buffer method. However, the choice of method for assay of urease activity should depend upon the purpose of the assay. If the purpose is to obtain an index of the urease activity under natural conditions, the non-buffer method described is obviously superior to the buffer method. On the other hand, if the purpose is to detect urease in soils or soil fractions, the buffer method should be preferred. The values obtained by the buffer method merely indicate the activity that occurs in the buffer selected and cannot be taken as an indication of the activity that occurs in the natural soil environment.

A detailed comparison was made between the non-buffer method and the isotope methods (Table 11). The isotope method where $^{14}CO_2$ released was taken as an index of urease activity, gave very low values compared to the non-buffer method. This probably may be due to the poor trapping of $^{14}CO_2$ in KOH. The second isotope method involved the determination of ^{14}C - urea remaining after the incubation period. This method has the advantage that the sample can be read in the liquid scintillation counter immediately after the extraction of the incubated soil. This method gave values comparable to that of non-buffer method not only for 5 h incubation but also for longer intervals of 10, 15, 20 and 24 h except in black soil. The urease activity values obtained for

black soil using the isotope method were rather low (less than 50 per cent of non-buffer method) for all the incubation periods. This may be due to the use of potassium acetate buffer which had a pH of 5.5. The use of this buffer in black soil which had an original pH of 8.0 might have retarded the hydrolysis of urea. The inclusion of this buffer in the isotope method could be an obvious disadvantage as it allows the assay to take place only under acidic conditions which is known to retard urease activity (Bremner and Mulvaney, 1978).

5.5.2 Isotope effect.

Though three different specific activities of ^{14}C - labelled urea were tried, there was not much difference in urea hydrolysis in the three soils studied (karappadam, kari and black). This disproves the belief that the use of ^{14}C - isotope for the assay of urease activity will lead to an underestimation of urease activity (Rabinowitz et al., 1956).

5.6 Molecular Absorption of Urea by Rice Plant

The main intention of taking up this experiment was to confirm whether urea is absorbed directly by the plant. This has a sppecial relevance in this study because it has been found that urea hydrolysis almost comes to a standstill when urea is added to soil submerged for more than 2 or 3 days. It was evident from the radioassay of rice plants which were allowed to absorb ^{14}C - urea that urea is being taken up in the molecular form itself. Autoradiograph of the plant also revealed clearly the pattern

of absorption (Plate 1). The labelled urea absorbed by the plant was translocated throughout the plant although ^{14}C - label was found concentrated mostly in the basal parts. The molecular absorption of urea has also been reported by serveral workers (Mitsui et al., 1960; Mitsui and Kurihara, 1962a,b).

Though it has been conclusively proved that rice plants do absorb molecular form of urea, the extent of its absorption is not known. This could be of special significance for rice plant grown under low land condition. The urea in its molecular form could be a potential source of nitrogen for the plant when urea is topdressed to flooded rice as its hydrolysis will be slow under anaerobic condition.

Summary

SUMMARY

An investigation was conducted at the College of Horticulture, Vellanikkara during the period 1987 - 89 to study the urease activity of six rice soils of Kerala, namely, laterite, karappadam, kari, kayal, kole and black soil. The following experiments were undertaken during the course of this investigation.

i) Kinetics and levels of soil urease activity of the six soil type under two different moisture regimes viz., soil at 60% WHC and under submergence.

ii) Effect of the following factors on soil urease activity.

a) Soil submergence.

b) Organic and inorganic reducing substances.

c) pH

d) Rice rhizosphere.

iii) Comparison of the three methods for the assay of the urease activity - buffer method, non-buffer method and isotope method.

iv) Molecular absorption of urea by rice plant.

The results of the investigation are summarised below.

There was marked variation in the urease activity of the different soil types both at 60% WHC and under submergence for 5 h incubation.

While the black soil exhibited the highest urease activity (321 µg urea hydrolysed g^{-1} soil), kari soil registered the lowest activity (175 µg urea hydrolysed g^{-1} soil). The urease activities of other soils did not show much variation after 5 h incubation period and the values ranged from 250 to 285 µg urea hydrolysed g^{-1} soil.

When soils were incubated with urea for periods longer than 5 h (both at 60% WHC and under submergence) the urea hydrolysis was complete within two days in the case of laterite, karappadam and kayal soils. For black and kole soils it took five days and for kari soil, 30 days for the completion of urea hydrolysis.

The kinetics of urease activity was worked out employing Lineweaver-Burk equation of double reciprocal plot for the three soil types, karappadam, black and kari using buffer and non-buffer methods. Without buffer, the K_m values obtained for karappadam, black and kari soils were 116, 130.2 and 714.3 µ moles of urea respectively. The corresponding V_{max} values obtained were 1.5, 2.6 and 7.3 µ moles of urea hydrolysed g^{-1} soil h^{-1} respectively. With buffer (pH 7.2) the K_m values obtained for karappadam, black and kari soils were 445.3, 55.1 and 2646.6 µ moles of urea and the corresponding V_{mqx} values were 4.2, 1.2 and 16.7 µ moles of urea hydrolysed g^{-1} soil h^{-1} respectively.

When urea was added to the soil which was in a reduced condition following flooding for periods ranging from 0 to 30 days, there was a

steady decrease in the urea hydrolysis. No urease activity could noticed in all the soil types upon flooding for periods longer than two days. With the longer submergence time there was an increase in pH and a corresponding decrease in Eh.

Significant positive correlation was observed between pH and urease activity. The effect of pH on urease activity was studied using buffers in laterite soil having a pH of 4.7 and found that maximum urease activity was at around pH 7. The urease activity was observed to be rather stable from pH 6 to 9.

The rice rhizosphere was found to have a positive influence on urease activity and the maximum urease activity in the rhizosphere was noticed at 60th day after planting.

Among the three methods compared for the assay of urease activity, the non-buffer and isotope method gave comparable values while the buffer method recorded fairly high values of urease activity. The isotope method used in this comparison involved the determination of ^{14}C urea remaining. The isotope method which involved the determination of $^{14}CO_2$ released gave very low values and did not give a good index of urease activity.

To study the isotope effect on urea hydrolysis three different specific activities of ^{14}C - labelled urea were used and found that there was no decrease in urease activity with increase in the specific activity.

The radioassay and the autoradiograph of rice plants which were allowed to absorb ^{14}C - urea, clearly revealed that urea was taken up by the plant in the molecular form itself.

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*Originals not seen

Appendices

Appendix I. Weather data (weekly average) for the cropping period (January 1989 to May 1989)

Week No.	Month and Date	Rainfall (mm)	Temperature (°C) Maximum	Minimum	Relative humidity Forenoon	Afternoon	Sunshine hours
4	January 22 - 28	--	33.6	21.2	71	26	7.4
5	January 29 - Feb. 4	--	34.9	20.3	59	17	4.1
6	February 5 - 11	--	36.1	19.1	67	17	10.4
7	February 12 - 18	--	36.8	21.8	84	23	9.8
8	February 19 - 25	--	36.6	23.6	73	28	9.6
9	February 26 - March 4	--	37.1	21.3	65	15	8.2
10	March 5 - 11	15.8	36.8	23.1	75	35	9.5
11	March 12 - 18	--	36.6	23.7	86	42	11.4
12	March 19 - 25	--	35.3	23.6	84	48	9.7
13	March 26 - April 1	15.5	36.8	24.6	82	42	9.4
14	April 2 - 8	--	35.7	25.2	86	48	8.9
15	April 9 - 15	1.0	36.4	25.6	83	50	9.4
16	April 16 - 22	47.3	34.5	24	88	55	6.5
17	April 23 - 29	4.3	34.3	25.3	89	56	7.9
18	April 30 - May 5	23.8	35.4	25	82	63	8.8

Appendix II. Results of paired t-test on soil urease activity in the rice rhizosphere at 30 DAP, 60 DAP and at harvest,calculated t-value, t-value from the table and their significance

Comparisons	t - value	
	Calculated	Table
With and without growing rice at 30 DAP	5.62*	2.15
With and without growing rice at 60 DAP	9.11*	2.15
With and without growing rice at harvest	9.4*	2.15
30 DAP and 60 DAP	2.75*	2.15
60 DAP and at harvest	5.41*	2.15
30 DAP and at harvest	0.0025	2.15

* Significance at 5 per cent level.

UREASE ACTIVITY IN RICE SOILS OF KERALA

By

P. SARASWATHI

ABSTRACT OF A THESIS

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ABSTRACT

An investigation on the urease activity of rice soils of Kerala was conducted at the College of Horticulture, Vellanikkara during the period 1987 - 1989. Representative soil samples were collected from six rice soils of Kerala, namely, laterite, karappadam, kari, kayal, kole and black soil. The kinetics of urease activity was worked out employing Lineweaver-Burk equation of double reciprocal plot. The effect of different factors like pH, soil submergence and rice rhizosphere on soil urease activity was investigated. A comparison of the efficiency of the different methods of urease assay was also made. The radioassay and autoradiograph of the plants which were allowed to absorb ^{14}C - urea were also done.

The kinetic studies revealed that the K_m and V_{max} values varied not only with the soil type but also with the method of assay employed. The highest level of urease activity was exhibited by black soil and the lowest by kari soil. When soils were incubated for periods longer than 5 h (Both at 60% WHC and under submergence) the urea hydrolysis was complete within two dayas in the case of laterite, karappadam and kayal soils. For black and kole soils it took five days and for kari soil, 30 days for the completion of urea hydrolysis.

When urea was added to the soil which was in a reduced condition, a steady decrease in urea hydrolysis was noticed and no urease activity could be noticed in all the soil types upon flooding for periods longer

than two days. The rice rhizosphere was found to have a significant influence on urease activity and the highest activity was noticed at 60th day after planting rice.

Among the three methods compared for the assay of urease activity, while the non-buffer and isotope method gave comparable values, the buffer method recorded fairly high values of urease activity. There was no isotope effect on urease hydrolysis as there was no decrease in the urease activity with increase in the specific activity of ^{14}C - urea.

The radioassay and the autoradiograph of rice plants which were allowed to absorb ^{14}C - urea clearly revealed that urea was taken up by the plant in the molecular form itself.