Urease

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The determination of urease activity in soil gives information about the activity of different fertilizers (refer to p. 722). The method described here was developed for the examination of soil[1,2], but it is also suitable for plant analysis.

Titrimetric Method

Principle

Urease catalyses the reaction:

(1) $$H_2N-CO-NH_2 + H_2O \longrightarrow CO_2 + 2\,NH_3$$

The increase in the amount of NH_3 with time is a measure of the urease activity. After the addition of alkali, the NH_3 is steam-distilled under reduced pressure at $<40°C$ from the reaction mixture and titrated.

Optimum Conditions for Measurements

The pH optimum of the urease from soil (acid loam; heavy clay; partly peaty and partly loamy sand; humus) is between 6.5 and 7.0. The optimum urea concentration is 1 g./assay mixture.

Reagents

1. Urea
2. Potassium dihydrogen phosphate, KH_2PO_4
3. Disodium hydrogen phosphate, $Na_2HPO_4 \cdot 2\,H_2O$
4. Sodium hydroxide, 20% (w/v) and 0.1 N
5. Sulphuric acid, 0.1 N
6. Toluene
7. Alizarin sulphonic acid (sodium salt)

Preparation of Solutions

I. Phosphate buffer (1 M; pH 6.7):

Dissolve 17.805 g. $Na_2HPO_4 \cdot 2\,H_2O$ in distilled water with warming and make up to 100 ml. Dissolve 13.61 g. KH_2PO_4 in distilled water and make up to 100 ml.
Mix the solutions and check the pH with a glass electrode.

II. Urea (10% w/v):

Dissolve 10 g. urea in distilled water and make up to 100 ml.

III. Sulphuric acid containing indicator (0.1 N H_2SO_4):

Dissolve a spatula tip of alizarin sulphonic acid (Na salt) in 1000 ml. 0.1 N H_2SO_4.

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[1] *Ed. Hofmann* and *W. Schmidt*, Biochem. Z. *324*, 125 [1953].
[2] *R. Thun*, *R. Hermann* and *E. Knickmann* in: Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik. Neumann, Radebeul 1955, Vol. I, p. 222.

Stability of the solutions

Providing that bacterial contamination is avoided all the solutions are stable practically indefinitely.

Procedure

Experimental material

Soil: Use air dried, sieved (mesh size: 1 mm.) soil.

Plants: Use dry preparations or glycerol extracts (see p. 906). The seeds of leguminosae are especially rich in urease.

Method

For each sample prepare a blank for the determination of the ammonia content, but only distill and titrate this blank.

Enzymatic reaction:

Add to a 50 ml. wide-necked Erlenmeyer flask:

10 g. dry, sieved soil
1 ml. toluene.

Mix thoroughly, allow to stand for 15 min. and then pipette in

10 ml. buffer (solution I)
10 ml. urea solution (II).

Mix thoroughly and incubate for 48 hours at 37° C; shake after 24 hours.

Distillation and titration:

Transfer the enzymatic reaction mixture to a 250 ml. distillation flask and add

20 ml. 2% NaOH.

Add*)

10 ml. 0.1 N H_2SO_4 (solution III)

to the distillation receiver. Steam-distill for 15 min. at reduced pressure and at less than 40° C.

Titrate the contents of the receiver with

0.1 N NaOH

until the colour changes from yellow to pink. Duplicate determinations should agree within 0.2 ml. 0.1 N NaOH.

Calculations

The amount of 0.1N H_2SO_4 neutralized by the ammonia distilled over gives the urease units. In the calculations the ml. of 0.1 N NaOH required to titrate the blank must be allowed for.

Therefore:

$[10 - (\text{ml. NaOH})_{\text{sample}}] - [10 - (\text{ml. NaOH})_{\text{blank}}] = (\text{ml. NaOH})_{\text{blank}} - (\text{ml. NaOH})_{\text{sample}}$ = urease units in 10 g. dry soil.

Evaluation of the results

According to[1] the following are typical values: loam (pH 4.15): 4.4 units/10 g., quaternary top loam (pH 6.55): 7.8 units/10 g. The urease activity decreases with the depth from which the soil was obtained (soil profile)[1]. Soil with vegetation contains more urease activity than that without[1]: soil without vegetation: 7.9 units/10 g., the same soil with a growth of grass; 16.8 units/10 g.

*) With soils especially rich in humus use 50 ml. 0.1 N H_2SO_4 (solution III).

Colorimetric Method[3]

Principle

See p. 913. Ammonia is determined colorimetrically after the formation of indophenolblue[4].

Reagents

1. Urea
2. Citric acid, A. R.
3. Potassium hydroxide, 1 N
4. Sodium hydroxide
5. Toluene
6. Phenol
7. Ethanol
8. Methanol
9. Acetone
10. Sodium hypochlorite
    containing at least 0.9% active chlorine*).
11. Ammonium sulphate, A. R.

Preparation of Solutions

I. Citrate buffer (pH 6.7):
Dissolve 368 g. citric acid in *ca.* 800 ml. distilled water. Dissolve 295 g. KOH in *ca.* 300 ml. distilled water. Combine the two solutions, cool, adjust to pH 6.7 with *ca.* 1 N KOH (glass electrode) and dilute to 2000 ml. with distilled water.

II. Urea (10% w/v):
Dissolve 10 g. urea in distilled water and make up to 100 ml.

III. Sodium phenate (12.5% w/v):
a) Dissolve 62.5 g. phenol in the smallest amount of ethanol, add 2 ml. methanol and 18.5 ml. acetone and dilute to 100 ml. with ethanol. Store the solution in a refrigerator.
b) Dissolve 27 g. NaOH in distilled water and make up to 100 ml.
Just before use mix 20 ml. of solutions a) and b) and make up to 100 ml. with distilled water.

IV. Sodium hypochlorite, NaOCl:
Dilute the commercial solution with distilled water so that it contains 0.9% active chlorine. The solution is stable.

V. Ammonium sulphate standard solution (1.21 mg. NH_3/ml.):
Dissolve 4.717 g. $(NH_4)_2SO_4$ in distilled water and make up to 1000 ml. Dilute 10 ml. of this solution to 1000 ml. with distilled water. 1 ml. of this solution contains 10 μg. N.

Procedure

Experimental material, see p. 914.

*) Active chlorine: determined, for example, according to[5] by addition of KI, acidification with HCl and back-titration with $Na_2S_2O_3$ solution.

[3] *G. Hoffmann* and *K. Teicher*, Z. Pflanzenernährung, Düngung, Bodenkunde *95 (104)*, 55 [1961].

[4] *A. B. Crowther* and *B. S. Large*, Analyst *81*, 64 [1956].

[5] *L. Medicus:* Massanalyse. 14 th. Ed. Steinkopff, Dresden 1952, p. 181.

Enzymatic reaction

For each sample prepare a blank containing distilled water instead of the urea solution (II). To 100 ml. wide-necked volumetric flasks add:

10 g. dry, sieved soil
1.5 ml.*) toluene.

Mix well, allow to stand for 15 min., then add:

10 ml. urea solution (II)
20 ml. citrate buffer (solution I).

Mix thoroughly, stopper with a rubber bung and incubate for 3 hours at 37° C in an incubator or in a water bath. Dilute to 100 ml. with distilled water (at 38° C), mix thoroughly by shaking and immediately filter through a fluted filter paper. Analyse the filtrate.

Colorimetric measurements

Wavelength: 630 or 580 mμ; light path: 1 cm.; room temperature. Measure against the blank. Pipette into 50 ml. volumetric flasks with thorough mixing:

1 ml. filtrate
9 ml. distilled water
4 ml. phenate solution (III)
3 ml. NaOCl solution (IV).

Allow to stand for 20 min. until the maximum colour is obtained. Dilute to 50 ml. with distilled water and mix thoroughly. Read the optical density within 60 min.

Standard curve

Prepare at least two standards containing 2 and 8 ml. $(NH_4)_2SO_4$ solution (V) (corresponding to 20 and 80 μg. ammonia nitrogen) instead of the filtrate and part of the distilled water. Measure in the same way as the sample. The standard curve (ordinate: optical density, abscissa: μg. N) is linear up to 100 μg. N. For the analysis of peaty soil prepare two further standards containing 6 and 9 ml. double strength $(NH_4)_2SO_4$ standard solution (corresponding to 120 and 180 μg. N) which fall on the flatter part of the standard curve[6].

Calculations

The "urease number" gives the amount of urease contained in 100 g. soil. The urease number 1 corresponds to the amount of enzyme which, under the conditions described here, hydrolyses 1 mg. of N as NH_3 from urea.

Obtain from the standard curve the μg. N corresponding to the difference in optical density between the sample and the reagent blank. As 1 ml. of the filtrate after the enzymatic reaction is equivalent to 100 mg. of the soil sample, the number of μg. N is the same as the mg.N/100 g. soil sample and therefore is equal to the urease number.

Multiplication of the urease number by 0.32 gives the urease units (see p. 914).

Evaluation of the results

See p. 914.

*) Take more with peaty soil, up to 5 ml.; sufficient toluene must be taken so that after dilution with water the toluene forms a layer floating on the surface.

6) *U. Bohnstedt*, Z. analyt. Chem. *163*, 415 [1958].