β-Glucosidase

Eduard Hofmann

The β-glucosidase activity found in cultivated land is due to bacteria and moulds[1]. The method described here was specially developed for the examination of soil[2,3], but in principle it is also applicable to plant material.

Principle

The β-glucosidase from micro-organisms and also the enzyme from sweet almonds (emulsin) hydrolyse aryl and alkyl-β-glucosides without the transfer of any groups[4]. In the method described here glucose is cleaved from the synthetic substrate β-D-glucosido-hydroquinone (arbutin), and the glucose is then determined volumetrically with *Fehling*'s solution.

Optimum Conditions for Measurements

The pH optimum of β-glucosidase in soil of different pH values (loamy sand, sandy loam, neutral soil) was found to be between 5.6 and 6.4 (mean 6.2)[2]. The optimum substrate concentration is 0.5 g. arbutin/assay mixture[2].

Reagents

For the enzymatic reaction:

1. Disodium hydrogen phosphate, $Na_2HPO_4 \cdot 12\ H_2O$
2. Acetic acid, A. R., 1 N
3. Toluene
4. β-D-Glucosido-hydroquinone (arbutin)*)

For the glucose estimation:

See the chapter on "Invertase", p. 904.

Preparation of Solutions

For the enzymatic reaction:

I. Buffer (pH 6.2):
   a) Dissolve 358 g. $Na_2HPO_4 \cdot 12\ H_2O$ with warming in distilled water, make up to 1000 ml. and cool to 25°C.
   b) Dilute 60 ml. acetic acid to 1000 ml. with distilled water.
   Mix 1 volume solution a) with 1.8 volumes solution b). Check the pH with a glass electrode and, if necessary, adjust with solution a) or b).

II. Arbutin (10% w/v):
   Dissolve 25 g. arbutin in distilled water and make up to 250 ml.

For the glucose estimation:

See solutions III to VI on p. 905.

*) Obtainable from E. Merck, Darmstadt, Germany.

1) *C. Neuberg* and *Ed. Hofmann*, Biochem. Z. *256*, 450 [1932]; *234*, 345 [1931]; *Ed. Hofmann*, Biochem. Z. *272*, 133, 426 [1934].
2) *Ed. Hofmann* and *G. Hoffmann*, Biochem. Z. *324*, 397 [1953].
3) Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik. Neumann, Radebeul 1955, Vol. I, p. 222.
4) *J. Larner* in *P. D. Boyer*, *H. Lardy*, and *K. Myrbäck:* The Enzymes. 2nd ed., Academic Press, New York, London 1960, Vol. 4, p. 369.

Stability of the solutions

See p. 902.

Procedure

Experimental material

As for the measurements of invertase activity, p. 905.

Enzymatic reaction

A blank is usually only necessary with peaty soil and plants. Prepare the blank in the same way as the unknown, but with water instead of the arbutin solution (II). As the activity of plant samples varies considerably, the first assay should be treated as a preliminary one to obtain the range. On repeating the assay take only sufficient sample so that about $^3/_4$ of the added arbutin is hydrolysed.

Add to 100 ml. volumetric flasks:

10 g. dry soil sample or plant sample after preliminary assay
1.5 ml. *) toluene.

Mix thoroughly by shaking and after 15 min. pipette in

15 ml. buffer solution (I)
5 ml. arbutin solution (II).

Stopper with a rubber bung and incubate for 95 hours in an incubator at 37°C. Shake occasionally, dilute to 100 ml. with

tap water at 37°C.

Shake and continue incubation at 37°C. After exactly 96 hours analyse the supernatant for glucose.

Glucose estimation[5]

Proceed as for the estimation of glucose in the chapter on "Invertase", p. 905. It should be noted that after the titration is complete the solution quickly becomes blue again, therefore take the first sharp colour change as the end-point. Humus is darkly coloured, so that the starch indicator should be added early.

Calculations

As in the chapter on "Invertase", p. 907.

Evaluation

See p. 722.

*) With peaty soil take more, up to 5 ml. Sufficient toluene should be added so that after the dilution with water the toluene forms a layer floating on the surface.

5) *N. Schorl* and *A. Regenbogen*, Z. analyt. Chem. *56*, 191 [1917].