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SHORT COMMUNICATION

Assay for soil urease activity

Summary

A procedure is described that allows assay of soil urease activity. The method uses a phosphate buffer (pH 8.8) and a urea substrate concentration of 0.007 *M*. Incubation for 4 h at 37°C is recommended and urease activity is estimated by determining the amount of ammonium produced by urea hydrolysis in soil. The method is precise, and compares favourably with other procedures.

Introduction

Urea is rapidly hydrolysed ($NH_2CONH_2 + H_2O \rightarrow 2NH_3 + CO_2$) in soil by soil urease. Recent work [4 5 14] has involved the use of urease inhibitors for retarding the rate of this reaction in soils, and to further these studies it is necessary that a satisfactory soil urease assay be developed.

When the work reported here was initiated, a number of methods had been reported [6 9 10 11 13 15 16] that allowed comparisons to be made between the urease activities of different soils. These methods differ greatly with regard to substrate concentration, time of incubation, product determination, buffer pH and composition and use of toluene and none has been satisfactorily evaluated. In a report [17] received while the present work was in progress, a method was proposed that seemed to overcome many of the problems of earlier procedures.

The purpose of this communication is to describe a simple and precise procedure for assay of soil urease activity, and to compare results obtained using this method with those obtained using the method proposed by Tabatabai and Bremner [17].

Materials and methods

Soils. The soils used (Table 1) were surface (0–15 cm) samples selected to give a range of properties. Before use, each sample was air dried and crushed to pass a 1 mm sieve. In the analyses reported in Table 1, pH was determined by a glass electrode (soil: water ratio 1 : 2.5), organic carbon by a Walkley-Black method [1], total nitrogen by a semi-micro Kjeldahl procedure [2], particle size distribution by a hydrometer method [7] and cation exchange capacity by an ammonium saturation technique [8].

Reagents. Phosphate Buffer (*M*/15) pH 8.8: Dissolve 24.0 g $Na_2HPO_4.12H_2O$ in 800 ml water and make up to 1 l. Similarly, dissolve

TABLE 1

Soil analyses

Soil	pH	Organic carbon %	Total nitrogen %	Clay %	Silt %	Sand %	Cation exchange capacity meq/100g soil
Dimboola lsa	7.0	2.07	.090	13.5	6.0	84.7	22.8
Keilor sacl	8.9	1.45	.159	24.6	16.0	51.8	12.3
Deer Park c	6.1	3.52	.301	54.0	15.0	27.3	29.7
Derrimut l	4.5	2.74	.293	23.0	29.0	44.0	18.4
Templestowe sacl	6.3	2.47	.195	15.6	21.0	61.4	9.2

lsa - loamy sand; sacl – sandy clay loam; l – loam, c – clay.

2.28 g KH_2PO_4 in 200 ml water and make up to 250 ml. Adjust the pH of the former solution to 8.8 by addition of KH_2PO_4 solution. (Approximately 30 ml is necessary).

Potassium chloride (2*M*) – phenylmercuric-acetate (PMA) solution: (2*M* KCl-PMA): Dissolve 150 g KCl in 800 ml water and 5 mg PMA in 100 ml hot water. Combine these solutions and make the volume to 1 l.

Standard urea solution: Dissolve 2.145 g urea in 800 ml water and dilute to 1 l. This solution contains 1000 μg urea – N per ml.

Magnesium oxide, sulphuric acid, boric acid indicator: As described previously [3].

Toluene: Analytical reagent (Ajax Chemicals, Melbourne).

All other reagents were analytical grade supplied by British Drug Houses Ltd., Poole, England.

Procedure

Place 3.0 g of soil in a 50-ml erlenmeyer flask, add 0.5 ml of toluene and stand for 10 min. Then add 12.0 ml of phosphate buffer and place the flask in a water bath at 37°C. After 10 min add 3.0 ml of urea solution, stopper the flask and swirl gently. After 4 h remove the flask from the water bath and add 15.0 ml 2*M* KCl-PMA solution. Shake for 5 sec, and then determine the ammonium content of a 10.0-ml portion of the supernatant by a steam distillation method described previously [3]. Obtain a blank value by following the procedure outlined above but add the urea solution to the flask after the addition of the KCl-PMA solution.

Comments

A preliminary incubation period of 10 min is necessary if the flask contents are to be at 37°C when the urea substrate is added. Addition of PMA to the 2*M* KCl solution ensures that soil urease activity is inhibited when this extractant is added to the soil after the 4 h incubation period. Silver sulphate,

as used in an alternative procedure [17], also effectively inhibits soil urease but great care must be taken in making up a 2*M* KCl-silver sulphate solution, as silver chloride may form due to its extremely low solubility product (1.2×10^{-10} at 25°C) and be precipitated. Blank determinations are necessary so that error caused by the presence of native ammonium in soil or ammonium that is formed by the slight (0.5 per cent) breakdown of urea during steam distillation is accounted for.

Results and discussion

In the development of the method, the following factors were considered; buffer pH and composition, time of incubation, substrate concentration, use of toluene and recovery of ammonium.

Buffer pH and composition. It was found that if the phosphate buffer described is used, the optimum pH for soil urease is 8.8. This is a similar pH optimum to that obtained by Tabatabai and Bremner [17] who used a THAM buffer, but differs considerably from the lower pH optima (5.5–7.2) in methods advocated by other workers [9 10 11 15 16].

The use of borate, diethylbarbiturate and glycine buffers proved to be unsatisfactory due to reduced levels of soil urease activity as estimated by urea hydrolysis in soil at pH 8.0. Both the THAM buffer and the phosphate buffer were found to have very small activating effects on soil urease. This was not marked enough to preclude the use of either buffer in a soil urease assay.

Time and temperature of incubation. A linear relationship is shown between ammonium-N released and time of incubation up to 24 h, which indicates that the method is estimating the activity of an enzyme and is not complicated by factors such as enzyme activation or microbial growth. The incubation time of 4 h ensures that the procedure recommended can be used for soils that have low urease activity.

An incubation temperature of 37°C is recommended, as other workers [10 11 16] have found this temperature to be convenient. However, soil urease activity was found to increase with temperature to a maximum at 70°C.

Substrate concentration and use of toluene. The choice of a substrate concentration of 0.007 *M* in the method described is satisfactory as

TABLE 2

Precision of assay

Soil	No. of samples	Urease activity		
		Range*	Mean*	Standard error
Derrimut	6	9.3–10.9	9.9	0.6
Dimboola	5	18.6–20.2	19.2	0.6
Keilor	6	31.1–31.9	31.5	0.3
Templestowe	5	34.3–35.9	35.3	0.7
Deer Park	6	55.9–60.7	58.0	1.8

* Urease activity expressed as μg NH_4^+–N released per g soil per h.

this is well above the level where this factor is affecting soil urease activity. This concentration corresponds to 1000 μg urea–N/g soil and is similar to that recommended in an alternative procedure [17].

Addition of 0.5 ml toluene to each sample in the method described had little effect on the results obtained for the soils studied, but the inclusion of this chemical is recommended so that unusually high microbial activities in some soils may be countered.

Recovery of ammonium. For soil urease assays based on determination of ammonium, several sources of error have been suggested [13]. One such error is caused by the conversion of urea to ammonium during steam distillation (found to be 0.5 per cent, which differs from the lack of conversion reported by other authors [17]). Blank determinations as described allow for this error. Other errors are possible if nitrification or fixation processes in soil cause significant decreases in the quantity of the hydrolysis product, ammonium, that is determined. Use of toluene should negate any significant nitrification that is liable to occur during the 4 h incubation period.

Fixation of ammonium is not likely to be a problem as studies show that fixed ammonium in soils did not increase during the course of typical assays.

Precision of method. The high precision of the method proposed is illustrated by Table 2 which gives the results of replicate analyses of 5 soils. Similar levels of soil urease activity were detected using either the present method or that of Tabatabai and Bremner [17].

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