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GAS CHROMATOGRAPHIC DETERMINATION OF UREASE ACTIVITY IN SOILS

A gas chromatographic method for assaying urease activity in soils is described. The method involves gas chromatographic determination of carbon dioxide released on incubation of 4 g soil with 5—10 mg of urea, at 35 °C for 2—3 hours. The method is compared with the one proposed by L. K. Porter. The method is sensitive and precise, allowing to assay the content of carbon dioxide in ca 10—15 soil samples per hour.

Introduction

The growing importance of urea as a nitrogen fertilizer in agriculture necessitates satisfactory and rapid methods of assaying urease activity in soils. Many methods have been used to assay urease activity, and numerous studies of locus of urease activity in soil have been reported. Most methods involve estimation of the ammonium released on incubation of soil with urea added (Hofmann, Schmidt, 1953; McGarity, Myers, 1967; Tabatabai, Bremner, 1972), but some involve estimation of the urea (Porter, 1965; Купревич, 1951; Zantua, Bremner, 1975) or carbon dioxide released (Skujins, McLaren, 1969), using C^{14}-urea, which is added in small quantities to C^{12}-urea.

No gas chromatographic method of estimation of urease activity in soils has been reported as yet. The investigation reported here was initiated to develop a comparatively simple and sensitive method for assaying urease activity in soils by gas chromatographic determination of carbon dioxide released on incubation of soil with urea added. The results were compared with those obtained by the method proposed by L. K. Porter (1965).

Material and methods

The soils used were surface (0—20 cm) samples. After air-drying at room temperature the soil samples were screened (2 mm sieve) and stored in plastic bags under refrigeration at 2—4° until needed.

In preliminary experiments for establishing the constant rate of hydrolysis of urea, we used 20 g of air-dry soil with 5 ml of buffer (pH 6.7) solution, 100 mg of urea with or without 0.5 ml of toluene added. The incubation was carried out in 120 ml bottles stoppered by serum bottle caps. In absence of urea, the evolution of carbon dioxide evolved by natural respiration of soil was measured. The difference of evolution of carbon dioxide in the presence or absence of urea was taken as carbon dioxide released by hydrolysis of urea. Gaseous samples were

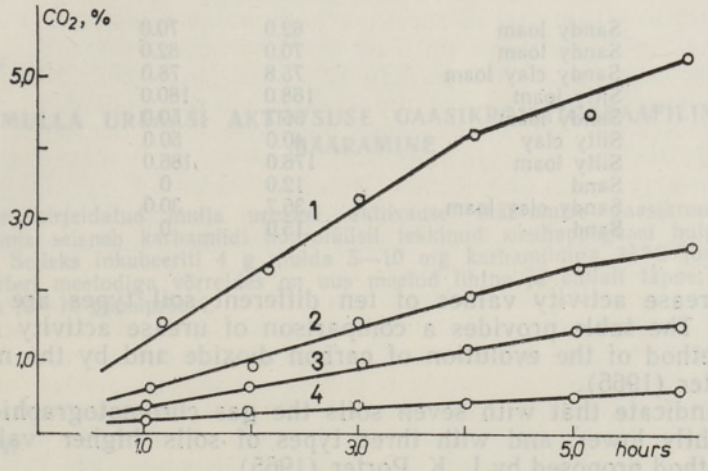
taken by a 1 ml syringe through the serum bottle cap and were analyzed by gas chromatography using a 6 m column of PORAPACK Q.

In experiments for assaying urease activity of soils we used 4 g of air-dry soil, 1 ml buffer solution (pH 6.7), 5 mg of urea and 0.1 ml of toluene in tests where it was necessary. For incubation we used 20 ml serum bottles, stoppered by serum bottle caps. The samples of soil were incubated for 3 hours at 35°. All incubation experiments were performed in duplicate or triplicate. Urease activity of soil was defined as the initial rate of hydrolysis of urea in the experiment.

Urease activity of soil, assayed by us after L. K. Porter (1965), involved calculation of urea content before and colorimetric determination of urea after incubation.

Results and discussion

The gas chromatographic method developed for assaying urease activity in soils involves estimation of the carbon dioxide released by hydrolysis on incubation of soil with urea. In the present work the effect of varying the substrate concentration was not studied because according to L. A. Douglas and I. M. Bremner (1971) and M. A. Tabatabai and I. M. Bremner (1972), the substrate concentration is not considered a limiting factor in the assay procedure. In assaying enzyme activity in soil it is important to use a method not requiring a long incubation time because the risk of error through microbial activity and through secondary processes increases with an increase in the time of incubation.



Evolution of carbon dioxide on incubation of sandy clay loam in the presence of urea (*1*, *2*) and toluene (*2*, *4*) or absence of urea (*3*, *4*) and toluene (*1*, *3*), respectively.

Preliminary experiments established (Fig.) that with all soils studied, there was a linear relationship between the time of incubation (up to 3—4 hours) and the amount of urea hydrolyzed. So it was ascertained that the formation of carbon dioxide at an assay of urease activity in soil was a zero-order reaction for 3 hours at least. Thus the incubation time of 2—3 hours insures that the measurement of carbon dioxide evolution takes place during the zero-order reaction time.

As it was established (Fig.), the sensitivity of gas chromatographic

method developed for assaying urease activity in soil makes it possible to reduce the incubation time to an hour or even to half an hour.

Toluene is commonly used to inhibit microbial growth and assimilation on enzymatic reaction products at assaying the enzymatic activity in soils. Investigations by M. I. Zantua and I. M. Bremner (1975, 1976) established that the results of soil urease activity are not significantly affected by an addition of toluene. However, R. C. Dalal (1975) reported that toluene greatly decreased the results obtained at assaying urease activity in some Trinidad soils by a non-buffer method, involving determination of the urea hydrolyzed on incubation of urea treated soils at 37° for 4 hours.

In the present investigation we studied the effect of toluene in the rate of evolution of carbon dioxide during the incubation of soil with or without urea. Experiments showed that the rate of evolution of carbon dioxide decreased in the presence of toluene (Fig.) approximately 40—50%.

Comparison of two methods of assaying urease activity in soils

Soil type	Urease activity µg/urea/h	
	Gas chromatographic method, incubated with toluene	Method of L. K. Porter (1965)
Sandy loam	62.0	70.0
Sandy loam	70.0	82.0
Sandy clay loam	75.8	78.0
Silty loam	168.0	180.0
Sandy loam	56.7	50.0
Silty clay	40.0	50.0
Silty loam	176.0	186.0
Sand	12.0	0
Sandy clay loam	36.7	30.0
Sand	15.0	0

The urease activity values of ten different soil types are given in the table. The table provides a comparison of urease activity measured by the method of the evolution of carbon dioxide and by the method of L. K. Porter (1965).

Data indicate that with seven soils the gas chromatographic method gave slightly lower, and with three types of soils higher values than by the method proposed by L. K. Porter (1965).

The results of the investigation confirmed that the determination of urease activity of soils using the method of assaying the amount of the carbon dioxide released by hydrolysis of urea, gives comparatively precise results and considerably simplifies the procedure.

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MULLA UREAASI AKTIIVSUSE GAASIKROMATOGRAAFILINE MÄÄRAMINE

Artiklis on kirjeldatud mulla ureaasi aktiivsuse määramise gaasikromatograafilist meetodit, mis seisneb karbamiidi hüdrolüüsil tekkinud süsihappegaasi hulga kindlakstegemises. Selleks inkubeeriti 4 g mulda 5—10 mg karbamiidiga 35 °C juures 3 tunni vältel. Porteri meetodiga võrreldes on uus meetod lihtne ja küllalt täpne; tunnis võib analüüsida 10—15 gaasiproovi.

Юри КАУП

ГАЗОХРОМАТОГРАФИЧЕСКОЕ ОПРЕДЕЛЕНИЕ УРЕАЗНОЙ АКТИВНОСТИ ПОЧВЫ

Настоящий метод заключается в определении двуокиси углерода, образующейся в почве во время инкубации; 4 *г* почвы с 5—10 *мг* мочевины при температуре 35 °С в течение 3 *ч*. Газохроматографическое определение уреазной активности почвы является более чувствительным способом по сравнению с методом Портера. Этот способ позволяет исследовать 10—15 газовых проб в час.